FINAL PROJECT REPORT

Project Title: Apple specific issues in fire blight management

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Cooperators:
Materials: Arysta Life Sciences, Croker's Fish Oil, Syngenta, Westbridge Agric. Products

Other funding sources: None

Total Project Funding: $ 39,200

Budget History:

Item	**2010**	**2011**	
Salaries FRA 3mo	10,000	10,300	
Benefits OPE 63%	6,300	6,489	
Wages			
Benefits			
Equipment			
Supplies	2,000	2,111	
Travel local	500	500	
Plot Fee	500	500	
Miscellaneous			
Total	19,300	19,900	

OJECTIVES:

1) Integration of a new material, kasugamycin, into blossom blight control programs for conventional orchards (this objective was funded from pear sources, but results are applicable to apple);

2) Evaluate fire blight suppression programs compatible with European organic certification standards;

3) Evaluate protection of apple rootstocks (ELMA 9, ELMA 26) from girdling fire blight infections via soil application of an inducer of systemic acquired resistance.

SIGNIFICANT FINDINGS:

- The product Kasumin 2L (kasugamycin) provided outstanding control of fire blight of apple; EPA registration is on track for 2012.

- Resistance management strategies for Kasumin -- i.e., mixtures with oxytetracycline and integration with biological control -- provided excellent fire blight control. These strategies should help to ensure longevity of the product.

- Effective non-antibiotic strategies for fire blight control were developed. These strategies are being implemented for apples exported under the International Organic Program standard.

- A yeast material, Blossom Protect, provided excellent control of fire blight, with registration and utilization within the International Organic Program expected in 2012.

- Pot drenches and trunk paints of the SAR inducer, ASM (acibenzolar-*S* methyl), slowed expansion of fire blight in inoculated shoots of potted apple rootstock cultivars ELMA 26 and Nic 29.

- ASM applied to potted 'Gala' on M26 provided a high level of protection of the rootstock after a high dose of the pathogen was inoculated directly into the graft union.

RESULTS

1) Integration of a new material, Kasumin, into blossom blight control programs for conventional orchards.

1. a) 2011 season. Treatments of Kasumin alone and in combination with other materials where tested in 2011, marking five years of evaluation. Trees used in the 2011 study averaged 587 flower clusters per tree. Fire blight risk as determined by the COUGARBLIGHT model was low during the bloom period but disease intensity was moderate with water treated trees averaging 78 blighted clusters per tree (14%) (Table 1). Each of the treatments significantly reduced ($P \leq 0.05$) incidence of infection and total number of infected flower clusters per tree compared to the water-treated control. Kasumin 2L and 8L (kasugamycin), Firewall (streptomycin), and Fireline (oxytetracycline) provided excellent disease control. The integrated program of Bloomtime in early bloom followed by Fireline, or Kasumin and Fireline at full bloom also provided excellent control. The pathogen inoculum used in the study was 50% streptomycin-resistant and 50% streptomycin-sensitive *E. amylovora.* Firewall (streptomycin) suppressed disease by 85%, whereas Kasumin 2L provided 93% control.

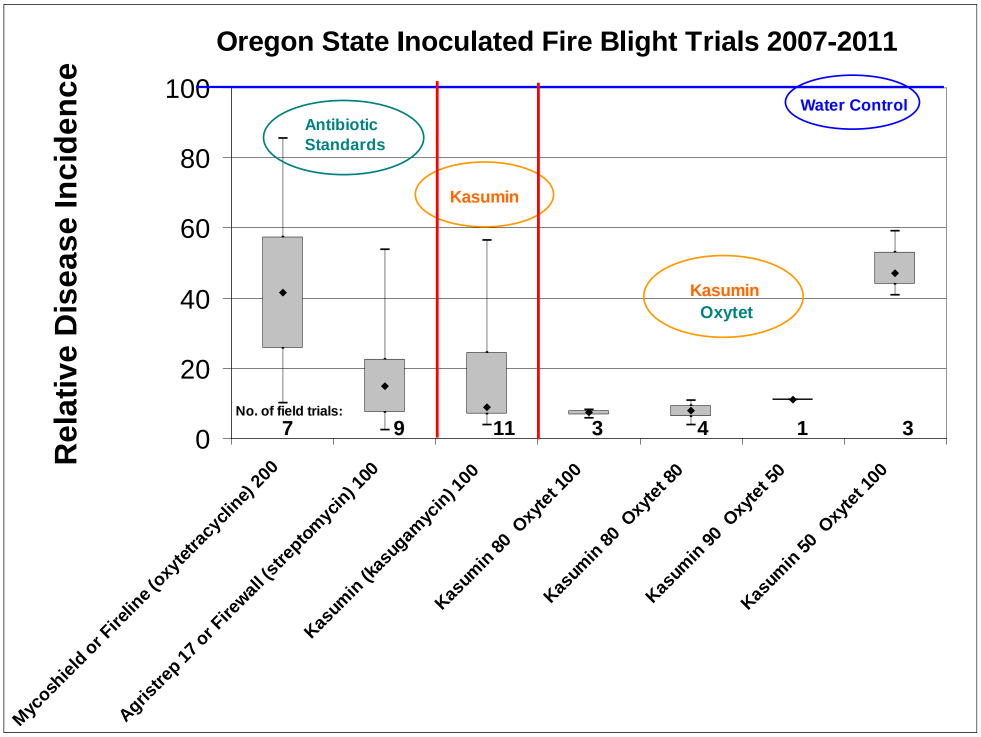
Table 1. Evaluation of Kasumin for suppression of fire bli8ght of Gala apple, 2011

