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

Project Title: Fire blight management in organic and conventional apple

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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

Total Project Funding: $44,660

Budget History:

Item	**2012**	**2013**	
Salaries Faculty Res. Assist.	11,000	11,330	
Benefits OPE 56%	6,160	6,345	
Wages undergrads	1,800	1,854	
Benefits OPE 8%	144	148	
Equipment			
Supplies	1,896	1,953	
Travel	500	515	
Miscellaneous			
Plot Fees	500	515	
Total	**$22,000**	**$22,660**	

Footnotes: Annually**,** FRA 3 mo plus fringe, 150 hr undergrad labor, 2K M&S, 1K local travel & plot fee, 3% inflation

OBJECTIVES

1) Understand the relative toxicity of fruit crop load (bloom) thinning materials to the fire blight pathogen, and to bacterial and fungal biological control agents.

2) Achieve an improved understanding of floral colonization by the yeast biological control agent, *Aureobasidium pullulans.*

3) In the field, evaluate an inducer of systemic acquired resistance for protection of apple from fire blight and as an aid to cutting of blight in scions of young apple trees.

SIGNIFICANT FINDINGS

- Oversprays of the bloom thinning agent, lime sulfur, suppressed populations of the fire blight pathogen and of biological control agents after their establishment on apple flowers.
- Treatment with *A. pullulans* (Blossom Protect) after lime sulfur and fish oil reduced fire blight infections by 91% compared with water only; this level of control level was similar to streptomycin against a strep-sensitive pathogen strain.
- The yeast, *A. pullulans* (Blossom Protect), is an excellent colonist of both the floral stigma and floral cup, which differentiates it from bacterial biocontrol agents that colonize only the stigma.
- In parallel trials at Corvallis, OR, Wenatchee, WA, and Lakeport, CA, *A. pullulans* colonized nearly 100% of flowers on trees treated once with Blossom Protect at early to mid-bloom.
- Over three seasons, the addition the systemic acquired resistance material, acibenzolar-S-methyl (Actigard) to antibiotic treatments significantly enhanced fire blight control.
- Paints of Actigard onto EMLA 26 rootstocks reduced canker size and tree death after inoculation of the rootstocks with the fire blight pathogen.

RESULTS & DISCUSSION

Obj. 1a. Effect of fruit crop load thinners on microbial populations. Compared to the untreated control (UTC), lime sulfur oversprays onto pre-established epiphytic microbe populations on Golden Delicious flowers reduced significantly ($P < 0.05$) the population sizes of the fire blight pathogen (*Erwinia amylovora)* and the biocontrol agents, *Pantoea agglomerans* (Bloomtime Biological) and *A. pullulans* (Blossom Protect), regardless of sampling date or the rate of lime sulfur applied (Fig.1). Similarly, for the first sampling date from Rome Beauty apple, epiphytic populations of *E. amylovora* and *A. pullulans* were significantly reduced by all rates of lime sulfur, but the effects of lime sulfur on the population size of *P. agglomerans* were inconsistent (Fig. 1). On the second sampling date, only the *E. amylovora* populations on the Rome Beauty flowers were suppressed significantly by the lime sulfur treatments.

Discussion. We have shown that lime sulfur partially suppresses fire blight (see Fig. 2 below). The reason is likely twofold: 1) the treatment causes flower abscission, which reduces the number of flower clusters that become diseased, and 2) lime sulfur is directly toxic to epiphytic microbes on the flowers. Recent surveys that we made on the detectability of epiphytic *E. amylovora* in pear and apple flowers sampled from commercial orchards found that the likelihood of positive pathogen detection is relatively small (< 5%) during early to mid-bloom when thinning agents are typically applied, but increases five- to 20-fold by petal fall. Consequently, because of its

antibacterial properties, lime sulfur is likely sufficient in most orchards to delay/suppress the epiphytic increase of *E. amylovora* in early bloom, and that the biological materials specifically registered for fire blight control can be implemented after the bloom thinning protocol is completed. The deleterious effects of lime sulfur oversprays onto biological antagonists (*P. agglomerans* and *A. pullulans*) also indicates that antagonist treatments should be delayed until after the bloom thinning protocol is complete.

