

Rapid detection of the fire blight pathogen by loop-mediated **isothermal** DNA amplification

Ken Johnson

Todd Temple

Oregon State Univeristy



AgriStrip

The rapid one-step assay



Erwinia amylovora
(Fire blight)

153083 AgriStrip Single strips 1 unit 100

Ea 153081 AgriStrip Complete kit 1 kit 25
153082 AgriStrip Single strips 1 unit 25

Specific antibodies were produced against a mixture of different isolates of *Erwinia amylovora* from Europe and the USA. The detection limit of the rapid assay is between 5×10^5 and 1×10^6 cfu/ml.



Simplification of LAMP

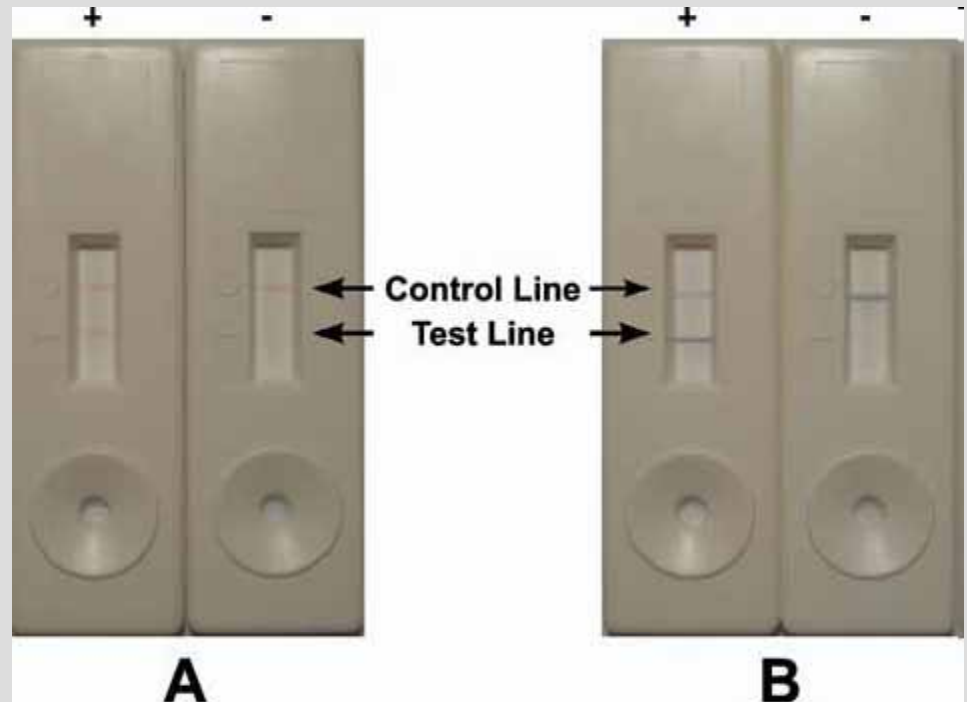
Example: Rapid Detection of *Phytophthora ramorum* ... by Two-Minute DNA Extraction Followed by Isothermal Amplification and ... Detection by ... Lateral Flow Device

“Lateral flow device (LFD) nucleic-acid-extraction method is completed in <5 min.”

“Results are generated in easy to interpret format in 1 h including DNA extraction.”

“Each of the 3 steps is sufficiently simple to allow LAMP to be performed on-site.”

From: Tomlinson, J. A., Dickinson, M. J., and Boonham, N. 2010. *Phytopathology* 100:143-149.



