FINAL PROJECT REPORT

Project Title: Rapid detection of fire blight pathogen

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Other Funding sources

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Budget 1:

Organization: OSU Agric Research Foundation		**Contract Administrator:** Dorothy Beaton	
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Item	**2007**	**2008**	**2009**
Salaries 6 mo. FRA	16,500	16,995	
Benefits 67%	11,055	11,387	
Wages			
Benefits			
Equipment			
Supplies	2814	2928	
Travel	250	250	
Miscellaneous plots	750	750	
Total	$31,369	$32,310	**no cost**

Objectives:

2007 to 2009:

1. Design a LAMP reaction to detect small quantities of the fire blight pathogen based on primers from *E. amylovora*-specific DNA sequences.
2. Determine specificity and sensitivity of the designed LAMP reaction against a diverse selection of microorganisms commonly found in pear and apple orchards.
3. Determine the sensitivity of LAMP reaction when one flower with a natural infection of *E. amylovora* is added to 100 flower clusters.
4. Use the LAMP reaction to detect *E. amylovora* in flower samples from inoculated and non-inoculated orchard trees.
5. Use the LAMP reaction to detect *E. amylovora* in flower samples from commercial orchards.
6. Optimize sampling protocols for implementation by growers or farm service providers.

Significant findings:

- **We developed two LAMP primer sets with high specificity to *E. amylovora*.** Two DNA primer sets are being used with field samples (from 45 that we designed and evaluated). One set is targeted to plasmid pEA29 and the other to the chromosomal gene *amsL*. LAMP reactions are highly specific for *E. amylovora*, and test negative with other bacteria recovered from flowers.

- **Positive LAMP reactions were attained using a gradient of pathogen mixed with a gradient of flowers.** *E. amylovora* was spiked into flower suspensions at 0, 500 and 5000 CFU per ml resulting in positive LAMP reactions if the pathogen was present. LAMP reactions were negative in the zero pathogen suspensions. Density of flowers in the wash had no effect on pathogen detection.

- **Mixed LAMP results were attained after adding a single flower infested with 10^5-10^7 CFU of *E. amylovora* to 100 floral clusters.** Single, pathogen-infested flowers when mixed in 1.5 L water yielded concentrations of 1×10^2 to 5×10^4 CFU per ml. LAMP reactions were positive when *E. amylovora* populations were $\geq 1 \times 10^3$ CFU per ml. Concentrating the wash with a filter improved detection.

- **Positive LAMP reactions were attained from 100 flower cluster samples taken from experimental apple and pear orchards inoculated with *E. amylovora*.** Moreover, LAMP reactions were negative for samples from non-pathogen-inoculated apple and pear orchards. Populations of indigenous bacteria in the washes ranged from 10^5 to 10^7 CFU/sample.

- **LAMP detected the fire blight pathogen in flower samples from commercial orchards.** A total of 43 commercial orchards from Oregon, Washington, California and Utah were surveyed. LAMP reactions were negative in 11 orchards with no blight developing in 9, and a few strikes in 2. Positive LAMPs were obtained in 30 orchards; 20 of which developed fire blight. In several cases, communication of positive LAMP test to orchardists resulted in intensified control efforts.

- **With in-state support, orchardists in Utah and California are using LAMP-based scouting in 2010.** Utah will use the technology industry-wide to time initiation of spray programs. California is using LAMP to re-evaluate the value of delayed dormant copper treatments for blight suppression.

Results and Discussion:

Objective 1. Design a LAMP reaction to detect small quantities of the fire blight pathogen based on primers from *E. amylovora*-specific DNA sequences.

Two DNA primer sets are being used with field samples (from 45 that we designed and evaluated). One set is targeted to plasmid pEA29 and the other to the chromosomal gene *amsL* (Table 1).

A positive LAMP reaction resulting in a white magnesium pyrophosphate precipitate (Fig. 1) in the PCR tube corresponded to dilution plate enumeration of $\geq$ 25 CFU of the pathogen. Pathogen cell concentrations below this level resulted in inconsistent precipitate formation in the PCR tube.

