

# **Clubroot Summit: Pathology and Surveillance**

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# Research Pillars

Integrated & Sustainable  
Strategies for Managing Clubroot on Canola

Pathology

*Improved understanding of  
the pathogen & resistance  
mechanisms, disease  
surveillance, pathotype  
monitoring &  
detection/diagnosis*

Disease  
Management

Breeding



# Outline of Presentation

- **Clubroot surveillance in 2011**
- **Studies on dispersal in dust & water**
- **Improved detection and quantification**
- **Resistance stewardship**
- **Additional research**
- **Summary**
- **Acknowledgments**

# Clubroot Surveillance

- **447 commercial canola crops in 21 counties visited in 2011**
  - **23 were confirmed to be cropped to resistant hybrids**
  - **424 cropped to susceptible hybrids or hybrids of unknown resistance**
- **Some counties also conducted their own surveys**

# Survey Findings

- **103 of 447 canola crops found to be clubroot-infested**
  - All were new records in the specific fields
- **Another 162 new records identified in independent surveys by Barrhead, Leduc, Parkland and Strathcona Counties**
- **Total of 265 new cases of clubroot identified in 2011**

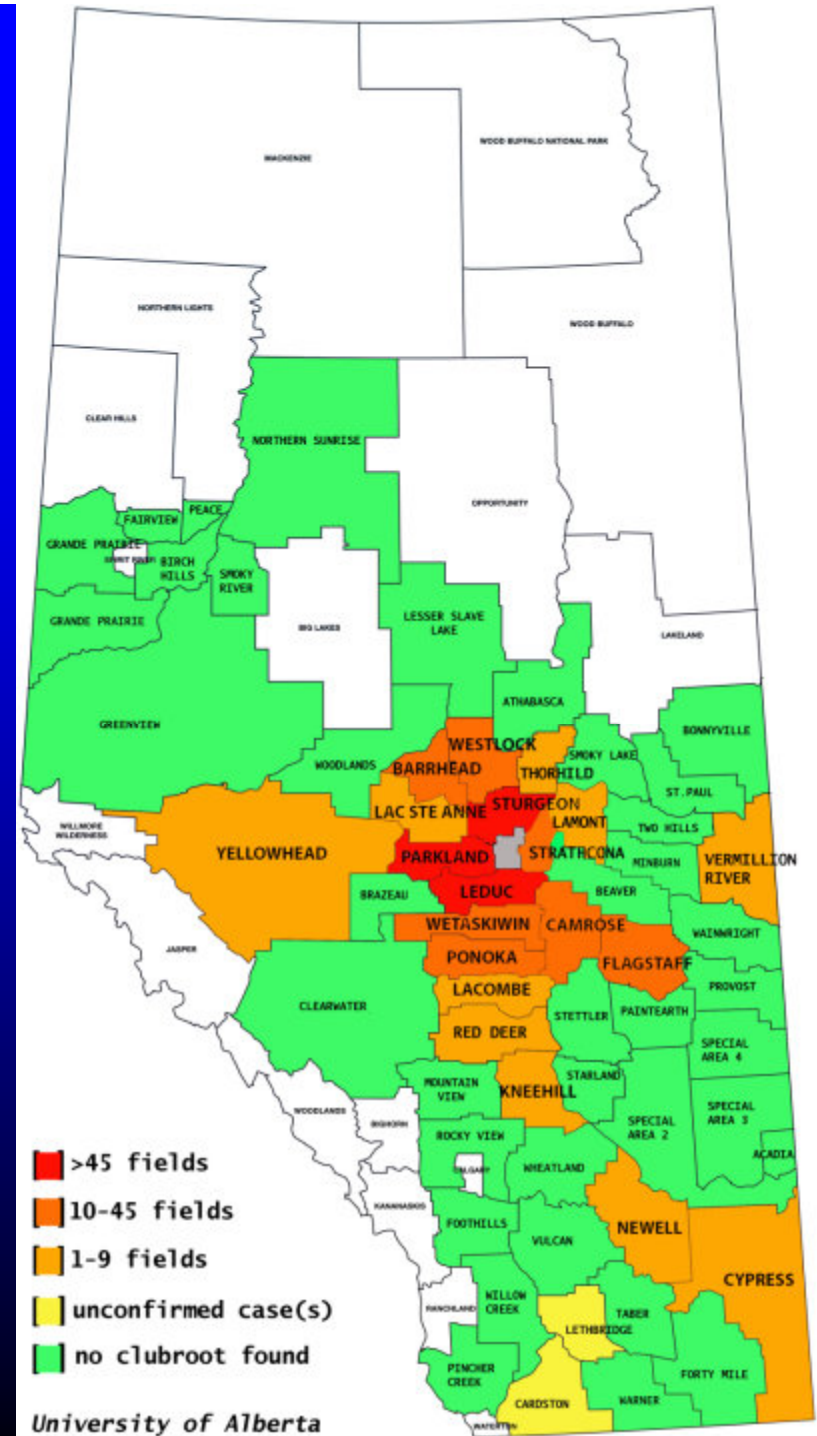
# Field Situation - 2011

- **Clubroot found in 9 of 23 crops sown to resistant hybrids & 94 of 424 crops sown to susceptible hybrids**
- **All genetically resistant canola products still fully effective in 2011**
  - **Disease severity on resistant canola crops was low (0.2 – 10.2%)**
  - **Severe clubroot found in many of the canola crops sown to susceptible cultivars (severity >60% in some)**

# Clubroot Situation (Fall 2011)

- 831 fields with confirmed *P. brassicae* infestations
- Mostly in central Alberta
  - Few cases in southern Alberta and Saskatchewan
  - A few infected plants in experimental plots in Elm Creek, MB (2005)

