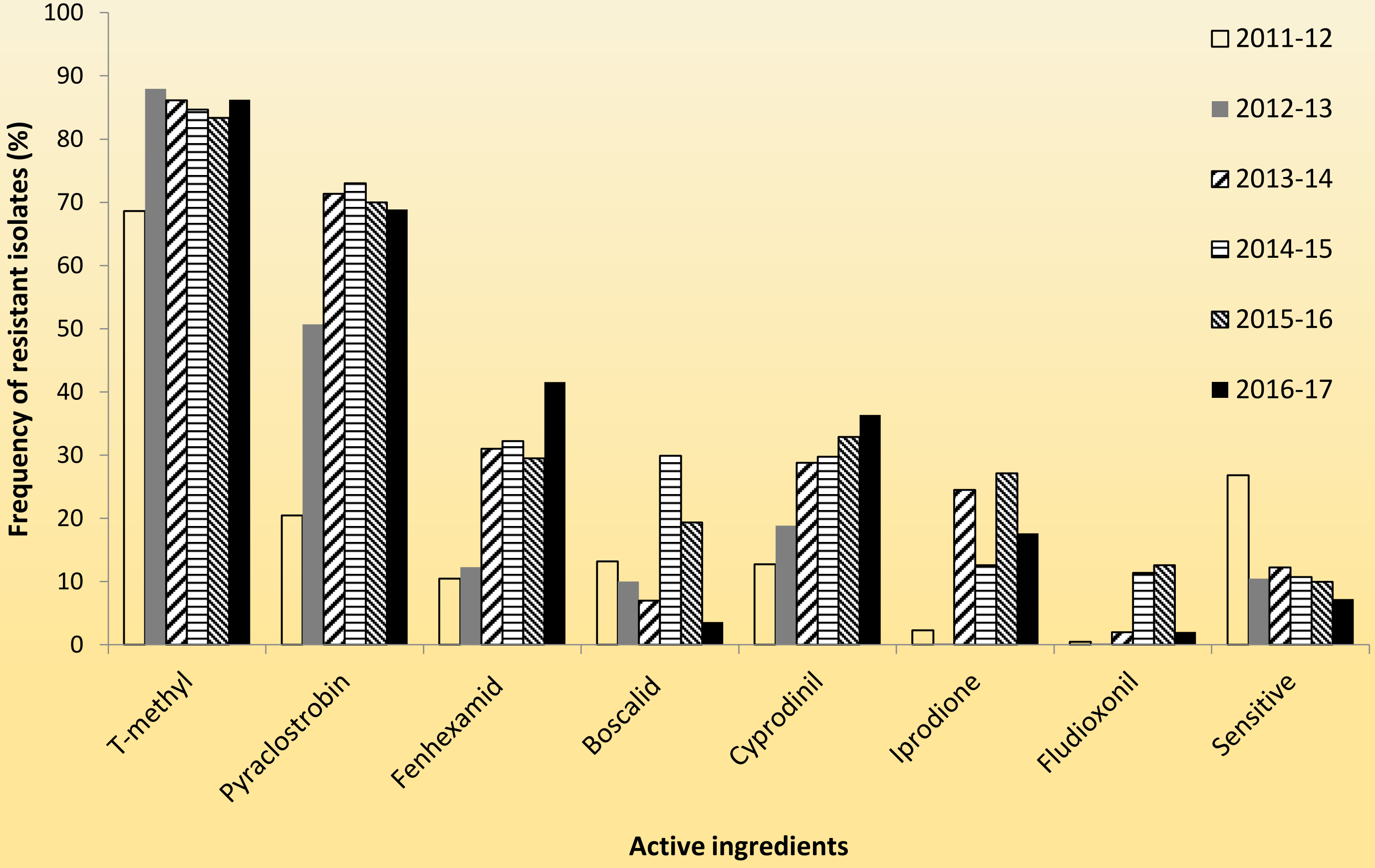
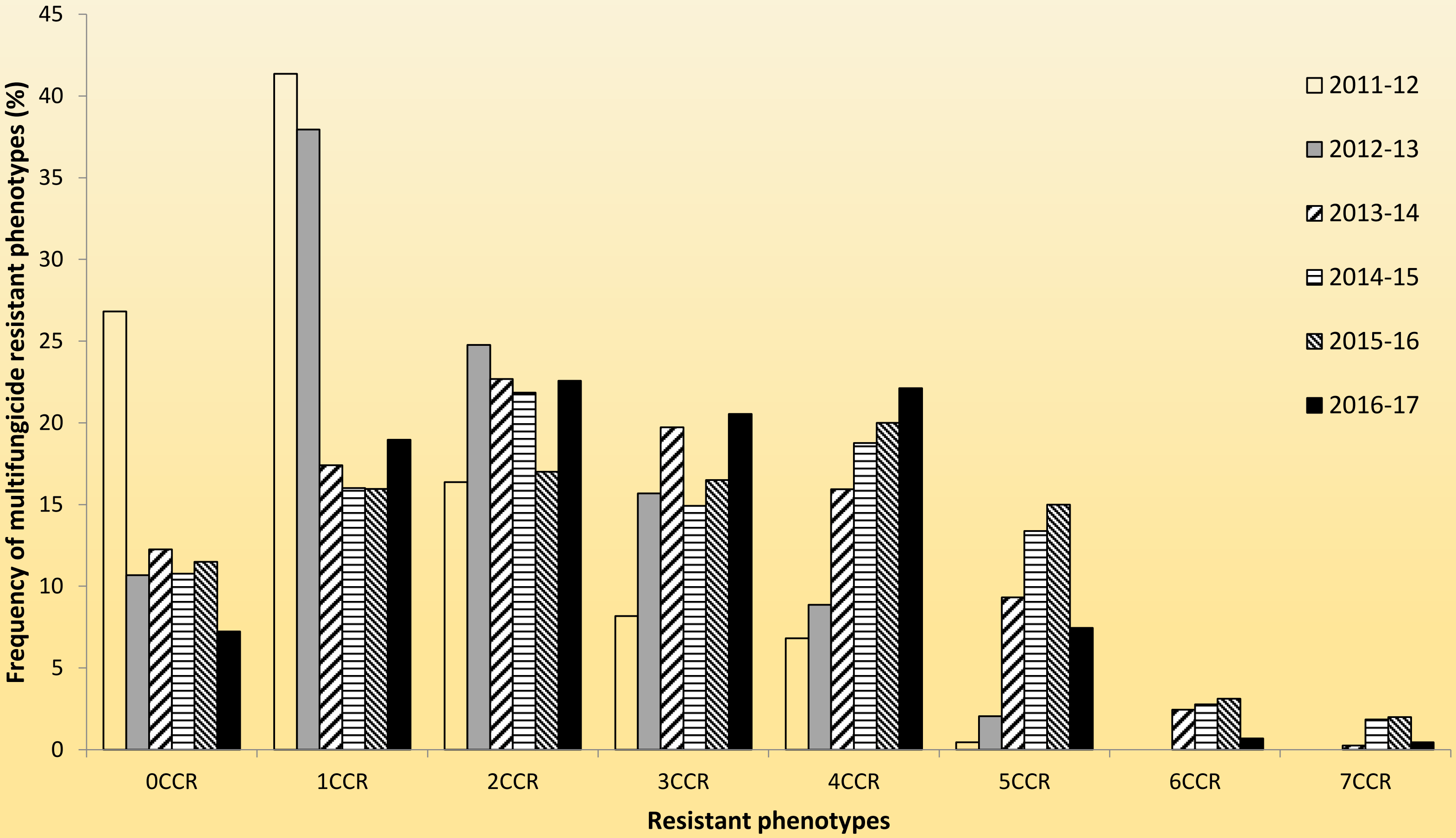
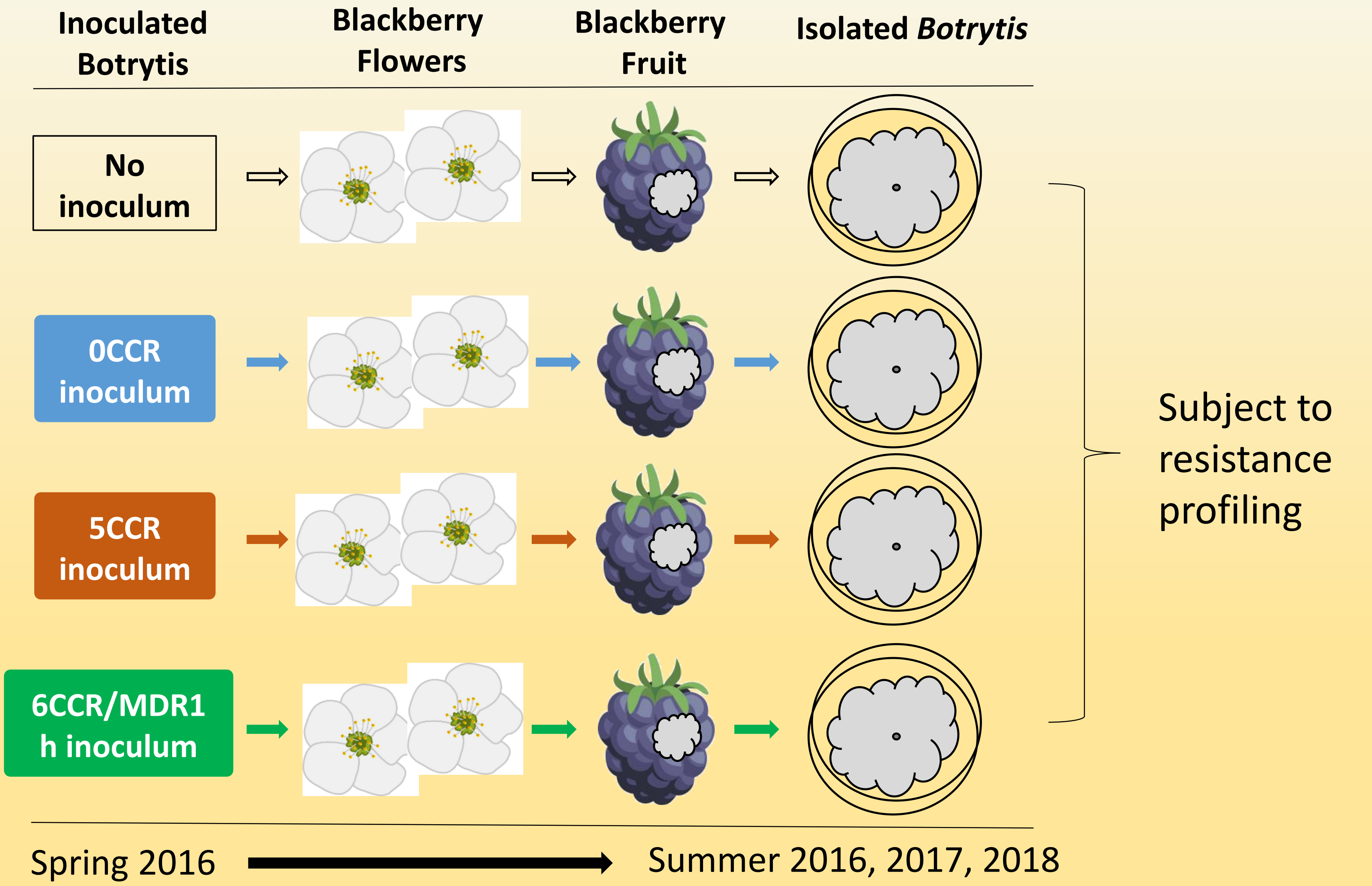
Frequency of Fungicide Resistance in B. cinerea from Strawberry Flowers from East Coast