Treatment	Rate per 100 gallons water	Date treatment applied* 4 May 30% bloom	6 May 70% bloom	10 May Full bloom	Number of blighted clusters per tree**		Percent blighted floral clusters ***	
Water control	**------**	**X**[§]	**X**	**X**	**78**	**a**[#]	**14.1**	**a**[#]
Agri-mycin 100 ppm	8 oz.	---	---	X	13	b	2.1	b
Bloomtime then	5 oz.	X	X	---	10	bc	1.6	bc
Kasumin 2L 90 ppm	52 fl oz.	---	---	X				
plus Fireline 50 ppm	4 oz.	---	---	X				
Bloomtime then	5 oz.	X	X	---	8	bc	1.4	bc
Fireline 200 ppm	16 oz.	---	---	X				
Kasumin 8L 100 ppm	16 fl. oz.	---	---	X	8	bc	1.4	bc
Kasumin 2L 100 ppm	64 fl. oz.	---	---	X	5	c	1.0	c

* Trees inoculated on 9 May with 5 x 10^5 CFU/ml of a 50:50 mix of *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) and strain Ea153S (streptomycin-resistant). ** Transformed log ($x + 1$) prior to analysis of variance; non-transformed means are shown. *** Transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown. [§] X indicates material was sprayed on that specific date; --- indicates material was not applied on that specific date. [#] Means within a column followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$.

1.b) Summary Kasumin field trials from 2007 to 2011. A total of nine orchard trials in apple and pear were conducted over the period. Data were summarized in box and whiskers plots as 'Relative Disease Incidence', which for each trial is the number of fire strikes in the Kasumin (or comparative)

Fig. 1



treatment divided by the number of fire strikes in the water-treated control (expressed as a percentage) (Fig. 1). Kasumin achieved a median reduction of fire blight of > 90%, which was equivalent to streptomycin (targeted to streptomycin sensitive performed strains of the fire blight pathogen) and better than the median 58% control obtained with oxytetracycline. [Note: trials in 2007, 2008, and 2009 had two treatments of each antibiotic, and had trials in 2010 and 2011 had one treatment.]

1.c) Compatibility of Kasumin with biological control. By first treating trees with a biological agent (BlightBan C9-1 or Bloomtime Biological) followed by Kasumin, an 'integrated strategy' reduces the likelihood of selection for kasugamycin-resistance in the pathogen. The mechanisms that reduce selection pressure are suppressed pathogen populations via competition with the biological agent and limitation of Kasumin use (e.g., to one application as opposed to the two applications often typical in commercial production). Over the 2007 to 2011 period, the orchard trials showed that there was no statistical difference between integrated control with Kasumin compared to Kasumin alone (Table 1 (above) and Table 2 (below)). We also hypothesized that use of Kasumin could have a negative impact on populations of the biological agent on flowers and that this effect could be overcome by use of a kasugamycin-resistant strain of *Pantoea vagans* strain C9-1S, thereby improving the efficacy of the biological component of the integrated strategy. In the field, data collected on disease incidence and on population sizes of the biological agents on flowers (data available on request) failed to support this hypothesis. We concluded that use of Kasumin 2-3 days after a biological treatment would be expected to have minimal impacts on populations of bacterial biological control agents. In this regard, the effect of Kasumin on non-target bacteria on flowers was more like that observed with oxytetracycline than observed with streptomycin.

Table 2.

