Understanding *Colletotrichum* species and their biology to improve chemical management options against grape ripe rot

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(Me-zoo-jo, rhyme with Idaho or Navajo)

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School of Plant and Environmental Sciences

Virginia Tech

Grapepathology.org

VA Wine Industry

Glen Manor vineyards, Browntown VA

- Over 300 active wineries and 350 – 400 associated vineyards
- $1.4 billion annual economic impact (Economic impact report, 2017)
- Ranked ~6th nationally in wine production
- Piedmont area in VA was selected as one of The World's Top 10 Wine Destinations for 2020 by VinePair.com

Quick background

- Ripe rot was not considered as a threat when I joined VT in 2009.
  - I have worked on viruses which seemed to be more important (and yes, it is important!)
  - Jones et al., (2015) EJPP, Jones and Nita (2016), EJPP, Jones and Nita (2019) PHP.
- However, I received a call from a grower in the eastern shore VA in 2011 who lost more than 30% (at least) of his crop 3-4 years in a row due to "berry shriveling" .

Cultivar Chardonnay with ripe rot at a commercial vineyard in eastern shore VA

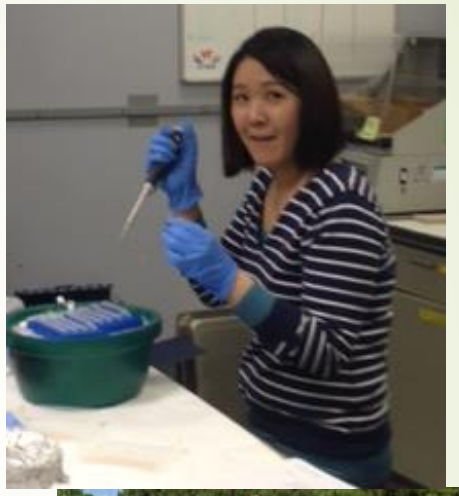
Confirmation of Shriveling berries from *Colletotrichum* inoculation

The majority of contents presented today was done by Dr. Charlotte Oliver

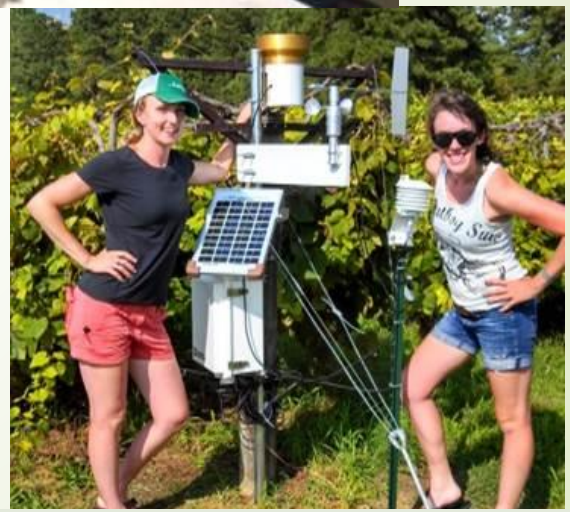
Mrs. Vanette Trumm
(2014-2017)

Dr. Diana McHenry
(2015-)

Dr. Charlotte Oliver
(2012-2018)



Akiko Mangan
(2014-)

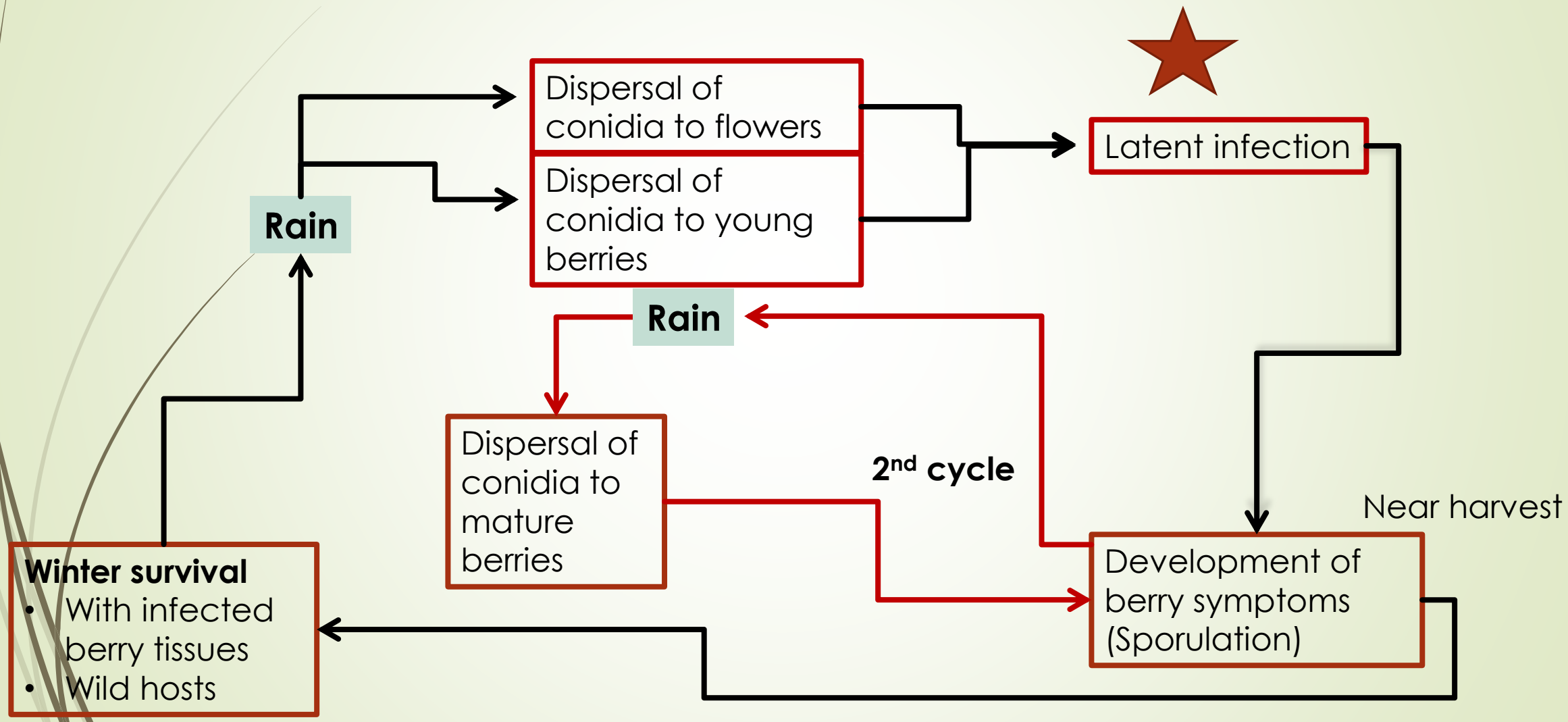


Ms. Amanda Bly and Ms. Sabrina Hartley
(2013-16)

Grape Ripe Rot

- Direct damage
- Indirect damage
  - Only 3% contamination can affect taste of wine (Meunier 2009)
- Two filamentous fungal species complexes:
  - *Colletotrichum acutatum*
  - *Colletotrichum gloeosporioides*
- Endemic to Virginia (Von Schrenk & Spaulding 1903)
- Prefers warm temperatures (25-30 °C) (Peres et al. 2005)
- Numerous acervuli with salmon-colored conidia in mass and berry shrivels as disease progresses

Ripe Rot Disease Cycle



Research Objectives

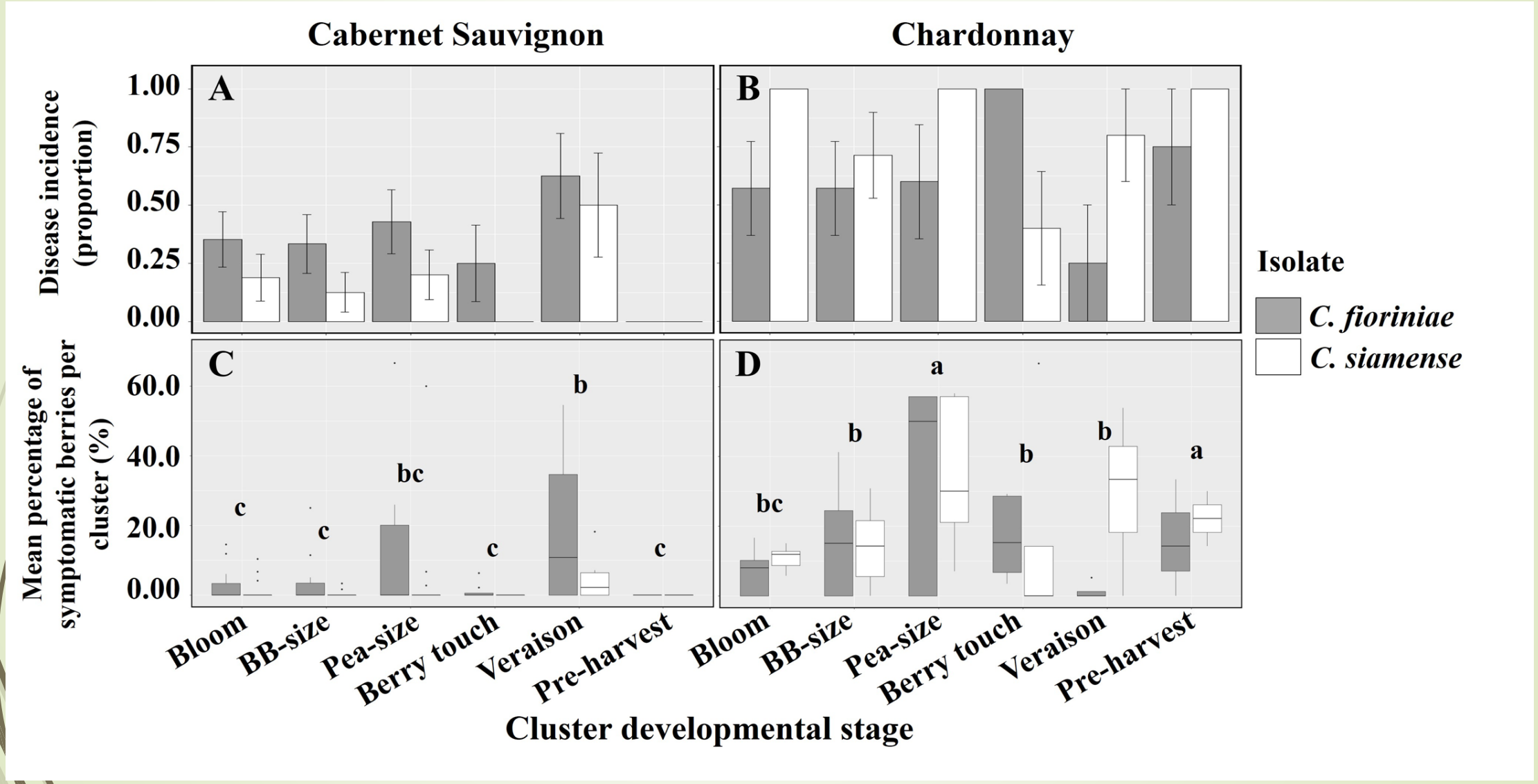
1. Investigate cluster maturity and varietal differences on disease incidence and severity
2. Investigate the infection process of *Colletotrichum* species in flower, leaf, and woody tissues of grape
3. Identification of *Colletotrichum* species isolated from grapes in VA
4. Screen fungicide modes of action to determine potential fungicides to be used.

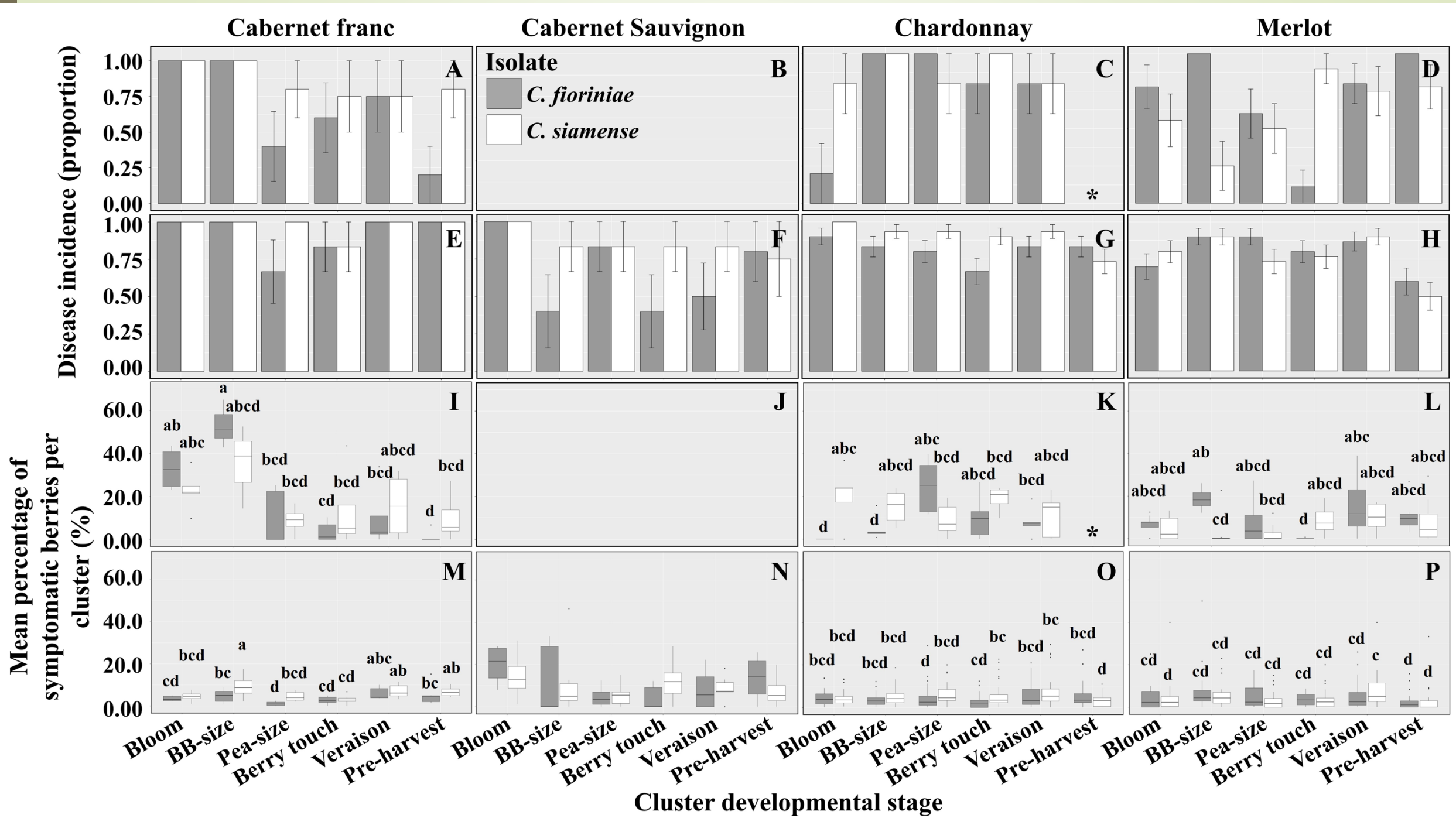
For the interest of time, I will not cover materials and methods in detail. Please see me if you have any questions.

Varietal and cluster maturity studies

- Field study 2012-2013
  - Cultivars: Chardonnay, Cabernet franc, Cabernet Sauvignon, and Merlot
- Potted vine study 2012-2013
  - Cultivars: Cabernet Sauvignon and Chardonnay
- Inoculation of *C. fioriniae* or *C. siamense* (10^5 spores/ml) was made based on cluster growth stage from bloom until two weeks after veraison
- Disease incidence and severity was visually estimated at harvest.

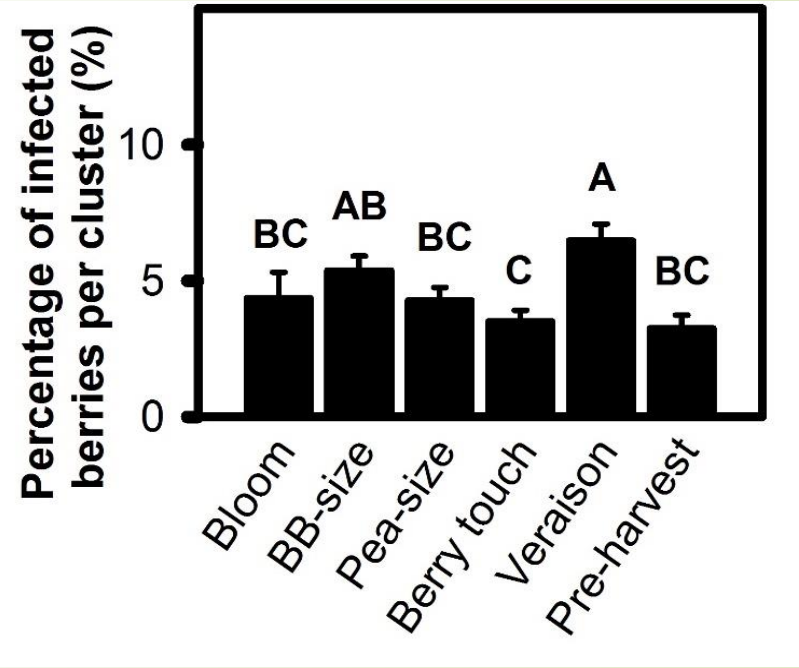
Results from potted vine study




Cabernet franc
Cabernet Sauvignon
Chardonnay
Merlot
Isolate
C. fioriniae
C. siamense
2013
2014
Disease incidence (proportion)
Mean percentage of symptomatic berries per cluster (%)
Bloom
BB-size
Pea-size
Berry touch
Veraison
Pre-harvest
Cluster developmental stage

