Empire State Producers Expo 2021

Fragile FRAC 7 Fungicides: Part I - Current status of Stemphylium leaf blight fungicide resistance in onion in New York.

Frank Hay[1], Christy Hoepting[2], Audrey Klein[1], Daniel Heck[1], Sarah Pethybridge[1], and Katrin Ayer[1]

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Thursday, January 14, 2021
Session Number 2
Session Name: **Onion Critical Issues**
Session Organized by: **Christy Hoepting, CCE Cornell Vegetable Program**

Stemphylium leaf blight has emerged as one of the main fungal foliar diseases of onion in NY over the last 10 years.

Causes premature death of leaves (‘onion dying standing up’).

Image: C. Hoepting Cornell Cooperative Extension.

Fungicide	FRAC group and active ingredient: 2	3	7	9	11	Year labelled in NY onion
Rovral®	iprodione					2003
Amistar®					azoxystrobin	2003
Endura®			boscalid			2005
Pristine®			boscalid		pyraclostrobin	2005
Scala®				pyrimethanil		2005
Tilt®		propiconazole				2008
Quadris Top®		difenoconazole			azoxystrobin	2010
Inspire Super®		difenoconazole		cyprodinil		2012
Viathon®		tebuconazole + potassium phosphite (33)				2013
Merivon Xemium®			fluxapyroxad		pyraclostrobin	2015
Luna Tranquility®			fluopyram	pyrimethanil		2016
Luna Experience®		tebuconazole	fluopyram			2016
Miravis Prime®			Pydiflumetofen* + fludioxonil (12)			2019

FRAC = Fungicide Resistance Action Committee. Fungicides are organized by FRAC code into modes of action. *Based on field performance only.

How do we identify fungicide resistance?

- Poor field control following fungicide application.

  (However, efficacy may also be affected by e.g. incorrect rate, poor water quality, poor application conditions, high disease pressure.)

- Shift in fungicide sensitivity in laboratory tests.

- Presence of genetic mutations known to be associated with resistance to particular modes of action.

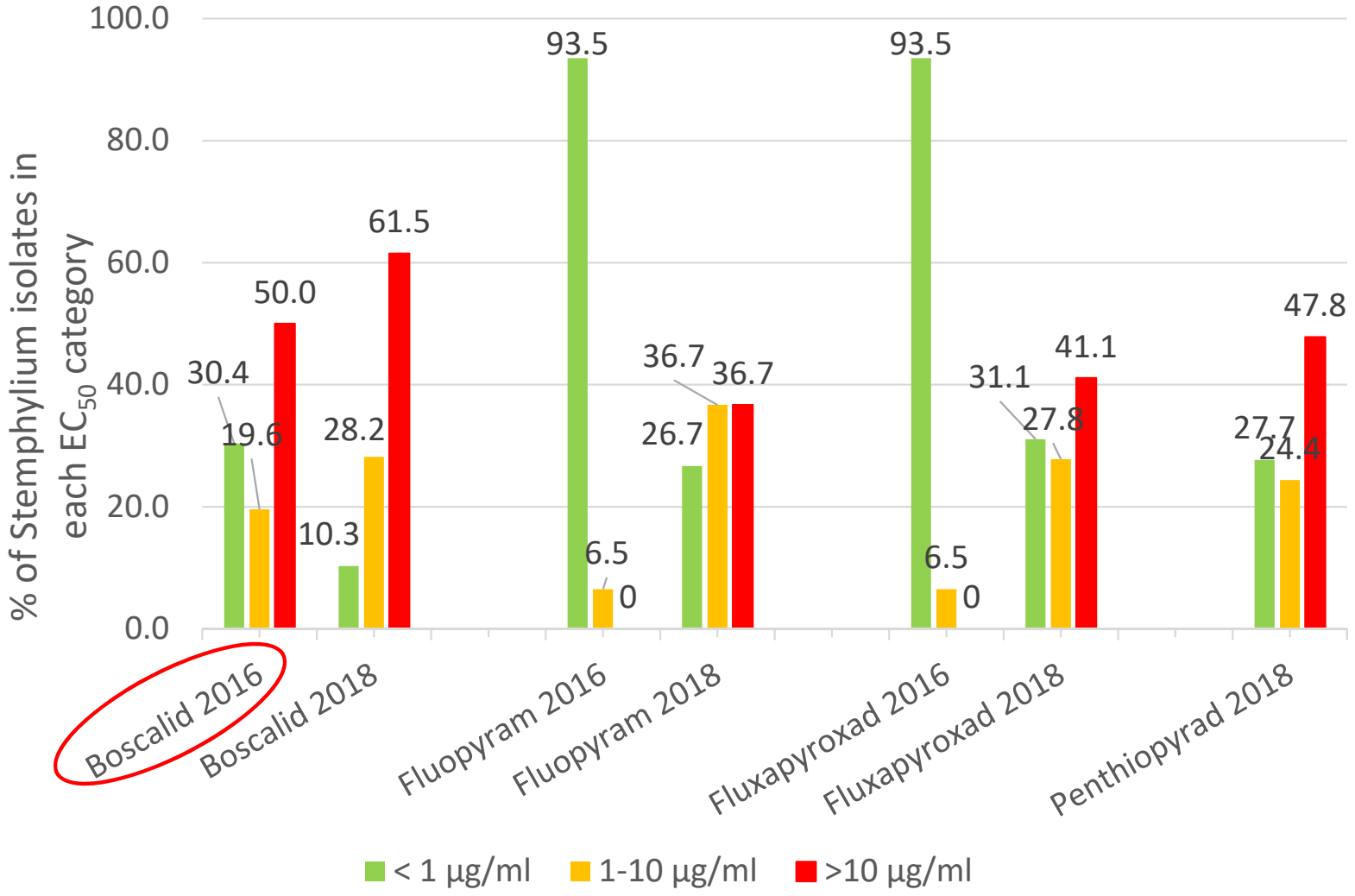
- In 2016 tested 46 isolates of Stemphylium collected from 19 onion crops: Orange (1), Wayne (2), Genesee (6), Seneca (1), Cattaraugus (1), Oswego (6), Ontario (1), Schuyler (1) counties against
  - Boscalid (e.g. Endura),
  - Fluopyram (e.g. Luna Tranquility),
  - Fluxapyroxad (Merivon)

- In 2018, tested isolates of Stemphylium from 23 crops: Elba (4), Oswego (5), Orange County (9), Yates (1), Wayne (4) against
  - Boscalid (e.g. Endura),
  - Fluopyram (e.g. Luna Tranquility),
  - Fluxapyroxad (Merivon),
  - Penthiopyrad (Fontelis).

  (Tested against 90 isolates except for boscalid which was tested against 30 isolates)

FRAC 7 (Fluopyram, Fluxapyroxad, Penthiopyrad)

Changes in sensitivity of Stemphylium isolates between 2016 and 2018.



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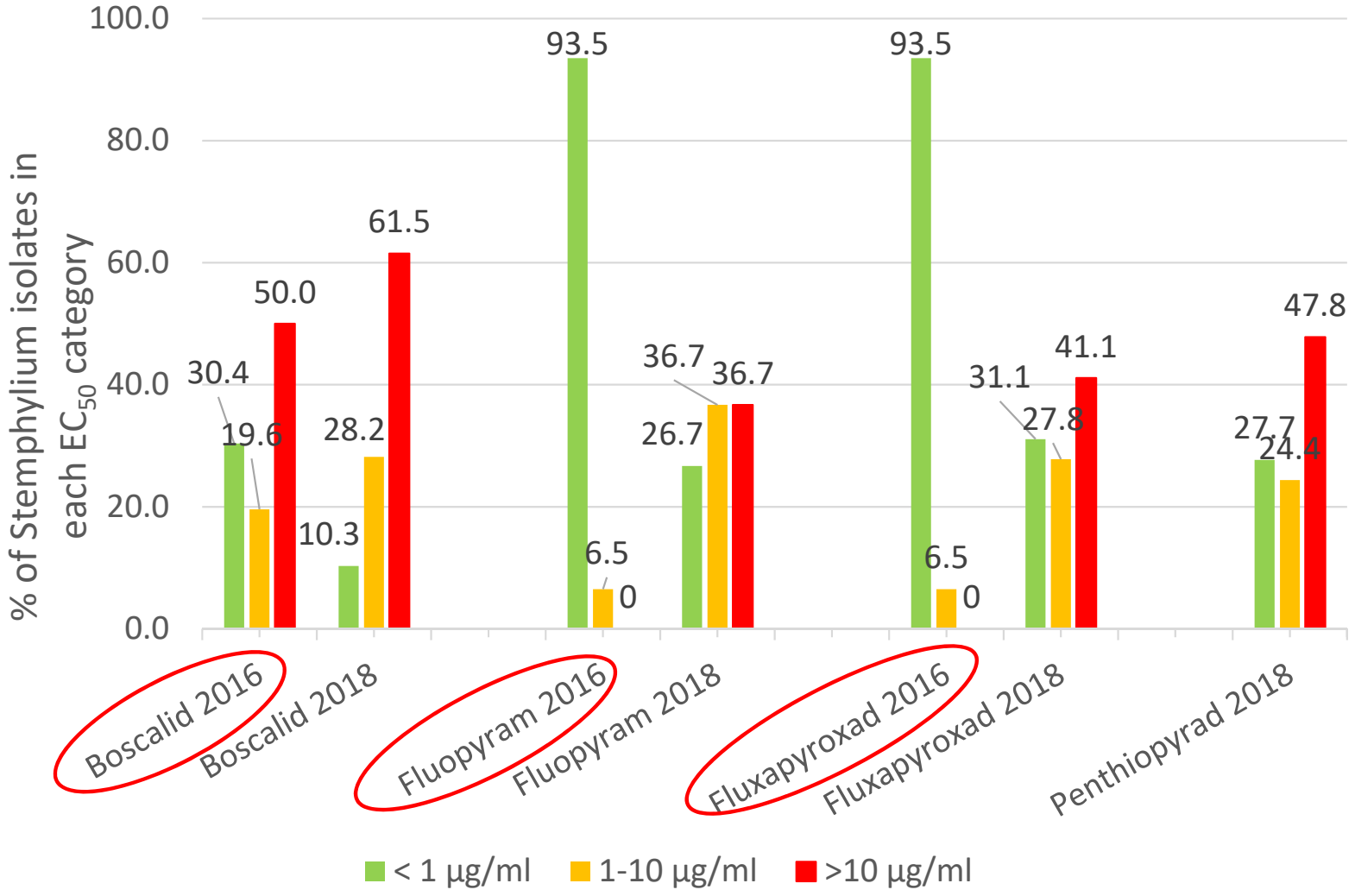

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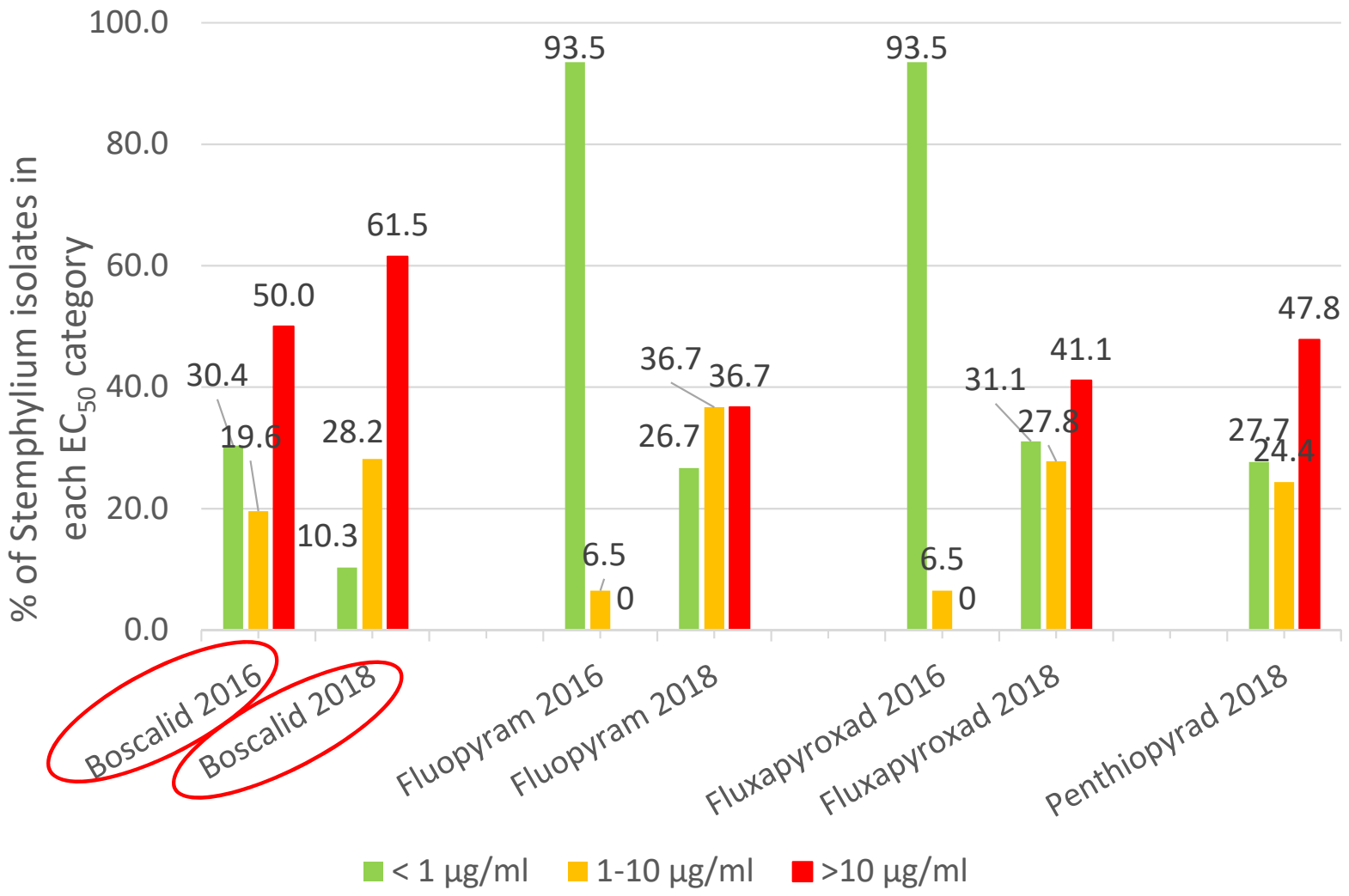

