Project Title: Pre-bloom resistance induction for fire blight suppression

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Report Type: Final Project Report

Project Duration: 2-Year

Total Project Request for Year 1 Funding: $ 34,455
Total Project Request for Year 2 Funding: $ 35,488

Other related/associated funding sources: Awarded
Funding Duration: 2021 - 2024
Amount: $240,000 (to Johnson)
Agency Name: USDA SCRI
Notes: PI, G. Sundin, Michigan State

WTFRC Collaborative Costs: none

Budget 1
Primary PI: Kenneth Johnson
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Item	**2020**	**2021**
Salaries Faculty Res. Assist. 3.5 mo	17,860	18,396
Benefits OPE 61%	10,895	11,221
Wages undergraduate $12.50/hr	1,111	1,143
Benefits OPE 8%	89	93
Equipment		
Supplies	1,000	1,030
Travel	1,500	1,545
Miscellaneous		
Plot Fees	2,000	2,060
Total	$34,455	$35,488

Footnotes: 3% inflation in year 2

OBJECTIVES

Obj. 1a: Determine if prohexadione-Ca or acibenzolar-S-methyl applied prior to bloom achieves blossom blight control, and 1b. determine if prebloom Ph-Ca enhances effectiveness of Actigard treatments during bloom.

Obj. 2. Determine if Regalia applied prebloom influences blossom blight suppression.

Obj. 3. In greenhouse, investigate synergy between Ph-Ca and Actigard for fire blight suppression, and resistance induction by Regalia for fire blight control.

SIGNIFICANT FINDINGS:

- Kudos/Apogee (prohexadione-Ca), Actigard, or Regalia applied at prebloom timings did not provide significant suppression of fire blight in flowers compared to a non-treated control.

- Kudos applied as a solitary treatment at timings of first pink, full pink and 10% bloom, did not reveal a pattern related to time of treatment and level of suppression.

- In 2020, Actigard sprayed three times during bloom reduced the incidence of infection compared the water-treated control (45 and 65 % reduction in pear and apple, respectively). In2020, a prebloom Kudos treatment appeared to enhance the level of suppression obtained by the Actigard treatment program. But in 2021, the degree of suppression obtained with a 3-spray Actigard program (75%) was not improved with a prebloom treatment of Apogee.

- In greenhouse, an Actigard spray reduced fire blight canker expansion in potted ELMA.26 by 28%, and the effect of Actigard was not improved with the addition of an Apogee spray. A single Actigard trunk paint treatment reduced canker expansion by 55% and was not improved with the addition of an Apogee spray.

- In greenhouse, among spray and trunk paint treatments of Apogee, Actigard, Regalia, and LifeGard, only Actigard trunk paint treatments showed sustained expression of apple defense genes, PR-1, and PR-2.

- In greenhouse, treatments with the organic materials, Regalia and LifeGard, had no effect on fire blight canker expansion in potted ELMA.26.

RESULTS

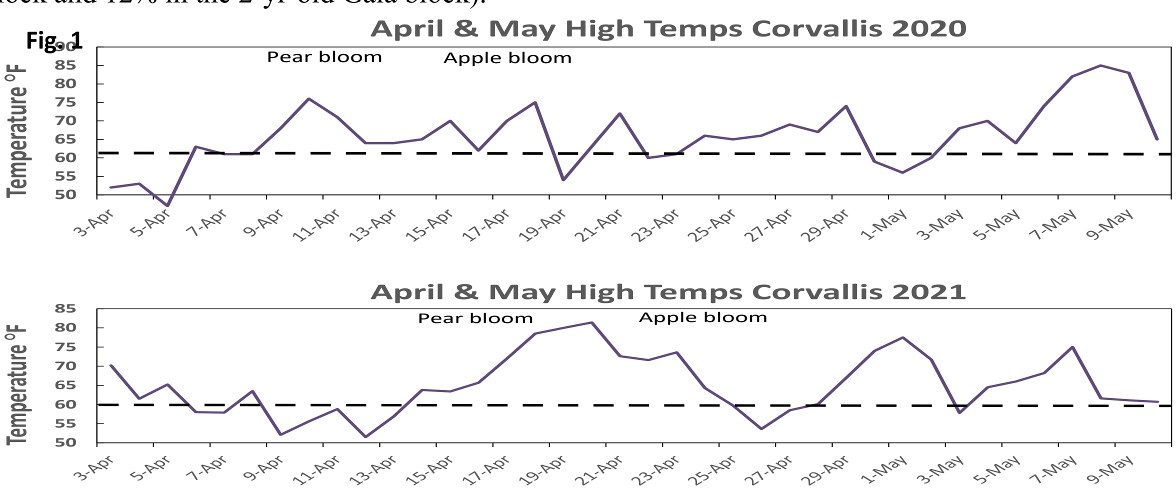
Experimental design for orchard trials. In 2020 and 2021, objectives 1 and 2 were conducted in a 6/7-yr-old and a 2/3-yr-old blocks of Gala apple located at the Oregon State University, Botany and Plant Pathology Field Laboratory near Corvallis. In 2020, additional treatments were evaluated in older (20-yr-old) blocks of Gala apple and Bartlett pear.

