Management of Clubroot in Canola

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2012 Clubroot Summit, March 7, 2012

1. Establishment of a consortium field nursery

Establishment of a consortium field nursery in 2010-2011

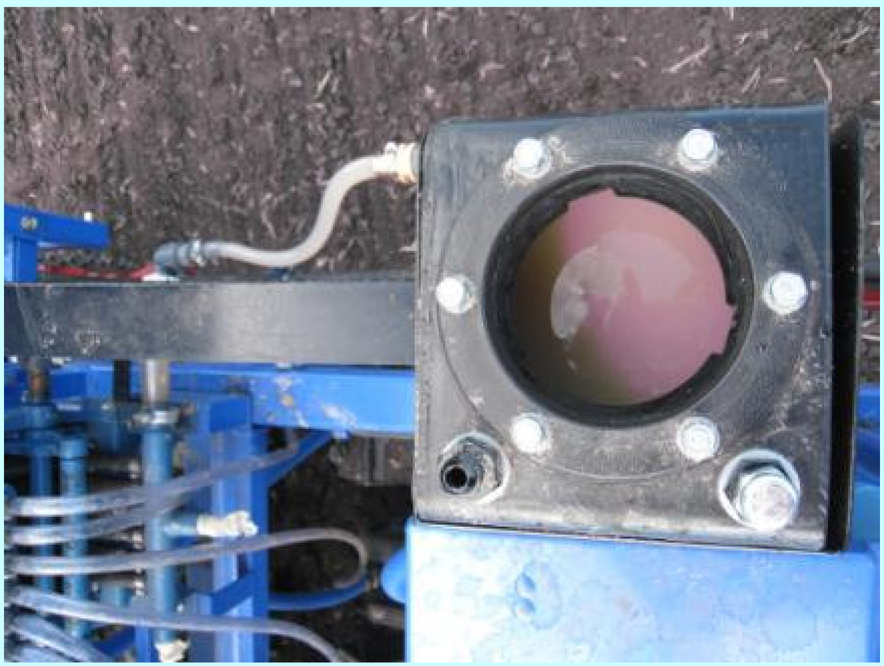
- 9.6 kg of clubroot galls were ground in a blender, the spores were suspended in water (10^8 spores/mL concentration)
- Canola (Female Parent A Sterile Seed) were planted along with clubroot spores over about 3 ha of the site in June.
- Irrigation line is in place to encourage disease development.
- 2, 4-D mixed with Roundup was sprayed in Oct. to stop the growth of the plants
- Plants were cut and worked into the soil in Nov.

Canola (Female Parent A Sterile Seed) were planted in 2010 and 2011.

George's dream comes true in 2010!

Derek's nightmare in 2007!




High-Tech harvesting in 2010

The inoculum level were tested at several sites throughout the field to ensure even distribution.

2. Industry representatives visited the nursery at various times throughout the summer.

3. Field testing – Edmonton, 2010-11

4. Soil treatments and amendments for amelioration of clubroot of canola

by

S. F. Hwang, S. E. Strelkov,

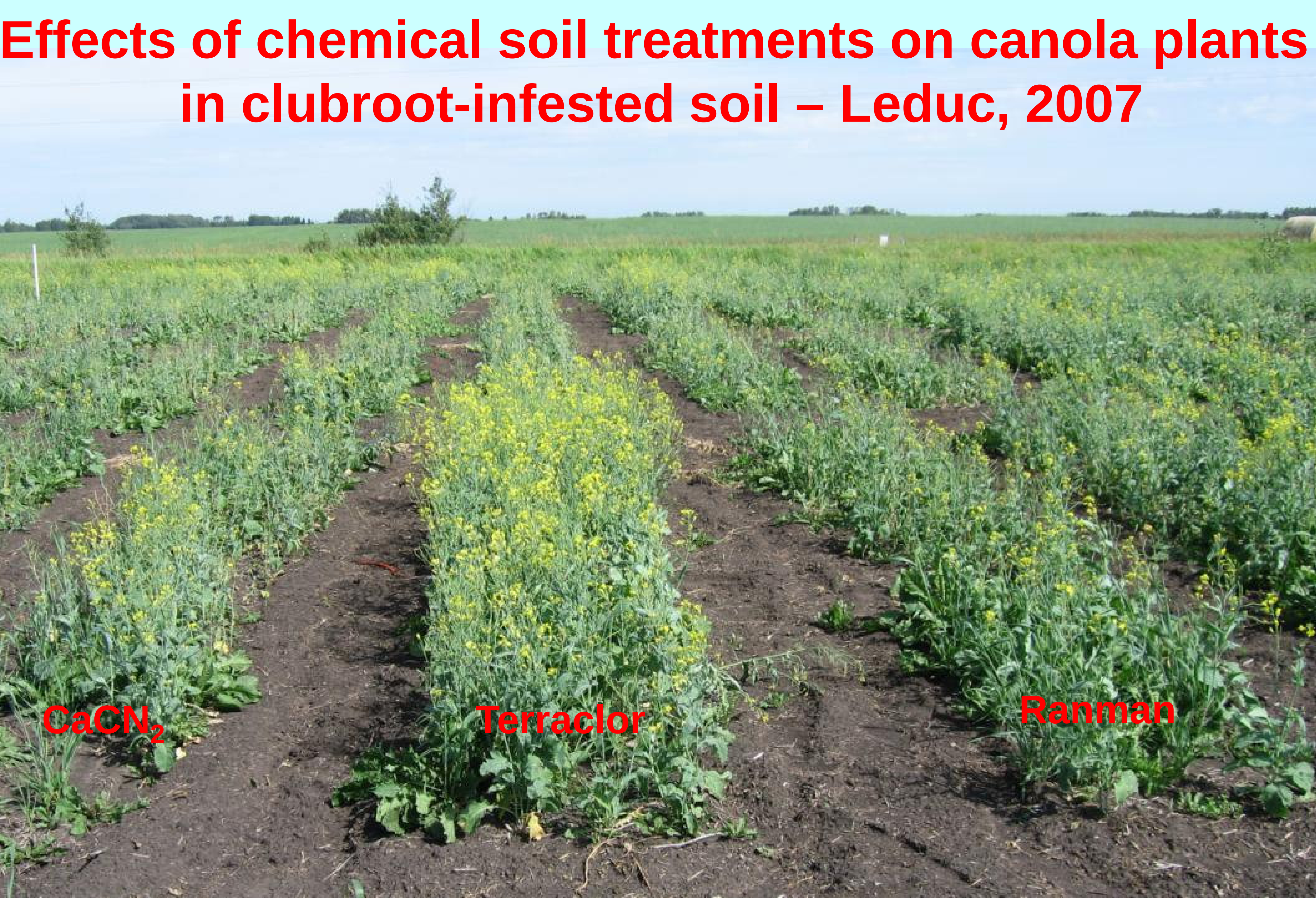
B. D. Gossen, G. D. Turnbull, H.U. Ahmed

and V.P. Manolii

Can. J. Plant Sci. (2011) 91: 999-1010.

Seeding and equipment sanitation 2007- 2008

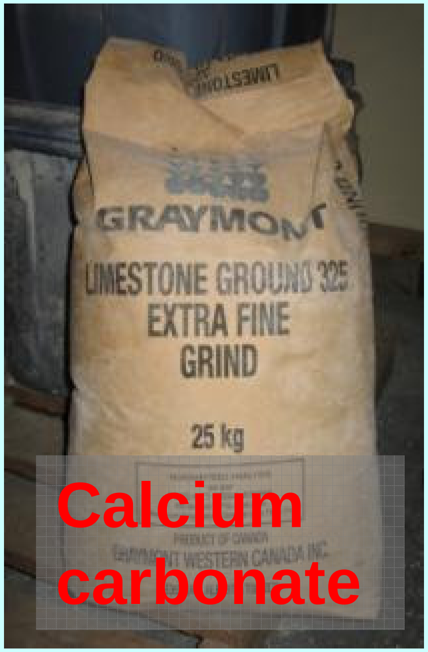
Effects of chemical soil treatments on canola plants in clubroot-infested soil – Leduc, 2007



Effects of soil amendments on canola plants in clubroot-infested soil – Leduc, 2008

Conclusions 2007-08:

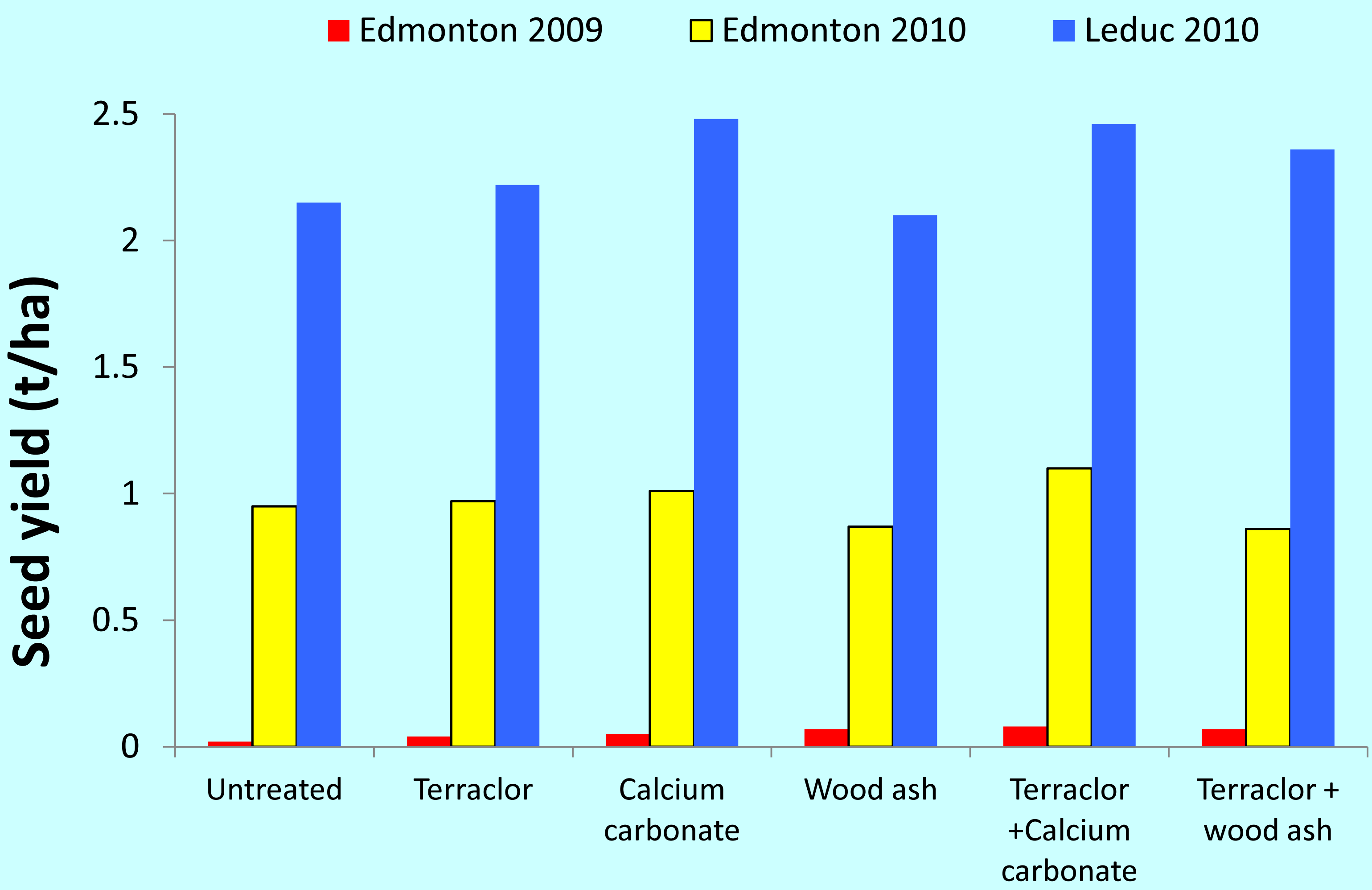
- Soil amendments such as calcium carbonate and wood ash, applied at 7.5 t/ha or more reduce the severity of clubroot and improve yield.
- As a chemical soil treatment, Terraclor applied at 90 kg/ha reduces the severity of clubroot, promotes growth, and improves yield. (At 90 kg/ha it costs $1100/ac).



2009-2010 Field Trials

- Locations: Leduc & Edmonton
- Five soil treatments applied in-row:
  - Terraclor (6.7 kg/ha)
  - Calcium Carbonate ($CaCO_3$, 67 kg/ha)
  - Wood ash (WA, 67 kg/ha)
  - Terraclor + $CaCO_3$ or WA
- Randomized Complete Block, 4 replicates

Effects of soil treatments on seed yield of canola in clubroot – infested soil

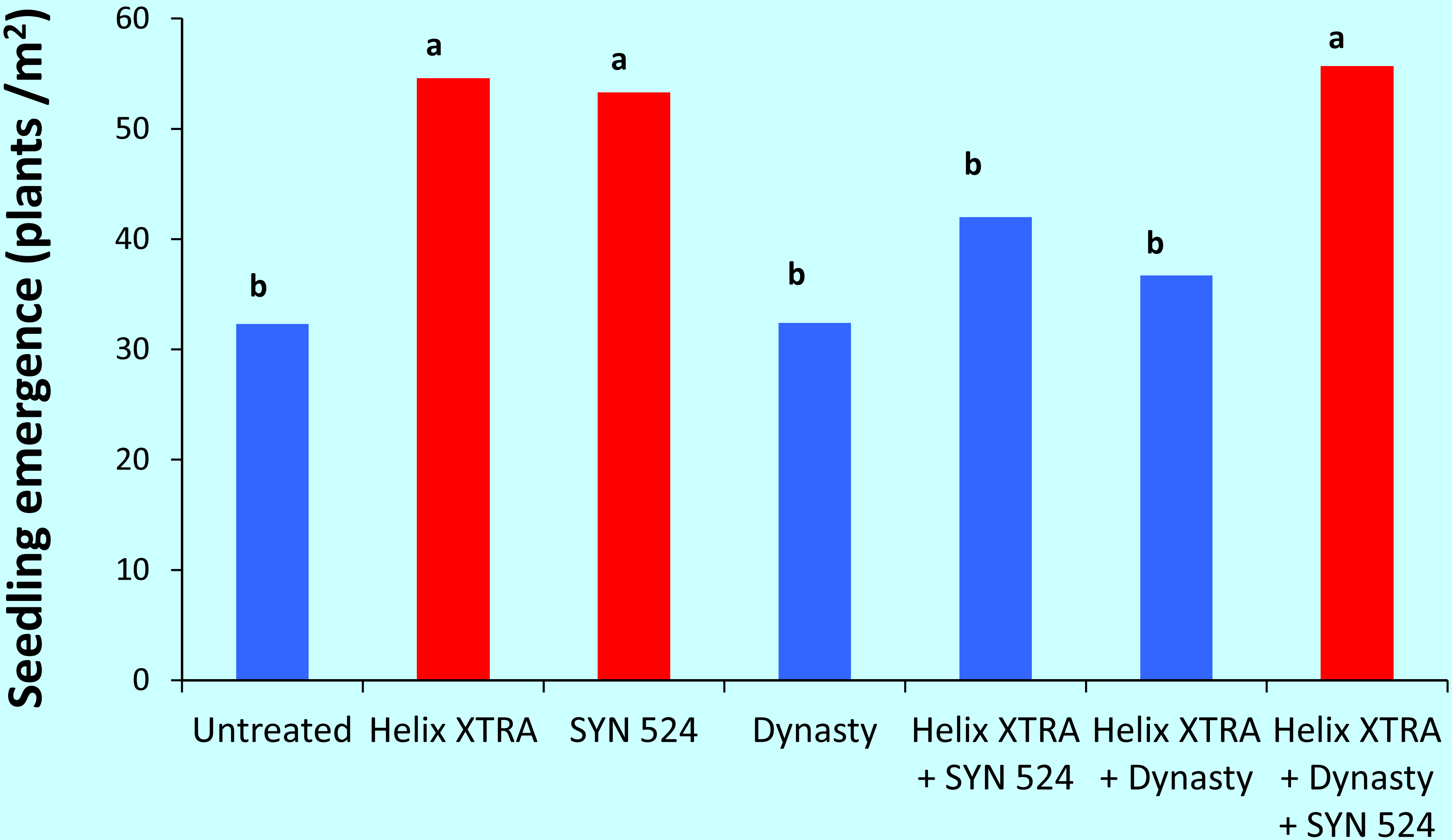


5. Effects of seed treatments on disease severity and yield of canola in clubroot infested soils 2009 – 2010

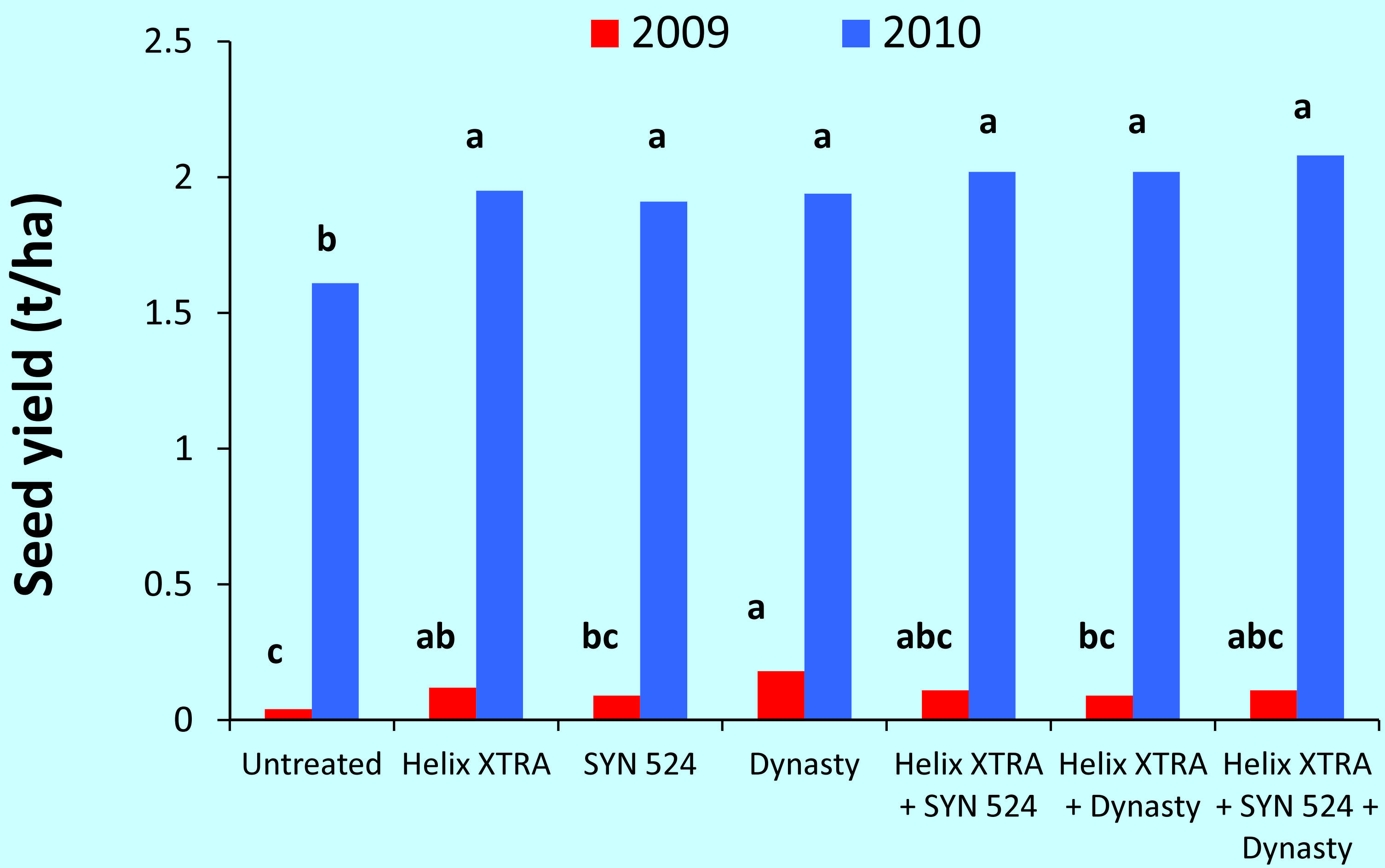
RCBD near Leduc and Edmonton

- **Helix Xtra** (difenconazole + fludioxonil)
- **SYN 524**
- **Dynasty** (azoxystrobin)
- **Helix Xtra+SYN 524**
- **Helix Xtra+Dynasty**
- **Helix Xtra+SYN 524+Dynasty**
- **Non-treated control**

Effects of seed treatments on emergence of canola in clubroot – infested soil in 2010



Effects of seed treatments on seed yield of canola in clubroot – infested soil



Conclusions:

- In-row application of lime, wood ash or Terraclor did not affect seed yield.
- Helix Xtra and Dynasty improved yield over the control in 2009.
- Helix Xtra, SYN 524 and Helix Xtra + SYN 524 +Dynasty improved emergence compared to the control
- All of the seed treatments improved yield over the control in 2010

6. Seedling age and inoculum density affect clubroot severity and seed yield in canola

by

S. F. Hwang, H. U. Ahmed, S. E. Strelkov,

B. D. Gossen, G. D. Turnbull, G. Peng

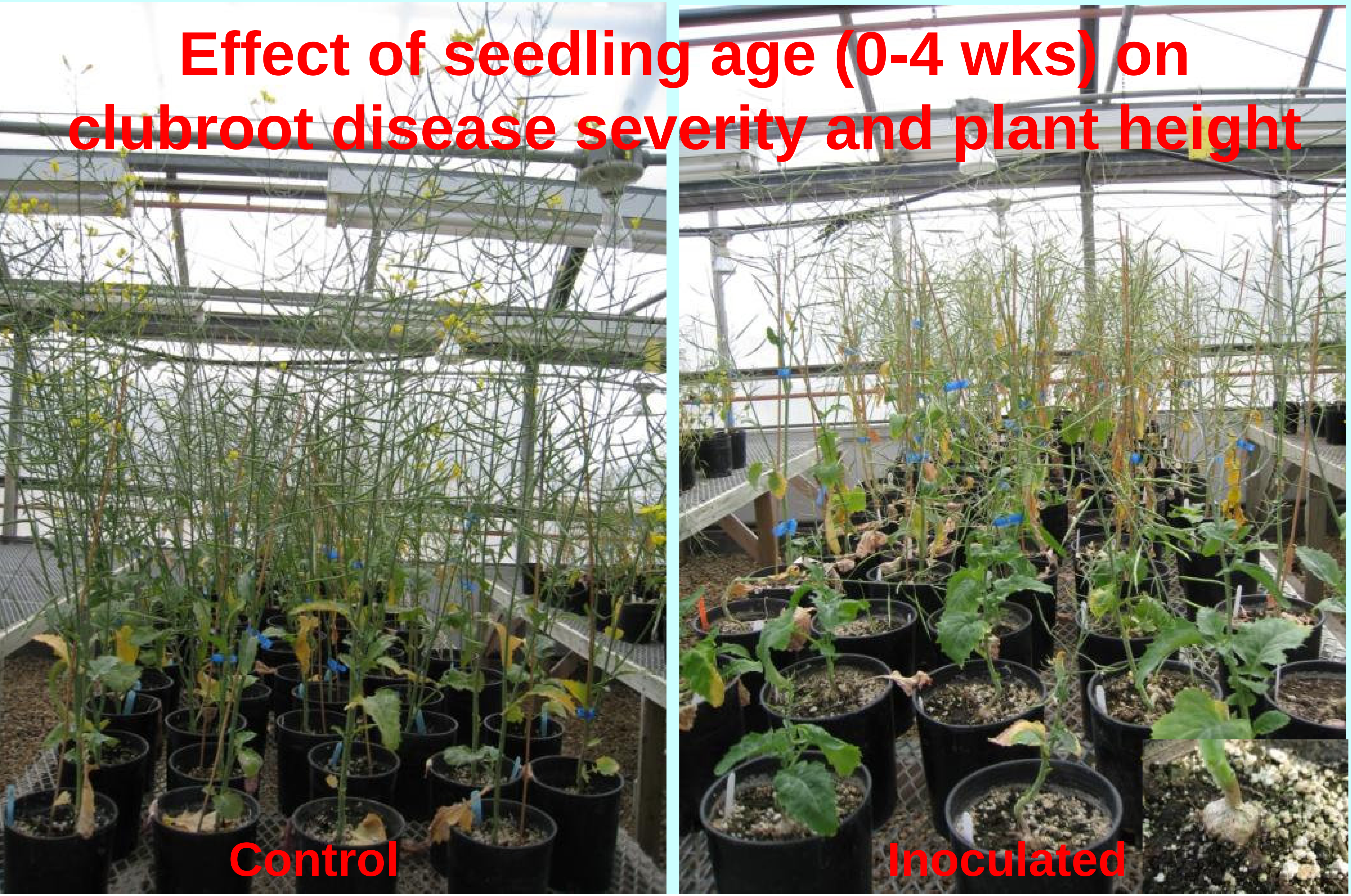
and R. J. Howard

Can. J. Plant Sci. 2011. 91: 183-190.

