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

Project Title: Fire blight epidemiology and improved post-infection control

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Total Project Request: **Year 1:** $15,667 **Year 2:** $16,137

Other funding sources

Agency: California Pear Advisory Board $46K (Elkins $25K, Johnson $12K, others $10K)
Syngenta Crop Protection (Johnson $15K)

Budget History:

Item	**2011-12**	**2012-13**	
Salaries Faculty Res. Assist.	7,337	7557	
Benefits OPE 56%	4,109	4232	
Wages undergrads	1,200	1236	
Benefits OPE 8%	96	99	
Equipment	0	0	
Supplies	1,925	1983	
Travel	500	515	
Miscellaneous		0	
Plot Fees	500	515	
Total	**$15,667**	**$16,137**	

Footnotes: Annually**:** FRA 2 mo + fringe, 130 hr undergrad labor, 2K M&S, 1K local travel & plot fee, 3% inflation

OBJECTIVES:

1a) Evaluate paints of an inducer of systemic acquired resistance as an aid to cutting of fire blight in diseased pear trees

1b) Evaluate an inducer of systemic acquired resistance in combination with antibiotics for protection of pear trees from fire blight

2a) Evaluate the effect of a delayed dormant, copper sanitation treatment on detectability of the fire blight pathogen in samples of flowers collect at various stages of pear bloom

2b) Evaluate new LAMP technologies that will facilitate widespread adoption of molecular scouting within regional fruit production districts.

SIGNIFICANT FINDINGS

- For a 3nd season, a paint of acibenzolar-S-methyl (Actigard) used in combination with cutting of blight reduced the severity of 're-ignited' fire blight cankers in pear.
- In pear, the addition of Actigard to antibiotic treatments enhanced fire blight control over antibiotics alone in both experimental and commercial orchards.
- For a 6th season, molecular scouting during the bloom period detected and characterized the build-up of fire blight pathogen populations in pear flowers with the pathogen detection being greatest near petal fall.
- For a 3rd season, the LAMP molecular scouting protocol demonstrated that a delayed-dormant timing of a copper bactericide suppressed positive pathogen detection in flower samples.
- The protocol and technologies used for molecular detection of the fire blight pathogen were refined and adapted for use with LAMP machines, which allow for the assay to be performed in the orchard.

RESULTS

Obj. 1a) Evaluate paints of an inducer of systemic acquired resistance as an aid to cutting of fire blight in diseased pear trees

Experiments were conducted in 2012 and 2013 to evaluate Actigard as an aid to cutting of blight in pear trees.

In 2012, in a 4-yr-old Bosc pear block, 1 to 5 fire blight strikes developed on each tree as a result of the pathogen inoculation at full bloom. Individual trees were then grouped into experimental blocks based on number of strikes per tree. Blight was cut on 6 June and if cuts re-ignited, repeated on 27 June. Cuts were made at 6-8" below the proximal margin of the canker. Immediately after each cutting, an Actigard paints (30 g/L [1 oz./qt.] in 2% Pentrabark) was

applied to 30-40 cm (12-16") of symptomless branch below the cut. Over the summer, cankers re-ignited in about half of the trees. Canker size and effect on tree health were evaluated in early October. Compared to cut only, the Actigard paint treatment significantly reduced ($P \leq 0.05$) severity of the re-ignited fire blight cankers (Fig.1).

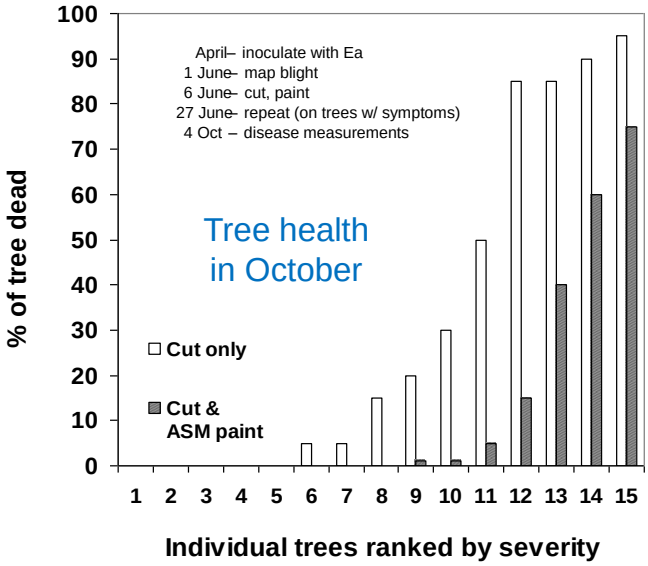


Fig. 1. Effect of an Actigard paint on re-ignited fire blight cankers in 4-yr-old 'Bosc' pear. Trees were inoculated on 27 April. Fire blight cankers were cut 15-20 cm (6-8") below canker margin on 6 and 27 June. Immediately after cutting, an Actigard paint (Actigard 30g/L in 2% Pentrabark) was applied to the 30-40 cm (12-16") of symptomless branch below the cut. Canker severity (weight) and % of tree dead from fire blight were assessed on October 4. Bars represent individual trees ranked in order of disease severity (% tree dead).