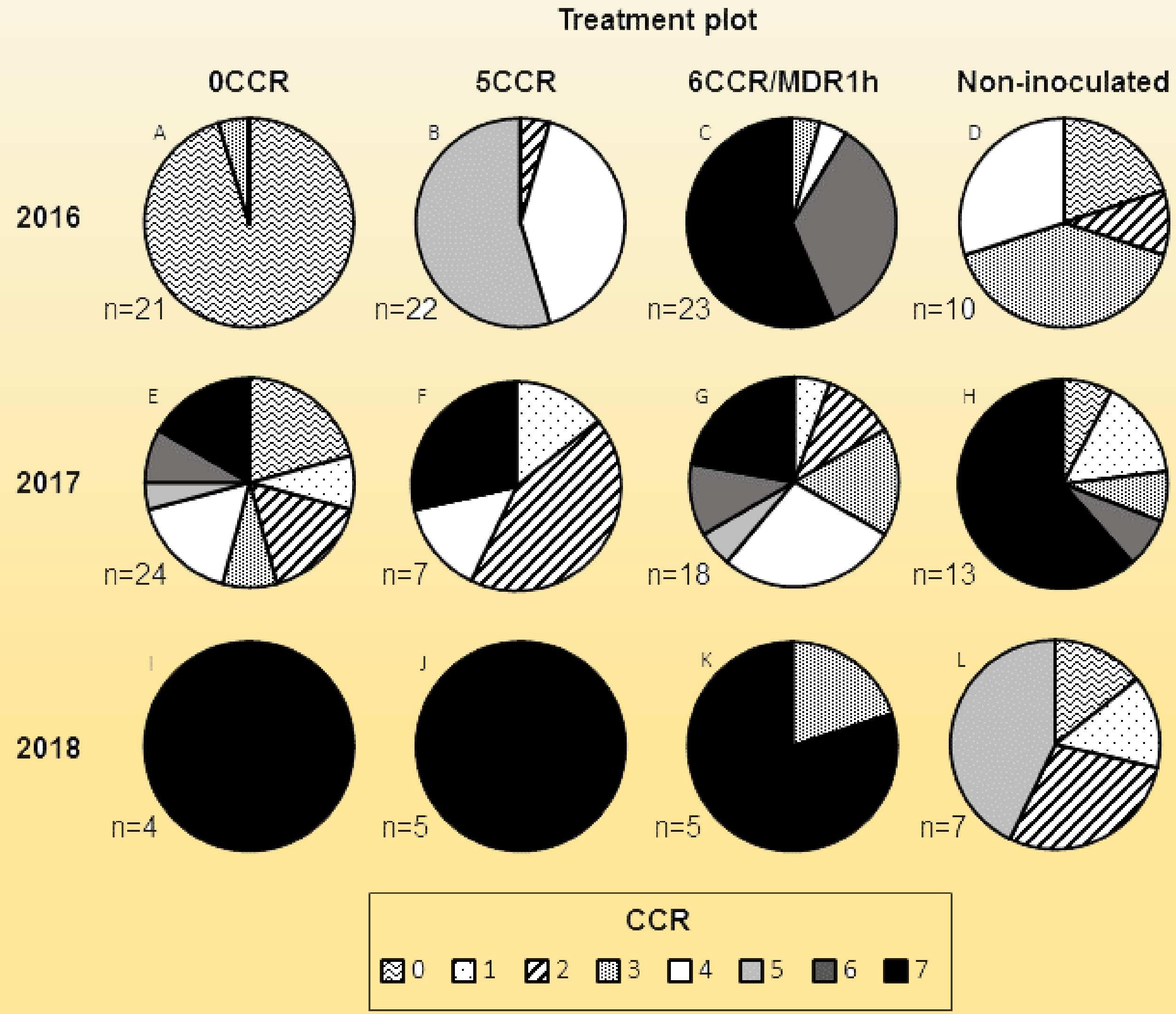
Multifungicide Resistance in the Same Isolates



Competitive ability of multi-fungicide resistant Botrytis isolates under field conditions



Frequency of fungicide resistance phenotypes of Botrytis spp. isolates recovered over three years



Fungicide resistance monitoring adjusts spray programs

- **2012-13**

  Topsin M (T-methyl), **Pristine (boscalid + pyraclostrobin)**, **Elevate (fenhexamid)** and others

  - **2013 - 14**

    Elevate, **Pristine**, **Switch,** and others

    - **2014 - 15**

      Switch, **Merivon,** **Elevate,** and others

      - **2015 -16**

        Captan, Thiram, **switch** and others

        - **2016 - now**

          Captan, Thiram, and tank mixing with others only when necessary (under high disease pressure)

Impact of resistance management tactics on resistance selection

	Increase selection	Decrease selection	No effect
Increase dose	**16**	**2**	**1**
Increase spray numbers	**6**	**0**	**0**
Split dose	**10**	**1**	**0**
Add mixture partner	**1**	**46**	**6**
Alternate	**1**	**9**	2
Adjust timing	**3**	**2**	**1**

Van den Bosch et al. 2014 Governing principles can guide resistance management tactics *Annual Review Phytopathology*

Resistance management strategies

a. The best fungicides are not as good as good weather conditions

- Spray as needed, minizine unnecessary sprays.

b. To maximize efficacy, fungicides are best to be applied prior to infection: Retaining their ability as protectants

c. *Primarily use multi-sites for controlling pathogens with high R risk*

d. *Use mixtures of a low-risk fungicide at high dose and a high-risk fungicide at low dose when disease pressure is high.*

e. *Limit each FRAC code to 2 applications per seasons*

COLLEGE OF AGRICULTURE & NATURAL RESOURCES

UNIVERSITY OF MARYLAND EXTENSION

Strawberry Anthracnose in the mid-Atlantic: Facts & Updates

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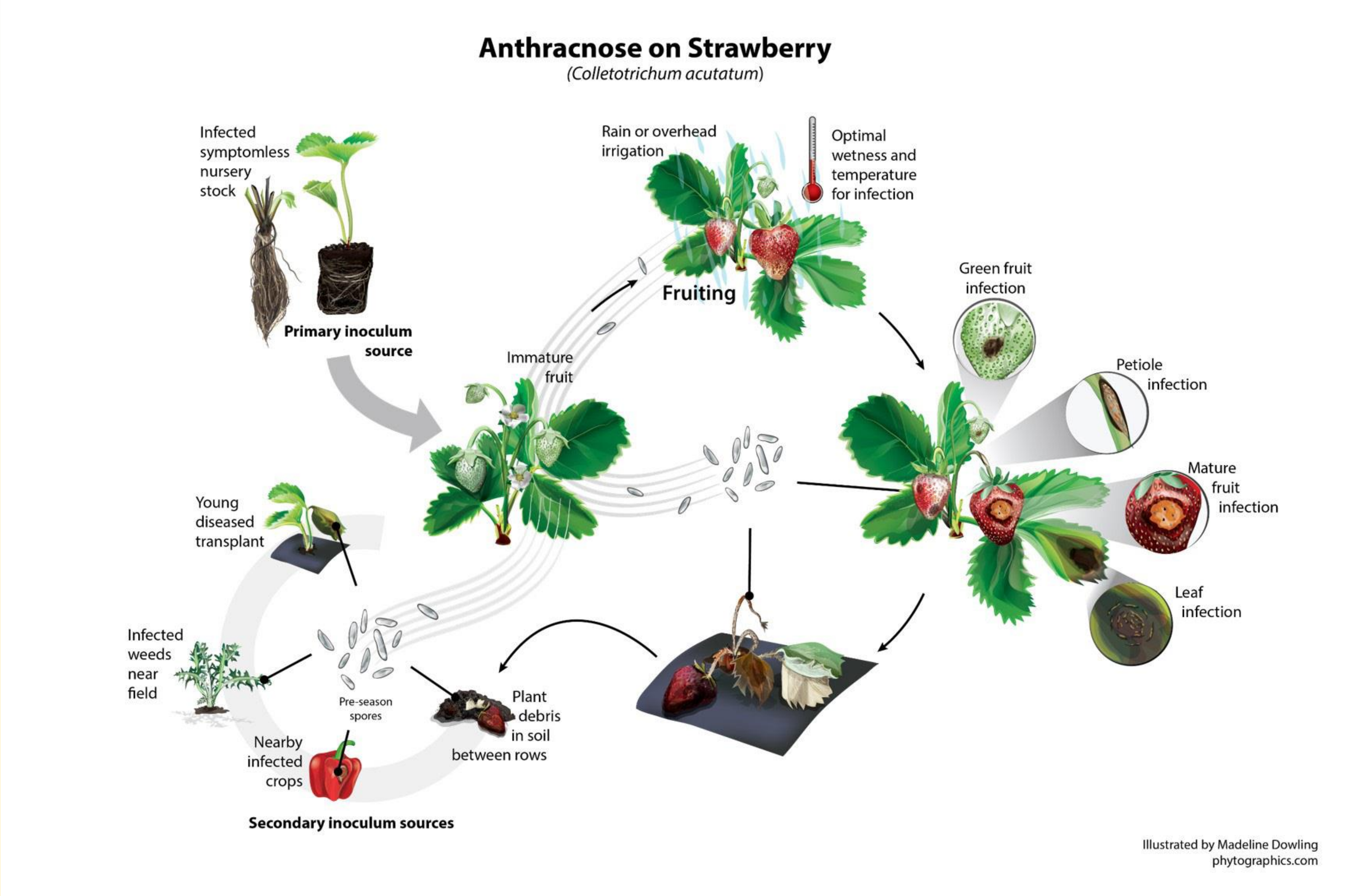
Life Cycle of Strawberry Anthracnose



