Molecular epidemiology of Cercospora leaf spot

Fungicide resistance and host adaptation in the Red River Valley

2026 Grower Seminar

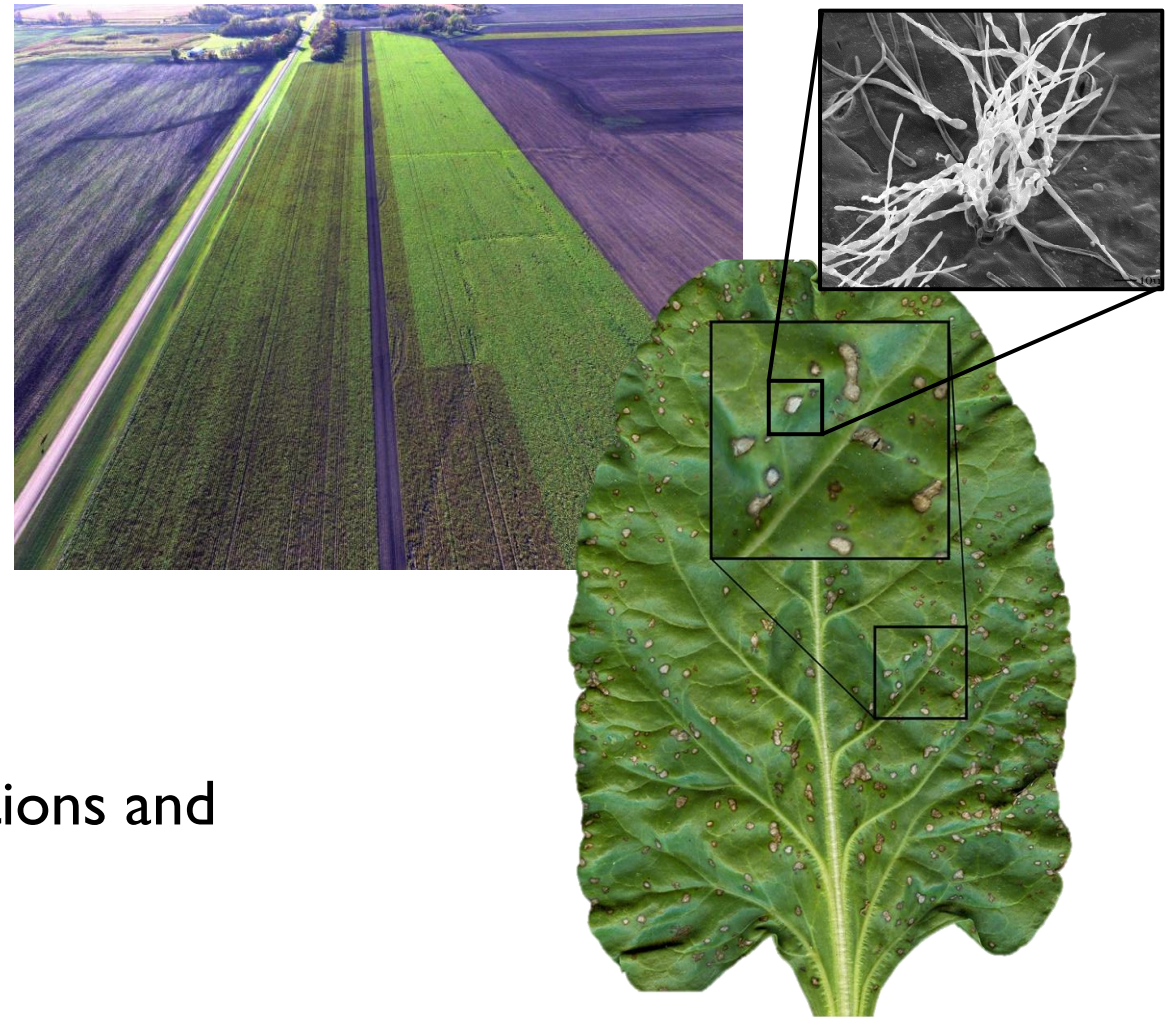
February 3rd 2025 – Grand Forks, ND

February 10th 2025 – Grafton, ND

February 17th 2025 – Fargo, ND

Cercospora beticola

- Cercospora leaf spot (CLS) on sugarbeet
- Hemibiotrophic fungus
  - Asymptomatic biotrophic phase
  - Symptomatic necrotrophic phase
- Polycyclic and genetically diverse
  - Cryptic sexual cycle
- Primarily controlled through fungicide applications and resistant sugarbeet varieties.



CLS disease cycle

PrePenetration

- Spore adhesion
- Sensory function
- Germination

Penetration

- Secrete effectors
- Locate stomata
- Penetrate stomata

Biotrophic phase

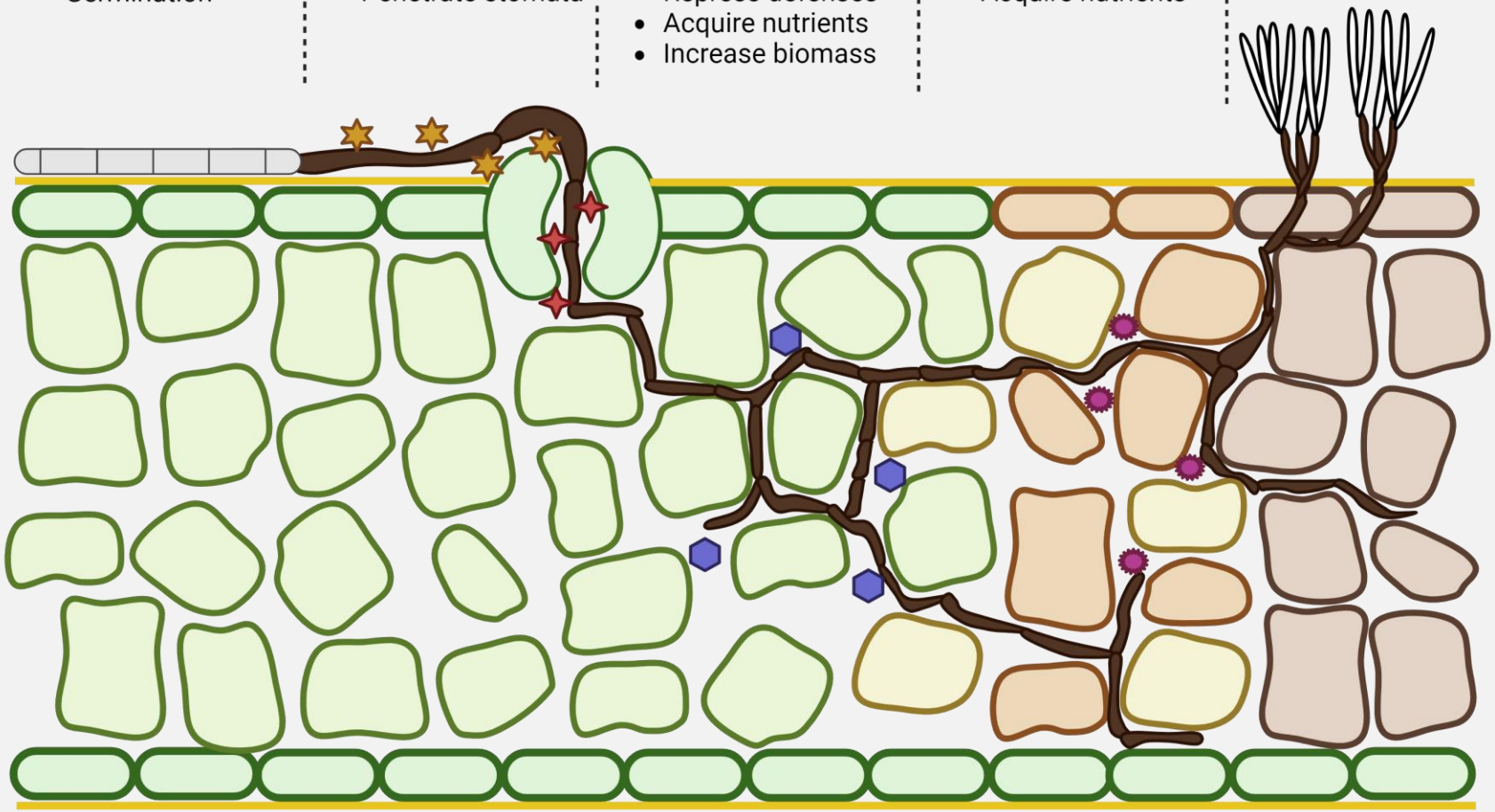
- Secrete effectors
- Avoid recognition
- Repress defenses
- Acquire nutrients
- Increase biomass

