Evaluation of biofungicides for control of clubroot on canola

G. Peng, B.D. Gossen, S.E. Strelkov, S.F. Hwang and M.R. McDonald.
Agriculture and Agri-Food Canada, Saskatoon, SK S7N 0X2, Canada. (S.E.S.) *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada. (*S.F.H.) *Crop Diversification Centre North, Alberta Agriculture and Rural Development, 17507 Fort Road, Edmonton, AB T5Y 6H3, Canada.* (M.R.M.) *Department of Plant Agriculture, University of Guelph, Guelph ON N1G 2W1, Canada.*

Abstract
Clubroot of canola, caused by the protist pathogen *Plasmodiophora brassicae* (Pb), is an emerging threat to canola production in western Canada. Effective/practical control options are currently lacking. This study was initiated to assess registered microbial fungicides for control of clubroot on canola. Selected biofungicides were initially applied as a soil drench and the fungicides Allegro and Ranman were also included for comparisons. Selected products were further evaluated at varying concentrations, soil drench volumes, and for seed treatment. At moderate disease pressure, the biofungicides Serenade and Prestop, as well as synthetic fungicides Allegro and Ranman were highly effective as a soil-drench treatment, reducing clubroot severity by 85–100% in controlled conditions. Biofungicide concentration appeared to be important while soil-drench volumes may be reduced. All products, however, were significantly less effective or ineffective under extremely high disease pressure. All products were less efficacious in trials using infested field soils, a circumstance that may be related to treatment timing. Results from seed-treatment trials were too variable to draw a conclusion but there was a strong indication that this approach be successful though more research is required on microbial formulations. Serenade, Prestop, Allegro, and Ranman should be further evaluated under field conditions for clubroot control.

Introduction
Since the first discovery near Edmonton in by Tewari et al. (2004), clubroot has been found in more than 15 counties in Alberta (Alberta Agriculture and Food 2008) and is becoming an emerging threat to the canola industry (Financial Post 2007). All current commercial canola cultivars are highly susceptible (Strelkov et al. 2006). The pathogen builds up rapidly on susceptible crops and can also persist in soils for many years when a suitable host is absent.

There is generally a lack of effective, practical control options against clubroot in canola. Although crop rotation reduces pathogen inoculum load in the soil (Klasse 1996), which may alleviate disease impact on those least susceptible varieties (Wallenhammar et al. 2000), rotation intervals will be long due to pathogen's ability to persist in the soil with resting spores and to infect many weed species in the mustard family. In western Canada, potential impact of the disease is huge due to the size and intensity of canola production. Most management strategies developed for other crop systems are impractical for canola due to prohibitive costs. Experiences show that finding resistant genes against multiple pathotypes will likely be difficult (Diederichsen et al. 2006; Hirai 2006). Fungicides occasionally provide disease suppression (Donald et al. 2006; McDonald et al. 2004) but the cost (up to $900/ha) and application methods (e.g. soil incorporation) make these practices unsuitable for canola. Soil liming (Tremblay et al. 2005), calcium cyanamide (Donald et al. 2006; McDonald et al. 2004), and a phosphonate product (Abbasi and Lazarovits 2006) have been shown to reduce

clubroot on cruciferous vegetables, but they are likely impractical under most circumstances in field crops; Calcium cyanamide costs at east $400/ha (Donald et al. 2004) and the water volume required for phosphonate drench is over 30,000 L/ha on muck soils.

Recent research on Chinese cabbage in Japan highlights the potential of using soil microbes to control clubroot (Arie et al. 1999, Narisawa et al. 1998). Microorganisms, especially those of plant endophytes or rhizosphere colonizers, may move with roots and potentially protect them. This mechanism may be particularly useful for protection against clubroot due to long infection period by the pathogen in the soil. Several biofungicides targeting other soil-borne diseases show the ability of colonizing roots of many horticultural plants and may be of the potential evaluated for control or suppression of clubroot on canola. The ability of colonizing plant roots may facilitate efficient delivery of biofungicides as "inoculants", through seed treatment or in-furrow application, to achieve long-term root protection. Several biofungicides have been registered in Canada for greenhouse and horticultural crops or registered in the US, including Actinovate, Mycostop, Prestop, Root Shield, Serenade, SoilGard, and Taegro. The potential of these products for clubroot control is unknown but most of them have showed a general ability to colonize plant roots, compete with or suppress other soil-borne pathogens. If proved effective, some of these products may be used readily in canola crops because of their registration status in Canada.

The objective of this study was to evaluate selected microbial fungicide products for control of clubroot on canola using common delivery approaches.

Materials and Methods

A conetainer system (**Figure 1**) was developed to produce canola plants for clubroot control efficacy trials carried out in a level-2 pathology containment facility at AAFC Saskatoon. This system allows canola roots and plants to develop normally for 6-8 wks, proving sufficient time for clubroot symptoms to develop after inoculation. Plastic pots (4") were used in greenhouse trials at Crop Diversification Centre north (CDC North), Alberta Agriculture and Rural Development.