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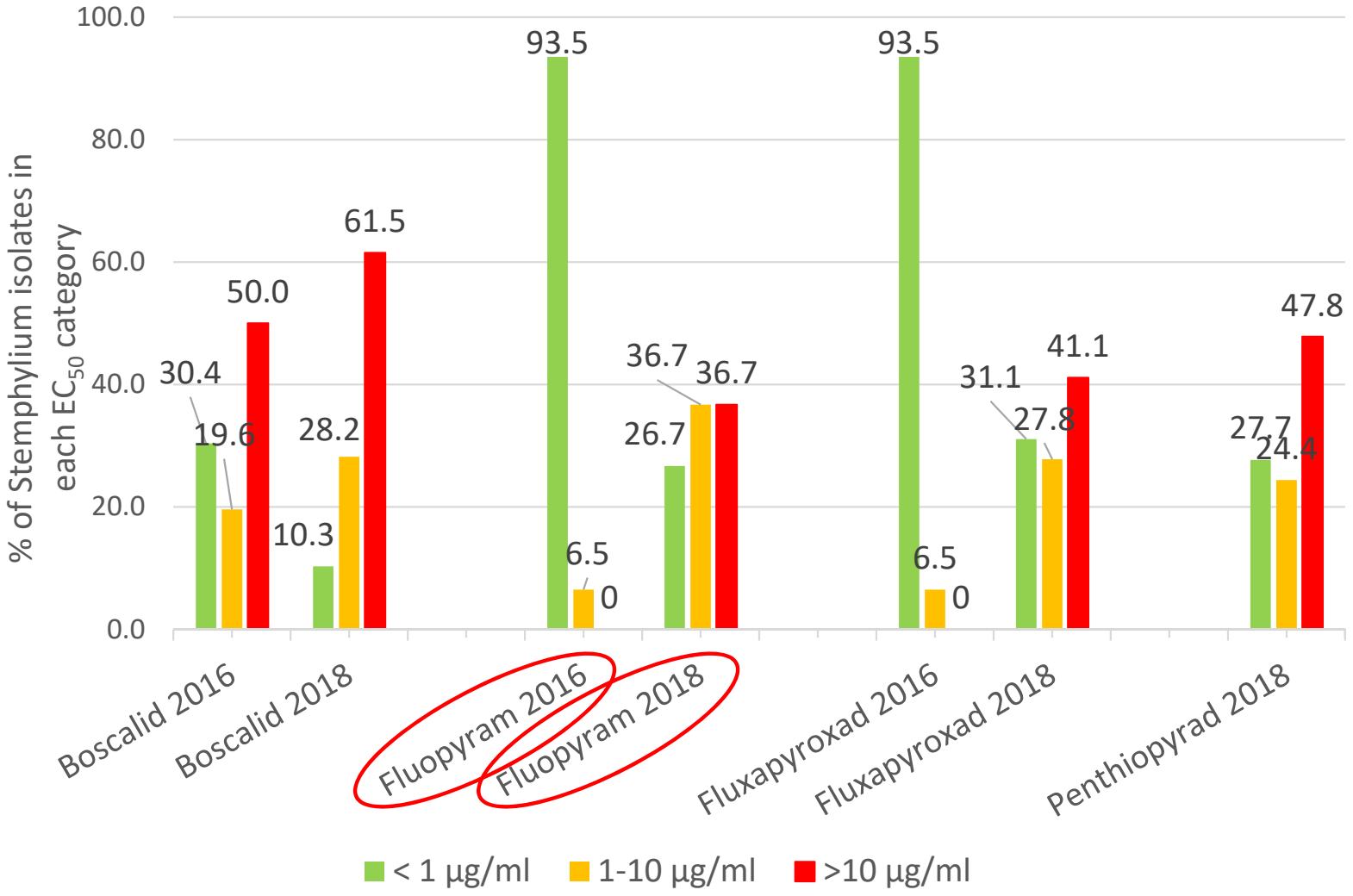

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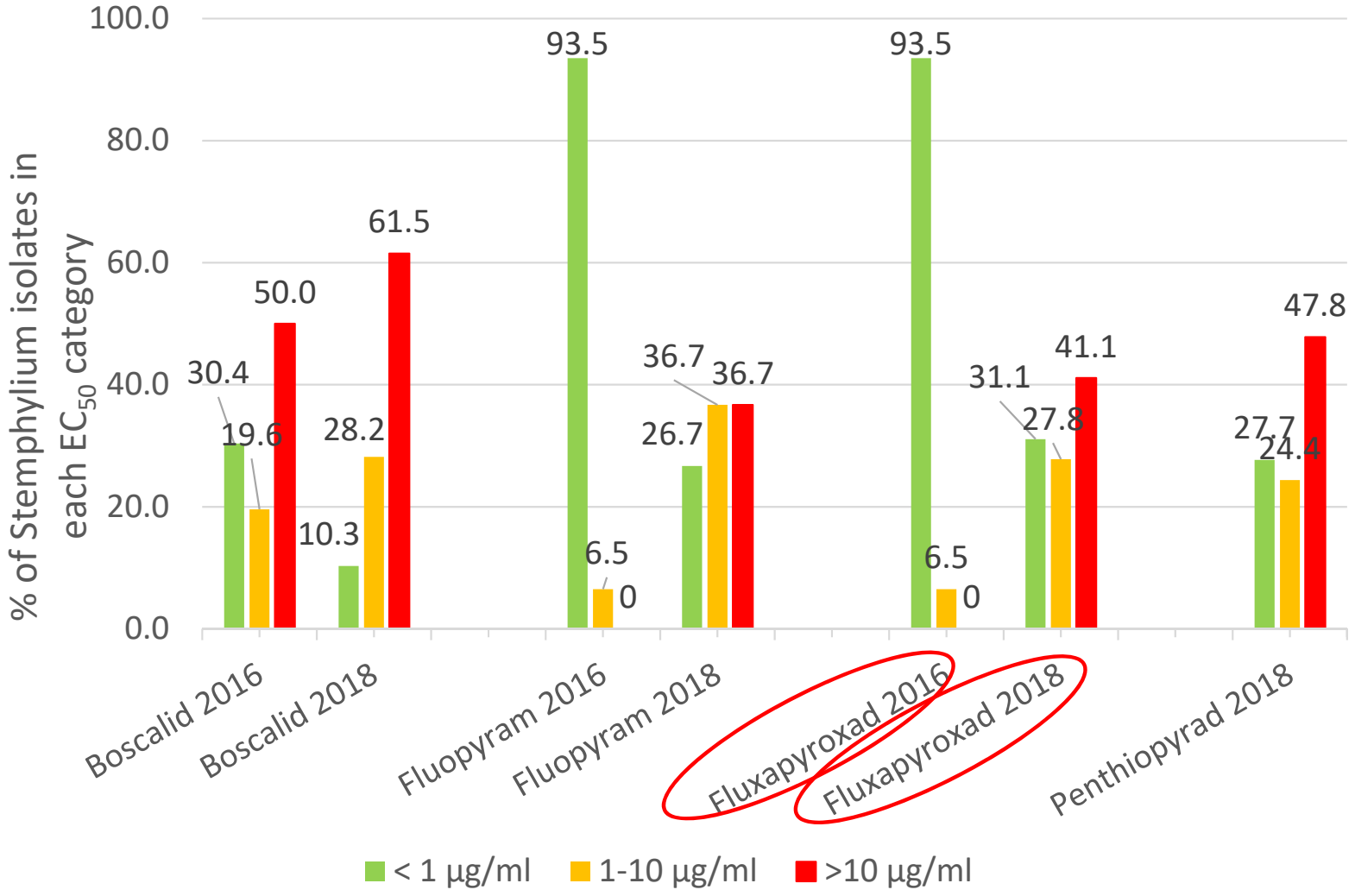

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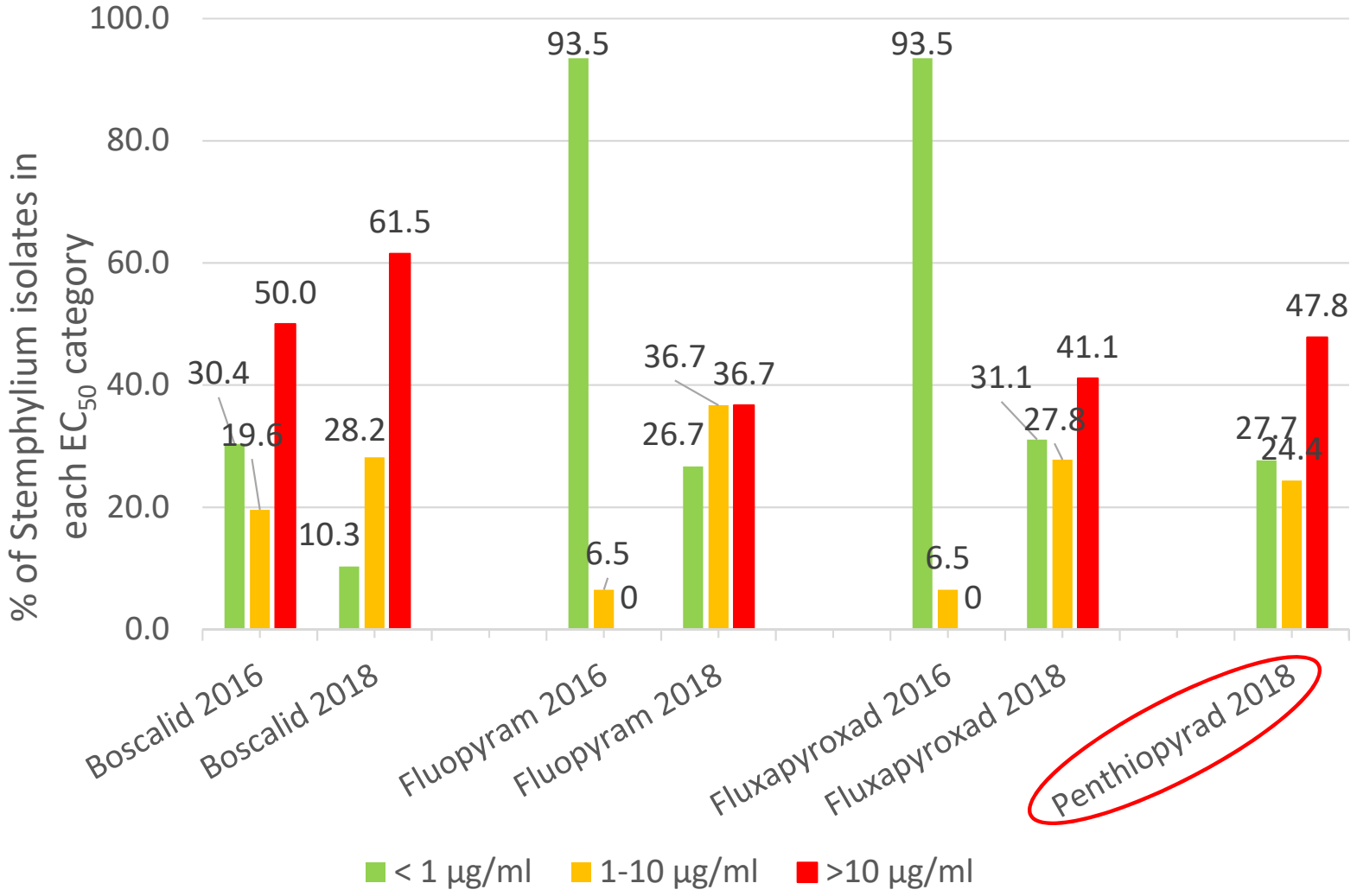
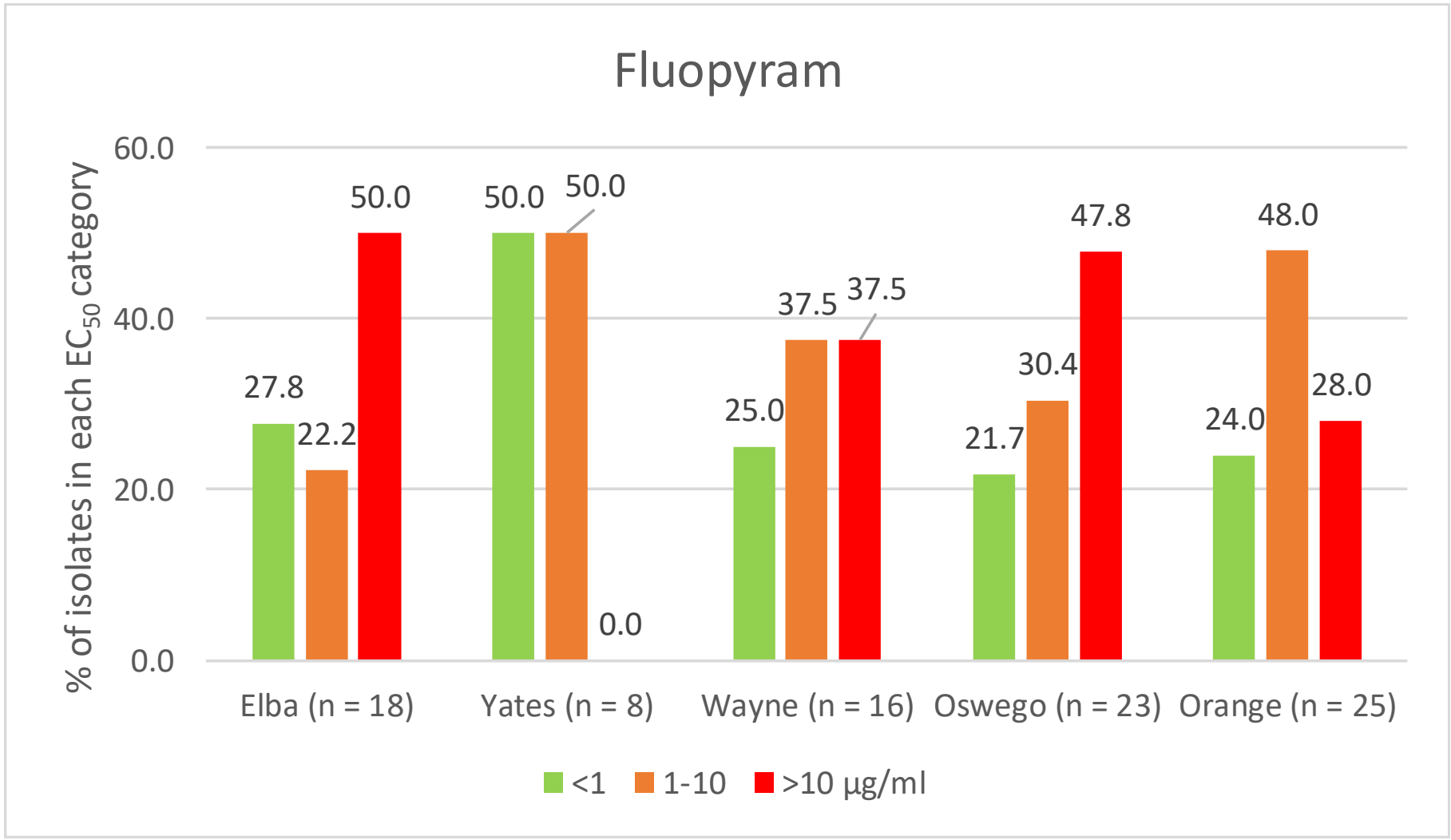


