Evaluation of Phloem-feeding Hemipteran Insects in the Spread of Grapevine red blotch–associated virus

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Vineyards with red blotch disease confirmed as Infected by Grapevine red blotch-associated virus

RB	Location	Clone	Virus + ve Year	Year block Removed
2008	Rutherford	Cab Sauvignon	!	2010
2009	Oakville	Cab Sauvignon	2011	2015
2009	Oakville	Cab Franc	2011	2014
?	St. Helena	Petite Sirah	2011	No access
?		Petit Verdot	2011	No access
2011	Napa	Cab Sauvignon	2011	2013
2011	Sonoma	Cab Sauvignon	2012	
2011	Napa-Oak Knoll	Merlot	2012	2013
?	Shandon	Merlot	2012	2013
		Mourvédre	2012	2013
2012		Cabernet Franc	2013	2013
2012	Rutherford	Cab Sauvignon	2012	2013 (!!!)

46.5% infected in 2011
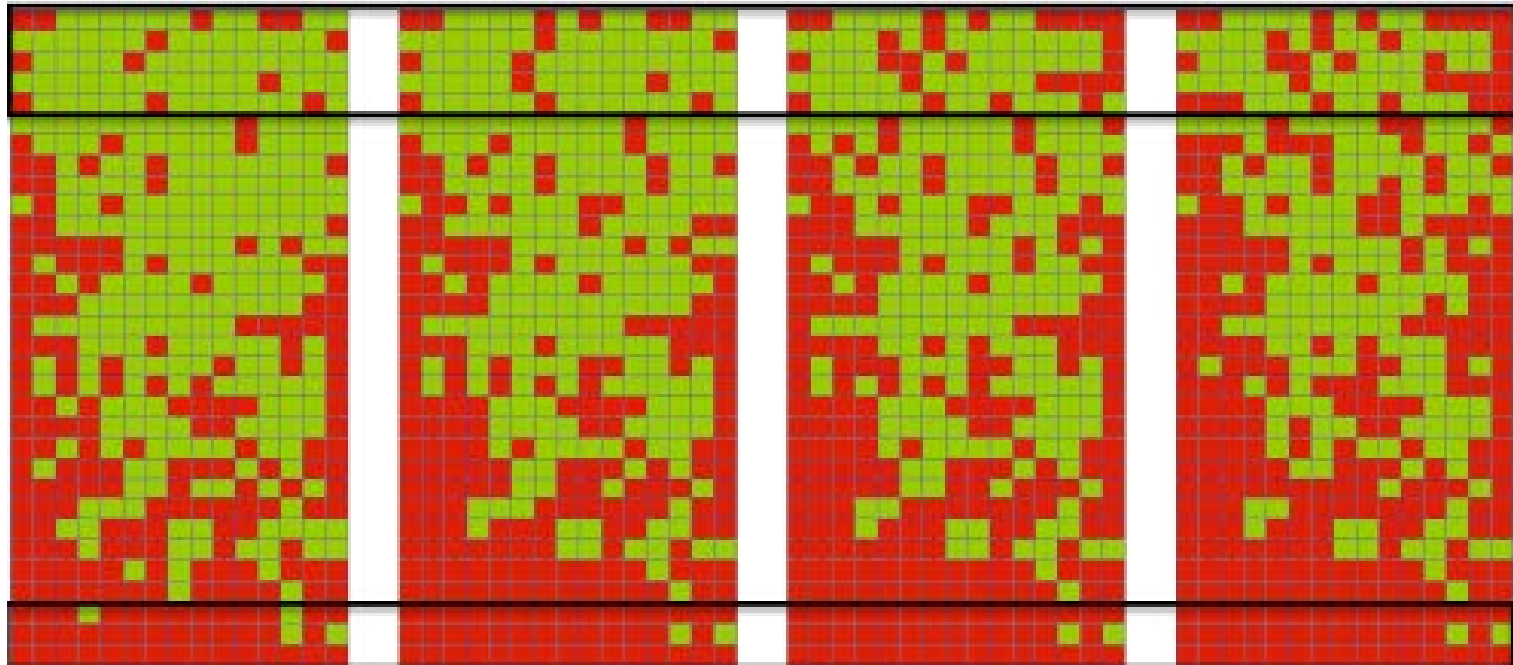
2011   2012   2013   2014
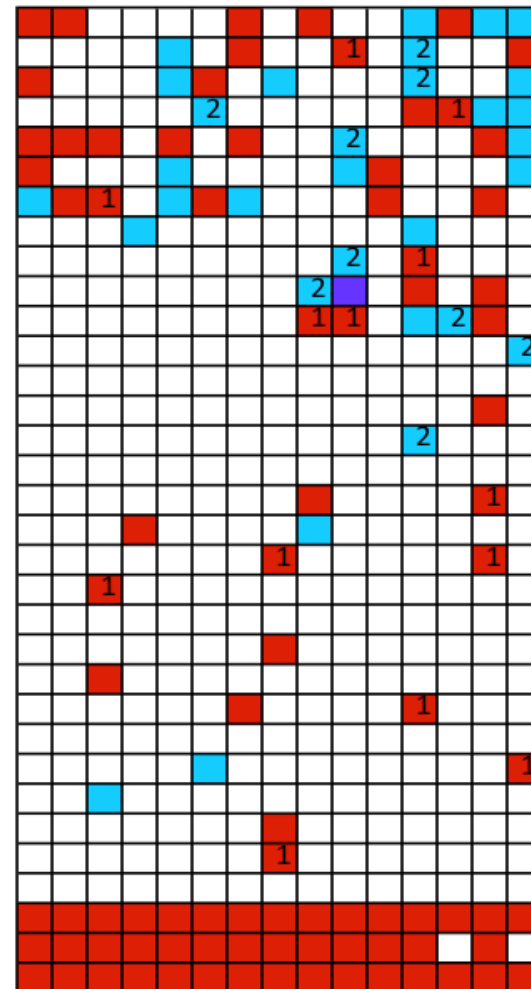
          4.4%   3.8%   5.2%