Integrated control with Kasumin and Kasumin-resistant BlightBan C9-1S

		Fire blight strikes per tree
2009	**Water**	**485 a**
Bartlett Pear	***P.v.* C91S^{kr} then Kasumin**	**38 b**
	***P.v.* C91S then Kasumin**	**42 b**
	Kasumin twice	**33 b**
2009	**Water**	**132 a**
Gala & Golden	***P.v.* C91S^{kr} then Kasumin**	**18 b**
Delicious Apple	***P.v.* C91S then Kasumin**	**16 b**
	Kasumin twice	**8 b**
2010	**Water**	**236 a**
Gala Apple	***P.v.* C91S^{kr} then Kasumin**	**14 b**
	***P.v.* C91S then Kasumin**	**16 b**
	Kasumin once	**20 b**

Means within cultivar and year followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$. Experiments conducted in orchards located near Corvallis, Oregon. *Pantoea vagans* C9-1S^{Kr} is a kasugamycin-resistant selection of *Pantoea vagans* C9-1S (the organism in BlightBan C9-1 and related to *Pantoea agglomerans* strain 325 in Bloomtime Biological).

When registered, Kasumin will enhance control and broaden the effective tool box for conventional fire blight management. Kasumin is not used in human medicine, and shows no cross resistance to streptomycin or oxytetracycline. Although not shown in our research, work by others researchers indicate Kasumin is slightly less effective than streptomycin (against sensitive strains) with tested under extreme disease pressure (very high inoculum levels). Speculating, Kasumin may not be absorbed into pear or apple tissue as readily as streptomycin, which is considered locally systemic. Analogous to oxytetracycline, sprays of Kasumin should be timed at full to late bloom beginning when moderate (as opposed to high) levels of disease risk have been forecasted.

In the years of this project, McGhee & Sundin (2011) characterized resistance in the fire blight pathogen to kasugamycin. Analogous to our results with biocontrol agent *Pantoea vagans*, selection of kasugamycin-resistant strains of the fire blight pathogen with the ability to grow at the maximum label rate of Kasumin (100 ppm) is a two-step mutation process. In contrast, a spontaneous mutation in these bacteria to resistant to the label rate of streptomycin is a one step process, and a spontaneous mutation to resistant to oxytetracycline has not been reported (even from the lab). Thus, the risk of selecting resistance in *E. amylovora* to Kasumin is apparently intermediate to the other registered antibiotics or fire blight control. A potential negative of Kasumin, reported rarely, is a rate-dependent phytoxicity to pear and apple (e.g., we have never observed a phytotoxic response in our use of Kasumin). Our results showing outstanding fire blight control with Kasumin at slightly reduced rates mixed with oxytetracycline (Fig. 1) may be a solution to a potential phytoxicity problem if it is ever observed (e.g., on a particular cultivar).

2) Evaluate control of blossom blight with programs acceptable for fruit export to the European organic markets.

2.a) Frequency of treatment. In orchard trials, for both pear and apple, increasing the frequency of treatment of biological products improved blossom blight control. For example, a *Pantoea agglomerans* product (Bloomtime Biological or BlightBan C9-1) applied at 30 and 70% bloom followed by two applications of the fermentation product of *Bacillus subtilis* QST 713 (Serenade Max) at full bloom and petal fall reduced the incidence of blighted flower clusters by an average of 71% (four orchard trials) compared to 47% when each component of this product combination was used only once.

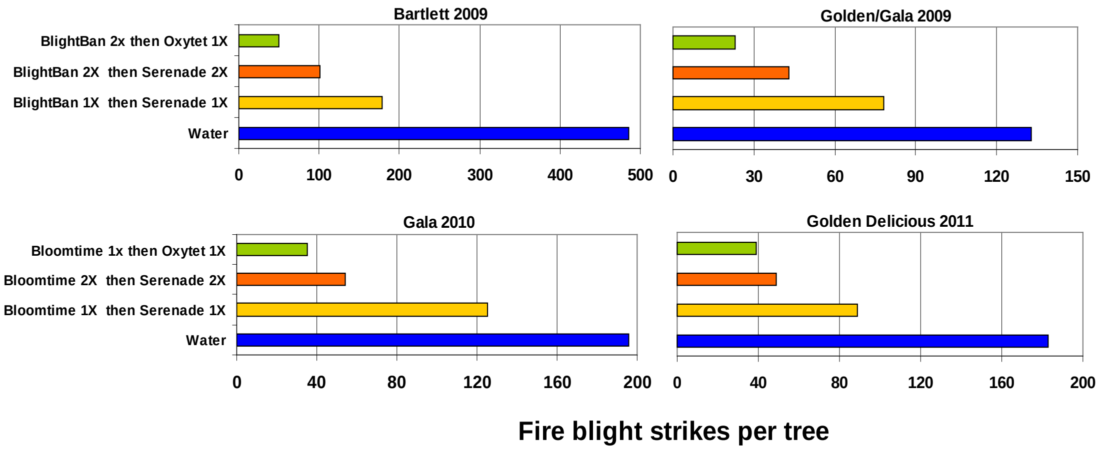


Fig. 2. Incidence of fire blight on pear and apple flower clusters as affected by integrated biological and antibiotic treatments in orchard trials conducted near Corvallis, Oregon from 2009 to 2011.

