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ADF Project Final Report Format

1. Project title and ADF file number.

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Extent of infestation and potential eradication of clubroot at sites in Saskatchewan

2. Name of the Principal Investigator and contact information.

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4. Abstract/ Summary: *This must include project objectives, results, and conclusions for use in publications and in the Ministry database. Maximum of 300 words in lay language.*

The objectives of this study were to 1) investigate the feasibility of eradicating clubroot from small-scale clubroot infestations to prevent establishment of the disease in new locations, 2) to assess selected actives on a range of soil types, and 3) to assess the horizontal and vertical distribution of resting spores in the soil profile at infested sites. Field trials were conducted from 2013 to 2015. Initial studies under controlled conditions demonstrated that several actives had excellent activity against resting spores. However, efficacy in field trials was consistently low, even when the fumigants were applied using professional-grade application equipment. One factor that increased efficacy was the quality of the cover applied after fumigation. A totally impermeable film (TIF) cover produced much higher efficacy than construction-grade plastics, but at a higher cost. No treatment totally eradicated the pathogen on any soil or in any year. Pathogen distribution was highly variable, both horizontally and vertically – samples only 0.5 m apart often contained very different levels of spores. At one infested site with a deep, uniform soil texture, spores occurred at detectable concentrations to a depth of at least 1 m (the deepest assessed). However, at two sites where the infestation was detected only recently, resting spores could only be detected at the soil surface. This indicates that spores can move downward slowly over time, likely in ground water. Studies to improve estimates of resting spore concentration with a competitive internal control were successful, as were studies to develop a technique for distinguishing between viable and dead resting spores using qPCR with a pre-treatment of propidium monoazide. These improvements represent a substantial contribution to knowledge of the biology and assessment of clubroot. However, the study demonstrated that resting spore numbers can only be reduced, but not totally eliminated, by application of fumigants.

5. Introduction: *Brief project background and rationale.*

Clubroot (*Plasmodiophora brassicae*) was identified on canola in western Canada for the first time near Edmonton AB in 2003, and has subsequently been identified from more than 2000 fields across Alberta. Studies on factors affecting the development of clubroot on canola demonstrated that the pathogen was difficult to control using conventional approaches (seed treatments, soil amendments), that canola production in most regions of the Canadian prairies was at risk of clubroot spread, that the pathogen moves readily on farm machinery and in wind and water, and that a breakdown in the resistance of resistant cultivars was already occurring. Therefore, clubroot represents a potentially serious threat to canola production in Saskatchewan. Development of a treatment that would eradicate clubroot from small infestations would benefit producers by preventing substantial losses in revenue and cropping options resulting from wider distribution of the infestation. However, drier conditions and more alkaline soils in Saskatchewan, together with the availability of new resistant cultivars, mean that pathogen populations in infested fields are likely to build up more slowly than they did in Alberta. As a result, producer groups were interested to determine if it might be possible to contain or even eliminate small areas of infestation if they are identified early enough. However, little is known about the distribution (horizontal and vertical) of resting spores in soil. Information on the vertical distribution on resting spores is needed to determine how deeply the fumigation treatments must penetrate to eliminate the infestation. There is little point in treating the surface if the pathogen is still active just below the treatment layer, but increasing treatment depth dramatically increases its cost and difficulty. Also, industries involved in large scale movement of soil (oil and gas, road construction) need to know if the soil that they are moving is contaminated with resting spores of clubroot. This study was conducted in collaboration with Dr. Hwang in Alberta to expand the scope of an existing project on the potential of fumigation to include additional actives, additional soil types and a wider range of weather conditions.

6. Methodology: *Include approaches, experimental design, methodology, materials, sites, etc.*

Fumigants:

Controlled environment – preliminary studies

Screening of potential fumigants under controlled conditions was conducted using small plastic tubs (39 x 26 x 23 cm) of naturally infested soil collected at the Muck Crops Research Station of the University of Guelph. The first study examined three rates (200, 400 and 800 kg/ha) of the fumigant metam sodium (41% sodium methyldithiocarbamat, trade name Busan 1236) to assess a range of rates for use in subsequent field trials: These rates represent a low, moderate and very high rate of application, respectively, based on label rates for field application. The experiment was conducted in a randomized complete block design with four replicates (tubs).

A second trial examined the fumigant chloropicrin (85%, trade name Pic Plus) at 162 L/ha and the biofumigant MustGrow (100% *Brasssica juncea*) at 2.2 T/ha. Chloropicrin is highly volatile and so is dangerous to work with in an enclosed space. Therefore, chloropicrin was applied at the label rate to the 0-15-cm depth of an infested field site at the Muck Crops Research Station (MCRS) using a tractor-pulled applicator (three hill fumigator). After injection, the study area was packed to seal the soil surface. When it was safe to re-enter the treated area (2 wk after treatment), soil from the treated area was collected and taken into the greenhouse for comparison with soil collected prior to treatment (control) and soil treated with MustGrow.

