Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Epidemiology and Management of Grapevine Viruses

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Summerland Research and Development Centre, AAFC

Canada

CCOVI Lecture Series, April 26, 2017.

Dr. Sudarsana Poojari

Peer-reviewed publications:

Poojari, S., D.T. Lowery, *et al*. 2017. Etiology and epidemiology of grapevine leafroll disease in British Columbia. Plant Disease (Under review)

Poojari, S., D.T. Lowery, *et al.* 2016. First report and prevalence of Grapevine fleck virus in grapevines (Vitis vinifera L.) in Canada. Plant Disease. Posted online February 25, 2016.

Poojari, S., D.T. Lowery, *et al.* 2017. Incidence, distribution and genetic diversity of Grapevine red blotch virus in British Columbia. Canadian Journal of Plant Pathology. DOI: 10.1080/07060661.2017.1312532.

Poojari, S., O.J. Alabi, *et al.* 2016. Development of SYBR Green I-based Quantitative Duplex Real-Time PCR for Detection and Discrimination of Grapevine Viruses. Journal of Virological Methods. doi:10.1016/j.jviromet.2016.05.013.

Poojari, S., T. Lowery, *et al.* 2016. Complete genome sequence analysis of a Canadian isolate of Grapevine Pinot Gris virus. Plant Disease. PDIS02160178PDN.

Presentations

Poojari, S., D.T. Lowery & J.R. Úrbez-Torres. 2017. Improvements in Grapevine virus diagnostics. CCOVI Lecture Series, Brock University. March 16 2017. (Invited Speaker).

Poojari, S., D.T. Lowery & J.R. Úrbez-Torres 2017. Epidemiology of major grapevine viruses in British Columbia. Kentville Research and Development Centre. March 12 2017. (Invited Speaker).

Poojari, S. D.T. Lowery, et al. 2016. Disease Diagnostics and Monitoring of Grapevine Red Blotch-Associated Virus in BC. 17th Annual Enology & Viticulture Conference, Penticton, BC Canada. July 18-19, 2016. (Oral Presentation).

Poojari, S., D.T. Lowery, *et al.* 2016. Monitoring for Mealybug and Scale Insect Populations in Vineyards of British Columbia. The 17th Annual Enology & Viticulture Conference, Penticton, BC Canada. July 18-19, 2016. (Poster Presentation).

Poojari, S., D.T. Lowery, et al. 2016. Status of Grapevine red blotch virus in Canada. Webinar entitled: Grapevine red blotch disease: What you need to know. UC Davis, USDA, FPS U.C Davis. Webinar (Invited Speaker).

Poojari, S., D.T. Lowery, et al. 2016. Prevalence of Grapevine red blotch associated virus in British Columbia, October 27-28. 2016. CPS – BC Regional Meeting. Summerland, BC, Canada. (Oral Presentation).

Poojari, S., D.T. Lowery, et al. 2016. Current Status of Grapevine Virus Diseases in British Columbia. APS Annual Meeting. August 5-9 2016, Tampa, FL, USA. (Poster Presentation).

Poojari, S., D.T. Lowery & J.R. Úrbez-Torres. 2016. British Columbia Grape Industry Needs a Comprehensive Approach for the Management of Grapevine Leafroll Disease. Summerland RDC Wine Grape Research Newsletter. November 2016.

Poojari, S. D.T. Lowery & J.R. Urbez-Torres. 2016. Summerland RDC-Grower Seminar Series. An Update on Sanitary Status of Grapevines in British Columbia. March 18, 2016 Summerland Research and Development Center.

Presentation Outline:

- Background and current status
- Primary virus infections
- Secondary spread by vectors
- Management
- Summary

Cabernet Franc infected with GLRaV-3

Grapevine Virus Research at AAFC Summerland:

Tom Lowery, Sudarsana Poojari & José Úrbez-Torres. Epidemiology and management of grapevine viruses. CCOVI Lecture Series, April, 2017.

Sudarsana Poojari, Tom Lowery & José Úrbez-Torres. Improvements in grapevine virus diagnostics. CCOVI Lecture Series, March, 2017.

José Úrbez-Torres. Demystifying the status of grapevine viruses in British Columbia. CCOVI Lecture Series, March, 2016.

Pat Bowen – vine physiology.
Carl Bogdanoff – winter hardiness, wine quality, etc.
Kevin Usher – aromatic and flavour compounds.

BC Wine Grape Council – Agri-Innovation Program, AAFC GF2

Enhancing the Sustainability of the BC Grape and Wine Sectors'

Title: Investigation of grapevine leafroll associated virus

PI: D.T. Lowery & J.R. Úrbez-Torres (SuRDC)
Collaborators: S. Poojari (NSERC-PDF), P. Bowen, C. Bogdanoff, K. Usher, & D. O'Gorman (SuRDC); A.-M. Schmidt & M. Rott (CFIA, Sidney)

Objectives:

3.1. Identification, molecular characterization, and distribution of GLRaVs in BC

3.2. Identification, abundance and control of potential GLRaV insect vectors

3.3. Determine insect vector efficiencies for the spread of GLRaVs in BC vineyards

3.4. Quantification of GLRaVs effects on vine growth, winter hardiness, fruit, and wine (Bowen, Bogdanoff, Usher)

AAFC A-Base Program

'Grapevine Pests/Disease Management and Enhanced Sustainability'

TITLE: Investigation of lesser known grapevine viruses in BC

PI: Tom Lowery (SuRDC)
Collaborators: J.R. Úrbez-Torres, S. Poojari, P. Bowen, C. Bogdanoff, K. Usher, D. O'Gorman (SuRDC), Anna-Mary Schmidt (CFIA, CPH)

Objectives:

3.1. Characterization, and distribution of GFLV, ArMV, GFkV, GRBaV

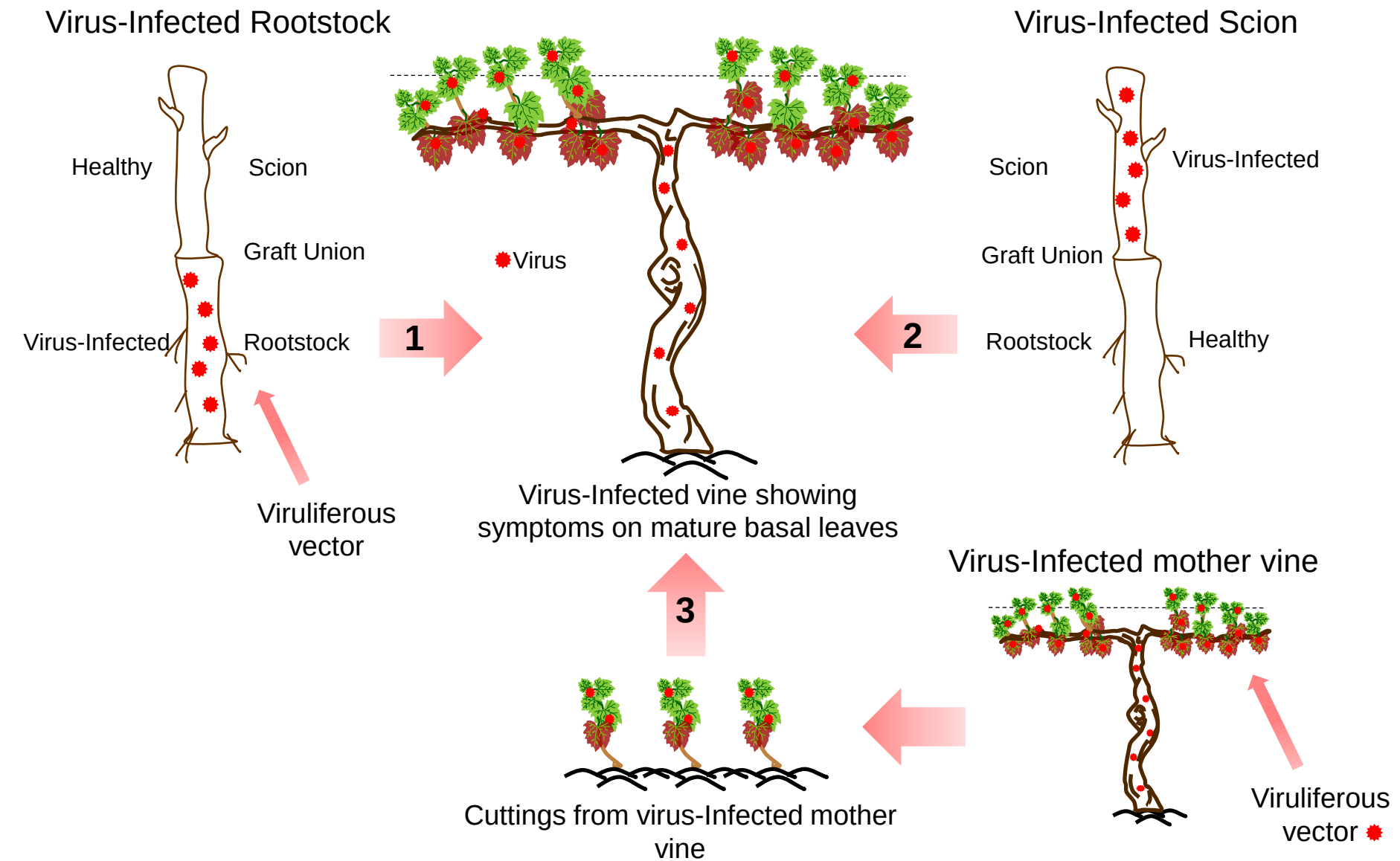
3.2. Identification, abundance and control of potential insect vectors in BC

3.3. Quantification of virus effects on vine growth, winter hardiness, fruit, and wine

Grapevines are known to host more infectious agents (viruses, viroids & phytoplasmas) than any other woody plant species!

- Long lived perennial.
- Vegetative production of new plants.
- Mixing of material by rootstocks, scions and top-working.
- Grown in many regions of the world.
- Long production history (China 7,000 BC; Georgia 5,400 BC).
- Nursery stock easily stored and transported.
- Wine and table grapes, and many wild *Vitis* species.
- Many major pests of grapes are also important virus vectors.

Infection of grapevine nursery material



Grapevine nursery material for BC sourced from France, Germany, Ontario, California, Washington, and elsewhere. Only a small amount is produced locally.

Map source: Wikipedia

Photo credit: Northwest Berry & Grape Information Network

Photo credit: plant-pest-advisory.rutgers.edu

Insects belonging to the Sub-Order Homoptera are the most important vectors of plant viruses. As pests of grapevines, in Canada some of these include:

Aphids:	Grapevine aphid, Grape phylloxera.
Leafhoppers:	Western grape leafhopper, Virginia creeper leafhopper, Eastern grape leafhopper, etc.
Treehoppers:	Buffalo and Three-cornered alfalfa treehoppers.
Sharpshooters:	Willow and poplar sharpshooters.
Mealybugs:	Grape mealybug.
Soft scale:	Cottony maple scale (Vine scale?), European fruit lecanium scale.
Psylla:	Hop-hornbeam psyllid.

