Red Blotch Disease

OWRI Red Blotch Team
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Corvallis, OR 97330

GRBV Update

The virus

The vectors?

The plant

The fruit

What to do?

The Virus

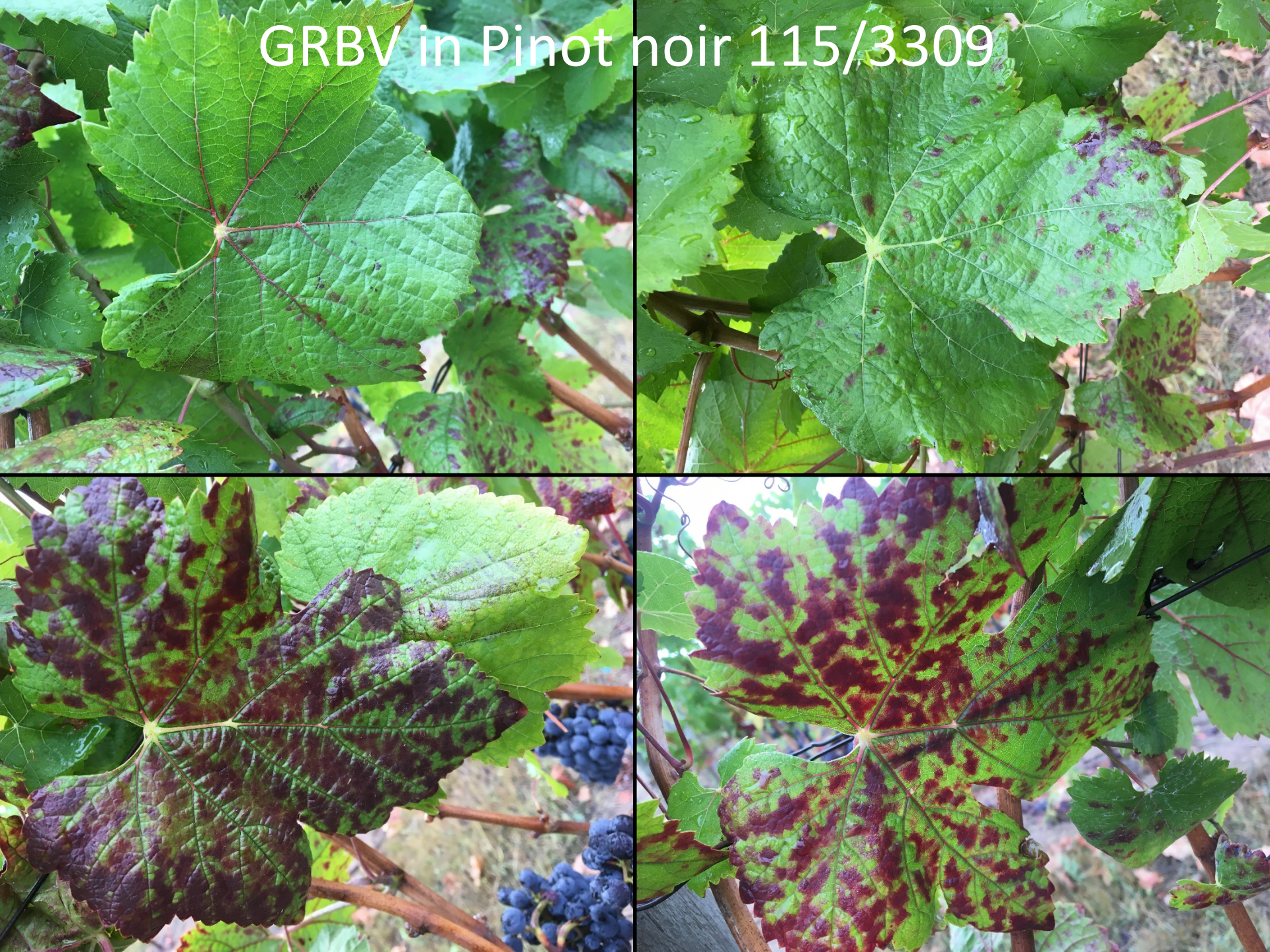
- Work on GRBD began at ARS and UC-Davis in 2008. Effect of red leaf disease of Cabernet sauvignon, and mitigation by delayed harvest & crop load adjustments (MS thesis, 2011)
- Virus associated with Red Blotch Disease reported at the ICVG meetings in Davis, CA in Oct. 2012 (Grapevine cabernet franc associated virus from New York and Grapevine red blotch associated virus from Davis, two diseases caused by same virus)
- Detection primers obtained from Sudarshana during the ICVG meetings.
- Tested archived grape samples from -80 freezer during winter of 2012-13, GRBV detected in widely in Oregon and from many cultivars (red and white)

GRBV - OR

- Initially sequenced isolate from Pinot noir exhibiting red leaf symptoms, using High Throughput Sequencing of small RNAs (2014)
- GRBV has been detected in all production areas in Oregon and in all cultivars tested, highest incidence in Southern OR, also considerable amount in the Willamette Valley
- Sequenced 16 isolates from different cultivars, locations and vineyard ages in Oregon
- Most sequenced isolates were very similar (oldest vineyard >35 years old with isolated GRBV infection, newest vineyard was two years old), typical for what found in rest of U.S.
- One unusual isolate detected based on HTS using small RNAs, confirmed by Sanger sequencing of variant region

Pinot noir 115 on 3309 Rootstock (WV)

GRBV in Pinot noir 115/3309



GRBV in Pinot noir 777/3309

Cabernet franc, GRBV (S. OR)

Cabernet franc, GRBV (S. OR)

