

**EFFECT OF GREEN MANURES AND ORGANIC AMENDMENTS ON  
VERTICILLIUM WILT OF POTATO IN MANITOBA**

BY

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## ABSTRACT

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*Verticillium dahliae* Kleb. is a soil-borne fungal pathogen of several crops and is broadly distributed worldwide. It is a very persistent soil-borne fungus in potato (*Solanum Tuberosum* L.) and responsible for the Verticillium wilt of potato. In Manitoba, potato fields planted with cv Russet Burbank have been found to be infested with highly pathogenic *V. dahliae* isolates, which can produce disease severity of up to 90% and reduce yield. Potato producers have then an increased interest on use of green manures and organic amendments to control Verticillium wilt. The objectives of this research were to evaluate selected green manure and organic amendments for their ability to reduce propagule density of *V. dahliae* in soil, incidence and severity of Verticillium wilt, and to enhance potato yield in Manitoba. In addition, a second study was conducted for the purpose of studying the potential of mustard green manure and seed meal at lower application rates than those previously recommended to inhibit the germination of microsclerotia. Our results for the first study showed that compost and seed-meal treatment reduced disease incidence to 30 and 40 % ( $P < 0.001$ ), but only seed meal reduced propagule density. Potato marketable yield increased only with application of compost. Overall, 1- or 2-years green manures were ineffective reducing propagule density or improving potato yield. Application of Vapam was partially effective reducing the propagule density only at the beginning of the potato season, but it did not reduce

disease incidence compared to control. Our results for the second study showed that 2-propenyl isothiocyanate (2-propenyl-ITC) was the only ITC present in amended soil with higher concentration in seed meal amendment. The germination of microsclerotia was mainly inhibited by 0.5 and 0.25% mustard seed meal treatments at four-day measurement. However, the effect of concentration of 2-propenyl-ITC at 22 nM g<sup>-1</sup> seems to be fungistatic, as germination of microsclerotia rebounded once the concentration of ITC dissipated. The results of this study suggest that the inhibition of germination of microsclerotia by oriental mustard plant tissue or seed meal is affected by soil with pH higher than 6.5, where the concentration of 2-propenyl-ITC is lower and dissipates rapidly. Findings from the current research suggest that one or two year of green manure does not appear to be an effective management tool for *Verticillium* wilt of potato in Manitoba. Composted beef cattle manure and oriental mustard seed meal amendments have promise as an alternative strategy for the control of *V. dahliae*. However, in terms of effective field implementation, higher concentration and residence time of 2-propenyl-ITC will be needed to have a fungitoxic effect on *V. dahliae* microsclerotia with mustard seed meal. Finally, only composted beef cattle manure reduced disease, increased potato yield and improved nutrient availability (P) in soil.

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## **FOREWORD**

This thesis has been prepared in manuscript format following the guidelines established by the Department of Soil Science at the University of Manitoba. The reference style used in this document is from the Plant Disease journal. Chapters 2, and 3 may be submitted to a peer-reviewed journal, to be decided in the future. For all papers, I will be the lead author and co-authorship will be decided accordingly.

## TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
FOREWORD.....	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES.....	x
LIST OF FIGURES.....	xi
ABBREVIATIONS.....	xiii
1. INTRODUCTION .....	1
1.1. <i>Verticillium dahliae</i> : a Soil-borne Pathogen .....	2
1.2. Green Manure and Organic Amendments.....	5
1.2.1. Green Manure and Organic Amendments: a tool for Management of Soil-borne Pathogens .....	6
1.2.2. Mechanisms of Disease Suppression of <i>V. dahliae</i> Associated to Green Manures and Organic Amendments.....	7
1.2.2.1. Promoting Suppressive Activity in Soil .....	7
1.2.2.2. Reaction Product of Glucosinolates Containing Materials.....	8
1.2.2.3. Nitrogen Transformation Products from Nitrogenous Amendments .....	11
1.3. Thesis Objectives .....	12
1.4. Literature Cited.....	13
2. FIELD STUDIES EXAMINING THE EFFECTS OF GREEN MANURE AND ORGANIC AMENDMENTS ON SURVIVAL OF MICROSCLEROTIA OF VERTICILLIUM DAHLIAE, VERTICILLIUM WILT AND POTATO YIELD.....	20
2.1. Abstract .....	20
2.2. Introduction .....	21
2.3. Materials and Methods .....	25
2.3.1. Experiment 1.....	25
2.3.2. Experiment 2 .....	31
2.3.3. <i>V. dahliae</i> Survival in Soil .....	33
2.3.3.1. Survival of <i>V. dahliae</i> Microsclerotia.....	33
2.3.3.2. <i>V. dahliae</i> Propagule Density in Soil.....	34
2.3.4. Disease Assessment .....	35
2.3.4.1. Verticillium Wilt Incidence and Severity.....	35
2.3.5. Plant and Soil Assessments.....	36
2.3.5.1. Leaf Chlorophyll Content.....	36

2.3.5.2. Soil Chemical Properties .....	36
2.3.5.3. Potato Yield and Quality .....	36
2.3.6. Statistical Analysis .....	37
2.4. Results .....	38
2.4.1. Experiment 1 .....	38
2.4.1.1. Survival of <i>V. dahliae</i> Microsclerotia.....	38
2.4.1.2. <i>V. dahliae</i> Propagule Density in Soil.....	40
2.4.1.3. Disease Incidence and Severity .....	42
2.4.1.4. Plant and Soil Assessment.....	43
2.4.1.4.1. Effects on Soil Properties .....	43
2.4.1.4.2. Marketable Yield and Tuber Quality.....	45
2.4.2. Experiment 2 .....	47
2.4.2.1. Effect on Propagule Density and Verticillium Wilt Incidence.....	47
2.4.2.2. Effect on Potato Marketable Yield and Tuber Quality.....	48
2.5. Discussion .....	49
2.5.1. Survival of <i>V. dahliae</i> Microsclerotia.....	50
2.5.2. Reduction of Propagule Density and Verticillium Wilt.....	53
2.5.3. Effect on Soil Properties and Potato Marketable Yield.....	58
2.6. Conclusions.....	61
2.7. Literature Cited.....	62
3. SURVIVAL OF <i>V. DAHLIAE</i> MICROSCLEROTIA IN THREE POTATO SOILS AMENDED WITH ORIENTAL MUSTARD TISSUE AND SEED MEAL .....	70
3.1. Abstract .....	70
3.2. Introduction .....	71
3.3. Materials and Methods .....	74
3.3.1. Plant Material for Amemendments .....	74
3.3.2. <i>V. dahliae</i> Inoculum Preparation .....	75
3.3.3. Microcosm Soil Preparation.....	75
3.3.4. Experimental Setup .....	76
3.3.5. <i>V. dahliae</i> Microsclerotia Analysis .....	77
3.3.6. Soil Chemical Analysis.....	78
3.3.6.1. Soil ITC Analysis .....	78
3.3.6.1.1. Soil Extraction.....	78
3.3.6.1.2. ITC Analysis.....	78
3.3.6.2. Ammonia, Nitrous Acid and pH Determinations .....	79
3.3.7. Statistical Analysis .....	80
3.4. Results .....	81
3.4.1. Effect of Mustard Tissues and Seed Meal on Survival of Microsclerotia .....	81
3.4.2. Glucosinolate Hydrolysis Products, Ammonia and Nitrous Acid in Soil.....	85
3.5. Discussion .....	89
3.5.1. Mechanisms Associated with Inhibition of <i>V. dahliae</i> Microsclerotia Germination .....	89
3.5.2. Soil Properties Related to Mechanisms Reducing <i>V. dahliae</i> Microsclerotia.....	92
3.6. Conclusions .....	95
3.7. Literature Cited.....	96



4. OVERALL SYNTHESIS .....	101
4.1. Reduction of Verticillium Population in Soil and Impact on Disease, Yield and Potato Quality .....	101
4.2. Mechanisms of Achieving Disease Reduction and Yield Increase without Decreasing Verticillium Propagule Density in Soil .....	103
4.3. Economic Considerations.....	104
4.4. Role of Soil Properties in Determining Efficacy of Green Manures and Amendments .....	106
4.5. Recommendations .....	107
4.6. Literature Cited.....	109
5. APPENDICES .....	112

## LIST OF TABLES

<b>Table 2.1.</b> Crop, green manure and organic amendment treatments used in this study at three sites in Manitoba. Plants other than wheat and potato were green manures.....	26
<b>Table 2.2.</b> Experiment 1. Effect of wheat, green manure, organic amendment and Vapam treatments on propagule density of <i>V. dahliae</i> (CFU g <sup>-1</sup> soil) and Verticillium wilt incidence at the Carberry site.....	41
<b>Table 2.3.</b> Experiment 1. Water soluble nitrate-N (NO <sub>3</sub> <sup>-</sup> -N), sodium bicarbonate extractable-P, ammonium acetate extractable-K, water soluble S (SO <sub>4</sub> <sup>-2</sup> ), pH and electrical conductivity (EC) in the soil following potato harvest, September 2008.....	44
<b>Table 2.4.</b> Experiment 1. Effect of wheat, green manure, organic amendments and Vapam treatment on marketable potato yield.....	46
<b>Table 2.5.</b> Experiment 2. Effect of wheat and green manure treatments on soil propagule density of <i>V. dahliae</i> (CFU g <sup>-1</sup> soil) and Verticillium wilt incidence...	48
<b>Table 2.6.</b> Experiment 2. Effect of wheat and green manure treatments on Verticillium wilt incidence and marketable yield of potato.....	49
<b>Table 3.1.</b> Characteristics of the three soils used in the study (depth: 0–15 cm). Data shown are the average of four replications.....	76

## LIST OF FIGURES

<b>Figure 1.1.</b> <i>Verticillium dahliae</i> . A) Microsclerotia. B) Colonies of <i>V. dahliae</i> developed from microsclerotia distributed on a plate. C) Germination of microsclerotia.....	3
<b>Figure 1.2.</b> <i>Verticillium</i> wilt disease cycle.....	4
<b>Figure 1.3.</b> Outline of hydrolysis of glucosinolates by the enzyme myrosinase. Structure of isothiocyanates most commonly encountered in soil amended with mustard green manure or seed meal .....	9
<b>Figure 2.1.</b> Location of the three study sites in relation to the towns of Carberry, Shilo and Miami, Manitoba. The Carberry site (●) was used in Experiment1, and the Shilo (▲) and Miami (■) sites used in Experiment2.....	25
<b>Figure 2.2.</b> Experiment 1. Green manures planted at the Carberry site. <b>A.</b> White mustard, <b>B.</b> Fall rye, <b>C.</b> Sorghum-sudangrass, <b>D.</b> Oriental mustard, <b>E.</b> Canada milk vetch, <b>F.</b> Oat/pea mix.....	28
<b>Figure 2.3.</b> <b>A.</b> Compost beef cattle manure applied to the soil surface on May 15, 2008 at the Carberry site, and <b>B.</b> Incorporation of compost in Experiment 1 .....	29
<b>Figure 2.4.</b> <b>A.</b> Field application of Vapam on October 15, 2007, immediately after wheat harvest at the Carberry site, and <b>B.</b> Rolling of Vapam treated plots to smooth and compact soil in Experiment 1 .....	30
<b>Figure 2.5.</b> <b>A.</b> Oriental mustard seed meal on the surface of a treated plot on May 15, 2008 at the Carberry site, and <b>B.</b> Incorporation of mustard seed meal in Experiment 1 .....	31
<b>Figure 2.6.</b> Bioassay results for the effect of mustard seed meal, Vapam and green manures on germination of <i>V. dahliae</i> microsclerotia, expressed as a percentage of germination of microsclerotia in wheat control plots at 1 week (□) and 3 weeks (■) after application at the Carberry site. MUST <sub>meal</sub> = mustard seed meal, VAP= Vapam, O/PEA= oat/peas mix, CMV= Canada milk vetch, SS2= 2-year sorghum-sudangrass, SS1= 1-years sorghum-sudangrass, RYE= fall rye, MUSTor= Oriental mustard, MUSTwh= white mustard, and ALF= alfalfa. Means followed by same letter are not significantly different ( $P < 0.05$ ) according to .....	39

<b>Figure 3.1.</b> Germination of <i>V. dahliae</i> microsclerotia in the three study soils amended with oriental mustard tissue at concentrations, 1.5% (■) and 3% (▲) w w <sup>-1</sup> , and mustard seed meal at concentrations, 0.25% (□) and 0.5% (○) w w <sup>-1</sup> , and nonamended (*). Data shown are the average of four replicates (+1 standard error). Means for the germination of microsclerotia 4 and 42 days after incorporation in each soil followed by the same letter are not significantly different ( <i>P</i> < 0.05) according to the Bonferroni's multiple comparison test.....	81
<b>Figure 3.2.</b> Photographs showing growth of <i>V. dahliae</i> microsclerotia on Petri plates with pectate–tergitol–agar four days after incorporation of mustard plant tissue and seed meal in the three study soils. The germination of microsclerotia is shown as formation of black colonies.....	84
<b>Figure 3.3.</b> 2-propenyl-isothiocyanate concentration in amendment and nonamended treatments. Data shown are the average of four replicates (+1 standard error). Means for the 2-propenyl isothiocyanate concentration in soil one hour after incorporation followed by the same letter are not significantly different ( <i>P</i> < 0.05) according to the Bonferroni's multiple comparison test .....	86
<b>Figure 3.4.</b> Linear regression of germination (%) of <i>V. dahliae</i> microsclerotia, at 4 days after incorporation, in relation to concentration of 2-propenyl isothiocyanate in soil (nM g <sup>-1</sup> soil) at one hour after incorporation.....	87
<b>Figure 3.5.</b> Ammonia and nitrous acid concentration in soil solution and soil pH in the three study soils following addition of oriental mustard tissue and seed meal. Data shown are the average of four replicates (+1 standard error).....	88

## ABBREVIATIONS

ANOVA	Analysis of variance
C	Celsius degrees
CC	container capacity
cv	cultivar
d	day
g	gram
GLS	glucosinolates
h	hour
HNO <sub>2</sub>	nitrous acid
ITC	isothiocyanate
min	minutes
mL	milliliter
mM	millimolar
nM	nanomolar
NH <sub>3</sub>	ammonia
NH <sub>4</sub> <sup>+</sup>	ammonium
NS	no significant
ppm	part per million
rpm	revolutions per minute
SE	standard error
tonne	1000 kilograms
Total N	total nitrogen in soil
var	variety

## 1. INTRODUCTION

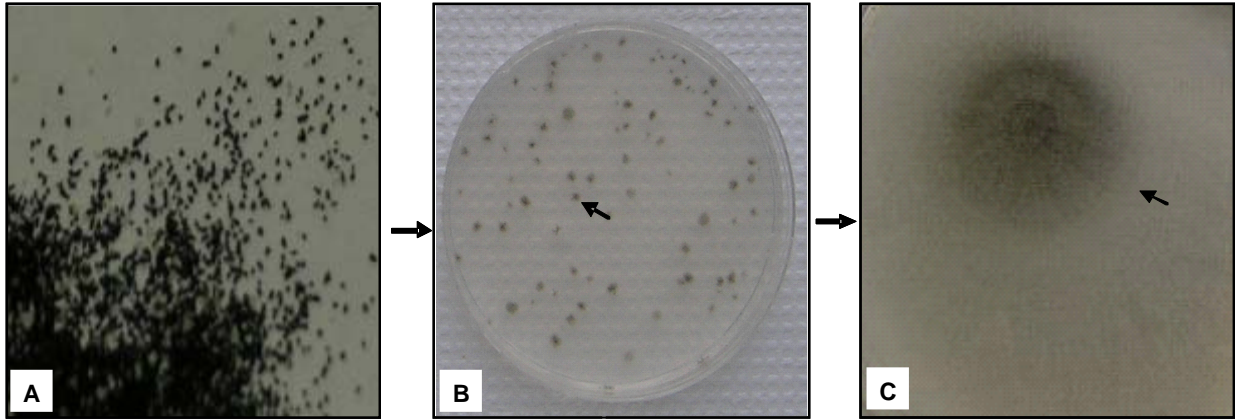
After the North American Free Trade Agreement (NAFTA) came into effect, potato production has become increasingly important to Canada (USDA 2002). The growing demand in North America and elsewhere for processed potatoes has resulted in expansion of potato processing and production on the Prairies (Agriculture and Agri-Food Canada 2007). This trend brings some environmental risk such as soil erosion and surface and groundwater contamination due to an increased use of fertilizer, insecticides and fungicides spraying. The environmental risk is often correlated to an increased pressure by plant pathogens. Verticillium wilt has been one of the most serious disease problems in most potato production areas in North America during the last 10 to 15 years. Potato growers have recognized the necessity of bringing alternative practices for management of Verticillium wilt. However, those practices have to be suitable to their soil and environmental conditions, which means that whatever the alternative approach to manage Verticillium wilt is, it needs to be evaluated before potato growers implement it.

Green manures and organic amendments are commonly used in crop production systems to increase soil nutrient availability, organic nitrogen and organic matter, which are associated with reduced diseases and higher tuber yields (Davis et al. 2001). The potential of green manures and organic amendments as means to reduce wilt diseases in Manitoba potato production areas is unknown, and research is needed to incorporate those practices in sustainable production systems in order to help farmers build soil, water and food quality for future generations.

### **1.1. *Verticillium dahliae*: a Soil-borne Pathogen**

*Verticillium dahliae* Kleb. is a soil-borne fungal pathogen of many crops and is broadly distributed worldwide (Katan 2000). It is responsible for serious disease damage in over 200 dicotyledonous species including herbaceous annuals, perennials and woody species (Fradin and Thomma 2006). *Verticillium* wilt symptoms are diverse and may be associated with other diseases or physiological problems. The first symptom is wilting and yellowing (chlorosis) of the lower leaves. The affected tissues die and the symptoms spread to younger leaves, thus resulting in the wilting of the plant, which in some cases begins from one side of the plant (Saeed et al. 1997; Vloutoglou et al. 2000; Fradin and Thomma 2006). *V. dahliae* is a soil-borne fungus of potato (*Solanum Tuberosum* L.) that is responsible for the *Verticillium* wilt disease (Rowe and Powelson 2002; Fradin and Thomma 2006). *Verticillium* wilt has developed slowly over many years in potato production areas of the Red River Valley in North America (Rowe et al. 1987). In Manitoba, since *Verticillium* wilt was first reported to have severe damage to potato in 1934 (Hoes and Zimmer 1968), many potato fields planted with cv Russet Burbank have been found to be infested with highly pathogenic *V. dahliae* isolates, which can produce disease severity of up to 90% (Uppal et al. 2007) and serious economic losses due to reduced yield and tuber quality (Johnson 1988; Shinnars-Carnelley et al. 2003).

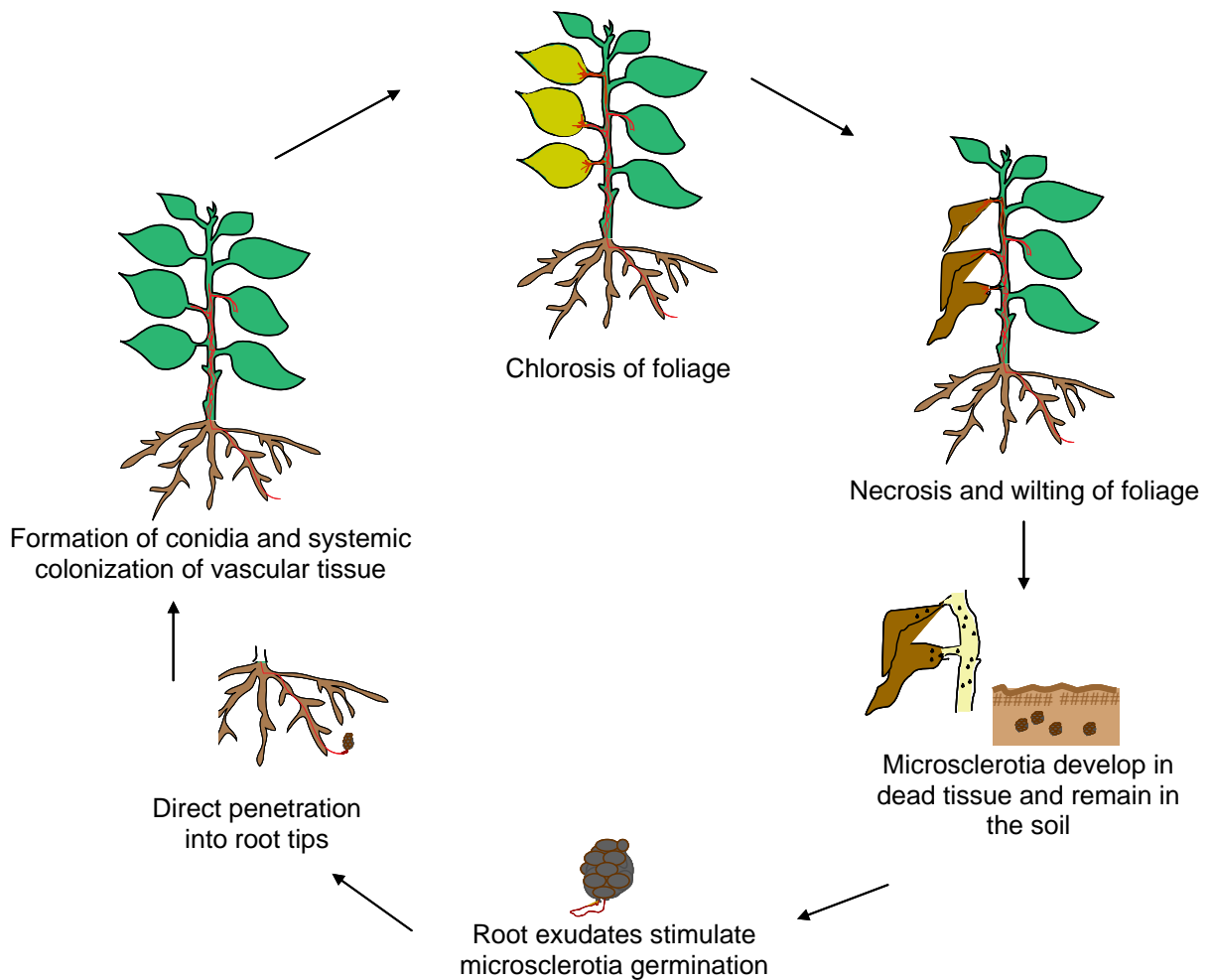
*V. dahliae* is a soil-borne deuteromycete fungus. It forms microsclerotia, thick-walled-melanized and multicelled resting structures (Lopez-Escudero et al. 2007) which are formed in senescent and dead tissue of many field crops (Mol and Scholte 1995) (Figure 1.1.).



**Figure 1.1.** *Verticillium dahliae*. A) Microsclerotia. B) Colonies of *V. dahliae* developed from microsclerotia distributed on a plate. C) Germination of microsclerotia.

Microsclerotia constitute the most important survival structure of this pathogen because they can survive up to 14 years in the field regardless whether the crop is a host or non-host (Wilhelms 1955). Microsclerotia are found either free or embedded in the plant debris, or in the vascular ring of the tuber (Rowe and Powelson 2002). The germination of microsclerotia is activated by root exudates in the rhizosphere of host plants. Then, the hyphae penetrate the plant roots and grow through the root cortex and into the vascular system causing vascular disruption (Fradin and Thomma 2006) (Figure 1.2).





**Figure 1.2.** Verticillium wilt disease cycle.

Management of *V. dahliae* is commonly achieved using chemical fumigants such as methyl bromide, vapam and chloropicrin (Rowe and Powelson 2002; Triky-Dotan et al. 2007). However, concern for potential health and/or environmental damage as well as cost have led to a search for alternative strategies to manage Verticillium wilt of potato (Davis et al. 1996).

