SELECTING WHEAT CULTIVARS FOR ORGANIC PRODUCTION

BY

ANNE PATRICIA KIRK

A Thesis
Submitted to the Faculty of Graduate Studies of
The University of Manitoba
In Partial Fulfilment of the Requirements
Of the Degree of

MASTER OF SCIENCE

Department of Plant Science
University of Manitoba
Winnipeg, MB

Copyright © 2009 by Anne Patricia Kirk

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES
*****
COPYRIGHT PERMISSION

Selecting Wheat Cultivars for Organic Production

By

Anne Patricia Kirk

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

Of

Master of Science

Anne Patricia Kirk©2009

Permission has been granted to the University of Manitoba Libraries to lend a copy of this thesis/practicum, to Library and Archives Canada (LAC) to lend a copy of this thesis/practicum, and to LAC's agent (UMI/ProQuest) to microfilm, sell copies and to publish an abstract of this thesis/practicum.

This reproduction or copy of this thesis has been made available by authority of the copyright owner solely for the purpose of private study and research, and may only be reproduced and copied as permitted by copyright laws or with express written authorization from the copyright owner.

ABSTRACT

Organic crop production systems produce a soil and weed environment different from that found in conventional crop production systems. Arbuscular mycorrhizal fungi (AMF) are important in organic cropping systems for assisting in the uptake of phosphorus from the soil. Due to the differing environments found between organic and conventional cropping systems it is suggested that crop performance could be improved in organic systems if cultivars were selected for organic growing conditions. Three studies were performed in 2007 and 2008 to examine wheat cultivar selection for organic crop production. The first two studies were carried out at two organically managed research sites in Southern Manitoba. The first study compared the hyphal, arbuscular and total AMF colonization of 26 hard red spring wheat lines. Percent colonization by AMF differed among wheat cultivars and between four of the parental combinations at one location. The second study compared AMF colonization and yield between modern and heritage spring wheat cultivars. At two of four site years modern cultivars had higher levels of colonization than heritage varieties. At one site year modern varieties yielded significantly higher than heritage varieties. No differences were found at the other site years. The third study compared yield, protein concentration and kernel weight of seven populations of wheat selected in both organic and conventional environments. The organic and conventionally selected populations were compared at four organic and four conventionally managed locations in Manitoba and Saskatchewan. Selection environment was found to affect yield, protein and kernel weight in both management environments. Direct selection in organic environments for organic agriculture was found to be superior to indirect selection in conventional environments.

ACKNOWLEDGEMENTS

I would like to thank my supervisors Dr. Stephen Fox and Dr. Martin Entz for their guidance, advice, support and patience; my committee members, Dr. Mario Tenuta and Dr. Anita Brûlé-Babel for their valuable assistance; and all those who provided much appreciated assistance with field work, in particular Keith Bamford. For help with AMF techniques I would like to thank Dr. Carla Zelmer. For first opening my eyes to the world of organic agriculture and the encouragement to think differently about the world we live in, as well as giving me the opportunity to attend many conferences and meetings I will always be thankful to Dr. Martin Entz. I would also like to thank the many summer students for their help, in particular Laura Wiebe who spent countless hours helping count mycorrhiza.

I am very thankful to have had the support of my family in the completion of my thesis and throughout my education. I am fortunate to have parents who have instilled in me a love of agriculture. I would also like to thank all of the plant and soil science graduate students making this process very enjoyable, in particular my good friend, lab mate and roommate Iris for all of the laughs, great times and late nights spend together in front of our computers.

This work was supported financially by the Canadian Wheat Board.

TABLE OF CONTENTS

ABSTRACT....ii

ACKNOWLEDGEMENTS....iii

TABLE OF CONTENTS....iv

LIST OF TABLES....vii

LIST OF FIGURES....xii

1. LITERATURE REVIEW.... 1

1.1 General Introduction....1
1.1.1 Organic Farming....1
1.1.2 Arbuscular Mycorrhizal Symbiosis....2
1.1.3 Wheat....3
1.2 Description of Arbuscular Mycorrhizal Fungi....5
1.2.1 Morphology of AMF....5
1.2.2 Colonization of Host Plants by AMF....8
1.2.3 Transfer and Uptake of Nutrients....10
1.3 Benefits of Mycorrhizal Symbiosis....13
1.3.1 Soil Aggregation....13
1.3.2 Environmental Stresses....14
1.3.3 Defense Against Disease-Causing Organisms....16
1.3.4 Environmental Benefits....17
1.4 Influence of Plant Ecology on AMF....18
1.4.1 Farming System....18
1.4.2 Crop Rotation....19
1.4.3 Cover Crops....19
1.5 Comparison of Organic and Conventional Farming Systems....20
1.5.1 Soil Fertility....20
1.5.2 Weed Management....22
1.5.3 Soil Microorganisms....22
1.5.4 Yield....23
1.6 Breeding for Organic Agriculture....24
1.6.1 Why Breed for Organic Agriculture?....24
1.6.2 Direct Selection....26
1.6.3 Desirable Plant Characteristics for Organic Production....27

2. VARIATION IN MYCORRHIZAL COLONIZATION OF PARENTAL SPRING WHEAT LINES IN ORGANIC GROWING CONDITIONS.....32

2.1 Abstract.....32
2.2 Introduction.....33
2.3 Materials and Methods.....35
2.3.1 Site Description and Treatments.....35
2.3.2 Root Sampling.....38
2.3.3 Mycorrhizal Colonization.....38
2.3.4 Statistical Analysis.....40
2.4 Results and Discussion.....40
2.5 Conclusions.....47

3. MYCORRHIZAL COLONIZATION IN HERITAGE AND MODERN WHEAT CULTIVARS.....48

3.1 Abstract.....48
3.2 Introduction.....49
3.3 Materials and Methods.....51
3.3.1 Site Description and Treatments.....51
3.3.2 Seeding.....54
3.3.3 Root Sampling.....55
3.3.4 Mycorrhizal Colonization.....55
3.3.5 Biomass Sampling.....55
3.3.6 Yield.....56
3.3.7 Statistical Analysis.....56
3.4 Results and Discussion.....57
3.4.1 Hyphal and Arbuscular Colonization.....57
3.4.2 Tissue Phosphorus.....60
3.4.3 Yield.....62
3.5 Conclusions.....70

4. COMPARISON OF ORGANIC AND CONVENTIONAL SELECTION ENVIRONMENTS.....71

4.1 Abstract.....71
4.2 Introduction.....71
4.3 Materials and Methods.....75
4.3.1 Site Description.....75
4.3.2 Experimental Design and Treatments.....78
4.3.3 Field Trial Management.....82
4.3.4 Data Collection.....83
4.3.5 Statistical Analysis.....85
4.4 Results and Discussion.....86
4.4.1 Yield.....86
4.4.2 Protein.....90

4.4.3 Kernel Weight.....94
4.4.4 Height.....98
4.4.5 Direct versus Indirect Selection.....100
4.5 Conclusions.....123

5. GENERAL DISCUSSION AND CONCLUSIONS.....124

5.1 Findings.....124
5.2 Strength of the AMF Association with Crop Plants.....125
5.3 AMF in Plant Breeding.....126
5.4 Organic Plant Breeding.....128

6. LITERATURE CITED.....130

7. APPENDICES.....140

Appendix I – Tables.....140
Appendix II – Procedure for Working with AMF.....148

LIST OF TABLES

Table 2.1 Soil association, texture, nutrient status, and previous crop history at each experimental site.....36

Table 2.2 Parental lines included in this study and date of release (based on actual date of release or 10 years after the cross was made).....37

Table 2.3 Checks included in this study and date of release.....37

Table 2.4 Environmental conditions during crop growth at each experimental site (MAFRI 2008).....38

Table 2.5 Mean percent arbuscular colonization and total colonization for the 26 lines at Glenlea and Carman tested in 2007 and 2008.....44

Table 3.1 Soil association, texture, nutrient status, and previous crop history at each experimental site.....53

Table 3.2 Environmental conditions during the growing season (May 1 – August 31) at each experimental site (MAFRI 2008), and long-term averages (Environment Canada 2004).....54

Table 3.3 Wheat cultivars included in this study, including date of cultivar release for commercial production.....54

Table 3.4 Schedule of field operations.....55

Table 3.5 Year of release, colonization by arbuscules, yield, and tissue P concentration for the 10 wheat cultivars at Glenlea and Carman in 2007.....66

Table 3.6 Year of release, colonization by arbuscules, yield, and tissue P concentration for the 10 wheat cultivars at Glenlea and Carman in 2008.....67

Table 3.7 Estimates and contrasts of the difference between heritage (1886-1969) and modern (1991-2006) varieties of wheat for average mycorrhizal colonization, yield, tissue P and biomass at the four sites.....68

Table 4.1 Management, location, soil association and soil texture at each experimental site.....75

Table 4.2 Soil nutrient status and previous crop history at each experimental site.....76

Table 4.3 Environmental conditions during the growing season (May 1 – August 31) at each experimental site (MAFRI 2008a and Environment Canada 2008), and long-term averages (Environment Canada 2004).....78

Table 4.4 Population size and selection intensity of BC crosses in organic and conventional selection environments.....81

Table 4.5 Wheat lines included in the study, including parents and the number of lines in each bulk population.....81

Table 4.6 Fertilizer and herbicide type, amount applied and the crop stage herbicides were applied at for the conventional experiments.....82

Table 4.7 Timing of seeding and harvest operations, plot size and row spacing for organic and conventional experiments.....83

Table 4.8 Yield LS means, ANOVA tests and contrasts for both organic and conventionally managed locations. ANOVA tests do not include check cultivars while contrasts compare check cultivars to all conventionally selected lines and check cultivars to all organically selected lines.....106

Table 4.9 ANOVA tests and contrasts for yield at all organic locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.....107

Table 4.10 ANOVA tests and contrasts for yield at all conventional locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under conventional management.....107

Table 4.11 Yield, protein, 1000 kernel weight (TKW) and height LS means for organic and conventionally managed sites and organic and conventional selection and check cultivars.....108

Table 4.12 Protein LS means, ANOVA tests and contrasts for both organic and conventionally managed locations. ANOVA tests do not include check cultivars while contrasts compare check cultivars to all conventionally selected lines and check cultivars to all organically selected lines.....110

Table 4.13 ANOVA tests and contrasts for protein at all organic locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.....111

Table 4.14 ANOVA tests and contrasts for protein at all conventional locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under conventional management.....111

Table 4.15 1000 kernel weight LS means, ANOVA tests and contrasts for both organic and conventionally managed locations. ANOVA tests do not include check cultivars while contrasts compare check cultivars to all conventionally selected lines and check cultivars to all organically selected line.....113

Table 4.16 ANOVA tests and contrasts for 1000 kernel weight at all organic locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.....114

Table 4.17 ANOVA tests and contrasts for 1000 kernel weight at all conventional locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under conventional management.....114

Table 4.18 Height LS means, ANOVA tests and contrasts for both organic and conventionally managed locations. ANOVA tests do not include check cultivars while contrasts compare check cultivars to all conventionally selected lines and check cultivars to all organically selected line.....116

Table 4.19 ANOVA tests and contrasts for height at all organic locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.....117

Table 4.20 ANOVA tests and contrasts for height at all conventional locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under conventional management.....117

Table 4.21 Yield, yield as a percent of check mean and ranking of the 20 treatments compared across organic and conventional management environments. Check mean includes all modern Canadian Western Red Spring wheat checks, and therefore does not include the check cultivar Red Fife. Italicizes font and dashed lines are populations selected under organic management. Underlined font and lines are the populations selected under conventional management. Bolded font and lines are the check cultivars.....119

Table 4.22 Protein concentrations, protein concentration as a percent of check mean and ranking of the 20 treatments compared across organic and conventional management environments. Check mean includes all modern Canadian Western Red Spring wheat checks, and therefore does not include the check cultivar Red Fife. Italicized font and dashed lines are populations selected under organic management. Underlined font and lines are the populations selected under conventional management. Bolded font and lines are the check cultivars.....120

Table 4.23 1000 kernel weight (TKW), TKW as a percent of check mean and ranking of the 20 treatments compared across organic and conventional management environments. Check mean includes all modern Canadian Western Red Spring wheat checks, and therefore does not include the check cultivar Red Fife. Italicized font and dashed lines are populations selected under organic management. Underlined font and lines are the populations selected under conventional management. Bolded font and lines are the check cultivars.....121

Table 4.24 Height, height as a percent of check mean and ranking of the 20 treatments compared across organic and conventional management environments. Check mean includes all modern Canadian Western Red Spring wheat checks, and therefore does not include the check cultivar Red Fife. Italicized font and dashed lines are populations selected under organic management. Underlined font and lines are the populations selected under conventional management. Bolded font and lines are the check cultivars.....122

Table 6.1 Contrasts of the percent arbuscular colonization at Glenlea of lines used as parents in previous crosses.....140

Table 6.2 Contrasts of the percent total colonization at Glenlea of lines used as Parents in previous crosses.....140

Table 6.3 Hyphal (HC), arbuscular (AC) and total (TC) percent colonization at Glenlea in 2007.....141

Table 6.4 Hyphal (HC), arbuscular (AC) and total (TC) percent colonization at Carman in 2007.....141

Table 6.5 Hyphal (HC), arbuscular (AC) and total (TC) percent colonization at Glenlea in 2008.....142

Table 6.6 Hyphal (HC), arbuscular (AC) and total (TC) percent colonization at Carman in 2008.....142

Table 6.7 Correlations between variety release date, arbuscular colonization, total colonization, yield, percent tissue P, and biomass at Glenlea in 2007. The top numbers are the Pearson Correlation Coefficients. The bottom numbers are the significance of the correlation.....143

Table 6.8 Correlations between variety release date, arbuscular colonization, total colonization, yield, percent tissue P, and biomass at Carman in 2007. The top numbers are the Pearson Correlation Coefficients. The bottom numbers are the significance of the correlation.....144
Table 6.9 Correlations between variety release date, arbuscular colonization, total colonization, yield, percent tissue P, and biomass at Glenlea in 2008. The top numbers are the Pearson Correlation Coefficients. The bottom numbers are the significance of the correlation.....145

Table 6.10 Correlations between variety release date, arbuscular colonization, total colonization, yield, percent tissue P, and biomass at Carman in 2008. The top numbers are the Pearson Correlation Coefficients. The bottom numbers are the significance of the correlation.....146

Table 6.11 Correlation between yield, protein, 1000 kernel weight (TKW) and height at all organic locations. The top number is the correlation between the two variables and the bottom number is the significance of the correlation.....147

Table 6.12 Correlation between yield, protein, 1000 kernel weight (TKW) and height at all conventional locations. The top number is the correlation between the two variables and the bottom number is the significance of the correlation.....147

LIST OF FIGURES

Figure 2.1 Arbuscules (black arrows) and hyphae (white arrows) of arbuscular mycorrhizal fungi in wheat roots under 250x magnification.....45

Figure 2.2 Percent arbuscular colonization of the parental lines at Glenlea.....46

Figure 3.1 Relationship between year of release and arbuscular colonization at Carman in 2007.....69

Figure 3.2 Relationship between arbuscular colonization and yield at Carman in 2008.....69

Figure 3.3 Relationship between release date and yield at Carman in 2007.....69

Figure 4.1 Selection procedures to identify superior lines for organic and conventional selection environments.....80

Figure 4.2 Average yield (kg/ha) of the seven organically (white) and conventionally (black) selected populations under organic management.....109

Figure 4.3 Average yield (kg/ha) of the seven organically (white) and conventionally (black) selected populations under conventional management.....109

Figure 4.4 Average protein concentration (%) of the seven organically (white) and conventionally (black) selected populations under organic management.....112

Figure 4.5 Average protein concentration (%) of the seven organically (white) and conventionally (black) selected populations under conventional management.....112

Figure 4.6 Average 1000 kernel weight (g) of the seven organically (white) and conventionally (black) selected populations under organic management.....115

Figure 4.7 Average 1000 kernel weight (g) of the seven organically (white) and conventionally (black) selected populations under conventional management.....115

Figure 4.8 Average height (cm) of the seven organically (white) and conventionally (black) selected populations under organic management.....118

Figure 4.9 Average height (cm) of the seven organically (white) and conventionally (black) selected populations under conventional management.....118

1. LITERATURE REVIEW

1.1 General Introduction

1.1.1 Organic Farming

Organic agriculture is an agricultural production system that prohibits the use of synthetic fertilizers, synthetic pesticides and genetically modified organisms, among other things (Mason and Spaner 2006). The Canadian General Standards Board defines organic agriculture as a holistic system designed to optimize the productivity and fitness of diverse communities within the agroecosystem (CGSB 2006). The general principles of organic agricultural production are to protect the environment, maintain long-term soil fertility and to maintain biological diversity within the system (CGSB 2006). In contrast, conventional agriculture uses a variety of synthetic inputs (fertilizer, pesticides) to maximize yield.

Producers have made the transition to organic agriculture for a variety of reasons, including lower crop input costs, grower independence, rising consumer demand (Entz *et al.* 2001) and for health and environmental benefits (Sahota 2006). Supporters of organic agriculture believe it to be a more sustainable production system than the current conventional alternatives, and demand for organic products has dramatically increased in recent years.

Globally more than 31 million hectares are managed organically, with the highest proportion of organic compared to conventionally managed land found in Europe (Yussefi 2006). As of 2005, Canada had over 3600 organic farms comprising about 1.5% of the total agricultural area in the country (Macey 2006). Approximately 530,919 ha are

under organic management in Canada, with wheat comprising the largest portion at 75,816 ha (Macey 2006). Globally, sales of organic food and drink increased by about 9% to 27.8 billion USD in 2004, with the North American market reporting the highest growth (Sahota 2006). Europe and North America account for 96% of global organic food revenues (Sahota 2006).

Organic agricultural production differs from conventional farming systems in terms of soil fertility, weed management and distribution of soil microflora (Entz *et al.* 2004). Organic producers tend to rely more heavily on diverse rotations, soil-fertility building crops, and mechanical weed control than their conventional counterparts since synthetic pesticides and fertilizers are prohibited (Lampkin *et al.* 2000). As a result, conversion to organic farming tends to provide opportunities for increased plant diversity, increased soil microbial activity and higher soil aggregate stability while minimizing nutrient losses and reducing the amount of fossil fuel energy used on farm (Hansen *et al.* 2001, Mäder *et al.* 2002). The soil microbial community plays an important role in nutrient cycling and soil fertility and is very important in organic cropping systems (Hansen *et al.* 2001).

1.1.2 Arbuscular Mycorrhizal Symbiosis

Arbuscular mycorrhizal fungi (AMF) are widespread in nature, forming mutually beneficial associations with the roots of approximately 85% of herbaceous plants (Dalpé 1997). In exchange for functioning as an extended root system, the fungal symbiont receives carbohydrates from the host plant (Redecker 2007). While most crop plants have a symbiotic relationship with arbuscular mycorrhizas, the Brassicaceae family is

non-mycorrhizal (Peterson *et al.* 2004). The term mycorrhiza comes from the combination of the Greek word *mikès* (fungus) and the Latin word *rhiza* (roots) (Dalpé 1997). Arbuscular mycorrhizal fungi are grouped into the order Glomales, with most species belonging to the family Glomaceae (Dalpé 1997). AMF consist of approximately 160 species belonging to 3 families (Dalpé 1997), Glomaceae, Gigasporaceae and Acaulosporaceae (Redecker 2007).

Mycorrhizas are known to be essential for terrestrial ecosystem function (Redecker 2007). Arbuscular mycorrhizas belong to the group Glomeromycota whose origin is estimated to be 600 to 620 million years ago (Redecker 2007). Recently, spores and hyphae strongly resembling AMF were found in 460 million year old dolomite rocks in Wisconsin (Redecker 2002). The first land plants were associated with AMF (Peterson *et al.* 2004), and it is thought that Glomeromycota were instrumental in the success of early land plants (Redecker 2002). Although plant associations with AMF are ancient, AMF studies are rather new. Arbuscular mycorrhizas may have been described as early as 1842, but identification of AMF structures occurred until the 1970s (Koide and Mosse 2004).

1.1.3 Wheat

Wheat (*Triticum aestivum* L.) evolved from wild grasses in the area of the Middle East commonly known as the Fertile Crescent. The remains of cultivated emmer (a relative of wheat) have been discovered at sites in Syria dating back to 7,500 BCE (Gill and Friebe 2007). Wheat is thought to be one of the first domesticated food crops, and has been an important staple food for the major civilizations of Europe, West Asia and

North Africa since its domestication (Curtis 2002). Wheat continues to be an important food source for humans, and is grown throughout the world. Cultivated wheat species of the genus *Triticum* are classified according to their ploidy, as diploid, tetraploid and hexaploid wheat species. Bread wheat is a hexaploid wheat (Gill and Friebe 2007).

Wheat is the most commonly grown crop in Canada, with the highest seeded area of all crops at 10,341 thousand hectares in 2008 (Statistics Canada 2008). In Canada most wheat production occurs in western Canada, and 61.9% of wheat produced in western Canada is Canadian Western Red Spring (CWRS) wheat (CWB 2008). Canadian Western Red Spring is a hard wheat that is characterized by high protein and gluten content which makes it particularly suited to bread baking (Alberta Agriculture 2001). Canada is known worldwide as a producer of high quality wheat. Canadian Western Red Spring wheat has a high export demand by countries looking to improve the baking qualities of their flour products (Curtis 2002). Almost 1.5 million tonnes of CWRS wheat is used domestically, but most CWRS produced in Canada is exported to Russia, the United Kingdom, China and Japan (Alberta Agriculture 2001).

Worldwide, wheat is grown on more than 240 million ha, a larger area than any other crop (Curtis 2002). Wheat is produced on so much land area worldwide due to its wide adaptation to diverse environmental conditions, making wheat the top provider of nutrients to the world population (Peña 2002). The top wheat producing countries are China, India, United States, Russia, and Canada. China has the largest wheat production at 112,024,000 tonnes, while Canada is number 5 worldwide at 26,337,000 tonnes (Pingali 1999).

1.2 Description of Arbuscular Mycorrhizal Fungi

1.2.1 Morphology of AMF

Although other mycorrhizal associations exist, the arbuscular mycorrhizal type is most widespread (Bolan 1991). The presence of extraradical mycelium, spores formed in the extraradical mycelium, intraradical hyphae, and arbuscules characterize arbuscular mycorrhizas (Peterson *et al.* 2004). Some fungal symbionts also form intercellular vesicles (Dalpé 2003), a storage organ for the fungi that becomes filled with lipid bodies (Peterson *et al.* 2004).

AMF grow by forming extraradical mycelium, or hyphae, a loose network of filaments in the soil (Dalpé 2003). These hyphae are very important for the formation, functioning, and perpetuation of mycorrhiza in disturbed and natural ecosystems (Abbott *et al.* 1992). Hyphae have several functions, the most important of which is the uptake and translocation of mineral nutrients from the soil solution to the roots (Peterson *et al.* 2004). The increased uptake and translocation of mineral nutrients, especially phosphorus (P), is possible because of the highly branched nature of the hyphae (Peterson *et al.* 2004), which act as an extended root system. In low P soil, it was calculated that with four hyphae entry points per millimeter of root length, and with hyphae extending 20 mm from the root surface, P uptake per unit area would be 60 times greater than if a symbiotic relationship had not been formed by the plant and mycorrhizal fungi (Koide and Mosse 2004).

Extraradical mycelium, or hyphae, originates from either an established hyphal network or from other propagules in the soil, such as spores or root pieces (Abbott *et al.*

1992). Glomeromycota are obligate symbionts; therefore, if no host root is found by the hyphae, growth will cease after some time (Redecker 2007). As the hyphae propagate in the soil, their filaments spread inside the cortical root tissues differentiating into various structures (Dalpé 2003), which will be described later in this section. Although the trademark of fungal phyla such as the Basidomycota and Ascomycota are septae, the hyphae of Glomeromycota fungi lack these regular cross walls (Redecker 2007).

Spores act as reserve and propagation organs and are therefore a very important stage in the lifecycle of AMF (Dalpé 1997). Although spores are thought to be a common feature of mycorrhizal fungi, there are thought to be species of AMF present in some soils that do not produce spores (Abbott and Robson 1991). There is no evidence the Glomeromycota reproduce sexually; therefore, it is generally assumed that spores are formed asexually (Redecker 2007). The spores of AMF are also important since they act as a reference structure for species identification (Dalpé 1997). Mature spores have layered walls that allow them to persist in soil, sometimes for many years (Peterson *et al.* 2004). New spores are formed on the hyphal mycelium within or outside the host root. These spores may be formed singly, in clusters, or in fruit bodies called sporocarps (Redecker 2007). Most spores are generally formed near the end of the growing season (Peterson *et al.* 2004).

Under favorable conditions Glomeromycotan spores germinate and produce hyphae. Many factors affect germination including dormancy, pH, temperature, moisture, aeration, light and radiation, inorganic ions, microorganisms, oxidizing agents, antibodies, and pesticides (Siqueira *et al.* 1985). Spores are usually induced to germinate when new plant roots come into the proximity of the spores (Peterson *et al.* 2004). The

initial hyphae formed from germinating spores are called germ tubes (Peterson *et al.* 2004). The production of germ tubes can occur by direct germination through the spore wall, peripheral compartments, or regrowth of hyphal attachments (Siqueira *et al.* 1985).

For the development of internal structures, fungal hyphae must contact the surface of the root or root hairs, and then penetrate the epidermal layer (Peterson *et al.* 2004). The intraradical mycelium is connected to the soil mycelium, and consists of hyphae, as well as arbuscules and possibly vesicles (Rillig 2004). There are two morphological types of intraradical hyphae, *Arum*-type and *Paris*-type (Peterson *et al.* 2004).

In *Arum*-type, the type formed in wheat roots, intercellular hyphae are formed between the cortical cells and intracellular arbuscules within the cortical cells, while extensive intracellular hyphal coils and arbuscule coils form in the root cortex of *Paris*-type (Cavagnaro *et al.* 2001). Plant species that develop *Paris*-type AM lack extensive intercellular spaces in the root cortex (Peterson *et al.* 2004). It has been suggested that the morphological type of AMF is largely dependent on the plant species, due to the presence or absence of extensive air-spaces in roots (Cavagnaro *et al.* 2001).

The intraradical hyphae initiate other fungal structures within the root tissues by differentiating into intracellular arbuscules and in some cases intercellular vesicles (Dalpé 2003). In *Arum*-type AMF a single branch from intercellular or intracellular hyphae penetrates the wall of a cortical cell and forms a trunk hyphae that branches repeatedly to form the tree-like arbuscules. The life cycle of arbuscules is completed in approximately 2.5-4 days, afterwards which they rapidly collapse and form clumps (Bonfante and Perotto 1995). Arbuscules are highly branched and provide a large surface area,

therefore it is expected that most nutrient exchange happens at this site (Peterson *et al.* 2004).

The formation of intercellular vesicles depends on the genera of the fungal symbiont (Dalpé 2003). Intraradical vesicles can develop within cells or in intercellular spaces of the root from the tips of hyphae or from lateral branches (Peterson *et al.* 2004). Vesicles that form within root cells frequently enlarge to occupy the entire volume of the cell and are important storage organs of the fungi, since they contain large amounts of lipids as well as many nuclei (Peterson *et al.* 2004). Vesicles have also been known to act as propagules for some species of AMF (Abbott and Robson 1991).

1.2.2 Colonization of Host Plants by AMF

Colonization of the host plant by AMF involves changes in both the mycorrhizal fungi and the host plant. Prior to colonization by AMF, the host plant and fungi must find each other in the soil. Nutrient deficient plants may send root-derived signals that redirect fungal growth and induce branching of fungal hyphae (Parniske 2004). Bécard and Piché (1989) found that fungal growth was stimulated eightfold by the presence of root volatiles and exudates. There is also evidence of a fungal signaling molecule that induces plant gene activation (Parniske 2004). The formation of appressoria, which is induced by root exudates, is one of the first morphological signs that recognition between the fungi and host plant has occurred (Bonfante and Perotto 1995).

Colonization is thought to be strictly under the control of the host plant through the opening of a cleft between two adjacent epidermal cells (Parniske 2004). This may restrict colonization by AMF when sufficient P is present in the soil, preventing a

parasitic relationship between the fungi and host plant. The opening of the cleft allows the hyphae to enter the root, and then appressoria are formed by the fungal hyphae to gain entry into root epidermal or exodermal cells (Parniske 2004). Hyphae are then produced which thread their way between the cortical cells of the root and quickly propagate (Daplé 1997). Combinations of both mechanical and enzymatic mechanisms are used to penetrate the host tissue (Bonfante and Perotto 1995).

Most AMF produce intercellular hyphae once inside the roots, which run within the air channels and then cross the wall of the cortical cells with the aid of penetration pegs to become intracellular (Bonfante and Perotto 1995). This results in the initiation of arbuscule formation. A single successful infection event can result in the colonization of large root sections (Parniske 2004).

During the process of colonization extensive changes occur in AMF, the most dramatic of which is the formation of arbuscules. A low to moderate supply of P is required for arbuscule formation. Arbuscule formation is inhibited at both a high and very low P supply due to the cost of maintaining this relationship to the plant; although, hyphal colonization can still occur (Ezawa *et al.* 2002).

AMF also induce morphological changes in the root system of the host plant. Variations occur in the branching pattern of colonized plants; both elongate and highly branched root systems have been observed in response to colonization (Berta *et al.* 1993). In leek, Berta *et al.* (1993) observed a denser root system with a higher number of shorter more branched adventitious roots. These adventitious roots had a greater diameter and a lower specific root length as compared to the control plants. Changes in root morphology may occur to allow the host plant to optimize its carbon investment. Since AMF acts like

an extended root system the host plant may focus less on nutrient acquisition and more on carbon uptake to feed the AMF, which in turn would increase nutrient acquisition.

1.2.3 Transfer and Uptake of Nutrients

Although there are many benefits of mycorrhizal symbiosis, nutrient uptake is most often discussed. Mycorrhizal fungi facilitate the uptake of copper, zinc, N, and P; although, the increased uptake of P is most often discussed. Phosphorus is immobile in soil and AMF are able to take up P more efficiently and transport P over longer distances than the plant root system, which helps to overcome P depletion in the rhizosphere (Ezawa *et al.* 2002). The increased uptake of P by AMF is largely attributed to an exploration of a larger soil volume and the diameter of mycorrhizal hyphae (Bücking and Shachar-Hill 2005). While P uptake by poorly dispersed root hairs may rapidly become limited by inter-hair competition, uptake by well-dispersed hyphae is probably not limited by depletion zones due to their small diameter (Jakobsen *et al.* 2005). Root hairs commonly have a diameter larger than 10 μm compared to the diameter of the hyphae of AMF, which ranges from 2-4 μm (Bolan 1991).

Arbuscular mycorrhizal fungi have other mechanisms for increased uptake of P, such as modification of the root environment, a lower threshold concentration for absorption, and a higher affinity for absorption of P (Bolan 1991). Arbuscular mycorrhizal fungi also produce organic acids and phosphatases that catalyse the release of P from organic complexes (Bücking and Shachar-Hill 2005) and can store larger amounts of absorbed P, which facilitates the continued movement of P into the roots (Bolan 1991).

