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

Project Title: Improved late- and post-bloom sanitation of fire blight pathogen

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Budget: **Year 1: $18,100 (apple) *** **Year 2: $18,462 (apple) ***

Other funding sources

Agency Name: USDA NIFA ORG
Amt. awarded: $495K to Johnson, Elkins, Granatstein and Smith 10/14 - 9/17
Notes: Objectives of this proposal are supplemental to objectives for the above 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	**2016-17**	**2017-18**	
Salaries Faculty Res. Assist. 2 mo.	9,200	9384	
Benefits OPE 58%	5,336	5443	
Undergraduate labor (&OPE 12%)	1064	1085	
Equipment			
Supplies	1,250	1275	
Local Travel	250	255	
Miscellaneous			
Plot Fees	1,000	1,020	
Total	**$18,100**	**$18,462**	

***Footnotes: Total Budget** **Year 1**: $36,200 **Year 2**: $36,924 (2% inflation)
50% by WTFRC Apple Crop Protection, 50% by FPC/WTFRC Pear.

OBJECTIVES

1) Evaluate EPA-registered materials for their ability to reduce epiphytic populations of the fire blight pathogen, *Erwinia amylovora*, on pear and apple trees, and to kill *E. amylovora* in laboratory-based dose-response experiments.

2) Evaluate the mineral material, alum ($KAl(SO_4)_2$), for fire blight control, to reduce epiphytic populations of *E. amylovora* on pear and apple trees, and to kill *E. amylovora* in laboratory-based dose-response experiments.

3) Evaluate and characterize the abilities of near-commercial preparations of *E. amylovora*-specific phage and protective amendments (sunscreens and carrier strains) for fire blight control, to reduce epiphytic populations of *E. amylovora* on pear and apple trees, and to kill *E. amylovora* in laboratory-based experiments dose-response experiments.

SIGNIFICANT FINDINGS

- In each of eight pear and apple trails conducted in 2016 and 2017, epiphytic populations of the fire blight pathogen on flowers increased after full bloom and reached a maximum at one week after petal fall.
- In general, materials that suppress infection also reduce pathogen inoculum on flowers. In 2016, under weather conditions highly conducive for fire blight, numerous materials were only fair at inoculum suppression including Bacillus-based biorationals (e.g., Serenade Opti), a three-quart rate of Cueva soluble copper, and experimental phage-based materials.
- Blossom Protect (*Aureobasidium pullulans*) provided very good fire blight control, but this material does not effectively suppress pathogen populations of flowers.
- Integrated programs that began with Blossom Protect and ended with a non-antibiotic chemical were more effective at suppressing pathogen populations than programs based on a single non-antibiotic material.
- Alum (1%, 8 lbs/100 gal) provided intermediate inoculum sanitation and excellent fire blight control.
- Among EPA-registered materials for non-antibiotic fire blight control, Previsto soluble copper stood out as an effective material for both infection suppression and inoculum sanitation.
- Late bloom (petal fall) treatments of lime sulfur (2 to 4 %) provided good inoculum sanitation, fire blight control and improved fruit finish.
- Acidifying oxytetracycline with buffer protect (pH 4.5) improved the level of inoculum sanitation and fire blight control from this antibiotic.

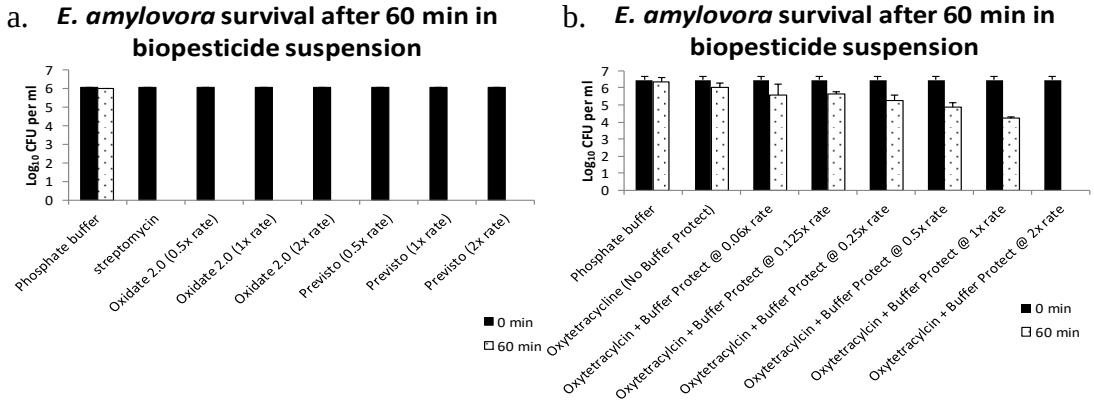
RESULTS & DISCUSSION

Obj. 1.a. Laboratory-based dose-response experiments to evaluate effect of EPA-registered materials on killing *E. amylovora* in vitro.

The purpose of this sub-objective was to develop laboratory-based assays to measure and compare the effects of fire blight-control materials on survival of *E. amylovora*. The assay exposed suspensions of pathogen cells (1×10^6 FU/ml) to a dose of a material for a period of time (e.g., 60 min). Pathogen cells were recovered from suspension by filtration, washed in phosphate buffer, then dilution plated on nutrient agar to determine survivorship relative to a non-treated control.

