PROJECT DETAILS

- **Funders:** Alberta Canola, SaskCanola
- **Research program:** Canola Agronomic Research Program (CARP)
- **Principal investigator:** Ron Howard
- **Collaborators/additional investigators:** Sharon Lisowski, Dustin Burke and Carol Pugh
- **Year completed:** 2012

Executive Summary
This project was undertaken to evaluate the efficacy of various disinfectants and thermal treatments against the resting spores of the clubroot pathogen *Plasmodiophora brassicae*, a disease of canola and other brassicaceous crops, under laboratory and greenhouse conditions. Effective treatments were also evaluated in commercial operations.

EcoClear™ Fast Acting Weed and Grass Killer, HyperOx® and Sodium Hypochlorite 10.8% were effective while Ag-Services Inc. General Storage Disinfectant, KleenGrow™, SaniDate® Disinfectant, Thymox™ and Virkon™ were marginally effective.

All thermal treatments of 40°C to 100°C proved effective at various minimum incubation times.

Cleaning, pressure washing and disinfection of farm and industrial equipment was effective at limiting the spread of the clubroot pathogen. Farm and industrial equipment has a great potential to spread soil from clubroot infested fields as indicated by the amount of soil recovered from a four-wheel drive tractor.

Introduction
Clubroot, caused by *Plasmodiophora brassicae*, is a serious disease of canola, mustard and cole crop vegetables (broccoli, Brussels sprouts, cabbage, cauliflower, Chinese cabbage, kale, kohlrabi, radish, rutabaga and turnip). Clubroot has been reported in a few cole crop garden plantings in Alberta in the past, but was first detected in canola near Edmonton in 2003 (Tewari et al., 2005). By 2007, clubroot had been confirmed in ten counties around Edmonton and one county in southern Alberta (Strelkov et al., 2008, Harding *et al.*, 2008). Yield losses in severely infested canola have ranged from 30 to100%. Clubroot has the potential to spread to most of the traditional canola growing areas of western Canada (Turkington *et al.*, 2004). Once a field becomes infested, it is difficult, if not impossible, to get rid of the pathogen because it produces long-lived resting spores. Resting spores are mainly spread via contaminated soil carried from field to field by equipment. Farm, oil field and construction machinery represent the greatest risk of spreading the disease as soil is frequently carried on these types of equipment.

In spring 2007, clubroot was added as a declared pest to Alberta's Agricultural Pest Act. The Alberta Clubroot Management Plan was developed to direct farmers and others working in infested fields on ways to prevent

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the build up and spread of clubroot (Hartman, 2007; Hartman *et al.*, 2008). Cleaning and sanitation of machinery and equipment is one of the key recommendations in the plan; however, we know very little about the relative efficiency of physical cleaning methods and the comparative efficacy of commercial chemical disinfectants. The evaluation of a wide variety of physical and chemical methods for reducing or eliminating clubroot spore contamination from hard surfaces will allow a direct comparison of their relative effectiveness. The findings from these studies will allow recommendations based on proven results. When these recommendations are put into practice they will enable agricultural producers, oil & gas and construction companies, and others to select practical and cost-effective methods for use in their respective situations, including those with low impacts on plants, soils and the environment. The result will be more effective management of the spread of clubroot to new fields. If the average yield loss due to clubroot was 10% and improved sanitation prevented spread of the disease by 20%, it would save the canola industry approximately $36 million per year. Improved sanitation is a rapid and cost-effective strategy to manage clubroot in canola and can be broadly applied by the agricultural, oil & gas and construction industries. Other efforts, such as the development of resistant varieties of canola, may take years to accomplish. The development and adoption of sound sanitation recommendations should give a very impressive return on investment for the management of clubroot in Alberta.

Project Overview

Objectives:

1. To compare the relative effectiveness of selective physical methods in removing soil and plant residues from surfaces and for killing spores of the clubroot pathogen.
2. To compare a variety of chemical methods in removing soil and plant residues from surfaces and for killing spores of the clubroot pathogen.

Deliverables:

1. Experimental protocols for critically assessing the relative effectiveness of physical and chemical treatments for cleaning and disinfesting machinery, equipment and tools.
2. Efficacy rankings and the relative advantages and disadvantages for the various cleaning and disinfection methods under test.
3. Development of sanitation protocols tailored to the specific needs of the agriculture, oil & gas and construction industries.

Project Team Members

Alberta Agriculture and Rural Development Crop Diversification Centre South, Brooks, Alberta

- Dr. Ronald Howard, Plant Pathology Research Scientist
- Mrs. Sharon Lisowski, Senior Plant Pathology Technologist
- Mr. Dustin Burke, Plant Pathology Technologist
- Ms. Carol Pugh, Plant Pathology Technologist

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Laboratory and Greenhouse Research Trials

Efficacy of Disinfectants and Thermal Treatments against Clubroot Resting Spores *In Vitro* and *In Vivo*, 2009

Abstract

An *in vitro* method was developed to treat suspensions of clubroot resting spores with various disinfectants, concentrations and exposure times as well as various temperatures and exposure times and subsequently use these treated resting spores to inoculate *Brassica napus* seedlings, observe disease development under greenhouse conditions and quantify symptoms (plant-bait assays). Treated spores were also observed directly and spore survival rate was quantified with bright-field microscopy (staining analysis).

To evaluate the efficacy of disinfectants, the plant-bait assays proved reliable while the staining analysis proved unreliable. To evaluate the efficacy of thermal treatments, the plant-bait assays proved reliable while the staining analysis proved marginally reliable with clubroot resting spores prepared from relatively fresh gall material, although the efficacy of the thermal treatments was underrepresented when compared to the plant-bait assays.

Bright-field microscopy staining analysis proved more reliable and less technically challenging that fluorescent microscopy staining analysis.

EcoClear™ Fast Acting Weed and Grass Killer, HyperOx® and Sodium Hypochlorite 10.8% proved effective at recommended label rates or less with recommended label exposure times as indicated by plant-bait assays. Ag-Services Inc. General Storage Disinfectant, KleenGrow™, SaniDate® Disinfectant, Thymox™ and Virkon™ proved effective at higher than recommended label rates with recommended label exposure times as indicated by plant-bait assays. Biostel® EO Anode Water and dutrion tablets proved ineffective at higher than recommended label rates with recommended label exposure times as indicated by plant-bait assays.

All thermal treatments of 40°C to 100°C proved effective at various minimum incubation times for each temperature as indicated by plant-bait assays.

Relatively small sample sizes and high seedling mortality due to bare-root transplanting created data with a high degree of variability. Improvements to protocols would include inoculating stem only to eliminate transplanting and the use of larger sample sizes with smaller increments of disinfectant concentrations and thermal incubation times to create dose-response curves with more statistical significance.

Objective

To evaluate the efficacy of plant-bait assays and staining analysis of clubroot resting spores treated with various disinfectants and thermal treatments and subsequently evaluate disinfectants and thermal treatments for efficacy against clubroot resting spores by the use of plant-bait assays and staining analysis.

Study Team

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Materials

Ag-Services Inc. General Storage Disinfectant (n-Alkyl [40% C_{12}, 50% C_{14}, 10% C_{16}] dimethyl benzyl ammonium chloride, 10.0%), Biostel® EO Anode Water (hypochlorous acid, 75, 90 and 100%), dutrion tables (chlorine dioxide, 0.2%), EcoClear™ Fast Acting Weed And Grass Killer (acetic acid, 250 g L^{-1}), HyperOx® (hydrogen peroxide, 25%; peracetic acid, 5%), KleenGrow™ (didecyl dimethyl ammonium chloride, 7.5%), SaniDate® Disinfectant (hydrogen peroxide, 27.11%; peracetic acid, 2.0%), Sodium Hypochlorite 10.8% (sodium hypochlorite, 10.8%), Thymox™ (thymol, 18%), Virkon™ (potassium peroxymonosulfate, 21.4%)

Methods

An *in vitro* method was developed to treat suspensions of clubroot resting spores with various disinfectants, concentrations and exposure times and subsequently use these treated resting spores to inoculate *Brassica* spp. seedlings, observe disease development under greenhouse conditions and quantify symptoms. Argentine canola spring hybrid cv. 45H26 (*Brassica napus*) was chosen as a susceptible canola variety which grows more upright and takes up less greenhouse space than susceptible Chinese cabbage cv. Granaat (*Brassica rapa* ssp. *Pekinesis*), which is typically used in clubroot research. Seedlings were transplanted into root trainers to allow the development of a larger taproot for subsequent early disease development. The following protocol was developed at CDC South:

1. Germinate a susceptible cultivar of canola seedlings in a greenhouse on coarse horticultural grade perlite in a seedling insert ca. 7-10 days before trial is anticipated so the seedlings are at the two leaf stage.

