The Impact of Clubroot Resistant Canola Cultivars on *Plasmodiophora brassicae* Resting Spore Concentrations in the Soil

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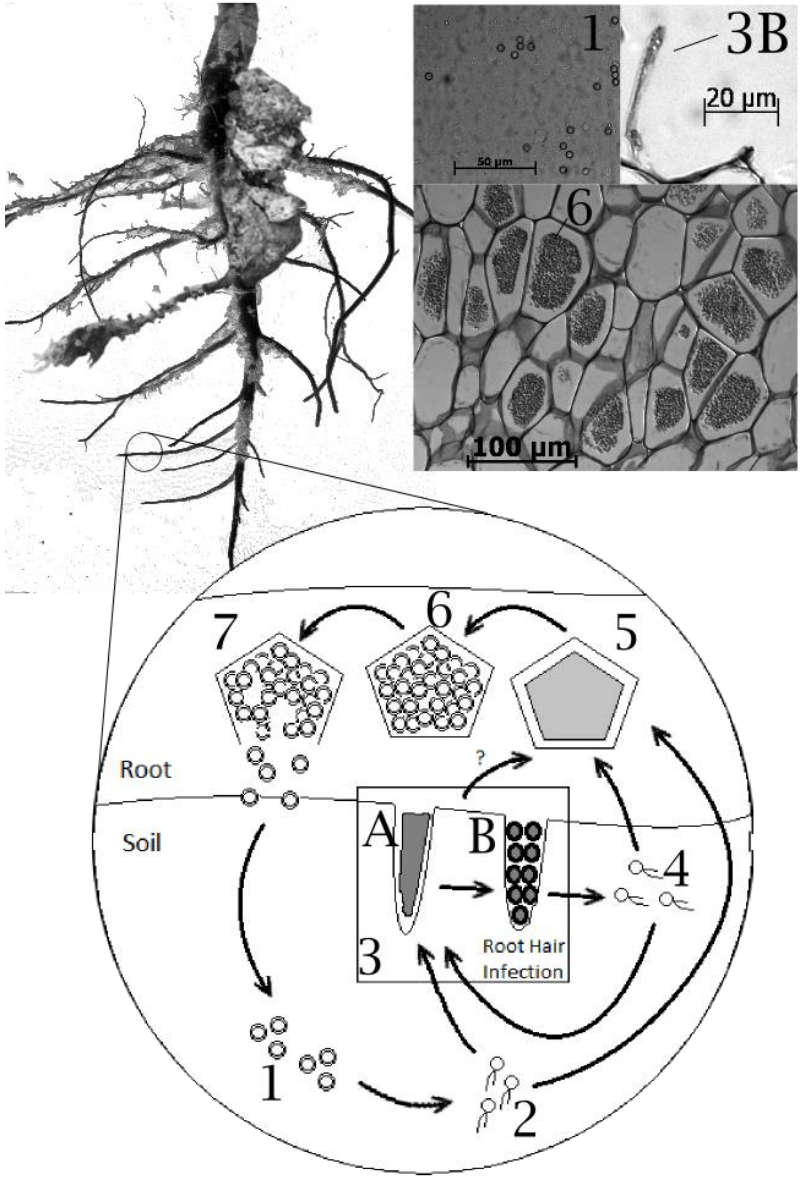
Presentation Outline

- Objectives/Hypotheses
  - *Plasmodiophora brassicae* background
  - *P. brassicae* resting spore dynamics
- Experimental Design and Materials/Methods
- Results & Conclusions:

  recommended best practices and potential future research

Plasmodiophora brassicae



- Soil-borne plasmodial endoparasite of cruciferous plants
  (Gibbs, 1932, Karling, 1968)
- Every species from all genera of the Brassicacea family are expected hosts of *P. brassicae*
  (Dixon, 2009)
- Host plants regardless of age are susceptible to *P. brassicae* infection when growth still occurs
  (Kunkel, 1918)

Background

- Clubroot resistant (CR) canola cultivars produce less galled root mass and less inoculum, than susceptible cultivars.

