Endophytic *Colletotrichum fioriniae* as a possible inoculum source for bitter rot of apple

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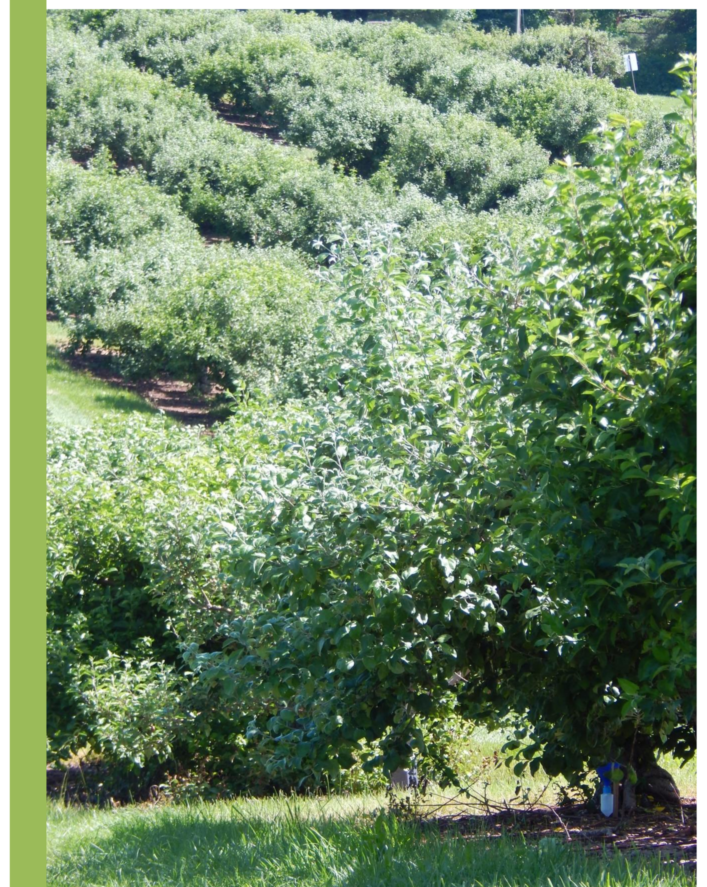
Background/ Hypothesis/ Central Questions

Colletotrichum fioriniae was historically identified as *C. acutatum* and is now considered a member of the *C. acutatum* species complex

C. fioriniae as a pathogen of apples

Major cause of summer rot of apples in the Northeast and Mid-Atlantic
Pennsylvania grows 23% of the region's 2 billion pound (~$500 million) annual apple crop
Historically a southern disease, bitter rot is an increasing challenge for apple growers in PA