LAMP-based scouting for the fire blight pathogen

Objective:

To develop an orchard sampling scheme that will detect pre-infection populations of *E. amylovora*

Sampling scheme in commercial orchards



Processing samples in the lab:

Wash blossom clusters

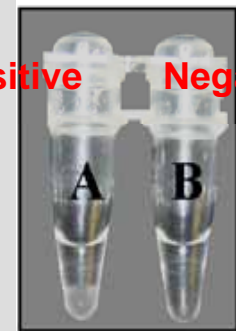


Concentrate wash and DNA extraction



Heat 45 min in hot water

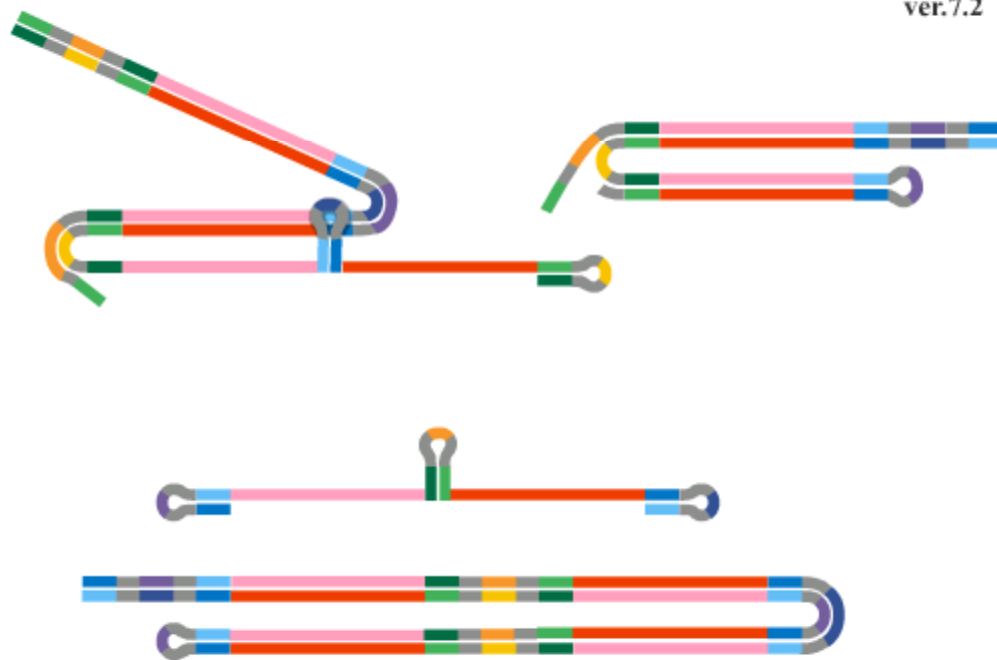
Positive Negative





Animation

ver.7.2



Restart

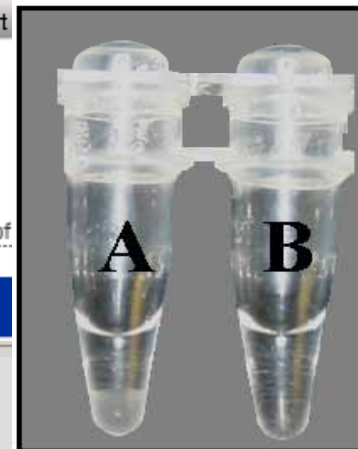
Start

Top of

Chemical Co., Ltd. All Rights Reserved.



Positive



Negative

LAMP sensitivity and specificity:

sensitivity

CFU per ml	Ea29	amsL B
	plasmid	chromosomal
5.00E+04	Y	Y
3.00E+03	Y	Y
2.00E+02	Y	Y
2.50E+01	Y	Y
2.00E+00	N	N