Canola plants

Seeds of a commercial Roundup-ready canola cultivar (Fortune RR in containment trials and cv. 34-6S RR in greenhouse trials) were sown in a soilless mix [1 part sand to approximately 12 parts of 1:2 sphagnum peat moss:vermiculite, amended with 1% (w/v) of 16-8-12 (N:P:K) control-released fertilizer]. Plants were kept at 23/18ºC (day/night) in a growth cabinet in the containment or in the greenhouse with 14 h supplementary daily lighting.

Plasmodiophora brassicae (Pb) inoculum

Galls of canola clubroot were collected from multiple fields in central Alberta, air dried, and stored at -15ºC until use. To extract resting spores for inoculum, about 3 g of dry galls were soaked in 150 ml water for 2 h to soften the tissue and macerated in a Waring blender at a high speed for 2 min. The resulting slurry was filtered through 4 layers of nylon cloth and spore concentrations estimated using a hemacytometer.

Inoculation and disease assessment
About one wk after seeding, each plant was inoculated with 1-5 ml of Pb inoculum at 10^6 or 10^7 resting spores/ml. Inoculated plants were watered daily with acidified water (pH 6.3) for the first week to keep the growth medium saturated, and thereafter with tap water (pH 8.0 – 8.5) when required. Clubroot development was rated 3 wks after inoculation using a 0-3 rating scale: 0= no galling; 1= small galls only, on less than 1/3 of roots; 2= small or medium-sized galls on 1/3 to 2/3 of roots; and 3= severe galling, medium to large-sized galls on more than 2/3 of roots (**Figure 2**). Disease severity index (DSI) was calculated based on the weight of each rating class observed

DSI (%) = $\sum$ (rating scale x No. of plants in the scale) x100 / (total No. of plants in the rep) x3

Figure 1. A conetainer system used for efficacy screening against clubroot root on canola

I. Disease response to Pb inoculum dose
This experiment was to determine the impact of pathogen inoculum pressure and identify a range of Pb doses that would cause a moderate severity of clubroot for biocontrol efficacy screening. Previous experiences indicated that extremely high disease pressure could overwhelm biocontrol treatments. Pb inoculum at the concentrations of 0 (control), 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7 resting spores/ml were applied as a drench to the growth medium at 1 to 5 ml per plant to inoculate canola seedlings. Inoculated plants were assessed for clubroot development using the 0-3 scale 3 to 5 wks after inoculation.

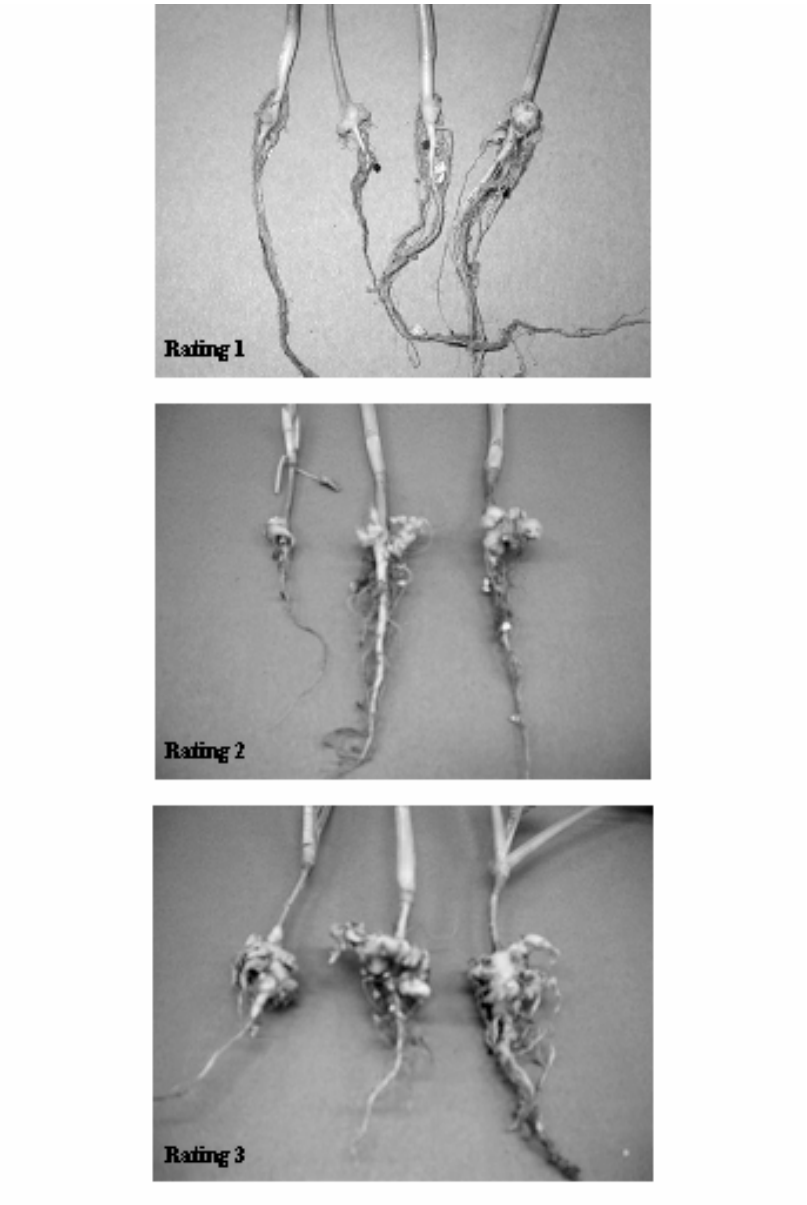

Figure 2. A pictorial rating scale for assessment of clubroot severity

II. Product efficacy – soil drench application

A. Trials in the containment at AAFC Saskatoon: Plants of canola cv. Fortune RR were produced in conetainers in growth cabinets (18–23ºC) and the following biofungicide or fungicide treatments were applied:

Treatments:

1. Control (blank)
2. Pb inoculation (pathogen check)
3. Mycostop® - *Streptomyces griseoviridis*, Verdera Oy

4. Prestop® - *Gliocladium catenulatum*, Verdera Oy
5. Root Shield® - *Trichoderma harzianum*, BioWorks Inc.
6. Actinovate® - *Streptomyces lydicus*, Natural Industries Inc.
7. Taegro™ - *Bacillus subtilis* var. *amyloliquefaciens*, Novozyme (registered in USA)
8. Serenade® ASO - *Bacillus subtilis*, AgraQuest Inc.
9. SoilGard®12 G – *Trichoderma virens*, Certis USA (registered in USA)
10. *Heteroconium chaetospira* (a fungal endophyte, provided by Dr. Narisawa, Japan)
11. Allegro 500F – Fluazinam (fungicide), ISK Biosciences Corp
12. Ranman® - Cyazofamid (fungicide), FMC Agricultural Products

Biofungicides (treatment 3-9) were applied at 5x label-rate concentrations and chemical fungicides (11 and 12) at 1x label rates. The treatment 10 (*H. chaetospira*) was a fungal endophyte that had demonstrated high efficacy against clubroot on Chinese cabbage in Japan. Conidia of *H. chaetospira* were produced on barley grains and conidial suspensions at10^6 spores/ml used. All treatments were applied as a soil drench 7-10 d after seeding at 50 ml/plant to saturate the growth medium. Biofungicide products/agents were applied 3 d prior to inoculation with the pathogen to allow the microorganisms to establish on canola roots whereas the two chemical fungicides were applied one h after the pathogen inoculation. Pb inoculum at10^6 resting spores/ml was applied at 5 ml /plant, and the plants were assessed 3 wks after inoculation.
These trials used a completely randomized design (CRD) with 7 replicates (canola plants) per treatment. A total of 3 trials (repetitions) were carried out between July and October of 2008. Data for individual trials (repetitions) were analyzed based on the disease rating (0-3), but data over 3 repetitions were analyzed using DSI calculated for each repetition. All data were subjected to ANOVA and, if significant (P=0.05), LSD was used to separate means.

B. Efficacy trials in greenhouse: Trials were conducted in a research greenhouse (20–22°C) at the CDC North, and the experimental protocol differed only slightly from the growth cabinet trials described above; canola cv. 34-6S RR was seeded in Pb-infested field soils in 4" pots or a non-infested soilless growth medium later inoculated with Pb resting spores. The following treatments were applied as a soil drench at 50 ml per pot:

Treatments
1. Pathogen control (check)
2. Mycostop® - Verdera Oy
3. Prestop® - Verdera Oy
4. Root Shield® - BioWorks Inc.
5. Actinovate® - Natural Industries Inc.
6. Serenade® ASO - AgraQuest Inc.
7. Allegro 500F – ISK Biosciences Corp
8. Ranman® - FMC Agricultural Products
9. Calcium Cyanamide (fungicide)

The biofungicides (treatments 2–6) were applied at 5x label rate and fungicides (treatments 7–9) at 1x label rate 1 week after seeding. The Pb inoculum was applied to non-infested growth medium 1 h after application of these treatments. Inoculated plants were kept in the greenhouse for 8 weeks to allow the development of clubroot symptoms.

Two trials were conducted between October 2008 and February 2009. In Trial 1, the infested field soil was mixed with soilless growth medium at a ratio of 1:1 (v/v) and the Pb inoculum (10^7 spores /ml) was applied to non-infested growth medium at 2 ml /pot. The Pb inoculum doses were reduced in Trial 2 by mixing the infested field soil with the soilless medium at a 1:2 ratio and applying Pb inoculum at 1 ml /pot to non-infested growth medium. The design of the experiment was a Randomized Complete Block Design with 4 replicates. Within a block, every treatment (including pathogen control) was applied to 10 plants.

III. Product efficacy - seed treatment

These trials were conducted at CDC north (two greenhouse trials, using 4" pots) and AAFC Saskatoon (one trial in a growth cabinet using the conetainer system). Canola seeds were immersed in a product solution/suspension for 5 min and air dried for 1 h prior to seeding. In the greenhouse trials, seeds were treated with biofungicides at 5x and fungicides at 1x label rate concentrations and planted into Pb-infested field soil (mixed with soilless growth medium at 1:1 and 1:2 rates in Trials 1 and 2, respectively) or a non-infested soilless growth medium inoculated later with Pb inoculum (10^7 spores/ml, 2 ml and 1 ml/pot for Trials 1 and 2, respectively). In the growth cabinet trial, all products were prepared at 10x label rate concentrations, and treated seeds were sown to a non-infested soilless growth medium later inoculated with Pb inoculum (10^7 spores/ml, 5 ml/plant). Treated plants were kept for 8 weeks for greenhouse trials and 3 wks for the growth cabinet trial, and then assessed for clubroot development. The eight products used in the Experiment III were evaluated in greenhouse trials but only Serenade, Prestop, Allegro and Ranman were tested for seed treatment in the growth cabinet trial, based on earlier soil-drench efficacy under the same condition.

