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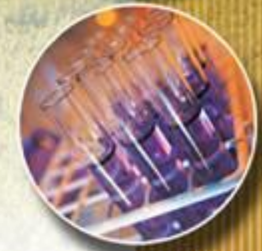


Patch Management for Clubroot Control

M. R. McDonald and B. D. Gossen

University of Guelph and AAFC, Saskatoon

Canola Discovery Forum

Canada

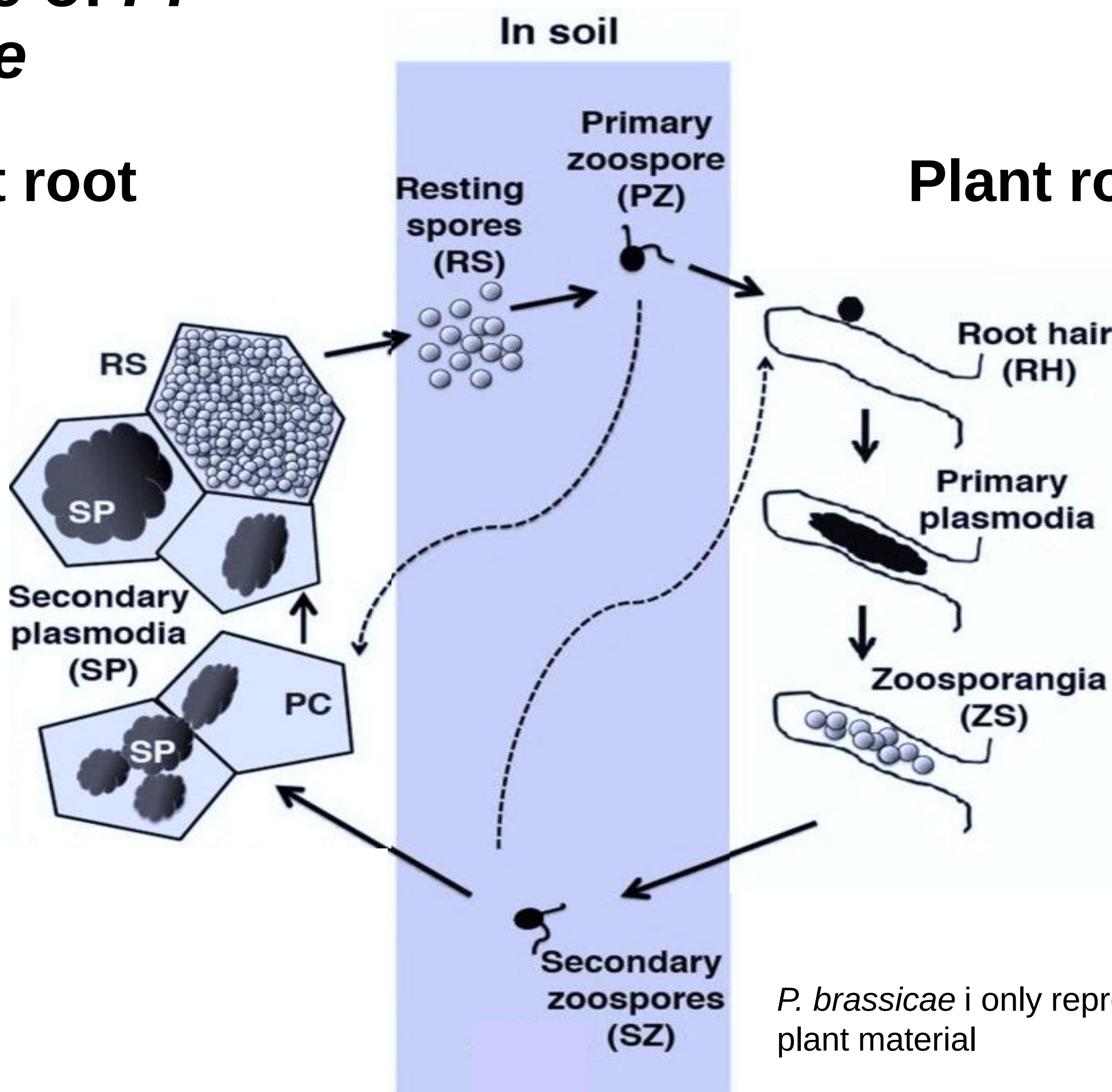
Clubroot on Brassica crops

- Cause: *Plasmodiophora brassicae* (Woronin)
- Needs a living host to reproduce
- Attacks most plants in the mustard (*Brassicacea)* family
- Causes stunting, delayed maturity, yield loss, and plant death
- Persistent resting spores, some survive many years in soil
- Many pathotypes that infect different crops and cultivars

Life cycle of *P. brassicae*

Plant root

Plant root



P. brassicae i only reproduces in living plant material

Arne Schwelm *et* al., 2015

Why Ontario?

- Clubroot on vegetables since 1920's
- Canola in Ontario in 2016
- Clubroot is not a regulated disease in Ontario
- Naturally occurring at the Muck Crops Research Station
- Hedging our bets: different locations with different weather

Canadian Canola Clubroot Cluster Pillar 3: Host-pathogen biology and interaction

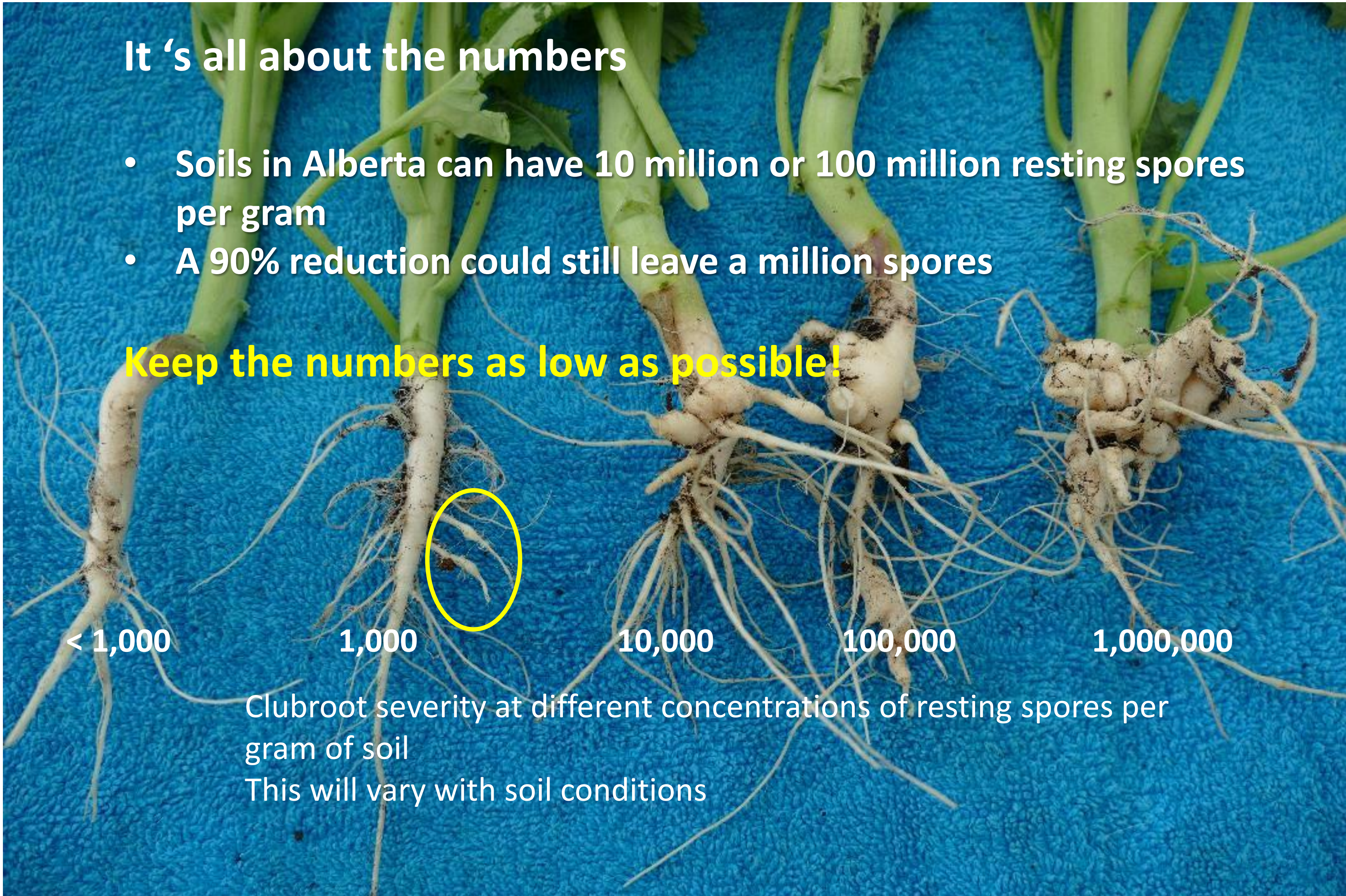
Objectives:

- **Identify methods to reduce resting spores and slow the spread of clubroot (Gossen / McDonald).**
- **Identify lines of Brassica species that carry multigene resistance compare to those with single gene resistance (McDonald, Gossen).**
- **Examine the mechanisms and role of Quantitative (multigene) Resistance in IPM for clubroot (Peng et al.).**
- **Examine the hormonal changes in canola during clubroot development (Strelkov / Hwang).**

It ‘s all about the numbers

- Soils in Alberta can have 10 million or 100 million resting spores per gram
- A 90% reduction could still leave a million spores

Keep the numbers as low as possible!