In each trial, a bioassay to assess efficacy was conducted using 10 plants of a highly susceptible line of Shanghai pak choy (*Brassica rapa*), a highly susceptible vegetable crop per plot or tub.Plants were watered with acidified water (pH 6.3) for the first 2 weeks and a high level of soil moisture was maintained throughout the experiment. The growth room was maintained at 23°/18°C with a 12-hr photoperiod. Plants were grown for 6 weeks, then removed from the soil and assessed for clubroot symptoms. Clubroot incidence and severity were assessed using a 0-3 scaling system, where 0 = no clubbing, 1 < 1/3 of tap root with clubbing symptoms, 2 = 1/3 to 2/3 of root area with clubs, and 3 > 2/3 clubbing of roots. A disease severity index (DSI) was also calculated as follows:

$$DSI = \frac{\sum (\text{class \#})(\text{\# of plants in class})}{(\text{total number of plants})(\text{\# of classes} - 1)}$$

Field trials - General

The studies were arranged in a random complete block design with three replicates. Each plot was 2.1 m × 13 m. Treatments were applied at 0–20 cm depth using a tractor-pulled fumigator with injectors spaced 16.5 cm apart to apply metam sodium and another tractor-pulled fumigator with injectors 18 cm apart for chloropicrin. At each site, the soil was worked thoroughly prior to application to ensure that the fumigator could provide uniform distribution and depth. Commercial-standard injection equipment was used in all of the later trials to further ensure uniformity and depth control. After injection, the study area was packed to seal the soil surface. In 2014, the chloropicrin treatments were further sealed with totally impermeable film (TIF), and in 2015 all plots were sealed with TIF. Clubroot incidence and severity were assessed on the susceptible Brassica crop that followed the fumigation treatment as described previously.

Viable resting spore levels were assessed by adding 1.0-1.5 g of dried soil to 100 mL of deionized water, homogenizing the suspension in a commercial blender for 2 min and filtering the suspension through 5-10 layers of cheese cloth to remove large particles. An aliquot (25 µL) of the filtered spore suspension was added to an equal amount of Evan's blue stain and allowed to sit at room temperature for 20-30 min. The suspension was diluted with 200 µL of 5% glycerol solution and thorough mixed. Then 25 µL of the final suspension was placed on a microscope slide and examined using a light microscope. Cell membranes of viable resting spores are impermeable to this vital stain, but dead spores take up the dye and ae stained blue. The viability of resting spores was also assessed using a pretreatment with 120 ppm of propodium monoazide before DNA extraction and qPCR. This treatment binds to the DNA of non-viable resting spores, such that they are not amplified in the qPCR reaction.

Field trials - Muck Crops Research Station (MCRS)

A field trial to test the effectiveness of chloropicrin on clubroot was conducted on a muck soil (pH 6.2, >70% OM) at the Muck Crops Research Station, Kettleby, Ontario in 2013. Chloropicrin was injected into the top 15–20 cm of soil in the field. Two weeks later, sub-plots of Shanghai pak choy, canola and cabbage crops were established in each main plot. Cabbage seedlings were transplanted individually, and canola and Shanghai pak choy seeds were sown at standard rates. Plants were grown to maturity, and roots were assessed for clubroot incidence (%) and severity rating using a standard 0–3 scale.

In 2014, the treatments were metam sodium (42%, trade name Vapam HL) at 150 and 300 kg a.i./ha, chloropicrin at 128 kg a.i./ha, and a nontreated control. Treatments were applied on July 14, 2014. After a 2-wk interval to ensure that the fumigant had dissipated, each plot was sown with four rows of the highly susceptible Shanghai pak choy cv. Mei Qing Choi on July 29, with 30 cm between rows. At 42 days after planting, each plant in the centre two rows of each plot was uprooted and the roots assessed for clubroot symptoms.

In 2015, a trial was conducted with four replicates. The fumigant treatments and rates were chloropicrin at 128, 164, 224, 280, and 336 kg ha^{-1} and metam sodium (Vapam) at 75, 150, and 300 kg ha^{-1}, applied on 12 June. After the treatments were applied, each plot was sealed using TIF, left for 14 days and uncovered. However, eight plots were sealed with only plastic tarps because the quantity of TIP supplied was not adequate. Also, the TIF covers for several plots blew off after only a few days and were not replaced. Each plot was seeded with four rows of Shanghai pak choi cv. Mei Qing Choi with 37 cm between rows using an Earthway 1001-B precision garden seeder with a 1002-9 disc on 8 July. At 6 weeks after seeding, clubroot incidence and severity were assessed on 30 plants per plot. Data were analyzed using Friedman's Chi-squared test (nonparametric) in SAS Version 9.2 (SAS institute, Cary, NC).

Field trials – Commercial field

In 2014, a study was conducted on a commercial field at a site near Hamilton, Ontario on mineral soil. The treatments were two formulations of metam sodium; Busan at 148 kg a.i./ha, and Vapam HL at 75, 150 and 300 kg a.i./ha, chloropicrin at 168, 224, 280 and 336 kg a.i./ha, and a nontreated control. The treatments were applied on July 15. At 14 days after treatment, about 2 L of soil from 0- 15-cm depth was collected for use in growth room trials.

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The field was then tilled and transplanted to broccoli cv. Everest on August 6, 2014 with a spacing of 90 cm between rows and 23 cm between plants. Clubroot incidence and severity were assessed on October 29.

In addition to the field bioassay with a brassica vegetable crop planted by the producer, a laboratory bioassay was conducted using treated soil from the field trial. Tall narrow plastic pots were filled with treated soil, with 12 pots per plot. Each pot received two seeds of Shanghai pak choy cv. Mei Qing Choi, and was thinned to one seedling per pot. Plants were watered daily with slightly acidic water (pH 6.3) for 42 days. Clubroot incidence and severity were assessed on 10 plants per plot. The growth room was maintained at 23°/18°C with a 12-hr photoperiod.

