Evaluation of Resistance, Hydrated Lime, and Weed Control to Manage Clubroot in Canola

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Plant Science

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ABSTRACT

Plasmodiophora brassicae Wor. is a soilborne parasite causing clubroot of canola (*Brassica napus* L.), a serious disease managed mostly by planting clubroot resistant (CR) cultivars. Recently, new pathotypes of *P. brassicae* have emerged that overcome resistance, highlighting the need for a greater understanding of resistance stewardship and an integrated approach to clubroot management. Replicated field experiments were conducted in Edmonton, AB, in 2018 and 2019, to evaluate the effect of lime application and weed management on clubroot severity, crop yield and various growth parameters in clubroot susceptible (CS) and CR canola cultivars. *P. brassicae* resting spore densities were also monitored by quantitative PCR for each treatment. When hydrated lime was applied to increase the soil pH from initial values of 5.2-5.5 to 7.2, clubroot severity decreased by 34-36% in the CS canola cultivar, while seed yield increased by 70-98%. On CR canola, clubroot severity was reduced by an average of 9% but yield was not significantly affected. The application of hydrated lime also decreased resting spore densities by 48-80%, relative to untreated controls, in plots where the CS cultivar was grown, while the lime application or management of weeds did not significantly affect spore densities in plots with the CR cultivar. While the management of weeds did increase seed yield by an average of 35% in the CR canola and 21-40% in the CS canola, this likely reflected reduced competition rather than a direct effect on *P. brassicae* spore levels or clubroot severity. The field trials were complemented by a greenhouse experiment in which CS and CR canola cultivars were grown in different combinations (CR/CR, CR/CS, CS/CR, CS/CS) in a canola-wheat-barley-canola rotation with different initial spore densities of *P. brassicae* (0, 1×10^2, 1×10^4, 1×10^6, 1×10^8 spores per g soil mix). Clubroot severity increased in CR canola when it was grown twice in the rotation, but

only when the initial spore concentrations were 1 x 10^6 or 1 x 10^8 spores/g soil mix. The results from the field trial suggest that the application of hydrated lime may be a useful strategy to manage clubroot, when used in combination with genetic resistance to reduce disease pressure. The greenhouse experiment suggested that there is a reduced risk of resistance erosion when a CR cultivar is deployed in mildly infested soils with a 2-year break, but this must be confirmed under field conditions. Ultimately, a combination of strategies will be required for sustainable clubroot management.

PREFACE

This thesis is an original work by Brittany Hennig. I conducted and analyzed all of the experiments described in this document, with assistance and training by others as noted below. I also wrote the first drafts of all six chapters, which were then reviewed and edited by my supervisors, Dr. Stephen Strelkov and Dr. Sheau-Fang Hwang. Victor Manolii and Ileana Strelkov provided me with the proper technical training to be successful in the field and laboratory, teaching me various important techniques for working with the pathogen, soil and plant material. George Turnbull (Alberta Agriculture and Forestry) provided the training and assistance necessary for the successful completion of the field trials, including plot preparation and maintenance. In addition, several summer students from the University of Alberta Plant Pathology Lab assisted me with routine but important duties such as weeding and watering in the field and greenhouse, respectively. No part of this thesis has been published, although preliminary findings have been presented at various conferences over the course of my graduate program. This research was supported by the Alberta Canola Producers Commission, and Agriculture and Agri-Food Canada and the Canola Council of Canada (CCC) through the Canadian Agricultural Partnership (CAP) Program. Alberta Agriculture and Forestry and the University of Alberta provided in-kind support.

DEDICATION

I would like to dedicate this work to my Papa Durie – as I will always treasure this shared passion for crops with you.

ACKNOWLEDGMENTS

First, I would like to thank Dr. Stephen Strelkov, who helped me discover my passion for pathology as an undergrad. I am thankful for your patience and support while I navigated my graduate studies. The desire to pursue a Master's in Science weighed heavily on having you as my supervisor. Your knowledge is endless, and I hope to continue learning from you as much and as long as possible.

Thank you to my co-supervisor, Dr. Sheau-Fang Hwang, whose excitement and dedication helped me develop my curiosity and interest in research. I am lucky to have met you during my graduate studies, as you have been instrumental to my future endeavours.

Special thanks to Dr. Linda Gorim, for her review of my thesis and serving as my arm's length examiner, and to Dr. Boyd Mori, for chairing my defense.

I could have never succeeded in this endeavour without the love and guidance of my family. Whether it was helping with watering, weeding, disease ratings, crop tours, or presentations – you did this with me. A support system I did not initially know how exhaustively I would need you. Thank you.

I want to thank the Canola Family; Ward Toma and Rick Taillieu, who have given me more opportunities these past few years than I could have imagined. All the staff at Alberta Canola who provided me with an encouraging and positive work environment. Clint Jurke, Autumn Barnes, Nate Ort, and staff from the Canola Council of Canada who gave me the desire to learn more about research extension. Breanne Tidemann, Gregory Sekulic, Larry Michielsen, Jennifer Zuidhof, Murray Hartman, and Scott Meers for keeping my spirits up when things got tough and always lending their best advice.

Thank you to everyone who played a part in helping me complete this achievement; Doug Penney, who took the time to explain how to correctly calculate the amount of hydrated lime to use in my field trial. I was able to use his thesis written on hydrated lime in 1973 to support mine! Samuel Robertson, for taking the time to help me enjoy and learn the process of statistical analysis. Keisha Hollman, for her support, friendship, and positive attitude – especially when the days were emotionally and physically challenging. Ileana Strelkov, for her patience and guidance in helping me learn laboratory techniques. Victor Manolii, for his continual help in the field and greenhouse. And, of course, all the graduate and summer students that lent a hand throughout this process.

Lastly, I would like to recognize COVID-19 for being a pain in my butt.

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LIST OF ABBREVIATIONS

AIC	Akaike's Information Criteria
ANOVA	Analysis of Variance
B	Barley
BIC	Bayesian Information Criteria
$CaCN_2$	Calcium Cyanamide
$CaCO_3$	Calicitic Limestone
$CaMg(CO_3)_2$	Dolomitic Limestone
CaO	Burned/Quick Lime
$Ca(OH)_2$	Hydrated Lime
CAP	Canadian Agricultural Partnership
CCC	Canola Council of Canada
CCD	Canadian Clubroot Differential
CCE	Calcium Carbonate Equivalent
CR	Clubroot Resistant
CR*a*	Dominant Resistant Gene
CS	Clubroot Susceptible
CY1	The first canola crop
CY4	The second canola crop
DNA	Deoxyribonucleic Acid
dpi	Day(s) Post-Inoculation
ECD	European Clubroot Differential
ENV	Effective Neutralizing Value
GSF	Germination Stimulating Factors
ID	Index of Disease
lme	Linear Mixed-Effects
PCR	Polymerase Chain Reaction
PMA-PCR	Porpidium Monoazide PCR
qPCR	quantitative PCR
REML	Restricted Maximum Likelihood
TNV	Total Neutralizing Value
W	Wheat
WCC/RRC	Western Canada Canola/Rapeseed Recommending Committee
1CY1	After the first canola crop, before gall reincorporation
2PW	After the wheat crop
3PB	After the barley crop
4CY4a	After the second canola crop, before gall reincorporation
5CY4b	After the second canola crop, after gall reincorporation

1.0 INTRODUCTION

1.1 WHAT IS CLUBROOT?

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is a soilborne disease of the Brassicaceae family. All species within this family are potential hosts of *P. brassicae* (Dixon, 2009), which causes estimated crop losses of 10-15% worldwide (Dixon, 2006). Although the Russian biologist Woronin first identified *P. brassicae* as the causal agent for clubroot in 1873 (Woronin 1878), historical accounts of the disease occur since at least Roman times (Strelkov and Dixon, 2014). Clubroot is increasingly threatening the production of cultivated brassica vegetables and non-vegetable oilseeds around the world. The severity of symptoms and crop losses are related in large part to the frequency of cultivation of susceptible crops, and therefore, clubroot is a 'disease of cultivation' (Dixon, 2009; Feng et al., 2014). The predominant visual symptom of clubroot is a swelling of the host roots, resulting in distinct galls or clubs on susceptible hosts. This swelling reflects hypertrophy and hyperplasia of the root tissues, which can in turn restrict nutrient and water movement within the host. Aboveground symptoms such as stunting, foliar wilting, leaf discoloration, and premature ripening appear as a result (Dixon, 2009).

1.2 IMPACT ACROSS CANADA

Clubroot is not a new disease to Canada, with symptoms on cabbage, cauliflower, Chinese cabbage, and turnip documented in the *Canadian Plant Disease Survey* by the 1920s. Throughout the 20th century, clubroot was a familiar disease affecting cultivated vegetables across British Columbia, Ontario, Quebec and the Maritimes (Howard *et al.*, 2010). However,

although there were isolated reports of clubroot on Brassica oilseed crops in Newfoundland (Creelman, 1967) and Quebec (Morasse *et al*., 1997), clubroot was not confirmed on canola (*Brassica napus* L.) in western Canada until 2003 (Tewari *et al*., 2005), when a dozen infected crops were found in central Alberta. Since 2003, clubroot has spread across most canola producing regions in the province, and now is also found with increasing frequency in Saskatchewan and Manitoba (Cao *et al.*, 2009; Strelkov and Hwang, 2014; Froese *et al.,* 2019; Ziesman *et al.,* 2019). Canola yields in affected crops can substantially decrease due to this disease, with the potential of complete yield loss (Pageau *et al., 2006*; Strelkov *et al*., 2007). The effective management of clubroot in canola is essential, as canola contributes approximately $26.7 billion CAD annually to the Canadian economy (LMC International, 2016). The canola industry supports 250,000 jobs and $11.2 billion in wages for Canadians (LMC International, 2016). In addition to canola being one of the most valuable crops in Canada (Statistics Canada, 2021), canola oil is considered a very affordable and healthy edible oil. Canola oil consumption may reduce cardiovascular disease risk due to its unsaturated fat content (Lin *et al*., 2013), and contributes to cleaner household air quality due to its high smoke point and fryer stability (Canola Eat Well, 2017). Therefore, proper clubroot management is important for the agricultural industry and all Canadians.

1.3 CLUBROOT IN ALBERTA

Due to its novelty when it was first discovered on canola in Alberta in 2003 (Tewari *et al*., 2005), few strategies for the management of clubroot were available to growers. In addition to a minimum 3-year break from cruciferous host crops, recommendations included the sanitization

of field equipment to prevent the spread of *P. brassicae* infested soil (Strelkov and Hwang, 2014). Unfortunately, farmers struggled with adopting longer rotations out of canola, due to various geographical, climatic, and economic limitations. Furthermore, the sanitization of machinery was also not practiced widely, given the amount of time required to properly clean-off soil from equipment (Strelkov *et al*., 2011; Hwang *et al*., 2014).

With such limited control measures, clubroot continued to spread across Alberta. The first clubroot resistant (CR) canola cultivar in western Canada was registered in 2009 ('45H29'), with additional resistant cultivars becoming available soon after. As *P. brassicae* spread and inoculum levels rose in infested fields, canola growers utilized CR cultivars heavily for effective clubroot management. While canola breeding companies did not disclose the genetic basis of the resistance in their cultivars, most of the resistant varieties released, at least initially, appeared to carry single-gene resistance (Rahman *et al*., 2014; Hwang *et al*., 2012a). The availability of CR canola enabled continued production of this crop even in fields with severe *P. brassicae* infestations.

In 2013, two fields near Edmonton planted to CR cultivars showed substantial symptoms of clubroot . Testing under controlled conditions confirmed that these symptoms were caused by the emergence of 'new' pathotypes of *P. brassicae*, capable of overcoming the resistance in may CR cultivars (Strelkov *et al.*, 2016). Since then, further surveillance and pathogenicity testing has found increasing number of fields with pathotypes able to break resistance (Strelkov *et al*., 2018; Hollman *et al*., 2020). The loss or erosion of resistance results from the selection pressure imposed by CR canola cultivars on *P. brassicae* populations, leading to pathotype shifts (LeBoldus *et al.*, 2012; Strelkov *et al.*, 2018). Recently, a new host differential system, the Canadian

Clubroot Differential (CCD) set, was developed by Canadian researchers to improve the identification of novel *P. brassicae* pathotypes (Strelkov *et al*., 2018). A total of 36 CCD pathotypes have been confirmed across Canada, of which 19 can overcome the resistance in at least some CR canola cultivars (Strelkov *et al*. 2018; Strelkov *et al*. 2020; Askarian *et al*. 2020; Hollman *et al*. 2020).

1.4 RESEARCH OBJECTIVES

As *P. brassicae* continues to spread across the Prairies and the loss of resistance becomes increasingly common, the adoption of multiple clubroot management strategies becomes critical for sustainable control of the disease. The aim of this project was to evaluate the effect of various management practices and approaches on *P. brassicae* inoculum levels and clubroot incidence and severity. The specific objectives were:

1. To determine whether there is a detriment to the early deployment of CR canola cultivars to mitigate *P. brassicae* inoculum levels,
2. To determine the effect of CR cultivars, when rotated with wheat and barley, on *P. brassicae* inoculum levels and clubroot incidence and severity,
3. To compare the effectiveness of the application of hydrated lime, weed management and the deployment of CR genetics, on clubroot severity, incidence and yield.

2.0 LITERATURE REVIEW

2.1 BIOLOGY OF *PLASMODIOPHORA BRASSICAE*

2.1.1 Life Cycle

The life cycle of *Plasmodiophora brassicae* is complex, which stymies the development of successful control strategies. It consists of three main stages: survival as resting spores in the soil, root hair infection, and cortical infection (FIGURE 2.1) (Ayers, 1944; Ingram and Tommerup, 1972; Naiki, 1987). Soil becomes infested with *P. brassicae* when resting spores of the pathogen are introduced, serving as primary inoculum (Kageyama and Asano, 2009). This introduction can occur from decomposing infected plant material or through the physical movement of infested soil by farm or other equipment (Cao *et al*., 2009; Hwang *et al*., 2014). Infested soil may also be carried and deposited by humans, animals, or environmental factors such as wind or water erosion, distributing spores and soil particles downwind or downstream (Rennie *et al*., 2015; Howard *et al*., 2010).

Resting spores can withstand degradation by the environment, surviving for up to 20 years in the soil (Braselton, 1995; Wallenhammar, 1996). Once stimulated to germinate by appropriate environmental conditions and the presence of plant root exudates (MacFarlane, 1970), the resting spore releases a biflagellate primary zoospore. Although short-lived, zoospores can swim in free water surrounding soil particles, increasing access to potential hosts (Feng *et al*., 2012). Once it contacts the surface of a root hair, the zoospore will encyst and penetrate the cell wall. This is the second stage of the life cycle, also known as the primary infection (Kageyama and Asano, 2009). Primary infections can occur in host and non-host plants (Feng *et al*., 2012)

and are not responsible for the visible gall formations on the root that leads to yield loss (Howard *et al*., 2010).

Once inside the root hair, the pathogen forms a primary plasmodium, which cleaves into a zoosporangium, releasing 4-16 secondary zoospores into the soil. The secondary zoospores then re-infect the host through the cortical root tissue. This is the final stage of the pathogen life cycle, also known as the secondary infection. The secondary phase of *P. brassicae* can infect both hosts and non-hosts, but secondary infection in non-hosts does not progress to gall formation (Feng *et al*., 2012), whereas in hosts, it causes disease (Howard *et al*., 2010). The proliferation of intracellular secondary plasmodia causes hormonal disturbances in the host, leading to the hypertrophy and hyperplasia of root tissues (Dixon 2009b; Kageyama and Asano, 2009). This expansion in both the size and numbers of resting spores in the root begins to restrict nutrient and water uptake, resulting in yield loss and, eventually, premature death of the plant. The secondary plasmodia eventually cleave into resting spores and are released back into the soil (Tommerup and Ingram, 1971; Kageyama and Asano, 2009). Hwang *et al*. (2013) estimates that there can be around 16 billion resting spores in 1 g of galled canola root.

P. brassicae can complete its lifecycle in a single growing season (Hwang *et al*., 2012a). However, infection itself requires much less time. Under controlled conditions, McDonald *et al*. (2014) observed primary root hair infection at 1-day post-inoculation (dpi), followed by secondary zoospores release at 3-5 days. Zamani-Noor and Rodemann (2018) observed clubroot symptoms 7 dpi on canola/oilseed rape grown in controlled conditions. Moreover, *P. brassicae* DNA was detected at 5 dpi by quantitative polymerase chain reaction (qPCR) analysis in greenhouse-grown canola (Cao *et al*., 2014). Soil temperatures cause discrepancies in infection

time between controlled conditions and field conditions in Canada. Temperatures < 17 ^{o}C greatly suppress clubroot symptoms, whereas temperatures between 24-26 ^{o}C result in the most severe symptoms (Gossen *et al*., 2012). Therefore, clubroot symptoms appear later in the field season as the soil warms. As the plant matures and dies, the resting spores are released back into the soil, mostly in the following spring (Ernst *et al*., 2019). These resting spores serve as inoculum for the coming years (Kageyama and Asano, 2009).

2.1.2 Taxonomy

A part of the protist supergroup Rhizaria, *P. brassicae* is classified in the phylum Cercozoa (Cavalier-Smith, 1993; Bass *et al*., 2009). The pathogen falls within the class Phytomyxea, grouped under one of two subphylas, Endomyxa (Cavalier-Smith and Chao, 2003). Due to the incomplete understanding of the *P. brassicae* life cycle, accurate taxonomic classification has been challenging (Donald and Porter, 2014). When or if karyogamy occurs to allow meiosis in haploid cells in not known (Braselton, 1995).

2.1.3 Host Range

P. brassicae is believed to be able to infect most of the 3,700 species in the family Brassicaceae (Dixon, 2009a). Since the prevalence of the pathogen is associated with the frequency of cruciferous crops grown, those hosts within the genera *Brassica* and *Raphanus* have been studied most extensively (Dixon, 2009a). The model plant *Aradiposis thaliana* (L.) Heynh. is also a host and has been the subject of many studies. In Canada, clubroot was first identified on cabbage (*Brassica oleracea* L. var. *capitata*), cauliflower (*Brassica oleracea* L. var. *botrytis*), Chinese cabbage (*Brassica rapa* L. var. *pekinensis*), and turnip (*Brassica rapa* L. var.

rapa) (Howard *et al*., 2010), before its relatively recent emergence on canola (*Brassica napus* L.) in western Canada (Tewari *et al.*, 2005).