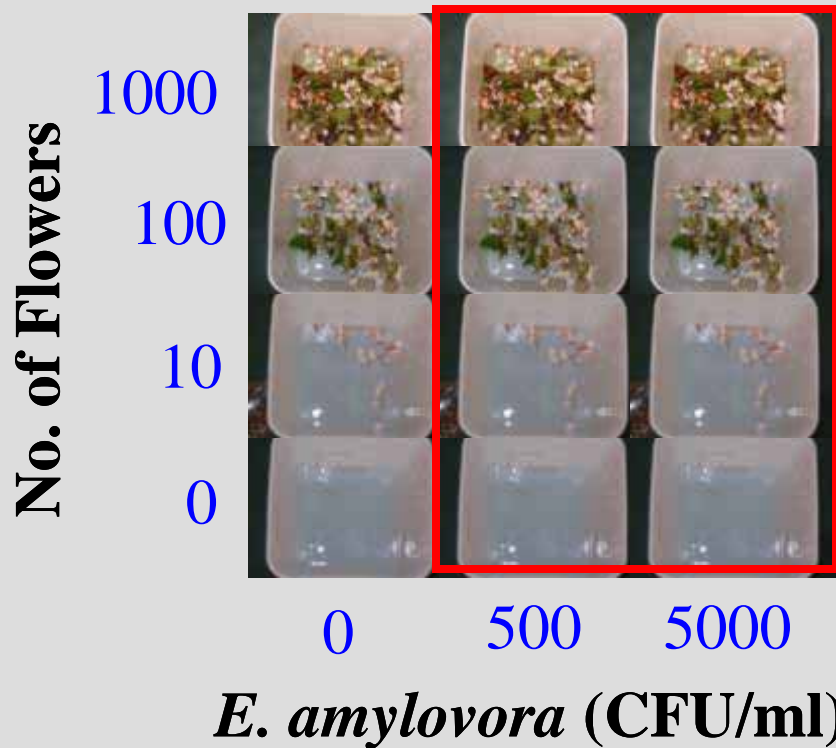
25 cells



specificity

Lab isolates	Ea29	amsL B
	plasmid	chromosomal
<i>E. amylovora</i>	Y	Y
<i>P. agglomerans</i>	N	N
<i>P. fluorescens</i>	N	N
<i>P. syringae</i>	N	N
water	N	N

All positive LAMPs



Flower density experiments:

The presence of flowers does not appear to effect the LAMP assay

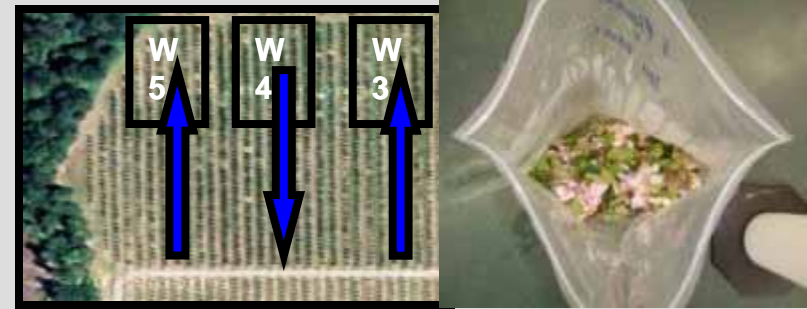
Cultivar	Wash sampled directly			Filter-concentrated wash		
	<i>E. amylovora</i> CFU/ml			<i>E. amylovora</i> CFU/ml		
	0	500	5000	0	500	5000
Aristocrat pear	0%	100%	100%	0%	100%	100%
Bartlett pear	0%	100%	100%	0%	100%	100%
Gala apple	0%	100%	100%	0%	100%	100%
	Log CFU	2.7	3.7			

