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Crop rotation, cultivar resistance, and biofungicide for clubroot control on canola

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Canada

Resistance is the cornerstone for clubroot management on canola

- ❖ Allowed canola to be grown again in fields with extremely high levels of pathogen inoculum only a few years ago
- ❖ Significantly better crops and higher yields than a cv. -in heavily infested fields
- ❖ Less amount of pathogen inoculum going back into the soil

Susceptible **Resistant**

Resistance ….but not "Immunity"

❖ *R* genes are race specific. May be eroded with shifting in pathogen race structure

❖ Clubroot severity increased when a *R* cv. was exposed repeatedly to same pathogen population (*LeBoldus et al.*, *2012*)

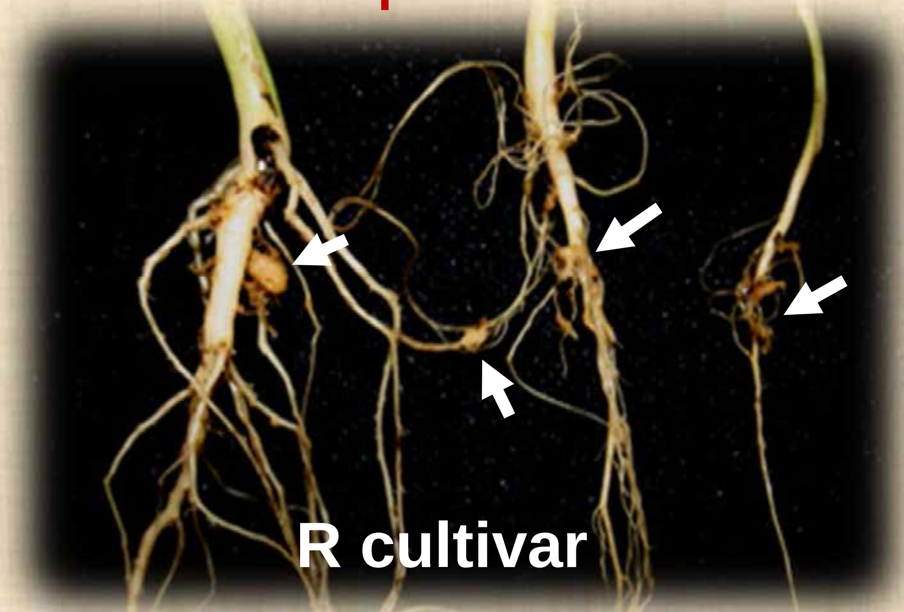
❖ small, spheroid, resistant-type galls (Osaki et al. 2008)

❖ Limited *R* sources

❖ Resistance stewardship

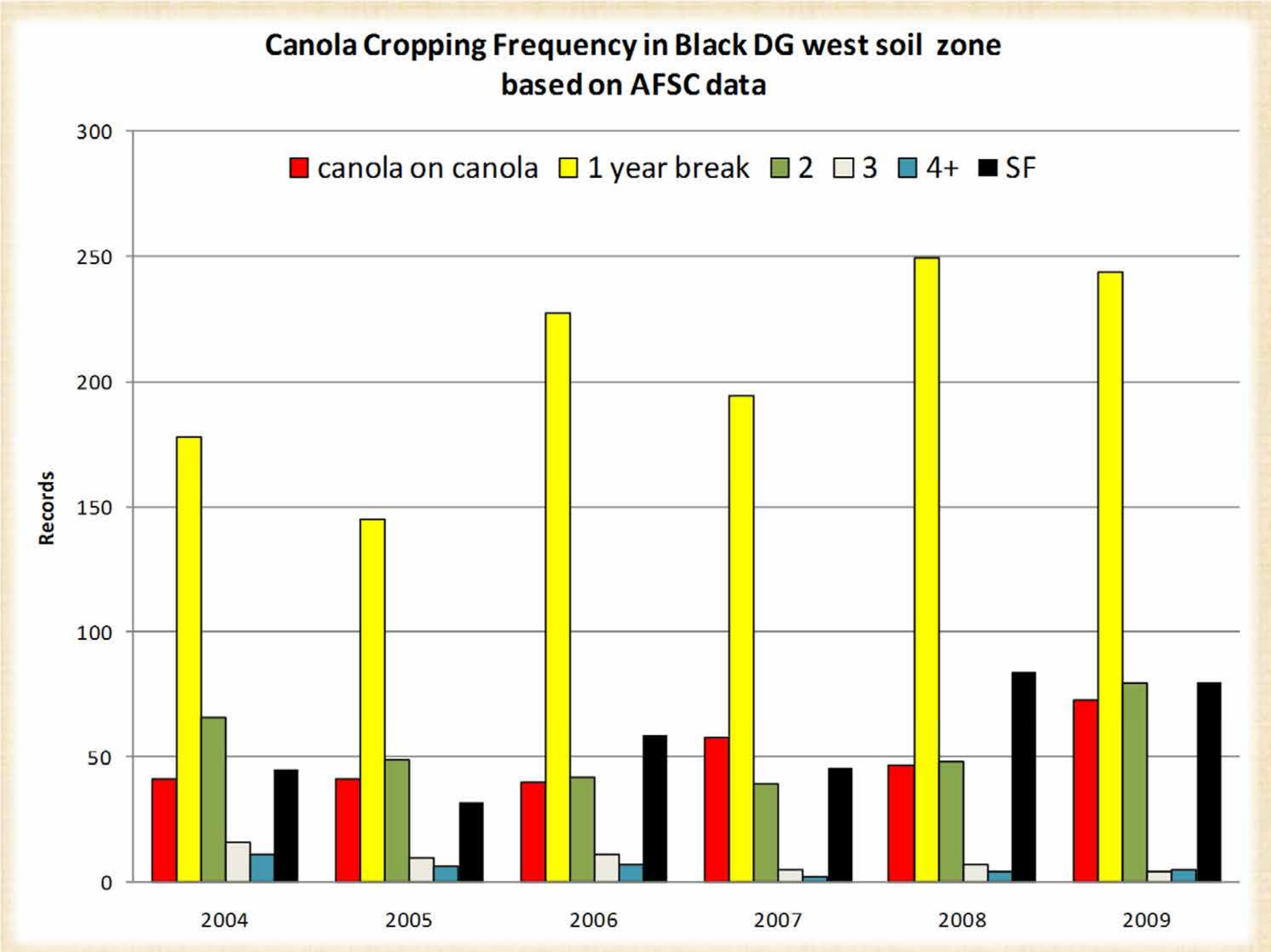
Additional measures helpful?

10^8 spores/mL



Crop Rotation

- Benefits to crop production are well recognized
- Important disease management tool for many field crops – for example, blackleg of canola in western Canada
- A 3-year rotation (canola – cereal - pulse) is considered sustainable (Cathcart *et al.*, 2006), but a 2-year rotation of canola with a cereal crop or even continuous canola is no longer uncommon (Hartman, 2012)
- Is 3- or 4-yr crop rotation effective for clubroot control?



Canola Cropping Frequency in Black DG west soil zone
based on AFSC data
canola on canola
1 year break
2
3
4+
SF
Records
0
50
100
150
200
250
300
2004
2005
2006
2007
2008
2009

Impact of crop rotation on *P. brassicae* resting spores in soils

- Based on bioassay results, the 'half life' of *P. brassicae* resting spores in field soils was estimated at about 4-5 years (Wallenhammar, 1996; Hwang *et al.*, 2013)
- In micro plots based on disease severity, a faster rate of decline of *P. brassicae* resting spores was indicated when non-host crops or fallow was used for 1-3 years (Robak, 1994)
- There has been no information on the effect of a break from canola to alleviate clubroot impact (crop development and yield) in field

    - sufficiently effective for reducing pathogen inoculum and clubroot severity?

- qPCR has been developed for direct enumeration of resting spores in soils (Wallenhammar *et al.,* 2012; Rennie *et al.*, 2011)

When the pathogen inoculum is reduced in the soil

- ❖ Reducing pathogen resting spores in the soil by 10-fold substantially lowered the clubroot severity under controlled conditions
- ❖ Can crop rotation result in such a significant reduction in pathogen inoculum under filed conditions?

5 x 10^6 spores/g soil

5 x 10^5 spores/g soil

Chemical/biological control?

