# Clubroot Management Update

## Clubroot Steering Committee

**April 30, 2020**

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# Clubroot Management:

- Whole genome sequencing and new virulent pathotypes (published)
- Effect of grass cover crops and rotation crops on resting spore concentrations in soil
- Fumigation and solarization

- Boron as a soil amendment with boron insensitive Brassicas

Whole genome sequencing to determine the genome similarity of single-spore isolates and field collections from locations in Canada, the USA, and China:

Sedaghatkish *et al. BMC Genomics* (2019) 20:744
https://doi.org/10.1186/s12864-019-6118-y

BMC Genomics

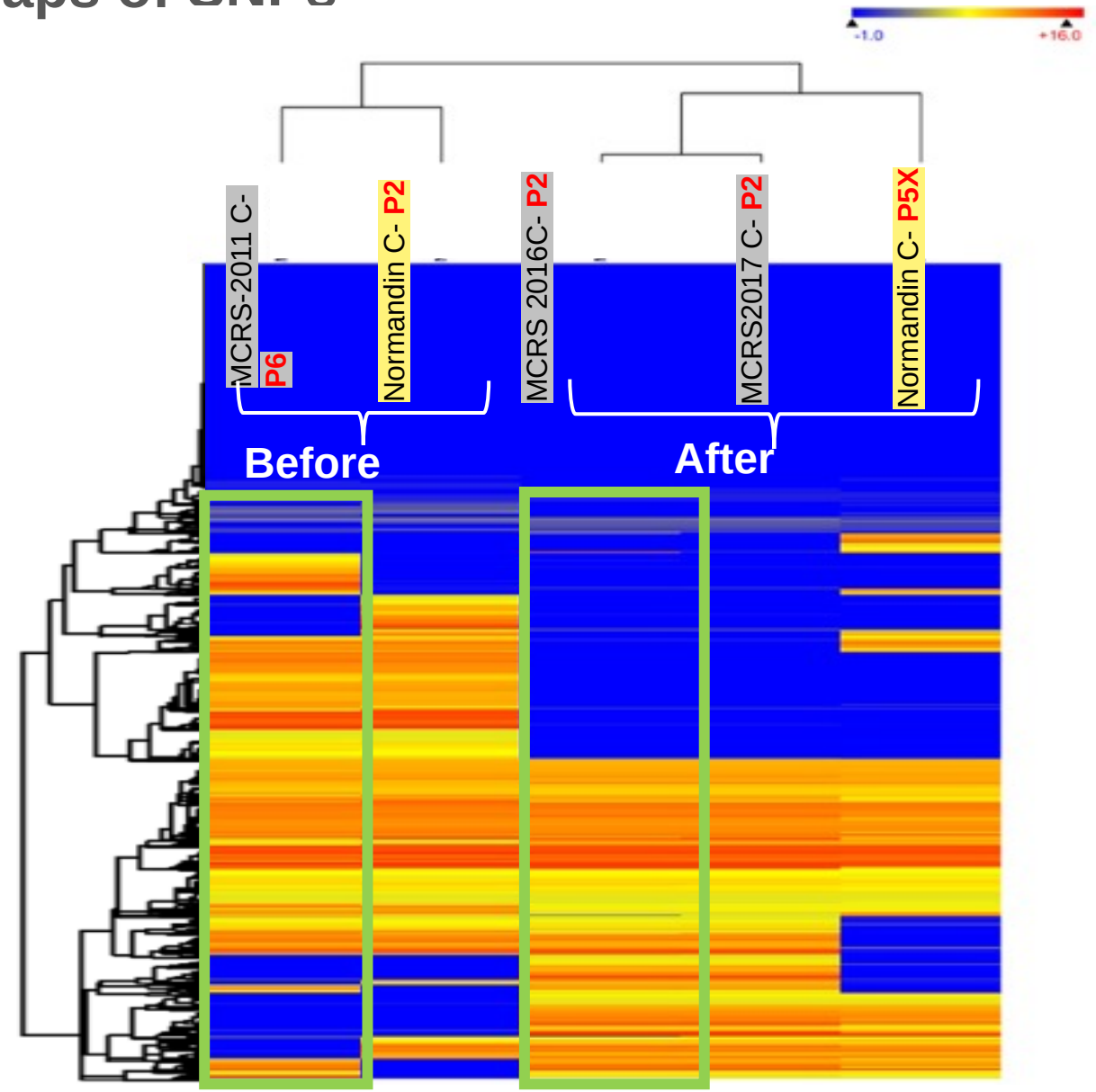
RESEARCH ARTICLE Open Access