Sensitivity of *Stemphylium vesicarium* to fluopyram (e.g. Luna Tranquility®) across regions (2018).



Where to from here?

- Identify what mutations are present in Stemphylium.
- Test fungicide sensitivity of Stemphylium isolates collected from the field in 2020.
- Investigate disease forecasting to reduce fungicide application to critical times in an effort to preserve fungicides.

Acknowledgements

- **AgriTech Staff:**
  - Audrey Klein, Daniel Heck, Sean Murphy, Sandeep Sharma.
- **CCE Staff**: Christy Hoepting, John Gibbons, Ethan Grundberg
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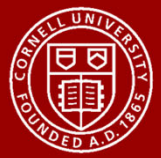
  - NYFVI SCBG SG16-008, SCG 18-003
  - USDA Hatch Project NYG-625445

USDA
United States Department of Agriculture
National Institute of Food and Agriculture

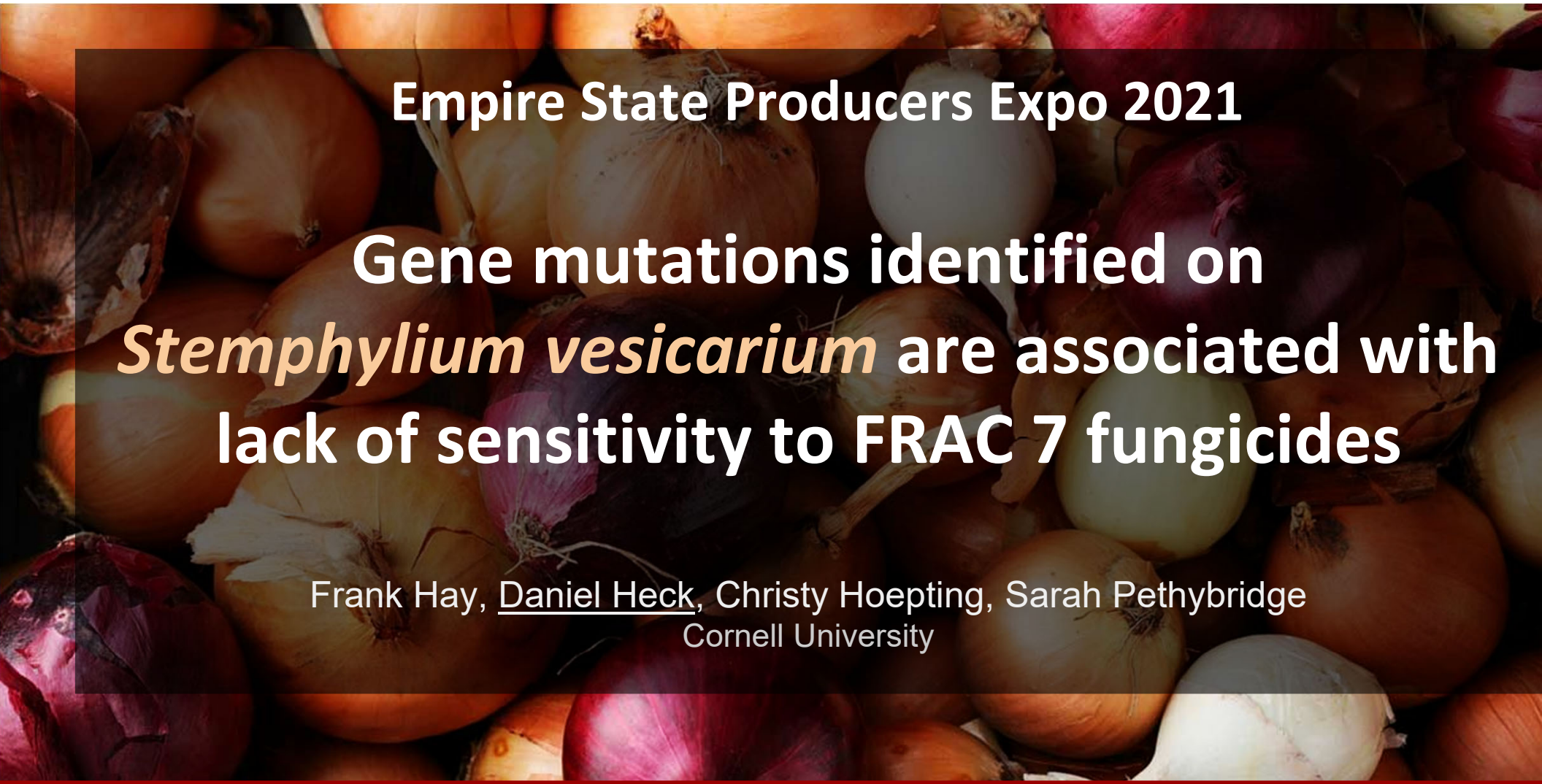
NY farm viability INSTITUTE

Cornell AgriTech

New York State Agricultural Experiment Station



Cornell CALS College of Agriculture and Life Sciences



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Gene mutations identified on *Stemphylium vesicarium* are associated with lack of sensitivity to FRAC 7 fungicides

Frank Hay, Daniel Heck, Christy Hoepting, Sarah Pethybridge
Cornell University

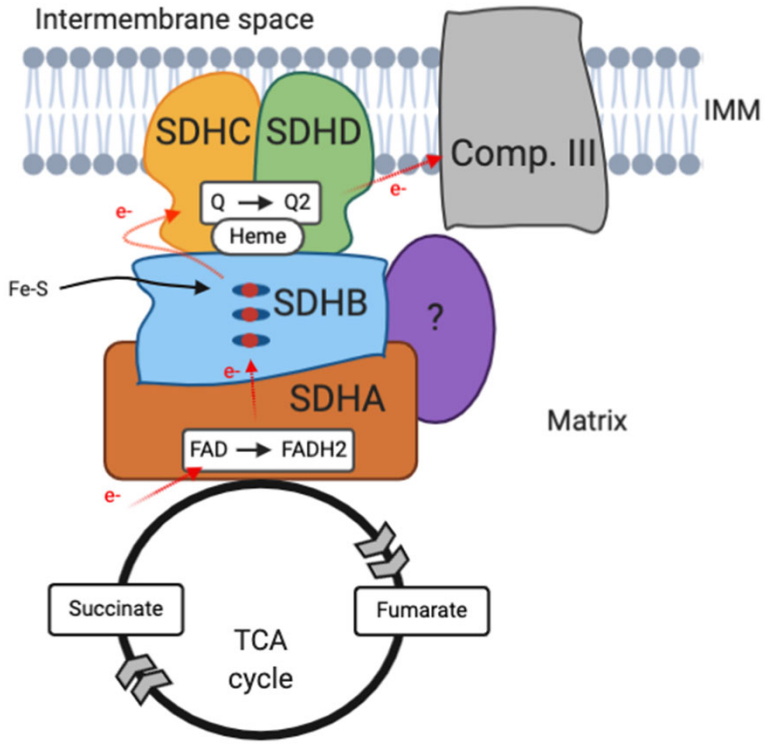
Mode of action and **molecular targets** of *SDHI* fungicides

*SDHI = **S**uccinate **De**Hydrogenase **Inhibitors***

MOA	TARGET SITE AND CODE	GROUP NAME	CHEMICAL OR BIOLOGICAL GROUP	COMMON NAME	COMMENTS	FRAC CODE
C. respiration	C2 complex II: succinate-dehydro-genase	SDHI (Succinate-dehydrogenase inhibitors)	phenyl-benzamides	benodanil flutolanil mepronil	Resistance known for several fungal species in field populations and lab mutants. Target site mutations in sdh gene, e.g. H/Y (or H/L) at 257, 267, 272 or P225L, dependent on fungal species. Resistance management required. **Medium to high risk.** **See FRAC SDHI Guidelines for resistance management.**	7
			phenyl-oxo-ethyl thiophene amide	isofetamid		
			pyridinyl-ethyl-benzamides	fluopyram		
			furan- carboxamides	fenfuram		
			oxathiin-carboxamides	carboxin oxycarboxin		
			thiazole-carboxamides	thifluzamide		
			pyrazole-4-carboxamides	benzovindiflupyr bixafen [illegible] fluxapyroxad [illegible] inpyrfluxam isopyrazam [illegible] penthiopyrad [illegible]		
			N-cyclopropyl-N-benzyl-pyrazole-carboxamides	isoflucypram		
			N-methoxy-(phenyl-ethyl)-pyrazole-carboxamides	pydiflumetofen		
			pyridine-carboxamides	boscalid		
			pyrazine-carboxamides	pyraziflumid		

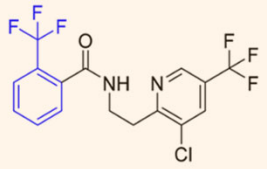
FRAC Code List (2020).

*SDH = **S**uccinate **De**Hydrogenase*

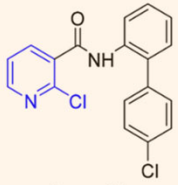


Developed on: Biorender (2021); Based on: Moosavi et al. (2019).

