Pathotypes of *Plasmodiophora brassicae* from clubroot resistant canola and assessment of amisulbrom for clubroot control

By

Keisha Hollman

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Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Clubroot, caused by *Plasmodiophora brassicae* Woronin, is an important soilborne disease of canola (oilseed rape; *Brassica napus* L.). In Canada, clubroot management relies heavily on the planting of resistant cultivars, but since 2013, resistance has been broken in an increasing number of fields. Prior to the introduction of resistance, *P. brassicae* pathotype 3H, as defined on the Canadian Clubroot Differential (CCD) set, was predominant in Alberta. In testing of pathogen collections from 2014-2016, however, pathotype 3A was most common, indicating rapid shifts in the pathogen population. Up-to-date knowledge of pathotype composition is important for effective resistance breeding and stewardship. Furthermore, strategies to supplement resistance, such as the application of fungicides, may also contribute to sustainable clubroot management. In this thesis, isolates of *P. brassicae* were obtained from 166 canola crops in Alberta, Saskatchewan and Manitoba, and evaluated for pathotype designation on the CCD set and the differentials of Somé et al. Seventeen pathotypes were detected on the CCD set, including the previously reported pathotypes 3A, 3D, 3H, 5L, 5X, 8E, 8N and 8P, plus the novel pathotypes 2C, 6D, 8D, 9A, 9B, 9C, 11A, 13A and 13B. Five pathotypes were identified on the hosts of Somé et al. including P_1, P_2, P_3, P_4 and P_5, with P_4 and P_5 reported here from Canada for the first time. The majority of the isolates, representing 39 fields in 2017 and 92 fields in 2018, could overcome genetic resistance. In a second study, the fungicide amisulbrom was tested for its efficacy in controlling clubroot in field trials conducted in 2018 and 2019 with the clubroot resistant canola cultivar 'CS2000' and the susceptible cultivar '45H31'. The results in 2018 indicated a significant effect of cultivar on clubroot severity, but the application of different rates of amisulbrom did not result in significant differences in disease level or plant growth parameters. Flooding of many of the plots in 2019 precluded the acquisition of meaningful results in that year. The field data were supplemented

with results from a greenhouse study conducted using the susceptible canola cultivar '45H31' at low (1×10^4 resting spores/g soil mix) and high (1×10^7 resting spores/g soil mix) *P. brassicae* inoculum levels. Treatment of the potting mix with three rates of amisulbrom (500 g active ingredient (ai)/ha, 1000 g ai/ha, and 1500 g ai/ha) resulted in significant declines in clubroot severity, and increases in plant height and aboveground weight, relative to the untreated control at both inoculum levels. Collectively, the results from this thesis suggest significant diversity in the virulence of *P. brassicae* populations and an increasing prevalence of resistance-breaking *P. brassicae* strains, as well as some potential for amisulbrom to reduce clubroot severity, at least under greenhouse conditions.

Preface

This thesis is an original work by me, Keisha Hollman. I conducted all of the experiments described in this document and wrote the first drafts of each chapter. The chapters were then edited and revised by Dr. Strelkov and Dr. Sheau-Fang Hwang, who then provided me with suggestions and edits to include in the document. Victor Manolii, a technician with our group, was instrumental in helping me to conduct and plan Chapter 3. I also received assistance from numerous summer students and other graduate students for routine tasks associated with the studies in Chapters 3 and 4, such as preparing potting mix and seeding and weeding my plots. Mr. George Turnbull (Alberta Agriculture and Forestry) also helped me with seeding and spraying of my field plots. Numerous graduate students and Victor Manolii also helped with data collection for the data chapters.

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Chapter 1 Introduction

1.0 Clubroot disease and canola

Plasmodiophora brassicae Woronin is an obligate parasite causing clubroot of canola (*Brassica napus* L.) and other crucifers. Clubroot is a soilborne disease associated with the formation of large galls on the roots of susceptible plants. Depending on the amount of inoculum present and the susceptibility of the host, these galls can range in size from small nodule-like malformations to large galls affecting the entire root system (Karling, 1968). Gall formation prevents the normal uptake of water and nutrients by infected plants, resulting in aboveground stunting, wilting, and premature ripening. Without proper management, extreme yield and quality losses may result (Dixon 2009a). On the Prairies, the main canola-producing region in Canada, clubroot was first identified on 12 canola crops in central Alberta in 2003 (Tewari et al. 2005). Since then, the disease has continued to spread. As of 2019, 3,353 fields were known to be infested with *P. brassicae* in Alberta (Strelkov et al. 2020b), and the disease is being identified with increasing frequency in Saskatchewan and Manitoba (Cao et al. 2009; Dokken-Bouchard et al. 2012, Manitoba Agriculture 2017). Clubroot now also occurs on canola in North Dakota in the United States (Chittem et al. 2014).

Clubroot management options are limited, particularly in canola cropping systems. Long-rotations out of canola can be effective at reducing soil inoculum levels, but many farmers rely on canola as a cash crop and hence are hesitant to extend their rotations (Peng et al. 2015). Chemical control strategies are either ineffective and/or prohibitively expensive for large acreage canola crops, and hence have not been widely used (Gossen et al. 2014). Given these limitations, there has been considerable interest in the identification and deployment of genetic resistance for clubroot management. The first clubroot resistant (CR) canola cultivar was released in 2009,

followed quickly by numerous other cultivars from various seed companies. Collectively, these CR canola cultivars soon became the most important clubroot management tool for Canadian farmers (Strelkov et al. 2018a), who grew them extensively throughout many regions at risk of the disease. In 2013, however, new strains of *P. brassicae* were identified that could overcome the resistance in most CR canola cultivars (Strelkov et al. 2016). Subsequent monitoring has identified an increasing number of canola crops where resistance to clubroot has been lost or eroded, resulting from the emergence of many new, virulent pathotypes of *P. brassicae* (Strelkov et al. 2018a). This loss of resistance represents one of the most important challenges to sustainable canola production in Canada.

1.1 Research objectives

In order to identify effective sources of clubroot resistance, it is important to monitor the pathotype composition of *P. brassicae* in regions where that resistance will be deployed. Furthermore, while genetic resistance is one of the most effective tools for clubroot management, it will need to be used as part of an integrated disease management strategy to ensure its durability and continued efficacy. The implementation of an integrated disease management strategy requires the identification of additional clubroot management methods, which can be used together with resistance for sustainable disease control.

My Master's project included two specific objectives: (1) to characterize isolates of *P. brassicae* recovered from canola fields in Alberta, Saskatchewan and Manitoba, for their virulence and pathotype classification, and (2) to evaluate the soil amendment fungicide, Amisulbrom, for its efficacy in reducing clubroot incidence and severity in greenhouse and field trials. This general aim of the research was to generate information to help improve our knowledge and management of clubroot of canola.

Chapter 2 Literature Review

2.0 Introduction

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Wor., is a soilborne disease of the Brassicaceae, and is associated with the development of large galls or 'clubs' on the roots of susceptible hosts. In Canada, the occurrence of clubroot has been reported for over a century in British Columbia, Ontario, and Quebec, where it occurs mainly on brassica vegetables in market gardens (Howard et al. 2010). In 2003, the disease was found on the Prairie canola (*Brassica napus* L.) crop for the first time, when it was identified in 12 fields near Edmonton, Alberta (Tewari et al. 2005). Since then, clubroot has continued to spread throughout the province, and now also occurs on canola in Saskatchewan, Manitoba and North Dakota (Cao et al. 2009; Dokken-Bouchard et al. 2012; Chittem et al. 2014; Manitoba Agriculture, 2017). Clubroot poses a major threat to canola, Canada's most economically important crop, with production estimated at 18.6 million tonnes and exports at $11.5 billion in 2019 (Statistics Canada 2019a, 2019b). *Plasmodiophora brassicae* is a difficult pathogen to manage, given its prolific rate of reproduction and the longevity of its soilborne resting spores. In an effort to contain the outbreak, *P. brassicae* was made a Declared Pest under the Alberta Agricultural Pests Act in April 2007 (Government of Alberta 2020a, Government of Alberta 2020b). Under this Act, enforcement of control measures was delegated to municipalities, which often developed clubroot management plans for their districts. While *P. brassicae* has continued to spread despite its status as a Declared Pest, this designation has helped to increase clubroot awareness in Alberta.

2.1 *Plasmodiophora brassicae*

2.1.1 Taxonomy

Plasmodiophora brassicae is the best known plant pathogen in the Plasmodiophorid group (Schwelm et al. 2016). Plasmodiophorids are characterized by their cruciform nuclear division and associated hypertrophy of parasitized cells (Braselton 1995). Other important plant pathogens belonging to the Plasmodiophorid group include *Spongospora subterranea* (Wallroth), *Polymyxa betae* (Keskin), and *Polymyxa graminis* (Ledingham) (Schwelm et al. 2016). The discovery of *P. brassicae* by Woronin in the 19th century was the first description of this novel group of microorganisms (Woronin 1878). They are further embedded within the Endomyxea in the Cercozoa, which is a sister group of the Foraminifera, which are within the monophyletic Eukarotic group, Rhizaria (Keeling 2001).

2.1.2 Lifecycle

Plasmodiophora brassicae goes through three main stages during its life cycle: the resting spore stage, root hair infection, and finally, cortical infection (Kageyama and Asano 2009). As noted above, *P. brassicae* produces long-lived resting spores, with a half estimated at 3.6-4.4 years (Wallenhammer 1996, Hwang et al. 2013). The resting spores form within infected root cells and are released into the soil as the root galls decompose. The pathogen can spread from location to location on infested field equipment, footwear, or even animals, and the resting spores also may be dispersed in wind-blown dust or surface water (Dixon 2009b, Rennie et al. 2015). The parasite draws resources away from normal plant processes to support its growth and resting spore production, contributing to yield losses. Moreover, the formation of the root galls interferes with normal water and nutrient uptake by infected hosts, resulting in wilting, stunting and premature senescence (Howard et al. 2010).

When a host is present, the resting spores will germinate, releasing primary zoospores that are spindle-shaped or pyriform, long and biflagellate (Kageyama and Asano 2009). There are two flagella on the zoospore. One is short with a blunt end, and the other is long and whip-like. These zoospores swim to the host in films of water, encysting on and penetrating the root hairs, resulting in primary infection. Once inside the root hair, primary plasmodia develop, followed by numerous nuclear divisions and finally cleavage into zoosporangia that form clusters in the root hairs or epidermal cells (Tommerup and Ingram 1971, Kageyama and Asano 2009). From these zoosporangia, groups of (4-16) secondary zoospores are released. These have the exact same appearance as the primary zoospores.

The secondary zoospores are believed to fuse in pairs and then penetrate the cortical tissue, initiating secondary infection (Tommerup and Ingram 1971). Studies have shown that secondary zoospores released from root hairs can actually re-infect them, producing more secondary zoospores. This means that cortical infection during the root hair infection stage may be amplified by this cycle (Naiki et al. 1984). Once inside the cortical tissue, growth and mitosis of the parasite occur, resulting in the formation of multinucleate secondary vegetative plasmodia (Tommerup and Ingram 1971, Kageyama and Asano 2009). This stage of the *P. brassicae* lifecycle is associated with hypertrophy and hyperplasia of the host tissues, resulting in the formation of the root galls. Nuclei in the secondary plasmodia fuse in pairs, and are thought to undergo meiosis, returning the plasmodia to a haploid state. The secondary plasmodia are then cleaved to form uninucleate, haploid resting spores, which are released back in the soil as survival structures (Tommerup and Ingram 1971, Kageyama and Asano 2009). The lifecycle of the pathogen occurs primarily in the soil and within the roots, underscoring the importance of proper soil stewardship and the reduction of soil movement from infested fields.

2.1.3 Host range

The host range of *P. brassicae* is vast and includes all cultivated and non-cultivated species in the family Brassicaceae, in which the pathogen is able to undergo both primary and secondary infection stages (Dixon 2009a). While there have been a number of studies on hosts within the Brassicaceae, little work has been done to determine other potential host families (Hwang et al. 2012a).

Using scanning electron microscopy (SEM), Ludwig-Müller et al. (1999) provided evidence that infection of non-host species by *P. brassicae* also may occur. Structures resembling *P. brassicae* were observed by SEM in the root cortex tissue of *Tropaeolum majus L., Carica papaya L., Reseda alba L.* and *Beta vulgaris L.,* all species outside of the Brassicaceae family. The structures resembling *P. brassicae* from *T. majus* and *B. vulgaris* were used to inoculate *Brassica rapa* L., resulting in the formation of galls. While this demonstrates that *P. brassicae* may in fact infect non-host species, the lifecycle does not appear to be completed in these non-hosts (Ludwig-Müller et al. 1999). Additional research in this area may be warranted.

Within the Brassicaceae, cultivated crops seem to be the most susceptible to *P. brassicae* infection and include *Brassica oleracea* L. (Brussels sprouts, cabbages, calabrese/green broccoli, cauliflower, culinary and fodder kale, kohlrabi), *Brassica rapa* L. (turnip, turnip rape, sarson, and a range of Oriental variants such as *B. rapa* var. *pekinensis* and *B. rapa* var. *chinensis*), and *B. napus* (swede (rutabaga), oil seed rape, and fodder rape, condiment (mustard), and vegetable crops derived from *Brassica carinata* (L.) Braun, *Brassica nigra* (L.) W.D.J. Koch, and *Brassica juncea* (L.) Czern) (Dixon 2009a). The rock garden plant (*Arabidopsis thaliana* (L.) Heynh.), often used as a scientific model, is also susceptible to *P. brassicae*, as are species in the genus *Raphanus* (Dixon 2009a).

The clubroot pathogen also affects host cruciferous weed species found on or near agricultural land, including flixweed (*Descurainia sophia* (L.) Webb ex Prant), stinkweed (*Thlaspi arvense* L.), shepherds purse (*Capsella bursa-pastoris* (L.) Medik.), volunteer canola (*B. napus*), and wild mustard (*Sinapis arvensis* L.) (Dixon 2009a). Control of cruciferous weed species in a field before gall formation can occur is imperative for reducing the number of resting spores in the soil. It is recommended that susceptible weeds be controlled within the first 3 weeks of emergence, in order to prevent infection and the production of resting spores. Ornamentals, such as flowering plants in the mustard family (*Matthiola* spp.) and wallflower (*Cheiranthus cheiri* L. Crantz), are also susceptible to *P. brassicae* (Dixon 2009a). Control or prevention of clubroot on these species is important for reducing soil inoculum levels and the potential for infested soil from gardens to be moved to cropland.

2.1.4 Physiologic specialization

Physiologic specialization, which refers to the occurrence of different forms of a pathogen that are morphologically identical but differ in their ability to infect different hosts, was first demonstrated to occur in *P. brassicae* by Honig (1931). Different forms of *P. brassicae* are referred to as ‘races’ or ‘pathotypes’ (see for example, Williams 1966 and Strelkov et al. 2006). Over the past few decades, however, the term ‘pathotype’ has become more common, since strains of *P. brassicae* are distinguished based mainly on their virulence phenotypes, with the genetics of the host-pathogen interaction not sufficiently well-defined to apply the concept of ‘races’ to this pathosystem (S.E. Strelkov, personal communication). Various sets of differential hosts have been proposed to identify *P. brassicae* pathotypes, four of which have been used most widely: the differentials of Williams (1966), the European Clubroot Differential (ECD) set (Buczacki et al.

1975), the differentials of Somé et al. (1996), and the Canadian Clubroot Differential (CCD) set (Strelkov et al. 2018a).

The differential set of Williams (1966) was originally created to identify differences in the virulence of *P. brassicae* collections from the United States. It consists of four hosts, namely the cabbages (*B. oleracea* var. *capitata*) 'Jersey Queen' and 'Badger Shipper' and the rutabagas (*B. napus* var. *napobrassica*) 'Laurentian' and 'Wilhelmsburger', allowing for the identification of a theoretical maximum of 16 pathotypes. Each pathotype (originally termed 'race' by Williams (1966)) is assigned a number based on its virulence pattern. Until recently, this system was the most widely used in Canada (Strelkov & Hwang 2014), but it cannot distinguish all pathotypes occurring on *B. napus* canola. As such, the hosts of Williams were recently incorporated into a new differential set, the CCD, as will be described below (Strelkov et al. 2018a).

The ECD set was established in an attempt to develop a standardized race or pathotype classification system for *P. brassicae* (Buczacki et al. 1975). This system was based on an earlier proposal by Habgood (1970), in which differential hosts were arranged in a fixed order and assigned a denary number (1, 2, 4, 8, 16 etc.), which then corresponded with a binary series (2^0, 2^1, 2^2, 2^3 etc.). Buczacki et al. (1975) gave a hypothetical example where the first, second, third and fifth differential hosts of a subset of five were susceptible to a field isolate or 'population' of *P. brassicae*. The corresponding values for each host in the binary were then summed ($2^0+2^1+2^2+2^4$ = 1+2+4+16= 23), resulting in a designation of 23 for that isolate. The ECD set expanded on the system by Habgood (1970) and includes five hosts each of *B. rapa*, *B. napus*, and *B. oleracea* (Buczacki et al.1975). The reactions of the host differentials in each of these subsets is scored separately, resulting in three numerical designations calculated as described in the example above. By convention, this is preceded by the acronym "ECD". As an illustration, the

pathotype ECD 16/15/12 would refer to a pathotype with a designation of 16 on the *B. rapa* subset, 15 on the *B. napus* subset, and 12 on the *B. oleracea* subset. If, for some reason one or more the subsets are not of interest for the particular sample, the designations on the species left out are replaced by a dash (e.g., ECD-/15/-). While the ECD set is still used occasionally in Europe, it did not gain widespread acceptance in Canada or elsewhere due to the complex nomenclature and the large number of differential hosts required.

The differential set of Somé et al. (1996) was originally developed to characterize *P. brassicae* field and single-spore isolates from France. It consists of three *B. napus* cultivars including 'Nevin' (also included in the ECD set as ECD 06), 'Wilhelmsburger' (ECD 10 and also included as a Williams' differential), and the spring oilseed rape 'Brutor'. Cultivars of *B. napus* were selected because it was easier to maintain genetically pure stocks of this inbreeding species. Based on their virulence patterns on the differentials, isolates were assigned pathotype designations sequentially, starting with P_1. The small number of hosts in the differential set of Somé et al. is a distinct advantage relative to the ECD set, reducing the space and number of inoculations needed to test isolates. Furthermore, the inclusion only of *B. napus* is an advantage when characterizing isolates recovered from this species. Nonetheless, the hosts of Somé et al. lack differentiating capacity, and cannot distinguish many of the *P. brassicae* virulence phenotypes that have been identified in Canada (Strelkov et al. 2018a; Askarian et al. 2020).

More recently, Canadian researchers have proposed the CCD set (Strelkov et al. 2018a) to identify pathotypes of *P. brassicae* from Canada. This system was developed to improve understanding of the virulence structure of the clubroot pathogen recovered from canola, and to distinguish pathotypes virulent or avirulent on clubroot resistant (CR) cultivars. The CCD set consists of 13 hosts, including the differentials of Williams (1966), Somé et al. (1996), selected

hosts of the ECD set (Buczacki et al. 1975), the CR winter oilseed rape (*B. napus*) 'Mendel', the spring oilseed rape 'Brutor', the open-pollinated spring canola 'Westar', and the CR hybrid canola '45H29' (Table 3.1). Inclusion of the differentials of Williams and Somé et al. allows researchers to obtain pathotype designations according to those systems as well, facilitating comparisons with earlier studies, although these designations did not constitute part of the CCD nomenclature. Initially, unique virulence patterns on the hosts of the CCD set were each assigned an uppercase letter (A, B, C, etc.) to designate them as different pathotypes (Strelkov et al. 2018a). Recently, however, the CCD pathotype nomenclature has been revised to include the Williams' number designation, followed by a letter indicating the CCD designation (Askarian et al. 2020), allowing the entire alphabet to be applied to distinguish multiple variants of a single Williams' pathotype (e.g., pathotypes 2A, 2B, 2C). Evaluation of *P. brassicae* collections made up until the end of 2016 on the CCD set indicated the presence of at least 17 pathotypes in Canada; in contrast, only 5 or 3 pathotypes, respectively, could be detected on the differentials of Williams and Somé et al. (Strelkov et al. 2018a). This highlights the increased differentiating capacity of the CCD set.

2.2 Clubroot management

Given that *P. brassicae* is a soilborne pathogen that produces long-lived resting spores that are released into the soil after root gall decay, clubroot management is focused on reducing the spread of infested soil to new areas, reducing soil inoculum levels, and planting resistant cultivars that suffer no or very limited yield loss. There are a number of chemical, biological, cultural and genetic resources available and recommended for clubroot control. The best form of control is prevention, which includes the deployment of management strategies before clubroot is present in a field or region. Once the disease is present, however, use of a combination of management strategies is imperative to mitigate its impact. No single management strategy results in complete

control, however, and strategies should be combined or 'integrated' for the successful, long-term management of clubroot. The exact approach to managing clubroot in a particular area may vary, depending on the specific situation and/or conditions. This section of the Literature Review will cover both existing and potential cultural, biological, and chemical strategies for the management of clubroot in Canada, and the future of genetic resistance in light of the increasing number of *P. brassicae* pathotypes able to overcome resistance.