Summary of Field and Potted Vine Studies

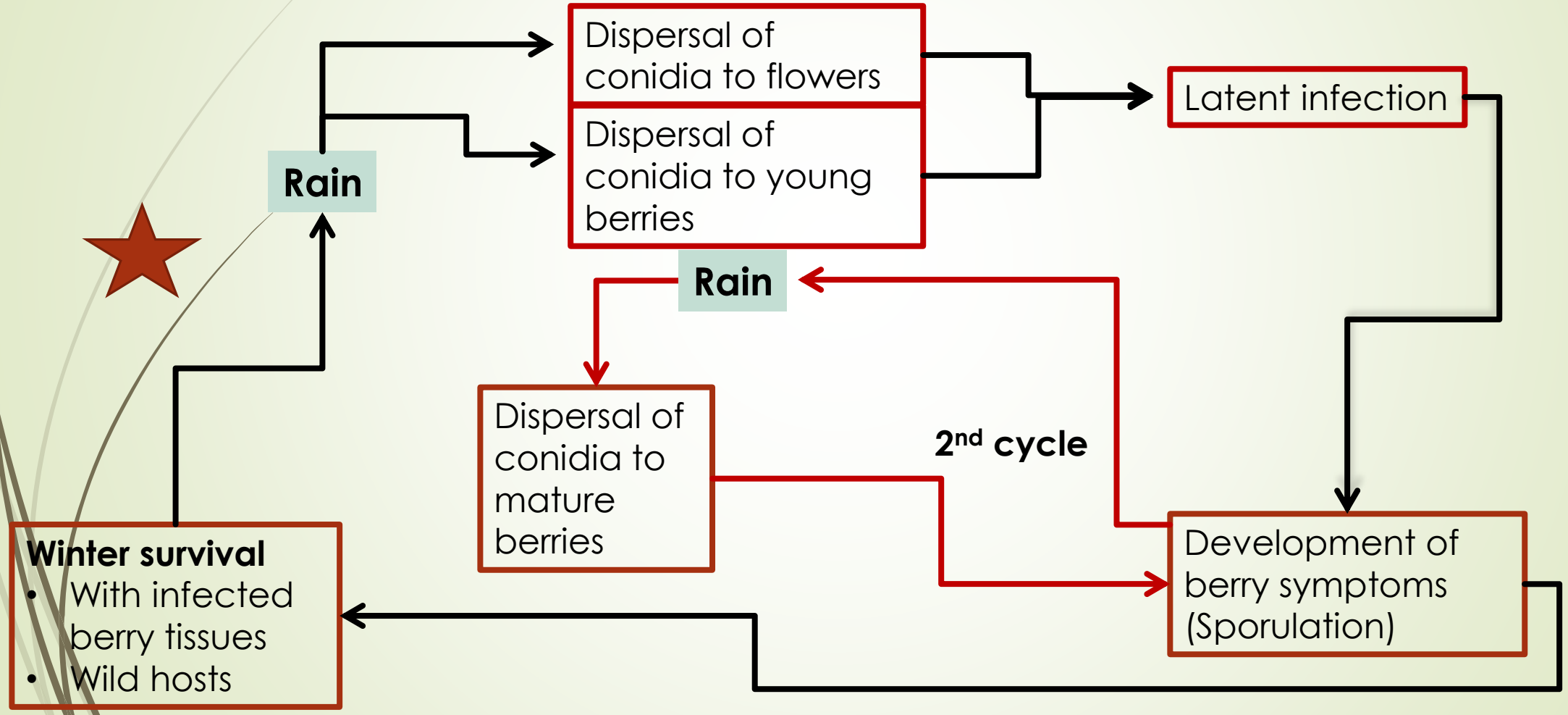
- There were differences among tested cultivars
  - Merlot<Cabernet Sauvignon<Cabernet franc = Chardonnay
  - However, even with Merlot, disease incidence was high
- There are differences between the inoculation time points
  - Tissues are susceptible from bloom until harvest
  - Significantly lower disease severity at berry touch, but still resulted in a certain level of disease development



Trying to fill in the gaps in ripe rot disease cycle

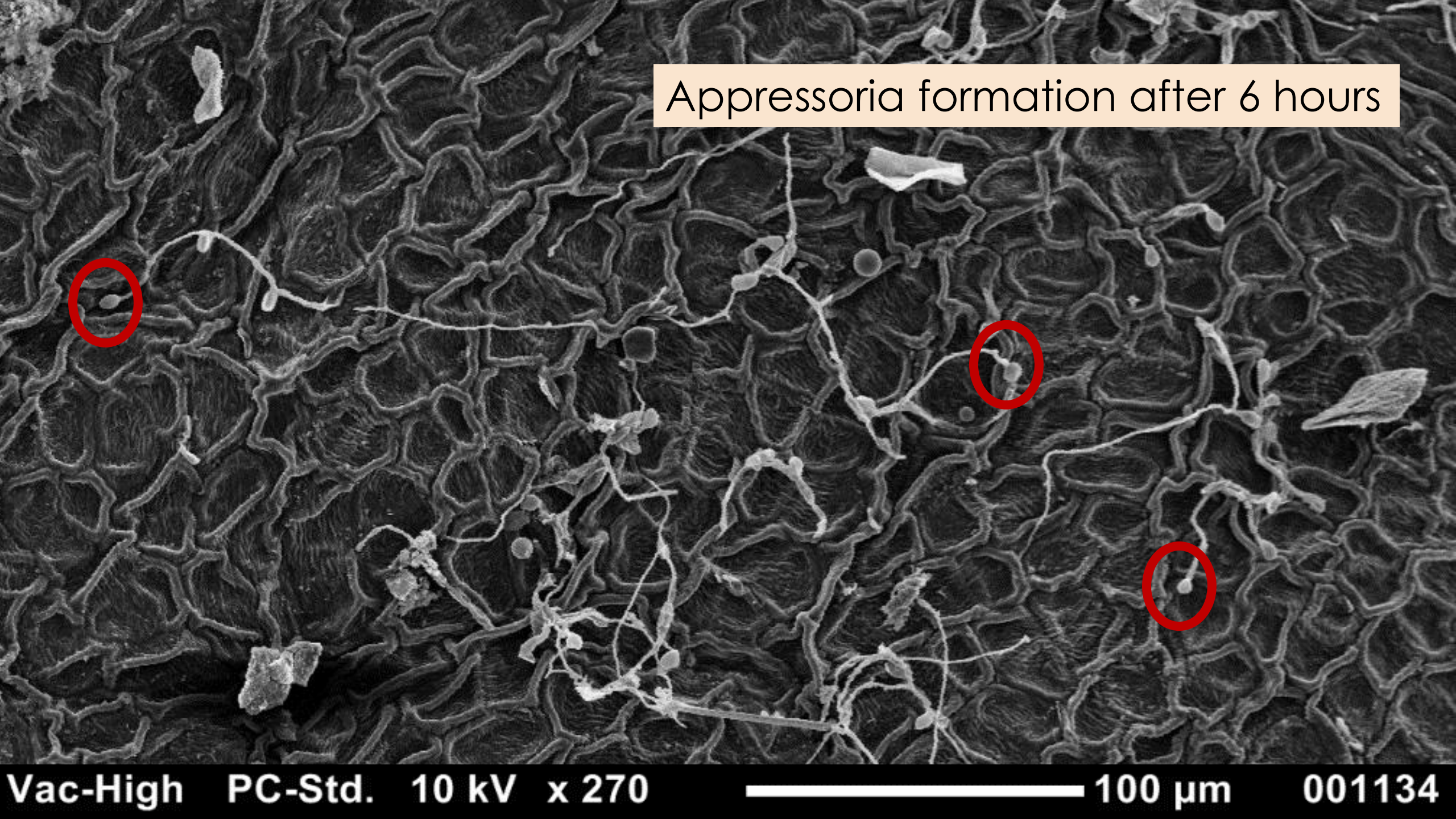
- It seems that there is a long time period between spore production at harvest (Aug – Oct) to the following year's bloom (June)
- Can they infect other parts of the grape while they are waiting for availability of flowers?
  - Leaves?
  - Can they infect woody tissues and produce spores from it?

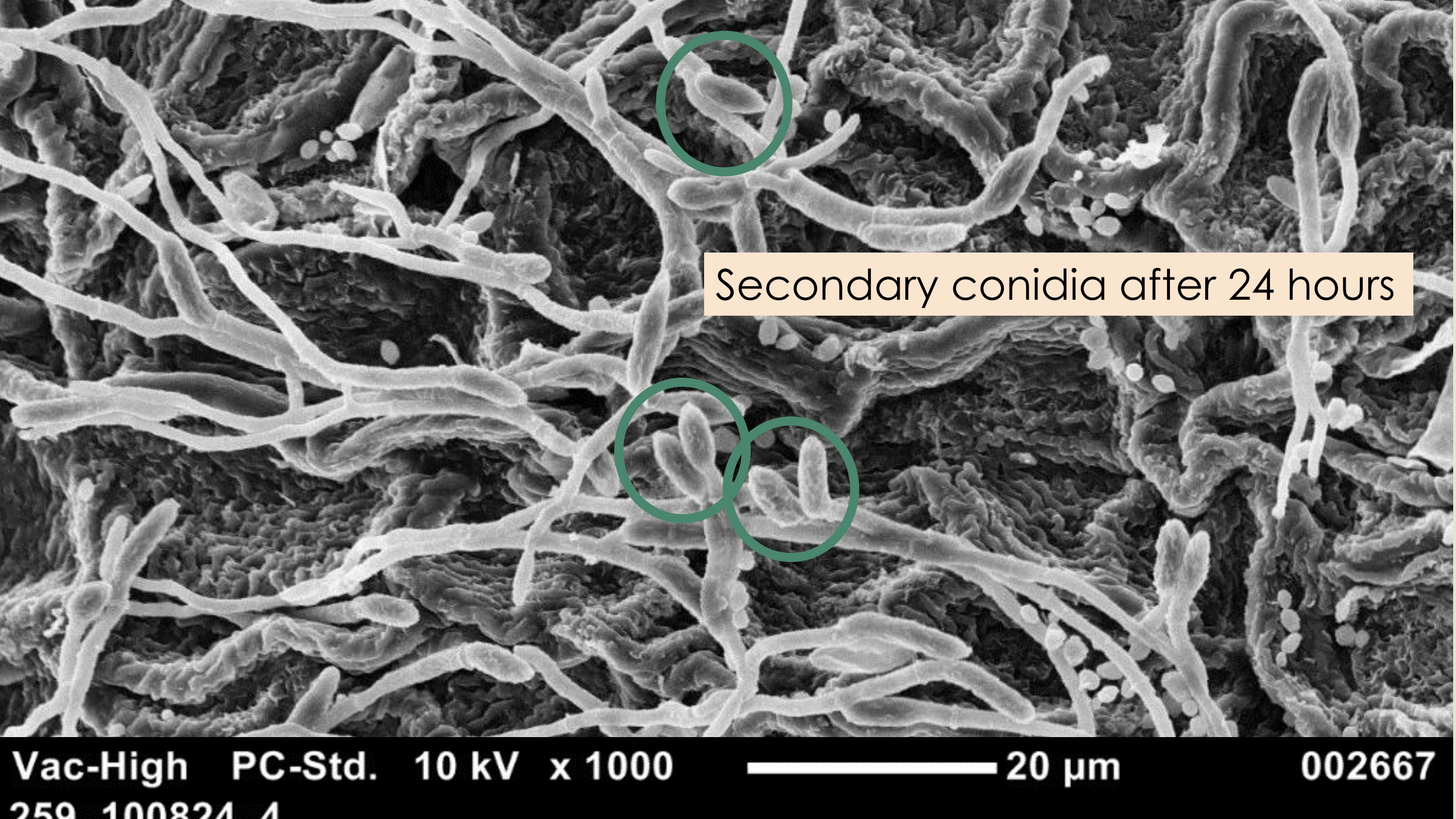
Ripe Rot Disease Cycle



Trying to fill in the gaps in ripe rot disease cycle: Leaf Histology Studies

- *C. fioriniae*
- One year old cv. 'Chardonnay' grafted grapevines
- Two 2 week old leaves per vine
  - Marked with a wax pencil
- 10 µL of a 5×10^5 conidia/mL
- Incubated for 3, 10, and 24 hr
- Samples were examined using the Scanning Electron Microscope

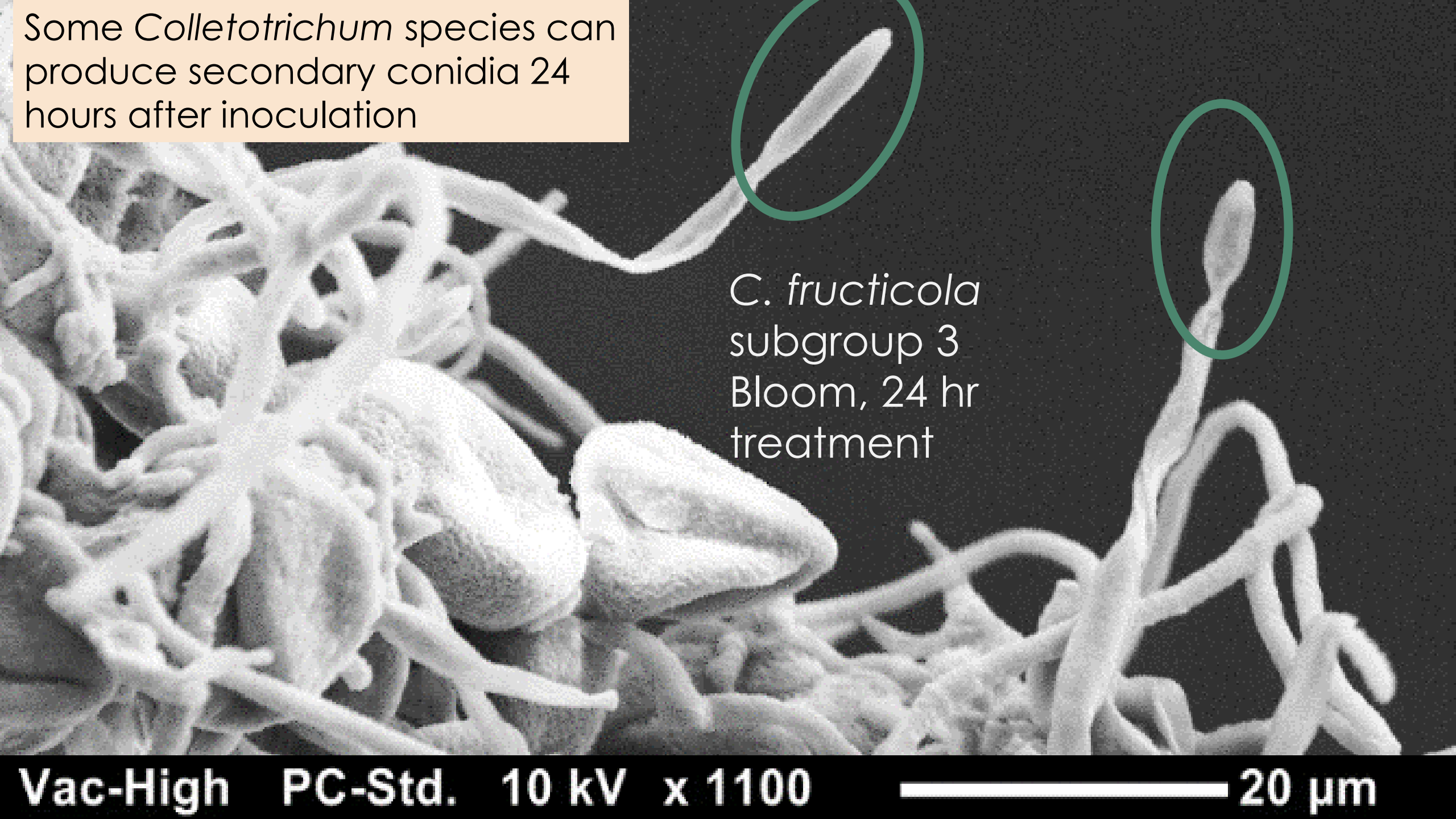

Appressoria formation after 6 hours
Vac-High PC-Std. 10 kV x 270
100 µm
001134

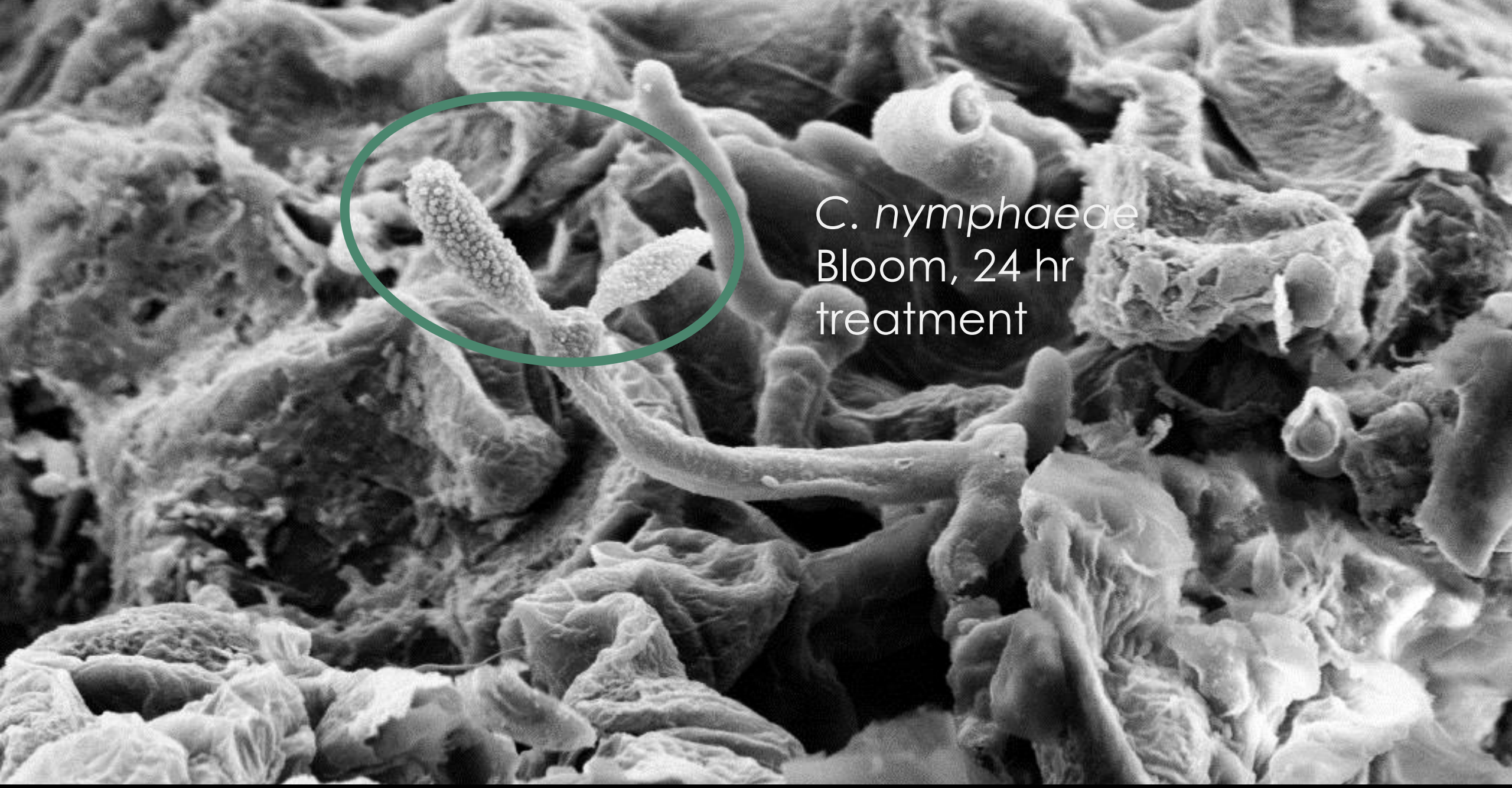

Secondary conidia after 24 hours
Vac-High PC-Std. 10 kV x 1000
20 µm
002667
259 100824 4