2. Fill 1.5 in. by 8 in. root trainers with peat-based growing medium (Sun Gro Horticulture Canada Ltd. Sunshine Professional Peat-Lite Mix no. 4 Aggregate).
3. Remove galls from canola roots that have been kept at ca. -20°C for no longer than ca. one year. Homogenize with laboratory mill using a coarse screen. Dilute ca. 3 g of ground gall in ca. 25 mL of 5% v/v glycerol in distilled water and agitate. Filter with a 40 µm cell strainer. Adjust spore concentration to ca. 1×10^7 spores mL^{-1} and store at ca. 4°C. Do not use a spore suspension that has been stored for more than ca. 24 hrs.
4. Transfer 5.00 mL of spore suspension to five mL vials. Use an untreated check when warranted and the appropriate number of replications.
5. Transfer appropriate amount of disinfectant and incubate for appropriate time.
6. After incubation time, filter 1.50 mL of suspension though a 47 mm × 0.8 µm microbiological analysis membrane filter. Do this in triplicate and transfer two membranes to a 13 × 100 mm culture tube with 5.0 mL of sodium phosphate buffer (pH = 8.0 and 10 mM) and one membrane to a 13 × 100 mm culture tube with 1.0 mL of sodium phosphate buffer.

7. In cases where a disinfectant is not meant to be diluted, filter 5.00 mL of spore suspension and add membrane filter directly to 5.00 mL of disinfectant.
8. Transfer contents of five mL culture tube to a disposable 35 mm Petri dish. Mix seedling root in the spore suspension for ca. 10 sec. and transplant. Roots may be trimmed for ease of transplanting. Ensure that soil is thoroughly wetted before transplanting to prevent seedlings from drying out. After transplanting, inoculate the base of the seeding by pipetting 200 µL of spore suspension into a small depression with the contents of the other five mL culture tube. Do not combine different products or rates in the same tray. Transfer to a growth chamber for ca. two weeks (16 hrs. at 23°C and maximum light intensity and 8 hrs. at 18°C and no light with ambient humidity) and then to a greenhouse (minimum of 16 hrs. of daylight at 23°C and 8 hrs. at 18°C with ambient humidity). Water with greenhouse nutrient solution only (225 ppm N and pH = 6.0) from the top only to prevent spores from spreading between root trainers.
9. Transfer 50 µL from the one mL culture tube to two micro centrifuge tubes. Incubate one tube with an equal volume of Evan's blue (20 mg mL^{-1}) stain (10-30 min.) and one tube with an equal volume of ethidium bromide (50 µg mL^{-1}) and calcofluor white M2R (100 µg mL^{-1}) stain (16 hrs.). Prepare the Evan's blue stain no longer than one month before use and prepare the ethidium bromide and calcofluor white M2R stain no longer than one week before use.
10. Observe Evan's blue stained cells with bright-field microscopy at 8 × ocular and 100 × objective. Observe ethidium bromide and calcofluor white M2R stain with fluorescent microscopy at 8 × ocular and 100 × objective. Rate a minimum of 100 spores per sample as unviable or viable.
11. After ca. six weeks after inoculation, rate galls using 0-3 scale where 0 = no galling; healthy plant; 1 = a few small galls; small galls on less than 1/3 of roots; 2 = moderate galling; small to 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) based on Kuginuki *et al.* (1999) as modified by Xue *et al.* (2008). Calculate disease severity by use of the following formula: D.S. = $(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3)/N_T$ where N_0 = number of plants with a rating of 0 … and N_T = total number of plants. Calculate index of disease (in order to convert disease severity to a percentage for ease of statistical analysis as data presented as small whole units often are not normally distributed) by the following formula: I.D. = $(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3)/(N_T \times 3) \times 100\%$ based on Horiuchi and Hori (1980) as modified by Strelkov *et al.* (2006).
12. Perform regression analysis on staining analysis and plant bait assays and correlation analysis comparing staining analysis and plant bait assays.
13. Stir or vortex between each transfer in the protocol. Label all culture tubes, vial and root trainers before proceeding.

The two staining assays were evaluated with thermal treatments at 40.0°C and 60.0°C at various incubation times and regression analysis was subsequently performed.

Ten commercial disinfectants were chosen for initial trials. Spore suspensions were exposed to each product at zero, one-half, one, two and five times manufacture's label rate. Spores were exposed for times indicated by product label or recommended by manufacture. Spore mortality was rated using the Evan's blue staining

analysis and the plant bait assay with regression analysis and correlation analysis subsequently performed.

Spore suspensions were prepared as described above and 5.0 mL aliquots were incubated in a digital dry bath at various times and temperatures. Spore mortality was rating using the Evan's blue staining analysis and plant bait assay previously described.

Results and Conclusions

Evaluation of the Evan's blue analysis by regression analysis proved to be a suitable assay as the LD_{50} value for 40°C (Figure 1) was 0.0 hrs. indicating that the spore mortality did not increase over time while the LD_{50} value for 60°C (Figure 2) was 2.4 hrs. indicating that spore mortality increased by one-half at 2.4 hrs. similar to previously published data. Evaluation of the ethidium bromide and calcofluor white M2R analysis did not give consistent results. Fluorescent microscopy also proved to be more technically challenging than bright-field microscopy.

For the disinfectant treatments, regression analysis of the staining analysis (Table 1) indicated that only KleenGrow™ had a significant LD_{50} value while regression analysis of the plant bait assay (Table 2) indicated that all products had significant LD_{50} values and were effective at increased rates with the exception of Biostel® EO Anode Water and dutrion tablets. EcoClear™ Fast Acting Weed and Grass Killer, HyperOx® and Sodium Hypochlorite 10.8% were the most effective followed by Virkon® followed by SaniDate® Disinfectant followed by Thymox™ followed by Ag-Services Inc. General Storage Disinfectant followed by KleenGrow™ (Figures 6-10). Correlation analysis between the staining analysis and plant bait assay (Table 3) indicated that KleenGrow™ had a highly statistically ($P \leq 0.01$) significantly strong correlation and Ag-Services Inc. General Storage Disinfectant had a statistically ($P \leq 0.05$) significantly weak correlation . Based on this data, it would appear that the staining analysis is not an effect method to evaluate the mortality of spores treated with disinfectants.

For the thermal treatments, regression analysis of the staining analysis (Table 4) indicated that only 80, 90 and 100°C (Figures 3 to 5) had an LD_{50} value less than the maximum exposure time while regression analysis of the plant bait assay (Table 5) indicated that 70, 80, 90 and 100°C had significant LD_{50} values while 80, 90 and 100°C had LD_{50} values of ca. 1.0 hrs. or less.

In general, with few exceptions, most disinfectants appeared to be highly effective at one or more rates and the higher rates of thermal treatments appeared to be highly effective.

Future disinfectant trials will not include the products that appeared to be ineffective based on the plant bait assay and will include more rates for products that appeared to be highly effective.

High seedling mortality due to bare-root transplanting proved to be problematic in subsequent trials. Further investigation concluded germinating seed directly in root trainers and inoculating the base of the stem at the two-leaf stage proved effective.

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Many of the treatment variances for much of the data were not homogeneous which may warrant larger sample sizes for future trials.

Table 1. Mortality of treated clubroot resting spores as indicated by a staining analysis.[1]

Rate[2]	Spore mortality (%)[3]									
	Ag-Services Inc. General Storage Disinfectant	Biostel® EO Anode Water[3]	dutrion tablets	EcoClear™ Fast Acting Weed and Grass Killer[3]	HyperOx®	KleenGrow™	SaniDate® Disinfectant	Sodium Hypochlorite 10.8%	Thymox™	Virkon™
0.0	12.9	13.8	10.8	20.8	17.3	10.3	17.1	11.6	9.2	14.6
0.5	15.9	11.6	15.0	24.8	12.5	9.2	20.0	18.1	9.3	10.9
1.0	18.9	19.4	17.3	16.4	14.7	16.6	15.3	14.4	8.2	16.0
2.0	18.2	19.1	12.1	18.8	12.4	31.2	20.1	15.7	12.9	16.2
5.0	22.8	-	12.3	21.2	18.9	84.4	16.8	14.9	10.5	16.0
LD_{50}	NS[4]	NS[4]	NS[4]	NS[4]	NS[4]	2.80	NS[4]	NS[4]	NS[4]	NS[4]
90% CL	-	-	-	-	-	2.6 3.0	-	-	-	-

[1]Results are the means of five replications. Probit-least squares dose-response regression analysis performed.
[2]Rates are multiples of manufacturer's label rates. Spores exposed for times indicated by label or recommended by manufacture.
[3]Rates are 0, 75, 90 and 100% Biostel® EO Anode Water and 0, 10, 20, 40 and 100% for EcoClear™ Fast Acting Weed and GrassKiller.
[4]Denotes non-significant.

