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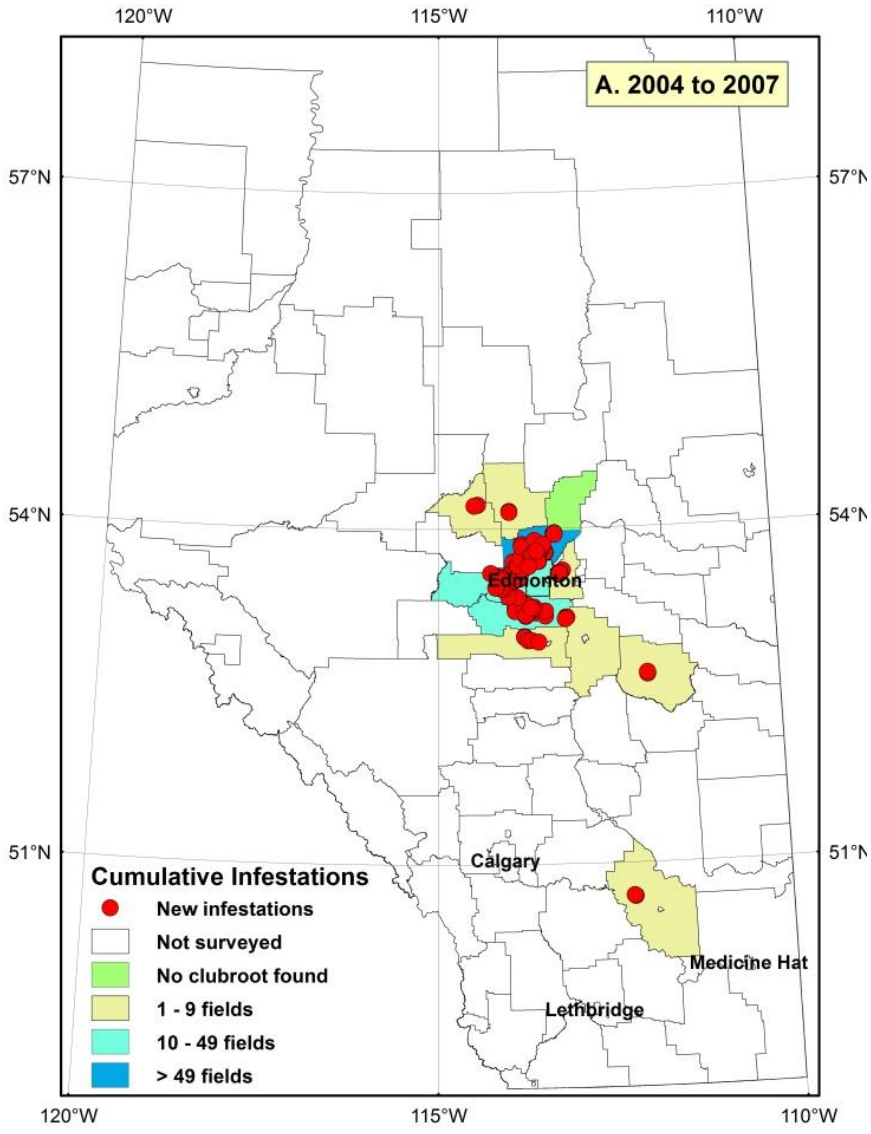
Management of clubroot: An overview of the challenges

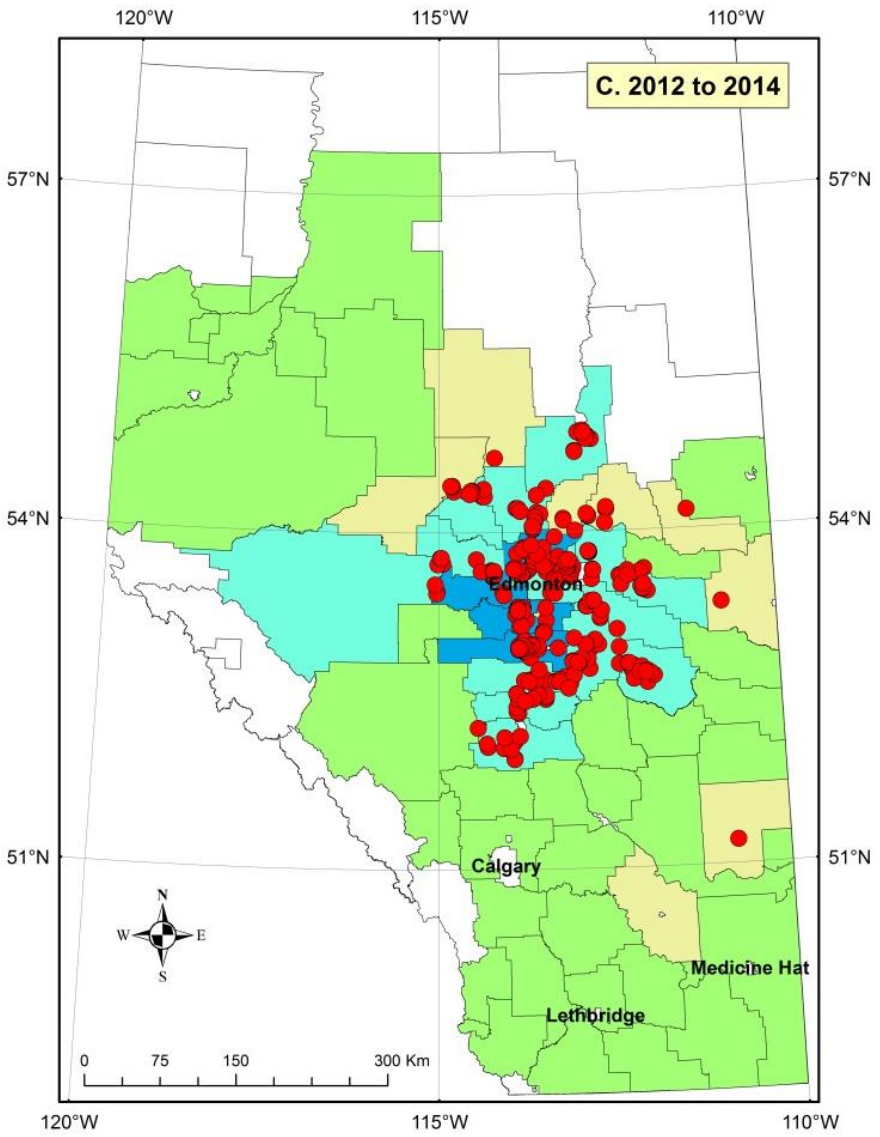
M. R. McDonald and B. D. Gossen

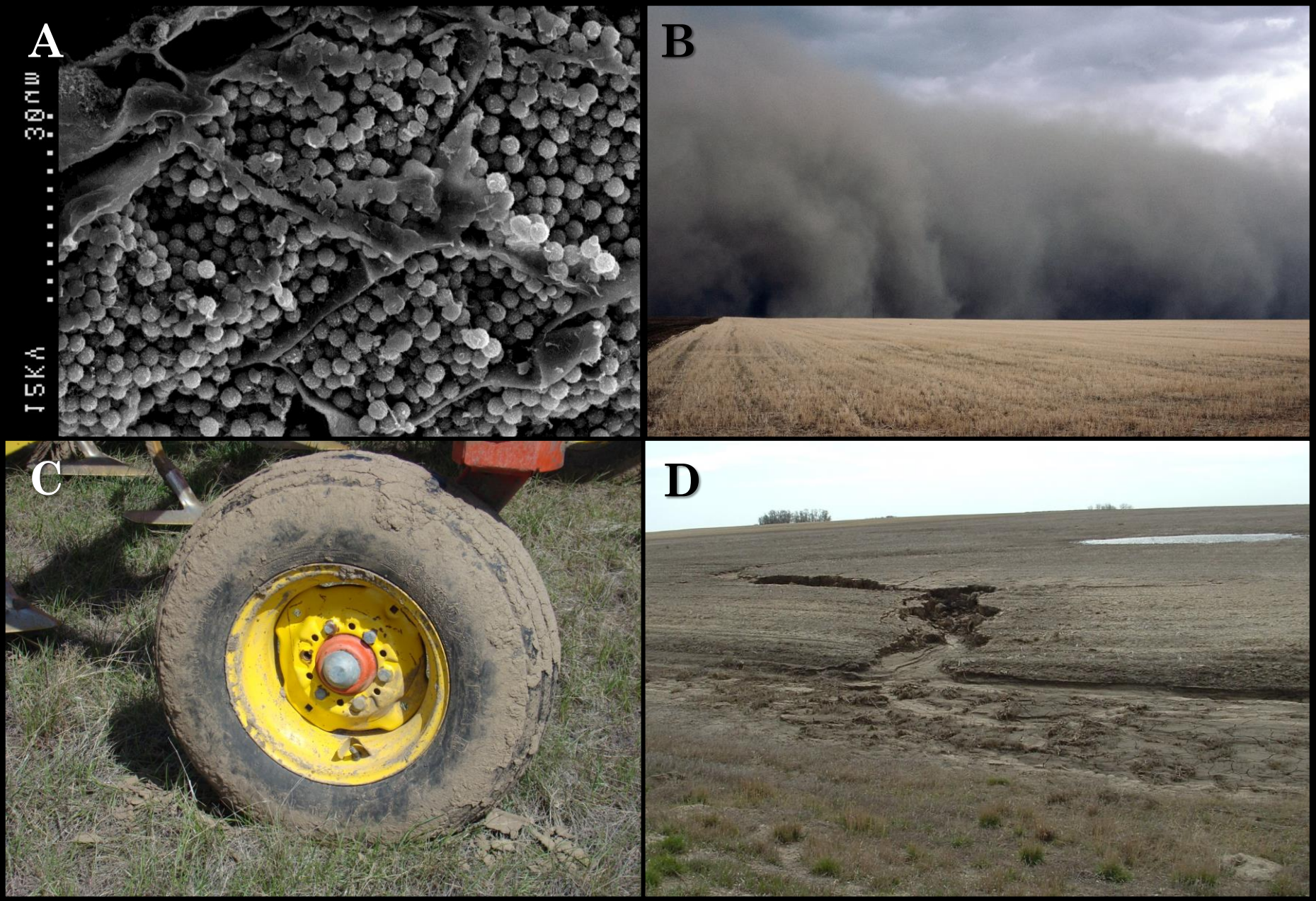
University of Guelph and AAFC, Saskatoon

International Clubroot Workshop, August 2018

Canada


A. 2004 to 2007
120°W
115°W
110°W
57°N
54°N
51°N
Edmonton
Calgary
Medicine Hat
Lethbridge
Cumulative Infestations
New infestations
Not surveyed
No clubroot found
1 - 9 fields
10 - 49 fields
> 49 fields


C. 2012 to 2014
120°W
115°W
110°W
57°N
54°N
51°N
Edmonton
Calgary
Medicine Hat
Lethbridge
N
W
E
S
0
75
150
300 Km


A
B
C
D

Factors Affecting Spread on Prairies

- Highly susceptible crop produced on > 8M ha each year, susceptible weeds also present.
- Large, contiguous fields.
- Trillions of spores in heavily infested fields.
- Short intervals between canola crops (0 or 1 yr).
- Many new pathotypes in heavily infested fields.

Virulence Patterns on Differentials

Genotype	Clubroot reaction: (+) Susceptible, (-) Resistant								
Westar	+	+	+	+	+	+	+	+	+
45H29	-	-	+	+	+	+	+	+	+
Mendel	-	-	-	-	+	-	+	+	-
ECD 02	-	-	-	-	-	-	-	-	-
ECD 05	+	+	+	+	+	+	+	+	+
ECD 06	+	+	-	-	+	+	+	+	+
ECD 08	+	+	+	+	+	+	+	+	+
ECD 09	+	+	-	-	+	+	+	+	+
ECD 10	-	-	-	-	-	-	-	-	-
ECD 11	-	-	-	-	-	-	-	+	-
ECD 13	+	-	-	+	+	+	+	+	-
Laurentian	+	-	-	-	+	-	+	+	+
Williams	3	5	5	6	3	6	3	2	8
Some et al.	P2	P2	P3	P3	P2	P2	P2	P2	P2

Alternatives to reliance on genetic resistance

- Seed treatments, fungicide drenches.
- Liming, soil amendments.
- Sanitation (keeping the pathogen out).
- Soil type, compaction.
- Biological control agents.
- Improved measures of spore conc.
- Crop rotation, bait crops.
- Partial / quantitative resistance.

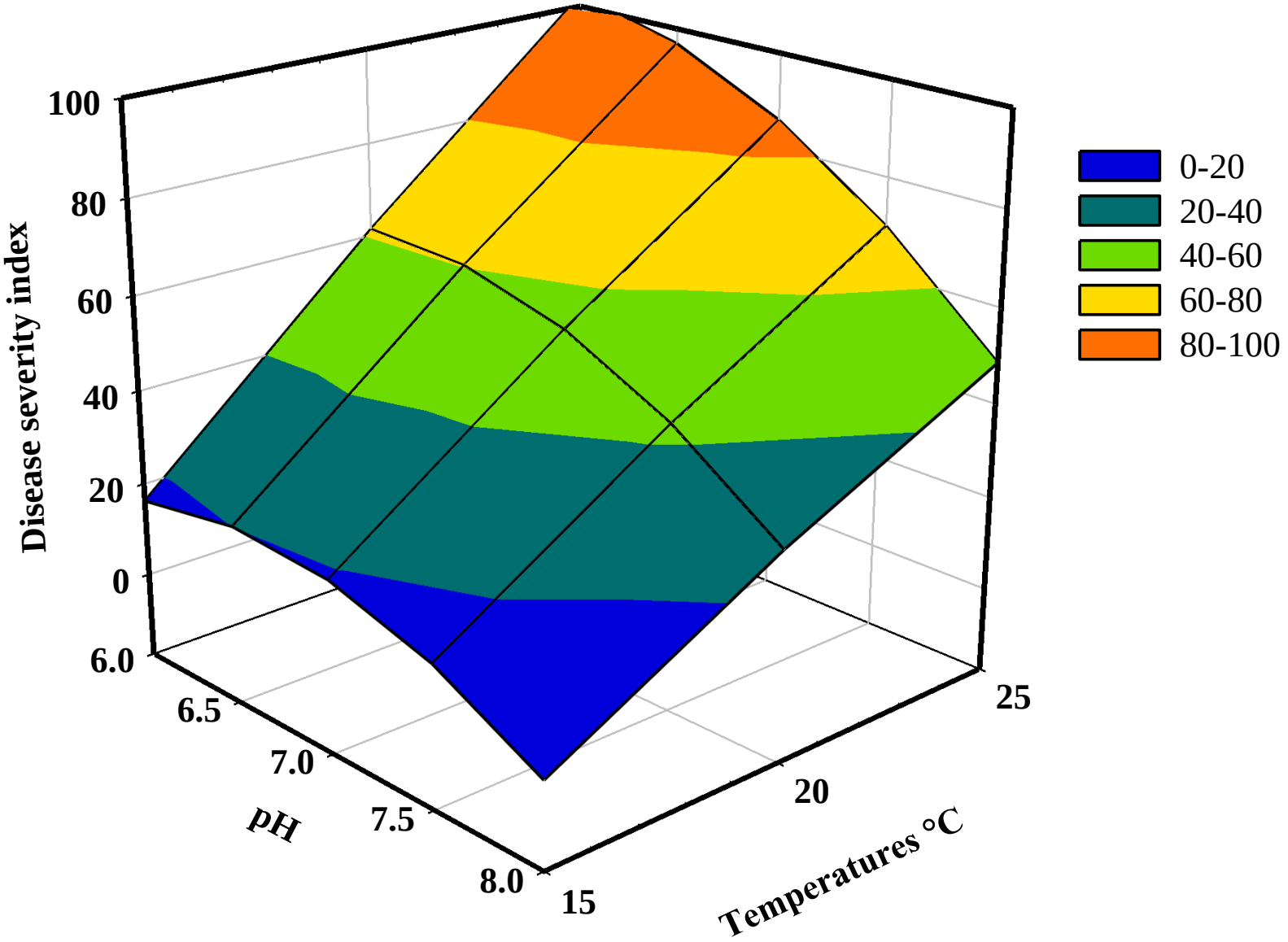
Alternatives to reliance on genetic resistance

- ✗ Seed treatments, fungicide drenches.
- ➡ Liming, soil amendments.
- ? Sanitation (keeping the pathogen out).
- ? Soil type, compaction.
- ✗ Biological control agents.
- ➡ Improved measurements of spore conc.
- ➡ Crop rotation, bait crops.
- ? Partial / quantitative resistance.

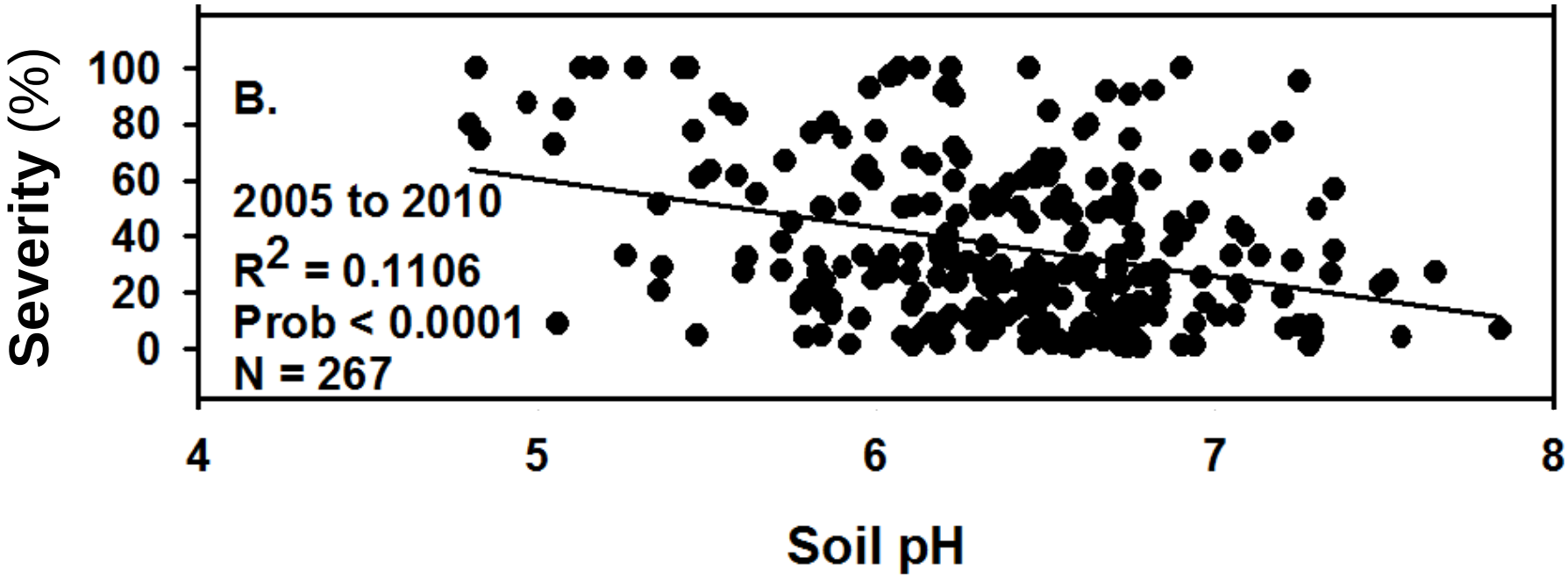
Management strategies for new pathotypes

- Few management options available.
  - Long rotations out of canola.
  - Equipment sanitization.
- N.B. Not popular with farmers and not widely adopted.