Experimental design for orchard trials. Experiments were arranged in randomized complete block designs with 9 to 14 replicates of single-tree plots in young tree blocks, and with 4 replicates of single-tree plots in older tree blocks. Prior to bloom, flower cluster density on individual trees was counted. These counts along with tree location in orchard were considered in the assignment of trees to blocks in the plot design. On the date of treatment, suspensions of each product in water were prepared at the specified rate and applied to near run-off with backpack sprayers in early morning under calm wind conditions. The amount of material suspension sprayed was 0.5 to 1.0 liter/tree in young tree trials, and 3 liters/tree in the older tree trials. On an evening near full bloom, re-suspended freeze-dried inoculum of pathogenic *E. amylovora* 153N (streptomycin and oxytetracycline sensitive pathogen strain) prepared at a concentration of 1 x 10^6 CFU/ml was fogged onto the trees. In the 2020 young tree trials, because of asynchronous bloom, the inoculation was repeated to ensure adequate disease pressure.

Disease assessment and data analysis. During bloom, treated trees were evaluated for phytotoxic responses from sprayed treatments. Beginning 2 to 3 weeks after petal fall, fire blight incidence was assessed by counting and removing blighted flower clusters from each tree. The number of blighted flower clusters was divided by the total number of clusters on each tree. Incidence of fire blight was subjected to analysis of variance (Analyze-It, v. 3.0, Leeds, UK).

Weather in spring 2020. Temperatures during primary bloom of both pear and apple were moderately favorable for fire blight development (Fig.1). Six days between 9 and 21 April had a maximum daily temperature > 70°F. On inoculated control trees, epiphytic pathogen populations were high with measured sizes exceeding 1 x 10^6 colony forming units (viable cells per flower) on flowers sampled near petal fall. These high populations on non-treated or water-treated control flowers resulted in infection incidences that averaged 31% of total clusters (the range was 14 to 41% among the four trials mentioned in this report). *Weather in spring 2021.* Temperatures during primary bloom of apple were moderately favorable for fire blight development. Four days between 21 and 30 April had a maximum daily temperature > 70°F; rain occurred on 24 and 25 April as apple trees entered full bloom. For the water-treated control, pathogen populations on flowers resulted in moderate incidences of infection averaged 9% of total clusters (the range was 5% in the 7-yr-old Gala block and 12% in the 2-yr-old Gala block).

Fig. 1



Objective 1a: Determine if Ph-Ca or Actigard applied prior to bloom achieves blossom blight control, and **Objective 2:** Investigate if Regalia applied prebloom influences blossom blight suppression.

Rationale. Wallis and Cox (2020) achieved very good suppression of fire blight from a single application of prohexadione-Ca (Ph-Ca, 6 oz. /100 gal.) prior to bloom. Questions that arose from their study include: 1) can this repeated under western conditions?, 2) what is the optimal timing of Ph-Ca treatment?, 3) could prebloom treatment with Ph-Ca would improve the efficacy of Actigard treatments during bloom (without antibiotics), and 4) could organic resistance-inducing materials applied prebloom, e.g., Regalia, achieve this suppression response.

Approach. In young tree trials in 2020, the timing of Kudos (prohexadione calcium, Fine Americas, Walnut Creek, CA) was varied from first pink to 10% bloom. In addition, Actigard 50WG (acibenzolar-S-methyl, Syngenta Crop Protection, Greensboro, NC), and Regalia (extract of

Reynoutria sachalinensis, Marrone Bio, Davis, CA) were each evaluated at the timing of full pink. In large tree trials in 2020, Kudos was applied at full pink and Actigard was applied at 70% bloom, full bloom, and petal fall. An additional treatment added Stimplex (0.01% cytokinin, 128 fl. oz. /100 gallons, Acadian Seaplants Ltd., Dartmouth, Nova Scotia) at timings of prebloom (with Kudos) and petal fall (with Actigard). Regalia was evaluated as a solitary treatment at full pink and petal fall.

In 2021, the only materials applied prebloom (full pink only) were Apogee (prohexadione calcium, BASF Ag, Research Triangle Park, NC) and Regalia. In addition, preboom treatments of Apogee were supplemented with bloom period sprays of Actigard, and prebloom Regalia treatments were supplemented with bloom period treatments of LifeGard (*Bacillus mycoides* isolate J, Certis Biologicals, Columbia, MD) gallons and an additional Regalia treatment at petal fall.

Observations in 2020. Trees used in the study averaged 82 flower clusters per tree in the 6-yr-old block and 57 flower clusters per tree in the 2-yr-old block. None of the materials (Kudos, Actigard, and Regalia) applied at prebloom timings produced symptoms of phytoxicity. Symptoms of fire blight were first observed on 5 May. For a pathogen-inoculated trial, incidence of infection was moderately high with non-treated control trees in the 6-yr-old block averaging 31 fire blight strikes per tree (41% of total clusters); in the 2-yr-old block, non-treated control trees averaged 23 strikes per tree (39% of total clusters) (Table 1). In both orchard blocks, while all treatments had fewer infections per tree than the non-treated controls, the overall suppression of infection from sprayed materials was only fair (17 to 39% in the 6-yr-old block) to poor (6 to 17% in the 2-yr-old block) and not statistically significant ($P > 0.05$) different from the non-treated control. Kudos, which was applied as a solitary treatment at timings of first pink, full pink and 10% bloom, did not reveal a pattern related to time of treatment and level of suppression.