The various forms of P in the soil are categorized into organic P (Po) and inorganic P (Pi). Vegetation, fertilizer history, microbial activity, cultivation and soil type affect the distribution of the two forms of P (Bolan 1991). Inorganic P can occur in soil solution or be adsorbed onto soil surfaces (Bolan 1991), and is a major component of the vacuolar P pool of AMF. The vacuolar P pool is made up of both Pi and polyphosphate (polyP) (Ezawa *et al.* 2002). Although only a small portion of total P in the soil is comprised of Pi, plants derive most of their immediate P requirements from this source (Bolan 1991). Organic P can be taken up by soil organisms or undergo mineralization to provide plant available Pi (Steward and Tiessen 1987).

Radioactive P (^{32}P) studies demonstrate that mycorrhizal and non-mycorrhizal plants obtain their P requirements from the same sources and forms of P, although mycorrhizal plants may obtain P that is unavailable to non-mycorrhizal plants (Bolan 1991). It is expected that AMF are able to solubilize poorly soluble P, since the addition of poorly soluble fertilizers results in the increased growth of mycorrhizal plants (Bolan 1991).

Long distance translocation of P occurs within the extraradical hyphae, where P is adsorbed from the soil solution (Uetake *et al.* 2002). Inorganic P, polyP, and possibly Po are the forms of P involved in long distance transport (Ezawa *et al.* 2002). Inorganic P is first incorporated into the cytosolic Pi pool following uptake. The concentration of Pi is kept constant to maintain various cell functions (Ezawa *et al.* 2002). Excess P is transported into the vacuoles if AMF are able to uptake more Pi than the amount required for the maintenance of cell functions (Ezawa *et al.* 2002). The presence of polyphosphate granules has been reported in vacuoles within the inter- and intra-cellular hyphae (Cox *et*

al. 1980, Uetake *et al.* 2002). The vacuolar pool may play an important role in the supply of P to the host plant (Ezawa *et al.* 2002). Cox *et al.* (1980) proposed that the transport of polyP-containing vacuoles by cytoplasmic streaming may be the method of P translocation from the soil mycelium to the root.

The host plant forms an interface compartment at the contact area between plant and fungal cell surfaces to allow for the two-way exchange of nutrients between the host plant and AMF (Bonfante and Perotto 1995). Since there is no direct continuity between the plant and the fungi, this membrane separates all intracellular structures from the root cytoplasm (Peterson *et al.* 2004). Both the carbon and mineral nutrients must pass through the interfacial apoplast before they can be absorbed (Bücking and Shachar-Hill 2005).

The contact areas between the plant and fungi are the arbuscules (Peterson *et al.* 2004). Based on indirect evidence, arbuscules are the site from which Pi is released to the interfacial apoplast and then absorbed by the plant cortical cell (Ezawa *et al.* 2002). It is expected that arbuscules are the site of Pi release and absorption due to the presence of high-affinity Pi transporters and P-ATPase genes expressed in arbuscule-containing host cells (Ezawa *et al.* 2002).

The branched tree-like structure of the arbuscules increases the surface area available for bi-directional transport (Peterson *et al.* 2004). Arbuscule formation, Pi liberation from the vacuolar P pool, and efflux through the tonoplast and plasma membrane of AMF to the interfacial apoplast may be the steps at which Pi transfer is regulated (Ezawa *et al.* 2002). Passive efflux of Pi and carbohydrates through the fungal and plant plasma membranes and then active absorption of nutrients by both symbionts

driven by H^+ATPase(s) is generally assumed to be the model for this process (Bücking and Shachar-Hill 2005).

1.3 Benefits of Mycorrhizal Symbiosis

1.3.1 Soil Aggregation

Arbuscular mycorrhizal fungi positively influence soil quality through their effects on soil aggregation, and therefore influence soil physical conditions and the soil microbial community (Hamel 2004). Aggregate stability is an important factor in soil quality, as it works to maintain good water infiltration rates, good tilth, and adequate aeration for plant growth (Wright and Upadhyaya 1998). Arbuscular mycorrhizal fungi are able to improve aggregate stability because they are abundant soil organisms that have direct access to plant carbon and do not have to compete for soil organic matter. The extraradical hyphae also function as stabilizing structures in the soil (Rillig 2004).

Another way that AMF are able to improve soil structure is through glomalin (Rillig *et al.* 2002). Glomalin is an immunoreactive glycoprotein that is produced by the hyphae of AMF (Wright and Upadhyaya 1998). Glomalin is sometimes referred to as glomalin-related soil proteins (GRSP) since the link between glomalin and soil protein fractions is not clearly established (Rillig 2004). There are many unknown aspects regarding glomalin, for example, its structure. Some research states that glomalin is produced by actively growing hyphae (Wright and Upadhyaya 1998), while others suggest that glomalin may be primarily contained in the hyphal and spore walls, and requires hyphal turnover for deposition into the soil (Rillig 2004). Although controversy exists regarding glomalin synthesis, it has been established that at least some portion of

GRSP originates as a result of AMF. Rillig (2004) showed that when AMF growth is eliminated from the soil, the concentration of GRSP declines.

Glomalin is found to be highly correlated with aggregate stability, and small increases in the easily extractable glomalin fraction could lead to significant increases in soil stability (Wright and Upadhyaya 1998). A well-aggregated soil is important in natural ecosystems and in agriculture, as it maintains aeration and moisture conditions required for optimal plant growth (Wright and Upadhyaya 1998).

1.3.2 Environmental Stresses

It has been suggested that AMF often only provide a benefit to the host plant during periods of environmental stress (Ryan and Ash 1996). The protection against environmental stress accrued to the host plant from AMF colonization is mainly due to enhanced drought tolerance, although AMF has also been reported to protect against chilling stress, salinity, and pollution (Dalpé 1997). Under drought stress, colonization of roots by AMF has been shown to improve the productivity of a variety of crop species (Al-Karaki *et al.* 2004).

The mechanisms by which AMF enhance drought resistance have not been well elucidated. Hyphae may be important in low water conditions as they can enter small pore spaces and may be able to take up water that is unavailable to the plant roots and root hairs. Possible mechanisms for higher water uptake rates of mycorrhizal roots could also be due to higher hydraulic conductivity, or a higher transpirational demand (George *et al.* 1992).

Increased drought resistance of mycorrhizal plants may also be the result of AMF enhancing nutrient uptake under drought conditions (Ryan and Ash 1996). Colonized plants have been shown to be better nourished and better adapted to their environment than non-colonized plants (Dalpé 1997). The ability of mycorrhizal plants to increase their uptake of nutrients could result in bigger plants with a longer rooting depth. Ellis *et al.* (1985) found that colonized plants had better rooting below 0.3 m compared to non-mycorrhizal plants, which resulted in increased soil water uptake. Plant resistance to drought could also be influenced by other factors associated with colonization of AMF such as changes in leaf elasticity, improved leaf water and turgor potentials, maintenance of stomatal opening and transpiration (Al-Karaki *et al.* 2004) and altered hormonal response (Ellis *et al.* 1985).

In a water stress study Ellis *et al.* (1985) found that over periods of water stress at 55, 63 and 70 days after transplanting the total dry weight and grain yield of plants inoculated with AMF were twice as great as for non-mycorrhizal plants. The plants not inoculated with AMF also showed water stress symptoms sooner than those that were inoculated (Ellis *et al.* 1985). Al-Karaki *et al.* (2004) demonstrated improved growth, yield and nutrient uptake in wheat plants inoculated with mycorrhiza under drought stress compared to control plants. Although the above studies found AMF to be beneficial under water stress, when severe drought stress naturally occurred in a study by Ryan and Ash (1996) it was unlikely that AMF made any contribution to reducing the water stress of the crop.

1.3.3 Defense Against Disease-Causing Organisms

Arbuscular mycorrhizal fungi have the ability to mediate interactions between disease-causing organisms and host plants (Ryan and Ash 1996). Colonization by AMF causes a consistent reduction of symptoms for nematodes such as *Rotylenchus*, *Pratylenchus*, and *Meloidogyne*, and for fungal pathogens such as *Phytophthora*, *Fusarium*, *Pythium*, *Sclerotium*, and *Verticillium* (Azcón-Aguilar and Barea 1996). There are 91 examples listed on the positive effects of AMF symbiosis against root pathogens on a wide range of plants, pathogens, and symbionts, in which 76% of the cases resulted in a significant reduction of disease (Fitter and Garbaye 1994).

Arbuscular mycorrhizal fungi species have differing abilities to enhance resistance or tolerance in roots, and protection is not effective for all pathogens. Soil and environmental conditions are also known to affect protection (Azcón-Aguilar and Barea 1996). Since interactions between AMF and other soil organisms are complex and poorly understood (Fitter and Garbaye 1994) the ability of AMF to reduce the incidence and harmful effects of pathogens is not well defined.

There are many mechanisms by which AMF could control root pathogens. Improvement in plant nutrition is one such way, as a greater availability of nutrients results in more vigorous plants that could be more resistant or tolerant to pathogen attack (Azcón-Aguilar and Barea 1996). Other mechanisms by which AMF may control pathogens include damage compensation, competition for host photosynthates, competition for infection/colonization sites, morphological changes in the root system, and through microbial changes in the mycorrhizosphere (Azcón-Aguilar and Barea

1996). Arbuscular mycorrhizal colonization may also induce an activation of metabolic pathways related to disease resistance mechanisms, although there is no clear evidence for this (Filion *et al.* 1999).

1.3.4 Environmental Benefits

The most obvious environmental benefit of AMF colonization of crop species is reduced fertilizer application. Producers could reduce their fertilizer input on mycorrhizal crop species without affecting crop yields, due to the capacity of AMF to improve plant nutrition (Daplé 2003). A reduction in fertilizer inputs, especially P, would lessen pollution of surface waters and decrease cost of crop production.

A second potential environmental benefit of AMF is in the remediation of soil contaminated with heavy metals; although, there are conflicting results on the accumulation of heavy metals within mycorrhizal plants (Khan *et al.* 2000). The anthropogenic sources of metals in soil include the addition of municipal sludge and atmospheric sources such as mining and smelting (Entry *et al.* 2002). The metal tolerance mechanisms of AMF colonized plants include adsorption onto plant or fungal cell walls, chelation by siderophores and metallothionens released by the fungi, sequestration by plant-derived compounds, or dilution due to increased root and shoot growth (Entry *et al.* 2002).

1.4 Influence of Plant Ecology on AMF

1.4.1 Farming System

Arbuscular mycorrhizal fungi colonization is shown to be higher in organically managed farming systems compared to conventional farming systems (Mäder *et al.* 2000, Entz *et al.* 2004, Douds *et al.* 1995). The main reason for higher colonization under organic systems is thought to be the lower availability of P in the soil. Mäder *et al.* (2000) found AMF colonization of winter wheat, vetch-rye, and grass-clover crops to be enhanced by 30-60% under organic farming compared to conventional crop production. While most agree that P fertilization reduces AMF colonization, others suggest that fertilization of naturally infertile soils increases colonization. There is little or no benefit to colonization of fertilizing naturally fertile soils (Gavito and Miller 1998).

Both fungicides and herbicides are thought to have a negative effect on AMF colonization. Siqueira *et al.* (1985) found that fungicide treatments reduced the viability of *Gigaspore margarita* azygospores, while others report that spore germination and germ tube growth of several AMF species was not affected by the application of pesticides to the soil at normal rates (Siqueira *et al.* 1985). Herbicides negatively affect AMF colonization by lowering spore viability and hyphal growth, as well as by eliminating weeds which can act as host plants for AMF (Mäder *et al.* 2000). Douds *et al.* (1993) found plots under low-input agriculture were covered with live plants 70% longer than conventional plots in an average year. Mäder *et al.* (2000) also found weed cover and diversity to be higher in organic plots compared to conventional plots.

1.4.2 Crop Rotation

Crop rotation is also shown to have an effect on the level of AMF colonization. Minimal rotation to non-host crops such as canola (*Brassica napus*) is thought to be favourable to colonization (Entz *et al.* 2004); although, there are contradictory results. Some studies have shown non-host plants to have no effect on mycorrhizal colonization, while others have found non-host crops to reduce colonization in subsequent crops (Gavito and Miller 1998).

When the previous crop planted was canola, Gavito and Miller (1998) found arbuscular and vesicular colonization to be greatly reduced compared to a previous crop of maize. Canola delayed colonization for more than one month into the next growing season (Gavito and Miller 1998). Entz *et al.* (2004) found colonization to be reduced in plots dominated with wild mustard (*Brassica kaber*) compared to plots which contained high populations of green foxtail (*Setaria viridis*), a weed known to be mycorrhizal.

1.4.3 Cover Crops

The viability of AMF can be reduced during fallow periods. Cover cropping with winter mycorrhizal plants helps to maintain or increase the inoculum potential for the succeeding crop (Kabir and Kodie 2002). During fallow periods, such as the spring and autumn, the energy available for the growth of AMF decreases due to the absence of a host crop. Extra-radical hyphae can also be physically disrupted by freezing and thawing of bare soil (Boswell *et al.* 1998).

Growth of cover crops in the autumn and spring supports the growth of extra-radical hyphae, which is beneficial to the following crop since rapid colonization of the

emerging seedlings can occur through contact with these extra-radical hyphae (Boswell *et al.* 1998). Although colonization is slower in cooler temperatures it has been shown that hyphal growth occurs even at temperatures close to freezing (Boswell *et al.* 1998). Kabir and Kodie (2002) showed that cover cropping with rye and oat significantly increased the yield of the following sweet corn crop compared to fallowing. Boswell *et al.* (1998) found a winter wheat cover crop to increase the inoculum potential of AMF in the following season.

1.5 Comparison of Organic and Conventional Farming Systems

1.5.1 Soil Fertility

Organic and conventional farming systems differ in soil fertility and nutrient management. While synthetic fertilizers are used to provide nutrients to growing crops in conventional farming systems, organic farmers must rely on legume and/or green manure based crop rotations, and additions of manure to maintain long-term soil fertility. A fertile soil is an important component of any agricultural system as it provides essential nutrients for plant growth, supports a diverse and active biotic community and promotes a good structured soil (Mäder *et al.* 2002).

Nitrogen deficiency is often a challenge during the transition years, but soil fertility levels can be maintained and improved by including green manures and grain legumes into crop rotations as soil fertility building crops (Watson *et al.* 2002). In a study of organic and conventional farming systems Pimentel *et al.* (2005) found that over a 22-year period N level in the organic animal and legume systems increased significantly, while soil N in the conventional system remained unchanged. Kelm *et al.*

(2008) compared 16 pairs of organic and conventional farms in Germany and found that many organic farms were N deficient. It was suggested that N management could be improved on these farms by adjusting rotations and more efficient utilization of manure.

Phosphorus deficiency is often a larger concern in organic farming systems due to the lack of readily available sources of P. In a study of 14 organic farms in the eastern portion of the Northern Great Plains the farms were generally sufficient in N, potassium and sulphur, but often deficient in P. The average nitrate-N recorded was higher than the Manitoba average while the average P level was substantially lower than that typically found in the province (Entz *et al.* 2001). In a 21-year study comparing farming systems, the organically managed soils had a negative P balance while the conventionally managed soils had a positive P balance (Oehl *et al.* 2002).

These studies raise questions about the sustainability of organic agricultural systems with regards to P fertility. If P removal exceeds P input then soil reserves or residual P from earlier fertilizer applications is providing the P required for plant growth (Oehl *et al.* 2002). There is a limit to how long mineralization can continue to provide P for cultivated crops without added P fertilizer (Hedley *et al.* 1982). In an organic cropping system it is evident that more than just plant available P needs to be accounted for when determining fertility. Welsh et al. (2009) found high yielding organic cropping systems to decrease plant-available soil P, but not recalcitrant forms of P. Relatively insoluble forms of P can be solubilized during crop growth by root processes and microbial activity (Hedley et al. 1982). During crop growth, root processes and microbial activity contribute to solubilizing relatively insoluble forms of P (Hedley *et al.* 1982).

1.5.2 Weed Management

Conventional farmers generally rely on synthetic pesticides to control weed populations while in organic cropping systems weeds are managed by employing diverse crop rotations, cover crops, and mechanical weed control. In organic farming systems, the approach to weed management often involves the whole system, with crop rotation being the primary focus (Bond and Grundy 2001).

Weed populations are often reported to be higher on organic farms compared to conventional farms. In the 21-year bio-Dynamic, bio-Organic and (K)conventional (DOK) trial in Switzerland, Mäder *et al.* (2002) found that organic farming allows the development of a relatively diverse weed flora with 9-11 weed species found on the organically managed plots compared to 1 species found in the conventionally managed plots. In a comparison study of organic and conventional cereal fields in Denmark, richness of wild plant species was highest in organic fields throughout the study. Crop biomass was 25% lower in organic fields compared with conventional, while the biomass of weed species was five times higher in the organic fields (Hald 1999).

1.5.3 Soil Microorganisms

Soil microorganisms contribute to maintaining soil fertility and productivity, and play a role in improving the functioning of the ecosystem (Stark 2008). Healthy ecosystems are generally characterized by high species diversity. Organically managed soils have been shown to exhibit greater biological activity than conventionally managed soils (Mäder *et al.* 2002, Pimentel *et al.* 2005). In the 21-year DOK trial, soil microbial biomass increased from conventional management to organic management. Activities of

dehydrogenase, protease, and phosphatase were higher in the organic systems which indicates higher overall microbial activity and a higher capacity to cleave Po, which would contribute to soil fertility (Mäder *et al.* 2002). Arbuscular mycorrhizal fungi also contribute to the success of organic systems and may also aid in biological control of pests (Pimentel *et al.* 2005).

Comparison studies have found organically managed systems to have higher soil microbial biomass and activity compared to their conventionally managed counterparts, but it has been suggested that these findings are more a result of management practices than system effects (Stark 2008). Farm yard manure additions were found to increase soil microbial biomass as well as have a positive impact on soil properties (Stark 2008, Lehocka *et al.* 2008). It has also been shown that there is a positive correlation between aggregate stability and microbial biomass, as well as that microbial biomass contributes to efficient resource utilization (Mäder *et al.* 2002).

1.5.4 Yield

Due to a lack of synthetic fertilizer additions and increased weed pressure, crop yields on organic farms tend to be lower than yields found on conventional farms. A 6-year study of 14 organic farms in the eastern portion of the northern Great Plains found hard red spring wheat, oat, flax and field pea yielded 77%, 73%, 78% and 67% of long-term conventional averages, respectively (Entz *et al.* 2001). In the first 22 years of the Rodale Farming Systems Trial organic corn yields were found to be similar to conventional corn yields after a five year transition period. It was concluded that organically managed crop yields can equal those from conventional agriculture,

depending on crop, soil and weather conditions; although, organic cash crops cannot be grown as frequently over time due to the cultural practices required to supply nutrients (Pimentel *et al.* 2005).

In the DOK trial, organic production systems were found to be efficient, since nutrient input (N, P, K) is 34-51% lower in organic farming systems compared to conventional systems, while mean crop yield was only 20% lower over a 21-year period (Mäder *et al.* 2002). Winter wheat yields in the DOK trial reached an average of 90% of the yield of conventional systems (Mäder *et al.* 2002), and the maximum yields recorded for cereal crops in the Entz *et al.* (2001) study were higher than the long-term average on conventional farms. Although crop yields under organic management are typically lower than what may be achieved under conventional management, high yields are possible in organic management systems, but possibly at a lower frequency.

1.6 Breeding in Organic Agriculture

1.6.1 Why Breed in Organic Production?

In Canada and the United States, organic producers are required to use certified organic seed and transplants when readily available (CGSB 2006). Organic seeds are thought to be essential to organic farming in order to maintain the integrity of the organic system; although, organic agriculture remains strongly dependent on conventional breeding. Organic farmers have been reliant on conventionally bred cultivars designed for crop production systems that use synthetic chemical fertilizers and pesticides. Crop performance in organic systems could be improved if cultivars were bred for typically organic conditions (Lammerts van Bueren *et al. 1999).*

Increased stress under organic management may affect cultivar performance to the extent where breeding specifically for organic environments is recommended (Mason and Spaner 2006). Breeding for specific environmental stresses such as P deficiency (Atlin and Frey 1989) and drought tolerance (Ceccarelli 1987) are good examples of environmental stresses that should be selected for directly in the stressful environment. An organic breeding program would focus on traits such as improved nitrogen use efficiency, adaptation to soil microbes, improved competitiveness against weeds, and resistance to insects and diseases (Murphy *et al.* 2007). Conventional breeders are also increasingly using gene technology to produce genetically modified cultivars which are banned in organic production (Lammerts van Bueren *et al.* 1999). Genetic modification is banned in organic crop production because it does not respect natural species authenticity and species characteristics (Lammerts van Bueren *et al.* 1999).

Breeding specifically for organic production is important if the cultivars with the highest yield and best quality under organic management differ from those that do best under conventional management. In a study comparing 35 soft white winter wheat advanced breeding lines over 5 locations under both organic and conventional production Murphy *et al.* (2007) found the rankings of high yielding genotypes in conventional systems to be unreliable predictors of the high yielding genotypes in organic systems. This study indicates the need for an organic breeding program, since under conventional selection most of the best yielding cultivars under organic management would have been dropped from the breeding program.

1.6.2 Direct Selection

Organic crop cultivars can be selected in organic or conventional management environments. In direct selection a plant breeder selects cultivars in the environment where the cultivar is to be grown in the future. For organic crop production this implies conducting plant selection in organic management environments. In indirect selection, a breeder selects for a secondary character when aiming to improve a primary character, for example, selection in the absence of stress to improve yield in the presence of stress. Studies have found that although progress exists at all input levels, genetic gain is lower under low-input levels (Brancourt-Hulmel *et al.* 2005), which is often the justification for selection in low-stress environments. Plant breeders also often aim for widely adapted cultivars. Although wide adaptation is considered in both a geographical sense and an environmental sense, environments where widely adapted cultivars are used are often made environmentally similar through the use of fertilizers and irrigation. In a study by Ceccarelli (1994) widely adapted lines yielded more than the best check in contrasting locations, but suffered a yield disadvantage of 10-30% when compared with lines selected for specific adaptation.

Regardless of genetic gain, studies show cultivars should be selected for in the environment in which they will be grown, due to the genotype x environment interaction. In a study comparing input levels of N and fungicides, Brancourt-Hulmel *et al.* (2005) found that indirect selection was never more efficient than direct selection. Atlin and Frey (1989) found similar results in a study looking at heat and P stress in oat cultivar selection. Response to direct selection was predicted to be more than twice as great as indirect selection, which indicates that high input environments are not necessarily

optimum for oat selection. Ceccarelli and Grando (1991) also found that breeding programs to increase yield under stress should rely on selection under stress. They found the entries selected in low yielding sites had a significantly higher grain yield in low yielding sites the following season than entries selected in high yielding sites.

Together these studies show that breeding programs targeted for organic production systems should include organic environments to maximize selection gains. When selection is carried out in organic growing conditions, the cultivars can adapt to organic conditions related to the soil, disease and weed pressure (Lammerts van Bueren *et al.* 1999).

1.6.3 Desirable Plant Characteristics for Organic Production

Organic land management produces a different environment than is generally found in conventional crop production. Although organic and conventional plant breeders may focus on some of the same desirable characteristics, such as efficient use and uptake of nutrients and water, resistance to diseases (Lammerts van Bueren *et al.* 1999, 2002), high yielding and yield stability, and good baking and nutritional quality (Wolfe *et al.* 2008), there are some plant characteristics that may be more desirable for organic crop production. Characteristics cited as more beneficial for organic crop production include ability to interact with beneficial soil microorganisms and ability to compete with weeds (Lammerts van Bueren *et al.* 1999, 2002).

The lack of inorganic fertilizer inputs in organic agricultural systems may result in lower nitrogen and phosphorus levels compared to conventional agriculture. As well as being adapted to lower nutrient levels, it would also be beneficial for cultivars to be

adapted to seasonal fluctuations in nutrient availability (Lammerts van Bueren *et al.* 1999) since organic farmers have fewer options to deal with nutrient deficiencies. In order to cope with lower nutrient levels, it would be beneficial for organic cultivars to be able to form and maintain a larger and more active root system for nutrient uptake (Lammerts van Bueren *et al.* 2002).

Phosphorus is the nutrient that is most often limiting in organic systems and is dependent on root characteristics for efficient uptake due to its immobility in the soil. In a study by Manske *et al.* (2000) root length density was found to be the most important trait for improved P absorption. Root length density is a hard trait to select for; therefore, breeders may want to rely on indirect selection for above ground traits that are found to be related to root growth. One example is the number of spikes m^{-2} which was found to be positively correlated with root length density by Manske *et al.* (2000). Rhizosphere acidification, root exudation of organic anions, root morphology, and symbiotic associations with mycorrhizal fungi are plant traits that are also found to be responsible for P uptake efficiency (Zhu *et al.* 2001).

Organic farming systems will depend more on interactions with beneficial soil microorganisms, such as mycorrhiza, than conventional systems. Mycorrhizal dependence is thought to be a genetic trait that is expressed in some wheat genotypes (Hetrick *et al.* 1992). There is a hypothesis that past selection of germplasm under fertilized conditions may have reduced the frequency of genes that promote mycorrhizal associations (Hetrick *et al.* 1992). Older cultivars and landraces may form stronger associations with mycorrhiza than modern cultivars.

In a study by Manske (1989) landraces were found to have a high mycorrhizal efficiency, and higher mycorrhizal dependency than modern cultivars. The theory that mycorrhizal dependence has been bred out of modern wheat cultivars is also supported by Hetrick *et al.* (1995), who found a stronger mycorrhizal dependence in cultivars released before 1975 than in modern semi-dwarf cultivars released after 1975. Hetrick *et al.* (1992) observed a general trend in which response to mycorrhiza was greatest in Asian landraces, lower in improved cultivars, and lowest in modern cultivars. A study by Zhu *et al.* (2001) also found that older cultivars (released before 1956) were generally more responsive to AMF than modern cultivars; although, the old cultivars had lower percent colonization than the modern cultivars.

Conventional herbicides are prohibited in organic farming systems. Crop cultivars with a superior ability to compete with weeds are beneficial in organic crop production. The competitive ability of a crop is based on two components: the crops ability to maintain yield in the presence of competition as well as the crops ability to suppress weeds (Lemerle *et al.* 1996). Cultivars that have rapid juvenile growth, plant architecture for early soil cover (Lammerts van Bueren *et al.* 1999) and are tolerant of mechanical weed control (Lammerts van Bueren *et al.* 2002) are beneficial for organic farmers. Plant characteristics such as height and tillering and crop characteristics such as rapid ground cover are important selection criteria when selecting for cultivars with weed suppressive abilities (Wolfe *et al.* 2008). Cosser *et al.* (1997) found older cultivars to grow taller, compete better for light, and accumulate more early dry matter than modern cultivars, indicating that older cultivars may be better suited to organic crop production.

Mason *et al.* (2007) found grain yield to be positively correlated with height at high weed pressure but not low weed pressure, indicating that tallness is an important characteristic of plants to be grown in organic conditions. Plant height and vigor were related to yield in a study by Carr *et al.* (2006), while other traits such as canopy closure and above ground biomass production were not consistently correlated with grain yield. The Carr *et al.* (2006) study does not support the hypothesis that older cultivars are better adapted to organic growing conditions, as it was found that cultivars developed before 1970 were generally lower yielding than several modern cultivars when grown under organic production.

In selecting an organic spring wheat cultivar, all-round disease resistance is important, since monogenetic resistance is fairly easily broken in organic production (Lammerts van Bueren *et al.* 1999). Disease resistance can be related to a plant architecture that will allow the ears to dry more rapidly, such as a longer stem and peduncle, an ear higher above the flag leaf, and a less compact spike (Lammerts van Bueren *et al.* 2002). That said, disease resistance may be less important in organic compared to conventional agriculture due to features of organic systems such as lower plant population densities and lower N levels (Wolfe *et al.* 2008).

Both conventional and organic production requires wheat cultivars with good baking and nutritional quality. Quality needs for organic crops vary depending on market use. Organic cereals entering the global market are generally required to conform to the industrialized milling and baking standards, which require high protein content and relatively strong gluten. In regional and local markets, there is generally more flexibility, as small bakers can often adjust the baking process to the quantity and quality of proteins

(Wolfe *et al.* 2008). In wheat, yield and protein content often exhibit an inverse relationship (Wolfe *et al.* 2008); therefore, lower yielding organic cultivars could be expected to have higher protein concentrations. This theory is not supported by Carr *et al.* (2006) who found modern HRS cultivars to have higher grain protein concentrations than older cultivars. A study by Garvin *et al.* (2006) found breeding for increased yield in hard red winter wheat has had a negative effect on seed zinc, iron, and selenium concentrations, indicating heritage wheat cultivars may have a higher nutritional value.

It is recommended that in following the principles of organic agriculture, organic plant breeding should follow the concept of naturalness by respecting natural reproductive ability and barriers, the plants relationship with the living soil, by avoiding the use of synthetic inputs, and by stimulating the self-regulatory ability of organic farming systems (Lammerts van Bueren *et al.* 2003). Breeding programs for organic cultivars will also benefit conventional farming systems by providing options for reduced use of nutrient and chemical inputs.

2. VARIATION IN MYCORRHIZAL COLONIZATION OF PARENTAL SPRING WHEAT LINES IN ORGANIC GROWING CONDITIONS

2.1 Abstract

Arbuscular mycorrhizal fungi (AMF) are important soil organisms for assisting in the uptake by plants of phosphorus (P) from soil. AMF are especially important in organic cropping systems where P is often deficient. This study compared the hyphal, arbuscular and total AMF colonization of 26 hard red spring wheat lines. The research was carried out at two organically managed research sites: the Glenlea Long-Term Rotation and the Organic Crops Field Laboratory, both located in Southern Manitoba. Of the 26 wheat lines studied, 7 were check cultivars and 19 were parental lines used in 12 different crosses, each of which were used to produce mapping populations of double haploids or recombinant inbred lines. At Glenlea, percent arbuscular colonization and percent total (arbuscular and hyphal) colonization differed significantly among the lines ($P < 0.05$); no significant differences in colonization were found at Carman. Site differences may be attributed to length of time under organic management, soil conditions and environmental conditions. Arbuscular and total colonization differed between 4 of the parental combinations at Glenlea ($P < 0.05$). The doubled haploid populations of these parental combinations may be appropriate for screening in the future to investigate the inheritance of mycorrhizal dependence. No relationship was observed between date of cultivar release and AMF activity. Thus evidence did not suggest that mycorrhizal dependency has been lost during the development of modern wheat cultivars.

2.2 Introduction

Arbuscular mycorrhizal fungi (AMF) form mutually beneficial associations with the roots of most herbaceous plants (Dalpé 1997). In low-input and organic agricultural systems, the main benefit of AMF is the increased capability to take up mineralized soil nutrients (Mäder *et al.* 2000). This is especially true for P, which is often deficient in organic systems (Entz *et al.* 2001). P is immobile in soil and AMF are able to take up P more efficiently and transport P over longer distances than the plant root system (Ezawa *et al.* 2002). The increased uptake of P by AMF is attributed to an increased surface area for nutrient absorption due to the presence of the extraradical fungal hyphae (Bücking and Shacher-Hill 2005). Although hyphae uptake and translocate mineral nutrients, arbuscules are thought to be the site of nutrient exchange (Peterson *et al.* 2004) and may be a better indication of the P acquisition of colonized plants. Other means by which AMF increase the uptake of P include modification of the root environment, a lower threshold concentration for absorption (Bolan 1991), and the production of organic acids and phosphatases that catalyse the release of P from organic complexes (Bücking and Shacher-Hill 2005).