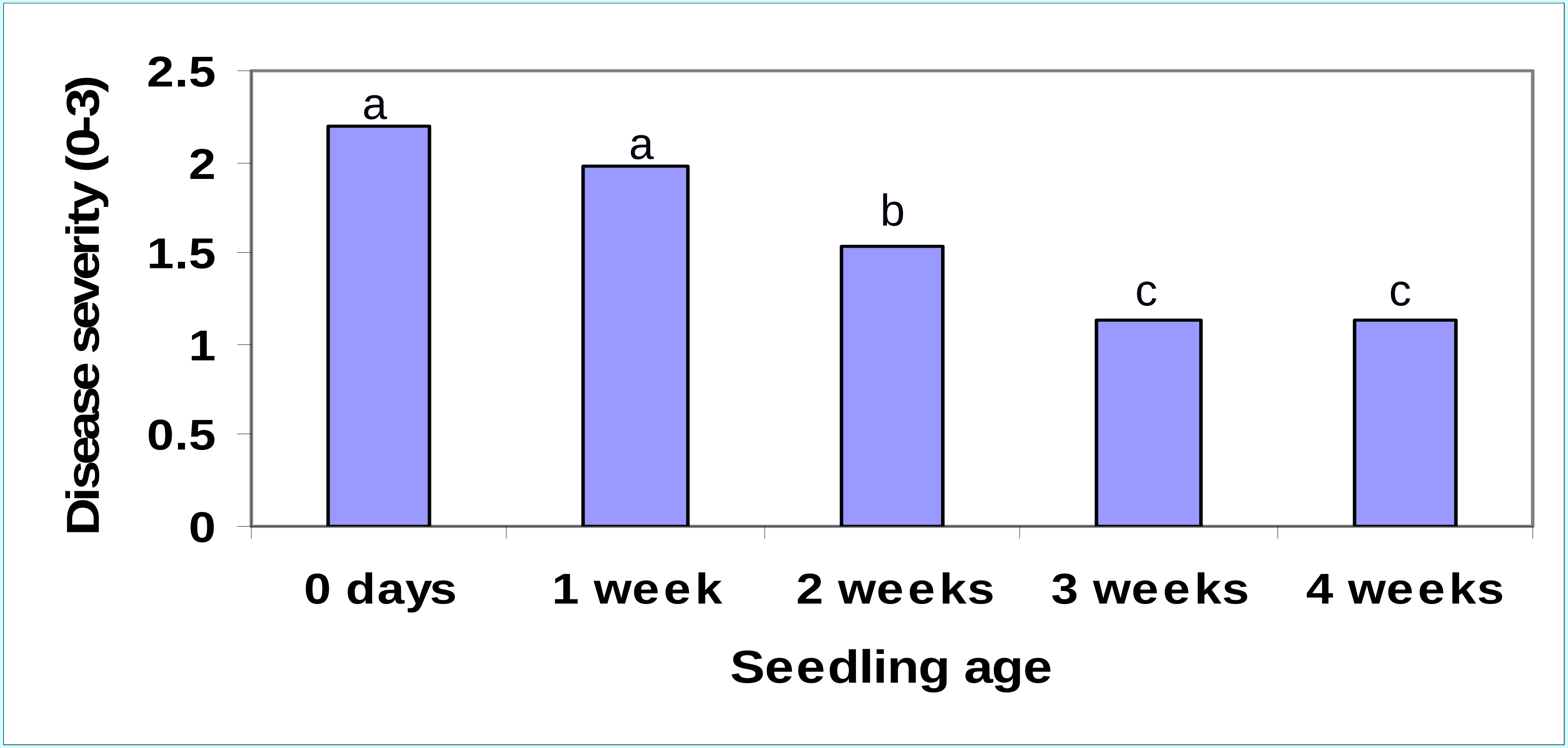
Introduction

- Plant disease development is regulated by the dynamic interaction of the host, the pathogen, and the environment.
- There is little or no data available regarding the impact of inoculum density and seedling age on clubroot disease development.

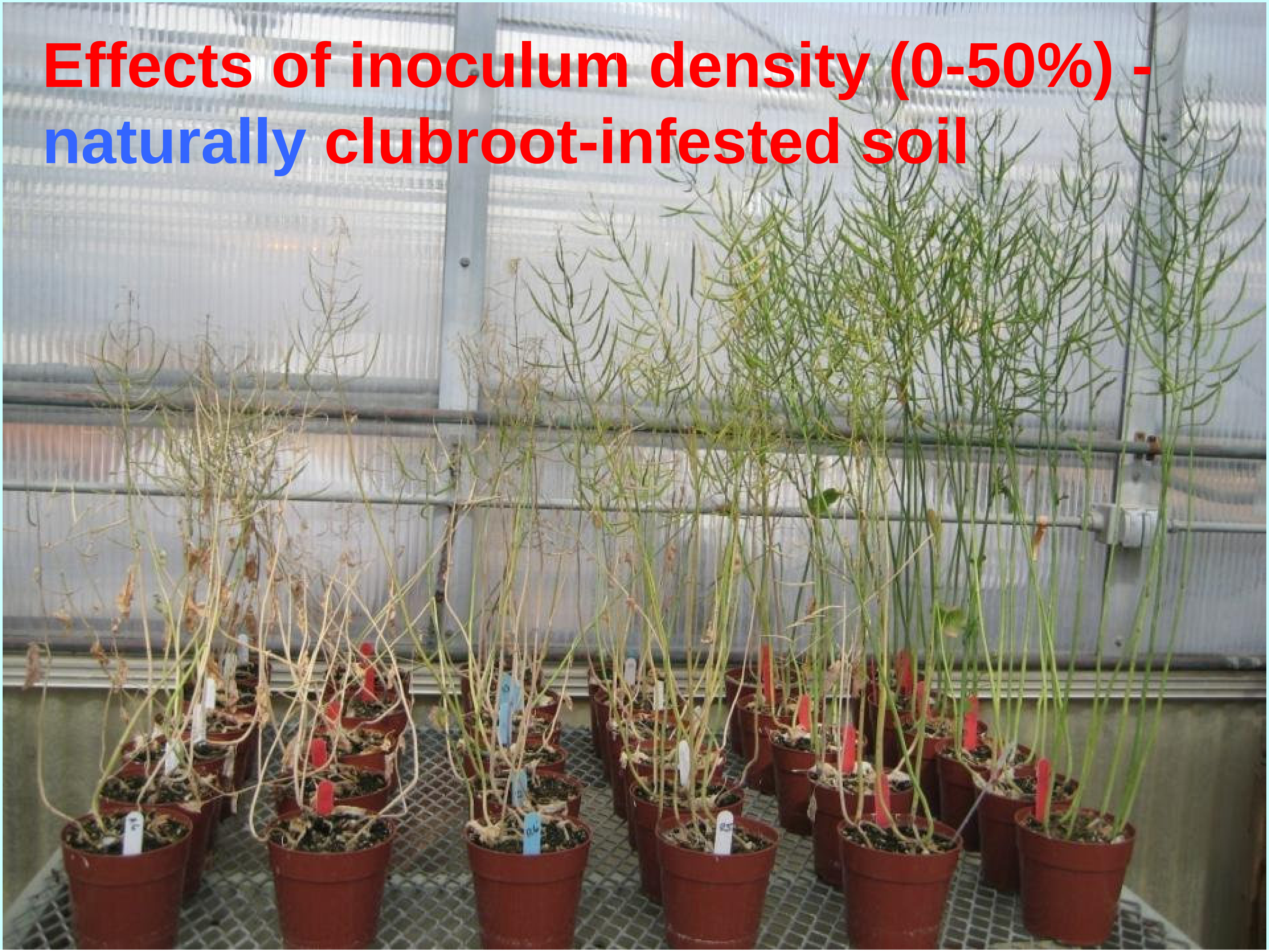
Effect of seedling age (0-4 wks) on clubroot disease severity and plant height

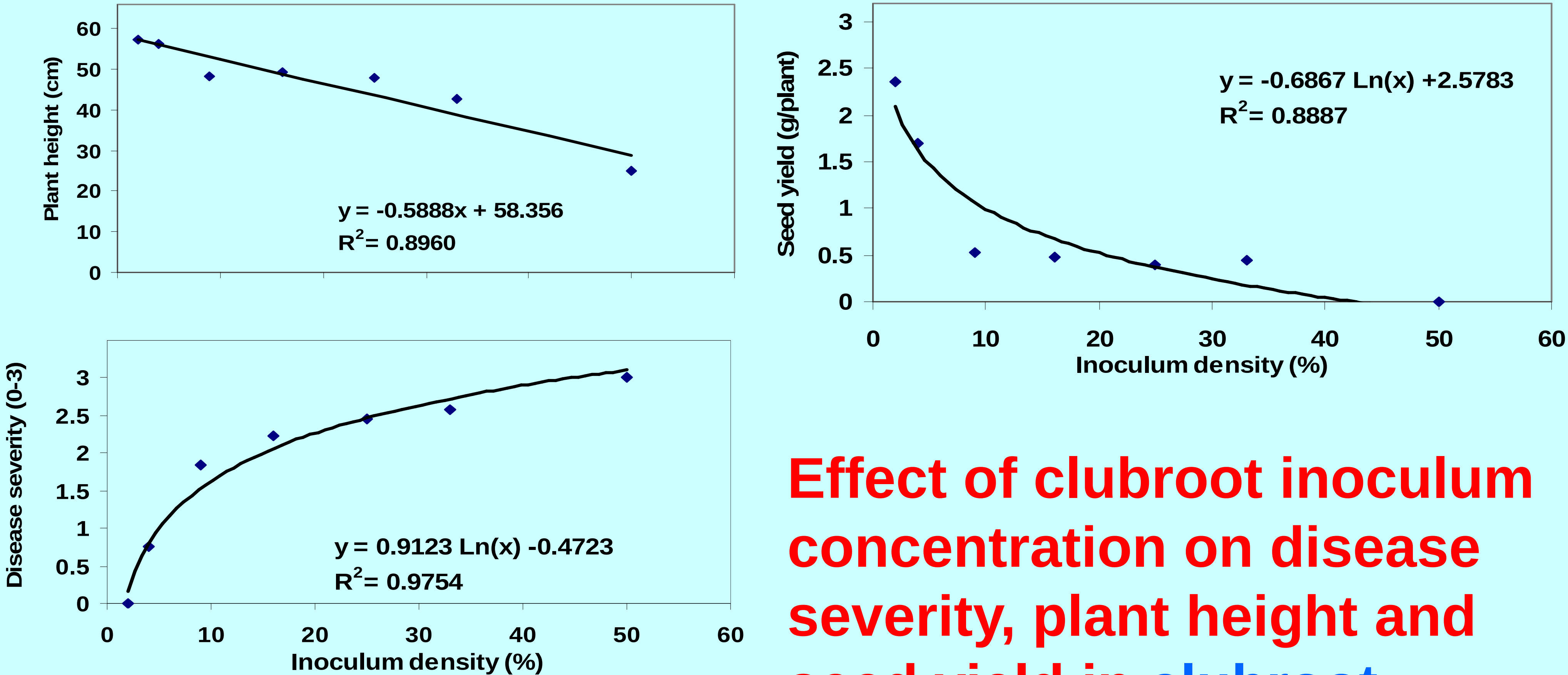


Effect of seedling age on clubroot disease severity under greenhouse conditions



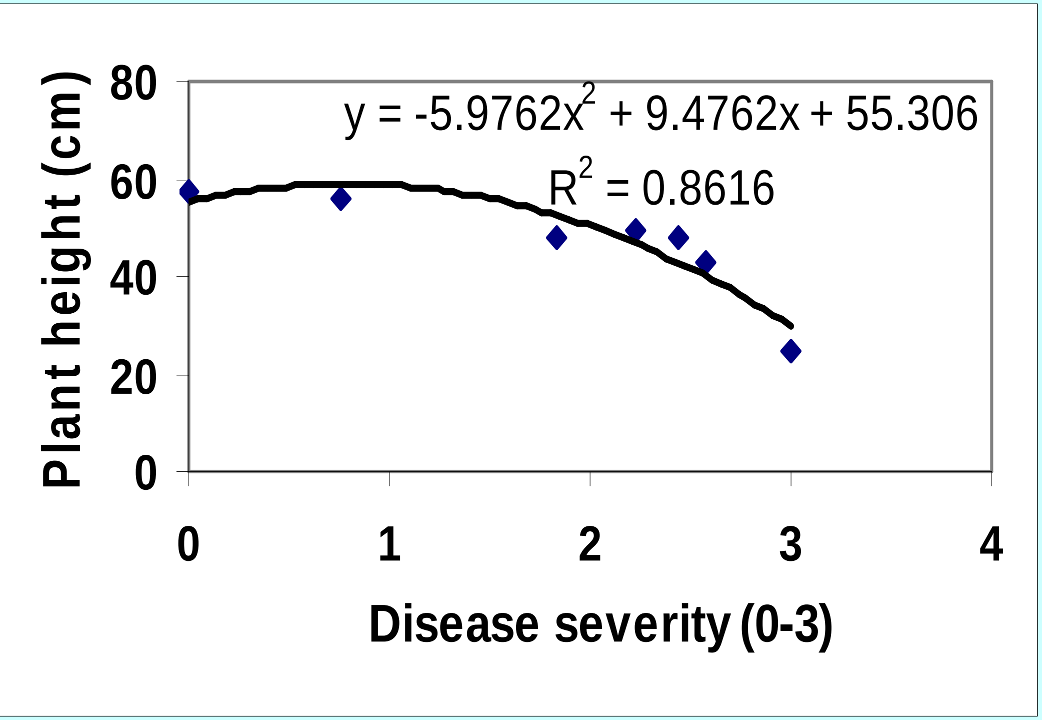
Effects of inoculum density (0-50%) - naturally clubroot-infested soil

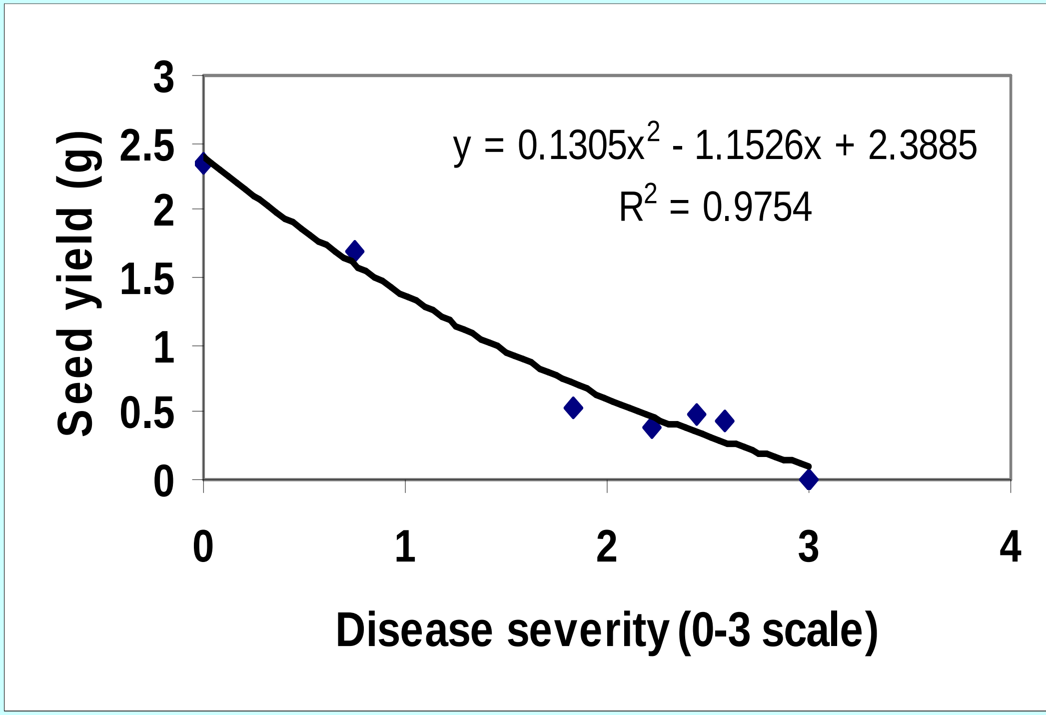




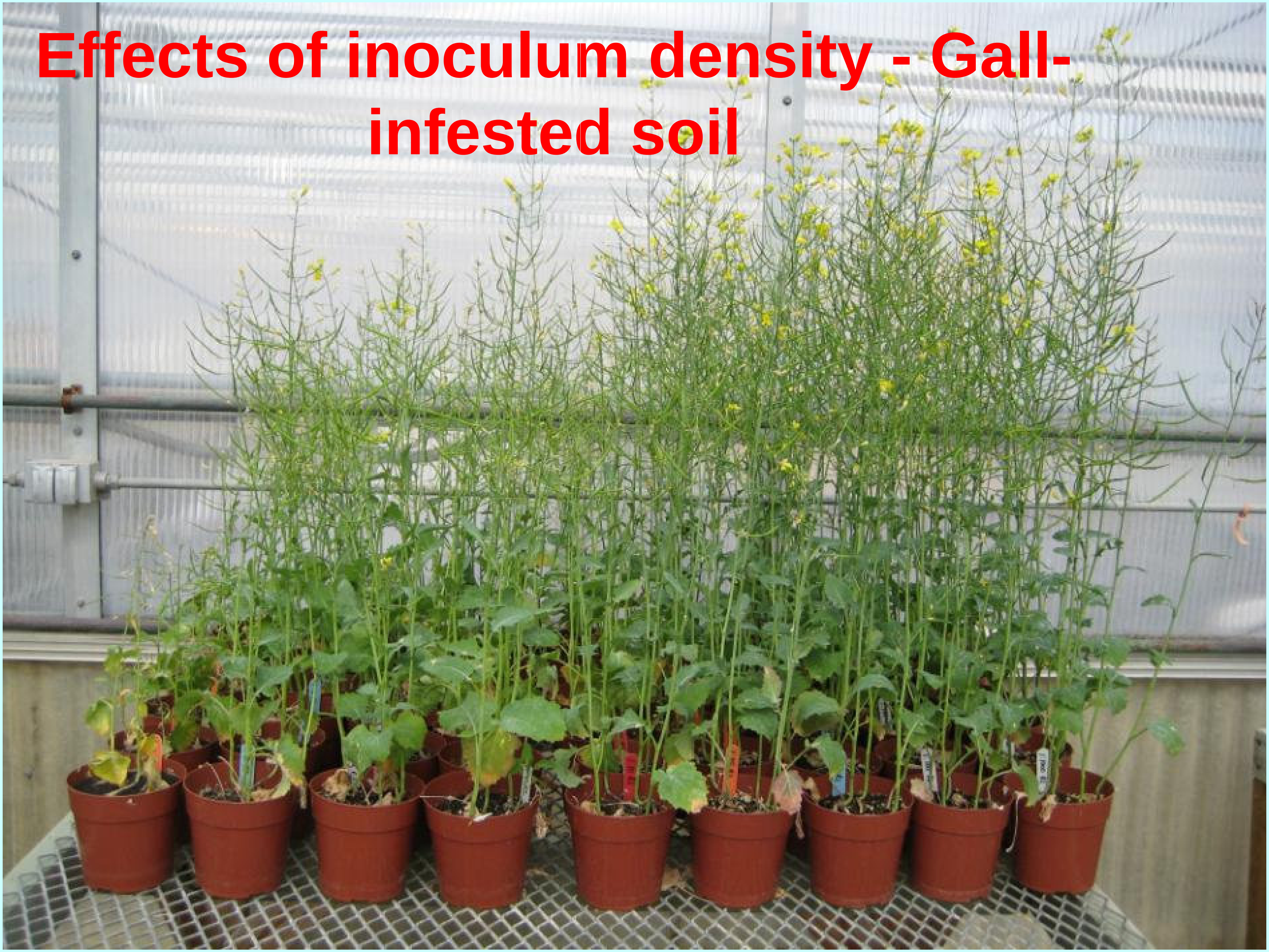
Effect of clubroot inoculum concentration on disease severity, plant height and seed yield in clubroot-infested soil (0 - 50%) under greenhouse conditions