Field trials – Alberta

Field and laboratory studies on the efficacy of Vapam fumigant were conducted on a heavy clay-loam soil at the clubroot nursery site, Crop Diversification Centre North near Edmonton, Alberta, in 2012-2014 in small plots. The fumigant was applied to these small plots using a hand-watering system (sprinkling can). Clubroot severity was examined at about 6 wk after seeding, as described above. A paper outlining the results was published in 2014. A second paper describing companion studies with Basamid fumigant has been submitted and is currently in review.

Interaction of fumigation and solarisation

Mineral soil from the commercial site used for the field assessment of fumigants was collected in the late summer of 2014. The soil was thoroughly mixed and placed in plastic tubs (39 x 26 x 23 cm) filled to the point of overflowing and watered to ensure that the soil was wet but not saturated. A small sample of soil from each container was taken for quantification of resting spores. The solarisation treatments were covered with TIF and the edges of the TIF were sealed to the plastic container using duct tape and left in the sun for 3 weeks. Nontreated containers were left open. HOBO temperature data loggers were used to record the soil temperature in representative containers of each treatment. After solarisation, metam-sodium (Busan 1236) at 74 kg a.i/ha (1/2X label rate) and 148 kg a.i./ha (label rate) in 750 mL of water was applied to the soil surface to mimic a drip application. The solarisation treatments were re-sealed with TIF film. The fumigation treatments are sealed by placing a tight plastic lid on the container. Each container was left in the sun for 3 weeks. The containers were opened and allowed to air for 1 week to allow the fumigants to dissipate, then soil samples were collected from top 4 cm of soil and bottom 4 cm of soil for analysis of resting spore viability. The soil from each treatment was bulked, thoroughly mixed, and about 2 L was used in a bioassay. The study was repeated in 2015 using heavily infested muck soil from the Muck Crops Research Station.

For both solarisation repetitions, a bioassay with 12 plants per experimental unit was conducted as described previously except that the soil was maintained at near-saturation levels for the first 4 weeks after seeding by standing the pots in water to maintain high levels of soil moisture. For the next 2 weeks, the pots were allowed to drain normally, and the young plants were watered from above. At 42 days after planting, clubroot incidence and severity were assessed on 10 plants per experimental unit. The fresh and dry weight of roots and shoots of the 10 bulked plants for each replicate of each treatment were assessed. Spore viability as assessed as previously described, except that three subsamples were assessed from each experimental unit.

Pathogen distribution:
In 2013, soil cores were collected from three sites naturally infested with *P. brassicae*; a high organic peat soil (MCRS, U of G), a mineral soil site in Ontario, and a mineral soil site near Bassano in southern Alberta. At each site, cores were collected to 56 cm depth (as deep as hand cores could readily be obtained) and divided into with seven ~8-cm-deep increments. In addition, cores were taken before and after application of a fumigant at the two sites in Ontario (chloropicrin at MCRS, MustGrow at the mineral soil site). The soil samples were dried at 40° C for 48 hr and stored until they could be assessed. The populations of resting spores in each sample were initially assessed using standard qPCR methods developed in Canada. There were problems with some of the estimates of resting spore concentrations in these samples, with several negative results from samples where spores were known to occur

based on plant symptoms. Initially, the problems were thought to be the result of discrepancies in technique, so a research associate spent a week in the laboratory where the qPCR techniques had been developed to improve / validate our method. However, unacceptable level of false negatives continued to occur. As a result, considerable effort was invested in the development of a Taqman multiplex competitive internal positive control (CIPC) protocol to estimate the level of amplification inhibition that was occurring in the samples. This modification substantially improved the accuracy and repeatability of estimates of resting spore concentration and reduced the number of false negatives. Therefore, it was used to re-assess each of the samples. A scientific paper describing the improved technique has been published (Deora et al. 2015). Additional samples to 1-m depth from an infested site in Ontario and two newly infested sites in Alberta were collected in the summer of 2014. At the site where the pathogen had been established for many years, resting spores could be detected down to 1m, which was the deepest depth assessed. However, resting spores could not be detected below the soil surface layer at the two sites where the pathogen had only recently been detected. A scientific paper has been prepared and submitted for review.

Initially, treatment with Evan's blue was used to assess the viability of resting spores, following the protocol of and previous studies in Asia and Australia. However, concurrent research on equipment sanitation at Brooks Alberta demonstrated that this methodology was confounded by interactions with many types of sterilant, which changed the permeability of the resting spore membrane without actually killing the spores (R. Howard, personal communication). Therefore, a replacement technique for assessment of viability using a pretreatment with 120 ppm of propidium monoazide was developed. This new protocol is being used to re-assess the estimates of spore viability from the field trials. Again, a scientific paper has been prepared and submitted to a journal for review.

7. Research accomplishments:

Objectives	**Progress**
1) Investigate the feasibility of eradicating clubroot from small-scale clubroot infestations to prevent establishment of the disease in new locations.	Field trials to assess the efficacy of fumigants were conducted in Ontario and Alberta from 2013-2015. At each site tested, application of fumigants reduced resting spore populations and generally reduced clubroot severity. However, efficacy was much lower and less consistent in the field than under controlled conditions. Treatments tarped with Totally Impermeable Film (TIF), which reduces losses to volatilization, had substantially higher efficacy than those tarped with construction-grade plastics or sealed by packing the soil surface. The study demonstrated that resting spore populations can only be reduced, not totally eliminated, by application of fumigants.
2) Assess selected actives on a range of soil types.	Application of chloropicrin often appeared to be more effective than metam sodium, but this difference was only apparent when the metam sodium treatments were not sealed with TIF. There were no differences between the two formulations of metam sodium that were assessed (Vapam and Busan). Basamid was also efficacious against resting spores of clubroot. No treatment totally eradicated the pathogen on any soil or in any year. However, there are no commercial applicators in Alberta or Saskatchewan, which makes this option untenable for many situations.
3) Assess the horizontal and vertical distribution of resting spores at infested sites.	The resting spore concentration in samples collected from three sites infested with *P. brassicae* in Ontario and three in Alberta were estimated using an improved qPCR technique developed in this project**.** Resting spores were present to deep in the soil profile in samples from the four fields where the pathogen had been established for many years, but only at the surface at two newly infested fields. This indicates that even eradication of the pathogen in the top 15 cm of soil would not eliminate it from established sites, but might have a large impact in newly infested sites. An improved technique for assessing resting spore viability using pretreatment with propidium monoazide was developed to replace Evan's blue vital stain.