In 2013, a 13-year-old Bartlett pear block was inoculated with E. amylovora as part of trial to evaluate treatments to prevent floral infection. After strikes were counted, we pulled the treatment flags and re-grouped the individual trees into experiment blocks based on number of strikes per tree; 14 paired replications of cut only and cut plus Actigard paint were organized in a randomized block design with an average of 9.5 strikes per tree. Blight was cut on 5 June; cuts were made at 6-8" below the proximal margin of the canker. Immediately after cutting, the Actigard treatment received a paint of this material (30 g/L) on the 30-40 cm (12-16") of symptomless branch below each cut. Over the summer, cankers re-ignited at about ¼ of the cuts; the number and sizes of these cankers were evaluated in late September. Compared to cut only, the Actigard paint treatment significantly reduced ($P \leq 0.05$) number and severity (weight) of the re-ignited fire blight cankers (Fig.2 **next page**).

Discussion of Actigard paints. Like apple, fire blight susceptible pear cultivars respond to treatments of the SAR inducer, acibenzolar-*S* methyl (Actigard), resulting in slowed canker expansion in diseased trees. In earlier greenhouse research, the effect of Actigard on suppression of fire blight was most dramatic when drenches were applied to potted trees, but in the field, Actigard drenches have not provided a significant response. Consequently, our experiments with SAR induction as an aid to the restoration of tree health has shifted to Actigard paint treatments applied to the symptomless branches after cutting.

Further rationale for the shift to paints was observed in 2011 greenhouse-grown apple (see January 2012 apple crop protection final report). Trunk paints of Actigard showed levels of disease resistance gene induction (termed 'PR-gene') that were on par with the levels of PR-gene induction achieved by pot drench. The measurement of PR-gene induction provides a marker on whether or not a SAR inducer is providing consistent induction of host defense responses (i.e., an enhanced ability to fend off pathogens). In contrast to pot drenches and trunk paints, foliar sprays have been less consistent in PR-gene induction.

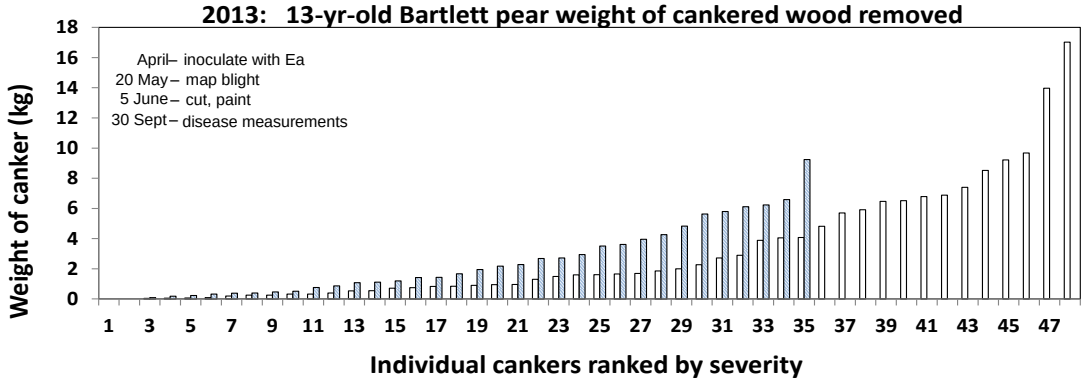


Fig. 1. Effect of branch paints the SAR-inducer, Actigard, on re-ignited fire blight cankers in 13-yr-old 'Bartlett' pear. Trees were inoculated with the fire blight pathogen on 9 April. Fire blight cankers were cut 15-20 cm (6-8") below canker margin on 5 June. Immediately after cutting, Actigard was applied by paint (Actigard 30g/L in 2% Pentrabark) to the 30-40 cm (12-16") of symptomless branch below the cut. Weight of cankered branches removed was assessed on September 30. Data are the ranked severity (weight) of individual cankers 'Actigard-painted' trees (hatched bar) compared to individual 'cut only' trees (open bar).

For the body of data collected from both pear and apple (see January 2012 apple and pear final reports and January 2014 apple crop protection final report), Actigard treatments applied by paint have been most suppressive when the pathogen was present but the amount of active disease in the tree was small. For example, in the greenhouse, paint or spray treatments made at the time of inoculation (pathogen present, small amount of disease) were more effective than treatments made one month prior (no pathogen) or one month after inoculation (increased amount of disease). In the field, Actigard paints applied to a symptomless branch below a surgical cut to remove a canker have provided a stronger response than trunk paints applied to trees where cankers were left to run.