2.b) Effect of bloom thinning on fire blight . In apple, fruit load (bloom) thinning with 2% lime sulfur and 2% fish oil at 30 and 70% bloom significantly ($P \leq 0.05$) reduced the proportion of blighted flower clusters in 3 of 4 orchard trials.

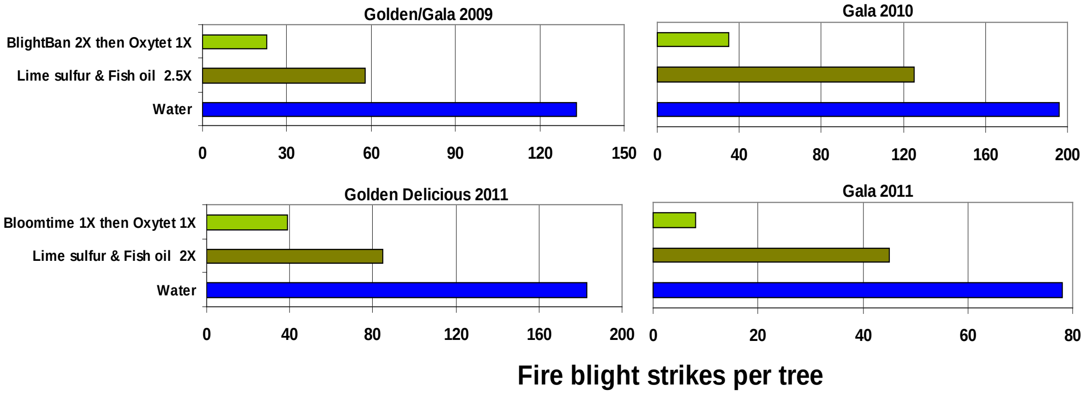


Fig. 3. Incidence of fire blight on pear and apple flower clusters as affected by as affected by the bloom thinning treatment, lime sulfur plus fish oil, in orchard trials conducted near Corvallis, Oregon from 2009 to 2011.

2.c. Integrated non-antibiotic control. Over three orchard trials, treatment with *Aureobasidium pullulans* (Blossom Protect) after LS+FO reduced the incidence of fire blight by an average of 91% blight control compared to trees treated with water only; this level of control was similar to treatment with streptomycin.

Table 3. Incidence of fire blight on apple flower clusters as affected by the bloom thinning treatment, lime sulfur plus fish oil, and subsequent biological treatments in orchard trials^{x} conducted near Corvallis, Oregon from 2009 to 2011.

	Orchard cultivar and year of trial		
Treatment	Golden Delicious 2010 (%)	Golden Delicious 2011 (%)	Gala 2011 (%)
Water	32.5 a (261)	34.9 a (183)	14.1 (78)
Lime sulfur plus fish oil twice	27.3 a	15.5 b	8.9 b
Lime sulfur plus fish oil twice then Blossom Protect twice	3.2 b	3.7 c	0.9 c
Lime sulfur plus fish oil twice then Bloomtime Biological combined with Blossom Protect once then Blossom Protect once	3.1 b	1.5 c	1.7 c
Bloomtime Biological twice then Blossom Protect twice	3.6 b	-	2.5 c

Bloomtime Biological once then oxytetracycline once	6.8 b	7.4 bc	1.4 c
Streptomycin sulfate once	2.5 b	-	2.1 c

[x] Single tree plots were arranged in a complete randomized block design with three to four replications per treatment. The bloom thinning treatment, lime sulfur (2%) plus fish oil (2%), was sprayed at 30 and 70% bloom. The biological materials following LS+FO were applied at full bloom and prior to petal fall. In the comparative standard (23), Bloomtime Biological was applied at 70% bloom and oxytetracycline was applied at full bloom.

[y] Within a column, means followed by the same letter do not differ significantly according to Fischer's protected least significance difference at *P* = 0.05. The arc-sine square root transformation was applied incidence data prior to analysis of variance.

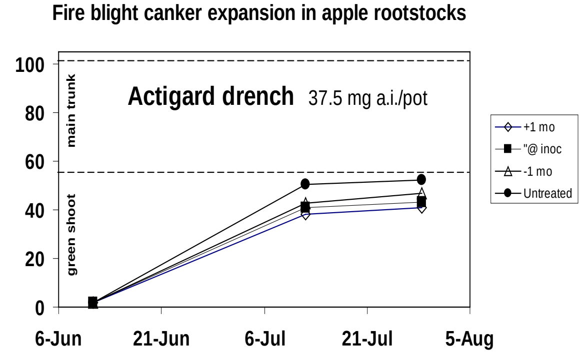
[z] Numbers in parentheses are the mean number of blighted flower cluster on water-treated trees. Incidence was computed by dividing number of blighted flower cluster by total number of cluster.