Necrotrophic phase

- Secrete effectors
- Induce cell death
- Acquire nutrients

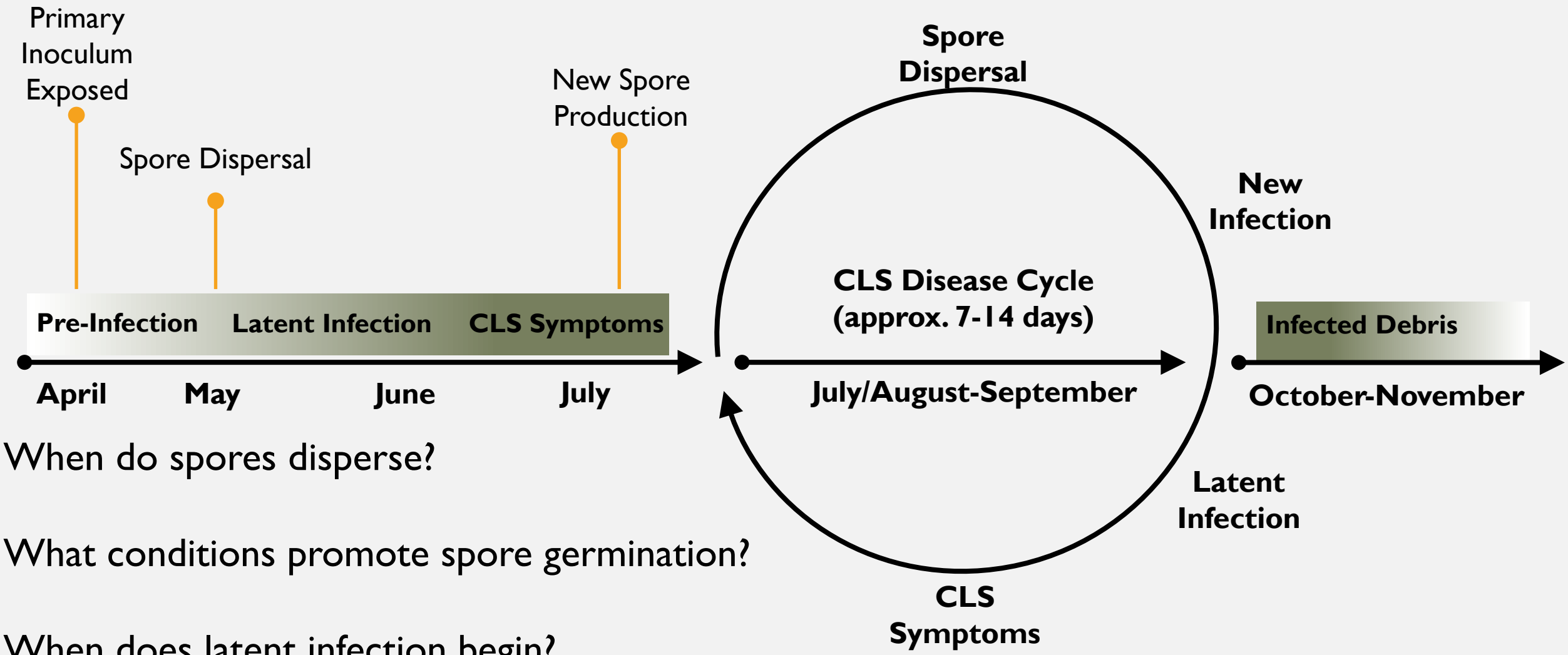
Sporulation

- Reproduce



Effectors:

CLS disease cycle



When do spores disperse?

What conditions promote spore germination?

When does latent infection begin?

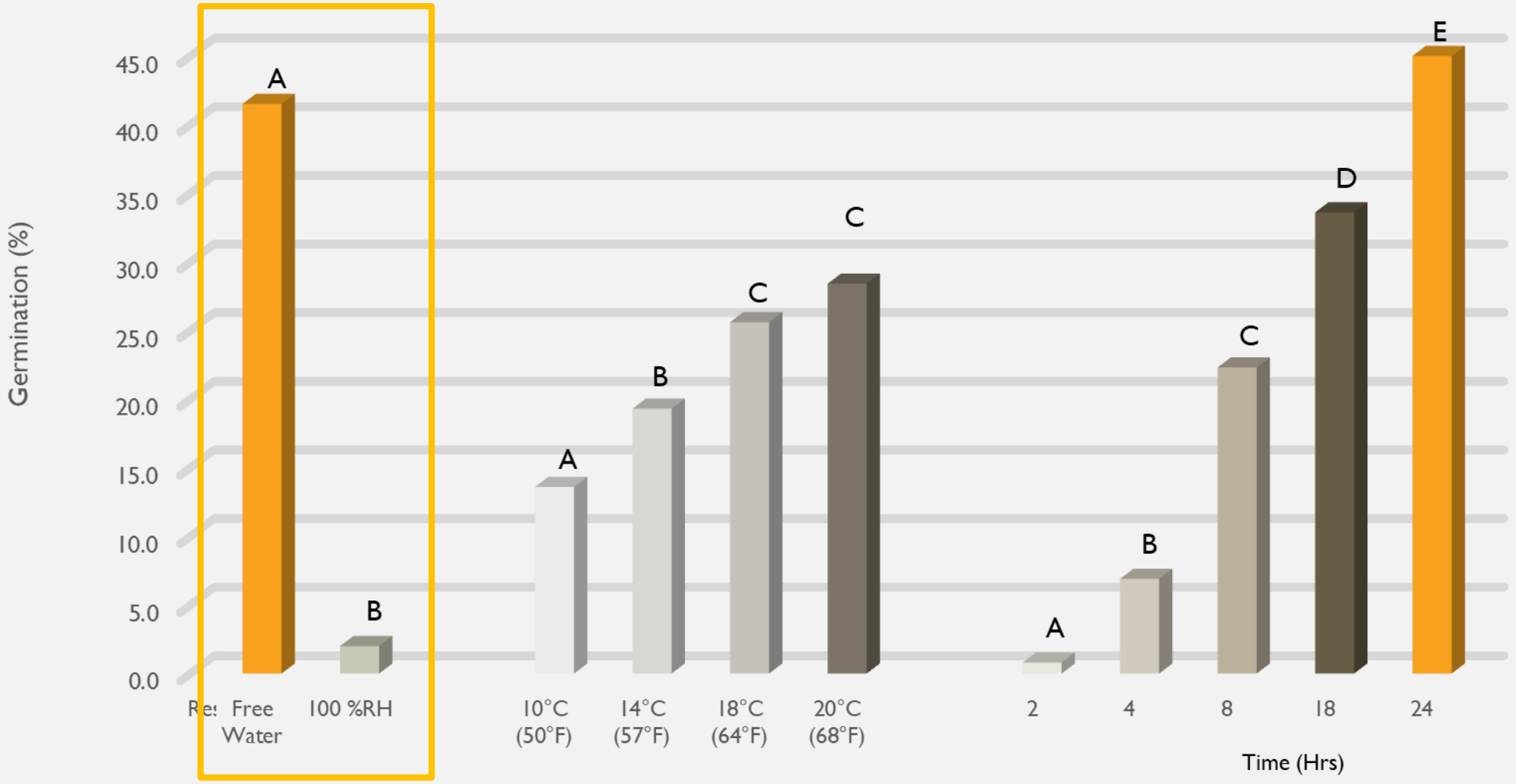
Spring spore dispersal

- Three year study 2021-2025
  - May - October
- **Six field sites** each year selected by agronomists
  - Adjacent to fields with sugar beet crop the previous year
- Cartridges collected three times weekly
- DNA from spores collected on the cartridge membranes was tested for the presence of *C. beticola* DNA by qPCR assay (Shrestha et al. 2020)

Spores detected at the earliest samples (May 1st) prior to planting.

