The role of ascospores in the epidemiology of citrus black spot

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Citrus black spot (*Phyllosticta citricarpa*)

- First reported in Australia 110 years ago
- Widespread in hot and humid summer areas
- Premature fruit drop causes yield loss
- Frequent fungicide application put up cost
- Reducing cosmetic value of fresh fruit

EU quarantine - Limited market access
Disease epidemiology - What is reported?

- Ascospores formed in leaf litter
  – considered most important
- Conidia readily formed
  – secondary inoculum
- Spermata readily formed
  – unknown role

Diagrammed by Miles, AK.
Epidemiological role of ascospores?

- Never been produced *in vitro*
- *MAT1-1* and *MAT1-2* identified*—heterothallic?*
- Existing knowledge based on field material

*Wang N. et al., 2016 and Amorim R. et al., 2017.*
Do ascospores really play a role?
Epidemiological role of ascospores?

1. Can *P. citricarpa* sexually reproduce *in vitro*?
2. Do spermatia play a role?
3. Can ascospores cause disease?
4. Are ascospores source of inoculum in the field?
Epidemiological role of ascospores?

1. Can *P. citricarpa* sexually reproduce *in vitro*?
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4. Are ascospores source of inoculum in the field?
Sexual reproduction of *P. citricarpa* in vitro?

- “Sandwich” mating technique

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**Sexual Reproduction in the Citrus Black Spot Pathogen, *Phyllosticta citricarpa***

Nga T. Tran, Andrew K. Miles, Ralf G. Dietzgen, Megan M. Dewdney, Ke Zhang, Jeffrey A. Rollins, and André Drenth
Epidemiological role of ascospores?

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I’m a little dumbbell-shaped spermatium
Do spermatia play a role?

Spermatia mating technique

Ascospores successfully produced


YEAH, I now know what I do. I fertilise the receptive element!
Are the ascospores recombinants?

**Multilocus genotyping**

1. Collect single ascospores
2. Grow into culture
3. DNA extraction
4. Multilocus analysis
5. PCR of:
   - 2 SSR loci
   - Mating type locus
6. Sequence

75% recombinant for cross 1
40% recombinant for cross 2
59% recombinant for cross 3

*P. citricarpa* sexual cycle completed *in vitro* after > 100 years!
Epidemiological role of ascospores?

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Are ascospores pathogenic?

Leaf inoculation
(‘Troyer’ citrange)

Fruit inoculation
(‘Murcott’ tangor)
Are ascospores pathogenic?

Symptom produced

Pathogen recovered from symptom

Recombinants recovered after ascospore inoculation

First time, Koch’s postulates have been fulfilled for *P. citricarpa* ascospores
Ascospores are more pathogenic than conidia?

Disease incidence caused by ascospores within the range of conidia

Tran, NT, Miles, AK. Smith, MW, Dietzgen RG, Drenth A. 2017. Pathogenicity of P. citricarpa ascospores on Citrus spp., Plant Dis. Submitted
Epidemiological role of ascospores?

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Are ascospores source of inoculum in the field?

### Timing of infection trial 2014-17

<table>
<thead>
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<th>Fruit exposure (week from fruit set)</th>
<th>Weeks after fruit set</th>
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*: fruit exposed (unsprayed)

++: first fungicide application and +: re-application to prevent infection

### Ascospore monitoring 2014-17

Spore trap operating in the field
Are ascospores source of inoculum in the field?

Peak ascospore did not correspond with infection event
So…What we’ve found?

- **Ascospores:**
  - Can be formed *in vitro*
  - Are hybrids

- **Role in epidemiology:**
  - Cause disease and produce spores
  - Pathogenicity within the range of conidia
  - Timing of peak ascospore release?

- **Importance of ascospores?**
Acknowledgements

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Malcolm Smith and the team
Megan Dewdney and the team

Horticulture Innovation Australia
We’ll be this PERFECT! Thanks Team Citrus!

Thank you for listening!