Where does it come from and how it spreads?

Dispersal

Conidia are typically **rain-splash dispersed**; On low-growing crops such as strawberry, conidia are **spread over short distances** (Peres et al. 2005).

Possible source of infection

- **Nursery transplants**
  - **Facts:** *Colletotrichum* specie have both biotrophic and necrotrophic stages. Symptoms may not develop for some time due to the biotrophic phase that typically occurs early in the infection process (Curry et al. 2002)
  - Soon after planting in fruiting fields, conidiation can occur on the surface of vegetative tissues when weather conditions are favorable, and this can serve to augment inoculum levels to infect flowers and fruit (Leandro et al. 2003)
  - **However,** infected transplants do not always result in the disease.

Photo by Madeline Dowling
http://phytographics.com/

Possible source of infection (continued)

- **Weeds**
  - **Facts:** the fungi seem to live on weeds as an endophyte or remain quiescent, which unlikely produce acervuli or conidia needed for dispersal
  - Even conidia are available from other hosts, they are limited in distances that they can spread as a rain- or water-splashing pathogen
- **Survivals in the soil**
  - **Facts:** not a typical soil-borne pathogen, but can survive in the soil for up to 12 months under dry conditions. Survival of conidia and sclerotia declined rapidly under moist conditions (Norman and Strandberg 1997).
  - At 11% soil moisture content, the time required for 95% loss of viability was 70 to 75 days. In soil at field capacity (22% moisture), a 95% reduction in population recorded within 4 to 10 days (Freeman et al. 2002)

Control of strawberry anthracnose

- **Chemical control**

- A major pillar in the IPM of strawberry anthracnose
- Strobilurin fungicides (QoIs; FRAC 11) are the most effective but **resistance** has been reported in the Southeast (Forcelini 2016, 2018).
- MBCs (FRAC 1) are effective against *C. gloeosporioides* only.
- Other fungicides such as captan and switch are effective to some extent.

- **Cultural-based control methods**

- Sanitation of infected plants/fruit: **may NOT be effective**
- Living mulches (such as wheat, rye, or rye grass) or organic mulches (wheat straw): **likely effective**
- Increasing plant density: **maybe effective**
- Anthracnose was found less severe when water is supplied to plants using drip irrigation rather than overhead irrigation (Madden, 1992; Smith and Spiers, 1986)

Colletotrichum species identified in Mid-Atlantic strawberry fields

Number of Colletotrichum spp. isolates from different states

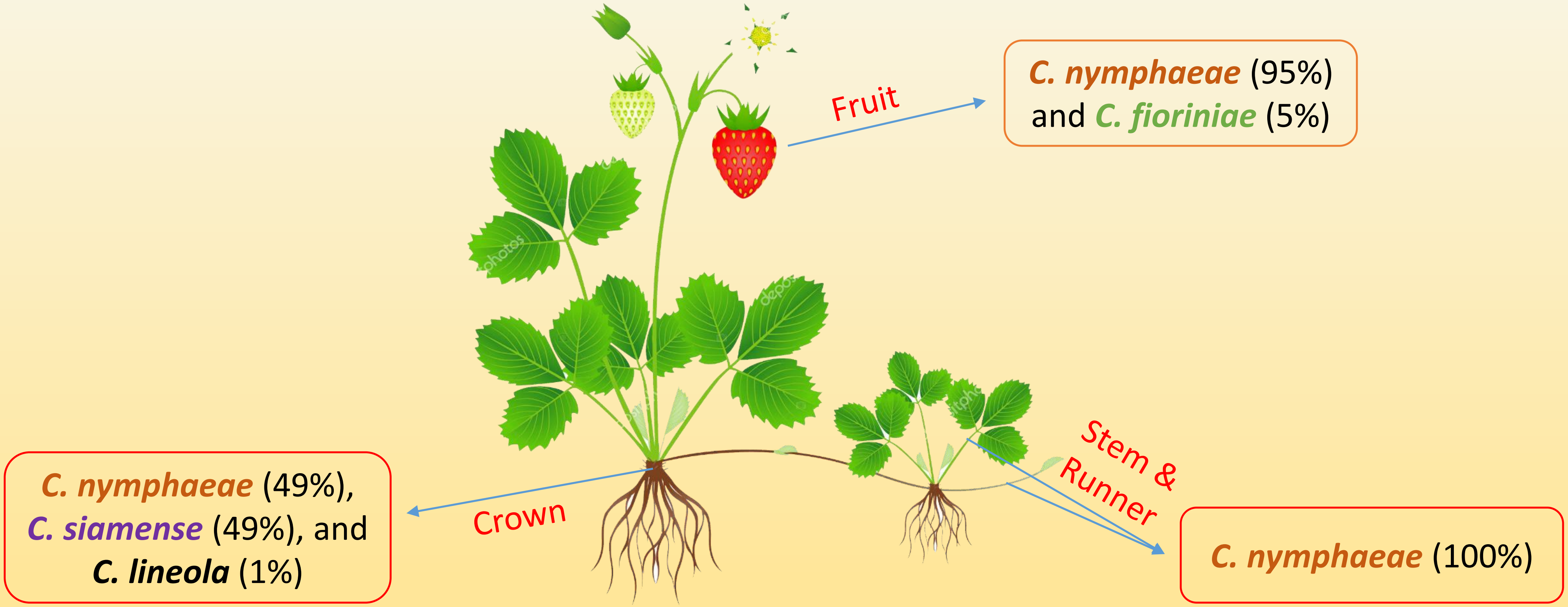
	***C. acutatum* complex**		***C. gloeosporides* complex**		
States	***C. nymphaeae***	***C. fioriniae***	***C. siamense***	***C. lineola***	**Total**
Maryland	121	6	2	0	129
Pennsylvania	36	2	3	1	42
Virginia	10	0	6	0	16
North Carolina	13	0	0	0	13
Total	180	8	11	1	**200**

Note:

Isolates were collected from the fruit, crowns, petioles, and runners

Species were identified through multi-locus sequence: ITS/G3PDH/CAL

Colletotrichum species recovered from different organs of strawberry plant

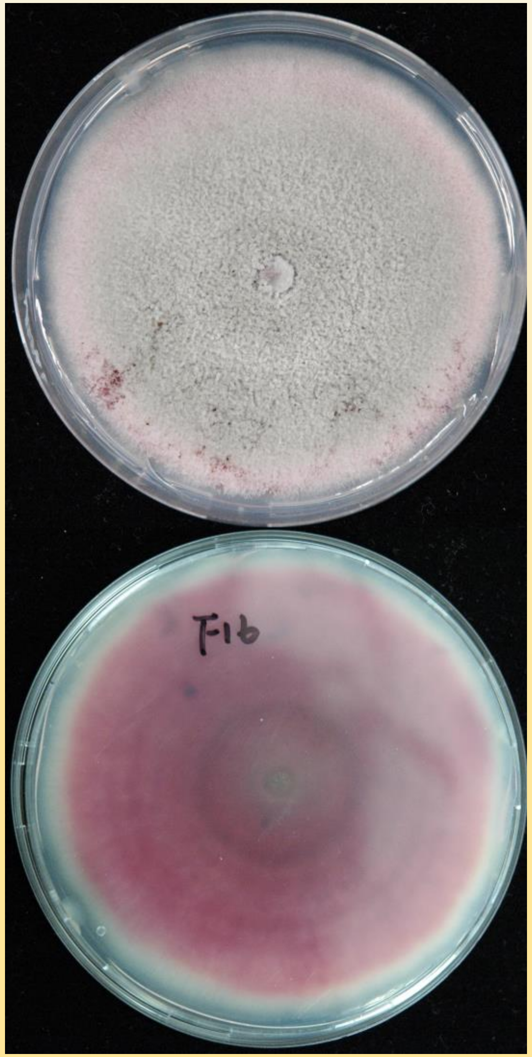


- Out of the 200 isolates, majority (85%) was obtained from strawberry fruit, whereas 12, 1.5, and 1.5% of the isolates were obtained from the crown, runner, and petioles.