(Hwang et al., 2012b, Hwang et al., 2015)

- Hwang et al. (2011) Suggested that: at low to medium inoculum density commercially available resistant canola cultivars may prevent further propagation of *P. brassicae* inoculum

- Risk: cruciferous weeds, canola volunteers and genetic off-types in seed lots will increase the inoculum levels

Objectives

- Determine the effect of resistant cultivars on *P. brassicae* soil inoculum loads:
  - at various initial levels of infestation
  - under various field conditions
  - within various crop rotations
- To determine the level of clubroot incidence and severity in CR cultivars within canola producing fields of Alberta

Materials & Methods



- Repetitive soil sampling at GPS marked locations, within *P. brassicae* infested fields, Pre-Seeding and Post-Harvest in 2010, 2011, 2012, and 2013.

- Post-Harvest soil sampling was accompanied by incidence and severity ratings, calculate ID%

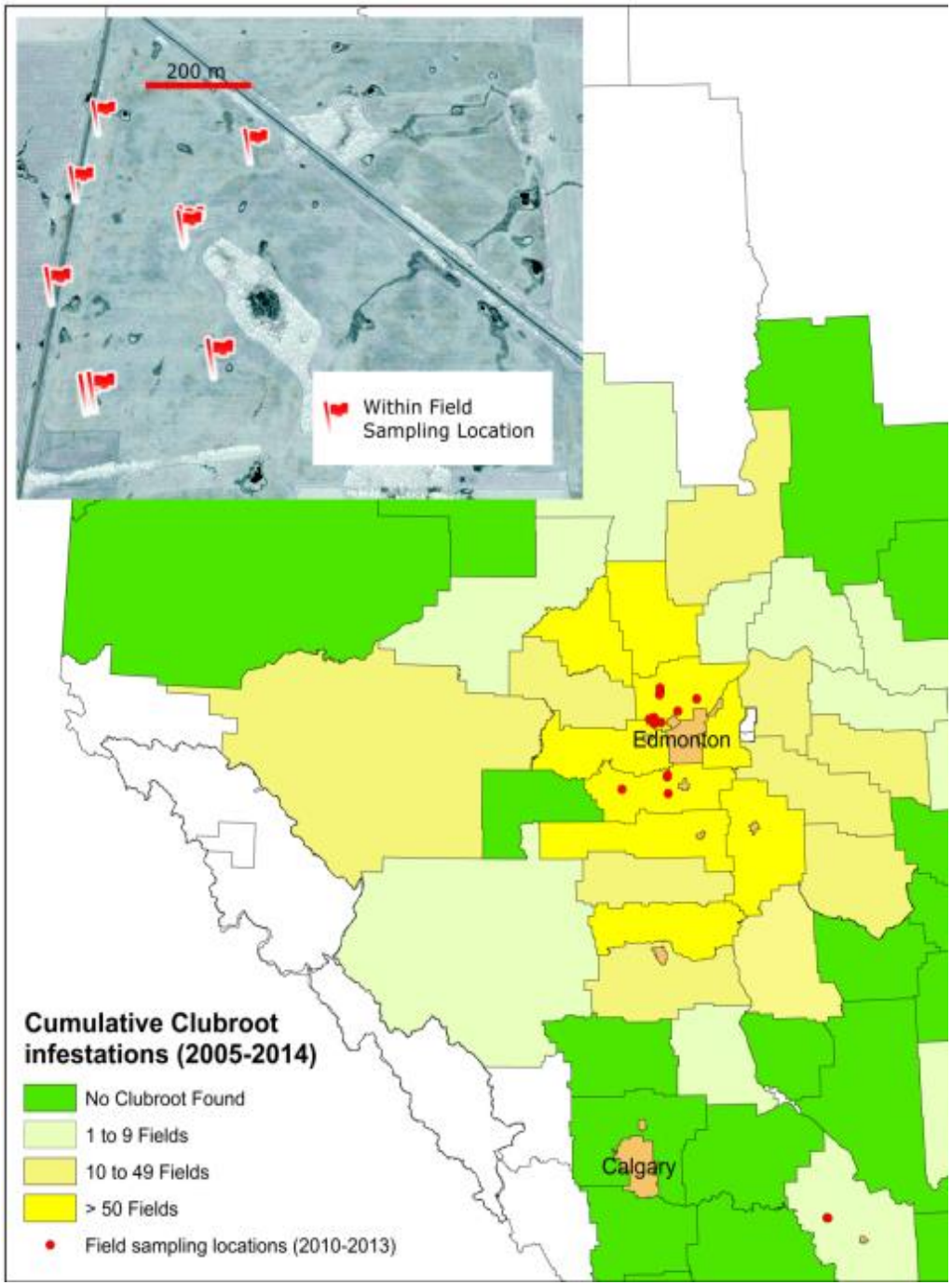


Figure - Distribution of fields monitored for *Plasmodiophora brassicae* resting spore concentration from 2010-2013 in Alberta, Canada.