8. Discussion: *Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks.*

Fumigants: In this study, several fumigants were identified that have activity against *P. brassicae*. Moderate rates of metam sodium (Vapam or Busan, Table 1) and basamid provided excellent control of clubroot under controlled conditions. Chloropicrin (Pic Plus), another effective fumigant identified in the study, is highly volatile, and so was only assessed in field trials due to applicator safety issues. In the field trials in Ontario, the actives were applied using an applicator that had been optimized for fumigants, which provided substantially more consistent results than the watering-can-sprinkler application approach used in Alberta.

In a field trial at the MCRS (Table 2), disease pressure was high, as evidenced by 90% clubroot severity (disease severity index, DSI) in the nontreated control. Metam sodium had no measurable effect on clubroot severity in this trial, but chloropicrin reduced severity and increased plant growth. In a trial on a commercial field, clubroot severity was low, possibly because the broccoli seedlings were not transplanted until July, and so matured under conditions that were too cool for development of clubroot symptoms. Clubroot severity in the nontreated control was only 3% at this site, and no clubroot was observed in the fumigant treatments (Table 3). However, it is interesting to note that plant growth was substantially higher at moderate rates of chloropicrin than in the control, which may indicate that chloropicrin reduced initial infection, even though subsequent symptom development was eliminated by cold temperatures.

In the fumigation trail at the MCRS in 2015, clubroot incidence and severity were very low in the first replicate block of the trial, likely due to a lower concentration of resting spores in the soil in this area. As a result, this block was excluded from subsequent analysis. Similarly, clubroot incidence and severity in the plots covered with plastic tarps were much higher than all adjacent plots (except the nontreated control) sealed with TIP. In contrast, the TIF plots where the covers were blown off had similar levels of clubroot to those of adjacent treatments. We concluded that the regular plastic was not effective in retaining the fumigant, so all of the treatments sealed with tarp were re-classified as nontreated controls. Analysis revealed differences in treatments for incidence and DSI ($P \leq 0.01$). All of the treatments, except metam sodium at 75 and 150 kg a.i. ha^{-1}, reduced clubroot incidence compared to the control. Chloropicrin at 164 kg a.i ha^{-1} was the most effective treatment, but was not replicated, so increased efficacy is not supported (not shown). All of the treatments except metam sodium at 75 kg a.i. ha^{-1} also reduced in clubroot severity on Shanghai pak choy (Fig. 1).

We conclude that use of TIF was extremely important to the efficacy of treatments; treatments sealed with packing the soil or covering with tarps were not effective. Maintaining the cover for the full 2-wk period was much less important for efficacy than applying the correct cover. It is important to note that recent changes to federal regulations, which came into effect in 2015, require that all fumigation applications be tarped, However, only limited amounts of TIF were available in 2014, so the metam sodium treatments were packed but not covered in the trials in 2014. As a result, the lower efficacy from metam sodium relative to chloropicrin in these trials (Table 2) may be related more to more rapid loss of the active with packing only compared to a TIF cover than to inherent differences in efficacy against clubroot.

Evan's blue is a vial stain that has been used in several studies to assess the viability of resting spores of P. brassicae. Living cells do not stain, but dead cells turn a deep blue. This standard technique was used initially to assess the viability of resting spores, and was compared with infection potential based on bioassays. Bioassays are not the preferred methodology for viability assessments because they are time consuming, resource-intensive, and can be strongly influenced by soil type, pH, nutrient content, watering regime and temperature. However, when conducted under carefully controlled conditions, they represent the gold standard for such assessments.

Despite evidence from the bioassays that the fumigants had reduced spore numbers and therefore reduced subsequent infection of a susceptible host, there were no differences among the treatments in the estimated number of viable spores in each treatment or the proportion of viable spores in the soil population in 2014 (Fig. 2). It is possible that changes in cell membrane produced by exposure to the fumigants altered their permeability to

Evan's blue, so that it is not an effective indicator of cell viability in this system. As a result of these problems with the standard method for assessing resting spore viability, another approach to assessment of resting spore viability was explored. A technique was developed that uses pretreatment with propidium monoazide to bind the DNA of nonviable resting spores so that it is not amplified in qPCR (ms describing the technique has been submitted for review). This technique is currently being used to re-assess the viability of all of the field samples, and the results from 2014 are presented in Fig. 2. The qPCR method (which does not distinguish between viable and dead spores) detected several times more spores than with Evan's blue. Estimates of spore numbers from the 120 µM pretreatment with PMA were substantially lower than with 0 µM PMA, which indicates that a high proportion of cells had been killed. However, estimates of spore numbers from the nontreated control were substantially lower than in the fumigant treatments, which did not correspond with results from the bioassays. This indicates that there may have been issues with representative sampling, uniformity of pathogen distribution, or even with the reproducibility of the PMA technique. The fumigation experiment was repeated at the Muck Crops Research Station in 2015, and as already described (Fig. 1), there were large differences in clubroot severity. Unfortunately, the assessments using PMA are still not completed for 2015. We are planning to repeat the fumigation trial at the Muck Crops Research Station in 2016 to provide uniform conditions of soil sealing and to provide additional samples that will allow for a rigorous assessment of the PMA protocol, but this sampling is outside the scope and time frame of the current report.

