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The relationship between blueberries and fungi: it is complicated

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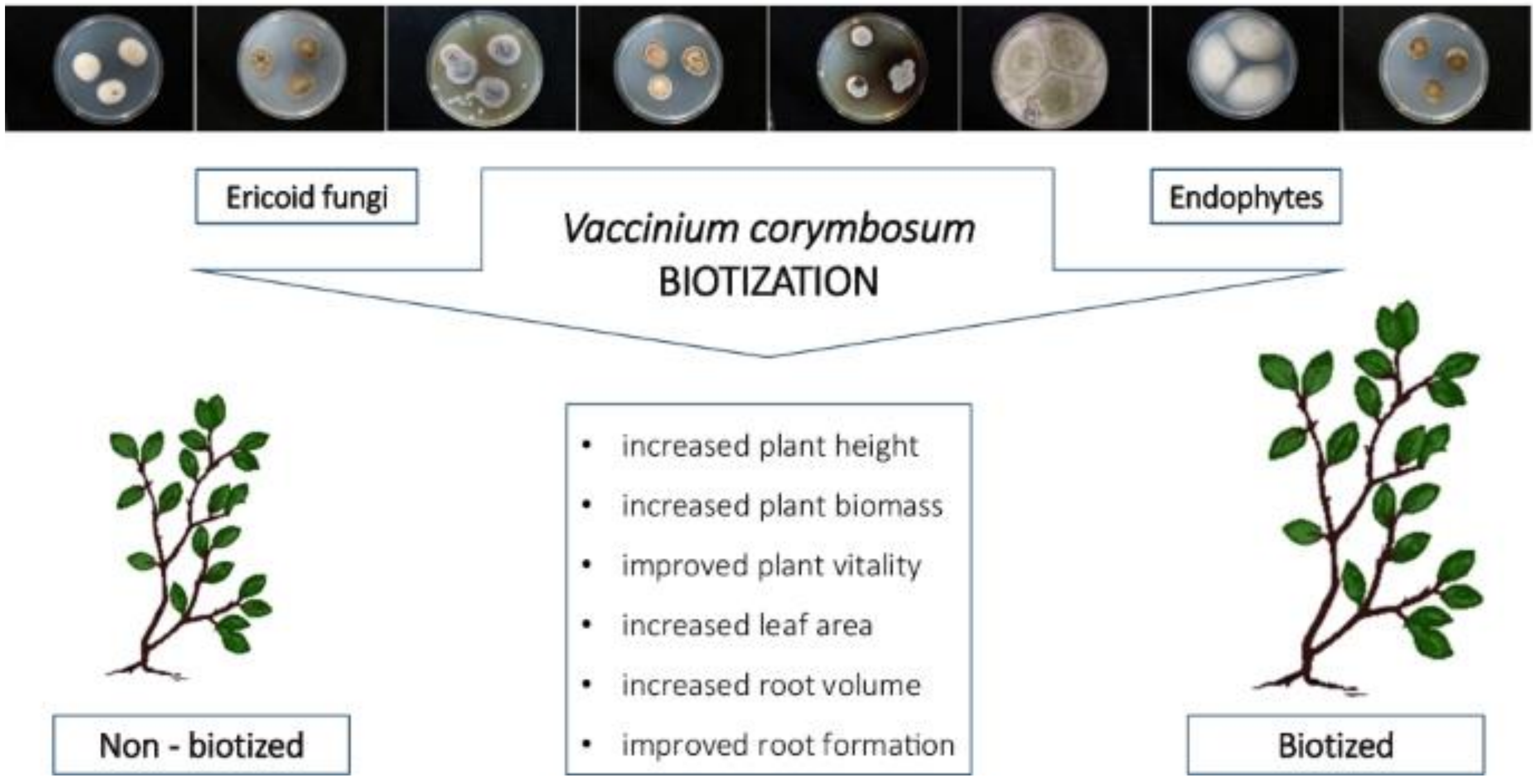
Photo by Rawpixel

Introduction

- Blueberry plants have complex relationships with fungi
- Some positive – mycorrhizae or endophytes



Ważny, R., Jędrzejczyk, R.J., Rozpądek, P. *et al.* . *Appl Microbiol Biotechnol* **106**, 4775–4786 (2022).

Introduction

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- Blueberry plants have complex relationships with fungi
- Some positive – mycorrhizae or endophytes
- Some negative – pathogens


Anthracnose


Rust

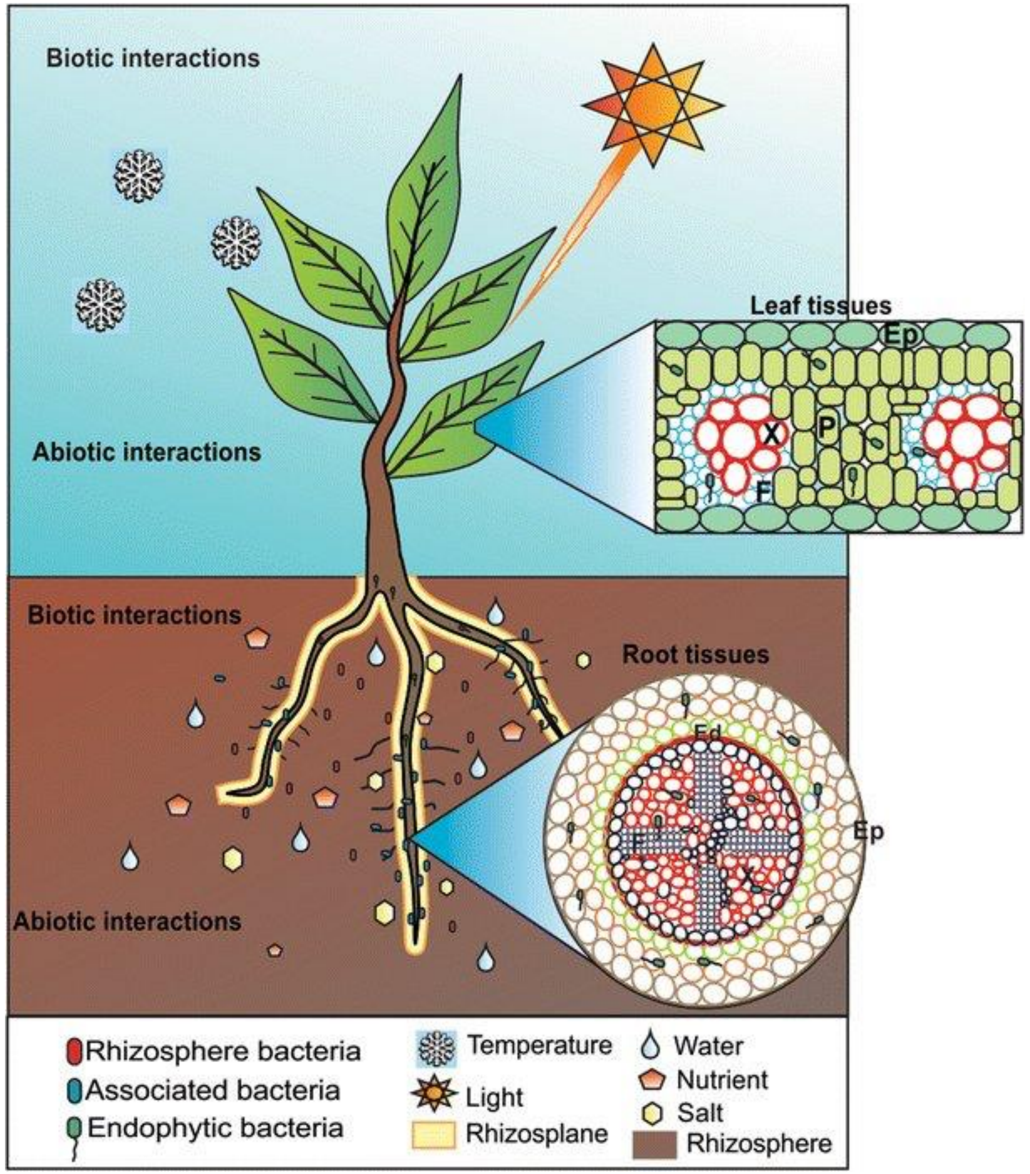

Botrytis


Powdery mildew


Alternaria rot

Introduction

- Understanding the scope of the biology and interactions in complex communities will aid in the management of these ecosystems