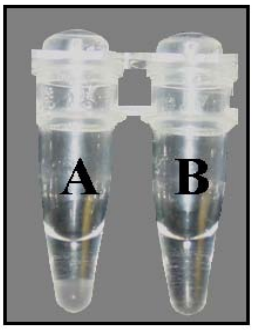

Figure 1: Comparison of positive and negative LAMP reactions. Tube (A): positive reaction seen as a cloudy white precipitate, and tube (B): negative reaction seen as clear liquid.

LAMP reactions run in a constant temperature heat block or water bath (65°C), and do not require expensive, precision instruments (a thermocycler followed by gel electrophoresis) to visualize the results.

Table 1. LAMP primers for detection of *Erwinia amylovora*. The full LAMP protocol is available from us upon request.

Primers to detect plasmid pEA29:

Name	Tm	5' to 3' primer sequence
Ea29 Fip	60°C	TCGTGGTTATGCGATAACGCGTCAGGAACTCCAGGGAGGTC
Ea29 Bip	60°C	TGTGTCACGATCCAGAGCACACGGTCATATGCAGGAGCAAGT
Ea29 F	59°C	ACGCAAGCCTTCTAAAGCT
Ea29 B	59°C	ATGGCCCGTGAAAAAGTCA
Ea29 Loop	60°C	GGGGGAGAGTCCATTTGGA

[a] Primers Fip and Bip were used at 2.4 µM, primers F and B at 0.2 µM, and Loop primer at 0.4 µM final concentrations .

Primers to detect amsL B:

Name	Tm	5' to 3' primer sequence
ALB Fip	60°C	CTGCCTGAGTACGCAGCTGATTGCACGTTTTACAGCTCGCT
ALB Bip	60°C	TCGTCGGTAAAGTGATGGGTGCCCAGCTTAAGGGGCTGAAG
ALB F	58°C	GCCCACATTCGAATTTGACC
ALB B	58°C	CGGTTAATCACCGGTGTCA

[a] Primers Fip and Bip were used at 2.4 µM, primers F and B at 0.2 µM final concentrations .

Objective 2. Determine specificity and sensitivity of the designed LAMP reaction against a diverse selection of microorganisms commonly found in pear and apple orchards.

Laboratory strains of *P. fluorescens*, *P. syringae*, and *P. agglomerans* were negative for precipitate formation in the LAMP reaction (data not shown). In addition, whole pear flowers, pear flower petals or pear flowers minus petals were negative for the LAMP reaction.

Freeze-dried cells of *E. amylovora* were suspended in water (a 3-L volume in food grade plastic pails) at concentrations of 2.5×10^2 and 1.8×10^3 CFU per ml (as determined by dilution

plating). Flowers of pear or apple were added to the pails as a treatment, which increased the population of indigenous (naturally occurring) bacteria in the suspensions to 10^2, 10^4, and 10^6 CFU per ml for pails with 10, 100 or 1000 flowers per 3 L, respectively. Indigenous bacteria were not recovered from wash suspensions without flowers.

For both Bartlett pear and Gala apple, 100% of LAMP reactions were positive if *E. amylovora* was spiked into flower suspensions (Table 1). The number of pear flowers in the suspension had no effect on the incidence of positive LAMP reactions. All LAMP reactions for wash suspensions containing no pathogen cells were negative.

Table 2. Percentage of positive LAMP detection as influenced *E. amylovora* concentration and a flower density in the wash.

Cultivar	*E. amylovora* concentration in suspension	Flower density in wash suspension [a]			
		0	10	100	1000
Bartlett pear	0	0% [b]	0%	0%	0%
	2.5×10^2 [b]	100%	100%	100%	100%
	1.8×10^3	100%	100%	100%	100%
Gala apple	0	0%	0%	0%	0%
	2.5×10^2	100%	100%	100%	100%
	1.8×10^3	100%	100%	100%	100%

[a] CFU per milliliter in 3 L volume of water.
[b] Average of 5 experiments.

