Factors influencing *Plasmodiophora brassicae* resting spore loads in soil and clubroot disease severity in canola (*Brassica napus*)

by

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Abstract

Clubroot, caused by *Plasmodiophora brassicae*, is an important soilborne disease of *Brassica napus* canola. Studies were conducted to increase understanding of the impact of various soil parameters and crop rotation regimes on *P. brassicae* inoculum levels and clubroot severity, respectively. In one study, the concentration of pathogen resting spores was assessed in 284 soil samples, collected from three clubroot-infested fields (Bassano, Edmonton, and Parkland County, Alberta), by quantitative PCR analysis. The samples also were analyzed for soil organic matter content, electrical conductivity, pH, and boron and calcium concentrations. Data analysis based on the *P. brassicae*-positive samples pooled from the three fields indicated that resting spore concentration was positively correlated with soil organic matter (r^2 =0.51, P <0.0001, N = 127), calcium (r^2 =0.44, P <0.0001, N = 127) and boron concentration (r^2 =0.11, P <0.0001, N = 127), but negatively correlated with soil pH (r^2 =0.09, P = 0.0004, N = 127) and electrical conductivity (r^2 =0.23, P <0.0001, N = 127), respectively. These correlations, while significant, were generally weak and suggest that other factors, such as cropping history, may affect resting spore concentrations in the soil. In a second study, the effects of six crop rotation regimes on clubroot severity were evaluated under greenhouse conditions. Rotations included: T1) continuous cropping of a clubroot-susceptible canola cultivar (S); T2) S –fallow (F) - S – F - S; T3) S – barley (B) - S – B – S; T4) S – resistant canola cultivar (R) - S – R – S; T5) continuous cropping of the same resistant cultivar; and T6) continuous cropping of different clubroot resistant canola cultivars. Index of disease (ID) was recorded along with other plant growth parameters for the canola at the

end of each cycle of a rotation. When galled root tissue collected from a treatment was incorporated back into the soil, the average ID of the susceptible canola cultivar included in T1 to T4 increased significantly ($p \leq 0.05$) from 93.1 % (after the first crop cycle) to 99.6 % (after the fifth crop cycle). The ID of the resistant canola cultivar included in T5 also increased significantly from 12.5 % to 22.3 %. In contrast, when different clubroot resistant canola cultivars were grown in succession in T6, the average ID decreased significantly from 12.5 % to 9.4 % (after the fifth cycle). The data suggest some erosion of resistance after continuous cropping of the same resistant cultivar, which was not observed when different resistant cultivars were rotated.

Dedication

This work is dedicated to Matisse and Valeria, you are always in my thoughts and I hope we will be together soon. As well, this is dedicated to the rest of my family Nicolas, Carmen, Johan, Mario, Sofia, Rodo, Ann, Christian, Stephen, Leslie, Ryland, Boyd and Ridley. Also, this is dedicated to my friends Cesar and family, Alejandro, Claudia and Steve. And I want to give a special thanks to Mohan Thiagarajah, who encouraged me to study this Masters. And further thanks to An Vo and Gabe Botar for your friendship and all your advice during this time at the University.

Preface

This is an original work by Mirko Tabori, who conducted the experiments with the assistance of others as outlined here, and wrote the first drafts of all chapters. Mr. Derek Rennie (technician) mentored Mr. Tabori in conducting the qPCR analysis, and assisted him with soil sample collection at the three field sites examined. Dr. Victor Manolii (technician) helped Mr. Tabori in setting up the greenhouse studies, including preparation of the soil and rating plants for clubroot symptom severity. Dr. Tiesen Cao (research associate) provided advice and guidance to Mr. Tabori as he conducted statistical analyses. Dr. Cao also proof-read the first draft of all of the chapters, providing suggestions for improvement prior to submission to Drs. Strelkov and Hwang (co-supervisors). The co-supervisors then read each chapter and provided additional suggestions and recommendations for improvement, which were in turn incorporated by Mr. Tabori.

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1.1 Clubroot in canola

1.1.1 History and main diseases of canola

The canola plant (*Brassica napus* L. and *Brassica rapa* L.) is a rapeseed type that has been modified, through classical breeding techniques, to produce high protein and oil for human consumption and livestock feeding. Canola belongs to the Brassicaceae family, which also includes broccoli, cauliflower, and mustard.

Rapeseed was first commercially produced in Canada during World War II (1942), with the oil used for lubricating the engines of naval vessels. At that time, controversy surrounded the suitability of rapeseed oil for human consumption, largely because of the high levels of erucic acid in the oil (Daun, 1993). Dr. Keith Downey (Agriculture and Agri-Food Canada) and Dr. Baldur Stefansson (University of Manitoba) started in 1966 to work with two species of rapeseed, the 'Argentine' (*B. napus* L.) and the 'Polish' (*B. rapa* L.), to create varieties of "double-low" rapeseed, which produced much lower levels of erucic acid and glucosinolates than other rapeseed. A few years later, in 1969, breeding research also began at the University of Alberta, initiated by Dr. Kondra. In 1974, the first ''double low'' canola variety with reduced levels of both erucic acid and glucosinolates was registered for commercial production (Stefansson and Kondra, 1975). The term "Canola" actually is a trade name derived from "Canadian Oil Low Acids", although it is now used generically to refer to the crop.

Since the late 1970s, canola has played a vital role in the success of the agriculture industry in Alberta and the prairie region as a whole. Rapeseed production in Canada was

approximately 14.2 million tonnes (MT) in 2012, approximately 20% of the world total (USDA 2012). Canola is the most important oilseed crop in Canada, with 7.9 million ha. seeded in 2013 alone (Statistics Canada, 2013). Canada is the single largest producer of canola in the world (Canola Council of Canada, 2013).

On the global market, rapeseed oil is the third most important source of vegetable oils in the world, after palm and soybean (Beckman, 2005). For western Canadian farmers, it provides some of the best economic returns of any of the crops that can be grown on the prairies. As a consequence, there is a constant propensity to increase canola production, through shortened rotations and increased acreage (Bartkowiak-Broda et al., 2005).

The intensification of canola production has resulted in the increased incidence and severity of various diseases, including damping-off, seedling blight, blackleg, Sclerotinia stem rot, Alternaria black spot or grey leaf spot, and clubroot (Thomas, 2003). Seedling diseases are caused mainly by the fungi *Rhizoctonia solani* Kühn*, Fusarium* spp. and *Pythium* spp. These parasites infect canola seedlings both before and after emergence, and cause symptoms such as seed decay, damping off, seedling blight, or seedling root rot (Gugel et al., 1987)

Blackleg disease is caused by the fungus *Leptosphaeria maculans* (Sowerby) P. Karst*,* which attacks the cotyledons, leaves, stems and pods. Foliar symptoms include the development of white, irregularly shaped spots, and the symptoms on the stems are usually found at the base and appear as brownish lesions or cankers (West et al., 1999)

Sclerotinia stem rot is caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, which attacks mainly the main stem and branches. The symptoms start with the

development of light brown lesions. On the stem, these lesions turn whitish, while the stem may begin to weaken and shred. The survival structures of the fungus, sclerotia, develop inside the stem. Infected plants may produce fewer pods and/or fewer seeds per pod or small pods (Gugel and Morral, 1986). If the main stem is infected, the entire plant may die.

Alternaria black spot or grey leaf spot is caused by a complex of fungi including *Alternaria brassicae* (Berk.) Sacc., *A. alternata* (Fr.) Keissl. and *A. raphani* J.W. Groves & Skolko. These fungi can attack cotyledons, leaves, stems, flowers, pods and seeds. Symptoms of infection may start as light brown lesions on the lower leaves, which can expand and turn a dark brown color, surrounded by chlorotic halos. The lesions may spread rapidly to the stem, flowers and pods (Bailey et al., 2008). *Brassica rapa* is more susceptible to Alternaria black spot than *B. napus*, and as most of the canola acreage in Canada is now *B. napus*, the economic impact of black spot in recent years has been relatively minor.

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, has emerged as an important soil-borne disease of canola on the Canadian prairies (Strelkov and Hwang, 2014). Infection by *P. brassicae* results in gall formation on the roots of susceptible plants, affecting water and nutrient uptake, and ultimately causing stunting, foliar wilting, chlorosis, and even premature death of plants when symptoms are severe. The persistence of clubroot disease and its continuous spread among commercial canola fields pose a significant threat to Canadian canola production (Strelkov and Hwang, 2014; Rempel et al., 2014).

1.1.2 Clubroot disease in Canada

Clubroot can be a devastating disease of canola and other cruciferous plants when the soil is heavily infested with *P. brassicae* and a susceptible cultivar is grown. Crop losses as a result of clubroot infestation were estimated to be at least 10% to 20% worldwide (Dixon, 2009a). In Canada, clubroot disease has been a problem in the production of cruciferous vegetables for more than a century in the Atlantic Provinces, Quebec, Ontario, and British Columbia (Howard et al., 2010). On the Prairies, however, there were only isolated cases of the disease, typically in home or market gardens, until 2003. In that year, clubroot was identified in 12 commercial canola crops near Edmonton, Alberta (Tewari et al., 2005). Annual surveys since then have revealed a rapid increase in the number of fields confirmed to be infested with *P. brassicae*, with nearly 1,500 confirmed infestations by 2013 (Strelkov et al., 2014). The clubroot outbreak remains most severe in central Alberta, although cases of the disease have been reported from southern Alberta, and also in Saskatchewan and Manitoba (Dokken-Bouchard et al., 2010; Cao et al., 2009; Strelkov and Hwang, 2014)

1.2 Biology of *Plasmodiophora brassicae*

1.2.1 Life cycle

Plasmodiophora brassicae is an obligate plant parasite, the taxonomy of which has been much debated over the past century. It is now generally regarded as a protist, in the Phylum Cercozoa, Sub-Phylum Endomyxa, Class Phytomyxea (Cavallier-Smith, 2003). Phytomyxeans include zoosporic species with two flagella of varied length. The zoospores (and dormant resting spores) are the only part of the life cycle outside of the

host, and can move short distances in the soil (Karling, 1968; Dixon, 2009).

The life cycle of *P. brassicae* consists of three stages: survival in the soil, root hair infection, and cortical infection (Kageyama and Asano, 2009). The resting spores of *P. brassicae* are durable and highly resistant structures, which allow this organism to endure adverse conditions. The half-life of the resting spores in the soil has been estimated as 3.6 years under Swedish conditions (Wallenhammer, 1996) and 4.4 years in the Edmonton area (Hwang et al., 2013). Despite the fact that they can remain inactive in the soil for many years, the resting spores are capable of responding quickly once a compatible host is available. The resting spores germinate to release primary zoospores, which can penetrate the cell walls of the root hairs. Once inside the root hairs, the pathogen forms a primary plasmodium (Kageyama and Asano, 2009). Another set of zoospores, termed secondary zoospores, develop from the plasmodia; it has not been clearly established whether or not the secondary zoospores are released back into the soil, where they re-infect the host tissue, or whether they move inside the root hair (or both possibilities) (Kageyama and Asano, 2009). Regardless, the infection by the secondary zoospores results in the development of secondary plasmodia, which develop inside cells of the root cortex. The plasmodia develop into the host vascular rays, vascular cambium and cortex (Asano and Kageyama, 2006; Kageyama and Asano, 2009). An active myxamoeboid phase also has been described by some researchers. The myxamoeba may enable the spread of the infection inside the host (Donald et al., 2008). Secondary infection is associated with the development of visible clubroot symptoms, as infection results in hormonal disturbances in the roots, causing hyperplasia and hypertrophy of the affected tissues (Kageyama and Asano, 2009). Eventually, the secondary plasmodia

cleave to produce a new generation of resting spores, which are released back into the soil as the root galls decompose.

1.2.2 Germination of resting spores

As noted above, the resting spores of *P. brassicae* can live in the soil for extended periods of time, with at least half of the resting spores surviving between 3 and 6 years after being produced and some surviving up to 17.3 years (Wallenhammar, 1996). The longevity of the resting spores creates significant challenges for the successful management of clubroot disease, which are exacerbated by the ability of *P. brassicae* to reproduce on a wide number of host plants, and by the genetic diversity that is often observed in pathogen populations (Howard et al., 2010).

It has been shown that plant root exudates from host and non-host species can stimulate *P. brassicae* resting spore germination (Kageyama and Asano, 2009). However, once germination occurs, they have to find a host soon in order to survive (Takahashi, 1994). For example, in highly infested fields in Alberta, the levels of disease incidence and severity were lower after the planting of a cruciferous bait crop versus a non-cruciferous bait crop (Ahmed et al., 2011).

Also, under conditions of high soil moisture, *P. brassicae* zoo spores have greater mobility. In high humidity soil with 60% - 80% moisture content, the incidence of clubroot is higher (Narisawa et al., 2005).

This has led researchers to explore the possibility of growing bait crops to reduce spore concentrations in the soil, by planting them in order to induce resting spore germination,

but then destroying the bait crop before *P. brassicae* has the time to generate a new generation of resting spores.

According to studies by Friberg et al. (2005), growing bait crops might be a good strategy for the management of clubroot because it stimulates resting spore germination in the absence of host plants. However, the results of studies from the field have been mixed (Hwang et al., 2006).

1.2.3 Pathotypes of *P. brassicae* in Canada

Single-spore and field isolates (populations) of *P. brassicae* can differ in their infectivity on specific host genotypes, and numerous races or pathotypes of the clubroot pathogen have been identified based on their virulence on a host differential set. Three sets of differential hosts have been used most commonly over the past few decades. These include the European Clubroot Differential (ECD) set (Buczacki et al., 1975), the differentials of Williams (1966), and the differentials of Somé et al. (1996). The ECD set consists of 15 host genotypes representing three subsets (*B. rapa*, *B. napus*, and *B. oleracea*) of host species (Buczacki et al., 1975). By contrast, the differentials of Williams (1966) consist of just two *B. napus* var. *rapifera* and two *B. oleracea* genotypes, while those of Somé et al. (1996) consist of three *B. napus* genotypes. Traditionally, the most extensively utilized differentials for the study of pathogenic diversity in *P. brassicae* in Canada have been the differentials of Williams (1966). Populations of *P. brassicae* obtained from Alberta were identified as pathotypes 3, 2 and 5. Pathotype 3 also was found in Nova Scotia and Saskatchewan, and pathotype 2 was identified in Quebec and Nova Scotia. Research also showed the presence of pathotype 6

in British Columbia and Ontario (Strelkov et al., 2006; Xue et al., 2008; Cao et al., 2009; Strelkov and Hwang, 2014). Pathotype 1 was also reported from Nova Scotia (Hildebrand and Delbridge, 1995).

Using single resting spore isolates instead of field populations, Xue et al. (2008) found that the pathotype composition of *P. brassicae* in Canada was more diverse. For example, while pathotype 3, as designated on the differentials of Williams (1966), represented 87% of the populations in Alberta, this percentage dropped to 72% when single-spore isolates were examined. Pathotypes 2, 5, 6, and 8 also were found in Alberta in lower frequencies (Strelkov et al., 2006, 2007; Xue et al., 2008). The fairly diverse pathotype composition in Alberta may represent a challenge for resistance breeding activities, since plant material must be screened against isolates or populations representing all known pathotypes of *P. brassicae*. Populations and single-spore isolates classified as pathotype 3 on the differentials of Williams (1966) were generally classifies as ECD 16/15/12 or P2 (Some et al. 1996; Strelkov et al., 2006; Strelkov et al., 2007; Cao et al., 2009).

1.2.4 Detection and quantification of *P. brassicae* in soil

The presence of *P. brassicae* was traditionally assessed by a soil bioassay to determine whether or not samples are infested with *P. brassicae*. The bioassay is performed by planting 1-week-old Chinese cabbage (*Brassica rapa* var. *pekinensis* cv. Granaat) seedlings or another highly susceptible host in the soil to be tested. The soil is often mixed with potting medium to favor good plant growth under greenhouse conditions. Six to eight weeks after planting, the plants are carefully dug out of the soil,

and the roots are washed with water and assessed for clubroot symptom development (Faggian and Strelkov, 2009).

In recent years, molecular diagnostic methods also have been commonly used to detect *P. brassicae* in soil and plant samples, usually based on PCR analysis. The polymerase chain reaction (PCR) is a cell-free method of DNA cloning, where defined target DNA sequences present within a source of DNA are amplified. The procedure was first introduced by Kerry Mullis in 1983 (Mullis, 1990). To allow selective amplification, a DNA sequence preceding the desired sequence is required (Dorak, 2007). This is used to create two primers specific for the target sequence that is approximately 15-25 nucleotides long. The deoxyribonucleotides (dATP, dCTP, dGTP and dTTP), which serve as the components of DNA, are added to the PCR along with the enzyme DNA polymerase, which synthesizes the DNA fragments. The PCR is conducted in three successive steps, including a denaturation step at about 93°C to 95°C, annealing at temperatures from about 50°C to 70°C, and extension of the DNA at about 70°C to 75°C.

PCR-based detection of *P. brassicae* requires an efficient method for DNA extraction from the soil (or tissue samples) (Faggian and Strelkov, 2009). A straightforward, PCR-based protocol for the detection of the clubroot pathogen in soil and plant samples was developed by Cao et al. (2007). Primers specific to *P. brassicae* were designed based on the ribosomal DNA region, and were confirmed as being highly specific only for the target organism. Indeed, under the PCR conditions developed, these primers did not generate any amplicons from non-infected plant hosts, or non-infested soil (Cao et al. 2007).

While conventional PCR analysis is useful for detecting the presence or absence of *P. brassicae*, it does not provide an accurate estimate of the amount of pathogen biomass in a test sample. For this purpose, real-time or quantitative PCR (qPCR) analysis is a more appropriate technique. Quantitative PCR, while also based on the selective amplification of highly specific DNA sequences, involves the constant compilation of fluorescent signals from one or more PCRs over a series of cycles, which can be used for the conversion of the fluorescent signals from each reaction into a numerical value for each sample (Dorak, 2007). A number of qPCR-based methodologies have been developed to measure *P. brassicae* in soil and plant samples (Wallenhammar et al., 2011; Sundelin et al., 2010). Standard curves can be developed to relate a specific amount of *P. brassicae* DNA with a certain number of pathogen resting spores, allowing the generation of estimates of resting spore concentrations in the soil (Rennie et al., 2011; Hwang et al. 2012). The availability of molecular methods to detect and measure *P. brassicae* in soil samples has greatly simplified work with this pathogen, facilitating surveillance activities and enabling research that would not otherwise have been possible.

1.3 Integrated management of *P. brassicae*

Integrated pest management (IPM) refers to a set of techniques or practices that are used to determine if a pest treatment is required, and to establish an action threshold to know when a pest population has to be controlled for the crop to not suffer an economic loss. A variety of strategies may be incorporated in order to effectively manage a pest, with the aim of more effective control while minimizing the environmental and economic costs associated with excessive use of pesticides.

Integrated pest management has been used successfully in some cases to reduce the impact of clubroot disease. In Australia, for example, the use of a combination of practices rather than a single approach for the management of clubroot in acidic soils increased the profit per hectare between $200 and $6,579 (Donald and Porter, 2009). This included the use of practices such as the application of lime to increase the soil pH, in addition to the application of chemical treatments such as the fungicide fluazinam. The Australian example reflects the experience with clubroot management in cruciferous vegetable crops, not canola, but nevertheless highlights the potential benefits of a holistic approach.

1.3.1 Cultural control

Cultural control refers to any practice that involves modification of the plant growth environment in order to reduce or avoid pests. A number of IPM practices have been suggested for the control of clubroot, including liming of the soil increase its pH, crop rotation, and sanitation practices (Strelkov et al., 2011).

