EFFECTS OF MONO-AMMONIUM PHOSPHATE AND PRECEDING CROP ON PLANT CADMIUM UPTAKE

BY

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ABSTRACT

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Cadmium is a potentially toxic trace element and food is a major route of Cd entry to humans. Phosphorus fertilization and crop rotation are two main agricultural management practices that can influence food crop Cd uptake. Therefore, two growth chamber studies were conducted to understand how: i) mono ammonium phosphate (MAP) affects durum wheat, flax or canola Cd uptake due to fertilizer induced changes in soil, soil solution and plant; ii) preceding canola and barley grown soils and the addition of crop residue of canola and barley affect the Cd uptake of subsequent durum wheat or flax crops.

Durum wheat and flax Cd concentration and durum wheat Cd accumulation were greater when grown on previous crop canola soil than when grown on previous crop barley soil regardless of what type of crop residue, if any, was added. The increase in Cd uptake for durum wheat and flax when on canola soil was probably due to increased availability of Cd in soil. In this experiment, the percentage of arbuscular mycorrhizal root colonization did not have an influence on the observed increase in durum wheat and flax Cd uptake.

Conversely, the application of mono-ammonium phosphate – reagent grade (RG) and the incorporation of preceding crop residue did not increase the crop Cd uptake of canola, durum wheat or flax and durum wheat or flax, respectively. Addition of MAP-RG did not affect Cd concentrations in soil solution or DTPA extractable Cd

concentrations in soil. The lack of increase in soil solution Cd and extractable soil Cd concentrations could be due to the lack of decrease in soil or soil solution pH, or an increase in soil or soil solution EC with MAP-RG addition. The lack of increase in plant Cd uptake with crop residue addition was probably due to the immobilization of added Cd in soil.

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FOREWORD

Chapter two of this thesis describes a study conducted to understand how mono-ammonium phosphate (MAP) affects Cd uptake in canola, durum wheat or flax due to the fertilizer induced changes in soil, soil solution and plant. Chapter three describes a study conducted to understand how canola and barley grown soils and canola and barley crop residue incorporation affect subsequent durum wheat and flax Cd uptake. During both growth chamber experiments described in Chapters 2 and 3, we used the technique of soil solution sampling by Rhizon soil moisture samplers to expand our understanding of the soil and plant processes which can affect plant Cd uptake. The soil solution samples required Inductively Coupled Plasma Mass Spectrometric analysis which, in turn, required that the samples be sent to Agriculture and Agri-Food Canada – Fredericton, NB. Analysis of the soil solution was completed for the first growth chamber study, reported in Chapter 2. However, the analysis of soil solution samples for the study reported in the third chapter is not completed yet. Therefore, we are looking forward to incorporate the soil solution data into a manuscript for publication when analysis is completed.

This thesis is prepared in manuscript format following the thesis guidelines of Department of Soil Science, University of Manitoba. The formatting of Chapters 2 and 3 is for the Journal of Environmental Quality.

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1. GENERAL INTRODUCTION

1.1 Introduction

Cadmium (Cd) is a nonessential potentially toxic trace element for plants and animals. Certain soils are naturally high in Cd content and, in addition, anthropogenic activities add Cd (Alloway and Steinnes, 1999). Food crops accumulate Cd and become a major route of Cd entry into humans (Kuboi et al., 1986). Cadmium is retained inside the body and can lead to chronic toxicity over time, if exposed to high concentrations. Cadmium toxicity can cause renal tubular damage, renal failures (Ryan et al., 1982; Nordberg et al., 2002), low bone density and bone fractures (Nordberg et al., 2002; Dong et al., 2007). Cadmium is also identified as a carcinogen by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO).

The maximum concentration of Cd proposed by Codex Alimentarius Commission of FAO/WHO is 0.1 mg kg^{-1} for cereal grain (excluding wheat) and 0.2 mg kg^{-1} for wheat, in international trade. Western Canada is a leading producer and exporter of wheat, canola and flax in the world. Durum wheat and flax produced in Canada risk exceeding the maximum permissible limits of Cd in seeds (Grant et al., 2002; Jiao et al., 2004). Therefore, it is vital to seek better crop management practices to maintain Cd content in Canadian grains at acceptable limits in order to ensure healthy consumption and exportability. Two crop management practices that influence plant Cd uptake are: i) P fertilizer application; ii) crop rotation.

