Managing Viruses in Washington Vineyards

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Thanks to:

Wine Research Advisory Committee of the Washington Wine Commission

Washington Association of Wine Grape Growers

WSU-CAHNRS Agricultural Research Center
Washington Grape and Wine Research Program
WSU Viticulture & Enology Program
CAHNRS Agricultural Extension
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Viticulture Consortium-West

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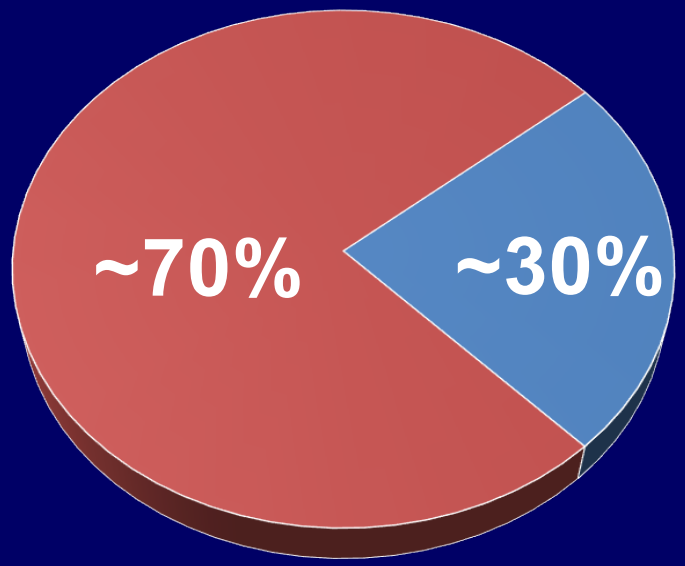
(2005-2016)

Other resources
$3,482,314



WRAC
$1,094,287

Altria - Chateau Ste. Michelle Wine Estates

Washington Wine Industry Foundation

Grape Virology Program

Aligned with industry's priorities

Grape and wine industry research priority

Management of viruses that impact vine health and fruit quality

Research on critical topics

- How many viruses are present in WA?
- Which ones are economically important ?
- How to manage virus diseases in vineyards ?

Translational Research

Strategic research
- Identification
- Genetic diversity
- Molecular biology
- Host-virus interactions

Applied research
- Impacts on:
  - vine health
  - fruit yield
  - fruit & wine quality
- Epidemiology
- Management

- **Make best use of the state-of-the-art technologies**
- **Collaborative multi-disciplinary teamwork**
- **Participatory approaches with stakeholders**

Industry-University partnerships

an opportunity to work together with grape growers for advancing sustainable growth of the grape and wine industry in Washington State

Grapevine is a ‘treasure trove’ of viruses

more than in any other perennial fruit crop !!!

AT LEAST 70 VIRUSES AND VIRUS-LIKE AGENTS REPORTED WORLDWIDE

Martelli GP. 2014. Directory of virus and virus-like diseases of the grapevine and their agents. *J. Plant Pathol.* 96 (Suppl. 1):1–136.

Do we have all of them or only a few?

- Conducted surveys in vineyards
- Optimized sampling strategies
- Tested samples using state-of-the-art diagnostic methods
  - PCR technology
  - Next-generation sequencing

Viruses documented in WA vineyards (as of 2015)

GLRaV-1
GLRaV-2
GLRaV-3
GLRaV-4
GLRaV-4 (strain -5)
GLRaV-4 (strain -9)

Leafroll complex

GRSPaV
GVA
GVB
GVE

Rugose wood complex

GFLV
TRSV

Fanleaf degeneration/decline

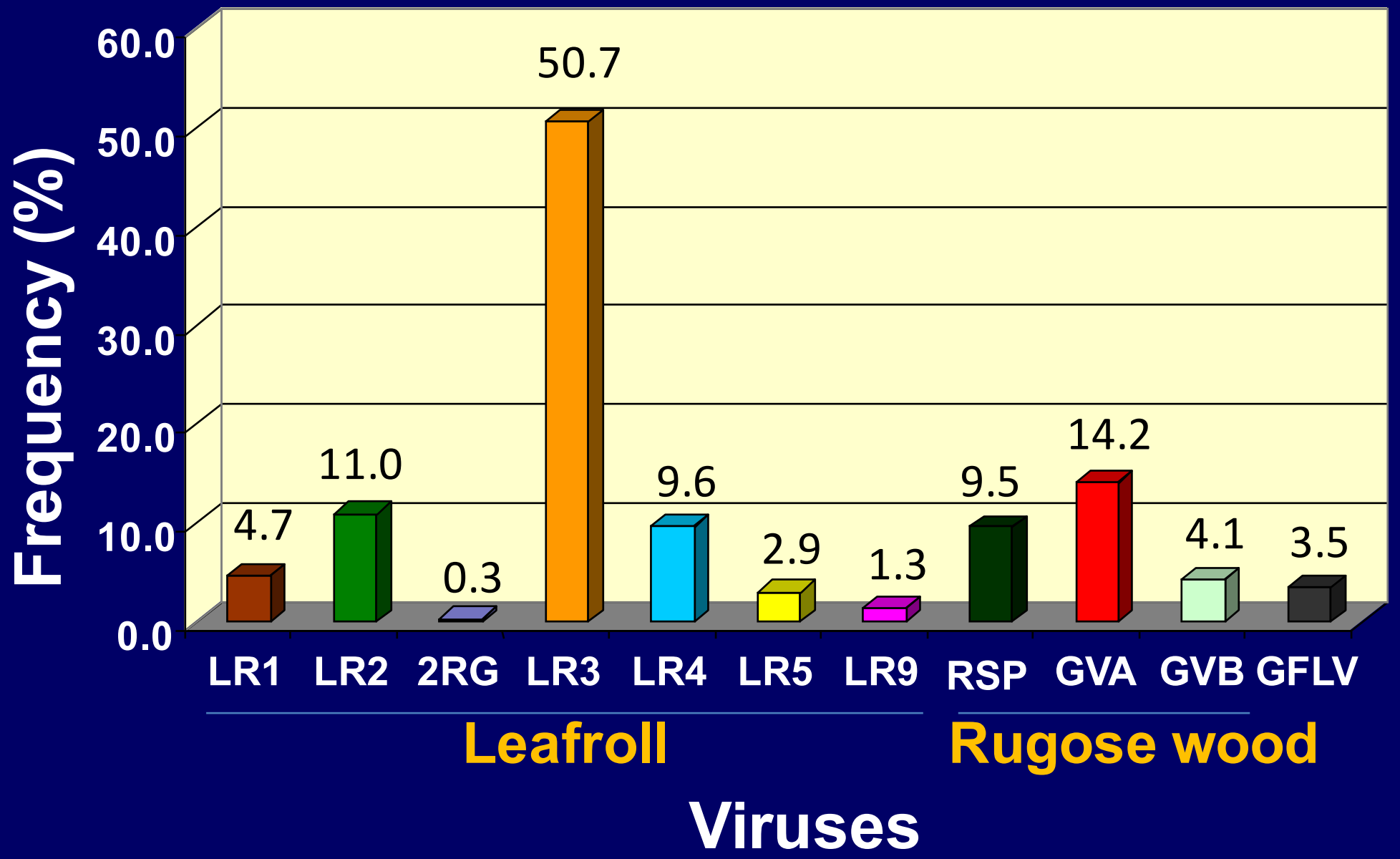
GFkV → **Fleck**

GSyV-1 → **Syrah decline??**

GRBaV → **Red blotch**

Other viruses?

Current status of grapevine viruses



Current status of virus diseases

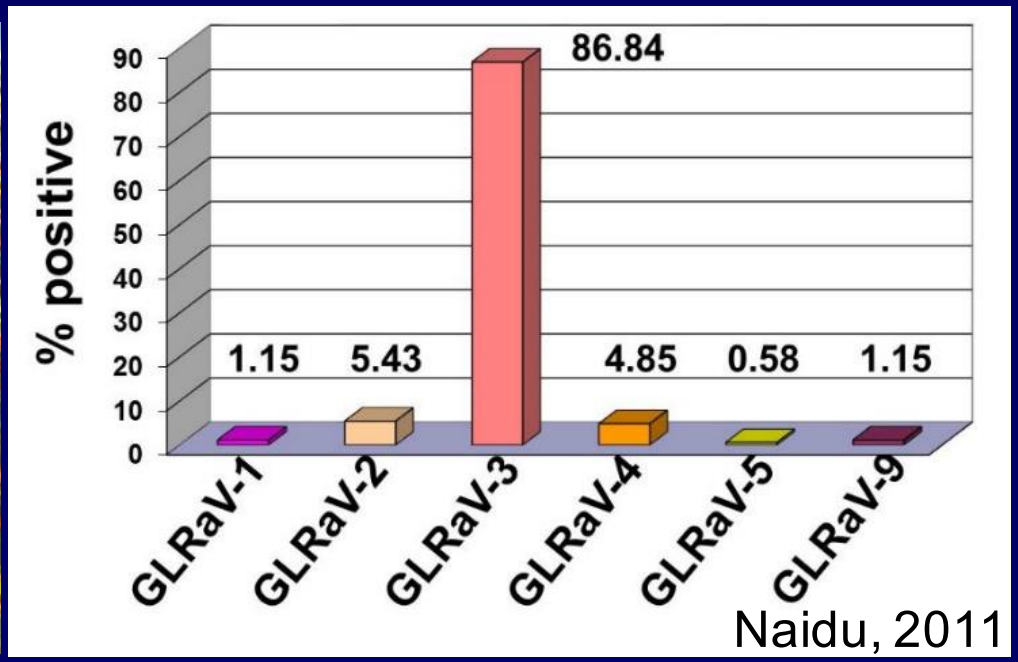
Established

- Leafroll

Emerging

- Red blotch
- Soil-borne

Grapevine leafroll disease



Virus	Cuttings	Mealybugs	Soft scales
GLRaV-1	Yes	Yes	Yes
GLRaV-2	Yes	No	No
GLRaV-3	Yes	Yes	Yes
GLRaV-4	Yes	Yes	Yes
GLRaV-7	Yes	No	No

Naidu et al., 2014. Plant Disease Plant Disease 98:1172-1185.