Strelkov et al. 2012

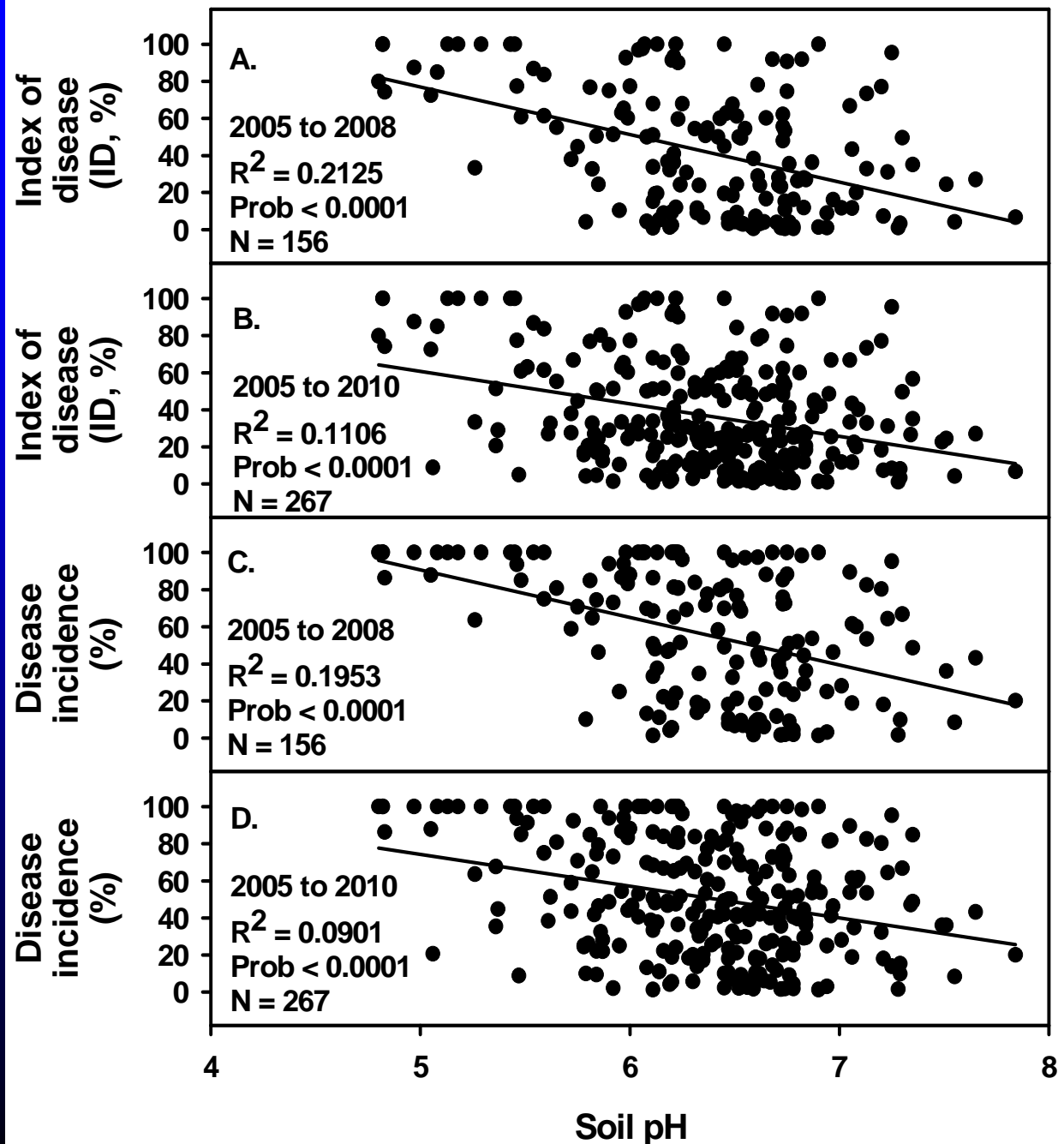


# Characteristics of Infested Fields

Compared clubroot severity & soil pH in 267 canola crops found to be clubroot positive between 2005-2010

While pH contributes to clubroot symptom severity, other factors appear to be involved

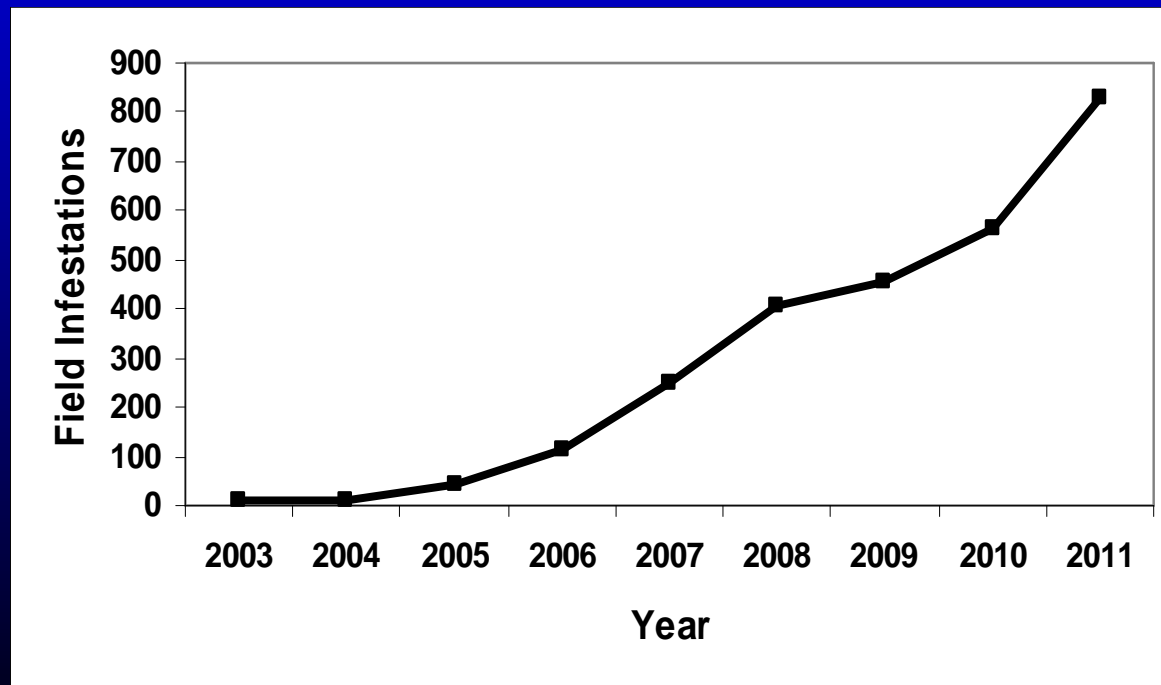
Cao et al.





# Clubroot in Alberta

- **Record number of new cases in 2011**
  - Favorable conditions early in the growing season
  - Continued spread of the disease



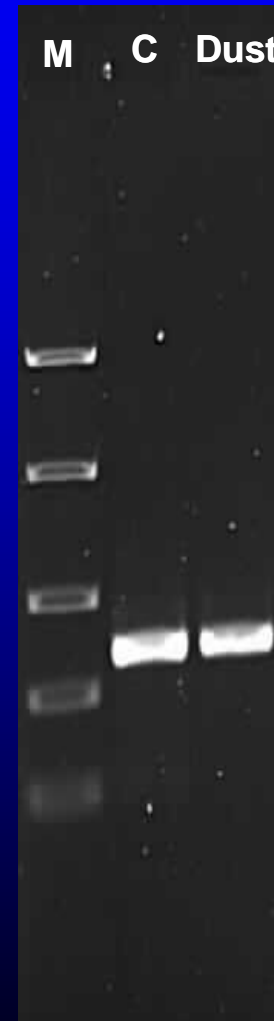
# Clubroot Dispersal

- **Main culprit is soil movement on machinery (Cao et al. 2009)**
- **Common, untreated seeds & tubers from infested fields may also serve as minor mechanism (Rennie et al. 2011)**
- **What about dispersal in dust and water?**