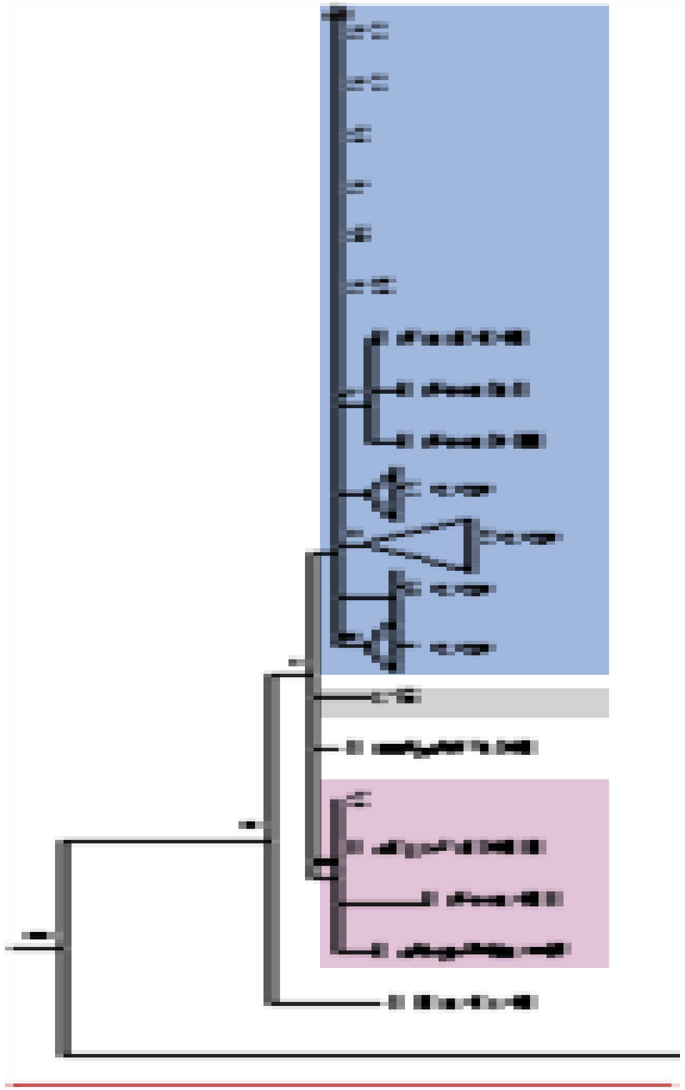
https://www.symplanta.com

From previous studies…

- The genus *Botrytis* is comprised of ascomycetous plant pathogenic fungi that are mostly specialists
- *B. cinerea* is a generalist pathogen that is believed to infect hundreds of plant species, including blueberries

- Has historically been classified as an exclusively necrotrophic pathogen

From previous studies…



- All isolates clustered with *B. cinerea* reference sequences, except two isolates (*B. pelargonii* and an unidentified *Botrytis*)

- No evidence that *B. cinerea* is an endophyte

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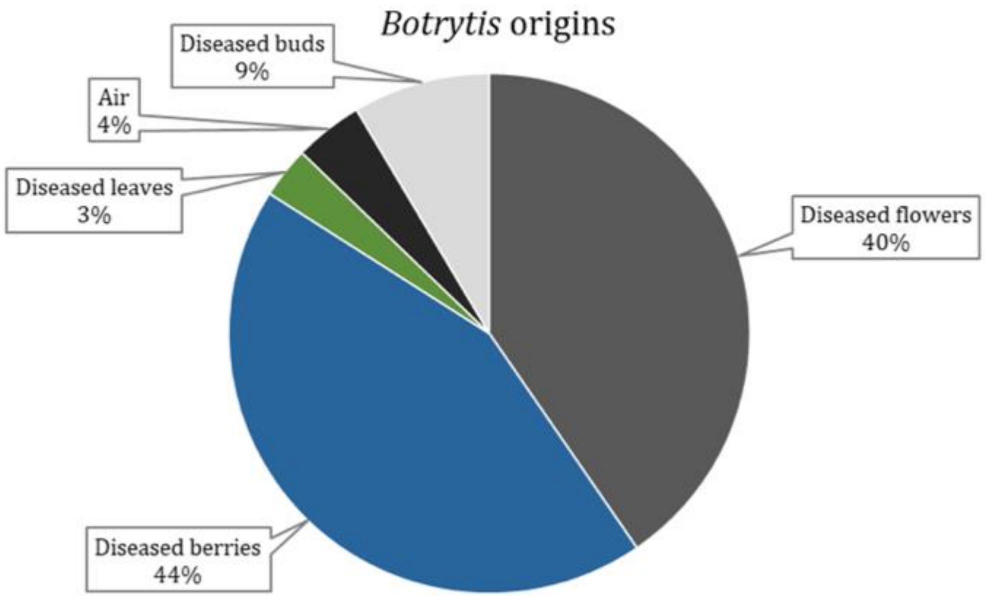

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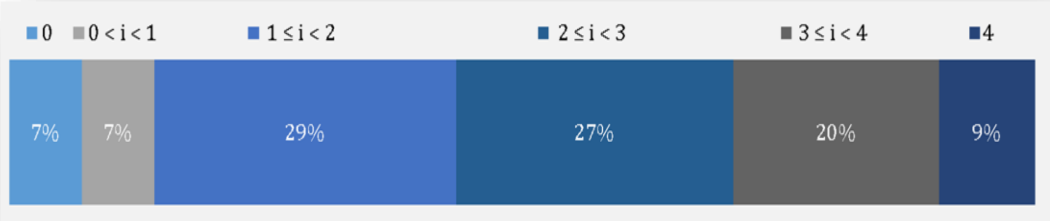

