POSTER ABSTRACTS

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1. Pathotype reaction in clubroot-resistant canola cultivars in Canada

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Clubroot (*Plasmodiophora brassicae*) was reported first on canola (*Brassica napus*) in western Canada in 2003. Since then, several clubroot-resistant cultivars have been developed. The timing and expression of resistance to each of the major pathotypes (pathotypes 2, 3, 5, 6, Williams' system) in Canada was examined in four resistant cultivars. Root hair infection occurred at high levels in each resistant cultivar, but developed more slowly than in the susceptible control. Secondary infection and development in cortical cells was inhibited in each resistant cultivar; only a few bi-nucleated plasmodia were observed at 12 days after inoculation (DAI), and plasmodia were rarely observed at 18 and 24 DAI. In contrast, development in the susceptible cultivar had progressed to resting spores by 24 DAI. In addition, a dense ring of reactive oxygen species (ROS) accumulated in and around the endodermis of non-inoculated controls and inoculated plants of each of the resistant cultivars. However, the ROS ring disappeared rapidly in infected plants of the susceptible control. No specific points of ROS accumulation or lignification were observed in any of the resistant cultivars; which indicates that a hypersensitive response did not occur. Resistance to clubroot is generally pathotype specific, so the uniform response of the resistant cultivars to several pathotypes is one line of evidence indicating that the resistance in these cultivars is conditioned by a gene(s) from a single source. If so, this may pose a threat to the durability of this resistance for clubroot management on canola in this region.

2. Screening germplasm in *Brassica oleracea* for breeding for resistance to clubroot (*Plasmodiophora brassicae*)

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Clubroot is a gradually increasing soilborne disease of cruciferous crops caused by *Plasmodiophora brassicae*, which has proven difficult to prevent and extirpate by cultural practices and chemical treatments. Resistant cultivars would therefore be desirable. To screen genetic resources resistant to clubroot in *Brassica oleracea*, a total of 436 varieties and lines of *B. oleracea*, comprising 240 cabbage accessions, 152 cauliflower accessions, 22 broccoli accessions and 22 accessions of somatic hybridization between cauliflower and black mustard, were tested in greenhouse for resistance to *P. brassicae* from a disease index evaluated on young plants artificially inoculated with a predominant pathotype 4 of *P. brassicae*. Pathotype 4, as classified on the differentials of Williams, is spread all over the country of China and is increasingly damaging to cruciferous crops. Some accessions of cabbage showed resistance to clubroot, very limited accessions of cauliflower and broccoli showed intermediate resistance to clubroot, and most accessions were susceptible. However, a high level of resistance was confirmed in the progeny of somatic hybridization between cauliflower and black mustard, which suggested the possibility of a new source of clubroot resistance for *B. oleracea*. These genetic resistance resources would be used for clubroot-resistance breeding stocks of *B. oleracea*.

3. Plasmodiophora brassicae resting spore load dynamics in clubroot-resistant canola (*Brassica napus*) cropping systems in Alberta, Canada

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Clubroot of canola (*Brassica napus*), caused by the biotrophic parasite *Plasmodiophora brassicae*, has proven difficult to manage due to the longevity of its resting spores, its ability to produce large amounts of inoculum, and the prohibitive costs of effective fungicides. Aside from a long rotation away from clubroot susceptible hosts, the cropping of resistant canola cultivars is one of the few effective strategies for clubroot management. There is evidence from greenhouse studies that resistant cultivars may induce resting spore germination, while supporting very limited production of new inoculum, thereby serving to deplete spore loads in the soil. In order to evaluate the impact of resistant cultivars on inoculum loads under field conditions, soil in fields that have natural clubroot infestations is being monitored for pre- and post-harvest *P. brassicae* resting spore concentration by quantitative PCR. Commercial fields in a number of crop rotation regimes have been included in the study. The main objective is to determine what effect resistant canola cultivars will have on *P. brassicae* soil inoculum loads in fields maintained under different rotations and with different levels of clubroot infestation. A second objective is to establish a threshold level of inoculum at which it is reasonable to plant resistant cultivars and expect no subsequent increase in spore load.