Objective 3. Determine the sensitivity of LAMP reaction when one flower with a natural infection of *E. amylovora* is added to 100 flower clusters.

Single apple or pear flowers on which *E. amylovora* had been inoculated and allowed to incubate for 24-72 hours were suspended 0.3 (2009) or 1.5 L (2008) of water. Populations of *E. amylovora* in the suspensions ranged from 8.9×10^2 to 4.7×10^6 CFU per ml per ml. Over this range of concentrations, LAMP reactions were a mix of positive and false negatives if populations of the pathogen were below 1×10^3 CFU per ml. Concentrating 30 ml of the wash suspension by embedding onto a 0.2 micron membrane and resuspending into a 1 ml volume of water increased pathogen cell density by one log unit (as determined by dilution plating). Also, DNA extraction with the InstaGene™ Matrix and a mini-elute column increased improved pathogen detection with LAMP.

Following this concentration and extraction protocol, LAMP yielded a positive result with all pathogen-inoculated flowers regardless if an additional (non-inoculated) 100-flower clusters were added to the wash suspension (Table 3). Indigenous bacteria were recovered in all wash volumes to which 100 flower clusters had been added (ranging from 2×10^2 to 4×10^6 CFU per ml). Water- or water and flower cluster only samples were negative for detection of *E. amylovora* by LAMP or dilution plating (Table 3.)

Table 3. Percentage of positive LAMP detections from single *E. amylovora*-colonized *flower* as influenced by presence or absence of pear or apple flowers in the wash[a].

	Treatment added to wash			
Cultivar	Nothing	100 flower clusters	Single flower colonized by *E. amylovora*	Single flower colonized by *E. amylovora* and 100 flower clusters
2008				
Bartlett pear	0%[b]	0%	100% (4.0 ± 0.96)[c]	100% (4.2 ± 0.34)
Gala apple	0%	0%	100% (3.4 ± 0.62)	100% (3.8 ± 2.05)
2009				
Bartlett pear	0%	0%	100% (4.8 ± 0.71)	100% (5.3 ± 1.92)
Gala apple	0%	0%	100% (5.3 ± 1.84)	100% (5.2 ± 1.20)

[a] 100 flower clusters per 0.3 (2009) or 1.5 L (2008) volume in a re-sealable plastic bag.
[b] Percentage of positive LAMP reaction is the average of 2 or 3 experiments each year.
[c] Average $\log_{10}$ population size (CFU per ml) and standard deviation of *E. amylovora* in the wash suspension after addition of a single pathogen-infested flower to 0.3 (2009) or 1.5 L (2008) water followed by concentrating 30 ml of the wash suspension onto a 0.2 micron membrane and resuspending into a 1 ml volume of water.

Objective 4. Use LAMP to detect *E. amylovora* in flower samples from inoculated and non-inoculated orchard trees.

In both 2008 and 2009, all 100-flower cluster samples from apple and pear orchards inoculated with *E. amylovora* 153N had positive LAMP reactions at full bloom and petal fall (Table 4). Pathogen populations in these samples, as estimated by dilution plating, ranged from 1.2×10^3 to 4.7×10^5 CFU per ml.

In nearby orchard blocks that were not inoculated with the pathogen, all 100-flowers cluster samples sampled processed in 2008 were negative for *E. amylovora* as determined by LAMP and by dilution plating. In 2009, floral clusters sampled at full bloom from non-pathogen-inoculated orchards also were negative, but the petal fall sample had positive LAMP reactions in 2 walks from a Bartlett pear block and 1 walk from a Braeburn apple block; these blocks were located < 100 meters from a inoculated pear block. *E. amylovora* was not detected by dilution plating method from any samples from the non-inoculated orchards (10^2 CFU per ml detection level). Populations of other bacteria in the flowers washes averaged approximately 1×10^6 CFU per ml (Table 4).

Table 4. LAMP results of 100 blossom cluster samples[a] from experimental pear and apple orchards inoculated with or without *E. amylovora*.