- Cheah LH et al. 2000. Biological control of clubroot on cauliflower with *Trichoderma* and *Streptomyces* spp. *NZ Plant Prot.* **53**, 18–21.
- Mitani et al. Effects of cyazofamid against Plasmodiophora brassicae Woronin on Chinese cabbage. Pest Man. Sci., 59, 287–293
- Narisawa K et al. 1998. Suppression of clubroot formation in Chinese cabbage by the root endophytic fungus, *Heteroconium chaetospira*. *Plant Pathol*. **47**, 206–210
- Townley & Fox. 2003. Control of clubroot disease using cyazofamid and fluazinam fungicides. In: *Proc 8th Int. Cong. Plant Pathol*. *Feb. 2–7, 2003, Christchurch, N.Z*.

No information on large-acreage crops like canola

Work conducted lately in Canada

- 5,000 indigenous soil microbes were assessed for the potential of clubroot control
- Applied as a soil drench, and efficacy compared with biological and synthetic fungicides registered in Canada or USA

Efficacy of indigenous microbes

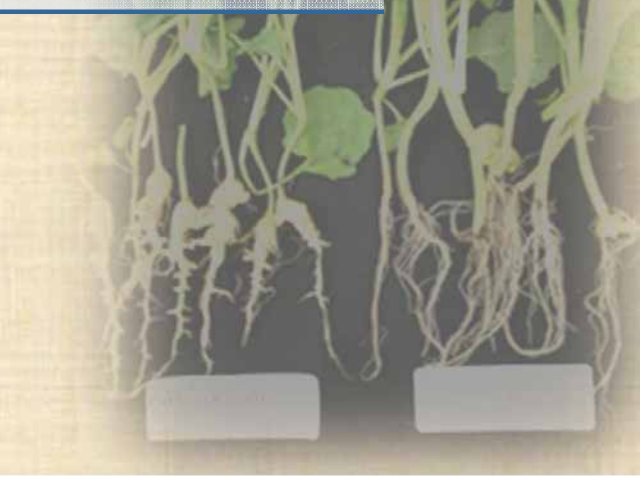
Efficacy of soil microbes against clubroot on canola

Isolates		Range of clubroot reduction (%)*		
		26-50	50-75	75-100
Fungi	Endophyte	7**	1	3
	Rhizosphere	13	2	0
Bacteria	Endophyte	7	1	0
	Rhizosphere	5	7	1

*Compared to the pathogen control in the same trial

**Number of isolates in the category

The indigenous candidates were less consistent than biofungicides under controlled conditions

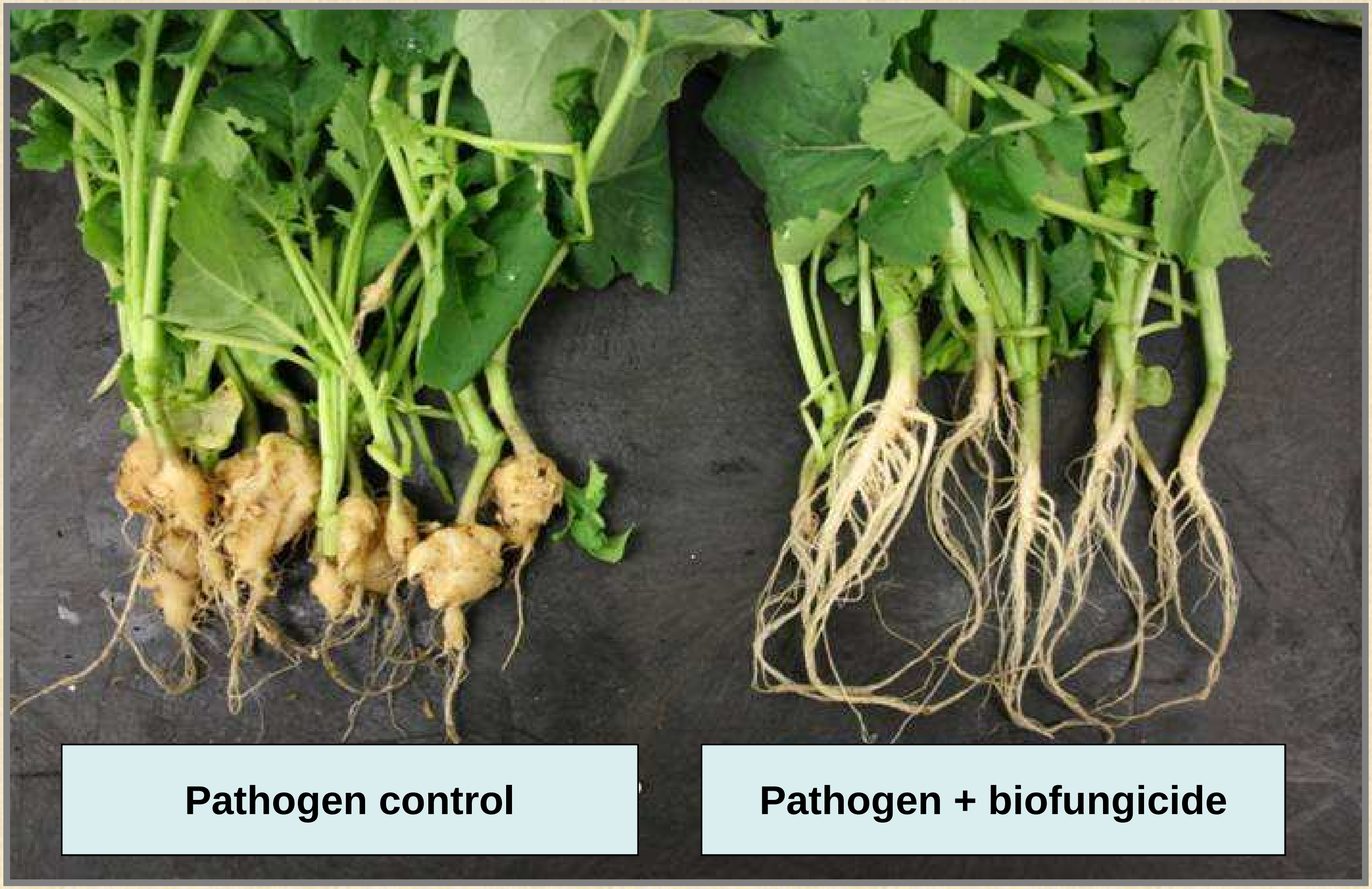


Biofungicides & fungicides

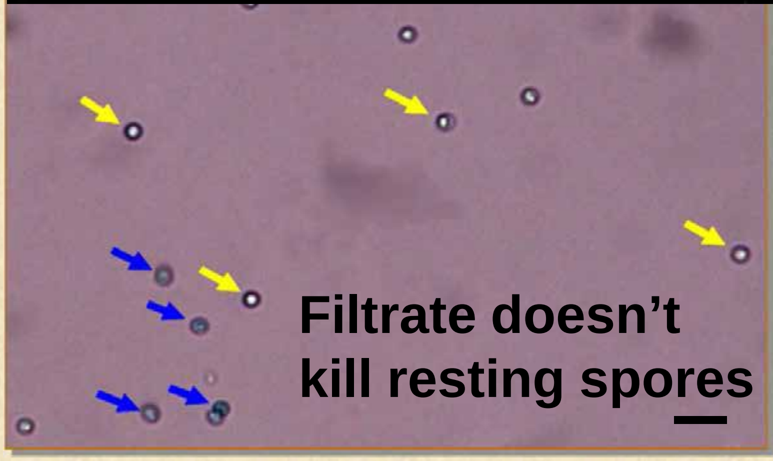
- **Serenade** (*Bacillus subtilis)*
- **Prestop** (*Clonostachys rosea*)
- **Allegro** (Fluazinam)
- **Ranman** (Cyazofamid)

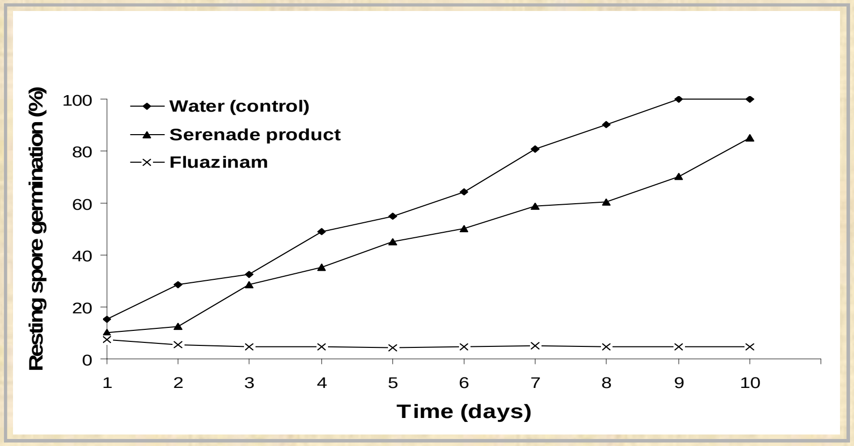
Effective when applied as a liquid under controlled-environment conditions