In addition to susceptible cultivated crops, there are many *P. brassicae*-susceptible cruciferous weeds. Common host weeds found across the prairies include volunteer canola (*B. napus* and *B. rapa*), field pepperwort (*Lepidium latifolium* L.), shepherd's purse (*Capsella bursa-pastoris* L.), stinkweed (*Thlaspi arvense* L.), and flixweed (*Descurainia sophia* L.) (Gibbs, 1932; Buczacki and Ockendon, 1979; Ren *et al*., 2016). Susceptible weeds are an important factor to consider when managing resting spore levels in non-host crop years (Dixon 2009b, Hwang *et al*., 2012a). The proper management of weeds is critical, as the plants can germinate throughout the growing season, increasing the risk of pathogen build-up (Zamani-Noor and Rodemann, 2018). Host plants are susceptible to infection by *P. brassicae* at all growth stages, although disease severity declines with plant age at the time of infection (Hwang *et al*., 2011b).

Varying degrees of susceptibility to *P. brassicae* have been observed. Ludwig-Müller *et al*. (1999) observed *P. brassicae*-like structures in the root cortex of non-brassica crops via scanning electron microscopy. Resting spores collected from the root tissues of garden nasturtium (*Tropaeolum majus* L.) and beet (*Beta vulgaris* L.) could infect *B. rapa*, which led to macroscopic symptoms. Alternatively, spores extracted from papaya (*Carica papaya* L.) and white mignonette (*Reseda alba* L.) were unable to cause macroscopic symptoms on *B. rapa*. Perennial ryegrass *Lolium perenne* L. has also been confirmed to be susceptible to primary infection. To add to the complexity of *P. brassicae*, MacFarlane (1952) did not observe any secondary infection on cruciferous crops infected with resting spores produced on *L. perenne*, while Feng *et al*. (2012)

did. These discrepancies are attributed to whether primary or secondary *P. brassicae* zoospores were used to infect their host (Feng *et al*., 2014).

2.1.4 Pathotypes

An understanding of physiological specialization in *P. brassicae* is important, since the efficacy of genetic resistance in host cultivars will depend on the predominant strains or pathotypes present in a region (Xue *et al.*, 2008). Pathotypes of *P. brassica*e are defined by their virulence patterns on specific groups of hosts, known as differential sets. Several differential systems have been proposed for the clubroot pathogen, with the hosts of Williams (1966), Somé *et al.* (1996), and the European Clubroot Differential (ECD) (Buczacki *et al.*, 1975) all used in at least some studies in Canada (Strelkov and Hwang, 2014). The differentials of Williams (1966) include two cabbages (*B. oleracea* var. *capitata*) and two rutabagas (*B. napus* var. *napobrassica*), whereas the differentials of Somé *et al*. (1996) consist of three genotypes of *B. napus*. Both differential systems include a limited number of hosts, reducing greenhouse space requirements and facilitating testing of larger numbers of samples. However, the differentials of Somé *et al*. lack differentiating capacity, and as such the system of Williams has until recently been most commonly used in Canada, particularly for extension purposes (Strelkov and Hwang, 2014; Strelkov *et al.*, 2018). The ECD set is larger, consisting of three subsets (*B. rapa*, *B. napu*s and *B. oleracea*) of five host genotypes each (Buczacki *et al.*, 1975). The large number of hosts and complex triplet code system used for pathotype nomenclature in the ECD system has, however, resulted in its limited adoption in the Canadian context.

The limitations of the existing differential systems were highlighted with the identification of the first isolates of *P. brassicae* able to overcome the resistance in CR canola in

2013 (Strelkov *et al.*, 2016). These isolates were classified as pathotype 5 on the system of Williams, or as pathotype P_3 on the hosts of Somé *et al.*, yet differed significantly from other pathotype 5 or P_3 isolates given their ability to cause severe clubroot on CR canola hosts (Strelkov *et al.*, 2016). As more isolates able to break resistance were found, it became clear that a new system would be required to distinguish and label new pathotypes, resulting in the development of the Canadian Clubroot Differential (CCD) set (Strelkov *et al.*, 2018). The CCD set consist of 13 Brassica genotypes, including the hosts of Williams, Somé *et al.*, and selected ECD differentials, as well as several *B. napus* varieties of particular relevance to Canadian canola production (Strelkov *et al.*, 2018). Since the CCD set also includes the differentials of Williams and Somé *et al.*, testing on this set also provides pathotype designations according to those systems, allowing continuity and comparison with earlier studies. The CCD set has significant differentiating capacity, and recent studies have enabled identification of 36 pathotypes across Canada, including 19 virulent on many CR canola hosts (Strelkov *et al.*, 2018, 2020; Askarian *et al.*, 2021; Hollman *et al.*, 2020). In the CCD system, each pathotype is assigned a number corresponding to its Williams classification, followed by a letter denoting its designation on the CCD hosts. Multiple variants of a single Williams' pathotype can be denoted by different letters (e.g., pathotypes 2A, 2B, 2C, etc.).

2.2 *P. BRASSICAE* DETECTION & QUANTIFICATION

2.2.1 Bioassays

A challenging aspect of working with *P. brassicae* is that it cannot be readily cultured *in vitro*. Therefore, as an obligate pathogen, the growing of susceptible plants in the test soil is a

useful method to detect *P. brassicae*. Due to their effectiveness, bioassays have been widely used to detect the pathogen in soil and plant samples (Samuel and Garrett, 1945; Foster, 1950; Wallenhammer, 1996; Strelkov *et al*., 2006). Foster (1950) used Chinese cabbage (cv. Chihli) as a host plant since it grows quickly and is highly susceptible to *P. brassicae*. The *Chinese cabbage* var. *pekinensis* (cv. Granaat) is universally susceptible and can also serve as a bait plant in bioassays (Faggian and Strelkov, 2009); seedlings are either pre-germinated and transplanted into the test soil, or directly sown there. As alluded to in the previous section, bioassays can also be used to identify pathotypes of in *P. brassicae*. In this case, seedlings of the differential hosts are grown in pathogen-free soil or potting mix and inoculated with resting spores of the *P. brassicae* isolate to be tested (see, for example, Strelkov *et al*., 2006 or Strelkov *et al*., 2018). The host reactions are monitored and the isolate being tested is assigned a pathotype classification based on its virulence patterns. In pathotyping studies, seedlings can be inoculated by dipping the roots in a spore suspension, by adding an aliquot of spore suspension to the seedlings in the potting mix, or by a combination of both methods.

Regardless of the inoculation method used, the seedlings require at least 5 weeks of growth under greenhouse conditions for macroscopic symptoms to appear (Colhoun, 1957). The roots are then cleaned with tap water and assessed for clubroot development (Faggian and Strelkov, 2009). Although widely used in *P. brassicae* research, bioassays are extremely laborious and require a substantial amount of greenhouse space (Faggian and Strelkov, 2009). To be detected, spore concentrations need to be excess of 1×10^3 spores per gram of dry soil (Cao *et al*., 2007). Additionally, there are discrepancies in quantifying *P. brassicae* spore loads based on the percentage of clubbed plants. Different soils with the same resting spore concentration can

show a different frequency or severity of infected plants due to various factors including soil structure, composition, and nutrient availability (Naumov, 1928; Colhoun, 1953; Karling, 1968; Wallenhammer, 1996).

2.2.2 Microscopy

Resting spores of *P. brassicae* can be visualized by compound microscopy, although this can be more difficult with soil samples where the presence of small particles and debris may hinder visualization. The resting spores can be readily extracted from plant tissues and quantified with a haemocytometer. Indeed, this is a routine way to determine and adjust inoculum concentration levels prior to host inoculation studies (see for example Hollman *et al*. 2020 and references therein). The haemocytometer is a thick, glass microscope slide with two calibrated counting chambers, each with a volume of 10^{-4} mL. Using the grid etched onto the slide, the number of *P. brassicae* spores per mL can be determined. Although this method is straightforward, it cannot distinguish between viable and dead resting spores. This limitation can therefore cause overestimations of the concentration of active inoculum. Staining of the resting spores with Evan's blue can help to assess spore viability (Tanaka *et al*., 1999; Rennie *et al*., 2011; Harding *et al.*, 2019). Evan's blue is a vital stain that is taken up only by non-viable spores, allowing visual differentiation from viable spores (Rennie *et al*., 2011; Harding *et al.*, 2019). Harding *et al*. (2019) observed an increase in the accuracy and consistency of measurements of viable *P. brassicae* resting spores by modifying the Evan's blue method by lengthening the staining time to 8 h or more. Despite their widespread use, microscopy-based techniques require relevant expertise and can be time-consuming.

2.2.3 Polymerase Chain Reaction (PCR) and quantitative PCR (qPCR)

The use of the polymerase chain reaction (PCR) to detect *P. brassicae* in soil and host tissues has become increasingly common (Faggian and Strelkov, 2009). The single-tube, non-nested PCR assay developed by Cao *et al*. (2007) is widely used across Canada for *P. brassicae* detection, and is the basis for several commercial tests offered by seed testing labs. This method is more rapid, with a lower risk of contamination than previous two-step PCR techniques (Ito *et al*., 1999; Faggian *et al*., 1999; Wallenhammer and Arwidsson, 2001). Unlike bioassays, reliable detection of *P. brassicae* is possible at quantities as low as 1×10^3 spores per gram of soil. Similarly, PCR can detect the pathogen in root tissues 3 days post-inoculation, compared with visual symptoms that are first expressed at around 24 days (Cao *et al*., 2007).

Although sufficient for detecting the presence or absence of the pathogen, conventional PCR analysis cannot quantify the amount of *P. brassicae* deoxyribonucleic acid (DNA) or spores. Quantification of pathogen levels is critical to understanding many aspects of host colonization and the plant-pathogen interaction, as well as the level of infestation of field soils. Various quantitative PCR (qPCR)-based methods have been developed for gene expression quantification, with SYBR green and TaqMan probes being the most common (Tajadini *et al*., 2013). SYBR green is an intercalating dye that binds to non-specific double-stranded DNA and emits fluorescence 1,000-fold greater than a solution free from DNA (Tajadini *et al*., 2013; Huang *et al*., 1995). Hydrolysis probes, like TaqMan, bind specifically to the target sequence which limits the expression of undesired DNA within the sample (Mullis, 1990; Mullis and Faloona, 1987). Tajadini *et al*. (2013) determined that the SYBR green and TaqMan method are comparable

when quantifying DNA, with the intercalating dye being more affordable. However, the SYBR green method must be completed with precision to equate to TaqMan (Tajadini *et al*. 2013).

Specific assays have been developed to determine the amount of *P. brassicae* in various media. Rennie *et al*. (2011) developed an assay to determine *P. brassicae* spore concentration on seeds and tubers, whereas Wallenhammer *et al*. (2012) quantified the genomic DNA of *P. brassicae* in naturally infested soil. Additionally, Cao *et al*. (2014) focused on an *in planta* assay. Recent protocols have increased the sensitivity of qPCR-based assays down to about 1×10^3 to 5×10^2 spores per gram of soil (Cao *et al*., 2014). Most qPCR protocols cannot distinguish between viable and dead resting spores, although propidium monoazide (PMA-PCR) can be added to inhibit DNA amplification from dead spores and thereby improve estimates of viable inoculum (Al-Daoud *et al*., 2017).

Given the capacity to estimate resting spore concentrations in the field, crucial decisions around crop rotation and resistant cultivar deployment are possible. A shortfall associated with quantifying *P. brassicae*, however, is the often-patchy distribution of the pathogen. Wallenhammar (1998) observed a within-field disease incidence variation ranging from 5%-95%. Therefore, results from PCR and qPCR tests must be used with care when assessing the overall threat of *P. brassicae* in a field. Sampling high-risk areas separately, such as field entrances, low pH areas, or wet spots, should be considered to avoid misrepresenting the field infestation level.

2.3 EVOLUTION OF MANAGEMENT STRATEGIES FOR CLUBROOT OF CANOLA IN CANADA

The effective management of clubroot is essential, given that canola contributes $26.7 billion CAD annually to the Canadian economy (LMC International, 2016). The short growing

season and large geographical area of the Canadian prairies directly affect each farm's practices and capabilities. Additionally, the challenging nature of the soil-borne clubroot pathogen, with its long-lasting spores and late-season expression, complicates the development of a successful management plan. Optimal conditions for *P. brassicae* resting spore germination include a soil temperature > 17°C, moist soil with a high water-holding capacity, and a soil pH < 7.2 (Gossen *et al*., 2014; Macfarlane, 1952; Karling, 1968). Once *P. brassicae* becomes established in a field, eradicating the pathogen becomes extremely difficult (Rennie *et al*., 2011). When clubroot was discovered on canola in Alberta in 2003, few practical strategies were available for its control (Strelkov *et al*., 2011). Due to the importance of clubroot as a 'new' disease of canola in Canada, extensive *P. brassicae* research continues. Studies on the development of effective disease management methods have examined many approaches. However, the integration of multiple strategies together could provide a long-term, practical plan for each farmer, tailored to their own operation's capabilities.

2.3.1 Sanitation

Farm equipment carrying infested soil is the primary method of spread for *P. brassicae* resting spores (Cao *et al*., 2009). Additionally, the pathogen can be disseminated by wildlife, wind erosion and water run-off (Rennie *et al*., 2015; Government of Alberta, 2019b). Eliminating or reducing the movement of infested soil between fields is the most effective way of slowing down the spread of clubroot. Unfortunately, the complete sanitation of equipment between each field is not a practical approach, particularly during seeding and harvest, due to the extensive time commitment required to sanitize machinery thoroughly. A three-step process is recommended: (1) the removal of large pieces of soil and debris through a rough cleaning, (2) a

thorough power-washing or fine-cleaning, and (3) application of a disinfectant solution to the cleaned surfaces to remove any residual inoculum (Canola Council of Canada, 2012; Government of Alberta, 2019a). On a 12-m cultivator, four or more hours are required to complete this 3-step process (Canola Council of Canada, 2012). A minimum 1.7% concentrated sodium hypochlorite solution is the most effective disinfectant, followed closely by "Spray Nine®," a product containing ethoxylated C9-C11 alcohols and dipropylene glycol monobutyl ether. Both products kill almost 100% of resting spores (Government of Alberta, 2019a). To minimize the spread of clubroot, a disinfectant must be applied to resting spores on equipment and tools, as the soil cannot be disinfected (Government of Alberta, 2019a).

Additionally, a proactive way to limit potential movement of *P. brassicae* resting spores involves the farmer assessing their clubroot risk. Clubroot risk mitigation protocols are encouraged for off-farm traffic such as custom operators, recreational vehicles, and hunters (Government of Alberta, 2019b; Canola Council of Canada, 2012). Limiting soil disturbance practices, such as tillage, discourages wind and water erosion and can limit the spread to neighbouring fields. During seeding and harvest, planning operations to progress from fields with low or undetectable levels of infestation to fields with high or detectable levels can prevent transfer of infested soil to the former (Rennie *et al*., 2015; Canola Council of Canada, 2012; Government of Alberta, 2009b). Farmers should also consider the origin of used or custom machinery. Equipment from districts with confirmed clubroot should be cleaned very thoroughly to remove any inoculum that might be present. Ultimately, the clubroot risk level determines the level of sanitation required. A farmer can use a combination of these strategies to minimize the

movement of potentially infested soil. The utilization of some level of sanitation is critical to slow the spread of *P. brassicae* spores.

2.3.2 Crop Rotation

The benefits of a diverse crop rotation are well known (St. Luce *et al*., 2015; Kutcher *et al*., 2011; Dosdall *et al*., 2012; Kutcher *et al*., 2013). Cook (2006) reviewed crop productivity and resilience against weeds, insects, and diseases and concluded that yield-robbing factors decrease with a longer rotation. Unfortunately, due to geographic, economic, and environmental limitations, one of the most common rotations across the Canadian prairies is CEREAL-CANOLA-CEREAL-CANOLA (Statistics Canada, 2020; Canola Council of Canada, 2020a). Given the longevity of *P. brassicae* resting spores, and the rapid build-up in inoculum levels in the presence of a suitable host (Strelkov *et al*., 2006; Hwang *et al*., 2010; Hwang *et al*., 2014; Peng *et al*., 2015; Ernst *et al*., 2019), clubroot severity can increase quickly under such tight rotations. A longer rotation away from host crops, while controlling host weeds in non-host crop years, is critical in managing clubroot disease (Dixon, 2009).

While the initial recommendation was to maintain a 3-year break between host crops (Government of Alberta, 2014), recent research supports a minimum 2-year break (Peng *et al*., 2015; Ernst *et al*., 2019). In field plot experiments conducted in Quebec, a 90% decrease in resting spore load was observed in a 2-year break from canola vs. a 1-year break or continuous canola (Peng *et al*., 2015). This reduction in *P. brassicae* inoculum level did not result in decreased clubroot severity or greater yields when susceptible or moderately resistant canola was grown, but the yield increased by at least 25% when a resistant variety was grown. Peng *et*

al. (2015) concluded that most *P. brassicae* resting spores disintegrate within the first two years without a host present.

Similarly, Ernst *et al*. (2019) also observed a substantial decline in *P. brassicae* spore loads following a 2-year break from canola, in an analysis of resting spore dynamics in commercial fields in Alberta. Additionally, the resting spore concentration peaked in spring following the cultivation of canola. The authors concluded that this reflected the decay of the galls (and subsequent release of resting spores) over the fall, winter, and early spring. Although a 2-year break can significantly reduce *P. brassicae* resting spore levels, a significant amount of inoculum may still be present if the initial infestation was very high. In such cases, a rotation > 2 years away from canola may be needed to allow further decreases in resting spore concentration (Ernst *et al.,* 2019). A robust rotation can play an essential role towards effective clubroot control, but its efficacy may be enhanced when deployed as part of an integrated management plan (Howard *et al*., 2010; Hwang *et al*., 2012b; Ernst *et al*., 2019).