Clubroot severity at different concentrations of resting spores per gram of soil
This will vary with soil conditions

Implications of high inoculum levels

- Yield reduction (of course)
  - Starts at 10^5 spores, 100% yield loss at 10^7 (Hwang et al. 2015)
  - Winter oilseed rape in the U K: 0.03 t/ha for every 1% increase in clubroot severity, therefore over 50% loss (McGrann et al 2016)

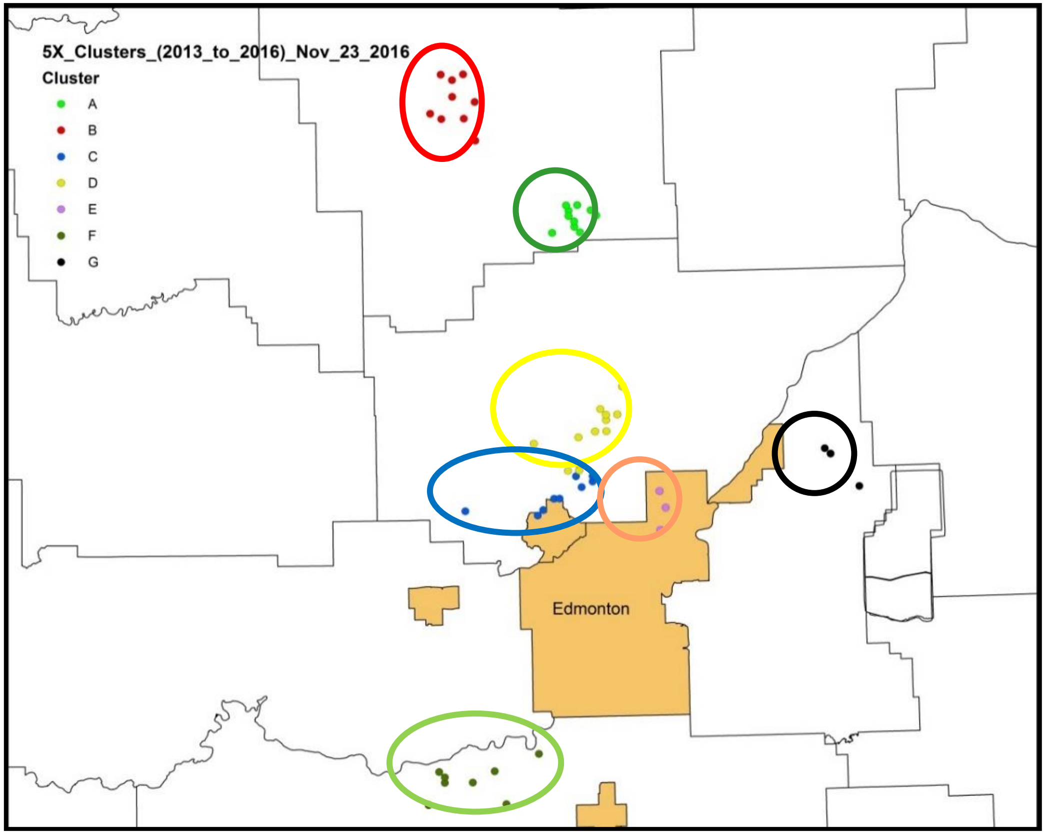
- Biological controls (and other methods) less effective
  - *Heteroconium* (Narisawa et al. 2005)
  - *Piriformospora indica*

- Lower yields (5 – 10%?) when resistant canola is grown in highly infested fields– metabolic cost of resistance

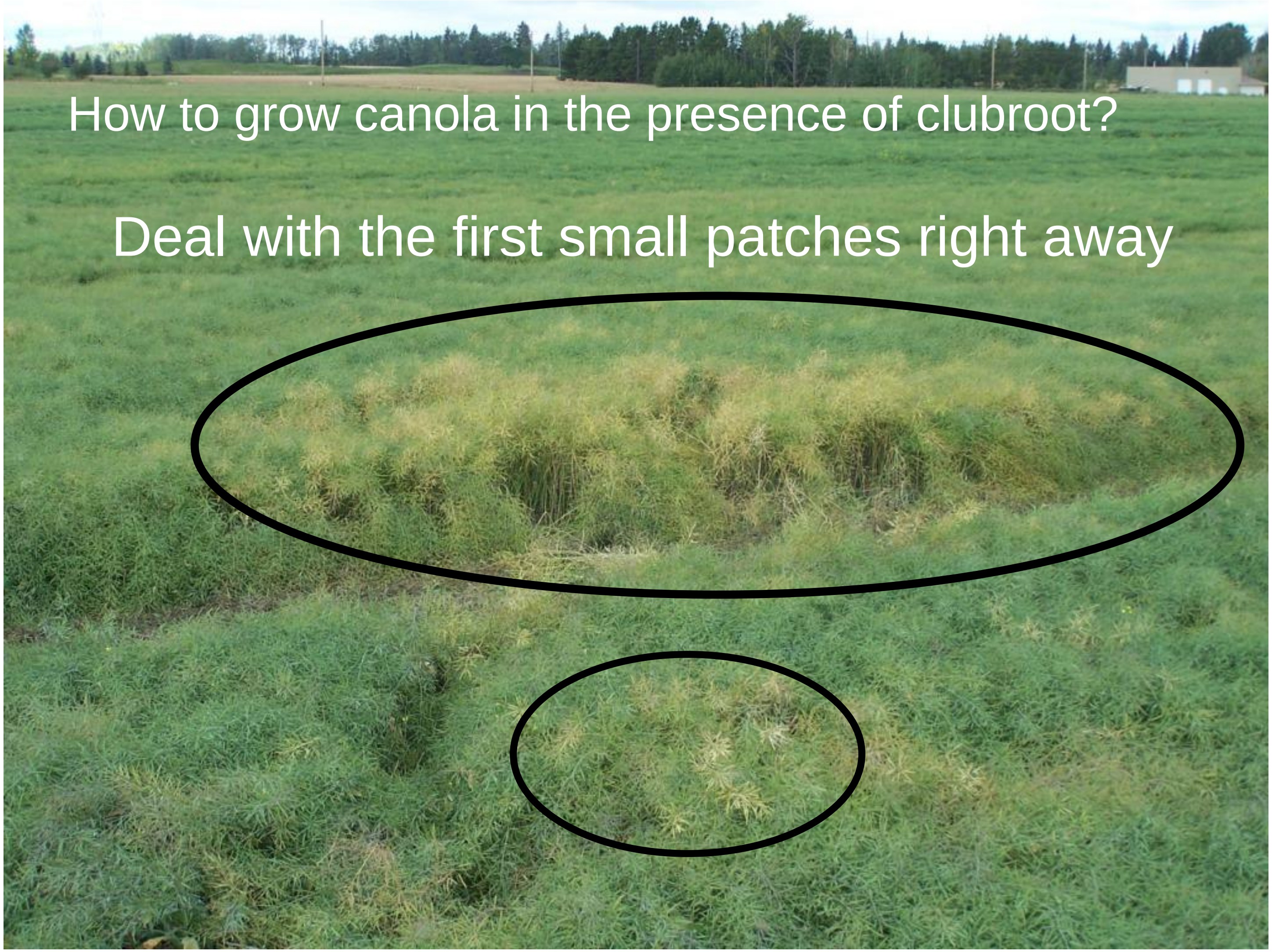
- **Selection of new virulent pathotypes**

Clusters of Fields with New Strains

- Clubroot resistant canola introduced in 2009
- New virulent pathotypes first found in 2013
- More found each year



Strelkov & Hwang

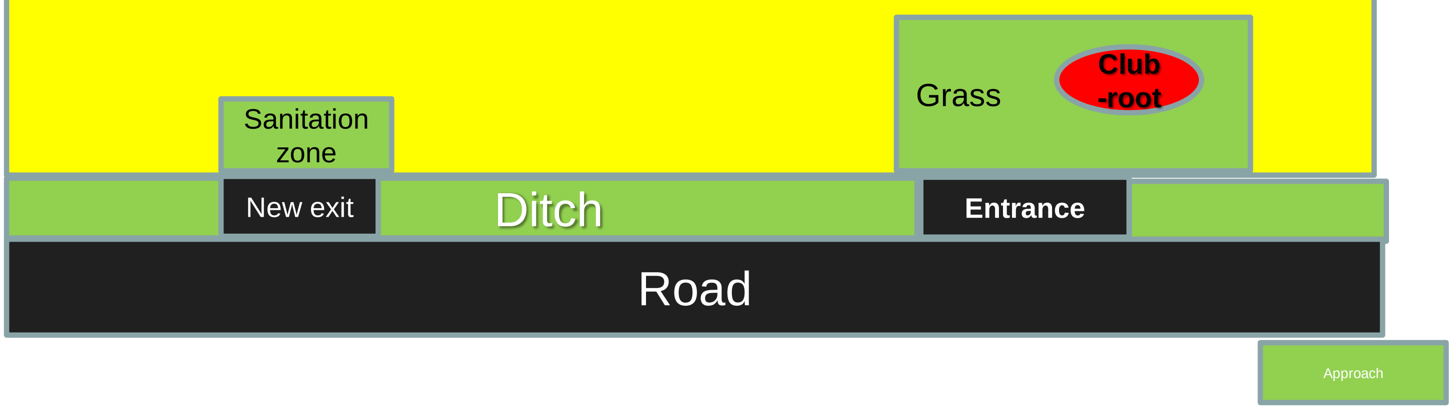

How to grow canola in the presence of clubroot?
Deal with the first small patches right away

CCC - Manage your patch!