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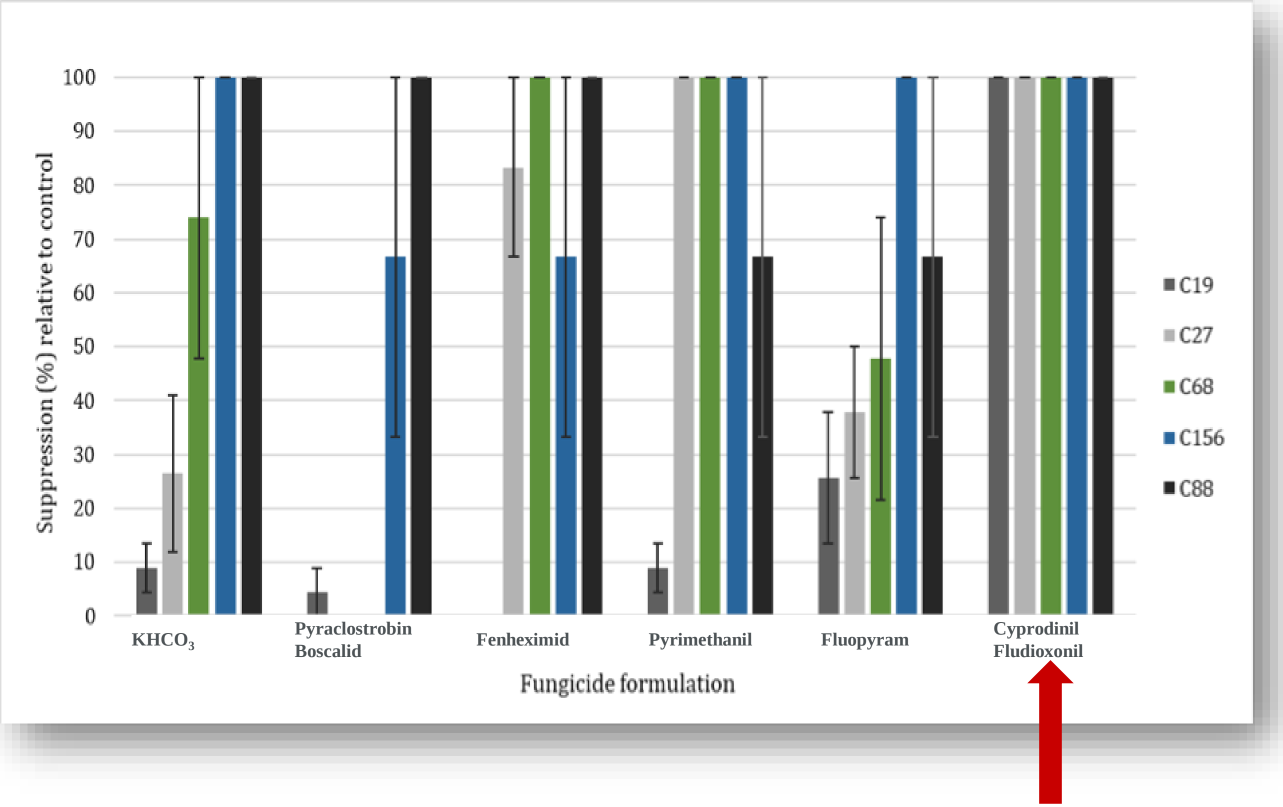
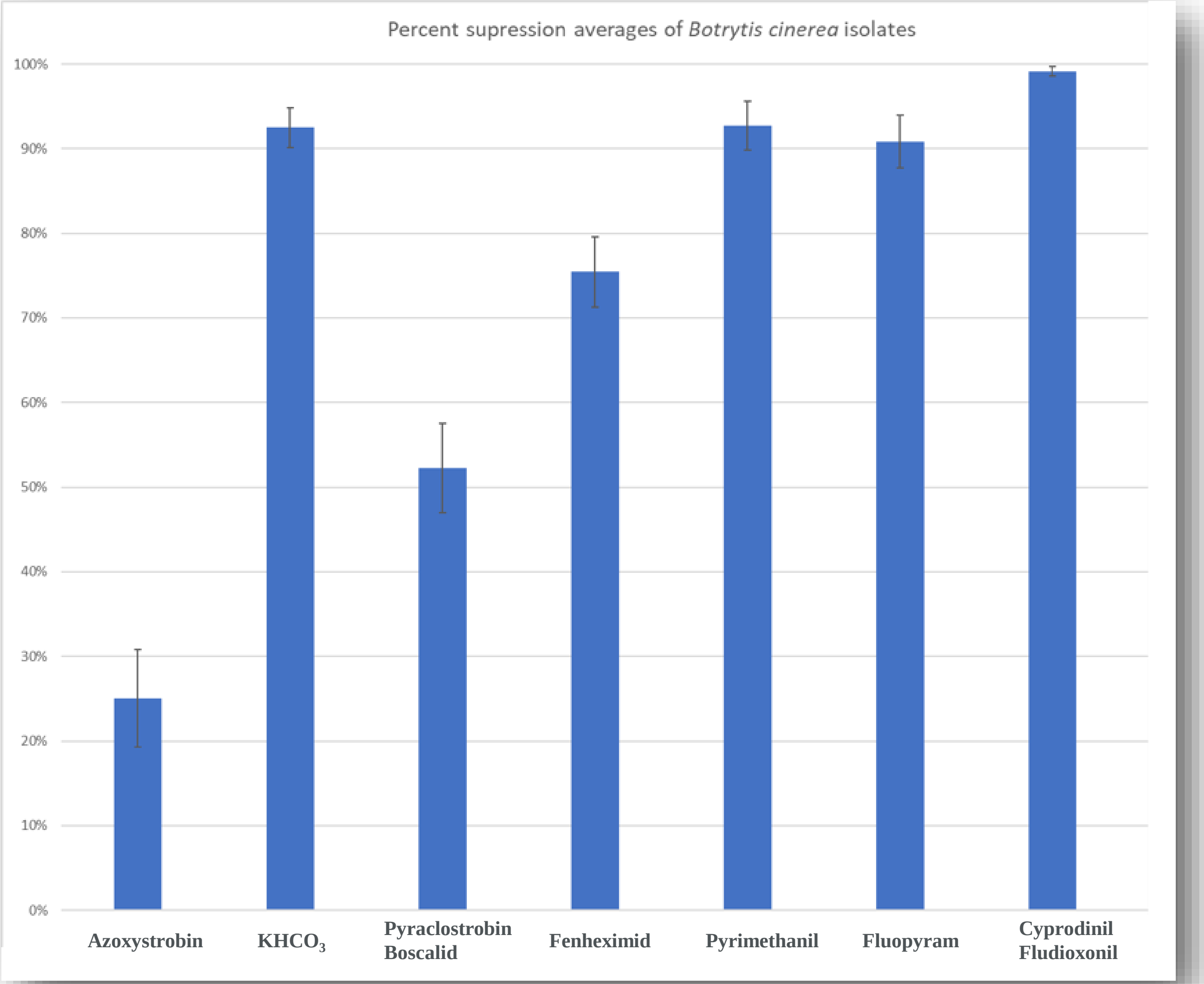
- No evidence that *B. cinerea* is an endophyte
- Most above-ground blueberry tissues are susceptible to infection by *B. cinerea*
- Majority of the strains collected from flowers and berries
- Majority of the strains are pathogenic

From previous studies…



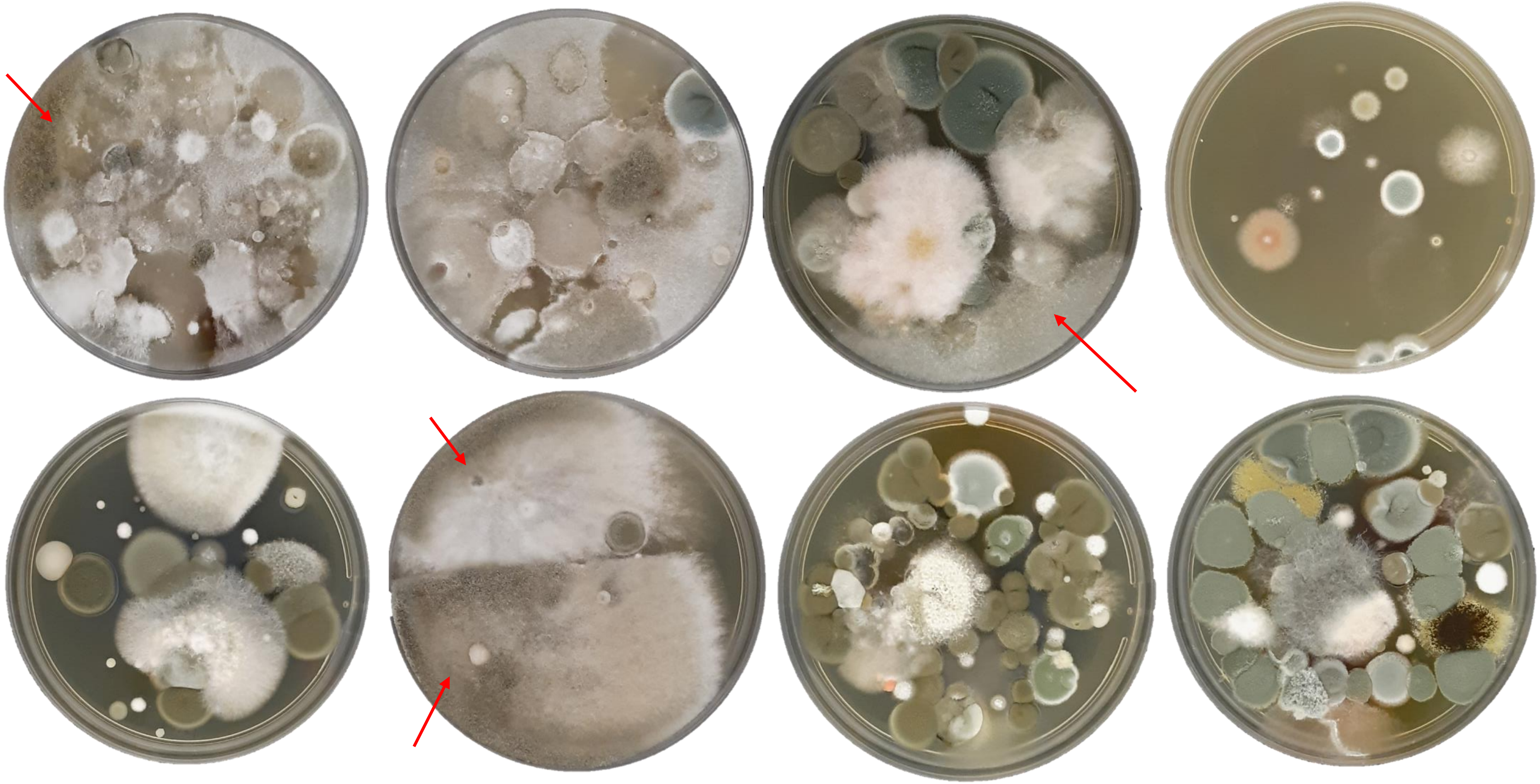
- Increasing frequency of multi-drug resistant *B. cinerea* strains
- Only one fungicide completely inhibited *B. cinerea* growth at the label recommended rates
  - Cyprodinil & Fludioxonil

Fungicide resistance



- Increased the number of strains
- Used a different methodology and added azoxystrobin
- Similar picture emerge
- Only one fungicide completely inhibited *B. cinerea* growth at the label recommended rates
  - Cyprodinil & Fludioxonil