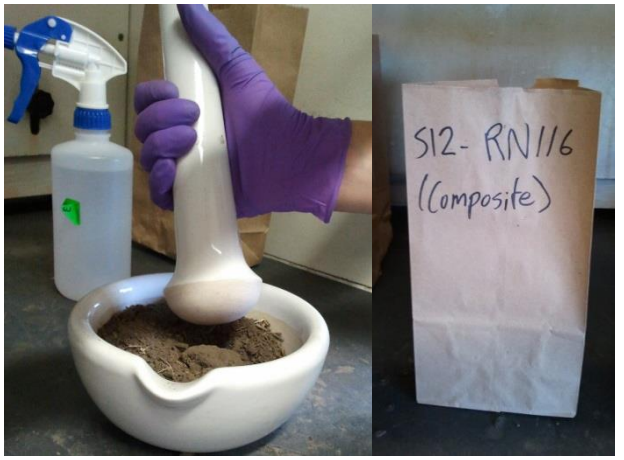
Embedded image (top left) illustrates the within-field distribution of sampling points for one sample field.

- sampling points had CR canola cultivated in various rotations
- control sampling points were closely associated with experimental points but remained fallow

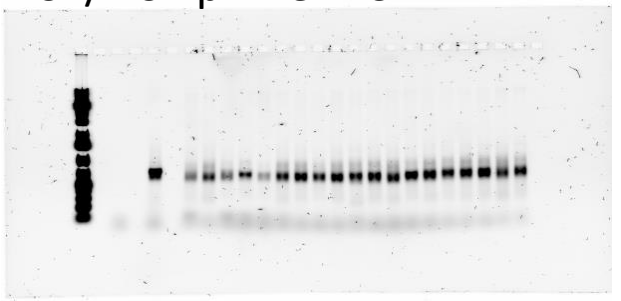
Cumulative infestations = total number of confirmed clubroot infestations in specific counties or municipalities (adapted from Strelkov and Hwang 2014).

Soil Preparation & Molecular Detection

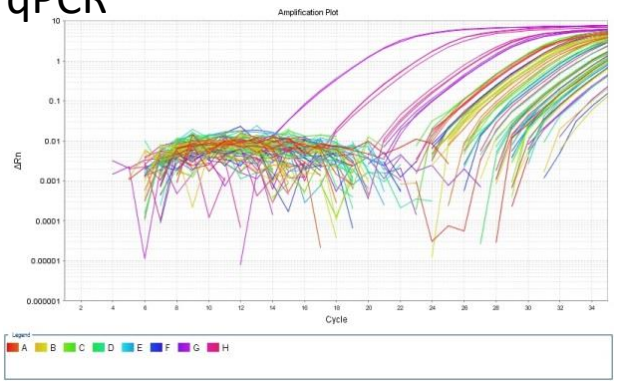
- Georeferenced Composite soil samples from each time period were dried and homogenized
- DNA extracted from soil samples
  - (PowerSoil DNA Isolation Kit, MO BIO Laboratories, Carlsbad CA, USA)
- Non-specific PCR
  - ITS1/ITS4 primers (Korabecna et al. 2007)
- Conventional PCR - *Pb* specific
  - TC1F/TC1R primers (Cao et al. 2007)
- qPCR - *Pb* specific
  - DC1F/DC1R primers (Rennie et al. 2011)



ITS1/ITS4 primer PCR



qPCR



Bio-Assays

- Inoculum potential of infested soil samples assessed via greenhouse bioassays.
- Naturally infested soil and potting medium
  - Volume 1:1 Ratio
- The susceptible cultivar Chinese cabbage (*Brassica rapa* ssp. *pekinensis* L.) cv. Granaat grown in infested soil and rated for clubroot severity and incidence after 6 weeks

Results

- Over 8500 soil samples collected
  - forming 895 composite samples
  - from 182 GPS marked locations
  - within 17 different fields situated across Alberta.

