ORAL PRESENTATION ABSTRACTS

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Clubroot (*Plasmodiophora brassicae* Woronin) – an agricultural and biological challenge worldwide

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Soil borne plant pathogens and their associated disease syndromes are especially challenging for both farmers and biologists. Disease only becomes apparent when crops start failing and finding the cause and its control is made difficult since the pathogen must be separated from the soil environment and identified from a huge and very varied microbial population. Clubroot disease and the causal microbe *Plasmodiophora brassicae* offer these challenges in abundance. The microbe is well fitted for its environment. It persists in soil as minute resting spores and actively grows and reproduces shielded inside the cells of host plants. That form of existence means it can only be studied when associated with host cells. The pathogen is outside the host for very short period as a vulnerable naked swimming primary zoospore. Consequently, scientific studies are challenging by the very biological nature of the host and pathogen interaction and the technology needed to penetrate that relationship. Controlling the disease challenges farmers, crop consultants and plant pathology practitioners because of the limited options available. Full symptom expression happens solely in members of the *Brassicaceae* family. Here few genes expressing strong resistance to *P. brassicae* are known and easily available for plant breeders. Agrochemical control is similarly limited by difficulties in molecule formulation which combine efficacy with environmental acceptability. Manipulation of husbandry encouraging changes in soil structure, texture, nutrient composition and moisture content can reduce populations of *P. brassicae*. Integrating such strategies with rotational and crop management will reduce but not eliminate this disease challenge. Additionally, there indications that forms of biological competition may be mobilised as part of integrated control strategies. The aim in this keynote presentation is to chart the development of scientific biological understanding of the host-pathogen relationships between *P. brassicae* and its hosts and attempts to grapple with Clubroot Disease which can devastate farmer's crops and profitability. This will set the scene for the presentations which follow.

Clubroot and the importance of canola in Canada

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Canola (*Brassica napus* L., *B. rapa* L. and *B. juncea* L.) produces one of the healthiest vegetable oils. Canola is a valuable crop for Canada. The canola industry in Canada is estimated at an annual average of C$15.4 billion between 2007-08 and 2009-10. Farm cash receipts of canola were valued at C$8.9 billion in 2012. The value of this crop is threatened by clubroot caused by *Plasmodiophora brassicae* (Woronin.), which has expanded dramatically since its discovery in canola in Alberta in 2003. Due to this pathogen moving with infested soil from field to field, control of this disease can be very costly to farmers. Even with new control options such as clubroot resistance, this disease will be a challenge for the canola industry's ability to stay ahead of it. Continued research into this disease is a necessity for the sustained success of the Canadian canola industry.

Pathology and epidemiology of clubroot in Canada

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Clubroot, caused by the obligate parasite *Plasmodiophora brassicae*, is an important soilborne disease of the Brassicaceae. In Canada, clubroot has long been established as a problem in cruciferous vegetables, but was not reported on the Prairie canola (*Brassica napus*) crop until 2003, when 12 clubroot-infested fields were identified in central Alberta. Continued surveillance has shown that the disease is spreading, and as of 2012 there were at least 1,064 fields with confirmed clubroot infestations in the province. While the outbreak is still mainly confined to central Alberta, isolated clubroot infestations and the presence of viable *P. brassicae* inoculum have been confirmed in southern Alberta, Saskatchewan, and Manitoba. Dissemination of the parasite appears to be predominantly through the movement of infested soil on farm and other machinery, although secondary mechanisms of spread, such as via wind and water erosion and in soil tags on seeds and tubers, also have been implicated. Given the significant economic value of the Canadian canola crop, the increased incidence of clubroot on canola has caused major concern and led to the initiation of a large, coordinated research effort aimed at understanding and managing this disease. The purpose of this presentation is to summarize the extent and nature of the clubroot outbreak in the Canadian canola crop, 10 years after it began, and to provide a context for the research and management strategies that have been developed over this period.