IV. Effect of product concentration and drench volume

Trials were conducted in the containment facility at AAFC Saskatoon. Canola plants were produced in conetainers and the following treatments were applied:

Treatments

1. Control (blank)
2. Pathogen (check)
3. Prestop® - Verdera Oy
4. Serenade® ASO - Agraquest Inc.
5. Allegro 500F (fungicide) – ISK Biosciences Corp
6. Ranman® (fungicide) - ISK Biosciences Corp

The biofungicides were applied at 1x and 5x label rate concentrations 5 d after seeding and fungicides at 1x label rate 8 d after seeding. All products were applied as a soil drench at 50 ml and 25 ml /plant, respectively. The Pb inoculum (10^7 resting spores /ml) was applied at 5

ml /plant 8 d after seeding, 1 h prior to the fungicides. Treated plants were kept in growth cabinets for 3 wks before rating.

Results

I. Disease response to Pb inoculum dose

Inoculation of canola seedlings with Pb resting spores resulted in clubroot symptoms within 3 weeks (**Table 1**). Disease incidence and severity generally responded to the Pb inoculum dose applied; concentrations higher than 10^5 spores/ml caused clubroot on more than 50% of the plants and a moderate level of disease was caused by inoculation with higher than 10^6 spores /ml. As a result, Pb resting spore suspensions ranging from 10^6 to 10^7 spores /ml were considered appropriate for efficacy evaluation trials.

Table 1. Incidence and severity of canola clubroot symptoms caused by *Plasmodiophora brassicae* (Pb) at different inoculum concentrations [a].

Pb spore concentration (ml)	10^7	10^6	10^5	10^4	10^3	10^2
Disease incidence (%)	67	93	50	44	61	24
Disease severity index (DSI)	39	42	17	15	21	8

[a] Applied at 1 ml per plant.

II. Product efficacy – soil-drench application

In three trial repetitions at AAFC Saskatoon (in containment), the biofungicides Serenade, Prestop, and Mycostop reduced clubroot significantly in all trials (**Figure 3, 4**), with a mean reduction in DSI by 91%, 81%, and 61%, respectively, when compared to pathogen checks (**Table 2**). The fungicides Allegro and Ranman were also highly effective, reducing DSI by 91%. Other biofungicides were moderately effective to ineffective. Serenade at 5× label rate concentration caused slight stunting of canola seedlings in two of the three trials.

In greenhouse trials at the CDC north, the disease pressure was extremely high in the Trial 1, causing 100% DSI in the pathogen check in both Pb inoculation scenarios (infested soil and artificial inoculation). Biofungicide efficacy was generally low under this high disease pressure, but the fungicides Allegro and Ranman were noticeably more effective, especially in artificial Pb inoculation (**Table 3**). In the Trial 2, the reduction of Pb inoculum dose lowered DSI slightly in pathogen controls. All treatments were significantly more effective than in the Trial 1. Serenade, Actinovate, and Prestop were the most efficacious biofungicides. The fungicides were highly efficacious, especially against the artificial Pb inoculation. No negative effect on canola plants was seen with any of the products applied.