Wine grape cultivars respond differently to leafroll disease

Red blotch

Grapes

New grape disease reduces yields, quality

New disease has symptoms similar to grapevine leafroll disease.

by Naidu Rayapati and Melissa Hansen

A new disease that threatens the health of grapevines nationwide highlights the need for stronger clean plant campaigns. The disease, first reported in a Napa Valley vineyard in 2008, has been identified in infected vines in the top three U.S. grape growing regions of California, Washington, and New York, as well as a few other East Coast and southern states, and Canada.

Washington State University researchers are tentatively calling the new disease grapevine redleaf disease due to the red to purple color on leaves of red varieties. Symptoms range from red veins and blotches to total reddening of the leaves in some red grape cultivars. Veins on the leaf undersides can be pink or red, or the major veins can still be green.

California and New York scientists have dubbed it "grapevine red blotch disease," but WSU scientists preferred to use the broader term "redleaf" to cover the full spectrum of symptoms, so that growers pay attention to all suspicious red leaves.

The difference in symptoms could be due to own-rooted vines being used in Washington and grafted vines in California, New York, and other places.

Misidentified in vineyards?

The symptoms of redleaf disease in red-fruited cultivars may look similar to those produced by grapevine leafroll disease, but the new disease is distinct in several respects. Like leafroll, redleaf symptoms show up just after veraison and look very similar to leafroll, which could be why growers and university researchers never paid much attention to it in the vineyard. Many just thought it was leafroll virus. But the epidemiological aspects are quite distinct.

It's like a person having the flu or West Nile virus. Symptoms can be similar, but the nature of the virus and mode of its spread are very different, and the same treatment doesn't work for both. With leafroll disease, growers have focused on controlling grape mealybug and scale infestations to slow the spread of the disease. But with redleaf, a new vector is suspected, and a different set of management tactics is required.

How does it spread?

WSU bench graft tests showed that redleaf disease is graft-transmissible. Symptoms on the scion (virus-free Cabernet Franc) were similar to those observed in the source vines used as the rootstock. Based on the

Good Fruit Grower. April 1, 2013. Vol. 64 (No. 7), pages 44-46.

Grapevine leafroll disease

- Green veins
- Interveinal reddening
- Downward rolling of leaf margins

Grapevine red blotch disease

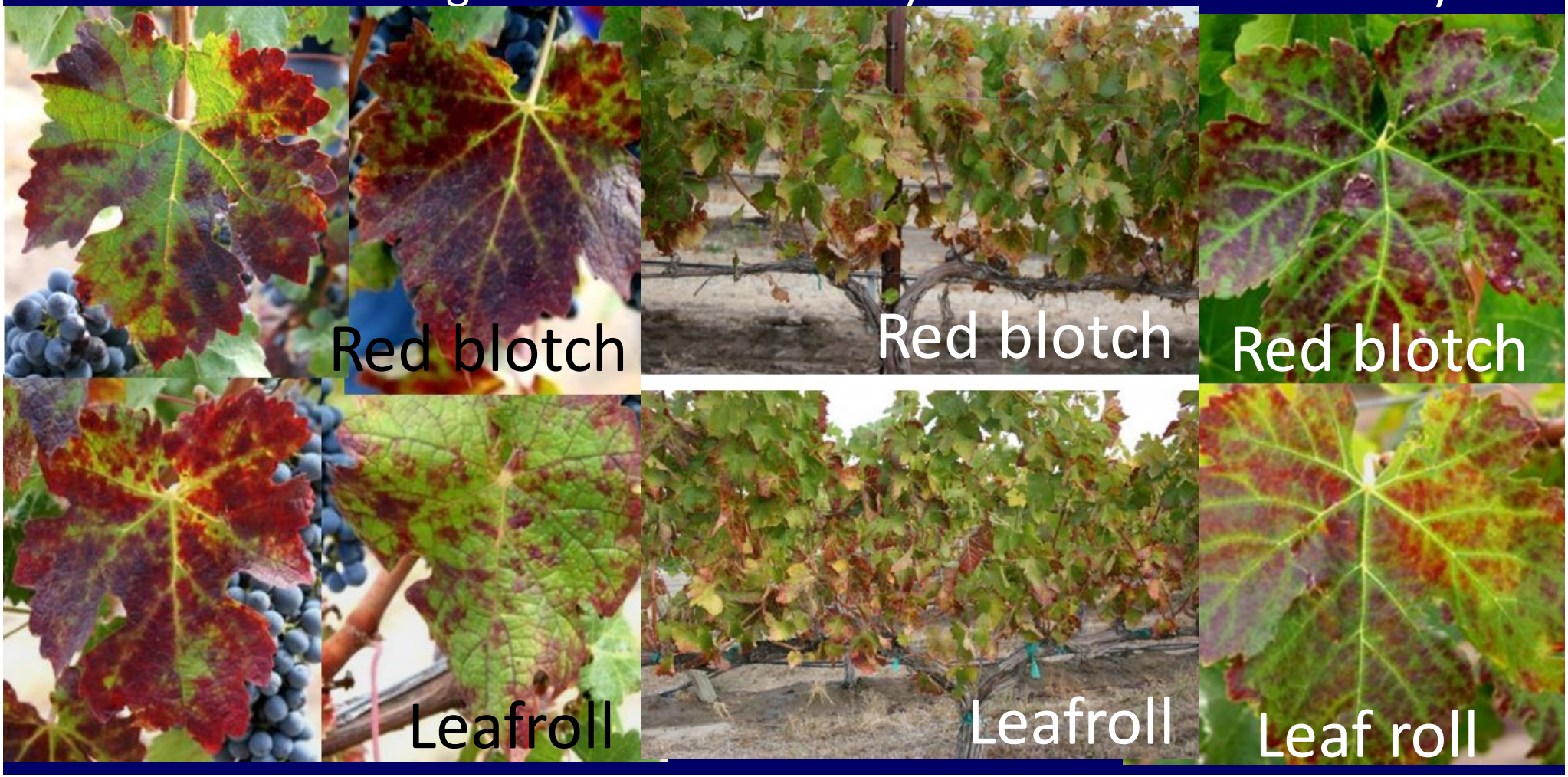
- Red veins
- Red blotches
- Interveinal reddening

Overlapping symptoms of leafroll and red blotch diseases in WA vineyards

Difficulty with symptom-based diagnosis

Have to use diagnostic assays for confirmation

cv. Cabernet Sauvignon | cv. Syrah | cv. Petit Syrah



http://www.washingtonwine.org

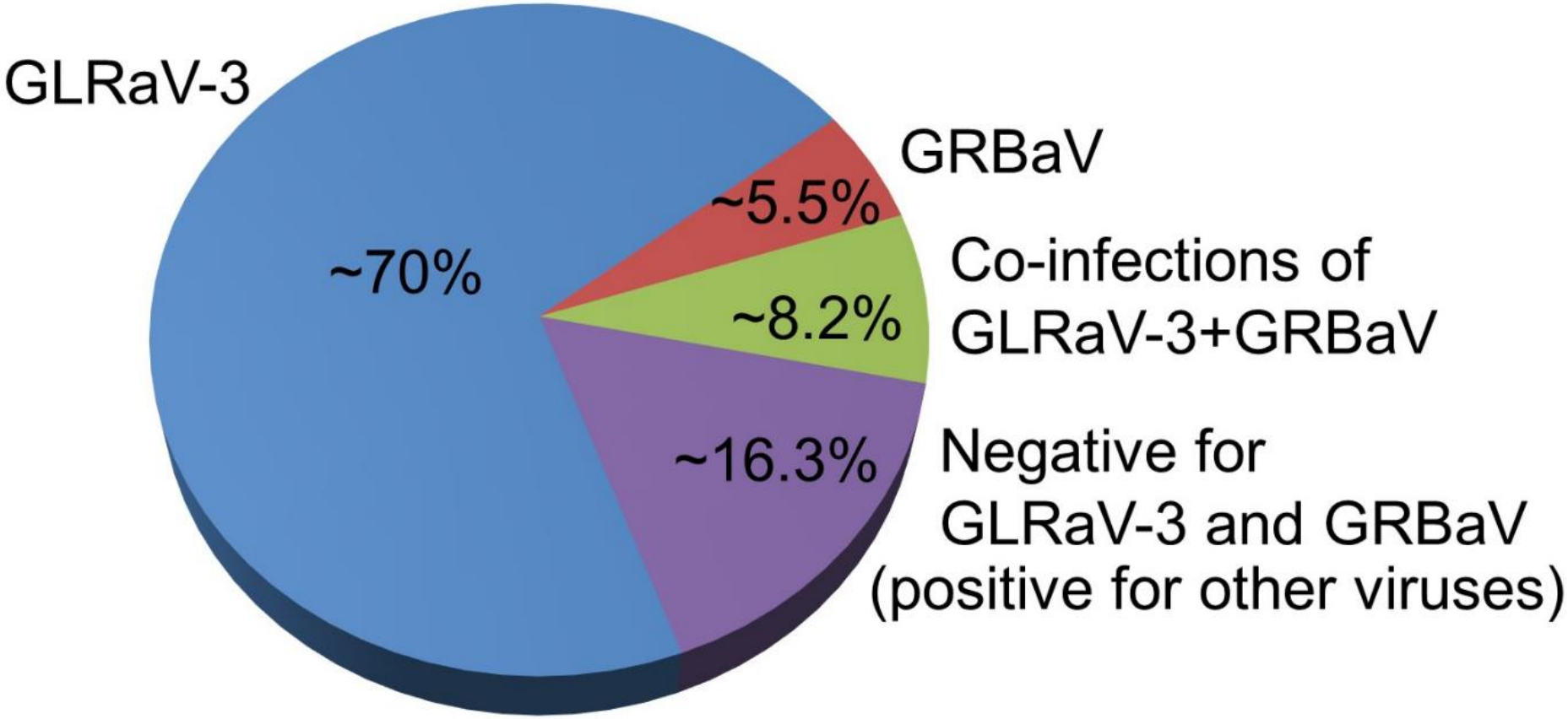
Leafroll (GLD) and red blotch (GRBD) in WA vineyards

- Survey conducted in 2014 & 2015 seasons in five AVAs.
- Red-berried cultivars: samples from grapevines exhibiting symptoms of GLD or GRBD and suspected for GLD- or GRBD-like symptoms.
- White-berried cultivars: random samples from grapevines due to the absence of visual symptoms of GLD and GRBD.

