# An assessment of the fumigant metam sodium and a Brassica juncea-derived biofumigant as management tools for clubroot (*Plasmodiophora brassicae*) of canola (*Brassica napus*)

by

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**Abstract**

Clubroot of crucifers, caused by the soilborne parasite *Plasmodiophora brassicae*, is spreading across canola (*Brassica napus*) fields in Alberta, Canada. Dissemination of the parasite is associated with the movement of infested soil on farm and other machinery, with the disease generally occurring first as localized patches near field entrances. The soil fumigant Vapam (metam sodium) was evaluated as a tool to manage foci of *P. brassicae* infestation. Replicated experiments at two field sites in central Alberta showed reductions in clubroot severity ranging from 9-51% following treatment with varying rates of Vapam. Some residual effects also were detected, as decreases in disease severity of up to 28% were observed in the year following Vapam treatment. In a second set of experiments, a commercial seed meal (*B. juncea*)-based biofumigant, MustGrow, was assessed for efficacy against clubroot under greenhouse conditions. No significant declines in clubroot severity were detected, and the concentration of *P. brassicae* resting spores in the soil, as measured by quantitative PCR analysis, did not decrease after treatment with the biofumigant. However, plant mortality was high, likely due to the presence of root rot pathogens in the soil, and the results of the biofumigant study should be interpreted with caution. While Vapam shows some potential as a clubroot management tool, additional research is needed to fully evaluate the efficacy of the biofumigant. An integrated approach will be required for the sustainable management of clubroot of canola.

## Preface

This thesis is an original work by me, Krista Ashley Zuzak. No part of this thesis has been published previously, except as preliminary conference abstracts corresponding to presentations made by me over the course of my Master's program. I conducted all of the experiments described in this document, and wrote the first drafts of all chapters. Each chapter was then reviewed by my supervisor, Dr. Stephen Strelkov, who provided me with editorial revisions and suggestions for improvement of the document. Following incorporation of these suggestions by me, each chapter was then reviewed by Dr. Sheau-Fang Hwang (co-supervisor), prior to inclusion in the thesis. While I conducted all of the experiments myself, I received assistance from summer students and other research personnel in routine tasks such as field plot preparation and weeding.

The work was funded by the Clubroot Risk Mitigation Initiative and the Growing Forward 2 Program (Canola Council of Canada & Agriculture and Agri-Food Canada), and by the Canadian Agricultural Adaptation Program (Agriculture and Food Council).

## Dedication

I dedicate my thesis, and all of the work which has gone into it, to my late grandparents, Annette and Steve Lebitko, and my late aunt Mary Ann Lebitko. I would like to thank my grandparents for teaching me about farming and inspiring my career journey and its ultimate destination. I would like to thank my aunt for her unconditional love and support and for my green thumb.

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# Chapter 1

## 1.0 General Introduction

### 1.1.1 Background

Clubroot is a soilborne disease of crucifers caused by *Plasmodiophora brassicae* Woronin, which causes development of the characteristic galled roots on infected plants. These root galls interfere with water and nutrient uptake from the soil, resulting in external symptoms such as a stunted growth habit and premature and uneven ripening. Ultimately, severe yield and quality losses can result from infection (Dixon 2009a). In canola (*Brassica napus* L., *Brassica rapa* L., and *Brassica juncea* L.), the plant produces fewer seeds with lower oil content (Pageau 2006). This literature review contains a summary of the impact of clubroot in the Canadian canola crop, the biology of the pathogen, and the management strategies employed to date. The specific objectives of the project are discussed in Section 5 of this Literature Review.

### 1.1.2 Importance of Clubroot Worldwide

The first reports of clubroot coincide geographically with the centre of origin of the genus *Brassica*, in the Mediterranean (Howard *et al.* 2010). The occurrence of clubroot was described on radishes (*Raphanus sativus* L.), turnips (*Brassica rapa* subsp*. rapa* L.) and rape (*Brassica napus* L.) in Italy as early as the fourth century AD (Watson & Baker 1969). A record from 1539 by Diaz de Isla in Spain centres on the presence of clubroot symptoms on cabbage, which he described as 'syphilitic' (Watson & Baker 1969, Karling 1968). In 1736, there were reports of clubroot on turnip in England, followed by other reports from continental Europe (Karling 1968, Howard *et al.* 2010). The presence and spread of

clubroot in St. Petersburg, Russia, led to devastating losses, prompting the identification of the causal agent by Woronin as *Plasmodiophora brassicae* (Woronin 1878, Karling 1968).

The first report of clubroot in North America is believed to have been near New York City in 1853 (Watson & Baker 1969). Some of the earliest reports of clubroot in Canada are documented in the *Canadian Plant Disease Survey*, a periodical on the occurrence of plant diseases throughout the country. The disease was reported on vegetable crops in the Maritimes, British Columbia, Quebec and Ontario between the 1920s and the 1950s (Howard *et al.* 2010).

### 1.1.3 Economic Importance of Clubroot

Plants infected with *P. brassicae* exhibit external symptoms such as a stunted growth habit, premature and uneven ripening, and the characteristic galled roots. There can also be severe yield and quality decreases associated with clubroot infection, while the value of clubroot infested land can be depressed (Dixon 2009a). In a Quebec study by Pageau et al. (2006), yield losses of 80 to 91% were reported in canola. The infected plants produced fewer seeds with lower oil content (Pageau et al. 2006). Clubroot is proving to be a serious concern for farmers as it spreads to new areas. Brassica crops are becoming increasingly important for both dietary and industrial applications, so more hectares are being grown, most of which are sown to varieties that are susceptible to clubroot (Dixon 2009a).

### 1.1.4 Importance of Clubroot in Alberta

Canola, a Brassica crop grown across the Canadian Prairies, was developed from oilseed rape through conventional breeding methods. Dr. Baldur Stephenson (University of Manitoba) and Dr. Keith Downey (Agriculture and Agri-Food Canada) worked to decrease the levels of erucic acid to below 2% and glucosinolates to below 30 μmol $g^{-1}$ to achieve this new crop, trademarked 'Canola'. The name canola stands for Canadian Oil Low Acid. Canola quality cultivars include genotypes of *B. napus*, *B. rapa*, and *B. juncea*. Currently, most canola cultivars are *B. napus*, commonly referred to as 'Argentine canola' (Rempel *et al.* 2014). Given the significant yield and quality losses that may be caused by clubroot, the disease is proving to be a serious concern for Canadian canola growers. The production of canola has been increasing in recent years, as a result of a strong demand for canola oil and good financial returns for producers. In 2009, the average yield in Canada was 1980 kg $ha^{-1}$, and the Canadian canola industry was valued at $15.4 billion between 2007-2008 and 2009-2010 (Rempel et al. 2014). The emergence of clubroot on canola threatens these gains (Rempel et al. 2014). Moreover, the intensive production of canola may exacerbate clubroot disease incidence and severity.

Despite its well documented occurrence in cruciferous vegetable crops in British Columbia and the eastern provinces of Canada since at least the early 20$^{th}$ Century, clubroot was not reported on canola anywhere on the Prairies until 2003. This changed when 12 canola crops in the Edmonton, Alberta, region were identified as having symptoms of clubroot. These represented the first cases of clubroot in Canadian canola (Strelkov *et al*. 2006). The initial foci of infection are suspected to have been home or

market gardens, where there had been occasional reports of clubroot on cruciferous vegetables as early as the 1970s (R.J. Howard and I.R. Evans, unpublished data). Clubroot poses a serious threat to the Canadian canola industry because of its potential to reduce yields. Plants infected with the clubroot pathogen exhibit reduced height on average and a slower rate of development compared to uninfected canola plants (Deora *et al.* 2012). Once soil becomes infested with *P. brassicae*, it is likely to stay that way. Clubroot resting spores exhibit extreme longevity, which contributes to the severity of this disease. The half-life of resting spores has been estimated to be 3.6 to 4.4 years, while they can survive in the soil for nearly 20 years (Wallenhammar 1996, Dixon 2009a, Hwang *et al.* 2013).

Farmers in many parts of the Prairies have limited cropping options, with canola representing one of the crops that provides the highest economic returns. This has resulted in increased cropping of canola in short rotations and over broad tracts of land, reaching 8.6 million hectares harvested in 2012 (Rempel et al. 2014). As will be discussed below, short rotations result in increased levels of *P. brassicae* inoculum in the soil. Moreover, large-scale agricultural operations require extensive use of large pieces of field equipment, such as tractors and cultivators, which can carry large amounts of *P. brassicae*-infested soil within and between fields (Hwang et al. 2014). Collectively, short rotations and movement of farm and other machinery represent a serious challenge to clubroot management (Dixon 2009a). This challenge is reflected in an increase from 12 to nearly 1,500 confirmed *P. brassicae*-infested fields in Alberta between its initial discovery in 2003 and 2013 (Strelkov et al. 2013). Indeed, *P. brassicae* and symptoms of clubroot have been detected in canola crops in Saskatchewan, Manitoba and the American state of North Dakota in recent years (Cao et al. 2009, Dokken-Bouchard et al. 2012, Chittem et al. 2014).

## 1.2 *Plasmodiophora brassicae*

### 1.2.1 Taxonomy

The order Plasmodiophorales is composed of only one family, Plasmodiophoraceae (Karling 1968). Historically, the Plasmodiophorales were composed of approximately 8 genera: *Plasmodiophora, Tetramyxa, Octomyxa, Sorosphaera, Sorodiscus, Spongospora, Ligniera and Polymyxa*. *Tetramyxa* included *T. parasitica*, *T. triglochinis*, and *T. elaeagani*. Octomyxa included *O. achlyae*, while *Sorosphaera* included *S. veronicae* and *S. radicalis*. *Sorodiscus* was composed of *S. callitrichis*, *S. radicicolus,* and *S. karlingii*. *Spongospora* contained *S. subterranea* and *S. campanulae*. *Ligniera* included *L. junci, L. pilorum, L. verrucosa, L. isoetes,* and *L. vascularum*. *Polymyxa* holds *P. graminis.* The genus *Plasmodiophora* encompasses *P. diplantherae*, *P. halophilae*, *P. fici-repentis* and *P. bicaudata*, in addition to *P. brassicae* (Karling 1968). The taxonomic classification of the family has evolved and expanded to include 35 species in 10 genera. Between 1968 and 1995, two genera, *Membranosorus* and *Woronina*, were added to the Plasmodiophoraceae (Braselton 1995).

Karling (1968) distinguished the genus *Plasmodiophora* from the other genera in Plasmodiophoraceae by what it lacked: a distinct cystosorus. Instead, the genus *Plasmodiophora* resting spores are contained loosely within the host cells as opposed to joined in a sorus. Additional characters of the plasmodiophorids include zoospores with two anterior flagella of unequal lengths, which function in a whiplash motion; multinucleated protoplasts forming plasmodia; and environmentally resistant resting spores (Braselton 1995). The plasmodiophorids also are classified by their cruciform

nuclear divisions and the associated hypertrophy of parasitized cells (Castlebury & Domier 1998). The developmental stages of the Plasmodiophorales include similarities to members of the Myxomycetes, Proteomyxa, Protozoa and some simple fungi (Braselton 1995).

According to Castlebury and Domier (1998), the plasmodiophorids are known parasites of both aquatic and terrestrial plants, Oomycetes and green and yellow algae. Some members of this group are parasites of agricultural crops. *Plasmodiophora brassicae* infects cruciferous plants and *Spongospora subterranea* (Wallr.), the causal agent of powdery scab, infects potatoes (*Solanum tuberosum* subsp. *tuberosum*) and watercress (*Nasturtium officinale* W.T. Aiton) (Castlebury & Domier 1998). *Plasmodiophora brassicae* and *S. subterranea* are the two most economically important members of Plasmodiophoraceae (Karling 1968; Braselton 1995).

More recently, based on three different sequencing methods, *P. brassicae* was placed at what is referred to as the "crown of the eukaryotic tree" by Castlebury & Domier (1998). This placement refers to *P. brassicae* currently occupying a position of divergence with animals, plants, alveolates, stramenophiles and the fungi. *Plasmodiophora brassicae* is currently located in the protist supergroup Rhizaria, within the class Phytomyxea (plasmodiophorids) (Hwang et al. 2012a). Phytomyxea is classified within the phylum Cercozoa and the Endomyxa (Neuhauser et al. 2011).

### 1.2.2 Host Range

*P.brassicae* is an obligate parasite, meaning that it cannot grow and reproduce in the absence of a living host organism (Braselton 1995). As a result, *P. brassicae* requires a

susceptible host to complete its life cycle. Hosts of the clubroot pathogen are members of the Brassicaceae family, specifically within the genera *Brassica, Raphanus,* and *Arabidopsis*. Some plant species that serve as hosts for the parasite include horseradish (*Armoracia rusticana* P.Gaertn., B.Mey. & Scherb), white mustard (*Brassica hirta* Moench), wild mustard (*Brassica kaber* (DC.) L.C.Wheeler ) and camelina or false flax (*Camelina sativa* (L.) Crantz) (Ahmed et al. 2011). Of greater economic concern, especially in areas of oilseed production, are the cultivated crop species that can be negatively impacted by *P. brassicae.* Potential hosts for this parasite of chief concern include canola and cruciferous vegetables.

Vegetable hosts include subspecies *of B. oleracea* such as Brussels sprouts (*Brassica oleracea* subsp. *gemmifera*), cabbage (*Brassica oleracea* subsp. *capitata* L.), cauliflower (*Brassica oleracea* subsp. *botrytis* L.), kale (*Brassica oleracea* subsp. *viridis* L.) and kohlrabi (*Brassica oleracea* subsp. *gongylodes* L.). Susceptible subspecies or varieties of *B. rapa* include turnip (*Brassica rapa* subsp. *rapa*) and Chinese cabbage (*Brassica rapa* subsp. *pekinensis*). The *B. napus* subspecies rutabaga or swede turnip and mustard also function as hosts of *P. brassicae* (Dixon 2009a, Howard et al. 2010, Hwang et al. 2012b). The initial reports of clubroot in Canada documented the occurrence of the disease on various vegetable crops including cabbage, cauliflower, rutabaga, broccoli, Brussels sprouts, Chinese cabbage and kale (Howard et al. 2010).