The small-scale studies of the interaction between solarisation and fumigation conducted over 2 years did not indicate any positive effect of solarisation or positive synergy with fumigation. This indicated that additional studies of solarisation are not warranted in Canada, where a short growing season and low air temperatures are likely not well suited for solarisation.

None of the fumigants completely eliminated the pathogen in the initial assessments at heavily infested field sites. It is important to note that the concentration of resting spores at each of the field sites was high (≥ 10^6 per g). It is highly likely that reductions in severity will be more consistent at newly infested sites where resting spore concentrations are lower and spores are still congregated close to the soil surface. The most important observation from these trials was that the use of TIF covers to retain the fumigant was the most important factor for increasing efficacy.

Pathogen distribution: A post-doctoral fellow received training on qPCR assessments of *P. brassicae* extracted from soil in Dr. S.E. Strelkov's lab at the University of Alberta in Edmonton in 2013. Dr. Strelkov has developed and published most of the molecular techniques for assessments of *P. brassicae* used in Canada. However, problems arose when in applying Dr. Strelkov's method, especially with some of the soils with high organic matter that are the main focus of clubroot infestation in Ontario. After considerable trial and error, we concluded that various inhibitors were suppressing amplification in many samples. To address these concerns, a multiplex qPCR system was developed that used amplification of low levels of the DNA coding for a green fluorescent protein (GFP) from jellyfish as an indicator of the success of amplification in the qPCR mixture. The multiplex system allowed for identification and re-testing (including re-purification) of samples where inhibition might have produced a false negative result from an infested soil. This new technique provided more accurate and reproducible estimates of resting spore populations than the previous standard approach. A manuscript describing this new method has been published. Recently, we were informed the CFIA has recommended this method to other countries as the preferred method for assessing clubroot infestation in food shipments destined for Canada, and so state government labs in California (and likely others) are using this technique for the 2016 field season.

The most striking feature of the pattern of resting spore concentration in soil cores was the variability, both vertically and horizontally, from site to site, and even core to core at the same site. For example, there are very substantial differences among the three replicate soil cores taken within 1 m^2 the site near Bassano in southern Alberta, and no consistent pattern of change in concentration as one moved down through the soil core (Table 4). In general, spore concentration declined with increasing depth down to about 20 cm depth, but were present to the bottom of the core at the four sites where the pathogen had been established for many years. This means that simply stripping off the plough layer will not remove all of the spores that are present in the soil column. The high variability means that

a few soil samples where the resting spores are not found may not indicate that the pathogen is not present, but rather that it is simply not detected. In contrast, resting spores only occurred at detectable levels near the soil surface at two sites where the pathogen had only recently been established. This indicates that resting spores move downward in the soil profile over time, likely carried by ground water.

Training: Two MSc students at the University of Guelph were supported by this project. One (Travis Cranmer) has recently defended his MSc thesis and graduated, and the second (Justin Robson) is completing his data collection and writing his thesis. In addition, two research associates (Drs. A. Deora and F. Al-Daoud) received training and substantially expanded their skills working on the project.

9. Conclusions and Recommendations: *Highlight significant conclusions based on the previous sections, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project.*

Several fumigants with strong activity against the resting spores of *P. brassicae* were identified / confirmed in this study. Also, the importance of adequately sealing the soil surface to retain the volatile active ingredients of the fumigants for even a couple of days after application for maximum efficacy was demonstrated. However, the high variability of resting spore concentration and the distribution vertically deep into the soil profile ensures that pockets of infested soil will remain even after a uniform application of an effective fumigant.

The observation that resting spores are present deep into the soil profile has little or no effect on producers because infection resulting from these deep spores will occur late and have little impact on crop development. However, they could impact companies involved in large-scale movement of soil, such as oil and gas companies and some construction activities (e.g., highways, housing). These industries in Saskatchewan have already been informed via their representatives in the Saskatchewan Clubroot Initiative.

Based on the high levels of inhibition observed in qPCR assessments of levels of *P. brassicae* from several soil types, it is possible (even likely) that low levels of infestation in many field soils have not been detected using the standard technique. We believe that the multiplex method developed in this project represents a substantial improvement over the existing method, especially for samples from sites where the soil type is not well characterized (e.g., in the provincial clubroot surveys conducted in SK and MB in 2015). Adoption of this improved technique should improve the accuracy of assessment of resting spore concentration in soil and reduce the potential for false negative errors. Also, more accurate assessments of resting spore concentration improve assessment of treatments that reduce spore numbers. For example, it was recently used to examine reductions in spore numbers over time associated with crop rotation in a in a long-term study.

Similarly, the development of a rapid and accurate assessment of resting spore viability will improve assessments of treatment efficacy against resting spores, which may eventually be used to identify effective treatments for soil amendments, sanitation and fumigation, where progress has been hampered by the need to conduct resource-intensive bioassays.