# Whole-genome DNA similarity and population structure of *Plasmodiophora brassicae* strains from Canada

Afsaneh Sedaghatkish[1,2], Bruce D. Gossen[2*], Fengqun Yu[2], Davoud Torkamaneh[1,3] and Mary Ruth McDonald[1]

Sequenced 43 collections, including 9 single spore isolates, mostly from Canada. They did not cluster by pathotype or host. Some clustered by geographic region.

# Heat maps of SNPs




Total of 9727 genes in *P. brassicae* genome

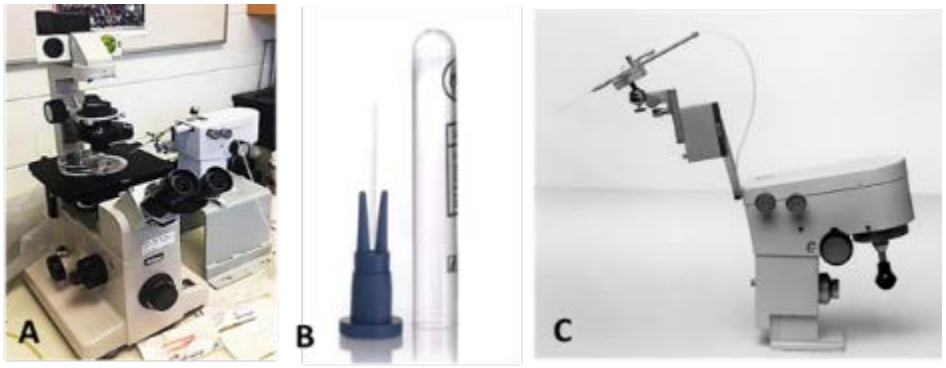
Selection, rather than single mutations responsible for the changes

Balancing selection

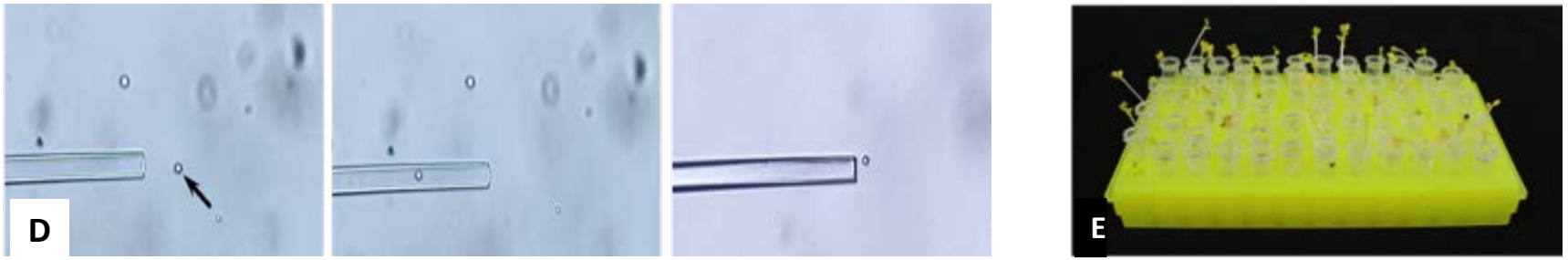
Normandin and MCRS collections before and after the change of pathotype
There is about a 50% difference in SNPs from before and after