4. Potential for degree day modeling of clubroot on canola

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There are a number of environmental factors that contribute to clubroot (*Plasmodiophora brassicae* Woronin) development in the field. Temperature affects all stages of clubroot development. A project was initiated to develop an equation based on temperature (degree days) and rainfall to predict clubroot severity on canola (*B. napus* L.). Cultivar 'InVigor 5030' was seeded at 2-wk intervals in 2011 and 2012, and plants were sampled and assessed for clubroot weekly. Additional data from previous trials on Chinese flowering cabbage (*B. rapa* subsp*. chinensis* (Rupr.) var. *utilis* Tsen and Lee) were included in the data set. With a T_{base} of 14 °C, the best predictive parameters during crop growth were accumulated air degree days (R^2 0.61) and soil degree days using a 1-wk delay (R^2= 0.54). The best predictive parameters at plant maturity was accumulated soil degree days over the last 2 weeks before sampling date plus rain in the first 2 weeks after seeding (R^2 = 0.45). The mean biases of the models were 2.8 %, -8.3 DSI, and 14 %, respectively. The bias of each analysis generally increased as severity increased. T_{base} of 12 and 17 °C were also assessed, but they resulted in lower R^2 or higher bias. This approach appears promising, but additional years of data are required to improve the model.

5. Limitations of qPCR to quantify gDNA of *Plasmodiophora brassicae* in young seedlings

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Microscopic examination of stained root hairs, cortical sectioning and image analysis, and qPCR were used to quantify the effects of temperature on development of *Plasmodiophora brassicae* Wor. There was a linear correlation between gDNA of *P. brassicae* and root hair infection at 4 and 8 days after infection (DAI) on cabbage (*Brassica oleracea* L.). However, there was no correlation between gDNA and root hair infection on canola (*B. napus* L.) at 10 DAI. In canola, gDNA of *P. brassicae* was highest at 10°, 12.5°, and 25° C, but low at 15°–22.5° C and 27.7° C. This result was unexpected based on visual assessment of total root hair infection, where development occurred more quickly with increasing temperature to a maximum at 25° C and then declined at higher temperature. The discrepancy between visual assessment and gDNA may be associated with a difference in assessment date. At 10 DAI and optimum temperatures, the pathogen is in transition between its primary (root hairs) and secondary (root cortex) infection cycles. Secondary zoospores are released from root hairs, but have not yet begun to colonize the root cortex. At this time, estimates of gDNA using qPCR can decline dramatically, only to increase quickly in subsequent assessments. Users need to be aware that assessments of gDNA during this transition period could substantially underestimate the infection potential of the pathogen in these young seedlings.

6. Cost of resistance to *Plasmodiophora brassicae* when inoculum pressure is high

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Canola (*Brassica napus*) is a major crop in Canada, valued at over $15 billion (CDN) per year. Clubroot (*Plasmodiophora brassicae*) is spreading rapidly on canola, but use of clubroot-resistant cultivars provides effective disease reduction. However, several lines of evidence indicate that the yield of resistant cultivars of canola and other Brassicas is substantially reduced when inoculum pressure is high. In growth cabinet trials, biomass was reduced and plant development was delayed in plans of resistant cultivars inoculated with an avirulent pathotype, relative to non-inoculated controls. Similarly, growth and yield of resistant cultivars of canola and several Brassica vegetables in field trials were substantially lower at sites where inoculum pressure was high compared to nearby sites where inoculum pressure was low. In a crop rotation study that compared 1-, 3- and 11-yr breaks from canola at a heavily infested site, development of resistant canola cultivars was delayed and yield was reduced by about 20% in the 1-yr break compared to the 3-yr break (severity in a susceptible cultivar was 100%, irrespective of cropping interval). Previous studies have shown that infection develops initially but does not persist in resistant canola cultivars, which indicates that resistance involves an active process of pathogen recognition and suppression. We conclude that there is a metabolic cost associated with expression of resistance when inoculum pressure is high that results in reduced plant size, delayed development, and reduced yield.

7. Genetics of resistance in rutabaga (*Brassica napus* var. *napobrassica*) to Canadian *Plasmodiophora brassiceae* pathotypes

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Clubroot disease, caused by *Plasmodiophora brassicae*, is an emerging threat to canola (*Brassica napus*) production in Canada. Canola cultivars with durable resistance are the key for effective management of this disease. Introgression of clubroot resistance into canola from rutabaga (*Brassica napus* var. *napobrassica*) and development of molecular marker(s) for use in marker-assisted breeding were the goals of this research. A rutabaga inbred line, Rutabaga-BF, resistant to *Plasmodiophora brassicae* pathotypes present in Canada, crossed to a clubroot susceptible spring canola line A07-29NI, and double haploid (DH) lines produced from the F_1 plants through application of microspore culture technique. The DH population tested in greenhouse for resistance to *P. brassicae* pathotypes 2, 3, 5, 6 and 8. Distribution of the DH population for resistance to these phenotypes was bi-modal. Of the DH lines showing resistance to pathotype 3, about 90% lines showed resistance to pathotypes 2, 5, 6 and 8 as well. This is also evident from strong correlation of resistance to pathotype 3 and resistance to other pathotypes. The result suggest that the same genomic region to be involved in the control of resistance in Rutabaga-BF.