In conducting these assays, we obtained results with some materials that correlated positively with what we observe in the field and results with other materials that were contradictory to (not predictive of) what we see in the field. After numerous assays, we concluded that this approach is not a useful expenditure of time and effort. For example in **Fig. 1a**, labeled rates of streptomycin, Previsto and OxiDate 2.0 were highly effective at killing *E. amylovora* in the lab-based assay, but in field trials we observed that while strep and Previsto are effective at suppressing the pathogen on apple and pear flowers, OxiDate has only a slight effect these same floral populations. Another example shows that oxytetracycline was relatively poor at killing *E. amylovora* after a 60 min exposure in the laboratory suspension (**Fig. 1b**) even when buffered at a lower pH with citrate (Buffer Protect). In contrast, in the field, oxytetracycline by itself shows intermediate suppression of *E. amylovora* populations on flowers, which was significantly enhanced in the field by the addition of Buffer Protect (data below). Potential reasons for lack of correlation between lab assay and field performance of a material likely include the disparities in length of effective residuals in the different environments, rates of material uptake by bacterial cells, and potential interactions of a material with the host surface.

Fig. 1. Laboratory-assay results that were not fully consistent with field observations.



Obj. 1.b. Effect of EPA-registered materials on late- and post-bloom sanitation of fire blight pathogen on flowers in apple and pear orchards.

In contrast to laboratory assays, the measurement of epiphytic pathogen populations on apple and pear flowers during the bloom period was insightful for understanding the efficacy of the various materials for fire blight control. As in 2016 (see previous report), the highest epiphytic populations were usually measured on the water-treated control. Also as in 2016, the highest pathogen populations were observed in samples taken at 'petal fall + one week' (when compared to samples taken at full bloom or petal fall). This latter observation suggests that extending spray programs into petal fall could have beneficial effects on late bloom sanitation and infection suppression.

For the most part, measured epiphytic populations of *E. amylovora* correlated positively with incidence of infection but there were exceptions. The figures that follow (**Figs. 2-5**) depict effects of control materials on pathogen populations of flowers in four 2017 orchard trials (see previous report for 2016 data). Materials that suppressed final pathogens populations to less than 10^5 cfu/flower (100,000 cells/flower) provided excellent infection suppression. In this regard, antibiotics (streptomycin, kasugamycin, and oxytetracycline) and soluble coppers (Previsto 3 qt and Cueva 4 qt) showed most consistent suppression of the pathogen. Materials that did not cause a large reduction in pathogen populations included Bacillus-based materials (e.g., Serenade Opti), which also gave relatively poor disease control. Blossom Protect plus Buffer Protect is an example of a treatment that had only slight effects of epiphytic *E. amylovora* populations but was effective for disease control. The addition of a half rate of Buffer Protect to oxytetracycline improved the ability of this antibiotic to suppress floral pathogen populations.

Fig. 2. Effect of treatments applied to Bartlett pear trees to suppress fire blight on populations of *E. amylovora* strain 153N on flowers during the bloom period of April 2017. The 58-yr-old orchard was located near Corvallis, OR with each treatment applied to four replicate trees. Pathogen populations were determined by bulk sampling five flower clusters (~25 flowers) from each replicate tree with each sample washed in 25-ml of sterile phosphate buffer followed by dilution plating on to nutrient agar plus nalidixic acid. Owing to cold weather in early bloom, the pathogen was detected only in the 'petal fall plus one week' sample; results of 'full bloom' sample not shown. Panel A: Antibiotics and Serenade Opti with and without a 75 oz. rate of Buffer Protect. Panel B: biologicals and alum. Horizontal dashed line in Panel A indicates the detection limit of the assay. Data depict mean and standard error of each treatment program on each sampling date.