		Date treatment applied*			**6-yr-old trees**	**2-yr-old trees**
Treatment	Rate **per 100 gal**	8 April First pink	11 April Full pink	14 April 10% bloom	% blighted floral clusters**	% blighted floral clusters**
Non-treated	-	--- §	---	---	**41** # (31 strikes)	**39** # (23 strikes)
Kudos y	6 oz.	X	---	---	25	34
Kudos y	6 oz.	---	X	---	34	36
Kudos y	6 oz.	---	---	X	28	26
Kudos y Actigard	2 oz. 3.2 oz.	---	X	---	31	32
Regalia z	256 fl. oz.	---	X	---	33	37

***On the evenings of 15 and 19 April, a motorized tank sprayer equipped with a hand wand was used to lightly fog a suspension of freeze-dried cells of *Erwinia amylovora* strain, which was prepared at 1 x 10^6 CFU per ml (0.1 to 0.2 liters per tree). ** 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 did not differ significantly ($P >$ 0.05) based on analysis of variance ($F > 0.05$). y Amended 1:1 with ammonium sulfate and Regulaid (16 fl. oz. per 100 gallons). z Amended with BioLink Spreader-Sticker: 4 fl. oz. per 100 gallons.**

In the 2020 large tree trials, the 20-yr-old Bartlett pear trees averaged 455 flower clusters per tree, and the 20-yr-old Gala apple trees averaged 430 flower clusters per tree. In pear, infection incidence was moderate with fire blight infections on water-treated trees averaging 67 strikes per tree (15% of total clusters); in apple, infection incidence was moderately high with water-treated trees averaging 126 strikes per tree (31% of total clusters) (Table 2). Based on percent blighted flower clusters and compared to the water-treated control, the solitary treatments of Kudos, Actigard or Regalia treatments did not reduce infection incidence significantly ($P < 0.05$). In contrast, in both trials, the combination program of Kudos (prebloom) and Actigard (during bloom) reduced significantly the incidence of infection compared the water-treated control (55 and 65% reductions in pear and apple, respectively). Regalia (1 gallon per 100 gallons of water) at the timings of pink and petal fall reduced incidence of infection (% blighted flower clusters) by 50% in the apple trial ($P < 0.05$).

Table 2. Evaluluation of prebloom PH-Ca and within-bloom Actigard treatments for fire blight control in 20-year-old Bartlett pear and Gala apple, Corvallis, 2020.

20-year-old Bartlett pear

Treatment	Rate per 100 gallons water	Date treatment applied*: 3 Apr Full white bud	7 Apr Mid-bloom	10 Apr Full bloom	14 Apr Petal Fall	Number of blighted clusters per tree**		Percent blighted floral clusters***	
Water	---	---	---	**X[§]**	**X**	**67**	**a [#]**	**14.5**	**a**
Actigard	2 oz.	---	X	X	X	52	a	11.8	ab
Kudos	6 oz.	X	---	---	---	74	a	19.1	a
Kudos	6 oz.	X	---	---	---	33	b	7.9	b
Actigard	2 oz.	---	X	X	X				
Kudos	6 oz.	X	---	---	---	32	b	8.7	b
Actigard	2 oz.	---	X	X	X				
Stimplex	128 fl. oz.	X	---	---	X				
Regalia	128 fl. oz	X	---	---	X	57	a	12.9	ab

20-year-old Gala apple

Treatment	Rate per 100 gallons water	*Date treatment applied**: 10 Apr Full-pink	14 Apr Mid-bloom	17 Apr Full bloom	21 Apr Petal Fall	Number of blighted clusters per tree**		Percent blighted floral clusters***	
Water	---	---	---	**X[§]**	**X**	**126**	**a [#]**	**30.7**	**a**
Kudos	6 oz.	X	---	---	---	137	a	35.5	a
Kudos	6 oz.	X	---	---	---	46	b	10.8	b
Actigard	2 oz.	---	X	X	X				
Kudos	6 oz.	X	---	---	---	57	ab	14.7	b
Actigard	2 oz.	---	X	X	X				
Stimplex	128 fl. oz.	X	---	---	X				
Regalia	128 fl. oz	X	---	---	X	66	ab	14.7	b

*** Trees inoculated on 8 (pear) or 15 (apple) April with 1×10^6 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain). ** Transformed $\log(x + 1)$ prior to analysis of variance; non-transformed means are shown. *** Transformed $\arcsin(\sqrt{x})$ 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.**

Observation in 2021. Trees used in the study averaged 138 flower clusters per tree in the 7-yr-old block and 149 flower clusters per tree in the 3-yr-old block. None of the materials (Apogee, Actigard, Lifeguard and Regalia) produced symptoms of phytoxicity. Symptoms of fire blight were first observed on 10 May. For a pathogen-inoculated trial, disease intensity was light to moderate with fire blight infections on water-treated control trees averaging 9 strikes per tree (5.4% of total clusters) in the 7-yr-old block and 17 strikes per tree (12.9% of total clusters) in the 3-yr-old block (Table 3). Averaged over both orchard blocks and compared to the water control, treatment with Actigard (or this material in a program with Apogee) provided a high level of infection suppression (75%). In contrast, the incidence of infection on trees treated with Apogee only was similar to those that received water. As solitary materials, Regalia (2 applications) and LifeGard (3 applications) provided intermediate levels of suppression (38 and 48%, respectively). The combination programs of Regalia and LifeGard increased the level of infection suppression to a mean of 59%. One application of FireWall (streptomycin) provided 92% infection suppression relative to the water-treated control.