Temperature x pH



Does moderate pH reduce clubroot?



Fields with clubroot in Alberta: pH above 7.5 reduces clubroot, but otherwise the relationship is quite weak

Effect of soil type on clubroot

Plant Pathology (2016) 65, 1238–1245 Doi: 10.1111/ppa.12510

Effect of soil type, organic matter content, bulk density and saturation on clubroot severity and biofungicide efficacy

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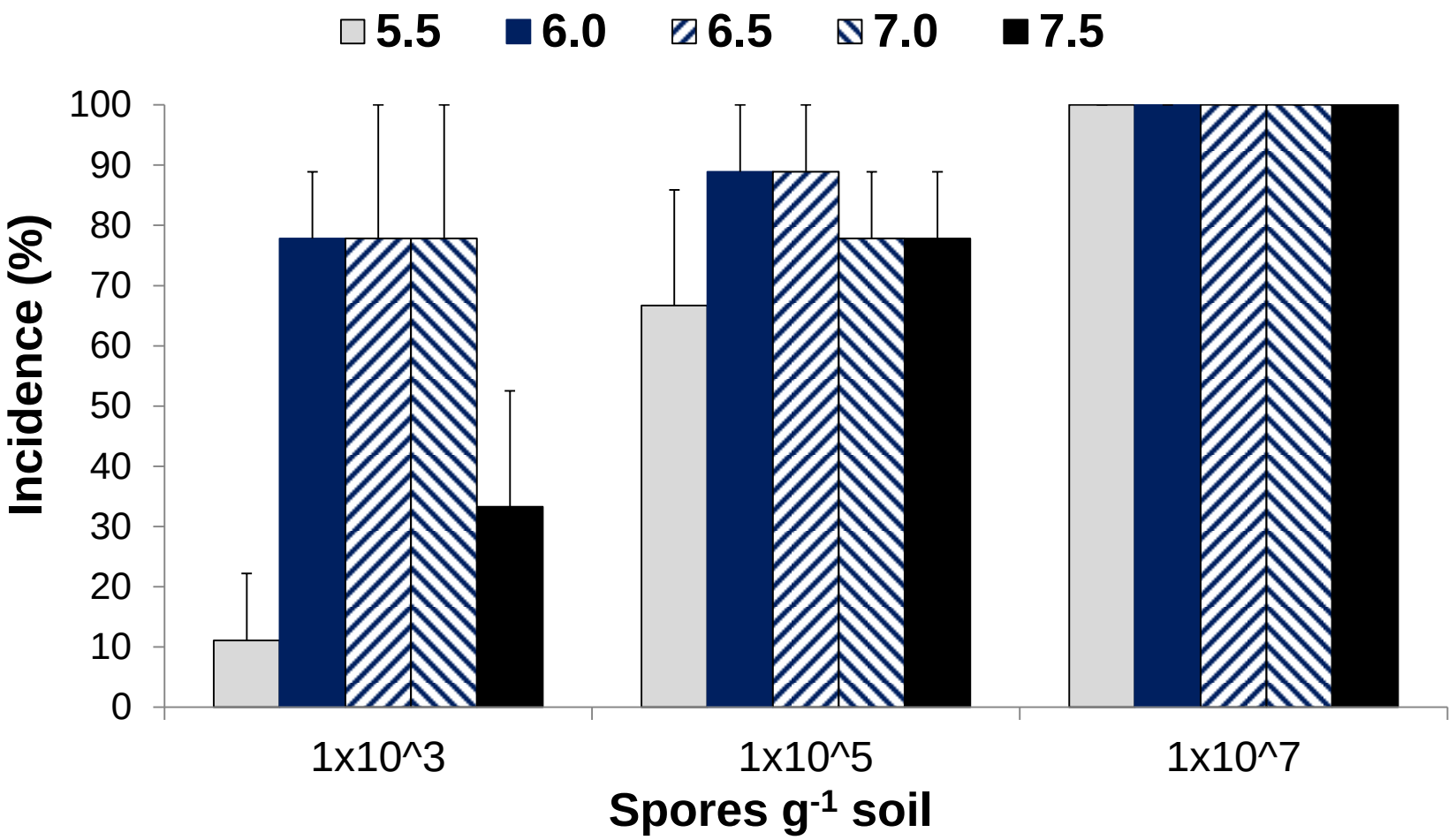
[a]*Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, S7N 0X2; and* [b]*Department of Plant Agriculture, University of Guelph, Guelph, ON, N1G 2W1, Canada*

Growth room experiments were conducted to assess the interaction of soil type, biofungicides, soil compaction and pathotype/host on infection and symptom development caused by *Plasmodiophora brassicae*, the cause of clubroot on *Brassica* spp. In two initial experiments, four soil types (peat soil, mineral soil, non-calcareous sand, soil-less mix), two biofungicides (*Bacillus subtilis*, *Clonostachys rosea*), and two pathotypes (3 and 6, Williams' differential set) were assessed. Differences in clubroot severity associated with soil type were unexpectedly small and variable. Prestop (*C. rosea*) was often more effective than Serenade (*B. subtilis*) at reducing clubroot levels on peat and mineral soils, but less effective than Serenade on sand. Inoculation with pathotype 3 often resulted in a slightly higher mean severity

Conclusions

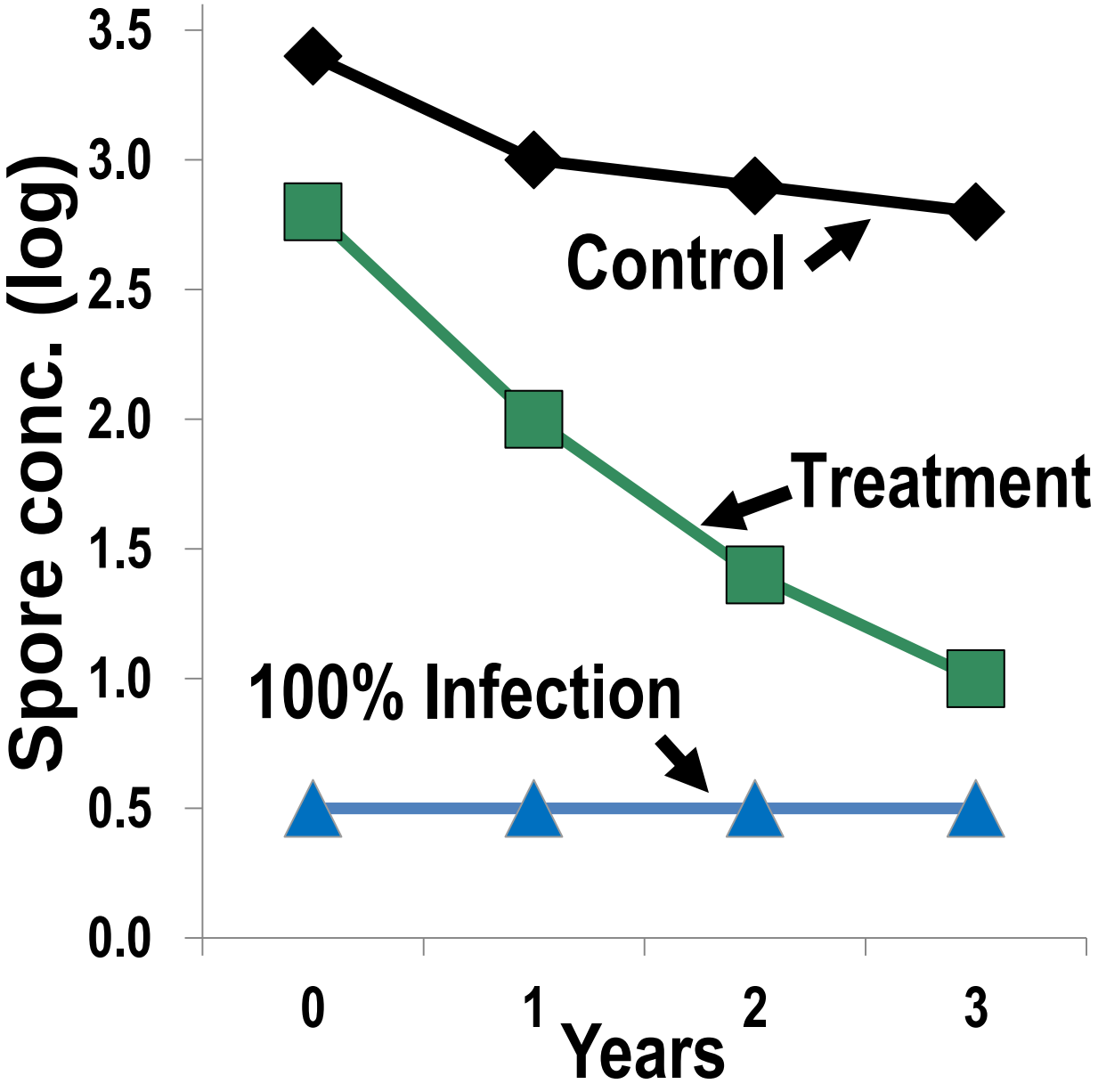
- Little effect of soil type or organic matter.
- No effect of saturation vs drained.
- Major impact of soil compaction.

Resting spore conc. and pH



High spore levels in soil are a problem!

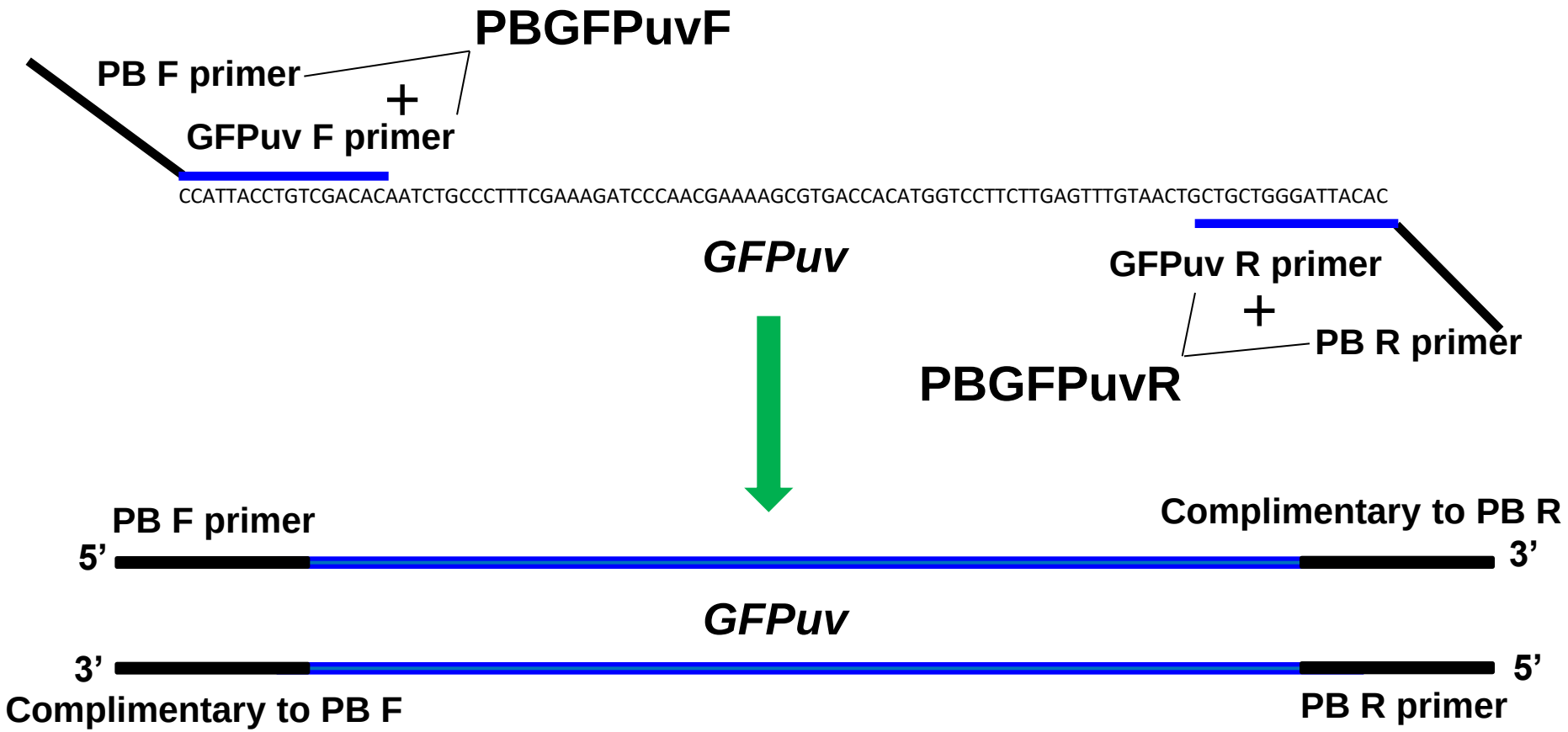
- Resistance always (?) breaks down first in fields with high spore concentration – high disease pressure, many spores for selection.
- Overwhelms cultural strategies such as liming, soil amendments, and biologicals.
- Overwhelms partial resistance (e.g., Invigor 5030).
- Spores can survive for many years in soil, and spore conc. is difficult to measure.


Spore conc. (log)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Control
Treatment
100% Infection
0
1
2
3
Years

Competitive Internal Positive Control

Plasmid: pDSK-GFPuv containing *GFPuv*

Attach Pb primers, so they will amplify *GFPuv* sequence.



CIPC qPCR

- One-step TaqMan qPCR assay for detection and quantification of *P. brassicae* DNA in soil.
- More accurate than standard PCR.
- Sensitivity – similar to standard (~800 spores/g) but more likely to detect false negatives.
- No need for extra run - saves time & resources.
- Suitable for wide range of soil types.

Deora A, Gossen BD, Amirsadeghi S, McDonald MR. 2015. A multiplex qPCR assay for detection and quantification of *Plasmodiophora brassicae* in soil. Plant Dis. 99: 1002–1009.

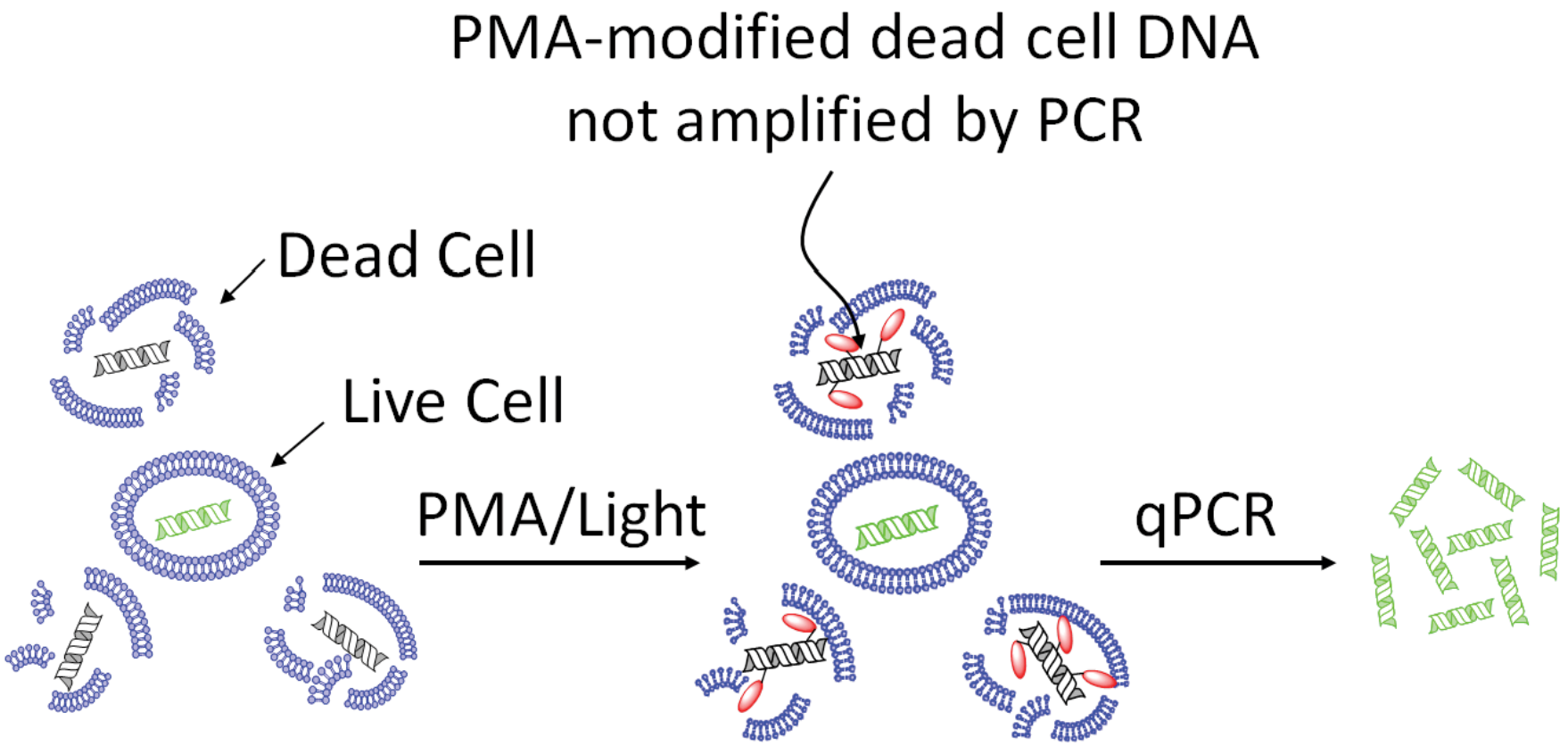
Propidium monoazide (viable spores)

Shake spores + PMA solution (300 rpm) in the dark at room temp (22–24 ºC) for 30 min

Put on ice and expose to 500 watt halogen light at 20 cm away for 15 min

Al-Daoud F, Gossen BD, McDonald MR. 2017. Propidium monoazide improves quantification of viable resting spores of *Plasmodiophora brassicae* with qPCR. Plant Dis. 101: 442–447

PMA treatment and photo-activation



Molecular methods being used to assess:

- The effect of cropping interval on resting spore concentration and longevity in soil.
- The distribution of spores in the soil profile and proportion of viable spores at depth.
- The efficacy of fumigation and solarisation treatments on spore concentration.
- The impact of specific crops (perennial grasses, cereals) on spore concentration.