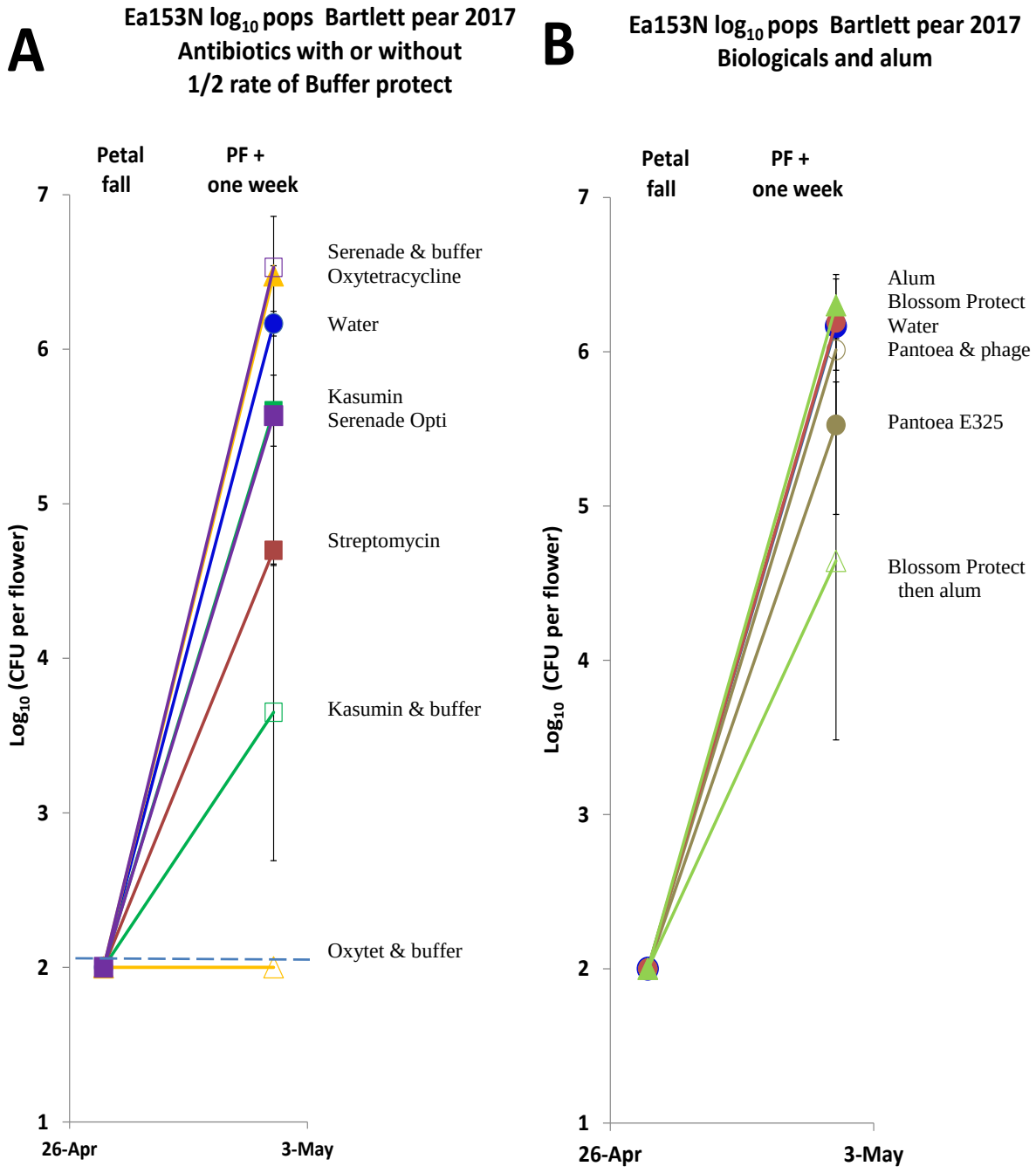


Fig. 3. Effect of treatments applied to Bartlett pear trees to suppress fire blight on populations of *E. amylovora* strain 153N on flowers during the bloom period of April 2017. The 17-yr-old orchard was located near Corvallis, OR with each treatment applied to four replicate trees. Pathogen populations were determined by bulk sampling five flower clusters (~25 flowers) from each replicate tree with each sample washed in 25-ml of sterile phosphate buffer followed by dilution plating on to nutrient agar plus nalidixic acid. Panel A: Integrated control programs. Panel B: streptomycin and selected soluble copper materials. Horizontal dashed line in Panel A indicates the detection limit of the assay. Data depict mean and standard error of each treatment program on each sampling date.