8. Breeding of clubroot resistant Ogura CMS New Variety CCR11242 on *Brassica campestris*

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The clubroot resistant self-incompatible Chinese cabbage variety CCK was backcrossed with Ogura CMS Chinese cabbage variety HGC for 6 generations to breed BC6 generations of clubroot resistant Ogrua CMS Chinese cabbage BIL line “CCR11239”. The clubroot resistant self-incompatible F6 generations maintainer line CCR11240 of CCR11239 has been bred through CCK self-crossed 6 generations. The clubroot resistant self-incompatible Chinese cabbage variety CCK was crossed with Yunnan large scale cultured variety 83-1 to breed clubroot resistant self-compatible F6 generation line CCR11241. New variety CCR11242 had been obtained through CCR11239×CCR11241. Through the testing of clubroot resistance, the disease index of this variety was revealed to be 5.63, much lower than for CK (83-1) 88.13. This indicates a high clubroot resistance. In the clubroot disease affected districts, after varieties were compared in the field, the yield of this variety was on average 4065kg/666.7m^2, representing an increase of 142% relative to CK in production.

9. Evaluating methods for cleaning and disinfesting equipment contaminated with clubroot

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The transportation of infested soil or infected crop residues from one field to another has the potential to spread the clubroot pathogen (*Plasmodiophora brassicae* Woronin). Farm machinery, especially tillage equipment, may carry hundreds of kilograms of soil and crop residues. Sanitization (sanitation) is the process of cleaning and disinfecting hard surfaces (machinery, equipment, vehicles, tools, footwear), seed, plant materials, water and/or soil infested with pathogens. It has been used for clubroot management in vegetable operations for many years, but its use in canola production systems has been a recent innovation. Equipment sanitization involves three key steps: i) rough cleaning using scraping, brushing or blowing to remove bulk soil and crop debris; ii) fine cleaning using pressure washing, scrubbing or compressed air to remove remaining residues; and iii) disinfection with an effective biocide applied to the cleaned surfaces with ≥20 minutes of contact time to ensure any remaining spores are killed. The most critical steps in sanitizing clubroot-infested farm machinery, equipment and vehicles are the rough and fine cleaning, which should render the surfaces free of visible soil and plant material and aim to remove up to 99% of the contamination. Various disinfectants and thermal treatments were evaluated for the final step in a sanitization protocol. Amongst 10 chemical disinfectants tested, sodium hypochlorite, hydrogen peroxide, acetic acid, and potassium peroxymonosulphate were the most effective. Thermal treatments of ≥80°C for 30 min were required to induce substantial resting spore mortality, but some even survived exposure to 100°C for up to 3 hr.

10. Study of clubroot resistance and Ogura CMS germplasm innovation on *Brassica* spp.

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In order to transfer clubroot resistance genes of *Brassica rapa* into *B. oleracea* and *B. napus*, the clubroot resistant Ogura CMS line C49-141 of *B. rapa* was crossed with the inbred line MWZS of *B. oleracea*. Embryo rescue techniques were used 12 days after pollination. In the medium MS+ 6-BA 1.0 mg/L + NAA 0.08 mg/L, we successfully obtained 1 plantlet from C49-141“1/1”×MWZS“3/1”. After propagation of this plantlet by tissue culture, the plantlets were tested for resistance to *Plasmodiophora brassicae* by soil inoculation, and shown to be shown to be highly resistant. Most showed morphological characteristics of *B. oleracea*, but some plantlets showed morphological characteristics of *B. napus* through chromosome doubling. All the plantlets showed characteristics of Ogura CMS based on observation of the flowers. By counting the chloroplast numbers of stomatal guard cells to identify chromosome ploidy level of the plants, we confirmed that we obtained Ogura CMS interspecific hybrid material of AC type chromosome and AACC type chromosome material of *B. napus.*

