What the Mildew?

Walt Mahaffee

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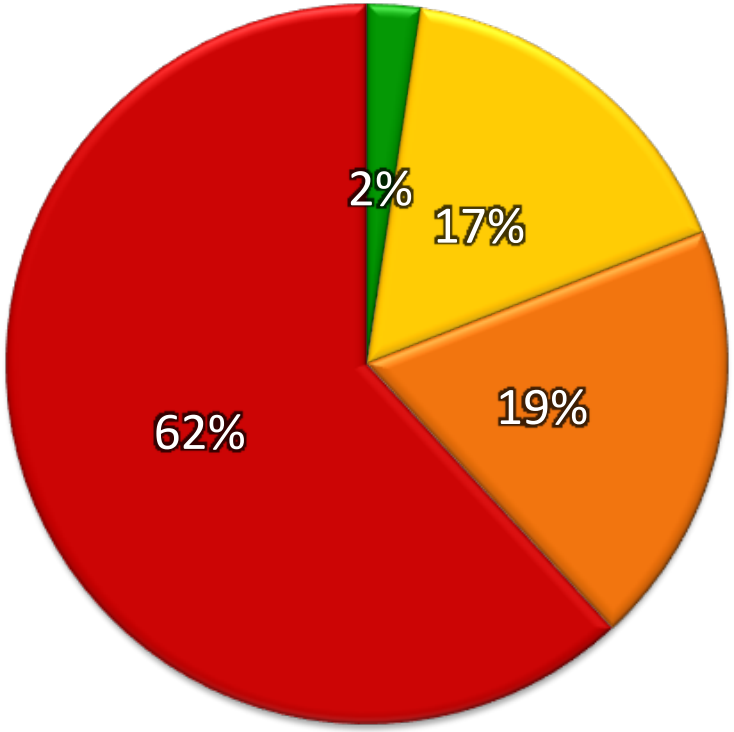
2017 Allele Frequency



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Where are we going today?

- Fungicide classes
- Factors affecting resistance development
- Assessing and Monitoring Fungicide Resistance
- Fungicide mobility
- Using Micronized Sulfur

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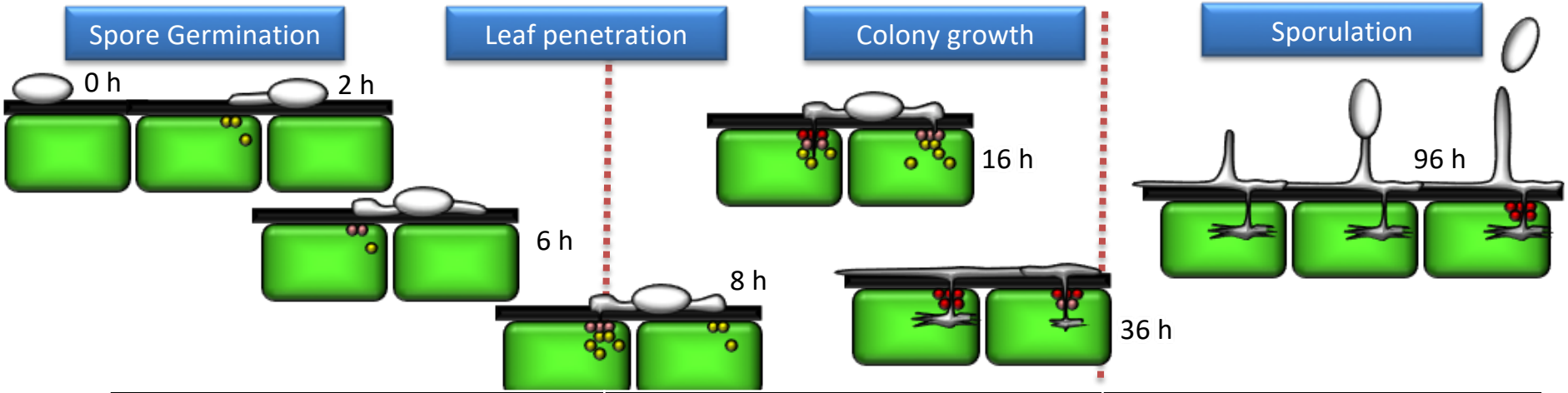
Fungicide Classes

Code	MOA	Example
3	DMI- Sterol biosynthesis in membranes (demethylation inhibitors)	Elite, Mettle 125ME, Rally, Rhyme, Vintage, Viticure,
7	SDHI- Respiration (succinate dehydrogenase inhibition)	Luna
11	QoI- Respiration (ubiquinol oxidase)	Abound, Sovran, Flint
13	Signal transduction (mechanism unknown)	Quintec
44	Microbial - Lipid synthesis and membrane integrity	Serenade, Sonata
M's	Multi-site modes of action	Copper, sulfur, ziram, captan
U's	Unknown	Vivando, Torino,
NC	Not classified	mineral oils, organic oils, potassium bicarbonate

Fungicide Modes of Activity



	Preventative	Curative	Knock Down
Activity	• Stop spore germination • Inhibit growth and penetration of the leaf	• Prevents sporulation • Stop growth and penetration of the leaf	• Physically destroy or smother established colonies • Arrest spread of the pathogen
Timing	Apply before infection occurs	Apply when disease pressure is high	Apply when infections are visible
Examples	FRAC 3: DMIS FRAC 13: Quintec FRAC M: Sulfur, bicarbonate, Copper FRAC 7: Luna, Aprovia,Endura FRAC 11: Flint, Sovran,Abound FRAC U#: Vivando, Torino	Horticultural/mineral oils FRAC 3: DMIs/SBIs	Horticultural/mineral oils?? FRAC 3: DMIs/SBIs??

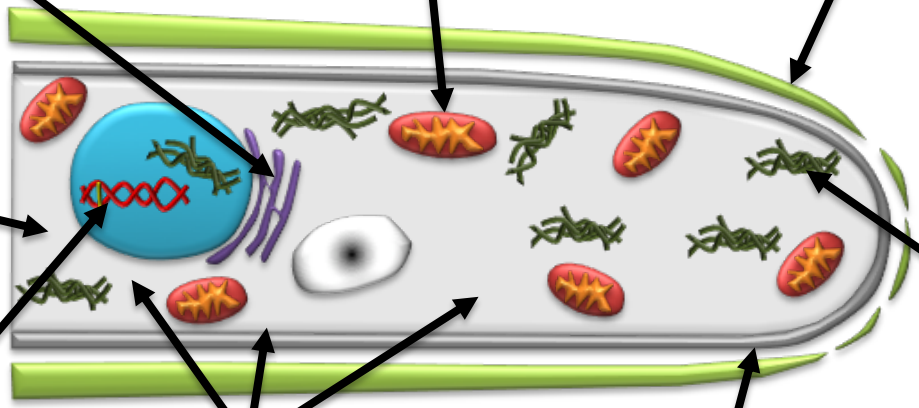
Fungicide Mode of Action

FRAC 9
Protein synthesis
- Benomyl
- Thiophanates

FRAC 7, 11
Respiration
- SDHI
- QoI

FRAC 19
Cell wall
- Polymyxin D

FRAC 2, 12, 13
Signal Transduction
- Dicarboximides
- Phenyl Pyrroles
- Quinoxyfen



FRAC 1
Microtubules
- Benomyl
- Thiophanates

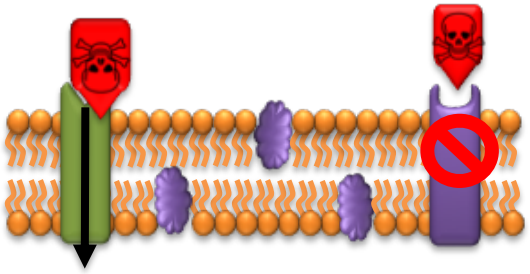
FRAC 4,8, 31, 32
Nucleic Acid
- None for GPM or Botrytis

FRAC M#
Multisite
- Copper (**01**)
- Sulfur (**02**)
- Mancozeb (**03**)
- Captan (**04**)

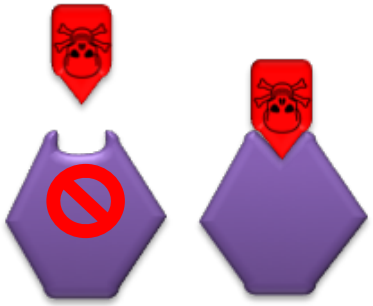
FRAC 3, 5, 17
Cell membrane synthesis
- DMI
- SBI
- Amines

Adapted from Latin, Richard. 2011. A Practical Guide to Turfgrass Fungicides

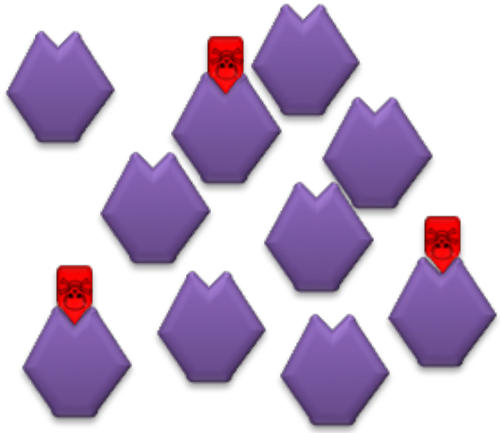
Mechanisms of Fungicide Resistance



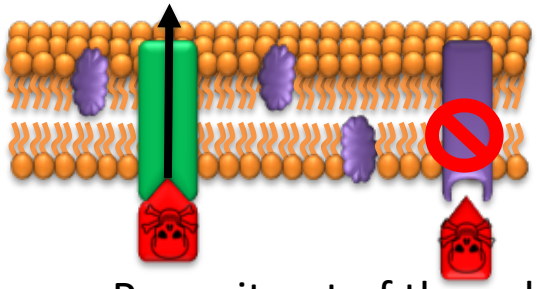
Reduce Uptake into Cell



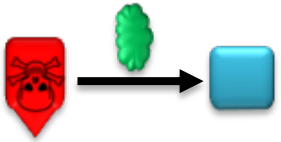
Alter binding site



Over Expression

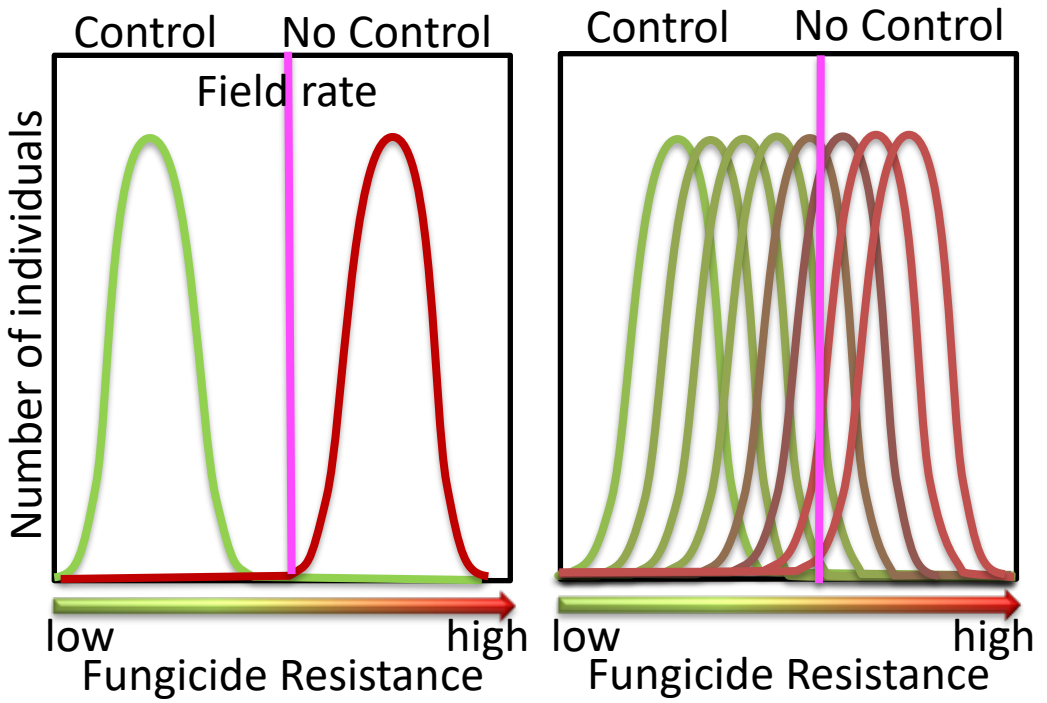


Pump it out of the cell



Detoxify

Types of resistance



Qualitative

(sudden)

QoI

Quantitative

(gradual/rate dependent)

DMI

QoI

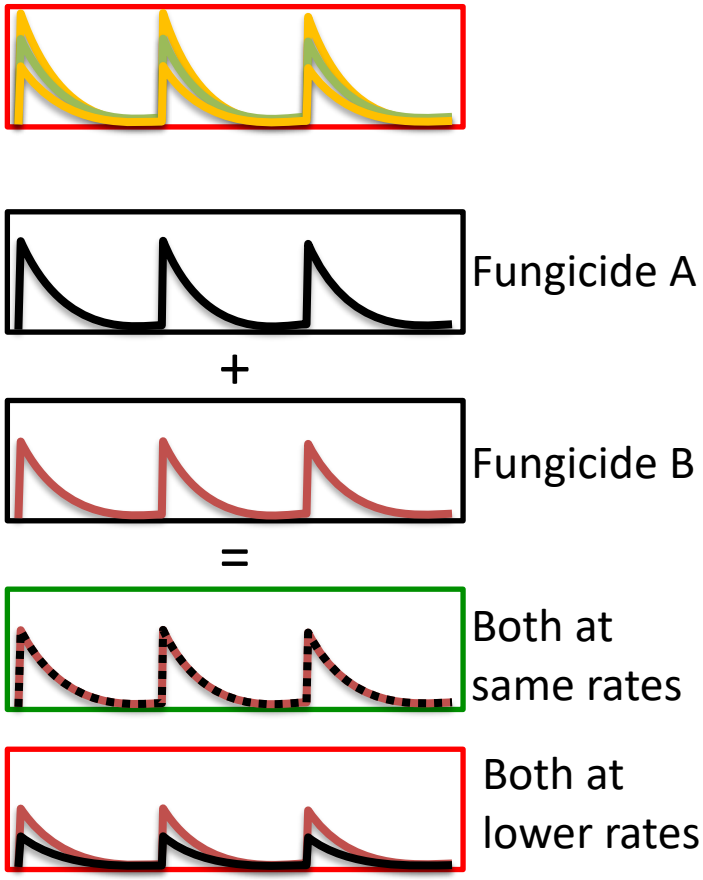
Abound (azoxystrobin)
Flint (trifloxystrobin)
Sovran (kresoxim-methyl)
Pristine (pyraclostrobin + boscalid)
Merivon (fluxapyroxad + pyraclostrobin)

DMI

Procure (triflumizole)
Rally (myclobutanil)
Vintage (fenarimol)
Elite (tebuconazole)
Inspire (cyprodinil + difenconazole)

Tactics for Managing Resistance

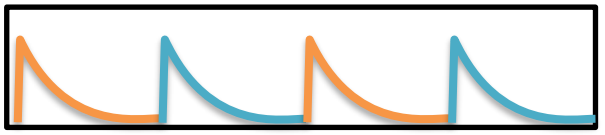
- **Adjust Dose**
  - increases resistance development
- **Tank Mixing with fungicide**
  - Same dose
    - decreases resistance in experiments
    - No effect in models unless coverage >95%
  - Reduce dose
    - Tends to increase resistance development



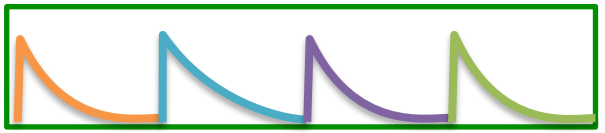
Adapted from van den Bosch et al. 2014. Annu. Rev. Phytopathol. 52:175-95

Tactics for Managing Resistance

- **Alternate fungicide classes** (Same number of Applications/season)
  - No effect



- **Alternate fungicide classes** (reduced number of Applications/season)
  - Decreases resistance development



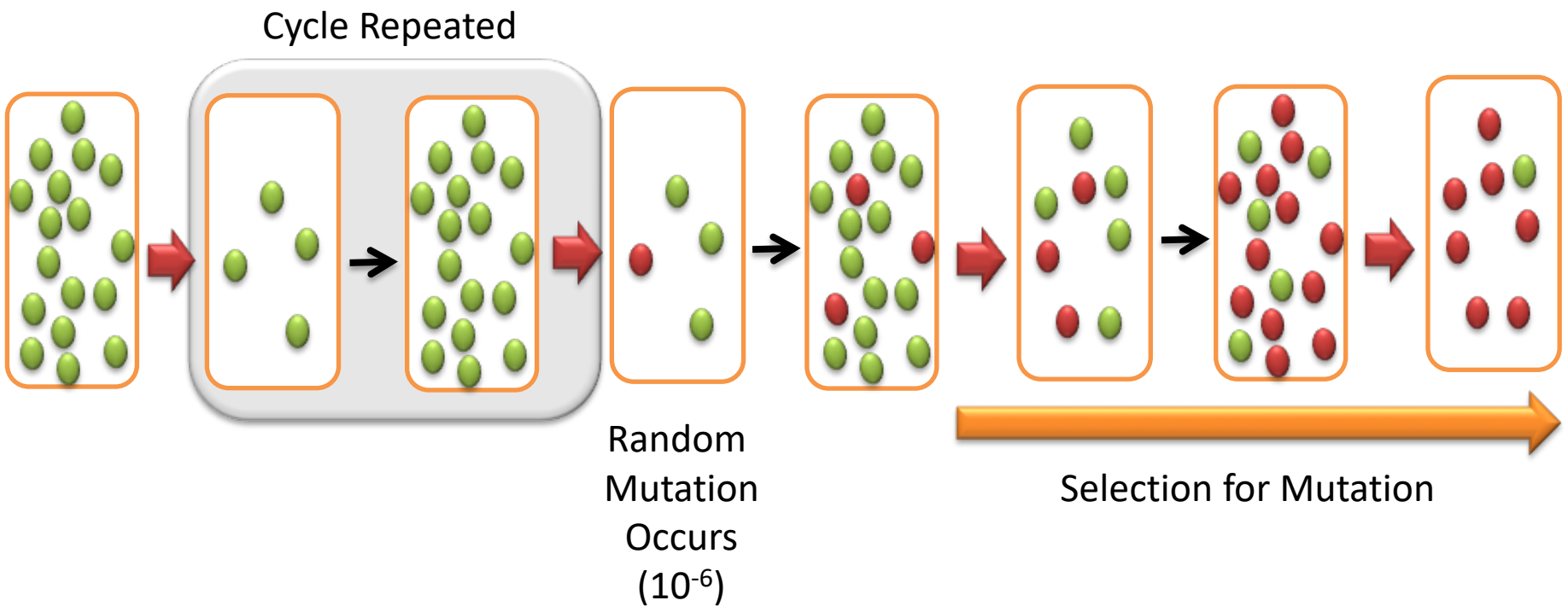
- **Split dose** (more applications with shorter intervals)
  - Increases resistance development

Adapted from van den Bosch et al. 2014. Annu. Rev. Phytopathol. 52:175-95

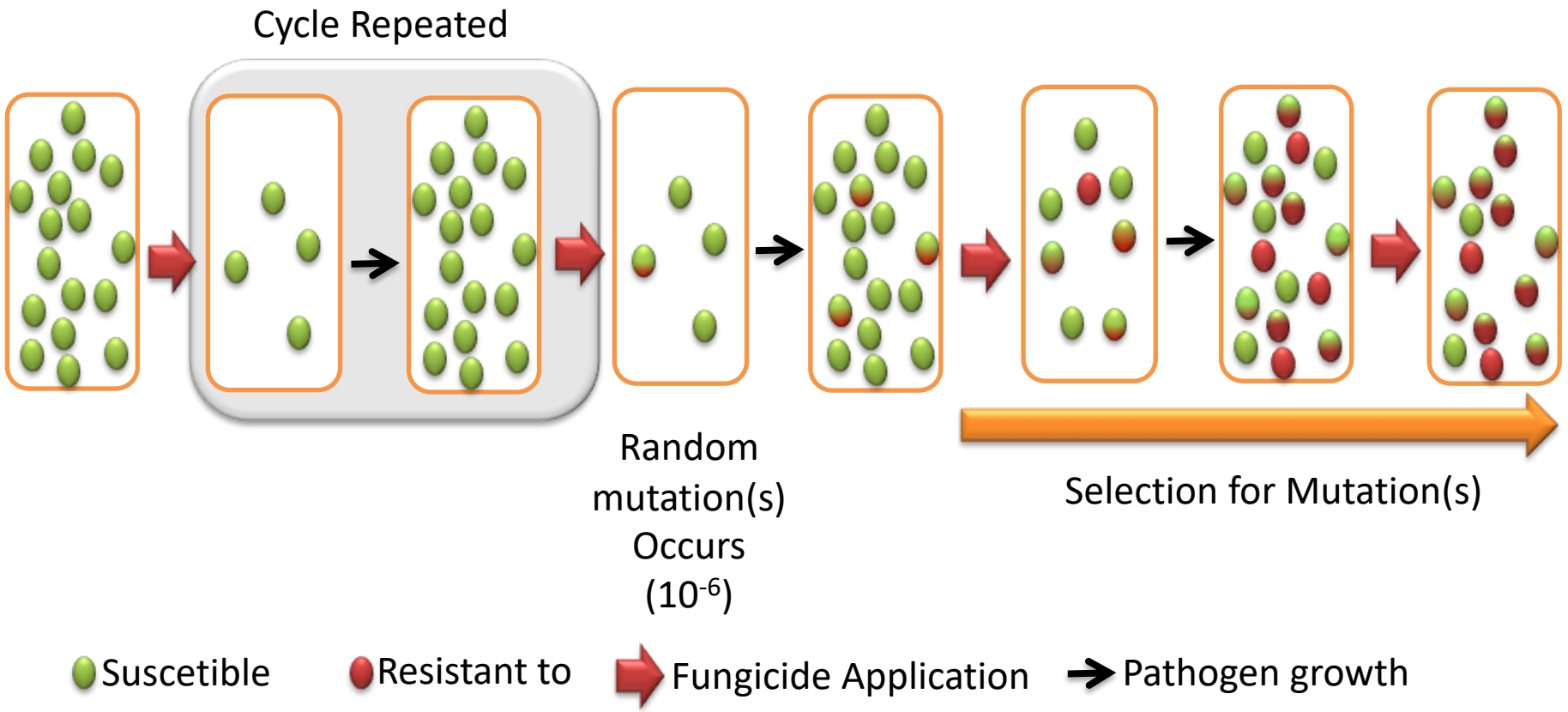
Pre-mixes

- **Pristine (FRAC 11 and FRAC 7)**
  - Pyraclostrobin + Boscalid
- **Quadris Top (FRAC 3 and FRAC 11)**
  - Difenoconazole + Azoxystrobin
- **Inspire Super (FRAC 3 and FRAC 9)**
  - Difenoconazole + Cyprodinil
- **Luna Tranquility (FRAC 7 and FRAC 9)**
  - Fluopyram + Pyrimethanil
- **Luna Experience (FRAC 7 and FRAC 3)**
  - Fluopyram + Tebuconazole