Mono-ammonium phosphate (MAP) is a common P fertilizer source in the Canadian prairies. Fertilizer grade MAP adds cadmium into agricultural soils as the fertilizer often contains Cd as an impurity and increases the risk of Cd accumulation by

food crops (Grant et al., 1998; Lambert et al., 2007). Increased Cd uptake due to MAP application, especially in the year of application, may not be entirely due to the addition of Cd into agricultural soils. The increase may also be due to indirect effects of MAP on plant Cd uptake via fertilizer induced changes in soil pH, ionic strength, Zn availability, rhizosphere chemistry, microbial activity and plant growth (Grant and Sheppard, 2008). Increased Cd concentration in durum wheat and flax due to indirect effects of MAP application has been suspected in several field and growth chamber studies (Choudhary et al., 1994; Grant et al., 2002; Jiao et al., 2004). In a growth chamber study, the shoot Cd concentration of durum wheat was significantly greater with the addition of MAP (P, 150 mg kg^{-1}) containing a low concentration of Cd (15 mg kg^{-1}) and with the addition of both MAP and NH_4NO_3 (P, 150 mg kg^{-1} and N, 200 mg kg^{-1}) than in the respective control where no MAP and no NH_4NO_3 were added. In the same experiment, the shoot Cd accumulation (i.e., concentration × biomass produced) of durum wheat was greater with the application of MAP (P, 150 mg kg^{-1} and) than with the application of NH_4NO_3, alone (at the rate of 74 mg N kg^{-1} soil). In a similar growth chamber study conducted by Choudhary et al. (1995), cadmium concentrations were 2.1 and 1.5 times greater in root, 1.9 and 1.6 times greater in leaf, 2.2 and 2.3 times greater in stem and 3.8 and 3.1 times greater in grain with MAP-RG addition (P, 100 mg kg^{-1} soil) than in the control (no addition of MAP) for durum wheat DT 627 and durum wheat Medora, respectively. In a field study conducted at 11 field sites in Manitoba and Alberta, Grant et al. (2002) observed an increase in durum wheat grain Cd concentration in the year of application with commercial grade MAP (Cd concentrations of 0.2, 7.8 and 186 mg kg^{-1} fertilizer). However, the increase in grain Cd was not related to the concentration of Cd present in each fertilizer. Also, Jiao et al. (2004) observed increases in the concentrations of Cd in

tissue and seeds of durum wheat and flax when reagent grade MAP was added (P, 100 mg kg^{-1} soil) in a growth chamber study conducted at Brandon, Manitoba.

In contrast, some other experiments reported that MAP with or without Cd impurity had no short term effect on Cd uptake by plants (Grant et al., 1998; Choudhary et al., 1994; McLaughlin et al., 1995). Potato tuber Cd concentrations in field plots receiving MAP (containing Cd at a rate of 6 and 95 mg kg^{-1} fertilizer) were not significantly different from tuber Cd concentrations in plots which did not receive any P fertilizer in five field sites (McLaughlin et al., 1995). The variation in tuber Cd concentration among field sites (which were different in soil physical and chemical characteristics) was larger than it was among treatments, indicating a strong influence of soil physical and chemical characteristics on tuber Cd concentration. A series of short term growth chamber studies were summarized by Grant et al. (1998) reporting that MAP-RG, MAP containing trace concentrations of Cd and MAP containing Cd at 15 mg kg^{-1} fertilizer did not significantly influence durum wheat tissue Cd concentration compared to respective control treatments. The addition of NH_4NO_3 increased Cd concentration in durum wheat shoot, but the addition of MAP (P, 150 mg P kg^{-1} containing Cd at a rate of 15 mg kg^{-1} fertilizer) along with NH_4NO_3 generally reduced Cd concentration in durum wheat shoot and may be due to the dilution of Cd within plant as plant growth increased with P nutrition (Choudhary et al., 1994).

Certain preceding crops in crop rotation can raise Cd uptake in the subsequent crop. However, there are few studies on this issue. In Australian studies, wheat grown after lupin had a significantly greater grain Cd concentration than wheat grown after wheat and wheat grown after wheat had greater grain Cd concentration than wheat grown after barley (Oliver et al., 1993). In saline soils of Iran, wheat grown after cotton

contained significantly more concentration of Cd in shoot and grain than wheat grown after sunflower in rotation (Khoshgoftarmanesh and Chaney, 2007). In a pot experiment, tobacco grown after a fallow period had greater shoot Cd concentration than tobacco grown after maize and tobacco (Mench, 1998). In a Manitoba field experiment, greater Cd concentration was observed in flax seed grown after canola than after durum wheat (Grant, 2003). In a growth chamber experiment with Manitoba soils, durum wheat on canola grown soil had a greater shoot Cd concentration than that on barley grown soil and there was no influence of preceding crop soil on shoot Cd concentration of flax (Eastley, 2008). The preceding crop influence over subsequent crop Cd uptake may be due to lasting alterations in soil chemical properties (Oliver et al., 1993; Khoshgoftarmanesh and Chaney, 2007) and/or may be due to crop residue, once incorporated.