Actigard could prove practical as aid to cutting blight in pear trees, either reducing severity of re-ignited cankers or reducing the incidence of re-ignition. A paint application of Actigard has been incorporated into the proposed pome fruit label, which is expected to receive an EPA registration in 2015. Nonetheless, although we have achieved positive results with the chosen rate and timing of Actigard paints, further exploration of both of these variables could improve our understanding on of this technology.

Obj. 1b) Evaluate an inducer of systemic acquired resistance in combination with antibiotics for protection of pear trees from fire blight

In 2013, Actigard (acetyl-S-methyl) was evaluated in plot trials and in commercial orchards for enhanced suppression of floral infection by the fire blight pathogen. In an experimental pear orchard, relative to the water-treated control, oxytetracycline alone significantly reduced ($P \leq 0.05$) incidence of infection and total number of infected flower clusters per tree. The addition of Actigard treatments in combination with oxytetracycline improved the control of fire blight compared to the antibiotics alone (Fig. 3). This result is consistent with apple trials (not shown) conducted 2011-2013 (see 2013 apple report).

Under the 2013 Actigard experimental use permit, 4 ha-sections (one pull tank) in commercial pear orchards located in northern California, southern and northern Oregon and north central Washington were treated at full bloom and petal fall with Actigard at 2 oz./A. During the bloom period, the orchardists also applied an antibiotic program to the entire orchard. In May, the Actigard treated area and an adjoining no-Actigard area were assessed for fire blight by counting strikes or by counting trees with blight (Medford only). Overall, the Actigard plus grower program had 37% fewer strikes than the adjoining area that received only the grower program (Fig. 4)

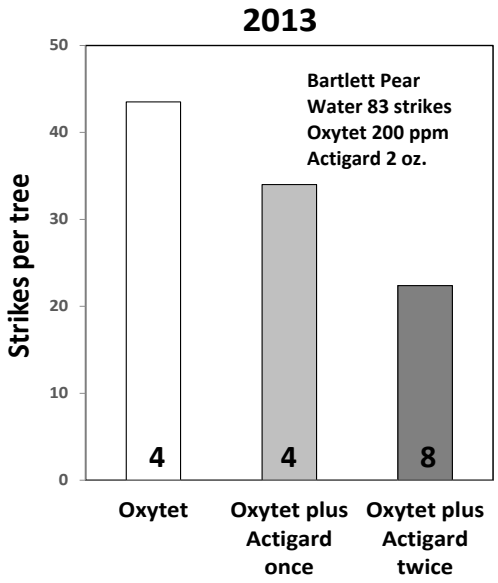


Fig. 3. Fire blight strikes per tree as affected by treatment with oxytetracycline once or in a program with 1 or 2 additional Actigard treatments. Trial conducted in an experimental Bartlett pear orchard near Corvallis, OR. The antibiotic was applied at full bloom; Actigard treatments varied from 30% bloom to petal fall. Numbers within bars are the number of replicate trees averaged for each mean

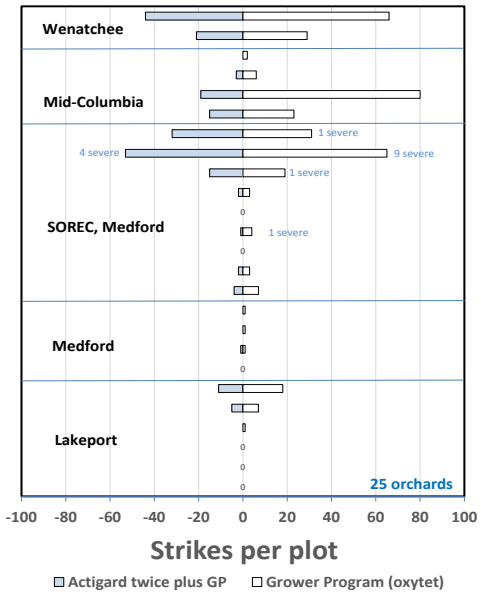


Fig. 4. Fire blight strikes per tree as affected by grower's antibiotic program alone or grower's program plus two additional Actigard treatments. Trials were conducted in commercial pear orchards at the locations shown. Actigard treatments were applied at full bloom and petal fall.

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 (just prior to or near time of infection, or after cutting). Actigard shows value as program partner with antibiotics during bloom, and we speculate it may be effective as long residual protectant for rattail, shoot infection or trauma blight phases of fire blight. In 2013, we also conducted a shoot blight trial on potted Concorde pear trees (data not shown). The potted trees were prayed with Actigard (2 oz./100 gal.) or untreated and then inoculated with high pathogen does (10^8 CFU/ml); under this extreme pressure, Actigard reduced shoot infection by 30%.