Cultivar	Inoculated [b]	No. samples per orchard with positive LAMP [c]	*E. amylovora* population [d] Log_{10}(CFU/ml)	Total bacteria population Log_{10}(CFU/ml)
2008				
Bartlett pear	No	0	Not detected	6.7 ± 0.10
Fuji apple	No	0	Not detected	5.7 ± 0.29
Jonathon apple	No	0	Not detected	5.7 ± 0.15
Bartlett pear	Yes	6	3.8 ± 0.70	6.3 ± 0.30
Gala apple	Yes	6	4.8 ± 0.53	5.5 ± 0.24
Golden Delicious apple	Yes	6	5.5 ± 0.16	6.1 ± 0.28

2009				
Bartlett pear	**No**	**2 (at petal fall)**	**Not detected**	**5.8 ± 0.41**
World pear	**No**	**0**	**Not detected**	**6.2 ± 0.16**
Braeburn apple	**No**	**1 (at petal fall)**	**Not detected**	**6.2 ± 0.08**
Bartlett pear	**Yes**	**6**	**5.3 ± 0.07**	**5.0 ± 0.24**
Red Delicious apple	**Yes**	**6**	**7.7 ± 0.05**	**6.9 ± 0.05**

[a] 100 flower clusters per sample were suspended in 0.3 (2009) or 1.5 L (2008) volume of water in a re-sealable plastic bag.
[b] Indicates if experimental orchard was inoculated with *E. amylovora* (1 x 10^6 CFU per ml).
[c] In each orchard, 6 samples were taken; 3 at full bloom and 3 at petal fall.
[d] Average $\log_{10}$ population size (CFU per ml) and standard deviation of *E. amylovora* or the total bacteria recovered recover in the floral washes.

Objective 5. Use the LAMP reaction to detect *E. amylovora* in flower samples from commercial orchards.

Selected commercial orchards were typically 3 to 5 hectares in size, and five 100 flower cluster samples were taken in each orchard on each sampling date. Each orchard was sampled three times: mid-bloom, full bloom, and petal fall. In 2008, the volume of water used to wash collected flower clusters was 1.5 L, whereas in 2009, this volume was reduced to 0.3 L. In addition, in 2009, 15 ml of the wash volume was concentrated on a 0.2 um filter, then the bacteria trapped on the filter were resuspended in 1 ml prior to DNA extraction. In 2008, extracted DNA was concentrated by high speed, low temperature evaporation.

2008:

Rogue Valley. The three commercial orchards sampled in the Rogue Valley of Oregon were all negative for detection of *E. amylovora* by LAMP or dilution plate, and for development of fire blight. Fire blight risk, as modeled by COUGARBLIGHT, was negligible during the mid-April sampling period.

Hood River Valley (Parkdale). Bloom at higher elevations in the Hood River Valley coincided with a period of extreme fire blight risk (Fig. 2). The first samples (30% bloom in pear) occurred at low risk, and *E. amylovora* was not detected. For the 3rd sample time (May 19), which occurred during the high risk period, *E. amylovora* was detected by LAMP in 4 of 5 orchards, all of which developed some fire blight (Table 3). Positive pathogen detection by LAMP in 3 of 4 orchards, however, required concentration of the extracted DNA by high speed, low temperature evaporation. This result was the reason the wash volume was reduced and further concentrated in 2009.

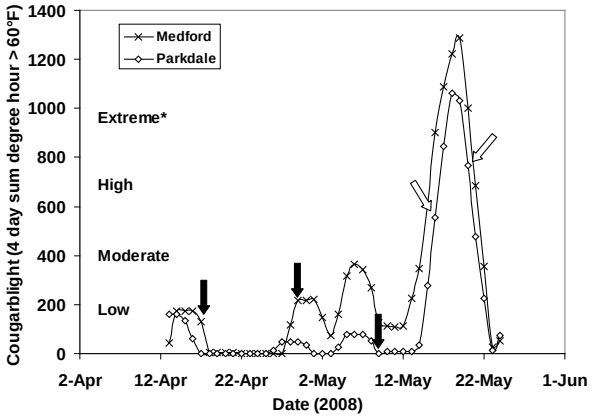

Fig. 2. Fire blight risk in spring 2008 based on temperatures measured at Medford and Parkdale, Oregon. Arrows indicate samples dates.