		Date treatment applied				**7-yr-old**		**3-yr-old**		**Combir**
Treatment	Rate **per 100 gal**	19 Apr Full pink	22 Apr 70% bloom	26 Apr Full bloom	29 Apr Petal fall	% blighted floral clusters		% blighted floral clusters		% blighte floral clustei
Water-treated	-	---[§]	X	X	X	**5.4**[#] 9 strikes	**b**	**12.9** 17 strikes	**a**	**9.1**
FireWall 50	2.3 oz.	---	-[w]-	X	-[w]-	0.8	e	0.7	f	0.7
Apogee [y]	12 oz.	X	-[w]-	-[w]-	-[w]-	9.7	a	8.0	b	8.7
Apogee [y]	6 oz.	X	---	---	---	3.2	bc	2.6	de	2.9
Actigard	2 oz.	---	X	X	X					
Apogee [y]	12 oz.	X	---	---	---	1.1	de	1.9	ef	1.6
Actigard	2 oz.	---	X	X	X					
Actigard	2 oz.	---	X	X	X	0.8	e	2.9	de	2.0
Regalia [z]	128 fl. oz.	X	-[w]-	-[w]-	---	4.0	bc	6.9	bc	5.7
Regalia [z]	64 fl. oz.	---			X					
Regalia [z]	128 fl. oz.	X	---	---	---	5.4	b	4.3	cd	4.8
Regalia [z]	64 fl. oz.	---	---	---	X					
LifeGard	13.5 oz.	---	X	X	X					
Regalia [z]	256 fl. oz.	X	---	---	---	1.0	de	4.2	cd	2.9
Regalia [z]	64 fl. oz.	---	---	---	X					
LifeGard	4.5 oz.	---	X	X	X					
LifeGard [z]	13.5 oz.	---	X	X	X	2.6	cd	6.3	bc	4.7

**** Transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown. 'X' indicates material was sprayed on that date; '---' indicates material was not applied on that specific date; and '-[w]-' indicates water was sprayed on that date. [#] Means within a column did not differ significantly ($P > 0.05$) based on analysis of variance ($F > 0.05$). [y]Amended 1:1 with ammonium sulfate and Regulaid (16 fl. oz. per 100 gallons). [z] Amended with BioLink Spreader-Sticker: 4 fl. oz. per 100 gallons.**

Box plots of the 2021 data are an alternative method to visualize infection incidence (Fig. 2). Relative to the water-treated control and to prebloom Apogee only, the response of individual trees to the Actigard treatments (with or without a prebloom Apogee treatment) was consistent and nearly as effective as streptomycin (Firewall). Moreover, with 14 replications, the responses observed from Regalia, LifeGard, and combination programs of these materials also showed a consistent response, although to a lesser degree of suppression than observed with Actigard.

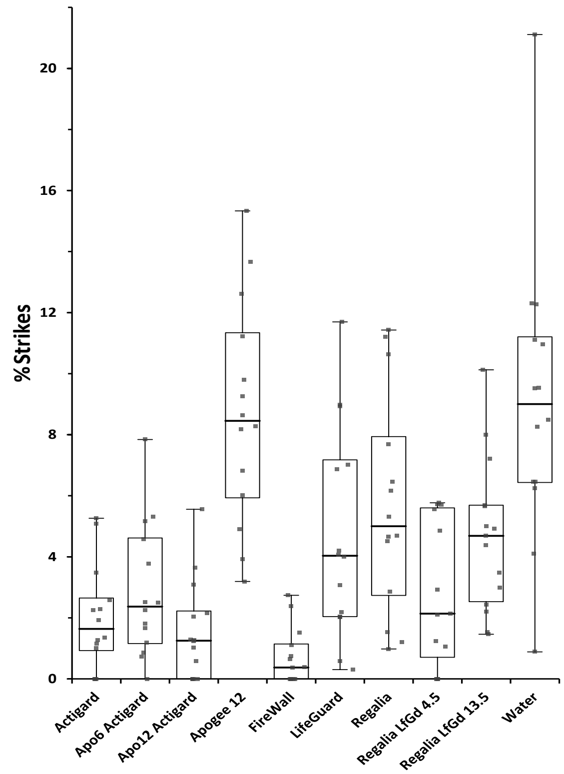
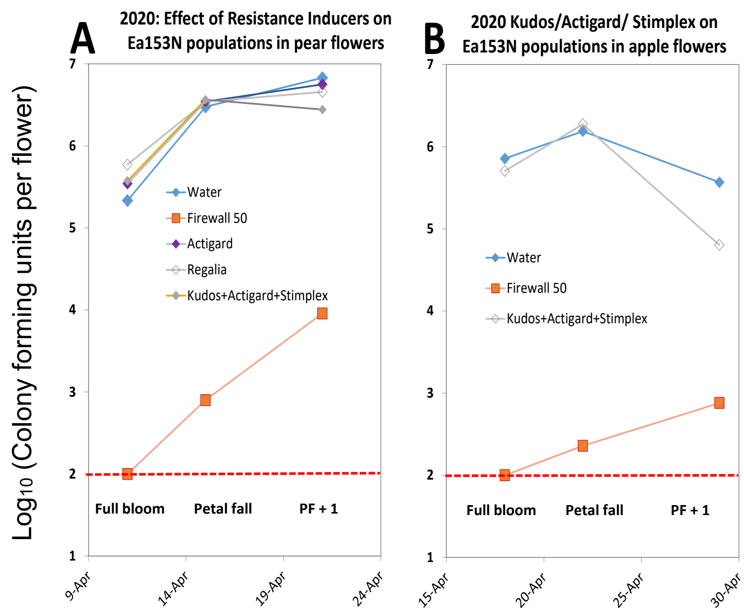