Obj. 2a. Evaluate the effect of a delayed dormant, copper sanitation treatment on detectability of the fire blight pathogen in samples of flowers collect at various stages of pear bloom

Treatment of pear trees with a copper-based bactericide at the 'green tip' phenological stage prior to flowering has been recommended historically to suppress primary inoculum of *Erwinia amylovora* associated with overwintering fire blight cankers. In the western United States, however, this practice became little used as treatment with effective antibiotic materials became common. We re-evaluated this practice by utilizing a primary bloom scouting protocol in conjunction with a loop-mediated isothermal DNA amplification assay (LAMP) for specific detection of *E. amylovora* in samples of flowers.

From 2010 to 2013, 4 ha-sections in commercial pear orchards located in northern California were split into two plots with the orchardist applying horticultural oil only to one plot and horticultural oil plus a fixed copper bactericide (6 lbs/A) to the other plot 2-4 weeks before first bloom. During the bloom period, the orchardists also applied their usual antibiotic program to the entire orchard.

Flowers from each 2 ha-plot were sampled at mid-bloom, at full bloom, and once or twice at petal fall. On each sampling date, three samples of 100-flower clusters (~ 600 flowers) were made in each 2-ha plot following a standard W-shaped scouting pattern with the LAMP protocol performed on the floral wash of each sample.

Overall, *E. amylovora* was detected rarely at mid-bloom (5% of samples) but detected commonly at petal fall (37% of samples) (Fig. 5). In 3 of 4 seasons, positive pathogen detection in flower samples increased more rapidly as a function of bloom stage in oil only plots compared to oil plus copper-treated plots. Among orchards, development of fire blight was sporadic but was decreased significantly ($P \leq 0.05$) by the copper treatment in two of 4 seasons. Among seasons, differences in detection of epiphytic *E. amylovora* by LAMP-based scouting indicated that orchardists could potentially benefit from 'point-of-care' LAMP testing in individual orchards each season.

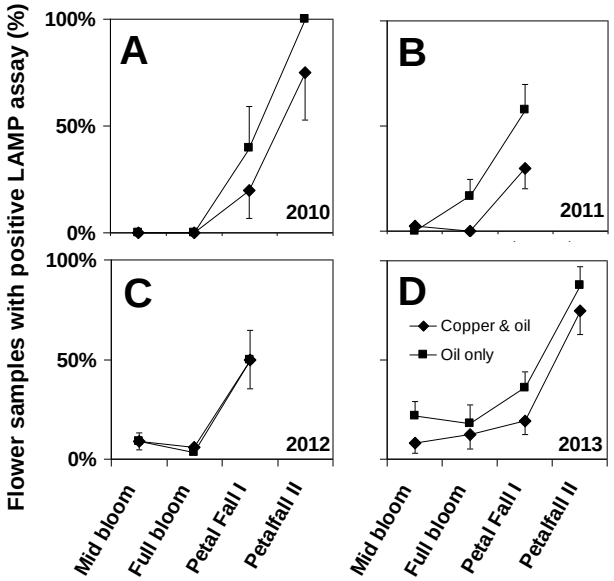


Fig. 5. Effect of delayed dormant timings of copper plus oil (♦) and oil only (■) on the detection of *Erwinia amylovora* in 100-flower cluster samples from commercial pear orchards in Yuba, Sacramento and Lake Counties in California. Detection of the fire blight pathogen was based on a loop mediated isothermal DNA amplification (LAMP) assay performed on washes of sampled flowers, which was confirmed by dilution plating of the same wash onto a selective culture medium. Each point is the mean of 29 to 41 100-flower cluster samples.

2012 was the season we failed to see an effect of delayed dormant copper on positive pathogen detection in pear flower samples. The spring of 2012 had a very cool beginning, which

compared to the other seasons resulted in a longer period (5-6 weeks) between the copper treatment and flowering (Fig. 6). Due to this longer period, we speculate that the copper residue had weathered (declined) to non-bactericidal levels at the beginning of the bloom period.