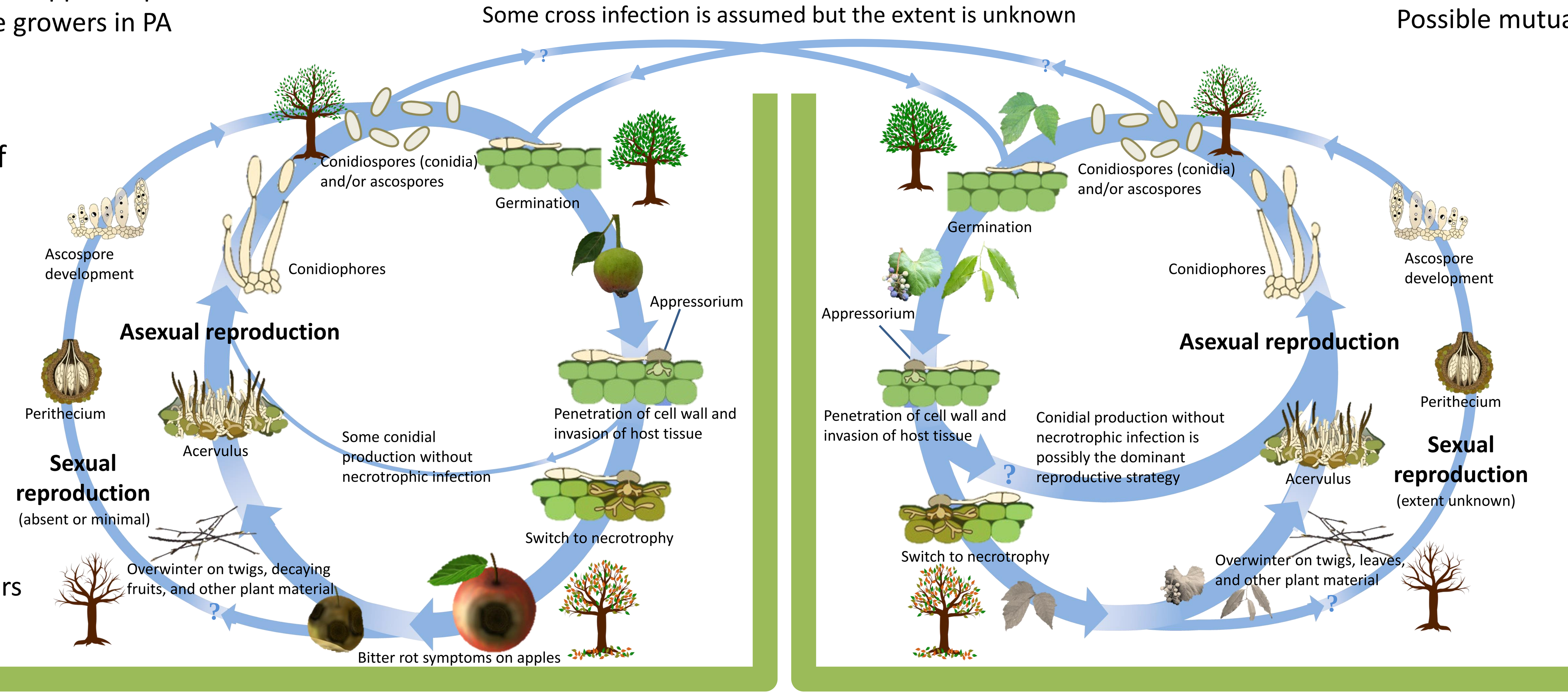


Pathogenic on a wide range of other fruits and vegetables

- Strawberries
- Celery
- Blueberries
- Cranberries
- Peaches
- Pepper
- Grapes
- Almonds
- Olives
- Tomato
- Weak pathogen on many others

Lifecycle on apples based on over a century of agricultural research

Lifecycle in the forest based mostly on observation



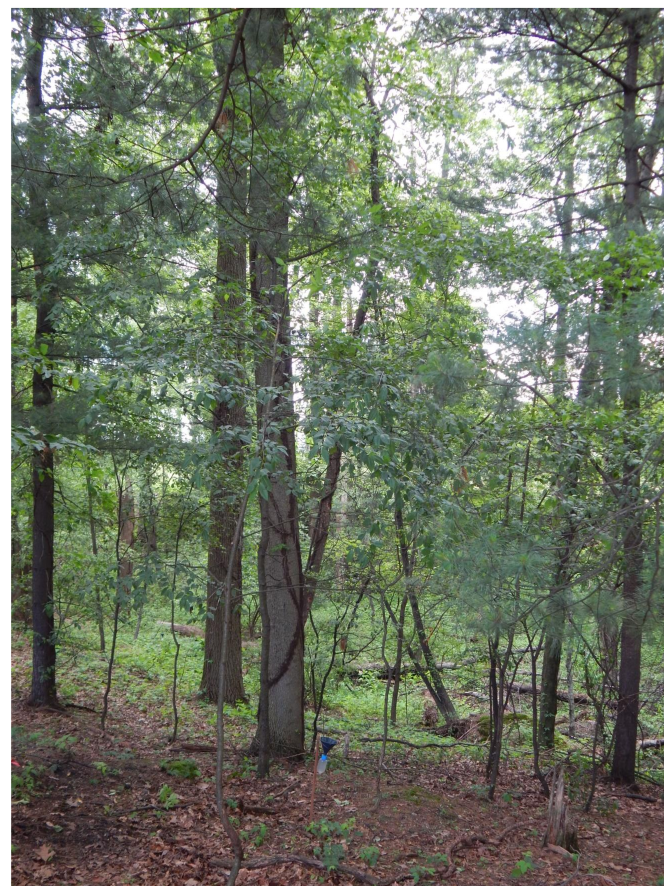
Based on the generalized *Colletotrichum* life cycle in De Silva et al. 2017

C. fioriniae as a forest endophyte

Natural role is largely unknown
Infects wild populations of the exotic scale insect *Fiorinae externa,* after which it is named
Possible mutualistic role with host plants as a defense against insect herbivory

Epiphytic and/or biotrophic on a wide range of broad leaved plants

- Dandelions
- Garlic Mustard
- Hemlock
- Poison Ivy
- Tulip Popular
- Asters
- Brambles
- Controlled studies show it can grow asymptomatically on most broad-leaved plants and some grasses