11. The incidence of *Plasmodiophora brassicae* in agricultural soils in Poland

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For many years, clubroot (*Plasmodiophora brassicae*) was not harmful to oilseed rape in Poland or it was a local problem at some sites in the western part of the country. In the last few years, a dramatic increase in clubroot incidence has been observed, which coincides with the rapid increase in oilseed rape cultivation. The aim of this study was to assess the incidence of *P. brassicae* in randomly collected samples of agricultural soils. Sampling was done using a soil auger at several places per each studied field. Collections were made from 697 fields in 280 out of the 380 districts of Poland. The presence of *P. brassicae* was studied under greenhouse conditions via a bioassay and in the laboratory using molecular detection techniques. In a bioassay test, 5-day old seedlings of *Brassica campestris* ssp. *pekinensis* and the Polish variety of oilseed rape (*B. napus*) Monolit were used as bait plants – they got infected in soils heavily infested with the pathogen. Molecular detection was correlated with the results of the bioassay. Samples originating from 29.3% of the districts were infested with *P. brassicae* at least at one or more spots. The pathogen was found in soil samples collected from most of the regions, including some sites considered to be free from clubroot. Most samples containing *P. brassicae* were obtained from the provinces located in the west and north-east of Poland. Based on these results, it can be concluded that *P. brassicae* is now widespread and it is necessary to monitor its incidence.

12. Clubroot in Europe – A survey of *Plasmodiophora brassicae* pathotypes occurring in the main European oilseed rape growing regions

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Clubroot caused by the obligate biotrophic protist *Plasmodiophora brassicae* is a serious soil-borne disease of cruciferous crops. It causes galls on roots leading to premature death of plants. Currently, due to increased oilseed rape acreage within the last decades, the number of infested fields is increasing. Numerous populations and races with differences in pathogenicity are present. For this reason breeding for resistance is difficult. To get more detailed information on the occurrence of different pathotypes and their implications for agricultural production, samples of infected plant material were taken from the main oilseed rape growing regions in Europe. The collection contained samples from the United Kingdom, France, Denmark, Poland, Czech Republic, Austria and Germany. These samples were analyzed under greenhouse conditions by using artificial inoculation and performing optical ratings of disease symptoms. The European Clubroot Differential Set (Buczacki et al. 1975, Trans. Br. Mycol. Soc. 65, 295-303) and the INRA differential set (Somé et al. 1996, Plant Pathol 45, 432-439) were used for the characterization, respectively. Results of these analyses show clearly that different pathotypes are present in Europe. The virulence of these pathotypes to the *Brassica rapa* genotypes of the ECD was rather low. For the *B. napus* and *B. oleracea* genotypes, the situation is different as considerable variation with respect to virulence to the different hosts within each group was detected. There was no clear correlation between pathotypes and the location they were derived from, but the frequency of the highly virulent pathotype P1 in Northern Europe was remarkable.

13. Reaction of canola to inoculation with primary and secondary zoospores of *Plasmodiophora brassicae*

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Plasmodiophora brassicae causes clubroot of canola (*Brassica napus*) and many other Brassica crops. Resting spores of the pathogen germinate and release primary zoospores that infect root hairs. Secondary zoospores are produced in root hairs, released, and then infect the root cortex. Studies were conducted to investigate the role of these two spore types on cortical infection and subsequent clubroot severity in canola cv. Zephyr. Plants were inoculated with resting spores (RS, as a source of primary zoospores) or secondary zoospores (SZ) of either a virulent (P3) or an avirulent (P6) pathotype, singly or with secondary zoospores of P3 added with resting spores of either P3 or P6. Percent area of the root cortex infected was assessed 10 days after inoculation and clubroot severity was assessed 42 days after inoculation. The pattern of response for cortical infection and severity were similar. Inoculation with RS-P6 (avirulent) resulted in almost no infection (0.1%) or severity (0%), but inoculation with SZ-P6 produced low levels of both infection (4%) and severity (31%). Inoculation with RS-P3 produced more infection (33% vs. 12%) and higher severity (67% vs. 100%) than SZ-P3. Adding SZ-P3 to RS-P3 did not increase cortical infection (34% vs. 33%), but adding RS-P3 + SZ-P6 produced lower infection (18% vs. 34%) and severity (84% vs. 100%) than RS-P3 + SZ-P3. These results indicate that pathogen effectors act at the root hair infection stage and suppress (P3) or induce (P6) resistance in the host.