Grape mealybug, *Pseudococcus maritimus*

Cottony maple scale tended by ants

A nation-wide survey for grapevine viruses was carried out by the CFIA in 1994-'95

Table 2. Aggregate summary of infected samples by region

	BC[a] (1,485)[b]	ON (9,779)	PQ (39)	NS (114)	Totals (11,417)
ArMV[c]	0.34% (5)	0.55% (54)	2.56% (1)	0	0.53% (60)
GFLV	0.06% (1)	0.32% (31)	0	0	0.25% (32)
GLRaV-I	1.28% (19)	1.75% (171)	0	0.87% (1)	1.67% (191)
GLRaV-III	2.15% (32)	12.2% (1,191)	5.12% (2)	1.75% (2)	10.8% (1,227)

[a] Province abbreviations: British Columbia (BC), Ontario (ON), Quebec (PQ), Nova Scotia (NS).
[b] Numbers in parentheses represent absolute numbers of samples tested per region or the number of samples found positive or suspect for each virus in each region.
[c] ArMV = arabis mosaic virus, GFLV = grapevine fanleaf virus, and GLRaV-I and -III = grapevine leafroll associated virus types I and III.

McKenzie et al., 1996

- A total of 11,417 samples from 637 vineyard blocks: *Vitis vinifera* and hybrids
- GLRaV-3 found both in *V. vinifera* (2.32 %) as well as hybrids (14.8 %)

Surveys for grapevine viruses began in BC in 2011 and expanded in 2014.

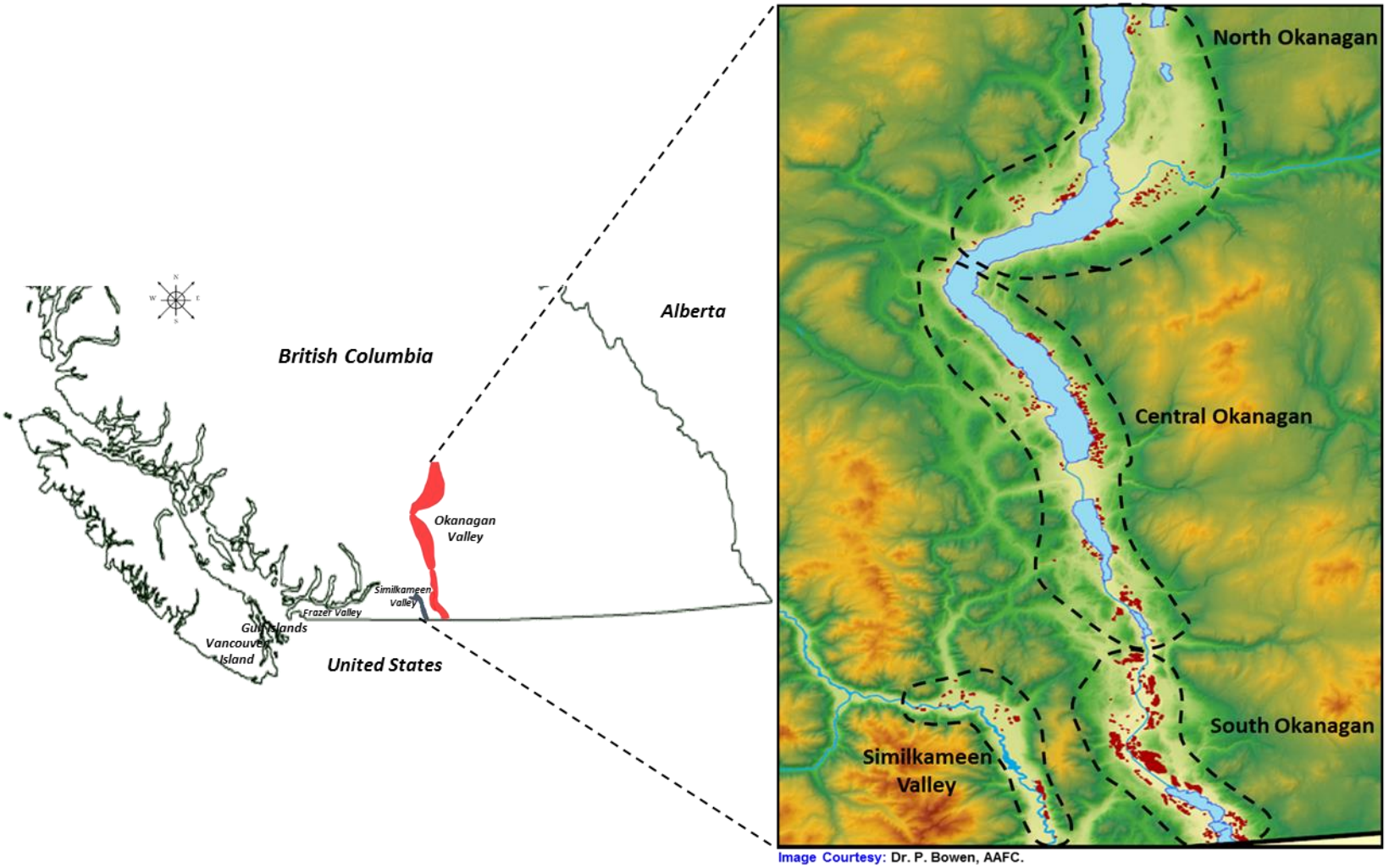
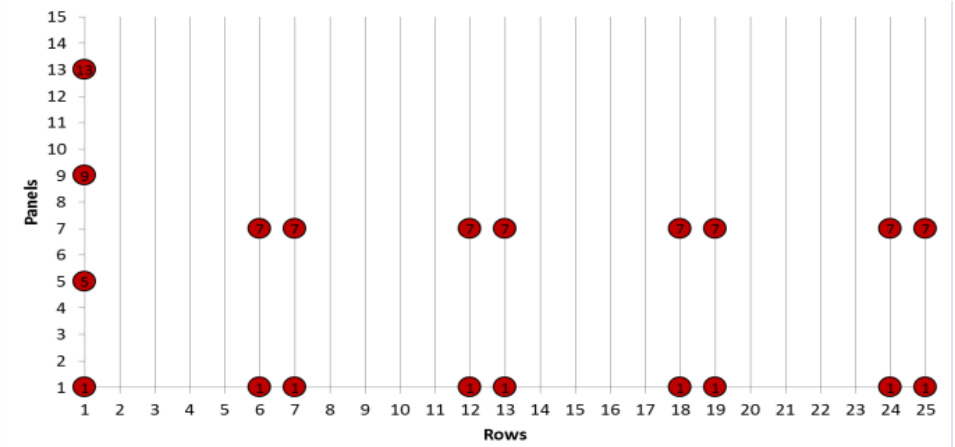
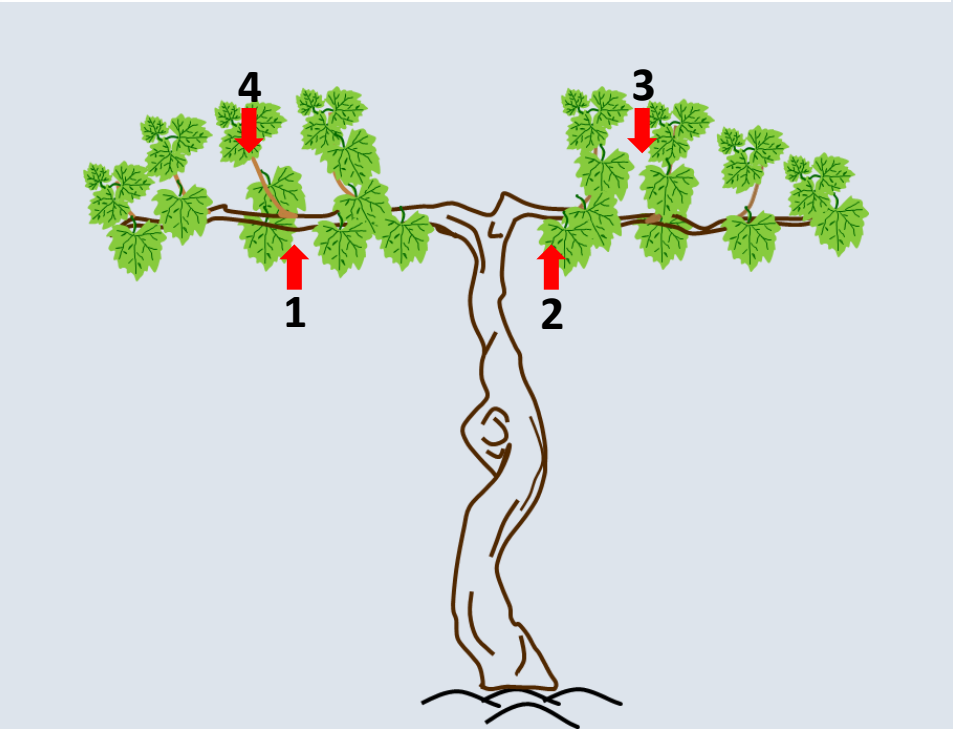


Image Courtesy: Dr. P. Bowen, AAFC.

Random Sampling Protocol





- 128 commercial vineyard blocks
- Six grape-growing regions
  - Okanagan Valley
  - Similkameen Valley
  - Fraser Valley
  - Vancouver and Gulf Islands
  - Kamloops region
- August - September : 2014 and 2015
- 4×5 quadrat sampling strategy (Fuchs et al. 2009)
- 20 composite samples/block
- composite samples = 4 leaves/vine from 5 consecutive vines
- Single vineyard block = 100 vines
- No priority given to vines with symptoms

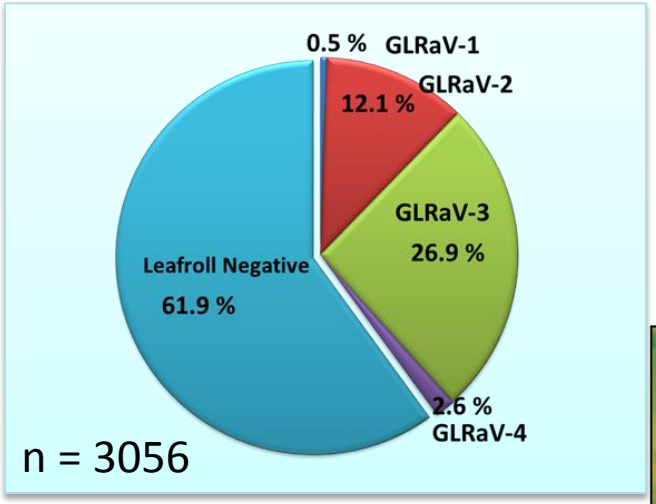
Random samples collected from 3,261 panels (5 vines/panel) in 241 cultivar blocks in BC showed little change in the incidence of **Grapevine fanleaf virus** or **Arabis mosaic virus** compared with the previous CFIA test results from 1994-’5.

