

## **Progress towards the Sustainable Management of Clubroot [*Plasmodiophora brassicae*] of Canola on the Canadian Prairies**

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### **Summary**

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, has recently emerged as an important disease of canola (*Brassica napus*) in central Alberta. Disease development is characterized by the formation of large galls on the roots of affected plants, which hinder water and nutrient uptake and lead to yield and quality losses. Over 560 clubroot infested fields have now been confirmed in the province, and while most cases of the disease are still found in central Alberta, clubroot appears to be spreading into southern counties. The primary mechanism for pathogen dispersal seems to be the movement of infested soil on field equipment, although secondary mechanisms, such as soil and wind erosion and infested seed, have also been suggested. Until recently, the main strategy for managing clubroot on canola was rotation out of susceptible crops for four or more years, although research is underway to evaluate the efficacy of numerous other control methods common in cruciferous vegetable production, including the application of soil amendments and fungicides, and the sanitation of vehicles, machinery and equipment. The potential for biological control is also being assessed. Six canola hybrids with genetic resistance to the predominant pathotypes of *P. brassicae* were recently released onto the Canadian market by several companies, collectively representing one of the most important new management tools available to growers. Genetic resistance will have to be carefully managed however, since regional populations of *P. brassicae* are fairly diverse and pathogen virulence patterns are known to shift quickly in response to selection pressure. As such, successful long-term management of clubroot on the prairies will require an integrated approach and widespread adoption of effective management strategies by canola growers and other stakeholders.

### **Introduction**

Clubroot, caused by *Plasmodiophora brassicae*, is an important soilborne disease of the family Brassicaceae. In Canada, clubroot has been a recurrent problem in the production of cruciferous vegetables in British Columbia and the eastern regions of the country<sup>1,2</sup>. The disease was reported only sporadically on the prairies until 2003, when it was first identified on canola (*Brassica napus* L.) in 12 fields in central Alberta<sup>3</sup>. This discovery was a cause for concern among growers, government, industry personnel and scientists, given the potential yield and quality losses associated with clubroot infection, and resulted in a coordinated research effort aimed at better understanding clubroot and its control. This paper provides a short review of the clubroot situation on the Canadian prairies seven years after the disease was first found on canola, with a particular emphasis on current and potential management strategies.

### **Symptoms of clubroot**

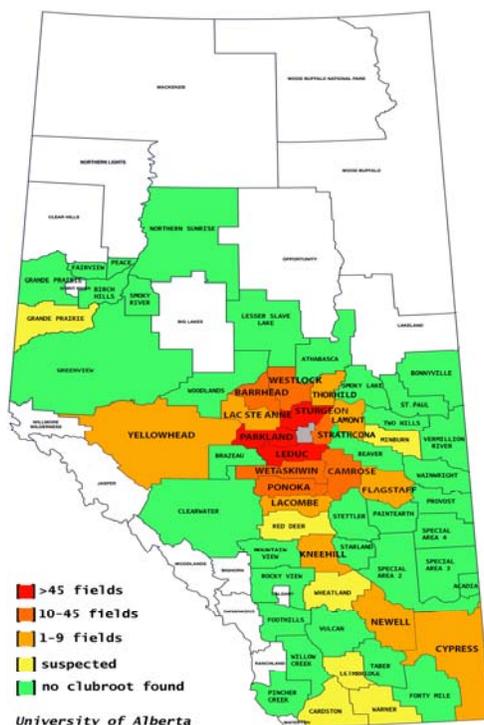
Clubroot is characterized by the development of large, club-shaped galls on the roots of susceptible plants, which give the disease its name. The extent of root galling can vary considerably depending on the amount of *P. brassicae* inoculum in the soil, the prevalent environmental conditions, and the degree of host resistance. Initially, the galls are firm and have a whitish-coloured appearance, but become soft, spongy and take on a brownish colour as they mature and decompose later in the season. The formation of galls hinders the capacity of the roots to take up water and nutrients from the soil. This results in the development of above-ground symptoms in affected plants, including wilting and stunting, as well as yellowing of the leaves and premature senescence. Grain yield and oil content can be significantly reduced in canola when symptoms are severe<sup>4</sup>.