14. Search for sources of resistance to *Plasmodiophora brassicae* in *Brassica* mutants and hybrids

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Screening for susceptibility/resistance to clubroot (*Plasmodiophora brassicae*) was carried out for 130 hybrids between *Brassica* species, 5 parental genotypes and 33 mutants of *Brassica napus*. The hybrids represented F3-F6 generations of crossings of *B. napus* x *B. carinata*, *B. napus* x *B. juncea*, *B. napus* x *B. campestris* ssp. *pekinensis* and *B. napus* x *B. campestris* ssp. *trilocularis*. The oilseed rape line used to obtain these hybrids was male sterile. The mutants of oilseed rape were obtained with chemical mutagens: MNUA and AS. The mutants included lines with increased tocopherols in seeds (400-500 ppm), lines with a decreased amount of indole glucosinolates (below 10 μM) and semi-dwarf lines. Screening was done using a biotest: small 5-day old seedlings were transplanted to the soil and inoculated with spore suspensions prepared separately for each of three isolates of *P. brassicae* of different origins. The isolates were obtained as a result of pathogen propagation in a glasshouse, in soil with decreased pH, which favors plant infection. The clubroot assessment was done 8 weeks after inoculation. A big variation in plant response to artificial inoculation was observed. Some genotypes differed in their reaction to particular isolates. The most susceptible genotypes showed disease symptoms in a field experiment conducted in south-west Poland. The experiment was done on a field infested by the pathogen and also in a distant field that was clubroot-free.

15. Clubroot on winter rape (*Brassica napus*) in the Czech Republic

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Winter rape is the second most important crop in the Czech Republic. Clubroot, caused by *Plasmodiophora brassicae*, was previously a problem in vegetable production, but the disease has begun to appear on rape recently. Infected stands were reported throughout the country in fall 2011. Serious infestations were found on 44 farms, mainly in the north and north-east of the country. Although the pathogen is probably widely spread in the whole country, its appearance in agricultural areas depends on appropriate conditions of weather in the sowing period in August. The damage on rape is not so high yet, because the amount of inoculum and the distribution of the pathogen within fields are not sufficiently great, but this is just the initial stage of colonization. The situation is serious, because there is not an easy, cheap and ecological way to protect against this pathogen. The research on *P. brassicae* in the Czech Republic is therefore really important and aimed at developing necessary management strategies against clubroot under Czech environmental conditions. Pilot experiments with clubroot resistant cultivars of winter rape started in 2012. Experiments were made in a greenhouse and under field conditions. However, the results obtained from both experiments are almost the same. One of the tested resistant cultivars is Alister, which shows no visible signs or symptoms of infection, although PCR analysis confirmed the presence of the pathogen in all cases. More experiments with the resistant cultivars will be carried out. For quantification of spore loads, qPCR analysis will be used and initial tests of qPCR have already started. The monitoring of clubroot pathotypes in the country is also planned.

16. The distribution, damage, biocontrol and physiological race identification of clubroot in Sichuan, China

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Clubroot, one of the most important diseases of the cruciferous crops, has been found in 40 cities and counties in the western and southern Sichuan Province, with especially severe damage in Guanghan, Pengzhou, Pixian, Meishan, Dayi, Xindu, Wenjiang and Xichang. It was discovered in Sichuan in1989 and the first report of infection in *Brassica napus* was in 1995. Clubroot causes big losses in the production of cruciferous crops because the galls on the roots affect the absorption of water and nutrients. In recent years, this disease has been spreading increasingly in Sichuan causing variable damage in different hosts: *Brassica rapa*＞*B. napus* ＞*B. oleracea*＞*Raphanus sativus*. After a series of screenings, two Actinomycetes isolates for the biocontrol of *Plasmodiophora brassicae* F-22 and F-24 were identified, with glasshouse control efficiencies of 52.78% and 55.37%, respectively. Field control efficiencies of 46.39% and 48.14% were also observed and mutant strains showed field control efficiencies of 59.99% and 58.93%; these have been grouped to *Streptomyces spp.* through morphological and molecular identification. Identification of physiological races is an important foundation work for breeding for disease resistance. Pathogen collections from some fields in Sichuan were identified with Williams' differential hosts. Most of the collections were identified to the same race, which was regarded as the dominant race. A few single-spore isolates were obtained through a series of modified single-spore isolation techniques. Primary analyses are underway to develop better identification methods in order to establish a standard and scientific identification procedure.

