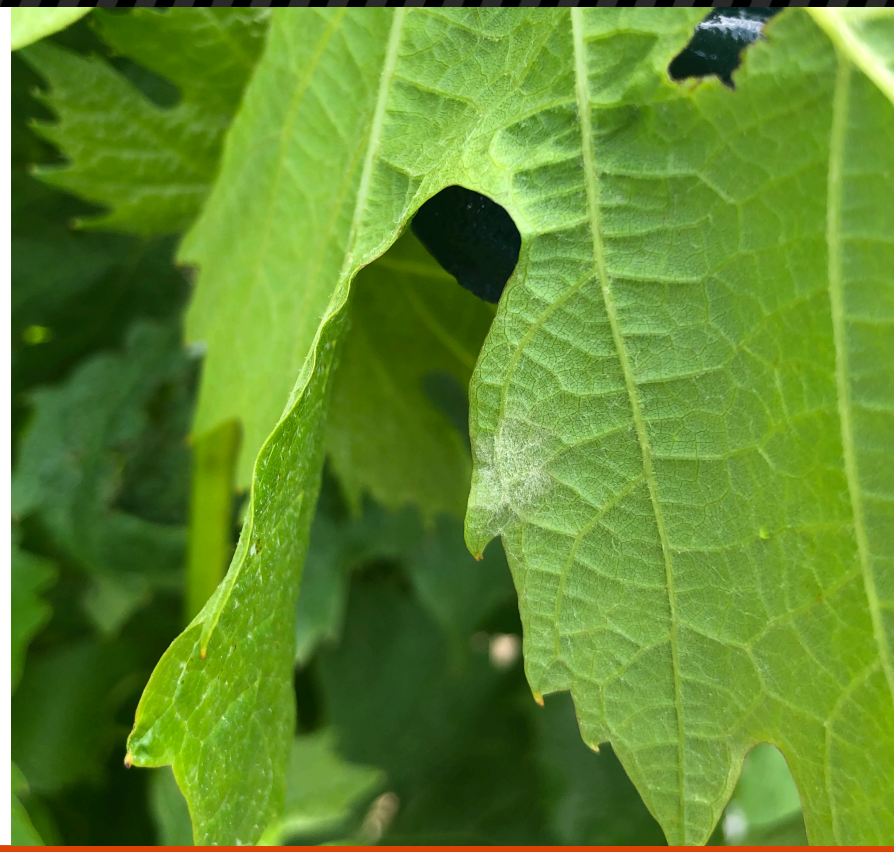
A Handy Tool: New methods in Monitoring Grape Powdery Mildew.



Sarah Lowder

lowders@oregonstate.edu

Oregon State University, Foliar Pathology Lab at USDA-ARS

Funded by: NIFA

Disease - Grape Powdery Mildew

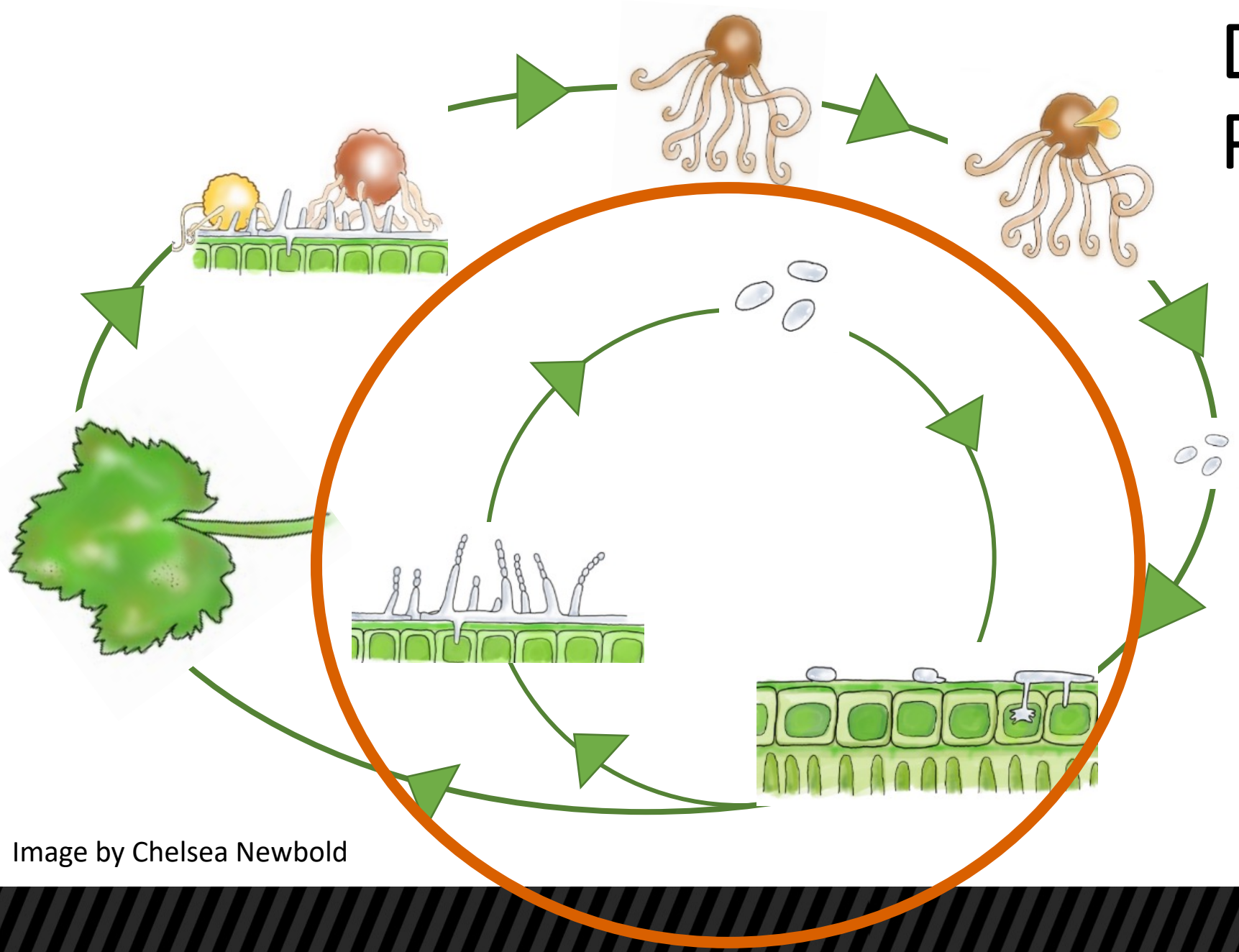


Image by Chelsea Newbold

Pathogen -

Erysiphe necator

‘Polycyclic’ disease

- A fresh disease colony can produce new spores ≥5 days

Grows on green, living tissue

Simulated disease development (untreated block)

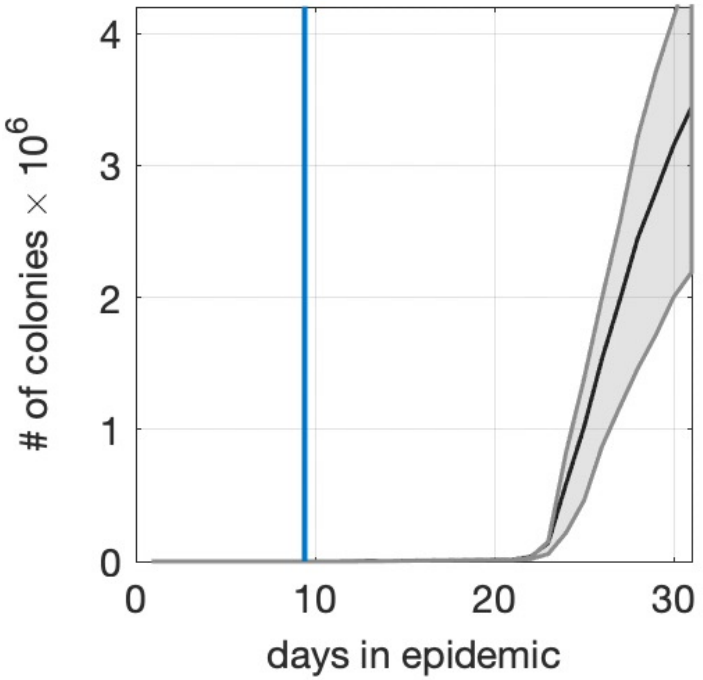
Pathogen spread simulation

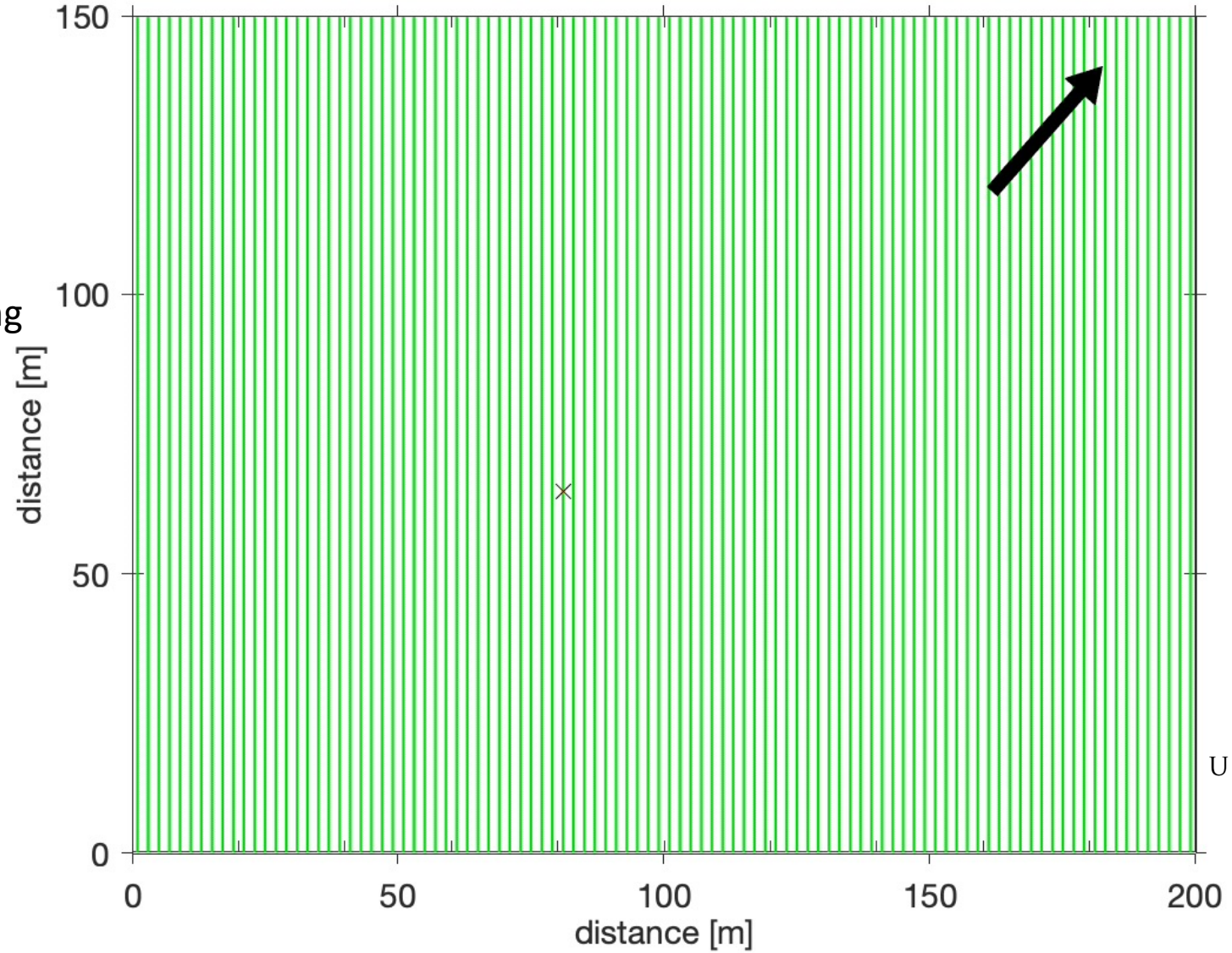
Day 9 Hour 9:00 of epidemic

Wind speed = 0.68 m/s

Wind direction = 221°

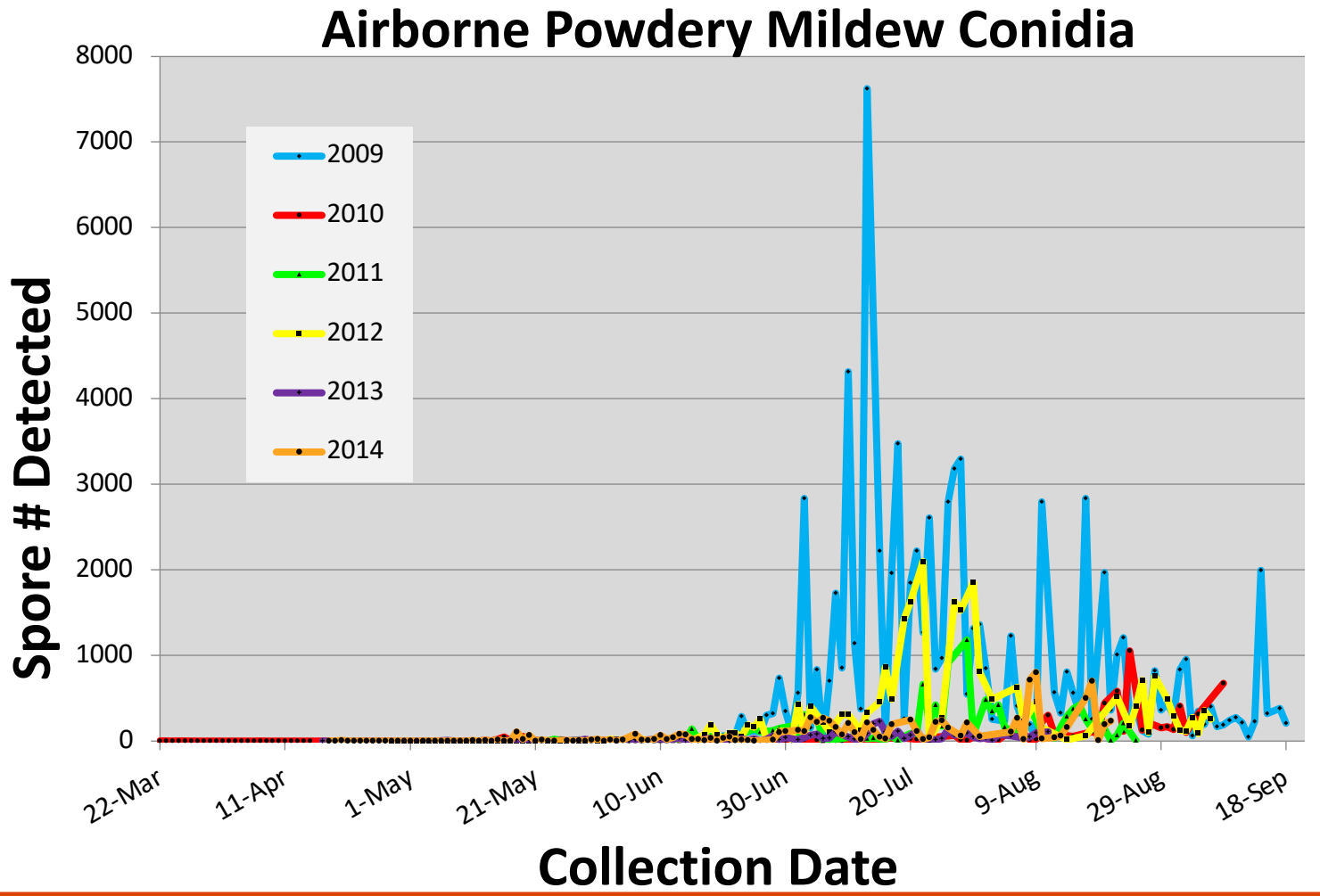
7.4-acre block with 5’ x 7’ vine spacing