GRBV in Chardonnay

Rhonda Smith, UCCE, Sonoma Co

GRBV in Chardonnay

Geoff Hall

GRBV Sauvignon blanc

GLRaV-2 plus RSPaV on grafted Pinot noir, Oregon

Leafroll 3 in Merlot

Grapevine leafroll 3 associated virus in Merlot

Leafroll 1, 2, and 3 in Chardonnay

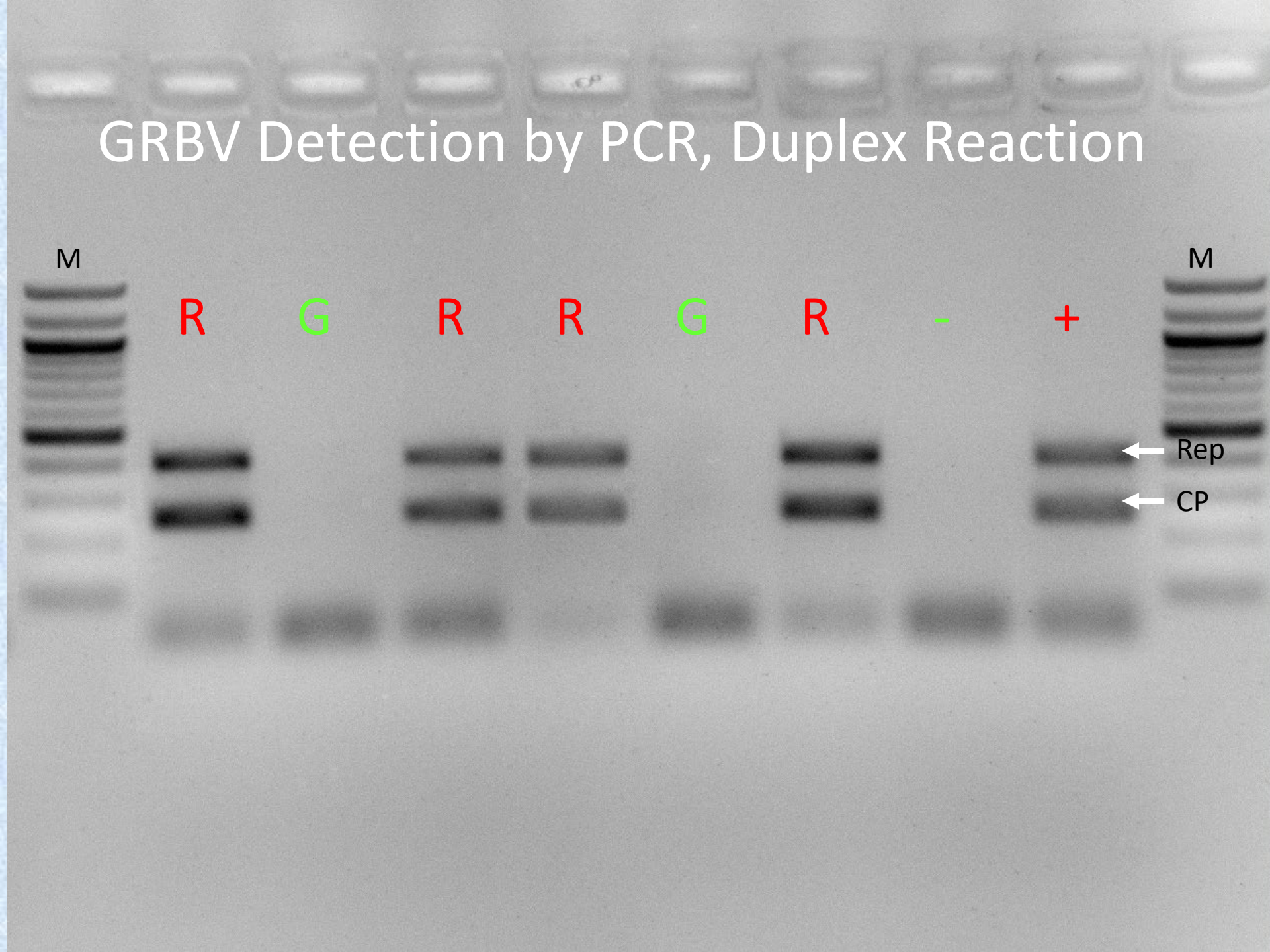
Mg Deficiency

Paul Schreiner

Phosphorous Deficiency

Paul Schreiner

GRBV Detection by PCR, Duplex Reaction



GRBV Detection

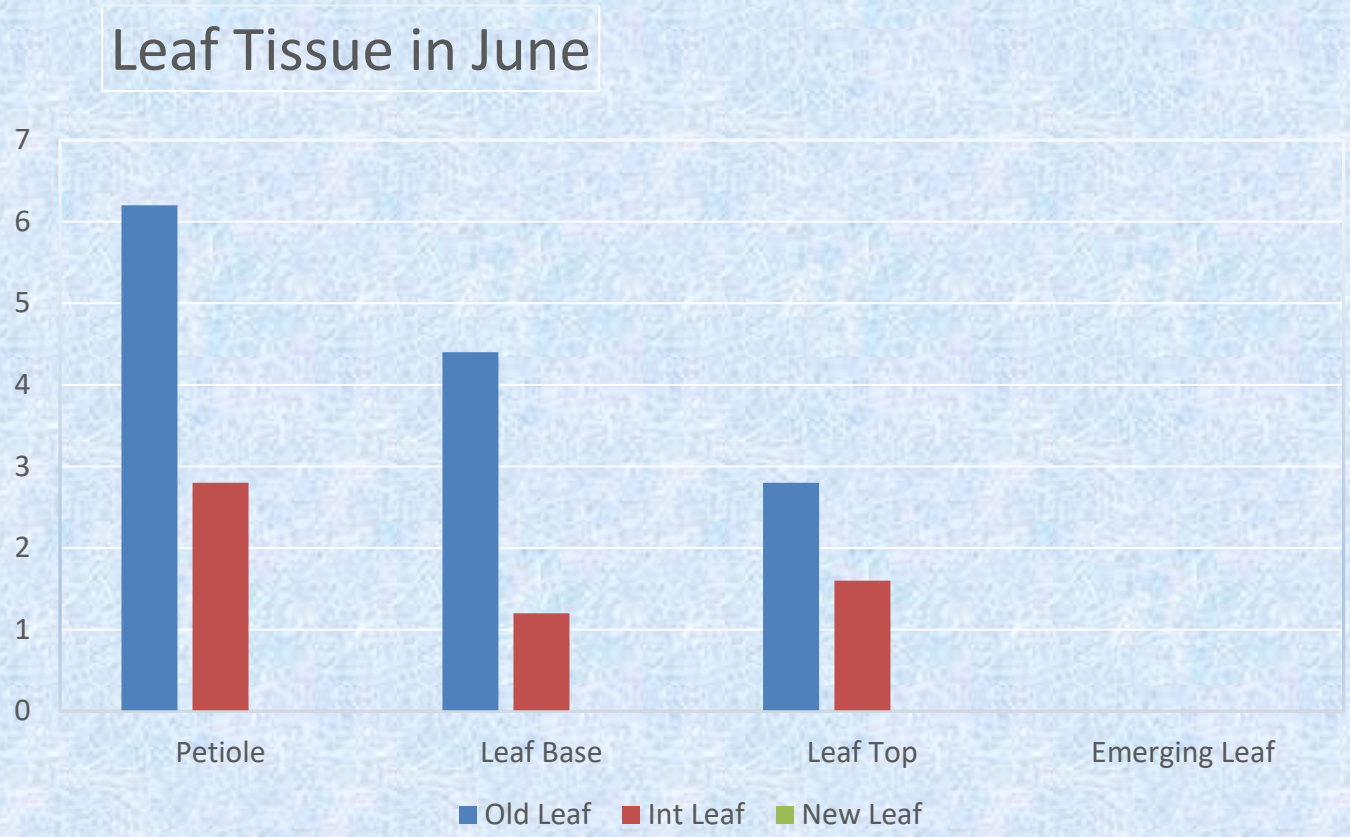
Detection is based on PCR, a laboratory test that is carried out by a number of private and public labs