# Dispersal in Dust & Water

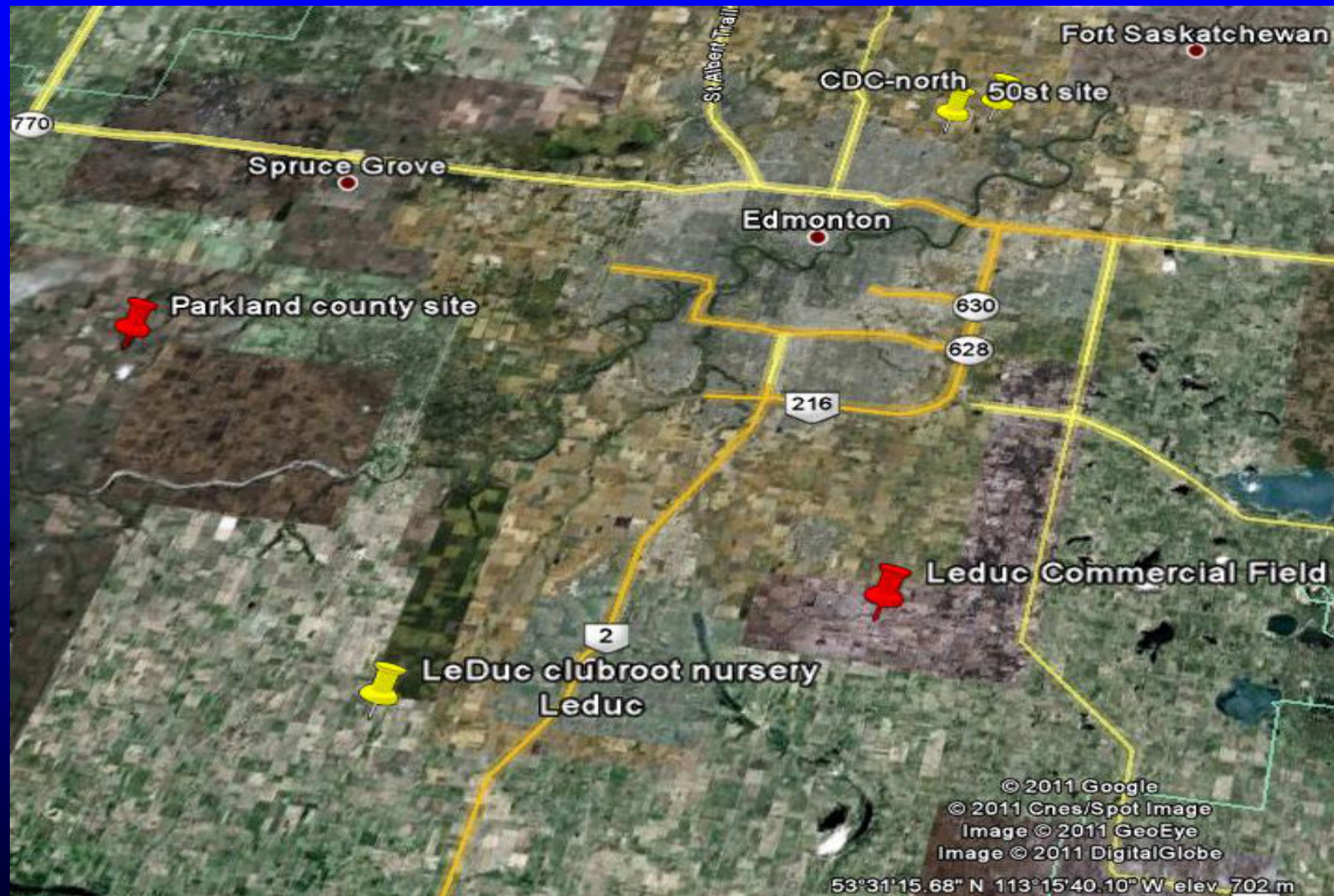
- Clubroot dispersal in dust and water may also occur
  - Extent of problem not well defined
- Epidemiological studies to track and quantify spread

Conventional PCR

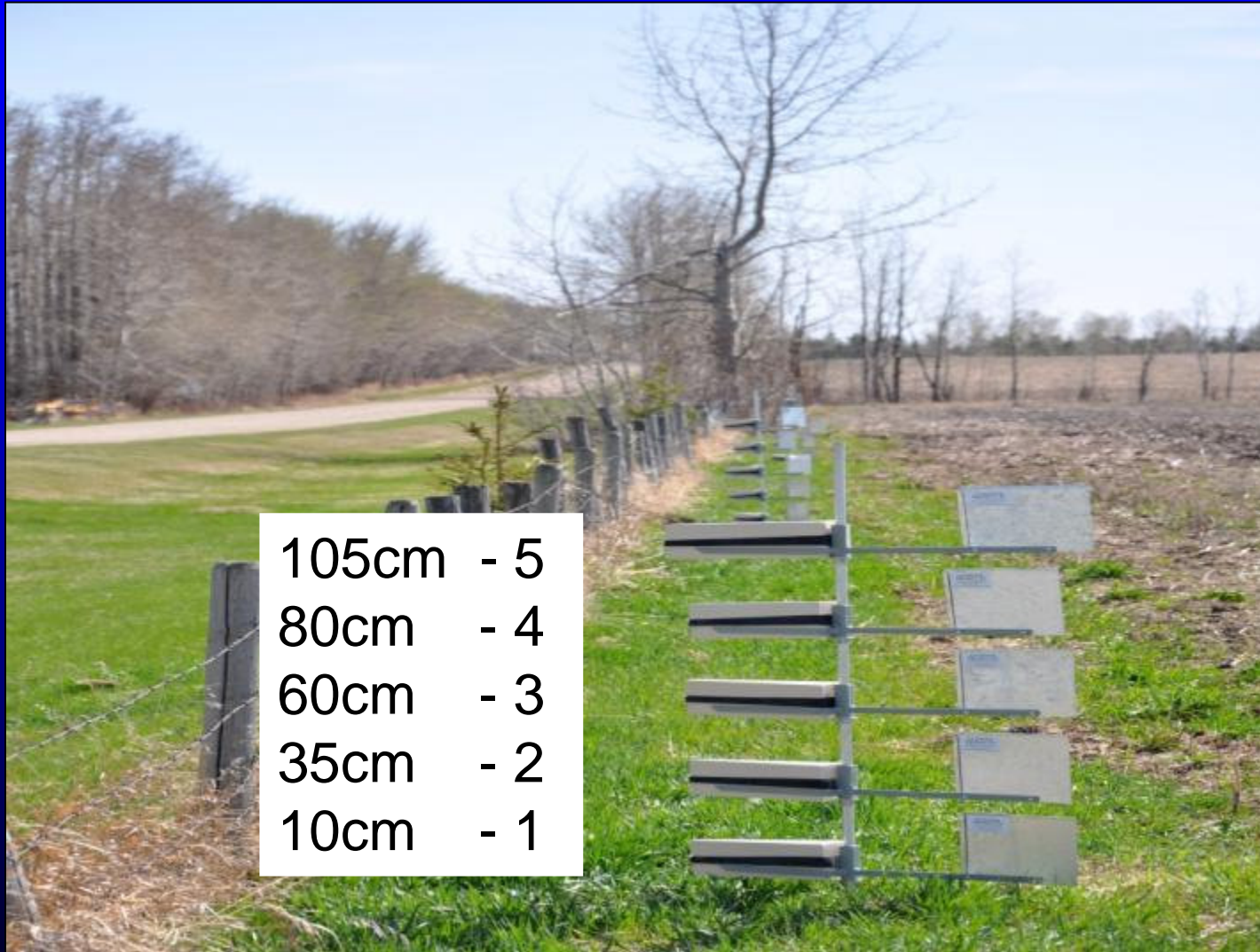


Rennie et al.

