Clubroot Management Update

Clubroot Steering Committee
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Clubroot Management:

- Whole genome sequencing and new virulent pathotypes (published)
- Effect of grass cover crops and rotation crops on resting spore concentrations in soil
- Fumigation and solarization
- Boron as a soil amendment with boron insensitive Brassicas
Whole genome sequencing to determine the genome similarity of single-spore isolates and field collections from locations in Canada, the USA, and China:

Sequenced 43 collections, including 9 single spore isolates, mostly from Canada. They did not cluster by pathotype or host. Some clustered by geographic region.
Heat maps of SNPs

Total of 9727 genes in *P. brassicae* genome

Normandin and MCRS collections before and after the change of pathotype

There is about a 50% difference in SNPs from before and after

Selection, rather than single mutations responsible for the changes

Balancing selection
Capturing single resting spores with a micromanipulator- a more efficient method to produce single spore isolates of *P. brassicae*

**Micromanipulation of a single spore**

(A) inverted microscope, (B) glass micropipette, C. micromanipulator, & D. isolation plate

(D) selection and collection of a single spore, and (E) placement of a single spore in Hoagland’s solution containing a 3-day old canola
Cover crops and rotation crops to stimulate the germination of resting spores

Materials & methods

• Soil with $5 \times 10^5$ resting spore per gram
• Crops grown for 8 weeks
• qPCR assessment of resting spores

Crops:

• Shanghai pak choi (Brassica rapa L.) susceptible check
• Smooth bromegrass (Bromus inermis L.) a common seed lot
• Meadow bromegrass (B. riparius R.) cv. Fleet
• Perennial ryegrass (Lolium perenne L.) cv.’s Norlea, All Star, and Fiesta

Afsaneh Sedaghatish Ph.D. thesis
Effect of grass species and cultivar on resting spore concentration of *P. brassicae* in soil (Based on qPCR, n = 6)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grass cultivar</th>
<th>Spore conc. (spores g⁻¹ soil)</th>
<th>Root dry wt. (g pot⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-plant soil</td>
<td></td>
<td>1.6 x 10⁶</td>
<td></td>
</tr>
<tr>
<td>No plant (control)</td>
<td></td>
<td>1.2 x 10⁶ a</td>
<td></td>
</tr>
<tr>
<td>Perennial ryegrass</td>
<td>Norlea</td>
<td>5.9 x 10⁵ a</td>
<td>6.35 a</td>
</tr>
<tr>
<td></td>
<td>All Star</td>
<td>4.9 x 10⁵ a</td>
<td>6.32 a</td>
</tr>
<tr>
<td></td>
<td>Fiesta</td>
<td>2.7 x 10⁵ b</td>
<td>2.73 b</td>
</tr>
<tr>
<td>Meadow bromegrass</td>
<td>Fleet</td>
<td>5.0 x 10⁵ b</td>
<td>6.44 a</td>
</tr>
<tr>
<td>Smooth bromegrass</td>
<td>Common</td>
<td>4.6 x 10⁵ b</td>
<td>3.85 b</td>
</tr>
</tbody>
</table>

The initial resting spore concentration was higher than intended
No correlation between resting spore concentration and root weight
## What about rotation crops?

### Resting spore concentration in soil with different crops

<table>
<thead>
<tr>
<th>Crop</th>
<th>Cultivar</th>
<th>Spore conc. g⁻¹ soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td></td>
<td>469,000 a</td>
</tr>
<tr>
<td><strong>No plant (control)</strong></td>
<td></td>
<td>310,000 b</td>
</tr>
<tr>
<td>Barley</td>
<td>Trochu</td>
<td>266,000 bc</td>
</tr>
<tr>
<td>Field pea</td>
<td>CDC Meadow</td>
<td>229,000 bc</td>
</tr>
<tr>
<td>Ryegrass</td>
<td>Norlea</td>
<td>183,000 bc</td>
</tr>
<tr>
<td>Wheat</td>
<td>AAC Connery</td>
<td>155,000 c</td>
</tr>
</tbody>
</table>

Plants grown for 8 weeks in the soil inoculated with $5 \times 10^5$ resting spores mL⁻¹ based on qPCR (n = 6).

Spring wheat is a good rotation crop and may help to reduce resting spore numbers. Still lots of variability in the data.
For a quicker effect: Fumigation and/or solarisation
Or boron?

Fumigated in late June or July
Chloropicrin (Pic Plus 164, 280 L/ha)
Metam sodium (Busan 150, 300 L/ha)
Immediately covered with totally impermeable film (TIF)

Uncovered check and untreated- tarped check

After 2 weeks, the tarp was removed, soil samples taken and a susceptible crop- pak choi – was seeded. Assessed 5 weeks later.
Bioassay with clubroot susceptible pak choi

Untreated untarped

Fumigated
Clubroot severity in pak choy following fumigation -2019

- **Solarisation?**
  - Ave temp in untarped plot 21.8 °C
  - Ave temp in tarped plot 29.1 °C
  - Temperatures are lower than published for solarization

- **Anaerobic soil disinfestation?**

**Severity (0-100)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>b</td>
</tr>
<tr>
<td>Busan high</td>
<td>a</td>
</tr>
<tr>
<td>TIF</td>
<td>a</td>
</tr>
<tr>
<td>Busan low</td>
<td>a</td>
</tr>
<tr>
<td>PicPlus low</td>
<td>a</td>
</tr>
<tr>
<td>PicPlus high</td>
<td>a</td>
</tr>
</tbody>
</table>

**Treatment explanation**
- **Busan** = metam sodium
- **PicPlus** = chloropicrin
Boron suppresses clubroot development
But can be phytotoxic
Use boron with boron insensitive varieties?
Effect of a drench application of boron at 8 kg/ha on clubroot severity in the field. Mean of 10 sensitive and 9 insensitive lines.

Next step: Assess plants in the synchrotron (Canada Light Source) to determine boron content of roots and leaves.
Boron K-edge XANES spectra collected on roots

Changes in spectra with added B indicate more boron-oxygen bonds
Clubroot Management: Conclusions

- Grass cover crops and rotation crops may reduce resting spores in soil faster than if soil was left fallow
  - However, the first results from field trials showed higher resting spores under perennial ryegrass

- New virulent pathotypes are selected from existing genotypes (not recent mutations)
- P. brassica exhibits balancing selection to preserve many genotypes
  - Continue to develop single spore isolates for research

- Solarization using totally impermeable film could be an approach to manage small patches of clubroot.

- Could boron be used to suppress clubroot using boron insensitive lines of B. napus?
Questions?
Acknowledgements

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