Biofungicide treatment (soil drench)

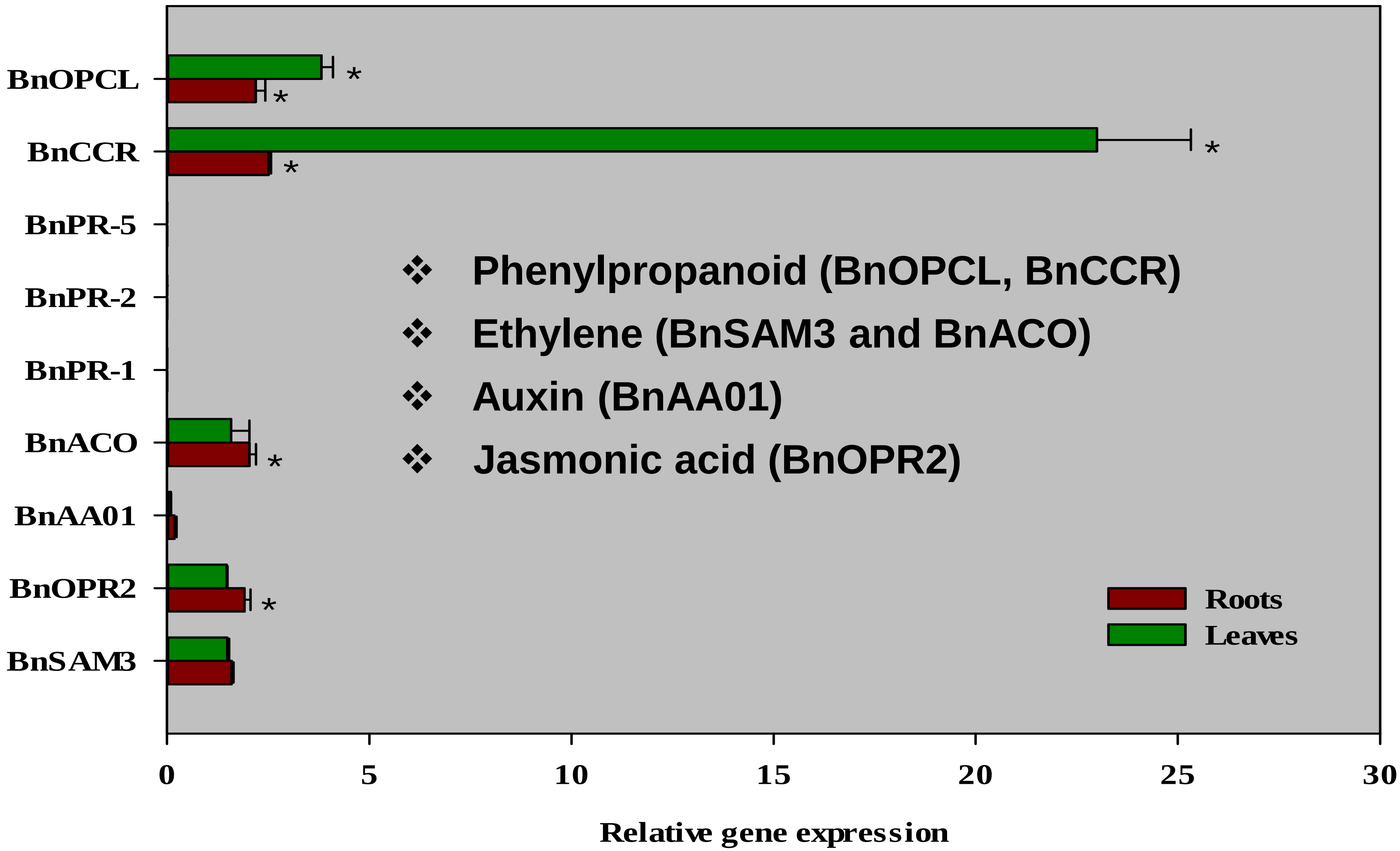


Modes of action for biofungicides

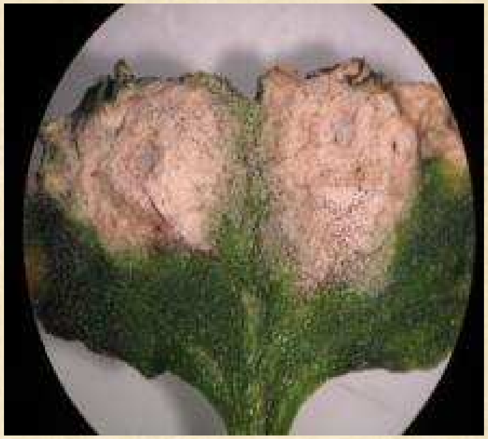


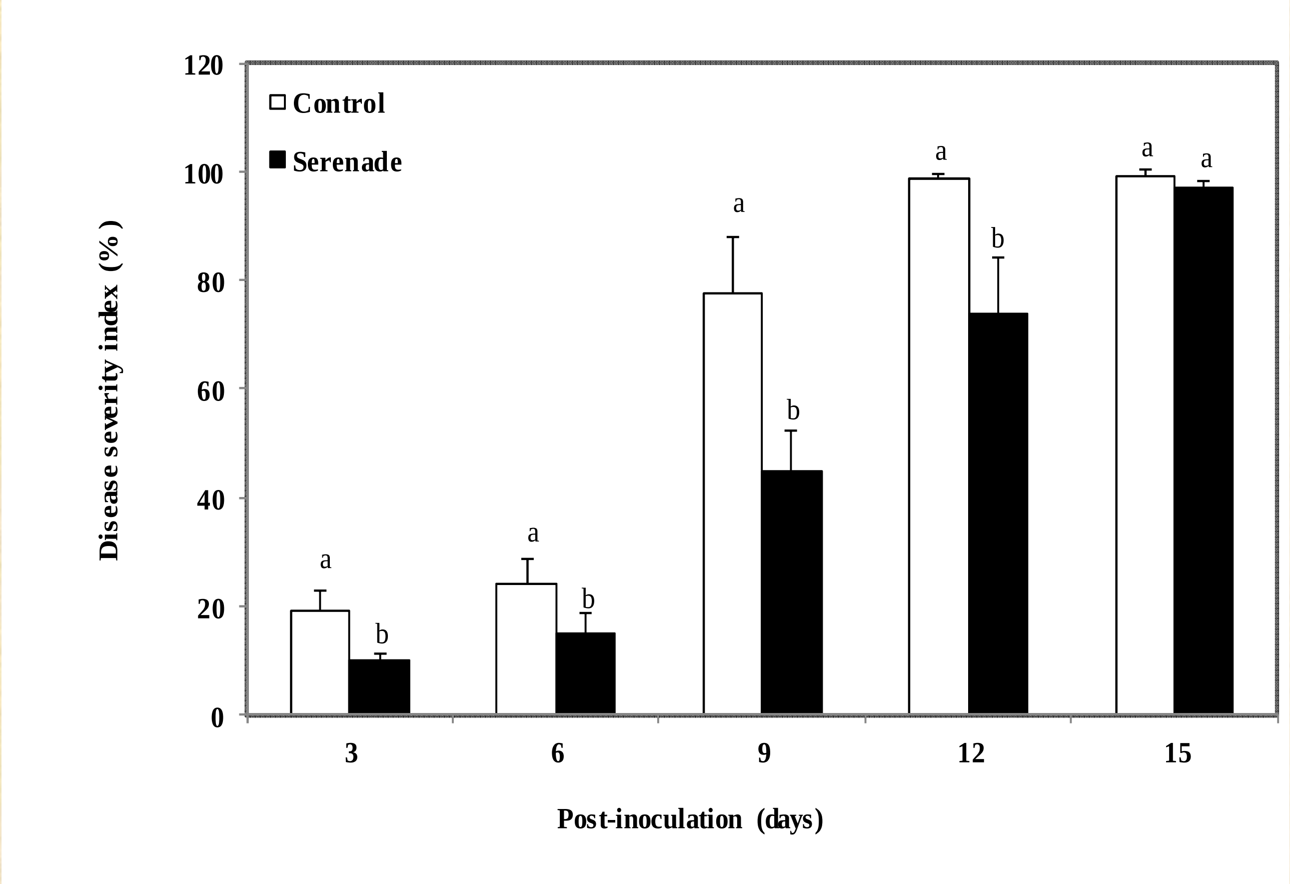


Up regulation: Phenylpropanoid (phenylalanine ammonia lyase- PAL), jasmonic-acid & ethylene pathways