- success of DNA extraction confirmed
  - PCR amplification with the non-specific primers ITS1 and ITS4

  (Korabecna et al. 2007)
  - DNA was amplified successfully from all samples tested

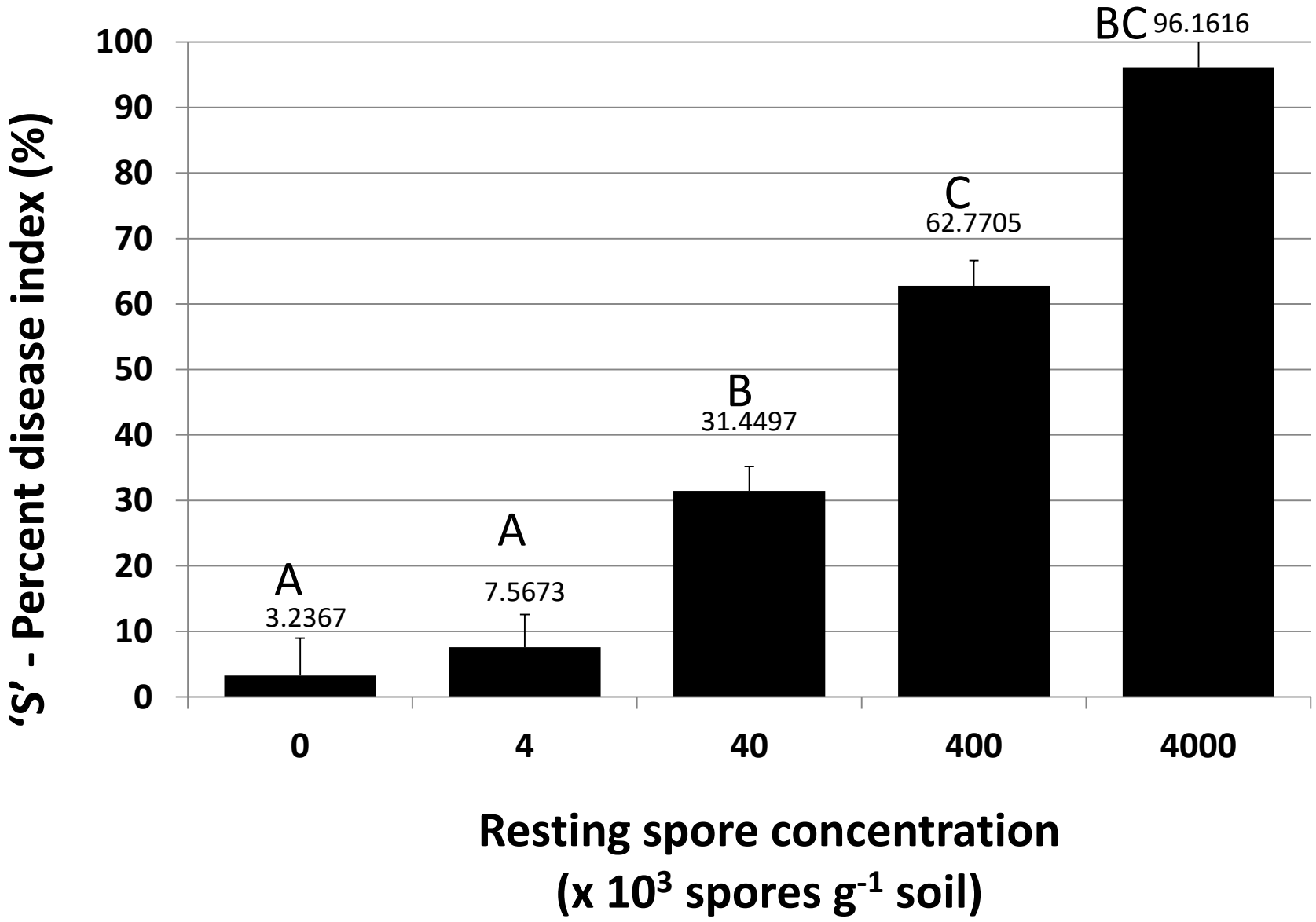


Figure - Relationship between *Plasmodiophora brassicae* resting spore concentration in infested soil as determined by quantitative PCR (qPCR) and index of disease (ID) on susceptible ('S') *Brassica napus* cv. Granaat in greenhouse bioassays.