Liming

Liming of the soil may increase the soil pH to an extent that creates adverse conditions for the development of clubroot disease. It is well known that clubroot is favored under acidic conditions, and hence any practice that raises the pH results in conditions that are less amenable to the disease. Liming of the soil is most effective when the *P. brassicae* infestation is mild, and a soil test is required to determine if such a treatment is necessary (Hwang et al., 2011a). The application of lime, however, may be

impractical or prohibitively expensive over the large numbers of hectares typically associated with canola production.

Crop rotation

Crop rotation involves rotating susceptible Brassica crops with non-hosts, which can help to reduce *P. brassicae* infestation levels in the soil (Friberg et al., 2006, Howard et al., 2010). Cereal grains, alfalfa, onions, peas, beans and carrots are some examples of non-host crops that could be included in a rotation (Howard et al., 2010). When the soil inoculum level is high, longer rotations are required to reduce the infestation to levels below a threshold where clubroot symptoms are unlikely to develop (Donald and Porter, 2009). In some cases, the length of rotations may be perceived as exceedingly long by farmers.

Baiting crops

Baiting crops refer to the planting of plants that will serve as 'bait' for *P. brassicae*, inducing germination of resting spores and thereby depleting inoculum levels in the soil. Clubroot incidence and severity on canola were shown to be reduced using a cruciferous bait crop versus a non-cruciferous crop or a cereal in highly infested soil under greenhouse conditions in Alberta (Ahmed et al., 2011). The root exudates of non-cruciferous crops, including leek (*Allium ampeloprasum* var. *porrum* (L.) Gay), rye (*Secale cereale* L.) and red clover (*Trifolium pratense* L.), also have been shown to stimulate resting spore germination. However, under field conditions, any of these non-cruciferous crops decreased the amount of *P. brassicae* in soils when they were bioassayed (Friberg et al., 2006).

Seeding date

The manipulation of seeding date has been explored as another method to control clubroot. For example, planting a crop when soil temperatures are cooler may inhibit disease development, since cooler temperatures are not favored by *P. brassicae* (Sharma et al., 2011). Early seeding, when soil temperatures were cooler, reduced clubroot disease severity by 10%-50%, and increased yields by 30%-58% (Gossen et al., 2012). Older plants also exhibit a reduced vulnerability to infection, and the infection that does occur is less likely to affect yield (Hwang et al., 2012).

Sanitation

This approach involves cleaning or disinfestation of tools and field equipment to reduce or eliminate the movement of *P. brassicae* infested soil. Soil on equipment that has been used on a *P. brassicae* infested field should be removed, first by knocking off large pieces of soil and then by washing with pressurized water or air. For risk-averse farmers, the machinery should then be disinfected with bleach or another suitable disinfectant (Howard et al., 2010). While the movement of *P. brassicae* as an external contaminant on seeds and tubers is likely to be rare, seed cleaning and or treatment with fungicides serves to greatly reduce any possible risk and also constitutes a form of sanitation (Rennie et al., 2011; Hwang et al., 2012).

1.3.2 Chemical control

For clubroot disease control, the main pesticides that have been used include alkylene bisdithiocarbamate, pentachloronitrobenzene, flusulfamide (MTF651, Nebijin),

and fluazinam (Shirlan or Omega) (Donald and Porter, 2009). In Canada, the fungicides registered for control of clubroot on Brassica crops are pentachloronitrobenzene (Adobe 75WP, Crusoe 75WP and Quintozene 75WP) and fluazinam (Allegro 500F). These products can be used as pre- or post-planting drenches on transplants (Howard et al., 2010). Fungicides that served to reduce or eliminate seed-borne inoculum under greenhouse conditions included Dynasty 100 FS (azoxystrobin), Helix Xtra (thiamethoxam+difenoconazole+metataxyl+fludioxonil), NebijinTM 5SC (flusulfamide), Prosper FX (clothianidin+carbathiin+ trifloxystrobin+metalaxyl), and Vitavax RS (carbathiin+thiram) (Hwang et al., 2012). The pesticides quintozene (PCNB) and fluazinam are registered for the control of clubroot on canola in Australia and several other countries (Donald and Porter, 2009), but not in Canada.

1.3.3 Biological control

Biological control refers to the use of microorganisms or natural extracts to control a pest. There have been reports that the rhizobacteria *Streptomyces graminifaciens* G11 and *Pseudominas fluorescens* H237 could reduce clubroot disease severity in canola by 15% to 20% in greenhouse experiments (Peng et al., 2014). Another report indicated that use of the extracts from the plant *Azadirachta indica* (A. Juss.) (Indian neem tree) reduced clubroot disease severity on Brassica crops when they were exposed to *P. brassicae* (Bhattacharya and Pramanik, 1998). In Canada, a study by Peng et al. (2014) evaluated 5,000 soil microorganisms indigenous to the Canadian prairies for activity against *P. brassicae*, and found that three endophytic fungal isolates and four rhizobacteria reduced clubroot disease severity by more than 75% when applied as soil drenches under controlled conditions (Peng et al., 2014).

1.3.4 Genetic resistance

Genetically resistant varieties can provide the most cost effective and valuable control of clubroot disease. Efforts to develop clubroot resistant canola varieties began soon after its initial identification on canola in Alberta. Pioneer Hi-Bred released the first clubroot-resistant canola hybrid, '45H29', in 2009 (Howard et al., 2010), following which a number of products from numerous seed companies have become available to Canadian farmers. In the UK and mainland Europe, the clubroot resistant white cabbage cvs. Tekila, Kilaton, and Kilaxy, and the cauliflower cv. Clapton, were released in 2005 (Donald and Porter, 2009). In Australia, the resistant cabbage cv. Maxfield and the cauliflower cv. Highfield were released in 2007 (Donald and Porter, 2009). The intensive use of resistant varieties under conditions of high disease pressure may result in a breakdown or erosion of resistance (Howard et al., 2010). This occurred in Australia, where continuous cropping of an older resistant Chinese cabbage ('Yuki') resulted in its resistance being overcome (Donald and Porter, 2009). Similarly, greenhouse studies with a Canadian population and single-spore isolate of *P. brassicae* showed that the continuous cropping of a resistant cultivar may result in a fairly rapid erosion of that resistance, as *P. brassicae* populations can adapt quickly to selection pressure (LeBoldus et al., 2012).

1.4 *P. brassicae* infested soils in Alberta

1.4.1 Types of soils in Alberta

Soil types in Alberta include Brown Chernozemic, Dark Brown Chernozemic, Black Chernozemic, Dark Gray Chernozemic, Dark Gray Chernozemic, Luvisolic, Brunisolic, and Cryosolic (Alberta Agriculture and Rural Development, 2005). Brown Chernozemic

soils are located in the driest or semi-arid region, in the southeast part of Alberta, and contain 3% to 4% organic matter. As the name implies, the color of this soil is light brown on the surface layer. One of the most important constraints in the semi-arid region is the lack of water, and many crops require irrigation systems. The Dark Brown Chernozemic soils contain about 4% to 6% organic matter, and are found mainly in the northern and western areas of the semi-arid region, and in a small area in the south-east of the province. This type of soil typically receives more moisture than the Brown Chernozems, but rainfall is still limited. Black Chernozemic soils contain 6% to 10% organic matter and are found to the north and west of the Dark Brown Chernozemic soils. As well, this soil type can be found in the north-west of the province in the Peace River region. This type of soil receives and holds greater amounts of moisture than the Brown and Dark Brown Chernozemic soils, and is ideally suited for production of agricultural crops. Dark Gray Chernozemic soils also contain 6% to 10% organic matter and are more commonly found in areas with long periods of frost mainly in the central west part of the province. Cropping options are more limited in this region.

Luvisolic soils contain a surface layer with material that is composed of forest matter, with the organic matter becoming more readily available after agricultural activities begin. These soils are also called grey wooded soils, mostly located in the north east region of the province. Brunisolic soils have a thick surface layer of organic matter with a dark brown or dark surface. They are situated mainly in drained and sloped areas (mountains), and the areas where they are located are usually extremely cold. Crop growth in these areas is limited due to the short growing seasons and slope of the terrain. Brunisolic Gray Luvisols are usually located in mountainous terrain and the foothills

region, on slopes of 2-15%, and are associated with coniferous mixed forests. Cryosolic soils have permafrost within one meter of the surface. Mainly forest trees grow in this region and also are associated with sloped lands. The surface layer is thick with an organic matter content of more than 30%. Organic Cryosolic soils are developed from material that has more than 17% organic carbon and are situated within one meter of the surface. Most of lands with this type of soil are palsas and areas containing peat moss (Alberta Agriculture Soils Branch, 1985). Vertisol soils are associated mainly with native vegetation consisting of xerophytic and mesophytic grasses. Regosolic soils do not contain a recognizable B horizon of at least 5 cm thickness, and consequently are referred to as being weakly developed. This deficiency with respect to a fully developed pedogenic B horizon may reflect various factors including the youthfulness of the material, recent alluvium, instability of the material, colluvium on slopes subject to mass wasting, and (or) the nature of the material and dry cold conditions. (Agriculture and Agri-Food Canada).

1.4.2 Soil characteristics

1.4.2.1 Organic matter

Organic matter refers to a wide variety of materials that originate primarily from plant and microbial residues. This matter also has been described as a combination of easily recognizable plant material undergoing decomposition, and humus, a dark substance associated with minerals and whose components are visually unidentifiable (Certini et al., 2006). Humus is important because it can influence water retention, provides nutrients used by plants to grow, and also retains macro- and micro-nutrients with positive charges in the soil for plant use (Singer et al., 1996). Organic matter is

mostly concentrated near the soil surface and varies in nature and quantity. Most of the organic content adheres strongly to the mineral particles, especially in clay soils (Gerrard, 2014).

The levels of organic matter are influenced by soil temperature, aeration and moisture. Soil acidity will not significantly affect the rate of losses in organic matter when soil pH values are between 5.5 and 7.5. Organic matter will be lost quickly when adequate levels of moisture are present with good aeration and high soil temperatures (Simpson, 1983).

1.4.2.2 pH

Hydrogen ion concentration in the soil solution influences most solid-liquid interactions in the soil. Soil pH values normally range from 4 to 10. A pH of 7 is considered neutral, and soil concentrations below 7 are considered acidic, while those above 7 are considered basic (Black, 1993; Coyne and Thompson, 2006). As a natural process, acidification is favoured by high rainfall, low evaporation and drainage. These oxidative biological activities produce acids and limited input of Ca^{++}, Mg^{++}, K^{+} and $Na^{+}$from the soil parental material (Gerrard, 2014). Soils with a pH value of 5.5 or lower can slow the growth of sensitive plant species. Roots are frequently the first organs to show injury as a result of soil acidity, the presence of Al^{+++}, or a deficiency in Ca^{++} (Singer et al., 1996). Percolated soils tend to be acidic and are usually associated with wet climates. Soils are more alkaline in drier regions (Gerrard, 2014).

1.4.2.3 Electric conductivity

Soil salinity can be measured as the electric conductivity of either a soil solution or a soil-water extract. The term salinity refers to the occurrence of the main dissolved inorganic solutes (i.e., Na^{+}, Mg^{++}, Ca^{++}, K^{+}, Cl^{-}, $SO4^{=}$, HCO_3^{-}, NO_3^{-} and $CO_3^{=}$) in liquefied samples (Rhoades et al., 1999). Salinity is quantified in terms of the total concentration of soluble salts or in terms of the electrical conductivity in the solution of a sample (US Salinity Laboratory Staff, 1954). Soil salinity is primarily associated with arid and semiarid locations. Under such conditions, there is insufficient water to leach out the soluble salts and consequently they build up within the soil (Gerrard, 2014). Consideration of soil types and their characteristics such as organic matter, pH, and electric conductivity with clubroot severity may help determine if the disease is more prominent in certain locations and in soils with certain characteristics.

1.5 Relationship between soil characteristics and clubroot disease development

Soil characteristics, such as soil pH, boron, and calcium, can influence the development of clubroot disease. In general, clubroot is favoured by acidic soil at high moisture and cool temperatures (Karling, 1968). The addition of calcium to the soil to increase pH may reduce clubroot disease (hence the liming of the soil as a clubroot management technique). It has been shown that sustaining a soil pH of 7.2 or higher, via the application of various soil amendments, including lime, can be an effective strategy for decreasing clubroot levels in horticultural crops (Murakami et al., 2002).

Lime amendments can be of various types in agriculture, including calcitic lime (calcium carbonate), dolomitic lime (calcium and magnesium carbonate), hydrated lime (calcium hydroxide), and quicklime (calcium oxide) (Howard et al., 2010).

Calcium can reduce the infective capacity of the *P. brassicae* resting spores, and also can affect the development of plasmodia in the root hairs (Webster and Dixon, 1991; Dixon and Page, 1998). On the other hand, lime also failed to control clubroot in a "lime nonresponsive" soil, even after the soil pH was increased to 7.7 (Donald and Porter 2009). Calcium can be applied as calcium sulphate ($CaSO_4$) or calcium carbonate ($CaCO_3$), or in combination with nitrogen as calcium cyanamide ($CaCN_2$). The application of calcium cyanamide has been demonstrated to raise the microbial population in the soil, thereby reducing the viability of *P. brassicae* (Dixon, 2009).

Boron also can reduce clubroot disease severity through pre-plant applications. In general, boron application has been suggested as a method to decrease clubroot on vegetable crops for more than 70 years (Dixon, 2009). Boron may delay clubroot development and increase vegetative growth compared with non-treated canola in organic soil (Deora et al., 2014). The application of boron in Brassica vegetables typically reduces the occurrence of clubroot by up to 50%, and increases yields by 30% (Dixon, 2009). Also, boron slowed the development of primary plasmodia in root hairs into sporangia, as well as the development of secondary plasmodia in the root cortex (Webster and Dixon, 1991). Boron has an important role in the structure of plant cell walls, and it also may interfere with the penetration of zoospores into the root hairs (Dixon, 1996). Although the application of boron into the soil has resulted in a reduction of clubroot disease in canola, it can also cause toxicity in plants when it is applied in high dosages (Deora et al., 2011).

1.6 Geographic information systems and clubroot

A geographic information system (GIS) is a computer-based system that collects, stores, and displays data related to geographical positions. A GIS can be a valuable tool that enables the creation of a data output that can be used for optimizing crop management (Dalsted, 2011), such as by preventing weed, insect, or disease infestations in crops. Such systems also can be used as tools to study the relationship between pest behavior and weather conditions, between soil types and mineral nutrients in the soil, and between soil characteristics and disease development.

A GIS can be used as a powerful tool in precision agriculture, including clubroot disease management. A good example of this potentially utility is illustrated in an experiment that was conducted in Japan in the early 1990s (Torige et al., 1993). In that experiment, digital satellite photographs of crops were converted to infrared images. The degree of moisture stress in the crop, which corresponded to the severity of clubroot symptoms, was assessed by the amount of infrared light reflected. This information was combined using GIS with data on soil characteristics, enabling the researchers to examine the relationship between the various parameters and conclude that soils with poor drainage were associated with increased clubroot disease (Torige et al., 1993).

1.7 Research objectives

The research presented in this thesis was intended to increase understanding of the impact of crop rotation and various soil parameters on clubroot severity and inoculum levels, respectively. Two separate studies were conducted to examine these topics. In the first study (Chapter 2), a large number of soil samples were collected from fields that were naturally infested with *P. brassicae*, and characterized for soil pH, organic matter,

electric conductivity, calcium, and boron content. The resting spore concentration in these same samples also was determined by qPCR analysis, and the relationship between inoculum level and each of the soil properties was then examined statistically to identify any trends or significant correlations. In the second study (Chapter 3), six crop rotation regimes were compared for their effects on clubroot severity and crop growth characteristics. Briefly, five cycles of different host and non-host plant genotypes were grown consecutively in *P. brassicae*-infested soil under greenhouse conditions, after which clubroot severity and parameters such as plant weight and height were assessed and compared in a final, susceptible canola crop. A short synopsis of the major findings from the studies is presented in Chapter 4, along with some general conclusions and suggestions for future work.

2 Evaluation of soil properties and *Plasmodiophora brassicae* resting spore concentrations in soils cropped to canola in Alberta, Canada

2.1 Introduction

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is an important soil-borne disease of canola on the Canadian prairies and other regions where cruciferous crops are grown. Infection by *P. brassicae* results in gall formation on the roots of susceptible plants, decreasing the efficiency of water and nutrient uptake and ultimately causing stunting, foliar wilting, chlorosis, and even premature death of plants when symptoms are severe. Crop losses as a result of clubroot infestation were estimated to be at least 10% to 20% worldwide (Dixon, 2009). In Alberta, Canada, clubroot was first identified on canola (*Brassica napus* L.) in 2003 in 12 commercial crops near Edmonton (Tewari et al., 2005). Since then, annual surveys have revealed a rapid increase in the number of clubroot infested fields, with 1482 confirmed infestations by 2013 (Strelkov et al., 2014). The clubroot pathogen produces long-lived resting spores, and once introduced into the soil, it is difficult to completely eradicate a *P. brassicae* infestation. This persistence and the negative effects of *P. brassicae* pose a significant threat to Canadian canola production (Rempel et al., 2014).

The development of clubroot is favoured by acidic soil with high moisture and cool temperature (Karling, 1968). Supplemental calcium and liming to increase soil pH may reduce disease pressure (Murakami et al., 2002). Pre-planting applications of boron may

delay clubroot development and increase vegetative growth and yield compared with non-treated canola in organic soil (Deora et al., 2014). Climate change also may contribute to the outbreak of certain diseases (Chakraborty et al., 2000). Therefore, a good understanding of the factors that are favourable for clubroot development may help to answer the question of why clubroot is more severe in some areas than others, which eventually may lead to the development of effective control measures for this disease in Canada.

In the current study, a total of 284 soil samples were randomly collected from three commercial canola fields in central Alberta for analyses of *P. brassicae* resting spore concentration, organic matter, pH, electrical conductivity, calcium and boron levels. The objective of this study was to investigate the relationship between resting spore concentration and these various soil parameters. Previously, a meta-analysis of field data collected from 2005 to 2010 revealed a significant negative correlation between clubroot severity and soil pH (Gossen et al., 2013), although this relationship was not as strong as expected.

2.2 Materials and methods

2.2.1 Experimental sites and soil sampling

The areas used for soil sampling in the study consisted of three different locations in Alberta where symptoms of clubroot disease had been identified over the past few years. Soil samples were taken from three commercial fields, including one field near Bassano in southern Alberta (Brown Chernozemic soil; 50° 47′ 3″ N, 112° 27′ 5″ W), one field in northeast Edmonton (Black Chernozemic soil;

53° 52′ 44″ N, 113° 2′ 13″ W), and a third field in Parkland County (Black Chernozemic soil; 53° 31′ 48″ N, 114° 0′ 23″ W) west of Edmonton. The specific points sampled in each field were selected using a random point generator in ArcGIS (ESRI Canada, Toronto, ON), and were collected with a soil auger. A total of 284 soil samples were collected including 95 samples from Bassano, 93 samples from northeast Edmonton, and 96 samples from Parkland County, with about 500 g soil contained in each sample. All of the soil samples were collected in the summer of 2012 from the top soil layer (0 to 30 cm) and were brought to the laboratory in brown paper bags for quantification of *P. brassicae* resting spore concentration and analysis of pH, electrical conductivity, organic matter, calcium, and boron levels.

2.2.2 Soil property analysis

Soil pH and electrical conductivity were measured with an AB200 pH/Conductivity Meter (Thermo Fisher Scientific Company, Waltham City, MA, USA). Briefly, 10 g of soil was placed in 20 mL water. This mix was agitated for 30 minutes on a laboratory shaker and then centrifuged for 1 min. The supernatant was decanted and transferred into a new tube. The probe tip was submerged into the solution until the meter reading stabilized, with the pH and E.C. values recorded. The tip of the probe was cleaned between readings with deionized water (Miller et al., 1982).