In years when clubroot resistant canola cultivars or non-host crops are grown, weed control is crucial for disease management, since many cruciferous weeds can serve as hosts for *P. brassicae*. Infected weeds can maintain inoculum levels in the soil, thereby reducing the efficacy of crop rotation or resistant host genotypes in providing a “break” away from

susceptible host plants (Dixon 2009b, Hwang et al. 2012a). Weeds such as stinkweed (*Thlaspi arvense* L.), shepherd's purse (*Capsella bursa-pastoris* (L.) Medik ), and volunteer plants also may be attacked by the pathogen and therefore, necessitate control measures (Dixon 2009a, Dixon 2009b, Hwang et al. 2012a).

### 1.2.3 Physiologic Specialization (Races or Pathotypes)

The clubroot pathogen is known to exhibit physiologic specialization, with strains of *P. brassicae* differing in their ability to infect particular host genotypes (Howard et al. 2010). A number of host differential sets have been proposed to identify and classify races or pathotypes of the pathogen. Among the most widely used differentials are those of Williams (1966), Somé et al. (1996), and the European Clubroot Differential (ECD) set (Buczacki et al. 1975). In Canada, all three sets of differentials have been used to characterize *P. brassicae* populations. Considerable efforts have been placed on characterizing pathogen populations from Alberta in particular, with the information being used to guide clubroot resistance breeding efforts in canola (Strelkov & Hwang 2014). This work has revealed that pathotype 3, as classified on the differentials of Williams (1966), is predominant in the central part of the province, where the clubroot outbreak began and is most severe. Nonetheless, other pathotypes also have been identified with lower frequency, including pathotypes 2, 5, 6 and 8 (Howard et al. 2010). In canola, pathotype 3 was found to cause more root hair infection as well as more extensive colonization of the cortex than pathotype 6 (Deora et al. 2012). In comparison with pathotype 6, plants inoculated with pathotype 3 caused reduced height and delayed plant development (Deora et al. 2012). A population of *P. brassicae* collected from field plots at the Crop

Diversification Centre North, Alberta Agriculture and Forestry, Edmonton, represents the first reported occurrence of pathotype 5 in Canada (Strelkov et al. 2006).

In Ontario, pathogen populations appear to be composed primarily of pathotype 6 (Cao et al. 2009). On the ECD set, the populations isolated from Ontario were virulent on the universal suscept (ECD 05) and the cabbage differentials ECD 13 and 14, further supporting the idea that clubroot originated from a vegetable host source (Cao *et al.* 2009). Pathotype 2 is predominant in the province of Quebec and pathotype 6 in pathogen populations from British Columbia (Williams 1966, Xue et al. 2008). An analysis of Nova Scotia pathogen populations revealed that pathotype 3 was predominant in that province (Hildebrand & Delbridge, 1995).

In general, pathotype 3 has been found to be more aggressive on canola than pathotype 6, as evaluated based on several factors including its ability to produce more severe symptoms of clubroot (Cao et al. 2009). Due to the diverse composition of pathotypes present across Canada, LeBoldus et al. (2012) suggested that there could be pathotype shifts in response to the selection pressure imposed by the planting of resistant host cultivars.

### 1.2.4 Life Cycle

The life cycle of *P. brassicae* has been studied extensively. *P. brassicae* is a soilborne pathogen. Soil becomes infested with *P. brassicae* when resting spores of the pathogen are released from decomposing host root tissue. These resting spores also can be dispersed to non-infested fields through the movement of infested soil and water (Dixon 2009b, Kageyama & Asano 2009), or perhaps even by wind-borne dust (Rennie *et al.* 2012).

*Plasmodiophora brassicae* resting spores exhibit extreme longevity, which contributes to the challenges posed in the management of clubroot. The half-life of resting spores has been estimated to be 3.6 to 4.4 years, while they can survive in the soil for nearly 20 years (Wallenhammar 1996, Dixon 2009a, Hwang et al. 2013). The cropping of host species in short rotation in *P. brassicae* infested soil results in increasing resting spore populations (Ahmed *et al.* 2011).

The primary inoculum of *P. brassicae* consists of the pathogen resting spores. Each resting spore germinates to release a primary zoospore, a biflagellate structure that encysts on and then penetrates the host root hairs. Primary plasmodia develop in the infected root hairs. Zoosporangia form after a series of nuclear divisions and cleavage, inside of which develop secondary zoospores. The secondary zoospores are released into the soil, where they then re-infect the roots by invading the cortex. The secondary zoospores are not distinguishable from the primary zoospores on a visual basis (Tommerup and Ingram 1971, Dixon 2009b, Kageyama & Asano 2009).

The secondary stage of infection takes place when cortical cells become invaded by secondary zoospores. A study by Myers and Campbell (1985) supported the idea that secondary zoospores must re-infect the host in order to cause clubbing symptoms. Secondary plasmodia then develop cells and multiply in the cortical cells, causing hormonal disturbances in the host which lead to hyperplasia and hypertrophy of the root tissues (Dixon 2009b, Kageyama & Asano 2009). Galling in roots results in a disruption of the tissue organization, causing vascular function and water transport to be impaired (Ludwig-Muller et al. 2009). Galls or clubs develop on the roots, and eventually contain millions of

resting spores, which arise from the cleaving of secondary plasmodia (Ahmed et al. 2011). The resulting galls decay at the end of the growing season, releasing the newly formed resting spores back into the soil and perpetuating the disease cycle (Dixon 2009b, Kageyama & Asano 2009). Hwang et al. (2013) estimated that as many as $1\times10^8$ resting spores can be obtained from 1 g of galled canola root tissue under field conditions.

### 1.2.5 Epidemiology

The most important method of pathogen spread is through the movement of *P. brassicae* infested soil. Agricultural machinery provides an ideal method of transport for the movement of *P. brassicae* infested soil from field to field, facilitating spread across borders and into previously uninfested regions (Dixon 2009a). On canola crops in western Canada, the highest clubroot infection frequencies were consistently found near the field entrances (Cao et al. 2009). The infection frequency was on average 0.901 at the entrance, and decreased at sampling points 150 m away (0.310 – 0.479) and 300 m away (0.155 – 0.296) from the field entrance (Cao et al. 2009). These results strongly suggest that *P. brassicae* is most commonly introduced into fields on farming equipment carrying infested soil, hence resulting in higher infection frequencies near the field entrance where the equipment first gains access to the land.

Despite the importance of machinery as a vector for the movement of *P. brassicae*, other mechanisms of dispersal also have been suggested. For example, there have been many anecdotal reports of the movement of *P. brassicae* as an external seedborne contaminant. Rennie et al. (2011) examined this possibility by measuring *P. brassicae* resting spore loads on pea, wheat and canola seeds, as well as potato tubers, harvested

from clubroot infested regions of Alberta. These workers found quantifiable levels of *P. brassicae* inoculum in some of the samples analyzed by quantitative PCR. Among the samples tested, wheat seeds were determined to have the highest level of inoculum present, at $3.43 \times 10^4$ resting spores per 10 g seed sample. One of the canola seed samples tested and one sample of potato tubers also had quantifiable levels of inoculum. The viability of the resting spores in all samples was assessed by staining and microscopic examination, which revealed that most resting spores were viable.

Nevertheless, in general, the total number of spores carried on the seeds and tubers, although in some instances sufficient to induce mild clubroot symptoms under greenhouse conditions, were very small compared with the number of spores that can be moved on soil carried by farm and other machinery. Seed cleaning was found to significantly reduce the likelihood that quantifiable levels of inoculum could be identified on the seeds (Rennie et al. 2011), suggesting that this is an effective strategy to reduce the possibility of seedborne dissemination of *P. brassicae*. Treatment of seeds with fungicides further reduced the risk of seedborne transmission of the pathogen (Hwang et al. 2012b). An additional mechanism of spread exists with the possibility of pathogen dissemination by soil erosion across infested fields. The severity of this contribution to disease spread would depend upon the amount of soil moved by wind, as well as the distance the dust is able to travel (Rennie et al. 2012, Rennie et al. 2015).

## 1.3. Methods for Pathogen Detection and Quantification

### 1.3.1 Bioassay

Due to the non-culturable nature of *P. brassicae*, detection of the pathogen in soil has been typically confirmed by bioassays. In the bioassay procedure, susceptible plant species are grown in the soil being tested. After sufficient time has elapsed to allow disease development, a visual assessment of root symptoms is conducted. For *P. brassicae*, the bioassay often takes a minimum of five to six weeks (Faggian & Strelkov 2009, Cao et al. 2007). In addition to enabling detection of the pathogen in a soil sample, bioassays also can be used to validate newer diagnostic procedures being developed (Faggian & Strelkov 2009).

The bioassay method requires dry soil samples where inoculum loads are in excess of 1000 spores per gram of soil, since this is generally the threshold minimum for clubroot symptom development under greenhouse conditions. Depending on the soil type and environmental conditions, there can be some variation in this generally accepted level (Faggian & Strelkov 2009). An estimate of spore loads in naturally infested soils can be obtained by comparing disease indices to soil inoculated with known spore concentrations (Cao et al. 2007). The bioassay method is reliable, but time-consuming and labour intensive. Non-molecular soil tests also require dedicated greenhouse space for maintaining the plants during testing (Siemens et al. 2009, Cao et al. 2007)

### 1.3.2 Root Hair Infection/Microscopy

A detection method requiring less time and space than the bioassay is microscopic examination of the host root hairs for signs of infection, and/or soil suspensions for the presence of *P. brassicae* resting spores. Microscopy based techniques rely on staining of root hairs or soil suspensions with various dyes or a single fluorochrome, respectively. The presence of plasmodia in the root hair indicates that inoculum is present in the soil. Similarly, the resting spores in a soil suspension can be visualized and counted when they fluoresce under a fluorescent microscope (Faggian & Strelkov 2009). Microscopy assays have been updated over time and now allow for improved spore detection and determination of spore viability (Faggian & Strelkov 2009, Rennie et al. 2011). Some methods have been refined to make use of two fluorochromes, enabling differential staining of viable versus nonviable resting spores. Like other detection methods, microscopy based techniques have can have challenges as well, including the requirement for trained personnel that are capable of recognizing plasmodia in the root hairs or fluorescing resting spores within a suspension of soil particles (Faggian & Strelkov 2009).

### 1.3.3 Polymerase Chain Reaction

The polymerase chain reaction (PCR) was developed to amplify a specific segment of deoxynucleic acid (DNA) using a DNA polymerase enzyme, nucleotides and primers in a buffer (Mullis & Faloona 1987). Customarily, PCR-based techniques have been used to detect fungal pathogens in plant tissue and soil samples. PCR analysis has been relied upon for obtaining sensitive, rapid results which are reliable (Cao et al. 2007). PCR techniques require only DNA, making the technique optimally suited to obligate parasites such as

*P.brassicae* (Faggian & Strelkov 2009). The PCR-based diagnostic assays that have evolved can now detect the presence of *P.brassicae* only 3 days after inoculation, whereas symptoms of the disease are not visible to the naked eye until approximately 21 days after inoculation. Another benefit of PCR is that it is now a routine technique in most laboratories, even those with minimal molecular capabilities, reducing the need for personnel with highly specific skills related to *P. brassicae* (Cao et al. 2007). More recently, quantitative PCR-based protocols have been developed that allow not only detection of *P. brassicae* in soil and tissue samples, but also enable measurement of the concentration of resting spores or amount of pathogen biomass in those samples (Wallenhammer et al. 2001, Rennie et al. 2011, Cao et al. 2014).

### 1.4. Clubroot Management

#### 1.4.1 Genetic Resistance

Researchers are attempting to broaden the spectrum of management tools available for clubroot, as the disease spreads and infections intensify. Currently, one of the most promising management recommendations is planting clubroot-resistant canola varieties on fields free of the disease (Hwang *et al.* 2012a). Unfortunately, however, single gene resistance has broken down quickly in winter canola as well as in other crops (Kuginuki et al. 1999). Because clubroot is a genetically diverse pathogen, relying on single gene resistance on a crop grown over a large number of hectares imposes a strong selection pressure for pathotypes able to overcome that resistance (Ahmed et al. 2011). This makes the breakdown of resistance more likely. Indeed, under greenhouse conditions, resistance

to *P. brassicae* was quickly eroded after repeated exposure of the same host genotypes to the same pathogen isolates and populations (LeBoldus et al. 2012).

The Canadian market has seen the release of a number of commercial canola cultivars with clubroot resistance, beginning in 2009 (Peng et al. 2014). These cultivars represent an important clubroot management tool for canola producers, especially in regions severely impacted by clubroot. The resistant cultivars that are currently available exhibit resistance to the predominant pathotypes of *P. brassicae* found in Canada (Strelkov & Hwang 2014). Genes imparting resistance to different pathotypes are also important based on the differing effects of pathotypes and their geographic distribution (Deora et al. 2012).

Sources of resistance against the predominant pathotypes of *P.brassicae* have been identified from the primary and secondary genetic pools of canola (*B. napus*). Sources of genetic resistance for canola cultivars include a dominant gene from the winter canola 'Mendel', which provides effective resistance against pathotype 3 of *P.brassicae*. Other sources of genetic resistance have been accessed from rutabaga and pak choi (Rahman et al. 2014).