# Capturing single resting spores with a micromanipulator- a more efficient method to produce single spore isolates of *P. brassicae*

## Micromanipulation of a singe spore




(A) inverted microscope, (B) glass micropipette, C. micromanipulator,& D. isolation plate




(D) selection and collection of a single spore, and (E) placement of a single spore in Hoagland's solution containing a 3-day old canola

# Cover crops and rotation crops to stimulate the germination of resting spores

## Materials & methods

- Soil with 5 x $10^5$ resting spore per gram
- Crops grown for 8 weeks
- qPCR assessment of resting spores

## Crops:

- Shanghai pak choi (*Brassica rapa* L. ) susceptible check
- Smooth bromegrass (*Bromus inermis* L.) a common seed lot
- Meadow bromegrass (*B. riparius* R.) cv. Fleet
- Perennial ryegrass (*Lolium perenne* L.) cv.'s Norlea, All Star, and Fiesta

Afsaneh Sedaghatish Ph.D. thesis

**Effect of grass species and cultivar on resting spore concentration of *P. brassicae* in soil** (Based on qPCR, n = 6)

| Treatment | Grass cultivar | Spore conc. (spores $g^{-1}$ soil) | Root dry wt. (g $pot^{-1}$) |
|---|---|---|---|
| Pre-plant soil | | $1.6 \times 10^6$ | |
| **No plant (control)** | | **$1.2 \times 10^6$ a** | |
| Perennial ryegrass | Norlea | $5.9 \times 10^5$ a | 6.35 a |
| | All Star | $4.9 \times 10^5$ a | 6.32 a |
| | Fiesta | **$2.7 \times 10^5$ b** | **2.73 b** |
| Meadow bromegrass | Fleet | **$5.0 \times 10^5$ b** | 6.44 a |
| Smooth bromegrass | Common | **$4.6 \times 10^5$ b** | **3.85 b** |



The initial resting spore concentration was higher than intended

No correlation between resting spore concentration and root weight

# What about rotation crops?

## Resting spore concentration in soil with different crops

| Crop | Cultivar | Spore conc. $g^{-1}$ soil |
|---|---|---|
| Soybean | | 469,000 a |
| **No plant (control)** | | **310,000 b** |
| Barley | Trochu | 266,000 bc |
| Field pea | CDC Meadow | 229,000 bc |
| Ryegrass | Norlea | 183,000 bc |
| **Wheat** | AAC Connery | **155,000 c** |

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

**Spring wheat is a good rotation crop and may help to reduce resting spore numbers. Still lots of variability in the data**