Adiputra et al., unpublished data

Leafroll and red blotch in WA vineyards

~1,500 samples during 2014 and 2015 seasons:
14 Red-fruited + 5 White-fruited
Tested by RT-PCR (GLRaV-3) and PCR (GRBaV)



Adiputra et al., unpublished data

Emerging problems

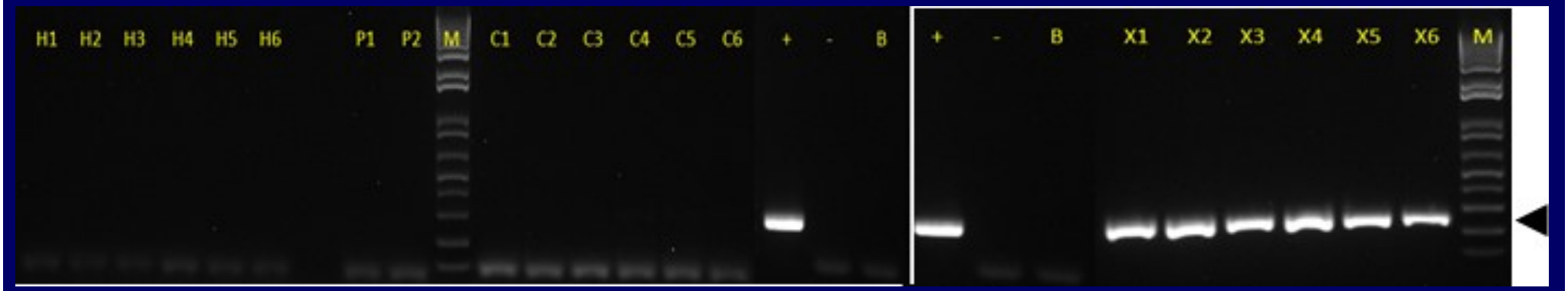
Pears – no obvious problem

Replaced with a wine grape cultivar – serious problem

An example of species jump – pears to wine grapes

Dagger nematode (*Xiphinema rivesi*) – a vector of TRSV ?

- Collected soil samples close to symptomatic vines.
- Isolated nematodes from soil samples.
- Separated individual species.
- Tested for the presence of TRSV using RT-PCR.
- Confirmed by sequence analysis.



H1-H6 : *Spiral nematode (*Helicotylenchus sp.)
P1,P2: *Lesion nematode (*Pratylenchus sp.)
C1-C6 : *Ring nematode (*Criconemoides sp.)
X1-X6 : *Dagger nematode (*Xiphinema rivesi)

+ : plant positive control
- : plant healthy control
B : Buffer control
M : 1kb+ DNA ladder
Amplification size- 254 bp

When do symptoms appear?

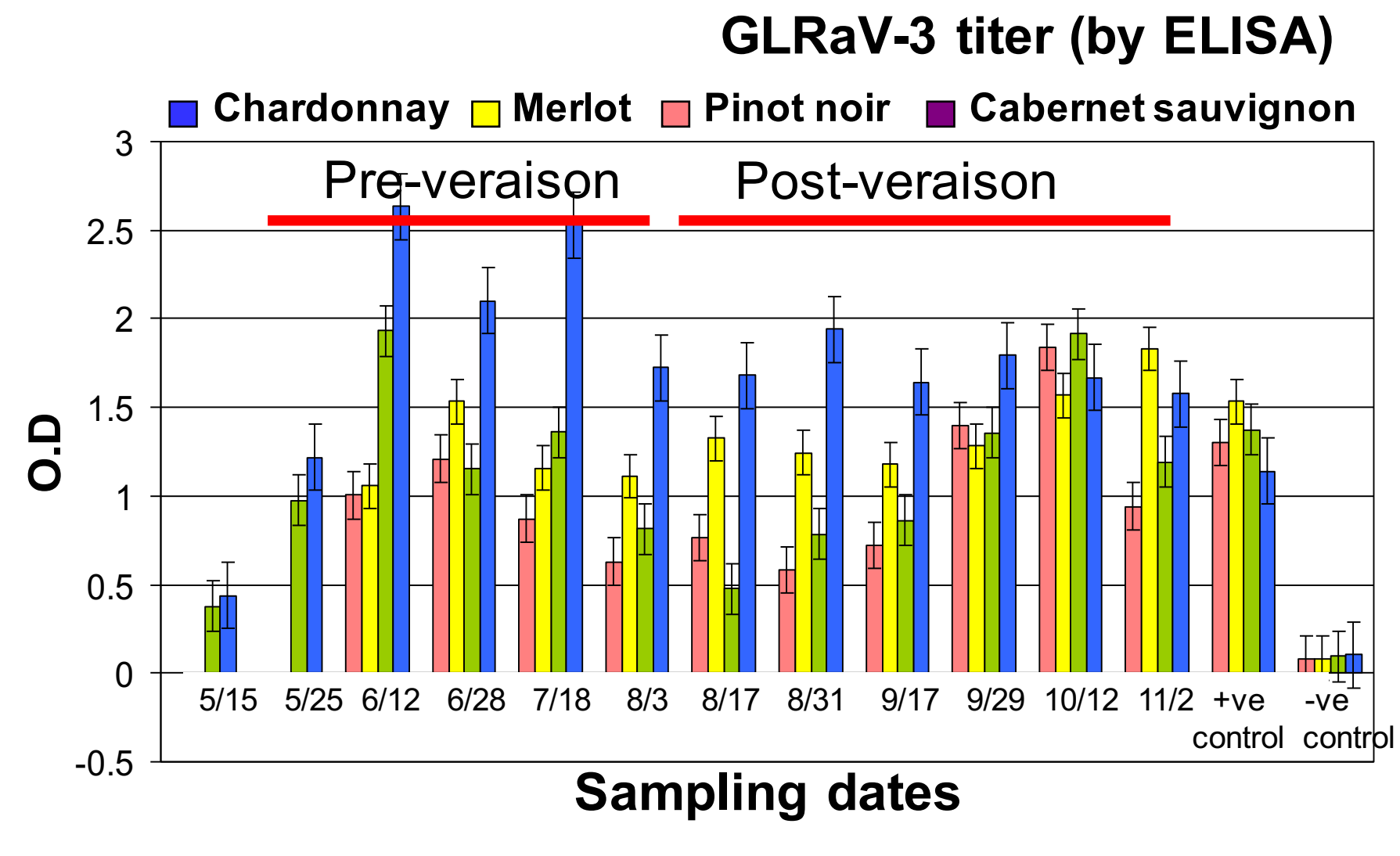
Leafroll

Pre-*Véraison*

Red blotch

Post-*Véraison*

Virus titer in wine grape cultivars



No correlation between presence of virus and time of symptom expression

2016: An unusual year for symptoms?

Leafroll

Red blotch

Current status of virus diseases

- Leafroll (GLRaV-3)
  - predominant & widely distributed
- Red blotch (GRBaV)
  - less predominant compared to GLRaV-3
- Soil-borne viruses (TRSV)
- Co-infections can occur

- Symptoms are similar, though not identical, in many red-berried cultivars
- Like leafroll, no apparent symptoms of red blotch in white-berried cultivars
- Symptom-based diagnosis is not reliable and diagnostic assays should be used for reliable ID

Take-home message

When can I test samples for viruses?

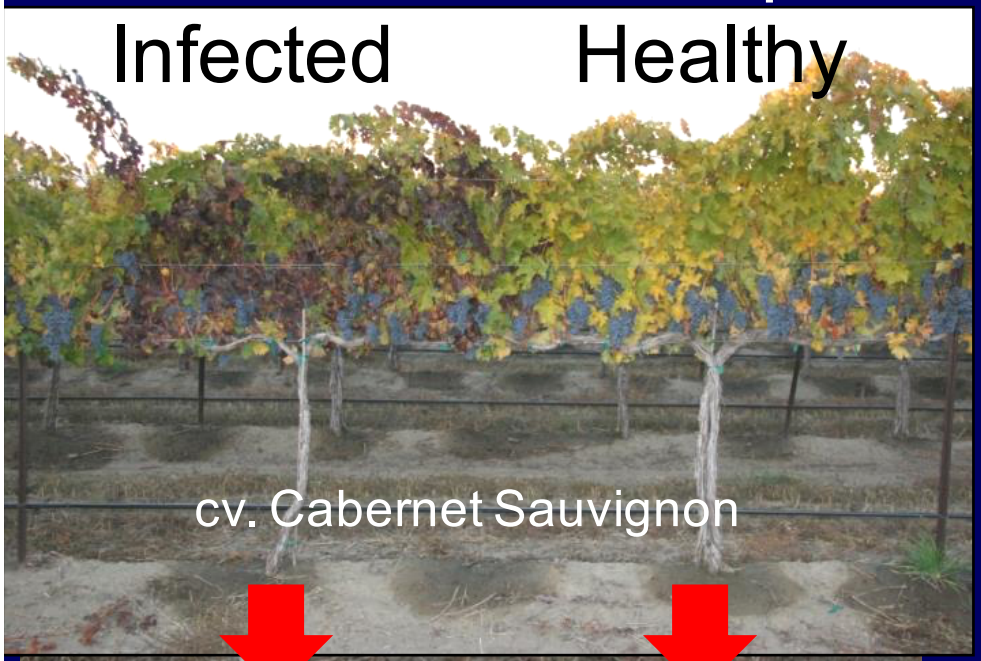
- Viruses distributed systemically in different parts (roots, canes, petiole and berry) of an infected grapevine.
- Viruses can be detected through out the season using leaf (petioles) and cane samples.
- Viruses can be detected using dormant canes in winter.