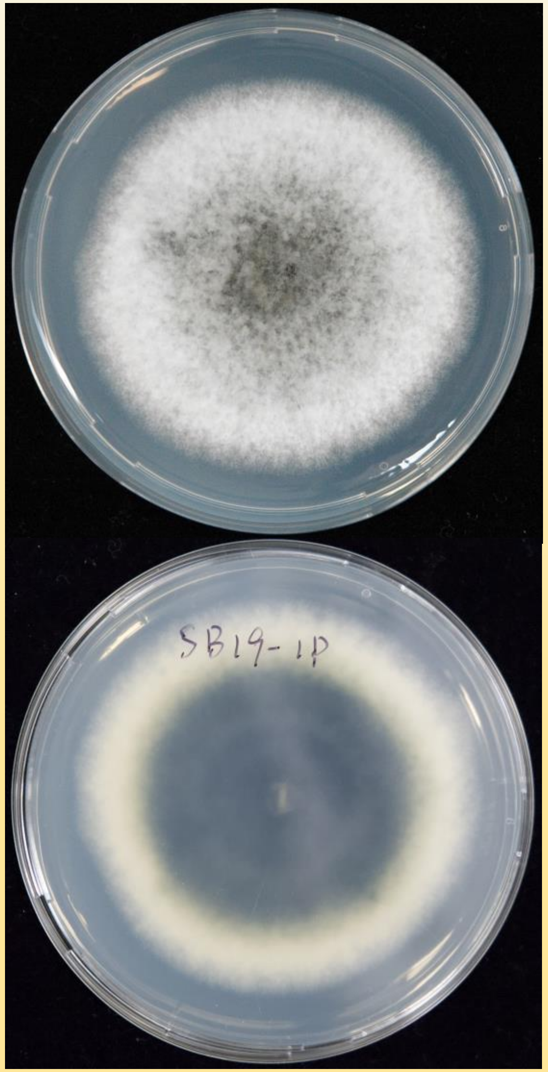
Colony morphology

C. nymphaeae

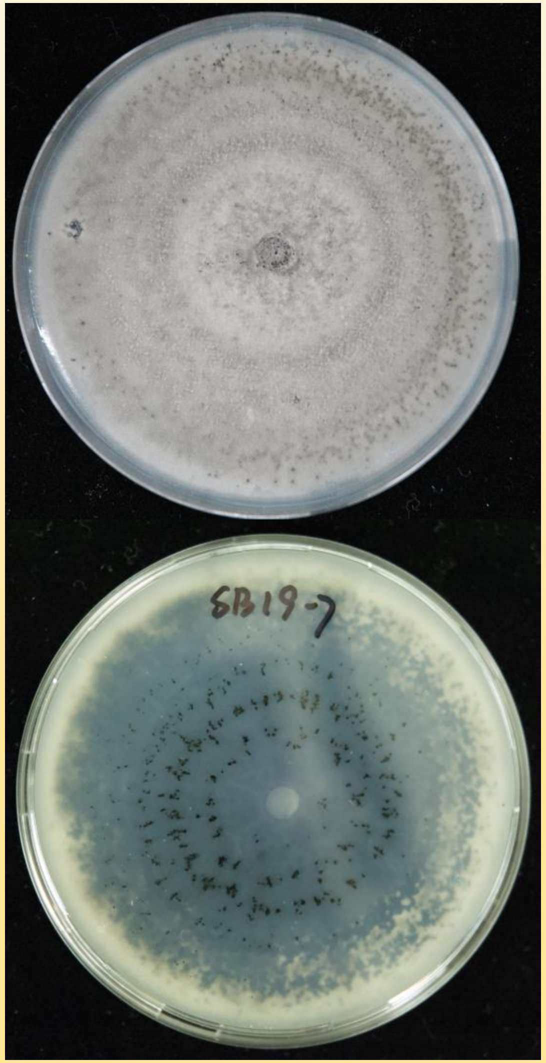
C. fioriniae



C. siamense



C. lineola



Fungicide Resistance Screening

Number of *Colletotrichum* isolates with different resistant phenotypes for azoxystrobin

Phenotype	***C. acutatum* *C. nymphaeae***	***C. fioriniae***	***C. gloeosporioides* *C. siamense***	**Total**
Resistant	71	0	6	76
Moderately Resistant	5	8	0	13
Sensitive	102	0	0	102
Total	178	8	6	192

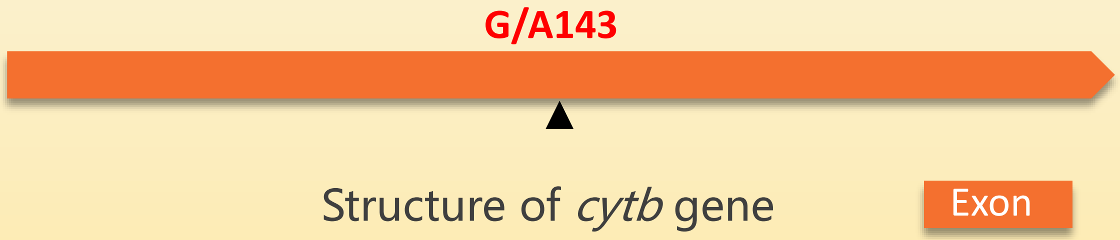
The overall resistance frequency is 39.6% for Azoxystrobin

Number of *C. siamense isolates* with different resistant phenotypes for thiophanate-methyl

Phenotype	***C. gloeosporides* *C. siamense***
Resistant	7
Moderately Resistant	0
Sensitive	4
Total	11

The overall resistance frequency is 63.6% for Thiophanate-methyl

Mutation Analysis– *Cytb* Sequence Analysis



G143A mutation in the *Cytb* was found to be linked to azoxystrobin resistance

Summary

01 **At least 4 *Colletotrichum* species were found associated with strawberry anthracnose**

- The majority of isolates from fruit was *C. nymphaeae*
- Both *C. nymphaeae* and *C. siamense* were frequently isolated from the crown
- *C. fioriniae* seemed to ONLY infect the fruit, with low occurrence (5%)

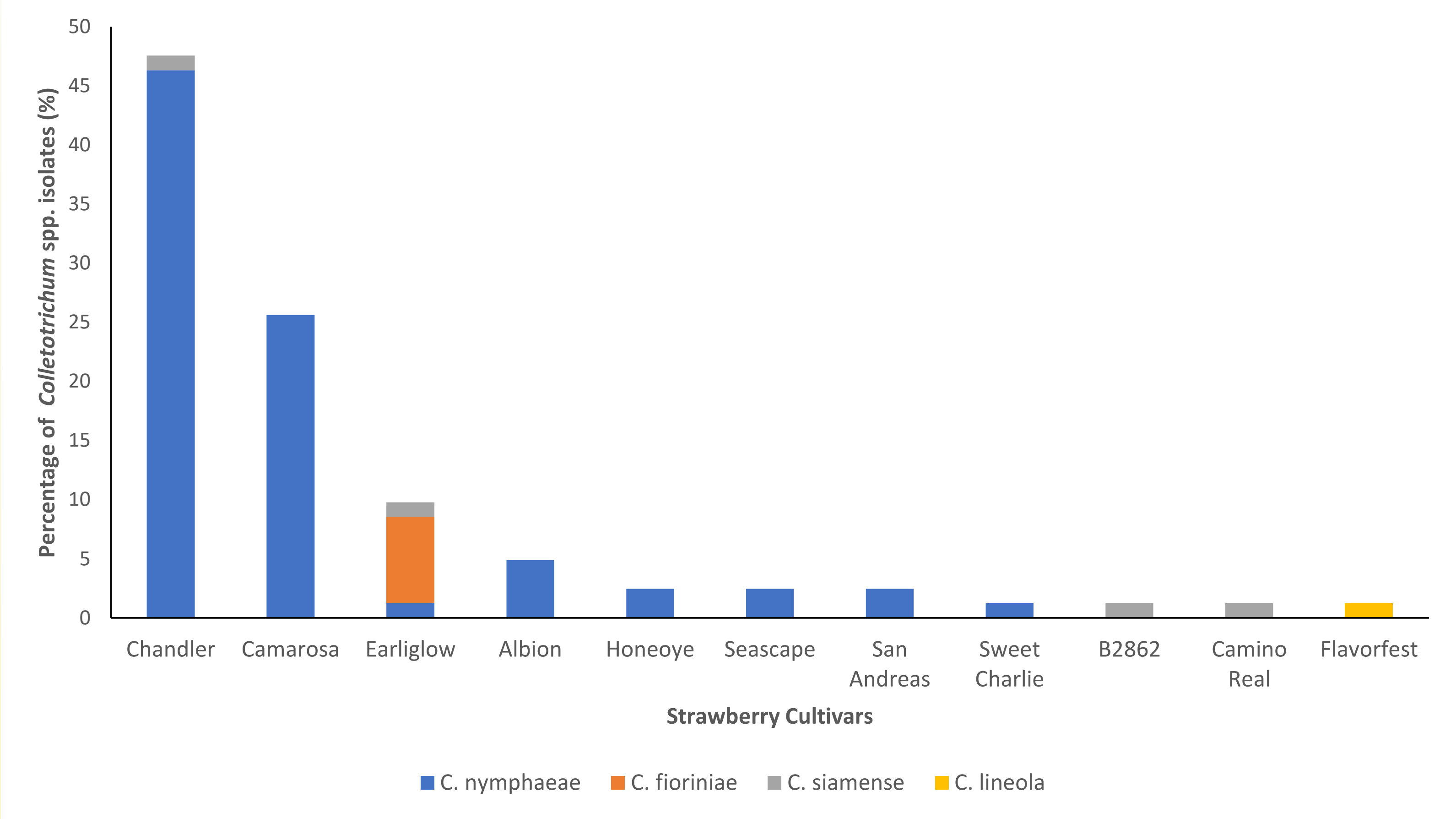
02 ***C. siamense was first found in the Mid-Atlantic region; C. lineola was not described as a cause of strawberry anthracnose previously.***

03 ***Resistance to QoI and MBC fungicides in Colletotrichum spp. is widespread, and resistant isolates were found from different plant organs.***

Are there any anthracnose resistant cultivars?