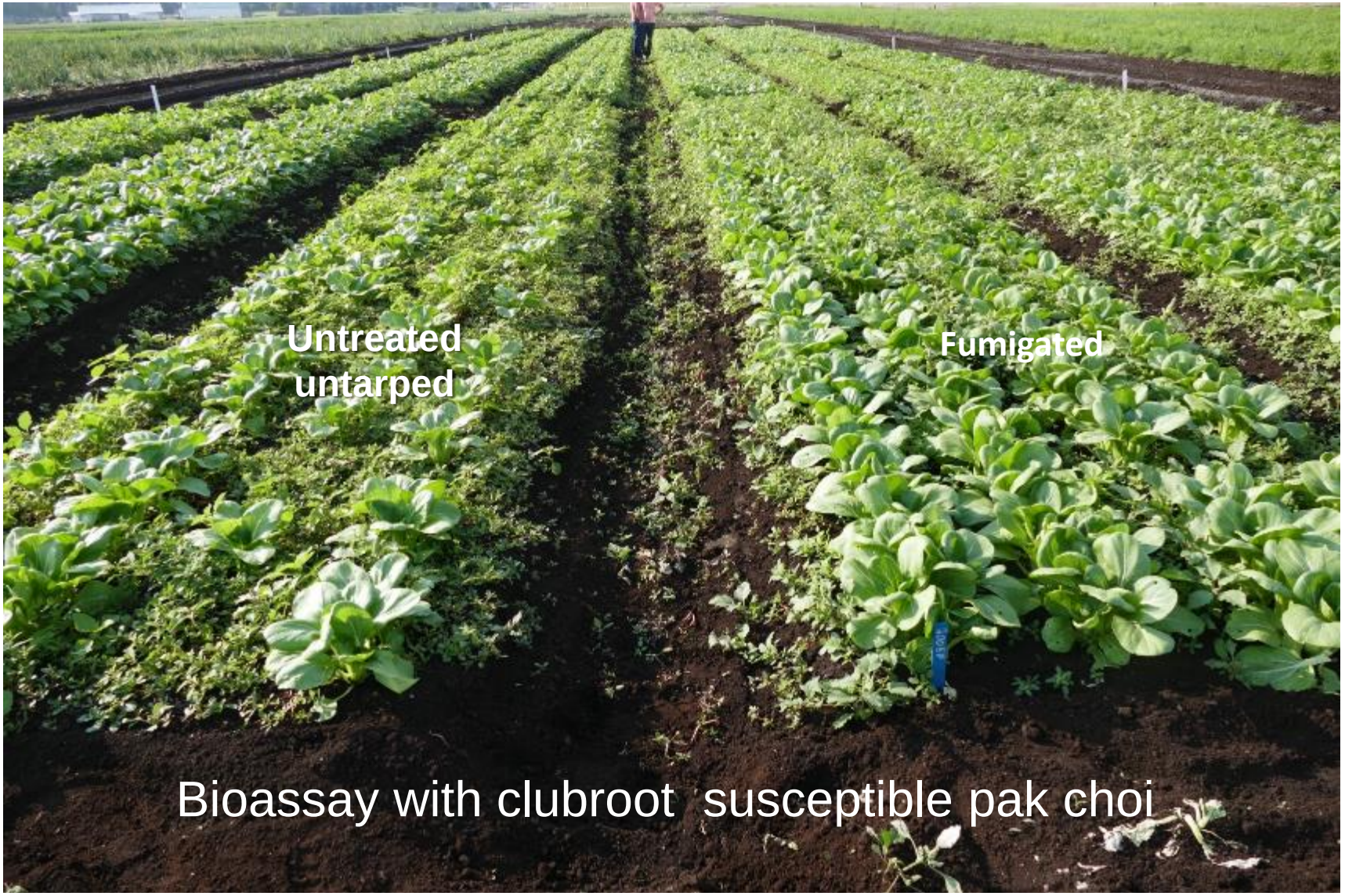
# For a quicker effect: Fumigation and/or solarisation Or boron?

Fumigated in late June or July
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