1.2 Soil pH

Soil pH affects Cd availability for plants and pH could be the most notable soil characteristic in this regard (He and Singh, 1993a; Grant et al., 1998; Grant et al., 1999; Adam et al., 2004; Grant and Sheppard, 2008). Decreasing soil pH favours Cd desorption from soil particles and increases the partition of Cd in the soil solution, making Cd more available for plants (Christensen, 1984; Levi-Minzi and Petruzzelli, 1984; Grant et al., 1998; Sauve et al., 2000; Grant and Sheppard, 2008).

The potential for pH reduction after MAP application has been observed in both laboratory and field studies (Lambert et al., 2007). Soil incorporation of MAP reduced soil pH and subsequently reduced Cd adsorption under laboratory conditions (Levi-Minzi and Petruzzelli, 1984) and it is assumed to reduce Cd adsorption and increase the fraction

of Cd that is plant available in agricultural soils (Grant et al., 1998; Grant et al., 2002; Grant and Sheppard, 2008).

Root exudation of protons and organic acids reduces soil pH, especially in the vicinity of active roots (Marschner, 1998; Clemens et al., 2002; Dakora and Phillips, 2002; Gunes et al., 2007). It is assumed that enhanced ability of preceding crop to acidify soil via root exudation may increase plant Cd uptake in the subsequent crop (Oliver et al., 1993; Grant et al., 1998). For example, significantly greater grain Cd concentration in wheat grown after lupin than after wheat was suspected to be due to soil acidification from release of protons by lupin (Oliver et al., 1993).

1.3 Ionic strength

An increase in ionic strength of a soil solution can increase desorption of Cd from soil exchange sites, increasing soil solution Cd concentration (Garcia-Miragaya and Page, 1976; Petruzzelli et al., 1985; Lorenz et al., 1994; Fotovat and Naidu, 1998). Cadmium may desorb when competitive electrolyte cations in soil solution exchange with Cd adsorbed on to soil surfaces. Cadmium may also form uncharged and negatively charged soluble complexes with anions in soil solution and ion pairs increasing Cd desorption and total concentration of Cd in solution (Garcia-Miragaya and Page, 1976).

Fertilizers including MAP can increase ionic strength in soil solution via addition of soluble salts (Lorenz et al., 1994; Mitchell et al., 2000) which may increase Cd availability for agricultural crops (Grant et al., 1998; Grant et al., 2002; Grant and Sheppard, 2008). Also, decomposition of soil incorporated crop residue might release soluble ions into soil solution, increasing ionic concentration in soil solution and Cd desorption. However, there is no information in literature about this process.

1.4 Cadmium, Zn and P interaction

Cadmium and Zn are chemically similar and compete with each other in soil and plant (Grant et al., 1998). An increase in Zn concentration in soil solution promotes desorption of Cd due to competition from Zn for Cd adsorption sited, increasing Cd availability for plants (Christensen, 1984; Moraghan, 1993; Norvell et al., 2000; Lambert et al., 2007). As a result, some researchers have found that Zn application increased plant Cd uptake (Williams and David, 1976; Norvell et al., 2000) and durum wheat grain Cd correlated positively ($r^2 = 0.52$) with DTPA extractable soil Zn concentration (Norvell et al., 2000).

However, other researchers have observed opposite results. For example, soil incorporation of Zn fertilizer was repeatedly shown to reduce Cd uptake in durum wheat and flax (Moraghan, 1993; Choudhary et al., 1994; Grant and Bailey, 1997; Jiao et al., 2004). Even though the increasing concentration of Zn in soil has been shown to increase the concentration of Cd in soil solution, these contrasting results may be due to the limited root uptake of Cd and limited translocation of Cd from root to shoot as Zn competes with Cd (Grant et al., 1998). As a result, in previous studies in Manitoba, flax seed Zn concentration was inversely associated with the seed Cd concentration irrespective of the factor that caused the change in seed Zn concentration, e.g., Zn fertilizer application, changes in soil Zn concentration and P fertilizer application (Grant and Bailey, 1997).

Phosphorus application often shows an inverse relationship with plant available soil Zn concentration and plant Zn concentration (Grant and Bailey, 1997; Grant et al., 2002; Jiao et al., 2004; Lambert et al., 2007; Ryan et al., 2008). Laboratory application of P fertilizer equivalent to 15 years of agronomic application decreased water extractable

soil Zn concentration (Lambert et al., 2007). The formation of Zn-P precipitates was suspected to be the reason for reduced Zn concentration. In Manitoba studies, concentration of Zn in flax seed was lower when P fertilizer was applied than when no P was applied (Grant and Bailey, 1997). Concentration of Zn in durum wheat grain was lower where P fertilizer was applied than where no P fertilizer was applied and the lowering of Zn concentration was proportional to the increase in rate of P applied (Grant et al., 2002). Flax and durum wheat tissue and seed Zn concentration was lower when P fertilizer was applied than in the control, where no P was applied (Jiao et al., 2004). The reduction in Zn concentration was attributed to direct interference from P for Zn uptake. Similarly, in Australian field studies, spring wheat grain Zn concentration was lower where P fertilizer was applied than in the control where no P fertilizer was applied (Ryan et al., 2008).