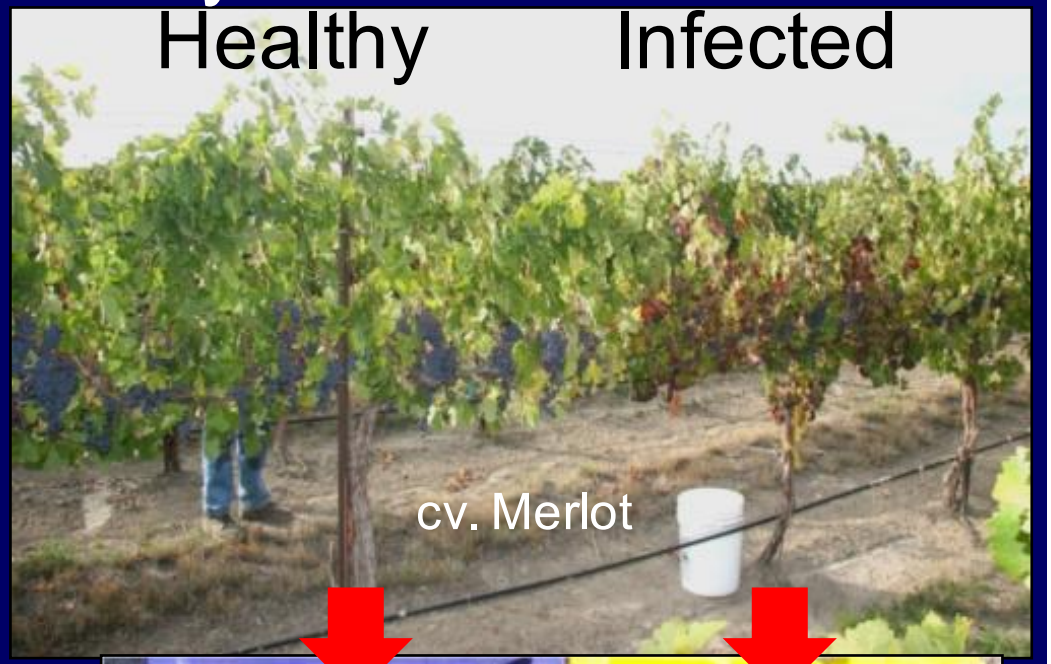
Impact of grapevine viruses

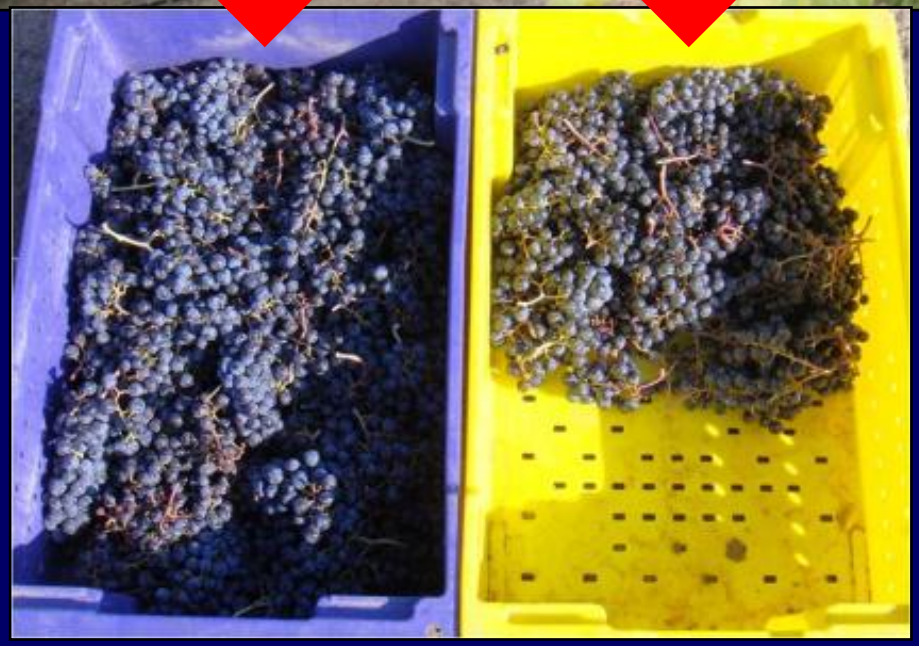
Leafroll
Red blotch

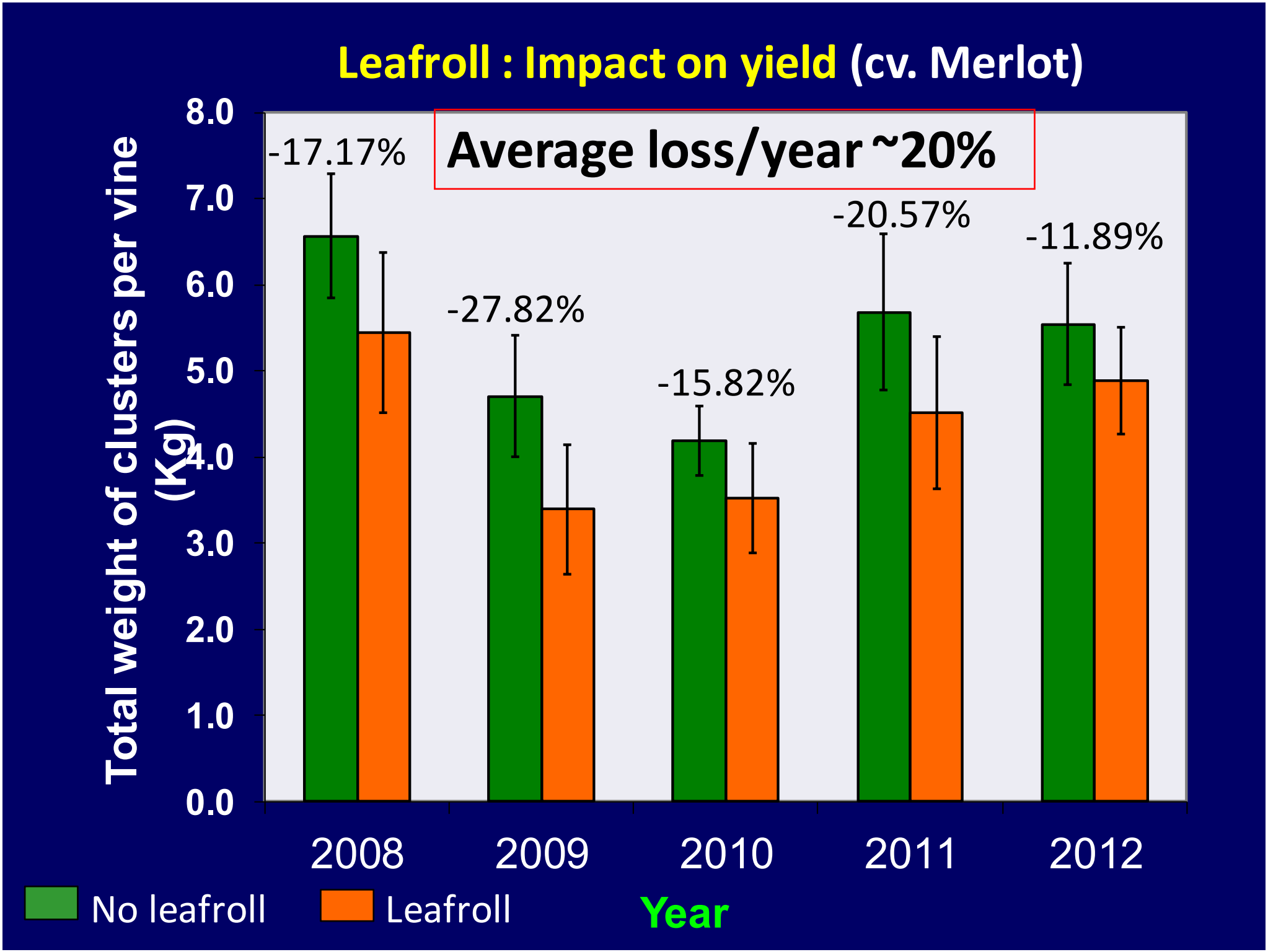
Grape yield and berry sugars:
the two most important parameters for growers and wine makers