Accurate assessment methods are essential for effective disease management in the field and for research

Distribution of resting spores in soil

Variation with:

- Time –number of years break from canola
- Depth – cores to 1 m below surface
- Horizontal distribution – research site west of Edmonton

Time: Crop Rotation and resting spore concentration

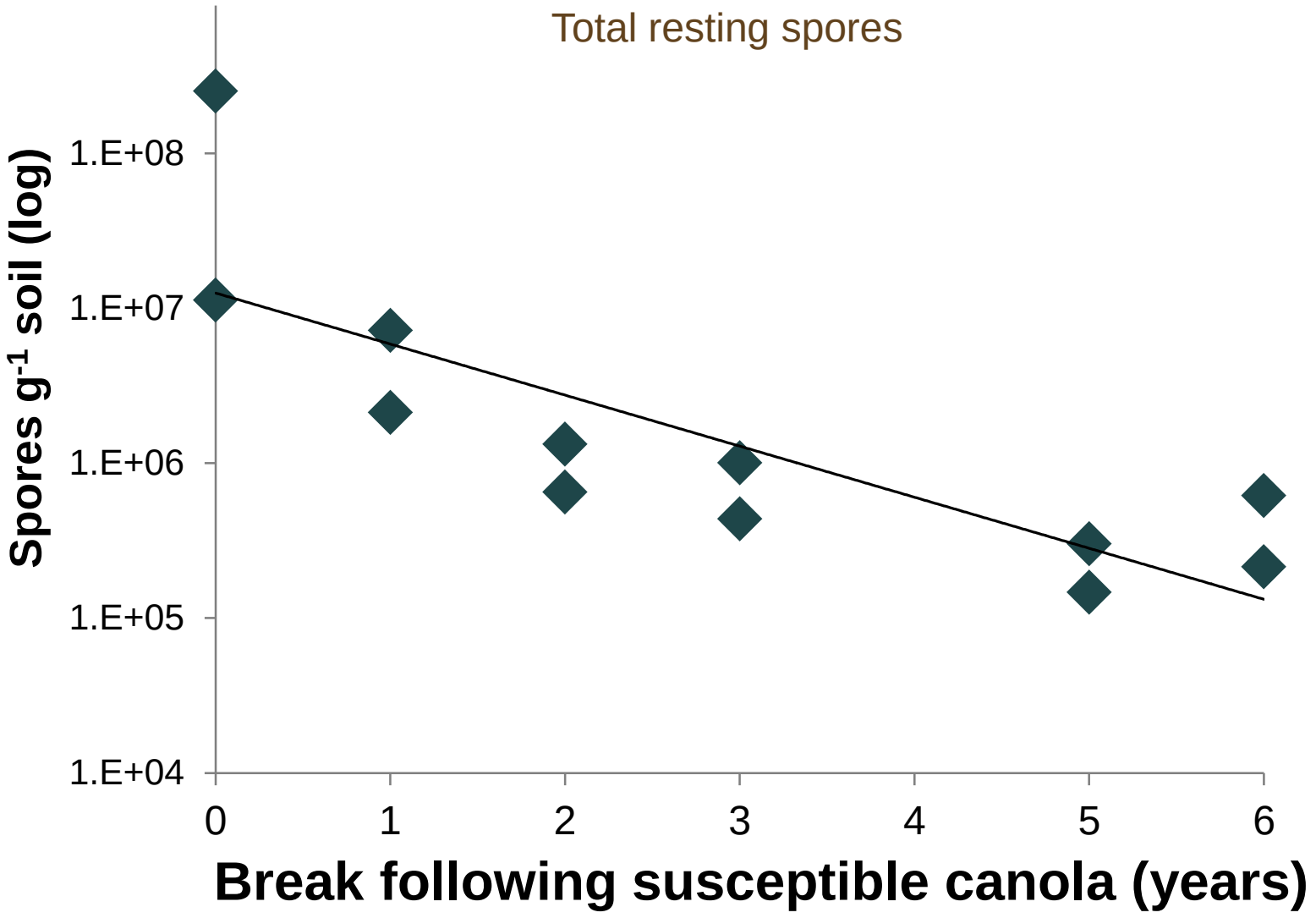
AAFC Research Farm, Normandin, QC, 48º 51’ N – 72º 32’ W
Labarre silty clay soil naturally infested with *P. brassicae*

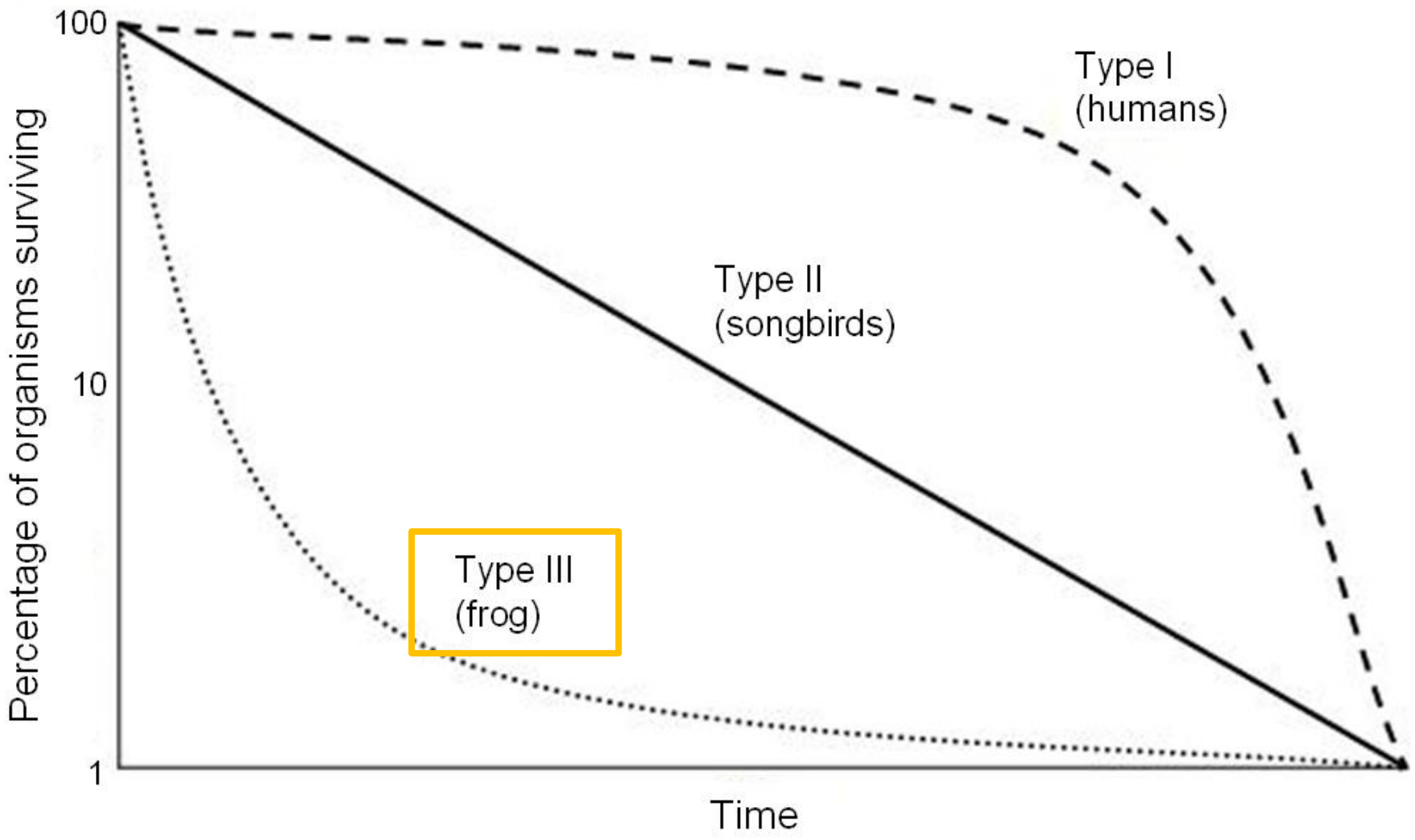
Continuous susceptible canola and break intervals of
1, 2, 3, 5, and 6 years following susceptible canola

Rotations during break intervals were barley and field pea
and fallow

AAFC Research Station Normandin, QC

Spore Conc. after Susceptible Canola

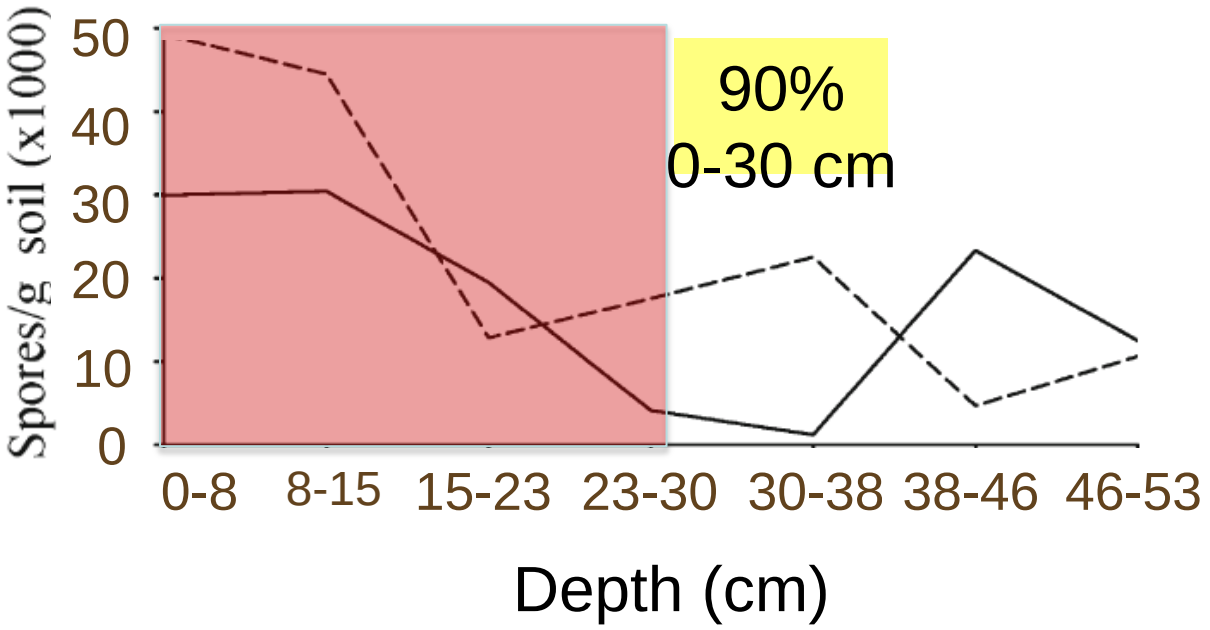




Three types of survival curves

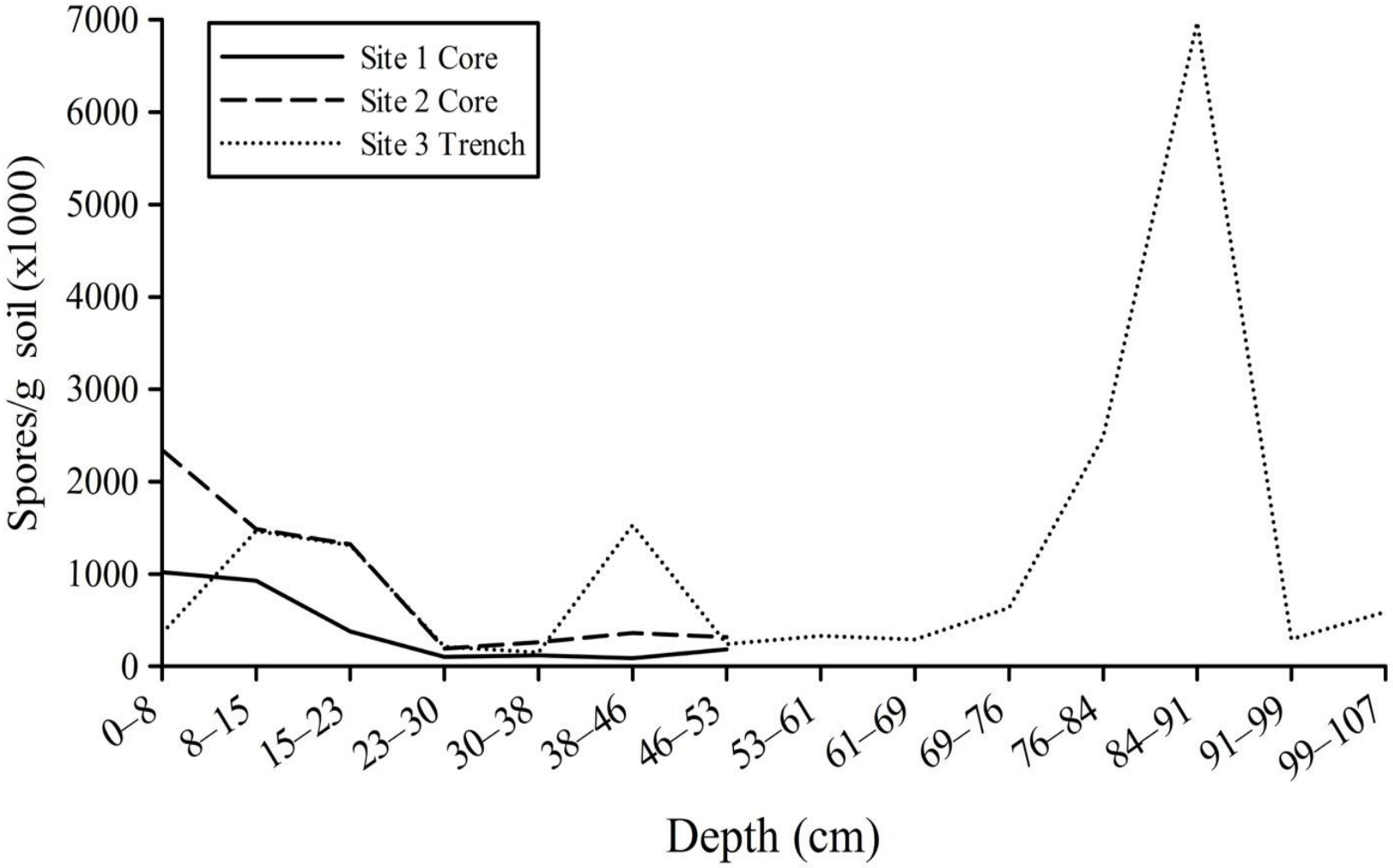
Vertical distribution of resting spores

- Trend of fewer spores with depth
- Spores detected in 100 cm soil samples
- Fields with clubroot history had deeper spores
- Highly variable
- Are deep spores viable?



Cranmer et al. (2017)

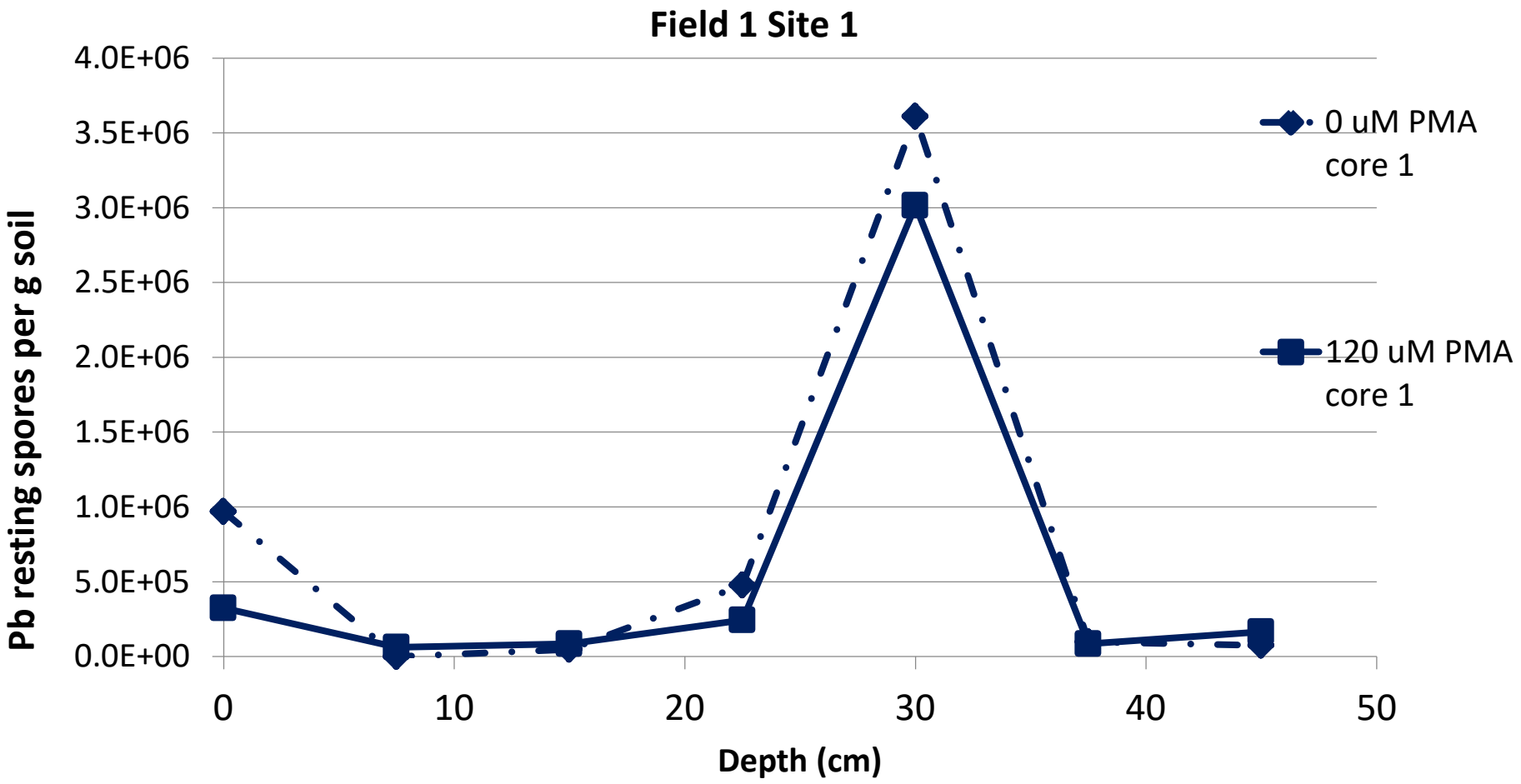
Distribution of resting spores with depth
Flamborough, Ontario



Research and demonstration site west of Edmonton

High clubroot severity

Resting spores at various soil depths- Alberta site



Total and viable spores- Alberta site

Total resting spores

2.E+05	3.E+05	2.E+05	3.E+05	9.E+03
2.E+04	6.E+04	7.E+05	2.E+05	2.E+05
8.E+03	1.E+04	2.E+05	2.E+05	1.E+04

Viable resting spores

2.E+04	4.E+05	8.E+05	3.E+05	1.E+04
3.E+04	5.E+05	5.E+06	9.E+05	6.E+04
8.E+03	2.E+04	2.E+05	2.E+05	2.E+04

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

Clubroot in soil

- Extremely variable in soil, even within a meter
- Challenges for growers, extension agents, researchers
- Helpful to be able to determine how many resting spores are alive
- Resting spores die off quickly but the remaining ones survive for a long time
- Levels of inhibition in the soil change over time

Distribution of resting spores in soil

- The Competitive Internal Positive Control to adjust for inhibition in soil
- PMA-PCR to quantify only viable resting spores
- Digital drop PCR is reported to be better for low concentrations of DNA BUT it appears to be less accurate for higher concentrations
- LAMP – best for identifying presence of the DNA, not very effective at quantifying DNA.