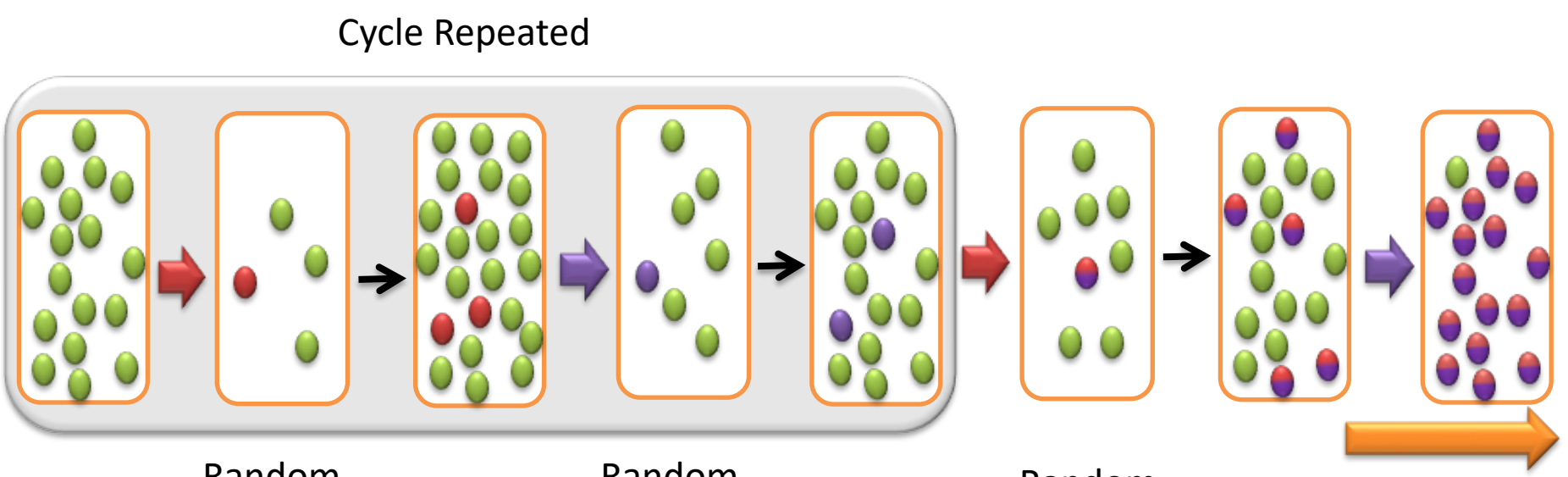
Fungicide Resistance Development
Qualitative Resistance



Fungicide Resistance Development
Quantitative Resistance



Effect of Rotation



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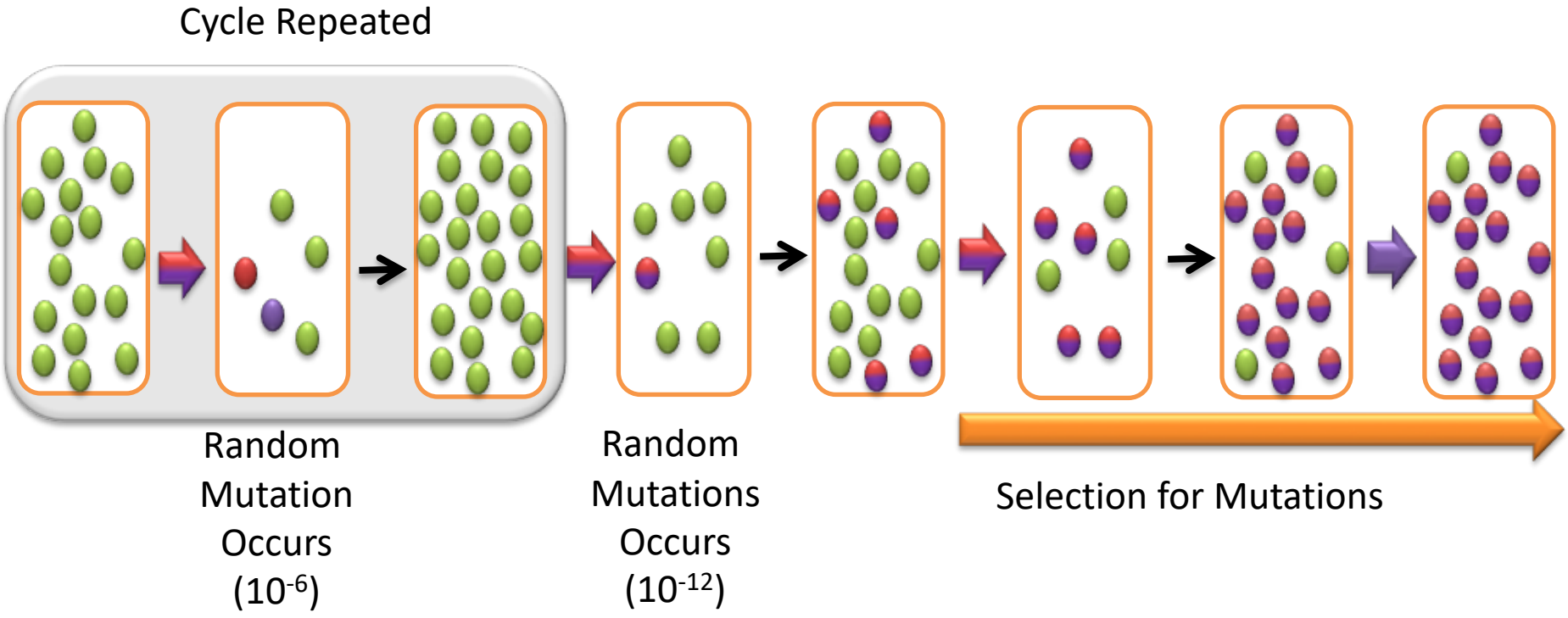
Effect of Tank Mixing



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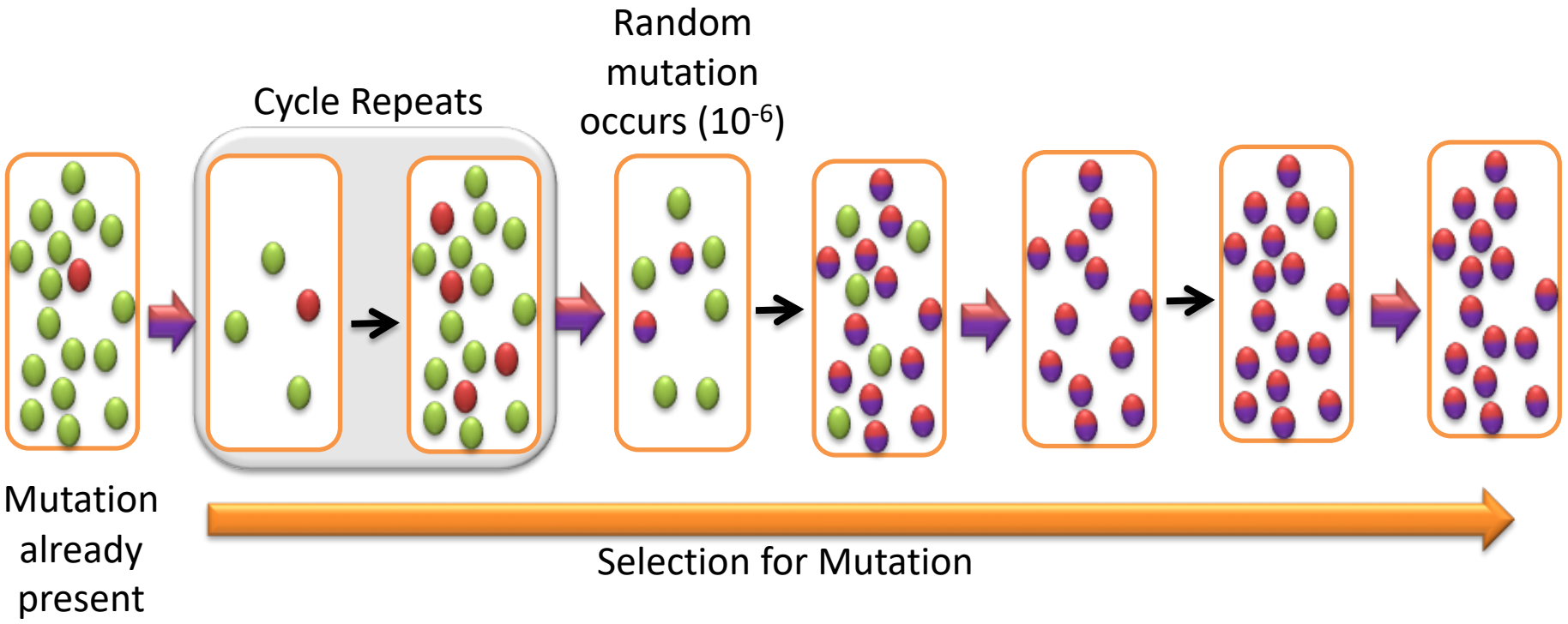
Tank Mixing with Pre-existing Resistance



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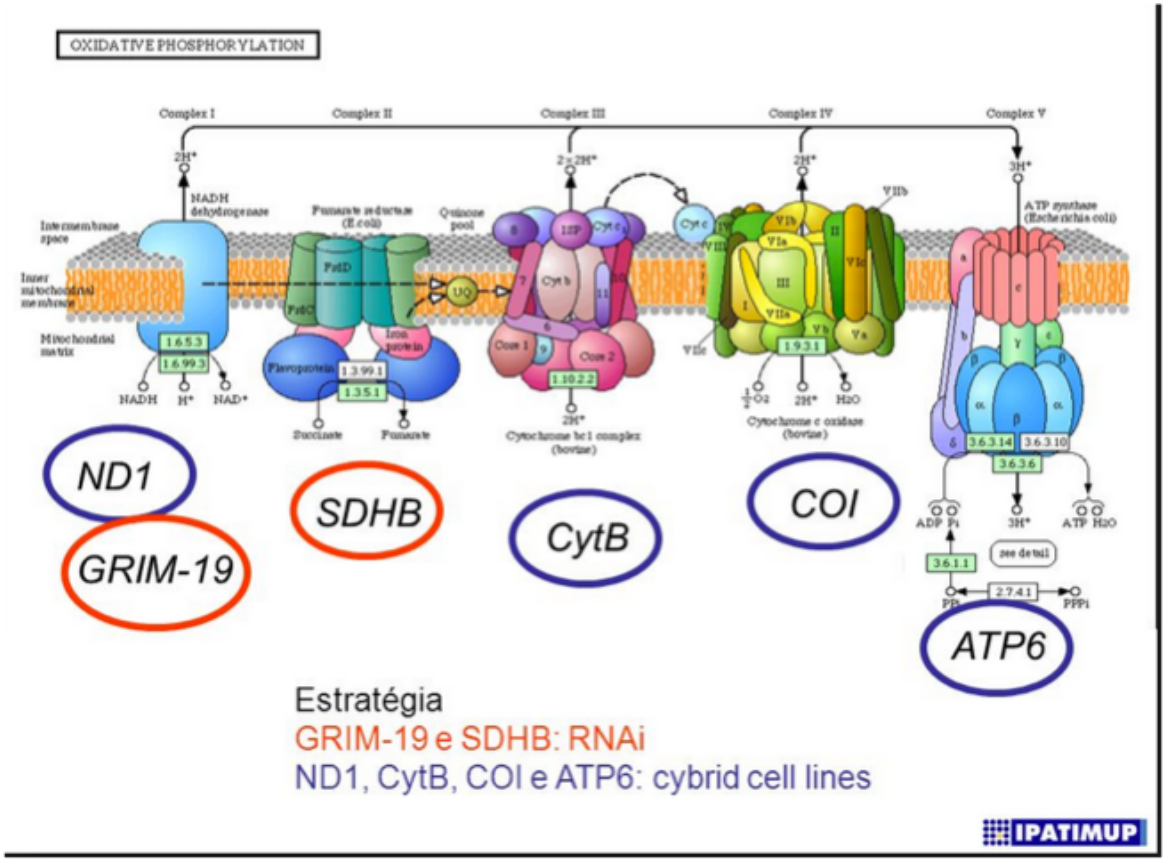
QoI (Stobilurin) Resistance FRAC 11

- Known in California and Eastern US since late 1990's
- Reports of uncontrollable disease development in July 2015



Survey of QoI Resistance in Oregon *Erysiphe necator* Populations

Field samples

- Fungal material directly from leaf and berry tissue
- DNA was extracted
- Sequencing

Isolates

- Single spore isolates
- Sequenced
- Bioassay and qPCR
- maintained for further testing

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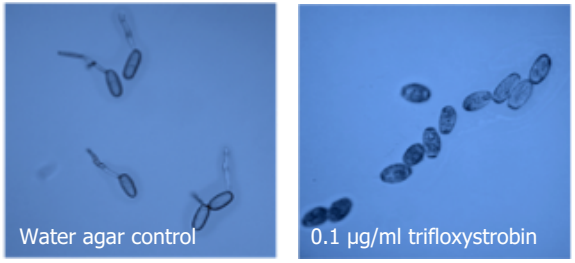
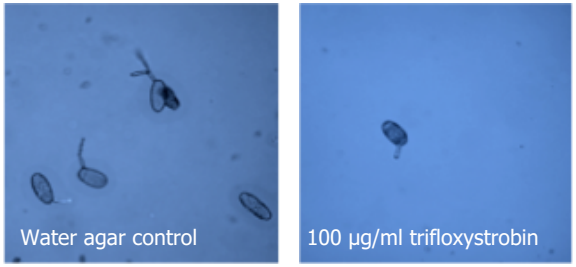
QoI Qualitative Resistance

- Fungicide amended 1.5% water agar
- After 48 h incubation germinated spores from 200 counted spores

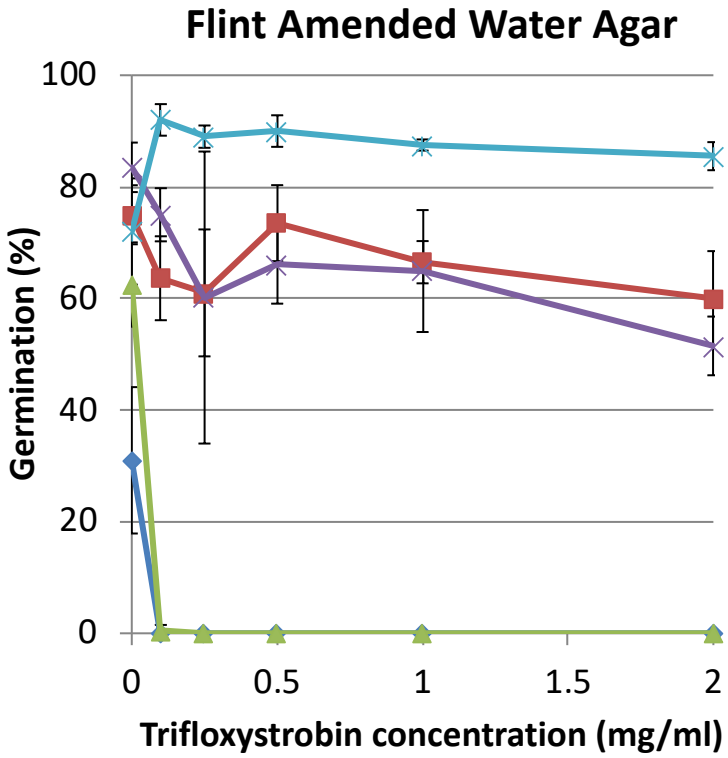
Susceptible isolate



Resistant isolate



L. Miles et al., 2012, Plant Dis.



- 0.1µg/ml was suitable discriminating dose
- Resistance isolates tolerate >20,000 times the sensitive isolates dose

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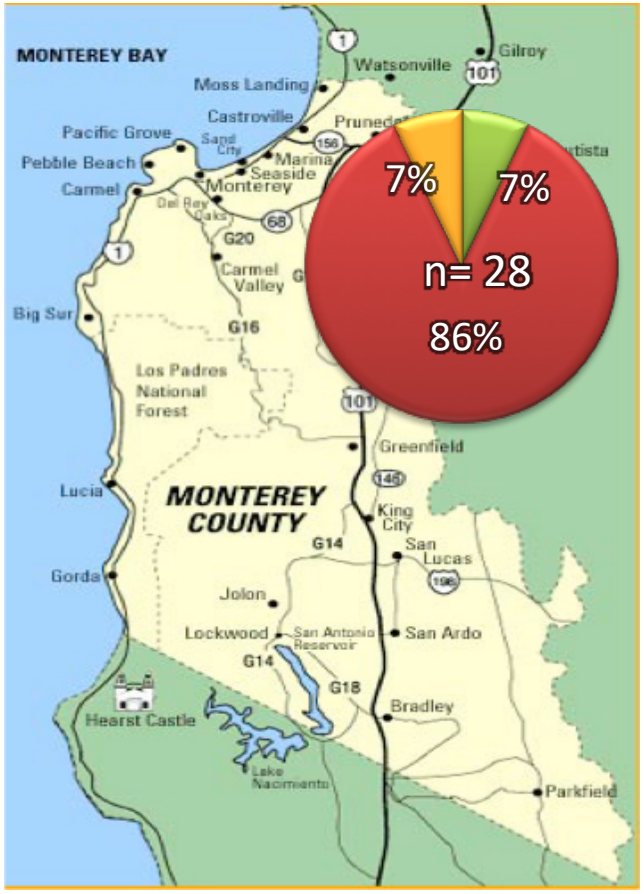
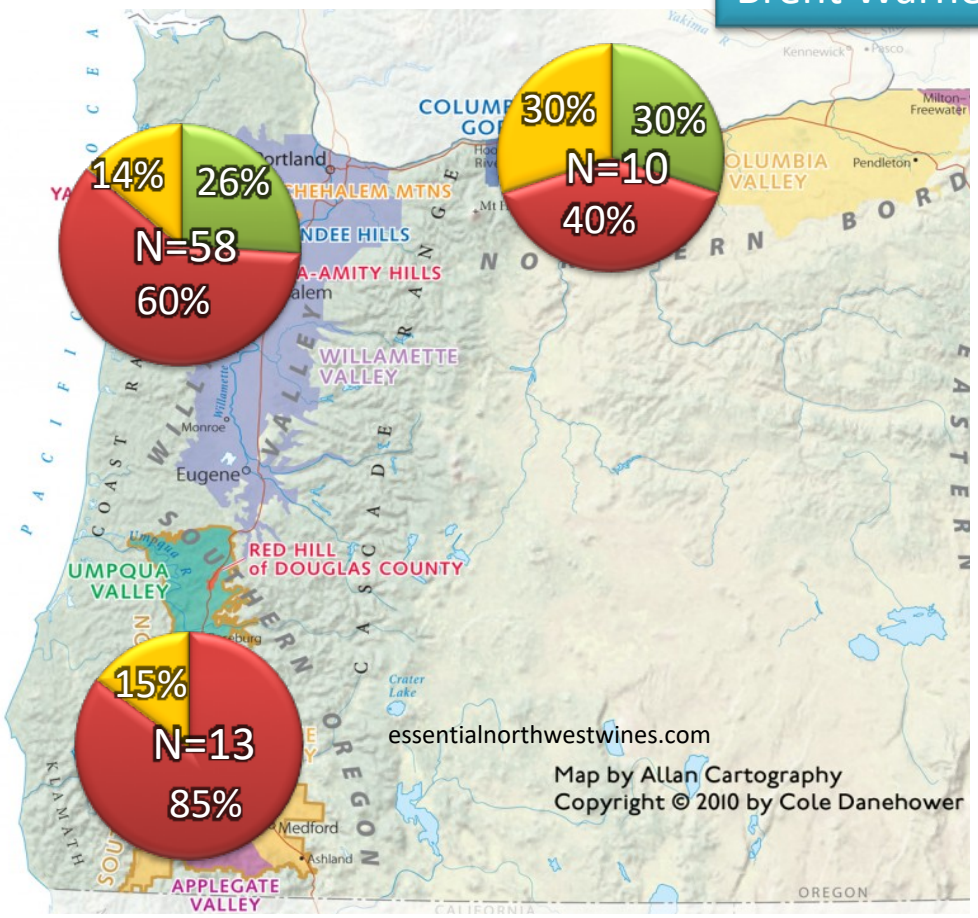
qPCR Analysis of QoI Resistance in Oregon 2015 *Erysiphe necator* Populations