In western Canada, clubroot usually shows up first near the field entrance and to the right

Field

Recommended to grass the area around the clubroot patch and create a new field entrance or exit

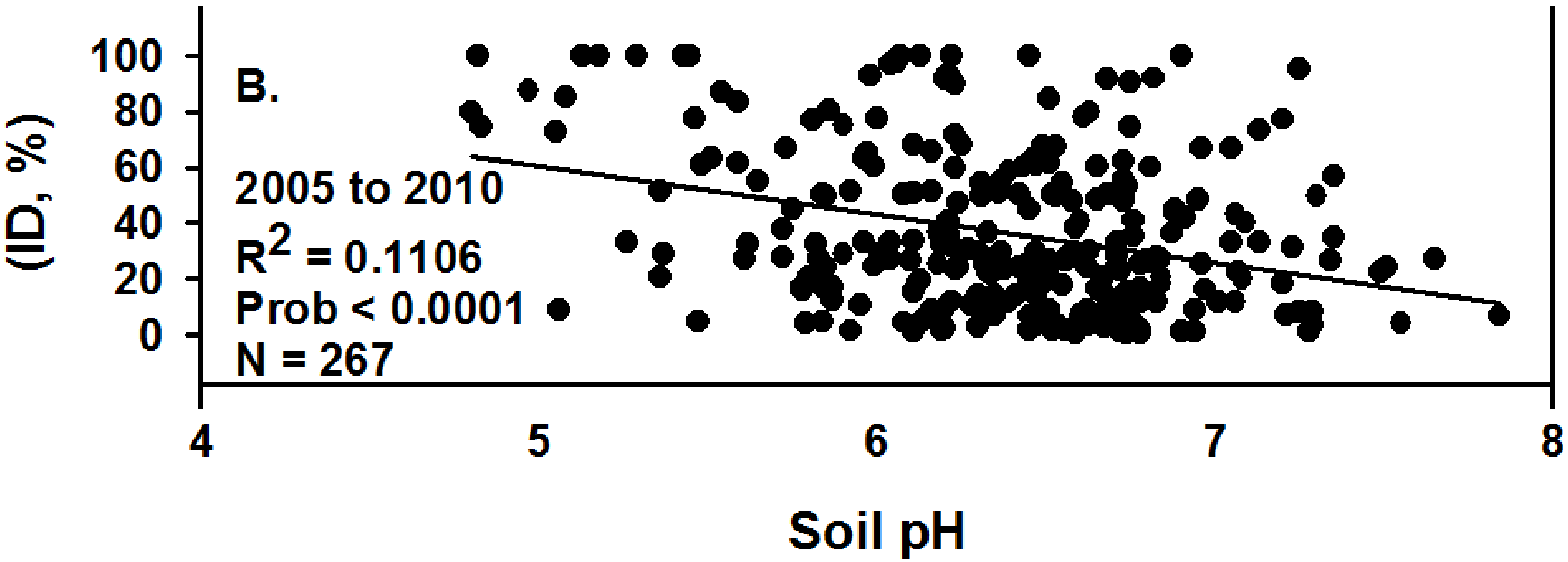


Patch Management Recipe for Clubroot

- Identify and mark infested area
  - Pull plants to confirm clubroot, pull others in an outward circle. **Destroy clubs**
  - Mark double the affected area (at least!)
  - No traffic on that area.

- Next
  - Incorporate lime to increase pH 7.5
    - (fumigate / solarize/ add boron?).
  - **Seed a grass cover crop**
  - When established, equipment can move through
- After 2 or 3 years: evaluate and terminate
  - Sample soil to check spore concentrations
  - When levels fall enough, break sod.
  - Use only clubroot-resistant cultivars.

Does high pH reduce clubroot?



Field assessments from 267 clubroot infested fields in Alberta

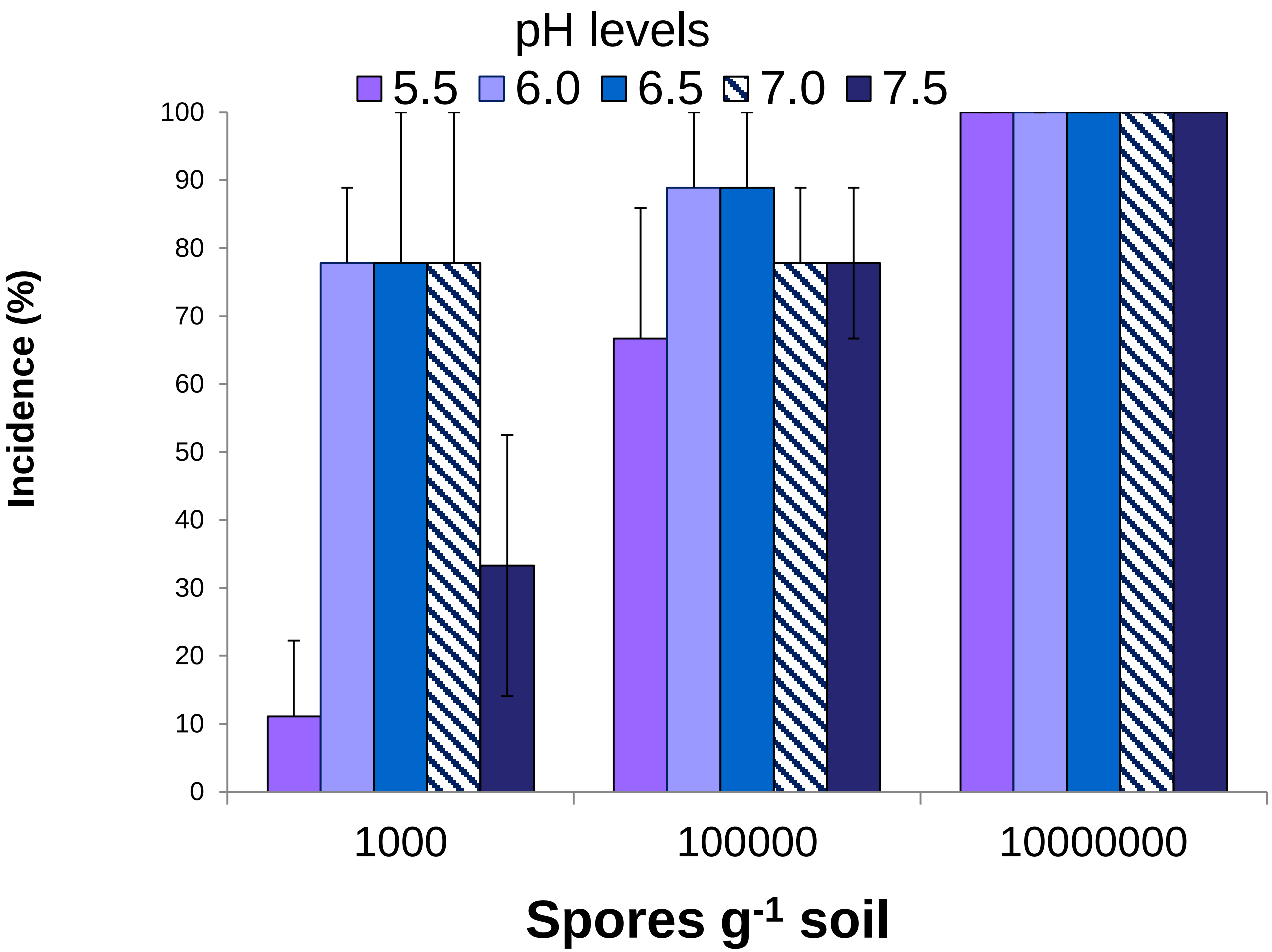
Data support the observations under controlled conditions; pH above 7.5 reduces clubroot, but otherwise the relationship is quite weak

(Gossen et al. 2013)