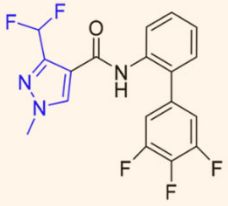
FRAC 7 fungicides



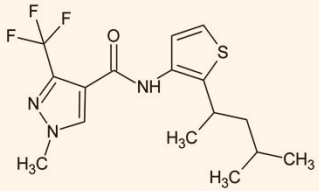
fluopyram



boscalid

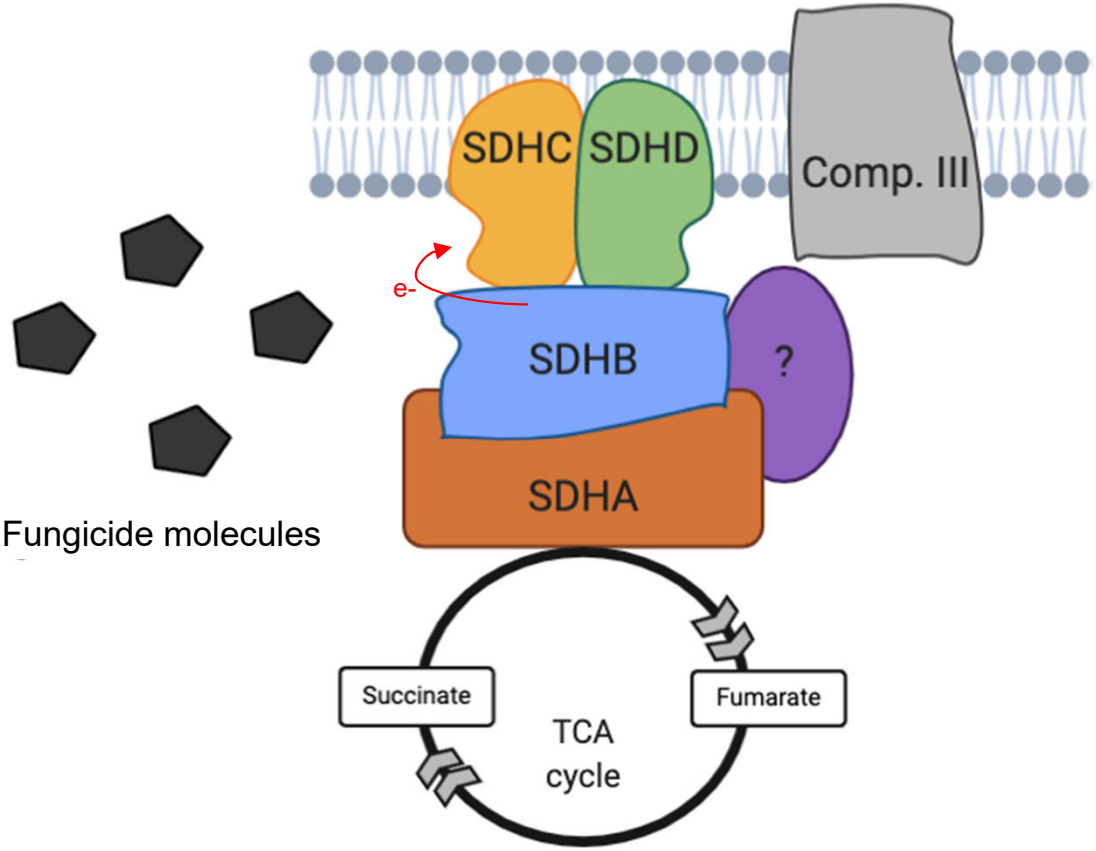


fluxapyroxad



penthiopyrad

Mode of action of *SDHI* fungicides



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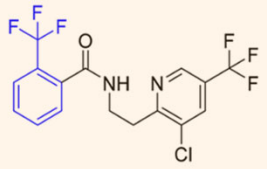
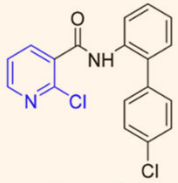
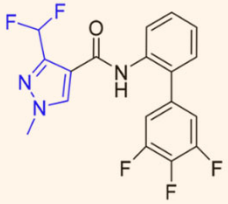
FRAC 7 fungicides



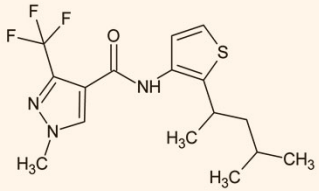
fluopyram



boscalid



fluxapyroxad



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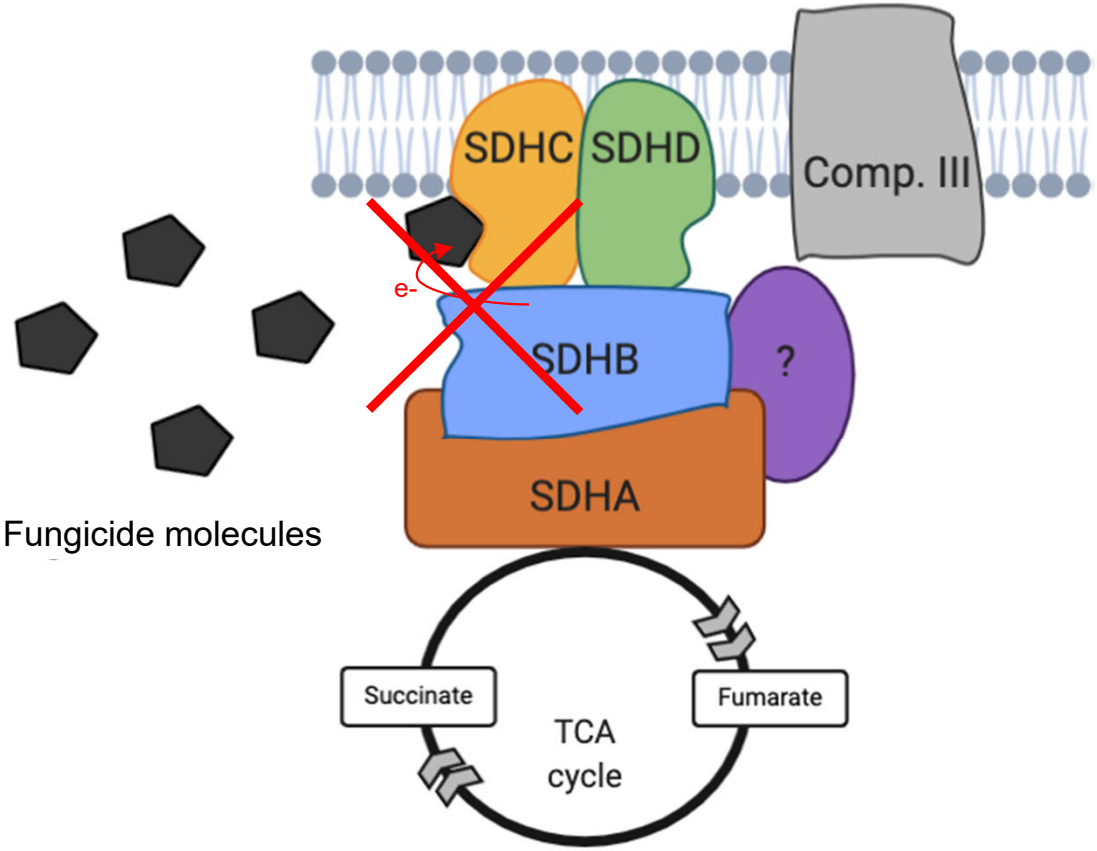

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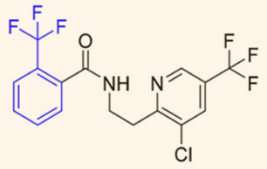
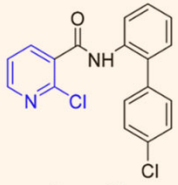
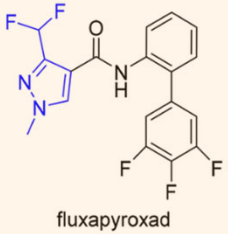
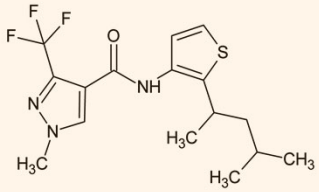
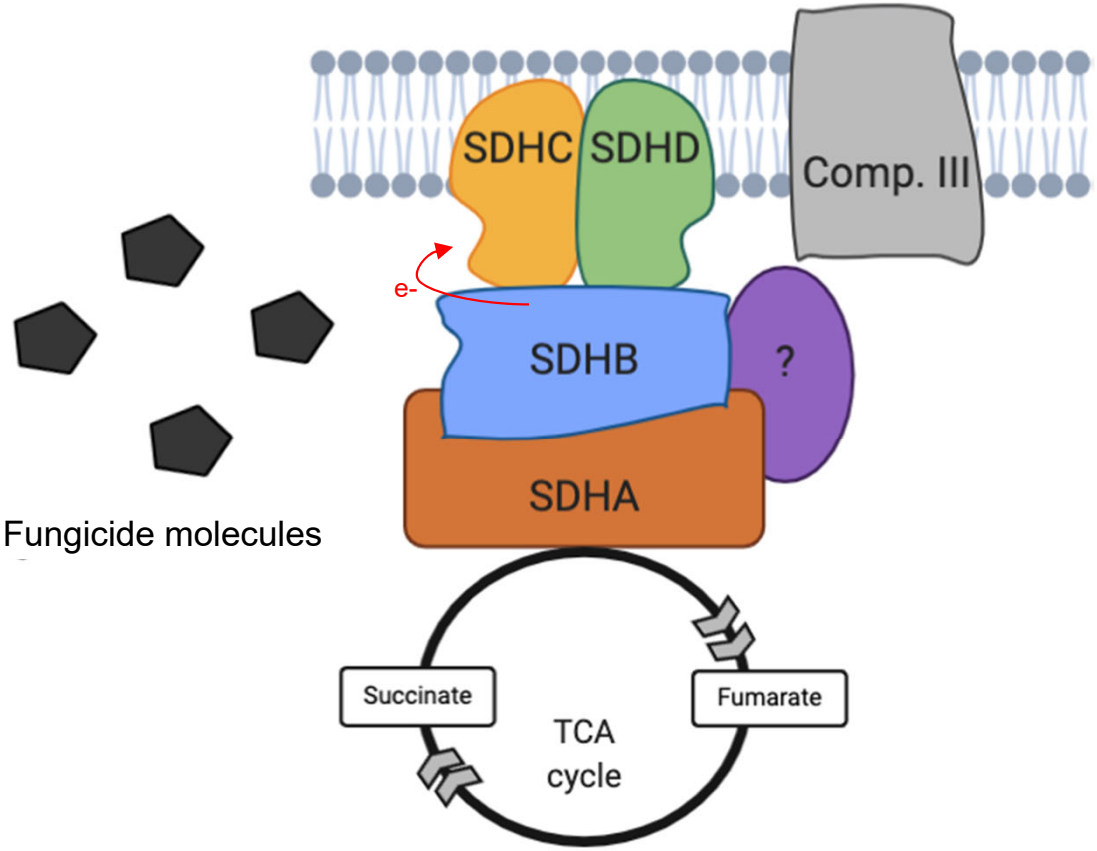








What happens if a gene mutation occurs?



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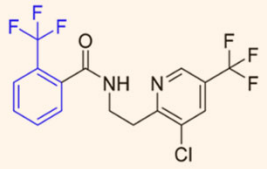
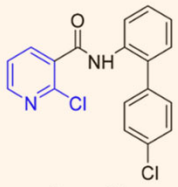
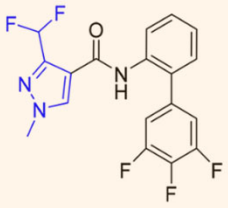


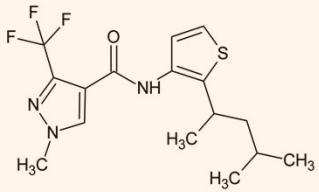
fluopyram



boscalid



fluxapyroxad



penthiopyrad

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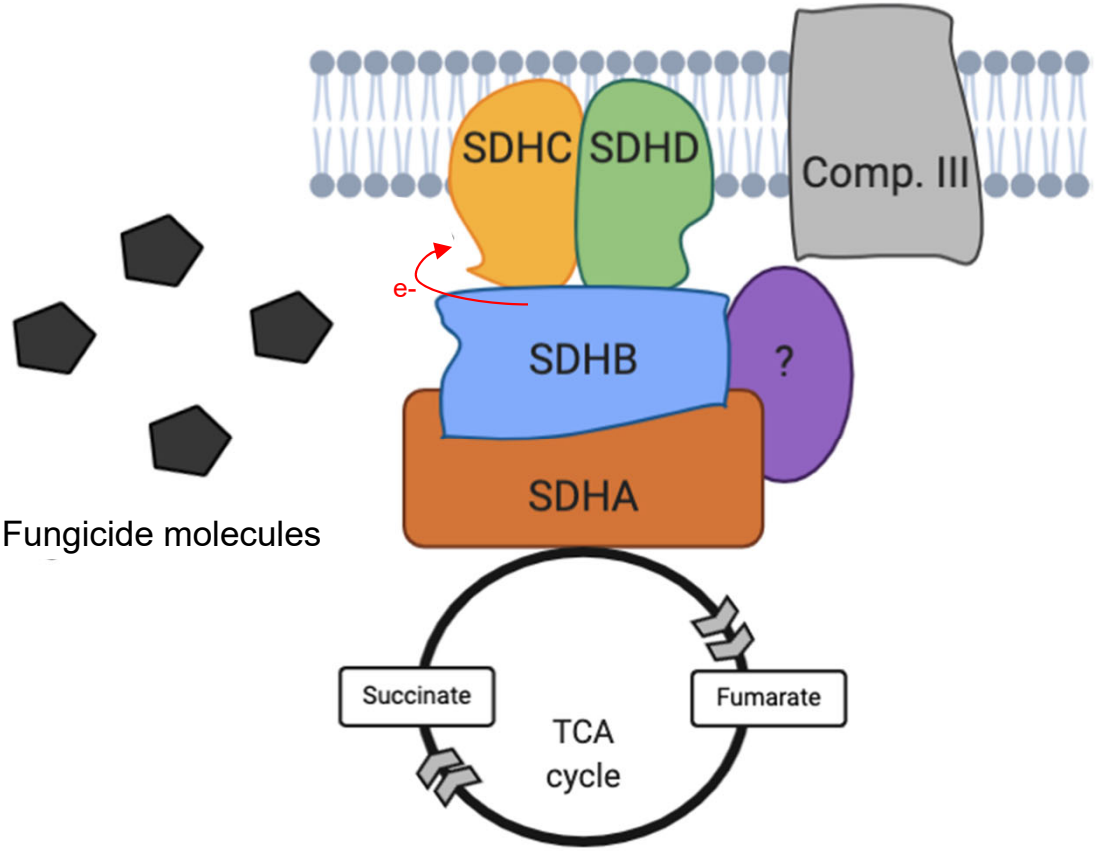