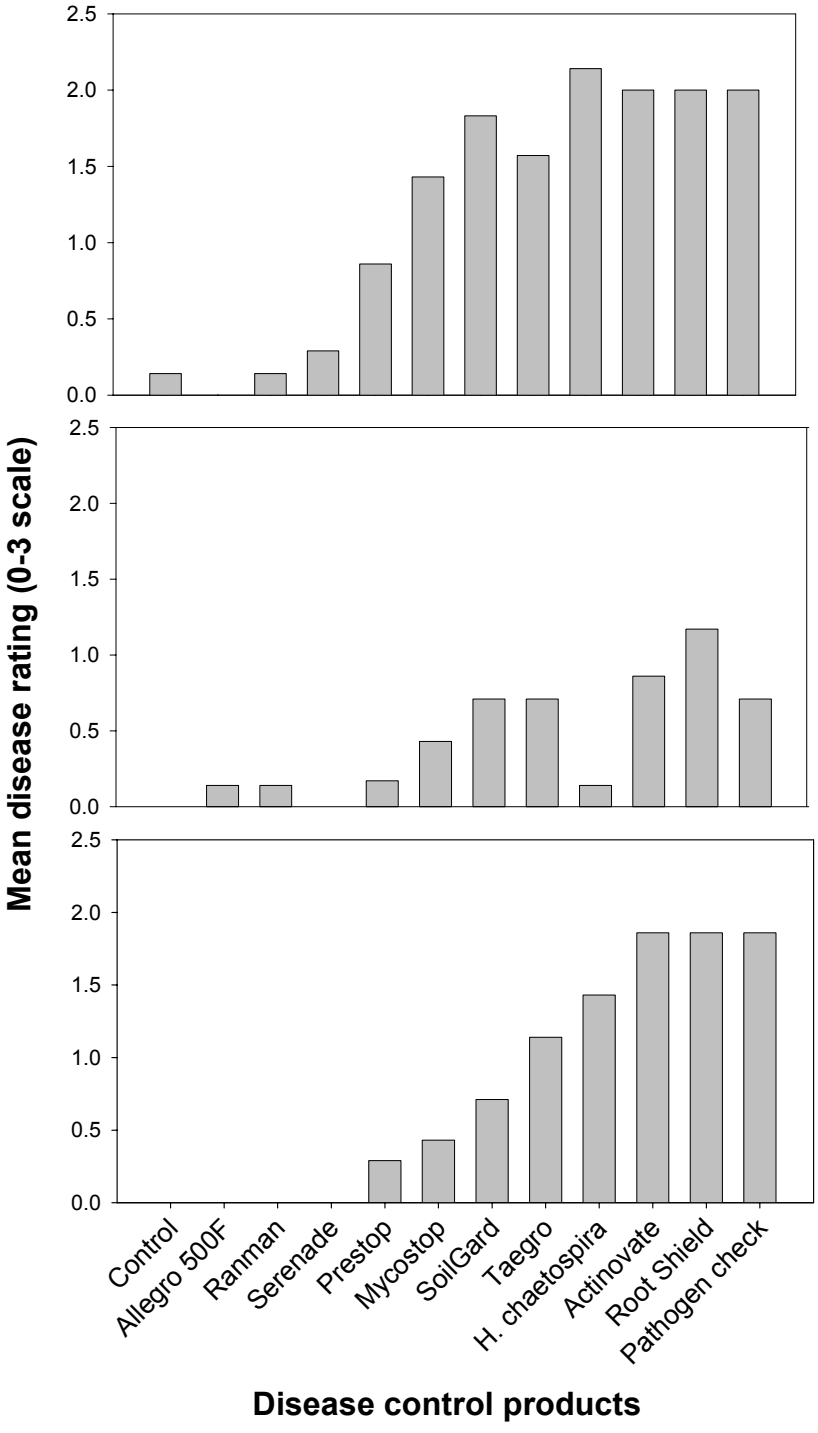

Figure 3. Efficacy of disease control products applied as a soil drench against clubroot on canola in three growth cabinet trials (from top to bottom: trial 1, 2, and 3).