Although the increased acquisition of mineral nutrients may be the most important role of the fungi, AMF also improve soil aggregate stability (Hamel 2004), protect the host plant from environmental stress (Daplé 1997) and enhance host plant resistance to specific disease-causing organisms (Azcón-Aguilar and Barea 1996; Fitter and Garbaye 1994). Colonization can be promoted by avoiding the use of fungicides (Siqueira *et al.* 1985) and herbicides (Mäder *et al.* 2000), minimal rotation to non-host crops (Entz *et al.* 2004) and maintaining ground cover with mycorrhizal cover crops

(Kabir and Kodie 2002). The principles of organic agricultural production either require or promote these beneficial practices; therefore, it is not surprising that AMF colonization is shown to be higher in organically managed farming systems compared to conventional farming systems (Douds *et al.* 1995; Entz *et al.* 2004; Mäder *et al.* 2000).

The general principles of organic agricultural production are to protect the environment, maintain long-term soil fertility and maintain biological diversity within the system (CGSB 2006). Organic production prohibits the use of synthetic fertilizers and pesticides. The maintenance of soil fertility is achieved by promoting optimal biological activity in the soil, conservation of soil resources, appropriate crop selection and crop rotation (CGSB 2006). The selection of crop cultivars that have strong associations with AMF may be important in mitigating inadequate soil fertility. Mycorrhizal dependence is thought to be a genetic trait that is expressed in some wheat genotypes (Hetrick *et al.* 1992). Older cultivars may form stronger associations with mycorrhiza than modern cultivars, since past selection of germplasm under fertilized conditions may have reduced the frequency of genes that promote mycorrhizal associations (Hetrick *et al.* 1992).

The objectives of this study were 1) to determine if levels of mycorrhizal colonization vary significantly among a group of 26 hard red spring wheat lines under organic management; 2) to establish whether significant differences in AMF colonization occurred between parental combinations under organic management; and 3) to determine if AMF colonization was related to wheat genotype date of release.

2.3 Materials and Methods

2.3.1 Site Description and Treatments

The research was conducted at the Glenlea Long-Term Rotation in Glenlea, Manitoba in 2007 and at the Organic Crops Field Laboratory on the Ian N. Morrison Research Farm in Carman, Manitoba in 2008. Glenlea is located 20 km south of Winnipeg, Manitoba (N 49,38,8 / W 97,8,6). Glenlea accumulates approximately 1,650 GDD (Base 5°C), averages 110 frost free days (MAFRI 1999) and receives approximately 531 mm of precipitation annually (Environment Canada 2004). The soil at Glenlea is a Rego Black Chernozem clay soil of Red River or Scantenbury series (Michalyna 1970). Carman is located 70 km south west of Winnipeg, Manitoba (N 49,29,57 / W 98,0,2). Carman averages the same GDD as Glenlea (1,650), but averages more frost free days (120) (MAFRI 1999) and receives less precipitation annually (483 mm) (Environment Canada 2004). The soil at Carman is an Orthic Black Chernozem fine sandy loam soil of the Hibsin series (Mills and Haluschak 1993).

The Glenlea Long-Term Rotation was established in 1992 and is Canada's oldest organic-conventional cropping comparison study. At the Glenlea site, this research was conducted under organic management in the forage-grain rotation on land that had been under organic management since the rotation was established. The crops grown in the forage-grain rotation are: flax (*Linum usitatissumum* L.) – alfalfa (*Medicago sativa* L.) – alfalfa – wheat (*Triticum aestivum* L.). The present study was seeded into a portion of one of the flax plots and the previous crop was wheat (Table 2.1). As shown in Table 2.1, soil P was high at Glenlea as a result of composted manure being applied to this plot

in 2000. The Organic Crops Field Laboratory consists of 4 hectares of land that have been under organic management since 2002. The land is divided into six parcels to accommodate the six-year crop rotation: oat/pea green manure – oilseed – cereal – oat/pea green manure – cereal – pulse crop. This trial was seeded within the cereal area following a green manure crop (Table 2.1). The research was conducted following organic principles and practices, including avoiding the use of synthetic fertilizers and pesticides.

Table 2.1 Soil association, texture, soil nutrient status, and previous crop history at each experimental site.

Site	Soil Association	Texture	Previous Crop	Sampling Depth (cm)	N*	P**	K	S***	pH
					kg/ha (ppm)				
Glenlea	Red River or Scantenbury	*clay*	Wheat	0-15	73 (18)	85 (21)	2463 (580)	30 (7)	7.8
				0-60	121 (30)	147 (36)	4458 (1050)	61 (14)	
Carman	Hibsin	*fine sandy loam*	Green Manure	0-15	113 (28)	50 (33)	194 (160)	34 (6)	5.4
				0-60	187 (46)	73 (48)	359 (296)	133 (24)	

*Nitrate-N, **Olsen-P, ***Sulfate-S

The experimental design was a randomized complete block, with four replicates. The treatments used in this study were 19 parental lines (Table 2.2) and seven check cultivars (Table 2.3), for a total of 26 treatments. All entries were spring growth habit except for Cheyenne. The 19 parental lines were chosen because they have been previously used as parents in Agriculture and Agri-Food Canada's wheat breeding program and genetics program. The parents were used in 12 different crosses, each used to produce a doubled haploid or recombinant inbred genetic population. The seven checks included heritage and modern cultivars of spring wheat. The wheat was hand

planted into 1 m rows. Sixty seeds were planted per 1 m row at a depth of approximately 4 cm. Rows were spaced 15 cm apart, and a border row of 5602HR was planted around the trial area.

Table 2.2 Parental lines included in this study and date of release (based on actual date of release or 10 years after the cross was made).

Parental line	Assumed Release Date
00H01*F57	2010
96B42-E3C	2006
98B25-AU5A01	2008
98B50-H4D	2008
99B60-EJ2G	2009
AC Domain	1992
AC Karma	1994
CDC Alsask	2004
Alsen	2000
Glenlea	1972
Infinity	2004
Kane	2006
AC Majestic	1995
ND744	2004
Opata M85	1985
RL4452	1983
SC8021V2	1991
Somerset	2005
Synthetic	1995

Table 2.3 Checks included in this study and date of release.

Checks	Release Date
CDC Teal	1991
Cheyenne	1933
Marquis	1911
McKenzie	1997
Neepawa	1969
Red Fife	1885*
Selkirk	1955

(* Introduced into Canada around 1885)

2.3.2 Root Sampling

The root samples were collected for analysis of mycorrhizal colonization. Four root samples were collected from each plot in both 2007 and 2008. In 2007, samples were collected on June 18, 321 GDD after seeding, while in 2008 samples were collected on July 2, 368 GDD after seeding (Table 2.4). The growth period was extended in 2008 to allow for a longer period in which AMF could colonize the wheat roots. A tulip bulb planter (7 cm diameter) was placed over two wheat plants and pressed down in the soil to a depth of approximately 10 cm. The four samples from each plot were combined and washed together to form one sample per plot. The root samples were soaked in buckets for 10-15 minutes to loosen the soil prior to being washed by hand. After removing the roots from the soil, only roots that were attached to a wheat plant were stored for later analysis. The clean root samples from each plot were stored in 70% ethanol solution.

Table 2.4 Environmental conditions during crop growth at each experimental site (MAFRI 2008).

Site Location	Year	Seeding Date	Sampling Date	Precipiation (mm)	Heat (GDD)
Glenlea	2007	May 17	June 19	161.3	321
Carman	2008	May 20	July 2	92.8	368

2.3.4 Mycorrhizal Colonization

Roots were rinsed with reverse osmosis (RO) water and placed into Petri dishes with a thin layer of RO water for ease of viewing. Using forceps all weed seeds, soil, and other plant material not including the wheat roots were removed from the sample. The remaining roots were then cut into 1-2 cm pieces. The clean samples were returned to

their bottles and clean 70% ethanol was added. A subsample of the root sections was rinsed with RO water and transferred to beakers. A 10% potassium hydroxide (KOH) solution was added to the beakers, and the roots were then autoclaved for 8 minutes to clear them of their internal structures. After clearing, the root segments were rinsed with RO water and transferred into small glass Petri dishes. Chlorazol Black E (0.05% w/v in 1:1:1 lactic acid, glycerol and water) solution was added and the dishes were placed in a 90°C oven for 90 minutes. The excess stain was removed from the roots by rinsing four times with deionised water. Glycerol was then added to the dishes and left to destain the roots for approximately 48 hours.

The stained roots were prepared for viewing by mounting approximately 25 root fragments on a glass slide and covering with a glass cover slip. Slides were viewed under a light microscope set at 250x magnification, or 400x magnification when further identification of AMF structures was required. Slides were scored using the magnified intersections method of McGonigle *et al.* (1990). The slide was moved up, down and across at regular intervals. Each intersection of the vertical cross-hair in the ocular lens and a root was scored as root, hyphae, or arbuscule. The intersection was scored as arbuscule when the crosshair intersected with one or more arbuscules or as hyphae when the crosshair intersected with AMF hyphae (distinguished by the presence of arbuscules). However, when both hyphae and arbuscules were intersected by the crosshair on the same root, only the arbuscules were scored. When the number of root sections evaluated reached 100, counting was stopped. Percent total colonization (arbuscular and hyphal colonization) and percent arbuscular colonization was then determined.

2.3.5 Statistical Analysis

Arbuscular and total colonization were compared using the Statistical Analysis Software program (SAS Institute 2001). Prior to the analysis, the data were tested for homogeneity of variance (Bartlett's) and for normality (Shapiro-Wilk). Data were analyzed using the mixed procedure (Proc Mixed, SAS Institute Inc.). The protected Least Significance Difference (LSD) test was used to determine significant differences ($P < 0.05$) among treatments. Contrasts were used to establish differences between parental combinations. Data was analyzed for linear correlations using the correlation procedure (Proc Corr, SAS Institute Inc.).

2.4 Results and Discussion

The first objective of this study was to determine if levels of arbuscular mycorrhizal colonization vary among wheat genotypes used in wheat crosses previously developed for genetic experiments. Percent root colonization by hyphae did not differ among wheat lines at Glenlea and Carman; however, percent arbuscular and total colonization differed significantly among the parental lines at Glenlea ($P < 0.05$) (Table 2.5). At Carman there were no significant differences in colonization among the parental lines. Arbuscular colonization ranged from 14.5 – 31.5 percent at Glenlea and 5.5 – 14 percent at Carman, while total colonization ranged from 20.25 – 40 percent at Glenlea and 11 – 21.5 percent at Carman (Table 2.5).

Significant effects of genotype on arbuscular colonization were demonstrated in 2007 at Glenlea (Table 2.5). Hyphae function in the uptake and translocation of mineral nutrients from the soil solution to the roots while arbuscules facilitate nutrient exchange

between the fungal symbiont and the plant cells (Peterson *et al.* 2004). Arbuscules are thought to be important for the exchange of nutrients due to the presence of ATPase activity and inorganic P (Pi) transporters in arbuscule-containing host cells (Ezawa *et al.* 2002) and due to their large surface area available for nutrient exchange (Peterson *et al.* 2004). Arbuscular colonization is considered more indicative of overall activity of AMF than hyphal colonization.

At Carman, no significant differences in hyphal, arbuscular or total colonization were observed (Table 2.5). Percent arbuscular and total colonization was approximately twice as high at Glenlea as at Carman. These differences between site years may be attributed to differing soil, environmental and management conditions at the two sites, with the most important difference being length of time under organic management. The Glenlea and the Carman sites have been managed organically for 16 and six years, respectively. Comparison studies elsewhere have shown that organically managed systems have higher soil microbial biomass and activity compared to their conventional counterparts (Mäder *et al.* 2002, Pimentel *et al.* 2005). Longer-term organic systems would be expected to have greater and differing soil biological diversity, which may be the reason for greater colonization at the older site, Glenlea.

Nutrient concentrations, based on soil test results at the time of seeding, were found to differ between locations. Total sodium bicarbonate extractable P was 147 kg/ha and 73 kg/ha at Glenlea and Carman, respectively. The high soil P level at Glenlea was not expected since 16 years of organic management would reduce plant available phosphorus concentrations, especially in the forage-grain rotation where plant matter is removed as forage. In a survey of organic farms throughout the Prairies, the average P

level on organic farms was found to be substantially lower than the average P level on conventional farms (Entz *et al.* 2001). High available soil P at Glenlea was the result of a composted manure soil amendment in 2000.

Differences in colonization between the two sites cannot be explained by soil test results. According to the literature, a higher level of soil P should result in lower colonization: if the plant has access to P, it has less need for AMF. It is known that a low to moderate supply of P is required for arbuscule formation. Arbuscular formation is inhibited at both a high and low P supply; although, hyphal colonization can still occur (Ezawa *et al.* 2002). Lower levels of Pi (plant available P) promote colonization by AMF and provide the crop access to organic P (Po), a residual form of P which is not measured by traditional soil tests (Bücking and Shachar-Hill 2006). The higher soil P level at Glenlea compared to Carman should have resulted in lower colonization at Glenlea; however the reverse was true.

Site differences may be attributed to environmental conditions. Although the growth period of the wheat was extended in 2008 at Carman to allow for higher levels of mycorrhizal colonization, the growing period GDD was similar between the two sites due to a cool spring in 2008. Carman also received less precipitation than Glenlea during the growing period prior to harvesting the wheat roots. The combination of these two factors may have resulted in lower colonization at Carman and no differences between lines.

A second study objective of this study was to determine if pairs of wheat parental lines used to create existing genetic populations differed in level of AMF colonization. Contrast analysis of arbuscular and total colonization showed that at the Glenlea site, four combinations of parents were found to have significant differences in both arbuscular and

total mycorrhizal colonization ($P < 0.05$) (Figure 2.1; Appendix I, Table 6.1 and 6.2). No differences between parental lines were observed at Carman. The four parental combinations with significant differences in arbuscular and total colonization at Glenlea are identified for future work in which the colonization levels of the doubled haploid populations created from the parental lines can be screened to elucidate inheritance of mycorrhizal dependency.

The final study objective was to test whether date of wheat cultivar release affected level of AMF colonization. This analysis was performed to test the hypothesis that mycorrhizal dependency has been lost during the development of modern wheat cultivars due to selection in environments not conducive to AMF colonization. Regression analysis showed no significant ($P > 0.05$) trend between estimated release date and arbuscular and total colonization (data not shown). This observation contradicts those by Hetrick *et al.* (1995) and Zhu *et al.* (2001), who found older cultivars to benefit more from AMF than modern cultivars. One limitation of the present study was the limited representation of older wheat cultivars. Although this study included lines with estimated release dates ranging from 1885 to 2010, only four of those lines were released prior to 1960. It is possible that no significant trend in release date and colonization was found since the majority of the cultivars were released in the last 25 years. It is also important to consider that the current study only measured percent colonization and not the benefit that the cultivars are receiving from colonization. To conduct a study on the benefit of AMF colonization plant growth would have to be compared in the presence and absence of AMF.

Table 2.5 Mean percent arbuscular colonization and total colonization for the 26 lines tested at Glenlea in 2007 and Carman in 2008.

Line	Assumed Release Date	Arbuscular Colonization		Total Colonization	
		Glenlea	Carman	Glenlea	Carman
00H01*F57	2010	23.25abcde[y]	8[z]	30.25abcdefg[y]	16.25[z]
99B60-EJ2G	2009	15.75e	11.25	22.25fg	21.5
98B50-H4D	2008	29abc	10.25	39.75ab	17.25
98B25-AU5A01	2008	19.5cde	6	27.75bcdefg	13.75
Kane	2006	22.75abcde	11.25	31.25abcdefg	18
96B42-E3C	2006	17.25de	13	24.75efg	25
Somerset	2005	26.75abcd	11.5	32.5abcdef	18.75
Infinity	2004	31.5a	10.25	40a	17
CDC Alsask	2004	21.25bcde	8.5	27.5cdefg	15.5
ND744	2004	19.25cde	11.5	27.25cdefg	17.75
Alsen	2000	15.75e	9	22.75fg	19
McKenzie	1997	22.5abcde	12	31.25abcdefg	20.75
AC Majestic	1995	16.75e	7.25	23.25efg	14
AC Karma	1994	30.5ab	7.75	39abc	15
AC Domain	1992	23.5abcde	12.25	30abcdefg	20
CDC Teal	1991	20cde	14	27.25cdefg	21.25
SC8021V2	1991	16.5e	11.25	23.25efg	17.5
Opata	1985	16.25e	11.75	22.75fg	20.75
RL4452	1983	14.25e	9	20.75fg	17
Glenlea	1972	15.25e	8.25	23efg	12
Neepawa	1969	20.25cde	11	26.75defg	17.25
Selkirk	1955	14.5e	9.25	20.25g	16.75
Cheyenne	1933	28.75abc	10.25	37.5abcd	15.5
Marquis	1911	15.5e	5.75	21.75fg	11.75
Red Fife	1885	17.75de	12.25	22.5fg	20
Synthetic	1995	23.5abcde	8.25	35abcde	16.25

[y]Means within the same site year followed by the same letter within a column are not significantly different (P>0.05) according to Fischer's protected LSD.

[z]Means are not significantly different (P>0.05) according to Fischer's protected LSD.

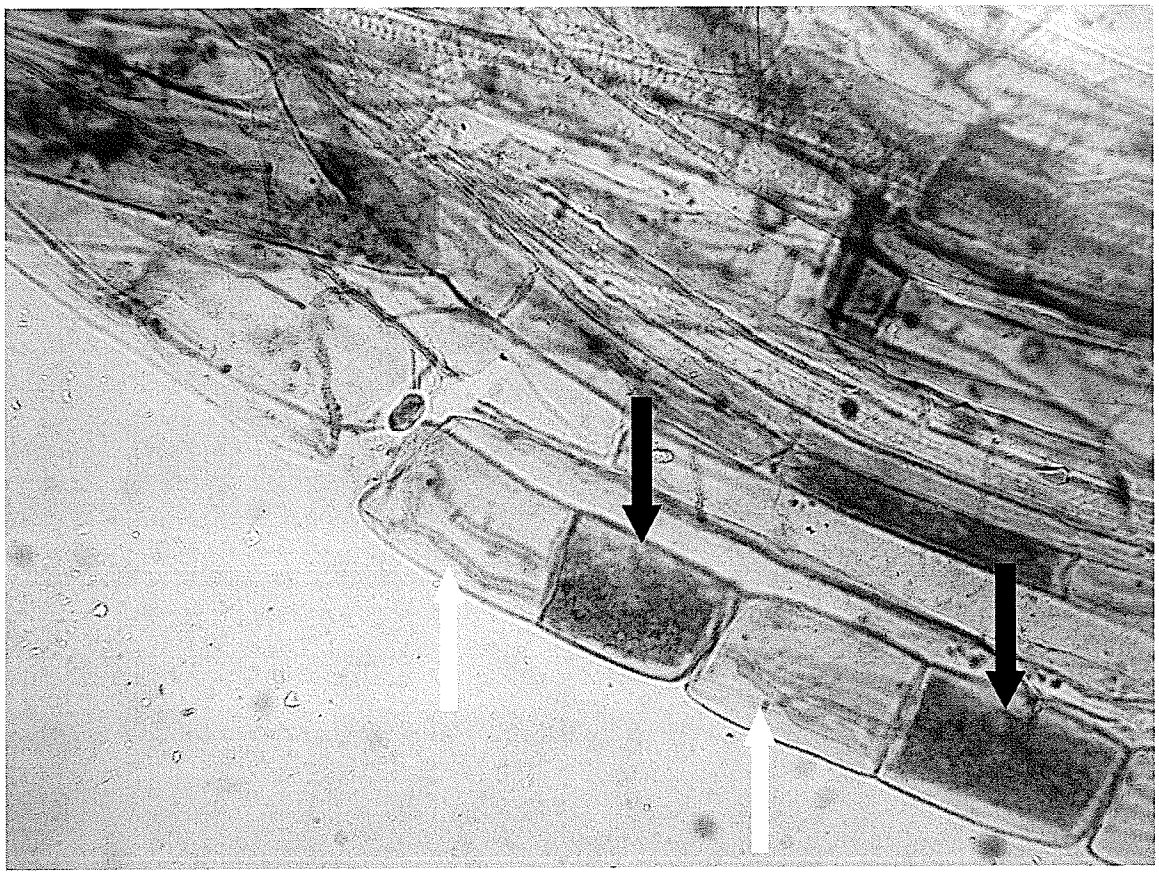

Figure 2.1 Arbuscules (black arrows) and hyphae (white arrows) of arbuscular mycorrhizal fungi in wheat roots under 250x magnification.

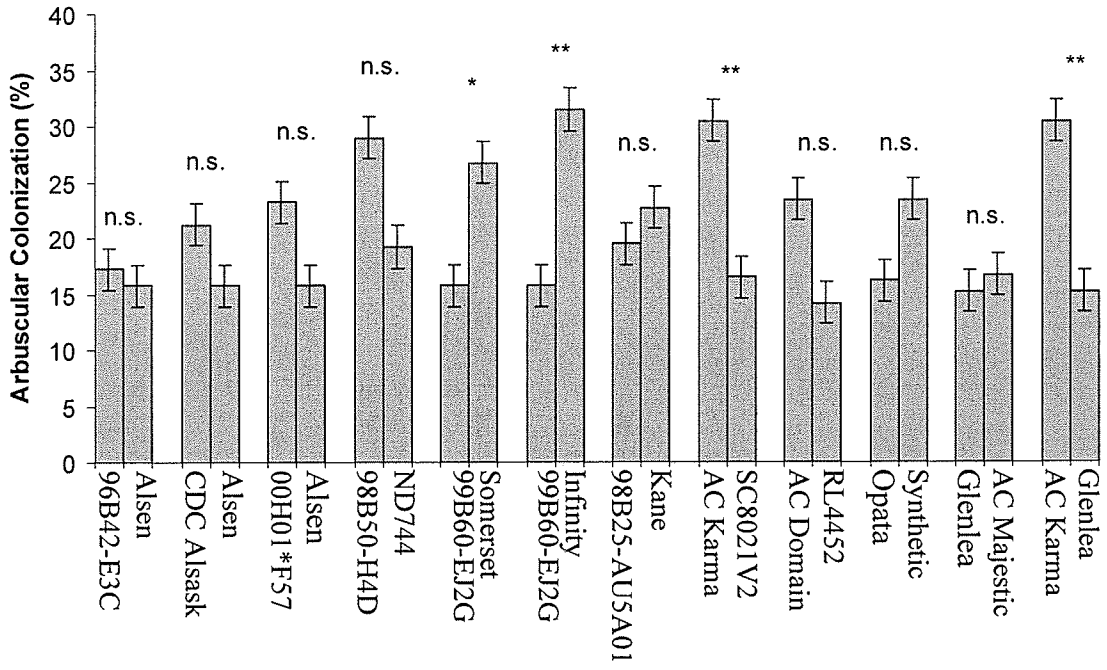

Figure 2.2 Percent arbuscular colonization of the parental lines at Glenlea. The parental lines are shown beside the line they had previously been crossed with. Four of the parental combinations were found to have significant differences in arbuscular colonization (*P<0.05, **P<0.01), while the remaining 8 parental combinations did not have significant (n.s.) differences in arbuscular colonization. The error bars represent ±1 standard deviation of four replicate values. The CV for this experiment was 34.

2.5 Conclusions

AMF colonization was found to differ significantly between the group of 26 hard red spring wheat lines at Glenlea, but not at Carman. Land management, including length of time under organic management and environmental conditions were most likely responsible for the lack of significant differences at the Carman location. Higher colonization was found at the Glenlea location compared to the Carman location, which was unexpected due to relatively higher soil P at Glenlea.

This study has identified significant differences in arbuscular mycorrhizal colonization in pairs of parental lines that may be appropriate for screening in the future to investigate the inheritance of mycorrhizal dependence. There were four pairs of parental lines at the Glenlea location that had significant differences in colonization.

Wheat cultivar date of release was not found to affect level of AMF colonization. The present study could not confirm or deny the hypothesis that mycorrhizal dependency has been lost during the development of modern wheat cultivars. More research needs to be conducted to determine the benefit the cultivars are receiving from AMF colonization with a better representation of older wheat cultivars.

3. MYCORRHIZAL COLONIZATION IN HERITAGE AND MODERN WHEAT CULTIVARS

3.1 Abstract

Arbuscular mycorrhizal fungi (AMF) are one of the most important soil organisms for assisting in nutrient uptake from soil, particularly phosphorus (P) in organic cropping systems. Mycorrhizal dependence varies between wheat genotypes. Past selection of wheat genotypes under fertilized conditions may have reduced the frequency of genes that promote mycorrhizal associations in modern cultivars; therefore, older cultivars may form stronger associations with AMF. This study compared AMF colonization, yield, and tissue P between five modern and five heritage spring wheat cultivars. The research was carried out at two organically managed research sites located in Southern Manitoba. Significant differences in arbuscular and total colonization occurred at the Carman research site, but not Glenlea. At two site-years (Carman in 2007 and 2008) modern cultivars had higher levels of colonization than heritage cultivars. Tissue P concentration did not differ between varietal groups. At Carman in 2007 modern cultivars yielded significantly higher than heritage cultivars; no differences in yield were found at the other site years. Yield differences between cultivars confirmed that organic farmers should be selecting different cultivars than conventional farmers. It cannot be conclusively stated that modern or heritage cultivars are better suited to organic management based on these results. Further work is needed to establish the benefit the cultivars are receiving from AMF colonization, since percent colonization is limited as a measure of mycorrhizal dependence.

3.2 Introduction

Arbuscular mycorrhizal fungi (AMF) form mutually beneficial associations with the roots of most herbaceous plants (Dalpé 1997). In low external input and organic systems an important benefit of AMF is the increased capability to take up mineralized soil nutrients (Mäder *et al.* 2000). This is especially true for P, which is often deficient in organic systems (Entz *et al.* 2001). P is immobile in soil and AMF are able to take up P more efficiently and transport P over longer distances than the plant root system (Ezawa *et al.* 2002). The increased uptake of P by AMF is attributed to an increased surface area for nutrient absorption due to the presence of the extraradical fungal hyphae (Bücking and Shacher-Hill 2005). Although the main role of hyphae is thought to be mineral nutrient uptake and translocation, arbuscules are thought to be the site of nutrient exchange (Peterson *et al.* 2004), and may be better indicators of P acquisition of colonized plants. Other means by which AMF increase the uptake of P include modification of the root environment, a lower threshold concentration for P absorption (Bolan 1991) and the production of organic acids and phosphatase enzymes that catalyse the release of P from organic complexes (Bücking and Shacher-Hill 2005).

The increased acquisition of mineral nutrients may be the most important role of AMF; however, the fungi also improve soil aggregate stability (Hamel 2004), protect the host plant from environmental stress (Daplé 1997) and enhance the host plant resistance to specific disease-causing organisms (Azcón-Aguilar and Barea 1996; Fitter and Garbaye 1994). Colonization can be promoted by avoiding the use of fungicides (Siqueira *et al.* 1985) and herbicides (Mäder *et al.* 2000), minimal rotation to non-host crops (Entz *et al.* 2004) and maintaining ground cover with mycorrhizal cover crops

(Kabir and Kodie 2002). The principles of organic agricultural production either require or promote these beneficial practices; therefore, it is no surprise that AMF colonization is shown to be higher in organically managed farming systems compared to conventional farming systems (Douds *et al.* 1995; Entz *et al.* 2004; Mäder *et al.* 2000).

The general principles of organic agricultural production are to protect the environment, maintain long-term soil fertility and to maintain biological diversity within the system (CGSB 2006). Organic production prohibits the use of synthetic fertilizers and pesticides; therefore, the maintenance of soil fertility is achieved by promoting optimal biological activity in the soil, conservation of soil resources, appropriate crop selection and crop rotation (CGSB 2006). The selection of crop cultivars that have strong associations with AMF may be important in providing adequate soil fertility to the crop.

Mycorrhizal dependence is thought to be a genetic trait that is expressed in some wheat genotypes (Hetrick *et al.* 1992). Older cultivars may form stronger associations with mycorrhiza than modern cultivars, since past selection of wheat genotypes under fertilized conditions may have reduced the frequency of genes that promote mycorrhizal associations (Hetrick *et al.* 1992). Arbuscule formation is inhibited at a high P supply, but hyphal colonization can still occur (Ezawa *et al.* 2002). Wheat selected under fertilized conditions may not have had to form strong associations with AMF.

The objectives of this study were 1) to determine if selecting wheat cultivars under conventional management (i.e., modern cultivars) has reduced their ability to colonize with AMF; 2) to determine if percent tissue P is a good indication of AMF colonization in wheat; and 3) to compare heritage and modern wheat cultivars on the basis of grain yield under organic management. Arbuscular and hyphal colonization,

tissue P, and yield were compared between five modern and five heritage cultivars at two organically managed locations, the Glenlea Long-Term Rotation and the Organic Crops Field Laboratory. It is hypothesized that modern cultivars will have lower levels of AMF colonization than heritage cultivars, and that heritage cultivars will yield higher than modern cultivars under organic management.

3.3 Materials and Methods

3.3.1 Site Description and Treatments

Field plots were located at the Glenlea Long-Term Rotation and at the Organic Crops Field Laboratory on the Ian N. Morrison Research Farm in Carman, Manitoba in 2007 and 2008. Glenlea is located 20 km south of Winnipeg, Manitoba (N 49,38,8 / W 97,8,6), accumulates approximately 1,650 GDD (Base 5°C), averages 110 frost free days (MAFRI 1999), and receives approximately 531 mm of precipitation (Environment Canada 2004) annually. The soil at Glenlea is a Rego Black Chernozem clay soil of the Red River or Scantenbury series (Michalyna 1970). Carman is located 70 km south west of Winnipeg, Manitoba (N 49,29,57 / W 98,0,2), averages the same GDD as Glenlea (1,650), but averages more frost free days (120) (MAFRI 1999) than Glenlea. On average Carman receives less precipitation annually (483 mm) than Glenlea (Environment Canada 2004). The soil at Carman is an Orthic Black Chernozem fine sandy loam soil of the Hibsin series (Mills and Haluschak 1993).

The Glenlea Long-Term Rotation was established in 1992 and is Canada's oldest organic-conventional cropping comparison study. At the Glenlea site, this research was conducted on land that had been under organic management since the rotation was

established. The Organic Crops Field Laboratory consists of 4 hectares of land that have been under organic management since 2002. The land is divided into 6 parcels to accommodate the six-year crop rotation: green manure – oilseed – cereal – green manure – cereal – pulse crop. This trial was seeded into the cereal section of the rotation following a green manure crop in both 2007 and 2008. See tables 3.1 and 3.2 for more information on the experimental sites.

Table 3.1 Soil association, texture, soil nutrient status, and previous crop history at each experimental site.

Site Location	Year	Soil Association	Texture	Previous Crop	Sample Depth (cm)	N*	P**	K	S***	pH
						kg/ha (ppm)				
Glenlea	2007	Red River or Scantenbury	*clay*	fallow	0-15	113 (13)	36 (4)	1460 (164)	20 (2)	7.2
					0-60	361 (40)	77 (9)	4537 (510)	61 (7)	7.3
Glenlea	2008	Red River or Scantenbury	*clay*	green manure	0-15	143 (37)	62 (40)	745 (561)	44 (11)	6.6
					0-60	220 (57)	89 (57)	1258 (947)	76 (19)	7.4
Carman	2007	Hibsin	*fine sandy loam*	green manure	0-15	41 (12)	102 (27)	996 (282)	24 (6)	6.1
					0-60	55 (16)	155 (41)	1399 (396)	45 (11)	7.9
Carman	2008	Hibsin	*fine sandy loam*	green manure	0-15	112 (30)	60 (37)	445 (275)	18 (4)	5.3
					0-60	168 (15)	83 (51)	618 (382)	27 (6)	7.2

*Nitrate-N, **Olsen-P, ***Sulfate-S

Table 3.2 Environmental conditions during the growing season (May 1 – August 31) at each experimental site (MAFRI 2008), and long-term averages (Environment Canada 2004).