### The life cycle of *P. brassicae*

Within the galls of infected roots, *P. brassicae* forms long-lived resting spores<sup>5</sup>, which are released into the soil as the galls decompose and serve as inoculum for future infections. These infections are initiated by the germination of the resting spores to yield primary zoospores, which encyst in the root hairs of the host and form primary plasmodia<sup>6</sup>. The primary plasmodia divide to produce zoosporangia, from which secondary zoospores are released and infect the root cortex. Secondary plasmodia develop within the infected cortical cells and eventually cleave to give rise to a new generation of resting spores<sup>6</sup>. It is the infection of the cortex by *P. brassicae* that results in the development of typical clubroot symptoms, which reflect pathogen-induced hormonal disturbances and hypertrophy of affected cells.

### Occurrence of clubroot on canola on the prairies

Clubroot was initially identified on 12 canola crops northwest of Edmonton, Alberta in 2003<sup>3</sup>. In order to assess the scope of the problem and track disease spread, annual clubroot surveys have been conducted since 2004. These surveys were initially focused on Alberta<sup>7,8</sup>, but were expanded in 2008 to include Saskatchewan and Manitoba<sup>9,10</sup>. The number of fields with confirmed *P. brassicae* infestations has increased steadily over the years. By October 2010, clubroot was confirmed in 566 fields distributed over 18 counties in Alberta, as well as in a rural area of northeast Edmonton<sup>11</sup>. Although some cases of the disease have been reported in southern regions of the province, the outbreak remains largely confined to central Alberta. The current distribution of clubroot in Alberta is illustrated in Fig. 1. Ironically, clubroot has only been observed on four farms growing cruciferous vegetables in Alberta since 2003<sup>12</sup>, and only one of these farms sustained economically significant disease losses.



**Figure 1.** The distribution of clubroot-infested fields in Alberta, Canada, as of fall 2010. Clubroot has been confirmed in a total of 566 fields representing 18 counties and a rural area of the City of Edmonton. In addition, suspected cases of clubroot have been reported from at least seven other municipalities.

In 2008, the presence of *P. brassicae* was detected (using both plant bioassays and a polymerase chain reaction (PCR)-based test<sup>13</sup>) in soil samples collected from a single canola field in west-central Saskatchewan<sup>14</sup>. While the pathogen itself was identified in the soil, and small galls were observed on canola and cabbage plants in the greenhouse bioassay, no symptoms of clubroot were observed on canola plants growing in the field. A few canola roots with very small galls, collected in 2005 from field plots in southern Manitoba, were also confirmed as clubroot infected<sup>8</sup>. However, PCR testing of additional soil samples recovered from the same location in 2009 yielded no positive results. Predictive models suggest that clubroot has the potential to become established as a serious pest of canola in various regions of the prairies beyond central Alberta, especially in southeastern Manitoba<sup>15</sup>. The extent of the possible impact of clubroot in these areas will vary depending on precipitation levels and temperatures<sup>16</sup>, while the presence of neutral or alkaline pH values in soils in these areas may reduce, but not eliminate the risk of clubroot. Long-term disruptions in weather patterns associated with climate change may have a significant impact on clubroot severity on a regional scale.

Most clubroot infestations identified in Alberta have been low to moderate in severity, although in some instances heavy infestations have been found. In at least one case, clubroot was so severe that the canola crop was not harvested<sup>17</sup>. While it is likely that the increasing number of clubroot infested fields reflects, in part, increased surveillance for the disease, clubroot also appears to be spreading. For instance, canola in a field that was first surveyed for clubroot in 2006 was free of the disease at that time, but cabbage and broccoli in that same field in 2010 were found to be infected with *P. brassicae*<sup>12</sup>. Some instances of clubroot along water runs and the edges of otherwise disease-free fields, which were adjacent to infested fields (S.E. Strelkov, unpublished data), also suggest continued dissemination of the disease. Given the widespread occurrence of clubroot in central Alberta in recent years, it is difficult to understand why the disease went undetected for so long in the past, unless its incidence and severity were much lower.