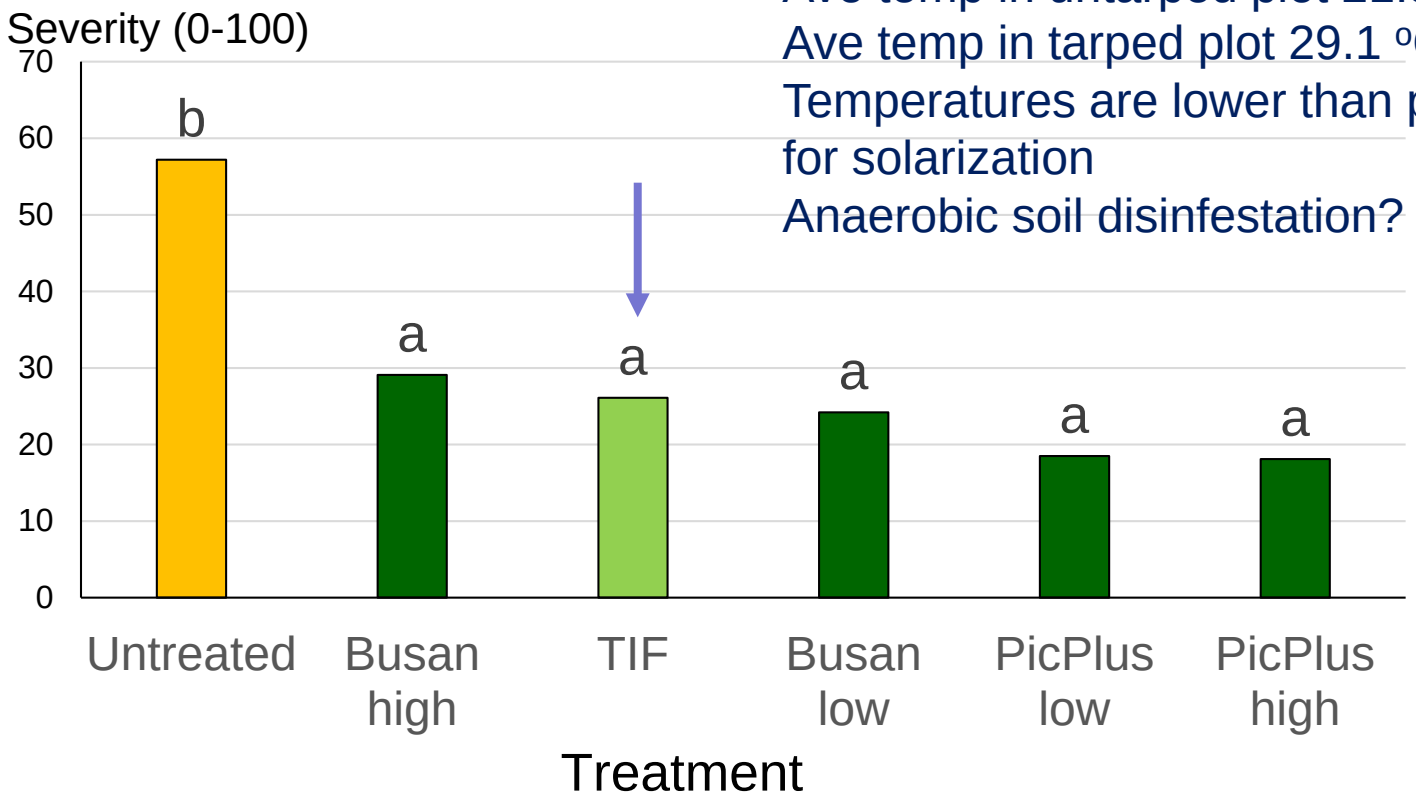
After 2 weeks, the tarp was removed, soil samples taken and a susceptible crop- pak choi – was seeded.  Assessed 5 weeks later.