For analysis of soil organic matter, soil samples were first air-dried and then sieved through 2 mm mesh. Approximately 20 g soil of each sample was placed in a crucible and kept in Tube Furnace 51441 (Thermal Product Solution Company, New Columbia,

PA, USA) for 16 h at 375^{o}C. Soil organic matter was expressed as the percentage of weight loss of the soil in the crucible after incineration (Martin, 2008).

Soil calcium and boron concentrations were analyzed with an inductively coupled plasma atomic emission spectrometer (iCAP™ 6000 Series ICP-OES, Thermo Fisher Scientific, Waltham City, MA, USA). Soil samples were ground to a powder and passed through a sieve. Fifty mL of concentrated HCl (for boron measurements) or 50mL of HNO_3 for (calcium) was added to 5 g aliquots of each soil sample. The mixture was warmed in a water bath at 90^{o}C for 1 h, allowed to cool, and then diluted with 1L of distilled water and filtered for the determination of isotope ratios with the spectrometer (Garbarino et al., 1994).

2.2.3 DNA extraction, PCR and quantitative PCR analyses

Approximately 250 mg air-dried soil was used for DNA extraction with a PowerMix Soil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA). The DNA concentrations of all the samples were determined using a NanoDrop (Thermo Fisher Scientific company, Waltham City, MA, USA), and then adjusted to 2 ng/μL before being subjected to PCR analysis. Conventional PCR was performed in a 25-μL reaction volume according to the protocol described by Cao et al. (2007) with the primers TC2F (5′-AAACAACGAGTCAGCTTGAATGCTAGTGTG-3′) and TC2R (5′-CTTTAGTTGTGTTTCGGCTAGGATGGTTCG-3′). Reaction conditions consisted of an initial heat denaturation step at 94ºC for 2 min, followed by 45 cycles of 94ºC for 30 s, 65ºC for 1 min, and 72ºC for 1 min, with a final extension at 72ºC for 10 min. The

amplicons were resolved on SYBR Green-stained agarose gels and visualized with a UV transilluminator (Bio-Rad, Mississauga. ON. Canada).

Samples testing positive for the presence of *P. brassicae* DNA by conventional PCR were further evaluated by quantitative PCR (qPCR) analysis, with reactions carried out in a 15-μL reaction volume as per the procedure of Rennie et al. (2011).

Standard curves for the quantification of *P. brassicae* resting spores were generated with DNA isolated from known quantities of spores. The standards were prepared from a serial dilution of 1×10^7 to 1×10^3 resting spores (Rennie et al., 2011), with the average threshold cycle (CT), linear regression coefficient (R^2), line equation and PCR efficiency (E) calculated from four independent sets of DNA. Quantitative PCR analysis was performed on undiluted DNA, or after dilution (1/2, 1/10, or 1/100 v/v) with sd-H_2O if the presence of PCR inhibitors was suspected. qPCR amplifications were carried out with a StepOnePlus Real Time PCR System (Applied Biosystems company, Carlsbad, CA, USA) in a 10 μL volume containing 2.5 μL of the template DNA, the primers DC1F (5'-CCTAGCGCTGCATCCCATAT-3') and DC1R (5'-CGGCTAGGATGGTTCGAAAA-3') (2.5 μL), and 5 μL Dynamite qPCR Mastermix (Molecular Biology Service Unit, University of Alberta, Edmonton, Canada) containing SYBR Green (Molecular Probes) as the detection dye. Reaction conditions consisted of an initial heat denaturation step at 95°C for 2 min, followed by 35 cycles of 95°C for15 s and 60°C for 60 s.

2.2.4 Statistical Analysis

Values were compared using the parametric Student's t-test and the data was checked for normality. Correlation analysis was performed between *P. brassicae* spore concentration and calcium, boron, organic matter, pH, and electrical conductivity. The data sets were transformed for homogeneity of variance using a log transformation test. All of the statistical analyses were performed with SAS 9.3 software (SAS Institute Inc., Cary, NC, USA).

2.2.5 Soil sampling location mapping with ArcGIS

The geocoordinates of each sampling location and the qPCR data on spore concentrations were integrated to generate an interpolation map in the ArcMap application using ArcGIS software (Esri Canada). This interpolation process is known as Kriging. Kriging is a geo-statistical technique similar to Inverse Distance Weighting (IDW) that uses a linear combination of weights at known points to estimate the value at an unknown point. This interpolation method was selected mainly because of its consideration of prediction confidence in error maps and spatial autocorrelation between sampling sites (Yang et al., 2004).

2.3 Results

2.3.1 Conventional PCR and qPCR analysis of soil samples

DNA was extracted from each soil sample. A total of 284 samples were tested by conventional PCR analysis, of which 132 samples (46%) tested positive and 152 (54%) tested negative for the presence of *P. brassicae* DNA. The positive samples were further tested by qPCR analysis, which showed that at the Parkland site, resting spore concentrations ranged from 1.4×10^4 to 2.0×10^6 spores/g soil, at Bassano, they ranged

from 3.0×10^2 to 3×10^5spores/g soil, and at the Edmonton site, they ranged from 4.5×10^4 to and 4.0×10^7 resting spores/g soil.

2.3.2 Comparison of calcium, boron, organic matter, pH, and electrical conductivity in soils with or without *P. brassicae* infestation

The average soil calcium and boron concentrations, organic matter, pH, and electrical conductivity were compared between soil samples testing positive or negative for the presence of *P. brassicae* at the three experimental sites. Statistical analysis revealed a significant difference ($P < 0.05$) in calcium concentration between *P. brassicae* positive (0.35 ± 0.01 %wt) and negative (0.44 ± 0.02 %wt) soil samples (Table 2-1). However, there were no significant differences with respect to boron, organic matter, soil pH, and electrical conductivity in *P. brassicae* positive (26.63 ± 0.86 mg/kg, 10.59 ± 0.30 %, 6.17 ± 0.04, 325.27 ± 19.22 μs/cm) and negative (24.98 ± 0.77 mg/kg, 10.25 ± 0.31 %, 6.21 ± 0.05, 309.46 ± 16.23 μs/cm) soil samples (Table 2-1).

2.3.3 Correlation between *P. brassicae* spore concentration and calcium, boron, organic matter, pH, and electrical conductivity

Analyses were conducted to identify whether there were any significant correlations between *P. brassicae* resting spore concentration and calcium and boron concentration, organic matter, pH, electrical conductivity in the *P. brassicae* positive samples. The results demonstrated a positive correlation between resting spore and calcium concentrations (r^2 =0. 44 P <0.0001, N = 127), and between resting spore and boron concentrations (r^2 =0.10, P <0.0001, N = 127) for all of the *P. brassicae* positive soil samples collected at the Bassano, Edmonton and Parkland sites (Fig. 2-1). The spore

concentration also was positively correlated with soil organic matter % (r^2 =0. 51, *P* <0.0001, N = 127) (Fig. 2-2). By contrast, the *P. brassicae* spore concentration was negatively correlated with soil pH (r^2 =0.09, *P* = 0.0004, N = 127) and electrical conductivity (r^2 =0.23, *P* <0.0001, N = 127).

A multiple correlation analysis also was conducted using *P. brassicae* spore concentration as the dependent variable, and soil organic matter, soil pH, electrical conductivity, soil calcium and boron concentrations as independent variables. The results indicated that organic matter was positively (*t* = 3.59, R^2 = 0.58, *P* = 0.0005, N = 127) correlated with *P. brassicae* spore concentration, and that electrical conductivity was negatively (*t* = -3.57, R^2 = 0.58, *P* = 0.0005, N = 127) correlated with spore concentration. Soil pH, calcium and boron concentrations were not significantly correlated with resting spore concentration. A stepwise regression analysis revealed that soil organic matter (partial R^2 = 0.52, *P* < 0.0001, N = 127) and electrical conductivity (partial R^2 = 0.054, *P* = 0.0003, N =127) were the only variables that were significant in the model.

2.3.4 Interpolation analysis between *P. brassicae* spore concentration and geo-coordinates of soil samples

The interpolation maps of the three field sites indicated the *P. brassicae* spore concentrations at each sampling point and their relative location to other sampling points within each field (Figs. 2-3, 2-4, 2-5). The sampling points with high *P. brassicae* concentrations in the Parkland County field were concentrated mainly in the central east area of the field (Fig. 2-3). By contrast, more than half of the sampling points within the Bassano field were heavily infested with *P. brassicae*, most of which were distributed in

the southeast area of the field (Fig. 2-4). At the Edmonton site, sampling points with high concentrations of *P. brassicae* resting spores occurred mainly in the southern area of the field (Fig. 2-5). At all three field sites, the highest spore concentrations occurred at the entrance of the field.

2.4 Discussion

The high percentage of soil samples that tested positive for the presence of *P. brassicae* suggested that the pathogen was fairly widespread in the three commercial fields. All of the samples that tested positive by conventional PCR also had quantifiable levels of *P. brassicae* spores. Correlation analyses revealed significant positive correlations between *P. brassicae* spore concentration and calcium and boron concentrations, which is not consistent with previous reports (Donald and Porter, 2009; Dixon, 2009b; Webster and Dixon, 1991). Given the very small slopes of these correlations, however, these results should be interpreted with caution. The overall levels of calcium and boron in Alberta soils are in the medium to adequate range (Alberta Agriculture and Rural Development, 1992; Canola Council of Canada, 2006), and these may not be major factors affecting *P. brassicae* spores in the soil. Nonetheless, the correlations observed, although small, suggest that calcium and boron concentrations may have influenced *P. brassicae* levels in the three fields described.

The current study showed a significant negative correlation between *P. brassicae* spore concentration and soil pH, which is consistent with previous reports (Karling, 1968; Gossen et al., 2013). However, given the fact that *P. brassicae* spores were found in 9 samples with a soil pH above 7.0, *P. brassicae* is not limited only to acidic soils in

Alberta. Interestingly, the current study also showed a positive correlation between *P. brassicae* spore concentration and soil organic matter. Resting spores of *P. brassicae* are found in higher numbers in galls of dead canola plants (organic matter); likely explaining high levels of organic matter were associated with high amounts of resting spores. In addition, the presence of *P. brassicae* resting spores in the soil also may contribute, at least partially, to the constitution of soil organic matter as determined by the weight loss procedure used in this study. This may contradict what is known about moisture holding capacity in organic matter soils (the higher the organic matter, the greater the holding capacity) and clubroot disease (the more moisture in the soil, the higher the incidence of the disease). However, this occurs mainly in soil with 60% - 80% moisture (Narisawa et al., 2005; Hamilton and Crete, 1978) where *P. brassicae* spores show increased mobility when there is greater water drainage in the soil.

A significant negative correlation between *P. brassicae* spore concentration and soil electrical conductivity was also found. Electrical conductivity is a measurement that is related to soil properties including soil texture, cation exchange capacity, drainage conditions, organic matter level, and salinity (Sarig et al., 1993; Wichem et al., 2006). Organic matter rich soils may have a stronger capacity to attract and retain cations (Brady et al., 2000; Caravaca et al.,1999; Helling et al.,1964), thus resulting in decreased electrical conductivity. Therefore, it is not surprising to see a negative correlation between *P. brassicae* concentration and electrical conductivity.

The ArcGIS software enabled the generation of interpolation maps to display soil sampling areas with low or high concentrations of *P. brassicae* spores. These maps are significant because they offer a graphical representation of *P. brassicae* resting spore

distribution. In the field in Parkland County, the distribution of resting spores was highly localized, as is often observed for soilborne plant pathogens. By contrast, at Bassano, the spores tended to be quite extensively distributed over the sampling areas. In the Edmonton field, the highest resting spore concentrations were found in the southern part of the field. Nevertheless, at all three sites, the highest concentrations of resting spores occurred at the field entrance, supporting earlier conclusions that most *P. brassica*e infestations are initiated near the field approach. This may signal the movement of infested soil on farm and other machinery (Cao et al., 2009). Beyond their utility in research, clubroot infestation maps also may help farmers to manage clubroot outbreaks. For example, such maps could be used to identify areas of a field that should be treated as *P. brassicae*-infested for equipment sanitization purposes, or to help decide on crop rotation strategies over a particular area.

In summary, the current study revealed positive correlations between *P. brassicae* spore concentration and soil calcium and boron concentrations and organic matter, but negative correlations between *P. brassicae* spores and soil pH and electrical conductivity. Interpolation maps demonstrated the uneven distribution or patchy nature of *P. brassicae* resting spores in the three fields tested in the study, but with infestation always associated with the field entrance.

Table 2-1. Comparison of calcium, boron, organic matter, soil pH, and electrical conductivity in *Plasmodiophora brassicae* positive and negative soils

Soils	Number of samples	Calcium (% wt)	Boron (mg/kg)	Organic matter (%)	pH	Electrical conductivity (μs/cm)
Clubroot positive	127	0.35 ± 0.01 A	26.63 ± 0.86 A	10.59 ± 0.30 A	6.17 ± 0.04 A	325.27 ± 19.22 A
Clubroot negative	157	0.44 ± 0.02 B	24.98 ± 0.77 A	10.25 ± 0.31 A	6.21 ± 0.05 A	309.46 ± 16.23 A

Means ± 1 standard error followed with the same letters are not significantly different at $P < 0.05$ according to the Student's *t*-test.

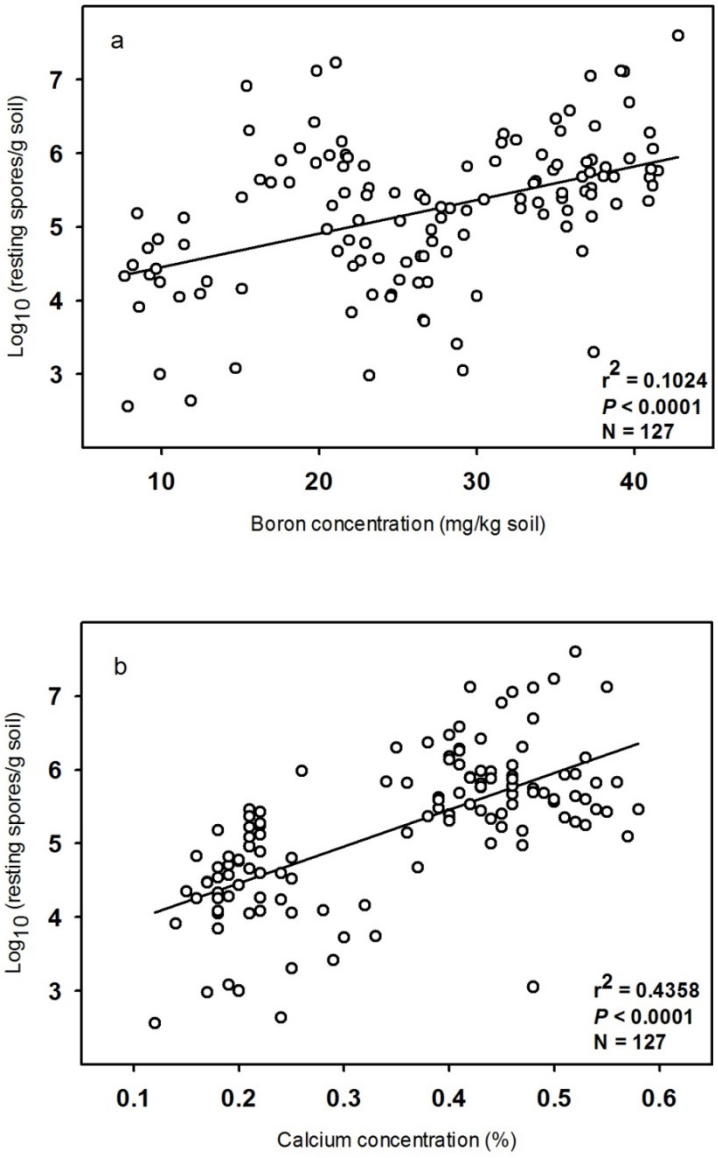

Figure 2-1. Correlation between *Plasmodiophora brassicae* resting spore concentration and soil calcium (a) and boron (b) concentrations at three *P. brassicae*-infested field sites in Alberta.

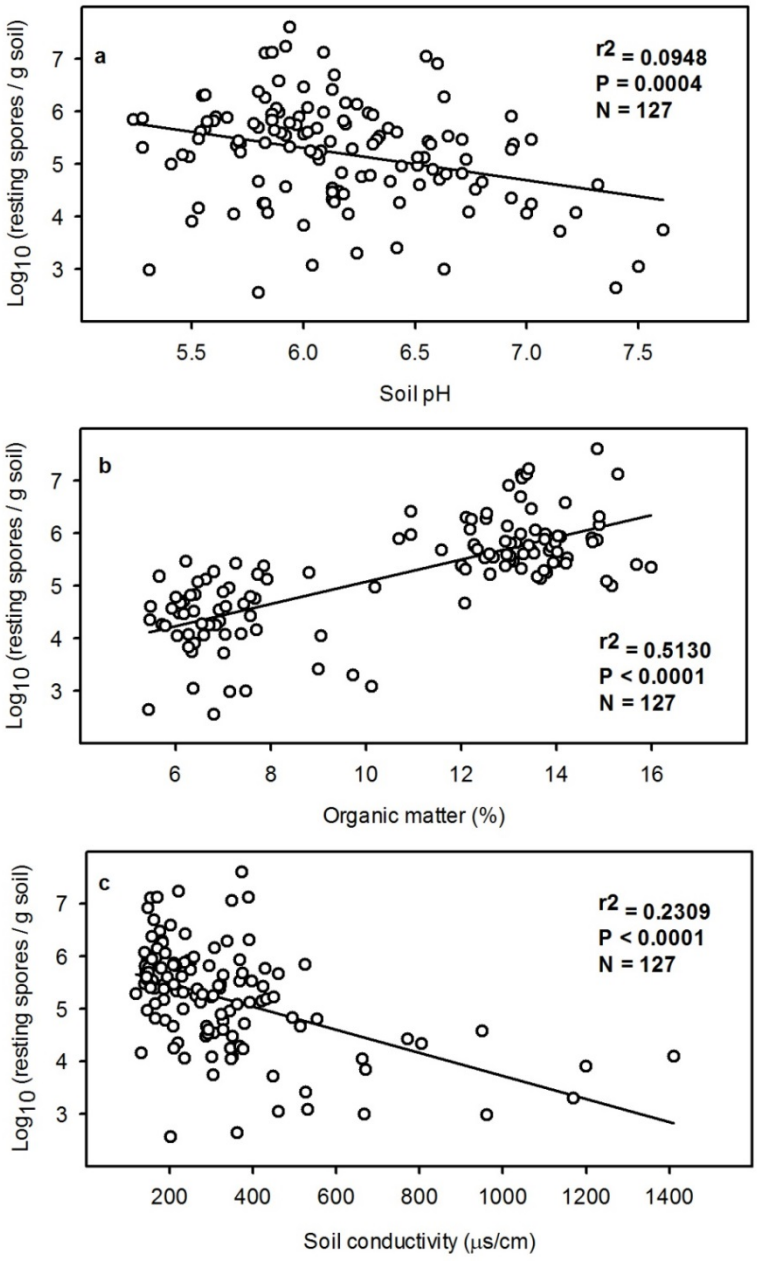

Figure 2-2. Correlation between *Plasmodiophora brassicae* resting spore concentration and soil pH (a), organic matter (b), and soil electrical conductivity (c) at three *P. brassicae*-infested field sites in Alberta.