These factors, together with the fact that potato resistance to *Verticillium* wilt has not been widely available (Rowe and Powelson 2002), have led the potato industry to searching for alternative management strategies. Recently, old practices such as incorporation of green manure, animal manure, compost, seed meals and other types of organic amendments have been used to control soil borne pathogens (Baley and Lazarovits 2003; Janvier et al. 2007).

### **1.2. Green Manure and Organic Amendments**

Organic amendments include a broad variety of inputs, including animal manure, compost and some other solid waste. Throughout the history of agriculture, organic amendments and green manures have been used to increase cropping sustainability (Haas and Defago 2005; Otten and Gilligan 2006; Raaijmakers et al. 2009) by improving soil organic matter, nutrient availability (Magdoff and Weil 2004), and plant health. Moreover, the incorporation of organic amendments enhance soil quality by increasing water-holding capacity and infiltration rates and lowering bulk density (Tester 1990; Werner 1997).

Green manure crops are any crop grown for the purpose of being plowed under while green or soon after maturity for soil improvement (SSSA 2008). Green manure can be a crop, crop-derived fertilizer, leguminous, or non-leguminous. It can be grown in situ or brought from outside as cuttings of trees and shrubs (green leaf manuring) (Singh et al. 1991). On the other hand, cover crops are “close-growing crops”, that provide soil protection, seeding protection, and soil improvement between periods of normal crop production, such as green manures. However, only when cover crops are plowed under and incorporated into the soil, cover crops may be referred to as green manure crops (Fageria 2007). The utilization of

green manure and organic amendments in agriculture has contributed immensely to converting poor fragile lands into stable productive areas. However, several factors may possibly restrict the potential benefits of green manures to crops such as the synchrony of nutrient release, during decomposition, with crop nutrient demand (Cobo et al. 2002). Also, when using green manure to reduce soil-borne pathogens, it is necessary to consider the type of materials, chemical composition, quantity and form of application into the soil (Lopez-Escudero et al. 2007; Ochiai et al. 2008).

### **1.2.1. Green Manure and Organic Amendments: a tool for Management of Soil-borne Pathogens**

The benefits of green manuring in agriculture are multifold (Sultani et al. 2007). Besides restoring soil productivity, green manures could reduce soil exposure to erosive processes and reduce nitrogen losses, and ultimately, green manure crops can be used to reduce weeds (Boydston and Hang 1995) and soil pathogen pressure (Davis et al. 1999; Larkin and Griffin 2007). Weed suppression has been reported with under-seeded biennial sweet clover (*Melilotus officianalis* L. Lam.) (Blackshaw et al. 2001), winter rye (*Secale cereale* L.), winter oilseed rape (*Brassica napus* L.) (Kruidhof et al. 2009) and some other species of the *Brassicacea* family (Kirkegaard and Sarwar 1999). Several reports have shown that green manures and some organic amendments can be highly effective in controlling a range of soil-borne diseases such as *Phytophthora* spp., *Fusarium* spp., *Verticillium* spp., *Rhizoctonia* spp, and *Sclerotinia* spp. (Hoitink and Boehm 1999; Noble and Coventry 2005; Bonanomi et al. 2007). The effectiveness of organic amendments in reducing disease

pressure is relatively variable among organic amendment types (Bonanomi et al. 2007), pathogen inoculum densities (Wiggins and Kinkel 2005b), and soil conditions (Tenuta and Lazarovits 2002a). This suggests that the positive effect of using green manures or organic amendments on soil-borne pathogens depends on the specific local crop-pathogen-environment interactions. However, in general the mechanisms involved in disease control are multiple and can vary with each pathosystem (Janvier et al. 2007). Green manures and organic amendments can reduce soil-borne pathogens through enhanced suppressive activity in soil (Mazzola 2004), or direct toxicity of fungitoxic compound and hydrolysis products from glucosinolates released from Brassicaceae residues incorporated into the soil (Subbarao and Hubbard 1996; Blok et al. 2000; Brown and Morra 2005; Wiggins and Kinkel 2005b; Matthiessen and Kirkegaard 2006) or mediated by the release of toxic nitrification products from high nitrogen amendments (Tenuta and Lazarovits 2002b).

### **1.2.2. Mechanisms of Disease Suppression of *V. dahliae* Associated to Green Manures and Organic Amendments**

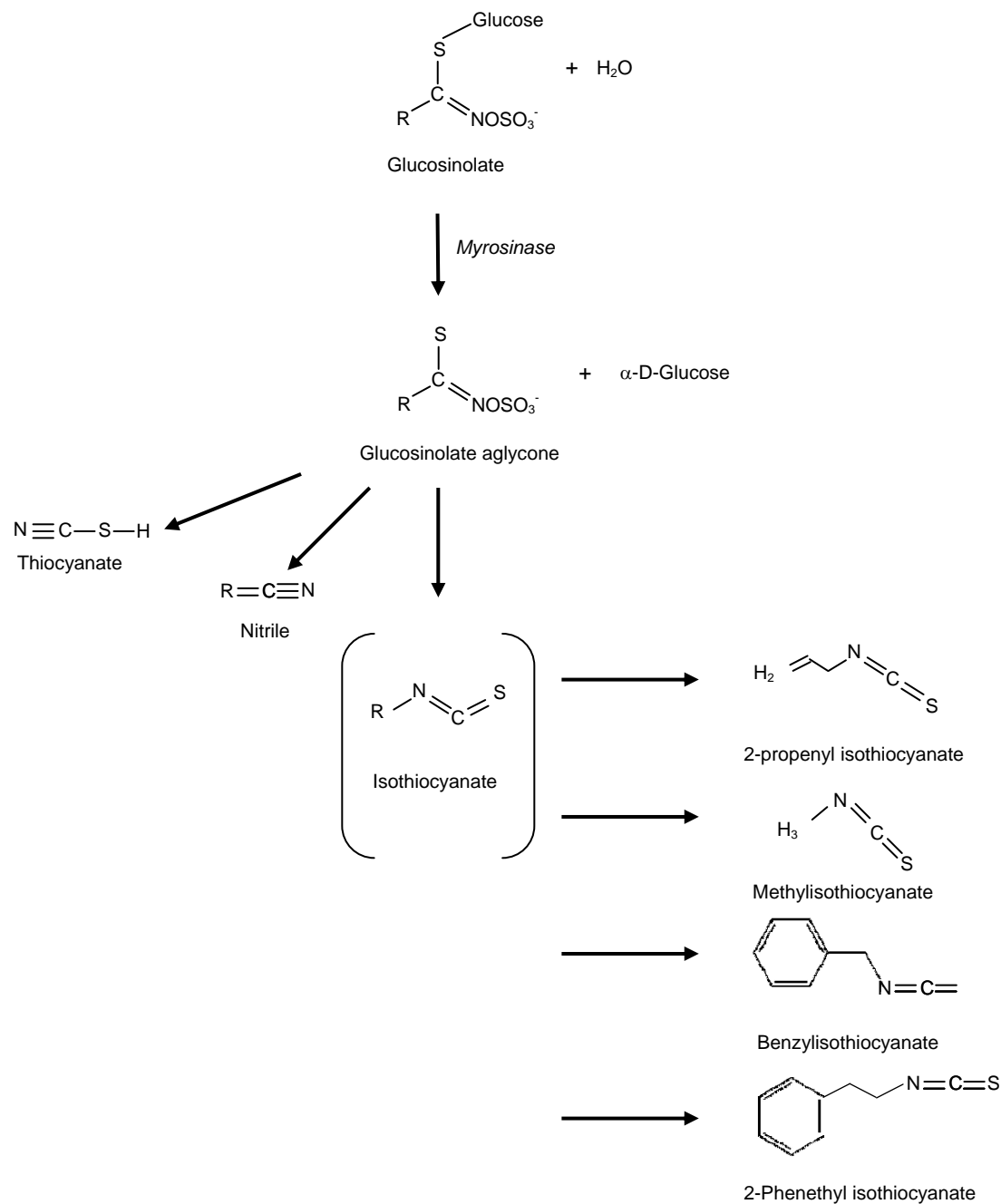
#### **1.2.2.1. Promoting Suppressive Activity in Soil**

The major benefit of organic amendments to soil is attributed to their labile carbon fractions, which are a source of energy necessary to increase soil microbial activity (Elliott and Lunch 1994; Stone et al. 2004), species richness (Janvier et al. 2007), and bacterial populations (Mader et al. 2002). Depending on the plant species used and cultivars, the increase in microbial biomass and microbial activity contributes to the disease suppressive capacity of the soil (Alabouvette et al. 2004; Dordas 2008). Besides this relationship, biological diversity and stability may be associated with soil health (van Bruggen and

Semenov 2000). It implies a connection between soil health, the capacity of the biological community to suppress soil-borne plant pathogens, the propagule density of soil-borne pathogens, and the disease incidence and severity (van Bruggen and Semenov 1996). By definition, a suppressive soil is one in which “*the pathogen does not establish or persist, establishes but causes little damage or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil*” (Stone et al. 2004). Furthermore, disease suppression can be an important function of a healthy soil, especially since the suppressive effect may be affected by environmental and soil conditions. Green manures and organic amendments promote soil suppression of soil-borne pathogens due to a range of several mechanisms such as competition and antagonism by the soil biota associated with microbial diversity and stability (Hoitink and Boehm 1999), volatile compounds from the organic matter (Noble and Coventry 2005), and enhancing availability of nutrients such as P, Mn and Zn, which can affect disease tolerance (Huber and Graham 1999).

#### **1.2.2.2. Reaction Product of Glucosinolates Containing Materials**

Brassicaceae plants such as cabbage, broccoli, and mustards are currently receiving renewed attention as an important source of sulphur secondary plant metabolites, glucosinolates (GSLs) (Matthiessen and Kirkegaard 2006). Glucosinolates naturally occur in plants in conjunction with a hydrolytic enzyme myrosinase, which catalyses their hydrolysis (Mikkelsen et al. 2002) (Figure 1.3.)



**Figure 1.3.** Outline of hydrolysis of glucosinolates by the enzyme myrosinase. Structure of isothiocyanates most commonly encountered in soil amended with mustard green manure or seed meal.

When the plant is physically disrupted, GSLs are hydrolyzed by the enzyme myrosinase to a number of products, most commonly found in soil isothiocyanates (ITCs), thiocyanates and nitriles (Brown et al. 1991). ITCs are generally regarded as a very toxic product against a wide range of soil organisms including nematodes, bacteria and fungi (Sarwar et al. 1998). ITCs are known to inhibit mycelial growth and germination of pathogen propagules (Olivier et al. 1999).

The effectiveness of biofumigation reducing soil-borne pathogen pressure depend on release efficiency and release rate of ITCs from plant tissues, susceptibility of the target species, soil texture, organic matter, pH and moisture content (Brown et al. 1991). Sorption of ITCs to soil particles and soil organic matter is an important mechanism in reducing the ITCs effectiveness (Matthiessen and Shackleton 2005). Also, high soil temperature has been correlated with an increased release rate of ITCs and greater inhibitory effect toward pathogenic fungi (Gamliel and Stapleton 1993). In addition, the soil moisture content directly affects production and retention time of ITCs. Under wet conditions, some ITCs with greater water solubility would have superior fungicidal effect compared to more volatile and less soluble ITCs (Frick et al. 1998). Increased water content correlates with ITC longevity in soil (Borek et al. 1995).

Soil factors such as pH or availability of ferrous ions are related with the type of product released after the hydrolysis of GSLs. For example, ITCs are usually produced at neutral pH values while nitrile production occurs at lower pH (Bones and Rossiter 1996).

Production of ITCs or other fungitoxic hydrolysis compounds is not the only cause for the suppression or reduction of soil-borne pathogens (Larkin and Griffin 2007). Cohen et al. (2005) found that suppression of *R. solani* by *Brassica napus* seed meal was associated with changes in soil microbial communities. Moreover, Matthiessen and Shackleton (2005) suggest that *Brassica* green manure incorporation increases soil organic matter and enhances soil structure and erosion control, which have been associated with reduction of soil-borne pathogens.

#### **1.2.2.3. Nitrogen Transformation Products from Nitrogenous Amendments**

High nitrogen-containing organic amendments and green manures have shown effective control of soil-borne pathogens (Gamliel et al. 2000; Tenuta and Lazarovits 2002b). Addition of high organic N amendments such as meat and bone meal, soy meal, oil seed meals and blood meal promote accumulation of volatile ammonia, which is toxic to many soil-borne organisms (Baley and Lazarovits 2003). Effectiveness of these high nitrogen-containing amendments have been often correlated with amendments with low C:N ratios (<10), such as oil seed meals, compost and green manures (Rodriguez-Kabana 1986).

*Verticillium dahliae* microsclerotia have been shown to be effectively reduced by accumulation of ammonia. Tenuta and Lazarovits (2002b) demonstrated that ammonia and nitrous acid from nitrogenous amendments and liquid swine manure inhibited microsclerotia germination. However, effectivity depends on accumulation of ammonia and



residence time. Such factors are controlled by the soil conditions. Bailey and Lazarovits (2003) stated that the pathogen suppressive effect of nitrogen-containing amendments is variable depending on the type of soil. Tenuta and Lazarovits (2002a) demonstrated that low organic carbon and high sand content is highly associated to NH<sub>3</sub> accumulation in soil. Such condition was found to be adequate for killing microsclerotia of *V. dahliae*.

### **1.3. Thesis Objectives**

The objectives of this thesis were:

- To evaluate selected green manure and organic amendments for their ability to reduce propagule density of *V. dahliae* in soil, incidence and severity of Verticillium wilt, and to enhance potato yield in Manitoba.
- To evaluate the potential of mustard green manure and seed meal in Manitoba soils at rates lower than those recommended; and to indentify soil conditions affecting the concentration and residence time of fungitoxic compounds released from oriental mustard plant tissue and seed meal, and thus, effectiveness to reduce survival of *V. dahliae* microsclerotia.

Field and laboratory studies (and analysis) were conducted in order to meet the above objectives. The data in this thesis present an approach to sustainable management of potato diseases in Manitoba. Chapter 2 describes the effects of green manure and organic

amendments on germination of *V. dahliae* microsclerotia, propagule density of *V. dahliae* in soil, and the effectiveness of selected green manure crops and organic amendments in reducing Verticillium wilt in potato. This chapter also highlights the effects of the treatments on marketable yield of potato and soil quality, in different potato soils of Manitoba. Chapter 3 presents results of a laboratory study examining the potential for mustard green manure and seed meal to reduce germination of *V. dahliae* microsclerotia in Manitoba soils, and the effectiveness of lower applications rates than those found to be effective, but economically impractical. In addition, this chapter discusses the effects of three different soils on the efficacy of ITCs, released from mustard green manure and seed meal. Chapter 4 is an overall synthesis discussing the general findings of the thesis and discusses the benefits of using green manures in plant pathogen control and concludes with recommendations.

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## **2. FIELD STUDIES EXAMINING THE EFFECTS OF GREEN MANURE AND ORGANIC AMENDMENTS ON SURVIVAL OF MICROSCLEROTIA OF VERTICILLIUM DAHLIAE, VERTICILLIUM WILT AND POTATO YIELD.**

### **2.1. Abstract**

Green manures and organic amendments can provide nutrients, improve soil quality and reduce disease pressure on crops. Mustard seed meal amendment, composted beef cattle manure and green manure crops oriental mustard, white mustard, oat/pea, alfalfa, sorghum-sudangrass, Canada milk vetch and fall rye were evaluated with two field experiments from 2006 to 2008, at three different locations in Manitoba for the control of *Verticillium dahliae*, and for their ability to enhance potato yield. Experiment 1 was a 3-year study consisting of 2-year green manure of sorghum-sudangrass grass and alfalfa, 1-year green manure of oat/pea, oriental mustard, yellow mustard, sorghum-sudangrass grass, Canada milk vetch, and rye, 2-years of composted beef cattle manure and 1-year oriental mustard seed-meal amendment, and a soil fumigant Vapam, as a chemical control and spring-wheat as a crop control treatment. Compost and seed-meal treatments reduced disease incidence to 25 and 13%. Additionally, compost increased marketable yield and mustard seed-meal provided the greatest reduction in *V. dahliae* propagules, whereas 1- or 2-years green manures were ineffective reducing propagule density or enhancing potato yield. Application of Vapam was partially effective in reducing propagule density only at the beginning of the potato season, but it did not reduce disease incidence compared to control. Experiment 2 was established on two commercial fields consisting of 1-year mustard mix (oriental and yellow mustard), oat/pea, sorghum-sudangrass and pear millet green manure. In both experiments, green manures did not

reduce the propagule density or *Verticillium* wilt incidence. Findings from the current studies suggest that one or two years of green manure does not appear to be an effective management tool for *Verticillium* wilt of potato in Manitoba. Composted beef cattle manure and oriental mustard seed meal amendments have promise as an alternative strategy for the control of *V. dahliae*. However, only compost beef cattle manure increased potato yield nutrient availability (P) in soil.

## **2.2. Introduction**

*Verticillium dahliae* Kleb., the causal agent of *Verticillium* wilt of potato, is a soil-borne fungal pathogen of potato in many growing regions around the world. Yield reduction in North America by *Verticillium* wilt can range from 10 to 50% (Powelson and Rowe 1993). Control of *Verticillium* wilt is extremely difficult due the formation of resting structures called microsclerotia, which can survive in soil for up to 10-20 years (Huisman and Ashworth 1976; Schnathorst 1981). Microsclerotia, mainly formed in the vascular system of a host, are the principal source of inoculum for wilt development (Mol and Scholte 1995). However, the ability of *V. dahliae* to colonize some weeds and even some crops, such as corn and wheat without causing disease (Lacy and Horner 1966), further allows the fungus to survive in soil (Joaquin et al. 1988). Threshold levels of around two microsclerotia per gram of soil are sufficient for 70-100% infection of potato stems (Nicot and Rouse 1987), and more than six microsclerotia per gram of soil can result in a large yield loss ( $\geq 20\%$ ) (Mol et al. 1996).

Management of Verticillium wilt of potato depends on a variety of strategies attempting to prevent the germination and/or reduce *V. dahliae* microsclerotia numbers to below a threshold at which disease does not cause significant yield losses. Currently, there is not an environmental friendly and agronomically effective strategy to control Verticillium wilt (Soltani et al. 2002). The most common control measures for reducing propagule density of *V. dahliae* are chemical and cultural practices (Easton et al. 1992; Davis et al. 1996). For example, soil fumigation with metam sodium alone or in combination with other fumigants has been an effective means of controlling Verticillium wilt of potato in North America (Rowe and Powelson 2002; Tsrer et al. 2005). However, concern for potential health and/or environmental damage from these pesticides as well as cost have led to a search for alternative strategies to manage Verticillium wilt of potato (Davis et al. 1996). Green manure is one cultural technique commonly recommended to reduce the number of *V. dahliae* microsclerotia in soil and to reduce Verticillium wilt incidence levels (Rowe and Powelson 2002).

Green manure, animal manure and compost are frequently used for improving soil quality, while providing nutrient to crops, and being suppressive to certain soil-borne pathogens such as *V. dahliae* (Davis et al. 1996; Lambert et al. 2005; Ochiai et al. 2008). Incidence of Verticillium wilt has been reduced with pea (*Pisum sativum*), oat (*Avena sativa*), broccoli (*Brassica oleracea*), sudan-grass (*Sorghum vulgare*) and corn (*Zea mays*) green manures (Davis et al. 1999; Wiggins and Kinkel 2005b; Ochiai et al. 2008), and addition of animal manures (Conn and Lazarovits 1999; Tenuta et al. 2002) and compost (LaMondia et al. 1999). However, incorporation of organic materials, such as

mustard or sudangrass green manure have also resulted in no reduction or even increased *V. dahliae* microsclerotia numbers in soil (Collins et al. 2005). Even though reduction of Verticillium wilt using organic amendments is often inconsistent and unpredictable, a variety of mechanisms responsible for killing *V. dahliae* microsclerotia have been associated with the incorporation of green manures, animal manures and composted materials (Conn and Lazarovits 1999; Tenuta et al. 2002). Suppression of Verticillium wilt by green manure and animal manures is attributed to reduction of microsclerotia in soil through fungitoxic compounds released during the decomposition of the amendment (Blok et al. 2000; Lopez-Escudero et al. 2007). Tenuta and Lazarovits (2002b) demonstrated that ammonia and nitrous acid from meat and bone meal and urea amendments killed microsclerotia in soil. Olivier et al. (1999) suggested that incorporation of glucosinolate containing plants, such as members of the Brassicaceae, reduce *V. dahliae* propagules due to the toxicity of hydrolysis products such as isothiocyanates (ITCs). Other studies have revealed that organic amendments can stimulate populations and activity of microorganisms that are antagonistic against *V. dahliae* (Lockwood 1988; Termorshuizen et al. 2006; Njoroge et al. 2008). In contrast, studies with sudan-grass and corn green manure suggest that reduction of Verticillium wilt incidence is not always associated with low propagule density of *V. dahliae* (Davis et al. 1996; Davis et al. 1999); it can also be attributed to improved soil fertility (NPK) (Rowe and Powelson 2002; Lambert et al. 2005) and soil quality (Ochiai et al. 2008).

Green manure and organic amendments have diverse and often unknown mechanisms by which *V. dahliae* can be controlled or killed. Mustard green manures and seed meal may

reduce *V. dahliae* microsclerotia numbers directly through fungitoxic compounds, such as ITCs (Olivier et al. 1999). Canada milk vetch green manure might reduce severity of Verticillium wilt due to the production of fungitoxic compounds or an induction of defense response mechanisms in the plant (Uppal et al. 2008). Oat/peas, fall rye, sorghum-sudangrass and alfalfa green manure and compost beef-cattle manure may also affect *V. dahliae* by changing soil conditions (N, total C and microbial biomass) (Ochiai et al. 2008).

In Manitoba, growers of processing potato are interested in controlling Verticillium wilt as it is believed to be one of the reasons why the provincial average yield is lower than in other potato growing regions. Manitoba potato fields planted with cv Russet Burbank have been found to be infested with highly pathogenic *V. dahliae* isolates, which can produce disease severity up to 90% (Uppal et al. 2007). This level of disease severity affects plant growth and depresses potato yield and ultimately reduces yield of potato. The effectiveness of green manures and organic amendments for reducing *V. dahliae* microsclerotia and Verticillium wilt has not been tested in Manitoba. Therefore, the objective of this study was to evaluate selected green manure and organic amendments for their ability to reduce *V. dahliae* microsclerotia density in soil, incidence and severity of Verticillium wilt, and to enhance potato yield under Manitoba growing conditions.

### 2.3. Materials and Methods

Two field experiments were performed from 2006 to 2008 at three sites located in Manitoba (Figure 2.1).



**Figure 2.1.** Location of the three study sites in relation to the towns of Carberry, Shilo and Miami, Manitoba. The Carberry site (●) was used in Experiment 1, and the Shilo (▲) and Miami (■) sites used in Experiment 2.

#### 2.3.1. Experiment 1

The first experiment was conducted on an experimental field located at the Canada-Manitoba Crop Diversification Centre (CMCDC) near Carberry, MB. (Figure 2.1), between 2006 to 2008. The soil was a Ramada loam ( $\text{pH}_{\text{water}}$  6.0, EC  $0.46 \text{ dSm}^{-1}$  and organic matter 5.1 %) naturally infested with *V. dahliae* ( $7 \text{ microsclerotia g}^{-1}$  soil) (0-15 cm depth). The field site had a history of moderate Verticillium wilt of potato (Dr. T. Shinnars-Carnelley, personal communication). It was planted to potato in the season (2005) preceding the start of this study. The experimental design was a randomized complete block with four blocks and 12 treatments (Table 2.1). A total of 48 individual plots, 18 m long x 6 m wide, were separated by 2 m of fallow soil between plots and 10 m between each block (Appendix I, Figure 5.1).