Two sets of primers developed for highly conserved areas of the virus genome, one in the coat protein gene and one in the replicase gene

We use these primers in a multiplex PCR, basically each sample is tested with both tests at the same time

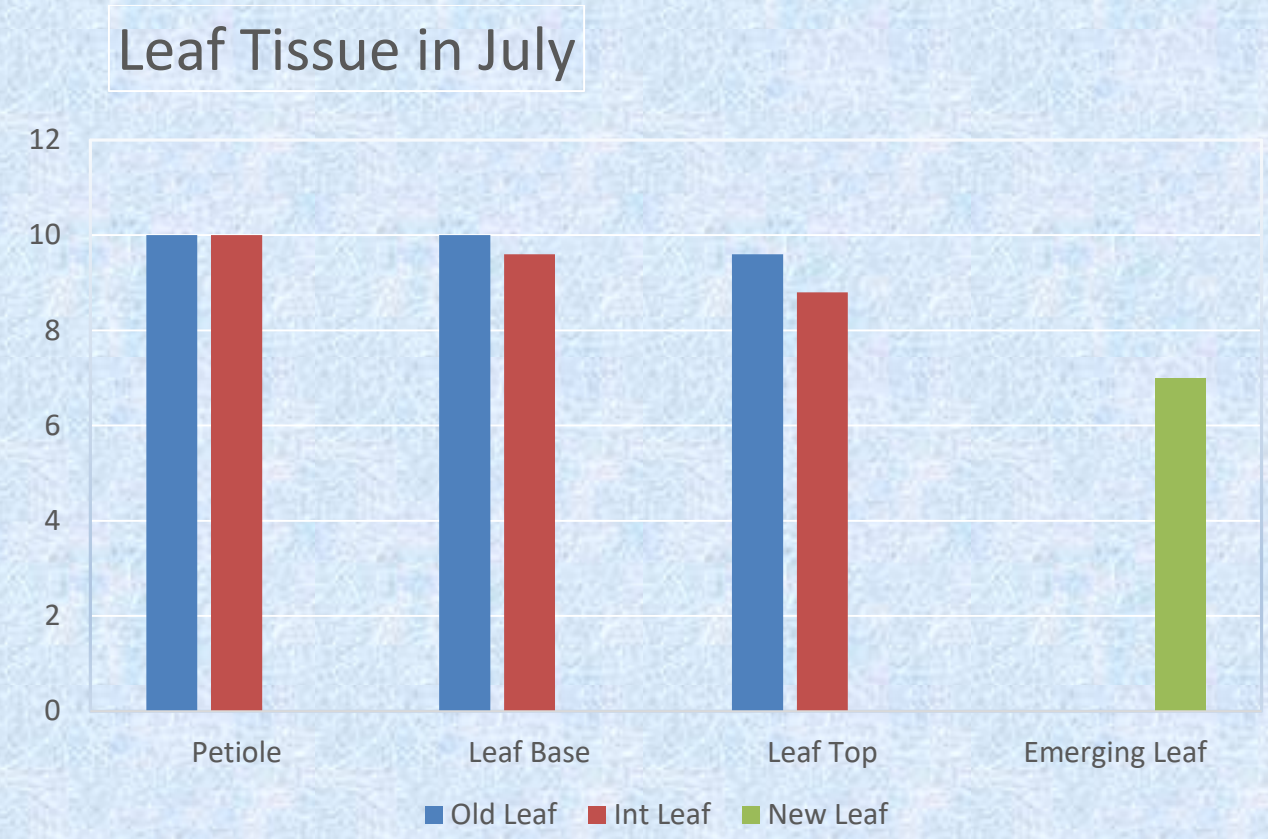
Currently running a “Ring Test” with 8 labs to evaluate the quality of the assays, but also the ability of different labs to detect the virus and not get false positives. 18 samples being evaluated with 0-8 viruses in samples. Total of 15 viruses plus the GLRaVs