2.3.3 Biological Control Agents

The use of biocontrol agents to manage clubroot has been of particular interest over the past few decades. Peng *et al*. (2011) tested the efficacy of several biofungicides registered for control of other soil-borne diseases. These products included Serenade® (*Bacillus subtilis*), Prestop® (*Gliocladium catenulatum*), Mycostop® (*Streptomyces griseoviridis*), Actinovate® (*S. lydicus*), and Root Shield® (*Trichoderma harzianum* Rifai). While some of these products provided excellent control under greenhouse conditions, their performance was inconsistent under field conditions. Zhou *et al*. (2014) identified three antagonistic strains of the bacterium *Lysobacter antibioticus* that had varying efficacy for suppressing *P. brassicae* resting spores. The timing of

inoculation of both the biocontrol agent and pathogen was critical in reducing disease severity. Lu *et al*. (2018) observed that a whole broth culture and seed coating with the biocontrol agent *L. capsci* ZSTI-2 resulted in significant suppression of resting spore germination *in vitro*. Recently, several potent strains of *Bacillus* spp. have also been identified that reduce clubroot severity in the greenhouse. *B. subtilis* XF-1 inhibits resting spore survival and germination if applied at an early growth stage of the host, whereas *B. velezensis* and *B. amyloliquefacians* can inhibit early infection and formation of secondary zoospores (He *et al*., 2019; Zhu *et al*., 2020). The endophytic fungus *Heteroconium chaetospira* can also suppress clubroot development in susceptible host plants (Narisawa *et al*., 2005). The fungal hyphae cover the root surface, penetrate the outer epidermal cells, and colonize the inner cortical tissues, reducing infection by *P. brassicae* zoospores (Usuki *et al*., 2002; Narisawa *et al*., 1998). Like the bacterial biocontrol agents, *H. chaetospira* can successfully control clubroot if *P. brassicae* resting spore loads do not exceed 1×10^5 spores/g of soil.

Given their potential to provide durable protection, the development of biocontrol agents for clubroot remains a priority for many researchers (Peng *et al*., 2014). Nonetheless, their application method, as well as the severity of the *P. brassicae* infestation being treated, can greatly influence the success of these biofungicides, as can environmental conditions and soil characteristics. Given these limitations, biofungicides are currently not a commercially feasible option for clubroot management on canola (Peng *et al*., 2014). Additionally, these microorganisms have a short growing season to become established, followed by a harsh winter. Conversely, biocontrol agents could be integrated with other strategies to reduce *P. brassicae*

inoculum levels. Whether this approach is economical to farmers would need to be explored further (Howard *et al.*, 2010).

2.3.4 Bait Crops

The zoospores released from germinated *P. brassicae* resting spores must find a living host in a short amount of time to continue their life cycle. Without a suitable host, the zoospores die and are unable to initiate the infection process. The germination of *P. brassicae* spores is enhanced by proximity to host and non-host plant roots (Friberg *et al*., 2006). This enhanced germination is hypothesized to reflect the presence of 'germination stimulating factors' (GSFs) released by host and certain non-host roots (Suzuki *et al*., 1992). Given this effect of root exudates, there has been interest in planting bait crops to accelerate resting spore germination and deplete inoculum levels, as the resulting zoospores either cannot complete their life cycle (non-host bait crops), or the infected seedlings are destroyed before resting spore formation (host bait crops) (Ahmed *et al*., 2011).

As previously mentioned, varying degrees of infection by *P. brassicae* are possible in non-brassica crops, including *T. majus* (Garden nasturtium), *B. vulgaris* (Beet), *C. papaya* (Papaya), *R. alba* (White Mignonette) and *L. perenne* (Perennial Ryegrass) (Ludwig-Muller *et al*., 1999; MacFarlane, 1952; Feng *et al*., 2012). Feng *et al*. (2012) confirmed that secondary infection could occur in a non-host plant, with about one-third of *L. perenne* plants becoming infected without gall development. Additionally, Feng *et al*. (2012) found that host plants could be infected by secondary zoospores produced on a non-host. Nonetheless, small clubs appeared on canola when inoculated with secondary zoospores from *L. perenne* compared with the development of large galls on canola when inoculated with secondary zoospores from canola. Interestingly, Liu *et*

al. (2020) recently suggested that non-hosts inhibit the progression of primary infection in the root epidermis, while resistant hosts inhibit the secondary infection phase in the cortex tissue.

The success of bait crops under field conditions has been underwhelming. Ahmed *et al*. (2011) reported a limited reduction in inoculum levels when using a bait crop on fields with a heavy infestation (> 1 x 10^6 resting spores per gram of soil), although it is possible that a greater effect would have been observed in a field with low to moderate levels of infestation. Nevertheless, the use of bait crops as a management method must be practical and economical if this strategy is to be adopted by growers. At present, there are many challenges to the use of bait crops, including seed cost, time to maturity, in-crop/post-crop weed management options, and possible end-product use. Moreover, some germination of resting spores can occur even in the absence of root exudates (Friberg *et al.*, 2005), meaning that this approach cannot fully eliminate a field infestation. Wallenhammar *et al*. (2014) cautioned against the use of host bait crops, since they could increase resting spore numbers if not timed properly.

2.3.5 Weed and Volunteer Management

Given that *P. brassicae* can infect most, if not all, species within the Brassicaceae (Dixon, 2009a), proper management of cruciferous weeds is critical. Common weeds that could serve as potential hosts across the prairies include field pepperwort (*L. latifolium*), shepherd's purse (*C. bursa-pastoris*), stinkweed (*T. arvense*), and flixweed (*D. sophia*) (Gibbs, 1932; Buczacki and Ockendon, 1979; Ren *et al*., 2016). In addition, it is also important to control volunteer canola in the years following a canola crop. Early destruction of cruciferous weeds and volunteer canola is necessary to prevent an increase in inoculum levels resulting from the proliferation of *P. brassicae* in these hosts (Zamani-Noor and Rodemann, 2018). Nonetheless, despite the potential

importance of weed control for clubroot management, there are very limited hard data available regarding the impact of weeds on *P. brassicae* in canola (or other) cropping systems.

2.3.6 Liming

Liming is one of the oldest and most common practices used to manage clubroot disease (Karling, 1942). *P. brassicae* is known to favor acidic soils, particularly between pH 6.0-6.5 (Karling, 1942). In an early study, Chupp (1928) observed a reduction in clubroot severity and incidence at a soil pH between 7.2-7.4, which later was shown to reflect slower germination of the resting spores (Karling, 1968). Niwa *et al*. (2008), however, questioned whether it is the increase in pH or the addition of calcium that actually affects resting spore viability. Further investigation is necessary to differentiate the effects of the two, although, it is known that the use of lime products create an unfavourable environment for the germination of *P. brassicae* resting spores.

Various factors affect the efficacy of soil liming, such as soil type, spore density, soil moisture, technique and timing of application, environmental conditions, and fertilizer requirements. Additionally, there are numerous liming materials available of varying efficacy (Karling, 1942; Donald and Porter, 2009; Cornell University, 2006b; Gossen *et al*., 2012). These materials consist of carbonates or oxides and hydroxides of calcium and magnesium (Cornell University, 2006c). Common agricultural liming materials include calcitic limestone ($CaCO_3$), burned/quick lime (CaO), dolomitic limestone ($CaMg(CO_3)_2$), and hydrated lime ($Ca(OH)_2$). Various quality standards differentiate these products, which include the total neutralizing value (TNV), calcium carbonate equivalent (CCE), fineness, and effective neutralizing value (ENV). The TNV represents the effectiveness of the lime material in neutralizing soil acidity – expressed as

the CCE. The CCE standard compares the liming material to the pure calcium carbonate form, which has a CCE exemplar of 100%. Fineness represents the particle size of the liming material and its ability to pass through various sized-mesh sieves. Particles able to pass through a 100-mesh sieve can react within the first year, whereas larger particles will take longer to react within the soil (Cornell University, 2006c). Due to the differences in CCE and fineness of liming materials, the ENV denotes the neutralizing capacity of a product within the first year of application (Cornell University, 2006c).

Calcium cyanamide ($CaCN_2$), calcitic dolomite, and calcium carbonate were all found to decrease disease index and resting spore levels in studies by Murakami *et al*. (2002). The application of powdery formulations of these products resulted in a greater reduction of disease index than granular formulations. In contrast, the dolomitic granular form was more effective than the powdery form. Applying the liming materials 2-weeks before seeding, rather than 4-weeks before seeding, also increased their effectiveness at suppressing disease severity. Similarly, Hwang *et al*. (2011a) found that high rates of wood ash and calcium carbonate tended to result in increased plant height and yields. Regardless of the rate, however, calcium cyanamide did not reduce clubroot severity (Hwang *et al*., 2011a). Conversely, Tremblay *et al*. (2005) observed a reduction in disease and a 14-fold increase in yield with fall-applied calcium carbonate followed by spring-applied calcium cyanamide 1-week before planting. A similar reduction in disease was seen with the solo application of spring-applied calcium hydroxide, but yields did not increase.

Despite the importance of pH in clubroot development, the disease can still occur in some cases in neutral or slightly alkaline soils, if conditions such as moisture, temperature, and

spore density, are favourable (Macfarlane, 1952; Colhoun, 1953; Gossen *et al*., 2013; Gossen *et al*., 2014). Moreover, macronutrients and micronutrients are most readily available around pH 6.5, and increasing soil pH could reduce their availability (Cornell University, 2006a). It is helpful, therefore, to consider liming as part of a wider clubroot (and crop) management plan.

2.3.7 Chemical Control

The use of synthetic chemicals, including fungicides and fumigants, to manage clubroot has been studied extensively. The mercury-based fungicide Calomel, for example, reduced clubroot severity while increasing yield in Chinese cabbage (Doyle and Clancy, 1987). However, this fungicide, while effective, was detrimental to the environment, leading to its ban across North America (Peng *et al*., 2014). The application of pentachloronitrobenzene (Terraclor® 75% WP) also resulted in a significant reduction in clubroot severity, but this product will likely also be phased out due to health concerns and longevity in soil (Hwang *et al*., 2008; Hwang *et al*., 2014).

Cyazofamid (Ranman™) and fluazinam (Allegro®, Omega®) have been popular in Canada as they are both commercially available. Although cyazofamid is not registered to control *P. brassicae*, Mitani *et al*. (2003) suggested that the fungicide directly inhibits resting spore germination, as there was no root-hair infection or gall formation observed following its application. Hwang *et al.* (2008) reported a significant reduction in clubroot severity with the application of cyazofamid to the soil before seeding, rather than in the seed furrow.

Fluazinam is registered to control *P. brassicae* on vegetable crops, inhibiting resting spore germination, root-hair infection, and cortical infection (Suzuki *et al*., 1995). This fungicide is effective in mildly infested soil, but its efficacy is reduced under high inoculum pressure (Tanaka *et al*., 1999; Ahmed *et al*., 2011). Donald *et al*. (2001) found band-incorporation of the fungicide

23 cm wide and 15-20 cm deep to be the most effective, while spot dench or continuous spray was equivalent to the untreated control.

Soil fumigation with metam sodium (Vapam®) has shown some promise in reducing clubroot severity on vegetables and canola (Papernik *et al*., 2004; Hwang *et al*., 2014; Hwang *et al*., 2018). Hwang *et al*. (2018) concluded that metam sodium limits both primary and secondary infection of roots by *P. brassicae* spores. In much the same fashion as lime application, the efficacy of metam sodium can be affected by soil type, moisture, pH, temperature, and organic matter (White and Buczacki, 1977). Additionally, the soil surface must be covered for a period after fumigation, to prevent rapid loss of the volatilized chemical and obtain a consistent reduction in clubroot severity (Papiernik *et al*., 2004; Hwang *et al*., 2018).

Although varyingly effective, many limitations surround the use of these chemicals to manage *P. brassicae* spore levels at the field level. The efficacy of both fungicides and fumigants depends on adequate water volumes, which results in reduced practicality and feasibility when applying them on a field scale (Donald *et al*., 2001; Peng *et al*., 2014). Metam sodium also requires covering of the soil after treatment, which is not feasible in large fields (Hwang *et al*., 2018). Additionally, since metam sodium is a non-selective chemical fumigant, it can be toxic to beneficial soil microbial communities, arthropods, and humans. Therefore, adequate training and substantial precautions are necessary for the applicator. Additionally, the control provided by fungicides was inconsistent in highly infested fields (Peng *et al*., 2014). If a fungicide could produce consistent results in fields with high infestation levels, it may be a more realistic option for farmers impacted by clubroot.

2.3.8 Resistance Genetics

Following the discovery of clubroot on canola in 2003, the first CR cultivar became commercially available in 2009 ('45H29'), with additional varieties available in the following seasons. Although the genetic basis in most CR canola cultivars has not been disclosed, it appears to be derived from the European winter rapeseed (*B. napus*) cv. 'Mendel' (Fredua-Agyeman *et al.*, 2018). The resistance in 'Mendel' was in turn derived from the European Clubroot Differential (ECD) 04, a *B. rapa* genotype (Diederichsen and Sacristan, 1996) containing three resistance genes. During the development of 'Mendel', which occurred prior to the introduction of molecular markers, two of these resistance genes appeared to have been lost (Diederichsen *et al*., 2006, 2009). As such, clubroot resistance in 'Mendel' and its CR canola derivatives may rely heavily on a single, dominant resistance gene (*CRa*) (Rahman *et al.*, 2014; Fredua-Agyeman and Rahman, 2016; Fredua-Agyeman *et al.*, 2018).

Major gene resistance can often be overcome by shifts in the virulence of pathogen populations. In the case of *P. brassicae*, short crop rotations with CR varieties have likely exerted significant selection pressure on the pathogen, increasing the proportion of the population virulent on these varieties (Strelkov *et al*., 2016; Cao *et al*., 2020). In greenhouse studies, LeBoldus *et al*. (2012) documented substantial decreases in the resistance of a CR canola cultivar after just two cycles of exposure to the pathogen. Cao *et al.* (2020) demonstrated that the amount of the virulent pathotype 5X increased rapidly when CR varieties were continuously cropped, relative to rotations where non-hosts were included. In other regions, the loss of resistance also has been documented in commercial cropping systems, including on winter oilseed rape (Oxley, 2007) and Chinese cabbage (Hatakeyama *et al*., 2006). Given this context, it

is perhaps not surprising that significant symptoms of clubroot were identified on CR canola in Alberta in 2013, just 4 years after introduction of the resistance trait (Strelkov *et al.*, 2016). Greenhouse testing confirmed the emergence of 'new' pathotypes of *P. brassicae* capable of overcoming the resistance in this 'first generation' of CR cultivars (Strelkov *et al.*, 2016).

Additional surveillance and testing in subsequent years has identified hundreds of fields where resistance has been compromised by highly virulent pathotypes (Strelkov *et al.*, 2018, 2020; Hollman *et al.*, 2020). To date, 19 of the 36 *P. brassicae* pathotypes identified in Canada can break resistance, with the pathotypes 3A and 3D found to be most common. Nearly all cases of resistance breakdown have been found in Alberta, which is consistent with the greater prevalence of clubroot in this province (Strelkov *et al.*, 2018; Hollman *et al.*, 2020). Nonetheless, pathotype 3A was recently also reported in Manitoba (Hollman *et al.*, 2020), the first time a resistance-breaking pathotype has been found outside Alberta. The loss of resistance poses a significant threat to sustainable canola production in Canada (Strelkov *et al.*, 2018), particularly given the importance of CR canola as a management tool. As such, resistance stewardship has been widely recommended, including longer rotations out of canola as discussed earlier (Strelkov and Hwang, 2014; Peng *et al.*, 2014). There have also been efforts to develop so-called "second generation" resistance (Canola Watch 2020; Hollman *et al.*, 2020), which can include stacked resistance genes in a single CR variety or the incorporation of novel sources of resistance (Rahman *et al.*, 2014).

Finally, CR varieties are not completely immune to clubroot, and may develop mild symptoms of the disease. Each canola variety with a claim to clubroot resistance must meet criteria specified by the Western Canada Canola/Rapeseed Recommending Committee

(WCC/RRC) (Canola Council of Canada, 2020b). A variety designated as 'Resistant (R)' is defined as one that develops a disease index that is <30% than that of a susceptible check. A variety with 'Intermediate (I)' resistance develops a disease index between 30% and 50% of the susceptible check. Finally, a 'Susceptible (S)' variety develops a disease index >50% of the susceptible check (Canola Council of Canada, 2020b). The fact that even CR canola cultivars may develop some galling means that they may contribute to increases in *P. brassicae* resting spore levels in the soil, as has been documented in CR canola cropping systems in Alberta (Ernst *et al.*, 2019). Hence, while CR canola remains a cornerstone of clubroot management, this tool should be used as part of an integrated approach to disease management.

2.3.9 Clubroot Patch Management

Given the patchy distribution of clubroot in most fields (Cao *et al.,* 2009), there has been a recent emphasis on the strategic management of these patches, in addition to altering practices across the entire field (Canola Council of Canada, 2020b). This method is called 'Patch Management.' While there is limited published research supporting its use, the technique collectively supports the general objective to identify methods that reduce the resting spore level of *P. brassicae* and slow its spread (McDonald and Gossen, 2019).

Patch management relies on identifying clubroot-infested areas in a field, by hand roguing of canola plants to identify symptomatic roots. Once identified, the infested area should be delineated to at least double the apparent size (McDonald and Gossen, 2019). This is to account for spores that have been spread mechanically over time. Given that billions of spores can be released from a clubroot gall, the strategy is to physically remove all infested plants from the patch, thereby preventing or limiting increases in inoculum concentration. All galled roots

are pulled from the soil, bagged, and the removed from the field or otherwise destroyed (Canola Council of Canada, 2020b). Roguing an entire patch can be laborious and time-intensive process and may not be practical for every situation. Nonetheless, removing as many galls as possible, then destroying them through landfill disposal or by incineration is encouraged (McDonald and Gossen, 2019). Air-drying the galls before burning could promote more intense and thorough combustion versus fresh, high-moisture galls, with subsequently greater destruction of spores (M.R. McDonald, *personal communication*). Following removal of the infested plants, the denuded patch can be seeded to grass to deter disturbance by tillage, harrowing, or seeding. Additionally, the grass will limit soil movement beyond the infested area and stimulate the germination of *P. brassicae* resting spores (MacFarlane 1952; Feng *et al*., 2012; Sedaghatkish thesis).