Clubroot in Europe

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Clubroot has been traditionally a major concern of European vegetable growers; however, recently the disease has a significant impact also on European oilseed rape (*Brassica napus* L.). Accordingly, many recent research projects on clubroot focus on different aspects of clubroot control in this crop. The release of the clubroot resistant winter oilseed rape cultivar 'Mendel' by a German breeding company has been a mile stone in clubroot control in oilseed rape and the efficacy of this resistance source is of key relevance not only in Europe. 'Mendel' is on the European seed market since twelve years and despite its race-specific resistance this cultivar and its successors which share the same resistance still give control in most growing areas. Virulent pathotypes can be found in Germany, Poland and in the UK. So far, no cases of 'Mendel' virulence have been reported by the French monitoring initiative. The number of virulent incidences on 'Mendel' is slowly increasing and displays a certain pattern. Most incidences occur in the North-East of Germany, other incidences seem to represent local events which are not related to the local frequency of 'Mendel' crops. There are indications that virulent isolates have been present in these areas before cultivar release. To be prepared for a loss of 'Mendel's efficacy new breeding material harboring a broader basis of clubroot resistance in modern oilseed rape types is under development.

Also in the kole crops (*B. oleracea*) the release of clubroot resistant cultivars like 'Kilaxy' or 'Clapton' has brought a significant change to the problem. Both types of resistance, i.e. the 'Mendel' and the 'Clapton' type, are based on introgression of dominant resistance genes from *B. rapa*, and interact in a similar pattern with different clubroot isolates.

To expand the IPM measures beyond the use of resistant cultivars a number of different attempts to control clubroot by agricultural means are pursued, such as the prevention of the strong multiplication of *Plasmodiophora brassicae* in rapeseed volunteers after harvesting or the control of clubroot also in oilseed rape by calcium cyanamide. The combination of resistant cultivars with other IPM measures should be pursued to achieve healthy crops and to prevent the erosion of the clubroot resistance.

Current incidence of clubroot on oilseed rape in East Europe

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The oldest reports of clubroot in Europe date back to the 13th century, but the cause of the disease – the protist *Plasmodiophora brassicae* - was described for the first time as late as in 1875. The pathogen identification was done by the Russian researcher Mikhail Woronin, as a result of his studies after a severe epidemic of clubroot in the region of St. Petersburg in the European part of north Russia. The scientist called the pathogen a "plasmodiophorous organism" and gave it the Latin name. The site where the disease was originally found is still severely contaminated by the pathogen. High incidence of clubroot is also reported in Kaliningrad region, located in the very north-west part of Russia. In the other zones of oilseed rape cultivation in Russia, such as the Central Chernozem and Kuban regions the disease is observed occasionally and has no economic significance. The moderate incidence of clubroot was reported close to Tomsk, Kemerovo and Novokuznetsk in Svierdlovskij region located in the Asiatic part of Russian Federation. The disease is occasionally observed in Lithuania and Latvia, as well as Estonia. Usually symptoms are visible as patches or rows of infected plants. In Belarus the disease started on oilseed rape about five years ago and since then a few outbreaks were severe. In Minsk district the disease was observed at 3 sites located in Minsk and Slutsk regions and 2 sites in Brest district - in Brest and Stolin regions. Current situation in farms growing oilseed rape in central and eastern Slovakia has not been recognized. In the western part of the country – around Topolčany, where oilseed rape is grown in big monocultures, the disease is practically unknown. In contrast, both in Poland and the Czech Republic clubroot is a damaging disease of oilseed rape and it is found in numerous areas of the country.

Clubroot, a permanent threat to Swedish oilseed rape production

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Brassica oilseeds were established as arable crops in Sweden in the early 1940s. Years of high market prices on vegetable oils resulted in intensive cultivation in various regions leading to problems with soilborne pathogens including *Plasmodiophora brassicae*. At present reports of clubroot outbreaks in Sweden are frequent, particularly from winter oilseed rape districts in south Sweden, with a trend on increasing disease incidences. BioSoM or Biological soil mapping is a multidisciplinary thematic research program between the Faculty of Natural resources at SLU, and eleven stakeholders. *P. brassicae* has a central role in the program that aims to provide scientific support to new services for farmers enabling detection and mapping of soilborne pathogens. Soil sampling procedures on field level, storage of samples and homogenizing methods are important factors to generate results using PCR. Since, 2012 DNA based soil analyses for the presence of *P. brassicae* are offered to farmers to improve the planning of their crop rotation practice. The effects of boron or different sources of nitrogen as means of limiting the damage caused by *P. brassicae* are presently

studied. To enhance understanding of the biology of *P. brassicae* and to improve future control measures, the sequencing of the *P. brassicae* genome has been initiated in Sweden. An increasing awareness of threats posed from *P. brassicae*, an urgent need for resistant cultivars adopted to Swedish conditions and a shift to longer Brassica oilseed rotations are routes to provide prerequisites for a sustainable oilseed production.