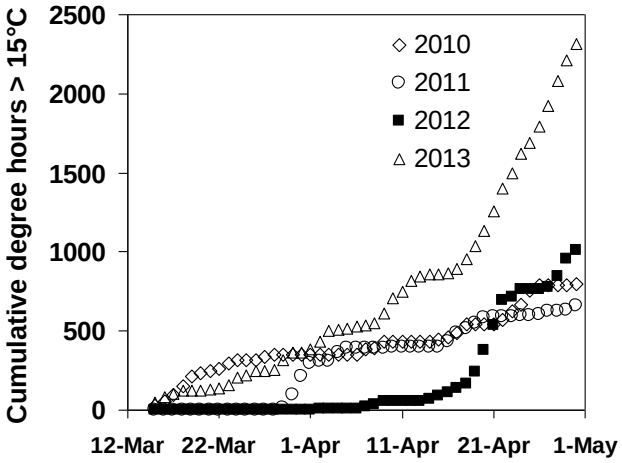
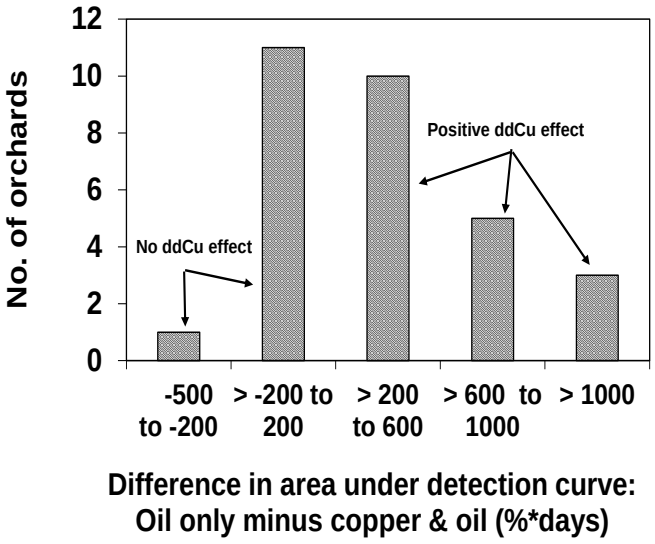


Fig. 6. Cumulative degree hours > 15°C for Lakeport, CA from March 15 to April 30 for the years 2010 to 2013.

For the seasons where we observed an effect of delayed-dormant copper on positive pathogen detection in pear flower samples (2010, 2011, and 2013), we evaluated the data from individual orchards first by calculating the 'area under the detection curve' (AUDC) for each treatment, and then secondly subtracting the area obtained for the oil plus copper treatment from the oil only treatment (Fig. 7). The units on AUDC are '% · days', and thus an AUDC value of e.g., 600, can be interpreted as an average of a 60% difference in Cu treated compared to untreated for a period of 10 days (60% · 10 days = 600). Overall, the magnitude of the difference between AUDC for oil plus copper subtracted from the oil only was > 200 (a positive delayed-dormant copper effect) for 2/3 of the orchards, and > 600 for 8 of the 30 orchards. Thus, delay dormant copper is an effective treatment when inoculum of the pathogen is expected to arise from within the orchard block.

Fig. 7. Histogram depicting the magnitude of the difference in area under the detection curve (AUDC) for *Erwinia amylovora* in 100-flower cluster samples collected from 2 ha sections of commercial pear orchards treated at a delayed dormant (green tip) timing with horticultural oil only or with oil plus copper. The orchards were located in Lake and Yuba Counties of California with 30 paired 2-ha sections treated and monitored during the years for 2010, 2011 and 2013. Each AUDC was comprised of three 100-flower cluster samples collected at each of several bloom stages: mid-bloom, full bloom, petal fall I, and petal fall II.



The overall performance of the LAMP assay over the four seasons of the California study in shown in Table 1. LAMP assay results were confirmed by plating the flower wash on culture media

semi-selective for the fire blight pathogen. Of 705 LAMP assays, there were 156 'true' positives for *E. amylovora* and zero 'false' negatives based on comparison to media isolation. There were also 16 positive LAMP assays that were not confirmed by media isolation. For this situation, the result could either be a false positive for LAMP or a false negative for media isolation. After working with LAMP and for 6 six years and media isolation for many more, we are more inclined to conclude the 16 non-corresponding data points are false negative for media isolation due to competing bacteria overgrowing the smaller *E. amylovora* colonies on the culture media; this concern is especially problematic when is a small number of pathogen cells in the sample.

Table 1. Comparison of the LAMP assay and dilution plating methods of detection of *Erwinia amylovora* washes of 100-flower cluster samples collected within commercial pear orchards in Lake and Yuba Counties, CA during the years of 2010 to 2013.