LAMP can detect a single flower infested with *E. amylovora*



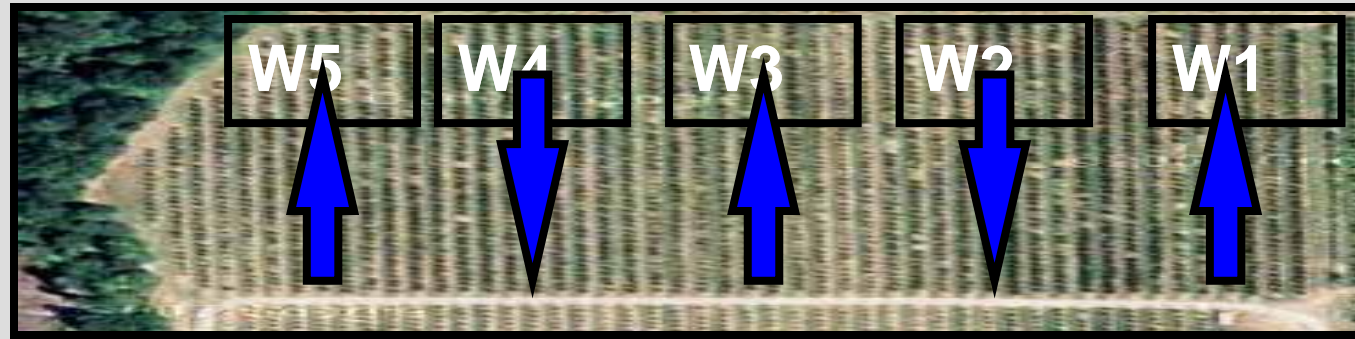
Cultivar	Treatment added to wash			
	Nothing	100 flower clusters	Single flower colonized by <i>E. amylovora</i>	Single flower colonized by <i>E. amylovora</i> and 100 flower clusters
2008				
Bartlett pear	0% ^b	0%	100% (4.0 ± 0.96) ^c	100% (4.2 ± 0.34)
Gala apple	0%	0%	100% (3.4 ± 0.62)	100% (3.8 ± 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)

Samples from OSU orchards used for fire blight trials



		2008		2009	
Willamette valley	Pathogen inoculated	LAMP	Media isolation (CFU/ml)	LAMP	Media isolation (CFU/ml)
Bartlett pear	N	N	N	N	N
Bosc, Bartlett, d'Anjou pear	N			N	N
Bartlett pear	Y	Y (9 of 9)	Y (1.1 x 10 ⁴)	Y (6 of 6)	Y (2 x 10 ⁵)
Golden delicious apple	Y	Y (9 of 9)	Y (3.5 x 10 ⁵)		
Gala apple	Y	Y (9 of 9)	Y (9.5 x 10 ⁴)		
Red Delicious apple	Y			Y (6 of 6)	Y (6 x 10 ⁵)
Jonathon apple	N	N	N	N	N
Braeburn apple	N			N	N
Fuji apple	N	N	N	N	N

Commercial Orchards_2008:



Rogue valley	LAMP	Media isolation	Disease
Bartlett pear	N	N	N
Bosc & Red d'Anjou pear	N	N	N
Bartlett pear	N	N	N
Hood River Valley			
Red d'Anjou pear	N	N	N
Bartlett, d'Anjou, Bosc pear	N/Y* (2 of 5)	Y 5.6 x 10 ² CFU/ml)	Y light
JonaGold Apple (30%)	N/Y* (2 of 5)	N	Y light
Bartlett & Bosc pear	Y (4 of 5)	Y (3.9 x 10 ³ CFU/ml)	Y mod
Gala apple (30%)	N/Y* (5 of 5)	N	Y mod

- LAMP detected *E. amylovora* in four of eight commercial pear or apple orchards surveyed in spring 2008.