Fig 2. Box plots of percent infected flower clusters in Gala apple trees treated prebloom with potential resistance-inducing materials. The 7-yr-old (6 replication) and 3-yr-old (8 reps) orchards were located near Corvallis, OR with each treatment applied to a total of 14 replicate trees during April 2021. Within each box, horizontal line is the treatment median.

Discussion. We viewed the quality of young-tree trials reported under this objective as excellent based on the number of replications (9 per trial in 2020 and 14 in 2021), bloom density on individual trees (very good), weather for pathogen growth in flowers and level of infection (not too much, not too little). Consequently, our inability to repeat results from solitary prebloom treatment of Ph-Ca in New York was disappointing (and follows prior trials (2018 and 2019) with similar poor results). In contrast to prebloom treatment only, in both years, treatments that sequenced resistance inducing sprays through the bloom period resulted in significant disease suppression. In 2020, in pear, fire blight suppression from Actigard sprays during bloom were apparently improved when preceded by a prebloom treatment of prohexadione-Ca, but this effect of the prebloom prohexadione-CA treatment on the Actigard program was not observed in Gala apple in 2021. It should be noted that resistance inducing materials do not have any effect on epiphytic populations of the fire blight pathogen in flowers (Fig. 3). Consequently, in conventional orchards, the approaches being evaluated here would need to be complemented by antibiotic treatments. In this context, the induced-resistance from an Actigard treatment program could be expected to lessen variability in antibiotic suppression of fire blight, which can result from less than perfect coverage, pathogen resistance to the material, and very short effective residuals of antibiotic materials. We plan to continue to trial Actigard bloom programs in 2022 to better understand the significance of application frequency.

With regard to the organic material, Regalia, applying it twice in 2020 to the older apple trees resulted in a better performance than its application as solitary prebloom treatment in the younger trees. In 2021, we evaluated Regalia in an organic induced-resistance program that included the resistance-inducing material, LifeGard. And (albeit based on one year's data), the three application/multiple material program achieved a somewhat higher level of suppression than either Regalia or LifeGard individually in two-spray programs. Combination programs of Regalia and LifeGard will be investigated in 2022 trials.

Fig. 3. Effect of resistance inducers applied to A) Bartlett pear and B) Gala apple trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers in 2020. Pathogen populations were measured by sampling five flower clusters (~25 flowers, bulked) from each replicate tree. Each sample was washed in 25-ml of sterile, de-ionized water followed by dilution plating onto nutrient agar plus nalidixic acid. Data for Firewall 50 (streptomycin) is shown for comparison. $Log_{10} = 2.0$ was the detection limit of the assay. Data depict mean of each treatment program on each sampling date.



Objective 3: In greenhouse, investigate further the potential synergy between Ph-Ca and Actigard for fire blight control.

Rationale. Although Kudos/Apogee (prohexadione-Ca), Actigard, Regalia and LifeGard are each marketed as materials that enhance host resistance to fire blight, to our knowledge only Actigard has been shown to increase expression of pathogenesis-related defense genes in apple. Consequently, under this objective, we attempted to slow fire blight canker expansion in potted, greenhouse-grown trees by treatment (sprays or trunk paints) of resistance inducing materials and measure if these treatments cause a change in defense gene expression.

Experimental design. In 2020, greenhouse experiments proposed did not occur owing to closure of the OSU greenhouse facility during early months of the COVID-19 pandemic. In 2021, plant material consisted of 400bareroot, 2-year-old ELMA.26 liners obtained in spring 2020 from TRECO (Woodburn, OR). The trees were greenhouse-grown in 2-gallon containers containing growth medium (Rexius Forest Products, Eugene, OR placed outside for winter 2020-21. During the summers, trees were fertilized monthly with Peters 20-10-20 (0.5 g).

The experiment was initiated when new shoots on potted trees were 12 to 14inches in length (early June 2021). Experimental design was a randomized block with 18 inoculated trees (replicates) per treatment (Table 4). Shoot tips were inoculated with a mixture of several *Erwinia amylovora* isolates suspended in water at concentration of 10^9 CFU per ml. The youngest and second youngest leaves on the actively growing shoot tip were split along mid-rib with a scissors that had been dipped in the pathogen suspension. Shoot tips were covered in a plastic re-sealable bag containing 1 ml of water for a period of 5 days. Incidence of infection, length (%) of vegetative shoot becoming necrotic, and whether or not the fire blight canker expanded into older trunk tissue were measured monthly. Relative proportion of a shoot diseased was calculated the ratio of canker length in current season growth divided by total length of the current season growth.