GRBV Detection – Tissue to Sample



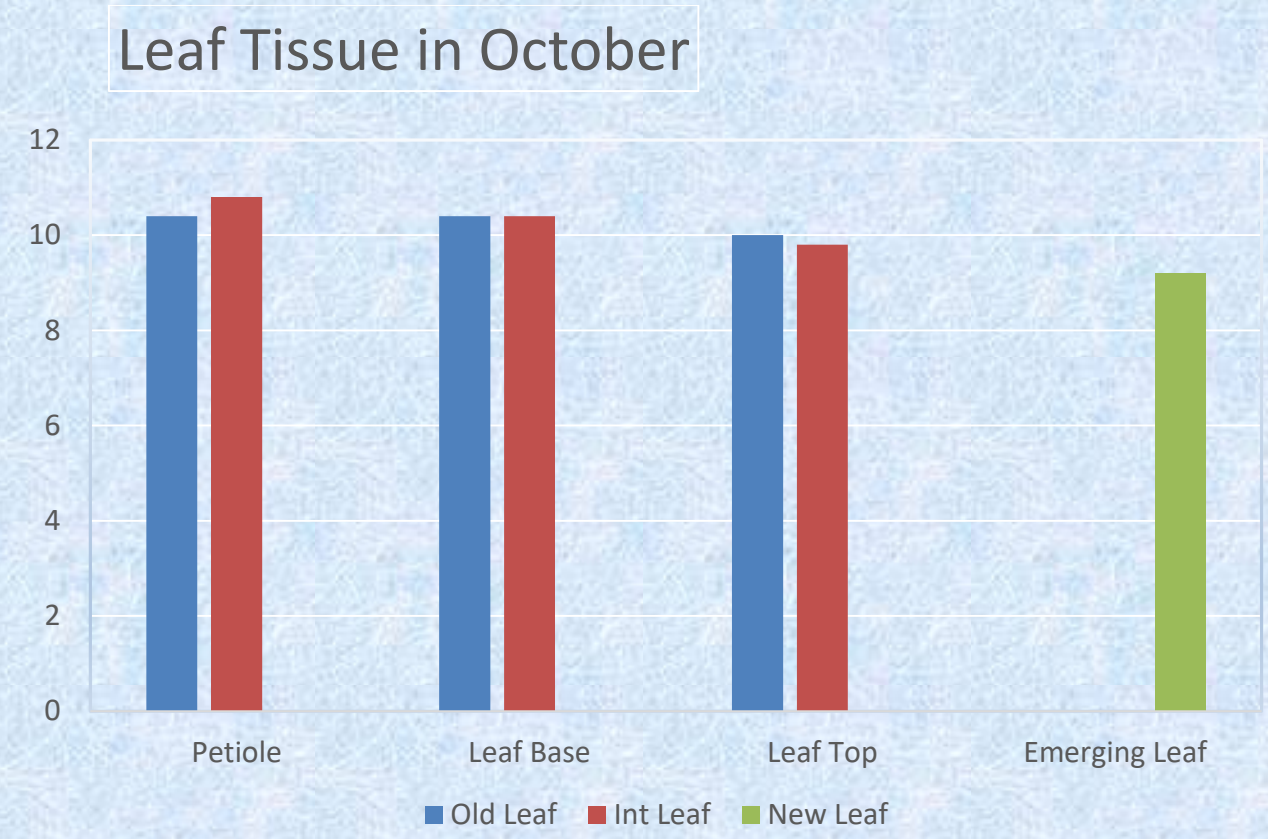
Setiono et al., November 2018

GRBV Detection – Tissue to Sample



Setiono et al., November 2018

GRBV Detection – Tissue to Sample



Setiono et al., November 2018

Vectors

Virginia Creeper Leafhopper (VCLH) reported to transmit GRBV in greenhouse studies (WA, 2013)

Three cornered alfalfa hopper (TCAH) reported to transmit GRBV in greenhouse studies (CA, 2016). Repeated in NY, but not published

With TCAH and the VCLH, repeating transmissions has been challenging

Is there something else that is transmitting this virus?

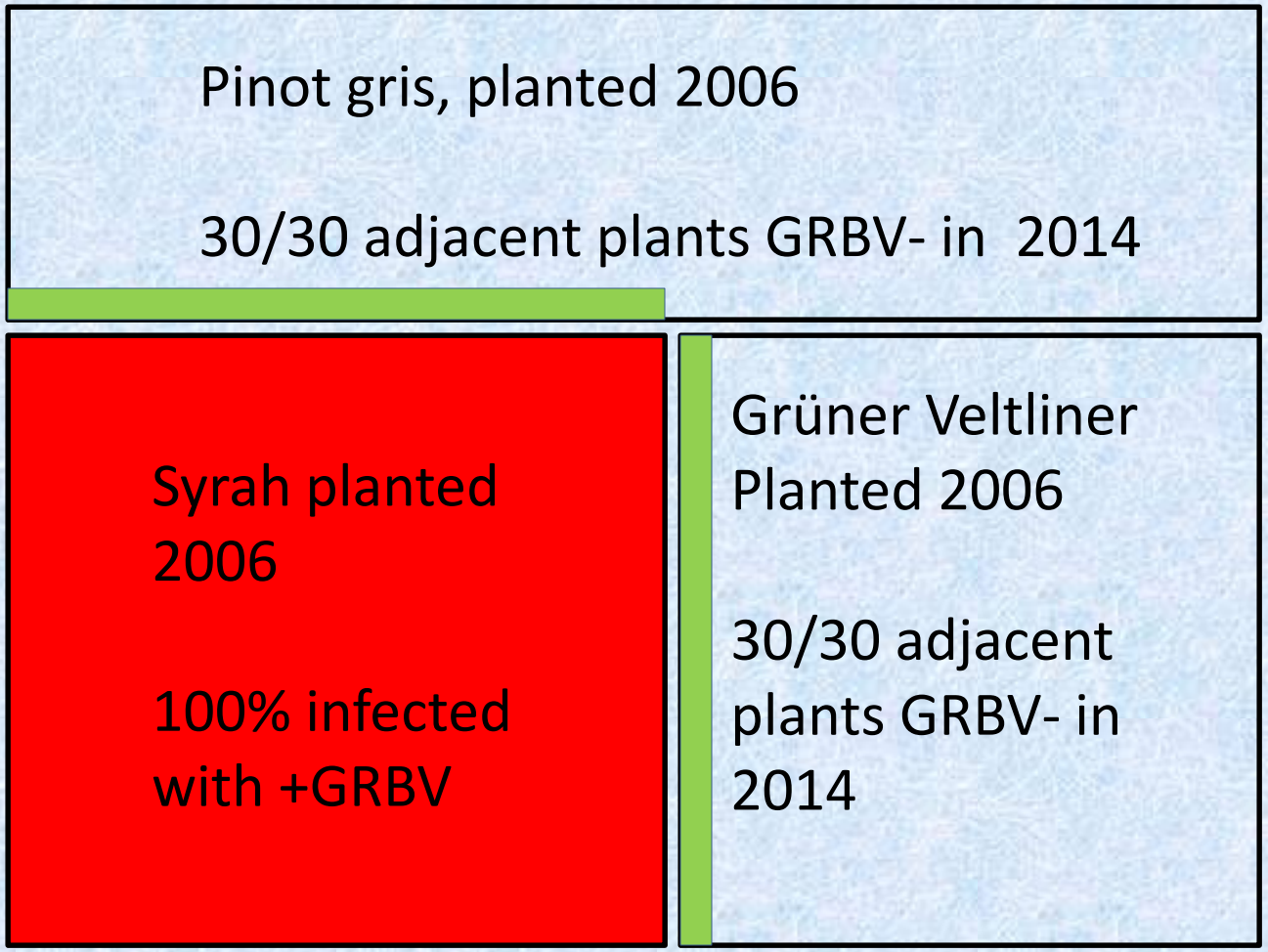
Are there biotypes of insects that are good vectors and others that are not? – happens with other persistently transmitted viruses

The Vector(s)?