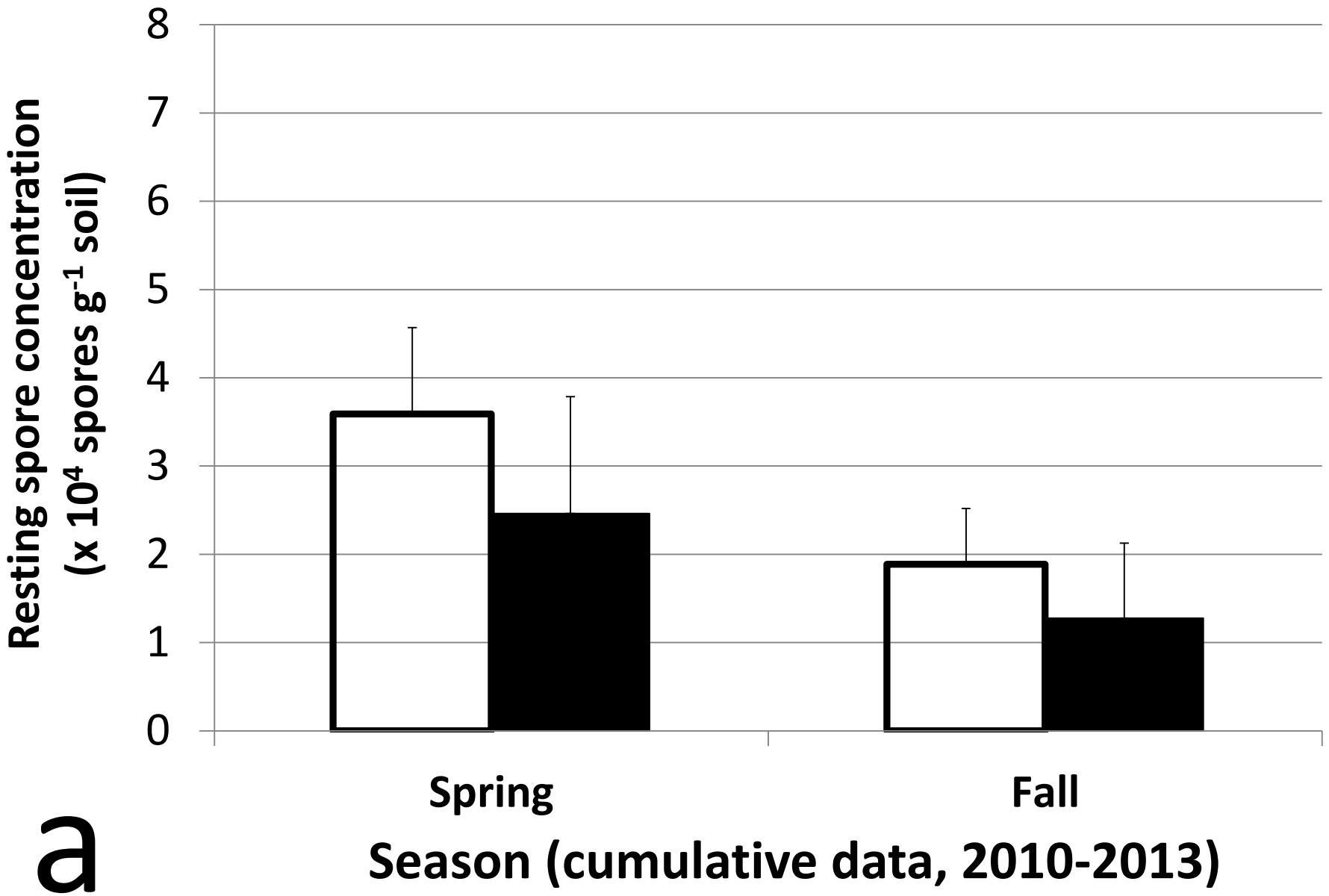


Figure - Cumulative reaction of *Plasmodiophora brassicae* resting spore concentration to the cultivation of CR canola (empty bars) and fallow (filled bars) within all fields of the study seeded to canola between 2010-2013, **No significant treatment, time, or treatment x time effects**

Conclusions 1

- DNA extraction was successful and reliable
- qPCR results reflected soil inoculum potential (i.e. the likelihood infection was observed on a susceptible cultivar during greenhouse bioassays)
- CR canola cultivars impact on *P. brassicae* resting spore germination = not significantly different from germination under fallow/non-host conditions, CR canola does not appear to function as a useful bait crop under field conditions in Alberta
  - corroborates Ahmed et al. (2011).