Leaves on non-inoculated but treated plants were sampled at 2, 4, and 7 weeks after the date of inoculation to measure expression of PR-gene indicators of resistance induction. Specifically, the relative expression of pathogenesis-related PR-genes, PR-1, PR-2, and PR-8, were quantified. Leaf samples were frozen in liquid nitrogen and stored at −80°C until processed. Leaves were processed by grinding in liquid nitrogen and followed by RNA extraction (RNeasy mini kit for plant tissue, Qiagen, Valencia, CA), and cDNA synthesis (Superscript II RNase H–Reverse Transcriptase, Gibco BRL, Grand Island, NY). PCR primers developed by Maxson-Stein et al. (2002) were used to quantify expression of PR proteins.

Table 4. 2021 treatments of resistance inducers applied to 2-year-old ELMA 26 trees.

Treatment	Prohexidione-Ca	Timing	ASM	Timing	inoculated	no inoc.
1	12 oz./100 gal.	foliar spray (-1)			18	
2	12 oz./100 gal.	foliar spray (-8)			18	3
3			3.2 oz./100 gal.	foliar spray (-1)	18	3
4	12 oz./100 gal.	foliar spray (-1)	3.2 oz./100 gal.	foliar spray (-1)	18	
5	12 oz./100 gal.	foliar spray (-8)	3.2 oz./100 gal.	foliar spray (-1)	18	3
6	12 oz./100 gal.	foliar spray (-1)	30 g/L	**trunk paint (-1)**	18	3
7			30 g/L	**trunk paint (-1)**	18	3
	Regalia	**Timing**	**LifeGard**	**Timing**		
8	1 gallon/100 gal.	foliar spray (-1)			18	
9	1 gallon/100 gal.	foliar spray (-8)			18	3
10			13.5 oz./100 gal.	foliar spray (-1)	18	3
11	1 gallon/100 gal.	foliar spray (-1)	13.5 oz./100 gal.	foliar spray (-1)	18	
12	1 gallon/100 gal.	foliar spray (-8)	13.5 oz./100 gal.	foliar spray (-1)	18	3
13	1 gallon/100 gal.	foliar spray (-1)	60 g/L	**trunk paint (-1)**	18	3
14			60 g/L	**trunk paint (-1)**	18	3
15	1:1 concentrate	**trunk paint (-1)**			18	3
16-17	**Inoc control**	-	-	-	36	
18	**No Inoc control**	-	-	-		12

In parentheses, the treatment timings '-1' and '-8' refers days before inoculation.

Observations on fire blight progression. In spring 2021, trees acquired for the study exhibited generally uniform leaf emergence and vegetative shoot production. At inoculation (9 June), lengths of the new shoots on the trees averaged 33 cm (13"). Inoculation of the terminal shoot tips with *E. amylovora* incited fire blight in 254 of 255 trees (99.7%). In the first month after inoculation, for control trees, the expanding canker consumed ~2/3 of the vegetative shoot tissue below the inoculation site (Fig. 1). From July to August, cankers in control trees continued to expand, extending into woody stem tissue on 64% of trees. Canker expansion slowed in the woody tissue. On 5 August, an average of 80% of shoot tissue on inoculated control trees showed symptoms of fire blight. Non-inoculated control trees did not show fire blight symptoms.

Treatments that suppressed canker expansion included Apogee sprays (15% suppression), Actigard sprays (with or without co-treatment with Apogee, 28% suppression), and Actigard trunk paints (with or without co-treatment with Apogee, 55% suppression) (Fig. 4). Organic inducers, whether applied as sprays or trunk paints in solitary or co-treatment, showed no significant reduction of canker expansion in new shoot tissue.

Observations on PR-gene expression. On the first leaf sampling date (22 June), most treatments (both organic and conventional) showed elevated expression of PR-1 and PR-2, although with the exception of Actigard trunk paint treatments, expression of defense genes relative to the untreated control was relatively small (< 10-fold) (Fig.5). For PR-2, Actigard trunk paints enhanced expression by 20 to 40-fold relative to the untreated control. On the second leaf sampling date (29 June) (Fig.5) and third leaf sample date (5 Aug) (data not shown), only the Actigard trunk paint treatments (with or without co-treatment with Apogee) showed elevated defense gene expression.