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Table 2. Disease severity of canola seedlings inoculated with treated clubroot resting spores.[1]

Rate[2]	Disease severity (0-3)[3]									
	Ag-Services Inc. General Storage Disinfectant	Biostel® EO Anode Water[4]	dutrion tablets	EcoClear™ Fast Acting Weed and Grass Killer[4]	HyperOx®	KleenGrow™	SaniDate® Disinfectant	Sodium Hypochlorite 10.8%	Thymox™	Virkon™
0.0	2.40	2.63	2.96	2.88	1.99	2.59	2.79	2.44	2.44	2.94
0.5	1.94	2.81	2.88	0.00	0.00	2.88	0.15	2.84	2.84	2.95
1.0	1.74	2.65	2.81	0.00	0.00	2.98	0.00	2.57	2.57	2.66
2.0	1.75	2.87	2.94	0.00	0.00	2.81	0.00	1.45	1.45	0.00
5.0	0.76	-	2.60	0.00	0.00	0.09	0.00	0.38	0.38	0.00
LD_{50}	1.64	0.98	NS[5, 6]	0.00	0.00	1.72[6]	0.00	0.89[6]	0.89[6]	0.38[6]
90% CL	0.66 6.18	NS[5]	NS[5]	NS[5, 7]	NS[5, 7]	1.12 3.58	NS[5, 7]	0.30 1.48	0.30 1.48	0.00 0.85

[1]Results are the means of five replications. Probit-least squares dose-response regression analysis performed.
[2]Rates are multiples of manufacturer's label rates. Spores exposed for times indicated by label or recommended by manufacture.
[3]Galls rated ca. six weeks after inoculation. Rating scale: 0 = no galling; healthy plant; 1 = a few small galls; small galls on less than 1/3 of roots; 2 = moderate galling; small to 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.
[4]Rates are 0, 75, 90 and 100% for Biostel® EO Anode Water and 0, 10, 20, 40 and 100% for EcoClear™ Fast Acting Weed and GrassKiller.
[5]Denotes non-significant.
[6]Treatment variances are not homogeneous. The assessment column failed Barlett's test for homogeneity and

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thus violates the ANOVA assumption of homogeneity of variance.
[7]The slope of the probit line is 0.00, therefore 90% confidence limits cannot be computed.

Table 3. Correlation analysis of spore mortality and disease severity.[1]

	Coefficient of determination and correlation coefficient									
	Ag-Services Inc. General Storage Disinfectant	Biostel® EO Anode Water	dutrion tablets	EcoClear™ Fast Acting Weed and Grass Killer	HyperOx®	KleenGrow™	SaniDate® Disinfectant	Sodium Hypochlorite 10.8%	Thymox™	Virkon™
r^2	0.1868	0.0008	0.0273	0.0008	0.0261	0.8584	0.0004	0.0865	0.0075	0.1162
r	-0.4323	0.0280	0.1652	0.0289	0.1616	-0.9265	-0.0201	-0.2942	-0.0864	-0.3409
P (r)	0.0309	0.9068	0.4300	0.8910	0.4404	0.0001	0.9239	0.1535	0.6815	0.0954

[1]Disease severity data was calculated as index of disease (%) = $(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3)/(N_T \times 3) \times 100$.

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Table 4. Mortality of thermally treated clubroot resting spores.[1]

Time (hrs.)	Spore mortality (%)						
	40°C	50°C	60°C	70°C	80°C	90°C	100°C
0.0	5.8	7.3	3.8	3.7	9.1	7.3	3.7
0.5	-	-	-	-	-	71.0	49.2
1.0	-	-	-	-	71.5	82.8	56.1
1.5	-	-	-	-	68.6	82.8	70.2
2.0	-	-	-	4.1	-	85.4	71.8
2.5	-	-	-	-	-	89.0	81.4
3.0	-	-	-	-	69.4	95.3	93.3
3.5	-	-	-	-	-	-	-
4.0	-	-	7.5	7.3	77.3	-	-
5.0	-	-	-	-	80.9	-	-
6.0	-	-	-	7.7	82.5	-	-
8.0	-	10.0	8.4	13.2	-	-	-
10.0	-	-	-	15.5	-	-	-
12.0	8.5	-	7.5	13.6	-	-	-
16.0	-	6.5	12.7	-	-	-	-
20.0	-	-	14.0	-	-	-	-
24.0	9.1	7.7	16.2	-	-	-	-
32.0	-	7.7	-	-	-	-	-
36.0	7.5	-	-	-	-	-	-
40.0	-	8.5	-	-	-	-	-
48.0	10.0	14.3	-	-	-	-	-
60.0	17.1	-	-	-	-	-	-
72.0	16.1	-	-	-	-	-	-
LD_{50}	1157[3]	NS[2]	517	64[3]	0.22	0.18	0.68[3]
90% CL	608 3261	-	244 1838	44 115	0.07 0.40	0.12 0.24	0.62 0.74

[1]Results are the means of five replications. Probit-least squares dose-response regression analysis performed.
[2]Denotes non-significant.
[3]Treatment variances are not homogeneous. The assessment column failed Barlett's test for homogeneity and thus violates the ANOVA assumption of homogeneity of variance.

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Table 5. Mortality of thermally treated clubroot resting spores.[1]

Time (hrs.)	Spore mortality (%)						
	40°C	50°C	60°C	70°C	80°C	90°C	100°C
0.0	20.9	15.1	13.0	12.4	14.2	13.1	10.4
0.5	-	-	-	-	-	36.4	45.7
1.0	-	-	-	-	66.2	51.9	54.4
1.5	-	-	-	-	64.5	46.5	60.6
2.0	-	-	-	59.3	-	72.9	66.3
2.5	-	-	-	-	-	92.4	75.8
3.0	-	-	-	-	67.2	89.0	72.1
3.5	-	-	-	-	-	-	-
4.0	-	-	20.6	32.9	87.2	-	-
5.0	-	-	-	-	65.2	-	-
6.0	-	-	-	64.5	64.0	-	-
8.0	-	21.2	30.8	52.4	-	-	-
10.0	-	-	-	66.3	-	-	-
12.0	19.7	-	30.8	67.2	-	-	-
16.0	-	19.3	27.3	-	-	-	-
20.0	-	-	25.9	-	-	-	-
24.0	21.2	15.7	32.2	-	-	-	-
32.0	-	25.3	-	-	-	-	-
36.0	14.7	-	-	-	-	-	-
40.0	-	23.6	-	-	-	-	-
48.0	17.6	36.8	-	-	-	-	-
60.0	21.7	-	-	-	-	-	-
72.0	22.5	-	-	-	-	-	-
LD_{50}	NS[2]	NS[2]	NS[2]	5.10	0.01	1.15	0.96
90% CL	-	-	-	4.17 6.08	NS[3]	1.10 1.20	0.86 1.05

[1]Results are the means of five replications. Probit-least squares dose-response regression analysis performed.
[2]Denotes non-significant.
[3]The slope of the probit line is 0.00, therefore 90% confidence limits cannot be computed.