2.2.1 Cultural control

Cultural disease management strategies, while not as widely used as chemical control and genetic resistance, can provide farmers with a solid foundation for effective clubroot management. The goal of cultural control is to create environments and situations that are unfavourable for disease development. Various cultural control methods have proven effective or promising for reducing the impact and severity of clubroot disease.

Crop rotation

Longer rotations and greater crop diversity have long been recommended and continue to be recognized for their benefits in disease control (Cathcart et al. 2006, Cook 2006). Indeed, when clubroot was first identified in western Canada, long rotations out of canola were one of the few management strategies available (Strelkov & Hwang 2014). Rotating out of a host crop for a number of years allows the *P. brassicae* resting spore populations to decline sufficiently to prevent significant disease when a host crop is grown again (Peng et al. 2015). As market demand for canola has increased and genetically resistant cultivars became available, however, shorter rotations have been widely practiced in the canola growing regions of Canada, precluding the adoption of more diverse rotations by producers. A typical rotation usually consists of canola and wheat in a continuous two-year cycle (Peng et al. 2015). In instances where fields show extremely

high disease pressure, or the emergence of resistance breaking pathotypes, rotations remain one of the most effective tools for clubroot management.

Since resting spores of *P. brassicae* can remain viable in the soil for many years, the question emerges of how long of a rotation is necessary to provide sufficient control. Peng et al. (2015) set out to determine whether a two-year rotation out of a host crop reduced *P. brassicae* resting spore populations to manageable levels, or whether longer breaks are required. The study, carried out under field conditions in Quebec, found that there was a significant decrease in clubroot severity after a 2-year break out of canola relative to a 1-year break. Furthermore, there was little difference between a 2-year break versus a 4-year break, suggesting a likely stabilization in resting spore populations after the steep decline observed during the first and second years out of a host crop. Similar results were reported in an independent evaluation of *P. brassicae* resting spore population dynamics in CR canola cropping systems (Ernst et al. 2019). This study by Ernst et al. (2019), which was conducted in commercial fields located in Alberta, also found that a 2-year break from a host crop led to a significant decline in *P. brassicae* resting spore populations. The findings of Peng et al. (2015) and Ernst et al. (2019), based on studies carried out in Canada, suggest a shorter longevity for most *P. brassicae* resting spores than what was reported in some earlier (mostly European studies), including by Wallenhammar et al. (1996) who reported a 3.6-year half-life for the resting spores. The length of rotation needed for spores to decline to a manageable level likely depends on the initial level of soil infestation (Ernst et al. 2019), and as such, additional management strategies may be necessary to achieve sufficient control.

Seeding date manipulation

An effective, low cost option for clubroot management is the manipulation of seeding date in order to give seedlings the best chance at survival before infection by *P. brassicae*. Younger

canola seedlings are more severely affected by clubroot than older seedlings, and clubroot development is favoured by high soil moisture and temperature conditions of 15-25 ºC (Hwang et al. 2011b; Karling 1968; Gossen et al. 2014). Hwang et al. (2012b) evaluated the effect of early vs. late seeding of canola on clubroot severity at the end of the season. Seedling emergence at all sites was lower in the early seeded plots, however, these plots saw the greatest reduction in clubroot severity and an increase in yield compared with the later seeded plots and the controls. The plants that were seeded early were able to establish in high moisture and low temperature conditions, which reduced their vulnerability to *P. brassicae* infection when temperatures increased. This form of cultural control is already (inadvertently) practiced to some extent on the Prairies, as canola is typically seeded early in order to maximize yield potential during the short growing season.

2.2.2 Bait crops and biological control

Bait crops consist of 'sacrificial' plants planted to attract pests and pathogens away from the crop of interest, and represent a form of biological control, which involves the introduction of another organism to control a pest through predation, parasitism, herbivory, or other natural mechanisms (Garrett 1956). Both bait crops and other forms of biological control have been evaluated for clubroot management.

Bait crops

Bait crops for clubroot control involve the use of non-host crops to stimulate the germination of *P. brassicae* resting spores, thereby depleting soil inoculum loads (Friberg et al. 2005). The germination of *P. brassicae* resting spores is stimulated by the proximity of various plant genotypes that release specific root exudates (Ahmed et al. 2011, Friberg et al. 2005). Non-host crops such as perennial ryegrass (*Lolium perenne* L.), leek (*Allium ampeloprasum* L. nom. cons.), rye (*Secale cereal* L.), and red clover (*Trifolium pratense* L.) stimulate germination of *P.*

brassicae through the release of root exudates (Friberg et al. 2005). Since the pathogen cannot complete its life cycle on these hosts, resting spore levels should decline, in theory at least, as the spores that germinated are not replenished. In addition, several host species, including canola and Chinese cabbage, have also been studied for use as bait crops. In the case of susceptible hosts, the infected plants are ploughed under or killed with herbicide before the pathogen is able to complete its life cycle (Friberg et al. 2005), reducing inoculum loads in the soil much more rapidly than use of a long crop rotation out of host species (Donald and Porter 2009, Friberg et al. 2005).

The success of bait crops has varied across numerous studies and calls into question the effectiveness of this method for clubroot control (Friberg 2006). Bait crops showed promising results under greenhouse conditions in studies done by Ahmed (2011) and Friberg et al. (2006). While the greenhouse results were promising, the impacts on inoculum potential and clubroot severity were too small and inconsistent to be effective in larger scale commercial situations. At the field scale, the Canadian climate poses a serious limiting factor for the use of bait crops, since the short summers and long winters mean that very few bait crop cycles can be completed in a single growing season. Nevertheless, there is potential for the use of bait crops in patch management for small areas in a field that are heavily infested, such as field entrances (Cao et al. 2009). Bait crops could also be effective when used in conjunction with other cultural management practices such as crop rotations.

Other biological control agents

Peng et al. (2011) looked at a number of potential biological control agents and their effectiveness for clubroot control in susceptible canola hosts. The most effective biological control formulation under greenhouse conditions was a mix of *Bacillus subtilis* (C.G. Ehrenberg), *Gliocladium virens* (J.H. Miller, J.E. Giddens & A.A. Foster), and *Streptomyces lydicus* (De Boer),

and reduced the incidence of disease by 61 to 91%. Other biological control agents were tested under controlled conditions but showed little to no effect in controlling clubroot. These biological control agents were also evaluated in field trials with canola, where there was little to no effect on clubroot severity. In field trials with Chinese cabbage, however, a disease suppression of 55 to 85% was observed (Peng et al. 2011). This was likely due to the fact that biocontrol agents are more effective at lower disease pressure, and in this study, clubroot was more severe in the canola vs. Chinese cabbage trial.

There are many other biological control agents that have proven effective at controlling clubroot, including *Heteroconium chaetospira* (Grove) M. B. Ellis, *Phoma glomerata* (Corda) Wollenq and Hochapfel, *Bacillus spp.* and *Pseudomonas* spp. (Garrett 1956). *Bacillus* spp. and *Pseudomonas* spp. reduced the overall survival ability of *P. brassicae* (Einhorn et al., 1991). A study by Narisawa et al. (2005) found that the root endophytic fungus *H. chaetospira* successfully suppressed *P. brassicae* in Chinese cabbage at moderate inoculum levels.

Generally, studies with biological control agents appear promising when conducted under controlled environment conditions, but little positive effect has been observed in the field, where the level of control has been unpredictable. The use of living organisms to control *P. brassicae* is difficult, and appears to be hampered by constraints such as winter survival of the biocontrol agent, high cost of application, accessibility to product, slow emergence and reproduction, and non-target selection (Peng et al. 2011).

2.2.3 Chemical control

Fungicides have been studied extensively as a control option for clubroot, with a few products demonstrating measurable success. Some of the chemicals that have shown potential and partial control as soil incorporations include the thiabendazole/iodophor complex, benomyl,

thiophanate methyl, carbendazim, calcium cyanamide, dazomet, manganese-zinc-iron-dithiocarbamate, the experimental fungicide WL105305, a phenolic mixture, sodium tetraborate and thiophanate-methyl (Buczacki 1973; Buczacki et al. 1976; Dixon & Wilson 1983, 1984, 1985; Humpherson-Jones 1993). A more recent study by Gossen et al. (2012) found that Ranman (cyazofamid) reduced clubroot on canola in spring and early summer when used as a drench application, but had no effect when inoculum levels of *P. brassicae* were low.

The fungicides Dynasty 100 FS (azoxystrobin), Nebijin 5SC (flusulfamide) and Helix Xtra (thiamethoxam+difenoconazole+metalaxyl+fludioxonil) were also assessed as a seed drench for effectiveness at controlling seedborne *P. brassicae* inoculum (Hwang et al. 2012b). All three fungicides were effective at controlling the incidence of disease when compared with the untreated check.

Fungicides can also be used to amend the soil when incorporated by rototiller. Multiple fungicides have been studied for their effectiveness at controlling clubroot as a soil treatment (Hwang et al. 2011a). Of 10 fungicides tested, Terraclor (quintozene) was shown to significantly reduce clubroot severity when compared with the control. Hwang et al. (2011a) reported that Ranman (cyazofamid) reduced clubroot severity when used as a soil amendment, similar to the results of Gossen et al. (2012), which demonstrated its effectiveness when used as a drench. At present, Ranman (cyazofamid) is the only fungicide registered for clubroot control on vegetable Brassica crops in Canada (Gossen et al. 2012).

Amisulbrom is a fungicide in the bromoindoles class that has been used historically to control late blight (*Phytophthora infestans* (Mont.) de Bary) and downy mildew (*Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni) of potato (U.S. National Library of Medicine 2020). Staniasek et al. (2008) obtained promising preliminary results for the use of amisulbrom to

manage clubroot in cabbage. There has been no research, however, on the use of amisulbrom for clubroot control in canola, suggesting the need for additional study. The evaluation of different rates of amisulbrom for clubroot management in canola under field and greenhouse conditions will be the focus of Chapter 3 of this thesis.

While fungicides may provide effective clubroot control in some cases, limiting factors such as high costs and potential environmental impacts exist. Nonetheless, fungicides may serve as potential tools for controlling small patches of clubroot, treating only a few infested hectares in a field, and could have potential for success when used in conjunction with other management strategies (Gossen et al. 2012). Proper stewardship of fungicides should also be practiced to extend their useful longevity and reduce the potential for pathogen resistance in the future. In general, fungicides should only be used when necessary and in conjunction with other disease management strategies whenever possible.

2.2.4 Soil amendments

Liming

Resting spores of *P. brassicae* prefer an acidic soil environment for germination (Karling 1968). Therefore, the application of lime to increase soil pH has been used as a strategy for the management of clubroot. A target pH of 7.2 resulted in the greatest reduction in spore germination without inducing nutrient disorders in the crop (Murakami et al. 2002). Lime is available in many different forms including agricultural lime (calcium carbonate and calcitic lime), dolomitic lime (calcium and magnesium carbonate), hydrated lime (calcium hydroxide) and quicklime (calcium oxide) (Hwang et al. 2014). Agricultural and dolomitic lime are slow acting and should be applied in the fall, whereas hydrated lime and quick lime are fast acting and should be applied in the spring. The application rate needed for sufficient control of clubroot also depends on soil type and starting

pH, and may require thousands of tonnes per hectare in order to raise the pH to desirable levels. A study by Colhoun (1953) determined that lime application might not be effective at all, if soil moisture and temperature favour *P. brassicae* development. The soil type and environment play an important role in whether or not liming will be effective for clubroot control (Dixon 2009b).

A study by (Fox 2019) tested the efficacy of hydrated lime products for control of clubroot in central Alberta. The study determined that moderate (6.7%; 8.0 T ha^{-1}) and high (11.4 T ha^{-1}) rates of hydrated lime under field conditions provided excellent control of *P. brassicae* and decreased clubroot incidence significantly. It was also observed that the application of hydrated lime at these rates increased aboveground plant biomass by 58-116%. Under greenhouse conditions, treatment with agricultural lime (calcium carbonate) eliminated disease completely at all inoculum concentrations, whereas hydrated lime decreased clubroot incidence only at the two lowest *P. brassicae* inoculum concentrations evaluated, 1×10^3 and 1×10^4 resting spores g^{-1} soil.

While liming holds promise for the control of clubroot, and has been used the management of the disease in Brassica vegetables, there are many factors limiting its widespread adoption in canola cropping systems (Hwang et al. 2014). The sheer amount of lime needed to raise soil pH over an entire quarter section is expensive, and the time and labour required to apply it may be prohibitive in the large fields typically associated with canola production in western Canada. Nonetheless, the use of lime for the management of clubroot in isolated patches or infection foci may hold some promise (Fox 2019).

Biochar

Biochar is a soil amendment used extensively for land reclamation and adopted as an amendment in agriculture (Knox et al. 2015). Biochar is similar to charcoal and when applied as a soil amendment, it can have a positive effect on soil fertility including nutrient use efficiency and

cation exchange capacity. Biochar can also alter the pH of a soil, making the soil environment less favourable for clubroot development. A study by Knox et al. (2015) outlined the effects of biochar on clubroot incidence and compared them with the effects of liming and to an untreated check. There was no significant decrease in incidence of disease from the application of biochar, although it was able to increase the soil pH from 5.9 to 7.3.

Boron

Boron has been used as a soil amendment for the control of clubroot on vegetable crops for many decades (Dixon 2009a). Boron is necessary for strengthening and forming cell walls in plants. Studies have shown that additional boron added as a soil amendment to *P. brassicae*-infested soils interrupts the pathogen lifecycle, by inhibiting the development of the plasmodium into a sporangium during the root hair infection stage (Webster & Dixon 1991). The application of boron can also increase overall plant biomass, plant health and cell function. Deora et al. (2011) observed that boron applied at a rate of 4 kg ha^{-1} was extremely effective at reducing the incidence of clubroot in canola, although higher rates were phytotoxic. Moreover, while boron has potential as a clubroot-management tool, soil type plays a large role in the level of disease control that can be achieved (Gossen et al. 2014).

Soil fumigants

Soil fumigants have been studied for their efficacy in controlling clubroot of canola in Canada (Hwang et al. 2014). While soil fumigants are not practical to apply on a large scale, due to the need to cover the soil for several days following application, they do show promise for the management of small patches of clubroot, such as at heavily infested field entrances (Hwang et al. 2014). Soil fumigants have been used for clubroot control in vegetable crop production, and it is expected that they will have similar capabilities in canola crops (Papiernik et al. 2004). A number

of different fumigants have potential for the control of clubroot, including basamid, 1,3-dichloropropene, chloropicrin, methyl bromide, propargyl bromide, and Vapam (metam sodium). The release of toxic volatiles, however, means that the application of fumigants requires appropriate safety measures and properly trained personnel.

Zuzak (2016) evaluated the efficacy of two soil fumigants, Vapam and a commercial seed meal-based biofumigant, MustGrow (derived from *B. juncea*) for the control of clubroot on canola in Alberta. The application of Vapam consistently reduced clubroot severity, although there was a wide range of effects depending on application rate. In contrast, there was no observable decrease in clubroot severity following the application of MustGrow. In another, as yet unpublished study (S.F. Hwang, personal communication), the application of Vapam prior to seeding provided significant control of clubroot and reduced overall disease severity.

2.2.5 Genetic clubroot resistance

The deployment of CR canola is the most cost effective and convenient option for the management of clubroot. The first CR canola cultivars became available on the Canadian market in 2009 and 2010 (Strelkov et al. 2018a), and currently there are 28 registered CR cultivars (Canola Council of Canada 2019). These varieties all have excellent resistance to pathotype 3, as designated on the differential system of Williams (1966), as well as pathotypes 2, 5, 6, and 8 (Strelkov and Hwang 2014). While the resistance in CR canola cultivars is not in the public domain, it appears that in most cultivars, it was derived from a CR winter oilseed rape, 'Mendel', produced in Europe (Fredua-Agyeman et al. 2018). This was based on major-gene resistance, making CR canola more prone to resistance loss through pathotype shifts. LeBoldus et al. (2012) showed that the clubroot resistance in various host genotypes, including the first CR canola cultivar in Canada, was quickly eroded after repeated exposure to the same field or single-spore isolate. The study by LeBoldus et

al. (2012), combined with earlier reports from the U.S., Japan and Europe, highlight the need for resistance stewardship (Tanaka & Ito 2013; Buczacki et al. 1975, Chittem et al. 2014).

Indeed, in 2013, just 4 years after the release of the first CR canola, significant levels of clubroot were found in two CR cultivars in central Alberta (Strelkov et al. 2016). Further testing confirmed that the pathogen populations in these fields included new virulence phenotypes, which could not be distinguished from 'old' pathotypes based on their designation on the differentials of Williams (1966). Additional surveillance from 2014 to 2016 identified 61 more fields in Alberta where clubroot resistance had been lost or eroded (Strelkov et al. 2018a). Isolates recovered from these fields exhibited multiple virulence patterns, leading to the development of the CCD set described earlier in this review to identify *P. brassicae* pathotypes. The 'old' pathotype 3, corresponding to CCD pathotype 3H, which had been predominant prior to the introduction of resistance, had been replaced by pathotype 3A in many fields (Strelkov et al. 2018a). The latter is highly virulent on many CR canola cultivars. The emergence of new pathotypes highly virulent on CR canola has led to the search for additional resistance sources, resulting in the recent release of new cultivars carrying so-called '2nd generation' resistance (Canola Watch 2020). The nature of this resistance remains unclear, apart from the observation that it is different to some extent to the 'Mendel'-derived resistance found in the original ('1st generation') CR canola cultivars. Characterization of the pathotype structure of *P. brassicae* and detection of shifts in the virulence of pathogen populations is important for monitoring the clubroot disease situation and guiding resistance breeding efforts (Karling, 1968). Chapter 3 of this thesis focuses on the characterization of pathotypes collected across the Prairies in 2017 and 2018, with an emphasis of isolates from Alberta.

Chapter 3 – Pathotypes of *Plasmodiophora brassicae* from clubroot resistant canola

3.0 Introduction

Plasmodiophora brassicae Woronin is an obligate parasite causing clubroot of canola (oilseed rape; *Brassica napus* L.) and other species in the Brassicaceae. Infection by *P. brassicae* is associated with the deformation of affected roots, resulting in yield losses estimated at 10-15% globally (Dixon 2006). The emergence of clubroot as a disease of the western Canadian canola crop is a major concern, as canola is worth $26.7 billion annually to the national economy (Canola Council of Canada, 2020). The number of confirmed clubroot infestations has been increasing rapidly, from just 12 fields in 2003 (Tewari et al. 2005) to more than 3,300 fields in Alberta by 2019 (Strelkov et al. 2020b). While the outbreak is most severe in Alberta, clubroot is also increasing in Saskatchewan and Manitoba, as well as in North Dakota in the United States (Cao et al. 2009; Chittem et al. 2014; Froese et al. 2019; Ziesman et al. 2019). Given its soilborne nature, the movement of vehicles and farm machinery can accelerate the spread of *P. brassicae*, particularly if proper sanitization practices are not in place (Strelkov & Hwang 2014).

Clubroot resistant (CR) canola cultivars were first introduced to the Canadian market in 2009. Although the genetic basis of this resistance is not in the public domain, most CR canola cultivars appear to derive their resistance from the oilseed rape 'Mendel' (Fredua-Agyeman et al. 2018). The first CR canola, '45H29', soon was followed by other cultivars released by various seed companies. These cultivars had excellent resistance to *P. brassicae* pathotype 3, as designated on the differentials of Williams (1966), which was predominant in Alberta, as well as to pathotypes 2, 5, 6, and 8, which prior to 2013 were the only other pathotypes reported from Canada (Strelkov and Hwang 2014). Given the effectiveness of this resistance, the planting of CR canola cultivars soon became the most effective clubroot management tool, allowing the production of this crop in severely infested fields without any significant impact on yields (Peng et al. 2014). The virulence

of *P. brassicae*, however, can shift quickly in response to host selection pressure (LeBoldus et al. 2012), and in 2013 severe symptoms of clubroot were found on two CR canola crops in central Alberta (Strelkov et al. 2016). Additional surveillance since 2013 has identified many 'novel' strains of the pathogen, capable of overcoming genetic resistance, in an increasing number of fields planted to CR canola (Strelkov et al. 2018a, 2020a). The Canadian Clubroot Differential (CCD) set was established recently to facilitate the detection and classification of these new strains (Strelkov et al. 2018a). It includes the *B. napus* cultivars 'Brutor', 'Mendel' and 'Westar', the Canadian CR cultivar '45H29', the differentials of Williams (1966) and Somé et al. (1996), as well as selected hosts of the European Clubroot Differential (ECD) set (Buczacki et al. 1975). In this system, isolates of the pathogen are assigned a number according to their Williams (1966) classification, followed by a letter based on their virulence on the CCD set (Strelkov et al. 2018a; Askarian et al. 2020). The 'original' pathotype 3, which does not overcome the resistance in CR canola, was reclassified as the CCD pathotype 3H; other variants of Williams' pathotype 3, which are highly virulent on CR canola, were also identified, with pathotype 3A found to be predominant (Strelkov et al. 2018a). On the differential hosts of Somé et al. (1996), originally developed to characterize *P. brassicae* collections from *B. napus* in France, most isolates were classified as pathotypes P_2 or P_3 (Strelkov et al. 2018a; Strelkov et al. 2020a). In western Canada, most of the pathotyping of *P. brassicae* from canola has focused on collections from Alberta, while little information is available with respect to the virulence of the pathogen in Saskatchewan and Manitoba.