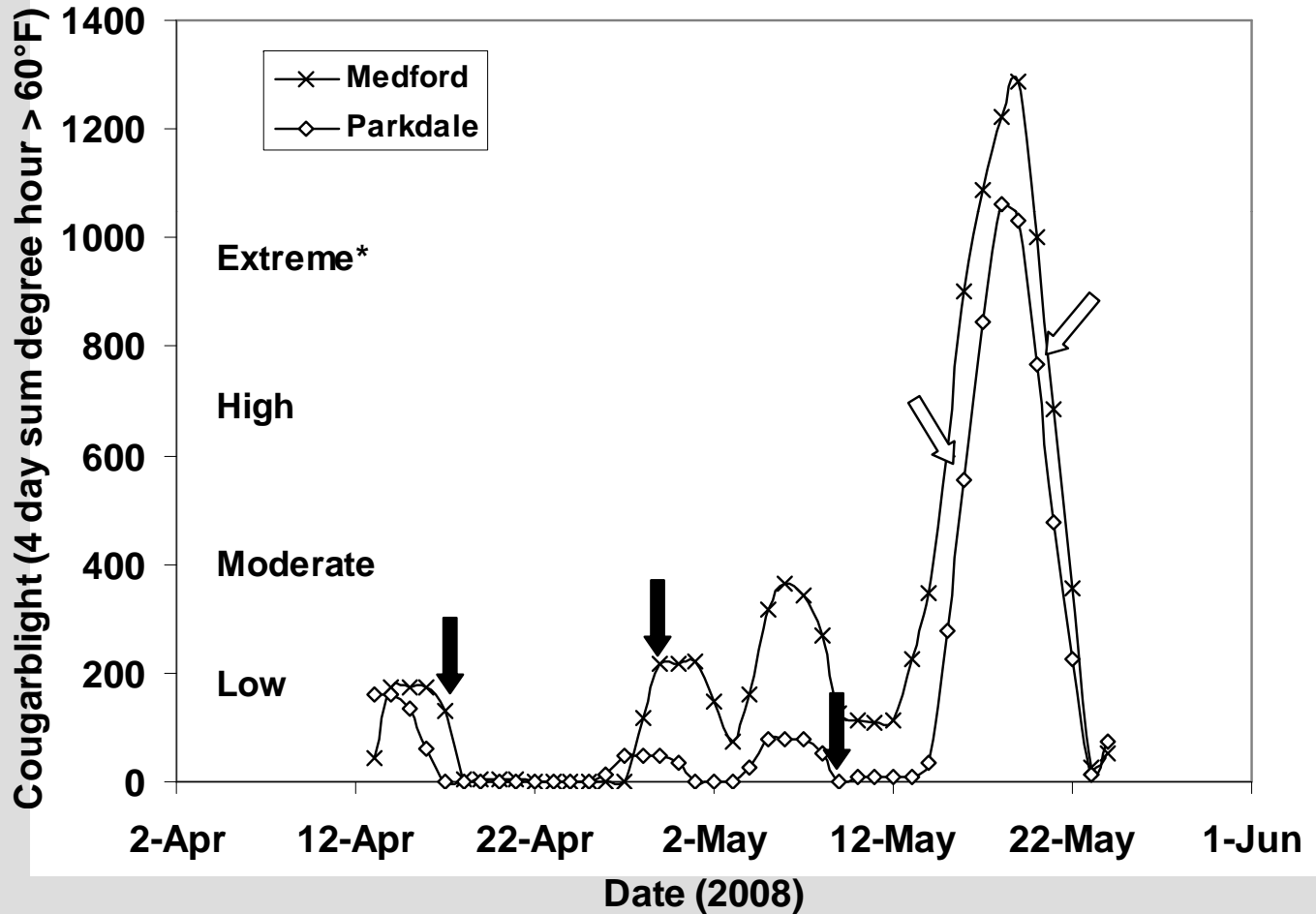
* Yes, after DNA concentrated with speedvac

Positive LAMP detection coincided with periods of COUGARBLIGHT risk.

Cougarblight model for 2008 spring blossom period.

Black arrows = no detection

White arrows = positive detection



Positive Negative

2009 Commercial Orchards:

**LAMP detected
the fire
blight
pathogen in
27 of 33
orchards in
WA, OR, CA
and UT.**

State	Cultivar	No. of samples with Positive LAMP			Media isolation ^b	Blight ^c	
		Mid-bloom	Full bloom	Petal fall	(Avg. Log10)		
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	0 of 5	0 of 5	1 of 5	No	no data
	Wenatchee	Pink Lady apple	no data	no data	1 of 1	Yes (5.0)	no data
		d'Anjou pear	0 of 5	0 of 5	0 of 5	No	No
	Okanogan	d'Anjou pear	0 of 5	0 of 5	0 of 5	No	No
		Bosc pear	0 of 4	0 of 6	2 of 4	Yes (5.3)	Yes light

+ 7 additional orchards in Utah

**2009
Commercial
Orchards:**

Grower was informed early of the presence of the fire blight pathogen, which caused him to intensify his control effort.

“Ken: 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.”