Available literature on the interaction of Zn, P and Cd suggests that the increase in Cd concentration after P fertilizer application may be due to the P-induced decrease in plant Zn concentration; i.e., the application of P fertilizer reduces Zn concentration in soil and plant. Cadmium will then have less competition from Zn for plant uptake and more Cd will be taken up by roots and translocated from root to shoot (Grant and Bailey, 1997; Grant et al., 2002; Jiao et al., 2004; Grant and Sheppard, 2008).

It should be noted; however, the influence of Zn on plant Cd uptake is variable and depends on Cd/Zn ratio in soil, soil properties and plant characteristics (Grant et al., 1998; Grant et al., 1999). For example, when Cd was not added to soil, seed Cd concentration in flax was decreased with the application of Zn compared to no application of Zn. In the same study, the application of Zn increased seed Cd concentration compared to no application of Zn, when Cd was added at 1 mg kg^{-1} soil (Moraghan, 1993).

1.5 Mycorrhizal fungi

Mycorrhizal fungi form a symbiotic association with living plant roots (Smith and Read, 1997). Durum wheat, barley and flax are mycorrhizal and canola is non-mycorrhizal (Gao et al., 2010). Mycorrhizae receive photosynthetic energy from plant and the plant acquires access to more nutrients in return (Smith and Read, 1997). Mycorrhizal hyphae penetrate into roots and form structures called vesicles and arbuscules inside the living root. Arbuscles are developed for solute exchange between root and fungal hyphae (Smith and Read, 1997). Hyphae reach smaller soil pockets and can extend larger distances into the soil, thereby accessing regions of the soil which plant roots alone cannot reach. Therefore, after associating with the fungi, plant roots are able to reach ions and solutes beyond commonly defined rhizosphere limits (Marschner, 1995; Marschner, 1998).

Mycorrhizae can increase P supply to the plant (Smith and Read, 1997). Mycorrhizal root colonization increases when plants are experiencing difficulties in P acquisition and AMF root colonization decreases when P availability is high for plants (Lu and Miller, 1989; Heggo and Angle, 1990; Smith and Read, 1997; Wong et al., 2007; Ryan and Angus, 2003; Ryan et al., 2008). At typical concentrations of soil Zn, mycorrhizae also increase Zn supply to the plant (Smith and Read, 1997; Liu et al., 2000; Ryan et al., 2008). However, at elevated soil Zn concentrations, plant uptake of Zn is reduced by AMF in order to provide plant protection (Galli et al., 1994; Wong et al., 2007).

A mycorrhizal host crop may produce greater mycorrhizal root colonization in the subsequent crop than a non-mycorrhizal preceding crop (Harinikumar and Bagyaray, 1988; Gavito et al., 1998; Arihara and Karasawa, 2000; Gao et al., 2010). The inoculum

potential of AMF fungi (i.e., hyphal density and spore count in soil) increases when mycorrhizal host crop is growing and that may increase subsequent crop AMF colonization (Lu and Miller, 1989; Kabir et al., 1998). In a Manitoba study, arbuscular colonization (AC) of durum wheat was 61% and 56% greater when the preceding crop was flax, instead of canola in the second and third years of a field study, respectively (Gao et al., 2010). In a field study conducted in India, AMF root colonization and AMF spore count in the subsequent crop of cowpea (a mycorrhizal crop) was significantly lower when the preceding crop was fallow or mustard (a non-mycorrhizal crop) than when cowpea (Harinikumar and Bagyaray, 1988).

There are only a few studies that have explored the influence of AMF on plant Cd uptake at Cd concentrations that are typical of agricultural soils. The body of literature still has wide inconsistencies based on plant species (Hetrick et al., 1994; Ryan et al., 2003; Ryan et al., 2008), mycorrhizal species/strain, soil and environmental conditions (Weissenhorn et al., 1995; Liu et al., 2000; Ryan et al., 2003; Gohre and Paszkow, 2006; Ryan et al., 2008) and soil trace element concentration (Heggo and Angle, 1990; Liu et al., 2000; Chen et al., 2004; Wong et al., 2007). Mycorrhizae could increase plant concentration of Cd via delivery of Cd through fungal hyphae (Guo et al., 1996; Smith and Read, 1997; Chen et al., 2004). Furthermore, AMF can also reduce plant uptake of Cd, especially at elevated soil Cd concentrations (Galli et al., 1993; Galli et al., 1994; Weissenhorn et al., 1995; Guo et al., 1996; Smith and Read, 1997; Chen et al., 2004). In contrast, some other studies did not report any impact of AMF on plant Cd uptake (Weissenhorn et al., 1995; Gao et al., 2010). The mechanism of Cd immobilization in Cd contaminated soils could be via secretion of metal chelating agents (e.g., glomalin) into soil (Gohre and Paszkow, 2006), screening of Cd at the fungal plasma membrane (Gohre

and Paszkow, 2006) and localization of Cd in fungal structures (Marschner, 1995; Khan et al., 2000; Smith and Read, 2000; Chen et al., 2005).