- GRBV spread in the Willamette Valley is quite variable, very slowly in two of the vineyards we have looked at) Vineyard 1 & 2
- Transmission reported in two others, all four of these vineyards are in Yamhill County
- Some vineyards it obviously came in on the planting stock (Vineyard #3)

- Rate of transmission varies greatly in Oregon:
  - Southern OR, 2 – 18 fold increase in 2 years
  - Willamette Valley, 2 fold increase in 2 years
  - Dalton et al., 2019 Plant Disease (In Press)

Vineyard 2 Willamette Valley (8 years old)



Vineyard 3 Willamette Valley (2 years old)

Pinot noir on 3309 – all negative for GRBV

Tempranillo on 3309 – all positive for GRBV

Tempranillo on 3309 – all positive for GRBV

Source of rootstock was same for the three blocks

GRBV map of vineyard in the Willamette Valley

Red dot indicates individual plant positive for GRBV in 2016, yellow dot indicates plants positive in 2017 that were symptomless in 2016

Timing Of GRBV Field Transmission In Oregon

Place trap plants in multiple vineyards at monthly intervals during two growing season (May thru Oct), Rogue and Willamette Valleys, 2016 and 2017

Treat plants with systemic insecticide after exposure

Hold plants in screenhouse and tested for GRBV at the end of the growing season (2016 and 2017)

Tested again fall of 2018, (will test again in 2019), work from Cornell suggested that detection can be unreliable for the first two years after infection

Plant Source

300 plants of Merlot/3309 were provided by Duarte Nursery in 4 inch pots (Feb 2016)

500 plants of Cabernet sauvignon on Schwarzman were provided by Duarte Nursery in Feb. 2017

In 2016, all plants were tested for GRBV by PCR prior to use, bark scrapings from dormant plants.

In 2017, 50 random plants were tested for GRBV before use

Potted in 3 gallon pots, plants maintained in screenhouse in Corvallis after field exposure

Vineyard 1, Southern Oregon

Vineyard 1 – Rogue Valley between Riparian Area and Vineyard with GRBV

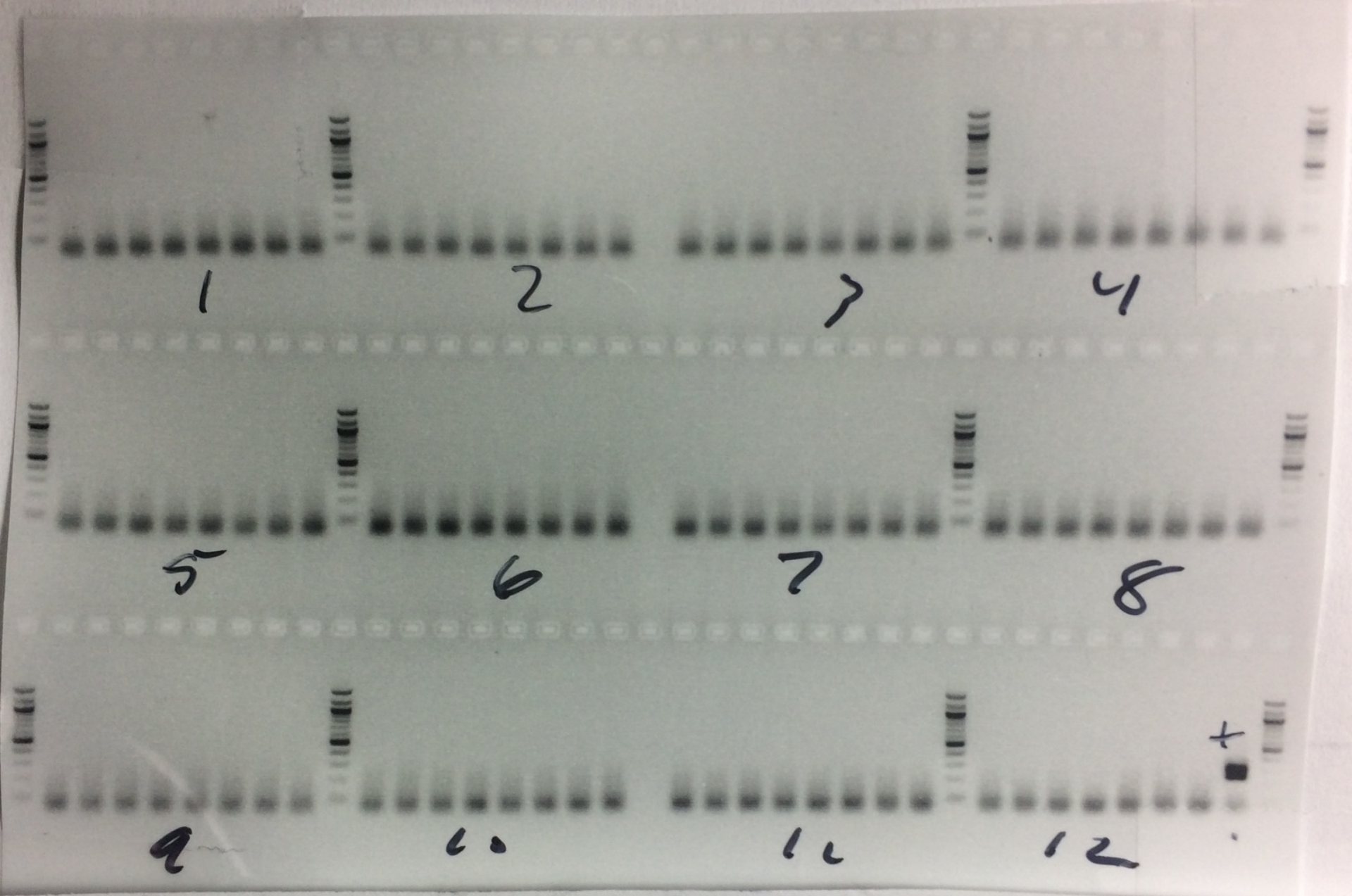
Vineyard 2 – Rogue Valley Adjacent to Alfalfa Field

Vineyard 2, Southern Oregon

Vineyard 3 – Yamhill County Pinot noir 777/44-53

Testing of Trap Plants

- Nov. 2016, tested all 300 trap plants for GRBV, all were negative
- May 2017, tested all 300 trap plants from 2016 field trials for GRBV, all were negative
- Nov. 2017, tested all 300 trap plants from 2016 field trials for GRBV, all were negative
- Nov. 2017, tested 200 of the 400 trap plants from 2017 field trials, all were negative
- Nov. 2018, tested all 700 trap plants from 2016 and 2017 trials (composites of three plants), one positive from 2016, vineyard 2 in Southern, OR
- Retested the 90 plants from this vineyard using bark scrapings, again one positive, plant in the field July 14-Aug 16, 2016.



Trap Plants GRBaV

GRBV Transmission Efforts 2018

Combined effort between Jana Lee (Entomologist USDA) and Bob Martin (Virologist USDA)

Collect insects from vineyard with high incidence of GRBV every two weeks started in June 2018

Insects collected from canopy, vineyard floor and surrounding vegetation using sweep nets, bug vac, and\or beat trays

Insects placed in cooler and brought back to lab

Insects sorted into major groups: froghoppers, bluegreen leafhoppers, small brown leafhoppers, aphids, etc.