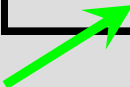
State	Cultivar	No. of samples with Positive LAMP			Media isolation ^b (Avg. Log10)	Blight ^c	
		Mid-bloom	Full bloom	Petal fall			
OR	Medford	Bartlett pear	1 of 5	0 of 5	0 of 5	Yes (2.3)	No
		Bosc & Red d'Anjou pear	0 of 5	0 of 5	1 of 5	Yes (6.0)	No
		Bartlett pear	4 of 5	1 of 5	2 of 5	Yes (6.0)	Yes 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
	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
WA	Wenatchee	d'Anjou pear	0 of 5	4 of 5	4 of 5	Yes (5.2)	Yes light
		d'Anjou pear	0 of 5	5 of 5	5 of 5	Yes (5.1)	Yes light
	Okanogan	Bosc pear	0 of 5	5 of 5	5 of 5	Yes (7.0)	Yes light
		Bosc pear	0 of 5	0 of 5	0 of 5	No	Yes light
	Okanogan	Bosc pear	0 of 5	no data	no data	No data	No
		Bosc pear	0 of 5	1 of 5	1 of 5	Yes (3.1)	Yes light
	Okanogan	Bosc pear	0 of 5	0 of 5	0 of 5	No	No
		Bosc pear	0 of 5	0 of 5	0 of 5	Yes (2.5)	No
	Okanogan	Bosc pear	0 of 5	0 of 5	0 of 5	No	Yes light
		Bosc pear	0 of 5	1 of 5	1 of 5	No	no data
Okanogan	Bosc pear	no data	1 of 1	1 of 1	Yes (5.0)	no data	
	Bosc 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	

Summary of survey

'08 & '09 commercial orchards

		Media isolation	
		-	+
LAMP	-	11	0
	+	6	25

Detection limit differential
or
Specificity problem?

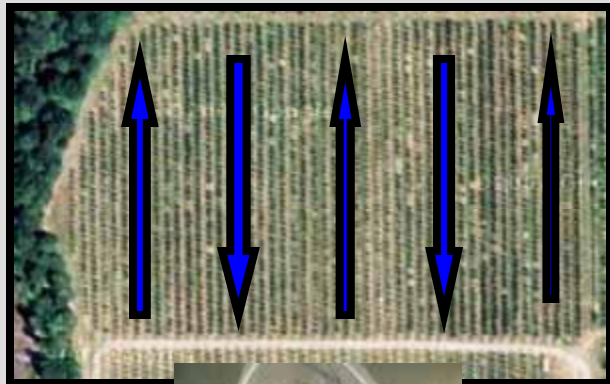


		Fire blight	
		-	+
LAMP	-	7	2 (light)
	+	9	19

***5 orchards had no blight data

Resistance to streptomycin 2009

LAMP-based scouting for early detection
of the fire blight pathogen



Positive

Negative

	2009 Percentage of Pop's Streptomycin Res or Sen			Blight ?
	1	2	3	
Zillah, WA	nd	nd	nd	no data
	nd	nd	nd	no data
	nd	nd	Res. (100%)	no data
Yakima, WA	nd	nd	nd	Y light
Wenatchee, WA	nd	nd	nd	N
	nd	nd	nd	N
Okanogan, WA	nd	nd	Res (100%)	Y light
Parkdale, OR apple	nd	nd	Sen (100%)	N
	nd	nd	Sen (100%)	Y light
Parkdale, OR pear	sen (100%)	nd	sen (100%)	N
	sen (100%)	nd	sen (100%)	N
	nd	nd	not det.	Y light
Hood River, OR	nd	nd	Sen (100%)	Y light
	nd	nd	nd	Y light
	nd	nd	nd	N
Medford, OR	Sen (100%)	nd	nd	N
	nd	nd	Res (100%)	N
	nd	nd	Res (100%)	Y light
	nd	nd	nd	N
Milton Freewater, OR	nd	Res (100%)	Sen (100%)	Y light
	nd	nd	nd	N
	nd	nd	Res (100%)	Y light
	nd	Sen (33%) Res (67%)	Res (100%)	Y light
Lake County, CA	sen (100%)	sen (100%)	sen (100%)	Y light
	nd	nd	nd	N
	nd	sen (100%)	nd	N

Red Bold are Resistant to Streptomycin and **Green bold** are sensitive to streptomycin.