Brent Warneke





Sensitive Resistant Mix

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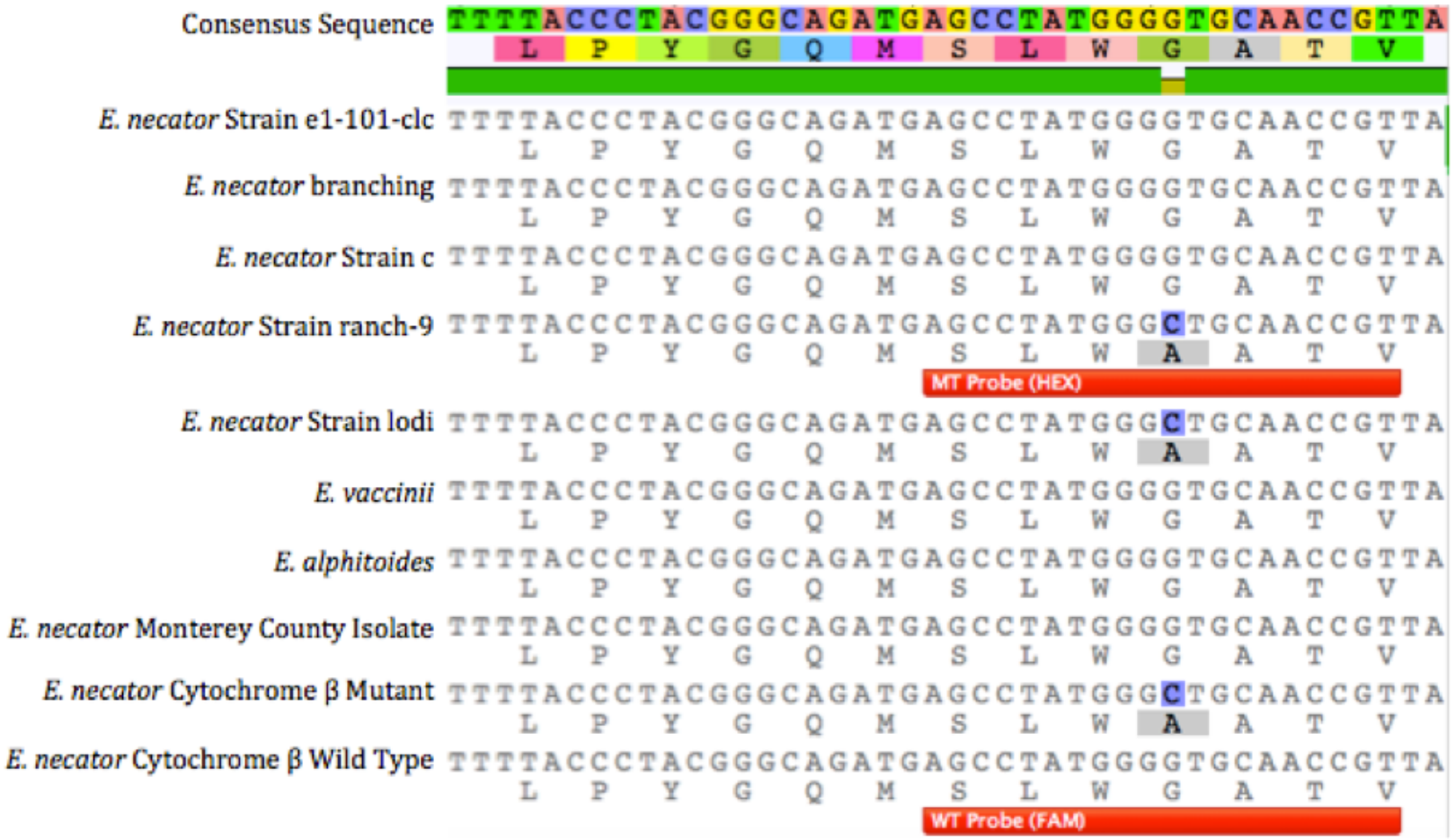
Comparative genomics



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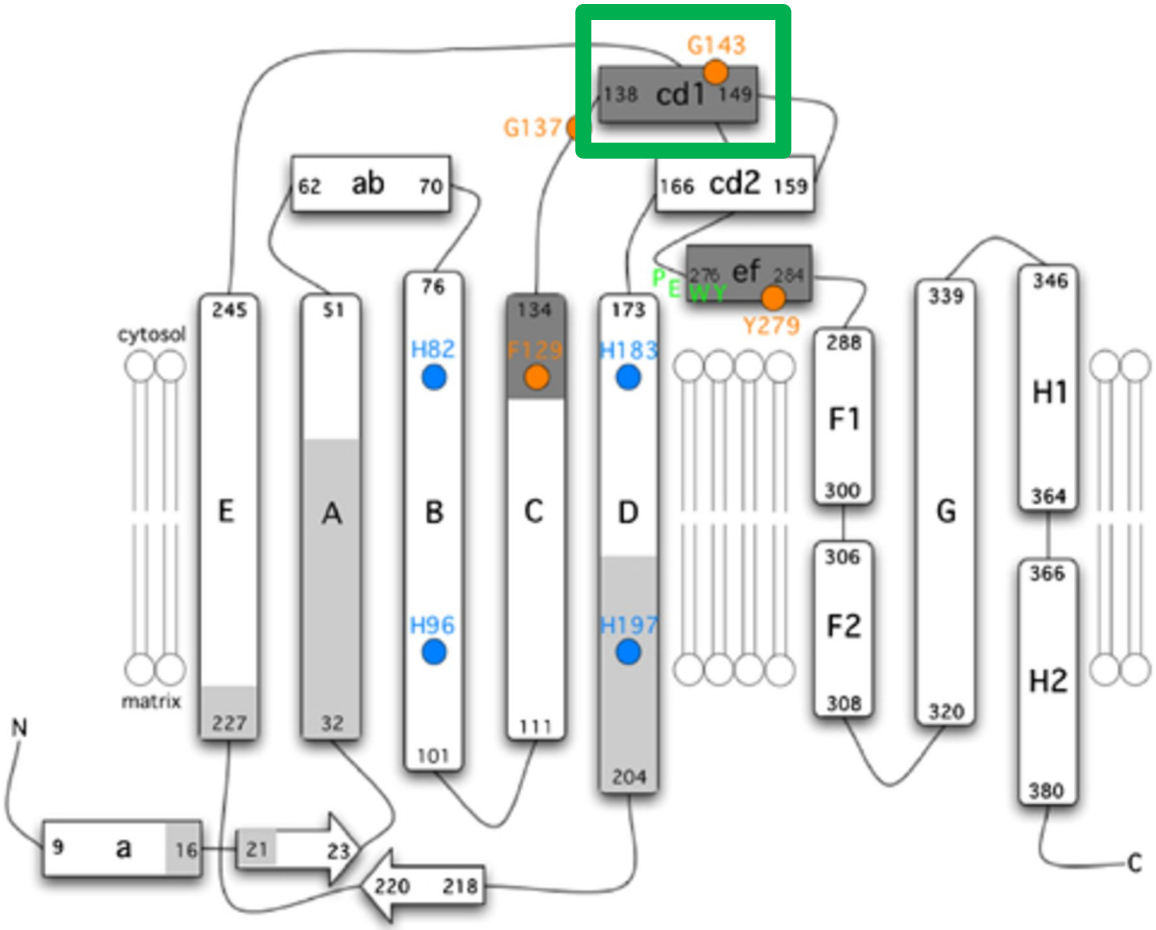
G143A only Mutation Found



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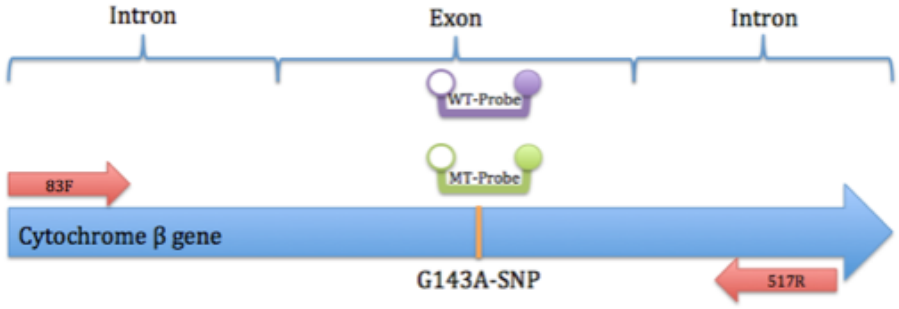
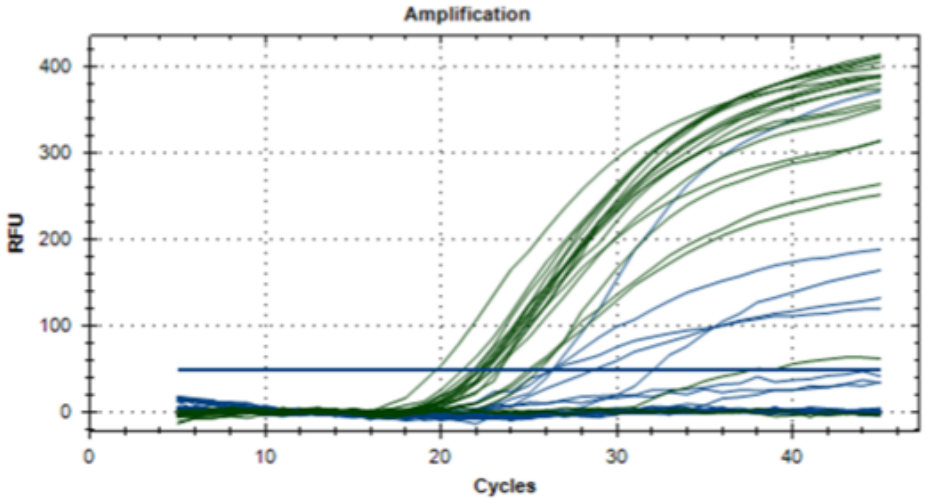
Molecular Detection of QoI Resistance

- **Single nucleotide mutation (G143A)**
- **Developed TaqMan qPCR competitive Assay.**
- **Sensitive to a single spore**

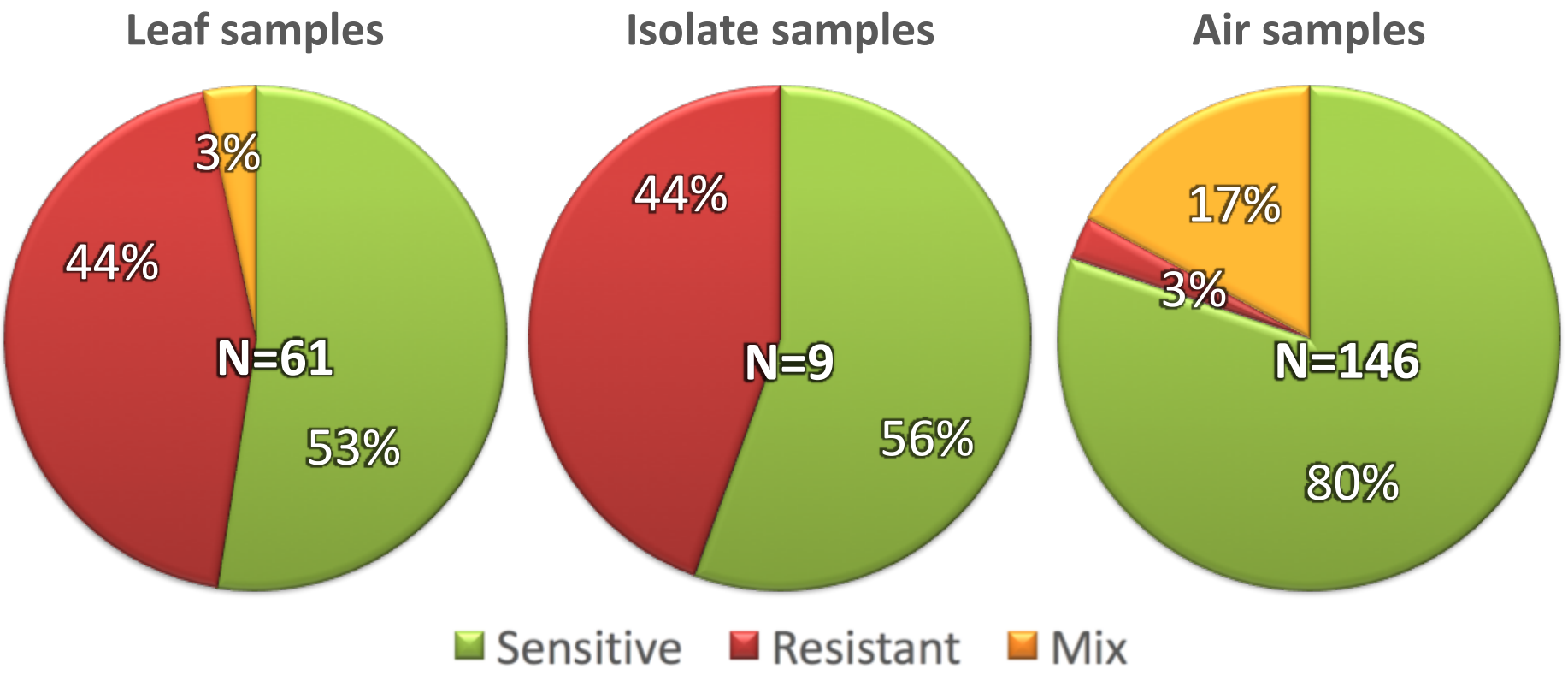




Rapid Sampling Methods

- **Fungal colonies**

2017 Fungicide Resistance Monitoring Oregon



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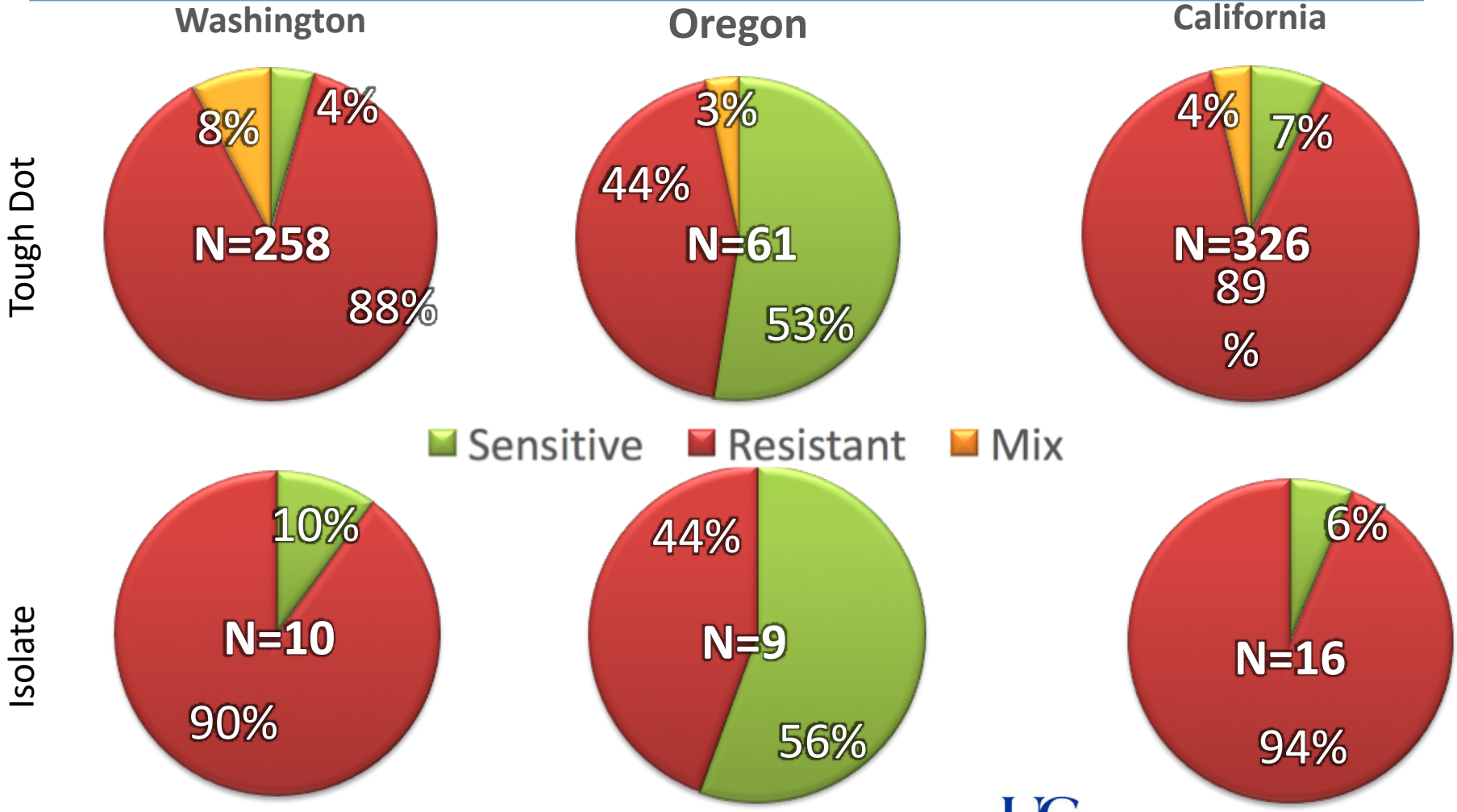
QoI Resistance Sampling 2017



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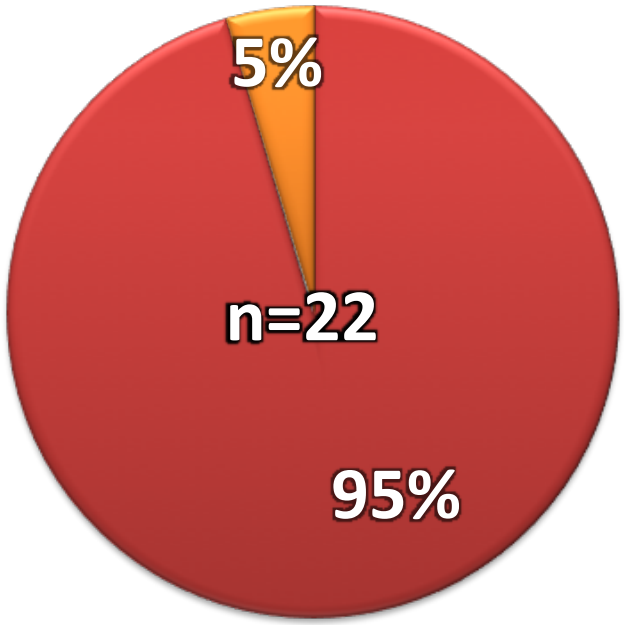
Santa Rosa G143A Frequency



Sampling Field Crew Gloves

Sarah Lowder

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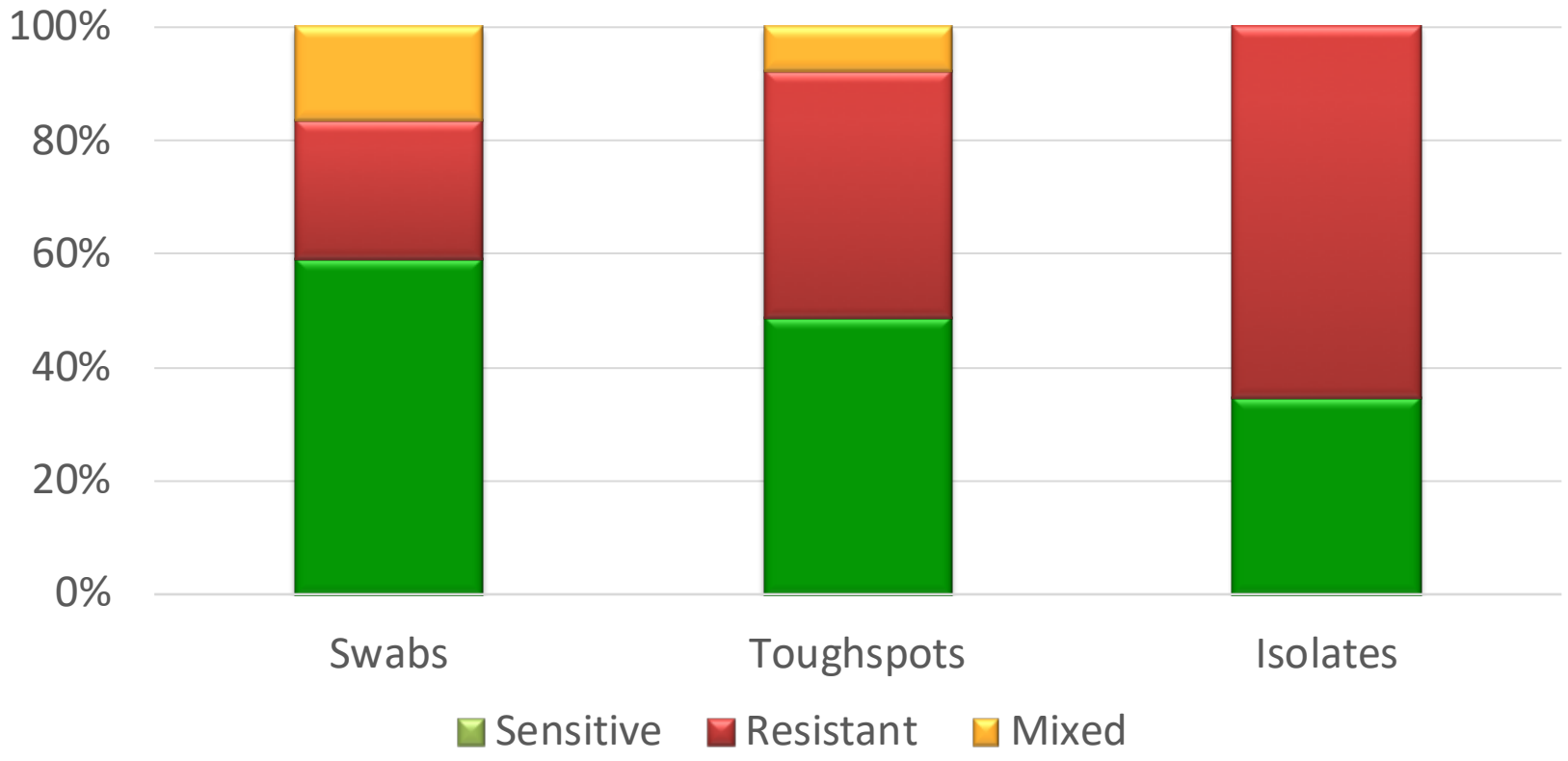
Comparison of Sampling Techniques