Grapevine leafroll disease
Impact on fruit yield





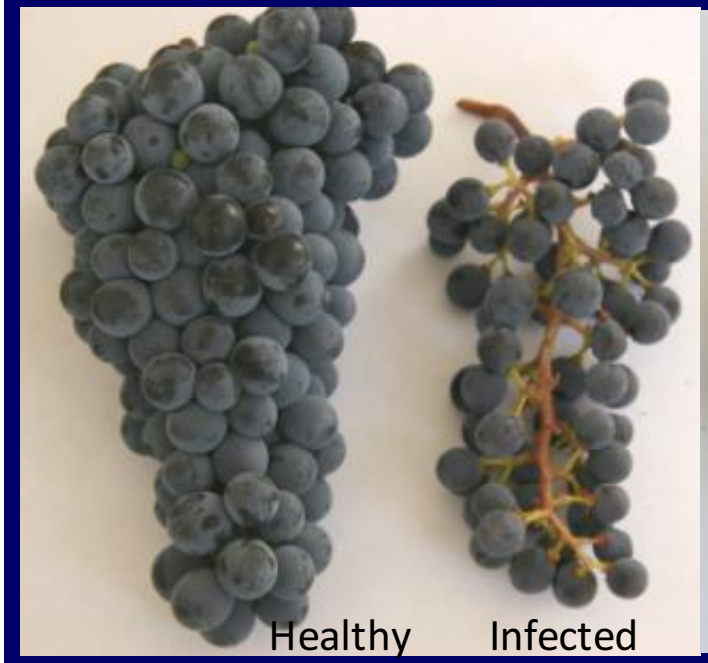



Leafroll : Impact on yield (cv. Merlot)
Average loss/year ~20%
-17.17%
-27.82%
-15.82%
-20.57%
-11.89%
Total weight of clusters per vine (Kg)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
2008
2009
2010
2011
2012
Year
No leafroll
Leafroll

Leafroll disease

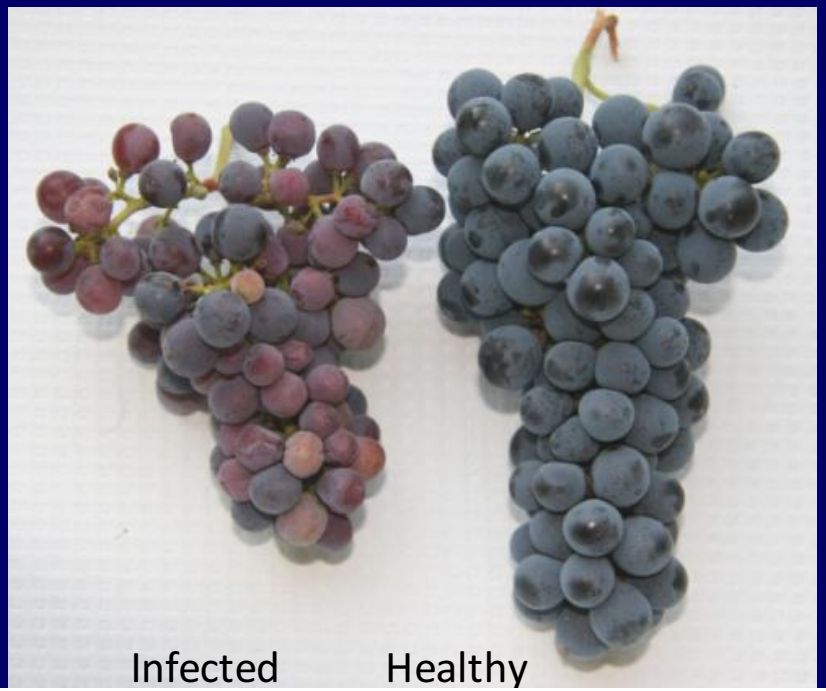
Impact on fruit quality

cv. Cabernet Sauvignon



cv. Pinot noir

cv. Cabernet franc



cv. Chardonnay

Leafroll : Impact on fruit quality

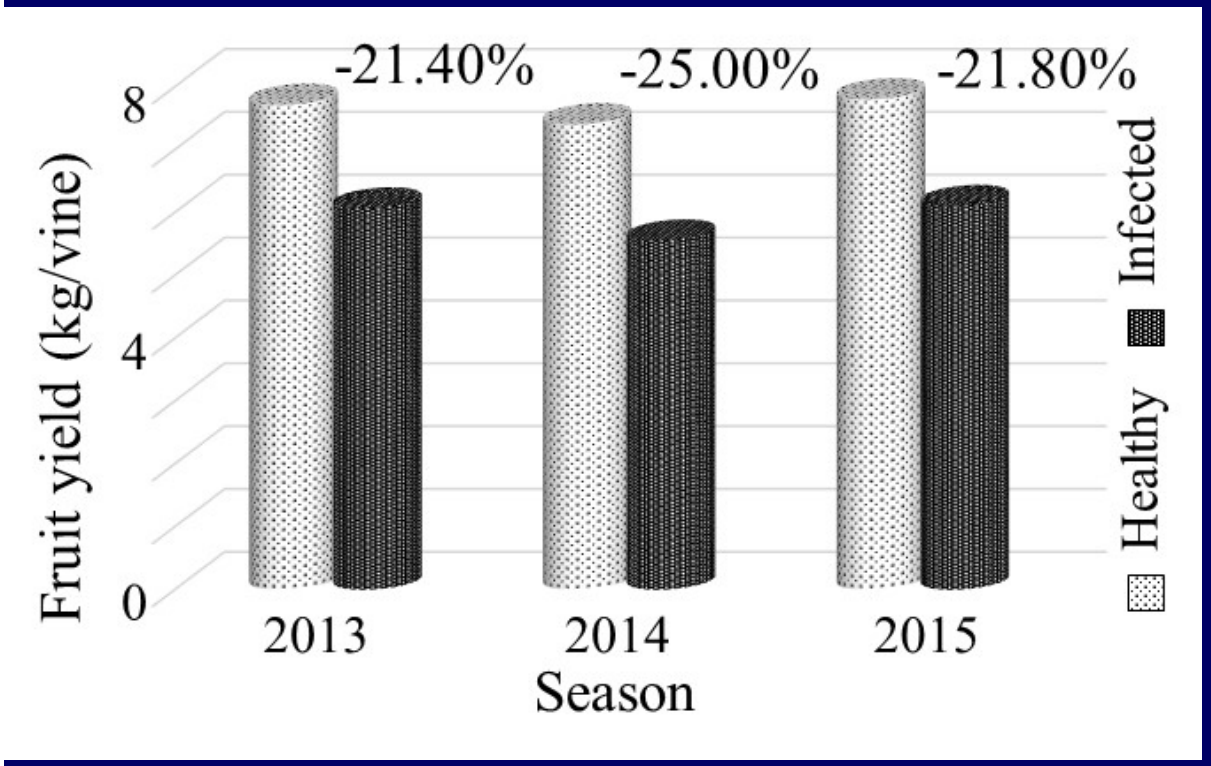
Total soluble solids (^{O}Brix) at harvest (cv. Merlot)

Year	Healthy	Leafroll	Difference
2009	24.83 ±0.09	23.30 ±0.10	- 6.16% ***
2010	25.03 ± 0.32	23.10 ±0.29	- 7.71% *
2011	23.50 ±0.06	22.53 ±0.15	- 4.13% *
2012	25.96 ±0.05	24.58 ±0.00	- 5.31% ***

*, p = 0.05-0.01; **, p = 0.01-0.002; ***, $p < 0.002$

Impacts of red blotch

Yield



cv. Merlot

Total soluble solids (°Brix)

Year	Healthy	Infected	
2013	24.18	21.00	-13.15%
2014	24.36	21.46	-11.90%
2015	25.32	22.78	-10.03%

Summary points

- Both leafroll and red blotch affect fruit yield and quality in the cv. Merlot examined.
- Impacts on berry sugars and anthocyanins more pronounced than grape juice pH and TA.

Future perspectives

- Study cultivar-specific responses
  (do leafroll & red blotch have similar impacts on red & white grape cultivars?)
- Study site-specific impacts
  (do we have similar impacts on grape cultivars in different appellations?)
- Variation between seasons
  (due to Genotype (G)-by-Environment (E) interactions)
- Are mixed virus infections cause more yield losses than single infection?

Spread of grapevine viruses

All viruses can be spread via planting materials

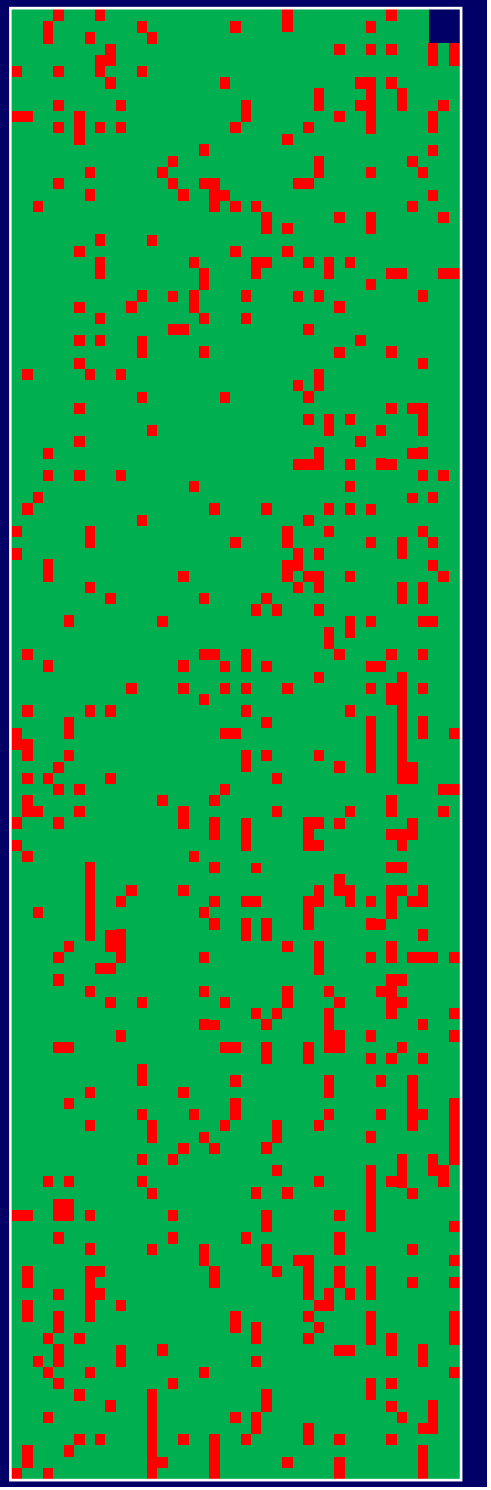
No chemicals to cure infection and make a sick plant healthy