Per 100-cluster sample: (n = 705)	**Results of media isolation for *E. amylovora***	
Result of LAMP assay for *E. amylovora*	negative	positive
negative	533	0
positive	16	156

Discussion of LAMP surveys. LAMP has proven to be an outstanding research tool, and has provided valuable information for understanding the impact of fire blight management treatments such as delay-dormant copper sanitation. Nonetheless, whether LAMP-based scouting will have a place in commercial IPM programs will not be known until the assay can be performed in the orchard (see sub-objective 2B). Degree-hour models, e.g. Cougarblight, Zoller 'California', and Maryblyt have evolved to be accurate in assessing conditions for inoculum presence and build-up. The results of the LAMP surveys, however, provide of a direct assessment the prevalence of pathogen inoculum, and can guide research on effective timings and materials for disease control. One result of LAMP-based scouting has been the observation that the detectability of the fire blight pathogen continues to increase in the late stages of bloom; this suggests that fire blight control programs could be enhanced by additional copper-based sanitation during bloom or by extending protective treatments into the period near and after petal fall.

2b) Evaluate new LAMP technologies that will facilitate widespread adoption of molecular scouting within regional fruit production districts

The rationale for this sub-objective is to improve and simplify the LAMP technology on which molecular scouting is based so that other individuals can perform the assay at the 'point-of-care', i.e., the orchard. Currently, for each flower-wash sample, our lab-based protocol requires i) concentration of the sample wash, ii) DNA extraction from concentrated wash, and iii) LAMP assay on extracted DNA. To perform the assay in the field, we need to eliminate DNA extraction and ideally, concentration of the sample (or simplify it). In the last few years, technology developed by Optigene, Ltd. (Horsham, West Surrey, England) has shortened the LAMP reaction time from 45 to 20 minutes, and their 'mastermix' has combined multiple reagents into a single reagent (liquid) that is added to the LAMP reaction tube. Optigene (and others) also have developed 'LAMP machines' (Optigene's machine is called 'Genie II') that run multiple assays at one time and potentially eliminate the need for DNA extraction.

Step 1: Adaption of LAMP to fluorescence-based machines. Figure 8 shows the adaption of our *E. amylovora* LAMP protocol to new Optigene technologies. To achieve these results, we designed and evaluated new primers (named 'Amy13'primers) targeted to chromosomal DNA of the fire blight pathogen. We also developed a highly-specific, fluorescent 'assimilation probe' that creates a machine-readable signal when DNA of the target (fire blight pathogen DNA) is amplified. The assimilation probe technology was first evaluated in flower samples collected in 2012, and used exclusively for the California flower samples analyzed in 2013 (see Obj. 2a above).

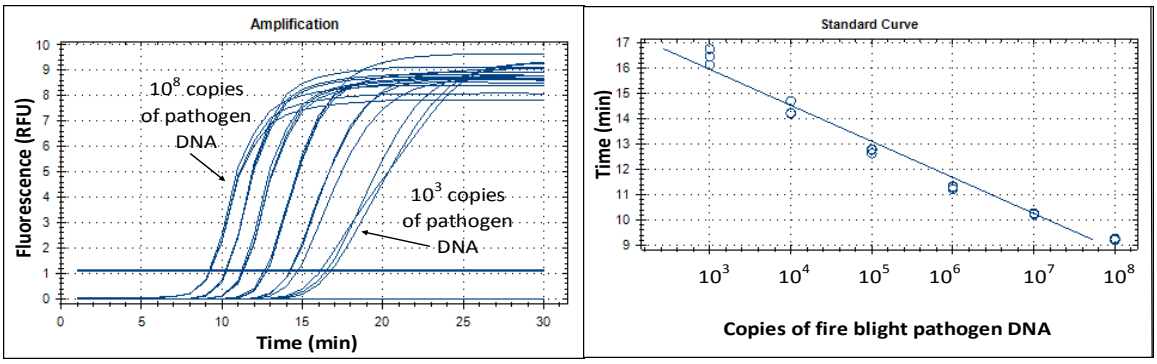


Fig. 8. Fluorescence-based loop-mediated isothermal amplification (LAMP 'ISO-001 polymerase) assay performed on DNA extracted from *Erwinia amylovora* cells grown in pure culture. Graph on left is cumulative fluorescence units emitted over time as influenced by a 1:10 dilution series of *E. amylovora* DNA (clustered groups of lines represent individual dilutions). Graph on right is time to achieve a 'positive' result as a function of amount of *E. amylovora* DNA.

Step 2: Evaluation of alternative polymerase, 'TIN', for amplification of DNA extracted from pathogen cells grown in per culture. LAMP reactions require an enzyme (a polymerase) that makes the chains of DNA after the pathogen-specific keys (primers) have located pathogen DNA in the sample. Elimination of the DNA extraction step requires the use of a heat tolerant polymerase (named 'TIN') that can withstand a 10 minute pre-heating of the sample to 95°C (203°F) to lyse cells prior to the LAMP reaction (which is then run at 65°C for 50-60 minutes). With extracted pathogen DNA, we first evaluated the TIN polymerase without pre-heating at 95°C. Compared to polymerase ISO-001, LAMP reactions with TIN required a few additional minutes (compare times Fig. 9 to Fig. 8) but sensitivity was not significantly altered by the switch to the heat-tolerant TIN-polymerase.