**Table 2.1. Crop, green manure and organic amendment treatments used in this study at three sites in Manitoba. Plants other than wheat and potato were green manures.**

Experiment	Site	Treatments	Year		
			2006	2007	2008 <sup>x</sup>
1	Carberry <sup>y</sup>	Control	Wheat	Wheat	Potato
		COM	Wheat	Compost / Wheat	Compost/Potato
		MUSTmeal	Wheat	Wheat	Seed meal/Potato
		VAP	Wheat	Wheat / Vapam	Potato
		O/PEA	Wheat	Oat / pea mix	Potato
		CMV	Wheat	Canada milk vetch	Potato
		SS2	Sorghum-Sudangrass	Sorghum-sudangrass	Potato
		SS1	Wheat	Sorghum-sudangrass	Potato
		RYE	Wheat	Fall Rye	Potato
		MUSTor	Wheat	Oriental mustard	Potato
		MUSTwh	Wheat	White mustard	Potato
ALF	Alfalfa	Alfalfa	Potato		
2	Shilo	Control	Wheat	Wheat	Potato
		O/PEA	Wheat	Oat / pea mix	Potato
		MUSTmix	Wheat	Mustard mix	Potato
		SS1	Wheat	Sorghum-Sudan	Potato
	Miami	Control	Corn	Wheat	Potato
		O/PEA	Corn	Oat / pea mix	Potato
		MUSTmix	Corn	Mustards mix	Potato
		MILLET	Corn	Pearl millet	Potato

<sup>x</sup> Carberry and Shilo sites planted to potato cv Russet Burbank, and Miami to cv Mozart.

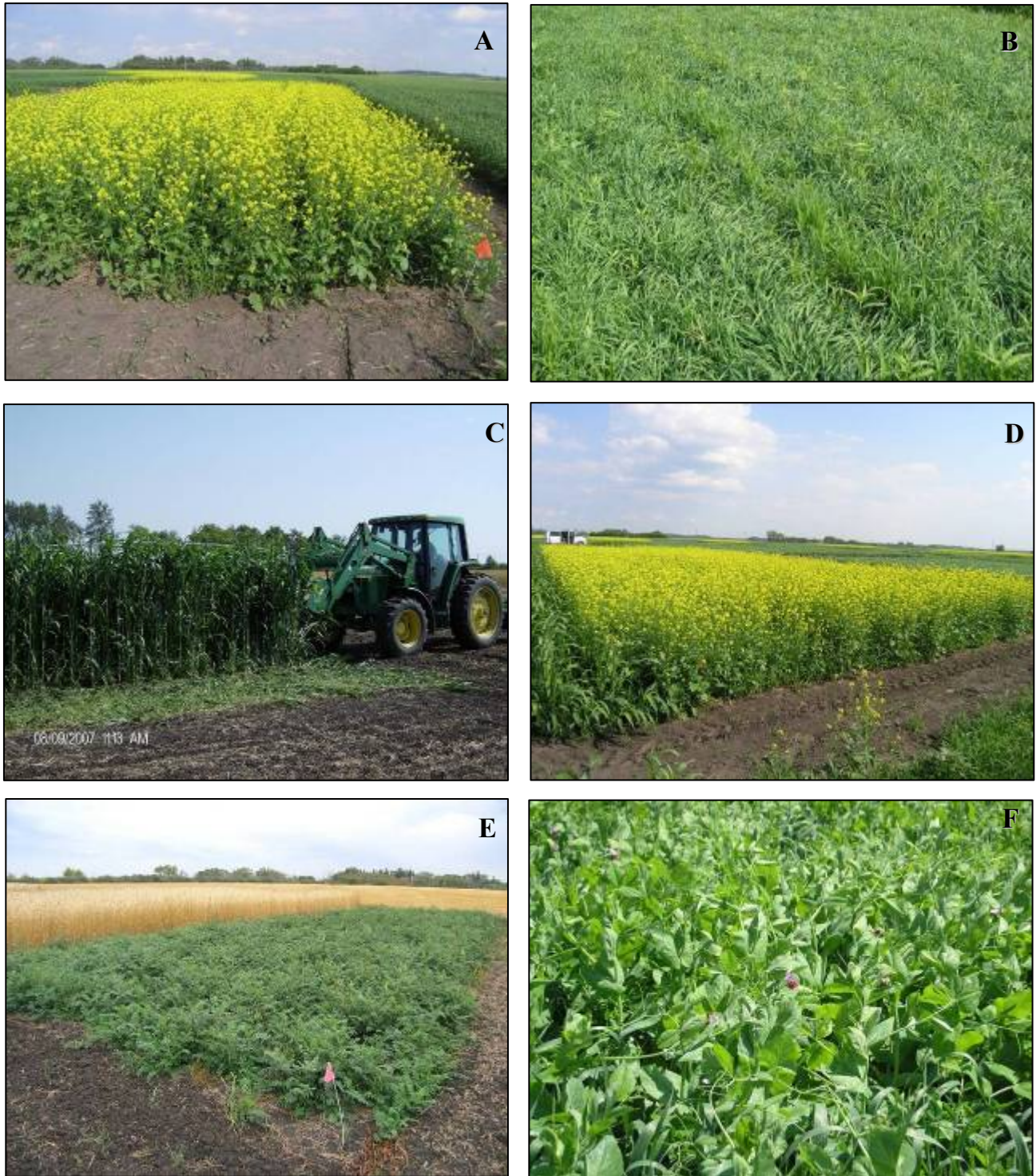
<sup>y</sup> Site planted to potato cv Russet Burbank in 2005.

In 2006, plots of ten treatments were planted (May 23) to spring wheat (*Triticum aestivum* L) var. AC Cora (123 kg ha<sup>-1</sup>). Plots of two treatments were planted (May 25) to sorghum-sudangrass hybrid (*Sorghum bicolor* L. Moench) Super Su 22 (29 kg ha<sup>-1</sup>) (SS2), and alfalfa (*Medicago sativa* L.) (9 kg ha<sup>-1</sup>) (ALF), respectively. The spring wheat was harvested and SS2 plots were disk soil incorporated to a depth of 15 cm on September 19, 2006.

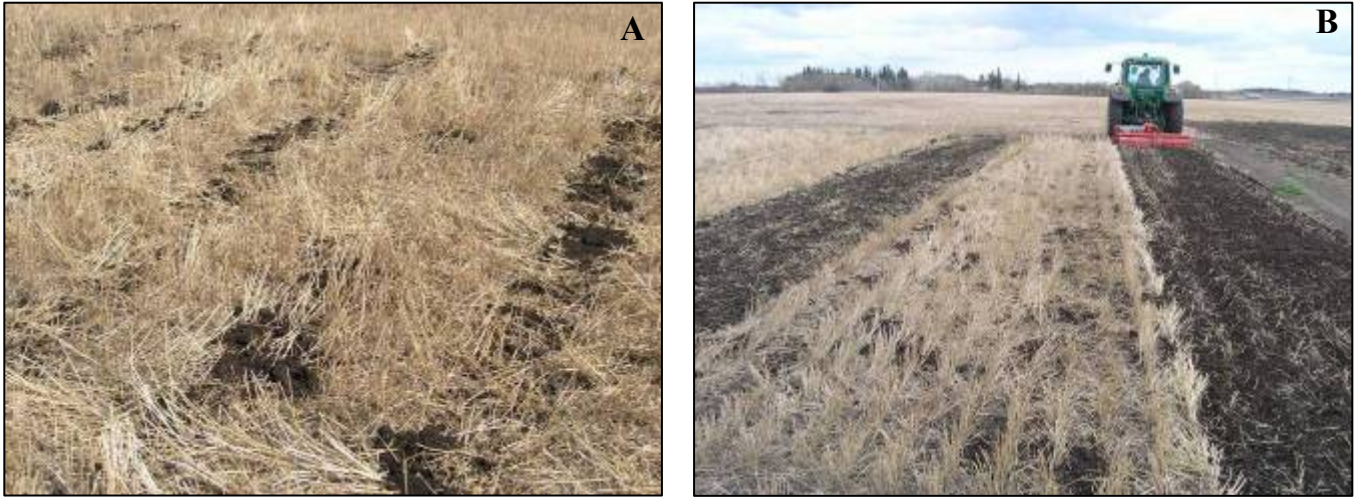
In 2007, for the SS2 treatment sorghum-sudangrass was re-planted on the same plots. Plots of six treatments were planted on May 29 to white mustard (*Sinapis alba* L.) var. Ace (9 kg ha<sup>-1</sup>) (MUSTwh); oriental mustard (*Brassica juncea* L.) var. Cutlas (5 kg ha<sup>-1</sup>) (MUSTor); oat (*Avena sativa* L.) var. Triple crown/pea (*Pisum sativum* L.) var. 40-10 Forage Pea (86 kg ha<sup>-1</sup>) (O/PEA); Canada milk vetch (*Astragalus canadensis* L.) (11 kg ha<sup>-1</sup>) (CMV); fall rye (*Secale cereale* L.) (81 kg ha<sup>-1</sup>) (RYE) and sorghum-sudangrass (SS1) (Figure 2.2). The remaining treatment plots were planted to spring wheat on May 30, 2007.

In 2007, compost beef cattle manure (COM) (Courtesy of Dr. K. Buckley, AAFC Brandon, MB) was hand applied, before wheat planting, at a rate of 44.5 wet tonne ha<sup>-1</sup> (42% organic matter, 1.28% N, 0.5% P) (Figure 2.3). Soil fumigation (VAP) with Vapam (United Agriproducts, Manitoba) was conducted on October 15, 2007, immediately after harvest of the spring-wheat. Before fumigation, plots were disked with a tandem disc followed by roto-tilling to loosen the soil to a depth of 15 cm. The fumigant was mixed with water and injected into plot areas, at 5000 L ha<sup>-1</sup> (762 L Vapam ha<sup>-1</sup>) (Figure 2.4).





**Figure 2.2.** Experiment 1. Green manures planted at the Carberry site. **A.** White mustard, **B.** Fall rye, **C.** Sorghum-sudangrass, **D.** Oriental mustard, **E.** Canada milk vetch, **F.** Oat/pea mix.



**Figure 2.3.** **A.** Compost beef cattle manure applied to the soil surface on May 15, 2008 at the Carberry site, and **B.** Incorporation of compost in Experiment 1.

Vapam treatment was used as a synthetic fungicide control treatment. Treatments with green manure crops were cut down and mechanically incorporated in soil to a depth of 15 cm, between August and September 2007, by either disc or rototiller. Treatments planted to spring wheat were harvested on October 15 (Figure 2.4). In addition, plots of one treatment were planted to spring wheat and used as crop control treatment (Control).



**Figure 2.4.** **A.** Field application of Vapam on October 15, 2007, immediately after wheat harvest at the Carberry site, and **B.** Rolling of Vapam treated plots to smooth and compact soil in Experiment 1.

On May 21, 2008, all plots were planted to potato (*Solanum tuberosum* cv Russet Burbank). Seed rows were 1 m apart, with six rows per plot, and within row spacing of potato plants of 0.25 m. Prior to planting, each plot received a broadcast application of nutrients based on soil fertility analysis. Treatments were grouped according to soil fertility levels as follows: Group 1. (Soil report 133 kg N ha<sup>-1</sup>, 12.5 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 265 kg ha<sup>-1</sup>) Control, VAP, COM, MUSTmeal, RYE, SS2 and ALF treatments received a basal application of 34 kg N ha<sup>-1</sup>, 45 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 78 kg K<sub>2</sub>O ha<sup>-1</sup> each plot. Group 2. (Soil report 82 kg N ha<sup>-1</sup>, 12.5 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 265 kg ha<sup>-1</sup>) O/PEA, MUSTwh, MUSTor and SS1 treatments received 90 kg N ha<sup>-1</sup>, 45 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 78 kg K<sub>2</sub>O ha<sup>-1</sup> to each plot. Soil was disced to 15 cm follow fertilizer application. Potato hilling was done on June 24.

Mustard seed meal (MUSTmeal) was applied prior to planting in the form of partially deoiled oriental mustard seed-meal containing a high level of glucosinolate sinigrin (0.85%), 25% protein, 8% moisture and 4-6% ash content (Mustard Capital Inc., Gravelbourg, SK). Mustard seed meal was broadcasted by hand to the soil surface at a concentration of 9000 kg ha<sup>-1</sup> and incorporated into soil with a rototiller on May 15 2008 (Figure 2.5).



**Figure 2.5.** **A.** Oriental mustard seed meal on the surface of a treated plot on May 15, 2008 at the Carberry site, and **B.** Incorporation of mustard seed meal in Experiment 1.

### 2.3.2. Experiment 2

The second experiment was established on two commercial fields. One field was located near Shilo and the other near Miami, MB. (Figure 2.1). The fields had a history of Verticillium wilt and, relatively high levels of *V. dahliae* microsclerotia in the 0-15 cm depth of soil (44 and 61 microsclerotia g<sup>-1</sup> soil, respectively). The Shilo site was established on a Wheatland sand with a pH<sub>water</sub> 7.0, EC 0.52 dSm<sup>-1</sup> and organic matter

3.6%. The year prior to the start of this study, spring wheat was grown in 2006. In 2007, four treatments were established: three green manure crops and a spring-wheat control (Table 2.1). Plots, 268 m long x 15 m wide, with alleyways between plots of 5 m, were established in a randomized complete block design with three blocks (Appendix I, Figure 5.2A).

One treatment was planted on May 15, 2007 to spring wheat to serve as a crop-control treatment (Control). On May 17, treatments of white mustard and oriental mustard mix (MUSTmix), oat and pea mix (O/PEA), and sorghum-sudangrass (SS) were planted. Plots to oat and pea mix were seeded at a rate of 62 kg ha<sup>-1</sup>, sorghum-sudangrass at 17 kg ha<sup>-1</sup>, and white and oriental mustard mix at 13 kg ha<sup>-1</sup>. The O/PEA treatment received 11 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 11 kg K<sub>2</sub>O ha<sup>-1</sup>. The MUSTmix received an application of 134 kg N ha<sup>-1</sup>, 22 kg S<sub>2</sub>O ha<sup>-1</sup>, 11 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 11 kg K<sub>2</sub>O ha<sup>-1</sup>, and the SS treatment 123 kg N ha<sup>-1</sup>, 11 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 11 kg K<sub>2</sub>O ha<sup>-1</sup>. On July 17 mustards were cut down and incorporated into the first 15cm of soil with a chisel plough. On August 2, oat peas and sorghum-sudangrass were disced right after mowing.

In 2008 all plots were planted to potato, cv Russet Burbank on May 12. Rows were spaced 1 m apart, with 15 rows per plot, and within row, spacing of potato plants was 0.25 m apart. Potato hilling was shortly after emergence, on June 16.

The Miami site was established on clay loam with a pH<sub>water</sub> 8.1, EC 0.64 dSm<sup>-1</sup> and organic matter 2.5%. This field was previously planted to corn, in 2006. In this study, three green manure treatments and spring-wheat (Control) were planted in 2007 in a

randomized completed block design, with three blocks (Table 2.1). Plots were 152 m long x 13 m wide (Appendix I, Figure 5.2B). At the site, a white mustard and oriental mustard mix (MUSTmix), oat and pea mix (O/PEA) and pearl millet (*Pennisetum glaucum* (L.) R Br.) cv Canadian forage pearl millet-101 (MILLET) were planted as green manure treatments on June 7, 2007, at seeding rates of 13, 62 and 10 kg ha<sup>-1</sup>, respectively. Spring-wheat was seeded at a rate of 106 kg ha<sup>-1</sup>, to serve as a control treatment (Control). The pearl millet was cut down 55 days after planting with a flail mower, to encourage new growth, and for the purpose of reducing weed pressure and increasing biomass accumulation. Forty-five days later, the pearl millet green manure was cut and disc incorporated into soil. The MUSTmix and O/PEA treatments were cut down at 50% of flowering, and incorporated into the first 15 cm of soil with a disc plough.

In 2008, all plots were planted to table potato, cv Mozart on May 17, 2008. Rows were spaced 1 m apart, with 12 rows per plot, and within row seed spacing of 0.25 m. Based on soil test analysis, 100 kg N ha<sup>-1</sup>, 78 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 22 kg S<sub>2</sub>O ha<sup>-1</sup> were applied to each plot.

### **2.3.3. *V. dahliae* Survival in Soil**

#### **2.3.3.1. Survival of *V. dahliae* Microsclerotia**

An in-situ soil bioassay was used in Experiment 1 to determine the survival of *V. dahliae* microsclerotia following green manure, seed meal and Vapam treatments. Microsclerotia from a highly pathogenic isolate of *V. dahliae* (vd-1396) to potato (Uppal et al. 2007)

was originally isolated by the Plant Pathology Laboratory, Winnipeg, Manitoba Agriculture and Food, Rural Initiatives. Microsclerotia of *V. dahliae* (vd-1396) were produced in the Soil Ecology Laboratory on semisolid Czapek-Dox medium for 3-4 weeks in the dark at 24°C (Hawke and Lazarovits 1994). The culture was poured through mesh screens to obtain microsclerotia of 75 to 106 µm diameter. The response of microsclerotia to the treatments was determined using microsclerotia mix with silica sand and the mixture added to nylon mesh bags (SAATILON® MONOFILAMENT NYLON) (Tenuta and Lazarovits 2002a). The mesh bags were placed in soil to a 10 cm depth immediately following treatment application and retrieved one and three weeks later. Bags were then dried at room temperature for 12 h and their contents spread onto a pectate–tergitol–agar plate using an Andersen Cascade Impactor (Andersen Instruments Inc., Smyrna, GA). The agar plates were incubated for 5-7 days in the dark at 24 °C and the germination of microsclerotia determined as the percentage of examined microsclerotia that germinated to form colonies (Tenuta and Lazarovits 2002a). Microsclerotia germination was normalized to the germination of the control treatment.

#### **2.3.3.2. *V. dahliae* Propagule Density in Soil**

To study density of *V. dahliae* in soil, a composite soil sample consisting of 20 soil cores were taken randomly from each plot with a 2.5 cm diameter soil probe to a depth of 15 cm, at four times: before green manure crops were planted (May, 2007), approximately two months after incorporation of green manures (September, 2007), prior to planting potato (April, 2009) and immediately after potato harvest (August, 2009). Soil cores from each plot were bulked, placed in polyethylene bags, and transported in an ice chest to the

University of Manitoba. The samples were mixed by hand and stored at 4°C until determination of *V. dahliae* propagule density in soil.

A hundred gram subsample from each soil sample was air-dried for seven days at room temperature and then placed into a paper bag. A 5 g subsample was diluted (1:50) with sterilized agar-water (1%), and agitated for two minutes using an orbital shaker at 60 rpm. A one mL aliquot was placed onto Sorensen's NP-10 medium (Sorensen et al. 1991) containing chloramphenicol, streptomycin sulphate and chlortetracycline HCl. Each sample was plated ten times. Plates were incubated for a minimum of 15 days in the dark at 22°C. Plates were then rinsed with tap water to remove soil particles from the agar surface. The number of germinated *V. dahliae* microsclerotia was counted using a stereomicroscope, and expressed as *V. dahliae* propagules per gram soil (CFU g<sup>-1</sup> soil).

#### **2.3.4. Disease Assessment**

##### **2.3.4.1. Verticillium Wilt Incidence and Severity**

Verticillium wilt incidence was determined late in the season (August 25 to 29, 2008) as the proportion of a number of plants examined with symptoms of Verticillium wilt in each plot. For disease severity, ten plants were dug from one row on each side of each plot. The plant material was transported in an ice chest to The University of Manitoba for analysis. Disease severity by *V. dahliae* was assessed by taking a stem portion from the upper, middle and lower section of the plant. Stem portions were then vertically split in three sections to estimate the percentage of vascular tissue discolored (wilt severity) from each section using reference pictures (Appendix II, Figure 5.3) with a predetermined



scale of 0 to 5, in which 0= 0%, 1= 1 to 10 %, 2= 11 to 30 %, 3 =31 to 50%, 4 = 51 to 75%, and 5 = 76 to 100% discoloration (Uppal et al. 2008).

### **2.3.5. Plant and Soil Assessments**

#### **2.3.5.1. Leaf Chlorophyll Content**

Leaf chlorophyll content was measured at the time of disease determinations, using a non-destructive portable chlorophyll meter (SPAD-502 Chlorophyll Meter, Minolta Camera Co., Ltd., Japan). The meter determined the difference in light attenuation wavelengths of 430 and 750 nm, and gives a numerical SPAD (Soil Plant Analysis Development) unit, ranging from 0 to 50, with 0 being completely chlorotic and 50 being strong green.

#### **2.3.5.2. Soil Chemical Properties**

For soil chemical analysis, three cores per plot were taken at two depths 0-15 cm for sodium bicarbonate extractable-P, ammonium acetate extractable K, pH and electrical conductivity, and 15-60 cm for the mobile nutrients,  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$ . The three soil cores from each plot were bulked, placed in polyethylene bags, and transported in an ice chest to the University of Manitoba, where each bag was mixed by hand and stored at 4°C. Samples were stored for no more than 24h before sending to the Bodycote Laboratory (Winnipeg, MB.) for analysis.

#### **2.3.5.3. Potato Yield and Quality**

For experiment 1, yield was expressed as metric tonne ha<sup>-1</sup>. Crop yield was determined by harvesting the two central rows of each plot. Experimental plots were mechanically harvested. Potato tubers were placed in open fiber bags, transported to the CMCDC station and stored at 5 °C until sample processing. Samples were then weighed to determine weight loss and washed. Based on tuber weight analysis, the size categories for marketable yield were: non-marketable tubers <85 g, regular 86-170 g, bonus 170.1-340 g and overweigh >340 g. Specific gravity was determined using the weight in air and water method, fry colour analysis by using USDA Fry Colour Chart, green colour, rot and hollow heart by tuber observation, and sugar end analyses by frying and comparing colour with a standard chart.

Potatoes were harvested manually for experiment 2. A three meter section from three central rows was dug by hand using a potato fork. Potato tubers were placed in open fiber bags, transported to the University of Manitoba, washed and weighed. Tubers were then manually graded into size categories: undersize <45 mm, marketable 45-50 mm and 51 mm diameter, for cv Russet Burbank; and small <55 mm, medium 55-88 mm and oversize >88 mm diameter for table potato cv Mozart. The difference in grading technique was because potato cv Russet Burbank was destined for processing and cv Mozart for table stock.

### **2.3.6. Statistical Analysis**

All statistical analyses were performed with the Statistical Analysis Software (SAS) (SAS Institute, Cary, NC; release 9.1 for Windows). Prior to analysis, *V. dahliae*

propagule density in soil, disease incidence and severity, and marketable yield data sets were checked for normality (PROC Univariate). Survival of microsclerotia was determined at different times due to the different incorporation time of the green manure and application of the seed meal amendment and fungicide. A control measurement was made for each time. Therefore, survival of microsclerotia is presented as percentage of the Control (microsclerotia germination of Control = 100%) in order to compare the reduction between treatments. The data were analyzed by analysis of variance (ANOVA-PROC MIXED). A mixed-model analysis for each individual sampling time was performed using PROC MIXED. Treatment was considered a fixed effect and block a random effect. Treatment means were separated using the Bonferroni's procedure if the F-test was significant ( $P < 0.05$ ). Contrasts were used to test the significance of differences between groups of treatments, particularly when significant interactions were found. Pearson correlation analysis was performed to examine the relationships between disease incidence, propagule density and measured soil characteristics.