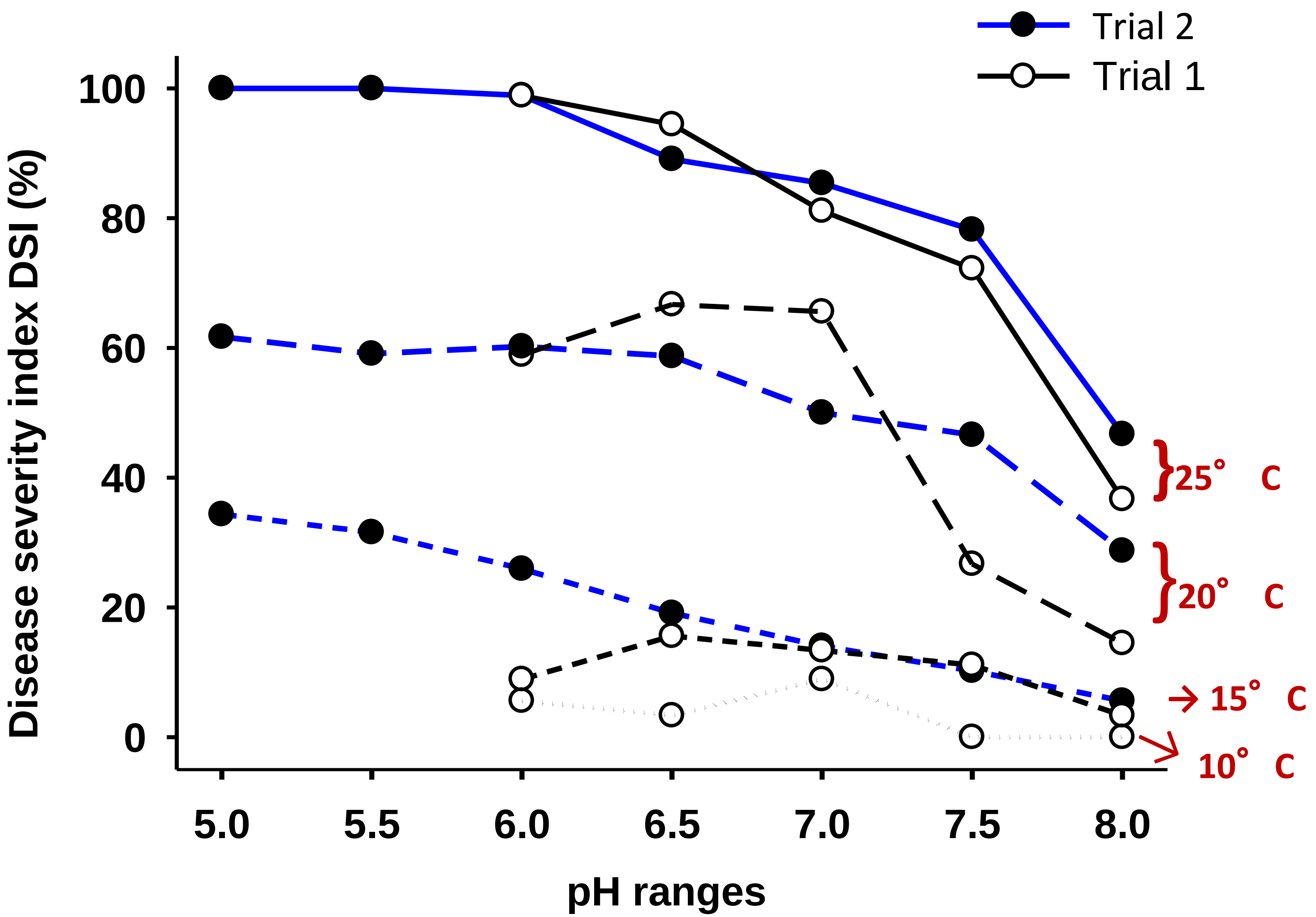
Resting spores, pH and clubroot

Resting spore concentration influences the response to pH

High spore concentration overcomes the effect of high pH in suppressing disease



Interaction of temperature and pH on clubroot on canola in growth room trials



Add Lime:
Raising soil pH to suppress clubroot
-Common in Europe
-Common for vegetables

Apply lime to increase soil pH to 7.2 (sometimes 7.5).
This can require several tonnes/ha

Several trials are underway:
Spruce Grove Alberta – 2018
Field trials in Saskatchewan and Manitoba in 2019
(Gossen, Froese, McDonald)
Keith Gabert and others

Patch management site established near Spruce Grove, AB in 2018.

- Lime (zero grind)
- Hydrated lime
- Lime + hydrated lime (half rates)
- Perennial ryegrass
- Untreated and not grassed

Soil pH and clubroot

- Use buffer pH to calculate rates of lime
- There are other factors affect clubroot development:
- It may appear that raising the pH has no effect, but this is difficult to tell without a check treatment
- pH may act separately from calcium
- Calcium content or calcium base saturation (over 70%?) may be important, but more research is needed

Spruce Grove site June 2019

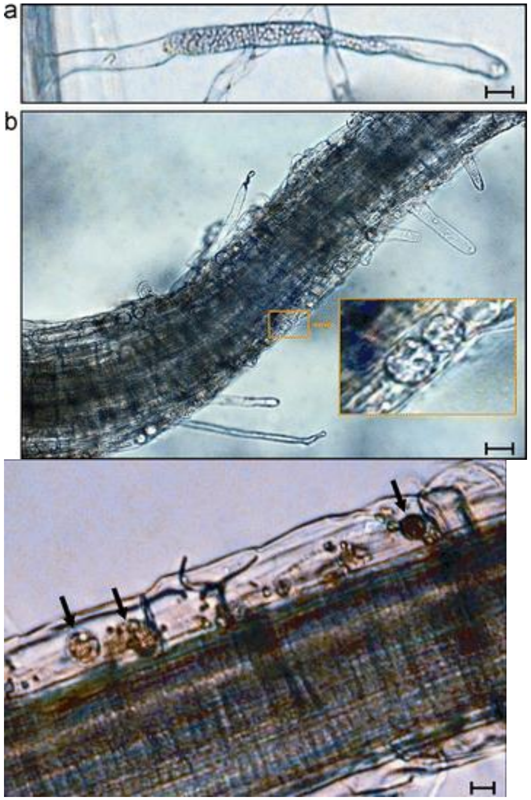
Treatment	pH
Hydrated lime	6.9
Lime	6.8
Lime+ H lime	6.7
Grass	5.8
Control	5.5

Seed Grass:
Can cover crops reduce resting spores in soil?

Grasses to hold soil in place
Germination of resting spores is stimulated by roots of grasses

- MacFarlane (1952) observed primary infection in non- host plants:
  - Perennial ryegrass

- Feng (2012) observed infection in ryegrass but resting spores were not produced.
- Trap crops not always effective, maybe because of high inoculum



Jie Feng *et* al.,2012

Resting spore decline with grasses - Methods

Crops:

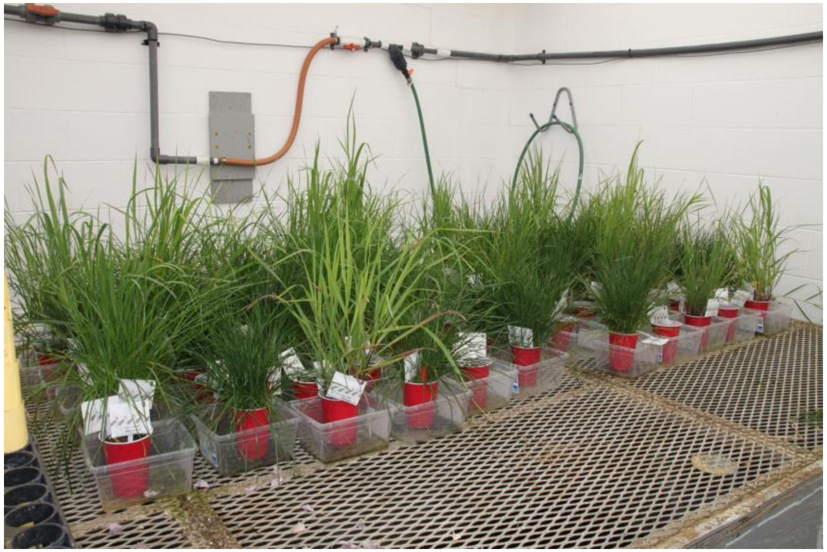
- Shanghai Pak Choy (*Brassica rapa*)
  susceptible check

- Perennial ryegrass (*Lolium perenne* L.)
  cv.s Norlea, All Star, and Fiesta

- Smooth bromegrass (*Bromus inermis* L.) Signal, Radisson, a common seed lot

- Meadow bromegrass (*B. riparius* R.) cv. Fleet

- Compared to no plants



Grown in inoculated field soil for 8 weeks (target 500,000 resting spores/gram and compared to bare soil

Resting spore concentration in soil with different crops

Crop	Cultivar	Spore conc. g^{-1} soil
Pre-plant		49,000 a
After 8 weeks		
Soil only (control)		30,000 ab
Perennial ryegrass	Norlea	31,000 a
Smooth bromegrass	Signal	**24,000 bc**
Smooth bromegrass	Radisson	**12,000 c**

Grass species grown for 8 weeks in the soil inoculated with $5x10^5$ resting spores mL^{-1} based on qPCR (n = 6).