13.4% increase from 2011

59.9% infection in 2014

Introduction of Genotype 2

- First detected in 2013
- Revealed through high resolution melting temperature analysis (HRMA)
- Genotype 1 = red
- Genotype 2 = blue
- Purple = mixed infection

Assessment of Potential Vectors

- 10 yellow sticky cards are placed at each vineyard where GBRaV is detected with some evidence of a spread and there is grower cooperation

- Monitored weekly for hempiteran insects moving within and into the vineyard

Assessment of Potential Vectors

- 2014: 2 field sites with 10 traps each
  - June to September
- 2015: 4 field sites with 10 traps each
  - June to September
- 25 different species of leafhoppers found associated with vineyard

Assessment of Potential Vectors

- Sub-sample of Hemipteran insects taken from traps, photographed and processed for DNA extraction

Assessment of Potential Vectors

- Standard PCR used to amplify a region of the COI gene to identify the insect species (if possible)

- qPCR used to detect GRBaV in nucleic acid extracts from insects

COI Gene

≈1,500 bp➔

1   2   3
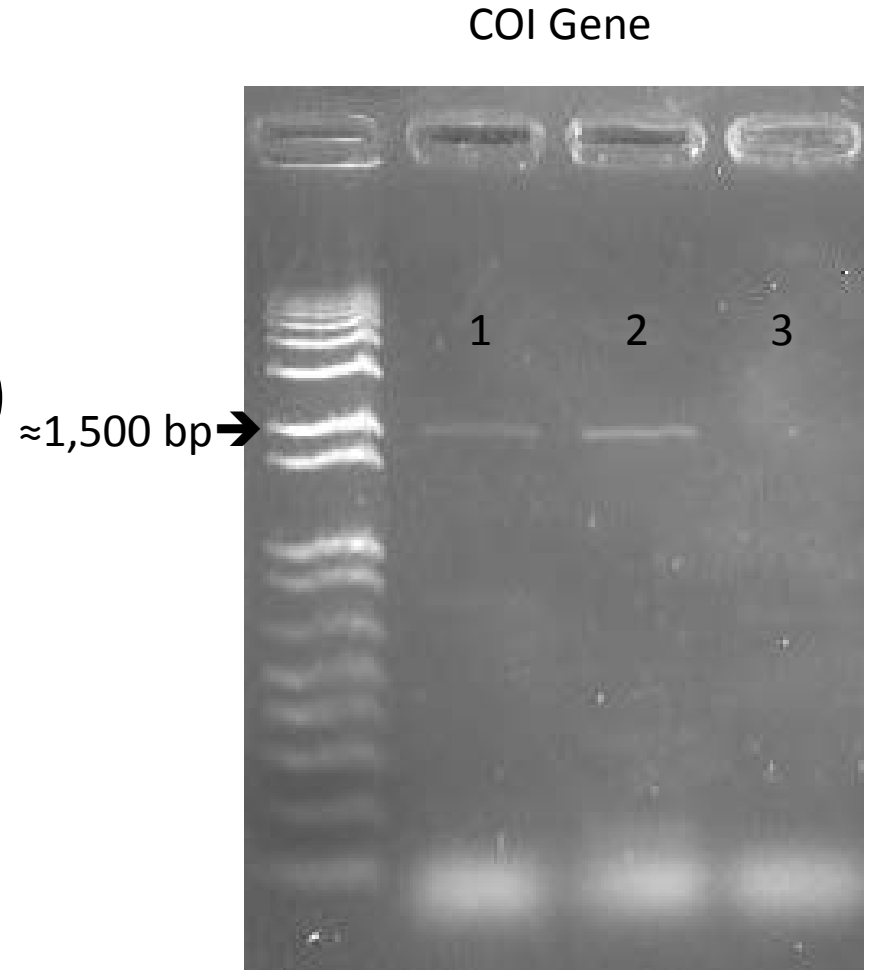
1=sample, 2=(+) control, 3=(-) control