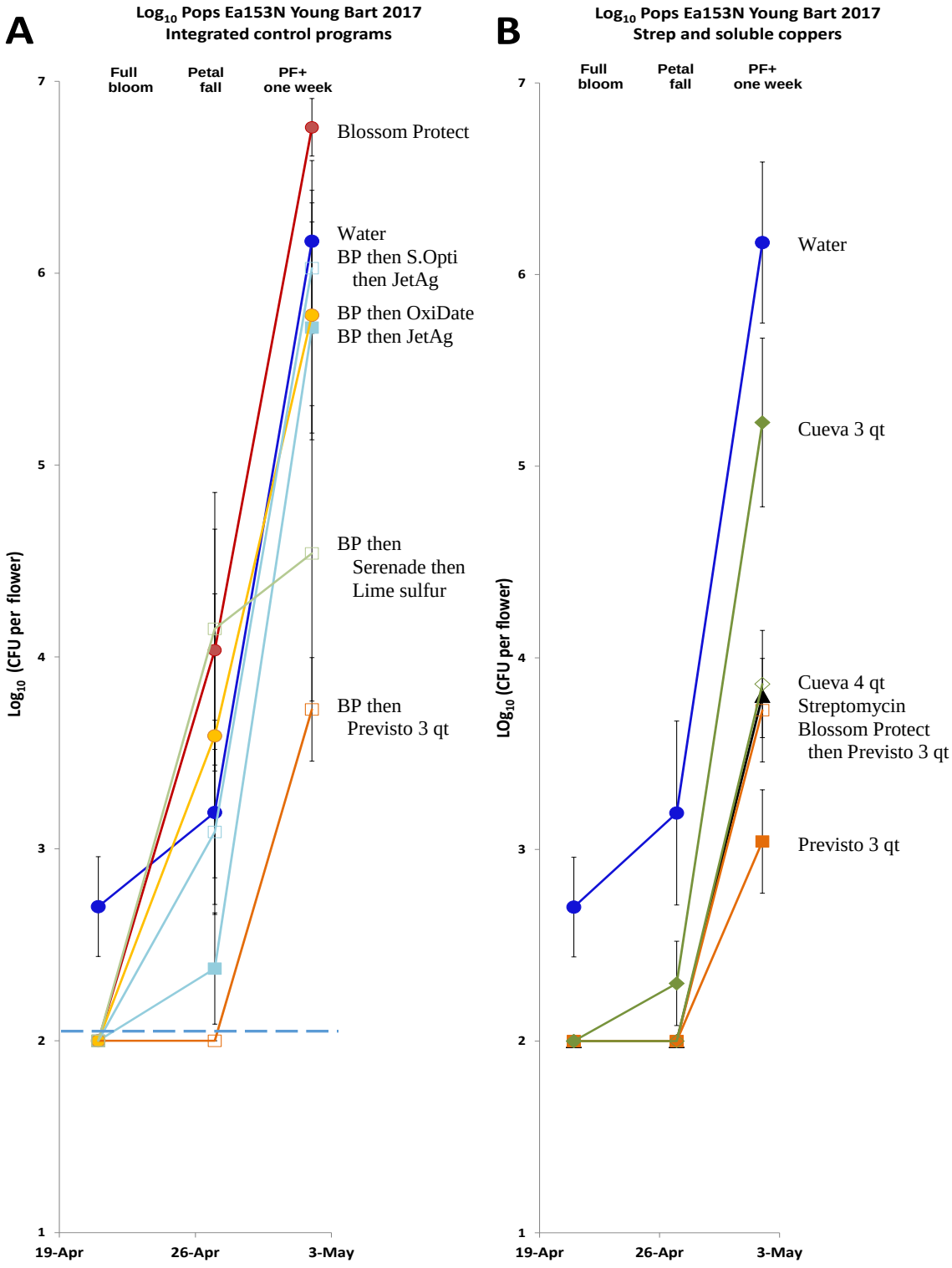


Fig. 4. Effect of treatments applied to Golden Delicious apple trees to suppress fire blight on populations of *E. amylovora* strain 153N on flowers during the bloom period of April and May 2017. The 37-yr-old orchard was located near Corvallis, OR with each treatment applied to four replicate trees. Pathogen populations were determined by bulk sampling five flower clusters (~25 flowers) from each replicate tree with each sample washed in 25-ml of sterile phosphate buffer followed by dilution plating onto nutrient agar amended with nalidixic acid. Panel A: FireLine (oxytetracycline) and Serenade Opti with and without a half label-rate of Buffer Protect. Panel B: Blossom Protect and Buffer Protect followed by alum, VP20, Previsto, or Serenade Opti then Rex Lime Sulfur; and solitary material treatments of Blossom Protect and Buffer Protect, alum, or VP20. Horizontal dashed line in Panel A indicates the detection limit of the assay. Data depict mean and standard error of each treatment program on each sampling date.