10. Success stories/ practical implications for producers or industry: *Identify new innovations and /or technologies developed through this project; and elaborate on how they might impact the producers /industry.*

Several fumigants with efficacy against the resting spores of *P. brassicae* were identified. Most producers on the prairies do not have access to the specialized application equipment and covers required to allow fumigants to work effectively, but fumigation may represent a viable option for some companies (e.g., oil and gas pipelines). Based on these results, a recipe that producers could use to manage small areas of clubroot infestation has been proposed and will (hopefully) be tested in one or more commercial field situations in 2016.

11. Patents/ IP generated/ commercialized products: *List any products developed from this research.*

NA

12. List technology transfer activities: *Include presentations to conferences, producer groups or articles published in science journals or other magazines.*

Presentations and Abstracts

2013

Cranmer, T., Gossen, B.D., and McDonald, M.R. 2013. Evaluation of fumigants for clubroot control on Shanghai pak choy. Muck Vegetable Cultivar Trial and Research Report, 62: 66–67.

Cranmer, T., Gossen, B.D., and McDonald, M.R. 2013. Susceptibility of Tillage Radish to different pathotypes of *Plasmodiophora brassicae*, the causal agent of clubroot. Muck Vegetable Cultivar Trial and Research Report, 62: 70–71.

Cranmer, T., Gossen, B.D., and McDonald, M.R. 2013. Evaluation of pic plus for clubroot control on Shanghai pak choy, canola and cabbage, 2013. Muck Vegetable Cultivar Trial and Research Report, 62: 67–68.

Gossen, B.D., and McDonald, M.R. 2013. Future plans for environment and clubroot risk 2013. Invited presentation, Planning Meeting, Clubroot in GF2, Edmonton, December 6, 2013.

Gossen, B.D., Deora, A., Peng, G., and McDonald, M.R. 2013. Update on environment and clubroot risk 2013. Invited presentation, Planning Meeting, Clubroot in GF2, Edmonton, December 5, 2013.

Hwang, S.F., Strelkov, S.E., Howard, R.J., Gossen, B.D., and Peng, G. Management of clubroot on canola in western Canada. Invited presentation, Planning Meeting, Clubroot in GF2, Edmonton, December 5, 2013.

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2014

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13. List any industry contributions or support received.

The TIF was provided by the manufacturer in 2015.

14. Is there a need to conduct follow up research? *Detail any further research, development and/or communication needs arising from this project.*

Although these studies did not provide an approach to completely eradicate *P. brassicae*, the problem of what to do with small infested areas in fields remains. Drs. Gossen and McDonald prepared a 'recipe' for dealing with small infestations, that outlines an approach to identify and delineate the infested area, treat it with fumigants (if available) or lime to minimize disease pressure, isolate it, plant it to grass to minimize soil movement out of the area,

and eventually to bring it back into production. This recipe needs to be tried at several sites to validate its efficacy. The Canola Council of Canada is currently seeking sites to conduct this work.

15. Acknowledgements. *Include actions taken to acknowledge support by the Ministry of Agriculture and the Canada-Saskatchewan Growing Forward 2 bilateral agreement.*

We thank Dr. S.E. Strelkov for providing training with the initial qPCR technique, the staff at the Muck Crops Research Station, who supervised the application of the fumigants at the sites in Ontario, D. Alma, the producer who donated an area of his field for a trial of fumigant efficacy under commercial conditions, J. Douglas for the TIS and to D. Burke, G.D. Turnbull, and N. Rauhala, wo assisted with collection of soil samples at the sites in Alberta.

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16. Appendices: *Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited*

Table 1. Fumigant study - Controlled environment.

Product	Rate (kg a.i. ha^{-1})	DSI	Viable Spore (%)	Fresh weight (g)		Dry weight (g)	
				Root	Shoot	Root	Shoot
Busan	**10**	**38 b**	**94 a**	**0.1 ns**	**0.3 d**	**0.1 ns**	**0.2 c**
	75	**0 c**	**79 b**	**0.1**	**0.6 cd**	**0.1**	**0.3 b**
	150	**0 c**	**77 b**	**0.2**	**1.0 ab**	**0.1**	**0.4 a**
	300	**3 c**	**75 b**	**0.1**	**0.8 abc**	**0.1**	**0.3 ab**
Vapam	**10**	**45 b**	**--**	**0.1**	**0.2 d**	**0.1**	**0.2 c**
	75	**2 c**	**85 ab**	**0.1**	**0.7 bc**	**0.1**	**0.3 ab**
	150	**0 c**	**--**	**0.2**	**1.0 a**	**0.1**	**0.4 a**
	300	**1 c**	**--**	**0.2**	**1.0 ab**	**0.1**	**0.4 a**
Control	**--**	**70 a**	**95 a**	**0.2**	**0.2 d**	**0.1**	**0.2 c**

Table 2. Clubroot severity (disease severity index, DSI) and plant growth of Shanghai pak choy following a field trial to assess the efficacy of two fumigants, MCRS in 2014.

Product	Rate (kg a.i. ha^{-1})	DSI	Fresh weight (g)	
			Root	Shoot
Chloropicrin	128	46 b	19 ns	310 ns
Metam sodium	150 [z]	84 a	25	230
	300	78 a	25	210
Control	--	89 a	22	190

[z] Label rate.

Table 3. Field trial to assess the efficacy of two fumigants against *Plasmodiophora brassicae* at a commercial field near Hamilton in 2014.