Assessment of Potential Vectors

	2014	2014	2015	2015	2015
Species	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
El. elegantula	942	1,310	163	37	n/a
El. variabilis	40	0	3	5	n/a
El. ziczac	0	5	0	0	n/a
Em. fabae	43	31	5	26	n/a
Sa. acutus	37	57	9	6	n/a
Ga. atropunctata	52	20	0	0	n/a

Assessment of Potential Vectors

Species	Insects Positive for GRBaV	Salivary Gland/Hemocoel
E. elegantula (Western Grape)	1/40	0/10
E. variabilis (Variegated)	0/40	0/10
E. ziczac (Virginia Creeper)	0/40	0/10
E. fabae (Potato)	2/40	0/10
S. acutus (Sharp-nosed)	5/20	n/a
Pl. citri (Citrus mealybug)	10/20	0/10
Ps. viburni (Obscure mealybug)	9/20	0/10
Melanoliarus sp. (Planthopper)	8/20	n/a
Unidentified treehopper	3/15	n/a
Unidentified mealybug	0/10	n/a
Unidentified whitefly sp.1	4/10	n/a
Unidentified whitefly sp.2	3/10	n/a
Unidentified pysllid	n/a	n/a

Assessment of Potential Vectors

- Colonies established to conduct transmission asssays
  - *E. elegantula*
  - *E. variabilis*
  - *E. ziczac*
  - *Ps. viburni*
  - *Ps. longispinus*
  - *Pl. citri*

Transmission Bioassays

Species	Transmission Results*
Western Grape Leafhopper	0/10
Variegated Leafhopper	0/10
Greenhouse Whitefly	0/10
Obscure Mealybug	0/10
Citrus Mealybug	0/10
Longtailed Mealybug	0/10
Potato Aphid	0/10
Grape Phylloxera	0/10
Unidentified mealybug	0/10
Unidentified treehopper	0/10
Unidentified leafhopper	0/10
Virginia Creeper LH (+) Control w/GRBaV	0/10
Obscure Mealybug (+) control w/ GLRaV	4/10

Assessment of Potential Vectors

- Light trapping to get live individuals of uncommon species for colony establishment and transmission assays

A New Look at Mealybugs

- Needed to identify specimens from red-blotch project
- Standard protocol for slide mounting
  - Make a hole and squeeze out body contents in KOH
  - Takes years of practice to master this technique
  - Lose DNA sequence data
- Standard protocol for DNA extraction
  - Macerate bug = loss of physical specimen

# Preparation of *Pseudococcus viburni*

1 mm

A New Look at Mealybugs

- Developed EPED protocol
  - EPED = extended proteinase K and extended detergent treatmeant
- Allows for extraction of DNA for analysis while maintaining a perfectly intact exoskeleton to slide-mount

Protocol

- Place mealybug in lysis buffer with proteinase K and allow to lyse at 56°C until specimen is cleared
  - In study, lysis ranged from 8 hours to 3 days
- Transfer liquid to new tube and proceed with DNA extraction protocol from manufacturer

Protocol

- Added more lysis buffer and detergent buffer to tube with exoskeleton.

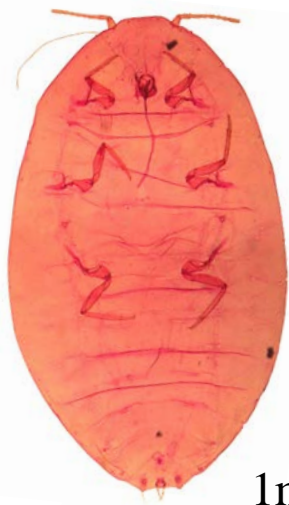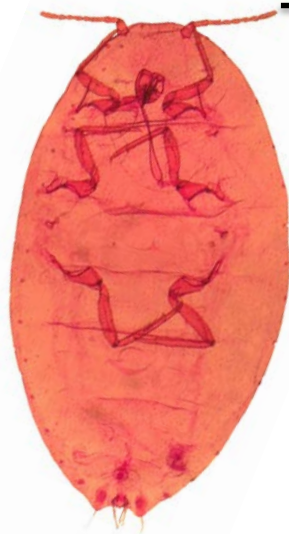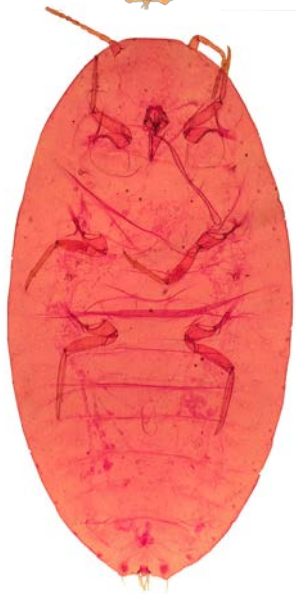- Placed at 96°C until fat bodies and wax dissolved from within specimen