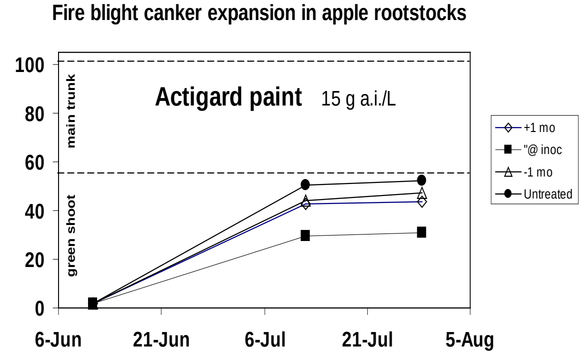
The integrated approach demonstrated by Stockwell et al. (2008) and Lindow et al. (1996) utilized a gram negative bacterial antagonist (e.g. *P. agglomerans* and/or *Pseudomonas fluorescens*) to suppress the pre-requisite epiphytic phase of *E. amylovora* on floral stigmas followed by an oxytetracycline treatment later in bloom to prevent infection in the nectary. A non-antibiotic program modeled on this strategy involved substitution of the biological product, Serenade Max, for oxytetracycline. In this role, Serenade Max proved to be inhibitory to the fire blight pathogen, but obtaining levels of fire blight control closer to that achieved with oxytetracycline after a bacterial antagonist required doubling the frequency of application of the biological products (Fig. 2). This result indicates that non-antibiotic programs for fire blight will likely be more expensive than programs utilizing antibiotics because satisfactory disease control will require more treatments in the orchard. Spraying more often also means that orchardists will need to be more preventative in their approach to fire blight control (i.e., sprays required every few days) as opposed to reactive, where a single antibiotic spray could be applied based on an imminent infection event forecasted by a disease warning model (CougarBlight).

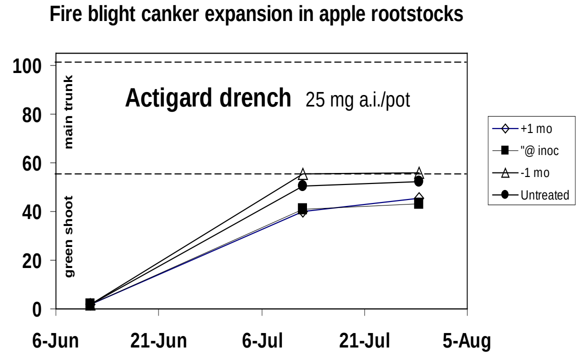
The yeast product, Blossom Protect, provided outstanding disease control when used either as part of an integrated program following *P. agglomerans* or following bloom thinning treatments of LS+FO (Table 3). In organic pome fruit production, the former program would have the highest value in pears where bloom thinning is not practiced routinely, whereas yeast treatments after LS+FO would be more practical in apples. A negative side effect of *A. pullulans* (the yeast organism in Blossom Protect) when used in a wetter climate has been a tendency to induce skin russet on the surfaces of developing fruit (Kunz 2008, Spotts & Cervantes 2002), which we also observed in our 2011 Gala apple trial when frequent rains occurred neared the end of the bloom period. Russeting of fruit surfaces can greatly reduce crop value, and thus an improved understanding of this potential in various climates should be obtained before this product is used extensively. EPA registration for Blossom Protect is on track for spring 2012.

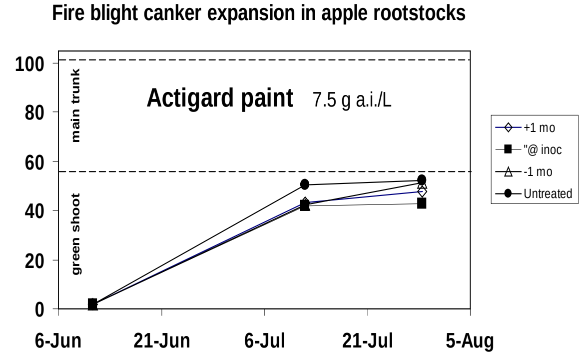
3) Evaluate protection of apple rootstocks (ELMA 9, ELMA 26) from girdling fire blight infections via soil application of an inducer of systemic acquired resistance.