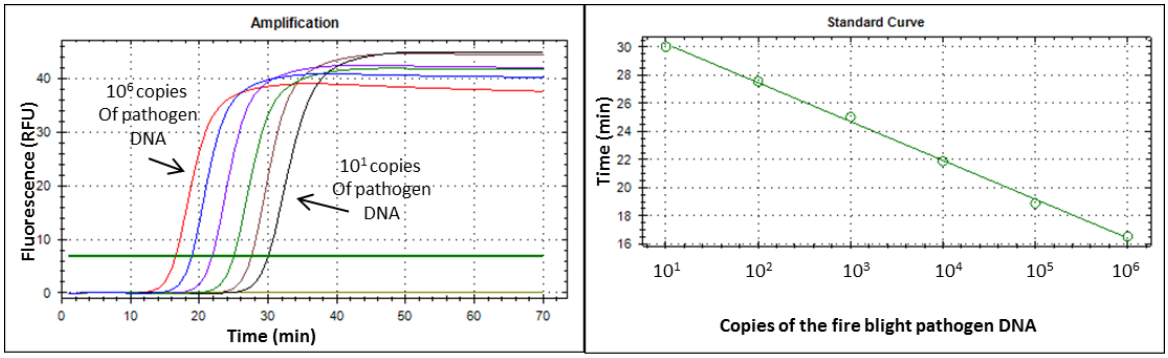


Fig. 9. TIN polymerase, fluorescence-based loop-mediated isothermal amplification (LAMP) assay performed on DNA extracted from *Erwinia amylovora* cells grown in pure culture. Graph on left is cumulative fluorescence units emitted over time as influenced by a 1:10 dilution series of *E. amylovora* DNA. Graph on right is time to achieve a 'positive' result as a function amount of *E. amylovora* DNA.

Step 3. Evaluation of alternative polymerase, 'TIN', for amplification of DNA in whole cells of the pathogen grown in per culture. The purpose of the additional 95°C treatment is to break open (lyse) pathogen cells and release the DNA. By lysing the cells in the preheating step (10 min), the LAMP reaction can be done in a single tube that is placed into the machine only once. Some organisms will lyse at 95°C without additional amendments to the sample; others require the addition of a lysing agent (e.g., NaOH:SDS or InstaGene matrix) to aid the DNA release. Initially, for whole cells of *E. amylovora*, reduced sensitivity of DNA amplification was observed in the 'Genie II' LAMP reactions when the cells were first pre-heated to treatment for 10 minutes. The addition of either NaOH:SDS or Instagene matrix to the LAMP reaction tubes resulted in positive amplification of cells down to 10^0 CFU per ml (i.e., assay sensitivity restored) (Fig. 10).

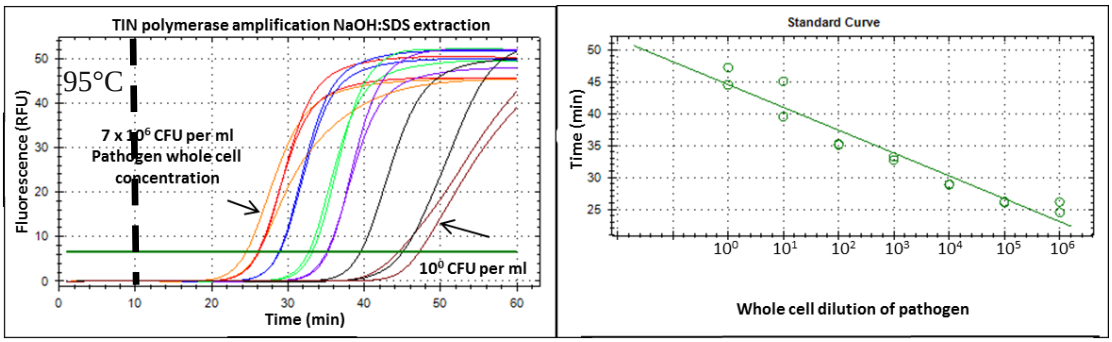


Fig. 10. TIN polymerase fluorescence-based loop-mediated isothermal amplification (LAMP) assay performed on whole cells of *Erwinia amylovora* grown in pure culture. Graph on left is cumulative fluorescence units emitted over time as influenced by a 1:10 dilution series of *E. amylovora* whole cells. NaOH:SDS was added to the reaction tube to aid in DNA release during the heating of cells to 95°C. Graph on right is time to achieve a 'positive' result as a function amount of *E. amylovora* whole cell concentration (CFU/ml).