Product	Rate (kg a.i. ha^{-1})	DSI	Fresh wt. per 10 plants (kg)	
			Root	Shoot
Pic Plus	168	0 b	0.5 ns	3.3 bc
	224	0 b	0.7	4.7 ab
	280	0 b	0.6	4.0abc
	336	0 b	1.6	5.8 a
Busan 1236	148	0 b	0.4	2.5 bc
Vapam HL	75	0 b	0.4	2.0 c
	150	0 b	0.5	3.2 bc
	300	0 b	0.6	3.5 abc
Control	--	3 a	0.2	2.0 c

Table 4. Vertical distribution of resting spores (of *Plasmodiophora brassicae* at a naturally infested site near Bassano in southern Alberta, collected in 2013.

	Site 1 (x1000, g^{-1})			Site 2 (x1000, g^{-1})		
Depth (cm)	**1**	**2**	**3**	**1**	**2**	**3**
0–7.9	41	18*	8.8*	46	160	8.8
8–15.9	9.2*	9.3	-	20	53	18
16–22.9	16	14	-	13	-	19
23–29.9	-	-	11	6.1	5.1*	9.3
30–37.9	-	-	21	-	-	25
38–45.9	-	2.3*	-	-	8.3*	-
46–53	-	7*	9.3	26	14	12*
Total	**66.2**	**50.6**	**50.1**	**111.1**	**240.4**	**92.1**

* Approximate # of resting spores detected, based on extrapolation.
– Unable to estimate spore numbers in initial assessments.

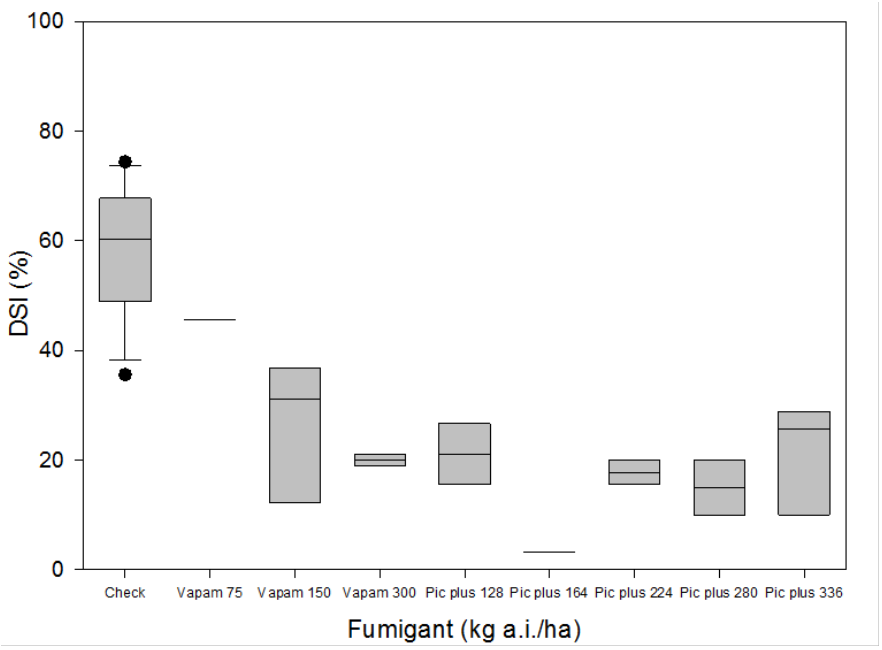

Fig. 1. Clubroot severity (disease severity index, DSI) on Shanghai pak choy planted after treatment with Pic Plus, Busan and Vapam fumigants at selected rates at the Muck Crops Research Station in 2015.

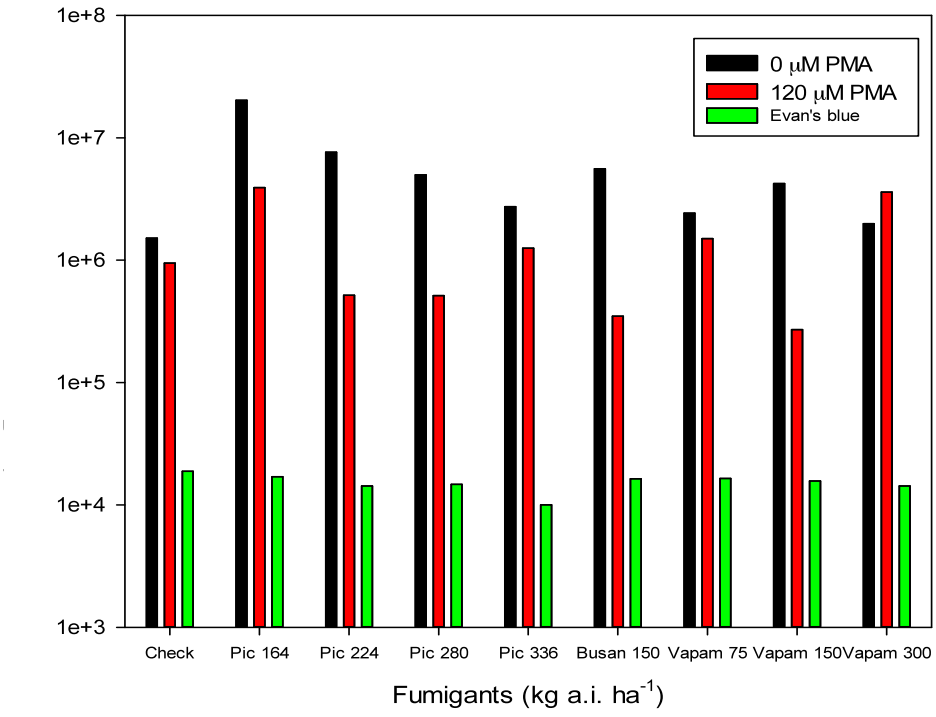

Fig. 1. Assessments of resting spore viability using Evan's Blue stain and 120 µM PMA compared to regular qPCR (0 µM PMA) after treatment with Pic Plus, Busan and Vapam fumigants at selected rates at the Muck Crops Research Station in 2014.