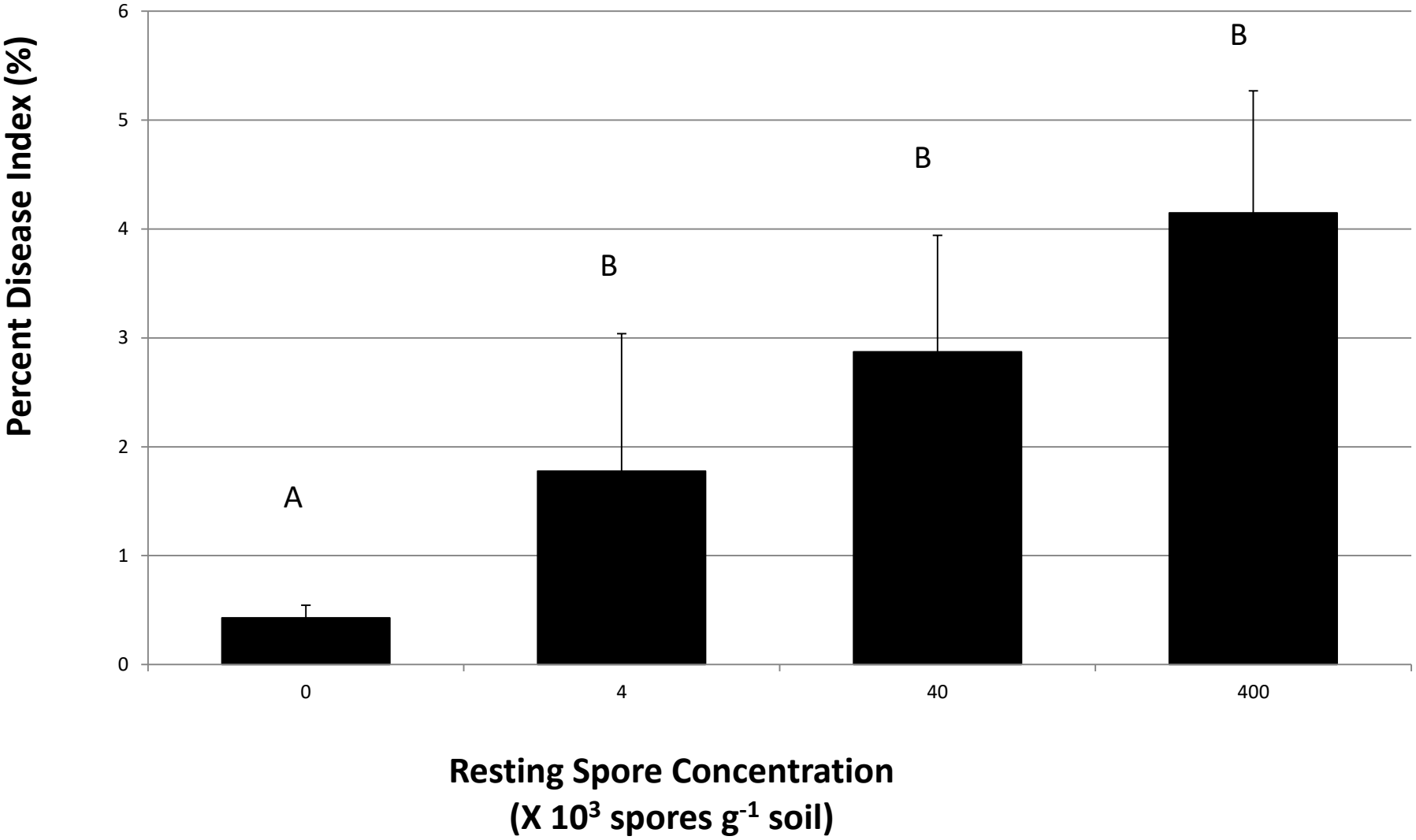


Figure 2-6. Index of disease (ID, %) in fields seeded with clubroot resistant canola when the independent variable 'initial *P. brassicae* resting spore concentration' is assessed categorically