To prolong the usefulness of resistant cultivars as a management tool, it is important that growers utilize an integrated approach including crop rotation as well as other available management tools (Strelkov & Hwang 2014).

### 1.4.2 Seeding Date Manipulation

Another clubroot management tool is the manipulation of seeding date for host crops. In a study by Gossen et al. (2012), Shanghai pak choy and Chinese flowering cabbage sown in July exhibited the highest levels of clubroot disease, while disease severity was lower when the crops were sown in June, and very low when sown in May, August or September. Clubroot development was slowed when temperatures were below optimum for disease development, generally between 20°C and 26°C. The mean ambient and soil temperatures for the May and September planting dates were below 17°C (Gossen et al. 2012). The decrease in disease severity is believed to be associated with lower temperatures at planting.

In a study with canola, the emergence of both susceptible and resistant cultivars was observed to improve with mid and late seeding compared with early seeding (Hwang et al. 2012b). A significant effect of seeding date on seedling emergence and canola yield was observed at both study sites. The canola yield was significantly higher in the early seeded plots. Interestingly, however, there was no significant effect of seeding date on symptom severity on the roots (Hwang et al. 2012b). As seeding date became later, both susceptible and resistant cultivars incurred a plant height decrease. Nonetheless, these results suggest that an earlier planting date has potential as a tool for clubroot management in canola. Planting canola slightly earlier in the season if at all possible could provide plants with an escape from disease pressure, allowing the crop to reap both height and yield benefits (Hwang et al. 2012b).

### 1.4.3 Bait Crops and Biological Control

In the absence of host plants, zoospores that emerge from the resting spores of *P. brassicae* survive only for short periods of time. The proximity of particular plant genotypes stimulates resting spore germination, and has been linked to the release of root exudates into the soil (Ahmed et al. 2011, Friberg et al. 2005). Studies have shown that resting spore germination is enhanced in the presence of root exudates from perennial ryegrass (*Lolium perenne* L.), leeks (*Allium ampeloprasum* L., nom. cons.), rye (*Secale cereale* L.) and red clover (*Trifolium pratense* L.) (Friberg et al. 2005, Friberg et al. 2006). This impact of root exudates on *P. brassicae* spore germination rates led to the suggestion of using bait crops as a clubroot management tool. It has been hypothesized that bait crops could be used to stimulate resting spore germination, thereby depleting spore loads in the soil. If a susceptible bait crop, such as canola or Chinese cabbage is grown, it could be ploughed under before the pathogen is able to complete its life cycle. If a non-host crop is grown, then resting spore germination would not be followed by successful infection of the roots and additional production of spores, and hence spore loads also would be depleted (Friberg et al. 2006). Perennial ryegrass, leek and winter rye all stimulated resting spore germination in the experiment by Friberg et al. (2006), whereas red clover did not. Nevertheless, despite its promise from a theoretical perspective, there have been conflicting results as to the effectiveness of bait crops as a practical clubroot management strategy (Ahmed et al. 2011, Friberg et al. 2006).

In a study by Ahmed et al. (2011), canola, Chinese cabbage, bentgrass, orchardgrass (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.), red clover (*Trifolium*

*pratense* L.), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) were assessed as bait crops to control clubroot in canola. Each crop was grown in a greenhouse study for 2 weeks at a time and uprooted at that point, twice consecutively, followed by canola planted in the same soil.

Under greenhouse conditions, taller canola resulted following the cropping of cruciferous bait crops as compared with non-cruciferous bait crops. Clubroot incidence and severity also were lower following cruciferous bait crops compared with being grown after other crops (Ahmed et al. 2011). Under field conditions, the population of viable *P. brassicae* resting spores was reduced when two cycles of a susceptible canola cultivar were grown for 6 weeks and then killed by herbicide treatment. Resting spore concentrations were slightly lower in bait treatments than in the control, but this was not a consistent finding (Ahmed et al. 2011). Although it is beneficial to have as many control options available as possible when managing a challenging disease such as clubroot, bait cropping practices may possess limited field potential due to the large hectarage of canola in Alberta and Canada as a whole. Friberg et al. (2006) reported that the application of bait crops as a clubroot management tool in commercial fields is limited by the small impact on inoculum potential and disease severity. Bait crops potentially are a more practical strategy in areas where inoculum levels are moderate to low (Friberg et al. 2006). It is also important to consider the Canadian environment and cropping practices, which are distinct from those in other regions such as northern Europe or Japan. The Canadian environment provides a limited growing season length relative to other regions where bait crop studies have been conducted. Moreover, on the Prairies, canola fields are generally very large (~65 ha),

posing an additional constraint to the use of treatments that may be costly to apply on a per hectare basis (Ahmed *et al.* 2011).

### 1.4.4 Fungicides

Fungicides have been tested for the control of *P. brassicae* for several decades. A study by Buczacki and Cadd (1976) evaluated 71 fungicides under greenhouse conditions for their impact on *P. brassicae* infection and clubroot severity in the cabbage 'Golden Acre'. In more recent research, a number of fungicides have been reported to be effective against *P. brassicae*. A drench application of the cyazofamid at seeding resulted in reduced clubroot levels at May, June and July planting dates (Gossen et al. 2012). However, the same drench fungicide application had no impact when clubroot levels were low (Gossen et al. 2012).

In a greenhouse study conducted by Hwang et al. (2012b), the efficacy of the fungicides Dynasty 100 FS (azoxystrobin), Nebijin 5SC (flusulfamide) and Helix Xtra (thiamethoxam+difenoconazole+metalaxyl+fludioxonil) was assessed against seed-borne inoculum of *P. brassicae*. Seeds of canola were artificially infested with high levels of pathogen resting spores, treated with the various fungicides and then sown, with an index of disease (ID) severity subsequently measured. Relative to the control that received no fungicide treatment, Dynasty 100 FS and Nebijin 5SC caused the greatest reductions in the index of disease (at 13.2% and 16.4%, respectively, vs. 68.1% for the control). While Helix Xtra also significantly reduced the index of disease compared with the control, this reduction was not as large (ID = 53.9%). Nebijin, which unlike the other products is not

registered as a seed treatment for canola, nonetheless also significantly reduced clubroot severity (Hwang et al. 2012b).

Fungicidal soil treatments also exist for the control of clubroot in canola. In a study by Hwang et al. (2011), the efficacy of 10 fungicide treatments was assessed after being incorporated by rototiller into the soil up to a depth of approximately 8 cm. Terraclor (quintozene) increased seed yield and reduced clubroot severity compared with the control treatment. An increased rate of Ranman (cyazofamid) also reduced clubroot severity. It is important to keep in mind, however, that fungicides and soil amendments used in horticulture have the potential to be cost-prohibitive for use in canola, considering the differences in application rates, the profit margins on each crop, and the average size of the fields these crops are grown on (Ahmed et al. 2011).

### 1.4.5 Soil Amendments

Soil amendments are materials or compounds added to soil to alter its properties, rather than a fungicide that targets the organism itself (Colorado State University Extension, 2015). In the case of clubroot control, soil additives are sought to make the soil environment less favourable for the pathogen and disease development.

Liming has been a historic measure for the control of clubroot in cruciferous crops (Walker & Larson 1935). In a field study by Hwang et al. (2011), three rates each of limestone and wood ash and two rates of calcium cyanamide were applied as soil amendments for the amelioration of clubroot in canola. The compounds were applied to the soil surface and incorporated, prior to seeding. It was found that limestone applied at 7.5 t $ha^{-1}$ reduced clubroot severity at both experimental sites in both years of the

experiment. Wood ash applied at the same rate also decreased disease severity at both sites in one year. Seed yield also increased with the application of 7.5 t $ha^{-1}$ of limestone or wood ash at both sites in at least one of the years. However, calcium cyanamide did not have an effect on any of the response variables measured in the study (Hwang et al. 2011). Calcium cyanamide applied to wet soil results in a hydrolysis reaction whereby $Ca(OH)_2$ and urea are produced. In uninfested soil, urea is ammonified, resulting in $Ca(OH)_2$ and $(NH_4)_2CO_3$ production and a subsequent increase in alkalinity or decreases in acidity (Walker & Lawson 1935).

Murakami et al. (2002) reported that the impact of lime on clubroot disease severity in infested soils depends on both the *P. brassicae* resting spore density and the dose-response relationship. They examined the impact of liming on disease severity as well as the density of resting spores in soil, using calcium cyanamide, dolomite ($CaMg(CO_3)_2$), and calcium carbonate. Amendment of plots with lime resulted in lower indices of disease than control plots without lime. Resting spore density rates also were impacted by the application of lime to soil. Calcium cyanamide application resulted in a 17%-31% reduction in resting spore density, compared with a reduction of 12%-29% for dolomite, and 20%-39% for calcium carbonate, all in comparison with the control without lime (Murakami et al. 2002). A protective value (PV) was calculated by Murakami et al. (2001) based on the disease index (DI). Reductions in DI by liming were attributed to the change in the density of resting spores in the soil, as the PV decreased when the inoculum concentration increased (Murakami et al. 2002).

The application of magnesium has been shown to inhibit all stages of clubroot development from infection to clubbing (Karling 1968, Myers & Campbell 1985). Calcium and magnesium in the lime itself are believed to have an effect on the disease development of *P.brassicae* (Hamilton et al. 1978, Fletcher et al. 1982). In an experiment by Myers & Campbell (1985), exchangeable calcium and magnesium levels in the soil were positively correlated with reduced clubroot severity when soils were limed. As levels of calcium and magnesium increased, plant infection and root galling were inhibited at certain concentrations, which also were affected by pH (Myers & Campbell 1985). Lime needs to be applied at rates of several tonnes per hectare to achieve a soil pH that results in reduced clubroot severity. Although effective, it can be impractical to source these quantities of lime, as well as apply and incorporate them across hundreds of hectares and multiple fields (Myers & Campbell 1985, Murakami et al. 2002).

### 1.4.6 Fumigants

#### 1.4.6.1 Chemical Fumigation

Soil fumigants have traditionally been used to control soilborne pests and pathogens in high-value crops such as vegetables (Papiernik et al. 2004). These compounds have several common characteristics that make them particularly effective, including relatively high vapour pressures, low boiling points, and high air-water partitioning coefficients (Papiernik et al. 2004). Numerous fumigants exist including a range of which have been assessed for clubroot control, such as basamid, 1,3-dichloropropene, chloropicrin, methyl bromide, propargyl bromide, and metam sodium (White & Buczacki 1977, Dungan &Yates 2003, Papiernik *et al.* 2004). The soil fumigant

Vapam (metam sodium) has low adsorption to soil particles and a comparatively slow diffusion rate within the soil. It also possesses a high rate of decomposition at high soil temperatures, and a relatively greater partition into water from air relative to some other fumigants (Smelt & Leistra 1974). These factors make Vapam a good candidate to assess as a soil fumigant for clubroot control.

Vapam is converted into an array of degradation products in the soil, including methyl isothiocyanate (MITC), carbon disulfide, carbonyl sulfide and hydrogen sulfide (Smelt & Leistra 1974, Saeed et al. 2000, Triky-Dotan et al. 2010). MITC is water soluble and toxic, with a relatively high vapour pressure (Saeed et al. 2000). It is the compound thought to have toxic effects on soilborne pests such as fungi, nematodes, weeds, and some soil arthropods (Smelt & Leistra 1974, Triky-Dotan 2010).

A study by Buczacki and White (1977) focused on testing soil partial sterilants for clubroot control in a glasshouse setting. Sterilants, including Vapam, were applied at 0.25, 0.50, 1.0, 2.0 and 4.0 times the basic dose (1.96L of 6.27% Vapam solution/$m^2$). Cabbage was grown in the sterilized soil as a susceptible host. The study found that all partial sterilants gave complete control of clubroot when tested at their basic rates (Buczacki & White 1977).

#### 1.4.6.2 Biological Fumigation

Biofumigation is defined as a method of controlling weeds and soil pests with biocidal compounds produced by other organisms. Of particular interest have been the glucosinolates, which are secondary metabolites produced by plants belonging to the order Brassicales (Kirkegaard & Sarwar 1998, Gimsing & Kirkegaard 2009). There are three

groups of glucosinolates, including the aromatic, aliphatic and indolyl glucosinolates (Mithen 2001). Plants that produce glucosinolates also produce the enzyme myrosinase, which is kept physically separated from the glucosinolates within the plant tissue (Mithen 2001, Szczyglowska et al. 2011). Although the glucosinolates and myrosinases are segregated in healthy cells, cell breakage causes them to mix and produce hydrolysis products, including isothiocyanates. Isothiocyanates are compounds that cause an irreversible chemical reaction with sulphur-containing groups in plant proteins. This toxic reaction is thought to be non-specific in its nature, but different chemical side-chains on isothiocyanates result in varying chemical and physical properties (Gimsing & Kirkegaard 2009). In biofumigation, it is desirable for the hydrolysis reaction to produce as much of the desired end-product, isothiocyanates, as possible. This can be more readily achieved by increasing cell breakage, adding more water to the reaction, and having a higher soil temperature (Gimsing & Kirkegaard 2009).

When selecting plants to use as biofumigants, the amount of glucosinolates contained within the plant must be considered. Plants belonging to the Brassicaceae family vary in the amount of glucosinolates they contain. Therefore, different species and varieties also possess their own biofumigant potential. The chemicals released during hydrolysis also may serve as sources of carbon, nitrogen, or sulfur for the plants being grown (Szczglowska *et al.* 2011).