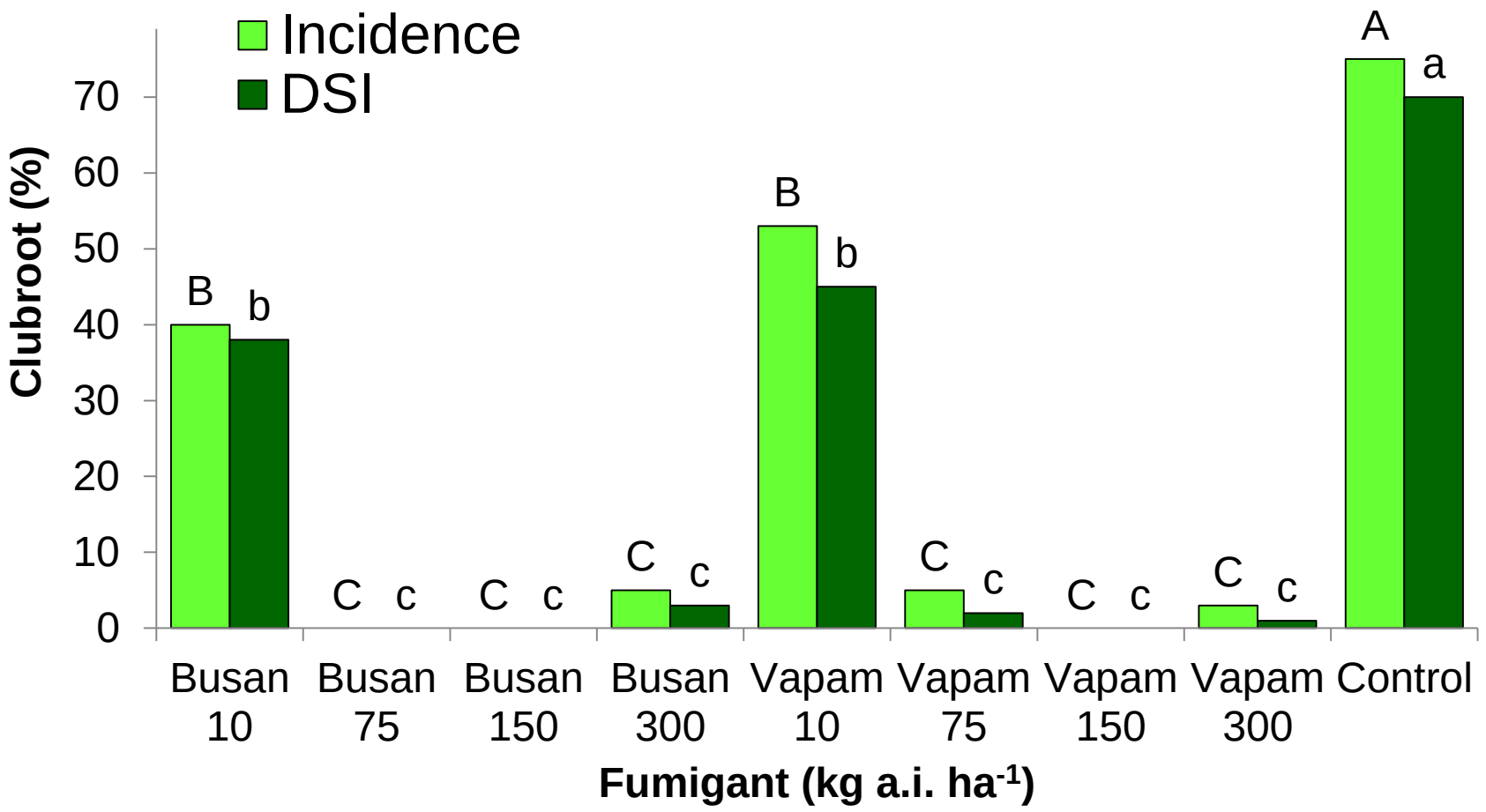
Disease management strategies

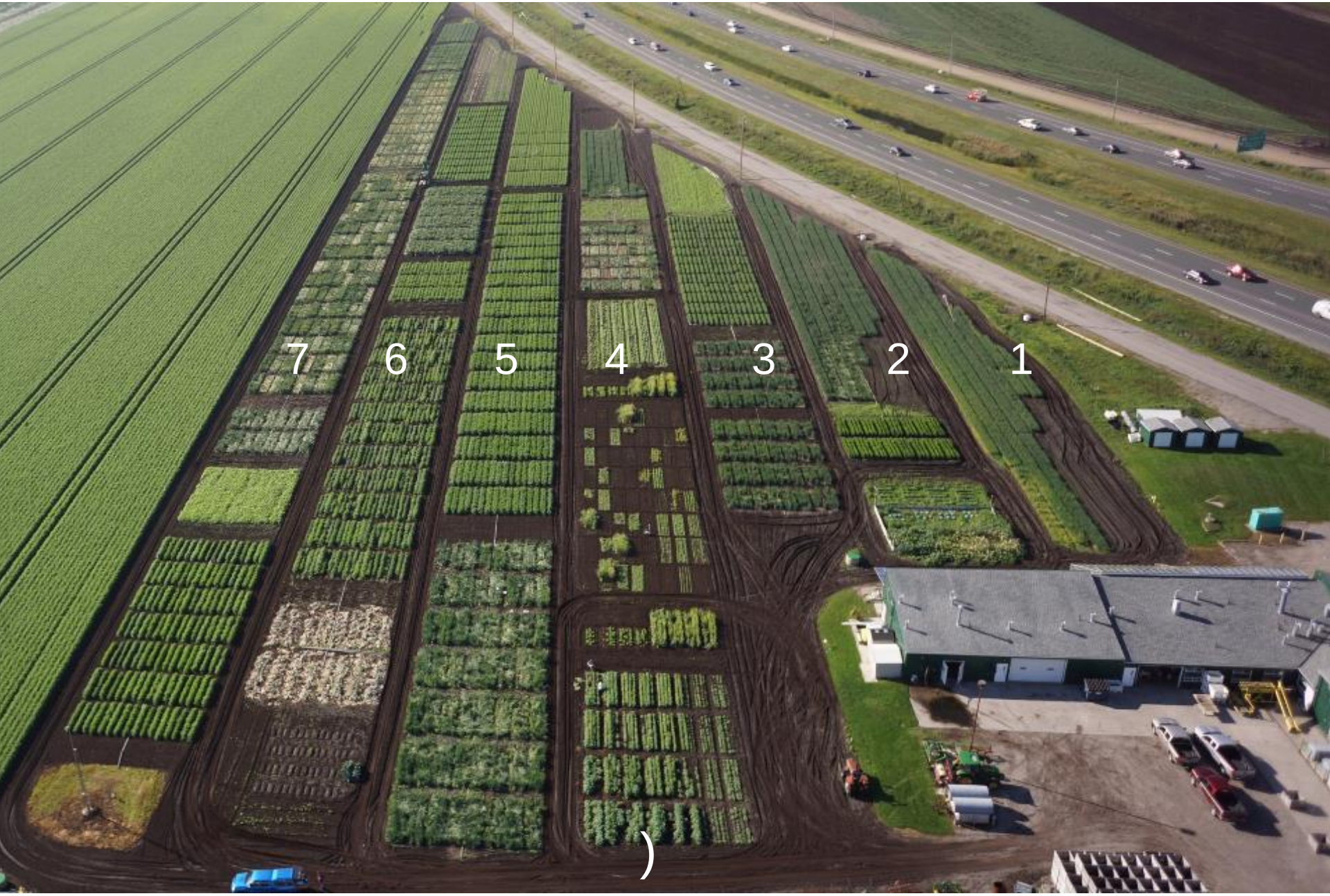
Fumigation: may be an approach to treat small areas. Not for large fields of canola

- Controlled environment trials
  - Fumigants applied to soil it bins, sealed for 2 weeks, bioassay with susceptible pak choy
- Field trials on naturally-infested, high organic matter soils.

- Metam sodium (Busan and Vapam)
- Chloropicrin (Pic Plus)
- Different types of tarp, including totally impermeable film (TIF) for 2 weeks

Fumigation - Controlled Environment 2014





Muck Crops Research Station

Tractor –drawn fumigation applicator. Fumigant injected into soil

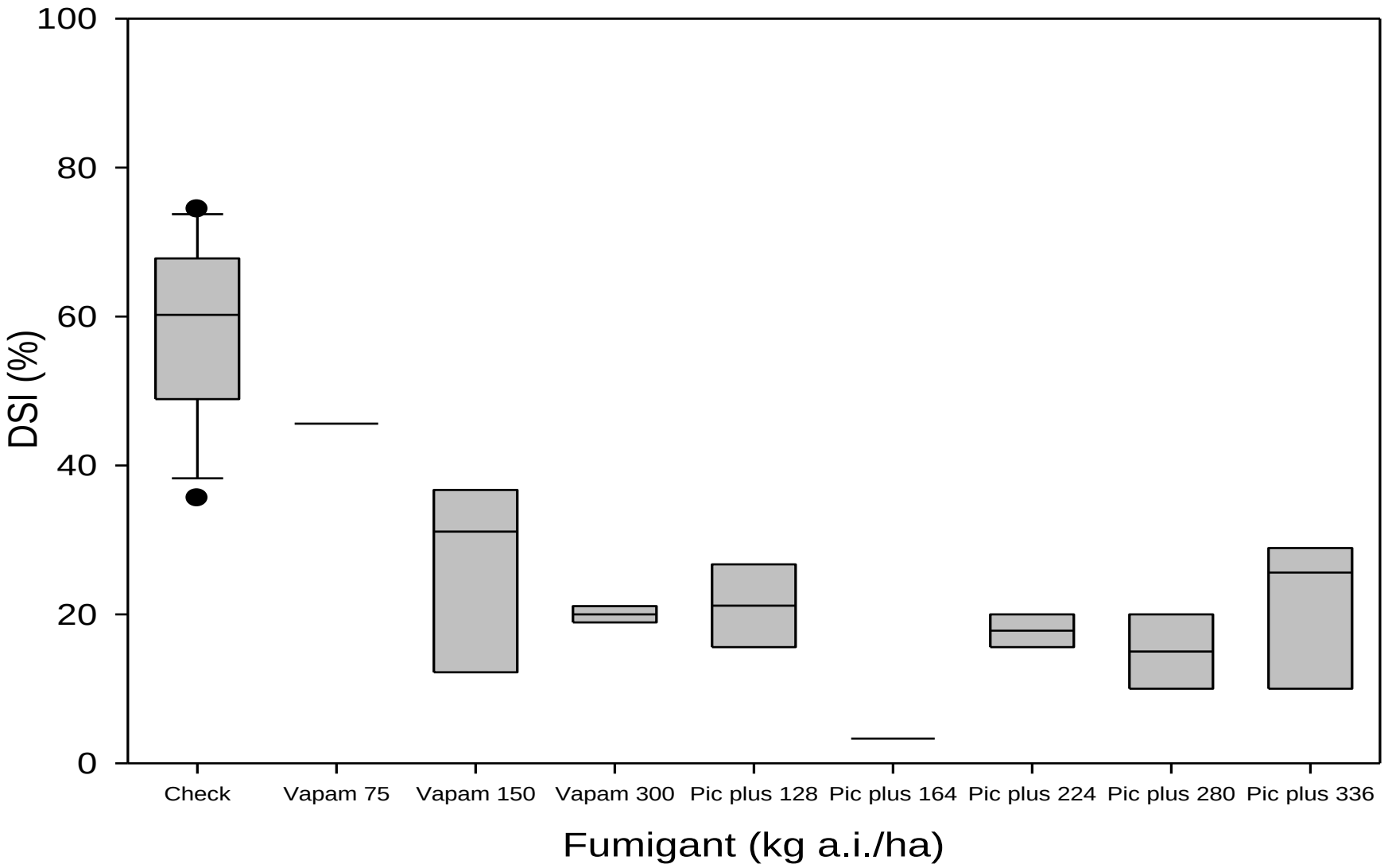
Some of the TIP tarps blew off within 3 days of treatment

New federal regulations now require that all fumigated soil be tarped following fumigation – 2015 trial on muck soil
Some treatments had to be considered as untreated checks

Fumigation trial, 2015

Disease severity index (0-100; 50 plants per experimental unit)

Effect of fumigant and rate on clubroot severity -2015

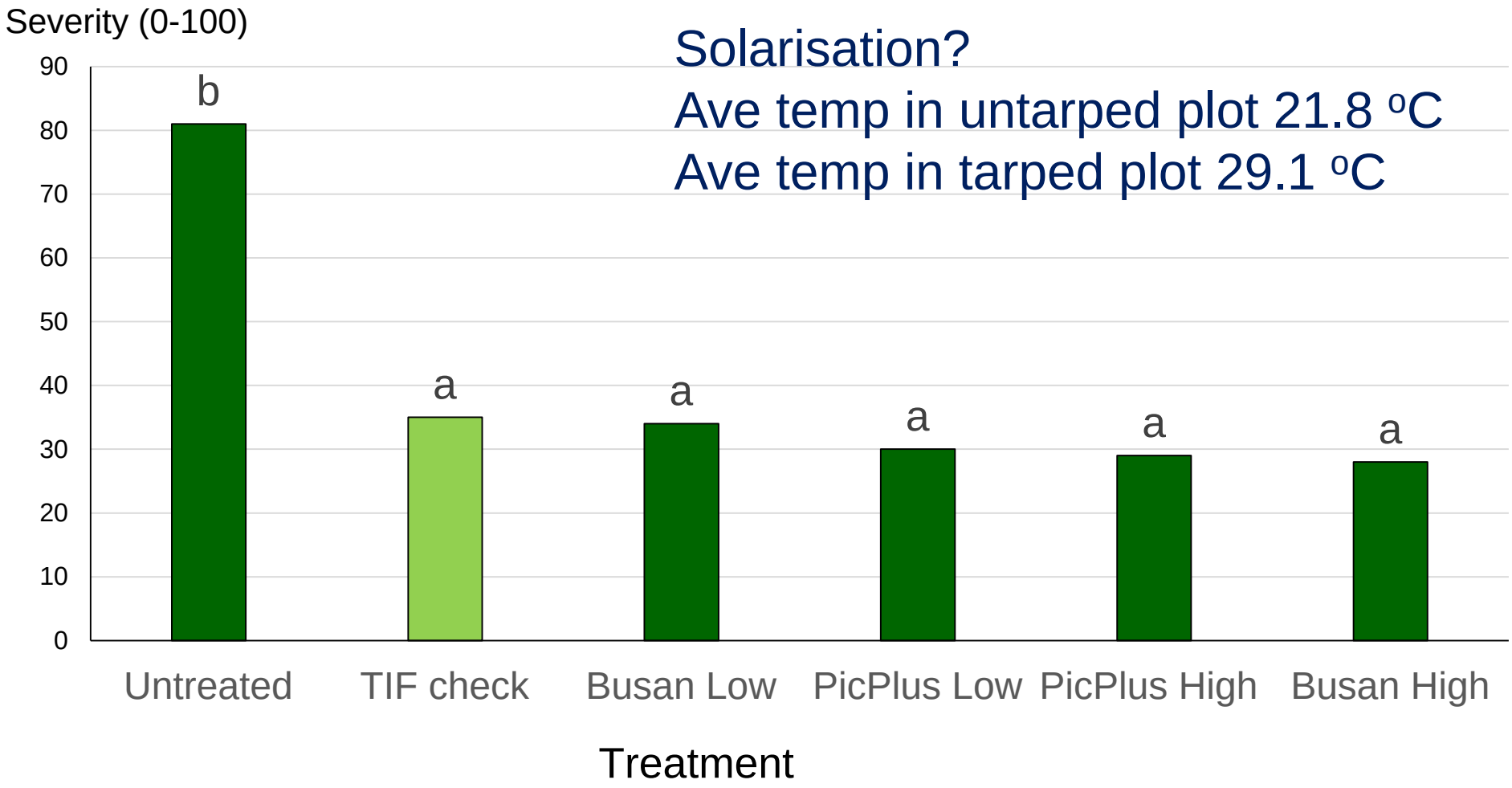



2016
Totally impermeable film
No clubroot in any of the treatments
Including the untreated check
Solarization?

2017 and 2018- untreated, uncovered check and untreated covered check

Bioassay with clubroot susceptible pak choi

Clubroot severity in pak choy following fumigation -2017



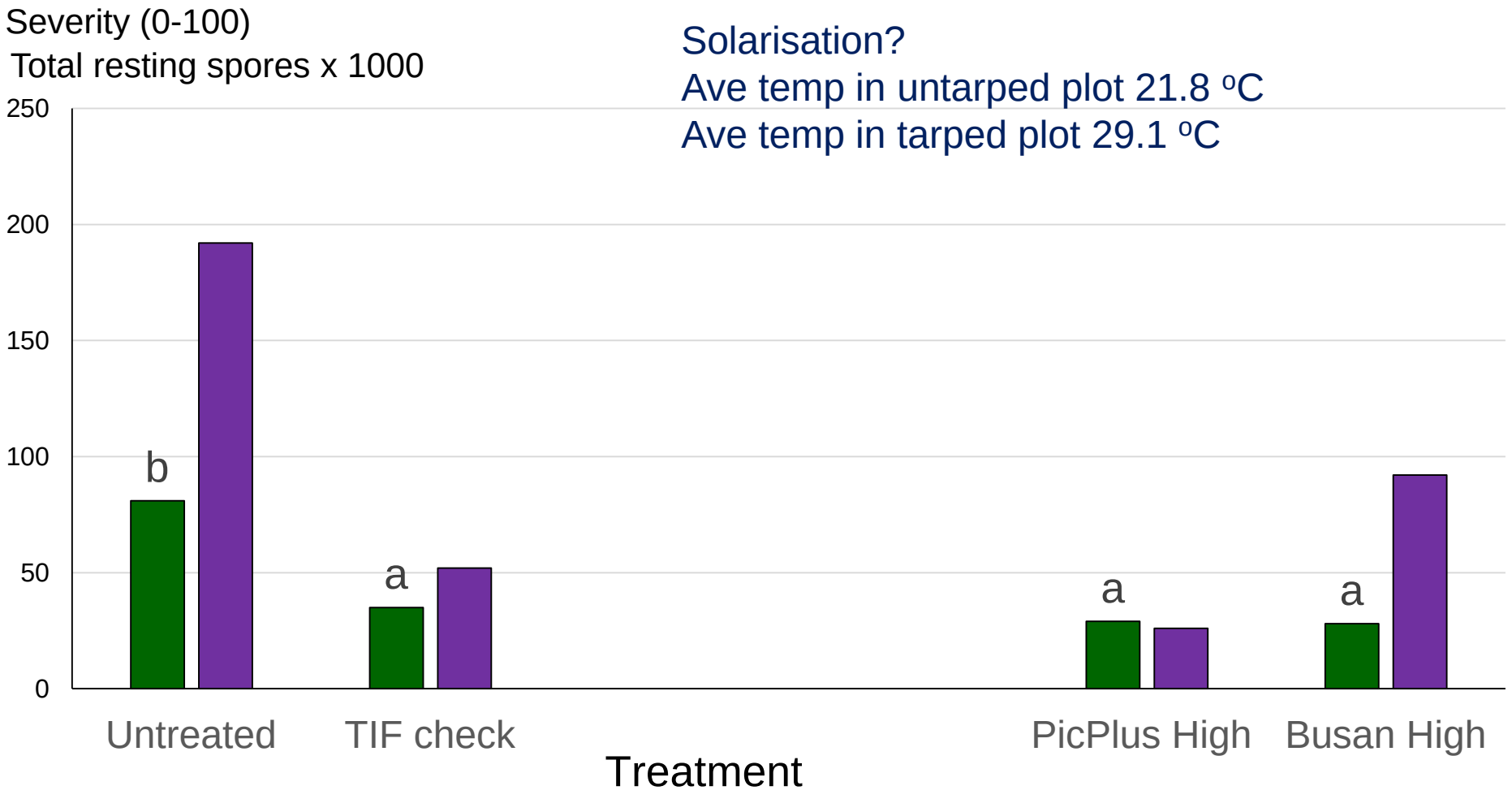
Solarisation?

