# The Effect of a Long-term Black Medic (*Medicago lupulina*) Cover Crop on Arbuscular Mycorrhizal Fungi Colonization and Nutrient Uptake of Flax (*Linum usitatissimum*)

BY

MARIE- SOLEIL TURMEL

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Plant Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

**The Effect of a Long-term Black Medic (*Medicago lupulina*) Cover Crop on Arbuscular Mycorrhizal Fungi Colonization and Nutrient Uptake of Flax (*Linum usitatissimum*)**

BY

MARIE-SOLEIL TURMEL

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

MASTER OF SCIENCE

UNIVERSITY OF MANITOBA | Faculty of Graduate Studies

# Master's Thesis/Practicum Final Report

The undersigned certify that they have read the Master's Thesis/Practicum entitled:

The Effect of a Long-Term Black Medic (Medicago lupulina) Cover Crop on Arbuscular Mycorrhizal Fungi Colonization and Nutrient Uptake of Flax (Linum usitatissimum)

submitted by

Marie-Soleil Turmel

in partial fulfillment of the requirements for the degree of

Master of Science

The Thesis/Practicum Examining Committee certifies that the thesis/practicum (and oral examination if required) is:

Approved

(Approved or Not Approved)

☑ Thesis ☐ Practicum

| | | |
|---|---|---|
| **Name/Unit:** | M.H. Entz (Plant Sci) (advisor) | **Signature:** |
| | J.C. Froese (Plant Sci) (Advisory Committee member) | |
| | M. Tenuta (Soil Sci) (Advisory Committee member) | |
| | J.G.P. Lafond(Soil Sci) (Advisory Committee member) | |

**Date:** April 27, 2007

fgs ADMIN

## ACKNOWLEDGEMENTS

I would like to express my appreciation to the following people and institutions whose contributions and support have made the completion of this project possible.

I wish to thank my supervisor, Dr. Martin Entz, for his guidance and encouragement throughout my studies.

Thank-you to the members of my examining committee, Dr. Mario Tenuta, Dr. Guy Lafond and Dr. Jane Froese, for their insightful comments that enhanced the quality of this thesis.

A special thanks to Keith Bamford for his advice and assistance with this research project.

I would also like to recognize and thank William May for managing the field trial at Indian Head, SK.

Thanks also to Dr. Terence McGonigle and Dr. Carla Zelmer for their advice and sharing their laboratory expertise.

I would like to acknowledge the importance of the institutional support that facilitated this study, particularly, NSERC, Western Grains Research Foundation and Agriculture and Agri-Food Canada.

Thanks to my friends, family and colleagues, especially to Cathy, Jackie, Jason, Sumi, Tim and Shathi, for their support, and encouragement.

"The health of soil, plant, animal and man is one and indivisible"

Lady Eve Balfour, 1943

# TABLE OF CONTENTS

# LIST OF TABLES

## LIST OF FIGURES

## LIST OF APPENDICES

## ABSTRACT

Turmel, Marie-Soleil. M.Sc., The University of Manitoba, April, 2007. The Effect of a Long-term Black Medic (*Medicago lupulina*) Cover Crop on Arbuscular Mycorrhizal Fungi Colonization and Nutrient Uptake of Flax (*Linum usitatissimum*). Major Professor; Martin H. Entz.

Cover cropping with black medic (*Medicago lupulina* cv. George) was investigated as a potential method of increasing arbuscular mycorrhizal fungi (AMF) colonization and early nutrient uptake in flax (*Linum usitatissimum*) by examining (1) early AMF colonization and nutrient uptake of flax in the field and (2) the effect of soil disturbance on early AMF colonization of flax grown after black medic in a growth chamber study. All samples (field study) and intact soil cores (growth chamber study, Manitoba) were collected from low and high soil test P long-term field experiments with the presence of black medic in a flax -wheat (*Triticum aestivum*) - oat (*Avena sativa*) rotation established in Manitoba and Saskatchewan in 2000 and 2002, respectively.

In the field study, a black medic cover crop had no influence on early AMF colonization by arbuscules or hyphal structures. When all site years were combined early flax concentrations of N, P, K and Zn were increased in the cover crop treatment, however, the cover crop had no effect on the total flax uptake of N, P, K, Mg, Zn, and Mn. The early uptake of S, Ca, Cu and Fe by flax was decreased in the black medic cover crop treatment. At one of the four site-years total flax P uptake was increased by the cover crop treatment though there was no effect on total N uptake. The results of the field study suggest that long-term cover cropping with black medic may have an effect on macro- and micro-nutrient uptake in flax that is not directly related to changes in the extent of AMF colonization.

In the growth chamber study, flax following black medic had higher early percent root length colonized by arbuscules than flax grown after soil that was left fallow ($P=0.0648$). Early flax hyphal colonization was not affected by the cover crop. Soil disturbance had no effect on the early flax root AMF colonization. The growth chamber experiment demonstrates that cover cropping with black medic is an effective method of increasing early AMF colonization.

## 1.1 INTRODUCTION

Cover cropping is a method of perennializing northern temperate agricultural systems by growing a crop in the fall, spring and winter between regular summer cropping intervals. In the present study, a unique cover crop system, one that involves a self-regenerating legume species, black medic (*Medicago lupulina* cv. George), was tested.

A potential benefit of cover cropping is the enhancement of arbuscular mycorrhizal fungi (AMF) symbiosis with the main crop (Kabir and Koide 2000; Boswell 1998; Sorensen et al. 2005). Arbuscular mycorrhizal fungi (AMF) are beneficial plant symbionts important for crop phosphorus and micronutrient uptake (Karagiannindis and Hadjisavva-Zinoviadi 1998; Liu et al. 2000). The increase in nutrient uptake is attributed to the AMF hyphal extension of the plant root system which increases the surface area of soil contact (Bolan 1991). The majority of studies have found that AMF colonization is increased following a cover crop (Galvez et al. 1995; Bosewell et al. 1998; Zak et al. 1998; Kabir and Koide 2000, 2002; Allen et al. 2001; Rutto 2002; Sorensen et al. 2005). AMF are dependent on plants for the growth and maintenance of their hyphal networks. Growing a mycorrhizal cover crop extends the time for AMF growth into the autumn, winter and spring. The extraradical mycelia are able to survive northern temperate winters providing rapid spring colonization and early season symbiosis (McGonigle and Miller 1999). Early season phosphorus supply is known to be critical for obtaining optimum crop yields. An inadequate phosphorus supply during early plant growth limits crop growth which cannot be recovered later in the season (Grant et al. 2001).

Increased AMF colonization has been correlated with an increase in plant uptake of phosphorus and micronutrients. Phosphorus uptake has been shown to increase in corn with increasing AMF colonization in field studies (Vivekanandan and Fixen 1991; Kabir and Koide 2000; Karasawa et al. 2002). However Bosewell et al. (1998) found that the increase in maize phosphorus concentration after cover cropping was not significantly correlated with colonization. Cover cropping has been shown to influence the uptake of other macro- and micro-nutrients such as potassium, calcium, zinc, copper, magnesium, iron, and manganese although results are inconsistent among studies (Bosewell et al. 1998; Kabir and Koide 2002).

The increase in mycorrhizal colonization and nutrient uptake found in crops following a cover crop may be largely attributed to an extension of the extraradical hyphal network (Boswell 1998). Disturbance of the soil and extraradical hyphal network has been shown to reduce the positive effect of cover cropping on mycorrhizal colonization (Boswell 1998). However, the research of McGonigle and Miller et al. (1990; 2000) has shown that the effect of soil disturbance on AMF colonization is inconsistent and may be dependent on the AMF inoculum density of the soil.

A field study was undertaken to investigate the influence a long term black medic cover crop on the AMF colonization and nutrient uptake of flax in low and high soil phosphorus conditions. Additionally, a growth chamber experiment was designed to investigate the influence of cover cropping combined with soil disturbance on the mycorrhizal colonization of the following flax crop.

The objectives of the field and growth chamber study were:

1. To determine if a long-term black medic cover crop increases early AMF colonization of flax. It is hypothesized that a black medic cover crop will increase the early AMF colonization of flax.
2. To determine if a long term black medic cover crop affects the early nutrient uptake of flax. It is hypothesized that the black medic cover crop will increase the uptake nitrogen, phosphorus, zinc and copper and influence the uptake of other micronutrients.
3. To determine if soil disturbance reduces the positive effect of cover cropping on the AMF colonization of the subsequent flax crop. It is hypothesized that soil disturbance will reduce the positive effects of cover cropping on the AMF colonization of the subsequent flax crop.

# 2. LITERATURE REVIEW

## 2.1 Introduction

### 2.1.1 Arbuscular Mycorrhizal Fungi Symbiosis

Arbuscular mycorrhizal fungi (AMF) are invaluable plant symbionts. They are ubiquitous among land plants suggesting that mycorrhizae were present in the early ancestors of extant land plants. This beneficial association may have even facilitated the colonization of land by plants (Read et al. 1976; Simon et al. 1993). AMF are thought to be ecologically important to most vascular plants. It has been said that it is easier to list the plants that do not form mycorrhizaes than those that do (Harley & Smith 1983). AMF are found in most species of herbaceous plants which have been studied and also in some tree species (Read et al. 1976; Harley & Smith 1983). Plants classified as bryophytes, pteridiophytes, gymnopsperms and angiosperms have been found to form mycorrhizaes (Harley and Smith 1983). The fossil record and molecular analysis shows that plants have formed mycorrhizaes for over 460 million years (Simon et al. 1993). The persistence of the relationship indicates that mycorrhizaes confer an evolutionary advantage to plants. Arbuscular mycorrhizae improve plant fitness and productivity directly through increasing uptake of phosphorus and insoluble micronutrients, and indirectly by improving soil quality parameters such as aggregate stability.

### 2.1.2 Cover crops

A cover crop is a living ground cover grown under the main crop or between regular cropping periods. Cover crops are also known as living mulches and green manures. Cover crops improve agroecosystems in many ways such as reducing nitrogen leaching, erosion and nitrogen volatilization, adding organic matter to the soil, nitrogen

fixation and enhancing beneficial microbial activity (Sarrantonio and Gallandt 2003). Perennial or self-seeding cover crops can be maintained to avoid the need for reseeding.

### 2.1.3 Black Medic (*Medicago lupulina* cv. George)

Black medic cv. George was developed at the Montana Agriculture Experimental Station, Bozeman, MT (Sims et al. 1985). Black medic is a small, short lived perennial plant from the family Leguminosae. Black medic is low growing, self-pollinated and regenerates well from seed. George is adapted to dry land farming conditions with 40 cm or more annual precipitation. Black medic is a nitrogen fixing crop, exhibiting low competitiveness, making it a potentially ideal understory cover crop (Moomaw 1995; De-Haan et al. 1997). It was developed to function as a green manure crop in "Australian Ley Farming" systems in North America (Sims et al. 1985).

### 2.1.4 Flax (*Linum usitatissimum*)

Flax is an annual crop plant in the genus *Linum*. Cultivated flax, *L. usitatissimum*, has varieties grown for oil and fiber. In Canada, mainly the oilseed flax varieties are grown (FCC, 2002). Flax is known to yield well after cereals and corn but not after canola (FCC, 2002). The response of flax to phosphorus fertilizers is low compared to other crops due to its poor chemotaxic ability and inability to proliferate roots in areas of high phosphate concentration. Thus, flax is highly dependant on AMF-mediated phosphorus uptake at low and intermediate soil phosphorus concentrations (Thingstrup et al. 1998).

## 2.2 The Influence of Plant Ecology on Arbuscular Mycorrhizal Fungi

Ecosystems are composed of many organisms interacting in a multitude of complex relationships with their environment and each other. Biological relationships are found on a scale from antagonistic to beneficial. Mycorrhizal symbiosis is a highly evolved beneficial relationship found between AMF and plants. Mycorrhizae are the most prevalent plant symbiosis known (Simon et al. 1993). They are ubiquitous across all ecosystems; 80% of all vascular plants are capable of forming mycorrhizae. In the past arbuscular mycorrhizal fungi were incorporated into the "black box" of soil microbial biomass and activities. The tremendous advancements in research on mycorrhizae physiology and ecology over the past 35 years have lead to a greater understanding of the multi-factorial role of AMF in the ecosystem and how human activities influence the efficacy of AMF symbiosis.

### 2.2.1 Plant Host Range and Specificity

The specificity, host range and degree of colonization of mycorrhizal fungi is difficult to analyze in the field due to the complexity of interactions between the AMF fungi within the root and soil system. There is no clear evidence that AMF exhibit specificity for colonization of potential AM host plant species as do fungal pathogens for their host plants (Smith & Read 2002). This may be due to the opposite selective pressures involved. In parasitic relationships the host plant benefits from mutations which prevent colonization, whereas in symbiotic relationships the plant benefits from mutations that allow for colonization by arbuscular mycorrhizal fungi (Smith & Read, 2002). However, plant species differ in the extent and dependence on colonization by

certain AM fungi. Some plants may be facultative mycotrophs while others may be obligate mycotrophs (Smith & Read 2002). Plants with tap-like roots with little branching and roots that are inefficient at seeking out phosphorus may receive the most benefit from mycorrhizal symbiosis. The ability of the same AM fungi to colonize many species of plants has ecological implications. Plants of different species may be linked underground by a common mycellial network (Smith & Read 2002). One plant may provide the photosynthate carbon for the establishment of the mycellial network which another plant of a different species will utilize for mineral uptake. This implies that arbuscular mycorrhizae are able to moderate below ground intra – and interspecific plant interactions which brings into question conventional ideas of plant competition (Smith & Read 2002).

### 2.2.2 Plant Influence on AMF Abundance and Diversity

Although AMF are thought to be generalists, there is evidence that plant species composition influences the structure of AM fungal communities (Harley and Smith 1983). The populations of AM fungi are greatest in plant communities with high diversity such as tropical rainforests and temperate grasslands where they have many potential host plants and can take advantage of their ability to colonize a broad host range (Smith & Read 2002). There is a lower incidence of mycorrhizal colonization in very arid or nutrient rich soils where their services may not be appreciated (Smith and Read 2002). Arbuscular mycorrhizal fungi species diversity is commonly determined by the spore phenotypic diversity and more recently by molecular genetic methods. When natural ecosystems are changed into arable land, the diversity of AMF spores often decreases (Schenck and Kinloch 1980). This is attributed to the lower diversity in plant species

found in agroecosystems compared to natural ecosystems. This phenomenon may also be applied to the levels of diversity found within agroecosystems. AMF species richness has been shown to decrease in crop monocultures compared to crop rotations (Hendrix et al. 1995). Bagayoko et al. (2000) found that cereals grown in rotation had higher early AMF colonization when compared to a monoculture. Baltruschat and Dehne (1988) also found that a continuous monoculture of wheat decreased the AMF inoculum potential of the soil compared to a more diverse rotation. Johnson et al. (1992) suggested that a continuous monoculture may select for rapidly growing AMF species which may be inferior mutualists.

Specific plant species have been shown to influence the population density and relative species abundance of AMF spores, although the effects are inconsistent (Black and Tinker 1979; Hendrix et al. 1995; Douds et al. 1997; Karasawa et al. 2002). Schenck and Kinloch (1980) found that AMF spore abundance and community structure was dependent on the previous plant species that was grown on newly cleared woodland soil. AMF spore populations are generally greater after mycorrhizal cover crops than after non-mycorrhizal cover crops although the effect is not consistent across all studies (Hayman et al. 1975; Douds et al. 1997; Karasawa 2002). Karasawa et al. (2002) also observed that the population density of AMF spores was greatly increased in the soil following sunflower (*Helianthus annus*), a mycorrhizal crop, compared to mustard (*Brassica nigra*), a non-mycorrhizal crop. Douds et al. (1997) also found that the relative AMF species abundance was influenced by plant species. However, Karasawa et al. (2002) found that dominant species of AMF in the soil did not change following sunflower or mustard. Hayman et al. (1975) found that spore populations were not

significantly reduced after one season of cropping with swede (*Brassica napobrassica*), a non-AMF host. Johnson et al. (1991) suggested that spore populations were affected by both the plant species and edaphic factors such as soil pH and temperature.

### 2.2.3 Plant Species Influence on Establishment of AMF Colonization

AMF ecology is directly influenced by the plant species and abundance of plants on the soil (Karasawa et al. 2002). In turn, AMF has a selective influence on the success of plants colonizing the soil. In natural systems, AMF diversity has been shown to increase plant species diversity as the potential number of associations increases. Dominant AMF species can prevent the invasion of non-mycorrhizal plants on land where they have established symbiosis while promoting their mycorrhizal hosts (Eriksson 2001).

### 2.2.4 Crop Rotation

Over the past 35 years, much research has been done to elucidate how plant species influence AMF colonization, nutrient uptake and growth of subsequent crops in agricultural systems. A crop rotation is a system of growing crop plants in a repeated, defined sequence (Harrier and Watson 2003). Crop rotation is a tool for managing nutrient supply, weeds, pest and disease. It is well known that the preceding crop will affect the growth of the subsequent crop (Karlen et al. 1994). This phenomenon, known as the "rotation effect", cannot be explained entirely by nutritional effects (Bourgeois and Entz 1996) and other factors, such as AMF, may play an important role in the success of crop rotations (Black and Tinker 1979; Hendrix et al. 1995).

It has been well established that AMF activity is decreased by non-AMF host plants (Black and Tinker 1979; Gavito and Miller 1998; Karasawa et al. 2002). Highly

mycorrhizal host crops have been found to increase AMF inoculum potential of the soil and colonization of the subsequent crops. For example, Karasawa et al. (2002) found an increase in AMF colonization and growth in maize following sunflower when compared to maize following mustard. Including non-mycorrhizal plants in the rotation reduces the rate of AMF colonization in following crops (Black and Tinker 1979; Gavito and Miller 1998). For example, Black and Tinker (1979) found that the rate of infection following the non-AMF host plant kale (*Brassica oleracea* L.) was lower than following the AMF host plant barley (*Hordeum distichon* L.). Gavito and Miller (1998) also observed delayed AMF colonization of maize (*Zea mays* L.) following canola (*Brassica napus* L.), a non-AMF host species, when compared to the colonization of maize following the AMF host species bromegrass (*Bromus inermis* Leys.) and alfalfa (*Medicago sativa* L.). The maize following canola had significantly lower AMF colonization for up to 62 days after planting, after which the colonization was equal to the maize following an AMF host species. These observations suggest that the AMF inoculum potential of the soil can be built up and the inhibitory effects of a non-mycorrhizal crop can be reduced after cropping with a mycorrhizal crop (Gavito and Miller 1998).