Larkin & Griffin (2007) conducted an experiment to evaluate the control of potato pathogens with canola, rapeseed, radish, turnip, yellow mustard, and Indian mustard. Canola and Indian mustard reduced powdery scab of potato by 15-40%. Canola and rapeseed decreased black scurf (*Rhizoctonia solani*) on potato by 70-80%. Indian mustard

reduced a variety of diseases by 80%-100%, including those caused by *R. solani* J.G. Kuhn, *Phytophthora erythroseptica* Pethybr. (1913), *Pythium ultimum* Trow (1901), *Sclerotinia sclerotiorum* (Lib.) de Bary 1884 and *Fusarium sambucinam* Fuckel (Larkin & Griffin 2007). In another experiment with cucumber (*Cucumis sativus* L.) in which horseradish was used as a treatment, a 100% inhibition of *R. solani* growth was observed (Larkin & Griffin 2007).

Friberg et al. (2009) examined the control of *R. solani* on carrot seedlings and *Fusarium oxysporum* Schltdl. 1824 on flax (*Linum usitatissimum* L. 'Astral') with by the incorporation of mustard residues in the soil. They found variable inhibition of *R. solani* and no definitive effect of mustard on *F. oxysporum*, but noted that both of these fungi can survive saprophytically in the soil. Thus, Friberg et al. (2009) hypothesized that biofumigation may have a greater disease-inhibiting potential when used to control obligate parasites. Based on this, it is likely that *P. brassicae*, as an obligate parasite, may be a good candidate to be controlled by biofumigation.

One of the benefits of biofumigation over chemical fumigation is a reduced environmental impact, since isothiocyanates do not persist long in the soil after they are absorbed and degraded. This approach can be thought of as a 'natural' alternative to products such as methyl bromide (Gimsing & Kirkegaard 2009). Methyl bromide was phased out starting in 2005, since it is a class 1 ozone depleting substance, as classified under the Montreal Protocol (EPA 2008). Additional fumigant options, including biofumigants are being sought after to replace methyl bromide as an effective management tool, especially in high-value horticulture crops (Ristaino & Thomas 1997).

It is not clear, however, whether biofumigation is a feasible disease management approach in large scale agricultural systems. It is important to recall that the control of

disease by biofumigation or green manure is often inconsistent or incomplete. In addition, large amounts of plant tissue are often required to provide sufficient amounts of isothiocyanates to provide satisfactory disease control (Lu et al. 2010).

## 1.5. Research Objectives and Hypotheses

### 1.5.1 Chemical Fumigation

The combination of increasing canola hectares combined with the continued spread of *P. brassicae* on the Canadian Prairies creates a need for effective and additional options for the management of clubroot. In an effort to prevent the establishment of the disease in regions currently free of the disease, or where it has been recently introduced, a control measure targeting *P. brassicae*-infested patches of a field could prove effective. Soil fumigation with a chemical such as Vapam could be utilized to target localized foci of infestation, before the pathogen becomes widespread in a field. Therefore, a primary objective of my M.Sc. program was to assess the efficacy of various Vapam concentrations in reducing or possibly eradicating localized infestations of *P. brassicae* in the soil. I hypothesize that soil fumigation with Vapam will result in a significant reduction in clubroot disease severity and gall weight, while increasing plant height, biomass and yield.

### 1.5.2 Biofumigation

Given the toxicity of Vapam and other chemical fumigants, a secondary objective of my M.Sc. program was to evaluate the efficacy of a commercial biofumigant as a treatment to reduce *P. brassicae* inoculum loads. I hypothesize that biofumigation will result in a significant reduction in clubroot severity relative to plants grown in non-treated growth medium.

# Chapter 2

## 2.0 Fumigation of Soil with Metam Sodium as a Clubroot (*Plasmodiophora brassicae*) Management Strategy in Canola

### 2.1 Introduction

*Plasmodiophora brassicae* Woronin is the causal agent of clubroot, a soilborne disease of the family Brassicaceae. As an obligate parasite, *P. brassicae* requires a living host for growth and completion of its life cycle. Potential hosts include cultivated crop species such as canola (*Brassica rapa* L. and *Brassica napus* L.), mustard (*Brassica hirta* Moench, *Brassica kaber* (DC.) L. C. Wheeler) and cruciferous vegetables, as well as weeds including stinkweed (*Thlaspi arvense* L.) and shepherd's purse (*Capsella bursa*-pastoris (L.) Medik.) (Dixon 2009a, Dixon 2009b, Hwang et al. 2012).

The pathogen spreads mainly through the movement of *P. brassicae*-infested soil and water (Dixon 2009b, Kageyama and Asano 2009), although significant levels of inoculum also have been identified in wind-borne dust (Rennie et al. 2015). In addition, quantifiable levels of resting spores have been found to occur as external contaminants of crop seeds and tubers, but spread as a seedborne contaminant may be effectively mitigated by seed cleaning and seed treatments (Rennie et al. 2011; Hwang et al. 2012). Resting spores of *P. brassicae* are extremely robust, prolonging pathogen survival in the soil. The half-life of the resting spores has been estimated to be 3.6 to 4.4 years, and they can survive in the soil for nearly 20 years (Wallenhammar 1996, Dixon 2009a, Hwang et al. 2013). The longevity of the resting spores can make clubroot management difficult. Host plants

infected with *P. brassicae* exhibit external symptoms, including poor above-ground plant growth, premature and patchy stand ripening, and the characteristic galled roots. Plants often display yellowed leaves, wilting, and may even succumb entirely to the disease. There can be severe yield and quality losses associated with *P. brassicae* infection, and the value of infested land may be depressed (Dixon 2009a). In canola, infected plants produce fewer seeds with lower oil content and quality (Pageau et al. 2006). Clubroot is proving to be a serious concern for farmers as it spreads to new areas. Brassica crops are becoming increasingly important for both the food market and industrial applications. As a result, more Brassicas are being grown, with many representing varieties that are susceptible to *P. brassicae* infection (Dixon 2009a).

In the Canadian canola crop, clubroot was not reported until 2003, when a dozen infested fields were identified in central Alberta. Previous reports of clubroot in Alberta were restricted to home and market gardens and this marked the first case of clubroot in canola on the Canadian Prairies (Strelkov et al. 2006). The intensive production of canola is associated with the use of large pieces of field equipment, which can act as vectors for the movement of infested soil. Machinery can therefore help spread *P. brassicae* from field to field, facilitating its dissemination across borders and into previously uninfested regions (Dixon 2009a). An increase in the area and intensity of canola cultivation, combined with the spread of the pathogen, has resulted in a sharp rise in the number of fields confirmed to be infested with *P. brassicae* over the past decade (Strelkov & Hwang 2014). While the first infestations were identified in central Alberta, where the outbreak remains most severe, isolated cases of clubroot have been reported with increasing frequency in other regions, including Saskatchewan, and Manitoba (Cao et al. 2009; Dokken-Bouchard et al. 2012;

Strelkov et al. 2012; Strelkov et al. 2014). Most recently, the first case of clubroot on canola in the United States was identified in North Dakota (Chittem et al. 2014).

Numerous management strategies have been recommended for clubroot. In the canola production systems of the Canadian Prairies, however, most farmers have relied on the cropping of clubroot-resistant canola cultivars (Rahman et al. 2014; Strelkov & Hwang, 2014). Genetic resistance, while often highly effective, does not eliminate soilborne *P. brassicae* inoculum. Moreover, repeated cropping of resistant varieties can cause shifts in the virulence of pathogen populations, which can result in a loss or erosion of resistance (LeBoldus et al. 2012). Strategies aimed at reducing the movement of *P. brassicae* inoculum, such as sanitization of field equipment, may help slow spread of the pathogen. Most farmers, however, do not regularly clean or sanitize equipment, citing costs, time and logistical concerns (Hwang et al. 2014). As such, the number of infested fields continues to increase.

Fungicides have been utilized in multiple ways to manage clubroot, and have been evaluated in canola as soil drench soil applications and as treatments against seed borne inoculum (Hwang et al. 2012; Gossen et al. 2012). Hwang et al. (2011) assessed 10 soil fungicides for their efficacy in controlling clubroot of canola, and found that both Ranman (cyazofamid) and Terraclor (quintozene) significantly reduced the severity of the disease. Various soil amendments, including lime, wood ash and calcium cyanimide, also have been evaluated for the management of clubroot (Murakami et al. 2002; Hwang et al. 2011) and, in some cases, reduced disease severity. Nevertheless, despite varying levels of efficacy with respect to disease control, neither fungicides nor soil amendments have been relied

upon as primary clubroot management tools in canola. While fungicides have been used with some success in higher-value crops, such as cruciferous vegetables (Donald & Porter 2014), the lower economic returns associated with canola, combined with the much larger scale in which this crop is typically grown, have made chemical control cost-prohibitive in most cases.

Soil fumigants and fungicides are both classified as pesticides, but fumigants differ from fungicides in that they produce vapours that are toxic to organisms in the soil. Fumigants are often more general in their target range than fungicides. Fumigation of the soil has been used as a strategy for the management of soilborne pests and pathogens in many high-value crops (Papiernik et al. 2004). Soil fumigants have several common characteristics that make them particularly effective, including relatively high vapour pressures, low boiling points, and high air-water partitioning coefficients (Papiernik et al. 2004). The soil fumigant Vapam (metam sodium; sodium N–methyldithiocarbamate) has a low adsorption to soil and a comparatively slow diffusion within soil. It also possesses a high rate of decomposition at high soil temperatures, and a relatively greater partition into water from air relative to some other fumigants (Smelt & Leistra 1974). These characteristics suggest that Vapam may be a good candidate for clubroot management in Canadian canola fields. Vapam degrades in the soil, yielding methyl isothiocyanate (MITC), carbon disulfide, carbonyl sulfide and hydrogen sulfide (Smelt & Leistra 1974, Saeed et al. 2000, Triky-Dotan et al. 2010). MITC is water soluble and toxic, with a relatively high vapour pressure (Saeed et al. 2000), and is the active ingredient postulated to have toxic effects on soilborne target organisms such as fungi, nematodes, weeds, and some soil arthropods (Smelt & Leistra 1974, Triky-Dotan 2010). Nonetheless, it is important to keep

in mind that fumigants are non-specific in their activity, so other organisms may be harmed unintentionally (Smelt & Leistra 1974).

The distribution of clubroot within infested fields is typically patchy, with foci of infection most often found around farm and field entrances (Cao et al. 2009; Strelkov & Hwang 2014; G.R. Dixon, cited in Strelkov & Hwang 2014). This has been postulated to reflect the introduction of *P. brassicae* to new fields on farm and other machinery. These localized infestations provide an opportunity to manage clubroot before *P. brassicae* spreads more widely within fields or to additional fields and new areas. Treatments that might not be practical or economical over an entire field, for example soil fumigation, may be feasible when carried out over a much smaller area, such as a small patch of infested soil. In this context, the objective of this study was to evaluate Vapam as a tool to eradicate or contain localized *P. brassicae* field infestations before they become widespread. Both the in-season and residual effects of Vapam on clubroot severity and associated plant growth traits were assessed.

## 2.2 Materials and Methods

### 2.2.1 Vapam Soil Fumigation

Trials to evaluate the impact of different concentrations of Vapam on clubroot severity and various plant growth parameters were established in 2012 and 2013 at two field locations in Edmonton, AB, which are naturally infested with *P. brassicae* (Henwood site: 53 38’ 48”N, 113 22’ 33”W; 50$^{th}$ Street site: 53 38’ 39”N, 113 24’ 41”W). The placement of the research plots within each field location was moved in the second year of the study, so that the plots were not placed exactly in the same spot as the previous year.

The soil at the Henwood and 50th Street sites is a black chernozemic loam, with pH 5.0 and 4.8 and organic content of 10% and 8%, respectively. Each location was prepared by cultivating the plot areas and measuring out the plot squares before treatment. All trials received their moisture strictly from rainfall. The experiments were arranged in a randomized complete block design with four replications. Each mini-plot was 1.4 m × 1.4 m, with a 1 m x 1 m treatment area in the center, 0.6 m spacing between plots, and a 0.6 m buffer between replications.

Vapam HL (42% sodium methyldithiocarbamate, AMVAC Chemical Corporation) was applied to the soil water as per the "watering can method" on the product label, typically used for small areas such as gardens, and which was practical for the small size of the plots. The recommended label rate for the watering can method of Vapam application is 74 mL $m^{-2}$ (31.1 ml active ingredient (AI)). Treatments of 10% (3.1 mL $m^{-2}$ AI), 25% (7.8 mL $m^{-2}$ AI), 50% (15.5 mL $m^{-2}$ AI), 100% (31.1 mL $m^{-2}$ AI) and 200% (62.2 mL $m^{-2}$ AI) of the recommended label rate of Vapam were selected. The treatments were applied in a plastic watering can as evenly as possible, with a sweeping side to side motion, in order to achieve uniform coverage of the soil in each mini-plot. Control plots were treated in the same manner, except that water without Vapam was applied.

After the soil was treated with the appropriate concentration of Vapam, each plot was covered with a black plastic tarp (approximately 1.2 m × 1.2 m), the edges of which were trenched approximately 10 cm deep into the soil to secure the covering and prevent volatilization. The tarps remained on the mini-plots for 48 to 72 hours and were then removed. After a minimum of two days without the tarps, the mini-plots were seeded with

the clubroot susceptible canola '73-15RR' (Dekalb, Monsanto Canada Inc., Winnipeg. MB, Canada). Mini-plots were hand-seeded, with four rows of 20 seeds at a depth of 2 cm, with the seeds spaced about 5 cm apart, representing a seeding rate of 80 seeds $m^{-2}$ plot. Row spacing was approximately 25 to 30 cm. The mini-plots at the Henwood site were fumigated on June 21st (2012) or May 16th (2013), and seeded on June 28th (2012) or May 28th (2013). At the 50th Street site, the mini-plots were fumigated on July 16th (2012) or May 17th (2013), and seeded on July 23rd (2012) or May 29th (2013).