Trying to fill in the gaps in ripe rot disease cycle: Cluster inoculation study

- Five *Colletotrichum* species
  - *C. aenigma, C. alienum, C. fructicola, C. fioriniae,* and *C. nymphaeae*
- Four cluster developmental stages & incubation times
  - 2016
    - BB/pea-size, berry-touch, veraison
    - 24hr, 72hr, 1 wk, 2 wk
  - 2017
    - Bloom, BB/pea-size, veraison
    - 24hr, 72hr, 2 wk
- Point inoculated 30 berries per species per time point

Some *Colletotrichum* species can produce secondary conidia 24 hours after inoculation

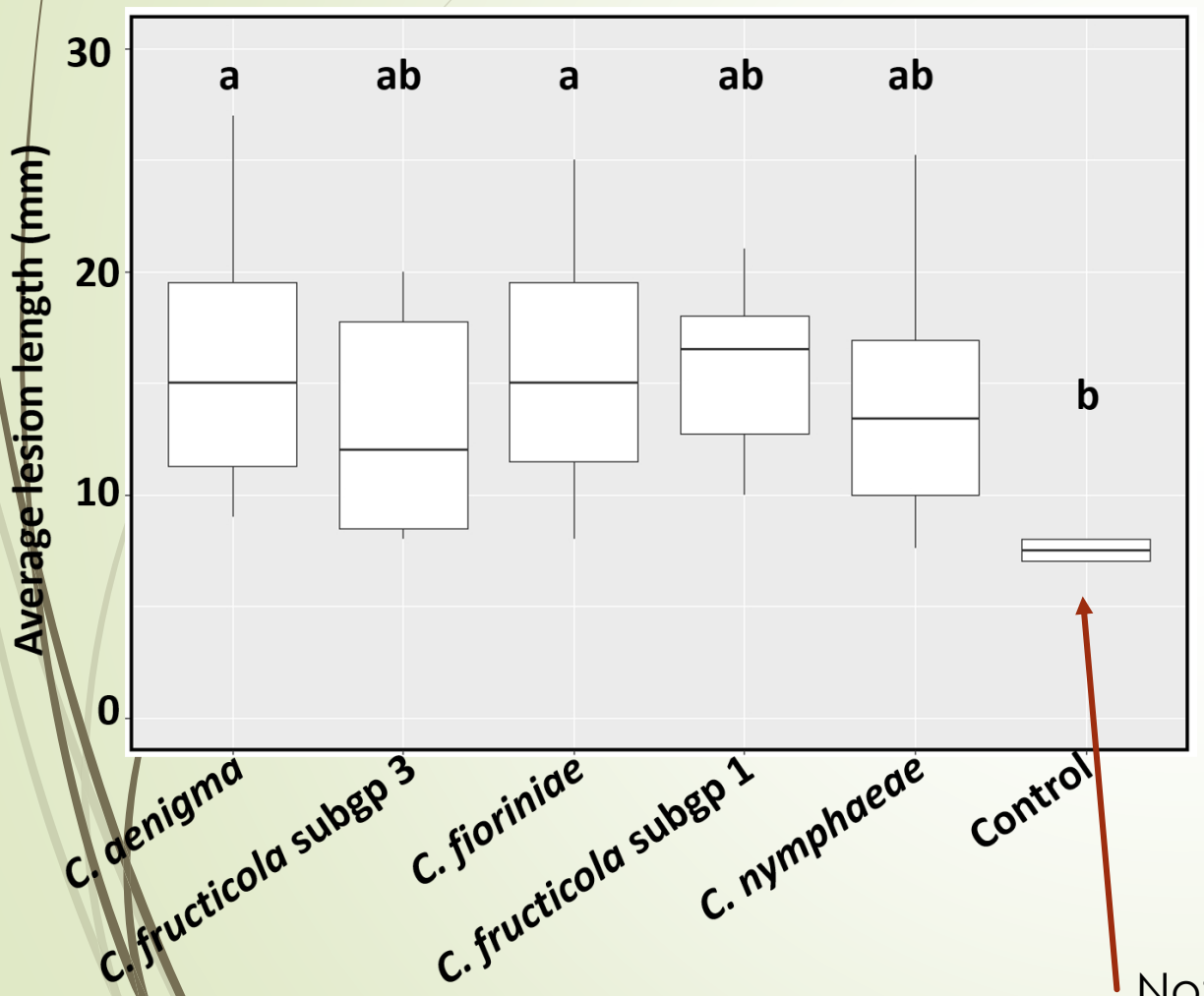



C. nymphaeae
Bloom, 24 hr
treatment
Vac-High PC-Std. 10 kV x 1700
10 µm
004399

Trying to fill in the gaps in ripe rot disease cycle: Cane inoculation study

- Two-month-old cv. 'Chardonnay' self-rooted cuttings
- Five isolates
  - *C. aenigma*, *C. fioriniae* subgroup 1, *C. fructicola* subgroups 1 and 3, and *C. nymphaeae* subgroup 1
- 3 mm agar core was placed in a 6 mm hole between 1st and 2nd internodes
  - WA agar for negative control
- Observed after 3 months

Cane Survival Summary

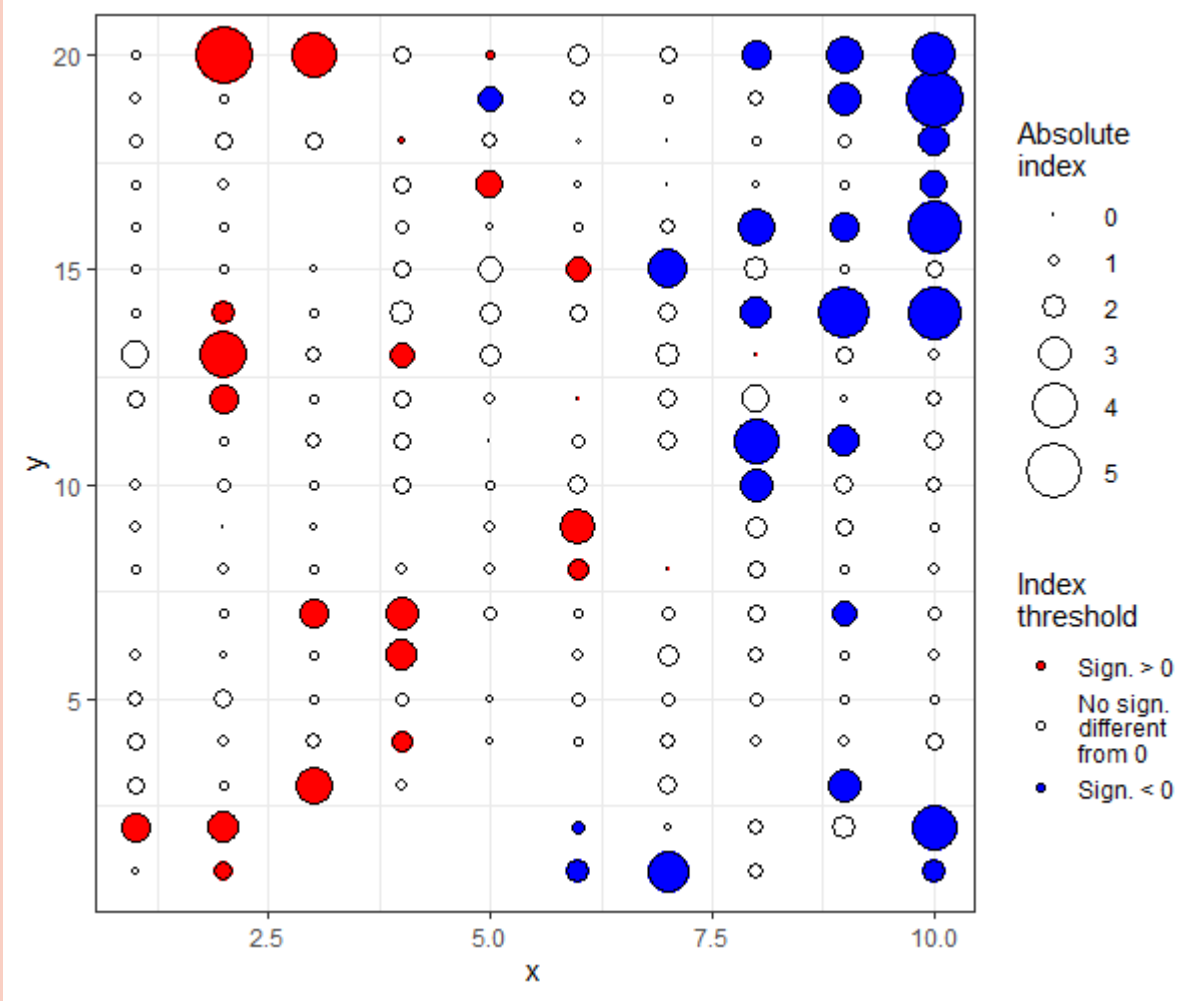


- *Colletotrichum* spp. can survive and form lesions in wounded canes
- C. *aenigma* and C. *fioriniae* produced larger lesions
- More trials required to confirm whether it leads to spore production or not.

Note: 6 mm was the size of the hole

Spatial-analysis of ripe rot showed the evidence of aggregation.

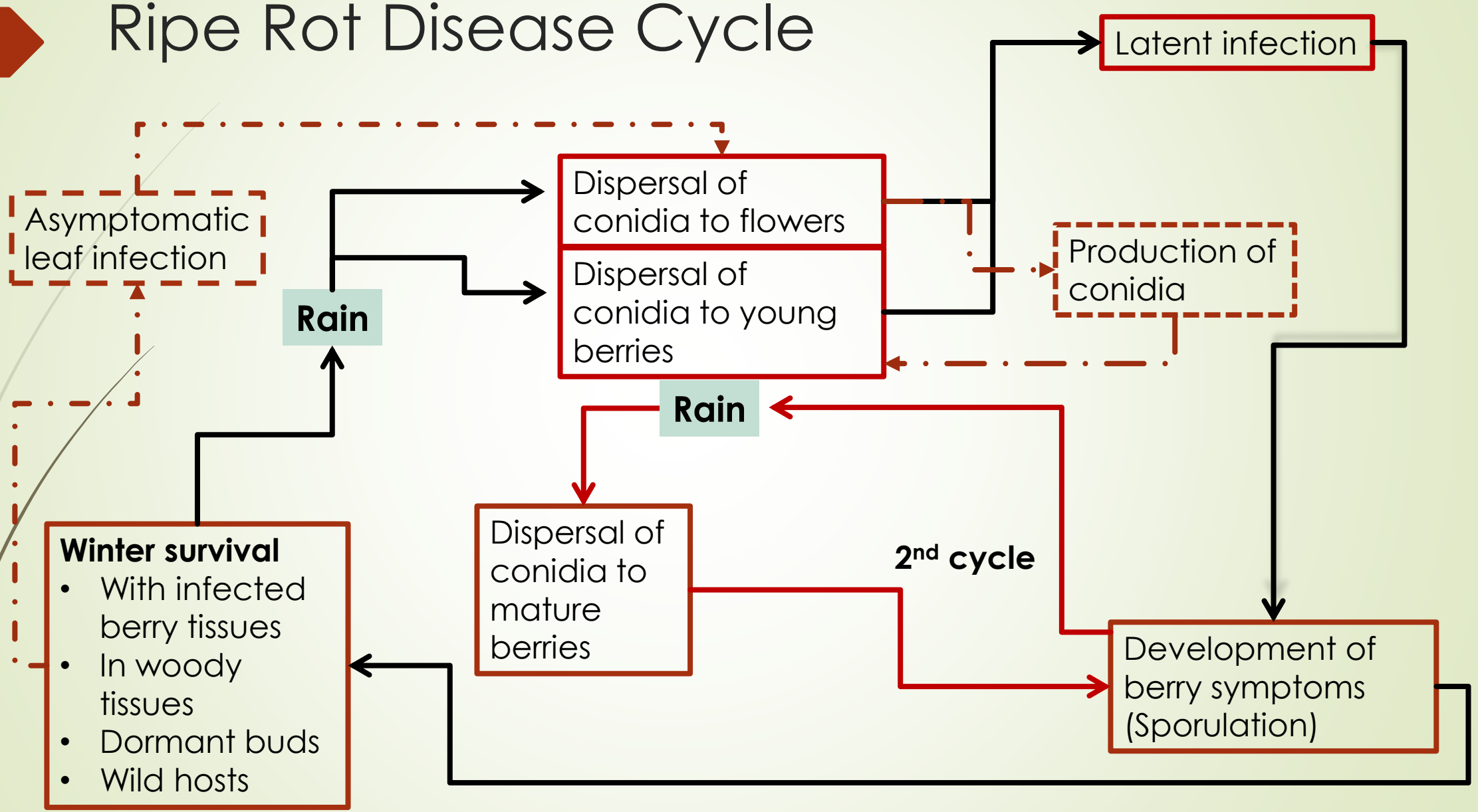
SADIE (Spatial Analysis by Distance Indices)
Index of aggregation
Ia = 1.48
P = 0.02
SWVA Block 1 2015



Grape tissue inoculation summary

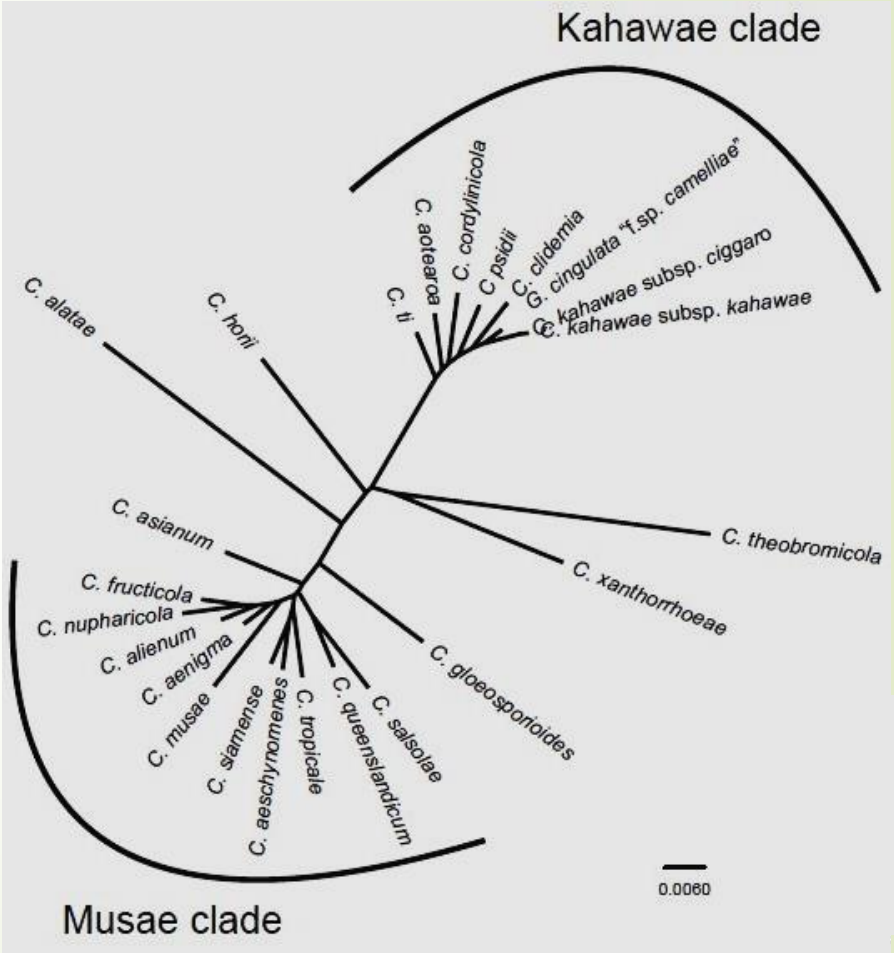
- *Colletotrichum* species can infect the bloom and leaf without showing any visible symptoms
  - Results in formation of secondary conidia
- These pathogens can also survive in the woody tissues.
  - Next step: confirm to see if they can produce spores

Ripe Rot Disease Cycle



Colletotrichum species complex survey in Virginia

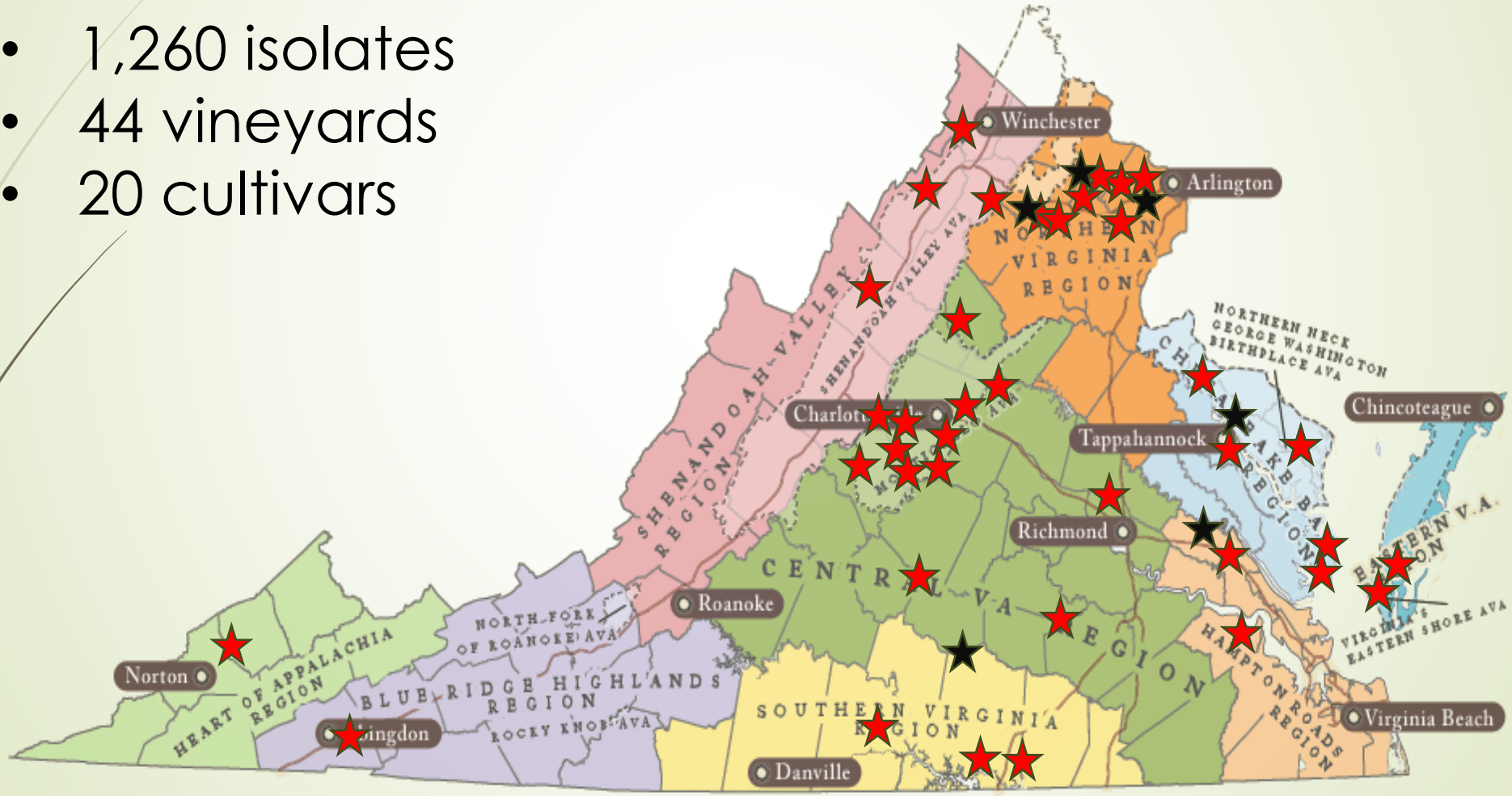
- *C. gloeosporioides* (Weir et. al, 2012)
  - 24 distinct species
- *C. acutatum* (Damm et. al, 2012)
  - 29 distinct species
- Overlapping morphology
- Multilocus sequence typing (MLST)



Weir, B. S., et.al. (2012). The *Colletotrichum gloeosporioides* species complex. *Studies in Mycology*, *73*, 115-180.