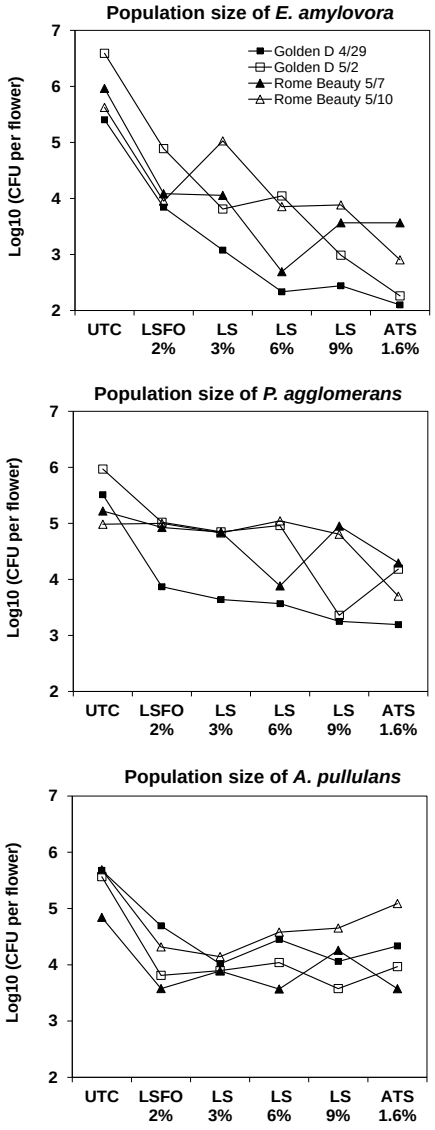

Fig. 1. Log_{10} (population size) of A) *Erwinia amylovora*, B) *Pantoea agglomerans* and C) *Aureobasidium pullulans* on apple flowers sprayed to runoff with the fire blight pathogen (1 x 10^6 CFU/ml) or with maximum labels rates Bloomtime Biological or Blossom Protect at 60 to 70% bloom (27 April and 2 May 2012 for cvs. 'Golden Delicious' and 'Rome Beauty', respectively) in experimental orchards located near Corvallis, OR. On the following day, inoculated trees were oversprayed to runoff with the fruit crop load thinning treatment lime sulfur (LS) (3, 6, or 9% v:v), with a mixture of lime sulfur and fish oil (LS+FO) (2%:2% v:v) or with ammonium thiosulfate (ATS) (1.6% v:v). Each point is the mean of three replications of five bulked flower clusters (~25 flowers per replicate) washed and dilution plated onto a semi-selective culture medium; standard errors for the points averaged 0.28 $\pm$ (s. d.) 0.21. Sample dates shown in the legend are 1 and 4 days after lime sulfur in Golden Delicious (squares) and 4 and 7 days after lime sulfur in Rome Beauty (triangles). UTC is the untreated control with respect to the fruit crop load thinning treatment.

Obj. 1b. Integrated fire blight control with lime sulfur thinning followed by Blossom Protect. We define 'integrated control' of fire blight as programs of different materials that when sprayed in sequence result in an improved (high) level of disease suppression. The improvement in control is achieved by suppression of the two distinct phases of the floral infection process: suppression the pathogen's prerequisite epiphytic phase on floral stigmas -- accomplished by a competing microorganism or by lime sulfur -- and suppression of infection by the pathogen via natural openings (nectarthodes) on the hypanthium -- accomplished with a chemical or by yeast colonization of this surface. For lime sulfur alone, treatment of apple trees at 30 and 70% bloom significantly ($P \leq 0.05$) reduced the incidence of blighted flower clusters (Fig. 2) compared to the water-treated control; the mean level of suppression achieved by LS+FO over all trials was 48 $\pm$ 10%. In the same orchard trials, an integrated program of two treatments of Blossom Protect (*A. pullulans*) following the bloom thinning protocol reduced the incidence of fire blight by an average of 91 $\pm$ 1% compared to trees treated with water only (Fig. 2). But within this four-spray program, the addition of Bloomtime Biological (*P. agglomerans*) to the full bloom Blossom Protect treatment did not improve control beyond that achieved by LS+FO followed by Blossom Protect alone (data not show). Overall, two applications of Blossom Protect after LS+FO provided a reduction of disease incidence that was

similar statistically to the level provided by the integrated program of *P. agglomerans* followed by oxytetracycline, and also to a single spray of streptomycin.