Prevention better than cure

- use ‘clean’ plants
- control vectors

Spread via compromised planting stock

5.5 acres:
11.8% vines
Positive for
GLRaV-3

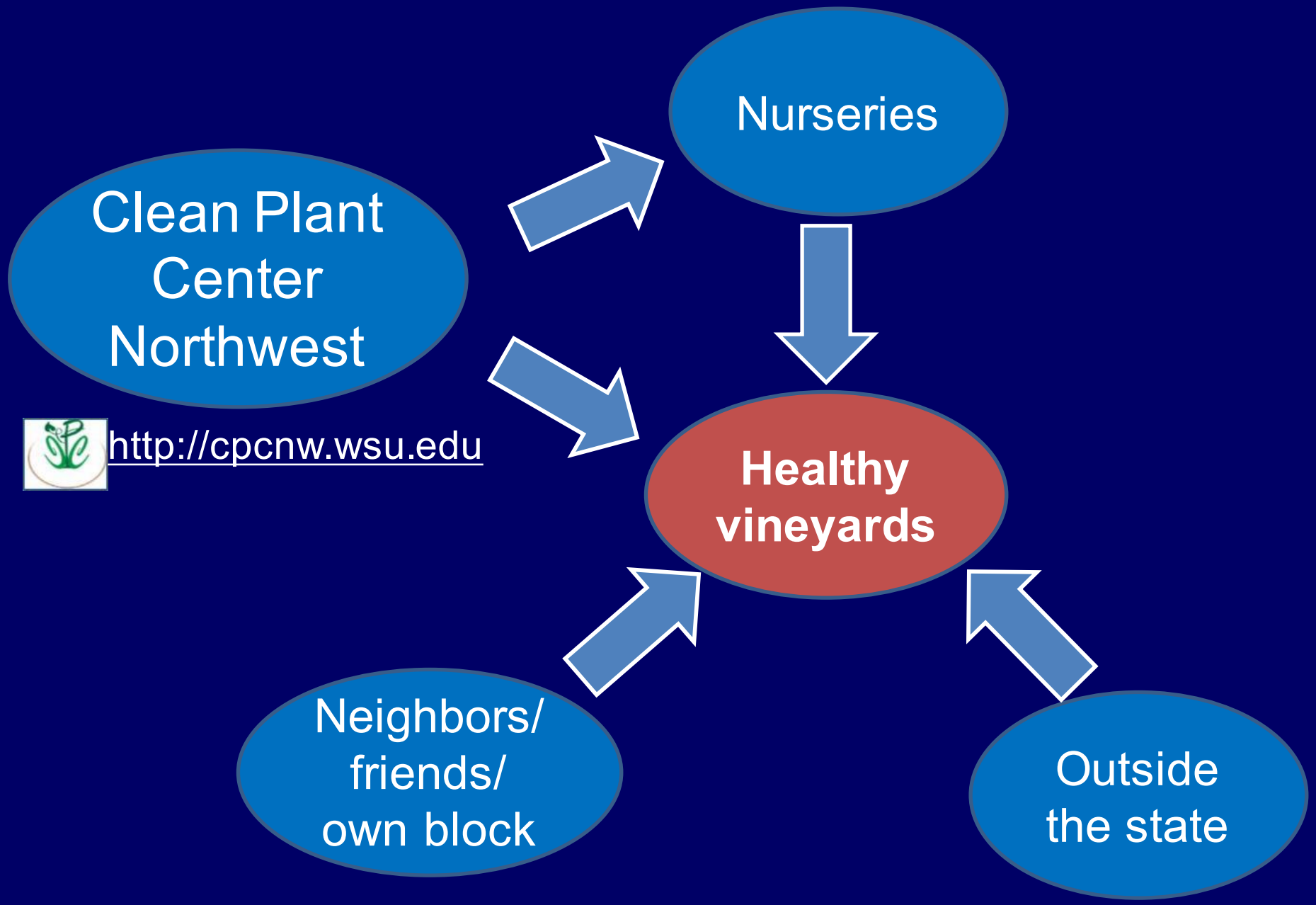


Virus spread via top-grafting

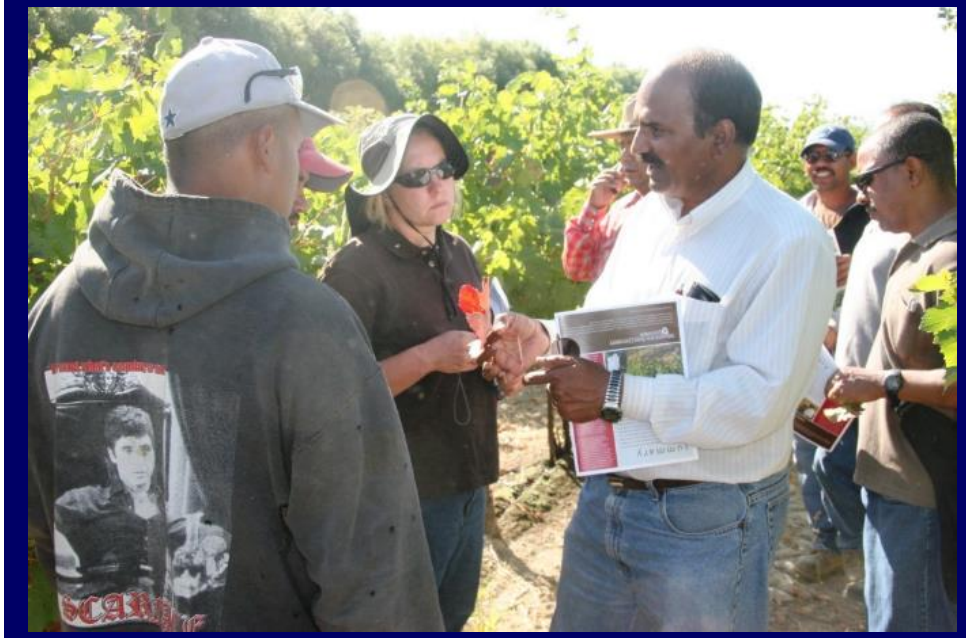
The ‘Mantra’ of ‘start clean’

Use virus-tested ‘clean’ plants as the first line of defense

Strengthening the grapevine supply chain



We are here to help you!



Share knowledge
Provide advise
Offer an action plan

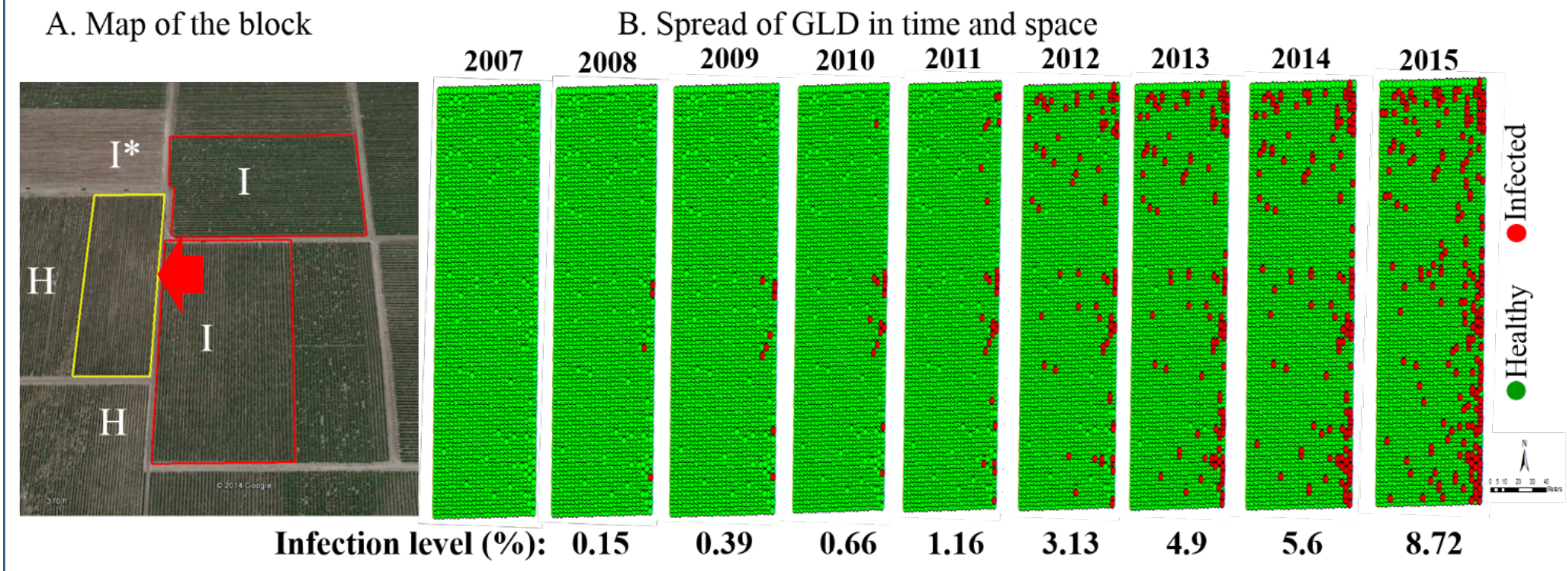
Should I offer diagnostic service to the industry ?

The ‘Mantra’ of ‘start clean, stay clean’

Do ‘clean’ plantings remain free from viral infections?