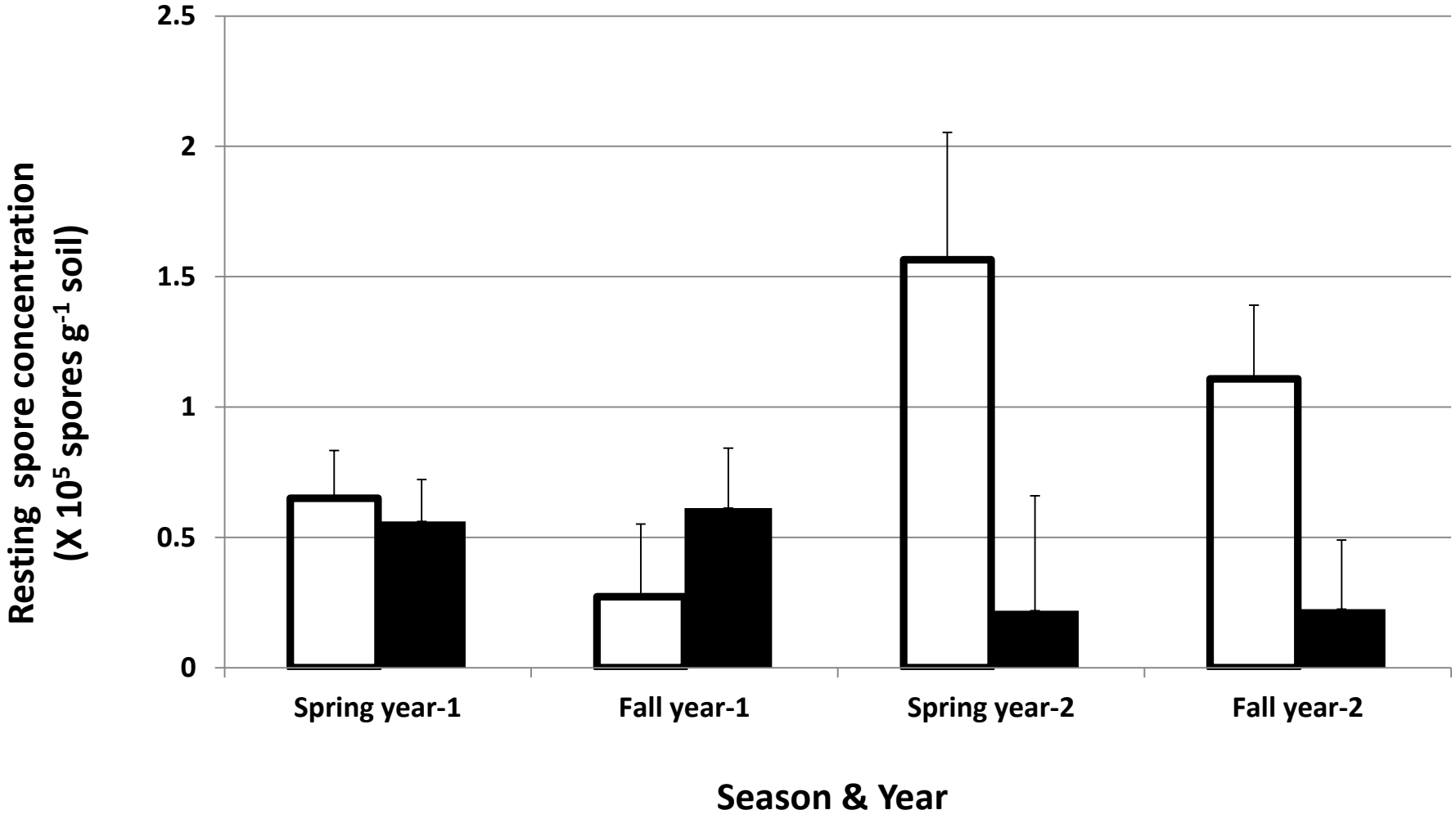
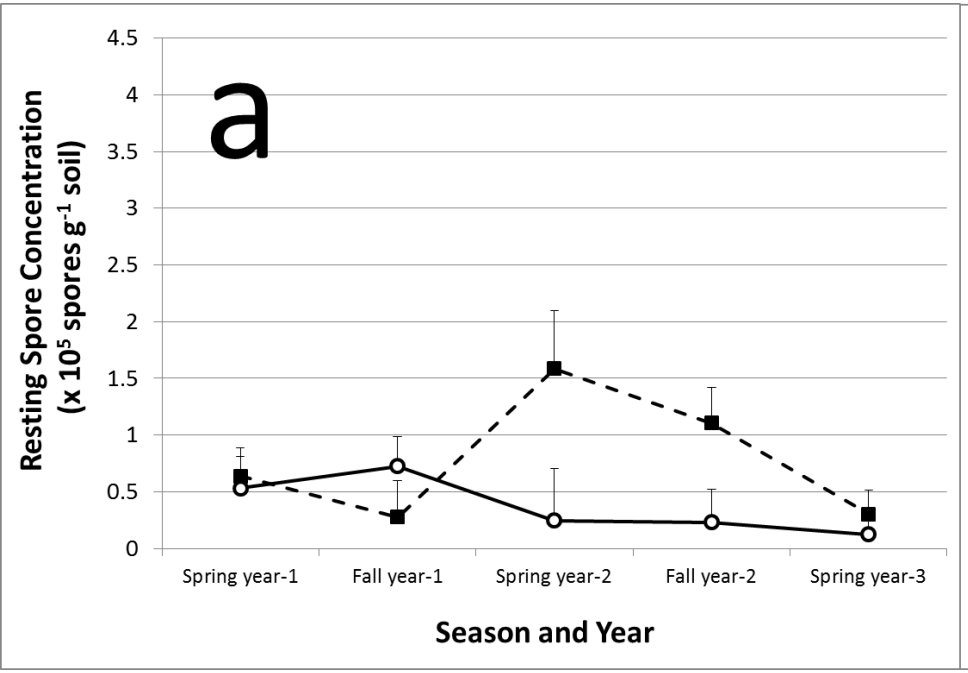