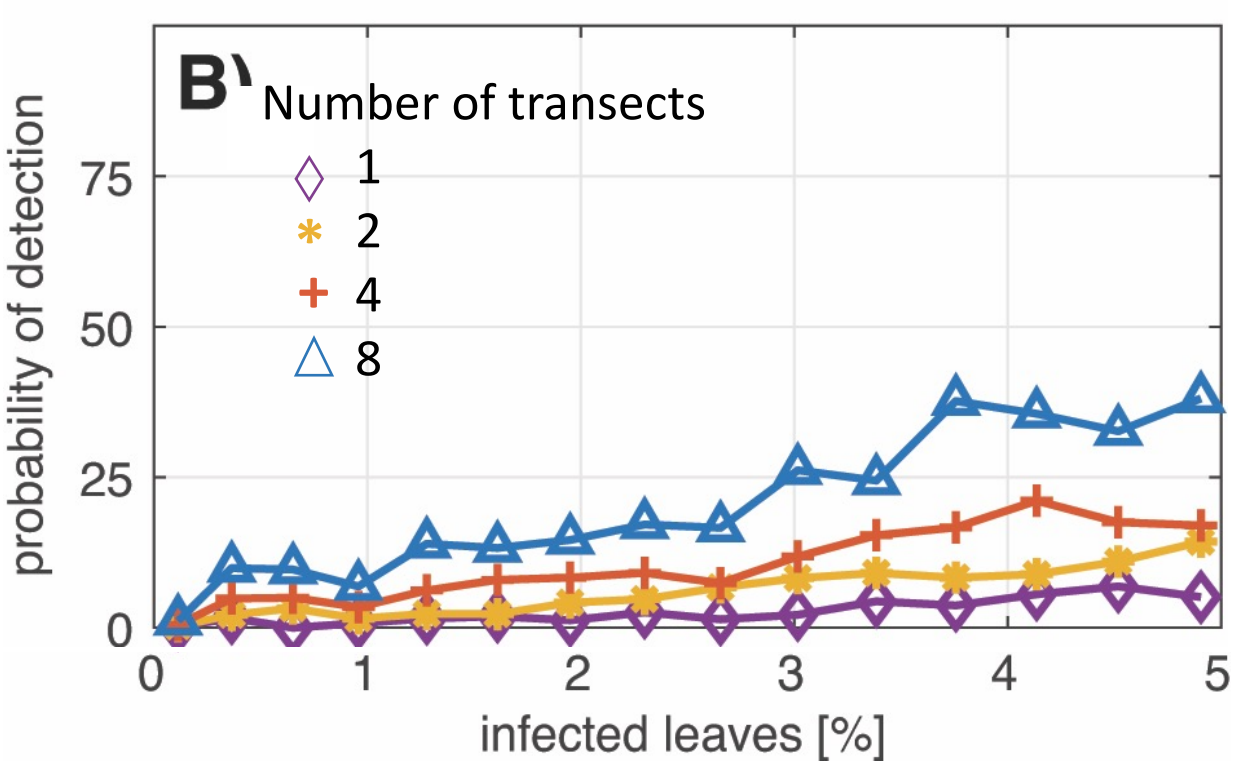


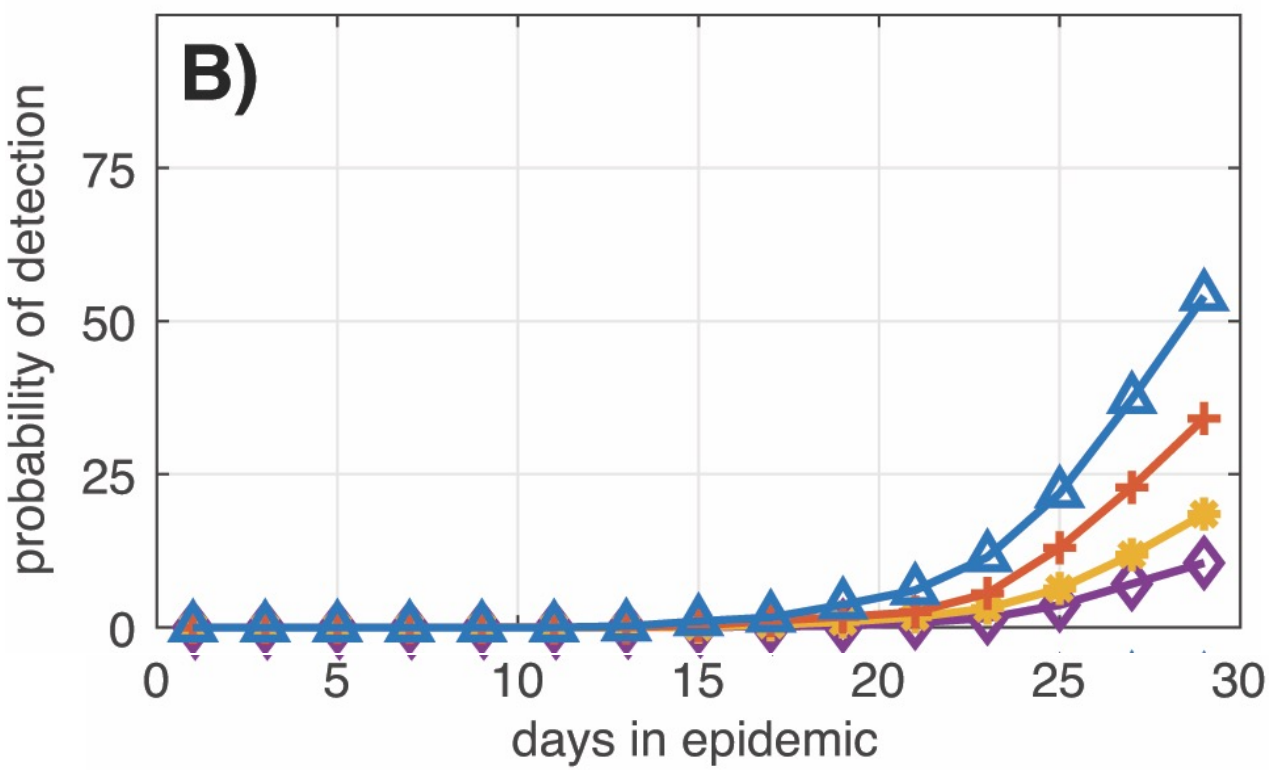
THE UNIVERSITY OF UTAH

Seasonal risk changes!



But it can be hard to find…

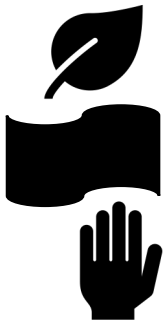




Want to play spot the mildew?

How do we increase our odds?

Types of spore samplers



- Leaf Swabs
- Spore Traps
- Glove Swabs

Types of spore samplers

- Leaf Swabs
- Impaction Traps
- Glove Swabs

“Leaf Swab” - After visual assessment, collection of purported *E. necator* from leaf tissue using a cotton swab

(+) Visual Assessment AND
(+) qPCR result
= (+) Leaf swab sample

Types of spore samplers

- Leaf Swabs
- Spore Traps
- Glove Swabs

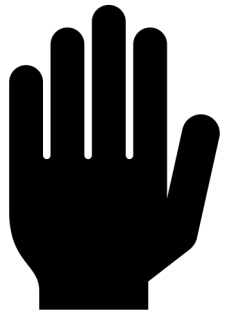
Impaction “Spore Trap”
air samplers
to collect airborne spores

- Commercially available service

Thiessen et al 2016, 2017

Types of spore samplers

- Leaf Swabs
- Spore Traps
- Glove Swabs



Workers manipulate the canopy throughout season

Question:

Can swabbing worker gloves can be a viable way to collect information on the disease quickly and inexpensively in the field?

Photo: Heather Daenitz

Types of spore samplers

- Leaf Swabs
- Spore Traps
- Glove Swabs

Photo: Heather Daenitz

<u>Leaf swab vs. Glove swab</u>

Collected 2018-2020 from 12, 24, and 7 blocks, respectively in OR, WA (2019), and CA (2019)

<u>Spore trap vs. Glove swab</u>

Collected 2019-21 from 12, 19, and 15 sites, respectively in OR.

How to take Glove Swabs:

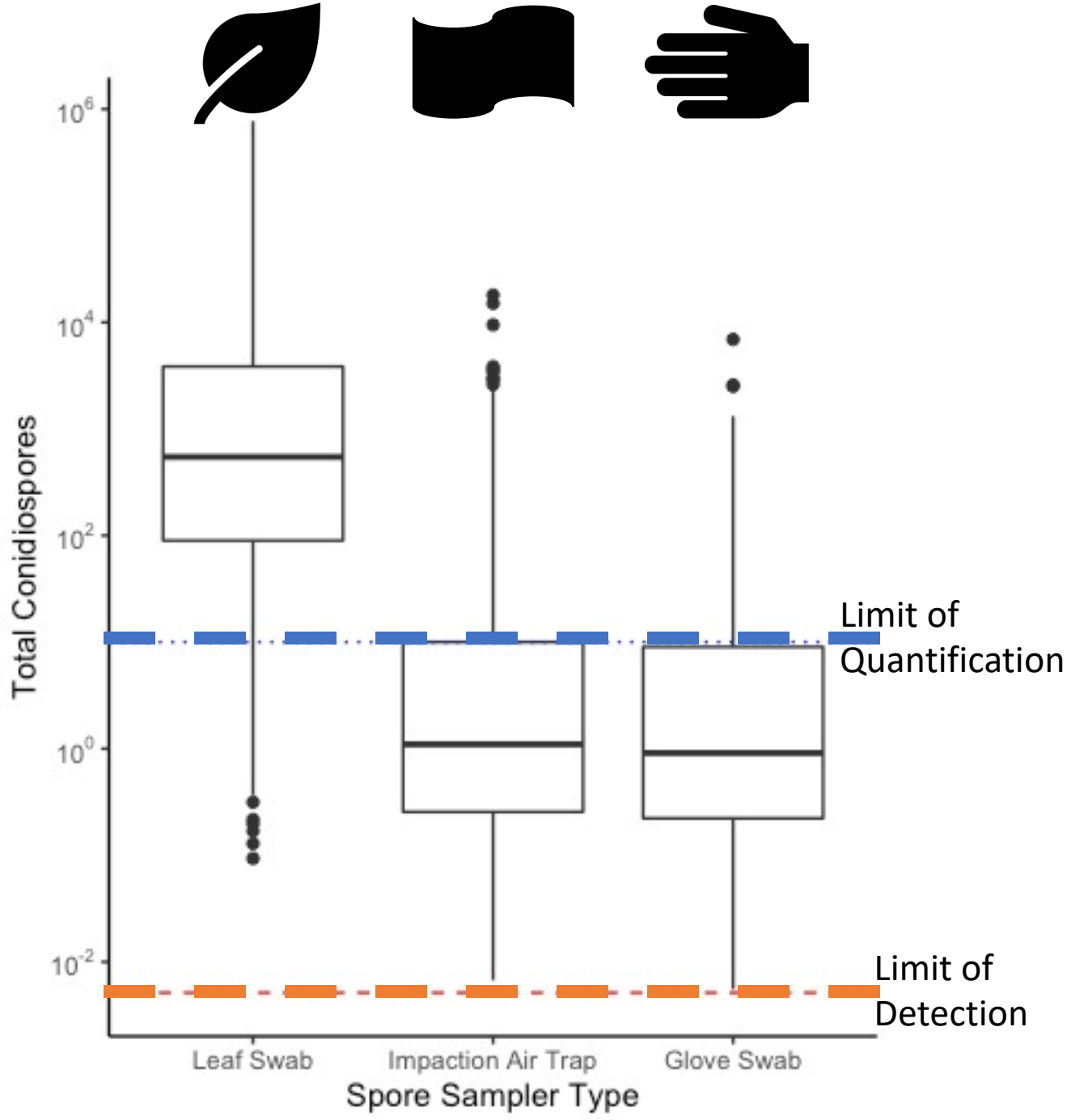
1. Rustle leaves along the row with your hands
2. Label sample
3. Push swab out
4. of protector and
5. rub cotton tip over hands
6. Rinse hands with spray bottle of water and dry
7. Repeat for additional glove samples

