Crop rotation, cultivar resistance, and biofungicide for clubroot control on canola

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Resistance is the cornerstone for clubroot management on canola

- Allowed canola to be grown again in fields with extremely high levels of pathogen inoculum only a few years ago
- Significantly better crops and higher yields than a cv. -in heavily infested fields
- Less amount of pathogen inoculum going back into the soil
Resistance ....but not “Immunity”

- \( R \) genes are race specific. May be eroded with shifting in pathogen race structure
- Clubroot severity increased when a \( R \) cv. was exposed repeatedly to same pathogen population (LeBoldus et al., 2012)
- small, spheroid, resistant-type galls (Osaki et al. 2008)
- Limited \( R \) sources
- Resistance stewardship

Additional measures helpful?
Crop Rotation

- Benefits to crop production are well recognized
- Important disease management tool for many field crops – for example, blackleg of canola in western Canada
- A 3-year rotation (canola – cereal - pulse) is considered sustainable (Cathcart et al., 2006), but a 2-year rotation of canola with a cereal crop or even continuous canola is no longer uncommon (Hartman, 2012)
- Is 3- or 4-yr crop rotation effective for clubroot control?
Canola Cropping Frequency in Black DG west soil zone based on AFSC data

- Red: Canola on canola
- Yellow: 1 year break
- Green: 2
- Blue: 3
- Light Blue: 4+
- Black: SF

Records

<table>
<thead>
<tr>
<th>Year</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
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<tbody>
<tr>
<td></td>
<td>150</td>
<td>100</td>
<td>250</td>
<td>200</td>
<td>250</td>
<td>150</td>
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</table>
Impact of crop rotation on *P. brassicae* resting spores in soils

- Based on bioassay results, the ‘half life’ of *P. brassicae* resting spores in field soils was estimated at about 4-5 years (Wallenhammar, 1996; Hwang *et al.*, 2013)

- In micro plots based on disease severity, a faster rate of decline of *P. brassicae* resting spores was indicated when non-host crops or fallow was used for 1-3 years (Robak, 1994)

- There has been no information on the effect of a break from canola to alleviate clubroot impact (crop development and yield) in field

  - sufficiently effective for reducing pathogen inoculum and clubroot severity?

- qPCR has been developed for direct enumeration of resting spores in soils (Wallenhammar *et al.*, 2012; Rennie *et al.*, 2011)
When the pathogen inoculum is reduced in the soil

- Reducing pathogen resting spores in the soil by 10-fold substantially lowered the clubroot severity under controlled conditions.
- Can crop rotation result in such a significant reduction in pathogen inoculum under fielded conditions?
Chemical/biological control?


**No information on large-acreage crops like canola**

**Work conducted lately in Canada**

- 5,000 indigenous soil microbes were assessed for the potential of clubroot control.
- Applied as a soil drench, and efficacy compared with biological and synthetic fungicides registered in Canada or USA.
### Efficacy of indigenous microbes

#### Efficacy of soil microbes against clubroot on canola

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Range of clubroot reduction (%)*</th>
<th>26-50</th>
<th>50-75</th>
<th>75-100</th>
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<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endophyte</td>
<td>7**</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Rhizosphere</td>
<td>13</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endophyte</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Rhizosphere</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
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</table>

*Compared to the pathogen control in the same trial

**Number of isolates in the category

The indigenous candidates were less consistent than biofungicides under controlled conditions.
Biofungicides & fungicides

- **Serenade** (*Bacillus subtilis*)
- **Prestop** (*Clonostachys rosea*)
- **Allegro** (Fluazinam)
- **Ranman** (Cyazofamid)

Effective when applied as a liquid under controlled-environment conditions
Biofungicide treatment (soil drench)

Pathogen control

Pathogen + biofungicide
Modes of action for biofungicides

- CK Filtrate Spores Product

Filtrate doesn’t kill resting spores

Water (control)  
Serenade product  
Fluazinam

Filtrate Spores Product

Resting spore germination (%) vs Time (days)
Up regulation: Phenylpropanoid (phenylalanine ammonia lyase- PAL), jasmonic-acid & ethylene pathways

- Phenylpropanoid (BnOPCL, BnCCR)
- Ethylene (BnSAM3 and BnACO)
- Auxin (BnAA01)
- Jasmonic acid (BnOPR2)
Defense responses were also induced in canola leaves where the infection by *Leptosphaeria maculans* was delayed for 12 days.
Field application of fungicides/biofungicides

- Liquid formulation
- in-furrow
- 500 L/ha

Poor efficacy against clubroot on canola
Biofungicide x cultivar resistance (n=8)

In controlled conditions

- Disease severity index (%)
  - Untreated
  - Serenade

Canola cultivar:
- Susceptible
- M. resistance
- H. resistance
Granular formulation of *Bacillus subtilis*

- Deliver maximum amounts of *Bacillus subtilis* “spores” (50 kg formulation/ha)
- Ease of application (with seeding)
- Cost effectiveness
I. Fungicide/biofungicide formulation x resistance (Leduc & Edmonton, AB; Normandin, QC)

- Cultivar resistance was highly effective: Clubroot severity was reduced and yield increased
- None of the fungicide or biofungicide treatments was effective, and there was no treatment by cultivar interaction
- The same trend was with all three trials
Biofungicide seed treatment