As noted earlier, clubroot patches may also be treated by targeted application of fumigants or lime (Canola Council of Canada, 2020b; McDonald and Gossen, 2019). Covering the soil surface post-fumigation to prevent loss of the fumigant can be more easily accomplished physically and economically on a small patch vs. a large field. Similarly, raising the soil pH in a discrete *P. brassicae*-infested patch to ≥ 7.2 is more feasible than in an entire field. Effective weed control is also necessary, regardless of whether the patch is within a canola crop or not. The continuous and timely management of susceptible hosts in the patch is critical to prevent gall formation, ultimately increasing *P. brassicae* resting spore levels (Zamani-Noor and Rodemann, 2018). The seeding of denuded patches to grass as noted above is helpful in this respect.

2.4 CONCLUSION

As *P. brassicae* continues to spread across the Prairies and reduces canola yields, the adoption of multiple clubroot management strategies becomes more critical. While the planting of CR canola cultivars is the most popular method to manage clubroot, the diversity in the virulence of the pathogen threatens the longevity of host resistance. As *P. brassicae* spores move with the soil, sanitation of equipment between fields is an effective strategy to limit clubroot spread. Sanitation, however, is arguably the most 'unrealistic' measure for farmers to adopt, given the time commitment required to properly clean machinery. Limiting soil disturbance by means of zero or reduced tillage minimizes the movement of infested soil due to erosion and equipment use, but does not completely prevent it. The identification of effective chemical fungicides and biological control agents to manage clubroot continues to be a priority in research, but thus far lacks large-scale success. Environmental conditions, soil characteristics, pathogen concentration, and affordability can affect the effectiveness of these products.

Minimizing gall development in field is vital, since it directly affects the spore population and, consequently, *P. brassicae* diversity. Removing the host from the disease triangle includes removing all species within the Brassicaceae family, which may serve as potential alternative hosts. Managing susceptible weeds, in addition to removing canola from rotation for at least two years, limits the host and pathogen interaction. Additionally, disrupting the conditions needed for optimal disease development can be achieved by the addition of lime products. Although not optimal for nutrient exchange, an increase in pH can impact the germination of *P. brassicae* resting spores in the soil.

Given the complex life cycle of *P. brassicae* and longevity of its resting spores, reactive measures can impede the successful management of clubroot disease. A collective and integrated implementation of control strategies is critical to minimizing the movement and expression of this disease. As such, the effect of various management practices and approaches must be evaluated, alone or in combination, to help reduce pathogen inoculum levels and clubroot incidence and severity.

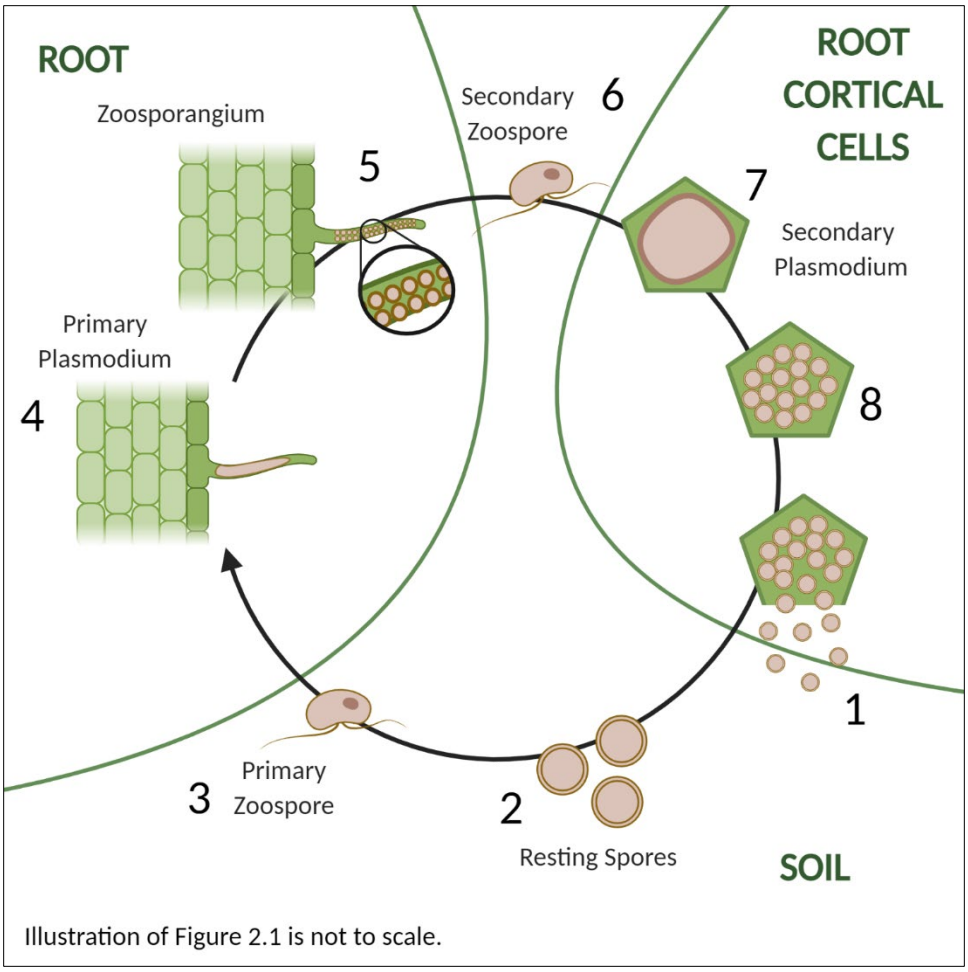

FIGURE 2.1: Life cycle of *Plasmodiophora brassicae* adapted from Kageyama and Asano (2009). Resting spores of the pathogen are introduced to the soil (1), which are then stimulated to germinate by the appropriate environmental conditions and plant root exudates (2). The resting spore releases a biflagellate primary zoospore (3) that can penetrate the cell wall of the plant, forming the primary plasmodium (4). The primary plasmodium cleaves into a zoosporangium (5), releasing 4-16 secondary zoospores back into the soil (6). The secondary zoospore re-infects the host through the cortical root tissue, forming a secondary plasmodium (7). As the secondary plasmodium matures, it proliferates intracellularly and produces resting spores (8). The life cycle is complete once the host roots decay to release the resting spores back into the soil (1).
Illustration created with BioRender.com

3.0 MATERIAL & METHODS

3.1 FIELD TRIAL

Replicated field trials were conducted in 2018 (site 1) and 2019 (sites 2 and 3) to study the effect of various combinations of clubroot management strategies (resistance, soil liming and weed control) on disease severity, yield, and *Plasmodiophora brassicae* spore density. The trials were located in a naturally infested clubroot nursery at the Crop Diversification Centre North (CDCN; 53°38'N, 113°21'W), Alberta Agriculture and Forestry, Edmonton, Alberta. The soil at this site consists of a Black Chernozem. Each trial included four replicates arranged in a randomized complete block design. There were eight different combinations of the management strategies, creating a block-split-split plot design. Each plot had an area of 9 m^2 (1.5 m x 6 m), with 4 rows per plot and a 2 m buffer between replicates.

The pre-treatment pH of the soil at site 1 (2018) was 5.3, while at sites 2 and 3 (2019), the soil pH values were 5.23 and 5.48, respectively. The amount of lime needed to reach the target pH of 7.2 was calculated based on recommendations from the Government of Alberta (2002) at site 1 in 2018, or following Penney (1973) at sites 2 and 3 in 2019. Hydrated lime ($Ca(OH)_2$, Graymont) was applied evenly, by hand, on May 28th, 2018 (14.09 T ha^{-1}, site 1) and May 31st, 2019 (11.49 T ha^{-1}, site 2; and 8.60 T ha^{-1}, site 3). The seedbed was prepped with a rototiller, which was used for a second time to incorporate the lime to a depth of 10 cm. Due to a lack of moisture in 2019 at the time of seeding, 30 L of water was applied per plot immediately following the lime application.

In 2018, a clubroot-resistant (CR) canola cultivar '45H29' (Dupont Pioneer, Mississauga, ON, Canada) and a clubroot-susceptible canola (CS) cultivar '45H31' (Dupont Pioneer) were

seeded at site 1. The development of clubroot galls was observed on the resistant variety during the growing season. Pathotyping of some of these galls indicated a virulence shift in this nursery from pathotype 3H to 3D, as defined on the Canadian Clubroot Differential set, the latter of which can overcome the resistance in '45H29' (Strelkov *et al*., 2018). Therefore, in 2019, '45H29' was replaced with '45CM39' (Dupont Pioneer), a canola cultivar with '2nd generation' resistance effective against pathotypes 2B, 3A, 3D and 5X. The trials were seeded on June 5th, 2018 and 2019, using a Fabro gravity seeder with double disk openers (Swift Current, SK, Canada) at a depth of 2.5 cm.

Plots that required weed control were hand-weeded every two weeks after the canola emerged until the end of July (2018 and 2019). Hand weeding ensured that no galls developed on any susceptible weeds, which could affect the *P. brassicae* spore densities. On July 6th, 2019, weed numbers were estimated in plots at site 2 and 3 where weeds were not managed. Briefly, a 0.25m^2 quadrat was placed at the front and back of each plot, avoiding the edges. All weed species were counted, separating the cruciferous from the non-cruciferous weeds. The counts were combined and multiplied by 2 to obtain the weed density per m^2 in each plot. Weed numbers were not quantified at site 1 in 2018. No herbicides were applied in either year at any site.

Total canola emergence was counted recorded at 7, 14 and 28 days after seeding. On day 16 at sites 2 and 3, high flea beetle feeding levels were observed, and the plots were sprayed with Decis 5EC insecticide (Bayer, Germany) on June 21st, 2019. Shoot height, shoot weight, and clubroot symptoms were evaluated on 10 plants per plot on July 31st, 2018 (site 1) and July 30th, 2019 (sites 2 and 3). Each root was rated on a 0 to 3 scale, where: 0 = no galling, 1 = small galling,

2 = moderate galling, and 3 = severe galling (Kuginuki *et al.*, 1999). Root and gall weights were also measured after the ratings were complete. The ratings were then used to calculate the index of disease (ID) for each plot, according to the formula of Horiuchi and Hori (1980) as modified by Strelkov *et al*. (2006):

$$\mathrm{ID}(\%) = \frac{\sum(n\,x\,0 + n\,x\,1 + n\,x\,2 + n\,x\,3)}{\mathrm{N\ x\ 3}}\ x\ 100$$

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

Reglone Ion (Syngenta, Guelph, ON, Canada) was applied as a desiccant to the plots on September 24th, 2018, and September 26th, 2019, at a rate of 6.10 L/ha to ensure proper dry down of the canola crop and weeds for harvest. The harvested seeds were cleaned and dried at air temperature before they were weighed to obtain yield estimates. In the spring of 2020, 10 soil samples per plot were collected from sites 2 and 3 with a 10 cm Dutch auger. To maintain consistency throughout sampling, five samples were taken within rows and five samples were taken between rows. The Dutch auger was washed with 80% ethanol between samples to prevent cross-contamination. The samples were stored at room temperature until used for quantitative PCR analysis of soil inoculum density. Unfortunately, at site 1, the plots were cultivated with a tractor shortly following the growth season, precluding the collection of soil samples in the following spring.

3.2 GREENHOUSE TRIAL

The CR cultivar '45H29' (Dupont Pioneer) and CS cultivar '45H31' (Dupont Pioneer) were grown in different combinations with wheat and barley in a soil mix inoculated with various levels of *P. brassicae* resting spores. Approximately 3,500 L of a Black Chernozemic soil was collected from a field in northeast Edmonton, AB, with no history of clubroot; to confirm the clubroot-free status of the soil, a composite subsample was tested for the presence of *P. brassicae* by Element Labs (Edmonton, AB). Once confirmed to be free of *P. brassicae* (at least at detectable levels), the soil was mixed with Sungro Professional Growing Mix (Sungro Horticulture, Seba Beach, AB, Canada) at a ratio of 65 parts soil to 35 parts growing mix. The soil mix was placed in 40 L plastic tubs and inoculated with *P. brassicae* resting spore suspensions to achieve final concentrations of 1×10^2, 1×10^4, 1×10^6, and 1×10^8 spores per gram of soil; checks were not inoculated (i.e., 0 resting spores per gram of soil). The inoculum was obtained from air-dried root galls following Strelkov et al. (2006), and the appropriate volume of the spore suspension was hand-incorporated into the top 16 L of soil mix in each tub to achieve the desired spore densities.

Crops were grown for 8-weeks each with a 4-week break between crops in the following rotations: CR canola (CR)-Wheat (W)-Barley (B)-CR; CR-W-B-CS canola (CS); and CS-W-B-CR and CS-W-B-CS. Each crop was planted to a target density of 50 plants per tub and then thinned to 40 plants per tub 2-weeks after seeding. For both the first and second canola crops in each rotation, 10 plants were selected randomly from each tub after 8-weeks. They were removed from the soil mix, and the roots were washed in water and rated for clubroot symptom severity. Severity ratings were made on a 0 to 3 scale (Kuginuki *et al*., 1999), where: 0 = no galling, 1 = a

few small galls, 2 = moderate galling, and 3 = severe galling. An index of disease (ID) was then calculated for each tub based on the individual ratings (Horiuchi and Hori, 1980; modified by Strelkov *et al*., 2006), as described above for the field trial. Shoot height, shoot weight and gall weight were also recorded for each plant. All remaining aboveground biomass in the tubs was harvested and discarded. Following the evaluations, the roots and galls from the sampled plants were combined with the remaining roots and galls of the canola plants in the tubs. The roots and galls were set aside for 4 weeks to air-dry for later re-inoculation. There were no measurements taken on the wheat or barley crops. After the cereals grew for 8-weeks, all biomass was removed, and the meristems were mechanically destroyed for desiccation.

Soil samples were taken over the course of the trial for resting spore quantification by PCR (see below). After the 4-week break following the first canola crop, the first soil sample was collected. This timeline mimics an in-field fall soil sample, largely prior to resting spore release from decomposing galls (Ernst et al. 2019). As the roots and galls were unable to decompose naturally in the soil with the short cropping intervals, they were re-incorporated into their respective tubs by blending the roots and galls with water. A second soil sample was collected 4-weeks after harvesting the wheat, and a third soil sample was collected 4-weeks after harvesting the barley. For the second canola crop in each rotation, two soil samples were collected: one was taken 4-weeks after harvest of the canola, prior to the re-incorporation of roots and galls, and a second was collected after the reincorporation of the homogenized root material back into the soil mix. All soil samples were collected from the top 5 cm of the soil mix.

3.3 PCR ANALYSIS

Soil samples collected from the field plots or greenhouse tubs were air-dried at room temperature and homogenized with an electric grinder (Waring Commercial, Stamford, CT) that was washed with an 80% ethanol solution between samples. Total genomic DNA was extracted from 0.25 g of each sample with a DNeasy® PowerSoil® Kit (Qiagen, Germantown, MD) according to the manufacturer's instructions. The concentration and quality of the DNA was estimated with a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA) and then stored at -20°C until preparation for PCR analysis.

The DNA samples were prepared for conventional PCR by dilution with nuclease-free water (Thermo Fisher Scientific Inc.) to a concentration of 2 ng μL^{-1}. Conventional PCR analysis was conducted following Cao *et al*. (2007) with the primers TC1F and TC1R. Negative controls included 5 µL nuclease-free water instead of a DNA template, while the positive controls included 10 ng of *P. brassicae* DNA. Reaction products were visualized on 2% agarose gels stained with 1X SYBR Safe (Invitrogen, Carlsbad, CA). Samples that tested positive for the presence of *P. brassicae* DNA by conventional PCR were analyzed further by quantitative PCR (qPCR). Briefly, the DNA samples were diluted 10-fold with nuclease-free water and subjected to qPCR analysis with the primers DR1F and DR1R as per Rennie *et al*. (2011) in a StepOnePlus Real Time PCR System (Applied Biosystems, Foster City, CA). The resting spore density in each sample was estimated relative to a standard curve generated with known amounts of *P. brassicae* inoculum (1×10^2, 1×10^3, 1×10^4, 1×10^5, and 1×10^6 resting spores g^{-1} soil; Rennie *et al.*, 2011).

3.4 STATISTICAL ANALYSIS

Data were analyzed with the packages 'tidyverse' and 'nlme' in RStudio Version 1.3.1093 (2009-2020 RStudio, PBC).

3.4.1 Field Trial

Field years were added as blocks to avoid fitting separate models. Plant response variables analyzed included *P. brassica*e resting spore density, index of disease, and yield. Residual plots were used to visualize the normality and homoscedasticity of the linear mixed-effects (lme) objects. The spore density data were log transformed, whereas yield and rating data were not. Three lme models were compared in an analysis of variance (ANOVA) for each response variable to determine the strongest model, based off Akaike's Information Criteria (AIC) and Bayesian Information Criteria (BIC) values. A chi-squared test of likelihood and weed correlation panel with associated r-values were performed to ensure correct model selection for the effects of weeds on spore density (FIGURE 3.1). The final models used the restricted maximum likelihood (REML) method for a better variance estimate. A type-II ANOVA was carried out on final response variable models, as a type-I ANOVA compares terms after removing the effects of the previous ones. The summary ( ) function provided the lme analysis. Differences were considered statistically significant if $P < 0.05$.

3.4.2 Greenhouse Trial

For the greenhouse data, a lme model was used on each response variable. Initial resting spore density, index of disease, shoot weight, shoot height, and gall weight were analyzed to evaluate the effects of crop rotations on various *P. brassicae* resting spore concentrations. The generalized least squares test was performed on all response variables to determine if the

random intercept was necessary. The difference between AIC values was not sufficient to remove the blocking factor; therefore, the lme models were selected to account for the variation between benches in the greenhouse and lack of trial duplication. Residual plots were used to visualize the normality and homoscedasticity of the data. Spore density data were log transformed, and the indices of disease and gall weights were non-normal. The anova( ) function provided the lme analysis. Additionally, a correlation panel was used to observe the interaction between the response variables.