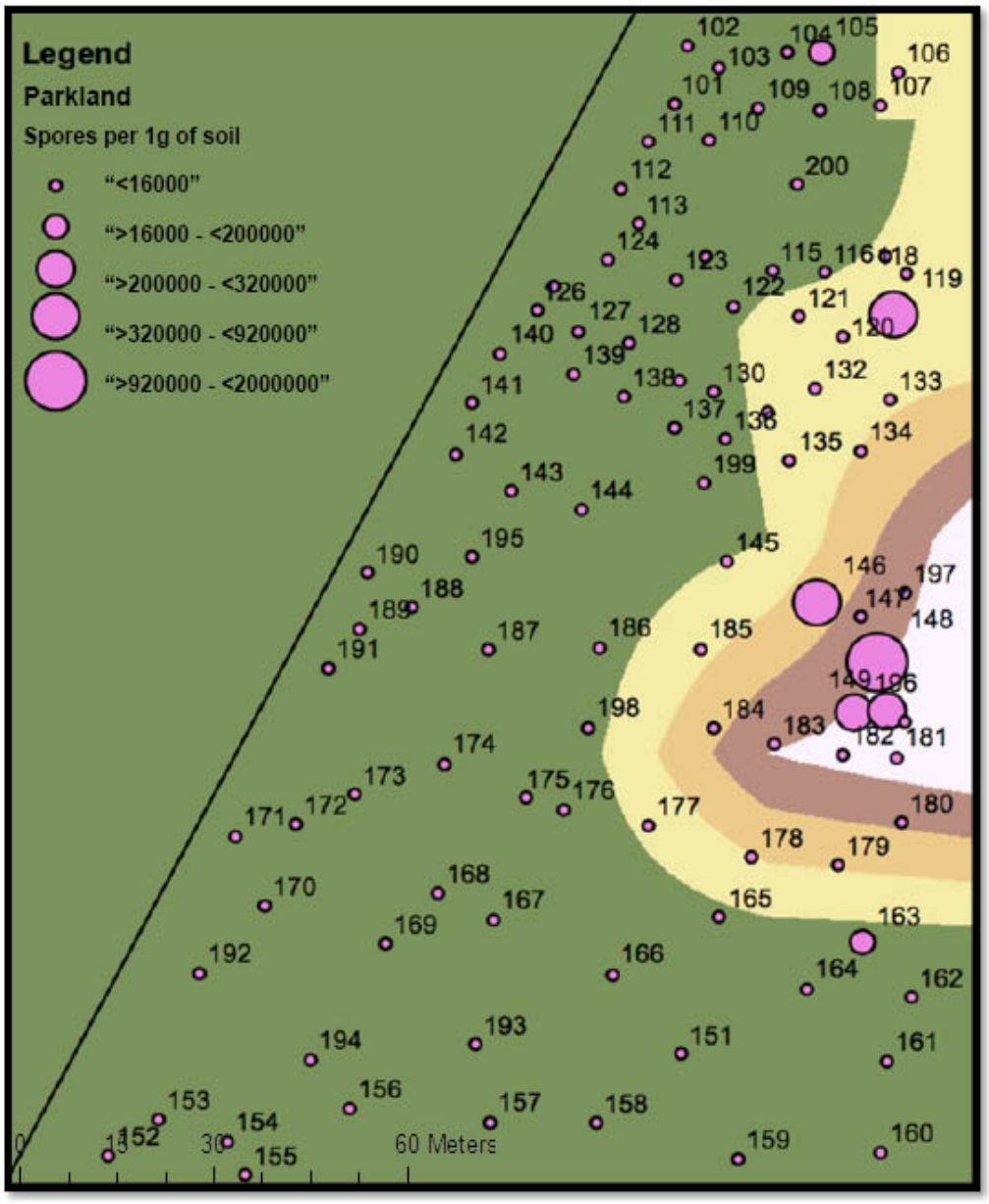

Figure 2-3. Interpolation analysis of *Plasmodiophora brassicae* resting spore concentration and location for 96 soil samples collected in a field (53° 31′ 48″ N, 114° 0′ 23″ W) in Parkland County, Alberta. Note that the highest resting spore concentrations are found in the east-central portion of the field, which corresponds to the field entrance.

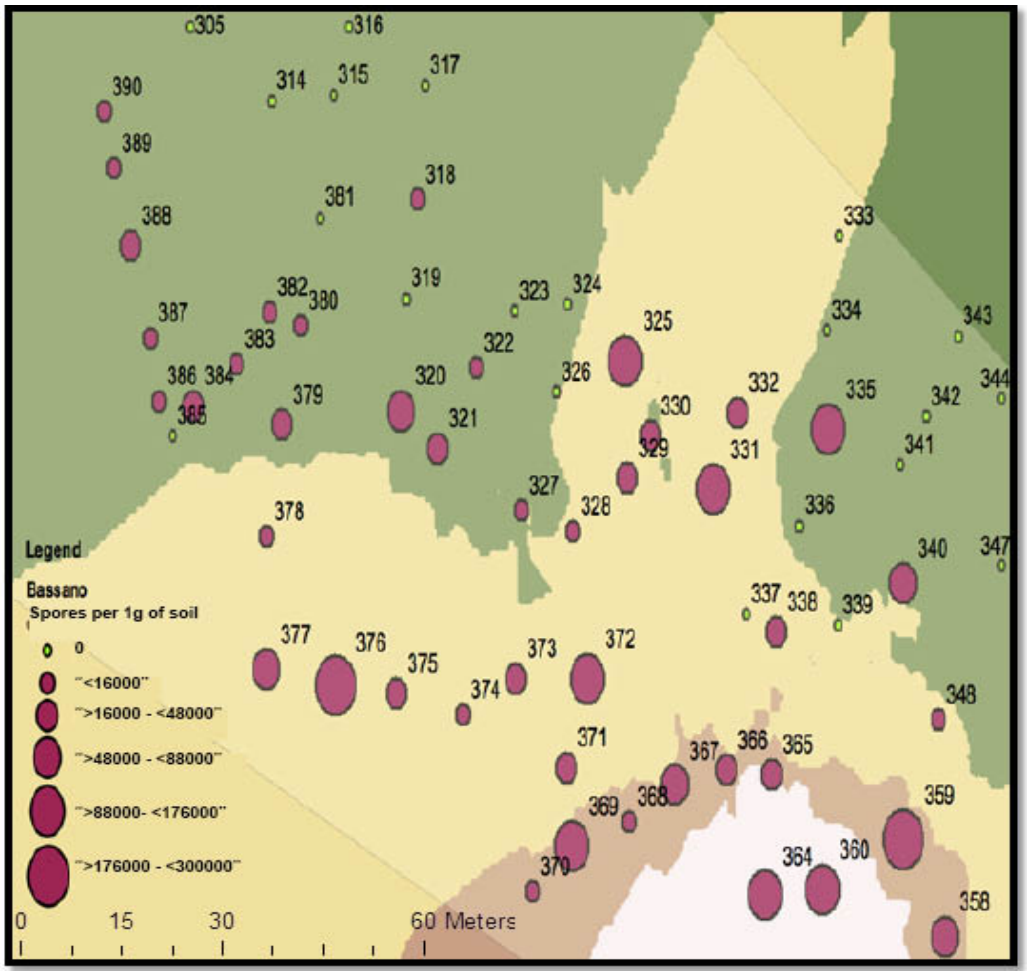

Figure 2-4. Interpolation analysis of *Plasmodiophora brassicae* resting spore concentration and location for 95 soil samples collected in a field (50° 47′ 3″ N, 112° 27′ 5″ W) near Bassano, Alberta. Note that the highest resting spore concentrations are found in the southeast portion of the field, which corresponds to the field entrance.

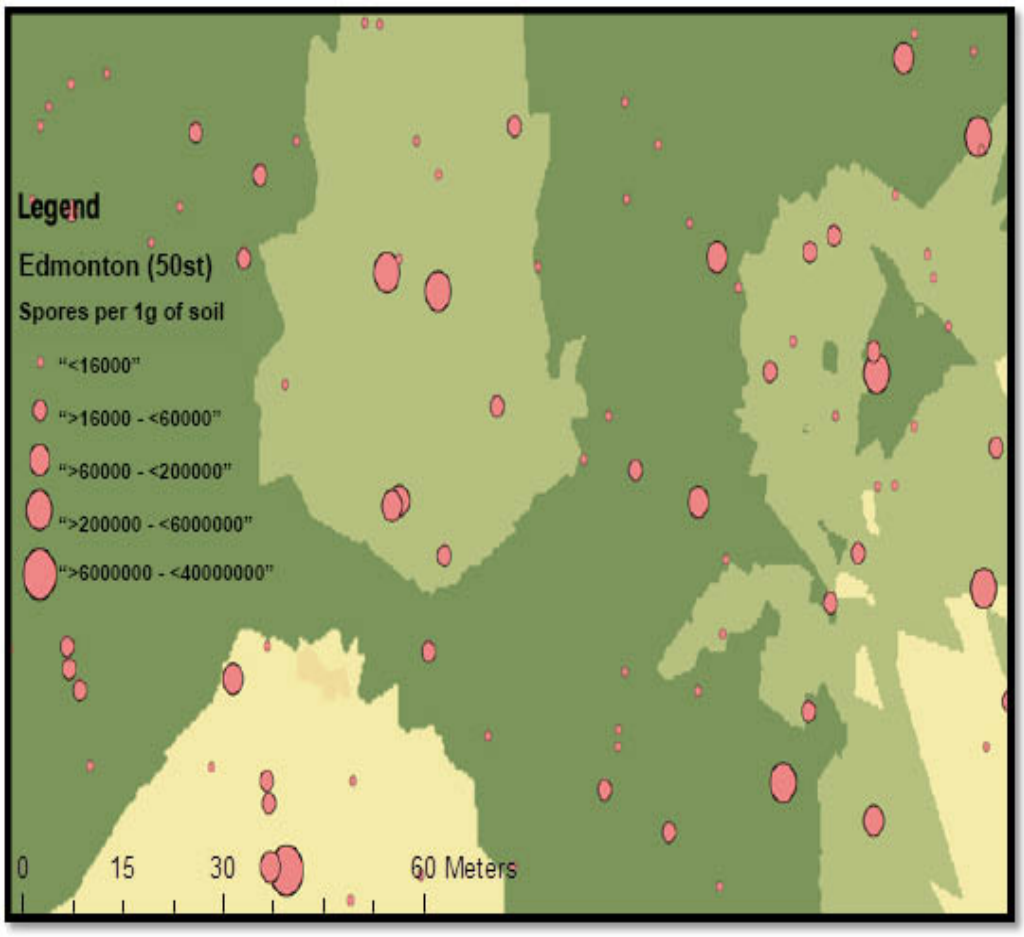

Figure 2-5. Interpolation analysis of *Plasmodiophora brassicae* resting spore concentration and location for 95 soil samples collected in a field (53° 52′ 44″ N, 113° 2′ 13″ W) in northeast Edmonton, Alberta. Note that the highest resting spore concentrations are found in the southwest portion of the field, which corresponds to the field entrance.

3 Evaluation of the impact of crop rotation on clubroot severity in *Brassica napus* canola under greenhouse conditions

3.1 Introduction

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is an important disease of canola (*Brassica napus* L) and other crucifers (Strelkov et al., 2012). Crop losses resulting from clubroot infestation have been estimated to be at least 10% to 20% worldwide (Dixon, 2006). Although it was long known as a disease of cruciferous vegetable crops in eastern Canada and British Columbia, clubroot was not identified on canola in the Prairie Provinces until 2003, when 12 commercial canola crops were found to be infested with the disease near Edmonton, Alberta (Tewari et al., 2005). The Prairie Provinces represent the most important region for the production of Canadian canola, and the identification of clubroot was a cause for concern (Curtis et al., 2014). Annual surveys for clubroot have revealed that the disease is spreading, and at least 1,482 commercial fields in Alberta were confirmed to be infested with *P. brassicae* by 2013, mainly in the central part of the province (Strelkov et al., 2014). Isolated cases of clubroot also have been identified in southern Alberta, Saskatchewan, and Manitoba (Strelkov and Hwang, 2014). The fairly rapid spread of clubroot in Alberta and its presence in Saskatchewan and Manitoba pose a major threat to the production of canola in the Prairies.

Infection by *P. brassicae* frequently causes galls on roots of susceptible host plants, disrupting water and nutrient uptake, and eventually resulting in stunted growth, foliar wilting, chlorosis, and even premature plant death when symptoms are severe. Millions of *P. brassicae* resting spores can be produced within a mature root gall, which in turn are released into the soil after the root galls decay. Control of clubroot is difficult because of the long persistence of the pathogen resting spores in the soil. Previous reports indicated that *P. brassicae* resting spores have a half-life of approximately four years (Wallenhammar, 1999; Hwang et al., 2013). The movement of infested soil on farm and other machinery is regarded as the most common method by which *P. brassicae* is disseminated from field to field (Cao et al., 2009; Strelkov et al., 2011). Most farmers do not routinely disinfect farm equipment, likely contributing to the rapid spread of the disease in Alberta. Once *P. brassicae* is introduced into a field, it is a difficult pathogen to manage effectively. Perhaps the most effective measure to manage clubroot is to plant clubroot resistant canola cultivars. However, continuous cropping of resistant cultivars may result in a loss of resistance to *P. brassicae*, as pathogen populations can adapt quickly to the selection pressure imposed by the planting of some resistant host genotypes (LeBoldus et al., 2012).

In the current study, six crop rotation regimes were compared for their effectiveness in the management of clubroot under greenhouse conditions in two different soils, collected near Bassano and Edmonton, Alberta. These soils were naturally infested with *P. brassicae*, and included a Brown Chernozem (Bassano) and a Black Chernozem (Edmonton).

3.2 Materials and Methods

3.2.1 Plant materials and soil collection

Two experiments were carried out under greenhouse conditions at the Crop Diversification Centre North (CDCN), Alberta Agriculture and Rural Development, Edmonton, Alberta. The plant materials used for the two experiments consisted of the canola cvs. 45H26, 45H29, 73-77, 9558C, L135C, and 10DC30622, and the barley (*Hordeum vulgare* L.) cv. McDiarmid. Canola cv. 45H26 (Dupont Pioneer) is susceptible to clubroot, while cvs. 45H29 (Dupont Pioneer) 73-77 (Monsanto), 9558C (Viterra), L135C (Bayer AG), and 10DC30622 (DL Seeds) are resistant to the predominant strains of the pathogen in Alberta. The plants were grown in Brown Chernozemic or Black Chernozemic soils collected from Bassano and Edmonton, Alberta, respectively.

3.2.2 Preliminary evaluation of soil inoculum levels

Before any experiments were conducted, a bioassay was carried out to verify that the soils collected from Bassano and Edmonton were severely infested with *P. brassicae*. Briefly, the bioassay was performed by planting 1-week-old Chinese cabbage (*Brassica rapa* var. *pekinensis* cv. Granaat) seedlings, at a rate of one seedling per pot, in 6 × 6 × 6 cm plastic pots (Kord Products Inc., Brampton, ON, USA) filled with either 1:1 (v:v) Bassano soil:Sunshine Professional Growing Mix (Sun Gro Horticulture Canada Ltd., Seba Beach, AB, Canada), or 1:1 (v:v) Edmonton soil: Sunshine Professional Growing Mix. A total of 140 seedlings were planted in each soil type. After planting, the seedlings were kept in a greenhouse at 23°C (day), 18°C (night) under a 16 h photoperiod with natural light supplemented by high pressure sodium light, with water and

fertilization provided when necessary. Five weeks after planting, the plants were carefully dug out of the soil, and the roots were washed with water and assessed for clubroot symptom development on a 0 to 3 rating scale, where: 0 = no galling, 1 = a few small galls, 2 = moderate galling, and 3 = severe galling (Strelkov et al., 2007). An index of disease (ID) was then calculated for the seedlings grown on each soil type, using the method of Horiuchi and Hori (1980) as modified by Strelkov et al. (2006):

$$ID\ (\%) = \{\Sigma(n\times 0 + n\times 1 + n\times 2 + n\times 3)/(N\times 3)\}\times 100\%$$

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. In the bioassay for Experiment 2 (see below), only the soil collected from Edmonton was assessed.

3.2.3 Experiment 1

Clubroot disease severity was compared under six different crop rotation regimes (treatments). The first crop rotation regime (T1) consisted of five consecutive crops of a susceptible (S) cultivar of canola (45H26), i.e., S – S – S – S – S. The second crop rotation regime (T2) consisted of S - fallow (F) – S – F – S. The third crop rotation regime (T3) consisted of S – barley (B) – S – B – S. The fourth crop rotation regime (T4) consisted of S – resistant canola cultivar (R, 45H29) – S – R – S. The fifth crop rotation regime (T5) was R – R – R – R – R (i.e., one resistant cultivar, 45H29). Finally, the last crop rotation regime (T6) included cropping resistant canola cultivars from different suppliers: 1 (R1, 45H29) – 2 (R2, 73-77) – 3 (R3, 9558C) – 4 (R4, L135C) – 5 (R5, 10DC30622) (Table 3-1).

Each of the six crop rotation regimes was tested on each of the two types of soils (i.e., Brown Chernozemic and Black Chernozemic) described above. Each treatment was replicated four times and arranged in a randomized complete block design. The experiment also was repeated in two greenhouses. In order to promote plant growth, the field soil was mixed with Sunshine Professional Growing Mix in a 1:1 ratio by volume. For each treatment, 100 seeds of each canola cultivar or barley were sown in four rows (25 seeds in each row) in a 14-litre container (42 × 33 × 16 cm), which was filled 2/3^{rds} of maximum capacity with the soil mix. After sowing, the soil mix in each container was watered from above with a fine sprinkler. Clubroot disease severity on the roots of the canola plants was measured five weeks after seeding, as described above for the bioassay. The height and dry weight of the canola plants also were recorded at the time of clubroot rating. All roots were removed from the soil, and no inoculum was re-introduced. The next crop in the rotation was seeded one week after the disease severity ratings were obtained for the previous crop.

3.2.4 Experiment 2

To better simulate field practices in Alberta, a second experiment was carried out. All of the six rotation regimes (treatments) were the same as in Experiment 1 (Table 3-1), except that 10 replicates were included for each rotation regime or treatment, and after each cycle of crop rotation, the root galls were homogenized in a blender with the homogenate integrated back into the same soil mix in each container. The containers used in Experiment 2 consisted of 34-litre tubs (60 × 40 × 32 cm), and only one type of soil (i.e., the Black Chernozemic) was used. A total of 100 seeds were sown into each of 60 containers as described above (i.e., 10 containers or replicates per treatment). After the

completion of each cycle of crop rotation (5 weeks per cycle), clubroot disease severity was assessed, and plant height and biomass were recorded as in Experiment 1.

3.2.5 Statistical analysis

Categorical data obtained from the disease severity ratings were analyzed with the CATMOD Procedure of SAS 9.3 (SAS Institute, Cary, NC), and plant height and biomass data were analyzed using the ANOVA Procedure of SAS 9.3. Differences were considered significant at $P \leq 0.05$.

3.3 Results

3.3.1 Experiment 1

The preliminary bioassays conducted prior to Experiment 1 revealed severe levels of clubroot on the susceptible Chinese cabbage genotype. An average ID (± 1 S.E.) of 99.8% ± 0.3% was obtained for the plants grown in the potting mix containing the Black Chernozemic (Edmonton) soil, and an ID = 99.5% ± 0.5% was obtained for the plants grown in potting mix containing the Brown Chernozemic (Bassano) soil, indicating that both types of soil were heavily infested with *P. brassicae*.

3.3.1.1 Variation in clubroot index of disease within each crop rotation regime

Analysis of variance using the CATMOD procedure revealed no significant effect between the two greenhouses (P = 0.19). Since the major focus of this experiment was to test the crop rotation regimes (treatments), the data obtained from the two greenhouses were pooled together for statistical analysis. Analysis of variance using the

CATMOD procedure revealed a significant effect of rotation (cycle) ($P < 0.0001$) for all six treatments.

Treatment 1 consisted of five consecutive cycles of the susceptible canola cv. 45H26. Among the five cycles, cycle 3 had the highest mean ID (97.3% ± 0.7%), which was significantly higher than those of cycles 1 (97.0% ± 1.2%), 2 (90.8 ± 3.2%), 4 (94.0% ± 4.3%), and 5 (83.0% ± 4.6%). Indices of disease did not differ significantly between cycles 2 and 4 (Fig. 3-1).