1.6 Rhizosphere

The concept of rhizosphere was first introduced by Hiltner in 1904. Rhizosphere is considered as the volume of soil around the root which is influenced by the activity of living plant roots (Darrah, 1993*;* Hinsinger et al., 2003; Gregory, 2006). Rhizosphere processes are actively involved in trace element uptake but they are often neglected in many studies. Agricultural management practices such as P fertilization and crop rotation affect rhizosphere soil processes and may therefore affect plant Cd uptake.

The rhizosphere receives large quantities of root exudates (rhizodeposits). Root exudation is the key process governing rhizosphere activity, which is unique to each plant (Marschner, 1995; Dakora and Phillips, 2002). Root exudation can influence Cd uptake by plants. Plants secrete organic ligands into the rhizosphere for metal chelation, which are commonly known as phytometallophores. Preceding crop secretion of phytometallophores may increase subsequent crop Cd uptake (Oliver et al., 1993). Chelated metals have high affinity for plant uptake (Mench and Martin, 1991; Marschner, 1995; Krishnamurti et al., 1997). For an example, phytosiderophores are a group of phytometallophores produced for iron chelation under iron deficiency conditions (Fan et al., 1997). Phytosiderophores have the ability to increase plant availability of several other trace elements such as Mn, Cu and Zn (Dakora and Phillips, 2002). The phytosiderophores may have a potential to increase plant availability of Cd, as well (Grant et al., 1998).

In contrast, certain other phytochelatins secreted by plants bind with trace elements and reduce phytoavailability through formation of organic-metal complexes. This is a mechanism of plant protection at elevated soil trace element concentrations (Schmoger et al., 2000; Clemens et al., 2002).

Definition of the rhizosphere boundary is still a matter of debate and it makes rhizosphere soil sampling a challenge. One to two millimetre thick soil layers surrounding the root are often identified as rhizosphere (Hojberg et al., 1996). Gregory (2006) stated that the rhizosphere boundary varies with processes that the researcher is interested in. He suggested that most of the soil in upper 10 cm in managed production systems can be considered as rhizosphere soil during a considerable part of the growing season when considering the dynamics of mobile nutrients, water and volatile compounds because distances between roots are small enough to consider the entire area as rhizosphere.

1.7 Crop residue incorporation

Crop residue management is an important factor determining Cd availability for the subsequent crop in a rotation. Recycling of crop residue adds Cd back into agricultural soils. The amount of Cd addition increases with increasing Cd concentration in residue and quantity of residue added. Decomposition of added residue releases Cd into soil, making Cd plant available (Grant et al., 1999). Over the long term, crop residue incorporation increases organic matter status of soil and soil organic matter can increase soil CEC (Grant et al., 1999; Grant and Sheppard, 2008). Increases in CEC reduce plant Cd uptake (Hinseley et al., 1982; Korcak and Fanning, 1985; He and Singh, 1993a). Soil organic matter (SOM) also has the ability to form organo-metal complexes with Cd. Some of these formations can increase Cd availability for plants while some others reduce

(Grant et al., 1999; Grant and Sheppard, 2008). Crop residue decomposition releases organic acids and protons and may have the potential to reduce soil pH (He and Singh, 1993b) and it is well known that soil pH is often inversely related to plant available soil Cd concentration and plant Cd uptake (Christensen, 1984; Levi-Minzi and Petruzzelli, 1984; Grant et al., 1998; Sauve et al., 2000; Grant and Sheppard, 2008).

Researchers have been looking at soil Cd concentration and plant Cd uptake by adding various types of organic amendments; yet, there are few studies on crop residue addition. Also, the findings on the effect of soil incorporation of organic matter on soil and plant Cd concentration are not consistent. In a study conducted in Norway, the incorporation of cow manure, hog manure and peat into an agricultural soil naturally high in Cd significantly decreased DTPA extractable soil Cd concentration, irrespective of its source (Narwal and Singh, 1997). Similarly, the incorporation of peat decreased NH_4OAc extractable Cd concentration in a clay and a loamy sand soil in Uppsala, Sweden (Eriksson, 1988). In contrast, incorporation of peat into sand, sandy loam and clay soil increased NH_4NO_3 and DTPA extractable soil Cd concentration in a study conducted in Norway (He and Singh, 1993a). Furthermore, He and Singh (1993b) did not find a relationship between Cd extracted (by NH_4NO_3 and DTPA) and organic matter added to soil. In terms of plant Cd uptake, a reduction in tissue Cd concentration was observed with farm yard manure incorporation in long term trials at Rothamsted research facility, UK (Jones and Johnston, 1989). Soil incorporation of peat decreased rye grass Cd concentration (He and Singh, 1993a). Shoot Cd concentration of maize was decreased with sludge application (Korcak and Fanning, 1985). In contrast, flax shoot Cd concentration was greater when canola residue and barley residue were incorporated than when no residue was incorporated. However, there were no differences in durum wheat

shoot Cd concentration among barley residue addition, canola residue addition and no residue addition (Eastley, 2008).