Representative insects from each group pinned for later identification

GRBV Transmission Efforts 2018

Insects placed on GRBV infected plants for 6 days

Add four healthy plants to each cage for 6 days

Fumigate

Remove healthy plants and treat with systemic insecticide

Air the infected plants 1-2 days

Repeat every two weeks until October

Plants stored in canyard near Corvallis 1 week after treated with systemic insecticide

Plants tested for GRBV in October of 2018

Plants will be held until fall of 2019 and retested

Repeat entire process in 2019

Strategy 2 – collect insects and feed on GRBV infected vines, test for transmission

Willamette Valley vineyard with significant GRBV increase from 2016 2017.

2016 + red,
2017 + yellow

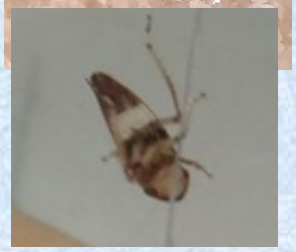
Collect insects from canopy, vineyard floor vegetation and surrounding vegetation, using bug-vac, beat-trays and sweep nets

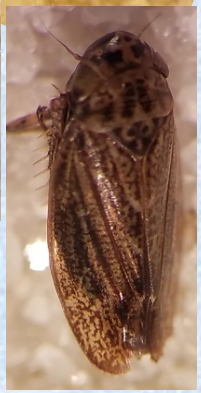
Collect every two weeks, mid-June through mid-October

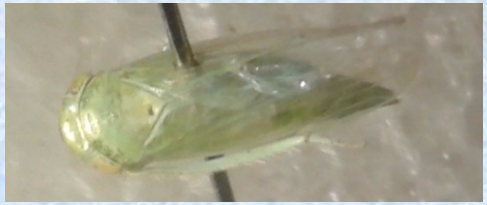
Vacuum samples

Sort insects by morphotype

- 2 aphid species
- 1 big-eyed bug (predator, but drinks from plant)
- 3 froghoppers







Sort insects by morphotype

- 2 aphid species
- 1 big-eyed bug
- 3 froghoppers
- 11 leafhoppers

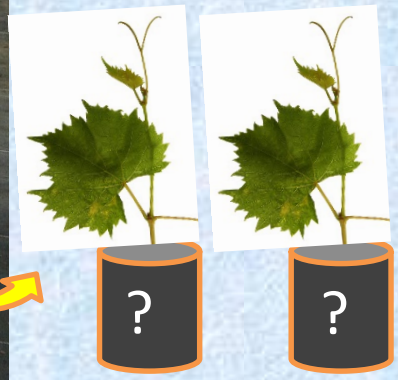
- 2 plant bugs (mirids)
- 1 hemipteran

- 1 stink bug (*Euschistus conspersu*

Run tests

1 type per cage with infected plant, leave for 6 days (AAP)

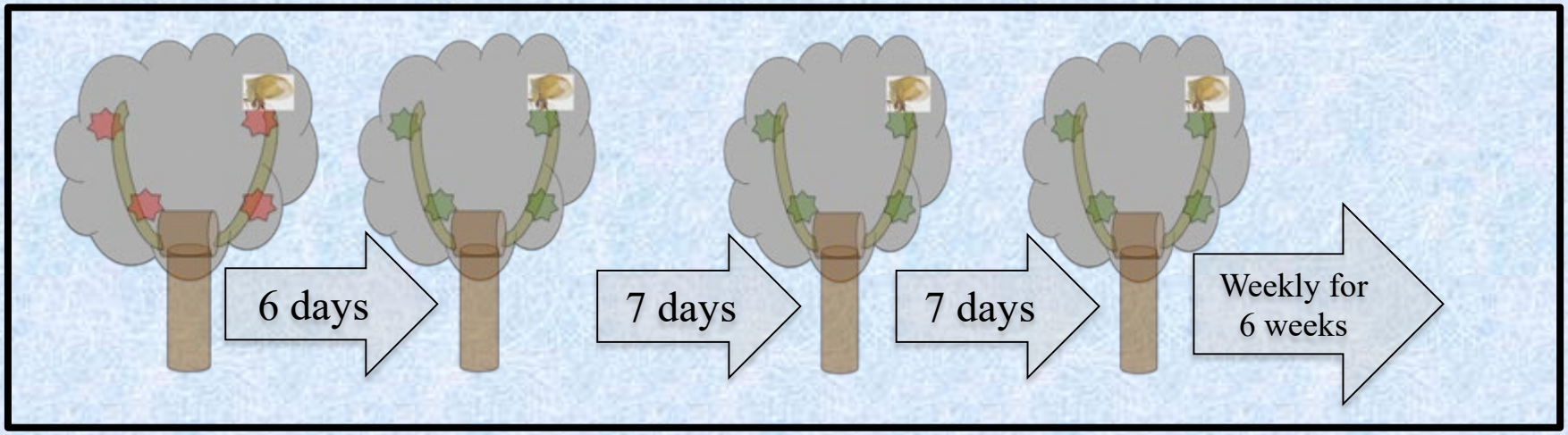
- Put in clean plants
- Insects free to move to them, leave for 6 days
- (IAP)