Treatment 2 consisted of growing the susceptible canola cv. 45H26 in cycles 1, 3, and 5, and maintaining the pots 'fallow' in cycles 2 and 4. Statistical analysis indicated that cycle 1 the highest mean ID (97.0% ± 1.3%), which was significantly higher than the IDs obtained in cycles 3 (92.7% ± 2.2%) and 5 (73.0% ± 7.3%). The ID in cycle 5 also was significantly lower than in cycle 3 (Fig. 3-1).

Treatment 3 consisted of growing the susceptible canola cv. 45H26 in cycles 1, 3 and 5, and barley in cycles 2 and 4. Statistical analysis indicated that cycle 1 developed the highest ID (98.1% ± 0.7%), which was significantly higher than the IDs obtained in cycles 3 (89.9% ± 3.5%) and 5 (74.0% ± 6.0%). Similarly to treatment 2, the ID in cycle 5 also was significantly lower than in cycle 3 (Fig. 3-1).

Treatment 4 consisted of growing the susceptible canola cv. 45H26 in cycles 1, 3, and 5, and the resistant canola cv. 45H29 in cycles 2 and 4. Statistical analysis indicated that among the five cycles, cycle 1 developed the highest ID (98.6% ± 0.7%), which was significantly greater than the IDs obtained in cycles 2 (11.7% ± 1.6%), 3 (71.3% ± 4.9%),

4 (28.0% ± 6.9%), and 5 (53.0% ± 7.0%). Index of disease did not differ significantly between cycles 4 and 5 (Fig. 3-1).

Treatment 5 consisted of growing the resistant canola cv. 45H29 in all five of the cycles. Statistical analysis indicated that among the five cycles, cycle 4 developed the highest mean ID (38.9% ± 1.9%), which was significantly greater than the IDs obtained in cycles 1 (15.0% ± 1.9%), 2 (14.5% ± 1.7%), 3 (30.6% ± 4.5%), and 5 (23.5% ± 5.8%). Index of disease did not differ significantly between cycles 1 and 2 (Fig. 3-1).

Treatment 6 consisted of growing five different resistant canola cultivars sequentially in the same pots, namely the cvs. 45H29, 73-77, 9558C, L135C and 10DC30622 in that order. Statistical analysis indicated that among the five cycles, cycle 4 (i.e., L135C) developed the highest ID (24.6% ± 7.3%), which was significantly greater than the IDs that developed in cycles 1 (45H29, 12.6% ± 2.2%), 2 (73-77, 14.3% ± 2.0%), 3 (9558C, 5.1% ± 1.2%) and 5 (10DC30622, 13.1% ± 5.2%). Index of disease did not differ significantly between cycles 2 and 5, or between cycles 1 and 5 (Fig. 3-1).

3.3.1.2 Variation in plant height and dry weight within each crop rotation regime

Analysis of variance revealed a significant effect of rotation (cycle) for all six of the treatments, based on the pooled data for plant height and dry weight obtained from the two greenhouses.

Among the five cycles in treatment 1, the greatest plant height was observed in cycle 4 (42.6 cm ± 5 cm), which was significantly greater than in cycles 1 (26.7 cm ± 1.3 cm), 2 (11.9 cm ± 0.3 cm), and 3 (35.2 cm ± 2.6 cm). Plant height did not significantly differ

between cycles 1 and 3, cycles 3 and 5 (37.3 cm ± 4.6 cm), or cycles 4 and 5 (Fig. 2). Similarly, the greatest plant dry weight was observed in cycle 4 (2.3 g ± 0.5 g), which was significantly greater than in cycle 2 (0.6 g ± 0.1 g). The dry weight was not significantly different between cycles 1 (1.8 g ± 0.2 g) and 3 (1.5 g ± 0.2 g), cycles 1 (1.8 g ± 0.2 g) and 4 (2.3 g ± 0.5 g), cycles 1 and 5 (1.5 g ± 0.2 g), cycles 2 and 3, cycles 2 and 5, cycles 3 and 4, cycles 3 and 5, or cycles 4 and 5 (Fig. 3-2).

In treatment 2, the greatest plant height was observed in cycle 5 (44.8 cm ± 5.5 cm), which was significantly greater than in cycles 1 (26.9 cm ± 1.3 cm) and 3 (33.9 cm ± 2.3 cm). The plant height did not significantly differ between cycles 1 and 3 (Fig. 3-2). The greatest dry weight was observed in cycle 1 (1.8 g ± 0.2 g), although dry weight did not significantly differ between cycles 1 and 3 (1.4 g ± 0.3 g), or cycles 1 and 5 (1.7 g ± 0.2 g) (Fig. 3-2).

In treatment 3, the greatest plant height was observed in cycle 5 (36.6 cm ± 4.5 cm), which was significantly greater than in cycles 1 (27.2 cm ± 1.2 cm) and 3 (31.1 ± 3.0 cm). Plant height did not significantly differ between cycles 1 and 3, or cycles 3 and 5 (Fig. 3-2). The greatest dry weight was found in cycle 3 (1.8 g ± 0.2 g), although the dry weight in cycle 3 did not differ significantly from the dry weights in cycles 1 (1.5 g ± 0.1 g) or 5 (1.3 g ± 0.2 g) (Fig. 3-2).

The patterns observed with respect to height in each of the crop cycles in treatments 4 and 5 were quite similar (Figs. 3-2). In both treatments, the smallest plant height was observed in cycle 2, while the greatest plant height was found in cycle 4. The plant height in cycle 4 of treatment 4 (46.8 cm ± 2.4 cm) was significantly greater than in cycles 1

(27.3 cm ± 1.4 cm), 2 (13.9 cm ± 0.3 cm), or 3 (33.9 cm ± 3.4 cm). No significant difference in plant height was observed between cycles 4 and 5 (46.8 cm ± 2.4 cm) (Fig. 2D) of treatment 4. In treatment 5, plant height in cycle 4 (46.9 cm ± 2.9 cm) was significantly different from cycle 2 (12.5 cm ± 0.5 cm), but was not significantly different from plant height in cycles 1 (34.8 cm ± 1.2 cm), 3 (41.3 cm ± 3.9 cm), and 5 (45.5 cm ± 5.2 cm) (Fig. 3-2).

With respect to plant dry weight, the greatest dry weight in treatment 4 was observed in cycle 1 (2.0 g ± 0.2 g), which was significantly greater than in cycle 2 (0.7 g ± 0.1 g). There were no significant differences in dry weight between cycles 1 and 3 (1.6 g ± 0.2 g), cycles 1 and 4 (1.8 g ± 0.2 g), cycles 1 and 5 (1.5 g ± 0.2 g), cycles 3 and 4, cycles 3 and 5, or cycles 4 and 5 (Fig. 3-3). In treatment 5, the greatest plant dry weight also was observed in cycle 1 (1.9 g ± 0.2 g), which was significantly greater than in cycles 2 (0.6 g ± 0.1 g) and 5 (1.1 g ± 0.1 g). The dry weight did not significantly differ between cycles 1 and 3 (1.6 g ± 0.2 g), cycles 1 and 4 (1.9 g ± 0.2 g), cycles 2 and 5, cycles 3 and 4, or cycles 3 and 5 (Fig. 3-3).

Among the cycles in treatment 6, the greatest plant height was observed in cycle 3 (52.8 cm ± 3.0 cm), which was significantly greater than in cycles 1 (35.8 cm ± 2.1 cm) and 2 (11.9 cm ± 0.4 cm), but not significantly different than in cycles 4 (46.7 cm ± 3.1 cm) and 5 (41.3 cm ± 4.9 cm) (Fig. 3-3). The greatest plant dry weight was found in cycle 1 (2.0 g ± 0.1 g), which was significantly greater than in cycles 2 (0.6 g ± 0.1 g) and 5 (0.9 g ± 0.1 g). The dry weight did not significantly differ between cycles 1 and 3 (1.8 g ± 0.1 g), cycles 1 and 4 (1.9 g ± 0.4 g), cycles 2 and 5, or cycles 3 and 4 (Fig. 3-3).

3.3.1.3 Variation in disease severity, plant height, and plant dry weight across treatments

Analysis of variance using the CATMOD procedure revealed a significant effect of treatments (P = 0.0001) when the average disease rating data were pooled together within each crop rotation regime. Treatment 1 developed the highest ID (92.4% ± 2.8 %), which was significantly greater than the IDs in treatment 2 (87.5% ± 3.6 %), treatment 3 (87.3% ± 3.4 %), treatment 4 (52.5% ± 4.2 %), treatment 5 (24.5% ± 3.9 %), and treatment 6 (13.9% ± 3.6%). The ID did not differ significantly between treatments 2 and 3. Treatment 6 developed the lowest ID (Fig. 3-4).

Analysis of variance using the PROC MIXED procedure revealed significant effects of treatments (P = 0.0004) when the average plant height data were pooled together within each crop rotation regime. The greatest plant height was observed in treatment 6 (37.7 cm ± 2.7 cm), which was significantly greater than in treatments 1 (30.7 cm ± 2.7 cm), 2 (35.2 cm ± 3 cm), 3 (31.6 cm ± 2.9 cm), 4 (32.2 cm ± 2.5 cm), or 6 (37.7 cm ± 2.7 cm). Plant height did not differ significantly between treatments 1, 3 and 4, or between treatments 2 and 5. (Fig.3-4).

Analysis of variance using the PROC MIXED procedure revealed no significant effects of the treatments (P = 0.31) when the average dry weight data were pooled together within each crop rotation regime. Treatment 2 had the greatest dry weight (1.6 g ± 0.3 g) (Fig.3-4).

3.3.1.4 Effect of soil type on clubroot severity

3.3.1.4.1 Clubroot severity between treatments

Analysis of variance using the CATMOD procedure revealed no significant differences between the Bassano soil and the Edmonton soil (P = 0.07), although the results were very close to significant when the average disease rating data were pooled together according to crop rotation regimes. As well, the Edmonton soil yielded slightly higher IDs than the Bassano soil in each crop rotation regime (Fig. 3-5).

3.3.1.4.2 Clubroot severity between rotations

Analysis of variance using the CATMOD procedure revealed no significant differences between the Bassano soil and the Edmonton soil (P = 0.05), although the results were very close to significant when the average disease rating data were pooled together within each crop rotation regime. Slightly higher IDs were obtained in the Edmonton soil in every cycle of rotation with the exception of cycle 1 (Fig. 3-6).

3.3.2 Experiment 2

The preliminary bioassay revealed the development of severe clubroot on the susceptible Chinese cabbage genotype, with an average ID (± 1 S.E.) of 99.7% ± 0.3% obtained in the potting mixture containing the Black Chernozemic (Edmonton) soil. The Edmonton area soil was the only soil included in Experiment 2. The severe clubroot that developed on Chinese cabbage confirmed the high level of *P. brassicae* infestation of that soil.

3.3.2.1 Variation in clubroot index of disease within each crop rotation regime

Analysis of variance using the CATMOD procedure revealed a significant effect of rotation ($P < 0.0001$) for all of the six treatments when the disease rating data were pooled together according to rotation and treatment.

Treatment 1 included five consecutive cycles of the susceptible canola cv. 45H26. The highest mean ID was obtained in cycle 5 (99.6% ± 0.3%), which was significantly greater than the IDs obtained in cycles 1 (91.6% ± 2.4%) and 2 (97.6% ± 1.1%). Indices of disease did not differ significantly between cycles 2 and 3 (99.1 ± 0.4%), 2 and 4 (99.1 ± 0.5%), 3 and 4, 3 and 5, or 4 and 5 (Fig. 3-7).

Treatment 2 consisted of growing the susceptible canola cv. 45H26 in cycles 1, 3 and 5, and leaving the pots fallow in cycles 2 and 4. Statistical analysis indicated no significant differences in ID between cycles 1 (97.5% ± 0.9 %) and 3 (99.1% ± 0.5 %), 1 and 5 (99.4% ± 0.3%), or 3 and 5 (Fig.3-7).

Treatment 3 consisted of growing the susceptible canola cv. 45H26 in cycles 1, 3 and 5, and barley in cycles 2 and 4. Statistical analysis indicated that cycle 5 developed the highest ID (99.9% ± 0.2%), which was significantly greater than the ID obtained in cycle 1 (97.5% ± 0.9%), but not significantly greater than the ID in cycle 3 (99.1% ± 0.5%) (Fig. 3-7).

Treatment 4 consisted of growing the susceptible canola cv. 45H26 in cycles 1, 3, and 5, and the resistant canola cv. 45H29 in cycles 2 and 4. Statistical analysis indicated that cycle 3 developed the highest ID (99.6% ± 0.2%), which was significantly greater than the IDs in cycles 1 (94.7% ± 1.5%), 2 (86.4% ± 1.9%), and 4 (86.4% ± 1.7%). Index of

disease did not differ significantly between cycles 2 and 4, or cycles 3 and 5 (99.3% ± 0.5%) (Fig. 3-7).

Treatment 5 consisted of growing the resistant canola cv. 45H29 in all five cycles. Statistical analysis indicated that cycle 5 developed the highest mean ID (22.3% ± 3.3%), which was significantly greater than the IDs in cycles 1 (12.5% ± 1.1%) and 3 (16.1% ± 3.1%). Index of disease did not differ significantly between cycles 1 and 3, cycles 2 (18.4% ± 4.6%) and 3, 2 and 4 (17.8% ± 1.3%), 2 and 5, 3 and 4, or cycles 4 and 5 (Fig. 3-7).

Treatment 6 consisted of growing five different clubroot resistant canola cultivars in sequential order: 45H29, 73-77, 9558C, L135C, and 10DC30622. The greatest ID was observed in cycle 2 (i.e., 73-77, 15.7% ± 4.6%), which was significantly greater than the IDs obtained in cycles 1 (45H29, 9.4% ± 1.5%) and 5 (10DC30622, 9.4% ± 1.7%). Index of disease did not significantly differ between cycles 1 and 3 (9558C, 11.9% ± 1.2%), 1 and 5, 2 and 3, 2 and 4 (L135C, 14.7% ± 2.5%), 3 and 4, and 3 and 5 (Fig. 3-7).

3.3.2.2 Variation in plant height and dry weight within each crop rotation regime

Among all of the cycles in treatment 1, the greatest plant height was observed in cycle 3 (34.7 cm ± 4.1 cm). Plant height did not differ significantly between cycles 3 and 1 (27.2 cm ± 2.7 cm), cycles 3 and 2 (32.2 cm ± 2.8 cm), cycles 3 and 4 (27.7 cm ± 3.9 cm), cycles 3 and 5 (27.2 cm ± 2.6 cm), cycles 1 and 2, cycles 1 and 4, cycles 1 and 5, cycles 2 and 4, cycles 2 and 5, or cycles 4 and 5 (Fig. 3-8). The greatest plant dry weight was observed in cycle 1 (3.3 g ±0.5 g). Dry weight did not differ significantly between cycles 1 and 2 (2.4 g ± 0.2 g), cycles 1 and 3 (2.6 g ± 0.4 g), cycles 1 and 4 (2.7 g ± 0.2

g), cycles 1 and 5 (2.2 g ± 0.2 g), cycles 2 and 3, cycles 2 and 4, cycles 2 and 5, cycles 3 and 4, cycles 3 and 5, or cycles 4 and 5 (Fig. 3-9).

In treatment 2, the greatest plant height also was observed in cycle 3 (33.1 cm ± 3.3 cm). Plant height did not differ significantly between cycles 3 and 1 (29.9 cm ± 3.9 cm), cycles 3 and 5 (27.4 cm ± 3.4 cm), or cycles 3 and 5 (Fig. 3-8). The greatest dry weight was also was found in cycle 3 (3.1 g ± 0.3 g). Dry weight did not significantly differ between cycles 3 and 1 (2.9 g ± 0.4 g), cycles 3 and 5 (2.4 g ± 0.4 g), or cycles 3 and 5 (Fig. 3-9).

Similarly, among the cycles in treatment 3, the greatest plant height was observed in cycle 3 (30.2 cm ± 4.4 cm). Plant height did not differ significantly between cycles 3 and 1 (29.4 cm ± 3.1 cm), cycles 3 and 5 (28.8 cm ± 2.5 cm), or cycles 3 and 5 (Fig. 3-8). The greatest dry weight was found in cycle 1 (3.1 g ± 0.3 g). Dry weight did not differ significantly between cycles 1 and 3 (2.8 g ± 0.3 g), cycles 1 and 5 (2.6 g ± 0.3 g), or cycles 3 and 5 (Fig. 3-9).

In treatment 4, the greatest plant height was observed in cycle 2 (33.6 cm ± 3.1 cm). The plant height did not differ significantly between cycles 2 and 1 (31.2 cm ± 3.7 cm), cycles 2 and 3 (25.5 cm ± 2.7 cm), cycles 2 and 4 (29.2 cm ± 4.5 cm), cycles 2 and 5 (27.7 cm ± 2.7 cm), cycles 1 and 3, cycles 1 and 4, cycles 1 and 5, cycles 3 and 4, cycles 3 and 5, or cycles 4 and 5 (Fig. 3-8). The highest dry was observed in cycle 1 (3.2 g ± 0.3 g). Dry weight did not differ significantly between cycles 1 and 2 (2.4 g ± 0.2 g), cycles 1 and 3 (2.1 g ± 0.3 g), cycles 1 and 4 (2.7 g ± 0.2 g), cycles 1 and 5 (2.9 g ± 0.4 g), cycles 2 and

3, cycles 2 and 4, cycles 2 and 5, cycles 3 and 4, cycles 3 and 5, or cycles 4 and 5 (Fig. 3-9).

In treatment 5, the greatest plant height was found in cycle 1 (33.5 cm ± 4.3 cm). The plant height did not significantly differ between cycles 1 and 2 (32.4 cm ± 3.6 cm), cycles 1 and 3 (28.9 cm ± 3.8 cm), cycles 1 and 4 (22.6 cm ± 1.7 cm), cycles 1 and 5 (25.9 cm ± 2.5 cm), cycles 2 and 3, cycles 2 and 4, cycles 2 and 5, cycles 3 and 4, cycles 3 and 5, or cycles 4 and 5 (Fig. 3-8). The greatest dry weight was observed in cycle 5 (3.1 g ± 0.4 g). Dry weight did not differ significantly between cycles 5 and 1 (2.6 g ± 0.6 g), cycles 5 and 2 (2.1 g ± 0.3 g), cycles 5 and 3 (2.8 g ± 0.4 g), cycles 5 and 4 (2.8 g ± 0.6 g), cycles 1 and 2, cycles 1 and 3, cycles 1 and 4, cycles 2 and 3, cycles 2 and 4, or cycles 3 and 4 (Fig. 3-9).

Finally, in treatment 6, the greatest plant height was observed in cycle 4 (32.7 cm ± 4.1 cm). Plant height did not differ significantly between cycles 4 and 1 (32.3 cm ± 3.5 cm), cycles 4 and 2 (31.4 cm ± 3.5 cm), cycles 4 and 3 (26.1 cm ± 2.7 cm), cycles 4 and 5 (29.8 cm ± 2.3 cm), cycles 1 and 2, cycles 1 and 3, cycles 1 and 5, cycles 2 and 3, cycles 2 and 5, or cycles 3 and 5 (Fig. 3-8). Also in treatment 6, cycle 3 had the highest dry weight (2.8 g ± 0.4 g). The dry weight did not significantly differ between cycles 3 and 1 (2.6 g ± 0.4 g), cycles 3 and 2 (2.7 g ± 0.3 g), cycles 3 and 4 (2.4 g ± 0.3 g), cycles 3 and 5 (2.3 g ± 0.3 g), cycles 1 and 2, cycles 1 and 4, cycles 1 and 5, cycles 2 and 4, cycles 2 and 5, or cycles 4 and 5 (Fig. 3-9).