Resistance
to
streptomycin
2009
Utah

Utah sample 1				sample 2				sample 3			
date	LAMP	Pops		date	LAMP	Pops		date	LAMP	Pops	
2-May	U1.1	Y	3.60E+03	3-May	U2.1	N	not det.	4-May	U3.1	N	nd
2-May	U1.2	Y	3.10E+05	3-May	U2.2	Y	2.10E+05	4-May	U3.2	Y	5.40E+05
2-May	U1.3	N	nd	3-May	U2.3	N	nd	4-May	U3.3	N	nd
2-May	U1.4	N	nd	3-May	U2.4	Y	nd	4-May	U3.4	Y	nd
2-May	U1.5	N	nd	3-May	U2.5	Y	nd	4-May	U3.5	N	nd
2-May	U1.6	Y	6.00E+04	3-May	U2.6	Y	8.70E+05	4-May	U3.6	Y	1.27E+06

sample 4				sample 5				sample 6			
date	LAMP	Pops		date	LAMP	Pops		date	LAMP	Pops	
5-May	U4.1	Y	3.00E+04	6-May	U5.1	Y	2.70E+05	7-May	U6.1	Y	6.40E+05
5-May	U4.2	Y	1.60E+06	6-May	U5.2	Y	4.40E+05	7-May	U6.2	Y	1.10E+05
5-May	U4.3	N	nd	6-May	U5.3	N	nd	7-May	U6.3	Y	1.30E+03
5-May	U4.4	N	nd	6-May	U5.4	Y	7.00E+02	7-May	U6.4	N	nd
5-May	U4.5	Y	nd	6-May	U5.5	Y	1.00E+02	7-May	U6.5	Y	1.12E+06
5-May	U4.6	Y	1.31E+06	6-May	U5.6	Y	1.50E+06	7-May	U6.6	N	nd
pos contol not working				6-May	U5.7	Y	9.00E+02	7-May	U6.7	Y	4.00E+05

sample 7				sample 8				sample 9			
date	LAMP	Pops		date	LAMP	Pops		date	LAMP	Pops	
8-May	U7.1	Y	6.30E+03	9-May	U8.1	Y	5.70E+03	10-May	U9.1	Y	1.90E+05
8-May	U7.2	Y	1.80E+05	9-May	U8.2	Y	2.00E+04	10-May	U9.2	N	nd
8-May	U7.3	Y	6.00E+02	9-May	U8.3	N	nd	10-May	U9.3	N	nd
8-May	U7.4	Y	8.00E+02	9-May	U8.4	N	nd	10-May	U9.4	Y	1.80E+03
8-May	U7.5	N	nd	9-May	U8.5	N	nd	10-May	U9.5	Y	1.00E+04
8-May	U7.6	Y	1.60E+06	9-May	U8.6	Y	6.95E+05	10-May	U9.6	Y	4.20E+05
8-May	U7.7	N	nd	9-May	U8.7	N	nd				

sample 10				sample 11				sample 12			
date	LAMP	Pops		date	LAMP	Pops		date	LAMP	Pops	
11-May	U10.1	Y	2.10E+05	12-May	U11.1	Y	8.20E+05	13-May	U12.1	Y	6.60E+05
11-May	U10.2	N	nd	12-May	U11.2	N	nd				
11-May	U10.3	N	nd	12-May	U11.3	Y	1.30E+03				
11-May	U10.4	Y	1.60E+05								
11-May	U10.5	N	nd								
11-May	U10.6	Y	6.10E+05								

Red Bold pop's are Resistant to Streptomycin and Green Bold pop's are sensitive to streptomycin.