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




Bioassay with clubroot susceptible pak choi

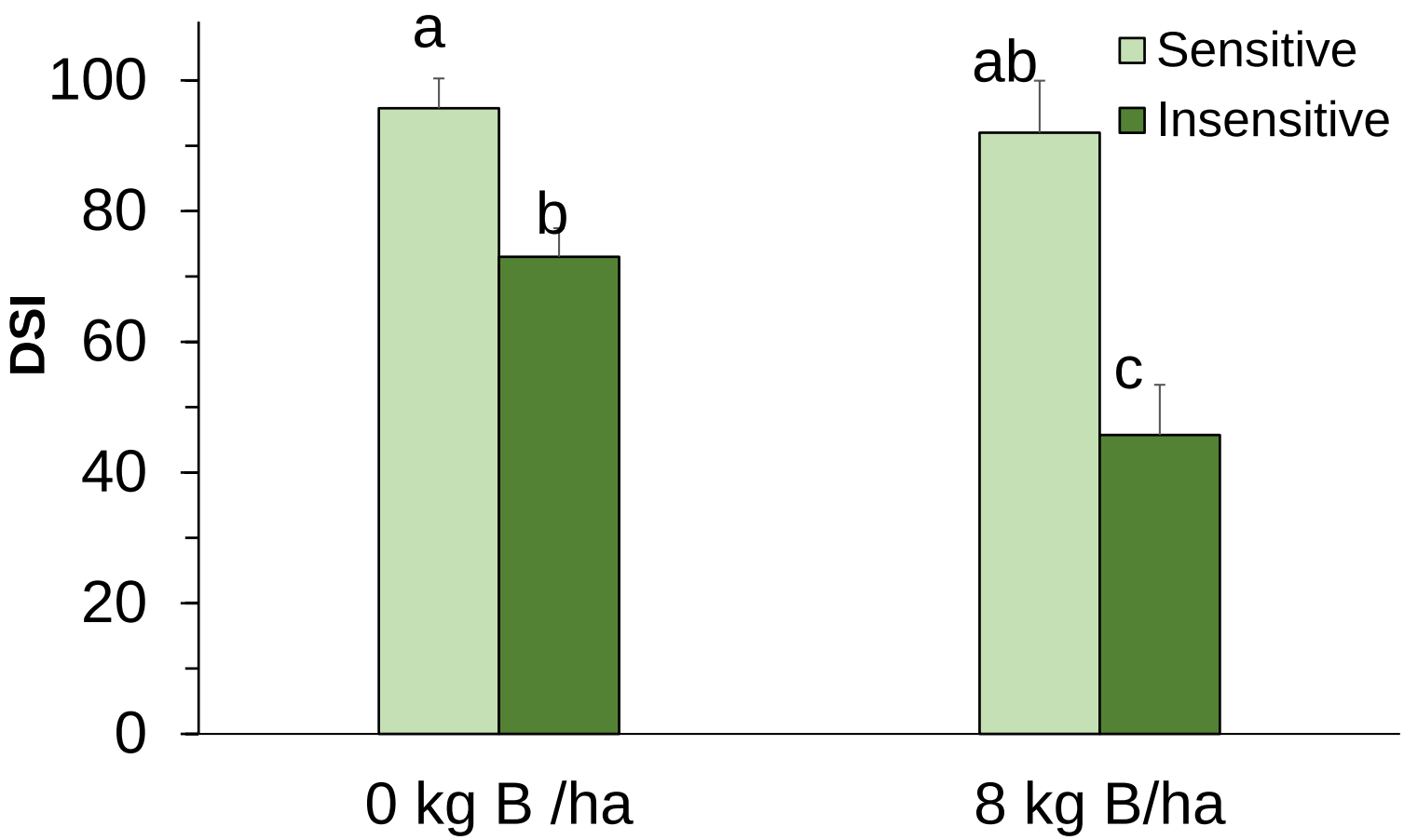
# Clubroot severity in pak choy following fumigation -2019




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?