Seedling emergence was recorded weekly for 3 weeks after the first seedlings emerged from the soil. The plants were grown for approximately 8 weeks after emergence, when they were dug out from the soil, and the roots were washed with water and rated for clubroot symptom development 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 . The harvest dates were selected to ensure clubroot gall development, but to avoid maturity to the point of gall decomposition in the soil. The mini-plots at the Henwood site were harvested on August 27th (2012) or August 20th (2013). The mini-plots at the 50th Street site were harvested on October 1st (2012) or August 20th (2013).

All plants within each mini-plot were assessed for clubroot severity and individual disease ratings were used to calculate an index of disease (ID) according to the formula of Horiuchi and Hori (1980) as modified by Strelkov et al. (2006):

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

Where: *n* is the number of plants in a class; *N* is the total number of plants in an experimental unit; and 0, 1, 2 and 3 are the symptom severity classes. Measurements also

were taken on fresh and dry gall weights, fresh and dry stem weight, plant height and pod count per plant.

### 2.2.2. Vapam Residual Effects in the Soil

A second study was conducted to assess if there were any residual effects from the application of Vapam HL on clubroot severity and plant growth traits in the first year following treatment (2013).

The mini-plots established at the Henwood and 50$^{th}$ Street sites in 2012 were maintained until the 2013 growing season, when they were tilled in preparation for planting of a new crop, without mixing the soil between plots. No additional Vapam was applied, and the mini-plots were seeded with the clubroot susceptible canola '73-15RR' at a density of 80 seeds m$^{-2}$ plot on May 9, 2013 at both sites. The plants were allowed to grow for approximately 8 weeks after emergence, when they were dug from the soil, washed with water, and rated for clubroot symptom development. Ten plants from each mini-plot were harvested on July 9 and 10, 2013, at the Henwood and 50$^{th}$ Street sites, respectively. Clubroot severity was assessed on the 0 to 3 scale described above (Kuginuki et al. 1999). Other measurements taken for each of the plants harvested included above ground plant height, fresh above ground plant weight, fresh below ground (gall) weight, dry above ground plant weight, and dry below ground (gall) weight. The remaining plants were left to mature past the point of gall symptom development to reach full maturity. Ten plants (if survival allowed) were harvested during the second week of July, without assessing disease symptoms. The seed was dried, cleaned and weighed for each treatment by replicate.

## 2.3 Statistical Analysis

Statistical analysis was performed with SAS Release 9.4, SAS Institute Inc., Cary, N.C. A mixed model analysis of variance was used to analyze the treatment effects on plant height, fresh above ground biomass per plant, number of pods per plant, fresh gall weight per plant, and dry gall weight per plant. A logarithmic transformation was applied to the fresh above ground plant weight, fresh gall weight, pod count, and dry gall weight data to correct for potential deviations from normality in both 2012 and 2013. A logarithmic transformation also was applied to the stem height data in 2012. Non-transformed means are presented for consistency, as normality was tested on residuals produced from the data.

The CATMOD procedure was used to analyze treatment effects for the disease severity data, as it performs modeling of categorical data. Clubroot severity is rated on the 0-3 scale and the CATMOD procedure allows severity data to be analyzed without transformation. For all analyses, differences were considered to be significant at $P<0.05$, unless otherwise stated. The majority of growth traits in the study on the residual effects of Vapam showed a significant interaction between site and treatment, meaning the treatment may have affected the plants differently, depending on the site growth environment. As a result, the data from the two trials are presented separately.

## 2.4 Results

### 2.4.1 Vapam Soil Fumigation

#### 2.4.1.1 *Disease Severity*

At the Henwood site in 2012, all treatments reduced clubroot disease severity when compared with the control treatment, which had an average index of disease of 89%. The resulting indices of disease severity in treated plots ranged from 39% to 80%. The 31.1 mL $m^{-2}$ rate resulted in a 51% reduction in disease severity, the greatest of the rates assessed (Table 2-1). However, there was not a significant difference in indices of disease as the rates increased from 7.8 mL $m^{-2}$ to 62.2 mL $m^{-2}$. Application of Vapam at rates of 7.8 mL $m^{-2}$ and above reduced the index of disease by 28% to 51%. At the 50$^{th}$ Street site in 2012, the control treatment had a lower average index of disease than at Henwood (56%). Indices of disease in the treated plots ranged from 23% to 42%, with the 62.2 mL $m^{-2}$ rate of Vapam giving the greatest reduction in index of disease relative to the control. It should be noted that despite the numerical decreases in index of disease, there was not a significant difference between treatments as the application rate increased from 7.8 mL $m^{-2}$ to 62.2 mL $m^{-2}$ (Table 2-1).

At the Henwood field site in 2013, all rates of Vapam resulted in a decrease in index of disease relative to the control treatment, which had an average index of disease of 95% (Table 2-2). Plots treated with rates of Vapam of 7.8ml $m^{-2}$ or above reduced index of disease by 27% to 33%. The 50$^{th}$ Street site experienced early and mid-season flooding in 2013, so the data were dropped from subsequent analyses.

#### 2.4.1.2 *Plant Growth Characters*

Soil treatment with Vapam in 2012 at the 7.8 mL $m^{-2}$ rate and above significantly increased plant height, pod numbers per plant and a decrease in fresh gall weight per plant at the Henwood site. The application of the label rate of Vapam, 31.1 mL $m^{-2}$, resulted in a 53% increase in above ground plant biomass and a 42% decrease in fresh clubroot gall weight. At the 50$^{th}$ Street site, there were no significant differences in plant height and fresh or dry gall weights in 2012. The significant differences in above ground plant biomass and pod numbers per plant were fewer than at Henwood (Table 2-3).

At the Henwood site in 2013, significant differences were observed in plant height, plant biomass and pod numbers between the control and plots treated with Vapam at 25% of the label rate (7.8mL $m^{-2}$) or greater (Table 2-4). There was not a statistically significant difference between the control and the 10% label rate (3.1 mL $m^{-2}$) treatment for plant height or the number of pods produced. Although not all differences between treatments were statistically significant, increases in plant height ranged from 14% to 24%, increases in plant biomass ranged from 63% to 150%, and increases in pod counts ranged from 62% to 105% relative to the control. The label rate of fumigant resulted in plants with an average stem height of 95.5 cm, compared with the control plot plants averaging 82.5 cm ($p=0.0197$). The average fresh biomass resulting from the label rate treatment was, on average, 52.7 g heavier than control plants ($p=0.0002$), whereas pod numbers for the label rate plots averaged approximately 114 pods, compared with only 62 pods ($p=0.0354$) per plant in plots where no Vapam was applied. There were no significant differences in fresh or dry gall weight.

### 2.4.2 Vapam Residual Effects in the Soil

#### 2.4.2.1 *Disease Severity*

At the Henwood site, index of disease values were significantly lower for clubroot susceptible canola plants grown in soil that had received Vapam at rates of 15.5 mL $m^{-2}$, 31.1 mL $m^{-2}$ and 62.2 mL $m^{-2}$ in the previous year, relative to plants grown in control plots that had not received any Vapam in the previous year (Table 2-5). The greatest reduction in index of disease (28% relative to the control) was observed in plants grown in soil that had received the 200% Vapam rate (62.2 mL $m^{-2}$) the year before ($p<0.0001$). At the 50$^{th}$ Street site, index of disease was significantly ($p=0.0004$) reduced only in those plots that had received the 31.1 mL $m^{-2}$ Vapam treatment in the previous year. These plants had an average index of disease of 53.3%, compared with 71.7% in the control plots. The plots that had received the 200% (62.2 mL $m^{-2}$) treatment rate did not exhibit a significant reduction in index of disease relative to either the control or the label rate of Vapam (31.2 mL $m^{-2}$).

#### 2.4.2.2 *Plant Growth Characters*

At the Henwood site, differences in stem height were observed between plants grown in plots that had been treated with 7.8 mL $m^{-2}$ or 62.2 mL $m^{-2}$ Vapam in the previous year ($p=0.0361$), as well as between plants grown in plots that had received the 15.5 mL $m^{-2}$ or 62.2 mL $m^{-2}$ treatment rates ($p=0.0113$). Significant differences also were found between these same treatments with respect to above ground plant biomass ($p=0.0297$, $p=0.0058$). Statistically significant differences in pod counts resulted from comparisons between the 7.8 mL $m^{-2}$ and 15.5 mL $m^{-2}$ rates ($p=0.0239$), and between the 15.5 mL $m^{-2}$

and 62.2 mL $m^{-2}$ rates (p=0.0500; Table 2-6). In contrast, no significant differences were observed for fresh or dry gall weight between any of the treatments, or between the treatments and the control plots for any of plant growth characters examined (Table 2-6). At the 50th Street site, no significant differences were observed for any of the plant growth traits between any of the treatments or control.

## 2.5 Discussion

Based on the efficacy of Vapam as a management tool for weeds, nematodes, insects and various soilborne diseases (Triky-Dotan et al. 2010), this fumigant may have potential as a management tool for clubroot disease of canola. However, it is important to note that Vapam is a non-selective toxic compound. Soil fumigation with a volatile chemical, which is also water soluble, poses threats to the adjacent environment at treatment sites as well as the applicator. It is of paramount importance for soil fumigation to be conducted in accordance with application regulations and label recommendations (AMVAC, 2005).

At the Henwood site in both 2012 and 2013, all treatments reduced clubroot disease severity when compared with the control treatment. At the 50th Street site in 2012, the control treatment had a lower average index of disease than at Henwood, indicating lower disease pressure. Moreover, as a result of flooding of the site in 2013, only the 2012 data from 50th Street could be included in the analysis. A study by Hwang et al. (2014) at different locations within the same field sites also assessed the efficacy of Vapam as a soil fumigant against clubroot. At the Henwood site, treatment with Vapam at a rate of 100 mL $m^{-2}$ resulted in a 62% decrease in clubroot severity relative to a non-treated control. At the 50th Street site, clubroot severity decreased by 54% with Vapam treatment. These results

suggested that Vapam may be effective for managing clubroot, perhaps by killing the resting spores of *P. brassicae*, thereby reducing the soil inoculum available to infect any susceptible plants. The treatment rates evaluated in the current study were different from those used by Hwang et al. (2014); in the earlier study, only one rate was examined, while in this study, multiple rates (above and below the recommended rate) were assessed. The label rate of 31.1 mL $m^{-2}$ and the 200% label rate of 62.2 mL $m^{-2}$ both resulted in decreases in clubroot severity at the Henwood and 50$^{th}$ Street sites in 2012. These decreases in disease severity, while lower than those observed by Hwang et al. (2014), were nevertheless significant. Moreover, Vapam appeared to have some residual effects, as significant decreases in clubroot severity were observed on canola grown in soil that had been treated with the fumigant in the previous year. This is consistent with the manufacturer's specifications that Vapam has residual activity.

The decreases in clubroot disease severity were sometimes reflected in significant reductions in root gall weight. This would be expected, since lower levels of disease would indicate a reduction in hyperplasia and hypertrophy of the root tissues. Consequently, the roots would be able to maintain more normal function, allowing the plants to produce taller stems and commit more energy to the production of above-ground biomass. Indeed, significant increases in various plant growth characters including plant height, fresh biomass, and pod number were observed at the Henwood site in 2012 and 2013. Interestingly, however, while Vapam was found to have a residual effect on disease symptom development on the roots of affected plants, and numerical increases in stem height, pod number and above ground biomass were observed for plants grown in soil treated with the fumigant in the previous year, these increases were in general not

significant. Therefore, the residual effects of Vapam may not be sufficient to adequately control clubroot on canola in the year following its application.

One of the reasons for evaluating multiple application rates of Vapam in the current study was to enable identification of the optimal rate for canola. Both the label and 200% rates provided comparable levels of clubroot control. Thus, the label rate seems most appropriate for several reasons. The first is from an environmental safety perspective, by limiting the amount of a toxic fumigant applied in a field. The second is from an economic perspective, as less fumigant would have to be purchased. The third, and perhaps most important reason from a disease management perspective, relates to the observation that higher levels of chemical applied did not always result in an increase in plant height, above ground plant biomass or pods per plant. High rates of Vapam have been associated with phytotoxicity in a study by White & Buczacki (1977). Similarly, Smelt and Leistra (1974) also recognized the considerable phytocidal activity of Vapam, and more recently Hwang et al. (2014) reported that higher rates of Vapam resulted in reduced seedling emergence. In the current study, a delay and reduction in plant emergence was noted in soil treated with the 200% label rate of Vapam, also suggesting some phytotoxic effects. When using Vapam on a field scale, even for smaller treatment areas, mild phytotoxicity could pose an issue for growers wanting to plant canola in the same season as the fumigation treatment. It is unknown what the effects would be on a crop other than canola following treatment with this product. An opportunity also exists for further studies on the management of clubroot based on soil fumigation in conjunction with various crop rotations.

The manufacturer label suggests that treated areas be kept covered for 48 hours after treatment to prevent product dissipation from occurring too soon (AMVAC 2005). The manufacturer also recommends seeding 14 to 21 days after fumigant application when the soil is covered or tarped over following treatment (AMVAC 2005). In the current study, the Vapam was allowed to dissipate for two days after the tarp coverings were removed from the plots and the canola was seeded, which may not have been sufficient time to allow the fumigant to dissipate. For the 200% label rate, introducing more chemical may have meant that more time should have been allotted between tarp removals and seeding. However, the size of the treated area is not specified in the manufacturer's instructions, or whether the amount of time differs for a field or a small localized area. A high rate of decomposition of Vapam was noted by Smelt & Leistra (1974), but the soil conditions in that study included higher temperatures (21°C) over a period of three weeks.