Defense responses were also induced in canola leaves where the infection by *Leptosphaeria maculans* was delayed for 12 days





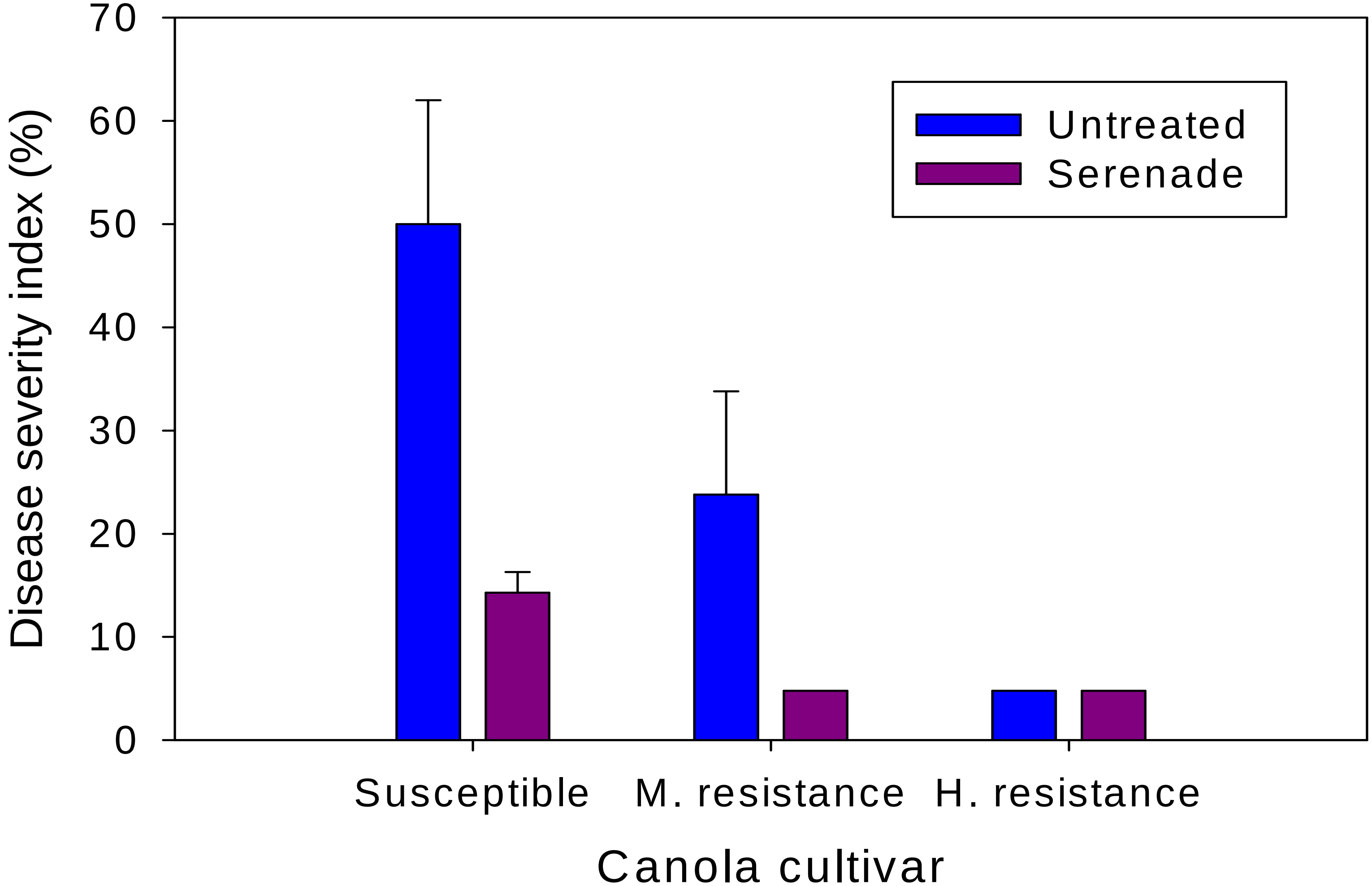
Field application of fungicides/biofungicides

- Liquid formulation
- in-furrow
- 500 L/ha

Poor efficacy against clubroot on canola

Biofungicide x cultivar resistance (n=8)

In controlled conditions



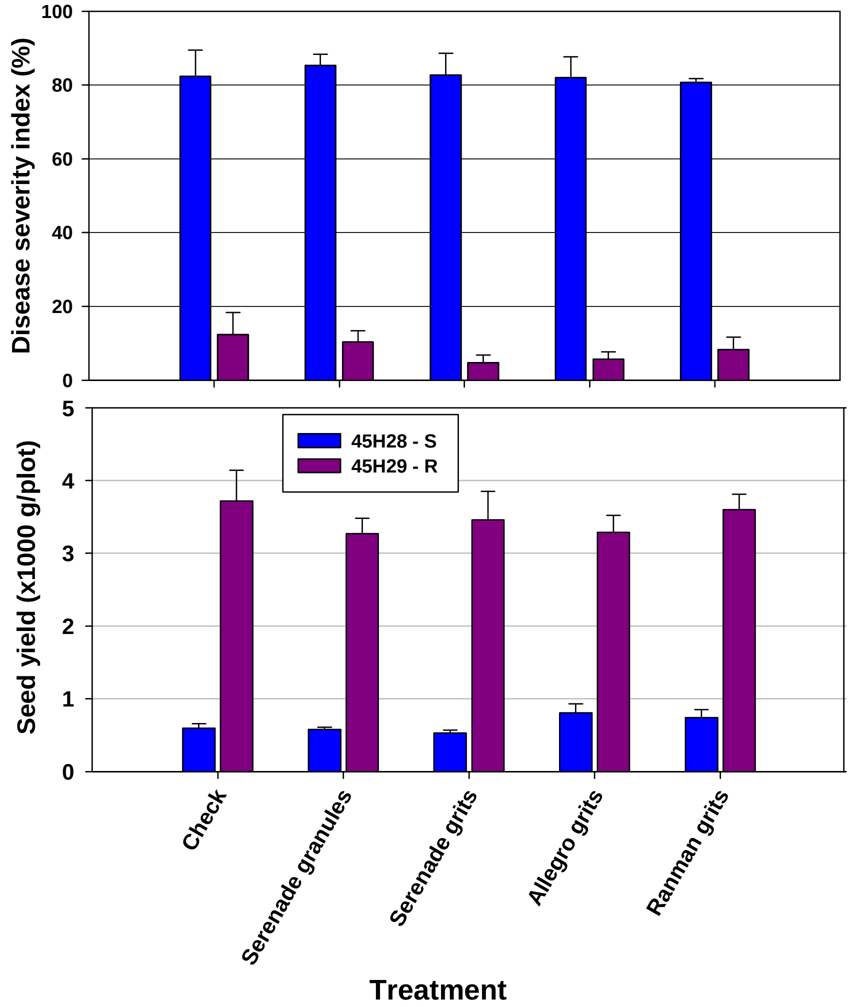
Granular formulation of *Bacillus subtilis*

- Deliver maximum amounts of *Bacillus subtilis* “spores” (50 kg formulation/ha)
- Ease of application (with seeding)
- Cost effectiveness

I. Fungicide/biofungicide formulation x resistance (Leduc & Edmonton, AB; Normandin, QC)

- ❖ Cultivar resistance was highly effective: Clubroot severity was reduced and yield increased
- ❖ None of the fungicide or biofungicide treatments was effective, and there was no treatment by cultivar interaction
- ❖ The same trend was with all three trials