3.3.2.3 Variation in disease severity, plant height, and plant dry weight across treatments

Analysis of variance using the CATMOD procedure revealed a significant effect of treatments ($P = 0.0002$) when the average disease rating data were pooled together within each crop rotation regime. Treatment 2 had the greatest ID (98.7% ± 0.6 %), which was significantly greater than the ID in treatments 5 (17.4 % ± 2.6 %) or 6 (12.2 % ± 2.3 %). Index of disease did not differ significantly between any of the other treatments (Fig. 3-10).

Analysis of variance using the PROCMIXED procedure revealed no significant effects of the treatments ($P = 0.69$) when the average plant height data were pooled together within each crop rotation regime, although had the greatest plant height was observed in treatment 6 (30.5 cm ± 3.2 cm) (Fig. 3-10).

Analysis of variance using the PROCMIXED procedure revealed no significant effects of the treatments ($P = 0.49$) when the average dry weight data were pooled together within each crop rotation regime, in spite of treatment 3 having the highest dry weight (2.9 g ± 0.3 g) (Fig. 3-10).

3.4 Discussion

Crop rotation is recommended as an important tool for plant disease management. However, its effectiveness against clubroot on canola has not been quantitatively assessed (Peng et al., 2014). In the current study, the effects of five crop rotation regimes on clubroot disease development were evaluated. In Experiment 1, root galls were removed from the soil after each cropping cycle in the rotation. This likely decreased the inoculum load available in the soil for the next crop cycle, since most galls were not decomposing at the time that they were collected (and hence, the amounts of

new resting spores introduced back into the soil would have been minimal). Therefore, it is not surprising that a decreasing trend was observed in terms of IDs for the crop rotation regimes (treatments) 1 to 4, in which susceptible canola cultivars were included. Treatment 5 involved the consecutive cropping of the same resistant cultivar '45H29'. Notably, the ID that developed on this cultivar was significantly greater in cycles 3, 4 and 5 than in cycles 1 and 2, suggesting an erosion of clubroot resistance by the third crop cycle, which is consistent with a previous report by LeBoldus et al. (2012).

In Experiment 2, the root galls were homogenized and incorporated back into the soil mix after the completion of each cycle of the crop rotation. Therefore, the inoculum concentration in the soil mix would have increased over the course of the experiment. This experimental set up more closely resembles the situation under field conditions in Alberta, since the roots (and the inoculum contained therein) remain in the soil following harvest of a canola crop. In Experiment 2, the IDs continued to increase over cycles 1 to 5 in treatments 1 to 5. It is worth noting that treatment 5, which included five consecutive cycles of the same resistant cultivar '45H29', once again showed a significant increase in IDs over the course of the experiment. As was the case in Experiment 1, this increase in ID in a resistant cultivar after multiple cycles of growth in the same soil suggests an erosion of clubroot resistance (LeBoldus et al., 2012). In Treatment 6, in which different clubroot resistant canola cultivars were grown consecutively in the same soil, the IDs were lower than in the other treatments. Moreover, there was no clear increase or decrease in the ID values over the span of the rotation. Since these are commercial cultivars, no information is available as to the sources of resistance in each, given proprietary concerns. It is possible that the presence

of different resistance genes or background effects in each cultivar could prolong the effectiveness of clubroot resistance.

The average IDs observed in treatments 1 to 4 were significantly greater than those observed in treatments 5 and 6 in both experiments 1 and 2. Most likely, this reflects the fact that treatments 1 to 4 included a susceptible canola cultivar in a least some of the crop cycles, whilst only resistant cultivars were included in treatments 5 and 6. Hwang et al. (2013) reported that rotation of susceptible canola cultivars intensified the severity of clubroot disease. Rotation of a non-host (barley) with a susceptible host (treatment 3) did not appear to be effective at reducing clubroot severity, likely because the break between susceptible cultivars was very short. Indeed, it was reported recently that rotations which include non-hosts such as barley, pea, or oat can have the greatest impact on clubroot severity, if they are of sufficient length (Hwang et al. 2015). While significant differences were observed with respect to the IDs that developed on canola in the various treatments, differences with respect to plant height and dry weight were in general not significant. This suggests that the differences in disease severity, while significant, were not necessarily reflected in the growth response of the host plants. This would be expected, given that clubroot severities were still very high in the susceptible cultivar (regarding of cycle or rotation sequence) and mild in the resistant cultivars.

Although not a component of the current study, a direct measurement of *P. brassicae* resting spore concentration in the soil, achieved through methods such as quantitative PCR analysis (Rennie et al., 2011; Wallenhammar et al., 2011; Cao et al., 2014), would have provided an additional assessment of the efficacy of various rotation regimes in managing this pathogen. Treatments that resulted in a reduction in resting spore

concentration presumably would be more effective for clubroot control. Nonetheless, the IDs measured in this study provided a more direct assessment of the impact of rotation regimes on clubroot development and severity.

Table 3-1. Crop rotation regimes compared in greenhouse experiments evaluating clubroot development in soils naturally infested with *Plasmodiophora brassicae.*

Rotation regime	Crop in each cycle of a rotation sequence				
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Regime 1	45H26 (S)	45H26 (S)	45H26 (S)	45H26 (S)	45H26 (S)
Regime 2	45H26 (S)	Fallow	45H26 (S)	Fallow	45H26 (S)
Regime 3	45H26 (S)	Barley	45H26 (S)	Barley	45H26 (S)
Regime 4	45H26 (S)	45H29 (R)	45H26 (S)	45H29 (R)	45H26 (S)
Regime 5	45H29 (R)	45H29 (R)	45H29 (R)	45H29 (R)	45H29 (R)
Regime 6	45H29 (R)	73-77 (R)	9558C (R)	L135C (R)	10DC30622 (R)

S-susceptible canola cultivar, R-resistant canola cultivar.

Experiment 1

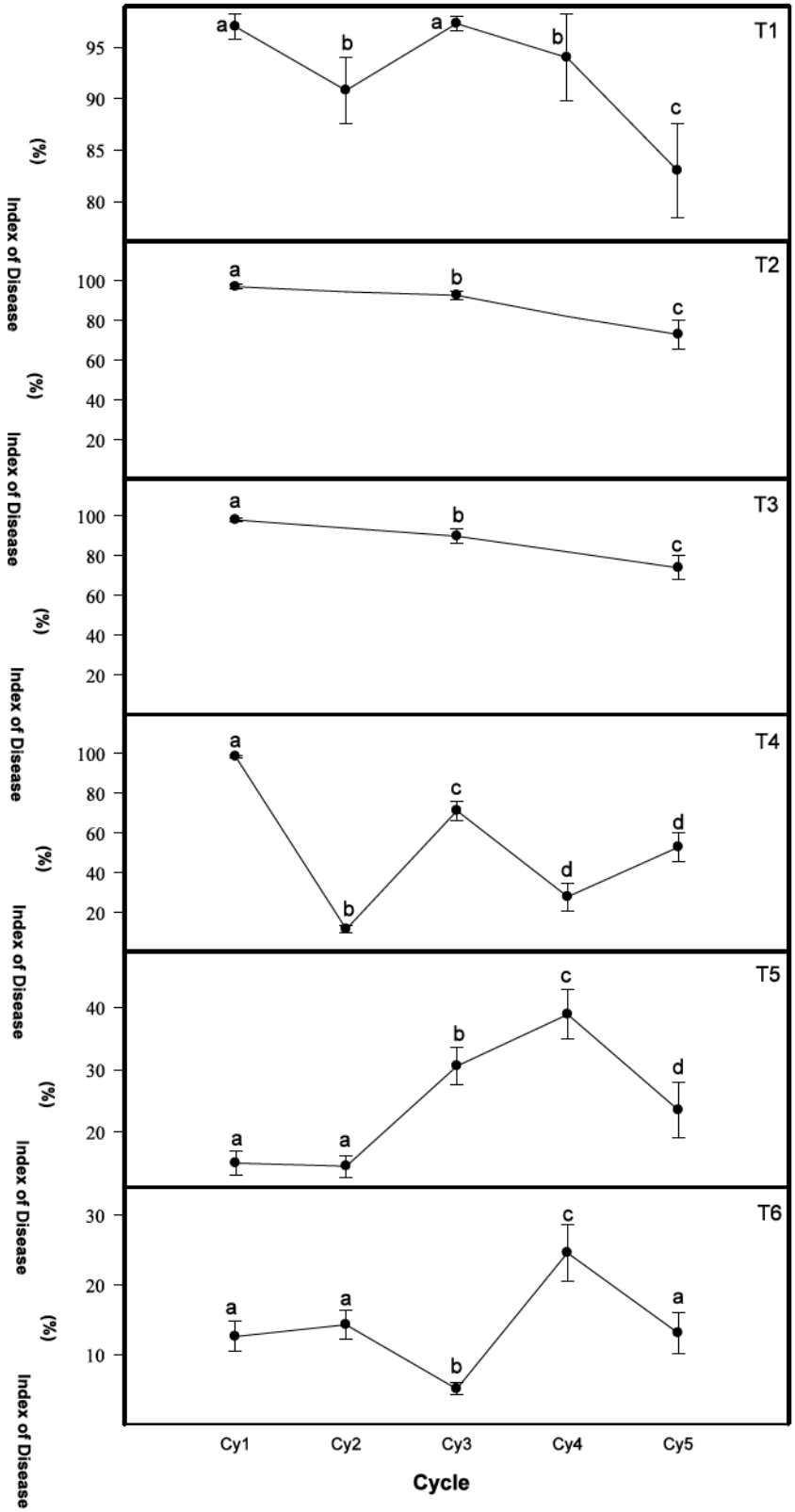

Figure 3-1. Index of disease in various clubroot resistant and susceptible *Brassica napus* canola cultivars grown in different sequences in a mixture of clubroot infested soil and potting medium under greenhouse conditions. Six crop rotation regimes (T1-T6) were assessed. (T1) consisted of five consecutive crops of a susceptible (S) cultivar of canola (45H26), i.e., S – S – S – S – S. (T2) consisted of a S - fallow (F) – S – F – S rotation. (T3) consisted of a S – barley (B) – S – B – S rotation. (T4) consisted of a S – resistant canola cultivar (R, 45H29) – S – R – S rotation. (T5) consisted of a R – R – R – R – R rotation (i.e., one resistant cultivar, 45H29). (T6) consisted of consecutive crops of clubroot resistant canola cultivars from different suppliers: 1 (R1, 45H29) – 2 (R2, 73-77) – 3 (R3, 9558C) – 4 (R4, L135C) – 5 (R5, 10DC30622). All canola root tissues were removed from the soil at the end of each crop cycle. The same lower case letter means no significant difference at $P < 0.05$ within a rotational regime or treatment.

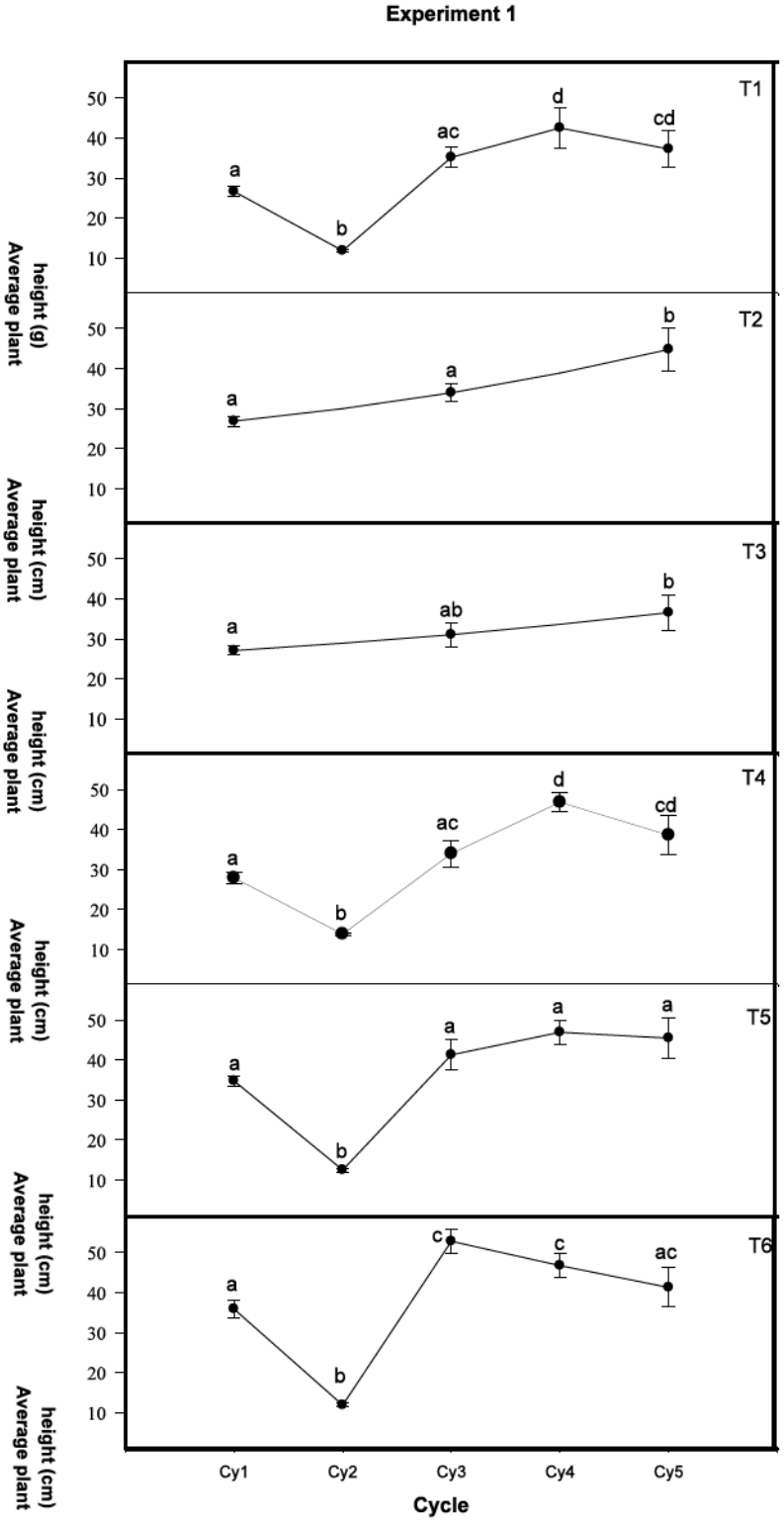
Experiment 1
T1
T2
T3
T4
T5
T6
Average plant height (g)
Average plant height (cm)
Cy1
Cy2
Cy3
Cy4
Cy5
Cycle

Figure 3-2. Average plant height in various clubroot resistant and susceptible *Brassica napus* canola cultivars grown in different sequences in a mixture of clubroot infested soil and potting medium under greenhouse conditions. Six crop rotation regimes (T1-T6) were assessed. (T1) consisted of five consecutive crops of a susceptible (S) cultivar of canola (45H26), i.e., S – S – S – S – S. (T2) consisted of a S - fallow (F) – S – F – S rotation. (T3) consisted of a S – barley (B) – S – B – S rotation. (T4) consisted of a S – resistant canola cultivar (R, 45H29) – S – R – S rotation. (T5) consisted of a R – R – R – R – R rotation (i.e., one resistant cultivar, 45H29). (T6) consisted of consecutive crops of clubroot resistant canola cultivars from different suppliers: 1 (R1, 45H29) – 2 (R2, 73-77) – 3 (R3, 9558C) – 4 (R4, L135C) – 5 (R5, 10DC30622). All canola root tissues were removed from the soil at the end of each crop cycle. The same lower case letter means no significant difference at $P < 0.05$ within a rotational regime or treatment.

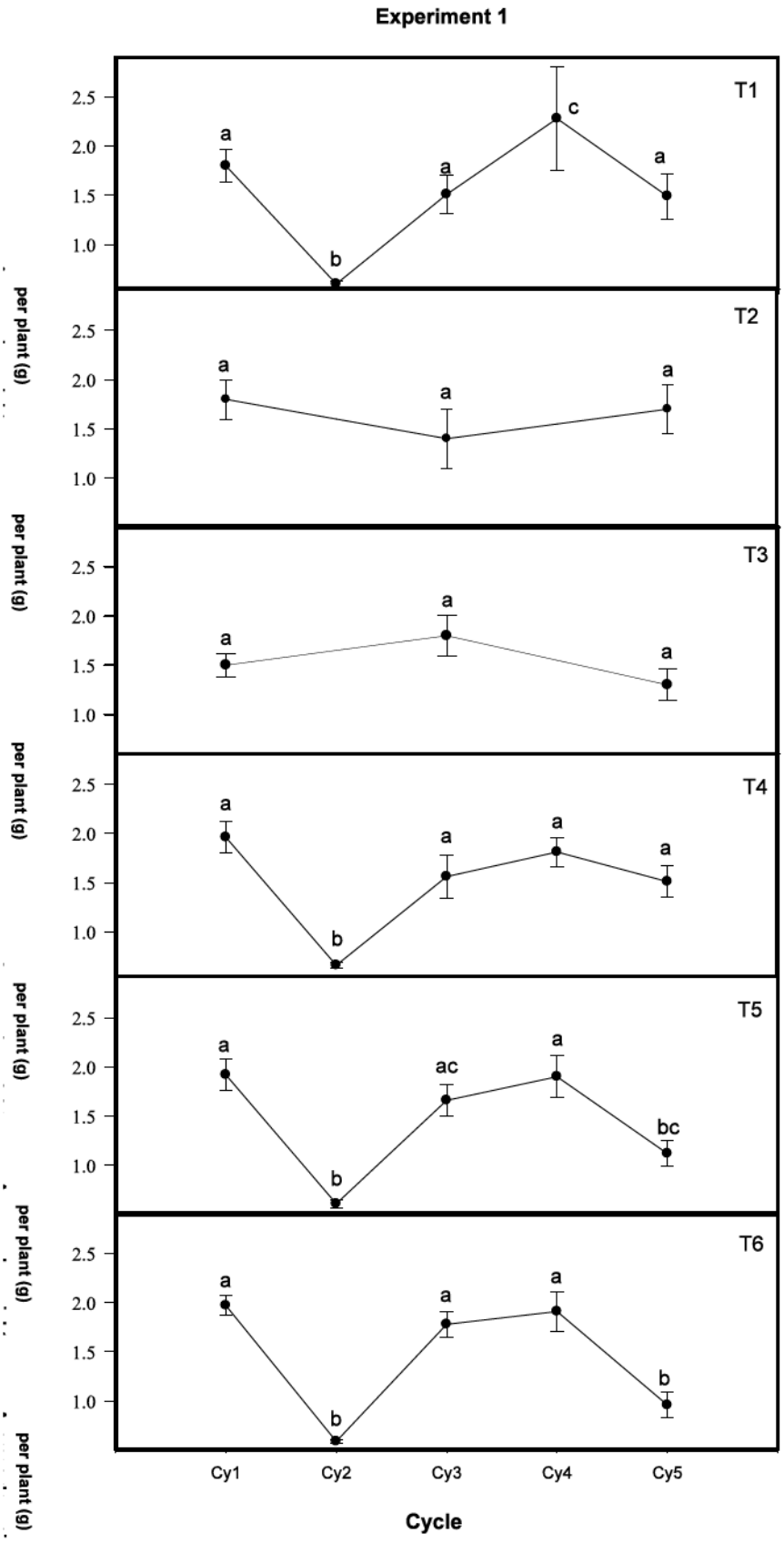
Experiment 1
T1
T2
T3
T4
T5
T6
per plant (g)
2.5
2.0
1.5
1.0
a
b
a
c
a
a
a
a
a
a
a
a
b
a
a
a
a
a
b
a
ac
a
bc
a
a
a
b
Cy1
Cy2
Cy3
Cy4
Cy5
Cycle

Figure 3-3. Average dry weight per plant in various clubroot resistant and susceptible *Brassica napus* canola cultivars grown in different sequences in a mixture of clubroot infested soil and potting medium under greenhouse conditions. Six crop rotation regimes (T1-T6) were assessed. (T1) consisted of five consecutive crops of a susceptible (S) cultivar of canola (45H26), i.e., S – S – S – S – S. (T2) consisted of a S - fallow (F) – S – F – S rotation. (T3) consisted of a S – barley (B) – S – B – S rotation. (T4) consisted of a S – resistant canola cultivar (R, 45H29) – S – R – S rotation. (T5) consisted of a R – R – R – R – R rotation (i.e., one resistant cultivar, 45H29). (T6) consisted of consecutive crops of clubroot resistant canola cultivars from different suppliers: 1 (R1, 45H29) – 2 (R2, 73-77) – 3 (R3, 9558C) – 4 (R4, L135C) – 5 (R5, 10DC30622). All canola root tissues were removed from the soil at the end of each crop cycle. The same lower case letter means no significant difference at $P < 0.05$ within a rotational regime or treatment.