17. The genome of a single-spore isolate of *Plasmodiophora brassicae*

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The soil-borne plant-pathogenic protist *Plasmodiophora brassicae,* belongs to the Plasmodiophorids inside the eukaryotic supergroup Rhizaria. *P. brassicae* is the causal agent of the clubroot disease, one of the most damaging diseases in the *Brassicaceae* plant family. Despite its agricultural importance, the biology of *P. brassicae* remains poorly understood. Due to its obligate biotrophic nature, *P. brassicae* remains impossible to grow in axenic culture and the typical experimental systems for working with *P. brassicae* are complicated. Molecular studies are especially challenging with approximately only 100 known genes. We recently succeeded in obtaining the whole genome sequence from a *P. brassicae* single-spore isolate. Our current assembly draft, obtained by Illumina sequencing (using a 250bp and 5kb insert library), suggests a total length of the genome sequence of 24 Mb, slightly larger than the previous estimation of a genome size of 18–20.3 Mb. Currently, 454 sequencing is in progress to validate and improve the *P. brassicae* genome assembly. Furthermore, transcriptome data were obtained from clubs and some life stage-specific states from which around 10,000 genes were predicted. Our *P. brassicae* sequence will be the first genome sequence of the plant pathogenic protist group Plasmodiophorids. The genome provides a solid reference to explore molecular differences between *P. brassicae* races in the future. The life stage-specific transcripts will help in understanding of the life cycle at a molecular basis, which will in the long run be helpful to understand and control clubroot disease. Further details on the genome and transcriptome are presented.

18. Molecular insight into *Plasmodiophora brassicae* pathogenesis in *Brassica napus*

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Damage to worldwide Brassica crops, as a result of infection by the protist *Plasmodiophora brassicae* Woronin (clubroot) is estimated to account for approximately 10-15 % of total Brassica production. *P. brassicae* life cycle dynamics, including primary and secondary infection, have been intensively studied, however, molecular insight into the pathogenesis of this protist is still a work in progress. As a result, very few ESTs of *P. brassicae* are currently available in Genbank. To investigate the molecular mechanism(s) of *P. brassicae* infection we have generated a cDNA library from *Brassica napus* (canola: Westar) roots infected by *P. brassicae* pathotype 3 (Dr. G. Peng: AAFC, Saskatoon). Galls from heavily infected plants were collected 35 days post inoculation and a cDNA library was generated from total mRNA. To date, EST sequencing and analysis has identified ~41 % of the library as putative *P. brassicae* clones with the vast majority previously undocumented. Of the 41 %, ~6 % are candidate secretory proteins, (SignalP 4.1), which may be involved in *P. brassicae* intracellular biotrophic parasitism. With 35 % of the clones containing canola ESTs a preliminary plant response network to *P. brassicae* infection is being developed. We are aiming to sequence 25000 clones, such that detailed molecular pathways of both the host and pathogen responses to clubroot formation should emerge.

19. Assessment of *Brassica rapa* and *Brassica napus* genotypes for detecting physiologic specialization in populations of *Plasmodiophora brassicae* from Canada

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The occurrence of physiologic specialization in *Plasmodiophora brassicae*, the causal agent of clubroot of crucifers, has long been known. In recent years, this parasite has emerged as an important constraint to canola (*Brassica napus*) production in the Canadian Prairies. As such, there has been strong interest in the development of a stream-lined system of differentials that can provide *P. brassicae* pathotype designations that are relevant to the canola crop. A collection of 11 *B. napus* and two *B. rapa* genotypes were tested for their suitability as potential hosts in a putative Canadian clubroot differential set. These included some of the genotypes from the European Clubroot Differential (ECD) set, the differentials developed by Somé *et al.*, and several commercially available Canadian cultivars of canola. This collection of hosts was tested against 11 populations and 7 single-spore isolates of *P. brassicae*, representing collections of the parasite from canola in the Prairies, canola in Quebec, and cruciferous vegetables from Canada, the United States and China. The putative differentials were able to distinguish five strains of *P. brassicae*, with some isolates and populations formerly classified as pathotypes 5 and 6 on the differentials of Williams separated into distinct groups. Several of the hosts gave similar reactions and could be represented by a single genotype in a new differential set. The data indicate that these hosts may constitute an effective foundation for the development of a Canadian clubroot differential set, aimed at better characterizing *P. brassicae* populations from this country.