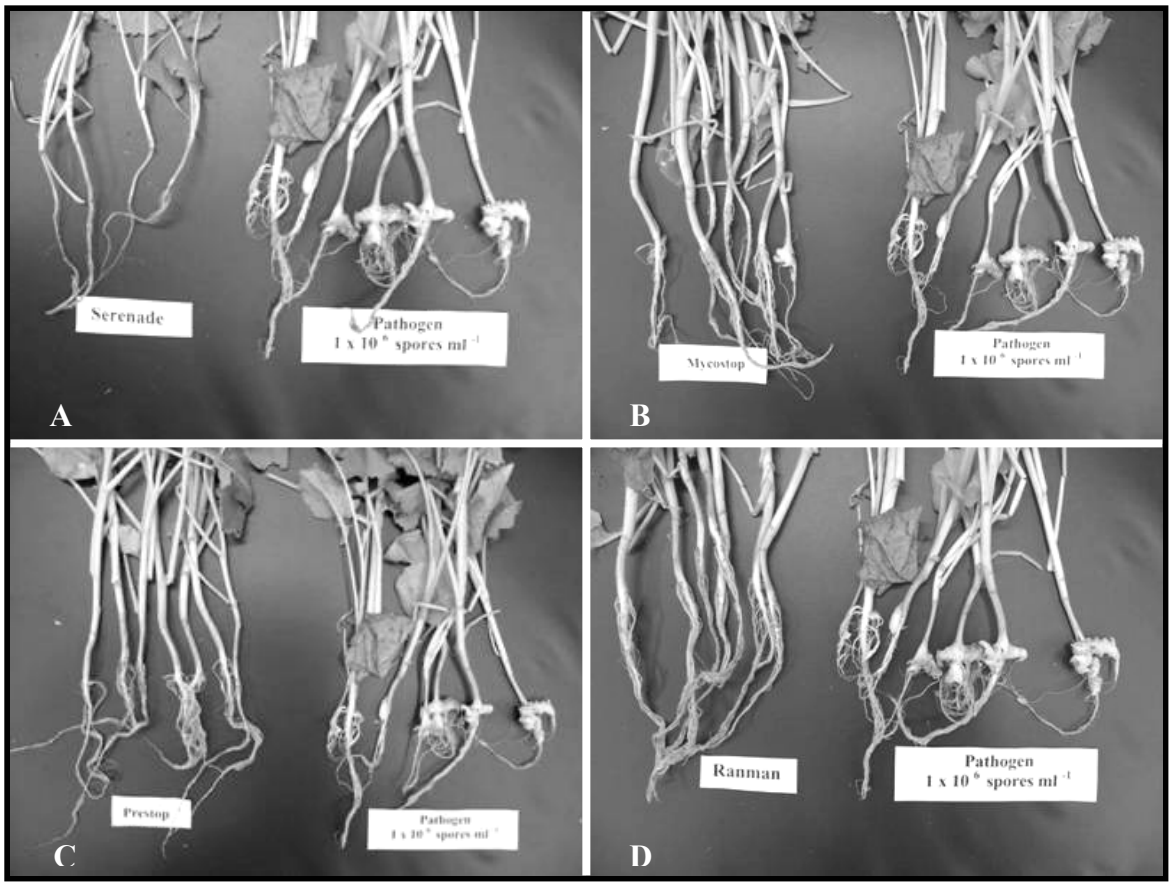

Figure 4. Efficacy of selected biofungicides and fungicides for control of clubroot on canola in controlled conditions. From A to D: Serenade, Mycostop, Prestop, and Ranman.

Table 2. Efficacy of selected products/agents against clubroot on canola (means of 3 trials)

Treatment	Disease severity index (%)	Clubroot reduction (%)
Untreated control	0 a	N/A
Serenade	3.2 ab	91.2
Ranman	3.2 ab	91.2
Allegro 500F	3.2 ab	91.2
Prestop	6.9 abc	81.1
Mycostop	14.3 abcd	60.8
Heteroconium chaetospira	19.1 bcde	47.8
SoilGard	23.8 cdef	34.8
Taegro	28.6 def	21.7
Pathogen check	36.5 efg	0.0
Actinovate	39.7 fg	- 8.7
Rootshield	46.6 g	- 27.6

Table 3. Effect of soil drench treatments on disease severity index (%) of clubroot on canola in two greenhouse trials (CDC, Edmonton)

Treatment	Trial 1		Trial 2	
	Pb-Infested field soil	Artificial Pb inoculation	Pb-Infested field soil	Artificial Pb inoculation
Pathogen check	100 a	100 a	98.3 a	75.8 a
Mycostop	93.4 ab	93.3 ab	76.7 b	33.3 b
Root Shield	85.8 bc	90.8 abc	45.0 cd	22.5 c
Serenade	90.0 abc	87.5 bc	68.3 b	2.5 e
Prestop	85.0 c	87.5 bc	36.1 de	13.1 cd
Actinovate	90.0 abc	85.8 bc	49.1 c	8.4 de
Calcium cyanamide	17.5 d	82.5 c	26.7 ef	1.7 e
Allegro 500	28.4 d	0 d	22.9 f	0 e
Ranman	23.4 d	0 d	10.7 g	0 e

III. Effect of seed treatment

As in the previous trials in greenhouse, extremely severe disease occurred in the Trial 1 and none of the treatments was effective (**Table 4**). The disease pressure was slightly lower in the Trial 2, and all treatments were noticeably efficacious, especially in the growth medium artificially inoculated with Pb (**Table 4**). The fungicides Allegro and Ranman were more effective than other treatments. The efficacy of seed treatment was lower than that of soil drench.

Table 4. Effect of seed treatment on disease severity index (%) of clubroot on canola in greenhouse trials (Edmonton)

Treatment	Trial 1		Trial 2	
	Pb-Infested field soil	Artificial Pb inoculation	Pb-Infested field soil	Artificial Pb inoculation
Pathogen check	100 a	100 a	80.0 a	75.0 a
Mycostop	95.8 ab	96.7 ab	55.6 bcd	34.3 bc
Root Shield	99.2 a	95.0 abc	68.4 ab	27.5 c
Serenade	94.2 abc	92.5 abcd	49.5 cd	43.0 b
Prestop	91.7 abc	88.4 bcd	61.1 bc	33.2 bc
Actinovate	90.8 abc	100 a	58.7 bc	35.3 bc
Calcium cyanamide	85.8 c	88.4 bcd	31.2 e	25.5 c
Allegro 500	89.2 bc	84.4 cd	40.1 de	6.7 d
Ranman	75.0 d	82.5 d	33.7 e	6.7 d

The seed treatment in the Saskatoon trial (growth cabinet) did not show clear efficacy (**Table 5**). Only Allegro reduced disease slightly (57%), and this efficacy was much lower than that seen in the greenhouse Trial 2.

Table 5. Effect of seed treatment on clubroot of canola in a growth cabinet trial (Saskatoon)

Treatment	Disease severity index (%)	Clubroot suppression (%)
Pathogen check	33.3	N/A
Serenade	38.1	0
Prestop	50.0	0
Allegro 500	14.3	57.0
Ranman	33.3	0

IV. Effect of product concentration and drench volume

The disease pressure in Trial 1 was too low for assessment of rate-volume effects, although all treatments showed lower level of disease than the pathogen check (**Table 6**). In Trials 2 and 3, 5x label rate concentration for biofungicides was more efficacious than the 1x label rate. Drench volume (25 ml vs. 50 ml) generally had no effect on the efficacy of fungicides or biofungicides.

Table 6. Effect of product concentration and soil-drench volume on canola clubroot severity.