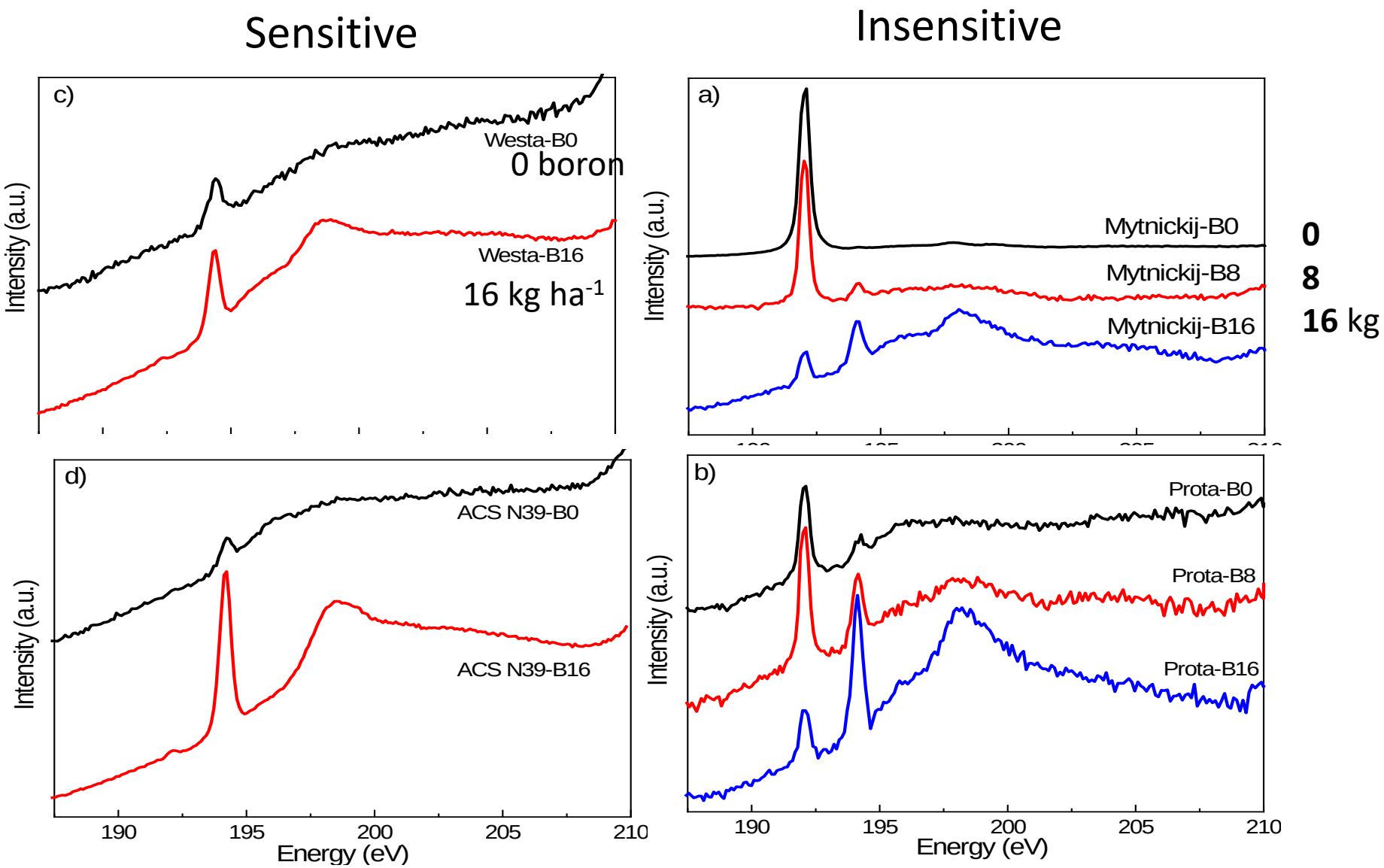
Busan = metam sodium, PicPlus = chloropicrin


Boron suppresses clubroot development
But can be phytotoxic
Use boron with boron insensitive varieties?
Boron
No boron




**Effect of a drench application of boron at 8 kg/ha on clubroot severity in the field** Mean of 10 sensitive and 9 insensitive lines

Next step: Assess plants in the synchrotron (Canada Light Source) to determine boron content of roots and leaves




Boron K-edge XANES spectra collected on roots

Changes in spectra with added B indicate more boron-oxygen bonds

# Clubroot Management: Conclusions

- Grass cover crops and rotation crops may reduce resting spores in soil faster than if soil was left fallow
  - However, the first results from field trials showed higher resting spores under perennial ryegrass

- New virulent pathotypes are selected from existing genotypes (not recent mutations)
- P. brassica exhibits balancing selection to preserve many genotypes
  - Continue to develop single spore isolates for research

- Solarization using totally impermeable film could be an approach to manage small patches of clubroot.

- Could boron be used to suppress clubroot using boron insensitive lines of B. napus?


Questions?

# Acknowledgements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- Ontario Canola Growers Association
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- Ontario Agri-Food Innovation Alliance