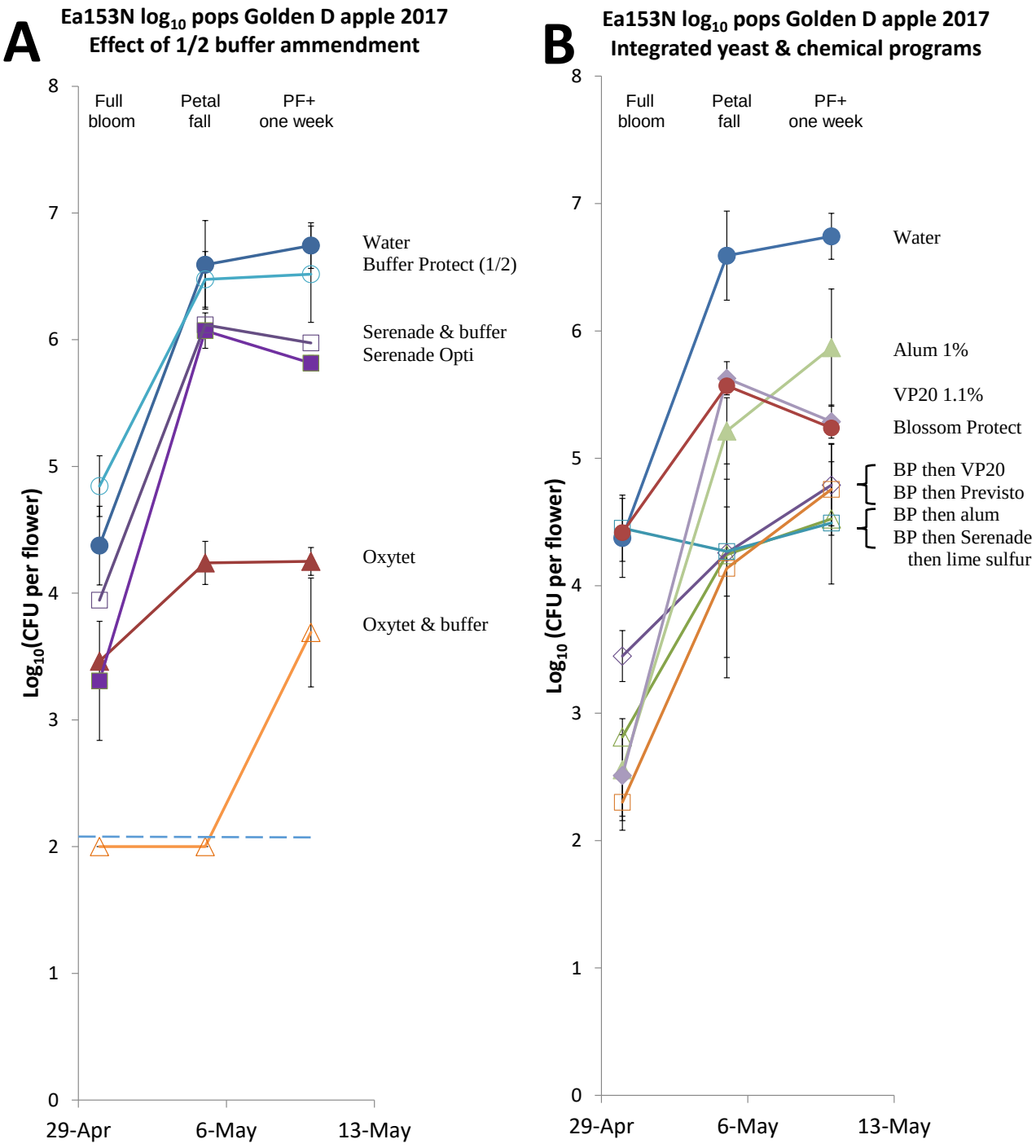
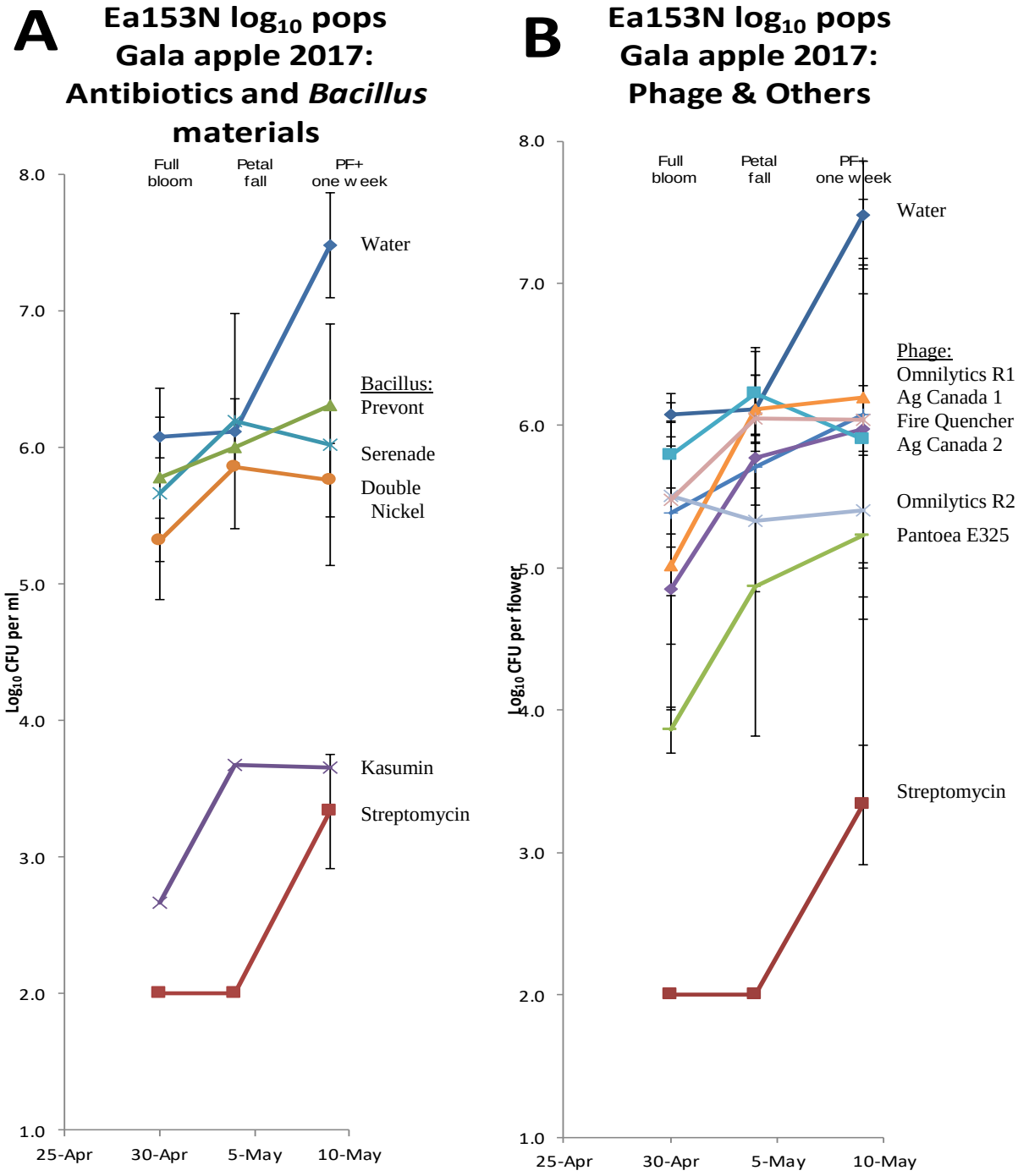


Fig. 5. Effect of treatments applied to Gala apple trees to suppress fire blight on populations of *E. amylovora* strain 153N on flowers during the bloom period (late-April to early-May 2017). The 17-yr-old orchard was located near Corvallis, OR with each treatment applied to four replicate trees. Pathogen populations were determined by bulk sampling five flower clusters (~25 flowers) from each replicate tree on each sample date with the sample washed in 25-ml of sterile phosphate buffer followed by dilution plating onto nutrient agar amended with nalidixic-acid (25 µg/L). Flowers were sampled on the days following the full bloom and petal fall sprays, and at 1-week after petal fall. Panel A: antibiotics and *Bacillus*-based materials; and Panel B: phage materials and *P. agglomerans* E325. Data depict mean and standard error of each treatment program on each sampling date.