Given the rapid pathotype shifts that can occur in *P. brassicae* and the increasing prevalence of clubroot in CR canola, continued monitoring of the virulence of pathogen populations is important for effective resistance breeding and stewardship. The focus of this study

was to evaluate the pathotype composition, as defined on the hosts of the CCD set and Somé et al., of *P. brassicae* collections made in Alberta, Saskatchewan and Manitoba in 2017 and 2018. This represents the most extensive analysis of the virulence and pathotype composition of *P. brassicae* in western Canada, including isolates from Saskatchewan and Manitoba where clubroot has only recently emerged as an issue.

3.1 Materials and methods

3.1.1 Collection of galled root material and extraction of spores

Canola roots with galls typical of clubroot were collected during annual disease surveys conducted across Alberta, Saskatchewan and Manitoba in 2017 and 2018 (Strelkov et al. 2018b, 2019b; Froese et al. 2019; Ziesman et al. 2019). In some cases, farmers and agronomists also forwarded galled roots for evaluation. The root samples represented 47 fields (44 in Alberta, 3 in Manitoba) in 2017 and 119 fields (102 in Alberta, 10 in Saskatchewan, 7 in Manitoba) in 2018. Nearly all root samples from Alberta were from CR canola cultivars (Supplementary Table 3.1), while the host cultivar was unknown or not reported in the samples from Saskatchewan and Manitoba (Supplementary Table 3.2). Resting spores of *P. brassicae* were extracted from 30-40 g of dried galls from each field following Strelkov et al. (2006), with each extraction representing one field isolate. The resulting spore suspensions were passed through eight layers of cheesecloth to remove any plant or soil debris, and the resting spore concentrations were estimated with a hemocytometer (VWR, Mississauga, ON). The concentration was adjusted to 1×10^7 spores mL^{-1} with sterile deionized water and the resting spore suspensions were used as inoculum as described below. All of the isolates included in this study are listed in Supplementary Tables 3.1 (Alberta) and 3.2 (Saskatchewan and Manitoba).

3.1.2 Host inoculation and disease assessment

One-week old seedlings of each host genotype were inoculated by the root dip method following Strelkov et al. (2006). Briefly, the seedlings were germinated on moistened filter paper in glass Petri dishes (9-cm diam.), after which their root systems were dipped into a *P. brassicae* resting spore suspension for 10 s. The inoculated seedlings were then planted in Sunshine LA4 potting mixture (Sunshine Growers, Vancouver, BC) in plastic pots (6 cm × 6 cm × 6 cm) at a density of one seedling per pot. An additional 1 mL of spore suspension was pipetted into the potting mix at the base of each seedling to ensure high exposure to inoculum. The pots were watered and moved to a greenhouse maintained at 20°C ± 2°C with a 16 h photoperiod under natural light supplemented by artificial lighting. The soil was kept saturated with slightly acidified water (pH 6.5) for the first week after inoculation, and then watered and fertilized with 20N: 20P: 20K as needed.

After 6 weeks, the plants were harvested and evaluated for the severity of clubroot symptoms on a 0-to-3 scale (Kuginuki et al. 1999), where: 0 = no galling, 1 = a few small galls (small galls on less than one-third of the roots), 2 = moderate galling (small to medium galls on one-third to two-thirds of the roots), and 3 = severe galling (medium to large galls on more than two-thirds of the roots). The individual severity ratings were then used to calculate an index of disease (ID) following Horiuchi & Hori (1980) as modified by Strelkov et al. (2006): ID (%) = $\{[\sum (n \times 0) + (n \times 1) + (n \times 2) + (n \times 3)]/ N \times 3\} \times 100$ %, where n is the number of plants in each class; N is the total number of plants; and 0, 1, 2, and 3 are the symptom severity classes. A mean ID was calculated by averaging the ID for each of the host-pathogen combinations across repetitions. A genotype was considered resistant if the mean ID was <50% and its associated 95% confidence interval (CI) did not overlap 50% (LeBoldus et al. 2012; Strelkov et al. 2016, 2018a).

The experiment was arranged in a completely randomized design replicated four times, with 12 seedlings per experimental unit.

3.1.3 Pathotype classification

The *P. brassicae* isolates were evaluated for pathotype classification on the 13 hosts of the CCD set (Strelkov et al. 2018a), which includes *Brassica rapa* L. ssp. *rapifera* line AAbbCC (European Clubroot Differential (ECD) 02), Chinese cabbage (*B. rapa* var. *pekinensis*) 'Granaat' (ECD 05), the fodder rapes (*B. napus*) 'Nevin' (ECD 06),'Giant Rape' selection (ECD 08) and New Zealand resistant rape (ECD 09), the rutabagas (*B. napus* var. *napobrassica*) 'Wilhemsburger' (ECD 10) and 'Laurentian', the cabbages (*B. oleracea* var. *capitata*) 'Badger Shipper' (ECD 11) and 'Jersey Queen' (ECD 13), and the canola/oilseed rape (*B. napus*) cultivars 'Brutor', 'Westar', 'Mendel' and '45H29'. Each CCD pathotype classification included a number indicating the Williams (1966) designation of each isolate, followed by a letter based on its virulence on the CCD set (Strelkov et al. 2018a; Askarian et al. 2020). Since the CCD set includes the full complement of differential hosts of Somé et al. (1966), pathotype designations according to that system also were recorded.

3.2 Results

3.2.1 Virulence of the isolates

All 47 isolates collected in 2017 were highly virulent on the universal suscept, ECD 05, and avirulent on ECD 02 (Supplementary Table 3.3). Almost all of the isolates were virulent on the *B. napus* genotypes 'Brutor', 'Westar' and ECD 08 with the exception of field isolate MB-11-17, which was avirulent on the latter. The majority of isolates were also virulent on ECD 06 and ECD 09, except for MB-SR-1-17, MB-SR-2-17, MB-11-17, which were avirulent on both of these hosts, and F.133-17 and F.118-17, which were avirulent on ECD 09. All of the isolates were virulent on the rutabaga 'Laurentian', and most were virulent on the cabbage ECD 13. In contrast,

a majority of the isolates collected in 2017 were avirulent on the rutabaga ECD 10, with only four (F.133-17, F.118-17, F.119-17 and MB-SR-1-17) classified as virulent on this host. Similarly, only two of the isolates collected in 2017 were virulent on the cabbage ECD 11, including PF-2-17 and MB-11-17. The CR oilseed rape 'Mendel' and CR canola '45H29' were susceptible to 37 and 39 of the 47 isolates, respectively, collected in 2017, indicating that most isolates were able to overcome resistance (Supplementary Table 3.3).

As with the isolates collected in 2017, all isolates from 2018 were also highly virulent on ECD 05 and avirulent on ECD 02 (Supplementary Table 3.3). Similarly, almost all of the isolates were virulent on the *B. napus* genotypes 'Brutor', 'Westar' and ECD 08; the only exceptions were D-5-18, which was avirulent on all three of these hosts, and MB-1-18, which was avirulent on ECD 08. The majority of isolates also were virulent on ECD 06 and ECD 09 with the exception of MB-3-18, which was avirulent on ECD 06; F.P.-73-18 and C.C.-3-18, which were avirulent on ECD 09; and S-11-18, D-5-18, MB-1-18, MB-6-18, SK-2018-CW-1 and SK-2018-RA-56, which were avirulent on both hosts. Unlike the isolates collected in 2017, not all of the isolates from 2018 were virulent on the rutabaga 'Laurentian', although the vast majority were, the only exceptions being S-11-18, D-5-18, MB-6-18, SK-2018-LLH-3 and SK-2018-RA-56. Most of the isolates collected in 2018 were also virulent on the cabbage ECD 13. In contrast, no field isolate was virulent on the cabbage ECD 11, and only two isolates (F.P.-73-18 and C.C.-3-18) were virulent on the rutabaga ECD 10. Just under half (53 of 119) of the isolates collected in 2018 were able to overcome the resistance in the CR oilseed rape 'Mendel', while most (92 of 119) were virulent on the CR canola '45H29'.

3.2.2 Pathotype classification

Of the 47 isolates collected in 2017, 33 were designated pathotype 3A, which is virulent on all of the CCD differential hosts except ECD 02, ECD 10, and ECD 11 (Table 3.1). Two isolates each were classified as pathotype 3D and the novel pathotype 9A. Pathotype 3D is distinguished from pathotype 3A by its avirulence on 'Mendel'. Four isolates represented the 'old' pathotype 3H, which is avirulent on both the CR canola '45H29' and the CR oilseed rape 'Mendel'. Pathotype 8N, which represents another of the 'old' pathotypes of *P. brassicae* from Canada (Strelkov et al. 2018a), and is also avirulent on 'Mendel' and '45H29', was identified from Sturgeon County, Alberta. A single field isolate from Two Hills, Alberta, represented pathotype 8P, which is distinguished from pathotype 3A by its avirulence on ECD 13. The remaining isolates represented novel virulence patterns identified from a single field each (Table 3.1). These included one isolate of each of pathotypes 2C, 9B and 13A from Manitoba, and pathotype 11A from Red Deer County in central Alberta. In 2017, four pathotypes were identified on the differential hosts of Somé et al. (1996), including P_1, P_2, P_3, and P_5 (Table 3.1). This was the first report of pathotype P_5 from Canada, while P_1 was previously identified from a single-spore isolate (Askarian et al. 2020).

As was the case in 2017, pathotype 3A was the most common pathotype among the pathogen collections made in 2018, representing 48 of 119 isolates tested, including an isolate from Manitoba. Nevertheless, pathotype 3D was recovered more frequently in 2018 than in 2017, and was the second most common pathotype (38 isolates). In 2018, a significant number (20) of the isolates tested were classified as pathotype 3H, including eight of 10 isolates collected from Saskatchewan (Table 3.1; Supplementary Table 3.2). One field isolate from Sturgeon County, Alberta, was designated pathotype 5X, which was the classification of the first *P. brassicae* strains found to overcome resistance in CR canola (Strelkov et al. 2016, 2018a). Three isolates

corresponding to pathotype 5L, which is not virulent on the CR *B. napus* 'Mendel' or '45H29', also were recovered from two fields in Saskatchewan and one in Manitoba (Table 3.1; Supplementary Table 3.2). A single isolate corresponding to pathotype 9A, first identified in 2017, was found again in 2018 in Red Deer County, Alberta (Supplementary Table 3.1). Pathotype 8N was identified in one field in Barrhead County, Alberta. Another previously reported variant of Williams' pathotype 8, the CCD pathotype 8P, also was identified in two fields in Barrhead County. Pathotype 8E was found in one field in Sturgeon County, which is adjacent to Barrhead. As was the case in 2017, several novel virulence patterns were identified among the isolates collected in 2018 and assigned new pathotype designations (Table 3.1). These included pathotypes 6D, 8D and 13B from Manitoba, and pathotype 9C from Rocky View County, in southern Alberta. Four pathotypes were identified on the differentials of Somé et al. (1996) in 2018, including P_1, P_2, P_3, and P_4 (Table 3.1); this was the first report of pathotype P_4 in Canada.

The prevalence of *P. brassicae* pathotypes across the Prairies and in the individual provinces of Alberta, Saskatchewan and Manitoba is illustrated in Fig. 3.1. The geographic distribution of pathotypes in Alberta, the source of most of the isolates, is shown in Fig. 3.2. Among the total 17 pathotypes identified in 2017-2018, eight (3A, 3D, 5X, 8E, 8P, 9A, 9C, 11A) can overcome the resistance in the CR canola '45H29'. An additional five pathotypes (3A, 5X, 8P, 9A, and 11A) are also virulent on the oilseed rape 'Mendel'. Unfortunately, the resistance-breaking pathotype 3A was identified outside Alberta for the first time, in the Municipality of Pembina, Manitoba (Supplementary Table 3.3). The resistance in '45H29' and 'Mendel' is still effective against the remaining pathotypes found in this study, including pathotypes 2C, 3H, 5L, 6D, 8D, 8N, 9B, 13A and 13B.

3.3 Discussion

Clubroot represents one of the most important threats to sustainable canola production in western Canada, with shifts in the virulence of *P. brassicae* populations complicating efforts to manage the disease via deployment of genetically resistant cultivars (Strelkov et al. 2016; Strelkov et al. 2018a). While most *P. brassicae* isolates characterized in the current study came from Alberta, the centre of the clubroot outbreak (Strelkov & Hwang 2014), 10 isolates also were analyzed from each of Saskatchewan and Manitoba, where the disease recently has been identified with increasing frequency (Froese et al. 2019; Ziesman et al. 2019). Hence, this is one of the most extensive evaluations of the pathotype composition of *P. brassicae* across the Canadian Prairies.

As was found in a previous study conducted with samples collected from 2014 to 2016 (Strelkov et al. 2018a), pathotype 3A was predominant among *P. brassicae* isolates recovered in 2017 and 2018. This pathotype is one of the most virulent on CR canola and can infect the oilseed rape 'Mendel', believed to be the source of resistance for most Canadian canola cultivars (Fredua-Agyeman et al. 2018). Nonetheless, pathotype 3H, which was predominant prior to the introduction of clubroot resistance (Strelkov & Hwang 2014), could still be identified, particularly among isolates collected in 2018. Since nearly all of the canola crops sampled in Alberta were resistant cultivars showing symptoms of clubroot, it is likely that our isolate collection was enriched for resistance-breaking pathotypes such as 3A (Strelkov et al. 2018a); pathotype 3H may still be predominant in fields where clubroot resistance remains effective, or where CR canola has not yet been grown. Indeed, eight of 10 isolates from Saskatchewan were classified as pathotype 3H. While the cultivars sampled in Saskatchewan were unknown, they were not necessarily CR cultivars, since until recently most of this province was assumed to be free of clubroot. In a new study of *P. brassicae* pathotypes in the Peace Country of northwest Alberta, another region where

clubroot recently has emerged as a problem, pathotype 3H also was found to be common (Strelkov et al. 2020a).

Despite the high prevalence of pathotype 3A, there was significant diversity in the virulence of the *P. brassicae* isolates, consistent with other recent reports from western Canada (Strelkov et al. 2018a; Askarian et al. 2020). Seventeen pathotypes could be distinguished on the CCD set, nine of which had not been reported previously. In Alberta, 10 pathotypes were identified among 146 isolates, with all except pathotypes 3A, 3D and 3H recovered from just one or two fields. As noted above, pathotype 3H was most common in Saskatchewan, with the only other pathotype identified, 5L, also found in Manitoba but not Alberta. The pathotype composition was most diverse in Manitoba, where nine different pathotypes were identified from just 10 isolates. Six of these pathotypes were found only in that province, including pathotypes 2C, 6D, 8D, 9B, 13A and 13B. Despite the limited number of samples tested, these results suggest a fairly distinct virulence profile for *P. brassicae* populations in Manitoba. A preliminary analysis of the pathotype composition in North Dakota also indicated the presence of pathotypes 2C and 8D (Chapara & Strelkov 2020), suggesting some similarity with the virulence of *P. brassicae* populations from Manitoba. Nonetheless, pathotype 3A was also identified from one field in Manitoba, the first time that a resistance-breaking pathotype has been detected on the Prairies outside of Alberta.

While the CCD set exhibits greater differentiating capacity among *P. brassicae* populations from Canada than the differentials of Williams (1966) or Somé et al. (1996) (Strelkov et al. 2018a; Askarian et al. 2020), the flux of 'new' virulence phenotypes associated with the introduction of CR canola is still detectable to some extent with the latter two systems. Pathotypes P_4 and P_5, as defined on the hosts of Somé et al., are reported here for the first time. Pathotype P_1, while recently

identified in a collection of single-spore isolates from Alberta (Askarian et al. 2020), is also reported here for the first time among field isolates of the pathogen. Similarly, this is the first report of variants of Williams' pathotypes 9, 11, and 13 from Canada, complementing the recent identification of pathotypes 4 and 7 among single-spore isolates (Askarian et al. 2020). Prior to the introduction of CR canola, only Williams' pathotypes 1, 2, 3, 5, 6 and 8 had been recorded in Canada (reviewed in Strelkov & Hwang 2014). Despite this apparent proliferation of pathotypes, there is genetic evidence to suggest that most of these 'new' pathotypes are not in fact new, and were present as minor components of *P. brassicae* populations before the cultivation of CR canola (Sedaghatkish et al. 2019; Strelkov et al. 2019).

The current analysis identified 131 isolates of *P. brassicae*, representing 130 fields in Alberta and one in Manitoba, able to overcome clubroot resistance in canola. This is consistent with an increasing trend in the number of fields where there has been a documented loss or erosion of resistance, starting with two fields in 2013 (Strelkov et al. 2016), an additional 61 fields from 2014 to 2016, and 133 fields in 2017 and 2018 (this study and Strelkov et al. 2020a). The resistance in most of these canola cultivars, which as noted earlier appears to be derived from the oilseed rape 'Mendel' (Fredua-Agyeman et al. 2018), is now often referred to as '1st generation' resistance (Canola Watch 2019). In recent years, as this resistance has come under pressure from the emergence of 'new' pathotypes of *P. brassicae*, there has been an effort to develop canola with novel resistance traits. This has resulted in the release of multiple cultivars possessing so-called '2nd generation' resistance (Canola Watch 2019). While reportedly different from 1st generation resistance, the genetic basis of 2nd generation resistance is not in the public domain, and may differ across cultivars. Nonetheless, it will important to develop an understanding of the virulence of *P. brassicae* pathotypes on hosts with 2nd generation resistance.

Since genetic resistance is the most widely used clubroot management tool by growers, the emergence and spread of resistance-breaking pathotypes of *P. brassicae* poses a particularly significant threat to canola production. It will be difficult for breeders to screen for resistance against all of the newly detected strains of the clubroot pathogen, and they will likely have to focus their efforts on the predominant and/or most virulent pathotypes. In fields infested with unique or very rare pathotypes, genetically resistant canola cultivars may not be an option for clubroot control. Moreover, in order to preserve genetic sources of resistance, proper stewardship, including longer rotations out of host crops and diligent scouting, will become increasingly important for the long-term management of clubroot.

Table 3.1 Pathotype classification of *Plasmodiophora brassicae* field isolates collected in 2017 and 2018 from Alberta, Saskatchewan and Manitoba.

	Number of isolates (year(s) found)																
	1 (2017)	81 (2017, 2018)	40 (2017, 2018)	24 (2017, 2018)	3 (2018)	1 (2018)	1 (2018)	1 (2018)	1 (2018)	2 (2017, 2018)	3 (2017, 2018)	3 (2017, 2018)	1 (2017)	1 (2018)	1 (2017)	1 (2017)	1 (2018)
	Pathotype designation[a,b]																
CCD	**2C**	3A	3D	3H	5L	5X	**6D**	**8D**	8E	8N	8P	**9A**	**9B**	**9C**	**11A**	**13A**	**13B**
Williams	2	3	3	3	5	5	6	8	8	8	8	9	9	9	**11**	**13**	**13**
Somé et al.	P_3	P_2	P_2	P_2	P_3	P_3	P_4	P_3	P_2	P_2	P_2	P_1	**P_5**	P_1	P_1	P_3	P_3
Differential Host[c]	**Reaction[d]**																
ECD 02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ECD 05	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ECD 06	-	+	+	+	-	-	-	-	+	+	+	+	-	+	+	-	-
ECD 08	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
ECD 09	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	-
ECD 10	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
ECD 11	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ECD 13	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	+
'Brutor'	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
'Laurentian'	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
'Mendel'	-	+	-	-	-	+	-	-	-	-	+	+	-	-	+	-	-
'Westar'	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
'45H29'	-	+	+	-	-	+	-	-	+	-	+	+	-	+	+	-	-

[a]Pathotype designations are based on the systems of the Canadian Clubroot Differential (CCD) Set, Williams (1966) and Somé et al. (1996); ECD 10, 11 and 13 are included in the differentials of Williams (1966), along with 'Laurentian', while the differentials of Somé et al. (1996) consist of ECD 06, 'Laurentian' and 'Brutor'. All hosts designated ECD are also members of the European Clubroot Differential Set (Buczacki et al. 1975), but ECD pathotype designations could not be obtained since not all ECD differentials were included in the test.

[b]Pathotypes in bold are reported for the first time in this study.