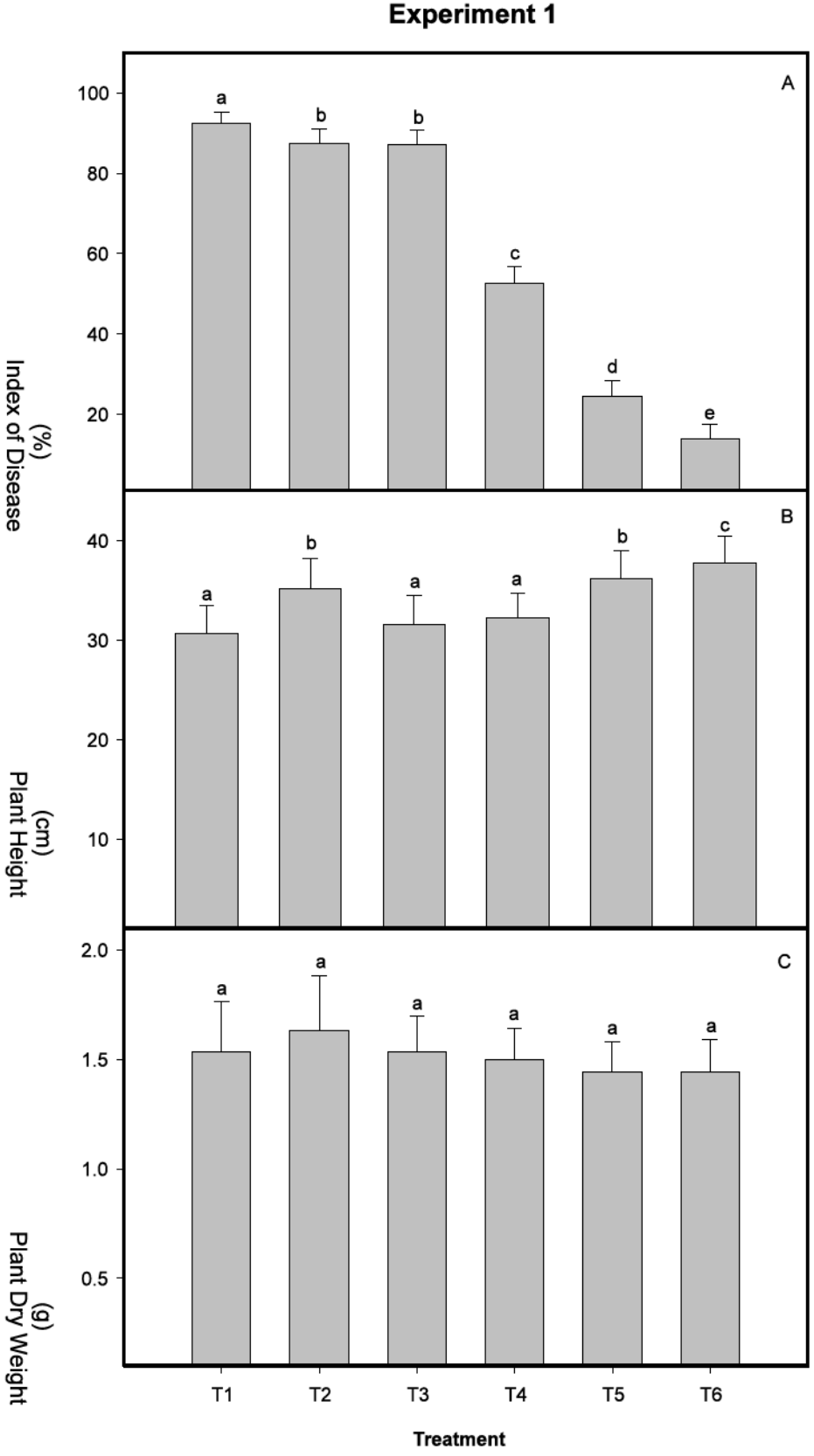
Experiment 1
A
B
C
Index of Disease (%)
Plant Height (cm)
Plant Dry Weight (g)
100
80
60
40
20
40
30
20
10
2.0
1.5
1.0
0.5
a
b
b
c
d
e
a
b
a
a
b
c
a
a
a
a
a
a
T1
T2
T3
T4
T5
T6
Treatment

Figure 3-4. Index of disease, plant height and plant dry weight across treatments in clubroot resistant and susceptible *Brassica napus* canola cultivars grown in different sequences in a mixture of clubroot infested soil and potting medium under greenhouse conditions. Six crop rotation regimes (T1-T6) were assessed. (T1) consisted of five consecutive crops of a susceptible (S) cultivar of canola (45H26), i.e., S – S – S – S – S. (T2) consisted of a S - fallow (F) – S – F – S rotation. (T3) consisted of a S – barley (B) – S – B – S rotation. (T4) consisted of a S – resistant canola cultivar (R, 45H29) – S – R – S rotation. (T5) consisted of a R – R – R – R – R rotation (i.e., one resistant cultivar, 45H29). (T6) consisted of consecutive crops of clubroot resistant canola cultivars from different suppliers: 1 (R1, 45H29) – 2 (R2, 73-77) – 3 (R3, 9558C) – 4 (R4, L135C) – 5 (R5, 10DC30622). All canola root tissues were removed from the soil at the end of each crop cycle. The same lower case letter means no significant difference at $P < 0.05$ for a particular parameter.

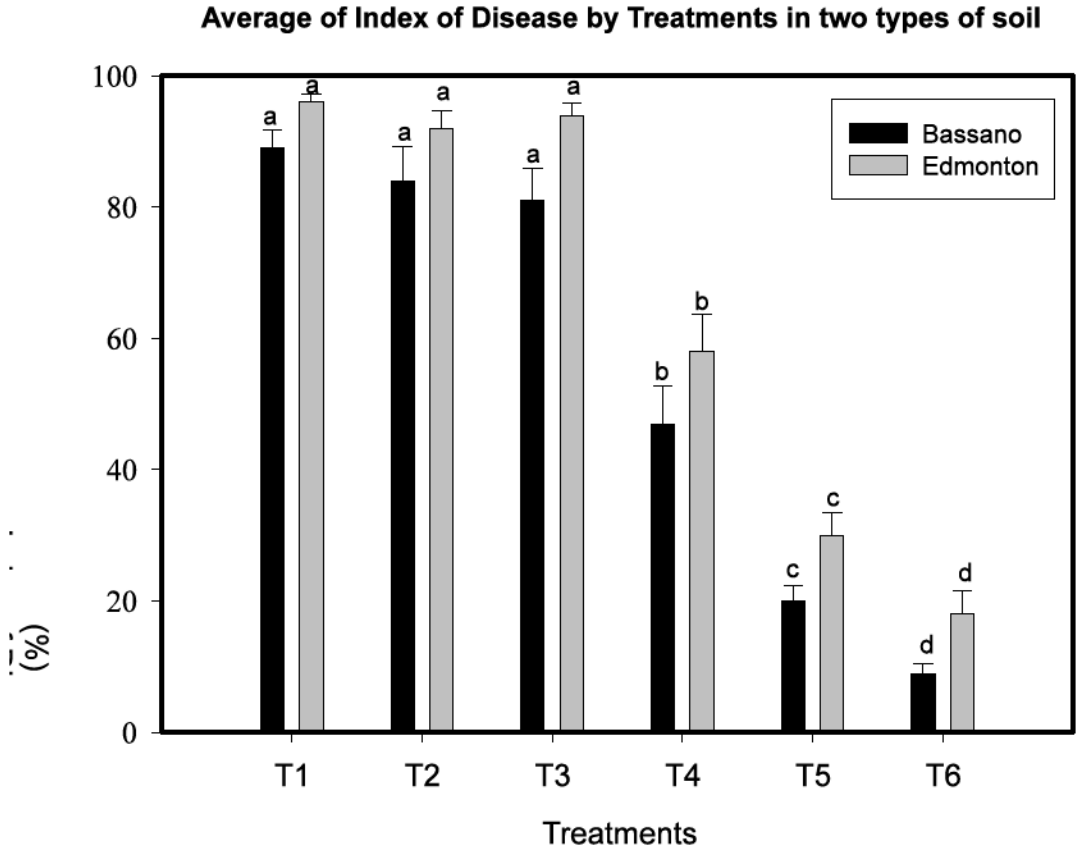

Figure 3-5. Comparison of index of disease in clubroot resistant and susceptible *Brassica napus* canola cultivars grown in different sequences in a mixture of clubroot infested soil collected from Bassano or Edmonton, Alberta, and potting medium under greenhouse conditions. Six crop rotation regimes (T1-T6) were assessed. (T1) consisted of five consecutive crops of a susceptible (S) cultivar of canola (45H26), i.e., S – S – S – S – S. (T2) consisted of a S - fallow (F) – S – F – S rotation. (T3) consisted of a S – barley (B) – S – B – S rotation. (T4) consisted of a S – resistant canola cultivar (R, 45H29) – S – R – S rotation. (T5) consisted of a R – R – R – R – R rotation (i.e., one resistant cultivar, 45H29). (T6) consisted of consecutive crops of clubroot resistant canola cultivars from different suppliers: 1 (R1, 45H29) – 2 (R2, 73-77) – 3 (R3, 9558C) – 4 (R4, L135C) – 5 (R5, 10DC30622). All canola root tissues were removed from the soil at the end of each crop cycle. Bars topped by the same letter are not significant different at $P < 0.05$.

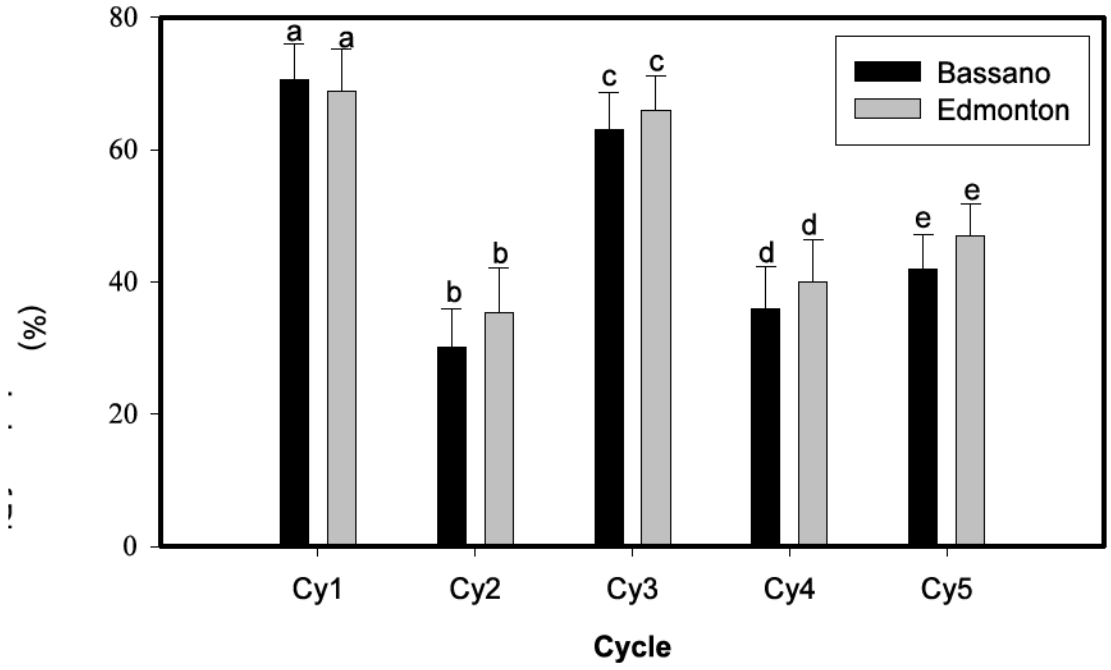

Figure 3-6. Comparison of index of disease in clubroot resistant and susceptible *Brassica napus* canola cultivars grown over five cycles in a mixture of clubroot infested soil collected from Bassano or Edmonton, Alberta, and potting medium under greenhouse conditions. The index of disease is averaged across treatments and cultivars for each crop cycle. All canola root tissues were removed from the soil at the end of each crop cycle. Bars topped by the same letter are not significantly different at $P < 0.05$.

Experiment 2

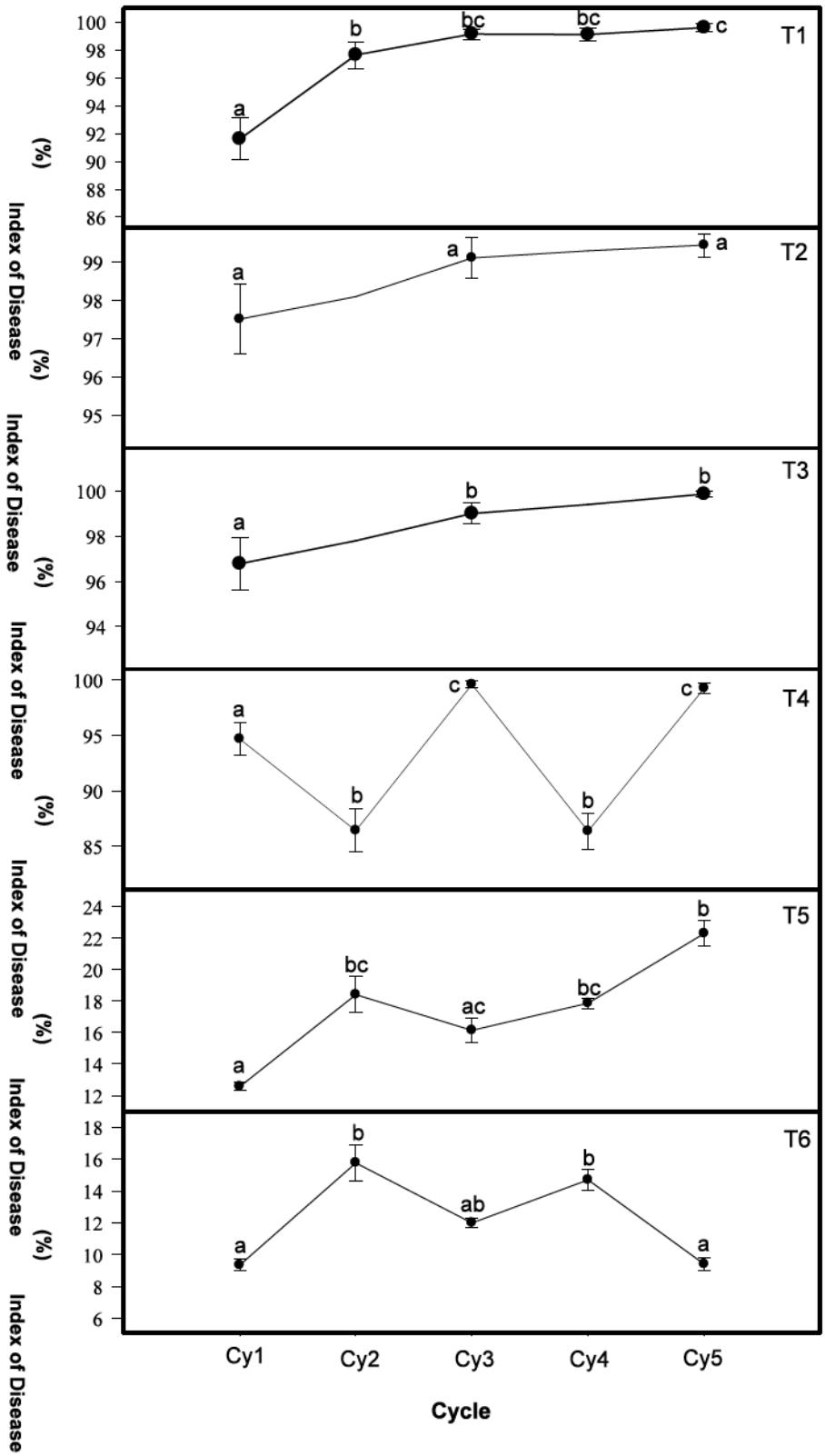

Figure 3-7. Index of disease in various clubroot resistant and susceptible *Brassica napus* canola cultivars grown in different sequences in a mixture of clubroot infested soil and potting medium under greenhouse conditions. Six crop rotation regimes (T1-T6) were assessed. (T1) consisted of five consecutive crops of a susceptible (S) cultivar of canola (45H26), i.e., S – S – S – S – S. (T2) consisted of a S - fallow (F) – S – F – S rotation. (T3) consisted of a S – barley (B) – S – B – S rotation. (T4) consisted of a S – resistant canola cultivar (R, 45H29) – S – R – S rotation. (T5) consisted of a R – R – R – R – R rotation (i.e., one resistant cultivar, 45H29). (T6) consisted of consecutive crops of clubroot resistant canola cultivars from different suppliers: 1 (R1, 45H29) – 2 (R2, 73-77) – 3 (R3, 9558C) – 4 (R4, L135C) – 5 (R5, 10DC30622). Canola root tissues were homogenized and incorporated back into the soil at the end of each crop cycle. The same lower case letter means no significant difference at $P < 0.05$ within a rotational regime or treatment.