Resting spore concentration in soil with different grass cover crops

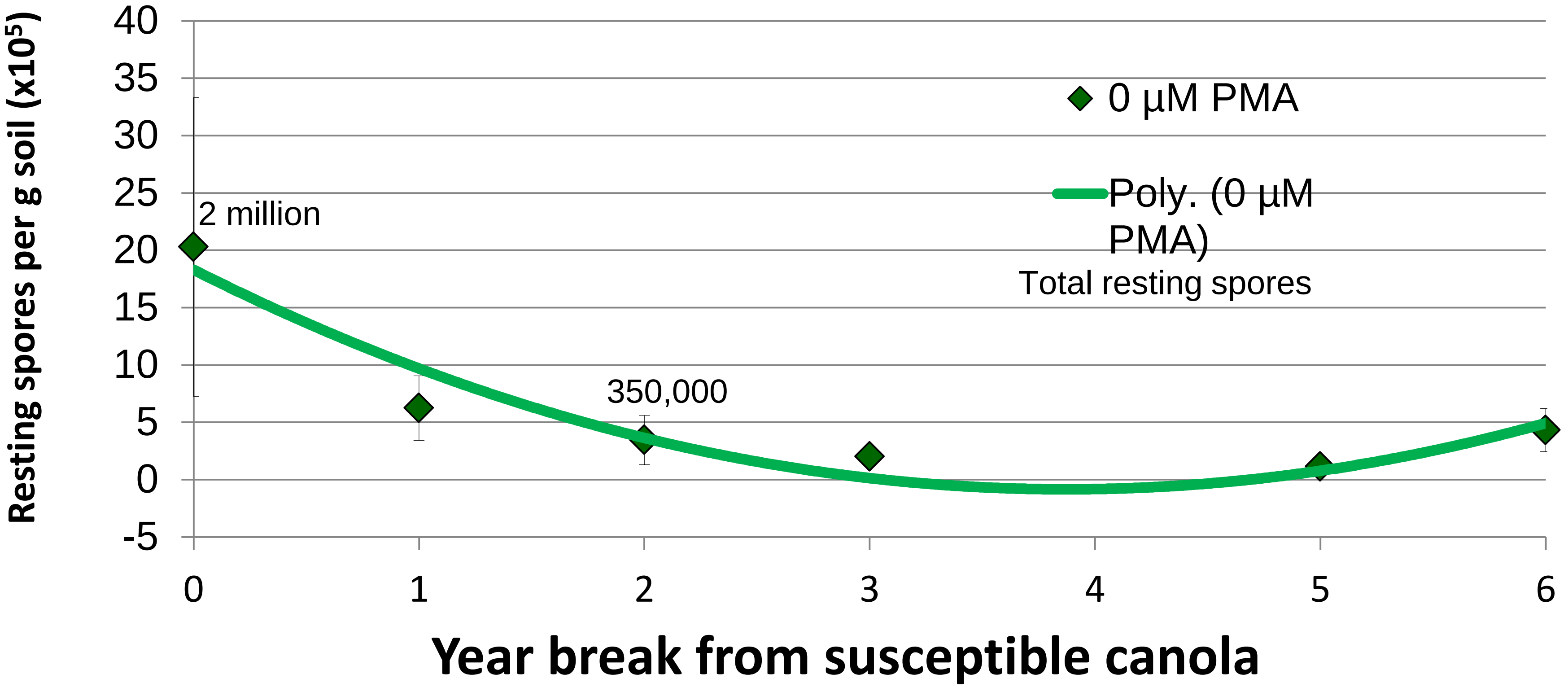
Crop	Cultivar	Spore conc. g^{-1} soil
Pre-plant		460,000
Soil only (control)		**260,000**
Meadow bromegrass	Fleet	230,000
Perennial ryegrass	All Star	220,000
Perennial ryegrass	Fiesta	140,000
Perennial ryegrass	Norlea	140,000
Smooth bromegrass	common seed	**110,000**

Plants grown for 8 weeks in the soil inoculated with $5x10^5$ resting spores mL^{-1} based on qPCR (n = 6).

No significant differences, but a trend.
Grass cover crops are important to prevent the movement of soil and may reduce resting spore numbers (smooth bromegrass?)

How long for rotation or a cover crop?

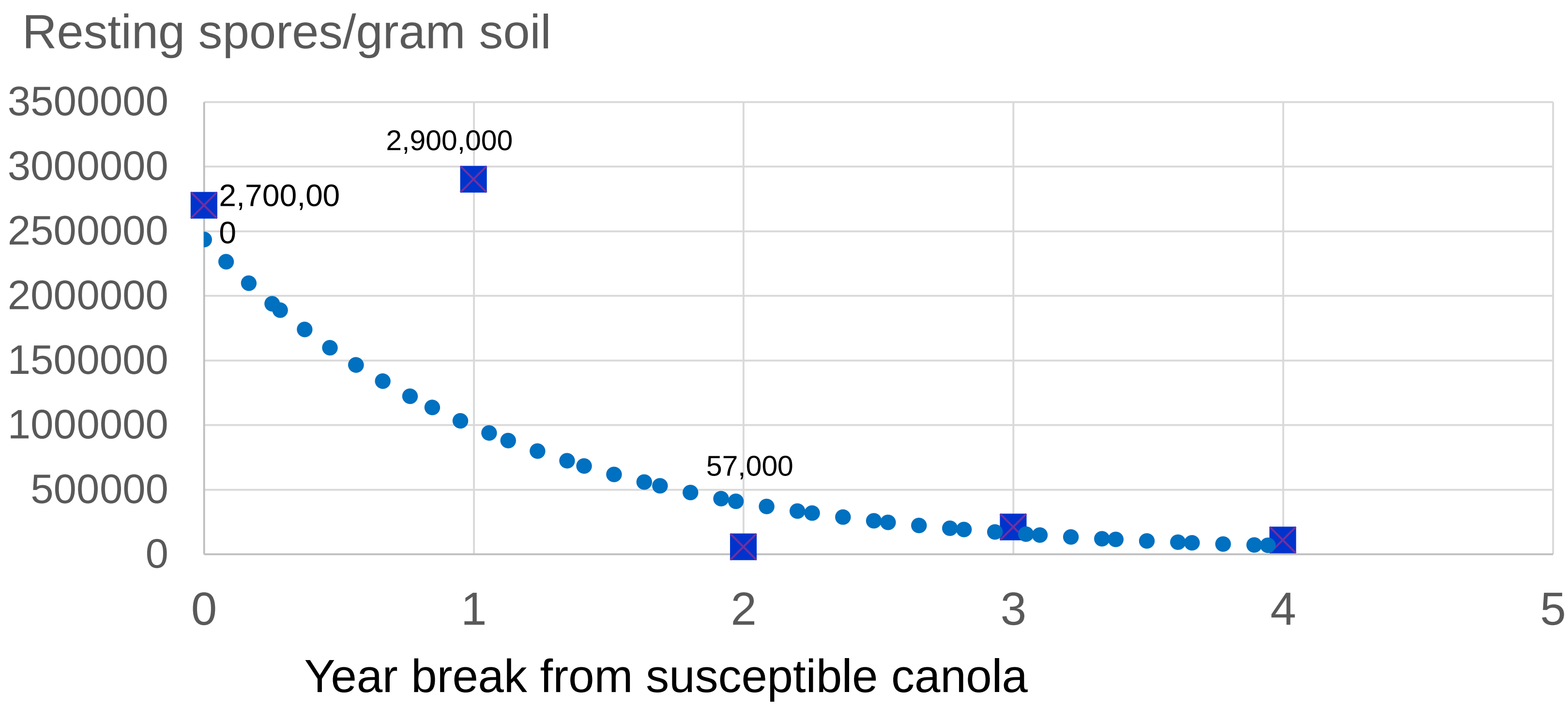
Resting spore counts in relation to crop rotation in soil, Normandin, Que



* Regression lines ($P < 0.05$)

Break from canola: soil samples also from Normandin

Peng et al. 2016



A two year break from canola results in a major drop in resting spore numbers

The numbers: Resting spores in a club

- **100 million** resting spores of *P. brassicae* in a gram of soil in a heavily infested field in Ontario, and in Alberta (Hwang et al 2013)

- 800 million *P. brassicae* resting spores in a gram of clubbed root
  x 20 g club

= **16 billion** resting spores from one club (Hwang et al 2017)

- Our work:
- 3 billion spores per club at 5 weeks after seeding
- **10- 81 billion** spores per club at 9 weeks
  - Clubs weighed 10.4 -11.8 g

- Optimum 50 – 60 canola plants per square meter
- = up to **600 billion to 4.9 trillion (4.9×10^{12}) resting spores per square meter**
- **Prevent these from spreading further**

Remove – and destroy- infected canola and weeds
What about spraying, mowing or disking?

Weeks after seeding	Resting spores per club (billions)
2017	
5	**3.1**
Tops removed at 5 wk (mowing), assessed at 7 wk	**2.7**
7	**5.6**
9	**10.0**
2018	
Glyphosate at 5 wk, assessed at 9 wk	**92.2**
9	**81.5**

What to do with clubroot infected plants?

- Mowing (cutting off the shoots) or spraying with herbicide did not kill the resting spores or stop development at 5 weeks after seeding.
- Plants have to be controlled or removed very early – 3 weeks? (Naznani-Noor 2018)

Does burning clubs using gasoline kill resting spores

3 reps burned (twice), 3 reps not burned as a control

Clubs of 7 week old pak choi harvested from the fumigation trial and left to dry for 5 days
Excess soil was removed by shaking several times in a sieve.