Airborne *Botrytis* spore load



Airborne *Botrytis* spore load

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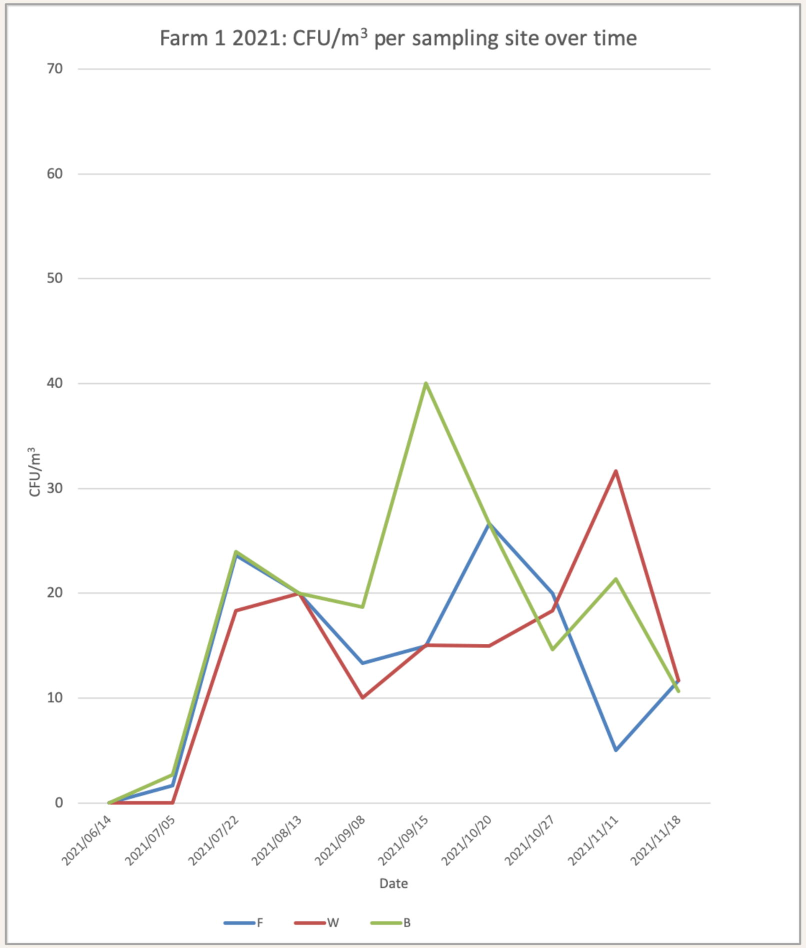