## **2.4. Results**

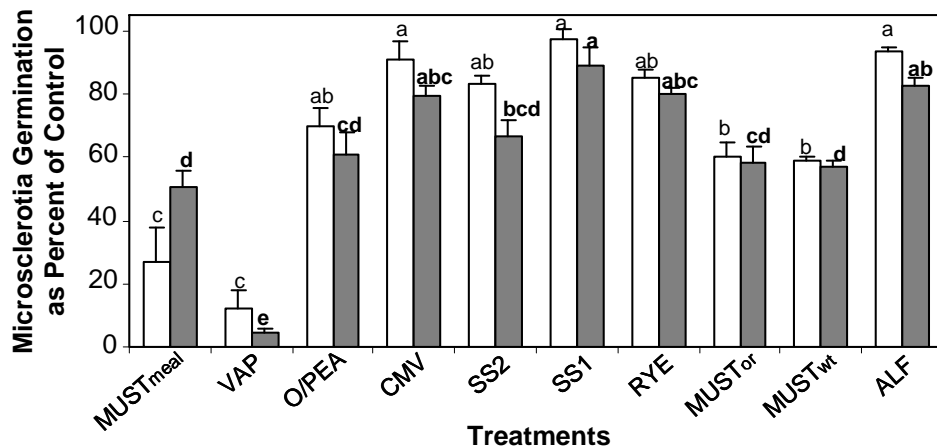
### **2.4.1. Experiment 1**

#### **2.4.1.1. Survival of *V. dahliae* Microsclerotia**

The bioassay results showed germination of *V. dahliae* microsclerotia was weak by treatment ( $P < 0.0001$ ) and time from treatment application ( $P < 0.006$ ). In general, percentage germination of microsclerotia relative to the control was lower at three weeks than one week post treatment, except for MUSTmeal treatment which increased slightly

after three weeks (Figure 2.6). VAP and MUSTmeal were the most effective treatments for reducing germination of microsclerotia ( $P < 0.0001$ ). One week following application, MUSTwh, MUSTor and O/PEA reduced germination of microsclerotia by about 40 % compared to the crop control. In contrast, CMV, SS1, SS2, RYE and ALF treatments did not affect the germination of *V. dahliae* microsclerotia (Figure 2.6).

Three weeks after application, Vapam was the most effective treatment reducing germination of microsclerotia to 5 % of the crop control ( $P < 0.0001$ ). In contrast, MUSTmeal treatment was only partially effective after three weeks with the germination of microsclerotia being 24% of the crop control (Figure 2.6)



**Figure 2.6.** Bioassay results for the effect of mustard seed meal, Vapam and green manures on germination of *V. dahliae* microsclerotia, expressed as a percentage of germination of microsclerotia in wheat control plots at 1 week (□) and 3 weeks (■) after application at the Carberry site. MUST<sub>meal</sub>= mustard seed meal, VAP= Vapam, O/PEA= oat/peas mix, CMV= Canada milk vetch, SS2= 2-year sorghum-sudangrass, SS1= 1-years sorghum-sudangrass, RYE= fall rye, MUST<sub>or</sub>= oriental mustard, MUST<sub>wh</sub>= white mustard, and ALF= alfalfa. Means followed by same letter are not significantly different ( $P < 0.05$ ) according to the Bonferroni's multiple comparison test. Error bars represent +1 standard error.

Germination of *V. dahliae* microsclerotia decreased slightly with green manure treatments ranging from 60 to 90% of the crop Control. However, germination of microsclerotia in O/PEA, SS2, MUSTor and MUSTwh treatments was not significantly different from the MUSTmeal treatment. Although the germination of microsclerotia observed in SS1, ALF, RYE and CMV treatments was not significantly different from other green manure treatments, it was significantly higher than MUSTmeal ( $P < 0.05$ ).

#### **2.4.1.2. *V. dahliae* Propagule Density in Soil**

Mean propagule densities of *V. dahliae* in spring and fall 2007 were 6 and 8 CFU g<sup>-1</sup> soil, respectively. Propagule density was not affected by incorporation of green manures in September 2007. However, contrast analysis showed lower density in MUSTwh and MUSTor compared to other green manure treatments ( $P = 0.0296$ ) (Table 2.2).

Propagule density in soil also differed with treatment in the spring and fall of 2008 ( $P = 0.0001$ ). In spring 2008, green manure treatments did not reduce the density of propagules in soil compared with the Control. MUSTwh, MUSTor and O/PEA treatments maintained low propagule density ranging from 3 to 7 CFU g<sup>-1</sup> soil. The application of Vapam in Fall-2007 reduced the density of propagules in soil from 9 to 3 CFU g<sup>-1</sup> soil. Propagule density in VAP plots was significantly lower compared to Control plots ( $P < 0.0001$ ) and green manure plots ( $P = 0.0006$ ) (Table 2.2).

**Table 2.2. Experiment 1. Effect of wheat, green manure, organic amendment and Vapam treatments on propagule density of *V. dahliae* (CFU g<sup>-1</sup> soil) and Verticillium wilt incidence at the Carberry site.**

Treatment	Propagule density (CFU g <sup>-1</sup> soil)				Incidence (%)
	2007		2008		2008
	May	Sept.	April	August	August
Control	3 ±1 <sup>v</sup>	5 ±2	13 ±3 a	27 ±2 ab	57 ±11 abc
COM	10 ±4	6 ±1	9 ±3 ab	12 ±3 cd	25 ±3 bc
MUSTmeal	10 ±6	11 ±2	15 ±2 a	10 ±1 d	13 ±7 c
VAP	4 ±2	9 ±5	3 ±1 b	24 ±3 abc	70 ±11 ab
O/PEA	11 ±5	9 ±2	7 ±2 ab	14 ±4 bcd	47 ±10 abc
CMV	6 ±2	12 ±6	12 ±4 ab	21 ±2 abcd	48 ±2 abc
SS2	4 ±3	4 ±1	11 ±2 ab	25 ±4 abc	93 ±7 a
SS1	6 ±3	11 ±7	14 ±3 a	18 ±2 abcd	62 ±7 ab
RYE	4 ±2	12 ±3	18 ±4 a	36 ±7 a	70 ±10 ab
MUSTor	8 ±3	7 ±4	7 ±2 ab	15 ±3 bcd	52 ±16 abc
MUSTwh	3 ±1	3 ±2	3 ±1 b	17 ±2 abcd	52 ±6 abc
ALF	7 ±1	7 ±3	12 ±2 ab	20 ±1 abcd	62 ±13 ab
<i>P</i> > <i>F</i>	ND	ND	0.0002 <sup>w</sup>	0.0001	0.0001
Selected contrasts (significant probability).					
(WHEAT) <sup>z</sup> vs Green manures	ND	ND <sup>x</sup>	0.0382	NS <sup>y</sup>	NS
(WHEAT) vs VAP	ND	ND	<0.0001	ND	ND
Vap vs Green manures	ND	ND	0.0006	NS	NS
Control vs COM	ND	NS	NS	0.0004	0.018
Control vs MUSTmeal	ND	ND	ND	<0.0001	0.0017
COM vs Green manures	ND	ND	ND	0.0022	0.0007
MUSTmeal vs Green manure	ND	ND	ND	0.0004	<0.0001
Mustards vs MUSTmeal	ND	ND	ND	0.0332	0.0014
Mustards vs Non-mustards	ND	0.0296	0.0002	0.0247	NS

<sup>v</sup> Value are means ± 1 standard error. Within columns means followed by the same letters are not significantly different ( $P \leq 0.05$ ) according to the Bonferroni's multiple comparison test.

<sup>w</sup> Treatment significant probability.

<sup>x</sup> ND, no determined.

<sup>y</sup> NS, no significant ( $P > 0.05$ ).

<sup>z</sup> Control= crop control, COM= compost, MUST<sub>meal</sub>= mustard seed meal, VAP= Vapam, O/PEA= oat/peas mix, CMV= Canada milk vetch, SS2= 2-year sorghum-sudangrass, SS1= 1-years sorghum-sudangrass, RYE= fall rye, MUSTor= Oriental mustard, MUSTwh= white mustard, and ALF= alfalfa. (WHEAT)= treatments not established, but planted with spring-wheat; mustards= white and oriental mustard; non-mustards= oat/peas, Canada milk vetch, 1- and 2-years Sorghum-sudangrass, fall rye and alfalfa.

After the potato season, the density of propagules in soil increased with all treatments, except for MUSTmeal and COM (Table 2.2). Contrast analyses showed that propagule density was lower with MUSTmeal ( $P < 0.0001$ ) and COM ( $P = 0.0004$ ) treatments compared to crop Control. The application of Vapam did not reduce the density of propagules compared to crop Control and green manure treatments. No significant difference was found between RYE, SS2, VAP, and Control treatments, which had the highest densities ranging from 24 to 36 CFU g<sup>-1</sup> soil (Table 2.2).

Propagule density in soil was lower in MUSTmeal and COM plots compared to Control plots ( $P = 0.0001$ ). Moreover, MUSTmeal plots had fewer CFU g<sup>-1</sup> soil at the end of the potato season compared to plots fumigated with VAP ( $P < 0.0001$ ) or green manure treatments ( $P = 0.0004$ ). Contrast analysis showed that COM treatment had significant lower density of propagules in soil compared to green manure treatments ( $P = 0.0022$ ).

#### **2.4.1.3. Disease Incidence and Severity**

Statistical analysis of wilt incidence data revealed significant effects of treatments ( $P = 0.0001$ ). Lowest disease incidence was 13 % and 25 % with MUSTmeal and COM treatments, respectively, and the highest was 93 % with SS2 treatment. Potato plants from plots fumigated with Vapam showed disease incidence levels of approximately 70 % which was not significantly less than in the crop Control treatment. Contrasts analyses showed significant differences between green manure treatments and MUSTmeal ( $P < 0.0001$ ) and COM ( $P = 0.0007$ ) treatments. Contrast analyses also showed significantly lower incidence in MUSTmeal than in Brassicaceae green manure treatments ( $P$

<0.0014). Incidence of Verticillium wilt was not reduced by any of the green manure treatments compared to the Control. Conversely, incidence increased with SS1, SS2, RYE, and ALF treatments (Table 2.2).

No green manure, organic amendment or chemical treatment reduced disease severity, at the lower, middle and upper section of the potato stems, compared to the crop Control ( $P < 0.05$ ). Severity as stem discoloration ranged from 11 to 45 %, 1 to 25 %, and less than 1 % for lower, middle and upper potato stem sections, respectively (Appendix II, Table 5.1). No significant difference was observed between treatments for chlorophyll concentration as determined with SPAD meter. Chlorophyll content ranged from 37 to 42 SPAD value (Appendix II, Table 5.1).

#### **2.4.1.4. Plant and Soil Assessment**

##### **2.4.1.4.1. Effects on Soil Properties**

In general, organic amendments and green manures promoted significant changes in soil fertility levels and potato yield. MUSTmeal treatment significantly increased  $\text{NO}_3^-$ -N ( $203 \text{ kg ha}^{-1}$ ) in soil by approximately 5 fold compared to the Control ( $P = 0.0001$ ) (Table 2.3). Plant available-P concentration was significantly greater in COM ( $65 \text{ kg ha}^{-1}$ ) than in green manure treatments ( $P < 0.0001$ ) or any other treatment except for MUSTmeal. MUSTmeal treatment also increased the availability of phosphorus in soil ( $45 \text{ kg ha}^{-1}$ ) compared to green manure treatments ( $P = 0.0105$ ). Total  $\text{SO}_4^{2-}$ -S concentration was greatest in MUSTmeal and VAP treatments ( $142$  and  $139 \text{ kg ha}^{-1}$ , respectively) compared to all other treatments ( $P = 0.0001$ ) (Table 2.3).



**Table 2.3. Experiment 1. Water soluble nitrate-N (NO<sub>3</sub><sup>-</sup>-N), sodium bicarbonate extractable-P, ammonium acetate extractable-K, water soluble S (SO<sub>4</sub><sup>-2</sup>), pH and electrical conductivity (EC) in the soil following potato harvest, September 2008.**

Treatment	NO <sub>3</sub> <sup>-</sup> -N	P	K	SO <sub>4</sub> <sup>-2</sup> -S	pH	EC
	kg ha <sup>-1</sup>					dS m <sup>-1</sup>
Control	49±6 b <sup>x</sup>	31±2 b	285±9	28±2 b	6.0±0.1	0.19±0.01 b
COM	73±19 b	65±11 a	455±81	61±9 b	6.7±0.2	0.35±0.05 ab
MUSTmeal	203±33 a	45±9 ab	388±74	142±28 a	5.9±0.2	0.52±0.10 a
VAP	66±17 b	31±3 b	307±8	139±21 a	6.1±0.1	0.27±0.02 ab
O/PEA	66±9 b	36±2 b	304±10	38±3 b	6.0±0.2	0.19±0.02 b
CMV	54±10 b	36±4 b	425±84	42±2 b	6.3±0.1	0.20±0.02 b
SS2	117±17 b	29±2 b	315±18	34±3 b	6.1±0.1	0.22±0.02 b
SS1	58±11 b	31±3 b	330±35	47±6 b	6.5±0.3	0.34±0.09 ab
RYE	88±11 b	29±1 b	351±28	36±3 b	6.3±0.2	0.26±0.03 ab
MUSTor	48±8 b	30±6 b	269±9	54±13 b	6.4±0.2	0.26±0.04 ab
MUSTwh	57±4 b	31±3 b	300±15	65±13 b	6.0±0.1	0.21±0.02 b
ALF	116±22 b	38±5 b	363±63	41±5 b	6.6±0.2	0.30±0.03 ab
<i>P &gt; F<sup>y</sup></i>	0.0001	0.0001	NS	0.0001	NS	0.0016
Selected contrasts (significant probability)						
VAP vs Green manures	NS	NS		<0.0001		NS
COM vs Green manures	NS	<0.0001		NS		NS
MUSTmeal vs Green manure	<0.0001	0.0105		<0.0001		<0.0001
Mustards vs MUSTmeal	<0.0001	0.0108		<0.0001		<0.0001
Mustards vs Non-mustards <sup>z</sup>	0.0094	NS		0.0406		NS

<sup>x</sup> Value are means ± 1 standard error. Within columns means followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to the Bonferroni's multiple comparison test.

<sup>y</sup> Treatment significant probability.

<sup>z</sup> Control= wheat-control, COM= compost, MUST<sub>meal</sub>= mustard seed meal, VAP= Vapam, O/PEA= oat/peas mix, CMV= Canada milk vetch, SS2= 2-year sorghum-sudangrass, SS1= 1-years sorghum-sudangrass, RYE= fall rye, MUSTor= Oriental mustard, MUSTwh= white mustard, and ALF= alfalfa. Mustards= white and oriental mustard; non-mustards= oat/peas mix, Canada milk vetch, 1- and 2-years Sorghum-sudangrass, fall rye and alfalfa.

Increases in plant available nutrients were correlated with Verticillium wilt development. Wilt incidence was significantly and negatively correlated (Pearson correlation analysis) with available-P ( $r=-0.49$   $P=0.0004$ ), K ( $r=-0.42$   $P=0.0027$ ) and  $\text{SO}_4^{2-}$ -S ( $r=-0.38$   $P=0.008$ ). In addition,  $\text{SO}_4^{2-}$ -S was inversely related to propagule density of *V. dahliae* ( $r=-0.30$   $P=0.04$ ). The incorporation of green manure and organic amendments into soil had no effect on soil pH (Table 2.3). No significant difference in EC was observed among green manure, COM and the Control treatments (EC ranged from 0.19 to 0.35 dS  $\text{m}^{-1}$ ). However, MUSTmeal treatment showed a significant increase (0.52 dS  $\text{m}^{-1}$ ) compared to the Control treatment ( $P=0.0016$ ) (Table 2.3).

#### **2.4.1.4.2. Marketable Yield and Tuber Quality**

A significant treatment effect was observed for total bonus and overweight marketable yield (Table 2.4), but not for tuber quality (fry colour, specific gravity and sugar ends of tubers) (Appendix III, Table 5.2).

**Table 2.4. Experiment 1. Effect of wheat, green manure, organic amendments and Vapam treatment on marketable potato yield.**

Treatment	Marketable yield (tonne ha <sup>-1</sup> ) <sup>v</sup>			
	Regular	Bonus	Overweight	Total
Control	11.0±0.9 <sup>w</sup>	16.1±1.1 ab <sup>y</sup>	3.7±1.3 ab	30.8±1.6b
COM	10.5±0.9	19.5±2.1 a	8.7±1.8 a	38.7±1.3 a
MUSTmeal	8.9±0.4	14.0±1.3 ab	4.3±0.6 ab	27.2±1.8b
VAP	13.3±1.0	16.6±1.1 ab	3.2±1.0 ab	33.1±1.6ab
O/PEA	9.5±0.8	16.9±0.8 ab	5.9±1.4 ab	32.3±1.2ab
CMV	10.9±0.3	15.2±0.9 ab	6.0±1.4 ab	32.0±0.3ab
SS2	11.7±0.3	11.4±1.2 b	2.9±1.6 b	26.0±1.6b
SS1	11.1±1.3	14.8±1.2 ab	4.9±0.4 ab	30.8±2.3b
RYE	11.0±0.9	14.5±1.6 ab	5.6±1.7 ab	31.2±2.6ab
MUSTor	11.7±1.4	15.5±1.5 ab	3.5±1.0 ab	30.6±1.4b
MUSTwh	11.7±0.8	15.4±1.1 ab	5.4±0.9 ab	32.6±1.7ab
ALF	10.1±0.4	15.4±0.9 ab	7.2±0.9 ab	32.6±1.2ab
<i>P</i> > <i>F</i> <sup>x</sup>	NS	0.0321	0.0365	0.0003
Selected contrasts (significant probability)				
Control vs Green manures		NS <sup>y</sup>	NS	NS
VAP vs Green manures		NS	NS	NS
COM vs Green manures		0.0016	0.0072	<0.0001
MUSTmeal vs Green manure		NS	NS	0.0200
Mustards vs MUSTmeal		NS	NS	0.0201
Mustards vs Non-mustards <sup>z</sup>		NS	NS	NS

<sup>v</sup> Potato tubers graded into weight categories for marketable yield: regular 86-170 g, bonus 170.1-340 g and overweight >340 g.

<sup>w</sup> Value are means ± standard error. Within columns means followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to the Bonferroni's multiple comparison test.

<sup>x</sup> Treatment significant probability.

<sup>y</sup> NS, no significant ( $P > 0.05$ ).

<sup>z</sup> Control= wheat-control, COM= compost, MUST<sub>meal</sub>= mustard seed meal, VAP= Vapam, O/PEA= oat/peas mix, CMV= Canada milk vetch, SS2= 2-year sorghum-sudangrass, SS1= 1-years sorghum-sudangrass, RYE= fall rye, MUSTor= Oriental mustard, MUSTwh= white mustard, and ALF= alfalfa. Mustards= white and oriental mustard; non-mustards= oat/peas mix, Canada milk vetch, 1- and 2-years sorghum-sudangrass, fall rye and alfalfa.

Total marketable yield of potato cv Russet Burbank in plots amended with COM treatment were 26 and 30 % higher than the crop Control and MUSTmeal treatments ( $P = 0.0003$ ). Contrasts analyses showed that the increase in total marketable yield with COM treatment ranged from 17 to 50% compared to green manure treatments (Table 2.4). Bonus marketable (170.1-360 g weight category), which bring a premium price, increased as a result of COM treatment, with increases ranging from 15 to 72 %, compared to other treatments (Table 2.4).

Contrast analysis indicated that bonus ( $P = 0.0016$ ) and overweight marketable ( $P = 0.0072$ ) tuber classes were significantly higher in COM compared to green manure treatments. Total marketable yield was correlated with some disease and soil factors. Marketable yield was inversely associated with incidence of *Verticillium* wilt ( $r = -0.39$ ,  $P < 0.0063$ ) and positively associated with available-P ( $r = 0.33$ ,  $P < 0.0221$ ) and K ( $r = 0.32$ ,  $P < 0.0243$ ). Tuber quality attributes (Green colour, rot damage, hollow heart, specific gravity, sugar end, dark end and fry colour) were not affected by the treatments (Appendix III, Table 5.2.).

## **2.4.2. Experiment 2**

### **2.4.2.1. Effect on Propagule Density and *Verticillium* Wilt Incidence**

The incorporation of green manures into soil did not affect propagule density of *V. dahliae* or disease incidence at both study sites (Table 2.5) Nonetheless, a time of sampling effect was observed at the Shilo ( $P < 0.05$ ) and Miami ( $P < 0.0001$ ) sites. At the Miami site, differences between treatments were observed at the beginning of the potato

season in April 2008 ( $P < 0.0001$ ), when the MUSTmix treatment reduced propagule density compared to the Control treatment (Table 2.5). *Verticillium* wilt incidence was not significantly affected by the treatments.

**Table 2.5. Experiment 2. Effect of wheat and green manure treatments on soil propagule density of *V. dahliae* (CFU g<sup>-1</sup> soil) and *Verticillium* wilt incidence.**

Site / potato cv.	Treatment	Propagule density (CFU g <sup>-1</sup> soil)				Incidence (%) 2008
		2007		2008		
		May	Sept.	April	August	August
	Control <sup>w</sup>	52±14 <sup>x</sup>	61±13	58±10	51±3	100±0
Shilo / Russet Burbank	MUSTmix	45±2	59±13	49±10	43±10	87±11
	O/PEA	47±8	56±9	51±8	43±2	93±5
	SS	49±1	73±21	64±15	49±15	78±10
	$P > F$ <sup>y</sup>	NS <sup>z</sup>	NS	NS	NS	NS
Miami / Mozart	Control	55±5	71±1	68±1 a <sup>z</sup>	73±2	63±6
	MUSTmix	54±2	49±1	37±4 b	66±6	55±12
	O/PEA	57±15	51±10	45±6 ab	58±8	50±10
	MILLET	67±3	59±2	50±3 ab	53±5	46±9
	$P > F$	NS	NS	0.0001	NS	NS

<sup>w</sup> Control= wheat-control, MUSTmix= white and oriental mustard mix, O/PEA= oat/peas mix, SS= sorghum-sudangrass, and MILLET= pearl millet.

<sup>x</sup> Value are means ± 1 standard error. Within columns means followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to the Bonferroni's multiple comparison test.

<sup>y</sup> Treatment significant probability.

<sup>z</sup> NS, no significant ( $P > 0.05$ ).

#### 2.4.2.2. Effect on Potato Marketable Yield and Tuber Quality

At both sites, yield of potato was generally unaffected by crop Control and green manure treatments. However, at the Shilo site, the SS treatment increased regular marketable yield by 50% compared to Control treatment ( $P < 0.02914$ ) (Table 2.6).

**Table 2.6. Experiment 2. Effect of wheat and green manure treatments on Verticillium wilt incidence and marketable yield of potato.**

Location / potato cv	Treatments	Yield (tonne ha <sup>-1</sup> ) <sup>v</sup>		
		Underweight	Regular	Overweight
Shilo / Russet Burbank	Control <sup>w</sup>	9.2±1.0 <sup>x</sup>	20.0±2.4 a <sup>x</sup>	4.7±1.4
	MUSTmix	13.2±1.0	28.3±0.7 ab	2.4±1.1
	O/PEA	12.4±0.6	28.3±3.2 ab	2.5±0.7
	SS	20.2±4.2	30.0±1.1 b	2.0±0.9
<i>P</i> > <i>F</i> <sup>y</sup>		NS <sup>z</sup>	0.0291	NS
Miami / Mozart		Small	Medium	Overweight
	Control	29.3±3.8	4.2±1.8	0±0
	MUSTmix	31.6±2.4	5.2±2.1	0.1±0.1
	O/PEA	30.5±0.8	5.4±1.2	0.4±0.4
	MILLET	32.0±2.9	7.5±0.7	0±0
<i>P</i> > <i>F</i>		NS	NS	NS

<sup>v</sup> Potato tubers graded into size categories for cv Russet Burbank: undersize 45 mm, marketable 45-51mm and 51 mm diameter; and tuber size categories for table potato cv Mozart: small <55 mm, medium 55.1-88 mm and oversize >88 mm.