- Seed dressing with the *Bacillus subtilis* biofungicide
- Moderately suppressive to clubroot at low pathogen inoculum pressure *(not a stand-alone option)*
- Using the commercial seed treatment formulation L1782
- Low to very high titre at 4 equal increment rates *(1 × 10^5 to 5 × 10^6 cfu/seed)*
II. Crop rotation x biofungicide

Crop rotations:
1. Canola-barley-canola (1-year break)
2. Canola-barley-field pea-barley-canola (3-year break)
3. Continuous barley (11-year break, for comparison only)

Biofungicide (B. subtilis) seed treatment
At low, medium, high, and very high rates to a susceptible cultivar

Assessment:
- Impact of crop rotation on resting spores in soil – Bioassay, qPCR
- Soil test/fertilization, seedling counts, flea beetle control
- Clubroot severity (0-3) at late flowering
- Impact on crop development (0-4) during ripening
- Seed yield
III. Crop rotation x cultivar resistance

Crop rotations:
1. Continuous canola (no break)
2. Canola-barley-canola (1-year break)
3. Canola-barley-pea-canola (2-year break)
4. Canola-barley-pea-barely-canola (3-year break)
5. Canola-barley-pea-barely-fallow (4-year break)

Canola cultivar:
1. 45H26 – susceptible (S)
2. 45H29 – resistant (R)
3. InVigor 5030 – moderately resistant (MR/MS)

Assessment:
- B. brassicae inoculum in soil – qPCR (direct quantification)
- Soil test/fertilization, seedling counts, flea beetle control
- Clubroot severity (0-3), crop impact (0-4), and seed yield
### Results

#### I. Effect of crop rotation on *P. brassicae* inoculum in soil

- **a. Bioassay of soil samples**
- **b. Early pathogen development in roots (qPCR, 2011)**

<table>
<thead>
<tr>
<th>Crop rotation (Years of break)</th>
<th>Bioassay (DSI%)</th>
<th>qPCR (ng/g fresh root)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Field trial 1</td>
<td>Field trial 2</td>
</tr>
<tr>
<td>1 year</td>
<td>74.8 a</td>
<td>11.6 a</td>
</tr>
<tr>
<td>3 years</td>
<td>47.0 b</td>
<td>7.3 b</td>
</tr>
<tr>
<td>11 years</td>
<td>28.3 c</td>
<td>8.7 b</td>
</tr>
</tbody>
</table>

*a* Soil samples were taken prior to trials and root samples were from non-treated control plots 4 weeks after seeding

**Both methods were indirect**
**Direct estimate of *P. brassicae* resting spores in soil using qPCR (2012)**

<table>
<thead>
<tr>
<th>A break from canola (year)</th>
<th>Resting spores /g soil $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$2.7 \times 10^6$ bc</td>
</tr>
<tr>
<td>1</td>
<td>$2.9 \times 10^6$ c</td>
</tr>
<tr>
<td>2</td>
<td>$5.7 \times 10^4$ a</td>
</tr>
<tr>
<td>3</td>
<td>$2.1 \times 10^5$ ab</td>
</tr>
<tr>
<td>4</td>
<td>$1.1 \times 10^5$ ab</td>
</tr>
</tbody>
</table>

$^a$ Based on 8 replicated blocks of each rotation in two trials

A > 2-year break from canola reduced *P. brassicae* resting spores in the soil by at least 10 fold relative to 0- or 1-year break
II. Crop rotation x biofungicide seed treatment

- Neither *B. subtilis* seed dressing (regardless of the rate) nor the crop rotation reduced clubroot severity substantially.

- In longer rotation plots, however, the galls were slightly smaller.
Clubroot impact on crop development

- *B. subtilis* seed dressing had no effect
- Longer rotation reduced clubroot impact
  (pooled data over all seed treatment rates)
Canola seed yield

- Biofungicide seed treatment showed no effect
- A >3-yr break from canola had higher yields for S cv.
- Overall, the yield was poor (<1 ton/ha) with S cv.
- Rotation alone was not enough to allow the S cv. to reach its yield potential
Ill. Crop rotation x cultivar resistance

Clubroot severity at flowering was reduced by R cv. but not by crop rotation on S or MS cvs.

Impact on crop development: A >2-yr break from canola reduced disease impact on S and MS cvs. No effect on R cv.
Continuous canola: There was hardly any S and MR/MS plants left, R looked thin.

1-year break: Not much different from 0-year break, R also looked thin.
Two- to 4-year breaks:
- Gradually increased stand for S and MR/MS, but crop was still much poorer than R
- R plots were fuller
- Plot appearance reflected the yield
A >2-yr break showed higher yields on S and MR, but overall yields were low (>0.5 T/ha). On the R cultivar, a >2-yr break had a 25% yield increase relative to continuous canola.
A >2-year break from canola reduced *B. brassicae* resting spores in the soil substantially.

Long rotation alone is not enough to allow a S or MS cv. to reach yield potential in heavily infested fields.

A resistant cultivar, in conjunction with a >3-year crop rotation may allow maximum yield potential in heavily infested fields, as well as reducing the pathogen inoculum loads in the soil.
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