Treatment	Trial 1	Trial 2	Trial 3
Water control	0.0	0 d	0 c
Pathogen check	16.7	66.7 a	85.7 a
1x Prestop, 25 ml drench	9.5	47.6 bc	85.7 a
1x Prestop, 50 ml	4.8	57.1 ab	88.9 a
5x Prestop, 25 ml	4.8	14.3 d	NT
5x Prestop, 50 ml	4.8	9.5 d	NT [a]
1x Serenade, 25 ml	4.8	33.3 c	77.8 b
1x Serenade, 50 ml	0.0	50.0 abc	81.0 ab
5x Serenade, 25 ml	4.8	5.6 d	16.7 c
5x Serenade, 50 ml	0.0	5.6 d	13.3 c
1x Allegro, 25 ml	4.8	4.8 d	9.5 c
1x Allegro, 50 ml	9.5	5.6 d	0 c
1x Ranman, 25 ml	0.0	0 d	0 c
1x Ranman, 50 ml	0.0	4.8 d	14.3 c

[a] The 5x Prestop label rate concentration was not tested because the product was used up.

Discussion and comments

The conetainer system is effective for efficacy screening against clubroot because inoculated plants can be maintained for weeks to allow for the disease to develop. It is also an efficient system for screening a large number of candidates rapidly without taking a huge growing space. It is particularly advantageous for trials in containment where space is limited.

The efficacy of biofungicide soil drench appears to be dependent on the product and pathogen inoculum pressure. Serenade and Prestop were frequently efficacious when disease pressure was moderate, but ineffective under high disease pressure. The other biofungicides, including Mycostop, Actinovate and Root Shield, were less effective or consistent. All products were noticeably less effective when applied to naturally infested field soils than to growth media inoculated artificially with the pathogen. This may be related to the timing of application; after germination, new roots can be infected once being in contact with pathogen inoculum in the infested soil, and treatments should also time this phase of infection to provide immediate protection.

Results from the seed-treatment trials were too variable to draw conclusions. However, it was encouraging that several biofungicides reduced clubroot substantially in one of the trials. The fungicides showed greater efficacy than biofungicides, but it is not clear if any formulation additives in these fungicides helped increase product retention on canola seeds. It is possible that the amount of products delivered with the seed-treatment method was too small to be consistently effective, and development of formulations specifically for microbial seed treatment may help pack greater doses of bioherbicides on relatively small canola seeds to enhance efficacy.

For biofungicide soil drench, product concentration appears to be important for efficacy, but drench volumes between 25 ml and 50 ml per plant showed little difference. It is possible to further reduce the drench volume by increasing product concentration and optimizing product placement. Low drench volumes will be practical due to limited ability to deliver products in a large liquid volume in canola fields.

Results of this study highlight the potential of using antagonistic microorganisms for control of clubroot. Because the biofungicide organisms tested in this study were isolated originally from other ecozones, the efficacy observed in controlled conditions should be validated under prairie field conditions. It may also be advisable to screen microorganism from canola roots on the prairies, and these indigenous candidates may adapt to prairie soil environments better than non-indigenous biofungicide agents.

Microorganisms in close association with canola roots, either as endophytes or rhizosphere inhabitants should be investigated extensively. Microbial endophytes are capable of beneficial infection and asymptomatic colonization of living tissues of their hosts. Recent studies in Japan have demonstrated the potential of the endophytic fungus *H. chaetospira* for control of clubroot on Chinese cabbage via induced disease resistance (Hashiba et al. 2003; Morita et al. 2003; Narisawa et al. 2002). Some endophytes can markedly increase stress tolerance in host plants (Redman et al. 2002; Rodriguez et al. 2004) or control other soil-borne diseases (Bacon and Hinton 2007). These features would be of additional benefit to canola production on the

Canadian prairies. Root endophytes and rhizosphere inhabitants are capable of moving with growing roots, which may be of practical value for clubroot control using microbial agents because their formulations, once developed, may be delivered efficiently as "inoculants", either through seed coating or in-furrow application, to provide long-term root protection. Eventually, this biocontrol strategy can be utilized with crop rotation and resistant canola cultivars for optimal management results.

In summary, the biofungicides Serenade and Prestop appear promising for control of clubroot on canola under moderate disease pressure. Product rates and application timing may affect efficacy of soil drench treatments. Seed treatment formulations should be developed to help pack more product materials on canola seeds and facilitate biocontrol activities in the soil. Several microbial and chemical fungicides should be tested further under field conditions to validate the efficacy against clubroot. Indigenous soil microorganisms should be investigated for better traits of interacting with canola roots against clubroot and other adversities.

Acknowledgement
Financial support from Pest Management Center of AAFC, Saskatchewan Canola Development commission, Alberta Canola Producers Commission to this research is gratefully acknowledged.

Thanks also go to the following people for technical assistant:
Linda McGregor and Margaret Molloy, technicians, AAFC Saskatoon
Dudley Chung, Jocelyn Reeve, Michelle Francisco, Coop students, AAFC Saskatoon
Robert Laprairie, FSWEP student, AAFC Saskatoon
Victor Manolii, Technician, University of Alberta
George Trumbull, CDC North, Aberta Agriculture and Rural Development

References cited

Abbasi, P. A. and G. Lazarovits. 2006. Effect of Soil Application of AG3 Phosphonate on the Severity of Clubroot of Bok Choy and Cabbage Caused by Plasmodiophora brassicae. Plant Dis. 90:1517-1522.