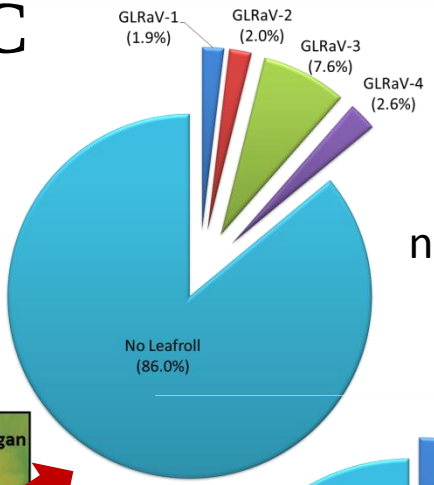
Levels of the **Grapevine leafroll associated viruses** were much higher in our tests; and these were the first records for **Grapevine red blotch virus** in BC.

Grapevine leafroll-associated virus-3

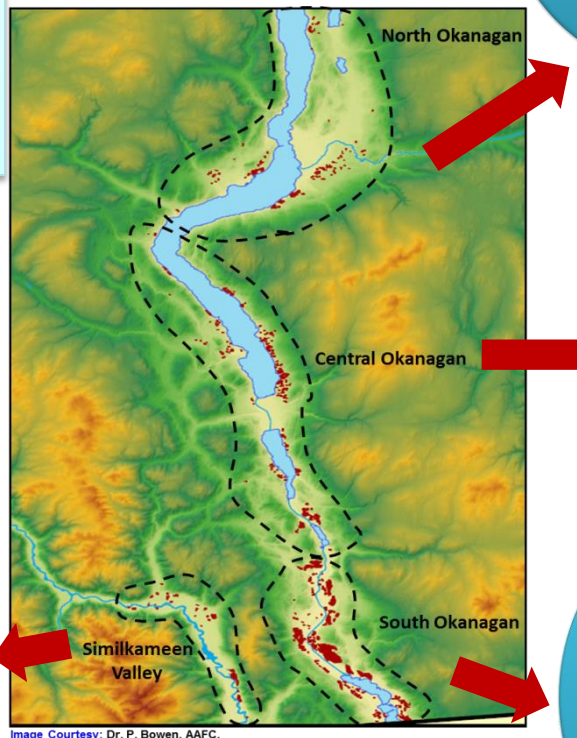
Grapevine red blotch virus

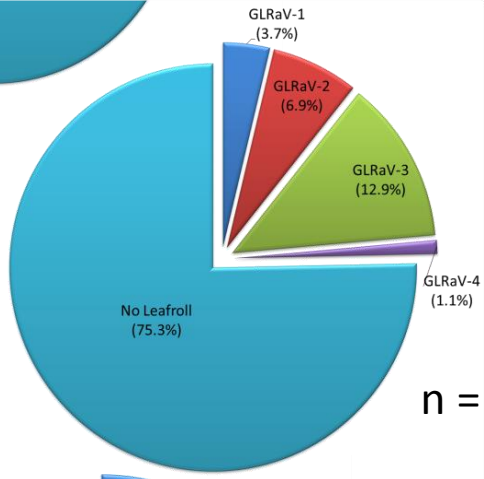
Incidence of GLRaVs in BC





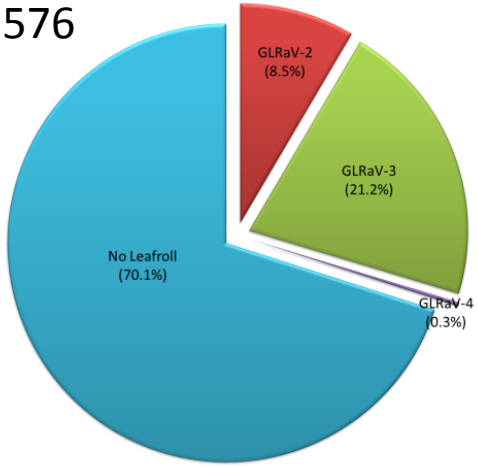
n = 858

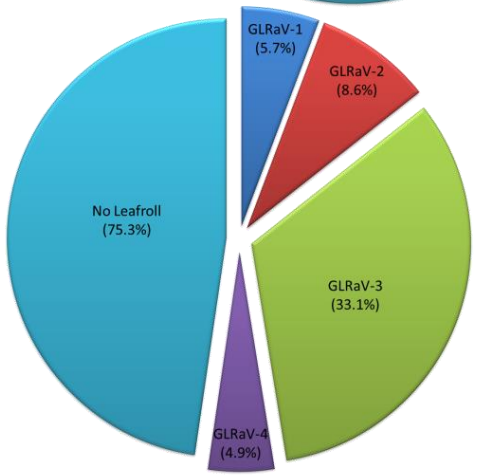




n = 385

n = 576





n = 1237

Versus 2.15% GLRaV-3 and 1.28% GLRaV-1 in 1995

Grapevine leafroll disease (GLRD)

Viruses colonize and reproduce in the phloem tissue disrupting the flow of nutrients to shoots, leaves and fruit.

Negative impact on fruit quality characteristics:

- Decreased berry weight and yield
- Delayed fruit maturity
- Reduced soluble solids (°Brix)
- Higher pH, lower titratable acidity

Infected vines cannot be cured.

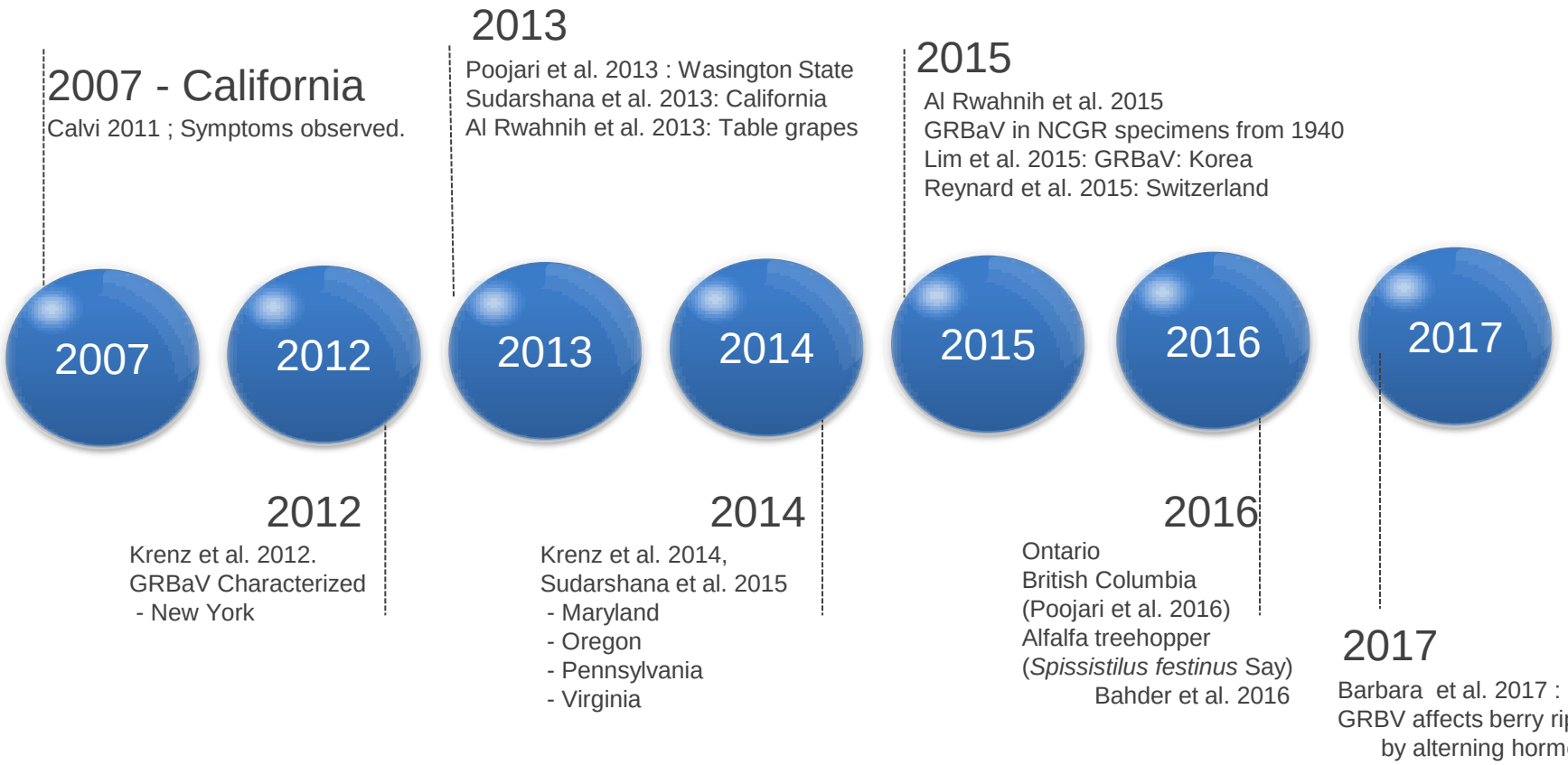
Grapevine leafroll-associated virus-3

Merlot

Chardonnay

Poojari et al. 2017. Plant Disease.

Grapevine Red Blotch Virus (GRBV) is a newly identified disease of grapevines. What we know so far….