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Table 6. Disease severity of canola seedlings inoculated with thermally treated clubroot resting spores.[1]

Time (hrs.)	Disease severity (0-3)[2]						
	40°C	50°C	60°C	70°C	80°C	90°C	100°C
0.0	0.30	2.66	2.93	2.65	1.97	2.17	2.16
0.5	-	-	-	-	-	0.00	0.00
1.0	-	-	-	-	0.00	0.00	0.00
1.5	-	-	-	-	0.00	0.00	0.00
2.0	-	-	-	0.03	-	0.00	0.00
2.5	-	-	-	-	-	0.00	0.00
3.0	-	-	-	-	0.00	0.00	0.00
3.5	-	-	-	-	-	-	-
4.0	-	-	0.00	0.03	0.00	-	-
5.0	-	-	-	-	0.00	-	-
6.0	-	-	-	0.06	0.00	-	-
8.0	-	0.90	0.13	0.00	-	-	-
10.0	-	-	-	0.00	-	-	-
12.0	0.33	-	0.00	0.00	-	-	-
16.0	-	0.63	0.00	-	-	-	-
20.0	-	-	0.00	-	-	-	-
24.0	0.00	0.00	0.00	-	-	-	-
32.0	-	0.20	-	-	-	-	-
36.0	0.00	-	-	-	-	-	-
40.0	-	1.53	-	-	-	-	-
48.0	0.00	1.00	-	-	-	-	-
60.0	0.00	-	-	-	-	-	-
72.0	0.00	-	-	-	-	-	-
LD_{50}	NS[3]	NS[3]	0.00	NS[3]	0.00	0.00	0.00
90% CL	-	-	NS[4]	-	NS[4]	NS[4]	NS[4]

[1]Results are the means of five replications. Probit-least squares dose-response regression analysis performed.
[2]Galls rated ca. six weeks after inoculation. Rating scale: 0 = no galling; healthy plant; 1 = a few small galls; small galls on less than 1/3 of roots; 2 = moderate galling; small to 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.
[3]Denotes non-significant.
[4]The slope of the probit line is 0.00, therefore 90% confidence limits cannot be computed.

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Table 7. Correlation analysis of spore mortality and disease severity.[1]

	Coefficient of determination and correlation coefficient						
	40°C	50°C	60°C	70°C	80°C	90°C	100°C
r^2	0.0000	0.0077	0.3032	0.3254	0.6917	0.4160	0.6199
r	-0.0066	-0.0880	-0.5507	-0.5704	-0.8317	-0.6450	-0.7873
P (r)	0.9699	0.6153	0.0006	0.0003	0.0001	0.0001	0.0001

[1]Disease severity data was calculated as index of disease (%) = $(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3)/(N_T \times 3) \times 100$.

Figure 1. Thermal treatment of sprores at 40 degrees Celsius.

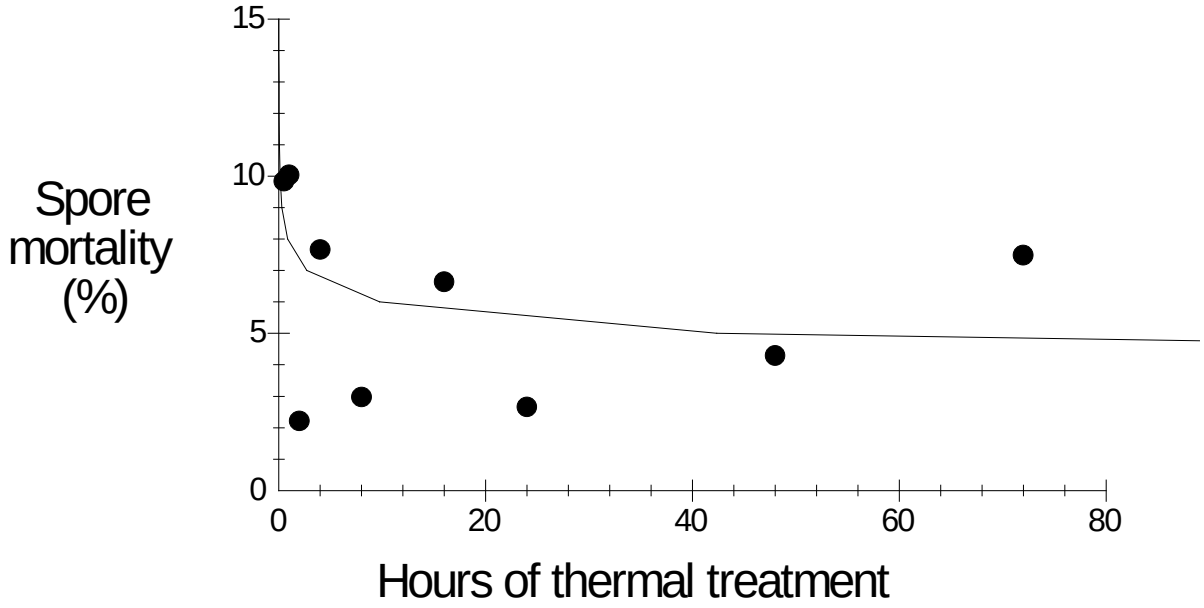

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Figure 2. Thermal treatment of spores at 60 degrees Celsius.

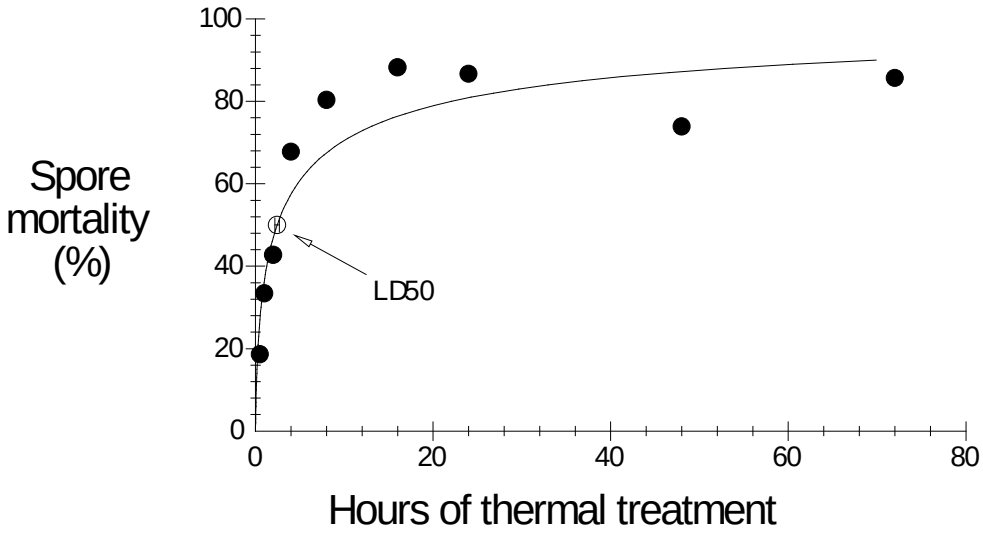

Figure 3. Thermal treatment of spores at 80 degrees Celsius.

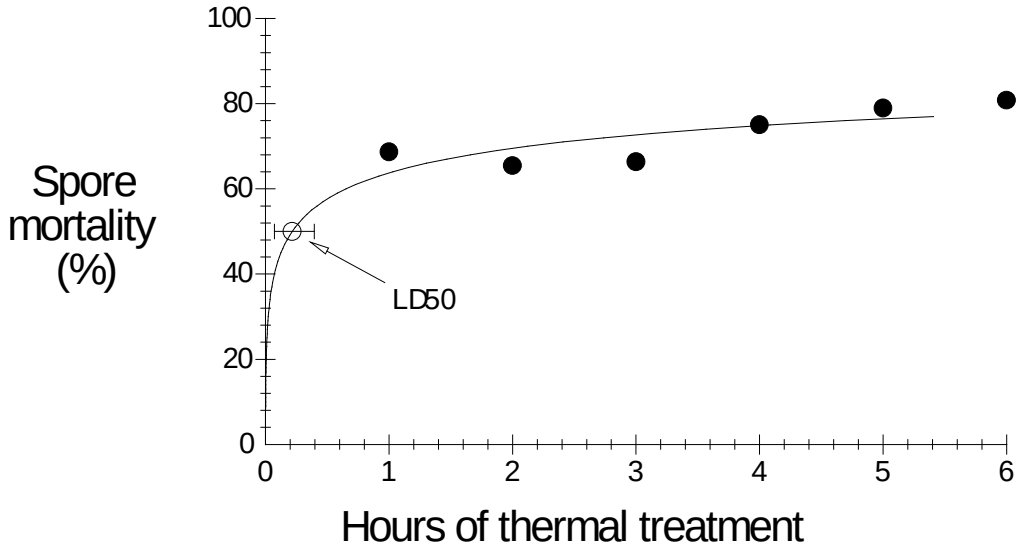

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Figure 4. Thermal treatment of spores at 90 degrees Celsius.