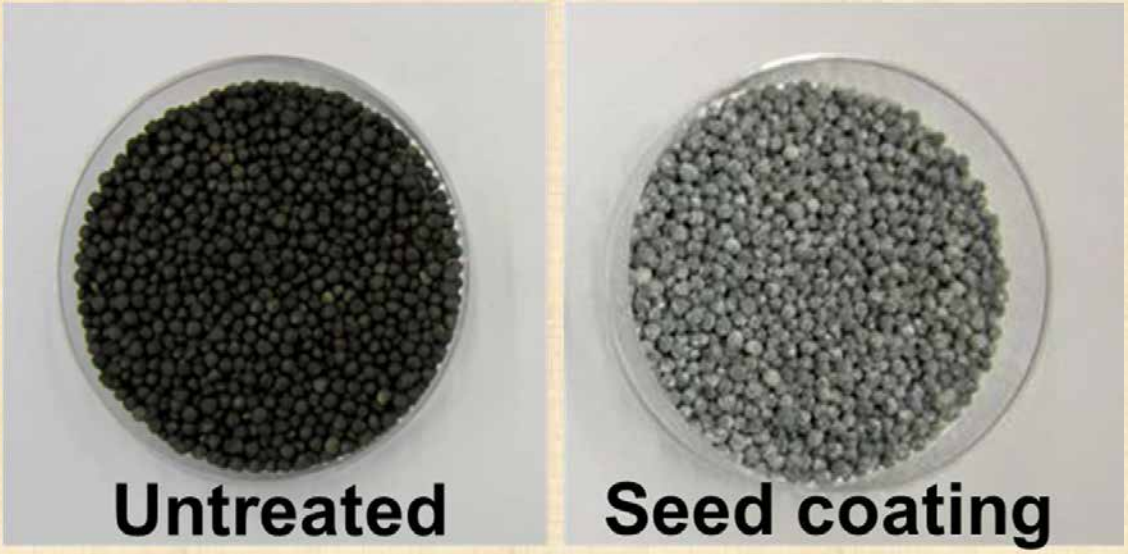
Leduc, AB (2011)



Seeding date: May 28, 2011

Biofungicide seed treatment

- ❖ Seed dressing with the ***Bacillus subtilis*** biofungicide
- ❖ Moderately suppressive to clubroot at low pathogen inoculum pressure (not a stand-alone option)
- ❖ Using the commercial seed treatment formulation L1782
- ❖ Low to very high titre at 4 equal increment rates (1×10^5 to 5×10^6 cfu/seed)



II. Crop rotation x biofungicide

Crop rotations:

1. *Canola-barley-canola* (1-year break)
2. *Canola-barley-field pea-barley-canola* (3-year break)
3. *Continuous barley* (11-year break, for comparison only)

Biofungicide (*B. subtilis*) seed treatment

At low, medium, high, and very high rates to a susceptible cultivar

Assessment:

- ❖ Impact of crop rotation on resting spores in soil –Bioassay, qPCR
- ❖ Soil test/fertilization, seedling counts, flea beetle control
- ❖ Clubroot severity (0-3) at late flowering
- ❖ Impact on crop development (0-4) during ripening
- ❖ Seed yield

III. Crop rotation x cultivar resistance

Crop rotations:

1. *Continuous canola* (no break)
2. *Canola-barley-canola* (1-year break)
3. *Canola-barley-pea-canola* (2-year break)
4. *Canola-barley-pea-barely-canola* (3-year break)
5. *Canola-barley-pea-barely-fallow* (4-year break)

Canola cultivar:

1. 45H26 – susceptible (S)
2. 45H29 – resistant (R)
3. InVigor 5030 – moderately resistant (MR/MS)

Assessment:

- ❖ *B. brassicae* inoculum in soil – qPCR (direct quantification)
- ❖ Soil test/fertilization, seedling counts, flea beetle control
- ❖ Clubroot severity(0-3), crop impact (0-4), and seed yield

Results

I. Effect of crop rotation on *P. brassicae* inoculum in soil

a. Bioassay of soil samples
b. Early pathogen development in roots (qPCR, 2011)[a]

Crop rotation	**Bioassay**	**qPCR (ng/g fresh root)**	
(Years of break)	(DSI%)	Field trial 1	Field trial 2
1 year	74.8 a	11.6 a	> 100 a
3 years	47.0 b	7.3 b	8.4 b
11 years	28.3 c	8.7 b	3.2 c

[a] Soil samples were taken prior to trials and root samples were from non-treated control plots 4 weeks after seeding

Both methods were indirect

Direct estimate of *P. brassicae* resting spores in soil using qPCR (2012)

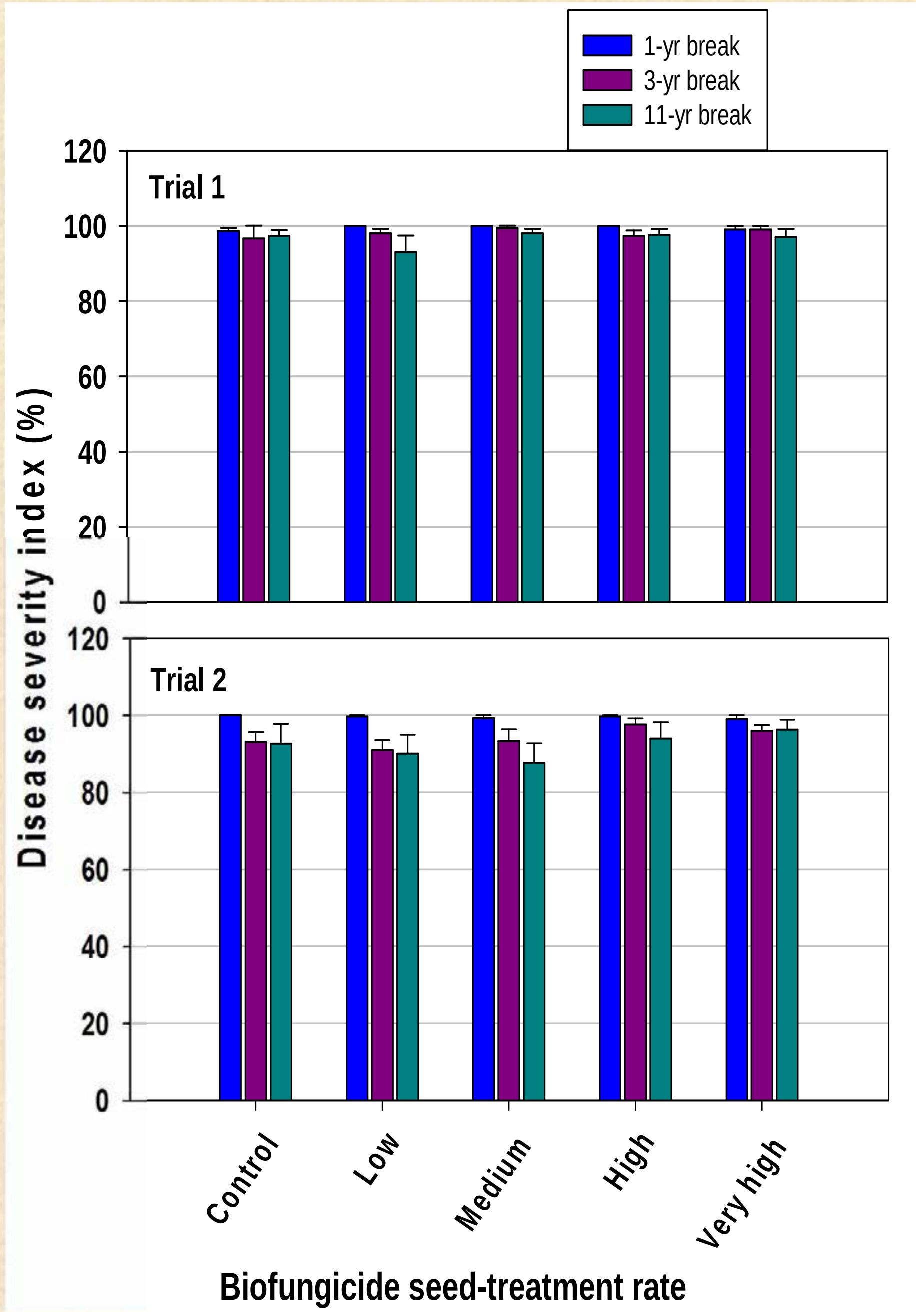
A break from canola (year)	**Resting spores /g soil** [a]
0	2.7×10^6 bc
1	2.9×10^6 c
2	5.7×10^4 a
3	2.1×10^5 ab
4	1.1×10^5 ab

[a] Based on 8 replicated blocks of each rotation in two trials

A > 2-year break from canola reduced *P. brassicae* resting spores in the soil by at least 10 fold relative to 0- or 1-year break