video tutorial: https://youtu.be/VuEBF-1Md08

Spore sampler, spore quantification

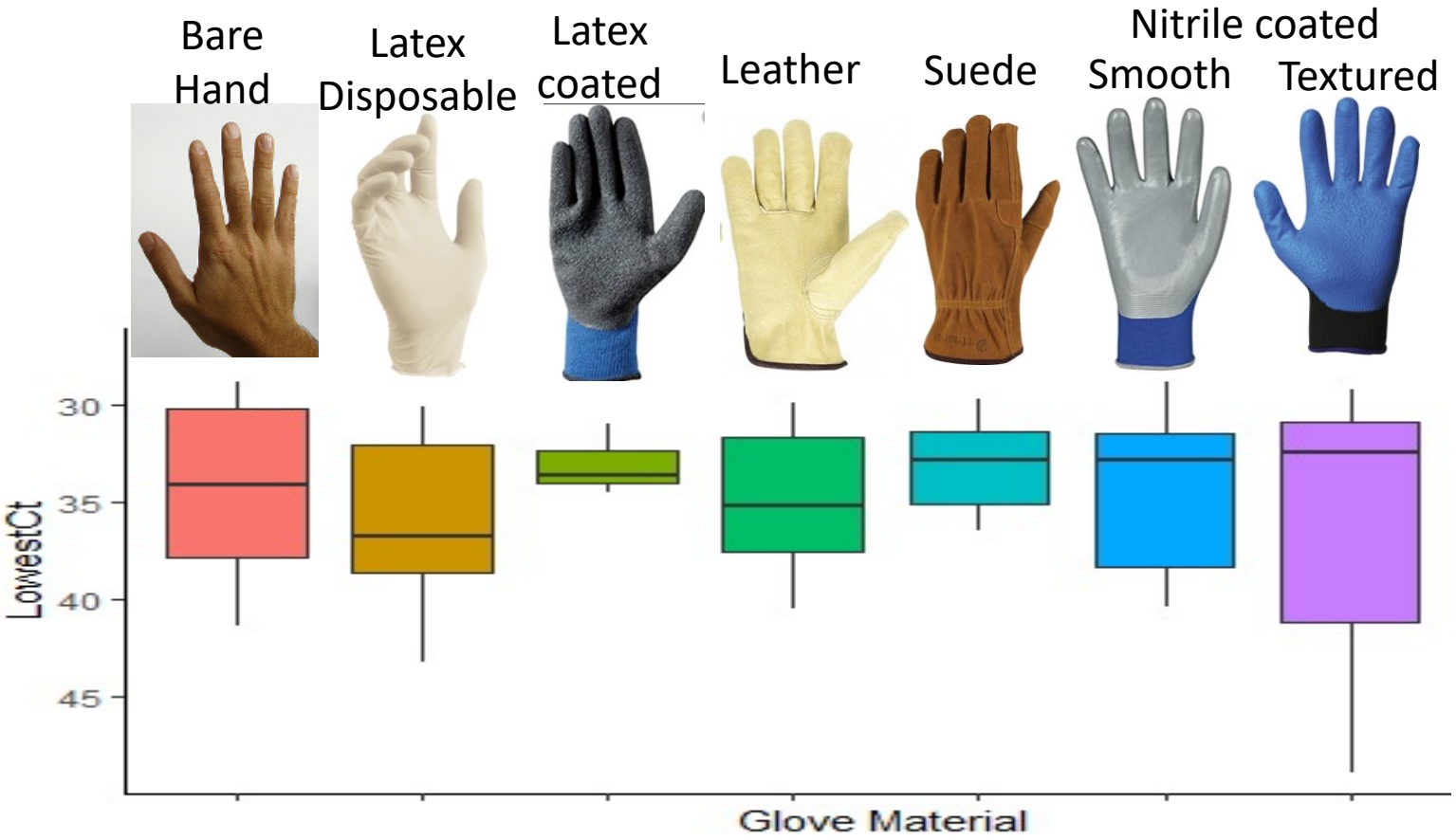
- Field collected sample comparison
- Subset of samples run on *Unc* qPCR assay (Thiessen et al., 2016)

- Quantification threshold is ~ 10 spores



Does the glove material matter?

- No significant difference between glove materials!
- Only differences came from location (ANOVA p=0.7)
- Less ripped and cleaner gloves may help, though!



Latent Class Analysis (LCA)

- Goal: Evaluate of diagnostic assays without known ‘true’ disease status

- LCA statistically constructs a reference standard to estimate:
  1. Latent class membership ← (*E. necator* presence OR absence)
  2. Technique sensitivity ←(rate of false negatives)
  3. Technique specificity ←(rate of false positives)

- Assumes the ‘true’ disease status are linked to diagnostic index tests.

Latent Class Analysis (LCA)

Year	Leaf Swab	Glove Swab	Count
2018	+	+	92
2018	+	-	9
2018	-	+	102
2018	-	-	123
2019	+	+	120
2019	+	-	7
2019	-	+	221
2019	-	-	110
2020	+	+	78
2020	+	-	8
2020	-	+	21
2020	-	-	24

1. Cannot estimate 2x2 tables
2. Year treated as independent populations
3. Disease incidence different each year, estimates for each year were allowed to be freely estimated

Latent Class Analysis (LCA) Glove vs. Leaf Swabs

Disease Incidence

Sensitivity and Specificity

				Leaf Swabs				Glove Swabs			
Year	n	LCA Est. Disease Incidence	SEM	Sensitivity	SEM	Specificity	SEM	Sensitivity	SEM	Specificity	SEM
2018	326	57%	25%	0.67	0.36	0.96	0.06	0.95	0.04	0.67	0.36
2019	458	51%	50%	0.51	0.46	0.97	0.06	0.97	0.05	0.51	0.46
2020	136	64%	15%	0.85	0.12	0.88	0.23	0.94	0.08	0.74	0.12

LCA Model fit statistics: $G^2 = 4.04$, AIC = 58.04, BIC = 188.15, df = 8

Latent Class Analysis (LCA)

Glove vs. Spore Trap

Disease Incidence

Year	n	LCA Est. Disease Incidence	SEM
2019	31	100%	-*
2020	131	66%	19%
2021	44	47%	25%

Sensitivity and Specificity

Spore Traps				Glove Swabs			
Sensitivity	SEM	Specificity	SEM	Sensitivity	SEM	Specificity	SEM
0.84 =	-	0.69 ↓	-	0.84 =	-	0.93 ↑	-
0.90 ↓	0.18	0.81 ↑	0.18	0.96 ↑	0.08	0.65 ↓	0.3
0.96 ↑	0.09	0.6 ↓	0.25	0.84 ↓	0.34	0.88 ↑	0.12

*Could not calculate

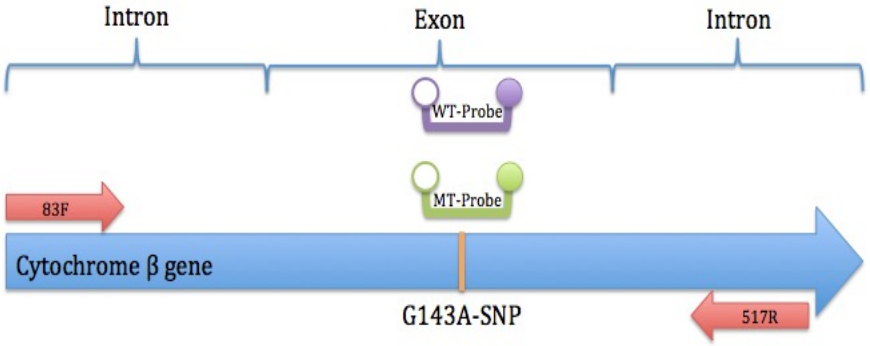
LCA Model fit statistics: $G^2 = 4.68$, AIC = 58.68, BIC = 148.66 , df = 8

Conclusions

- Glove swabs are more sensitive than leaf swabs
- Glove swabs provide very similar to information impaction spore traps

Molecular identification – G143A for QoI/Strobilurin fungicide resistance

- G143A mutation is the only mutation found in GPM for FRAC 11 resistance
- 100% agreement with QoI resistance bioassays
- Sensitive to a single spore



Resistant

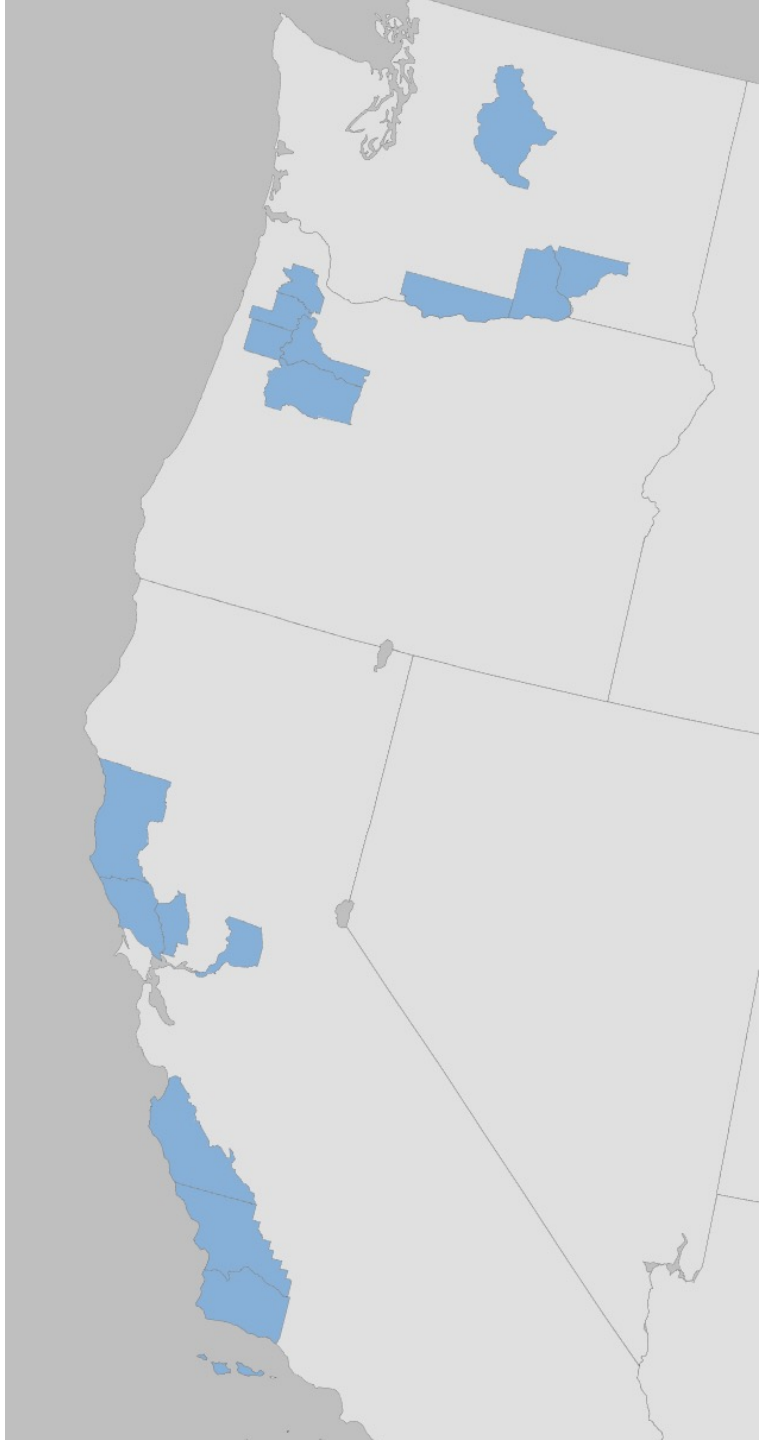
Mixed (Resistant AND Susceptible)

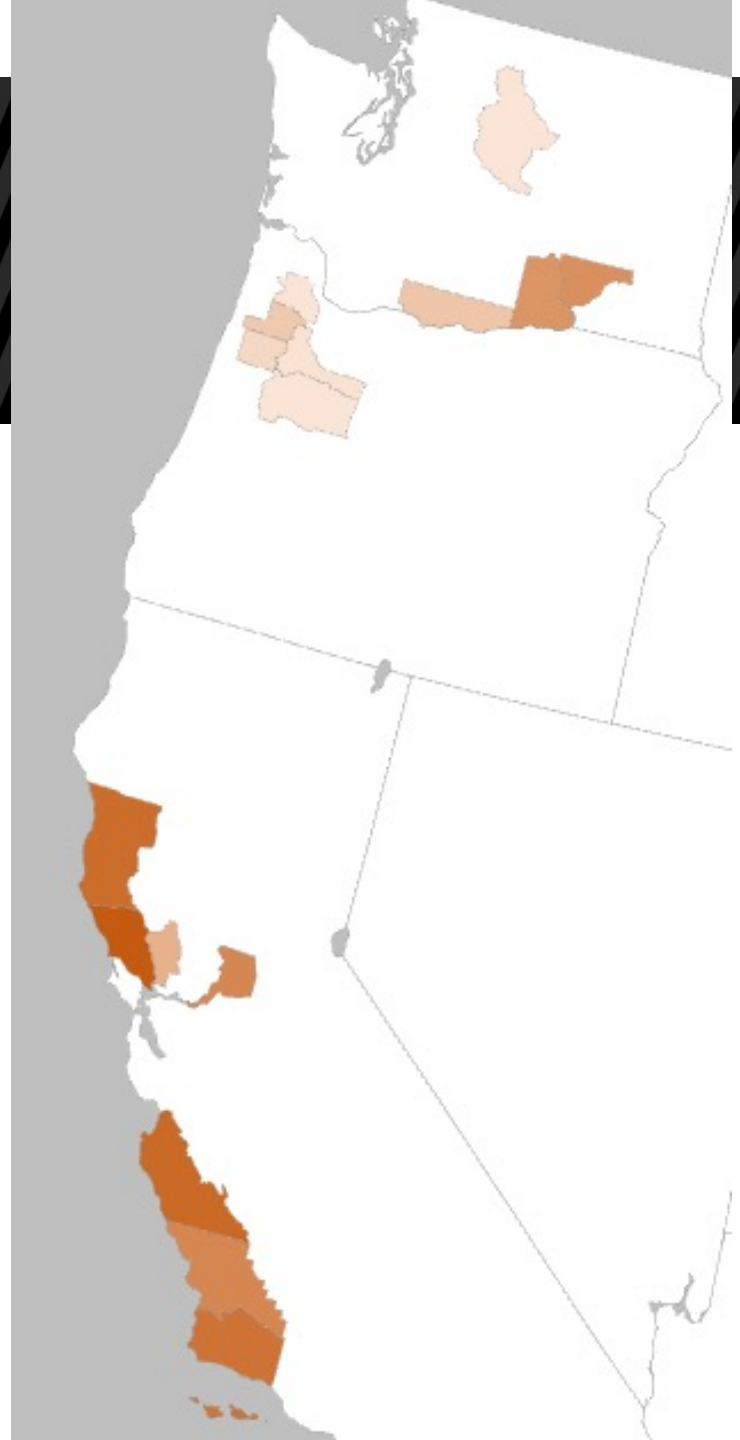
Susceptible

Miles, et. al. 2021

Glove Swab Samples

State	County	Vineyards	# Organic	Years Sampled
Washington	Benton	14	1	2019-20
	Chelan	4	2	2019-20
	Franklin	5	1	2019-20
	Klickitat	1		2019
	Morrow	1		2019
Oregon	Linn	2		2019-20
	Marion	17		2018-20
	Polk	12	6	2018-20
	Washington	1		2019
	Yamhill	11	2	2018-20
California	Mendocino	1		2019
	Monterey	6		2019
	Napa	3		2019
	Sacramento	1		2019
	San Luis Obispo	12		2019-20
	Santa Barbara	16		2019-20
	Sonoma	3		2019