There is evidence that some plant species may reduce the population of a specific AMF species known to have a negative influence on the growth of the subsequent crop (Hendrix et al. 1995). The AMF species *Glomus macrocarpum* has been found to have a negative effect on tobacco (*Nicotiana tabacum* L.). This phenomenon is referred to as mycorrhizal stunt disease. Cropping tall fescue (*Festuca arundincea* Screb cv. Johnstone) before tobacco has been shown to decrease the populations of the *G. macrocarpum*, reducing its negative effect on tobacco (Hendrix et al. 1995). Management

of mycorrhizal fungi by cropping system design may be a promising way to improve the efficacy of AMF symbiosis.

### 2.2.5 Intercropping

The effect of intercropped plants on their mutual AMF colonization is likely species-specific (Hayman et al. 1979; Ocampo et al. 1980). Hayman et al. (1979) found that when onion (mycorrhizal) was grown with swede, a non-mycorrhizal plant, the onion became less colonized than when grown alone. Conversely, Ocampo et al. (1980) found that intercropping with a non-mycorrhizal plant did not reduce the colonization of mycorrhizal plants.

### 2.2.6 The Effects of Fallow on AMF

Fallow is the practice of leaving the land idle or uncropped. There are several kinds of fallow. Summer fallow was once a widespread practice on the Canadian prairies that aimed to accumulate mineral nitrogen in the soil and conserve moisture. This practice has largely been abandoned as the negative impacts of fallow on soil organic matter and fertility become more recognized (Janzen 2001). Long fallow (1 year or more) is sometimes adopted in very arid climates to accumulate enough moisture to grow a crop (Thompson 1994). In temperate climates, the soil is left fallow over fall and winter between regular spring and summer cropping intervals. Green fallow is an alternative to summer fallow in which a legume is grown over the summer and plowed into the soil to return organic matter and nitrogen to the soil (Janzen 2001).

Fallowing without AMF host cover has a detrimental effect on AMF symbiosis with the following crops. Arbuscular mycorrhizal fungi are dependent on carbon input

from their plant host for growth and reproduction. When the soil is cleared of AMF host plants, the AMF population decreases and hyphal networks deteriorate. Thus, mycorrhizal colonization and phosphorus uptake of host plants decreases with increasing length of the preceding fallow (Black and Tinker 1979; Vivekanadan and Fixen 1991; Kabir et al. 1999; Kabir and Koide 2000). When the soil is left bare, the viable hyphal network decreases over time which reduces the mineral uptake and growth of the subsequent mycorrhizal crop (Kabir et al. 1999). Long fallowing has lead to severe phosphorus and zinc deficiency in crops in Australia (Thompson 1994). Even a short fallow can reduce mycorrhizal activity and nutrient uptake of subsequent crops. For example, in a pot study by Kabir et al. (1999), a 90 day fallow was found to decrease active hyphae by 57%, AMF colonization of maize by 33% and the uptake of phosphorus, zinc and copper by 19%, 54% and 61% respectively. Clearly, fallowing is a practice that is detrimental to AMF-plant symbiosis.

### 2.3 Cover Cropping Effects on AMF Activity, Nutrient Uptake and Yield

Cover cropping is the practice of growing a crop, usually in the fall, between regular cropping intervals. Cover cropping with mycorrhizal crops perennializes the cropping system, avoiding the negative effects of fallowing on AMF, by maintaining a viable plant host throughout the growing season (Boswell et al, 1998; Sorensen et al., 2005). Cropping system effects on AMF colonization are usually observed early in the growing season (Bagayoko et al. 2000; Gavito and Miller 1998). Early colonization is crucial to providing nutritional benefits to the plant (Black and Tinker 1979). Mycorrhizal cover crops may be effective in increasing early AMF colonization, early

phosphorus and nutrient uptake and yield the main crop (Bosewell et al. 1998; Kabir and Koide 2000, 2002).

### 2.3.1 Arbuscular Mycorrhizal Fungi Colonization

The development of AM fungi prior to root colonization, known as presymbiosis, consists of three stages: spore germination, hyphal growth, as well as host recognition and appressorium formation (Douds & Nagahashi 2000). Spores are thick walled multi-nucleate resting structures (Wright, 2005). AMF spores may germinate given suitable conditions of the soil matrix, temperature, carbon dioxide concentration, pH and phosphorus concentration (Douds & Nagahashi 2000). The germination of AMF spores is not thought to be under direct control of the plant as spores have been germinated under experimental conditions in the absence of plants both *in vitro* and in soil. However, the rate of spore germination can be increased by plant host root exudates (Douds & Nagahashi 2000).

The growth of arbuscular mycorrhizal hyphae through the soil is controlled by host root exudates and soil phosphorus concentration. AMF colonization is higher in nutrient poor soils and decreases with the addition of phosphate fertilizer (Vivekanandan and Fixen 1991; Hayman et al. 1975; Read et al. 1976). Low soil phosphorus concentrations increase hyphal growth and branching as well as induce plant exudation of compounds which control hyphal branching intensity (Nagahashi et al. 1996, Douds & Nagahashi 2000). Arbuscular mycorrhizal fungi also have chemotaxic abilities which enable hyphal growth toward the roots of a potential host plant (Sbrana & Giovannetti 2005).

Once an arbuscular mycorrhizal fungal hypha encounters the root of a host plant, an appressorium is formed on the root epidermis from which the hyphae can penetrate into the host's parenchyma cortex (Gianinazzi-Pearson 1996). Once inside the parenchyma, the fungi forms highly branched structures for nutrient exchange with the plant, known as arbuscules (Gianinazzi-Pearson 1996). Arbuscules are the sites of exchange for phosphorus, carbon, water and other nutrients (Wright 2005). The host plant exerts control over the intercellular hyphal proliferation and arbuscule formation (Gianinazzi-Pearson 1996).

There are two other types of morphologically distinct hyphae which originate from the colonized host plant root. Once colonization has occurred, short lived runner hyphae grow from the plant root into the soil. These are the hyphae that take up the phosphorus and micronutrients which are transferred to the plant. AM fungal hyphae have a high surface area to volume ratio making their absorptive ability greater than that of plant roots (Tuomi et al. 2001). AMF hyphae are also finer than plant roots and can enter into pores of the soil that are inaccessible to roots (Bolan 1991).

The other type of AMF hyphae grow from their host's roots and colonize other host plant roots (Bolan 1991). Colonized roots and extraradical hyphae are an important source of inoculum in the soil (Read et al. 1976; Bosewell et al. 1998; Smith and Read 2002). A living extraradical mycellial network is important in the rapid colonization of seedlings (Read et al. 1976; Bosewell et al. 1998).

### 2.3.2 Mechanism for Increased AMF Colonization in Cover Crop Systems

AM fungi are obligate symbionts; they are dependent on their host plants for growth, maintenance and reproduction (Harley & Smith 1983). AMF take up their plant

host's products of photosynthesis such as hexoses, fructose and sucrose. The transfer of carbon from the plant to the fungi may occur through the arbuscules or intraradical hyphae (Pfeffer et al. 1999). Up to 50% of the host plant's photosynthate carbon may be transferred to the AM fungi (Pfeffer et al. 1999). This represents a considerable carbon investment in the extraradical hyphal network by the host plant. Growing a cover crop extends the time for carbon input into AMF growth and reproduction over the autumn, winter and spring (Galvez et al. 1995). The increase in colonization found in cover cropping systems may be attributed to the increase in density of infective spores, colonized roots and particularly the extraradical hyphal network (Boswell et al. 1998). The viability of AMF spores decreases over winter, which suggests that they may not be the main source of inoculum for early AMF colonization (Read et al. 1976).

An important source of inoculum may be AMF-colonized root fragments and extraradical hyphae (Read et al. 1976; Boswell et al. 1998). In cover crop systems, the density of colonized roots and hyphae is increased, which may lead to a greater soil inoculum potential (Kabir and Koide 2002). Cover cropping with AMF host plants is a soil management practice that maintains viable AMF mycelium, providing rapidly forming mycorrhizal infection (Boswell et al. 1998). Boswell et al. (1998) demonstrated that the increase in AMF colonization after a winter wheat cover crop was reduced when the extraradical hyphal network was disturbed by tillage. This indicates that the mycorrhizal colonization increase found in cover crop systems may be largely attributed to an increase in the extraradical hyphal network that can colonize the roots of the establishing crop (Boswell et al. 1998). AMF extraradical mycelia are able to survive the winter providing rapid spring colonization and early season symbiosis (McGonigle and

Miller 1999; Allen et al. 2001). This early symbiosis allows plants to tap into the well established hyphal network and be supplied with adequate phosphorus nutrition during establishment (Read et al. 1976). However, the extent of root colonization does not always correspond with phosphorus uptake (Pacovsky et al., 1986; Bosewll et al., 1998; McGonigle and Miller 2000) and the extent of the intact hyphal network may be of more importance to phosphorus uptake than the extent of root colonization (Kabir et al. 1999).

### 2.3.3 Cover Crop Species Effect on Early AMF Colonization

As discussed previously, mycorrhizal colonization is affected by the history of plant species in the agroecosystem (Karasawa et al. 2002; Gavito and Miller 1998). The majority of investigations have concluded that early AMF colonization is increased by cover cropping with mycorrhizal plants as summarized in Table 2.1 (France et al. 1985; Galvez et al. 1995; Boswell et al. 1998; Zak et al. 1998; Kabir and Koide 2000, 2002; Allen et al. 2001; Rutto 2003; Sorensen et al. 2005). Planting non-mycorrhizal cover crop (i.e., rape and oil seed radish) as a green manure or catch crop decreases the AMF inoculum in the soil (Baltruschat and Dehne 1988)

**Table 2.1.** Summary of research findings on cover crop effects on AMF colonization, uptake of phosphorus (P) and other nutrients as well as yields of the main crop (Significant increase, *; No significant effect, ns; Increase, + ; Decrease, -).

| Source | Cover Crop | Bioassay Plant | AMF Colonization | P | Other Nutrients | Yield |
|---|---|---|---|---|---|---|
| France et al. 1985 (Southern USA) | white clover | *Liquidambar styraciflua* | * | | | * |
| | corn | " | * | | | * |
| | crowder pea | " | ns | | | * |
| | lespedeza | " | ns | | | * |
| | millet | " | * | | | * |
| | milo | " | ns | | | * |
| | peanut | " | ns | | | * |
| | sorghum | " | ns | | | * |
| | soybean | " | * | | | * |
| | red clover | " | * | | | * |
| | oats | " | * | | | * |
| | ryegrass | " | * | | | * |
| | wheat | " | * | | | * |
| Galvez et al. 1995 (Pennsylvania, USA) | hairy vetch | bahai grass | * | | | |
| Boswell et al. 1998 (Pennsylvania, USA) | winter wheat | maize | * | * | Mg, + ; Fe, - | * |
| Zak et al. 1998 (Texas, USA) | wheat | cotton | * | | | |
| Kabir and Koide 2000 (Pennsylvania, USA) | winter wheat | maize | * | * | | ns |
| | dandelion | | * | * | | * |
| Allen et al. 2001 (Idaho, USA) | wheat | dry bean | * | * | Zn,+; Mn,+; Cu,+; K,+; Mg,+; Ca,+; Fe, ns | * |
| | wheat | sweet corn | * | ns | ns | ns |
| Kabir and Koide 2002 (Pennsylvania, USA) | oats, rye and both | sweet corn | * | * | N, ns | * |
| | rye | sweet corn | * | * | N, ns | * |
| | rye and oat | sweet corn | * | * | N, ns | * |
| Rutto 2003 (Ehime, Japan) | milk vetch | *Prunus persica* | * | | | |
| | hairy vetch | " | * | | | |
| | narrow vetch | " | * | | | |
| | white clover | " | * | | | |
| | red clover | " | * | | | |
| | indigenous weeds | " | * | | | |
| Baumgartner et al. 2005 (California, USA) | rye and triticale/ | grapes | ns | | | |
| | triticale | grapes | ns | | | |
| Sorensen et al. 2005 (Denmark) | black medic | leeks | * | ns | Zn, ns; Cu, ns | ns |

Cover cropping with mycorrhizal plants has been shown to increase spore densities when compared to fallowing (Kormanik 1980; France 1985; Galvez et al. 1985). However, spore populations are not always correlated with the extent of AMF colonization (Read et al. 1976; Hayman et al. 1979). France (1985) found that the cover crops effectively increased spore populations but this increase was not correlated to increased colonization of the subsequent crop.

Cover crop species differ in their to ability increase the AMF colonization of the subsequent crop (Table 2.1). There may be a species-specific interaction between the cover crop, AMF, and main crop (France et al. 1985; Allen et al. 2001). France et al. (1985), working with many summer and winter cover crops, found that soybean, millet and corn increased the AMF colonization of the hardwood *Liquidambar styraciflua* seedlings while white clover, crowder pea, lespedeza, milo, peanut and sorghum/sudan were less effective (Table 2.1). Rutto (2003) studied the effect of various clover, vetches and indigenous weed cover crops and found that all cover crops increased the colonization of the subsequent *Prunus persica* crop when compared with no cover crop. Galvez et al. (1995) found that a hairy vetch cover crop increased the colonization of bahaigrass. Boswell et al. (1998) and Kabir and Koide (2000) found that winter wheat was effective at increasing the AMF colonization of maize. Similarly, Allen et al. (2001) found that wheat increased the early AMF colonization of sweet corn and dry bean. Cotton was also observed to have higher colonization when planted into a terminated winter wheat cover crop (Zak et al. 2005). Kabir and Koide (2002) found that both oats

and rye were effective at increasing the AMF colonization of sweet corn, however, Baumgartner et al. (2005) found that rye and triticale cover crops had no effect on the AMF colonization of grapes. A combination of both rye and oat produced a higher AMF colonization in sweet corn than rye and oat alone, suggesting that species diversity in cover crops may be beneficial (Kabir and Koide 2002). These findings are summarized in Table 2.1.

Previous research demonstrates that cover cropping may be an effective method increasing rapid AMF colonization of the following crop. The research suggests that species-specific interactions between the cover crop, AMF and following crop may play a role in the extent of colonization increase (France et al. 1985; Allen et al. 2001; Kabir and Koide 2002).

### 2.3.4 Management of Weeds as Cover Crops to Increase AMF Colonization

Management of indigenous AMF host weed species may be an effective means for promoting AMF symbiosis (Kabir and Koide 2000). There is evidence to suggest the presence of mycorrhizal weeds maintains a diverse AMF population which promotes highly effective symbiosis with the crop plant. Feldman and Boyle (1998) found that the AMF benefits to maize yield from maintaining a diverse weed cover crop outweighed any yield penalty due to competition. Kabir and Koide (2000) compared a winter wheat cover crop to a dandelion cover and found that dandelion produced higher AMF colonization in the following maize crop. Sorensen et al. (2005) found that a black medic cover crop was effective at increasing AMF colonization in leeks.

## 2.4 Early Phosphorus Uptake

### 2.4.1 Nutrient Uptake and Exchange

The benefit of AMF to plants is mainly attributed to an increase in nutrient uptake, especially phosphorus. Early season phosphorus supply is known to be critical for obtaining optimum crop yields. An inadequate phosphorus supply during early plant growth limits crop growth which cannot be recovered later in the season (Grant et al. 2001). Phosphorus concentration has been shown to increase up to four times in mycorrhizal plants (Karagiannidis and Hadjisavva-Zinoviadi 1998). This increase in uptake may be due to an increase in surface area of soil contact, increased movement of nutrients into mycorrhizae, a modification of the root environment and increased storage (Bolan 1991). Mycorrhizal hyphae prove to be much more efficient than plant roots at taking up phosphorus. Phosphorus travels to the roots via diffusion and AMF hyphae reduce the distance required for diffusion thus increasing uptake. The rate of inflow of phosphorus into mycorrhizae can be up to six times that of the root hairs (Bolan 1991). In some cases the role of phosphorus uptake can be completely taken over by the mycorrhizal network and all of the plant's phosphorus may be of hyphal origin (Smith et al. 2003). An additional mechanism by which AMF may increase the available phosphorus concentration in the root zone is by lowering the rhizosphere pH due to selective uptake of $NH_4^+$ and release of $H^+$ ions (Hamel 2004).

An increase in the carbon supplied by the plant host to the AM fungi increases the uptake of phosphorus and the transfer of phosphorus from fungi to plant (Bucking & Shaker-Hill 2005). Hence, phosphorus uptake and transfer is lowered when the photosynthate supplied to the fungi is decreased. Species of AMF differ in their abilities to supply the plant with phosphorus (Pacovsky et al. 1986; Smith et al. 2003). In some

cases arbuscular mycorrhizae are poor symbionts providing little phosphorus while taking relatively high amounts of carbon (Smith et al. 2003).

### 2.4.2 Plant effect on AMF mediated P uptake

Early AMF mediated plant phosphorus uptake is higher in crops following mycorrhizal plants compared to fallow or non-mycorrhizal plants. For example, Karsawa et al. (2002) found that AMF colonization and phosphorus concentration in maize was increased following a mycorrhizal crop compared to a non-mycorrhizal crop. Vivekanandan and Fixen (1991) found that early colonization and early phosphorus concentration in corn was greater in a soy-corn rotation compared to a corn-fallow rotation. Greater early phosphorus uptake has been correlated with an increase in mycorrhizal inoculum potential and extraradical network in cover crop systems (Boswell et al. 1998; Kabir and Koide 2000, 2002; Allen et al. 2001)

### 2.4.3 Cover crop effect on AMF mediated phosphorus uptake

Plant phosphorus uptake is not always correlated with the extent of mycorrhizal colonization. Boswell et al. (1998) found the increase in early maize phosphorus concentration after a winter wheat cover crop was not significantly correlated with colonization. The authors proposed the increase in maize phosphorus concentration could have been due to a more extensive hyphal network in the cover crop system (Boswell et al. 1998). On the other hand, Kabir and Koide (2000) found that the increase in phosphorus concentration in maize following winter wheat and dandelion cover crops was in fact highly correlated with AMF colonization. However, the authors also found an increase in soil aggregate stability in the cover crop treatment compared to fallow

indicating the possibility that the hyphal density may have also increased due to the cover crop (Kabir and Koide 2000). Kabir and Koide (2002) followed this up by measuring the hyphal density in cover crop systems compared to fallow. They discovered that hyphal density was in fact increased in the cover crop system and was highly correlated with an increase in the early phosphorus concentration of the following maize crop (Kabir and Koide 2002). This provided evidence that the increase in early phosphorus uptake observed in crops following a cover crop may be due to an increase in the extraradical hyphal network (Boswell et al. 1998; Kabir and Koide 2002)

Not all studies have found a positive phosphorus uptake response due to cover cropping (Allen et al. 2001; Sorensen et al. 2005). Allen et al. (2001) found that a wheat cover crop was effective at increasing phosphorus concentration in dry bean but not in sweet corn although early AMF colonization was higher after wheat in both crops. Sorensen et al. (2005) also found that although AMF colonization of leeks was increased following a cover crop, there was no difference in root or shoot phosphorus concentrations compared to fallow. The authors attributed this result to the high soil phosphorus concentrations in the field (Sorensen et al. 2005). Phosphorus uptake may also be correlated with the soil N level rather than AMF colonization (Baltruschat and Dehne 1988). This factor is of importance in cover crop–AMF studies since nitrogen fixing plants are commonly used as the cover crop and may increase nitrogen availability and uptake.