Experiments and results

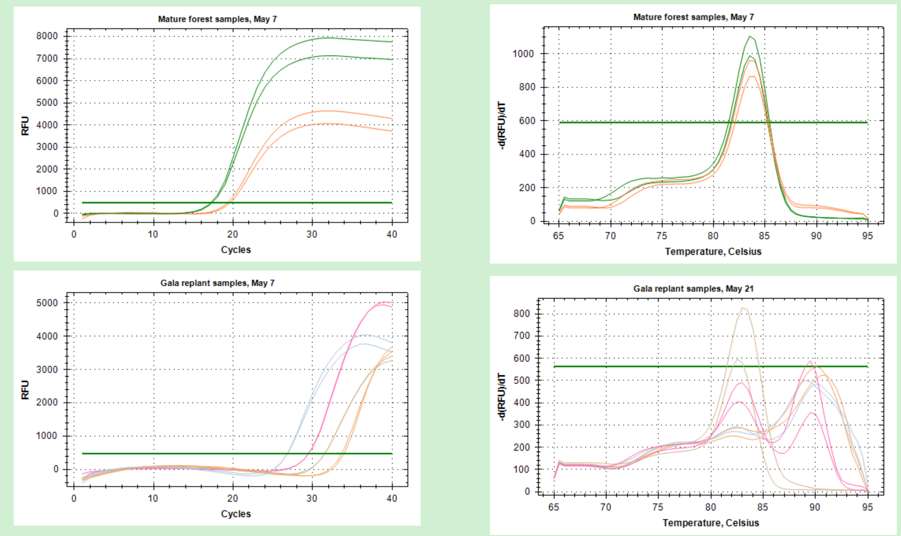
Detection of rain dispersed *C. fioriniae* conidia in orchard and forest environments with SYBR-Green q-PCR

Methods

1. Design and test q-PCR primers specific to *C. fioriniae*
2. Use the specific primers to detect *C. fioriniae* in orchard and forested environments
   a) Collect spores in rainwater
   b) Extract DNA
   c) Quantify with primers

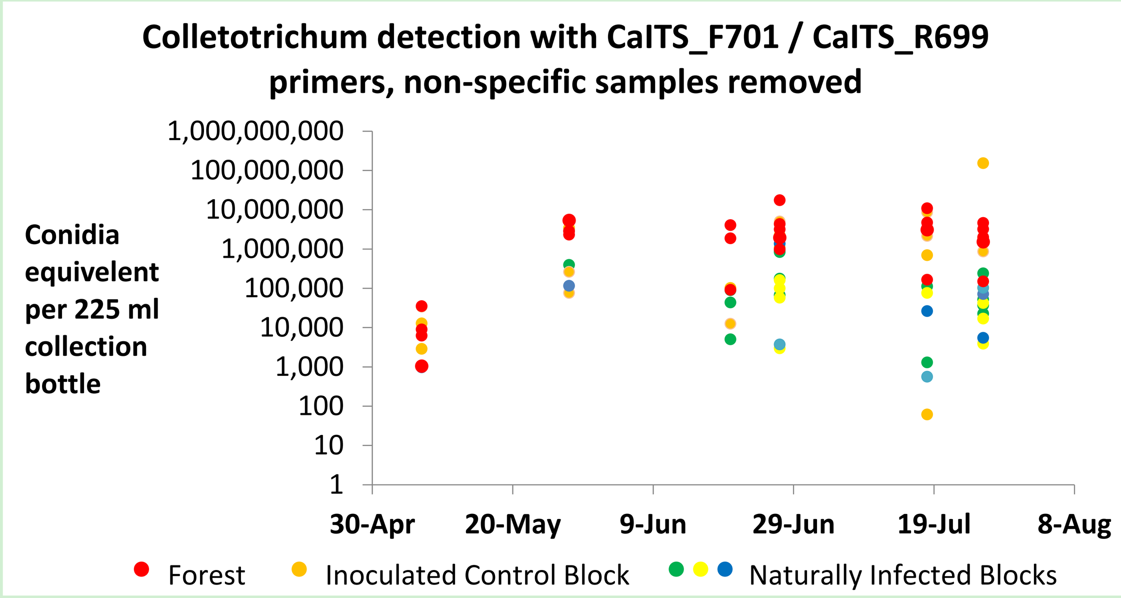
Results: Primer specificity

Non-specific binding with high Cq detections in all primers



Conclusion: •Design Taq-man assay instead of SYBR Green
• Use CaITS primers to get preliminary results
• Save environmental samples for retesting

Results: *C. fioriniae* in orchard and forest environments



Conclusion: Large amounts of conidia are dispersed in the forest. Forest plants should be sampled to determine the conidia source