Sarah Lowder



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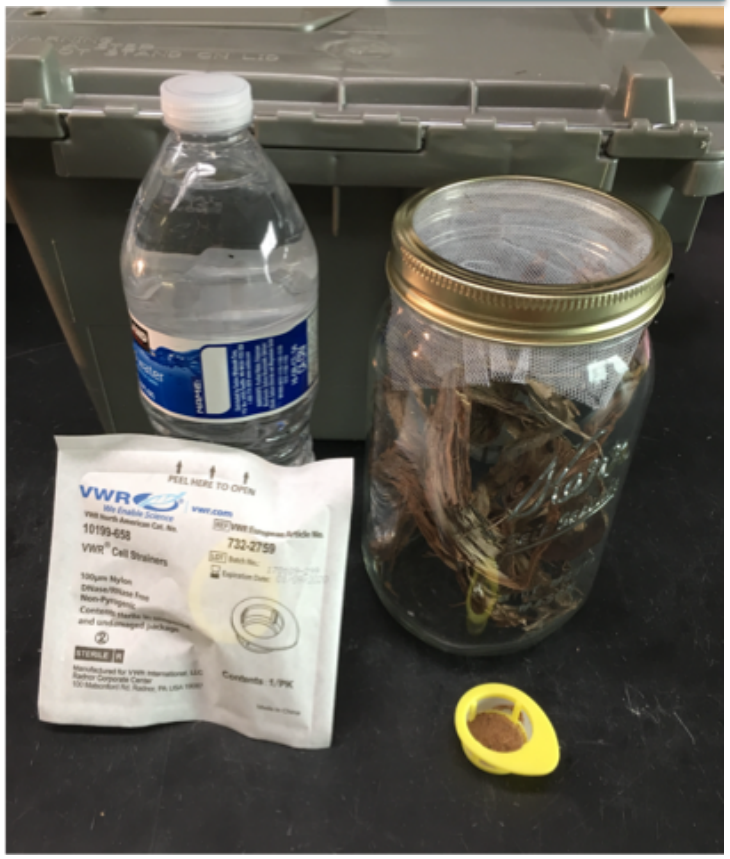
Rapid Sampling Methods

Sarah Lowder

- **Chasmothecia/cleistothecia**
  - Can detect chasmothecium in 55 g of dry bark
  - Currently testing sensitivity and reducing costs

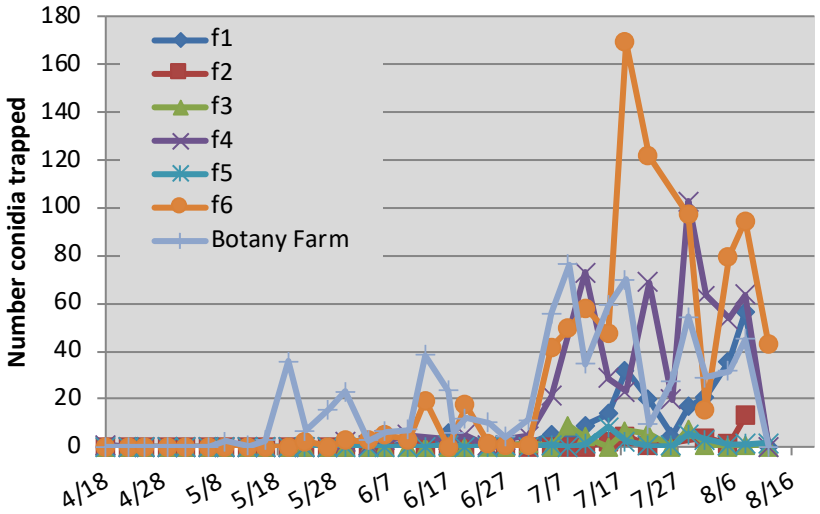


What Does the Sampling Mean?

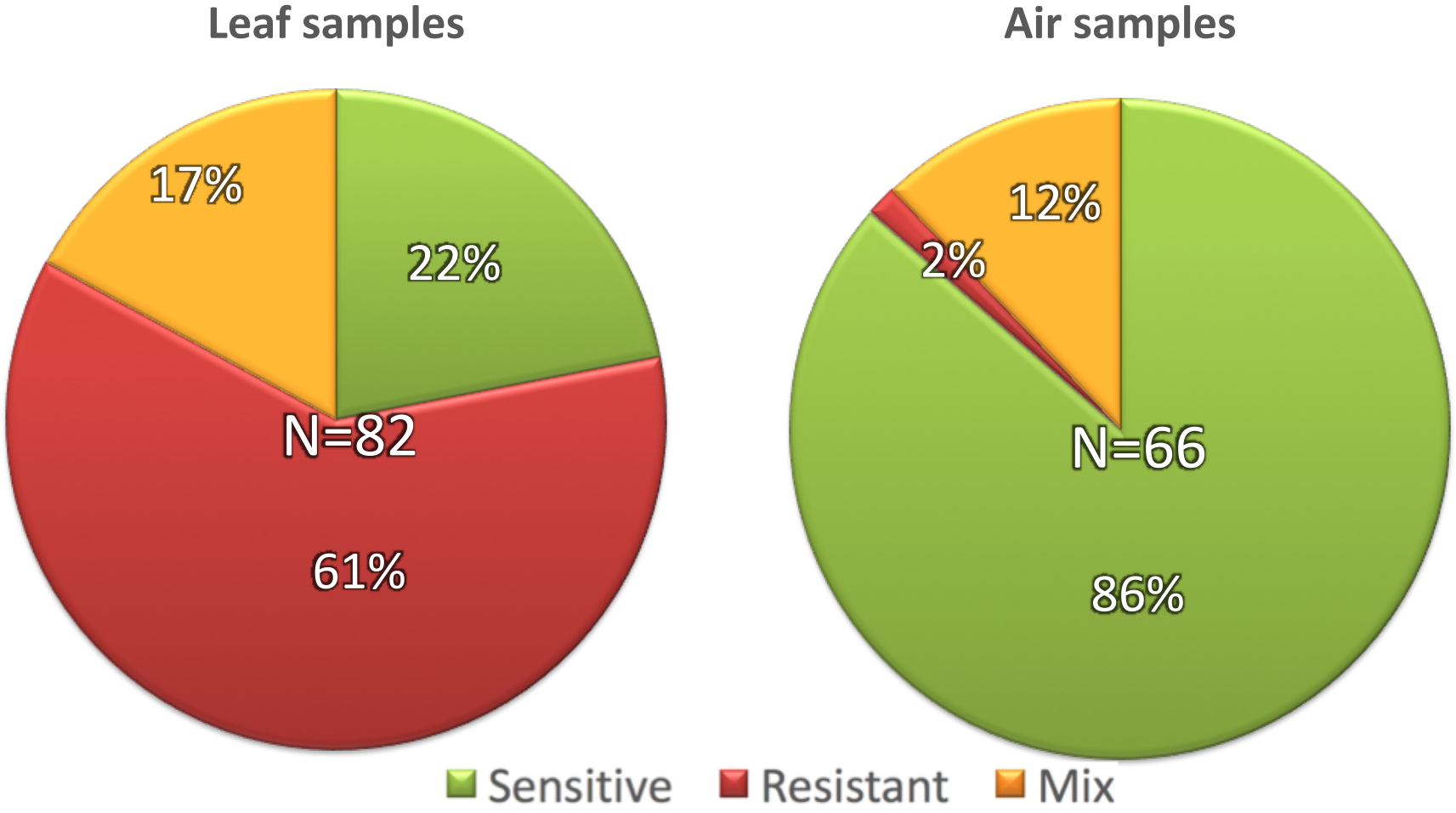
- **Samples often coming from vineyards with management concerns**
- **None random thus not able to extrapolate out to the industry as a whole**
- **How many samples needed to characterize a vineayrd?**

Inoculum Detection as a Decision Aid

- An average 2.3 in fungicide applications were saved when initiating fungicide program based on detection
- An additional 1.9 applications were saved when adjusting application interval
- Level of disease control not significantly different between treatments
- Commercially available



2015 Fungicide Resistance Monitoring Oregon



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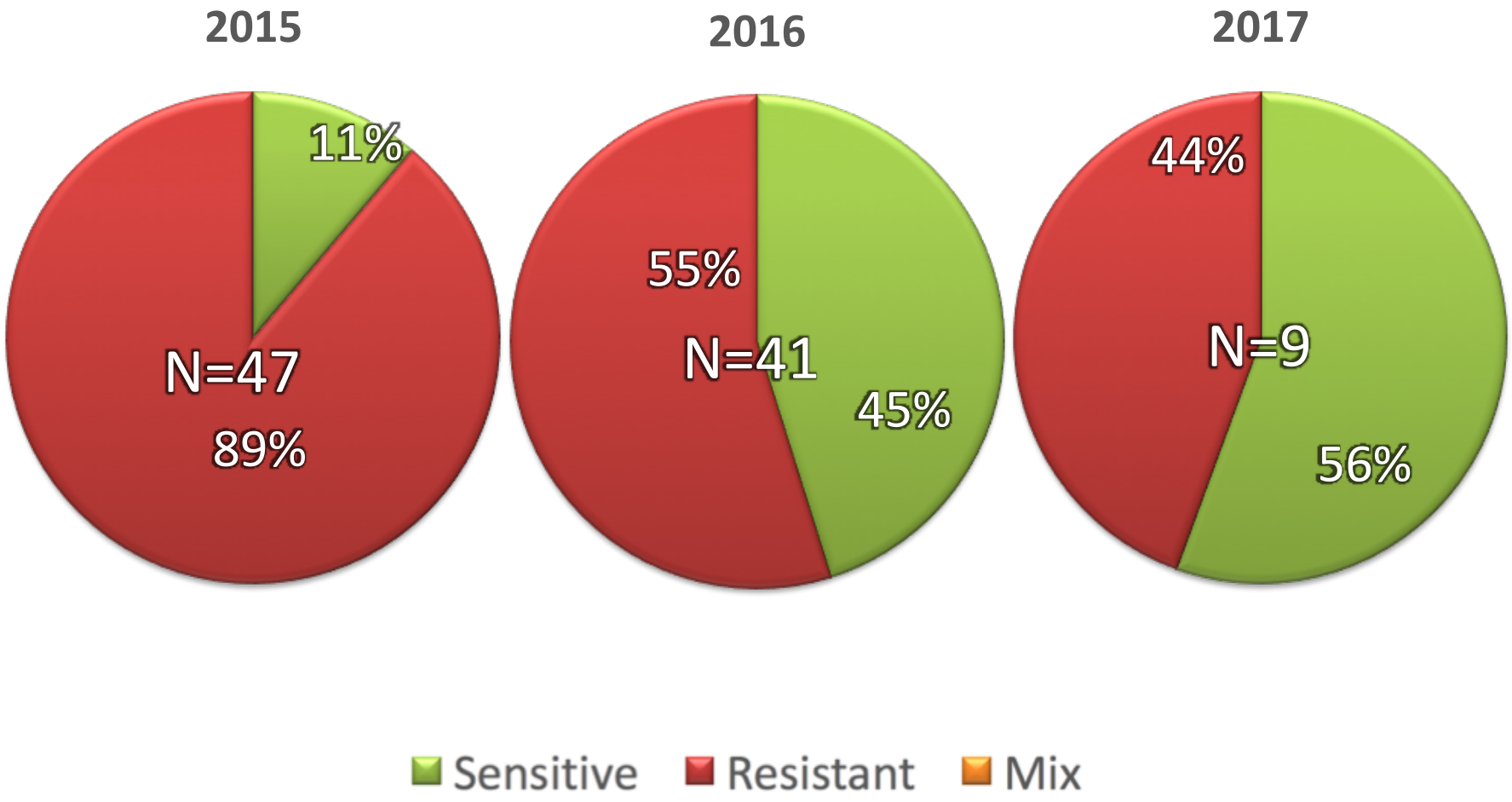
Fungicide Resistance Monitoring Oregon Isolate Samples



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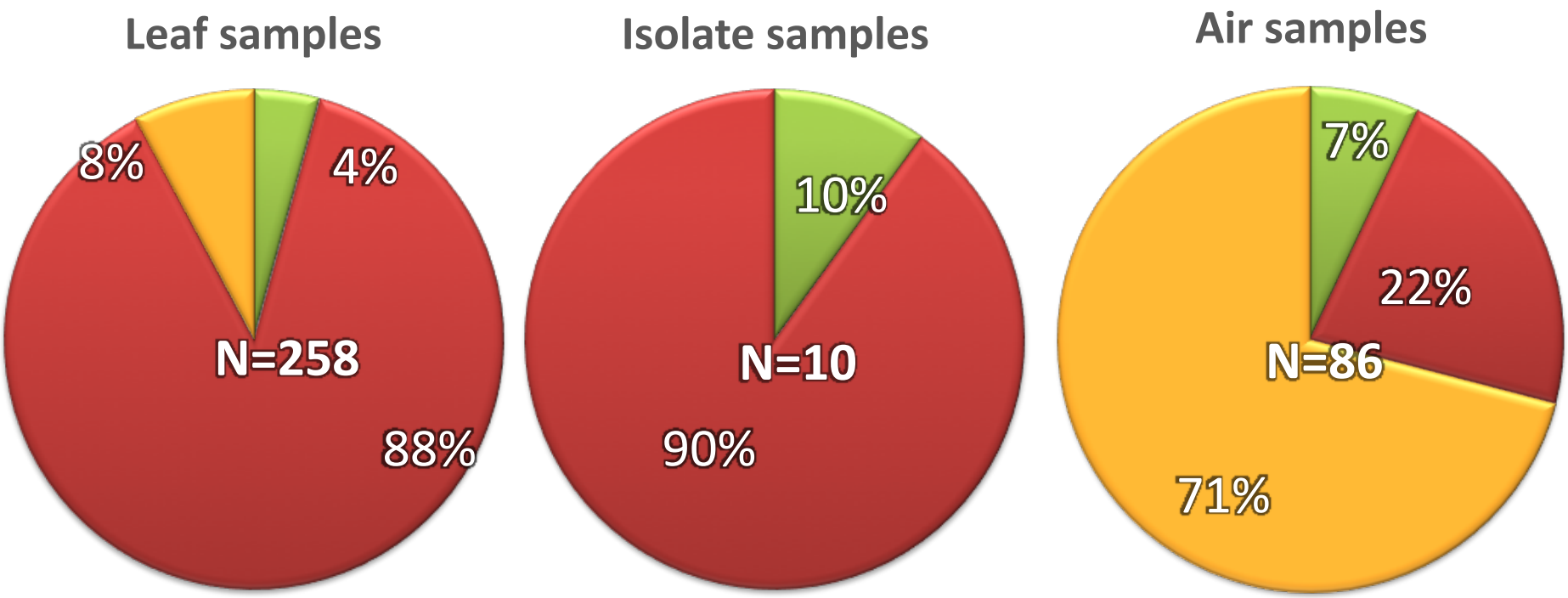
2017 Fungicide Resistance Monitoring Washington



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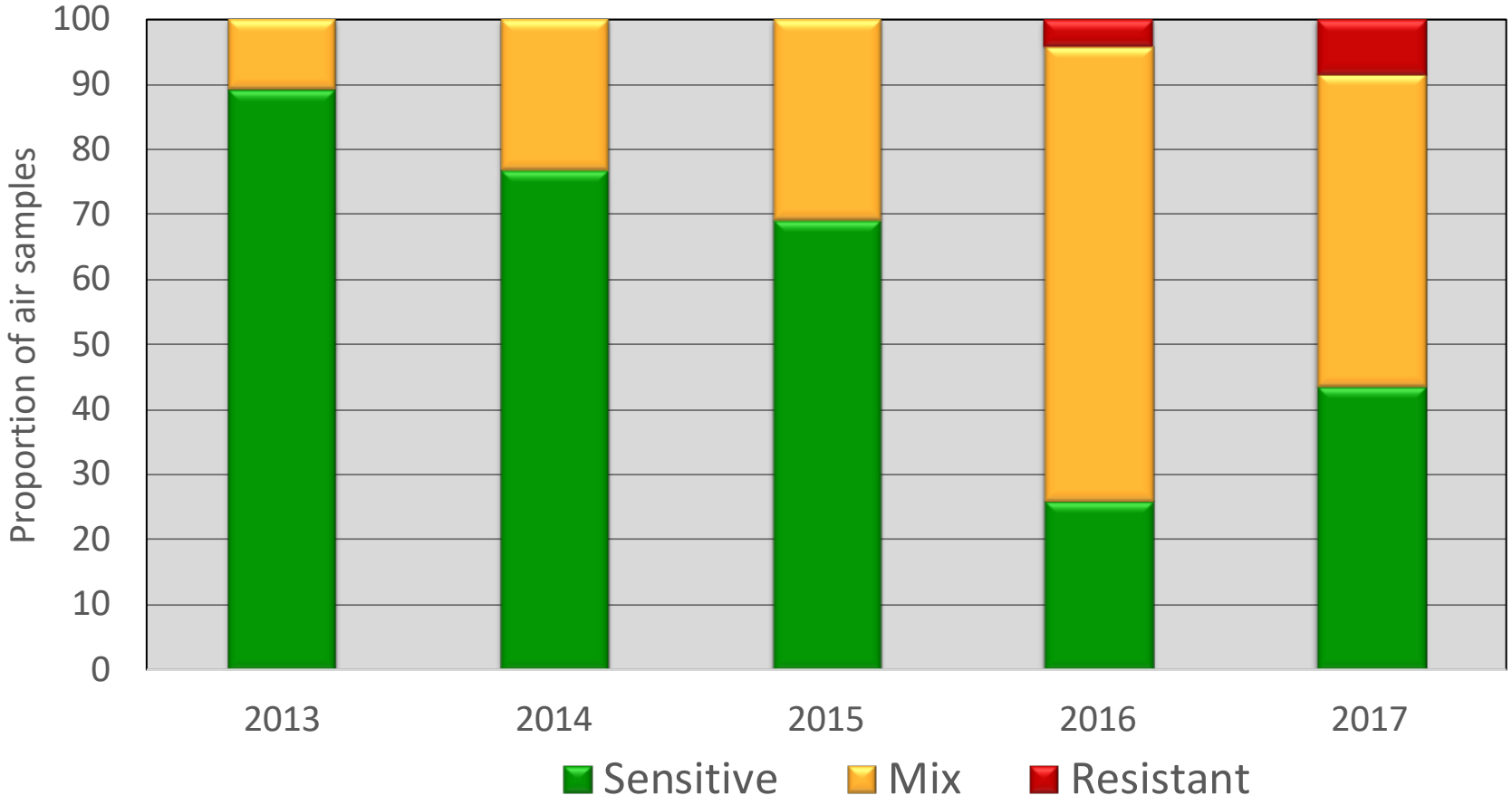
Allele frequency in Oregon Vineyard Air Samples



Summary of samples from 9 vineyards that used QoIs prior to 2016

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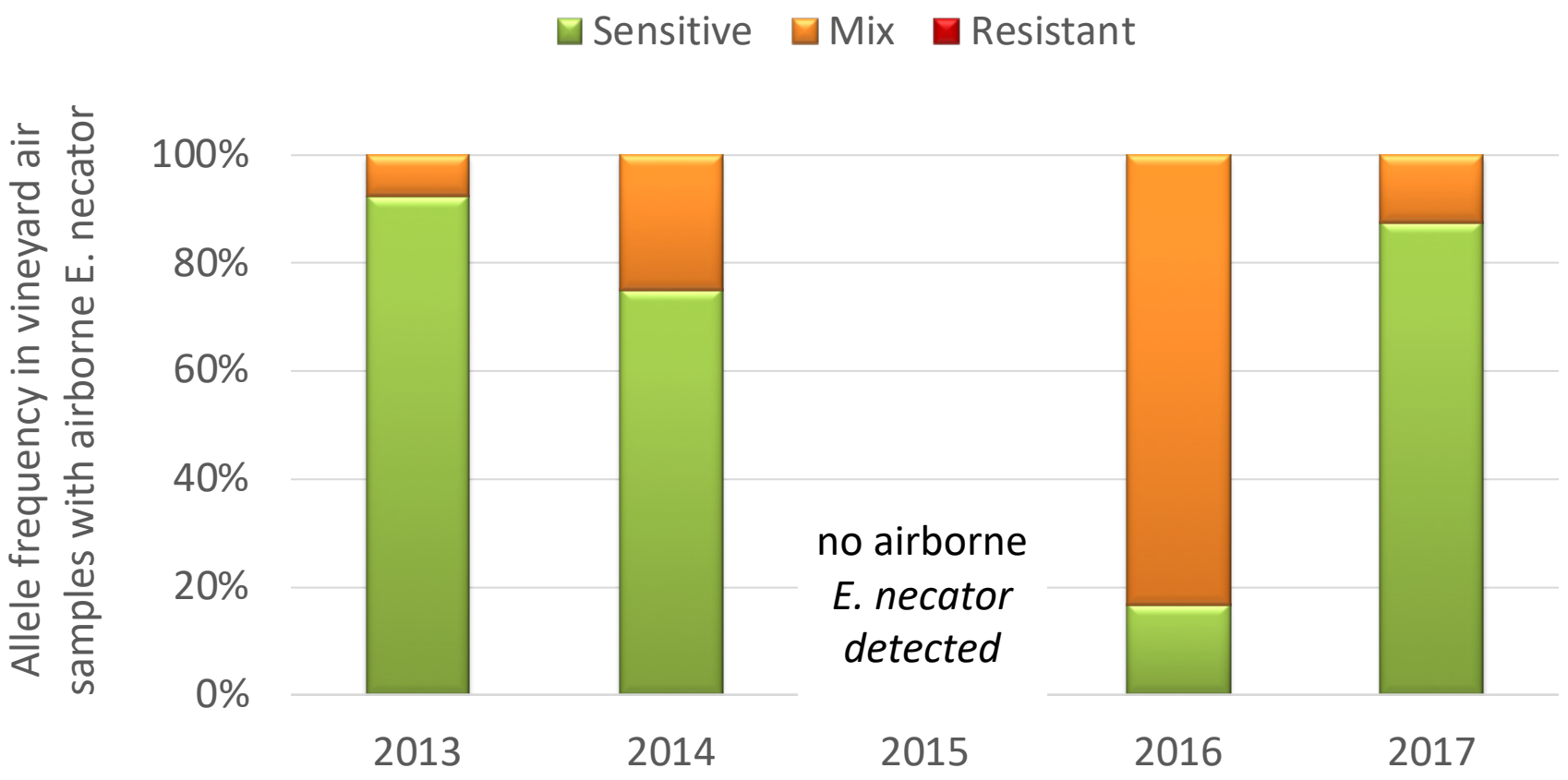
QoI Resistance over Time