2009:

The survey was expanded to apple and pear production areas in four states: Oregon, California, Washington, and Utah (Table 5). With the exception of Utah, all samples were processed at Oregon State University.

In Oregon, a total of 10 pear and 6 apple orchards were sampled from Hood River, Medford, Milton-Freewater, and Parkdale. Positive LAMP reactions were obtained from 14 of 16 orchards, Summer fire blight evaluation revealed light disease development in 8 of the 16 orchards, 7 of which were positive for LAMP (Table 3).

In California, three pear orchards were sampled from Lake County at mid-bloom, full bloom, and petal fall. Two of three orchards had positive LAMP reactions with light disease developing in one of the two positive orchards (Table 3). *E. amylovora* was isolated on culture media in only one orchard with an average population of 2.6×10^4 CFU per sample.

In Washington, a total of 3 pear and 3 apple orchards were sampled; these orchards were located in the Yakima, Zillah, Wenatchee, and Okanogan districts. Positive reactions were obtained in three of six orchards; light disease developed in one orchard in which *E. amylovora* was detected, and in one orchard in which it was not detected (and disease data were not obtained for 2 of the six orchards).

In Utah, 7 apple orchards located south of Provo were sampled. At this location, the orchards were sampled from 4 to 12 days in a row with 6 orchards being sampled at least 10 days in a row (Table 3). Positive LAMP reactions resulted from all orchards with populations of *E. amylovora* that ranged from 2.4×10^3 to 3.2×10^7 CFU per sample. Stigma imprints were performed on 4 (one orchard) or 8 (6 orchards) of 10 sample days and resulted in detection of *E. amylovora* in 4 of the 7 orchards (Table 3). In all orchards, fire blight developed in degrees varying from light to heavy (Table 3).

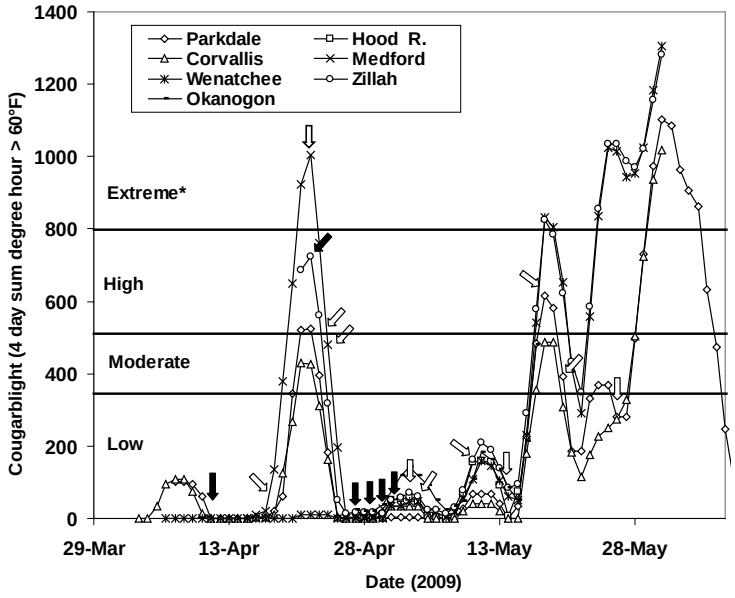

Figure2: Cougarblight model consisting of 4-day sum of degree hours greater than 15°C (60°F) plotted against dates in spring in 2009. Risk of disease outbreak is based on the assumption of "blight present in the region but not near the orchard last year" where 0 to 350 is low risk, 350 to 500 is moderate risk, 500 to 800 is high risk, and 800+ is extreme risk. Black arrows indicate negative detection and white arrows indicate positive detection of *E. amylovora* by loop mediated isothermal amplification.