Research status of clubroot disease of cruciferous crops in China

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China is a main cruciferous crop-producing country, including 6,700,000 ha, 2,609,300 ha, 898,500 ha, 574,100 ha, 850,000 ha for canola, Chinese cabbage, cabbage, pakchoi, tuber mustard, respectively. However, these crops have been infected, by *Plasmodiophora brassicae*, since 1950s when the disease was found in Taiwan, Jiangxi, and Hunan provinces sequentially. Since 1990s, especially the end of 1990s, the disease has broken out in the southwest and northeast regions, transmitted by seeds, soil, and the floating system of establishing seedlings in water. At present, clubroot is distributed all around China, although it is more severe in the southwest, northeast and middle regions than other regions, infecting 1 million ha of cruciferous crops annually It occurs in Chinese cabbage, cabbage, pakchoi, tuber mustard, stem mustard, even medicinal crops such as *Radix Isatidis*. In the recent years, it has spread into canola in Anhui, Sichuan, and Hubei provinces and into Chongqing City. In some areas, it destroys vegetable bases (planting beds) resulting in cruciferous crops not being planted anymore.

In order to control clubroot, the Ministry of Agriculture in China set up a nationwide team under a program in 2010 titled “Research and Demonstration of Control Technologies for Clubroot Disease of Cruciferous Crops (201029030)”. This program is involved with 16 public institutions, such as the agricultural universities of Yunnan, Sichuan, Huazhong, Hunnan, China, Shenyang, Anhui and Tibet, comprehensive universities of Southwest, Zhejiang, and Science and Technology of East China and the agricultural academies of Yunnan, Jiangxi, China (CAAS), Beijing and Liaoning. A total of 25 principal scientists are involved into the program under the leadership of Yunnan Agricultural University. Their duties are to investigate the pathogenic variation and physiological race distribution, understand the factors affecting epidemics, screen resistant germplasm, breed resistant Chinese cabbage, cabbage and canola, and develop and demonstrate integrated management strategies which include chemical, physical, agricultural and biological methodologies in farmer fields.

Since their establishment, the clubroot team has made a lot of achievements in pathogenic variation, resistance breeding, biological and chemical controls, agricultural management, and biocontrol agent formulation. The aim in this keynote presentation is to chart a brief history of the disease research in China, of their main achievements, and of the authors own laboratory’s results in biofumigation, biological control and controlling agent formulation.

Hormone signaling during the development of the clubroot disease in *Arabidopsis thaliana* roots

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The clubroot disease symptoms of Brassicaceae, caused by the soilborne obligate biotrophic pathogen *Plasmodiophora brassicae*, are determined by the modulation of plant hormones such as auxins and cytokinins. *Arabidopsis thaliana* is used as a model host to understand the molecular biology underlying these processes. The biosynthesis of auxin, likely via the nitrilase-pathway, is upregulated in infected roots, especially in plasmodia-containing cells as demonstrated by different methods. Here, auxin is involved in cell elongation and subsequently hypertrophy of the pathogen-containing cells. It could be demonstrated that the auxin signal is perceived via two different auxin receptor classes and that the auxin-inducible *GH3* family genes are targets for one signaling pathway. The *GH3*-gene family is involved in the conjugation of auxin and jasmonate to amino acids, thereby inactivating free auxin possibly as detoxification mechanism of the plant. In addition to auxin, cytokinin modulates cell division and thereby gall size. Consequently, in plants with reduced cytokinin levels the galls were much smaller. Also, the growing root gall constitutes a strong metabolic sink, which could be induced by cytokinins. Targets for the induction by cytokinin could be invertases, which were shown to be involved in disease development. A model to explain the function of plant hormones and their targets in clubroots will be presented.