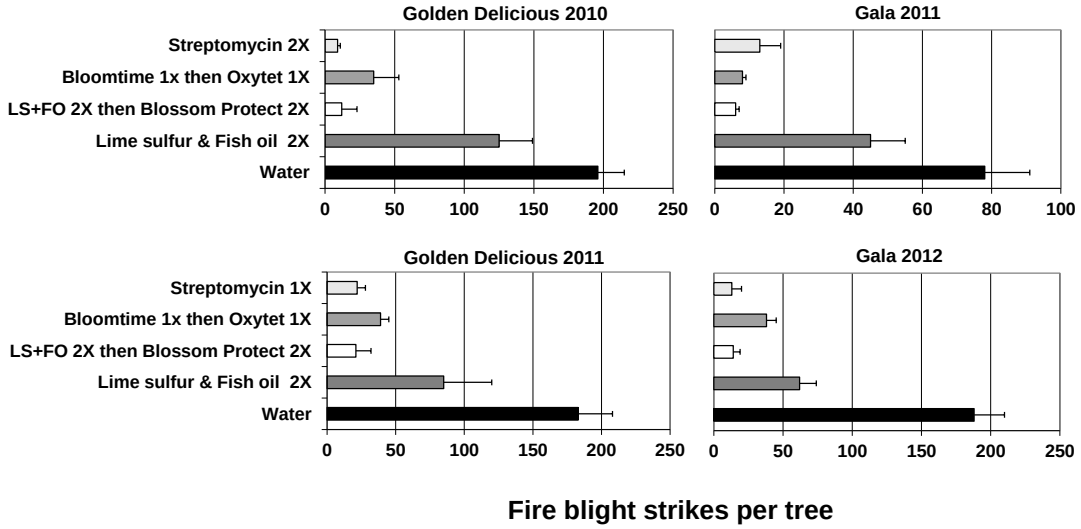

Fig. 2. Incidence of fire blight in Gala apple trees as affected by chemical and biological treatments sprayed onto the trees to suppress infection. The orchards were inoculated with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) at full bloom. Lime sulfur and fish oil (2%:2% v:v) treatments were applied at 30 and 70% bloom. Bloomtime Biological and the first Blossom Protect treatment were applied at 80% bloom. Antibiotics and the second Blossom Protect treatment were applied 1 to 3 days after the pathogen inoculation. Error bars associated with each larger bar represent plus/minus one standard error of the mean.

Obj. 2a. Improved understanding of floral colonization by *Aureobasidium pullulans.*

Parts of the flower colonized by the yeast. In 2011 and 2012, overspray of a 80% bloom timing of Bloomtime Biological (*P. agglomerans*) with Blossom Protect (*A. pullulans*) at full bloom and petal fall revealed differences in the colonization of the flowers by these microbes. On stigmas, the incidence of both microbes was high (2011: 63 and 98% for *P. agglomerans* and *A. pullulans*, respectively; 2012: 71 and 94% for *P. agglomerans* and *A. pullulans*, respectively) (Fig. 3A, B). In contrast, for floral cups (hypanthial surface), the yeast again was recovered from 98% (2011) to 100% (2012) of sampled flowers but *P. agglomerans* was detectable on 9% and 18% of washed hypanthia in 2011 and 2012, respectively (Fig. 3C, D).