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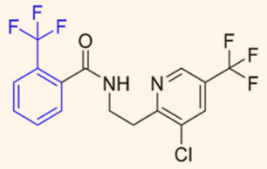
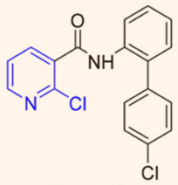


fluopyram



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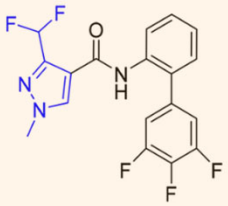
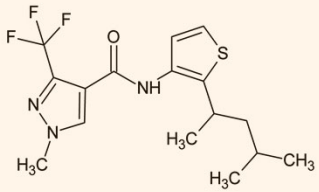


fluxapyroxad



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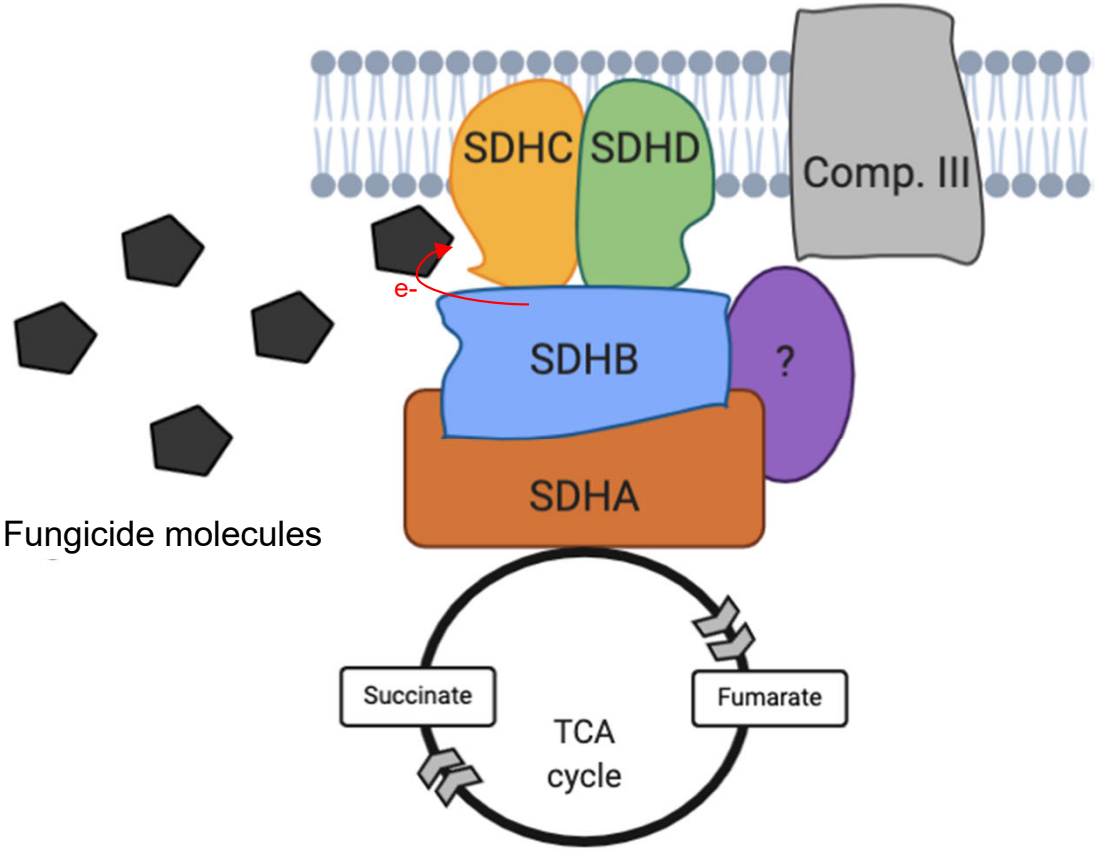

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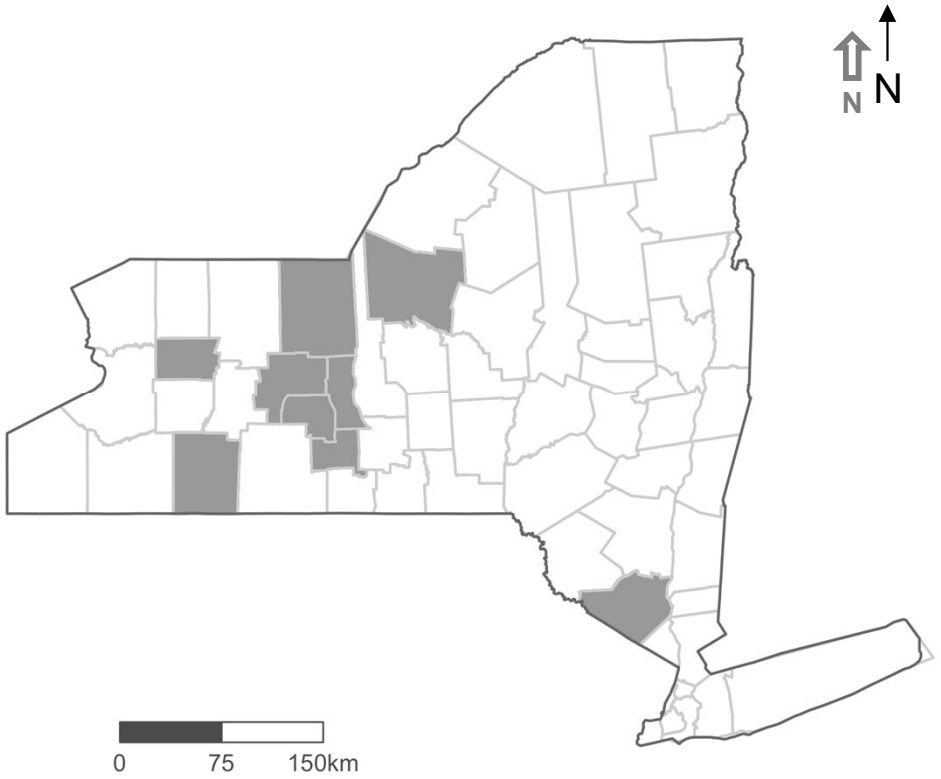
How the work was done?

Stemphylium vesicarium

2016

Where:

Allegany
Elba
Ontario
Orange
Oswego
Schuyler
Seneca
Wayne
Yates



2016 and 2018: fungicide sensitivity (lab conditions) + sequencing of genes;
2020: sequencing of genes;

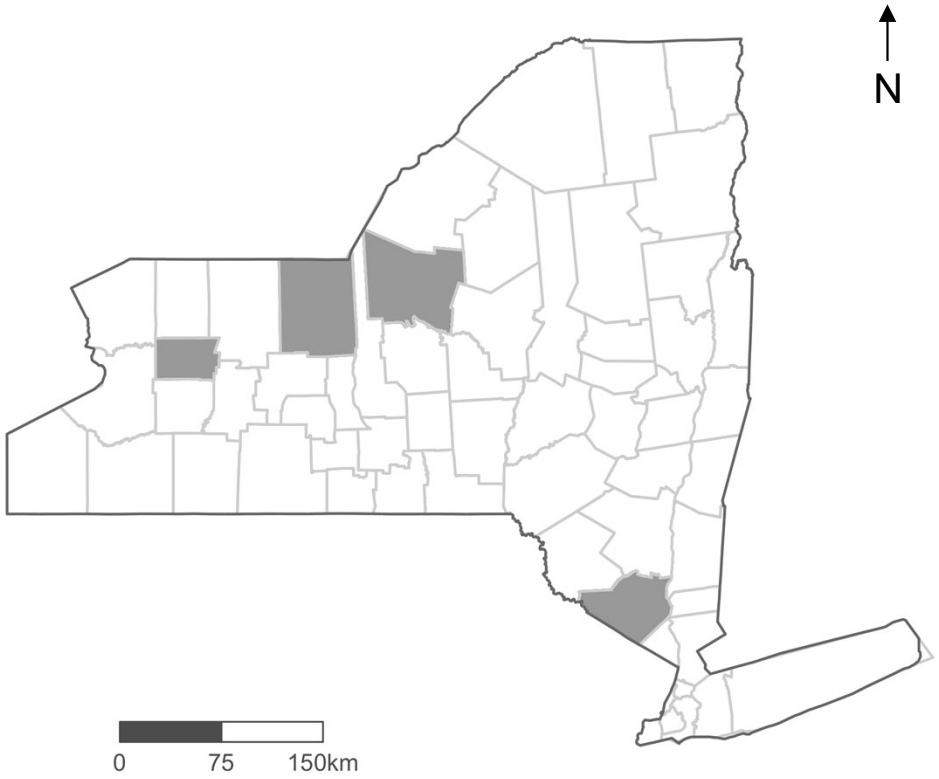
How the work was done?

Stemphylium vesicarium

2018
and
2020

Where:

Allegany
Elba
Ontario
Orange
Oswego
Schuyler
Seneca
Wayne
Yates

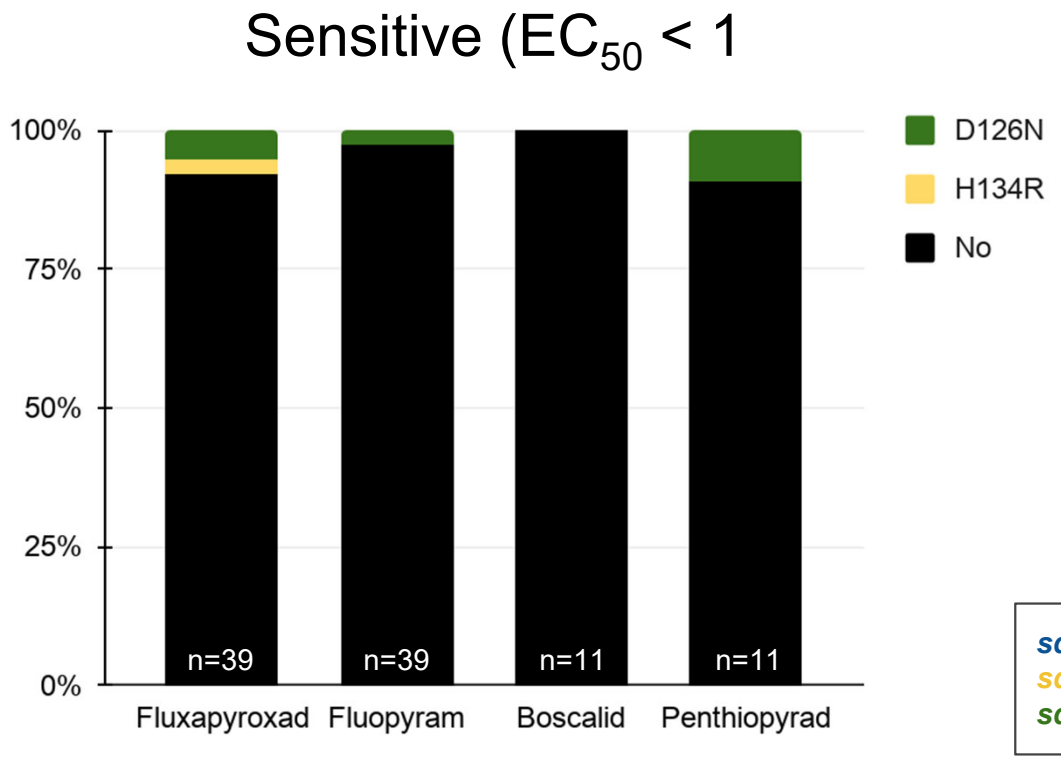


2016 and 2018: fungicide sensitivity (lab conditions) + sequencing of genes;
2020: sequencing of genes;

Which of the *sdh* **gene mutations** explained the observed **FRAC 7** resistance?

Frequency of *sdh* gene mutations:

Fluxapyroxad (Merivon®):	**8%**
Fluopyram (Luna Tranquility®):	**3%**
Boscalid (Endura®):	**0%**
Penthiopyrad (Fontelis®):	**9%**



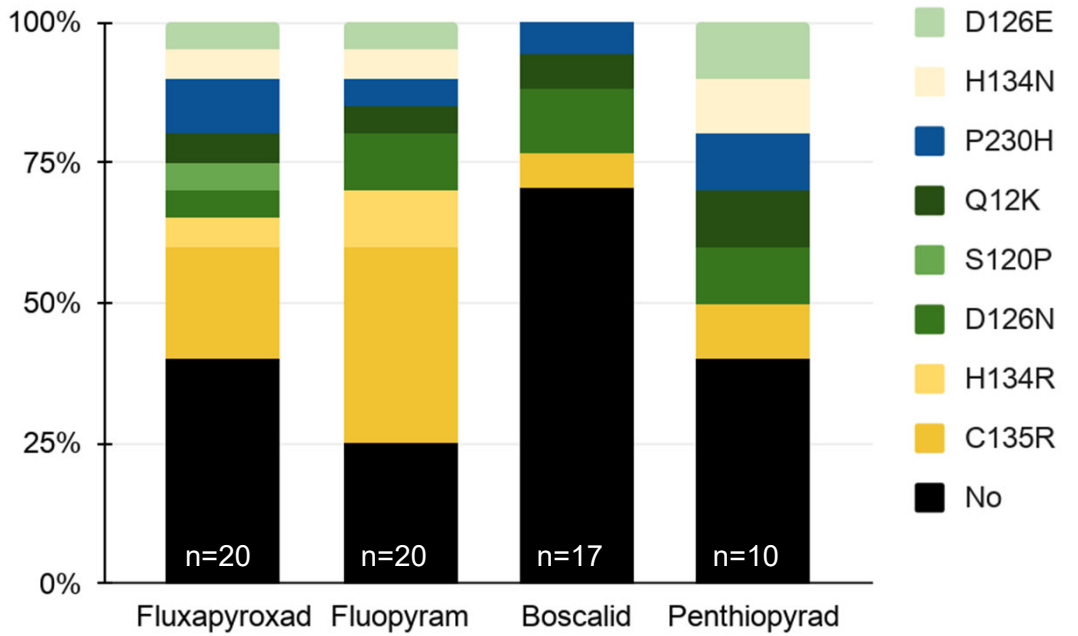
Which of the *sdh* **gene mutations** explained the observed **FRAC 7** resistance?