### 2.5 Early Uptake of other Nutrients

Arbuscular mycorrhizal fungi may increase plant uptake of other soil nutrients that, like phosphorus, have low mobility in the soil. As with phosphorus, increased

nutrient uptake by AMF is thought to be due to the increased surface area for uptake provided by the extraradical hyphal network. A decrease in mycorrhizal colonization due to high soil phosphorus levels has been reported to lead to plant deficiencies in other micronutrients that require mycorrhizal mediated uptake such as copper and zinc (Lambert et al. 1979; Timmer and Leyden 1980; Pacovsky et al. 1986). Liu et al. (2000) and Lambert et al. (1979) have also reported that the effect of AMF on micronutrient uptake by plants is highly dependent on the concentration of the nutrient in the soil.

### 2.5.1 Copper and Zinc

Several studies have found that shoot concentrations of zinc and copper were increased in mycorrhizal plants when compared to un-inoculated plants (Lambert et al. 1979; Pacovsky et al. 1986; Liu et al. 2000). Plant zinc concentrations have been shown to increase by 1.7 to 3.8 times in mycorrhizal maize shoots (Kothari et al. 1991). Kothari et al. (1991) found that copper concentrations were higher in the roots of mycorrhizal plants but not the shoots. Conversely, Karagiannidis and Hadjisavva-Zinoviadi (1998) found that the micronutrient concentrations were lower in mycorrhizal plants.

Boswell et al. (1998) found that a winter wheat cover crop had no effect on the copper and zinc concentrations in maize although the mycorrhizal colonization was increased. Similarly, Allen et al. (2001) found that a wheat cover crop did not increase the copper and zinc uptake of maize when compared to fallow. This observation corresponded to no change in maize AMF colonization. They did, however, observe an increase in the zinc and copper uptake of soybean following the wheat cover crop which corresponded to an increase in AMF colonization. Sorensen et al. (2005) found no difference in leek zinc and copper concentration after a black medic cover crop; although

an increase in AMF colonization was observed. Thus, an increase in AMF colonization is not always correlated with an increase in copper and zinc uptake.

### 2.5.2 Iron and Manganese

Iron and manganese concentrations have been found to be lower in mycorrhizal plants compared to the un-inoculated controls (Pacovsky et al. 1986; Kothari et al. 1991). There is evidence that the effect of AMF on the plant uptake of these nutrients is highly dependent on their soil concentration (Lambert et al. 1979; Liu et al. 2000). AMF may affect the manganese-reducing bacteria in the rhizosphere by changing the reduction potential of $Mn^{+5}$. AMF may interact with the microbial production of ferrated siderophores, reducing plant available iron (Kothari et al. 1991). These observations could also be due to complex interactions between phosphorus and micronutrients or micronutrient retention in fungal storage organs (Pacovsky et al. 1986; Liu et al. 2000). Liu et al. (2000) studied AMF mediated uptake of iron and manganese when different amounts of micronutrients were applied to the soil. They found that the manganese uptake was reduced in mycorrhizal plants at high soil manganese levels but at the lower concentration there was no difference compared to the un-inoculated control. Iron uptake was increased with mycorrhizae at the lower soil levels and decreased compared to the control at high soil levels (Liu et al. 2000). Lambert et al. (1979) observed an increase in plant iron concentrations in mycorrhizal plants only when soil phosphorus levels were below 25 ppm.

The effect of cover crops on AMF mediated uptake of iron and manganese are inconsistent. Boswell et al. (1998) found a decrease in iron concentrations in maize after a winter wheat cover crop which corresponded to an increase in colonization. The cover

crop had no effect on the maize manganese concentration. Allen et al. (2001) found that the iron and manganese concentrations in maize following a cover crop were no different from the maize following fallow. However, in soybean following a wheat cover crop, Allen et al. (2001) observed an increase in manganese but no change in iron concentration.

### 2.5.3 Potassium, Calcium and Magnesium

Mycorrhizae can favor the uptake of potassium relative to calcium and magnesium since potassium diffuses faster in the soil than calcium or magnesium (Lambert et al. 1979). The interactions between mycorrhizae and potassium, calcium, and magnesium are not consistent between crops (Lambert et al. 1979). Boswell et al. (1998) found that a winter wheat cover crop increased magnesium concentration in the flag leaves of corn which corresponded to an increase in mycorrhizal colonization. The cover crop had no effect on the maize potassium and calcium concentrations. Allen et al. (2001) found that a winter wheat cover crop increased the concentration of calcium and magnesium in soybean but observed no change in maize.

## 2.6 Crop Yield

### 2.6.1 AMF and Crop Yield

It has been well established that arbuscular mycorrhizal symbiosis increases crop yields. A survey of 78 published field trials found that increased AMF colonization resulted in an average yield increase of 37% percent (McGonigle et al. 1988). Another meta-data analysis of 290 published field and greenhouse studies determined that increased AMF colonization resulted in a 23% yield increase (Lekberg and Koide 2005).

Early season phosphorus supply is known to be critical for obtaining optimum crop yields. An inadequate phosphorus supply during early plant growth limits crop growth which cannot be recovered later in the season (Grant et al. 2001). Thompson (1994) found that mycorrhizal flax had higher seed yield than an un-inoculated control. The dry weight of flax seed was linearly dependent on the degree of early AMF colonization (Thompson 1994). However, there is not always a direct relationship between an increase in AMF colonization and phosphorus uptake in mycorrhizal plants and an increase in biomass production (Karagiannidis and Hadjisavva-Zinoviadi 1998). There is no consistent correlation between yield increase and the extent of AMF colonization (France 1985; McGonigle 1988; Baltruschat and Dehne 1988). The degree to which AMF increases yields is also dependent on the soil type, nutrient status and crop (Karagiannidis and Hadjisavva-Zinoviadi 1998). Karagiannidis and Hadjisavva-Zinoviadi (1998) found the effect of the AMF species *Glomus mosseae* on biomass of durum wheat (*Triticum turgitum* var. *durum*) in 10 different soils ranged from 11.6 to 3.6 times higher than the un-inoculated control. Nonetheless, all yields were found to be increased by inoculation with AMF in all 10 soils (Karagiannidis and Hadjisavva-Zinoviadi 1998).

### 2.6.2 Cover cropping effect on AMF mediated increase in Crop yield

There is substantial evidence that cover cropping is an effective method of increasing the yield of the following crop (France 1985; Bosewell et al. 1998; Kabir and Koide 2000, 2002; Allen et al. 2000). However, the results are mixed as to whether yield increase can be correlated with an increase in mycorrhizal colonization or early phosphorus uptake. France (1985) experimented with nine different cover crops and

found that seedling growth of the hardwood tree *Liquidambar styraciflua* was increased by all cover crops when compared to fallow. The increase in biomass corresponded to an increase in spore density but not percent root colonization. Bosewell et al. (1998) observed an increase in height, ears/plant, and grain dry weight/plant of maize cropped after a winter wheat cover crop when compared to fallow. The increase in yield corresponded to an increase in AMF colonization but not phosphorus uptake. The authors suggested that yield increase due to AMF may not necessarily be mediated through an increase in phosphorus uptake (Boswell et al. 1998). Conversely, Kabir and Koide (2000) did not observe an increase in maize grain weight/plant following a winter wheat cover crop although there was an increase in colonization and phosphorus concentration at 8 days after emergence. However, in the same experiment, they observed a significant yield increase following a dandelion cover crop which corresponded to an increase in colonization and phosphorus uptake at 8 and 25 days after emergence (Kabir and Koide 2000). Allen et al. (2001) found that dry bean yields increased after a winter wheat cover crop compared to fallow. The increase was correlated with both colonization and the uptake of zinc, copper, and phosphorus. They did not find a change in maize yield after a winter wheat cover crop (Allen et al. 2001). Kabir and Koide (2002) found that rye and oat cover crops increased maize yield and the combination of rye and oat produced the highest yield when compared to fallow. The yield was significantly correlated with shoot phosphorus content at 27 days after emergence which disagrees with the findings of Boswell et al (1998). Sorensen et al. (2005) found no yield increase in leeks cropped after a black medic cover crop although there was an increase in AMF colonization.

## 2.7 Conclusion

Over hundreds of millions of years plants and arbuscular mycorrhizal fungi have evolved complex signaling and physiological processes allowing for symbiosis. The ubiquitous symbiotic relationship between AMF and vascular plants has been in place on earth for at least 460 million years - as long as terrestrial plants have existed. The role of arbuscular mycorrhizal fungi in agricultural ecosystems and the human impacts on AMF is increasingly being recognized. Cover cropping may be a way to mimic natural systems by providing perennial plant cover of mycorrhizal hosts. This improves the AMF inoculum potential and extent of the extraradical hyphal network providing rapid colonization and effective symbiosis to plants during establishment. Early symbiosis increases plant nutrition resulting in improved crop yields. Cover crops may be an essential element of successful low input production systems which rely on arbuscular mycorrhizal symbiosis for nutrient uptake rather than heavy agrochemical inputs.

# 3. THE INFLUENCE OF A LONG-TERM BLACK MEDIC COVER CROP ON ARBUSCULAR MYCORRHIZAL FUNGI COLONIZATION AND NUTRIENT UPTAKE IN FLAX GROWN UNDER ZERO-TILLAGE MANAGEMENT

## 3.1 Abstract

Cover cropping was investigated as a potential method of increasing arbuscular mycorrhizal fungi (AMF) colonization and early macro- and micro-nutrient uptake in flax (*Linum usitatissimum*). The presence of black medic (*Medicago lupulina* cv. George) in a flax -wheat (*Triticum aestivum*) - oat (*Avena sativa*) rotation was tested in long-term field experiments established in Manitoba and Saskatchewan in 2000 and 2002, respectively. The experimental design was a RBC replicated three times. Flax roots and aboveground tissue were collected in June of 2005 and 2006 from both medic and non-medic plots. Percent root length colonized by AMF arbuscules and hyphae and nutrient concentration and uptake were determined. The black medic cover crop had no influence on the early AMF colonization by arbuscules or hyphal structures. When all site years were combined, early flax concentrations of N, P, K and Zn were increased in the cover crop treatment, however, the cover crop had no effect on the total flax uptake of N, P, K, S, Ca, Mg, Zn, and Mn. The early flax uptake of Cu and Fe was decreased in the black medic cover crop treatment. At one of the four site years, total flax P uptake was increased by the cover crop treatment though there was no effect on total N uptake. This research suggests that long-term cover cropping with black medic has an effect on macro- and micro-nutrient uptake in flax that is not directly related to changes in the extent of AMF colonization of roots.

### 3.2 Introduction

Cover cropping is a method of perennializing northern temperate agricultural systems by growing a crop in the fall, winter and spring in between regular summer cropping intervals. In the present study, a unique cover crop system that involves a self-regenerating legume species, black medic (*Medicago lupulina* cv. George) was tested as a potential method of increasing arbuscular mycorrhizal fungi (AMF) colonization and macro- and micro-nutrient uptake of flax.

A potential benefit of cover cropping is the enhancement of arbuscular mycorrhizal fungi (AMF) symbiosis with the main crop (Kabir and Koide 2000; Boswell 1998; Sorensen et al. 2005). AMF are beneficial plant symbionts important for crop phosphorus and micronutrient uptake (Cu, Zn) (Karagiannindis and Hadjisavva-Zinoviadi 1998; Liu et al. 2000). This increase in nutrient uptake is attributed to the AMF hyphal extension of the plant root system which increases the surface area of soil contact (Bolan 1991). AMF are dependent on plants for the growth and maintenance of their hyphal networks. Growing a mycorrhizal cover crop extends the time for AMF growth into the autumn, winter and spring. The extraradical mycelia are able to survive the winter providing rapid spring colonization and early season symbiosis (McGonigle and Miller 1999). Early season phosphorus supply is known to be critical for obtaining optimum crop yields. An inadequate phosphorus supply during early plant growth limits crop growth which cannot be recovered later in the season (Grant et al. 2001).

The majority of studies have found that AMF colonization is increased following a cover crop (Galvez et al. 1995; Bosewell et al. 1998; Zak et al. 1998; Kabir and Koide 2000, 2002; Allen et al. 2001; Rutto 2003; Sorensen et al. 2005). However, France et al.

(1985) experimented with 13 different plant species as cover crops and found that only 5 of the 8 crops were effective at increasing the AMF colonization of the following crop.

Increased AMF colonization has been correlated with an increase in plant uptake of phosphorus and micronutrients. Phosphorus uptake has been shown to increase in corn with increasing AMF colonization in field studies (Vivekanandan and Fixen 1991; Kabir and Koide 2000; Karasawa et al. 2002). However Bosewell et al. (1998) found that the increase in maize phosphorus uptake after cover cropping was not significantly correlated with colonization. Cover cropping has been shown to influence the uptake of other macro and micro-nutrients such as potassium, calcium, zinc, copper, magnesium, iron, and manganese although results are inconsistent among studies (Bosewell et al. 1998; Kabir and Koide 2002). This field experiment was designed to investigate the influence of cover cropping with black medic on the AMF colonization and nutrient uptake in flax. The objectives of this study were:

1. To determine if a long term black medic cover crop increases the early AMF colonization of flax. It is hypothesized that a black medic cover crop will increase the early colonization of flax.
2. To determine if a long term black medic cover crop affects the early nutrient uptake of flax. It is hypothesized that a black medic cover crop will increase the uptake of nitrogen, phosphorus, zinc and copper and influence the uptake of other micronutrients.

## 3.3 Materials and Methods

### 3.3.1 Field Trial Management

Field experiments were conducted at the Department of Plant Science Research Station, Winnipeg, MB (Winnipeg) and Agriculture and Agrifood Canada Research Farm, Indian Head, SK (Indian Head) in 2005 and 2006. Precipitation data was obtained from the weather stations at the Winnipeg and Indian Head sites. The Winnipeg field trial was established in 2000. The trial had a fully phased winter wheat, oat, and flax crop rotation until 2002. In 2003, the winter wheat plot was chemical fallowed due to crop failure. The flax followed oat in 2005 and 2006. The Indian Head trial was established in 2002. This trial has a fully phased crop rotation of winter wheat, oat and flax. The experimental design at both locations is a randomized complete block design with 3 blocks. A black medic cover crop is the main plot effect. Plot dimensions at Winnipeg were 4 x 8 m and 4 x 10.7 m at Indian Head. Flax was used as the indicator species in this study because it is highly dependent on AMF mediated-phosphorus uptake at low and intermediate soil phosphorus concentrations (Thingstrup, et al. 1998). The flax cultivar used in this study was CDC Bethune.

The Winnipeg trial flax was seeded June 2, 2005 and May 23, 2006 at a seeding rate of 45 kg/ha and a depth of 2 cm with 15.2 cm row spacing using an zero-till disk drill experimental plot seeder (Fabro Industries, Swift Current). Indian Head 2005 flax was seeded April 28, 2005 and May 5, 2006 at a rate of 63 kg/ha and a depth of 2.5 cm with 30.45 cm row spacing using a zero-till disk hoe seeder (Conserva Pak). The black medic re-emerged after flax emergence and grew under the flax (Figure 3.1).

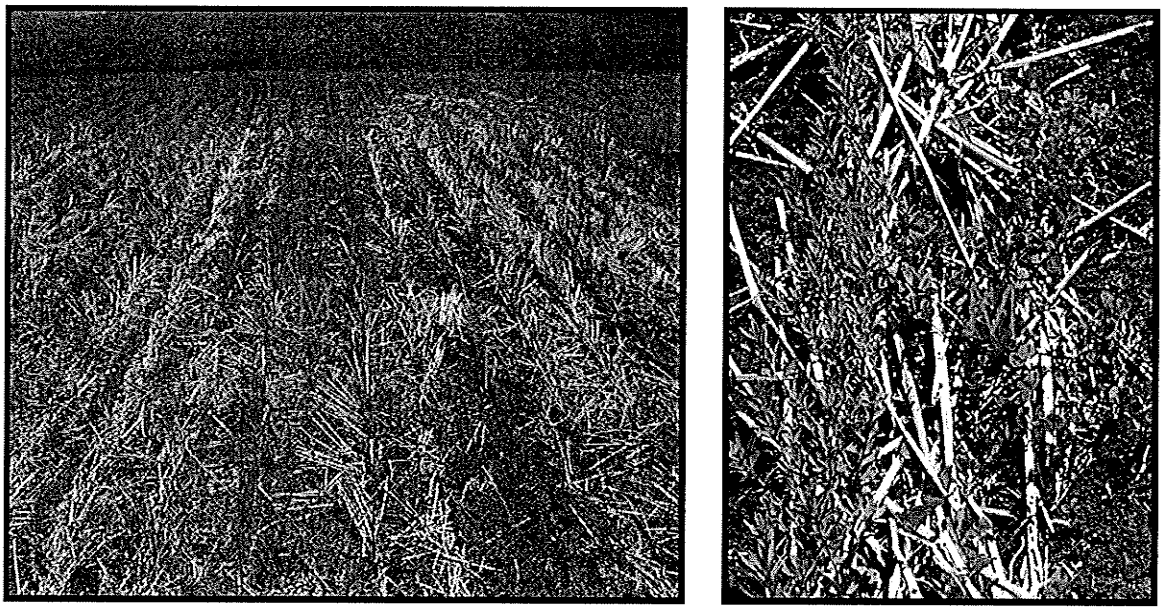

**Figure 3.1.** Indian Head 2006 flax crop with black medic cover crop 3 weeks after flax emergence (flax, f; medic, m).

The Winnipeg trial soil was mapped as a Riverdale silty clay soil. The site had historically high soil test phosphate levels. In 2004 the soil had 134 kg phosphate-P/ha (Olsen (60 ppm)) and 123 kg nitrate-N/ha. No nitrate or phosphate fertilizer was added to the Winnipeg trial in 2005 or 2006. On May 18, 2006, soil samples from each plot of the Winnipeg trial were collected from a depth of 0-12 cm analyzed individually for nitrate-N, Olsen P, Zn and Cu to determine if the medic cover crop had an effect on the early season availability of these nutrients. The soil type at Indian Head was an Indian Head heavy clay, Rego Black Chernozem (Mitchell et al. 1944). At Indian Head 2005, the residual soil nitrate-N was 19 kg/ha and phosphate-P (Olsen) was 15 kg/ha (4 ppm). At Indian Head 2005, 25 kg/ha Urea-N, 10.6 kg/ha-P, 3 kg/ha-K, and 3 kg/ha-S was added to the plots. In 2006 the residual soil N was 7.5 kg/ha and 14.5 kg/ha Urea-N, 9.2 kg/ha-

P, 2.6 kg/ha K and 2.6 kg/ha S was added to the plots. Fertilizer was placed below and to the side of the seed.