Resistance to streptomycin 2009

Utah

Utah sample 1				sample 2			sample 3				
date	LAMP	Pops		date	LAMP	Pops	date	LAMP	Pops		
2-May	U1.1	Y	3.60E+03	3-May	U2.1	N	not det.	4-May	U3.1	N	nd
2-May	U1.2	Y	3.10E+05	3-May	U2.2	Y	2.10E+05	4-May	U3.2	Y	5.40E+05
2-May	U1.3	N	nd	3-May	U2.3	N	nd	4-May	U3.3	N	nd
2-May	U1.4	N	nd	3-May	U2.4	Y	nd	4-May	U3.4	Y	nd
2-May	U1.5	N	nd	3-May	U2.5	Y	nd	4-May	U3.5	N	nd
2-May	U1.6	Y	6.00E+04	3-May	U2.6	Y	8.70E+05	4-May	U3.6	Y	1.27E+06

sample 4				sample 5			sample 6				
date	LAMP	Pops		date	LAMP	Pops	date	LAMP	Pops		
5-May	U4.1	Y	3.00E+04	6-May	U5.1	Y	2.70E+05	7-May	U6.1	Y	6.40E+05
5-May	U4.2	Y	1.60E+06	6-May	U5.2	Y	4.40E+05	7-May	U6.2	Y	1.10E+05
5-May	U4.3	N	nd	6-May	U5.3	N	nd	7-May	U6.3	Y	1.30E+03
5-May	U4.4	N	nd	6-May	U5.4	Y	7.00E+02	7-May	U6.4	N	nd
5-May	U4.5	Y	nd	6-May	U5.5	Y	1.00E+02	7-May	U6.5	Y	1.12E+06
5-May	U4.6	Y	1.31E+06	6-May	U5.6	Y	1.50E+06	7-May	U6.6	N	nd
pos contol not working				6-May	U5.7	Y	9.00E+02	7-May	U6.7	Y	4.00E+05

sample 7				sample 8			sample 9				
date	LAMP	Pops		date	LAMP	Pops	date	LAMP	Pops		
8-May	U7.1	Y	6.30E+03	9-May	U8.1	Y	5.70E+03	10-May	U9.1	Y	1.90E+05
8-May	U7.2	Y	1.80E+05	9-May	U8.2	Y	2.00E+04	10-May	U9.2	N	nd
8-May	U7.3	Y	6.00E+02	9-May	U8.3	N	nd	10-May	U9.3	N	nd
8-May	U7.4	Y	8.00E+02	9-May	U8.4	N	nd	10-May	U9.4	Y	1.80E+03
8-May	U7.5	N	nd	9-May	U8.5	N	nd	10-May	U9.5	Y	1.00E+04
8-May	U7.6	Y	1.60E+06	9-May	U8.6	Y	6.95E+05	10-May	U9.6	Y	4.20E+05
8-May	U7.7	N	nd	9-May	U8.7	N	nd				

sample 10				sample 11			sample 12				
date	LAMP	Pops		date	LAMP	Pops	date	LAMP	Pops		
11-May	U10.1	Y	2.10E+05	12-May	U11.1	Y	8.20E+05	13-May	U12.1	Y	6.60E+05
11-May	U10.2	N	nd	12-May	U11.2	N	nd				
11-May	U10.3	N	nd	12-May	U11.3	Y	1.30E+03				
11-May	U10.4	Y	1.60E+05								
11-May	U10.5	N	nd								
11-May	U10.6	Y	6.10E+05								

Red Bold pop's are Resistant to Streptomycin and Green Bold pop's are sensitive to streptomycin.

Bottom line:
if you want to use strep, use it only once and mix it with a full rate of oxytet

Conclusions:

- **LAMP amplification of DNA is a viable technology for detection of *E. amylovora*.**
- **LAMP-based scouting will be most efficient when used with COUGARBLIGHT and orchard history.**
- **A sample size of ~600 flowers has worked well. Results can be obtained as fast as a few hours after sampling.**
- **Negative: We'd like the technique to be somewhat simpler – it's not ready for a grower's kitchen table.**