[c]European Clubroot Differential (ECD) 02 = *Brassica rapa* ssp. *rapifera* line AAbbCC; ECD 05 = *B. rapa* var. *pekinensis* 'Granaat'; ECD 06 = *Brassica napus* 'Nevin'; ECD 08 = *B. napus* 'Giant Rape' selection; ECD 09 = *B. napus* New Zealand resistant rape; ECD 10 = *B. napus* var. *napobrassica* 'Wilhemsburger'; ECD 11 = *Brassica oleracea* var. *capitata* 'Badger Shipper'; ECD 13 = 'Jersey Queen'; Brutor = *B. napus* 'Brutor'; 'Laurentian' = *B. napus* var. *napobrassica* 'Laurentian'; 'Mendel' = *B. napus* 'Mendel'; 'Westar' = *B. napus* 'Westar'; and 45H29 = *B. napus* '45H29'.
[d]Plus (+) and minus (-) signs denote susceptible and resistant host reactions, respectively. A host was considered resistant if the mean index of disease was <50% and its associated 95% confidence interval did not overlap 50%

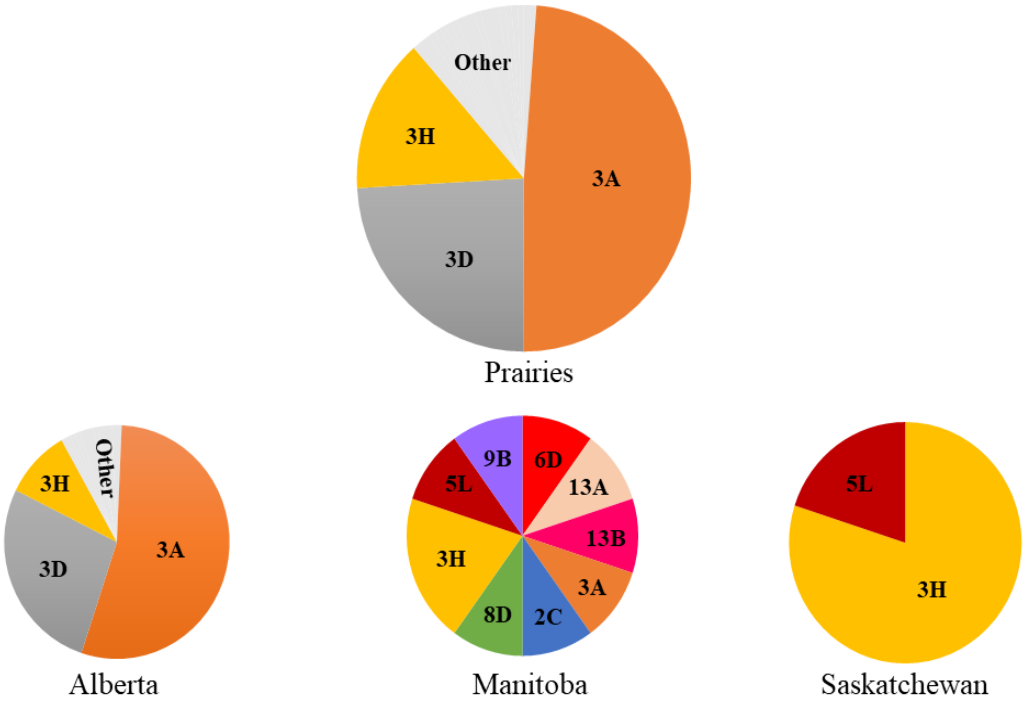
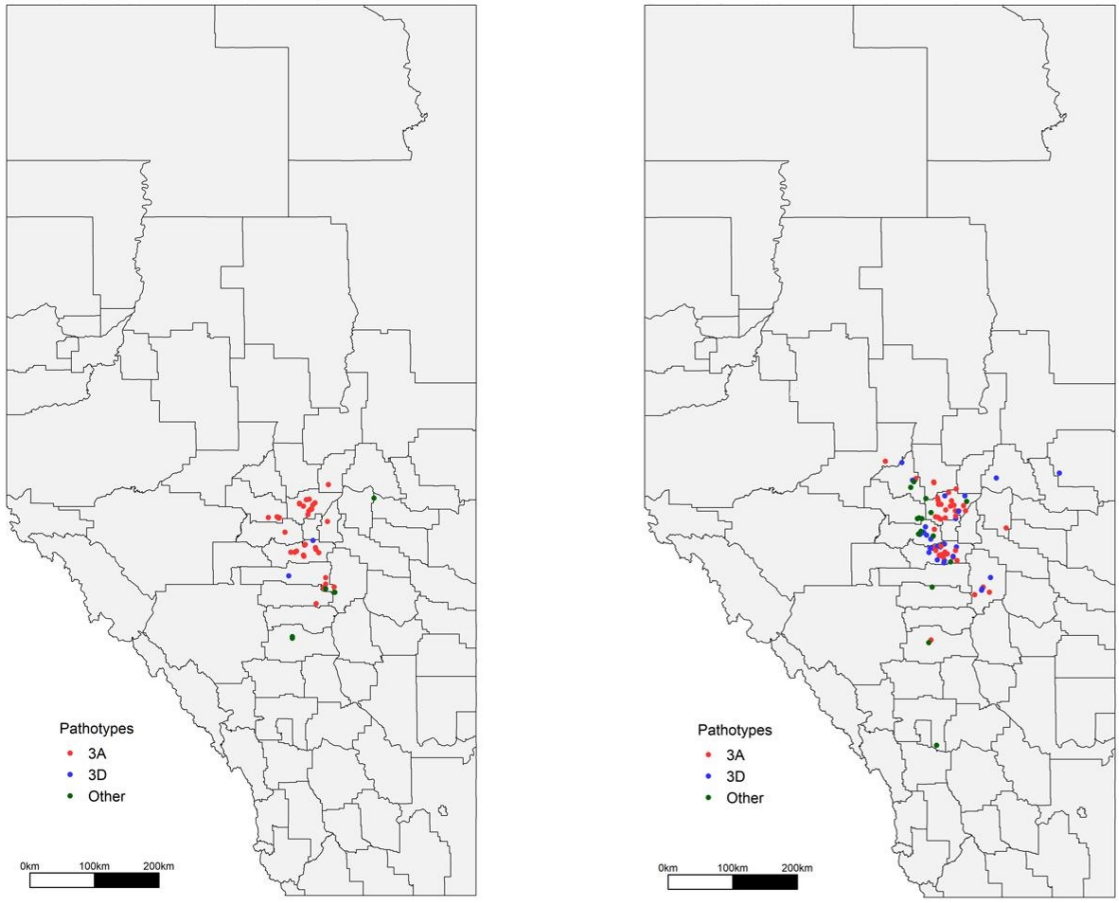


Fig. 3.1 Prevalence of *Plasmodiophora brassicae* pathotypes across the Canadian Prairies and in the individual provinces of Alberta, Manitoba, and Saskatchewan, based on collections made from canola crops in 2017 and 2018. One hundred sixty-six *P. brassicae* field isolates were tested, including 146 from Alberta, and 10 each from Manitoba and Saskatchewan. Pathotype classifications are according to the Canadian Clubroot Differential set.



2017 **2018**

Fig. 3.2 Distribution of resistance-breaking pathotypes identified among *Plasmodiophora brassicae* field isolates collected from clubroot resistant canola crops in Alberta in 2017 and 2018. Pathotype classifications are according to the Canadian Clubroot Differential set. The pathotype distribution in Saskatchewan and Manitoba is not shown, given the limited number of samples from each of those provinces.

Supplementary Table 3.1 Origin of *Plasmodiophora brassicae* field isolates collected in Alberta, Canada, in 2017 and 2018, from galls of clubroot resistant canola plants. Each isolate represents a different field.

Year Collected	Population	Host[a]	Origin[b]	Pathotype[c]
2017	F.3-17	L241C	Wetaskiwin	3D
	F.112-17	L241C	Wetaskiwin	3A
	F.113-17	L241C	Wetaskiwin	9A
	F.118-17	L135C	Red Deer	9A
	F.119-17	L135C	Red Deer	11A
	F.203-17	75-42CR	Two Hills	8P
	F.253-17	L241C	Lac Ste. Anne	3A
	F.254-17	1020RR	Lac Ste. Anne	3A
	F.261-17	L241C	Lac Ste. Anne	3A
	Strathcona-1-17	L135C	Strathcona	3A
	Camrose-2-17	45CS40	Camrose	3A
	C-1-17	VR9562GC	Ponoka	3A
	C-2-17	L241C	Sturgeon	3A
	C-3-17	L241C	Sturgeon	3A
	PF-1-17	L241C	Wetaskiwin	3A
	PF-2-17	L241C	Wetaskiwin	3A
	P-1-17	45CS40	Sturgeon	3A

P-2-17	75-42CR	Sturgeon	3A
P-3-17	Unknown	Sturgeon	8N
S-1-17	L241C	Westlock	3A
S-2-17	L241C	Parkland	3A
S-3-17	CS2000	Sturgeon	3A
S-4-17	L241C	Sturgeon	3A
S-5-17	L241C	Sturgeon	3A
S-6-17	L241C	Sturgeon	3A
EA-1-17	45CS40	Sturgeon	3A
EA-2-17	45H33	Sturgeon	3A
EA-3-17	45CS40	Sturgeon	3A
EA-4-17	45CS40	Lac Ste. Anne	3A
Leduc-1-17	45CS40	Leduc	3A
Leduc-2-17	45H29	Leduc	3A
Leduc-3-17	45H33	Leduc	3A
Leduc-4-17	L241C	Leduc	3D
Leduc-5-17	L241C	Leduc	3A
Leduc-6-17	45CS40	Leduc	3A
Leduc-8-17	CS2000	Leduc	3A
Leduc-9-17	L241C	Leduc	3A

	Leduc-10-17	L135C	Leduc	3A
	Leduc-11-17	L135C	Leduc	3A
	Leduc-12-17	45H29	Leduc	3A
	Greenview-1-17	Unknown	Greenview	3H
	Greenview-2-17	Unknown	Greenview	3H
	Greenview-3-17	Unknown	Greenview	3H
	Wainwright-9-17	Unknown	Wainwright	3H
2018	N. Sunrise c. 1-18	45H29	Northern Sunrise	3H
	CDCN-2-18	L241C	Sturgeon	3A
	CDCN-3-18	75-42CR	Sturgeon	3D
	CDCN-4-18	L241C	Sturgeon	3D
	LSAC-4-18	45H29	Lac Ste. Anne	3H
	LSAC-5-18	45CS40	Lac Ste. Anne	3H
	LSAC-6-18	L241C	Lac Ste. Anne	3H
	C-1-18	V14-1	Leduc	3A
	Lamont c.1-18	45CM36	Lamont	3H
	Lamont c.2-18	PV581GC	Lamont	3A
	F.P.-1-18 (Westlock)	75-42CR, 45H33, 45CM36, 45CS40	Westlock	3A
	F.P.-5-18 (C.C.)	45H29	Parkland	3A

F.P.-6-18 (Woodland)	45CS40	Woodlands	3A
F.P.-8-18	45H29	City of Edmonton	3D
F.P.-25-18 (C.C.)	L241C	Westlock	3A
F.P.-26-18 (C.C.)	L241C	Smoky Lake	3D
F.P.-73-18	Susceptible	Rocky View	9C
F.P.-91-18	L157H	Bonnyville	3D
F.P.-144-18	L241C	Red Deer	3A
Parkland c.-1-18	L241C	Parkland	3D
Parkland c.-2-18 (F.P.-14-18)	CS2000	Parkland	3D
Parkland c.-3-18 (F.P.-16-18)	PV581GC	Parkland	3D
Parkland c.-4-18 (F.P.-17-18)	PV581GC	Parkland	3D
Parkland c.-5-18 (F.P.-18-18)	45H33	Parkland	3D
Parkland c.-6-18 (F.P.-22-18)	45H29	Parkland	3D
Parkland c.-7-18 (F.P.-23-18)	45CS40	Parkland	3D
Parkland c.-8-18 (F.P.-15-18)	PV581GC	Parkland	3D
C.C.-1-18	L255PC	Sturgeon	3A
C.C.-2-18	L241C	Sturgeon	3A

C.C.-3-18	CS2000	Red Deer	9A
Camrose c.-1-18	L241C	Camrose	3H
Camrose c.-2-18	L241C	Camrose	3A
Camrose c.-3-18	L241C	Camrose	3A
Camrose c.-4-18 (P-1-18)	45H33	Camrose	3D
Camrose c.-5-18	L241C	Camrose	3D
Camrose c.-6-18	45H33	Camrose	3H
Camrose c.-7-18	CS2000	Camrose	3A
Camrose c.-8-18	CS2000	Camrose	3D
S-1-18	L241C	Sturgeon	8E
S-2-18	75-42CR	Sturgeon	3A
S-3-18	L241C	Sturgeon	3A
S-4-18	L255PC	Sturgeon	3A
S-5-18	75-42CR	Sturgeon	3A
S-6-18	6076CR	City of Edmonton	3H
S-7-18	L255PC	Sturgeon	3A
S-8-18	L241C	Sturgeon	3A
S-9-18	L241C	Sturgeon	3A
S-10-18	L255PC	Sturgeon	3A
S-11-18	CS2000	Sturgeon	5X

S-12-18	L241C	City of Edmonton	3A
S-13-18	45H29	Sturgeon	3A
S-14-18	L241C	Sturgeon	3A
S-15-18	L241C	Sturgeon	3A
S-16-18	L241C	Lac Ste. Anne	3H
S-17-18	L241C	Lac Ste. Anne	3D
S-18-18	L241C	Lac Ste. Anne	3A
S-19-18	6076CR	Westlock	3H
S-20-18	75-42CR	Westlock	3A
S-21-18	45CS40	Sturgeon	3A
S-22-18	1026RR	Sturgeon	3A
S-23-18	L255PC	Sturgeon	3D
S-24-18	L241C	Sturgeon	3A
S-25-18	1026RR	Sturgeon	3A
S-26-18	1026RR	Sturgeon	3A
F.P.-4-18 (Leduc c1-18)	75-42CR	Leduc	3D
Leduc c.1-18	75-42CR	Leduc	3A
Leduc c.3-18	L255PC	Leduc	3D
Leduc c.4-18	45CS40	Leduc	3D

Leduc c.5-18	CS2000	Leduc	3D
Leduc c.6-18	45H29	Leduc	3D
Leduc c.7-18	6076CR	Leduc	3A
Leduc c.8-18	45CS40	Leduc	3A
Leduc c.9-18	L255PC	Leduc	3A
Leduc c.10-18	CS2000	Leduc	3D
Leduc c.11-18	PV581GC	Leduc	3D
Leduc c.12-18	L241C	Leduc	3A
Leduc c.13-18	45H29	Leduc	3A
Leduc c.14-18	45CS40	Leduc	3A
Leduc c.15-18	1026RR	Leduc	3A
Leduc c.16-18	75-42CR	Leduc	3A
Leduc c.17-18	CS2000	Leduc	3A
Leduc c.18-18	L231C	Leduc	3A
Leduc c.19-18	PV581GC	Leduc	3A
Leduc c.20-18	L255PC	Leduc	3A
Leduc c.21-18	75-42CR	Leduc	3D
Leduc c.22-18	L241C	Leduc	3D
Leduc c.23-18	45H29	Leduc	3D
Leduc c.24-18	L255PC	Leduc	3D

D-1-18 (Leduc c.25-18)	45H29	Leduc	3D
D-2-18 (Leduc c.25-18)	45CM36	Leduc	3D
D-3-18 (Leduc c.25-18)	CS2000	Leduc	3D
D-4-18 (F.P.-5-18; C.C.)	6066CR	Leduc	3A
Henwood (C. of Edmonton)	45H29	City of Edmonton	3D
U of A-1-18	45H29	City of Edmonton	3D
U of A-2-18	45H29	City of Edmonton	3D
Barrhead c.-1-18	D3155C	Barrhead	3D
Barrhead c.-2-18 (F.P.-28-18)	45CS40	Barrhead	3D
Barrhead c.-3-18 (F.P.-29-18)	45H29	Barrhead	8N
Barrhead c.-4-18 (F.p.-30-18)	45CS40	Barrhead	3A
Barrhead c.-5-18	L241C	Barrhead	3D
Barrhead c.-6-18	45H29	Barrhead	8P
Barrhead c.-7-18	45CS40	Barrhead	8P

[a]The hosts represent the canola cultivars from which each *P. brassicae* isolate was obtained; unless listed as 'susceptible', all were considered resistant to isolates of the pathogen collected prior to 2013.
[b]Names listed refer to counties or municipal districts with the exception of the City of Edmonton.
[c]Pathotype designations are based on the Canadian Clubroot Differential set.

Supplementary Table 3.2 Origin of *Plasmodiophora brassicae* field isolates collected in Manitoba and Saskatchewan, Canada, in 2017 and 2018, from galls found on canola roots. Each isolate represents a different field.

Year Collected	Population	Host[a]	Origin[b]	Pathotype[c]
2017	MB-SR-1-17	Unknown	Swan River, MB	9B
	MB-SR-2-17	Unknown	Swan River, MB	13A
	MB-11-17	Unknown	Manitoba, MB	2C
2018	MB-1-18	Unknown	Carman, MB	13B
	MB-2-18	Unknown	Pembina, MB	3A
	MB-3-18	Unknown	Pembina, MB	8D
	MB-4-18	Unknown	Louise, MB	3H
	MB-5-18	Unknown	Louise, MB	3H
	MB-6-18	Unknown	Louise, MB	5L
	D-5-18	Unknown	Winnipeg, MB	6D
	SK-2018-DB-1	Unknown	Saskatchewan	3H
	SK-2018-DB-2	Unknown	Saskatchewan	3H
	SK-2018-DB-3	Unknown	Saskatchewan	3H
	SK-2018-DB-4	Unknown	Saskatchewan	3H
	SK-2018-DM-1B	Unknown	Saskatchewan	3H
	SK-2018-CN-2	Unknown	Saskatchewan	3H
	SK-2018-CP-62	Unknown	Saskatchewan	3H

SK-2018-CW-1	Unknown	Saskatchewan	5L
SK-2018-LLH-3	Unknown	Saskatchewan	3H
SK-2018-RA-56	Unknown	Saskatchewan	5L

[a]The canola cultivars from which each *P. brassicae* isolate was obtained was either not known or not provided with the samples.
[b]For the samples from Manitoba (MB), the names listed refer to municipalities with the exception of the City of Winnipeg; no municipality or regional information was provided with the samples from Saskatchewan.
[c]Pathotype designations are based on the Canadian Clubroot Differential set.

Supplementary Table 3.3 Reaction of Brassica host differential genotypes to inoculation with *Plasmodiophora brassicae* field isolates collected from the Canadian Prairies, in 2017 and 2018.

	Host Genotype[a,b]												
Isolate	ECD 02	ECD 05	ECD 06	ECD 08	ECD 09	ECD 10	ECD 11	ECD 13	Brutor	Laur.	Mendel	Westar	45H29
2017													
F.3-17	0.0	100.0	78.7	95.6	83.0	19.8	10.5	58.3	100.0	83.0	36.1	100.0	100.0
		±0.0	±2.57	±1.66	±1.75	±5.52	±3.89	±7.96	±0.0	±2.34	±5.88	±0.0	±0.0
F.112-17	0.0	100.0	85.6	100.0	87.3	24.9	5.7	70.2	100.0	92.2	50.7	100.0	100.0
		±0.0	±2.57	±0.0	±3.24	±4.89	±2.13	±3.41	±0.0	±2.70	±7.16	±0.0	±0.0
F.113-17	0.0	100.0	95.8	57.8	22.7	100.0	0.0	38.4	100.0	100.0	85.6	100.0	100.0
		±0.0	±3.51	±3.99	±2.74	±0.0		±5.09	±0.0	±0.0	±4.52	±0.0	±0.0
F.118-17	0.7	100.0	100.0	50.0	22.5	100.0	0.0	39.8	100.0	100.0	84.3	100.0	100.0
	±1.36	±0.0	±0.0	±1.21	±1.25	±0.0		±4.77	±0.0	±0.0	±4.22	±0.0	±0.0
F.119-17	2.1	100.0	97.9	96.5	89.5	96.5	3.5	63.9	100.0	100.0	88.0	100.0	100.0
	±2.61	±0.0	±4.08	±3.41	±4.48	±5.15	±1.32	±8.01	±0.0	±0.0	±3.19	±0.0	±0.0
F.203-17	0.7	100.0	87.3	97.9	89.2	29.0	9.7	29.0	88.6	80.9	52.8	88.4	86.6
	±1.36	±0.0	±3.24	±2.61	±5.43	±1.73	±3.51	±1.73	±4.66	±4.25	±5.88	±4.44	±5.08
F.253-17	13.7	100.0	82.6	97.2	94.9	18.4	15.0	85.5	100.0	82.0	70.1	100.0	100.0
	±3.21	±0.0	±4.65	±2.23	±4.30	±1.29	±4.39	±1.89	±0.0	±5.23	±7.16	±0.0	±0.0
F.254-17	2.1	100.0	82.3	100.0	86.9	11.2	8.5	82.5	100.0	84.4	67.4	100.0	100.0
	±4.08	±0.0	±1.32	±0.0	±4.98	±4.25	±3.01	±7.10	±0.0	±3.41	±3.43	±0.0	±0.0
F.261-17	2.1	100.0	74.6	100.0	85.8	19.3	7.8	59.8	100.0	65.3	51.4	100.0	100.0
	±1.41	±0.0	±6.24	±0.0	±2.31	±4.71	±2.49	±7.31	±0.0	±4.79	±1.57	±0.0	±0.0
Strathcona-1-17	2.1	100.0	100.0	100.0	100.0	46.5	43.1	92.1	100.0	100.0	95.1	100.0	100.0
	±4.08	±0.0	±0.0	±0.0	±0.0	±2.31	±2.31	±3.38	±0.0	±0.0	±2.31	±0.0	±0.0
Camrose-2-17	2.1	100.0	96.5	98.5	99.3	31.1	13.2	78.0	100.0	100.0	88.9	100.0	100.0
	±2.63	±0.0	±3.41	±1.65	±1.36	±6.29	±4.65	±3.88	±0.0	±0.0	±2.22	±0.0	±0.0
C-1-17	2.1	100.0	97.9	98.6	95.8	31.3	19.9	89.5	98.6	96.5	75.7	100.0	100.0