Burning lasted 1.5 to 4.5 minutes during the first burn.
After 10 minutes more gasoline was applied. Burning lasted about 2.5 minutes the second time.

Assessed for resting spore viability after 2 days

Four clubs per tray
First burn 1.5- 4.5 min
Second burn 2.5 min

Non-burned clubs

Effect of burning on resting spore survival

Treatment Burned	Burning time minutes	Viable resting spores (%)	Bioassay Percent	Bioassay Severity
Rep 3	6.5	16	13	12
Rep 1	5.5	26	0	0
Rep 2	3.5	63	47	20
Unburned check	0	93	97	73

Clubs after second burn

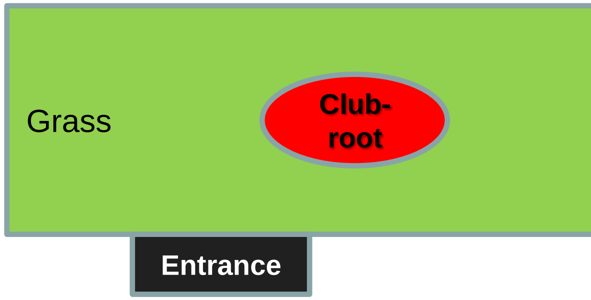
What to call this?

- **Incinerate the inoculum**
- **Roast the ‘root**
- **Clubroot campfire?**

CCC - Manage your patch!

- Check near field entrance and scout for patches

- Pull and destroy!
- Burning? A longer burn is better



- Apply lime – hydrated lime or zero grind

- Grass the area with a perennial cover crop
  Smooth bromegrass? Perennial ryegrass?
- Leave for at least 2 years, 3 years better
- Assess soil for resting spores, break and seed clubroot resistant canola
- Other options for killing or reducing resting spores are in the research stage

Clubroot on Brassica crops
It is all about the numbers!

- Resting spores can build up to huge numbers (billions, trillions and quadrillions!) in one season in a heavily infested crop
- Everything works better under low disease pressure
- Keep spore numbers low
  - reduce soil movement
  - grow resistant crops,
  - grass field entrances,
  - deal with small patches
- Crop rotation is important – 2 year break
- **Never forget selection pressure: more spores = more new pathotypes**

Acknowledgements

- Canola Council of Canada
- Agriculture and Agri-Food Canada
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- Ontario Canola Growers Association
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- Fadi Al-Daoud
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Also many publications by Steve Strelkov and Sheau-Fang Hwang, Univ. of Alberta, and Gary Peng, AAFC Saskatoon


Questions?

For a quicker effect: Fumigation and/or solarisation Or boron?

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

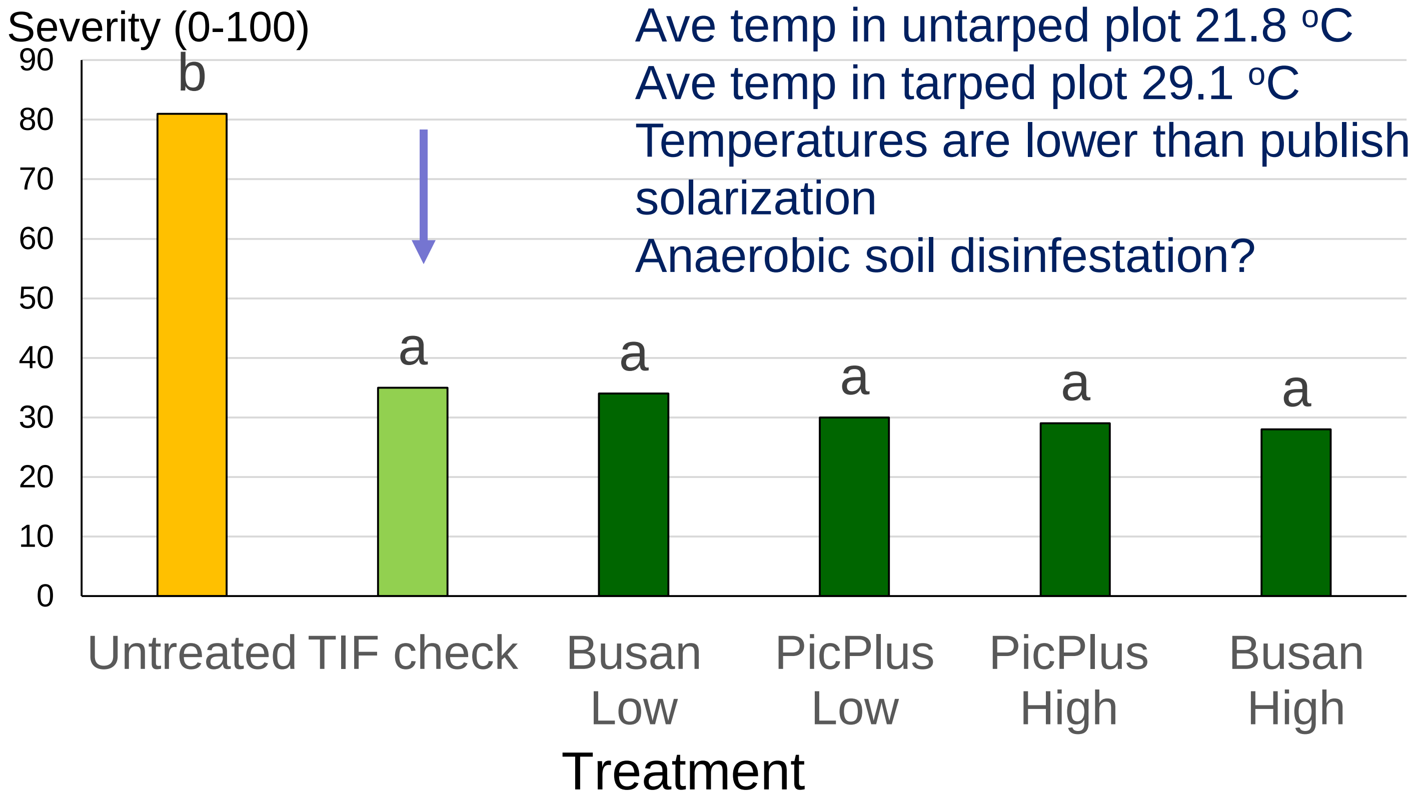
Uncovered check and untreated-tarped check

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

2017 field plot in Ontario with naturally infested high organic matter soil

Clubroot severity in pak choy following fumigation -2017

Solarisation?
Ave temp in untarped plot 21.8 ^{o}C
Ave temp in tarped plot 29.1 ^{o}C
Temperatures are lower than published for solarization
Anaerobic soil disinfestation?