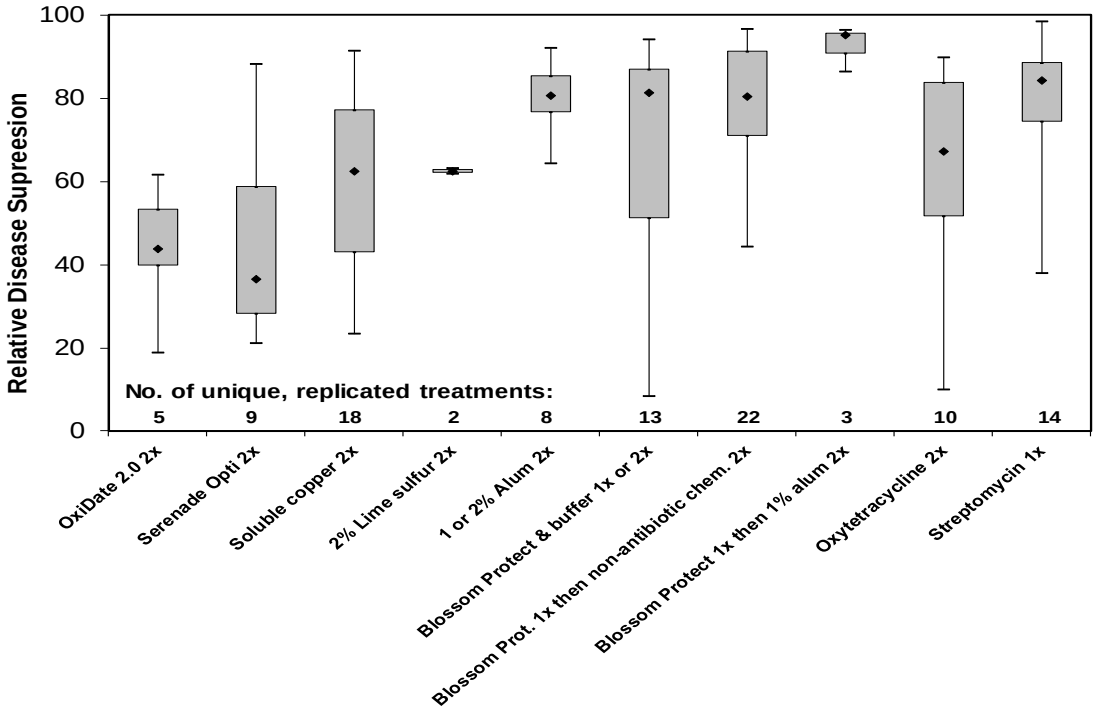


Obj. 1.c. Effect of EPA-registered materials on fire blight control in in apple and pear orchards.

Rather than show only 2017 data, a summary of important fire blight control treatments from 2013 to 2017 orchard trials is depicted below. The amount of fire blight in control trees of individual trials ranged from 7 infections per tree in Bartlett pear in 2017 to 673 infection per tree in Bartlett pear in 2014. Over all trials, the water-treated control averaged 147 infections per tree, which represented a mean of 26% of total flower clusters on the trees. As measured by the CougarBlight fire blight risk model, the conduciveness of the temperatures for epiphytic growth of *E. amylovora* varied by season with 2016 trials experiencing the most favorable conditions (extreme risk), and the 2017 season experiencing the least favorable (low risk in pear to moderate in apple) conditions. For other years, the conduciveness of temperatures for epiphytic growth was intermediate (moderate to high infection risk conditions). Primary conclusion is that non-antibiotic materials when applied as solitary treatments are less effective than antibiotics, but that integrated programs that begin with Blossom Protect (yeast) followed by a non-antibiotic chemical material can achieve a level of control on par with antiobiotics.

Fig. 6. Box and whiskers plot of relative fire blight suppression from 17 pathogen-inoculated pear and apple orchard trials conducted near Corvallis, Oregon from 2013 to 2017. Relative disease incidence was calculated for each treatment program by dividing mean number of infected flowers clusters observed on treated trees by the mean number of infected clusters on corresponding water-treated control. For each treatment, based on the trials in which it was present, the diamond is median relative disease suppression, the box is the range of the two quartiles of observations nearest the median, and the whiskers are the minimum and maximum observations. Each treatment consisted of one to three spray applications during the bloom period as indicated by the number preceding the 'x' in the axis label.

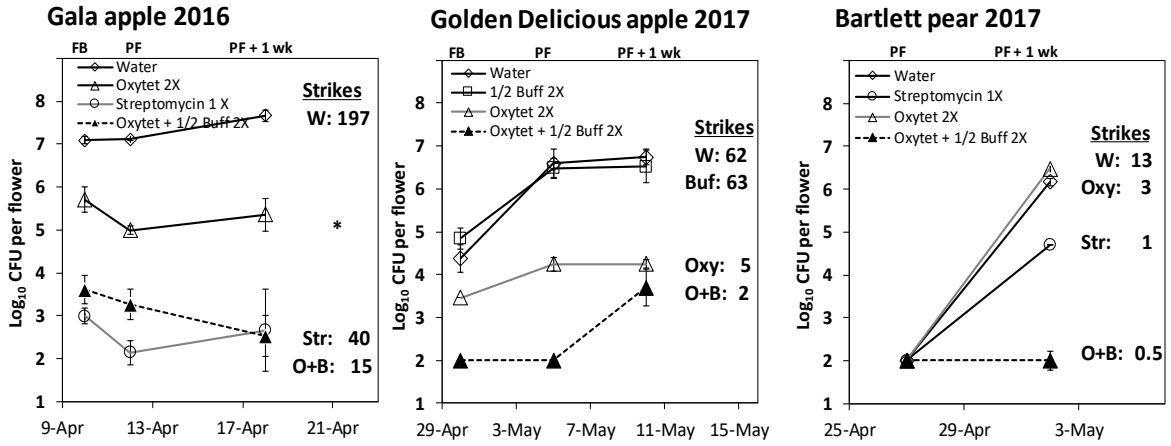
For the treatment 'Blossom Protect then non-antibiotic chemical', those chemicals were either Serenade Opti, a soluble copper (Previsto or Cueva), or OxiDate.