Frequency of *sdh* gene mutations:

Fluxapyroxad (Merivon®):	**60%**
Fluopyram (Luna Tranquility®):	**75%**
Boscalid (Endura®):	**30%**
Penthiopyrad (Fontelis®):	**60%**

sdhB;
sdhC;
sdhD

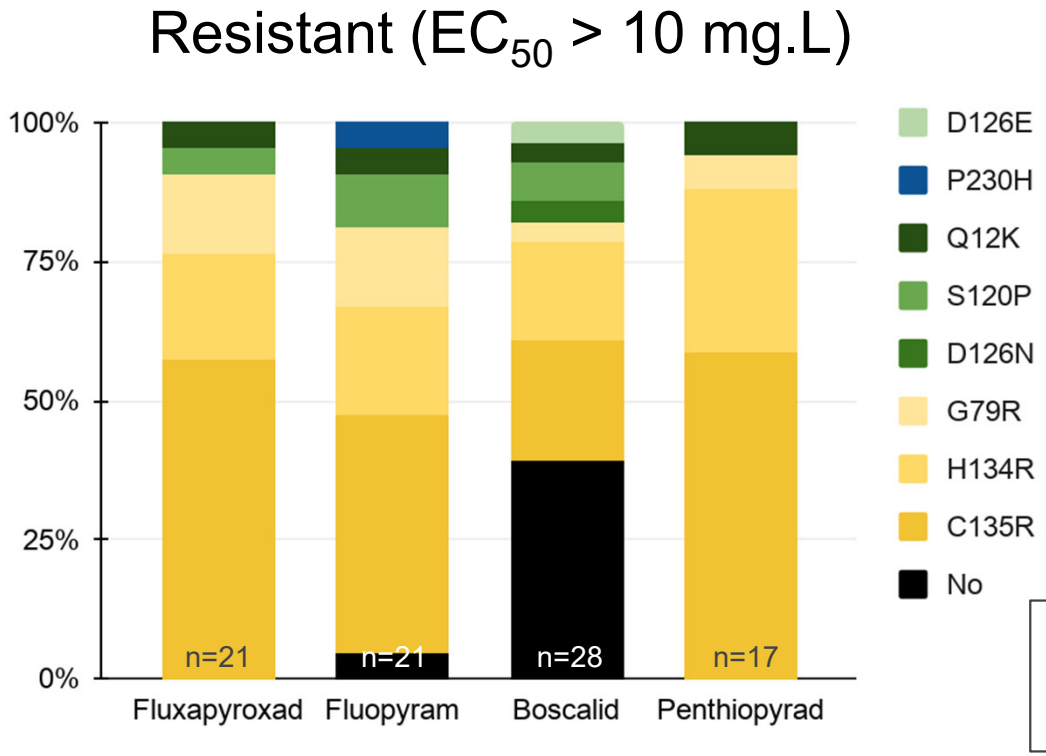
Moderately resistant (EC_{50} 1-10 mg.L)



Which of the *sdh* **gene mutations** explained the observed **FRAC 7** resistance?

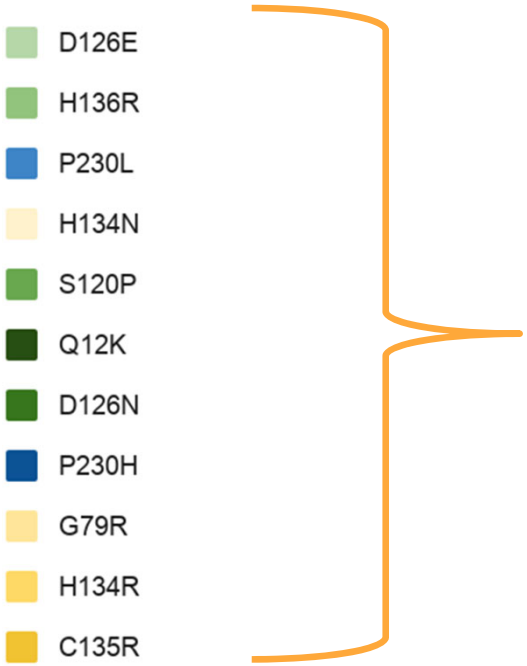
Frequency of *sdh* gene mutations:

Fluxapyroxad (Merivon®):	**100%**
Fluopyram (Luna Tranquility®):	**95%**
Boscalid (Endura®):	**57%**
Penthiopyrad (Fontelis®):	**100%**



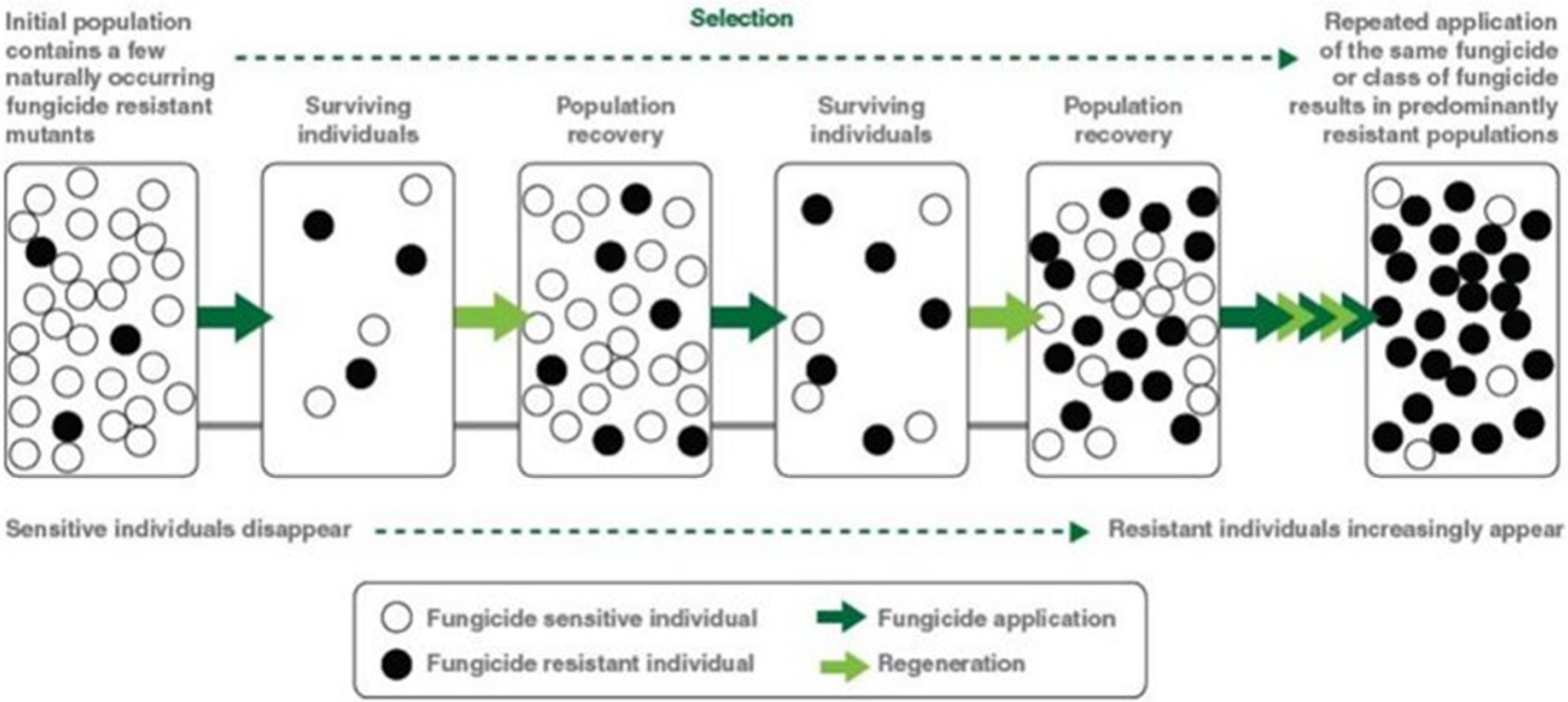
Which of the *sdh* **gene mutations** explained the observed **FRAC 7** resistance?

sdhB;
sdhC;
sdhD



Fungicide	Frequency	X^2 (*P*-value)	Cramer's V
Fluxapyroxad	**36**/80 isolates	49.4 (<0.001)	0.786
Fluopyram	**36**/80 isolates	57.1 (<0.001)	0.845
Boscalid	**22**/56 isolates	13.2 (0.001)	0.486
Penthiopyrad	**24**/38 isolates	23.8 (<0.001)	0.791

QUALITATIVE RESISTANCE BUILD-UP



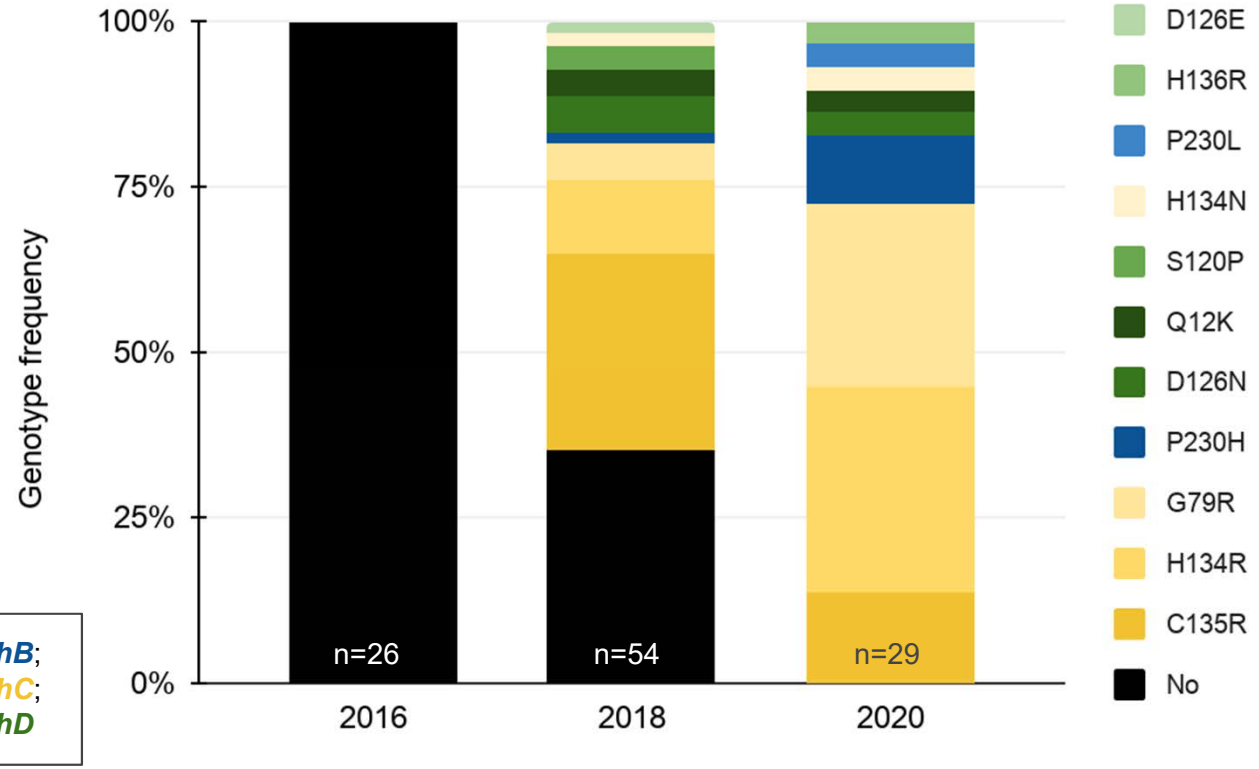
Qualitative resistance: Pathogen population changing from a sensitive pathogen strain to an insensitive pathogen strain. (Modified from Hewitt,1998)

How was the frequency of the *sdh* **gene mutations** since 2016?

