CROP MANAGEMENT IMPACTS ON MYCORRHIZAL COLONIZATION AND CADMIUM AVAILABILITY IN AGRICULTURAL CROPS

BY

MST. FARDAUSI AKHTER

A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements
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University of Manitoba
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ABSTRACT

Akhter, Fardausi M. M.Sc., The University of Manitoba, September 2008. Crop management impacts on mycorrhizal colonization and cadmium availability in agricultural crops. Major Professor: Dr. Mario Tenuta.

Cadmium is a naturally occurring element in mineral soils. It may accumulate in crops to levels that are of concern in human diets. The study aimed at determining the effect of selected crop management practices on above ground cadmium concentration and accumulation in durum wheat and flax. Management practices included tillage (conventional and reduced), crop sequence (flax-durum wheat and canola-durum wheat) and phosphorus fertilization (low and high level). The approach used was to determine single and interactive effects of these management practices on cadmium concentration and accumulation in the above ground tissues and whether arbuscular mycorrhizal fungal colonization of roots had a role.

Among crop management practices studied, tillage and high level of P fertilizer containing Cd as impurities increased above ground tissue Cd concentration the most. In the case of high Cd loading to soil through P fertilizer with high concentration of Cd, Cd concentration and accumulation of flax and durum wheat tissue was simply related to DTPA extractable Cd in soil. Cd concentration was higher in above ground flax tissues compared to durum wheat substantiating flax as high Cd accumulating crop. Conventional tillage and canola as a proceeding crop reduced arbuscular mycorrhizal

fungal colonization in durum wheat although only the former was related to increased Cd concentration of durum wheat. Phosphorus fertilizer reduced arbuscular mycorrhizal fungal colonization in durum wheat but not in flax. Thus, a clear effect of arbuscular mycorrhizal colonization on Cd uptake was not evident in this field study. However, multiple-linear regression analysis revealed that root mycorrhizal colonization variables (either percent arbuscular root colonization, arbuscular colonization density, or mycorrhizal Cost:Benefit) to be negatively associated with Cd concentration of above ground tissue for both durum wheat and flax. This indicated potential association of mycorrhizal fungi in restricting Cd transfer to above ground tissues.

In conclusion, above ground Cd concentration and accumulation most clearly increased with addition rates of Cd laden phosphorus fertilizer. Conventional tillage was the other management practice examined that increased Cd concentration and accumulation; however, the effect was small. Mycorrhizal colonization of roots was reduced with canola as a previous crop, conventional tillage and increasing P addition. Relation of mycorrhizal colonization to Cd concentration and uptake was weak. The study indicates basis to detail the relationship between mycorrhizal colonization to Cd uptake of crops under controlled environment conditions where confounding field conditions are reduced and the impact of absence and presence of colonization can be determined.

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1. GENERAL INTRODUCTION

1.1 Cadmium in Soils and Plants

Cadmium (Cd) is a non-essential trace element to plants, animals and human. This element has attracted much attention in soil science and plant nutrition, because (i) it is toxic to humans at concentrations lower than those lethal to plants, (ii) it is more mobile and bioavailable than many other metals, and (iii) its effects on humans are chronic (McLaughlin and Singh, 1999). The two main causes of Cd in soils are geological parent materials and inputs from extraneous sources, which for the most part are anthropogenic in origin. Soils derived from Cd rich parent materials have concentrations up to 24 mg total Cd kg^{-1}(Alloway and Steinnes, 1999). Anthropogenic means of increased Cd in agricultural soils include atmospheric deposition from industrial sources, fertilizers, manures and municipal sewage wastes. These anthropogenic sources may exceed the release of this metal from natural sources by two-fold or more (Nriagu and Pacyna, 1988). In urban and industrial areas, most soil Cd comes from atmospheric deposition. A long-term experiment conducted in the UK clearly showed gradual increase in soil Cd over time with farmyard manure and fertilizer application (McLaughlin and Singh, 1999). In many agricultural systems, the main sources of Cd inputs to soils are fertilizers, soil amendments, manures and sewage biosolids (Lugon-Moulina et al., 2006; Manciulea and

Ramsey, 2006; McLaughlin et al., 1996), which are also slowly increasing with time by applying either mineral or organic fertilizers (Alloway and Steinnes, 1999).

The Cd concentration of Manitoba soil ranges from 0.1 to 7.9 mg kg^{-1} (Haluschak et al., 1998). The Cd content in Manitoba soil is highly related to texture. The average Cd content in coarse textured soil was 0.1 mg kg^{-1}, whereas it was four times higher in fine textured soil (Haluschak et al., 1998). The highest concentrations occured in soils derived directly from shale bedrock or those in which a large content of weathered shale particles comprise the soil parent material (Haluschak et al., 1998). Upon weathering, these shales release Cd^{2+} into solution. This ionic species is the form that Cd is taken up by plants, although concentration in solution will be low, because Cd^{2+} forms complexes and organic chelates in soil.

The cadmium cycle is fairly complex in agricultural soils (Figure 1.1). The accumulation of soil Cd in food crops is dependent on a number of physical, chemical and biological processes that control the solubility and form of Cd in the soil solution, especially in the rhizosphere (Welch and Norvell, 1999). There are a myriad of reactions and interactions of Cd with soil components, other nutrients, metals, plant, climate and management factors, which may affect Cd transfer through the food chain. For a sustainable Cd management strategy, it is important to control inputs of Cd to the soil as well as transfer to plants and into the food chain.

1.2 Mechanism of Cadmium Uptake and Translocation in Plants

1.2.1 Mechanisms of Cadmium Uptake

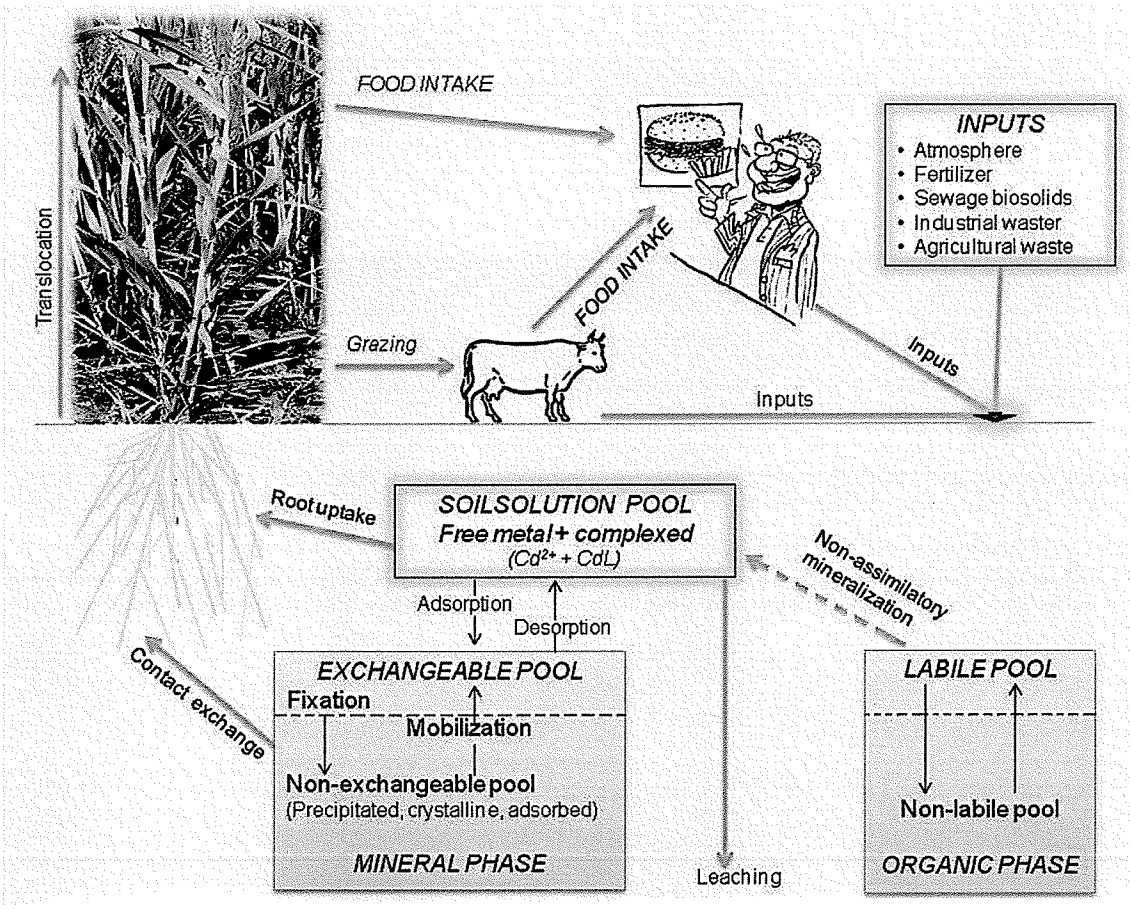

Figure 1.1 Fluxes of Cd in soils, plants and the food chain (reproduced from McLaughlin and Singh, 1999, with kind permission of Springer Science and Business Media)

Cd can be taken up by plants either by foliar or root absorption and then transfered to different plant parts (Liu et al., 2003; Cobb et al., 2000; Florijn and Van Beusichem, 1993). Root absorption is the dominant pathway for aqueous Cd uptake and accumulation to the plants. Other forms of Cd in the soil solution are in the forms of inorganic or organic complexes, and some of these complexes may participate directly in root uptake (Welch and Norvell, 1999).

There are a number of root processes that increase the solubility of Cd in the rhizosphere that may also have large effects on the amount of Cd diffusing to root

surfaces. These root processes include root-cell efflux of H^+ ions, release of respired carbon dioxide, root efflux of organic acid and reductants (e.g. phenolic compounds like lignin, flavonoids etc.), the uptake of macro- and micronutrient cations and anions and Cl^- (Welch and Norvell, 1999). These processes affect Cd availability and absorption to varying degrees depending on plant species, genotype and environmental conditions.

1.2.2 Intra-cellular Homeostasis: Complexation, Compartmentation and Efflux

The fate of Cd^{2+} after influx across the root-cell plasma membrane is complex. Cd^{2+} is highly reactive and preferentially binds to sulphhydryl ligand groups rather than to other ligands (e.g. carboxyl, hydroxyl, amino-N etc.). As a result, Cd^{2+} competes with other sulphur binding metals such as Zn, Ni and Cu for active functional binding sites in essential cytosolic metabolites of plants (e.g. glutathione and other sulphhydryl-containing proteins). In the cytosol, a set of low molecular weight cysteine-rich polypeptide (phytochelatins or cadystins) form complexes with Cd^{2+} (Rauser, 1995). Wu et al. (1995) reported that Cd^{2+} phytochelatins can be transported into vacuoles directly and transfer their Cd to higher molecular weight metallothionein-type proteins (>10,000 daltons) for storage of Cd in inactive forms in the vacuole. Phytometallophores (e.g. nicotianamine, mugineic acid) could also play a role in controlling Cd^{2+} activity in cytosol and intra- and intercellular symplastic transport of Cd^{2+}(Welch and Norvell, 1999), however to my knowledge there is no published reports in support of this assumption. Organic acids (e.g., citric acid) are also reported to complex Cd^{2+} in the cytosol (Senden et al., 1995). Cd^{2+} can also be transported into cell organelles via a membrane transport protein Cd^{2+}/H^+ antiporter (Salt and Wagner, 1993). This protein

exchanges H^+ for Cd^{2+} during Cd^{2+} transport into the vacuole. Among all the Cd complexers, glutathione (GSH) is the most important which can be exposed to non-growth inhibiting levels of Cd whereas Cd(II)-phytochelatins are only important at higher Cd exposures (> 5 μM Cd) (Vögeli-Lange and Wagner, 1996).

1.2.3 Cadmium Transport

The entry of Cd into edible portions of crops involves transfer from roots to shoots. The transfer starts as a membrane transport process in parenchyma cells that adjoin xylem vessels within the endodermis of roots (Welch and Norvell, 1999). Here, xylem elements load Cd from symplastic pools in parenchyma cells. The chemical composition of xylem sap consists of a pH ranging from 5.0-6.0 with low concentrations of organic compounds, such as sugars, peptides and proteins. Cd can move in xylem sap as an inorganic cation (Cd^{2+}) as well as in complexes with organic molecules. Cd prefers to form bonds with sulphhydryl ligand groups, it also binds to N and O ligand groups. Cysteine and other sulphhydryl containing compounds (e.g., phytochelatins, glutathione) and various organic compounds, such as citrate and amino acids in xylem sap, can be important in transporting Cd from root to shoot (Welch and Norvell, 1999). Within the xylem, Cd moves with the transpiration stream and eventually unloaded from the xylem sap into leaf mesophyll cell apoplasmic spaces. After this, Cd must cross a plasma membrane to again enter the symplasm. In order to enter seed or grains, Cd crosses a companion cell plasma membrane adjacent to a sieve tube element to enter the phloem sap for transport. This means of transport is important because there are no mature xylem

in tissues that can provide mineral elements directly to developing seeds and grains within a reproductive organ.

The chemical composition of phloem sap consists of a pH ranging from 7.0 - 8.0 with high concentrations of organic ligands (e.g. organic acids, amino acids, sugars, peptides and proteins). The redox potential of phloem sap is low and provides stability to sulphhydryl- containing ligands, which are the carriers of Cd. The Cd complexes in phloem sap may include phytometallophores, such as nicotianamine and the class 3 metallothioneins, the phytochelatins, as well as glutathione, cysteine and other sulphhydryl-containing molecules (Welch and Norvell, 1999). However, to my knowledge, there is no direct evidence for any specific Cd complexes in phloem sap. Once xylem transfer of Cd at phloem unloading sites within a reproductive organ occurs, Cd transports across a plasma membrane of cells associated with the developing embryonic tissues of the developing seed or grain begins (Welch, 1995). Therefore, Cd crosses numerous cell membrane barriers to enter edible plant organs, especially seeds and grains.

1.3 Factors Affecting Cadmium Availability to Plants

Cd availability to plants is affected by crop genetics and Cd activity in soil solution (Grant et al., 2008; Ozkutlu, et al., 2007; Dunbar et al., 2003; Ozturk, et al., 2003; Grant et al., 1999). Cd concentration in plant edible parts can be reduced by manipulating crop selection and crop and soil management practices (Grant et al., 2008). Cd concentration in soil solution is affected by plant growth, soil pH, cation-exchange capacity, rhizosphere chemistry, microbial activity, soil organic matter, ionic strength of the soil solution,

competing or complexing ions, total and plant available Cd content of the soil (Grant and Sheppard, 2008; Carrillo-González et al., 2006; Bolan, et al., 2003; McBride, 2002; Peijnenburg et al., 2000; Grant et al., 1999). Important crop management practices which may impact on Cd concentration in the soil solution by affecting soil chemical properties or direct addition of Cd to the soil, or on crop growth and rooting distribution, are discussed below.

1.3.1 Phosphate Fertilizer Application

Most Cd in nature occurs as an atomic substitution for Zn in minerals (Mineral Information Institute, 2007). Only a few relatively pure Cd minerals are known (e.g. cadmium sulphide, CdS). In addition, Cd can occur as an impurity in phosphate minerals (Mineral Information Institute, 2007). Some natural phosphate ores may contain several hundred parts per million (ppm) of Cd (Mineral Information Institute, 2007), and so phosphate fertilizers manufactured from these ores may contain high concentrations of cadmium (Alloway and Steinnes, 1999). Phosphate fertilizers can contain Cd as a contaminant at levels varying from trace amount to as much as 300 mg Cd kg^{-1} of dry product (Alloway and Steinnes, 1999; Grant and Sheppard, 2008). All soils receiving these fertilizers will have an input of Cd, but how much will depend on the source of rock phosphate used for its manufacture and the amounts applied (Grant and Sheppard, 2008; McLaughlin et al., 1996). Phosphate fertilization can influence Cd phytoavailability directly through addition of Cd as an impurity or indirectly through immediate and long-term effects on soil characteristics (Grant and Sheppard, 2008; Lambert et al., 2007;

Zaccheo et al., 2006; Zhao et al., 2005; Kashem and Singh, 2002; Mitchell et al., 2000; Grant and Bailey, 1998; McLaughlin et al., 1995). Application of phosphate fertilizer can greatly influence Cd speciation and complexation and can also affect crop rhizosphere characteristics, root distribution and general crop growth (Grant et al., 1999). Mulla et al. (1980) reported a 14-fold increase in Cd concentration in surface soils as a result of long-term high-rate phosphorus fertilizer application. The effect of this high surface soil Cd on crop accumulation is not clear. Some investigators showed increased crop Cd accumulation from increased surface soil Cd from phosphate fertilizers, whereas others failed to get any significant increase (Jiao et al., 2004; Adams et al., 2004; Kashem and Singh, 2002; Grant et al., 2002; Mclaughlin et al., 1995; He and Singh, 1993).

1.3.2 Soil Tillage

Tillage may impact soil physical, chemical and biological properties such as soil pH, organic matter, soil micro-fauna, soil microclimate and nutritional stratification in the soil profile (Adams et al., 2004; Grant and Bailey, 1994). In addition, tillage may impact root distribution and influence crop growth dynamics. It is assumed that these effects may influence crop Cd uptake. There are contradictory results of the effect of tillage practices on crop Cd accumulation. Some researchers reported higher concentrations of Cd in crops grown under direct drilling into stubble (Oliver et al., 1993). They suggested that restricted rooting under zero-tillage might encourage crop Cd accumulation from surface soil where Cd concentration is higher than at lower soil depths. In contrast, a number of studies showed opposite results where comparatively lower Cd accumulation was reported in durum wheat and canola under zero tillage than conventional tillage (Grant et

al., 1999). The reasons for the differences might be due to crop variety, soil type, tillage, type and amount of fertilizers used. However, detailed experiments are required in order to confirm the nature of tillage effects on crop Cd accumulation.

1.3.3 Crop Rotation

Crop rotation may impact crop Cd concentration and accumulation, although this has not been thoroughly investigated. Crop species and cultivars differ widely in their ability to absorb, accumulate and tolerate Cd (Grant et al., 1999) and may influence Cd accumulation in subsequent crops. Oliver et al. (1993) found higher Cd concentration in wheat grain when grown in rotation after lupins than after cereals. They suggested that the increase in Cd concentration may be partially, but not solely, attributed to the acidifying effect of the legume and the subsequent mobilisation of Cd for uptake by the subsequent crop. However, crop rotation effect also depends on residue management practices. In the case of high Cd accumulating crops, crop residues left in the field can affect the Cd accumulation of subsequent crops. An understanding of the impact of high and low Cd accumulating crops, complete crop removals (e.g. Alfalfa) and residue effect of phytoavailable Cd is unknown.