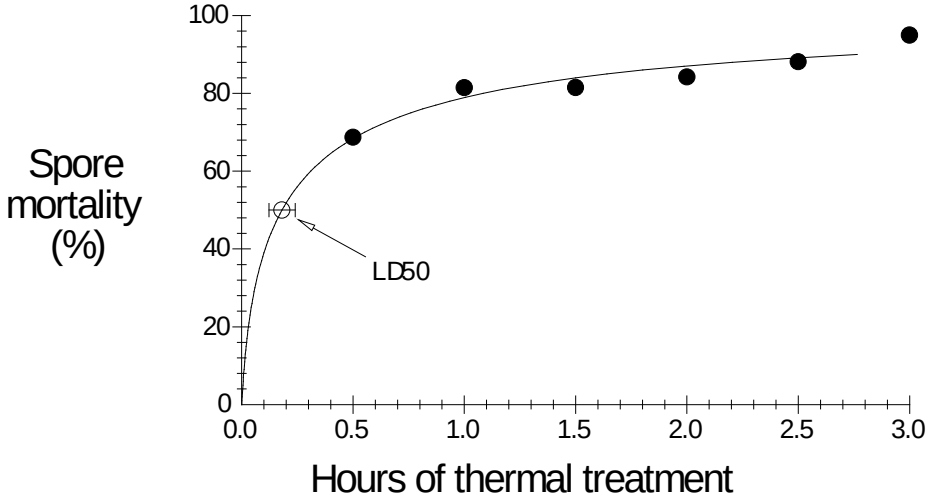

Figure 5. Thermal treatment of spores at 100 degrees Celsius.

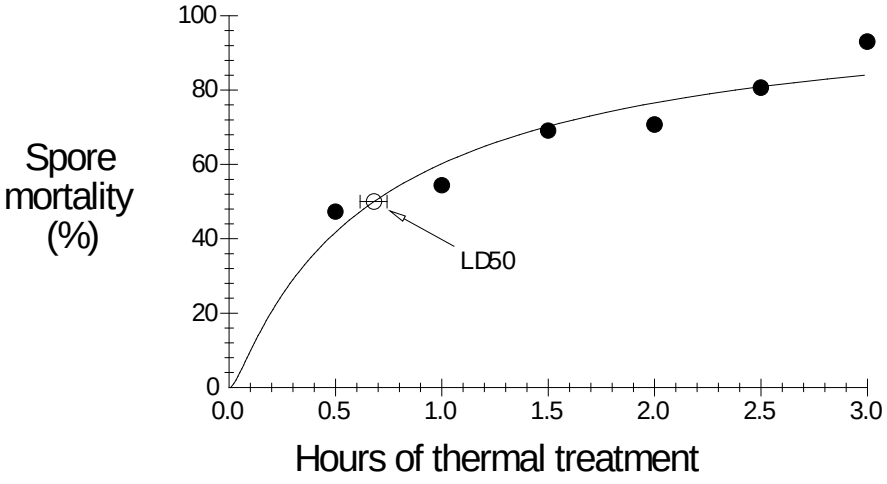

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Figure 6. Spores treated with Ag-Services Inc. General Storage Disinfectant.

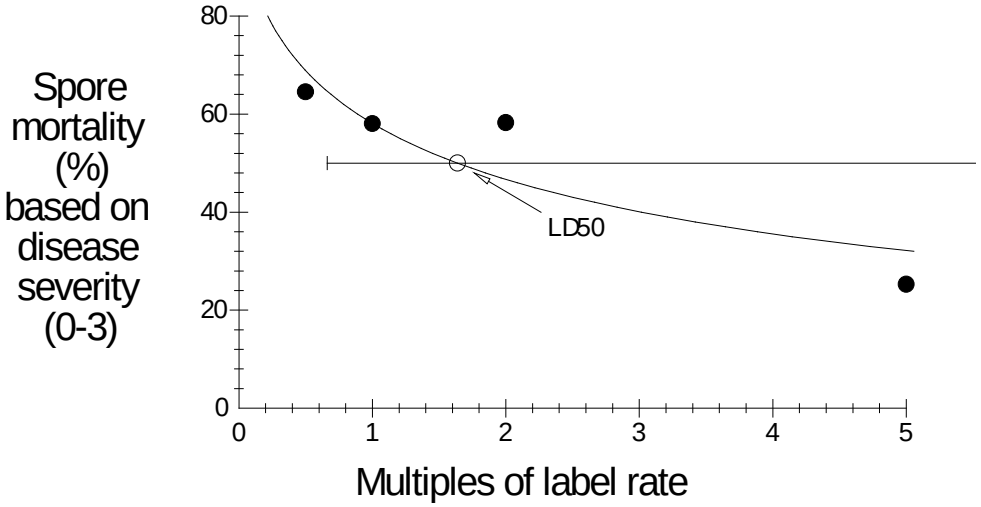

Figure 7. Spores treated with KleenGrow.

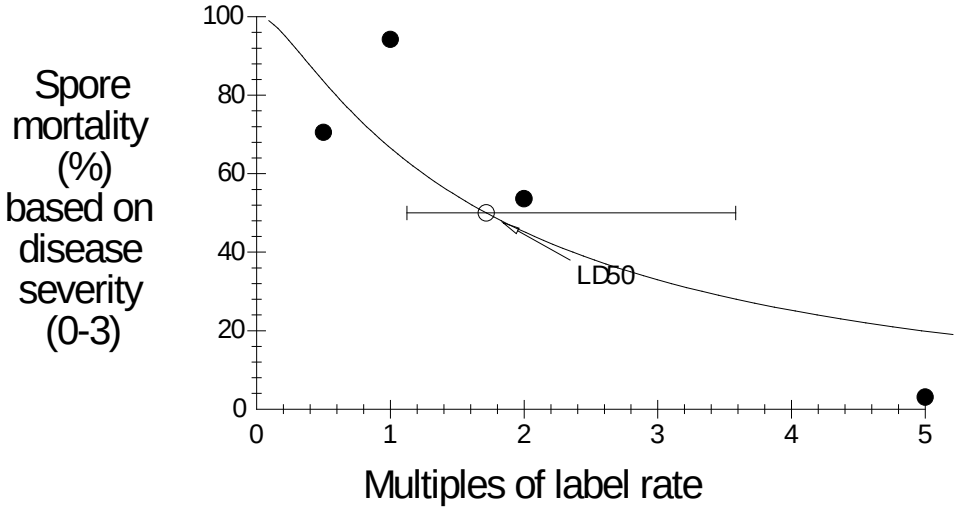

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Figure 8. Spores treated with SaniDate Disinfectant.

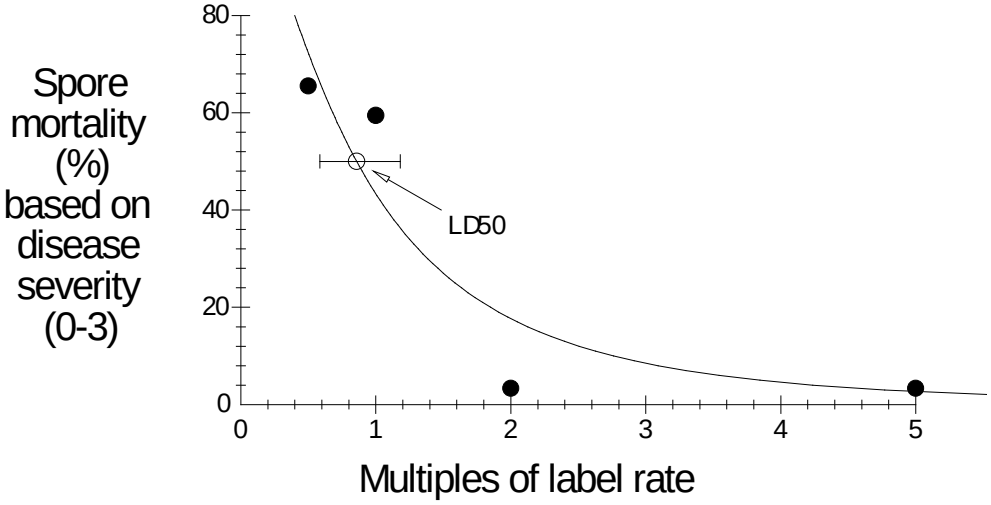

Figure 9. Spores treated with Thymox.