	±4.08	±0.0	±4.08	±2.72	±3.51	±4.65	±2.39	±5.03	±2.72	±4.08	±4.08	±0.0	±0.0
C-2-17	1.4	100.0	97.9	100.0	100.0	46.8	43.1	86	100.0	100.0	97.9	100.0	100.0
	±2.72	±0.0	±4.08	±0.0	±0.0	±1.67	±4.71	±6.84	±0.0	±0.0	±1.41	±0.0	±0.0
C-3-17	1.4	100.0	97.9	100.0	100.0	22.2	29.2	100	100.0	100.0	86.8	100.0	100.0
	±2.72	±0.0	±4.08	±0.0	±0.0	±2.22	±5.21	±0.0	±0.0	±0.0	±1.36	±0.0	±0.0
PF-1-17	5.7	100.0	97.9	100.0	100.0	40.3	31.3	90.7	100.0	100.0	95.8	100.0	100.0
	±3.86	±0.0	±4.08	±0.0	±0.0	±5.67	±4.65	±3.81	±0.0	±0.0	±1.50	±0.0	±0.0
PF-2-17	3.6	100.0	95.8	100.0	100.0	32.6	50.0	100.0	100.0	100.0	95.1	100.0	100.0
	±2.74	±0.0	±4.71	±0.0	±0.0	±3.43	±2.22	±0.0	±0.0	±0.0	±2.54	±0.0	±0.0
P-1-17	1.4	100.0	95.8	100.0	100.0	22.0	31.3	79.2	100.0	100.0	82.6	100.0	100.0
	±1.57	±0.0	±4.71	±0.0	±0.0	±2.28	±2.61	±2.72	±0.0	±0.0	±5.15	±0.0	±0.0
P-2-17	0.0	100.0	95.6	100.0	98.5	39.6	42.4	90.2	100.0	100.0	91.6	100.0	100.0
		±0.0	±3.81	±0.0	±1.65	±4.65	±4.08	±6.39	±0.0	±0.0	±4.34	±0.0	±0.0
P-3-17	0.0	100.0	71.5	82.6	82.6	9.7	2.1	40.3	91.0	69.4	6.4	86.1	6.3
		±0.0	±4.65	±2.61	±2.61	±3.51	±4.08	±8.16	±2.61	±2.22	±7.89	±6.67	±7.82
S-1-17	0.0	100.0	95.6	100.00	97.9	31.3	22.2	83.3	100.00	100.00	72.2	100.0	100.0
		±0.0	±4.94	±0.0	±4.08	±1.36	±4.97	±6.67	±0.0	±0.0	±5.88	±0.0	±0.0
S-2-17	0.0	100.0	85.9	91.6	86.5	29.0	14.8	50.7	100.0	80.9	61.7	100.0	100.0
		±0.0	±2.89	±1.65	±2.78	±6.64	±4.33	±1.36	±0.0	±4.25	±14.57	±0.0	±0.0
S-3-17	0.0	100.0	88.0	100.0	87.9	11.6	14.1	65.3	100.0	86.8	50.00	100.0	100.0
		±0.0	±3.89	±0.0	±3.40	±3.95	±2.89	±10.1	±0.0	±2.61	±2.22	±0.0	±0.0
S-4-17	0.0	100.0	92.0	100.0	98.5	46.1	8.0	62.2	100.0	100.0	58.3	100.0	100.0
		±0.0	±2.57	±0.0	±1.65	±2.99	±2.57	±6.92	±0.0	±0.0	±8.01	±0.0	±0.0
S-5-17	0.0	100.0	87.5	100.0	86.6	29.2	14.1	70.8	100.0	89.4	54.6	100.0	100.0
		±0.0	±2.72	±0.0	±3.95	±3.51	±1.84	±3.51	±0.0	±3.97	±10.01	±0.0	±0.0
S-6-17	0.0	100.0	75.1	100.0	78.6	5.7	12.8	55.6	100.0	91.1	50.7	100.0	100.0
		±0.0	±2.03	±0.0	±4.43	±2.13	±1.35	±7.70	±0.0	±2.15	±1.36	±0.0	±0.0
EA-1-17	5.7	100.0	96.5	100.0	100.0	36.7	13.4	79.2	100.0	100.0	95.1	100.0	100.0

	±2.13	±0.0	±4.08	±0.0	±0.0	±4.76	±3.80	±8.46	±0.0	±0.0	±2.54	±0.0	±0.0
EA-2-17	0.7	100.0	97.9	100.0	100.0	23.4	28.5	94.9	100.0	100.0	91.5	100.0	100.0
	±1.36	±0.0	±4.08	±0.0	±0.0	±2.62	±2.61	±2.60	±0.0	±0.0	±2.25	±0.0	±0.0
EA-3-17	0.0	100.0	90.8	100.0	90.2	36.8	11.6	56.3	100.0	100.0	57.6	100.0	100.0
		±0.0	±2.57	±0.0	±3.33	±5.61	±1.96	±8.99	±0.0	±0.0	±6.81	±0.0	±0.0
EA-4-17	2.1	100.0	70.1	96.4	84.6	20.8	6.4	84.7	100.0	78.8	59.5	100.0	100.0
	±4.08	±0.0	±4.65	±4.17	±8.86	±4.71	±1.30	±5.21	±0.0	±2.98	±6.70	±0.0	±0.0
Leduc-1-17	0.0	100.0	87.3	100.0	90.0	17.6	12.6	59.0	100.0	81.7	66.7	100.0	100.0
		±0.0	±3.24	±0.0	±3.24	±4.22	±4.48	±3.31	±0.0	±5.28	±2.22	±0.0	±0.0
Leduc-2-17	5.7	100.0	63.2	91.5	81.6	13.4	12.1	51.4	100.0	63.0	56.9	100.0	100.0
	±4.04	±0.0	±9.53	±6.68	±1.29	±2.39	±2.98	±1.57	±0.0	±3.77	±6.48	±0.0	±0.0
Leduc-3-17	7	100.0	77.3	100.0	83.4	26.8	13.9	73.7	100.0	87.2	68.1	100.0	100.0
	±2.60	±0.0	±2.43	±0.0	±1.98	±6.31	±4.97	±6.82	±0.0	±2.60	±3.55	±0.0	±0.0
Leduc-4-17	0.0	100.0	69.7	88.7	90.0	26.2	21.8	70.9	97.2	81.7	40.7	100.0	97.9
		±0.0	±14.65	±1.93	±4.51	±4.80	±6.69	±10.33	±3.85	±5.28	±6.13	±0.0	±2.61
Leduc-5-17	1.4	100.0	75.1	100.0	97.9	10.6	10.1	82.4	100.0	92.2	58.3	100.0	97.9
	±2.72	±0.0	±2.03	±0.0	±4.08	±2.43	±2.50	±5.26	±0.0	±2.49	±5.88	±0.0	±1.41
Leduc-6-17	0.7	100.0	83.4	100.0	85.8	13.3	11.3	69.4	100.0	84.3	59.7	100.0	100.0
	±1.36	±0.0	±3.13	±0.0	±2.31	±6.62	±1.93	±5.88	±0.0	±3.77	±5.67	±0.0	±0.0
Leduc-8-17	3.5	100.0	81.7	100.0	94.2	24.3	18.4	80.7	100.0	96.3	66.7	100.0	100.0
	±1.32	±0.0	±5.28	±0.0	±3.30	±6.04	±3.06	±8.46	±0.0	±2.72	±4.97	±0.0	±0.0
Leduc-9-17	7.8	100.0	72.9	97.0	88.0	14.5	8.5	62.5	97.9	71.6	64.5	100.0	97.9
	±1.53	±0.0	±6.04	±2.43	±3.19	±1.89	±3.01	±3.51	±2.61	±2.54	±9.63	±0.0	±1.41
Leduc-10-17	3.5	100.0	83.1	100.0	93.5	20.1	8.6	59.0	100.0	79.9	68.1	100.0	100.0
	±4.08	±0.0	±6.37	±0.0	±2.71	±4.65	±2.04	±11.63	±0.0	±4.08	±8.46	±0.0	±0.0
Leduc-11-17	2.8	100.0	88.7	100.0	100.0	8.5	26.4	90.3	100.0	100.0	84.3	100.0	100.0
	±2.23	±0.0	±3.69	±0.0	±0.0	±2.03	±2.72	±4.71	±0.0	±0.0	±6.89	±0.0	±0.0
Leduc-12-17	0.0	100.0	76.8	100.0	81.1	9.8	8.5	51.1	100.0	63.9	50.8	100.0	100.0

		±0.0	±5.43	±0.0	±1.89	±3.33	±2.25	±1.32	±0.0	±2.22	±2.38	±0.0	±0.0
Greenview-1-17	0.0	100.0	91.5	96.4	98.5	26.4	19.9	75.7	100.0	97.9	7.8	100.0	10.6
		±0.0	±4.79	±2.74	±1.65	±3.51	±6.72	±7.82	±0.0	±2.61	±1.53	±0.0	±3.9
Greenview-2-17	0.0	100.0	95.6	100.0	100.0	36.8	27.1	77.2	100.0	100.0	8.6	100.0	11.6
		±0.0	±4.94	±0.0	±0.0	±8.43	±4.65	±7.05	±0.0	±0.0	±3.75	±0.0	±5.37
Greenview-3-17	0.0	100.0	95.1	100.0	100.0	38.9	31.8	94.9	100.0	100.0	21.2	100.0	12.7
		±0.0	±4.08	±0.0	±0.0	±3.85	±3.71	±1.34	±0.0	±0.0	±4.33	±0.0	±4.51
MB-SR-1-17	0.0	94.4	0.0	88.01	0.0	79.42	6.31	38.19	82.5	84.9	6.65	95.1	10.6
		±1.13		±4.9		±1.74	±1.28	±5.12	±4.62	±2.19	±0.6	±1.3	±2.03
MB-SR-2-17	0	95.1	1.39	90.28	0	48.17	7.27	54.9	90.8	91.5	1.39	95.0	10.0
		±1.3	±1.39	±1.79		±4.39	±1.33	±8.62	±1.73	±1.15	±1.39	±1.77	±2.3
MB-11-17	0.69	95.8	2.08	4.17	8.52	2.08	84.28	100	100.0	91.7	7.41	100.0	5.7
	±0.69	±1.79	±2.08	±2.41	±3.41	±2.08	±3.51	±0.0	±0.0	±1.13	±0.8	±0.0	2.1
Wainwright-9-17	2.08	100.0	69.44	100	90.59	14.46	34.03	75	100.0	97.2	5.74	100.0	8.5
	±2.08	±0.0	±1.6	±0.0	±1.37	±2.0	±4.73	±1.13	±0.0	±1.6	±2.06	±0.0	±3.41
2018													
N. Sunrise c. 1-18	0.0	97.9	89.6	97.9	97.9	20.4	3.5	72.5	97.9	88.6	4.4	98.5	2.1
		±2.61	±7.82	±2.61	±2.61	±5.55	±3.41	±6.46	±2.63	±3.54	±4.94	±1.65	±4.08
CDCN-2-18	0.0	100.0	85.2	100.0	91.5	12.0	15.5	60.6	100.0	92.1	50.4	100.0	100.0
		±0.00	±7.63	±0.00	±6.68	±2.29	±3.15	±2.57	±0.00	±4.38	±0.74	±0.00	±0.00
CDCN-3-18	0.0	97.9	80.9	95.6	84.8	4.4	2.8	51.5	95.8	85.2	20.1	95.8	97.9
		±2.63	±5.91	±3.80	±4.02	±1.66	±2.23	±2.10	±3.49	±2.19	±1.36	±3.51	±2.61
CDCN-4-18	0.0	97.9	97.9	97.9	94.4	6.4	2.1	65.9	97.2	92.8	19.4	97.2	97.9
		±2.61	±4.08	±2.61	±3.07	±1.30	±2.61	±2.67	±2.23	±3.53	±5.30	±2.23	±2.61
LSAT-4-18	0.0	100.0	89.4	100.0	92.9	12.7	1.5	54.7	100.0	95.8	7.8	100.0	6.4
		±0.00	±3.97	±0.00	±3.36	±3.24	±1.65	±5.91	±0.00	±3.49	±6.76	±0.00	±7.89
LSAT-5-18	0.0	100.0	95.6	100.0	94.3	28.3	11.6	79.8	100.0	100.0	6.4	100.0	19.1
		±0.00	±4.94	±0.00	±4.04	±2.94	±1.96	±4.01	±0.00	±0.00	±4.22	±0.00	±3.90

LSAT-6-18	1.4	100.0	95.8	100.0	97.9	13.6	7.9	63.8	100.0	100.0	4.4	100.0	13.1
	±2.72	±0.00	±3.48	±0.00	±4.08	±2.69	±2.35	±3.85	±0.00	±0.00	±4.94	±0.00	±6.00
C-1-18	0.0	100.0	89.4	100.0	100.0	12.8	17.4	72.0	100.0	100.0	55.6	100.0	100.0
		±0.00	±3.97	±0.00	±0.00	±1.35	±1.36	±8.31	±0.00	±0.00	±4.14	±0.00	±0.00
Lamont c.1-18	0.0	100.0	89.0	100.0	93.7	17.0	3.7	61.6	100.0	90.0	4.2	100.0	4.2
		±0.00	±3.75	±0.00	±4.02	±2.34	±1.20	±4.30	±0.00	±2.92	±4.71	±0.00	±4.71
Lamont c.2-18	0.0	100.0	100.0	100.0	100.0	16.0	12.8	73.2	100.0	100.0	76.4	100.0	100.0
		±0.00	±0.00	±0.00	±0.00	±5.00	±1.35	±8.03	±0.00	±0.00	±7.86	±0.00	±0.00
F.P.-1-18 (Westlock)	0.7	100.0	91.0	100.0	94.2	22.6	10.5	63.9	100.0	87.4	65.8	100.0	99.3
	±1.36	±0.00	±6.80	±0.00	±5.07	±5.83	±9.45	±5.44	±0.00	±11.73	±12.68	±0.00	±1.36
F.P.-5-18 (C.C.)	0.0	95.0	62.5	82.6	82.1	2.1	5.1	78.7	85.8	83.0	55.4	86.9	94.3
		±3.47	±5.21	±1.55	±1.55	±2.61	±1.34	±3.40	±3.90	±5.72	±3.69	±5.72	±4.04
F.P.-6-18 (Woodland)	0.7	97.9	98.6	91.5	97.9	12.1	31.3	78.7	95.6	80.1	92.8	97.9	97.9
	±1.36	±2.61	±2.72	±2.25	±2.61	±2.98	±8.13	±5.91	±1.66	±5.94	±3.53	±2.61	±2.61
F.P.-8-18	0.0	100.0	91.5	100.0	97.9	14.3	6.5	65.3	100.0	90.0	19.8	100.0	100.0
		±0.00	±6.68	±0.00	±2.61	±5.86	1.2	±6.53	±0.00	±3.24	±5.52	±0.00	±0.00
F.P.-25-18 (C.C.)	1.4	97.7	90.1	97.7	95.6	18.0	2.8	60.0	99.3	98.6	51.0	99.3	99.3
	±2.72	±1.51	±2.62	±2.84	±4.94	±3.40	±2.23	±2.90	±1.36	±2.72	±3.05	±1.36	±1.36
F.P.-26-18 (C.C.)	0.0	100.0	87.3	100.0	97.9	4.4	5.9	61.6	100.0	97.0	35.4	100.0	100.0
		±0.00	±4.51	±0.00	±4.08	±1.59	±2.18	±1.54	±0.00	±2.24	±4.65	±0.00	±0.00
F.P.-73-18	0.0	100.0	82.8	100.0	5.8	77.0	2.1	15.8	85.9	89.4	6.5	100.0	93.6
		±0.00	±6.40	±0.00	±3.30	±4.13	±4.08	±5.29	±3.64	±3.97	±1.22	±0.00	±2.57
F.P.-91-18	0.0	100.0	97.9	100.0	96.5	18.1	10.2	73.2	100.0	100.0	2.2	100.0	55.3
		±0.00	±4.08	±0.00	±4.08	±2.98	±5.30	±9.08	±0.00	±0.00	±2.61	±0.00	±7.72
F.P.-144-18	0.0	100.0	91.5	100.0	94.9	23.2	4.9	56.3	100.0	87.0	91.5	100.0	100.0
		±0.00	±6.68	±0.00	±4.30	±3.84	±2.54	±4.65	±0.00	±2.44	±6.68	±0.00	±0.00
Parkland c.-1-18	4.4	100.0	89.6	100.0	97.9	31.8	10.7	60.4	100.0	100.0	27.0	100.0	100.0
	±4.94	±0.00	±7.82	±0.00	±4.08	±5.14	±1.60	±4.65	±0.00	±0.00	±2.50	±0.00	±0.00

Parkland c.-2-18 (F.P.-14-18)	0.0	98.5	87.9	92.0	91.5	13.5	3.7	63.1	93.5	90.5	15.2	94.3	95.1
		±1.65	±3.40	±1.29	±2.25	±3.56	±1.25	±5.14	±2.71	±2.84	±3.06	±2.24	±2.54
Parkland c.-3-18 (F.P.-16-18)	0.0	100.0	88.8	100.0	100.0	16.3	4.9	55.6	100.0	92.9	11.4	100.0	100.0
		±0.00	±6.57	±0.00	±0.00	±0.74	±2.54	±4.44	±0.00	±3.36	±2.28	±0.00	±0.00
Parkland c.-4-18 (F.P.-17-18)	0.0	97.9	86.6	95.0	97.2	14.6	10.8	78.1	98.5	97.9	19.6	98.5	98.5
		±2.61	±5.41	±2.48	±2.23	±2.61	±3.29	±5.23	±1.65	±2.61	±3.21	±1.64	±1.65
Parkland c.-5-18 (F.P.-18-18)	0.0	100.0	90.5	100.0	97.9	15.2	2.8	70.5	100.0	100.0	32.6	100.0	100.0
		±0.00	±1.77	±0.00	±2.61	±2.67	±2.23	±6.50	±0.00	±0.00	±2.61	±0.00	±0.00
Parkland c.-6-18 (F.P.-22-18)	0.0	97.9	88.1	85.4	93.0	13.9	5.0	77.3	97.9	89.4	7.8	96.4	97.2
		±2.61	±5.43	±6.04	±2.60	±3.85	±1.46	±2.43	±2.63	±2.43	±2.49	±2.74	±2.23
Parkland c.-7-18 (F.P.-23-18)	0.0	100.0	83.6	89.4	98.5	15.5	8.6	78.0	97.9	88.6	22.7	97.9	97.9
		±0.00	±3.60	±4.55	±1.65	±3.15	±2.04	±2.28	±2.63	±4.66	±4.55	±2.61	±2.61
Parkland c.-8-18 (F.P.-15-18)	0.0	100.0	89.4	100.0	100.0	16.0	4.9	58.2	100.0	92.7	13.2	100.0	100.0
		±0.00	±3.97	±0.00	±0.00	±4.09	±2.55	±3.74	±0.00	±1.68	±1.36	±0.00	±0.00
C.C.-1-18	1.4	100.0	90.8	100.0	100.0	17.8	40.7	100.0	100.0	100.0	100.0	100.0	100.0
	±2.72	±0.00	±5.59	±0.00	±0.00	±3.15	±3.46	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00
C.C.-2-18	1.4	100.0	93.8	100.0	100.0	20.1	21.7	77.4	100.0	100.0	92.2	100.0	100.0
	±2.72	±0.00	±7.82	±0.00	±0.00	±2.61	±2.49	±4.46	±0.00	±0.00	±3.49	±0.00	±0.00
C.C.-3-18	1.4	100.0	96.5	52.0	12.9	77.1	0.0	43.9	100.0	100.0	55.1	100.0	100.0
	±2.72	±0.00	±3.43	±2.58	±5.18	±5.50		±2.43	±0.00	±0.00	±4.48	±0.00	±0.00
Camrose c.-1-18	0.0	92.2	51.5	93.6	80.9	6.7	10.7	54.6	94.2	90.5	2.1	92.7	6.4
		±2.49	±3.06	±2.82	±3.90	±1.18	±4.38	±2.57	±2.01	±4.44	±2.61	±1.68	±4.22
Camrose c.-2-18	0.0	100.0	86.7	98.5	92.0	15.5	16.2	68.1	100.0	100.0	57.4	100.0	100.0
		±0.00	±4.76	±1.74	±2.57	±3.15	±4.33	±1.65	±0.00	±0.00	±4.82	±0.00	±0.00
Camrose c.-3-18	2.1	100.0	93.6	100.0	100.0	9.9	8.6	58.2	100.0	100.0	100.0	100.0	100.0
	±2.61	±0.00	±4.22	±0.00	±0.00	±1.39	±2.04	±2.03	±0.00	±0.00	±0.00	±0.00	±0.00
Camrose c.-4-18 (P-1-18)	0.0	100.0	86.6	100.0	98.5	20.5	7.1	58.0	100.0	100.0	8.5	100.0	91.7
		±0.00	±3.08	±0.00	±1.65	±1.99	±1.44	±2.04	±0.00	±0.00	±2.25	±0.00	±6.67