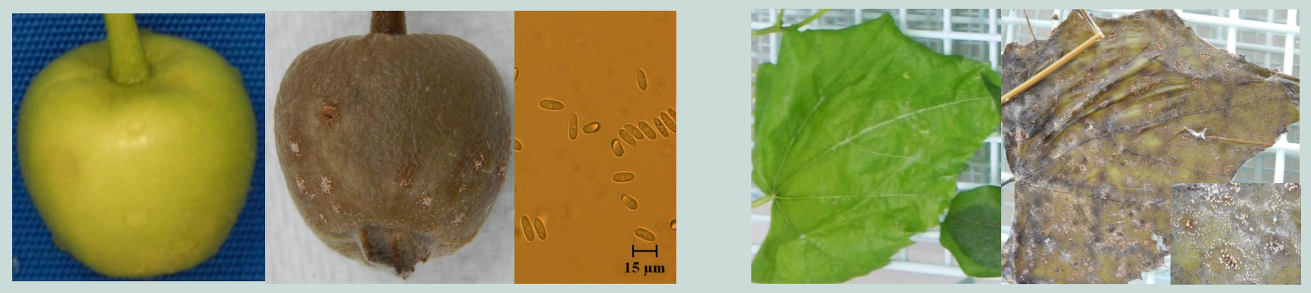
Detection of biotrophic *C. fioriniae* infections with disinfestation and freezing

Method:

1. Surface disinfest with bleach and ethanol
2. Freeze to kill the plant cells
3. Incubate in warm moist container
4. Observe for signs of *Colletotrichum* spp.

Plants tested June 2018	Results
Young apple fruits	Present
Poison Ivy leaves	Present
Japanese Honeysuckle leaves	Present
Wild Grape leaves	Present
Virginia Creeper leaves	Absent
Wild Cherry leaves	Absent

Conclusion: *Colletotrichum* spp. are present asymptomatically on many forest plants.



Apples (above left) and wild grape leaves (above right) before and after freezing and incubation

Japanese honeysuckle (left) and poison ivy leaves (right) after freezing and incubation

Experiments and results

Detection of biotrophic *C. fioriniae* infections with disinfestation and freezing
Are apple leaves a host of *Colletotrichum fioriniae?*

June 26th 2019, small scale study, 25 leaves each from inoculated untreated and un-inoculated fungicide treated trees.

Results:

% leaves with *Colletotrichum* spores	
On non-inoculated fungicide treated trees	0%
On inoculated untreated trees	42%

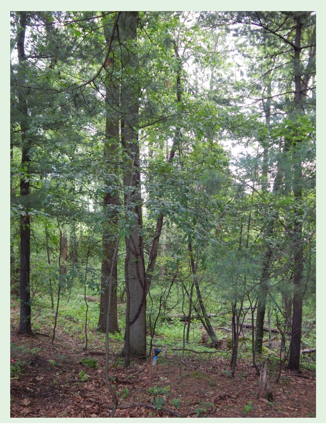
Discussion: Apple leaves are capable of hosting *Colletotrichum.* Fungicides appear to limit this, but more testing is needed to confirm.

Discussion

C. fioriniae as a forest epiphyte

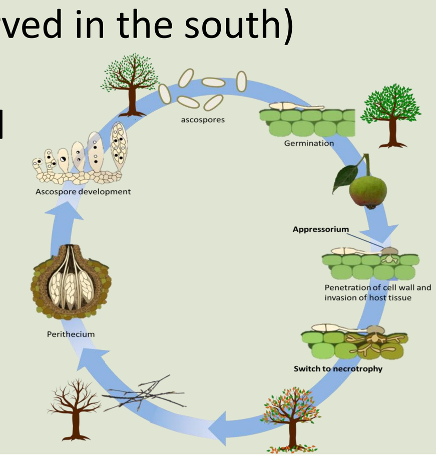
Natural role is largely unknown

Could have a role in the seed dispersal dynamics of poison ivy. The John Jelesko Lab at Virginia Tech discovered that poison ivy seedlings from seeds not scarified by passage through a birds digestive system often die from *C. fioriniae* infection.



Sexual reproduction in *C. fioriniae*

- Rarely if ever observed in nature
- Rarely observed in cultures
- Likely present at a low level in nature
- Could play an important role in the population structure
- Possibly temperature dependent (sexual stages in *Colletotrichum* are more frequently observed in the south)
- Importance could be inferred based on population allele frequencies



Metagenomic amplicon sequencing; a useful tool to elucidate *C. fioriniae's* role in the environment?

Feasibility

- *C. fioriniae* has a unique sequence variant in the ITS2 gene, making detection theoretically possible.

Requirements

- DNA sequencing with errors of < 1%.
- Single sequence variation data analysis

Advantages

- Placement of *C. fioriniae* in the context of the fungal population.
- Comparisons with other fungi.
- Identification of antagonists or symbionts.

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