1.7 Objective for current studies

Mono-ammonium phosphate has the capacity to increase plant uptake of Cd even without Cd as an impurity in fertilizer. However, the mechanism for this process is not known. Therefore, the overall objective of experiment one was to understand how reagent grade MAP increases plant Cd uptake in durum wheat, flax and canola by the fertilizer induced changes in soil, soil solution and plant. For that, a growth chamber study was conducted with two rates of P fertilizer application (i.e., 0 and 80 mg P kg^{-1} soil) and three test crops (i.e., canola, durum wheat and flax) along with an unplanted control.

Certain preceding crops also increase subsequent crop plant Cd uptake but that mechanism, too, is not well understood. The increases could be via chemical changes made in soil by the preceding crop and/or by release of Cd during crop residue decomposition and/or by the changes in AMF fungi root colonization. Therefore, another growth chamber study was conducted to understand how soil, soil solution and durum wheat and flax are affected by preceding crop soil (i.e., canola and barley grown soils) and crop residue addition (i.e., canola residue, barley residue and no addition of crop residue).

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2. MONO-AMMONIUM PHOSPHATE INFLUENCE ON THE CONCENTRATION OF CADMIUM IN SOIL AND CADMIUM UPTAKE OF CANOLA, DURUM WHEAT AND FLAX

2.1 ABSTRACT

Cadmium is a potentially toxic trace element and food is a major route of Cd entry to humans. In previous studies, mono-ammonium phosphate (MAP) has been reported to increase food crop Cd uptake via fertilizer induced changes in soil and plant. A growth chamber study was conducted to understand how MAP-reagent grade (RG) (at 80 mg P kg^{-1} soil) influences Cd uptake in canola, durum wheat and flax.

There was no significant increase in shoot or root Cd concentration in durum wheat, flax or canola when MAP-RG was added to a Newdale clay loam soil (Orthic Black Chernozem). The lack of increase in shoot or root Cd concentration with MAP-RG addition was probably due to the lack of increase in soil solution and soil extractable Cd concentration with MAP-RG addition in pots planted to canola, durum wheat or flax. Furthermore, the lack of marked decrease in soil solution pH and/or soil pH or increase in soil solution EC and/or soil EC with MAP-RG addition could be key reasons for the lack of increase in soil Cd concentration with MAP-RG addition. Arbuscular mycorrhizal fungi (AMF) root colonization percentage was significantly decreased with MAP-RG addition. However, changes in AMF root colonization percentage did not influence the Cd uptake of durum wheat and flax.

Overall, the observations in this study indicate that the application of MAP at rates within the typical range of agricultural application may not increase plant available Cd

concentration or plant Cd uptake due to fertilizer induced changes in soil and plant, especially when soil pH and EC remain relatively stable.

2.2 Introduction

Cadmium (Cd) is a potentially toxic trace element. Certain soils are naturally high in Cd content. In addition, anthropogenic activities add Cd into soil (Alloway and Steinnes, 1999) and food crops accumulate cadmium from soil (Kuboi et al., 1986). Consumption of food crops containing cadmium in concentrations beyond acceptable concentrations may lead to accumulation of Cd in human body over time causing chronic toxicity (Ryan et al., 1982; Nordberg et al., 2002; Dong et al., 2007). The maximum permitted concentration of Cd for international trade is 0.2 mg kg^{-1} for wheat (Codex Alimentarius Commission of FAO/WHO, 2009). Phosphorus fertilization is an important agricultural management practice which has the potential to affect crop Cd concentration and it is important to understand its influence in detail in order to reduce Cd in food crops.

Mono-ammonium phosphate (MAP) is a common P fertilizer source in alkaline soils of Western Canadian prairies. This fertilizer adds Cd into agricultural soils as it often contains Cd as an impurity and increases the risk of Cd accumulation by food crops (Grant et al., 1998; Lambert et al., 2007). However, the effect of MAP on food crop Cd uptake, especially in the year of application, may not be entirely due to the addition of Cd into agricultural soils. Mono-ammonium phosphate may have an indirect effect on plant Cd uptake via fertilizer induced changes in soil pH, ionic strength, Zn availability,

rhizosphere chemistry, microbial activity and plant growth (McLaughlin et al., 1995; Grant et al., 2002; Grant et al., 1998; Grant and Sheppard, 2008).