Table 5. LAMP results of 100 blossom cluster samples[a] taken from commercial orchards in the Pacific Northwest of the United States of America.

			No. of samples with Positive LAMP			Media isolationb	
State		Cultivar	Mid-bloom	Full bloom	Petal fall	(Avg. Log10)	Blight[c]
2008							
OR	Medford	Bartlett pear	0 of 5	0 of 5	0 of 5	No	No
		Bosc & Red d'Anjou pear	0 of 5	0 of 5	0 of 5	No	No
		Bartlett pear	0 of 5	0 of 5	0 of 5	No	No
		Red Bartlett pear	0 of 5	0 of 5	0 of 5	No	No
	Parkdale	Red d'Anjou pear	0 of 5	0 of 5	0 of 5	No	No
		Bartlett, d'Anjou, & Bosc pear	0 of 5	2 of 5 *	5 of 5 *	Yes (3.3)	Yes Moderate
		JonaGold apple	2 of 5 *	no data	no data	No	Yes Moderate
		Bartlett & Bosc pear	0 of 5	1 of 5 *	2 of 5 *	Yes (2.7)	Yes light
		Gala apple	5 of 5 *	no data	no data	No	Yes light
2009							
OR	Medford	Bartlett pear	1 of 5	0 of 5	0 of 5	Yes (2.3)	No
		Bosc & Red d'Anjou pear	0 of 5	0 of 5	1 of 5	Yes (6.0)	No
		Bartlett pear	4 of 5	1 of 5	2 of 5	Yes (6.0)	Yes light
		Red Bartlett pear	0 of 5	1 of 5	0 of 5	No	No
	Parkdale	Red d'Anjou pear	2 of 5	0 of 5	2 of 5	Yes (4.0)	No
		Bartlett, d'Anjou, & Bosc pear	2 of 5	0 of 5	1 of 5	Yes (3.7)	No
		JonaGold apple	0 of 5	2 of 5	2 of 5	Yes (4.0)	No
		Bartlett & Bosc pear	0 of 5	0 of 5	0 of 5	N	Yes light
		Gala apple	0 of 3	0 of 3	2 of 3	Yes (4.0)	Yes light
	Milton-Freewater	Gala apple	0 of 5	1 of 5	4 of 5	Yes (5.5)	Yes light
		Gala apple	1 of 5	0 of 5	1 of 5	No	No
		Pink Lady apple	0 of 5	0 of 5	4 of 5	Yes (5.2)	Yes light
		Pink Lady apple	0 of 5	3 of 5	5 of 5	Yes (5.1)	Yes light
	Hood River	Forelle pear	2 of 5	4 of 5	5 of 5	Yes (7.0)	Yes light
		Bartlett pear	1 of 5	2 of 5	0 of 5	No	Yes light
		Bartlett pear	0 of 5	0 of 5	no data	No data	No
CA	Lake County	Star Crimson pear	1 of 5	1 of 5	1 of 5	Yes (3.1)	Yes light
		Bartlett pear	0 of 5	0 of 5	0 of 5	No	No
		Bartlett pear	2 of 5	1 of 5	0 of 5	Yes (2.5)	No
WA	Yakima	Gala apple	0 of 5	0 of 5	0 of 5	No	Yes light
	Zillah	Gala apple	0 of 5	0 of 5	1 of 5	No	no data
		Pink Lady apple	no data	no data	1 of 1	Yes (5.0)	no data
	Wenatchee	d'Anjou pear	0 of 5	0 of 5	0 of 5	No	No