- Very few cultivars are resistant to anthracnose crown rot (ACR)
- Some cultivars are known to be more susceptible to anthracnose fruit rot (AFR; e.g. Chandler, Albion, and Camarosa)
- Cultivars grown in the Mid-Atlantic have not been evaluated in depth for their AFR and ACR susceptibility

Colletotrichum spp. isolates collected from different strawberry cultivars in the Mid-Atlantic



Any new or existing fungicides that may offer some efficacy?

***Colletotrichum* spp. affecting strawberries**

- ***Colletotrichum acutatum*** [a, b]

  -*C. nymphaeae*

  Resistant to FRAC 11

  -*C. fioriniae*

  Resistant to FRAC 11

  [a]Inherently resistant to FRAC 1

- ***Colletotrichum gloeosporioides*** [b]

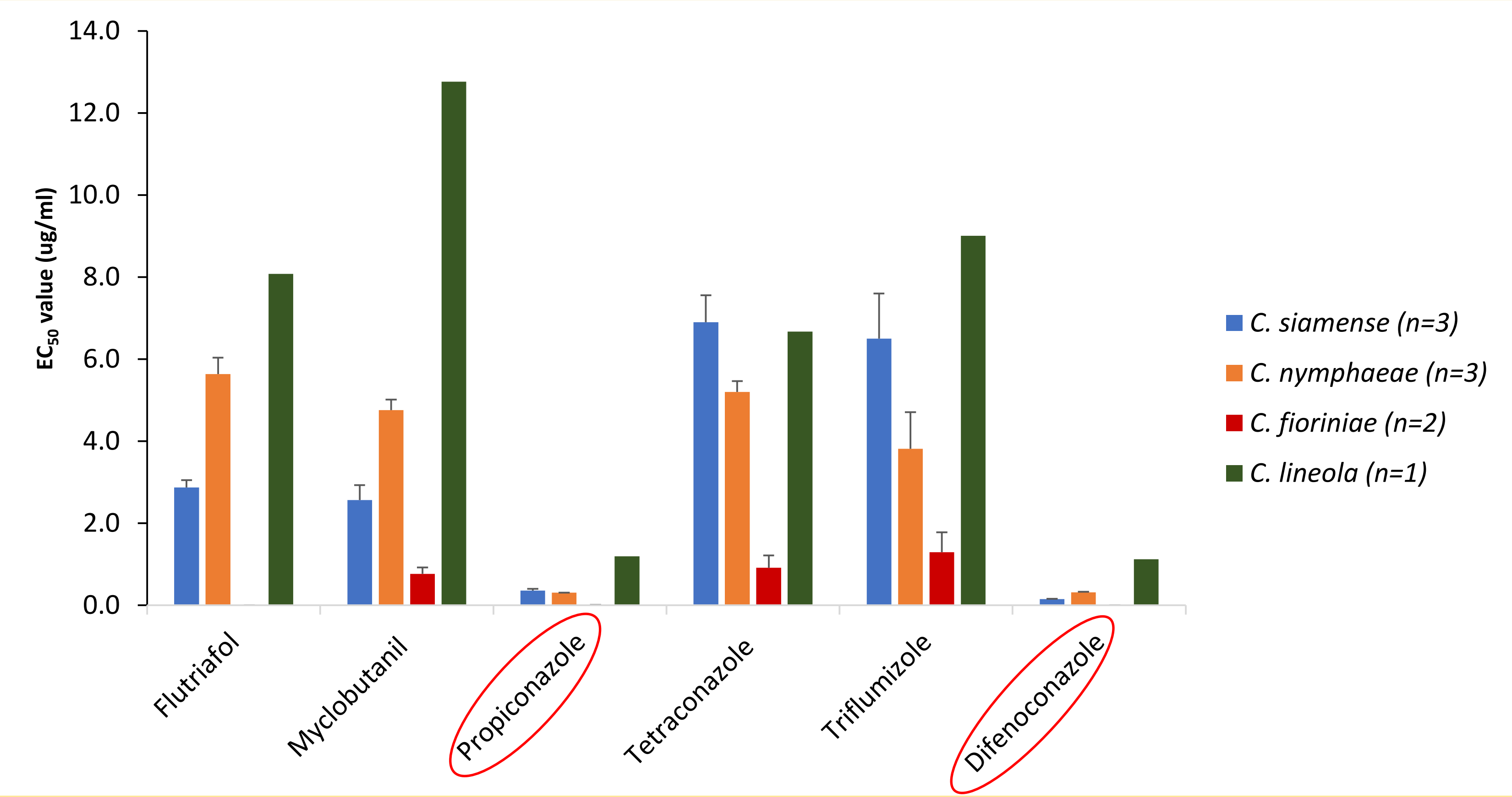
  -*C. siamense*

  Resistant to FRAC 1 & FRAC 11

Major chemical classes of fungicides labelled on strawberry

- ~~FRAC 1: (Thiophanate-methyl; Topsin M)~~
- ~~FRAC 2: (Iprodione; Rovral)~~
- FRAC 3: (a variety of a.i. available; Rally, Tilt, Mettle etc.)
- FRAC 7: (multiple a.i. available; Pristine, Fontelis etc.)
- ~~FRAC 11: (multiple a.i. available; Pristine, Abound etc.)~~
- FRAC 12: (fludioxonil; Switch)

Sensitivity of Colletotrichum Species to FRAC 3 Fungicides



Anita and Hu (unpublished data)

Any new or existing fungicides that may offer some efficacy?

Sensitivity of *Colletotrichum* isolates to FRAC 7 (SDHI) fungicides (mycelial growth)

Species and isolates	EC_{50} (μg mL^{-1}) of fungicide:				
	Bos-	**Fluxapy-**	**Penthio-**	**Fluopy-**	**Benzovin-**
C. gloeosporioides					
Niitaka 3	>100	>100	2.6	>100	0.2
5-2-1	>100	>100	1.9	>100	<0.1
5-2-2	>100	>100	1.8	>100	<0.1
Nagasaki 1	>100	>100	0.8	>100	<0.1
Nagasaki 2	>100	>100	0.7	>100	<0.1
19002	>100	>100	1.6	>100	<0.1
Cg_RR12-1	>100	>100	1.2	>100	<0.1
Cg_SE12-2	>100	>100	1.1	>100	<0.1
Cg_EY12-2	>100	>100	2.6	>100	<0.1
Cg_RR12-4	>100	>100	1.1	>100	<0.1
Ca_EY12-1	>100	>100	2.0	>100	<0.1
C. acutatum					
GC2-1	>100	>100	0.3	>100	<0.1
AAU811-3	>100	>100	0.5	>100	<0.1
CO4-35	>100	>100	1.2	>100	<0.1

- Bos: Pristine
- Fluxapy: Merivon
- Penthio: Fontelis
- Fluopy: Luna series
- Benzovin: Aprovia (not labeled on strawberry)

Ishii et al., 2016 (Pest Manag. Sci.)

Take-Home Message

- Resistance to QoI (FRAC 11) or MBC (FRAC 1) is common, use of these two fungicide classes may no longer be effective
- Captan should be included in every sprays during fruit ripening
- Certain DMIs (i.e. Tilt and Quadris Top) and Fontelis may be useful, but their efficacy need to be validated under field conditions.
- Avoid growing highly susceptible cultivars in open-field condition.
- Any practices that keep water/rain off the plant WILL be of great benefit
- Do not keep strawberries in the “permanent” crop areas, especially when soils are on dry side.

A Glimpse into Weather Variables and Disease Risk at Strawberry Canopy

Disease prediction for AFR and BFR

- Infection risk can be predicted using disease models based on leaf wetness duration and temperature (Bulger et al. 1987, Wilson et al. 1990)
- Strawberry Advisory System (StAS) developed in Florida (Pavan et al. 2011, MacKenzie and N. A. Peres 2012)
  - On average 40% reduced fungicide use compared to (weekly) calendar sprays
  - No significant differences in marketable yield

