Clubroot development and quantification of *Plasmodiophora brassicae* DNA in canola root tissue following treatment with lime products

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History of Clubroot In Alberta

• 2003 - introduction (12 fields)
• 2009/2010 – resistant cultivars available *45
• 2013 – genetic resistance breakdown (1st findings (2 fields))
• 2018 – 3,044 field infestations

Strelkov et al. 2017
History of Clubroot In Alberta

• 2003 - introduction (12 fields)
• 2009/2010 – resistant cultivars available
• 2013 – genetic resistance breakdown (1st findings (2 fields))
• 2018 – 3,044 field infestations
  ➢ 170 fields with new strains confirmed
  = genetic resistance breakdown

Strelkov et al., unpublished
Genetic resistance is vulnerable to pathotype shifts

✓ incomplete resistance to some pathotypes
✓ off-types in seed lots
✓ gene segregation
✓ presence of susceptible cruciferous weeds
✓ Wind
✓ Other Pathotype shifting pressures
Etc.
“Clubroot management strategies that aim to reduce inoculum pressure will contribute to the stewardship of cultivar resistance.”

- Peng et al., 2015
Project Outline:
How does variable lime rates and products affect clubroot disease:

Greenhouse Project:
✓ Test varying rates of two lime products at multiple inoculum concentrations

Lab Project:
✓ Quantify *P. brassicae* ability to cause root hair infection under varying treatments
Greenhouse Project:

Treatment list:
- **Inoculum level:** (4) - $10^{3,4,5,6}$ spores/g soil
- **Lime Products:** (2)
  1. High calcium hydrated lime
  2. High calcium limestone
- **Lime rate:** (5) - 0.7, 1.2, 1.7, 2.2 increase in pH from control)
- **Cultivar:** (2)
  1. Susceptible
  2. Moderately resistant
YEAR 1

Hydrated Lime

YEAR 2

Susceptible cultivar
YEAR 1

Hydrated Lime

YEAR 2

Moderately resistant cultivar
Control (no lime) $10^6$ spores/g soil

Lowest rate of H-lime  

Highest rate of H-lime
YEAR 1

Limestone

Susceptible cultivar

YEAR 2
**YEAR 1**

**Limestone**

**YEAR 2**

Moderately resistant cultivar

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**Disease Index c.v. 9558 - Zero Grind**

- **No Lime (pH 5.3)**
- **4.7 ZG (pH 6.0)**
- **8.1 ZG (pH 6.5)**
- **11.4 ZG (pH 7.0)**
- **14.8 ZG (pH 7.5)**

**Disease Index c.v. 9558 (MR) Zero Grind**

- **No Lime (pH 5.3)**
- **4.7 ZG (pH 6.0)**
- **8.1 ZG (pH 6.5)**
- **11.4 ZG (pH 7.0)**
- **14.8 ZG (pH 7.5)**
Greenhouse Trail Conclusions:

- Plant parameters (plant height, shoot and root weight) followed closely with the level of disease control.

Factors affecting the parameters:

- **Cultivar**: affects disease incidence and influences pathotype selection
- **Inoculum level**: affected limestones ability to control disease
- **Lime product**: affected the level of disease control
  - Hydrated lime effectively eliminated disease, and although rate was not significant, it would likely be in the field.
- **Year**: inoculum source (i.e. viability/strength) gave different effect size
Root hair colonization (lab study):
What effects lime has on the ability for clubroot to colonize the root hairs of young, highly susceptible hosts.

• 10 day old seedling roots collected from each treatment regime:
  1. DNA isolation (Macherey-Nagel kit)
  2. *P. brassicae* DNA presence in the root hairs (PCR)
  3. Quantify root hair infection (qPCR)
Evaluation 2: Root samples

**PCR reaction components:**
- sdH20: 13.3 µL
- 10X PCR buffer: 2.5 µL
- dNTPs (2.5 nM): 2 µL
- MgCl2 (50 nM): 1 µL
- TC1F (10 mM): 1 µL
- TC1R (10 mM): 1 µL
- Taq Polymerase: 0.2 µL
- DNA template: 5 µL
- DNA fragments were separated by running 1% agarose gel at 90 V for 40 min.

**qPCR reaction components:**
- SYBR green master mix
- **Primers:** DR1F & DR1R
- 2.5µL 1:10 diluted sample

94°C (2 min) – 94°C (30s)- 65°C (90s) – 72°C (30s) – 72°C (10 min) – 4°C
35 Cycles

94°C (2 min) – 94°C (30s)- 65°C (90s) – 72°C (30s) – 72°C (10 min) – 4°C
45 Cycles

YEAR 1  Hydrated Lime
Susceptible cultivar

spores/g root tissue Hydrated lime (S c.v.)
YEAR 1

Hydrated Lime

Susceptible cultivar

YEAR 2

Spores/g root tissue Hydrated lime (S c.v.)

Spores/g root tissue HL c.v. 45H31
YEAR 1

Hydrated Lime

Moderately resistant cultivar

YEAR 2
YEAR 1
Limestone
Moderately resistant cultivar

YEAR 2

Spores/g root tissue Limestone MR c.v.

Spores/g root tissue ZG c.v. 9558
Root hair colonization Conclusions:

Factors affecting the ability of *P. brassicae* to cause root hair infection:

- **Year**: inoculum source (i.e. viability/strength) produced significantly different results

**Year 1:**
- **Inoculum level**: affected the rate required to eliminate infection
- **Rate**: increased with increasing inoculum concentration
- **Lime product**: affected the level of disease control

**Year 2:**
- All factors, including cultivar
Conclusions:

❖ Liming as a mean to control clubroot has a very complicated interaction involving many factors.

❖ There may never be a specific rate recommendation.

❖ Recommendations of lime product and rate may need to be based on inoculum concentration and its viability, as well as initial pH.

✓ Liming shows promising results to be an effective IPM tool to control clubroot disease, inoculum level and pathotype pressure; therefore, contributing to resistant cultivar stewardship.
Thank you!

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