Experiment 2

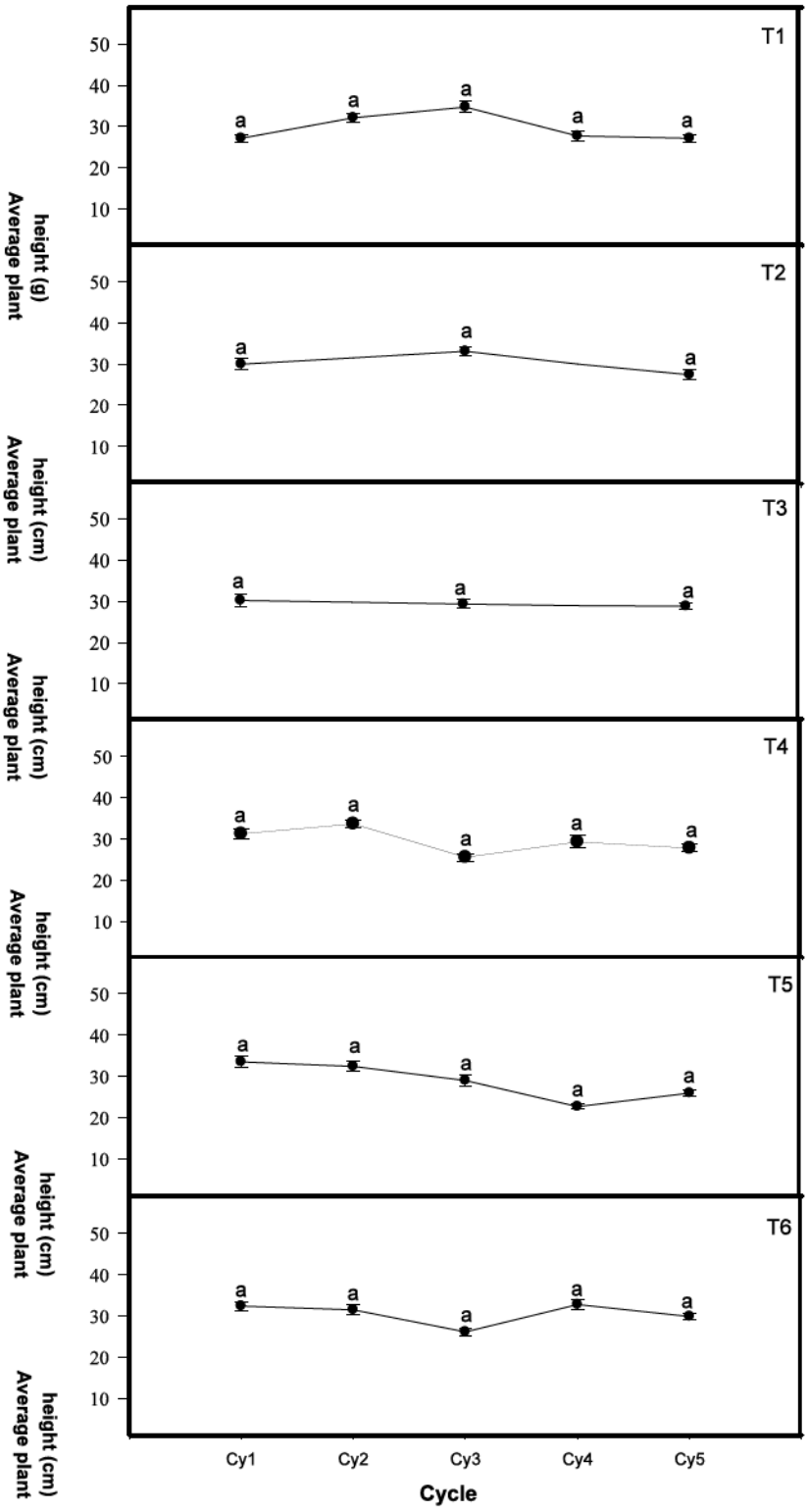

Figure 3-8. Average plant height in various clubroot resistant and susceptible *Brassica napus* canola cultivars grown in different sequences in a mixture of clubroot infested soil and potting medium under greenhouse conditions. Six crop rotation regimes (T1-T6) were assessed. (T1) consisted of five consecutive crops of a susceptible (S) cultivar of canola (45H26), i.e., S – S – S – S – S. (T2) consisted of a S - fallow (F) – S – F – S rotation. (T3) consisted of a S – barley (B) – S – B – S rotation. (T4) consisted of a S – resistant canola cultivar (R, 45H29) – S – R – S rotation. (T5) consisted of a R – R – R – R – R rotation (i.e., one resistant cultivar, 45H29). (T6) consisted of consecutive crops of clubroot resistant canola cultivars from different suppliers: 1 (R1, 45H29) – 2 (R2, 73-77) – 3 (R3, 9558C) – 4 (R4, L135C) – 5 (R5, 10DC30622). Canola root tissues were homogenized and incorporated back into the soil at the end of each crop cycle. The same lower case letter means no significant difference at $P < 0.05$ within a rotational regime or treatment.

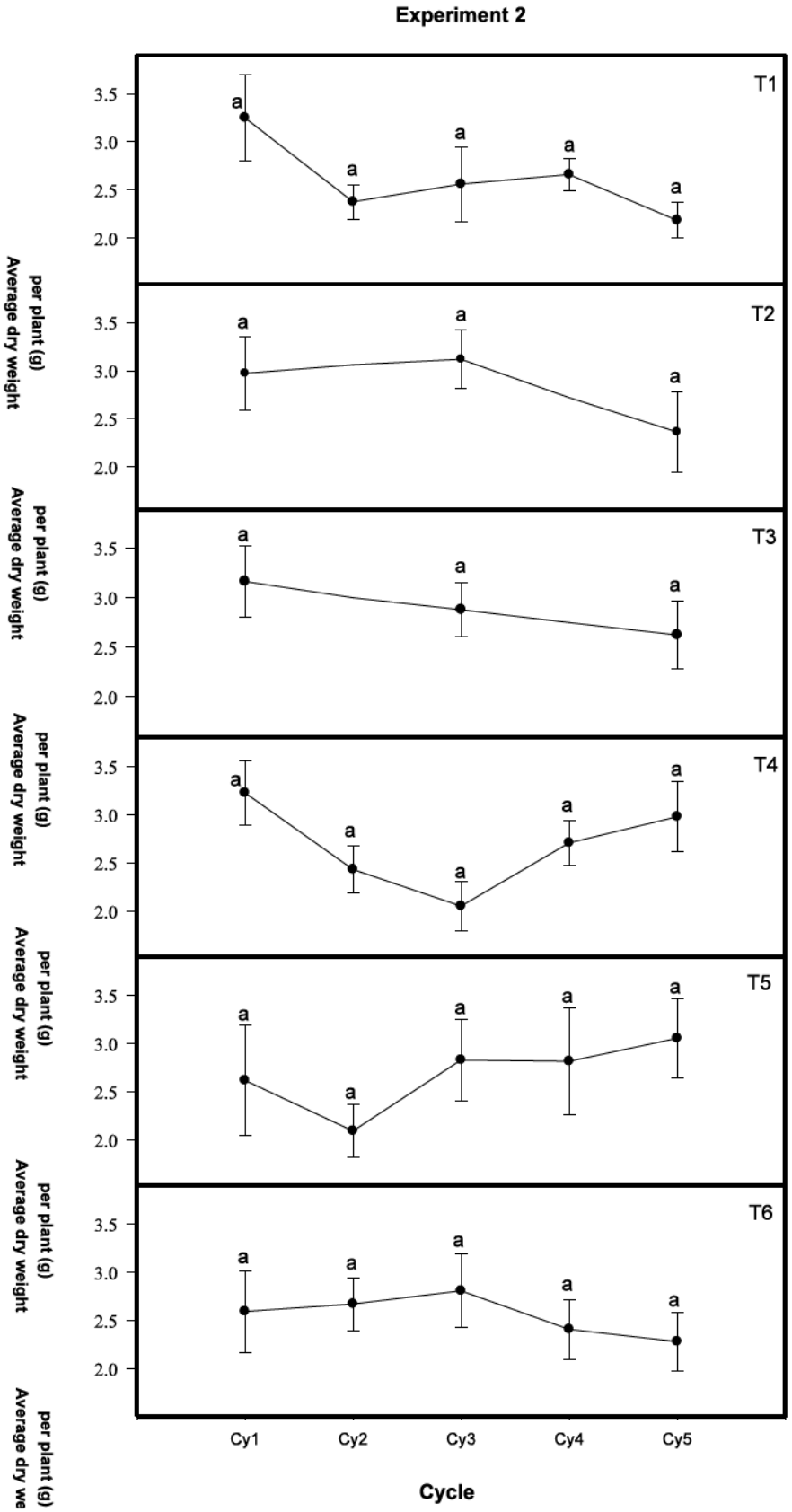
Experiment 2
T1
T2
T3
T4
T5
T6
3.5
3.0
2.5
2.0
a
Average dry weight per plant (g)
Cy1
Cy2
Cy3
Cy4
Cy5
Cycle

Figure 3-9. Average dry weight per plant in various clubroot resistant and susceptible *Brassica napus* canola cultivars grown in different sequences in a mixture of clubroot infested soil and potting medium under greenhouse conditions. Six crop rotation regimes (T1-T6) were assessed. (T1) consisted of five consecutive crops of a susceptible (S) cultivar of canola (45H26), i.e., S – S – S – S – S. (T2) consisted of a S - fallow (F) – S – F – S rotation. (T3) consisted of a S – barley (B) – S – B – S rotation. (T4) consisted of a S – resistant canola cultivar (R, 45H29) – S – R – S rotation. (T5) consisted of a R – R – R – R – R rotation (i.e., one resistant cultivar, 45H29). (T6) consisted of consecutive crops of clubroot resistant canola cultivars from different suppliers: 1 (R1, 45H29) – 2 (R2, 73-77) – 3 (R3, 9558C) – 4 (R4, L135C) – 5 (R5, 10DC30622). Canola root tissues were homogenized and incorporated back into the soil at the end of each crop cycle. The same lower case letter means no significant difference at $P < 0.05$ within a rotational regime or treatment.

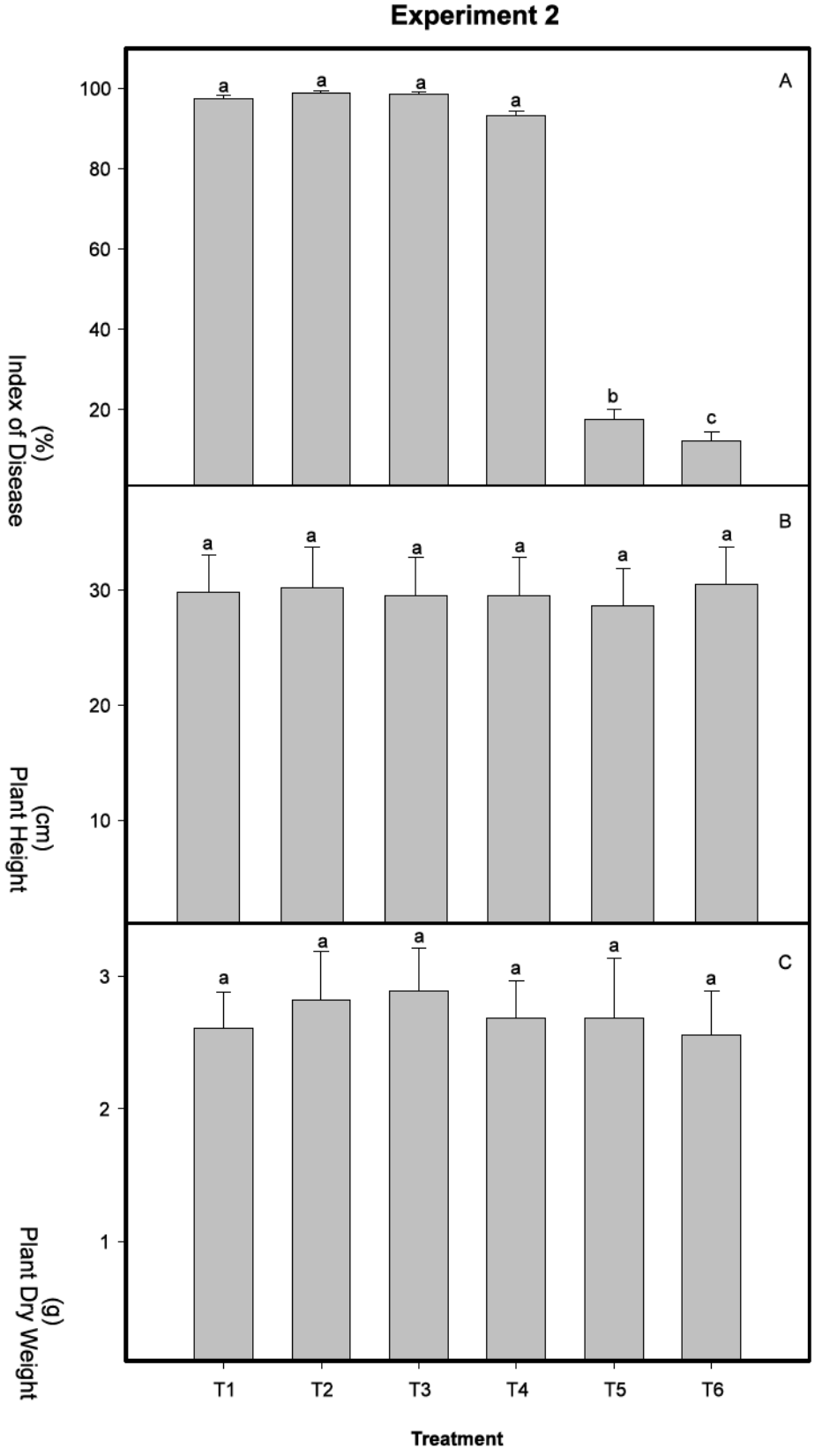
Experiment 2
A
B
C
Index of Disease (%)
Plant Height (cm)
Plant Dry Weight (g)
100
80
60
40
20
30
10
3
2
1
a
b
c
T1
T2
T3
T4
T5
T6
Treatment

Figure 3-10. Index of disease, plant height and plant dry weight across treatments in clubroot resistant and susceptible *Brassica napus* canola cultivars grown in different sequences in a mixture of clubroot infested soil and potting medium under greenhouse conditions. Six crop rotation regimes (T1-T6) were assessed. (T1) consisted of five consecutive crops of a susceptible (S) cultivar of canola (45H26), i.e., S – S – S – S – S. (T2) consisted of a S - fallow (F) – S – F – S rotation. (T3) consisted of a S – barley (B) – S – B – S rotation. (T4) consisted of a S – resistant canola cultivar (R, 45H29) – S – R – S rotation. (T5) consisted of a R – R – R – R – R rotation (i.e., one resistant cultivar, 45H29). (T6) consisted of consecutive crops of clubroot resistant canola cultivars from different suppliers: 1 (R1, 45H29) – 2 (R2, 73-77) – 3 (R3, 9558C) – 4 (R4, L135C) – 5 (R5, 10DC30622). Canola root tissues were homogenized and incorporated back into the soil at the end of each crop cycle. The same lower case letter means no significant difference at $P < 0.05$ for a particular parameter.

4 Conclusion

Clubroot, caused by *Plasmodiophora brassicae* Woronin, is an emerging disease of canola (*Brassica napus* L.) in Alberta, Canada. In this thesis, two studies were conducted to improve understanding of how environmental and cropping factors influence clubroot development on canola.

4.1 Evaluation of soil properties and *P. brassicae* spore concentrations in soils cropped to canola in Alberta, Canada

The first study focused on the relationship between the amount of spores in soil samples and the corresponding soil characteristics, which was different from previous studies (Karling, 1968; Dixon, 2009 and Deora et al., 2014) that focused on the relationship between incidence of the disease and soil characteristics. A total of 284 soil samples were randomly collected from three commercial canola fields in Alberta (Bassano, Edmonton, Parkland County) for analyses of *P. brassicae* spore concentration, organic matter, pH, electric conductivity, calcium and boron levels. Previously, a meta-analysis of field data collected from 2005 to 2010 revealed a significant negative correlation between clubroot disease severity and soil pH (Gossen et al., 2013), although this relationship was not as strong as expected. In addition, preplant application of boron could delay clubroot development and increase vegetative growth and yield compared to non-treated canola in soils heavily infested with *P. brassicae* (Deora et al., 2014). Supplemental applications of calcium and liming to increase soil pH also were found to reduce disease pressure (Murakami et al., 2002).

The presence of *P. brassicae* DNA in each sample was first evaluated by conventional PCR analysis, followed by a quantitative PCR assay to measure resting spore concentration if the conventional PCR yielded a positive result. Results indicated that 56% of the samples from Bassano, 8% of the samples from Parkland County, and 72% of the samples from Edmonton were positive for the presence of *P. brassicae* DNA and had quantifiable spore loads. Positive correlations were found between *P. brassicae* spore concentration and calcium concentration (r^2 =0. 44, P <0.0001, N = 127) and boron concentration (r^2 =0.10, P <0.0001, N = 127). A positive correlation also was found between resting spore concentration and soil organic matter (r^2 =0. 51, P <0.0001, N = 127). In contrast, *P. brassicae* spore concentration was negatively correlated with soil pH (r^2 =0.09, P = 0.0004, N = 127) and electrical conductivity (r^2 =0. 23, P <0.0001, N = 127).

In the canola field sampled in Parkland County, *P. brassicae* spores were mainly found within a localized area of the field. In the field sampled near Bassano, however, *P. brassicae* spores tended to be distributed fairly extensively over the sampling areas. At the Edmonton location, *P. brassicae* spores appeared to be most heavily concentrated in the south area of the field. Based on the more widespread distribution of the resting spores in the canola field near Bassano, it appears that this was the most heavily infested of the three fields analyzed. Nonetheless, the highest resting spore concentrations were found at the entrance of each of the three fields, confirming the results of an earlier report that infestations most often tended to be found at the entrance of canola fields (Cao et al. 2009). The interpolation maps that were generated using the field sampling data highlighted the generally patchy distribution of *P. brassicae* resting spores, which is a

common feature of soilborne plant pathogens. The generation of field-specific inoculum maps could represent a tool to assist farmers or researchers in developing appropriate disease management plans (for example, by allowing a farmer to avoid cultivation of highly infested patches within a field).

4.2 Evaluation of the impact of crop rotation on clubroot severity in Brassica napus canola under greenhouse conditions

The second study focused on the use of crop rotation as a tool for clubroot management. Six crop rotation regimes were tested with respect to their effectiveness in clubroot control in soil that was collected from canola fields near Edmonton. These rotations included: (1) continuous cropping of a clubroot-susceptible canola cultivar (S); (2) S –fallow (F) - S – F - S; (3) S – barley (B) - S – B – S; (4) S – resistant canola cultivar (R) – S – R – S; (5) continuous cropping of the same resistant cultivar; and (6) continuous cropping of different resistant cultivars. Two greenhouse experiments were conducted to assess the effects of the six crop rotation regimes on clubroot disease development. In experiment 1, root galls were removed out from the soil after the completion of each crop cycle, which in turn decreased the inoculum load in the soil for the next crop rotation. As a consequence, the index of disease (ID) declined in rotations 1 to 4 when a susceptible canola cultivar was cropped. Crop rotation regime 5 involved consecutive cropping of the same resistant cultivar over five cycles. In this case, a significant increase in ID was observed in cycles 3, 4, and 5 relative to cycles 1 and 2, suggesting an erosion of clubroot resistance in this cultivar over a period of three or more cropping cycles; this finding is consistent with a previous report by LeBoldus and coworkers (2012), who found that repeated re-inoculation with the same single-spore

isolate or population of *P. brassicae* could cause declines in the resistance of Brassica host genotypes. In experiment 2, the root galls were homogenized and incorporated into the soil after completion of each cropping cycle, and thus the inoculum concentration kept increasing during the course of the experiment. This provided a better representation of what would actually occur under field conditions, where the root galls would remain in the soil. Under this experimental protocol, the ID values continued to increase over each crop cycle in the rotation regimes 1 to 5. As in the first experiment, repeated cropping of a clubroot resistant canola cultivar resulted in a significant increase in disease severity in that cultivar after each cycle, again suggesting an erosion of resistance. Collectively, these experiments highlight the potential utility of crop rotation and appropriate crop choice in managing clubroot of canola, although additional studies, incorporating a wider set of crops within the rotations, are needed to fully understand this potential.

4.3 Future studies

This thesis has provided the basis for possible future studies to further increase understanding of the soil factors influencing clubroot development and the effectiveness of crop rotation as a management strategy. The detailed analysis of field characteristics and their relationship to *P. brassicae* resting spore concentrations could be supplemented by additional studies in which clubroot incidence and severity also were monitored. This could be accomplished through greenhouse bioassays with the soil collected from the field, or directly by planting canola cultivars of interest in the fields in question, and monitoring disease development over the growing season at the sampling points. Similarly, the results obtained in the crop rotation study, although valuable, would be strengthened by assessing several of the rotational treatments under field conditions in

replicated experiments. Ideally, the rotational trials would be conducted at a government run nursery, such as the clubroot nursery at the Crop Diversification Centre - North, Edmonton, to ensure appropriate biosafety protocols were in place. Additional research will ensure that understanding of the biology of clubroot disease continues to increase, facilitating the development of effective management strategies.

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