3.a). Rootstock only experiment. Drenches and paints of the a SAR material, ASM (acibenzolar-*S* methyl) slowed expansion of fire blight in one year old, potted apple rootstock cultivars, EMLA 29 and Nic 29 apple (Fig. 4). In non-treated trees, the expanding canker consumed nearly all of the current season growth, but generally did not expand into the woody tissue. Green shoots on SAR-treated trees had smaller cankers relative to the non-treated trees, but the effects of the SAR treatment on apple rootstocks was smaller than was observed previously in pear (see 2009 pear report). In pear, the marked effect of ASM on canker expansion occurred mostly in woody tissue. For apple rootstock cultivars, we attribute the smaller effect of ASM to the fact that cankers were limited to the current season shoots. Interestingly, relative to drench and spray treatments, the ASM paint treatment (15 g a.i./L) applied at the at inoculation showed the largest reduction in canker size (top right panel).









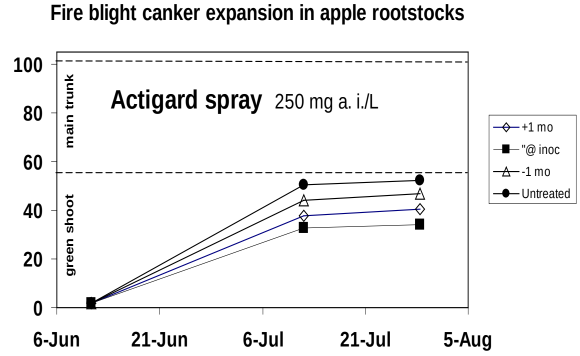
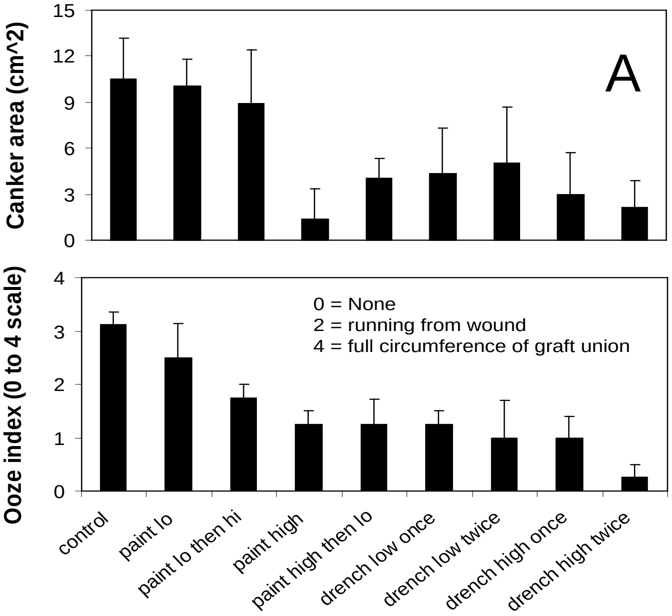


Fig. 4. Effect of pot drenches, trunk paints, and foliar sprays of the SAR-inducer, ASM, on expansion of fire blight cankers in apple rootstock cvs. EMLA 26 and Nic 29. All trees were inoculated with the fire blight pathogen on June 10. Treatments were made 1 mo prior, at, or 1 mo after inoculation. Each point is the mean of 12 trees (6 of EMLA 26 and 6 of Nic 29).

3.b). Rootstock Scion experiment. Drenches of ASM, but also some ASM-paint treatments, provided significant control of canker expansion in wound-inoculated, EMLA 26 rootstock (under Gala) measured in August of 2010 (Fig. 5 A). In particular, two drenches (May 21 and July 2) of ASM (50 mg a.i. per plant) provided nearly complete suppression of canker development. By October, scions of several of the untreated trees were dead as a result of the girdling fire blight canker in the rootstock. In contrast, none of the ASM-treated trees were girdled. The re-assessment of these treatments in May 2011 showed that canker size had-increased from August 2010, and that drenches and several paint treatments still showed significant suppression of canker size (Fig. 5B). This greenhouse experiment was repeated in 2011 with EMLA 26 and Nic 29 rootstocks (under Cameo). Preliminary observations show responses similar to what was observed for the 2010 experiment, but measurement of canker size was delayed to spring 2012. A field trial similar to the greenhouse experiment also was conducted in 2011 (EMLA 26 under Gala) but our inoculations failed, due to drying and cracking of the trunk wraps placed over the inoculation wounds. Field experiments will be initiated again in 2012.



Ooze of the fire blight pathogen on untreated EMLA 26 (Gala).