### **Dissemination of clubroot**

As a soilborne pathogen, the intra- and inter-field spread of *P. brassicae* has generally been regarded as slow. The disease is usually monocyclic, and zoospore movement in the soil is limited<sup>2</sup>. However, any activity that results in transport of soil from one point to another has the potential to disseminate clubroot. An examination of the distribution of *P. brassicae* infected canola plants within clubroot infested fields revealed that the highest frequency of infection occurred at the field entrances, and dropped significantly at sampling points 150 m and 300 m from the entrance<sup>8</sup>. This finding suggests that pathogen inoculum was introduced on farm or other machinery as it entered the field, presumably in the form of resting spores present in soil transported from clubroot infested fields.

While the dissemination of clubroot on field equipment appears to be the primary method of pathogen spread, other mechanisms have also been implicated. There is some evidence of the movement of clubroot via soil erosion, although this is largely anecdotal and/or preliminary in nature. Collaborative research between the University of Alberta, Agriculture and Agri-Food Canada and Alberta Agriculture and Rural Development is currently underway to examine this hypothesis and to quantify both the amount of inoculum that can be moved in this manner, as well as the distance over which it may spread. In addition, *P. brassicae* resting spores have been detected as external contaminants of seeds and tubers of various field crops grown in clubroot infested fields<sup>18</sup>. Although the numbers of spores (as measured by quantitative PCR) were generally much lower than what could be carried on machinery, seedborne inoculum may have the potential to initiate new infestations under favourable conditions. Hence, the dissemination of clubroot on propagative materials may represent another secondary mechanism of field-to-field spread. It is suspected that passenger vehicles, transport trucks and heavy equipment associated with activities such as oil and gas well drilling and servicing, pipelining, earthmoving and custom agricultural work also have the potential to move clubroot-infested soil both short and long

distances, but this risk has not been critically assessed. Nevertheless, many companies engaged in these activities have adopted risk mitigation strategies<sup>19</sup>.

### **Clubroot management**

The occurrence of clubroot on canola represents a major challenge to farmers. While methods exist to control clubroot on cruciferous vegetables, these are not necessarily practical or economically feasible in canola cropping systems. The tools and information needed to effectively manage clubroot in Canadian canola were almost completely lacking when the disease was first identified in 2003. While clubroot continues to represent a major constraint to canola production, a concerted research effort has resulted in improved management options, most notably genetically resistant cultivars, which have enabled growers to better cope with the disease.

**Cultural strategies to manage clubroot.** A number of cultural strategies are available to help reduce the spread and impact of clubroot on canola. Among the most important of these is sanitation of farm machinery and other equipment that may have come into contact with clubroot infested soil, a practice that is meant to slow the spread of the disease. As discussed above, the movement of pathogen resting spores by machinery represents the principal mechanism by which clubroot has been disseminated throughout central Alberta<sup>8</sup>. Proper sanitation of machinery requires three main steps: (1) removal of bulk soil and crop debris, (2) pressure washing or scrubbing to remove any residual soil, and (3) application of a recommended disinfectant to the scrubbed surfaces<sup>2</sup>. While many agricultural and oil and gas companies that regularly enter farmers' fields have adopted rigorous sanitation protocols as part of their standard operating procedures, the adoption of such strategies by farmers themselves has been less common. The main reason for this appears to be the amount of time and effort required to properly clean and disinfest equipment. Nonetheless, as a bare minimum, farmers should remove bulk soil and crop debris from their machinery and vehicles, even if they do not pressure wash or disinfest them, since this is where most of the pathogen resting spores would be expected to be found. Rotation out of susceptible crops represents an important cultural strategy for mitigating the impact of clubroot in fields where the disease is

already present. It is well-established that the severity of clubroot symptoms is correlated with the concentration of resting spores in the soil, and that a reduction in spore density will therefore reduce disease severity. Since the resting spores of *P. brassicae* are particularly long-lived, with an estimated half-life of 3.6 years<sup>20</sup>, long rotations out of susceptible hosts are required for the pathogen inoculum to decline to an acceptable level. Conversely, repeated cultivation of a susceptible host results in a rapid increase in the number of resting spores in the soil. The appropriate frequency of canola cultivation within rotations depends largely on the initial level of infestation in a particular field, as well as environmental factors, although long-term studies in

the western Canadian context are still lacking. In Alberta, recommendations include a minimum rotation of four years between susceptible canola cultivars, or three years between resistant cultivars<sup>19</sup>. However, a long rotation out of canola is not a popular option among many farmers, given the higher commodity price and economic returns typically associated with this crop compared to cereals and pulses, and the fairly limited cropping options available in many parts of the prairies.