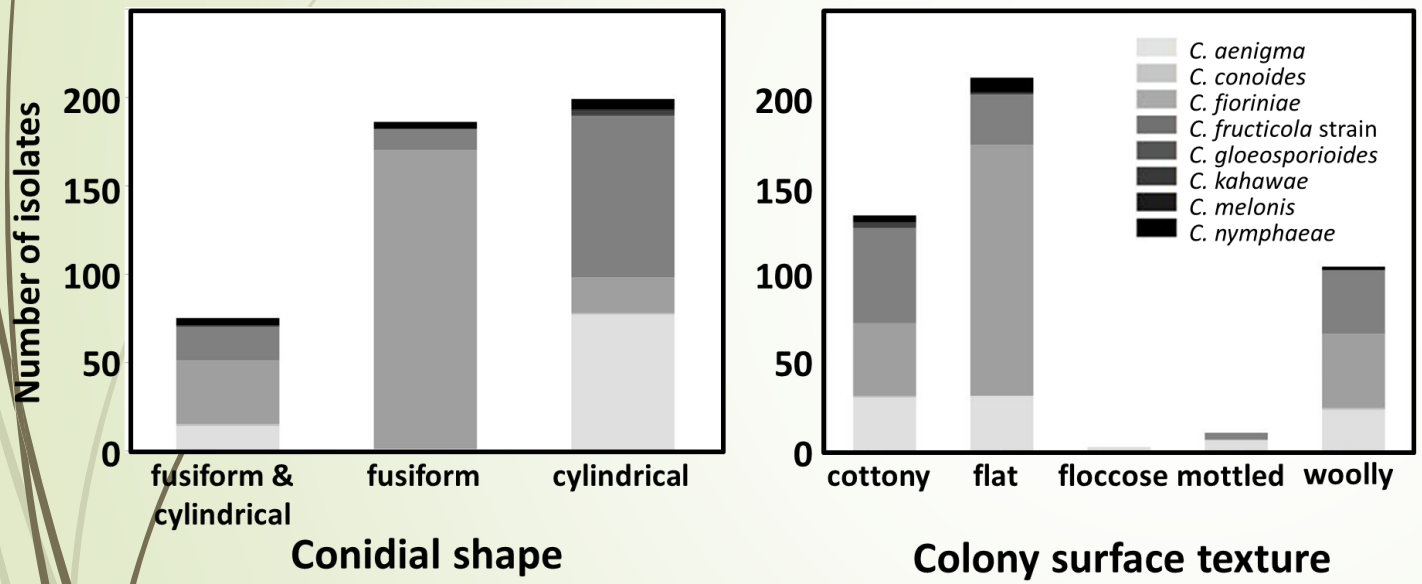
The majority of the survey was conducted in 2013 and 2014

- 1,260 isolates
- 44 vineyards
- 20 cultivars

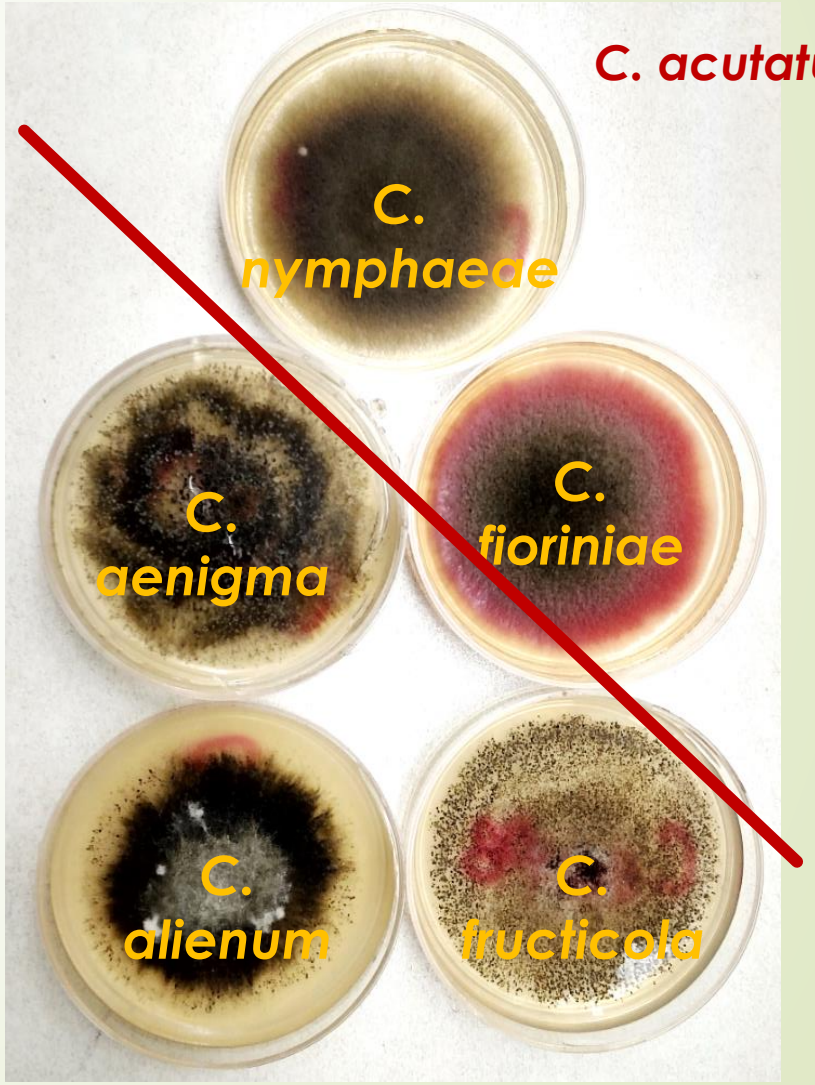


Provided by Virginia Wine Marketing Office

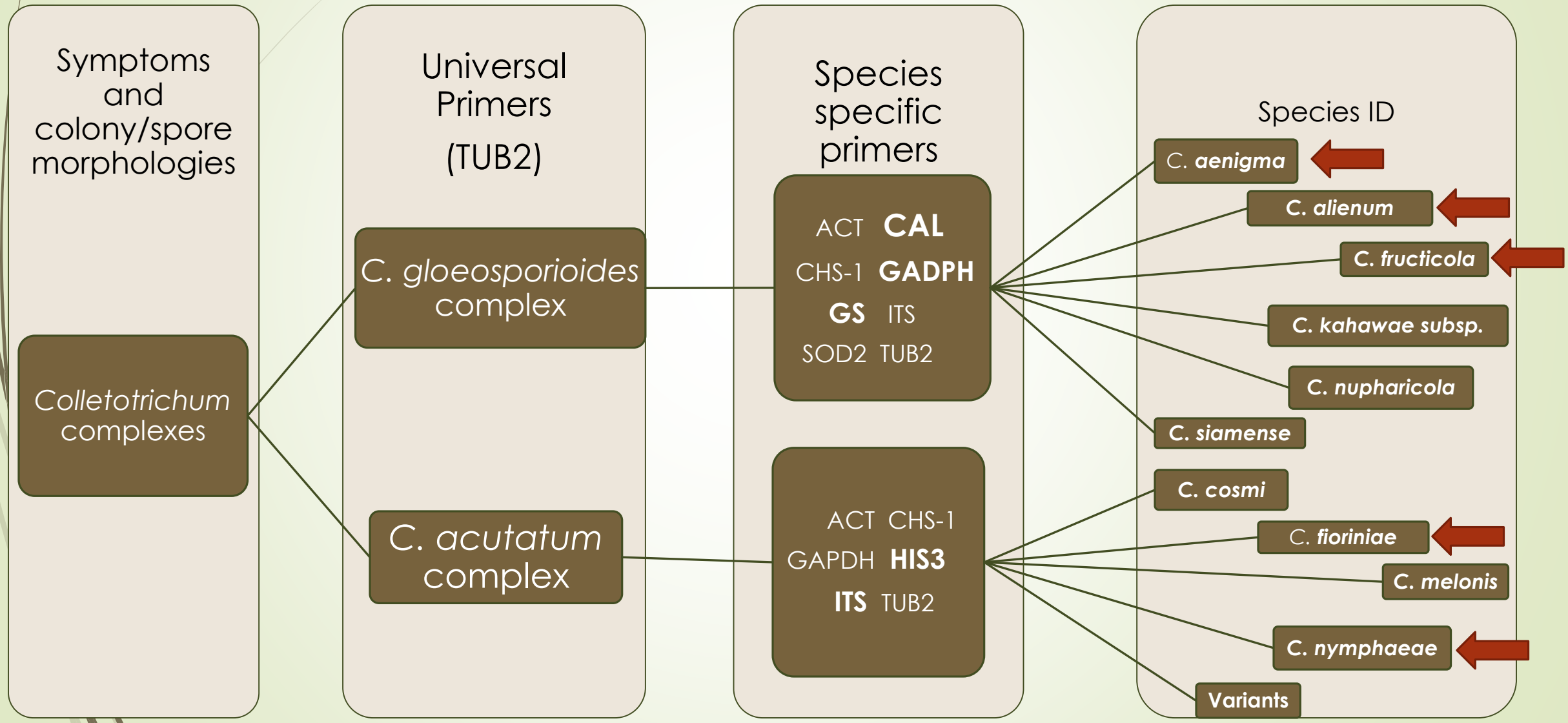
Morphological Identification was challenging