Research Site	Precipitation			Heat		
	Long-term Average[1]	2007	2008	Long-term Average[1]	2007	2008
	------ *mm* ------			--- *Growing Degree Days* ---		
Carman		271	210		1,467	1,239
Glenlea	304	247	280	1,455	1,455	1,213

[1]30-year long-term averages.

The design of this experiment was a randomized complete block, with four replicates. Ten treatments were used including 5 modern (1991-2006) and 5 heritage (1885-1969) cultivars of wheat (Table 3.3). Plot dimensions were 0.61 x 3 m at Glenlea, 0.61 x 4.5 m at Carman in 2007, and 0.61 x 4 m at Carman in 2008.

Table 3.3 Wheat cultivars included in this study, including date of cultivar release for commercial production.

Cultivar	Release Date
FBC Dylan	2006
5602 HR	2004
McKenzie	1997
AC Barrie	1994
CDC Teal	1991
Neepawa	1969
Selkirk	1955
Mida	1944
Marquis	1911
Red Fife	1885*

(* Introduced to Canada around 1885)

3.3.2 Seeding

Prior to seeding the ground was cultivated to provide a smooth seed bed. Plots were seeded using a disk drill (Fabro Industries, Swift Current) at a rate of 333 kernels/m^2 to account for over seeding. Field trials were conducted on organic land, and

were managed following organic principles and practices throughout the experimental period.

Table 3.4 Schedule of field operations.

Operation	Carman 2007 (GDD)*	Glenlea 2007 (GDD)	Carman 2008 (GDD)	Glenlea 2008 (GDD)
Seeding	May 10	May 3	May 6	May 8
Root Sampling	June 6 (198)	June 5 (263)	June 16 (260)	June 18 (273)
Biomass Collection	July 24 (867)	July 24 (937)	July 14 (566)	July 14 (606)
Harvest	August 22	August 30	August 19	August 36

GDD base 5 °C

3.3.3 Root Sampling

Root samples were collected for analysis of mycorrhizal colonization at approximately the four leaf stage in 2007 and 2008. Root samples were collected at a later date in 2008 to allow for a longer period in which AMF could colonize the wheat roots, but due to a cool spring the GDD to sampling did not differ much between sites and years (Table 3.4). See page 38 for more information on root sampling.

3.3.4 Mycorrhizal Colonization

See page 38 for a thorough description of the assessment of mycorrhizal colonization.

3.3.5 Biomass Sampling

Above ground wheat biomass was collected for analysis of phosphorus concentration. Biomass samples were collected on July 24 in 2007 at approximately the

soft dough stage and on July 14 in 2008 at booting (Table 3.4). Samples were collected from two 1 meter lengths of row. Plant tissue samples were stored in paper bags and sorted to remove plant material that was not wheat. Samples were dried in an oven at 50°C (in 2008, 70 °C degrees) for 48 hours and then ground into a fine powder. Plant tissue phosphorus concentration was conducted by Bodycote Laboratories (Winnipeg, MB) by acid digest followed by analysis on an Inductive Coupled Plasma Spectrophotometer (ICP).

3.3.6 Yield

Prior to harvesting, plots were trimmed and measured in order to calculate yield. In 2007 wheat was harvested at Carman with a Wintersteiger plot combine and at Glenlea with a Hege plot combine. In 2008 wheat was harvested at Carman and Glenlea using a Wintersteiger (Laval, QC) plot combine. Samples were dried on a forced air drying bed then cleaned. In 2007 chaff and weed seeds were removed by putting samples through an air blower followed by shaking through a #5 rectangular mesh screen. In 2008, samples were cleaned with an air blower to remove chaff and then put through a dockage tester to remove weed seeds (Carter Dockage Tester 31624/W-3301). The dockage tester was equipped with a no. 1 riddle, 9/64 tri double cut screen and a S-909 S1/2 164 R.086 screen. Clean weights were measured, and yield was calculated based on plot area.

3.3.7 Statistical Analysis

Arbuscular and total colonization, tissue phosphorus and yield were compared using the Statistical Analysis Software program (SAS Institute 2001). The data were first

tested for homogeneity of variance (Bartlett's) and for normality (Shapiro-Wilks) and then analyzed using the mixed procedure (Proc Mixed, SAS Institute Inc.). The protected Least Significance Difference (LSD) test was used to determine significant differences ($P < 0.05$) among treatments. Contrasts were used to establish differences between modern and heritage cultivars. Data was analyzed for linear correlations using the correlation procedure (Proc Corr, SAS Institute Inc.).

3.4 Results and Discussion

3.4.1 Hyphal and Arbuscular Colonization

The first objective of this study was to determine if selecting wheat cultivars under conventional management has reduced their ability to colonize with AMF compared with older cultivars. Hyphal and arbuscular colonization data were used to investigate the hypothesis that lines selected under fertilized conditions have a weaker association with AMF. Site years differed in colonization with wheat at Carman showing higher colonization levels than wheat at Glenlea. This was especially evident in 2008 when colonization was extremely low at Glenlea (Table 3.6). Differences in AMF colonization between sites and years may be attributed to differing soil, environmental and management conditions at the two sites. For example, higher colonization in 2007 may be a result of warmer temperatures compared to what was experienced in 2008. While the growth period in 2008 was extended to allow for higher levels of mycorrhizal colonization; cooler spring conditions and less accumulated GDD throughout the 2008 season (Table 3.2) resulted in slower morphological development in 2008.

Another possible reason for differences between sites may be crop rotation history. The previous crop at Carman was a green manure whereas Glenlea was left fallow. During fallow periods, the energy available for the growth of AMF decreases due to the absence of a host crop (Boswell *et al.* 1998). However, the very low AMF colonization at Glenlea in 2008 cannot be easily explained. Many factors can affect AMF germination including dormancy, pH, temperature, moisture, aeration, light and radiation, inorganic ions, microorganisms, oxidizing agents, antibodies and pesticides (Siquera *et al.* 1985).

Percent colonization by hyphae did not differ among any of the treatments, while arbuscular and total colonization differed between treatments at only one location (Appendix I, Tables 6.3-6.6). At Carman in 2008, FBC Dylan, 5602HR and AC Barrie had significantly higher arbuscular colonization than the other seven cultivars (Table 3.6). Fewer differences in hyphal colonization may be expected since the arbuscules are thought to be the site of nutrient exchange (Peterson *et al.* 2004), and therefore may be considered to be more indicative of the overall activity of AMF than hyphae. Hyphae function in the uptake and translocation of mineral nutrients from the soil solution to the roots, while arbuscules facilitate nutrient exchange between the fungal symbiont and the plant cells (Peterson *et al.* 2004). Arbuscules are thought to be important for their large surface area available for nutrient exchange (Peterson *et al.* 2004) and for the exchange of nutrients due to the presence of ATPase activity and inorganic P (Pi) transporters in arbuscule-containing host cells (Ezawa *et al.* 2002).

Root weights were not found to differ significantly between treatments (data not shown) or between modern and heritage cultivars (Table 3.7). Although there were no

significant differences in root weights, it is important to consider that a dilution effect may have occurred. Two cultivars with differing root weights could have had the same number of arbuscules in total, but fewer arbuscules may have been counted in the cultivar with a larger root system.

Contrast analysis of arbuscular and total colonization showed that the five heritage and five modern cultivars performed differently at Carman, but not at Glenlea. At Carman in 2007 and 2008 modern wheat cultivars (1991-2006) were found to have significantly higher arbuscular and total colonization than the heritage wheat cultivars (1886-1969) (Table 3.7). These results agree with Zhu *et al.* (2001) who found modern wheat cultivars had higher AMF colonization than the older cultivars. Arbuscular colonization was found to be dependent on release date only at Carman in 2007, with higher colonization observed in the most recently released cultivars (Figure 3.1). These results show that the hypothesis that modern cultivars will have lower levels of AMF colonization than heritage cultivars is incorrect.

Although modern cultivars were found to have higher levels of AMF colonization than heritage cultivars at two site years, this may not be the best way to identify the cultivars most efficient at taking up P. Hetrick *et al.* (1993) found no relationship between the degree of root colonization and the degree of benefit from AMF symbiosis. A better measure of the AMF benefit would be to look at the cultivars response to mycorrhizal colonization. Response to colonization would be measured by comparing plant growth in the presence and absence of AMF. Some researchers have found that efficiency or response to AMF is greater in older cultivars than modern cultivars (Hetrick *et al.* 1993; Zhu *et al.* 2001), supporting the theory that mycorrhizal dependence has been

bred out of modern wheat cultivars. Hetrick *et al.* (1995) found stronger mycorrhizal dependence in cultivars released before 1975 than in modern semi-dwarf cultivars released after 1975. The study by Zhu *et al.* (2001) found that although the older cultivars (released before 1956) had lower percent colonization than the modern cultivars, the older cultivars were generally more responsive to AMF than modern cultivars. More research is needed to assess the benefit the cultivars are receiving from mycorrhizal symbiosis.

3.4.2 Tissue Phosphorus

A second study objective was to determine if plant tissue P concentration differed between modern and heritage cultivars and whether plant P uptake may be a good indication of AMF colonization in wheat. Significant differences in tissue P concentration were observed across treatments at all site years except Carman in 2008. In 2007 AC Barrie was observed to have the highest tissue P concentration, and McKenzie the lowest (Table 3.5); however, at Glenlea in 2008 AC Barrie had the lowest tissue P concentration (Table 3.6). When contrasts were performed on the tissue P concentration of the heritage and modern cultivars no differences were observed between the two groups at any location (Table 3.7). The observed differences did not have a consistent trend across locations so it is unlikely that the suitability of cultivars for organic management can be chosen based on tissue P concentration.

Tissue P was not found to be a good indication of mycorrhizal colonization in wheat. At three of four sites no significant correlation with colonization was observed (Table 6.7 – 6.9). At the final site (Carman in 2008), the correlation between tissue P

concentration and both arbuscular and total colonization was actually found to be negative (Table 6.10). AMF facilitate the uptake of P into the plant, and increased colonization was expected to increase tissue P. P uptake per unit area of root was calculated to be 60 times greater with four hyphal entry points per millimeter of root length than if a symbiotic relationship had not been formed between the plant and mycorrhizal fungi (Kodie and Mosse 2004).

Tissue P concentration was negatively correlated to plant biomass in all years except for Glenlea in 2007 (Appendix I, Tables 6.7-6.10). More biomass production resulted in lower tissue P concentration. There was also a positive correlation between yield and biomass at all site years except for Carman in 2007; although, yield and tissue P were observed to be negatively correlated only at the 2007 sites (Appendix I, Tables 6.7-6.10). The negative correlation between tissue P and yield, and tissue P and biomass show that there is a dilution effect. An increased AMF benefit may allow the plants root system to take up more P, which in turn may allow the plant to grow bigger. The amount of P that the plant took up would then be dispersed throughout a presumably larger plant, causing a dilution effect.

Across all site years, tissue P concentrations were found to be low to marginal (Table 3.5 and 3.6) according to cereal crop nutrition requirements (Alberta Agriculture 1998). Soil P was found to be adequate and yields did not appear to suffer as a result of P. High tissue P would be beneficial since P in plant tissue results in seed P. Zhu and Smith (2001) found that high seed P reserves increased plant biomass compared to plants grown from seeds with low P reserves. Although tissue P was not positively correlated

with yield and biomass, P in the plant tissue is transferred to the seed, which is beneficial for the next crop grown.

3.4.3 Yield

The final objective of this study was to determine if heritage wheat cultivars yield more under organic management than modern cultivars that were selected under conventional management. Yield differences were observed between sites and years, with Carman yielding marginally higher than Glenlea in both years (Table 3.5 and 3.6). Both sites yielded more in 2008 than in 2007. Averaged across locations 2007 yields were 56% of 2008 yields. The yield differences between sites and years can be explained by differing soil and environmental conditions. Low yields at Glenlea in 2007 were attributed to the very weedy location, most likely due to prior management practices. The more favourable previous crop of a green manure in 2008 would have allowed for a higher yield in that year compared to the previous crop of tilled fallow in 2007. Low yields in 2007 at Carman were attributed to N deficiency at that location (Table 3.1); while in 2008 there was sufficient N for crop growth, resulting in higher yields.

Yield differences were observed across treatments at all site years except Glenlea in 2008. At Glenlea in 2007, the heritage cultivar Red Fife yielded the highest while AC Barrie yielded the lowest (Table 3.5). Although weed and disease pressure were not rated, it was observed that weed and disease pressure were high at Glenlea in that year, and it is speculated that the heritage cultivar Red Fife likely performed well under these stressful conditions due to its height and later maturity. It has been found that older cultivars grow taller, compete better for light, and accumulate more early dry matter than

modern cultivars, indicating that older cultivars may be better suited to organic crop production (Cosser *et al.* 1997). A study by Carr *et al.* (2006) also found plant height and vigor to be related to yield. Although disease measurements were not taken, it is thought that AC Barrie performed poorly in 2007 due to high levels of leaf rust (*Puccinia triticina* f. sp. *tritici*), which it is susceptible to.

The highest yielding cultivar in both years at Carman was the modern cultivar FBC Dylan (Table 3.5 and 3.6). This line was bred specifically for organic conditions, highlighting the importance of varietal development for organic crop production. FBC Dylan was selected under organic growing conditions by organic farmers and may have been better adapted to organic growing conditions than the other modern cultivars. Comparisons between FBC Dylan and the other modern cultivars included in this study should be interpreted with caution since it is not known if FBC Dylan is of the same market class as the other cultivars. However, selection under organic growing conditions may have imparted beneficial characteristics on FBC Dylan.

Characteristics such as the ability to interact with beneficial soil microorganisms and the ability to compete with weeds are cited as being more beneficial for organic than conventional crop production (Lammerts van Bueren *et al.* 1999, 2002). In the breeding process for FBC Dylan these and other beneficial characteristics may have been selected for. Ceccarelli (1994) found widely adapted lines to suffer a yield disadvantage of 10-30% when compared with lines selected for a specific adaptation, as FBC Dylan was. FBC Dylan did not yield as high at Glenlea in 2007 most likely due to its short stature and the large weed populations it had to compete against.

Contrast analysis of yield showed significant differences between modern and heritage cultivars at one of the site years; however, at all site years the modern cultivars yielded higher than the heritage cultivars (Table 3.7). A higher yield with modern cultivars opposes the hypothesis that heritage cultivars would yield higher under organic management since they were selected under organic conditions. When selection is carried out in organic growing conditions, the cultivars can adapt to organic conditions related to the soil, disease conditions and weed pressure (Lammerts van Bueren *et al.* 1999). Organic agriculture also prohibits the use of synthetic fertilizers and pesticides, and new crop cultivars have been primarily tested under high input conventional systems, which selects for cultivars that perform well with conventional agricultural products. Regardless of selection under conventional growing conditions, the results of this study show that modern cultivars yield higher than heritage cultivars. This suggests that modern cultivars are better suited to organic growing conditions than heritage cultivars.

Modern wheat cultivars may not be fully adapted to organic management; however, there are important reasons why modern cultivars performed well in these experiments. The time span of selection in conventional environments could be expected to lead to some yield advantage of modern cultivars in organic production situations that is independent of advantages associated with adaptation to organic soil conditions. Over the past 50 years, global wheat breeding efforts have largely focused on improving yield potential and end-use quality of wheat cultivars (Mason and Spanner 2006). Breeding efforts have also focused on improving resistance to diseases, insects and lodging.

The relationship between grain yield and AMF colonization was tested using regression analysis. Significant relationships were observed only at Carman in 2008,

where yield was positively related to arbuscular colonization (Figure 3.2). AMF colonization is very important in organic cropping systems since they facilitate the uptake of phosphorus (Ezawa *et al.* 2002), a nutrient often found to be deficient in organic cropping systems. The lack of a relationship between yield and colonization at three of the four site years is surprising given that AMF would be expected to have a positive relationship with yield in low P conditions. It is possible that there was adequate P for wheat growth at the 3 site years, and AMF colonization did not provide an additional yield benefit. These results support the theory that arbuscular colonization is not a good indication of the benefit the wheat is receiving from AMF.

Yield was found to be dependent on release date of the cultivars at only one site (Carman in 2007), where yield increased with release date (Figure 3.3). It is especially difficult to make comparisons between release dates and yield since in this study the modern cultivars spanned 15 years, while the cultivars considered to be the heritage cultivars had release dates spanning 83 years. In order to make stronger conclusions a study observing more cultivars across the time span and more data to evaluate yield, both of which are beyond the capacity of this project, would be needed.

Table 3.5 Year of release, colonization by arbuscules, yield, and tissue P concentration for the 10 wheat cultivars at Glenlea and Carman in 2007.

Cultivar	Year of Release	Arbuscular Colonization (%)		Yield (kg/ha)		Tissue P (%)	
		Glenlea	Carman	Glenlea	Carman	Glenlea	Carman
McKenzie	1997	8.5[z]	21[z]	1,793ab[y]	1,729b[y]	0.135d[y]	0.182e[y]
CDC Teal	1991	8	19.5	1,478bc	1,424cd	0.145bcd	0.2cd
FBC Dylan	2006	9.8	19	1,382cd	2,307a	0.155abc	0.2cd
AC Barrie	1994	6	14.5	1,137d	1,113e	0.17a	0.24a
5602 HR	2004	8	13.3	1,859a	2,054a	0.14cd	0.195cde
Selkirk	1955	7.8	13.3	1,314cd	1,210de	0.16ab	0.225ab
Red Fife	1886	9	13	1,892a	1,516bc	0.155abc	0.2cd
Neepawa	1969	5.8	12	1,351cd	1,318cde	0.155abc	0.225ab
Mida	1944	11.3	11.3	1,283cd	1,589bc	0.15bcd	0.192de
Marquis	1911	8.3	10.3	1,443cd	1,218de	0.16ab	0.21bc

[y]Means within the same site year followed by the same letter within a column are not significantly different (P>0.05) according to Fischer's protected LSD.
[z]Means are not significantly different (P>0.05) according to Fischer's protected LSD.

Table 3.6 Year of release, colonization by arbuscules, yield, and tissue P concentration for the 10 wheat cultivars at Glenlea and Carman in 2008.

Cultivar	Year of Release	Arbuscular Colonization (%)		Yield (kg/ha)		Tissue P (%)	
		Glenlea	Carman	Glenlea	Carman	Glenlea	Carman
FBC Dylan	2006	0.7[z]	21.8a[y]	2,590[z]	4,132a[y]	0.18ab[y]	0.18[z]
5602 HR	2004	0.6	16.5ab	2,703	3,004bc	0.16b	0.173
AC Barrie	1994	0.3	15abc	2,465	2,965bc	0.168b	0.178
Mida	1944	1.0	12.3bcd	2,496	3,781ab	0.165b	0.173
Red Fife	1886	0.6	11.3bcd	2,560	2,325c	0.18ab	0.183
CDC Teal	1991	0.47	10.8bcd	2,464	2,654c	0.178ab	0.175
Selkirk	1955	1.1	10.3bcd	2,438	2,806c	0.17b	0.17
McKenzie	1997		7.3cd		2,338c		0.179
Neepawa	1969	0.13	6.3cd	2,578	3,182abc	0.178ab	0.185
Marquis	1911	0.6	5.3d	2,092	2,328c	0.198a	0.18

[y]Means within the same site year followed by the same letter within a column are not significantly different (P>0.05) according to Fischer's protected LSD.
[z]Means are not significantly different (P>0.05) according to Fischer's protected LSD.

Table 3.7 Estimates and contrasts of the difference between heritage (1886-1969) and modern (1991-2006) cultivars of wheat for average mycorrhizal colonization, yield, tissue P and biomass at the four sites.

Location	Est./Contrast	Arb. Col (%)	Total Col. (%)	Root Weight (g)	Yield (kg/ha)	Tissue P (%)	Biomass (g)
Glenlea 2007	Heritage	8.4	15.5	1.21	1456	0.16	173
	Modern	8.1	14	1.02	1530	0.15	155
	Difference	0.4	1.5	0.19	-73	0.01	18
	Significance	n.s.	n.s.	n.s.	n.s.	n.s.	*
Carman 2007	Heritage	12	20	0.87	1370	0.21	242
	Modern	17.5	27.8	0.87	1725	0.2	253
	Difference	-5.5	-7.8	-0.02	-355	0.01	-11
	Significance	**	**	n.s.	***	n.s.	n.s.
Glenlea 2008	Heritage	0.69	1.4	2.41	2433	0.18	101
	Modern	0.51	1.2	2.29	2555	0.17	100
	Difference	0.19	0.2	0.13	-123	0.01	1
	Significance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Carman 2008	Heritage	9.1	13.1	1.97	2884	0.18	155
	Modern	14.3	19.3	2.13	3019	0.18	151
	Difference	-5.2	-6.2	-0.16	-134	0	4
	Significance	*	*	n.s.	n.s.	n.s.	n.s.

n.s. (not significant at $P<0.05$), *($P<0.05$), **($P<0.01$), ***($P<0.001$)

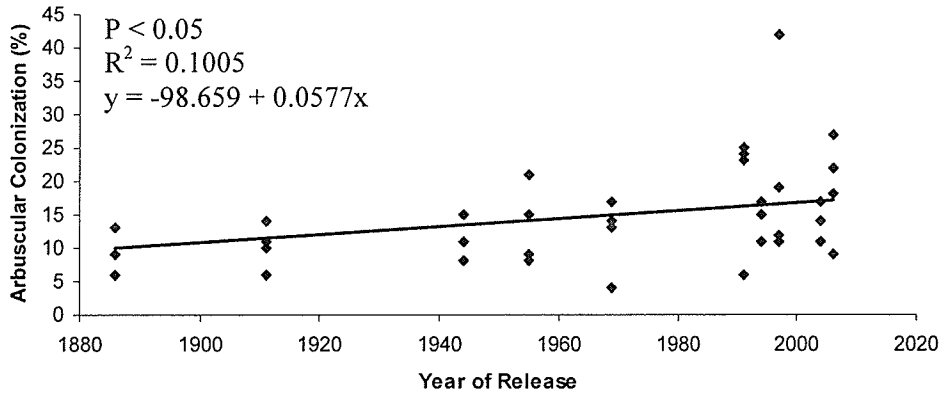

Figure 3.1 Relationship between year of release and arbuscular colonization at Carman in 2007.

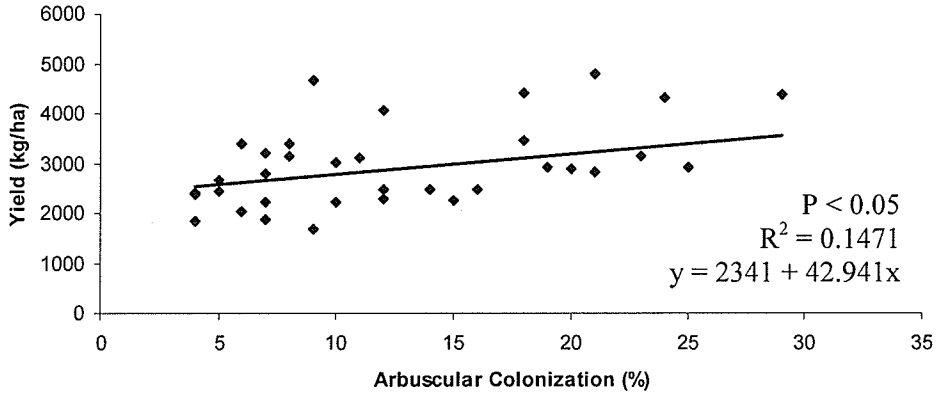

Figure 3.2 Relationship between arbuscular colonization and yield at Carman in 2008.

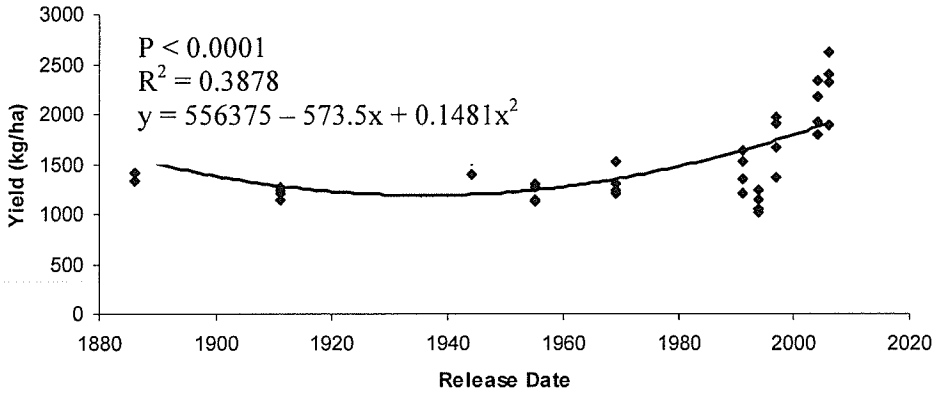

Figure 3.3 Relationship between release date and yield at Carman in 2007.

3.5 Conclusions

Modern cultivars had higher arbuscular and total colonization than heritage cultivars in all site years, although only significant at Carman. This is the opposite result as was expected, and there may be no relationship between percent root colonization and the benefit from AMF symbiosis. This study did not confirm the theory that selecting wheat cultivars under conventional management has reduced their ability to colonize with AMF. More research needs to be conducted to determine the benefit the cultivars are receiving from AMF instead of only looking at percent colonization.

Tissue P was not found to be a good indication of AMF colonization in wheat. Significant differences in tissue P concentration were observed across treatments, although the results were not consistent across site years. Tissue P concentration and colonization had a negative correlation at one site year. No correlations between tissue P and colonization were observed in the other three site years.

Yield differences between cultivars suggest that organic farmers should be selecting different cultivars than conventional farmers in some environments. Characteristics associated with different cultivars may prove to be more useful in certain environmental and management situations. At Glenlea the heritage cultivar Red Fife likely performed well due to its ability to compete with weeds. At Carman the organically selected cultivar FBC Dylan may have been better adapted to organic soil conditions. Modern cultivars yielded higher than heritage cultivars at all site years, although only significant at Carman 2007; however, some modern cultivars performed better than others from the same time period. It can not be conclusively stated that modern cultivars are better suited to organic management based on these results.

4. COMPARISON OF ORGANIC AND CONVENTIONAL SELECTION ENVIRONMENTS

4.1 Abstract

Organic crop production systems produce a soil and weed environment that is different than the one found in conventional wheat breeding programs. An important research question is whether wheat cultivars for organic production should be selected under organic field conditions. This study compared the yield, protein concentration, kernel weight, and height of seven populations of wheat selected in both organic and conventional growing conditions (termed selection environments). The organic and conventionally selected populations were compared at four organic and four conventionally managed locations in Manitoba and Saskatchewan (termed management environments). The selection environment was found to affect yield, protein, kernel weight and height in both management environments. Direct selection resulted in higher yield than indirect selection at all but one location. Populations selected under organic management resulted in higher protein concentrations and kernel weights under both organic and conventional management environments. Direct selection under organic growing conditions is recommended when selecting wheat lines for organic agriculture, since the lines with the highest yield and best quality under organic management may differ from those that perform best under conventional management.

4.2 Introduction

As organic farming gains popularity in Canada and throughout the world, a major challenge is to maintain yield levels and produce high quality crops while using

sustainable agricultural practices. The requirement of organic producers to use certified organic seed and transplants when readily available (CGSB 2006), coupled with rising input prices and consumer demand has increased interest in breeding specifically for organic crop production. Organic farmers have been reliant on conventionally bred cultivars designed for crop production systems that use synthetic chemical fertilizers and pesticides. Crop performance in organic systems could be improved if cultivars were bred for typically organic conditions (Lammerts van Bueren *et al.* 1999), such as increased weed pressure and lower levels of readily available soil nutrients.

More yield limiting factors, and a higher magnitude of crop stress is expected under organic crop production compared to conventional (Burger *et al.* 2008). The increased crop stresses experienced under organic management may affect cultivar performance to the extent where breeding specifically for organic environments is recommended (Mason and Spaner 2006). Although organic and conventional plant breeders may focus on some of the same desirable characteristics, such as yield and quality, characteristics related to competition for nutrients and improved competition against weeds are of more importance in organic production (Murphy *et al.* 2007). The inclusion of these traits in a crop cultivar may make it more suitable for organic crop production and result in higher yields than the conventionally selected cultivars currently in use.

A lack of inorganic fertilizer inputs in organic agricultural systems may result in lower N and phosphorus (P) levels compared to conventional agriculture. It would therefore be beneficial for cultivars to be adapted to lower nutrient levels and seasonal fluctuations in nutrient availability by maintaining larger and more active root systems

(Lammerts van Bueren *et al.* 1999, 2002) since farmers have fewer options to deal with nutrient deficiencies. Organic farming systems will also depend more on interactions with beneficial soil microorganisms to maintain fertility levels. Mycorrhizal dependence is thought to be a genetic trait expressed in some wheat genotypes (Hetrick *et al.* 1992); therefore cultivars with a greater response to mycorrhizal colonization may be identified.

Crop cultivars with a superior ability to compete with weeds are beneficial in organic crop production since synthetic herbicides are prohibited in organic farming systems. The competitive ability of a crop is based on two components: the crops ability to maintain yield in the presence of competition as well as the crops ability to suppress weeds (Lemerle *et al.* 1996). Traits that contribute to a cultivars ability to compete with weeds include rapid juvenile growth (Lammerts van Bueren *et al.* 2002, Lemerle *et al.* 1996), plant architecture for early soil cover and more light competition (Lammerts van Bueren *et al.* 2002; Wolfe *et al.* 2008), tallness (Lemerle *et al.* 1996; Mason *et al.* 2007a; Vandeleur and Gill 2004; Wolfe *et al.* 2008), and resistance to mechanical weed control (Lammerts van Bueren *et al.* 2002).

Cultivars for organic crop production can be selected in organic or conventionally managed environments. Direct selection occurs when a plant breeder selects cultivars in the environment where the cultivar is to be grown in the future. Indirect selection refers to selection for yield in one system when the other system is the target environment (Murphy *et al.* 2007). Wide adaptation is a common goal in plant breeding, with wide adaptation referring to both geographical and environmental adaptation. Ceccarelli (1994) found widely adapted lines to yield more than the best check in contrasting locations, however widely adapted lines suffered a yield disadvantage of 10-30% when

compared with lines selected for specific adaptation. Direct selection for low-input environments was found to be more efficient than indirect selection in studies by Brancourt-Hulmel *et al.* (2005), Atlin and Frey (1990), Ceccarelli (1994), Ceccarelli and Grando (1991), and Murphy *et al.* (2007). These studies show that breeding programs targeted for organic production systems should include organic environments to maximize selection gains, as cultivars selected in organic growing conditions can adapt to organic conditions related to the soil, disease, and weed pressure (Lammerts van Bueren *et al.* 1999).

The objectives of this study were 1) to determine if selection in organically managed field conditions offers advantages to indirect selection in conventionally managed field conditions; and 2) to compare rank changes of populations and check cultivars between the two different management environments to determine if the populations with high yield, protein and thousand kernel weight (TKW) differ between organic and conventional growing environments. To achieve these objectives yield, protein concentration, TKW and height of 7 populations of wheat selected under organic and conventional environments, grown in organic and conventionally managed field trials were compared. The wheat lines and check cultivars were grown at four organically managed locations and four conventionally managed locations over a period of two years. The hypothesis is that direct selection in organically managed field conditions will offer advantages to indirect selection, and that rank changes will occur between management environments, indicating the need for cultivar selection under organic conditions.