# Research Sites



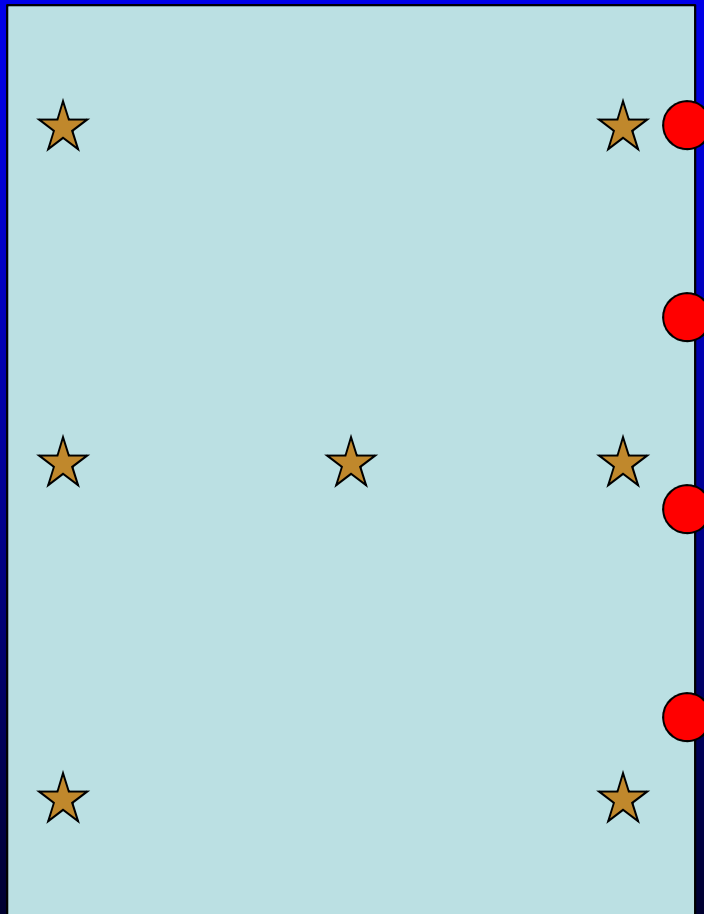
# BSNE (Dust) Samplers



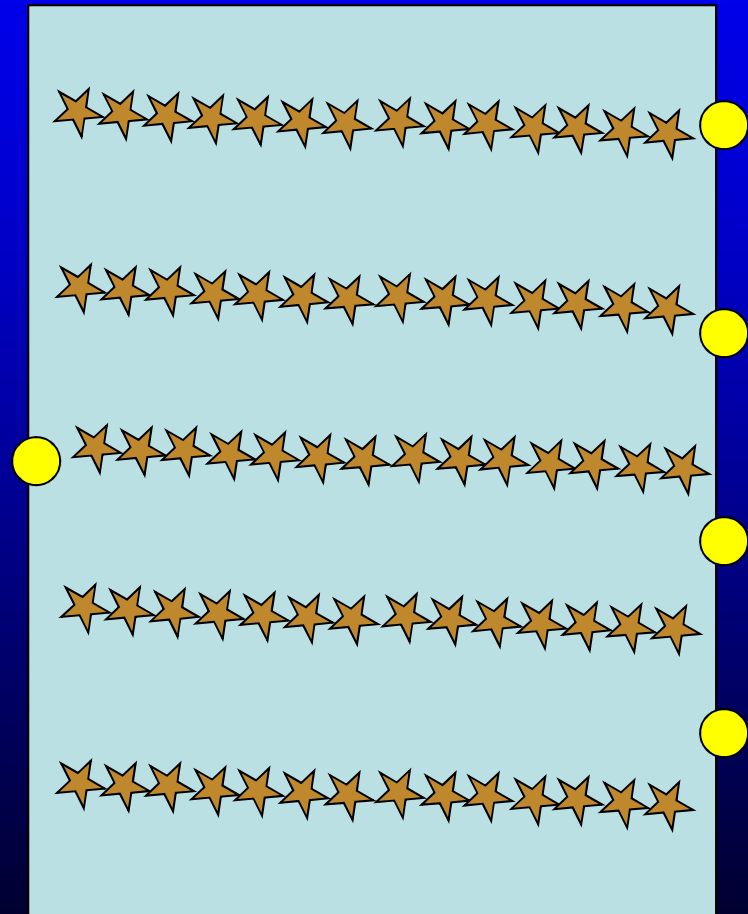
# Sampling

Wind direction  
→

## Commercial Fields

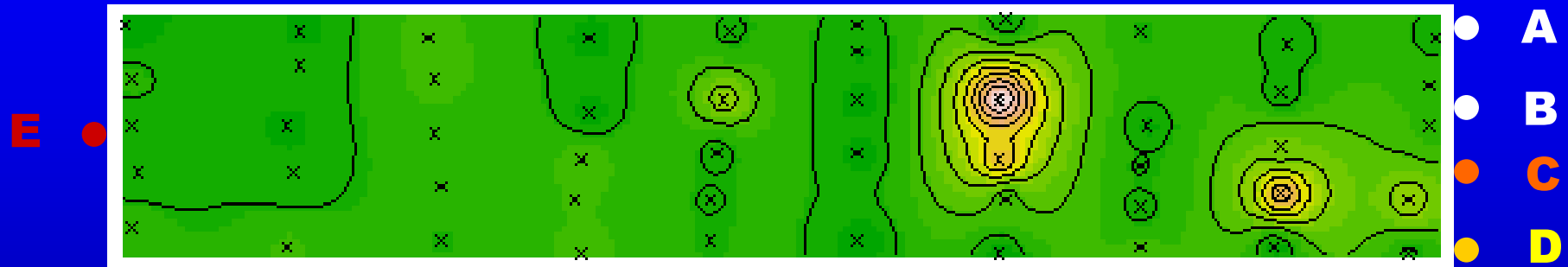


## Research Plots



Field Entrance

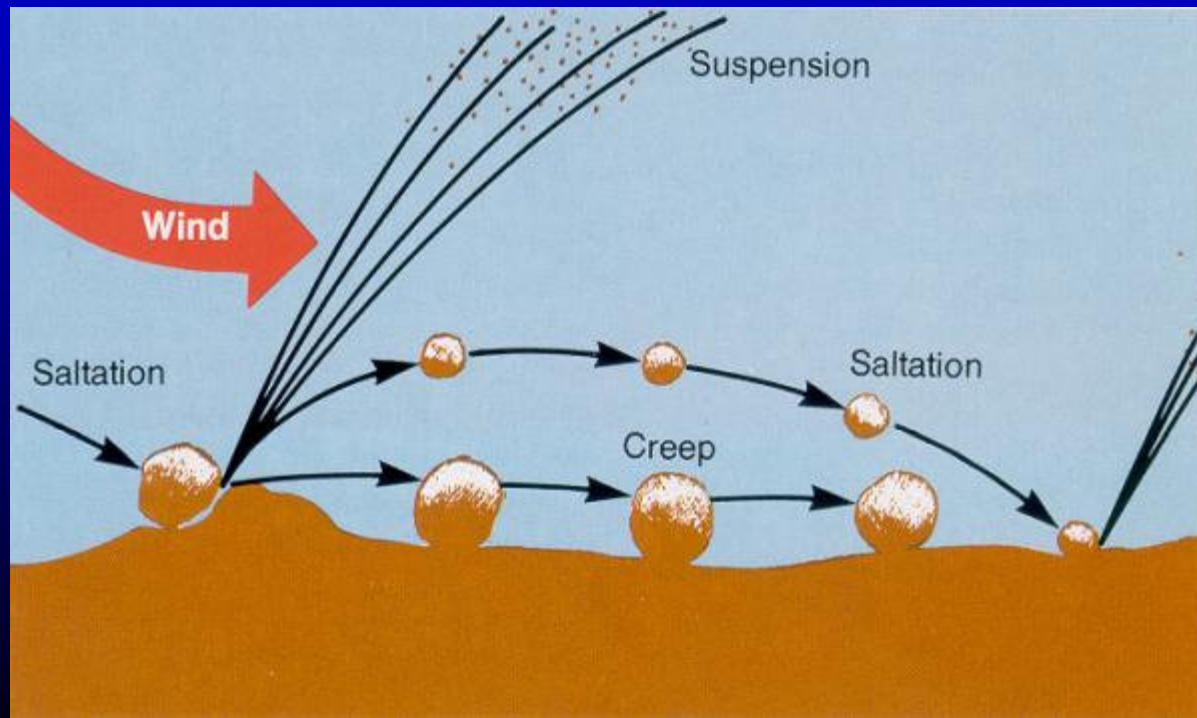
Wind direction



Sampler	Concentration of spores in dust	Amount of dust collected
A, B	No spores detected at any height	
D (10 cm)	$7.7 \times 10^2$ spores per g	3.16 g
D (105 cm)	$8.8 \times 10^2$ spores per g	0.370 g
C (10 cm)	$1.7 \times 10^3$ spores per g	2.33 g
E (80 cm)	$1.6 \times 10^4$ spores per g	0.260 g