Alberta Agriculture and Rural Development. 2008. Clubroot disease of canola and mustard. http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex8593 (Retrieved November 24, 2008).

Arie, T., Kobayashi, Y., Okada, G., Kono, Y. and Yamaguchi, I. 1998. Control of soilborne clubroot disease of cruciferous plants by epoxydon from *Phoma glomerata*. Plant Pathology 47: 743-748.

Bacon, C.W. and Hinton, D.M. 2007. Potential for control of seedling blight of wheat caused by *Fusarium graminearum* and related species using the bacterial endophyte *Bacillus mojavensis*. Biocontrol Science and Technology 17:81-94.

Diederichsen, E., J. Beckmann, J. Schondelmeier, and F. Dreyer. 2006. Genetics of clubroot resistance in Brassica napus 'Mendel'. Acta horticulturae 706:307-311.

Donald, E. C., I. J. Porter, R. Faggian, and R. A. Lancaster. 2006. An integrated approach to the control of clubroot in vegetable Brassica crops. Acta horticulturae; 2006 Apr, no 706; 283 300.

Financial Post. 2007. http://www.financialpost.com/story.html?id=ed70cd9c-1144-4042-80b1-d54db0903236&k=83822.

Hashiba, T, Morita, S, Narisawa, K. and Usuki, F 2003. Mechanisms of symbiosis and disease suppression of root endophytic fungus. Nippon Bogeikagaku Kaishi - Japan Society for Bioscience, Biotechnology and Agrochemistry 77:130-133.

Hinton, D.M. and Bacon, C.W. 1995. *Enterobacter cloacae* is an endophytic symbiont of corn. Mycopathologia 129: 117-125.

Hirai, M. 2006. Genetic analysis of clubroot resistance in Brassica crops. Breeding Science 56:223-229.

Klasse, H.-J. 1996. Calcium cyanamide – An effective tool to control clubroot – A review. Acta Horticulturae 407: 403-409.

McDonald, M. R., B. Kornatowska, and A. W. McKeown. 2004. Management of clubroot of Asian Brassica crops grown on organic soils. Acta Horticulturae; 2004 Mar (635); 25 30.

Morita, S., Azuma, M., Aoba, T., Satou, H., Narisawa, K. and Hashiba, T. 2003. Induced systemic resistance of Chinese cabbage to bacterial leaf spot and *Alternaria* leaf spot by the root endophytic fungus, *Heteroconium chaetospira*. Journal of General Plant Pathology 69: 71-75.

Narisawa, K., Kawamata, H., Currah, R.S. and Hashiba, T. 2002. Suppression of *Verticillium* wilt in eggplant by some fungal root endophytes. European Journal of Plant Pathology 108:103-109.

Narisawa, K., Shimura, M., Usuki, F., Fukuhara, S. and Hashiba, T. 2005. Effects of pathogen density, soil moisture, and soil pH on biological control of clubroot in Chinese cabbage by *Heteroconium chaetospira*. Plant Disease 89: 285-290.

Narisawa, K., Tokumasu, S. and Hashiba, T. 1998. Suppression of clubroot formation in Chinese cabbage by the root endophytic fungus, *Heterocronium chaetospira*. Plant Pathol. 47: 206-210.

Ohki, T., Masuya, H., Yonezawa, M., Usuki, F., Narisawa, K. and Hashiba, T. 2002. Colonization process of the root endophytic fungus *Heteroconium chaetospira* in roots of Chinese cabbage. Mycoscience 43: 191-194.

Redman, R.S., Sheehan, K.B., Stout, RG, Rodriguez, R.J. and Henson, J.M. 2002. Thermotolerance generated by plant/fungal symbiosis. Science 298:1581.

Rodriguez, R.J., Redman, R.S. and Henson, J.M. 2004. The role of fungal symbiosis in the adaptation of plants to high stress environments. Mitigation and Adaptation Strategies for Global Change 9: 261-272.

Strelkov, S.E., Tewari, J.P., and Smith-Degenhardt E. 2006. Characterization of *Plasmodiophora brassicae* populations from Alberta, Canada. Canadian Journal of Plant Pathology 28, 467-474.

Tewari, J. P., D. Orchard, M. Hartman, R. Lange, T. K. Turkington, and S. Strelkov. 2004. First report of clubroot of canola caused by Plasmodiophora brassicae in the Canadian prairies. Can. J. Plant Pathol. 26:228-229.

Tremblay, N., C. Belec, J. Coulombe, and C. Godin. 2005. Evaluation of calcium cyanamide and liming for control of clubroot disease in cauliflower. Crop protection 24:798-803.

Usuki, F. and Narisawa, K. 2007. A mutualistic symbiosis between a dark septate endophytic fungus, *Heteroconium chaetospira*, and a nonmycorrhizal plant, Chinese cabbage. Mycologia 99(2): 175-184.

Wallenhammar, A.-C., Johnsson, L., and Gerhardson, B. 2000. Agronomic performance of partly clubroot-resistant spring oilseed turnip rape lines. Journal of Phytopathology 148: 495-499.