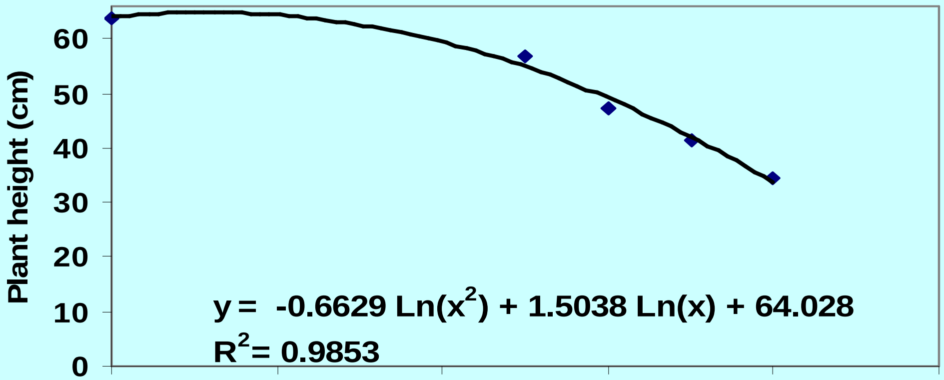
Effect of clubroot severity on plant height and seed yield of canola in clubroot-infested soil

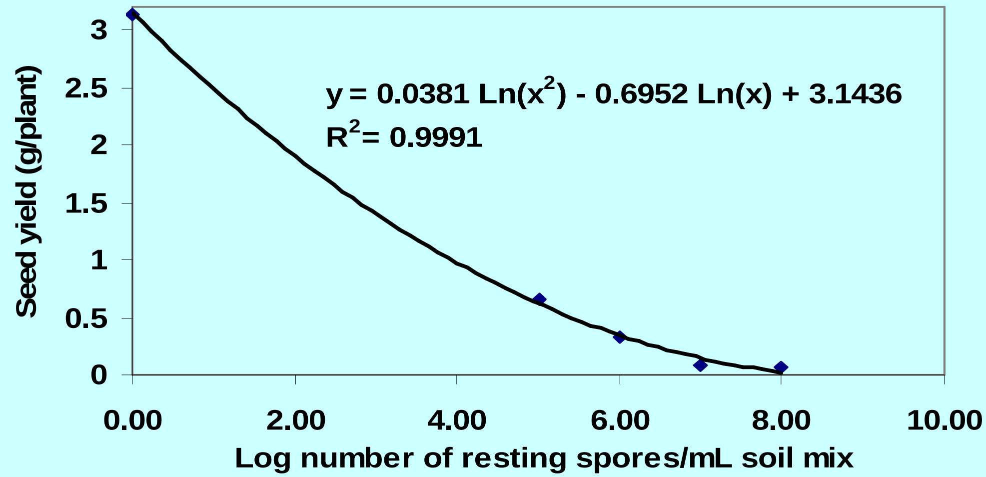


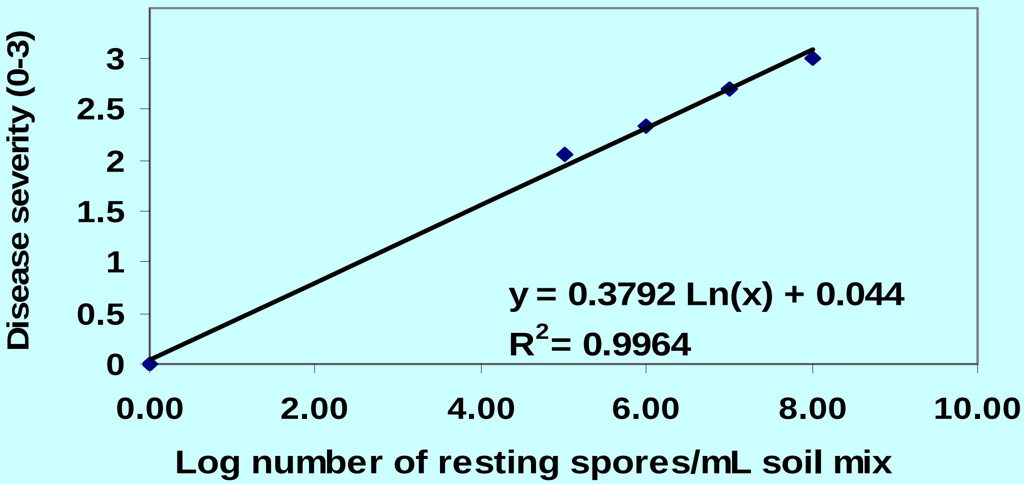


Effects of inoculum density - Gall-infested soil



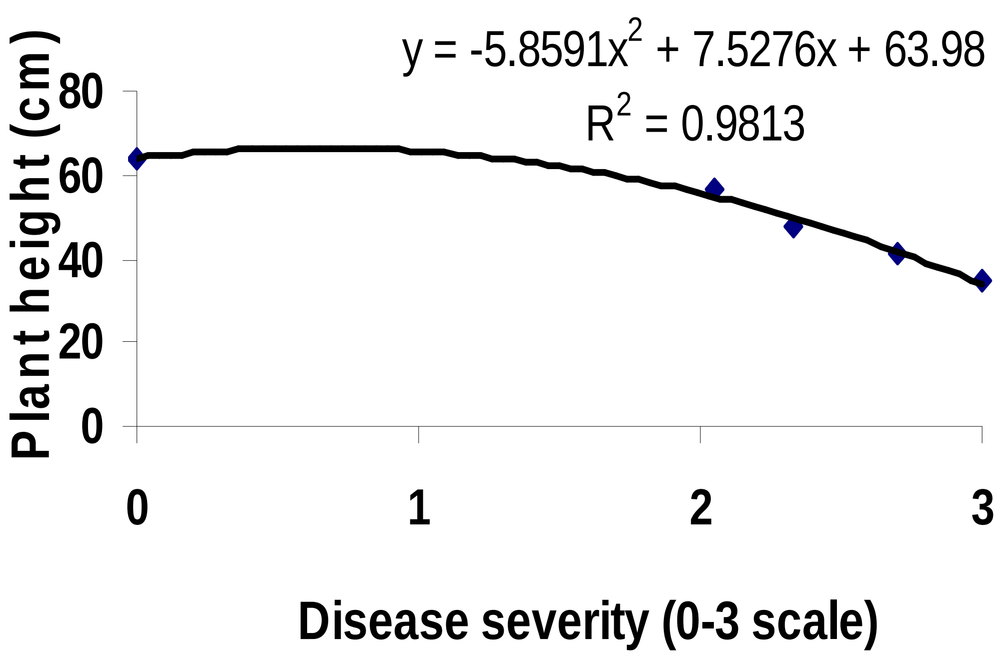


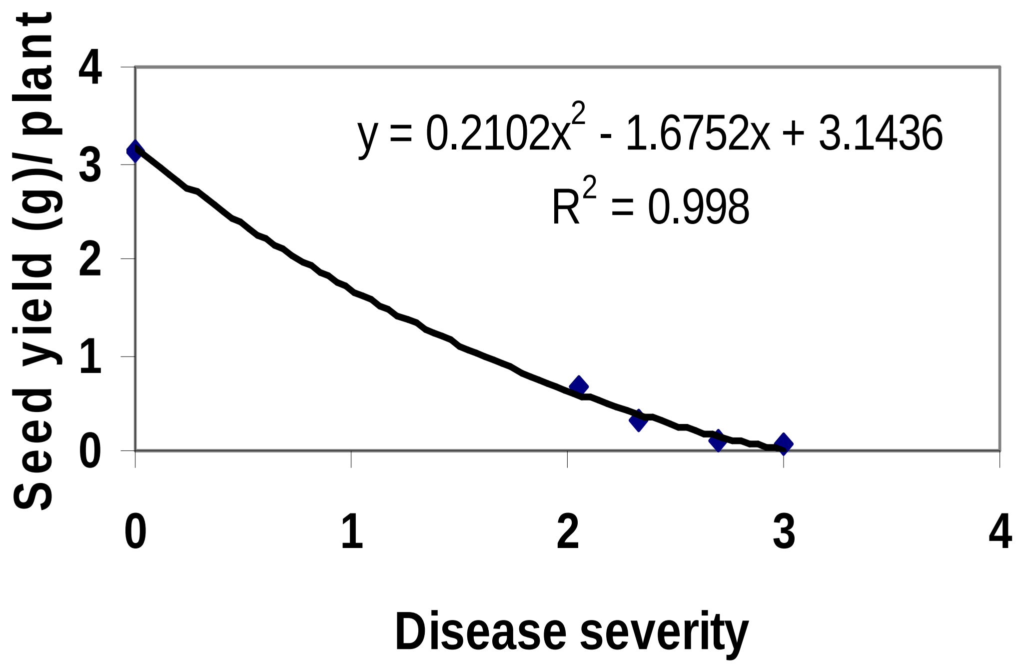




Effect of clubroot spore populations on disease severity, plant height and seed yield in gall-infested soil under greenhouse conditions

Effect of clubroot severity on plant height and seed yield of canola in soil inoculated with root galls





Conclusions

- Clubroot severity increased and plant height and seed yield decreased with increasing inoculum density.
- The young seedlings had higher clubroot severity, shorter plants and lower yield than inoculation of older seedlings.
- These results indicate that seed treatments with a long residual period (4 weeks or more) may be useful for management of clubroot.

7. Assessment of bait crops to reduce inoculum of clubroot (*Plasmodiophora brassicae*) of canola

by

H.U. Ahmed, S. F. Hwang, S. E. Strelkov,

B. D. Gossen, G. Peng,

R.J. Howard and G. D. Turnbull

Can. J. Plant Sci. (2011) 91: 545-551.

Introduction

- Use bait crop as a component of an integrated clubroot management program.
- A crop that stimulates resting spore germination could be planted and then ploughed down before the pathogen completes its life cycle, thereby reducing resting spore populations in heavily infested fields.

Conclusions

- Both host and non-host crops reduced clubroot incidence in greenhouse studies.

- Bait crops did not reduce spore populations or clubroot severity in field studies.

- Use of bait crops is unlikely to be an important component of an IPM program for clubroot of canola.

8. Infection of canola by secondary zoospores of *P. brassicae* produced on a nonhost

By

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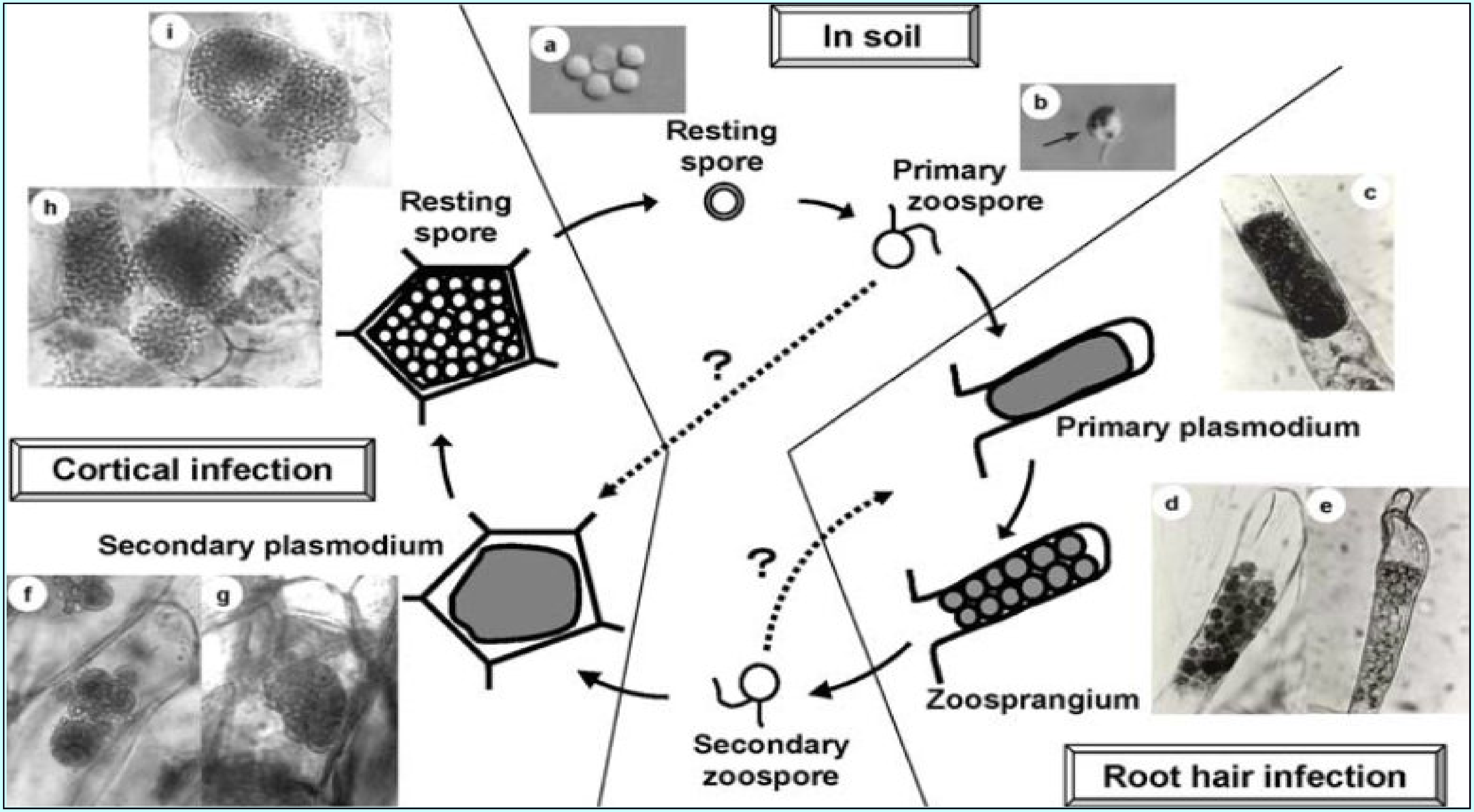
S.E. Strelkov and B.D. Gossen

Eur. J. Plant Pathol.

DOI 10.1007/s10658-011-9875-2

Causal agent: *P. brassicae*

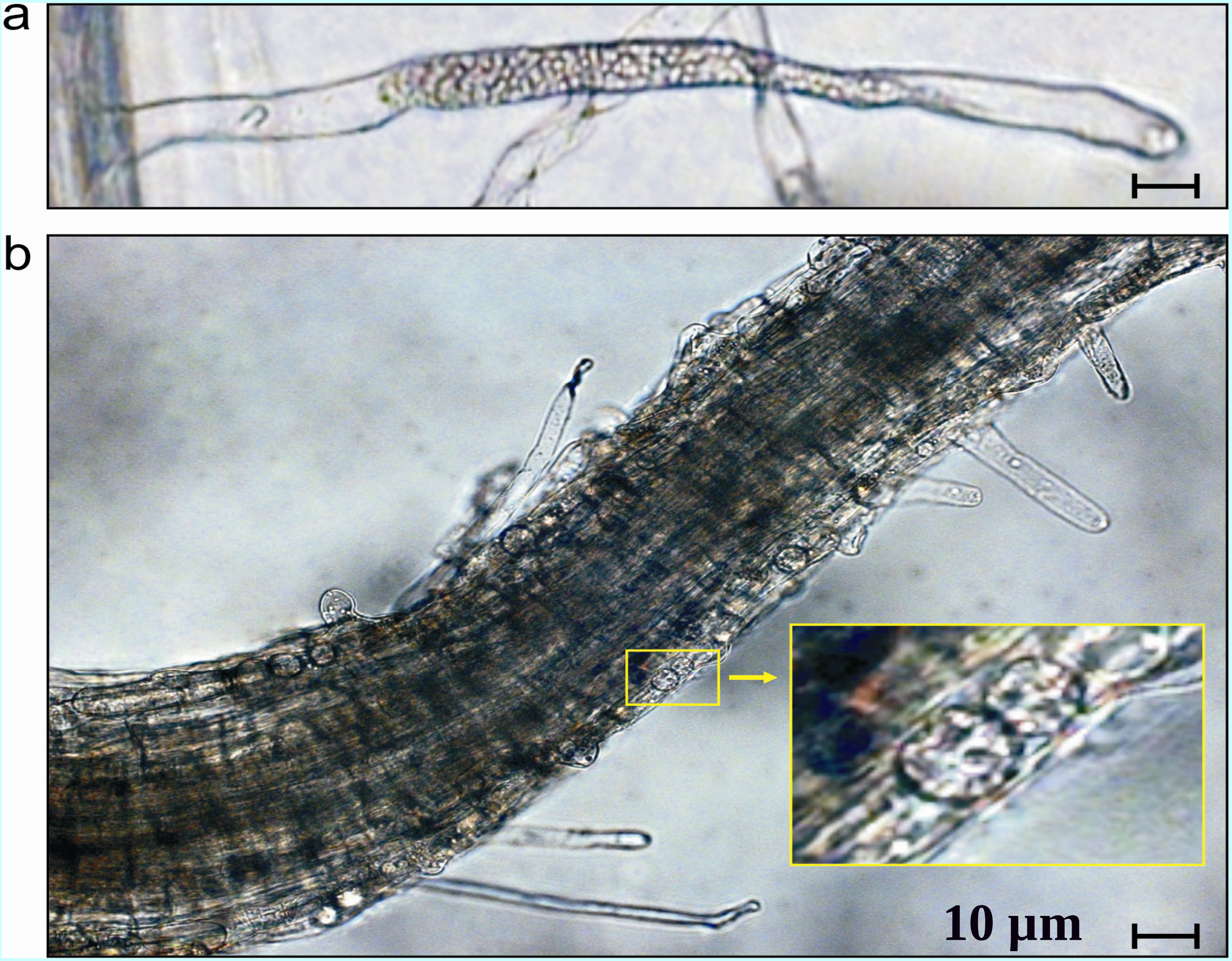


Kageyama and Asano 2009

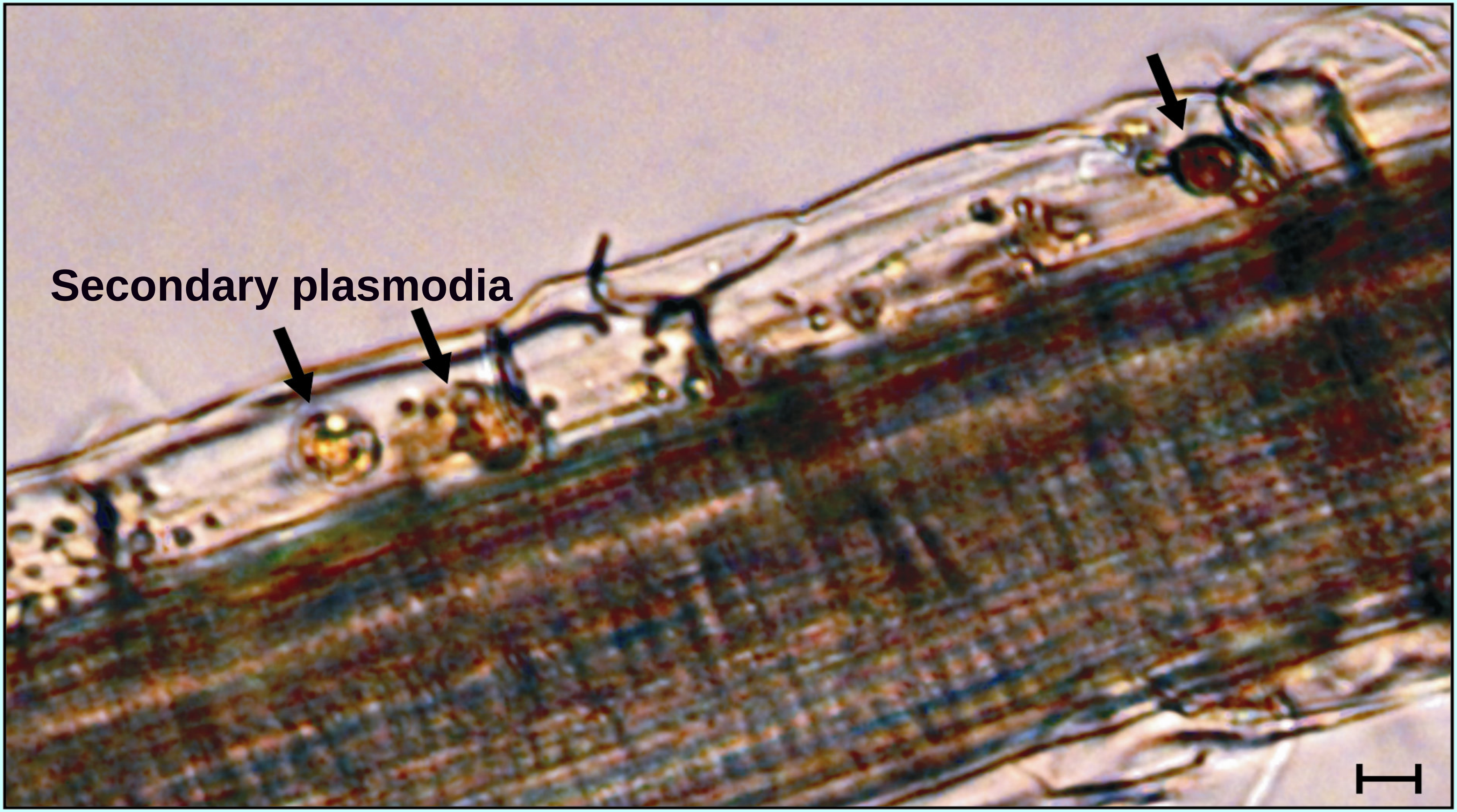
Secondary Zoospore Cross Infection Study

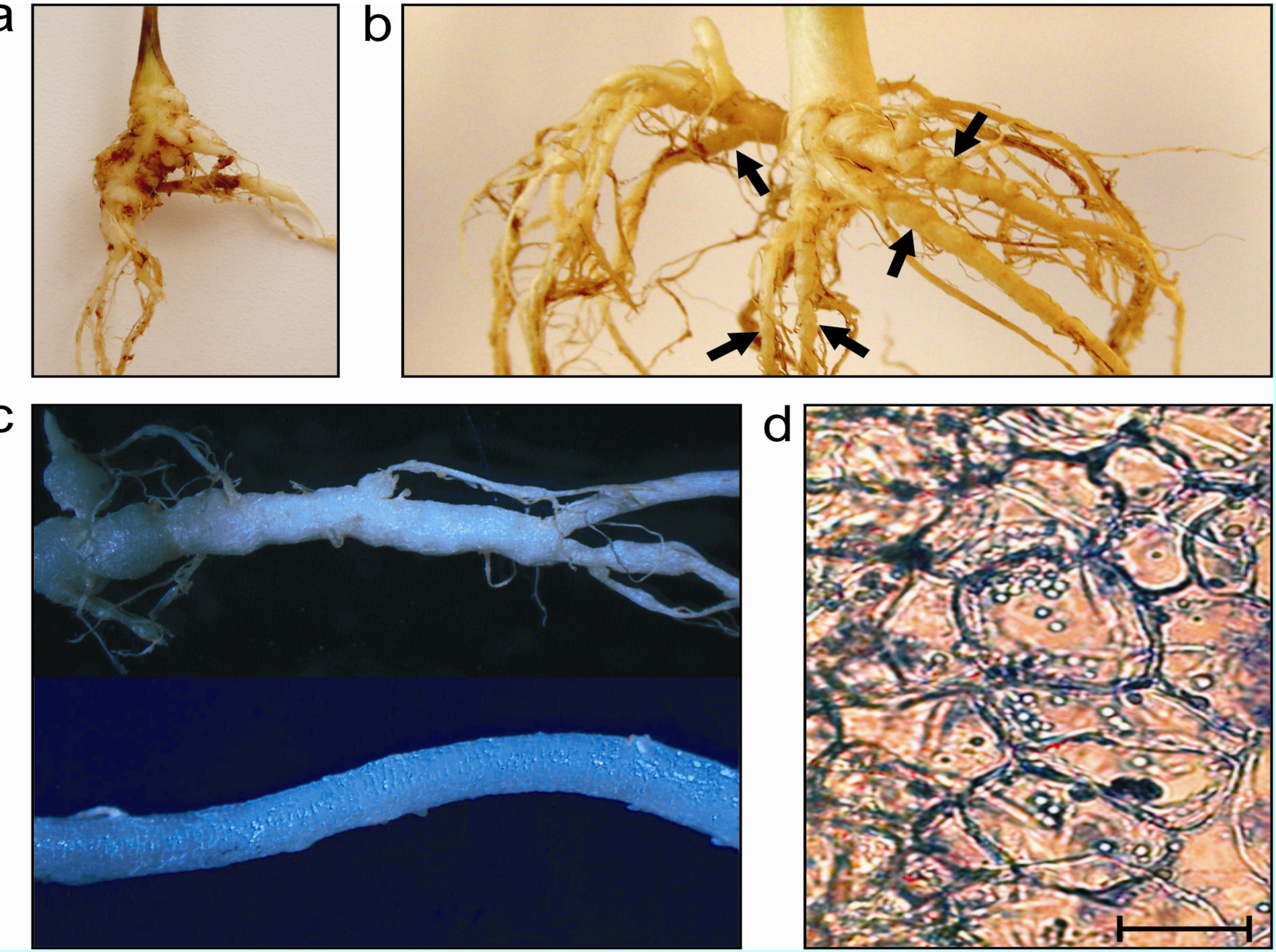
- **Canola inoculated by 2nd spore produced**
  - **from canola C^C**
  - **from ryegrass C^R**
- **Ryegrass inoculated by spore produced**
  - **from canola R^C**
  - **from ryegrass R^R**

Primary and secondary infection of ryegrass - 5 days after inoculation with secondary zoospores from canola.