20. Evaluation of the impact of crop rotation on clubroot severity in canola in Alberta, Canada

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Clubroot, caused by *Plasmodiophora brassicae*, is an emerging disease of canola in the Canadian Prairies. A greenhouse experiment was conducted to evaluate the effects of six crop rotation regimes on clubroot severity. These included: T1) continuous cropping of a clubroot-susceptible canola cultivar (S); T2) S –fallow (F) - S – F - S; T3) S – barley (B) - S – B – S; T4) S – resistant canola cultivar (R) - S – R – S; T5) continuous cropping of the same resistant cultivar; and T6) continuous cropping of different resistant cultivars. Index of disease (ID), plant height and dry mass were recorded at the end of each rotation. Over the course of the experiment, the range of IDs in the susceptible canola cultivar grown in T1 to T4 decreased significantly from 97.0% - 98.6% (after the first crop) to 52.8% – 82.5% (after the fifth crop). In contrast, the IDs in the resistant canola cultivar grown in T5 increased significantly form 15.0% to 23.5%. In T6, in which different resistant cultivars were grown in succession, no significant changes in ID were observed. At the end of the experiment, the plant height of the canola plants in T5 and T6 was significantly higher than in T1 to T4, reflecting the much greater root infection in the susceptible canola cultivars. The data suggest some erosion of resistance after continuous cropping of the same resistant cultivar, which was not observed when different resistant cultivars were rotated. Resistance stewardship will have to be an important component of a sustainable clubroot management approach.

21. Life cycle of *Plasmodiophora brassicae* and auxin homeostasis in plant-*P. brassicae* interaction

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Plasmodiophora brassicae, a soilborne parasite, induces club-shaped tumor-like growths on the host root system and hypocotyls after infection. The symptom developments are reported to be associated with alterations in the levels of phytohormones, especially auxins and cytokinins. In this study, an axenic dual culture system of *P. brassicae* with its hosts was established in the absent of exogenous plant hormone. The inoculum from calli induced from clubroot harboring *P. brassicae* retains infectious ability on both canola and Arabidopsis plants growing on medium plates. At higher inoculation pressure (5×10^7 to 10^8 spores/ml), the pathogen finished its life cycle with all development stages and cause gall formation. The details of growth and development stages of *P. brassicae* with its host were studied by fluorescence microscopy. Furthermore, transgenic Arabidopsis lines with GFP tagged auxin efflux proteins (PIN1-GFP, PIN2-GFP, PIN3-GFP, PIN4-GFP, PIN7-GFP) are used to investigate the regulation of change in auxin homeostasis at the infection site by confocal microscopy. Also, mutants defective in auxin biosynthesis and transport are investigated to decipher the genetic and molecular mechanisms of clubroot symptom developments. In summary, our established pathosystem has provided a new platform for studying the cellular interactions between *P. brassicae* and its host plants.

22. Field volunteer destruction strategies to manage clubroot disease

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Clubroot, caused by the root-infecting biotrophic pathogen *Plasmodiophora brassicae*, is a destructive disease of cultivated crucifers, and is an emerging threat to German oilseed rape (*Brassica napus*) production. Previous studies demonstrated that oilseed rape volunteers and weeds play a critical role in predisposing disease incidence and severity. In the present greenhouse study, a clubroot-susceptible oilseed rape cultivar was used to examine the efficacy of foliar application of the herbicide glyphosate and mechanical destruction of rapeseed volunteers in reducing clubroot disease severity. Plants were raised until growth stage 12 and then inoculated with resting spores of *P. brassicae*. To determine the effect of timing of applications, plants were treated early (seven days after inoculation, dpi) or late (21 dpi). Disease severity was assessed on 35 dpi. Changing the time of treatment had a significant impact on control efficacy. Relative to untreated controls, plants in the early application of glyphosate as well as the early mechanical destruction had limited the clubroot symptoms. Especially, early glyphosate treatment caused a reduction of disease incidence and severity in treated plants. Hypothetically, these treatments interrupted the pathogen's development, and suppressed its establishment and survival of the resting spores. Early plant destruction has the potential to reduce inoculum build-up of *P. brassicae* supposedly because host plants act as trap crops for this obligate pathogen. In the absence of other management options, early destruction of volunteers could be recommended as pathogen-suppressing integrated pest management tool.

23. Clubroot (*Plasmodiophora brassicae*) control in the Canadian canola (*Brassica napus*) crop using Vapam as a soil fumigant

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Plasmodiophora brassicae is an obligate parasite responsible for causing clubroot, an economically important soilborne disease of the family Brassicaceae. In Alberta, clubroot was first reported in 12 canola (*Brassica napus*) fields in 2003, and as of 2012, there were more than 1,000 fields with confirmed clubroot infestations in the province. Isolated cases of the disease have also been identified in Saskatchewan and Manitoba. Given the yield and quality losses associated with clubroot of canola, it is important to consider control measures to limit the spread of this disease. Soil fumigation could prove to be an effective tool to eradicate localized clubroot infestations and new infection foci. Soil-applied Vapam is a liquid metam sodium solution traditionally applied to control weeds, nematodes, insects and soil-borne diseases in crops. Two heavily infested field locations in Edmonton, Alberta, were selected to analyze the efficacy of various Vapam concentration rates for the control of clubroot of canola. A clubroot-susceptible canola cultivar was grown in soil treated with Vapam and plants were assessed for disease severity, gall weight, and plant weight and height. Preliminary results from one of the field locations suggest that Vapam may effectively reduce clubroot severity when applied at label rates or higher. The same sites will be sown to the same canola cultivar in 2013 to assess the residual effects of the Vapam treatments. New field sites will also be included to replicate the initial experiment using the same concentrations of Vapam.