Camrose c.-5-18	0.0	100.0	95.8	100.0	97.9	19.1	16.3	70.3	100.0	97.9	13.4	100.0	100.0
		±0.00	±4.71	±0.00	±2.61	±2.31	±2.34	±2.74	±0.00	±2.61	±2.39	±0.00	±0.00
Camrose c.-6-18	0.0	99.2	97.9	99.2	98.6	24.8	4.9	65.3	100.0	98.5	6.4	100.0	6.3
		±1.48	±4.08	±1.48	±2.72	±2.25	±2.54	±4.79	±0.00	±1.65	±4.22	±0.00	±7.82
Camrose c.-7-18	0.0	100.0	97.9	99.3	97.2	19.6	4.5	77.1	100.0	95.1	51.1	100.0	100.0
		±0.00	±4.08	±1.36	±3.85	±1.22	±1.50	±4.65	±0.00	±4.65	±3.41	±0.00	±0.00
Camrose c.-8-18	0.0	97.2	83.0	97.2	95.0	9.9	6.0	52.5	98.5	93.7	12.0	98.5	98.5
		±2.23	±0.74	±3.14	±3.47	±1.39	±2.17	±0.62	±1.65	±5.99	±3.89	±1.74	±1.65
S-1-18	1.4	100.0	53.5	59.7	77.8	0.0	0.0	24.3	80.6	85.2	9.2	83.6	84.3
	±2.72	±0.00	±3.42	±7.20	±7.70			±11.20	±2.22	±2.19	±1.29	±1.75	±3.05
S-2-18	0.0	100.0	89.2	100.0	97.9	23.9	6.3	55.4	100.0	94.4	50.7	100.0	100.0
		±0.00	±3.85	±0.00	±4.08	±2.69	±2.50	±3.62	±0.00	±2.12	±1.36	±0.00	±0.00
S-3-18	2.1	100.0	95.6	100.0	100.0	11.3	6.4	68.8	100.0	100.0	61.7	100.0	100.0
	±4.08	±0.00	±4.94	±0.00	±0.00	±3.69	±2.82	±1.41	±0.00	±0.00	±4.68	±0.00	±0.00
S-4-18	0.0	100.0	97.2	100.0	100.0	16.9	19.4	75.9	100.0	100.0	54.6	100.0	100.0
		±0.00	±3.85	±0.00	±0.00	±6.01	±5.30	±3.40	±0.00	±0.00	±3.40	±0.00	±0.00
S-5-18	0.0	100.0	85.2	100.0	92.2	19.7	20.8	90.0	100.0	90.0	62.5	100.0	100.0
		±0.00	±2.19	±0.00	±2.49	±5.43	±3.51	±4.51	±0.00	±1.90	±5.21	±0.00	±0.00
S-6-18	0.0	100.0	91.5	100.0	93.8	8.5	9.2	77.4	100.0	100.0	2.1	100.0	2.1
		±0.00	±6.68	±0.00	±7.82	±2.03	±1.29	±3.16	±0.00	±0.00	±4.08	±0.00	±4.08
S-7-18	0.0	100.0	81.3	100.0	100.0	18.2	7.3	84.5	100.0	100.0	80.4	100.0	100.0
		±0.00	±4.26	±0.00	±0.00	±3.54	±1.68	±3.15	±0.00	±0.00	±3.21	±0.00	±0.00
S-8-18	0.0	100.0	89.4	100.0	97.9	17.0	6.3	78.9	100.0	98.6	62.2	100.0	100.0
		±0.00	±3.97	±0.00	±2.61	±2.83	±4.65	±7.46	±0.00	±1.57	±6.07	±0.00	±0.00
S-9-18	0.0	100.0	91.5	100.0	100.0	5.8	16.5	81.5	100.0	84.0	55.2	100.0	100.0
		±0.00	±6.68	±0.00	±0.00	±2.01	±4.15	±3.83	±0.00	±1.82	±9.31	±0.00	±0.00
S-10-18	0.0	100.0	89.4	100.0	100.0	9.2	5.7	84.4	100.0	100.0	100.0	100.0	100.0
		±0.00	±3.97	±0.00	±0.00	±1.29	±2.13	±1.32	±0.00	±0.00	±0.00	±0.00	±0.00

S-11-18	0.0	94.3	1.4	61.0	0.0	0.0	0.0	11.5	91.2	5.1	51.8	91.4	95.1
		±2.24	±2.72	±3.86				±2.71	±4.11	±4.26	±2.57	±2.04	±3.43
S-12-18	2.1	100.0	80.7	100.0	100.0	20.1	17.0	74.7	100.0	100.0	79.4	100.0	100.0
	±4.08	±0.00	±7.39	±0.00	±0.00	±9.26	±2.83	±8.39	±0.00	±0.00	±7.07	±0.00	±0.00
S-13-18	0.0	100.0	93.6	100.0	95.6	23.5	21.0	84.2	100.0	100.0	90.8	100.0	100.0
		±0.00	±4.22	±0.00	±4.94	±4.58	±1.87	±6.09	±0.00	±0.00	±1.29	±0.00	±0.00
S-14-18	0.0	100.0	92.2	97.2	98.5	11.4	8.6	88.7	100.0	100.0	54.9	100.0	100.0
		±0.00	±5.65	±3.85	±1.65	±0.49	±3.99	±3.69	±0.00	±0.00	±2.61	±0.00	±0.00
S-15-18	0.0	97.2	85.2	96.5	87.8	8.5	5.8	66.7	97.9	95.0	52.1	97.2	97.2
		±2.23	±2.19	±2.59	±3.14	±2.03	±2.01	±2.32	±2.61	±3.47	±1.36	±2.22	±2.61
S-16-18	2.1	100.0	89.9	100.0	100.0	27.1	22.3	86.3	100.0	100.0	2.1	100.0	4.4
	±4.08	±0.00	±2.50	±0.00	±0.00	±2.61	±7.24	±4.49	±0.00	±0.00	±4.08	±0.00	±4.94
S-17-18	0.0	97.9	92.9	96.5	98.6	10.7	9.2	61.0	96.4	92.2	39.0	96.4	97.9
		±2.63	±4.80	±2.59	±1.57	±1.60	±1.29	±5.89	±2.74	±2.49	±2.24	±2.73	±2.61
S-18-18	0.0	100.0	73.6	100.0	98.6	19.8	9.5	64.6	100.0	98.6	55.2	100.0	100.0
		±0.00	±5.67	±0.00	±2.72	±1.67	±2.07	±2.61	±0.00	±2.72	±5.54	±0.00	±0.00
S-19-18	0.0	100.0	93.8	100.0	97.9	29.7	32.6	84.9	100.0	100.0	5.9	100.0	2.1
		±0.00	±7.82	±0.00	±4.08	±2.74	±1.36	±3.54	±0.00	±0.00	±4.04	±0.00	±4.08
S-20-18	1.4	100.0	92.2	100.0	98.6	8.5	16.7	76.4	100.0	100.0	66.0	100.0	100.0
	±2.72	±0.00	±6.84	±0.00	±2.72	±0.37	±4.61	±3.51	±0.00	±0.00	±3.43	±0.00	±0.00
S-21-18	2.1	100.0	100.0	100.0	91.5	14.7	15.5	86.4	100.0	100.0	79.0	100.0	100.0
	±4.08	±0.00	±0.00	±0.00	±6.68	±6.59	±2.23	±4.54	±0.00	±0.00	±8.97	±0.00	±0.00
S-22-18	0.0	96.5	83.8	95.7	93.1	7.3	12.5	69.4	94.3	93.6	53.5	95.1	95.8
		±2.61	±2.97	±3.59	±7.20	±1.68	±3.51	±3.85	±2.24	±2.82	±2.61	±2.54	±1.50
S-23-18	2.1	100.0	89.4	95.0	97.2	10.0	9.5	63.1	100.0	100.0	43.8	100.0	100.0
	±4.08	±0.00	±3.97	±1.46	±2.23	±4.51	±1.77	±3.41	±0.00	±0.00	±7.16	±0.00	±0.00
S-24-18	0.0	100.0	90.2	92.9	92.2	7.3	4.2	68.6	100.0	100.0	77.1	100.0	100.0
		±0.00	±3.33	±3.63	±1.53	±1.68	±3.48	±2.61	±0.00	±0.00	±2.61	±0.00	±0.00

S-25-18	0.0	100.0	100.0	100.0	100.0	14.8	13.5	80.8	100.0	100.0	58.3	100.0	100.0
		±0.00	±0.00	±0.00	±0.00	±4.33	±4.75	±1.84	±0.00	±0.00	±7.03	±0.00	±0.00
S-26-18	4.2	100.0	93.8	100.0	95.8	24.7	16.3	88.7	100.0	100.0	67.4	100.0	100.0
	±5.21	±0.00	±7.82	±0.00	±3.51	±5.44	±1.42	±3.69	±0.00	±0.00	±4.13	±0.00	±0.00
F.P.-4-18 (Leduc c1-18)	0.0	100.0	72.9	97.2	91.5	2.8	12.4	83.1	100.0	94.2	29.9	100.0	100.0
		±0.00	±3.42	±2.23	±2.25	±2.23	±1.87	±4.62	±0.00	±2.24	±6.81	±0.00	±0.00
Leduc c.1-18	0.0	100.0	89.4	100.0	100.0	27.0	11.4	82.4	100.0	100.0	58.3	100.0	100.0
		±0.00	±3.97	±0.00	±0.00	±2.57	±2.28	±4.22	±0.00	±0.00	±4.97	±0.00	±0.00
Leduc c.3-18	5.7	96.5	64.5	87.7	77.3	7.2	6.7	53.0	97.2	70.3	8.6	97.9	97.9
	±4.04	±2.59	±3.44	±2.68	±4.34	±3.24	±1.18	±2.10	±2.23	±5.92	±3.75	±2.61	±1.41
Leduc c.4-18	0.0	97.0	56.9	97.9	97.1	11.3	3.5	76.7	97.9	93.0	23.2	97.9	98.5
		±2.43	±6.48	±2.63	±3.29	±1.93	±2.59	±4.83	±2.61	±3.42	±6.28	±2.61	±1.65
Leduc c.5-18	0.0	93.5	60.8	96.3	90.5	10.4	2.8	50.4	98.5	89.8	2.1	97.2	95.8
		±3.87	±4.26	±2.72	±5.51	±2.61	±2.23	±2.97	±1.74	±1.72	±4.08	±2.23	±4.71
Leduc c.6-18	0.0	100.0	88.9	100.0	95.6	14.1	14.8	70.1	100.0	100.0	43.6	100.0	100.0
		±0.00	±3.85	±0.00	±4.94	±1.84	±2.19	±4.08	±0.00	±0.00	±2.35	±0.00	±0.00
Leduc c.7-18	1.4	100.0	94.9	100.0	100.0	8.5	5.8	68.1	100.0	100.0	51.1	100.0	100.0
	±2.72	±0.00	±4.30	±0.00	±0.00	±2.25	±2.01	±1.57	±0.00	±0.00	±4.97	±0.00	±0.00
Leduc c.8-18	0.0	100.0	84.4	100.0	100.0	18.1	19.8	82.0	100.0	100.0	67.2	100.0	100.0
		±0.00	±1.32	±0.00	±0.00	±1.98	±1.67	±5.23	±0.00	±0.00	±7.95	±0.00	±0.00
Leduc c.9-18	0.0	100.0	90.7	100.0	100.0	12.2	7.0	75.3	100.0	95.5	52.5	100.0	100.0
		±0.00	±4.94	±0.00	±0.00	±3.14	±3.42	±2.04	±0.00	±1.87	±2.89	±0.00	±0.00
Leduc c.10-18	0.0	100.0	87.3	100.0	100.0	14.1	7.1	68.1	100.0	100.0	37.2	100.0	100.0
		±0.00	±4.51	±0.00	±0.00	±1.84	±1.44	±3.55	±0.00	±0.00	±6.03	±0.00	±0.00
Leduc c.11-18	0.0	100.0	100.0	100.0	100.0	6.4	2.1	73.0	100.0	97.9	41.0	100.0	100.0
		±0.00	±0.00	±0.00	±0.00	±1.30	±2.63	±4.02	±0.00	±2.63	±4.65	±0.00	±0.00
Leduc c.12-18	0.0	100.0	94.3	99.3	98.5	13.5	10.6	71.0	100.0	95.8	57.4	100.0	100.0
		±0.00	±4.04	±1.36	±1.65	±2.78	±2.43	±2.81	±0.00	±3.48	±2.57	±0.00	±0.00

Leduc c.13-18	0.0	100.0	95.6	100.0	97.1	3.6	2.8	67.5	100.0	97.8	50.1	100.0	100.0
		±0.00	±4.94	±0.00	±3.29	±2.74	±2.23	±3.66	±0.00	±2.67	±2.73	±0.00	±0.00
Leduc c.14-18	0.0	100.0	95.6	100.0	97.9	10.4	14.5	79.9	100.0	95.6	50.8	100.0	100.0
		±0.00	±4.94	±0.00	±4.08	±1.59	±1.89	±4.26	±0.00	±4.94	±2.84	±0.00	±0.00
Leduc c.15-18	0.0	100.0	97.9	100.0	97.9	10.6	2.9	76.5	100.0	96.4	52.1	100.0	100.0
		±0.00	±4.08	±0.00	±2.61	±2.43	±2.24	±3.33	±0.00	±2.74	±2.61	±0.00	±0.00
Leduc c.16-18	0.0	100.0	93.2	100.0	100.0	8.6	2.1	69.5	100.0	97.9	49.0	100.0	100.0
		±0.00	±2.64	±0.00	±0.00	±2.04	±2.61	±2.23	±0.00	±2.61	±2.09	±0.00	±0.00
Leduc c.17-18	0.0	100.0	91.7	100.0	100.0	6.0	13.4	75.4	100.0	94.3	68.1	100.0	100.0
		±0.00	±9.43	±0.00	±0.00	±2.32	±2.86	±2.65	±0.00	±2.13	±3.80	±0.00	±0.00
Leduc c.18-18	1.4	100.0	93.5	100.0	100.0	12.3	7.8	75.4	100.0	97.9	72.3	100.0	100.0
	±2.72	±0.00	±2.71	±0.00	±0.00	±1.13	±2.49	±0.43	±0.00	±2.63	±3.79	±0.00	±0.00
Leduc c.19-18	0.0	100.0	89.2	100.0	97.9	11.6	7.1	77.5	100.0	97.9	44.4	100.0	100.0
		±0.00	±3.85	±0.00	±4.08	±1.96	±3.31	±1.25	±0.00	±2.61	±6.67	±0.00	±0.00
Leduc c.20-18	0.0	99.3	90.8	99.2	96.4	7.3	2.8	72.7	99.3	100.0	59.5	98.6	100.0
		±1.36	±1.29	±1.48	±4.17	±1.68	±2.23	±9.39	±1.36	±0.00	±7.21	±2.72	±0.00
Leduc c.21-18	0.0	100.0	88.3	100.0	100.0	9.6	2.8	77.5	100.0	97.7	37.9	100.0	100.0
		±0.00	±4.56	±0.00	±0.00	±1.17	±2.23	±2.55	±0.00	±2.84	±2.04	±0.00	±0.00
Leduc c.22-18	0.0	100.0	88.7	100.0	92.3	7.0	0.0	62.6	100.0	81.6	28.1	100.0	100.0
		±0.00	±3.69	±0.00	±7.44	±3.42		±5.32	±0.00	±1.29	±5.11	±0.00	±0.00
Leduc c.23-18	0.0	97.9	75.8	98.5	92.0	5.1	5.7	56.1	97.7	94.4	42.4	98.4	98.5
		±2.63	±3.27	±1.74	±1.65	±1.34	±2.13	±2.43	±2.84	±3.85	±2.61	±1.80	±1.65
Leduc c.24-18	0.0	97.1	74.6	97.9	89.1	11.0	2.8	51.7	96.4	91.3	22.7	97.2	97.8
		±2.24	±3.77	±2.61	±1.25	±2.04	±2.23	±2.10	±2.74	±2.26	±6.74	±3.14	±1.45
D-1-18 (Leduc c.25-18)	0.0	97.9	70.8	97.8	89.8	8.8	7.4	51.1	95.5	89.5	27.5	94.8	96.5
		±2.61	±5.21	±1.47	±4.43	±1.81	±1.53	±1.32	±1.50	±2.20	±3.23	±2.81	±2.59
D-2-18 (Leduc c.25-18)	0.0	100.0	73.9	97.9	90.7	8.1	5.9	54.8	100.0	96.3	25.0	100.0	100.0
		±0.00	±6.17	±2.63	±2.34	±2.44	±2.18	±7.40	±0.00	±2.72	±6.67	±0.00	±0.00

D-3-18 (Leduc c.25-18)	0.0	98.5	70.1	94.7	87.5	9.8	5.8	55.0	91.7	80.3	25.7	91.4	94.1
		±1.65	±4.65	±1.68	±2.87	±3.33	±2.01	±4.18	±2.22	±1.24	±2.61	±2.04	±2.44
D-4-18 (F.P.-5-18; C.C.)	0.0	98.5	51.9	97.9	61.6	2.8	2.8	51.6	98.5	95.8	51.9	97.8	97.9
		±1.65	±4.33	±2.63	±5.98	±2.23	±2.23	±6.51	±1.65	±1.50	±3.91	±2.82	±2.61
D-5-18	0.0	91.5	5.1	0.0	11.3	7.8	4.4	100.0	36.4	0.0	10.1	6.6	2.9
		±2.25	±1.34		±3.69	±2.49	±1.66	±0.00	±3.43		±4.59	±2.59	±3.29
Henwood (C. of Edmonton)	0.0	97.7	78.1	97.1	89.2	9.6	6.5	61.7	99.3	98.5	8.6	99.2	99.3
		±2.84	±2.75	±3.29	±2.23	±1.17	±2.71	±4.61	±1.36	±1.74	±2.04	±1.48	±1.36
U of A-1-18	1.4	97.2	54.9	98.5	88.0	14.1	3.7	54.2	98.6	92.1	7.1	98.6	92.9
	±2.72	±2.23	±7.82	±1.65	±3.89	±1.84	±1.25	±6.35	±1.57	±3.38	±5.74	±2.72	±1.81
U of A-2-18	0.0	96.5	51.4	92.8	72.4	5.9	3.0	51.8	97.0	83.0	7.4	97.0	97.8
		±4.08	±1.57	±1.29	±2.13	±2.13	±2.24	±2.19	±2.43	±4.51	±3.71	±2.43	±2.82
Barrhead c.-1-18	0.0	100.0	90.7	95.6	84.5	4.2	2.1	83.0	97.9	89.3	5.7	98.5	98.6
		±0.00	±5.50	±1.66	±2.23	±1.50	±2.61	±5.72	±2.61	±4.38	±4.45	±1.65	±1.57
Barrhead c.-2-18 (F.P.-28-18)	0.0	96.3	87.3	96.4	92.9	12.2	2.8	63.2	95.1	91.5	17.7	97.2	97.2
		±2.72	±3.24	±2.74	±3.36	±2.22	±2.23	±4.08	±2.54	±4.79	±5.60	±3.14	±2.23
Barrhead c.-3-18 (F.P.-29-18)	0.0	97.9	86.2	97.0	94.9	9.9	2.8	18.9	95.8	90.5	2.8	95.0	4.4
		±2.63	±4.26	±2.24	±4.26	±2.62	±2.23	±1.89	±3.51	±2.84	±2.23	±3.47	±4.94
Barrhead c.-4-18 (F.P.-30-18)	0.0	100.0	86.9	100.0	95.6	6.9	8.8	59.5	100.0	100.0	54.1	100.0	100.0
		±0.00	±4.98	±0.00	±1.66	±1.57	±1.81	±1.81	±0.00	±0.00	±2.57	±0.00	±0.00
Barrhead c.-5-18	4.4	97.2	75.1	98.4	90.6	5.9	7.6	56.5	97.9	97.1	24.9	98.5	98.6
	±4.94	±2.23	±2.03	±1.80	±1.17	±2.18	±2.54	±1.72	±2.63	±3.29	±4.34	±1.65	±1.57
Barrhead c.-6-18	0.0	97.9	86.9	98.6	96.5	5.7	4.2	40.4	100.0	97.9	50.0	100.0	100.0
		±2.61	±4.05	±1.57	±4.08	±2.13	±1.50	±1.44	±0.00	±2.63	±5.44	±0.00	±0.00
Barrhead c.-7-18	0.0	97.9	91.5	99.3	98.5	5.7	1.5	31.7	96.4	95.8	49.7	97.9	98.5
		±2.63	±2.25	±1.36	±1.74	±2.13	±1.65	±5.99	±2.74	±3.48	±1.75	±2.61	±1.65
MB-1-18	0.0	93.7	2.1	2.1	2.3	1.4	48.6	52.5	94.1	92.9	7.6	96.3	8.5
		±4.02	±4.08	±4.08	±4.45	±2.72	±3.89	±1.84	±4.04	±3.46	±6.81	±1.20	±6.68