# Dispersal in Soil and Water

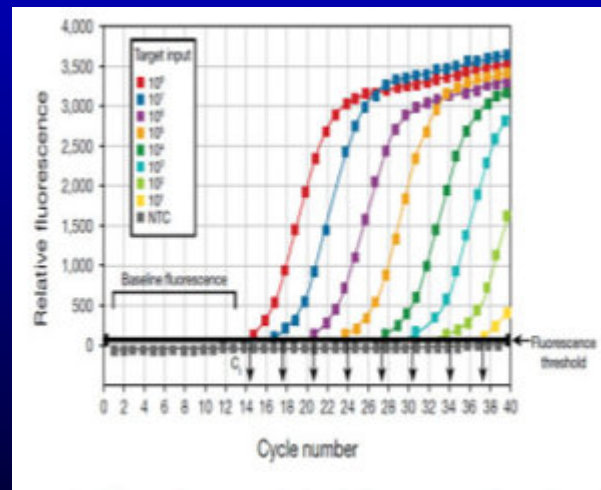
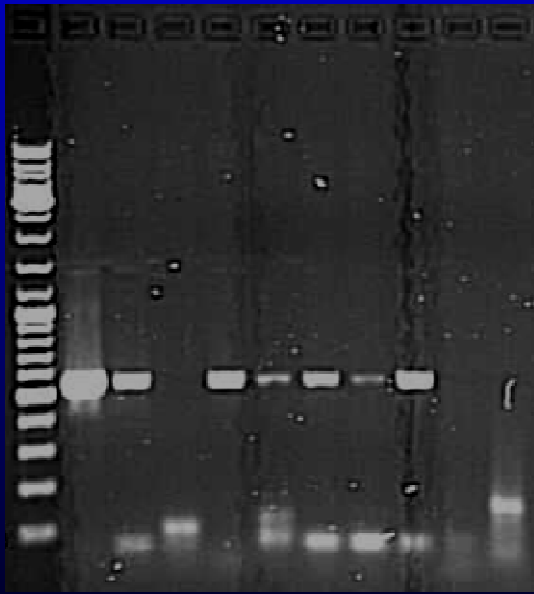
- Work ongoing in 2012 at multiple sites
  - Dust and water run-off
- Will also examine surface creep





# Additional Surveillance Activities

- Also screening hundreds of soil samples collected from SK and MB for presence of *P. brassicae*



(1) Conventional PCR

(2) Quantitative PCR

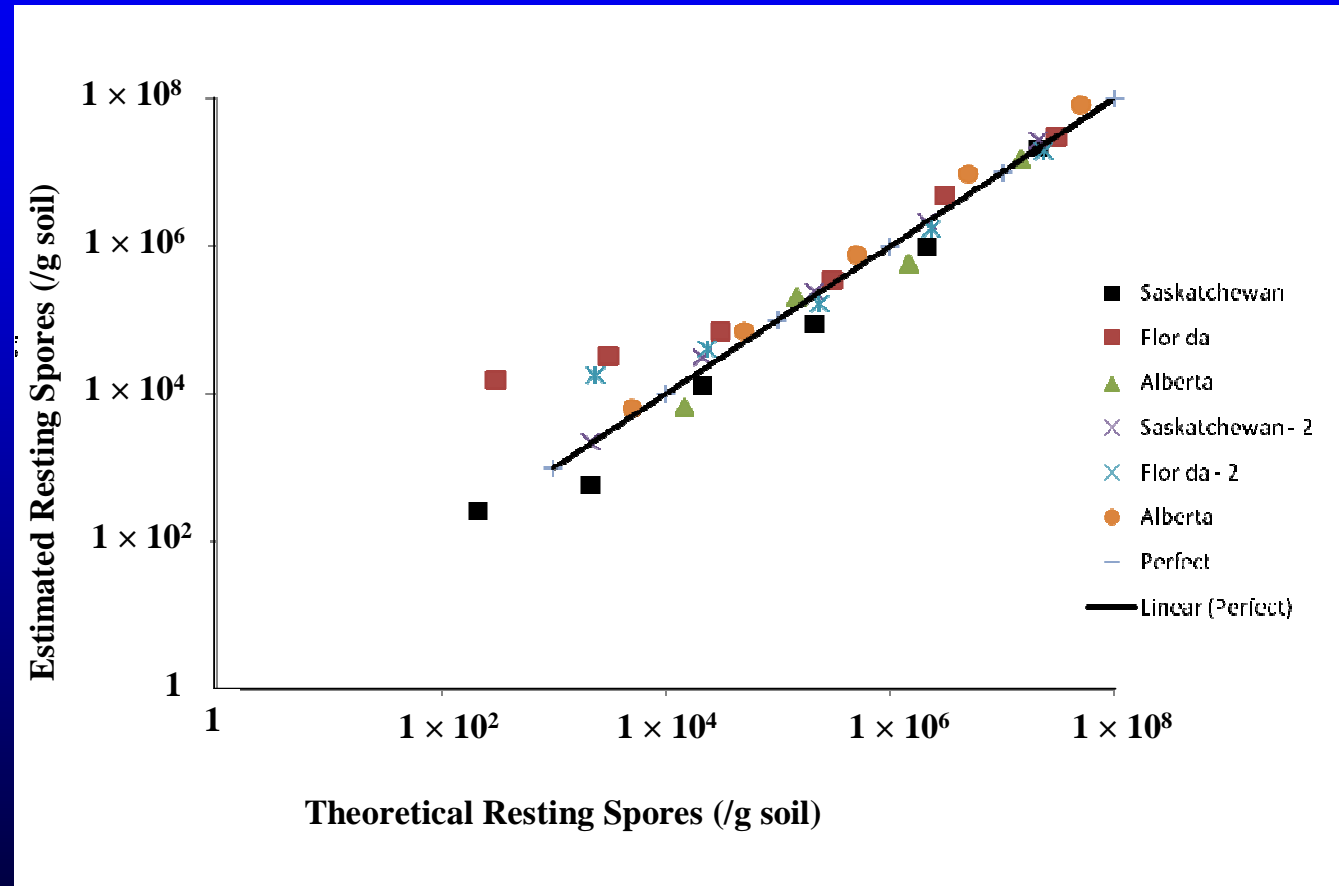
(3) Bioassays

# **Improved Clubroot Detection and Quantification**

- **Clubroot research facilitated by development of improved methods for pathogen detection and quantification**
- **Conventional PCR (Cao et al. 2007)**
  - **Soil and plant tissue**
- **Quantitative PCR (qPCR)**
  - **Seeds and tubers (Rennie et al. 2011)**
  - **Soil (Rennie et al.)**
  - **Root tissue (Cao et al.)**