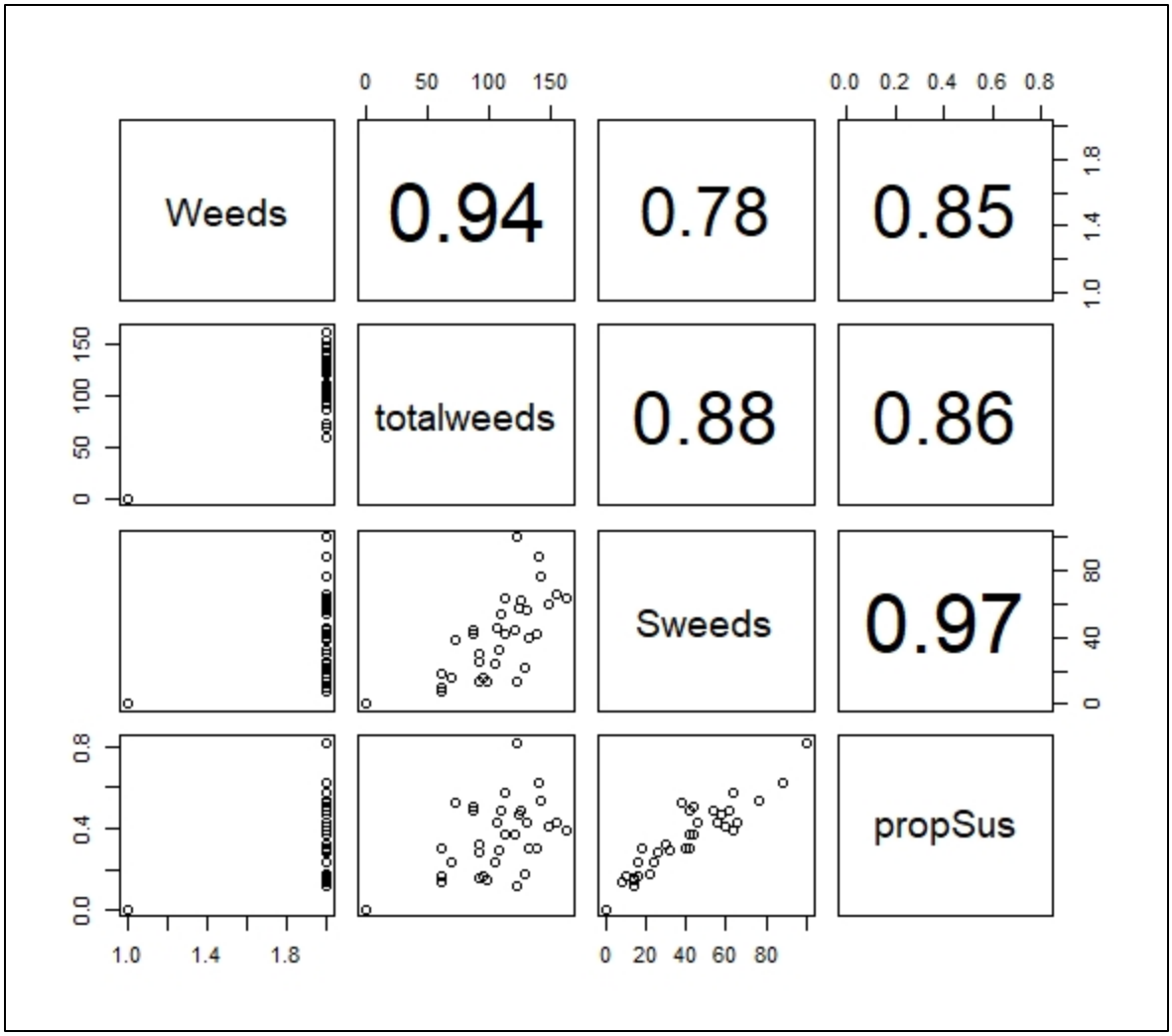

FIGURE 3.1: A weed correlation panel was used to choose the best model for the impact of weeds on spore density from sites 2 and 3 in Edmonton, AB, Canada, in 2019. A 0.25m^2 quadrat was placed at the front and back of each plot ,avoiding the edges. All weed species were counted, separating the cruciferous from the non-cruciferous weeds. The counts were combined and multiplied by 2 to obtain a weed density per m^2 in each plot. The AIC on the spore density model using susceptible weeds was slightly better than the model using weed presence/absence. However, a change in spore density could have been affected by susceptible weeds in total weeds as there is a high correlation between the presence or absence of weeds (Weeds), total weeds (totalweeds), total susceptible weeds (Sweeds), and proportion of susceptible weeds (propSus).

4.0 RESULTS

4.1 FIELD TRIAL

4.1.1 Resting Spore Density

The effect of canola cultivar on *P. brassicae* resting spore density was significant (P=0.0023; TABLE 4.1), along with the interaction between the application of hydrated lime and weed management (P=0.0481; TABLE 4.1; FIGURE 4.2). Spore densities in the treatments that included the CR cultivar '45CM39' and the application of hydrated lime or weed management were all significantly lower than in the treatments that included the susceptible cultivar'45H31' without the application of hydrated lime (TABLE 4.2; FIGURE 4.1). None of the other treatments was significantly different.

4.1.2 Clubroot Severity

The effect of canola cultivar on clubroot disease severity was significant (P=0.0005; TABLE 4.1), as was the interaction between cultivar and the use of hydrated lime (P=0.0028; TABLE 4.1). Within the treatments that included the CR cultivar '45CM39', the only significant difference in clubroot severity was between the treatments using hydrated lime without weed management, and those not using hydrated with weed management (TABLE 4.2; FIGURE 4.1). Within the treatments using the susceptible cultivar '45H31', clubroot severity in plots with hydrated lime was significantly lower than in those without hydrated lime (TABLE 4.2 FIGURE 4.1).

4.1.3 Yield

The effect of canola cultivar, the management of weeds, and the interaction between cultivar and the application of hydrated lime on yield were all statistically significant (P=0.0151, P<0.0001, P=0.0012; TABLE 4.1; FIGURE 4.2). The treatments that included the CR cultivar '45CM39' with managed weeds showed the largest difference with the treatments that included the susceptible cultivar '45H31' without the application of hydrated lime (TABLE 4.2; FIGURE 4.1). Yields in the treatments using the CR cultivar '45CM39' with unmanaged weeds were not significantly different from treatments that included the susceptible cultivar '45H31' with the application of hydrated lime (TABLE 4.2).

4.1.4 Growth Parameters

The only significant effect on gall weight was the interaction between the use of canola cultivar and the application of hydrated lime (P=0.0054; TABLE 4.1). The greatest difference was observed between treatments that included a CR cultivar and treatments that used a susceptible cultivar without the application of hydrated lime (TABLE 4.2). Gall weight was significantly lower when hydrated lime was applied to treatments using a susceptible cultivar (TABLE 4.2).

Canola cultivar, the application of hydrated lime, and the management of weeds all had significant effects on shoot weight (P=0.0045, P=0.0363, and P=0.0079, respectively; TABLE 4.1). Treatments using a CR canola cultivar with the application of hydrated lime and/or managed weeds were similar, and had significantly greater shoot weights relative to all other treatments. In contrast, when the CR cultivar was grown in the absence of hydrated lime or weed management, shoot weight was not statistically different from treatments with a susceptible

canola cultivar when either no hydrated lime was applied or the weeds were not managed (TABLE 4.2).

The effects of canola cultivar, the interaction between canola cultivar and the application of hydrated lime, and the interaction between the application of lime and the management of weeds were significant on shoot height (P= 0.0478, P= 0.009, P=0.0408; TABLE 4.1). There was no significant difference between treatments when using a CR cultivar, although they were significantly higher than treatments with a susceptible cultivar and no application of hydrated lime (TABLE 4.2). Finally, the treatment that included the CR cultivar with no application of hydrated lime and managed weeds was significantly higher than all treatments with a susceptible canola cultivar (TABLE 4.2).

4.2 GREENHOUSE TRIAL

4.2.1 Spore Densities

The length of the crop rotation (CANOLA-WHEAT-BARLEY-CANOLA), rotation between the clubroot susceptible '45H31' and resistant '45H29' canola cultivars (CR-W-B-CR; CR-W-B-CS; CS-W-B-CR; CS-W-B-CS), and the initial spore density all significantly (P<0.0001) affected the change in resting spore density over time (TABLE 4.3). Additionally, the interaction between the length of crop rotation and canola cultivar rotation, and the interaction between the canola cultivar rotation and initial *P. brassicae* spore load, both significantly affected (P=0.0008, P=0.0006) the change in spore density over time (TABLE 4.3). When the starting resting spore density was 1 x 10^6 spores g^{-1} of soil, the only significant difference was observed after the galls

of the second canola crop (5CY4b) were reincorporated into the soil mix in the CS-W-B-CS rotation (TABLE 4.4, FIGURE 4.4). In contrast, when the starting spore density was 1 x 10^8 spores g^{-1} of soil, significant differences were observed in the CR-W-B-CR and CS-W-B-CS rotations (TABLE 4.4, FIGURE 4.4). Within the CR-W-B-CR rotation, a significant decrease in spores was observed following the wheat crop (2PW) (TABLE 4.4, FIGURE 4.4). However, after the reincorporation of the roots and galls back into the soil mix following the final canola crop (5CY4b), the resting spore density returned to levels similar to those prior to the planting of the wheat crop (TABLE 4.4, FIGURE 4.4). In the CS-W-B-CS rotation, the *P. brassicae* resting spore density increased significantly following the second canola crop, prior to gall reincorporation (4CY4a) (TABLE 4.4, FIGURE 4.4).

4.2.2 Clubroot Severity

All factors in the greenhouse trial had significant effects on clubroot severity, including: the length of crop rotation (P=0.0001), the rotation of the CS '45H31' and CR '45H29' cultivars (P<0.0001), the initial spore density (P<0.0001), the interaction between the length of the rotation and the rotation of canola cultivars (P<0.0001), the interaction between the length of the rotation and the initial spore density (P<0.0001), the interaction between the rotation of canola cultivars and the initial spore density (P<0.0001), and the interaction between the length of rotation, the rotation of canola cultivars and the initial spore density (P<0.0001) (TABLE 4.3). There were no significant differences between clubroot severity ratings within the canola crop rotations at initial spore densities of 0 (check), 1 x 10^2, and 1 x 10^4 spores g^{-1} of soil (TABLE 4.5; FIGURE 4.3). When analysing the change in clubroot severity in the first vs. second canola crop included in the CR-W-B-C rotations at a starting resting spore density of 1 x 10^6 spores g^{-1},

significant differences were observed (TABLE 4.5; FIGURE 4.3). The treatments with a starting resting spore density of 1 x 10^8 spores g^{-1} also showed similar differences when starting with a CR crop, as well as in the CS-W-B-CR rotation (TABLE 4.5; FIGURE 4.3). All other treatments were non-significant.

4.2.3 Growth Parameters

Most factors in the greenhouse trial had significant effects on the shoot height, including: the length of crop rotation (P<0.0001), the rotation of the CS '45H31' and CR '45H29' cultivars (P<0.0001), the initial spore density (P<0.0001), the interaction between the length of the rotation and the rotation of canola cultivars (P<0.0001), the interaction between the rotation of canola cultivars and the initial spore density (P=0.0001), and the interaction between the length of rotation, the rotation of canola cultivars, and the initial spore density (P=0.484) (TABLE 4.3). At starting resting spore densities of 0 and 1 x 10^8 spores g^{-1} soil, the rotations CR-W-B-CR, CS-W-B-CR, and CS-W-B-CS all had significantly higher shoot heights in the second canola crop compared with the first (TABLE 4.5). Treatments with a starting spore density of 1 x 10^2 and 1 x 10^4 spores g^{-1} also had significantly higher shoot heights in the second canola crop compared with the first, across all rotations (TABLE 4.5). In the rotation CS-W-B-CS, significantly lower shoot heights were observed in the second canola crop compared with the first at a starting spore density of 1 x 10^6 spores g^{-1} (TABLE 4.5).

Many factors in the greenhouse trial had significant effects on the shoot weight, including: the length of crop rotation (P=0.0004), the rotation of the CS '45H31' and CR '45H29' cultivars (P=0.0024), the initial spore density (P<0.0001), the interaction between the length of the rotation and the rotation of canola cultivars (P=0.0033), the interaction between the rotation

of canola cultivars and the initial spore density (P=0.0206), and the interaction between the length of rotation, the rotation of canola cultivars, and the initial spore density (P=0.0147) (TABLE 4.3). The CS-W-B-CS rotation with a starting resting spore density of 0 spores g^{-1} had significantly lower shoot weights in the second canola crop compared with the first (TABLE 4.5). The CR-W-B-CS rotation with a starting resting spore density of 1 x 10^2 spores g^{-1} had significantly lower shoot weights on the second canola crop compared with the first (TABLE 4.5). The CS-W-B-CR rotation with a starting spore density of 1 x 10^6 spores g^{-1} had a significantly higher shoot weight in the second canola crop compared with the first, whereas the CS-W-B-CS rotation was significantly lower (TABLE 4.5). Within treatments with a starting resting spore density of 1 x 10^8 spores g^{-1}, the CS-W-B-CR and CS-W-B-CS rotations had significantly higher shoot weights on the second canola crop compared with the first (TABLE 4.5).

Similarly, most factors in the greenhouse trial had significant effects on root gall weight, including: the rotation of the CS '45H31' and CR '45H29' cultivars (P=0.373), the initial spore density (P<0.0001), the interaction between the length of the rotation and the rotation of canola cultivars (P=0.0001), the interaction between the rotation of canola cultivars and the initial spore density (P=0.003), and the interaction between the length of rotation, the rotation of canola cultivars, and the initial spore density (P=0.0001) (TABLE 4.3). The CR-W-B-CS and CS-W-B-CR rotations with a starting spore density of 1 x 10^8 spores g^{-1} had significantly lower gall weights in the second canola crop compared with the first (TABLE 4.5).

TABLE 4.1: Two-way ANOVA for field trials evaluating the effects of clubroot resistance, soil liming and weed control for the management of *Plasmodiophora brassicae* on canola at three naturally infested field sites in Edmonton, AB, Canada, in 2018 and 2019.

	Gall Weight			Shoot Weight			Shoot Height		
	denDF	F-value	P-value	denDF	F-value	P-value	denDF	F-value	P-value
(Intercept)	44	0.01754	0.8952	44	159.614	<0.0001	44	973.885	<0.0001
Genetics[a]	11	6.80717	0.243	11	12.62348	0.0045	11	4.9601	0.0478
Lime[b]	22	0.15227	0.7001	22	4.96933	0.0363	22	2.9111	0.1021
Weeds[c]	44	0.00425	0.9483	44	7.75091	0.0079	44	0.9534	0.3342
Genetics:Lime	22	9.52315	0.0054	22	0.32934	0.5719	22	8.2165	0.009
Genetics:Weeds	44	1.36735	0.2486	44	0.64137	0.4275	44	0.0011	0.9739
Lime:Weeds	44	0.00561	0.9406	44	0.96424	0.3315	44	4.4428	0.0408
Genetics:Lime:Weeds	44	0.28471	0.5963	44	0.39678	0.532	44	0.417	0.5218
	Index of Disease Rating			Yield			Spore Count (Site 2 & 3 only)		
	denDF	F-value	P-value	denDF	F-value	P-value	denDF	F-value	P-value
(Intercept)	44	2.14677	0.15	44	125.751	<0.0001	28	361.778	<0.0001
Genetics[a]	11	23.9626	0.0005	11	8.26119	0.0151	7	21.807	0.0023
Lime[b]	22	2.66388	0.1169	22	0.30847	0.5842	14	0.5426	0.4735
Weeds[c]	44	0.9503	0.335	44	26.7119	<0.0001	28	0.319	0.5767
Genetics:Lime	22	11.3242	0.0028	22	13.8795	0.0012	14	3.521	0.0816
Genetics:Weeds	44	0.26954	0.6062	44	3.17616	0.0816	28	0.3948	0.5349
Lime:Weeds	44	0.05047	0.8233	44	0.00913	0.9243	28	4.2711	0.0481
Genetics:Lime:Weeds	44	0.57625	0.8114	44	0.01152	0.915	28	3.3805	0.0766

[a] Genetics is the canola genetic cultivar grown (clubroot susceptible '45H31' or clubroot resistant '45H29' site 1 & '45CM39' sites 2 & 3)
[b] Lime refers to either the application of hydrated lime or no application
[c] Weeds refers to either the management or non-management of weeds

TABLE 4.2: Effect of clubroot resistance, hydrated lime application, and weed management on growth parameters, clubroot index of disease, canola yield, and *Plasmodiophora brassicae* spore density in canola field trials at three naturally infested sites in Edmonton, AB, Canada, in 2018 and 2019.

Treatment			Fresh shoot weight (g) 10 plants	Fresh gall weight (g) 10 plants	Shoot height (cm)	Index of Disease Rating (%)	Yield (g plot^{-1})	Spore load (spores g^{-1} of soil) Sites 2 & 3 only
Genetics[a]	Lime[b]	Weeds[c]						
Resistant	Lime	No Weeds	1860.2 a	2.3 c	120.7 abc	6 cd	2167.8 a	1.7 x 10^5 c
Resistant	Lime	Weeds	1581.2 ab	1.6 c	122.3 ab	3 d	1594.0 b	2.5 x 10^5 c
Resistant	No Lime	No Weeds	1599.0 ab	9.5 c	124.2 a	15 c	2250.1 a	9.5 x 10^4 c
Resistant	No Lime	Weeds	1180.8 cde	7.8 c	120.9 abc	12 cd	1661.3 b	7.2 x 10^6 bc
Susceptible	Lime	No Weeds	1436.6 bc	51.5 b	116.1 cd	35 b	1684.6 b	8.5 x 10^6 bc
Susceptible	Lime	Weeds	1271.1 cd	33.9 bc	117.8 bc	29 b	1390.6 b	1.4 x 10^7 bc
Susceptible	No Lime	No Weeds	1080.3 de	139.8 a	111.4 d	69 a	986.6 c	4.4 x 10^7 a
Susceptible	No Lime	Weeds	901.9 e	110.3 a	110.3 d	65 a	701.4 c	2.7 x 10^7 ab

* Means followed by the same letter do not differ at $P < 0.05$ within each column

[a] Genetics is the canola genetic cultivar grown (clubroot susceptible '45H31' or clubroot resistant '45H29' site 1 & '45CM39' sites 2 & 3)

[b] Lime refers to either the application of hydrated lime (Lime) or no application (No Lime)

[c] Weeds refers to either the management (No Weeds) or non-management (Weeds) of weeds

TABLE 4.3: Two-way ANOVA for a trial evaluating different *Plasmodiophora brassicae resting* spore densities and crop rotations and on clubroot index of disease and canola growth parameters under greenhouse conditions.