1.4 Soil Cadmium as a Threat to Human Health

The main Cd exposure routes to humans are inhalation, ingestion and to a lesser extent absorption through skin (Grant and Sheppard, 2008; Podar and Ramsey, 2005; Browning, 1961). Inhalation is more related with occupational hazards, whereas ingestion occurs through consuming food. Wagner (1993) reported 70% of the Cd taken up by

humans originates from plant foods. One example is the excessive Cd accumulation and subsequent exposure to Itai-Itai disease in some Japanese farm families who consumed rice grown on contaminated soils with Zn mine waste (Simmons et al., 2005; Ogawa et al., 2004). Studies from Japan and China reported adverse health effects from soil Cd even at a lower concentration of 2 mg total Cd kg^{-1} (Cai et. al., 1990), whereas no Cd diseases were observed in other cases where people consumed garden crops grown on soils with 50-150 mg total Cd kg^{-1} (Chaney et al., 1999). When people have a high concentration of Cd in their dietary foods, part of this Cd is initially absorbed into the intestinal mucosa and held in the intestinal turnover pool for a prolonged period. Later on, this Cd is absorbed into the circulation and deposited into tissues. According to FAO/WHO, a provisional tolerable weekly intake of 400-500 μg Cd per person is recommended (approximately 1 μg Cd kg^{-1} body weight d^{-1}). The average dietary intake of Cd by Canadians is 0.21μg kg^{-1} d^{-1} (Dabeka and Mckenzie, 1992). Cereals alone can contribute up to 30 to 36 percent of the total dietary intake of Cd per day. Among everyday foods, fish, vegetables, breads and cereals contain the highest Cd levels (Dabeka and McKenzie, 1992). Durum wheat is routinely grown on Canadian prairie soils and rarely can contain as much as 3.8 mg Cd kg^{-1}, however, 0.28 mg Cd kg^{-1} being the average concentration in Canadian durum wheat grain (Garrett et al., 1998). The voluntary CODEX limit for grains is set at 0.2 mg Cd kg^{-1} (Joint FAO/WHO Food Standards Programme CODEX Alimentarius Committee, 2008). Therefore, along with health risks from contaminated food, it is assumed that high grain Cd concentrations may hinder exportability of grain and grain products.

Under conditions of no fertilizer supplementation, Cd is present in amounts equivalent to 1 mg kg^{-1} in many plants and animal tissues (Browning, 1961). Cd contained in the harvested portions of grains increase the potential for human exposure, while further Cd can enter the food chain through animal feed (Kubota et al., 1992). If the crops are not grown in highly localized areas such as mining sites, they do not elicit a phytotoxic response. However, levels may be high enough to contribute to significant amount of Cd to daily human diet (Wagner, 1993).

Cd has no beneficial effect to human health. The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) has classified Cd as a Class 1 carcinogen (Chaney et. al., 1999). An exposure to significantly higher Cd level is reported to cause Itai- Itai disease, renal tubular dysfunction, reproductive failure and possibly even infertility and a combination of osteomalacia and osteoporosis (Åkesson et al., 2006; Alfvén et al., 2004; Ogawa et al., 2004; Ismail et al., 2002). Other health effects that can be caused by excessive Cd intake are damage to central nervous system, damage to immune system, physiological disorder, possibly DNA damage or cancer development, aggression and anxiety. However, most of these studies were tested on animals, and detailed work is required to confirm toxicity level to humans (Barański, 1986; Barański et al., 1983).

1.5 Project Background

This study is part of the Canadian Research Network in Toxicology programme, Metals in the Human Environment (MITHE). The study started in 2005 with the aim to determine the impact of crop management practices on cadmium availability of selected

agricultural crops. Crop management practices include tillage, rotation and phosphorus (P) fertilization. The approach used was to determine their single and interactive effects on Cd concentration and total Cd accumulation in two crops (flax and durum wheat) and whether arbuscular mycorrhizal fungi association of these two crops are associated with restricted Cd uptake from soils. The study consisted of two field experiments. The first trial, examined the effect of tillage, rotation and fertilization practices. Tillage practices chosen were conventional tillage and reduced tillage. Crop rotations included flax-durum wheat and canola-durum wheat, considering the fact that flax is highly mycotropic whereas durum wheat is intermediate and canola is non-mycotropic. Fertilization practices consisted of two levels of P fertilizer (0 and 30 kg P_2O_5 per hectare). Monoammonium phosphate (MAP) used as a phosphate fertilizer source came from Kapuskasing, ON and had low amount of Cd as impurities in it. The second trial, examined in more detail levels of P fertilization with a high Cd laden fertilizer source on arbuscular mycorrhizal fungi colonization and Cd accumulation in durum wheat and flax. Four levels of phosphorus (0, 20, 40 and 80 kg P per hectare) were added. Monoammonium phosphate (MAP) used as a P fertilizer source came from Idaho and had high amount of Cd as impurities in it. The unique aspect of this thesis study is the examination of the association of management practices, arbuscular mycorrhizal fungi and crop Cd concentration and accumulation in the field.

1.6 Aim and Outline of the Thesis

This thesis aimed at identifying soil and plant management factors that affect Cd accumulation in durum wheat and flax. This was done by answering the following two

research questions: (1) what are the effects of tillage, crop rotation and P fertilizer on Cd accumulation?, and (2) are arbuscular mycorrhizal fungi able to restrict Cd accumulation in the crops. The findings of the study were summarized in a model to interpret different management practices studied and their potential mechanisms that may affect Cd accumulation to crops.

The complete thesis is written by the leading author. The thesis is comprised of two manuscripts. The first, "Crop management alters arbuscular mycorrhizal fungal colonization and cadmium accumulation in durum wheat" determined the impacts of crop management practices generally used on the Canadian Prairies on arbuscular mycorrhizal fungi colonization and crop Cd accumulation in durum wheat. The second, "Long-term high Cd-containing phosphate fertilizer impacts on mycorrhizal colonization and cadmium concentration and accumulation in durum wheat and flax" detailed the prospective application of different rates of P fertilizer containing high level of Cd on Cd accumulation of durum wheat and flax and association to arbuscular mycorrhizal fungi colonization. Finally, the thesis ends with a general discussion and conclusions chapter. Discussion is made of the relationships between the two studies in answering the two research questions given above.

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2. CROP MANAGEMENT ALTERS ARBUSCULAR MYCORRHIZAL FUNGAL COLONIZATION AND CADMIUM ACCUMULATION IN DURUM WHEAT

2.1 Abstract

Cadmium (Cd) accumulation in crops is becoming a growing concern due to potential health risks from contaminated foods. In the present study, cadmium concentration and accumulation in durum wheat was investigated in an intensively managed tillage-previous crop-fertilization experiment at AAFC, Brandon. This study was designed to test the hypothesis that non-host previous crop, tillage and phosphorus fertilization reduce arbuscular mycorrhizal fungi colonization and therefore increase Cd accumulation in durum wheat. Plots were either planted to a crop dependent upon arbuscular mycorrhizal fungi (flax), or a crop not dependent upon arbuscular mycorrhizal fungi (canola). In the following year all plots were planted with durum wheat. The two tillage practices were conventional (CT) and reduced tillage (RT). The crops received either 0 or 30 kg P_2O_5/ha as monoammonium phosphate (MAP) containing low Cd as impurities in it. Soil cores and above ground plant biomass were collected to analyze plant available phosphorus (P) and Cd concentration in the soil, crop physical growth, root arbuscular mycorrhizal fungi colonization and above ground Cd, Zn and P concentration. The results showed no significant tillage and previous crop effect on Olsen P and DTPA Cd concentration in the soil. Tillage had a weak effect on above ground Cd concentration and total Cd accumulation at second sampling time. Previous crop had no

effect on above ground Cd concentration and Cd accumulation across the study years. A P fertilizer effect on above ground Cd concentration and total Cd accumulation was found inconsistent across the study years. Multiple-linear regression analysis of the potential factors affecting crop Cd accumulation showed that root arbuscular mycorrhizal fungi colonization was one of the most important variables that controlled above ground Cd concentration and accumulation. The percentage of arbuscular mycorrhizal fungi total colonization showed negative correlation with above ground tissue Cd concentration in conventional tillage plots throughout the study period except at first sampling in 2007. The result showed that above ground Cd concentration decreased as root mycorrhizal colonization increased. The findings indicated arbuscular mycorrhizal fungi association in restricting Cd concentration and accumulation in above ground tissues.

2.2 Introduction

Cadmium (Cd) is a non-essential trace element naturally present in soils. Additional sources of cadmium to soil are fertilizer, manure, sewage sludge and aerial deposition (Alloway and Steinnes, 1999). The uptake of this element into plants can be a concern to human health. According to the FAO/WHO, a provisional tolerable weekly intake of 400-500 μg Cd per person is recommended (approximately 1 μg Cd kg^{-1} body weight d^{-1}). The average dietary intake of Cd by Canadians is 0.21μg kg^{-1} d^{-1} (Dabeka and Mckenzie, 1992). Cereals alone can contribute up to 30 to 36 percent of the total dietary intake of Cd per day. Among the daily foods, fish, vegetables, breads and cereals contains the highest Cd concentration (Dabeka and McKenzie, 1992). Irrespective of additional sources to soil, Cd is present in amounts equivalent to 1 mg kg^{-1} in many

plants and animal tissues (Browning, 1961). Cd contained in the harvested portions of grains is a means of direct exposure to plant uptake of Cd, while Cd is also realized through diets of livestock and soil residue on food (Kubota et al., 1992). Durum wheat is an important export grain grown on Canadian prairie soils. Concentration of Cd in Canadian grown durum wheat ranges from 0.10 - 0.50 mg kg^{-1} with an average being 0.28 mg kg^{-1} depending on the cultivar (Garrett et al., 1998). The voluntary CODEX limit of grains is 0.2 mg Cd kg^{-1} (Joint FAO/WHO Food Standards Programme CODEX Alimentarius Committee, 2008). Therefore, along with health risks from contaminated food, it is assumed that high grain Cd concentrations may hinder exportability of grain and grain products.

The economic implications coupled with the perceived health risk have prompted multiple research initiatives which attempt to characterize and understand the mechanisms that control Cd accumulation and distribution in food. One of the approaches that has been taken is to determine the potential use of arbuscular mycorrhizal fungi (AMF) in restricting accumulation of Cd in grains. These arbuscular mycorrhizal fungi are ubiquitous in agricultural soil and are known to have an important role in plant nutrition (Smith and Read, 2002). A major group of crops form a symbiotic relationship with this fungus (Smith and Read, 2002). In this relationship, arbuscular mycorrhizal fungi aid the plant to absorb low mobility nutrients such as phosphorus (P) and zinc (Zn) that otherwise would be inaccessible to the plant. In return, the fungi are supplied with available carbon (C) from the plant (Hamel, 2007). External hyphae of arbuscular mycorrhizal fungi extend from the rhizosphere soil and beyond the phosphorus depletion zone to access a greater volume of undepleted soil than the root alone (Hamel, 2007).

Also, the small diameter mycorrhizal hyphae (20-50 μ) allow the fungi to access soil pores that cannot be explored by roots. Therefore, mycorrhizal roots have a greater effective surface area to absorb nutrients and explore a greater volume of soil than non-mycorrhizal ones.

There is considerable diversity of mycorrhizal fungi in metal contaminated soils (Cairney and Meharg, 1999) which is in contrast to the above ground floral diversity (Macnair, 1993). A number of studies have shown that arbuscular mycorrhizal fungi confer plants with tolerance against non-essential trace metal stress (Meharg, 2003; Joiner et al., 2000; Hartley et al., 1997). Different mechanisms are proposed to be involved in this activity, including metal binding to the hyphae (Joiner et al., 2000), organic acid exudation (Meharg, 2003), intracellular compartmentalisation (Joiner and Leyval, 1997) and competition with other nutrients.

Agricultural practices are reported to modify the level of arbuscular mycorrhizal fungal colonization of roots. Some studies showed reduced arbuscular mycorrhizal fungi colonization in roots following a non-mycorrhizal previous crop (Kabir et al., 1998; Lu and Miller, 1989) whereas others reported no significant effects of non-host crops on arbuscular mycorrhizal fungi colonization (Ocampo and Hayman, 1981; Hayman et al., 1975). The contradictory results are not surprising considering the complexity of mycorrhizal systems and the myriad of undefined variables and interactions inherent in field conditions.

Other than host crops, soil tillage and phosphorus fertilization were reported to reduce AMF colonization (McGonigle et al., 2003; Kabir et al., 1999; Kabir et al., 1998; Vivekanandan and Fixen, 1991). Phosphorus fertilizers are frequently applied to improve

the P nutrition of crops. Since P fertilizers can increase P concentration in plant tissue, they can depress mycorrhizal colonization (Kahiluoto et al., 2000, 2001; Liu et al., 2000).

Phosphorus fertilizers contain cadmium Cd as an impurity at levels varying from trace amounts to in excess of 300 mg kg^{-1} on a total dry weight basis, depending on the concentration available in the rock from which the fertilizer was manufactured (Alloway and Steinnes, 1999). The use of Cd containing P fertilizers is a major anthropogenic source of Cd in agricultural soils (Sheppard et al., 2007). In a number of long-term field studies, an increase in soil Cd from phosphorus fertilizer application was associated with increased crop Cd accumulation (Adams et al., 2004; Kashem and Singh, 2002).

In addition to Cd, phosphorus fertilizers may also contain Zn as an impurity (Alloway and Steinnes, 1999). Continued P fertilizer addition can also result in Zn accumulation in the soil (Lambert et al., 2007). Zn and Cd are chemically similar and may compete for binding sites in soil and uptake sites within the plant (Jiao et al., 2004; Grant et al., 2000; Grant et al., 1999; Christensen and Huang, 1999; Grant and Bailey, 1997). Zn is not a strong competitor with Cd for binding sites in soils. However, a high concentration of Zn in the soil solution may increase Cd desorption from soil (Christensen, 1984).

The experiment reported here aimed at determining if arbuscular mycorrhizal fungi colonization is affected by management practices and, in turn, if this alters Cd accumulation in above ground plant tissue of durum wheat. The management practices examined to alter arbuscular mycorrhizal fungi colonization were previous crop, tillage and P fertilization. In addition to Cd, Zn and P concentrations as well as total

accumulations in the crop shoots were determined to see if they are related to above ground cadmium concentration.

2.3 Materials and Methods

2.3.1 Site Description and Treatments

The study was conducted at the Agriculture and Agri-Food Canada, Brandon Research Centre, located in Brandon, Manitoba (N49^{0}54'0", W99^{0}57'0"). The study plot encompasses portions of section 27 in Township 10, Range 19 west of the prime meridian. The surface geological deposits which form the parent materials of the soils on the research centre have been derived mainly as a result of glacial lake and stream water deposition and erosion (Fitzmaurice et al., 1999). The mean annual maximum and minimum air temperature of the site was 9.7°C and - 3.06°C respectively for the period of January 2005 to December 2007. The mean annual precipitation in 2005, 2006 and 2007 was 582mm, 465mm and 460mm, respectively, with about 433mm, 283mm and 280mm occurring during the May to September growing season. It was observed that 2005 was a very wet year compared to 2006 and 2007. The soil at the study site was mapped as Ramada series (Orthic Black soil developed on well drained, moderately calcareous, fine loamy CL, lacustrine sediments), non-eroded to moderately eroded, nearly level to very gently sloping, non-stony, non-saline, Rego Black soil (Fitzmaurice et al., 1999). The experiment was started in 2005 as a three-year tillage by previous crop-fertilization trial to determine the impact of metal uptake of crops. The experimental design was a split plot. The plots were 5m long by 2m wide. The whole plot factors were previous crop: flax or canola and tillage system: conventional or reduced tillage. The split factor was

phosphate fertilizer with two levels: 0 and 30 kg P_2O_5 ha^{-1} as MAP side banded. MAP was purchased locally and it was assumed that the fertilizer was originated from Kapuskasing, ON. Fertilizer metal analyses showed that on average it contained 3.7 mg kg^{-1} Cd in 2006 and 2007. In case of Zn, the MAP used contained 67 and 199 mg kg^{-1} Zn in the years 2006 and 2007, respectively. The cultivar used was AC Avonlea durum (durum wheat) at a seeding rate of 100 kg ha^{-1}. All plots received a total of 100 kg N ha^{-1} as urea side-banded.

2.3.2 Above Ground Plant Dry Weight

From 2005 to 2007, above ground plant biomass was harvested approximately at four weeks and eight weeks after germination. In the first year, harvesting was done once in June 2005. In the second and third years, first harvesting was done at June 29, 2006 and June 14, 2007 and second harvesting was done at July 28, 2006 and August 13, 2007 respectively. A second harvesting was included to measure above ground Cd concentration in the durum wheat before it was ready to harvest. During both samplings, four plant cuttings in 20 cm long sections were taken at the base of the plants from each plot, with two cuttings taken 1 m apart from both the front and back of the plots on either side. The samples were then weighed, rinsed in RO water and dried in a drying room for two weeks in paper and/or drying bags. After drying, the samples were weighed again prior to grinding and then stored in sealed bags for P, Zn and Cd analysis.

2.3.3 Root Sampling

Root samples were collected at the same time as above ground plant biomass. These samples were used for analysis of root length density and AMF colonization. A tulip bulb planter (7 cm diameter) was used in root sampling. The planter was placed over top of a single plant and pressed down in the soil to a depth of 10 cm. In this way, an equal volume of soil was confirmed in each sample and could be used to calculate soil volume, bulk density and root density. During each sampling, four root and soil samples were collected from each plot. Two samples were taken 1 m away from both the front and back of the plots and one row in from either side. They were mixed in a polyethylene bag and stored in a cooler (4˚C) immediately upon return from the field. The samples were washed within four to five days using a root washer (Gillison's Variety Fabrication Inc., GVF Root Washer) and then stored in 70% ethanol until AMF analysis. The samples were soaked overnight (in water at 4˚C) prior to washing to facilitate separation of fine roots from the fine textured soil.

2.3.4 Root Density Determination

The root density in each sample was estimated quantitatively following the method outlined by Newman (1966) with modifications from Tennant (1975). At first, a rectangular tray (24.5×17.5×4.5 cm) was filled with water. Transparency of 2×2 cm grid squares was placed on the bottom of the tray. The roots were then cut into 2 cm long fragments and submerged in water on the tray. Root intercepts with the vertical and horizontal grid lines were counted to calculate total root length (R) using the formulae R= 11/14 × No. of intercepts (N) × 2 (grid unit). The average root density in each sampled plot was calculated by dividing the root length by the bulk volume of soil removed by the

tulip bulb planter. A random sub sample of the root fragments were then preserved in 75% ethyl alcohol (Phillips and Hayman, 1970) until processing for AMF colonization.

2.3.5 Determination of Mycorrhizal Colonization

Mycorrhizal colonization was measured using the method as described by McGonigle et al. (1990). At first, the preserved roots were removed from the ethyl alcohol solution and rinsed with water. Roots were then autoclaved in 10% KOH solution for 10 minutes, well rinsed with deionized water and stained with Chlorazol Black E (0.03% w/v in 1:1:1 lactic acid, glycerol and water) at 90°C for 90 minutes. The roots were rinsed again and glycerol was added for destaining overnight. The root segments were mounted on slides for viewing under a compound microscope at 250x magnification. The percentage of mycorrhizal colonization was measured according to the magnified intersections method of McGonigle et al. (1990). Total fractional colonization (TC) was separated into specific colonization's of arbuscules (only arbuscule or both arbuscule and hyphae present in the intersection, AC), only vesicle (vesicle colonization, VC) and only hyphae (hyphal colonization, HC). To estimate mycorrhizal benefit to crop production, fractional colonization was multiplied by root length density to give colonized root length density (Brundrett et al., 1996). If the colonization density appeared small compare to percent colonization, the plant biomass is likely small and therefore crop production may not benefitted from the association. The ratio of AC/TC was calculated to estimate the cost: benefit ratio of mycorrhizal colonization in the different treatments (Dekkers and van der Werff, 2001).