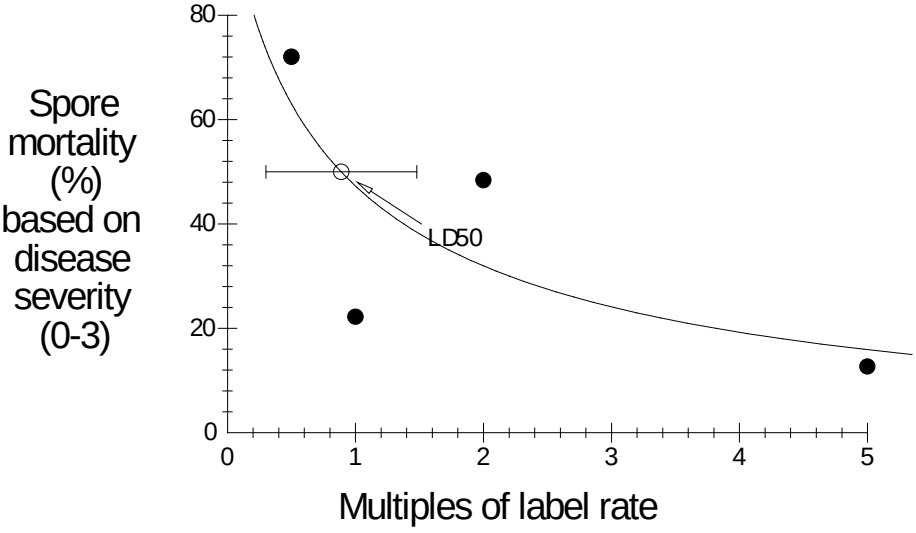

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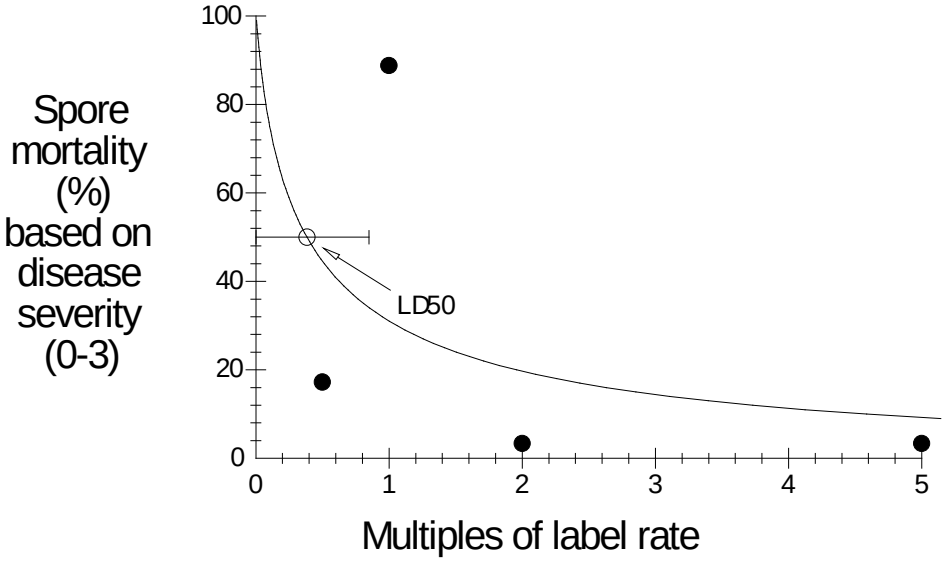
Figure 10. Spores treated with Virkon.



Field Research Trials

Efficacy of cleaning and sanitizing farm and industrial equipment to prevent clubroot field infestation, 2009-2010

Abstract

A chisel plow used in a clubroot infested commercial field was pressure washed and disinfected with four products. Sampling was subsequently done with sterile cellulose sampling sponges which were subsequently processed and used for bright-field microscopy analysis and plant-bait assays of clubroot resting spores. A four-wheel-drive tractor and grain cart were sampled and analyzed in a similar manner. Sterile cellulose sampling sponges used to sample industrial equipment were obtained from Swift Environmental Equipment Ltd. and processed and analyzed in a similar manner.

The presence of clubroot resting spores was indicated by bright-field microscopy analysis and plant-bait assays although interpretation of the results regarding pressure washing and pressure washing and disinfecting was inconclusive.

Objective

To assess the efficacy of pressure washing and disinfecting of agricultural and industrial equipment for the control of the clubroot pathogen.

Study Team
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Materials
HyperOx® (hydrogen peroxide, 25%; peracetic acid, 5%), KleenGrow™ (didecyl dimethyl ammonium chloride, 7.5%), Sodium Hypochlorite 10.8% (sodium hypochlorite, 10.8%), Virkon™ (potassium peroxymonosulfate, 21.4%)

Methods
In May of 2010, a chisel plow used to cultivate a clubroot infested commercial field (Hutterian Brethren of Lathom) was sampled to determine the efficacy of pressure washing followed by various disinfectants applied at recommended product label rates to shovels and shanks. The treatments consisted of pressure washing alone and pressure washing and disinfecting with one of four disinfectants with five replications. The field (SW 24-20-17 W4 County of Newell no. 4) was naturally infested with *Plasmodiophora brassicae*.

On May 12, 2010, after pressure washing and after pressure washing and disinfecting for a minimum of 20 min., the entire surface of each shovel and shank was swabbed with a sterile cellulose sampling sponge (pre-moistened with 10 mL of neutralizing buffer).

The sterile cellulose sampling sponges were mixed with 12.5 mL of sodium phosphate buffer (pH = 8.0 and 10 mM) and processed with a stomacher lab blender and 2.0 mL of this sample was subsequently filtered through a 47 mm × 0.8 µm microbiological analysis membrane filters and transferred to two 13 × 100 mm culture tubes with 5.0 mL of sodium phosphate buffer and one 13 × 100 mm culture tube with 1.0 mL of sodium phosphate buffer.

Clubroot susceptible Argentine canola spring hybrid 45H26 seeds were germinated on filter paper for 7-10 days. The roots were inoculated with the processed samples and transplanted to root trainers with Sunshine Professional Peat-Lite Mix no. 4 Aggregate growing media. The base of each stem was inoculated with an additional 200 µL of processed sample.

Inoculated seedlings were transferred to a growth chamber for ca. two weeks (16 hrs. at 23°C and maximum light intensity and 8 hrs. at 18°C and no light with ambient humidity) and then to a greenhouse (minimum of 16 hours of daylight at 23°C and 8 hours at 18°C with ambient humidity) and irrigated with a continuous feed of nutrient solution fertilizer at 225 ppm N and pH = 6.0.

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The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

Roots were washed and rated after ca. eight weeks using a rating scale. Disease incidence, disease severity and index of disease were calculated where: 0 = no galling; healthy plant; 1 = a few small galls; small galls on less than 1/3 of roots; 2 = moderate galling; small to 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) based on Kuginuki *et al.* (1999) as modified by Xue *et al.* (2008). Disease severity was calculated by use of the following formula: D.S. = $(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3)/N_T$ where N_0 = number of plants with a rating of 0 … and N_T = total number of plants. Index of disease were calculated (in order to convert disease severity to a percentage for ease of statistical analysis as data presented as small whole units often are not normally distributed) by the following formula: I.D. = $(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3)/(N_T \times 3) \times 100\%$ based on Horiuchi and Hori (1980) as modified by Strelkov *et al.* (2006).

Bright-field microscopy analysis consisted of staining processed samples with equal volumes of Evan's blue (20 mg mL^{-1}) to differentiate between viable and unviable spores. Total spore count and percentage of unviable spores was recorded.

On September 30, 2010, a four-wheel-drive tractor and grain cart used from the same field previously described was sampled and analyzed in a similar manner previously described.

In October of 2009, industrial equipment from Swift Environmental Equipment Ltd. used in a clubroot infested field was pressure washed and disinfected and subsequently sampled and analyzed in a similar manner previously described.

Results and conclusions

For the samples obtained from the chisel plow and industrial equipment from Swift Environmental Equipment Ltd., bright-field microscopy analysis was ineffective for determining efficacy of pressure washing and pressure washing and disinfecting as the absence of observable spores made interpretations difficult as to whether spores were removed in the pressure washing process or if the spore suspension was too dilute to rate effectively (Tables 1 and 5). For the same samples, as indicated by plant-bait assays, pressure washing and pressure washing and disinfecting did not appear to give good control of clubroot spore contamination (Tables 2 and 6). It is unclear as to whether these results were invalid do to cross-contamination in the growth chamber and greenhouse.

For the samples obtained from the four-wheel-drive tractor and grain cart, bright-field microscopy analysis indicated positive results at relatively low levels (Table 3). For the same samples as indicated by plant-bait assays, all results were negative (Table 4).

The presence of clubroot resting spores was indicated by bright-field microscopy analysis and plant-bait assays although interpretation of the results regarding pressure washing and pressure washing and disinfecting was inconclusive.