Ave temp in untarped plot 21.8 ^{o}C

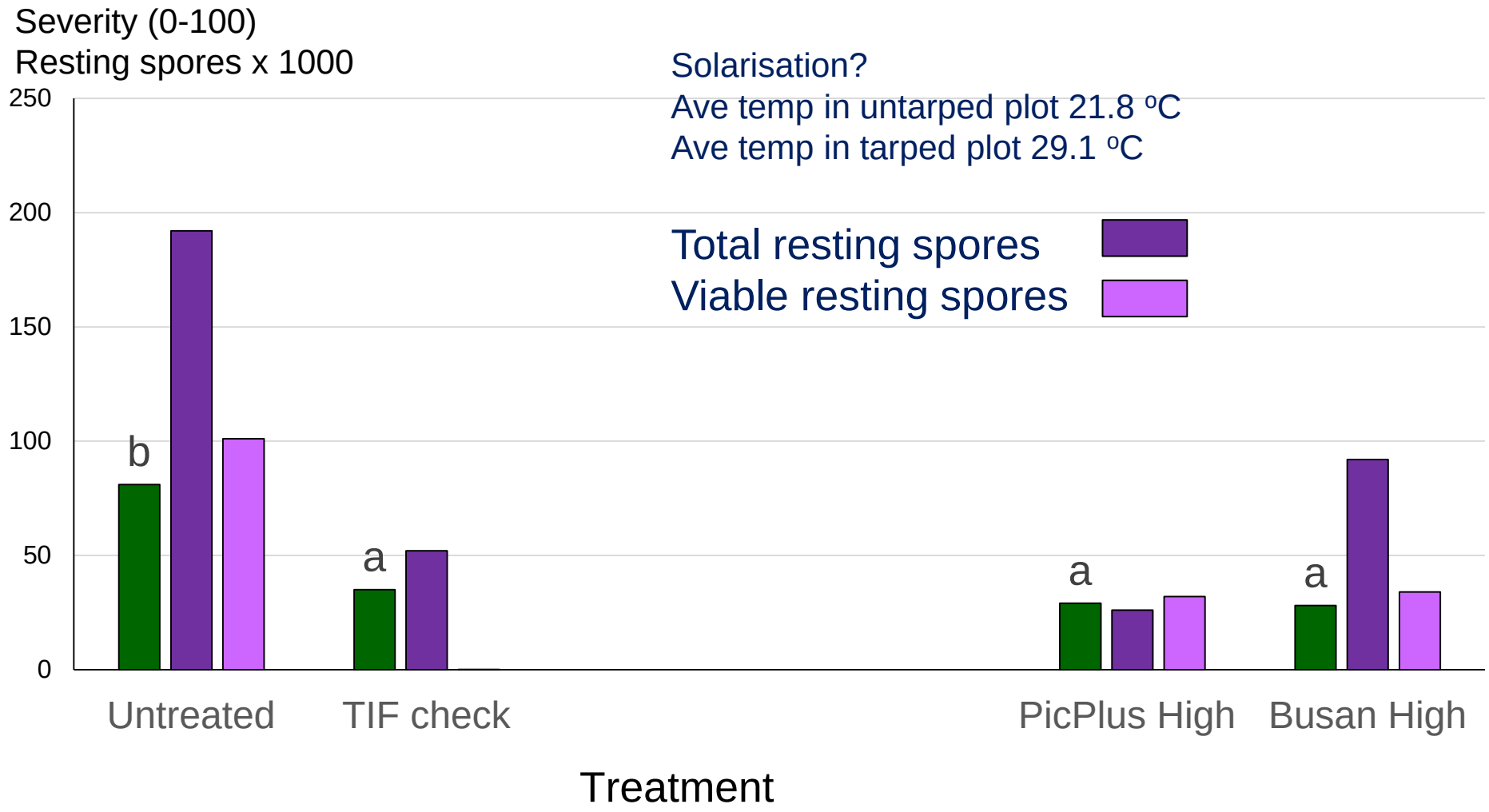
Ave temp in tarped plot 29.1 ^{o}C

Untreated and untarped check Mostly 3’s (0- 3 scale)

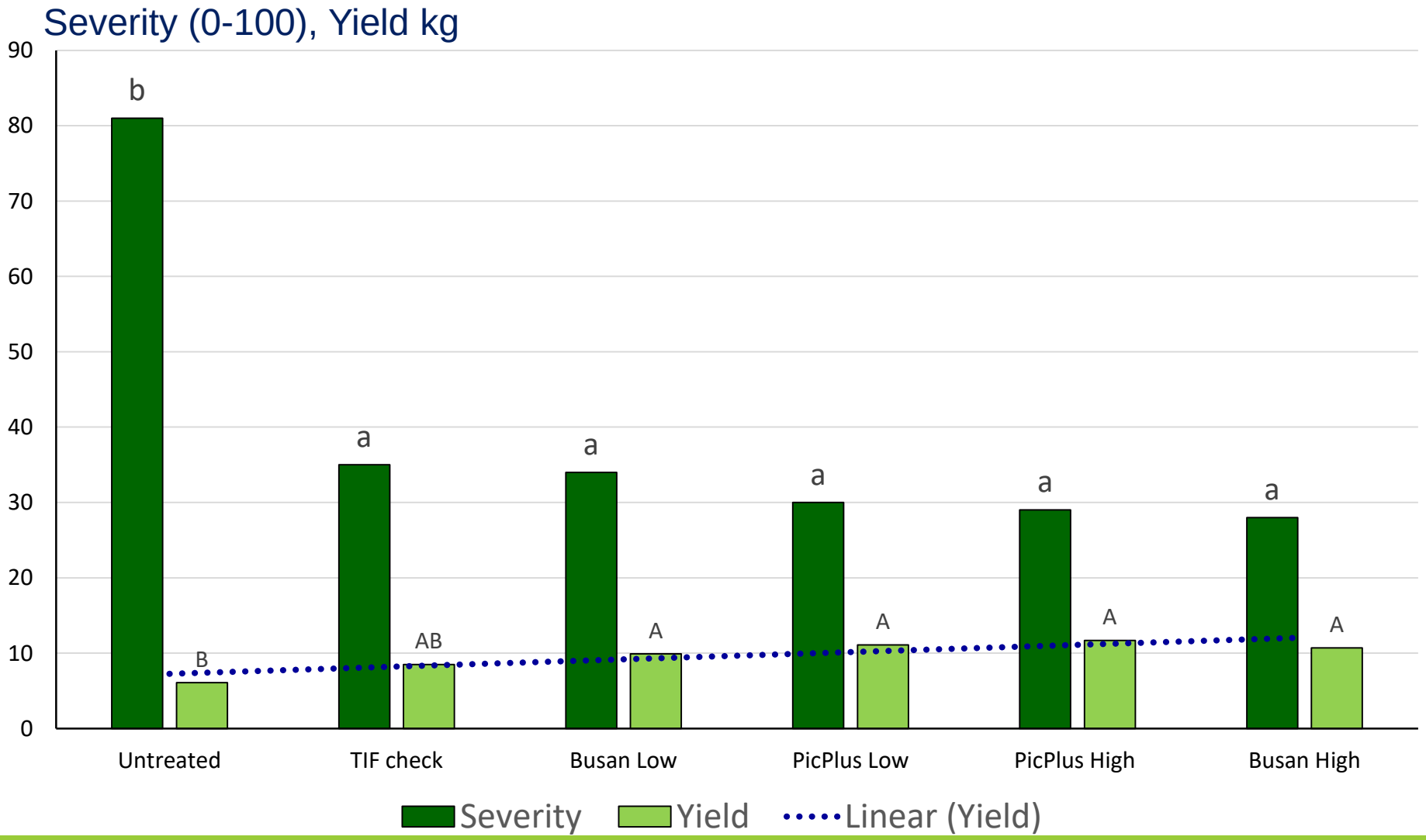
Clubroot severity in pak choy following fumigation -2017



Clubroot severity in pak choy following fumigation -2017



Clubroot severity and yield of pak choy following fumigation and solarisation



Fumigation and solarization

- Fumigants can reduce clubroot severity
- Solarization alone may be effective fro clubroot control
- Can we use cheaper tarps?
- Is there an advantage to using low rates of fumigant with solarization
- Timing will be site specific
- Repeating the trial in 2018 with TIF and greenhouse grade plastic

Cover crops hold the soil in place but can also stimulate resting spore germination

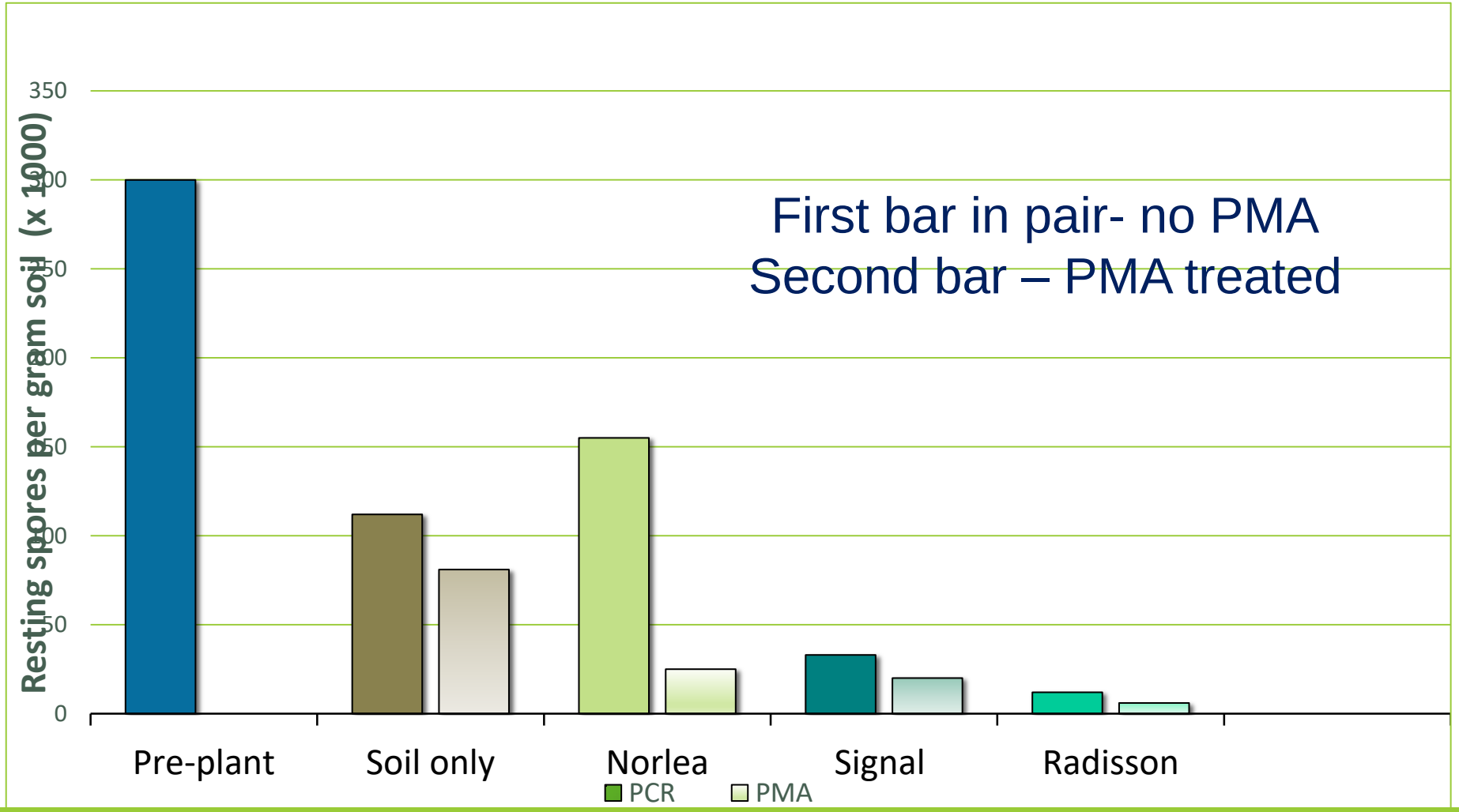
Effectiveness of grass cover crops to reduce resting spore numbers by stimulating germination
- controlled environment study

Crops:

- Smooth bromegrass (*Bromus inermis* L.) cv.'s Signal, Radisson
- Perennial ryegrass (*Lolium perenne* L.) cv. Norlea
- Shanghai pak choy (*Brassica rapa* L. ssp. chinensis L.)
- No-plant check (but some weed seedlings)
- Field soil 500,000 resting spores per gram added
  - (300,000 actual)
- Grown for 8 weeks, total and viable resting spores quantified

Perennial grasses in the growth room

Number of resting spores of *P. brassicae* per gram of soil, treated with and without PMA, 8 weeks after seeding.



Grasses to reduce resting spore in soil

- Important to test for viable resting spores
- Grasses appear to stimulate resting spore germination
- Perennial grasses will hold the soil in place
- Variability – weeds ?
- Repeating the trial with more crops and cultivars

Durable resistance? Resistance and partial resistance to P. brassicae in cabbage

Objective:

- Examine the development of clubroot in resistant, partially resistant and susceptible cabbage cultivars to determine when resistance occurs during secondary infection.

  - Follow up on the work published in Gludovacz et al. 2014

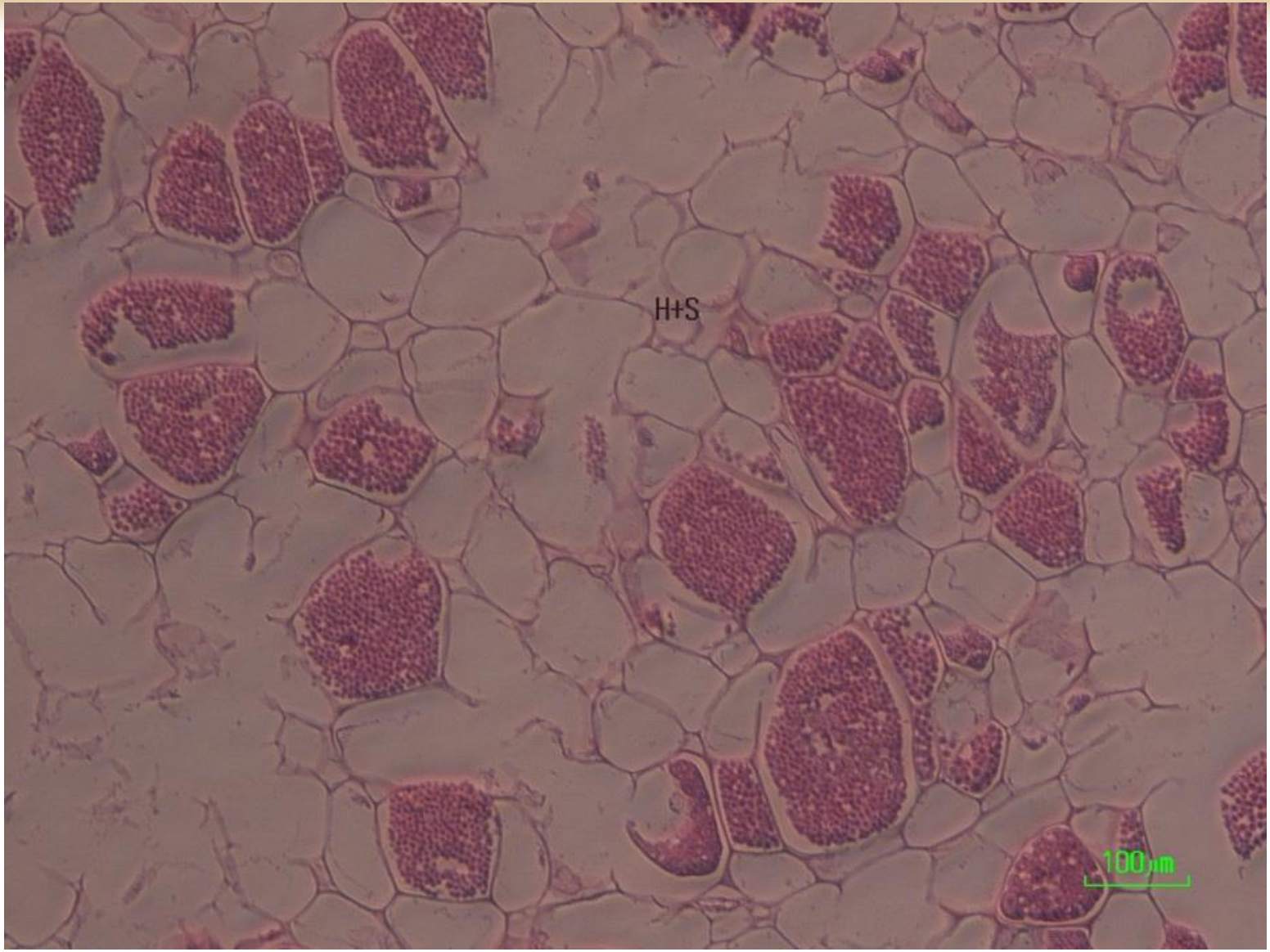
Cortical Colonization by Plasmodiophora brassicae in Susceptible and Resistant Cabbage Cultivars

T. V. Gludovacz & A. Deora & M. R. McDonald & B. D. Gossen

- Eur J Plant Pathol (2014) 140:859–862
- DOI 10.1007/s10658-014-0492-8

Clubroot incidence and severity in field grown cabbage cultivars with different levels of resistance, Muck Crops Research Station

	High inoculum site		Low inoculum site	
Cultivar	Clubroot incidence (%)	Disease Severity Index	Clubroot incidence (%)	Disease Severity Index
Klimaro (S)	100 a[1]	100 a	72 a	27 a
Bronco (S)	100 a	100 a	71 a	24 a
B-2819 (MS)	98 b	53 b	11 a	4 a
Kilaherb (R)	0 c	0 c	0 b	0 b



Cells infected with P. brassicae. Resting spores have developed

K. Sharma

P. brassica development in cabbage 28 days after inoculation

Cultivar	% Area infected	# Young plasmodia	# Mature plasmodia	# Resting spores	Total cells infected
Bronco (S)	26 a	69 a	32 a	34 a	135 a
B-2819 (MS)	9 b[1]	66 a	26 b	3 b	95 b
Kilaherb (R)	7 c	42 b	9 c	0 c	51 c

P. brassica development in cabbage 28 days after inoculation

Cultivar	% Area infected	# Young plasmodia	# Mature plasmodia	# Resting spores	Total cells infected
Bronco (S)	26 a	69 a	32 a	34 a	135 a
B-2819 (MS)	9 b[1]	66 a	26 b	3 b	95 b
Kilaherb (R)	7 c	42 b	9 c	0 c	51 c

Conclusions: resistant cabbage

- Resistance in ‘Kilaherb’ was expressed much later in cortical colonization than in canola.
- The intermediate resistance in ‘B-2819’ appears to restrict the growth of secondary plasmodia prior to development into resting spores.
- Both responses are different from resistance in canola
- Useful contribution to durable resistance?

Cloubroot management

- Accurate and consistent assessment of resting spores.
- Accurate soil sampling is difficult
- Keep resting spore concentration low!
- Crop rotation is important. Many resting spores die within the first 1-2 years, remaining spores survive for a long time (Type 3 survival curve)
- Durable resistance? Quantiative restistance?
- Soil treatments: lime, solarization, fumigation

No single approach provides control!