2.3.6 Nutrient and Trace Metal Analysis

Olsen's method for alkaline, neutral or calcareous soil was used for the determination of plant available phosphate in 0.5M $NaHCO_3$ soil extracts in the range 0-3 mg P l^{-1} (Olsen and Sommers, 1982). Then 0.5 g NaOH was added to adjust the pH to 8.5. A few drops of dilute NaOH/HCl was required to bring the pH 8.5. This solution was used as the 0.5 M $NaHCO_3$ extracting solution. During extraction, a 1.0 g sample of air-dried soil (ground to <2 mm) was placed in a 50 ml centrifuge tube. Then 20 ml of $NaHCO_3$ solution and 0.25 g charcoal (DARCO G-60 powder, Sigma-Aldrich, Catalogue no. 242276) was added to each tube. The tubes were caped and shaken for 30 minutes on a reciprocating shaker at 120 strokes per minutes. The extract was then filtered through Whatman no. 42 filter paper into 20 ml scintillation vials. The samples were then stored at 4°C and analyzed within 72 hours.

The ascorbic acid method (Murphy and Riley, 1962) was used for colorimetric determination of phosphorus in soil extracts. During analysis phosphate reacted with ammonium molybdate in an acid medium to form molybdophosphoric acid which was then reduced to a molybdenum blue complex by reaction with ascorbic acid. The presence of antimony allowed for a rapid development of color. Absorbance was measured at 882 nm using a spectrophotometer (Ultrospec 2100 pro UV/Visible Spectrophotometer). A standard curve constructed from absorbance readings of standards (at known P concentration) was used to convert absorbance values of samples to phosphate concentration in solution.

The DTPA extraction method was used for the determination of plant available cadmium in diethylene triamine penta-acetic acid (DTPA) soil extracts. At first 3.93 g

DTPA is weighed into a 2 L Erlenmeyer flask containing a small amount of water. Then 29.8 g (26.5 ml) TEA (triethanolamine) was added and the solution diluted to 1900 ml with deionized water. After that 2.94 g $CaCl_2.2H_2O$ was added and the solution was thoroughly mixed. The pH was then adjusted to 7.3 using 6N HCl. The solution was made to 2L with deionized water. During extraction, 10 g air dried soil (ground to <2 mm) was placed into a 50 ml centrifuge tube. Then 20 ml extracting solution was added to each tube. In each set of 40 samples, a sample repeat, reagent blank and reference soil was included. The tubes were placed on reciprocating shaker for 2 hours. The extract was then filtered through Whatman no. 42 filter paper into 20 ml scintillation vials and stored at 4°C until sent for analysis. The samples were analyzed using a graphite furnace atomic absorption spectrophotometer (GFAA, Varian Spectra 400) at the Agriculture and Agri-Food Canada, Brandon Research Centre. Certified reference soil from SCP SCIENCE was used to check the reliability of Cd determinations.

Above ground dry matter was weighed and grounded prior to wet digestion for mineral and trace element determination of plant above ground biomass. At first 1 g of ground sample was digested in a temperature of 100-150°C (first phase 100°C, second phase 130°C and third phase 150°C) in 10 ml of concentrated HNO_3. Digested samples were transferred to volumetric flasks and then diluted to 50 ml. Zinc in plant digests was analyzed using flame atomic absorption spectrophotometer (Perkin-Elmer PC5100). Cadmium and phosphorus were analyzed using graphite furnace atomic absorption (Perkin-Elmer Simultaneous Multi-Element Atomic Absorption Spectrometer, SIMAA 6100). The analyses were done at the Canadian Grains Commission, Winnipeg, Manitoba..

2.3.7 Statistical Analysis

Above- ground dry matter, root length density, AMF colonization and nutrients were compared using a split-plot design and the Statistical Analysis Software programme (SAS Inc., version 9.0). Before the analysis, the data were tested for normality using the Proc Univariate Procedure of SAS (Shapiro-Wilks). Treatment mean and standard errors of the mean were calculated manually in Microsoft Excel. Tests of fixed effects of tillage, previous crop, phosphorus fertilizer and their interaction effects were calculated using the "Mixed Procedure" of the SAS system. All analysis were performed at α=0.10 significance level and using replicate data for each sample occasion. Multiple linear regression analysis was done to determine measured variables related to Cd concentration in above ground biomass. A forward multiple linear regression procedure was used with variables having correlations of $P < 0.10$ being added and retained to the model using the software programme, SigmaStat 3.5 (SysStat Software Inc.). Multiple-linear regression analysis was done using average data for the four replicates.

2.4 Results

2.4.1 Stand Density

Effect of tillage, previous crop and P fertilization on stand density of durum wheat in years 2005, 2006 and 2007 are presented in Appendix I. Tillage effect was found inconsistent across the study periods. There was no significant previous crop effect on stand density, though in 2006 it was found numerically higher when grown after canola than flax. In case of P fertilization, no differences were observed between fertilized and

Control treatments. There were no significant treatment interaction effects on seedling stand density.

2.4.2 Grain Yield

Effect of tillage, previous crop and phosphorus fertilization on grain yield of durum wheat in 2005, 2006 and 2007 are presented in Appendix II. There was a significant tillage effect for durum wheat yield ($P < 0.05$) in 2006 (Appendix II); however, the result was found inconsistent with other years. When durum wheat was grown after canola, yields were either equal or numerically higher under conventional tillage than reduced tillage (data not shown). P fertilization did not significantly influence grain yield, although yield was numerically equal or higher with P than in the Control. There were no significant treatments interactions effects on grain yields across the study period.

2.4.3 Above Ground Plant Dry Weight

Effect of tillage, previous crop and P fertilization on above ground dry weight of durum wheat in 2005, 2006 and 2007 are presented in Appendix III. The above ground dry weight was not affected by tillage and previous crop across the study period. In 2005, above ground weight was found to be little higher in reduced tillage system having flax as a previous crop; however, the result was found in consistent in the following years. In the case of P fertilizer, significant treatment effect was observed at first sampling in 2007. P fertilization increased above ground dry weight in June 2007. A similar trend was observed at first sampling in June 2006, however, statistical analysis showed the effect

insignificant. No treatment interaction effects were observed in all three years. In 2007, first sampling was done early three weeks after germination, which was why above ground dry weight was very low in June 2007 (Appendix III).

2.4.4 Plant Available Phosphorus and Cadmium in the Soil

The main and interaction effects of tillage system and previous crop on concentration of DTPA Cd and Olsen P in the soil in 2005, 2006 and 2007 are given in Appendix IV and Appendix V. There were no significant tillage and previous crop effect on DTPA extractable-Cd in the soil. Though the highest cadmium concentration was found in 2006 in conventional tillage system having canola as a previous crop; statistical analysis shows the difference insignificant. A similar result was found in Olsen P level in the soil. Olsen-P exhibited no significant differences in concentrations among treatments across the study period. The interaction effects of the treatments on DTPA-Cd and Olsen-P concentrations were found insignificant.

2.4.5 Above Ground Cd Concentration

The summary of analysis of variance showing significance levels of tillage, previous crop and P fertilizer and their interaction effects for above ground Cd concentration and total Cd accumulation in durum wheat was given in Table 2.1. The result showed that tillage has weak effect on above ground Cd concentration and total Cd accumulation at second sampling time. The result was found consistent across study years with lower Cd concentration and accumulation in reduced tillage plots compared to conventional tillage plots (Appendix VI, VII). The second sampling was very important

in agronomic point of view in the present study considering tissue dilution effect of Cd in the above ground durum wheat and grain Cd concentration. The effect was found insignificant at first sampling except in year 2007 where tillage had weak effect on Cd concentration and Cd accumulation. Previous crop had no significant effect on above ground Cd concentration and Cd accumulation across the study years. P fertilizer moderately affected above ground Cd concentration at second sampling in 2006 and strongly affected Cd accumulation at first sampling in 2007. However the effect was found to be inconsistent with other study years and there were no specific pattern of P fertilizer effect on above ground Cd concentration and Cd accumulation. The interaction effect of tillage and previous crop was found to have weak effect on above ground Cd concentration at first sampling in 2007; however, the result was found to be inconsistent with other years. The interaction effect of tillage, previous crop and P fertilizer was found to have no effect on Cd concentration at first sampling across the study period. A moderate interaction effect was found on Cd concentration at second sampling in 2006; however, the result was not consistent with other study years.

2.4.6 Root Mycorrhizal Colonization

The summary of analysis of variance showing significance levels of tillage, previous crop and P fertilizer and their interaction effects for root arbuscule and total colonization of arbuscular mycorrhizal fungi in durum wheat was given in Table 2.2. The effects of tillage and previous crop on arbuscule and total colonization were found to be very highly significant at first sampling in 2006 and 2007. It was found that reduced

Table 2.1 Summary of analysis of variance showing significance levels of treatment and interaction effects for Cd concentration and accumulation in above ground durum wheat as affected by tillage, previous crop and P fertilizer

Source of variation	Cd concentration			Cd accumulation		
	2005	2006	2007	2005	2006	2007
First sampling						
Tillage (T)	0.79	0.82	0.23	0.96	0.19	**0.10**
Previous crop (PC)	0.86	0.35	0.37	0.58	0.53	0.22
P fertilizer (P)	0.94	0.15	0.18	0.61	0.11	**0.001**
T × PC	0.49	0.70	**0.07**	0.47	0.53	0.20
T × PC × P	0.97	0.94	0.99	0.95	0.71	0.27
Second sampling						
Tillage (T)	NA[1]	**0.09**	**0.07**	NA	**0.06**	**0.08**
Previous crop (PC)	NA	0.67	0.15	NA	0.13	0.56
P fertilizer (P)	NA	**0.02**	0.42	NA	0.19	0.98
T × PC	NA	0.20	0.28	NA	0.42	0.18
T × PC × P	NA	**0.05**	0.94	NA	0.44	0.77

[1] NA = data not available

Table 2.2 Summary of analysis of variance showing significance levels of treatment and interactions effects for arbuscule and total colonization of arbuscular mycorrhizal fungi in durum wheat as affected by tillage, previous crop and P fertilizer

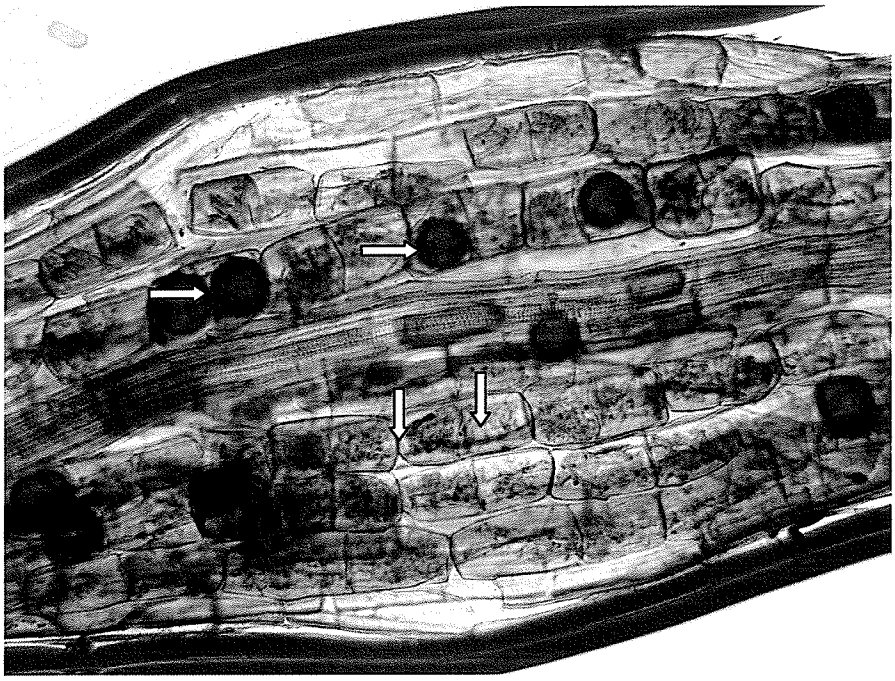
Source of variation	Arbuscule Colonization			Total Colonization		
	2005	2006	2007	2005	2006	2007
First sampling						
Tillage (T)	0.16	**0.0001**	**0.01**	**0.10**	**0.001**	**0.01**
Previous crop (PC)	0.69	**0.0001**	**0.01**	**0.06**	**0.001**	**0.01**
P fertilizer (P)	0.31	0.84	0.70	0.18	0.98	0.23
T × PC	**0.02**	**0.07**	**0.01**	**0.03**	**0.09**	**0.07**
T × PC × P	0.41	0.23	0.88	0.94	0.26	0.74
Second sampling						
Tillage (T)	NA[1]	**0.0001**	0.52	NA	**0.0001**	0.97
Previous crop (PC)	NA	**0.0001**	**0.02**	NA	**0.0001**	0.74
P fertilizer (P)	NA	0.98	0.24	NA	0.23	0.19
T × PC	NA	**0.02**	0.95	NA	**0.04**	0.21
T × PC × P	NA	**0.07**	0.71	NA	**0.10**	0.88

[1] NA = data not available

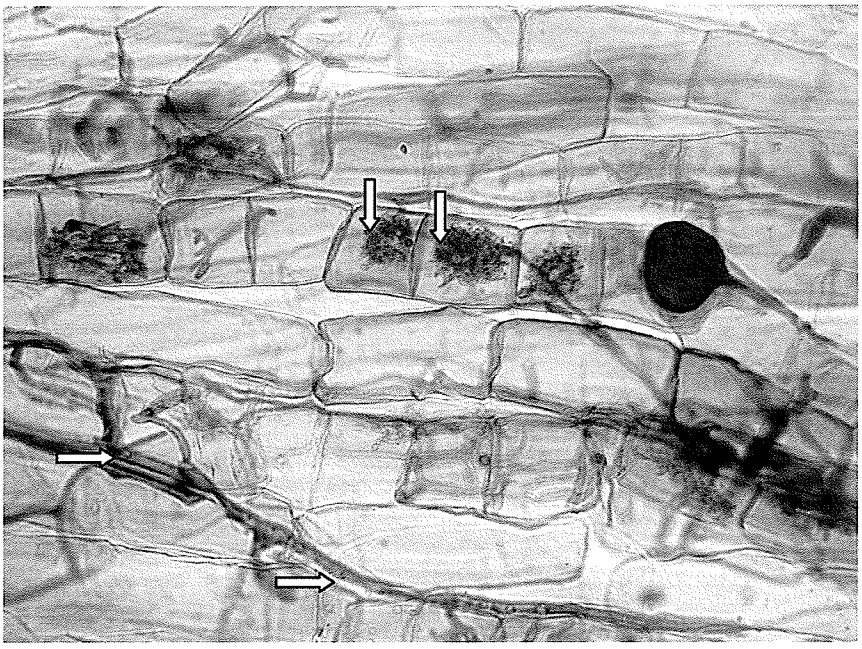
Table 2.3 Summary of analysis of variance showing significance levels of treatment and interaction effects for arbuscule density of arbuscular mycorrhizal fungi and ratio of arbuscular root length to total colonized root length (Cost:Benefit) in durum wheat as affected by tillage, previous crop and P fertilizer

Source of variation	Arbuscule Colonization Density			Cost:Benefit		
	2005	2006	2007	2005	2006	2007
First sampling						
Tillage (T)	0.61	**0.0005**	**0.001**	0.51	0.22	0.47
Previous crop (PC)	0.20	**0.0009**	**0.001**	0.15	0.80	0.41
P fertilizer (P)	0.37	**0.04**	0.15	0.51	0.21	0.59
T× PC	0.31	**0.03**	**0.001**	**0.09**	0.36	**0.03**
T × PC × P	0.40	0.40	0.12	0.39	0.71	0.65
Second sampling						
Tillage (T)	NA[1]	**0.0001**	0.18	NA	**0.001**	0.34
Previous crop (PC)	NA	**0.0001**	**0.04**	NA	**0.001**	**0.004**
P fertilizer (P)	NA	0.85	0.22	NA	0.42	0.36
T × PC	NA	0.79	0.34	NA	0.45	0.58
T × PC × P	NA	0.68	0.30	NA	0.51	0.64

[1] NA = data not available



a



b

Figure 2.1 a) Good colonization of arbuscules (vertical arrow) and vesicles (horizontal arrow) from reduced tillage plot and b), poor colonization of arbuscules (vertical arrow) and hyphae (horizontal arrow) from conventional tillage plot at eight weeks after emergence of durum wheat in year 2006

tillage and previous crop flax increased both root arbuscule and total colonization in durum wheat (Appendix VIII, IX). The result was found to be consistent at second sampling in 2006; however, in 2007, tillage and previous crop moderately affected arbuscule colonization and did not affect total colonization. In 2005, tillage and previous crop did not affect arbuscule colonization; however, it weakly affected total colonization at first sampling (Table 2.2, Appendix X). It was assumed that 2005 was a very wet year that might affect root arbuscular mycorrhizal fungi to form symbiosis with durum wheat roots. The effect of P fertilizer was found insignificant across study period. The interaction effects of tillage and previous crop on arbuscule and total colonization was moderately significant at first sampling. The result was found to be consistent across the study period; however, it was inconsistent with second sampling. The interaction effects of tillage, previous crop and P fertilizer was insignificant at first sampling across the study period and at second sampling it weakly affected arbuscule and total colonization in 2006. The interaction effect was found to be inconsistent with 2007.

The differences in arbuscule between systems showed mostly the same trend when multiplied by the density of the durum wheat roots. The ratio of arbuscular colonization to total colonization (cost:benefit) was not significantly different between conventional tillage and reduced tillage system except at second sampling in 2006 (Table 2.3). In most of the sampling times, reduced tillage had a higher cost: benefit ratio than conventional tillage, however statistical analysis found the differences significant only at second sampling in 2006 (Appendix XI, XII, XIII). The ratio was found to be significantly different between plots having flax as a previous crop at second sampling in 2006 and 2007 (Table 2.3). Previous crop flax was found to increase cost:benefit ratio than previous crop canola, indicating that durum wheat plots having canola as a previous

crop may not have been benefitting from arbuscular mycorrhizal fungi colonization to the same extent as the plots having flax as a previous crop. Fertilization was found to have no effect on mycorrhizal cost: benefit ratio across sampling years (Table 2.3). The interaction effects of tillage and previous crop were found to differ for mycorrhizal cost:benefit ratio at first sampling in 2007 (Table 2.3), however the result was found to be inconsistent with other years.

2.4.7 Relationship between Root Mycorrhizal Colonization and Above Ground Cd Concentration

It was observed in Table 2.1 that tillage was the most important factor among all the management practices studied in the present study that affected above ground Cd concentration. In the case of root mycorrhizal colonization, it was observed that tillage was also one of the important factors that high significantly affected root colonization in the field. Based on these two findings, the association between root arbuscule colonization of arbuscular mycorrhizal fungi and above ground Cd concentration was taken into consideration to examine their inter relationship under tillage condition. It was found that the relationship mostly showed a negative trend in conventional tillage plots across the study period; although it was only found weakly significant at second sampling in 2006 (Figure 2.2). The result was found to be inconsistent with first sampling in 2007 where the relationship was found high significantly positive in the conventional tillage plots. The reason for the difference might be due to the early sampling in 2007 and it was assumed that the seedlings might uptake more Cd during early stages of growth. The relationship between root arbuscule colonization and above ground Cd concentration in

reduced tillage plots mostly didn't show any trend except at first sampling in 2007 where a weak positive relationship was observed.

The association between root total mycorrhizal fungi colonization and above ground Cd concentration mostly showed the same trend where a negative association was observed in conventional tillage plots, except at first sampling in 2007 (Figure 2.2). The relationship was found to be highly significant at second sampling in 2007. The result showed that above ground Cd concentration decreased as root mycorrhizal colonization increased. In the case of reduced tillage plots, a weak positive association was recorded at first sampling in 2007; however, the result was not consistent in other years.