24. Reduction of clubroot (*Plasmodiophora brassicae*) symptoms associated with using calcium cyanamide fertilizer Perlka® in field crops

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Calcium Cyanamide has been known for decades to be associated with suppressive effects for clubroot (*Plasmodiophora brassicae*). Recent field trials show how to use this fertilizer in brassica production making most profitable use of these suppressive effects. Trial work has been done in Germany with transplanted cauliflowers. Applications of 120 kg/ha N in form of Calcium Cyanamide Perlka® one week before planting and of 80 kg/ha N as a top dressing 14 days after planting enabled the successful growth of cauliflowers even on highly infested fields. These suppressive effects are thought to be associated with increased soil microbial flora which is antagonistic to *Plasmodiophora brassicae*. For autumn sown oil seed rape the highest risk for clubroot infections is in the first weeks after sowing as at the end of summer the soil is still quite warm. Reductions in clubroot infections have been observed associated with applications of 50 kg/ha N as Calcium Cyanamide Perlka® made immediately before sowing and without incorporating the fertilizer into the soil. This application the fertilizer is thought to stimulate soil microbial flora which affect the germination of clubroot resting spores in the top soil horizons just where the rape seeds are going to germinate.

25. Identification and mapping of clubroot resistance genes.

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Clubroot, caused by *Plasmodiophora brassicae* Woronin, is a serious disease in *Brassica* crops worldwide, and a threat to canola production in western Canada. Host resistance is one of the most effective strategies for clubroot control. In preliminary screening, 35 *Brassica* spp. accessions, including *B. rapa, B. nigra, B. oleracea* and *B. napus*, showed substantial resistance against *P. brassicae* pathotype 3 (predominant on canola). Selected resistant plants were crossed with susceptible doubled haploid (DH) lines of the same species, and segregating populations (test-cross, F_2 or BC_1) were used to identify and map clubroot resistance (CR) genes. DNA samples from segregating populations were analyzed with microsatellite markers. A single dominant CR gene was identified in *B. rapa* var. *chinensis* (pak choy), herein designated *Rpb1*, and mapped to a genomic region on the *B. rapa* linkage group A3. Molecular markers closely linked to this CR gene were developed and validated to facilitate marker-assisted selection (MAS) for resistance breeding. This pak choy cultivar also showed high resistant to five *P. brassicae* pathotypes found in Canada. Introgression of *Rpb1* into canola germplasm is in progress, using MAS.

26. In-field distribution of *Plasmodiophora brassicae* measured using quantitative real-time PCR and the influence of soil physiochemical parameters on disease development

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Clubroot in Brassica crops, caused by *Plasmodiophora brassicae* Woronin, is recognised as a serious soil-borne disease, associated with appreciable yield losses, and is considered one of the most economically important diseases of cruciferous crops. The disease is found world-wide throughout the growing areas of oilseed rape and vegetable Brassicas, with the reported outbreak of clubroot in parts of the rape-growing districts of western Canada of particular concern. In Sweden, outbreaks of clubroot in recent years have been reported to be more frequent in winter oilseed rape districts, and severe attacks have once again been reported from spring oilseed rape districts where severe outbreaks of the disease occurred 25 years ago. A protocol using real-time PCR for the direct detection and quantification of *P. brassicae* in soil samples previously developed, was used to determine the spatial distribution in a 25 ha field of spring oilseed rape (*Brassica napus)* sampled after harvest in central Sweden 2010.

The results show an overall spatial trend in the variation of the amount of *P. brassicae* DNA detected, with the lowest amounts of *P. brassicae* detected in the eastern part of the field, where also the pH values determined were on a higher level compared to that of the sampling points in the western part of the field. A correlation was found between pH value and amount of *P. brassicae* DNA. The amount of *P. brassicae* DNA detected ranged from 225 to 33098 fg plasmid DNA g^{-1} soil, that is of a considerably higher magnitude than previously determined in fields of winter oilseed rape. Analyses of soil type and other soil physiochemical parameters are underway. Continuous sampling in the following years will enable determining a measure of rate of decline of *P. brassicae* DNA.