- Remove test plants
- Treat with insecticides
- Monitor for GRBV

Transmission biology studies
Walton Lab

Acquisition → Inoculation → Persistence of infectivity



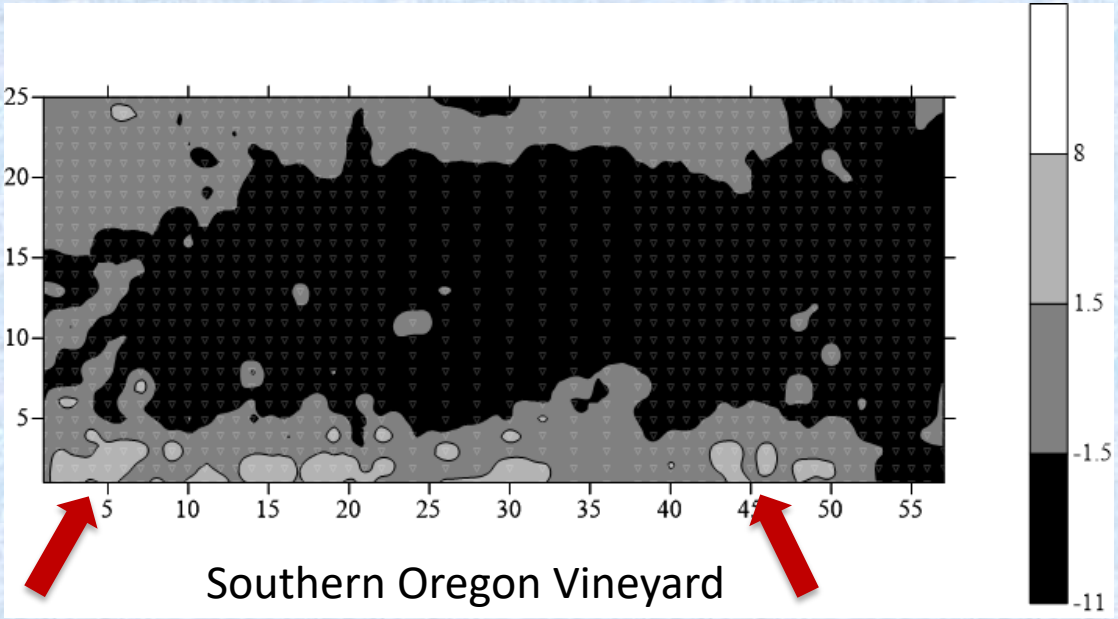
Five adult insects on Red Blotch positive vine

Five adult insects on Red Blotch negative vine

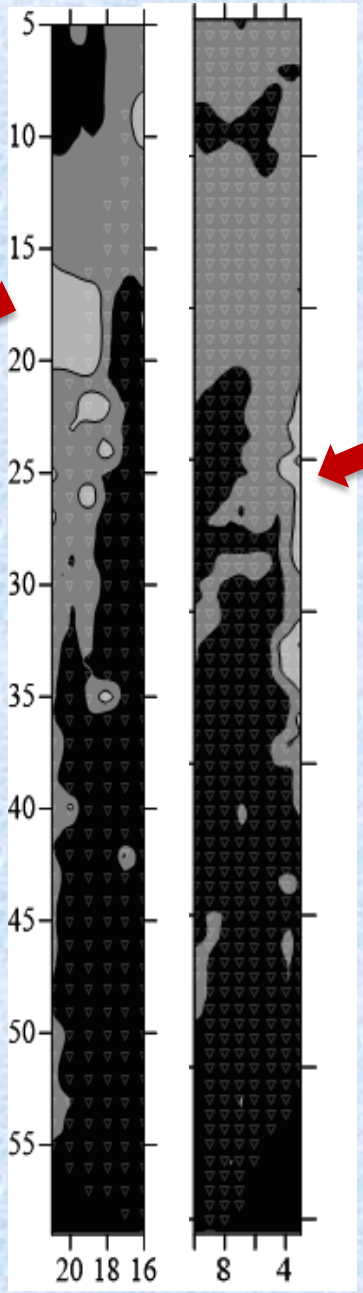
S. festinus, T. albidosparsus and *T. wickhami* used

Oregon State University

Mapping Treehopper Injury in Vineyards
Walton, Hilton Labs



Southern Oregon Vineyard



Willamette Valley Vineyard

Other Possible Vectors Based on PCR Positive Insects

Colladonus reductus
(Variegated hopper)

*Osbornellus s*pp.
(Variegated hopper)

Melanoliarus sp.
(Planthoppers)

Scaphytopius magdalensis
Blueberry leafhopper

Empoasca spp.

Graphocephala atropunctata
Blue-green sharpshooter

Other species from these genera known to vector viruses and phytoplasma, some were PCR positive for GRBV when collected from infected plants in CA (Cieniewicz et al. 2018)


The picture on vectors
is still not complete

The Plant

Skinkis

In low stress vineyard, high elevation and with irrigation, there were very minimal differences between GRBV+ and GRBV- vines in terms of photosynthetic activity, also very mild symptoms in 2017

In 2018 – vineyard under higher stress:

Infected vines had lower photo-assimilation than healthy vines

Decline in photosynthesis occurred before leaf reddening

ABA treatment had no effect on fruit ripening

Early season leaf removal can enhance fruit color and phenolics

Fruit ripening was not changed by earlier leaf removal

The Fruit

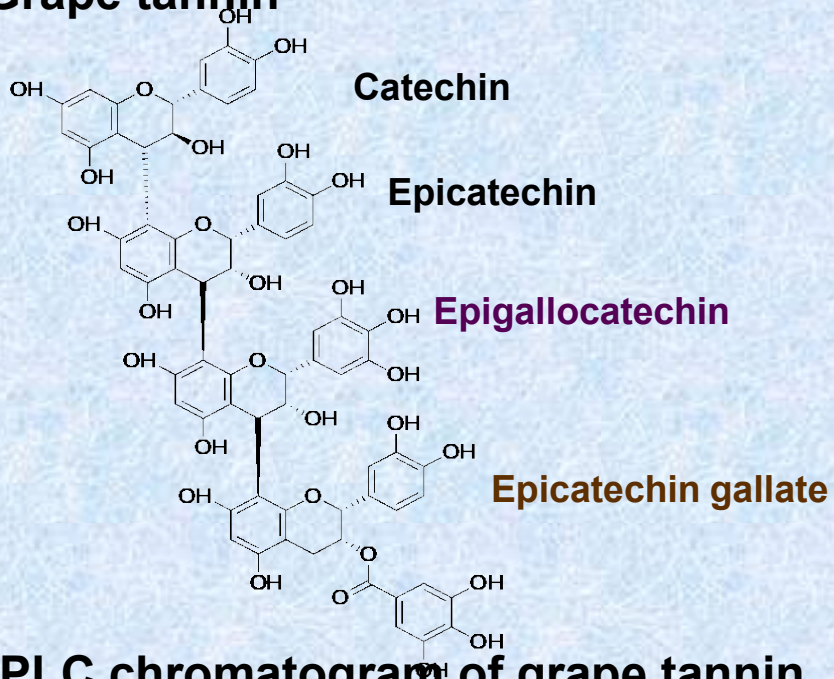
GRBV Impact on Pinot noir – S. Oregon
Levin, Osborne

- Pinot noir in Rogue Valley (2017)
  - RB+ with deficit irrigation, 1.5 lower Brix
    lower anthocyanins, 30%?
  - irrigation at 100 Et, little difference with RB- and RB+ in Brix, but reduced anthocyanins