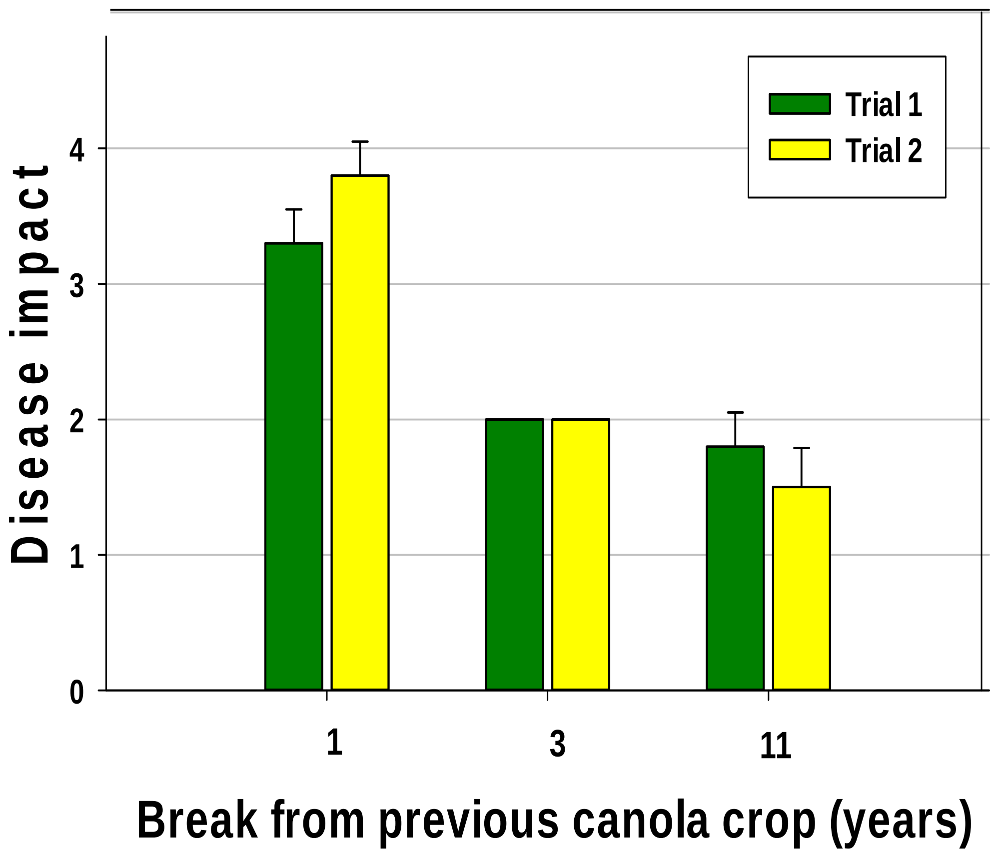
II. Crop rotation x biofungicide seed treatment

- Neither *B. subtilis* seed dressing (regardless of the rate) nor the crop rotation reduced clubroot severity substantially
- In longer rotation plots, however, the galls were slightly smaller



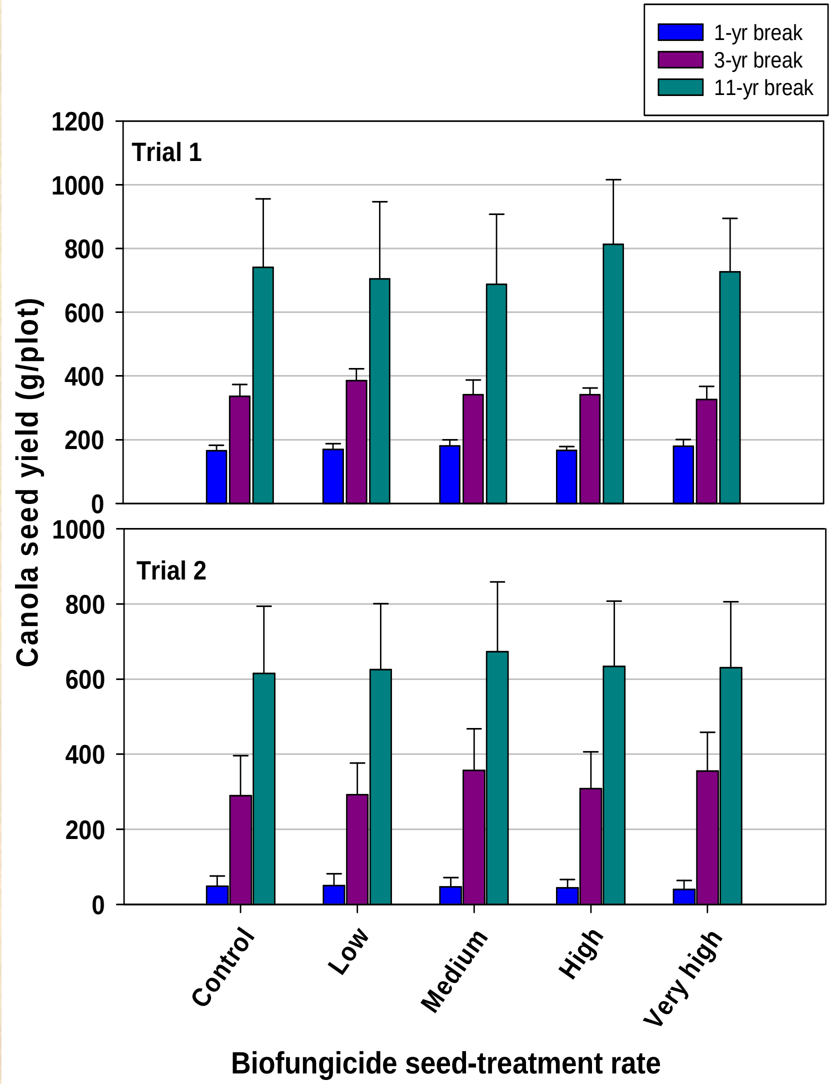
Clubroot impact on crop development

- *B. subtilis* seed dressing had no effect
- **Longer rotation reduced clubroot impact**
  (pooled data over all seed treatment rates)



Canola seed yield

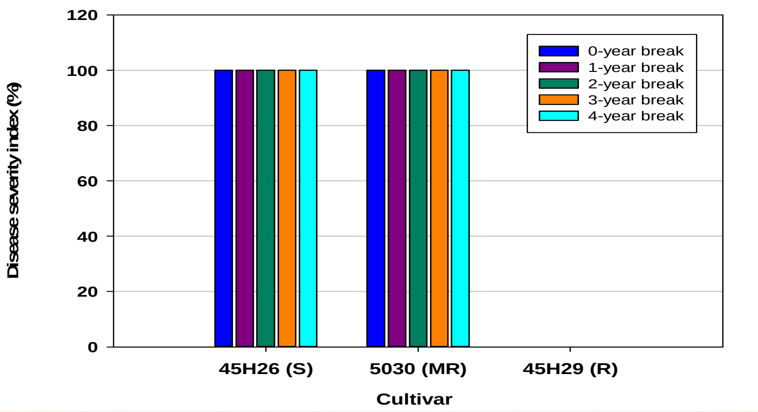
- Biofungicide seed treatment showed no effect
- A >3-yr break from canola had higher yields for S cv.
- Overall, the yield was poor (<1 ton/ha) with S cv.
- Rotation alone was not enough to allow the S cv. to reach its yield potential



III. Crop rotation x cultivar resistance

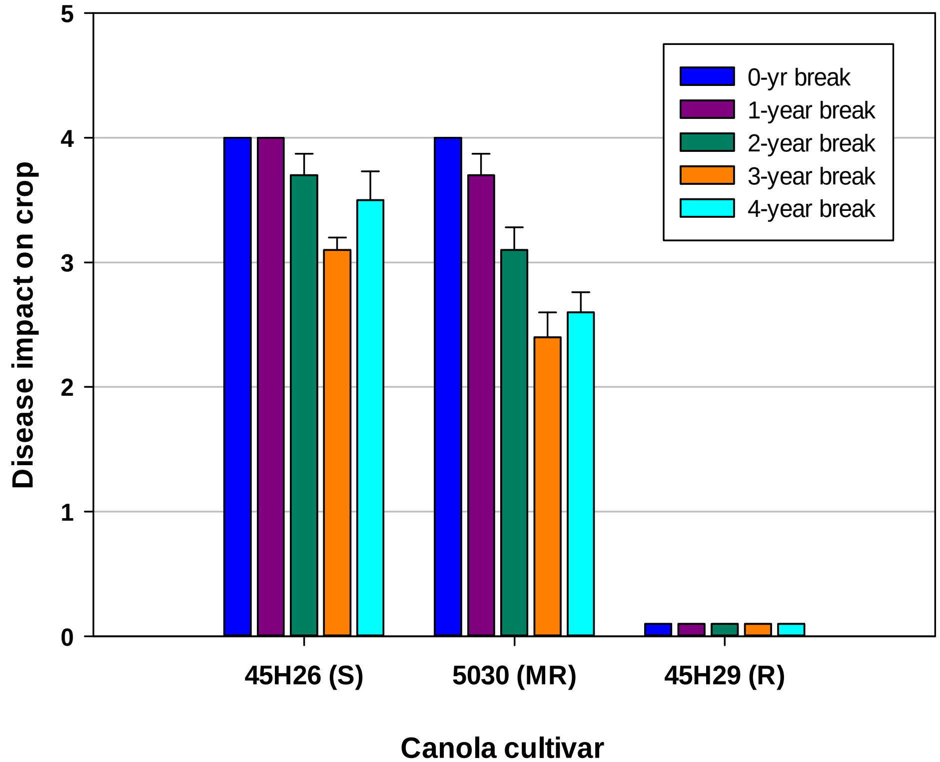
Clubroot severity at flowering was reduced by R cv. but not by crop rotation on S or MS cvs.