Secor et al. Unpublished

C. beticola spore germination

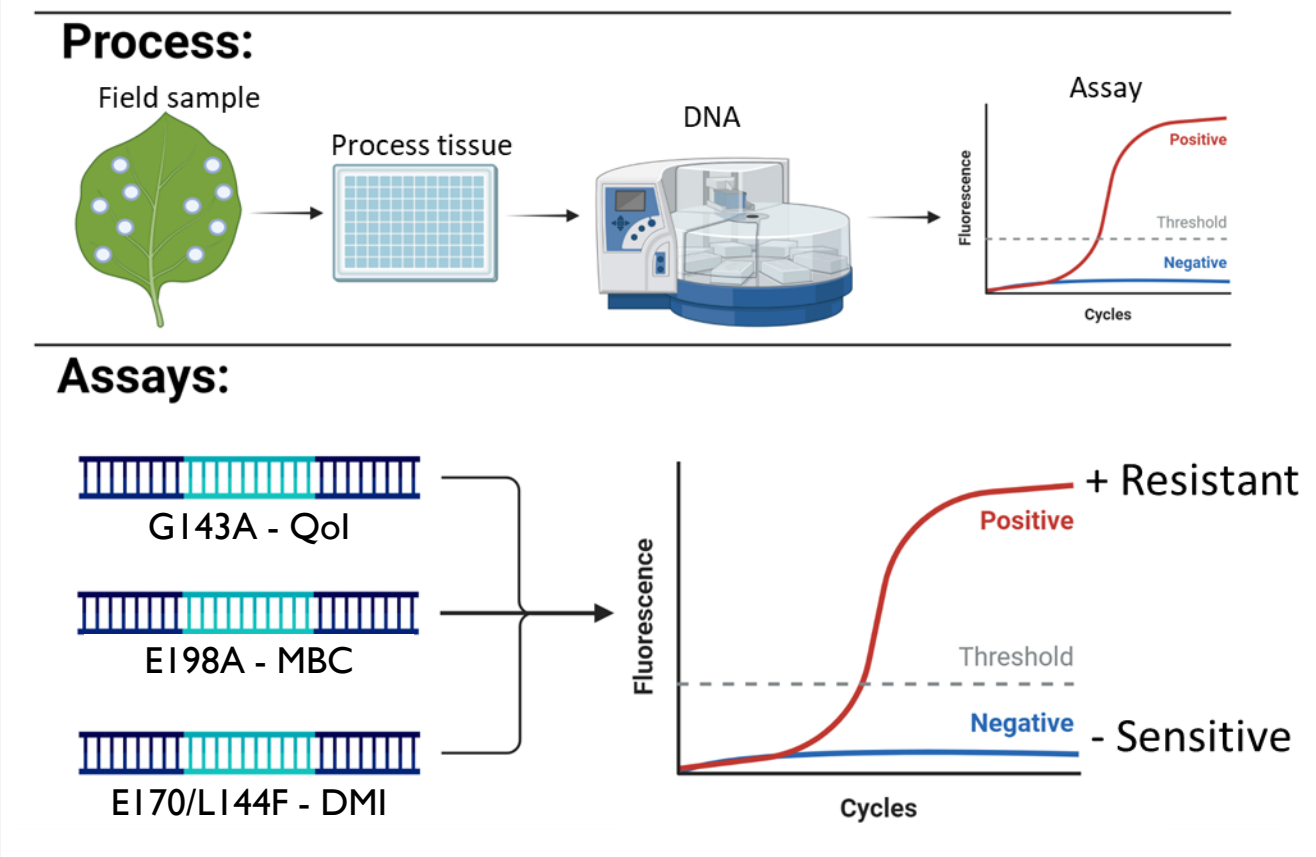


Data Provided by Dr. Gary Secor

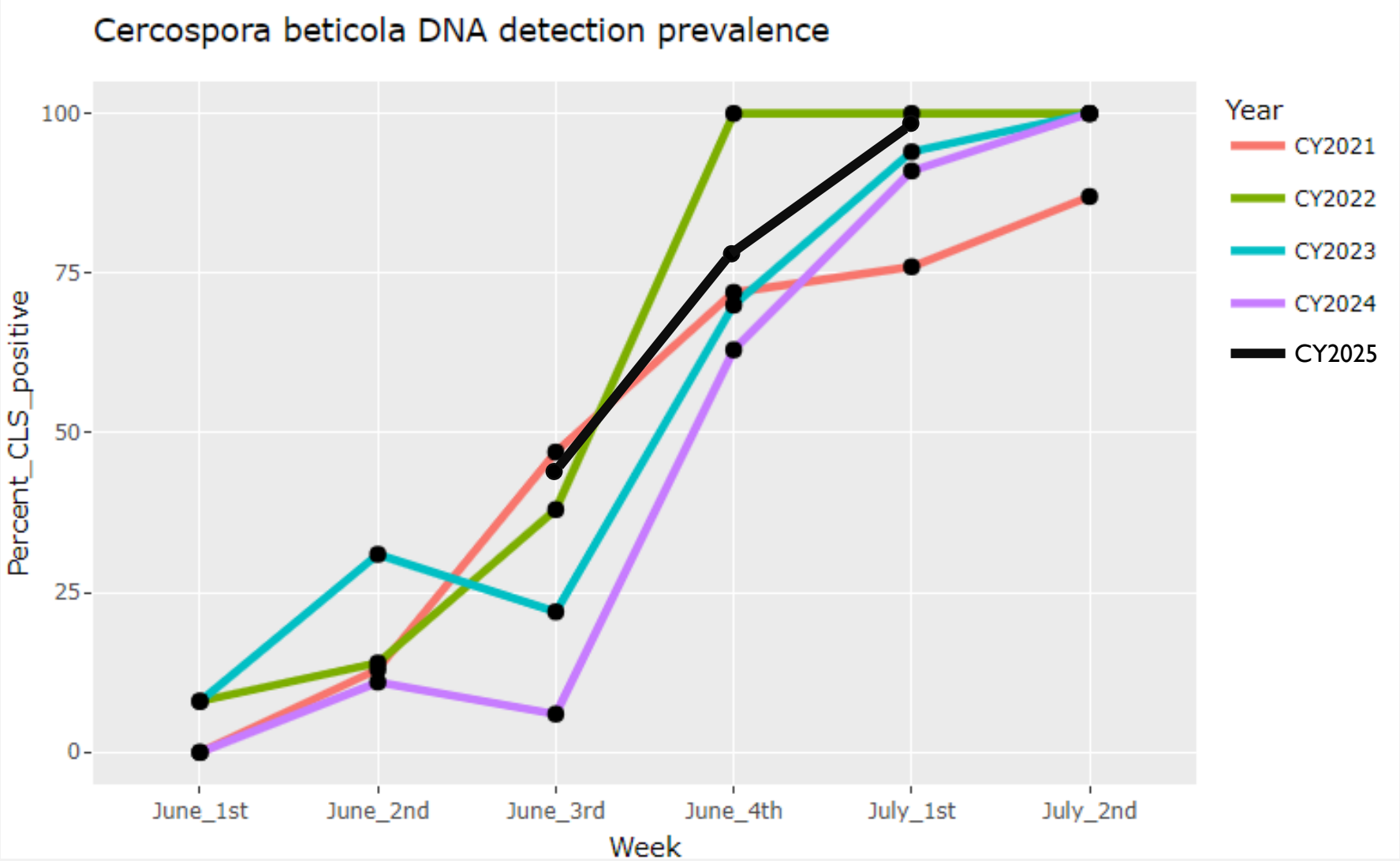
Latent infection screening

Survey of commercial sugarbeet fields

- Conducted in 2021 – 2025
- 280 commercial sugarbeet fields
  - Weekly sampling starting at the 4-6 leaf stage
- Targeting field locations with high CLS likelihood
- 2026 will report on fungicide sensitivity/resistance ratios.

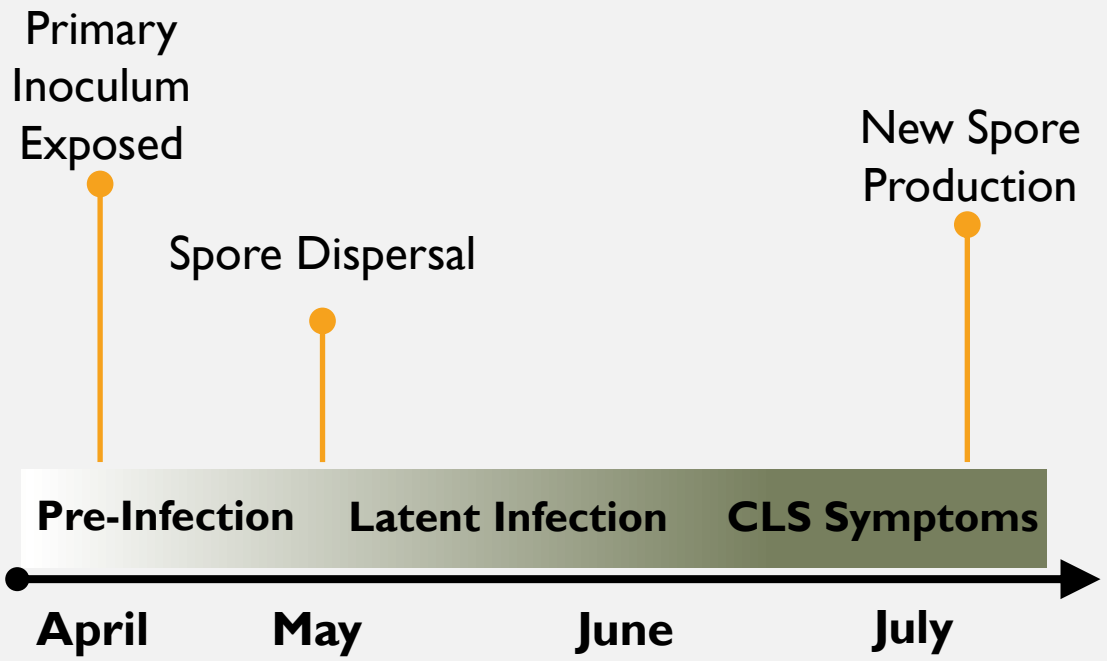


2021-2024 Latent CLS prevalence



Wyatt et al. Unpublished data

CLS disease cycle



<u>Presymptomatic CLS model:</u>
Temperature thresholds bracketed for increasing temps above 50°F.

Highly weighted for free moisture availability:

- Rain events greater
- Dew formation

Minor factors:

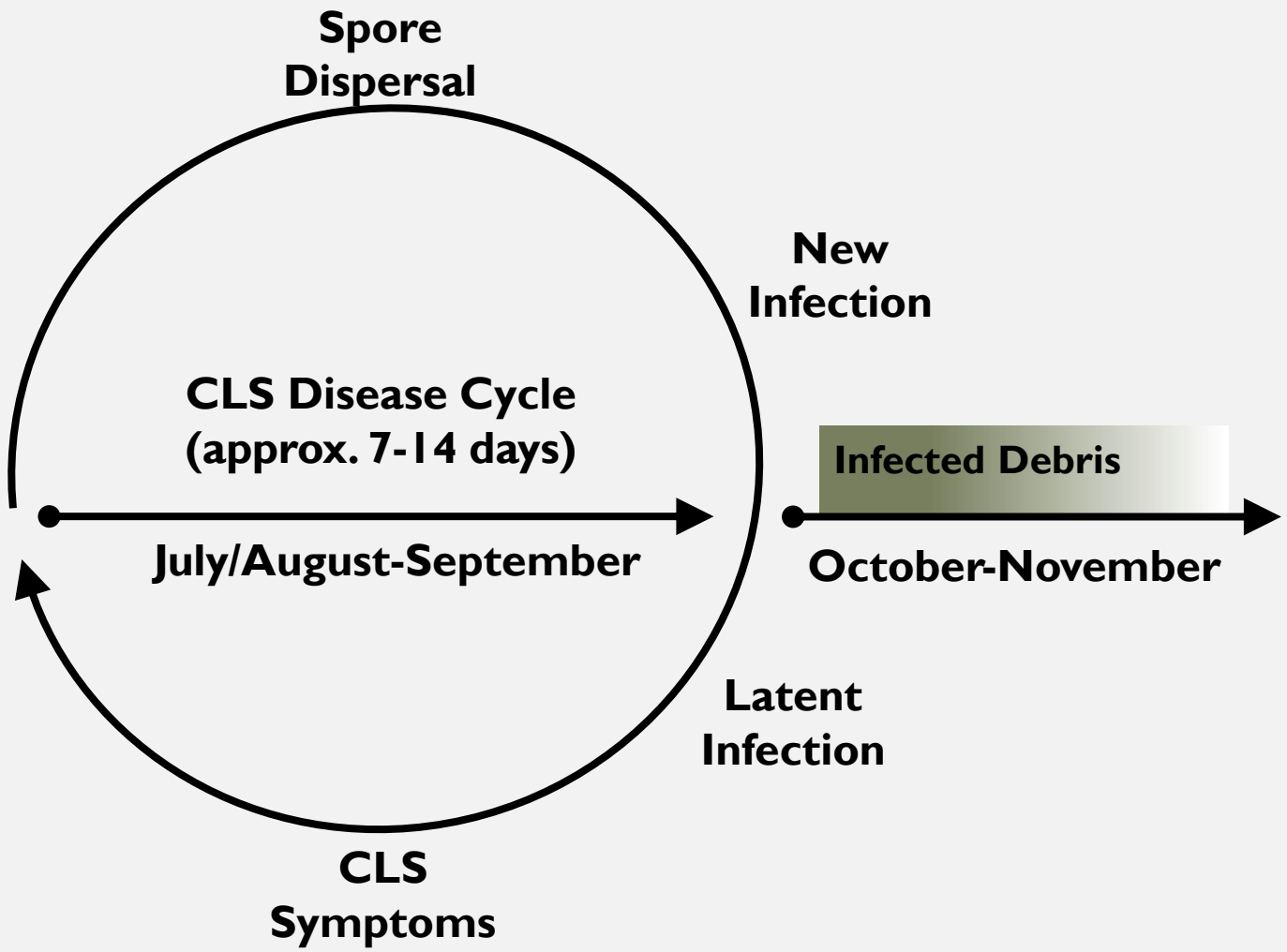
- Wind speed and duration
- Soil moisture

CLS disease cycle

Shane and Teng model with modification:

Increased weight on free moisture

Confirmation of CLS risk with spore trapping data throughout the season.

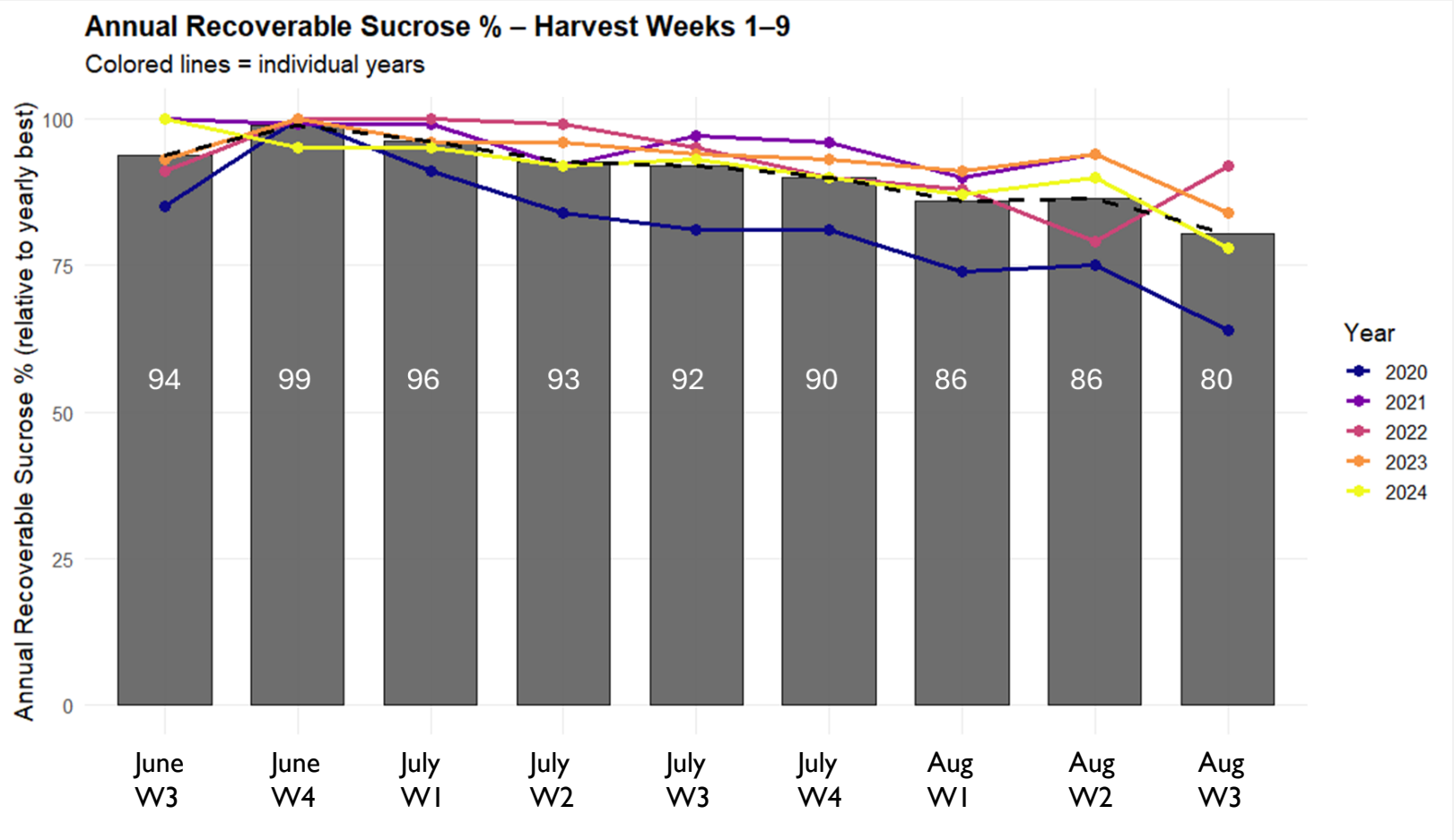


Why focus on asymptomatic latent infection?

Why do we care if there are no symptoms?

Primary results

- Highest average annual RSA obtained when CLS fungicide control began in the last week of June.
- ~5% RSA reduction from June Week 4 to July Week 1
- 2020: High CLS pressure year
- 2022: Low CLS pressure year



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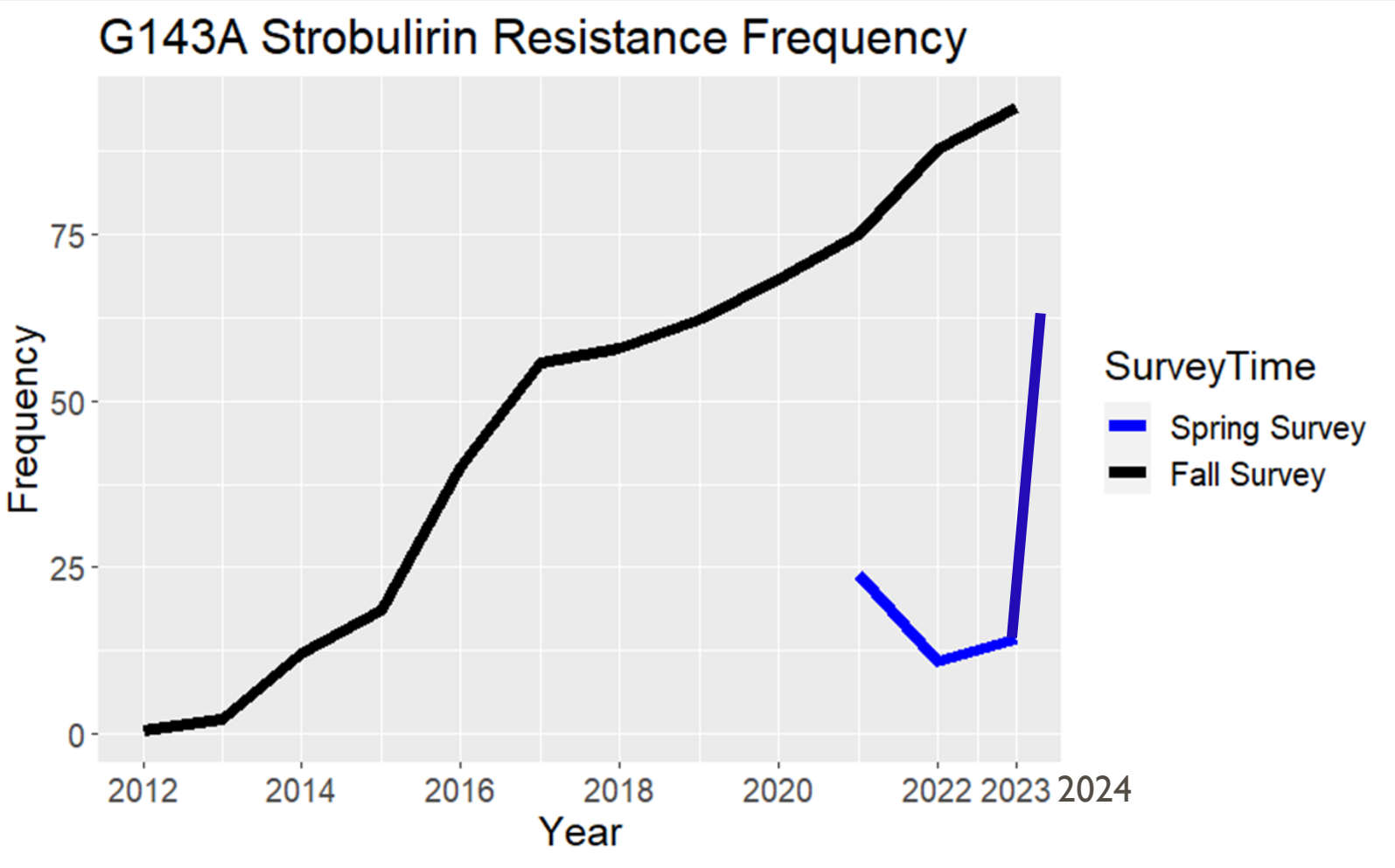
C. beticola population dynamics: Fungicide resistance