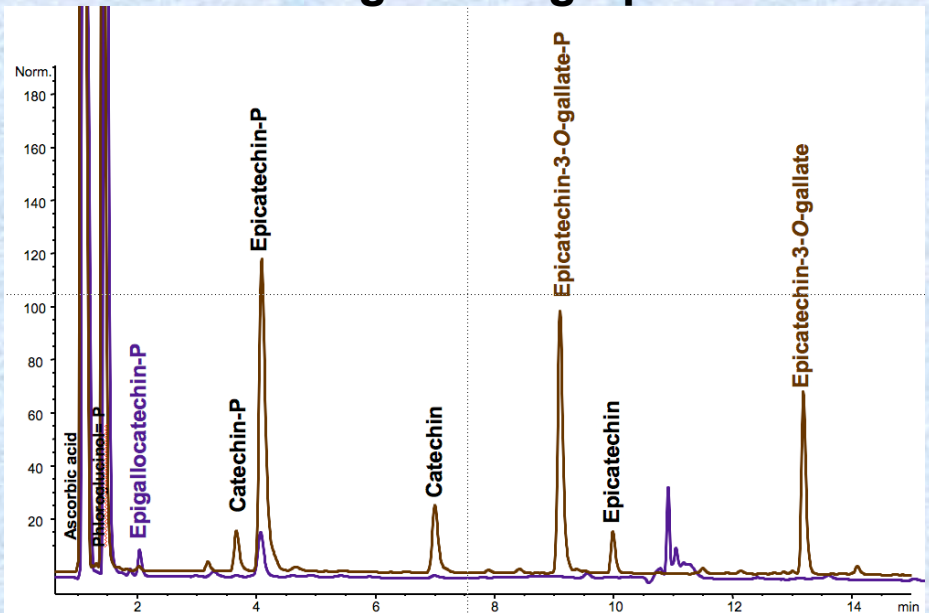
Wine phenolic analysis

Jungmin Lee
(USDA)

Grape tannin



HPLC chromatogram of grape tannin



Wines from 2018 and 2019 –

Phenolic monomers and polymers will be analyzed by HPLC

Table 2. Fresh Weight, Berry Composition of Grapes Collected at Two Sampling Times in 2016 (before Symptoms Were Visible and at Harvest) from Healthy or Red Blotch Diseased-Vines, RB(−) and RB(+), and Two Rootstocks, 110R and 420A Cabernet Sauvignon, Napa County, CA

		110R		420A	
	date	RB(−)	RB(+)	RB(−)	RB(+)
Berry weight (g)	19/08/2016	1.19 ± 0.04	1.17 ± 0.05	1.28 ± 0.06	1.15 ± 0.04
	20/09/2016	1.14 ± 0.04	1.03 ± 0.03	1.21 ± 0.05	1.00 ± 0.04 ++
TSS	19/08/2016	21.67 ± 0.23	18.75 ± 0.43 **	20.62 ± 0.15	18.63 ± 0.45 ++
	20/09/2016	27.00 ± 0.15	22.08 ± 0.59 **	25.80 ± 0.26	23.22 ± 0.56 ++
pH	19/08/2016	3.41 ± 0.01	3.37 ± 0.03	3.38 ± 0.01	3.29 ± 0.03 +
	20/09/2016	3.75 ± 0.03	3.68 ± 0.03	3.64 ± 0.02	3.55 ± 0.03 +
titratable acidity	19/08/2016	7.93 ± 0.07	9.17 ± 0.5 *	7.98 ± 0.1	9.17 ± 0.54 +
(g L^{-1})	20/09/2016	5.85 ± 0.15	7.15 ± 0.29 **	6.32 ± 0.34	6.83 ± 0.19

RB = red blotch. * and ** for P values <0.05 and 0.01 for 110R_RB(−) versus 110R_RB(+). + and ++ for P values <0.05 and 0.01 for 420A_RB(−) versus 420A_ RB(+).

Martinez-Luscher et al., 2019 J. Agric. Food Chem.

WHAT TO DO?

Oregon Grapevine Quarantine and Certification Changes

Harmonize the grapevine quarantine and certification for the PNW

Quarantine would include: GFLV, GLRaVs, GRBV, GVA, GVB, Phylloxera, Vine mealybug, European grapevine moth, Xylella and X. index

Certification would require all grapevines coming into the state be from certification programs approved by ODA (2023)

ODA is working on writing the new rule and it should be available for comment in April (OWB and OWRI will send out email alerts to grape industry when it is available for comment)

OR and WA hope to have new rules in place by summer 2019, Idaho is a legislative process and may take 2 years

Oregon Grapevine Quarantine and Certification Changes

Transition Period

Beginning **January 1, 2021**, all grape plants or cuttings entering Oregon must be from an approved certification program, OR derived from mother vines that have been tested and found free from pathogens that are of regulatory and economic concern within the previous year. Testing must be done by a laboratory and using methods approved by the department.

Fully Implemented

Beginning **January 1, 2023** only grape plants or cuttings originating from an approved certification program are eligible for entry into Oregon. The approved certification program must be comparable to the grapevine certification adopted by the Oregon Department of Agriculture. An approved certification program, at a minimum, must include testing for pathogens that are of regulatory and economic concern.

GRBV – How Big a Threat to Oregon Wine Industry?

There is still a lot to learn on how to control the virus and if there are ways to manage its affects on yield and quality

What is the primary vector(s) of the virus in Oregon?

Are there cultural practices that will mitigate its impact?

Are some rootstocks or cultivars more vulnerable?

Some clones of cultivars?

Highly variable reports on the impact of GRBV on juice chemistry, from no difference to unacceptable

What is your target market?

Virus Management

1. **START CLEAN** (Harmonize efforts in PNW)
2. Then work to STAY CLEAN
3. Understand the threats, what is in the area?
4. Do we know the vectors?
5. Manage the vectors (NEED MORE INFO)
6. **Early detection** and removal of infected plants if incidence below some threshold level (30%) (Ricketts et al., AJEV 2017). Walk vineyard annually after harvest starting with new vineyards
7. TAG, TEST and ROGUE

Virus Management

8. Test, Don't Guess – there are look alikes out there (LR, Nutrition, Other Stressors – 2018 lots of vole damage)
9. If top-working a vineyard, be sure the budwood is certified or tested before use: topworking a virus-infected vineyard, still have a virus-infected vineyard
10. Viruses can come from rootstock or scion wood, be sure both are certified
11. Ask for virus test results from supplier of certified plants

Virus Diseases of Grapevine

All viruses of grapevine are transmitted through vegetative cuttings

All are transmitted by grafting

Grafting infected vines can result in virus complexes with more severe symptoms

Suitcase importations allow for transmission anywhere in the world very quickly, and they could put existing vineyards at risk!

Red blotch is on everyone's radar now, but don't forget about the other **70** viruses that infect grapevine

The Importance of Proper Identification Test Don't Guess

Questions
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