Resistance Frequency across the Western US Counties sampled

107 vineyards
4857 samples
17 Counties in CA, OR, WA
2018-2020

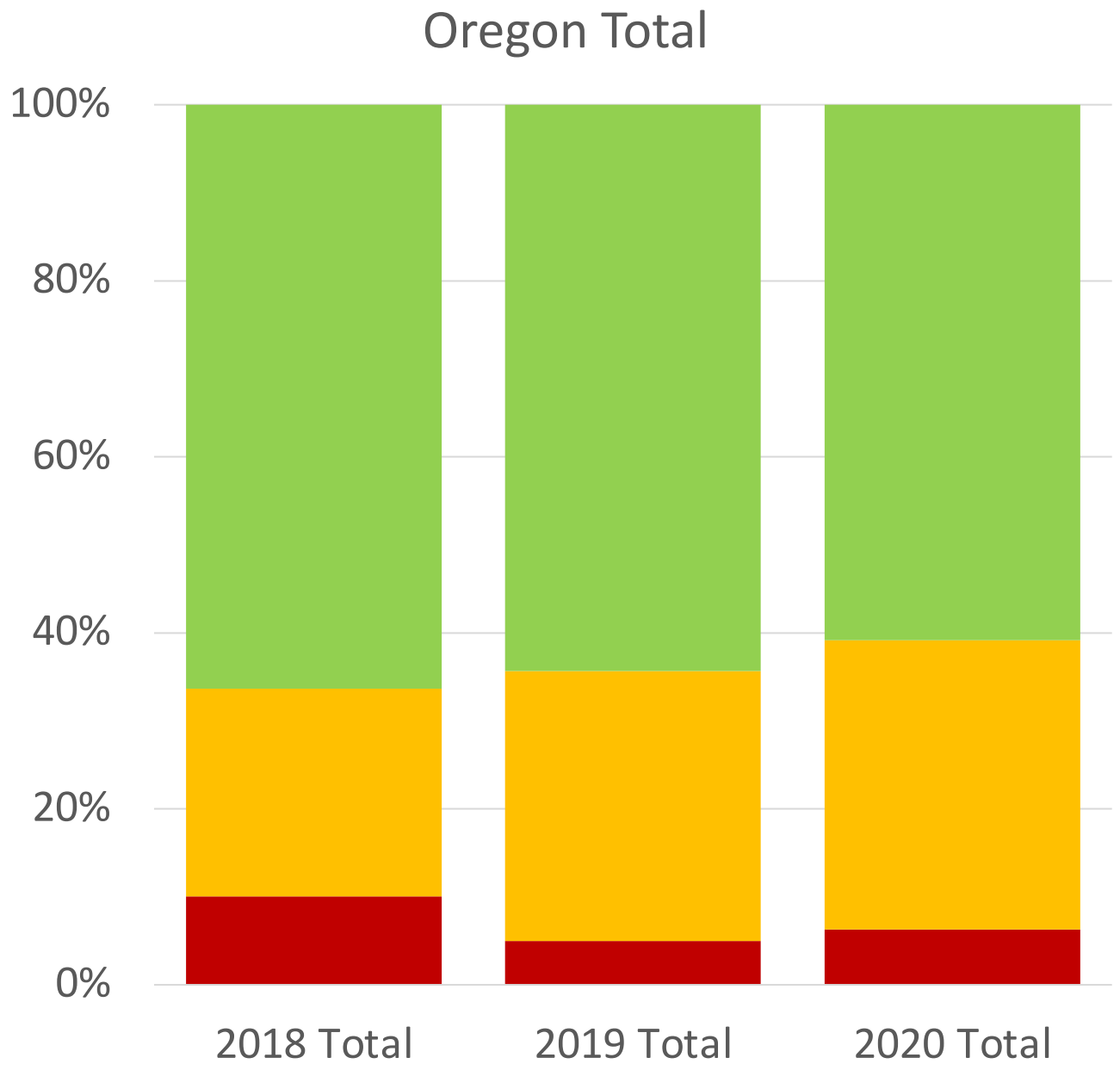
Frequency Resistant

0 0.75

G143A Frequency over the years in Oregon

11 vineyards

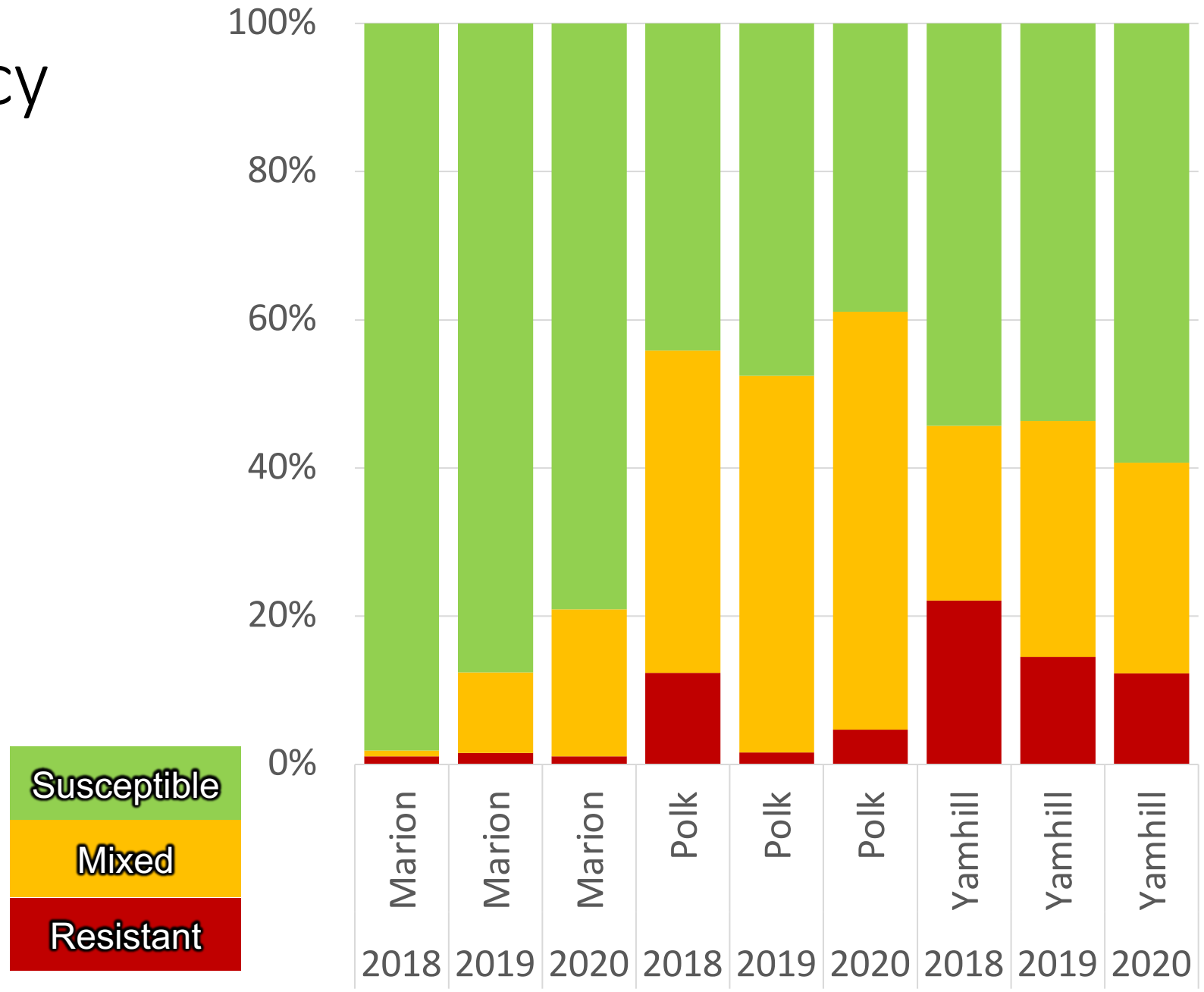
N = 2418



G143A Frequency over the years in Oregon

11 vineyards

2418 samples



G143A Frequency over the years

Washington:

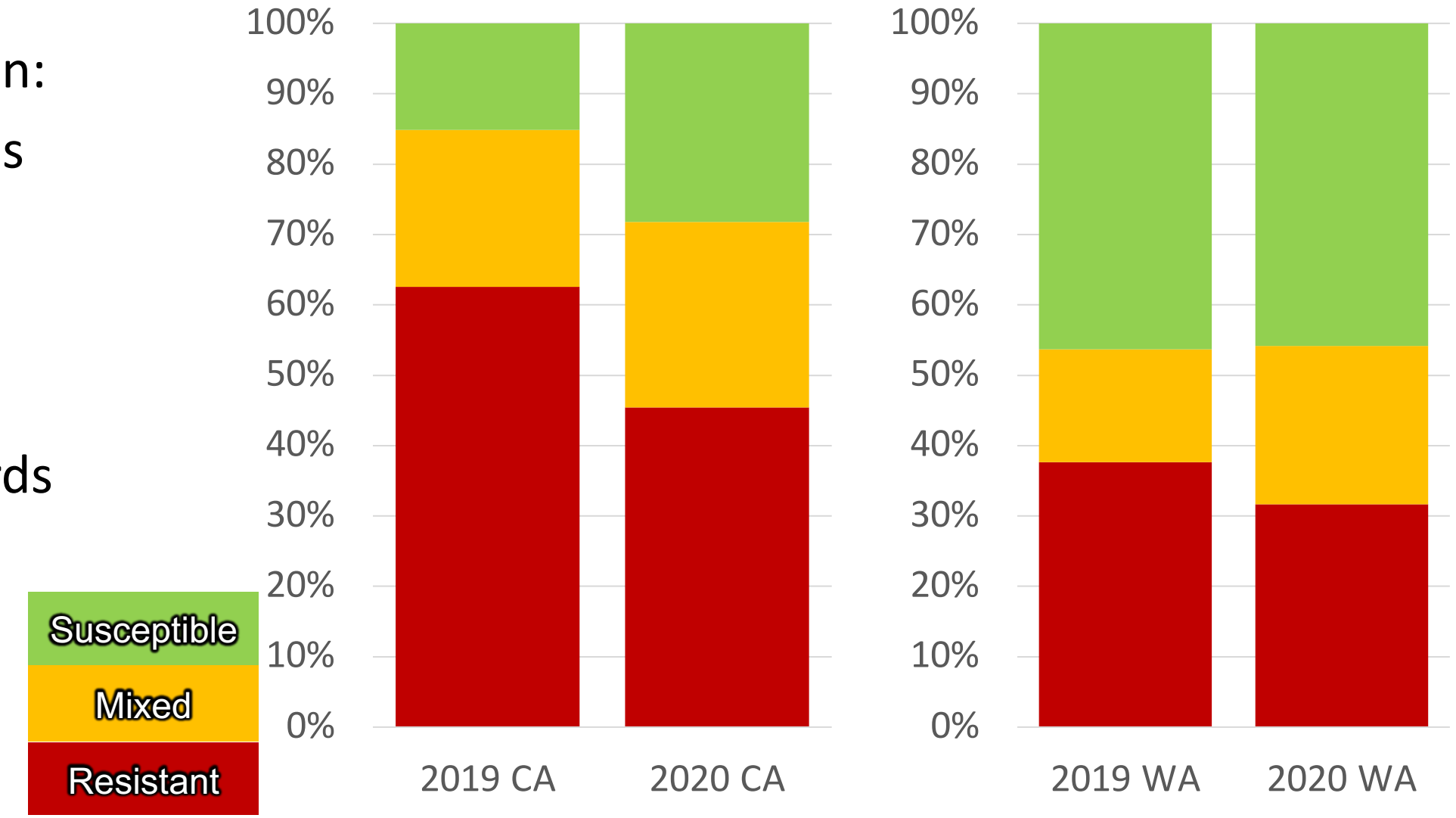
5 Vineyards

N = 250

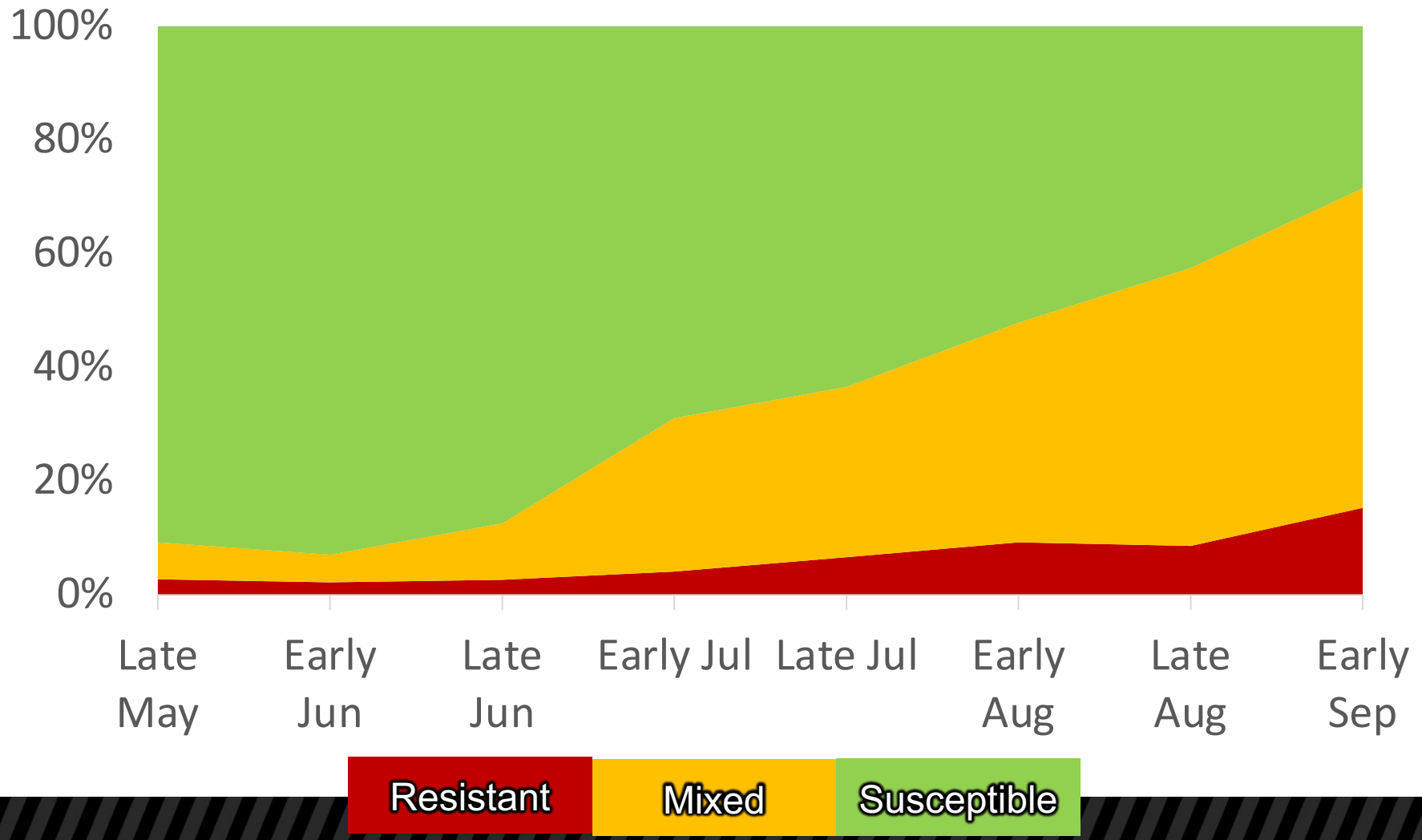
California:

12 Vineyards

N = 493

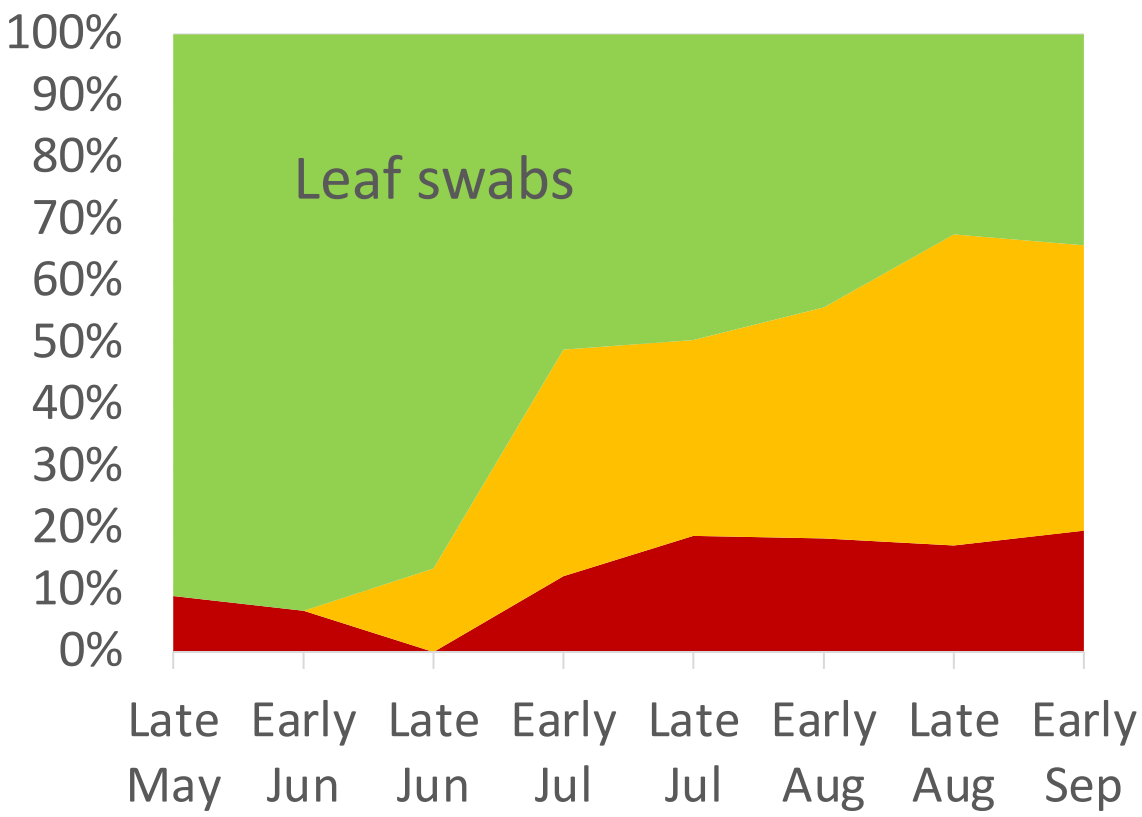


G143A Frequency within the season

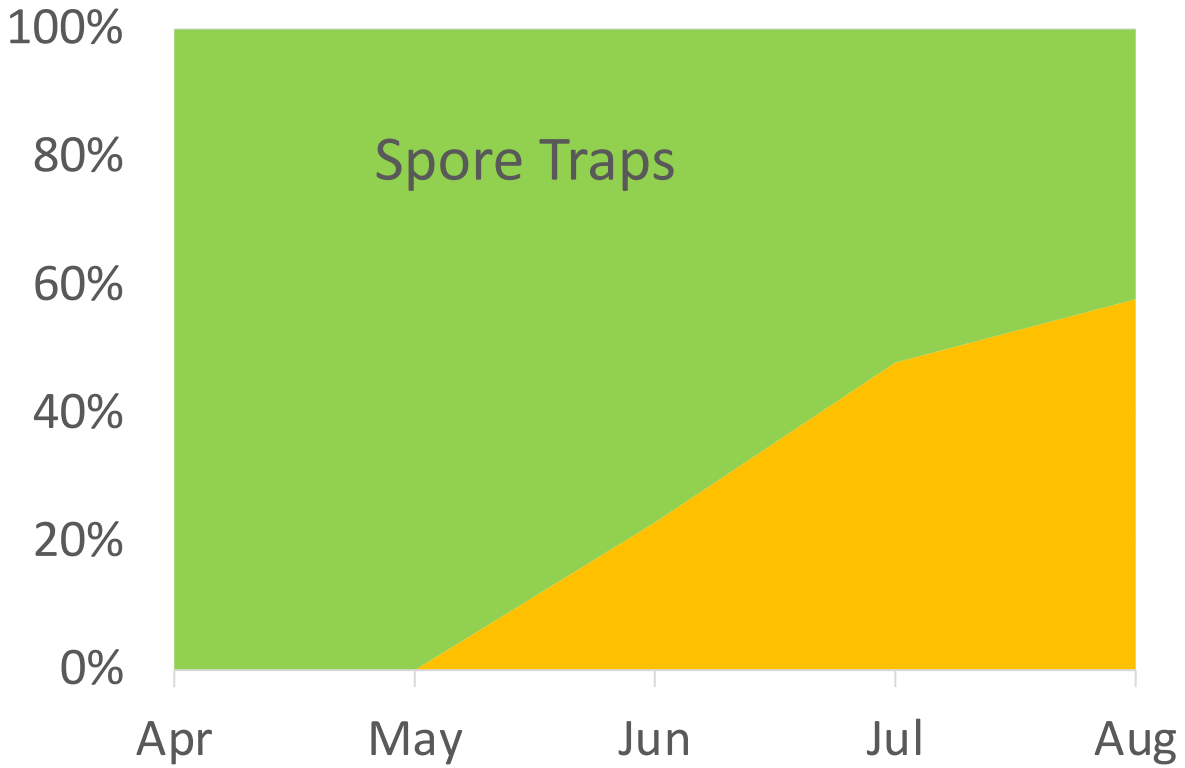


All of Oregon Samples
N=3117

G143A Frequency within the season by sample type



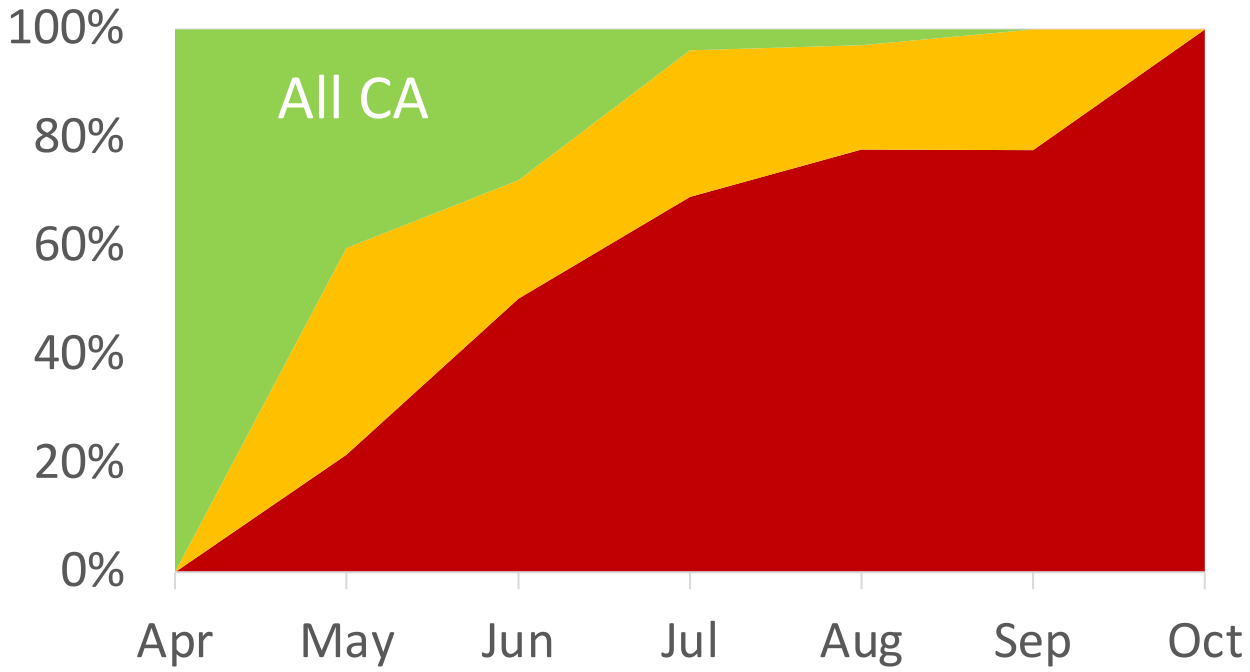
N=1179

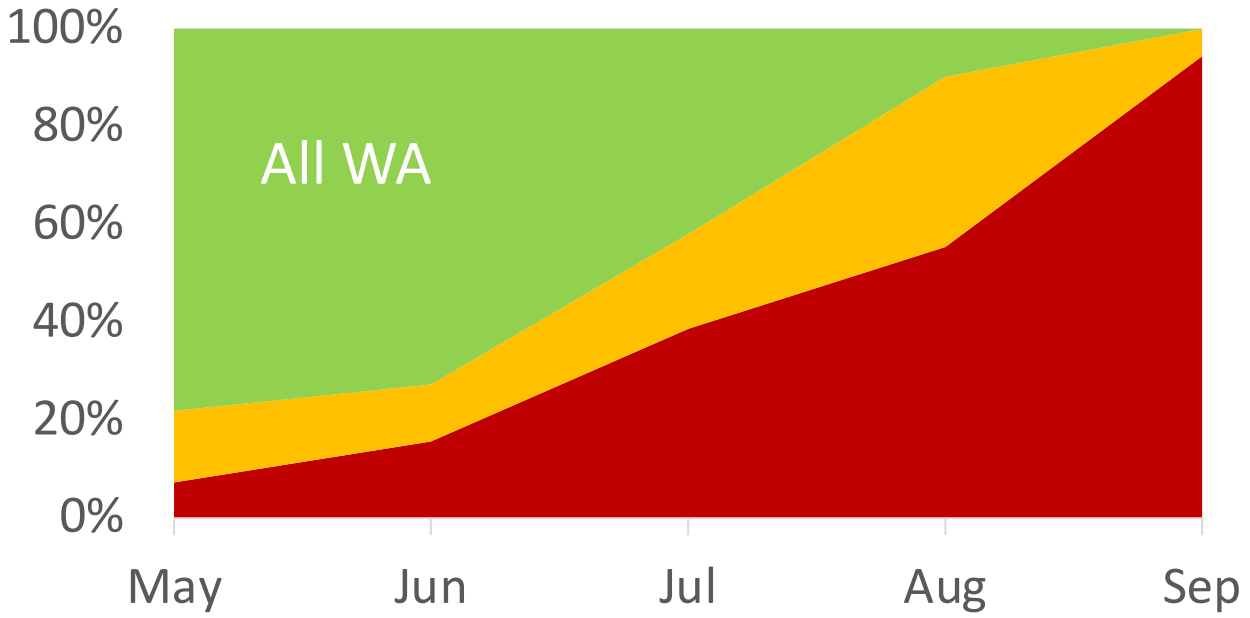


N=86

Resistant Mixed Susceptible

G143A Frequency within the season





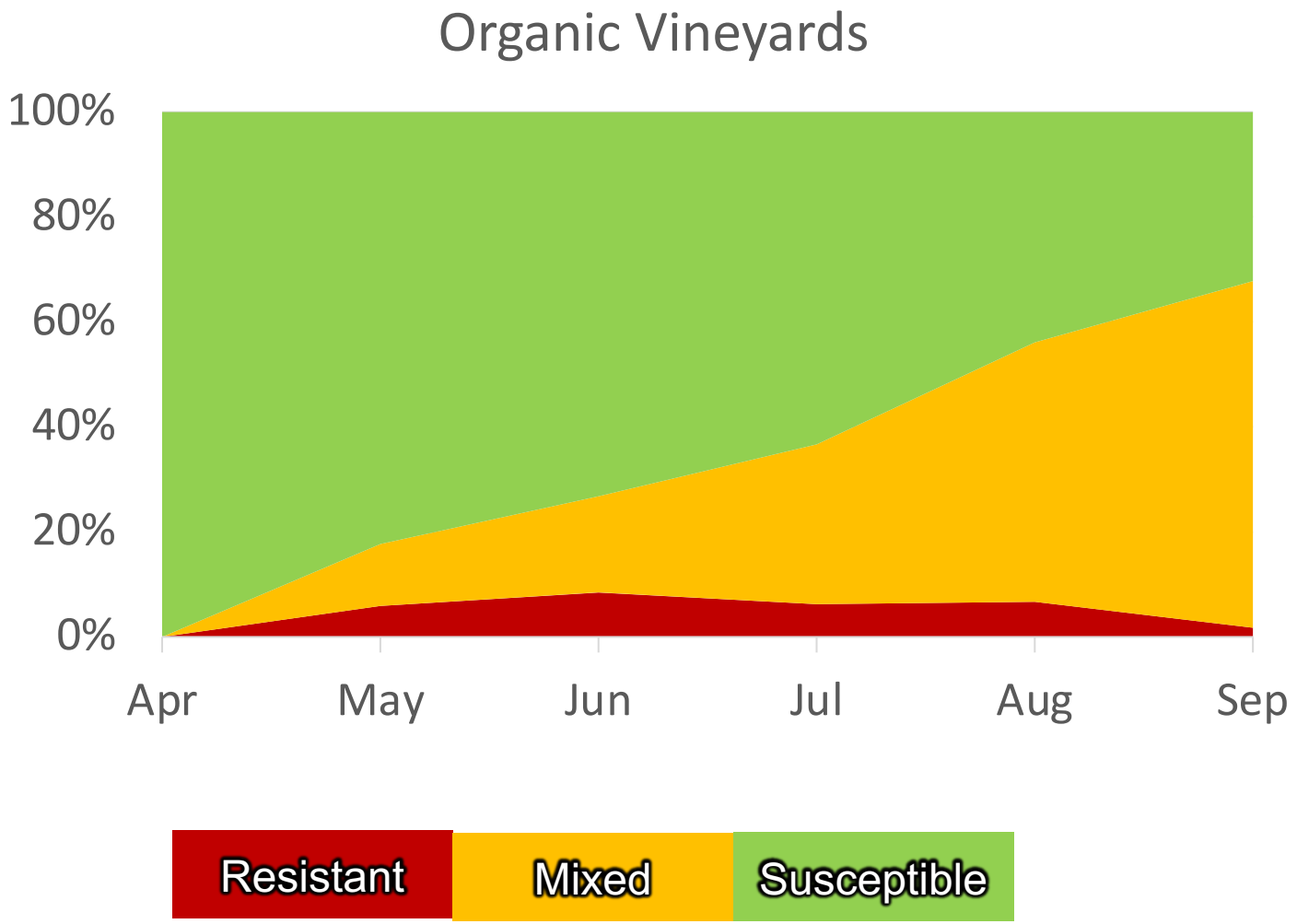
Resistant | Mixed | Susceptible

G143A Frequency within the season

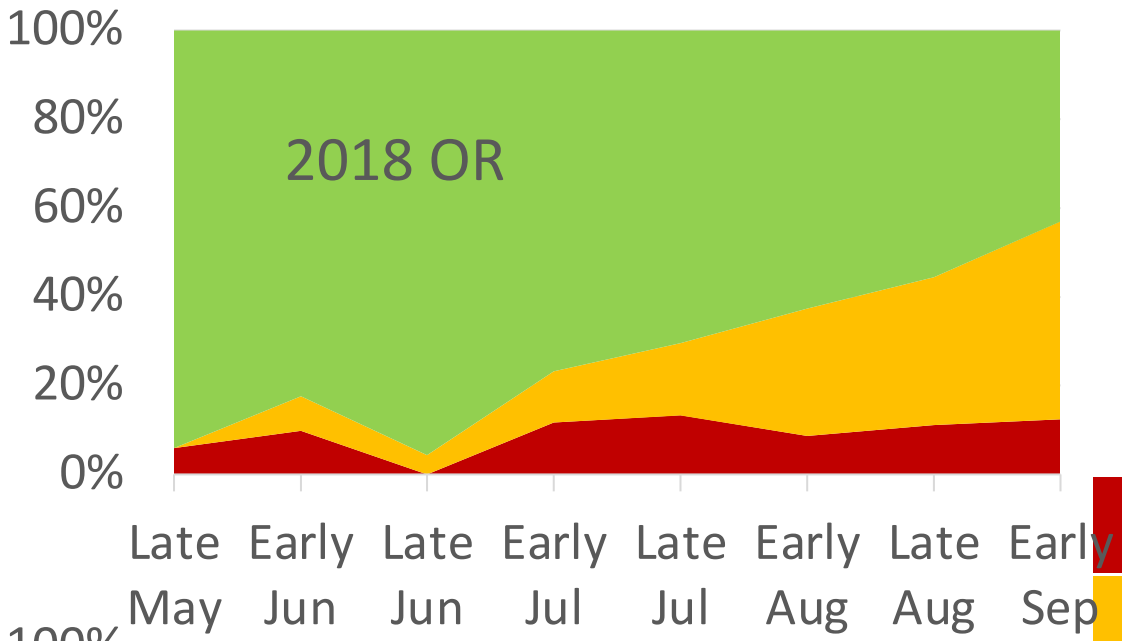
8 vineyards in OR

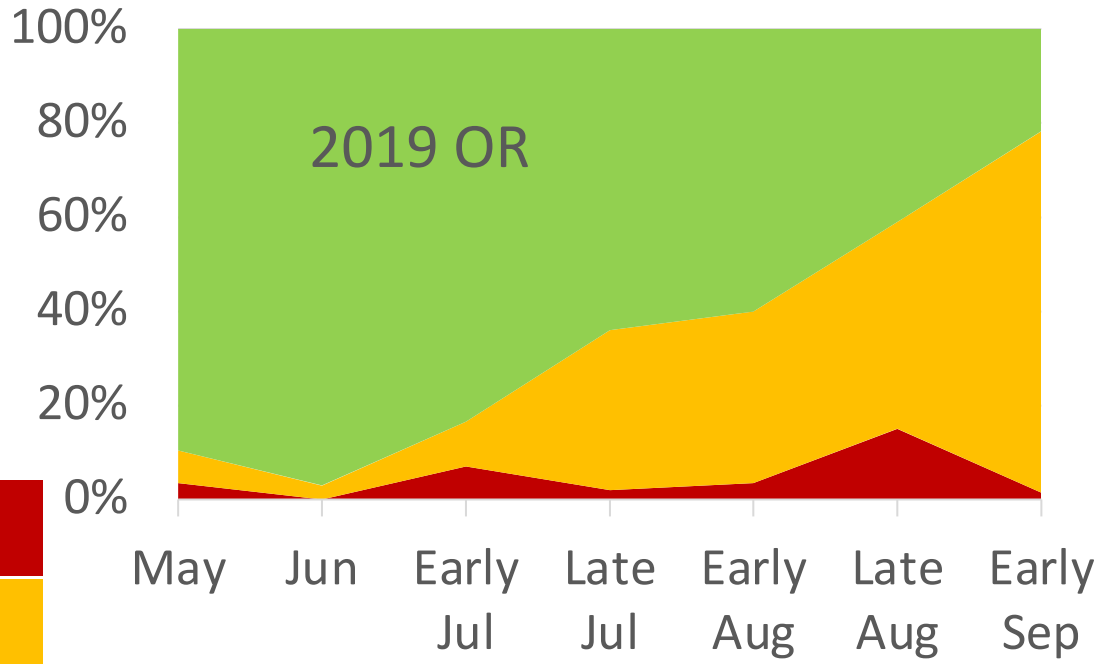
4 vineyards in WA

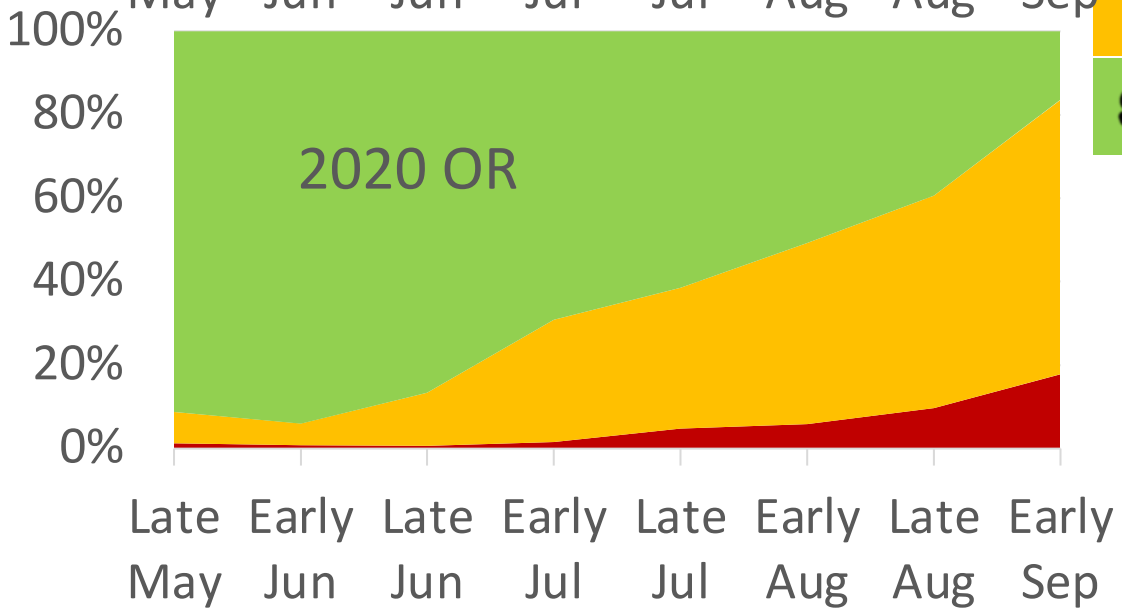
729 samples



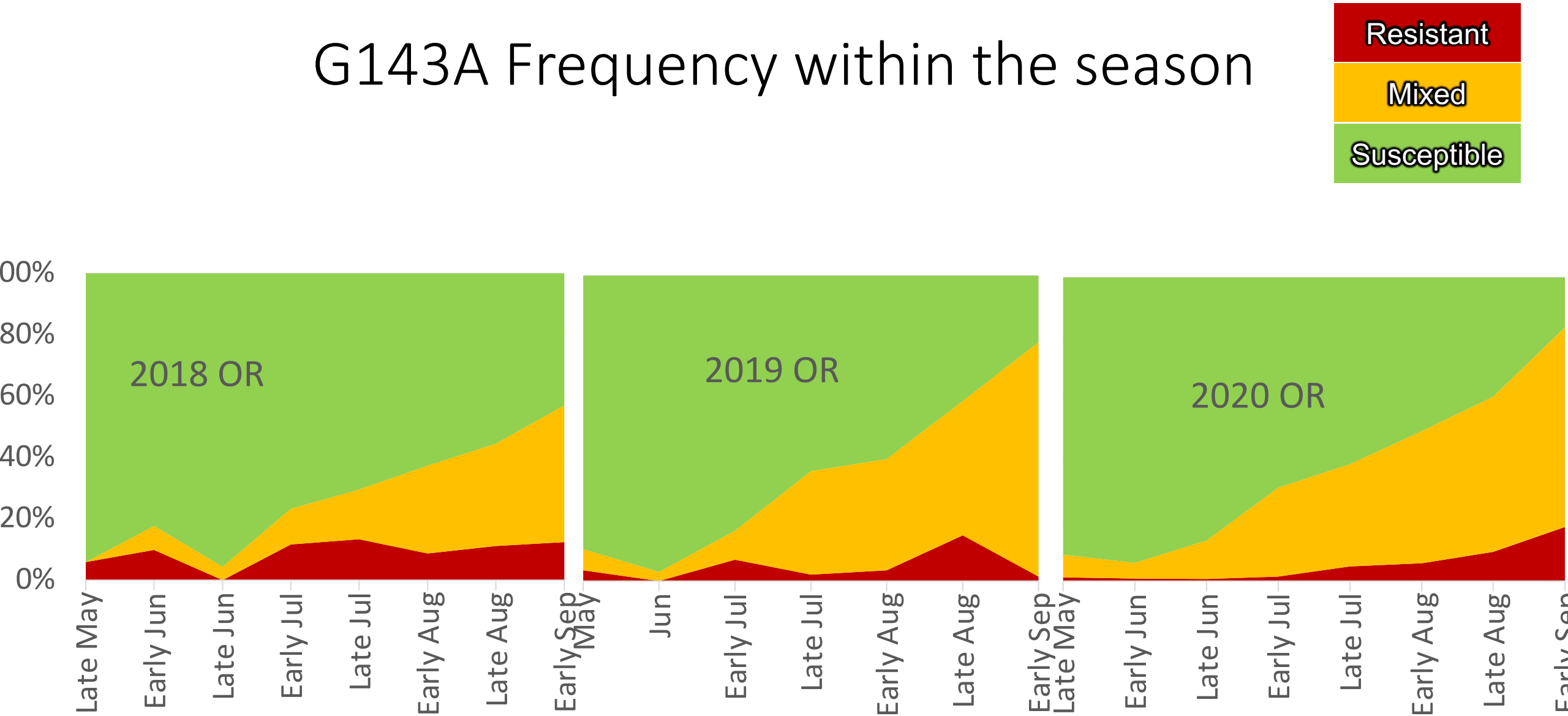