- Registered fumigants do not eliminate the pathogen from infested sites, but do reduce viable resting spores.
- Solarization may enhance or replace fumigation
- Strategies for reducing resting spore concentration are generally too expensive to be applied to entire fields.
- Producers need an effective way to minimize movement of soil out of infested patches and to hasten reduction in resting spore numbers – coming presentation

Acknowledgements:

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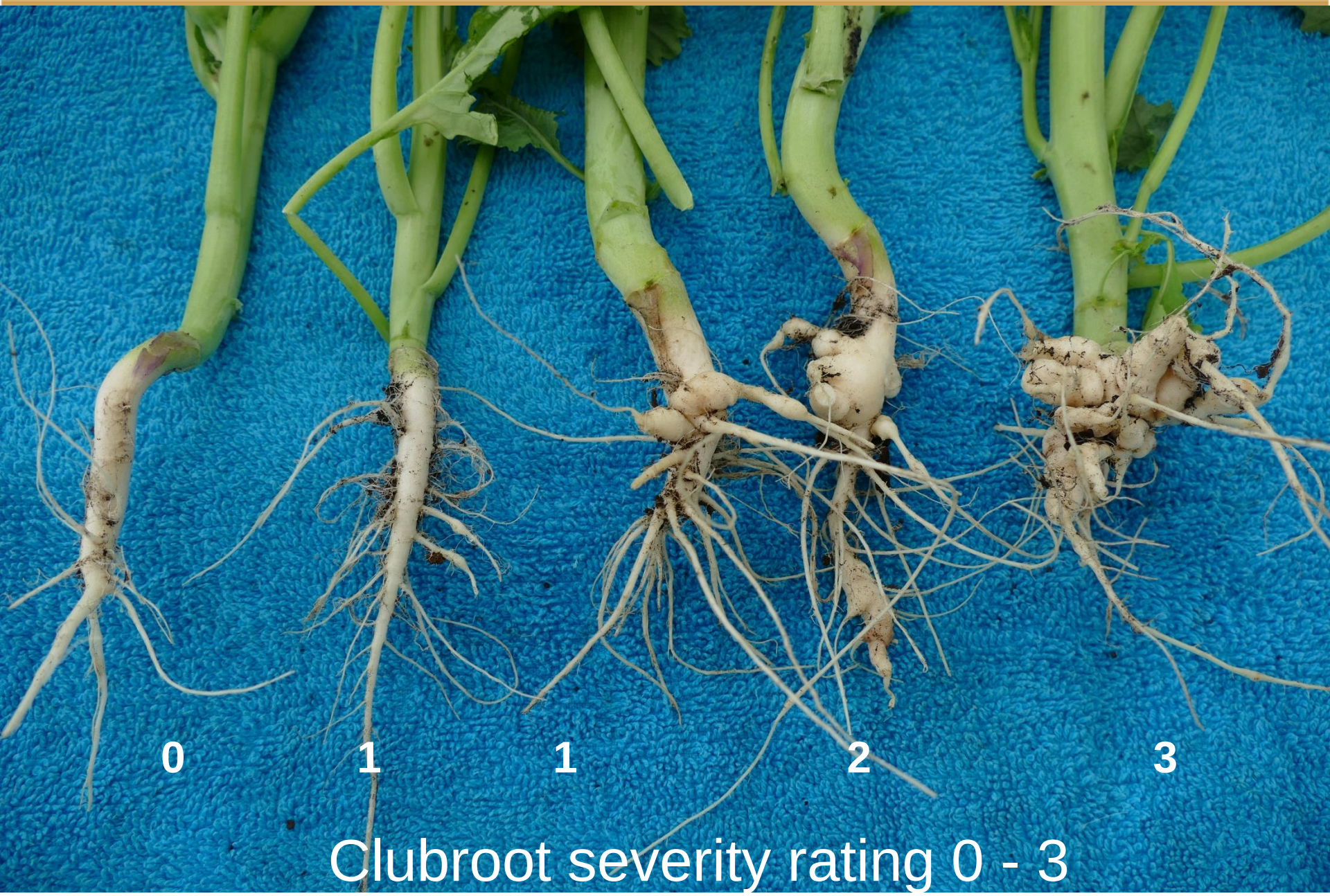
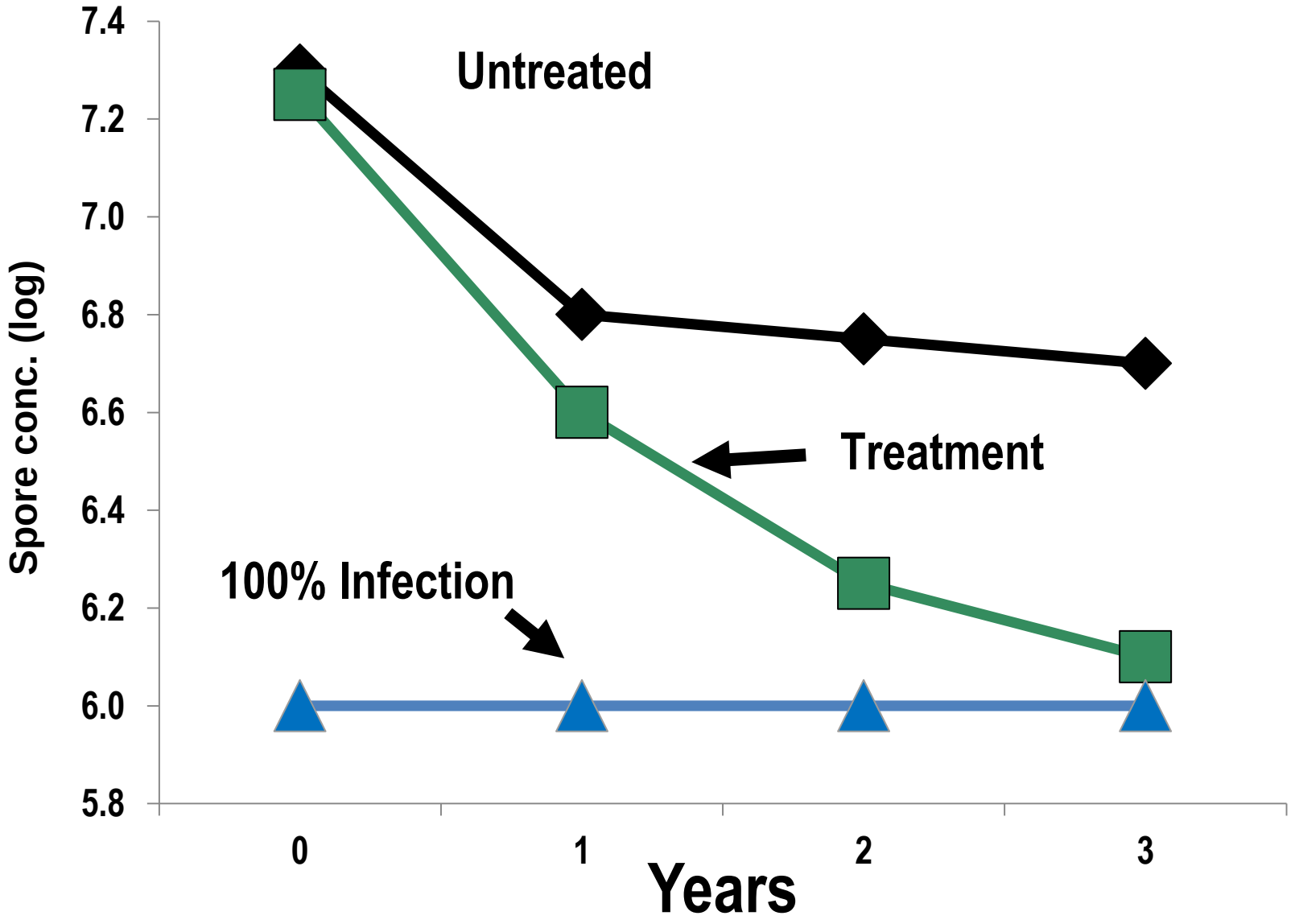
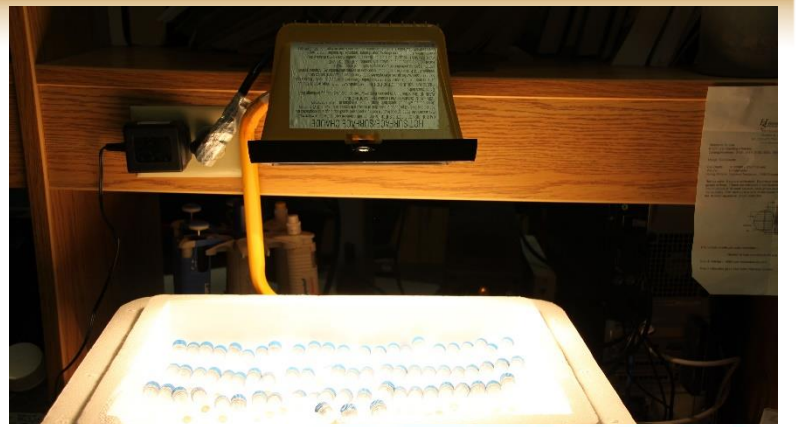
- Clubroot Risk Mitigation Initiative and GF2 (AAFC and the Canola Council of Canada)
- SaskCanola
- Western Grains Research Fund
- Agriculture Development Fund of Saskatchewan
- OMAFRA/University of Guelph Partnership


Questions?
Canada

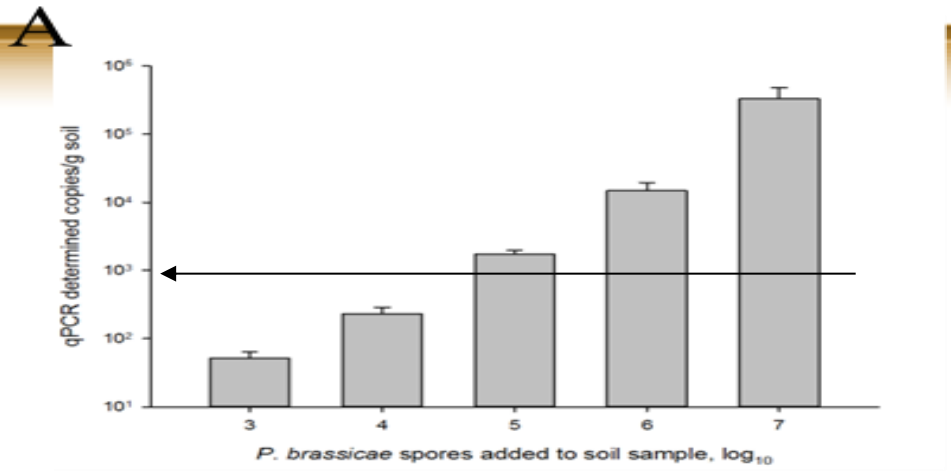


Clubroot severity rating 0 - 3

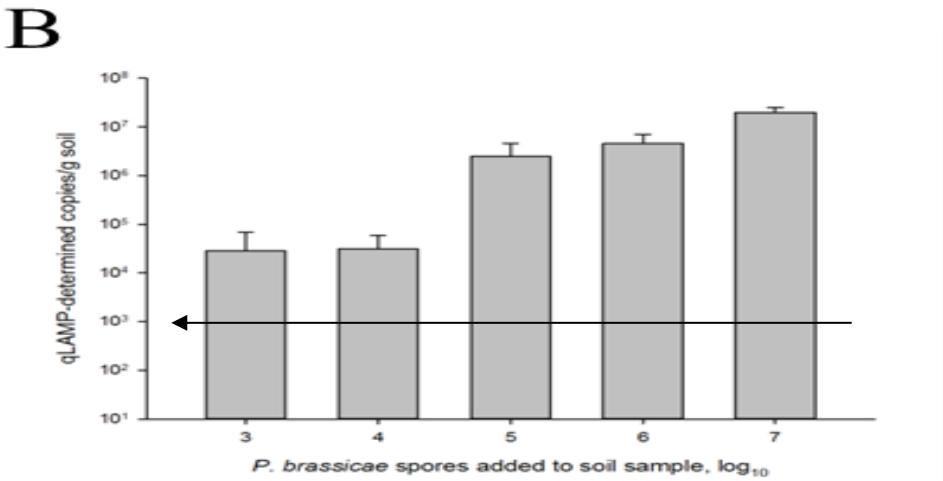

Untreated
Treatment
100% Infection
Spore conc. (log)
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
0
1
2
3
Years



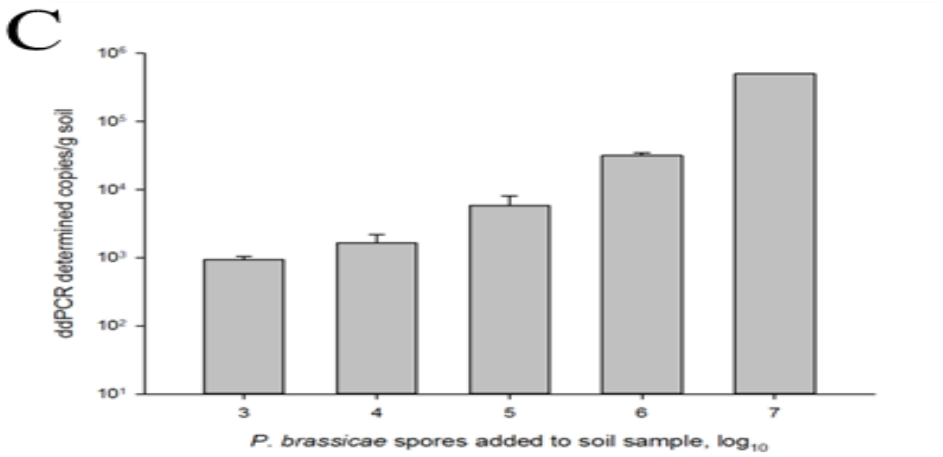
qPCR



LAMP



ddPCR



Plasmodiophora brassicae resting spores in soil at known levels 10^3 – 10^7.

Clubroot in soil

- Extremely variable within a field
- Challenges for growers, extension agents, researchers
- Can be found quite deep in soil – important?
- Very helpful to be able to determine how many resting spores are alive
- ie. After one - two years of crop rotation, most remaining resting spores are alive

Clubroot in soil

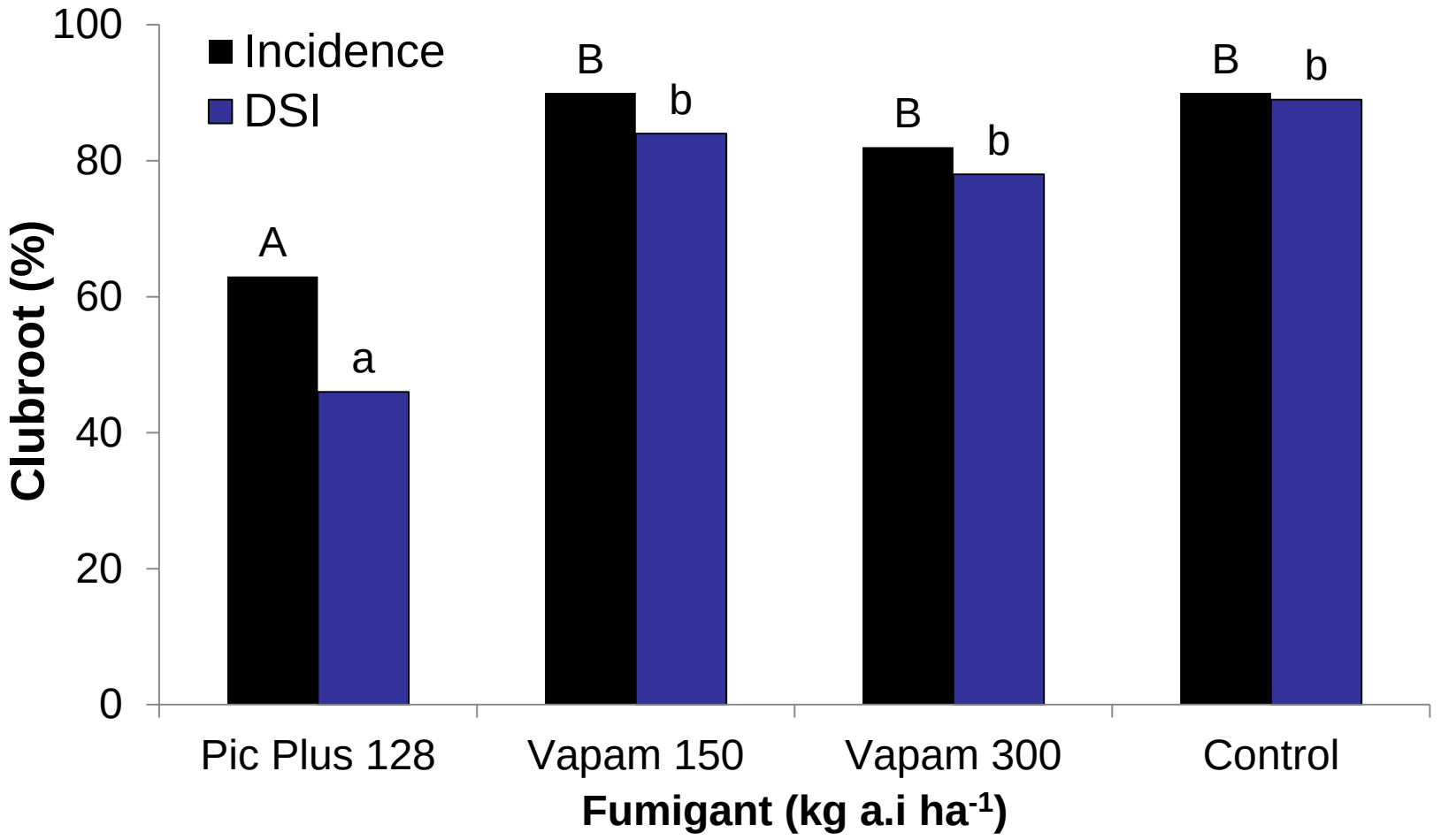
- Extremely variable
- Challenges for growers, extension agents, researchers
- Helpful to be able to determine how many resting spores are alive
- ie. After two years of crop rotation, numbers of resting spores are lower, but most remaining resting spores are alive