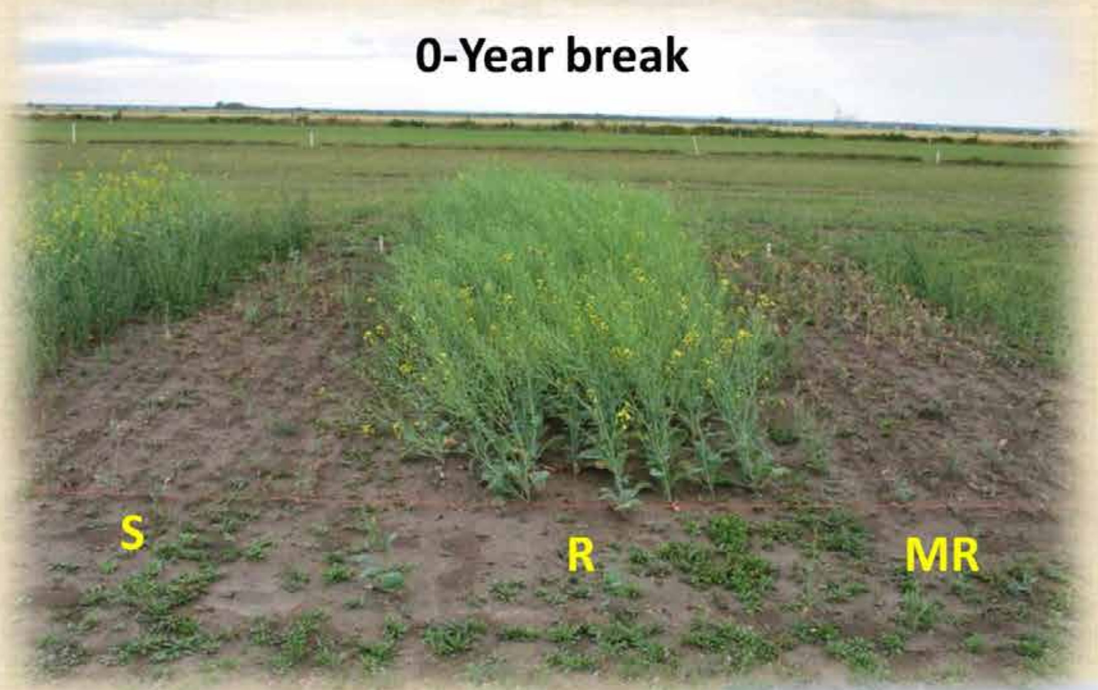
(two trials)



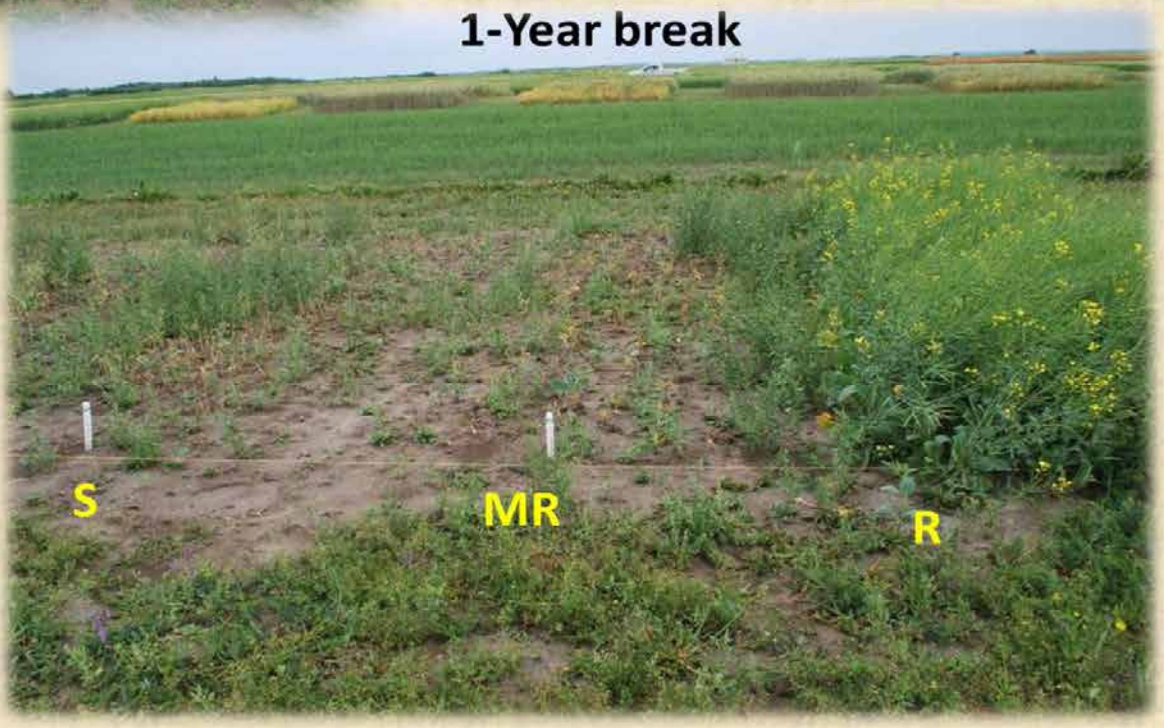
Impact on crop development: A >2-yr break from canola reduced disease impact on S and MS cvs. No effect on R cv.

(two trials)

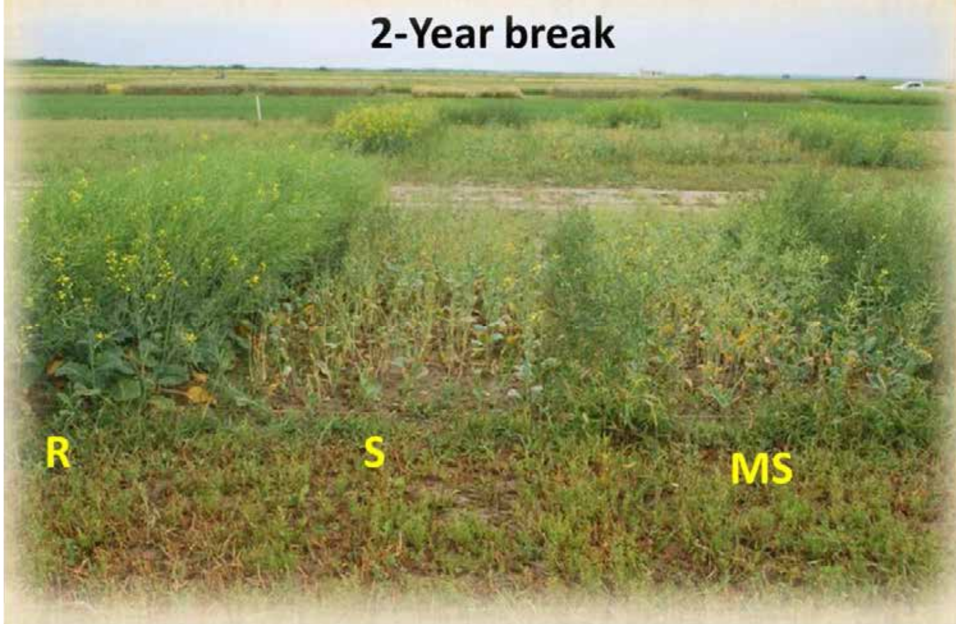




Continuous canola:
There was hardly any S and MR/MS plants left, **R looked thin**