C. gloeosporioides

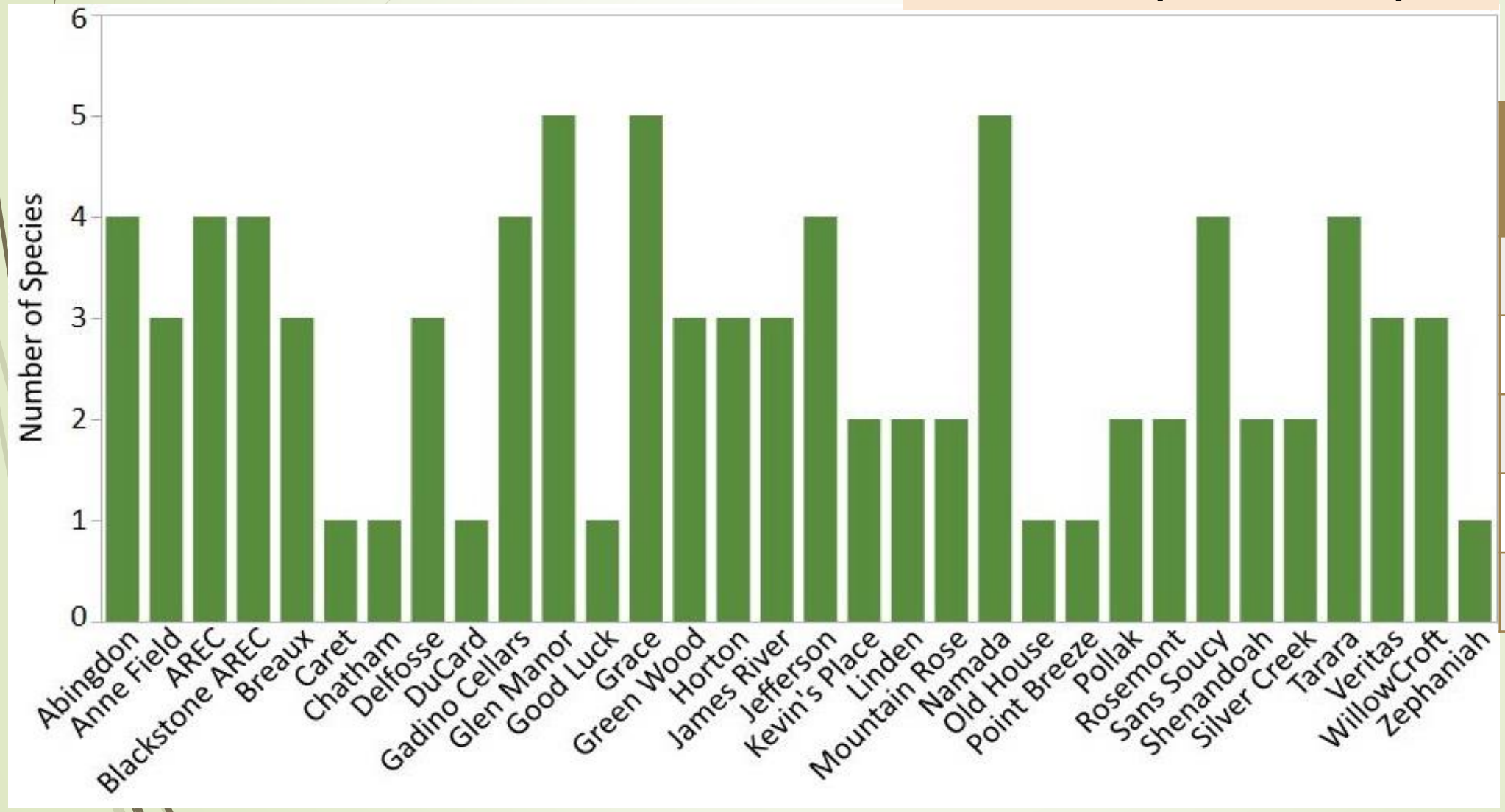


MLST flowchart for *Colletotrichum* species ID



Species in a Vineyard

Ave. = 2.75 species/ vineyard

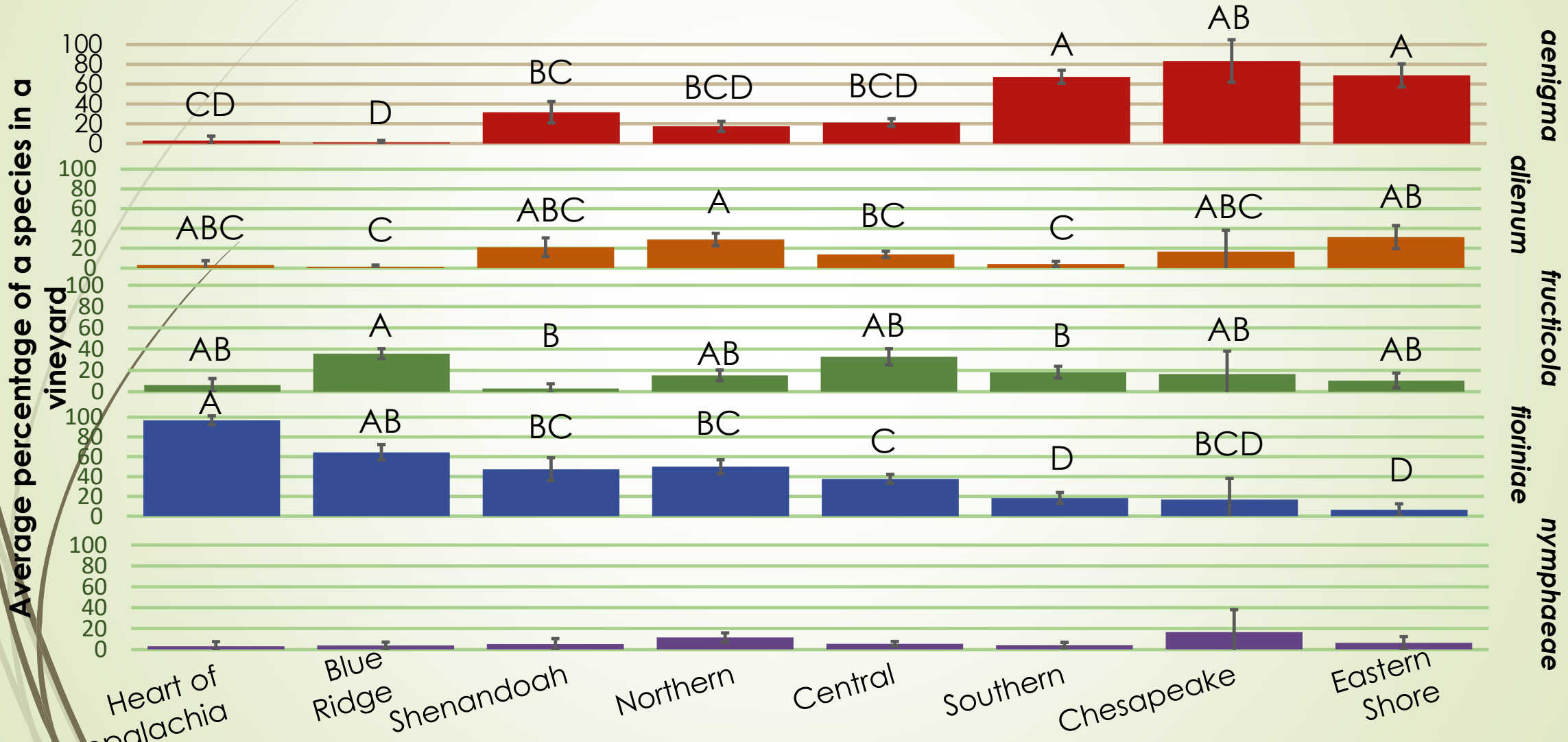


It seems to be a function of the sample number

# of spp	# of vineyards
1	7
2	7
3	8
4	7
5	3

Spatial Distribution

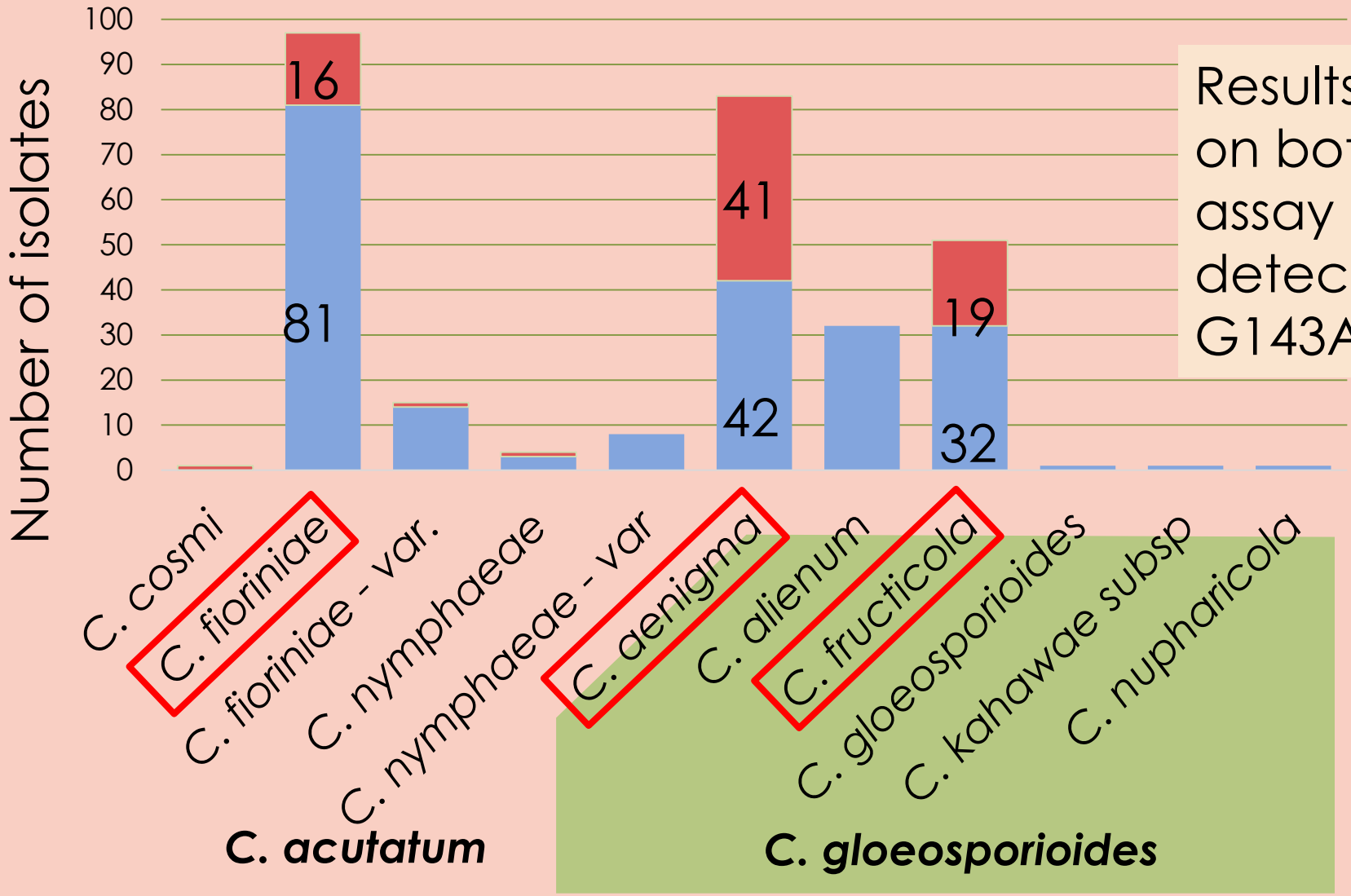
Based on the generalized linear mixed model results (GLIMMIX in SAS 9.4)



Pathogen ID summary

- A statewide survey from 43 vineyards (19 cultivars) showed that we have up to eight different *Colletotrichum* species.
- Multiple species can be present in a vineyard
  - Av number of species is 2.75 per vineyard
- Five main species in VA are
  - ***C. aenigma, C. alienum, C. fructicola, C. fioriniae, and C. nymphaeae***

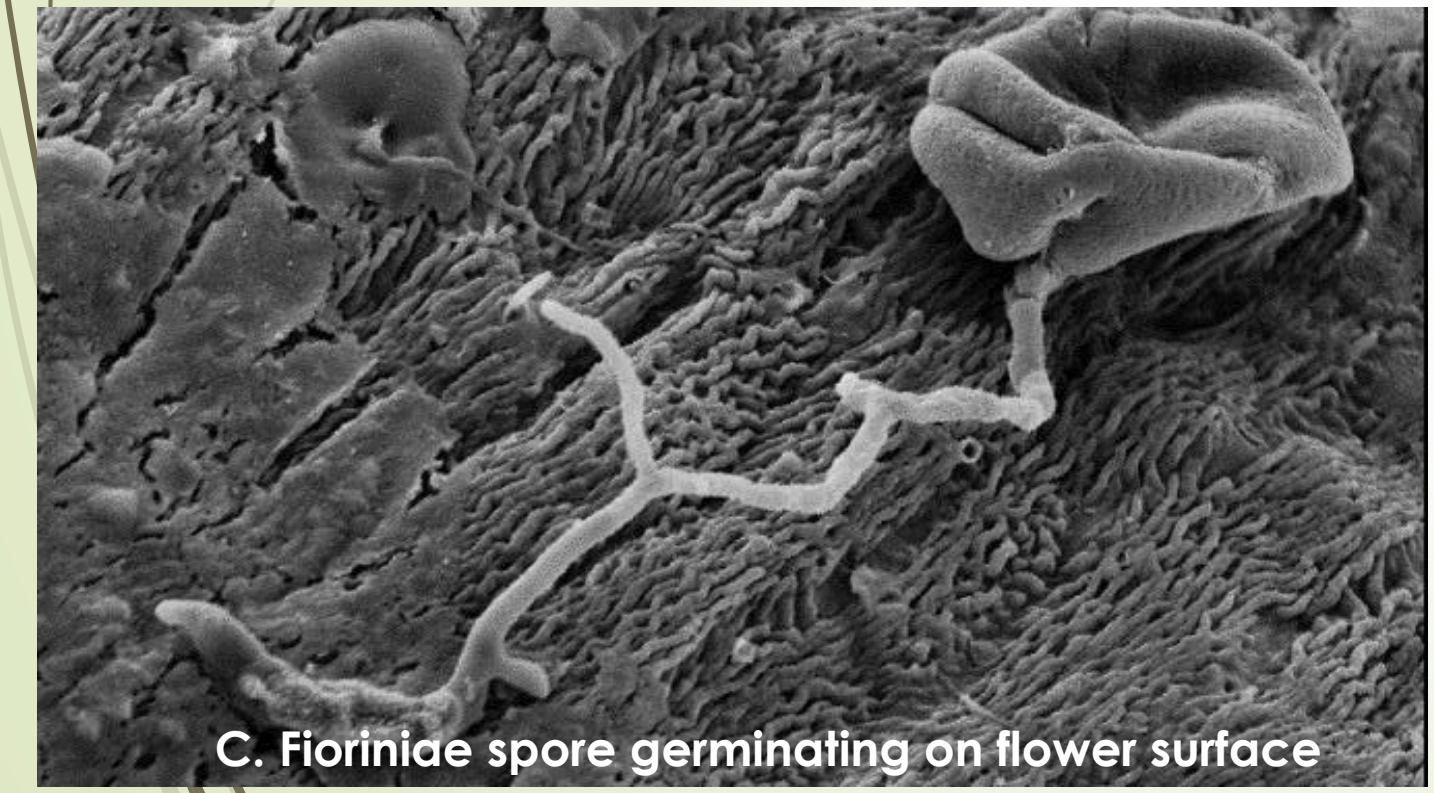
25% of our isolates were resistant to QoI (Abound), and more prevalent species had higher percentages of QoI-resistant isolates



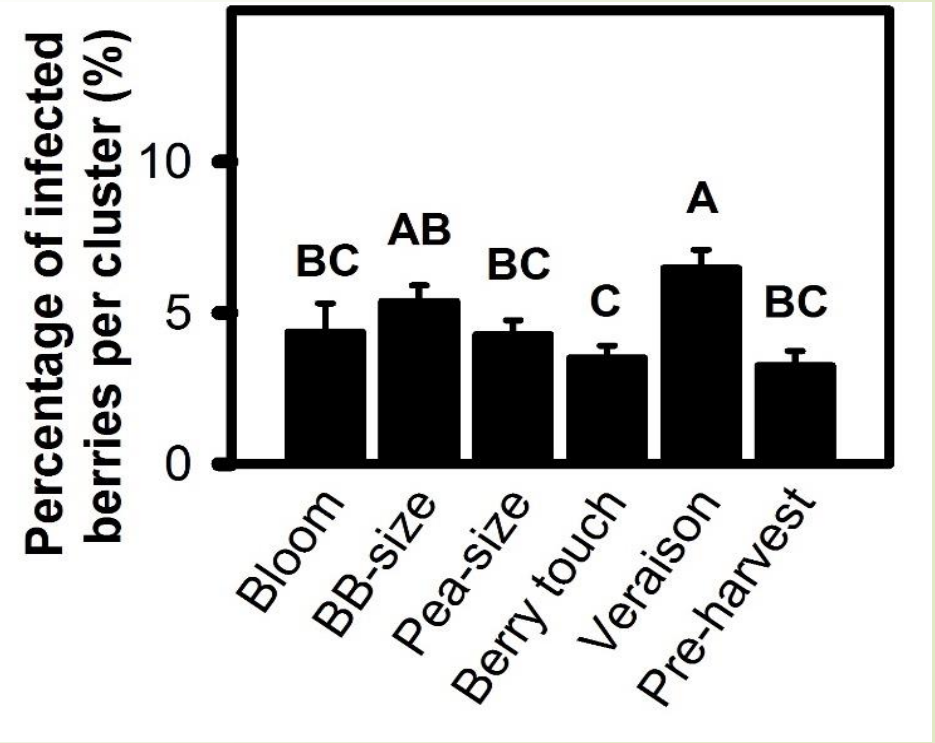
Results are based on both a plate assay and the detection of G143A mutation

Ripe rot field trials 2015-17

- Recommended products: mancozeb, captan, and QoI
- Locations: Winchester, VA (AREC), Abingdon VA (Southwestern VA)
- Treatments were applied at bloom, berry touch, and veraison



C. Fioriniae spore germinating on flower surface



Newer fungicides tested in ripe rot trials
SDHIs

Aprovia (Syngenta)

- SDHI (FRAC = 7)
- A.I. = benzovindiflupyr
- Botrytis not listed on the label

Miravis (Syngenta)

- SHDI (FRAC = 7)
- A.I. = Pydiflumetofen
- Note: Miravis Prime (7 + 12) became available for grape in 2018 (Botrytis is listed on the label)

Newer fungicides tested in ripe rot trials DMI and Polyoxin-D

Viathon

- Introduced to the US market in 2019
- A mixture of Prophyt and tebuconazole (Elite, etc.)

Ph-D (Arysta lifescience)

- FRAC = 19
- A.I. = Polyoxin D zinc salt (11.3%)
- List many different pathogens

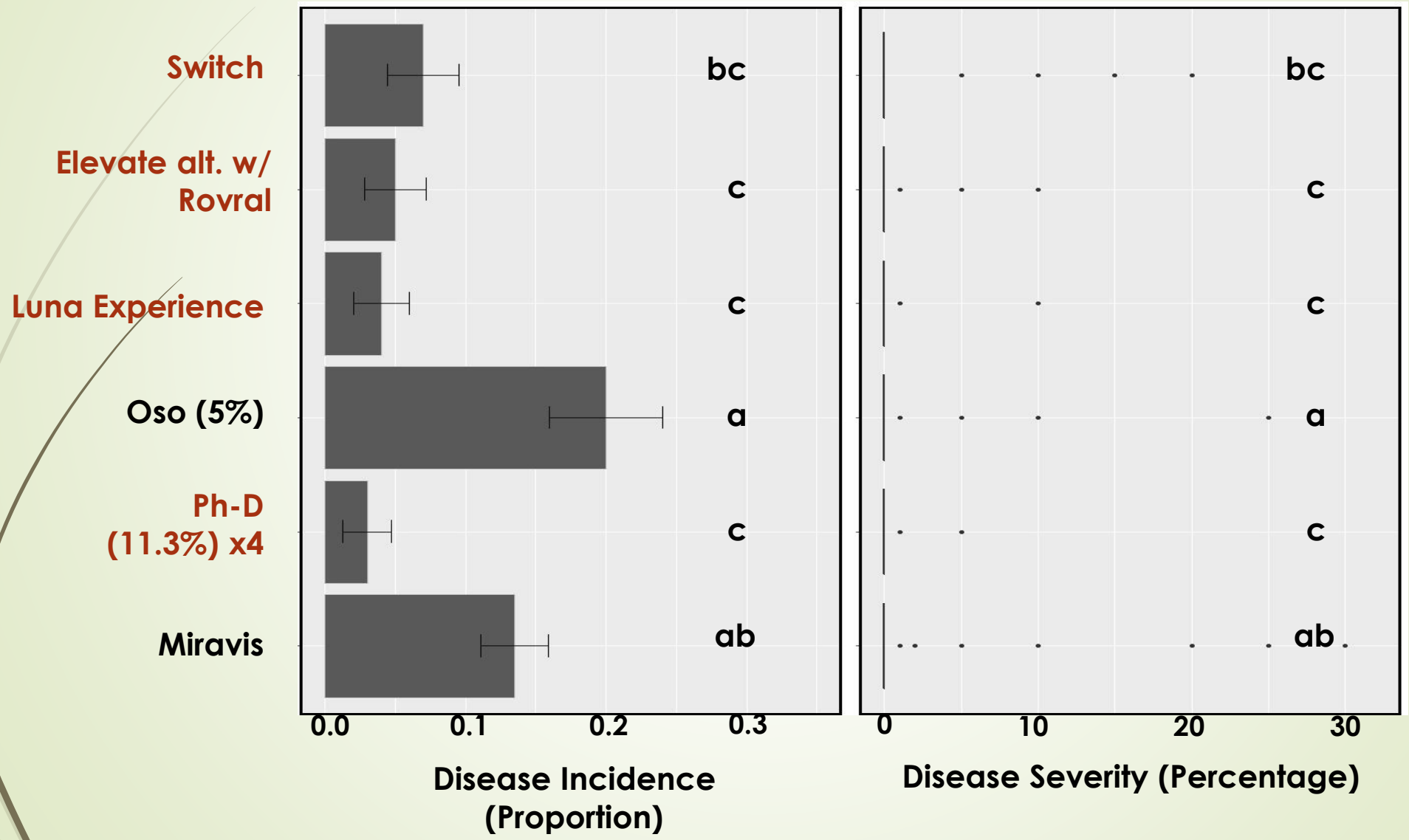
Oso (Certis)

- Polyoxin D, but in lower % (5%)

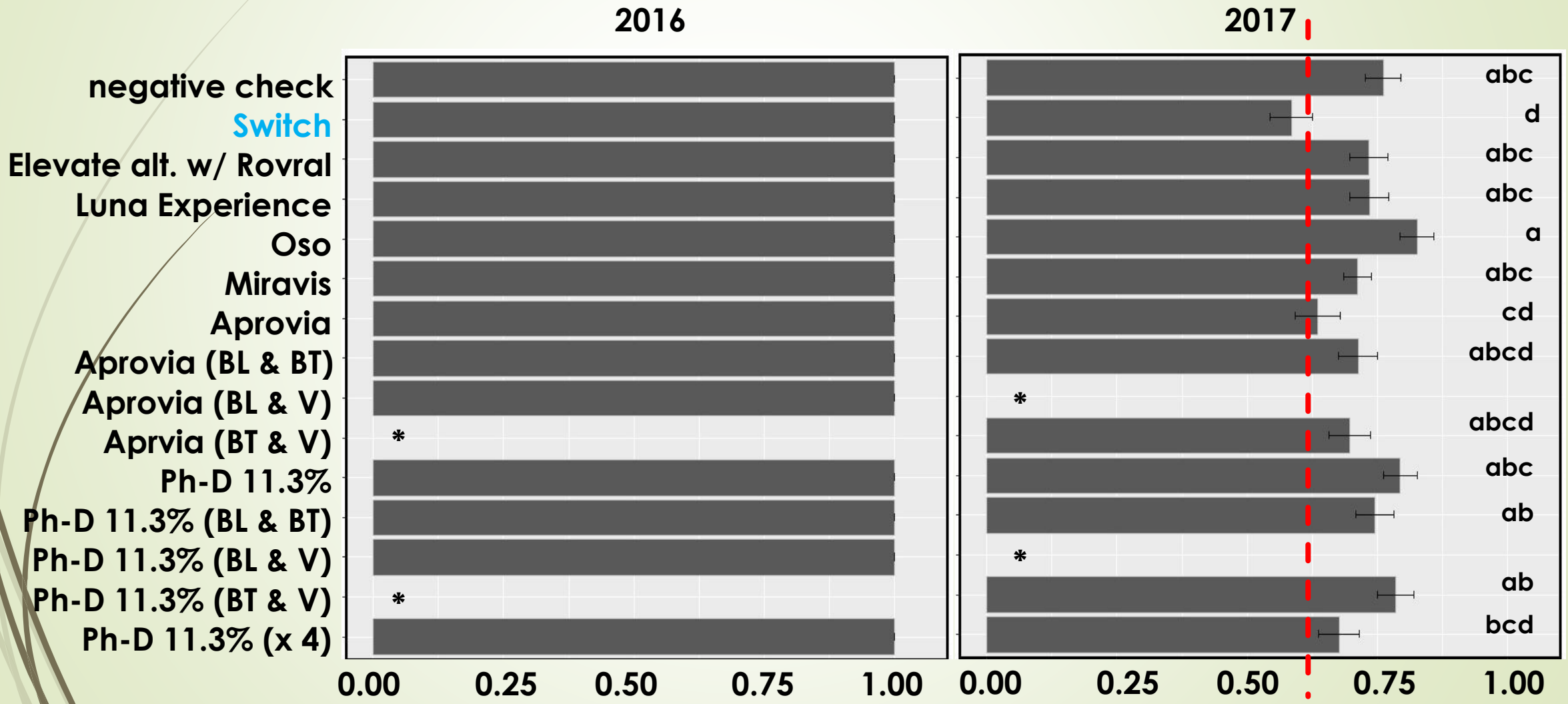
AHS AREC Spray Program

Year	Active ingredient	Commercial products	MOA	Application time
2015	cyprodinil + fludioxonil	Switch® 62.5 WG	9, 12	BL, BT, V
	fenhexamid alt. w/ iprodione	Elevate® 50 WDG & Rovral®	17, 2	BL, BT, V
	fluopyram + tebuconazole	Luna® Experience	7, 3	BL, BT, V
	polyoxin-D	Oso™ 5%SC	19	BL, BT, V
	polyoxin-D	Ph-D®	19	BL, BT, V, LM
	pydiflumetofen	Miravis®	7	BL, BT, V
Added	benzovindiflupyr	Aprovia®	7	BL, BT, V
2016	benzovindiflupyr	Aprovia®	7	BL, BT
& 2017	benzovindiflupyr	Aprovia®	7	BL, V
	benzovindiflupyr	Aprovia®	7	BT, V
	polyoxin-D	Ph-D®	19	BL, BT, V,
	polyoxin-D	Ph-D®	19	BL, BT
	polyoxin-D	Ph-D®	19	BL, V
	polyoxin-D	Ph-D®	19	BT, V