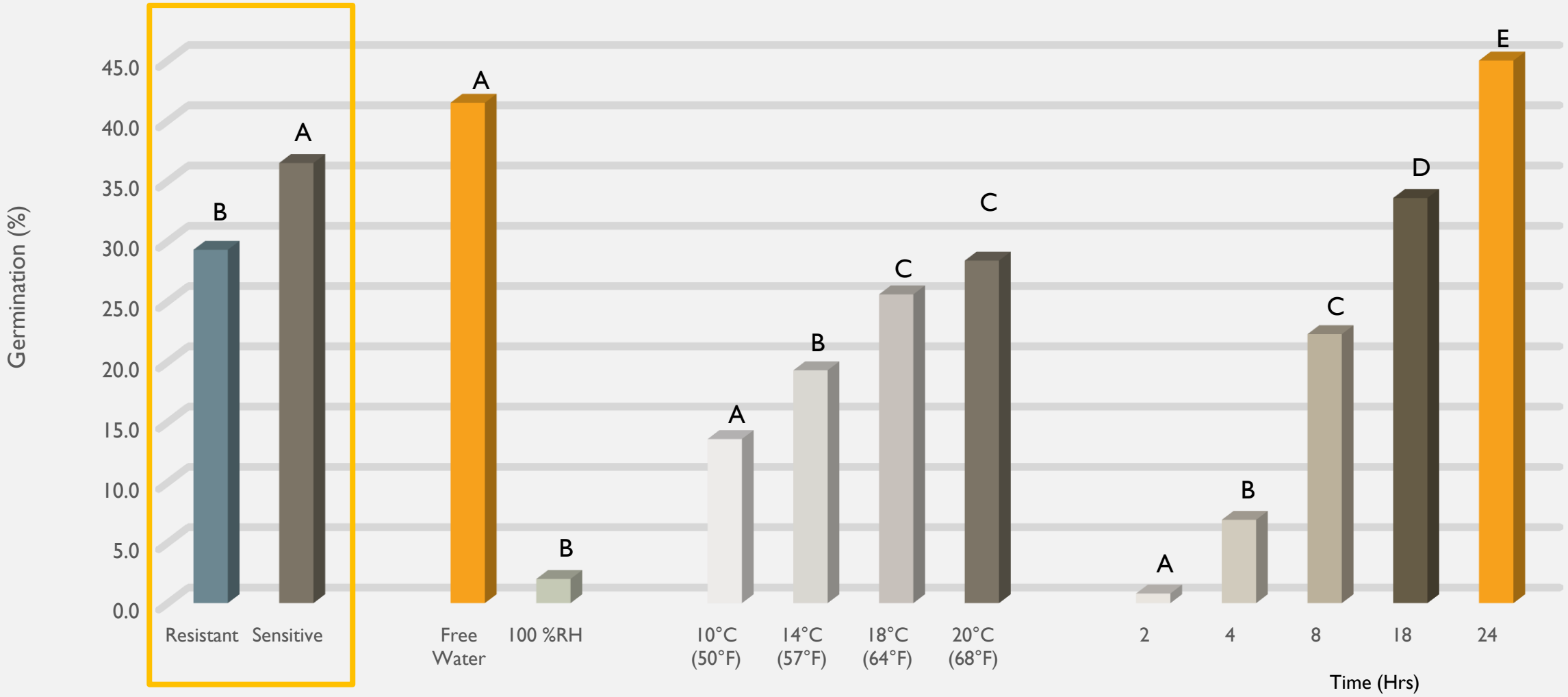
Annual Strobilurin Resistance Fluctuations



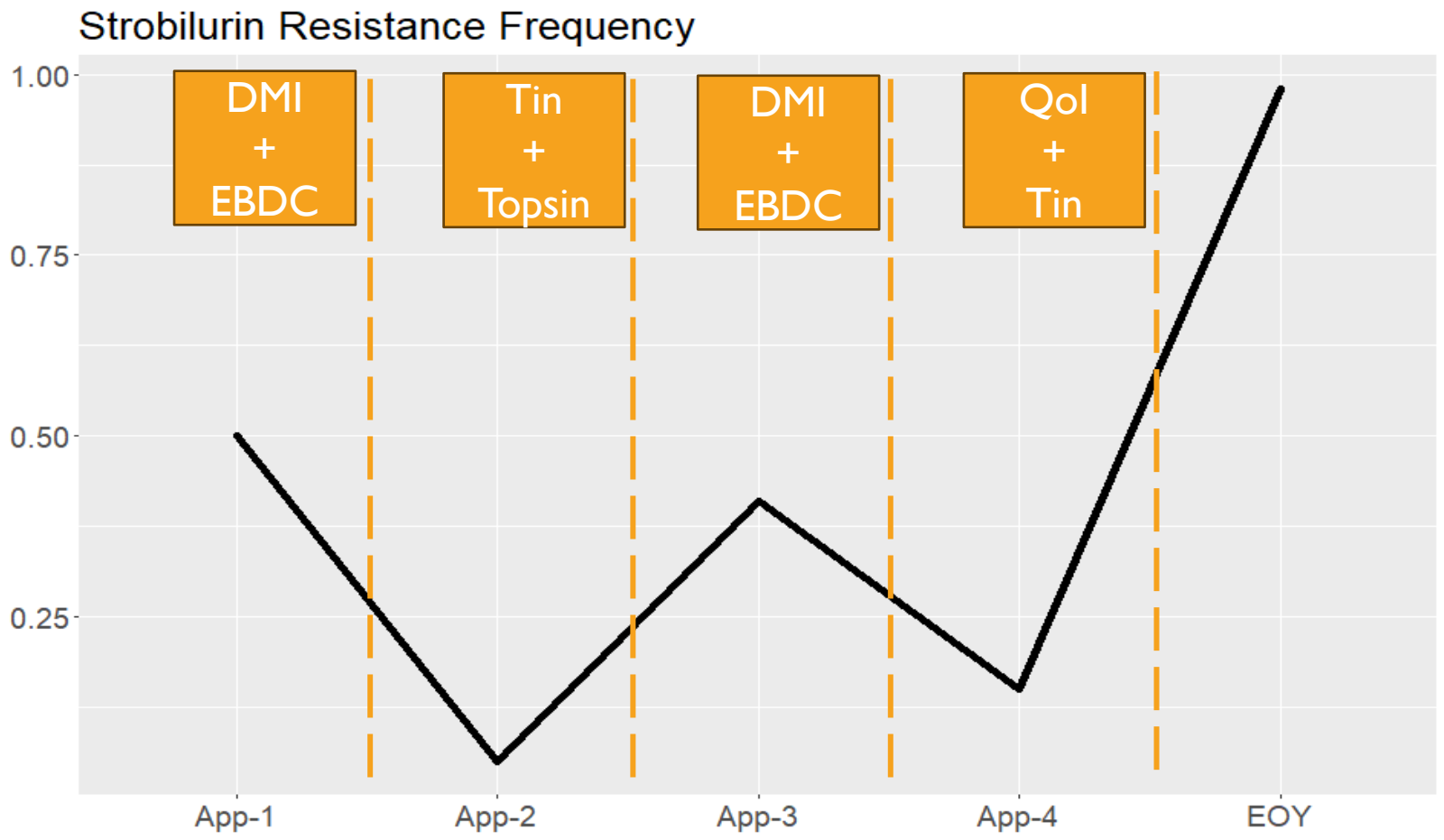
Primary results

- Stobilurin resistance is lower in the spring
- Spring survey based on Spore trap data and Latent infection data
- What caused the increase in 2024 Strobilurin resistance in the spring