Status of GRBV in BC Vineyards

Cultivar	# of Blocks Tested	# of Random Composite Samples Tested	# of GRBV Positives
Whites			
Bacchus	1	10	0
Chardonnay	9	90	0
Gewurztraminer	1	10	0
Pinot gris	3	30	0
Riesling	6	60	0
Sauvignon Blanc	4	35	0
Semillion	2	20	0
Pinot Blanc	2	20	0
Kerner	1	10	0
Reds			0
Cabernet Franc	2	30	3(1)
Cabernet Sauvignon	1	10	0
Merlot	7	70	0
Pinot Noir	9	90	0
Syrah	1	10	0
Tempranillo	1	10	0
Gamay Noir	1	10	0
Zinfandel	**1**	10	0
Total	**52**	**525**	**03**

2014

- **52 vineyard blocks**
- **525 Random composite samples**
  - **Represent >2600 vines**
- **PCR based diagnostics**
- **Most of the blocks aged >10yrs**

Poojari et al. 2017. CJPP: ID TCJP-2016-0192

Status of GRBV in BC Vineyards

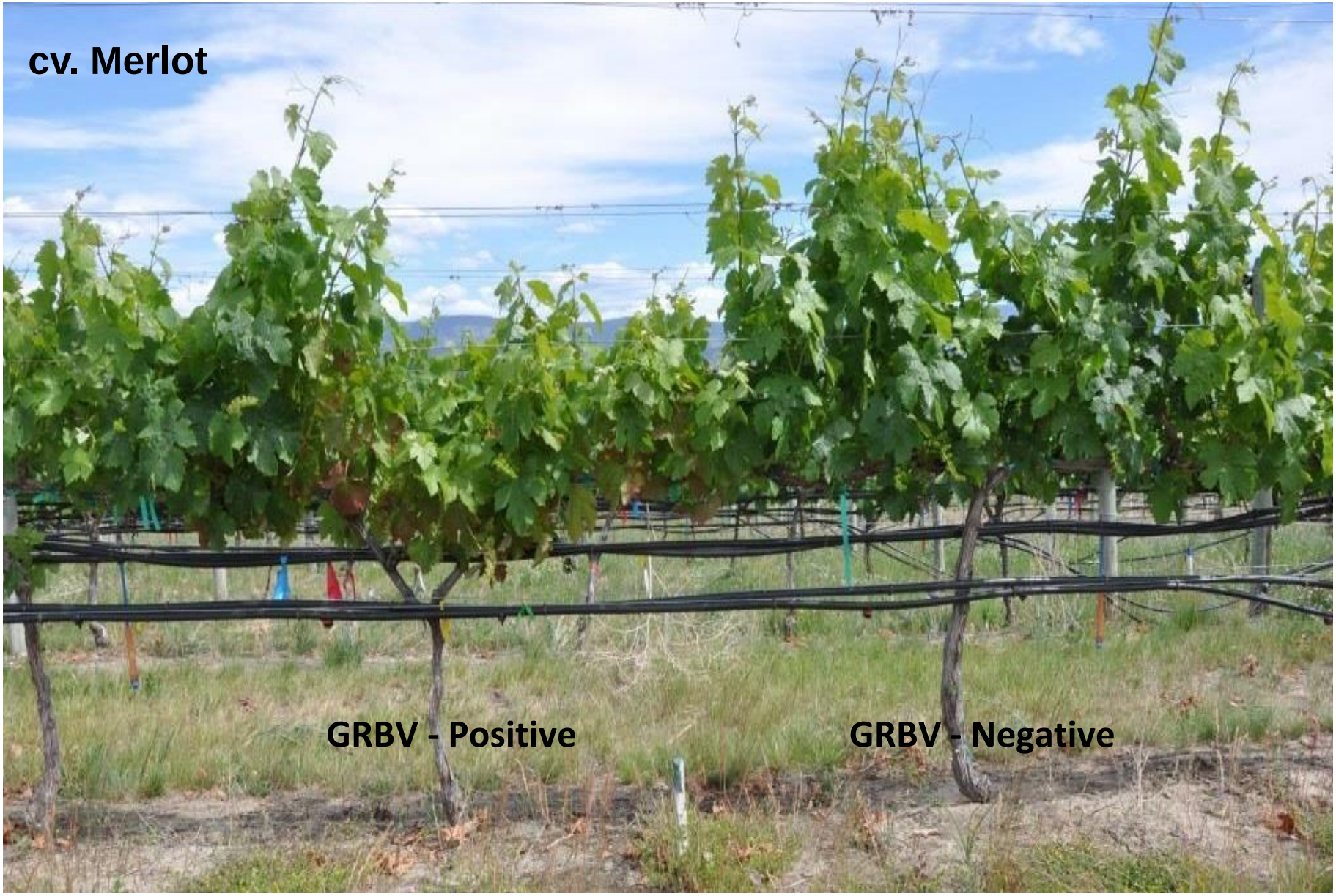
Cultivar	# of Blocks	# of Random Composite Samples Tested	# of GRBV Positives
Whites			
Chardonnay	9	180	1
Gewurztraminer	3	60	0
Pinot gris	7	140	0
Riesling	6	120	0
Sauvignon Blanc	2	40	0
Pinot Blanc	1	20	0
Viognier	1	20	0
Muscat	3	60	13 (1)
Pinot Meunier	1	20	0
Reds			
Cabernet Franc	8	160	8 (1)
Cabernet Sauvignon	3	50	0
Merlot	12	220	0
Pinot Noir	15	285	0
Syrah	2	40	5 (1)
Zinfandel	2	40	1
Petit Verdot	1	20	1
Total	**76**	**1475**	**29 (5)**

2015

- **76 vineyard blocks**
- **Vines: 2 to 8yrs of age**
- **1475 Random composite samples**
  - **Represent >7000 vines**
- **Higher incidence in young vines reflects recent appearance**

Poojari et al. 2017. CJPP: ID TCJP-2016-0192

Grapevine red blotch virus



Grapevine leafroll disease and Grapevine red blotch virus cause similar symptoms and damage, but there are important differences.

Grapevine leafroll disease consists of several unrelated RNA viruses.

GLRaV-1,3,4,5,6,10	Ampelovirus	mealybug and soft scale vectors
GLRaV-2	Closterovirus	no known vector
GLRaV-7	Velarivirus (?)	parasitic dodder (*Cuscuta* sp.)

Grapevine red blotch virus is a Geminivirus (DNA). Geminiviruses are vectored by phloem-feeding homopteran insects such as whiteflies, leafhoppers, treehoppers, etc.

Introduction of grapevine viruses into a vineyard (primary infection) occurs through:

- Planting of infected nursery material
- Infected interplants
- Infected scions when top-working
- Movement of infected leaves and vectors on equipment
- Long-distance movement of infectious vector

Vector species are responsible for secondary spread throughout the vineyard

Prevention of primary infections.

1) Certified virus-free planting material.

- GLRaV's and other grapevine viruses were deregulated after 1995
- Testing and certification of nursery material
- Progress toward a National Clean Plant Program for grapes

2) Inspection and removal of infected vines

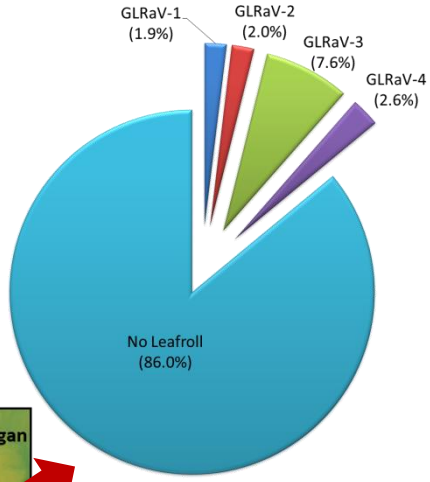
3) Restrictions on movement of equipment and workers.

Photo credit: OxboInternational

Incidence of GLRaVs in BC

Roguing of infected vines or interplants will work in the north Okanagan where the incidence of GLRaV is low and there are few vectors.

A co-ordinated area-wide approach with removal of entire blocks or vineyards and establishment of buffer zones is required in some areas where the incidence is high and spread is rapid.



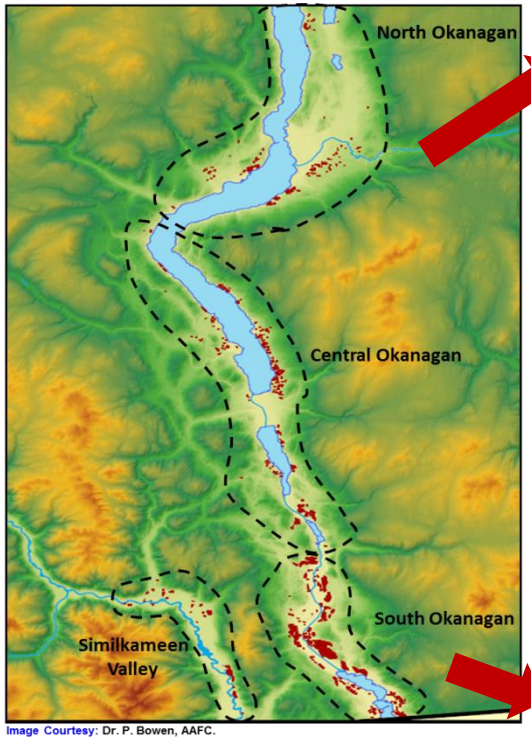
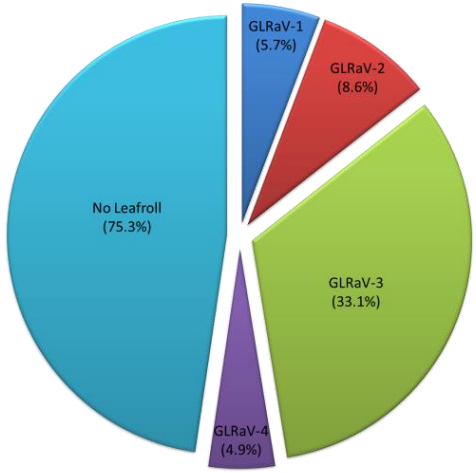


Image Courtesy: Dr. P. Bowen, AAFC.