AHS AREC 2015
Ripe rot data from Botrytis trial
(i.e., no negative check **+ applied with mancozeb at bloom**)



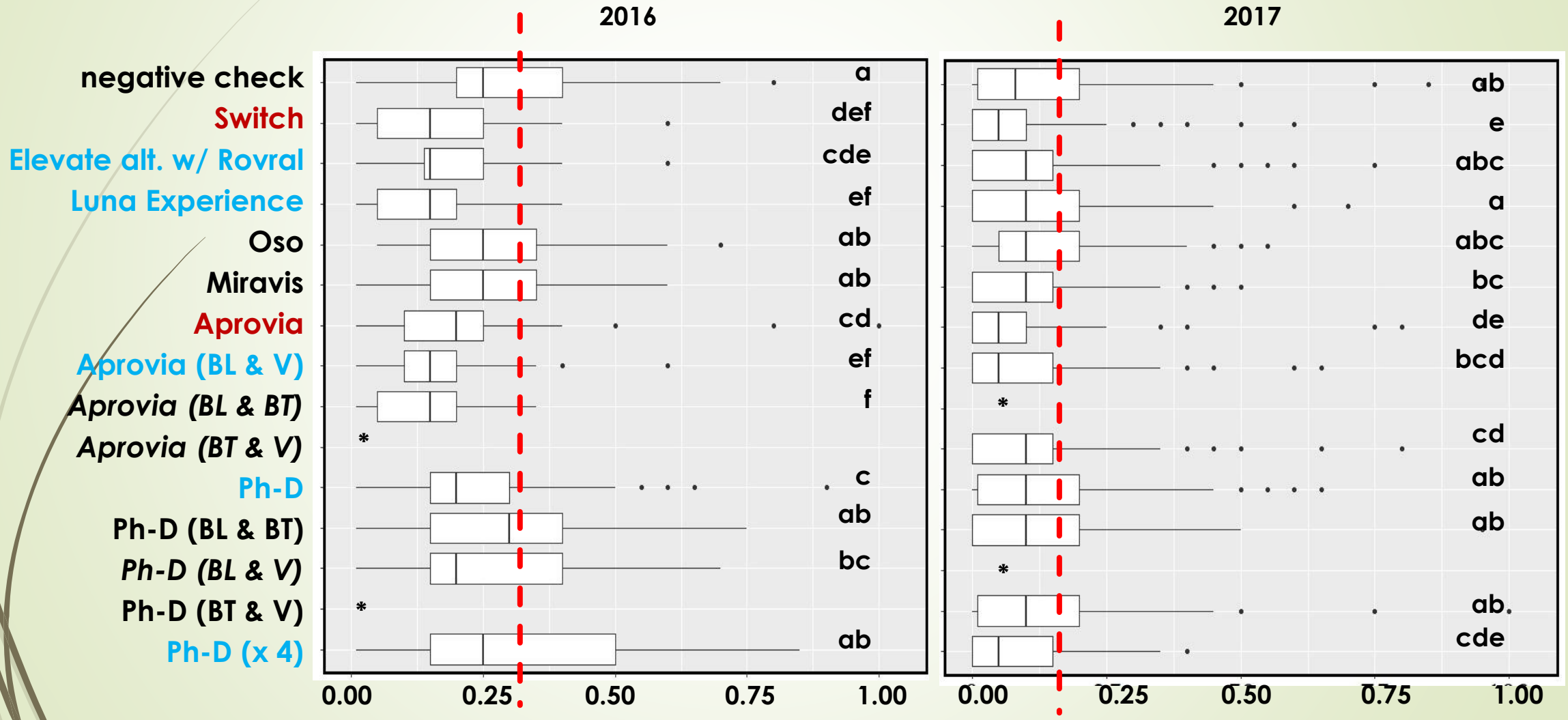
AHS AREC: Cluster disease incidence (Yes/No per cluster)
Single product trial: negative check = Revus and Vivando



AHS AREC – Cluster disease Severity (% area infected)

Red = significant reduction in both years

Blue = significant reduction in one year



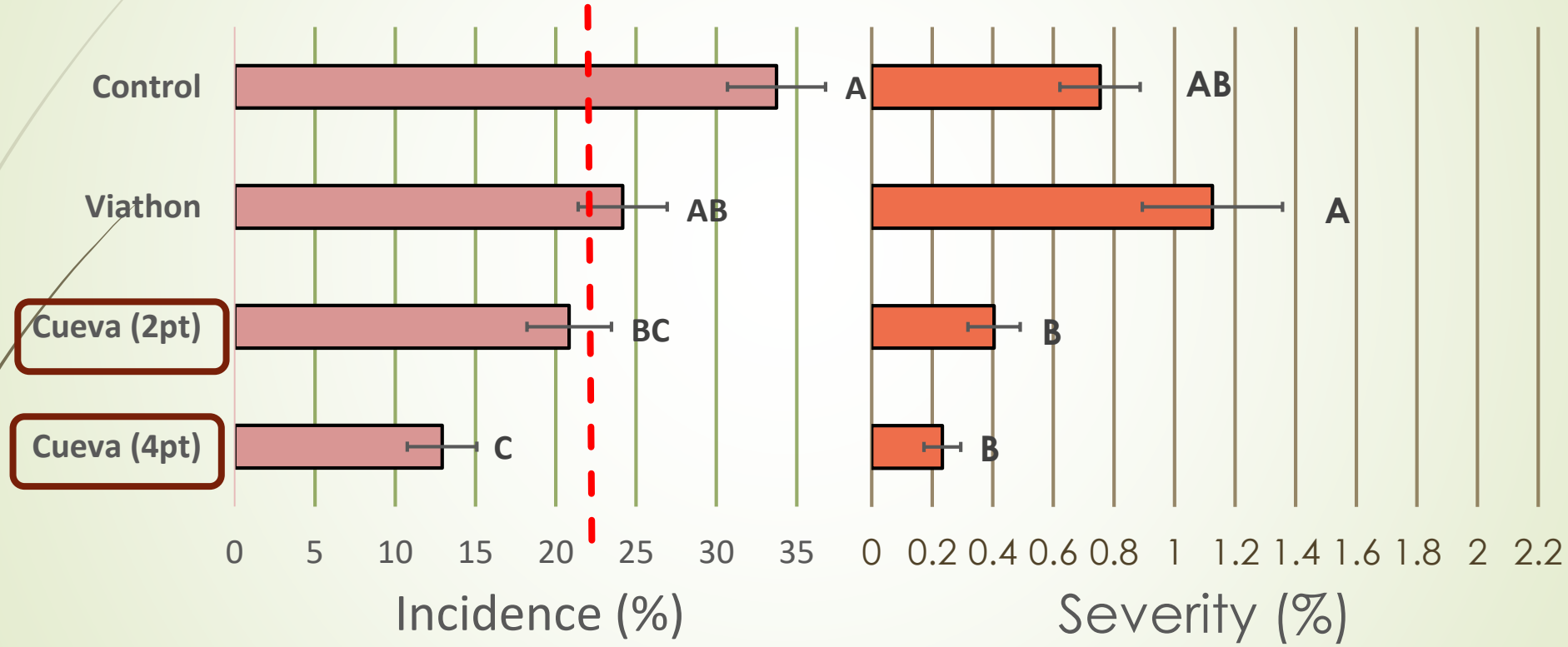
Commercial vineyard in southwestern VA

Treatments were applied in addition to regular fungicide applications

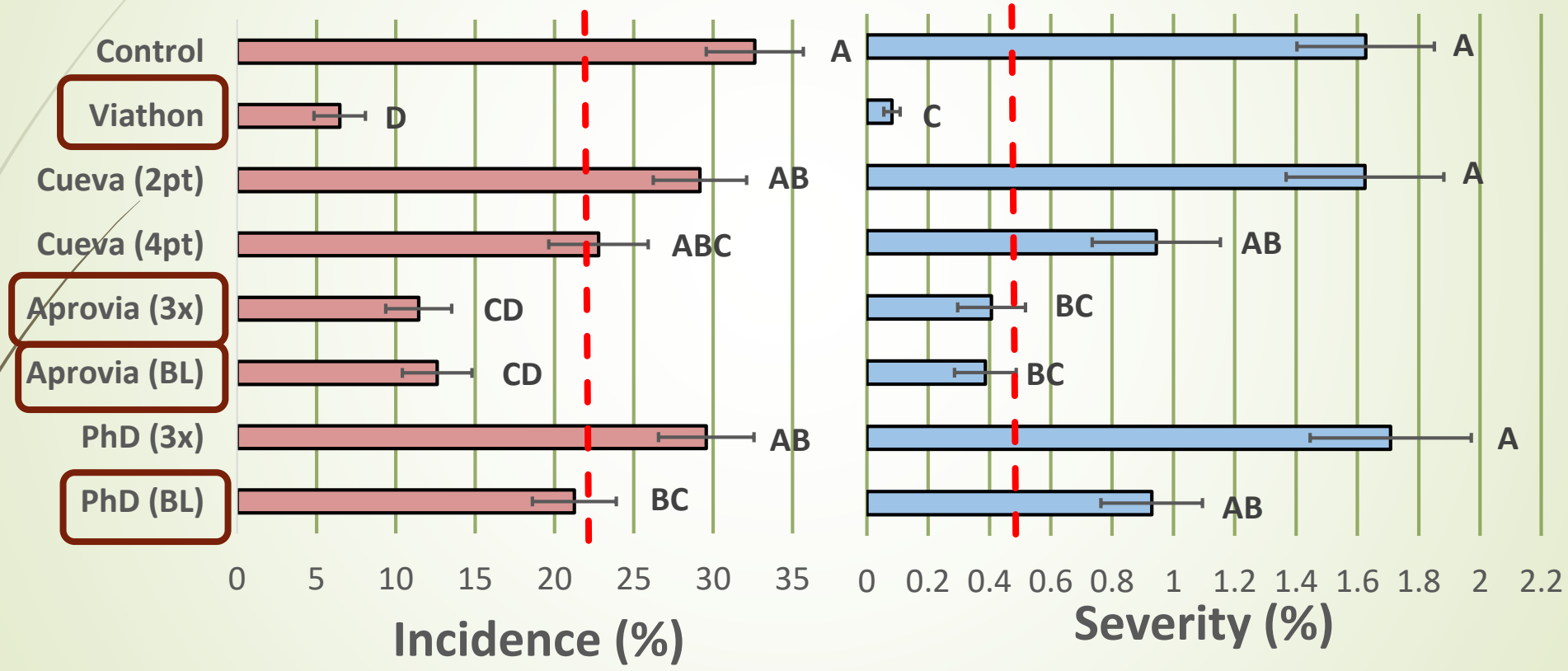
2015		2016 & 2017	
Treatment (rate/A, 100 gal)	Timing	Treatment (rate/A, 100 gal)	Timing
Control		Control	
Viathon (2pt)	BL, BT, V, LM	Viathon (2pt)	BL, BT, V
Cueva (2 pt)	BL, BT, V, LM	Cueva (2 pt)	BL, BT, V
Cueva (4 pt)	BL, BT, V, LM	Cueva (4 pt)	BL, BT, V
		Aprovia (9 fl oz)	BL, BT, V
		Aprovia (9 fl oz)	BL
		PhD (6.2 oz)	BL, BT, V
		PhD (6.2 oz)	BL

Viathon is a mixture of Prophyt and tebuconazole (Elite)