Applicator for Vapam

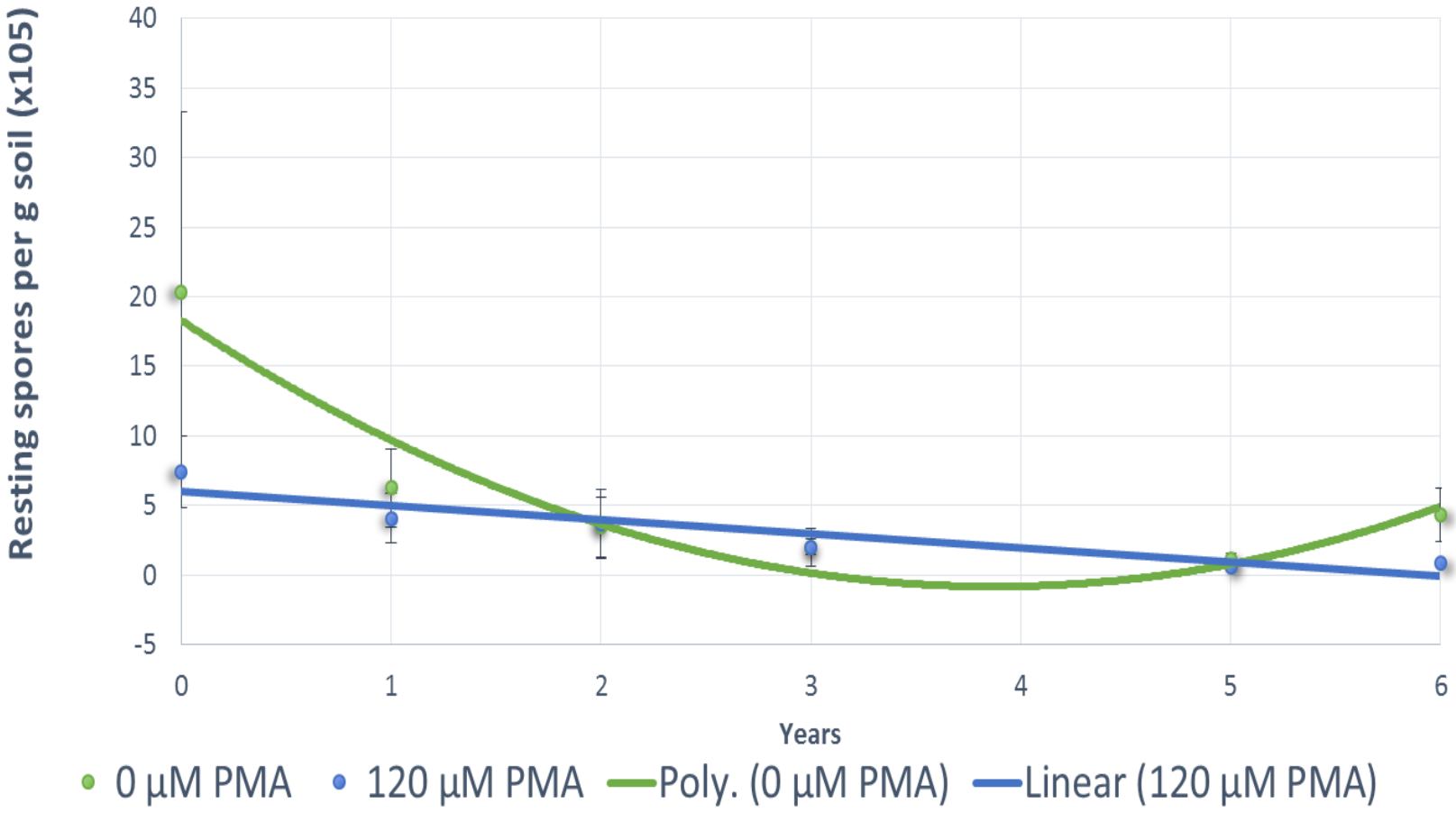
Fumigation trial, 2015

Disease severity index (0-100; 50 plants per experimental unit)

Field Trial, 2014



Results: PMA-PCR on crop rotation soil



* Regression lines ($P < 0.05$)

Spatial distribution of resting spores

Site west of Edmonton **Total resting spores**

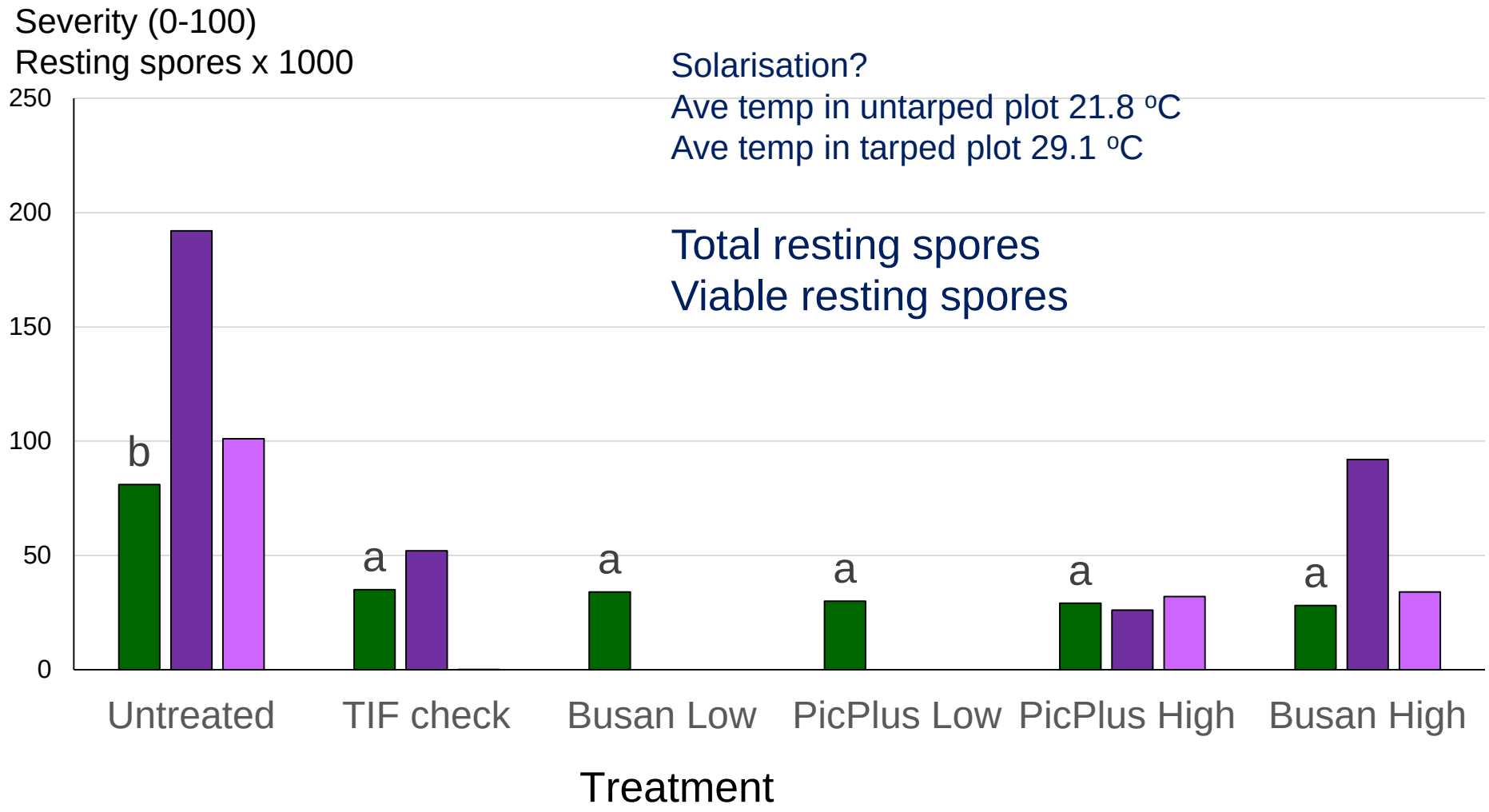
Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
137,097	3,783,380	85,987	1,073,189	2,529
3,290	698,927	1,463,110	123,188	56,916
14,411	0	117,508	735,706	14,832

12m x 12m

5 cores per plot

15 mm deep

Clubroot severity in pak choy following fumigation -2017



Concentration of resting spores at the Alberta site

PCR results
total resting spores

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PMA PCR results
- viable resting spores

	Rep 1	Rep 2	Rep 3	Rep 4	Rep 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

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Spatial distribution of resting spores

Site west of Edmonton **Viable resting spores**

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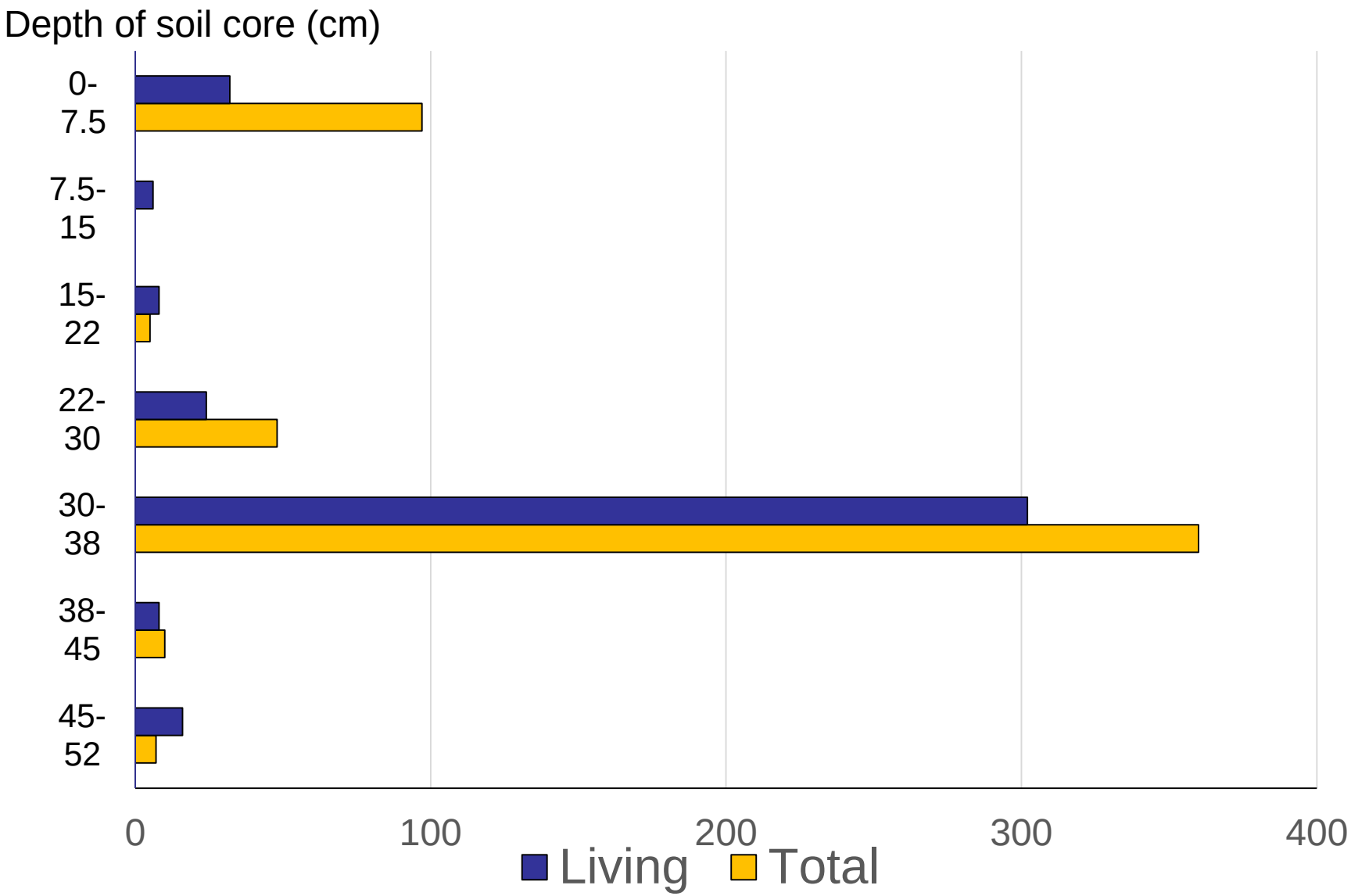
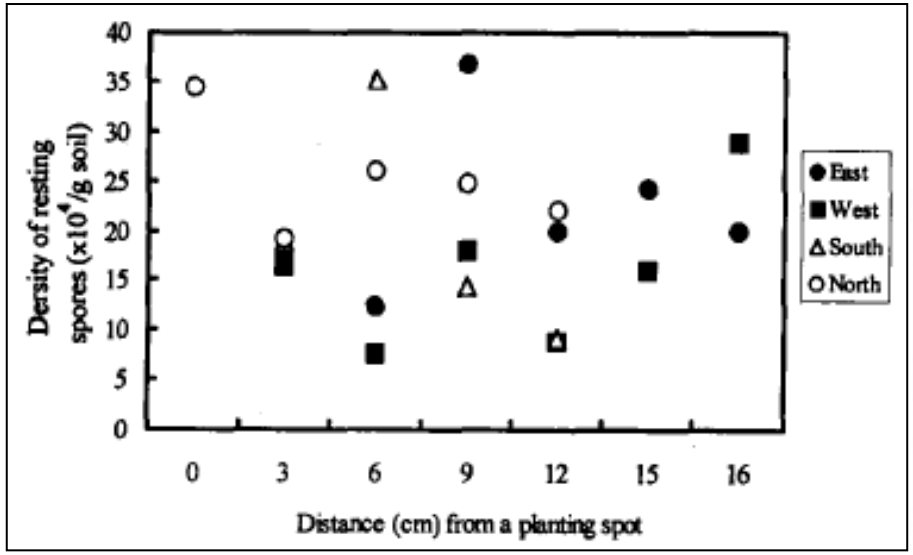
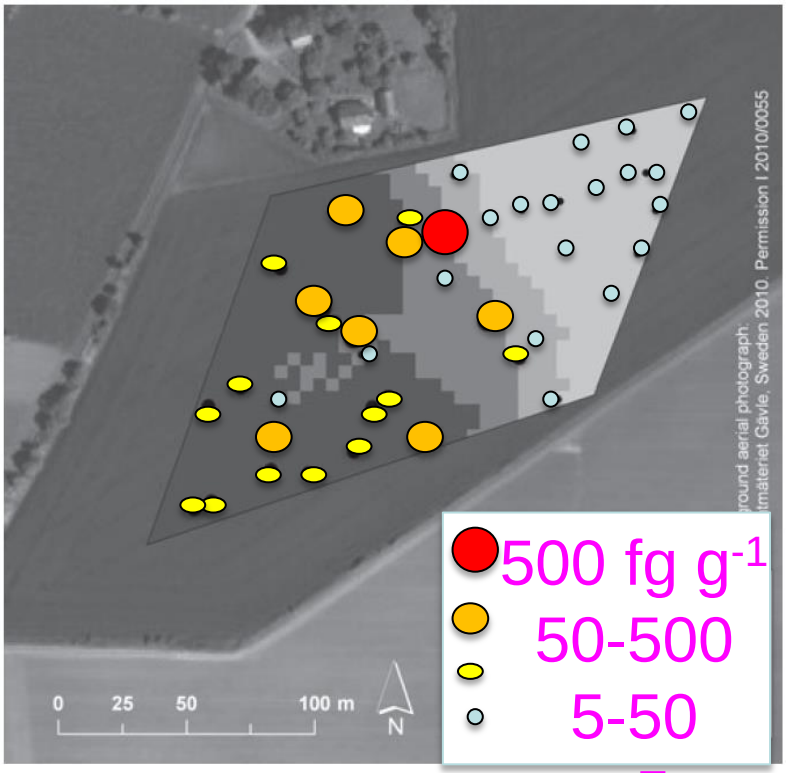
Distribution of resting spores with depth – Alberta site



Introduction – horizontal spore distribution

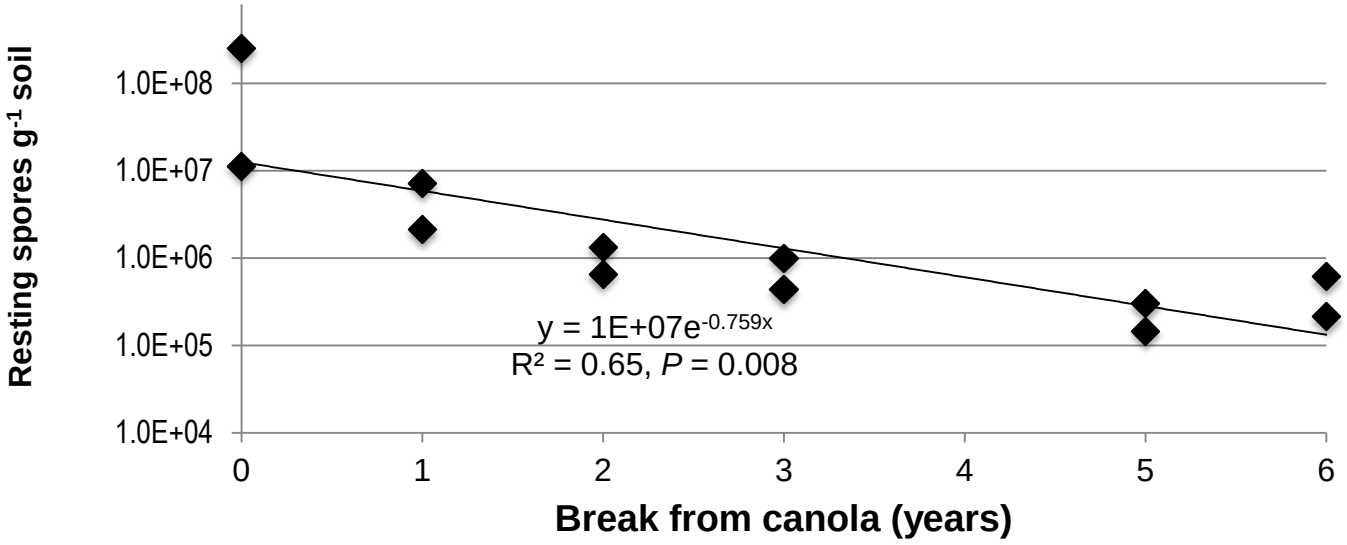


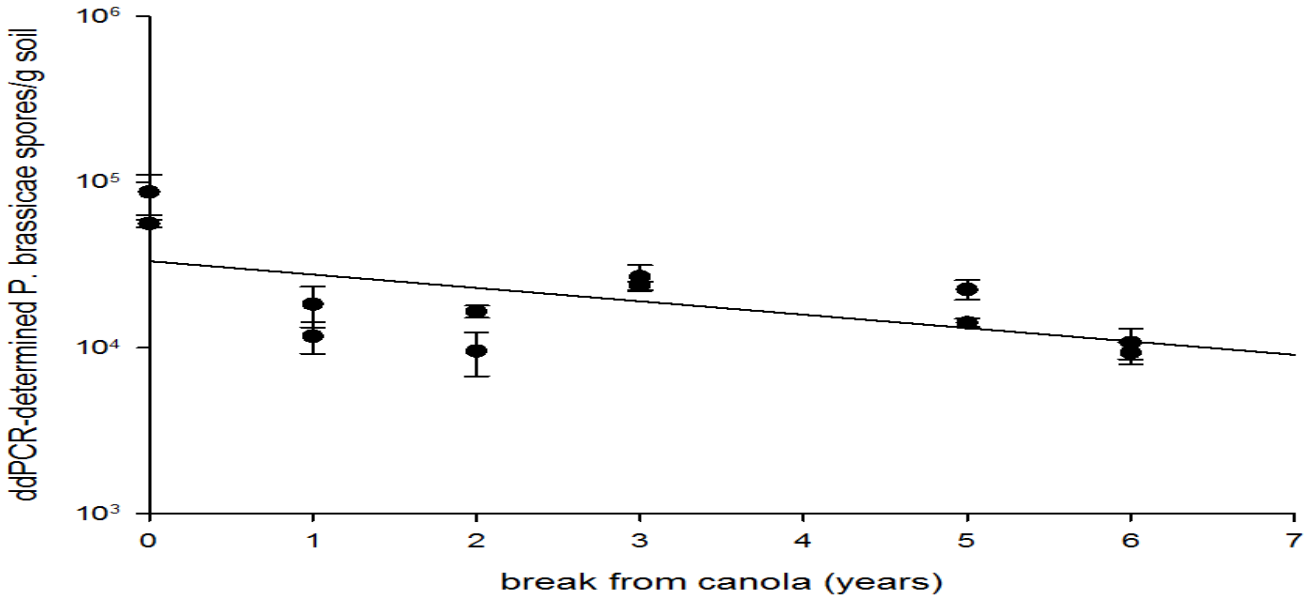
Kim et al. (2000), microscopy



Wallenhammar (2012), real-time P

- Highly variable
- Patchy


Resting spores g-1 soil
1.0E+08
1.0E+07
1.0E+06
1.0E+05
1.0E+04
y = 1E+07e-0.759x
R² = 0.65, P = 0.008
0
1
2
3
4
5
6
Break from canola (years)