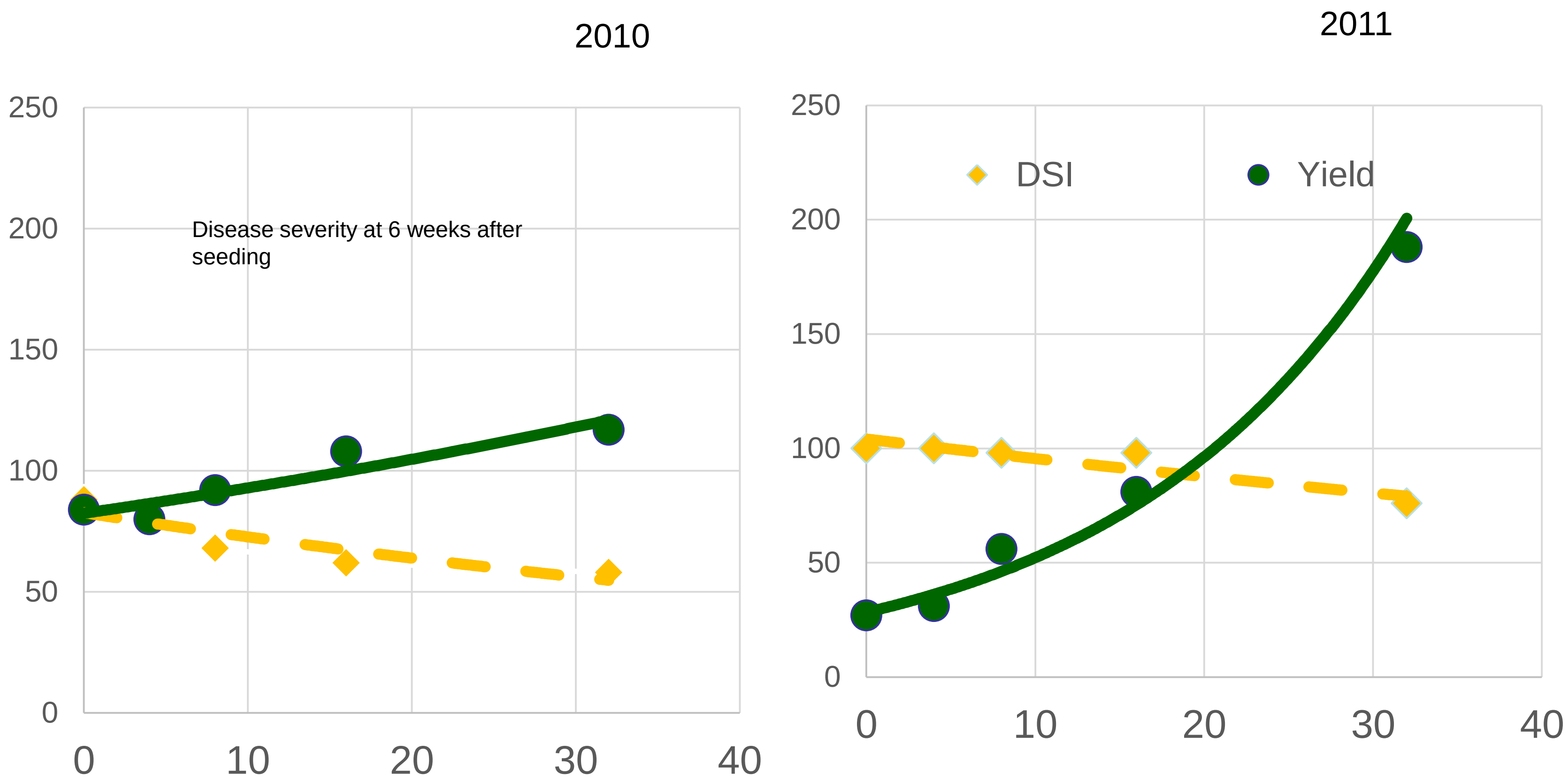


Calcium and boron

- Calcium (calcium carbonate) and boron (1- 3 kg/ha, sometimes more) are routinely applied to brassica vegetables.
- Currently boron is not recommended for canola on the prairies, as no yield increase is expected. This is being revisited by some companies.
- Calcium, boron and nitrate nitrogen, delay or prevent the development of the pathogen in root hairs and the release of secondary zoospores
- Calcium inhibits the germination of resting spores (Dixon and Page 1998)
- Boron enhances the effects of calcium (Dixon and Page, 1998)
- **Both Ca and B appear to have direct effects on the pathogen and on the host, but the actual modes of action are not well understood** (Dixon 2009)

Effect of rates of boron on clubroot and yield- field trials

Disease severity and yield (x 10kg/ha)



Rates of boron kg/ha
(0, 4, 8, 16, 32)

Deora et al. 2014)


Boron
No boron

The numbers: Plasmodiophora

- Our work:
- 3 billion spores per club at 5 weeks after seeding
- 8-10 billion spores per club at 9 weeks
- Clubs weighed 10.4 -11.8 g

- 16 billion to 100 billion (1×10^{11}) resting spores per clubbed root
- Optimum 50 – 60 canola plants per square meter
- = up to **5 - 6 trillion (6×10^{12}) resting spores per m^2**


Hybrid seed
production
Male & female lines

Clubroot in Ontario

PATCH MANAGEMENT FOR CLUBROOT CONTROL

Mary Ruth McDonald and Bruce D. Gossen

How can we grow canola in the presence of clubroot?

Avoid selection pressure: Keep spore numbers low

Methods to reduce the numbers of resting spores

- Crop rotation
- Trap crops
- Soil treatments- calcium cyanamide
- Fumigation and solarisation/anaerobic soil disinfestation

Lower clubroot even with high resting spores

- Temperature
- Lime and pH, calcium, boron
- Fungicides

Untreated check clubs 2 m away from wheelbarrow with burning clubs

Crop rotation, canola and clubroot

- There are many benefits to crop rotation beyond clubroot management
- Greatest reduction in viable resting spores in the first year, some decrease in the second year, then very slow decline
- The spores that survive the first year or two are viable and very persistent
- Recommend a two year break from canola – but there could still be high numbers of resting spores in the soil

Objective from the Canola Council of Canada:
How can we grow canola in the presence of clubroot?

Avoid selection pressure: Keep spore numbers low

Methods to reduce the numbers of resting spores

- Crop rotation
- Trap crops
- Soil treatments- calcium cyanamide
- Fumigation and solarisation/anaerobic soil disinfestation

Lower clubroot even with high resting spores

- Temperature
- Lime and pH, calcium, boron

Canola vegetables

- High area
- Low value relative to vegetables but high value for a field crop
- Farm gate $1,431- 1,776/ha
- Some crop rotation
- Relatively low inputs- no liming or micronuntrients
- Some fungicides or insecticides
- Custom farm operations common
- No-til
- Seed harvested at maturity
- Direct seeded
- Most canola in Canada is spring canola

Brassica

- Low area
- High value per area:

Farm gate broccoli- $16,288/ha

- Longer rotations?
- Many inputs: lime, boron and other micronutrients
- Many fungicides and insecticides
- Little custom farm work
- Tillage
- Immature shoot or flower buds harvested
- Many grown from transplants

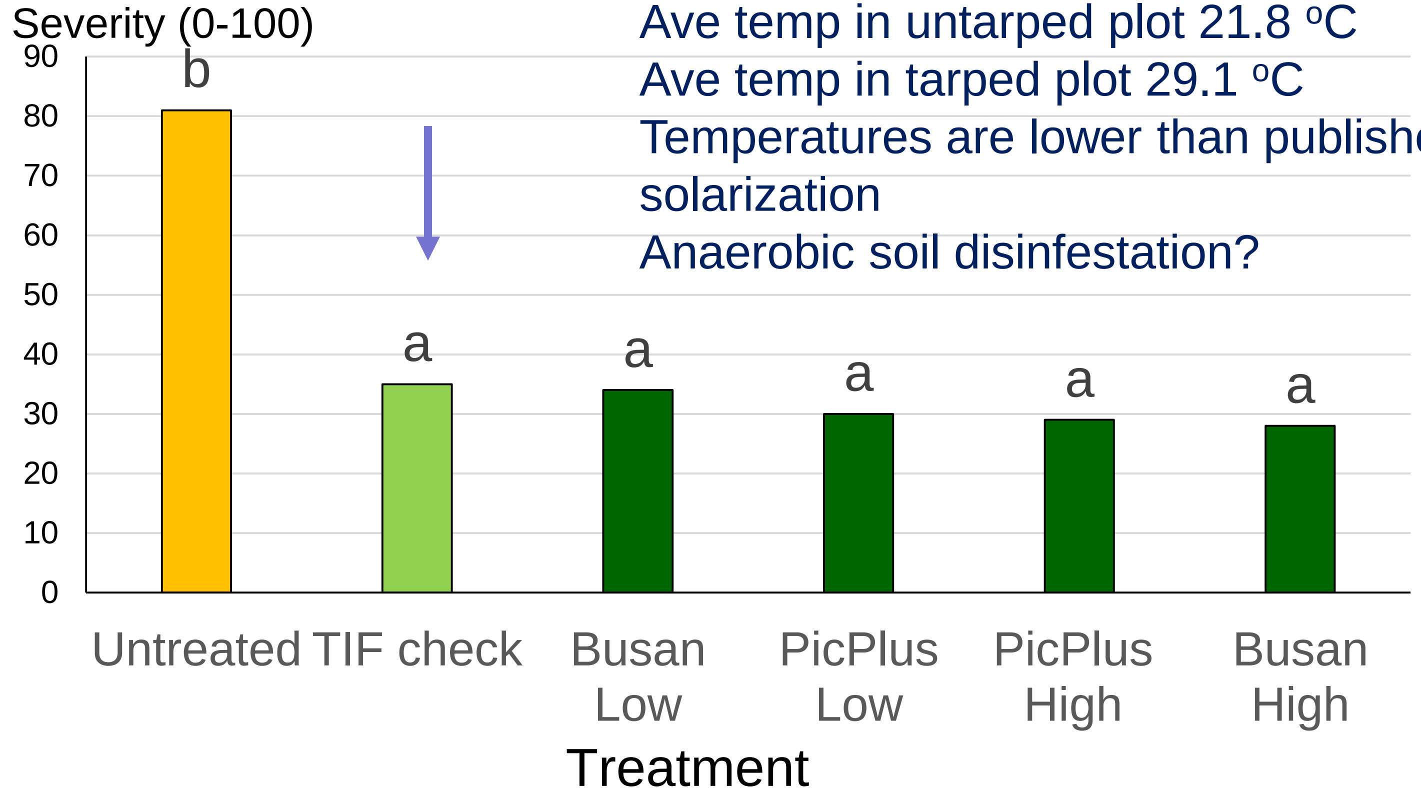
Resting spore concentration in soil with rotation crops

Crop	Cultivar	Spore conc. g^{-1} soil
Soil only (control)		$1x10^7$ a
Perennial ryegrass	Norlea	$9x10^6$ ab
Perennial ryegrass	All Star	$4x10^6$ ab
Perennial ryegrass	Fiesta	$2x10^6$ b
Smooth bromegrass	common	$4x10^6$ b
Meadow bromegrass	Fleet	$3x10^6$ b

Crops grown for 8 weeks in the soil inoculated with $5x10^5$ resting spores mL^{-1} based on qPCR (n = 6).

rops have the potential to reduce resting spore numbers in the soil while preventing the movement of infested soil within or between fields.