Both sites were managed in a zero-tillage production system. The Winnipeg trial was sprayed with glyphosate (3.7 L/ha) May 31, 2005. An incrop herbicide application of 0.47 L/ha Poast Ultra (sethoxydim) and 1 L/ha BuctrilM (bromoxynil + MCPA ester) with Merge surfactant (1 L/100 L) was applied on July 5, 2005. In 2006, the Winnipeg trial was sprayed with glyphosate (5 L/ha) on May 19 and Poast Ultra (0.47 L/ha) and BuctrilM (1 L/ha) with Merge surfactant (1 L/100 L) June 30. At Indian Head 2005, BuctrilM (1L/ha) with Merge surfactant (1 L/100 L) was applied on June 6, 2005. At Indian Head 2006 a pre-seeding application of 2.8 L/ha Curtail (clopyralid + 2,4-D) was sprayed on the non-medic plots. On June 1, BuctrilM (1 L/ha) with Merge surfactant (1 L/100 L) was applied. Glyphosate (3.7 L/ha) was applied on August 18, 2006 at Indian Head.

### 3.3.2 Plant Nutrient Concentration and Uptake

In 2005, above ground flax biomass samples were collected from the Indian Head site on June 14 and from Winnipeg on July 12, approximately 5 weeks after flax emergence. In 2006 samples were collected approximately 3 weeks after flax emergence at Indian Head (June 6) and Winnipeg (June 22). Samples were collected from 3, 1 meter lengths of plant row. Plant tissue samples were stored in paper bags and dried in an oven at 50°C. Plant tissue nutrient analyses were conducted by Envirotest Laboratories (2005) and Agvise (2006). Plant tissue N content was determined by nitrogen combustion analyses. Plant tissue K, Ca, Mg, S, B, Zn, Fe, Cu, and Mn content was determined by digesting the sample with 30% hydrogen peroxide and nitric acid with heat and then

analyzing on an Inductive Coupled Plasma Spectrophotometer (ICP). Total nutrient uptake was calculated using the early flax biomass and nutrient concentration data.

### 3.3.3 AMF Colonization

Flax root samples were collected from Indian Head on June 14, 2005 and Winnipeg on July 12, 2005 approximately 5 weeks after flax emergence at the same time as aboveground tissue sampling. In 2006 samples were collected approximately 3 weeks after flax emergence at Indian Head (June 6) and Winnipeg (June 22). Flax roots were sampled to a depth of approximately 12cm using a modified bulb planter (Figure 3.2a). Samples consisted of a composite of 6 randomly collected cores per plot. Approximately 30 flax plants were contained in each sample. Black medic roots from Indian Head were also randomly sampled to confirm their mycorrhizal status. The samples were soaked for 0.5 – 2.0 hours in water to loosen the roots from the soil. Roots that were not attached to a flax root system were removed. The flax roots were rinsed free of soil and cut from the above ground portion of the plant. The roots were then preserved by submerging in a 70% ethanol solution.

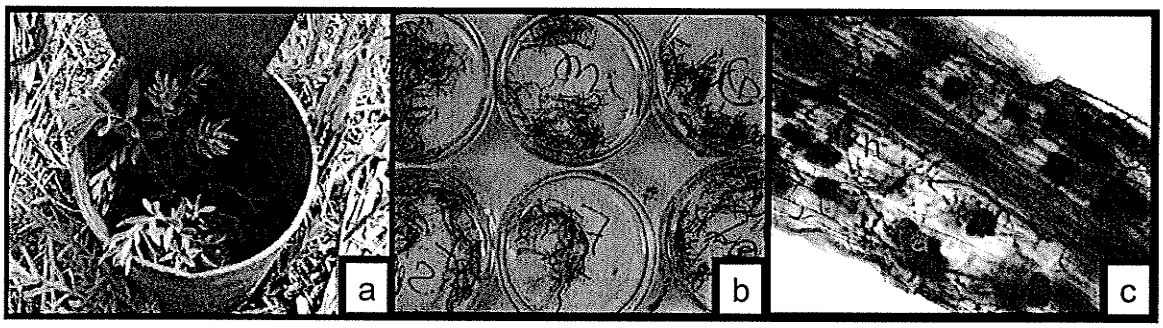


**Figure 3.2.** (a) Flax root sampling. (b) Stained flax roots. (c) Flax root highly colonized by AMF (arbuscules, a; hyphae, h; 100x mag.).

The flax roots were later prepared for AMF observation. The roots were removed from the 70% percent ethanol solution and rinsed with de-ionized (DI) water. Roots were cut with scissors into fragments approximately 2cm in length. Roots were then fixed by soaking in formaldehyde acetic acid solution (95% ethanol: 28% glacial acetic acid: 37% formaldehyde; 18:1:1) solution for 24 hours. The roots were then rinsed with DI water. Root cellular contents were then cleared by autoclaving in 10% KOH for 10 minutes and then rinsing a third time with DI water. Roots were then submerged in 0.05% Chlorozal Black E stain (Chlorozal Black E, 85 % lactic acid, glycerol and water) and put in a 90°C oven for 90 minutes. The excess stain was then rinsed from the roots. The roots were allowed to de-stain in glycerol for approximately 18 hours (Figure 3.2b). Stained roots were prepared for viewing by mounting, in glycerol, approximately 25 root fragments horizontally on a glass slide and covering with glass.

The AMF colonization of the flax roots was quantified by observation of the roots at 100x magnification. Roots were observed at 400x magnification when further identification of AMF structures was required. AMF structures were counted using the magnified intersections method (McGonigle et al. 1990). Slides were scanned up and down at regular intervals. Each intersection of the vertical eyepiece crosshair and a root was scored as root, hyphae or arbuscule (Figure 3.2c). The intersection was scored as root when no AMF structures were observed, hyphae when only AMF hyphae were observed and arbuscule when the crosshair intersected with one or more arbuscules. This was repeated until 100 intersections were scored and a measure of percent root length colonized by arbuscules and percent root length colonized by AMF (arbuscules and

hyphae) was determined. The treatment identity of each slide was concealed at the time of counting to ensure the objectivity of the study.

### 3.3.4 Yield

Winnipeg flax yield samples were collected from 3, $1m^2$ quadrants on September 27, 2005 and belt threshed and dried on a forced air drying bed. In Winnipeg 2006 flax was harvested using a Kincaid experimental plot combine and dried on a forced air drying bed. Indian Head 2005 flax was harvested using an MF 300 combine on September 6, 2005. Indian Head 2006 flax was harvested using the same combine on August 22, 2006. Dry weight of the flax seed yield was determined.

### 3.3.5 Statistical Analysis

All data were visually examined for normality and homogeneity of residuals using residuals and probability plots. Data were analyzed by analysis of variance for a randomized complete block design using the general linear model procedure (Proc GLM, SAS Institute Inc.) Data was analyzed for linear correlations using the correlation procedure (Proc Corr SAS Institute Inc.). *P* values less than 0.1 and 0.05 were considered significant.

## 3.4 Results and Discussion

### 3.4.1 Soil Nutrients

There was no significant difference in the soil concentration of nitrate-nitrogen, Olsen-phosphorus, and copper in the long-term black medic cover crop treatment when compared to the control treatment (Table 3.1). There was, however, a significant increase in soil zinc concentration in the medic treatment when compared to the control (Table

3.1). This may be due to an increase in the organic matter deposition and bio-cycling of zinc in the topsoil in the medic treatment. Pegoraro et al. (2005) observed an increase in organic acids from cover crop decomposition which increased the availability of zinc in the topsoil.

**Table 3.1** Soil-available nitrate-nitrogen, Olsen-phosphorus, zinc and copper from Winnipeg 2006 medic and no medic plots at a depth of 0-12 cm on May 18, 2006.

| Treatment | Nitrate - N (kg/ha) | Olsen- P (ppm) | Zn (ppm) | Cu (ppm) |
|---|---|---|---|---|
| Medic | 22.03 | 52.33 | 4.46 | 2.14 |
| No Medic | 21.28 | 47.67 | 3.68 | 2.02 |
| Significance | ----------*P*---------- | | | |
| | 0.8658 | 0.4422 | 0.0356 | 0.4707 |

### 3.4.2 Plant Density and Biomass

The medic cover crop had no influence on flax plant density at Winnipeg 2005 or 2006 (Table 3.2). This is possibly because the medic was eliminated with a pre-seeding glyphosate application before flax seeding. There was no difference in the early flax above ground biomass in Winnipeg 2005 (Table 3.2). However, in 2006 there was significantly lower early flax above ground biomass with the medic cover crop (Table 3.2). The difference in biomass may be explained by competition for water between the flax and the medic during the 2006 early summer drought. In 2006 the Winnipeg area experienced a severe drought (Appendix A) and flax plant growth was visibly reduced in the treatment with the medic cover crop.

**Table 3.2.** Flax stand density and early flax biomass at field sites five (2005) and three (2006) weeks after flax emergence.

| Site | Year | Treatment | Plant Density (plants/m$^2$) | Biomass (kg/ha) |
|---|---|---|---|---|
| Winnipeg | 2005 | Medic | 606 | 196 |
| | | No Medic | 708 | 229 |
| | 2006 | Medic | 458 | 188 |
| | | No Medic | 486 | 305 |
| Indian Head | 2005 | Medic | 696 | 288 |
| | | No Medic | 1264 | 265 |
| | 2006 | Medic | 647 | 216 |
| | | No Medic | 878 | 224 |
| All Site Years | | Medic | 602 | 222 |
| | | No medic | 834 | 256 |
| Significance | Site | Year | ----------*P*---------- | |
| | Winnipeg | 2005 | 0.4064 | 0.1265 |
| | | 2006 | 0.3608 | 0.0224 |
| | Indian Head | 2005 | 0.0477 | 0.4768 |
| | | 2006 | 0.4335 | 0.7522 |
| | All Site Years | | 0.0224 | 0.0845 |

The medic cover crop significantly reduced the flax stand density at Indian Head in 2005 ($P$=0.0477) although the early flax biomass was not affected (Table 3.2). The reduced flax stand may have been due to the absence of a pre-seeding glyphosate application and the high population of medics present in 2005 which likely imposed additional water stress on the flax (Appendix A). In Indian Head 2006 the medic had no influence on the flax plant density or early flax biomass (Table 3.2). The higher early flax plant density at Indian Head compared to Winnipeg may be explained by the higher seeding rate used at the Indian Head trial.

Results from these two field sites over two different years indicate that the medic reduced early flax plant density ($P$=0.02), early flax biomass accumulation ($P$=0.08). In

most site years (Winnipeg 2005 and Indian Head 2005, 2006) the flax biomass was only an average of 6 kg/ha lower in the medic plots. However, under the drought conditions experienced in Winnipeg 2006, the presence of the medic had a more negative effect on early flax biomass accumulation (Table 3.2).

### 3.4.3 Arbuscular Mycorrhizal Fungi Colonization

The medic cover crop had no influence on the percent flax root length colonized by AMF or the percent flax root length colonized by arbuscules (Fig. 3.3). The AMF colonization was high at the Winnipeg site despite high soil test phosphorus levels (Table 3.3 & 3.3). In Winnipeg 2005 the flax grown with a medic cover crop had 47% of its root length colonized by arbuscules which was not significantly different from colonization in flax grown without a cover crop (56%). Total colonization did not differ in flax grown with and without a medic cover crop at Winnipeg 2005 with both having 82% of the root length colonized by AMF. In Winnipeg 2006, there was again no difference in the arbuscular colonization: both medic and non-medic treatments had 51% colonization. Again, the total root length colonized by AMF in Winnipeg 2006 was not significantly affected by the presence of a cover crop. Results at Indian Head were similar to those observed at the Winnipeg site with no significant effect of the medic on either arbuscular or total AMF colonization (Fig. 3.3).

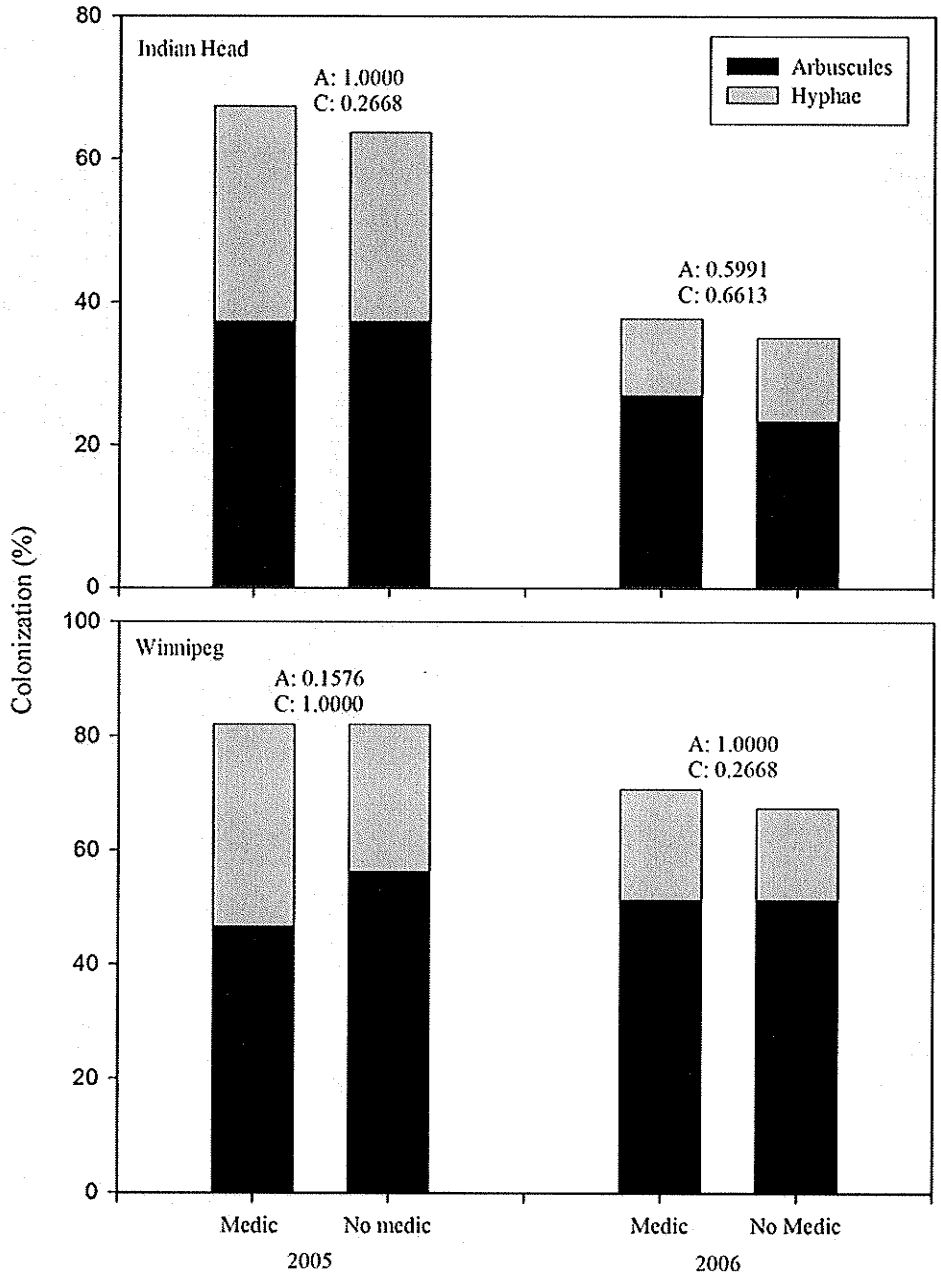


**Figure 3.3.** Arbuscular and total (arbuscules plus hyphae) AMF colonization of early flax roots at Winnipeg 2005 and 2006 and Indian Head in 2005 and 2006 as influenced by a black medic cover crop. The statistical significance (*P* value) of the medic influence on percent arbuscular colonization (A) and total colonization (C) is shown for each site year.

When all site years were analyzed together it was found the medic cover crop had no influence on either the percent root length colonized by arbuscules or total

colonization (Table 3.3). Colonization was higher in 2005 compared to 2006 (Figure 3.3). Reduced colonization in 2006 was attributed to the fact that samples were collected two weeks earlier in 2006 than 2005. Colonization differences due to previous treatment are usually most pronounced in the early season after which time the colonization among treatments approaches equal (Gavito and Miller 1998; Bagayoko et al. 2000). In 2005 no differences in AMF colonization were observed between the medic and non medic treatments so sampling was done earlier in 2006 in an effort to detect a treatment effect. However, the medic had no influence on early AMF colonization when we sampled two weeks earlier (Table 3.3). Black medic roots collected from Indian Head 2006 were highly colonized by AMF at the time of flax root sampling. Thus, the mycorrhizal status of black medic was confirmed. The mycorrhizal status of black medic has not been previously documented. However, black medic is part of the Leguminosae family of which the members are generally capable of forming mycorrhizal symbiosis (Harley and Smith 1983).