4.3 Materials and Method

4.3.1 Site Descriptions

Field experiments were located on both organic and conventionally managed land. Conventionally managed experiments received herbicide and fertilizer additions as necessary while organically managed experiments were managed in accordance with the Canadian organic standards (CGSB 2006). In 2007 the organically managed experiments were located at the University of Manitoba's Glenlea and Carman research stations, and the conventionally managed experiments were located at Agriculture and Agri-Food Canada's Glenlea and Brandon research stations. In 2008 the organically managed experiments were located at the University of Manitoba's Glenlea and Carman research stations, and on a certified organic farm in Oxbow, Saskatchewan. The conventionally managed experiments were located at Agriculture and Agri-Food Canada's Glenlea, Portage and Saskatoon research stations in 2008. Flooding in 2007 caused the conventional experiment at Glenlea to be abandoned. Site characteristics are outlined in table 4.1 and 4.2.

Table 4.1 Management, location, soil association and soil texture at each experimental site.

Site Name	Management	Latitude (N)	Longitude (W)	Soil Association *(texture)*
Carman	organic	49° 29'	98° 0'	Hibsin *(fine sandy loam)*
Glenlea	organic	49° 38'	97° 8'	Red River or Scantenbury *(clay)*
Oxbow	organic	49° 13'	102° 10'	Oxbow *(loam)*
Glenlea	conventional	49° 38'	97° 8'	Red River or Scantenbury *(clay)*
Brandon	conventional	49° 50'	99° 57'	Fairland *(loam)* and Ramada *(clay loam)*
Portage	conventional	49° 58'	98° 17'	Neuhorst *(clay loam)*
Saskatoon	conventional	52° 8'	106° 41'	Sutherland (clay loam)

Table 4.2 Soil nutrient status and previous crop history as each experimental site.

Site Location	Management	Year	Previous Crop	Sample Depth (cm)	Nitrate-N	Olsen-P	K	Sulfate-S	pH
					kg/ha (ppm)				
Carman	Organic	2007	green manure	0-15	41 (12)	102 (27)	996 (282)	24 (6)	6.1
				0-60	55 (16)	155 (41)	1399 (396)	45 (11)	7.9
Glenlea	Organic	2007	fallow	0-15	41 (4)	45 (5)	1365 (154)	13 (1)	7.4
				0-60	137 (15)	66 (7)	3225 (360)	45 (5)	7.7
Carman	Organic	2008	green manure	0-15	112 (30)	60 (37)	445 (275)	18 (4)	5.3
				0-60	168 (15)	83 (51)	618 (382)	27 (6)	7.2
Glenlea	Organic	2008	green manure	0-15	143 (37)	62 (40)	745 (561)	44 (11)	6.6
				0-60	220 (57)	89 (57)	1258 (947)	76 (19)	7.4
Oxbow	Organic	2008	green manure	0-15	104 (25)	14 (10)	459 (339)	835 (182)	7.5
				0-60	183 (44)	24 (17)	760 (561)	1752 (382)	7.7
Brandon	Conventional	2007		0-15	43	20	725	29	7.8
				0-30	143	25		117	7.5
Glenlea AAFC[1]	Conventional	2008		-					
				-					
Portage[1]	Conventional	2008		-					
				-					
Saskatoon	Conventional	2008	fallow	0-15	34	52	>572	16	6.8
				0-30	65			28	7.4

*Nitrate-N, **Olsen-P, ***Sulfate-S

[1] Soil nutrient status unavailable

The three organically managed locations were managed according to organic production principles. The Glenlea Long-Term Rotation was established in 1992 and is Canada's oldest organic-conventional cropping comparison study. At the Glenlea site this research was conducted on land that had been under organic management since the rotation was established. The Organic Crops Field Laboratory consists of 4 hectares of land that have been under organic management since 2002. The land is divided into 6 parcels to accommodate the six-year crop rotation: green manure – oilseed – cereal – green manure – cereal – pulse crop. The third organic location, at Oxbow, Saskatchewan, was located on Ian Cushon's 777 ha organic farm, which has been under certified organic management for over 20 years.

The conventionally managed experiments were located on Agriculture and Agri-Food Canada research stations at Brandon, Glenlea, and Portage, Manitoba, as well as Saskatoon, Saskatchewan. Fertilizer application was based on soils tests and herbicides were used to control weeds as necessary.

Weather data collected by Manitoba Agriculture, Food and Rural Initiatives (MAFRI 2008a), Environment Canada's climate data online (Environment Canada 2008), and 30-year averages (Environment Canada 2004) are presented in Table 4.3. The six research sites used for this research were chosen in an attempt to provide a range of weather conditions typically experienced on the Canadian Prairies. Although the Saskatchewan sites would be expected to receive less precipitation than the Manitoba sites, in 2008 Oxbow received an above average amount of precipitation.

Table 4.3 Environmental conditions during the growing season (May 1 – August 31) at each experimental site (MAFRI 2008a and Environment Canada 2008), and long-term averages (Environment Canada 2004).

Research Site	Precipitation			Heat		
	Long-term Average[1]	2007	2008	Long-term Average[1]	2007	2008
	------ *mm* ------			-- *Growing Degree Days* --		
Carman	282	271	210	1,427	1,467	1,239
Glenlea	304	247	280	1,455	1,455	1,213
Oxbow	243		271	1,449		1,320
Brandon	270	267		1,334	1,356	
Glenlea AAFC	304	247	280	1,455	1,455	1,213
Portage	267		309	1,445		1,288
Saskatoon	195		183	1350		1270

[1]30-year long-term averages.

4.3.2 Experimental Design and Treatments

Populations of wheat from the same crosses were grown and selected in separate organic and conventional breeding programs. Crosses were made at Agriculture and Agri-Food Canada's Cereal Research Centre in Winnipeg, MB. Differences occurred between the organic and conventional selection procedures, as outlined in Figure 1. Conventionally selected material was grown in breeding nurseries exposed to artificially induced epidemics of leaf rust (*Puccinia triticina*), stem rust (*Puccinia graminis*), and fusarium head blight (*Fusarium graminearum*). Organically managed breeding nurseries were dependent on naturally occurring disease infection. The F_3 generations from both selection environments were grown in contra-season nurseries in New Zealand, while only the conventionally selected F_5 generation was grown in New Zealand.

Selection occurred in the F_2 generation for the conventional material and the F_3 generation for the organic material, resulting in $F_{2:6}$ conventional populations and $F_{3:6}$ organic populations. The difference in the generation of selection presumably gives an

advantage to the organically selected material since the proportion of fixed desirable alleles would be 25% through conventional selection and 37.5% through organic selection. Population sizes and selection intensity also varied between methods of selection (Table 4.4). Although it is thought that the organically selected populations would have an advantage due to selection in the F_3 generation when selection for specific traits is possible, it should also be noted that the greater population sizes in the conventional F_2 nurseries (Table 4.4) would have created an advantage for the conventionally selected populations. As there was no active selection for any of the quantitative traits except for height (not too tall or too short), it is not clear that selection in one later generation would provide detectable differences in outcomes.

Populations from seven crosses were identified where there were at least four F2:6 or F3:6 lines remaining from each cross selected in respective conventional and organic management environments (Figure 4.1). To provide a uniform seed source for this experiment, lines used to develop the population bulks and check cultivars were increased together at the same time and location. Lines contributing to a bulk were mixed on an equal weight basis. These bulks were used as treatments in a randomized complete block experiment, with three replicate blocks. Five modern CWRS cultivars and the heritage cultivar Red Fife were included as check treatments in this experiment.

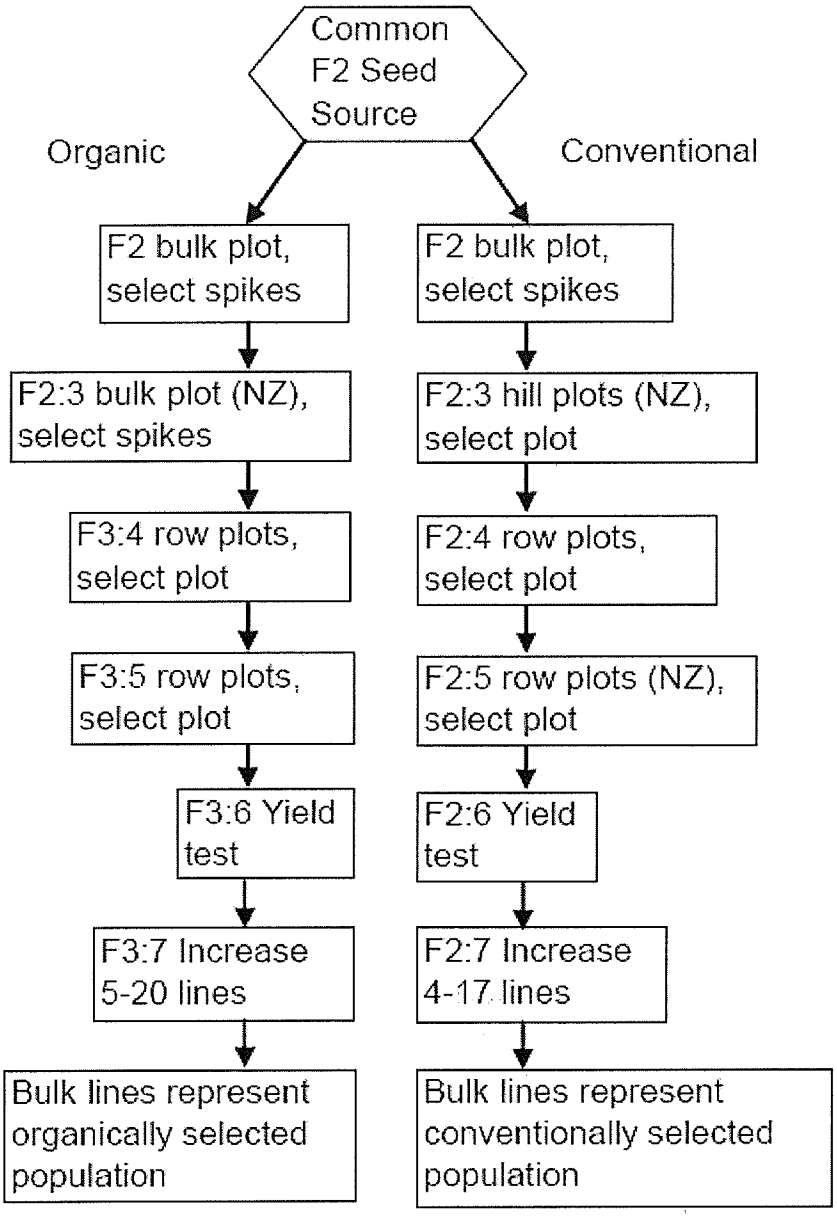

Figure 4.1 Selection procedures to identify superior lines for organic and conventional selection environments. NZ indicates that selection took place in contra-season nurseries in New Zealand. (From: Fox *et al.* 2008).

Table 4.4 Population size and selection intensity of BC crosses in organic and conventional selection environments.

Cross	Selection Environment	Population Sizes and Selection Intensity (SI)								
		F2 pop	F2 SI	F3 pop	F3 SI	F4 pop	F4 SI	F5 pop	F5 SI	F6 pop
BC07	ORG	2000	1.00	2000	0.10	200	0.21	42	0.48	20
BC07	CON	4000	0.05	186	0.60	112	0.35	39	0.44	17
BC10	ORG	2000	1.00	2000	0.10	206	0.17	35	0.37	13
BC10	CON	4000	0.05	212	0.60	127	0.20	25	0.48	12
BC11	ORG	2000	1.00	2000	0.10	201	0.10	20	0.25	5
BC11	CON	4000	0.05	211	0.42	88	0.23	20	0.40	8
BC23	ORG	2000	1.00	2000	0.10	199	0.10	19	0.84	16
BC23	CON	4000	0.05	188	0.61	114	0.28	32	0.31	10
BC37	ORG	2000	1.00	2000	0.10	190	0.07	13	0.54	7
BC37	CON	4000	0.04	151	0.61	92	0.20	18	0.44	8
BC41	ORG	2000	1.00	2000	0.10	193	0.06	12	0.50	6
BC41	CON	4000	0.04	176	0.44	78	0.14	11	0.45	5
BC42	ORG	2000	1.00	2000	0.09	184	0.07	13	0.38	5
BC42	CON	4000	0.05	186	0.51	95	0.18	17	0.24	4
		Average Selection Intensity								
	ORG		1.00		0.10		0.11		0.48	
	CON		0.05		0.54		0.22		0.39	

Table 4.5 Wheat lines included in the study, including parents and the number of lines in each bulk population.

Line	Parentage	No. in Bulk
McKenzie	Columbus/Amidon	
Superb	Grandin*2/AC Domain	
AC Barrie	Neepawa/Columbus/BW90	
5602HR	AC Barrie/Norpro	
Waskada	BW278/2*BW252	
Red Fife	Reselection from Ukrainian Introduction	
BC07-ORG	98B25-AS6D01/ND744	20
BC07-CON	98B25-AS6D01/ND744	17
BC10-ORG	98B25-AG3C7/ND744	13
BC10-CON	98B25-AG3C7/ND744	12
BC11-ORG	98B25-DF2B04/ND744	5
BC11-CON	98B25-DF2B04/ND744	8
BC23-ORG	98B34-T4B/98B26-N1C01	16
BC23-CON	98B34-T4B/98B26-N1C01	10
BC37-ORG	97B64-E9B5/96B32-AN3C	7
BC37-CON	97B64-E9B5/96B32-AN3C	8
BC41-ORG	BW301/Alsen//94B18-G1E1/9606-EJ03B	6
BC41-CON	BW301/Alsen//94B18-G1E1/9606-EJ03B	5
BC42-ORG	BW301/Alsen//94B18-G1E1/9606-EJ03B	5
BC42-CON	BW301/Alsen//94B18-G1E1/9606-EJ03B	4

4.3.3 Field Trial Management

Conventional experiments were managed according to standard tillage, fertilization and herbicide use as decided by managers at the respective Agriculture and Agri-Food Canada centres. The organic experiments were managed according to organic standards by farmers or University of Manitoba technicians. Fertilizer was applied to conventional experiments as recommended by soil test results (Table 4.6). All fertilizers were applied in the spring prior to seeding, with the exception of Glenlea where anhydrous ammonia was applied the previous fall. Fertilizer was broadcast and incorporated in the spring at Portage, and deep-banded at Brandon and Saskatoon. Pesticides were applied to the conventional sites as needed, following crop stage and rate recommendations of the Guide to Field Crop Production (MAFRI 2007, MAFRI 2008b) (Table 4.6).

Table 4.6 Fertilizer and herbicide type, amount applied and the crop stage herbicides were applied at for the conventional experiments.

Location	Fertilizer				Herbicides		
	Type	N (lb/ac)	P_2O_5 (lb/ac)	S (lb/ac)	Type	Amount	Crop Stage
Brandon	10-30-0	6.6	20		Everest	14.1 g/ac	1-4 leaf
	82-0-0	49			Refine Extra	8 g/ac	1-4 leaf
					Accord	27 g/ac	1-4 leaf
Glenlea							pre-
	82-0-0	49			Roundup	0.9 L/ac	emergence
					Achieve	0.2 L/ac	5 leaf
					Buctril M	0.4 L/ac	5 leaf
Portage	11-52-0	4	21		Refine Extra	8 g/ac	1-4 leaf
	46-0-0	25			Puma Super	0.31 L/ac	1-4 leaf
Saskatoon	30-25-0-10	35	29	12	Prestige A	0.32 L/ac	1-4 leaf
					Prestige B	0.80 L/ac	1-4 leaf
					Horizon	0.093 L/ac	1-4 leaf
					Score (adjuvant)	0.8L/100L H_2O	1-4 leaf

Prior to seeding the soil was cultivated to provide a smooth seed bed. Plots under organic management were seeded using a disk drill (Fabro Industries, Swift Current) while plots under conventional management were seeded using a press drill. All locations were seeded at a rate of 333 viable seeds/m^2. Organic experiments were harrowed with a Lely flex-tine harrow at approximately the four leaf stage. See table 4.7 for more information on seeding and harvest operations.

Table 4.7 Timing of seeding and harvest operations, plot size and row spacing for organic and conventional experiments.

Site Location	Management	Year	Plot Area (m^2)	Row Spacing (cm)	Seeding	Harvest
Carman	Organic	2007	2.75	15.24	15-May	22-Aug
Glenlea	Organic	2007	1.83	15.24	17-May	30-Aug
Carman	Organic	2008	2.44	15.24	6-May	19-Aug
Glenlea	Organic	2008	2.44	15.24	8-May	26-Aug
Oxbow	Organic	2008	2.44	15.24	13-May	10-Sep
Brandon	Conventional	2007	3.68	22.86	27-May	15-Sep
Glenlea AAFC	Conventional	2008	3.08	15.24	17-May	11-Sep
Portage	Conventional	2008	3.08	15.24	24-May	12-Sep
Saskatoon	Conventional	2008	3.35	15.24	30-May	30-Sep

4.3.4 Data Collection

Relative maturity ratings were taken at all sites in mid-August once the lines showed signs of maturing. Maturity ratings were done on a scale of 1-5, with 1 being early maturing and 5 being late maturing. The maturity ratings of the lines were taken in comparison to the checks, with McKenzie being used at an early maturing check, AC Barrie as an intermediate maturing check, and Superb being a late maturing check. Relative maturity ratings were not taken at Oxbow due to the distance to the site.

Height measurements were taken once stem elongation had completed. Height was measured at the distance from the soil surface to the tip of the spike, ignoring the

awns. Two height measurements were taken per plot, with plant height for each plot being the average of the two measurements.

Data was not collected on weed density in each plot. In general weed populations were found to be relatively uniform across site years with the exception of Carman in 2008, in which wild oat density was found to be very high in some locations and not others. The Carman site in 2008 was not taken into consideration in this study due to the uneven weed populations.

Prior to harvesting, plots were trimmed and the harvestable area was measured in order to accurately calculate yield. All experiments were harvested with a Wintersteiger plot combine, with the exception of Glenlea in 2007 which was harvested with a Hege plot combine. Samples were dried on a forced air drying bed then cleaned. In 2007 chaff and weed seeds were removed by putting samples through an air blower then a shaking through a #5 rectangular sieve. In 2008 samples were put through an air blower to remove chaff, and then put through a dockage tester to remove weed seeds (Carter Dockage Tester 31624/W-3301). The dockage tester contained a no. 1 riddle, 9/64 tri double cut sieve, and a S-909 S1/2 164 R.086 sieve. Clean weights were taken and yield was calculated based on plot area.

Kernel weight was calculated was calculated by weighing approximately 20 grams of wheat per plot then pouring the kernels through a seed counter (Agriculex Inc. ESC-1). Thousand kernel weight (TKW) was then calculated based on the number of kernels that made up the 20 gram sample.

Protein was measured with a grain spectrometer (Foss NIRSystems, Inc.).

4.3.5 Statistical Analysis

Relative maturity, height, yield, TKW, and protein were compared among the populations and methods of selection using the Statistical Analysis Software program (SAS Institute 2001). Prior to the analysis, the data were tested for homogeneity of variance (Bartlett's) and for normality (Shapiro-Wilks). When the variances differed across groups the mixed procedure was altered to account for heterogenous variances. Square root and log10 data transformations were used when the error deviations were found to be not normally distributed. Non-normality was found in the kernel weight analysis, therefore 3 outliers (>2 standard deviations from the mean) were removed from the analysis. All data was analyzed using the mixed procedure (Proc Mixed, SAS Institute Inc.).

The eight locations were first analyzed on an individual basis with and without the check cultivars included in the analysis. Each experiment was analyzed individually with checks removed from the analysis to establish if there were significant differences among the seven populations, two methods of selection, and the interaction between the populations and methods of selection. In the second analysis of the individual locations, checks were included and contrasts were used to establish differences between the checks and organic or conventional methods of selection.

Another analysis was performed in which the four organic locations were combined and the four conventional locations were combined. Excluding the checks, experiments from the same management environment (i.e. Conventional vs. organic) were combined to establish if there were significant differences between the populations, method of selection, and the site years across the environments. In a further analysis with

the management environments combined the checks were included, and contrasts were used to establish if there were significant differences between the checks and method of selection.

4.4 Results and Discussion

The first objective of this study was to determine if selection in organically managed field conditions offers advantages to indirect selection in conventionally managed field conditions. Data on yield, protein concentration, TKW and height were used to support the hypothesis that different characteristics are being selected for in organic compared to conventional breeding nurseries.

4.4.1 Yield

On average the organic locations yielded 68% of the conventional locations, although yields at the conventional locations Brandon and Glenlea were similar to the yields at the organic locations Carman, Glenlea and Oxbow in 2008 (Table 4.8). The lower yields in organic management are likely due to a lack of synthetic fertilizer additions and increased weed pressure. Across the eastern portion of the northern Great Plains, organic hard red spring wheat was found to yield 77% of conventional long-term averages (Entz *et al.* 2001). The later seeding dates of the conventional locations (Table 4.7) may have resulted in a yield disadvantage for the conventional locations, potentially resulting in less of a yield difference between management environments than if similar seeding dates had occurred. Yield differences were observed between sites due to differing soil and environmental conditions throughout the site years. The lower yields at

the organic Glenlea 2007 site may be partially explained by the lack of a previous green manure crop on that site. Green manure crops are used to build soil fertility in the rotation (Watson *et al.* 2002); therefore wheat planted into green manure would be expected to have more available nutrients than wheat planted into tilled fallow.

Significant yield differences between the seven populations were found at all locations (Table 4.8). Crop yields were significantly affected by method of selection at three locations, but not at the other five locations. At Carman (organic management) and Brandon (conventional management) in 2007 the populations selected in organic environments yielded higher than those selected in conventional environments, while the reverse was true at the conventional location Saskatoon in 2008 (Table 4.8). Although it may be thought that selection in organic environments would favour high yields compared to selection in conventional environments due to the generation of selection this is most likely not the case since yield alleles were not being actively selected for.

At the organic experiments direct selection in organic systems produced yields 9%, 3%, 3% and 0% higher (Table 4.8) than yields resulting from indirect selection, at Carman 2007, Glenlea 2007, Glenlea 2008 and Oxbow 2008, respectively. The differences between direct and indirect selection are smaller than those reported by Murphy *et al.* (2007), who found direct selection in organic systems to produce yields 5-31% higher than indirect selection in conventional systems. Differences in crop rotation and field management may have resulted in less yield differences seen in the current study. In most instances the yield of the check cultivars did not differ significantly from the populations (Table 4.8), indicating that the two breeding programs are at least meeting current yield standards.

Significant interactions between population, method of selection and site year indicate that the different soil and environmental conditions found in the various site years cause the different populations and methods of selection to yield differently across site years. When organic experiments were combined and conventional experiments were combined significant population by site year and method by site year interactions were found throughout the organic and conventional site years. The significant population by site year interactions in both management environments suggest that the different plant characteristics associated with each population result in some populations being better suited to one environment over another. The same is also true for method of selection. Each population is genotypically different, and the two methods of selection resulted in visual differences in the organic and conventionally selected lines from each population.

Studies have shown that when selection is carried out in organic growing conditions, the lines adapt to organic conditions related to the soil, disease and weed pressure (Lammerts van Bueren *et al.* 1999), which may include characteristics such as efficient nutrient use, vigorous early growth and high leaf area index (Wolfe *et al.* 2008). These plant characteristics, and the characteristics associated with each population and selection method, would be more beneficial in some site years than others depending on soil temperature, soil type, weed pressure, and a host of other conditions.

The four site years in each management environment were combined to see if method of selection was significant under each management environment. Although the soil and weather conditions differed across the site years, resulting in differing yields, the site years were combined due to the similar management practices employed throughout

the growing season. When the organically managed experiments were combined, significant yield differences were found between the two methods of selection, with organically selected lines yielding significantly higher than conventionally selected lines (Table 4.9 and 4.10). These results show evidence that genotypes selected under organic management performed better under organic conditions, and are in agreement with studies by Ceccarelli and Grando (1991) and Burger *et al.* (2008).

Contrasts were performed on the organic and conventionally selected lines from each population, and only BC11 was found to have significant yield differences between methods of selection (Figure 4.2). Contrasts showed no significant difference between the yield of the check cultivars and the organic or conventionally selected lines (Table 4.9).

Method of selection was not found to have a significant effect on yield when the four conventionally managed locations were combined (Table 4.11). A significant yield difference was found only for BC11 when contrasts were performed on the organic and conventionally selected lines from each population (Figure 4.3). Figure 4.3 also illustrates the significant interaction between population and method of selection (Table 4.11); not all populations reacted similarly to a change in method of selection. Under conventional management the yield of the conventionally selected lines did not differ significantly from the check cultivars, while the organically selected lines yielded significantly less than the check cultivars (Table 4.10 and 4.11). It was expected that the organically selected lines would yield lower than the check cultivars under conventional conditions since the check cultivars were selected for conventional environments and the organically selected lines were not. Also, the organically selected lines are populations

of the best material from each selection scheme (Table 4.5) but the likelihood of the majority of the lines forming each population being superior to the checks would be expected to be small.

An important concern that will require further work is the separation of normal genotype by environment interactions from the impact of management. One approach will be to investigate stability of the populations within each management type and overall to provide some insight.

4.4.2 Protein

Organic and conventional management environments produced wheat with comparable protein concentration, 14.6% and 14.8%, respectively (Table 4.11). Organic cropping systems are expected to have lower N availability than conventional systems, which may result in lower protein concentrations; however, Mason *et al.* (2007b) also observed similar protein concentrations from organic and conventional management. In the current study, as well as the study by Mason *et al.* (2007b), protein concentrations were considered to be high, with both studies observing protein concentrations over 14%. This is in contrast to other studies which found organic cereal crops to have lower protein content, but a higher quality protein than conventional cereals (Magkos *et al.* 2003; Worthington 2001).

A possible reason for comparable protein between management environments is the 'protein dilution' effect, due to the inverse relationship between grain yield and protein content in wheat (Wolfe *et al.* 2008). Lower yielding sites are expected to have higher protein concentrations. This relationship was observed under both organic and

conventional management environments. As yield increased, protein concentration decreased (Table 5.3 and 5.4). Organically managed locations had lower yields than conventional locations; therefore organic locations had higher protein concentrations.

Protein concentration is an important factor to consider for organic cereals entering the global market, since these cereals are generally required to conform to industrialized milling and baking standards, which require high protein content and relatively hard gluten (Wolfe *et al.* 2008). Less of an emphasis on a standardized protein concentration would be placed on cereals entering local markets. Although high protein is intuitively though to be a desirable characteristic, high protein content is usually associated with softer gluten, which reduces baking quality (Wolfe *et al.* 2008).

Average protein concentration differed significantly between populations at all locations but Oxbow (Table 4.12). The organic location Glenlea 2008 and the conventional location Portage 2008 had protein concentrations approximately 3% lower than the other locations (Table 4.12). Glenlea had high levels of N, and Portage was fertilized to meet plant requirements. Weather and soil conditions do not explain low protein concentrations at these sites either. Although site to site differences were observed, average protein concentrations did not differ between management environments.

Protein concentration was significantly affected by method of selection at the organic sites Carman 2007 and Glenlea 2008, and at the conventional site Brandon in 2007 (Table 4.12). Organically selected lines had higher protein concentration than conventionally selected lines in all cases but the conventional experiments Portage and Glenlea, where organic and conventional selection resulted in the same protein

concentration (Table 4.12). This suggests that selection under organic management may be increasing the N uptake efficiency and utilization. In organic agriculture the potential for grain protein production has to be higher than in conventional agriculture to compensate for the lower N availability in organic systems (Wolfe *et al.* 2008). Lines selected under organic management also had significantly higher protein concentrations than the check populations at all locations but the conventional location Glenlea (Table 4.12). Conventionally selected lines had significantly higher protein concentrations than the check cultivars at only three locations (Table 4.12).

Site years within each management system were combined even though significant interactions between site year and population for both management environments, and a significant site year by method of selection interaction for conventional management were detected (Table 4.13 and 4.14). These interactions suggest that there are characteristics associated with each population and method of selection that may result in differing protein concentrations across environments. Each experiment contained differing levels of soil N and soil types. The different populations and lines from each method of selection would have had variations in their ability to take up and convert the N into protein from the different sites.

When the four organically managed locations were combined the method of selection was found to have a significant effect on protein concentration (Table 4.13), with organically selected lines having a higher protein concentration than the conventionally selected lines (Table 4.11). Conventionally selected lines averaged 98% of the protein of organically selected lines. When selecting a cultivar for organic production direct selection is thought to be superior to indirect selection (Ceccarelli

1994). The higher protein concentrations observed in wheat selected in organic environments shows the superiority of direct selection. Characteristics that may be beneficial for extracting N from the soil and converting it to protein include; improved N use and uptake efficiency, adaptation to seasonal fluctuations in nutrient availability, or a larger and more active root system for nutrient uptake (Lammerts van Bueren *et al.* 1999, 2002). Plants selected as superior in organic growing conditions would be thought to contain more of these beneficial characteristics as they are more important in organic conditions than fertilized conventional environments.

Lines from both organic and conventional selection environments were compared to check cultivars at the organic experiments. Results showed that lines from both management environments had significantly higher protein concentrations than the check cultivars (Table 4.13 and 4.11). Contrasts were performed on the organic and conventionally selected lines from each population, and four of the seven populations were found to differ significantly for method of selection (Figure 4.4). Figure 4.4 shows that not all populations respond the same to method of selection, indicating that direct selection is more important in some populations than others. For example, BC42 has a strong response to direct selection in organic growing conditions, where as the protein concentration in BC41 is approximately the same regardless of selection environment (Figure 4.4).

When the four conventionally managed locations were combined the method of selection was found to have a significant effect on protein concentration (Table 4.14). Results showed that the organically selected lines had a significantly higher protein concentration than both the conventionally selected lines and check cultivars (Table 4.11

and 4.14). Therefore, organically selected lines showed a protein advantage under both organic and conventional management in this study.

Contrasts showed significant differences in protein concentration between the two methods of selection for all populations with the exception of two (Figure 4.5). This figure shows a significant population by method of selection interaction (Table 4.14), indicating that all populations did not respond the same way to method of selection.

The dilution effect between protein concentration and yield cannot explain the higher protein concentrations in organically selected wheat. Organically selected lines had higher yields under organic management than conventionally selected lines, and organically and conventionally selected lines had similar yields under conventional management. The efficient use of nutrients is a required characteristic in breeding for organic agriculture (Wolfe *et al.* 2008) due to the nature of organic environments. It has also been suggested that plants selected in organic environments are better adapted to seasonal fluctuations in nutrient availability and uptake nutrients when available (Lammerts van Bueren *et al.* 1999; Wolfe *et al.* 2008).

4.4.3 Kernel Weight

Conventionally managed environments averaged higher TKW than the organically managed environments (Table 4.15). Higher kernel weights in conventional locations are expected since a significant positive correlation was found between kernel weight and yield (Table 6.11 and 6.12), and conventional locations had a higher average yield than the organic locations. Average TKW differed significantly between populations at all locations (Table 4.15). Although TKW and yield are correlated when

averaged across environments, this correlation does not appear to hold true for each experiment. The lower yields at the organic location Glenlea are not reflected in lower TKW (Table 4.15). Kernel weight is expected to differ across experiments, since different environmental conditions, soil conditions, and disease pressure were observed at each site and year.