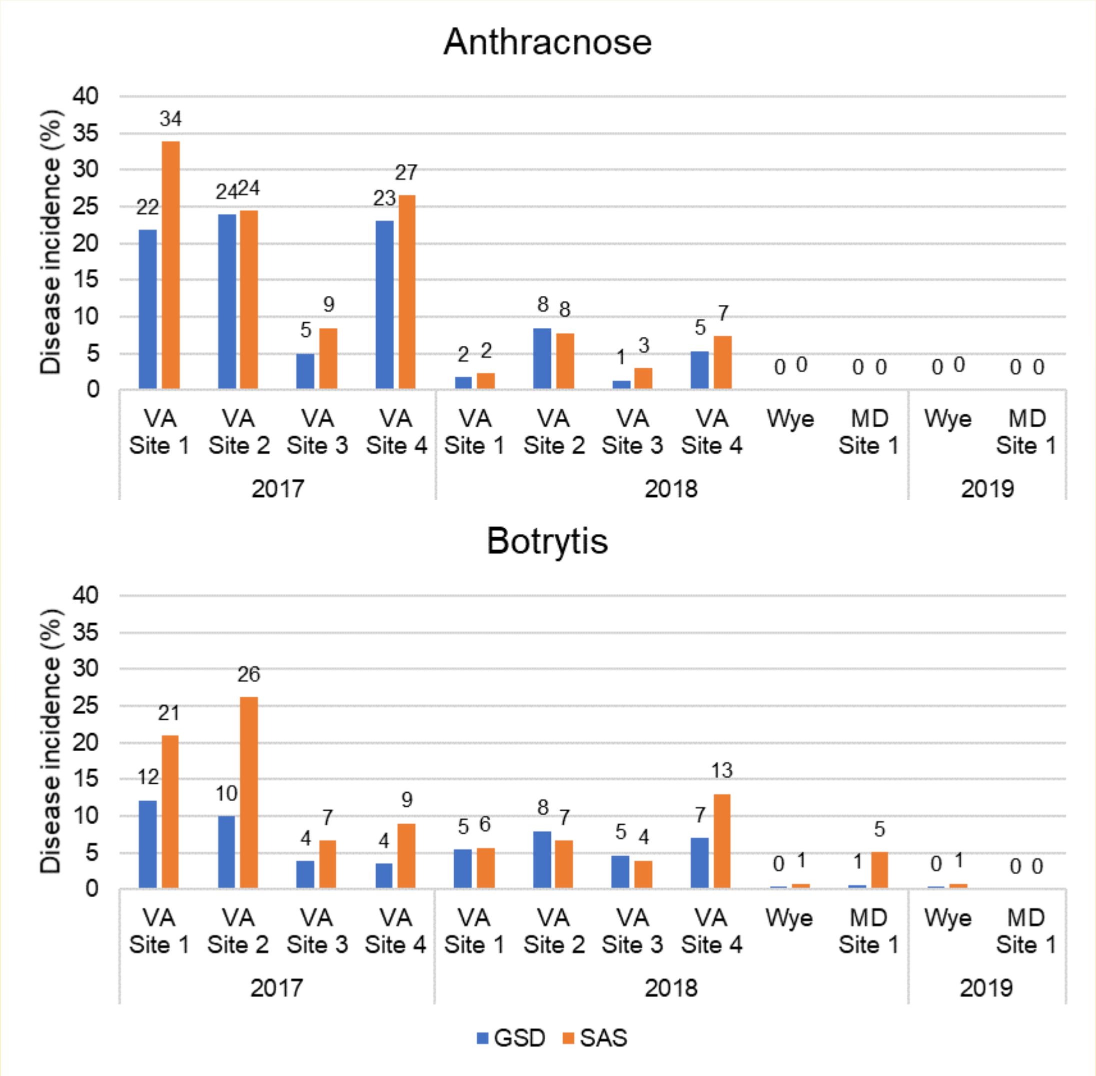
Different growing practices and soils in the Mid-Atlantic

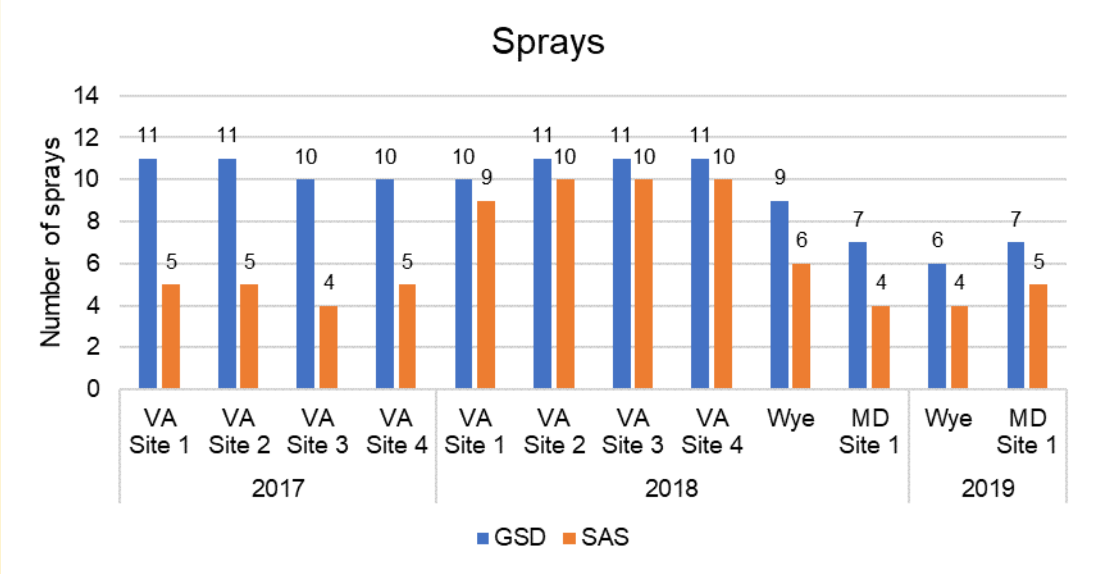
Matted-row (perennial)

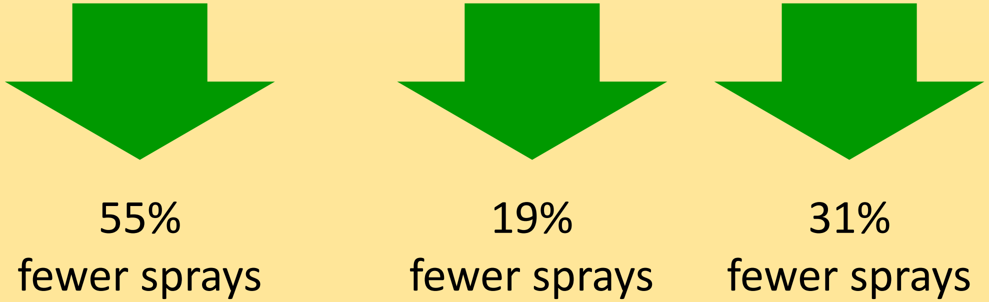
Plasticulture (annual)

Fall and winter row covers

StAS trials in the Mid-Atlantic







Goal and assumptions

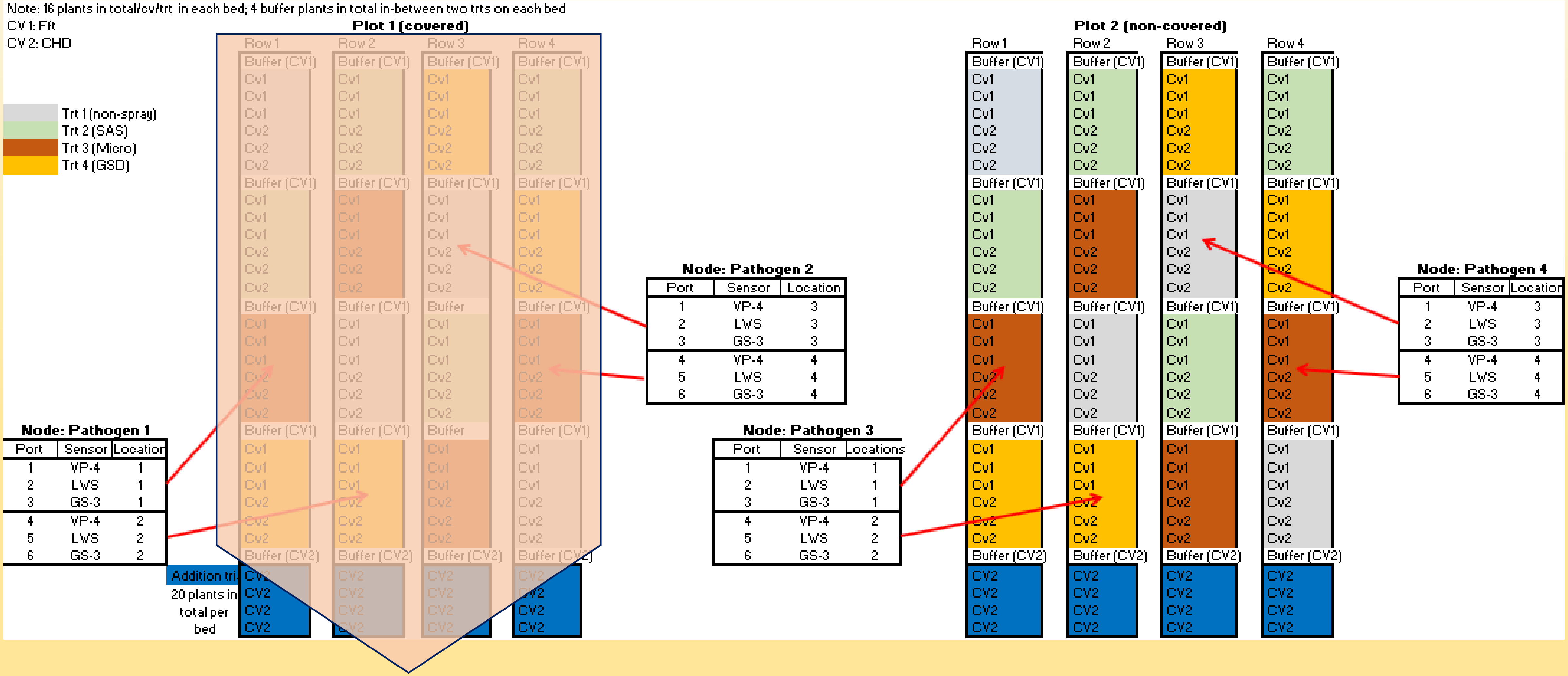
Can we decrease the number of sprays by improving precision of the StAS prediction while increasing / maintaining yield and disease control?

- On-farm StAS weather stations do not account for microclimatic conditions in the canopy and under row covers:
  - Higher air temperature and relative humidity?
  - Increased leaf wetness durations?
  - Favorable conditions for AFR and BFR infections?