	Gall Weight			Shoot Weight			Shoot Height			Index of Disease			Spore Density		
	numDF	F-value	P-value	numDF	F-value	P-value	numDF	F-value	P-value	numDF	F-value	P-value	numDF	F-value	P-value
(Intercept)	1	235.76113	<0.0001	1	1917.8721	<0.0001	1	20368.809	<0.0001	1	926.4479	<0.0001	1	16642.049	<0.0001
Crop[a]	1	0.52058	0.472	1	13.0576	0.0004	1	629.218	<0.0001	1	15.8031	0.0001	4	9.176	<0.0001
Rotation[b]	3	2.91414	0.0373	3	5.0849	0.0024	3	31.71	<0.0001	3	13.6168	<0.0001	3	10.576	<0.0001
Spore density[c]	4	105.74935	<0.0001	4	16.2031	<0.001	4	63.457	<0.0001	4	362.2352	<0.0001	1	168.478	<0.0001
Crop:Rotation	3	7.39482	0.0001	3	4.8419	0.0033	3	39.751	<0.0001	3	11.0912	<0.0001	12	0.979	0.4731
Crop:Spore density	4	0.20306	0.9362	4	1.2634	0.2884	4	0.877	0.48	4	7.5084	<0.0001	4	5.068	0.0008
Rotation:Spore density	12	2.703	0.003	12	2.1187	0.0206	12	3.596	0.0001	12	9.4589	<0.0001	3	6.252	0.0006
Crop:Rotation:Spore density	12	3.71449	0.0001	12	2.2225	0.0147	12	1.847	0.0484	12	4.4019	<0.0001	12	0.58	0.8547

[a] Crop refers to the two canola crops; first canola crop (CY1) and second (CY4) grown in rotation
[b] Rotation refers to the genetic rotation surrounding wheat (W) and barley (B); Clubroot Resistant (CR)-W-B-CR; CR-W-B-Clubroot Susceptible (CS); CS-W-B-CR; CS-W-B-CS
[c] Spore density refers to the initial resting spore density: 0 (check), 1 x 10 , 1 x 10 , 1 x 10 , 1 x 10 spores per gram of soil mix

TABLE 4.4: Effect of crop rotation and clubroot resistance on *Plasmodiophora brassicae* resting spore densities under greenhouse conditions.

Treatment		Initial Spore Density of 1×10^6	Initial Spore Density of 1×10^8
Rotation[a]	Crop[b]		
CR-W-B-CR	1CY1	5.4 x 10^4 c	1.0 x 10^7 ab
	2PW	1.5 x 10^5 c	2.1 x 10^6 b
	3PB	3.8 x 10^4 c	1.1 x 10^6 b
	4CY4a	1.0 x 10^6 c	4.9 x 10^6 b
	5CY4b	2.4 x 10^6 bc	1.2 x 10^7 ab
CR-W-B-CS	1CY1	2.5 x 10^5 c	5.9 x 10^6 b
	2PW	1.8 x 10^5 c	1.7 x 10^6 b
	3PB	2.6 x 10^4 c	1.0 x 10^6 b
	4CY4a	3.6 x 10^5 c	6.4 x 10^6 ab
	5CY4b	1.4 x 10^6 c	1.1 x 10^7 ab
CS-W-B-CR	1CY1	6.0 x 10^5 c	9.4 x 10^6 ab
	2PW	1.9 x 10^6 c	7.1 x 10^6 ab
	3PB	4.0 x 10^5 c	2.7 x 10^6 b
	4CY4a	1.7 x 10^6 c	9.7 x 10^6 ab
	5CY4b	1.1 x 10^7 a	1.9 x 10^6 b
CS-W-B-CS	1CY1	2.4 x 10^5 c	8.3 x 10^6 ab
	2PW	2.0 x 10^6 c	4.4 x 10^6 b
	3PB	1.9 x 10^5 c	3.5 x 10^6 b
	4CY4a	1.3 x 10^6 c	1.7 x 10^7 a
	5CY4b	9.2 x 10^6 ab	9.6 x 10^6 ab

* Means followed by the same letter do not differ at P > 0.05 within each column

[a] Rotation refers to the genetic rotation surrounding wheat (W) and barley (B); Clubroot Resistant (CR)-W-B-CR; CR-W-B-Clubroot Susceptible (CS); CS-W-B-CR;CS-W-B-CS

[b] Crop refers to when the soil sample was collected; after the first canola crop before gall reincorporation (1CY1), after wheat (2PW), after barley (3PB), after the second canola crop before gall reincorporation (4CY4a), after the second canola crop after gall reincorporation (5CY4b).

TABLE 4.5: Effect of crop rotation and clubroot resistance at various initial *Plasmodiophora brassicae* resting spore densities on canola growth parameters and clubroot index of disease under greenhouse conditions.

Treatment		Fresh shoot weight (g) 10 plants	Fresh gall weight (g) 10 plants	Shoot height (cm)	Index of Disease (%)
Crop [a]	Rotation [b]				
CHECK : 0 spore g^{-1} soil					
CY1	CR-W-B-CR	292.3 abc	0 a	93.6 c	0 a
CY4	CR-W-B-CR	322.5 abc	0 a	125.7 a	0 a
CY1	CR-W-B-CS	339.5 abc	0 a	99.0 bc	0 a
CY4	CR-W-B-CS	278.8 bc	0 a	109.9 b	0 a
CY1	CS-W-B-CR	390.0 a	0 a	80.8 d	0 a
CY4	CS-W-B-CR	351.7 abc	0 a	130.4 a	0 a
CY1	CS-W-B-CS	366.0 ab	0 a	76.8 d	0 a
CY4	CS-W-B-CS	260.5 c	0 a	110.1 b	0 a
1 x 10^2 spore g^{-1} soil					
CY1	CR-W-B-CR	363.3 ab	0 a	91.0 c	0 a
CY4	CR-W-B-CR	286.8 bc	0 a	123.8 a	0 a
CY1	CR-W-B-CS	369.8 a	0 a	87.2 cd	0 a
CY4	CR-W-B-CS	269.0 c	0 a	109.1 b	0 a
CY1	CS-W-B-CR	369.8 a	0 a	82.5 cd	0 a
CY4	CS-W-B-CR	293.5 abc	0 a	123.9 a	0 a
CY1	CS-W-B-CS	329.3 abc	0 a	80.2 d	0 a
CY4	CS-W-B-CS	2663.25 c	0.3 a	108.0 b	0.8 a
1 x 10^4 spore g^{-1} soil					
CY1	CR-W-B-CR	279.8 a	0 a	84.0 de	0 a
CY4	CR-W-B-CR	296.0 a	0 a	120.3 ab	0 a
CY1	CR-W-B-CS	298.8 a	0.3 a	91.7 d	0.83 a
CY4	CR-W-B-CS	280.5 a	0 a	106.8 c	0 a
CY1	CS-W-B-CR	334.3 a	0 a	79.3 e	0 a
CY4	CS-W-B-CR	321.5 a	0 a	125.7 a	0 a
CY1	CS-W-B-CS	309 a	0.3 a	84.1 de	0.83 a
CY4	CS-W-B-CS	253.3 a	0 a	109.3 bc	0 a
1 x 10^6 spore g^{-1} soil					
CY1	CR-W-B-CR	249.8 a	5.3 bc	84.2 c	14.2 d
CY4	CR-W-B-CR	212.5 abc	14.5 bc	118.0 a	45.8 c
CY1	CR-W-B-CS	242.3 ab	3.3 c	89.3 c	10.8 d
CY4	CR-W-B-CS	186.3 bcd	26.0 abc	104.8 b	65.8 bc
CY1	CS-W-B-CR	172.0 cd	47.0 a	52.3 d	85.8 ab
CY4	CS-W-B-CR	252.5 a	30.0 ab	112.0 ab	70.0 abc
CY1	CS-W-B-CS	256.5 a	42.5 a	63.2 d	67.5 abc
CY4	CS-W-B-CS	144.5 d	41.5 a	32.6 c	97.5 a

		1 x 10^8 spore g^{-1} soil			
CY1	CR-W-B-CR	359.0 a	45.3 abc	68.4 c	69.2 bc
CY4	CR-W-B-CR	277.0 abc	66.0 ab	105.5 b	95.8 a
CY1	CR-W-B-CS	364.8 a	32.5 bc	70.4 c	49.2 c
CY4	CR-W-B-CS	211.8 bc	72.3 a	80.8 c	94.2 a
CY1	CS-W-B-CR	175.0 c	74.5 a	44.0 d	88.3 ab
CY4	CS-W-B-CR	341.0 ab	20.0 c	119.5 a	59.2 c
CY1	CS-W-B-CS	144.3 c	56.0 ab	43.4 d	95.8 a
CY4	CS-W-B-CS	150.0 c	66.5 ab	68.8 c	98.3 a

* Means followed by the same letter do not differ at P > 0.05 within each column

[a] Crop refers to the two canola crops; first canola crop (CY1) and second (CY4) grown in rotation

[b] Rotation refers to the genetic rotation surrounding wheat (W) and barley (B); Clubroot Resistant (CR)-W-B-CR; CR-W-B-Clubroot Susceptible (CS); CS-W-B-CR; CS-W-B-CS

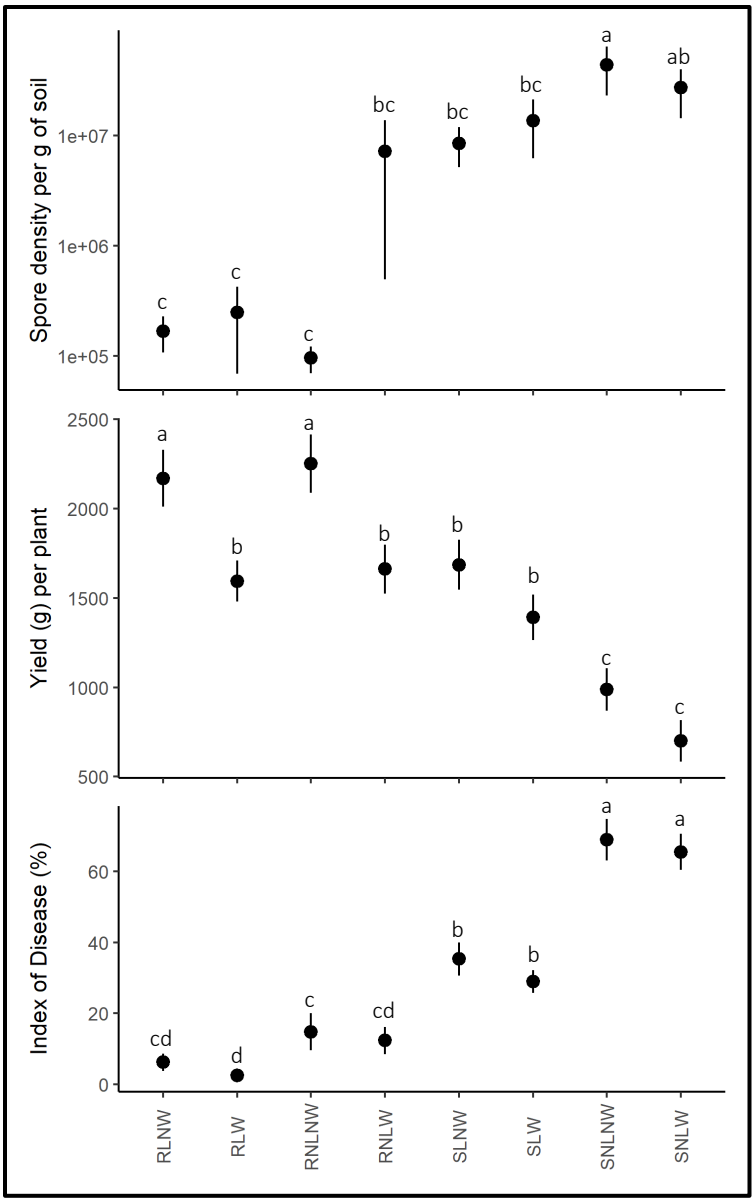

FIGURE 4.1: Clubroot index of disease, canola yield, and amount of *Plasmodiophora brassicae* in the soil in field plot experiments conducted at three field sites in Edmonton, AB, in 2018 and 2019. A clubroot resistant (R) cultivar '45H29' (site 1) / '45CM39' (sites 2 and 3) or clubroot susceptible (S) cultivar '45H31' was grown in soil treated with hydrated lime (L) to reach a soil pH of 7.2 or in soil where lime was not applied (NL). In plots where weeds were managed (NW), weeds were hand weeded 2 weeks after emergence. Weeds were not removed in plots with weeds (W). Resting spore densities were evaluated by quantitative PCR analysis of soil samples collected from the replicated treatments. Canola seed was dried and weighed at harvest to estimate yield (g) per plot. Index of disease was calculated at 8-weeks and ranges from 0% (no clubroot symptoms) to 100% (all roots severely galled). Differences in means are denoted by different letters at $P < 0.05$.

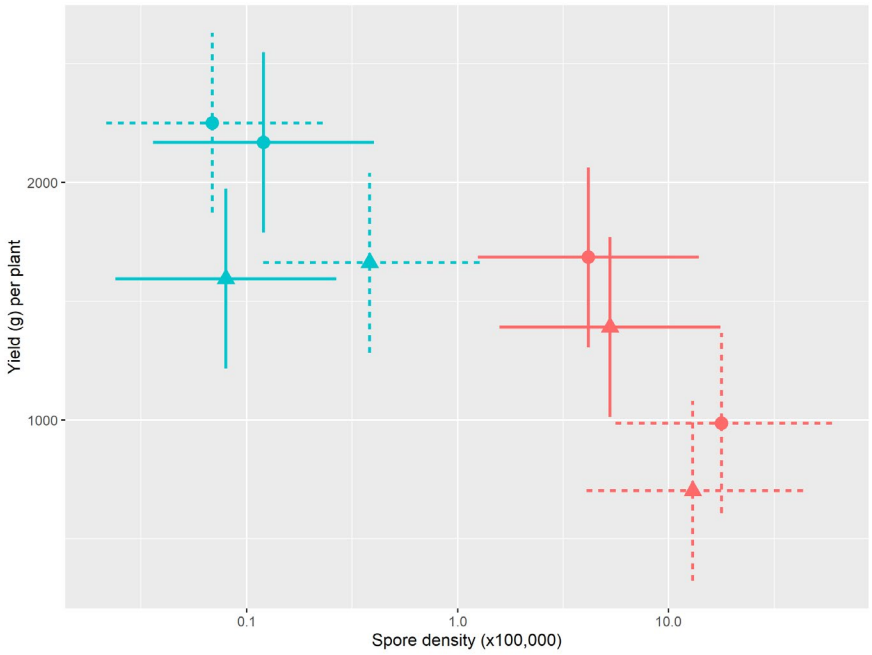

FIGURE 4.2: Canola yield and the amount of *Plasmodiophora brassicae* in the soil in field plot experiments conducted at three field sites in Edmonton, AB, in 2018 and 2019. A clubroot resistant (✦) cultivar '45H29' (site 1) / '45CM39' (sites 2 and 3) or a clubroot susceptible (✦) cultivar '45H31' was grown in soil treated with hydrated lime (+) to reach a soil pH of 7.2, or in soil where lime was not applied (⁘). In plots where weeds were managed (●), weeds were hand weeded every 2 weeks after emergence. Weeds were not removed in plots with weeds (▲). Resting spore densities were evaluated by quantitative PCR analysis of soil samples collected from the replicated treatments. Canola seed was dried and weighed at harvest to estimate yield (g) per plot. Lines represent a standard deviation +/- 1.96 for the 95% confidence interval.