Table 1. Analysis of clubroot resting spores sampled from a chisel plow used in a clubroot infested commercial field, 2010.[1]

Product	Spore mortality (%)	
	Pre-disinfection	Post-disinfection
HyperOx®	-	-
KleenGrow™	-	-
Sodium Hypochlorite 10.8%	-	-
Virkon™	-	-
Positive control[2]	18.5	-
Negative control[3]	-	-

[1]Results are the means of five replications.
[2]Mature clubroot resting spores in sodium phosphate buffer.
[3]Sodium phosphate buffer only.

Table 2. Disease incidence, disease severity and index of disease of canola plant-bait assays of clubroot resting spores sampled from a chisel plow used in a clubroot infested commercial field, 2010.[1]

Product	Pre-disinfection			Post-disinfection		
	DI (%)	DS (0-3)	ID (%)	DI (%)	DS (0-3)	ID (%)
HyperOx®	0	0.00	0.0	61	0.96	31.9
KleenGrow™	34	0.59	19.8	30	0.55	18.4
Sodium Hypochlorite 10.8%	31	0.41	13.6	61	1.04	34.7
Virkon™	11	0.13	4.4	3	0.07	2.2
Positive control[2]	49	0.68	22.7	-	-	-
Negative control[3]	3	0.07	2.3	-	-	-
Uninoculated check	1	0.01	0.4	-	-	-

[1]Results are the means of five replications with 18 plants.
[2]Mature clubroot resting spores in sodium phosphate buffer.
[3]Sodium phosphate buffer only.

Table 3. Analysis of clubroot resting spores sampled from a four-wheel-drive tractor and grain cart used in a clubroot infested commercial field, 2010.[1]

Unit	Dead spores	Total spores	Spore mortality (%)
Grain cart tire	2	2	100
Grain cart box	2	2	100
Four-wheel-drive tractor steps	0	1	0
Four-wheel-drive tractor tire	4	5	80
Four-wheel-drive tractor engine housing	3	4	75
Positive control[2]	13	102	13
Negative control[3]	0	0	-

[1]Results obtained from one sample.
[2]Mature clubroot resting spores in sodium phosphate buffer.
[3]Sodium phosphate buffer only.

Table 4. Disease incidence, disease severity and index of disease of canola plant-bait assays of clubroot resting spores sampled from a four-wheel-drive tractor and grain cart used in a clubroot infested commercial field, 2010.[1]

Unit	DI (%)	DS (0-3)	ID (%)
Grain cart tire	0	0.00	0.0
Grain cart box	0	0.00	0.0
Four-wheel-drive tractor steps	0	0.00	0.0
Four-wheel-drive tractor tire	0	0.00	0.0
Four-wheel-drive tractor engine housing	0	0.00	0.0
Positive control[2]	0	0.00	0.0
Negative control[3]	0	0.00	0.0

[1]Results obtained from one sample.
[2]Mature clubroot resting spores in sodium phosphate buffer.
[3]Sodium phosphate buffer only.

Table 5. Analysis of clubroot resting spores sampled from industrial equipment from Swift Environmental Equipment Ltd., 2009.[1]

Unit	Spore mortality (%)	Unit	Spore mortality (%)
1455 (dirty)	-	210029-2289 (August 30, 2009)	-
1455 (clean)	-	210029-2289 (September 10, 2009)	-
1458 (clean)	-	210029 (September 26, 2009)	-
336	-	210029-2289 (September 26, 2009)	-
4054	-	210029 (October 1, 2009)	-
4416B	-	210029-2289 (October 1, 2009)	-
X159-78	-	Positive control[2]	26
PW46	-	Negative control[3]	-
2021	-	-	-

[1]Results are the means of five replications.
[2]Mature clubroot resting spores in sodium phosphate buffer.
[3]Sodium phosphate buffer only.

Table 6. Disease incidence, disease severity and index of disease of canola plant-bait assays of clubroot resting spores sampled from industrial equipment from Swift Environmental Equipment Ltd., 2009.[1]

Unit	DI (%)	DS (0-3)	ID (%)
1455 (dirty)	33	0.61	20.4
1455 (clean)	33	0.61	20.4
1458 (clean)	28	0.39	13.0
336	22	0.39	13.0
4054	17	0.50	16.7
4416B	11	0.11	3.7
X159-78	0	0.00	0.0
PW46	0	0.00	0.0
2021	0	0.00	0.0
210029-2289 (August 30, 2009)	11	0.28	9.3
210029-2289 (September 10, 2009)	11	0.17	5.6
210029 (September 26, 2009)	0	0.00	0.0
210029-2289 (September 26, 2009)	28	0.61	20.4
210029 (October 1, 2009)	0	0.00	0.0
210029-2289 (October 1, 2009)	6	0.06	1.9
Positive control[2]	76	1.96	65.2
Negative control[3]	0	0.00	0.0

[1]Results are the means of five replications with 18 plants.
[2]Mature clubroot resting spores in sodium phosphate buffer.
[3]Sodium phosphate buffer only.

Sampling and Testing Soil and Crop Residues Obtained From the Surfaces of Agricultural and Industrial Equipment to Detect Resting Spores of the Clubroot Pathogen, 2011

Abstract
A center pivot, chisel plow and four-wheel-drive tractor from a clubroot infested commercial field were cleaned, pressure washed and disinfected with various products and sampled with sterile cellulose sampling sponges. Soil and crop residues were collected intermittently during the cleaning process. Soil collected from the four-wheel-drive tractor and chisel plow was quantified and used for plant-bait assays. The sterile cellulose sampling sponges were subsequently processed and used for plant-bait assays. Sterile cellulose sampling sponges used to sample industrial equipment and water samples obtained from Swift Environmental Equipment Ltd. were processed in a similar manner.

All plant-bait assays were negative and the amount of soil collected from the four-wheel-drive tractor was estimated at ca. 300 kg.

Objective
To assess the efficacy of cleaning, pressure washing and disinfecting of agricultural and industrial equipment for the control of the clubroot pathogen and to quantify the amount of contaminated soil transferred on agricultural equipment.

Study Team
R.J. Howard[1], D.A. Burke[1], S.L.I. Lisowski[1], S.L. Mobbs[1], G.C. Daniels[1] and S. Hannigan[2]
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301 Horticultural Station Road East, Brooks, Alberta T1R 1E6
[2]Swift Environmental Equipment Ltd.
2-51331 Range Road 224, Sherwood Park, Alberta T8C 1H3

Materials
Ag-Services Inc. General Storage Disinfectant (n-Alkyl [40% C_{12}, 50% C_{14}, 10% C_{16}] dimethyl benzyl ammonium chloride, 10.0%), HyperOx® (hydrogen peroxide, 25%; peracetic acid, 5%), KleenGrow™ (didecyl dimethyl ammonium chloride, 7.5%), SaniDate® Disinfectant (hydrogen peroxide, 27.11%; peracetic acid, 2.0%), Sodium Hypochlorite 10.8% (sodium hypochlorite, 10.8%), Thymox™ (thymol, 18%), Virkon™ (potassium peroxymonosulfate, 21.4%)

Methods
In October of 2011, a center pivot, chisel plow and four-wheel-drive tractor from a clubroot infested commercial field (Hutterian Brethren of Lathom) were sampled to determine the efficacy of cleaning, pressure washing and disinfecting with various products at recommended product label rates. The field (SW 24-20-17 W4 County of Newell no. 4) was naturally infested with *Plasmodiophora brassicae*.

On October 20 and 21, after cleaning and after cleaning and pressure washing and after cleaning, pressure washing and disinfecting for a minimum of 20 min., ca. 100 cm^2 of each surface was swabbed with a sterile cellulose sampling sponge (pre-moistened with 10 mL of neutralizing buffer).

The sterile cellulose sampling sponges were mixed with 12.5 mL of sodium phosphate buffer (pH = 8.0 and 10 mM) and processed with a stomacher lab blender and 2.0 mL of this sample was subsequently filtered through a 47 mm x 0.8 µm microbiological analysis membrane filters and transferred to two 13 x 100 mm culture tubes with 5.0 mL of sodium phosphate buffer.

Clubroot susceptible Argentine canola spring hybrid 45H26 seeds were germinated in root trainers with Sunshine Professional Peat-Lite Mix no. 4 Aggregate growing media ca. two weeks prior to inoculation with the base of each stem inoculated with 1.0 mL of the processed samples.

Inoculated seedlings were transferred to a growth chamber for ca. two weeks (16 hrs. at 23°C and high light intensity and 8 hrs. at 18°C and no light) and then to a greenhouse (minimum of 16 hours of daylight at 23°C and 8 hours at 18°C with ambient humidity) and irrigated with a continuous feed of nutrient solution fertilizer at 250 ppm N and pH = 6.0.