2.4.8 Factors Affecting Above Ground Cd Uptake

Multiple-linear regression models were used to predict the factors most related to above ground durum wheat Cd concentration and accumulation for the first and second sampling times across the study years. The models are summarized in Figure 2.4 and Table 2.4. It was found that root arbuscular mycorrhizal fungi colonization was one of the most important variables that controlled above ground Cd concentration and accumulation. Other variables included durum wheat root length density, above ground biomass and Zn concentration. The first model showed that above ground Cd concentration was significantly affected by root length density, above ground Zn concentration and cost:benefit ratio of arbuscular mycorrhizal fungi colonization in the roots (Figure 2.4). Among these variables, above ground Cd concentration was positively affected by root length density and negatively by above ground Zn concentration as well as cost: benefit ratio of root arbuscular mycorrhizal symbiosis. The result was found consistent at the second sampling where above ground Cd concentration was mostly

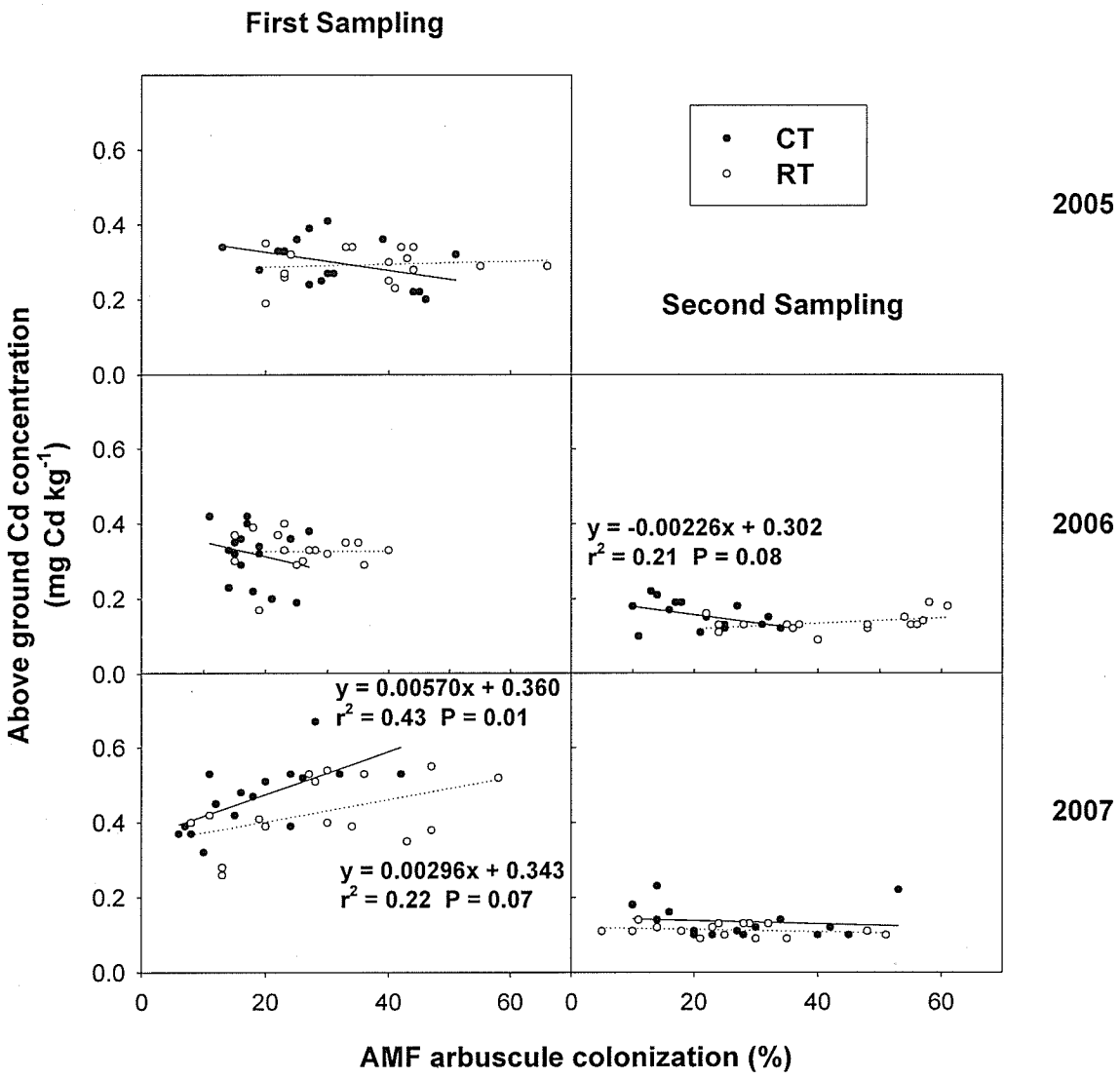


Figure 2.2 Relationship between percentage of root arbuscule colonization and above ground Cd concentration of durum wheat for first and second sampling times and for conventional (CT) and reduced (RT) tillage treatments across study years

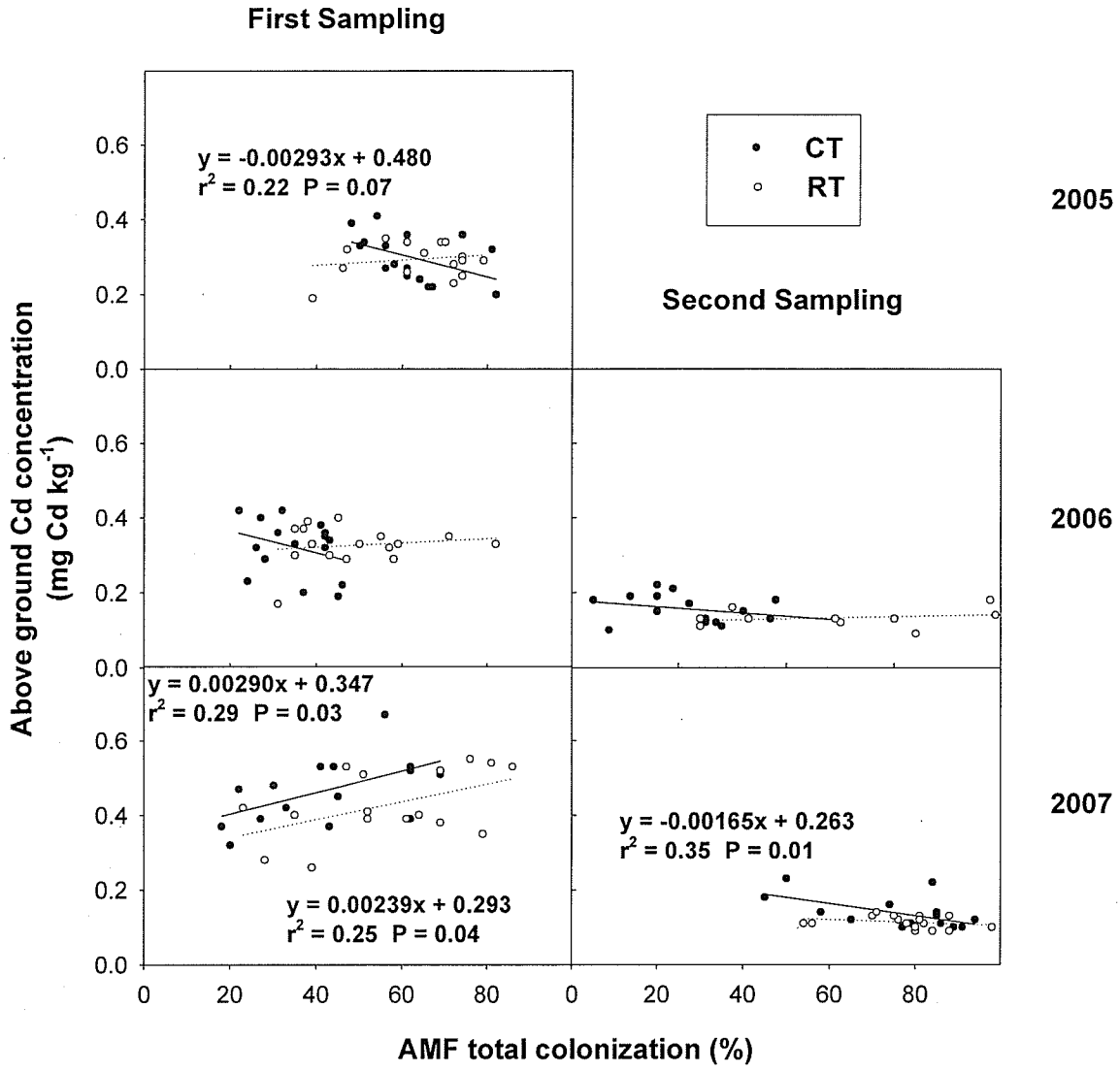


Figure 2.3 Relationship between percentage of root arbuscular mycorrhizal fungi total colonization and above ground Cd concentration of durum wheat for first and second sampling times and for conventional (CT) and reduced (RT) tillage treatments across study years

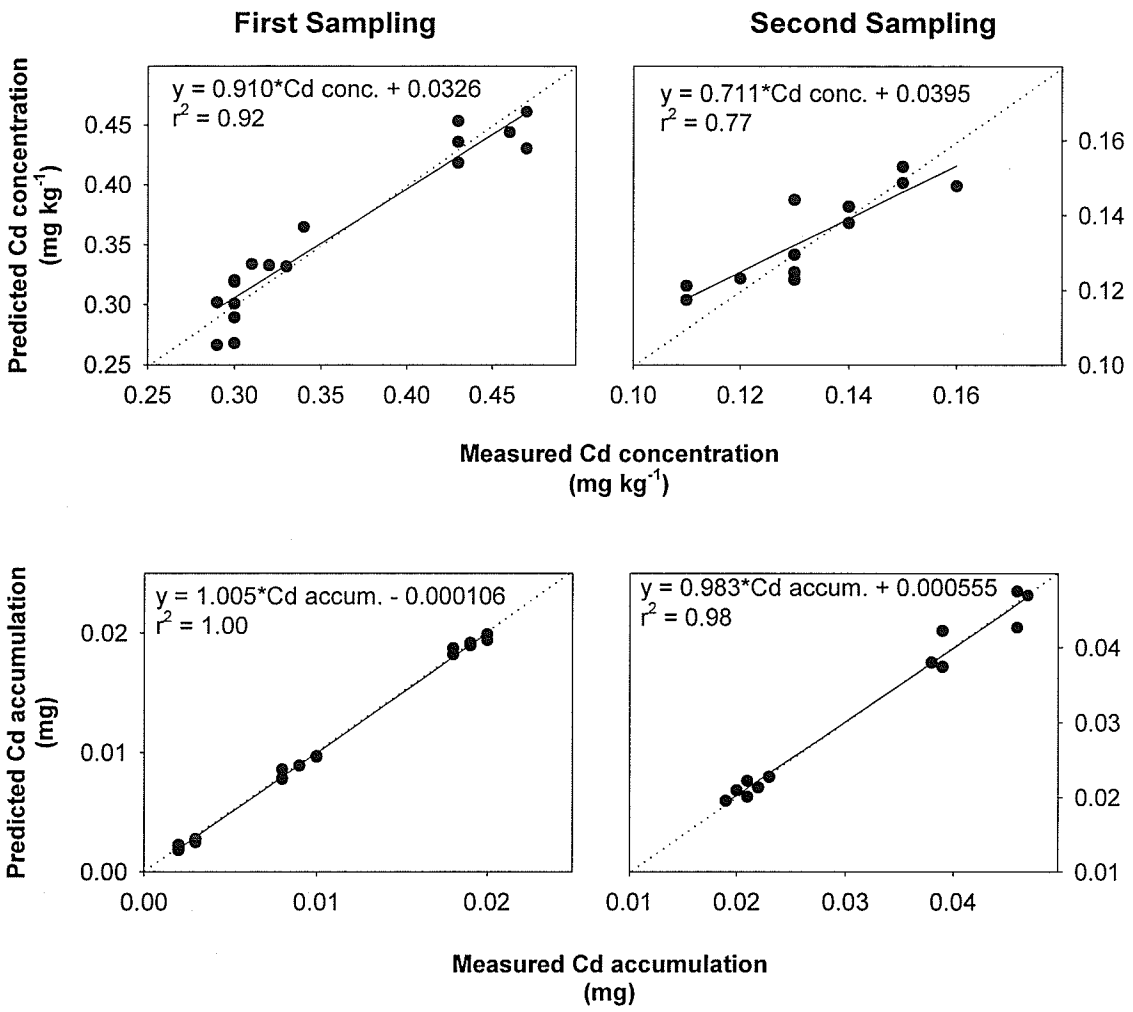


Figure 2.4 Summary of multiple-regression models for prediction of Cd concentration and accumulation in above ground biomass for first and second sampling time across study years. Actual models for prediction of Cd concentration and accumulation are given in Table 2.4.

Table 2.4 Summary of multiple-linear regression models for prediction of Cd concentration and accumulation in above ground shoot biomass for first and second sample occasions. Models are across study years for first sampling (2005-2007) and second sampling (2006-2007)

Sampling	Variable	Coefficient	r^2	P
First sampling				
Cd (mg kg^{-1})			0.92	<0.001
	RLD	0.0363		<0.001
	Zn (mg kg^{-1})	-0.00569		0.001
	Cost:Benefit	-0.401		0.062
	Constant	0.511		
Cd (mg)			1.00	<0.001
	Shoot biomass	0.329		<0.001
	% total AMF	-0.0000639		0.001
	AMF density	0.000474		0.018
Second sampling				
Cd (mg kg^{-1})			0.82	<0.001
	Cost:Benefit	0.0868		0.005
	% total AMF	-0.000474		0.026
	Constant	0.131		
Cd (mg)			0.98	<0.001
	Shoot biomass	0.284		<0.001
	RLD	0.000758		0.037
	Constant	-0.0520		

affected by cost:benefit ratio of mycorrhizal symbiosis and percentage of total mycorrhizal colonization. Considering the results from both sampling times, it was seems that arbuscular mycorrhizal fungi were the most significant variable that affected above ground Cd concentration.

In the case of above ground Cd accumulation, the model showed that the most significant variables were above ground biomass, percentage of total mycorrhizal colonization and arbuscular mycorrhizal fungi density. Figure 2.4 showed that when these three variables were taken into consideration, the model showed the best fitness in terms of measured Cd accumulation with predicted Cd accumulation. The result was found to be inconsistent with second sampling time where above ground biomass was not affected by root mycorrhizal colonization; however, it was still affected by above ground biomass. Considering all these four models it was assumed that percentage of total mycorrhizal colonization, cost: benefit ratio of mycorrhizal symbiosis, above ground biomass, root length density and above ground Zn concentration were the important variables that affected above ground durum wheat Cd concentration and accumulation in the present study.

2.5 Discussion

2.5.1 Tillage Effect on Above Ground Cd Concentration

The results showed that tillage significantly affected above ground Cd concentration in durum wheat tissues. It was found that above ground tissue Cd concentration was higher when durum wheat was grown in conventional tillage plots compared to reduce tillage plots. There might be a number of reasons working behind the increased above ground Cd concentration in conventional tillage plots including tillage

effect on soil pH, cation exchange capacity and decomposition of crop residue from previous crop. However, in the present study it was also found that tillage significantly affected arbuscular mycorrhizal fungi colonization in the durum wheat roots. It is assumed that tillage might be affecting above ground tissue Cd concentration by altering root mycorrhizal colonization. The result showed that conventional tillage plots significantly reduced root arbuscular mycorrhizal fungi colonization. This happened due to the soil disturbance from conventional tillage system that might damage arbuscular mycorrhizal hyphae in the field. In countries where soil temperature goes below freezing condition, the only propagules that likely survive winter are hyphae (Miller, 2000). Under conventional tillage system, hyphal fragmentation would reduce the length of the hyphal fragments that can survive before the next crop is germinated (Kabir et al., 1999, 1997). As a result, delayed colonization may occur, particularly when there is winter in between two crops, in soil with medium to low level of colonization.

2.5.2 Interaction between Mycorrhizal Colonization and Above Ground Cd Concentration

The summary of the results in the multiple-regression models showed that above ground tissue Cd concentration was negatively associated with percentage of arbuscular mycorrhizal colonization and cost:benefit ratio of the fungal symbiosis in the roots. The percentage of arbuscular mycorrhizal fungi total colonization showed negative association with the above ground tissue Cd concentration in conventional tillage plots throughout the study period except at first sampling in 2007. The first samples in 2007 were collected a week earlier compared to other years and that might be the reason for higher Cd concentration in the above ground tissues. The negative association between

percentage of arbuscular mycorrhizal fungi total colonization and above ground tissue Cd concentration might indicate mycorrhizal tolerance and efficiency in restricting Cd transfer from roots to the above ground tissues. The tolerance might either be reduced mobilization of toxic metals through decreased organic exudation, or immobilizing toxic metals through precipitation with organic acids, or detoxify toxic metals by complexing them (Meharg, 2003). Some of the organic acids, such as citric, malic, oxalic etc. are readily utilizable organic substrate (Jones, 1998) and their affinities for particular metal ions may play an important role in their cycling and their ability to mobilize-immobilize ions.

A possible mechanism of arbuscular mycorrhizal fungal restriction of Cd to the above ground tissue might be related with increased Zn uptake from fungal symbiosis. Along with P, arbuscular mycorrhizal fungi also help plant in uptaking Zn in Zn-deficient soils. In the present study soil Zn concentration was not determined. So, there are possibilities that in favourable conditions arbuscular mycorrhizal fungi might increase above ground Zn uptake. These favourable conditions may include reduced tillage and mycorrhizal host previous crop (flax) in the field. In the present study, previous crop affected above ground Zn concentration in the durum wheat tissues. It was found that mycorrhiza host flax increased above ground Zn concentration compared to mycorrhiza non-host canola (Appendix XIV). In natural condition, the mobility of Zn in soils is very low and its uptake is diffusion limited. Mycorrhizal fungi can extend beyond root zone by extending hyphal network and can help plant in uptaking Zn that would otherwise be inaccessible to the root. The increased Zn might compete with Cd in the root uptake sites and reduced above ground transport of Cd.

Another possible mechanism of mycorrhizal restriction of Cd to the above ground tissue might be Cd sequestration in the hyphal wall. Electron energy loss spectroscopy showed greater accumulation of Cd in the mycorrhizal structures (hyphae, vesicles) than in the root cells themselves (Chen et al., 2001). It was suggested that sequestration of Cd by polyphosphate in the mycorrhiza might have been important in minimizing transfer to the plant, however this requires confirmation. In another study Joiner and Leyval (1997) conducted experiments with only the AMF hyphae having access to a soil compartment containing radioactive Cd (^{109}Cd). They found that AMF colonization enhanced Cd assimilation by the plant, but restricted transfer of this Cd to the shoot, with most Cd staying in the root. It was assumed that arbuscular mycorrhizal fungi immobilized Cd in the roots. Some other studies suggested that metal cations can be complexed by metal chelators such as dicarboxilic organic acids, amino acids, phytochelatins (PCs) and metallothioneins (MTs) in the vacuoles, but evidence to confirm this assumption is still scanty. Another possibility that Cd accumulation might result in damage to the fungi has also been addressed. Weissenhorn et al. (1994) showed that prolonged exposure to Cd can result in the development of tolerance in *Glomas* spp., but again the mechanism is not known.