A

1 mm

B

1 mm

Results

1 mm
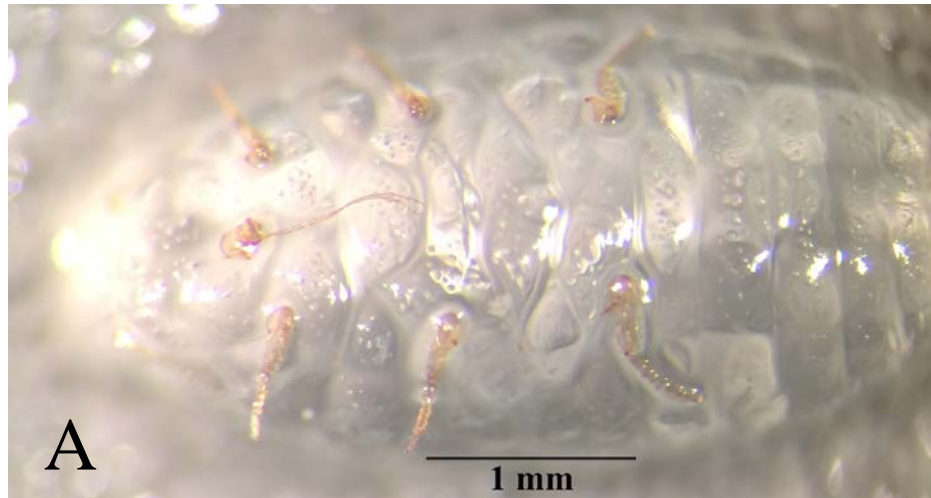
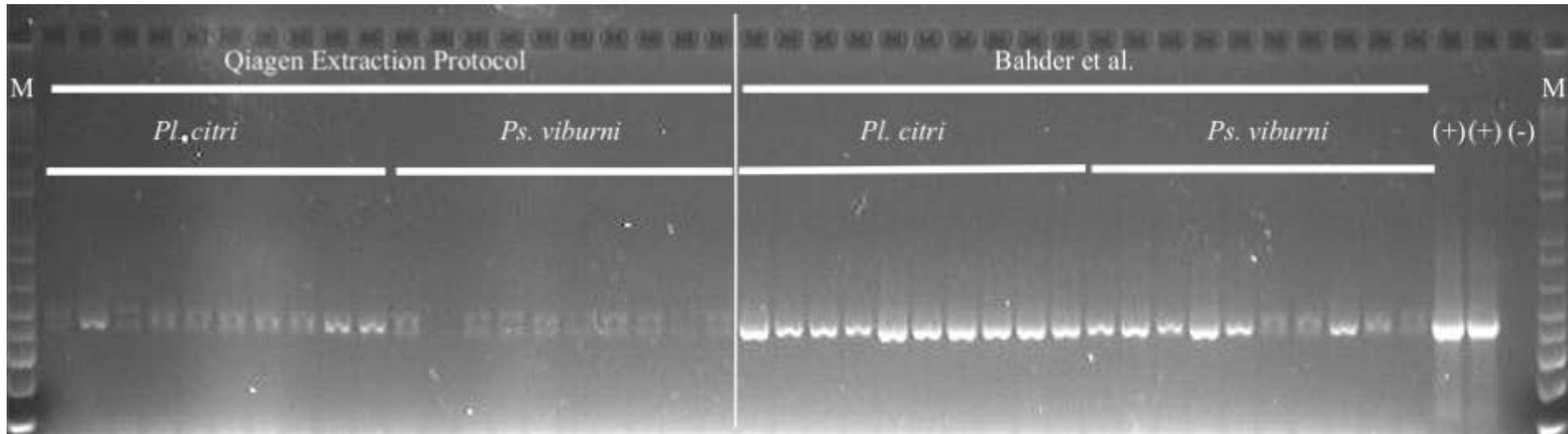A   B

1mm

Results

DNA Yield: 19.25±3.1 ng/µl
Purity: 5.79±2.1

DNA Yield: 32.9±3.9 ng/µl
Purity: 1.94±0.03

Optimal purity should be between 1.8 and 2.0

Contact

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