C. beticola spore germination



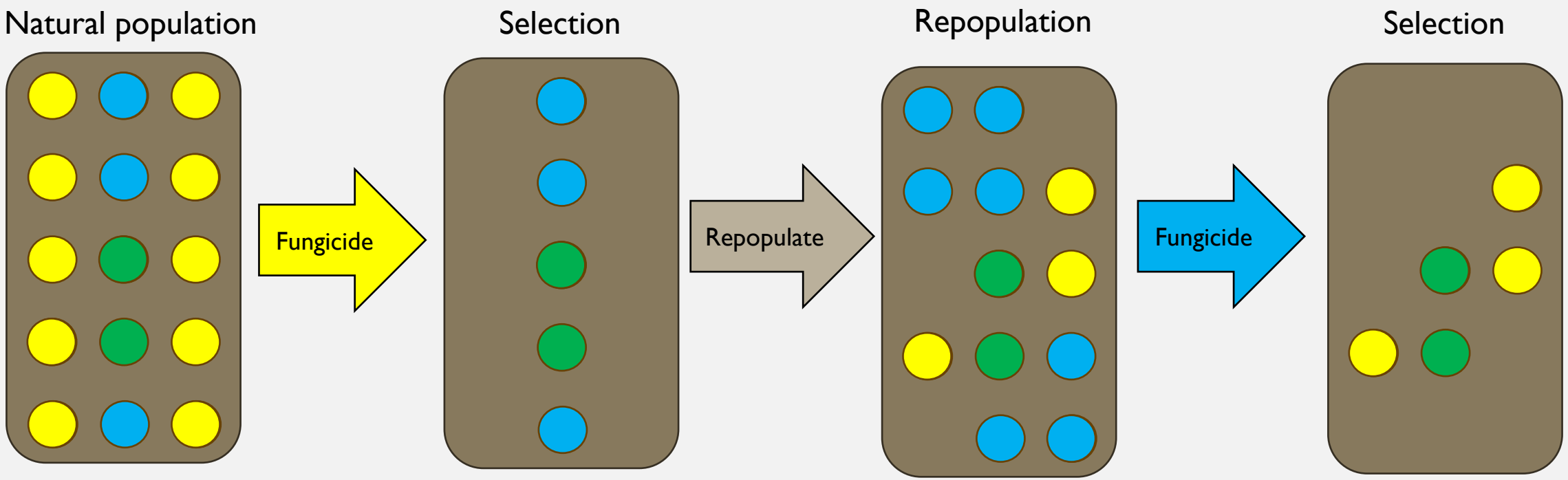
Annual Strobilurin Resistance Fluctuation



Primary results

- Strobilurin resistance changes throughout the year in response to management practices
- DMI applications show potential to reduce strobilurin resistance in the field.

Management is selection



Resistant isolate

Yellow sensitive isolate

Blue sensitive isolate

Fungicide cross resistance

Fungicide	Commercial product	Headline	Topsin	Domark	Proline	Inspire	TPTH
Strobilurin	Headline	1.00					
Benzimidazole	Topsin	0.18	1.00				
Triazole	Domark	0.69	0.33	1.00			
Traizole	Proline	0.53	0.41	0.92	1.00		
Triazole	Inspire	0.51	0.37	0.59	0.60	1.00	
Tin	TPTH	0.40	0.21	0.48	0.54	0.43	1.00

- Cross resistance scored from 0.00 to 1.00 with higher values indicating higher degrees of cross resistance.

Primary results

- Cross resistance is relatively low between different chemistries
- Tank Mixing multiple chemistries as an effective strategy
- Currently recommended Tank Mixes (ACSC):

Fungicide cross resistance

Fungicide	Commercial product	Headline	Topsin	Domark	Proline	Inspire	TPTH
Strobilurin	Headline	1.00					
Benzimidazole	Topsin	0.18	1.00				
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- Cross resistance scored from 0.00 to 1.00 with higher values indicating higher degrees of cross resistance.

Primary results

- Cross resistance is relatively low between different chemistries
- Tank Mixing multiple chemistries as an effective strategy
- No EBDC resistance has been detected.

C. beticola population dynamics

Fungicide	Commercial name	Mutation	2016	2017	2021	2023	2024
Strobilurin	Headline	G143A	29%	31%	20%	25%	33%
Triazole	Domark/Proline	E170	81%	46%	72%	62%	75%
Triazole	Inspire/Provysol	L144F	47%	45%	51%	53%	55%
Benzimidazole	Topsin	E198A	30%	14%	23%	15%	29%
Organotin	SuperTin	GST	31%	22%	35%	30%	30%

Frequency of fungicide resistance mutations in whole genome sequenced *C. beticola* isolates collected at the end of season survey.

Though the incidence of fungicide resistance is high across fields for any level of resistance, the individuals in those fields show lower resistance levels.

Example: Most fields sampled in 2021 had isolates that were Tin resistant.

BUT not all of the isolates in any one field were resistant.

C. beticola population dynamics: Host adaptation

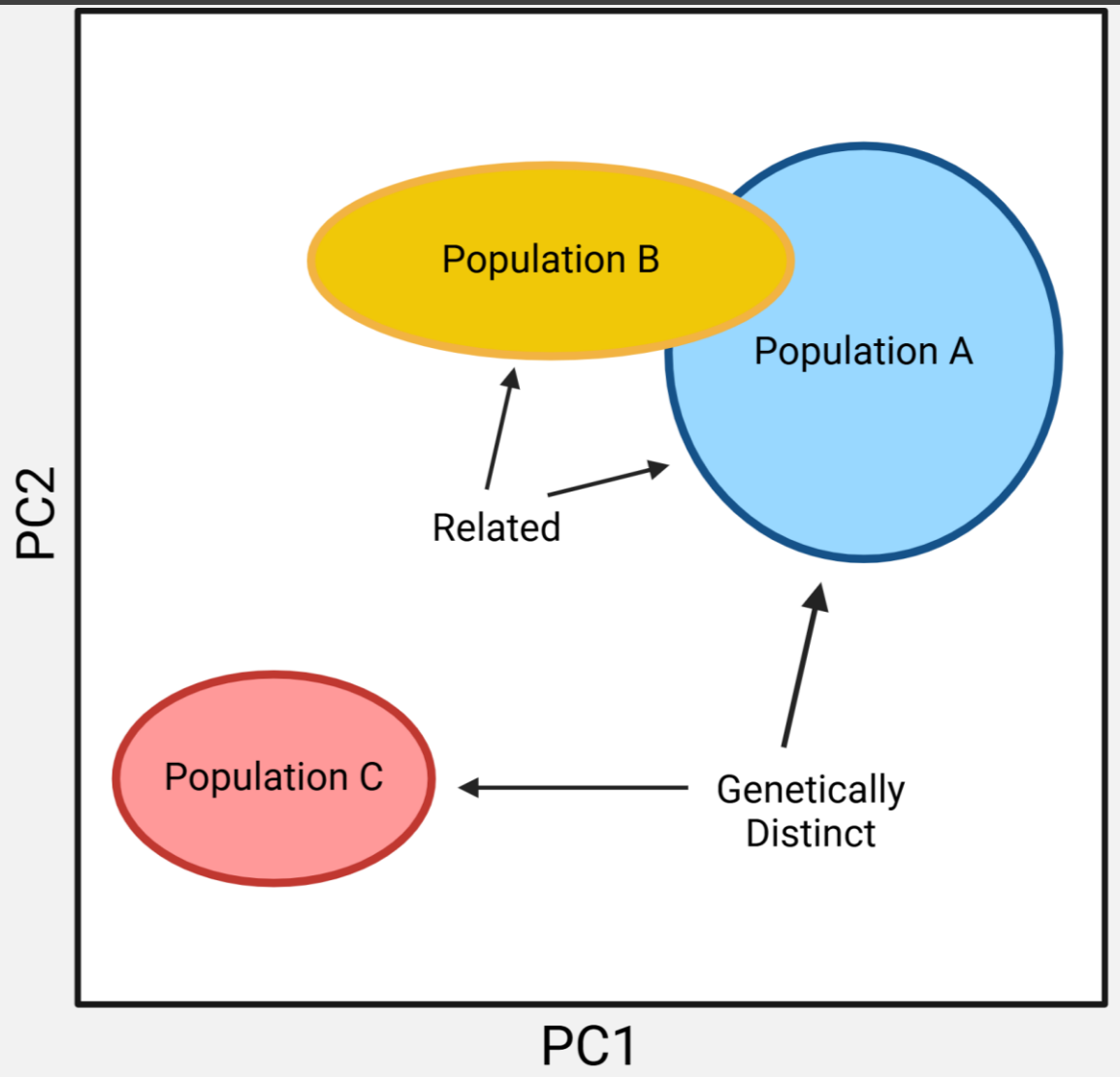
WGS and PCA Primer

Whole genome sequencing of RRV populations to identify mutations.