Breeding for clubroot resistance at the University of Alberta

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Since finding of clubroot disease (causal agent *Plasmodiophora brassicae*) in canola (*Brassica napus* L.) fields in Alberta in 2003, research in various discipline was conducted by different researchers to manage this disease in an effective way from a long-term perspective. At that time, all Canadian canola cultivars lack resistance to this disease. However, screening of germplasm of the six Brassica species, collected from different parts of the world including from the gene banks, identified several accessions carry resistance to different *P. brassicae* pathotypes, including the pathotype 3 which is most virulent and prevalent in Alberta. In case of diploid species, resistance found most often in turnip (*B. rapa var. rapifera*) and *B. nigra*; while in case of the amphidiploid species, resistances present in Rutabaga (*B. napus var. napobrassica*) and winter B. napus canola cv. Mendel are quite effective against different pathotypes including the pathotype 3. A dominant gene in Mendel primarily controls resistance to this pathotype; while in the case of Rutabaga, a simple to complex genetic control of resistance was found. Based on Mendel and Rutabaga resistances, several canola quality spring type lines developed. The lines carrying resistance to pathotype 3 often showed resistance to the other pathotypes present in Alberta, such as pathotype 3, 5, 6 and 8. Use of resistance of these unadapted germplasm in breeding also introduces several undesired traits into Canadian spring canola; intensive or repeated cycle of breeding often needed to meet all agronomic and seed quality standards in the resistant lines. The above-mentioned resistances may give immediate solution; however, stacking of multiple resistance genes would be needed for long-term management of this disease.

Identification of clubroot-resistance genes and development of clubroot resistant canola germplasm

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Clubroot, caused by *Plasmodiophora brassicae*, poses a serious threat to canola production in western Canada. Genetic resistance is considered to be the most efficient method for control of this disease. Two hybrids of oriental vegetable (*Brassica rapa*), FN (var. *chinensis*) and JNC (var. *pekinensis*), were highly resistant to all pathotypes of *P. brassicae* found in Canada. Mapping of clubroot resistance (CR) genes was carried out in these *B. rapa* genotypes using AAFC microsatellite markers, and two dominant CR genes, *Rpb1* in FN and *Rpb2* in JNC, were genetically mapped to different genomic regions of the *B. rapa* linkage group A3. The candidate *Rpb1* and *Rpb2* genes, which encode toll interleukin 1 receptor (TIR)-nucleotide binding site (NBS)-leucine-rich repeat (LRR) proteins, were isolated using *in silico* map-based cloning. These CR genes have been transferred into *B. napus* canola via agrobacterium-mediated transformation. Molecular markers tightly linked to these CR genes were developed using *B. rapa* sequencing information, facilitating the introgression of CR genes into canola breeding lines through marker-assisted selection. To speed up the CR *B. napus* germplasm development, the genetic composition of the A genome and the number of C-genome chromosomes were analyzed using genome-wide SNP markers (6K Illumina Infinium SNP array) on BC_1 resistant plants, and individuals with the least amount of CR donor's genetic background and a C-genome complement most similar to the recurrent *B. napus* parental line were selected for further backcrossing.

Mapping clubroot resistance genes in Chinese cabbage and ECD accessions of *Brassica rapa*

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There are various clubroot resistant sources within *Brassica* species, while the European turnips are identified as the best ones and commonly used for the development of resistant cultivars in Chinese cabbage and canola. To use clubroot resistant sources effectively, it is necessary to map and clone clubroot resistance genes, so molecular markers inside or linked closely to these resistance genes can be developed. Through molecular marker assisted selection, the clubroot resistance genes can be effectively transferred from cultivars to cultivars and from species to species. In our clubroot research program, we started gene mapping using Chinese cabbage and the turnip accessions of the European clubroot differential (ECD) set. We have so far mapped one clubroot resistance locus in five Chinese cabbage cultivars and two clubroot resistance loci in ECD1, ECD2, ECD3 and ECD4, We also found that there is one more locus in ECD3 and mapping the third clubroot resistance locus is underway. Molecular markers which are linked closely to all mapped clubroot resistance loci have been developed and used to develop near isogenic lines for the mapped loci in Chinese cabbage, ECD1, ECD2, ECD3 and EDC4. We have produced all near isogenic lines contain single clubroot resistance locus in the same genetic background and will use these near isogenic lines to perform gene interaction analysis with different clubroot pathotypes.