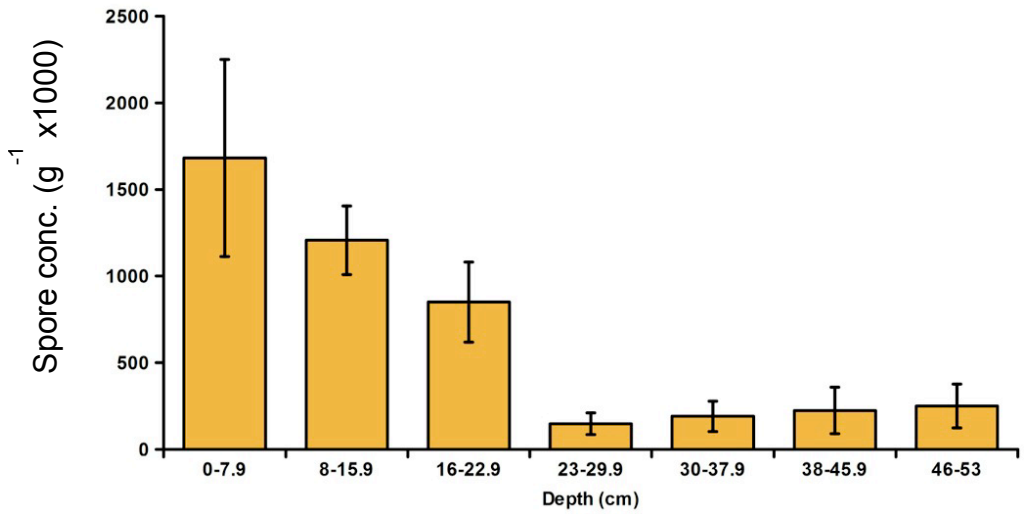

Fig. 2. Vertical distribution of resting spores within the soil at a location in Ontario naturally infested with *Plasmodiophora brassicae*.

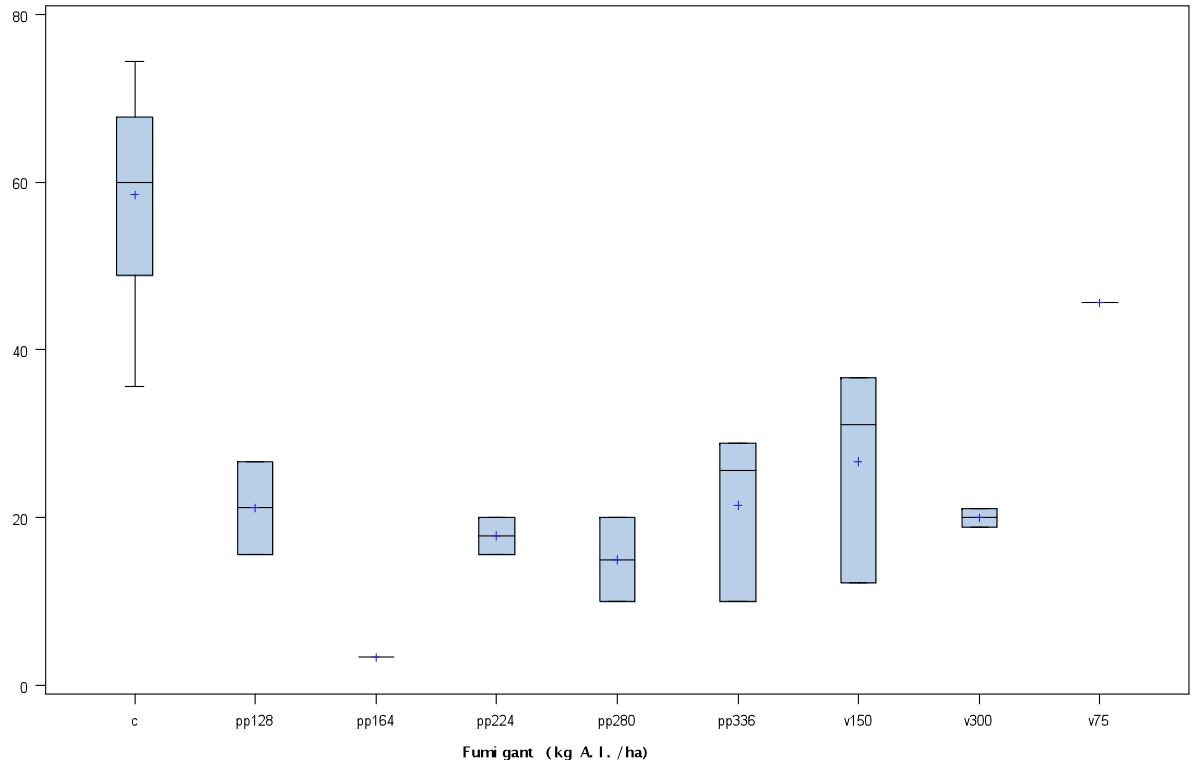

Fig. 3. The effect of fumigant and rate (Pic Plus (pp) at 128, 164, 224, 280, 336 kg ha^{-1}; Vapam (v) at 75, 150, 300 kg ha^{-1}) on subsequent clubroot severity on Shanghai pak choi cv. Mei Qing Choi at the Muck Crops Research Station, 2015. .