**Table 3.3.** Arbuscular and total (arbuscular + hyphal) AMF colonization of flax roots collected from field sites five (2005) and three (2006) weeks after flax emergence.

| | | Winnipeg | | Indian Head | | All Site Years |
|---|---|---|---|---|---|---|
| Colonization Type | Treatment | 2005 | 2006 | 2005 | 2006 | |
| | | ----------percent root length colonized---------- | | | | |
| Arbuscules | Medic | 47 | 51 | 37 | 27 | 41 |
| | No medic | 56 | 51 | 33 | 23 | 42 |
| Total | Medic | 82 | 71 | 67 | 38 | 64 |
| | No medic | 82 | 67 | 64 | 35 | 62 |
| Significance | | ----------*P*---------- | | | | |
| | Arbuscules | 0.1576 | 0.267 | 1.000 | 0.599 | 0.6083 |
| | Total | 1.000 | 1.000 | 0.267 | 0.661 | 0.1857 |

The overwhelming conclusion from this study is that the medic cover crop had no influence on arbuscular and total colonization of flax roots. Therefore, results from the present study do not agree with previous studies on the influence of cover cropping on AMF colonization. For example, a majority of studies have found that cover cropping increases the AMF colonization of the following corn, cotton, leek, bahiagrass, and tree crops (Table 2.1: Galvez et al. 1995; Boswell et al. 1998; Zak et al. 1998; Kabir and Koide 2000; Allen et al. 2002; Rutto 2003; Sorensen et al. 2005). One possible reason for the unique results in the present study was the several agronomic management techniques known to promote mycorrhizal colonization, such as zero-tillage, planting mycorrhizal crops and low phosphate fertilizer addition (Miller et al. 1995; McGonigle and Miller 1999; Mozafar et al. 2000; Black and Tinker, 1979; Gavito and Miller 1998; Karasawa et al. 2002; Grant et al. 2005) employed in this study. In both treatments with and without the cover crop the early flax roots were highly colonized with AMF (Fig. 3.3) compared to other studies (Bosewell et al. 1998; Sorensen et al. 2005)

Only one previous study has investigated the effect of a black medic cover crop on the AMF colonization of subsequent crops. Contrary to our results, Sorensen et al. (2005) found that a black medic cover crop significantly increased AMF colonization of a following leek (*Allium porrum*) crop. This inconsistency in the effect of a black medic cover crop on AMF colonization may be due to the difference in the previous crop and tillage regime used in the studies. In the Sorensen et al. (2005) experiment the colonization of the control was low (25%) and was increased to 62% by the black medic cover crop pre-treatment. In the present experiment, on the other hand, the early AMF colonization in the control treatment without a cover crop was high (62%) and was not

significantly increased with the addition of the medic cover crop. In the Sorensen et al. study, the previous crop was a non-mycorrhizal crop, canola (*Brassica napus* L.) while in the present study, the previous crop was a mycorrhizal crop, oat (*Avena sativa*). Non-mycorrhizal crops have been shown to significantly decrease AMF colonization of the following crop (Black and Tinker 1979; Gavito and Miller 1998; Karasawa et al. 2002). Thus, in the Sorensen et al. (2005) study, the previous canola crop presumably lowered the AMF inoculum potential of the soil while in the present study the oat maintained the AMF inoculum potential. This presumably made the influence of the cover crop on the AMF colonization of the subsequent crop less substantial in the present study. Additionally, in the Sorensen et al. (2005) study, plots were tilled before the establishment of the medic, whereas our trials were managed using zero-tillage for four (Indian Head) and five (Winnipeg) years at the time of samples collection. Tillage is known to decrease the AMF colonization of the following crop (McGonigle and Miller 1993). Thus, although the extent of colonization with a black medic cover crop was comparable between these two studies, Sorensen et al. (2005) presumably found a significant black medic cover crop influence because of previous management.

Consideration must also be given to the observational methods used in the present study. Although the root length colonized by AMF was not influenced by the presence of the cover crop, the medic may have contributed to the development of a more extensive extraradical hyphal network of the flax plants. Elsewhere it has been shown that cover crops increase the AMF extraradical hyphal network (Kabir et al. 1999) and that the extent of this network may be of even greater agronomic importance than an increase in

colonization (McGonigle and Miller 2000). Measurement of the hyphal network would be required to test this conjecture.

Additionally, the black medic cover crop may have had an influence on the AMF species diversity and community structure that could not be detected by the methods used in this study. The addition of a continuous black medic crop into the crop rotation may have altered the mycorrhizal community by promoting a particular AMF species. Crop species are known to influence the abundance of certain AMF species (Schenck and Kinloch 1980). Addition of the black medic cover crop increased plant species diversity compared to the control which may have had an influence on the AMF species diversity. Increased plant diversity is known to increase the diversity of mycorrhizal species (Grime 1987; van der Heijden 1998). Also, perennializing the cropping system by growing a cover crop in the spring and fall may have selected for certain AMF species. Cropping systems with short periods of vegetative cover select for rapidly sporulating species of AMF which may be inferior mutualists (Johnson et al. 1992; Ohel et al. 2003). Extending the period of AMF plant host availability by cover cropping allows for the slower growing and late sporulating species to complete their reproductive cycle (Ohel et al. 2003). Methods of spore identification and molecular genetic methods of *in situ* identification would be required to determine how cover cropping influences the mycorrhizal community structure.

### 3.4.4 Nutrient Concentrations and Uptake

Early flax nutrient concentrations were measured to determine the influence of a black medic cover crop on early flax nutrient uptake.

*Nitrogen*

The black medic cover crop increased early flax nitrogen concentration in one of the four site years. In Winnipeg 2005, there was an increase in early flax nitrogen concentration in the medic treatment compared to the control (Table 3.4). However, there was no significant increase in the total early flax nitrogen uptake in the medic compared to the control (Table 3.5). In Winnipeg 2006, the medic cover crop decreased total early flax nitrogen uptake (Table 3.5). This may have been due to the extremely low precipitation accumulated in Winnipeg 2006 (Appendix A) resulting in less growth and nitrogen uptake of the flax in the medic plots. The medic was presumably competing with flax for water rather than nitrogen. De-Haan et al. (1997) found that black medic (cv. George) has a low nitrogen requirement and did not compete with corn for available soil nitrogen.

**Table 3.4.** Nutrient concentration of above-ground flax tissue collected from field sites five (2005) and three (2006) weeks after flax emergence.

| | | | N | P | K | S | Ca | Mg | Zn | Cu | Fe | Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | (%) | | | | | | (ppm) | | | |
| Winnipeg | 2005 | Medic | 2.823 | 0.577 | 2.863 | 0.273 | 1.170 | 0.423 | 28.3 | 8.0 | 280.0 | 65.3 |
| | | No Medic | 2.310 | 0.543 | 2.583 | 0.293 | 1.203 | 0.423 | 27.7 | 8.3 | 440.0 | 72.7 |
| | 2006 | Medic | 2.900 | 0.677 | 2.667 | 0.310 | 0.973 | 0.497 | 45.7 | 7.7 | 95.7 | 67.7 |
| | | No Medic | 2.733 | 0.590 | 2.467 | 0.303 | 0.993 | 0.493 | 40.7 | 7.3 | 94.0 | 72.7 |
| Indian Head | 2005 | Medic | 3.663 | 0.437 | 2.817 | 0.240 | 1.127 | 0.420 | 23.0 | 8.0 | 286.7 | 112.0 |
| | | No Medic | 3.383 | 0.390 | 2.627 | 0.223 | 1.100 | 0.413 | 20.0 | 8.3 | 383.3 | 100.0 |
| | 2006 | Medic | 3.667 | 0.357 | 2.833 | 0.307 | 1.067 | 0.503 | 26.3 | 7.3 | 396.0 | 149.7 |
| | | No Medic | 3.633 | 0.387 | 2.867 | 0.303 | 1.113 | 0.493 | 23.0 | 8.0 | 511.7 | 159.7 |
| All Site Years | | | 3.263 | 0.512 | 2.795 | 0.283 | 1.084 | 0.461 | 30.8 | 7.8 | 264.6 | 98.7 |
| | | | 3.015 | 0.478 | 2.636 | 0.281 | 1.103 | 0.456 | 27.8 | 8.0 | 357.3 | 101.3 |
| Significance | | | *P* | | | | | | | | | |
| | Winnipeg | 2005 | 0.0305 | 0.3468 | 0.0691 | 0.0742 | 0.6710 | 1.0000 | 0.5286 | 0.6667 | 0.0165 | 0.0258 |
| | | 2006 | 0.3377 | 0.0506 | 0.2254 | 0.7538 | 0.5101 | 0.8600 | 0.1628 | 0.4226 | 0.8995 | 0.4941 |
| | Indian Head | 2005 | 0.1926 | 0.0848 | 0.2786 | 0.1994 | 0.7189 | 0.7538 | 0.4830 | 0.6667 | 0.5800 | 0.0351 |
| | | 2006 | 0.8845 | 0.4830 | 0.8075 | 0.9284 | 0.5135 | 0.5799 | 0.1835 | 0.1835 | 0.6185 | 0.6547 |
| All Site Years | | | 0.0120 | 0.0651 | 0.0214 | 0.8607 | 0.4885 | 0.5313 | 0.0186 | 0.3388 | 0.1254 | 0.6189 |

**Table 3.5.** Nutrient uptake of above-ground flax tissue collected from field sites five (2005) and three (2006) weeks after flax emergence.

| | | | N | P | K | S | Ca | Mg | Zn | Cu | Fe | Mn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | (Kg/ha) | | | | | | | | | |
| Winnipeg | 2005 | Medic | 5.603 | 1.143 | 5.678 | 0.543 | 2.286 | 0.828 | 0.00563 | 0.00160 | 0.05432 | 0.01304 |
| | | No Medic | 5.309 | 1.256 | 5.949 | 0.676 | 2.786 | 0.979 | 0.00641 | 0.00192 | 0.10181 | 0.01713 |
| | 2006 | Medic | 5.524 | 1.261 | 4.997 | 0.583 | 1.817 | 0.927 | 0.00854 | 0.00146 | 0.01761 | 0.01291 |
| | | No Medic | 8.357 | 1.799 | 7.580 | 0.936 | 3.029 | 1.508 | 0.01244 | 0.00225 | 0.02859 | 0.02190 |
| Indian Head | 2005 | Medic | 10.551 | 1.256 | 8.118 | 0.689 | 3.242 | 1.205 | 0.00660 | 0.00230 | 0.08224 | 0.03225 |
| | | No Medic | 8.932 | 1.021 | 7.007 | 0.589 | 2.918 | 1.087 | 0.00544 | 0.00221 | 0.10830 | 0.02675 |
| | 2006 | Medic | 7.889 | 0.773 | 6.075 | 0.653 | 2.275 | 1.087 | 0.00573 | 0.00156 | 0.08379 | 0.03215 |
| | | No Medic | 8.113 | 0.867 | 6.418 | 0.680 | 2.485 | 1.104 | 0.00514 | 0.00179 | 0.11691 | 0.03593 |
| All Site Years | | Medic | 7.3918 | 1.1083 | 6.2171 | 0.6168 | 2.4047 | 1.0119 | 0.006624 | 0.001729 | 0.05949 | 0.02259 |
| | | No Medic | 7.6776 | 1.2357 | 6.7384 | 0.7203 | 2.8046 | 1.1693 | 0.007356 | 0.002043 | 0.08890 | 0.02543 |
| Significance | | | *P* | | | | | | | | | |
| | Winnipeg | 2005 | 0.5606 | 0.4959 | 0.6630 | 0.1087 | 0.1916 | 0.2275 | 0.3194 | 0.3256 | 0.0383 | 0.1180 |
| | | 2006 | 0.0259 | 0.0202 | 0.0351 | 0.0229 | 0.0131 | 0.0148 | 0.0078 | 0.0044 | 0.1177 | 0.0140 |
| | Indian Head | 2005 | 0.2147 | 0.0675 | 0.3800 | 0.2555 | 0.5543 | 0.3598 | 0.4500 | 0.8395 | 0.6700 | 0.1050 |
| | | 2006 | 0.8550 | 0.4964 | 0.4470 | 0.7020 | 0.4511 | 0.9036 | 0.5959 | 0.1551 | 0.4978 | 0.3454 |
| | All Site Years | | 0.6365 | 0.2002 | 0.3073 | 0.0902 | 0.0825 | 0.1059 | 0.3151 | 0.0410 | 0.0734 | 0.1445 |

In Indian Head 2005 and 2006 the medic had no influence on early flax nitrogen concentration or uptake (Tables 3.4 & 3.5). It was expected that nitrogen uptake would be increased with the medic because of the potential long-term accumulation and decomposition of nitrogen rich medic plant material. When the data from all sites was analyzed together, the presence of medic resulted in significantly higher early flax nitrogen concentration (Table 3.4). However, the flax biomass was lower ($P$=0.08) in all site years leading to no difference in the overall uptake of nitrogen. This observation provides some evidence that the medic enriched the soil available nitrogen early in the growing season (Tables 3.4). Legume-based cropping systems have previously been shown to increase the nitrogen supplying power of the soil (Drinkwater et al. 1998).

*Phosphorus*

The black medic cover crop increased total phosphorus uptake in one of the four site years. In Winnipeg 2005, the medic cover crop had no influence on early flax phosphorus concentration or uptake (Figs. 3.4 & 3.5). In Winnipeg 2006, the medic significantly increased the early flax phosphorus concentration from 0.590% (no medic) to 0.677% (medic) (Table 3.4). However, Winnipeg 2006 total early flax phosphorus uptake was lower in the medic (1.261 kg/ha) compared to the control (1.799 kg/ha) (Fig. 3.5). In Indian Head 2005, the early flax phosphorus concentration was higher in the medic than the control (Table 3.4) and the phosphorus uptake was also higher in the medic treatment (Table 3.5). In Indian Head 2006, the medic had no significant influence on either early flax phosphorus concentration or uptake (Tables 3.4 & 3.5).

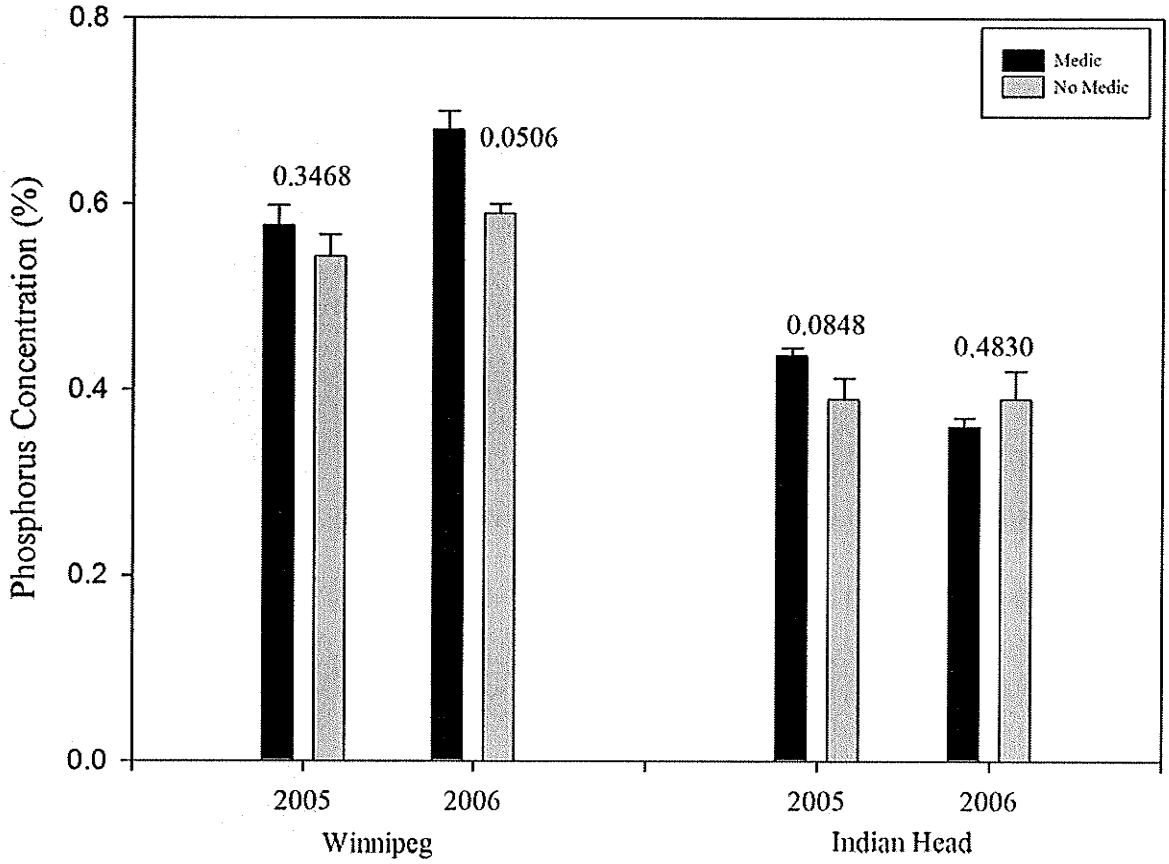


**Figure 3.4.** Early phosphorus concentration of above-ground flax tissue at Winnipeg and Indian Head 2005 and 2006 as influenced by a black medic cover crop. The statistical significance (*P* value) of the medic influence on phosphorus concentration is shown for each site year.

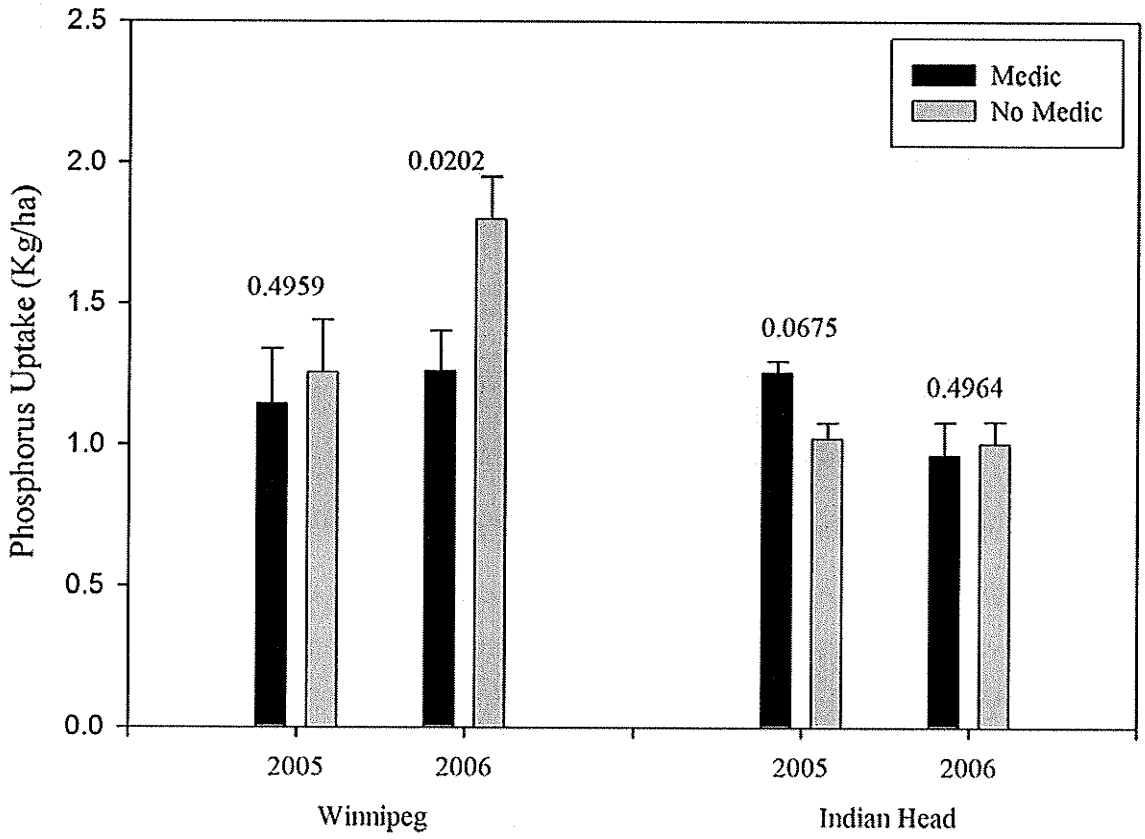


**Figure 3.5.** Early phosphorus uptake of flax at Winnipeg and Indian Head in 2005 and 2006 as influenced by a black medic cover crop. The statistical significance (*P* value) of the medic influence on phosphorus uptake is shown for each site year.