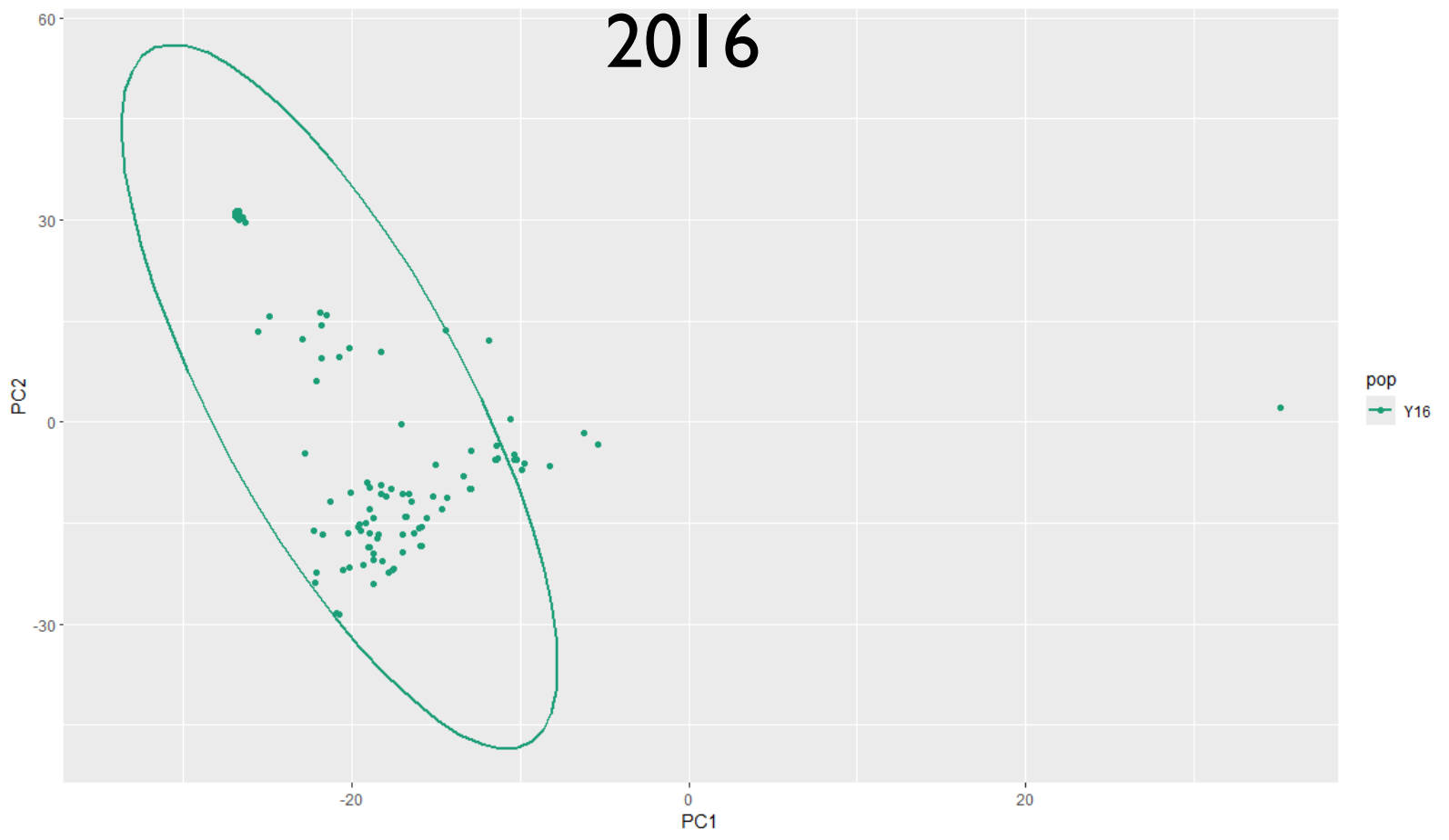
Principle component analysis can be used to identify patterns of genetic variation among individuals/populations.

Factors commonly influencing population structure:

- Geography
- Sampling timeline
- Environmental conditions
- Specific selective pressures
  - i.e. Management practices