G143A Frequency within the season







G143A Frequency within the season



Monitoring after QoI use

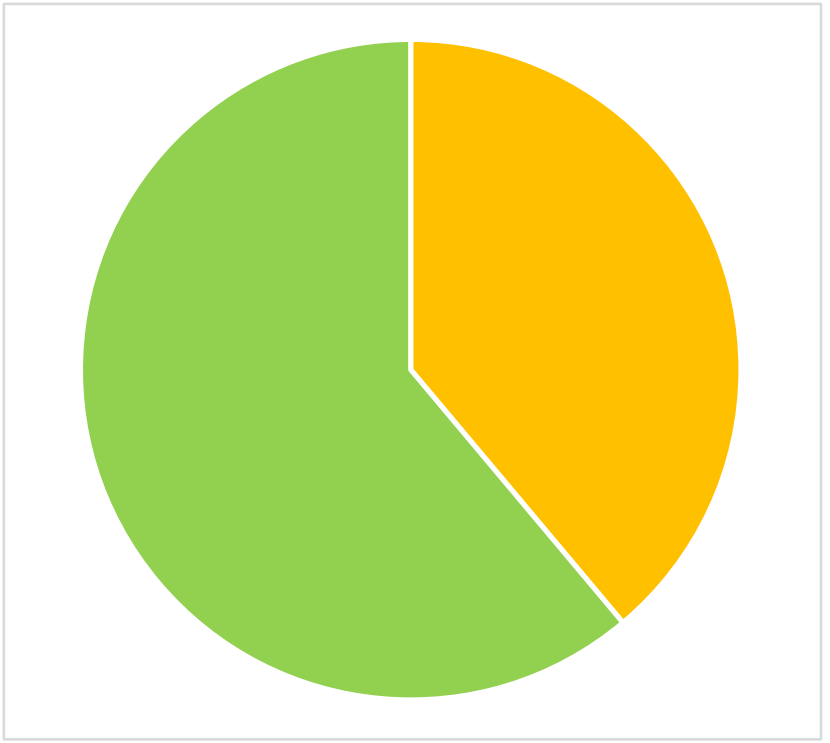
- 2020 Flint Extra was sprayed on July 7th, on blocks 1 and 2, with Vivando on blocks 3 and 4

Resistant

Mixed

Susceptible

Field 2019 Resistance

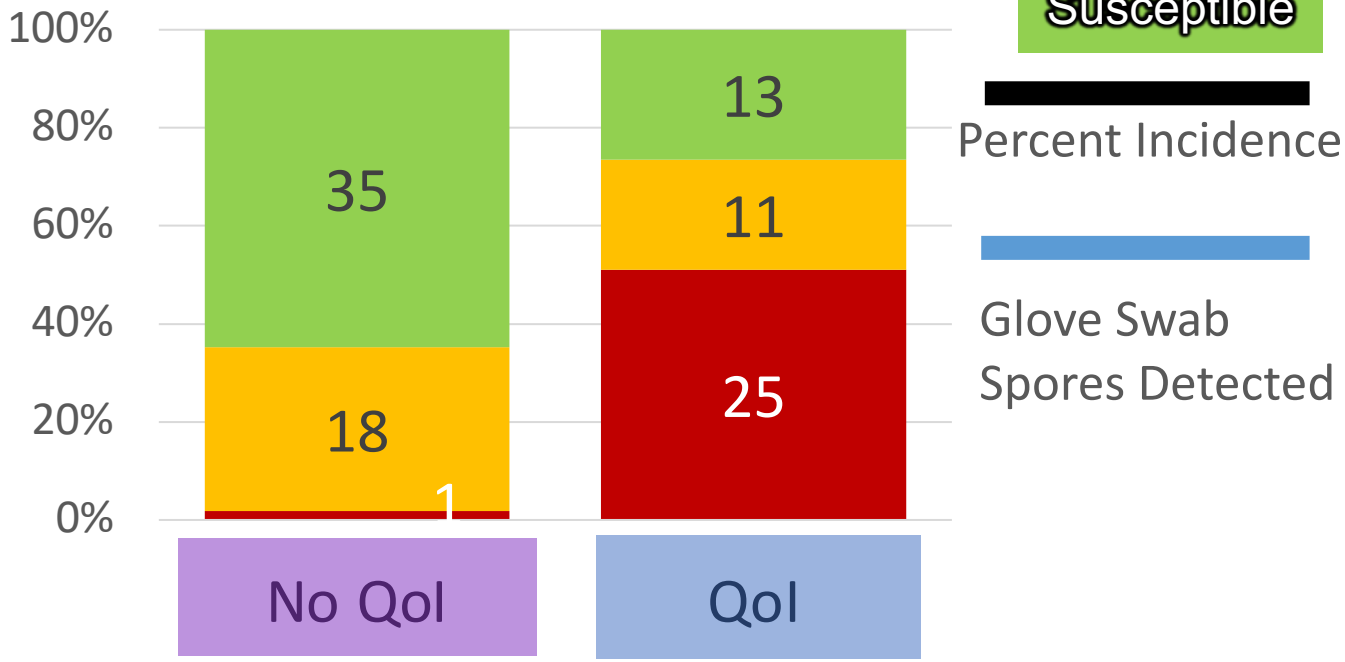


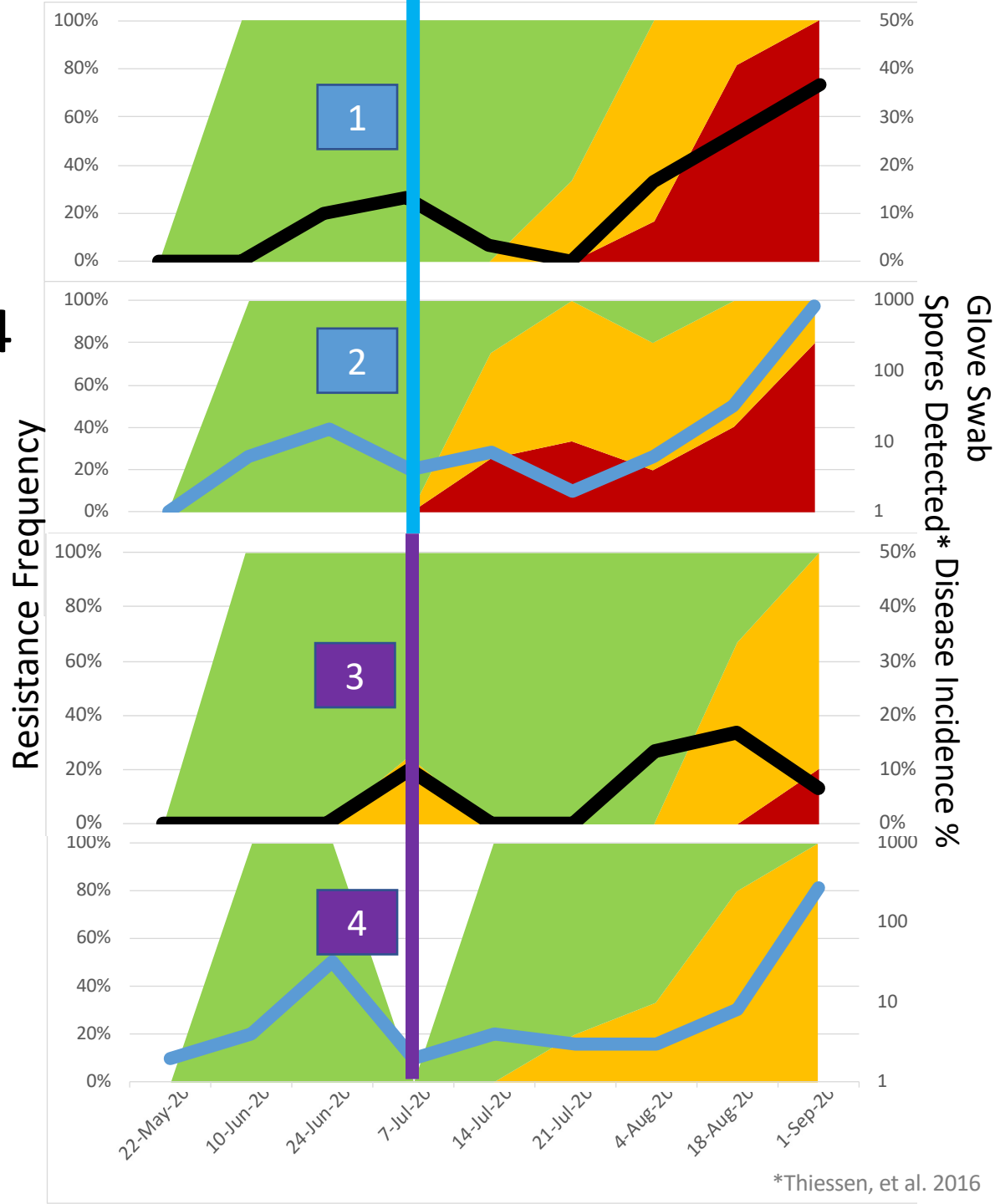
N=18

Tank mixed with 3lbs of sulfur

Monitoring after QoI use

- Flint Extra was sprayed on blocks 1 and 2, with Vivando on blocks 3 and 4
- Disease was very low in all the blocks

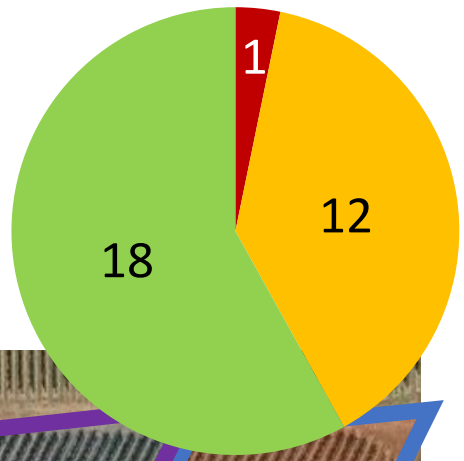


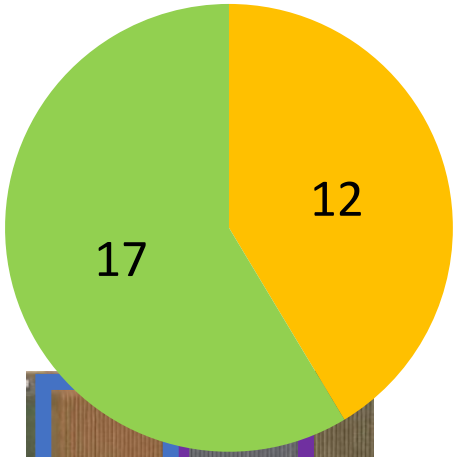


*Thiessen, et al. 2016

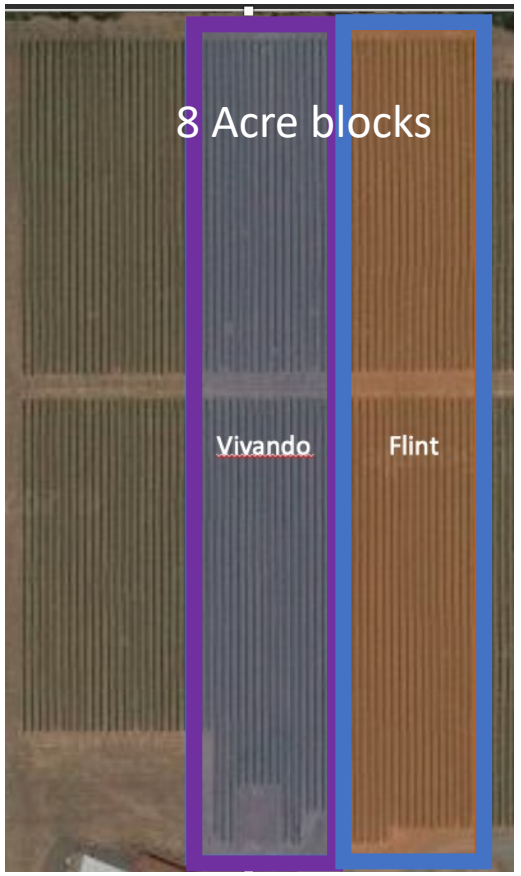
What happens when you spray a QoIs? 2021