The present experiment could not confirm the results of previous studies (Galvez et al. 1995; Boswell et al. 1998; Zak et al. 1998; Kabir and Koide 2000, 2002) which showed that cover cropping increases mycorrhizal mediated phosphorus uptake. Results presented here show that the effect of the black medic cover crop on early flax phosphorus uptake varied with site and year.

The difference in early flax phosphorus concentration and uptake found in Winnipeg 2006 may have been due to water stress imposed by the medic cover crop and lower flax biomass in the medic treatment compared to the control (Table 3.2) was likely

responsible for the higher tissue phosphorus concentration. Higher total biomass production in the control resulted in the total early flax phosphorus uptake being higher in the control compared to the medic treatment (Table 3.5).

At Indian Head 2005, there was a significant increase early phosphorus concentration and uptake in the medic treatment compared to the control although there was no change in AMF colonization (Tables 3.3, 3.4 & 3.5). Total phosphorus uptake was 1.26Kg/ha with the medic cover crop compared to 1.02Kg/ha in the control (Table 3.5). The higher early flax phosphorus concentration and uptake in the medic cover crop was not related to any difference in AMF colonization due to the cover crop (Table 3.3). Although the flax plant density was lower in the medic treatment in Indian Head 2005, it was interesting that the higher total phosphorus uptake was also not related to total biomass, which was the same across both treatments.

The present study did not provide any evidence that the increase in phosphorus uptake by flax at Indian Head in 2005 was due to a medic-related increase in mycorrhizal colonization. However, as previously discussed, the black medic cover crop may have altered the nature of the AMF symbiosis in ways that could not be detected in this study such as the extent of the extraradical hyphal network and AMF species diversity. In fact, Kabir and Koide (2002) found that phosphorus uptake is more highly correlated with the density of the extraradical hyphal network rather than the percent root length colonization. However, when the data from all site years were combined there was a weak significant increase in early flax early phosphorus concentration with the medic compared to the control (Table 3.4).

Higher early flax phosphorus uptake in Indian Head 2005 in the medic system may be attributed to other non-mycorrhizal means. The medic had no influence on early flax nitrogen uptake, ruling out the possibility that nitrogen was responsible for higher phosphorus uptake (Table 3.5). One plausible reason for greater phosphorus uptake by flax in the medic plots is increased phosphorus mineralization and bioavailability. There is evidence that green manures increase soil phosphorus availability through increased organic phosphorus mineralization (Randhawa et al. 2005). A second reason may be intercrop facilitation. Intercrops are known to increase phosphorus availability to the main crop by excretion of extracellur phosphatases (Hauggaard-Nielson and Jensen 2005). However, further experimentation would be required to determine if black medic has these effects on soil phosphorus availability.

*Copper and Zinc*

The medic had no influence on early flax copper and zinc concentration and uptake in Winnipeg 2005. In Winnipeg 2006, there was no change in early copper and zinc concentration between the medic treatment and control. However, the total uptake of copper and zinc was decreased by the medic cover crop in Winnipeg 2006 presumably due to the drought effects discussed earlier. The medic cover crop had no influence on early flax concentration and uptake of zinc and copper in Indian Head 2005 and 2006.

When all site years were analyzed together, the early flax zinc concentration was found to be significantly higher in the medic treatment compared to the control (Table 3.4). However, there was no significant difference in total zinc uptake (Table 3.5). The

copper concentration was not significantly different in the medic compared to the control. In fact, the flax uptake of copper was significantly lower in the medic treatment.

It was hypothesized that a black medic cover crop would increase early flax zinc and copper uptake due to an increase in early AMF colonization. It has been well established that AMF are important for the early uptake of copper and zinc (Lambert et al. 1979; Pacovsky et al. 1986; Liu et al. 2000), because like phosphorus, these nutrients are non-mobile in soil. However, in previous cover crop studies, positive correlations between AMF colonization and zinc and copper uptake have not been found as consistently as with phosphorus uptake (Table 2.1; Boswell et al. 1998; Kabir and Koide 2000; Sorensen et al. 2005). In the present study, medic had no influence on AMF or zinc uptake and copper uptake was lower in the medic system (Table 3.5). Medic did not influence the early flax zinc uptake, which is consistent with the AMF colonization results (colonization was not increased by the presence of the medic, Fig. 3.3).

In the combined analysis, it was found that total copper uptake was decreased in the medic treatment (Table 3.4). These findings do not agree with the results of Sorensen et al. (2005) who found the black medic cover crop had no effect on copper uptake in leek. The results of the present study do, however, support the findings of Bosewell et al. (1998) who found a non-significant decrease in copper concentration in corn following a winter wheat cover crop.

At Winnipeg 2006, soil test results showed that soil zinc concentrations were higher in the medic plots compared to the control (Table 3.1). The increase in early flax zinc concentration may be due to enhanced soil zinc availability in the medic plots. This could be a result of greater accumulation of zinc in topsoil from an increase in plant

biomass deposition in the medic treatment. Pegoraro et al. (2005) found that the addition of 18t/ha of millet plant materials to the soil increased the diffusion of Zn by 73%. Low molecular weight organic acids from root exudates and decomposing organic residues can coat clay particles reducing adsorption and increasing diffusion of nutrients to the root (Pegoraro et al. 2005). This conjecture would need to be tested with further experimentation.

*Potassium, Sulfur, Calcium and Magnesium*

The black medic cover crop affected potassium, sulfur, calcium and magnesium uptake at only one of the four site years (Winnipeg 2006). In Winnipeg 2005, the medic cover crop increased early flax potassium concentration ($P$=0.069; Table 3.4). Sulfur concentration was decreased by the presence of the medic ($P$=0.074; Table 3.4). The medic had no influence on the early flax concentration of calcium or magnesium (Table 3.4). There was no significant effect of the medic on flax uptake of potassium, sulfur, calcium or magnesium in Winnipeg 2005 (Table 3.5). The medic had no influence on the early flax concentration of potassium, sulfur, calcium, and magnesium in Winnipeg 2006 (Tables 3.4 & 3.5). The total uptake of potassium, sulfur, calcium, and magnesium was decreased by the medic cover crop in Winnipeg 2006 presumably due to the drought effects discussed earlier (Table 3.5). In Indian Head 2005 and 2006, the medic cover crop had no influence on the early flax concentration and uptake of potassium, sulfur, calcium, and magnesium (Tables 3.4 & 3.5).

When all site years were combined, the flax in the medic cover crop treatment demonstrated higher early potassium concentration ($P$=0.021; Table 3.4). This may be

related to the increase in nitrogen uptake observed (Table 3.5). The concentration of sulfur, calcium and magnesium were not affected by the medic cover crop. There was a decrease in the early flax uptake of sulfur and calcium in the medic plots which was driven by the results of Winnipeg 2006 (Table 3.5). There was no difference in the uptake of potassium and magnesium when all site years were combined.

Previous studies have provided evidence that AMF influence the uptake of potassium, sulfur, calcium, and magnesium by their host plants (Lambert et al. 1970; Boswell et al.; Allen et al. 2001). The present study did not provide any evidence that the increase in early flax potassium concentration uptake by flax was related to any increase in mycorrhizal colonization due to the medic cover crop.

*Iron and Manganese*

In one of the four site years (Winnipeg 2005) the early flax iron and manganese concentration was decreased by the medic cover crop when compared to the control (Table 3.4). Early flax iron uptake was significantly lower in the medic treatment in Winnipeg 2005 (Table 3.5). There was no difference in the early flax uptake of manganese between the medic treatment and the control in Winnipeg 2005 (Table 3.4). In Winnipeg 2006, the medic had no influence on the early flax concentration of iron and manganese (Table 3.4). The medic had no influence on the flax uptake of iron in Winnipeg 2006; however, the early flax uptake of manganese was significantly reduced in the medic treatment (Table 3.5). In Indian Head 2005, the medic significantly increased the early flax concentration of manganese compared to the control (Table 3.4). There was no difference in the flax manganese concentration in the medic treatment

compared to the control in Indian Head 2006 (Table 3.4). The medic had no influence on the early flax uptake of manganese in Indian Head 2005 or 2006 (Table 3.5). The medic had no significant influence on the early flax iron concentration and uptake in Indian Head 2005 or 2006 (Table 3.5).

When all site years were analyzed together there was a significant ($P$=0.073) decrease in early flax iron uptake in the medic cover crop treatment (Table 3.5). However, there was no significant difference in the flax iron concentration in the medic treatment compared to the control (Table 3.4). The medic had no influence on the early flax manganese concentration or uptake when data from all site years was combined (Tables 3.4 & 3.5).

It was expected that the black medic cover crop would have an influence on AMF mediated uptake of iron and manganese. The cover crop significantly decreased the early flax uptake of iron when all site years were combined and in Winnipeg 2005 alone. However, our experiment did not provide evidence that the change in iron or manganese uptake was related to a change in AMF colonization due to cover cropping.

Our experiment supports the results of Bosewell et al. (1998) who found that cover cropping decreased the uptake of iron in the following corn crop (Table 2.1). The decrease in iron uptake by flax grown with a black medic cover crop found in Winnipeg 2005 and the combined analysis may be due to increased competition for iron in the rhizosphere. Iron containing proteins are essential for the nitrogen-fixing symbiosis between *Rhizobium* and legumes. Thus, nodulating legumes have a high demand for iron (Guerinot 1991). Intercropping with black medic may have presented an increased competition for iron. However, this may not be an accurate explanation considering

Boswell et al. (1998) found a decrease in iron uptake after cover cropping with winter, wheat which is not a nitrogen-fixing crop. It is possible that cover crop transpiration reduced soil moisture, which could have decreased the availability of iron in the rhizosphere (Havlin et al. 2005). The decrease in flax iron uptake observed in the present study may be due to another cover crop effect that is unexplainable given the variables measured in this study. The medic cover crop also had a significant yet unexplainable effect on early flax concentration of manganese: it was decreased in Winnipeg 2005 and increased in Indian Head 2005.

### 3.4.5 Flax Seed Yield

The flax seed yield at Winnipeg was very low in both 2005 and 2006 (Appendix B). This can be attributed to the flood conditions at Winnipeg 2005 and drought in Winnipeg 2006 (Appendix A). The medic cover crop significantly increased the flax seed yield in Winnipeg 2005. The medic had no influence on final seed yield in Winnipeg 2006 despite the presumed water competition between the flax and medic and lower early flax biomass. The medic cover crop had no influence on grain yield in Indian Head 2005 and 2006.

It was expected that the presence of the medic would increase flax yields due to both increases in nitrogen and mycorrhizal mediated nutrient uptake. However, it was found that that the medic did not increase total nutrient uptake in early flax. Therefore, the yield results agree with the colonization and combined nutrient uptake data which suggest that the medic did not increase the early nutrient uptake in flax. It is important to note the medic did not present any competition detrimental to final yield under low soil

nitrate-N conditions of the experiment. This agrees with other studies that have found that intercropping black medic did not reduce crop yields (Moomaw 1995).

Combining all site years (Winnipeg 2005, Winnipeg 2006, Indian Head 2005 and Indian Head 2006) yielded a significant correlation between early nitrogen, phosphorus, potassium, zinc and manganese concentration with percent root length colonized by arbuscules. This agrees with previous studies on the effect of AMF colonization on early phosphorus concentration and uptake (Kabir and Koide 2000). However, examination of the plotted data indicated that the significant correlation can be attributed to the clustering of data points in two groups based on site (Indian Head and Winnipeg) rather than a continuous relationship between nutrient uptake and percent arbuscular colonization.

### 3.5 Summary and Conclusions

The black medic cover crop had no influence on the early AMF colonization of flax. This study demonstrated that under zero-tillage management with only mycorrhizal crops in rotation, the AMF colonization is very high and the addition of a cover crop has no effect mycorrhizal colonization. However, it is possible that cover cropping would have a positive effect in cropping systems that are not so favorable to AMF. Considering that canola is a major crop on the prairies and tillage is still practiced, further research to determine the effect of a black medic cover crop on AMF colonization in a canola-dominant or tilled prairie cropping system is warranted.

Early flax above ground tissue concentrations of nitrogen, phosphorus, potassium and zinc were increased in the black medic cover crop treatment. However, cover cropping with black medic had no effect on the total flax uptake of nitrogen, phosphorus,

potassium, magnesium, zinc, and manganese. The early flax uptake of sulfur, calcium, copper and iron was decreased the black medic cover crop treatment.

Total flax phosphorus uptake was increased in the cover crop treatment in only one site year (Indian Head 2005). Thus, the black medic cover crop may have influenced phosphorus availability by a non-mycorrhizal related mechanism.

Winnipeg 2006 demonstrated that under extremely arid conditions that are often experienced on the northern prairies, a medic cover crop can exert some competition with the main crop. However, although the yields were extremely low, the medic cover crop had no influence on flax grain seed yield.

This research suggests that long-term cover cropping with black medic may have an effect on macro- and micro-nutrient uptake in flax that is not directly to a cover crop influence on AMF colonization.

# 4. INCREASED ARBUSCULAR MYCORRHIZAL FUNGI COLONIZATION OF FLAX DUE TO A MEDIC COVER CROP IS NOT AFFECTED BY SOIL DISTURBANCE

## 4.1 Abstract

The effect of cover cropping and soil disturbance on arbuscular mycorrhizal fungi (AMF) colonization of flax (*Linum usitatissimum*) was investigated in a growth chamber experiment. The objective of this study was to determine if a black medic (*Medicago lupulina* cv. George) cover crop increases AMF colonization of the following flax crop and if any change in AMF colonization would be reduced by soil disturbance. Intact soil cores with and without cover crops were collected in the spring from a 5 year old black medic field trial in Winnipeg, MB, Canada. Flax grown following black medic had higher early percent root length colonized by arbuscules than flax grown after soil was left fallow ($P$=0.0648). Early flax hyphal colonization was not affected by the cover crop. Soil disturbance had no effect on the early flax root AMF colonization. This experiment demonstrates that cover cropping with black medic is an effective method of increasing early AMF colonization in flax.

## 4.2 Introduction

Arbuscular mycorrhizal fungi are dependent on photosyntate inputs from their plant host for growth, maintenance and reproduction (Smith and Read 2002). When the soil is left fallow or devoid of AMF host plants, the AMF population decreases and the hyphal network deteriorates (Kabir et al. 1999). Thus, mycorrhizal colonization and phosphorus uptake of the following host plant decreases with increasing length of the preceding fallow (Black and Tinker 1979; Vivekanadan and Fixen 1991; Kabir et al. 1999; Kabir and Koide 2000). In a pot study by Kabir et al. (1999), a 90 day fallow was

shown to decrease active hyphae by 57%, AMF colonization of maize by 33% and the uptake of phosphorus, zinc and copper by 19%, 54%, 61% respectively. Clearly, fallowing, even for a short time, is a practice that is detrimental to the AMF-plant symbiosis.

Growing a cover crop extends the time for carbon input into AMF growth and reproduction and maintenance of the extraradical hyphal network into the autumn, winter and spring (Galvez et al. 1995). The mycorrhizal colonization increase found in crops following a cover crop may be largely attributed to an increase in the extraradical hyphal network (Boswell et al. 1998). Disturbance of the soil and extraradical hyphal network has been shown to reduce the positive effect of cover cropping on mycorrhizal colonization (Boswell et al. 1998). However, the research of McGonigle and Miller et al. (1990; 2000) has shown that the effect of soil disturbance on AMF colonization is inconsistent and may not always be decreased.

This controlled experiment was designed to investigate the influence of cover cropping combined with soil disturbance on the mycorrhizal colonization of the following flax crop. The objectives of this study were:

1. To determine if cover cropping with black medic increases the AMF colonization of the subsequent flax crop. It is hypothesized that a black medic will increase the AMF colonization of the subsequent flax crop.
2. To determine if soil disturbance reduces the effect of cover cropping on the AMF colonization of the subsequent flax crop. It is hypothesized that soil disturbance will reduce the positive effects of cover cropping on the AMF colonization of the subsequent flax crop.

## 4.3 Materials and Methods

### 4.3.1 Cover Crop Field Trial

Soil cores were taken from the black medic cover crop field trial at the Department of Plant Science Research Station, Winnipeg, MB (Winnipeg). The field trial was established in 2000 and had a fully phased winter wheat, oat, and flax crop rotation until 2002. In 2003, the winter wheat plot was chemical fallowed due to crop failure. The flax followed oat in 2005 and 2006. The field trial design was a randomized complete split-plot block design consisting of three blocks. A black medic cover crop was the main plot effect and the addition of nitrogen fertilizer was the sub-plot effect. Plot dimensions were 4 x 8m. The soil was mapped as a Riverdale silty clay soil. The soil from the medic trial had 21.65 kg/ha of nitrate-N and 50 ppm Olsen-P in the spring of 2006.

### 4.3.2 Soil Core Collection

Intact soil cores were collected from the Winnipeg black medic trial on May 16 2006 before herbicide application and seeding. The black medic was approximately 2cm in height above ground and at the first true leaf stage. Cores were sampled from all the plots to be planted to flax in 2006; 12 plots in total. The previous crop on these plots was oats. Cores collected from the plots with the medic cover crop contained at least 5 living black medic plants. Cores were taken to a depth of 9 cm using aluminum pipes 10 cm in length and 5 cm in diameter. Pipes were lubricated with linseed oil to prevent the soil from adhering to the aluminum and compacting. Cores were taken in pairs at 2 locations in each plot; 4 cores from each plot and 48 cores in total. The cores were capped with

plastic at the bottom end to prevent soil and water loss. The soil-filled cores weighed between 285 and 310g.