Roots were washed and rated after ca. eight weeks using a rating scale. Disease incidence, disease severity and index of disease were calculated where: 0 = no galling; healthy plant; 1 = a few small galls; small galls on less than 1/3 of roots; 2 = moderate galling; small to 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) based on Kuginuki *et al.* (1999) as modified by Xue *et al.* (2008). Disease severity was calculated by use of the following formula: D.S. = $(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3)/N_T$ where N_0 = number of plants with a rating of 0 … and N_T = total number of plants. Index of disease were calculated (in order to convert disease severity to a percentage for ease of statistical analysis as data presented as small whole units often are not normally distributed) by the following formula: I.D. = $(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3)/(N_T \times 3) \times 100\%$ based on Horiuchi and Hori (1980) as modified by Strelkov *et al.* (2006).

Sterile cellulose sampling sponges used to sample industrial equipment and water samples were obtained from Swift Environmental Equipment Ltd. and subsequently processed and used for plant-bait assays in the manner previously described.

Results and conclusions

All plant-bait assays were negative (Tables 1-7) and the amount of soil collected from the four-wheel-drive tractors was estimated to be ca. 300 kg.

Find more information on this project and many other relevant canola studies on the Canola Research Hub.
The Canola Research Hub is funded through the substantial support of the Canadian Agricultural Partnership and the canola industry, including Alberta Canola, SaskCanola, Manitoba Canola Growers and the Canola Council of Canada.

Table 1. Disease incidence, disease severity and index of disease of canola plant-bait assays to assess the efficacy of cleaning, pressure washing and disinfection of center pivot tires from a clubroot infested commercial field, 2011.[1]

Stage	Product	DI (%)	DS (0-3)	ID (%)
	Ag-Services Inc. General Storage Disinfectant	0	0.00	0.0
	KleenGrow™	0	0.00	0.0
Pre-wash	SaniDate® Disinfectant	0	0.00	0.0
	Thymox™	0	0.00	0.0
	Untreated check	0	0.00	0.0
	Ag-Services Inc. General Storage Disinfectant	0	0.00	0.0
	KleenGrow™	0	0.00	0.0
Pressure wash	SaniDate® Disinfectant	0	0.00	0.0
	Thymox™	0	0.00	0.0
	Untreated check	0	0.00	0.0
	Ag-Services Inc. General Storage Disinfectant	0	0.00	0.0
	KleenGrow™	0	0.00	0.0
Disinfection	SaniDate® Disinfectant	0	0.00	0.0
	Thymox™	0	0.00	0.0
	Untreated check	0	0.00	0.0
Positive control[2]	-	0	0.00	0.0
Negative control[3]	-	0	0.00	0.0
Uninoculated check	-	0	0.00	0.0

[1]Results are the means of three replications with ten plants.
[2]Mature clubroot resting spores in sodium phosphate buffer.
[3]Sodium phosphate buffer only.

Table 2. Disease incidence, disease severity and index of disease of canola plant-bait assays to assess the efficacy of cleaning, pressure washing and disinfection of chisel plow tires from a clubroot infested commercial field, 2011.[1]

Stage	Product	DI (%)	DS (0-3)	ID (%)
	HyperOx®	0	0.00	0.0
	KleenGrow™	0	0.00	0.0
Pre-wash	Sodium Hypochlorite 10.8%	0	0.00	0.0
	Virkon™	0	0.00	0.0
	Untreated check	0	0.00	0.0
	HyperOx®	0	0.00	0.0
	KleenGrow™	0	0.00	0.0
Pressure wash	Sodium Hypochlorite 10.8%	0	0.00	0.0
	Virkon™	0	0.00	0.0
	Untreated check	0	0.00	0.0
	HyperOx®	0	0.00	0.0
	KleenGrow™	0	0.00	0.0
Disinfection	Sodium Hypochlorite 10.8%	0	0.00	0.0
	Virkon™	0	0.00	0.0
	Untreated check	0	0.00	0.0
Positive control[2]	-	0	0.00	0.0
Negative control[3]	-	0	0.00	0.0
Uninoculated check	-	0	0.00	0.0

[1]Results are the means of three replications with ten plants.
[2]Mature clubroot resting spores in sodium phosphate buffer.
[3]Sodium phosphate buffer only.

Table 3. Disease incidence, disease severity and index of disease of canola plant-bait assays to assess the efficacy of cleaning, pressure washing and disinfection of chisel plow shovels from a clubroot infested commercial field, 2011.[1]

Stage	Product	DI (%)	DS (0-3)	ID (%)
	HyperOx®	0	0.00	0.0
	KleenGrow™	0	0.00	0.0
Pre-wash	Sodium Hypochlorite 10.8%	0	0.00	0.0
	Virkon™	0	0.00	0.0
	Untreated check	0	0.00	0.0
	HyperOx®	0	0.00	0.0
	KleenGrow™	0	0.00	0.0
Pressure wash	Sodium Hypochlorite 10.8%	0	0.00	0.0
	Virkon™	0	0.00	0.0
	Untreated check	0	0.00	0.0
	HyperOx®	0	0.00	0.0
	KleenGrow™	0	0.00	0.0
Disinfection	Sodium Hypochlorite 10.8%	0	0.00	0.0
	Virkon™	0	0.00	0.0
	Untreated check	0	0.00	0.0
Positive control[2]	-	0	0.00	0.0
Negative control[3]	-	0	0.00	0.0
Uninoculated check	-	0	0.00	0.0

[1]Results are the means of three replications with ten plants.
[2]Mature clubroot resting spores in sodium phosphate buffer.
[3]Sodium phosphate buffer only.

Table 4. Disease incidence, disease severity and index of disease of canola plant-bait assays to assess the efficacy of cleaning, pressure washing and disinfection of four-wheel-drive tractor tires from a clubroot infested commercial field, 2011.[1]

Stage	Product	DI (%)	DS (0-3)	ID (%)
Pre-wash	Sterile cellulose sampling sponge	0	0.00	0.0
		0	0.00	0.0
	Sterile cotton sampling pad	0	0.00	0.0
		0	0.00	0.0
Pressure wash	Sterile cellulose sampling sponge	0	0.00	0.0
		0	0.00	0.0
	Sterile cotton sampling pad	0	0.00	0.0
		0	0.00	0.0
Disinfection	Sterile cellulose sampling sponge	0	0.00	0.0
		0	0.00	0.0
	Sterile cotton sampling pad	0	0.00	0.0
		0	0.00	0.0

[1]Results are the means of three replications with ten plants. Disinfectant was 1% sodium hypochlorite.

Table 5. Disease incidence, disease severity and index of disease of canola plant-bait assays to assess the efficacy of cleaning, pressure washing and disinfection of a center pivot from a clubroot infested commercial field, 2011.[1]

Stage	Part	DI (%)	DS (0-3)	ID (%)
	Frame	0	0.00	0.0
Pre-wash	Strut	0	0.00	0.0
	Span	0	0.00	0.0
	Frame	0	0.00	0.0
Pressure wash	Strut	0	0.00	0.0
	Span	0	0.00	0.0
	Frame	0	0.00	0.0
Disinfection	Strut	0	0.00	0.0
	Span	0	0.00	0.0

[1]Results are the means of five replications with ten plants. Disinfectant was 1% sodium hypochlorite.

Table 6. Disease incidence, disease severity and index of disease of canola plant-bait assays of soil collected from a four-wheel-drive tractor and chisel plow from a clubroot infested commercial field, 2011.[1]

Sample	DI (%)	DS (0-3)	ID (%)
Four-wheel-drive tractor	-	-	-
Chisel plow	-	-	-

[1]Results are the means of ten plants.

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Table 7. Disease incidence, disease severity and index of disease of canola plant-bait assays to assess sterile cellulose sampling sponges and water samples from industrial equipment from Swift Environmental Equipment Ltd., 2011.[1]

Sample	DI (%)	DS (0-3)	ID (%)
Norwest Energy NL unit 138 (June 16, 2010)	0	0.00	0.0
Norwest Energy NL unit 4528 (June 16, 2010)	0	0.00	0.0
D & D Oilfield Equipment water sample 1 (November 12, 2009)	0	0.00	0.0
D & D Oilfield Equipment water sample 1 (May 20, 2009)	0	0.00	0.0

[1]Results are the means of three replications with ten plants.

Acknowledgements
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County of Newell no. 4

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