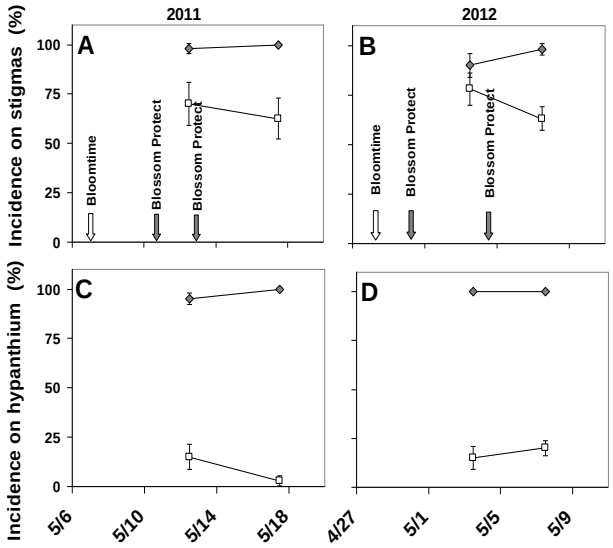

Fig. 3. Incidence of detection of *Pantoea agglomerans* (open squares) and of *Aureobasidium pullulans* (gray diamonds) on floral stigmas (A, B) and on hypanthia (C, D) by date of sampling from Gala apple trees treated with Bloomtime Biological at 80% bloom (open arrow) and with Blossom Protect at full bloom and prior to petal fall (hatched arrows) in an experimental orchard located near Corvallis, OR in 2011 and 2012. On each sampling date, incidence was determined by dilution plating dissected stigma and hypanthium subsamples from 10 flowers from each of four replicate trees. Error bars associated with each point represent plus/minus one standard error of the mean.

Environmental influences on yeast establishment in flowers. In 2012, at Corvallis and Wenatchee, treatment with Blossom Protect at 20, 80 or at both 20 and 80% bloom resulted in recovery of *A. pullulans* from nearly every flower sampled between full bloom and petal fall (Fig. 4A,C,D). Flowers sampled from the untreated control trees also had a measureable incidence of *A. pullulans* on flowers that ranged from 26 (apple Corvallis) to 80% (pear Corvallis). At Lakeport CA, pear trees treated with Blossom Protect in early bloom were sampled in mid-June when fruit were thumb-sized. Calyx ends of these fruit were washed and subjected to dilution plating. Nearly every calyx-end of sampled pear fruit had a recoverable population of *A. pullulans* (Fig. 4B). A repeat of the Lakeport experiment in 2013 had similar results (Fig. 5).

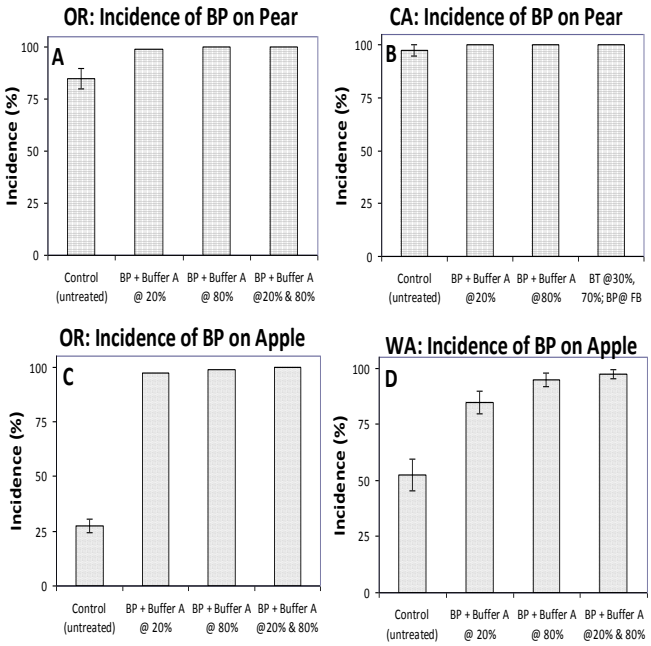

Fig. 4. 2012 experiments: Incidence of detection of *Aureobasidium pullulans* on pear (A, B) and apple (C, D) flowers after treatment with Blossom Protect at 20, 80 or both 20 and 80% bloom in orchards located near Corvallis, OR (A,C), Lakeport, CA (B), and Wenatchee, WA (D) in 2012. In Corvallis and Wenatchee, flowers were sampled twice between full bloom and petal fall. On each sampling date, incidence was determined by dilution plating 10 flowers from each of four replicate trees; the average incidence for the two sampling dates is presented in the figure. In Lakeport, samples were taken once when pear fruit were thumb-sized. The calyx ends of 10 pear fruit from each of four replicate trees fruit were washed and dilution plated. Error bars associated with each point represent plus/minus one standard error of the mean.