### 4.3.3 Cover Crop Pre-treatment

Cores were incubated for 18 days in a 21°C growth chamber with 16 hours of sunlight. The black medic plants in the cores from field plots with medic were allowed to grow while the cores from the plots without medic were left bare. All cores were given 35 mL of water approximately every three days to keep soil moist. Weeds that emerged were pulled out and placed back on top of the soil. On June 2, 2006, medics were harvested by cutting the stem at the soil surface. The medics were dried in a 55°C oven and weighed. The above ground medic biomass was not returned to the soil.

### 4.3.4 Soil Disturbance

Soil cores were disturbed to break up the AMF extraradical hyphal network. Cores were incubated for 10 days after medic harvest. On June 12, 2006, one soil core from each pair was disturbed using a biologically inert titanium wire. The wire was threaded through the length of the core and then passed back and forth across the diameter of the core. In this manner, the soil core was sliced into approximately 1cm cubes without disturbing the vertical soil core profile.

### 4.3.5 Flax Seeding

Flax was then seeded two days after soil disturbance on June 14, 2006. The flax variety used for this study was Bethune. Seeds were placed at a depth of 3cm; 6 seeds per core. Cores were incubated in a 21°C growth chamber with 16 hours of sunlight. The

flax began to emerge on June 19, 2006 and was thinned to 3 plants per core on June 23, 2006. Cores were given 35mL of water approximately every three days to keep soil moist.

### 4.3.6 Flax Harvest

Flax roots and above ground tissue were harvested 25 days after emergence on July 13, 2006. Cores were soaked for 24 hours and then removed from the aluminum tubes. Cores were then soaked for an additional 2 hours to loosen the soil from the flax roots. The remaining soil was removed from the flax roots by spraying with water. Clean roots were then cut from the above ground portion of the plant and submerged in FAA (95 % ethanol: 28% glacial acetic acid: 37% formaldehyde; 18:1:1) fixing solution. The above ground portion of the flax plants were dried in a 55°C oven and weighed.

### 4.3.7 Root Colonization

Roots were later prepared for AMF observation. Fixed roots were rinsed with de-ionized water. Roots were cut with scissors into fragments approximately 2cm in length. Cellular contents were then cleared by submerging in 10% KOH and autoclaving for 10 minutes. The roots were rinsed a third time in DI. Roots were then submerged in 0.05% Chlorozal Black E stain (Chlorozal Black E, 85 % lactic acid, glycerol and water) and put in a 90°C oven for 90 minutes. The excess stain was then rinsed from the roots and they were allowed to de-stain in glycerol for 20 hours. Stained roots were prepared for viewing by mounting, in glycerol, approximately 25 root fragments horizontally on a glass slide and covering with glass.

Flax roots were observed for quantification of AMF structures at 100x magnification. Roots were observed at 400x magnification when further identification of AMF structures was required. AMF structures were counted using the magnified intersections method (McGonigle et al. 1990). Slides were scanned up and down at regular intervals. Each intersection of the vertical eye piece crosshair and a root was scored as root, hyphae or arbuscule. The intersection was scored as root when no AMF structures were observed, hyphae when only AMF hyphae were observed and arbuscule when the crosshair intersected with one or more arbuscules. Counting proceeded until 100 intersections were scored giving a measure of percent root length colonized by arbuscules and percent root length colonized by AMF (arbuscules and hyphae). The treatment identity of each slide was concealed at the time of counting.

### 4.3.8 Statistical Analysis

All data were visually examined for normality and homogeneity of residuals using residuals and probability plots. The effects of the medic cover crop, previous nitrogen fertilization, and soil disturbance on early flax biomass and AMF colonization was analyzed. Data was analyzed by analysis of variance for a randomized complete block design using the general linear model procedure (Proc GLM, SAS Institute Inc.). *P* values were considered significant at the 0.1 and 0.05 level.

## 4.4 Results and Discussion

### 4.4.1 Medic Biomass

There was a weakly significant increase in the medic biomass in the cores from plots historically fertilized with nitrogen and those without ($P$ = 0.0950). It was noted

that black medic plants in the area of the field trial were at the same stage as the medics in the greenhouse at the time of medic removal indicating that growing conditions in the growth chamber were similar to those experienced in the field.

### 4.4.2 Flax Biomass

The early flax biomass was significantly greater in cores taken from the plots with historical nitrogen fertilization (Table 4.1). The medic cover crop had no direct effect on the early flax biomass (Table 4.1). Disturbance alone did not have a significant effect on early flax biomass. There was, however a significant interactive effect of the medic cover crop and soil disturbance on flax biomass (Table 4.1). The highest flax biomass was found in the cores that previously had a medic cover crop and received soil disturbance. This was presumably due to increased decomposition and nitrogen mineralization of the medic residues after soil disturbance. Nitrogen mineralization and availability of a green manure crop has been shown to increase after soil disturbance. (Sarrantonio et al. 1988; Drinkwater et al. 2000).

**Table 4.1.** Early flax biomass and arbuscular and total AMF (arbuscules and hyphae) colonization of flax roots. Flax biomass and roots were collected 25 days after flax emergence from soil cores grown with and without a black medic cover crop and with and without soil disturbance.

| Treatment | | Flax Biomass (g) | Arbuscules (%) | Colonization (%) |
|---|---|---|---|---|
| Medic | | | | |
| | Nitrogen | 0.0745 | 37.3 | 61.3 |
| | Disturbance | 0.0753 | 32.3 | 55.7 |
| | N + Dist. | 0.0960 | 38.3 | 59.0 |
| | no N + no Dist | 0.0775 | 30.3 | 58.7 |
| | Average | 0.0808 | 34.6 | 58.7 |
| No Medic | | | | |
| | Nitrogen | 0.0654 | 23.0 | 45.0 |
| | Disturbance | 0.0635 | 33.0 | 68.0 |
| | N + Dist. | 0.0713 | 23.3 | 52.7 |
| | no N + no Dist | 0.0752 | 26.7 | 52.0 |
| | Average | 0.0689 | 26.5 | 54.4 |
| Significance | | ----------*P*---------- | | |
| | Medic | 0.3856 | 0.0648 | 0.1188 |
| | Nitrogen | 0.0249 | 0.9835 | 0.9372 |
| | Medic*Nitrogen | 0.3220 | 0.1229 | 0.1982 |
| | Disturbance | 0.4903 | 0.5521 | 0.3945 |
| | Medic*Disturbance | 0.0594 | 0.8203 | 0.6055 |
| | Nitrogen*Disturbance | 0.2355 | 0.6655 | 0.6273 |

### 4.4.3 AMF Colonization

The black medic cover crop increased early percent root length colonized by arbuscules in the following flax roots compared to flax grown in the fallow soil ($P$=0.0648) (Figure 4.1). Early flax root colonization increased from 26.5% root length colonized by arbuscules in the fallow pre-treatment to 34.6% in the cover crop pre-treatment. This agrees with a previous controlled study that found higher AMF colonization in crops when grown in soil with a cover crop compared to soil that had been fallowed (Kabir et al. 1999). The results of the present study verify the results of several field experiments demonstrating that maintaining a mycorrhizal host plant on the soil increases the AMF colonization of subsequent crops compared to fallow (Black and Tinker 1979; Vivekanadan and Fixen 1991; Kabir and Koide 2002). The black medic pre-

treatment presumably provided a host plant for the AMF and initiated the growth of the extraradical hyphal network. The flax was then planted into the pre-established hyphal network which allowed for rapid colonization. Whereas, in the control treatment, the flax was planted into soil lacking an active AMF hyphal network. Thus, slower colonization would have occurred from spores and over wintered AMF colonized root fragments.

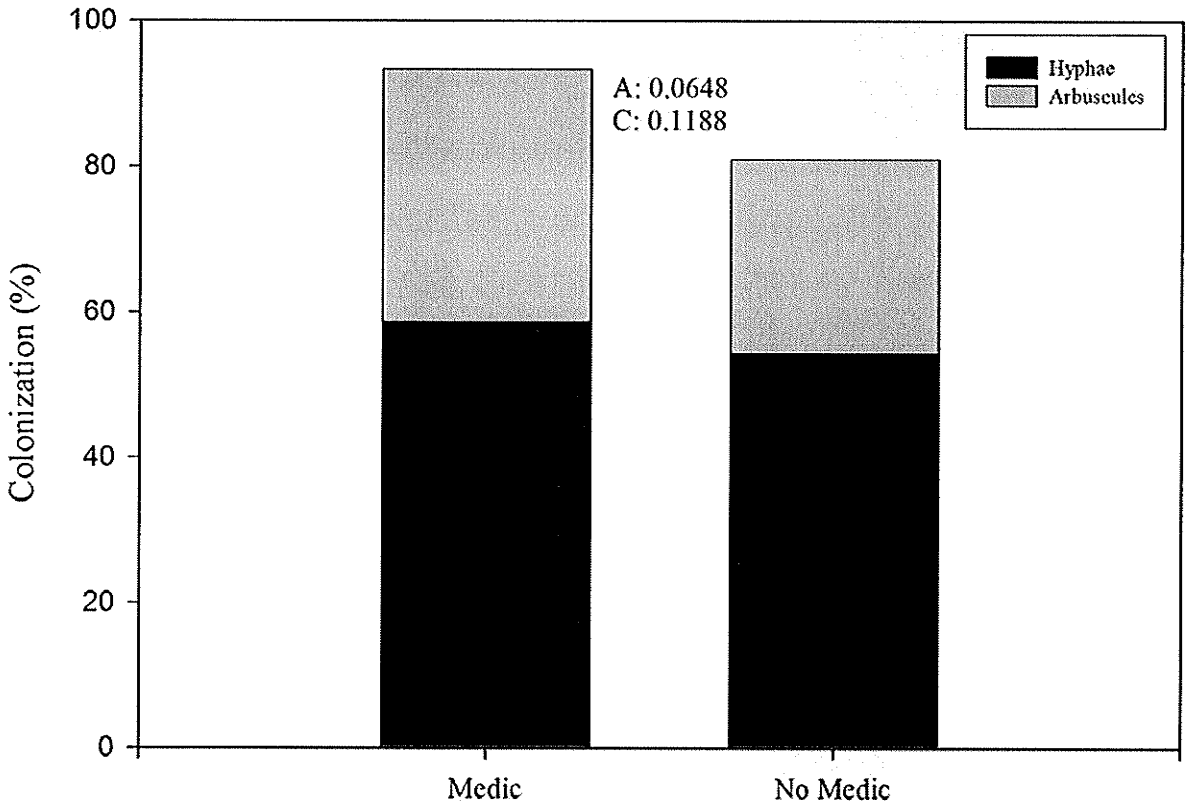


**Figure 4.1.** Early arbuscular mycorrhizal colonization of flax roots grown in intact soil cores from field plots with and without a black medic cover crop. The statistical significance (P value) of the medic cover crop influence on percent arbuscular colonization (A) and total colonization (C) is shown for each site year.

Disturbance of the soil cores had no influence on arbuscular colonization or total AMF colonization (Table 4.1). This disagrees with a field study by Boswell et al. (1998) who found that soil disturbance decreased the AMF colonization of corn following a

cover crop. They attributed the increase in AMF colonization after cover cropping to an increase in the extraradical hyphal network. When the extraradical hyphal network was disturbed, AMF colonization was reduced. However, in a pot study by McGonigle et al. (1990), colonization was not affected by soil disturbance which is similar to findings of the present study. McGonigle and Miller (2000) suggested that the inconsistent effect of soil disturbance on AMF colonization could be explained by the amount of AMF inoculum present in the soil. In soil with high AMF inoculum density, colonization may not be affected by soil disturbance, whereas, when inoculum is low colonization may be reduced by soil disturbance. It is possible that because of the small volume of the cores used in the present study, the medic root density would have been high. Thus the colonized root inoculum density would have also been high within the core. Early flax roots would have been in close proximity to an inoculum source regardless of disturbance of the extraradical hyphal network.

The historical nitrogen fertilization had no influence on mycorrhizal colonization of early flax roots (Table 4.1). This agrees with previous studies that have determined only very high levels of nitrogen fertilization have a negative effect on AMF colonization (Treseder 2004; Bradley 2006).

### 4.5 Summary and Conclusions

The results of this growth chamber experiment suggest that a black medic cover crop can effectively increase the early AMF colonization of the following flax crop. This study provides evidence that winter and spring fallow can significantly reduce the mycorrhizal inoculum potential of the soil and cover cropping with AMF host plants is a soil management practice that encourages rapidly forming AMF colonization. This

experiment also demonstrated that a previous black medic cover crop in combination with soil disturbance increases the early biomass accumulation of flax.

In the present, study soil disturbance did not reduce the increase in colonization of flax following a black medic cover crop. This suggests that the major source of AMF inoculum may not have been the pre-existing extraradical hyphal network but rather colonized black medic root fragments and AMF spores. However, although early flax colonization was not affected by soil disturbance, early flax phosphorus uptake could have been decreased. The extent of mycorrhizal colonization is not always correlated with plant phosphorus uptake (McGonigle et al. 1990). An increase in AMF-mediated phosphorus uptake may be more closely related to the extent of the intact AMF hyphal network and thus disturbance of the AMF network can reduce phosphorus uptake (Boswell et al. 1998). Thus, it may be more agronomically important to measure the influence of soil disturbance after cover cropping on phosphorus uptake and hyphal density rather than colonization only. In the future experiments, plant tissue phosphorus and hyphal density should also be analyzed to further explore this question.

# 5. GENERAL DISCUSSION

## 5.1 AMF Colonization in Field and Growth Chamber Study

In the field study the black medic cover had no effect on the early AMF colonization of flax roots. However, in the growth chamber study, the previous medic cover crop increased the early AMF colonization of the following flax crop. There are several possible explanations for the discrepancy in results between the field and growth chamber studies. For example, the black medic was grown for two weeks longer in the growth chamber study than in the field. This would have provided additional time for the black medic root system and the AMF extraradical hyphal network to develop compared to the field study. Thus the flax roots may have been provided with a higher density of AMF inoculum in the growth chamber study than what was experienced in the field. It is also possible that the newest flax root growth may not have been captured with the root sampling method used in the field study whereas the whole root system was recovered from the soil cores in the growth chamber experiment. A difference in AMF colonization may have only existed in the newest roots. In the future, larger and deeper samples should be taken to capture the whole root system and avoid this potential source of inaccuracy. A third plausible explanation is the presence of weeds. In the growth chamber study, the medic cover crop treatment was compared to a control treatment in which all the weeds were removed. Whereas, in the field study, weeds were present on the control plots in the early spring. The weeds on the control plots may have provided an early host for AMF thus increasing the early inoculation potential of the soil to a level similar to the medic cover crop treatment.

The growth chamber experiment provided evidence that a previous black medic cover crop in combination with soil disturbance increases early biomass accumulation of

flax. This increase in biomass was attributed to greater nitrogen availability in the disturbed medic soil. The medic presumably enriched the soil nitrogen status by nitrogen fixation and later the decomposition roots and above ground biomass. Disturbance likely increased the rate of nitrogen mineralization and nitrogen availability to the flax (Sarrantonio et al. 1988; Drinkwater et al. 2000). Further research on the nitrogen supplying power of soils cover cropped to black medic is currently being undertaken.

Flax was used as the bio-indicator crop in both the field and growth chamber study. Flax is known to be highly dependant on AMF for the uptake of phosphorus (Thingstrup et al. 1998). Perhaps the results of this experiment would have been different with another crop such as corn that has previously been found to respond to previous cover cropping (Bosewell 1998; Kabir and Koide 2000, 2002).

### 5.2 Future Research and the Value of Cover Crops in Cropping Systems

In future studies on the effect of cover cropping and other agronomic practices on AMF symbiosis, it may be more informative to measure AMF hyphal density and root colonization rather than root colonization alone. Previous studies on tillage and AMF have shown that at a high AMF inoculum density, the percent root length colonization may not be affected by soil disturbance even though the extraradical hyphal network maybe affected (McGonigle and Miller 2000). Other research has shown that the benefit of cover cropping to AMF mediated phosphorus uptake can be attributed to the extension of the AMF extraradical hyphal network (Kabir et al. 1999). Thus, measurement of the AMF in the crop roots is not enough to determine if an agronomic practice has an effect on AMF symbiosis. Measurement of the extraradical hyphal network is required to fully access the impact of an agronomic practice has on AMF symbiosis.

The present field study demonstrated that under zero-tillage management with only mycorrhizal crops in rotation, early AMF colonization is very high and the addition of a cover crop did not further elevate AMF colonization of flax. However, cover cropping may have an influence on the early AMF colonization of crops in management systems that are not so favorable to AMF. For example, canola is a non-mycorrhizal crop that is common in prairie crop rotations. A yield reduction occurs when mycorrhizal crops, such as flax, follow canola which is attributed to a reduction in early AMF symbiosis (Johnston et al. 2005). The potential of mycorrhizal cover crops intercropped with or following non-mycorrhizal crops to alleviate the negative effects on AMF colonization of the following crops provides an interesting subject for future research.

Cover crops may not increase early AMF colonization of the following crops under the management system used in the present study; however, there are many other benefits of cover crops that were not thoroughly investigated in this study. The benefits of cover cropping include the addition of nitrogen and organic matter to the soil, prevention of soil erosion and nutrient loss (Drinkwater et al. 1998; Sarrantonio and Gallandt 2003). There is also evidence that cover cropping promotes the activity of microorganisms, other then AMF, in the soil (Mendes et al. 1999; Schutter and Dick 2002). Cropping systems in southern Manitoba generally have enough residual moisture and growing degree days to capture some of these benefits by growing a late season cover crop (Thiessen Martens et al. 2001).

The major conclusion of this study is that cover cropping has an influence on the uptake of macro and micronutrients by the following crop that is not related to increase AMF colonization. The present study provided evidence that a long term black medic

cover crop increases early phosphorus uptake and nitrogen and zinc concentration and decreases iron and copper uptake. Further studies are needed investigate the potential phosphorus solubilizing abilities of black medic and the role of black medic in enriching soil organic matter and micronutrients (Zn). Micronutrient competition (Fe and Cu) between the main crop and a black medic intercrop may also be of importance in future research.

## 6. LITERATURE CITED

Allen B.L., Jolley V.D., Robbins C.W., Freeborn L.L., 2001. Fallow versus wheat cropping of unammended soils related to mycorrhizal colonization, yield, and plant nutrition of dry bean and sweet corn. *Journal of Plant Nutrition* 24 (6): 921-943.

Bagayoko M., Buerkert A., Lung G., Bationo A., Romheld V., 2000. Cereal/legume rotation effects on cereal growth in Sudano-Sahelian West Africa: Soil mineral nitrogen, mycorrhizae and nematodes. *Plant and Soil* 218: 103-116.