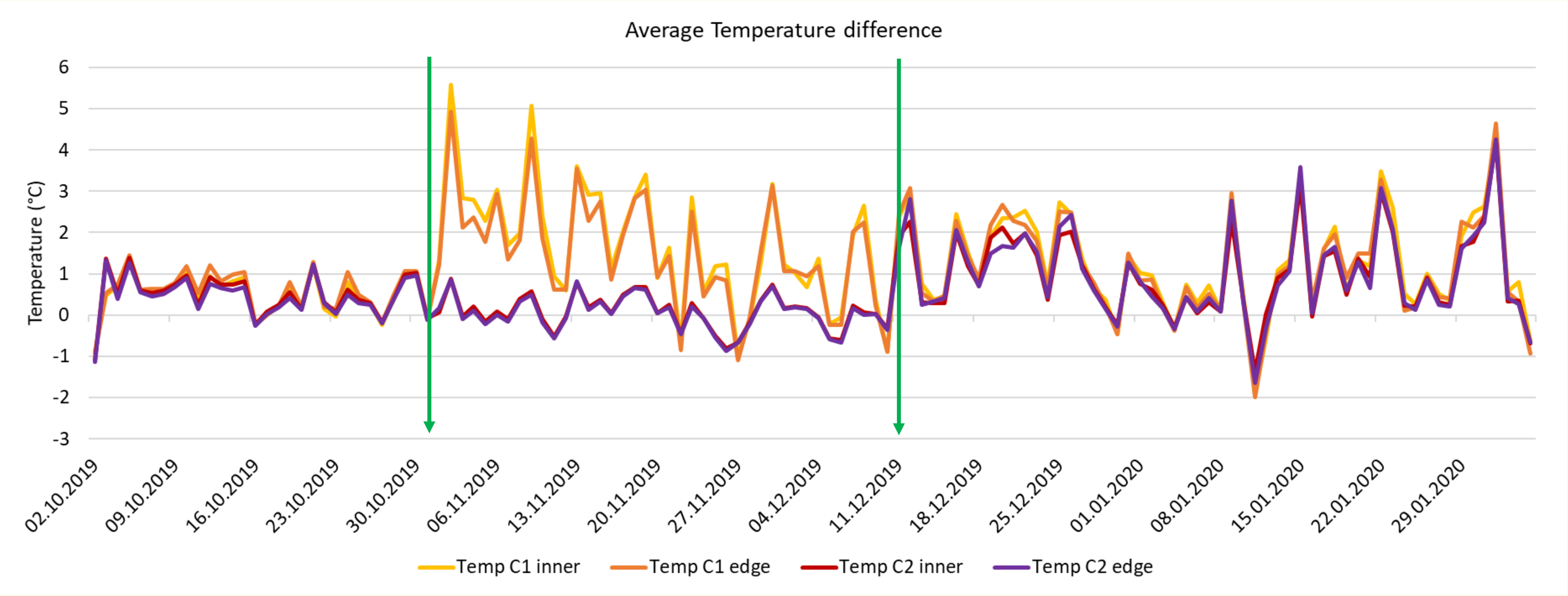
➢ **Hypothesis:** Monitoring canopy-level environmental variables will significantly improve disease prediction precision

Photo: John D. Lea-Cox

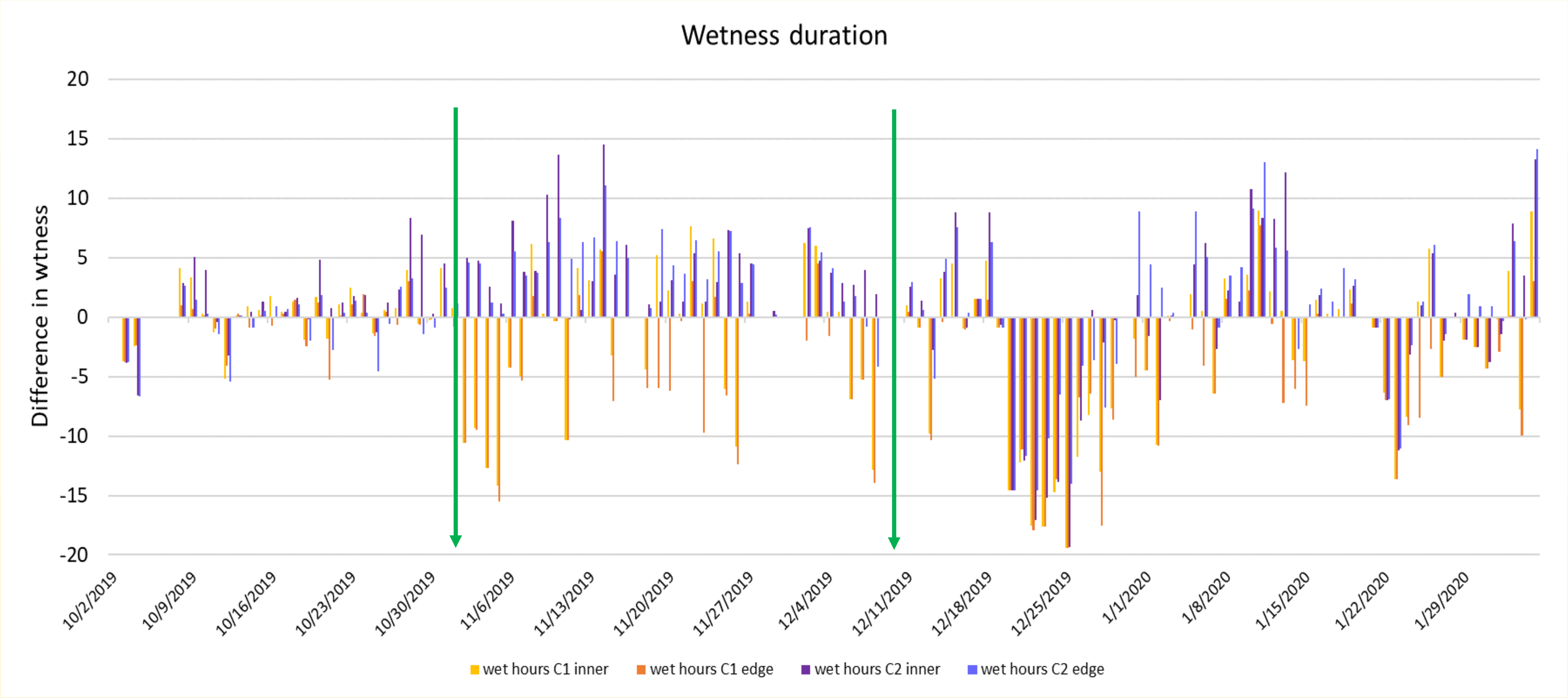
Experimental setup at the Wye REC



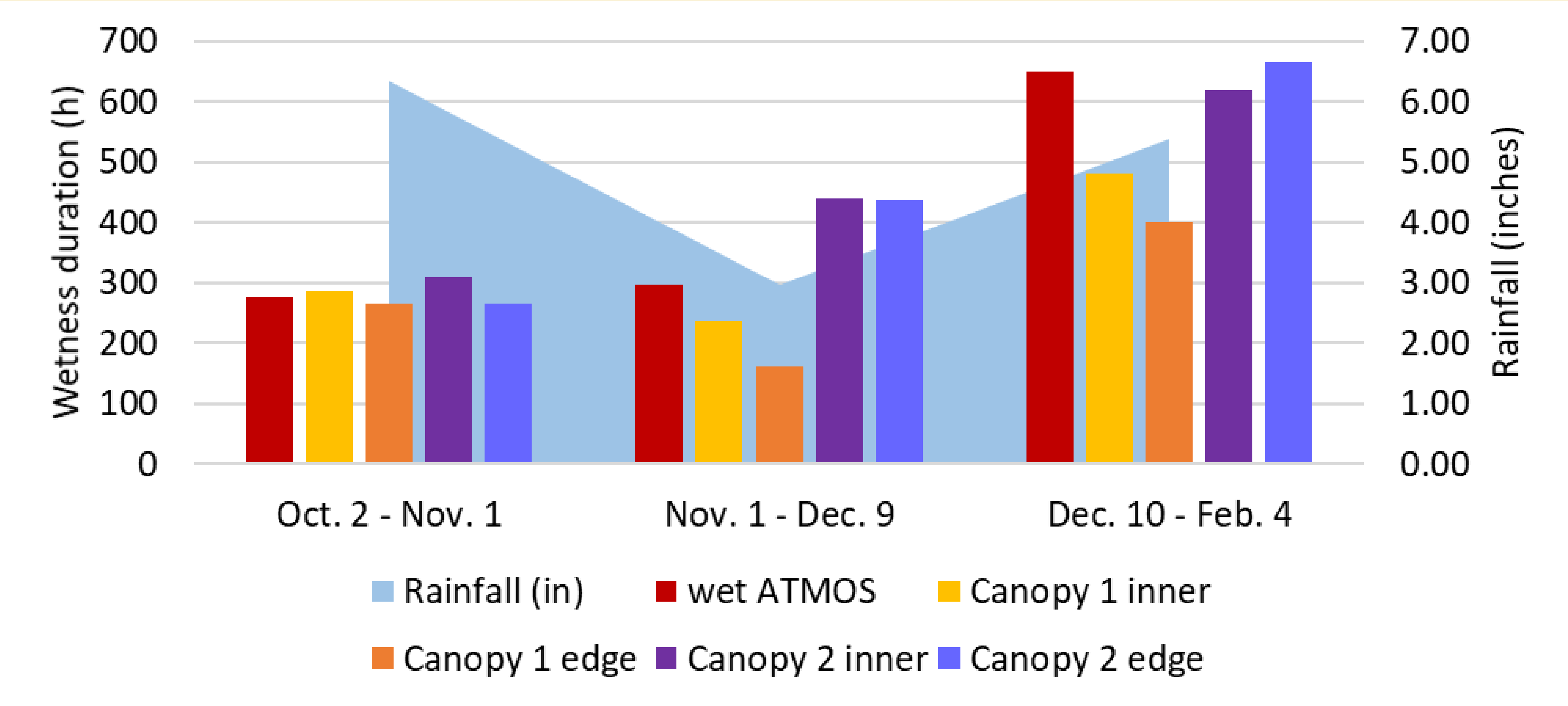
Average temperature higher under fall cover