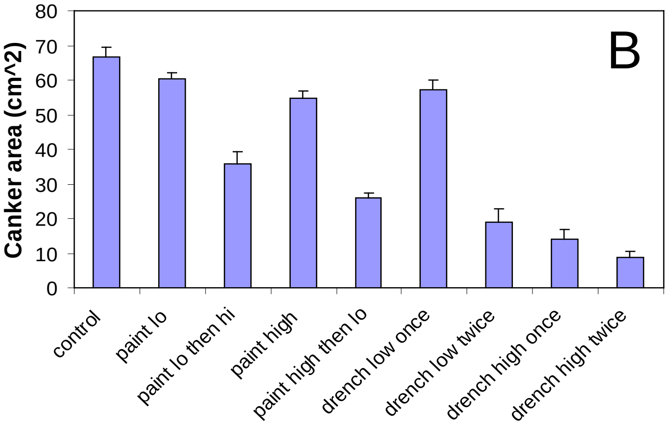


Fig. 5. Effect of pot drenches, trunk paints, and foliar sprays of the SAR-inducer, ASM, on expansion of fire blight cankers in apple rootstock cv EMLA 26 (under 'Gala'). Wounds on rootstocks trees were inoculated with the fire blight pathogen on July 8. Treatments were made on May 30 and June 21. Each bar is the mean of 6 trees. A) Measurement recorded 9 August 2010; B) measurements recorded 22 May 2011.

3.c.) SAR induction of pathogenesis-related proteins. Treatment with ASM induces genes involved with host defense against pathogens, and after induction, these genes produce products collectively known as 'pathogenesis-related proteins' (PR-proteins). Whether or not a treatment with a SAR inducer has had an effect on host defenses can be evaluated by measurement of the level of expression of PR-protein genes in the treated plant (the measurements are done with reverse-transcriptase, real time-polymerase chain reaction (rt-qPCR)). In the greenhouse experiments, we measured PR-protein expression in apple leaves after application of ASM by the methods of pot drench, trunk paints and foliar sprays. Data on gene induction were not always conclusive but generally leaf samples from trees that received ASM by drench or by paint mostly showed higher levels of expression of genes PR-1, Pr-2, and PR-8 (Fig. 6). Spray treatments of ASM , however, did not show a high level of induction and/or the induction effect was less persistent than observed with drenches and paints.