Weather conditions may have been less ideal for Vapam treatment as well. Cold soil temperatures can cause slower conversion to MITC, the primary bioactive ingredient (AMVAC 2005). Turner and Corden (1963) found that the rate of metam-sodium transformation to MITC in soils was increased by both lower moisture content and higher temperature. In 2012, the plots were treated later in the season and temperatures were in the range of 23°C -24°C in June and July. The plots were treated earlier in 2013 when temperatures were lower on average, but still within the recommended window for soil fumigation. Another factor that could have an effect on the efficacy of Vapam treatment is the product application method. Depending on the particular crop and where it is grown, various methods may be utilized for the application of fumigants. In California and Florida, for example, fruit growers use chloropicrin and 1,3-dichloropropene as alternatives to

methyl bromide (Chellemi et al. 2013), which they apply by shank injection, or more effectively and safely, through drip irrigations systems. More effective application methods allow for less of the active ingredient to be released into the atmosphere. The amount of ingredient leaving the soil surface is influenced by the rate of diffusion and degradation (Dungan & Yates 2003). In the current study, rototilling the chemically treated soil may have resulted in more effective incorporation of the product and more pronounced treatment effects (Hwang et al. 2014). Similarly, additional watering also may have improved the efficacy of the Vapam treatments, since soil fumigant activity will not move past the point of the water front, either horizontally or vertically within the soil (White & Buczacki 1977). Water volumes applied were based on the amount of water saturating the ground at a test site. Field conditions in each plot may have differed and been drier. The volume of water applied with the chemical also may play a role in the erratic and inconsistent control of clubroot in Brassica crops using Vapam (White & Buczacki 1977).

While the rates of Vapam evaluated in the current study were not sufficient to completely eradicate the disease, they could limit symptom development and possibly the production of new inoculum. The field sites assessed in this study were heavily infested with *P. brassicae*, and it is possible that clubroot severity could be reduced to negligible levels at field sites where low levels of inoculum have been recently introduced. As such, the application of Vapam may represent a useful tool to contain localized clubroot infestations within fields, and/or to prevent more widespread spread of the disease in regions where it is not endemic. Fumigation could be used in conjunction with other tactics, such as the sanitization of field machinery and the planting of resistant canola cultivars, to reduce the impact of clubroot. Nonetheless, there are constraints to its

application, including the safety risks posed to the environment and applicator. Covering the treated area to prevent loss of the chemical to volatilization also may prove prohibitively expensive or impractical in many circumstances. Of additional concern is the non-specific nature of metam sodium, which could result in significant reductions in the populations of non-target or beneficial soil organisms (Smelt & Leistra 1974). A cost/benefit analysis should be conducted prior to the application of Vapam or other fumigants in specific fields. Ultimately, multiple approaches will be needed for the sustainable management of clubroot on canola.

**Table 2-1. Effect of Vapam (metam sodium) on clubroot disease severity under field conditions at two sites (Henwood and 50th Street) near Edmonton, AB, Canada in 2012**

| Site | Vapam active ingredient* (mL $m^{-2}$) | Index of disease (ID; %) |
|---|---|---|
| Henwood | 0 | 89.0 a** |
| | 3.1 | 80.2 b |
| | 7.8 | 60.9 c |
| | 15.5 | 59.4 c |
| | 31.1 | 37.6 c |
| | 62.2 | 39.1 c |
| 50th Street | 0 | 55.8a |
| | 3.1 | 41.8 b |
| | 7.8 | 33.4 c |
| | 15.5 | 31.3 cd |
| | 31.1 | 26.0 de |
| | 62.2 | 22.8 e |

*Recommended rate for the watering can application of Vapam is 31.1 mL active ingredient $m^{-2}$

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

**Table 2-2. Effect of Vapam (metam sodium) on clubroot severity under field conditions at one site (Henwood) near Edmonton, AB, Canada in 2013**

| Vapam active ingredient* (mL $m^{-2}$) | Index of disease (ID; %) | Reduction in ID relative to control (%) |
|---|---|---|
| 0 | 95.0 a** | - |
| 3.1 | 81.7 b | 13.3 |
| 7.8 | 65.0 c | 30.0 |
| 15.5 | 70.0 c | 25.0 |
| 31.1 | 68.4 c | 26.6 |
| 62.2 | 61.7 c | 33.3 |

*Recommended rate for the watering can application of Vapam is 31.1 mL active ingredient $m^{-2}$

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

**Table 2-3. Effect of Vapam (metam sodium) on plant growth characters and yield components under field conditions at two sites (Henwood and 50th Street) near Edmonton, AB, Canada in 2012**

| Site | Vapam active ingredient* (mL $m^{-2}$) | Plant height (cm) | Fresh biomass (g)/ plant | No. pods/ plant | Fresh gall weight (g)/ plant | Dry gall weight (g)/ plant |
|---|---|---|---|---|---|---|
| Henwood | 0 | 82.5 a** | 47.0 ab | 62.3 ab | 14.1 a | 3.2 a |
| | 3.1 | 83.6 a | 43.9 a | 60.4 b | 11.5 a | 2.6 ab |
| | 7.8 | 92.5 b | 62.1 abc | 86.3 c | 9.5 b | 2.2 bc |
| | 15.5 | 92.1 b | 65.3 bc | 83.4 ac | 13.8 b | 2.3 c |
| | 31.1 | 95.5 b | 99.7 c | 114.2 c | 8.2 c | 2.4 c |
| | 62.2 | 93.3 b | 96.8 c | 126.0 c | 8.6 bc | 2.5 c |
| 50th Street | 0 | 87.0 a | 76.6 ab | 73.1 ab | 9.2 a | 2.1 a |
| | 3.1 | 81.8 a | 59.3 a | 82.1 ab | 5.7 a | 1.9 a |
| | 7.8 | 89.1 a | 74.4 ab | 80.2 a | 6.3 a | 1.8 a |
| | 15.5 | 85.0 a | 61.6 ab | 67.9 ab | 5.3 a | 1.8 a |
| | 31.1 | 87.8 a | 86.3 ab | 52.1 b | 7.0 a | 2.2 a |
| | 62.2 | 84.6 a | 90.3 b | 81.1 ab | 8.0 a | 2.4 a |

*Recommended rate for the watering can application of Vapam is 31.1 mL active ingredient $m^{-2}$

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

**Table 2-4. Effect of Vapam (metam sodium) on plant growth characters and yield components under field conditions at one site (Henwood)near Edmonton, AB, Canada in 2013**

| Vapam active ingredient* (mL $m^{-2}$) | Plant height (cm) | Fresh biomass (g/ plant) | Pods/ plant | Fresh gall weight (g/ plant) | Dry gall weight (g/ plant) |
|---|---|---|---|---|---|
| 0 | 59.2 a** | 19.3 a | 33.4 a | 12.1 a | 2.2 a |
| 3.1 | 67.6 ab | 31.5 b | 54.0 ab | 11.5 a | 2.1 a |
| 7.8 | 71.7 b | 38.7 bc | 64.0 b | 9.8 a | 1.8 a |
| 15.5 | 73.3 b | 43.0 bc | 68.4 b | 13.5 a | 2.5 a |
| 31.1 | 71.8 b | 44.8 c | 62.4 b | 15.2 a | 2.8 a |
| 62.2 | 69.5 ab | 48.3 c | 58.7 b | 11.0 a | 2.2 a |

*Recommended rate for the watering can application of Vapam is 31.1 mL active ingredient $m^{-2}$

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

**Table 2-5. Effect of Vapam (metam sodium) applied the previous year on clubroot severity under field conditions at two sites (Henwood and 50th Street)near Edmonton, AB, Canada in 2013**

| Site | Vapam active ingredient* (mL $m^{-2}$) | Index of disease (ID; %) |
|---|---|---|
| Henwood | 0 | 83.3 a** |
| | 3.1 | 76.7 ab |
| | 7.8 | 84.2 a |
| | 15.5 | 72.5 b |
| | 31.1 | 72.5 b |
| | 62.2 | 55.0 c |
| 50th Street | 0 | 71.7 a |
| | 3.1 | 68.3 a |
| | 7.8 | 70.0 a |
| | 15.5 | 64.2 a |
| | 31.1 | 53.3 b |
| | 62.2 | 60.8 ab |

*Recommended rate for the watering can application of Vapam is 31.1 mL active ingredient $m^{-2}$

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

**Table 2-6. Effect of Vapam (metam sodium) applied the previous year on plant growth characters and yield components under field conditions at two sites (Henwood and 50th Street) near Edmonton, AB, Canada in 2013**

| Site | Vapam active ingredient* (mL $m^{-2}$) | Plant height (cm) | Fresh biomass (g)/ plant | No. pods/ plant | Fresh gall weight (g)/ plant | Dry gall weight (g)/ plant |
|---|---|---|---|---|---|---|
| Henwood | 0 | 68.38 ab** | 44.18 ab | 20 ab | 9.70 a | 1.91 a |
| | 3.1 | 67.89 ab | 58.03 ab | 24 ab | 13.32 a | 2.08 a |
| | 7.8 | 63.50 a | 35.51 a | 33 a | 12.28 a | 2.12 a |
| | 15.5 | 61.14 a | 34.90 a | 13 b | 10.61 a | 1.94 a |
| | 31.1 | 69.83 ab | 48.04 ab | 25 ab | 12.20 a | 2.50 a |
| | 62.2 | 77.03 b | 72.11 b | 28 a | 9.21 a | 1.87 a |
| 50th Street | 0 | 71.75 a | 57.42 a | 33 a | 10.48 a | 1.85 a |
| | 3.1 | 60.11 a | 39.86 a | 24 a | 10.04 a | 1.67 a |
| | 7.8 | 66.19 a | 43.25 a | 14 a | 6.95 a | 1.25 a |
| | 15.5 | 67.76 a | 39.31 a | 26 a | 7.91 a | 1.63 a |
| | 31.1 | 70.64 a | 52.52 a | 21 a | 7.97 a | 1.55 a |
| | 62.2 | 68.63 a | 43.42 a | 14 a | 7.03 a | 1.35 a |

*Recommended rate for the watering can application of Vapam is 31.1 mL active ingredient $m^{-2}$

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

# Chapter 3

## 3.0 Evaluation of *Brassica juncea* Meal as a Biofumigant for the Management of Clubroot (*Plasmodiophora brassicae*) of Canola

### 3.1 Introduction

Clubroot is a soilborne disease of crucifers caused by the obligate parasite *Plasmodiophora brassicae* Wor. The disease causes the development of large galls or clubs on the roots of susceptible hosts. These malformations interfere with normal water and nutrient uptake by the plant, leading to above ground symptoms that can include shorter, stunted plants, early and uneven ripening, and reduced and shrivelled pods. In canola (*Brassica napus* L.), the yield losses associated with severe *P. brassicae* infection were reported to range from 80% to 91% in a study conducted in Quebec (Pageau et al. 2006). In commercial canola crops in Alberta, yield losses as high as 30% to 100% have occasionally been observed (Strelkov & Hwang 2014). Clubroot also causes declines in seed quality, reducing oil content and seed weight (Pageau et al. 2006; Hwang et al. 2010).

The most widely used strategy for the management of clubroot in canola is the planting of genetically resistant cultivars (Rahman et al. 2014). These cultivars generally have high levels of resistance to the predominant pathotypes of *P. brassicae*, and enable the production of good yields in fields that are heavily infested with the pathogen. However, repeated cropping of resistant varieties can cause shifts in the virulence of *P. brassicae* populations, which can result in an erosion or even total loss of resistance in some cultivars (LeBoldus et al. 2012). Indeed, clubroot resistance already has been overcome in some fields in Alberta (Strelkov et al. 2016), threatening the effectiveness of resistance as a management tool.

Various fungicides have been assessed for their efficacy against clubroot in canola, as part of studies aimed at developing additional tools to control this disease. Soil amendments, including lime, wood ash and calcium cyanimide, also have been considered for the management of clubroot (Murakami et al. 2002; Hwang et al. 2011) and, in some cases, successfully reduce disease severity. Despite some reports of adequate efficacy, however, neither fungicides nor soil amendments have been adopted as clubroot management tools in canola. The costs associated with application of these products, particularly over the large fields typically associated with canola production, make their use uneconomical and often impractical (Gossen et al. 2013).

Soil fumigants, like fungicides, are utilized for pest control, but fumigants rely on toxic vapours to impact soil organisms, and are more general in their target range than fungicides. Fumigants have been used traditionally to control soilborne pests and pathogens in high-value crops such as vegetables (Papiernik et al. 2004). In recent studies, the fumigant metam sodium has been explored as a tool for clubroot management in canola (Chapter 2, Hwang et al. 2014). However, fumigants such as metam sodium also can have negative effects on non-target organisms, given their non-specific activity (Smelt & Leistra 1974, Triky-Dotan 2010). As a result, it is important to examine environmentally "friendlier" options to such products, including the use of biofumigants and biofumigation.

Biofumigation is defined by Gimsing & Kirkegaard (2009) as a method of controlling weeds and soilborne pests by incorporating plant tissue containing glucosinolates into the soil. Lu et al. (2010) includes the incorporation of fresh plant material (as in green manure), seed meals (by-products of seeds from oil extraction), or dried plants under the

umbrella of biofumigation. The Brassicaceae are a plant family known to have high glucosinolate levels, and as such are good candidates for application as biofumigants (Omirou et al. 2011). Plants producing glucosinolates also contain the enzyme myrosinase, located in myrosin cells (Lu et al. 2010, Szczyglowska et al. 2011). The glucosinolates and myrosinases are segregated in different cells, but cell breakage caused by pests or injury causes these chemicals to mix, resulting in the release of hydrolysis products, including indoles, thiocyanates, nitriles and isothiocyanates, which have antimicrobial activity (Omirou et al. 2011, Szczyglowska et al. 2011). Several studies have found that biofumigation can alter the structure and function of soil microbial communities, including bacteria and fungi (Omirou et al. 2011, Matthiessen & Shackleton, 2005). The aim of biofumigation is to create a hydrolysis reaction which produces as much of the desired end-product, isothiocyanates, as possible. The production of isothiocyanates can be enhanced most readily by increasing cell breakage, adding more water to the reaction, and applying under higher soil temperatures (Gimsing & Kirkegaard 2009). One of the benefits of biofumigation over chemical fumigation is a reduced environmental impact, since isothiocyanates do not persist long in the soil. Thus, biofumigants can be thought of as natural alternatives to chemical fumigants (Gimsing and Kirkegaard 2009).