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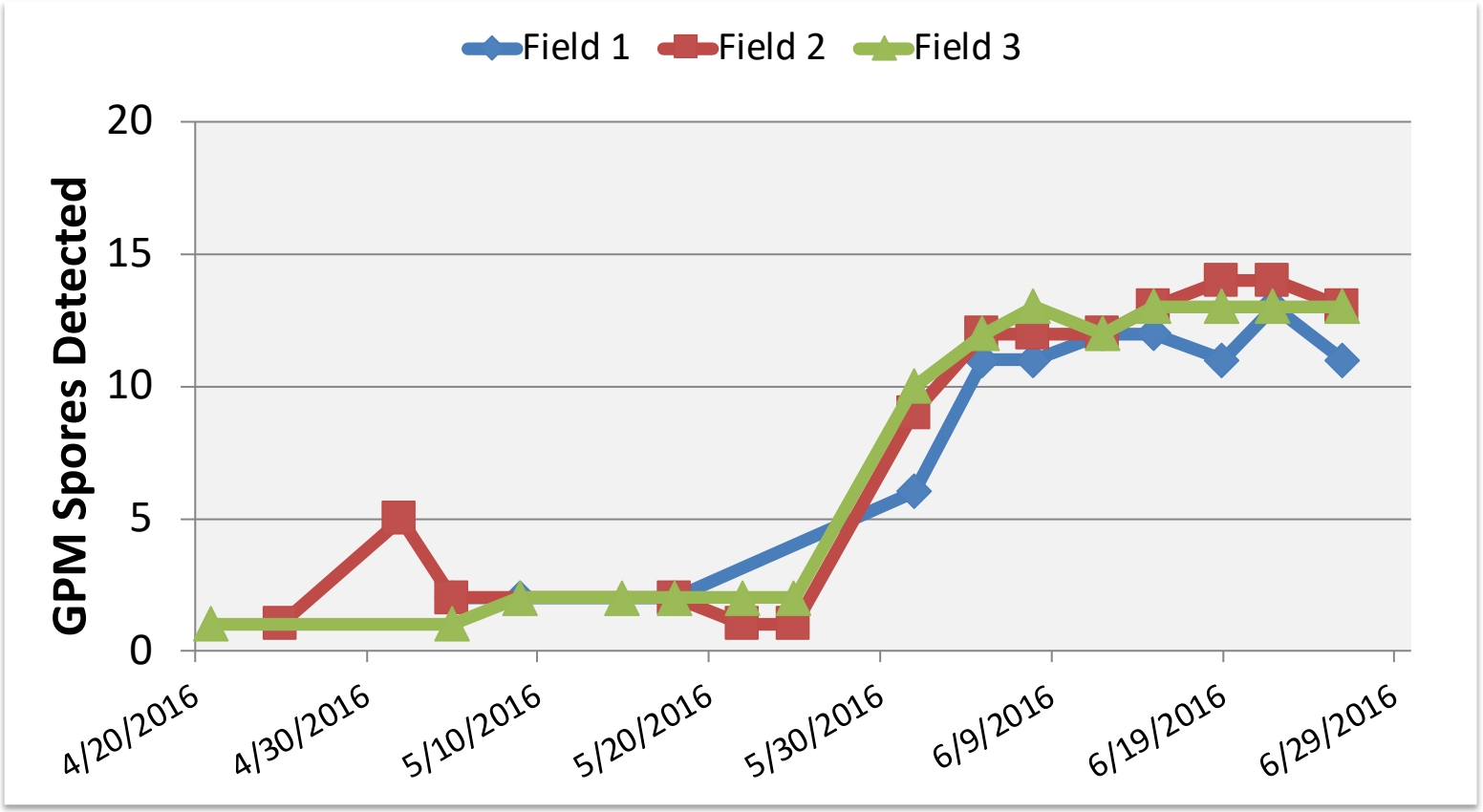
2016 QoI Field Resistance Monitoring



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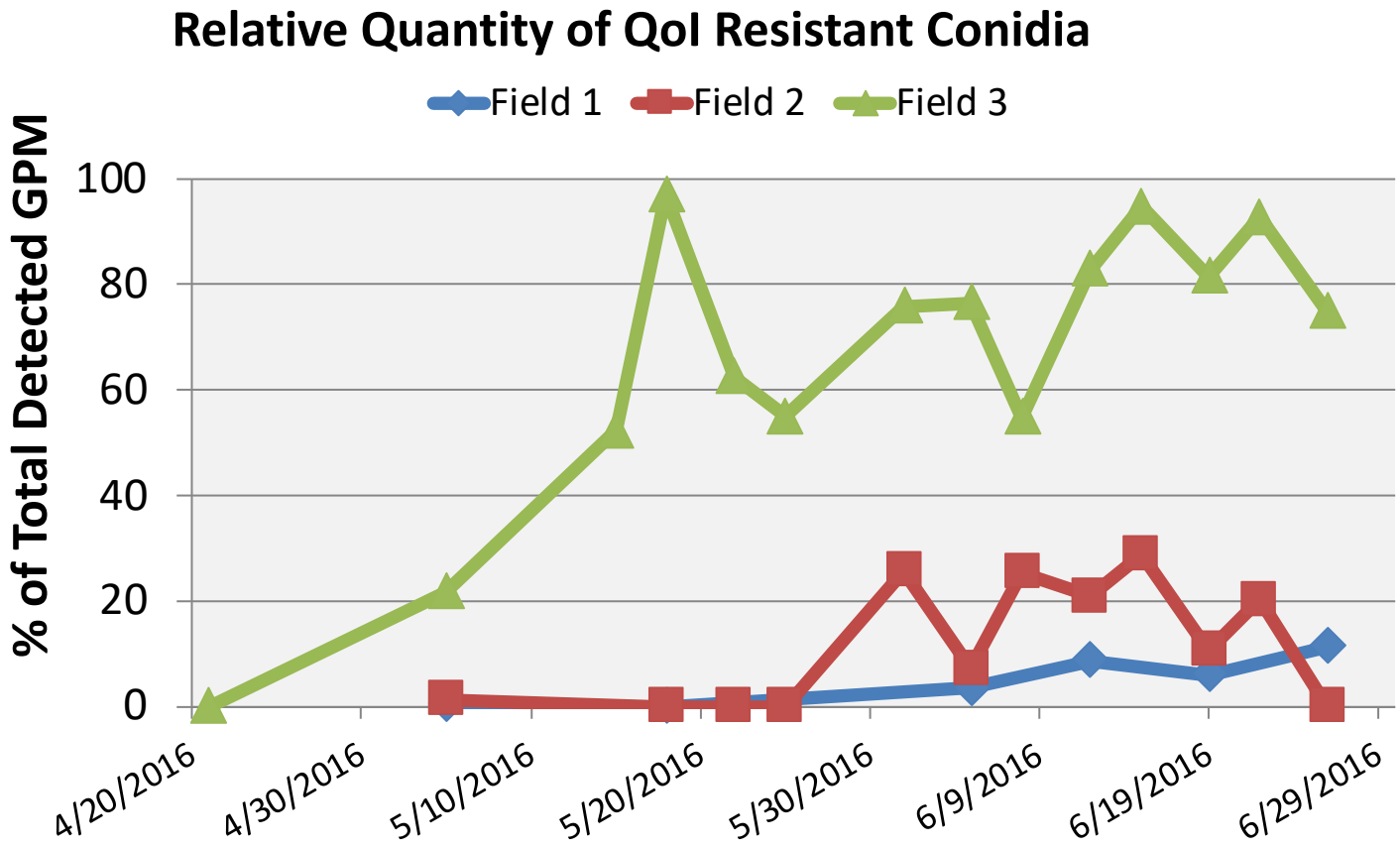
2016 QoI Field Resistance Monitoring



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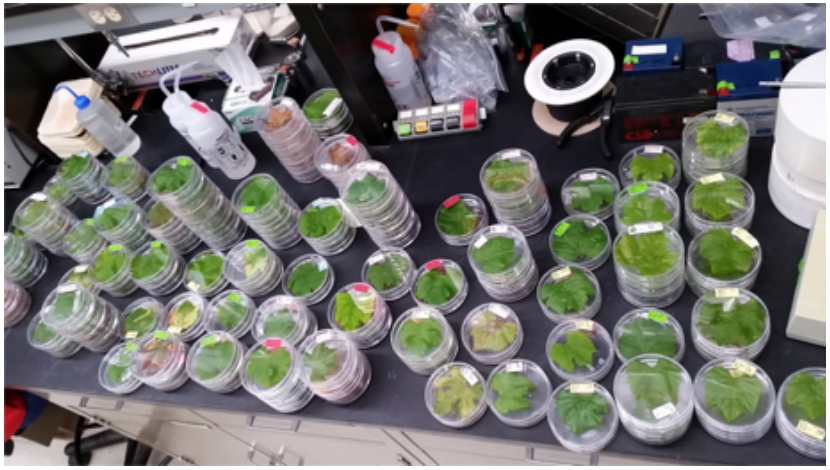
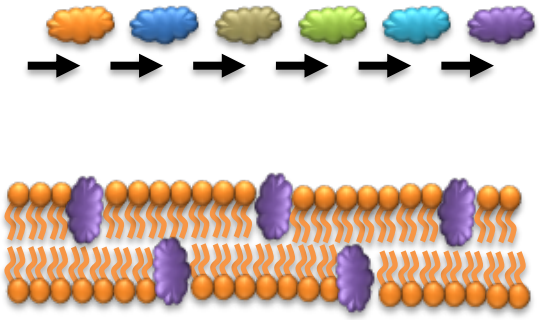
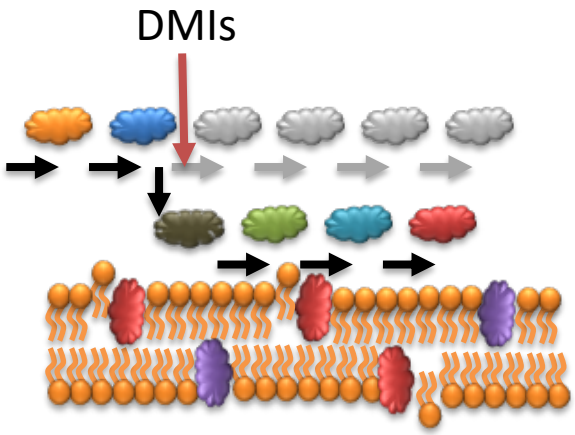
Sterol Biosynthesis Inhibitors FRAC 3

- Known in California and Eastern US
- Suspected to occur in Oregon and Washington but no clear evidence of control failure
- Multiple independent copies of genes involved
- Multiple mechanisms

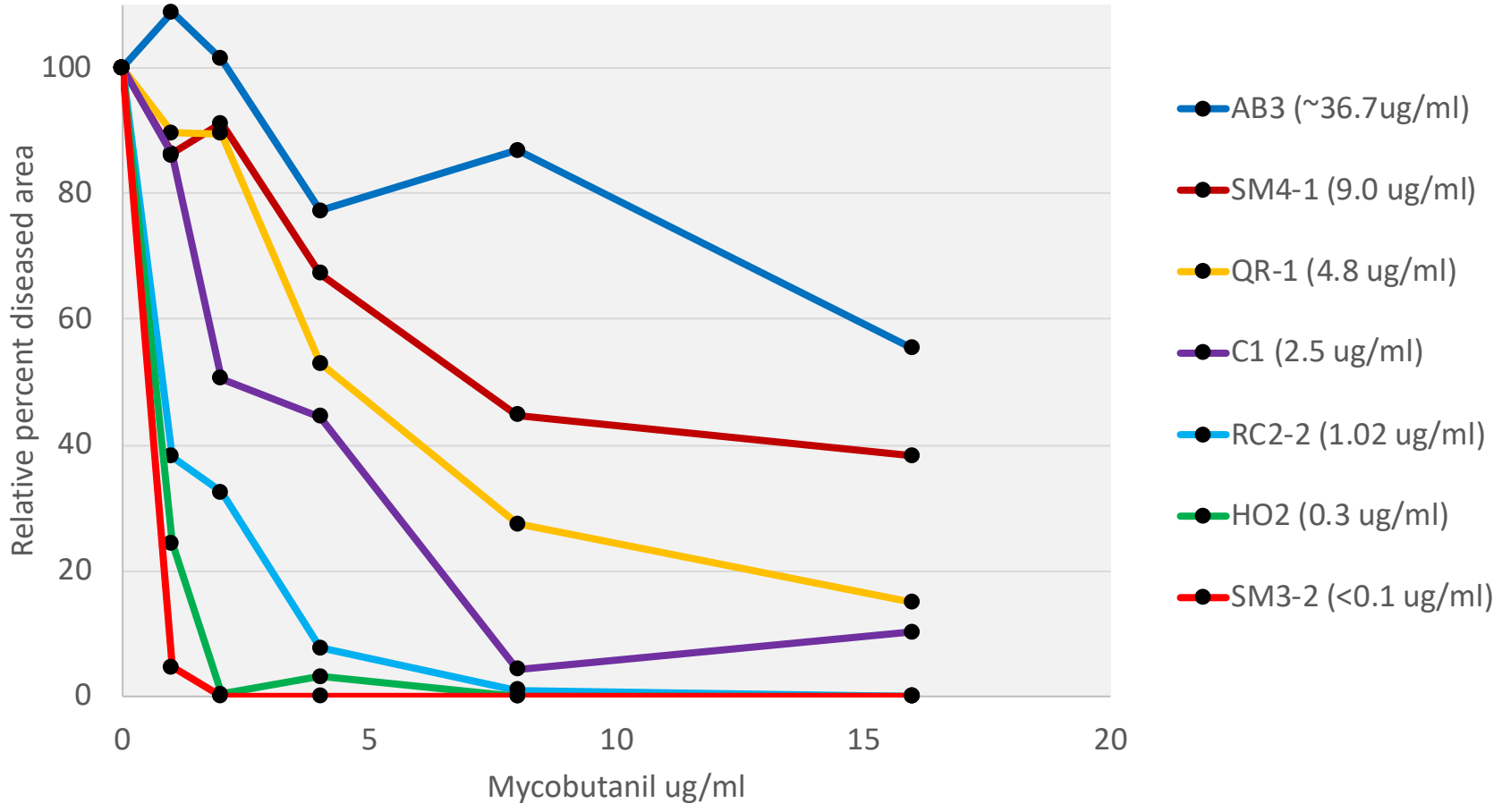






Nature Reviews Microbiology 6, 187-198

LD50 of Rally (Myclobutanil)



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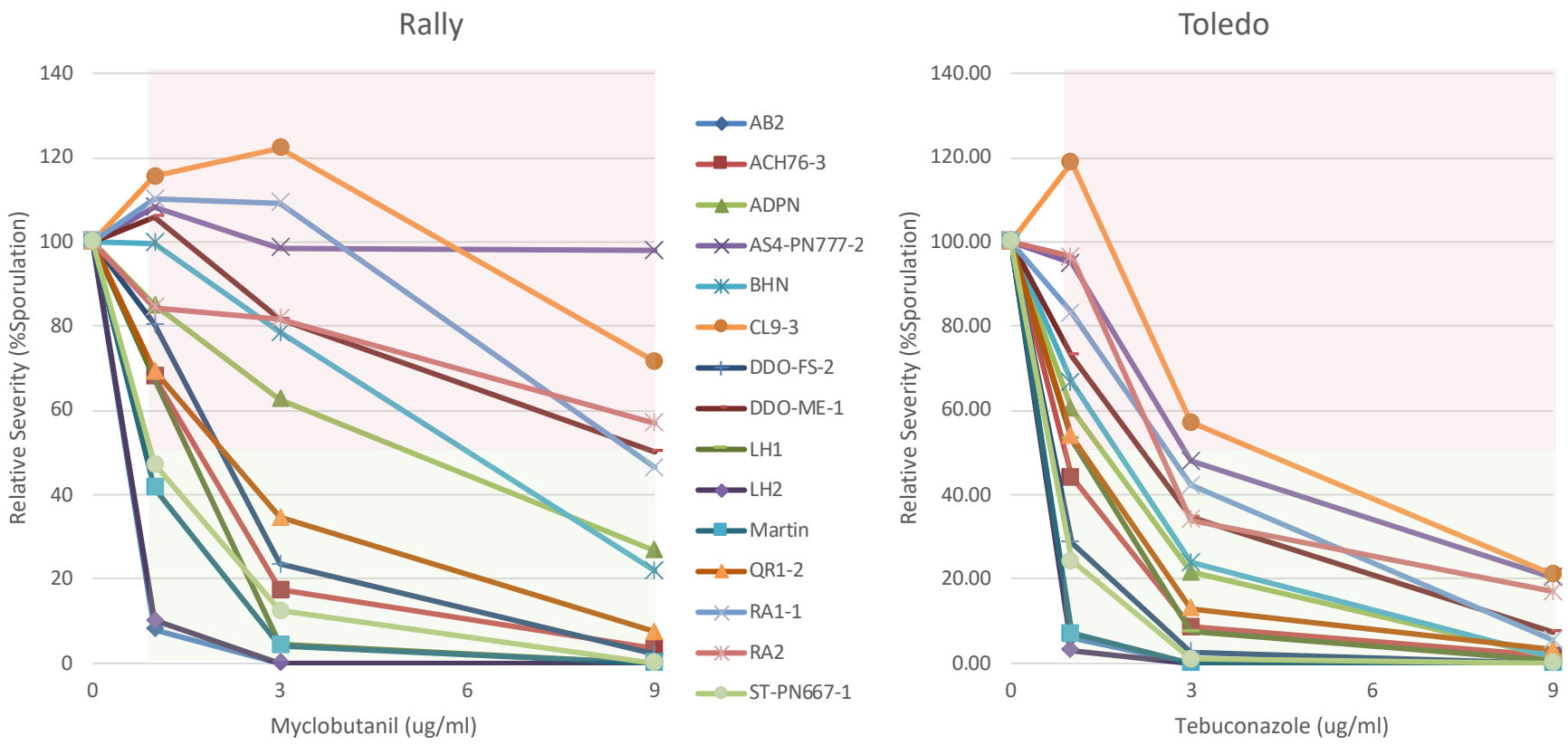
Discriminating dose



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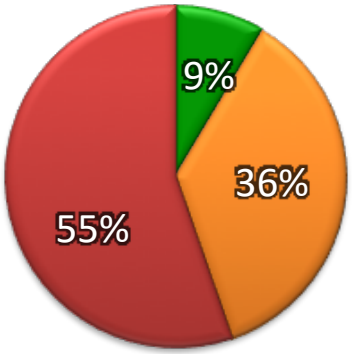
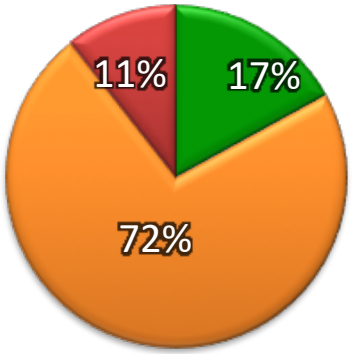
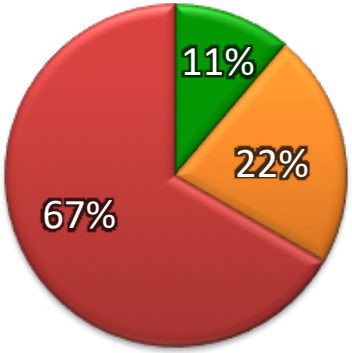
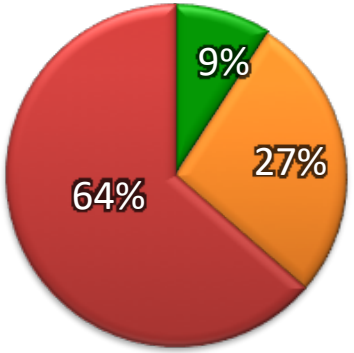
SBI Qualitative Resistance