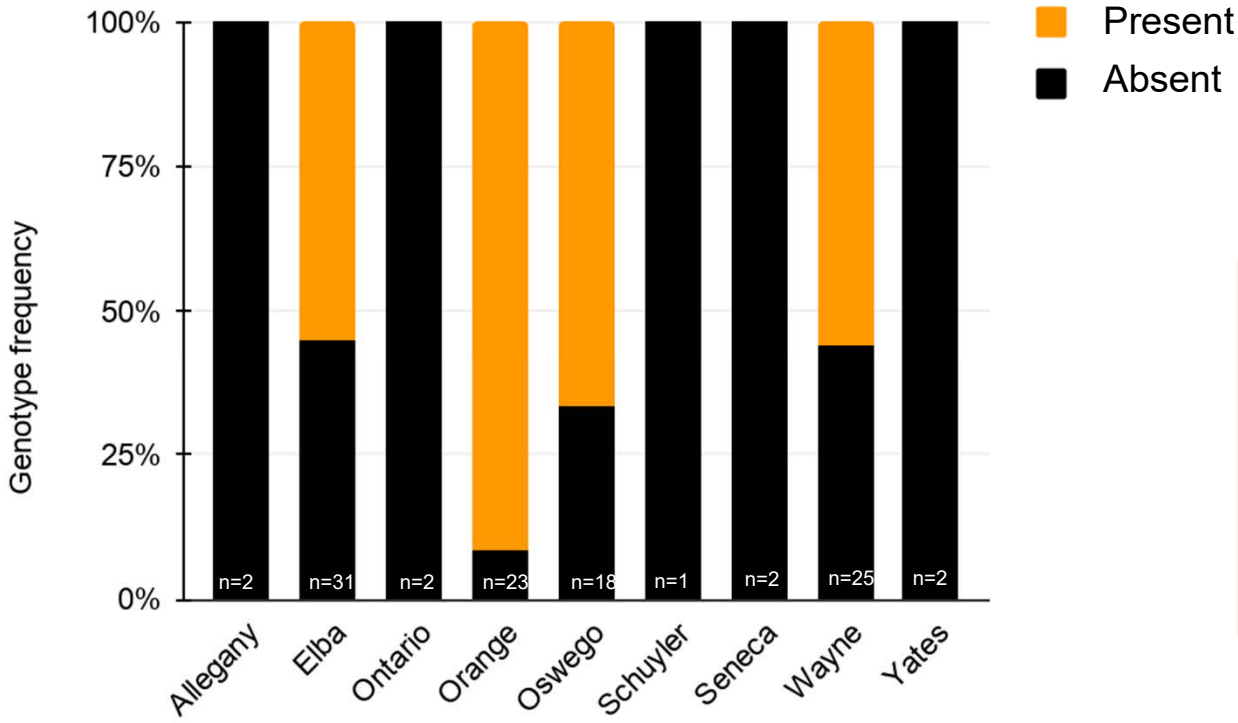
Frequency of mutations in *sdh* genes:

2016: 0%
2018: 65%
2020: 100%

Increase in the frequency of mutations in *sdhC* from 40% in 2018 to 70% in 2020.



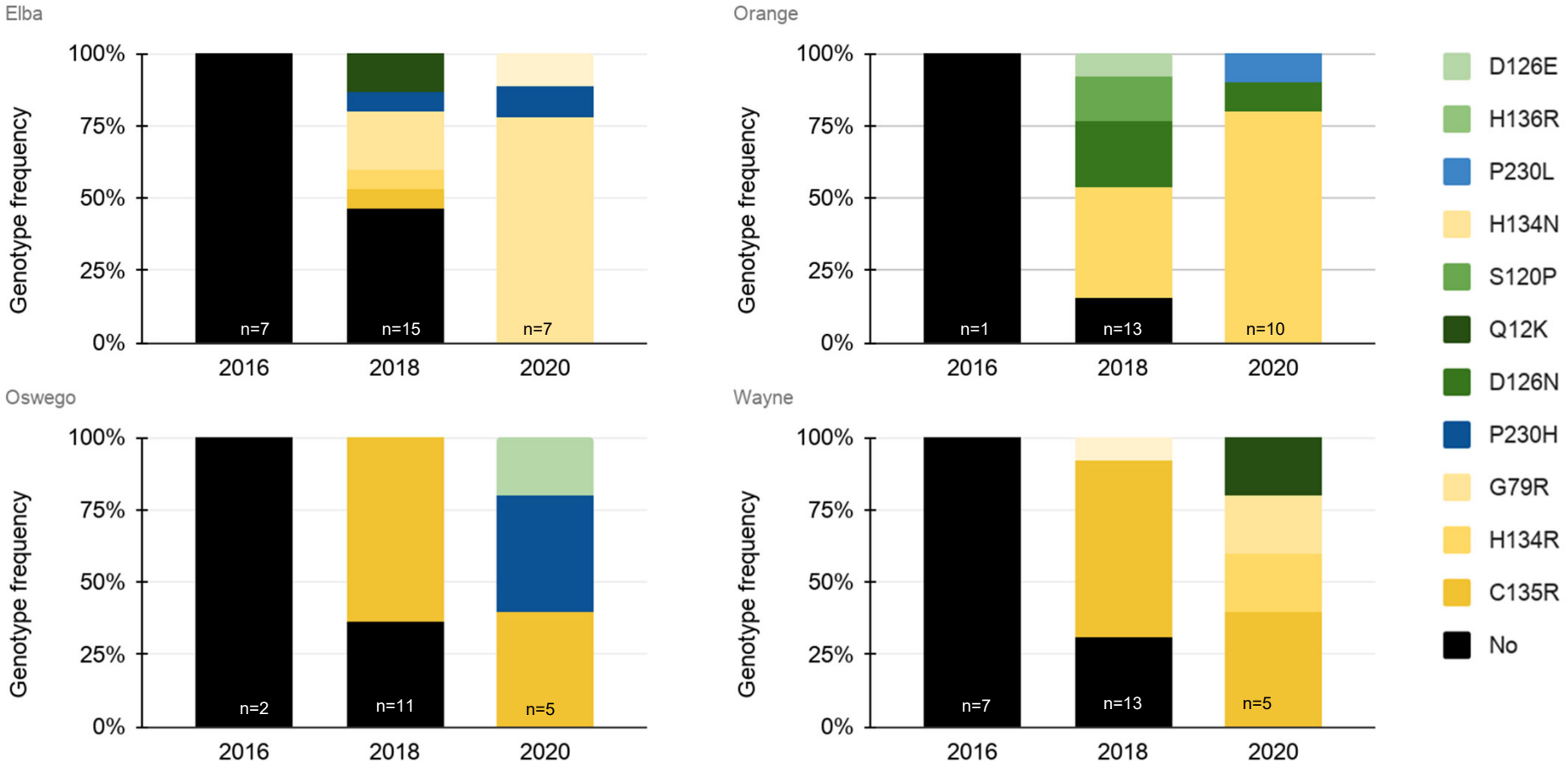
What is the **distribution** of the **mutant genotypes** across **regions**?



Frequency of mutations in *sdh* genes:

Allegany:	0%
Elba:	55%
Ontario:	0%
Orange:	90%
Oswego:	65%
Schuyler:	0%
Seneca:	0%
Wayne:	55%
Yates:	0%

… across **regions?** … and over **time?**



sdhB;
sdhC;
sdhD

Summary

All mutations found in the *sdh* genes were associated with **moderately** and/or **resistant fungicide** phenotypes;

The mutations are associated with **cross-resistance** to **boscalid** (Endura®), **fluopyram** (Luna tranquility®, Luna experience ®), **fluxapyroxad** (Merivon®) and **penthiopyrad** (Fontelis®);

The frequency of FRAC 7 fungicide resistant isolates **increased over time**, and **different patterns of** are being observed between regions;

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USDA

United States Department of Agriculture
National Institute of Food and Agriculture

NY farm viability INSTITUTE

Cornell AgriTech
New York State Agricultural Experiment Station

Fragile FRAC 7 Fungicides: Part I - Current status of Stemphylium leaf blight fungicide resistance in onion in New York

Effect of Fungicide Application Rate on Stemphylium Leaf Blight Resistance Development Over Time

Katrin Ayer[1], *Kerik Cox*[1], *Frank Hay*[1], *Daniel Heck*[1], *and Christy Hoepting*[2]

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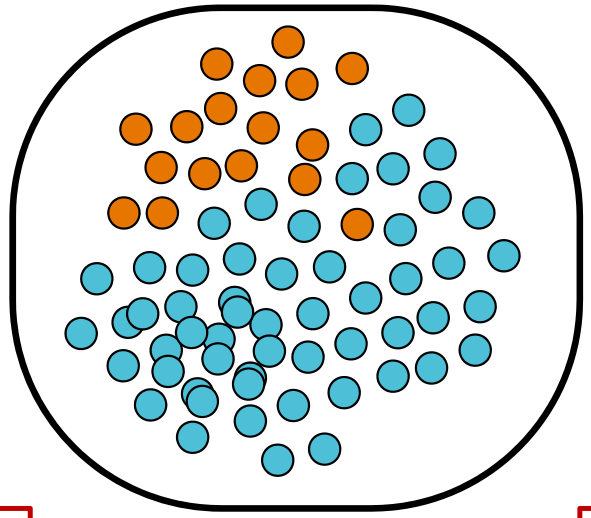
Phases of Resistance Development

1. Emergence*
2. Selection
3. Establishment



Pathogen Population
- Sensitive Isolate
- Resistant Isolate

(adapted from van den Bosch et al 2011)

*Fungicides are not inherently mutagenic, mutations are **pre-existing**

*Advantageous mutations occur **infrequently**

Application of a fungicide does not cause emergence, rather will select for further establishment

How can we delay development of fungicide resistance?

Goal: Identify fungicide application practices that slow down selection for establishment of fungicide resistance over time

Effect of fungicide rate on resistance

General Work Flow

Fungicide Applications → **Isolate Collection** → **Sensitivity Testing**

Christy Hoepting
CCE

Tested on group 7 active ingredient

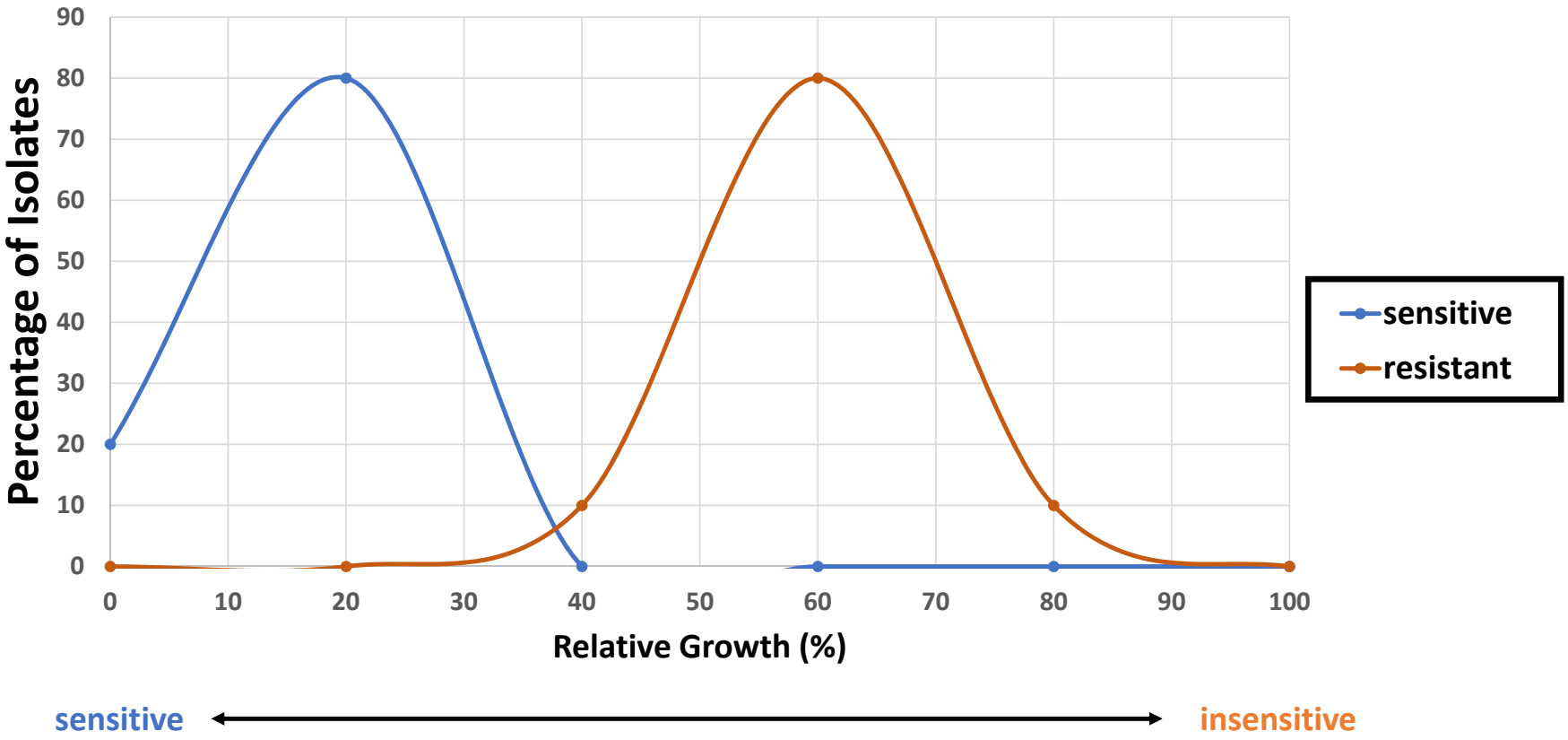
Fungicide Applications

Treatment	FRAC Groups
Untreated	-
Luna Tranquility 12 fl oz	7+9 (Fluopyram & Pyrimethanil)
Luna Tranquility 16 fl oz	7+9 (Fluopyram & Pyrimethanil)
Merivon 5.5 fl oz	7+11 (Fluxapyroxad & Pyraclostrobin)
Merivon 9 fl oz	7+11 (Fluxapyroxad & Pyraclostrobin)