# qPCR Assay to Measure Spores in Soil

- Robust technique
- Adapted from our protocol for seeds
- Multiple soil types and pathogen strains



## ***In Planta* Quantification of *P. brassicae***

- **Methodology also developed for quantification of *P. brassicae* in roots of plants**
- **Will not only facilitate biological studies, but also has potential to be used as a resistance screening tool**

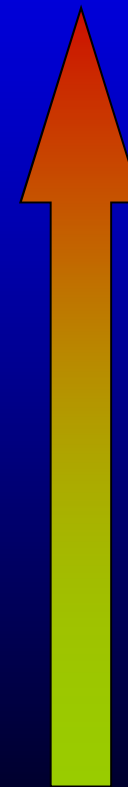
# qPCR for Resistance Screening

- **If amount of *P. brassicae* DNA in plant roots shortly after inoculation is well-correlated with eventual clubroot reaction, qPCR could be used to screen out material that will likely be susceptible**

# Plant Material

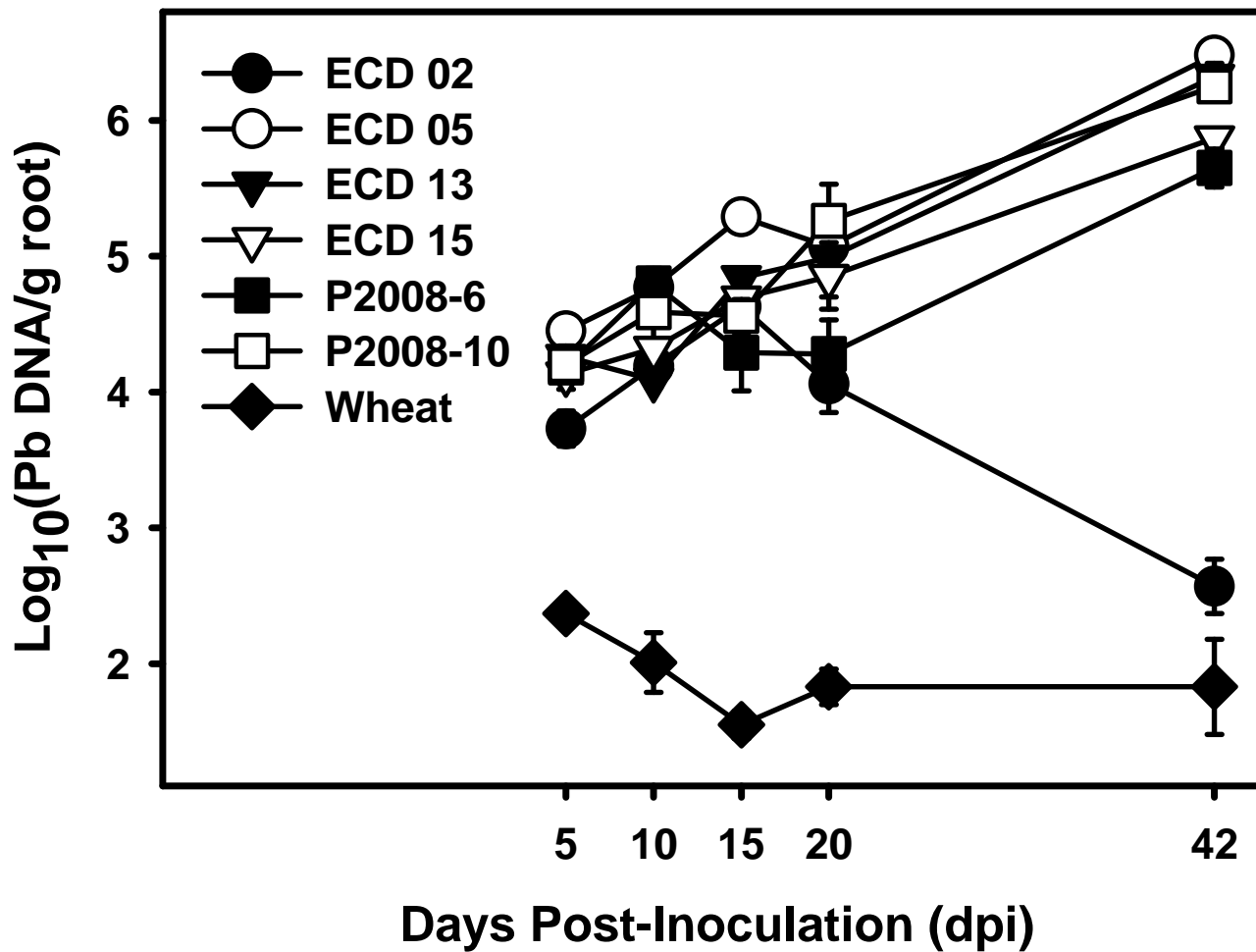
Plant Genotype	Abbreviation	Clubroot reaction ID $\pm$ S.E. (%)
<i>B. rapa</i> var. <i>pekinensis</i> cv. Granaat (Chinese cabbage)	ECD 05	99.1 $\pm$ 0.9
<i>B. oleracea</i> var. <i>capitata</i> cv. Jersey Queen (Cabbage)	ECD 13	81.5 $\pm$ 3.1
<i>B. oleracea</i> var. <i>capitata</i> subvar. <i>laciniata</i> cv. 'Verheul' (Kale)	ECD 15	37.6 $\pm$ 5.6
<i>B. napus</i> L. line P2008-10 (Canola line)	P2008-10	29.6 $\pm$ 2.9
<i>B. napus</i> L. Line P2008-6 (Canola line)	P2008-6	13.0 $\pm$ 4.0
<i>Brassica rapa</i> subsp. <i>rapifera</i> line AAbbCC (Polish rape)	ECD 02	0.0 $\pm$ 0.0
<i>Triticum aestivum</i> L. cv. Harvest (Wheat)	Wheat	0.0 $\pm$ 0.0