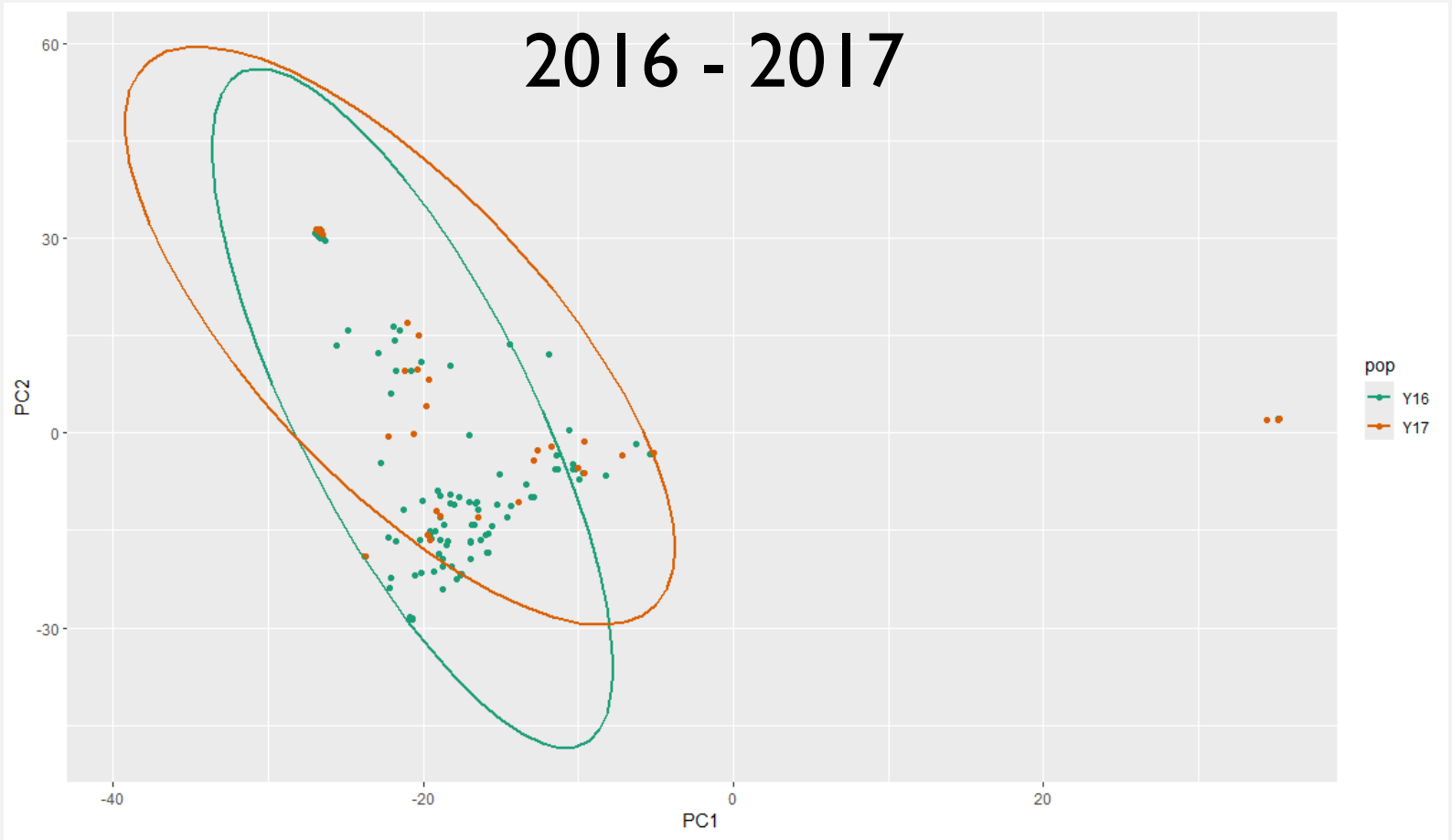
C. beticola population dynamics



Percent of isolates adapted to CR+ in 2016: 0.0%

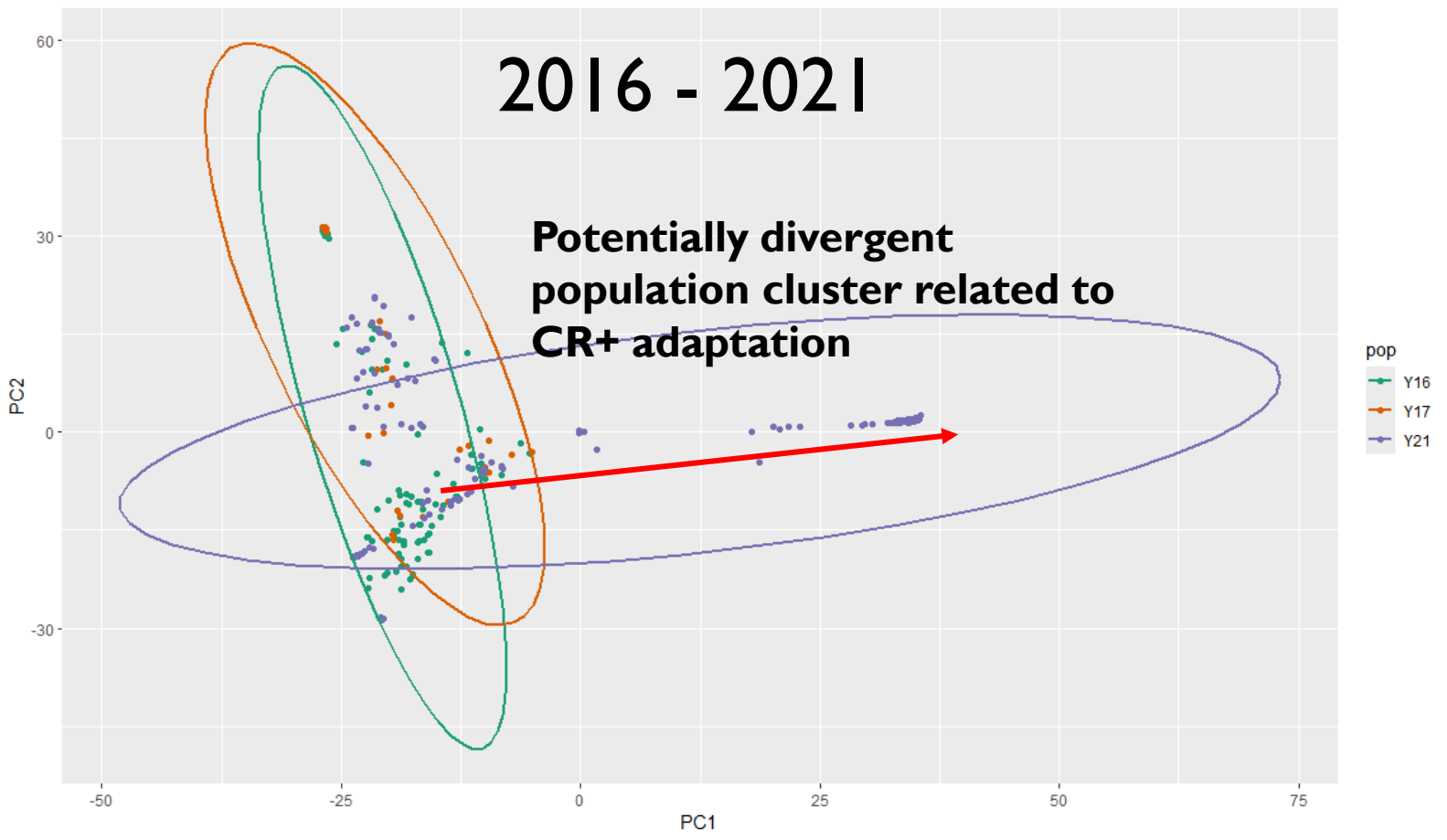
Wyatt et al. Unpublished data

C. beticola population dynamics



Percent of isolates adapted to CR+ in 2017: 0.0%

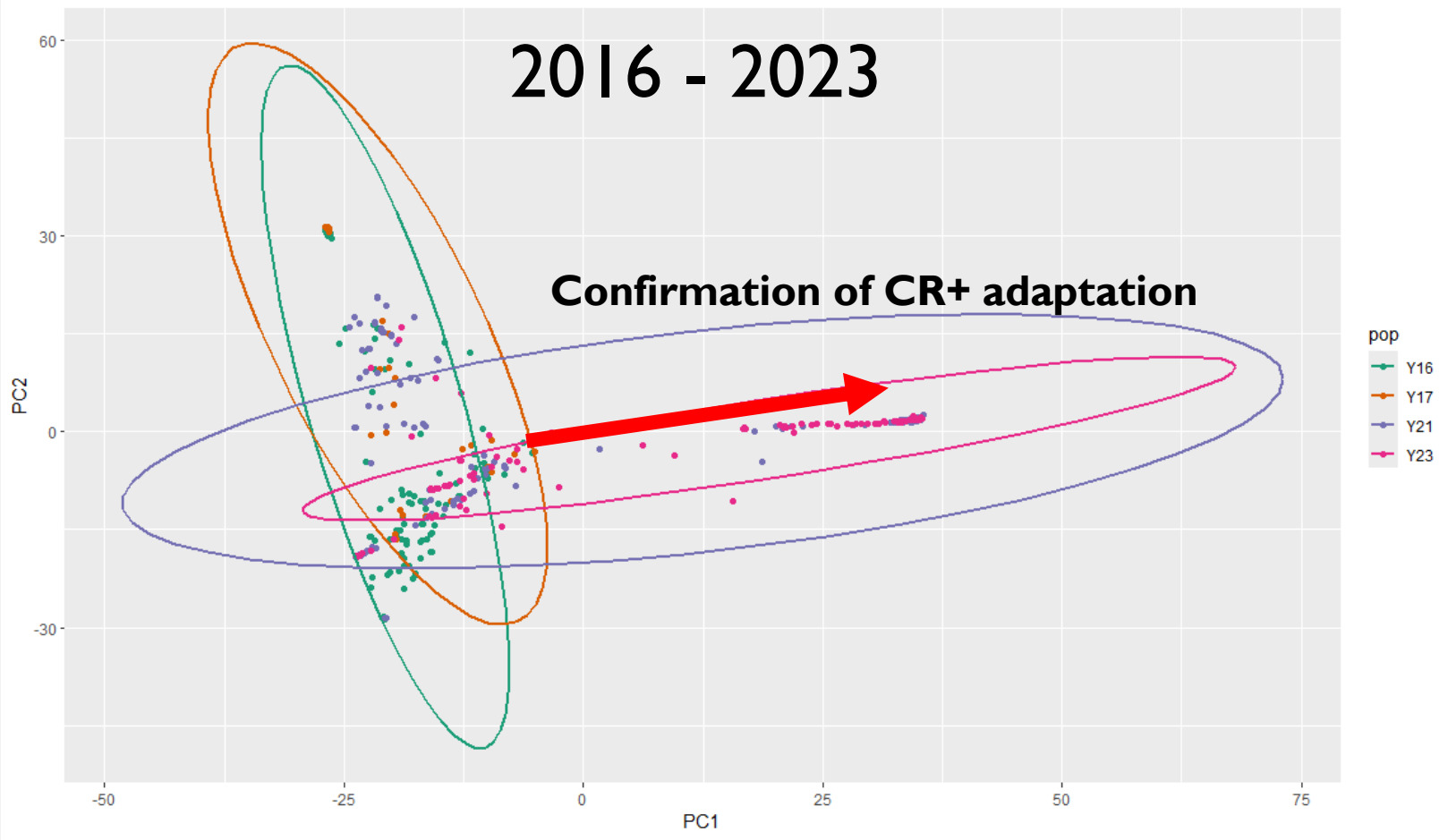
C. beticola population dynamics



Percent of isolates adapted to CR+ in 2021: **11.2%**

Wyatt et al. Unpublished data

C. beticola population dynamics



Percent of isolates adapted to CR+ in 2023: **37.4%**

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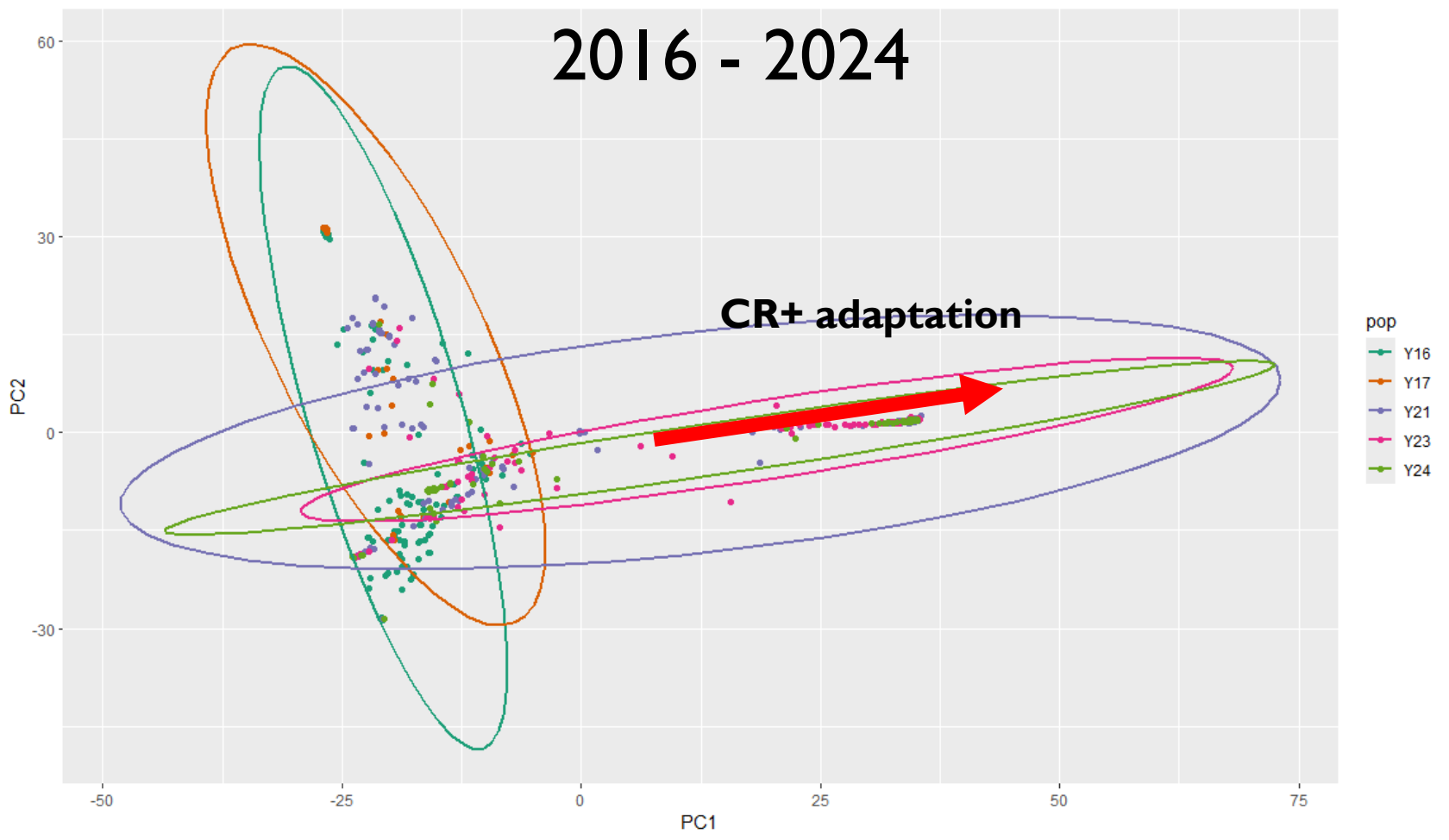
C. beticola population dynamics



Percent of isolates adapted to CR+ in 2024: **76.3%**

Wyatt et al. Unpublished data

Ongoing research

- 2026 Latent infection surveys will included % of fungicide resistance
- Currently finalizing an expanded CLS risk model to aid in management decisions.
  - Two part model (presymptomatic and post-symptomatic model).
- PCR based marker validation is in progress for both CR+ and Tin resistance mutations.
- Continuing *C. beticola* whole genome sequencing of isolates collected in 2025 and 2026.
- Actively sequencing *C. beticola* isolates collected 1997 to present to examine population dynamics relevant to changing management practices.
  - Triazole and strobilurin introduction in early 2000's.
  - Tank mixing popularity
  - Narrowing genetic diversity of the commercial sugar beet varieties.

Acknowledgements

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Sugarbeet Research & Education Board

BEET SUGAR DEVELOPMENT FOUNDATION

NDSU NORTH DAKOTA STATE UNIVERSITY

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Thank you for your attention!