Year	Rally (myclobutanil)	Elite (tebuconazole)
2015		
2016		

Sensitive Moderate Resistant

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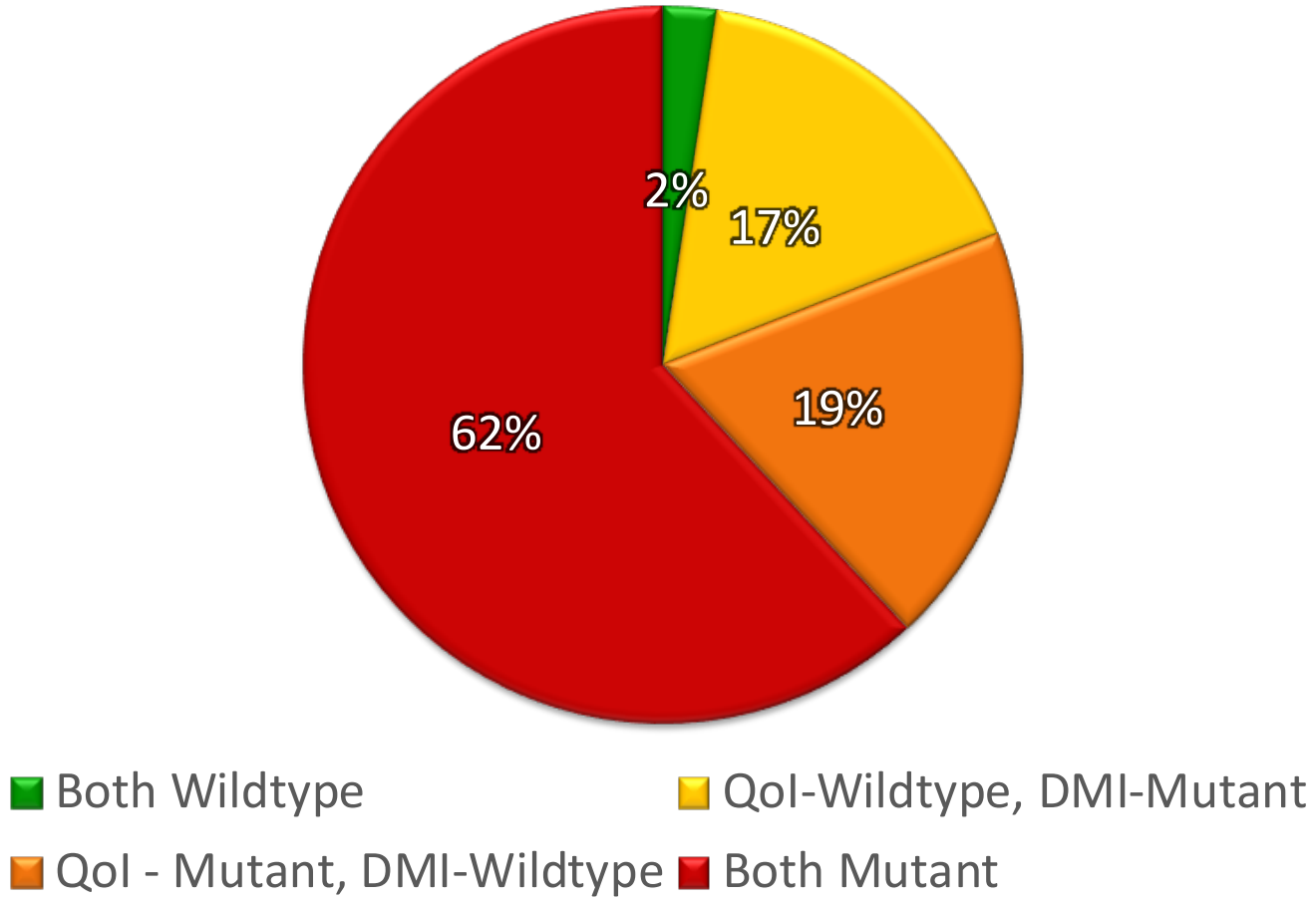
2017 Allele Frequency



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Simulating Disease Development

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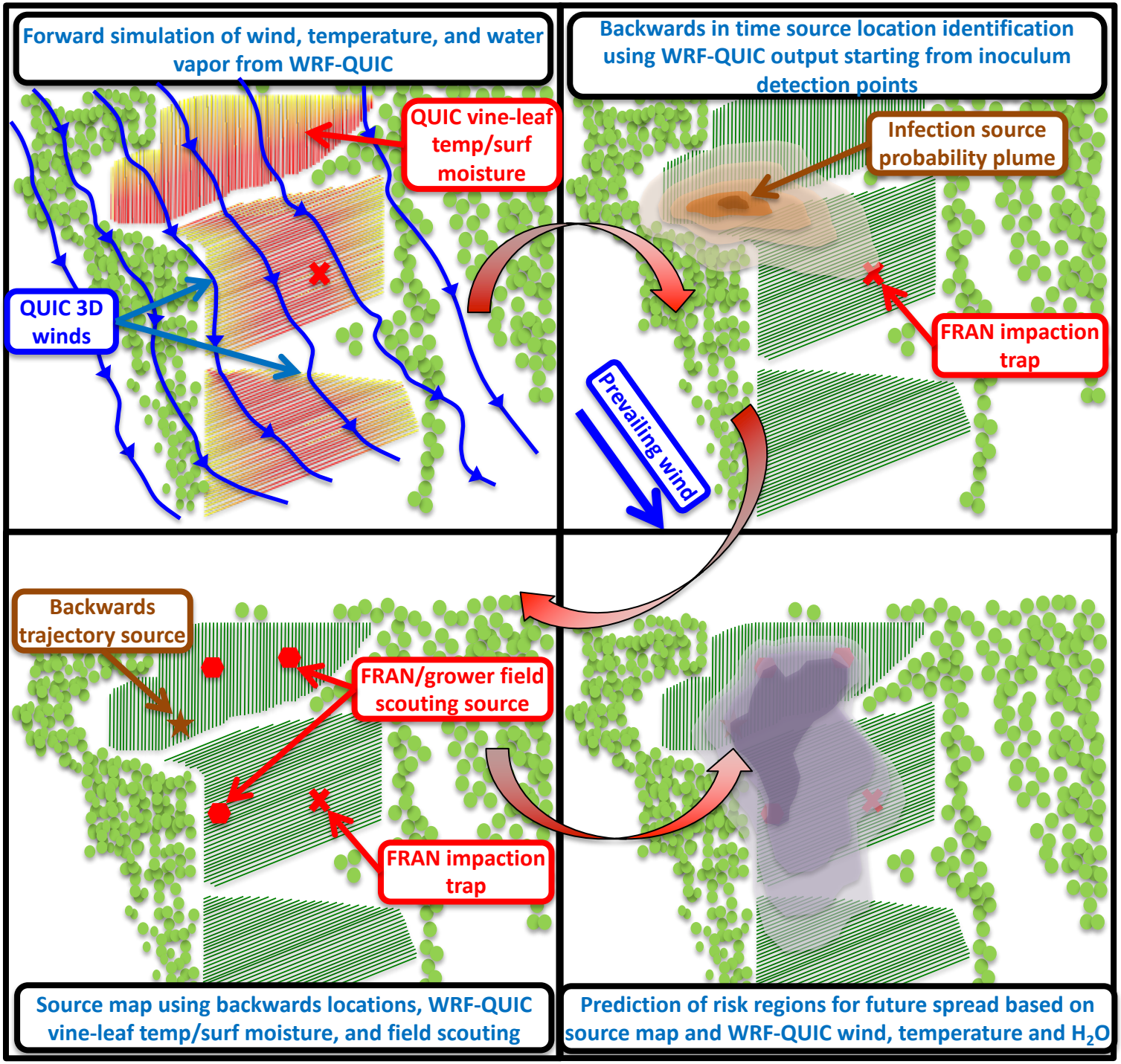
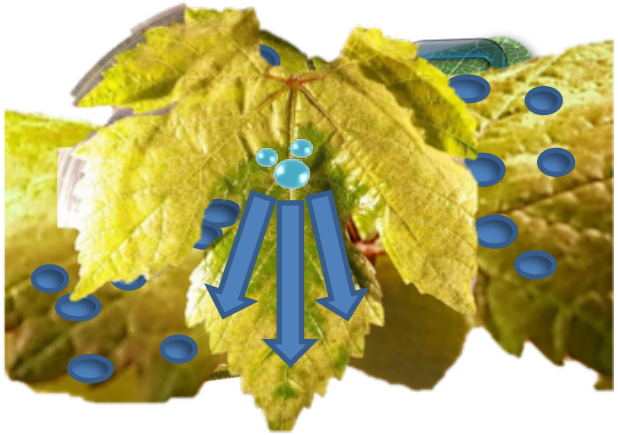
UC CE University of California Cooperative Extension
UC DAVIS University of California
Washington State University
The University of Utah
California State University Monterey Bay
Oregon State University


Forward simulation of wind, temperature, and water vapor from WRF-QUIC
QUIC vine-leaf temp/surf moisture
QUIC 3D winds
Backwards in time source location identification using WRF-QUIC output starting from inoculum detection points
Infection source probability plume
FRAN impaction trap
Prevailing wind
Backwards trajectory source
FRAN/grower field scouting source
FRAN impaction trap
Source map using backwards locations, WRF-QUIC vine-leaf temp/surf moisture, and field scouting
Prediction of risk regions for future spread based on source map and WRF-QUIC wind, temperature and H_2O

Fungicide Mobility

- **Fungicides have attributes which influence their activity**
- **Mobility**
  - Contact
  - Systemic
  - Translaminar
  - Vapor phase

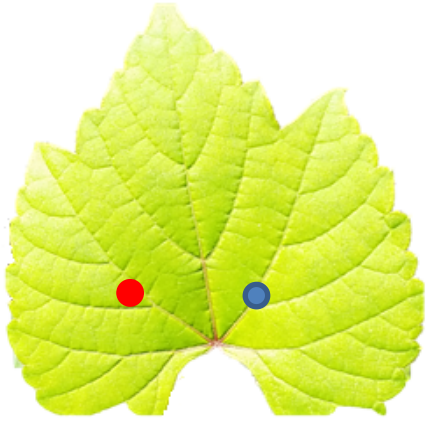


Detached Leaf Fungicide Mobility

Brent Warneke

- **Fungicides applied to pre-determined spots on the leaf**
- **Leaf inoculated with settling tower for even deposition**
- **Inhibition area measured after 7-10 days**
- **Completely randomized design with 4 replicate leaves per treatment**

Setup



Not to scale

Key

Control disc placement

Treatment disc placement

Data Collection

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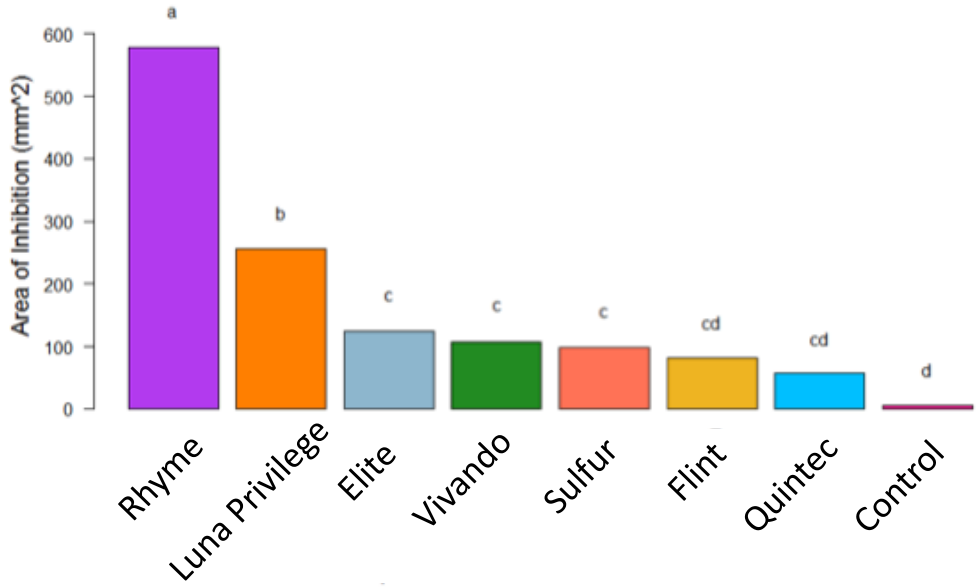
Xylem Movement

Brent Warneke

- **Fungicide treated filter disk applied to upper surface**

Luna Privilege | Elite | Flint



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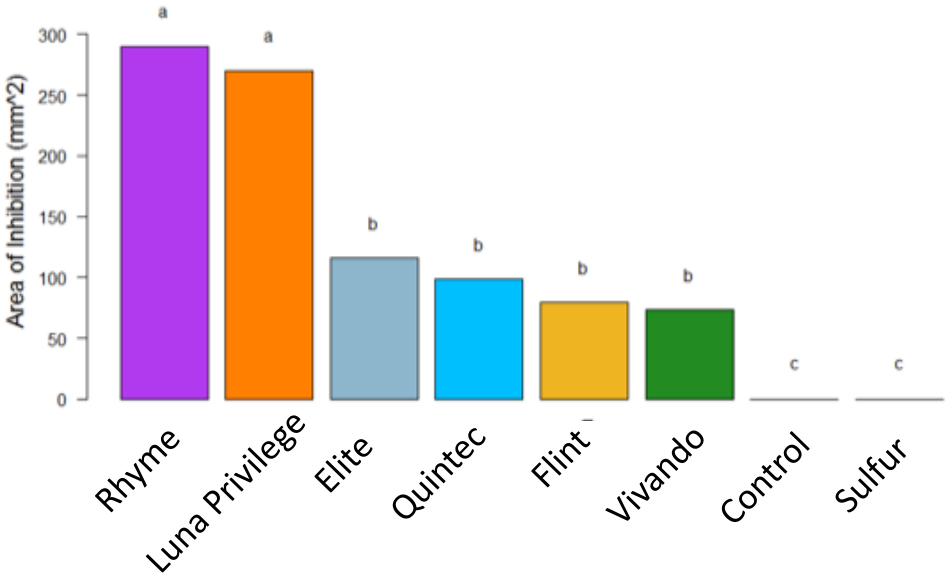
Translaminar Movement

Brent Warneke

- **Fungicide treated filter disk applied to the lower surface**

Rhyme Luna Privilege Vivando



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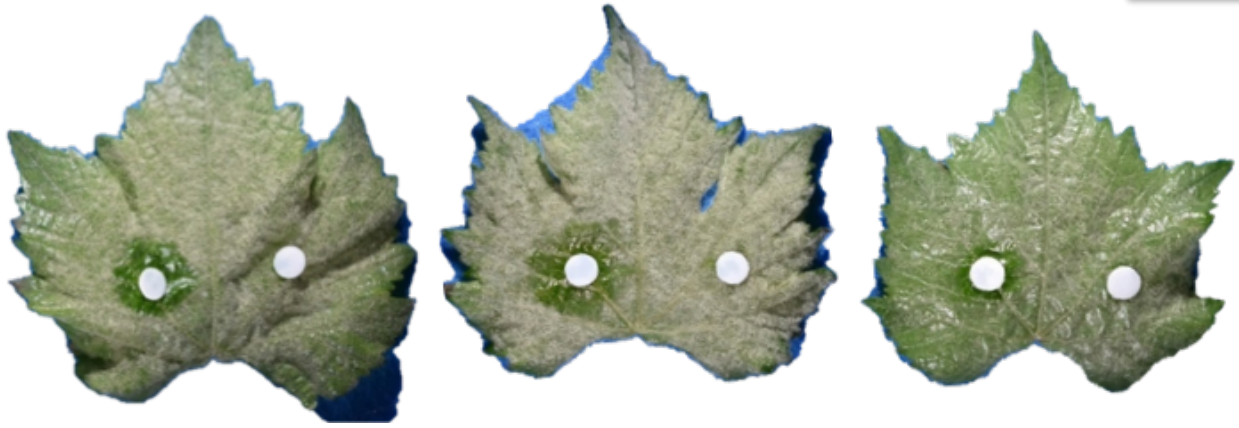
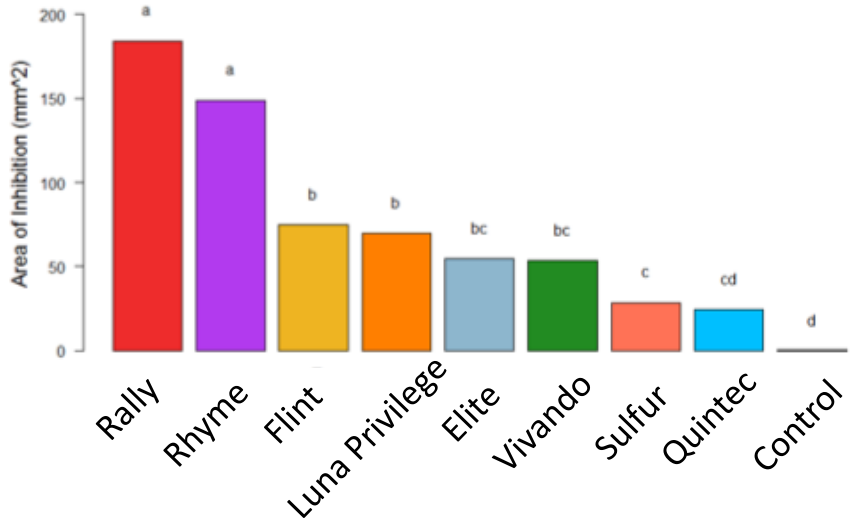
Vapor Phase Movement

Brent Warneke

- **Fungicide applied to an impermeable Teflon disc**



Luna Privilege Flint Sulfur



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Mobility Summary

- **Most modern fungicides have some form of mobility**
- **The amount and type of mobility varies widely among products**
- **All fungicides tested exhibited vapor phase mobility**

Fungicide Phenological Timing

Primary Infection

Flowering and early cluster development

Secondary Infection

Timing applications to critical fruit development stages increases disease control efficiency

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Managing Fruit Infection

- **Motivations**
  - When scouting we often find disease first on inflorescences or clusters
  - Various chemistries claim mobility to unprotected tissues

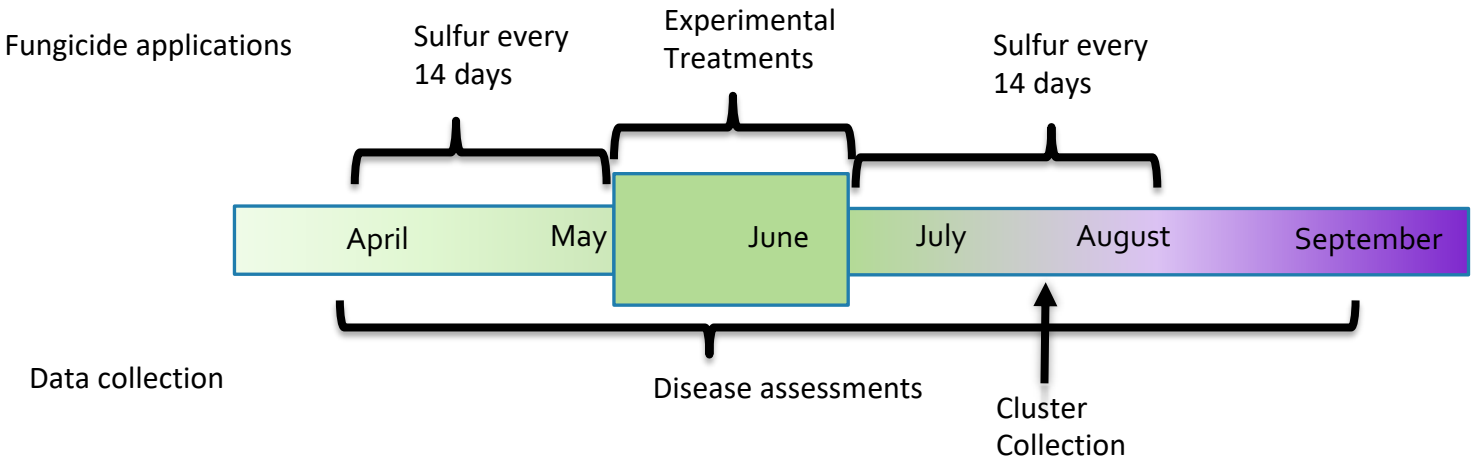
Inflorescence elongation

50% Bloom

Berry set

Experiment Timeline

Brent Warneke



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Fungicides

Brent Warneke

Fungicide	FRAC Group	Activity	Rate per acre
Quintec	13	xylem mobility and volatilization	4 fl oz
Elite 45	3	xylem mobile	4 oz
Luna Privilege	7	locally systemic	4 fl oz
Flint	11	locally systemic	2 oz
Microthiol	M2	non-systemic, volatilization	3 lb