Method of selection significantly affected kernel weight at five locations; however, at all locations lines selected under organic management had higher kernel weights than lines selected under conventional management (Table 4.15). Organically selected lines had TKW 1-3% higher than lines selected under conventional management. This suggests that selection in organically managed environments results in desirable characteristics. It is interesting to note that although direct selection in the absence of stress for conventional management most often resulted in higher yields for the individual sites (Table 4.8), indirect selection under stress resulted in higher TKW for conventionally managed environments (Table 4.15).

Site years within each management system were combined even though significant interactions between site year and population for both management environments, and a significant site year by method interaction occurred for organic management was detected (Table 4.16 and 4.17). These interactions suggest that there are characteristics associated with each population and method of selection that may result in differing protein concentrations for the populations and methods across environments. Each experiment contained varying levels of disease pressure, soil nutrients and moisture, which affected plant health and therefore kernel size. Genotypic

differences in disease resistance and nutrient uptake would make certain populations and methods of selection more suitable to certain environments.

When the four organically managed locations were combined the method of selection was found to have a significant effect on TKW (Table 4.16), with organically selected lines having higher kernel weights than the conventionally selected lines (Table 4.11). Conventionally selected lines averaged 98% of the TKW of organically selected lines in the organic experiments. These results are consistent with those of Ceccarelli and Grando (1991) who observed that lines selected for high grain yield in low yielding conditions had larger kernels under low yielding conditions than lines selected for high grain yield under high yielding conditions. The higher kernel weights suggest a change in the distribution of yield components of organically selected lines to favour seed size over spikes/m^2.

Lines from both organic and conventional selection environments were compared to check cultivars at the organic experiments. Conventionally selected lines had significantly lower TKW than the checks, while organically selected lines did not have a significantly different TKW than the check cultivars (Table 4.11 and 4.16). This observation suggests that conventionally selected lines in this study are less adapted to deal with the stress conditions found in organic management than currently registered cultivars, with the exception of the heritage cultivar Red Fife. Contrasts were performed on the organic and conventionally selected lines from each population, and three of the seven populations were found to differ significantly for method of selection (Figure 4.6), with organic selection resulting in higher kernel weights.

When the four conventionally managed locations were combined method of selection was found to have a significant effect on TKW (Table 4.17), with organically selected lines having a significantly higher TKW than conventionally selected lines (Table 4.11). Therefore, organically selected lines showed a kernel weight advantage under both organic and conventional management in this study. Contrasts performed on the organic and conventionally selected lines from each population showed significant TKW differences for four populations in conventional experiments (Figure 4.7). Figure 4.6 shows that each population responds differently to direct and indirect selection.

The populations BC41 and BC42 had high kernel weights at both management environments, while the populations BC23 and BC37 had comparatively low kernel weights (Figure 4.6 and 4.7). Taking the positive correlation between yield and TKW into consideration, it is interesting to note that although yields appear to be similar across populations (Figure 4.2 and 4.3), there are large differences in TKW of the seven populations (Figure 4.6 and 4.7). Kernel weight may be related to a population's susceptibility to disease, since disease pressure would be expected to result in a less healthy plant with lower kernel weights. The plant architecture may also play a role in kernel weight, since disease resistance can be related to plant architecture. Lammerts van Bueren *et al.* (2002) suggest a plant architecture that will allow the spikes to dry more rapidly, such as a longer stem and peduncle, a spike higher above flag leaf, and a less compact spike, will result in higher levels of disease resistance.

4.4.4 Height

Increased plant height was observed at the conventional locations compared to the organic locations overall, although the conventional locations Portage and Glenlea had similar heights to the organic locations (Table 4.18). Increased height was made possible at conventional locations due to an abundance of nutrients and less weed competition. There were significant height differences between the seven populations at all locations but Oxbow (Table 4.18). Fewer differences may have been seen at Oxbow due to the specific environmental conditions at that site. Plant height differences between the experiments can be attributed to the different environmental, soil fertility and weed conditions at each site.

Method of selection significantly affected height at the organic location Carman and the conventional locations Brandon and Glenlea, in which the organically selected populations were significantly taller than the conventionally selected populations (Table 4.18). Height is thought to be an important characteristic in maintaining grain yield, as tall cultivars may allow the crop to intercept more solar radiation above the weed layer (Mason *et al.* 2007a). Few significant height differences between the check cultivars and the organic or conventionally selected lines were found (Table 4.18). It should be noted that the heights of the check cultivars Superb and Red Fife, a short and tall cultivar, respectively, caused some sites to have non-normal distributions. Removing these cultivars from the analysis resulted in normality, but they were kept in the analysis for comparison purposes.

Site years within each management system were combined even though a significant interaction between site year and population, and a significant site year by

method interaction occurred for conventional experiments (Table 4.20). No significant interactions between site year, population, and method of selection were observed at organic experiments (Table 4.19). Populations and methods of selection responded differently under the various conventionally managed locations, which is interesting because populations displayed much more variable height in organic locations (Figure 4.8) than conventional locations (Figure 4.9).

When the four organically managed locations were combined, there were no significant height differences between the two methods of selection (Table 4.19). These results are in contrast to Ceccarelli and Grando (1991) who observed that lines selected for high grain yield in low yielding conditions were taller under low yielding conditions than entries selected for high yield in high yielding conditions. The shorter heights overall may have resulted in less differences seen at the organic locations. Although no significant height differences were found between methods of selection overall, contrasts performed on the organic and conventionally selected lines from each population showed three populations to have significant height differences between methods of selection (Figure 4.8). Figure 4.8 also shows the significant interaction between population and method of selection (Table 4.19), suggesting that the importance of height in competitive ability differs across populations.

When the four conventionally managed locations were combined, significant height differences between the two methods of selection were observed (Table 4.20), with the organically selected lines being significantly taller than the conventionally selected lines (Table 4.11). No significant difference was seen between the height of organically selected lines, conventionally selected lines, and the check cultivars (Table

4.11). Contrasts between the organic and conventionally selected lines from each population showed significant height differences for method of selection for only one population (Figure 4.9), although overall method of selection was significant. In contrast to the organic experiments, Figure 4.9 shows that there was no significant population by method of selection interactions at the conventional experiments (Table 4.20). This indicates that height plays a larger role in the competitive ability of populations under organic management than conventional management.

A positive correlation was found between grain yield and plant height at conventional locations (Table 6.12) but not organic locations (Table 6.11). This is surprising since grain yield is expected to be related to height under organic management, since plant height is used as a selection factor when selecting cultivars for weed suppressive abilities (Wolfe *et al.* 2008). Mason *et al.* (2007a) found grain yield to be positively correlated with height at high weed pressure. Other studies have found plant height to be an important factor in the competitive ability of cultivars, with increased height resulting in decreased weed biomass (Champion *et al.* 1998; Lemerle *et al.* 1996; Vandeleur and Gill 2004). In the present study taller lines were selected in organic selection compared to conventional selection, although only significantly taller when grown under conventional management (Table 4.11).

4.4.5 Direct versus Indirect Selection

An important study objective was to test if rank changes of populations and check cultivars occurred between the two management environments. Rankings across environments were performed to test the theory that direct selection is more effective

than indirect selection. Line rankings were used to demonstrate the importance of having separate breeding programs for organic and conventional crop production systems. Lines were ranked by percent of check mean for the variables yield, protein, TKW and height. Although statistics could not be performed on rank correlations between the two environments and methods of selection, populations that are responsive to selection environment can be identified by observing the rank changes across environments. Populations were considered to be responsive to selection environment if rank changes occurred for the two methods of selection between the two management environments. Populations were considered to be unresponsive to selection environment if there was no rank change or a very slight rank change between management environments. Ranking of check cultivars was also performed in an attempt to establish whether certain commercial wheat cultivars may be better suited to organic production than others.

For yield, the populations BC07, BC37 and BC42 were found to be responsive to selection environment (Table 4.21). Line rank changes occurred between management environments in the direction that would be expected, meaning populations selected under organic management yielded higher under organic management than conventional management, and populations selected under conventional management yielded higher under conventional management than organic management (Table 4.21).

Rank changes for yield occurred between management environments for check cultivars as well, indicating that some cultivars may be better suited for organic management than others. The check cultivar 5602HR had the highest yield under conventional management, but ranked near the bottom under organic management, while McKenzie performed well in both management environments (Table 4.21). McKenzie

has previously been identified as a cultivar with superior weed suppressive abilities (Mason *et al.* 2007a). Rank changes in check cultivars highlight the importance of proper varietal selection in organic agriculture. The most suitable cultivars for conventional agriculture cannot be expected to also be the most suitable for organic agriculture. This observation is in agreement with Ceccarelli and Grando (1991) and Murphy *et al.* (2007) who observed that identifying high yielding cultivars in conventional systems is not an efficient criterion to identify high yielding cultivars in organic systems.

More variation in yield was seen under organic management than conventional management. For example, lines yielded 75-115% and 82-108% of check mean under organic and conventional management, respectively (Table 4.12). In organic management the lines out yielded the check cultivars by a larger percentage than in conventional management. This observation has important implications as it suggests that selection under organic management has the potential to increase yields of organically-grown crops.

The lines with the highest protein concentration had small or no rank changes across management environment, and therefore were found to be unresponsive to selection environment (Table 4.22). Two populations, BC37 and BC11, were found to be responsive to selection environment, and of these only BC11 was found to interact with the check mean (Table 4.22). Both of the responsive populations had higher protein concentrations under organic management when organically selected than their conventionally selected counterparts.

Two of the check cultivars had large rank changes between management environments. AC Barrie was found to have a higher protein concentration under

conventional management, while Waskada was found to have a higher protein concentration under organic management than conventional management (Table 4.22). The other four check cultivars had relatively stable protein concentrations across management environment.

No populations were found to be responsive to selection environment for TKW. Many populations ranked the same across management environments, or had very small rank changes (Table 4.23). For height, on the other hand there were three populations that were found to be responsive to selection environment (Table 4.24). For the populations BC10 and BC41 the lines selected under organic management were taller under conventional management and the lines selected under conventional management were taller under organic management (Table 4.24).

The ranking tables show that direct selection under organic management resulted in higher performance for several traits when grown in organic systems. It is important to note that significant differences in method of selection are not found as often when individual locations were looked at. Combining locations increases the power of the statistical test and may be accentuating small differences. That said the results show that cultivar selection for organically managed environments should be conducted under organic growing conditions. Several studies have shown that cultivar selection for low-input environments should be conducted in low-input environments (Atlin and Frey 1989; Brancourt-Hulmel *et al.* 2005; Burger *et al.* 2008; Ceccarelli 1994; Murphy *et al.* 2007), making a case for separate breeding programs for low-input environments.

Direct selection in organic environments for organic environments resulted in higher yield, protein and TKW (Table 4.11). This indicates that in organic environments

different plant characteristics are being selected for that are more beneficial in organic growing conditions. This is supported by Lammerts van Bueren *et al.* (1999), who found that when selection is carried out under organic growing conditions, the cultivars can adapt to organic conditions related to the soil, disease and weed pressure.

Selection in organic environments was found to result in significantly higher protein concentrations and TKW for lines grown in conventional environments than direct selection at the conventional locations. Different results were seen for yield; the conventional location Brandon was the only location in which indirect selection in organic environments resulted in higher yields than direct selection in conventional environments. These results contrast those of Ceccarelli (1994), who found direct selection in conventional environments for conventional production to be more efficient than indirect selection in organic environments. Similar to the yield results for this study, Murphy *et al.* (2007) found direct selection to result in higher yields for conventional production only 2 out of 5 times. It is possible that selection under organic conditions results in the selection of characteristics that are desirable for both conventional and organic production.

Murphy *et al.* (2007) states that an organic breeding program would focus on traits such as improved nitrogen use efficiency, adaptation to soil microbes, and improved competitiveness against weeds. Organic selection is conducted under greater stress levels than conventional selection, therefore it is expected that more competitive plants will be selected in organic growing conditions, which may result in greater TKW and protein concentration. Although less of an emphasis is placed on competitive plants in conventional crop production it is still an important trait. The increased protein

concentration and TKW of organically selected lines indicate that these lines are more competitive and are therefore better able to uptake the nutrients available in the soil. The potential for grain protein production in organic agriculture has to be higher than in conventional to compensate for the lower N availability in organic systems (Wolfe *et al.* 2008). Lines selected under organic environments may also have had stronger associations with AMF, which are thought to facilitate increased uptake of N through the maintenance of soil fertility (Stark 2008) and access to nutrients lower in the soil profile (Ellis *et al.* 1985).

Table 4.8 Yield LS Means, ANOVA tests and contrasts for both organic and conventionally managed locations. ANOVA tests do not include check cultivars while contrasts compare check cultivars to all conventionally selected lines and check cultivars to all organically selected lines. The CV's for the organic locations are 8, 14, 7 and 6 for Carman 2007, Glenlea 2007, Glenlea 2008 and Oxbow, respectively. The CV's for the conventional locations are 5, 4, 17 and 5 for Brandon, Portage, Glenlea and Saskatoon, respectively.

			Organic Locations				Conventional Locations			
		df	Carman 07	Glenlea 07	Glenlea 08	Oxbow 08	Brandon 07	Portage 08	Glenlea 08	Sask 08
LS Means	Org Selection		2,476	1,781	2,746	2,240	2,490	3,427	2,856	4,457
	Con Selection		2,262	1,719	2,675	2,250	2,323	3,507	2,806	4,663
	Checks		2,189	1,790	2,816	2,283	2,220	3,664	2,973	4,915
ANOVA Tests	Population[1]	6	****	*	****	****	****	****	***	****
	Method[2]	1	***	ns	ns	ns	****	ns	ns	***
	Population x Method	6	ns	ns	**	ns	ns	**	ns	***
Contrasts	Check vs. Conv	1	ns	ns	ns	ns	ns	ns	ns	ns
	Check vs. Org	1	*	ns	ns	ns	ns	**	ns	**

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

Table 4.9 ANOVA tests and contrasts for yield at all organic locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.

ANOVA Tests	df	F Value	Pr > F	Significance
Population[1]	6	11.92	<0.0001	****
Method[2]	1	6.56	0.0117	**
Population x Method	6	0.89	0.5044	ns
SY[3]	3	59.53	<0.0001	****
Variety x SY	18	2.51	0.0016	***
Method x SY	3	2.68	0.0501	*
Contrasts	**df**	**T Value**	**Pr > T**	**Significance**
Check vs. Conv	215	-0.87	0.3869	ns
Check vs. Org	215	0.79	0.4311	ns

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment, [3] Site year
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

Table 4.10 ANOVA tests and contrasts for yield at all conventional locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.

ANOVA Tests	df	F Value	Pr > F	Significance
Population[1]	6	19.74	<0.0001	****
Method[2]	1	0.14	0.7119	ns
Population x Method	6	2.29	0.0396	**
SY[3]	3	88.53	<0.0001	****
Variety x SY	18	6.6	<0.0001	****
Method x SY	3	4.76	0.0036	***
Contrasts	**df**	**T Value**	**Pr > T**	**Significance**
Check vs. Conv	220	-1.47	0.1422	ns
Check vs. Org	220	-1.69	0.0924	*

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment, [3] Site year
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

Table 4.11 Yield, protein, 1000 kernel weight (KWT) and height LS means for organic and conventionally managed sites and organic and conventional selection, and check cultivars.

	Yield		Protein		KWT		Height	
	Management		Management		Management		Management	
Selection Method	Org	Conv	Org	Conv	Org	Conv	Org	Conv
Organic	2312a	3307a	14.86a	15.09a	32.46ac	34.25a	89.61a	99.32a
Conventional	2232b	3325ab	14.59b	14.91b	31.78b	33.57bc	88.75a	98.28b
Checks	2271c	3443b	14.34c	14.45c	32.8c	34.70c	89.5a	98.79ab

Means within the same management environment followed by the same letter within a column are not significantly different (P>0.05) according to Fischer's protected LSD.

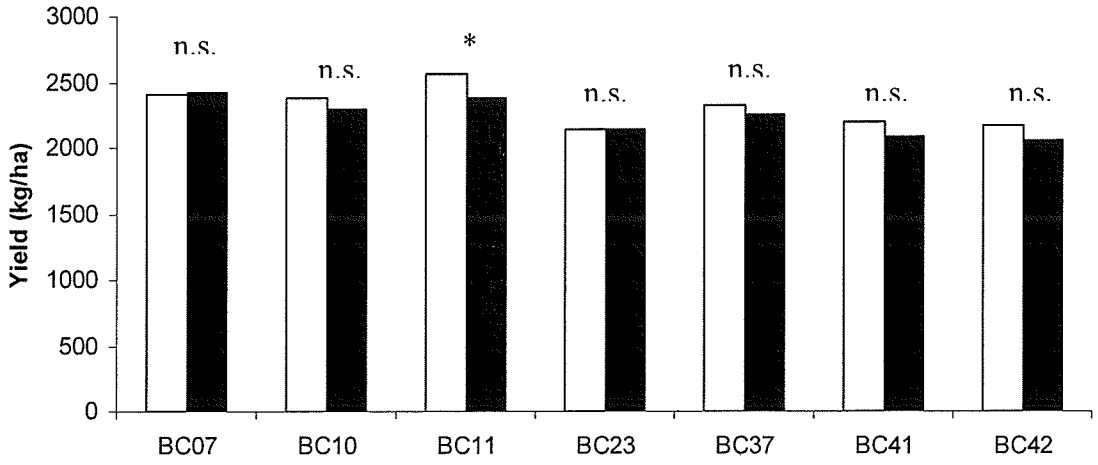

Figure 4.2 Average yield (kg/ha) of the seven organically (white) and conventionally (black) selected populations under organic management. Although the organically selected populations yielded higher overall than the conventionally selected populations, when contrasts were performed on the organic and conventionally selected lines from each population, only BC11 was found to differ significantly (*) ($P < 0.05$). No difference (n.s.) between method of selection was found for the other six populations ($P < 0.05$).

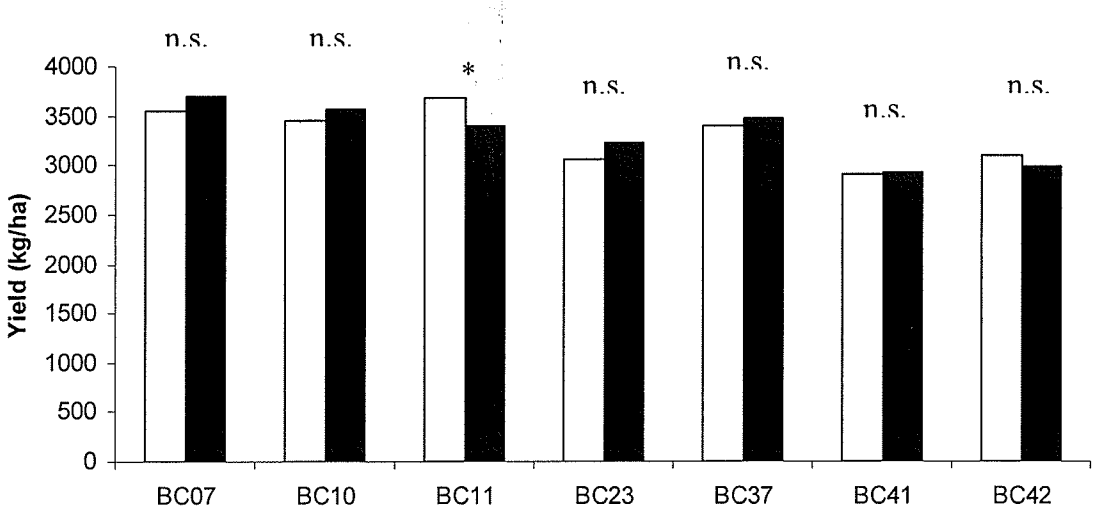

Figure 4.3 Average yield (kg/ha) of the seven organically (white) and conventionally (black) selected populations under conventional management. Contrasts were performed on the organic and conventionally selected lines from each population. A significant yield difference (*) was found for the population BC11, while no significant difference (n.s.) was found for the other six populations ($P < 0.05$).

Table 4.12 Protein LS Means, ANOVA tests and contrasts for both organic and conventionally managed locations. ANOVA tests do not include check cultivars while contrasts compare check cultivars to all conventionally selected lines and check cultivars to all organically selected lines.

			Organic Locations				Conventional Locations			
		df	Carman 07	Glenlea 07	Glenlea 08	Oxbow 08	Brandon 07	Portage 08	Glenlea 08	Sask 08
LS Means	Org Selection		16.7	16	12.5	14.2	17.4	12.8	14.7	15.4
	Conv Selection		16.3	15.7	12.4	14	16.9	12.8	14.7	15.2
	Checks		16.4	15.5	11.9	13.6	16	12.3	14.8	14.9
ANOVA Tests	Population[1]	6	****	**	****	ns	****	****	****	****
	Methods[2]	1	****	ns	*	ns	****	ns	ns	ns
	Population x Method	6	****	ns	**	ns	****	ns	ns	**
Contrasts	Check vs. Conv	1	ns	ns	**	ns	***	***	ns	ns
	Check vs. Org	1	*	*	***	***	****	***	ns	**

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

Table 4.13 ANOVA tests and contrasts for protein at all organic locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.

ANOVA Tests	df	F Value	Pr > F	Significance
Population[1]	6	15.08	<0.0001	****
Method[2]	1	14	0.0003	****
Population x Method	6	2.6	0.0211	**
SY[3]	3	332.62	<0.0001	****
Variety x SY	18	3.4	<0.0001	****
Method x SY	3	1.62	0.1891	ns
Contrasts	**df**	**T Value**	**Pr > T**	**Significance**
Check vs. Conv	220	2.21	0.0281	**
Check vs. Org	220	4.62	<0.0001	****

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment, [3] Site year
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

Table 4.14 ANOVA tests and contrasts for protein at all conventional locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.

ANOVA Tests	df	F Value	Pr > F	Significance
Population[1]	6	45.8	<0.0001	****
Method[2]	1	8.36	0.0045	***
Population x Method	6	9.28	<0.0001	****
SY[3]	3	134.47	<0.0001	****
Variety x SY	18	5.27	<0.0001	****
Method x SY	3	5.91	0.0008	****
Contrasts	**df**	**T Value**	**Pr > T**	**Significance**
Check vs. Conv	220	3.8	0.0002	****
Check vs. Org	220	5.31	<0.0001	****

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment, [3] Site year
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

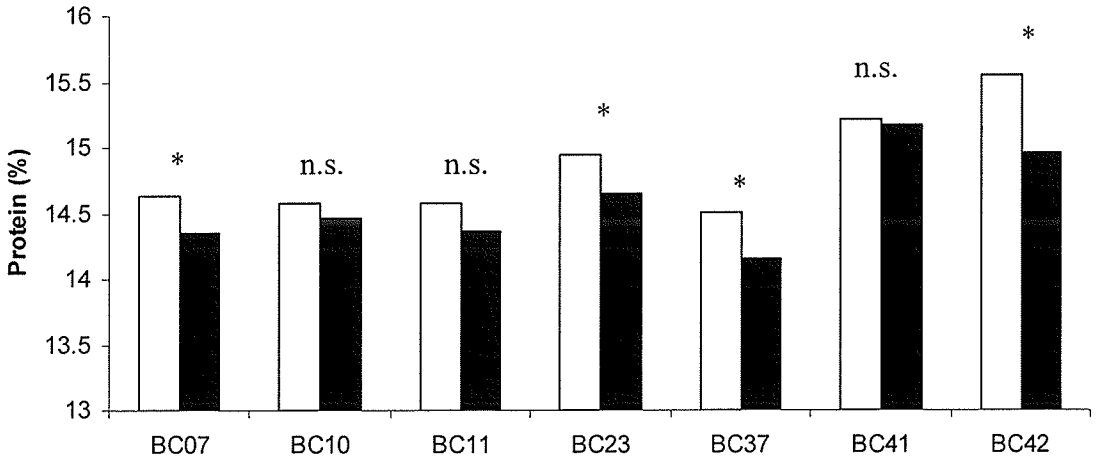

Figure 4.4: Average protein concentration (%) of the seven organically (white) and conventionally (black) selected populations under organic management. Significantly different protein concentrations (*) were found for four of the populations, BC07, BC23, BC37 and BC42, when contrasts were performed on the organic and conventionally selected lines, while no significant differences (n.s.) were found when comparing method of selection for the other three populations ($P < 0.05$).

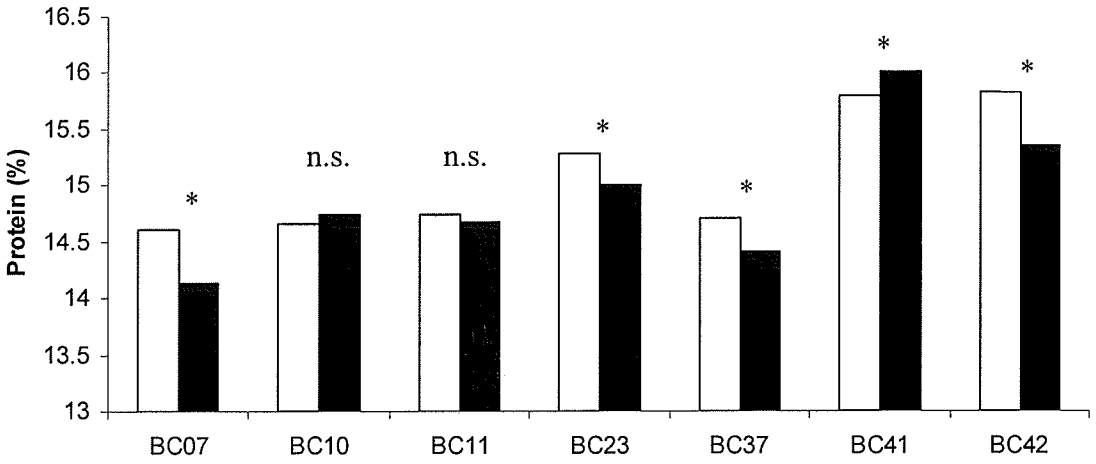

Figure 4.5: Average protein concentration (%) of the seven organic (white) and conventionally (black) selected populations under conventional management. Contrasts were performed on the organic and conventionally selected lines from each population. Significant protein concentration differences (*) were found for five of the seven populations, while BC10 and BC11 did not differ significantly (n.s.) between method of selection ($P < 0.05$).

Table 4.15 One thousand kernel weight LS Means, ANOVA tests and contrasts for both organic and conventionally managed locations. ANOVA tests do not include check cultivars while contrasts compare check cultivars to all conventionally selected lines and check cultivars to all organically selected lines.

			Organic Locations				Conventional Locations			
		df	Carman 07	Glenlea 07	Glenlea 08	Oxbow 08	Brandon 07	Portage 08	Glenlea 08	Sask 08
LS Means	Org Selection		31.8	31.5	33.7	32.9	28.6	37.4 (37.6)	34	36.9
	Conv Selection		30.9	30.9	32.6	32.7	28.2 (27.6)	36.9	33.8	36
	Checks		31.7	30.9	34.6	34.1	28.4	38.6	34.9	37
ANOVA Tests	Population[1]	6	****	****	****	****	****	****	****	****
	Methods[2]	1	**	ns	****	ns	***	***	ns	**
	Population x Method	6	ns	ns	ns	ns	ns	ns	ns	***
Contrasts	Check vs. Conv	1	ns	ns	***	**	ns	***	**	ns
	Check vs. Org	1	ns	ns	ns	*	ns	*	ns	ns

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

Table 4.16 ANOVA tests and contrasts for 1000 kernel weight at all organic locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.

ANOVA Tests	df	F Value	Pr > F	Significance
Population[1]	6	91.28	<0.0001	****
Method[2]	1	25.8	<0.0001	****
Population x Method	6	2.67	0.0182	**
SY[3]	3	16.67	0.0008	****
Variety x SY	18	2.07	0.0109	**
Method x SY	3	4.49	0.005	***
Contrasts	**df**	**T Value**	**Pr > T**	**Significance**
Check vs. Conv	220	-3.2	0.0016	***
Check vs. Org	220	-1.06	0.2884	ns

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment, [3] Site year
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

Table 4.17 ANOVA tests and contrasts for 1000 kernel weight at all conventional locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.

ANOVA Tests	df	F Value	Pr > F	Significance
Population[1]	6	113.95	<0.0001	****
Method[2]	1	27.14	<0.0001	****
Population x Method	6	3.65	0.0023	***
SY[3]	3	572.66	<0.0001	****
Variety x SY	18	7.03	<0.0001	****
Method x SY	3	1.75	0.1601	ns
Contrasts	**df**	**T Value**	**Pr > T**	**Significance**
Check vs. Conv	217	-3.11	0.0021	***
Check vs. Org	217	-1.35	0.1785	ns

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment, [3] Site year
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

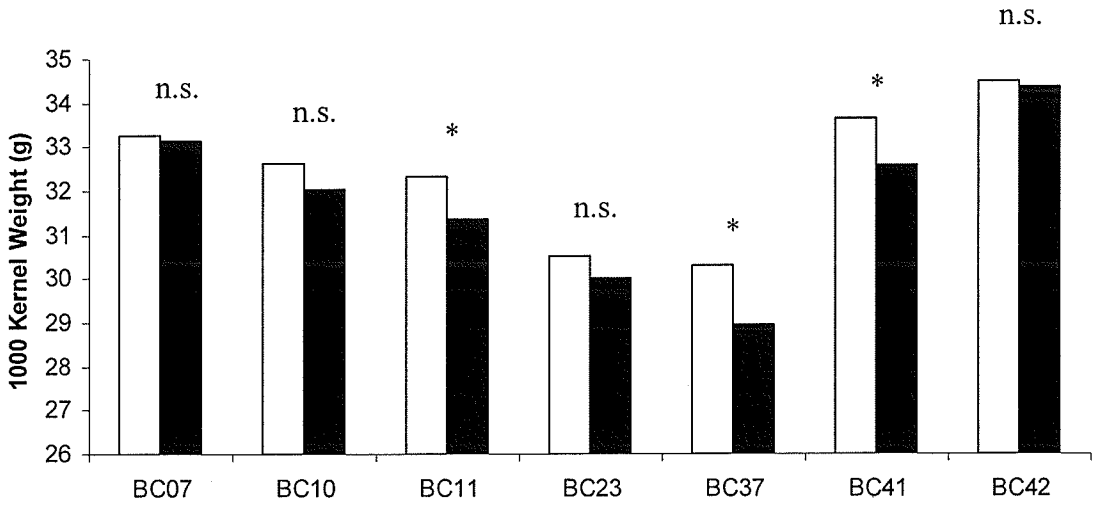

Figure 4.6 Average 1000 kernel weight (g) of the seven organic (white) and conventionally (black) selected populations under organic management. Contrasts were performed on the organic and conventionally selected lines from each population, and significant differences (*) were found for the populations BC11, BC37 and BC41 ($P < 0.05$). The difference in 1000 kernel weight between organic and conventional method of selection for the populations BC07, BC10, BC23 and BC42 were not significant (n.s.) ($P < 0.05$).