Figure - Mean concentration of *Plasmodiophora brassicae* resting spores in the soil over any two year period within 2010-2013.



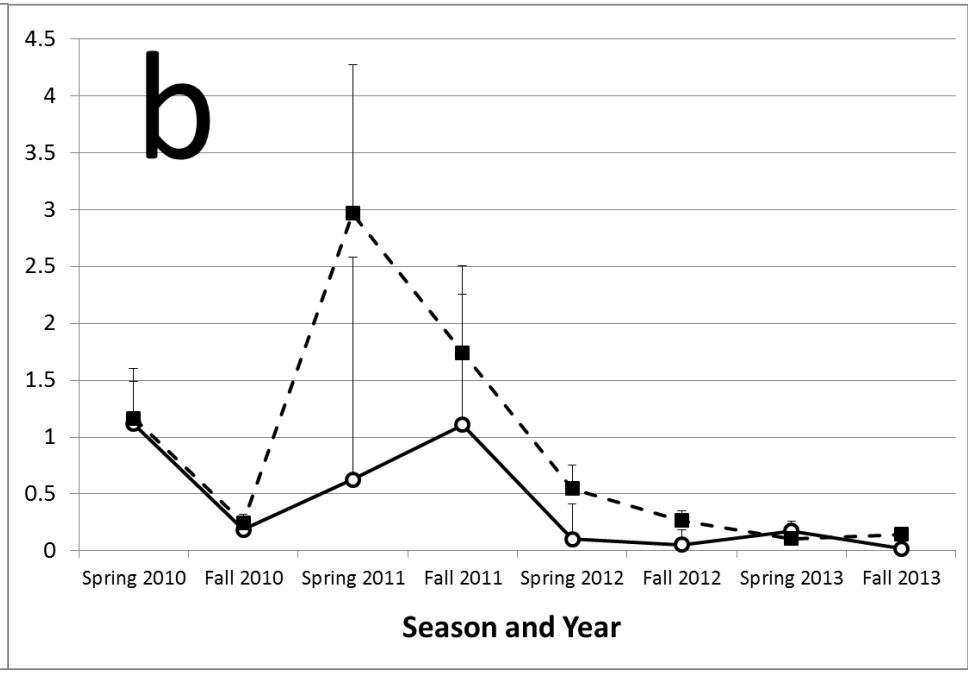


Figure - Concentration of *Plasmodiophora brassicae* resting spores in the soil of fields with a rotation that includes clubroot resistant (CR) canola grown in a 1-in-2 year rotation (a) as well as a 1-in-4 year rotation (b) compared to control plots. Rotation in both graphs = CR canola year-1 → non-host crop in subsequent years, represented by filled-black squares; Fallow year-1 → non-host crop in subsequent years, represented by empty circles

Conclusions 2

- Generally low clubroot incidence and severity within observed CR canola cultivated fields of Alberta between 2010-2013
  - ID generally < 4.15%

- There is a potential lag in the release of new mature *P. brassicae* resting spores into the soil after CR canola cultivation.
  - Significant increases in resting spore concentrations were detected the year following cultivation of CR canola.

- Minimum ≥2-year break from CR canola in infested fields
  - a 1-year break from CR canola cultivation can reduce *P. brassicae* concentration to initial levels **(HOWEVER, enriched with virulent pathotypes???** Likely**)**
  - Large declines in resting spore concentration can be achieved with a ≥2-year break from *Brassica* cultivation.

Thanks

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QUESTIONS ?