Clubroot severity in pak choy following fumigation -2017



Solarisation?
Ave temp in untarped plot 21.8 ^{o}C
Ave temp in tarped plot 29.1 ^{o}C
Temperatures are lower than published for solarization
Anaerobic soil disinfestation?

Recommendations for clubroot management

Canola

- Avoid infested fields
- Prevent introduction
- Resistant cultivars
- Crop rotation 2 to 4 years
- Intensive management of new outbreaks (new recommendations to manage small patches to reduce spreading)

Vegetables

- Avoid infested fields
- Prevent introduction
- Resistance not always available
- Crop rotation – 5 to 7 years (old recommendation) Currently: crop rotation is not effective
- Lime to pH 7.2 or above
- Fungicides at planting

Yield penalty and metabolic cost of resistance:

Some data from trials that were not established to look at the effect of resistance on plant growth, but showed the same trend.

- Hwang et al. 2011. Plant Pathology 60:820-829
- Deora et al. 2013. Annals of Applied Biology 163:56-71

- Controlled environment:
- resistant canola had somewhat reduced growth when exposed to *P. brassicae*, as compared to the same plants that are not exposed to the pathogen.

•In the field, these effects are difficult to separate from crop rotation effects on canola growth

- •There can be a 20% yield increase after a 2 year break from canola (Canola Council of Canada)

Concentration of resting spores at the Alberta site

PCR results
total resting spores

	plot 1	plot 2	plot 3	plot 4	plot 5
Block 1	**137,097**	3,783,380	85,987	1,073,189	2,529
Block 2	3,290	698,927	1,463,110	123,188	56,916
Block 3	14,411	0	117,508	735,706	14,832

PMA PCR results
viable resting spores

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Block 1	**78,451**	166,382	36,500	133,946	-
Block 2	3,745	614,987	660,078	199,654	6,164
Block 3	26,284	12,773	150,027	443,903	57,692

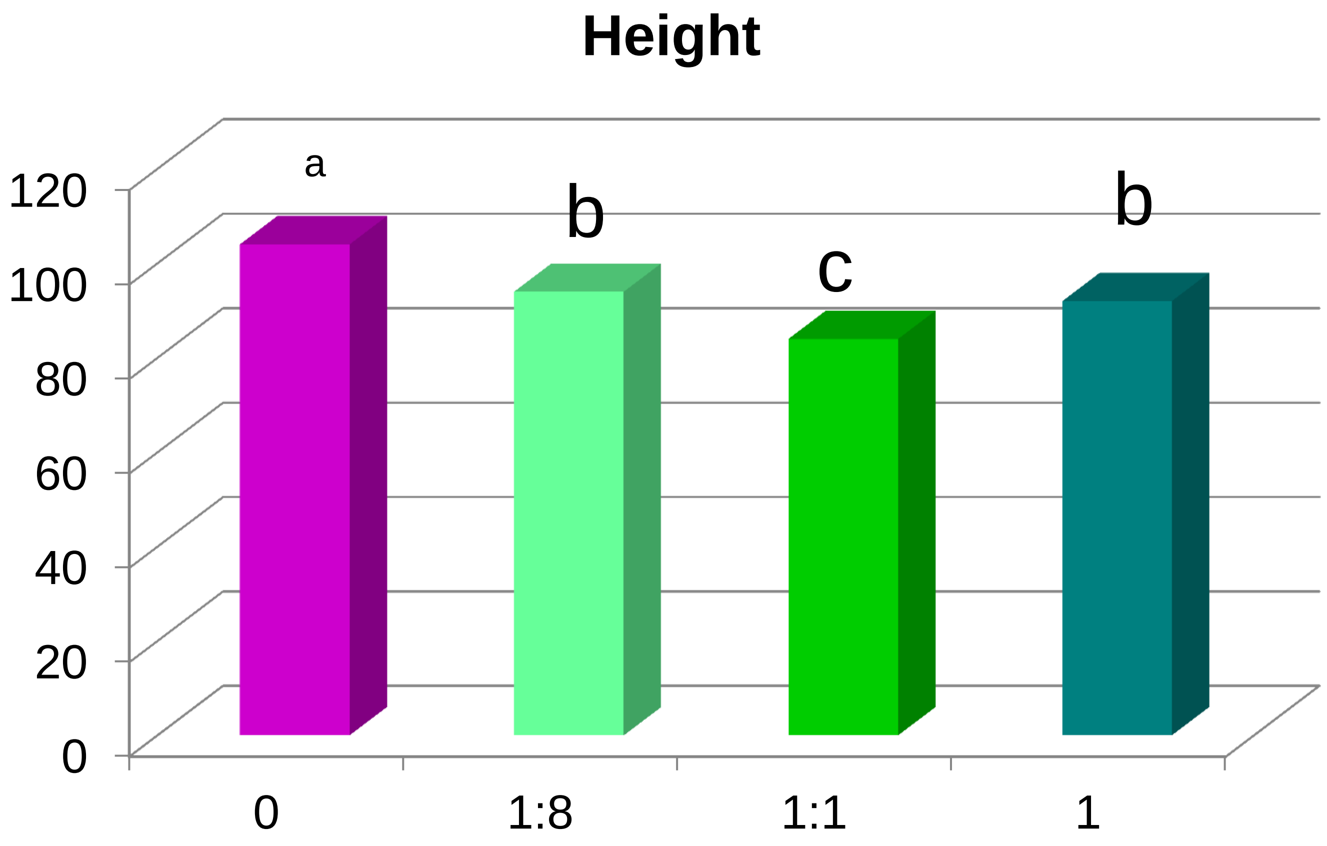
Each square is 12 x 12 m. Five soil cores to 15 cm were sampled per plot and combined for assessment. In most cases, PMA qPCR showed fewer viable resting spores
-still needs some fine tuning

Height of resistant canola 45H29 exposed to different concentrations of *P. brassicae* inoculum

(Hwang et al. 2011 Plant Pathology)

Controlled environment: 0 to 100% soil with P. brassicae resting spores

All plants exposed to resting spores were shorter



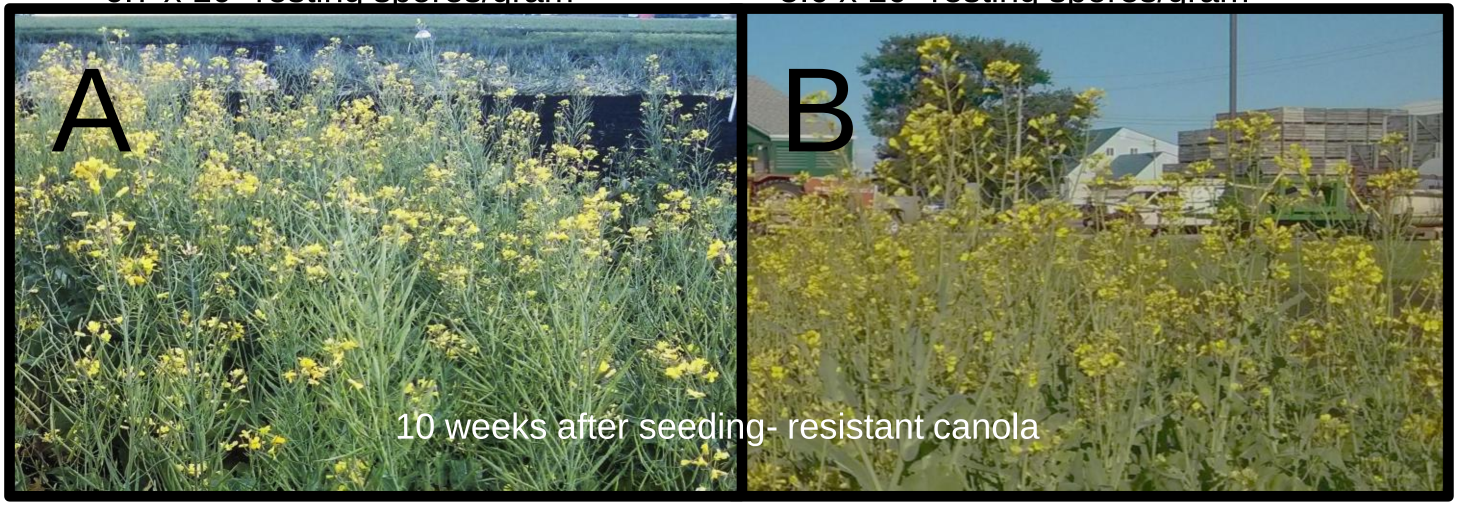
All plants exposed to inoculum were shorter than the non-inoculated check (0)

2014 Field Trials: metabolic cost of resistanc

No clubs on resistant canola, 100% disease severity on susceptible canola

6.7 x 10^7 resting spores/gram

5.0 x 10^8 resting spores/gram



Lower spore load- 60% with pods
with pods
Area under growth curve 1000
curve 600

Higher spore load- 15%

Area under growth

Slower development and smaller plants in the presence of high numbers of resting spores, even with no symptoms of disease




HIGH CALCIUM HYDRATED LIME
HIGH CALCIUM HYDRATED LIME

Numbers of resting spores

4 weeks after seeding	
5 weeks after seeding	3 billion spores per club
9 weeks after seeding	
Total per single club	16 billion - 156 billion
Total per square meter (	9.3 trillion