The effectiveness of *Brassica juncea* as a biofumigant against soilborne pests has been examined in various studies. Zasada et al. (2009) found that *B. juncea* meal had the highest nematode-suppressing activity of all of the treatments they evaluated, reducing soilborne populations of *Pratylenchus penetrans* and *Meloidogyne incognita* by more than 90%. Similarly, in a study of the management of potato pathogens, Szczyglowska et al. (2011) reported that *B. juncea* could reduce diseases caused by the fungi *Rhizoctonia solani*,

*Phytophthora erythroseptica*, *Pythium ultimum*, *Sclerotinia sclerotiorum* and *Fusarium sambucinam* by 80-90%. In contrast, Friberg et al. (2009) reported variable and inconsistent results in an assessment of *B. juncea* as a biofumigant against *R. solani* on carrots. It is possible that the variety of *B. juncea* selected as a source of the seed meal, or the manner in which the meal extract is prepared, can influence its effectiveness as a biofumigant.

MustGrow (MPT Mustard Products & Technologies Inc., Saskatoon, SK) is a fumigation product manufactured from the seeds of *B. juncea*. It is a dry granular product which is applied and incorporated into the soil pre-planting for the control of soilborne nematodes and microbial pathogens. MustGrow was initially registered for the suppression of red stele in strawberries, phytophthora root rot in caneberries, and root lesion nematodes in strawberries and caneberries (Pest Management Regulatory Agency 2016). Since then, registration in Canada has expanded to include additional pests on more crops, such as soil-borne *Pythium* and *Fusarium* species on root, tuber, and leafy vegetables, including Brassica leafy vegetables. The Canadian registration also now includes registration for nematode and disease control on legume vegetables, fruiting vegetables, some stone fruits, tree nuts and tobacco (Pest Management Regulatory Agency 2016). It is not currently registered on canola (*Brassica napus*).

Unlike liquid fumigants, MustGrow is not mixed with water prior to application. The product is applied to a dry soil surface and water is then added to the treated soil. According to the manufacturer's website, MustGrow contains a mixture of glucosinolates and myrosinases in a concentrated and stable form for treatment (MustGrow 2015). The

addition of water causes a hydrolysis reaction and the production of the active ingredient, allyl isothiocyanate (AITC). Allyl isothiocyanate is the soluble volatile gas responsible for toxic effects on the target organisms. About 70% of the gas created dissolves in the carrier water, while the remaining 30% is active as a gas (MustGrow 2015).

The purpose of this study was to evaluate the efficacy of *B. juncea* meal (MustGrow) applied as a pre-plant soil treatment for the control of clubroot of canola. The effect of treatment with this product on clubroot symptom severity, and on plant growth characters, including plant height, stem and gall weight and seed yield, were evaluated under greenhouse conditions. The ultimate aim of the research was to increase knowledge of possible strategies that could be used as part of an integrated system for the sustainable management of clubroot of canola.

## 3.2 Materials and Methods

### 3.2.1 Pathogen Material

Soil infested with *P. brassicae* was collected from a field nursery in Edmonton, AB (the 'Henwood site', 53 38' 48"N, 113 22' 33"W), in 2013. The soil at this site is a black chernozemic loam, pH 5.0, with an organic content of 10%. The soil was allowed to air dry and then measured into a large re-sealable plastic bag. Aliquots of 1.75 L (~1.2 kg) of soil were used for each treatment and control as described below. The soil was mixed to create a homogenous blend, and a 250 mL volume was collected from each aliquot as a pre-treatment sample for testing for the presence and amount of *P. brassicae* resting spores via conventional polymerase chain reaction (PCR) and quantitative PCR analysis, as described below. The remaining 1.50 L (~1.0 kg) of soil in the bag was treated with MustGrow (MPT

Mustard Products & Technologies Inc., Saskatoon, SK). Briefly, the treatment granules were added to the 1.50 L of infested soil in the plastic bag and incorporated by sealing and shaking the bag. In the control treatment, no biofumigant was added. After the pellets were incorporated into the soil as evenly as possible, 250 mL of water was added and the soil was mixed again. Three MustGrow treatment rates were evaluated: low (1.11 g MustGrow/L soil); medium (2.22 g MustGrow/L soil); and high (4.44 g MustGrow/L soil). The application rates were determined empirically based on those used in earlier preliminary trials (data not shown). The manufacturer's recommended rates consist of a range of 1121-2240 kg/ha for the control of specific nematodes and some pathogens, such as *Verticillium*, or a single rate of 2240kg/ha for soil-borne pathogens such as *Pythium* and *Fusarium* species or certain nematodes (Pest Management Regulatory Agency 2016). The lowest rate applied in the current study was equivalent to approximately 1153 kg/ha, the medium rate was equivalent to about 2313 kg/ha, and the highest rate to 4625kg/ha, corresponding to the lowest recommended rate, the highest rate and a rate double the maximum recommendation, respectively.

### 3.2.2 Greenhouse Assays

After treatment, the bags of treated soil were poured into 12 cm x 12cm x 12cm pots. The pots of treated soil were seeded 14 days after the biofumigant was incorporated into the soil samples to allow any excess fumigant to dissipate. A clubroot susceptible canola cultivar, 45H26(Pioneer, Caledon, ON), treated with Helix Xtra (Syngenta, Guelph, ON) was planted in each pot at a rate of 10 seeds per pot. Six samples were included for each of the four treatments, for a total of 24 pots. The trial was replicated twice in the same greenhouse, so two blocks in this experiment were created. In the greenhouse, plant

growth conditions were maintained at 24°C and approximately 30% relative humidity under natural light supplemented with artificial lighting (16 hours day/8 hours night).

Greenhouse experiments were conducted at the Crop Diversification Centre North, Alberta Agriculture and Forestry, Edmonton, AB, Canada. The plants were sown on March 21st and April 11th, 2014 for the first and second blocks, and harvested on May 2nd and May 23rd, 2014, respectively. At harvest, the plants were carefully dug out from the soil, with the roots washed with tap water and evaluated for clubroot symptom severity. All plants in each pot were assessed.

The roots of each plant were rated on a 0 to 3 scale, where 0= no galls, 1= a few small galls, 2= moderate galling and 3= severe galling (Kuginuki et al. 1999). The individual disease ratings were used to calculate an index of disease (ID) according to the formula of Horiuchi and Hori (1980) as modified by Strelkov et al. (2006):

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

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

In addition to evaluating clubroot symptom severity on the roots, the height of the main stem, fresh above ground plant biomass, seed yield, and fresh and dry gall weights also were measured and recorded. After the plants were harvested, a 250mL soil sample was collected from each pot as a post-treatment sample for analysis via conventional and qPCR analysis.

### 3.2.3 PCR Analysis

Total genomic DNA was extracted from 250 mg aliquots of the soil samples collected before and after the growth of the plants using a Powersoil DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA), as per the manufacturer's instructions. The DNA concentration was determined with a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA). Samples were stored at 4°C and prepared for PCR analysis. For conventional PCR analysis, DNA samples were diluted with pure water to a concentration of 2 ng $\mu L^{-1}$. Samples were prepared as a 1:10 (v:v) dilution for the qPCR assays.

All soil samples were tested for the presence of *P. brassicae* DNA by conventional PCR analysis. Conventional PCR was conducted as per Cao et al. (2007) using the *P. brassicae*-specific primers TC1R and TC1F. Samples that tested positive for the presence of DNA of the clubroot pathogen by conventional PCR (as determined by the presence of a single amplicon of the expected size in an agarose gel) were further analyzed by qPCR according to the method of Rennie et al. (2011) with the DR1R/DR1F primer set. The concentration of resting spores in a sample was calculated by comparison with a standard curve generated with DNA samples from known *P. brassicae* spore concentrations (Rennie et al. 2011).

## 3.3 Statistical Analysis

Statistical analysis was performed with SAS v.9.4 (SAS Institute Inc., Cary N.C. 2013). A mixed model analysis of variance was used to evaluate the treatment effects on plant height, fresh above ground biomass per plant, fresh gall weight per plant, dry gall weight

per plant, and yield per pot. A logarithmic transformation was applied to the fresh above ground plant weight, fresh gall weight, and dry gall weight data to correct for potential deviations from normality seen in the residual data. Non-transformed means are presented for consistency.

The CATMOD procedure was used to analyze treatment effects for the disease severity data. For all analyses, differences were considered to be significant at $P< 0.05$ unless otherwise stated. The two experiments were conducted in the same greenhouse and based on the inference space of our study, assessing the effect of this biofumigant across conditions, the two blocks were pooled. Since this study was intended to evaluate how true the treatment effect would be, sample, treatment within sample, the interaction of block and treatment, and treatment within the block-sample interaction, were all considered random. A t-test was performed to evaluate whether resting spore concentrations in the soil were significantly different before and after each treatment was applied.

## 3.4 Results

### 3.4.1 *Disease Severity*

Some plant mortality was observed across treatments in the first block, but was most severe in the control pots, where all plants in 5 of the 6 reps died. The greatest number of surviving plants was observed in the highest treatment rate (4.44 g MustGrow /L soil). All measurements were therefore based on a single pot of plants for each treatment, and as such, results from the first block should be treated with caution. In the second block, similar but less serious issues with plant survival in the control also were

observed, as 2 out of the 6 reps had no surviving plants. Seedling mortality appeared to be the result of root rot, as decomposition of the roots and lower stems was observed. Clubroot symptoms were visible on the roots of plants harvested from all the treatments. However, in this experiment, no treatments resulted in an index of disease which was significantly different from the control (Table 3-1). The index of disease for the control was 79.2%. Relative to the control, plants grown in soil treated with each of the three rates of MustGrow developed numerically lower ID values, but these were not significantly different from the control (Table 3-1).

### 3.4.2 *Plant Growth Characters*

Over the course of this experiment, there were no significant differences observed in plant height at any of the rates of MustGrow, relative to the control or to the other treatment levels (Table 3-2). No significant differences were observed between any of the treatments or the control with respect to the amount of fresh biomass produced per plant. Similarly, no significant differences were observed with respect to fresh or dry gall weights.

### 3.4.3 *Seed Yields*

Seed yield in the control treatment was 0.00 g/pot. Although some yield was recorded for all of the treatments that received MustGrow, the amounts harvested were not significantly different from zero (Table 3-2).

### 3.4.4 *Resting Spore Concentration*

Most treatments did not result in significant changes in the *P. brassicae* resting spore concentration in the soil (Table 3-3). In soil treated with the highest rate of

MustGrow, the spore concentration increased from 7.04 x $10^3$ to 4.6 x $10^5$ spores/g soil, which was the only statistically significant change in spore loads between the pre- and post-treatment soil samples($P$ = 0.0003) (Table 3-3).

### 3.5 Discussion

The Brassicaceae hold promise as biofumigants because of their high glucosinolate content (Omirou et al. 2011). Nonetheless, plants in this family vary in the amount of glucosinolates they contain, with those genotypes producing more glucosinolates having better biofumigant capacity (Szczglowska et al. 2011). Kirkegaard and Sarwar (1999) assessed 22 Australian canola (*B. napus*) and 15 Indian mustard (*B. juncea*) genotypes for their glucosinolate profiles. The *B. juncea* genotypes produced higher concentrations of aromatic and aliphatic glucosinolates in their roots than did the *B. napus* genotypes, although *B. napus* produced more indolyl glucosinolates. These findings suggest that both species may be useful as sources of biofumigants. On the pathogen side, obligate parasites may represent the best targets for biofumigant activity, since the ability of non-obligate parasites to survive saprophytically in the soil could impact the effectiveness of these products (Friberg et al. 2009). Given the nature of *P. brassicae* as an obligate parasite, a *B. juncea*-based biofumigant may represent a useful tool for the management of clubroot. There are relatively few reports in the literature regarding the use of biofumigants to manage clubroot infestations.

In one study, Cheah et al. (2001) grew Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) seedlings in *P. brassicae*-infested soil that had been mixed with turnip (*B. rapa* subsp. *rapa*) leaf and stem tissue. After five weeks, clubroot development on the seedlings

was found to be significantly lower than on control plants grown in non-treated soil (Cheah et al. 2001). In a second study, turnips were grown in *P. brassicae* infested soil, and then were tilled into the soil and allowed to decompose. Chinese cabbage seedlings subsequently grown in the same soil were found to develop less clubroot than the controls (Cheah et al. 2001). In a more recent study, Friberg et al. (2009) carried out greenhouse and field experiments using *B. juncea*, the same species as in MustGrow, as a biofumigant against *R. solani* on carrots and *F. oxysporum* f. sp. *lini* on flax. While a reduction in *R. solani* inoculum density was detected initially under greenhouse conditions, there was an increase in disease levels above those seen in controls later in the trial (Friberg et al. 2009). Under field conditions, treatment with *B. juncea* meal did not cause detectable effects on *R. solani* soil inoculum levels when assessed 4 and 8 months after treatment, and was associated with increased inoculum levels after 11 months. Based on these results, Friberg et al. (2009) concluded that they could not consider their mustard treatment an effective option for the management of *R. solani*, and cautioned it could actually pose a risk for increasing disease. In trials with *F. oxysporum*, no treatment effects were detected on the density or population structure of *Fusarium* species in the soil (Friberg et al. 2009).