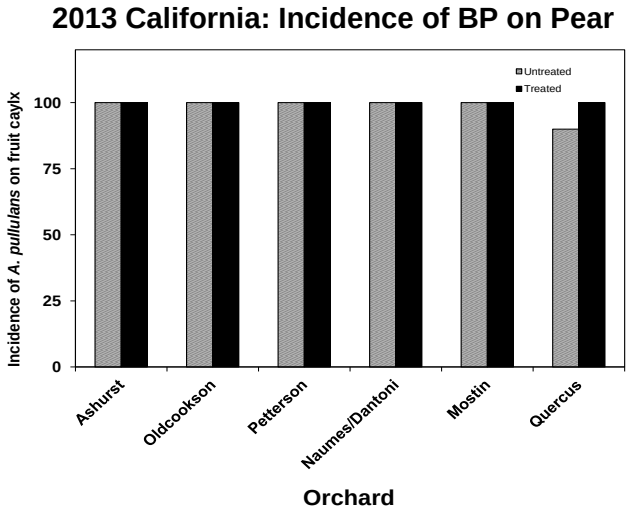

Fig. 5. 2013 experiment: Incidence of detection of *Aureobasidium pullulans* on Bartlett pear fruit sampled from six orchards in Lakeport, CA. Trees were treated with Blossom Protect once at 80-90% bloom. Samples were taken in mid-June when pear fruit were thumb-sized. The calyx ends of 10 pear fruit from each of four replicate trees per orchard were washed and dilution plated.

Discussion. The yeast, A. *pullulans*, is an excellent colonist of both the stigma and the hypanthium, whereas bacterial biocontrol agent, *P. agglomerans*, is only a good colonist of stigmas. The ability of *A. pullulans* to colonize the hypanthium may be a primary mechanism by which this

organism provides outstanding fire blight suppression (the hypanthial surface is the infection site for *E. amylovora*). Certainly, for yeasts used as biocontrol agents of postharvest fruit rots, the effectiveness of these antagonists is attributed to an ability to rapidly utilize nutrients available at the site of infection. As a biological product, Blossom Protect is produced to an excellent quality standard, which results in a high number of viable colony forming units (spores) in the spray tank. For three environments (Corvallis, Wenatchee and Lakeport), *A. pullulans* became established in nearly all pear and apple flowers to which Blossom Protect was applied. Moreover, this microorganism apparently spread flower-to-flower after initial establishment as evidenced by the high recovery of *A. pullulans* from flowers treated at 20% bloom, and from flowers and fruit sampled from the untreated control trees. After several years of orchard trials, non-antibiotic programs that utilize Blossom Protect continue to be the most effective and consistent for fire blight control.

Obj. 2b. Integrated fire blight control with Blossom Protect followed by Previsto copper. One potential drawback (expressed to us by growers) of a spray program where Blossom Protect follows lime sulfur is a *complete* reliance on a biological product (living microorganism) for fire blight control during the higher risk, late bloom period. A second potential drawback of Blossom Protect is a known ability of *A. pullulans* to russet fruit surfaces if extended wet periods occur during the period from late bloom to a few weeks after petal fall (Note: fruit russet caused by *A. pullulans* is generally unlikely on apples produced in semi-arid areas of central Washington). Consequently, we evaluated an alternative integrated program that began with one treatment of Blossom Protect (which could follow lime sulfur thinning in a commercial setting) followed by a treatment of Gowan's Previsto copper product, which is the (as yet unregistered) copper in ammonium/alginate complex that has shown less potential to russet fruit than other copper-based bactericides.