Other cultural management strategies are also being studied for clubroot on canola in western Canada. The manipulation of seeding dates, for instance, has been shown to be a promising approach for reducing this disease. Preliminary results indicated that early seeding significantly reduced the severity of clubroot at one of two field sites and increased canola yields by up to 58%<sup>21</sup>. These findings are consistent with what has been observed in seeding date experiments conducted with cruciferous vegetables<sup>22</sup>, and likely reflects less favourable conditions for the pathogen at the time of initial infection.

**Soil amendments.** Since clubroot development is generally favoured by acidic soils, amendments which increase the soil pH may serve to reduce symptoms of the disease<sup>23</sup>. Lime amendments have long been used to control clubroot on vegetable Brassicas. However, the effectiveness of lime treatments can be influenced by the form of lime used<sup>24</sup>, the timing of the application<sup>25</sup>, and the type of soil being treated<sup>26</sup>. Repeated applications of large amounts of lime may

also be required to raise the soil pH to a value of 7.2 or greater, which is considered optimal for clubroot control. While this may be feasible in market gardens producing cruciferous vegetables, it is likely not possible or economical in canola cropping systems, since field sizes are much larger and financial returns are lower per unit area. Moreover, while a significant negative correlation was observed between soil pH and clubroot severity in canola in central Alberta, severe infestations were also observed in soils with a neutral or even basic pH, in fields under intensive canola production and high moisture levels<sup>7</sup>. Therefore, high soil pH alone may not be sufficient to ensure effective clubroot control.

Calcium cyanamide is another amendment that has long been used to control clubroot in the vegetable Brassicas<sup>27</sup>. This compound and its break-down products increase soil pH, possess fungicidal properties, and serve as a source of nitrogen for the crop. Calcium cyanamide breaks down to yield hydrogen cyanamide and hydrated lime (calcium hydroxide), followed by urea, ammonia and nitrate<sup>27</sup>. Unfortunately, shipping and application costs make calcium cyanamide prohibitively expensive for routine use in Canadian canola production, although there may be potential for spot treatments in cases where clubroot occurs in localized areas of a field.

Another method to mitigate the impact of clubroot is the application of boron to the soil, which has been a recommended practice in cruciferous vegetable production for more than 70 years<sup>27</sup>. Boron inhibits the morphogenic change from plasmodium to sporangium during infection of the root hairs by *P. brassicae*, thereby interfering with pathogen development<sup>28</sup>. However, while greenhouse assessments and field testing at a small number of locations revealed that boron could reduce both the incidence and severity of clubroot on canola, phytotoxic effects were also observed, even at moderate rates of application<sup>29</sup>.

**Fungicides.** In Canada, a number of fungicides, including pentachloronitrobenzene (PCNB; Adobe 75WP, Crusoe 75WP, and Quintozene 75WP) and fluazinam (Allegra 500F) are registered for control of clubroot on cruciferous vegetables, and can be applied as pre- or post-planting drenches on transplants. In

addition, the soil fumigant sodium methylthiocarbamate (Vapam HL) can be used to treat the transplant propagation beds. None of these products are registered for canola, and research is underway to evaluate their utility on this crop. In field trials conducted in Alberta, the application of PCNB (Terraclor 75% WP) as a soil drench resulted in a significant reduction in clubroot severity on canola, which in turn led to reduced seedling mortality and increased plant cover and height<sup>30</sup> (Hwang et al. 2008). In less heavily infested soils, cyazofamid (Ranman) also had a positive effect on these parameters. These results suggest that PCNB and cyazofamid may be useful tools to reduce the impact of clubroot on canola, but additional work is needed to optimize application rates and strategies. Indeed, the rates found to be effective on canola would not have been cost effective for this crop.