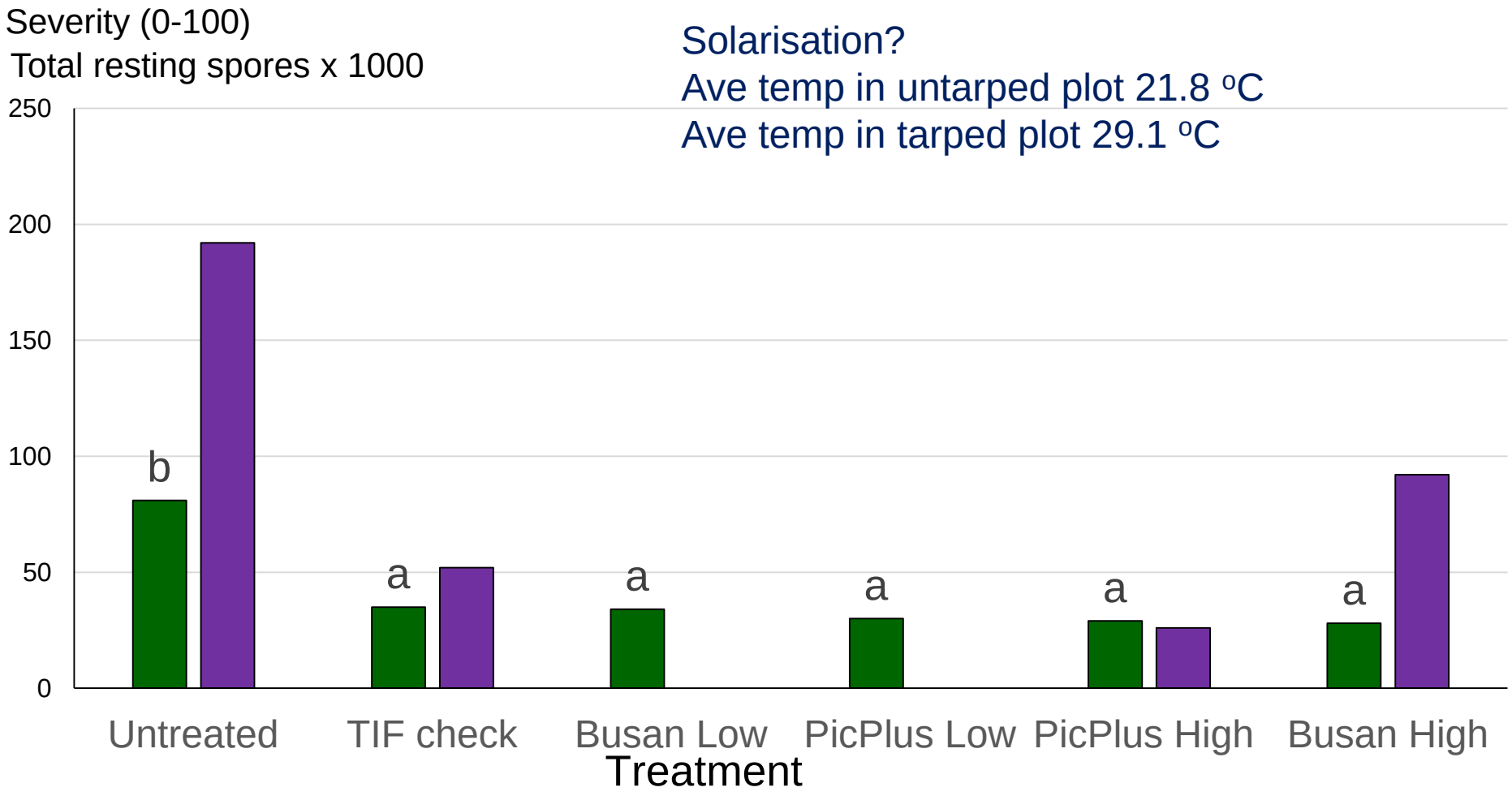

ddPCR-determined P. brassicae spores/g soil
10⁶
10⁵
10⁴
10³
0
1
2
3
4
5
6
7
break from canola (years)

Acknowledgements

Funding for the research was provided by:

- ✓ Clubroot Risk Mitigation Initiative and Growing Forward 2 (AAFC & Canola Council of Canada).
- ✓ SaskCanola.
- ✓ Western Grains Research Fund.
- ✓ Agriculture Development Fund of Saskatchewan
- ✓ OMAFRA/University of Guelph Partnership.

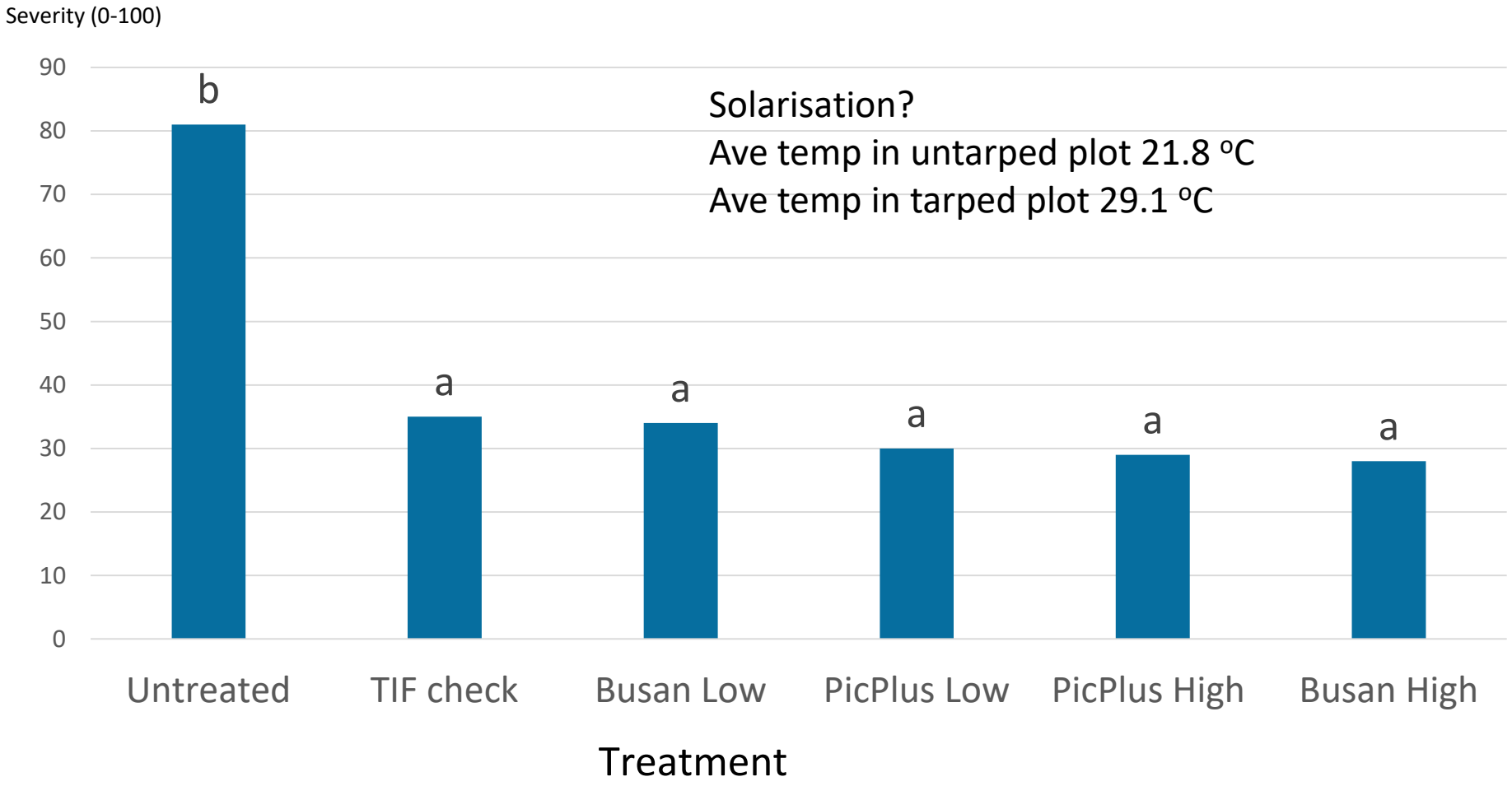
Clubroot severity in pak choy following fumigation -2017



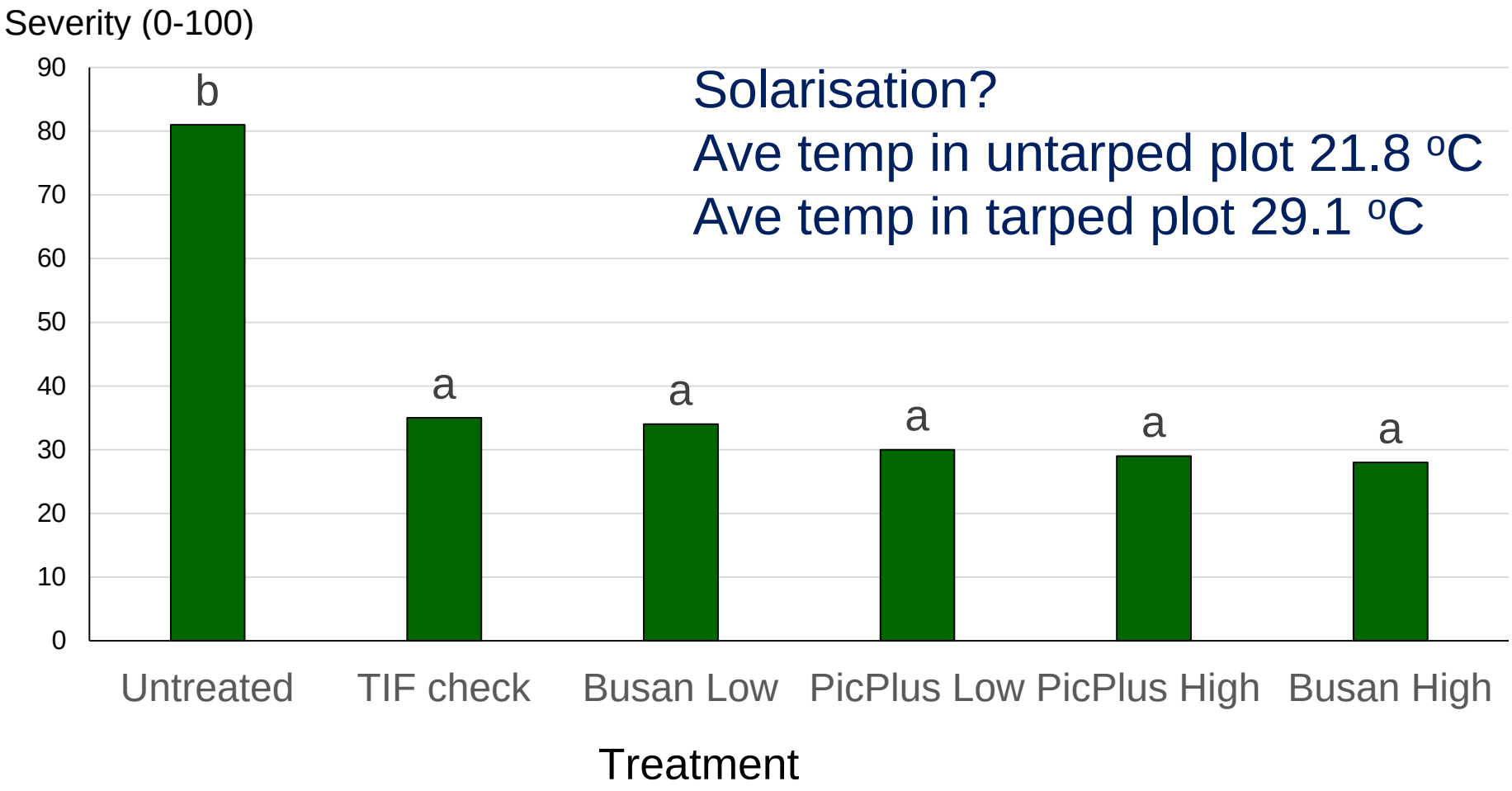
Spatial distribution of resting spores

- Site west of Edmonton

Clubroot severity in pak choy following fumigation



Clubroot severity in pak choy following fumigation



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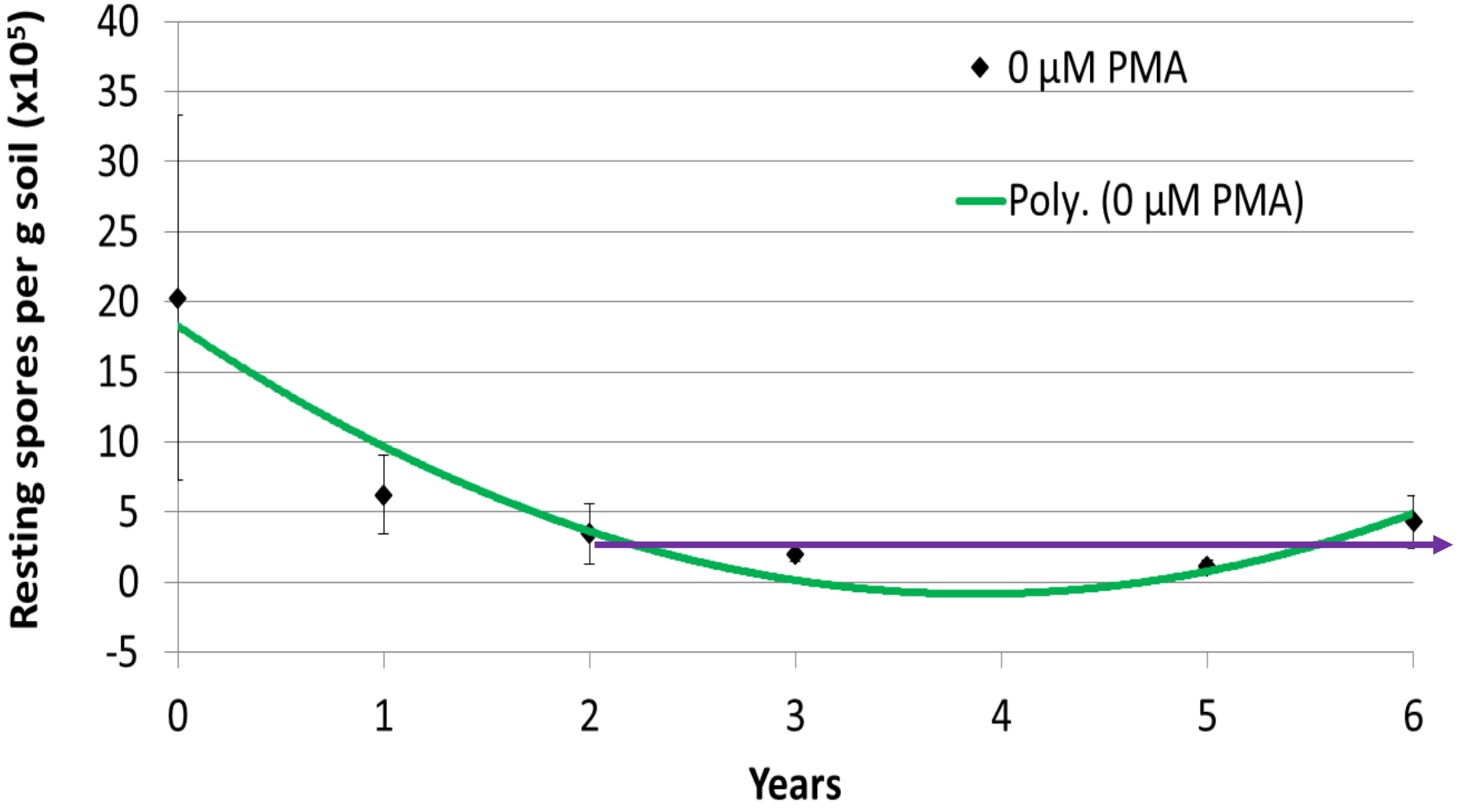
PMA-PCR after 0 – 6 year break from canola



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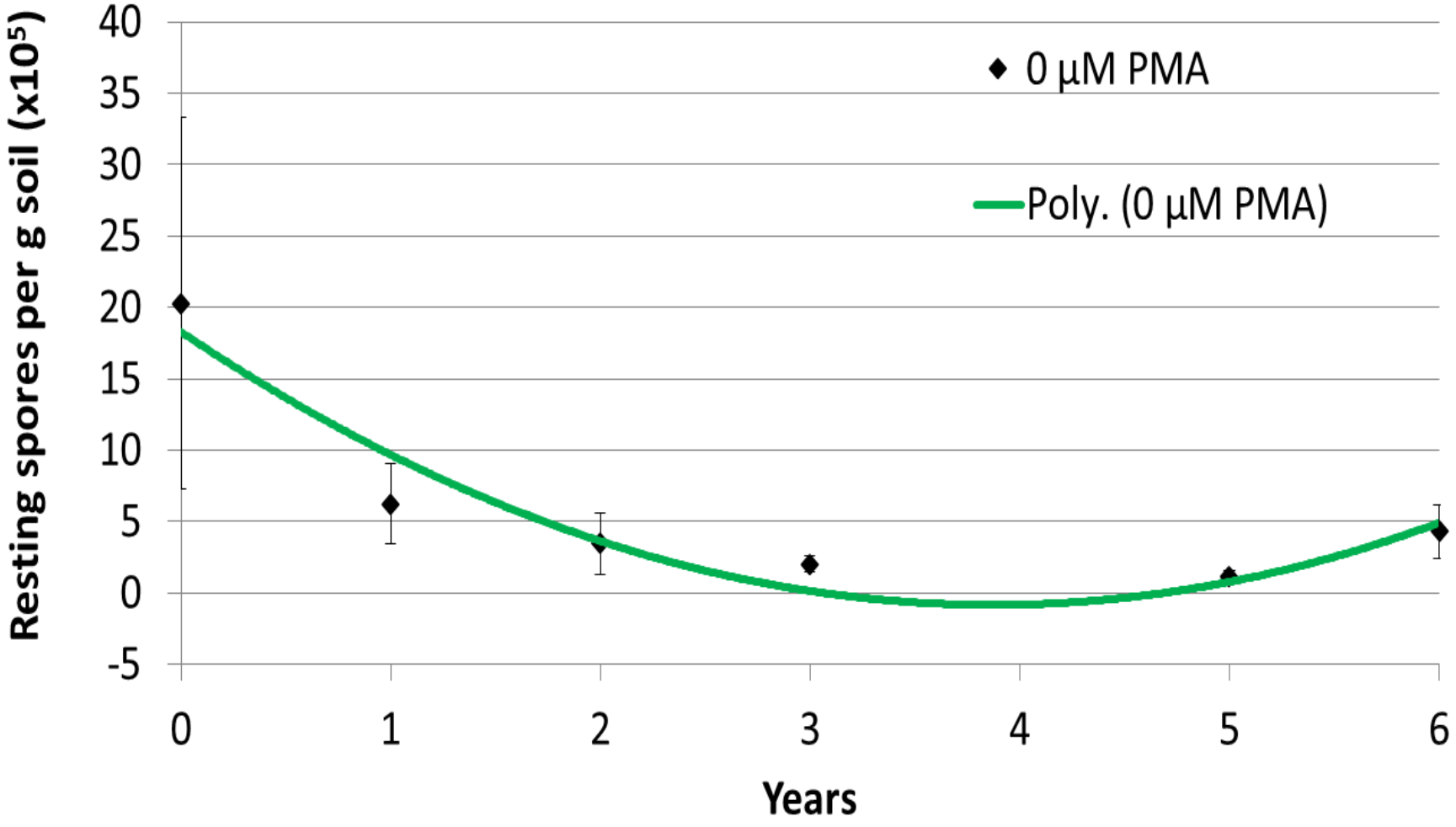

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