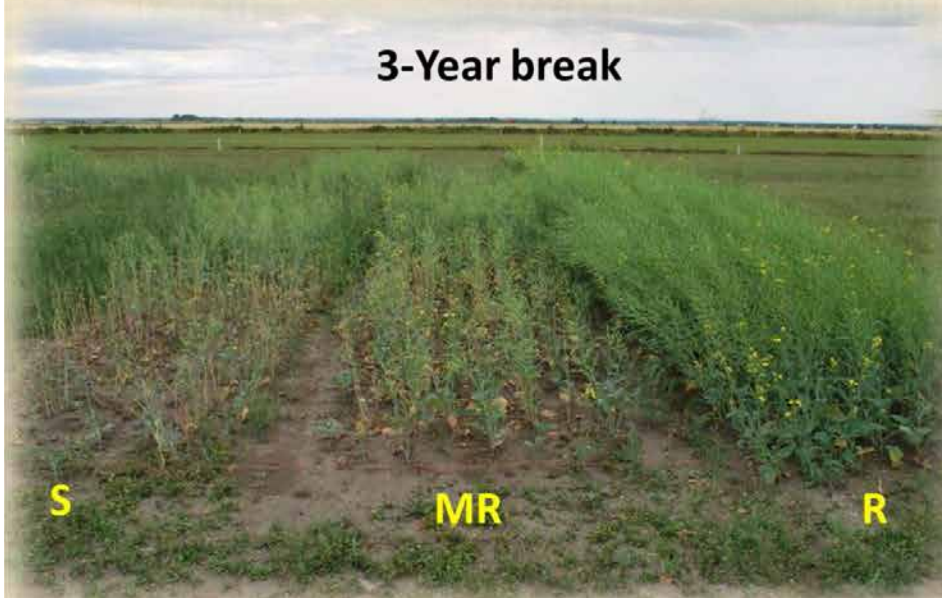


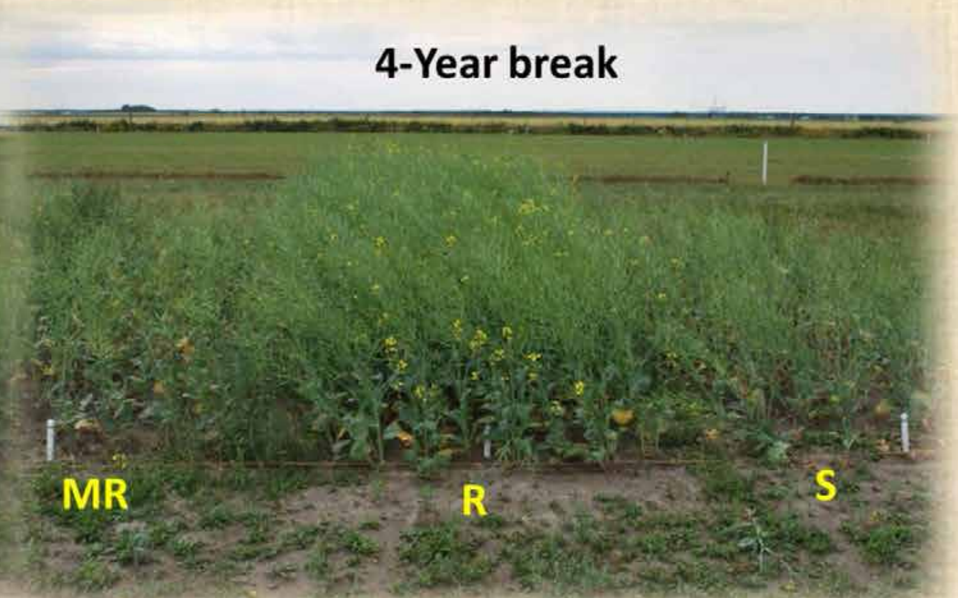
1-year break:
Not much different from 0-year break, **R also looked thin**



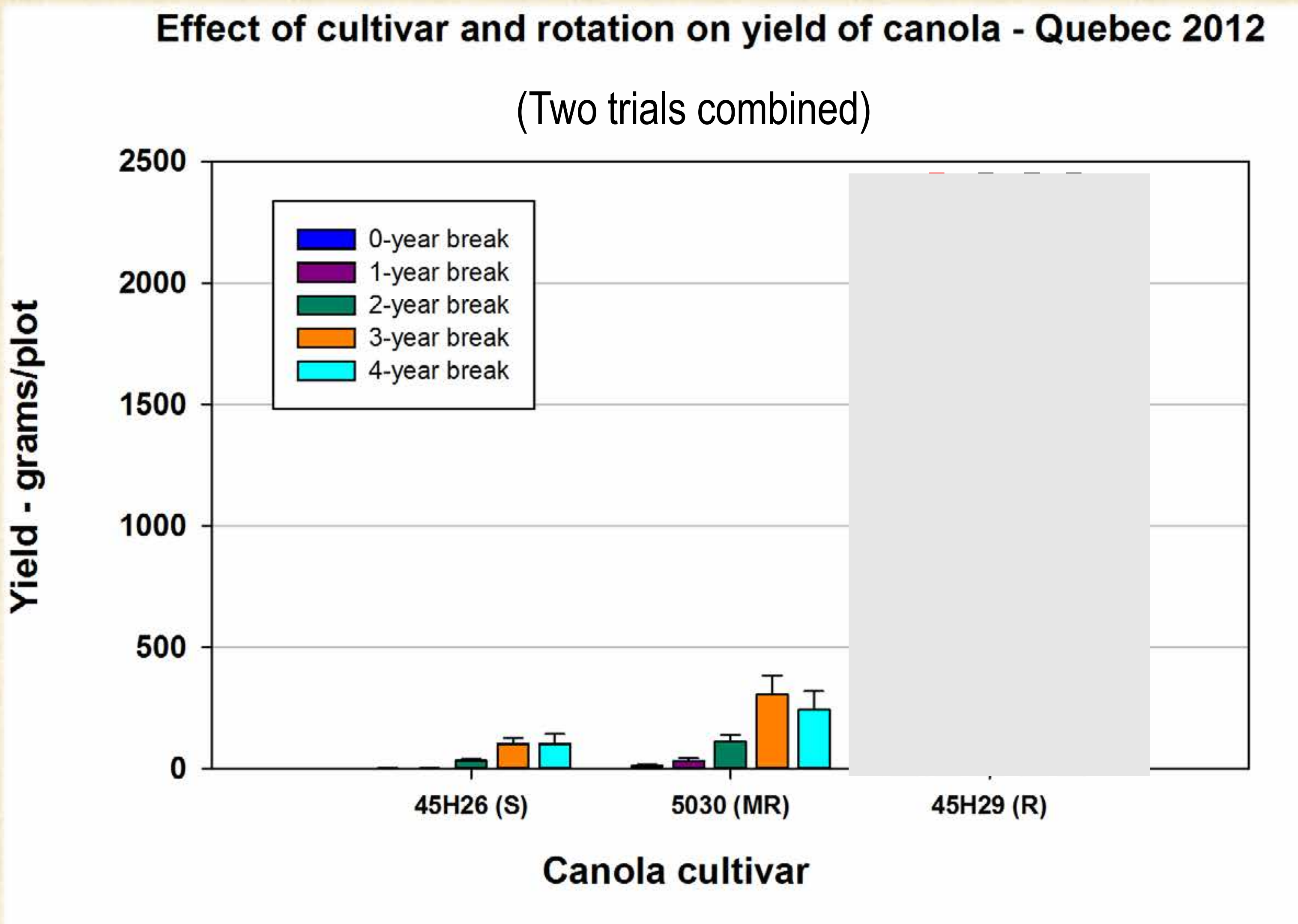
Two- to 4-year breaks:

- Gradually increased stand for S and MR/MS, but crop was still much poorer than R
- **R plots were fuller**
- Plot appearance reflected the yield





A >2-yr break showed higher yields on S and MR, but overall yields were low (>0.5 T/ha). On the R cultivar, a >2-yr break had a **25%** yield increase relative to continuous canola



Summary

- ❖ A >2-year break from canola reduced *B. brassicae* resting spores in the soil substantially
- ❖ Long rotation alone is not enough to allow a S or MS cv. to reach yield potential in heavily infested fields
- ❖ A resistant cultivar, in conjunction with a >3-year crop rotation may allow maximum yield potential in heavily infested fields, as well as reducing the pathogen inoculum loads in the soil

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