**Biological control.** In addition to cultural and chemical strategies to manage clubroot, there has been a considerable amount of interest in the biological control of this disease. A number of microorganisms have been shown to have good potential as biocontrol agents for *P. brassicae* (reviewed in<sup>27</sup>). To date, however, no biological control agents have been registered for clubroot management in Canada, although various microbial fungicides [including Serenade (*Bacillus subtilis* (Ehrenberg) Cohn), RootShield (*Trichoderma harzianum* Rifai) and Prestop (*Gliocladium catenulatum* J.C. Gilman & E.V. Abbott)] are available for other soilborne diseases in Canada. These commercial biofungicides have been evaluated as both soil drenches and seed treatments for control of clubroot<sup>31</sup> (Peng et al. 2009). Under controlled-environment conditions, the efficacy of Serenade and Prestop was similar to that of the chemical fungicides, with a reduction in clubroot severity of more than 80% relative to pathogen-inoculated controls. Under high disease pressure, however, the biofungicides were less effective than the chemical fungicides. Microbial control of clubroot is attractive because certain soil microbes could colonize the host roots and/or rhizosphere, thereby providing durable protection. The best control was obtained when the biofungicides were applied as drench applications rather than seed treatments, suggesting that these biocontrol agents did not colonize the roots

or rhizosphere to give long-lasting clubroot control. In addition to research with commercially available biofungicides, work is also underway to identify indigenous soil microorganisms that could serve as effective biocontrol agents for clubroot of canola (G. Peng, *personal communication*).

**Genetic resistance.** Genetic resistance to clubroot can vary from broad-spectrum resistance effective against numerous races or pathotypes of *P. brassicae*, to highly specific resistance effective only against particular strains of the pathogen<sup>32,33</sup>. The durability of a resistance source is, therefore, influenced by the number and relative prevalence of pathotypes within the region(s) over which this resistance is intended for deployment. Analysis of *P. brassicae* populations from Alberta has revealed a fair amount of pathogenic diversity in the parasite, with at least five different pathotypes (2, 3, 5, 6 and 8) identified on the Williams<sup>33</sup> differential set<sup>35,7,36,8</sup>. A concerted effort to produce clubroot resistant canola hybrids, led by various private companies and public breeders, has resulted in the recent release of six cultivars into the Canadian market (Pioneer '45H29' and 'D3152', Dekalb '73-67RR' and '73-77RR' and Canterra '1960' and Proven '9558C'). While the availability of these cultivars represents one of the most important new developments in the management of clubroot on the Canadian prairies, the deployment of resistant canola will have to be carefully managed to maintain durability. The pathotype composition of *P. brassicae* can shift rapidly in response to selection pressure, and previous experience in other countries has shown that genetic resistance can quickly break down<sup>37,38</sup>. In an attempt to preserve the effectiveness of the clubroot resistance trait, a break of three years is recommended between resistant canola cultivars on clubroot infested fields<sup>19</sup>. Resistance stewardship is complicated however, by a lack of knowledge on the nature of, and relationship among, sources of resistance in commercial hybrids.

#### **Regulatory approaches to clubroot management.**

Clubroot was declared a pest under the *Agricultural Pests Act* (APA) of Alberta in April 2007. The APA is the foundation legislation for the enforcement of control measures for pests in Alberta. The decision to

add clubroot to the APA was taken in consultation with stakeholders, and was supported by Agricultural Service Boards and the Alberta Canola Producers Commission. Under the APA, the province has developed a Clubroot Management Plan, which is interpreted and enforced by individual municipalities as they see fit. The Alberta Clubroot Management Plan has served as a model for similar plans adopted in Saskatchewan and Manitoba. The plan continues to evolve to incorporate novel information and management tools as they become available, and is an important tool for raising clubroot awareness among growers.

#### **Conclusions**

In less than a decade, clubroot has emerged as one of the most important diseases of canola in central Alberta. The disease is now endemic to this region, and while methods such as sanitation of equipment may help to slow the dissemination of clubroot, it will likely continue to spread. As such, sustainable canola production on the prairies will depend on effective disease management approaches. The research efforts currently underway have greatly increased understanding of clubroot of canola and have led to a number of potential control strategies. Genetically resistant canola hybrids may be among the most important of these tools. However, successful control of clubroot will require an integrated approach, and continued collaboration and consultation among stakeholders.

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