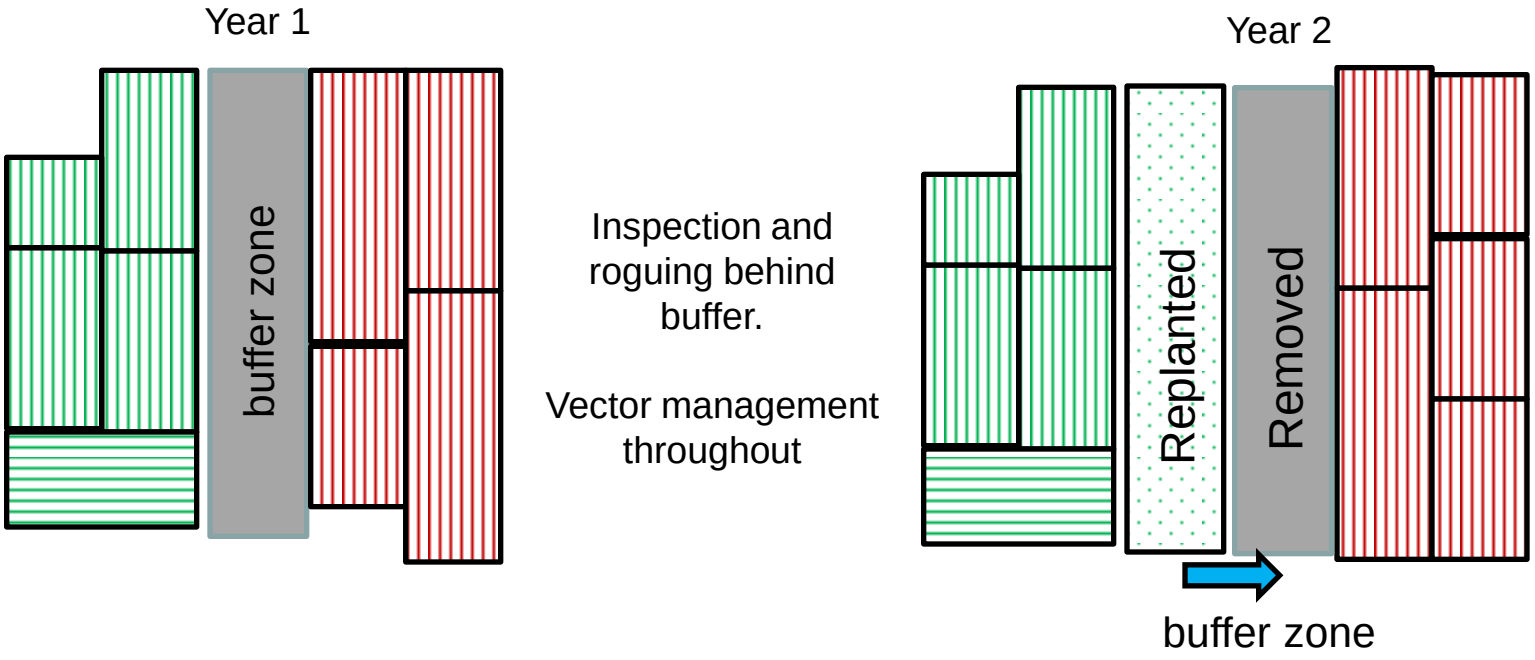
Cottony maple scale tended by ants



Roguing or replanting of individual blocks is not practical in some areas with very high rates of infection, large vector populations, and resulting rapid rates of spread.

Area-wide management program for GLRaV:

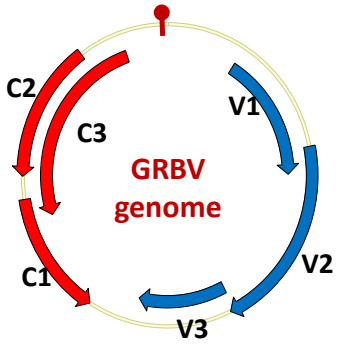
- Requires the cooperation of many producers.
- Value of the crop and temporary loss of revenue.
- How wide does the sliding buffer zone need to be?
- Vigorous hybrids are asymptomatic and largely unaffected.
- Effective control of vectors a necessity.



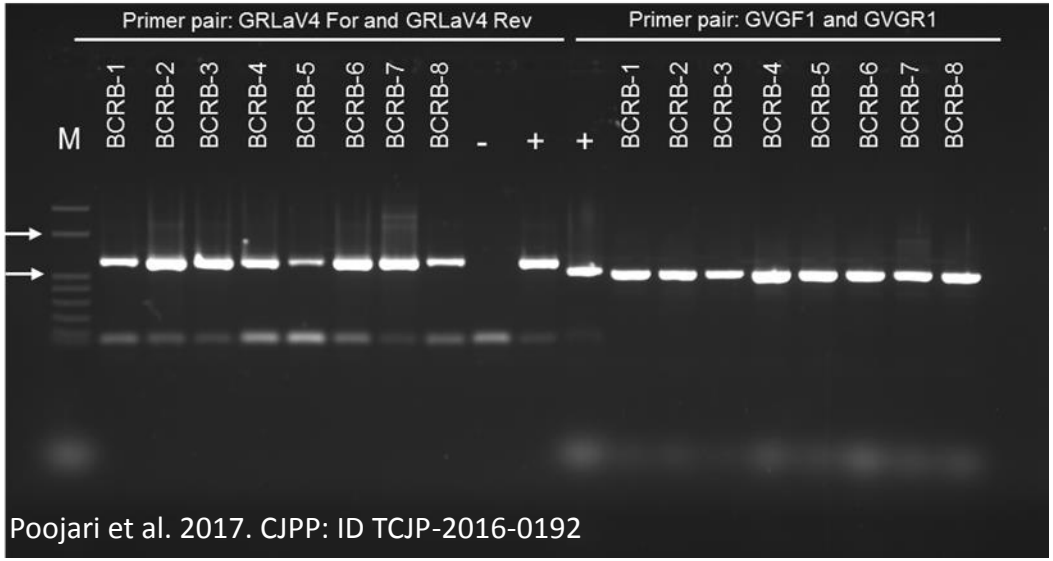
Testing a large number of vines is a necessary component of an effective virus control program.

Leaf with petiole

DNA Extraction



PCR



Poojari et al. 2017. CJPP: ID TCJP-2016-0192

PCR based Diagnostics for GRBV

Secondary spread of virus by insect vectors

Soft Scale Vectors of GLRD

Virus	
GLRaV-1	**Cottony vine scale** (*Pulvinaria vitis)* **European fruit lecanium scale** (*Parthenolecanium corni)* **Cottony maple scale** (*Neopulvinaria innumerabilis)*
GLRaV-3	**Cottony vine scale** (*Pulvinaria vitis)* **Cottony maple scale** (*Neopulvinaria innumerabilis)* **European fruit lecanium scale** (*Parthenolecanium corni)* **Brown soft scale** (*Coccus hesperidum)* Long brown scale (*Coccus longulus)* Nigra scale (*Parasaissetia nigra)* Fig wax scale (*Ceroplastes rusci)*
GLRaV-4 and its strains -5, -6, and -9	Fig wax scale (*Ceroplastes rusci)*

Cottony maple scale tended by ants

Mealybug Vectors of GLRD

Virus	Vectors
GLRaV-1	Bohemian mealybug (*Heliococcus bohemicus*)
	Apple mealybug (*Phenacoccus aceris*)
	Obscure mealybug (*Pseudococcus viburni*)
	Citrophilous mealybug (*Pseudococcus calceolariae*)
	Grape mealybug (*Pseudococcus maritimus*)
	Comstock mealybug (*Pseudococcus comstocki)*
GLRaV-3	Bohemian mealybug (*Heliococcus bohemicus*)
	Vine mealybug (*Planococcus ficus*)
	Citrus mealybug (*Planococcus citri*)
	Longtailed mealybug (*Pseudococcus longispinus*)
	Citrophilous mealybug (*Pseudococcus calceolariae*)
	Grape mealybug (*Pseudococcus maritimus*)
	Obscure mealybug (*Pseudococcus viburni*)
	Comstock mealybug (*Pseudococcus comstocki*)
	Apple mealybug (*Phenacoccus aceris*)
GLRaV-4 and its strains -5, -6, and -9	
	Vine mealybug (*Planococcus ficus*)
	Longtailed mealybug (*Pseudococcus longispinus*)
	Apple mealybug (*Phenacoccus aceris*)

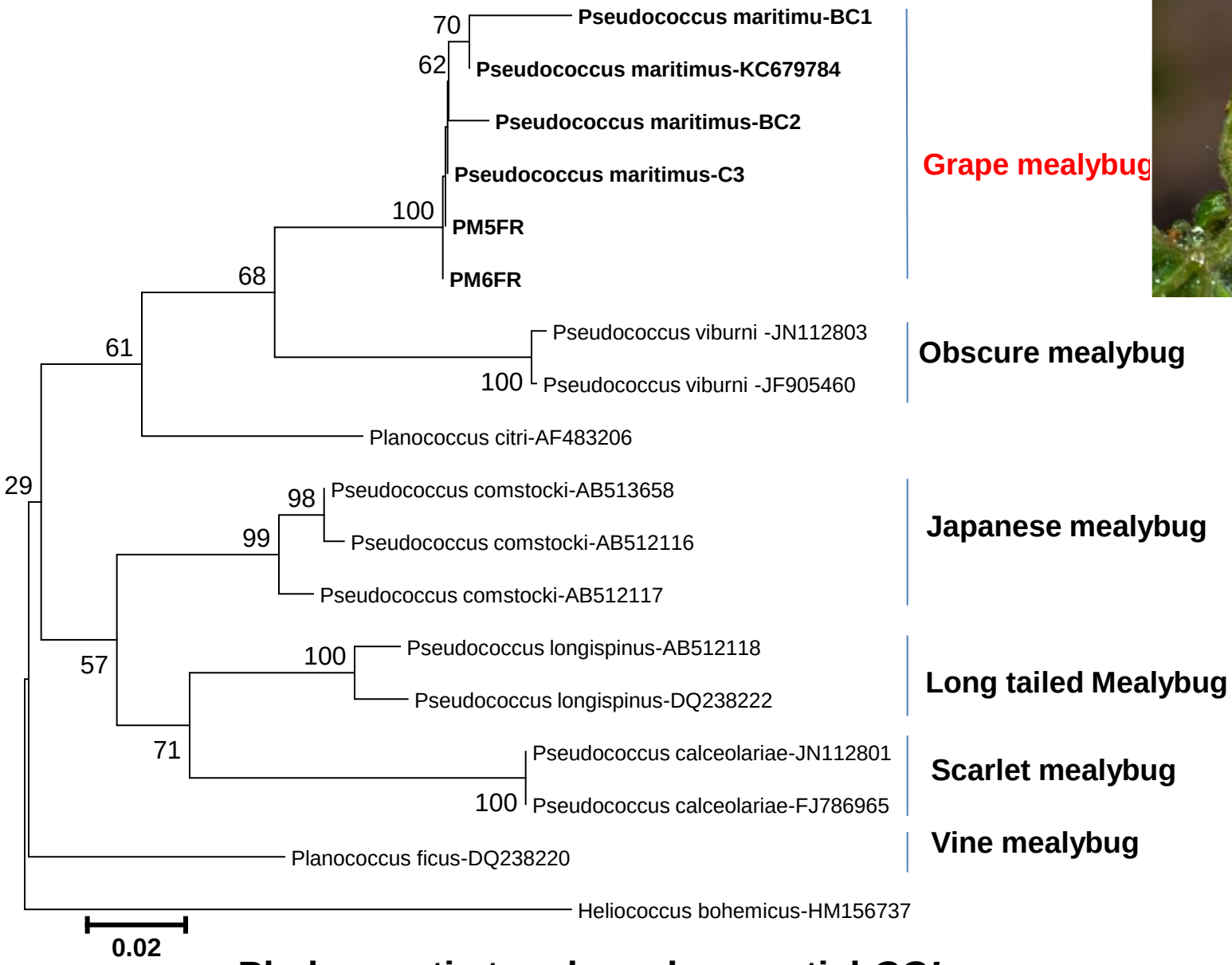
Grape mealybug (*Pseudococcus maritimus*)

DNA barcoding results for mealybug and soft scale specimens collected from vineyards in BC.