Integrated management of clubroot on canola with crop rotation, cultivar resistance, and fungicides/biofungicides

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Host resistance is a cornerstone for management of clubroot on canola. Studies on biofungicides and fungicides, used alone or with cultivar resistance or crop rotation, were reviewed for possible integrated management of the disease. More than 5000 soil microbial isolates were screened, but none showed consistent efficacy against clubroot. The biofungicides Serenade® and Prestop®, however, suppressed clubroot on canola substantially under controlled conditions via antibiosis and induced host resistance. The fungicides pentachloronitrobenzene, fluazinam and cyazofamid also showed promise. Granular and seed-treatment formulations were developed to facilitate the delivery of biofungicide in field conditions. Under heavy infestation, these biofungicides and fungicides were generally ineffective regardless of cultivar resistance. They occasionally reduced clubroot on Chinese cabbage when applied as a soil drench at high water volumes. Resistant cultivars consistently limited the development of clubroot and increased the yield of canola in field trials. Crop rotation reduced the pathogen inoculum load in the soil; although a 1-year break from canola did not lower the pathogen population substantially based on qPCR testing, a 2-year break reduced the inoculum by 90% relative to that in the 1-year break. This inoculum reduction, however, was not enough to allow a susceptible canola cultivar to yield normally, despite the fact that the disease impact was alleviated. For resistant cultivars, a >2-year break increased the yield by up to 25% when compared to no break. Therefore using a resistant cultivar with a 3-year crop rotation is recommended for control of clubroot and maximum yield of canola.

Management of clubroot on canola in western Canada

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Following its discovery on canola (*Brassica napus* L.) in Alberta in 2003, studies of management of clubroot (*Plasmodiophora brassicae*) initially focused on approaches developed for the vegetable industry, where clubroot is also an important disease. Treatments such as liming or otherwise amending the soil to increase soil pH, seed treatments, and bait crops to reduce the concentration of resting spores in soil were assessed. The efficacy of several of these treatments was sufficient for use in intensive production of short-season vegetable crops, but they were too expensive and not effective enough for use in the large-scale production of canola. Manipulation of seeding date, application of fungicides or fumigants, and rotation with non-hosts can be effective in specific situations, but are not, by themselves, sufficient to reduce spore populations to acceptable levels. Sanitization of equipment to prevent the spread of resting spores is time consuming and costly, but also has merit in some situations. Genetic resistance has been shown to be a practical option for clubroot management on canola. However, resistance is unlikely to be durable if canola crops are grown on a large acreage under short cropping rotations, only one source of resistance is utilized, and high populations of resting spores occur in pathogen-infested fields. Resistance stewardship that involves crop rotation, early seeding, sanitation and other best management practices will be required to maintain the performance and longevity of genetic resistance.

Environmental parameters that affect clubroot risk on the Canadian prairies

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Clubroot (*Plasmodiophora brassicae* Wor.) on canola (*Brassica napus* L.) spread rapidly across a large area of the province of Alberta following its initial discovery in 2003. A critical area of research was to assess the risk that clubroot would continue to spread rapidly across the canola production area on the Canadian prairies. The effect of factors such as temperature, pH, soil type and micronutrients on clubroot development, together with assessment of the importance of soil moisture, was examined. This information was needed to make informed assessments of risk. Temperatures below 17° C were shown to inhibit or delay development of all stages of the life cycle of *P. brassicae.* Alkaline pH also reduced infection and symptom development, but the impact of pH in both controlled environment and field situations was smaller than had been anticipated. The impact of soil type in controlled environment studies was also unexpectedly small, but a strong interaction with soil moisture under field conditions is extremely likely. Differences in micronutrient concentration are unlikely to limit the spread of clubroot. Soil moisture, especially in the rhizosphere during primary and secondary infection, is likely to have an important impact on clubroot development, but is the most difficult factor to manipulate or control. We conclude that clubroot has the potential to spread and become endemic across large portions of the prairie region.

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.

Clubroot management

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When Clubroot emerged as a disease of canola in Alberta in 2003, there were very few control options available to canola producers. But since that time, clubroot research has expanded dramatically, and now armed with a better understanding of this disease, there are many more clubroot management options. Dan and Clint will discuss some of the latest clubroot research data and how these apply to the agriculture sector in Canada. They will also examine the keys to managing clubroot successfully for canola growers and for anyone else that comes into contact with soil.