Secondary infection on ryegrass – 35 days after inoculation with secondary zoospores from ryegrass. Bar = 10 µm.




a
b
c
d

Conclusions

- Secondary zoospores produced on a nonhost can infect a host species.
- Secondary infection can occur in a nonhost plant species.
- Pb can proliferate by cycling within root hairs prior to secondary infection.
- Resistance to secondary infection in ryegrass is induced during primary infection.

9. Effects of Seeding Date and Cultivar Resistance on Clubroot Severity, Seedling Emergence and Yield of Canola

By

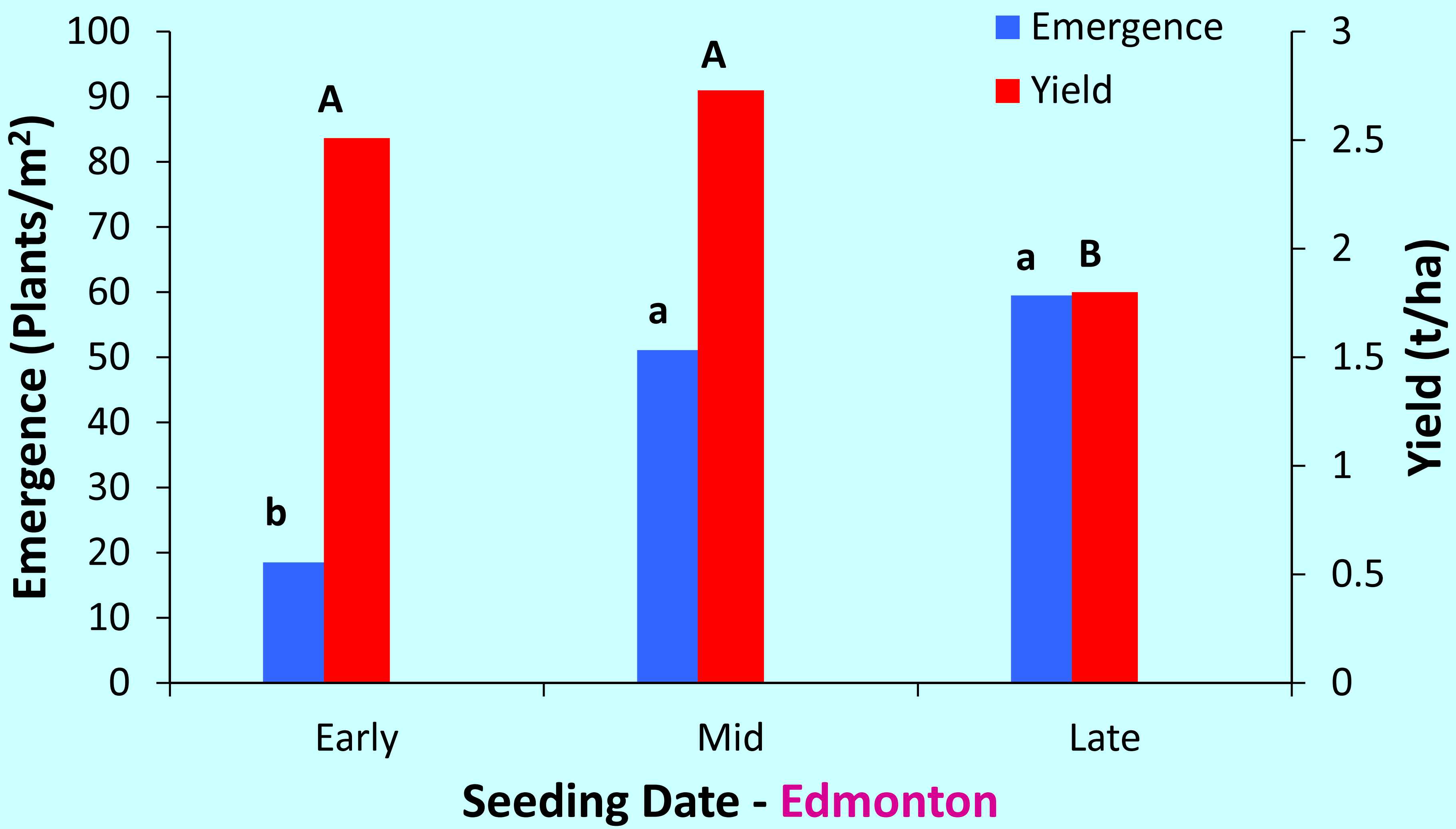
S.F. Hwang, T. Cao, Q. Xiao, H.U. Ahmed, V.P. Manolii,
G.D. Turnbull, B.D. Gossen, G. Peng and
S.E. Strelkov,

Can. J. Plant Sci. 2012 (in press)

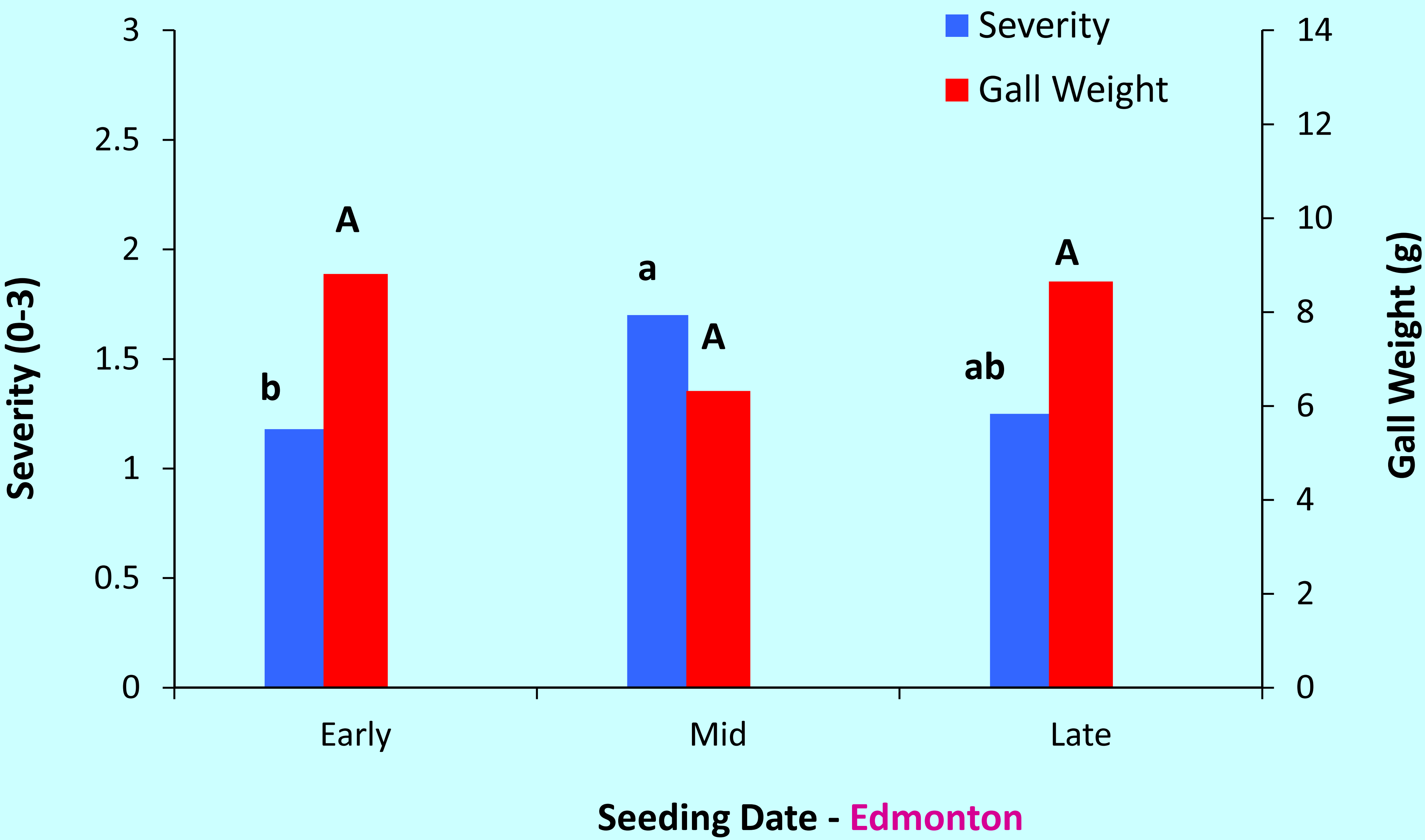
Field Studies - Interacting effects of seeding date and cultivar resistance (2010-11)

- Canola cultivars 45H26 (S) and 45H29 (R) serve as main plots
- Seeding dates (Early, Mid, Late) in sub-plots
- Plots were assessed for emergence, clubroot severity, yield and gall weight

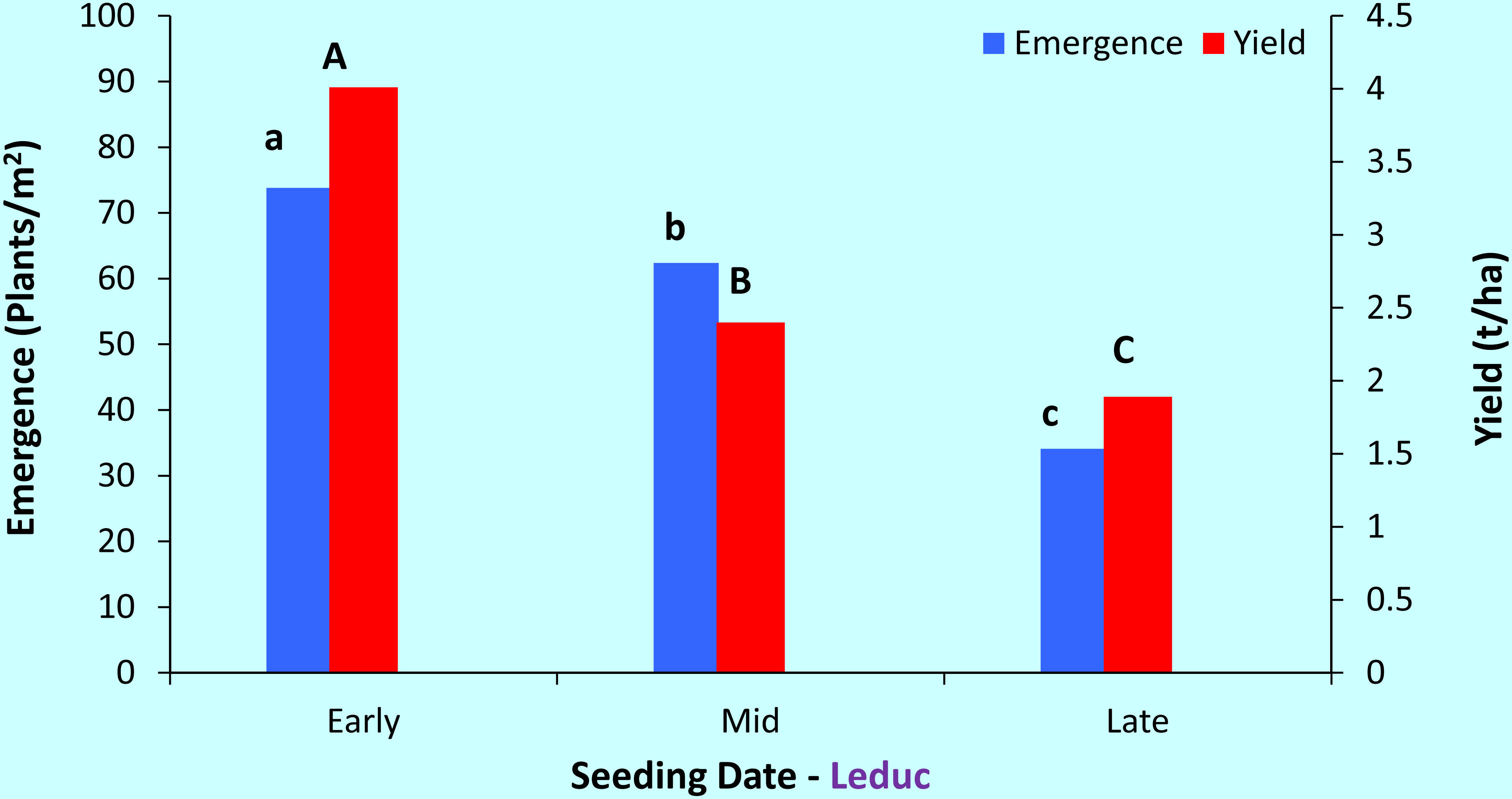
Effects of seeding dates on emergence and seed yield of canola in clubroot – infested soil



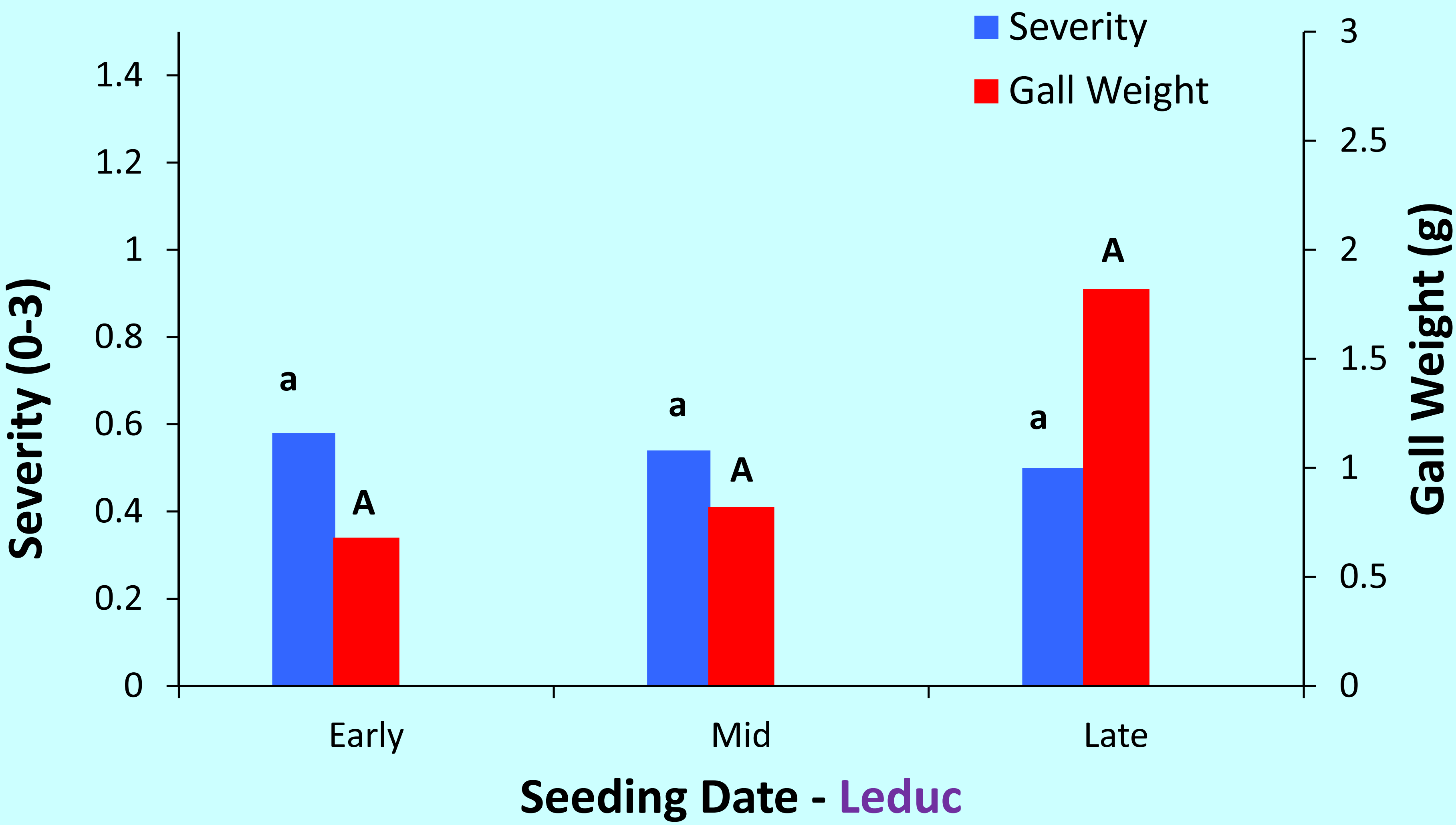
Effects of seeding dates on clubroot severity on canola in clubroot – infested soil



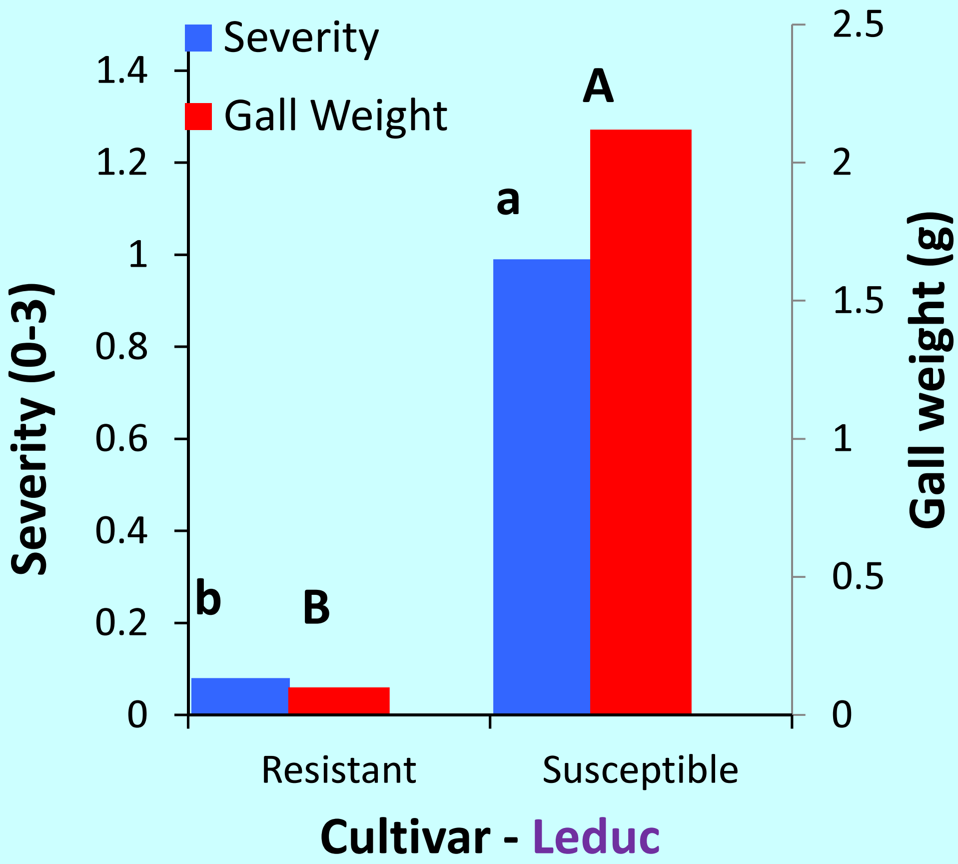
Effects of seeding dates on emergence and seed yield of canola in clubroot – infested soil

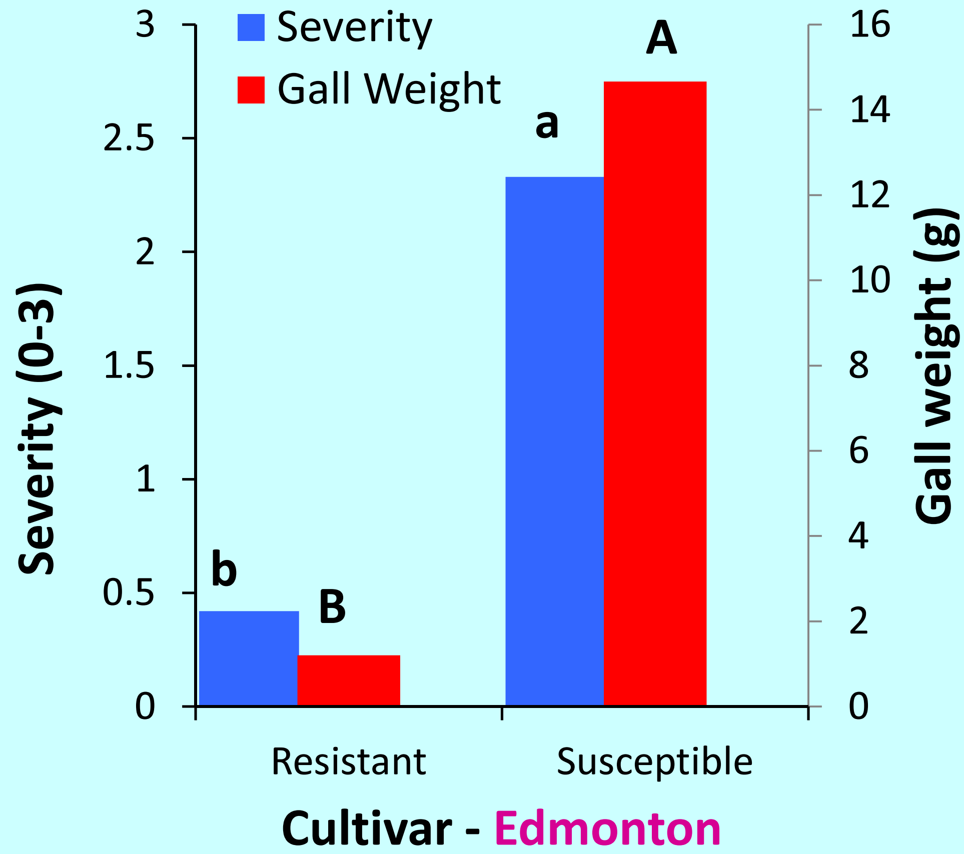


Effects of seeding dates on clubroot severity on canola in clubroot – infested soil