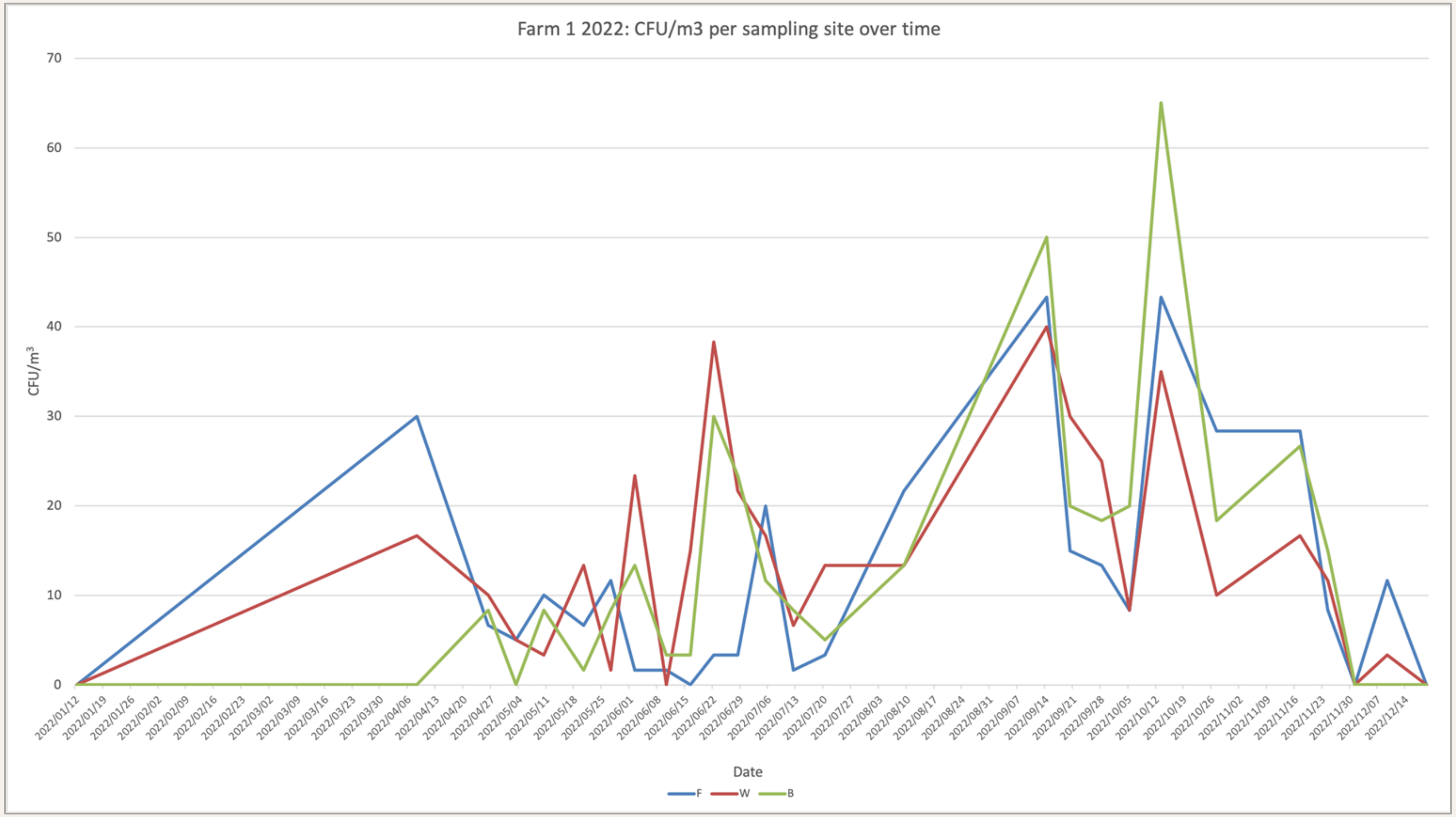

Airborne *Botrytis* spore load

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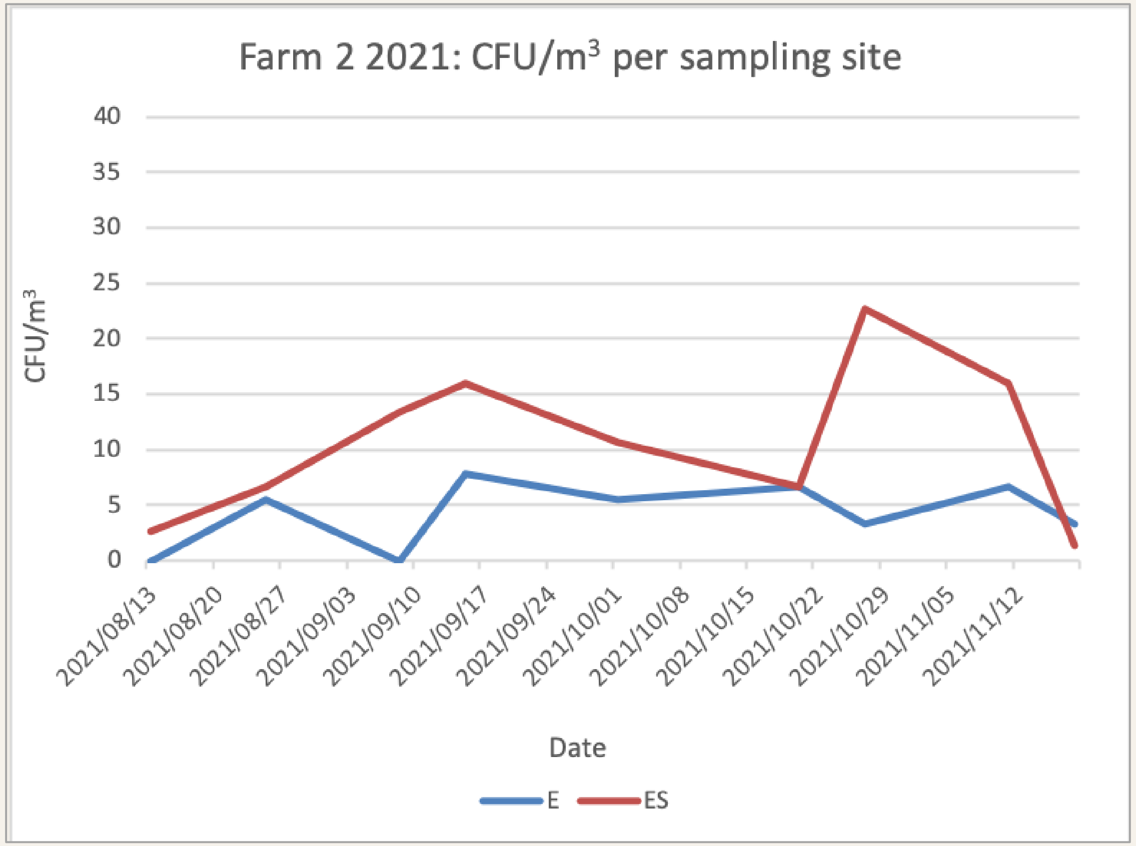
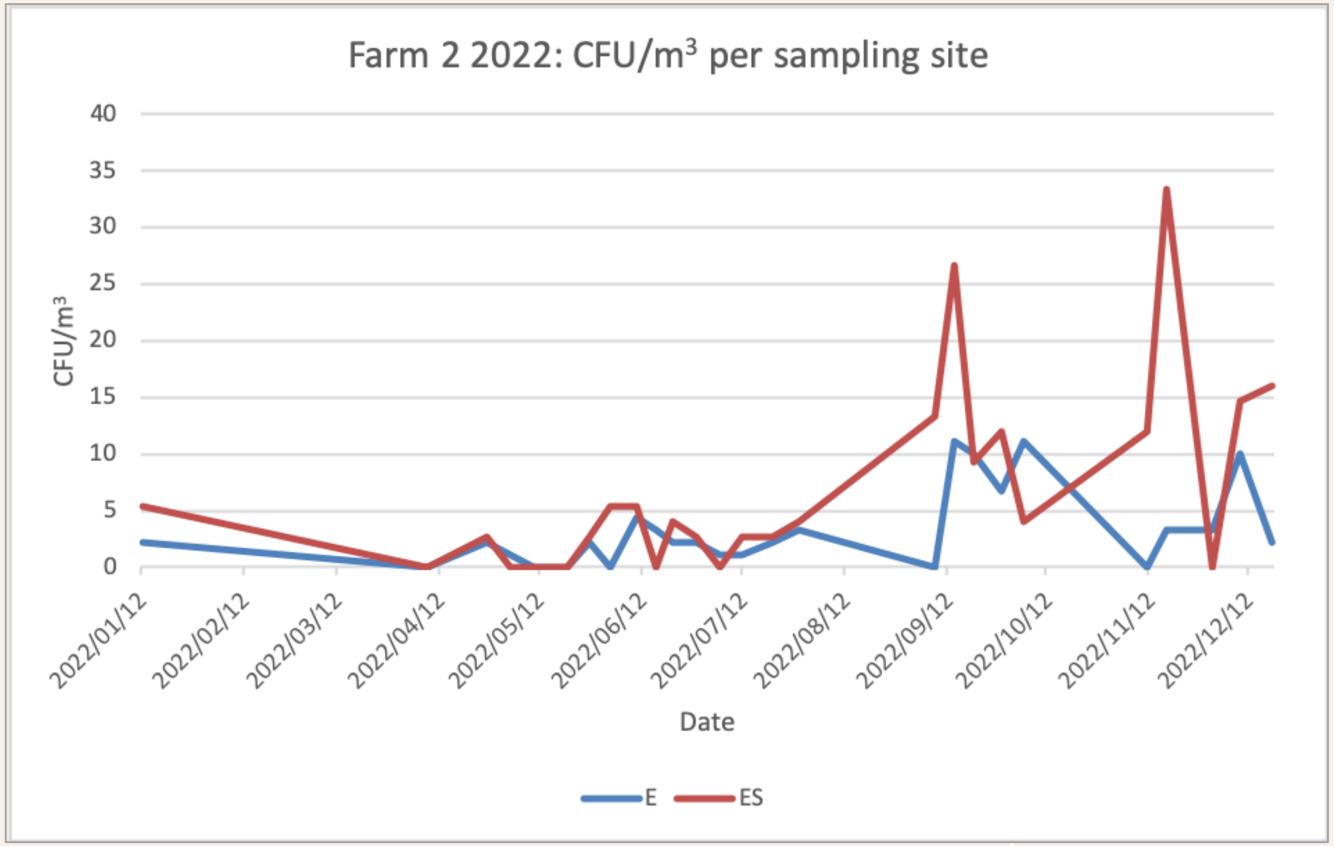


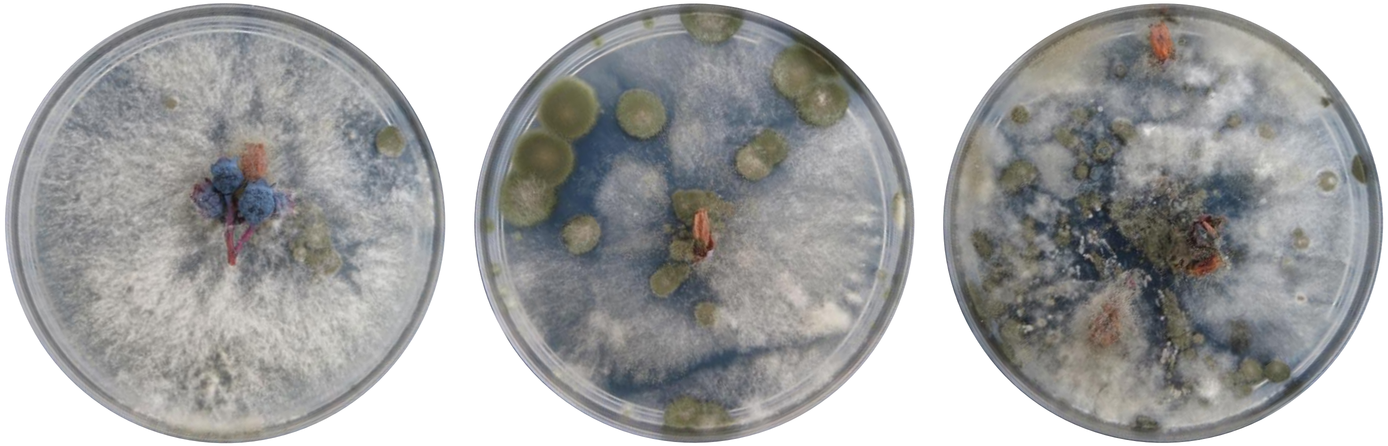


Looking ahead …..

- Develop a rapid screen for the presence of *Botrytis* spores on berries
- High through-put molecular screen for disease resistance using specific markers:
  - SdhB (succinate dehydrogenase B), SdhD (succinate dehydrogenase D) and CytB (cytochrome C) genes
- Understand the population genetics of *Botrytis* on blueberries

What is the story with *Cladosporium*?

- Consistent recovery of both *Botrytis* and *Cladosporium* isolates from the same diseased material