<sup>w</sup> Control= wheat-control, MUSTmix= white and oriental mustard mix, O/PEA= oat/peas mix, SS= sorghum-sudangrass, and MILLET= pearl millet.

<sup>x</sup> Value are means ± 1 standard error. Means followed by the same letters within each site and yield category are not significantly different ( $P \leq 0.05$ ) according to the Bonferroni's multiple comparison test.

<sup>y</sup> Treatment significant probability.

<sup>z</sup> NS, no significant ( $P > 0.05$ ).

## 2.5. Discussion

Green manure, and organic amendments such as compost and mustard seed meals have been proposed as alternative method to chemical control of soil-borne plant pathogens (Janvier et al. 2007). These strategies aim to prevent or decrease the development of disease problems caused by soil-borne pathogens, by the provision of soil conditions that are optimal for crop growth and unsuitable for pathogen survival (Litterick et al. 2004). In this research, a variety of green manure crops and organic amendments, such as

mustard seed meal and composted beef cattle manure, were assessed for their ability to kill *V. dahliae* microsclerotia, to reduce Verticillium wilt incidence and ultimately, to increase potato yield.

### **2.5.1. Survival of *V. dahliae* Microsclerotia.**

VAP and MUSTmeal were the most effective treatments reducing germination of microsclerotia within one week after incorporation. The efficacy of the soil fumigant to reduce germination of *V. dahliae* in soil planted to potato has been reported by Fravel (1996). The effect of Vapam on microsclerotia has been also correlated with decreased growth rates and reduced pathogenicity of *V. dahliae* (Engelkes and Fravel 1997). The efficacy of Vapam reducing germination of microsclerotia depends on its conversion in the soil to very active and toxic ITCs compounds. In moist soil, Vapam decomposes rapidly to methyl-isothiocyanate (methyl-ITC), which constitutes 90% of the conversion products in soil (Leistra et al. 1974).

The suppressive activity of the MUSTmeal treatment may be associated with the presence of glucosinolate hydrolysis products. The partially deoiled mustard seed meal used contained sinigrin glucosinolate, which decomposes into 2-propenyl isothiocyanate (2-propenyl-ITC). One of the most biologically active forms of ITCs, 2-propenyl-ITC has been shown to kill soil pathogens like *V. dahliae* (Olivier et al. 1999). Although MUSTmeal treatment reduced the germination of microsclerotia 1 week after incorporation, the germination increased 30% 2 weeks later.

This results suggest that fungitoxicity of ITCs could be induced by increasing concentration or exposure time of microsclerotia to ITCs. When the microsclerotia were recovered from soil and taken to the laboratory for growth on selective media, it was expected that the media would contain all necessary requirements to stimulate cell growth. However, it is common that some organisms weakened by sublethal treatments remain viable but not culturable, which means that some cells will require special stimuli to return to the culturable state (Weichart and Kjelleberg 1996). This condition is often observed in injured organisms, which fail to grow under selective isolation conditions. However, when injured organisms are removed from the sublethal conditions, the microorganisms restore their ability to grow in culture (Weichart and Kjelleberg 1996). For example, exposure of microsclerotia to sublethal concentrations of liquid swine manure (LSM) or volatile fatty acids (VFA) (Tenuta et al. 2002),  $\text{NH}_3$  and  $\text{HNO}_2$  (Tenuta and Lazarovits 2002b, 2004) followed by immediate plating onto germination medium resulted in a lower germination of microsclerotia. However, placement of the microsclerotia in soil free of LSM, VFA,  $\text{NH}_3$  or  $\text{HNO}_2$  for about seven days allows them to recover from the presumed stress of sublethal concentrations, resulting in a moderate rebound of germination of microsclerotia upon retrieval.

The findings reported here provide evidence that exposure to sublethal concentrations of ITC sufficiently weaken microsclerotia that they do not germinate on selective media. Once the ITC concentration dissipated in soil, microsclerotia regained attributes to tolerate placement onto plate medium and grow. Similar results were suggested by Smolinska et al (2003) who found that *F. oxisporum* isolates exposed to low



concentrations of pure 2-propeny-ITC (0.3  $\mu$ l) for 24 h did not grow on plate medium until the medium was cleared of ITCs.

MUSTwh, MUSTor and O/PEA treatments inhibited germination of microsclerotia to 60% of the control. These results suggest that these green manures show some promise as a means to reduce *V. dahliae* propagule pressure in soil. Reduction of germination of artificially produced microsclerotia has been observed with several green manures (Lopez-Escudero et al. 2007). Brassicaceae green manure crops have been recently used to reduce soil populations of *V. dahliae* and incidence of Verticillium wilt in potato (Rowe and Powelson 2002) due to the release of fungicidal compounds during the hydrolysis of glucosinolates, most reported, ITCs. The efficacy of Brassicaceae green manures to kill the pathogen depends on the concentration ITC released from the plant material (shoot tissue, roots or seed meal) (Olivier et al. 1999). Although concentration of glucosinolates in plant shoot is higher at the start of flowering and declines at seed filling and end of flowering (Sarwar and Kirkegaard 1998), the concentration is usually much higher in seed (26 mg g<sup>-1</sup>) than in other part of the plant (5.3 mg g<sup>-1</sup>) (Zrybko et al. 1997). High concentration of ITC in soil is expected in soils where high glucosinolate-containing tissues are added (Morra and Kirkegaard 2002). However, the most important factor limiting ITC concentration in soil is thus not glucosinolate concentration in the amendment itself, but release from the amendment to the soil. High release of ITC in soil occurs when cell membranes are broken (Gimsing and Kirkegaard 2009). The level of physical disruption necessary to maximize ITC is generally impossible with green manure crops (Matthiessen and Kirkegaard 2006). However, such a limitation does not

exist when using seed meal amendments since the seed crushing procedure has resulted in extensive cellular disruption (Brown and Morra 2005). Therefore, the concentration of ITCs released by the mustard seed meal may be 10 times higher than from intact vegetative plant tissue (roots and aerial parts) (Kirkegaard et al. 1996; Brown and Morra 2005). Therefore, we can hypothesize that the higher concentration of ITCs released by the MUSTmeal was responsible for greater inhibition of germination of *V. dahliae* microsclerotia in soil.

Fungitoxic compounds other than ITCs also likely contribute to lowering of germination of *V. dahliae* microsclerotia. Oat/pea green manure may reduce propagule density of soil-borne plant pathogens in potato by releasing fungitoxic compounds such as avenacin, saponins and civine. Avenacin and saponins are fungitoxic compounds released from oat tissue whereas civine is found in pea tissue (Pavlík et al. 2002; Wiggins and Kinkel 2005b). Those compounds can produce lysis and inhibition of germination of soil-borne pathogens (Deacon and Mitchell 1985; Pavlík et al. 2002).

### **2.5.2. Reduction of Propagule Density and Verticillium Wilt.**

In general, green manure crops did not reduce the germination of microsclerotia or propagule density of *V. dahliae*, compared to the Control. In experiment 2, green manure treatments did not reduce propagule density or Verticillium wilt incidence. In fact, some green manure treatments such as RYE and SS2 increased propagule density and Verticillium wilt incidence. The lack of effect of the green manure treatments to reduce

propagule density of *V. dahliae* and Verticillium wilt incidence may be due to several issues. Factors such as number of green manure cycles (Rowe and Powelson 2002) or insufficient physical disruption of plant tissue during plow down that lead to low released of toxic compounds (Morra and Kirkegaard 2002). These factors are relatively important on long-term survival structures of plant pathogens in soil, particularly, microsclerotia of *V. dahliae*, which are the major means of soil survival for this pathogen (Rowe and Powelson 2002).

These results are consistent with those of Davis et al. (1996) who found that one or two consecutive years of sudangrass, oat, rye, or corn green manure did not reduce Verticillium wilt incidence in potato. Green manure crops can reduce density of propagules in soil and Verticillium wilt incidence in potato (Lopez-Escudero et al. 2007; Ochiai et al. 2008). However, reduction of Verticillium wilt of potato with green manure requires more than two consecutive years of incorporation (Davis et al. 1996; Rowe and Powelson 2002).

Several mechanisms other than ITCs could be responsible for reducing Verticillium wilt. Green manure can also release toxic compound like hydrogen cyanide (HCN) (Widmer and Abawi 2000), that can be toxic to specific organisms, such as nematodes. LaMondia (1999) suggest that one or two year of green manure might reduce Verticillium wilt in soils where the disease is greatly increased by co-infection of *V. dahliae* and the root lesion nematode, *Pratylenchus penetrans* by reducing populations of the nematode. Several other studies have shown that short periods of green manure are effective against

nematodes. Sorghum-sudangrass has been effective to prevent damage of plants by the nematode *Meloidogyne* spp (Mojtahedi et al. 1993). Wiggins and Kinkel (2005a) found that HCN was the primary factor responsible for the suppression of *M. hapla* with one year of sorghum-sudangrass green manure. The root lesion nematode *Pratylenchus neglectus* was killed in a short-term greenhouse study by Brassicaceae green manure (Potter et al. 1998).

Although microsclerotia partially restored their ability to germinate three weeks after incorporation of MUSTmeal, germination was reduced by 50%. Moreover, Verticillium wilt incidence and propagule density of *V. dahliae* were effectively reduced by MUSTmeal. It can be hypothesized that glucosinolate hydrolysis products could reduce germination of microsclerotia, as well as *V. dahliae* propagule concentration in soil. Soil pathogen suppression using seed meal has been attributed to the toxicity of glucosinolate hydrolysis products (Brown and Morra 1997). However, the mechanisms by which Brassicaceae green manure and seed meals may suppress soil-borne pathogens are varied and often unknown (Wiggins and Kinkel 2005a). MUSTmeal might reduce propagule density indirectly by influencing indigenous microbial population through the compounds released directly from the meal upon addition or during its decomposition. For example, Njoroge et al. (2008) detected ITCs in amended soil with Brassicaceae materials and observed increased population densities of fluorescent pseudomonads, an antifungal bacteria antagonist against *V. dahliae* (Pegg and Brady 2002).

Incorporation of Brassicaceae seed meals significantly increase populations of *Streptomyces* spp (Cohen et al. 2005), which, are associated with reduction of *V. dahliae* (Krechel et al. 2002). The antagonistic effect of *Streptomyces* spp. on soil pathogens has been attributed to their release of antibiotics and other secondary metabolites (Cohen et al. 2005). This mechanism of control could explain the consistent reduction of propagule density observed before and after the potato season in plots amended with mustard seed meal.

The application of Vapam reduced propagule density of *V. dahliae* at the beginning of the potato season in 2008. However, severe incidence of Verticillium was observed on potato, as well as an increased number of propagules after the potato harvest with Vapam treatment. The biocidal activity of Vapam is provide by methyl isothiocyanate (methyl-ITC) (Gerstl et al. 1977), a very active ITC with high vapor pressure (16.0 mmHg at 20°C), and thereby elevated potential for volatilization (Zheng et al. 2006). This suggests that even with a higher application rate (762 L ha<sup>-1</sup>) than those being used in potato for control of Verticillium wilt at 600 L ha<sup>-1</sup> (Tsrer et al. 2005), the Vapam treatment was not effective for pathogen control. The lack of effectivity is possibly due to the volatilization loss of methyl-ITC in the upper soil profile, where a large amount of wheat residues (Figure 2.3), may prevent proper sealing of the soil, therefore methyl-ITCs could escape to the atmosphere. Ideal conditions for effective control of pathogens due optimum distribution of methyl-ITC in soil, is rarely achieved with current application practices (Duniway 2002).

Composted materials have been effective for reducing *Verticillium* wilt of potato in previous (Entry et al. 2005). In this study, composted cattle manure reduced incidence of *Verticillium* wilt and maintained low propagule density of *V. dahliae* in soil. Organic soil amendments might be responsible for changes in soil properties that may disrupt the ability of *V. dahliae* to recognize a potential host, propagules to germinate, grow, or colonize a host (Ochiai et al. 2008). Davis et al. (1990) suggested that reduction of *Verticillium* wilt may be mediated by improved soil fertility, particularly by optimum available phosphorus in soil. Compost can improve plant health due to increased soil nutrient concentration and improvement of soil physiochemical properties (Corti et al. 1998). Composted soil amendments can build a pathogen suppressive soil environment, where biological control of soil-borne pathogens is promoted through antagonism, microbial competition, hyperparasitism and antibiosis (Hoitink and Fahy 1986). Kuter et al. (1983) reported reduction of propagule density of *R. solani* in soil amended with composted cattle manure due to enhanced activity of antagonistic microorganism populations.

Biological control of plant pathogens has been observed in several studies (Noble and Coventry 2005). Baker (1987) describes biological control as “the action of parasites, predators, or pathogens in maintaining another organism’s population at a lower average than would occur in their absence”. A similar definition is used to describe suppressive soils, in which disease development is minimal even in the presence of a pathogen and susceptible host (Mazzola 2007). In this context, composts based on cattle manure or in combination with plant residues have been used effectively to develop soil

suppressiveness in potting medium (Hoitink et al. 1977; Gorodecki and Hadar 1990; Yogeve et al. 2009).

This result suggests that reduction of *Verticillium* wilt incidence is not necessarily dependent of reduction of propagule density of *V. dahliae*. This agrees with the findings of others that *Verticillium* wilt may be controlled without reducing the number of propagules in soil (Davis et al. 1999; Ochiai et al. 2007). Thus, reduction of *Verticillium* wilt of potato may be linked with other mechanism, in addition to reduction of propagule density. Ochiai et al. (2007) suggested that low pH and high total organic carbon may be related to reduction of *Verticillium* wilt severity.

### **2.5.3. Effect on Soil Properties and Potato Marketable Yield.**

Potato systems in which green manures, animal manure, composts, or any types of organic amendment have been applied to soil can improve soil nutrient availability, soil tilth, water holding capacity and plant health (LaMondia et al. 1999; Stark and Porter 2005). In this study, composted cattle manure significantly increased marketable potato yield, and neither VAP nor any of the green manure treatments resulted in improvement of marketable yield compared with the crop Control treatment. In addition, only the COM treatment yield (38.7 tonnes ha<sup>-1</sup>) was higher than the crop Control treatment (30.8 tonnes ha<sup>-1</sup>). This increment represents a significant improve of potato yield if comparing to the production average for Manitoba (31.4 tonnes ha<sup>-1</sup>) (Agriculture and Agri-Food Canada 2007).

The lack of significant impact of SS1, SS2, RYE, and MUSTor treatments in Experiment 1, and O/PEA, and MUSTmix treatments in Experiment 2, on yield of potato might be explained by the high *Verticillium* wilt incidence at the study sites. However, factors other than high *Verticillium* wilt pressure also likely contributed to lowering of potato yield. In this study, not all treatments which significantly reduced disease incidence and number of propagules in the soil resulted in increased potato yield. Despite the reduced germination of microsclerotia and reduced density of propagules in soil, the MUSTmeal treatment did not increase potato yield. Incorporation of the mustard seed meal led to significantly higher concentration of N in soil (203 kg N ha<sup>-1</sup>), but did not significantly increase extractable P concentration. Mazzola et al (2007) documented that mustard seed meal usually contains from 5.5 to 6.8 % nitrogen. Therefore, mustard meal added at 9000 kg ha<sup>-1</sup> added approximately 281 kg N ha<sup>-1</sup>. In this study, this N content of oriental mustard seed meal was not taken into account to reduce fertilizer application rates. This additional nitrogen from the seed meal added with the MUSTmeal treatment could have led to more vegetative growth and less tuber development. Adequate management of N is critical for optimal potato yield (Sincik et al. 2008), whereas excessive available N in soil results in reduced yields (Lauer 1986; Neeteson and Zwetsloot 1989).

Soil amendment with composted materials also delays decline of photosynthesis in leaves that expand early in the season, increasing the number of leaves and leaf area (Gent et al. 1999) and ultimately, reducing yield losses caused by *Verticillium* wilt (LaMondia et al. 1999). By this mechanism compost may help potato to defend against the detrimental effect of *Verticillium* wilt, which is believed to decrease leaf surface area as well as photosynthesis of the plant and ultimately, the supply of assimilates required for later



tuber bulking growth (Bowden and Rouse 1991). Improvement in soil fertility N, P, K by application of composted materials have been responsible for increase in tuber initiation over tuber bulking, with more tubers per plant, although considerable more tubers also reached marketable size (>100g) (Sillitoe 1996).

Disease suppression by compost is associated with increased microbial activity as well as changes in concentration of N, P, Ca, and soil organic matter of treated soils (Hoitink and Fahy 1986). In potato fields of Manitoba, increased soil organic matter has been associated with reduction of *Verticillium* wilt incidence (Briar et al. in preparation; Tenuta et al. in preparation). Compost amendments are a source of organic carbon, and when added to the soil should increase soil organic carbon (Zinati et al. 2001; Magdoff and Weil 2004). In this study, the carbon applied with composted beef cattle manure was equivalent to an addition of 1.4% of total soil mass, which is enough to reduce *Verticillium* wilt according to previous work of Tenuta et al. (in preparation). The increase of soil organic matter serves as a source of carbon and energy for microorganisms (Weil and Magdoff 2004), and consequently, reduces propagule density due to parasitism, predation and competition for nutrients and carbon resources by beneficial soil microorganisms (Hoitink and Fahy 1986). Soil organic matter is also a major source of plant nutrients, such as N and P (Weil and Magdoff 2004). Optimum nutrient (NPK) availability for potato yield is also associated with reduced *Verticillium* wilt incidence (Lambert et al. 2005).

## **2.6. Conclusions.**

One or two years of green manure seems to be ineffective in reducing Verticillium wilt of potato. However, the results of this study suggest that oriental mustard, white mustard and oat/peas mix green manure have potential to reduce germination of *V. dahliae* and soil propagule density. Therefore, multiple years of green manure may be needed to reduce Verticillium wilt in potato field of Manitoba.

Addition of oriental mustard seed meal has the potential to be an important part of Verticillium wilt management programs. Results of this study suggest that oriental mustard seed meal amendments have promise as an alternative strategy for the control of Verticillium wilt of potato, as well as a potential source of N to crops. The role of mustard seed meal in nutrient management programs needs further investigation for growers who seek a nonchemical alternative to fumigation. However, critical issues remain which must be addressed before mustard seed meal can be considered a viable option. For example, the rates used in this study would be economically unaffordable for potato producers; therefore, it is necessary to investigate whether lower application rates can achieve the desired control of disease.

The findings of this study indicate that incorporation of composted beef cattle manure to potato fields planted to the susceptible cv Russet Burbank increases potato yield, reduces Verticillium wilt incidence, and improves nutrient availability. The mechanism responsible for the reduction of Verticillium wilt incidence was not examined here; however, it is hypothesized that improvement of nutrient availability and SOM is

possibly involved. Further examination of the factors controlling the suppressive activity of compost against *Verticillium* wilt of potato is required.

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### **3. SURVIVAL OF *V. DAHLIAE* MICROSCLEROTIA IN THREE POTATO SOILS AMENDED WITH ORIENTAL MUSTARD TISSUE AND SEED MEAL**

#### **3.1. Abstract**

Addition of high-glucosinolate containing Brassicaceae materials are known to reduce *Verticillium dahliae* Kleb. Control has been attributed to different mechanisms in particular to the release of toxic isothiocyanates (ITCs), but specific soil properties could affect the concentration of ITCs accumulated in soil. However, the soil properties affecting the biocidal activity of fungitoxic compounds against the fungal-wilt pathogen, *V. dahliae* are poorly understood. Therefore, we investigated the effect of three soils on the accumulation of ITCs and toxicity to *V. dahliae* microsclerotia from *Brassica juncea* L. plant tissue (1 and 3% w/w) and seed-meal (0.25 and 0.5% w/w) as soil amendment in a microcosm experiment. The only ITC present in amended soil with tissue and seed meal amendment was 2-propenyl isothiocyanate (2-propenyl-ITC). The highest concentration of 2-propenyl-ITC was measured 1 hour after incorporation of 0.5% seed-meal. At 24 h, the concentration of 2-propenyl-ITC was markedly reduced in soil of pH higher than 6.5 compared to soils with lower pH. The germination of microsclerotia was mainly inhibited by 0.5 and 0.25% seed meal treatments at four-day measurement. However, the effect of concentration of 2-propenyl-ITC at 12 nM g<sup>-1</sup> seems to be fungistatic as germination of microsclerotia rebounded once the concentration of ITC dissipated. The results of this study suggest that the inhibition of germination of microsclerotia by oriental mustard plant tissue or seed meal is affected by soil with pH higher than 6.5, where concentration and residence time of 2-propenyl-ITC was lower

than 12 nM g<sup>-1</sup> soil and 1 day, respectively. In terms of practical field implementation, higher concentration and residence time of 2-propenyl-ITC may be needed to have a fungitoxic effect on *V. dahliae* microsclerotia.

### **3.2. Introduction**

*Verticillium dahliae* Kleb. is a soil-borne pathogen of potato (*Solanum Tuberosum* L.) that is responsible for the Verticillium wilt disease (Rowe and Powelson 2002; Fradin and Thomma 2006). Verticillium wilt has developed slowly over many years in potato production areas of the Red River Valley in North America (Rowe et al. 1987). In Manitoba, since Verticillium wilt was first reported to have severe damage to potato in 1934 (Hoes and Zimmer 1968), many potato fields planted with cv Russet Burbank have been found to be infested with highly pathogenic *V. dahliae* isolates, which can produce disease severity of up to 90% (Uppal et al. 2007) and serious economic losses due to reduced yield and tuber quality (Johnson 1988; Shinnars-Carnelley et al. 2003). *V. dahliae* forms microsclerotia, thick-walled-melanized and multicelled resting structures (Lopez-Escudero et al. 2007) which are the most important survival structure in soil for this pathogen. Microsclerotia can survive up to 14 years in field soil regardless of whether suitable host crops have been grown (Wilhelms 1955).

Management of *V. dahliae* is commonly achieved by using chemical fumigants such as methyl bromide, vapam and chloropicrin (Rowe and Powelson 2002; Triky-Dotan et al. 2007). However, concern for potential health and/or environmental damage as well as cost have led to a search for alternative strategies to manage Verticillium wilt of potato

(Davis et al. 1996). Consequently alternative methods to control soil-borne pathogens are required. One approach with potential to reduce *V. dahliae* damage to potato is the use of Brassicaceae green manure or seed meal amendments (Ochiai et al. 2007). Members of the order Brassicaceae have been shown to contain sulfur compounds known as glucosinolates that produce active allelochemicals against a variety of weeds and soil-borne plant pathogens (Gimsing and Kirkegaard 2009). Glucosinolates are nitrogen and sulphur-rich organic anions, with specific side chains derived from amino acids, that distinguish one glucosinolate from another (Brown and Morra 1997; Mithen 2001b). Glucosinolates can be divide in three major groups; aromatic, aliphatic and indoyl (Mithen 2001b). All plants containing GSLs also contain the enzyme myrosinase, which is stored in separated compartments of cells. In the presence of water and after tissue disruption the myrosinase comes into contact with the glucosinolates to form a number of hydrolysis products (Mithen 2001a, 2001b). Of the possible hydrolysis products, the most related to soil-borne pathogen control are isothiocyanates (ITCs), oxazolidinethiones, ionic thiocyanate and organic cyanides (Morra and Kirkegaard 2002).