Species	Region (number of blocks)				% identity
	North Okanagan (6)	Central Okanagan (9)	South Okanagan (46)	Similkameen Valley (10)	
Mealybug					
Pseudococcus maritimus	0	3	35	0	99.53-99.53
Soft scale					
Parthenolecanium corni	7	21	57	19	99.21-99.22
Pulvinaria sp.	3	21	30	45	82.00-95.00

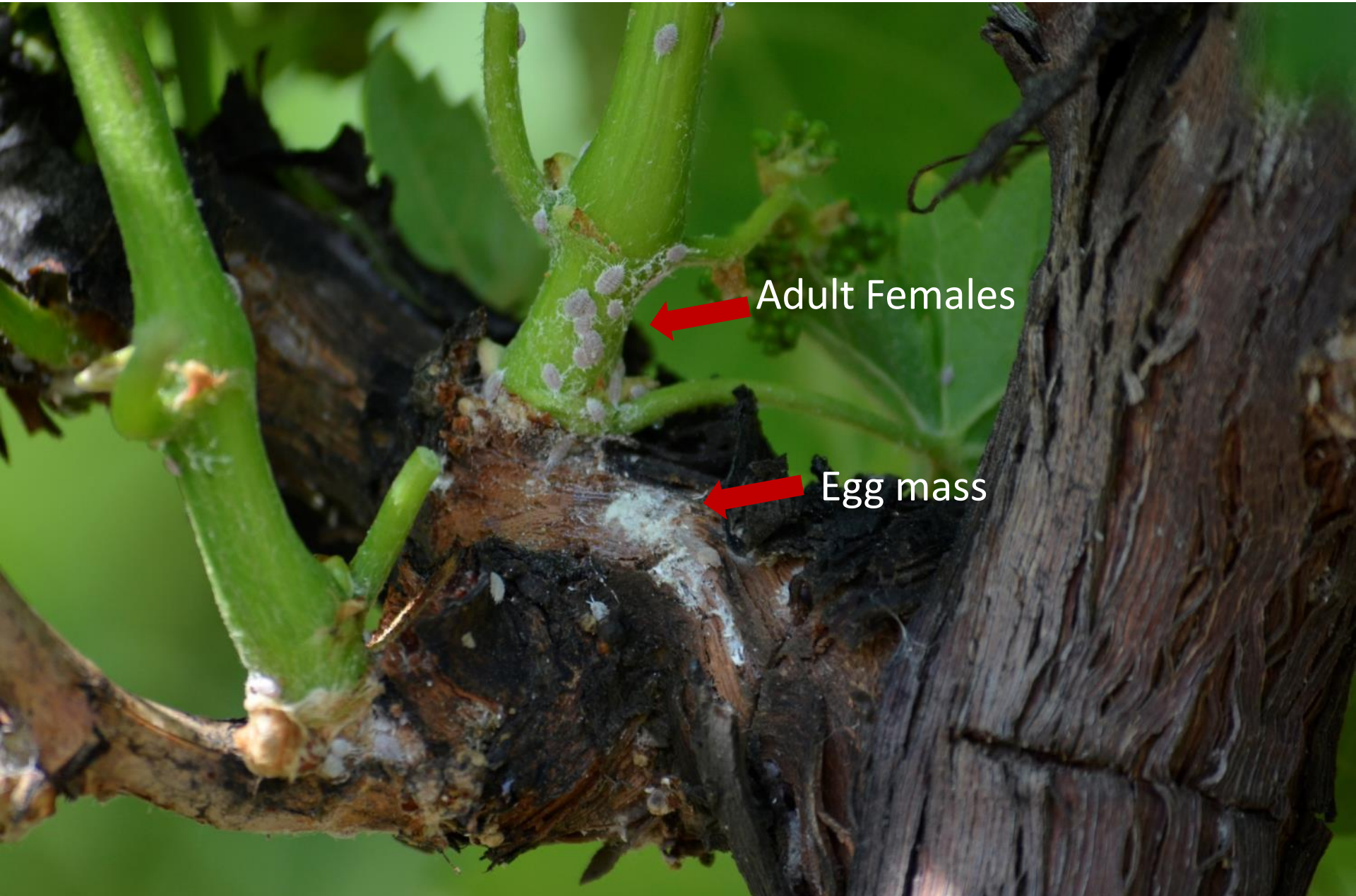
Other species of soft scale possibly present in BC vineyards. Genetic sequences for the cottony maple scale and cottony vine scale are not available in the database. Further morphological and molecular confirmations are necessary

Grape mealybug (*Pseudococcus maritimus*)



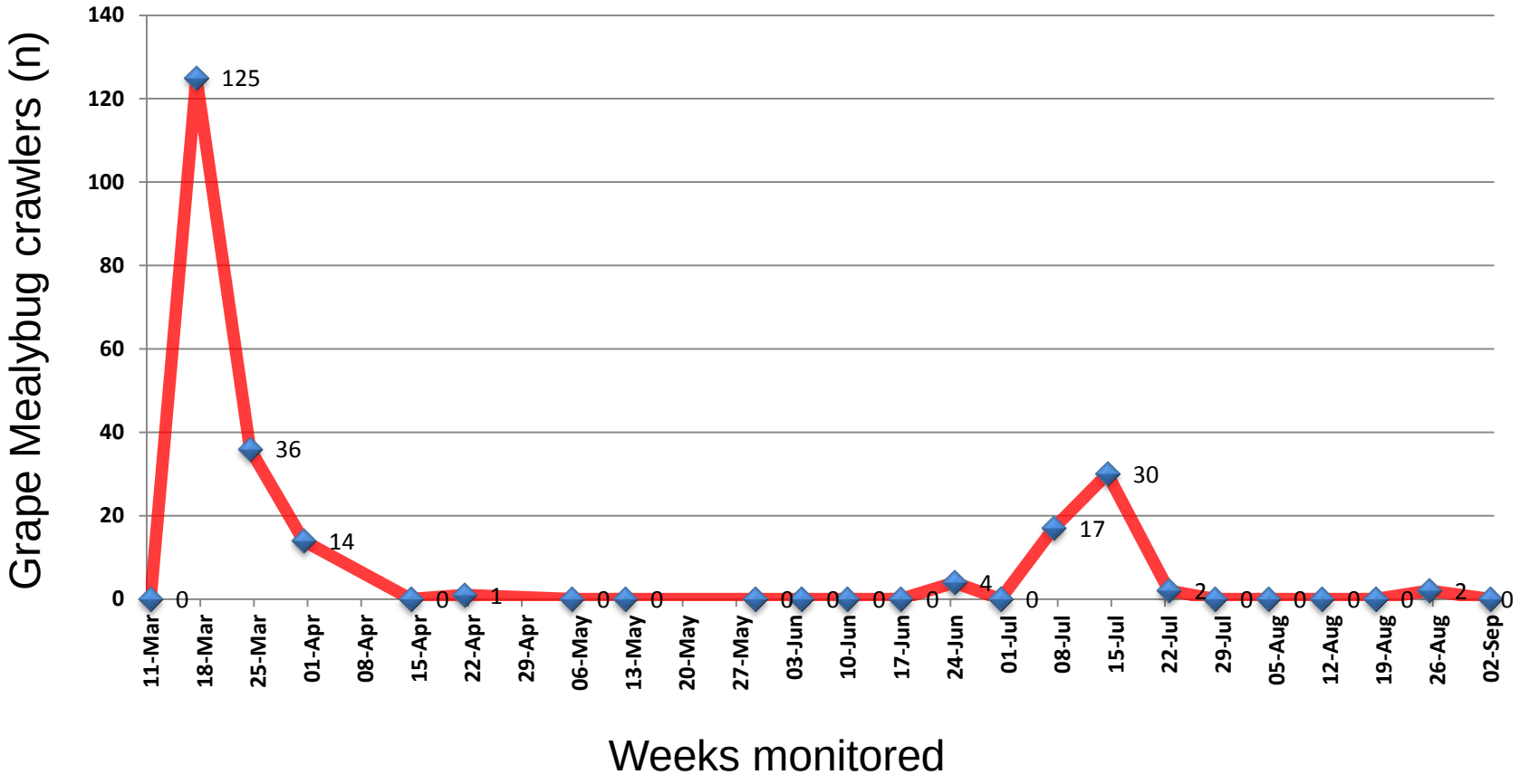
Phylogenetic tree based on partial *COI* gene

Grape mealybug (*Pseudococcus maritimus*)