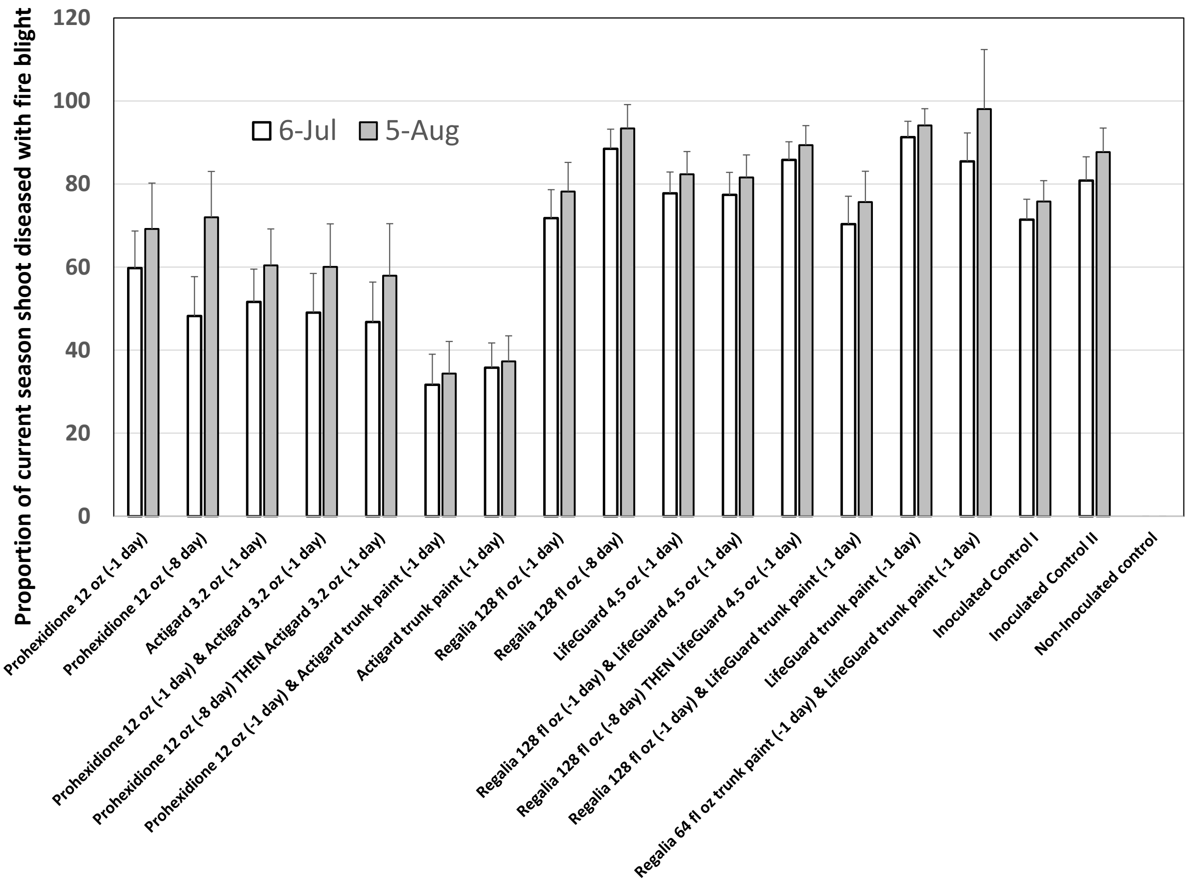


Fig. 4. Effect of applied resistance inducers on the proportion of ELMA.26 shoots diseased with fire blight. A shoot tip on the potted 2-year-old, greenhouse trees were inoculated with *Erwinia amylovora* in early June. Treatments were either a SPRAY applied to runoff at 8 or 1 day before inoculation, or a TRUNK PAINT applied to runoff at one day before inoculation. The date of inoculation was 9 June; data for 6 July (white bar) and 5 August (gray bar) are shown. Data are expressed as length of the shoot canker divided by current season shoot length. Smaller bars are the standard error of the mean (n = 18 trees). See Table 3 for additional information related to experimental design.

Discussion. From past experience and literature reports, we know that both Actigard and Ph-Ca (Kudos, Apogee) when applied to trees as solitary treatments slow/prevent the development of shoot blight during late spring and early summer. Also known are the mechanisms of suppression of Ph-Ca (inhibition of gibberellin synthesis) and Actigard (induction of systemic acquired resistance). Perhaps unsurprisingly, our experiment confirmed that both materials suppress canker expansion to a partial degree, and that Actigard induced defense gene expression and Ph-CA did not. The higher degree of defense gene induction by Actigard trunk paints relative to sprays is a result we have demonstrated previously. Consequently, the 'new information' revealed by this experiment was limited, but it did confirm our previous understanding of Ph-CA and Actigard.

For both orchard and greenhouse data, none of the experiments demonstrated additivity or synergy from combinations of Actigard and Ph-Ca. Of the two materials, Actigard results were more intriguing because the degree ofsuppression was stronger, and the understanding of its full potential in fire blight management is still incomplete. It would behoove the industry to continue to investigate the degree to which Actigard can impact the fire blight development in susceptible cultivars. This includes a better understanding how rate and frequency of sprays contributes to suppression, as well as further investigation of concentrated trunk applications for blight prevention. Currently, trunk applications of Actigard are limited by the material cost and the EPA label. These constraints could potentially be modified if the biological data can further demonstrate the material's value.