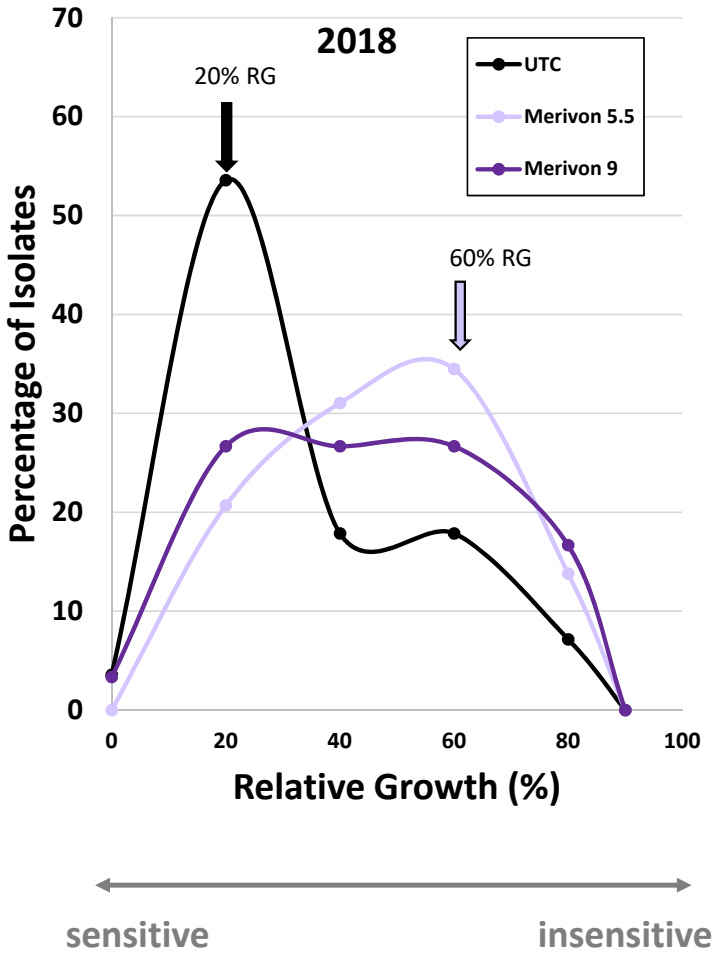
Applied 6-8 times throughout season

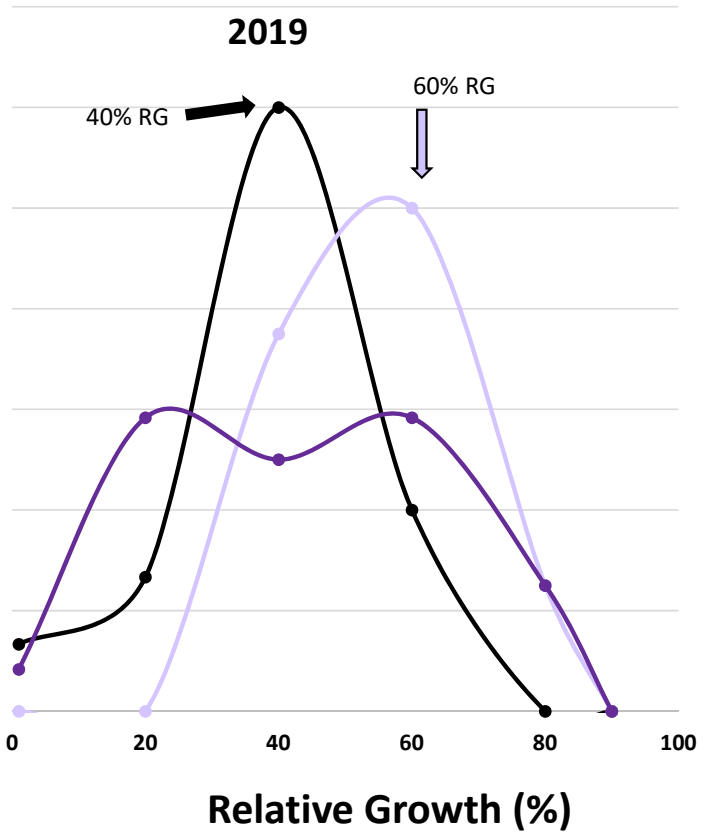
2018, 2019, and 2020 in Elba, NY

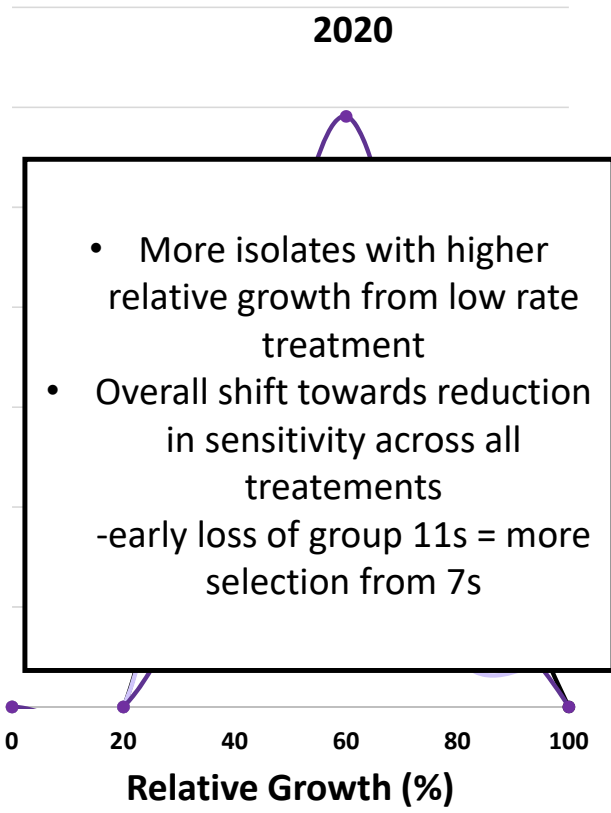
Example Graph



Effect on Fungicide Sensitivity- Merivon (Group 7 & 11)





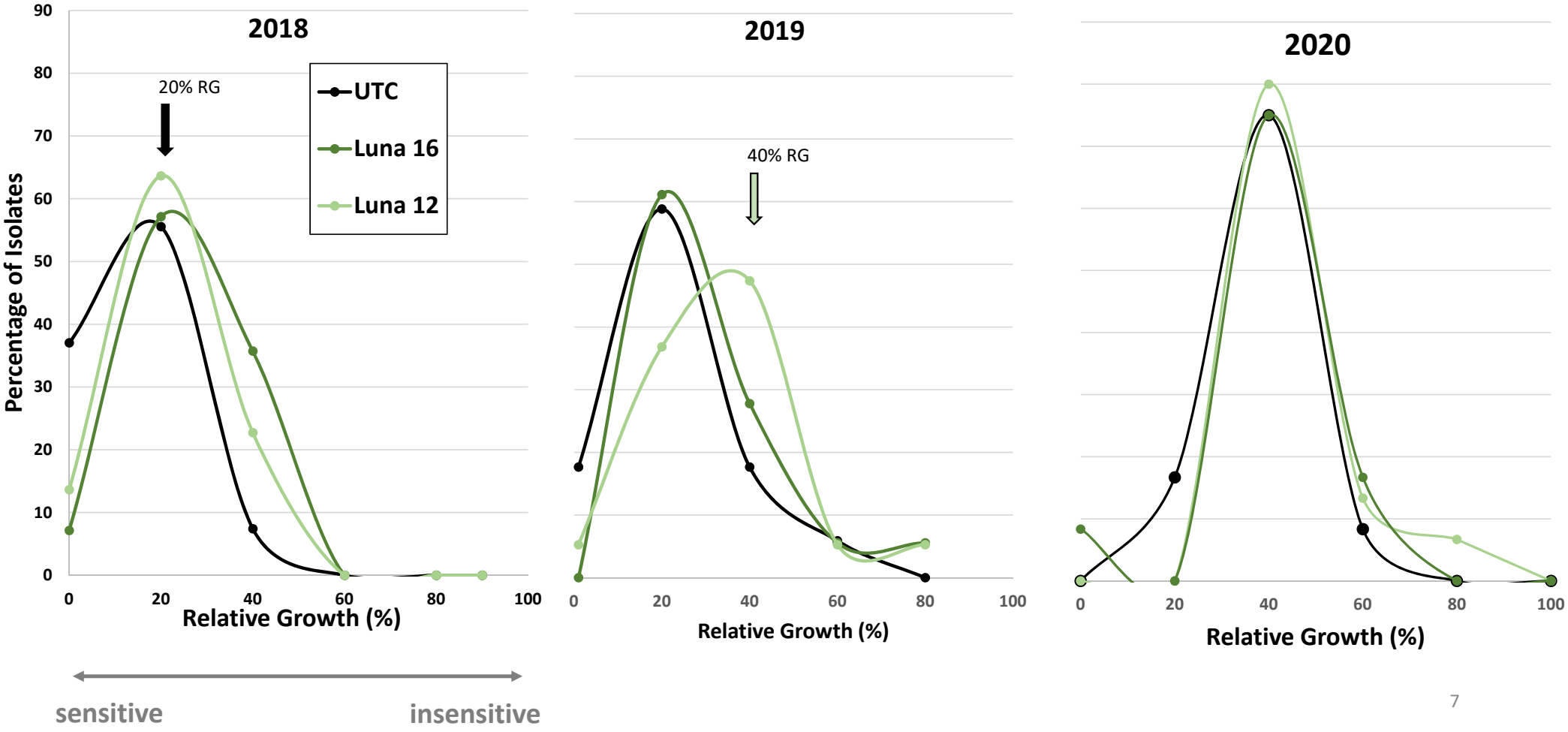


- More isolates with higher relative growth from low rate treatment
- Overall shift towards reduction in sensitivity across all treatements

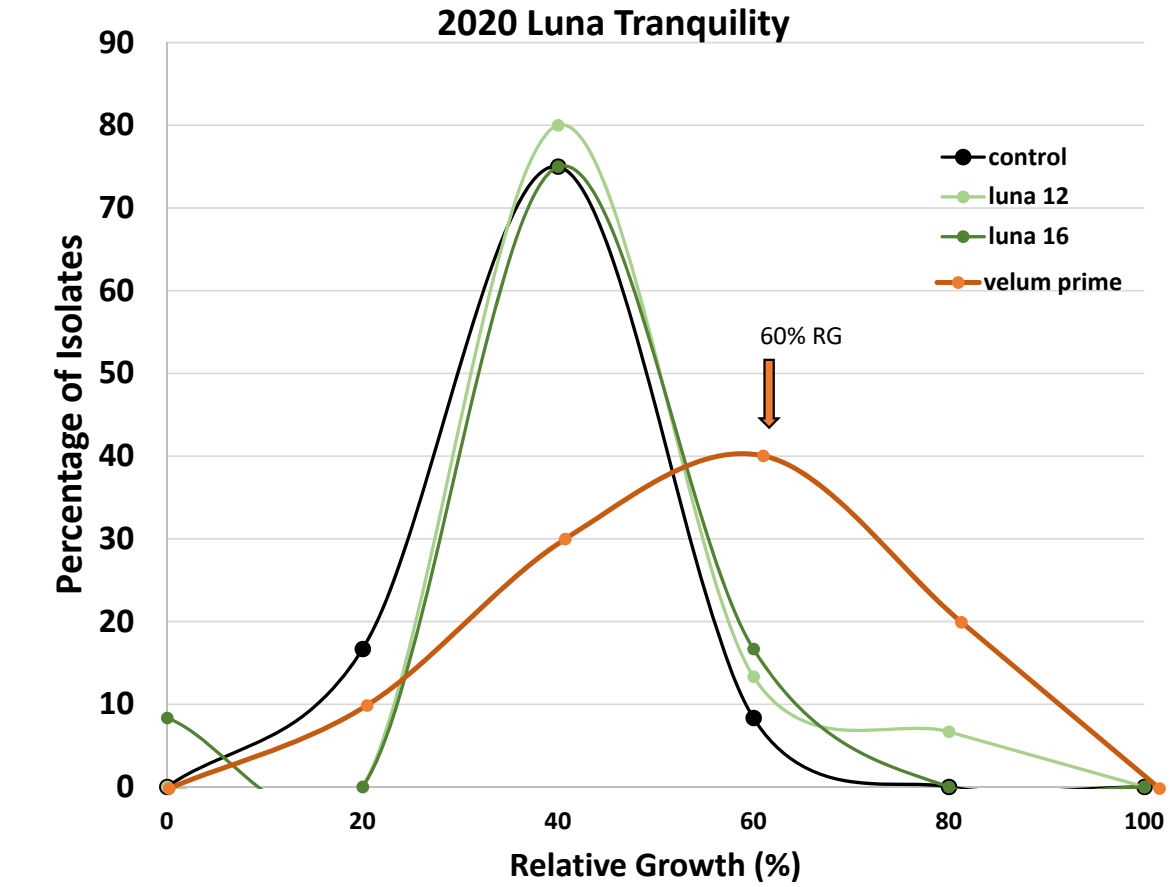
-early loss of group 11s = more selection from 7s

sensitive ⟷ insensitive

Effect on Fungicide Sensitivity- Luna Tranquility (Group 7 & 9)



Effect on Fungicide Sensitivity- Luna Tranquility (Group 7 & 9)



Luna Tranquility= Fluopyram & Pyrimethanil (Group 7 + 9)

Velum Prime = Fluopyram alone (Group 7)

Importance of mixing/rotating ingredients

Conclusions

Important to remember: Results here are due to continuous single product use, rate of sensitivity loss is increased. Sample size is small

But… gives insight in how to best use these products to preserve them

- Importance of use of two effective active ingredients
- Shifts in sensitivity over time, limiting applications in same group
- High rates potentially "held on" to sensitivity longer
- Importance of knowing resistance status in field

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- Daniel Heck

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Questions?

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