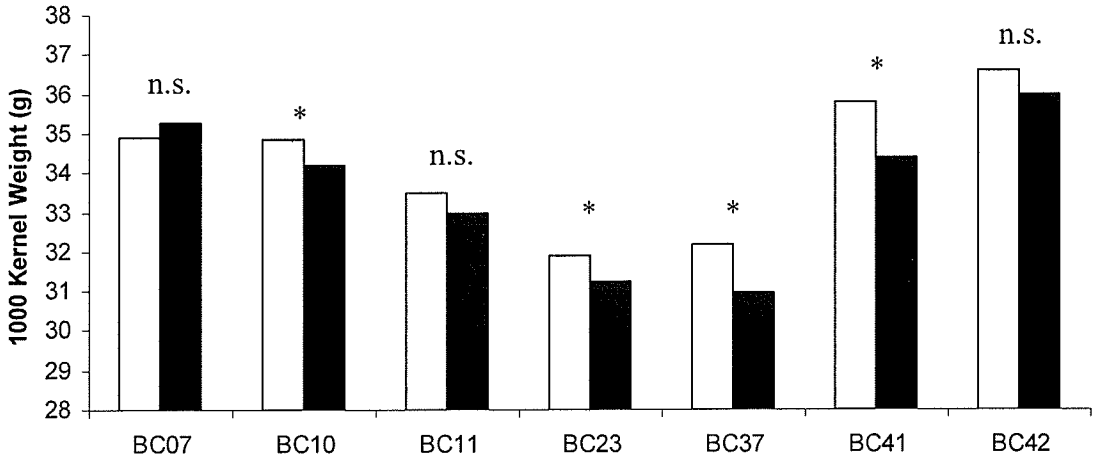

Figure 4.7 Average 1000 kernel weight (g) of the seven organic (white) and conventionally (black) selected populations under conventional management. Contrasts were performed on the organic and conventionally selected lines from each population, and significant differences (*) were found for the populations BC10, BC23, BC37 and BC41 ($P < 0.05$). There were no significant differences (n.s.) between the organic and conventionally selected lines in the remaining three populations ($P < 0.05$).

Table 4.18 Height LS Means, ANOVA tests and contrasts for both organic and conventionally managed locations. ANOVA tests do not include check cultivars while contrasts compare check cultivars to all conventionally selected lines and check cultivars to all organically selected lines.

			Organic Locations				Conventional Locations			
		df	Carman 07	Glenlea 07	Glenlea 08	Oxbow 08	Brandon 07	Portage 08	Glenlea 08	Sask 08
LS Means	Org Selection		99.2	94.1	85.2	79.6	103.7	96.5	87.6	109.4
	Conv Selection		97.7	91.7	84.9	80.6	102.5	95.4	84.8	110.3
	Checks		100.5	98.3	89.4	81.8	102.4	96.6	87.1	108.9
ANOVA Tests	Population[1]	6	****	**	****	ns	****	****	****	****
	Methods[2]	1	*	ns	ns	ns	*	ns	**	ns
	Population x Method	6	***	ns	*	ns	**	ns	ns	ns
Contrasts	Check vs. Conv	1	ns	**	*	ns	ns	ns	ns	ns
	Check vs. Org	1	ns	ns	*	ns	ns	ns	ns	ns

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

Table 4.19 ANOVA tests and contrasts for height at all organic locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.

ANOVA Tests	df	F Value	Pr > F	Significance
Population[1]	6	11.24	<0.0001	****
Method[2]	1	1.96	0.1644	ns
Population x Method	6	3.7	0.0021	***
SY[3]	3	34.09	<0.0001	****
Variety x SY	18	0.96	0.5147	ns
Method x SY	3	1.1	0.3535	ns
Contrasts	**df**	**F Value**	**Pr > F**	**Significance**
Check vs. Conv	208	0.9	0.3441	ns
Check vs. Org	208	0.02	0.8864	ns

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment, [3] Site year
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

Table 4.20 ANOVA tests and contrasts for height at all conventional locations. ANOVA tests do not include check cultivars, while contrasts are comparing the check cultivars to all conventionally selected lines and check cultivars to all organically selected lines under organic management.

ANOVA Tests	df	F Value	Pr > F	Significance
Population[1]	6	39.09	<0.0001	****
Method[2]	1	6.34	0.0131	**
Population x Method	6	1.29	0.2666	ns
SY[3]	3	224.29	<0.0001	****
Variety x SY	18	1.76	0.0376	**
Method x SY	3	3.08	0.0299	**
Contrasts	**df**	**F Value**	**Pr > F**	**Significance**
Check vs. Conv	208	0.35	0.5560	ns
Check vs. Org	208	0.37	0.5424	ns

[1] A bulk of genetically similar material, [2] Organic or conventional selection environment, [3] Site year
n.s. (not significant at P<0.1), *(P<0.1), **(P<0.5), ***(P<0.01). ****(P<0.001)

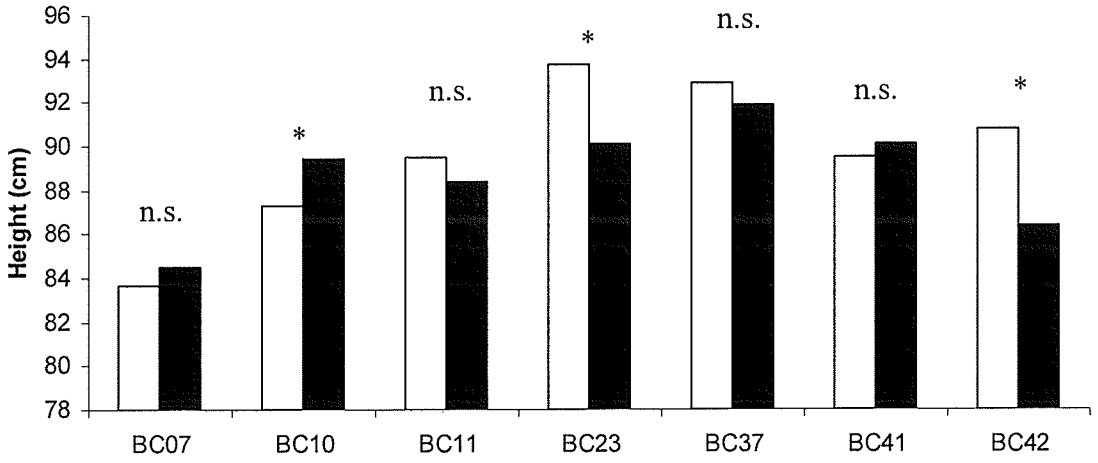

Figure 4.8: Average height (cm) of the seven organic (white) and conventionally (black) selected populations under organic management. Contrasts were performed on the organic and conventionally selected lines from each population, and significant differences (*) were found for the populations BC10, BC23 and BC42, while no difference (n.s.) was found for the remaining four populations ($P < 0.05$).

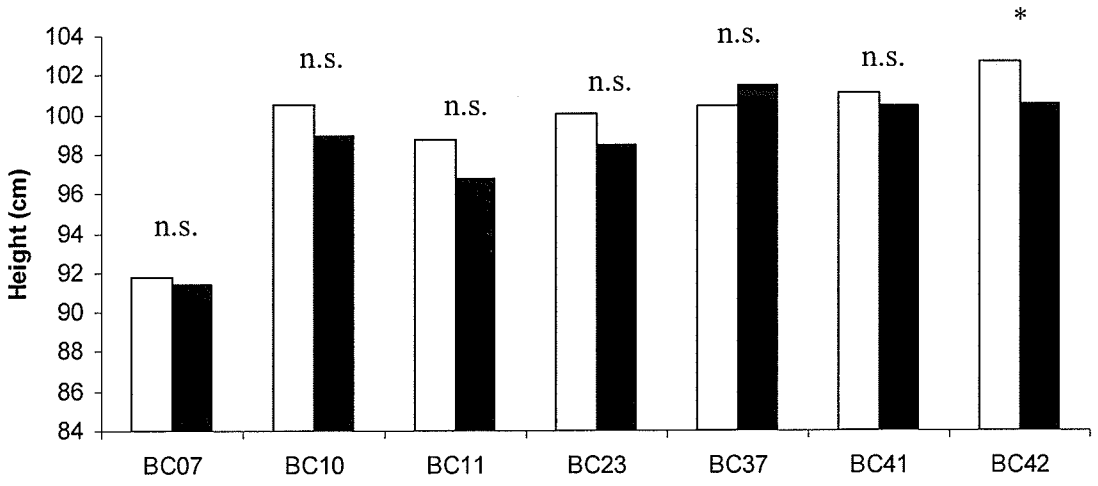

Figure 4.9: Average height (cm) of the seven organic (white) and conventionally (black) selected populations under conventional management. Contrasts were performed on the organic and conventionally selected lines from each population. A significant height difference was found for the population BC42 (*), while no significant difference (n.s.) was found for the other six populations.

Table 4.21 Yield, yield as a percent of check mean and ranking of the 20 treatments compared across organic and conventional management environments. Check mean includes all modern Canadian Western Red Spring wheat checks, and therefore does not include the check cultivar Red Fife. Italicized font and dashed lines are the populations selected under organic management. Underlined font and lines are the populations selected under conventional management. Bolded font and lines are the check cultivars. Lines highlight the change in rank between the populations when grown under two management environments. BC07, BC37 and BC42 are considered to be responsive to selection environment since rank changes occurred for the two methods of selection between the two management environments.

Organic Management				Conventional Management			
kg/ha	% of check mean	Rank	Line	Line	Rank	% of check mean	kg/ha
2594	114.4	1	**McKenzie**	**5602HR**	1	108.4	3830
2579	113.7	2	*BC11*	**McKenzie**	2	106.0	3745
2470	108.9	3	**Waskada**	BC07	3	105.9	3742
2399	105.8	4	*BC07*	*BC11*	4	104.2	3683
2386	105.2	5	**Superb**	**Superb**	5	101.7	3594
2361	104.1	6	BC11	**Waskada**	6	100.1	3537
2339	103.1	7	BC10	**Check Mean**	7	100.0	3534
2321	102.4	8	**Red Fife**	BC10	8	99.6	3521
2313	102.0	9	*BC37*	BC37	9	99.4	3511
2312	101.9	10	*BC10*	*BC07*	10	99.2	3505
2295	101.2	11	BC07	*BC10*	11	99.0	3499
2268	100.0	12	**Check Mean**	BC11	12	96.1	3395
2260	99.7	13	BC37	*BC37*	13	95.1	3360
2210	97.4	14	*BC42*	BC23	14	89.8	3173
2195	96.8	15	*BC41*	*BC23*	15	88.3	3120
2193	96.7	16	**5602 HR**	*BC42*	16	87.3	3086
2154	95.0	17	*BC23*	BC42	17	84.9	2999
2133	94.0	18	BC23	**Red Fife**	18	84.6	2989
2091	92.2	19	BC41	**AC Barrie**	19	83.8	2963
2018	89.0	20	BC42	BC41	20	83.0	2931
1696	74.8	21	**AC Barrie**	*BC41*	21	82.0	2898

Table 4.22 Protein concentrations, protein concentration as a percent of check mean and ranking of the 20 treatments compared across organic and conventional management environments. Check mean includes all modern Canadian Western Red Spring wheat checks, and therefore does not include the check cultivar Red Fife. Italicized font and dashed lines are the populations selected under organic management. Underlined font and lines are the populations selected under conventional management. Bolded font and lines are the check cultivars. Lines highlight the change in rank between the populations when grown under two management environments. BC11 and BC37 are considered to be responsive to selection environment since rank changes occurred for the two methods of selection between the two management environments.

Organic Management				Conventional Management			
Protein	% of check mean	Rank	Line	Line	Rank	% of check mean	Protein
15.65	107.7	1	*BC42*	*BC41*	1	109.2	16.01
15.21	104.7	2	BC41	BC41	2	107.6	15.78
15.17	104.4	3	*BC41*	*BC42*	3	107.6	15.78
15.02	103.3	4	BC42	BC42	4	105.1	15.41
14.98	103.1	5	**5602HR**	*BC23*	5	104.0	15.25
14.96	102.9	6	*BC23*	**AC Barrie**	6	103.2	15.14
14.64	100.7	7	*BC07*	BC23	7	102.6	15.05
14.63	100.7	8	BC23	**5602HR**	8	101.5	14.88
14.62	100.6	9	*BC37*	BC11	9	100.8	14.79
14.55	100.1	10	**Waskada**	BC10	10	100.5	14.74
14.54	100.0	11	**AC Barrie**	*BC10*	11	100.0	14.67
14.534	100.0	12	**Check Mean**	*BC37*	12	100.0	14.67
14.52	99.9	13	*BC11*	**Check Mean**	13	100.0	14.666
14.45	99.4	14	*BC10*	*BC07*	14	99.8	14.63
14.43	99.3	15	BC10	*BC11*	15	99.8	14.63
14.43	99.3	16	BC11	**McKenzie**	16	99.5	14.59
14.35	98.7	17	BC07	BC37	17	98.5	14.45
14.33	98.6	18	**Superb**	**Superb**	18	98.5	14.44
14.27	98.2	19	**McKenzie**	**Waskada**	19	97.4	14.28
14.04	96.6	20	BC37	BC07	20	96.3	14.12
13.39	92.1	21	**Red Fife**	**Red Fife**	21	91.0	13.34

Table 4.23 1000 kernel weight (KWT), KWT as a percent of check mean and ranking of the 20 treatments compared across organic and conventional management environments. Check mean includes all modern Canadian Western Red Spring wheat checks, and therefore does not include the check cultivar Red Fife. Italicized font and dashed lines are the populations selected under organic management. Underlined font and lines are the populations selected under conventional management. Bolded font and lines are the check cultivars. Lines highlight the change in rank between the populations when grown under two management environments. No populations were considered to be responsive to selection environment.

Organic Management				Conventional Management			
kwt (g)	% of check mean	Rank	Line	Line	Rank	% of check mean	kwt (g)
35.52	109.5	1	**Superb**	**Red Fife**	1	108.3	37.06
34.67	106.9	2	**Red Fife**	**Superb**	2	106.7	36.49
34.65	106.8	3	*BC42*	*BC42*	3	106.7	36.49
34.42	106.1	4	BC42	BC42	4	105.5	36.1
33.71	103.9	5	*BC41*	*BC41*	5	104.6	35.78
33.24	102.5	6	*BC07*	BC07	6	103.3	35.35
33.21	102.4	7	BC07	*BC10*	7	102.2	34.97
32.58	100.5	8	**Waskada**	*BC07*	8	101.8	34.82
32.54	100.3	9	BC41	**Waskada**	9	100.8	34.47
32.47	100.1	10	*BC10*	BC41	10	100.5	34.39
32.42	100.0	11	**Check Mean**	**Check Mean**	11	100.0	34.212
32.33	99.7	12	**5602HR**	BC10	12	99.7	34.1
32.31	99.6	13	*BC11*	**AC Barrie**	13	99.6	34.09
31.96	98.6	14	BC10	**5602HR**	14	97.9	33.51
31.4	96.8	15	BC11	*BC11*	15	97.9	33.5
31.2	96.2	16	**McKenzie**	BC11	16	96.3	32.96
30.52	94.1	17	**AC Barrie**	**McKenzie**	17	95.0	32.5
30.5	94.0	18	*BC23*	*BC37*	18	94.5	32.33
30.36	93.6	19	*BC37*	*BC23*	19	93.1	31.86
30.03	92.6	20	BC23	BC23	20	91.1	31.17
28.92	89.2	21	BC37	BC37	21	90.1	30.84

Table 4.24 Height (cm), height as a percent of check mean and ranking of the 20 treatments compared across organic and conventional management environments. Check mean includes all modern Canadian Western Red Spring wheat checks, and therefore does not include the check cultivar Red Fife. Italicized font and dashed lines are the populations selected under organic management. Underlined font and lines are the populations selected under conventional management. Bolded font and lines are the check cultivars. Lines highlight the change in rank between the populations when grown under two management environments. BC10, BC37 and BC40 are considered to be responsive to selection environment since rank changes occurred for the two methods of selection between the two management environments.

Organic Management				Conventional Management			
cm	% of check mean	rank	line	line	rank	% of check mean	cm
108.54	121.27	1	**Red Fife**	**Red Fife**	1	116.70	112.17
94.58	105.68	2	**Waskada**	*BC42*	2	106.64	102.50
93.33	104.28	3	*BC23*	BC37	3	105.48	101.38
93.13	104.05	4	*BC37*	*BC41*	4	105.04	100.96
91.67	102.42	5	BC37	*BC10*	5	104.90	100.83
90.63	101.26	6	*BC42*	BC42	6	104.82	100.75
90.63	101.26	7	**McKenzie**	BC41	7	104.60	100.54
90.42	101.02	8	BC23	*BC37*	8	104.56	100.50
90.21	100.79	9	**5602 HR**	*BC23*	9	104.34	100.29
89.79	100.33	10	BC41	**Waskada**	10	103.91	99.88
89.79	100.33	11	BC10	*BC11*	11	102.74	98.75
89.79	100.33	12	*BC41*	BC10	12	102.57	98.58
89.79	100.33	13	*BC11*	BC23	13	102.18	98.21
89.50	100.00	14	**Check Mean**	**AC Barrie**	14	101.53	97.58
88.13	98.46	15	BC11	BC11	15	100.62	96.71
88.13	98.46	16	**AC Barrie**	**5602 HR**	16	100.53	96.63
87.71	98.00	17	BC42	**McKenzie**	17	100.23	96.33
86.25	96.37	18	*BC10*	**Check Mean**	18	100.00	96.12
84.38	94.27	19	*BC07*	BC07	19	95.50	91.79
83.96	93.81	20	**Superb**	*BC07*	20	95.11	91.42
83.75	93.58	21	BC07	**Superb**	21	93.81	90.17

4.5 Conclusions

Selection environment was found to affect yield, protein, TKW, and height in both organic and conventional management environments. At most locations, Brandon was the exception, direct selection resulted in higher yield than indirect selection. This observation supports similar conclusions by previous researchers.

Organically selected lines produced higher protein concentrations and TKW under both organic and conventional management environments. Selection in organic environments can be considered to be selection under stress, and may result in more competitive lines better adapted to lower nutrient levels, which may be beneficial for both organic and conventional crop production. In the case of genotypes targeted to organic agriculture, selection in organically managed field conditions offers advantages to indirect selection in conventionally managed field conditions. Organic farmers would benefit from organic breeding programs.

This study has shown that the lines with the highest yield and best quality under organic management may differ from those that perform best under conventional management. Populations responsive to selection environment can be identified for all variables but TKW. Rank changes also occurred in registered cultivars, indicating that organic and conventional farmers should be choosing different cultivars. Direct selection was found to be more effective for lines to be grown in organically managed environments, while the results are inconclusive for conventionally managed environments. Direct selection may be of less importance in conventional compared to organic agriculture because conventional growing environments are made similar through the use of fertilizer, herbicides, and fungicides.

5. GENERAL DISCUSSION AND CONCLUSIONS

5.1 Findings

This study was designed to answer questions pertaining to cultivar selection in organic agricultural production. The studies on arbuscular mycorrhizal fungal colonization were performed to discover what wheat cultivars were colonized by AMF and possibly more suitable for the low nutrient environments associated with organic crop production systems. AMF colonization was found to differ between cultivars, indicating that some cultivars may form stronger associations with mycorrhiza and may be better suited to organic agriculture. The hypothesis that heritage cultivars would have higher AMF colonization than modern cultivars was shown not to be true. Although modern cultivars had higher colonization than heritage cultivars, colonization was not found to be strongly related to wheat cultivar date of release or yield performance.

The study on organic and conventional crop selection environments demonstrated that there is value in breeding wheat for organic production. Selection environment affected yield, protein, 1000 kernel weight and height of the lines. Direct selection in organic environments for organic cropping systems resulted in higher yields, protein concentration and thousand kernel weight than indirect selection in conventional environments. The lines with the highest yield and protein under organic management differed from those with the highest yield and protein under conventional management. These apparent interactions between selection outcomes with selection environment suggest organic farmers could benefit from organic plant breeding programs.

5.2 Strength of the AMF Association with Crop Plants

Assessments of a range of parental wheat breeding lines and modern and heritage wheat cultivars did not support the theory that AMF colonization is greater in older wheat cultivars. The theory that mycorrhizal responsiveness has been bred out of modern wheat cultivars has been proposed by Hetrick *et al.* (1995), who found evidence that responsiveness to AMF is a heritable trait. The Hetrick *et al.* (1995) study found mycorrhizal responsiveness to be strong in cultivars released prior to 1975, while cultivars released after 1975 lacked a significant response to AMF. An earlier study found mycorrhizal responsiveness to be greatest in Asian landraces, less in older, introduced or improved wheat cultivars, and lowest in modern cultivars of wheat (Hetrick *et al.* 1992). This theory suggests that crop selection under the phosphorus rich conditions associated with conventional agriculture is selecting for crops that do not form strong associations with AMF.

With the advent of synthetic phosphorus fertilizer, the AMF association may have become less important for crops grown on land with readily available P fertilizer. It is also possible that modern cultivars have better phosphorus uptake and use efficiencies than heritage cultivars.

AMF is an ancient symbiosis that is thought to have been associated with the first land plants (Peterson *et al.* 2004) and instrumental in the success of early land plants (Redecker 2002). Since land plants have been associated with AMF since their beginnings, it is unlikely that in the comparatively short time period synthetic fertilizers have been in use crops would lose the genetic ability to form strong associations with AMF.

It would be beneficial to investigate how root architecture and rooting depth have changed throughout the course of plant selection. Cultivars with larger and more fibrous root systems that are able to explore larger soil volumes would have less need for a symbiotic relationship with AMF than cultivars with a more compact root system. The shift towards semi-dwarf wheat varieties has resulted in cultivars with lower levels of mycorrhizal responsiveness (Hetrick *et al.* 1995), which may be associated with changes in the plants root system. However, FBC Dylan may be a semidwarf yet it demonstrated high colonization and good performance in organic growing conditions (Tables 3.6 and 3.7). The discovery of patterns in plant responsiveness to AMF proves difficult.

5.3 AMF in Plant Breeding

Organic and conventional agricultural systems would benefit from crops that are able to form strong association with AMF to increase their uptake of nutrients. Prior to incorporating AMF into a breeding program a better way to quantify the cultivars response to colonization is required as well as yield response due to colonization. Percent colonization by arbuscules does not account for the benefit the plant may be receiving from AMF colonization. Further studies investigating cultivar response to colonization by comparing plant growth in the absence and presence of AMF would be beneficial. Although two cultivars may have similar percent colonization they may respond differently to colonization. A limitation of this research is the inability to conduct it under field conditions since growing plants in the absence of AMF requires sterilized soil.

A second important consideration is the length of time required to assess AMF in the roots of plants. Due to the labour required to collect, wash, stain roots and count AMF structures under a microscope, and compound this with the large number of lines in a breeding program, characterizing colonization is beyond the capacity of most breeding programs. It would be beneficial to find some form of indirect selection technique to ensure that breeding lines are receiving a large benefit from AMF colonization. Alternatives to assessing colonization in each line may include looking at parental lines and how response to colonization is inherited, the use of molecular markers, or finding plant traits with strong associations to mycorrhizal colonization that can be easily quantified.

The value of AMF colonization is difficult to demonstrate under field conditions since AMF colonization was not found to be a reliable predictor of yields. In the second study AMF colonization was only associated with higher yields in one site year out of four (Table 6.10). In the second study McKenzie was found to have approximately 50% of the level of colonization as 5602HR and yields between the two cultivars were not significantly different (Table 3.6), while in the organic conventional comparison experiments 5602HR was significantly lower yielding than McKenzie in organically managed test sites (Table 4.20). A stronger correlation between colonization and yield or a better way to assess the benefit a cultivar is receiving from AMF are needed in order to rationalize spending the time and money assessing AMF colonization in wheat cultivars. It is possible that plant characteristics such as root architecture and nutrient use efficiency may confound detection of response to AMF colonization. The relative importance of AMF to wheat compared to that of other plant characteristics needs to be

further considered. Given the inconclusive value that AMF colonization may have on performance of spring wheat and the expense required to assess this trait, selection for AMF colonization in wheat is unlikely to become a breeding objective in the near future.

5.4 Organic Plant Breeding

Organic and conventional farmers should be using different crop cultivars, and organic agriculture would benefit from breeding programs tailored specifically to organic growing conditions. The question of what crop characteristics are most important for organic farming remains. More research is required to investigate traits such as height, plant architecture, rooting density and depth, AMF colonization, tolerance to in-crop harrowing, weed competitiveness and how these characteristics interact and contribute to a superior organic crop cultivar. The below ground plant characteristics are the hardest to investigate but may have the largest affect on the competitive ability of a crop cultivar and specifically address where the primary differences between organic and conventional farming systems differ.

A challenge of crop selection in organic growing environments is the lack of uniform weed pressure across a site. Although experimental sites are chosen to be as uniform as possible certain weed species such as Canada Thistle (*Cirsium arvense*) and Wild Oat (*Avena sativa*) are prevalent southern Manitoba and tend to occur in patches. Replicated yield trials are preferred since a replicate could be discarded in the event of an unexpected and patchy weed problem. There is no immediate solution to this problem considering that the ability of the crop to compete with weeds is an important breeding objective and requires this research to be conducted on organic land.

Crop breeding in Canada is primarily done by large institutions, public and private depending on crop type. It has been suggested that plant breeding efforts for organic agriculture would also benefit from more producer participation (Ceccarelli and Grando 2007). Conducting research in producer's fields with farmer participation would ensure that the crop cultivars being developed are relevant to the needs of the farmer and well adapted to real life growing conditions. Years of on-farm observation and knowledge passed down throughout generations make farmers the obvious choice for good plant selection. Farmers involved in a participatory breeding program could receive a segregating population from a research centre, rogue unfavourable plants throughout the growing season, harvest the best plants, and then plant the next generation of the population and continue to select the best plants. This form of crop selection would increase the stability of the system by reducing genetic homogeneity and increasing diversity, while increasing the efficiency of a breeding program because larger numbers of early generation breeding lines could be assessed in more environments than are typically available to a typical western Canadian breeding program. Currently the highly regulated nature of wheat production from seed to sales would necessitate an assessment of farmer breeding line selections by accredited plant breeders that use approved testing procedures that are required for registration of wheat cultivars in Canada.

6. LITERATURE CITED

Abbott, L.K. and Robson, A.D. 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agric. Ecosys. Environ. **35**: 121-150.

Abbot, L.K., Robson, A.D., Jasper, D.A., and C. Gazey. 1992. What is the role of VA mycorrhizal hyphae in soil? Pages 37-41 *in* D.J. Read, D.H. Lewis, A.H. Fitter, and I.J. Alexander, eds. Mycorrhizas in ecosystems. C.A.B. International

Alberta Agriculture, Food and Rural Development. 2001. Wheat Utilization. [Online] Available: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/crop1274 [2008 Apr 17].

Alberta Agriculture, Food and Rural Development. 1998. Crop nutrition and fertilizer requirements. [Online] Available: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex3791 [2009 Aug 8].

Al-Karaki, G., McMichael, B., and Zak, J. 2004. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza **14**: 263-269.

Atlin, G.N. and Frey K.J. 1990. Selecting oat lines for yield in low-productivity environments. Crop Science **30**: 556-561.

Atlin, G.N. and Frey K.J. 1989. Predicting the relative effectiveness of direct versus indirect selection for oat yield in three types of stress environments. Euphytica **44**: 137-142.

Azcón-Aguilar, C. and Barea, J.M. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. Mycorrhiza **6**: 457-464.

Baon, J.B., Smith, S.E., and Alston, A.M. 1993. Mycorrhizal responses of barley cultivars differing in P efficiency. Plant Soil **157**: 97-105.

Bécard, G., and Piché, Y. 1989. Fungal growth stimulation by CO_2 and root exudates in vesicular-arbuscular mycorrhizal symbiosis. Appl. Environ. Microbiol. **55**: 2320-2325.

Berta, G., Fusconi, A., and Trotta, A. 1993. VA Mycorrhizal Infection and the Morphology and Function of Root Systems. Environmental and Experimental Botany. **33**: 159-173.

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

Bond, W. and Grundy, A.C. 2001. Non-chemical weed management in organic farming systems. Weed Research **41**: 383-405.

Bonfante, P. and Perotto, S. 1995. Strategies of arbuscular mycorrhizal fungi when infecting host plants. New Phytol. **130**: 3-21.

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

Brancourt-Hulmel, M., Heumez, E., Pluchard, P., Beghin, D., Depatureaux, C., Giraud, A., and Le Gouis, J. 2005. Indirect versus direct selection of winter wheat for low-input or high-input levels. Crop Sci. **45**: 1427-1431.

Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N. 1996. Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. Canberra, Australia. 374 pp.

Bücking, H. and Shacher-Hill, Y. 2005. Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability. New Phytol. **165**: 899-912.

Burger, H., Schloen, M., Schmidt, W. Geiger, H.H. 2008. Quantitative genetic studies on breeding maize for adaptation to organic farming. Euphytica. **163**: 501-510.

Canadian General Standards Board (CGSB). 2006. Organic Production Systems – General principles and management standards. Publication CAN/CGSB-32.310-2006. [Online] Available: http://www.tpsgc-pwgsc.gc.ca/cgsb/on_the_net/organic/032_0310_2006-e.pdf [2008 Oct 29].

Canadian Wheat Board. 2008. 2008 Canadian wheat board variety survey. [Online] Available: http://www.cwb.ca/public/en/farmers/surveys/variety/08-09/pdf/0809results.pdf [5 Nov 2008].

Carr, P.M., Kandel, H.J., Porter, P.M., Horsley, R.D., and Zwinger, S.F. 2006. Wheat cultivar performance on certified organic fields in Minnesota and North Dakota. Crop Sci. **46**: 1963-1971.

Cavagnaro, T.R., Gao, L-.L., Smith, F.A., and Smith, S.E. 2001. Morphology of arbuscular mycorrhizas is influenced by fungal identity. New Phytol. **151**: 469-475.

Ceccarelli, S. 1994. Specific adaptation and breeding for marginal conditions. Euphytica **77**: 205-219.

Ceccarelli, S. 1987. Yield potential and drought tolerance of segregating populations of barley in contrasting environments. Euphytica **36**: 265-273.

Ceccarelli, S. and Grando, S. 1991. Environment of selection and type of germplasm in barley breeding for low-yielding conditions. Euphytica **57**: 207-219.

Ceccarelli, S. and Grando, S. 2007. Decentralized-participatory plant breeding: an example of demand driven research. Euphytica **155**: 349-360.

Champion, G.T., Froud-Williams, R.J., and Holland, J.M. 1998. Interactions between wheat (*Triticum aestivum* L.) cultivars, row spacing and density and the effect on weed suppression and crop yield. Ann. Appl. Biol. **133**: 443-453.

Cosser, N.D., Gooding, M.J., Thompson, A.J., and Froud-Williams, R.J. 1997. Competitive ability and tolerance of organically grown wheat cultivars to natural weed infestations. Ann. Appl. Biol. **130**: 523-535.

Cox, G., Moran, K.J., Sanders, F., Nockolds, C., and Tinker, P.B. 1980. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. III. Polyphosphate granules and phosphorus translocation. New Phytol. **84**: 649-659.

Curtis, B.C. 2002. Wheat in the world. *In* B.C. Curtis, S. Rajaram, and H. Gómez Macpherson, eds. Bread wheat improvement and selection. Food and Agriculture Organization of the United Nations. [Online] Available: http://www.fao.org/DOCREP/006/Y4011E/y4011e04.htm#bm04 [4 Nov 2008].

Dalpé, Y. 2003. Arbuscular mycorrhizae. Agriculture and Agri-food Canada. [Online] Available: http://res2.agr.gc.ca/ecorc/mycor/index_e.htm [2007 Jan 20].