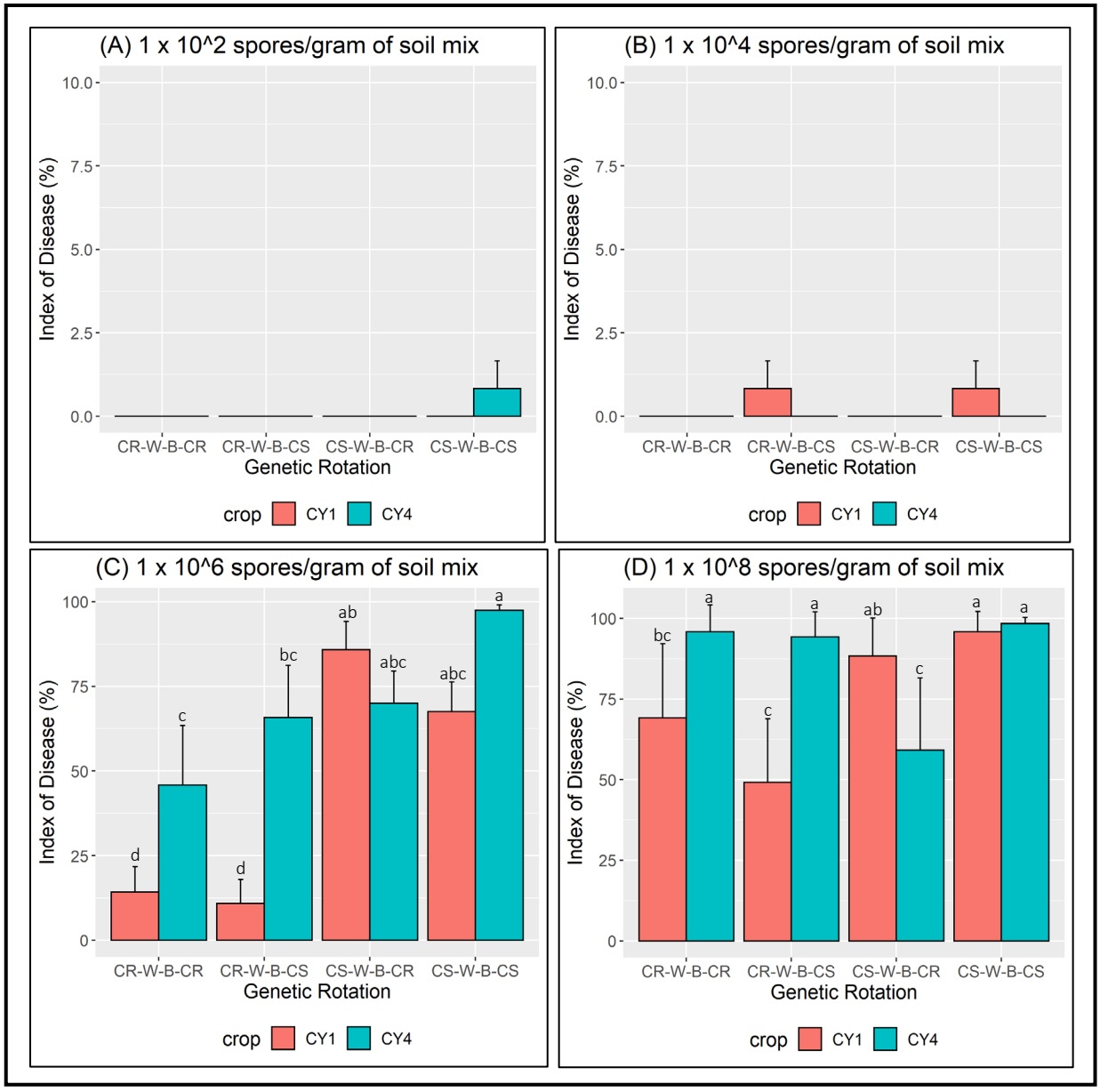

FIGURE 4.3: Clubroot index of disease for the first (CY1) and second (CY4) canola crops grown in a soil/potting medium mixture inoculated with *Plasmodiophora brassicae* resting spores at densities of (A) 1×10^2, (B) 1×10^4, (C) 1×10^6, and (D) 1×10^8 spores per gram soil mix ; negative controls were not inoculated (i.e., 0 resting spores per gram of soil) and did not develop any symptoms (not shown). Crops were grown for 8-weeks each with a 4-week break between crops in the following rotations: clubroot resistant canola (CR)-Wheat (W)-Barley (B)-CR; CR-W-B-clubroot susceptible (CS) canola; CS-W-B-CR and CS-W-B-CS. The CR canola cultivar grown was '45H29', and the CS canola cultivar was '45H31'. An index of disease was calculated at harvest and ranges from 0% (no clubroot symptoms) to 100% (all roots severely galled). These results are from a single run of this experiment. Different letters denote significant differences in the means at $P < 0.05$; means with no letters or the same letters do not differ.

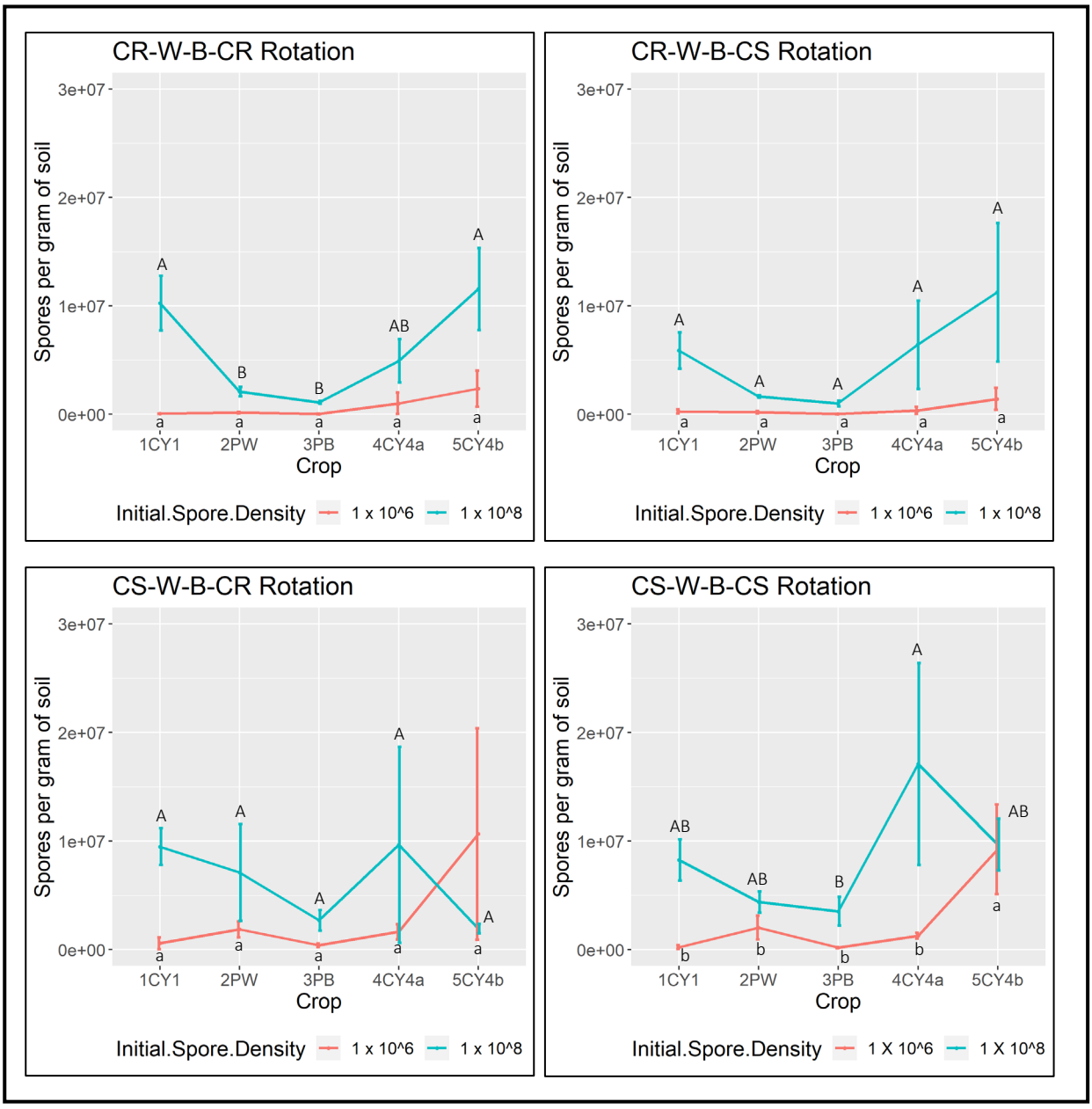

FIGURE 4.4: *Plasmodiophora brassicae* resting spore density in greenhouse trials as estimated by quantitative PCR analysis. Crops were grown for 8-weeks each with a 4-week break between crops in the following rotations: clubroot resistant canola (CR)-Wheat (W)-Barley (B)-CR; CR-W-B-clubroot susceptible (CS) canola; CS-W-B-CR and CS-W-B-CS. Crops were grown in a soil/potting medium mixture inoculated with *Plasmodiophora brassicae* resting spores at densities of 1×10^2, 1×10^4, 1×10^6, and 1×10^8 spores per gram soil mix ; negative controls were not inoculated (i.e., 0 resting spores per gram of soil). The CR canola cultivar grown was '45H29', and the CS canola cultivar was '45H31'. Soil samples were collected after each crop ; the first canola crop prior to gall reincorporation (1CY1), wheat (2PW), barley (3PB), the second canola crop prior to gall reincorporation (4CY4a) and after gall reincorporation (5CY4b). Resting spores were not detected in the negative, 1×10^2 and 1×10^4 spores per gram soil mix treatments and were therefore not included. These results are from a single run of this experiment. Means differences are denoted by different letters at $P < 0.05$. Lowercase letters placed beneath error bars denoted significance for treatments with initial spore densities of 1 x 10^6 spores per gram of soil, and uppercase letters placed above error bars denote significance for treatments with initial spore densities of 1 x 10^8 spores per gram of soil.

5.0 DISCUSSION

The main objective of this project was to evaluate the effects of various clubroot management strategies on disease incidence and severity, *P. brassicae* inoculum level, and canola yield. In the field experiments, this included the application of hydrated lime to increase the soil pH to 7.2, the management of weeds to eliminate clubroot susceptible (CS) hosts, and the deployment of clubroot resistant (CR) cultivars. The field data were supplemented with results from a greenhouse trial to investigate further the effect of various rotations of CR and CS canola cultivars, grown at different *P. brassicae* inoculum concentrations, on clubroot development and resting spore dynamics.

5.1 FIELD TRIAL

Based on the results of the field experiments, it appears that genetic resistance in the host remains the most effective tool for managing clubroot of canola in *P. brassicae*-infested soil. The clubroot index of disease (ID) was reduced by an average of 41%, relative to the CS cultivar, when the CR cultivar was grown. This is consistent with previous studies, which have shown that resistance significantly reduced clubroot development in canola (Hwang *et al*., 2011b; Peng *et al*., 2014). Nonetheless, the efficacy of genetic resistance will depend on the predominant pathotypes of *P. brassicae* present in a field (Xue *et al.*, 2008). Many 'novel' pathotypes of *P. brassicae* have emerged in recent years, about half of which can overcome the resistance in most CR canola cultivars (Strelkov *et al.,* 2018, 2020; Hollman *et al.*, 2020). While the genetic basis of the resistance in commercial cultivars is not generally in the public domain, it appears to be similar in most varieties introduced after the initial identification of clubroot (Fredua-Agyeman *et al.* 2018), and is now often referred to as '1st generation' resistance (Hollman *et al*.

2020). The CR cultivar '45CM39' included in this study, however, is among a select group of newer genotypes carrying '2nd generation' resistance. The resistance in these cultivars is reportedly distinct from 1st generation resistance and was developed to manage pathotypes of *P. brassicae* that can overcome the latter (Canola Council of Canada, 2020). The presence of pathotype 3D, which is one of the main pathotypes able to break 1st generation resistance (Strelkov *et al.*, 2018), has been confirmed in the disease nursery where these trials were conducted (see Chapter 3). Thus, the significant reduction in clubroot severity on '45CM39' likely reflects appropriate cultivar selection and the efficacy of its clubroot resistance. The reduced clubroot symptom development in '45CM39' was reflected in improved overall plant health and yield; the resistant variety showed significant increases in shoot height, shoot weight, and amount of harvested seed relative to the CS cultivar. The selection of an appropriate CR cultivar allows the canola plant to establish, grow, and produce seed with minimal impact from *P. brassicae*.

Cropping of the CS cultivar resulted, on average, in 2.1 x 10^7 more resting spores per gram of soil relative to a CR cultivar in the spring following cultivation. Similar results were reported in earlier comparisons of the resting spore contributions from CS and CR canola (Hwang *et al.*, 2011b; Hwang *et al.*, 2017). This highlights the positive impact of growing a CR cultivar with respect to *P. brassicae* inoculum management. Gall development is greatly reduced on resistant hosts, and as such, the production of new resting spores that can be released back into the soil is also reduced (Zamani-Noor and Rodemann, 2018; Ernst *et al.*, 2019). Nonetheless, the spores that are produced on CR canola, while less in number, may be enriched for pathotypes able to overcome the resistance, and may contribute to virulence shifts (Ernst *et al.*, 2019).

There was a significant interaction between lime treatment and cultivar. The most notable differences were observed for the CS cultivar, where treatment with lime resulted in a decline in clubroot severity of about 35%, while the yield almost doubled. This is consistent with the results of earlier studies, which showed some potential for the application of lime to mitigate clubroot (Karling, 1942; Tremblay *et al*., 2005; Fox *et al*., 2021). Lime could be a particularly useful tool to manage clubroot in patches where resistance-breaking pathotypes are predominant and resistance is no longer effective. It could also serve as a tool to supplement genetic resistance before it is lost or eroded, since the application of lime resulted in a decrease in clubroot severity even on the CR cultivar. This suggests that combining genetic resistance with lime could slow increases in *P. brassicae* inoculum in the soil, and hence extend the durability of resistant varieties. Hydrated lime, however, did not have a significant effect on yield in the CR cultivar; indeed, while not significant, some numerical decreases were observed in the yield of the CR variety following lime treatment. This could reflect changes in soil nutrient availability for the host because of increased pH (Binkley and Vitousek, 1991; Cornell University, 2006a). Tremblay *et al*. (2005) also observed reduced clubroot severity on cauliflower (*Brassica oleracea* L. var. *botrytis*) without a concomitant yield increase following spring application of calcium hydroxide. Collectively, the results from this study suggest that the application of hydrated lime should target a soil pH of 7.2.

The management of weeds resulted in significantly higher yield for the CR canola cultivar, but the effect of weed management on yield of the CS cultivar was not significant. Weeds compete with crops for nutrients, water, and space (Oerke, 2006). As such, weed control is part of a good crop management plan and can result in higher yields. In the case of the CS canola

cultivar, weed control did not affect clubroot severity among treatments with similar cultivar and lime regimes. The negative effects associated with severe clubroot development may have prevented the CS cultivar from taking full advantage of the increased resources available with weed control.

The CR cultivar treatments without weed management had yields that were statistically similar to the treatments that included a CS cultivar with hydrated lime. While the cropping of a CS cultivar in a *P. brassicae*-infested soil is not recommended, since variation in environmental conditions or timing of the lime treatment can still result in development of disease (Fox et al. 2021), these results do highlight the potential effectiveness of combining strategies for clubroot management. By reducing disease pressure, control measures such as the application of lime may help to prolong the effectiveness of resistance, thereby contributing to the stewardship of this important resource.

Resting spore densities were highest when the CS cultivar was grown without hydrated lime and with weed management. These results suggest that a more acidic soil increased the number of resting spores produced in the more severely infected host tissues. Additionally, it is possible that the absence of weeds increased access to the canola roots by the resting spores, promoting more secondary infections, since no weed roots would have been present. A challenge associated with measuring the effects of weeds in the field can be the uneven distribution of weed species and quantity. Clubroot susceptible weeds were found across sites 2 and 3, including shepherd's purse (*Capsella bursa-pastoris* L.), stinkweed (*Thlaspi arvense* L.), and flixweed (*Descurainia sophia* L.). However, there were 15.75% more clubroot susceptible weeds across site 3 than site 2 (TABLE A.1). Furthermore, across site 3, the proportion of susceptible

weeds varied from as low as 15% to as high as 82% in the plots. At site 2, the highest proportion of clubroot susceptible weeds in any plot was 57%. As the linear mixed-effects model used total weeds rather than susceptible weeds, no conclusions can be made on the effects of clubroot susceptible weeds on inoculum levels. However, we hypothesize that the hydrated lime inhibited spore germination, which suppressed clubroot severity on susceptible weeds, affecting the spore density (Niwa *et al*., 2008). In the plots where weeds were managed, frequent hand weeding was completed as necessary to prevent an increase in inoculum levels resulting from the proliferation of *P. brassicae* in these hosts (Zamani-Noor and Rodemann, 2018).

5.2 GREENHOUSE

The primary purpose of the greenhouse trial was to determine whether there was a detrimental effect associated with the early deployment of CR cultivars for the management of clubroot. It has been hypothesized (Diederichsen *et al*., 2009) that the planting of CR canola or oilseed rape, before clubroot has been identified in a field, could favor the erosion of that resistance and its loss for use in heavier infestations. However, waiting to identify clubroot visually in a field before planting a CR cultivar may result in an increase in *P. brassicae* inoculum levels, as the pathogen rapidly proliferates on susceptible hosts. This increase in inoculum could also favor pathotype shifts when a CR cultivar is eventually introduced (LeBoldus *et al*., 2012; Strelkov *et al*., 2018). Given the risk of selecting for resistance-breaking pathotypes, an understanding of the timing of CR cultivar deployment in *P. brassicae* infested fields is critical.

To interpret the greenhouse results accurately, a few shortcomings in the experiment must be addressed. The root galls used to generate the inoculum for the greenhouse trials were collected from a clubroot nursery where pathotype 3H had previously been predominant. After the experiments were underway, however, we confirmed that there had been a shift in the virulence of the pathogen field population towards pathotype 3D, which overcomes 1st generation resistance (Strelkov et al. 2018). Hence, '45H29', the CR cultivar chosen for the greenhouse study and which carries 1st generation resistance, was susceptible to the virulent, resistance-breaking pathotype that represented at least some fraction of the inoculum. This resulted in a more rapid increase in ID than would likely have occurred if a cultivar with effective ('2nd generation') resistance to both pathotypes 3H and 3D had been grown. Additionally, quantitative PCR analysis indicated lower spore densities than expected given the initial amount of inoculum applied. This may reflect the fact that the initial spore suspensions used to inoculate the greenhouse treatments were quantified with a haemocytometer, but no staining was conducted to determine the viability of the resting spore suspensions. Staining with a vital stain such as Evan's blue (Harding *et al.*, 2019) may have provided a more accurate estimate of the viable, rather than total, resting spores in the inoculum suspensions. This means that the initial effective spore concentrations in each of the treatments may have been lower than estimated, if a significant portion of the inoculum represented non-viable resting spores. Unfortunately, technical issues, including a change of the greenhouse illumination to a non-compatible light source, precluded the re-running of this trial as part of the current project.

The predominant visual symptom associated with clubroot is the swelling of the host roots. The belowground nature of this symptom, combined with the patchy in-field distribution

of the disease, makes the timely management of *P. brassicae* challenging. Usually, the first indication that a crop is infected is the appearance of aboveground symptoms, such as premature ripening, stunting, leaf discoloration, and foliar wilting (Dixon, 2009). In this greenhouse trial, however, it was difficult to determine the severity of clubroot visually based solely on aboveground symptoms; shoot height and shoot weight had to be quantified and compared statistically to identify differences (which were not necessarily visually striking). The only clear, aboveground visual symptom was the shorter height of the second canola crop in the CS-W-B-CS rotation with an initial spore density of 1 x 10^8 spores per gram of soil mix. The soil inoculum level after the first and second canola crops was, however, statistically similar. Furthermore, regarding the second canola crop, the CR-W-B-CR rotation with an initial spore density of 1 x 10^8 spores per gram of soil produced significantly taller plants than x-W-B-CS, with a statistically similar ID. These results could reflect higher tolerance towards *P. brassicae* infection in the CR cultivar compared with the CS cultivar. Additionally, the CR cultivar could have delayed the response to secondary infection caused by *P. brassicae*, resulting in less severe effects on growth parameters.