Application Timing

Brent Warneke

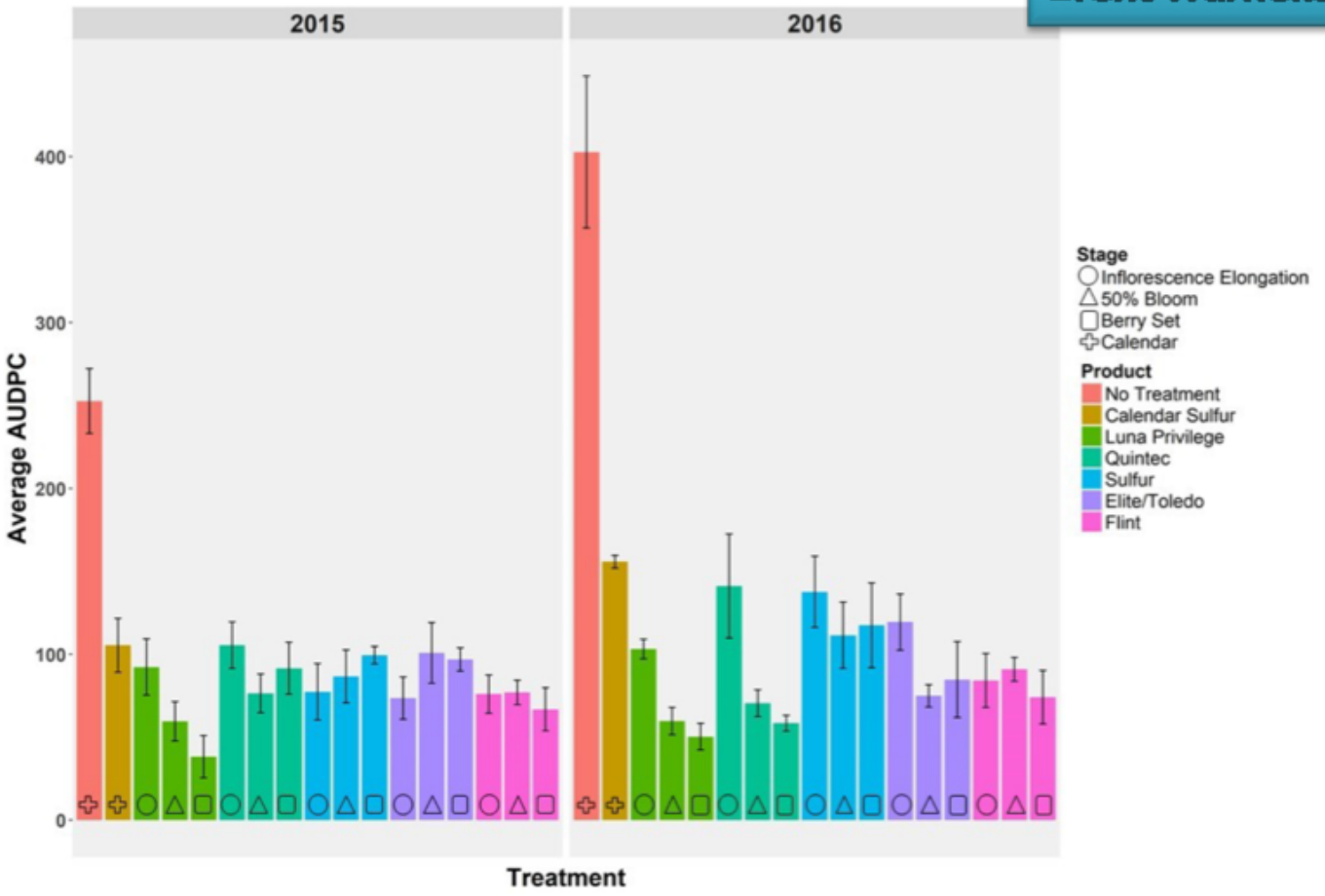
Inflorescence elongation

50% Bloom

Berry set

Leaf Disease Development

Brent Warneke



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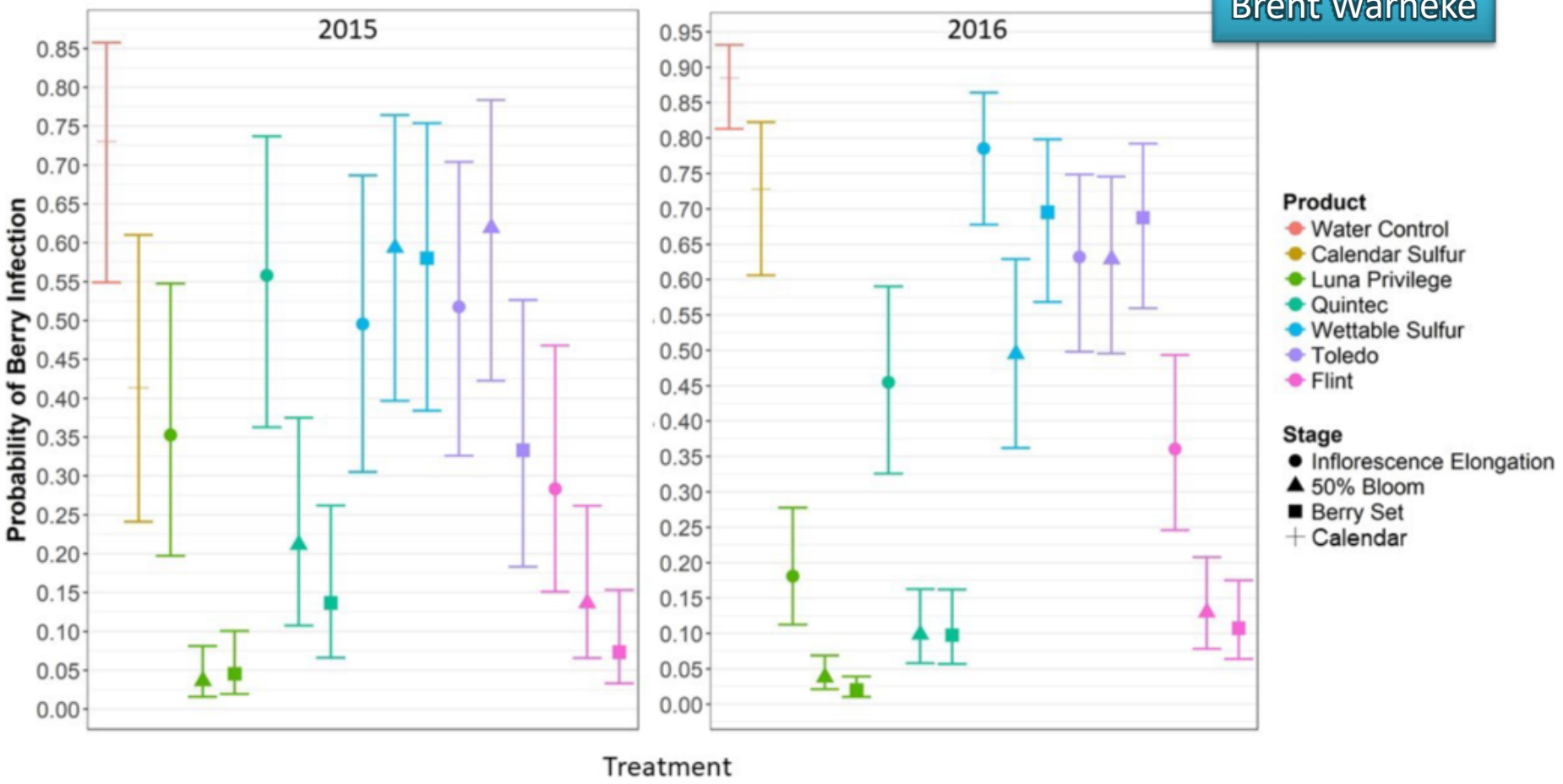
Berry Disease Development

Brent Warneke



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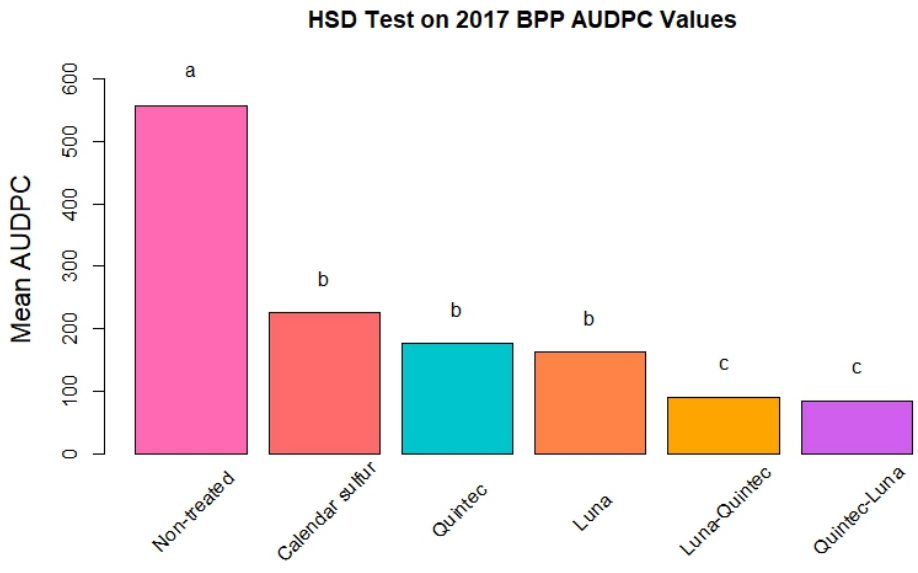
Program Integration

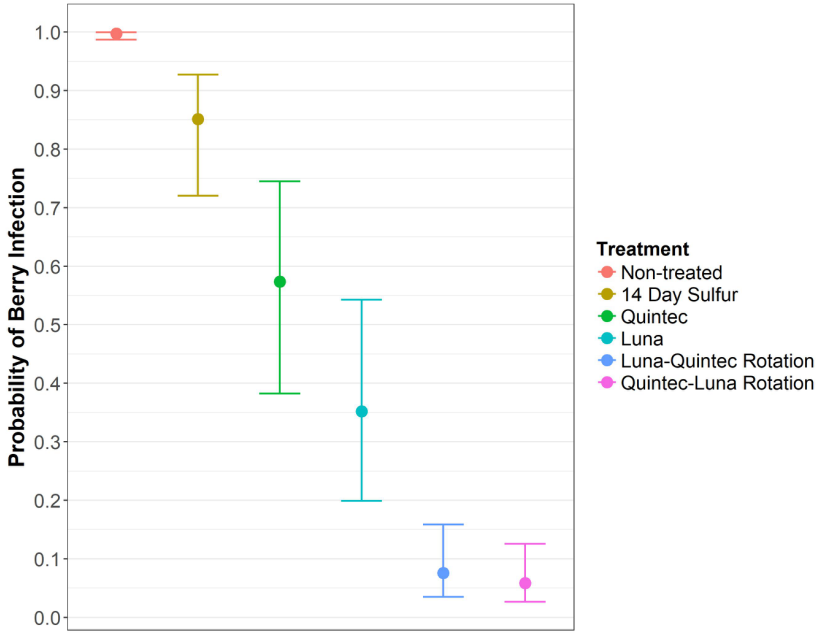
Brent Warneke

- **Season long wetable sulfur at 14 day intervals except Luna or Quintec applications beginning Bloom**

Leaf Disease



Berry Disease



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Sulfur Use

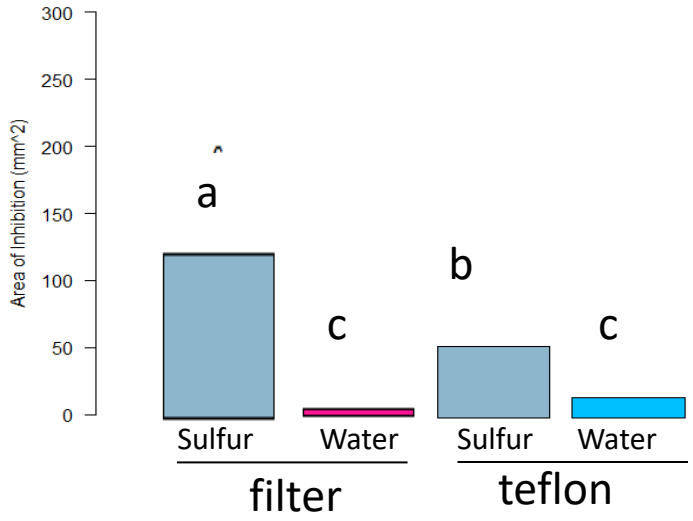
- 3-17 applications per season depending on region
- More in organic, LIVE, biodynamic, etc
- Negative Impact on beneficial insects
- Increases mite movement and populations
- Residues thought to be an issue in the winery

Hop Powdery Mildew

- **Hops managed with same rates of sulfur but have 3× the canopy**
- **Large aggregates appeared to extend the longevity of disease control**

Sulfur Redistribution

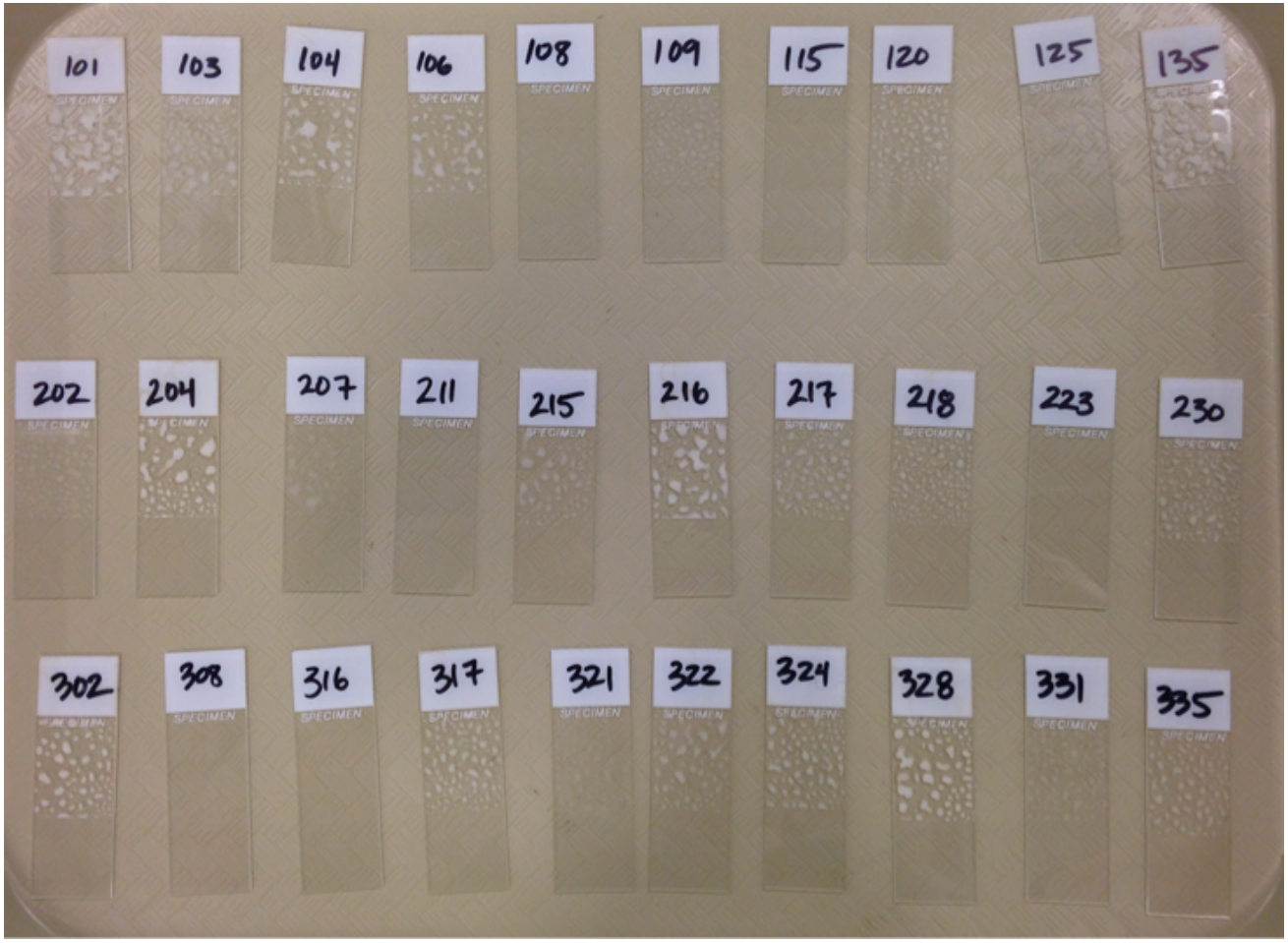
- Diffusion
- Vapor



Effect of Sulfur Proximity on Conidia Germination

- Glass slides with ½ covered with tape sprayed with sulfur.
- Tape removed and incubated at 70°F
- Slides were placed in settling tower and inoculated with *E. necator* spores
- Incubated at 72°F for 48 h then observed microscopically
- Germinated and ungerminated
  - 1, 5, 10, and 15mm away from the sulfur on the sulfur-free half
  - 5 mm into the sulfur zone

Aggregate size



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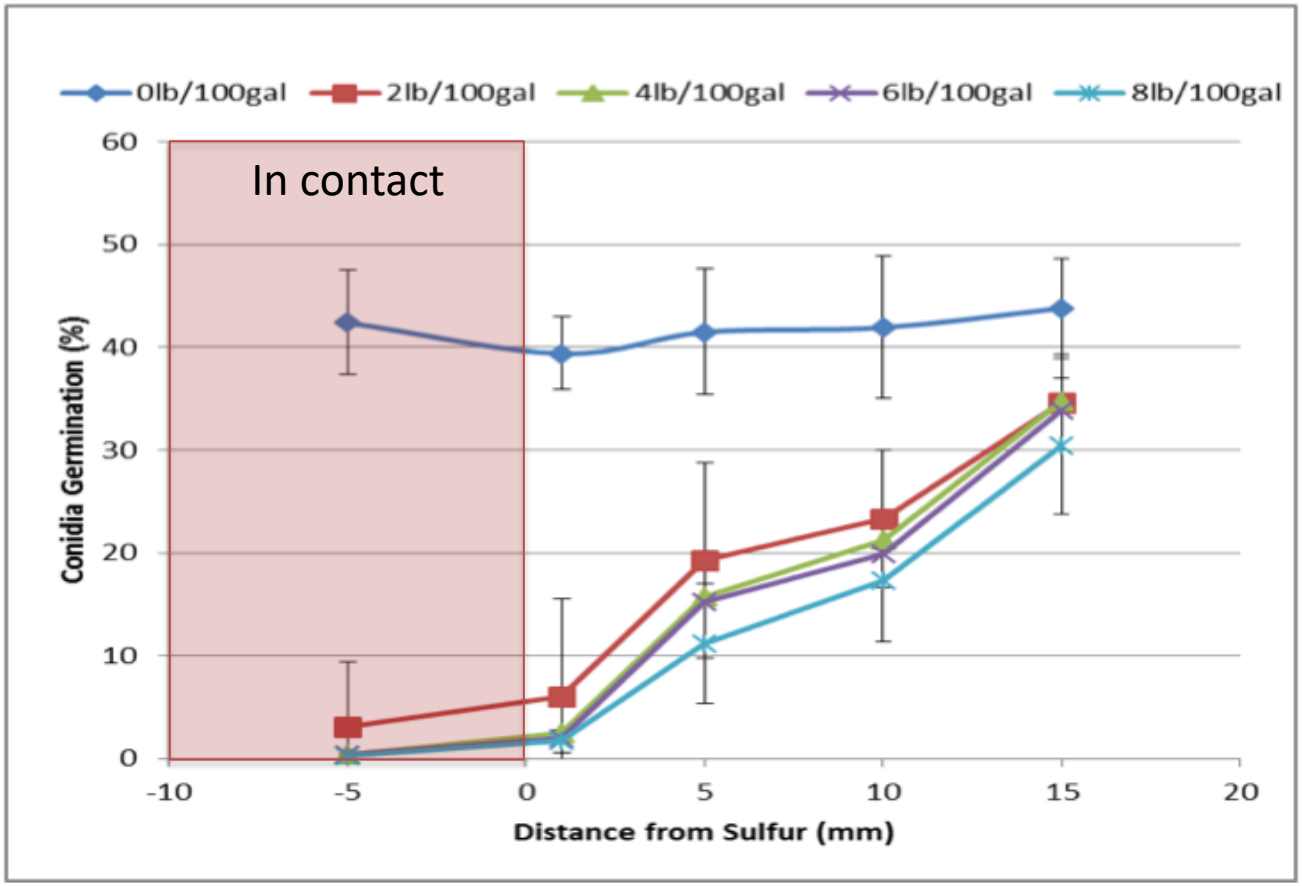
Effect of Sulfur Proximity on Conidia Germination



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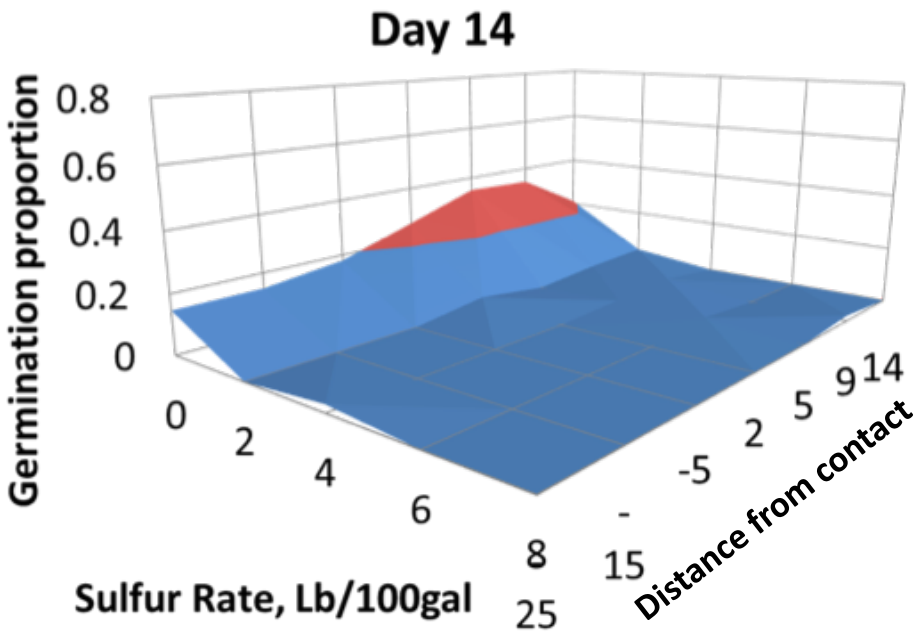
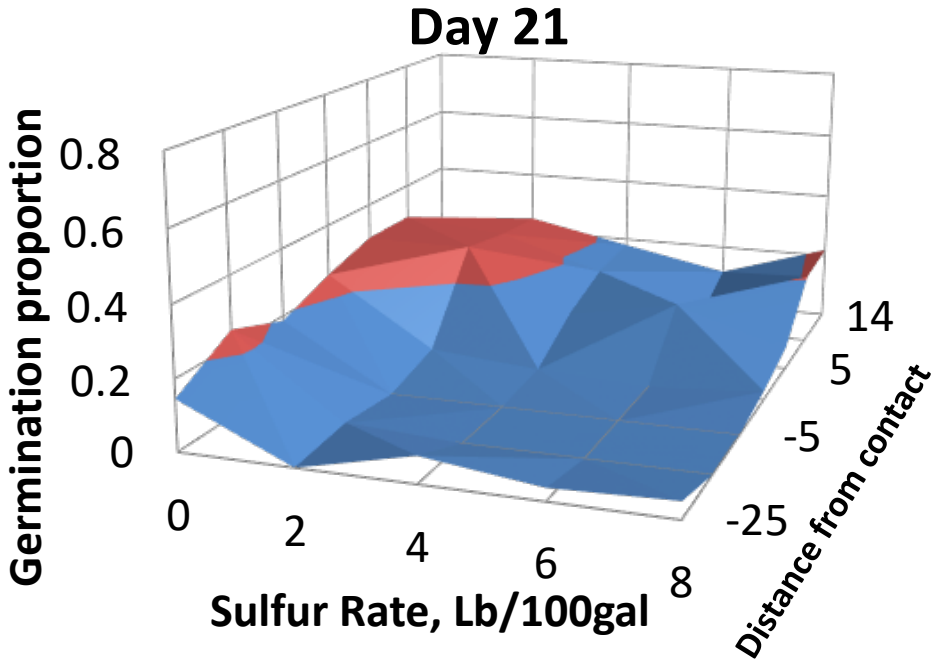
Effect of Sulfur on Conidia Germination Over Time