An increase in Cd concentration of durum wheat and flax due to indirect effects of MAP application has been observed in previous field and growth chamber studies (Choudhary et al., 1994; Grant et al., 2002; Jiao et al., 2004). In a field study conducted at 11 field sites in Manitoba and Alberta, Grant et al. (2002) observed an increase in durum wheat grain Cd concentration in the year of application with three sources of commercial grade MAP (Cd concentrations of 0.2, 7.8 and 186 mg kg^{-1}). The increase in grain Cd in that study was not related to the concentration of Cd present in each fertilizer and it was attributed to fertilizer induced changes in soil and plant. In a growth chamber study, conducted at Brandon, Manitoba, with Newdale clay loam soil (Orthic Black Chernozem), the concentration of Cd in tissue and seed of durum wheat and flax were greater with the addition of MAP-RG (P, 100 mg kg^{-1} soil) than without (Jiao et al., 2004). In another growth chamber study, the shoot Cd concentration of durum wheat was significantly greater with addition of MAP (P, 150 mg kg^{-1}) containing a low concentration of Cd (15 mg kg^{-1}) and with addition of both MAP and NH_4NO_3 (P, 150 mg kg^{-1} and N, 200 mg kg^{-1}) than in the respective control (no MAP and no NH_4NO_3) (Choudhary et al., 1994). The shoot Cd accumulation (i.e., concentration*biomass produced) of durum wheat was also greater with the addition of MAP (P, 150 mg kg^{-1}) than with the addition of NH_4NO_3 alone (at the rate of 74 mg N kg^{-1} soil). In a similar growth chamber study conducted by Choudhary et al. (1995), the Cd concentrations were 2.1 and 1.5 times greater in root, 1.9 and 1.6 times greater in leaf, 2.2 and 2.3 times greater in stem and 3.8 and 3.1 times greater in grain with MAP-RG addition than in the

control (no addition of MAP) for durum wheat DT 627 and durum wheat Medora, respectively.

In contrast, some other short term studies on the effect of MAP on plant Cd uptake (with or without Cd in fertilizer) reported that MAP has no effect on Cd uptake by plants (Grant et al., 1998; Choudhary et al., 1994; McLaughlin et al., 1995). Potato tuber Cd concentrations in field plots receiving MAP (containing Cd at a rate of 6 and 95 mg kg^{-1}) were not significantly different from tuber Cd concentrations in plots which did not receive any P fertilizer in the year of application (McLaughlin et al., 1995). The variation in tuber Cd concentration among field sites was larger than among treatments, indicating a strong influence of soil physical and chemical characteristics on tuber Cd concentration. A series of short term growth chamber studies summarized by Grant et al. (1998) reported that MAP-RG, MAP containing trace concentrations of Cd and MAP containing Cd at 15 mg kg^{-1} fertilizer did not significantly influence durum wheat tissue Cd concentration compared to respective control treatments. The plant Cd concentration for MAP + NH_4NO_3 was lower than the plant Cd concentration for NH_4NO_3 alone at equivalent concentrations of N (Choudhary et al., 1994). The reduction of plant Cd concentration with MAP addition may have been due to the dilution of Cd concentration inside the plant as dry matter production increased with P addition.

Soil pH has been shown to affect Cd availability for plants and it could be the most notable soil characteristic in this regard (He and Singh, 1993; Grant et al., 1998; Grant et al., 1999; Adam et al., 2004; Grant and Sheppard, 2008). Decreasing soil pH favours Cd desorption from soil particles and increases the partition of Cd into the soil solution, making Cd more available for plants (Christensen, 1984; Levi-Minzi and Petruzzelli, 1984; Grant et al., 1998; Helmke, 1999; Sauve et al., 2000; Grant and

Sheppard, 2008). The potential for pH reduction after MAP application was shown in both laboratory and field studies (Lambert et al., 2007). Soil incorporation of MAP reduced soil pH and subsequently reduced Cd adsorption under laboratory conditions (Levi-Minzi and Petruzzelli, 1984) and it is suspected to reduce Cd adsorption and increase the plant available soil Cd fraction in agricultural soils (Grant et al., 1998; Grant et al., 2002; Grant and Sheppard, 2008).

The increase in ionic strength of a soil solution can increase desorption of Cd from soil exchange sites, increasing soil solution concentration (Garcia-Miragaya and Page, 1976; Petruzzelli et al., 1985; Lorenz et al., 1994; Fotovat and Naidu, 1998; Helmke, 1999). Cadmium may desorb when competitive electrolyte cation in soil solution exchange with Cd adsorbed onto soil surfaces. Cadmium may also form uncharged and negatively charged soluble complexes with anions in soil solution and ion pairs, increasing Cd desorption and total concentration of Cd in soil solution (Garcia-Miragaya and Page, 1976). As a result, fertilizers, including MAP, can increase ionic strength in soil solution via addition of soluble salts (Lorenz et al., 1994; Mitchell et al., 2000) which may increase Cd availability for plants in agricultural soils (Grant et al., 1998; Grant et al., 2002; Grant and Sheppard, 2008).