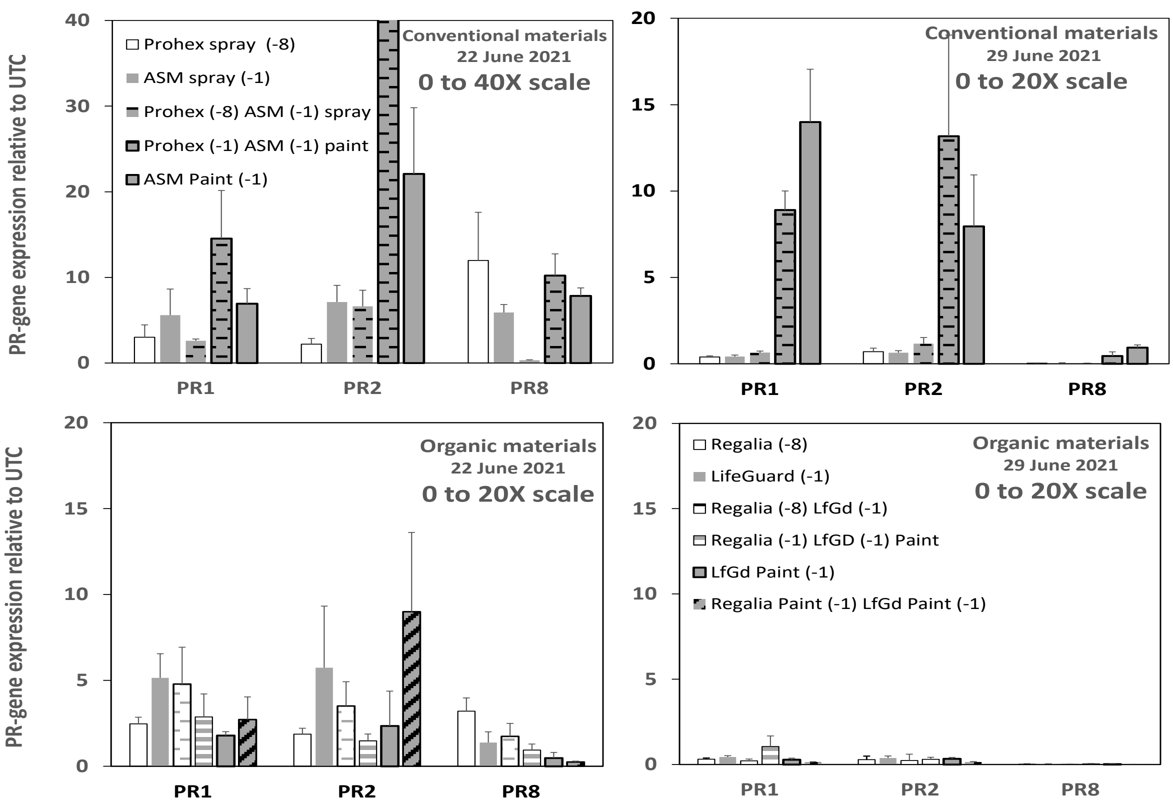


Fig. 5. Effect of applied resistance inducers on expression of pathogenesis-related (PR) genes PR-1, PR-2, and PR-8 in ELMA.26 trees. Treatments were either a SPRAY applied to runoff at 8 or 1 day before inoculation, or a TRUNK PAINT applied to runoff at one day before inoculation. Relative levels of PR protein gene expression were quantified by quantitative reverse-transcription polymerase chain reaction performed on c-DNA prepared from total RNA extracts from apple leaves sampled approximately 3, 4 and 8 weeks after the pathogen inoculation using expression of actin as the reference gene. The date of inoculation was 9 June, and data for 22 June (left panels) and 29 June (right panels) are shown. Data are expressed as length of the trunk canker divided by the height of the tree. Smaller bars are the standard error of the mean (n = 3 trees). See Table 3 for additional information related to experimental design.

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 resistance-inducing materials was investigated. Solitary, prebloom treatment of Kudos/Apogee (prohexadione-Ca), Actigard, or Regalia were of little value. Three-spray, bloom period programs of Actigard only provided up to 75% infection suppression. Organic, bloom period spray programs that combined the materials, Regalia and LifeGard, provided 59% infection suppression.

SIGNIFICANT FINDINGS:

- Kudos/Apogee (prohexadione-Ca), Actigard, or Regalia applied at prebloom timings did not provide significant suppression of fire blight in flowers compared to a non-treated control.
- Kudos applied as a solitary treatment at timings of first pink, full pink and 10% bloom, did not reveal a pattern related to time of treatment and level of suppression.
- In 2020, Actigard sprayed three times during bloom reduced the incidence of infection compared the water-treated control (45 and 65 % reduction in pear and apple, respectively). In2020, a prebloom Kudos treatment appeared to enhance the level of suppression obtained by the Actigard treatment program. But in 2021, the degree of suppression obtained with a 3-spray Actigard program (75%) was not improved with a prebloom treatment of Apogee.
- In greenhouse, an Actigard spray reduced fire blight canker expansion in potted ELMA.26 by 28%, and the effect of Actigard was not improved with the addition of an Apogee spray. A single Actigard trunk paint treatment reduced canker expansion by 55% and was not improved with the addition of an Apogee spray.
- In greenhouse, among spray and trunk paint treatments of Apogee, Actigard, Regalia, and LifeGard, only Actigard trunk paint treatments showed sustained expression of apple defense genes, PR-1, and PR-2.
- In greenhouse, treatments with the organic materials, Regalia and LifeGard, had no effect on fire blight canker expansion in potted ELMA.26.

FUTURE DIRECTIONS

- Within current labeling restrictions, Actigard requires further investigation including how rate and frequency of sprays contributes to preventative blossom blight suppression.
- Similarly, combination bloom period spray programs of Regalia and LifeGard require further investigation.
- Without regard to practicality, industry-sponsored research should learn as much as possible about the degree to which Actigard could impact the development of fire blight in susceptible apple cultivars (i.e., out-of-the-box thinking with little consideration to current material cost, pesticide labeling or application rate or technology).