2.5.3 Interactions between Above Ground Zn and Cd Concentration

The summary of multiple-linear regression models showed that above ground Zn concentration affected tissue Cd concentration at the first sampling. It was found that above ground Zn concentration was negatively associated with tissue Cd concentration. This might happen due to the competitive interaction of these two metals in the uptake sites. In terms of chemical behavior, Cd had the greatest similarity with Zn and it was

assumed that these two metals might enter root cells via a common transport system. The competitive interaction between Cd and Zn could be the result of the role of Zn in maintaining root-cell plasma membrane integrity. In Zn-deficient condition, root-cell plasma membrane disrupts in such a way that leads to the accumulation of Cd. It was estimated that, correcting Zn deficiency would then lower crop Cd accumulation by restoring the integrity of root-cell plasma membranes (Welch and Norvell, 1999).

2.5.4 Conclusion

Among crop management practices studied in the present study, tillage affected above ground tissue Cd concentration. Conventional tillage increased above ground tissue Cd concentration in compared to reduced tillage. Conventional tillage also reduced arbuscular mycorrhizal fungi colonization that was found to have a significant negative association with above ground tissue Cd concentration. It was suggested that mycorrhizal fungi either directly immobilizing Cd in the root system to restrict transfer to the shoot or indirectly increasing above ground tissue Zn concentration that will compete with Cd in their common transport system. However, confirmation of these assumptions will require further study in controlled environment in growth chamber or green houses to clearly investigate mycorrhizal role in crop Cd uptake. There are opportunities to conduct laboratory study where mycorrhizal hyphae and crop roots would be separated in a root-compartmented system to confirm the mechanisms of arbuscular mycorrhizal fungi in limiting above ground tissue Cd concentration.

2.6 Acknowledgements

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3. LONG-TERM HIGH Cd-CONTAINING PHOSPHATE FERTILZER IMPACTS ON MYCORRHIZAL COLONIZATION, CADMIUM CONCENTRATION AND ACCUMULATION IN DURUM WHEAT AND FLAX

3.1 Abstract

Phosphorus (P) fertilizers are essential to obtain high crop productivity, however they can contain Cd as an impurity and therefore can be a major source of Cd input to human food chains. The present study investigated Cd uptake in durum wheat and flax from different levels of P fertilizer applied in the field and potential association of arbuscular mycorrhizal (AM) fungi in restricting Cd accumulation. The study was designed to examine to what extent does above ground Cd concentration in flax and durum wheat increase in situation of high loading of Cd as impurities in P fertilizer and low root arbuscular mycorrhizal fungi colonization. High rates of P fertilizer addition was done to providing Cd loading and repressed AM fungal colonization. The experimental design was a randomized complete block with four replications. The four fertilizer treatments were 0, 20, 40 and 80 kg P ha^{-1}. Soil cores and above ground biomass were collected to analyze plant available P and Cd concentration in the soil, yield, AM fungal colonization and shoot Cd, Zn and P uptake. The result showed no significant treatment effect on yield and root length density. Plant available P and Cd concentration in the soil were higher in fertilized compared to Control treatments. DTPA soil Cd and above ground Cd concentration was positively associated with each-other. Arbuscule colonization decreased with fertilized durum wheat; however, no such effects were

observed in flax. Shoot Cd concentration and total Cd accumulation increased with addition of P fertilizer to both durum wheat and flax. Such an increase was a function of the rate of fertilizer application. It was observed that Cd concentration was higher in the above ground flax tissues compared to durum wheat tissues substantiating flax as a high Cd accumulating crop. It was found that mycorrhizal colonization and shoot Cd concentration were negatively associated to each-other in both durum wheat and flax. Cd concentration of soil was most related to above ground biomass concentration of Cd. However, addition of high levels of P fertilizer laden with Cd decrease the activity and function of mycorrhizal fungi for P and Zn transfer to the shoot that seems to have contributed slightly to higher Cd accumulation in flax and durum wheat.

3.2 Introduction

Phosphorus is one of the major plant nutrients that affect all biological processes (Ragothama, 2005). Plants require adequate P from the very early stage of growth for optimum production (Grant et al., 2001). The uptake of P early in the growth of plants is influenced by soil P, P applications and by soil and environmental conditions that affect P availability to the plant. In low available soil P condition, arbuscular mycorrhizal (AM) fungi association with roots may help to improve early season P nutrition (Bucher, 2007). The external hyphae of AM fungi can extend from the root surface to the soil beyond the P depletion zone and can access a greater volume of undepleted in average P soil than the root alone (Hamel, 2007). In highly managed agricultural systems, P fertilizers are commonly applied to ensure that sufficient P is available to optimize crop growth and

yield. For many crops, P is applied once before seed sowing and may have detrimental effects on colonization of AM fungi and crop growth (Smith and Read, 2002).

The interaction between P fertilization and AM fungal colonization is complex and it is hard to make simple generalization from published studies. A number of field studies showed that P fertilizers decreased AM fungal colonization (Treseder, 2004; Kahiluoto et al., 2000; Vivekanandan and Fixen, 1991); however, other field experiments showed the opposite (Johnson, 1993; Gryndler et al., 1990). The contradictory results are not surprising considering the complexity of mycorrhizal systems and the myriad of undefined variables and interactions inherent in field conditions. Some of those variables include the original fertility and organic matter content of the soil, balance of nutrients within the fertilizer and mycorrhizal dependency of the crop species or cultivars used (Grant et al., 2005).

P fertilizers contain Cd as an impurity at levels varying from trace amounts to in excess of 300 mg kg^{-1} on a total dry weight basis, depending on the concentration in the rock from which the fertilizer was manufactured (Alloway and Steinnes, 1999). The use of Cd laden P fertilizers is a major anthropogenic source of Cd in agricultural soils (Sheppard et al., 2007; McLaughlin et al., 1996). In a number of long-term field studies, an increase in soil Cd from P fertilizer application was associated with increased crop Cd accumulation (Adams et al., 2004; Kashem and Singh, 2002; Jones and Johnston, 1989). However, Cd concentration in crop is largely dependent on source of P fertilizer, amount of Cd concentration present in the fertilizer and the rate of application.

If Cd concentration in applied fertilizer is low, long-term P fertilizer application may not result in increases in soil Cd (Jeng and Singh, 1995; Mortvedt, 1987; Andersson and Hahlin, 1981). However, increase in soil Cd as a result of addition of P fertilizers has been reported in many studies (Jeng and Singh, 1995; He and Singh, 1993a; Andersson and Siman, 1991; Mulla et al., 1980). Singh (1991) estimated that Cd input from fertilizers was 2-3 times higher than crop removal rate and resulted in Cd accumulation in soil; however, in this situation it was not clear how soil Cd is affecting Cd accumulation by crops. A number of studies reported higher Cd uptake by the crops from the application of Cd containing P fertilizers (Andersson and Siman, 1991; Jones and Jonston, 1989; Jones et al., 1987; Mulla et al., 1980). In other studies, no increase in crop Cd uptake was reported from increased application of Cd containing P fertilizers (He and Singh, 1993 b,c; Baerug and Singh, 1990; Andersson and Hahlin, 1981).

In addition to the long-term effects of P fertilizer application on Cd accumulation in soils and crops, P fertilizer may also effect Cd concentration in the year of application (Grant et al., 1999). In a series of growth chamber studies, higher Cd concentration was recorded in a range of crops from the application of P fertilizers containing Cd (Jiao et al., 2004; Grant et al., 1999; Choudhary et al., 1994). However, the result was found to be different in field studies where Cd concentration in P fertilizer did not influence the Cd concentration of the crops in the year of application (He and Singh, 1995; Singh and Myhr, 1998; Guttormsen et al., 1995). Grant et al. (2002) showed that Cd concentration in durum wheat in several locations across Canadian Prairies increased by application of P in the year of application; however, the increase was not correlated to the concentration of Cd present in the P fertilizer.

In addition to Cd, P fertilizers may also contain Zn as an impurity, though unlike Cd, Zn is an essential plant nutrient (Alloway and Steinnes, 1999). When P fertilizer is applied over many years, Zn may accumulate in soil along with Cd (Lambert et al., 2007). Zn and Cd are chemically similar and may compete for binding sites in the soil system and uptake sites within the plant (Christensen and Huang, 1999). Although Zn was not a strong competitor with Cd for binding sites in soils, increasing Zn concentration in the soil solution may increase Cd desorption from soil (Christensen, 1984), resulting in increased Cd concentration in soil solution. It was suggested that although desorption could increase crop Cd availability, the competition between Zn and Cd for uptake and transfer by the plant may reduce accumulation of Cd (Zhao et al., 2005; Jiao et al., 2004; Grant et al., 2000; Grant et al., 1999; Grant and Bailey, 1997; Choudhary et al., 1995).

In the previous study of this thesis, it was found that low level of P fertilizer did not increase above ground Cd concentration in the durum wheat shoot (chapter two). Based on this finding the present study was designed to examine to what extent does above ground Cd concentration in flax and durum wheat increase in situation of high loading of Cd as impurities in P fertilizer and low root arbuscular mycorrhizal fungi colonization caused by high P addition. Arbuscules, hyphae and total colonization density were compared between plots receiving different levels of P fertilizers containing high Cd as impurities. A cost:benefit ratio of arbuscular mycorrhizal fungi colonization was calculated to determine if colonization is helping or hindering crop yields. Above ground Cd, Zn and P concentrations and total accumulations in the crop shoots were analyzed to determine whether P fertilizer was increasing crop Cd accumulation and if there was a

significant association between above ground Cd concentration and arbuscular mycorrhizal fungi colonization in the roots.

3.3 Materials and Methods

3.3.1 Site Description and Treatments

The study was conducted at the Agriculture and Agri-Food Canada, Brandon Research Centre, Brandon, Manitoba (N49054'0", W99057'0"). The study plot encompasses portions of section 27 in Township 10, Range 19 west of the prime meridian. The surface geological deposits which form the parent materials of the soils on the research centre have been derived mainly as a result of glacial lake and stream water deposition and erosion (Fitzmaurice et al., 1999). The mean annual maximum and minimum air temperature of the site was 9.7°C and - 3.06°C respectively for the period of January 2005 to December 2007. The mean annual precipitation in 2005, 2006 and 2007 was 582mm, 465mm and 460mm respectively with about 433mm, 283mm and 280mm was occurring during the May to September growing season. It was observed that 2005 was a very wet year compared to 2006 and 2007. The soil was mapped as Harding series that consists of imperfectly drained Gleyed Black soils developed on moderately to strongly calcareous, silty clay to clay, lacustrine deposits (Fitzmaurice et al., 1999).

The experiment was started in 2002 with wheat and flax grown in alternate years. The experiment was conducted for six years; however, in this study, the last three years were investigated having flax (2005 and 2007) and durum wheat (2006). The effect of P fertilization on AM fungal colonization and Cd concentration in the above ground tissues

were determined by measuring field colonization levels of arbuscular mycorrhizal fungi, average soil and plant Cd and nutrition concentrations in 2005, 2006 and 2007. The experimental design was a randomized complete block with four replications. The plots were 5 m long by 2 m wide. The four fertilizer treatments were 0, 20, 40 and 80 kg P ha^{-1} and they were randomized in the main plot. For 20P, 46 kg P_2O_5 ha^{-1} was applied as Monoammonium phosphate (MAP) side-banded as starter fertilizer. For 40P, 92 kg P_2O_5 ha^{-1} was applied as MAP of which half was side-banded as starter fertilizer and the rest broadcasted. For 80P, 46 kg P_2O_5 ha^{-1} (as MAP) was side banded as starter fertilizer and 138 kg P_2O_5 ha^{-1} (as MAP) was broadcasted. MAP used as a phosphate fertilizer source came from Idaho and had high amount of Cd (211 mg kg^{-1}) and Zn (3,500 mg kg^{-1}) as impurities in it. Nitrogen was balanced to 50 kg ha^{-1} for each treatment in flax and 90 kg ha^{-1} for each treatment in durum wheat as urea as a pre-plant band. The crop cultivars used were AC Avonlea (durum wheat) and AC Lightning (flax).

3.3.2 Above Ground Plant Dry Weight

Crop performance was measured as above ground dry weight from 2005 to 2007. Above ground plant biomass was harvested approximately four weeks and eight weeks after emergence in 2005 and 2006. In the first year, sampling was done once in June 2005. In the second and third years, first sampling was done on June 29, 2006 and June 14, 2007 and second sampling was done on July 28, 2006 and August 13, 2007. Sampling procedure was followed that previously given in section 2.3.2.

3.3.3 Root Sampling

Root samples were collected at the same time along with the above ground plant biomass. The sampling procedure was followed that previously given in section 2.3.3. These samples were used for analysis of root length density and AM fungal colonization.

3.3.4 Root Density Determination

The root density in each sample was estimated quantitatively following the method outlined by Newman (1966) with slight modifications of Tennant (1975). The procedure was followed that previously given in section 3.4.

3.3.5 Determination of Mycorrhizal Colonization

AM fungal colonization was measured using the method as described by McGonigle et al. (1990). The procedure was followed that previously given in section 2.3.5.

3.3.6 Nutrient and Trace Metal Analysis

Olsen's method for alkaline, neutral or calcareous soil was used for the determination of plant available phosphate in 0.5M $NaHCO_3$ soil extracts in the range 0-3 mg P l^{-1} (Olsen and Sommers, 1982). The ascorbic acid method (Murphy and Riley, 1962) was used for colorimetric determination of P concentration of extracts. The diethylene triamine penta-acetic acid (DTPA) extraction method was used for the determination of plant-available Cd concentration in soil extracts. In the present study only the lowest (0 kg P ha^{-1}) and the highest (80 kg P ha^{-1}) fertilizer P treatments were sampled and analyzed for DTPA extractable soil Cd. data was shown. Above ground dry matter was weighed and grounded prior to wet digestion for mineral and trace element determination of plant above ground biomass. Zn in plant digests was analyzed using

flame atomic absorption spectrophotometer (Perkin-Elmer PC5100). Cd and P were analyzed using graphite furnace atomic absorption (Perkin-Elmer Simultaneous Multi-Element Atomic Absorption Spectrometer, SIMAA 6100). The procedure was followed that previously given in section 2.3.6.

3.3.7 Statistical Analysis

Analysis of variance was done on data on above ground dry matter, root length density, AM fungal colonization in the root and P, Cd and Zn concentration in the shoot. Means were compared using LSD ($P < 0.10$) in the Statistical Analysis Software programme (SAS Inc., version 9.0). Before the analysis, the data were tested for normality using the Proc Univariate Procedure of SAS (Shapiro-Wilks). Treatment mean and standard errors of the mean were calculated manually in Microsoft Excel. Multiple linear regression analysis was done to determine measured variables related to Cd concentration in above ground biomass. A forward multiple linear regression procedure was used with variables having correlations of $P < 0.10$ being added and retained to the model using the software programme, SigmaStat 3.5 (SysStat Software Inc.). Multiple-linear regression analysis was done using average data for the four replicates.

3.4 Results

3.4.1 Above Ground Plant Dry Weight

The above ground dry weight was not significantly affected by P fertilization in all three years (Appendix XIV). In the case of flax, above ground weight was found a

little higher in fertilized plots compared with the Control; however, statistical analysis showed the difference insignificant. In the case of durum wheat, a similar trend was observed in June 2006 where higher above ground dry weight was recorded in P fertilized plots (Appendix XIV). In 2007, the first sampling was done early compare with 2005 and 2006, which was why above ground dry weight was very low compared to the previous two years (Appendix XIV).

3.4.2 Plant Available P and Cd in the Soil

The effect of P fertilization on concentration of DTPA-Cd and Olsen-P in the soil in 2004, 2005, 2006 and 2007 is given in Figure 3.1. In the present study the soils were collected from the Control and 80 kg P ha^{-1} treatments and hence results were shown from these two treatments only. Soil level of readily available Cd and P were found to be higher in fertilized compared to the Control treatment. The average of available P in the Control was 20 mg kg^{-1} which was four times lower than that for fertilized with 80 kg P ha^{-1}. The concentrations of available Cd in the fertilized ranged between 180 μg kg^{-1} and 230 μg kg^{-1} which lowered to 130 μg kg^{-1} in the Control treatment.

A very strong positive correlation was found between plant available P and Cd (Figure 3.2). The results presented in Figure 3.3 suggest that accumulation of Cd follows the same trend as of P. The r^2 value of soil P and Cd in 2005, 2006 and 2007 were 0.92, 0.91 and 0.94 respectively. It is assumed that application of P fertilizer could elevate Cd levels in the surface soil layer.

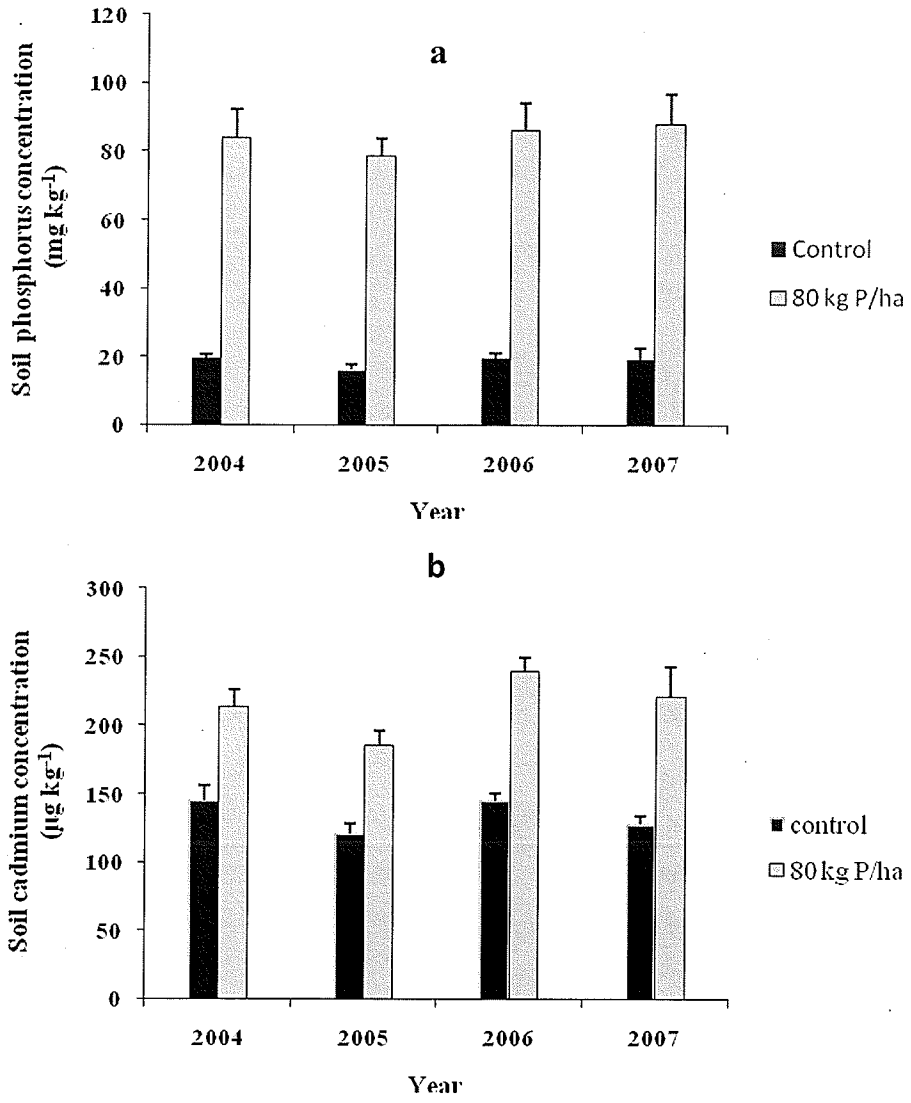

Figure 3.1 Effect of P fertilizer application on (a) $NaHCO_3$ extractable P (Olsen-P) and (b) DTPA extractable Cd from soil.