Reduction of soil-borne pathogens by Brassicaceae amendments is believed to be due to the release of ITCs, which are the most bioactive and toxic of the glucosinolate reaction products (Morra and Kirkegaard 2002). However, there may also be alternative mechanism of control which is not linked to ITCs production. Brassicaceae green manure and seed meals are also nitrogen rich materials, which means that ammonia and nitrous acid accumulation following incorporation may be another possible mechanism responsible for reducing soil pathogen levels (Gimsing and Kirkegaard 2009). Tenuta and

Lazarovits (2002b) reported that the accumulation of ammonia and nitrous acid following degradation of nitrogenous amendments were fungitoxic to *V. dahliae* microsclerotia.

Pesticidal activity of the glucosinolate reaction products is short lived due to their short residence time from 20 to 60 hours in soils (Borek 1995). Control of a pathogen, is dependent upon the concentration of the reaction product released from the glucosinolate containing material. Gimsing and Kirkegaard (2006) suggest that pathogen suppression is achieved at ITC levels of  $100 \text{ nM g}^{-1}$ . Accumulation of nitrogen products in soil determines the efficacy of an amendment in killing a pathogen. For example, accumulation of ammonia in soil amended with meat and bone meal become fungitoxic to *V. dahliae* microsclerotia at levels higher than  $20 \text{ mM}$  (Tenuta and Lazarovits 2002a, 2002b).

Soil properties themselves affect the concentration and residence time of ITCs, ammonia and nitrous acid, and thus, their effectiveness for killing pathogens. A number of soil factors including soil texture, pH, soil organic matter (SOM), nutrient concentration, moisture content and temperature influence degradation and volatilization of ITCs (Gimsing and Kirkegaard 2009). A lack of efficacy of Brassicaceae material killing soil-pathogens is also associated with sorption of ITCs to clay and organic matter in soil (Brown and Morra 2005; Matthiessen and Shackleton 2005; Matthiessen and Kirkegaard 2006). Tenuta and Lazarovits (2004) also report accumulation of ammonia to occur in soil having low organic carbon content ( $<1.4\%$ ), high pH (9) and high sand content,

whereas nitrous acid toxicity was promoted by soil acidity (pH<6.0) and rapid nitrification.

Although previous research has demonstrated the potential of Brassicaceae plants to reduce soil-borne pathogens in potato, the recommended application rates are often too high to be practical (> 20 tonne ha<sup>-1</sup> green manure dry weight) (Ochiai et al. 2007), and the effectiveness is often inconsistent, due to the high variability of concentration and residence time of glucosinolate reaction products among different soils (Kirkegaard and Sarwar 1998; Olivier et al. 1999). The objectives of this study were to evaluate the potential of mustard green manure and seed meal in Manitoba soils at rates lower than those recommended; and to identify soil conditions affecting the concentration and residence time of fungitoxic compounds released from oriental mustard plant tissue and seed meal, and thus, effectiveness to reduce survival of *V. dahliae* microsclerotia.

### **3.3. Materials and Methods**

#### **3.3.1. Plant Material for Amendments**

Oriental mustard (*Brassica juncea*) var. Cutlass and oriental mustard seed meal from Canadian oriental #1 were used as soil amendment because these materials have a high concentration of 2-propenyl glucosinolate (2-propenyl-GSL) around 1.8 mg g<sup>-1</sup> of dry plant tissue (Olivier et al. 1999). Oriental mustard plants for the amendments were grown in a growth chamber at the Soil Science Department at the University of Manitoba, during winter 2008/2009; plants were grown under a 16 h photoperiod with regulated temperature 20±2 °C and humidity 50-60%. Plants were grown in a potting mix soil and

watered daily. The leaves and stems were harvested by hand when plants were 42 days old and flowering. The vegetative plant material was then chopped into small pieces, homogenized using a coffee grinder, and applied immediately to the soil. The mustard seed meal was a free flowing powder milled from Canadian Oriental #1 seed (Mustard Capital Inc., Gravelbourg, SK). The meal had been partially deoiled and contained 0.85% Sinigrin, 25% protein, 8% moisture and 4-6% ash content. The seed meal was stored at 4 °C until use.

### **3.3.2. *V. dahliae* Inoculum Preparation**

An isolate of *V. dahliae* (Vd-1396), initially obtained from The Plant Diagnostic Laboratory of Manitoba Agriculture, Food, and Rural Initiatives, was selected based on its high pathogenicity to potato (Uppal et al. 2007). The isolate was maintained as a culture on potato dextrose agar medium at 4 °C. Microsclerotia of *V. dahliae* (vd-1396) were produced in the Soil Ecology Laboratory on semisolid Czapek-Dox medium for 3-4 weeks in the dark at 24°C (Hawke and Lazarovits 1994). The culture was poured through mesh screens to obtain microsclerotia of 75 to 106 µm diameter. Microsclerotia mix with silica sand and the mixture added to nylon mesh bags (SAATILON® Monofilament Nylon) (Tenuta and Lazarovits 2002a).

### **3.3.3. Microcosm Soil Preparation**

The microcosm study was conducted using surface soils (0–15 cm) collected from three different sites near the towns of Carberry (Soil A), Portage La Prairie (Soil B) and Winkler (Soil C), Manitoba. Each soil had been cropped to spring wheat (*Triticum*



*aestivum* L) prior to sample collection. Soil was collected at random from four different positions in each field site to provide replicate soil samples. Soil from each position was stored in a polyethylene bag at 4°C until use. A sub-sample was taken from each sample and analyzed for mobile nutrients, NO<sub>3</sub><sup>-</sup>, sodium bicarbonate extractable-P, Ca, pH<sub>water</sub>, organic matter, and soil particle size distribution at the Bodycote Laboratory (Winnipeg, MB.). Characteristics of the soils used are given in Table 3.1.

**Table 3.1. Characteristics of the three soils used in the study (depth: 0–15 cm). Data shown are the average of four replications.**

Parameter	Sand	Silt	Clay	Texture	NO <sub>3</sub> -N	P	Ca	pH	OM (%)
	(%)				(mg kg <sup>-1</sup> )				
Soil A	43	37	19	Loam	37.8	13.8	2875	6	5.2
Soil B	86	9	5	Sand	21.8	40.3	2700	7.3	1.8
Soil C	48	35	16	Loam	27.8	17.8	5025	8	3.6

### 3.3.4. Experimental Setup

Collected soil was air-dried for 12 hours to reduce soil moisture content, before being passed through a 2 mm mesh screen. After 12 hours, moisture content of the soil was 19, 7 and 16 %, for Soil A, B and C, respectively. The gravimetric moisture content of each soil at 60% container capacity was determined to be 25% in Soil A, 18% in Soil B and 26% in Soil C.

Survival of microsclerotia of *V. dahliae* was studied using a laboratory microcosm experiment. Five treatments, consisting of two rates of oriental mustard plant tissue, 9 tonne ha<sup>-1</sup> dry weight (TISSUE 1.5%) and 18 tonne ha<sup>-1</sup> dry weight (TISSUE 3%), two different rates of oriental mustard seed meal 4.7 tonne ha<sup>-1</sup> (MEAL0.25%) and 9.3 tonne ha<sup>-1</sup> (MEAL0.5%), and one nonamended treatment, as a control (Control), were added to

each soil with four replications per treatment. Twenty g of treatment soil was placed into a 50 mL conical tube (25 by 150 mm) (VWR International) and immediately after each addition, tubes were covered with a polyethylene cap (VWR International) that allowed for exchange of air. Sufficient tubes were prepared for each treatment to allow destruction sampling at 1, 6, 12 hours and 1,7,14, 21, 28, 42 days.

### **3.3.5. *V. dahliae* Microsclerotia Analysis**

Reduction of microsclerotia germination was determined using microsclerotia in a silica sand mixture in nylon mesh bags (SAATILON® MONOFILAMENT NYLON) (Tenuta and Lazarovits 2002b) buried into soil. The mesh bags were placed into the tubes, previously prepared with soil amendment treatments. A mesh bag in each tube was placed in centre of height of the soil in the tube (two cm from the surface). Three aliquots of deionized water were added to bring the moisture content of each soil to 60% of soil container capacity. Microcosms were watered regularly with deionized water during the experimental period to replace lost by evaporation.

A group of four mesh bags were analyzed for germination of microsclerotia at the beginning of the experiment to represent initial germination of microsclerotia (T0). The bags of microsclerotia were retrieved from tubes after 1, 7, 14, 21, 28 and 42 days. Mesh bags were dried at room temperature for 12 h in the dark, and the contents spread onto a pectate–tergitol–agar plate with an Andersen cascade impactor (Andersen Instruments Inc., Smyrna, GA). The agar plates were incubated for 5-7 days in the dark at 24°C and survival of microsclerotia was determined as the percentage of microsclerotia that germinated to produce colonies (Tenuta and Lazarovits 2002b). Plates containing fungal

colonies were scanned systematically at 20X magnification until one hundred microsclerotia had been examined and scored for the formation of colonies.

### **3.3.6. Soil Chemical Analysis**

#### **3.3.6.1. Soil ITC Analysis**

##### **3.3.6.1.1. Soil Extraction**

Soil (5 g) was obtained from each tube after 1 h, 6 h, 12 h, 1 d and 4 d after incorporation for ITC analysis. Isothiocyanates were extracted by adding 5 mL cold 80% methanol to the sample in a polypropylene conical centrifuge tube (50 mL). The methanol was used to trap any volatile compound released from the mustard material (Angus et al. 1994). The mixture was agitated on a reciprocal shaker (Eberbach Corp., MI) at 150 excursions per minute at room temperature for 1 h. This procedure prevented hydrolysis of remaining glucosinolates that might occur during the extraction procedure (Morra and Kirkegaard 2002). The mixture was vortexed and centrifuged (10 min, 4000 x g). Then, a 2 mL aliquot of the clear methanol supernatant was removed and stored at -20 °C prior to analysis ITCs by HPLC (Zasada and Ferris 2004).

##### **3.3.6.1.2. ITC Analysis**

Analysis for ITC concentration of extracts was conducted using a Waters 2695 HPLC system (Waters Corporation, Milford, MA). The HPLC was equipped with an

autosampler and a Waters 996 photodiode array detector, and fitted with a 5  $\mu\text{m}$  LiChrospher 100 RP-18 guard column and a reverse-phase 5  $\mu\text{m}$  250-4 LiChrospher 100 RP-18 analytical column. Results were processed using Millennium Software, version 3.2 (Waters Corporation). The column was eluted at a flow of 1  $\text{mL min}^{-1}$  with a gradient of A: HPLC grade methanol and B:  $\text{H}_3\text{PO}_4^-$  acidified water. The gradient used to carry the analysis was as follows: (time [min]/A [%]/B [%]) = 001/0/100, 8/7/93, 12/15/85, 30/55/40, 35/92/8, 40/92/8, 45/1.5/98.5, 52/0/100. Sample (20 mL) was introduced to the HPLC and absorbance of ITC determined from 200 to 400 nm. A calibration curve was constructed using 2-propenyl-ITC, methyl-ITC, 2-phenethyl-ITC and benzyl-ITC as external standards (Sigma). Identification of ITCs was determined by matching the elution time of standards and peaks from samples ( $\text{nM g}^{-1}$  soil).

### **3.3.6.2. Ammonia, Nitrous Acid and pH Determinations**

Ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), and pH were analyzed at time zero and 1, 7, 14, 28 and 42 days of incubation. Soil in each tube was homogenized by mixing the contents with a 15 cm long flat-spoon spatula. A 6 g sample was added to 30 mL cold distilled water ( $4^\circ\text{C}$ ) placed in a conical polypropylene centrifuge tube (50 mL). The tube was capped and the slurry mechanically disrupted with an Eberbach reciprocal shaker for one hour at 150 excursions per minute at  $4^\circ\text{C}$ . Then, the tubes were centrifuged for 10 min at  $12,500 \times g$  at  $4^\circ\text{C}$ , to set the particulates. Clear supernatant (18 mL) was transferred to a labeled scintillation vial. The cleared solution was stored at  $-20^\circ\text{C}$  if not analyzed within a week after extraction. Nitrite analysis was done within 48 hours of extraction to prevent loss. The pH of the bioassay solutions was determined using a pH

meter (Orion 720A, Thermo ATC probe, Orion, Montreal, QC). Nitrate was analyzed using a Technicon<sup>TM</sup> Autoanalyzer II System (Pulse Instruments, Saskatoon, SK) where it underwent cadmium reduction before being analyzed for nitrite. Nitrite analysis of extracts was done similarly except for omitting the reduction step. The concentration of ammonium in extract solutions were determined by using the automated phenate method also using a Technicon<sup>TM</sup> Autoanalyzer II System. The concentrations of ammonium and nitrous acid are reported as millimolar (mM) in soil solution. Ammonia and nitrous acid concentrations were estimated using the Henderson-Hasselbalch equation and the pH, temperature, soil moisture content and ammonia or nitrite concentration (Tenuta and Lazarovits 2002b).

### **3.3.7. Statistical Analysis**

Statistical analyses were conducted using the Statistical Analysis Software (SAS Institute, Cary, NC; release 9.1 for Windows). Germination of microsclerotia was arcsine transformed and ITC power-transformed after the addition of a common coefficient of 1 in order to improve both the normality and homogeneity of the variances prior to analysis of variance. One-way ANOVA (PROC MIXED) was used to analyze each variable. Due to the high concentration of ITC immediately after treatment incorporation that caused trends on germination of microsclerotia, ANOVA was conducted for the first date of incubation for ITC and 4 d and 42 d incubation for germination of microsclerotia. Means were separated according to the Bonferroni's procedure when the F-test was significant ( $P < 0.05$ ). Moreover, a relationship between concentration of ITC and germination of microsclerotia was determined using linear regression analysis (PROC REG).

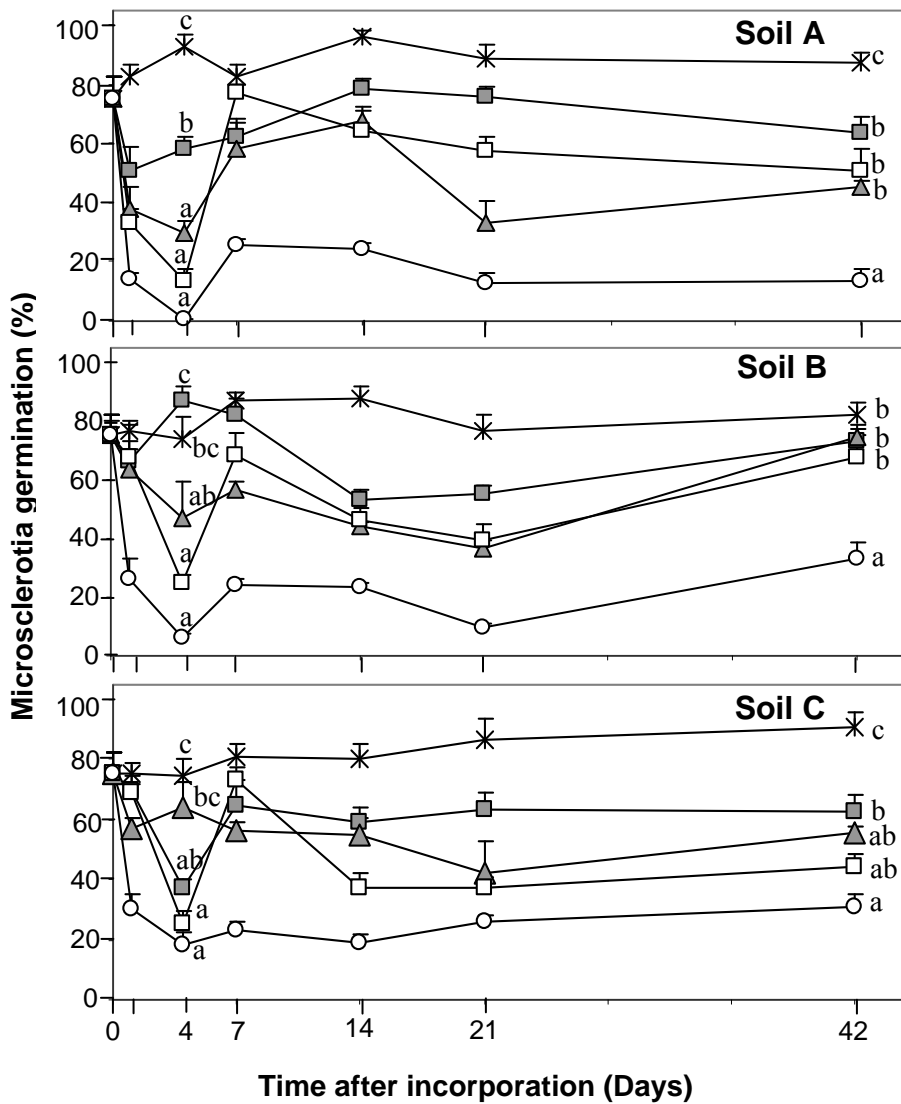
### **3.4. Results**

#### **3.4.1. Effect of Mustard Tissues and Seed Meal on Survival of Microsclerotia**

The incorporation of oriental mustard tissue and seed meal reduced the germination of microsclerotia in all soils. In the Control treatment, the germination of *V. dahliae* microsclerotia was higher than 75% in all soil during the whole experiment. The effect of soil on germination of microsclerotia was not significant ( $P > 0.05$ ) (Figure 3.1).

All amendment treatments reduced the germination of microsclerotia one and four days following incorporation, except for TISSUE1.5% at day four in Soil B (Figure 3.1). At day four, MEAL0.5% and MEAL0.25% consistently resulted in the larger decrease in germination of microsclerotia in all three soils compared to the Control (Figure 3.1., 3.2).

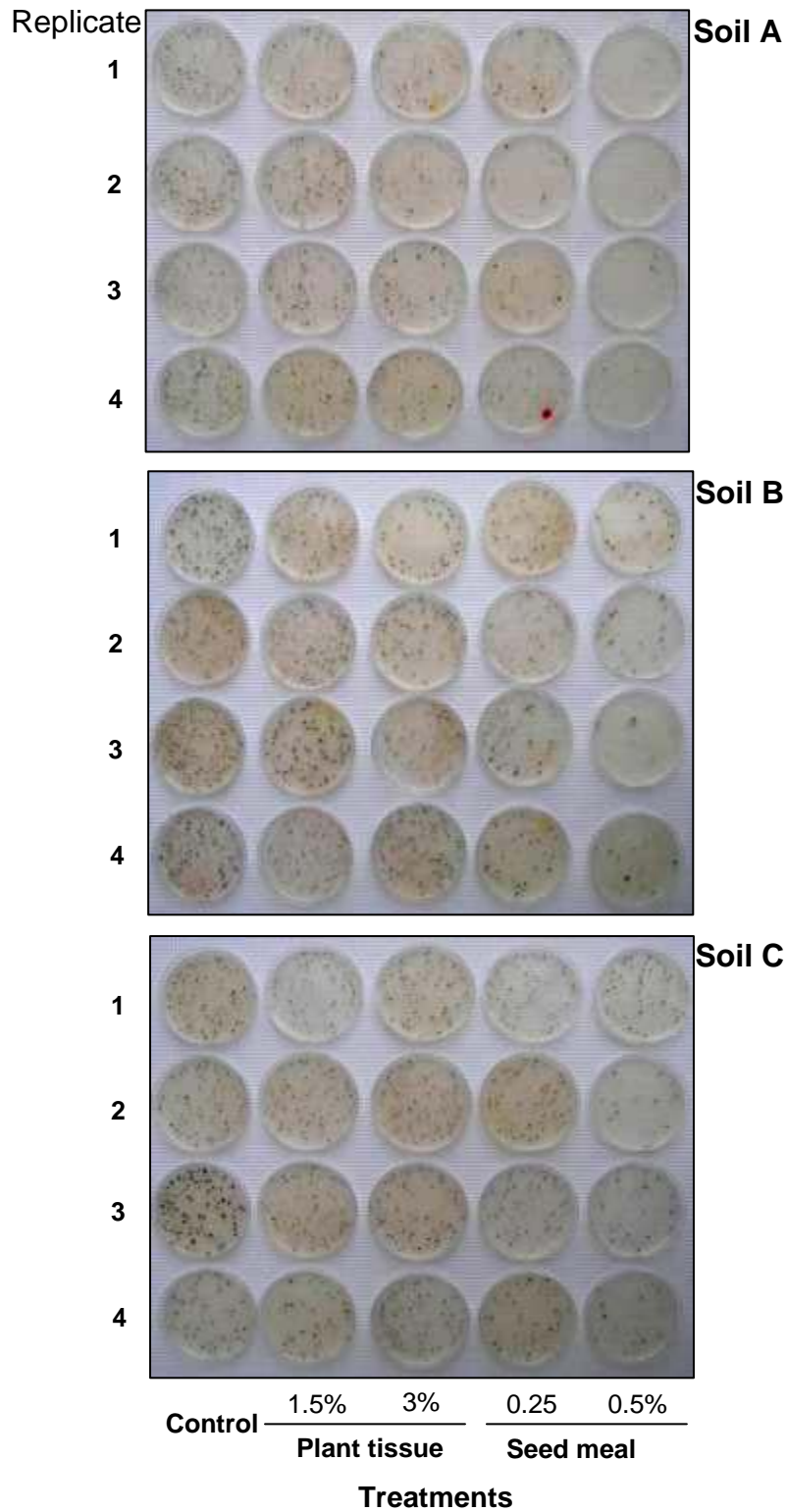
In contrast, MUST1.5% and MUST3% treatments reduced microsclerotia germination at day four only for Soil A. This initial reduction in germination of microsclerotia was followed by an increase at day seven for all amendment treatments and soils, except MEAL3% in Soil C (Figure 3.1).



**Figure 3.1.** Germination of *V. dahliae* microsclerotia in the three study soils amended with oriental mustard tissue at concentrations, 1.5% (■) and 3% (▲)  $w w^{-1}$ , and mustard seed meal at concentrations, 0.25% (□) and 0.5% (○)  $w w^{-1}$ , and nonamended (\*). Data shown are the average of four replicates (+1 standard error). Means for the germination of microsclerotia 4 and 42 days after incorporation in each soil followed by the same letter are not significantly different ( $P < 0.05$ ) according to the Bonferroni's multiple comparison test.

An ANOVA conducted at the end of the experiment (42 days) showed a significant treatment amendment treatment effect on germination of *V. dahliae* microsclerotia in each soil ( $P < 0.0001$ ). Germination of microsclerotia was significantly lower for the MEAL0.5% treatment compared to the Control treatment in all soils. The others amendment treatments resulted in microsclerotia germination values that were lower than the Control. However, in soil B, only the MEAL0.5% treatment reduced germination of microsclerotia compared to the Control treatment. In soil C, all amendment treatments reduced germination of microsclerotia compared to the Control treatment; the only difference among amendments was observed when germination in the MEAL0.5% treatment was lower than in the TISSUE1.5% treatment ( $P < 0.0001$ ) (Figure 3.1).