MB-2-18	0.0	97.9	89.4	98.6	97.2	4.4	2.8	50.3	98.5	95.7	59.2	99.3	99.3
		±2.63	±3.97	±2.72	±3.85	±1.66	±2.23	±1.75	±1.65	±3.59	±7.01	±1.36	±1.36
MB-3-18	0.0	97.9	8.0	88.0	83.7	1.5	1.5	16.6	97.9	93.5	2.1	97.9	6.3
		±2.61	±1.65	±3.89	±2.34	±1.65	±1.65	±3.13	±2.63	±3.87	±4.08	±2.63	±7.82
MB-4-18	0.0	98.6	59.5	98.5	96.5	13.4	18.8	58.3	98.5	95.7	6.4	98.5	12.7
		±1.57	±4.80	±1.65	±4.08	±2.39	±2.87	±4.34	±1.65	±1.73	±7.89	±1.65	±4.51
MB-5-18	1.4	97.9	78.1	98.5	88.3	10.7	4.5	63.8	98.5	94.3	5.9	97.1	9.2
	±2.72	±2.63	±3.54	±1.65	±5.09	±1.60	±1.50	±7.08	±1.65	±3.86	±4.04	±3.29	±5.05
MB-6-18	0.0	97.1	11.3	89.9	13.4	0.0	0.0	12.1	88.7	25.6	2.1	82.8	5.8
		±3.29	±1.93	±1.14	±2.39			±1.28	±3.69	±4.58	±4.08	±6.40	±2.01
SK-2018-DB-1	0.0	100.0	68.1	100.0	89.4	6.6	5.9	51.0	100.0	95.6	2.1	100.0	19.1
		±0.00	±3.51	±0.00	±3.97	±2.59	±3.67	±5.86	±0.00	±1.59	±4.08	±0.00	±4.25
SK-2018-DB-2	0.0	99.3	63.7	97.2	87.2	10.9	5.2	52.5	99.3	91.4	3.6	98.5	4.4
		±1.36	±2.43	±2.23	±4.65	±3.54	±2.81	±3.90	±1.36	±3.63	±4.17	±1.74	±4.94
SK-2018-DB-3	0.0	100.0	79.5	100.0	85.9	9.3	5.2	60.4	100.0	96.3	4.4	100.0	10.8
		±0.00	±3.72	±0.00	±2.89	±2.34	±1.42	±5.99	±0.00	±2.72	±4.94	±0.00	±3.85
SK-2018-DB-4	1.4	98.5	60.2	97.7	93.6	15.2	7.3	59.3	98.5	92.7	11.0	97.1	6.3
	±2.72	±1.65	±5.00	±2.84	±4.63	±3.06	±1.14	±5.44	±1.74	±1.68	±3.75	±3.29	±7.82
SK-2018-DM-1B	0.0	96.3	73.8	97.0	82.3	17.0	5.1	71.1	97.2	87.2	0.0	97.9	19.1
		±2.72	±2.87	±2.43	±3.41	±1.75	±1.34	±3.87	±3.14	±6.15		±2.63	±5.29
SK-2018-CN-2	0.0	96.4	60.2	97.0	88.8	8.6	8.0	57.7	98.6	96.3	2.1	99.3	4.4
		±2.74	±2.82	±2.24	±3.05	±2.04	±2.21	±5.37	±1.57	±1.25	±4.08	±1.36	±4.94
SK-2018-CP-62	0.0	98.5	86.2	97.8	90.8	12.8	5.1	58.9	99.3	97.2	3.5	99.2	6.3
		±1.65	±2.54	±2.82	±1.29	±1.35	±2.40	±6.76	±1.36	±2.23	±4.08	±1.63	±7.82
SK-2018-CW-1	0.0	97.9	0.0	87.9	9.4	0.0	0.0	17.2	98.5	0.0	5.7	98.6	18.1
		±2.63		±1.28	±5.39			±5.40	±1.74		±4.04	±1.57	±1.57
SK-2018-LLH-3	0.0	100.0	59.0	100.0	85.8	8.2	5.8	52.9	100.0	98.5	4.4	100.0	12.0
		±0.00	±7.11	±0.00	±2.31	±1.59	±2.01	±1.22	±0.00	±1.74	±4.94	±0.00	±3.89

SK-2018-RA-56	0.0	92.2	6.5	54.7	8.5	1.5	0.0	19.6	92.5	15.5	11.0	92.9	2.1
		±6.24	±2.71	±2.29	±3.74	±1.65		±4.26	±2.02	±3.15	±8.17	±6.32	±4.08

[a]European Clubroot Differential (ECD) 02 = *Brassica rapa* ssp. *rapifera* line AAbbCC; ECD 05 = *B. rapa* var. *pekinensis* 'Granaat'; ECD 06 = *Brassica napus* 'Nevin'; ECD 08 = *B. napus* 'Giant Rape' selection; ECD 09 = *B. napus* New Zealand resistant rape; ECD 10 = *B. napus* var. *napobrassica* 'Wilhemsburger'; ECD 11 = *Brassica oleracea* var. *capitata* 'Badger Shipper'; ECD 13 = 'Jersey Queen'; Brutor = *B. napus* 'Brutor'; Laur. = *B. napus* var. *napobrassica* 'Laurentian'; Westar = *B. napus* 'Westar'; and 45H29 = *B. napus* '45H29'.

[b]The mean index of disease (ID, %) ± standard error is shown for each host genotype/pathogen combination. A host was considered resistant if the mean ID was <50% and the 95% confidence interval (CI) did not overlap 50%. The numbers in bold indicate susceptible reactions; those reactions in which the mean ID <50% but the 95% CI overlapped 50% are denoted with an asterisk (*)

Chapter 4 – Assessment of amisulbrom as a clubroot management tool in canola

4.0 Introduction

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Wor., is an important disease of the Brassicaceae family (Dixon 2009a). Disease development is associated with the formation of large galls on the roots of susceptible plants, which interfere with water and nutrient uptake and can result in severe yield and quality losses. In western Canada, clubroot has emerged as a major disease of canola (*Brassica napus* L.) in recent years (Strelkov & Hwang 2014). While farmers rely on the deployment of clubroot resistant (CR) canola cultivars as the main strategy to manage the disease (Peng et al. 2015), novel, resistance-breaking pathotypes of *P. brassicae* have been detected with increasing frequency in recent years (Chapter 3; Strelkov et al. 2016, 2018a). These pathotypes likely reflect the selection pressure imposed on *P. brassicae* populations by CR canola (LeBoldus et al. 2012; Strelkov et al. 2016), and highlight the need for an integrated approach to clubroot management.

One of the main challenges associated with the effective control of *P. brassicae* is the ability of the pathogen to produce very large numbers of long-lived resting spores in galled root tissue; these spores are released into the soil as the galls decompose. As many as 8×10^8 resting spores can be produced from an infected canola plant (Hwang et al. 2012c), leading to rapid increases in soil inoculum levels. While the proliferation of *P. brassicae* on CR canola is more limited, it still occurs, with the resulting resting spores likely enriched for components of the pathogen population able to overcome resistance (Ernst et al. 2019). Management methods that can reduce disease pressure are important for supplementing and promoting the longevity of clubroot resistance. Numerous such methods have been evaluated in recent years, including the application of lime and other soil amendments, often with mixed results (Hwang et al. 2014).

Fungicides in particular represent a chemical alternative for clubroot control, with Dynasty® 100FS (azoxystrobin), Helix Xtra® (thiamethoxam + difenconazole + metalaxyl + fludioxonil), Prosper™ FX (clothianidin + carbathiin + trifloxystrobin + metalaxyl), Vitavax® RS (carbathiin + thiram) and Nebijin® (flusulfamide) studied for the management of the disease on canola in western Canada (Hwang et al. 2011c). Most of these products, while effective at reducing the severity of clubroot under low disease pressure or controlled conditions, did not provide sufficient control under high disease pressure in the field.

Amisulbrom is an active ingredient in the sulfonamide chemical class discovered in 1999 by Nissan Chemical Industries Ltd. (Tokyo, Japan). This fungicide is a mitochondrial electron transport system complex III Qi inhibitor (Qii) (Nissan Chemical 2019) and provides good control of oomycete diseases (Dawson et al. 2008). In Japan, amisulbrom is registered for use in controlling various diseases of agricultural and horticultural crops. The fungicide has shown great promise in the control of *P. brassicae* in a variety of Brassica crops (Dawson et al. 2008), since it affects both the primary and secondary zoospores. There is, however, no information on the efficacy of this product on clubroot of canola.

The objectives of this study were (1) to evaluate the efficacy of varying rates of amisulbrom in reducing clubroot severity on canola under field conditions with high *P. brassicae* inoculum pressure, and (2) to evaluate the efficacy of varying rates of amisulbrom applied in two different ways (in-furrow vs. broadcast application) under greenhouse conditions.

4.1 Materials and methods

4.1.1 Field trials

Amisulbrom (GWN 10440; 20% soluble concentrate) was obtained from Nissan Chemical Corp. (Tokyo, Japan) through a local supplier. Replicated field trials were conducted in 2018 and

2019 to evaluate the efficacy of different product rates on clubroot disease severity and a variety of plant growth parameters including yield. The trials were located at the Crop Diversification Centre - North (CDC-N), Alberta Agriculture and Forestry, in Edmonton, Alberta, in a naturally infested *P. brassicae* field nursery (53 38' 48"N, 113 22' 33"W). The soil at this site is a Black Chernozemic loam. Three rates of amisulbrom were evaluated, 500 g active ingredient (ai)/ha, 1000 g ai/ha, and 1500 g ai/ha. The rates were applied to two canola cultivars, '45H31' (clubroot susceptible) and 'CS 2000' (moderately resistant), and compared with an untreated control. The treatments were arranged in a randomized complete block design (RCBD) with four replicates. Plots were 1.5 m × 6 m with a 0.5 m buffer between each plot and a 2 m buffer between each replicate. There were four rows per plot, which were seeded and treated by hand. The location of the plots was moved to a different spot in the nursery in 2019, to avoid any residual effects of the amisulbrom.

The plots were treated and seeded on June 6, 2018, and June 13, 2019. Prior to seeding, a small-plot seeder was used to compact the seedbed and create aligned seed furrows. The product was diluted with water to achieve the respective treatment rates, which were applied in a volume of 1000 L/ha with a backpack sprayer. Immediately afterwards, 0.7 g of seed of each cultivar was sown evenly along each furrow and covered before the product could dry. A volume of 1000 L/ha of water, without any amisulbrom, was applied to the untreated checks. The plots received approximately 189 mm of rain in the 2018 growing season and 358 mm in the 2019 growing season (Environment Canada). No irrigation was applied. The higher than average rainfall in 2019 partially submerged the experimental site, leading to plant death and limited emergence in a few of the plots that were in lower areas. The plots were treated with Decis 5EC (Bayer, Germany) on June 14, 2018, to control cutworms.

Ten plants per plot were collected 8-weeks after seeding in each year of the study for clubroot disease assessment (see below). The plants were pulled gently or dug from the soil so as not to damage the roots. Plant height for all sampled plants was also measured along with the aboveground biomass, root weight, and gall weight. For yield evaluation, the plots were harvested on October 18, 2018 and October 11, 2019. The harvested seeds were dried and cleaned prior to weighing.

4.1.2 Greenhouse trials

The greenhouse trials included the same rates of amisulbrom (0 g ai/ha (UTC), 500 g ai/ha, 1000 g ai/ha, and 1500 g ai/ha) as in the field. Two application methods were compared, an in-furrow application and a broadcast application incorporated into the top layer of soil. The efficacy of the treatment rates and application methods was evaluated at two *P. brassicae* resting spore concentrations: low (1×10^4 resting spores g^{-1}) and high (1×10^7 resting spores g^{-1}) on the clubroot-susceptible canola '45H31'. Briefly, a mixture of 50% field soil, collected from a non-infested site at CDCN, and 50% Sungro Professional Growing Mix (Sungro Horticulture, Seba Beach, AB), was inoculated with pathotype 3H of the clubroot pathogen (Strelkov et al. 2018a), at the low or high spore concentrations. Following inoculation, the potting mix was placed in small plastic tubs (11.2 L). For the in-furrow application of amisulbrom, the different rates were applied to each of four rows or 'furrows' in each tub using a calibrated syringe. For the broadcast application, the top 5-cm of potting mix in each tub was placed in a Ziploc® bag (S.C. Johnson & Son, Brantford, ON), treated with the appropriate rate of amisulbrom, mixed thoroughly, and then placed back in the tub on top of the untreated potting mix. Controls were treated with water. Canola seeds were sown at a density of 12 seeds per row along each of four rows in each tub and covered with a thin layer of potting mix.

The tubs were maintained at approximately 24°C with 30% relative humidity under natural light supplemented with artificial lighting (16 h day/8 h night). The potting mixture was top-watered daily with slightly acidified water (20 mL HCl in 200 L water), in order to create a favourable environment for clubroot development, and fertilized 2-weeks after seeding with a 0.1% solution of 20:20:20 (N:P:K) fertilizer. Seedlings were thinned to 10 plants per row after emergence (40 plants per tub), and treatments were replicated four times (one tub per replicate). The entire experiment was repeated, with the repetitions referred to as run one and run two. The roots were collected 6-weeks after seeding for clubroot symptom assessment as described below. Ten plants were randomly selected per tub, carefully dug out from the potting mix, and gently washed under standing water. Individual plant height, aboveground biomass, root weight and gall weight also were recorded.

4.1.3 Disease assessment

The roots of each plant were rated for clubroot severity on a 0-to-3 scale (Kuginuki et al. 1999), where: 0 = no galling, 1 = a few small galls (small galls on less than one-third of the roots), 2 = moderate galling (small to medium galls on one-third to two-thirds of the roots), and 3 = severe galling (medium to large galls on more than two-thirds of the roots). The individual severity ratings were used to calculate an index of disease for each replicate according to the formula of Horiuchi & Hori (1980) as modified by Strelkov et al. (2006):

$$ID\ (\%) = \frac{\sum(n\times0+n\times1+n\times2+n\times3)}{N\times3} \times 100\%$$

Where n is the number of plants in each class; N is the total number of plants; and 0, 1, 2, and 3 are the symptom severity classes.

4.1.4 Statistical analysis

All statistical analyses were performed with RStudio Version 1.1.442 (2009-2018 RStudio, Inc). Differences were considered statistically significant if $P < 0.05$. The normality of the field data was tested using the Shapiro-Wilk test, and both the 2018 and 2019 data were found to be normal. The homogeneity of the data was tested using visual assessment of the residuals and random effects. The response variables were transformed using the square root function to meet normality when necessary. The least squared means function was then used for Post-hoc comparisons. A one-way ANOVA test was conducted to determine the significance of the treatments and seed types and their interactions. The plant parameters analyzed included plant height, shoot weight, root weight, gall weight, yield and clubroot severity (ID) to evaluate the effects of cultivar ('45H31' vs. 'CS2000') and different rates of amisulbrom vs. non-treated controls.

A crossed factorial design was used to assess the greenhouse data. The generalized least squares test was performed to determine if a blocking factor was necessary, and it was determined that it could be removed for both independent runs of the experiment. Normality of the data was tested using the Shapiro-Wilk test for normality, and the data from both run one and run two of the greenhouse trials were normal. Homogeneity of the variances was tested using the Bartlett test and both runs demonstrated homoscedaticity of variances. The linear model was used to determine the significance of the treatment effects, and a three-way ANOVA was carried out to determine the significance of the treatments and their interactions. Tukey's test was then used for Post-hoc comparisons, which indicated significant differences among statistical parameters. The plant parameters analyzed included plant height, shoot weight, root weight, gall weight, and clubroot

severity (ID) to evaluate the effects of application method (in furrow vs. broadcast) and different treatment rates of amisulbrom vs. non-treated controls.

4.2 Results

4.2.1 Field trials

Disease severity

In 2018, the effect of canola cultivar on clubroot disease severity (ID) was statistically significant ($P = 0.0001$; Table 4.1), with the moderately resistant genotype 'CS2000' developing very little disease in any of the treatments including in the untreated control (ID = 2.5%), and the susceptible cultivar '45H31' showing moderate levels of disease (Table 4.2). Given the near absence of clubroot symptoms on 'CS2000', this cultivar was not included in the greenhouse trials (see below). In the case of '45H31', the application of amisulbrom resulted in numerical decreases in ID, from 30.0% in the untreated control to 15.0%, 13.3% and 10.8%, respectively, at the low (500 g ai/ha), mid (1000 g ai/ha) and high (1500 g ai/ha) rates, but these declines were not statistically significant ($F = 1.7137$, $P = 0.1909$) (Table 4.2).

In 2019, due to very high rainfall after seeding and throughout the season, part of the trial was submerged in water for multiple days. This flooding appeared to diminish clubroot development in the susceptible cultivar '45H31', with IDs ranging from 3.3% to 6.7% across rates of amisulbrom, including the untreated control (Table 4.3). In the case of the moderately resistant cultivar 'CS2000', the ID on the control was 20.8%, which was not significantly different ($F = 0.8603$, $P = 0.4752$) from the ID observed at any of the application rates (Table 4.3). The effect of cultivar in 2019 on ID was significant ($P = 0.0029$; Table 4.4), although it was the opposite of what was expected based on resistance ratings.

Yield and plant growth parameters

In 2018, there was no significant difference among the amisulbrom treatments for shoot weight, root weight, gall weight, plant height, or yield in either cultivar (Table 4.2). There was, however, a statistically significant interaction between ID and root weight (F = 19.6167, P = 0.0002) and ID and gall weight (F = 89.0421, P = 0.0001) for both cultivars. Although not significant at $P < 0.05$, there was a numerical trend of decreasing root gall weight with increasing rate of amisulbrom (from 37.3 g in the control to 5.5 g at the highest rate) in the susceptible cultivar (Table 4.2). Similarly, in 2019, there were no statistically significant differences for shoot weight, root weight, gall weight, plant height or yield as a result of the amisulbrom treatments. There was, however, a statistically significant interaction between ID and gall weight (F = 113.7864, P = 0.0001) as well as ID and yield (F = 4.9102, P = 0.0369) for both cultivars.

4.2.2 Greenhouse trials

Application method

Amisulbrom application method (in-furrow vs. broadcast) did not have a significant effect on clubroot severity or any of the plant growth parameters in either run of the greenhouse experiment, and hence the data were pooled for application method when analyzing each run.

Disease severity

Highly significant differences were observed with respect to amisulbrom application rates, inoculum levels and clubroot severity in both repetitions of the experiment. In the first run, both treatment (P = 0.0004; Table 4.5) and inoculum concentration (P = 0.0074; Table 4.5) were highly significant. Plants grown in 1×10^4 resting pores g^{-1} potting mixture developed an ID of 48.3% in the control treatment, vs. 21.3%, 13.3% and 8.8% at the low, moderate and high rates of amisulbrom, respectively (Table 4.6). Similarly, plants grown in 1×10^7 spores g^{-1} potting mixture

developed an ID of 73.8% in the control treatment, vs. IDs of 42.9%, 25.8% and 30.4% at the low, moderate and high rates of amisulbrom, respectively (Table 4.6). There were no statistical interactions between the different inoculum concentrations and the different treatment levels. In the second run of the greenhouse experiment, there were also highly significant differences among the rates of amisulbrom across both resting spore concentrations ($P = 1.43 \times 10^{-6}$; Table 4.7), although the resting spore concentrations (1×10^4 spores g^{-1} and 1×10^7 spores g^{-1}) ($P = 0.1556$; Table 4.7) themselves were not statistically significant. The plants grown in 1×10^4 spores g^{-1} medium developed an ID of 100% in the control, vs. IDs of 67.5%, 56.7% and 59.2%, respectively, at the low, moderate and high rates of amisulbrom (Table 4.8). In the case of the plants grown in 1×10^7 spores g^{-1}, the ID was 100% in the control, 74.6% at the lowest rate of amisulbrom, 78.3% at the medium rate, and 58.3% at the highest rate (Table 4.8). There were no statistical interactions between the different inoculum concentrations and the different treatment levels.

Plant growth parameters

In the first run of the greenhouse experiment, in the case of plants grown in 1×10^4 spores g^{-1} growing medium, there was a statistical significance for amisulbrom rates overall (F = 3.6697, $P = 0.0263$). There was, however, no statistical significance between ID and shoot weight (F = 2.5978, $P = 0.1191$) and ID and plant height (F = 0.0154, $P = 0.9022$). There was a highly significant correlation between ID and gall weight (F = 333.9990, $P = 2.33 \times 10^{-16}$), which translated into a significant correlation between ID and root weight (F = 115.4912, $P = 4.64 \times 10^{-11}$). Similarly, for the plants grown in 1×10^7 spores g^{-1} medium, there was a statistical significance between treatment concentrations overall (F = 3.7149, $P = 0.0252$), but not between ID and shoot weight (F = 0.9707, $P = 0.3336$) or ID and plant height (F = 3.3903, $P = 0.0775$). There was a

highly significant correlation between ID and gall weight (F = 25.2715, $P = 3.13 \times 10^{-05}$), which translated to a significant correlation between ID and root weight (F = 23.4363, $P = 5.11 \times 10^{-05}$).