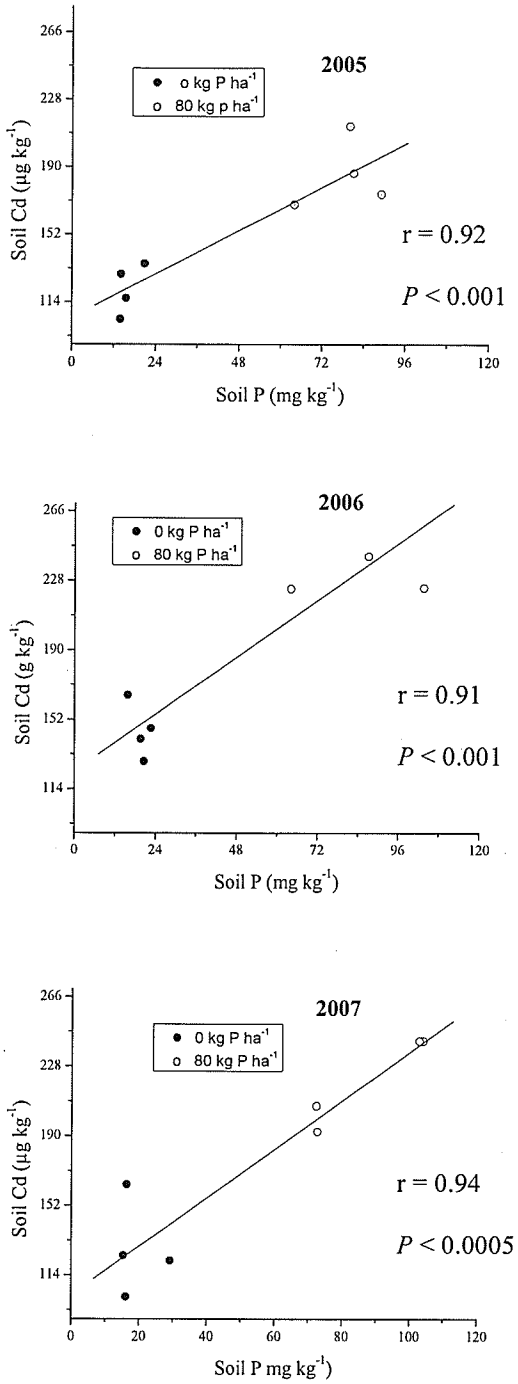

Figure 3.2 Relationship between plant available soil P (Olsen-P) and DTPA extractable Cd in 2005, 2006 and 2007.

3.4.3 Above Ground Cd Concentration

P fertilization had increased above ground Cd concentration in flax and durum wheat tissues (Table 3.1). It was found that above ground tissue Cd concentration increased as level of P fertilizer application increased in the soil. In the case of flax, an average of 1.31 mg kg^{-1} Cd was determined at first sampling in 2007 from plots fertilized with 80 kg P ha^{-1} which was nearly double that of the Control. Cd concentration in flax tissues was lower at second sampling compared to the first sampling indicating tissue dilution of Cd from increased plant growth. In the case of durum wheat, average Cd concentration at first sampling was 0.17 mg kg^{-1} for the Control which increased up to 0.25 mg kg^{-1} with 80 kg P ha^{-1} fertilizer application. P fertilizer did not significantly affect above ground dry weight. So, P fertilizer effect on above ground flax and durum wheat total Cd accumulation showed the same response as of above ground tissue Cd concentration.

In the case of above ground Cd concentration between flax and durum wheat, it was found that Cd concentration was higher in flax compared to durum wheat. The differences between above ground Cd concentration between first and second sampling were also higher in flax compared to drum wheat. It was found that above ground tissue Cd concentration was more or less equal between sampling times in durum wheat whereas it was more than three times lower at second sampling compared to first sampling in above ground flax tissues. It was assumed that flax was a high Cd accumulator crop compared to durum wheat and accumulated more Cd at the early stages of growth.

Table 3.1 Effects of different levels of P fertilizer on Cd concentration and accumulation in above ground flax and durum wheat

Fertilizer rate kg P ha^{-1}	Cd concentration mg kg^{-1}			Cd accumulation mg		
	2005 Flax	2006 Durum wheat	2007 Flax	2005 Flax	2006 Durum wheat	2007 Flax
First sampling						
0	0.45 (0.06)b[1]	0.17 (0.02)b[1]	0.74 (0.10)c	0.02 (0.00)b	0.01 (0.00)b	0.002 (0.00)c
20	0.47 (0.04)b	0.22 (0.02)a	0.92 (0.10)bc	0.02 (0.00)b	0.02 (0.00)ab	0.002 (0.00)bc
40	NA[2]	0.23 (0.02)a	1.10 (0.10)ab	NA	0.02 (0.00)ab	0.003 (0.00)a
80	0.63 (0.03)a	0.25 (0.01)a	1.31 (0.20)a	0.03 (0.00)a	0.02 (0.00)a	0.003 (0.00)ab
Second sampling						
0	NA	0.15 (0.02)c	0.25 (0.06)ab	NA	0.04 (0.00)c	0.018 (0.00)a
20	NA	0.18 (0.02)bc	0.22 (0.03)b	NA	0.05 (0.01)bc	0.015 (0.00)a
40	NA	0.22 (0.03)ab	0.32 (0.06)a	NA	0.06 (0.01)ab	0.023 (0.01)a
80	NA	0.23 (0.01)a	0.32 (0.04)a	NA	0.06 (0.00)a	0.022 (0.01)a

[1] Values shown are the average of four replicate values with standard error of the average given in parenthesis. Different letters denote significant differences at $P < 0.05$

[2] NA = Data not available

3.4.3 Relationship between DTPA Extractable Soil Cd and Cd Concentration in the Above Ground Tissues

Relationship between DTPA soil Cd and Cd concentration in the above ground flax and durum wheat tissues across the study years was presented in Figure 3.3. It was found that in all the cases DTPA soil Cd and above ground tissue Cd concentration was positively associated with each-other. The figure showed that as DTPA soil Cd concentration increased, then above ground tissue Cd concentration was also increased. The high soil Cd in the present experiment came of addition of high levels of P fertilizer containing high levels of Cd as impurities in it. So, it was assumed that the more Cd was loaded in the soil along with P fertilizer, the higher it was transferring to the above ground tissues. The bi-plot in the Figure 3.3 showed that amount of above ground Cd concentration was higher in the flax in both sampling times compared to durum wheat indicating flax as a high Cd accumulator crop. The figure also showed that the association was stronger at the first sampling period in both flax and durum wheat compared to second sampling indicating higher uptake of Cd during early stages of growth (Figure 3.3). Later on, Cd diluted in the later stages of growth that lowered Cd concentration in the above ground tissues.

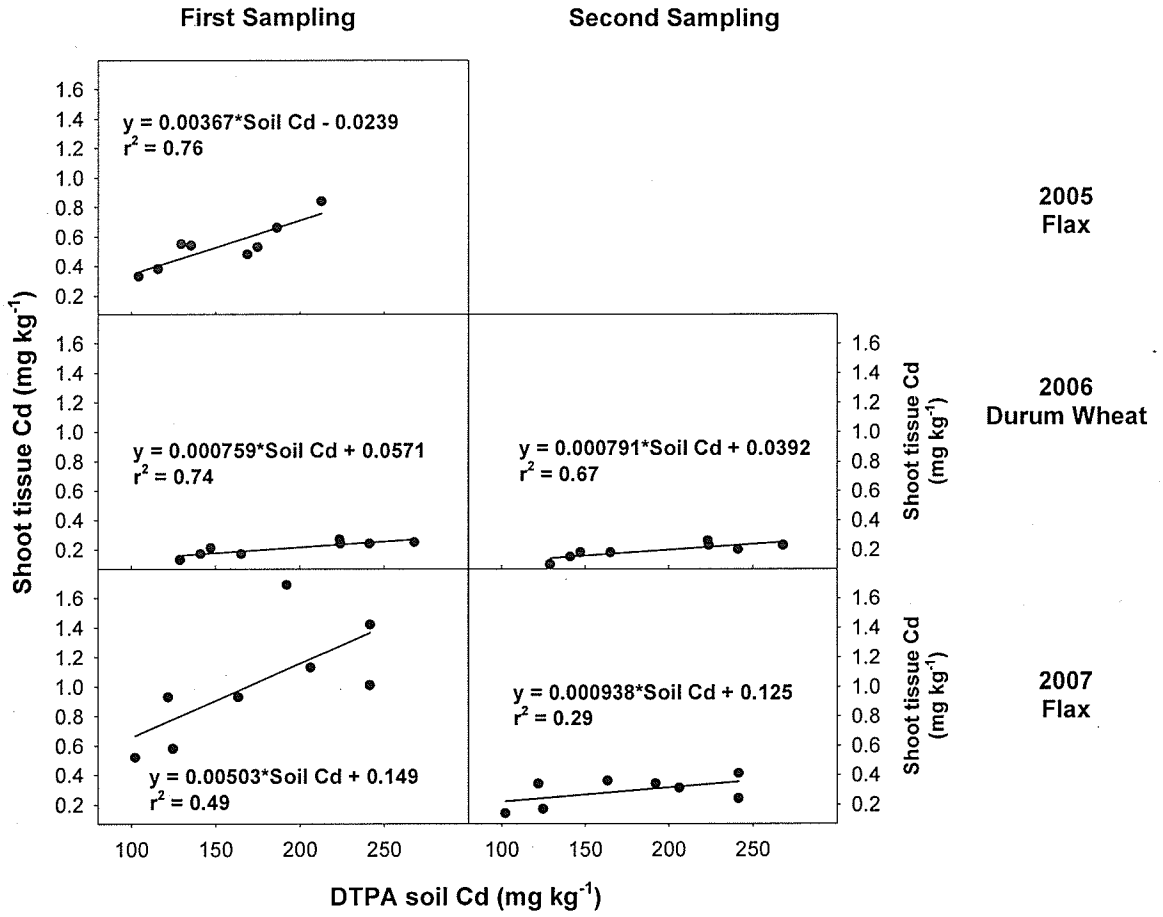

Figure 3.3 Relationship between DTPA extractable soil Cd and Cd concentration in the above ground tissues of flax and durum wheat in both first and second sampling across the study years.

3.4.4 Root Arbuscular Mycorrhizal Fungi Colonization

Effects of P fertilizer on root arbuscular mycorrhizal fungi colonization in flax and durum wheat in 2005, 2006 and 2007 are presented in Table 3.2. The result showed differences in P fertilizer effect between flax and durum wheat. No significant effect of P fertilizer on flax AM fungal colonization was observed except at the first sampling in 2007 where colonization was found the lowest in the plots fertilized with 20 kg P ha^{-1}. In the case of durum wheat, a significant decrease in arbuscule and total colonization was observed at both first and second sampling in 2006 (Table 3.2). An average of 78% total colonization was recorded from unfertilized (Control) durum wheat plots during early sampling which decreased to 55% in plots fertilized with 80 kg P ha^{-1} (Table 3.2). There was not a significant treatment effect on hyphae colonization (data not shown). The differences in arbuscule and total colonization between P fertilizer treatments showed mostly the same trend when multiplied by the density of the flax and durum wheat roots in the plots. In the case of durum wheat, the arbuscular colonization density decreased with increasing P fertilizer level in the soil (Table 3.2). Similar trend was observed in total colonization density though statistical analysis showed the differences insignificant (data not shown).

The ratio of arbuscular colonization to total colonization (Cost:Benefit) was not significantly different between P fertilizer treatments in flax (Table 3.2). The ratio was found different between P fertilizer treatments for durum wheat (Table 3.2). P fertilizer was found to significantly decrease cost:benefit ratio than Control, indicating that P fertilized durum wheat plots may not have been benefiting from arbuscular mycorrhizal fungi colonization to the same extent as the unfertilized plots.

Table 3.2 Effect of different levels of P fertilizer on percentage of arbuscule (AC), total colonization (TC), arbuscule density (ACD) and Cost:Benefit of root arbuscular mycorrhizal fungi in flax and durum wheat

Fertilizer rate kg P ha^{-1}	2005 – Flax				2006 - Durum wheat				2007- Flax			
	AC %	TC %	ACD cm ml^{-1}	Cost: Benefit	AC %	TC %	ACD cm ml^{-1}	Cost: Benefit	AC %	TC %	ACD cm ml^{-1}	Cost: Benefit
First Sampling												
0	62 (4)a[1]	78 (2)a	3 (0.2)a	0.8 (0.0)a	55 (5)a	78 (4)a	5 (0.9)a	0.7 (0.1)a	48 (4)a	74 (4)a	3 (0.4)a	0.7 (0.1)a
20	52 (2)ab	75 (3)a	2 (0.4)a	0.7 (0.0)a	45 (2)ab	71 (6)a	4 (0.9)ab	0.6 (0.1)ab	37 (5)b	60 (6)a	2 (0.3)a	0.6 (0.1)a
40	NA[2]	NA	NA	NA	35 (3)bc	64 (4)ab	2 (0.3)bc	0.5 (0.0)bc	44 (4)ab	74 (4)a	3 (0.3)a	0.6 (0.1)a
80	53 (5)ab	75 (2)a	2 (0.6)a	0.7 (0.0)a	25 (3)c	55 (6)b	2 (0.2)c	0.5 (0.1)c	41 (2)ab	74 (5)a	2 (0.4)a	0.6 (0.1)a
Second sampling												
0	NA	NA	NA	NA	64 (4)a	86 (7)a	8 (1.3)a	0.8 (0.1)a	54 (4)a	86 (7)a	15 (1.3)a	0.6 (0.0)a
20	NA	NA	NA	NA	51 (2)b	79 (5)ab	8 (0.8)a	0.7 (0.0)ab	48 (9)a	79 (4)a	12 (3.7)a	0.6 (0.1)a
40	NA	NA	NA	NA	45 (3)b	79 (6)ab	7 (0.7)a	0.6 (0.0)ab	49 (10)a	85 (6)a	12 (2.9)a	0.6 (0.1)a
80	NA	NA	NA	NA	31 (3)c	61 (7)b	6 (0.9)a	0.5 (0.1)b	47 (7)a	83 (6)a	14 (2.5)a	0.6 (0.1)a

[1] Values shown are the average of four replicate values with standard error of the average given in parenthesis. Different letters denote significant differences at $P < 0.05$

[2] NA = Data not available

3.4.5 Factors Affecting Above Ground Cd Concentration

The summary of multiple-linear regression models for prediction of Cd concentration for the first and second sample occasions for both flax and durum wheat across the study period is given in Figure 3.4 and Table 3.3. It was found that factors affecting tissue above ground Cd concentration vary between flax and durum wheat. Above ground tissue P concentration significantly affected flax shoot Cd concentration at first sampling time (Table 3.3). The association was found highly positive with an r^2 value of 0.53. For the second sampling occasion the factor that most significantly related tp above ground flax Cd concentration was cost:benefit ratio of AM fungi symbiosis in the roots. The association was found highly negative with an r^2 value of 0.46. The result showed that the less flax was benefitted from mycorrhizal symbiosis, the more Cd concentrated in the above ground tissues. This might be related to addition of high levels of P fertilizer in the soil with high levels of Cd as impurities in it. It was assumed that in the presence of high levels of P in the soil, flax needed not to be dependent on AM fungi for P nutrition; however, it still supplied carbon for the fungus. The extra carbon cost tends to be higher the cost and in return the fungi didn't supply P to the flax. As a result net benefit went lower and more Cd was transferred to the flax tissues. On the other hand, in the case of durum wheat the most significant factor that affected above ground tissue Cd concentration at both first and second sampling was root arbuscule density of arbuscular mycorrhizal fungi (Figure 3.4, Table 3.3). It was found that above ground Cd concentration was high negatively associated with root arbuscule density with r^2 values of 0.43 and 0.35 for the first and second sampling respectively (Figure 3.4, Table 3.3). It was assumed that above ground durum wheat tissue Cd concentration decreased as root

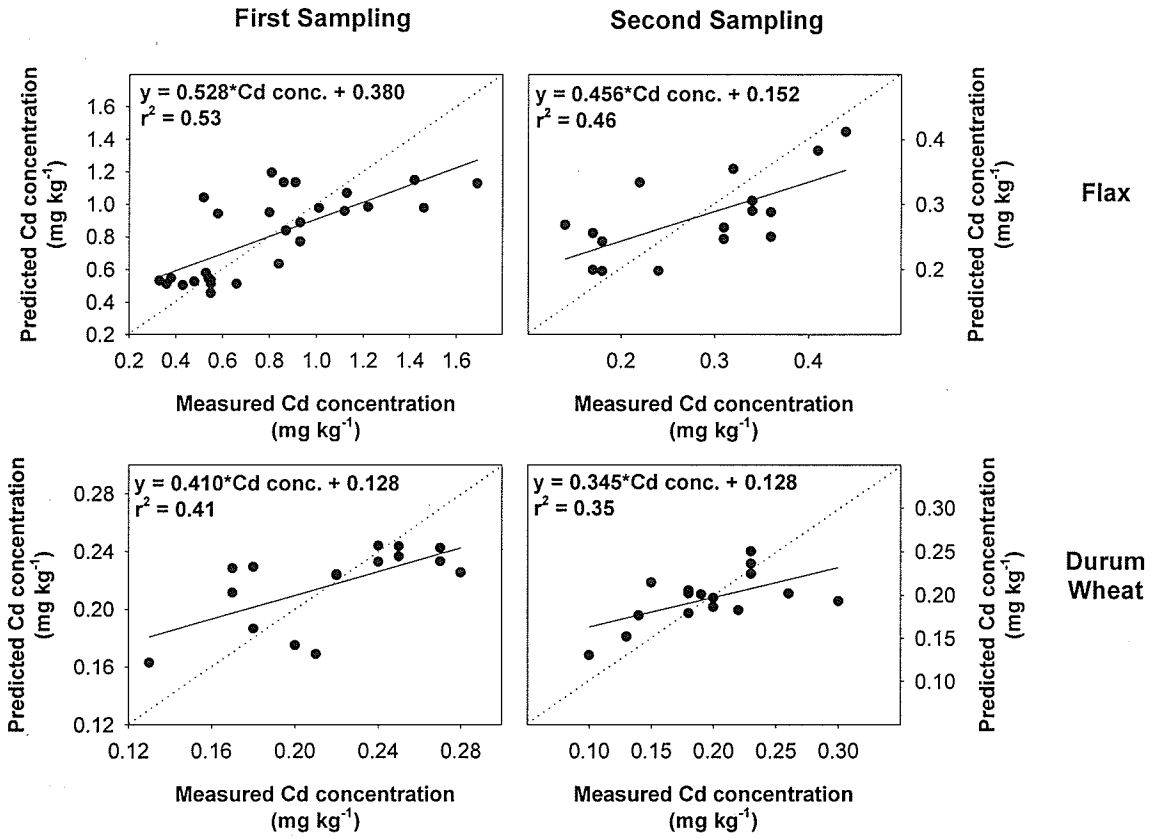

Figure 3.4 Summary of multiple-linear regression models for prediction of Cd concentration for first and second sampling occasion in flax and durum wheat.