The integrated program of one treatment of Blossom Protect (*A. pullulans*) followed by Previsto copper reduced the incidence of fire blight by an average of 91 ± 2% compared to trees treated with water only (Fig. 6). Overall, this treatment program was similar to a single spray of streptomycin and superior statistically to the level of suppression provided by the integrated program of Bloomtime Biological (*P. agglomerans*) followed by oxytetracycline.

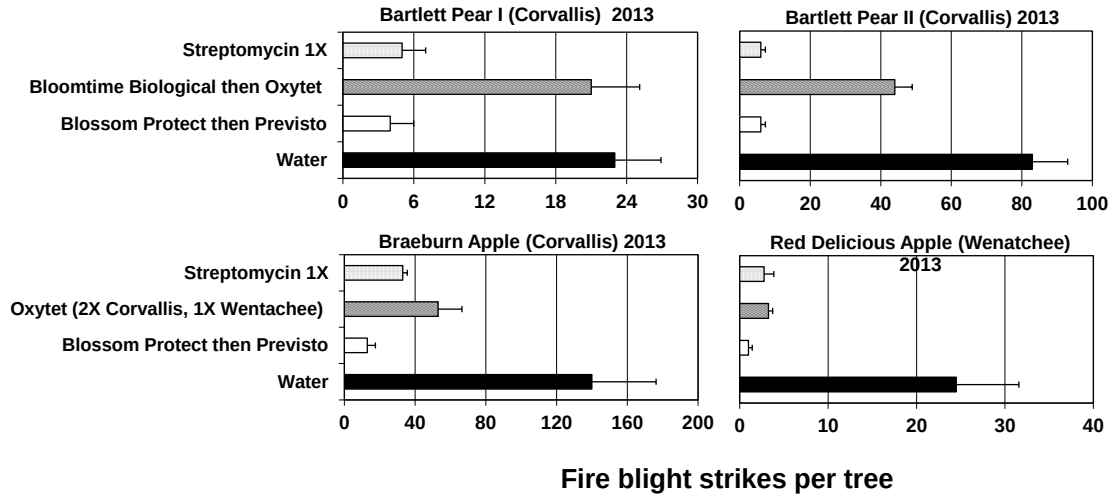

Fig. 6. Incidence of fire blight in experimental pear and apple orchards as affected by chemical and biological treatments sprayed onto the trees to suppress infection. The orchards were inoculated with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) at full bloom. Biological treatments were applied at 70% bloom. Antibiotics and Previsto copper were applied 1 to 3 days after the pathogen inoculation. Error bars associated with each larger bar represent plus/minus one standard error of the mean.

An additional rationale to follow Blossom Protect with Previsto is that the copper product could suppress the yeast populations and potentially prevent yeast-induced fruit russet if excessively wet conditions were to occur in late bloom. In 2013, in a preliminary effort, we measured yeast populations on flowers oversprayed with Previsto. We observed a decline in the proportion of flowers with detectable yeast populations after treatment with Previsto copper (Fig. 7), which we have not observed when Blossom Protect was the last treatment sprayed onto the trees (see Figs. 4 and 5). Interestingly, however, we observed a larger decline in flowers with detectable yeast populations after treatment with Serenade Optimum. Serenade also has provided excellent fire blight control when sprayed after Blossom Protect (data not shown), is commonly used as a fungicide in other crops, and is considered fruit safe (D. Sugar, *unpublished data*). Further investigation of the response shown in Fig. 7 is an objective of our 2014-15 research proposal.

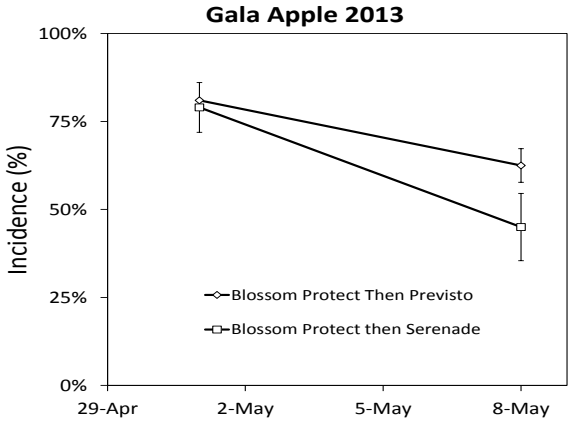

Fig. 7. Incidence of detection of *Aureobasidium pullulans* by date of sampling from Gala apple trees treated with Blossom Protect at 80% bloom and with Previsto or Serenade Optimum at full bloom in an experimental orchard located near Corvallis, OR in 2013. Flowers were sampled just prior to and six days after the Previsto or Serenade treatment.