Dynamics of grape mealybug crawler populations

Year: 2015



Two generations in a growing season

Potential GLRaVs vectors in BC

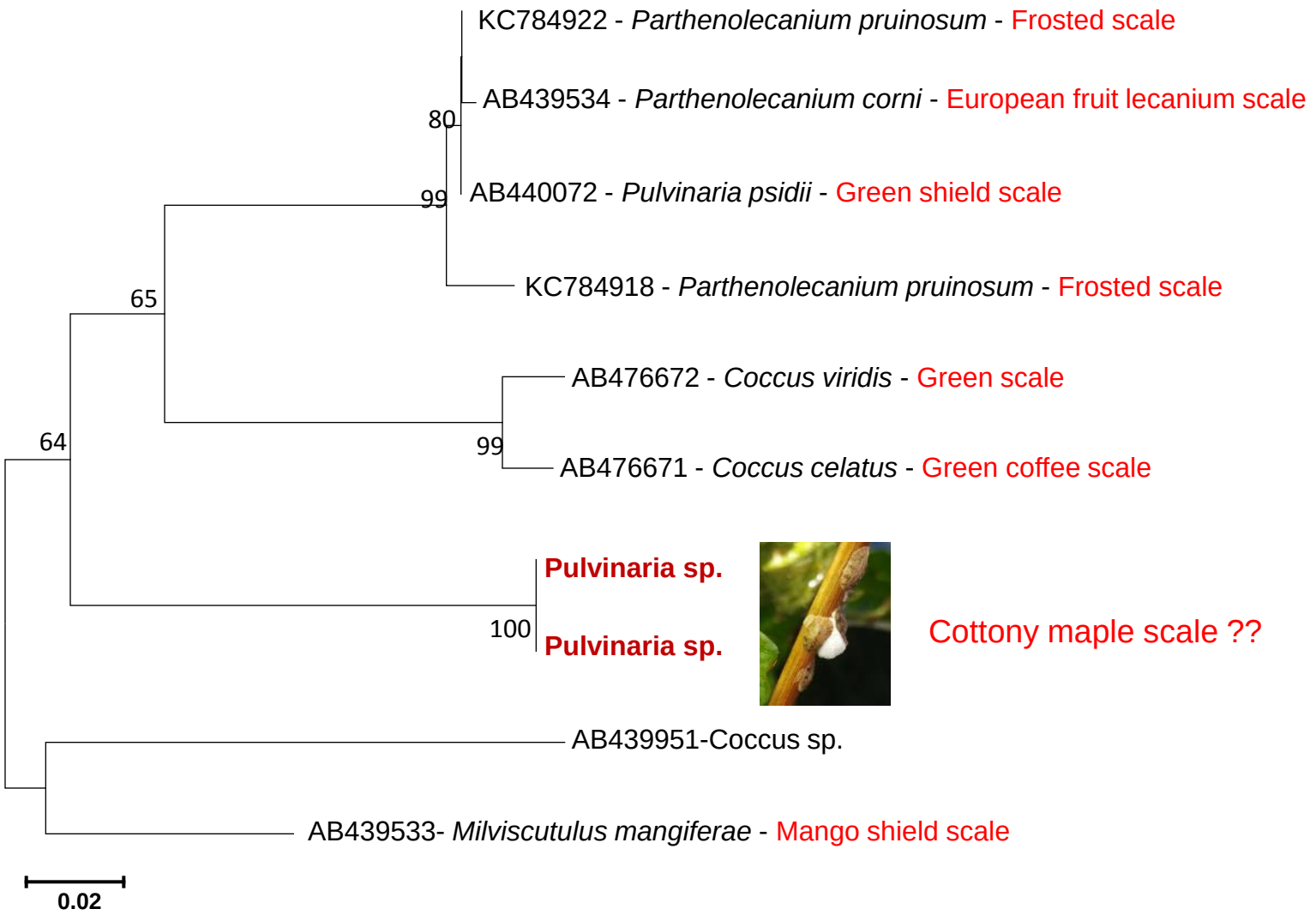
European fruit lecanium scale

Photos: S. Poojari (SuRDC)

Cottony maple scale

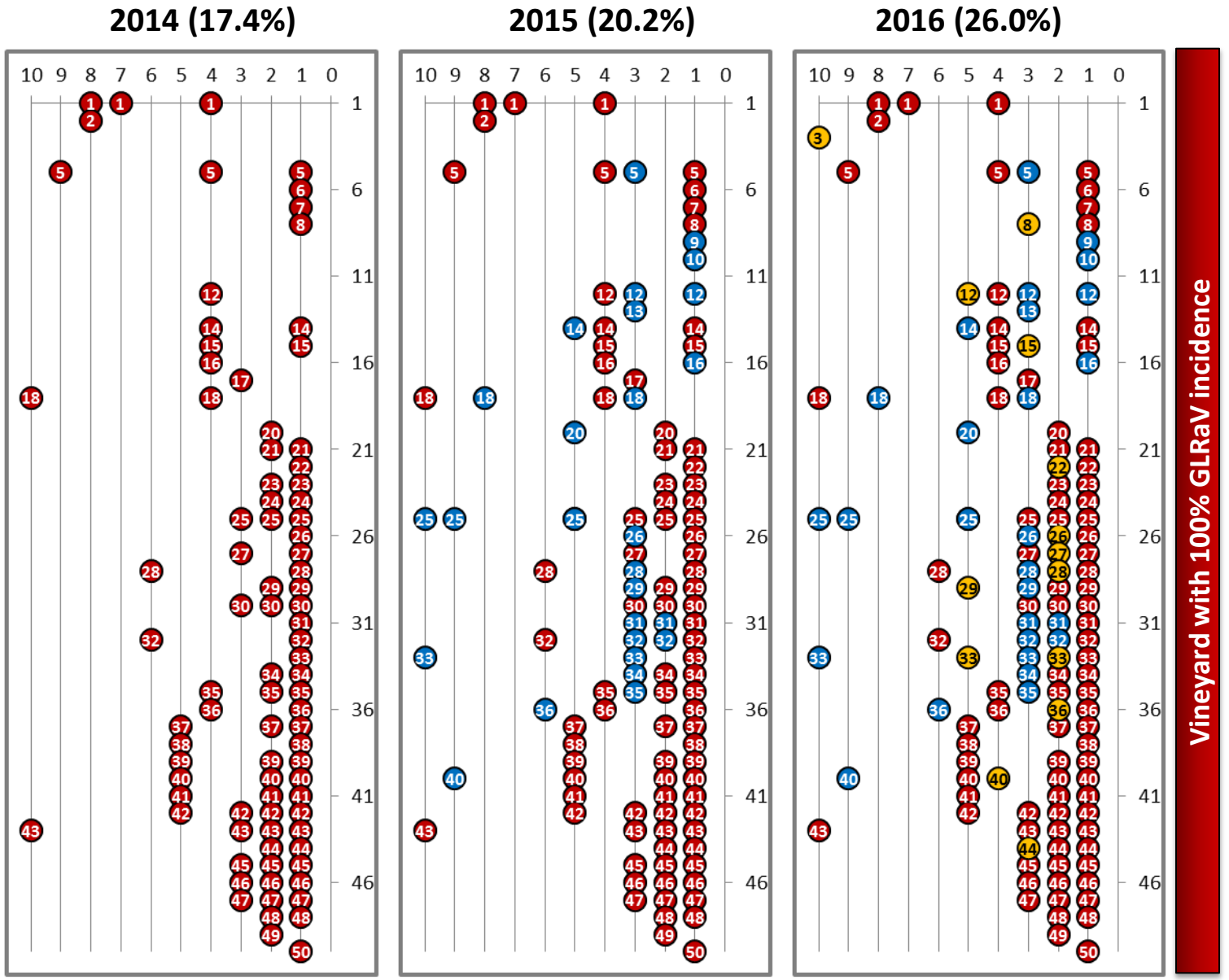
Photo: J.R. Úrbez-Torres (SuRDC)