Table 3.3 Summary of multiple-linear regression models for prediction of Cd concentration for first and second sample occasions. Models are using replicate data and for Flax First Sampling using results from 2005 and 2007, Flax Second Sampling for 2007, Durum Wheat First and Second Samplings 2006

Sampling	Crop	Variable	Coefficient	r^2	P
First	Flax			0.53	<0.001
		AGB P mg kg^{-1}	0.130		<0.001
		Constant	0.184		
Second	Flax			0.46	<0.004
		Cost:Benefit	-0.446		<0.004
		Constant	0.544		
First	Durum wheat			0.41	0.006
		Arb density	-0.000162		0.006
		Constant	0.268		
Second	Durum wheat			0.35	0.017
		Arb density	-0.000142		0.017
		Constant	0.299		

AGB = above ground biomass; Arb density = arbuscular root colonization density

arbuscule colonization increased. Based on the results it could be said that the factors affected above ground tissue Cd concentration were shoot P concentration and cost: benefit ratio of mycorrhizal symbiosis for flax and arbuscular density for durum wheat.

3.5 Discussion

3.5.1 Impact of High Levels of P fertilizer Laden with Cd on Cd Uptake

High levels of P fertilizer may increase above ground tissue Cd concentration. In the present study it was found that addition of 80 kg P ha^{-1} greatly increased DTPA extractable Cd concentration in the soil (Figure 3.1 and 3.2) compared to the Control. The increase in DTPA extractable Cd in the soil likely came from P fertilizer containing 211 mg kg^{-1} Cd as impurities in it. Plants can uptake DTPA extractable Cd directly into their root system. So, there was strong possibility that increased DTPA soil Cd might transfer to the above ground flax and durum wheat tissues which was observed in Figure 3.3. It was found that above ground flax and durum wheat tissue Cd concentration was highly positively associated with DTPA extractable Cd concentration in the soil (Figure 3.3). Based on the finding it can be said that P fertilizer containing high level of Cd as impurities were the source of above ground Cd in flax and durum wheat tissues. It is assumed that P fertilizer might influence Cd phytoavailability directly through addition of Cd as impurities or indirectly through immediate and long-term effect on soil characteristics. A number of other studies also reported elevated soil Cd concentration resulted from addition of Cd containing P fertilizer and subsequent transfer to the above

ground tissues (Jeng and Singh, 1995; He and Singh, 1993a; Anderson and Siman, 1991; Mulla et al., 1980; Jones et al., 1987).

Placement of P fertilizer may also influence Cd accumulation in crops (Grant et al., 1999). In the present study MAP was side-banded in low fertilized plots and broadcasted in highly fertilized plots. The high ionic strength, reduced pH and enhanced root development associated with the micro-region surrounding the fertilizer band may increase crop Cd availability. Higher Cd accumulation in flax with banded application was reported in comparison with broadcast application of MAP (Grant and Bailey, 1997). Although banding increase crop Cd accumulation compared to broadcast application of an equivalent amount of fertilizer, banding increased P use efficiency and decreased the amount of fertilizer required to optimize crop production, leading to lower long-term addition of Cd to the soil.

3.5.2 Interaction between Mycorrhizal Colonization and Above Ground Cd Concentration

The summary of multiple-linear regression models showed that along with above ground tissue P concentration cost:benefit ratio of AM fungal symbiosis affected above ground Cd concentration in flax tissues. In the case of durum wheat it was found that it was arbuscular density of arbuscular mycorrhizal fungi that highly affected above ground tissue Cd concentration. Based on the model, it can be said that arbuscular mycorrhizal fungi was highly associated with above ground tissue Cd concentration in both flax and durum wheat. The association was found highly negative in both first and second sampling times across the study period and it was assumed that above ground tissue Cd

concentration increased when flax and durum wheat were not benefitted from arbuscular mycorrhizal symbiosis. In the present experiment high levels of P fertilizer were used and it was found that these high levels of P fertilizer significantly decreased durum wheat arbuscule colonization. It was assumed that when arbuscular density decreased in the durum wheat roots, it might increase above ground tissue Cd concentration. The mechanism might be increased Zn uptake through mycorrhizal hyphae and subsequent transfer to the above ground tissue through nutrient exchange zone in mycorrhizal arbuscules. Zn and Cd have similarity in chemical behaviour and it was assumed that these two metals may compete each-other for the uptake sites in the root and transfer zones.

In the case of flax, it was found that above ground tissue Cd concentration was affected by shoot Cd concentration and cost:benefit ratio of mycorrhizal colonization in the roots. Flax is a highly mycotropic crop and it needed P at the very early stages of growth. In the present experiment, high levels of P fertilizer was applied and hence flax seedlings needed not to be dependent on AM fungi for additional P supply to the above ground tissues. However, the P fertilizer applied in the flax field had high levels of Cd as impurities in it. So, along with supplying sufficient P to the above ground tissues, high levels of P also transferred high amount of Cd to the shoot. These might be the reason for the positive association between above ground tissue Cd concentration and tissue P concentration in flax. On the other hand, when flax was not benefitting from mycorrhizal fungi in terms of P nutrition, it was still supplying carbon to the fungus that accounted for cost to the flax. This high carbon cost might lead flax to uptake more P as well as Cd from the soil and eventually the above ground Cd concentration went up.

Based on the findings it could be said that AM fungi might be involved in transferring Cd to the above ground flax and durum wheat tissues. The mechanism might be AM fungi association in the roots in supplying Zn to the above ground tissues and interactions between Zn and Cd in the root uptake sites. Other studies also showed that in a Zn deficient condition, root-cell plasma membrane integrity disrupts in a way that may lead to the accumulation of Cd to the above ground tissues (Welch and Norvell, 1999). It is assumed that correcting Zn deficiency may lower crop Cd accumulation by restoring the integrity of root-cell plasma membrane.

3.5.3 Conclusion

The study was designed to examine to what extent does above ground Cd concentration in flax and durum wheat increase in situation of high loading of Cd as impurities in P fertilizer and low root AM fungal colonization. It was found that when high Cd was added as impurities in P fertilizer, even low level of P fertilizer (20 kg P ha^{-1}) increased above ground Cd concentration in flax and durum wheat tissues. When high levels of P fertilizer containing high levels of Cd as impurities was used, plant available Cd in the soil increased thus increasing the risks of high levels of Cd transfer in flax and durum wheat. When high levels of P fertilizer were added to soil, root arbuscular mycorrhizal fungi colonization decreased in durum wheat and above ground Cd concentration increased. In the case of flax, high levels of P fertilizer didn't decrease root arbuscular mycorrhizal fungi colonization, however, it affected cost:benefit ratio of the fungal symbiosis in the roots to indicate less benefit to the plant. It was assumed that root arbuscular mycorrhizal fungi colonization was affected by either application of high levels of P fertilizer or by addition of high levels of Cd as impurities in it. Whatever it is,

it was found that when root arbuscular mycorrhizal fungi colonization was low, above ground Cd concentration was found to increase in both flax and durum wheat tissues. So, considering the findings from the present study it can be concluded that P fertilizer containing high levels of Cd increase Cd concentration and uptake by loading of Cd to soil with the Cd available for plant uptake. Less important, a decrease in AM fungal colonization was weakly associated with increased Cd uptake.

3.6 Acknowledgements

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4. GENERAL DISCUSSION AND CONCLUSIONS

This thesis project was designed to answer some questions pertaining to potential problems with crop management practices in the Prairie Region. First there is a severe problem associated with cadmium accumulation in crops, which raises the question whether crop management practices influence cadmium accumulation in the agricultural soil and crops. Another important question being do management practices affect AM fungal colonization in the field and if so, which management practices are the most detrimental for AM fungi survival and colonization? These two research questions lead into another question and that is if AM fungi are able to assist crops in restricting Cd transfer to the above ground tissues? These questions will be discussed further in the light of the results of the two studies presented here. Future research aims regarding reduction of cadmium accumulation in crops are also presented.

4.1 Effect of Management Practices on Crop Cd Uptake

Among the management practices tested, tillage and P fertilizer increased above ground Cd concentration in flax and durum wheat. When P fertilizer containing low level of Cd as impurities was applied, it did not increase above ground Cd concentration in durum wheat. However, when P fertilizer containing very high concentration of Cd as impurities was applied, it significantly increased above ground Cd concentration in both

flax and durum wheat. Monoammonium phosphate (MAP) used in the study presented in Chapter 2, had low Cd concentration, being 3.7 mg kg^{-1}. It was observed that using this fertilizer at a rate of 30 kg P_2O_5 ha^{-1} it did not affect durum wheat tissue Cd concentration. It is assumed that using MAP at this rate and low Cd concentration in fertilizer does not increase Cd transfer to the above ground tissues. The source of MAP used in the study presented in Chapter 3 had comparatively very high (211 mg kg^{-1}) Cd concentration compared to that of Chapter 2. The higher Cd containing MAP source resulted in increased concentration of Cd in above ground tissues of flax and durum wheat. Even at a low rate of the high Cd containing MAP, 20 kg P ha^{-1}, above ground Cd concentration in flax and durum wheat increased. Therefore, my results indicate that at rates of P addition similar to that in which farmers use, Cd concentration in the P fertilizer used determines more Cd concentration in crops than the P fertilizer rate.

For the study presented in Chapter 3, very high additions of P fertilizer were used which is not generally applied by the farmers. The purpose of this study was to determine to what extent does above ground Cd concentration increase in situation of high loading of Cd as impurities in P fertilizer. It was found that rates of P fertilizer containing high Cd as impurities significantly increased both flax and durum wheat above ground Cd concentration; however, the concentration was higher in flax compared to durum wheat. This is not surprising because flax is known to be a greater accumulator of Cd than durum wheat (Moraghan, 1993; Marquard et al., 1990)

Results of multiple-linear regression analysis showed a number of variables affected above ground tissue Cd concentration. Comparing the consistency of the models

and r^2 value, it was observed that AM fungi was the most consistent factor that affected above ground Cd concentration at both first and second sampling in both flax and durum wheat. Arbuscular mycorrhizal fungi in terms of percentage arbuscule and total colonization, density of arbuscule and total colonization and cost:benefit ratio of fungal symbiosis in the flax and durum wheat roots showed a negative association with above ground tissue Cd concentration. Results from the study presented in Chapter 2 demonstrated that tillage and non-host previous crop canola significantly reduced AM fungal colonization in durum wheat. By reducing mycorrhizal colonization in the field, these two management factors also indirectly affected above ground tissue Cd concentration in the durum wheat. Separating the direct effect of AM fungal colonization on above ground tissue Cd concentration is confounded by tillage and non-host previous crop (canola) also affecting AM fungal colonization. In the study presented in Chapter 3, high levels of P fertilizer addition reduced mycorrhizal colonization in durum wheat and did not show any effect on flax; however, high level of above ground Cd concentration was still found for both crops. The multiple-linear regression analysis showed that it was arbuscule density of arbuscular mycorrhizal fungi that affected above ground Cd concentration in durum wheat and in the case of flax, both above ground P concentration and cost:benefit ratio of mycorrhizal symbiosis affected above ground Cd concentration.

4.2 Mycorrhizal Association in Restricting Cd Accumulation

To separate P fertilizer and AM fungi effect, a situation was considered where AM fungal colonization was reduced without P fertilizer addition. This was the case for

canola as a previous crop and when tillage was practiced. The result showed a slight increase in Cd concentration and accumulation with decreased AM fungal colonization from tilled plots. However, canola as a previous crop did not affect above ground Cd concentration even though it also decreased AM fungal colonization. The results obviously seem contrary.

I examined the results of the two studies using multiple-linear regression to determine which measured variables most influence Cd concentration of above ground tissues. Multiple-linear regression models showed that mycorrhizal parameters were the most consistent variables that affected above ground tissue Cd concentration. The mechanism of Cd restriction by AM fungi may be related to above ground Zn concentration and total Zn accumulation by the crops though not very likely as this variable was not very useful in predicting above ground tissue concentration. It is known that along with P, arbuscular mycorrhizal fungi also help plant in Zn accumulation. These two metals (Cd and Zn) have similar chemical behaviour and they may compete each-other for binding sites in the soil and for uptake and transfer sites within the plant (Christensen and Huang, 1999). However, this mechanism might not always work depending on bioavailable Cd and Zn concentration in the soil. When low level of P fertilizer was added to the soil, bioavailable Cd and Zn was low and arbuscular mycorrhizal fungi were fully functional. In this situation, arbuscular mycorrhizal fungi helped crops to accumulate more Zn along with P from the soil. Accumulated Zn competed with Cd and reduced total Cd accumulation. On the other hand, when high level of P fertilizer was added, bioavailable Cd and Zn was higher in the soil and arbuscular mycorrhizal fungi colonization was lower and/or not functional. This

happened because in abundance of P fertilizer in the soil, crops needed not to be dependent on arbuscular mycorrhizal fungi for P uptake as they had abundant supply from P fertilizer. In this situation, high level of bioavailable Cd suppressed Zn uptake and as a result total Cd accumulation increased.

If P concentration in the soil is low, mycorrhizal fungi can function actively to supply nutrients (mainly P and Zn) to the crop as well as restrict uptake of Cd in roots. However, if soil P concentration is high, it might not reduce mycorrhizal colonization to the trace level, but may affect their active participation in nutrient uptake and may affect crop Cd accumulation. A schematic diagram of the routes of Cd uptake, transport and deposition within crops; possible association of AM fungi fungi in restricting Cd transfer within fungal biomass and competition between Cd^{2+} and Zn^{2+} for their common uptake and transport sites is presented in Figure 4.1.

The next logical step from the present study is to set up experiments under controlled environment conditions where confounding field conditions are reduced to investigate AM fungi mediated Cd uptake by durum wheat and flax and the effects of the soil factors P, Zn and Cd addition levels. This could be done using metal tolerant AM fungi species and different combinations of P and Zn fertilizers. This method will make it possible to investigate the impact of absence and presence of AM fungi colonization and whether the AM fungi could influence Cd transfer from root to shoot.

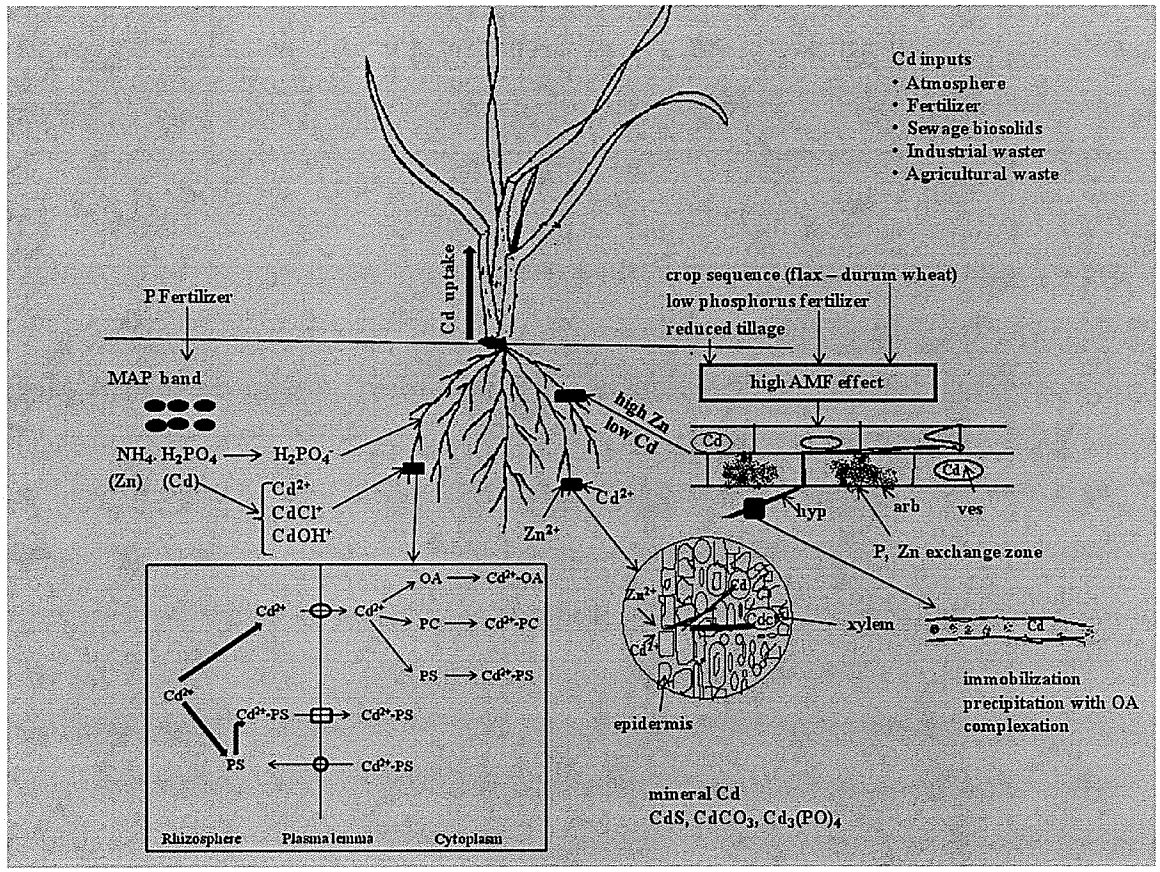

Figure 4.1 Model diagram of the possible mechanisms of Cd uptake, arbuscular mycorrhizal (AM) fungal association in restriction and competition between Cd and Zn for the uptake sites. OA represents organic acid; PC represents phytochelatins; PS represents phytosiderophores.

4.3 Conclusions and Recommendations

Do tillage, previous crop and P fertilizer application influence above ground cadmium concentration in durum wheat and flax?

Findings:

- Tillage increase above ground Cd concentration in durum wheat.
- Previous crop has no effect on above ground Cd concentration in durum wheat
- Low level of P fertilizer containing low Cd as impurities didn't affect above ground Cd concentration in durum wheat; however, when P fertilizer contained high Cd as impurities, it significantly increased above ground tissue Cd concentration.

Recommendations:

- Reduced tillage instead of conventional tillage is recommended to lower Cd transfer to the above ground tissues
- Effective soil test is recommended in fall to have idea about plant available and total P level in the soil
- Rate of P fertilizer application in different crops should be determined according to the guidelines recommended in the Manitoba Soil Fertility Guide (source: Manitoba Soil Fertility Guide, 2007)

Are arbuscular mycorrhizal fungi able to assist crops in restricting Cd transfer to the shoot?

Findings:

- The negative association between root arbuscular mycorrhizal fungi colonization and above ground tissue Cd concentration is an indication of mycorrhizal involvement in restricting Cd transfer to the shoot.
- Arbuscular mycorrhizal fungi might help flax and durum wheat in transferring Zn from the soil. Increased Zn might compete with Cd in the root uptake sites and decreased above ground Cd transfer.