Dalpé, Y. 1997. Biodiversity of mycorrhizal fungi. Agriculture and Agri-food Canada. [Online] Available: http://res2.agr.gc.ca/ecorc/mycor/bio_sols_e.htm [2007 Jan 20].

Douds, D.D., Galvez, L., Janke, R.R., and Wagoner, P. 1995. Effects of tillage and farming system upon populations and distribution of vesicular-arbuscular mycorrhizal fungi. Agric. Ecosystems Environ. **52**: 111-118.

Douds, D.D., Janke, R.R., and Peters, S.E. 1993. VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. Agric. Ecosystems Environ. **43**: 325-335.

Ellis, J.R., Larsen, H.J., and Boosalis, M.G. 1985. Drought resistance of wheat plants inoculated with vesicular-arbuscular mycorrhizae. Plant Soil **86**: 369-378.

Entry, J.A., Rygiewicz, P.T., Watrud, L.S., and Donelly, P.K. 2002. Influence of adverse soil conditions on the formation and function of *Arbuscular mycorrhizas*. Advances in Environmental Research 7: 123-138.

Entz, M.H., Guilford, R., and Gulden, R. 2001. Crop yield and soil nutrient status on 14 organic farms in the eastern portion of the northern great plains. Can. J. Plant Sci. **81**: 351-354.

Entz, M.H., Penner, K.R., Vessey, J.K., Zelmer, C.D., and Thiessen Martens, J.R. 2004. Mycorrhizal colonization of flax under long-term organic and conventional management. Can. J. Plant Sci. **84**: 1097-1099.

Environment Canada. 2004. Canadian climate normals 1971-2000. [Online] Available: http://www.climate.weatheroffice.ec.gc.ca/climate_normals/index_e.html [2008 Dec 3].

Environment Canada. 2008. National climate data and information archive. [Online] Available: http://climate.weatheroffice.ec.gc.ca/climateData/canada_e.html [2008 Dec 3].

Ezawa, T., Smith, S.E., and Smith, F.A. 2002. P metabolism and transport in AM fungi. Plant Soil **244**: 221-230.

Filion, M., St-Arnaud, M., and Fortin, J.A. 1999. Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytol. **141**: 525-533.

Fitter, A.H. and Garbaye J. 1994. Interactions between mycorrhizal fungi and other soil organisms. Plant Soil **159**: 123-132.

Fox, S.L., Kirk, A.P., and Entz, M.H. 2008. Comparison of selecting spring wheat in conventional and organic environments. *In R. Appels, R. Eastwood, E. Lagudah, P. Langridge, M. Mackay, L. McIntyre, and P. Sharp, eds. Breeding for a Changing World. Proc. 11th International Wheat Genetics Symposium.* [Online] Available: http://hdl.handle.net/2123/3495 [2009 June 04].

Garvin, D.F., Welch, R.M., and Finley, J.W. 2006. Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. J Sci Food Agric. **86**: 2213-2220.

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

George, E., Häussler, K., Kothari, S.K., Li, X.-L., and Marschner, H. 1992. Contribution of mycorrhizal hyphae to nutrient and water uptake of plants. Pages 42-47 *in* D.J. Read, D.H. Lewis, A.H. Fitter, and I.J. Alexander, eds. Mycorrhizas in ecosystems. C.A.B.I. Publishing.

Gill, B.S. and Friebe, B. 2007. Cytogenetics, phylogeny and evolution of cultivated wheats. [Online] Available: http://www.fao.org/DOCREP/006/Y4011E/y4011e07.htm#bm07 [2007 Feb 12].

Hald, A.B. 1999. Weed vegetation (wild flora) of long established organic versus conventional cereal fields in Denmark. Ann. Appl. Biol. **134**: 307-314.

Hamel, C. 2004. Impact of arbuscular mycorrhizal fungi on N and P cycling in the root zone. Can. J. Soil Sci. **84**: 383-395.

Hansen, B., Fjelsted, Alrøe, H.F., Kristensen, E.S. 2001. Approaches to assess the environmental impact of organic farming with particular regard to Denmark. Agr. Ecosyst. Environ. **83**: 11-26.

Hedley, M.J., Stewart, J.W.B., and Chauhan, B.S. 1982. Changes in inorganic and organic soil phosphorus fraction induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. **46**: 970-976.

Hetrick, B.A.D., Wilson, G.W.T., and Cox, T.S. 1992. Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. Can. J. Bot. **70**: 2032-2040.

Hetrick, B.A.D., Wilson, G.W.T., Gill, B.S., and Cox, T.S. 1995. Chromosome location of mycorrhizal responsive genes in wheat. Can. J. Bot. **73**: 891-897.

Jakobsen, I., Baodong, C., Munkvold, L., Lundsgaard, T., and Zhu, Y-.G. 2005. Contrasting phosphate acquisition of mycorrhizal fungi with that of root hairs using the root hairless barley mutant. Plant, Cell and Environment **28**: 928-938.

Kabir, Z. and Kodie, 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 Soil **238**: 205-215.

Kelm, M., Loges, R., and Taube, F. 2008. Comparative analysis of conventional and organic farming systems: Nitrogen surpluses and nitrogen losses. Pages 312-315 *in* D. Neuhoff, N. Halberg, T. Alföldi, W. Lockeretz, A. Thommen, I. Rasmussen, J. Hermansen, M. Vaarst, L. Lueck, F. Caporali, H. Jensen, P. Migliorini, and H. Willer, eds. Cultivating the Future Based on Science. Proc. 2nd Scientific Conference of the International Society of Organic Agriculture Research.

Khan, A.G., Kuek, C., Chaudhry T.M., Khoo, C.S., and Hayes W.J. 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere **41**: 197-207.

Koide, R.T. and Mosse, B. 2004. A history of research on arbuscular mycorrhiza. Mycorrhiza **14**: 145-163.

Lammerts van Bueren, E.T., Hulscher, M., Haring, M., Jongerden, J., van Mansvelt, J.D., den Nijs, A.P.M., Ruivenkamp, G.T.P. 1999. Sustainable organic plant breeding. Final report: a vision, choices, consequences and steps. Louis Bolk Instituut, Driebergen, The Netherlands, 60 pp.

Lammerts van Bueren, E.T., Struik, P.C., and Jacobsen, E. 2002. Ecological concepts in organic farming and their consequences for an organic crop ideotype. Neth. J. Agric. Sci. **50**: 1-26.

Lammerts van Bueren, E.T., Struik, P.C., Tiemens-Hulscher, M., and Jacobsen, E. 2003. Concepts of Intrinsic Value and Integrity of Plants in Organic Plant Breeding and Propagation. Crop Sci. **43**: 1922-1929.

Lampkin, N., Padel, S., and Foster, C. 2000. Organic Farming. Pages 221-238 *in* F. Brouwer and P. Lowe, eds. CAP regimes and the European countryside – Prospects for the integration between agricultural, regional and environmental policies. CABI Publishing.

Lehocka, Z., Klimekova, M., and Bielikova, M. 2008. Soil quality indicators in organic and conventional farming systems in Slovakia. Pages 44-47 *in* D. Neuhoff, N. Halberg, T. Alföldi, W. Lockeretz, A. Thommen, I. Rasmussen, J. Hermansen, M. Vaarst, L. Lueck, F. Caporali, H. Jensen, P. Migliorini, and H. Willer, eds. Cultivating the Future Based on Science. Proc. 2nd Scientific Conference of the International Society of Organic Agriculture Research.

Lemerle, D., Verbeek, B., Cousens, R.D., and Coombes, N.E. 1996. The potential for selecting wheat varieties strongly competitive against weeds. Weed Res. **36**: 505-513.

Macey, A. 2006. Certified organic production in Canada 2005. Prepared for Canadian Organic Growers, Aug 2006. [Online] Available: http://www.cog.ca/documents/certifiedorganicproduciton05E_000.pdf. [2008 Jan 23].

Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., and Niggli, U. 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. Biol. Fertil. Soils **31**: 150-156.

Mäder, P., Fließbach, A., Dubois, D., Gunst, L., Fried, P., and Niggli, U. 2002. Soil fertility and biodiversity in organic farming. Science **296**: 1694-1697.

MAFRI (Manitoba Agriculture, Food and Rural Initiatives). 1999. Agricultural climate of Manitoba. [Online] Available: http://www.gov.mb.ca/agriculture/climate/waa50s00.html [2008 Dec 3].

MAFRI (Manitoba Agriculture, Food and Rural Initiatives). 2007. Manitoba soil fertility guide. [Online] Available: http://www.gov.mb.ca/agriculture/soilwater/nutrient/fbd02s00.html [2009 March 2].

MAFRI (Manitoba Agriculture, Food and Rural Initiatives). 2008a. Manitoba Ag-Weather Program. [Online] Available: http://tgs.gov.mb.ca/climate [2008 Dec 3].

MAFRI (Manitoba Agriculture, Food and Rural Initiatives). 2008b. Guide to field crop protection: weeds, plant diseases, insects.

Magkos, F., Arvaniti, F., and Zampelas, A. 2003. Organic food: nutritious food or food for thought? A review of the evidence. Int. J. Food Sci. Nutr. **54**: 357-371.

Manske, G.G.B. 1989. Genetical analysis of the efficiency of VA mycorrhiza with spring wheat. Agric. Ecosys. Environ. **29**: 273-280.

Manske, G.G.B., Ortiz-Monasterio, J.I., van Ginkel, M., González, R.M., Rajaram, S., Molina, E., and Vlek, P.L.G. 2000. Traits associated with improved P-uptake efficiency in CIMMYT's semidward spring bread wheat grown on an acid Andisol in Mexico. Plant Soil **221**: 189-204.

Mason, H., Goonewardene, L., and Spaner, D. 2007a. Compatitive traits and the stability of wheat cultivars in differing natural weed environments on the northern Canadian Prairies. Journal of Agricultural Science **146**: 21-33.

Mason, H., Navabi, A., Brick, B., O'Donovan, J., Niziol, D., and Spaner, D. 2007b. Does growing Canadian Western Hard Red Spring wheat under organic management alter its breadmaking quality? Renewable Agriculture and Food Systems **22**: 157-167.

Mason, H.E. and Spanner, D. 2006. Competitive ability of wheat in conventional and organic management systems: a review of the literature. Can. J. Plant Sci. **86**: 333-343.

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

Michalyna, W. 1970. Report of the detailed soil survey of Glenlea Research Station. Canada-Manitoba Soil Survey. Township 8, Range 3E.

Mills, G.F. and Haluschak, P. 1993. Soils of the Carman Research Station N1/2 23-6-5W. Special report series 93-1. Manitoba Soil Survey Unit and Manitoba Land Resource Unit. Agriculture Canada.

Murphy, K.M., Campbell, K.G., Lyon, S.R., and Jones, S.S. 2007. Evidence of varietal adaptation to organic farming systems. Field Crops Research **102**: 172-177.

Oehl, F., Oberson, A., Tagmann, H.U., Besson, J.M., Dubois, D., Mäder, P., Roth, H.-R., and Frossard, E. 2002. Phosphorus budget and phosphorus availability in soils under organic and conventional farming. Nutr. Cycl. Agroecosys. **62**: 25-35.

Parniske, M. 2004. Molecular genetics of the arbuscular mycorrhizal symbiosis. Current Options in Plant Biology **7**: 414-421.

Peña, R.J. 2002. Wheat for bread and other foods. *In* B.C. Curtis, S. Rajaram, and H. Gómez Macpherson, eds. Bread wheat improvement and selection. Food and Agriculture Organization of the United Nations. [Online] Available: http://www.fao.org/DOCREP/006/Y4011E/y4011e0w.htm#bm32 [4 Nov 2008].

Peterson, R.L., Massicotte, H.B., and Melville, L.H. 2004. Mycorrhizas: anatomy and cell biology. NRC Research Press. Ottawa, Canada. 173 pp.

Pimentel, D., Hepperly, P., Hanson, J., Douds, D., and Seidel, R. 2005. Environmental, energetic, and economic comparisons of organic and conventional farming systems. Bioscience **55**: 573-582.

Pingali, P.L. (ed.). 1999. CIMMYT 1998-99 World wheat facts and trends. Global wheat research in a changing world: challenges and achievements. Mexico, D.F.; CIMMYT.

Redecker, D. 2002. Molecular identification and phylogeny of arbuscular mycorrhizal fungi. Plant Soil **244**: 67-73.

Redecker, D. 2007. Glomeromycota: Arbuscular mycorrhizal fungi and their relative(s). [Online] Available: http://www.tolweb.org/Glomeromycota [2007 Jan 18].

Rillig, M.C. 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. Can. J. Soil Sci. **84**: 355-363.

Rillig, M.C., Wright, S.F., and Eviner, V.T. 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant Soil **238**: 325-333.

Ryan, M.H. and Ash, J.E. 1996. Colonization of wheat in southern New South Wales by vesicular-arbuscular mycorrhizal fungi is significantly reduced by drought. Australian Journal of Experimental Agriculture **36**: 563-569.

Sahota, A. 2006. Overview of the global market for organic food and drink. Pages 69-73 *in* H. Willer and M. Yussefi, eds. The world of organic agriculture: Statistics and emerging trends 2006. FiBL and IFOAM Publication [Online] Available: http://soel.de/inhalte/publikationen/s/s_74_08.pdf#page=24 [2008 April 16].

Siqueira, J.O., Sylvia, D.M., Gibson, J., and Hubbell, D.H. 1985. Spores, germination, and germ tubes of vesicular-arbuscular mycorrhizal fungi. Can. J. Microbiol. **31**: 965-972.

Stark, C.H. 2008. Are soil biological properties and microbial community structure altered by organic farm management? Pages 36-39 *in* D. Neuhoff, N. Halberg, T. Alföldi, W. Lockeretz, A. Thommen, I. Rasmussen, J. Hermansen, M. Vaarst, L. Lueck, F. Caporali, H. Jensen, P. Migliorini, and H. Willer, eds. Cultivating the Future Based on Science. Proc. 2nd Scientific Conference of the International Society of Organic Agriculture Research.

Statistics Canada. 2008. Acreage of crops in Canada. [Online] Available: http://www40.statcan.ca/l01/cst01/prim11a.htm [2008 Oct 28].

Steward, J.W.B. and Tiessen, H. 1987. Dynamics of soil organic phosphorus. Biogeochemistry **4**: 41-60.

Talbert, L.E., Smith, L.Y., and Blake, N.K. 1998. More than one origin of hexaploid wheat is indicated by sequence comparison of low-copy DNA. Genome **41**: 402-407.

Uetake, Y., Kojima, T., Ezawa, T., and Saito, M. 2002. Extensive tubular vacuole system in an arbuscular mycorrhizal fungus, *Gigaspore margarita*. New Phytol. **154**: 761-768.

Vandeleur, R.K. and Gill, G.S. 2004. The impact of plant breeding on the grain yield and competitive ability of wheat in Australia. Aust. J. Agric. Res. **55**: 855-861.

Watson, C.A., Atkinson, D., Gosling, P., Jackson, L.R., and Ryans, F.W. 2002. Managing soil fertility in organic farming systems. Soil Use Manag. **18**: 239-247.

Welsh, C., Tenuta, M., Flaten, D.N., Thiessen-Martens, J.R., and Entz, M.H. 2009. High yielding organic crop management decreases plant-available but not recalcitrant soil phosphorus. Agron. J. **101**: 1027-1035.

Wolfe, M.S., Baresel, J.P., Desclaux, D., Goldringer, I., Hoad, S., Kovacs, G., Löschenberger, F., Miedaner, T., Østergård, H., and Lammerts van Bueren, E.T. 2008. Developments in breeding cereals for organic agriculture. Euphytica **163**: 323-346.

Worthington, V. 2001. Nutritional quality of organic versus conventional fruits, vegetables, and grains. J. Altern. Complementary Med. **7**: 161-173.

Wright, S.F. and Upadhyaya, A. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil **198**: 97-107.

Yussefi, M. 2006. Organic farming worldwide 2006: Overview and main statistics. Pages 23-35 *in* H. Willer and M. Yussefi, eds. The World of Organic Agriculture Statistics and Emerging Trends 2006. IFOAM, Bonn, Germany and Research Institute of Organic Agriculture FiBL, Frick, Switzerland.

Zhu, Y.-G. and Smith, S.E. 2001. Seed phosphorus (P) content affects growth, and P uptake of wheat plants and their association with arbuscular mycorrhizal (AM) fungi. Plant Soil **231**:1 05-112.

Zhu, Y-.G., Smith, S.E., Barrit, A.R., and Smith, F.A. 2001. Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. Plant Soil **237**: 249-255.

6. APPENDICES

Appendix I – Tables

Table 6.1 Contrasts of the percent arbuscular colonization at Glenlea of lines used as parents in previous crosses.

Parent 1		Parent 2		Difference	Pr > F	Significance
Line	Col (%)	Line	Col. (%)			
96B42-E3C	17.25	Alsen	15.75	1.5	0.7636	ns
Alsask	21.25	Alsen	15.75	5.5	0.2719	ns
00H01*F57	23.25	Alsen	15.75	7.5	0.1354	ns
98B50-H4D	29	ND 744	19.25	9.75	0.0534	ns
99B60-EJ2G	15.75	Somerset	26.75	-11	0.0299	*
99B60-EJ2G	15.75	Infinity	31.5	-15.75	0.0022	**
98B25-AU5A01	19.5	Kane	22.75	-3.25	0.5151	ns
AC Karma	30.5	SC8021V2	16.5	14	0.0062	**
AC Domain	23.5	RL 4452	14.25	9.25	0.0666	ns
Opata	16.25	Synthetic	23.5	-7.25	0.1487	ns
Glenlea	15.25	Majestic	16.75	-1.5	0.7636	ns
AC Karma	30.5	Glenlea	15.25	15.25	0.003	**

n.s. (not significant at P<0.05), *(P<0.05), **(P<0.01)

Table 6.2 Contrasts of the percent total colonization at Glenlea of lines used as parents in previous crosses.

Parent 1		Parent 2		Difference	Pr > F	Significance
Line	Total Col. (%)	Line	Total Col. (%)			
96B42-E3C	24.75	Alsen	22.75	2	0.7482	ns
Alsask	27.5	Alsen	22.75	4.75	0.4466	ns
00H01*F57	30.25	Alsen	22.75	7.5	0.2308	ns
98B50-H4D	39.75	ND 744	27.25	12.5	0.0476	*
99B60-EJ2G	22.25	Somerset	32.5	-10.25	0.1029	ns
99B60-EJ2G	22.25	Infinity	40	-17.75	0.0055	**
98B25-AU5A01	27.75	Kane	31.25	-3.5	0.5746	ns
AC Karma	39	SC8021V2	23.25	15.75	0.0133	*
AC Domain	30	RL 4452	20.75	9.25	0.1404	ns
Opata	22.75	Synthetic	35	-12.25	0.0521	ns
Glenlea	23	Majestic	23.25	-0.25	0.968	ns
AC Karma	39	Glenlea	23	16	0.0119	*

n.s. (not significant at P<0.05), *(P<0.05), **(P<0.01)

Table 6.3 Hyphal (HC), arbuscular (AC) and total (TC) percent colonization at Glenlea in 2007.

Cultivar	Year of Release	HC (%)	AC (%)	TC (%)
Mida	1944	6.5[z]	11.3[z]	17.8[z]
FBC Dylan	2006	6.5	9.8	16.3
Red Fife	1886	6.5	9	15.5
McKenzie	1997	6.3	8.5	14.8
Marquis	1911	7.3	8.3	15.5
5602 HR	2004	6.5	8	14.5
CDC Teal	1991	4.5	8	12.5
Selkirk	1955	8.5	7.8	16.3
AC Barrie	1994	6	6	12
Neepawa	1969	6.5	5.8	12.3

[z]Means are not significantly different (P>0.05) according to Fischer's protected LSD.

Table 6.4 Hyphal (HC), arbuscular (AC) and total (TC) percent colonization at Carman in 2007.

Cultivar	Year of Release	HC (%)	AC (%)	TC (%)
McKenzie	1997	11.5[z]	21[z]	32.5[z]
CDC Teal	1991	7.3	19.5	26.8
FBC Dylan	2006	11.8	19	30.8
AC Barrie	1994	9	14.5	23.5
5602 HR	2004	12.3	13.3	25.5
Selkirk	1955	7	13.3	20.3
Red Fife	1886	8.3	13	21.3
Neepawa	1969	8.3	12	20.3
Mida	1944	10	11.3	21.3
Marquis	1911	6.8	10.3	17

[z]Means are not significantly different (P>0.05) according to Fischer's protected LSD.

Table 6.5 Hyphal (HC), arbuscular (AC) and total (TC) percent colonization at Glenlea in 2008.

Cultivar	Year of Release	HC (%)	AC (%)	TC (%)
Selkirk	1955	1[z]	1.1[z]	1.9[z]
Mida	1944	1.3	1.0	1.6
FBC Dylan	2006	1.3	0.7	1.8
Red Fife	1886	0.3	0.6	1.1
5602 HR	2004	0.5	0.6	1.2
Marquis	1911	1	0.6	1.7
CDC Teal	1991	0.5	0.5	0.7
AC Barrie	1994	1	0.3	0.8
Neepawa	1969	0.8	0.1	0.6
McKenzie	1997			

[z]Means are not significantly different (P>0.05) according to Fischer's protected LSD.

Table 6.6 Hyphal (HC), arbuscular (AC) and total (TC) percent colonization at Carman in 2008.

Cultivar	Year of Release	HC (%)	AC (%)	TC (%)
FBC Dylan	2006	7.5[z]	21.8a[y]	29.3a[y]
5602 HR	2004	6.3	16.5ab	22.8ab
AC Barrie	1994	4.3	15abc	19.3abc
Mida	1944	4.8	12.3bcd	17bcd
Red Fife	1886	3.5	11.3bcd	14.8bcd
CDC Teal	1991	4.5	10.8bcd	15.3bcd
Selkirk	1955	6	10.3bcd	16.3bcd
McKenzie	1997	2.5	7.3cd	9.8cd
Neepawa	1969	4	6.3cd	10.3cd
Marquis	1911	1.8	5.3d	7d

[y]Means within the same site year followed by the same letter within a column are not significantly different (P>0.05) according to Fisher's protected LSD.
[z]Means are not significantly different (P>0.05) according to Fischer's protected LSD.

Table 6.7 Correlations between cultivar release date, arbuscule colonization, total colonization, yield, percent tissue P, and biomass at Glenlea in 2007. The top numbers are the Pearson Correlation Coefficients. The bottom numbers are the significance of the correlation.

	Release Date	Arbuscule Colonization	Total Colonization	Yield	Tissue P	Biomass
Release Date	1 -	-0.09 n.s.	-0.11 n.s.	-0.12 n.s.	-0.21 n.s.	-0.40 *
Arbuscule Colonization	-0.09 n.s.	1 -	0.90 ***	-0.09 n.s.	-0.23 n.s.	-0.05 n.s.
Total Colonization	-0.11 n.s.	0.90 ***	1 -	-0.10 n.s.	-0.27 n.s.	-0.02 n.s.
Yield	-0.12 n.s.	-0.09 n.s.	-0.10 n.s.	1 -	-0.37 *	0.59 ***
Tissue P	-0.21 n.s.	-0.23 n.s.	-0.27 n.s.	-0.37 *	1 -	-0.25 n.s.
Biomass	-0.40 *	-0.05 n.s.	-0.02 n.s.	0.59 ***	-0.25 n.s.	1 -

n.s. (not significant at P<0.05), *(P<0.05), **(P<0.01), ***(P<0.001)

Table 6.8 Correlations between cultivar release date, arbuscule colonization, total colonization, yield, percent tissue P, and biomass at Carman in 2007. The top numbers are the Pearson Correlation Coefficients. The bottom numbers are the significance of the correlation.

	Release Date	Arbuscule Colonization	Total Colonization	Yield	Tissue P	Biomass
Release Date	1 -	0.32 *	0.37 *	0.40 *	-0.03 n.s.	0.14 n.s.
Arbuscule Colonization	0.32 *	1 -	0.91 ***	0.24 n.s.	-0.21 n.s.	0.21 n.s.
Total Colonization	0.37 *	0.91 ***	1 -	0.36 *	-0.25 n.s.	0.19 n.s.
Yield	0.40 *	0.24 n.s.	0.36 *	1 -	-0.51 ***	0.27 n.s.
Tissue P	-0.03 n.s.	-0.21 n.s.	-0.25 n.s.	-0.51 ***	1 -	-0.32 *
Biomass	0.14 n.s.	0.21 n.s.	0.19 n.s.	0.27 n.s.	-0.32 *	1 -

n.s. (not significant at P<0.05), *(P<0.05), **(P<0.01), ***(P<0.001)

Table 6.9 Correlations between cultivar release date, arbuscule colonization, total colonization, yield, percent tissue P, and biomass at Glenlea in 2008. The top numbers are the Pearson Correlation Coefficients. The bottom numbers are the significance of the correlation.

	Release Date	Arbuscule Colonization	Total Colonization	Yield	Tissue P	Biomass
Release Date	1 -	-0.09 n.s.	-0.09 n.s.	0.30 n.s.	-0.35 *	0.13 n.s.
Arbuscule Colonization	-0.09 n.s.	1 -	0.92 ***	0.12 n.s.	0.19 n.s.	0.12 n.s.
Total Colonization	-0.09 n.s.	0.92 ***	1 -	-0.08 n.s.	0.17 n.s.	0.14 n.s.
Yield	0.30 n.s.	0.12 n.s.	-0.08 n.s.	1 -	-0.25 n.s.	0.38 *
Tissue P	-0.35 *	0.19 n.s.	0.17 n.s.	-0.25 n.s.	1 -	-0.35 *
Biomass	0.13 n.s.	0.12 n.s.	0.14 n.s.	0.38 *	-0.35 *	1 -

n.s. (not significant at P<0.05), *(P<0.05), **(P<0.01), ***(P<0.001)

Table 6.10 Correlations between cultivar release date, arbuscule colonization, total colonization, yield, percent tissue P, and biomass at Carman in 2008. The top numbers are the Pearson Correlation Coefficients. The bottom numbers are the significance of the correlation.

	Release Date	Arbuscule Colonization	Total Colonization	Yield	Tissue P	Biomass
Release Date	1 -	0.29 n.s.	0.31 *	0.32 n.s.	0.03 n.s.	-0.01 n.s.
Arbuscule Colonization	0.29 n.s.	1 -	0.97 ***	0.38 *	-0.34 *	0.23 n.s.
Total Colonization	0.31 *	0.97 ***	1 -	0.36 *	-0.33 *	0.19 n.s.
Yield	0.32 n.s.	0.38 *	0.36 *	1 -	-0.15 n.s.	0.48 **
Tissue P	0.03 n.s.	-0.34 *	-0.33 *	-0.15 n.s.	1 -	-0.43 **
Biomass	-0.01 n.s.	0.23 n.s.	0.19 n.s.	0.48 **	-0.43 **	1 -

n.s. (not significant at $P<0.05$), *($P<0.05$), **($P<0.01$), ***($P<0.001$)

Table 6.11 Correlation between yield, protein, 1000 kernel weight (KWT) and height at all organic locations. The top number is the correlation between the two variables and the bottom number is the significance of the correlation.

	yield	protein	kwt	height
yield	1 -	-0.52 *	0.4 *	-0.12 ns
protein	-0.52 *	1 -	-0.36 *	0.45 *
kwt	0.4 *	-0.36 *	1 -	-0.28 *
height	-0.12 ns	0.45 *	-0.28 *	1 -

n.s. (not significant at P<0.05), *(P<0.05)

Table 6.12 Correlation between yield, protein, 1000 kernel weight (KWT) and height at all conventional locations. The top number is the correlation between the two variables and the bottom number is the significance of the correlation.

	yield	protein	kwt	height
yield	1 -	-0.32 *	0.58 *	0.32 *
protein	-0.32 *	1 -	-0.63 *	0.29 *
kwt	0.58 *	-0.63 *	1 -	-0.01 ns
height	0.32 *	0.29 *	-0.01 ns	1 -

n.s. (not significant at P<0.05), *(P<0.05)

Appendix II – Procedure for Working with AMF

6.1 Mycorrhizal Colonization

6.1.1 Materials – Refer to MSDS for more information

Fisherbrand* Graduated Polypropylene Laboratory Bottles	(Fisher Cat. # 03-405-31)
100mL Pyrex* Vista* Griffin Beakers	(Fisher Cat. # 07-250-054)
Pyrex* Vista* Petri Culture Dishes	(Fisher Cat. # S95184B)
BD Plastic Disposable Transfer Pipet	(Fisher Cat. # 13-669-12)
Fine Forceps	(Fisher Cat. # 08-880)
Dissecting Scissors	(Fisher Cat. # 08-951-20)
Microscope Slides	(Fisher Cat. # 22-267013)
Coverslips	(Fisher Cat. # 12-547)
Ethanol 95% Denatured	(Fisher Cat. # 573979)
Potassium Hydroxide	(Fisher Cat. # P250-1)
Glycerol	(Fisher Cat. # G33-1)
Chlorazol Black E.	(Fisher Cat. # AC404401000)
Autoclave	
Laboratory Oven	
Light Microscope	

6.1.2 Chemical Formulae

<u>70% Ethanol:</u>

737ml 95% Ethanol: 263ml Water

10% Potassium Hydroxide:

100 g Solid Potassium Hydroxide: 1000ml Water

Chlorazol Black E: (0.05% w/v in 1:1:1 lactic acid, glycerol and water)

0.45g Chlorazol Black E: 300ml Lactic acid: 300ml Glycerine: 300ml Water

6.1.3 Colonization Determination

The clearing and staining of mycorrhizal roots follows the methods of Brundrett *et al.* (1996). Take a random sample of 1-2 cm root pieces from the ethanol solution and rinse with deionized water in a fine sieve. Place the root samples in 100 mL beakers and add 10% Potassium Hydroxide (KOH) solution. Cover the beakers with tin foil and autoclave for 10 minutes to clear the roots of their internal structures. After clearing, rinse the root segments in a fine sieve and transfer to small glass Petri Dishes. Add Chlorazol Black E (0.05% w/v in 1:1:1 lactic acid, glycerol and water) solution to the dishes, just enough to cover the roots. Place the dishes in a 90°C oven for 90 minutes. Remove the excess stain with disposable Transfer Pipets and dispose in a properly labeled container according to MSDS regulations. Rinse the roots four times with deionized water and add glycerol to destain for 48 hours.

Mycorrhizal colonization was scored using the magnified intersections method of McGonigle *et al.* (1990). Arrange approximately 25 root segments horizontally on a slide with fine forceps and cover with a cover slip. Press down on the cover slip and carefully soak up excess glycerol from around the cover slip. Apply clear nail polish around the edges of the cover slip to preserve the root samples. Mount the slide under the microscope at 250x magnification and move the slide up, down and across at regular

intervals. As the slide is moved up or down count when the vertical line of the cross-hair in the ocular lens bisects a root, hyphae or arbuscule. Count as an arbuscule when the vertical line intersects with one of more arbuscules and hyphae when the vertical line intersects with hyphae (distinguished as mycorrhizal hyphae by the presence of arbuscules). When both hyphae and arbuscules are intersected by the vertical line on the same root only count the arbuscule. A higher magnification may be required for identification of AMF structures if they are unclear. When the number of root sections evaluated reaches 100 stop counting. The percent hyphal and arbuscular colonization can now be determined using the number of hyphae and arbuscules intersected in the 100 root intersections.