		d'Anjou pear	**0 of 5**	**0 of 5**	**0 of 5**	**No**	**No**
	Okanogan	**Bosc pear**	**0 of 4**	**0 of 6**	**2 of 4**	**Yes (5.3)**	**Yes light**
UT				**LAMP[d]**	**Stigma imprint[e]**		
	Provo	**Gala apple**		**10 of 12**	**8 of 8**	**Yes (4.8 ± 1.00)**	**Yes heavy**
		Gala apple		**8 of 10**	**4 of 8**	**Yes (5.3 ± 0.71)**	**Yes heavy**
		Fuji apple		**2 of 10**	**0 of 8**	**Yes (2.9 ± 0.24)**	**Yes light**
		Gala apple		**5 of 10**	**5 of 8**	**Yes (3.6 ± 1.12)**	**Yes moderate**
		Fuji apple		**5 of 10**	**0 of 8**	**Yes (4.0 ± 2.02)**	**Yes moderate**
		Jonathon apple		**9 of 10**	**8 of 8**	**Yes (6.0 ± 0.24)**	**Yes heavy**
		Gala apple		**2 of 4**	**0 of 4**	**Yes (4.3 ± 1.87)**	**Yes light**

[a] 100 flower clusters per sample were washed in 0.3 (2009) or 1.5 L (2008) of water in a re-sealable plastic bag.
[b] Average $\log_{10}$ population size of *E. amylovora* (CFU per ml) recovered from floral washes.
[c] Whether or not fire blight developed in the orchard, and if yes, the disease rating applied to the orchard: light = 1 strike per tree, moderate =2 to 5 strikes per tree, and heavy $\geq$ 6 strikes per tree).
[d] Incidence of positive LAMP reaction is the average of up to 12 floral samples in Utah taken daily from orchards from mid-bloom to petal fall.
e Incidence of positive isolation of *E. amylovora* from imprinting stigmas of pear or apple flowers onto CCT media.

In summary, *E. amylovora* was detected in 30 of 41 commercial orchards, 20 of which developed fire blight. Detection of *E. amylovora* in commercial orchards typically coincided with full bloom to petal after heat units had begun to accumulate on a COUGARBLIGHT risk curve. Nonetheless, E. amylovora was detected in 9 orchards at the early (mid-bloom) sample. In several cases, information that *E. amylovora* was present in flowers in an orchard intensified the orchardist's fire blight management activities.

Discussion:

Given the sensitivity of LAMP and our preliminary results, we expected that our sampling scheme would readily detect *E. amylovora* at high levels of infestation, which proved true. In addition, through refinement of the method we used to wash bulk flower samples, detection of *E. amylovora* at lower levels of infestation also was improved.

The important question raised by the data concerns whether or not LAMP-based scouting for *E. amylovora* is worth the effort. In our view there are several answers to this question:

a) In cases where we detected either a high-infestation levels of the pathogen (mostly Utah in 2009) or the pathogen was detected early in the bloom period (Hood River and Parkdale, OR and Lake County, CA in 2009), orchardists responded to positive LAMP results by intensifying their control efforts. This intensification following the information provided by LAMP was in our view the most beneficial aspect of early scouting for *E. amylovora.* It is likely that through early knowledge of the pathogen's presence, at least some orchardists reduced fire blight damage.

b) In numerous orchards we detected the pathogen but late in bloom and in only one or two samples from an orchard (i.e., a relatively low level of infestation, which was also evidenced by relatively low levels of blight during the summer). The later bloom samples were taken at generally higher CougarBlight heat unit accumulations, and thus knowledge of fire blight risk was available using a simpler and cheaper method. In these cases, it is unlikely that LAMP based scouting provided value beyond that provided by CougarBlight. Nonetheless, one grower expressed a level of 'peace-of-mind' from negative results:

> "The information we received from the 2009 fire blight program was invaluable. Knowing that we had fire blight in the orchard but, more importantly, knowing where it was, saved us money. We didn't just spray all the pears like we usually do. Besides saving money, resistance might be further delayed. We would be interested in participating in the 2010 program also."