Completely Susceptible

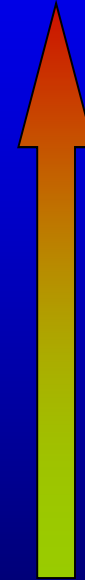


Non-Host

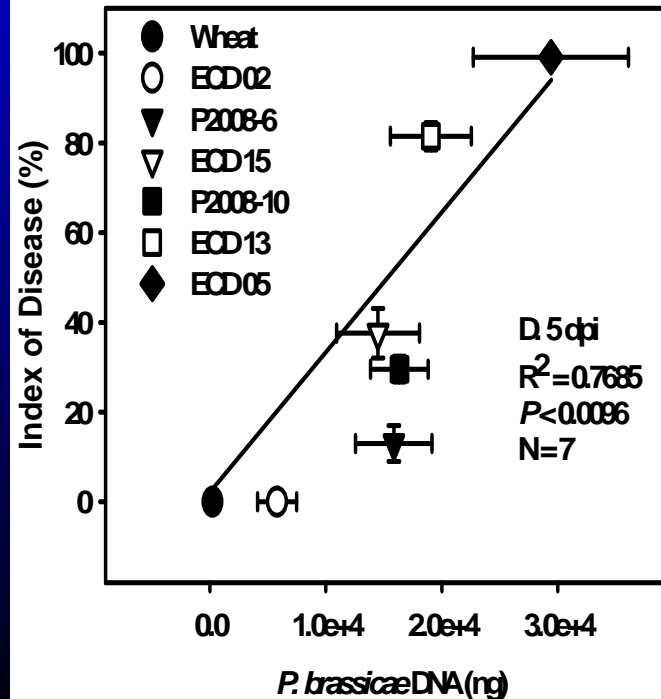
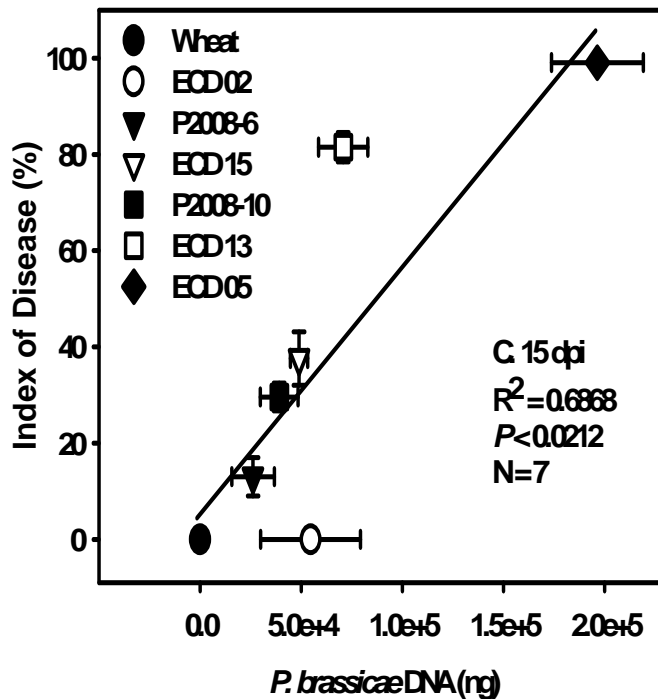
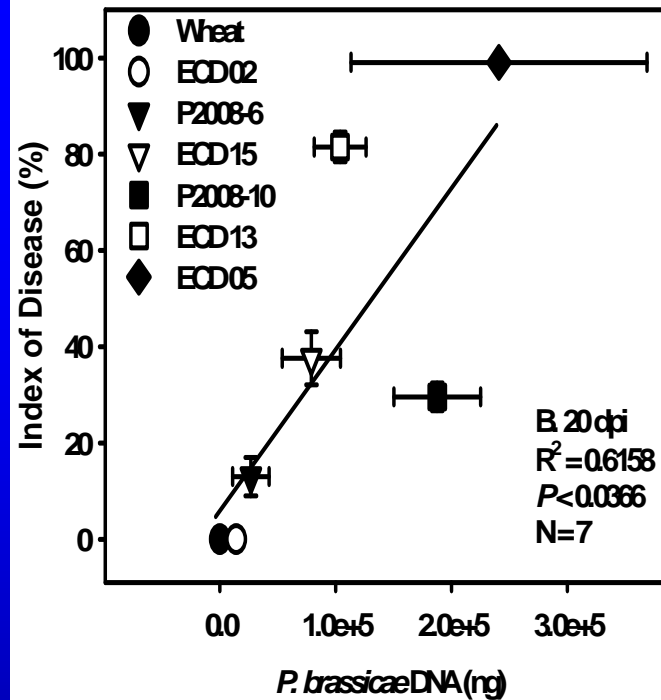
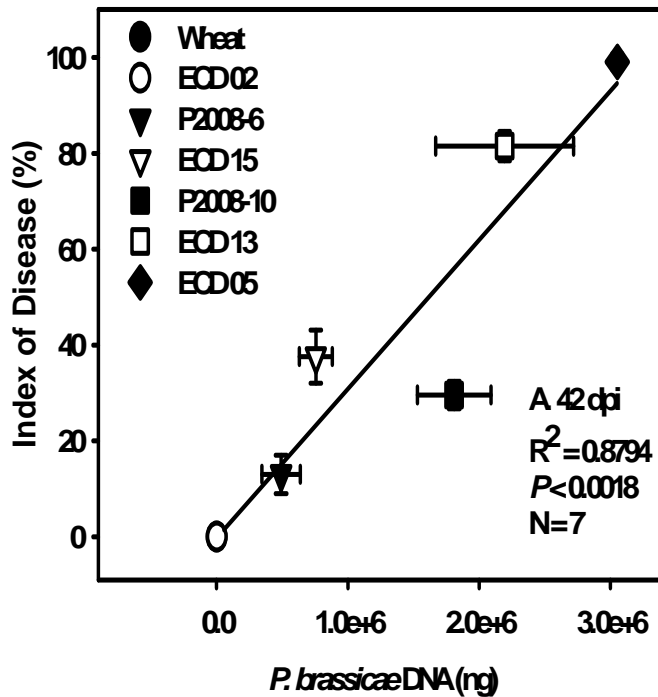
# Amount of *P. brassicae* DNA in Roots



Completely  
Susceptible



Non-Host



- **Good correlations between clubroot severity at 42 days, and amount of pathogen DNA at 5, 15, 20 and 42 dpi**

- **Amount of DNA as early as 5 dpi could be used as predictor of eventual clubroot response**



# Clubroot Resistance Stewardship

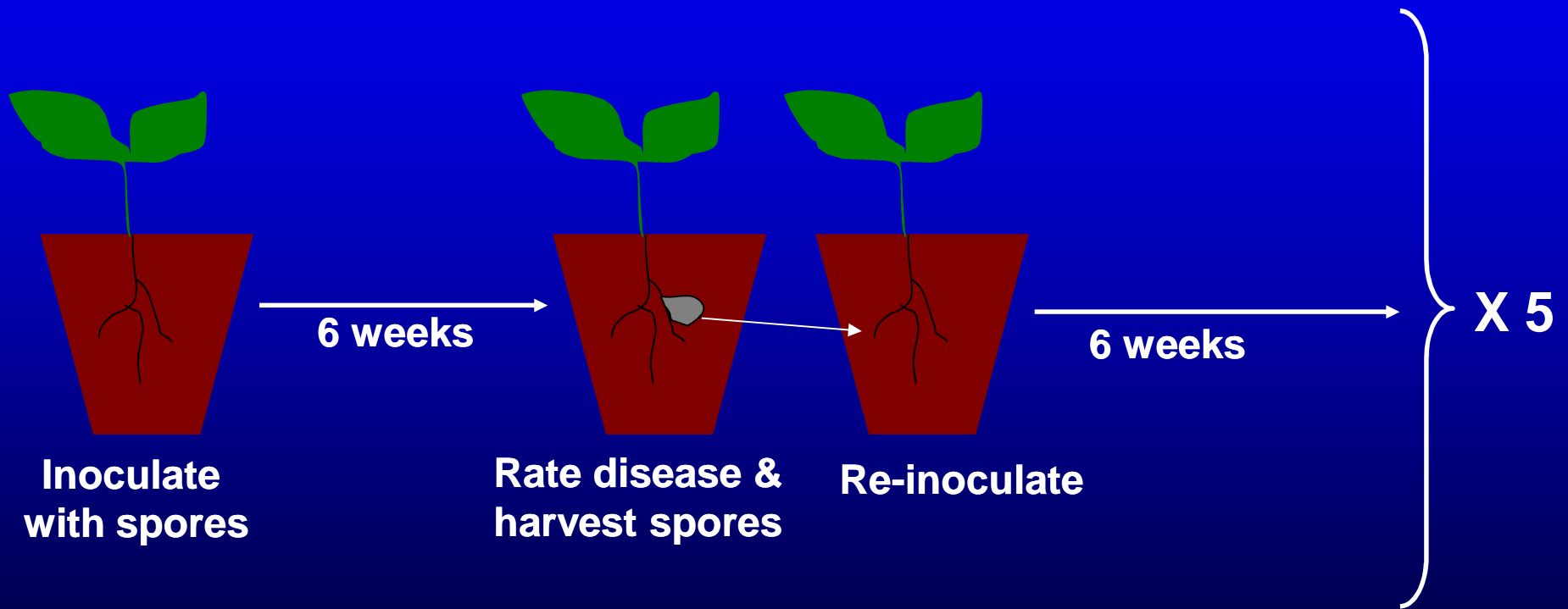
- Genetic resistance holding up well as of 2011, but will have to be well-managed!
- Conducting series of studies looking at adaptive potential of *P. brassicae*