Step 4. Evaluation of polymerase, TIN', for amplification of whole pathogen cells of the pathogen in a floral wash. Whole cells of *E. amylovora* were serially diluted and mixed with a wash of field-sampled flowers void of pathogen DNA. After adding NaOH:SDS and preheating the sample at 95°C, positive amplification of pathogen cells was observed down to 10^2 CFU per ml (*data not shown*). As expected, however, the time to obtain a positive amplification was increased by 10-15 minutes compared to cells suspended in water. This increase in reaction time reduced sensitivity is due to the presence (competition/interference) of DNA and other biochemicals from other organisms in the sample.

Step 5. Evaluation polymerase, 'TIN', for amplification of E. amylovora in naturally infested, non-concentrated wash samples collected in spring 2013. A subset of 30 floral washes from the California study (above) were saved (frozen). Previously, with the lab-based LAMP protocol and media isolation, 24 of these samples were found to be positive for *E. amylovora* DNA and 6 were negative. These saved floral washes were 'non-concentrated', meaning we omitted the step of our lab protocol where 30 ml of floral wash is embedded onto a filter membrane and then resuspended in 1 ml of liquid. When samples were incubated at 95°C for 10 minutes in either NaOH:SDS or InstaGene matrix, amplification occurred in 92 and 100% of positive samples, respectively. No amplification was observed in the 6 negative samples. Amplification required up to 70 minutes, suggesting that the omitted concentration step has a significant impact on time to get a positive signal.

Discussion of new LAMP technologies. New LAMP technologies have greatly simplified the process of performing a LAMP assay for specific detection of *E. amylovora*. Moreover, compared to the original technology based on the visualization of turbidity (precipitate formation), the new florescence-based technology is better suited to quantification of the *amount* of target DNA present in a sample (Figs. 8-10). Compared to LAMP assays performed on lab-grown pure cultures, samples of flowers from orchards show more variability in the estimated amount of pathogen, but it should be possible to distinguish between those samples with greater amounts of pathogen (fast time to reaction) vs. those with small amounts of pathogen (long time to reaction). We have also streamlined the handling and preparation of flower samples for LAMP, such that multiple samples can processed within a shorter period of time.

We have not purchased a LAMP machine because the companies have overpromised the availability of models designed for filed use (e.g., Optigene initiated a re-design of model Genie III before it was ever marketed). The Genie II machine used for this research was on loan, and it is not designed for field-use. Optigene's website is indicating the field-designed, Genie III LAMP machine, will be marketed sometime in 2014 (http://www.optigene.co.uk/instruments/instrument-genie-iii/). We also were working with another company's (diagenetix.com/product-and-technology/smart-dart-platform) inexpensive field-based LAMP machine. In our evaluation of their prototype, we concluded that they have several technical issues to overcome before the machine can be used reliably as an aid to decision making.

All-in-all, we are close to an ability to run LAMP-based scouting in the orchard, but this ability will require a reliable LAMP machine (Optigene's Genie III fulfills this requirement but cost will be a consideration). Once a field-based machine is in hand, there are still a few technical issues to iron out in how samples are processed, but these issues are minor.

EXECUTIVE SUMMARY

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

SIGNIFICANT FINDINGS:

Systemic acquired resistance:

- For a 3nd season, a paint of acibenzolar-S-methyl (Actigard) used in combination with cutting of blight reduced the severity of 're-ignited' fire blight cankers in pear.
- In pear, the addition of Actigard to antibiotic treatments significantly enhanced fire blight control in both experimental and commercial orchards.

Industry implications: Actigard, with its unique mode-of-action (induction of host defense genes), shows value as program partner with antibiotics for fire blight prevention during bloom. Even with very good products for prevention of fire blight, 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 pear trees.

LAMP-based molecular scouting:

- For a 6th season, molecular scouting during the bloom period detected and characterized the build-up of fire blight pathogen populations in pear flowers with pathogen detection being greatest near petal fall.
- For a 3rd season, molecular scouting for the fire blight pathogen in commercial orchards demonstrated that a delayed-dormant timing of a copper bactericide suppressed positive pathogen detection in flower samples.
- The protocol and technologies used for molecular detection of the fire blight pathogen were refined and adapted for use with LAMP machines, which will allow for the assay to be performed in the orchard.

Industry implications: Having an ability to quickly detect the fire blight pathogen in flowers has to potential to improve the timing of treatments for fire blight control and to lessen the impact of this disease. For example, in a 4-yr study that utilized molecular scouting in commercial pear orchards, we demonstrated that a copper bactericide applied at the 'green tip' phenological stage delayed the build-up the fire blight pathogen in flowers during the bloom period. The technology that we used to detect the pathogen, loop-mediated isothermal amplification of DNA, is very new and rapidly evolving. We are keeping pace with this evolution with a goal of developing molecular scouting for wide-spread use in orchard IPM programs.