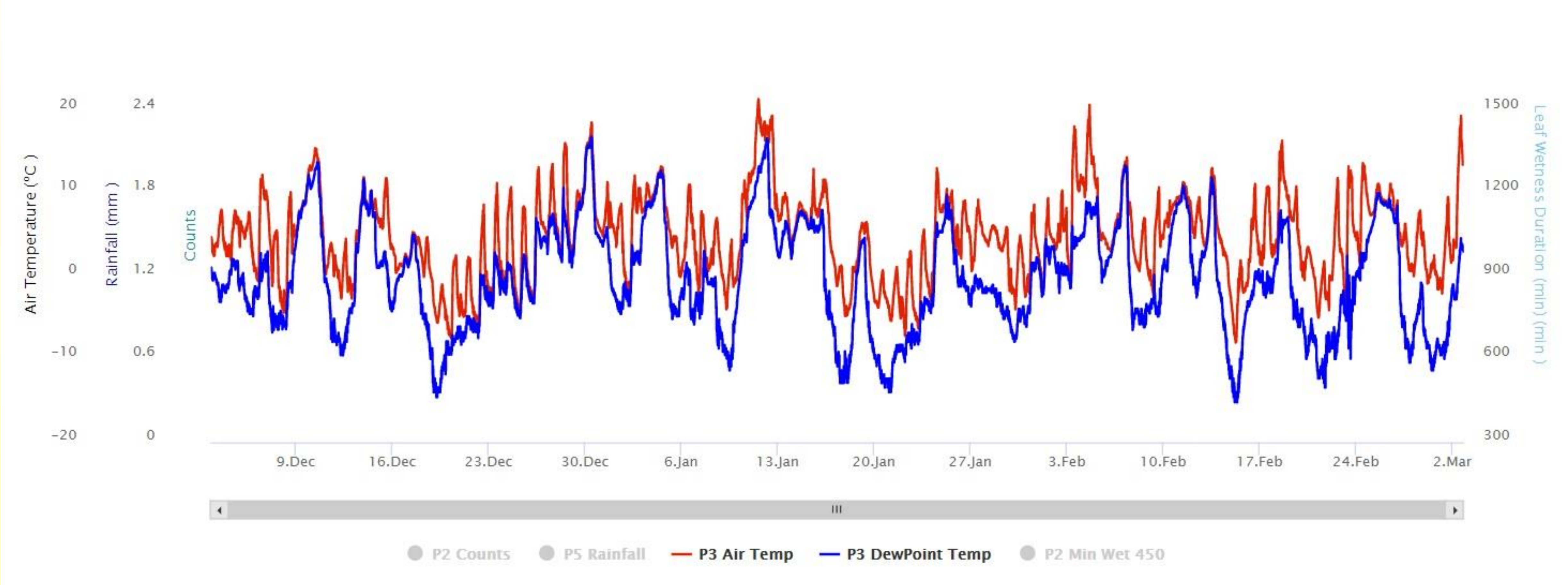
… but less prolonged wetness under cover …



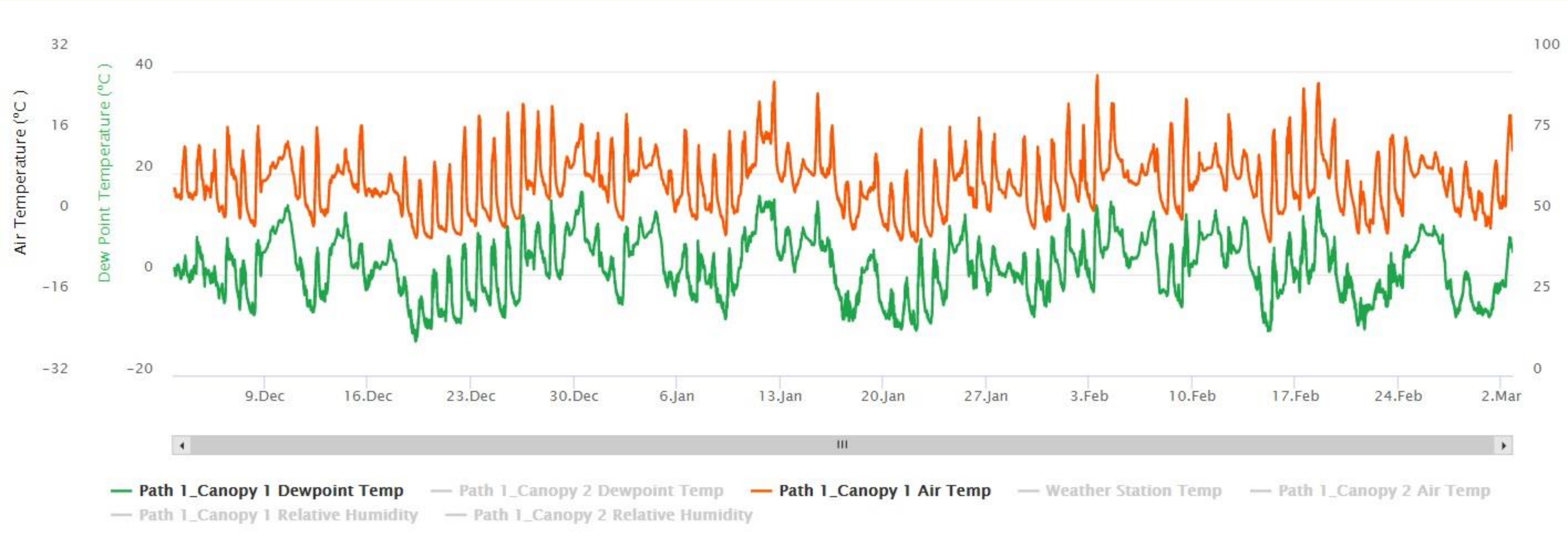
Wetness duration and rainfall Wye



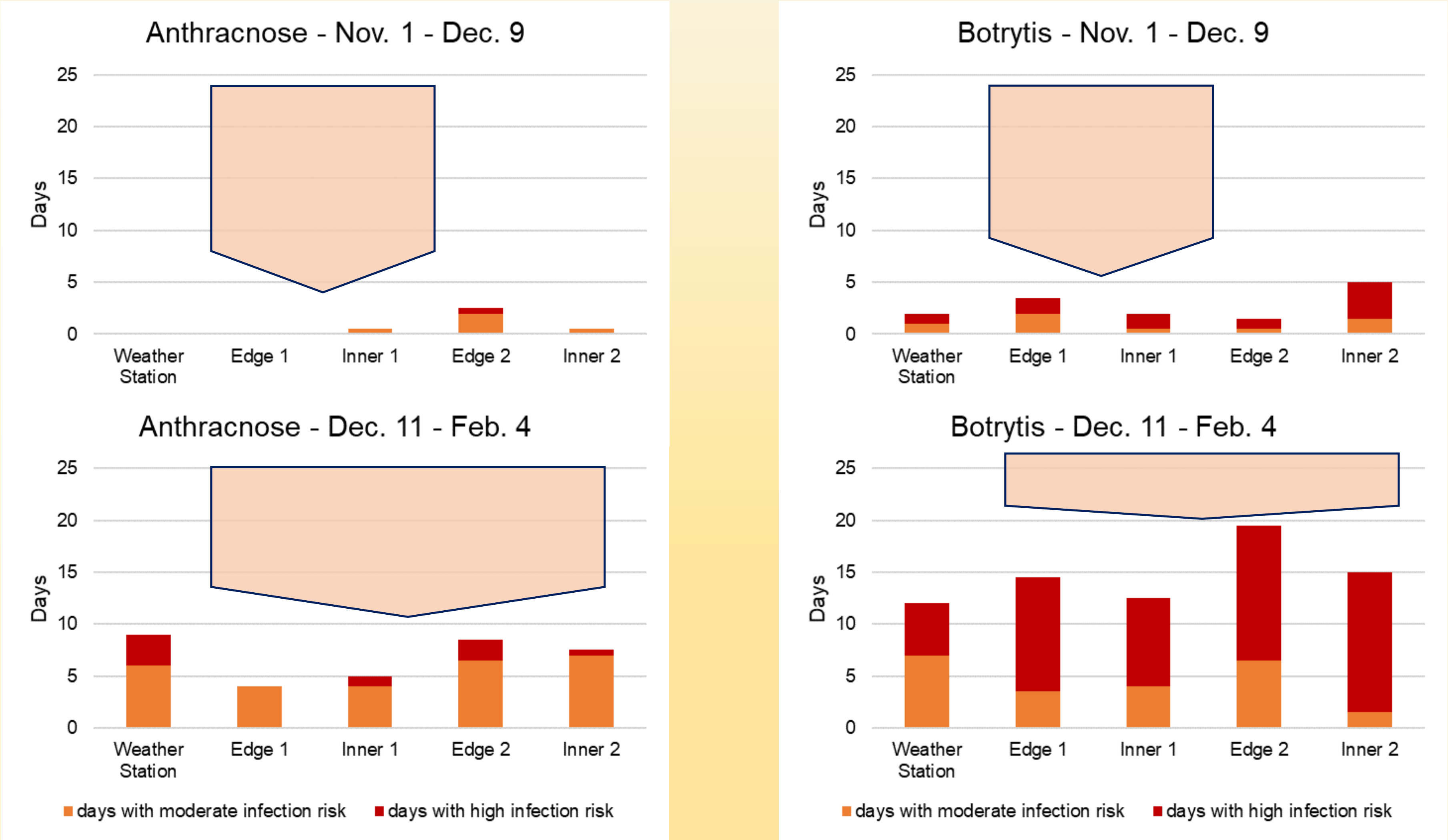
Air and dewpoint temperature (Weather station)



Air and dewpoint temperature (Canopy Sensors)



Comparison disease models Wye



What we learned so far…

- With the row-cover during late-fall (Nov 1 to Dec 9; in comparison with ATMOS):
    1. Increased average temperature (by up to 9 F)
    2. Decreased wetness duration (by up to 15 h)
    3. Disease risk seems largely unaffected, but timing varied occasionally.

- Similar observations for the winter row-cover treatment (Dec 10 to Feb 4)

- Dew events seemed to be less at canopy-level, indicating a possible drier environment under the cover.

- Comprehensive analysis of is needed to further understand such dynamic.

Acknowledgements

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agZOOM