In the above chart (**Fig. 6**), fire blight was suppressed significantly ($P \leq 0.05$) by non-antibiotic or antibiotic treatments most of the time. The mean 'percent disease suppression relative to the control' (S_{rc}) for all evaluated non-antibiotic treatment programs was 65% $\pm$ (s.d.) 24 (n = 88). In contrast, for the antibiotic controls (streptomycin and oxytetracycline), mean S_{rc} for all treatments 72% $\pm$ (s.d.) 22 (n = 24). Box and whisker plots showed that specific NOP-approved, non-antibiotic materials (OxiDate, Serenade Opti, lime sulfur or the soluble coppers, Cueva and Previsto) tended to be only partially effective for fire blight suppression (median S_{rc}–values ranging from 35 to 62%) when sprayed as the only material in the program. For Blossom Protect and its companion buffer, a median level of control was 81%, which was intermediate to streptomycin and oxytetracycline (median S_{rc}-values of 84 and 65%, respectively). The experimental material, alum, and integrated programs that began with Blossom Protect followed by a NOP-approved non-antibiotic material also provided high levels of suppression with median S_{rc}-values of 81%. Integrated programs that consisted of Blossom Protect followed by another material showed less variability in suppression when compared to treatment programs comprised of a single material (**Fig. 6**).

A final consistent and potentially significant result from 2016-17 trials was enhanced late-bloom sanitation and disease suppression with pH-buffered oxytetracycline (i.e., the addition of a half label rate of Blossom Protect to the oxytet suspension) (**Fig. 7**). This result requires additional research to determine the optimal rate of a buffer amendment.

Figure 7. Effect of pH-buffering of oxytetracycline on fire blight-pathogen populations on apple and pear flowers sampled from orchards near Corvallis, OR. Each treatment was applied to four replicate trees. Y-axis is log scale: a value of '2.0' is 100 pathogen cells/flower (the detection limit) and a value of '6.0' is one million cells per flower.



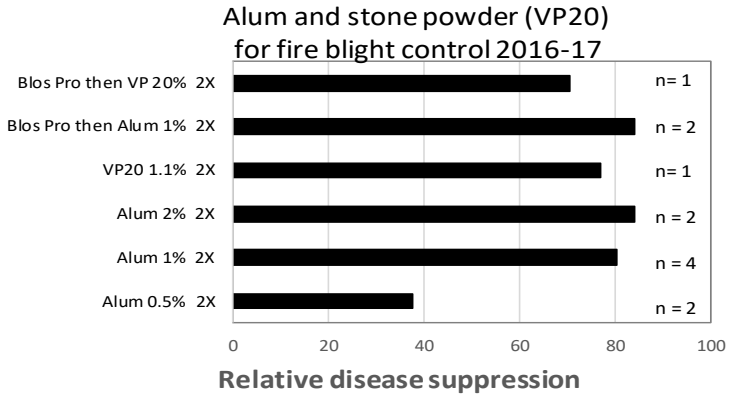
Obj. 1.d. Effect of EPA-registered materials on fruit russeting.

Because of Corvallis' wet spring environment, fruit russeting is typically moderate to severe regardless of treatment, especially for pears. For the last few seasons, we have collected fruit russeting data from selected fire blight control treatments. The results generally confirm the materials that have an enhanced risk of inducing fruit russeting (e.g., soluble coppers, and to a lesser extent, Blossom Protect). Most other materials (alum, Bacillus-based materials, biologicals, oxidizing agents) have not shown levels of russeting that are different from the water treated control. For two seasons, lime sulfur has shown a consistent reduction in fruit russeting, which we attribute to suppression of natural yeast populations (including *Aureobasidium pullulans*) (data available by request).

Obj. 2. Effect of mineral material, alum on late-bloom sanitation of fire blight pathogen on flowers and on fire blight control in apple and pear orchards.

Alum ($KAl(SO_4)_2$) provided only an intermediate level of pathogen-population suppression (**Figs. 2B and 4B**) but an outstanding level of disease control (**Fig. 6**). Alum's best fit in organic spray programs would be as a full bloom to petal fall treatment(s) after Blossom Protect. Effective rate is ~1% (8 lbs/100 gal). Alum is not currently approved for use in organic agriculture but a preliminary (and positive) OMRI-assessment was completed to utilize it as an manure amendment: http://www.ams.usda.gov/sites/default/files/media/Aluminum%20Sulfate%20Petition.pdf , and http://www.ams.usda.gov/sites/default/files/media/Aluminum%20Sulfate%20TR.pdf .
The alum containing stone powder we evaluated is an organic crop protection product sold in Europe under the name Mycosin (BIOFA AG, Münsingen, Germany, http://www.biofa-profi.de/en/about-us.html). We brought the material into the U.S. with help from Michael Braverman of IR-4 (Rutgers), who it was gave it the code name, 'VP20'.