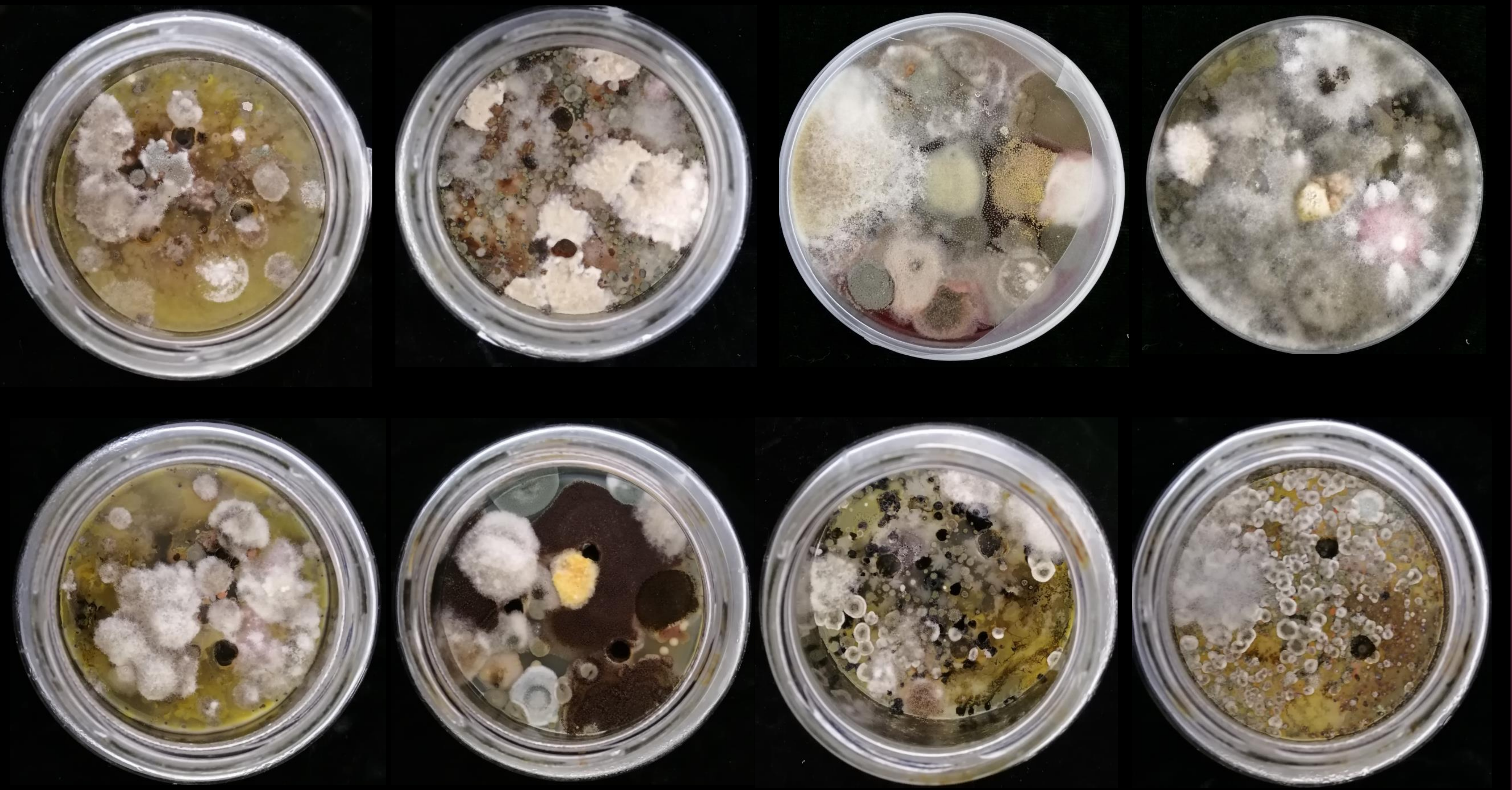
- This co-abundance suggests some form of interaction
  - commensalism?
  - mutualism?
  - antagonism?

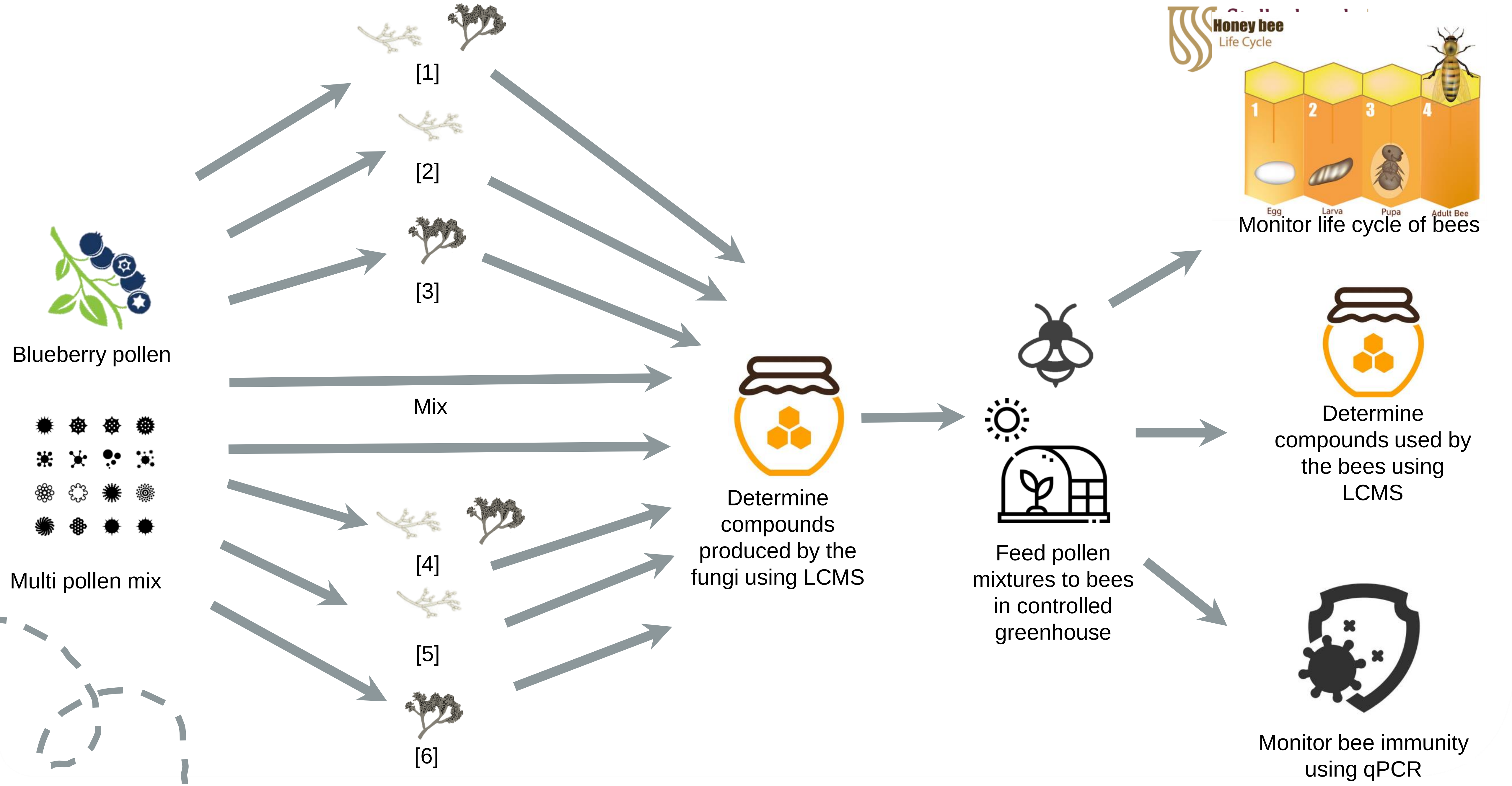
Some questions….

Are *B. cinerea* and *Cladosporium* spp. part of the external microbiome of bees foraging on blueberries?

How are *B. cinerea* and *Cladosporium* dispersed by bees and how does it compare with another key environmental factor namely wind?

Are there benefits for bees in collecting the *B. cinerea* and *Cladosporium* spores?





Blueberry pollen
Multi pollen mix
[1]
[2]
[3]
Mix
[4]
[5]
[6]
Determine compounds produced by the fungi using LCMS
Feed pollen mixtures to bees in controlled greenhouse
Honey bee
Life Cycle
1
2
3
4
Egg
Larva
Pupa
Adult Bee
Monitor life cycle of bees
Determine compounds used by the bees using LCMS
Monitor bee immunity using qPCR