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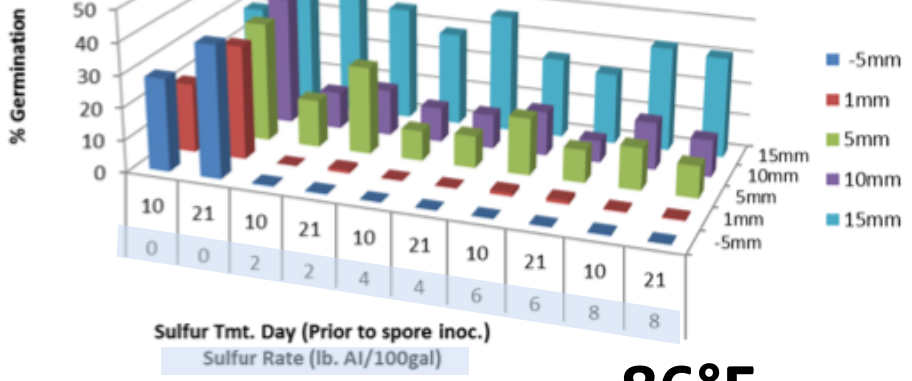
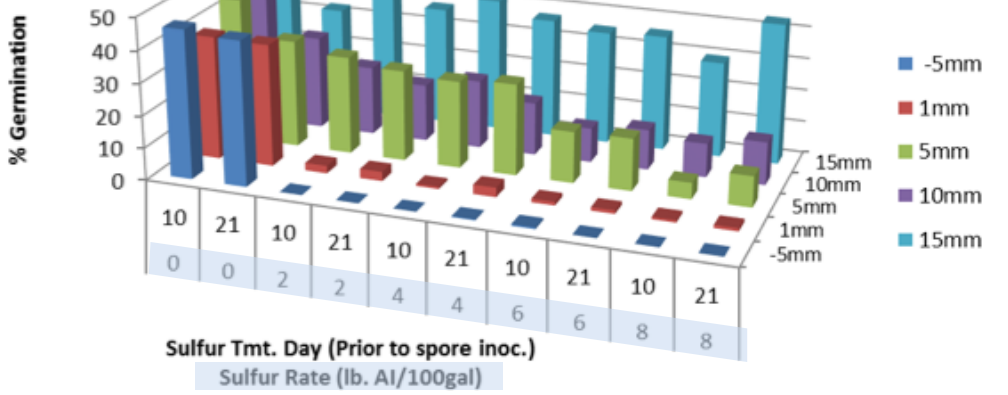
Temperature and Sulfur Activity

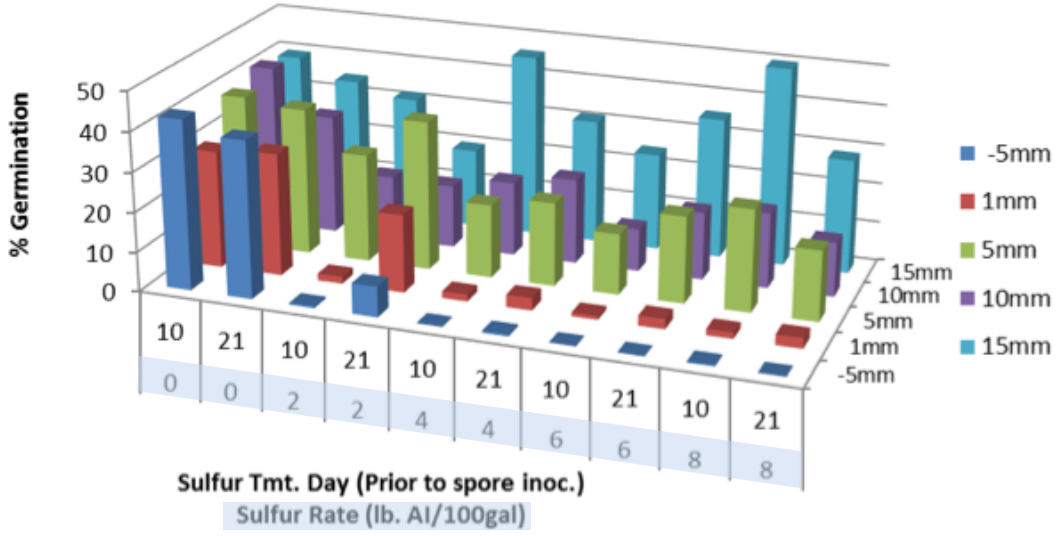
50°F



68°F



86°F



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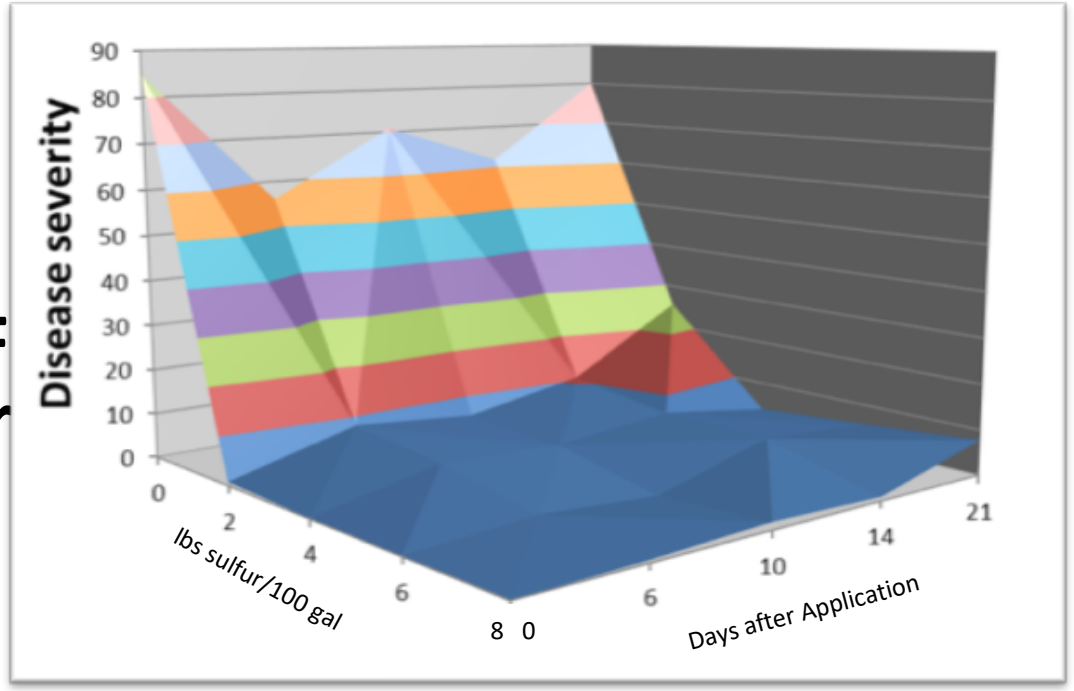
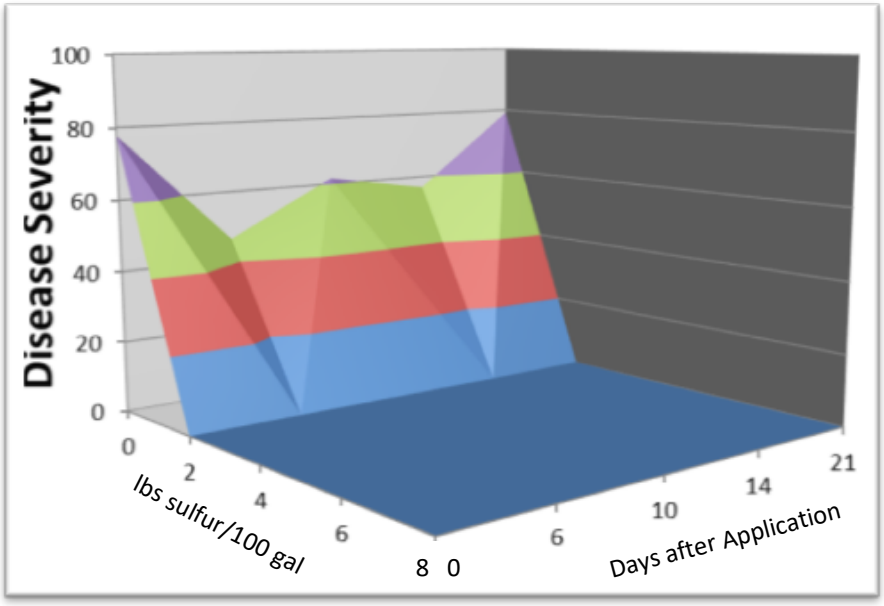
Sulfur and Leaf Age Interactions

- **Chardonnay seedlings sprayed with sulfur**
- **Incubated at 70°F for 0, 6, 10, 14, or 21 days**
- **Inoculated with *E. necator* spores**
- **Incubated at 70°F 10 to 12 days**

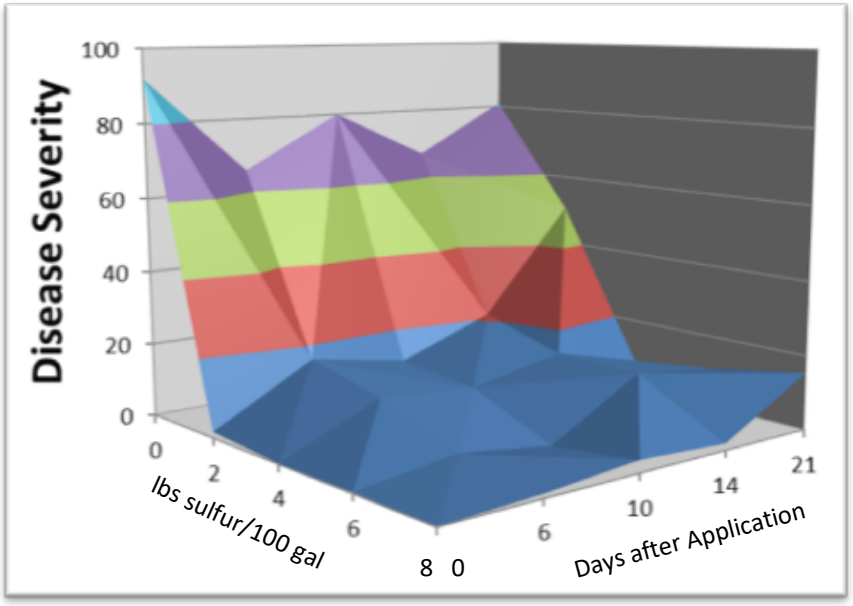


Sulfur and Leaf Age Interactions

Fully Expanded Leaves



Expanding Leaves



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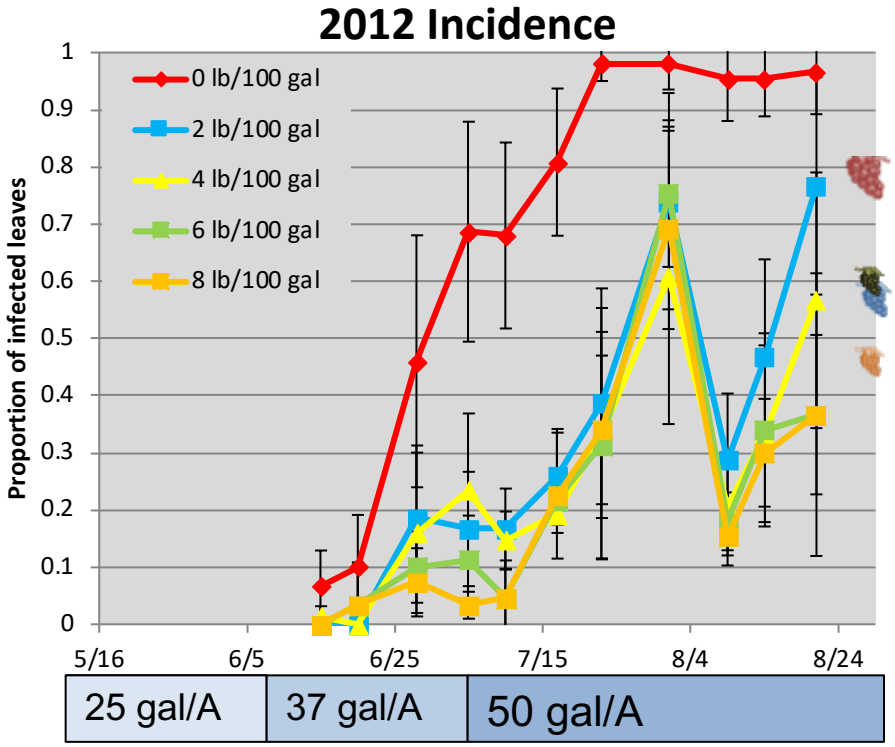
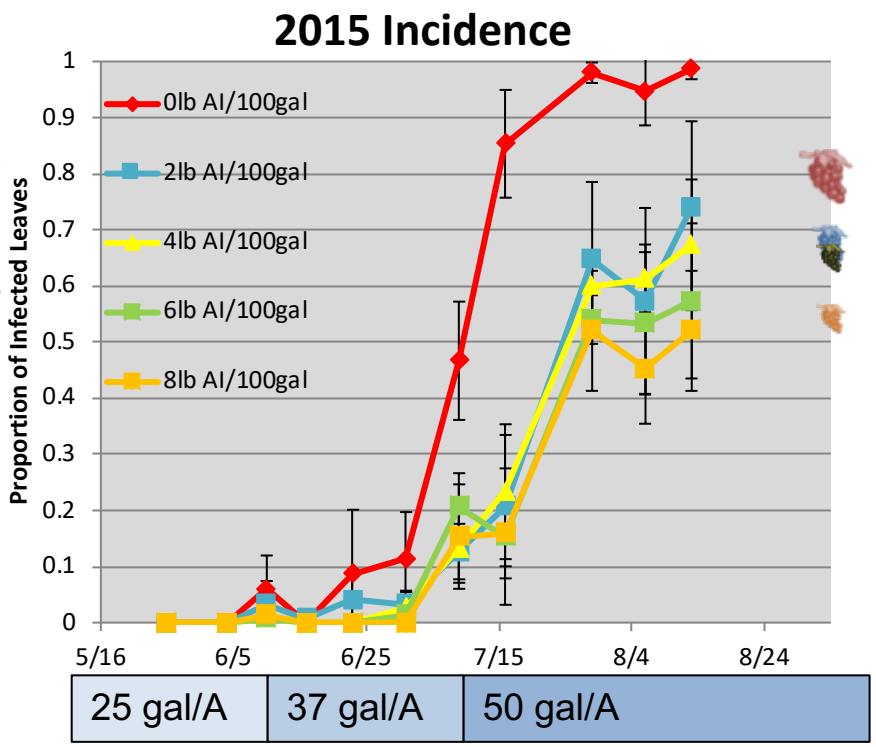
Field Efficacy

- Five vine plots
- Pinot Noir clone 2A on 420A rootstock
- 5’ x 6’ spacing bilateral canes with VSP
- Sulfur applications initiated inoculum was detected.
- Rates were 0, 2, 4, 6, or 8 pounds sulfur mixed with 100 gallons water. Volume applied varied based on canopy growth
- Applied using a ducted over the row sprayer with venturi nozzles
- 10 leaves from 5 vines assessed every 2 weeks.
- 10 cluster/vine sampled just prior to véraison and disease incidence on 25 berries/cluster

Efficacy of Micronized Sulfur for Powdery Mildew Management





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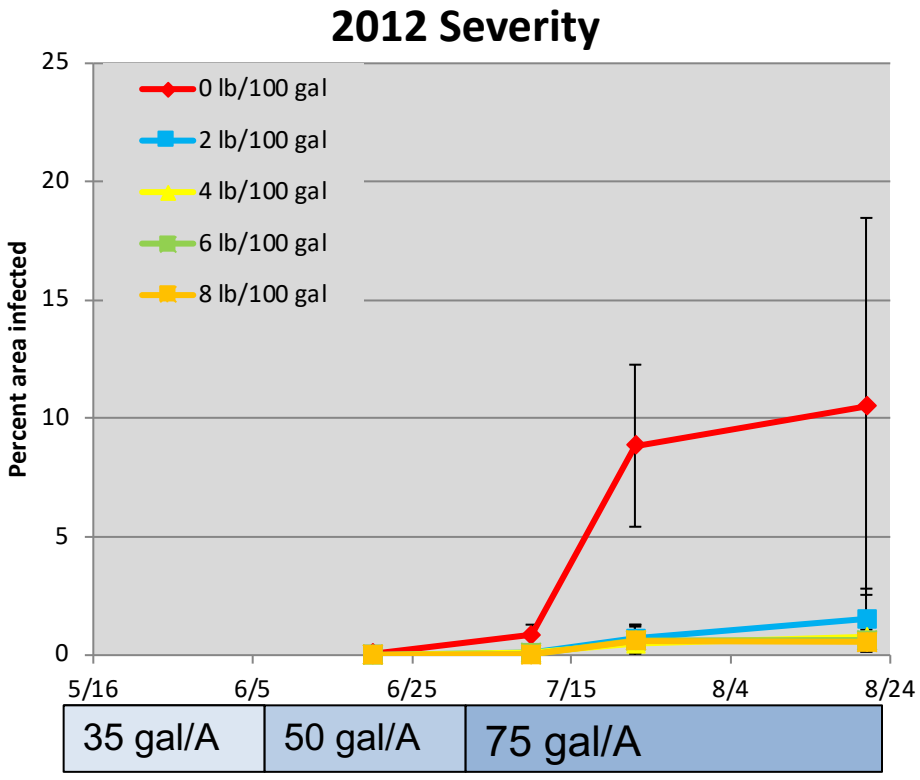
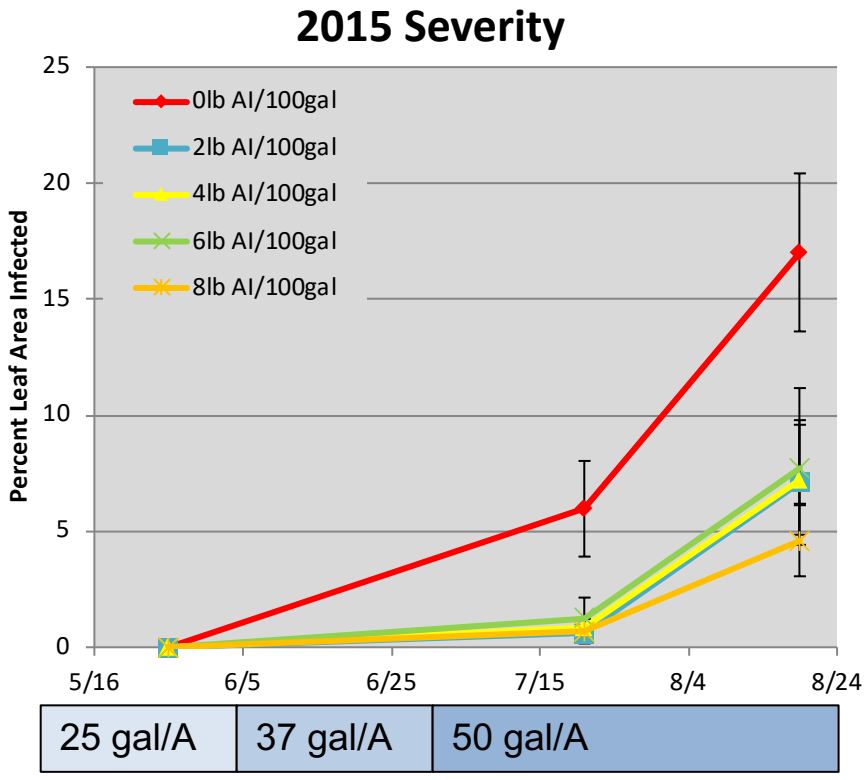
Efficacy of Micronized Sulfur for Powdery Mildew Management





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Foliar Pathology Lab 2017

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Graduate Students, Oregon State University

Carley Allen, Jack Blackham, Baily Williams, & Katlyn Thrall:
Undergraduates, Oregon State University

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Ioannis Stergiopoulos

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