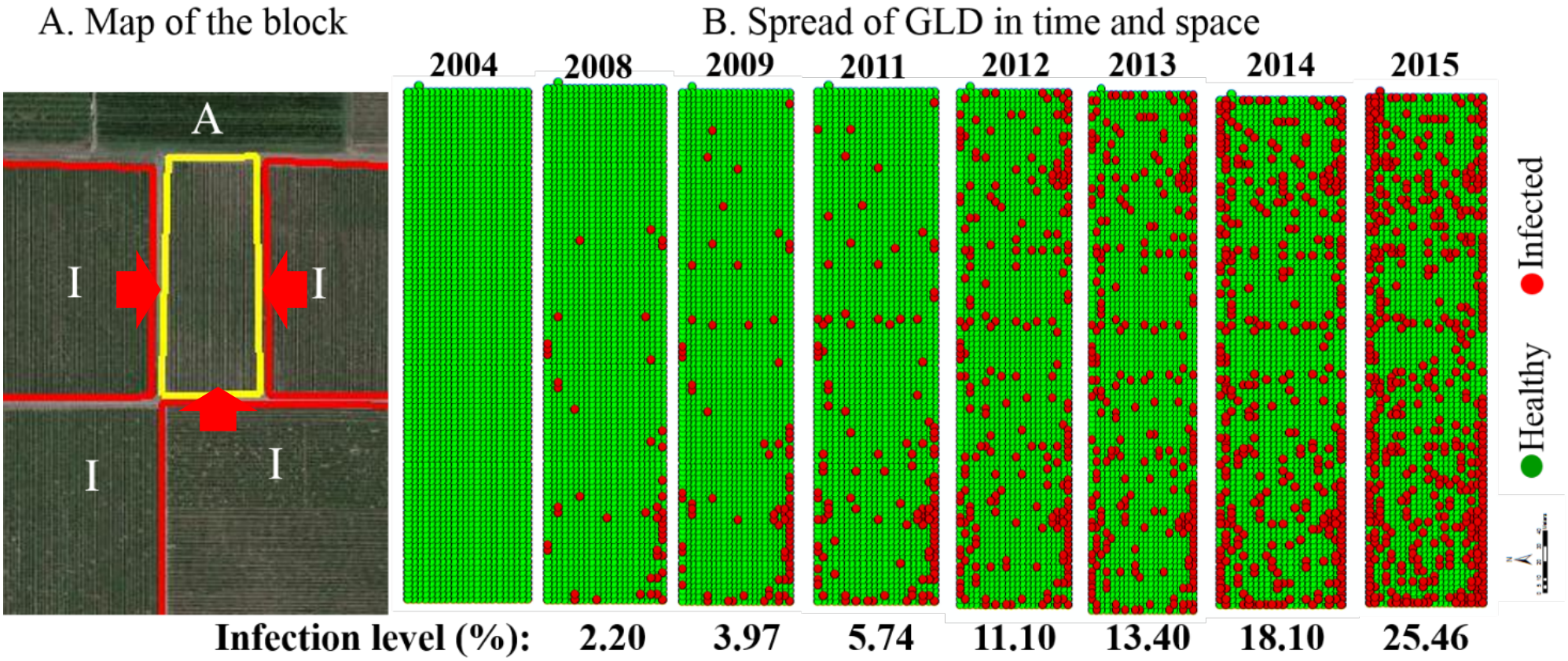
‘Clean’ plantings are vulnerable to leafroll

cv. Cabernet Sauvignon



- Increased number of symptomatic vines each season.
- A gradient of infected vines – indication of initial spread from heavily infected old blocks.
- Clustering of symptomatic vines with time – indication of secondary spread.

‘Clean’ plantings are vulnerable to leafroll cv. Syrah



- Increased number of symptomatic vines each season.
- A gradient of infected vines – indication of initial spread from heavily infected old blocks.
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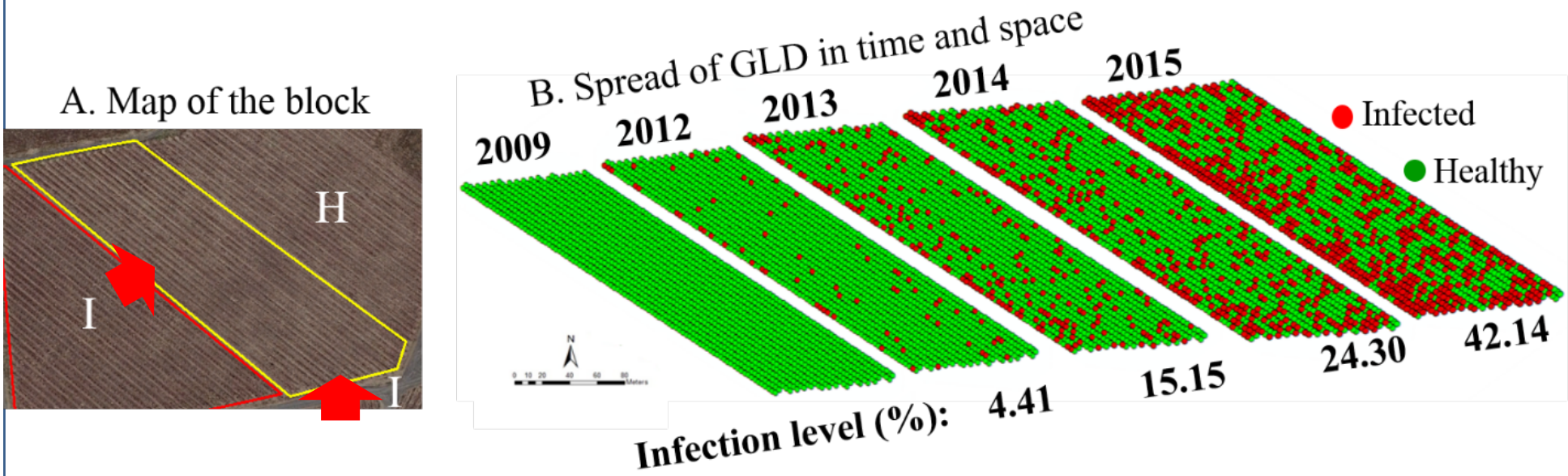
‘Clean’ plantings are vulnerable to leafroll

cv. Petit Syrah



- Increased number of symptomatic vines each season.
- A gradient of infected vines – indication of initial spread from heavily infected old blocks.
- Clustering of symptomatic vines with time – indication of secondary spread.

Summary points

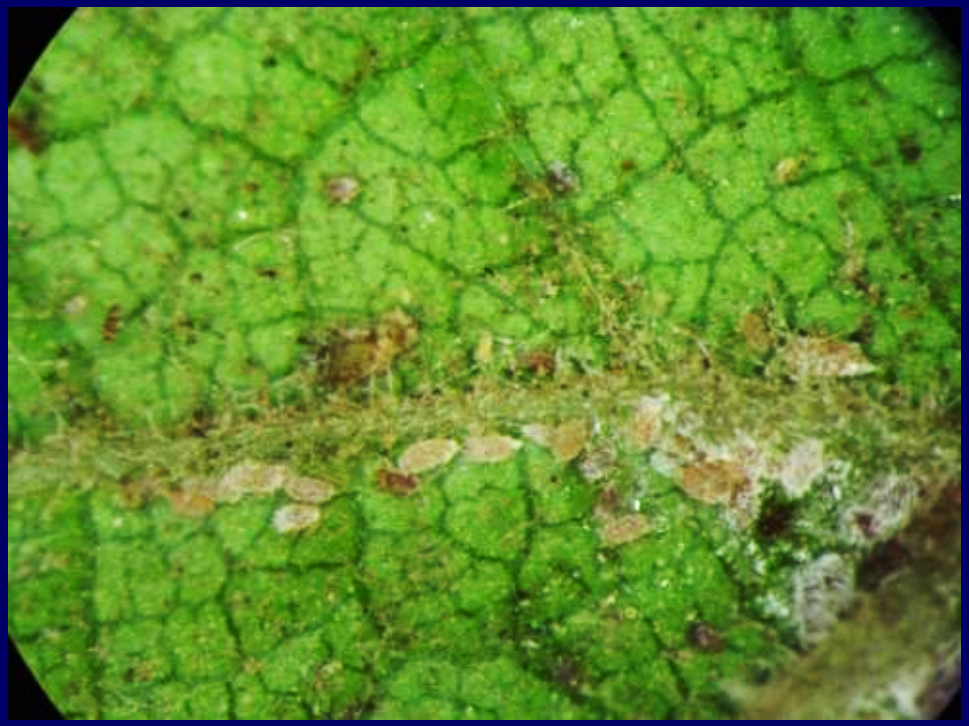
Multi-year field studies on the spread of GLD in three wine grape cultivars have provided convincing evidence that:

- Young vineyards planted with certified ‘clean’ stock can become infected with GLD.
- Rate of spread may depend on site-specific influences (viz. proximity to infected blocks, weather-driven factors, vector species composition, virus strains, etc.).

Blame the bugs?

Vectors of leafroll in WA

Grape mealybugs
(Pseudococcus maritimus)



Soft scale insects
(*Parthenolecanium corni*)

Bahder, et al. Environmental Entomology 42: 1292-1298 (2013).

Post-planting strategies

Pesticides (e.g. imidacloprid) used for chemigation and foliar application (Movento or Neuprid) to control mealybugs.

Even with an effective pest-management program, the spread of GLD was observed in vineyards!!!

Replanting of vineyards

Key issues while replacing/replanting of old block for management of viral diseases.

- Via cuttings used for new plantings
- Spread by vectors from old blocks
- Suckers from residual roots of infected vines
- Potential risk of spread of leafroll viruses from residual roots of infected vines via root-grafting.
- Spread by insect vectors (e.g. mealybugs) surviving on residual roots of infected vines in the soil.