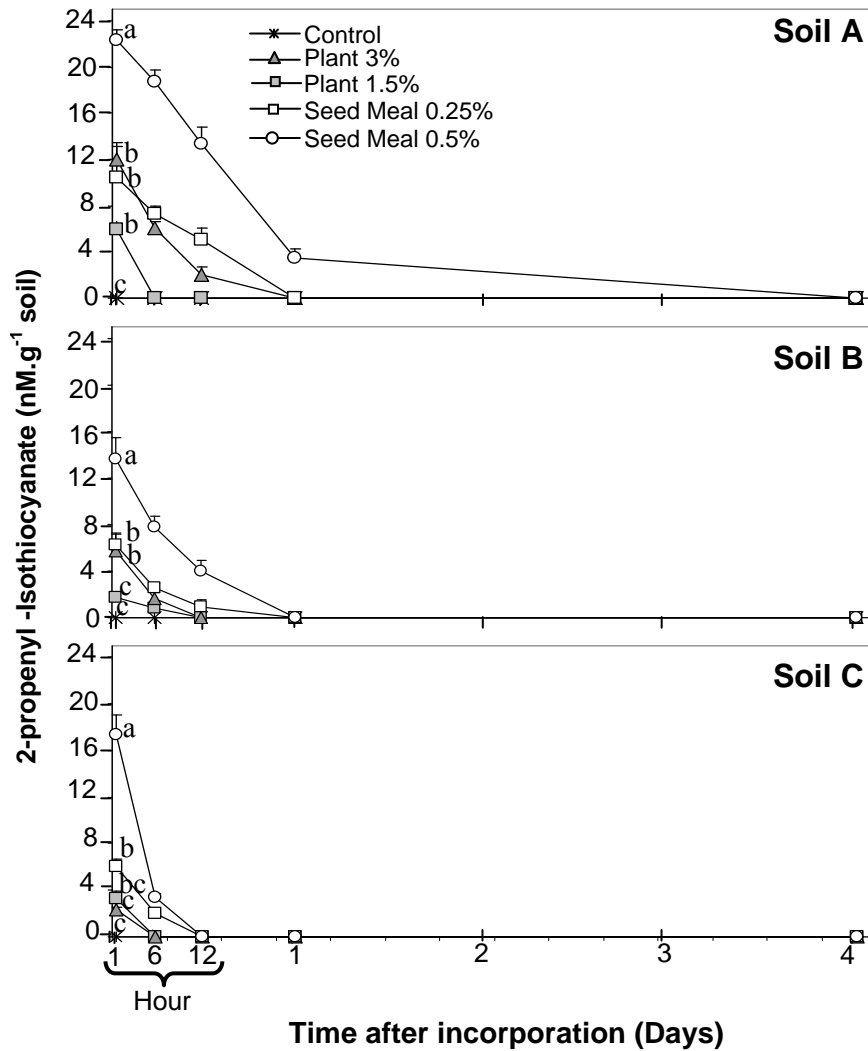


**Figure 3.2.** Photographs showing growth of *V. dahliae* microsclerotia on Petri plates with pectate-tergitol-agar four days after incorporation of mustard plant tissue and seed meal in the three study soils. The germination of microsclerotia is shown as formation of black colonies.

### 3.4.2. Glucosinolate Hydrolysis Products, Ammonia and Nitrous Acid in Soil

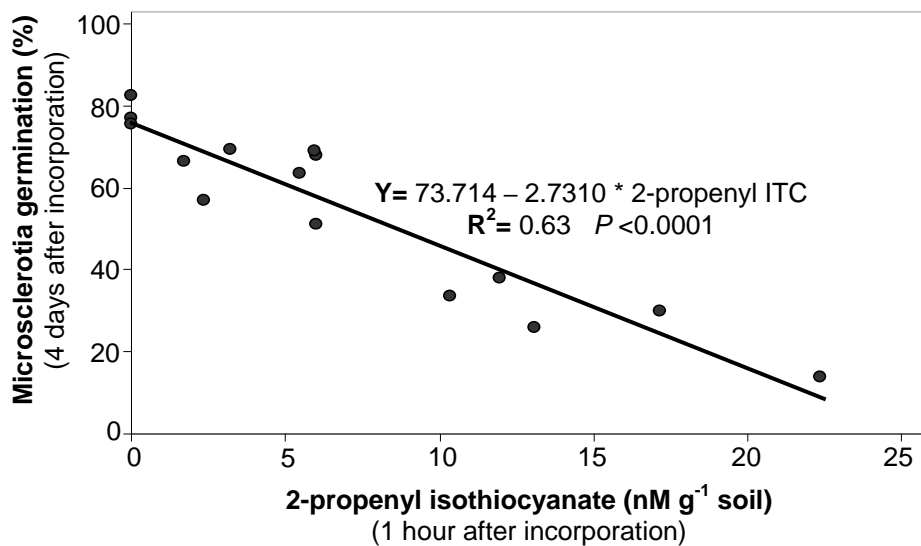
2-propenyl isothiocyanate (2-propenyl-ITC) was the only glucosinolate hydrolysis product detected in soils amended with oriental mustard tissue or seed meal. The statistical analysis showed a significant amendment treatment effect in all soils ( $P < 0.0001$ ), and significant differences between soils ( $P < 0.001$ ). The effect of the treatments was different among the soils, as evident by a treatment\*soil interaction ( $P < 0.0001$ ) (Figure 3.3).

The highest concentration of 2-propenyl-ITC was found after one hour of incorporation in all three soils. Beyond this time, the decline in 2-propenyl-ITC concentration was faster in Soils B and C with the compound being undetected at 24 and 12 hours after incorporation, respectively. The concentration of 2-propenyl-ITC for all amended treatments was also higher in Soil A than in Soil B and Soil C (Figure 3.3). In general, the concentration of 2-propenyl-ITC was twice higher in the seed meal treatment (MEAL0.5%) than in the tissue treatment (TISSUE3%). At one hour after incorporation in Soil A, the concentration of 2-propenyl-ITC decreased in the order: MEAL0.5% > MEAL0.25%, TISSUE3% and TISSUE1.5% > Control treatment ( $P < 0.0001$ ). While in Soil B the concentration of 2-propenyl-ITC decline in order of MEAL0.5% > MEAL0.25% = TISSUE3% > TISSUE1.5% = Control ( $P < 0.0001$ ). The concentration of 2-propenyl-ITC in Soil C, was MEAL0.5% > MEAL0.25% = TISSUE1.5%, MEAL0.25% > TISSUE3% = Control ( $P < 0.0001$ ) (Figure 3.3).



**Figure 3.3.** 2-propenyl-isothiocyanate concentration in amendment and nonamended treatments. Data shown are the average of four replicates (+1 standard error). Means for the 2-propenyl isothiocyanate concentration in soil one hour after incorporation followed by the same letter are not significantly different ( $P < 0.05$ ) according to the Bonferroni's multiple comparison test.

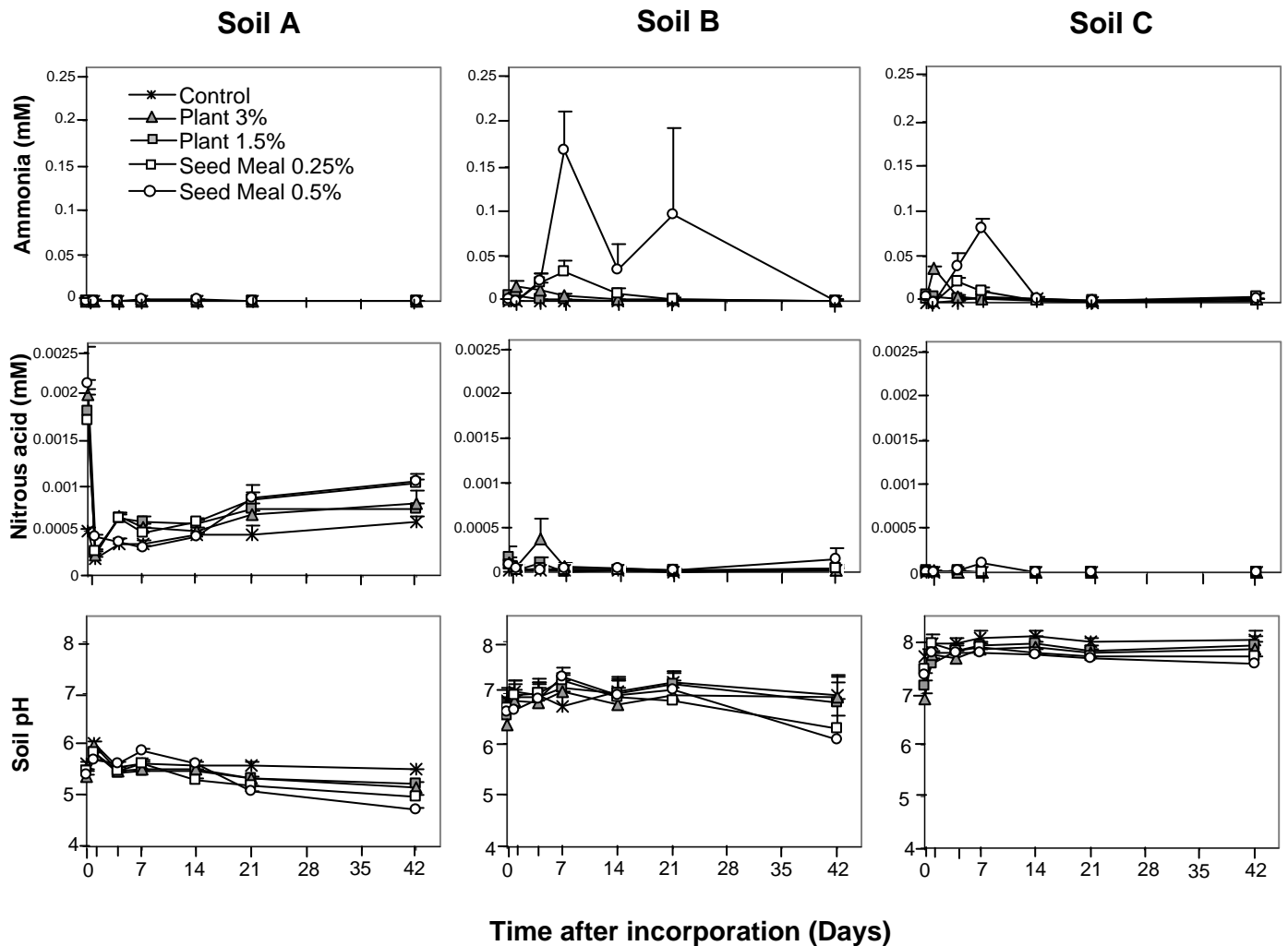
The association between 2-propenyl-ITC concentration in soil and germination of microsclerotia was assessed using linear regression analysis. The analysis indicated that accumulation of 2-propenyl-ITC one hour after incorporation of mustard materials was an effective predictor of germination of *V. dahliae* microsclerotia four days after incorporation of mustard amendments (Figure 3.4).



**Figure 3.4.** Linear regression of germination (%) of *V. dahliae* microsclerotia, at 4 days after incorporation, in relation to concentration of 2-propenyl isothiocyanate in soil (nM g<sup>-1</sup> soil) at one hour after incorporation.

Additions of mustard plant tissue or seed meal at rates evaluated did not result in biological significant concentrations of ammonia or nitrous acid in soil (Figure 3.5). Ammonia was present in Soil B amended with mustard plant tissue or seed meal during day 1 through day 42, whereas the highest concentration of nitrous acid was 0.002 mM at time T0 in Soil A.

Soil pH was not significantly affected by addition of mustard tissue or seed meal. However, at the end of the experiment, in Soil A, the pH dropped slightly from the start of the experiment until 42 day with MEAL0.5% and MEAL0.25% treatments from 5.4 to 4.7 and from 5.5 to 5.0, respectively. In Soils B and C, the pH was not affected by treatments. Soil pH for Soil B and C, ranged from 6 to 7 and 7 to 8, respectively (Figure 3.5).



**Figure 3.5.** Ammonia and nitrous acid concentration in soil solution and soil pH in the three study soils following addition of oriental mustard tissue and seed meal. Data shown are the average of four replicates (+1 standard error).

### **3.5. Discussion**

The results of this study confirm the ability of oriental mustard green manure and seed meal to kill potato pathogens in soil such as *V. dahliae*, and demonstrate the effectiveness of the materials to be dependent on the rate of application and soil. The effectiveness of glucosinolate reaction products in soil was dependent upon the concentration of ITC to inhibit germination of microsclerotia.

#### **3.5.1. Mechanisms Associated with Inhibition of *V. dahliae* Microsclerotia**

##### **Germination**

Results from this study show that mustard plant tissue and seed meal when incorporated into soil have a moderate level of fungicidal activity against microsclerotia of *V. dahliae*. However, the level of inhibition of germination of microsclerotia from mustard plant tissue and seed meal was different between the materials, rates of application and the soils used. Based on the germination of microsclerotia at 42 days after incorporation, approximately 50% or more of *V. dahliae* microsclerotia was able to germinate in amended soil with 1.5 % and 3% plant tissue or seed meal at 0.25%. In contrast, the largest reduction in microsclerotia germination was achieved with incorporation of oriental mustard seed meal at 0.5%. Oriental mustard plants and seed meal have been effectively used to reduce soil-borne pathogens and diseases in potato (Larkin and Griffin 2007). Control of soil-borne pathogens by Brassicaceae materials is often attributed to reactive compounds release during the hydrolysis of glucosinolates containing in the material (Gamliel and Stapleton 1993; Angus et al. 1994; Morra and Kirkegaard 2002).

In vitro studies have confirmed that pure glucosinolate hydrolysis product can prevent growth of soil-borne fungal pathogens (Bending and Lincoln 1999).

Recently, it has been suggested that ammonia and nitrous acid accumulation following incorporation of Brassicaceae plants (green manure) and seed meals may be responsible for reducing soil pathogen levels (Gimsing and Kirkegaard 2009). The results of this study show that the incorporation rates of mustard plant tissue and seed meal used did not promote accumulation of ammonia and nitrous acid to concentrations expected to be fungitoxic. The accumulation of nitrous acid was less than 0.002 mM, which is very low compared with the 0.01 mM threshold for killing *V. dahliae* microsclerotia (Tenuta and Lazarovits 2002b). Similarly, Tenuta and Lazarovits (2002a) found that NH<sub>3</sub> concentration of 25 mM were necessary to kill microsclerotia in soil, much greater than the peak 0.2 mM found in this study.

The only ITC product detected by HPLC analysis in amended soil during the study was 2-propenyl-ITC. That finding is consistent with that of Potter et al. (1998) and Matthiessen and Shackleton (2005) who reported that above ground oriental mustard tissue produced almost entirely 2-propenyl ITC when incorporated to soil. 2-Propenyl-ITC is inhibitory to a number of different organisms, including weeds (Rice et al. 2006), nematodes (Zasada and Ferris 2004), and soil-borne pathogens (Brown and Morra 1997; Olivier et al. 1999; Smolinska et al. 2003). However, the threshold for significant pathogen reduction with ITC is approximately nM g<sup>-1</sup> soil (Gimsing and Kirkegaard 2006). In the current study, peak of 2-propenyl-ITC was much lower, 22.2 nM g<sup>-1</sup> soil but

these relatively low concentration appeared to reduce germination of microsclerotia in soil.

The germination of *V. dahliae* microsclerotia was effectively reduced with 2-propenyl-ITC concentrations of 22 nM g<sup>-1</sup> soil and less. However, some of this effect seems to be fungistatic because in some treatments and soils the germination returned when concentration of 2-propenyl-ITC dissipated. Concentrations of ITC less than 100 nM g<sup>-1</sup> soil appear to be fungitoxic, but might not kill microsclerotia. However, these sublethal concentrations may weaken microsclerotia to facilitate lysis by antagonistic communities in the soil. Compounds released directly from the meal or during its decomposition can stimulate populations and activity of antagonistic microorganisms (Lockwood 1988; Njoroge et al. 2008) such as *Streptomyces* (Cohen et al. 2005), which are associated with suppression of *V. dahliae* (Krechel et al. 2002). However, this was likely not the case as germination of microsclerotia rebounded in many cases once ITC concentration dissipated. Rather a situation often observed for animal and human pathogens, referred as viable but not culturable (Sigstad et al. 2002), condition of microorganisms likely occurred. To survive in hostile environments some bacteria enter in this state of very low metabolic activity where bacterial cells do not form colonies or grow in medium. Bacteria then require a “resuscitation” treatment (ex. placement in milk protein solution) before being able to grow in medium (Keep et al. 2006). Microsclerotia during exposure to ITC in soil may be weakened sufficiently to not survive handling processing, and transfer to plate medium. However, the fact that the microsclerotia remain in soil for six or more



days after the ITC dissipated allowed some of them to recover from the presumed stress of sublethal concentrations of ITCs prior to placement onto germination medium.

### **3.5.2. Soil Properties Related to Mechanisms Reducing *V. dahliae* Microsclerotia.**

The suppression effect of MEAL0.5 % treatment was consistently higher than for other treatments in this study. However, this amendment's inhibition of germination of microsclerotia 42 days after incorporation was higher in Soil A than Soil B and C. When mustard materials are incorporated into soil, the accumulation of ITC is dependent upon soil moisture content and other soil characteristics (Brown and Morra 2005). In Soil A, 2-propenyl-ITC concentration peaked at 1 h after incorporation, and then fell markedly within 1 day. This agrees with results of Brown et al. (1991) and Mattner et al. (2008) that showed ITC accumulation reached a maximum at 2 h after incorporation and then declined by more than 90% within 1 d. In this study, significant differences in concentration and residence time of 2-propenyl-ITC were observed between soils, being lower for Soil B and C, than A. Soil B and C had higher pH than in Soil A (Table 3.1).

The concentration and residence time of ITCs in soil is dependent upon losses resulting from microbial degradation, volatilization, and sorption onto soil components (Matthiessen and Kirkegaard 2006; Gimsing and Kirkegaard 2009). Previous studies have showed that reduction of ITC concentration in soil is due to sorption to soil particles and organic matter in soil (Brown and Morra 2005; Mattner et al. 2008; Gimsing and Kirkegaard 2009). However, Matthiessen and Shackleton (2005) reported that aliphatic ITCs, such as 2-propenyl-ITC and methyl-ITC, are less sorb to organic matter and soil

particles than aromatic ITC, which suggest that sorption of 2-propenyl-ITC was likely not the mechanism responsible for the reduced concentration of 2-propenyl-ITC in some soils of the current study. Gimsing et al. (2009) found that 2-propenyl-ITC is less sorbed to soil organic matter than other types of ITCs such as benzyl-isothiocyanate, commonly found in *Brassica napus*, due to the low  $K_d$  ( $0.7 \text{ L kg}^{-1}$ ) and  $K_{oc}$  ( $41 \text{ L kg}^{-1}$ ) values for 2-propenyl-ITC, and because benzyl-isothiocyanate is more hydrophobic than 2-propenyl-ITC. High soil pH in particular are associated with elevated biodegradation rates of methyl-ITC (Matthiessen and Kirkegaard 2006), which is the active degradation product of metam sodium (Vapam) and very similar to 2-propenyl-ITC release from oriental mustard. During the first four days of this study, the pH averaged 5.6, 6.8 and 7.6 in Soil A, B and C, respectively, for all amended treatments (Figure 3.5), suggesting that concentration and residence time of ITC are higher in soil with low pH than in soil with pH values between 6.8 and 7.6. This agrees with results of Smelt et al. (1989) that reported minimal biodegradation of ITC in soil with pH between 4.7 and 5.3, while rapid biodegradation was observed in soils with pH value of 7.3, where calcium is an important nutrient for bacteria that form spores or resistant resting stages such as *Bacillus* spp. and actinomycetes, which have been identified as responsible for biodegradation of aliphatic ITCs (Warton et al. 2001).

The results of this study suggest that the inhibition of germination of microsclerotia by oriental mustard plant tissue or seed meal is affected by soil conditions controlling biological degradation of 2-propenyl-ITC in soil. In terms of practical field implementation, mustard seed meal application seems to have more potential for reducing

germination of microsclerotia in soils with low pH. The greatest challenge in using mustard seed meal is to reduce the rate of application, which is the major obstacle for the adoption of this practice. Inhibition of pathogen with Brassicaceae seed meals applied at high rates equivalent to about 20 tonne ha<sup>-1</sup> in laboratory and greenhouse conditions are effective (Chung et al. 2002) but such rates in the field are not practical. High amendment rates equivalent to 24 tonne ha<sup>-1</sup> dry biomass have been also used in studies with Brassicaceae green manure. The cost of purchase as well as, shipping, application and incorporation of such large amounts of meal or green manure is economically unfeasible.

The goal of this study was to test lower rates (ex.  $\leq 10$  tonne ha<sup>-1</sup>) of application, identify soil conditions affecting the accumulation of production of fungitoxic compounds, and ultimately, how to suggest cost-effective use of Brassicaceae amendments to control Verticillium wilt. Mustard seed meal seems to be a very expensive option, since application of the lowest rates (0.25%) could cost about \$8000USD ha<sup>-1</sup> (1.51 CAD kg<sup>-1</sup> Mustard Capital Inc.). In comparison, the cost of the soil fumigant metam sodium (Vapam), commonly used in potato systems to control soil-borne plant pathogens, is approximately \$1400 USD ha<sup>-1</sup> (based on 2008 prices and rate of application of 700 L ha<sup>-1</sup>) (Wick, R. cited by Mahran et al. (2008)). However, it is also necessary to account for potential benefits such as reduction of weeds (Rice et al. 2006), insects and nematode populations (Brown and Morra 2005) and improved crop yields due increased plant-available nitrogen when using Brassicaceae materials. Nutrient content of mustard seed meal is high, particularly nitrogen being 5.6 to 6.8% N, phosphorus (1.2 to 1.4%) and potassium (1.1 to 1.5%) (Mazzola et al. 2007). The estimated nutrient value of

applications of 0.25% mustard seed meal is approximately \$700 USD ha<sup>-1</sup> (N=\$0.88, P=1.06 and K=\$0.54 USD kg<sup>-1</sup> (Oehmke et al. 2008). Developing cost-effective solutions for disease management is a significant challenge. Using seed meal to effectively control Verticillium wilt in potato still requires better understanding of the fate of the hydrolysis products in soil (Gimsing and Kirkegaard 2009). This study will help to predict the lowest effective application rate of oriental mustard seed meal that need to be used to reduce the survival of *V. dahliae* microsclerotia by determining the soil properties affecting the concentration and residence time of ITCs.

### **3.6. Conclusions**

Application of oriental mustard seed meal at rates lower than 0.5% seems to be effective reducing germination of *V. dahliae* microsclerotia. However, the effect was partially fungitoxic as germination of some microsclerotia rebounded once the concentration of ITC dissipated.

Despite the toxicity of the mustard seed meal at 0.25 and 0.5% being inherently greater than mustard green manures, effectiveness of seed meal against germination of *V. dahliae* microsclerotia may be reduced in soils with pH higher than 6.5. This is important help to develop realistic application rates to achieve the desired disease control. The rates used in this study still remain economically impractical for potato producers. However, some advantages of using mustard seed meal such as reduction of weed and pests, other than soil-borne fungal pathogens, were not included in the economic analysis.

Ammonia accumulation may be another means by which oriental mustard green manure or seed meal amendment can suppress soil-borne plant pathogens (Gimsing and Kirkegaard 2009). However, low concentration and low application rates of mustard tissue and seed meal may be not sufficient to promote accumulation of ammonia or nitrous acid in soil.

### 3.7. Literature Cited

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## 4. OVERALL SYNTHESIS

Controlling soil-borne pests and diseases is difficult to achieve within the constraints of farm economics. Soil is a heterogeneous medium of complex biological, chemical and physical interactions. Soil-borne pathogens are also microscopic with some producing very long-lived resting structures in soil making sampling, monitoring and management a challenge..

In Manitoba, growers of processing potato are interested in controlling *Verticillium* wilt as it is believed to be a reason for lower yields than in other potato growing regions (Dr. M. Tenuta, personal communication). In addition, the processing potato industry is requesting less pesticide use; for example the fast-food chain, McDonald's Corp., is reviewing pesticides used for the production of potato in its food products (REUTERS 2009). The results of my thesis have contributed to an improved understanding of *Verticillium* wilt and Potato Early Dying, potato yield, and soil quality management in Manitoba.