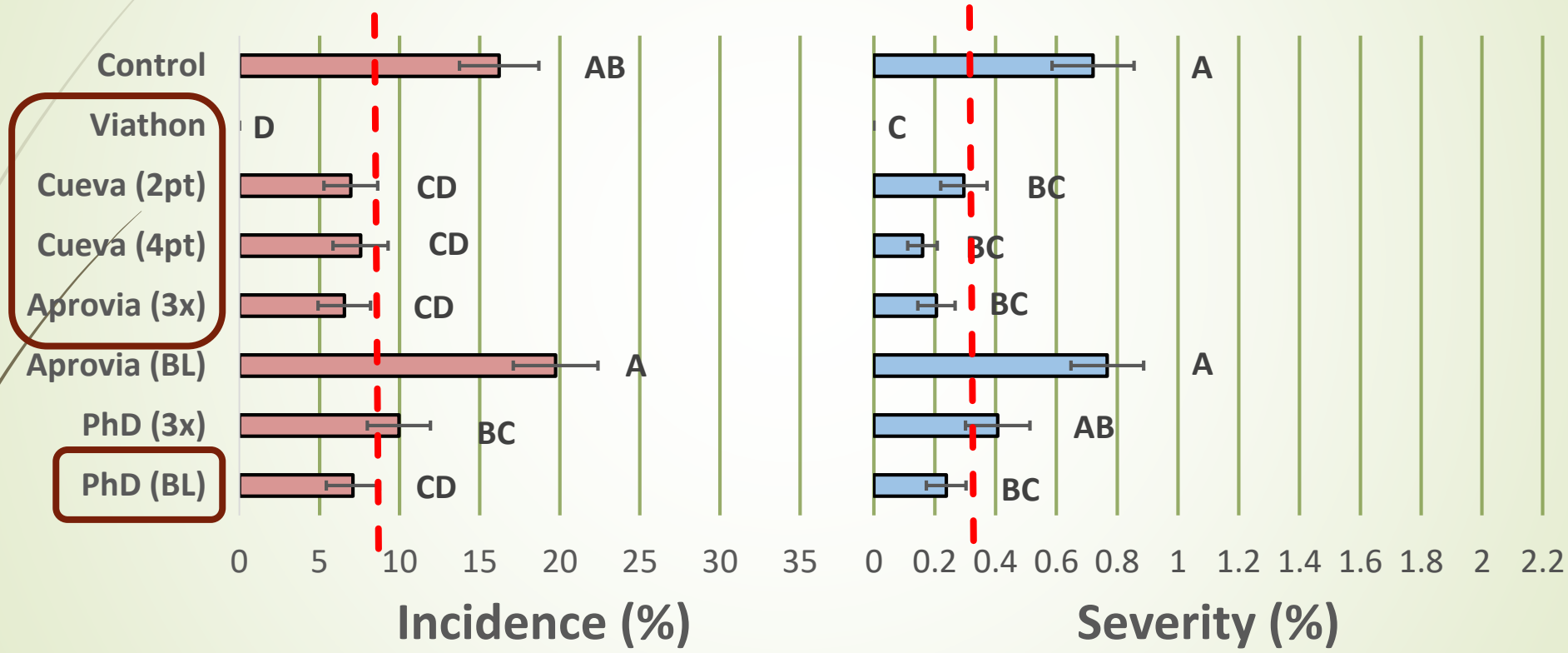
Southwestern VA, 2015



Southwestern VA, 2016

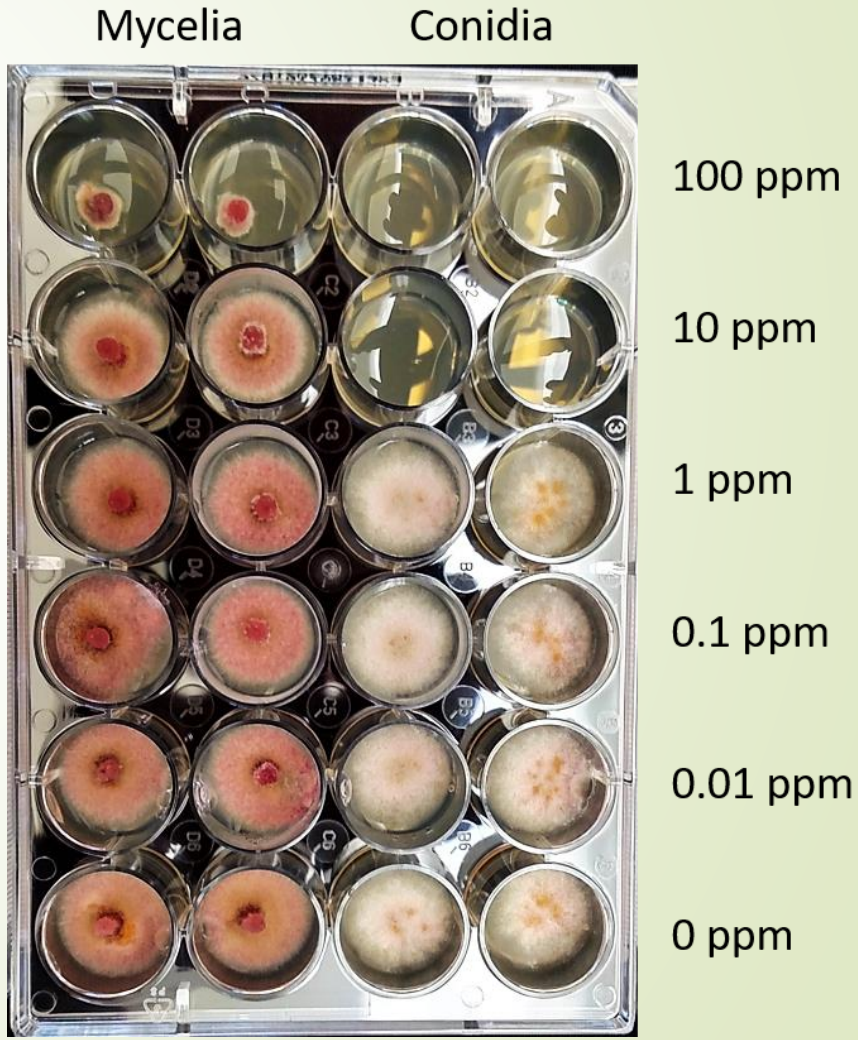


Southwestern VA, 2017



Looking for other options: Fungicide lab assay

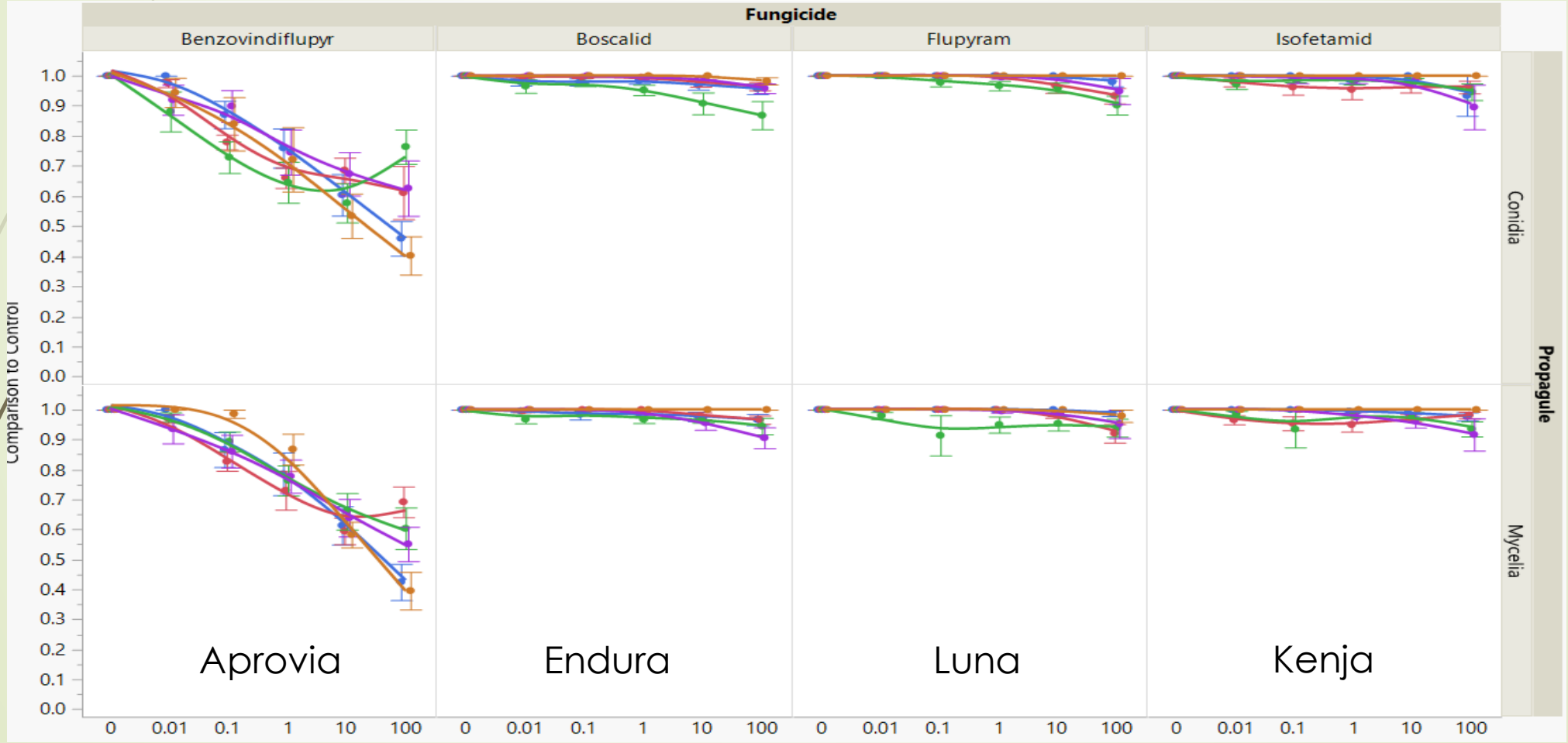
- Plate preparation
  - Fungicides were suspended in sterile distilled water before dilution
  - Media was allowed to cool to 55 °C before fungicide addition
  - All plates were used within 5 days of production
- Incubated at 25 ºC with a diurnal light cycle
- Data was collected for both propagules after 72 hr
  - Diameter of colony (mm) in two directions
- EC50 calculations



Looking for candidate product to be used in the field: plate assay
Options seem to be limited…

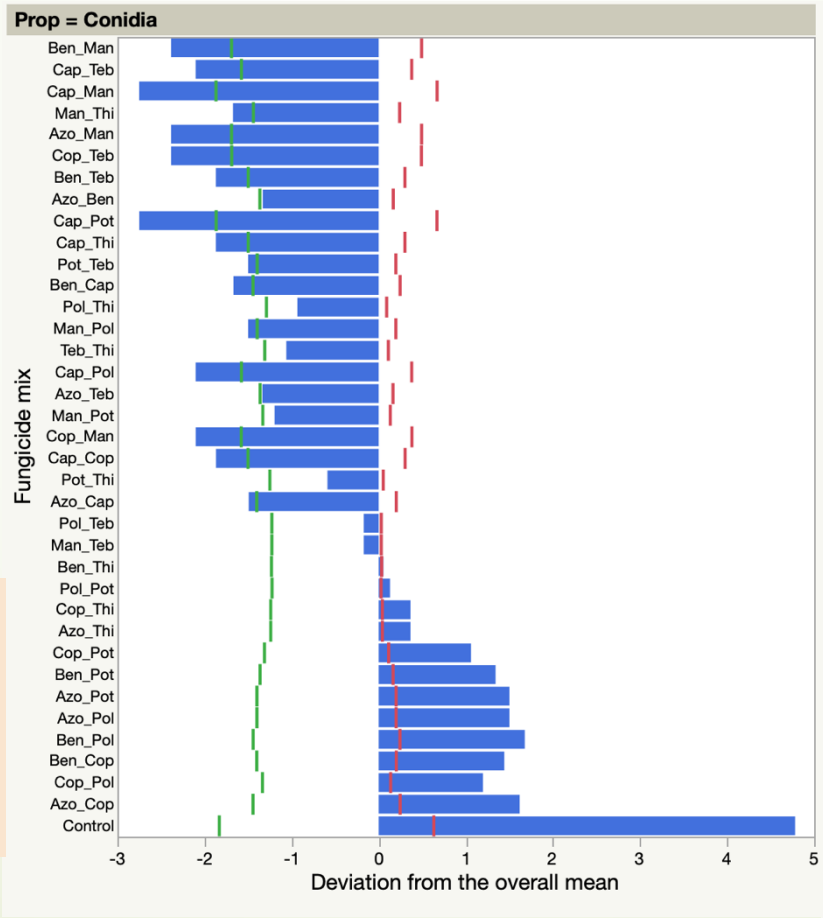
	C. siamense [Z]											
Active ingredient	**Link[Y]**	**Intercept[X]**		**Slope[X]**		**EC50[W]**		**Link[Y]**	**Intercept[X]**		**Slope[X]**		**EC50[W]**	
Azoxystrobin	CLL	3.7	*	-1.6		472.8	+	probit	3.6	**	-1.4	**	385.3	+
Boscalid	logit	16.5		0.0		NC	+	logit	11.3		-3.4		NC	+
Captan	probit	1.8	**	-1.8	**	**8.9**		probit	2.7	**	-2.2	**	**16.6**	
Copper hydroxide	probit	5.6	**	-3.3	**	**48.3**		logit	4.0	**	-2.9	**	**35.5**	
Copper octanoate	probit	2.7	*	-1.7	**	**53.7**		probit	3.1	*	-2.0	**	**43.6**	
Mancozeb	logit	21.6		-32.0		**3.5**		CLL	2.2		-3.1	*	**11.1**	
Potassium phosphite	probit	5.1	**	-2.8	**	118.9		CLL	18.0	**	-10.2	**	87.7	
Pyriofenone	CLL	4.8		-1.6		1140.9	+	CLL	1.1	**	-0.2		7.1×10^9	+
Tetraconazole	probit	3.8	**	-2.4	**	**39.5**	+	probit	2.5	**	-2.0	**	**22.8**	+
Thiophanate-methyl	CLL	6.2	**	-2.7	*	281.4		logit	7.5	**	-3.2	**	238.3	

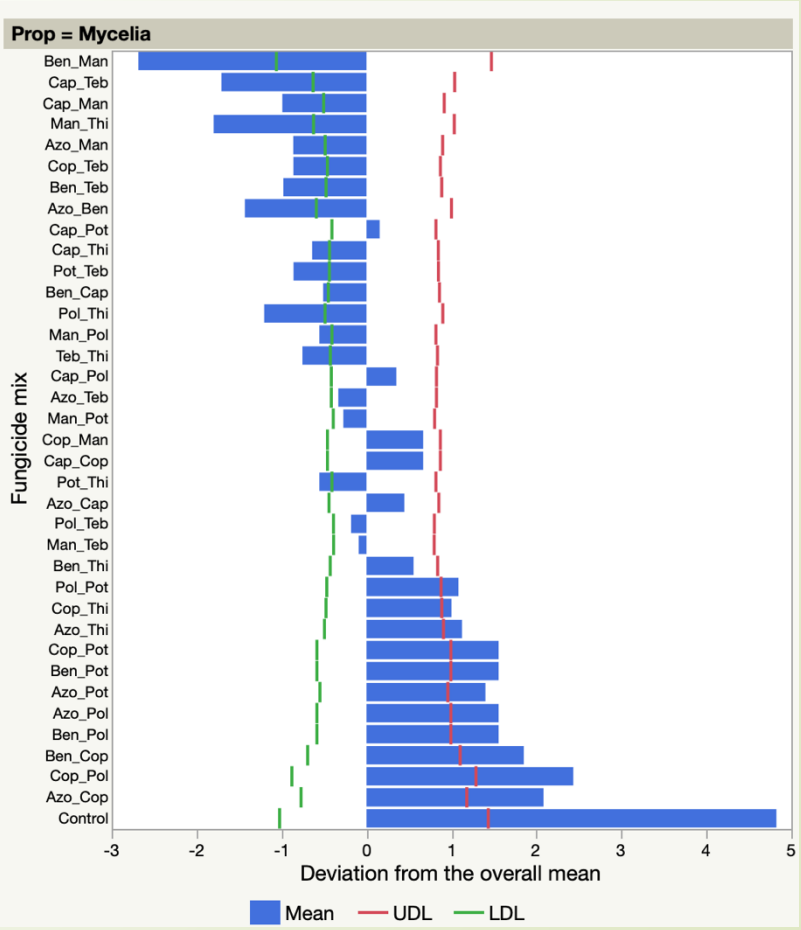
Among SDHIs, only Aprovia showed a sign of its efficacy based on results from a plate assay



More on two modes of action (MOA) Plate assay (2018)

- Nine different MOA are tested at 100 ppm to see if they can inhibit the growth of five *Colletotrichum* species





Results are based on the analysis of means from the generalized linear regression model in JMP Pro 14

Conidia			Mycelia	
MOA 1	MOA 2		MOA 1	MOA 2
Aprovia	Mancozeb		**Aprovia**	Mancozeb
Captan	**Tebuconazole**		Captan	**Tebuconazole**
Captan	Mancozeb		Captan	Mancozeb
Mancozeb	**Topsin-M**			
			Mancozeb	**Tebuconazole**
Abound	Mancozeb		Abound	Mancozeb
Copper	**Tebuconazole**		**Copper**	**Tebuconazole**
Aprovia	**Tebuconazole**		**Aprovia**	**Tebuconazole**
			Abound	**Aprovia**
Captan	**Prophyt**			
Captan	**Topsin-M**		Captan	**Topsin-M**
Prophyt	**Tebuconazole**		**Prophyt**	**Tebuconazole**
Aprovia	Captan		**Aprovia**	Captan
			Polyoxin-D	**Topsin-M**
Mancozeb	**Polyoxin-D**		Mancozeb	**Polyoxin-D**
Captan	**Polyoxin-D**			
Copper	Mancozeb			
Copper	Captan			
Abound	Captan			
			Tebuconazole	**Topsin-M**
			Prophyt	**Topsin-M**

Summary of 2-MOA plate assay

- Mancozeb, Captan, and Abound (QoI) are working (good confirmation!)
- Aprovia, tebuconazole, and Topsin-M are commonly included
- With Polyoxin-D, the effect was not very strong.
- Topsin-M and Tebuconazle may affect mycelial growth
- Prophyt is showing here and there, but it is probably due to tebuconazole

Summary: Fungicide field and lab studies

- Captan (M4) and Mancozeb (M3) were effective
  - Pros: Cost effective, little or no resistance issues
  - Cons: Insensitivity of some C. *gloeosporioides* to Captan
- Switch ((cyprodinil (9) + fludioxonil (12)) was also effective
  - But better with mancozeb
- Rovral/Metor (iprodione (2)) has suppressive effect on Colletotrichum (shown in other studies)
- Variability of Viathon (Potassium phosphite (P07) + tebuconazole (3)) between years and locations
  - Dese potassium phosphite have any effect?
  - Viathon did not provide any efficacy at one of tested locations.

Summary

- SDHIs were generally not effective
  - Aprovia (Benzovindiflupyr (7)) seemed to work in the field, especially with additional mancozeb or captan sprays.
  - PHI = 21 days!
- Abound (Azoxystrobin (11)) and Topsin M (Thiophanate methyl (1)) suppressed fungal growth only with another MOA
  - Resistance issues with QoIs and Topsin M
  - Should not be applied by itself!!!

Mixing multiple MOA is probably the key for ripe rot management

Mixing partners for mancozeb/ziram or captan (Timing: bloom, berry touch, veraison)		
Moderate level of reduction	**Low level of reduction**	**No or limited effect**
• **Aprovia** (Benzovindiflupyr, FRAC= 7) • **Cueva** (Copper (M1)) • **Intuity** (mandestrobin, (11)) • **Viathon** (Phos acid (33) + tebuconazole (3)) • **Switch** (cyprodinil (9) + fludioxonil (12))	• **Elevate** (fenhexamid (7)) alt. w/ **Rovral** (iprodione (2)) • **PhD** (polyoxin-D (19)) • **Luna Experience** (Fluopyram (7) + tebuconazole (3))	• **Endura** (Boscalid (7)) • **Oso** (polyoxin-D (19)) • **Rally** (myclobutanil (3)) • **Miravis** (Adepidyn (7))

The same MOA provided different level of control…

Mixing partners for mancozeb/ziram or captan (Timing: bloom, berry touch, veraison)		
Moderate level of reduction	Low level of reduction	No or limited effect
• **Aprovia** (Benzovindiflupyr, FRAC= 7) • **Cueva** (Copper (M1)) • **Intuity** (mandestrobin, (11)) • **Viathon** (Phos acid (33) + tebuconazole (3)) • **Switch** (cyprodinil (9) + fludioxonil (12))	• **Elevate** (fenhexamid (7)) alt. w/ **Rovral** (iprodione (2)) • **PhD** (polyoxin-D (19)) • **Luna Experience** (Fluopyram (7) + tebuconazole (3))	• **Endura** (Boscalid (7)) • **Oso** (polyoxin-D (19)) • **Rally** (myclobutanil (3)) • **Miravis** (Adepidyn (7))

Resistance issue!!

Overall summary

- Grape clusters are susceptible throughout the season.
  - Also, it can cause asymptomatic infection on leaves that can produce spores
- Cultivar susceptibility varies
  - Susceptible: Chardonnay, Traminette, Cabernet Sauvignon, Cabernet franc
  - Less susceptible: Merlot, Petit Manseng
  - However, even with less susceptible cultivar, outbreak can occur…
- Very inconsistent field testing results when we rely on one product
  - Probably due to multiple species in the vineyard *plus*, these pathogens are generally less sensitive to many fungicides
  - Tank mixing Switch, Aprovia, tebuconazole, or iprodione with a relatively less resistant-prone material (mancozeb or captan) is probably the best approach at this point.

What's next?

On-going

- Field trials using plant defense activators and nutrient (Calcium)
- Effect of high relative humidity on germination

Future

- Look into more DMIs using plate and field assays
- Investigate the use of Copper and Topsin-M as a mixing partner
  - Both are not used as much as used to be.
- Determine when the pathogens become active
  - Spore collection and detection

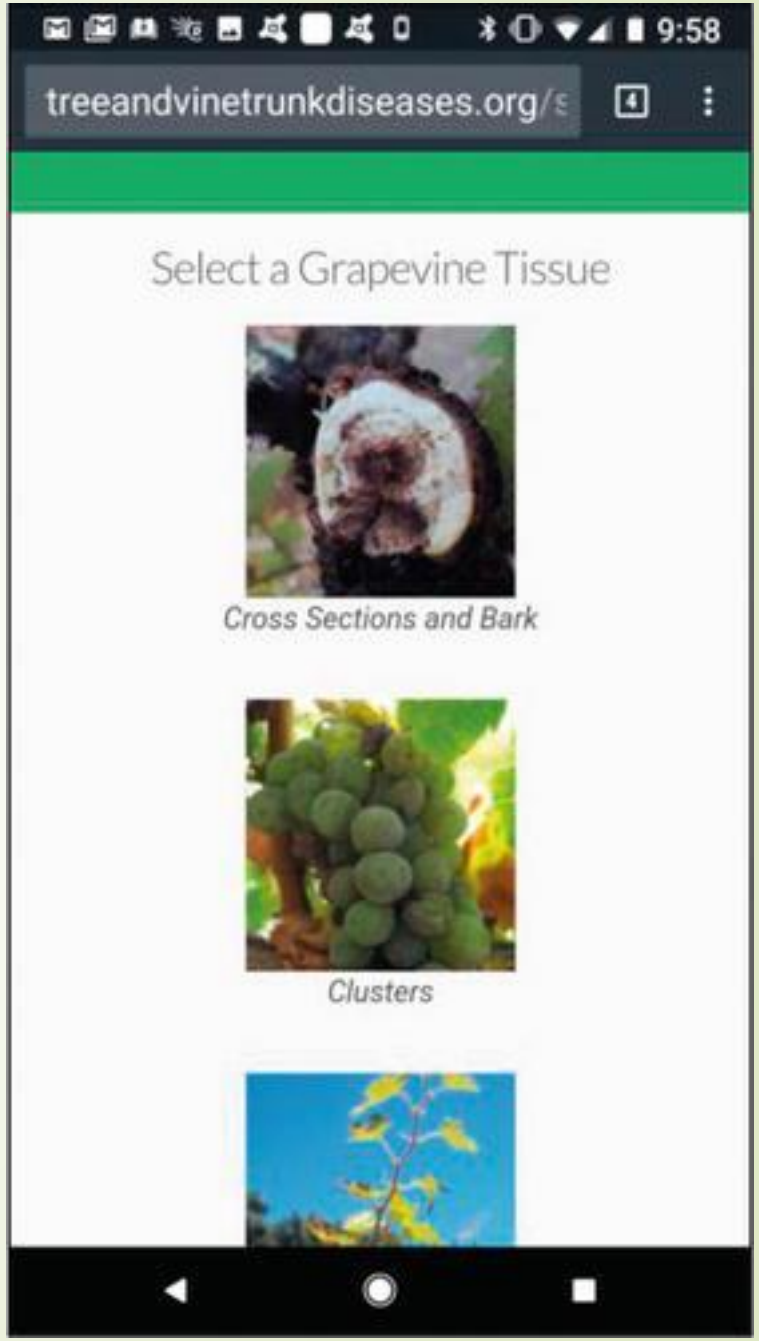
Funding sources

- USDA/NIFA HATCH project (2013-18)
- Virginia Wine Board (2013-2018)
- VDACS/USDA SCRI Block (2015-17)
- Southern Region Small Fruit Consortium (2013-15)