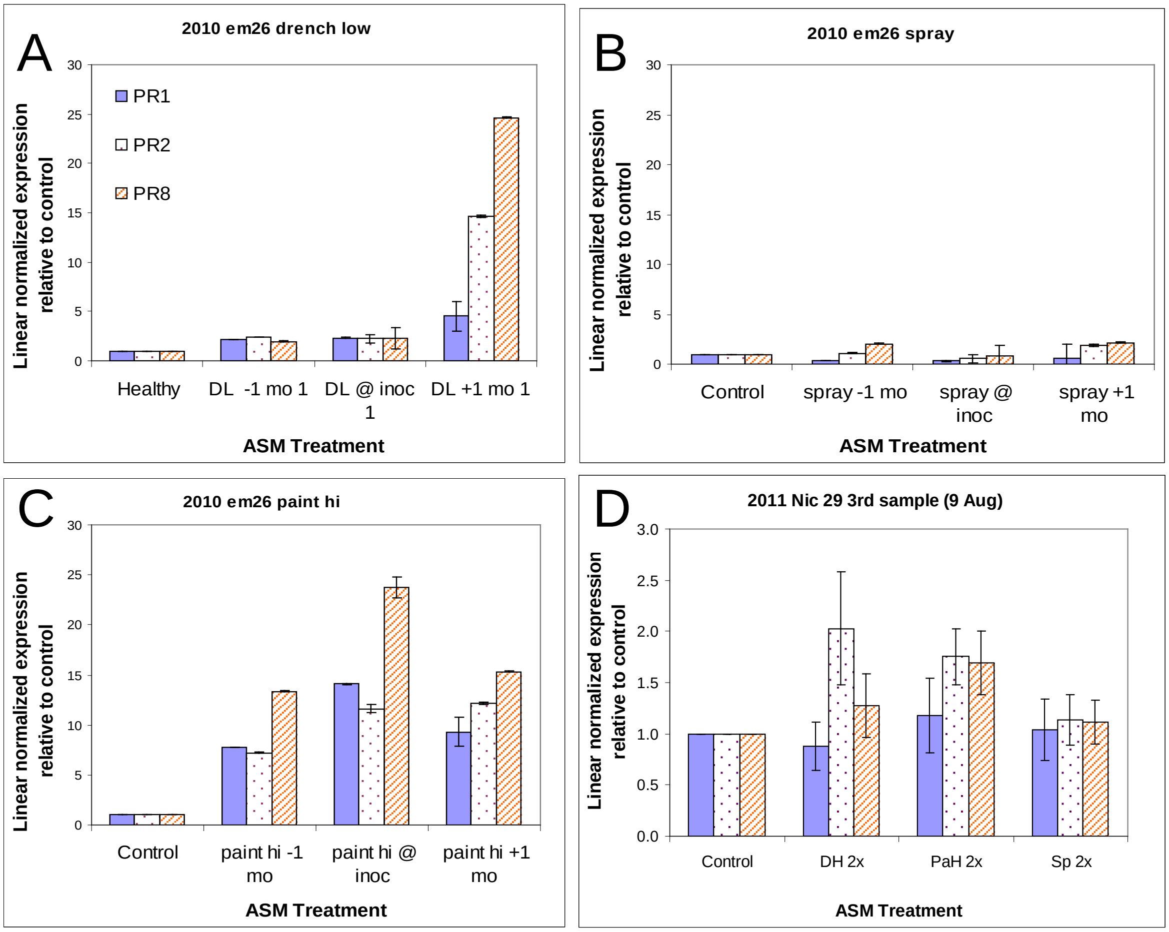


Fig. 6. Effect of pot drenches, trunk paints, and foliar sprays of the SAR-inducer, ASM, on expression of pathogenesis-related proteins PR-1, PR-2 and Pr-8 in greenhouse-grown apple rootstock cvs. EMLA 26 and Nic 29. In A-C), trees were inoculated with the fire blight pathogen on 10 June; ASM treatments were made 1 mo prior, at, or 1 mo after inoculation and leaves were sampled for analysis on 29 September. In D), the trees were not inoculated with the pathogen; ASM treatments were made on 14 June and 5 July and leaves were sampled for analysis on 9 August. DL (2010) and DH (2011) = drench of 50 mg Actigard in 500 ml water; PaH = trunk paint of Actigard (30g/L) in 2% Pentrabark, and Sp = Actigard (500 mg/L) to runoff.

Discussion of SAR. Like pears, fire blight-susceptible apple rootstock cultivars respond to drenches and paints of the SAR inducer, acibenzolar-*S* methyl, resulting in slowed canker expansion in diseased trees. The effect of ASM on suppression of fire blight was most dramatic when woody tissue of EMLA 26 was inoculated with *E. amylovora* just below the graft union. We are making progress in understanding the effective rates of ASM for the various methods of application. In the future, we intend to focus on paints in rescue-type treatments as shown above, because this method of of application will likely provide the greatest responses in the field environment.

EXECUTIVE SUMMARY

Project Title: Apple specific issues in fire blight management

Investigator: Ken Johnson, Oregon State University

SIGNIFICANT FINDINGS:

Kasumin:

- Over five years of testing, the product Kasumin 2L (kasugamycin) provided outstanding control of fire blight of apple; EPA registration is on track for 2012.
- Resistance management strategies for Kasumin -- i.e., mixtures with oxytetracycline and integration with biological control -- provided excellent fire blight control. These strategies should help to ensure longevity of the product.

Industry implications: When registered, Kasumin will enhance control and broaden the effective tool box for protection of apple flowers from fire blight in conventionally managed orchards. Kasumin is more effective than oxytetracycline, and we expect it to have a positive impact on fire blight management, particularly in high disease risk situations.

Organic fire blight control:

- Effective non-antibiotic strategies for fire blight control were developed. These strategies are being implemented for apples exported under the International Organic Program standard.
- A yeast material, Blossom Protect, provided excellent control of fire blight, with registration and utilization within the International Organic Program expected in 2012.

Industry implications: The information we have been generating has been immediately implemented by growers in the International Organic Program (IOP). Furthermore, the issue of non-antibiotic control of fire blight has increased in importance because the USDA National Organic Program (NOP) has set a 2014 sunset on use of streptomycin and oxytetracycline under the NOP standard.

Systemic acquired resistance:

- Pot drenches and trunk paints of the SAR inducer, ASM (acibenzolar-*S* methyl), slowed expansion of fire blight in inoculated shoots of potted apple rootstock cultivars ELMA 26 and Nic 29.
- ASM applied to potted 'Gala' on M26 provided a high level of protection of the rootstock after a high dose of the pathogen was inoculated directly into the graft union.

Industry implications: Even with excellent products for prevention of fire blight, the disease still occurs and its clean-up can be difficult, especially in young orchards. Systemic acquired resistance (SAR) is an induced defense response in a tree, which when induced in apple and pear has the potential to slow/stop fire blight progression. Commercial products that induce SAR in apple have potential to be used as aids in cutting of fire blight to prevent re-ignition of advancing cankers, to protect graft unions from the rootstock blight phase of fire blight, and to enhance protective sprays when mixed with antibiotics.