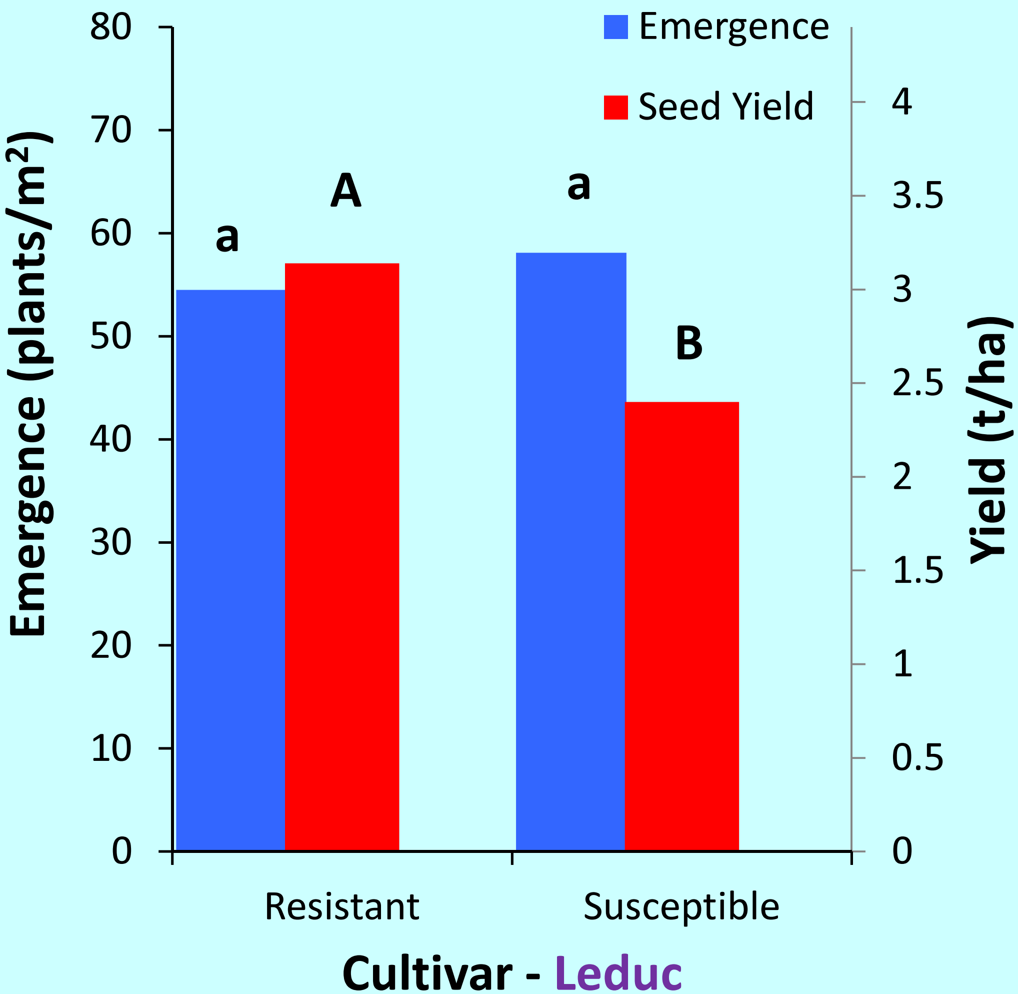


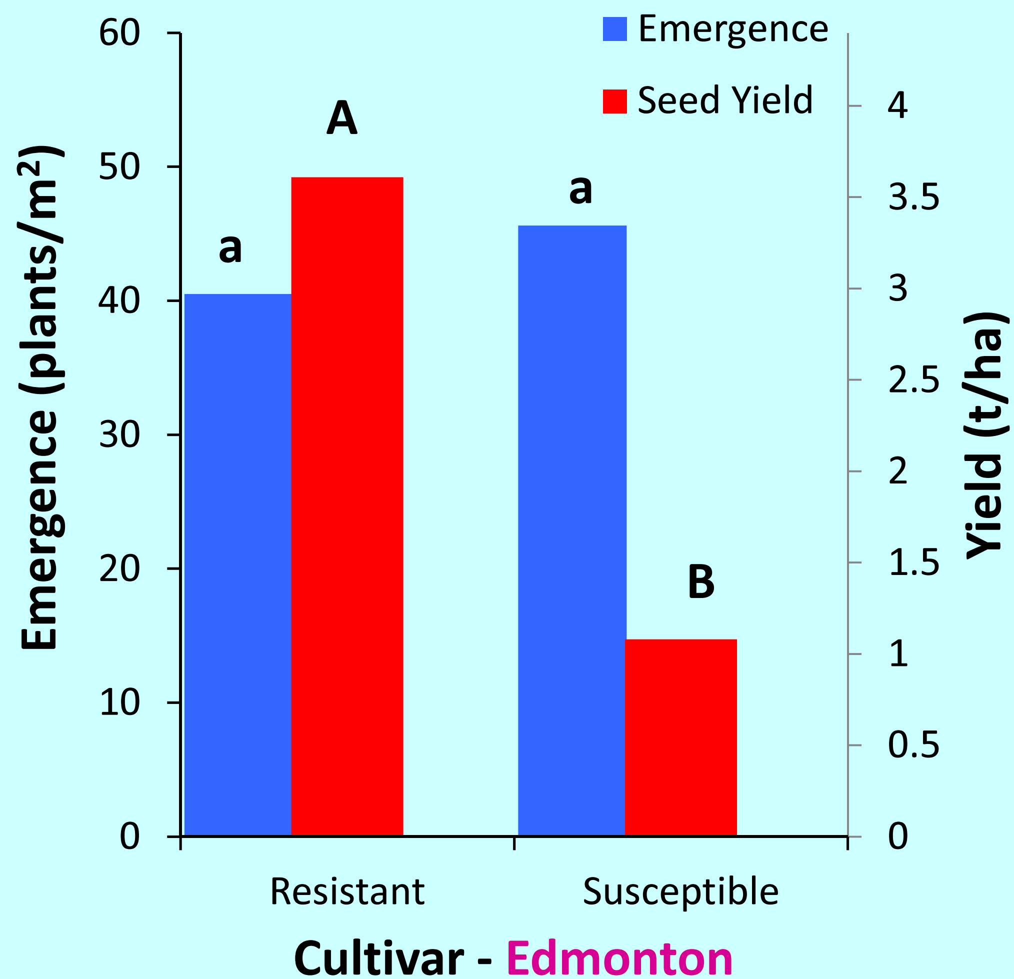
Effects of cultivar resistance on clubroot severity on canola in clubroot – infested soil





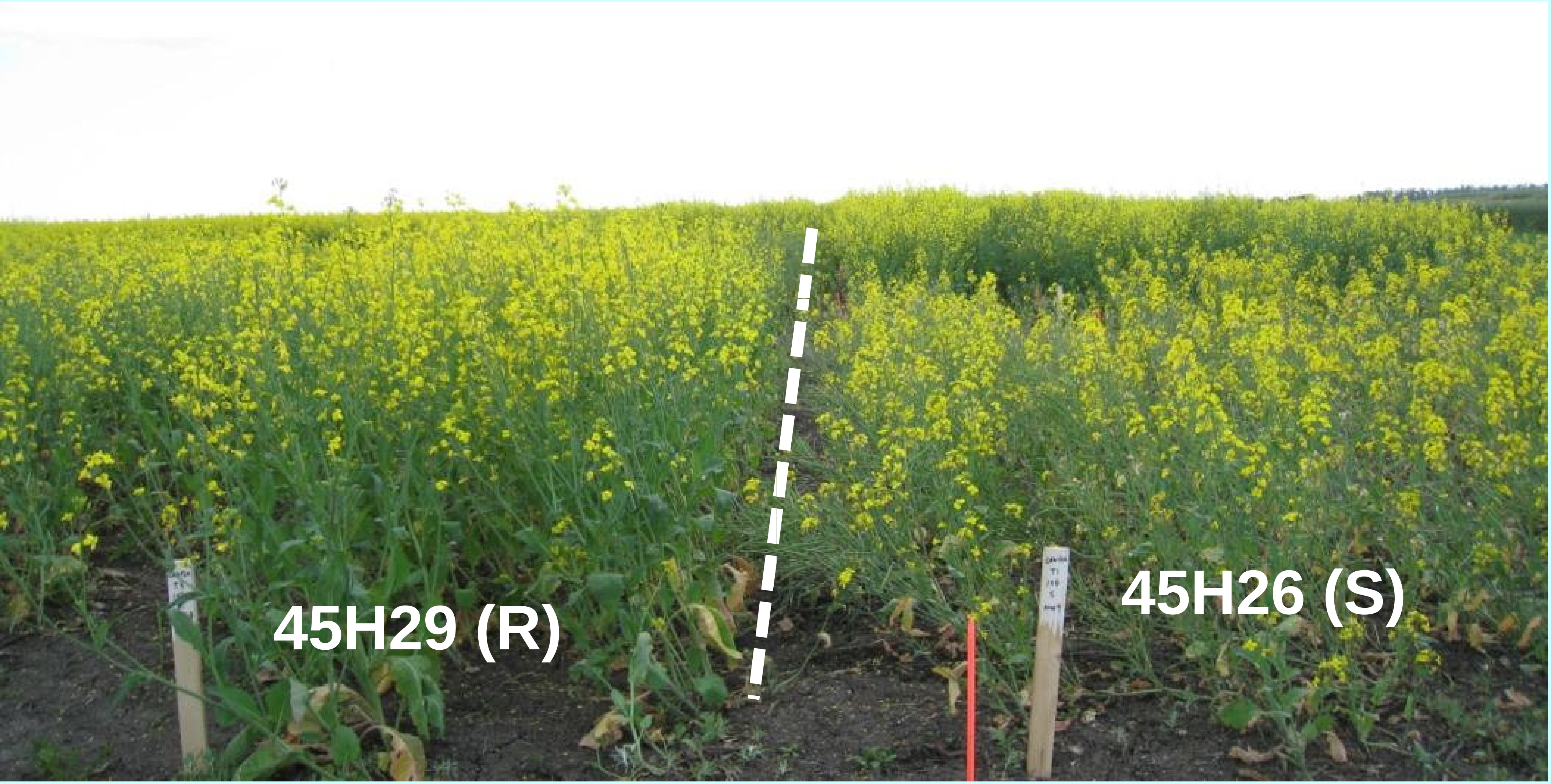
Effects of cultivar resistance on emergence and yield of canola in clubroot – infested soil



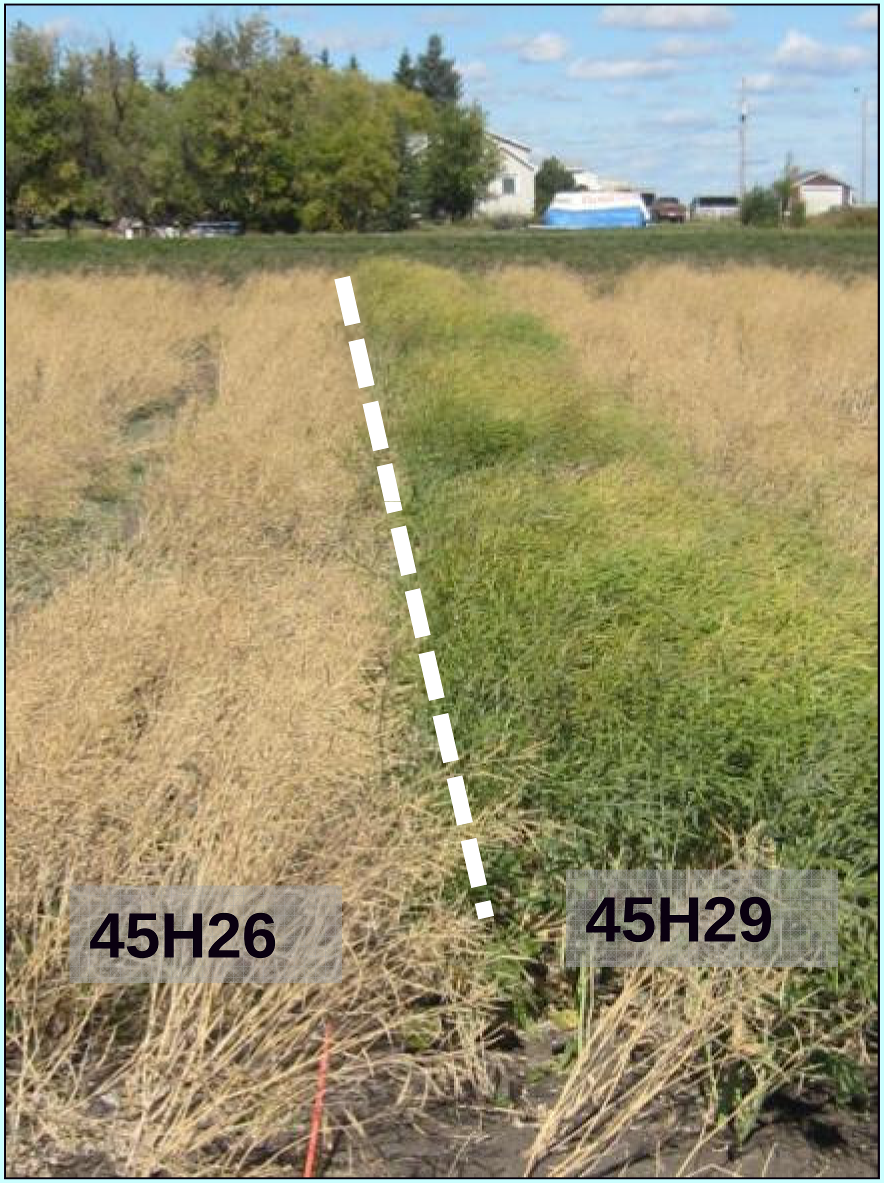


Cultivar Effect on Clubroot - 2010

Cultivar Effect on Clubroot - 2011



Cultivar Effect on Clubroot – Sept. 20, 2011



Late season clubroot galls

Conclusion – Seeding Date

- Manipulation of seeding date and the cropping of clubroot resistant canola cultivars can be used as additional tools in a clubroot management program
- Younger seedlings suffered greater disease severity and a greater reduction in plant height and yield than older seedlings in both the resistant and susceptible canola cultivars
- Clubroot resistant canola cultivar 45H29 is not immune to the disease

10. Influence of cultivar resistance and inoculum density on root hair infection of canola by Plasmodiophora brassicae

by

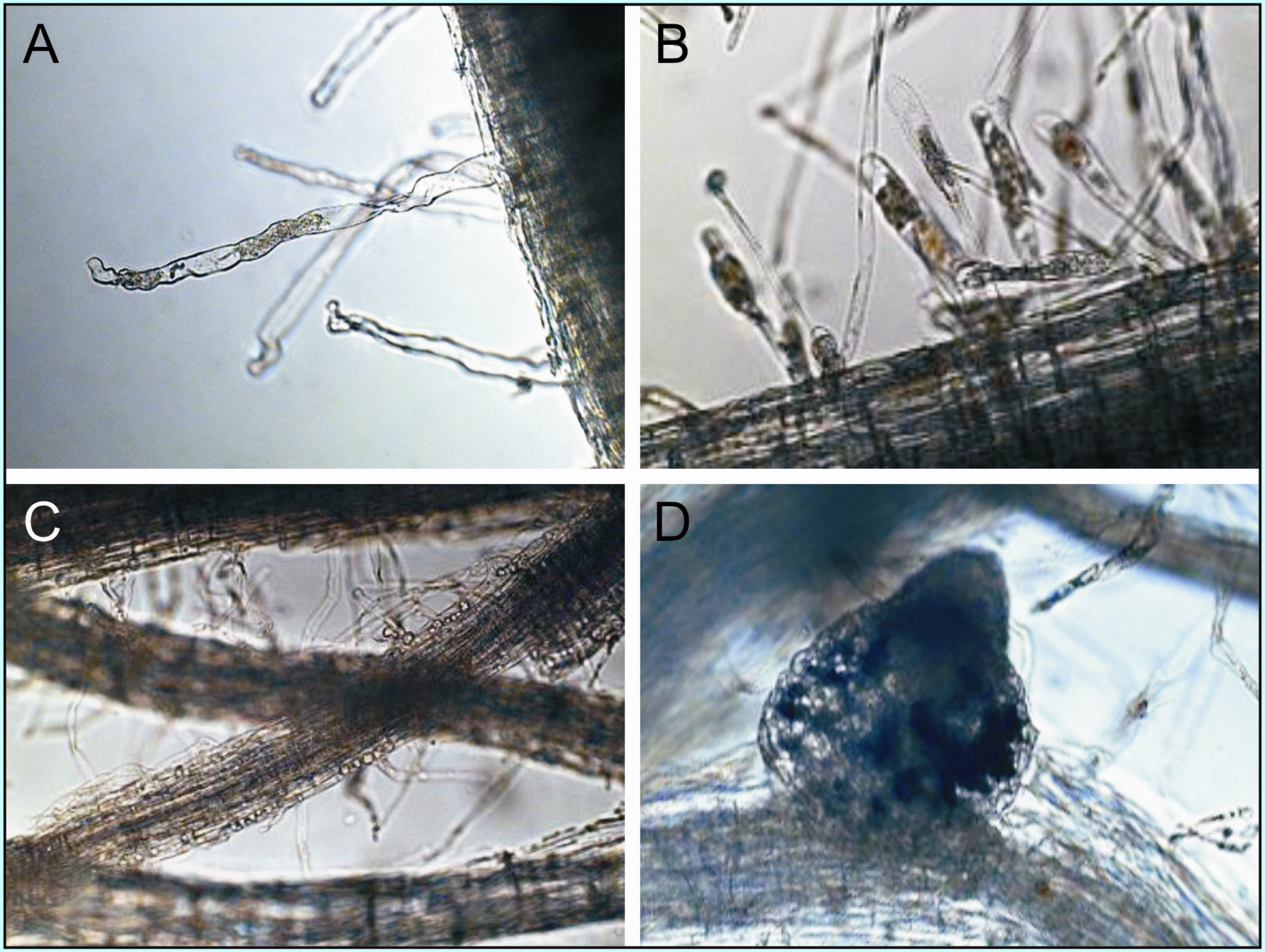
S.F. Hwang, H.U. Ahmed, Q. Zhou, S.E. Strelkov, B.D. Gossen, G. Peng and G.D. Turnbull

Plant Pathology (2011) 60: 820-829.

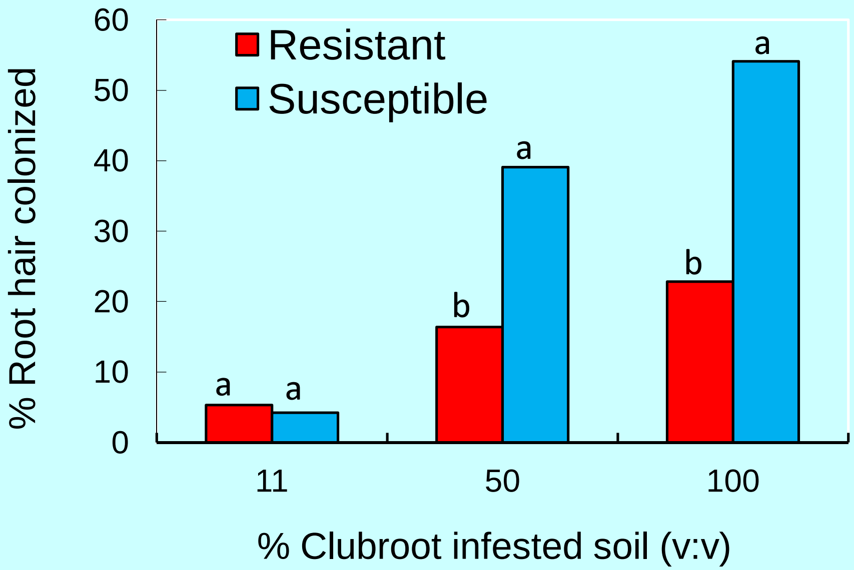
Effects of soil inoculum density on growth, disease and yield of canola

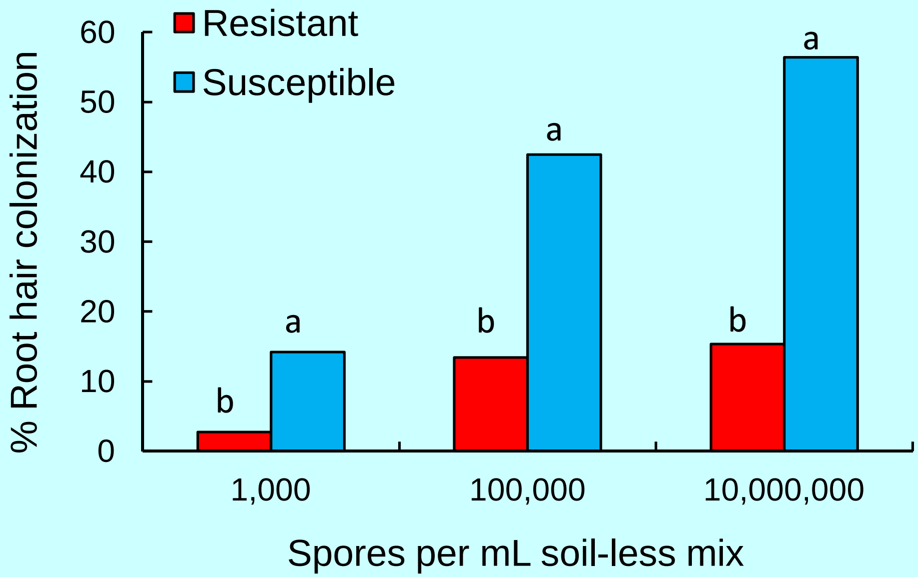
Soil Dilutions	Height (cm)	Emergence (%)	Yield (g/pot)	Disease Index
Clubroot-Resistant (45H-29)				
0:1 (infested soil: soil-less mix)	104 a	72 a	2.79 a	0
1:8 (infested soil: soil-less mix)	94 b	58 a	2.26 ab	0
1:1 (infested soil: soil-less mix)	84 c	54 a	1.77 b	0
1:0 (infested soil: soil-less mix)	92 b	18 b	0.60 c	5.6
Mean	94 A	51 A	1.85 A	**1.4 B**
Clubroot-Susceptible (45H-26)				
0:1 (infested soil: soil-less mix)	105 a	72 a	2.67 a	0 c
1:8 (infested soil: soil-less mix)	94 b	42 b	0.78 b	28 b
1:1 (infested soil: soil-less mix)	54 c	28 bc	0.01 c	90 a
1:0 (infested soil: soil-less mix)	40 d	22 c	0.01 c	100 a
Mean	73 B	41B	0.86 B	**54.5 A**