Clubroot development was limited across rotations with initial spore densities below 1 x 10^6 spores per gram of soil mix. Nonetheless, CR canola cultivars are not immune to the disease (Canola Council of Canada, 2020b), and very mild symptoms of clubroot were observed on the resistant host '45H29', even at low spore concentrations. These symptoms may also have reflected the presence of at least some inoculum of pathotype 3D, as noted above, which could infect the CR host. While the number of resting spores released back into the soil is minimal when clubroot symptoms are mild, these spores may consist of enhanced numbers of resistance-

breaking pathotypes, which would be the most likely to be able to reproduce on a CR genotype (Ernst *et al*., 2019). As such, these resting spores could be particularly important to consider when developing resistance stewardship strategies.

In both the CR-W-B-CR and CR-W-B-CS rotations, a significant increase in ID on the second canola crop was observed in treatments with initial spore densities of 1×10^6 and 1×10^8 spores per gram of soil mix. Interestingly, while the inoculum consisted of at least some pathotype 3D, which can overcome the resistance in the CR cultivar '45H29', this host still developed lower IDs in the first cycle of rotation. This CR cultivar also developed a significantly lower ID in the CS-W-B-CR rotation when the initial spore density was 1×10^8 spores per gram of soil mix. These observations support the presumption that multiple pathotypes exist within a gall (Ernst *et al*., 2019), and indicate that while there may have been a shift to pathotype 3D in the initial inoculum source, pathotype 3H was still present and effectively controlled by '45H29.' It is likely that additional exposure of this resistance source to the same inoculum over multiple cycles would have resulted in an increase in clubroot severity, as has been reported previously in experiments with single-spore and field isolates of *P. brassicae* (LeBoldus et al. 2012).

With the exception of the CR-W-B-CR rotation with an initial spore density of 1×10^6 spores per gram of soil mix, which showed a 30% reduction in resting spores following the 2-cereal crop break, all other treatments with initial resting spore densities of 1×10^6 and 1×10^8 spores per gram of soil mix in the CR-W-B-x rotations saw an average reduction of 87% in resting spores. This finding is consistent with previous studies, which have shown a 90% decrease in *P. brassicae* resting spore load after the cultivation of a CR cultivar followed by a 2-year break from canola (Peng *et al*., 2015; Ernst *et al*., 2019). Nonetheless, if initial spore densities are sufficiently

high, even a 90% reduction in spore numbers could still result in a high inoculum load. For instance, a 90% reduction in an infested soil with 100,000,000 (1×10^8) spores per gram of soil would result in 10,000,000 (1×10^7) spores per gram of soil remaining, which is still more than sufficient to cause severe clubroot (Zamani-Noor and Rodemann, 2018; Ernst *et al*., 2019; Fox *et al*., 2021).

While a similar trend was observed in this trial compared to previous studies (Peng *et al*., 2015; Ernst *et al*., 2019), a 90% reduction in *P. brassicae* resting spore load after the cultivation of a CR cultivar followed by a 2-year break from canola was not observed across all CR-W-B-x rotations. This inconsistency may reflect differences in environmental conditions and shorter crop cycles in the greenhouse experiment relative to earlier field-based studies (Peng *et al*., 2015; Ernst *et al*., 2019). In those studies, the resting spores had at least 16 weeks to complete their life cycle, with environmental factors promoting gall degradation over the winter and spring. In the greenhouse, the resting spores had 8 weeks to infect their host and reproduce, with mechanical destruction to break down the clubroot galls. These artificial conditions could have affected inoculum production and viability.

The only notable change in spore load among CS-W-B-x rotations was in the CS-W-B-CS rotation with an initial spore density of 1×10^6 spores per gram of soil mix. While the level of inoculum was consistent throughout the rotation, the reincorporation of the root galls significantly increased the spore density. In other treatments that included highly susceptible hosts, the reincorporation of root galls did not significantly affect spore densities, perhaps because much of the inoculum was already in the soil mix. Given that 1 g of galled canola root can produce billions of resting spores (Hwang *et al*., 2013), rotations that include susceptible

canola genotypes have significant potential to contribute to increases in inoculum loads. This underscores the importance of excluding clubroot susceptible hosts from *P. brassicae*-infested soils as part of a proactive disease management plan.

Collectively, while there were significant changes in the IDs and resting spore densities in some of the treatments, these were not generally reflected in the aboveground appearance of the canola plants. Moreover, given the minimal disease development on the CR and CS cultivars at initial spore densities below 1×10^6 spores per gram of soil mix, relative to the higher spore densities, the results suggest that there is a larger risk of pathogen proliferation and resistance erosion associated with the delayed deployment of CR canola. The increases in clubroot incidence, severity, and inoculum levels associated with the planting of CS canola likely represent a greater threat to sustainable canola production in the long run, as the pathogen proliferates and the chances for the emergence of resistance-breaking pathotypes increase. Early detection of *P. brassicae*, the appropriate use of CR cultivars, and the timely deployment of resistant genetics are essential for successful canola production in *P. brassicae*-infested soils.

6.0 CONCLUSIONS & FUTURE RESEARCH

6.1 GENERAL CONCLUSIONS

Clubroot continues to threaten the production of canola across the Canadian prairies. Short cropping rotations with canola have contributed to the increased prevalence of this disease (Hwang *et al*., 2014), as inoculum levels can increase exponentially on infected hosts. Approximately 16 billion resting spores of *Plasmodiophora brassicae* can be produced in 1 g of galled canola root tissue (Hwang *et al*., 2013). Currently, the planting of clubroot resistant (CR) cultivars is the predominant strategy for disease management. These CR cultivars, however, can exert selection pressure on *P. brassicae* populations, resulting in the development of significant levels of clubroot on previously resistant cultivars (Strelkov *et al*., 2016). The loss or erosion of genetic resistance reflects the emergence of 'new' *P. brassicae* pathotypes, which can be distinguished based on their virulence patterns on the Canadian Clubroot Differential (CCD) set (Strelkov *et al*., 2018). To date, 36 pathotypes have been identified on the CCD set, of which 19 can overcome resistance in at least some CR canola cultivars (Strelkov *et al*., 2018; Strelkov *et al*., 2020; Askarian *et al*., 2020; Hollman *et al*., 2020).

The increasing prevalence of novel *P. brassicae* pathotypes highlights the diversity in the virulence of this pathogen. While genetic resistance can provide excellent control of clubroot, proper stewardship of this resource is imperative to maintain its effectiveness and longevity. Since the resting spores produced on CR canola are likely enriched for pathotypes that can overcome resistance (Ernst *et al*., 2019; Cao *et al.*, 2020), the continued reliance solely on CR genetics to manage clubroot may not be sustainable, as breeding efforts cannot keep up. Additionally, the longevity of the pathogen resting spores, and their movement on field

machinery (Cao *et al.*, 2009) and even windborne dust (Rennie *et al*., 2015; Botero-Ramirez *et al*. 2021), further complicate control of this disease. The integration of multiple strategies in clubroot management plans, beyond a reliance solely on genetic resistance, is imperative to minimizing the movement and build-up of the disease (Hwang *et al.*, 2014). Ultimately, this will reduce the risk of virulence shifts in *P. brassicae* populations and improve the longevity of resistance (Peng *et al*., 2013, Peng *et al*., 2015). The main objective of this thesis project was to evaluate the effect of various management practices and approaches on *P. brassicae* inoculum levels and clubroot incidence and severity.

Determining whether there is any detrimental effect to the early deployment of CR canola cultivars, before clubroot is identified in a field, is important, particularly given concerns that such an approach could contribute to the rapid loss of resistance (Diederichsen *et al.*, 2009). In this project, under greenhouse conditions, resting spores could not be detected following various crop rotation sequences when the initial resting spore density was 1×10^2 or 1×10^4 spores per gram of soil mix. This suggests that at low initial spore densities, there is limited production of additional resting spores when resistant and/or susceptible canola is grown in rotation with other, non-host crops. If resting spore numbers can be kept low, the likelihood of pathotype shifts will be lower, since there will be a smaller pathogen population upon which host selection pressure can be exerted. Inoculum densities did increase in treatments with an initial resting spore density equal to or greater than 1×10^6 spores per gram of soil mix. While the pathotype composition was not tested throughout the trial, and likely would affect the rate of inoculum increase, the results suggest that cultivation of CR cultivars in soils infested with spore

concentrations of 1×10^4 spores per gram or less may be associated with a reduced risk of resistance erosion.

In addition to the timely deployment of CR canola, an understanding of the rotations favoring the prolonged effectiveness of resistance is necessary. Based on the greenhouse results, some of the trends observed with respect to *P. brassicae* resting spore densities over time aligned with previous studies (Ernst *et al*., 2019; Peng *et al*., 2015). A 2-crop break from canola was found to result in decreases of up to 90% in the spore concentration when a CR cultivar had been grown in an initial spore density equal to or greater than 1×10^6 spores per gram of soil mix. A decrease in spore density after a 2-crop break was also observed with the clubroot susceptible (CS) cultivars, although to a lesser extent. Surprisingly, the differences were not significant among rotations. As noted earlier, caution must be exercised when interpreting the results of this greenhouse trial, as the rotations were grown over 11 months, as opposed to the 48 months that would have been required under field conditions. Additional long-term field trials may be warranted to further explore the effect of the crop rotations examined in these greenhouse trials.

A first-generation CR cultivar was selected for the greenhouse trial. Unfortunately, the pathotyping of the galls used as inoculum was completed after planting, and it was determined that the inoculum, initially thought to be pathotype 3H (which is controlled by first-generation resistance) had shifted towards pathotype 3D (which can overcome first-generation resistance). Therefore, this trial simulated conditions where CR erosion is occurring. Cultivar selection did not affect the disease severity or incidence among any rotation at the low initial spore densities. Minimal galling was observed, potentially contributing to an enrichment of virulent pathotypes,

but posing less risk for high selection pressure due to the limited amounts of inoculum produced. The CR cultivars could suppress the susceptible pathotypes at higher spore densities during the first cropping year, but due to the high inoculum levels and a predominant CR-breaking pathotype, clubroot incidence and severity increase in the final cropping year. These results align with previous studies that suggest a quick increase in inoculum levels could favour pathotype shifts when a CR cultivar is introduced (LeBoldus *et al*., 2012; Strelkov *et al*., 2018). Overall, no rotation appeared better than the next among those evaluated in the greenhouse trial when canola was grown on highly infested potting mix in the presence of virulent pathotypes. While CR cultivars will provide some benefit in the short term, they may succumb to resistance loss or erosion under high selection pressure. To prolong the effectiveness of CR cultivars, they must be introduced to fields with low *P. brassicae* spore densities and grown in rotations that include a minimum 2-year break from host plants. For a 90% reduction in resting spore density to be of benefit, the remaining 10% must be at a level that minimizes the potential for erosion of the resistance in the CR cultivars (Ernst *et al*., 2019). As such, if the initial spore concentration is very high, a longer rotation may be required to achieve reductions in inoculum to satisfactory levels.

While CR cultivars represent the first line of defence against clubroot for canola growers, it is evident that additional strategies are necessary to manage this disease. *P. brassicae* has proven its resiliency and diversity; therefore, other techniques to disrupt the disease triangle using various control methods are required. In the field experiments conducted as part of this thesis, the application of hydrated lime and weed management were evaluated individually and collectively to assess their effectiveness in reducing clubroot severity and incidence, *P. brassicae* inoculum levels, and increasing canola yield, on CS and CR cultivars.

The application of calcium products to increase soil pH ≥ 7.2 slows the germination of resting spores, reducing clubroot severity and incidence (Chupp, 1928; Karling, 1968; Donald and Porter, 2009; Fox *et al*., 2021). In a recent study (Fox *et al*., 2021) evaluating lime for clubroot management in canola, hydrated lime was found to be more effective at managing high levels of *P. brassicae* inoculum than common limestone. In the current thesis, the most notable effects of the application of hydrated lime were observed on the CS cultivar, where clubroot severity decreased and seed yield almost doubled. Although not as substantial, a reduction in clubroot severity was also observed on the CR cultivar with the application of hydrated lime. While there were limited differences detected among spore densities with the application of hydrated lime, this product could serve as a tool in mitigating the erosion of clubroot resistance, or for managing clubroot patches with resistant-breaking pathotypes. A major challenge facing the adoption of hydrated lime by prairie canola growers is its cost; at $320 CAD per tonne, applying hydrated lime to an entire field is likely not economical (Donald and Porter, 2009; Fox *et al*., 2021). Additionally, determining the amount of hydrated lime required to manage clubroot effectively without affecting crop performance may be challenging, given irregular soil types, spore densities and soil moisture across a field. Therefore, at present, the use of hydrated lime should be considered mainly as a strategy for clubroot patch management.

P. brassicae can infect nearly all species within the Brassicaceae family (Dixon, 2009a). Some common prairie cruciferous weed species that serve as potential hosts include field pepperwort (*Lepidium latifolium* L.), shepherd's purse (*Capsella bursa-pastoris* L.), stinkweed (*Thlaspi arvense* L.), and flixweed (*Descurainia sophia* L.). It is also necessary to consider volunteer canola as a weed, since it contributes to the proliferation of *P. brassicae* resting spores

(Zamani-Noor and Rodemann, 2018). However, gathering hard data on the impact of cruciferous weeds on clubroot disease in canola is challenging. In addition to the patchy distribution of *P. brassicae* resting spores, the distribution of weed species and their quantity is also highly variable. As such, more research is required to quantify the contribution that cruciferous weeds make to *P. brassicae* inoculum levels. Nonetheless, weed control is an integral part of good crop management. This was evident from the field trials in this project, where treatments that included managed weeds and the CR cultivar had the greatest yields. In contrast, the lowest canola yields were observed on the CS cultivar in the absence of hydrated lime. The severe clubroot development on the CS cultivar could have negated any benefits provided by the absence of weeds.

The necessity and importance of a proactive and integrated clubroot management plan is well established (Donald and Porter, 2009; Howard *et al*., 2010; Hwang *et al*., 2014; Dixon, 2014; Peng *et al*., 2014). Results from this thesis reinforce why CR cultivars have been the first line of defence against clubroot across the prairies. When the appropriate CR cultivar is selected for the predominant pathotype in a field, clubroot may seem to be 'completely controlled'. However, this can provide growers with a false sense of security. Given the rapid emergence of novel pathotypes able to overcome resistance (Strelkov *et al.*, 2016; Strelkov *et al.*, 2018; Hollman *et al.*, 2020), the control afforded by CR canola may soon be lost, along with the most used and effective clubroot management tool. Proper stewardship will prolong the longevity of CR canola cultivars. Stewardship measures include identifying clubroot in the field early, deploying CR genetics before the disease becomes established, and reducing the number of times the pathogen is exposed to susceptible hosts and resistant genetics. Individually, rotations, hydrated

lime, and weed management will not provide the same short-term results as CR cultivars. However, using these strategies collectively to limit exposure to susceptible hosts and to create an unfavourable environment for the pathogen could help to protect resistance. Such an integrated approach will reduce clubroot disease incidence and severity, help to reduce *P. brassicae* inoculum levels, and ultimately increase canola yield for Canadian canola growers.

6.2 FUTURE RESEARCH

As more growers face the challenges posed by clubroot and resistant-breaking pathotypes of *P. brassicae*, there has been an increased interest in liming as a clubroot management tool. However, limited research exists on which lime product to select for different soil types and inoculum levels, as well as on the optimal rate of lime to apply. In this thesis, hydrated lime was evaluated as a treatment because a previous study had confirmed its effectiveness in the same clubroot nursey (Fox *et al*., 2021). Future studies will need to determine which lime products are best suited for the various soil types found across the Canadian prairies, and how to assess appropriately the optimal amount of lime required. As liming can be costly, growers need more regional and applied research to ensure their efforts at clubroot management are successful, without negatively affecting their canola crops. More research is also needed to understand the longevity of various liming products in the soil. Due to their different quality standards and associated costs of lime products, knowledge of the differences with respect to the frequency of application is important to encourage their use as another clubroot management tool.

The influence of cruciferous weeds on *P. brassicae* also requires more research. Weeds grow continuously throughout the cropping season. Therefore, it would be beneficial to determine when the weed is most susceptible to infection and if *P. brassicae* spores are equally virulent throughout the growing season. Additionally, studying whether cruciferous weeds are susceptible to all pathotypes equally will enhance our knowledge of pathotype spread and proliferation. If there are weed species that are naturally resistant to specific pathotypes, the basis for resistance in these weeds could be investigated for potential integration into canola for more durable CR genetics. Non-host weeds should also be studied to understand their contribution to *P. brassicae* inoculum levels. Ultimately, a comprehensive research approach, taking into consideration the needs of growers and making use of all available tools, will help to move our understanding of the clubroot pathosystem and its management forward.

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APPENDIX

TABLE A.1 : Average total weed count per m^2 and proportion of clubroot susceptible weeds per m^2 at two field sites in Edmonton, Alberta, 2019, treated with hydrated lime for clubroot management.

Treatment			Average total weeds per m^2		Proportion of susceptible weeds (%) per m^2	
Genetics[a]	Lime[b]	Weeds[c]	Site 2	Site 3	Site 2	Site 3
Resistant	Lime	No Weeds	0 b	0 d	0 c	0 b
Resistant	Lime	Weeds	90 a	93 c	29 ab	50 a
Resistant	No Lime	No Weeds	0 b	0 d	0 c	0 b
Resistant	No Lime	Weeds	111 a	145 a	37 a	47 a
Susceptible	Lime	No Weeds	0 b	0 d	0 c	0 b
Susceptible	Lime	Weeds	95 a	119 b	20 b	34 a
Susceptible	No Lime	No Weeds	0 b	0 d	0 c	0 b
Susceptible	No Lime	Weeds	113 a	108 bc	29 ab	47 a

* Means followed by the same letter do not differ at $P > 0.05$ within each column

[a] Genetics is the canola genetic cultivar grown (clubroot susceptible '45H31' or clubroot resistant '45CM39')

[b] Lime refers to either the application of hydrated lime (Lime) or no application (No Lime)

[c] Weeds refers to either the management (No Weeds) or non-management (Weeds) of weeds