Baltruschat H. and Dehne H.W., 1988. The occurrence of vesicular-arbuscular mycorrhiza in agro-ecosystems. I. Influence of nitrogen fertilization and green manure in continuous monoculture and in crop rotation on the inoculum potential of winter wheat. *Plant and Soil* 107: 279-294.

Baumgartner K., Smith R.F., Bettiga L., 2005. Weed control and cover crop management affect mycorrhizal colonization of grapevine roots and arbuscular mycorrhizal fungal spore populations in a California vineyard. *Mycorrhiza* 15: 111-119.

Black, R. and Tinker P.B., 1979. The development of endomycorrhizal root systems. II. Effect of agronomic factors and soil conditions of the development of vesicular-arbuscular mycorrhizal infection in barley and on the endophyte spore density. *New Phytologist* 83: 401-413.

Bolan, N.S. 1991. A critical review of the role of mycorrhizae fungi in the uptake of phosphorus by plants. *Plant and Soil* 134: 189-207

Boswell E. P., Koide R.T., Shumway D.L., Addy H.D. 1998. Winter Wheat cover cropping, VA mycorrhizal fungi and maize growth and yield. *Agriculture, Ecosystems and Environment* 67: 55-65.

Bourgeois L., Entz M.H., 1996. Influence of previous crop type on yield of spring wheat: analysis of commercial field data. *Canadian Journal of Plant Science* 76: 457-459.

Bradley K., Drijber R.A., Knops J., 2006. *Soil Biology and Biochemistry* 38: 1583-1595.

Bucking H., Shacker-Hill Y., 2005. Phosphate uptake, transport and transfer by arbuscular mycorrhizal fungus is increased by carbohydrate availability. *New Phytologist* 165: 889-912.

Dee-Haan R.L., Sheaffer C.C., Barnes D.K., 1997. Effect of annual medic smother plants on weed control and yield in corn. *Agronomy Journal* 89(5): 813-821.

Douds Jr. D.D., Galvez L, Franke-Snyder M., Reider C., Drinkwater L.E., 1997. Effect of compost addition and crop rotation point upon VAM fungi. *Agriculture, Ecosystems and Environment* 65: 257-266.

Douds, D.D. and Nagahashi, G., 2000. Signalling and Recognition Events Prior to Colonisation of Roots by Arbuscular Mycorrhizal Fungi. In *Current Advances in Mycorrhizae Research.* Ed. Podila G., Douds D.D. Minnesota: APS Press. Pp 11-18.

Drinkwater L.E., Wagoner P. and Sarrantonio M., 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396: 263-265.

Drinkwater L.E., Janke R.R., Rossoni-Longnecker L., 2000. Effects of tillage intensity on nitrogen dynamics and productivity in legume based grain systems. *Plant and Soil* 227: 99-113.

Eriksson, A., 2001. Arbuscular mycorrhizae in relation to management history, soil nutrients and plant diversity. *Plant Ecology* 155: 129-137.

Feldman F., Boyle C., 1998. Weed-mediated stability of arbuscular mycorrhizal fungi effectiveness in maize monocultures. *Journal of Applied Botany* 73: 1-5.

Flax Council of Canada (FCC), 2002. *Growing Flax: Production, Management and Diagnostic Guide*. Flax Council of Canada, Winnipeg.

France R. C., 1985. Cover crops to increase inoculum in the field. *Proceedings of the 6th North American Conference on Mycorrhizae*. Pp. 92-94.

Galvez L., Douds D.D. Jr., Wagoner P., Longnecker L.R., Drinkwater L.E., Janke R.R., 1995. An overwintering cover crop increases inoculum of VAM fungi in agricultural soil. *American Journal of Alternative Agricutlure* 10 (4): 152-156.

Gavito M.E., Miller M.H., 1998. Changes in mycorrhiza development in maize induced by crop management practices. *Plant and Soil* 198: 185-192.

Gianinazzi-Pearson V. 1996. Plant cell responses to arbuscular mycorrhizae fungi: getting to the roots of symbiosis. *The Plant Cell* 8: 1871-1883

Grime J.P., Mackey J.M.L., Hillier S.H., Read D.J., 1987. Floristic diversity in a model system using experimental microcosms. *Nature* 328: 420-422.

Grant C.A., Flaten D.N., Tomasiewicz D.J., Sheppard S.C., 2001. The importance of early season phorphorus nutrition. *Canadian Journal of Plant Science* 81: 211-224.

Grant C., Bittman S., Montreal M., Plenchette C. and Morel C. 2005. Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. *Can. J. Plant Sci.* 85: 3–14.

Guerinot M.L., 1991. Iron uptake and metabolism in rhizobia/legume symbioses. *Plant and Soil* 130: 199-209.

Hamel C. 2004. Impact of arbuscular mycorrhiza fungi on N and P cycling in the root zone. *Canadian Journal of Soil Science*. 84:383-395.

Harrier L.A. and Watson C.A., 2003. The Role of Arbuscular Mycorrhizal fungi in Sustainable Cropping Systems. *Advances in Agronomy* 20: 185-224.

Harley J.L., Smith S.E., 1983. *Mycorrhizal Symbiosis*. Academic Press: London.

Hauggaard-Nielsen H. Jensen E.S., 2005. Facilitative root interactions in intercrops. *Plant and Soil* 274: 237-250.

Hayman D.S., Johnson A.M., Ruddlesdin I., 1975. The influence of phosphate and crop species of endogone spores and vesicular-arbuscular mycorrhiza under field conditions. *Plant and Soil* 43: 498-495.

Hendrix J.W., Guo B.Z., An Z-Q., 1995. Divergence of mycorrhizal fungal communities in crop production systems. *Plant and Soil* 170: 131-140.

Havlin J.L., Beaton J.D., Tisdale S.L., Nelson W.L., 2005. *Soil Fertility and Fertilzers*. New Jersey: Prentice Hall.

Janzen H.H., 2001. Soil Science on the Canadian Prairies – Peering into the future from a century ago. Canadian Journal of Soil Science 81: 489-503.

Johnson N.C., Pfeger F.L., Crookston R.K., Simmons S.R., Copeland P.J., 1991. Vesicular-Arbuscular Mycorrhizas Respond to Corn and Soybean Cropping History. *New Phytologist* 117 No.4: 657-663.

Johnson N.C., Copeland P.J., Crookston R.K., Pfedger F.L., 1992. Mycorrhizae – Possible explanation for yield decline with continuous corn and soybean. *Agronomy Journal* 84 (3): 387-390.

Johnston A. M., Kutcher H. R., and Bailey K. L., 2005. Impact of crop sequence decisions in the Saskatchewan Parkland. Canadian Journal of Plant Science 85: 95-102.

Kabir Z., O'Halloran I.P, Hamel C., 1999. Combined effects of soil disturbance and fallowing on plant and fungal components of mycorrhizal corn (*Zea mays* L.). *Soil Biology and Biochemistry* 31: 307-314.

Kabir Z. and Koide R.T., 2000. The effect of dandelion or a cover crop on mycorrhiza inoculum potential, soil aggregation and yield of maize. *Agriculture, Ecosystems and Environment* 78: 167-174.

Kabir Z. and Koide R.T., 2002. Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient uptake and yield of sweet corn in Pennsylvania, USA. *Plant and Soil* 238: 205-215.

Karagiannidis, N., Hadjisavva-Zinoviadi S., 1998. The mycorrhizal fungus *Glomus mosseae* enhances the growth, yield and chemical composition of duram wheat in 10 different soils. *Nutrient Cycling and Agroecosystems* 52: 1-7.

Karasawa T. Y., Takebe K. M., 2002. Differences in growth responses of maize to preceding cropping caused by fluctuation in the population of indigenous arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* 34: 851-857.

Karlen D.L., Varvel G.E., Bullock D.G., Cruse R.M., 1994. Crop Rotations for the 21st Century. *Advances in Agronomy* 53:1-45.

Kormanik P.P., 1980. Increasing endomycorrhizal fungus inoculum in forest nursery soil with cover crops. *Southern Journal of Applied Forestry* 4: 151-153.

Kothari S.K., Marchner H., Romheld V., 1991. Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. *Plant and Soil* 131: 177-185.

Lambert D.H., Baker D.E., Cole H. Jr., 1979. The role of mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. *Soil Science Society of America Journal* 43: 976-980.

Lekberg Y., Koide R.T., 2005. Is plant performance limited by an abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988-2003. *New Phytologist* 168: 189-2004.

Liu A., Hamel C., Hamilton R.I., Ma B.L., Smith D.L., 2000. Acquisition of Cu, Zn, Mn, and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza* 9: 331-336.

Mendes, I.C., Bandick A.K., Dick R.P. and Bottomley P.J., 1999. Microbial Biomass and Activities in Soil Aggregates Affected by Winter Cover Crops. *Soil Science Society of America Journal* 63:873-881

McGonigle T.P. 1988. A numerical analysis of published field trials with vesicular-arbuscular mycorrhizal fungi. *Functional Ecology* 2: 473-478.

McGonigle T.P., Miller M.H., Evans D.G., Fairchild G.L., Swan J.A., 1990a. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist*: 115: 495-501.

McGonigle T.P., Evans D.G., Miller M.H., 1990b. Effect of soil disturbance on mycorrhizal colonization and phosphorus absorption by maize in growth chamber and field experiments. *New Phytologist* 116: 629-636.

McGonigle T.P., and Miller M.H., 1993. Mycorrhizal development and phosphorus absorption in maize under conventional and reduced tillage. *Soil Science Society of America Journal* 57: 1002-1006.

McGonigle T.P, Miller M.H., 1999. Winter Survival of extraradical hyphae and spores of arbuscular mycorrhizal fungi in the field. *Applied Soil Ecology* 12: 41-50.

McGonigle T.P., Miller M.H., 2000. The inconsistent effect of soil disturbance on colonization of roots by arbuscular mycorrhizal fungi: a test of the inoculum density hypothesis. *Applied Soil Ecology* 14: 147-155.

Miller M.H., McGonigle T.P., Addy H.D. 1995. Functional ecology if vesicular arbuscular mycorrhizas as influenced by phosphate fertilization and tillage in an agricultural ecosystem. *Critical Reviews in Biotechnology* 15: 241-255.

Mitchell J., Mess H.C., Clayton J.S., and Edmunds F.H., 1944. Soil survey of southern Saskatchewan from Township 1-48 inclusive. Soil Survey Rep. No. 12. University of Saskatchewan, Saskatoon, SK.

Moomaw R.S., 1995. Selected crops established in early soybean growth stage. *Journal of Soil and Water Conservation* 50 (1): 82-86.

Mozafar A., Anken T., Ruh R., Frossard E., 2000. Tillage intensity, Mycorrhizal and non mycorrhizal fungi and nutrient concentrations in maize, wheat and canola. *Agronomy Journal* 92: 1117-1124.

Nagahashi G., Douds D. D., Abney G.D., 1996. Phosphorus amendment inhibits hyphal branching of VAM fungus Gigaspora margarita directly and indirectly through its effect on root exudation. *Mycorrhizae* 6: 403-408.

Ocampo J.A., Martin J., Hayman D.S., 1980. Influence of Plant Interactions on Vesicular-Arbuscular Mycorrhizal Infections. I. Host and Non-Host Plants Grown Together. *New Phytologist* 84: 27-35.

Oehl F., Sieverding E., Ineichen K., Mäder P., Boller T., Wiemken A., 2003. Impact of land use intensity of the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and Environmental Microbiology* 69 (5): 2816-2824.

Pavkovsky R.S., Bethlenlfalvay G.J., Paul E.A., 1986. Comparisons between P-fertilized and Mycorrhizal Plants. *Crop Science* 26: 151-156.

Pegoraro RF, Silva IR, Novais RF, Mendonca ES, Alvarez VHV, Nunes FN, Fonseca FM, Smyth TJ., 2005 Diffusive flux of cationic micronutrients in two oxisols as affected by low-molecular-weight organic acids and cover-crop residue. *Journal of Plant Nutrition and Soil Science* 168 (3): 334-340.

Pfeffer P., Douds D., Becard G., Shachar-Hill Y. 1999. Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. *Plant Physiology* 120: 587-598.

Randhawa P.S., Condron L.M., Di H.J., Sinaj S., McLenaghen R., 2005. Effect of green manure addition on soil organic phosphorus mineralization. *Nutrient Cycling in Agroecosystems* 73: 181-189.

Read D.J., Koucheki H.K., Hodgson J., 1976. Vesicular-Arbuscular Mycorrhiza in Natural Vegetation Systems. *New Phytologist* 77: 641-653.

Rutto K.L, Mizutani F., Moon D., Cho Y., Kadoya K., 2002. Seasonal Fluctuations in Mycorrhizal Spore Populations and Infection Rates of Vineyard Soils Planted with Five Legume Cover Crops. *Journal of the Japanese Society for Horticultural Science*. 72 (4): 262-267.

Sarrantonio M., Scott T.W., 1988. Tillage effects on availability of nitrogen to corn following a winter green manure. *Soil Science Society of America Journal* 52(6): 1661-1688.

Sarrantonio M. and Gallandt E., 2003. The Role of Cover Crops in North American Cropping Systems. In *Cropping Systems: Trends and Advances*, ed., A. Shrestha, New York: Food Products Press, pp. 53-74.

Sbrana C., Giovannetti M., 2005. Chemotropism in the arbuscular mycorrhizal fungus *Glomus mosseae*. *Mycorrhizae* 15: 539-545.

Schenck N.C. and Kinloch R.A., 1980. Incidence of mycorrhizal fungi on 6 field crops in monoculture on a newly cleared field site. *Mycologia* (72) 3: 445-456.

Schutter M.E., and Dick R.P., 2002. Microbial community profiles and activities among aggregates of winter fallow and cover-cropped soil. *Soil Science Society of America Journal* 66: 142-153.

Simon L., Bousquet J., Levesque C., Lalonde M., 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature* 363: 67-69.

Sims J.R., Koala S., Ditterline R.L., Wiesner L.E., 1985. Registration of 'George' Black Medic. *Crop Science* 24(4): 709-710.

Smith S.E., Read D.J. *Mycorrhizal Symbiosis*. 2002. Academic Press: London.

Smith S., Smith A., Jakobsen I., 2003. Mycorrhizal fungi can dominate phosphorus supply to plant irrespective of growth response. *Plant Physiology* 133: 16-20.

Sorensen J.N., Larsen J., and Jakobsen I., 2005. Mycorrhizae formation and nutrient concentration in leeks (*Allium porrum*) in relation to previous crop and cover crop management on high P soils. *Plant and Soil* 273: 101-114.

Thiessen Martens J.R., Hoeppner J.W., Entz M.H., 2001. Legume Cover Crops with Winter Cereals in Southern Manitoba. *Agronomy Journal* 93: 1086-1096.

Thingstrup I., Rubaek G., Sibbensen E. and Jakobsen I., 1999. Flax (*Linum usitatissimum* L.) depends on arbuscular mycorrhizal fungi for growth and P uptake at intermediate but not high soil P levels in the field. *Plant and Soil* 203: 37-46.

Thompson J.P., 1994. Inoculation with vesicular-arbuscular mycorrhizal fungi from cropped soil overcomes long-fallow disorder of linseed (*Linum Usitatissium* L.) by improving P and Zn uptake. *Soil Biology and Biochemisty* 26 (9): 1133-1143.

Timmer L., Leyden R., 1980 The relationship of mycorrhizal infection to phosphorus-induced copper deficiency in sour orange seedlings. *New Phytologist* 85: 15-23.

Treseder K.K., 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus and atmospheric $CO_2$ in field studies. *New Phytologist* 164: 347-355.

Tuomi J., Kytoviita M., Hardling R., 2001.Cost efficiency of nutrient acquisition of mycorrhizal symbiosis for the host plant. *Oikos* 92:62-70.

Van der Heijden M.G.A., Klironomos J.N., Ursic M., Moutoglis P., Streitwolf-Engel R., Boller T., Wiemken A., Sanders I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69-72.

Vivekanandan M., Fixen P.E., 1991. Cropping Systems Effects on Mycorrhizal Colonization, Early Growth, and Phosphorus Uptake. *Soil Science Society of America Journal* 55:136-140.

Wright S.F. Management of Arbuscular Mycorrhizal Fungi. 2005. In *Roots and Soil Management: Interactions between roots and the soil.* Ed. Zobel, R.W., Wright, S.F. USA: American Society of Agronomy. Pp 183-197.

Zak J.C., McMichael B., Dhillion S., Friese C., 2005. Arbuscular-mycorrhizal colonization dynamics of cotton (*Gossypium hirsutum* L.) growing under several production systems on the Southern High Plains, Texas. *Agriculture, Ecosystems and Environment* 68: 245-254.

# APPENDICES

**Appendix A.** Monthy precipitation (mm) (2005-2006) at Winnipeg and Indian Head field sites.

| Site | Year | Jan | Feb | Mar | Apr | May | June | July | Aug | Sept | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Winnipeg | 2005 | - | 6.0 | 1.9 | 30.4 | 72.9 | 176.3 | 198.5 | 22.8 | 47.6 | 37.5 | 19.3 | 9.4 |
| | 2006 | 9.3 | 0.3 | 32.6 | 7.7 | 22.0 | 22.0 | 15.1 | 76.8 | 42.5 | 13.1 | 5.5 | 5.2 |
| Indian Head | 2005 | 37.0 | 6.8 | 29.0 | 6.8 | 57.6 | 99.2 | 59.2 | 98.0 | 4.0 | 6.6 | 34.9 | 24.7 |
| | 2006 | 13.1 | 6.3 | 10.6 | 73.2 | 39.0 | 80.4 | 4.4 | 11.6 | 54.8 | | | |

**Appendix B.** Flax seed yield from Winnipeg and Indian Head field sites in 2005 and 2006.

| Site | Year | Treatment | Yield (Kg/ha) |
|---|---|---|---|
| Winnipeg | 2005 | Medic | 45.56 |
| | | No Medic | 30.90 |
| | 2006 | Medic | 83.70 |
| | | No Medic | 75.34 |
| Indian Head | 2005 | Medic | 1386.88 |
| | | No Medic | 1445.31 |
| | 2006 | Medic | 1199.44 |
| | | No Medic | 1088.58 |
| All Site Years | | Medic | 540.89 |
| | | No medic | 607.34 |
| Significance (P) | Site | Year | |
| | Winnipeg | 2005 | 0.0206 |
| | | 2006 | 0.7574 |
| | Indian Head | 2005 | 0.6167 |
| | | 2006 | 0.5997 |
| | All Site Years | | 0.3112 |