Recommendations:

- Favourable condition should be created for arbuscular mycorrhizal fungi in the field so that the fungi can function actively in terms of increase nutrient uptake and restrict Cd transfer to the shoot
- Exclude non-host crops in rotation
- Reduce tillage
- Avoid applying excess P fertilizer in the field
- Identification of AM fungi communities in the field that are tolerant to high Cd concentration in the soil

4.4 Literature Cited

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5. APPENDICES

Appendix I. Stand density of durum wheat as affected by previous crop, tillage and P fertilizer at the tillage-previous crop-fertilization study at Brandon Research Centre

Treatment	[1]Stand density (plants m^{-2})		
	2005	2006	2007
Tillage system			
Conventional	121 (3)[2]	147 (5)	183 (7)
Reduced	121 (4)	137 (4)	165 (5)
Previous crop			
Flax	121 (4)	139 (4)	174 (6)
Canola	121 (3)	145 (5)	173 (6)
P Fertilizer			
0 kg/ha	118 (3)	138 (3)	170 (6)
30 kg/ha	124 (3)	145 (6)	177 (7)
Tillage (T)	n.s.	n.s.	*
Previous crop (PC)	n.s.	n.s.	n.s.
P fertilizer (P)	n.s.	n.s.	n.s.
T × PC	n.s.	n.s.	n.s.
T × PC × P	n.s.	n.s.	n.s.

* significant at $P < 0.05$; n.s. = not significant at $P < 0.05$

[1]stand density was calculated from 2×1 m rows after emergence

[2]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis

Appendix II Grain yield of durum wheat as affected by previous crop, tillage and P fertilizer at the tillage-previous crop-fertilization study at Brandon Research Centre

Treatment	Grain yield (t ha^{-1})		
	2005	2006	2007
Tillage system			
Conventional	3.7 (0.1)[1]	3.6 (0.3)	2.4 (0.1)
Reduced	3.4 (0.2)	4.1 (0.1)	2.3 (0.1)
Previous crop			
Flax	3.5 (0.1)	3.8 (0.2)	2.4 (0.1)
Canola	3.7 (0.1)	4.0 (0.2)	2.4 (0.1)
P Fertilizer			
0 kg/ha	3.5 (0.2)	3.8 (0.2)	2.4 (0.1)
30 kg/ha	3.7 (0.1)	4.0 (0.2)	2.4 (0.1)
Tillage (T)	n.s.	*	n.s.
Previous crop (PC)	n.s.	n.s.	n.s.
P fertilizer (P)	n.s.	n.s.	n.s.
T × PC	n.s.	n.s.	n.s.
T × PC × P	n.s.	n.s.	n.s.

* significant at $P < 0.05$; n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis

Appendix III Effects of tillage, previous crop and phosphorus fertilization on above ground dry weight (g) of durum wheat at the tillage-previous crop-fertilization study at Brandon Research Centre

Treatment	Above ground dry weight				
	2005	2006		2007	
	First Sampling	First Sampling	Second sampling	First sampling	Second sampling
Tillage system					
Conventional	62.3 (3.6)[1]	23.0 (2.2)	311.4 (18.9)	5.5 (0.4)	174.2 (8.4)
Reduced	64.0 (5.3)	28.8 (2.2)	279.1 (15.0)	4.5 (0.4)	167.4 (6.6)
Previous crop					
Flax	66.2 (4.9)	25.8 (1.8)	276.0 (11.9)	5.2 (0.5)	165.1 (6.6)
Canola	60.1 (4.0)	26.0 (2.8)	314.5 (20.7)	4.8 (0.4)	176.4 (8.3)
P Fertilizer					
0 kg/ha	64.7 (5.2)	23.4 (2.2)	295.3 (18.6)	4.1 (0.3)	175.9 (8.2)
30 kg/ha	61.7 (3.7)	28.3 (2.3)	295.2 (16.5)	5.9 (0.4)	165.6 (6.7)
Tillage (T)	n.s.	n.s.	n.s.	n.s.	n.s.
Previous crop (PC)	n.s.	n.s.	n.s.	n.s.	n.s.
P fertilizer (P)	n.s.	n.s.	n.s.	***	n.s.
T × PC	n.s.	n.s.	n.s.	n.s.	n.s.
T × PC × P	n.s.	n.s.	n.s.	n.s.	n.s.

*** Significant at $P < 0.001$; n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis.

Appendix IV Main and interaction effects of tillage and previous crop on concentration of DTPA-Cd in the soil at the tillage-previous crop-fertilization study at Brandon Research Centre

Management	Cd concentration in soil (ppb)		
	2005	2006	2007
Tillage system			
Conventional	150.2 (9.5)[1]	192.2 (14.6)	176.6 (8.9)
Reduced	153. 6(5.1)	186.6 (6.8)	161.4 (12.4)
Previous crop			
Flax	156.2 (6.8)	187.8 (9.3)	174.1 (11.7)
Canola	147.7 (8.1)	190.9 (13.2)	163.9 (10.3)
Tillage (T)	n.s.	n.s.	n.s.
Previous crop (PC)	n.s.	n.s.	n.s.
T×PC	n.s.	n.s.	n.s.

n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis. Soil samples were collected in spring before P fertilizer was added to the field.

Appendix V Main and interaction effects of tillage and previous crop on concentration of Olsen-P in the soil at the tillage-previous crop-fertilization study at Brandon Research Centre

Treatment	Phosphorus concentration (mg kg^{-1})		
	2005	2006	2007
Tillage system			
Conventional	41.2 (2.9)[1]	22.1 (3.1)	15.4 (1.0)
Reduced	40.8 (3.5)	20.5 (1.9)	14.8 (1.3)
Previous crop			
Flax	41.3 (3.2)	21.0 (2.3)	14.5 (1.4)
Canola	40.7 (3.3)	21.6 (2.8)	15.6 (0.9)
Tillage (T)	n.s.	n.s.	n.s.
Previous crop (PC)	n.s.	n.s.	n.s.
T×PC	n.s.	n.s.	n.s.

n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis. Soil samples were collected in spring before P fertilizer was added to the field.

Appendix VI Effects of tillage, previous crop and phosphorus fertilization on above ground cadmium concentration (mg kg^{-1}) in durum wheat tissues at the tillage-previous crop-fertilization study at Brandon Research Centre

Treatment	2005	2006		2007	
	First sampling	First sampling	Second sampling	First Sampling	Second sampling
Tillage system					
Conventional	0.30 (0.02)[1]	0.32 (0.02)	0.15 (0.01)	0.47 (0.02)	0.13 (0.01)
Reduced	0.30 (0.01)	0.32 (0.01)	0.14 (0.01)	0.43 (0.02)	0.11 (0.00)
Previous crop					
Flax	0.29 (0.01)	0.31 (0.01)	0.14 (0.01)	0.46 (0.02)	0.13 (0.01)
Canola	0.30 (0.02)	0.33 (0.01)	0.15 (0.01)	0.43 (0.03)	0.12 (0.00)
P Fertilizer					
0 kg/ha	0.29 (0.01)	0.30 (0.01)	0.13 (0.01)	0.43 (0.02)	0.12 (0.01)
30 kg/ha	0.30 (0.01)	0.34 (0.01)	0.16 (0.01)	0.47 (0.02)	0.13 (0.01)
Tillage (T)	n.s.	n.s.	n.s.	n.s.	n.s.
Previous crop (PC)	n.s.	n.s.	n.s.	n.s.	n.s.
P fertilizer (P)	n.s.	n.s.	*	n.s.	n.s.
T × PC	n.s.	n.s.	n.s.	n.s.	n.s.
T × PC × P	n.s.	n.s.	*	n.s.	n.s.

* significant at $P < 0.05$; n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis

Appendix VII Effects of tillage, previous crop and phosphorus fertilization on cadmium accumulation (mg) in above ground durum wheat tissues at the tillage-previous crop-fertilization study at Brandon Research Centre

Treatment	2005	2006		2007	
	First Sampling	First Sampling	Second sampling	First sampling	Second sampling
Tillage system					
Conventional	0.019 (0.002)[1]	0.007 (0.001)	0.047 (0.004)	0.003 (0.000)	0.023 (0.002)
Reduced	0.019 (0.001)	0.009 (0.001)	0.037 (0.002)	0.002 (0.000)	0.019 (0.001)
Previous crop					
Flax	0.020 (0.001)	0.008 (0.001)	0.039 (0.001)	0.002 (0.000)	0.022 (0.001)
Canola	0.018 (0.001)	0.009 (0.001)	0.046 (0.004)	0.002 (0.000)	0.020 (0.001)
P Fertilizer					
0 kg/ha	0.020 (0.002)	0.007 (0.001)	0.039 (0.003)	0.002 (0.000)	0.021 (0.002)
30 kg/ha	0.018 (0.001)	0.009 (0.001)	0.046 (0.003)	0.003 (0.000)	0.021 (0.001)
Tillage (T)	n.s.	n.s.	n.s.	n.s.	n.s.
Previous crop (PC)	n.s.	n.s.	n.s.	n.s.	n.s.
P fertilizer (P)	n.s.	n.s.	n.s.	***	n.s.
T × PC	n.s.	n.s.	n.s.	n.s.	n.s.
T × PC × P	n.s.	n.s.	n.s.	n.s.	n.s.

*** significant at $P<0.001$; n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis. Samples were collected from four 20 cm lengths along the rows in each plot.

Appendix VIII Arbuscular (AC), Hyphal (HC) and total (TC) percent colonization in durum wheat for the main and interaction effects of tillage, previous crop and P fertilization at the tillage-previous crop-fertilization study at Brandon Research Centre sampled in 2006

Treatment	First sampling			Second sampling		
	AC	HC	TC	AC	HC	TC
Tillage system						
Conventional	18 (1)[1]	17 (2)	35 (2)	21 (2)	22 (2)	43 (3)
Reduced	26 (2)	23 (2)	49 (4)	43 (3)	38 (3)	81 (6)
Previous crop						
Flax	26 (2)	24 (2)	50 (3)	41 (4)	35 (3)	75 (7)
Canola	18 (1)	17 (1)	35 (2)	23 (2)	25 (2)	49 (5)
P Fertilizer						
0 kg/ha	22 (2)	20 (2)	42 (4)	32 (4)	32 (3)	64 (7)
30 kg/ha	22 (2)	20 (2)	42 (3)	32 (4)	28 (2)	60 (6)
Tillage (T)	***	*	***	***	***	***
Previous crop (PC)	***	**	***	***	***	***
P fertilizer (P)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
T × PC	n.s.	n.s.	n.s.	**	*	**
T × PC × P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*, **, *** significant at $P < 0.05$, $P<0.01$, $P<0.001$, respectively; n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis

Appendix IX Arbuscular (AC), Vesicular (VC), Hyphal (HC) and total (TC) percent colonization in durum wheat for the main and interaction effects of tillage, previous crop and P fertilization at the tillage-previous crop-fertilization study at Brandon Research Centre sampled in 2007

Treatment	First sampling			Second sampling			
	AC	HC	TC	AC	VC	HC	TC
Tillage system							
Conventional	19 (3)[1]	23 (3)	42 (4)	28 (3)	6 (2)	44 (3)	78 (4)
Reduced	29 (4)	27 (3)	57 (5)	25 (3)	6 (3)	46 (3)	78 (3)
Previous crop							
Flax	30 (4)	27 (3)	57 (5)	32 (4)	5 (2)	40 (2)	77 (4)
Canola	18 (2)	24 (3)	42 (4)	22 (2)	7 (2)	50 (3)	78 (3)
P Fertilizer							
0 kg/ha	25 (3)	28 (3)	53 (5)	29 (4)	3 (1)	48 (3)	81 (3)
30 kg/ha	23 (3)	23 (3)	46 (5)	24 (3)	9 (3)	42 (3)	74 (4)
Tillage (T)	**	**	**	n.s.	n.s.	n.s.	n.s.
Previous crop (PC)	**	**	**	*	n.s.	**	n.s.
P fertilizer (P)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
T × PC	**	**	n.s.	n.s.	n.s.	n.s.	n.s.
T × PC × P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*, ** significant at $P < 0.05$, $P<0.01$, respectively; n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis

Appendix X Arbuscular (AC), Hyphal (HC) and total (TC) percent colonization in durum wheat for the main and interaction effects of tillage, previous crop and P fertilization at the tillage-previous crop-fertilization study at Brandon Research Centre sampled in June, 2005

Treatment	AC	HC	TC
Tillage system			
Conventional	31 (3)[1]	31 (2)	62 (3)
Reduced	37 (3)	27 (2)	64 (3)
Previous crop			
Flax	35 (3)	31 (1)	66 (2)
Canola	33 (3)	27 (2)	60 (3)
P Fertilizer			
0 kg/ha	35 (3)	28 (1)	64 (3)
30 kg/ha	33 (3)	29 (2)	62 (2)
Tillage (T)	n.s.	n.s.	n.s.
Previous crop (PC)	n.s.	n.s.	n.s.
P fertilizer (P)	n.s.	n.s.	n.s.
T × PC	*	*	*
T × PC × P	n.s.	n.s.	n.s.

*significant at $P < 0.05$; n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis.

Appendix XI Arbuscular (ACD), Hyphal (HCD) and total (TCD) colonized root length density and ratio of arbuscular root length to total colonized root length in durum wheat as affected by tillage, previous-crop and P fertilizer treatments sampled in June, 2005

Treatment	ACD (cm ml^{-1})	HCD (cm ml^{-1})	TCD (cm ml^{-1})	AC:TC
Tillage system				
Conventional	0.9 (0.1)[1]	0.9 (0.1)	1.9 (0.2)	0.5 (0.0)
Reduced	0.9 (0.1)	0.6 (0.1)	1.4 (0.2)	0.6 (0.0)
Previous crop				
Flax	0.8 (0.1)	0.8 (0.1)	1.6 (0.2)	0.5 (0.0)
Canola	1.0 (0.2)	0.7 (0.1)	1.7 (0.2)	0.6 (0.0)
P Fertilizer				
0 kg/ha	0.8 (0.1)	0.6 (0.1)	1.4 (0.2)	0.6 (0.0)
30 kg/ha	1.0 (0.1)	0.9 (0.1)	1.7 (0.2)	0.5 (0.0)
Tillage (T)	n.s.	*	n.s.	n.s.
Previous crop (PC)	n.s.	n.s.	n.s.	n.s.
P fertilizer (P)	n.s.	*	n.s.	n.s.
T × PC	n.s.	n.s.	n.s.	n.s.
T × PC × P	n.s.	n.s.	n.s.	n.s.

* significant at $P < 0.05$; n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis

Appendix XII Arbuscular (ACD), Hyphal (HCD) and total (TCD) colonized root length density and ratio of arbuscular root length to total colonized root length in durum wheat as affected by tillage-previous-crop and P fertilizer treatments sampled in 2006

Treatment	First sampling (cm ml^{-1})				Second sampling (cm ml^{-1})			
	ACD	HCD	TCD	AC:TC	ACD	HCD	TCD	AC:TC
Tillage system								
Conventional	1.1 (0.1)[1]	1.0 (0.1)	2.2 (0.2)	0.5 (0.0)	3.0 (0.3)	3.1 (0.4)	6.1 (0.6)	0.5 (0.0)
Reduced	2.0 (0.2)	1.8 (0.3)	3.7 (0.5)	0.5 (0.0)	6.1 (0.6)	5.4 (0.5)	11.5 (1.0)	0.5 (0.0)
Previous crop								
Flax	1.9 (0.2)	1.8 (0.3)	3.7 (0.5)	0.5 (0.0)	5.9 (0.6)	5.2 (0.6)	11.1 (1.1)	0.5 (0.0)
Canola	1.1 (0.1)	1.0 (0.1)	2.2 (0.2)	0.5 (0.0)	3.1 (0.4)	3.4 (0.3)	6.5 (0.7)	0.5 (0.0)
P Fertilizer								
0 kg/ha	1.3 (0.2)	1.3 (0.2)	2.6 (0.3)	0.5 (0.0)	4.5 (0.6)	4.4 (0.6)	8.9 (1.1)	0.5 (0.0)
30 kg/ha	1.7 (0.2)	1.6 (0.3)	3.3 (0.5)	0.5 (0.0)	4.6 (0.7)	4.1 (0.5)	8.7 (1.0)	0.5 (0.0)
Tillage (T)	***	**	***	n.s.	***	***	***	***
Previous crop (PC)	***	**	***	n.s.	***	**	***	***
P fertilizer (P)	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
T × PC	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
T × PC × P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*, **, *** significant at $P < 0.05$, $P<0.01$, $P<0.001$, respectively; n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis

Appendix XIII **Arbuscular (ACD), Hyphal (HCD) and total (TCD) colonized root length density and ratio of arbuscular colonized root length to total colonized root length in durum wheat as affected by tillage-previous-crop and P fertilizer treatments sampled in 2007**

Treatment	First sampling (cm ml^{-1})				Second sampling (cm ml^{-1})				
	ACD	HCD	TCD	AC:TC	ACD	VCD	HCD	TCD	AC:TC
Tillage system									
Conventional	1.7 (0.2)[1]	2.2 (0.3)	3.9 (0.4)	0.5 (0.1)	9.5 (1.3)	1.7 (0.5)	14.3 (1.4)	25.5 (1.4)	0.4 (0.03)
Reduced	2.9 (0.4)	2.6 (0.3)	5.6 (0.5)	0.5 (0.0)	7.8 (1.1)	1.9 (0.8)	14.7 (1.0)	24.5 (1.3)	0.3 (0.03)
Previous crop									
Flax	3.0 (0.4)	2.7 (0.3)	5.7 (0.6)	0.5 (0.1)	11.1 (1.4)	1.5 (0.7)	13.9 (0.9)	26.6 (1.5)	0.4 (0.03)
Canola	1.7 (0.2)	2.2 (0.3)	3.8 (0.4)	0.5 (0.0)	6.4 (0.6)	2.1 (0.6)	14.9 (0.9)	23.4 (0.9)	0.3 (0.02)
P Fertilizer									
0 kg/ha	2.2 (0.3)	2.6 (0.3)	5.0 (0.5)	0.5 (0.0)	9.6 (1.5)	1.0 (0.3)	15.2 (0.9)	25.7 (1.5)	0.4 (0.04)
30 kg/ha	2.3 (0.4)	2.2 (0.4)	4.7 (0.6)	0.5 (0.1)	7.9 (0.9)	2.6 (0.9)	13.8 (1.0)	23.3 (1.2)	0.3 (0.02)
Tillage (T)	***	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Previous crop (PC)	***	n.s.	***	n.s.	*	n.s.	*	n.s.	***
P fertilizer (P)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
T × PC	***	n.s.	***	*	n.s.	n.s.	n.s.	n.s.	n.s.
T × PC × P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*, **, *** significant at $P < 0.05$, $P<0.01$, $P<0.001$, respectively; n.s. = not significant at $P < 0.05$

[1]values shown are the average of sixteen replicate values with 1 standard error of the average given in parenthesis

Appendix XIV Effects of different levels of phosphorus fertilizer application on above ground dry weight (g) of flax and durum wheat at the fertilization study at Brandon Research Centre

Fertilizer rate	Flax 2005	Wheat 2006		Flax 2007	
kg P ha^{-1}	First sampling	First sampling	Second sampling	First sampling	Second sampling
0	38.2 (3.5)a[1]	58.9 (5.4)a	279.8 (10.4)a	2.4 (0.1)a	71.3 (3.6)a
20P	43.0 (5.5)a	84.0 (15.1)a	275.3 (11.8)a	2.0 (0.2)a	65.3 (5.5)a
40P	-	78.5 (10.7)a	275.9 (9.7)a	2.7 (0.2)a	68.5 (5.0)a
80P	45.7 (4.1)a	82.6 (18.8)a	275.2 (7.3)a	2.2 (0.3)a	77.3 (7.3)a

[1]Values shown are the average of four replicate values with 1 standard error of the average given in parenthesis. Same letters denote no significant differences at $P < 0.05$. Samples were collected from four 20 cm lengths along the rows in each plot.