### 4.1. Reduction of *Verticillium* Population in Soil and Impact on Disease, Yield and Potato Quality

Propagule density of *V. dahliae* in soil declined with mustard seed meal and Vapam treatments, whereas the incidence of *Verticillium* wilt was reduced with mustard seed meal, and compost treatments, but not with Vapam. The soil fumigant, Vapam, was partially effective to lower propagule density as the density was reduced only at the

beginning of the potato season. In general, green manure treatments did not reduce propagule density of *V. dahliae* or the incidence of Verticillium wilt in potato.

The in-situ soil bioassay used in Experiment 1 presented in Chapter 2, showed that Vapam and mustard seed meal had an inhibitory effect on germination of *V. dahliae* microsclerotia one and three weeks after application. However, beyond that time several factors could stimulate or reduce the inhibitory effect. Effectiveness of Vapam and mustard green manure or seed meal controlling soil-borne pathogens depends on concentration and residence time of ITCs in soil. If the concentrations are lower than those needed to be fungitoxic, I hypothesize the microsclerotia remain viable but not culturable. However, once accumulation of ITC dissipated, microsclerotia recovered ability to grow in culture. Moreover, lower inhibition of germination of microsclerotia with Brassicaceae green manures was probably due to the lower concentration of ITC compared to soil amended with mustard seed meal (Chapter 3). Other fungitoxic compounds such as avenacin and saponins in oat tissue, and civine in pea (Pavlík et al. 2002; Wiggins and Kinkel 2005b), can produce lysis and inhibition of germination of soil-borne pathogens (Deacon and Mitchell 1985; Pavlík et al. 2002) though these are untested for *V. dahlia* microsclerotia.

Except for the mustard seed meal treatment, potato yield was lower in treatments with high Verticillium wilt incidence and high propagule density at the end of the potato season. The mustard seed meal treatment effectively reduced both propagule density and disease; however, yield was affected probably by other factors different to disease like

excessive available soil N (Lauer 1986; Neeteson and Zwetsloot 1989). In contrast, the application of compost during two consecutive years contributed effectively to maintain low number of propagules in soil during the potato season and reduced Verticillium wilt incidence, which ultimately could contribute to increased potato yield. The results of Experiment 1 presented in Chapter 2 of this thesis show that the reduction of Verticillium wilt incidence observed in plots treated with compost was not necessarily related to a reduction of propagule density of *V. dahliae*. Thus management of Verticillium wilt seems to be achievable by means other than reducing propagule density.

#### **4.2. Mechanisms of Achieving Disease Reduction and Yield Increase without Decreasing Verticillium Propagule Density in Soil**

The mechanisms of achieving disease reduction and yield increase without decreasing propagule density of *V. dahliae* in soil remains unclear (Ochiai et al. 2007). Several mechanisms have been proposed such as improved N and P availability (Davis et al. 1990), increased soil organic matter (Ochiai et al. 2007), soil suppressiveness (Hoitink and Fahy 1986), changes in soil physical properties (LaMondia et al. 1999) and possible systemic-acquired-resistance (LaMondia 2006). Recently, Ochiai (2008) proposed that addition of organic soil amendments may alter soil properties leading to the disruption in ability of *V. dahliae* to recognize a host, and propagules to germinate or grow (Ochiai et al. 2008).

The results of this thesis suggest that that improved soil quality due to increased availability of plant nutrients P and N, and soil organic matter contributed to reduced

disease and improved potato yield. The total carbon applied with composted beef cattle manure was equivalent to an addition of 1.4% of soil organic matter. From a study examining *Verticillium* wilt incidence in 24 commercial potato fields and another examining intra-field variability of four commercial potato farms in Manitoba, an increase in soil organic matter of 1.4% was associated with a decrease in disease incidence of 20 to 50% (Briar et al. and Tenuta et al., in preparation). Based on the results of Experiment 1 presented in Chapter 2, the same composted cattle manure was applied in 2009 to soil at the CMCDC Winkler field station ( $40 \text{ t ha}^{-1}$ ) with the result of having increased yield of cv Russet Burbank and cv Umatilla Russet (Cavers et al., unpublished). Soil organic matter serves as a source of carbon and energy for microorganisms (Weil and Magdoff 2004) resulting in a decline in density of soil-borne pathogen propagules due to parasitism, predation and competition for nutrients and carbon resources (Hoitink and Fahy 1986; Entry et al. 2005). Thus, several factors and mechanisms may have contributed to composted beef cattle manure having reduced disease and improved yields. The adoption of beneficial management practices for use of composts to control *Verticillium* wilt will depend upon identifying soil factors and mechanisms controlling the efficacy of the product.

### **4.3. Economic Considerations.**

The results of Experiment 1 presented in Chapter 2 indicates that composted beef cattle manure has the potential to substitute for the need to fumigate soil for the control of *Verticillium* wilt and supply important amounts of nutrients for crop production. Though

potato yield increased, the economic benefit of this increase must be considered relative to costs. Overall, addition of compost in a potato production system creates direct costs of application and incorporation. Further, acquiring the product may have costs associated with purchase and transportation. Unlike the situation in the province of Alberta where cattle feed lots provide a plentiful source of manure for compost production, Manitoba has few cattle feed lots. Procuring composted cattle manure in Manitoba may thus be costly. A large amount of product at an affordable price has led compost application to be a common practice for Alberta potato growers (Dr. F. Larney, personal communication). Other considerations are important when considering the economic benefit of compost addition such as improved yield of future crops, nutrient value of the product and possible improved water use efficacy.

Mustard seed meal has the potential to be an important part of *Verticillium* wilt management programs. However, more work is needed to determine if effective disease reduction and yield benefit can be obtained at rates lower than 5 t ha<sup>-1</sup>. The greatest challenge in using mustard seed meal is to reduce the rate of application, which is the major economic obstacle for the adoption of this practice to control soil pathogens. Inhibition of pathogens with Brassicaceae seed meals applied at high rates equivalent to about 20 t ha<sup>-1</sup> in laboratory and greenhouse conditions are effective (Chung et al. 2002) but such rates in the field are not economically affordable. The importance of achieving lower rates lies in the high purchase cost (1.51 CAD kg<sup>-1</sup>) of the material as well as cost for shipping, application and incorporation of such a large rate of product. Additional

benefits from the mustard seed meal such as N and S release may increase the advantage of using mustard seed meal in potato systems.

#### **4.4. Role of Soil Properties in Determining Efficacy of Green Manures and Amendments**

There is increasing evidence that the use of green manures and organic amendments can help farmers to maintain or improve crop health through enhancement of soil quality and through direct and indirect control of soil-borne plant pathogens (Davis et al. 1996; Rowe and Powelson 2002; Ochiai et al. 2008; Goicoechea 2009). However, soil properties play an important role in determining efficacy of green manures and organic amendments. Verticillium wilt reduction with pea, broccoli or sudan grass green manure has been associated with a number of soil chemical properties, including: decreased pH or calcium and increased potassium or magnesium (Ochiai et al. 2008).

Soil properties themselves affect the concentration and residence time of ITCs, ammonia and nitrous acid, and thus, effectiveness to kill pathogens. A number of soil factors including soil texture, pH, soil organic matter (SOM), nutrient level, moisture content and temperature influence degradation and volatilization of ITCs (Gimsing and Kirkegaard 2009). A lack of efficacy of Brassicaceae materials for killing soil-pathogens is associated with sorption of ITCs to clay and organic matter in soil (Brown and Morra 2005; Matthiessen and Shackleton 2005; Matthiessen and Kirkegaard 2006). Tenuta and Lazarovits (2004) also report accumulation of ammonia to occur in soil having low

organic carbon content (<1.4%), high pH (9) and high sand content, whereas nitrous acid toxicity was promoted by soil acidity (pH<6.0) and rapid nitrification.

The results of this study suggest that considering soil properties is a means to select what amendment such as mustard seed meal or green manure could be used to reduce soil-borne plant pathogen pressure. Efficacy of mustard green manure and seed meal to reduce *Verticillium* wilt was associated with the concentration of 2-propenyl-ITC in soil and the soil conditions affecting this concentration. In terms of practical field implementation, mustard seed meal application seems to have better potential for reducing germination of microsclerotia in soils with low pH, where the concentration of ITC in soil was potentially more toxic to the pathogen (Chapter 3).

#### **4.5. Recommendations**

Farmers in Manitoba using green manures to control *Verticillium* wilt of potato need to re-evaluate their plan for disease management. Green manures were not effective in reducing propagule density or *Verticillium* wilt. Previous studies recommend that effective control with green manure need at least two or more consecutive years of green manure; however, longer cycles are an unviable approach for management due to the loss of income.

The soil fumigant Metam sodium (Vapam) was also ineffective for the control of *Verticillium* wilt. Application of Vapam in the fall prior to spring planting had a limited



effect on propagule density, which was reduced until potato planting, and but had no effect on *Verticillium* wilt incidence.

Reduction of *Verticillium* wilt with composted beef cattle manure was not necessarily achieved due to a reduction in propagule density of *V. dahliae*. In contrast, mustard seed meal treatment reduced the number of propagules in soil, and reduced disease incidence. The reduction of the number of propagules in soil with mustard seed meal provides evidence that toxic compounds in the amendment, or generated following incorporation were involved. However, it is still unclear what mechanisms are involved in the reduction.

The effectiveness of an organic amendment in reducing disease or increasing potato yield is dependent upon properties of the soil treated. Soil characteristics may play different roles on the mechanisms of achieving disease reduction and yield increase in other potato growing areas of Manitoba. Studies at other potato growing areas in Manitoba will contribute to understand how soil properties impact the ability of organic amendments like compost, to achieve disease control and what mechanisms are responsible for the reduction.

It is still unknown at what rates of compost addition or how often is best to reduce disease. Studies with compost at different rates and sites will contribute to know the mode of action, and ultimately, improve the effectiveness of compost to reduce disease and increase potato yield. If those mechanisms can be determined, farmers could plan and

refine the application of compost in terms of timing, rates and number of applications for best control.

In Ontario, hog manure have been recently used to kill soil-borne pathogens, particularly, the root lesion nematode *Pratylenchus* spp. (Mahran et al. 2008). Composting hog manure could be an alternative manure management practice to export P from municipalities in Manitoba having excess P. Although this practice may represent a more labour intensive and perhaps more expensive than conventional liquid manure handling, composting of liquid manure would have the benefits of controlling storage and spreading odours. If composted hog manure can control soil-borne plant pathogens it would provide incentive to hog producers to invest in dry manure handling systems and be a means of exporting P to P deficient areas in Manitoba.

#### 4.6. Literature Cited

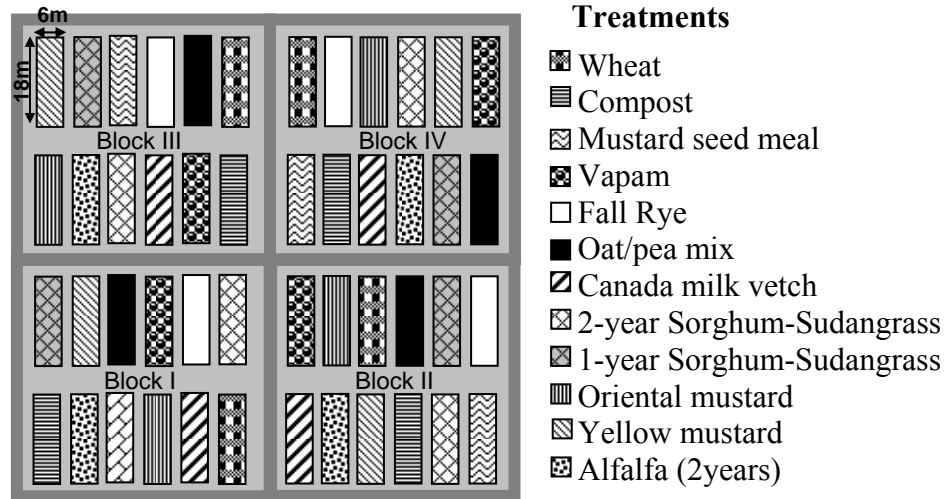
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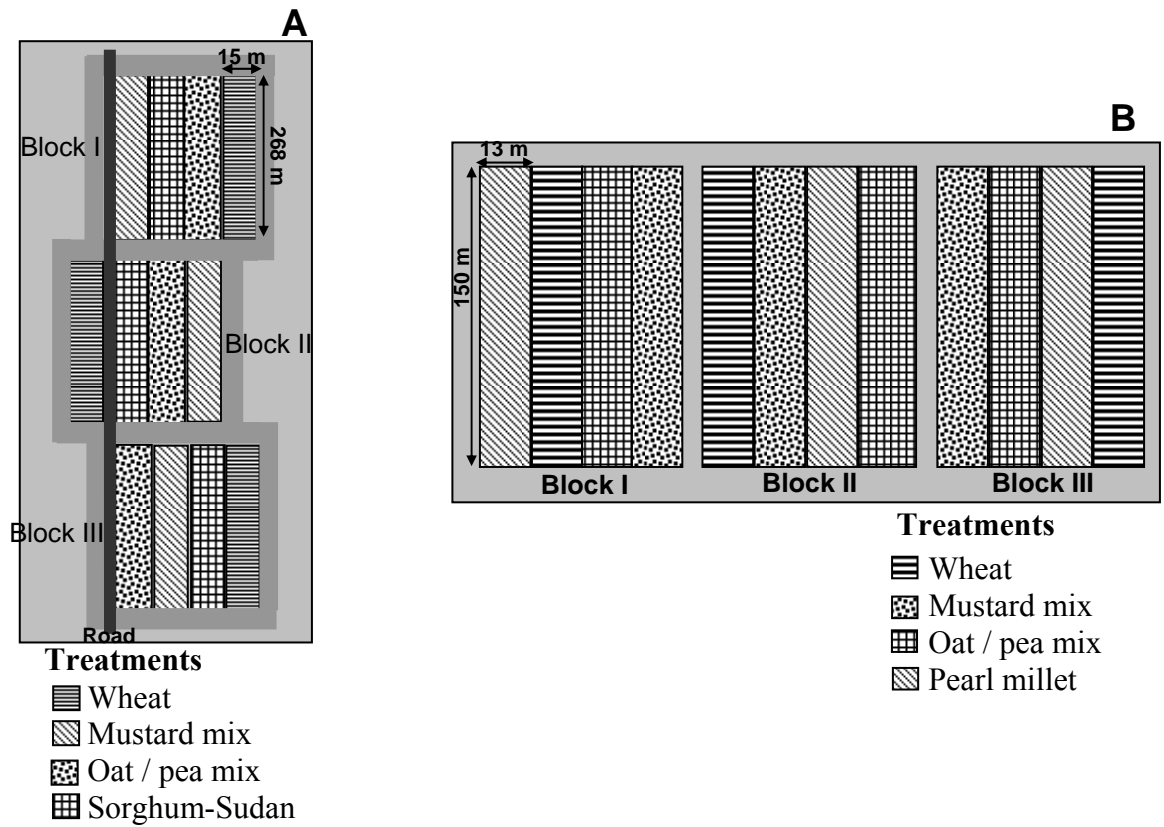
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## 5. APPENDICES

### Appendix I. Experiment 1. Field experiments.

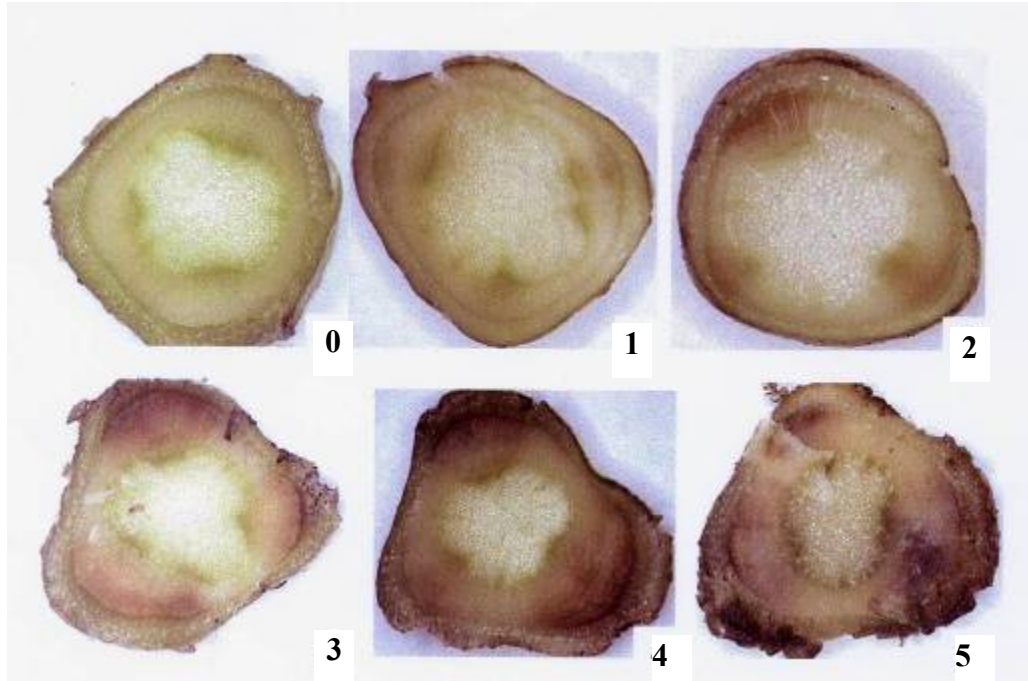


**Figure 5.1.** Experiment 1. Plot and treatment layout at the Carberry site.



**Figure 5.2.** Experiment 2. Plot and treatment layout at the Shilo (**A**) and Miami (**B**) sites.

**Appendix II.** Verticillium wilt severity in potato.



**Figure 5.3.** Rating scale to evaluate the severity of vascular discoloration of potato stems caused by *Verticillium dahliae*. 0, no vascular discoloration; 1, trace to less than 9% of the stem cross-section showing a vascular discoloration; 2, 10–24% of the stem cross-section with a vascular discoloration; 3, 25–49% of the stem cross-section showing vascular discoloration; 4, 50–74% of the stem cross-section exhibiting vascular discoloration; and 5, 75–100% of the stem cross-section displaying vascular discoloration.

**Table 5.1. Experiment 1. Effect of wheat, green manure, organic amendments and Vapam treatments on disease severity of Verticillium Wilt and chlorophyll content of potato leaves (SPAD value), August 2008.**

Treatment	Disease severity <sup>v</sup>			SPAD value <sup>w</sup>
	Lower	Middle	Upper	
Control <sup>x</sup>	1.9 ±0.3 <sup>y</sup>	0.7 ±0.1	0.1 ±0.1	40 ±0.5
COM	2.4 ±0.3	1.3 ±0.2	0.3 ±0.1	37 ±0.7
MUSTmeal	2.8 ±0.2	1.8 ±0.5	0.5 ±0.2	42 ±0.7
VAP	2.2 ±0.2	0.7 ±0.4	0.2 ±0.2	37 ±1.3
O/PEA	2.0 ±0.2	0.9 ±0.3	0.3 ±0.1	40 ±0.2
CMV	2.0 ±0.3	1.3 ±0.3	0.3 ±0.0	40 ±1.5
SS2	2.4 ±0.4	1.3 ±0.2	0.1 ±0.1	37 ±2.7
SS1	1.9 ±0.2	1.2 ±0.4	0.5 ±0.2	39 ±0.4
RYE	2.7 ±0.3	1.3 ±0.3	0.3 ±0.1	37 ±1.5
MUSTor	2.2 ±0.4	1.3 ±0.5	0.4 ±0.2	38 ±1.9
MUSTwh	2.4 ±0.3	1.1 ±0.2	0.3 ±0.2	39 ±0.4
ALF	2.0 ±0.2	1.5 ±0.5	0.8 ±0.2	39 ±0.6
<i>P</i> > <i>F</i> <sup>z</sup>	NS	NS	NS	NS

<sup>v</sup> Percentage of vascular tissue discoloured based on a scale of 0 to 5. (0= no discoloration, 1= 1 to 10%, 2= 11 to 30%, 3 =31 to 50%, 4 = 51 to 75%, and 5 = 76 to 100% discoloration).

<sup>w</sup> SPAD value. Chlorophyll meter values taken with the Minolta SPAD-502.

<sup>x</sup> Control= wheat-control, COM= compost, MUST<sub>meal</sub>= mustard seed meal, VAP= Vapam, O/PEA= oat/peas mix, CMV= Canada milk vetch, SS2= 2-year sorghum-sudangrass, SS1= 1-years sorghum-sudangrass, RYE= fall rye, MUSTor= Oriental mustard, MUSTwh= white mustard, and ALF= alfalfa.

<sup>y</sup> Value are means ± 1 standard error.

<sup>z</sup> Treatment significant probability. NS, no significant (*P*>0.05).



**Appendix III.** Experiment 1. Tuber quality of potato cv Russet Burbank.

**Table 5.2. Experiment 1. Effect of wheat, green manure, organic amendment and Vapam applications on potato quality attributes at the Carberry site.**

Treatment	Green colour	Rot (%)	Hollow heart	Specific gravity (g ml <sup>-1</sup> )	Sugar end (%)	Dark end (%)	USDA Mean fry colour <sup>w</sup>
Control <sup>x</sup>	0 ± 0 <sup>x</sup>	0 ± 0	2 ± 1	1.095 ± 0.003	0 ± 0	6 ± 1	0.04 ± 0.03
COM	0 ± 0	0 ± 0	0 ± 0	1.088 ± 0.003	0 ± 0	0 ± 0	0.05 ± 0.04
MUSTmeal	0 ± 0	0 ± 0	1 ± 1	1.087 ± 0.005	0 ± 0	1 ± 1	0.09 ± 0.03
VAP	0 ± 0	0 ± 0	1 ± 1	1.092 ± 0.001	0 ± 0	12 ± 6	0.07 ± 0.02
O/PEA	1 ± 1	0 ± 0	5 ± 4	1.093 ± 0.002	1 ± 1	5 ± 2	0.06 ± 0.03
CMV	0 ± 0	1 ± 1	5 ± 5	1.089 ± 0.003	0 ± 0	2 ± 2	0.1 ± 0.03
SS2	0 ± 0	0 ± 0	5 ± 3	1.093 ± 0.002	1 ± 1	18 ± 8	0.06 ± 0.03
SS1	0 ± 0	0 ± 0	5 ± 4	1.096 ± 0.002	0 ± 0	12 ± 4	0.05 ± 0.02
RYE	1 ± 1	0 ± 0	2 ± 1	1.092 ± 0.001	0 ± 0	9 ± 4	0.04 ± 0.02
MUSTor	0 ± 0	0 ± 0	3 ± 2	1.092 ± 0.005	1 ± 1	12 ± 8	0.03 ± 0.02
MUSTwh	0 ± 0	0 ± 0	5 ± 4	1.093 ± 0.001	0 ± 0	8 ± 2	0.05 ± 0.02
ALF	0 ± 0	0 ± 0	0 ± 0	1.091 ± 0.005	0 ± 0	4 ± 3	0.02 ± 0.01
<i>P</i> > <i>F</i> <sup>y</sup>	NS <sup>z</sup>	NS	NS	NS	NS	NS	NS

<sup>w</sup> Fry colour analysis based on the USDA French fry colour chart.

<sup>x</sup> Control= wheat-control, COM= compost, MUST<sub>meal</sub>= mustard seed meal, VAP= Vapam, O/PEA= oat/peas mix, CMV= Canada milk vetch, SS2= 2-year sorghum-sudangrass, SS1= 1-years sorghum-sudangrass, RYE= fall rye, MUSTor= Oriental mustard, MUSTwh= white mustard, and ALF= alfalfa.

<sup>y</sup> Value are means ± 1 standard error.

<sup>z</sup> Treatment significant probability. NS, no significant (*P*>0.05).