Colonization of canola root hairs

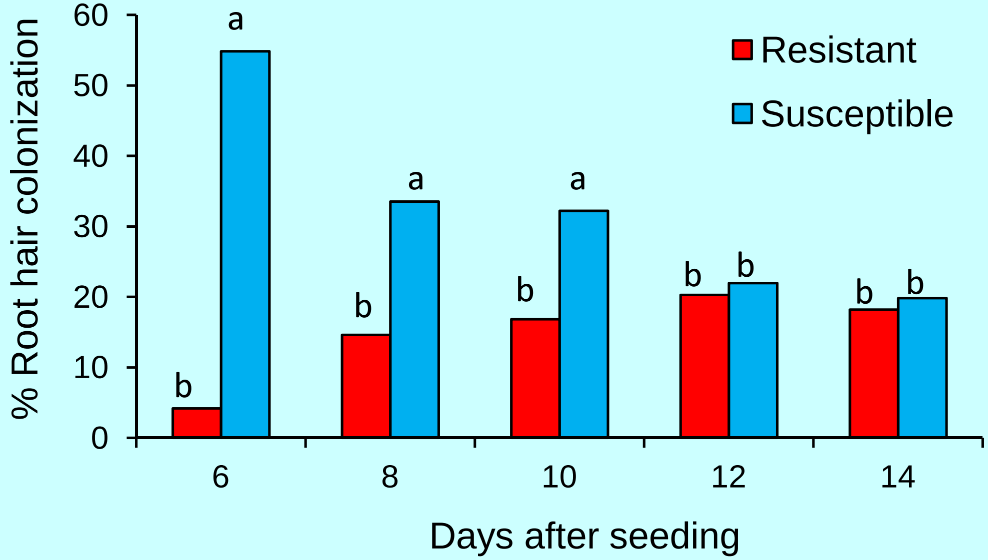


Effects of soil inoculum level on root hair colonization

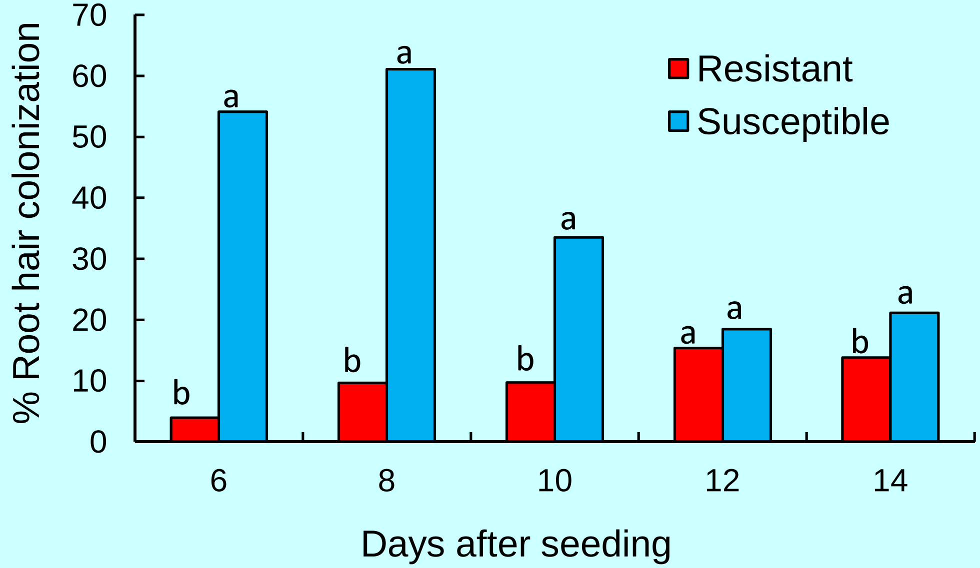




Effects of incubation period on root hair colonization

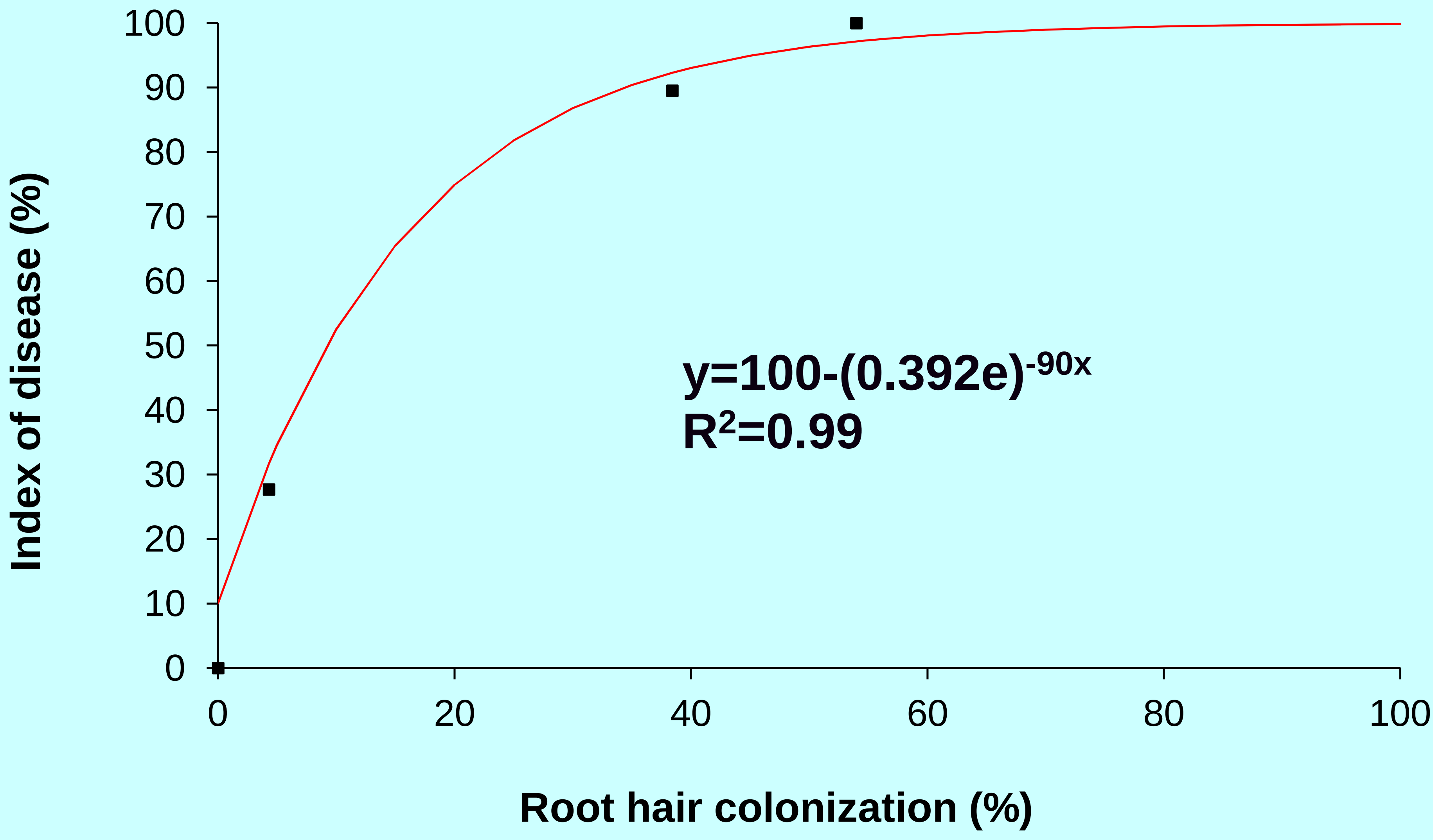


Naturally infested soil



Spores inoculated in soilless mix (10^6/g)

Effects of root hair colonization on disease index of clubroot



11. Root Hair Colonization of Resistant and Susceptible Canola by *Plasmodiophora brassicae*

By

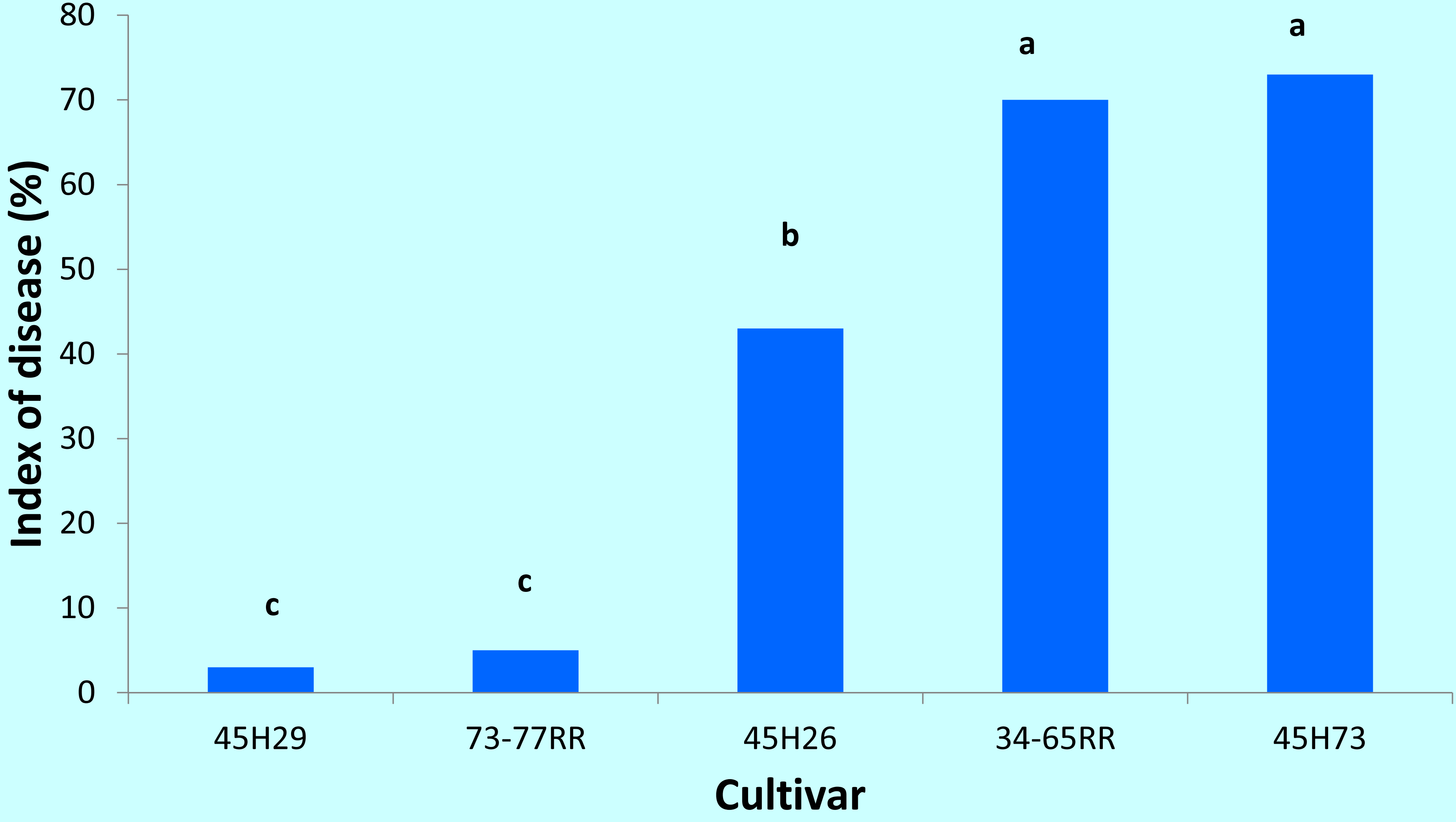
S.F. Hwang, H.U. Ahmed, Q. Zhou, S.E. Strelkov,
B.D. Gossen, G. Peng, and G.D. Turnbull

Plant Pathology 2012
(Doi: 10.1111/j.1365-3059.2011.02582.x)

Objective:

- To examine the relationship between root hair infection and *P. brassica* DNA detected by q PCR.

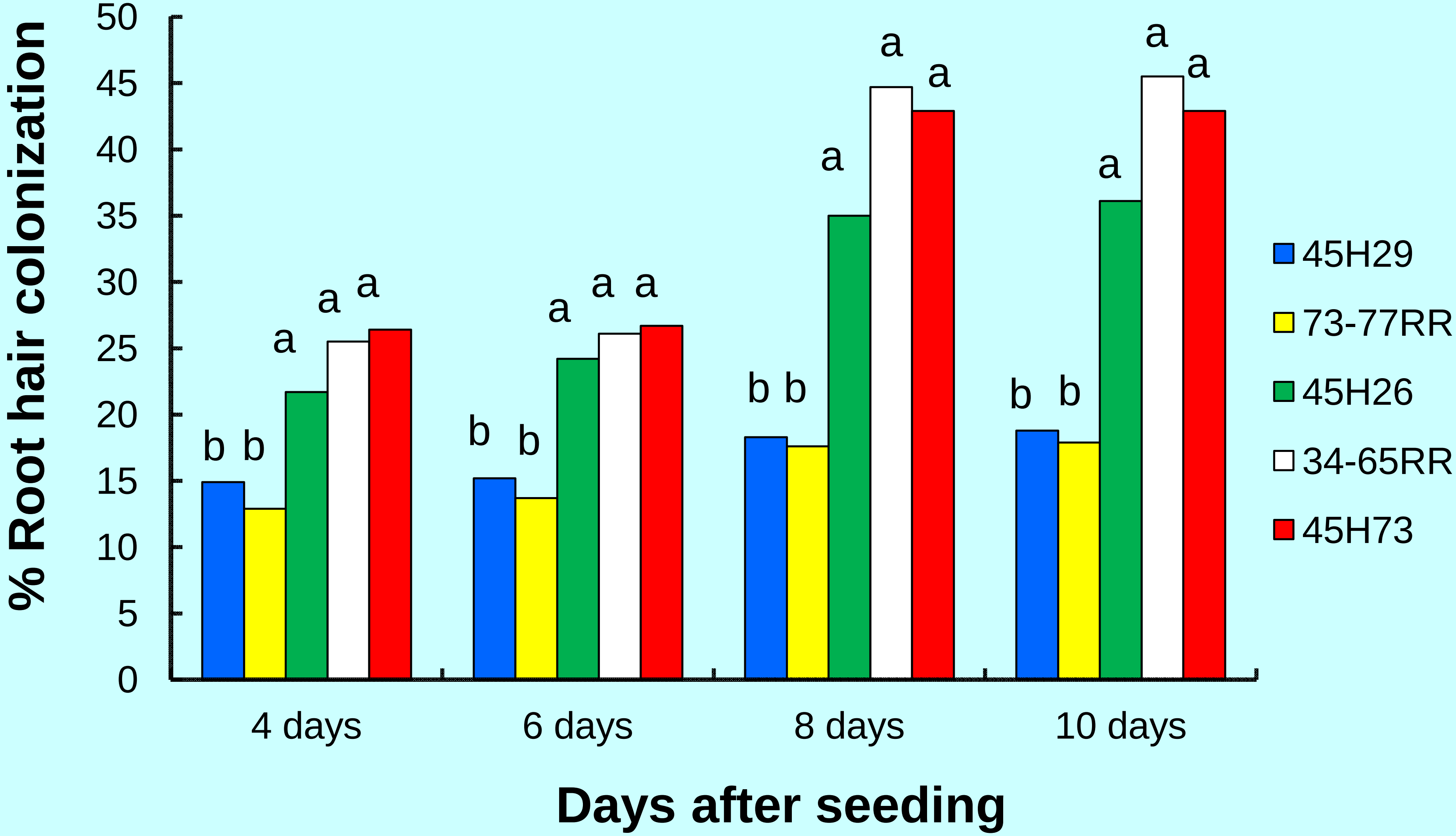
Effect of cultivar resistance on the index of disease in five canola cultivars



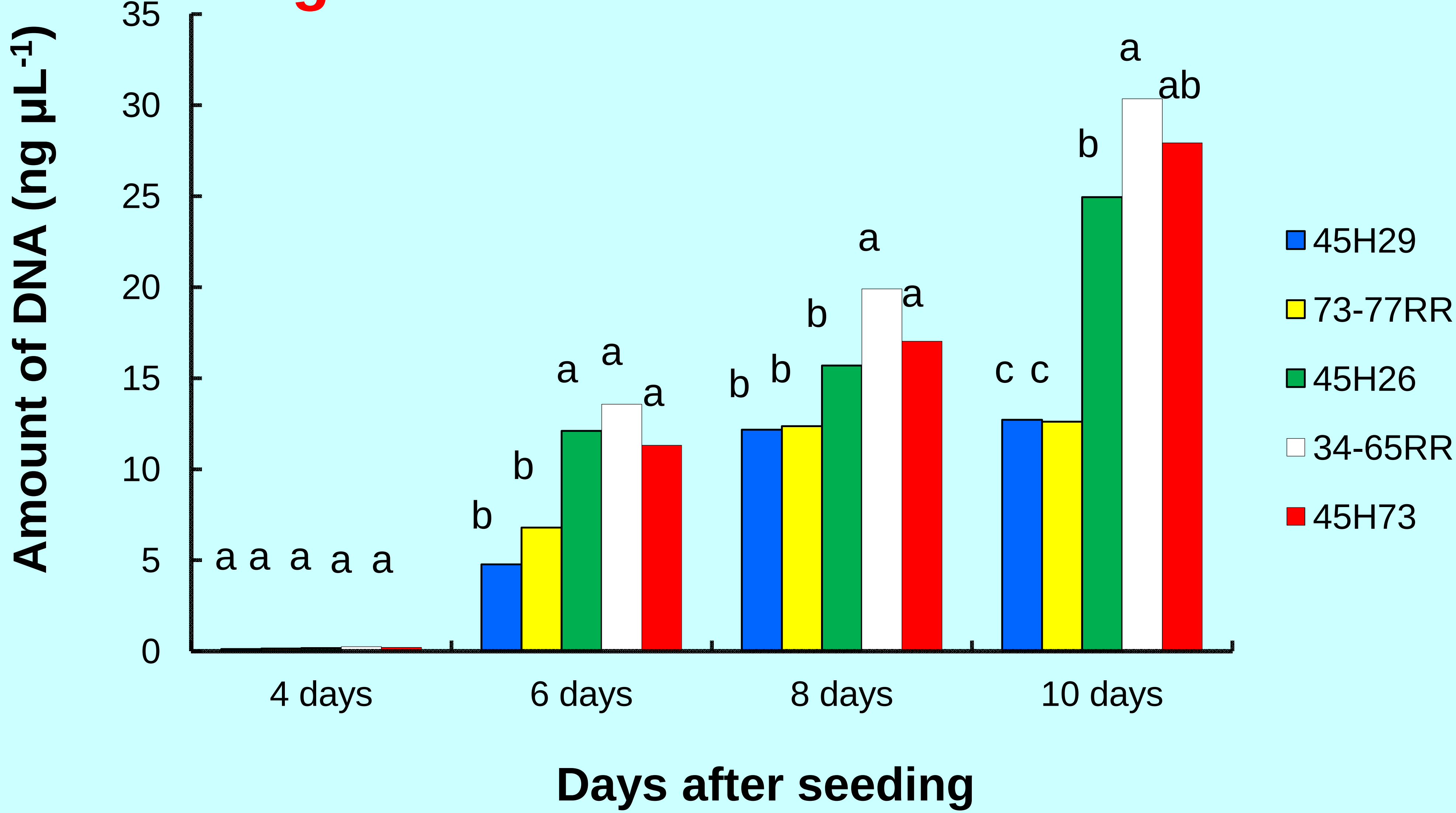
Comparison of bioassay and qPCR analysis

- **Five canola cultivars, 45H29, 45H26, 73-77RR, 34-65RR, and 45H73 were planted in cups.**
- **Each cultivar was sampled at 4, 6, 8, and 10 days after sowing.**
- **Half of the plants were fixed in FAA for root hair analysis; half were stored for qPCR analysis.**
- **Weight of *P. brassicae* DNA was estimated using qPCR analysis.**

Root hair colonization in five canola cultivars grown in clubroot-infested soil

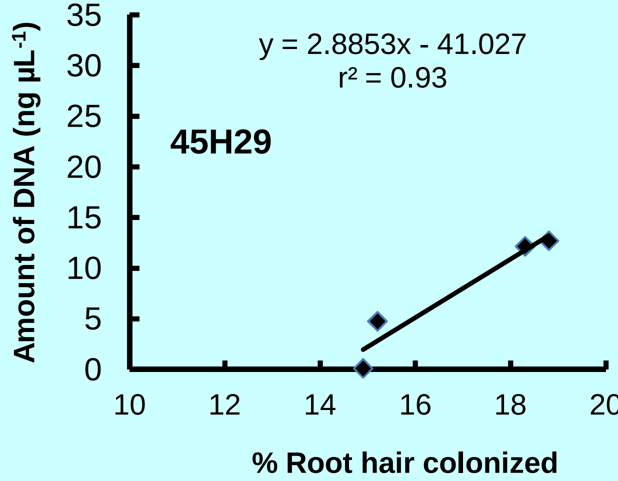


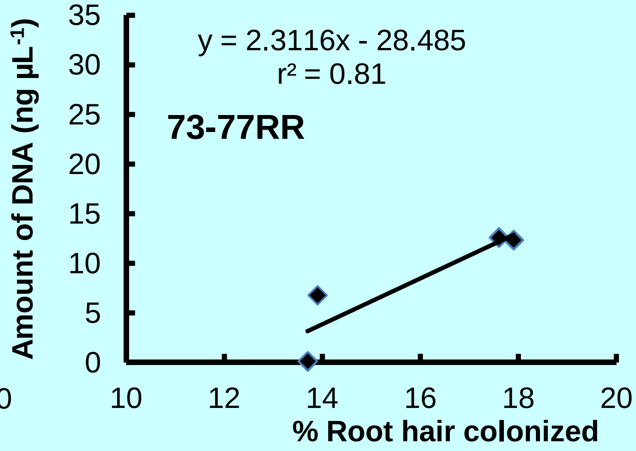
P. Brassicae DNA found in five canola cultivars grown in clubroot-infested soil