Pulvinaria sp. : partial *COI* gene

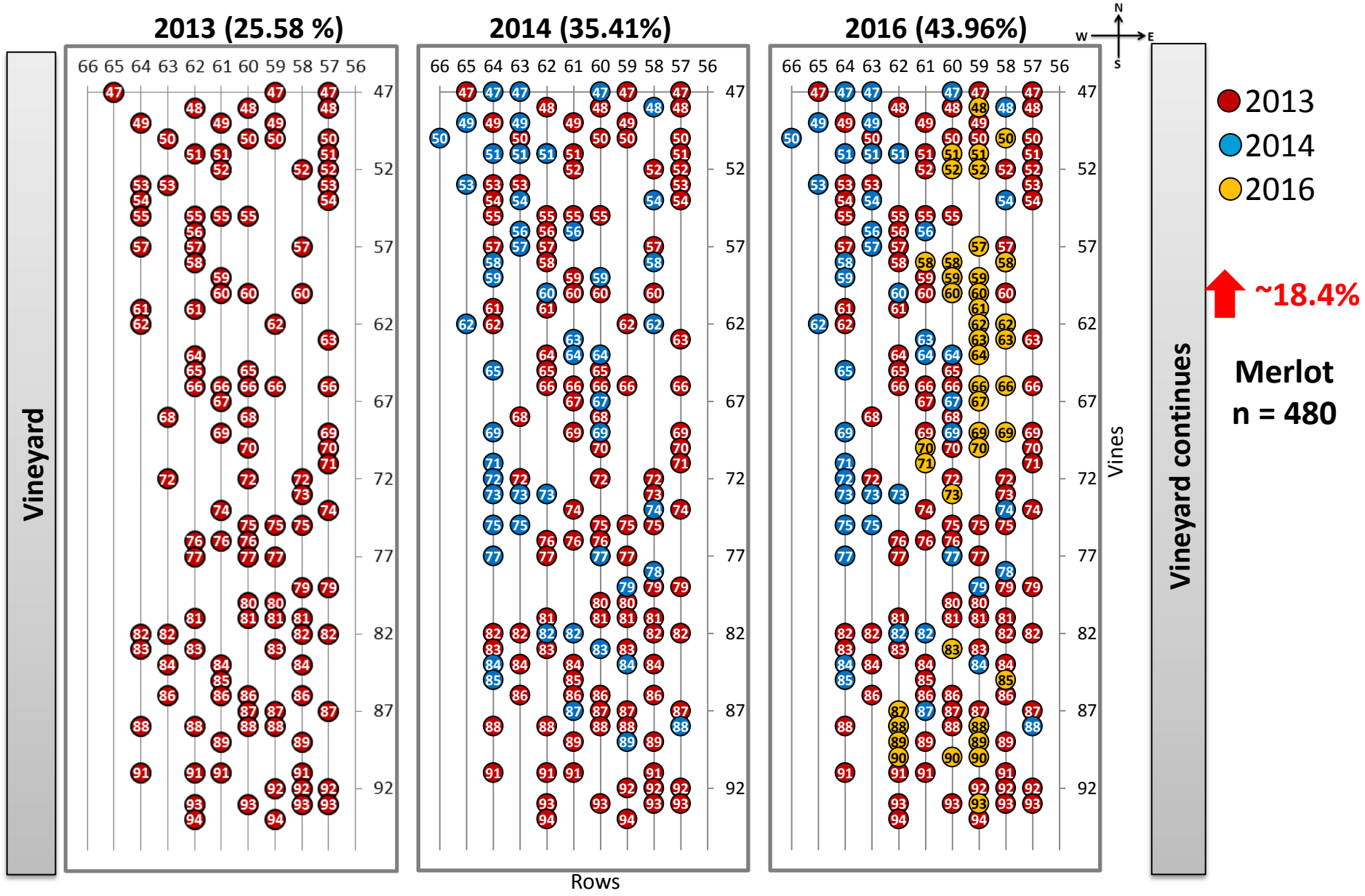


Further morphological and molecular confirmations are necessary

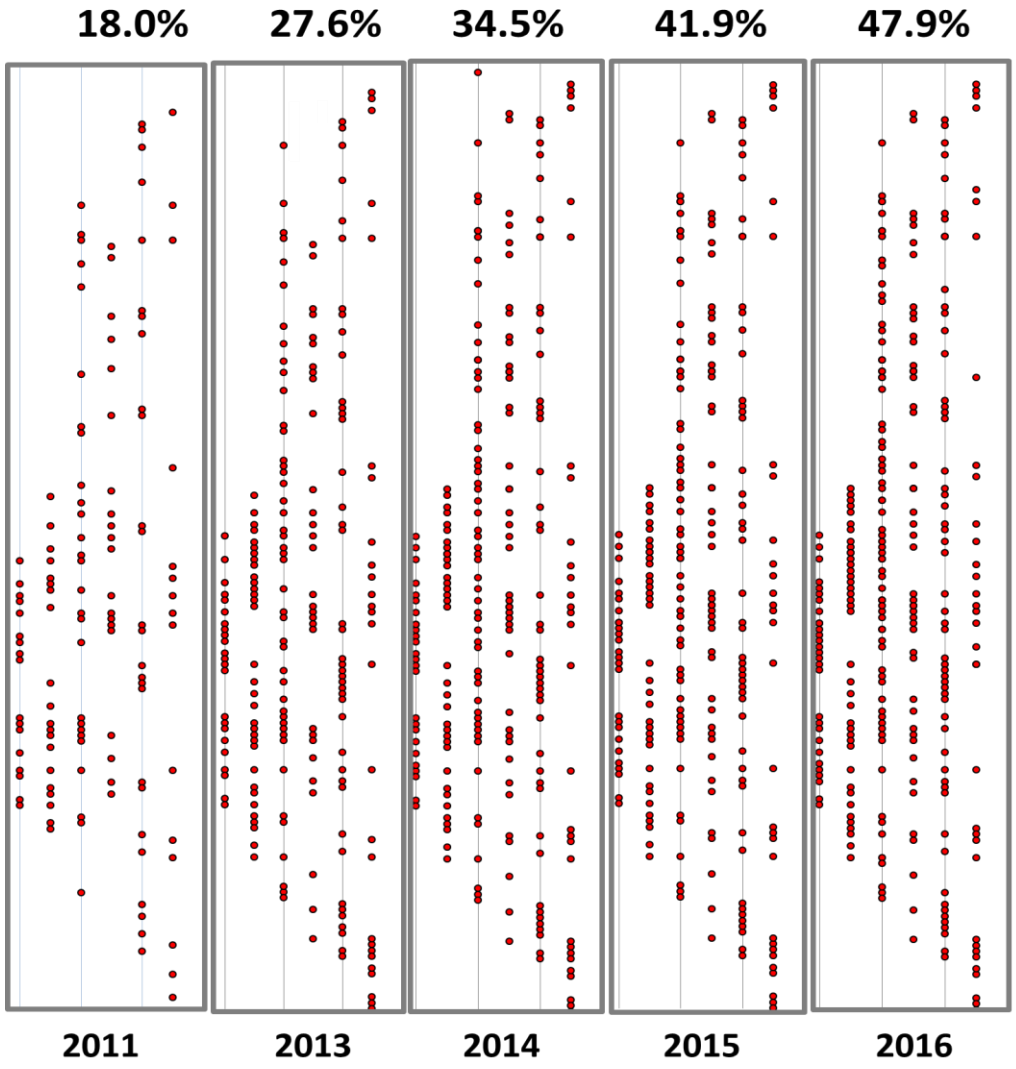
Spread of grapevine leafroll disease : Block A



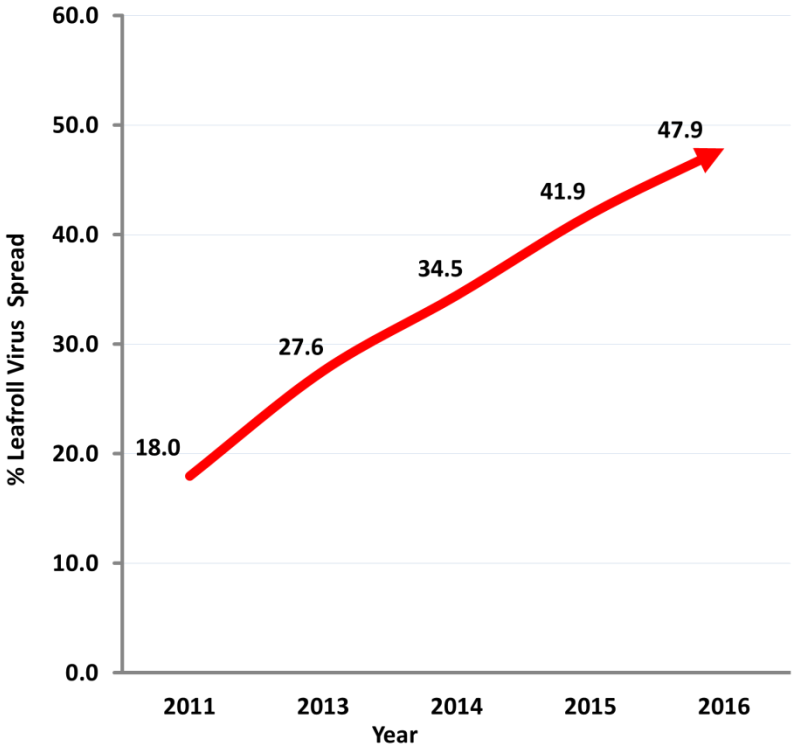
Spread of grapevine leafroll disease : Block B



Spread of grapevine leafroll disease : Block C



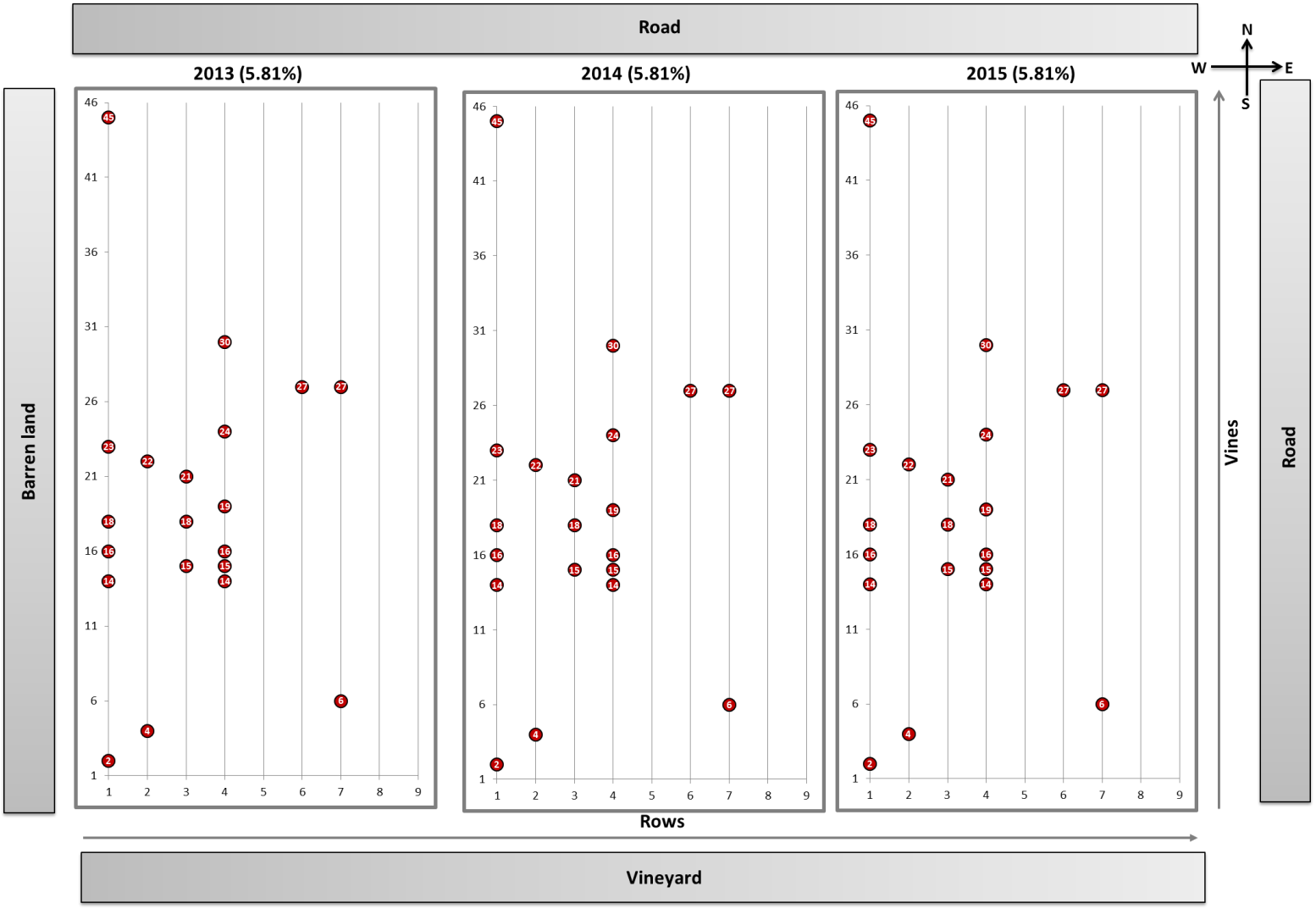
cv. Cabernet Franc, n = 689



~30% increase in 5 years

Spread of Grapevine leafroll disease : Block D

No vector incidence No Leafroll spread



Vectors of Grapevine red blotch virus

- Secondary spread of GRBaV was recorded in California

"The incidence of diseased plants increased by 1-2% annually"

Cieniewicz E.J. et al. 2017. Spatiotemporal spread of grapevine red blotch-associated virus in a California vineyard, *Virus Research.* http://dx.doi.org/10.1016/j.virusres.2017.03.020

- Alfalfa treehopper (*Spissistilus festinus* Say) (Bahder et al. 2016) – under controlled laboratory conditions
- GRBaV found in field-collected insect species belonging to the Membracidae (*Ceresa festina*), Cicadellidae (*Colladonus reductus* and *Osbornellus* sp.) and Cixiidae (unknown species) (Fuchs et al. 2016)
- No sign (yet) of GRBV spread in a BC vineyard
- Studies of insect vector capabilities are underway in BC

Bahder: Three cornered alfalfa treehopper

Chemical control of grapevine vectors

Insecticides can be effective due to the prolonged feeding times required for virus acquisition and transmission.

Dormant sprays of lime sulphur or oil against overwintering eggs and nymphs.

Foliar sprays (crawlers/nymphs)

- Insecticidal soap – mealybug, scale
- Movento (spirotetramat) – mealybug, scale
- Malathion – mealybug, scale
- Clutch (clothianidin) - mealybug

Chemical control of grapevine vectors

- Sprays for other grape pests can impact vector populations. In BC, large outbreaks of mealybug and scale recorded 1-2 years after sprays of neonicotinoids applied for the control of leafhoppers, presumably due to the lasting negative effects on mealybug and scale parasitoids. These vectors uncommon in organic vineyards.
- The situation in Ontario vineyards is likely different.
- Alternative, effective and selective spray materials would be of benefit.

Summary

- We have shown a significant increase in the amount of GLRaV since the previous survey (1996), and a low incidence of GRBV in BC vineyards.
- GLRaV-3 is predominant and widespread in certain areas of the south Okanagan where mapping studies have shown high yearly rates of increase.
- Vectors of GRBV recorded, but no documented spread as yet.
- At least 3 species of GLRaV-3 vectors present in BC vineyards; populations vary by region.
- Effective virus management requires **technology transfer**, **virus-free planting material**, **roguing**, and **vector management**.
- A co-ordinated area-wide approach would be of benefit where virus and vector levels are high.

Thank you

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