Obj. 3. Systemic acquired resistance for protection of apple.

Actigard combined with antibiotics. From 2011 to 2013, Actigard (acetyl-S-methyl) was evaluated in combination with antibiotics for enhanced suppression of floral infection by the fire blight pathogen. Relative to the water-treated control, antibiotics alone significantly reduced ($P \leq 0.05$) incidence of infection and total number of infected flower clusters per tree. In general, the addition of Actigard treatments in combination with streptomycin or oxytetracycline improved the control of fire blight compared to the antibiotics alone. (An additional 2013 pear trial (not shown) had results similar to Gala apple in 2011 and 2012.)

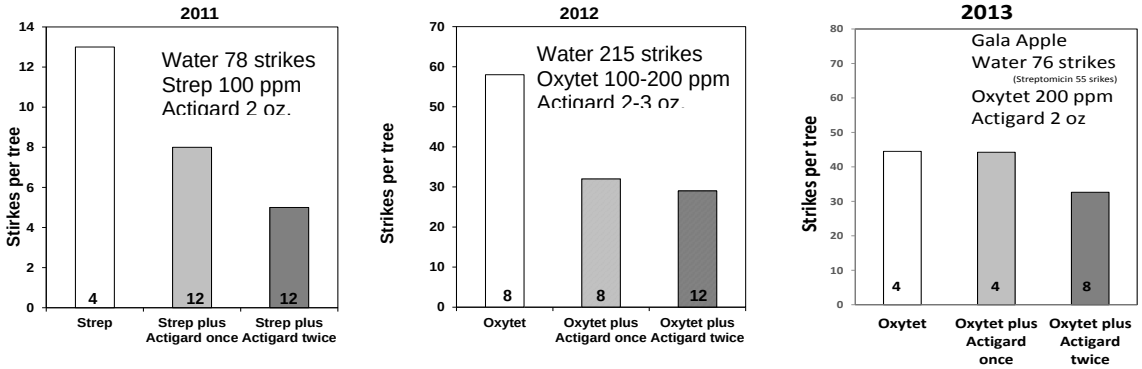

Fig. 7. Fire blight strikes per tree as affected by treatment with streptomycin (2011) or oxytetracycline (2012 and 2013) once or in a program with one or two additional Actigard treatments. Trials were conducted in a Gala apple orchard near Corvallis, OR. The antibiotic treatment was made at full bloom; timings of Actigard treatments varied from 30% bloom to petal fall. Numbers within bars are the number of replicate trees averaged for each mean.

Actigard as aid to cutting of blight in scions of young apple trees. Experiments were conducted in 2012 and 2013 to evaluate Actigard as an aid to cutting of blight in scions of young trees. In both years, the cutting experiment failed in apple because fire blight infections did not run in our young trees of cultivar 'Cameo' ('Red Delicious' parentage). We have removed the Cameo block and are re-planting with Gala and Pink Lady to continue this research. Several Actigard aid-to-blight-cutting experiments in pear have been successful (see pear report).

Actigard for protection of apple rootstocks from fire blight. We demonstrated previously that Actigard applied to potted trees of cv. 'Gala' on EMLA 26 provided a high level of protection from rootstock blight after the pathogen was inoculated directly below the graft union. In 2012 and 2013, we ran similar experiments in the field on 3- to 4-year-old 'Gala' on ELMA 26. In both seasons, the rootstocks were treated twice with an Actigard paint (30-45 g/L plus 1-2% Pentrabark, early and late June) prior to an early July inoculation of a high pathogen dose directly into the rootstock tissue. In these trials, cankers have formed on both the Actigard and untreated rootstocks, with a slight reduction in canker size on trees treated with Actigard. A larger difference among the treatments, however, has been observed in the appearance and health of the scion. For the 2012 experiment, 70% the untreated trees died in spring 2013 compared to 36% of the trees treated with Actigard; rootstock cankers in the Actigard trees had stopped expanding and had begun to heal. Similarly, for the trees inoculated in 2013, 45% of untreated trees showed decline of the scion in September (yellow foliage, early fall senescence and defoliation) compared to 10% of scions on Actigard treated trees. These trees will be evaluated again in the spring of 2014.