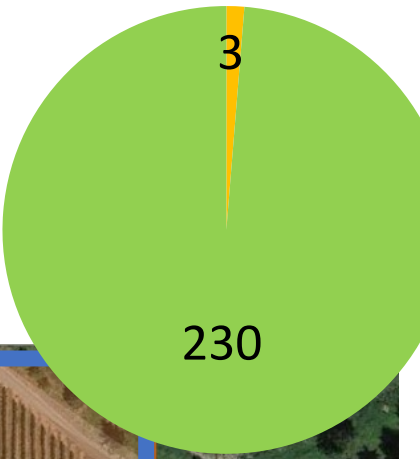
2020 Field Resistance

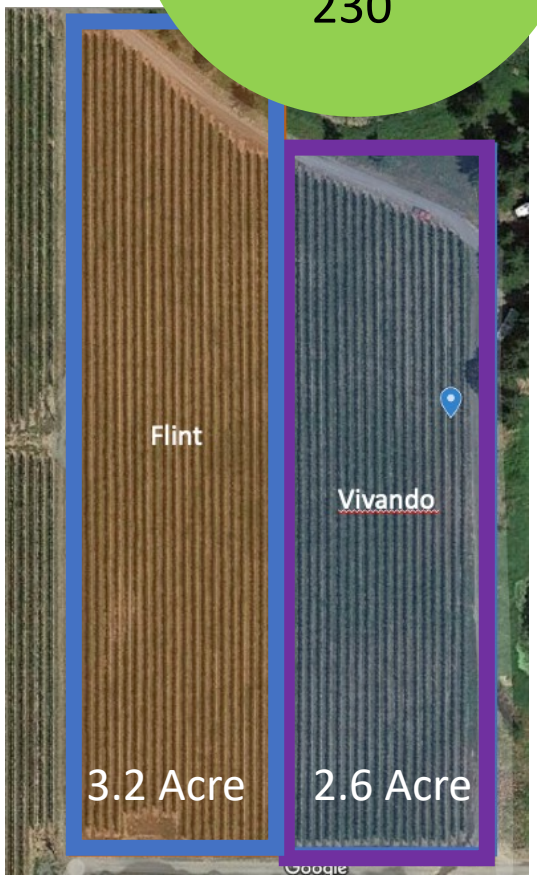




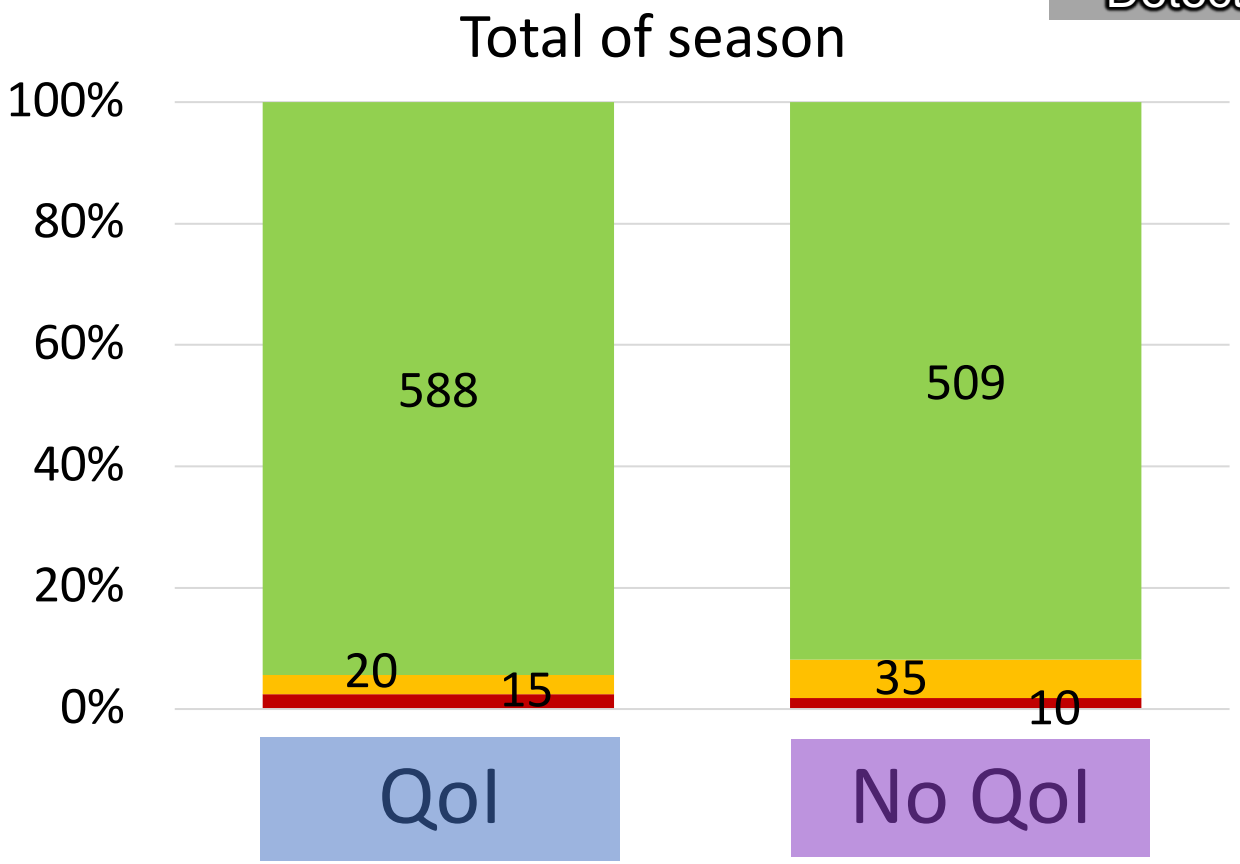
No 2020 data for this field

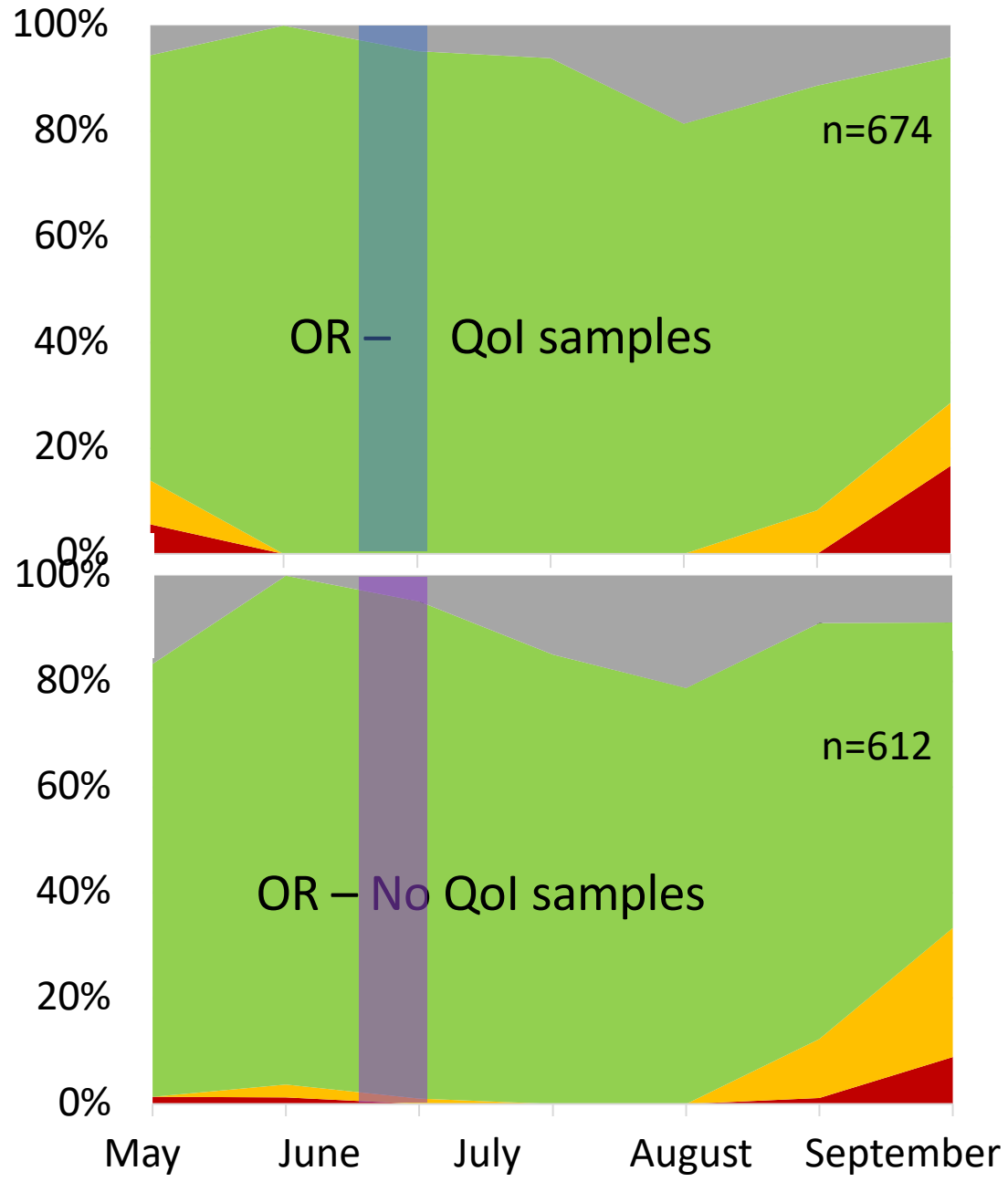






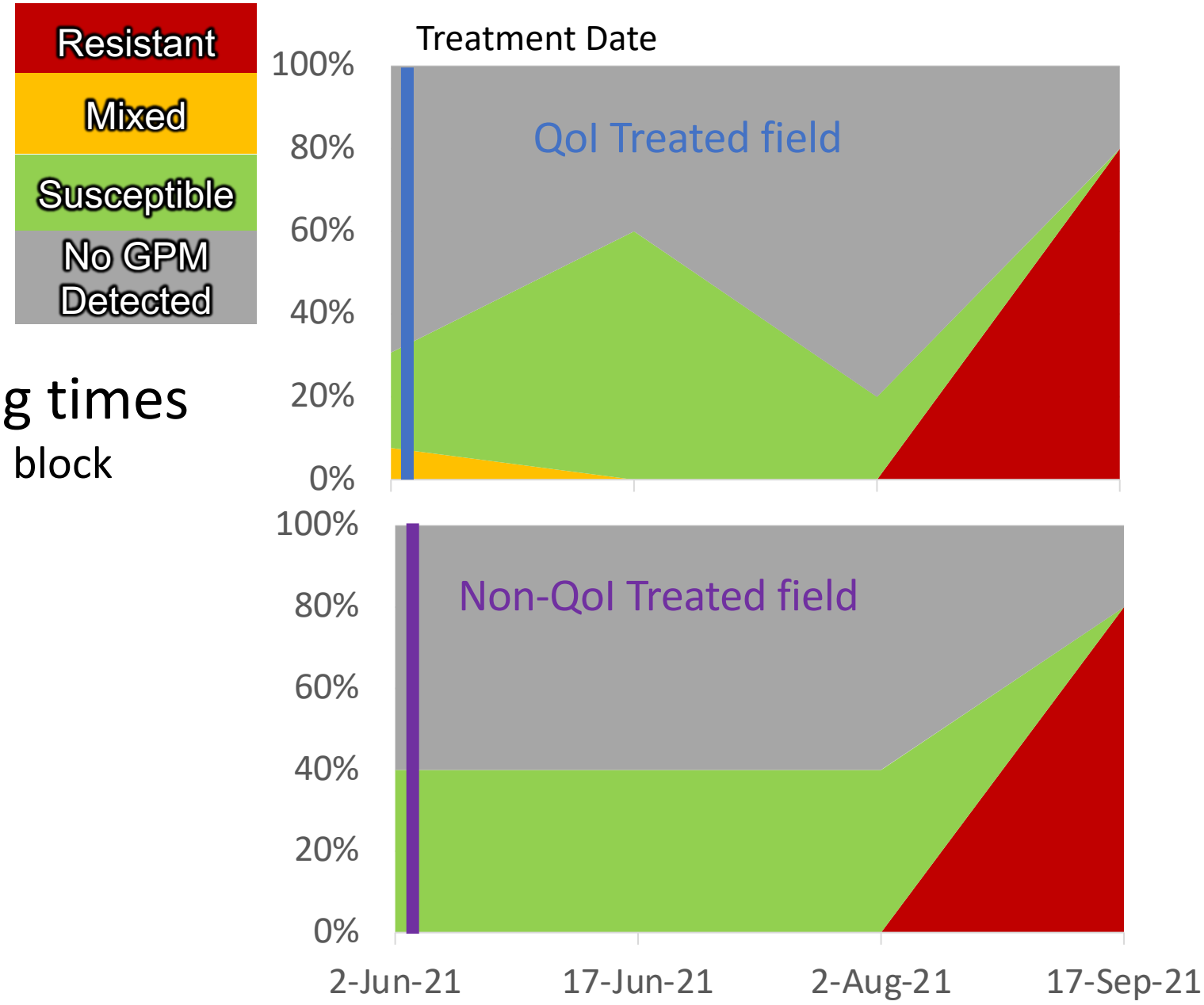
2021 OR Blocks – 4 fields combined





San Joaquin, CA field site

- 5 glove swabs at 4 sampling times
  + a couple leaf swabs on 2 Jun QoI block
- Pristine
  - Boscalid FRAC 7
  - Pyraclostrobin FRAC 11
- Inspire Supra
  - Cyprodinil FRAC 9
  - Difenoconazole FRAC 3
- Blocks >12 Acres each



Samples collected by Paul Walgenbach, Bayer, Inc.

Take Home Messages:

- Oregon has the lowest levels of QoI/group 11 resistance (so far)
- QoI resistance increases within a field season, but there is a decline during dormancy for not much visible change between field seasons
- QoIs can still be used successfully! But you may want to monitor your resistance levels

Foliar Pathology Lab 2022

Walt Mahaffee, Hannah Soukup and Tara Neill: USDA-ARS

Alex Wong, Kate Baldino - Grad Students

Savanah Espinosa, Eliza Allen, Kiersten Brophy, Haylee Garrett, Destiny Perkins, Danielle Scutero - Undergrads

COLLABORATORS

Rob Stoll, Nate Miller

Kam Leang and Eric Pardyjak

Cynthia Schwartz,

Chad Higgins

Michelle Moyer

Charlotte Oliver

Tim Miles

Shunping Ding

Paul Walgenbach, Bayer

Monica Copper, Mark Battany, Larry Bettiga, Glenn McGourty

David Gadoury

José Úrbez-Torres

Brian Bailey,

Ioannis Stergiopoulos

Amy Peetz

Funding

USDA-ARS, Washington Wine Commission, Oregon Wine Board, American Vineyard Foundation, Erath Family Foundation Northwest Center for Small Fruit Research, USDA-NIFA-SCRI, National Grape Research Alliance

Grower Collaborators

Questions?

lowders@oregonstate.edu