# Pathogen Cycling Experiment

- **Objective:** To assess the effect of multiple infection cycles on the virulence of *P. brassicae*
- **Methodology:**
  - Population and single-spore isolate representing pathotype 3
  - Cycled 5× on a selection of R, MR and S host genotypes

# Methodology

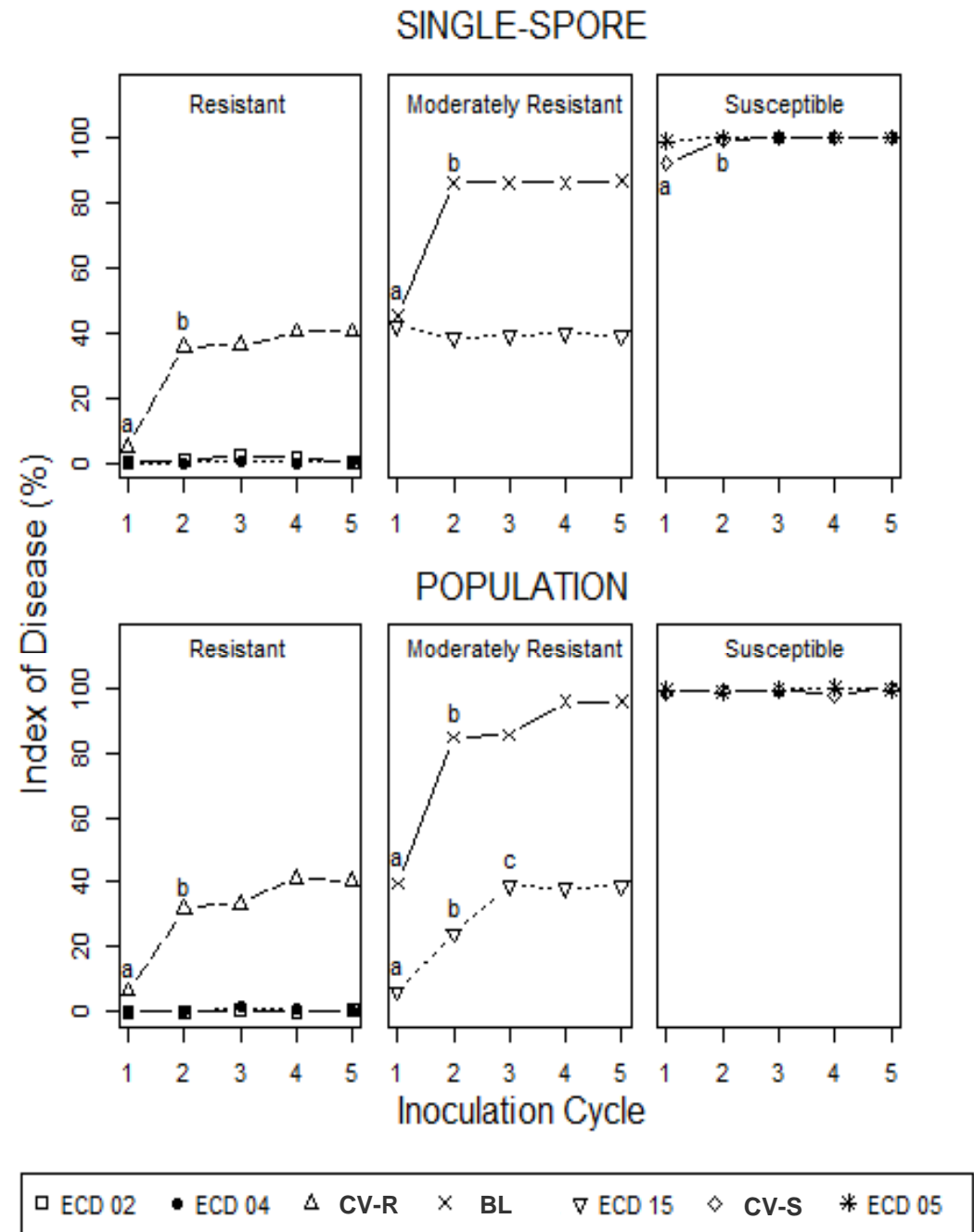


**GREENHOUSE STUDY**

# Pathogen Cycling

Repeated cropping of a resistance source can erode the effectiveness of that resistance

Resistance stewardship is important!



# Cross-Infectivity Experiments

- **Objective:** To assess whether various commercial canola cultivars carry the same or different sources of resistance
- **Methodology:**
  - Cross-inoculate canola cultivars with *P. brassicae* populations cycled on other *Brassica* hosts
- **Rationale:**
  - If same source of resistance, then pathogen populations cycled on one cultivar should show increased infectivity on other cultivars

# Cross-Infectivity Experiments

**Pathogen populations cycled on one host did not show equivalent increases in virulence on other hosts**

Canola host	Cycled populations			
	CV-R	BL	ECD 05	ECD 15
<b>W</b>	5.5±9.4	1.9±7.7	4.6±8.9	5.5±9.4
<b>X</b>	8.6±2.9	0.0±0.0	0.0±0.0	0.0±0.0
<b>Y</b>	1.9±7.7	0.0±0.0	0.0±0.0	0.0±0.0
<b>Z</b>	11.1±9.5	0.0±0.0	0.0±0.0	0.0±0.0

LeBoldus et al. 2012

# Rotation of Resistance Sources

- **Cross-infectivity experiments suggest that some cultivars may be carrying different sources of resistance**
- **Potential for rotation of resistance sources?**
- **Further work is ongoing**



## Other Activities



- **A wide breadth of other research is also currently underway**
  - **Clubroot and soil foraging by roots**
  - **Resting spore survival in dust**
  - **Molecular diversity of pathogen populations, markers for strain differentiation**
  - **Histopathology & host-pathogen interactions**
  - **Development of a Canadian Clubroot Differential system... *discussion later today!***



# Summary

- **Extensive research under the Pathology Pillar**
- **Focused on various streams: pathogen surveillance and dispersal, development of improved clonotype quantification tools, resistance stewardship & other areas**
- *Striving to meet the aim of improved understanding of the pathogen & resistance mechanisms, disease surveillance, pathotype monitoring & detection/diagnosis*

# Acknowledgments

- **Collaborators**
- **Students & other research personnel**
- **Funders:** Canola Council of Canada through AAFC Clubroot Risk Mitigation Initiative, Alberta Crop Industry Development Fund, Alberta Innovates – Bio Solutions, ACPC, Canadian Seed Growers Association, SaskCanola, MCGA and other industry partners