Discussion. We have made significant progress in understanding effective rates of Actigard for the various methods of application. Induction of systemic acquired resistance appears to have its greatest protective effect when blight symptoms are minimal (prior to or near time of infection, or after cutting). Actigard shows value as program partner with antibiotics during bloom, and perhaps more significant, it may be effective as long residual protectant for rattail and shoot infection phases of fire blight (e.g., in long blooming cultivars like 'Pink Lady'). Use of Actigard paints continues to show promise, although we are not convinced that protection of apple rootstocks with Actigard paints will be practical in commercial orchards given that the rates of Actigard required to obtain a response are high in relation to the probability of a tree developing a rootstock infection. Nonetheless, in pear, we continue to achieve promising results with Actigard as an aid to cutting of blight in scions of young trees (see pear report). It is likely that apple scions with running fire blight cankers would benefit from similar Actigard paint treatments to healthy tissue below the cut.

EXECUTIVE SUMMARY

Project Title: **Fire blight management in organic and conventional apple**
Investigator: Ken Johnson, Oregon State University

SIGNIFICANT FINDINGS:

Integration of fruit crop load thinning with fire blight control:

- Oversprays of the bloom thinning agent, lime sulfur, suppressed populations of the fire blight pathogen and of biological control agents after their establishment on apple flowers.
- Treatment with *A. pullulans* (Blossom Protect) after lime sulfur and fish oil reduced fire blight infections by 91% compared with water only; this level of control level was similar to streptomycin against a strep-sensitive pathogen strain.

Industry implications: Chemical fruit crop load thinning with lime sulfur has become common practice in both organic and conventional apple production. Timing of these thinning treatments can coincide with treatments to prevent fire blight. Because of its antibacterial properties, lime sulfur is likely sufficient in most orchards to delay/suppress the epiphytic increase of *E. amylovora* in early bloom, and that the biological and chemical materials specifically registered for fire blight control can be implemented immediately after the second thinning treatment at 70% bloom.

Understanding floral colonization by the yeast biocontrol agent, *Aureobasidium pullulans*:

- *A. pullulans* (Blossom Protect) is an excellent colonist of both the stigma and floral cup, which differentiates it from other biocontrol agents that colonize only the floral stigma.
- In parallel trials at Corvallis, OR, Wenatchee, WA, and Lakeport, CA, *A. pullulans* colonized nearly 100% of flowers on trees treated once with Blossom Protect at early to mid-bloom.

Industry implications: *A. pullulans* ability to colonize the floral cup (hypanthium) may be a primary mechanism by which this yeast provides outstanding fire blight suppression. As a product, Blossom Protect is produced to an excellent quality standard, which results in a high number of viable yeast spores in the spray tank. This yeast became established and spread to nearly all apple and pear flowers on trees treated with Blossom Protect regardless of the trial environment or the timing of the treatment. Spray programs that utilize Blossom Protect continue to be among the most effective for fire blight control.

Systemic acquired resistance:

- Over three seasons, the addition the systemic acquired resistance material, acibenzolar-S-methyl (Actigard), to antibiotic treatments significantly enhanced fire blight control.
- Paints of Actigard onto EMLA 26 rootstocks reduced canker size and tree death after inoculation of the rootstocks with the fire blight pathogen.

Industry implications: Actigard, with its unique (host defense gene-inducing) mode-of-action, shows value as program partner with antibiotics for fire blight prevention during bloom. With regard to Actigard paints, even with very good products for fire blight prevention, the disease still occurs and its clean-up can be difficult, especially in young orchards. We continue to achieve promising results with Actigard as an aid to clean-up of blight in scions and rootstocks of young trees.