In the current biofumigation study, many of the plants did not produce any seeds or died over the course of the experiment. In treatments where most of the plants died, it is likely that this mortality resulted from the presence of root rot pathogens in the soil, since some decomposition of seedling roots was observed. The plants were grown in field soil that had not been sterilized, and which therefore may have contained other saprophytic and pathogenic microorganisms in addition to *P. brassicae*. The possible impact of

MustGrow on other soil microbes was not examined. In this context, the results of this study must be interpreted with caution.

Disease severity (ID) and soil resting spore concentrations did not improve with MustGrow treatment. These results may have reflected sample sizes that were too small to make accurate assessments. Nonetheless, it is also possible that the addition of *B. juncea* meal to the soil increased soil organic matter, which improved the nutritional status of the host plants and favoured clubroot development (and formation of resting spores by the pathogen), offsetting any benefits associated with the treatment. In earlier experiments with broccoli (*B. oleracea*) as a biofumigant, Omirou et al. (2011) observed an increase in soil respiration following treatment, which they attributed to an increase in organic matter associated with the incorporation of the broccoli residues. MustGrow itself has a guaranteed content of 5% total nitrogen, 1% available phosphate and 1% soluble potash (MustGrow 2015), and could serve as a potential fertilizer.

The rates of MustGrow applied in this study were equivalent to those recommended by the manufacturer under field conditions, or in the case of the highest treatment rate, greater than those recommended by the manufacturer. While no impact of *B. juncea* meal (MustGrow) on clubroot severity was found, additional studies may be required to fully evaluate the potential utility of this and other biofumigants. The limitations inherent in this study made it difficult to draw any sweeping conclusions. As such, *B. juncea* meal and other environmentally 'friendly' products should continue to be studied as clubroot management tools. A broad spectrum of strategies will be needed to enable sustainable production of canola in *P. brassicae*-infested fields.

**Table 3-1. Effect of MustGrow (*Brassica juncea* meal) on clubroot disease severity under greenhouse conditions in Edmonton, AB, Canada in 2014**

| Treatment Level | MustGrow Rate (g/L soil) | MustGrow (g) | Index of disease (ID; %) |
|---|---|---|---|
| Control | 0.00 | 0.00 | 79.2 a |
| Low | 1.11 | 1.67 | 70.0 a |
| Medium | 2.22 | 3.33 | 65.9 a |
| High | 4.44 | 6.66 | 72.6 a |

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

**Table 3-2. Effect of MustGrow (*Brassica juncea* meal) on plant growth characters and yield components under greenhouse conditions in Edmonton, AB, Canada in 2014**

| MustGrow Rate (g/L soil) | MustGrow (g) | Plant height (cm) | Fresh biomass (g)/ plant | Fresh gall weight (g)/ plant | Dry gall weight (g)/ plant | Yield (g) / pot |
|---|---|---|---|---|---|---|
| 0.00 | 0.00 | 41.26 a | 6.95 a | 6.29 a | 1.44 a | 0.00 a |
| 1.11 | 1.67 | 57.64 a | 20.82 a | 9.60 a | 2.24 a | 0.31 a |
| 2.22 | 3.33 | 71.02 a | 23.77 a | 22.97 a | 4.40 a | 0.65 a |
| 4.44 | 6.66 | 60.02 a | 14.00 a | 10.27 a | 2.02 a | 0.41 a |

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

**Table 3-3. Effect of MustGrow (*Brassica juncea* meal) on *Plasmodiophora brassicae* resting spore loads under greenhouse conditions in Edmonton, AB, Canada in 2014**

| MustGrow rate (g/ L soil) | Average spore load before treatment (spores/g soil) | Spore load range before treatment (spores/ g soil) | Average spore load after treatment (spores/ g soil) | Spore load range after treatment (spores/ g soil) |
|---|---|---|---|---|
| 0 | $1.22x10^4$ | $1.6x10^3 – 2.7x10^4$ | $3.0x10^4$ | $2.3x10^3 – 2.0x10^5$ |
| 1.11 | $1.55x10^4$ | $4.8x10^2 – 4.6x10^4$ | $1.3x10^6$ | $6.0x10^3 – 1.4x10^7$ |
| 2.22 | $1.04x10^4$ | $1.0x10^3 – 3.3x10^4$ | $1.2x10^6$ | $1.7x10^3 – 7.2x10^6$ |
| 4.44 | $7.04x10^3$ | $7.8x10^2 – 1.5x10^4$ | $4.6x10^5$* | $4.0x10^3 – 1.1x10^6$ |

*Resting spore concentrations after treatment that are designated with an asterisk (*) are significantly different from the concentration before treatment for a particular rate of MustGrow based on a t-test at $P<0.05$

# Chapter 4

# 4.0 General Conclusions

## 4.1 Synthesis and Implications

The primary objective of this thesis was to evaluate the efficacy of the fumigant Vapam (metam sodium) as a tool to reduce or possibly eradicate localized infestations of *Plasmodiophora brassicae* in the soil. A secondary focus, given the toxicity of Vapam and other chemical fumigants, was to examine the efficacy of a biofumigant (*Brassica juncea* meal, MustGrow) in reducing *P. brassicae* inoculum potential and disease severity.

While many clubroot management strategies have been assessed since the disease was first identified on Canadian canola (*Brassica napus*), the most widely used control strategy has been the planting of clubroot-resistant cultivars (Rahman et al. 2014). The high level of control achieved with clubroot-resistant canola makes it appealing to growers, but does not eliminate the underlying cause of the disease (i.e., the presence of *P. brassicae* resting spores in the soil). While resistant cultivars contribute fewer resting spores back into the soil compared with susceptible varieties, they do not eradicate the soilborne *P. brassicae* inoculum (Hwang et al. 2011). Moreover, the repeated growing of resistant canola crops in *P. brassicae* infested fields may cause shifts in the virulence of pathogen populations, which can result in an erosion of effective resistance, as has been observed already both in greenhouse experiments (LeBoldus et al. 2012) and in commercial fields (Strelkov et al. 2016) . Additional strategies are needed to complement genetic resistance and continue to ensure sustainable canola production in *P. brassicae* infested fields.

Fungicides and soil amendments have been considered for the management of clubroot, and have been shown to provide variable levels of control (Murakami et al. 2002; Hwang et al. 2011). While the use of fungicides for clubroot management is common in higher-value crops such as cruciferous vegetables (Donald & Porter 2014), these products have not been adopted as tools for the control of clubroot on canola. Similarly, soil amendments need to be applied at such high rates (Myers & Campbell 1985, Murakami et al. 2002, Hwang et al. 2014) that the cost and logistics discourage growers from using them as management tools.

Given the challenges associated with clubroot management, additional tools and strategies need to be considered. One of these is the fumigation of infested soil. Chemical fumigants have been used as a management tool for soilborne insects, weeds, nematodes and pathogens in various crops (Papiernik et al. 2004). In the context of this thesis and the clubroot situation on canola, the fumigant Vapam was selected for study in Chapter 2. It was found to significantly reduce clubroot severity in field trials when applied prior to planting of the crop. In addition, Vapam was observed to have some residual effects, as plants planted in plots that had been treated with Vapam the year before exhibited significant decreases in clubroot severity. These findings showed potential for the use of Vapam as a clubroot management tool, particularly for spot applications to contain localized patches of infestation within otherwise disease-free fields.

Despite its potential, Vapam did not eradicate *P. brassicae* from treated field plots, as confirmed by the fact that some clubroot development still occurred. It is important to note, however, that the experimental field sites in Chapter 2 were very heavily infested

with *P. brassicae*. As such, even significant reductions in inoculum levels may not have been sufficient to completely eradicate either the pathogen or disease symptoms. It is possible that at sites where *P. brassicae* resting spore concentrations are lower, treatment with Vapam could reduce inoculum to negligible levels. Vapam may then represent a new option to contain localized clubroot infection foci within fields, and/or to prevent the spread of the disease in regions where it is not widespread. Fumigation could be used in conjunction with other management strategies, such as longer rotations and the planting of resistant canola cultivars, to prevent *P. brassicae* from becoming established in a field or area.

Fumigants such as metam sodium also can have negative effects on non-target organisms, given their non-specific activity (Smelt & Leistra 1974, Triky-Dotan 2010). It is desirable, therefore, to identify less environmentally harmful tools to achieve the same goals. In this context, the efficacy of the biofumigant MustGrow (derived from *B. juncea* meal) was assessed in Chapter 3 as a clubroot management tool under greenhouse conditions. Unfortunately, treatment with MustGrow did not decrease the severity of clubroot symptoms or the *P. brassicae* resting spore concentrations in the soil. As discussed in Chapter 3, there are several possible explanations for these results, including the fact that sample sizes may have been too small to detect clear trends. It is also possible that the addition of *B. juncea* meal into the soil improved host plant nutritional status, favoring enhanced growth, while at the same time favouring development of clubroot symptoms and spore formation by *P. brassicae*. As an obligate parasite, *P. brassicae* development is favored to a certain extent by a host capable of meeting its nutritional

needs. Additional work with MustGrow and/or other biofumigants is needed to fully explore their potential as clubroot management tools.

### 4.2 Questions and Future Work

The promising results obtained with Vapam have generated interest in the canola industry as to its use as a clubroot management tool (S. Strelkov, Personal Communication). However, further studies are needed before any specific recommendations can be made. These studies should be conducted on a larger scale than the mini-plots used in Chapter 2. In addition, different application methods need to be assessed. The trials presented in this thesis, due to their small scale, made use of the "watering can method" of application. Application is also possible with shank injection, irrigation, rotary tiller, or rotvator and roller (AMVAC 2005). Because this chemical is currently not registered on canola, it is important to not only analyze its efficacy, but also to optimize the rates of application for canola production.

Across the Prairies and throughout Canada, soil types vary. Vapam is formulated to take into account the soil type as well as the percent soil moisture (AMVAC, 2005). Experiments in which Vapam is applied at different rates across various soil types could further increase the relevance of the results obtained. In this thesis, only one soil type (typical of central Alberta) was represented in the field trials. While this approach was justified, given the fact that the clubroot outbreak is still centered in this region, as clubroot continues to spread to other regions, more information on soil type on the efficacy of Vapam will be of value. Additional studies could examine the impact of increasing or decreasing water volumes on the ability of Vapam to permeate the soil spaces. Water

volume could help explain the occasionally erratic and inconsistent control of clubroot in Brassica crops using Vapam (White & Buczacki 1977). Indeed, additional watering may have improved the efficacy of the Vapam treatments in Chapter 2, since soil fumigant activity cannot exceed the reach of the water, either horizontally or vertically within the soil (White & Buczacki 1977).

Future research also could examine the phytotoxic effects of Vapam in greater detail, particularly in western Canadian cropping systems. Studies by White and Buczacki (1977) and Smelt and Leistra (1974) have addressed the potential risk of phytocidal Vapam activity. Similarly, Hwang et al. (2014) reported reduced canola seedling emergence associated with higher Vapam application rates. In Chapter 2 of this thesis, plant emergence was delayed and reduced in soil treated with the 200% label rate of Vapam, also suggesting some phytotoxicity. Phytotoxic effects could represent an issue for growers wanting to plant canola in the same season as the fumigation treatment. Therefore, the impact of fumigation on canola and other crops should be better characterized. Finally, additional research on Vapam for clubroot management should include evaluation of the effect that different treatments have on *P. brassicae* resting spore concentrations in the soil. The impact of treatments on the pathogen itself was not evaluated in Chapter 2 of this thesis, but was examined in Chapter 3 when MustGrow was assessed. In retrospect, having a direct measure of Vapam treatment effects on pathogen inoculum levels in Chapter 2 would have provided valuable information.

As noted above, additional research also may be worthwhile with the biofumigant MustGrow, especially additional replication of the greenhouse trial. This is particularly

important since there was high plant mortality, particularly in one of the trials presented in Chapter 3. It appears that many seedlings were killed by microbial pathogens other than *P. brassicae* that were present in the soil samples. The impact of the MustGrow treatments on other soil microbes was not examined, and the soil samples were not characterized for species composition prior to use in the greenhouse studies. The soil was not sterilized prior to use, as the aim was to use naturally occurring *P. brassicae* inoculum from the field. An alternative approach to prevent this problem in future experiments would be to sterilize the soil, and then re-inoculate it with the desired concentration of *P. brassicae* resting spores, prior to use. An evaluation of the impact of MustGrow treatment on other soilborne pathogens, while beyond the scope of this thesis, might have identified other potential targets for control with this compound.

Additional studies with MustGrow could be conducted under field conditions instead of the greenhouse. The field represents the ultimate litmus test of the efficacy of any treatment, and may have facilitated inclusion of additional treatment rates and application timings. MustGrow is not registered on canola, so additional optimization of treatments may be required to identify the best way in which to use this product. From a broader perspective, it would also be valuable to assess additional biofumigants, particularly those that are not based on *B. juncea*, in order to compare their effectiveness and identify compounds that may be more useful.

The successful and sustainable management of clubroot of canola, especially in light of new threats such as the emerging pathotypes of *P. brassicae* (Strelkov et al. 2016), will require the evaluation of novel ideas and strategies. The volume of clubroot research is

increasing as more management options are sought to keep up with the increasing pressure placed by this disease on the canola industry. Through the research presented in this thesis, a potential niche for two new tools - the chemical fumigant Vapam and the biofumigant MustGrow (*B. juncea* seed meal) - has been identified. These may represent much-needed additional management options for clubroot of canola. While these products are not currently registered for use on this crop, it may be worthwhile to pursue such registration if their efficacy can be confirmed. Future studies with Vapam and MustGrow may provide this confirmation, along with specific recommendations for the most effective ways in which to use these products to manage clubroot. The identification of new management tools is a step in the right direction towards an integrated approach to mitigating the impact of clubroot in the Canadian canola crop.

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