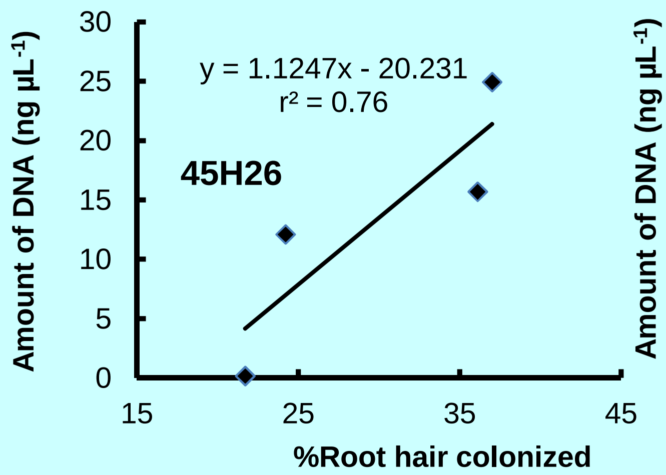


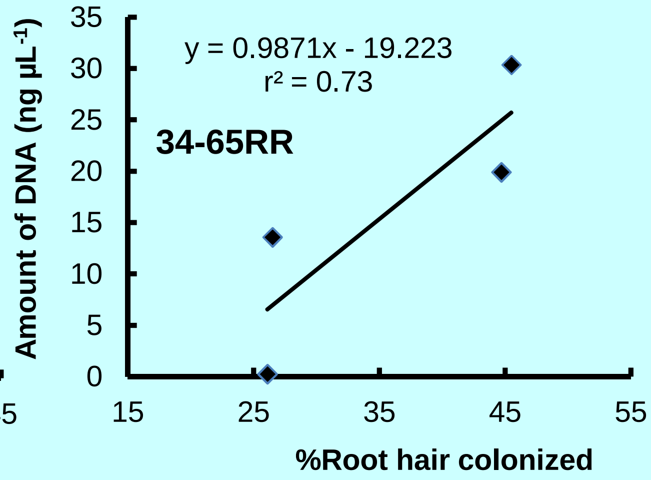
Relationship between amount of *P. Brassicae* DNA and root hair colonization in five canola cultivars

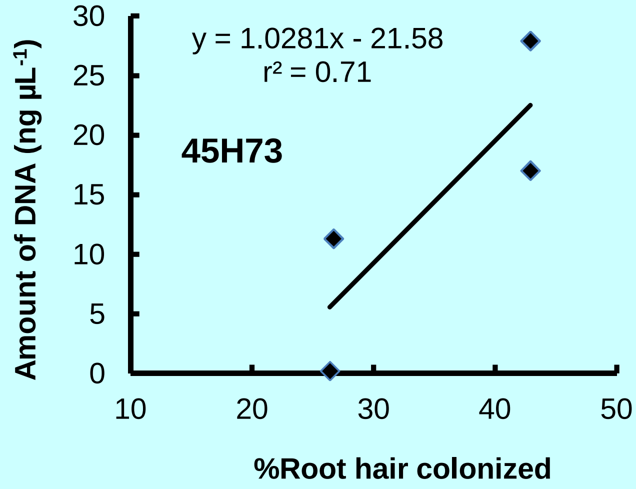
Resistant cultivars





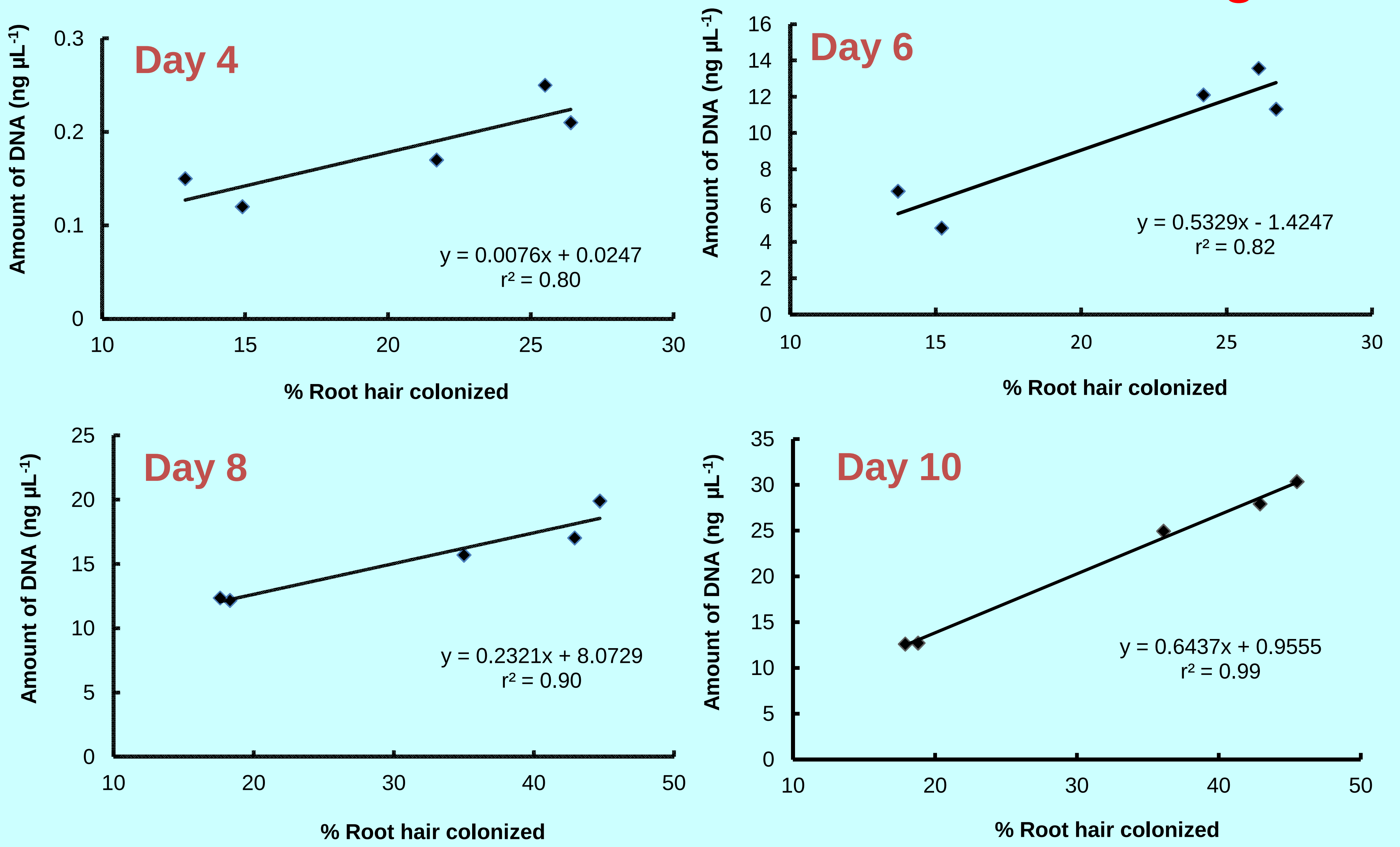




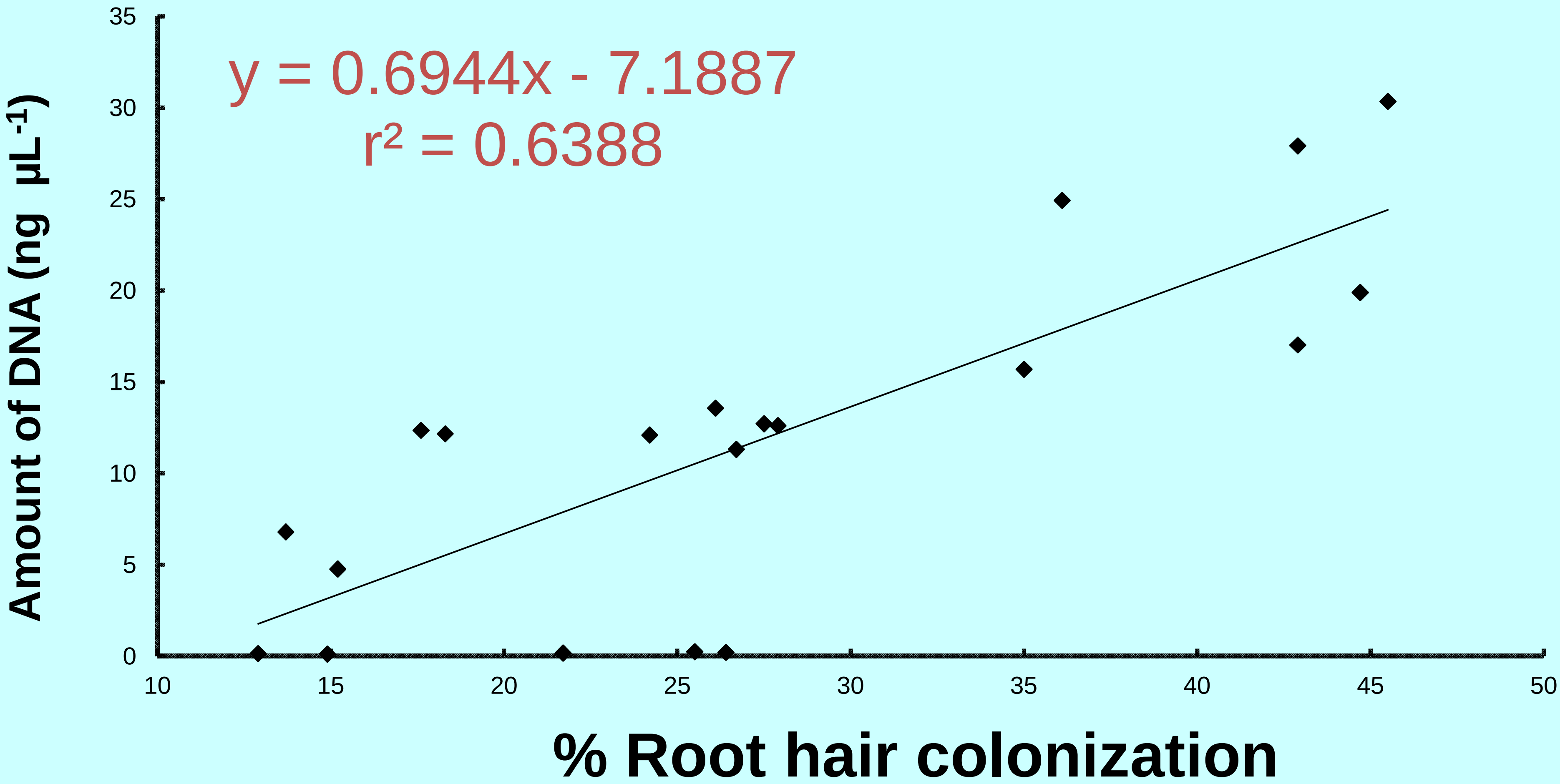


Susceptible cultivars

P. Brassicae DNA found in canola grown in clubroot-infested soil at four intervals after seeding



Relationship between amount of *P. Brassicae* DNA and root hair colonization in five canola cultivars at four sampling dates



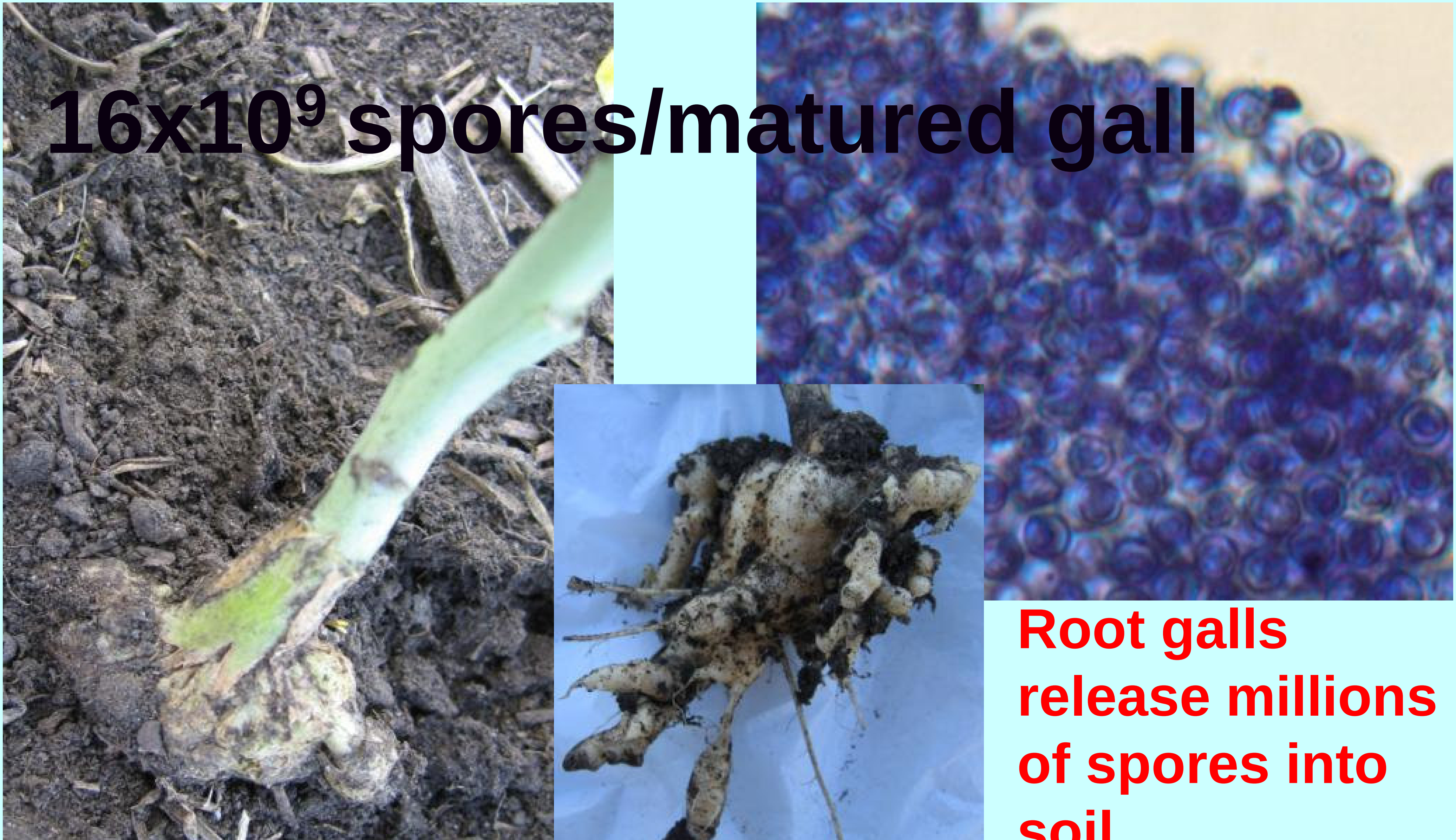
Results:

- A strong linear relationship was found between root hair infection and the amount of pathogen DNA.
- In susceptible cultivars the amount of pathogen DNA rose more sharply than in the resistant cultivars.
- Height of both susceptible and resistant cultivars was reduced after inoculation with the pathogen.

Effects of growing resistant cultivars on spore populations

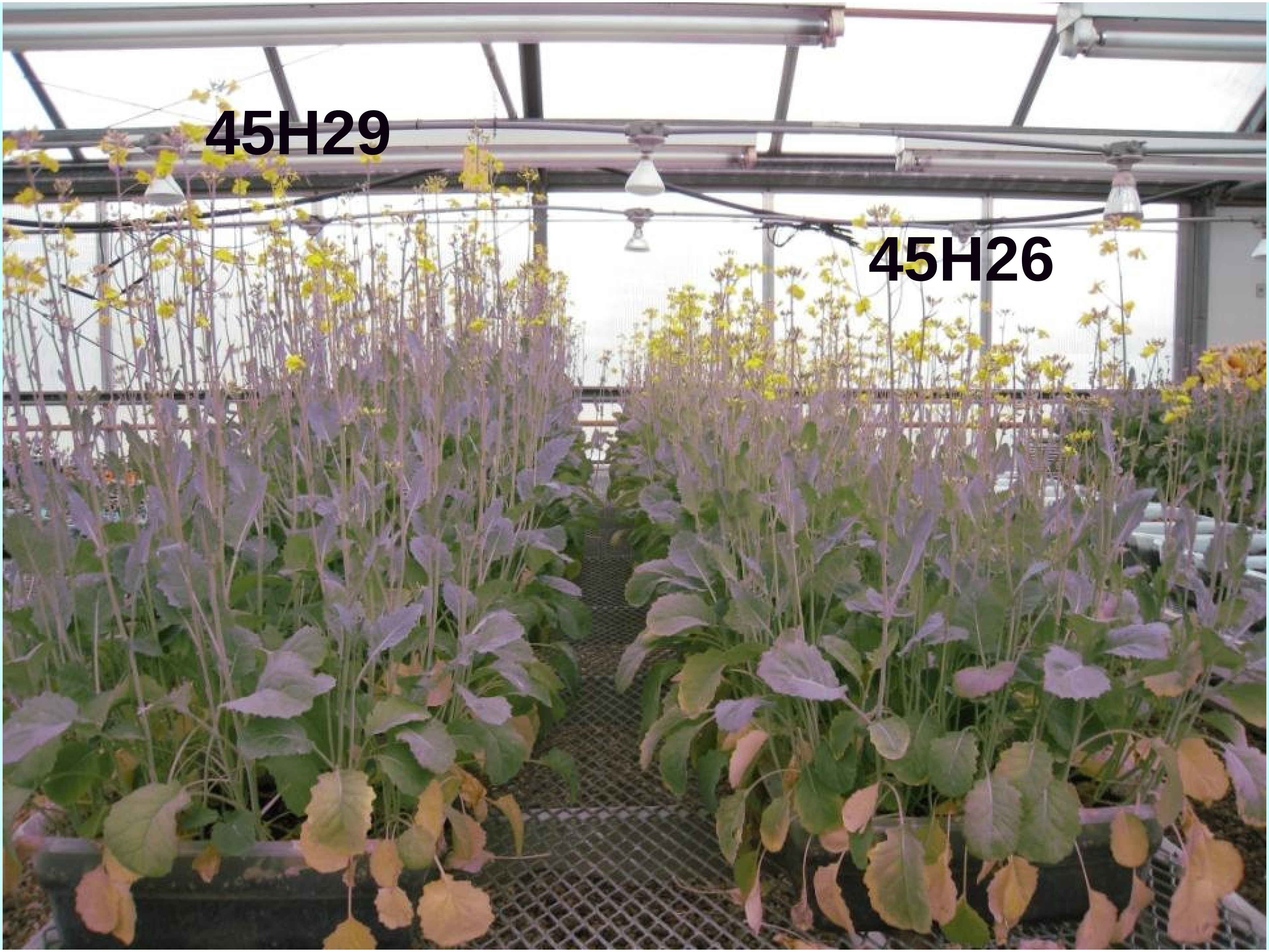
Widespread release of genetically resistant canola hybrids in 2010

16×10^9 spores/matured gall



Root galls release millions of spores into soil

≈ 800×10^6 spores/g gall, ≈ 20 g/gall of matured plant


45H29
45H26

Quantification of P. brassicae by microscopy and qPCR analysis

Treatment	Resting spore (g)-1 soil	Ct Value	DNA (ng) -µL
Resistant cultivar	1.0×10^{8} b	24.75 a	0.338 b
Susceptible cultivar	2.0×10^{8} a	20.18 b	6.248 a
Fallow control	9.2×10^{7} c	25.24 a	0.215 b

DNA was extracted from 0.5 g soil after adding macerated gall tissue after the first cycle of cropping

12. Effects of Cropping Clubroot-Resistant Canola Plants on Plasmodiophora brassicae Resting Spore Populations in the Soil

By

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Plant Pathology 2012 (accepted)

Effects of Cycles of Resistant Canola Lines on Clubroot Spore Populations in Infested Soil

Objective:

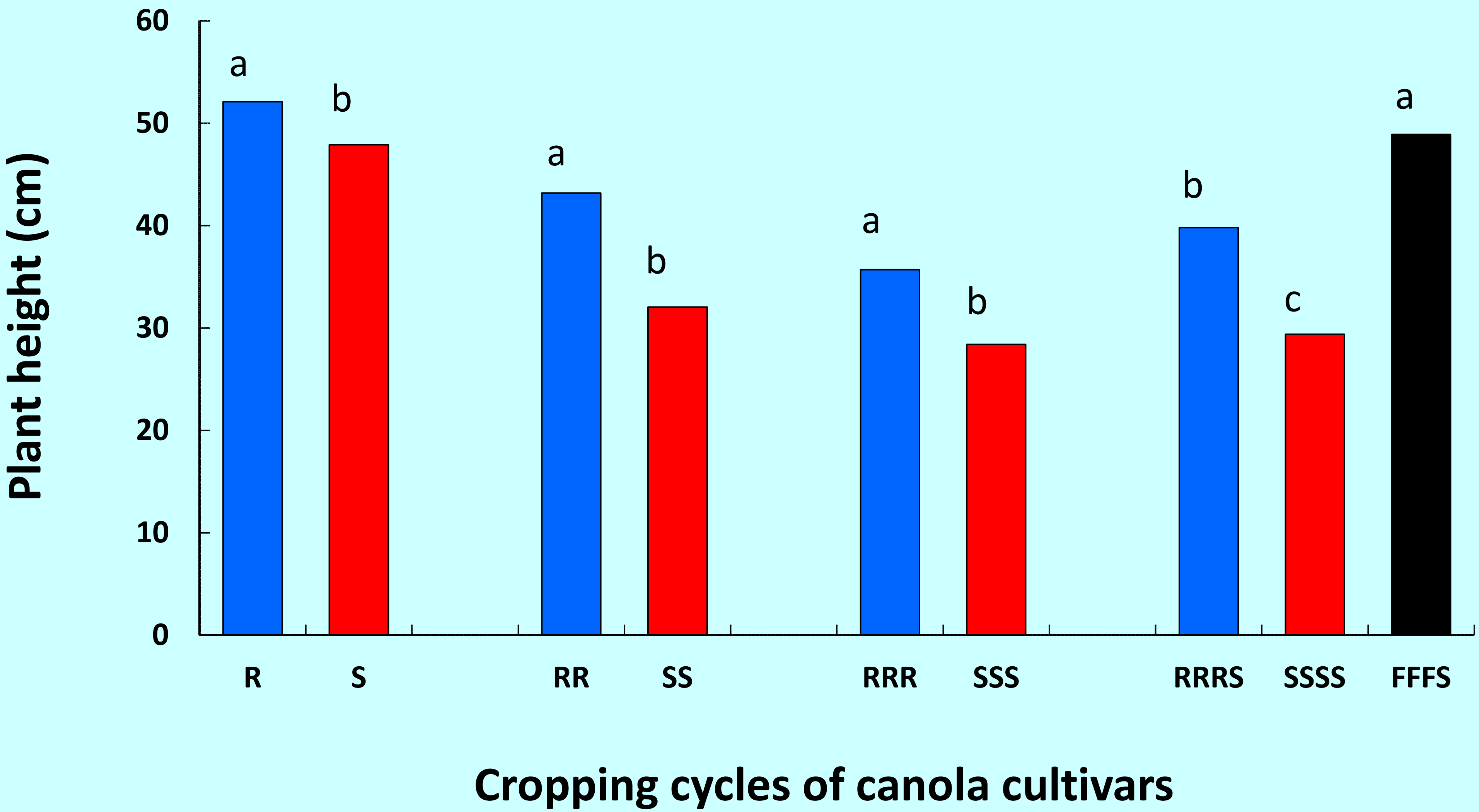
To evaluate the effects of repeatedly growing the same resistant cultivar on:

- **Resting spore populations of *P. brassicae***
- **Subsequent severity of clubroot in susceptible canola.**

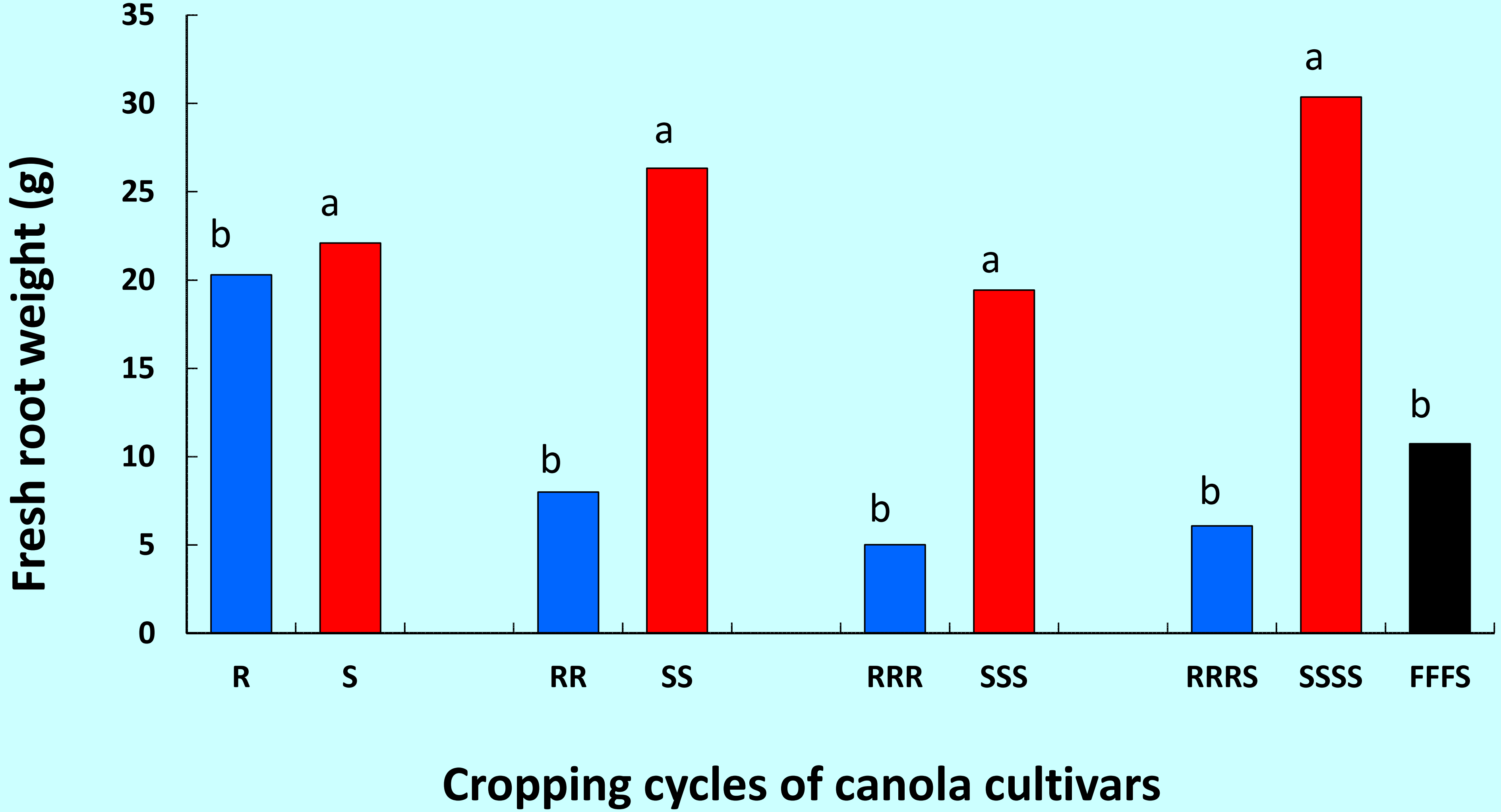
A. Effects of growing resistant cultivars on clubroot severity in subsequent crops

- Canola cvs 45H29 (R) and 45H26 (S) were grown in inoculated soilless mix. A fallow control (F) was added .
- After 4 wk, roots were re-incorporated into the soil.
- A new crop (same cultivar) was replanted into the soil.
- Three treatments: RRRS, SSSS, FFFS
- Root weight, plant height, clubroot incidence and severity, and resting spore populations were recorded after each cycle.

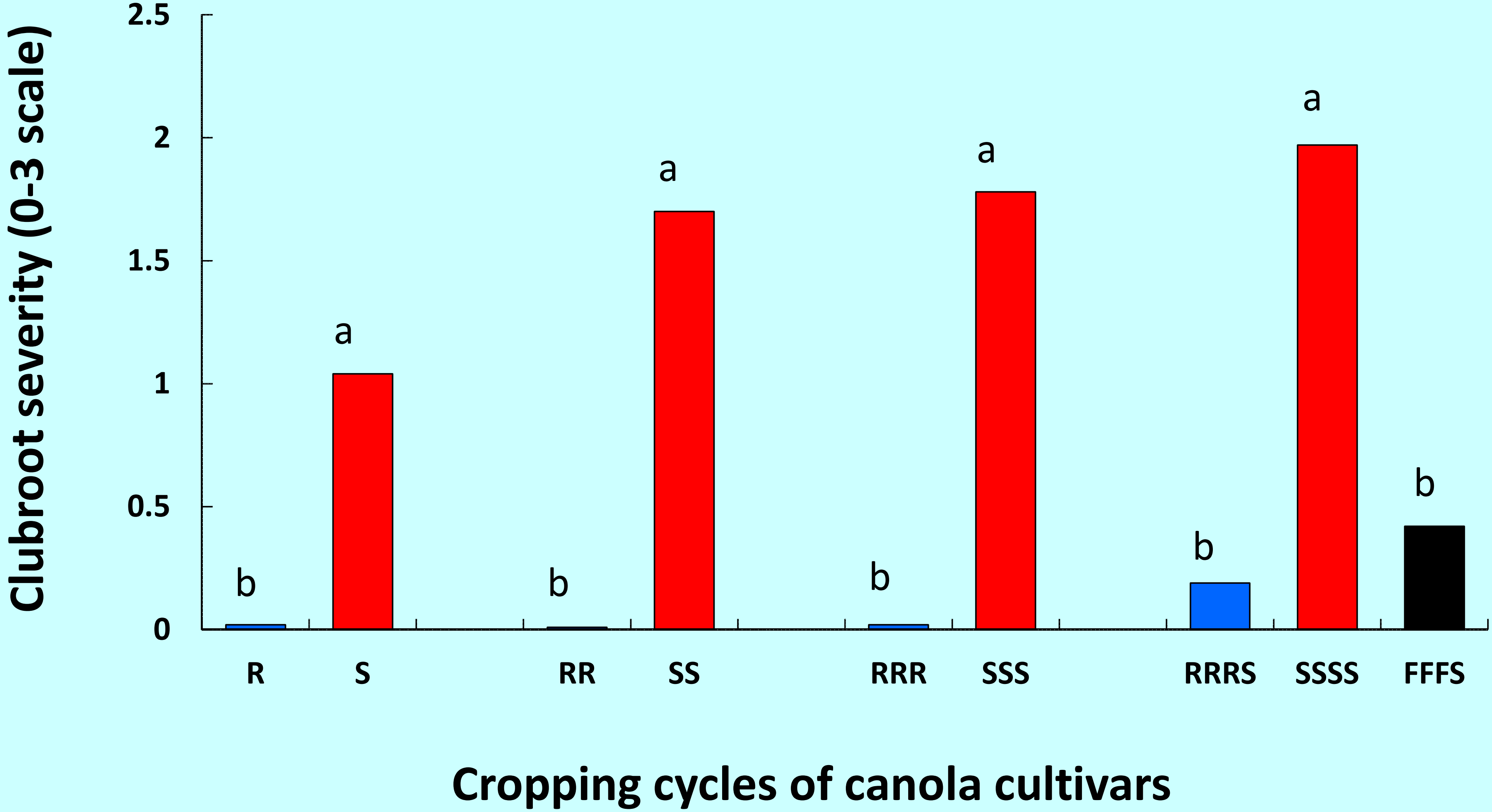
Effect of sequential growth of resistant and susceptible canola cultivars on plant height



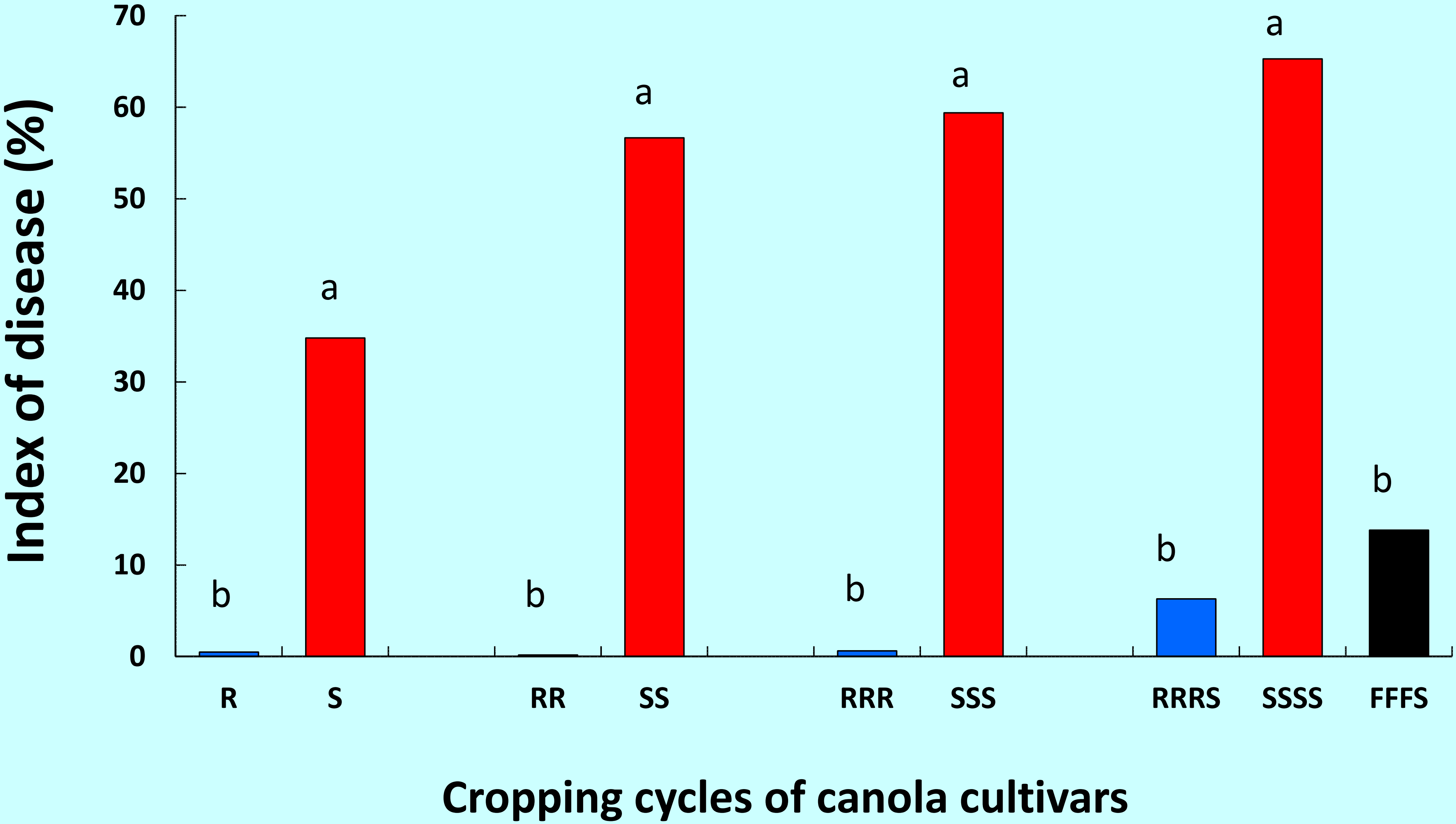
Effect of sequential growth of resistant and susceptible canola cultivars on root biomass



Effect of sequential growth of resistant and susceptible canola cultivars on clubroot severity

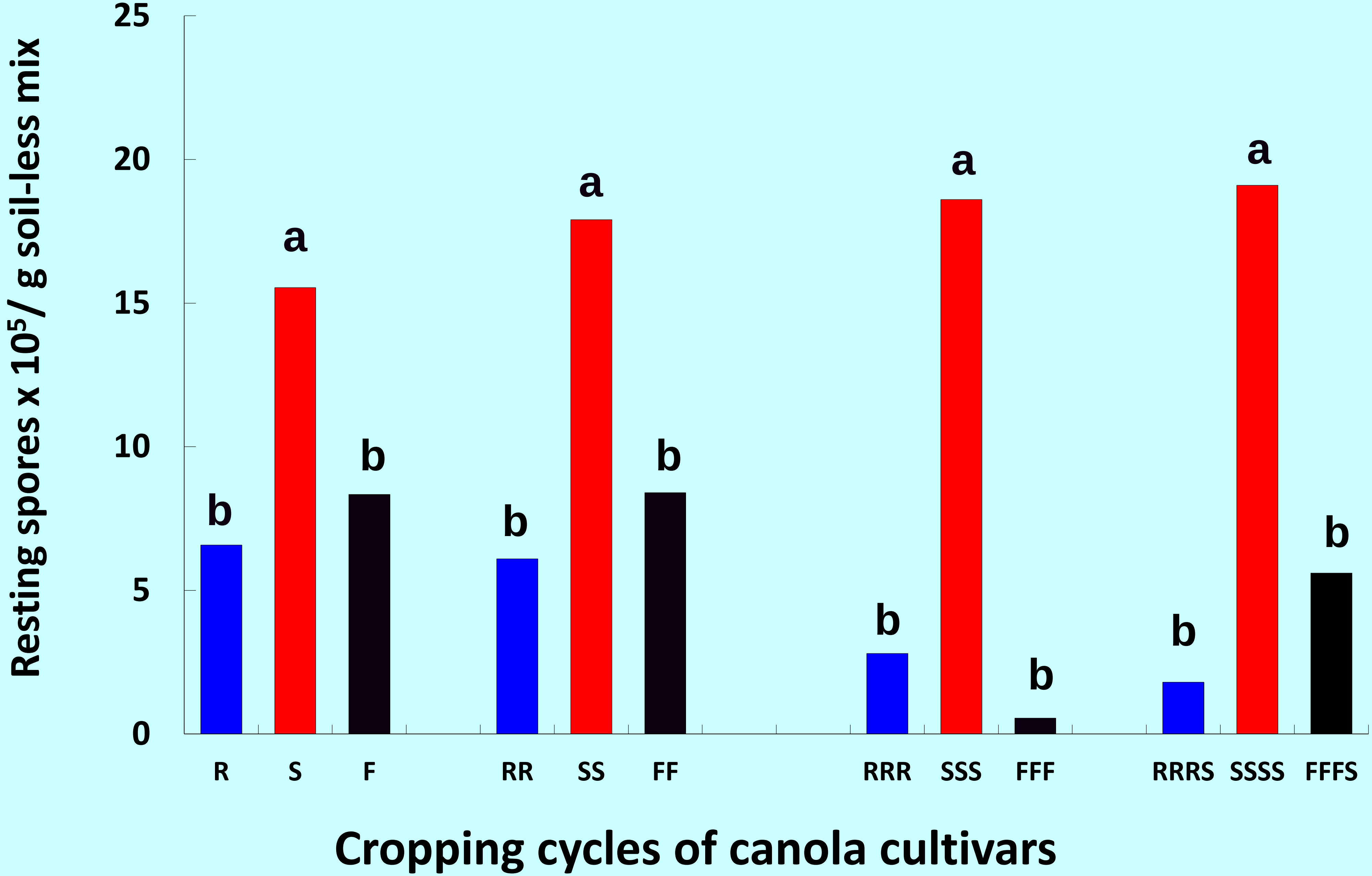


Effect of sequential growth of resistant and susceptible canola cultivars on index of disease



Results - Effects of growing resistant cultivars on clubroot severity in subsequent crops and resting spore population

- Plant height: FFFS>RRRS>SSSS
- Greater root mass in the susceptible cultivar resulted from gall formation.
- At the end of fourth cropping cycle, the disease severity on a susceptible canola cultivar grown in the potting mixture was 10-fold lower in the RRRS compared to the SSSS cropping sequence.
- The clubroot severity in FFFS sequence was also very low compared to the SSSS sequence.



Resting spores x 10^5/ g soil-less mix
25
20
15
10
5
0
b
a
b
b
a
b
b
a
b
b
a
b
R
S
F
RR
SS
FF
RRR
SSS
FFF
RRRS
SSSS
FFFS
Cropping cycles of canola cultivars

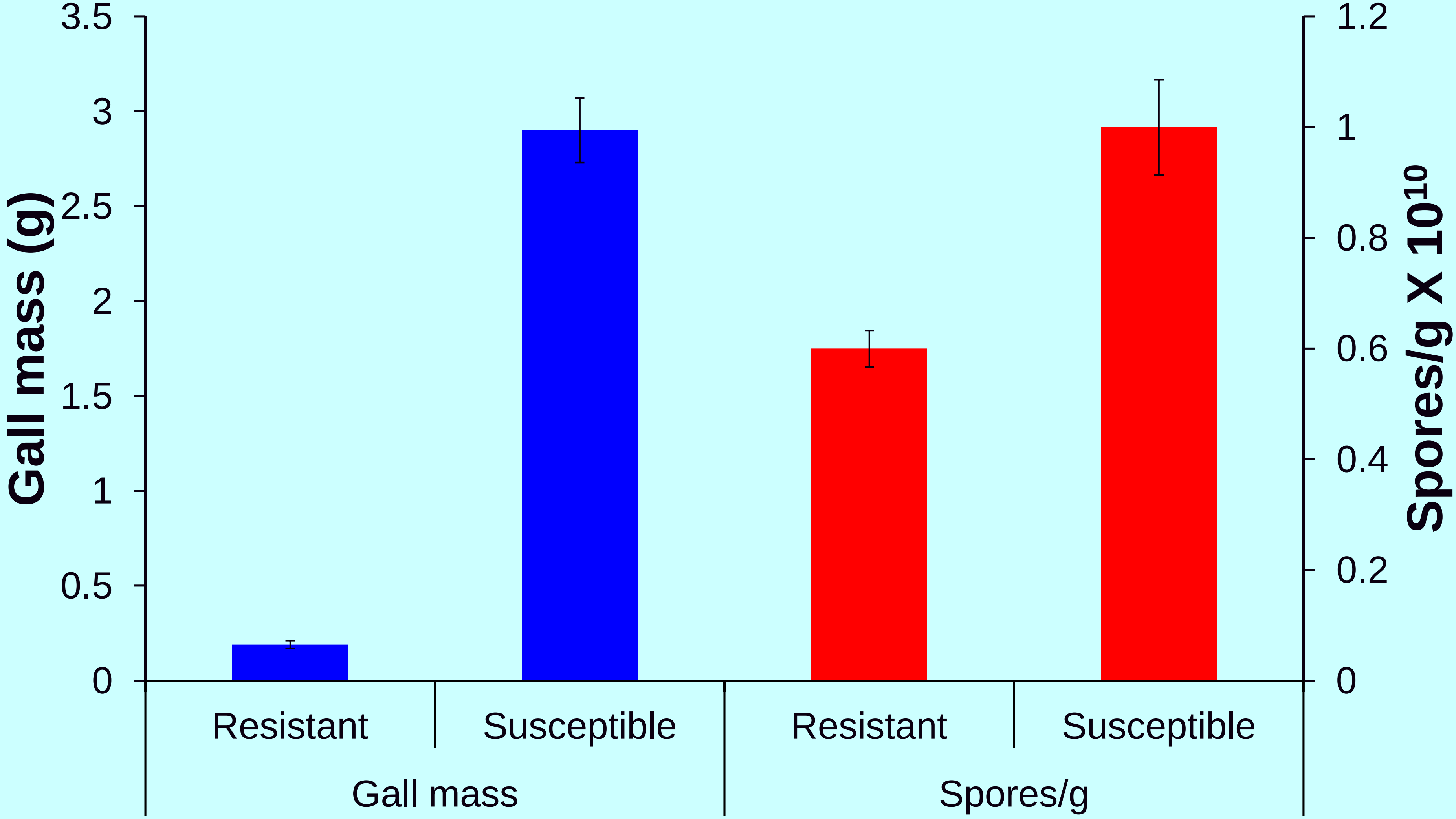
Results - Effects of resistant cultivars on resting spore population

- The number of resting spores following the SSSS sequence was **5 and 15-fold higher** than in the FFFS and RRRS sequences, respectively.
- After each cycle of cropping of susceptible canola (S, SS, SSS and SSSS) the inoculum density gradually increased.
- The resting spore density in the Susceptible sequence was greater relative to the Resistant or Fallow sequences.

B. Resting spore populations after cropping resistant and susceptible canola

- 45H29 (R) and 45H26 (S) canola cultivars were grown at 2 sites in heavily infested field soil.
- On August 16, 20 plants per replicate of a cultivar were uprooted and washed
- Gall mass and spores per gram of gall tissue were recorded.

Spore production in resistant and susceptible canola cultivars



Results - Resting spore contribution due to cropping resistant and susceptible canola

- The gall mass produced by the susceptible canola cultivar was 14-fold greater compared to the resistant canola cultivar.
- 14% of 45H29 were infected with clubroot; 100% of 45H26 plants were infected.
- Galls from the susceptible canola produced 10^{10} spores /g gall while those from the resistant canola produced 0.6 x10^{10} spores /g gall.

Conclusions

- Growing susceptible canola contributed more resting spores into the soil population than growing the resistant cultivar.
- Repeated growing of resistant canola and fallowing both reduced resting spore populations in the soil.
- However, repeated cultivation of a resistant cultivar may result in selection for pathogen phenotypes that can overcome this source of resistance.
- **Resistance Stewardship is needed.**

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Alberta Agriculture
Teamwork Recognition Award 2011

Thanks for your attention!