Results

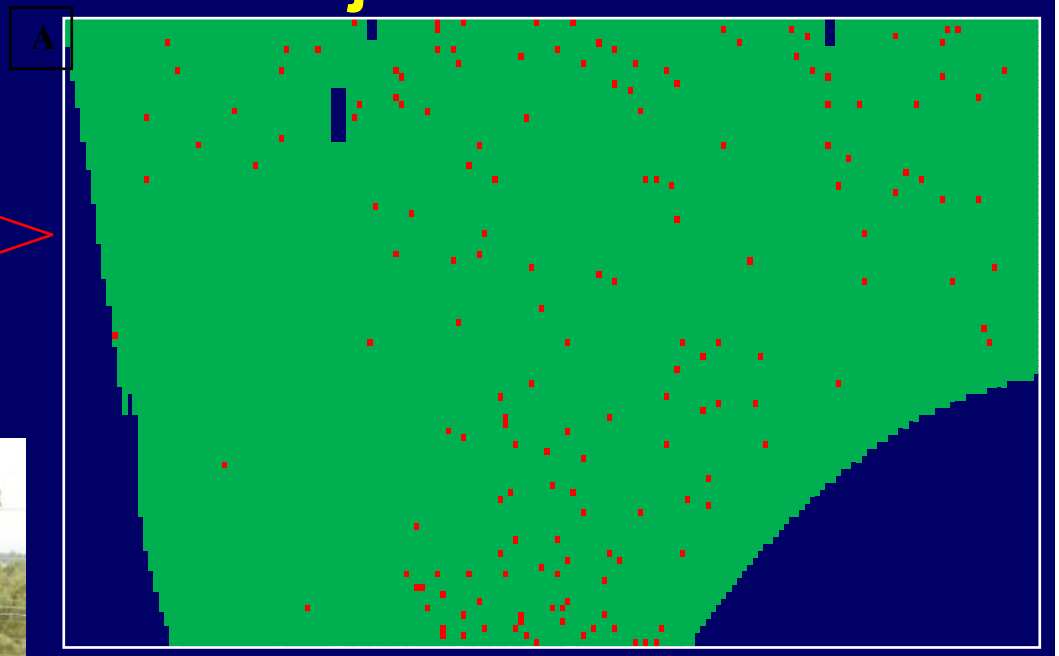
- No suckers from residual roots, if old vines are totally uprooted.
- Residual rootlets of infected old vines are unlikely to serve as a source of virus infection to newly planted vines.

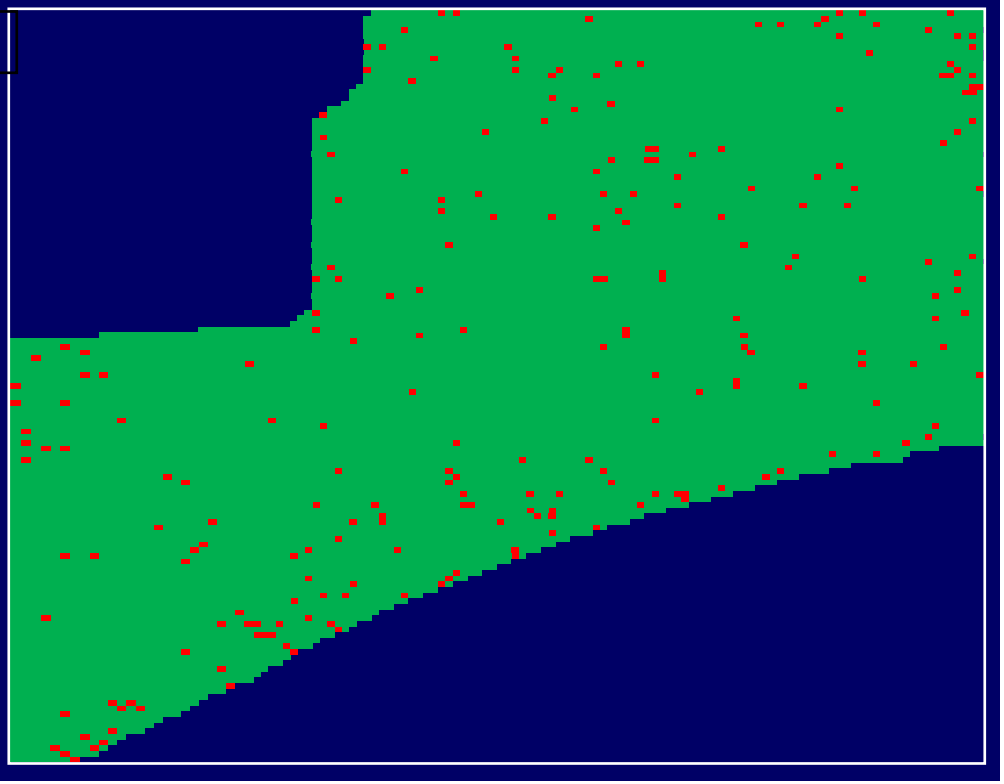
Spread via planting stock is still the major concern

19 acres
1.1% Symptomatic vines
GLRaV-3

Cabernet Sauvignon

13 acres
2.2% Symptomatic vines
GLRaV-3





Post-planting strategies

Roguing (within 5 years post-planting) and replanting as a key component of management strategy for reducing GLD spread in new plantings.

GOOD TO KNOW

Is 'Grape Virus Tax' hitting your pocketbook?

The Washington State grape and wine industry has recognized management of viral diseases as one of the highest priorities if the industry is to maintain its growth trajectory.

Viral diseases affect vine health, fruit yield, and quality and lead to poor quality wines. Thus, the industry must instill concerted and collaborative approaches among the stakeholders, researchers, and regulatory agencies to thwart the increasing threat of viral diseases in vineyards. This is of paramount importance for advancing sustainable growth of an industry that continues to make significant contributions to the state economy.

A new study estimates the economic losses from grape leafroll disease.

by Naidu Rayapati and Doug Walsh, Washington State University

One aspect of viral diseases that remains unresolved is the effect that they can have on the profitability and sustainability of Washington's vineyards. To address this question, a project was funded by the Washington State Department of Agriculture's Specialty Crop Block Grant Program to determine the economic impacts of viral diseases on growers' pocketbooks.

Washington State University scientists teamed up with agricultural economists, Trent Ball and Ray Folwell of Agri-Business Consultants, to estimate the operating returns lost by a grower due to viral diseases. The team has initially focused on the economic impact of grapevine leafroll disease, by far the most insidious viral disease in Washington vineyards.

The study used yield and fruit quality data obtained from previous WSU research that studied a commercial Merlot vineyard from 2009 through 2012. In that trial, yield was reduced from 12 percent to 28 percent during the four years. Further, Brix, which is a measure of sugar in the fruit, dropped below 23.5°, the desired contract minimum in seasons with below average growing degree-days (heat accumulation). Brix was measured at 23.1° and 22.5° in 2010 and 2011, respectively. There can be as much as a 2 percent price reduction penalty for each 0.1° Brix reduction below the 23.5° minimum.

Yield and quality losses

Data from the Merlot study were used to answer two questions:

1. How much is lost (in terms of operating returns) when fruit yield is reduced by 10 percent, 20 percent, or 30 percent?
2. How much is lost (in terms of operating returns) from a combination of reduced fruit yield and price penalty imposed by wineries when grapes have less than expected sugar levels?

A base-line scenario for a Merlot vineyard was created using an average annual yield of 4.5 tons per acre and a three-year average price of $1,127 per ton. The associated variable costs were calculated using the Northwest Grape Costs of Production Calculator using 2014 prices and production practices for a vineyard in full production. Industry stakeholders participated in discussions and validation of costs estimated for grape production. Annual operating returns (excluding fixed costs) were calculated for a Merlot vineyard that had no virus infection as the base-line scenario.

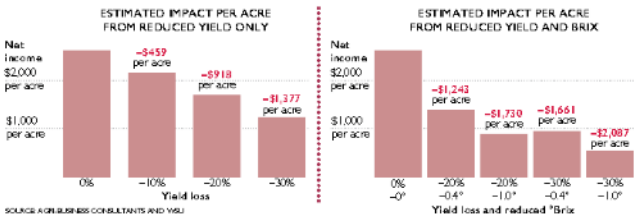
The base-line operating returns of $2,619 per acre were compared to the operating returns for vineyards managed under various virus infection scenarios of crop losses and reduction in fruit quality, especially sugars measured as Brix. *(See Figure 1.)* The study used two Brix

COURTESY WASHINGTON STATE UNIVERSITY

The Merlot vines on the right, with red leaves, are infected with grape leafroll disease. Notice the smaller clusters in the infected vine compared to a healthy vine on the left.

FIGURE 1

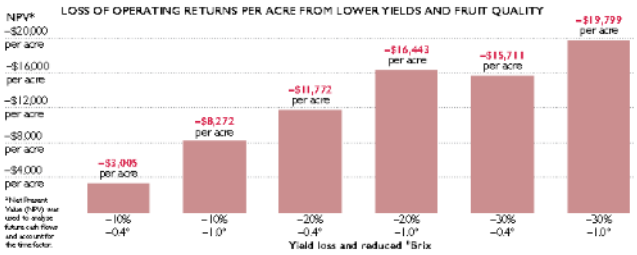
Annual losses from grape leafroll virus



SOURCE: AGRI-BUSINESS CONSULTANTS AND WSU

FIGURE 2

Losses from grape leafroll virus over 20-year period



*Net Present Value (NPV) was used to analyze future cash flows and account for the time factor.

SOURCE: AGRI-BUSINESS CONSULTANTS AND WSU

Estimated economic impact of leafroll, if no control measures are implemented (using Merlot as a case study):

from nearly <u>$1,836 per acre to $23,000 per acre over a 25-year period</u>, depending on the rate of spread.

Good Fruit Grower May 15, 2015. Vol. 66 (No. 10), pages 10-11.

Our goal

Lab Members

Thank You