In the second run of the greenhouse experiment, there was high statistical significance between amisulbrom rates overall (F = 11.4210, $P = 7.57 \times 10^{-05}$) in plants grown in 1×10^4 spores g^{-1} potting mix. There were also statistically significant relationships between ID and shoot weight (F = 11.4506, $P = 0.0023$), ID and plant height (F = 9.4514, $P = 0.0050$) and ID and gall weight (F = 9.5736, $P = 0.0047$). The latter translated into a significant correlation between ID and root weight (F = 4.9170, $P = 0.0355$). For the plants grown in 1×10^7 spores g^{-1} potting mix, there was statistical significance between treatment concentrations overall (F = 5.0188, $P = 0.0077$) and between ID and gall weight (F = 6.0553, $P = 0.0208$). There were, however, no statistical significant relationships between ID and shoot weight (F = 0.1803, $P = 0.6746$), ID and root weight (F = 2.5956, $P = 0.1192$), and ID and plant height (F = 0.3894, $P = 0.5380$).

4.3 Discussion

Amisulbrom shows some promise for the management of clubroot in canola. In 2018, a general trend was observed of decreasing clubroot severity (ID) and root gall weight with increasing rate of amisulbrom, and while at $P = 0.1909$ the results were not significant, they do suggest that additional study may be warranted. Nonetheless, the resistant canola 'CS2000' developed significantly lower levels of disease than the susceptible '45H31', underscoring the importance of genetic resistance as one of the most important clubroot management tools (Peng et al. 2014; Rahman et al. 2014). It seems that as long as resistance remains effective in a field, other management strategies are not nearly as effective or convenient. Unfortunately, heavy rainfall in 2019 caused flooding of some of the plots and had an adverse effect on plant health generally. Stressed plants generally do not support much clubroot development, with *P. brassicae* preferring

otherwise healthy hosts (Gossen et al. 2014). Indeed, clubroot development is reduced under the saturated or anoxic conditions associated with waterlogged soils (Dobson et al. 1982). Consequently, there was very little clubroot symptom development observed in 2019. Unexpectedly, ID was significantly higher in the resistant vs. susceptible cultivar in 2019, although this likely reflected the low levels of disease observed overall.

To explore the potential utility of amisulbrom for clubroot management further, the field studies were complemented by greenhouse trials. In these trials, two methods of applying amisulbrom were compared, an in-furrow application vs. a broadcast application in the top layer of the potting mix. No significant differences were detected with respect to application method, suggesting that they provided equivalent levels of control. If these results are confirmed in field trials, they could influence the likelihood that products such as amisulbrom are integrated into on-farm clubroot management plans. In Alberta, zero-till systems have been widely adopted and accepted to conserve and protect the soil (Lafond et al. 1996), and conservation or zero-till farming practices are now prevalent across Canada (Environment Canada 2019). While this may make broadcast application of amisulbrom more challenging, it could still be an option in a conservation tillage system where other approaches are limited. Moreover, in-furrow application may also pose a challenge, since equipment able to band a liquid formulation together with seed or fertilizer is not common and may add costs for the farmer (Exapta, 2019).

Regardless of application method, the results from the greenhouse trials suggested further promise for amisulbrom as a clubroot management tool under both low (1×10^4 resting spores g^{-1} potting mix) and high (1×10^7 resting spores g^{-1} potting mix) inoculum levels. There was a trend of decreasing ID with increasing rate of amisulbrom, in most cases significant, across both runs of the experiment at both inoculum levels. Similarly, plant height and shoot weight were greater, and

root and gall weight were lower, in amisulbrom treated vs. control plants. These results indicate less severe clubroot and healthier plants because of amisulbrom application. While this fungicide is not commonly used for clubroot control at present, it has shown efficacy against certain oomycete diseases in Japan. Amisulbrom reduced late blight of potato caused by *Phytophthora infestans*, with zoospore release by the pathogen inhibited for up to 28 days after its application (Honda et al. 2008). The fungicide also significantly reduced the production of zoosporangia, which in turn suggested the inhibition of secondary infection by *P. infestans*. While *P. brassicae* is not an oomycete, but rather a protist, there are similarities in the life cycle with *P. infestans*, including zoospore production and its soilborne nature (Andrivon 1995; Kageyama and Asano 2009).

The greater effect of amisulbrom treatment in the greenhouse vs. field may reflect the greater disease pressure under the controlled conditions, as reflected by higher IDs. This enhanced disease pressure may have been influenced by higher inoculum levels in the greenhouse, where we applied known amounts of *P. brassicae* resting spores, as well as conditions more conducive to clubroot development. Indeed, the heavy rainfall in 2019 and the flooding of many plots precluded the possibility of making any meaningful from the field trials that year. Given the promising greenhouse data, and the positive trends observed with respect to some parameters in the 2018 field trial, it may be worthwhile to explore the use of amisulbrom for clubroot management further. While genetic resistance can be a very effective tool for controlling clubroot, the identification of increasing numbers of resistance breaking pathotypes (Chapter 3; Strelkov et al. 2016, 2018a), along with rapid shifts in the virulence of *P. brassicae* (LeBoldus et al. 2012; Cao et al. 2020), suggest that resistance cannot be used in isolation. There is a growing emphasis on the need for an integrated management plan, in which multiple strategies are combined in order

to better control clubroot (Donald & Porter 2009). Amisulbrom may have potential as part of such an integrated approach, contributing to sustainable clubroot management.

Table 4.1 One-way ANOVA for the 2018 field season.

	numDF	denDF	F-value	p-value
Intercept	1	24	34.81	<0.0001
Seed Type	1	24	20.76	0.0001
Treatment	3	24	1.71	0.1909

Table 4.2 Effect of amisulbrom application rate on clubroot index of disease and canola yield and growth parameters under field conditions in Edmonton, AB, Canada, 2018.

Year 1 (2018) - '45H31' Susceptible						
Amisulbrom Rate (g ai/ha)	**Index of disease (%)**	**Fresh Shoot Weight (g) 10 plants**	**Fresh Root Weight (g) 10 plants**	**Fresh gall Weight (g) 10 plants**	**Plant Height (cm)**	**Yield g/plot**
0.0	30.0 a	1190.0 a	107.0 a	37.3 a	112.6 a	1324.5 a
500.0	15.0 a	1026.0 a	93.3 a	22.8 a	110.3 a	1396.0 a
1000.0	13.3 a	1112.3 a	76.0 a	5.8 a	112.2 a	1380.0 a
1500.0	10.8 a	843.8 a	61.0 a	5.5 a	112.5 a	1531.5 a
Year 1 (2018) - 'CS2000' Moderately Resistant						
Amisulbrom Rate (g ai/ha)	**Index of disease (%)**	**Fresh Shoot Weight (g) 10 plants**	**Fresh Root Weight (g) 10 plants**	**Fresh gall Weight (g) 10 plants**	**Plant Height (cm)**	**Yield g/plot**
0.0	2.5 a	1184.3 a	75.0 a	2.5 a	115.2 a	1349.0 a
500.0	2.5 a	1074.0 a	71.8 a	5.5 a	113.3 a	1297.0 a
1000.0	2.5 a	1197.5 a	70.8 a	0.5 a	115.6 a	1418.0 a
1500.0	4.2 a	1494.8 a	96.3 a	4.8 a	113.6 a	1344.5 a

*Means followed by the same letter do not differ according to Tukey test at $P > 0.05$

Table 4.3 Effect of amisulbrom application rate on clubroot index of disease and canola yield and growth parameters under field conditions in Edmonton, AB, Canada, 2019.

Year 2 (2019) - '45H31' Susceptible						
Amisulbrom Rate (g ai/ha)	**Index of disease (%)**	**Fresh Shoot Weight (g) 10 plants**	**Fresh Root Weight (g) 10 plants**	**Fresh gall Weight (g) 10 plants**	**Plant Height (cm)**	**Yield g/plot**
0.0	6.7 a	358.8 a	39.0 a	1.5 a	103.3 a	1163.8 a
500.0	3.3 a	322.0 a	31.8 a	0.8 a	99.7 a	1058.8 a
1000.0	5.0 a	369.0 a	38.8 a	2.3 a	93.6 a	1167.3 a
1500.0	3.3 a	383.3 a	38..8 a	0.8 a	121.9 a	1010.5 a
Year 2 (2019) - 'CS2000' Moderately Resistant						
Amisulbrom Rate (g ai/ha)	**Index of disease (%)**	**Fresh Shoot Weight (g) 10 plants**	**Fresh Root Weight (g) 10 plants**	**Fresh gall Weight (g) 10 plants**	**Plant Height (cm)**	**Yield g/plot**
0.0	20.8 a	350.5 a	45.0 a	6.5 a	88.6 a	1881.0 a
500.0	15.8 a	319.8 a	31.8 a	4.5 a	84.4 a	1684.3 a
1000.0	10.0 a	268.8 a	28.8 a	1.3 a	86.3 a	1499.8 a
1500.0	12.5 a	293.0 a	31.5 a	4.8 a	82.2 a	1666.0 a

*Means followed by the same letter do not differ according to Tukey test at $P > 0.05$

Table 4.4 One-way ANOVA for the 2019 field season.

	numDF	denDF	F-value	p-value
Intercept	1	24	39.64	<0.0001
Seed Type	1	24	11	0.0029
Treatment	3	24	0.86	0.4752

Table 4.5 Three-way ANOVA table for the first run of the greenhouse experiment.

	DF	Sum Sq	Mean Sq	F-Value	P-Value
Incorporation Method	1	9.0	8.5	0.01	0.9204
Concentration	1	6602.0	6601.6	7.82	0.0074
Treatment	3	18355.0	6118.3	7.25	0.0004
Incorporation Method: Concentration	1	1567.0	1567.2	1.86	0.1793
Incorporation Method: Treatment	3	1017.0	339.0	0.40	0.7524
Concentration: Treatment	3	363.0	121.0	0.14	0.9334
Incorporation Method: Concentration: Treatment	3	2720.0	906.6	1.07	0.3689
Residuals	48	40507.0	843.9		

Table 4.6 Effect of amisulbrom application on clubroot index of disease and canola ('45H31') growth parameters under greenhouse conditions (first run of experiment) and *Plasmodiophora brassicae* resting spore levels.

Application Rate (g active ingredient/ha)	Index of Disease (ID %)		Plant Height (cm)		Shoot Weight (g)		Root Weight (g)		Gall Weight(g)	
	Resting spores g^{-1} medium		Resting spores g^{-1} medium		Resting spores g^{-1} medium		Resting spores g^{-1} medium		Resting spores g^{-1} medium	
	1×10^4	1×10^7	1×10^4	1×10^7	1×10^4	1×10^7	1×10^4	1×10^7	1×10^4	1×10^7
0.0	48.3 a	73.8 a	33.7 b	30.0 b	60.5 b	39.2 b	9.1 a	8.6 a	7.1 a	8.0 a
500.0	21.3 b	42.9 ab	36.8 a	36.7 a	71.9 a	63.5 a	6.4 a	5.5 a	4.1 a	3.9 b
1000.0	13.3 b	25.8 b	37.0 a	36.0 a	68.9 b	57.3 a	5.8 a	4.5 a	3.1 a	3.0 b
1500.0	8.8 b	30.4 ab	39.6 a	35.1 a	80.6 a	51.6 a	5.0 a	4.8 a	2.4 b	3.5 b

*Results from in-furrow and broadcast application methods were pooled, due to lack of statistically significant differences between the two

**Means followed by the same letter do not differ according to Tukey test at $P > 0.0$

Table 4.7 Three-way ANOVA table for the second run of the greenhouse experiment.

	DF	Sum Sq	Mean Sq	F-Value	P-Value
Incorporation Method	1	39.1	39.1	0.10	0.7481
Concentration	1	779.3	779.3	2.08	0.1556
Treatment	3	15346.4	5115.5	13.66	1.43E-06
Incorporation Method: Concentration	1	351.6	351.6	0.94	0.3374
Incorporation Method: Treatment	3	2692.2	897.4	2.39	0.0796
Concentration: Treatment	3	1301.9	434	1.16	0.3351
Incorporation Method: Concentration: Treatment	3	868.6	289.5	0.77	0.5146
Residuals	48	17969.4	374.4		

Table 4.8 Effect of amisulbrom application on clubroot index of disease and canola ('45H31') growth parameters under greenhouse conditions (second run of experiment) and *Plasmodiophora brassicae* resting spore levels.

Application Rate (g active ingredient/ha)	Index of Disease (%)		Plant Height (cm)		Shoot Weight (g)		Root Weight (g)		Gall Weight(g)	
	Resting spores g^{-1} medium		Resting spores g^{-1} medium		Resting spores g^{-1} medium		Resting spores g^{-1} medium		Resting spores g^{-1} medium	
	1×10^4	1×10^7	1×10^4	1×10^7	1×10^4	1×10^7	1×10^4	1×10^7	1×10^4	1×10^7
0.0	100.0 a	100.0 a	24.3 b	23.3 b	60.6 b	56.8 b	40.8 a	40.3 a	40.8 a	40.3 a
500.0	67.5 b	74.6 ab	30.5 a	28.9 ab	102.4 ab	77.9 ab	35.8 a	34.3 a	29.6 a	30.8 a
1000.0	56.7 b	78.3 ab	32.6 a	33.0 a	104.6 ab	101.0 ab	36.1 a	45.1 a	28.8 a	42.1 a
1500.0	59.2 b	58.3 b	32.9 a	32.4 a	111.8 a	109.1 a	30.6 a	33.1 a	21.5 a	22.6 a

*Results from in-furrow and broadcast application methods were pooled, due to lack of statistically significant differences between the two

**Means followed by the same letter do not differ according to Tukey test at $P > 0.05$

Chapter 5 – Conclusions

5.0 General conclusions

Clubroot poses a major threat to producers in western Canada. In 2009, the first clubroot resistant (CR) cultivar became available commercially, followed soon afterwards by many other CR cultivars. Resistance quickly became the most widely used tool by producers in controlling clubroot in canola crops (Peng et al. 2015). In 2013, with the discovery of two fields near Edmonton, Alberta, positive for clubroot on CR cultivars, it became evident that there was an erosion of resistance, and since 2013, there has been an increasing number of fields with clubroot on CR cultivars (Chapter 3; Strelkov et al. 2016, 2018a). Given the discovery of resistance erosion, characterizing the virulence of *P. brassicae* populations has become increasingly important for many reasons, including focusing breeding efforts, determining pathotype locations and spread, and helping producers to update on-farm management plans (Strelkov et al. 2018a).

The Canadian Clubroot Differential Set (CCD), which combines the differentials of Williams (1966), Somé et al. (1996), selected hosts of the European Clubroot Differential (ECD) (Buczacki et al. 1975), and hosts of particular importance in Canada, is a robust system to characterize existing and new pathotypes of *P. brassicae* (Strelkov et al. 2018a). As discussed in Chapter 3 of this dissertation, there are now 36 unique pathotypes known in Canada, 19 of which overcome 1st generation clubroot resistance (derived from the *B. napus* 'Mendel'; Fredua-Agyeman et al. 2018), and 17 of which are still controlled by this resistance. This underscores the vast diversity in the virulence of *P. brassicae*. The virulence of these pathotypes on recently released canola cultivars that contain '2nd generation' clubroot resistance is still not clear, and should be the focus of future studies (see below).

Clubroot continues to spread, with the first cases identified in Saskatchewan, Manitoba, the Peace Country of northwest Alberta, and North Dakota over the last decade (Strelkov & Hwang 2014; Strelkov et al. 2020b; Chapara & Strelkov 2020). Pathotype 3A, as designated on the CCD set, continues to be predominant among the resistance-breaking pathotypes and is now the focus of breeding efforts by most seed companies (Chapter 3; Strelkov et al. 2018a). Another resistance-breaking pathotype, 3D is the second most common, and as discussed in Chapter 3 of this dissertation, is now also a focus of breeding efforts. Pathotype 3H, the predominant pathotype on canola prior to the introduction of clubroot resistance (Strelkov & Hwang, 2014), is still common and predominant in Saskatchewan (Chapter 3).

With the discovery of many new pathotypes, and the likely identification of additional pathotypes in the coming years, it will become difficult for breeders to keep up. This fact underlines the importance of an integrated management strategy, in which clubroot management is not focused solely on genetic resistance. While genetic resistance provides an excellent means for clubroot control, without proper genetic stewardship, the erosion of resistance is likely to continue. In some areas or certain fields with unique pathotypes, genetically resistant cultivars may no longer be an option, as breeding focuses primarily on the most common pathotypes. As such, there has been an enhanced interest on different methods for clubroot control, which give producers the opportunity to develop an integrated disease management plan. Incorporation of various strategies for clubroot control, if correctly executed, could decrease *P. brassicae* resting spore loads, which in turn would decrease disease pressure and increase the longevity of resistant cultivars by lowering the likelihood of pathotype shifting (Diederichsen et al. 2003).

Amisulbrom, discovered in 1999 by Nissan Chemical Industries Ltd. (Tokyo, Japan), has shown promise for the control of oomycete diseases in Japan (Honda et al. 2008). In Chapter 4,

the efficacy of this product for the control of clubroot of canola in Alberta was explored. While most results for the field component of the study were not statistically significant, there were still some numerical trends, including a decrease in clubroot severity with increasing rates of amisulbrom. In the greenhouse component, significant reductions in clubroot severity were observed following amisulbrom treatment at two levels of *P. brassicae* inoculum. Two amisulbrom incorporation methods were compared in the greenhouse experiment, in-furrow vs. broadcast application, which did not result in statistically significant differences. A mechanical or broadcast application to the top soil layer likely poses difficulties for on farm use, as tillage is not as commonly practiced in Canada as it once was, although it may be an option in conservation tillage situations (Lafond et al. 1996; Environment Canada 2019). The promising results obtained with amisulbrom in the greenhouse suggest that it may be an option for clubroot patch management, helping to decrease spore loads, particularly at field entrances where the disease is usually most prevalent (Cao et al. 2009). If its potential efficacy reported in Chapter 4 can be confirmed at the field level with further testing, amisulbrom could be a good addition to an integrated on-farm management plan.

5.1 Future studies and questions

Chapter 3 of this dissertation described the identification of new *P. brassicae* pathotypes, which shows the importance of continued monitoring of pathogen populations. Pathotyping helps to determine which pathotypes are predominant, identifies pathotype diversity 'hot spots', and suggests areas of spread between provinces and in some case across country borders. Annual surveys and the submission of diseased root samples by municipalities, agricultural fieldmen, and agronomists will continue to be important in determining pathotype composition over time. It will also be important to continue mapping of the distribution of pathotypes, helping growers and

agronomists to determine pathotype prevalence and occurrence in their regions, and allowing for informed clubroot management decisions. The development and use of molecular markers for pathotype identification would greatly facilitate high-throughput testing of *P. brassicae* isolates, allowing many more samples to be evaluated for pathotype each year. The availability of pathotype-specific markers is limited at present, although efforts are underway to develop RNase H2-dependent PCR (rhPCR) and SNaPshot assays based on diagnostic polymorphic regions identified through genomic analysis of the pathogen (S.E. Strelkov, personal communication).

With many pathotypes able to overcome 1st generation resistance, multiple companies have released cultivars that carry what is generally referred to as '2nd generation resistance' (Canola Watch 2020). While the nature of this resistance is not in the public domain, it is generally understood to be something different from 1st generation, 'Mendel'-derived resistance, in the future, it will be important to screen these new cultivars for reactions to many of the novel and prevalent pathotypes of *P. brassicae*, especially any pathotypes recovered from 2nd generation CR hosts. By understanding the response of 2nd generation resistance to *P. brassicae* pathotypes in their region, growers may be able to make better decisions regarding rotation of resistance sources and/or the incorporation of other management methods.

It may also be possible to improve the consistency of pathotyping results by refining the CCD set itself. At present, one of the CR canola hosts in the CCD set, '45H29', is a hybrid, seed of which may become limited in the future. It would be preferable to replace this variety with a non-hybrid *B. napus* genotype carrying the same resistance source, and initial work on identifying a suitable replacement has already began (S.E. Strelkov, personal communication) and should continue. The Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) 'Granaat', which serves as the universal suscept, should also be replaced with a *B. napus* susceptible check, since *P. brassicae* in

Canada mainly occurs on *B. napus* canola. Careful screening of any candidate differential genotypes should be conducted before any changes are made, to ensure their suitability for inclusion in the CCD set.

Amisulbrom showed some promise during the field and greenhouse studies performed as part of this dissertation. Since this is one of the first instances of this product being tested for its efficacy at controlling clubroot of canola, more field testing will be necessary to get a better understanding of its efficacy and a more robust data set. Flooding of some of the plots in the field trials conducted in Chapter 4 resulted in poor plant health and decreased disease pressure, likely due to the saturated, anoxic soil conditions. Therefore, more field studies across various growing seasons and soil zones and types will help to determine whether this product will be effective at controlling clubroot on canola, not just in central Alberta, but also across the Canadian Prairies. It will also be useful to compare different formulations of amisulbrom, including potentially a granular formulation or fertilizer impregnated with the product, which will be easier for producers to apply with equipment they already have. The efficacy of such different formulations would also have to be tested.

Since 2003, clubroot has emerged as one of the most important diseases of canola in Canada. Its soilborne nature, hardy resting spores, and shifts in virulence make the clubroot pathogen particularly challenging to control. Growers, agronomists and others with a stake in canola production will need to remain vigilant and apply an integrated approach, making use of all available tools, to ensure the sustainable long-term management of this disease.

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