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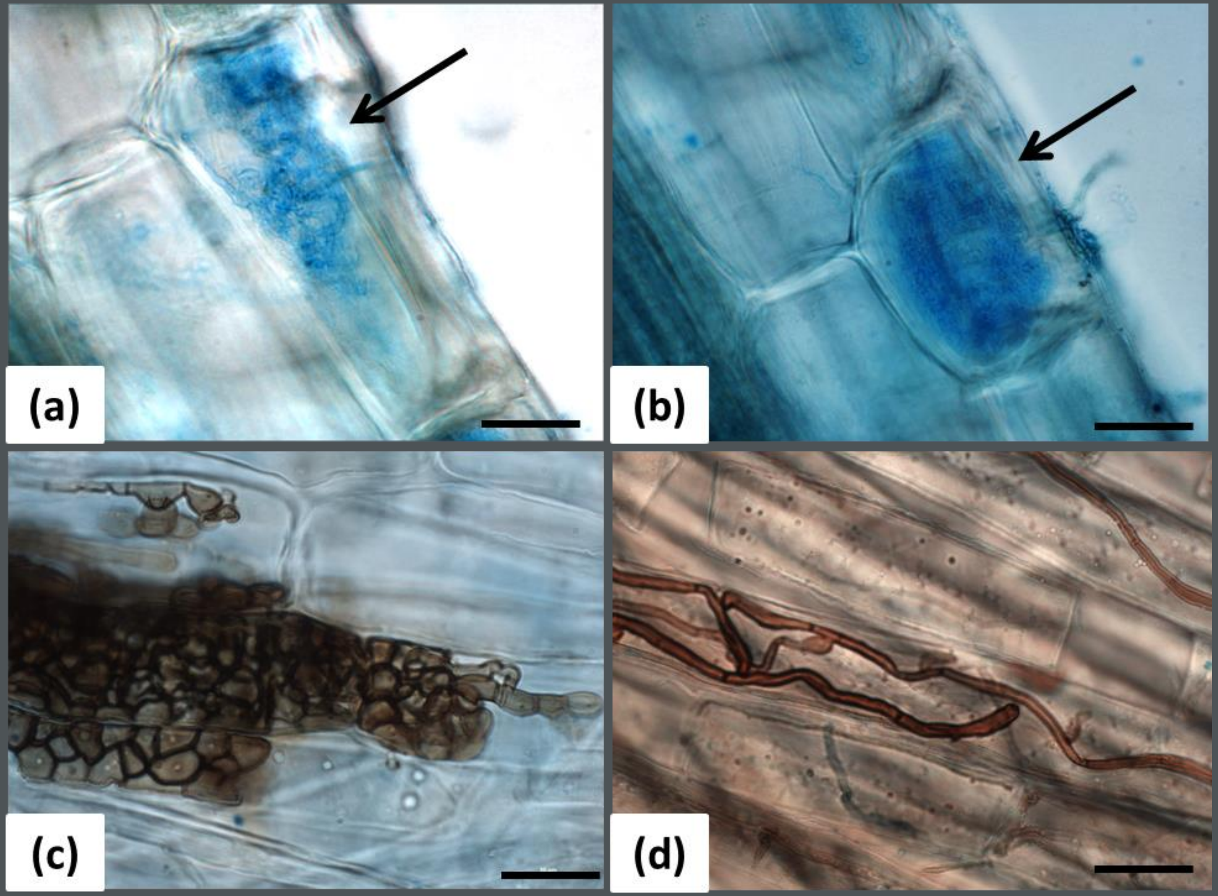
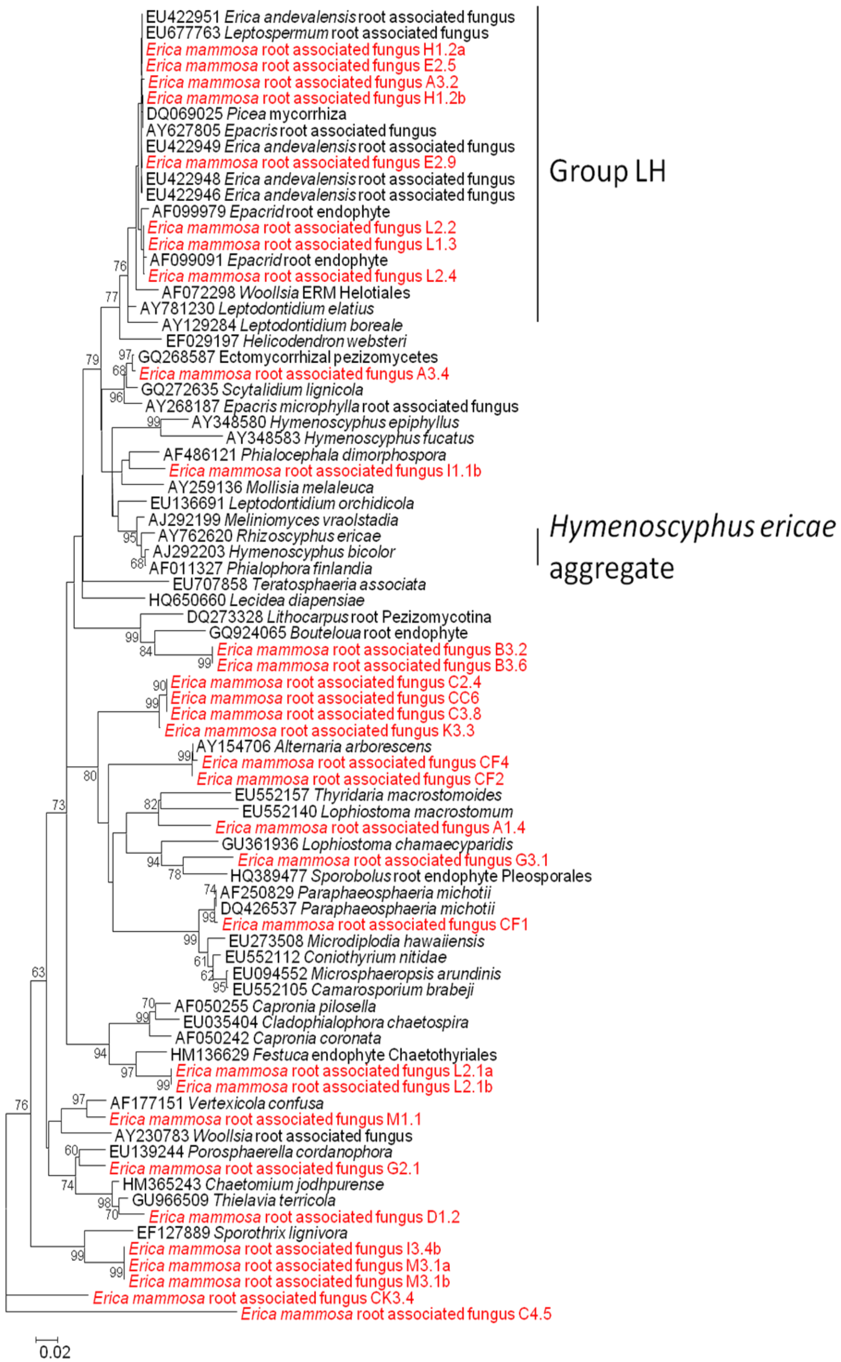
What about the roots?

- Healthy roots sustain yields and growth of the plant
- Mycorrhizal fungi are crucial in providing nutrients to the roots in nutrient poor soils
- Ericoid mycorrhizae are abundant in Fynbos soils
- Can be grown in pure culture