This statement shows potential for additional value from LAMP-based scouting; however, in our opinion, we think the LAMP scouting database is still too small to make the judgment "to not spray all the trees like we usually do."

c) Finally, both a) and b) are conditioned on the current state of molecular-based detection technology (in this case LAMP) and its relative ease of use. Currently, we feel that the LAMP protocol to detect the fire blight pathogen in flower samples needs to be done by an individual who is trained and experienced with the methods and aware of the potential problems (such as minimizing molecular contamination, and inclusion/interpretation of controls). However, it is likely that in the not-to-far-off future, advances in technology will make assays like LAMP easier to deploy at an on-site location by a less experienced user. An example that coincides with the submission of this report is:

> Tomlinson, J. A., Dickinson, M. J., and Boonham, N. 2010. Rapid detection of *Phytophthora ramorum* and *P. kernoviae* by two-minute DNA extraction followed by isothermal amplification and amplicon detection by generic lateral flow device. Phytopathology 100:143-149.

Results summary:

- We developed two LAMP primer sets for specific detection of *E. amylovora*.
- The detection limit with pure cultures is ~25 pathogen cells per ml. Practical detection limit with field samples is ~10,000 cells per 100 flower cluster sample.
- We consistently detect *E. amylovora* in spiked washes, and inoculated field trials.
- *E. amylovora* was detected in commercial orchards using a sample size of 100 flower clusters (sampled into a re-sealable plastic bag) taken at a frequency of one sample per hectare (typically 5 samples per orchard).
- Consistent detection of *E. amylovora* was achieved when 100-flower cluster samples were washed in 0.3 L water, and 15 ml of this wash was concentrated to 1 ml prior to DNA extraction.
- *E. amylovora* was detected in 30 of 41 commercial orchards, 20 of which developed fire blight. Detection of *E. amylovora* in commercial orchards coincided with full bloom after heat units had begun to accumulate on a COUGARBLIGHT risk curve. In several cases, information that *E. amylovora* was present in flowers in an orchard intensified the orchardist's fire blight management activities..

Executive Summary:

We have developed a LAMP-PCR method for detection of the fire blight pathogen, *Erwinia. amylovora*, from pure cultures, laboratory experiments in floral washes, and from bulked floral samples obtained from experimental and commercial orchards.

Early detection of the fire blight pathogen in commercial orchards involves sampling bulked, 100-flower cluster samples (~ 1 per hectare) and processing the sample wash with LAMP, which requires 1–2 hr to complete. The method reliably detects a single pathogen-colonized flower in a sample of 100 clusters (~600 flowers). In three experimental orchards inoculated with *E. amylovora*, positive LAMP reactions were attained from nine of nine 100-flower cluster samples.

A two year study evaluated LAMP-based scouting for the fire blight pathogen in 41 pear and apple orchards in of Oregon, Washington, California and Utah. *E. amylovora* was detected by LAMP in flower samples from 30 orchards, of which 20 developed fire blight. In another eleven orchards, all floral washes were negative for *E. amylovora* by LAMP and by dilution plate; of these, light disease was observed in two orchards during the summer.

Overall, detection in commercial orchards coincided with full bloom after heat units had begun to accumulate on a COUGARBLIGHT risk curve, indicating that the heat unit model works well to forecast fire blight risk, and may well be a sufficient measure of risk for many orchardists. On the other hand, several growers were able to use information provided by LAMP- based scouting to intensify or modify their control practices. For example, one grower cooperator wrote:

> "The information we received from the 2009 fire blight program was invaluable. Knowing that we had fire blight in the orchard but, more importantly, knowing where it was, saved us money. We didn't just spray all the pears, like we usually do. Besides saving money, resistance might be further delayed. We would be interested in participating in the 2010 program also."

Implementation of LAMP 'on-site' (e.g., an orchardist's kitchen) is not a feasible currently, but use by regional extension or a field station unit is a viable option. For example, in 2010, Utah through cooperative extension personnel will implement LAMP technology industry-wide to time initiation of spray programs. Growers and extension personnel in Lake Co., CA are using LAMP in 2010 to re-evaluate the value of delayed dormant copper treatments for blight suppression. The ease of implementing LAMP-based detection on-site is expected to improve in the coming years.