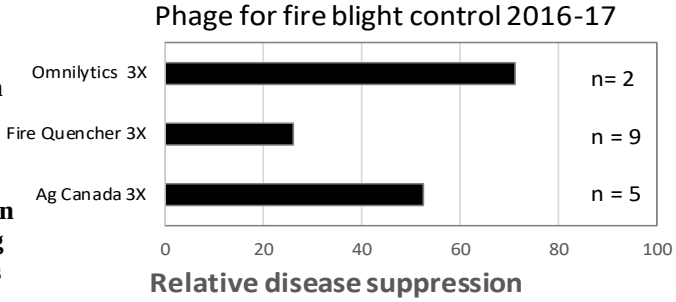
Fig. 8. Relative fire blight suppression from pathogen-inoculated pear and apple orchard trials conducted near Corvallis, Oregon from 2016 to 2017. Relative disease incidence was calculated for each treatment program by dividing mean number of infected flowers clusters observed on treated trees by the mean number of infected clusters on corresponding water-treated control. 'n' is number of times the treatment was trialed.



Obj. 3. Effect of *E. amylovora*-specific phage on late-bloom sanitation of fire blight pathogen on flowers and on fire blight control in apple and pear orchards.

Phage are viruses that attack bacteria, with which several groups are attempting to develop commercial products for fire blight management. In our hands, phage treatments have provided only intermediate levels of control (even after three applications) (**Fig. 8**), and generally poor levels of late-bloom sanitation (**Fig. 5B**). Disease suppression from phage treatments was better in 2017 than 2016, perhaps because we changed the protocol to apply the first treatment within an hour of the pathogen inoculation (how does a grower do this?). The primary drawback of phage is that they are very short-lived (µV sensitive) if their host (the fire blight pathogen) is not present at the time of treatment**.** Every season the formulations of evaluated phage materials have been modified from the previous season. Therefore, there is still a chance that one of the groups developing a product will hit on a formulation with improved efficacy.

Fig. 8. Relative fire blight suppression from pathogen-inoculated pear and apple orchard trials conducted near Corvallis, Oregon from 2016 to 2017. Relative disease incidence was calculated for each treatment program by dividing mean number of infected flowers clusters observed on treated trees by the mean number of infected clusters on corresponding water-treated control. 'n' is number of times the treatment was trialed.



EXECUTIVE SUMMARY

Project Title: Improved late- and post-bloom sanitation of fire blight pathogen
Investigator: Ken Johnson, Oregon State University

SIGNIFICANT FINDINGS

- In each of eight pear and apple trails conducted in 2016 and 2017, epiphytic populations of the fire blight pathogen on flowers increased after full bloom and reached a maximum at one week after petal fall.
- In general, materials that suppress infection also reduce pathogen inoculum on flowers. In 2016, under weather conditions highly conducive for fire blight, numerous materials were only fair at inoculum suppression including Bacillus-based biorationals (e.g., Serenade Opti), a three-quart rate of Cueva soluble copper, and experimental phage-based materials.
- Blossom Protect (*Aureobasidium pullulans*) provided very good fire blight control, but this material does not effectively suppress pathogen populations of flowers.
- Integrated programs that began with Blossom Protect and ended with a non-antibiotic chemical were more effective at suppressing pathogen populations than programs based on a single non-antibiotic material.
- Alum (1%, 8 lbs/100 gal) provided intermediate inoculum sanitation and excellent fire blight control.
- Among EPA-registered materials for non-antibiotic fire blight control, Previsto soluble copper stood out as an effective material for both infection suppression and inoculum sanitation.
- Late bloom (petal fall) treatments of lime sulfur (2 to 4 %) provided good inoculum sanitation, fire blight control and improved fruit finish.
- Acidifying oxytetracycline with buffer protect (pH 4.5) improved the level of inoculum sanitation and fire blight control from this antibiotic.

Industry implications: In the non-antibiotic era that began in 2015, the materials now used for organic fire blight control possess diverse modes of action, which are not completely understood. In particular, while it is possible to assign a ranking to material effectiveness for infection suppression during primary bloom, little has been known about how well these materials reduce (kill) floral pathogen populations that can carry over into the post-bloom period. The reason this distinction is significant relates to the fact that PNW apple and pear orchards frequently escape primary bloom infection, but develop fire blight in late and secondary flowers, and in rapidly growing shoots in warmer, unsettled weather of late spring. The late- and post-bloom period is also the period of high sensitivity to chemical-induced fruit russeting, which restricts choice of materials available for late- and post-bloom sanitation.

Results of this project showed that a) antibiotics are better at for inoculum sanitation than non-antibiotic materials, b) integrated non-antibiotic programs that begin with Blossom Protect followed by a non-antibiotic chemical is a valid strategy for fire blight control and offers an intermediate level of inoculum sanitation, and c) acidifying spray suspensions by buffering can potentially improve inoculum sanitation; this result needs further research. The material, alum (/organic stone powder) should be considered for commercial implementation. Materials based on bacteriophages (viruses that infect and kill the fire blight pathogen) were not particularly effective at reducing pathogen inoculum and likely are not commercially useful at this time.