Mycorrhizae

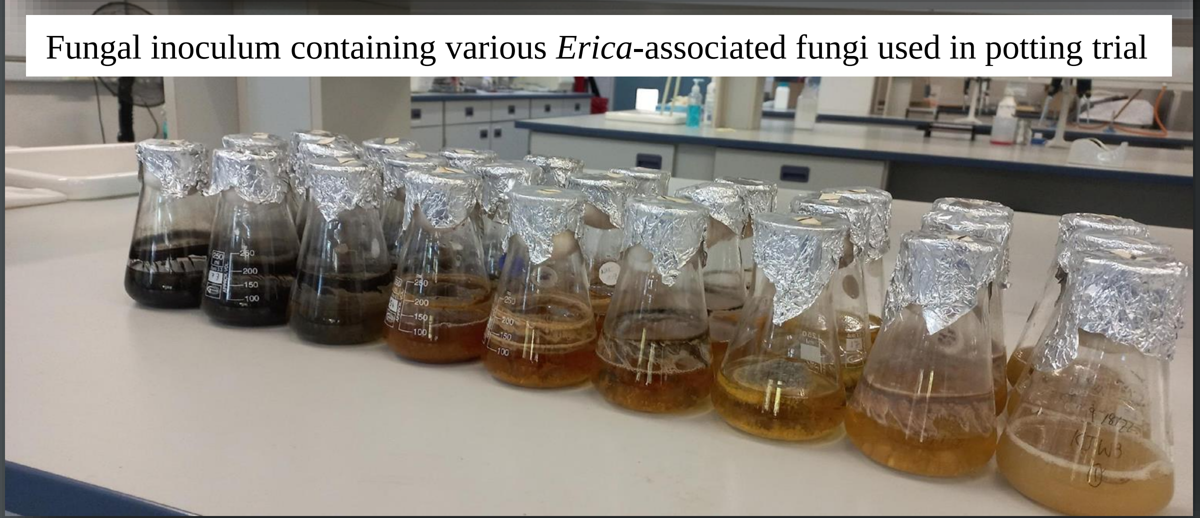




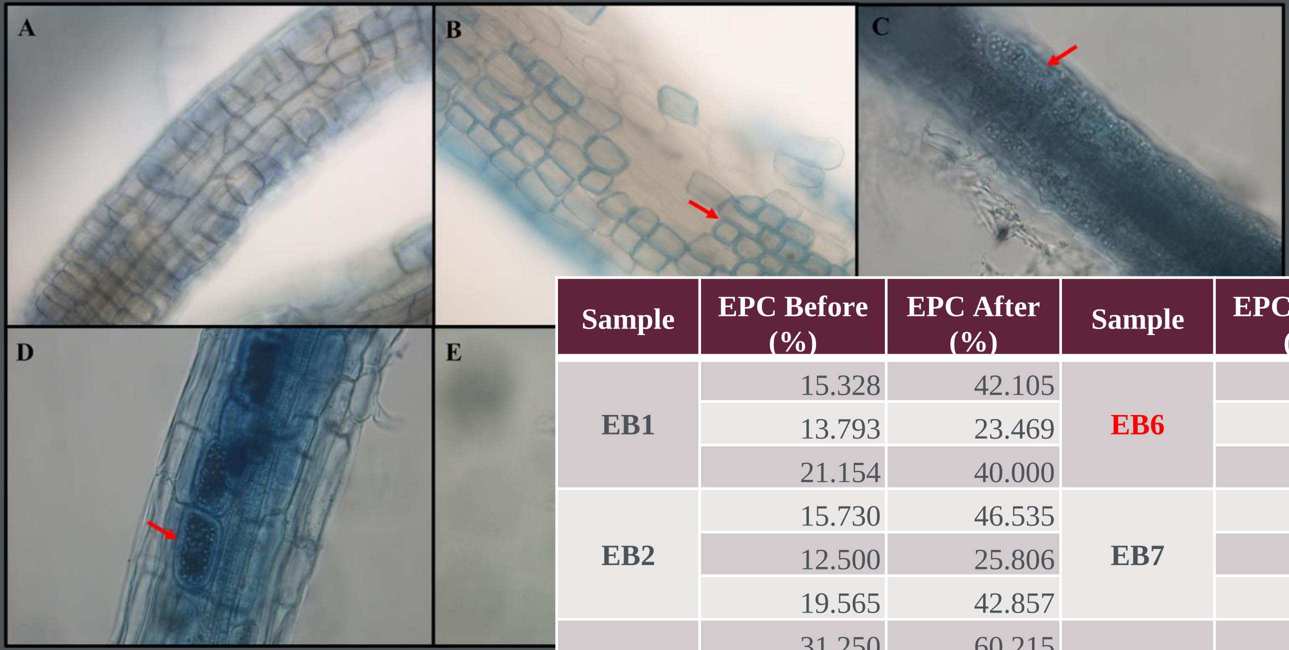
So we gave it a try….

- *Vaccinium corymbosum* 'Legacy' plants inoculated in triplicate with fungal inoculum isolated from the roots of *Erica* sp. The potting trial took place over 6 weeks
- Root samples taken before and after inoculation
- Estimated percentage colonisation (EPC) manually calculated using microscopic analysis


Vaccinium corymbosum 'Legacy' saplings used for the potting trial


Fungal inoculum containing various *Erica*-associated fungi used in potting trial

- EB3 (p<0,034), EB5 (p<0,017), EB6 (0,018) and EB9 (0,011) showed significant differences in their EPC compared to the control group



Photographs taken of root sections

Sample	EPC Before (%)	EPC After (%)	Sample	EPC Before (%)	EPC After (%)
EB1	15.328	42.105	EB6	17.857	53.922
	13.793	23.469		12.500	46.602
	21.154	40.000		7.595	41.509
EB2	15.730	46.535	EB7	12.281	31.395
	12.500	25.806		12.048	38.028
	19.565	42.857		8.511	44.792
EB3	31.250	60.215	EB8	22.430	45.455
	9.434	64.835		9.735	58.252
	14.706	36.735		9.649	22.449
EB4	20.000	44.318	EB9	16.514	48.760
	20.755	40.206		14.961	49.020
	11.429	34.146		23.622	43.617
EB5	9.483	46.154	EB10 (Control)	18.987	29.730
	19.626	53.333		10.204	22.340
	6.863	42.000		20.755	37.500

EPC for the blueberry replicates before and after inoculation of the roots.

Consider the ecology…

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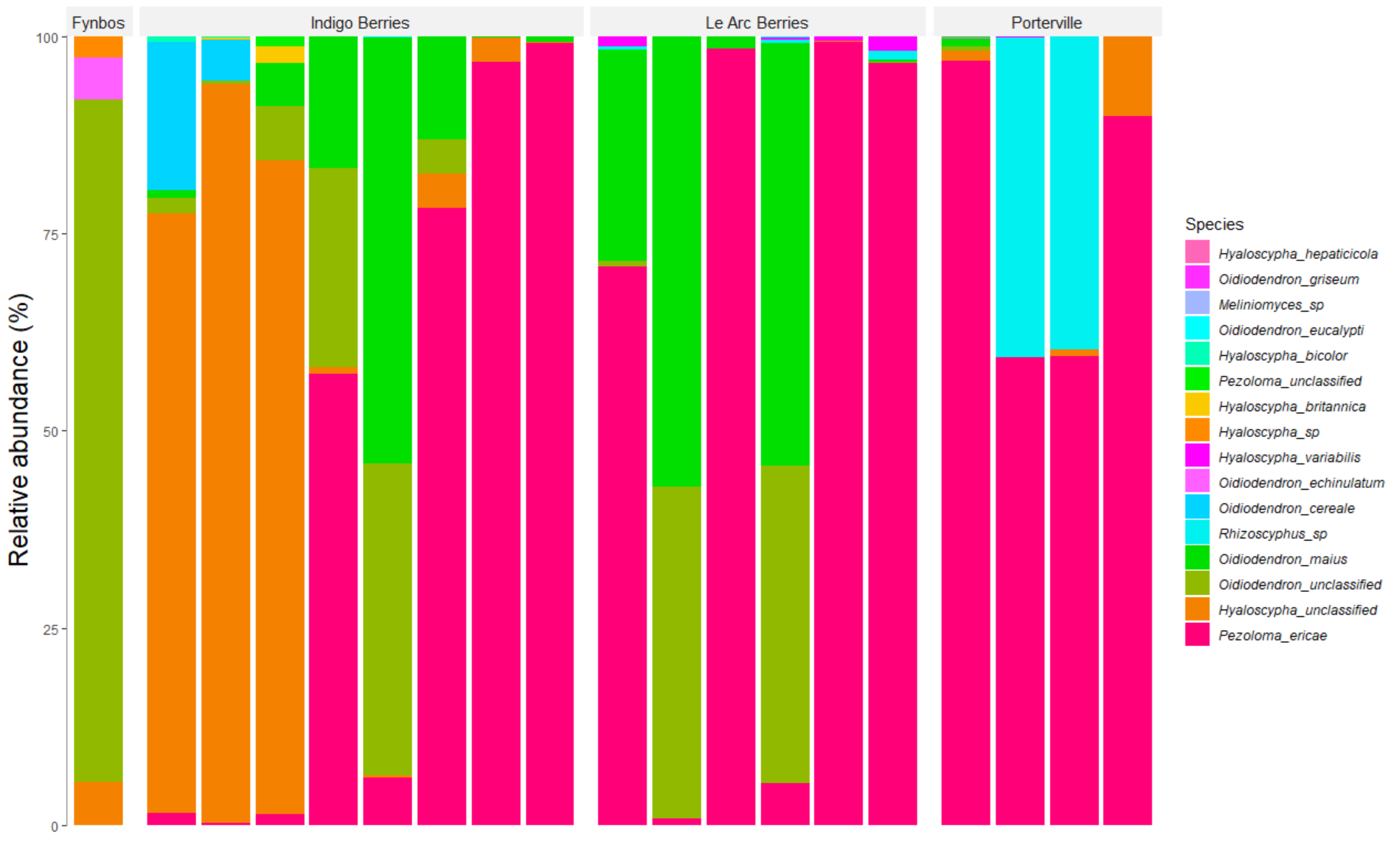
- Rhizosphere soil taken from blueberry orchards in the Western Cape
- Three different farms were sampled from various cultivars



Conclusion

- Relationship between blueberry and fungi is complex
- Use an ecological approach to understand this ecosystem
- Development of rapid detection methods are vital
- Provide a tool to manage the system for optimal growth and yield

https://lovepik.com/images/png-blueberries.html">Blueberries Png vectors by Lovepik.com

Thank you
Enkosi
Dankie