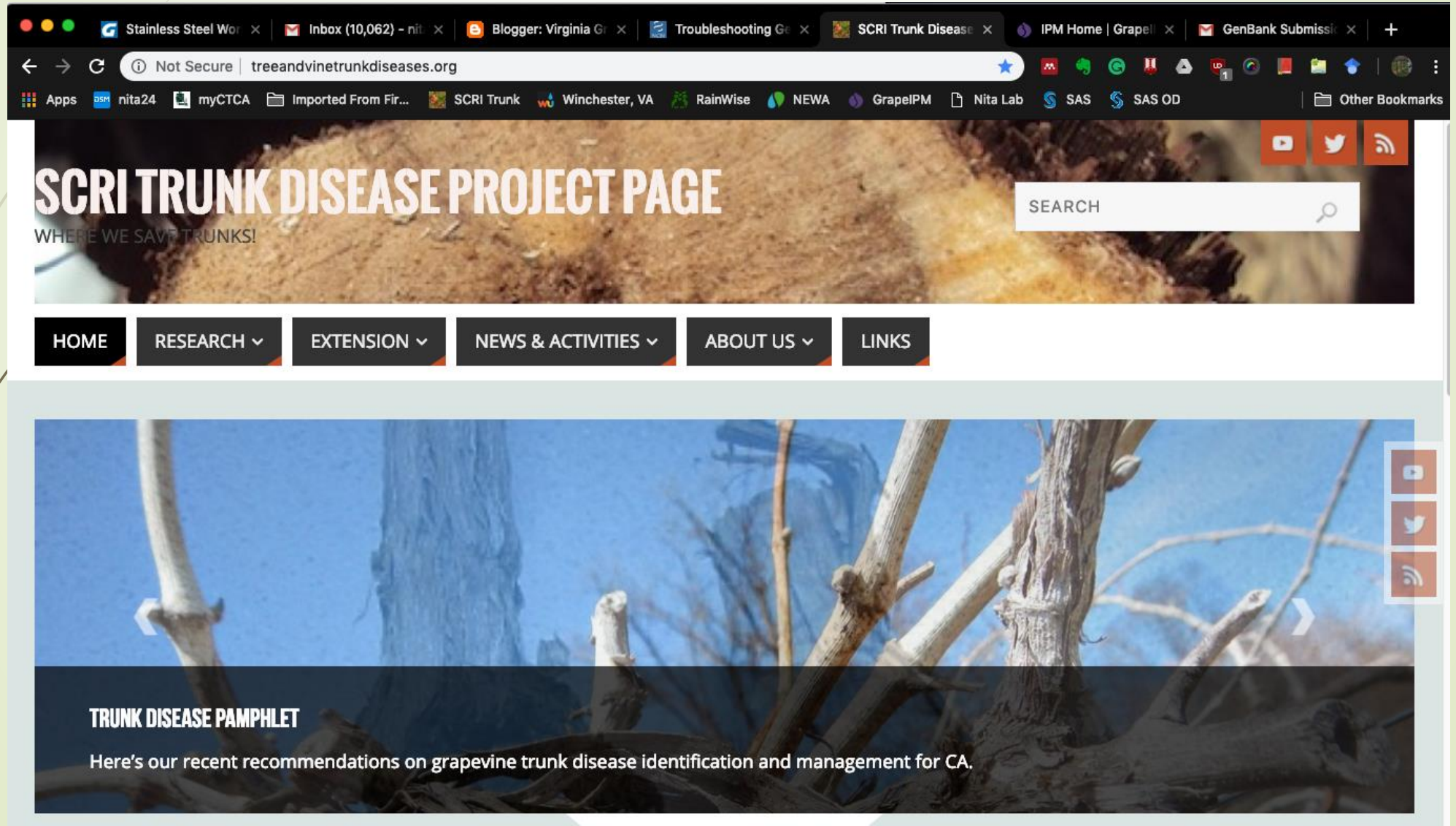
HATCH project
SCRI Block Grant

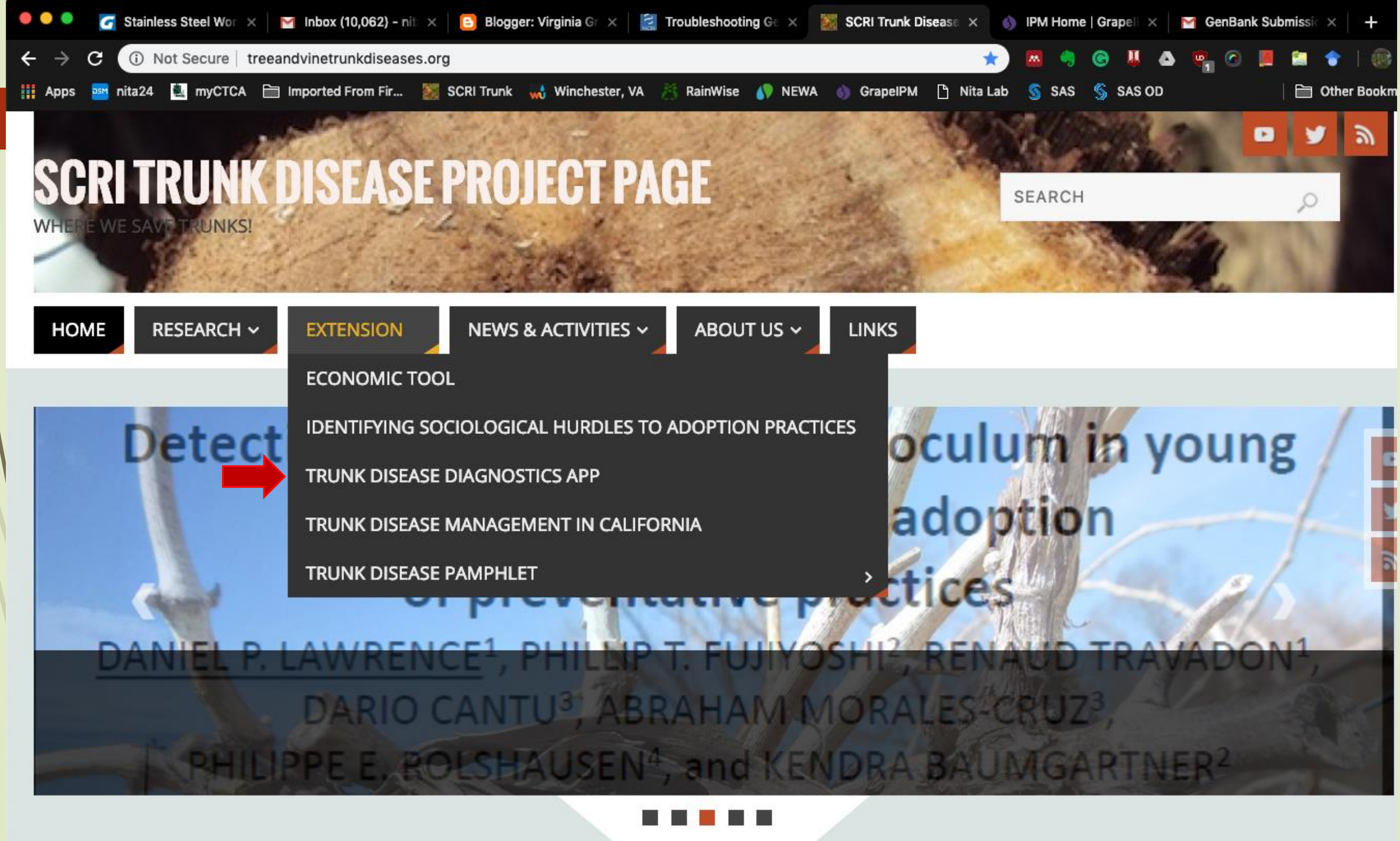
Trunk disease diagnostic aid application

- Online application to help you identify trunk diseases and other disorders that are associated with trunk/vascular tissue related issues.
- Mobile ready
- https://www.intechopen.com/books/advances-in-plant-pathology/developing-an-online-grapevine-trunk-disease-diagnostic-aid
- Introduced in 2016, and currently I am making more adjustments.



Treeandtrunkdiseases.org (find a link from **grapepathology.org**)

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LINKS
ECONOMIC TOOL
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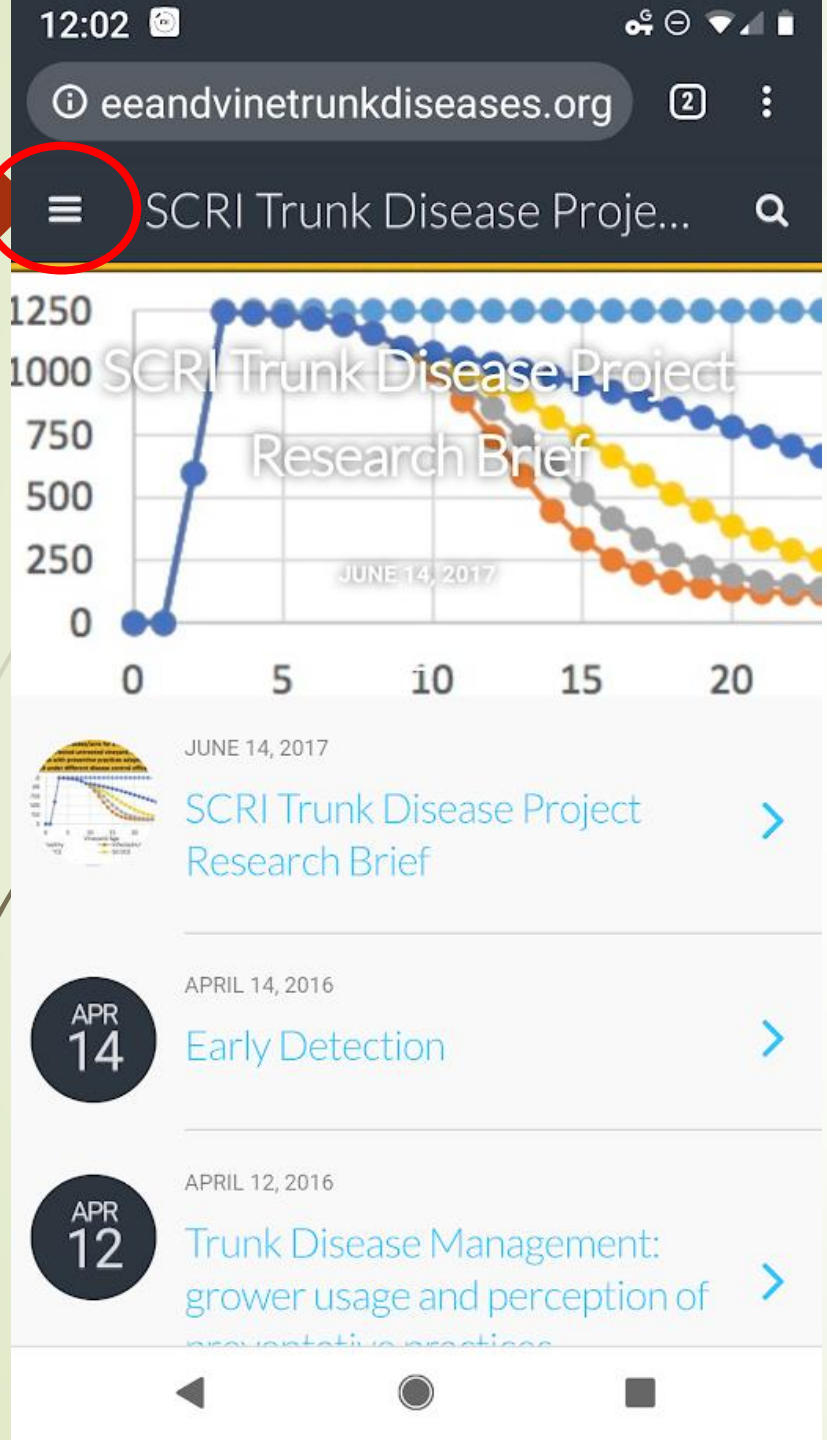
PHILIPPE E. ROLSHAUSEN[4], and KENDRA BAUMGARTNER[2]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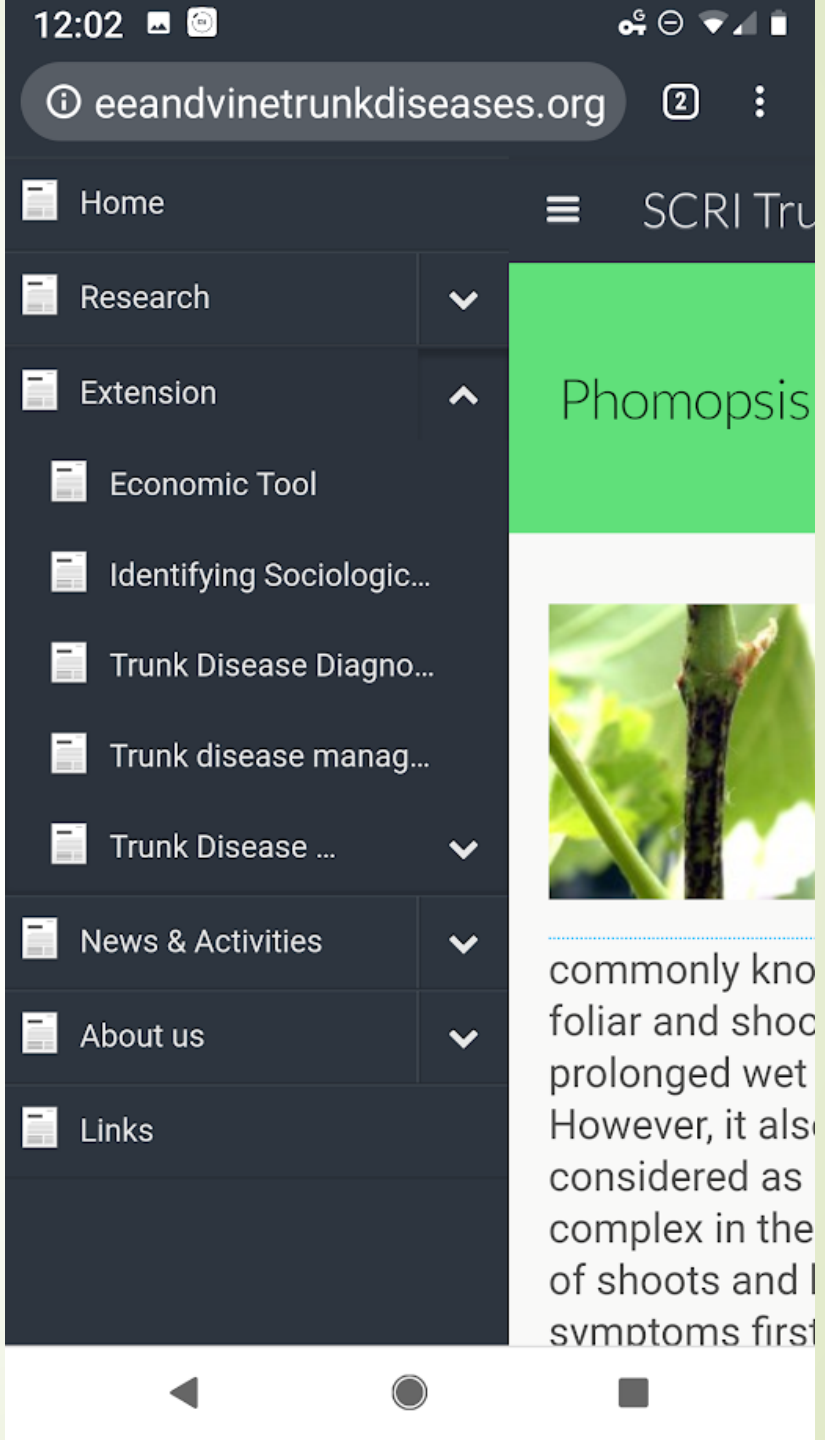
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Apps | nita24 | myCTCA | Imported From Fir... | SCRI Trunk | Winchester, VA | RainWise | NEWA | GrapeIPM | Nita Lab | SAS | SAS OD | Other Bookmarks

SCRI TRUNK DISEASE PROJECT PAGE

WHERE WE SAVE TRUNKS!

SEARCH

HOME | RESEARCH | EXTENSION | NEWS & ACTIVITIES | ABOUT US | LINKS

TRUNK DISEASE DIAGNOSTICS APP



This web application will help diagnosis of a trunk disease based on symptoms, and provide management options for the infected vine(s). The results and following recommendations are based on the responses you provide through the image survey of symptomatic tissues. To begin, select the tissue where you find the most prominent symptoms.

Let's Get Started!

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Not Secure | treeandvinetrunkdiseases.org/select-a-grapevine-tissue

Apps | nita24 | myCTCA | Imported From Fir... | SCRI Trunk | Winchester, VA | RainWise | NEWA | GrapeIPM | Nita Lab | SAS | SAS OD | Other Bookma

TRUNK DISEASE DIAGNOSTIC APP

Select a Grapevine Tissue

Leaves

Shoots

Clusters

Cross Sections and Bark

FOLIAR SYMPTOM GALLERY

Select the leaf symptom that best resembles yours.

Marginal Burning (photo credit: Mizuho Nita)

Yellow Specks (photo credit: Mizuho Nita)

Tiger Striping (photo credit: Mizuho Nita)

Chlorotic Cuppled Leaves

LEAF SYMPTOMS

Do these symptoms resemble your vine?




(Click to enlarge)

YES NO

DO YOU SEE ONE OR MORE OF THESE SYMPTOMS?

Click for image details

YES NO

ESCA

Esca, also known as grapevine measles, petri disease, or black goo, can slowly plague a vineyard into decline. Esca pathogens excrete toxins that trigger an array of symptoms. Measles-like symptoms (dark spots with purple halos) appear on fruit, especially on white-fruited cultivars, anytime from fruit set to harvest. Foliar toxin expression of esca appears as a tiger stripe pattern of interveinal necrosis with yellow or red margins. Cross section of the infected trunk, cordon, and cane reveals black spots in the vascular system that may secrete black goo. Severe cases exhibit shoot/tendril dieback and "apoplexy" or complete defoliation and fruit abortion. Spores enter pruning wounds and colonize the vascular system anytime from November to April. New infections can have symptom expressions in the same year, and symptoms become more prevalent in years of wet springs and hot summers.

Tiger Striping

Causal agent: Several genera of pathogens can cause Esca in grapevines. *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp. and *Toginia* spp. are common species.

Management: The prevention at the time of winter pruning is the best approach for the management. Double pruning, protection of pruning wound using paint with boron (trade name: B-Lock), as well as chemical control, such as use of thiophanate-methyl (trade name: Topsin-M) and/or myclobutanil (trade name: Rally) to protect pruning wounds are known to reduce risk of infection to pruning wounds. Also, it is advised to prune vines on a day followed with several days of sunny condition. (I.e., avoid pruning before the rain) If you decided to use aforementioned fungicides, make sure to obtain the label for the specific uses. Although symptom appears in the vineyard after 5-10 years of planting, young vines are as susceptible as the old vines; therefore, it is advised to implement a management plan from early years of the vineyard.

For infected unproductive vines, remove infected trunk or cordon arm 12 inches below canker, and move the infected woods out from the vineyard for burying or burning. Vine health is extremely important for the recovery after the major pruning of cordon or trunk. Provide sufficient water and nutrients to encourage healthy vegetative growth.

Links:

Grapevine Measles. eXtension