Cadmium and Zn are chemically similar and compete with each other in soil and plants (Grant et al., 1998). Soil incorporation of Zn fertilizer was repeatedly shown to reduce Cd uptake in durum wheat and flax (Moraghan, 1993; Choudhary et al., 1994; Grant and Bailey, 1997; Jiao et al., 2004). This reduction may be due to limited root uptake of Cd as Zn competes for uptake sites in root and also may be due to limited Cd translocation from root to shoot by favouring Cd retention in root (Grant et al., 1998). Grant and Bailey (1997) claimed that the effect on plant Zn concentration is inversely

associated with the effect of plant Cd concentration irrespective to the factor causing the change in plant Zn concentration.

Phosphorus application often shows an inverse relationship with plant available soil Zn concentration and plant Zn concentration (Grant and Bailey, 1997; Grant et al., 2002; Jiao et al., 2004; Lambert et al., 2007; Ryan et al., 2008). Laboratory application of P fertilizer equivalent to 15 years of agronomic application decreased water extractable soil Zn concentration (Lambert et al., 2007). The formation of Zn-P precipitates was suspected to be the reason for reduction in Zn concentration. In Manitoba field studies, the concentration of Zn in flax seed was lower when P fertilizer was applied than when no P fertilizer was applied (Grant and Bailey, 1997). In another set of field studies conducted in Manitoba and Alberta, the concentration of Zn in durum wheat grain was lower where P fertilizer was applied than where no P fertilizer was applied and the lowering of Zn concentration was proportional to the increase in rate of P applied (Grant et al., 2002). In a growth chamber study conducted in Brandon, Manitoba, flax and durum wheat tissue and seed Zn concentration was lower when P fertilizer was applied than in the control where no P was applied (Jiao et al., 2004). The reduction in Zn concentration was attributed to the direct interference from P for Zn uptake. Similarly, in field studies conducted in Australia, spring wheat grain Zn concentration was lower where P fertilizer was applied than in the control where no P fertilizer was application (Ryan et al., 2008).

Available literature on the interaction of Zn, P and Cd suggests that the increase in Cd concentration after P application may be due to the P induced decrease in plant Zn concentration: i.e., the application of P fertilizer reduces Zn concentration in soil and plant. Cadmium will then have a less competition from Zn for plant uptake and more Cd

will be taken up by roots and translocated from root to shoot (Grant and Bailey, 1997; Grant et al., 2002; Jiao et al., 2004; Grant and Sheppard, 2008).

Nevertheless, the presence of Zn at higher concentrations in soil solution could promote desorption of Cd due to the competition from Zn for soil exchange sites, increasing Cd availability for plants (Christensen, 1984; Moraghan, 1993; Norvell et al., 2000; Lambert et al., 2007). Furthermore, in some cases, the factors reducing soil solution Zn concentration (such as P fertilizer application) may reduce Cd availability for plants. However, an increase in Zn concentration may still reduce Cd concentration due to the competition between Cd and Zn during root uptake and translocation inside the plant (Grant et al., 1998).

Mycorrhizal fungi form a symbiotic association with living plant roots (Smith and Read, 1997). Durum wheat and flax are mycorrhizal and canola is non mycorrhizal (Gao et al., 2010). Mycorrhizae receive photosynthetic energy from plant and the plant acquires access to more nutrients, in return (Smith and Read, 1997). Hyphae reach smaller soil pockets and larger distances, thus exploring portions of the soil that plant roots can not reach. Therefore, after associating with the fungi, plant roots have the opportunity to reach ions and solutes beyond commonly defined rhizosphere limits (Marschner, 1995; Marschner, 1998). Mycorrhizal hyphae penetrate into roots and form vesicles and arbuscules inside the living root. Arbuscles are developed for solute exchange between root and fungal hyphae and vesicles are assumed to be storage organs (Smith and Read, 1997).

Mycorrhizae can increase P supply to plants (Smith and Read, 1997). Mycorrhizal root colonization increases when plants are experiencing difficulties in P acquisition and AMF root colonization decreases when the P availability is high for plants (Lu and

Miller, 1989; Heggo et al., 1990; Li et al., 1991; Smith and Read, 1997; Wong et al., 2007; Ryan and Angus, 2003; Ryan et al., 2008). At typical concentrations of Zn in agricultural soils, mycorrhizae can increase Zn uptake by plants (Smith and Read, 1997; Liu et al., 2000; Ryan et al., 2008). However, at very high soil Zn concentrations, plant uptake of Zn is reduced by AMF in order to provide plant protection (Galli et al., 1994; Wong et al., 2007).

The influence of AMF on plant Cd uptake is still a matter of debate because the existing body of literature has wide inconsistencies based on plant species (Hetrick et al., 1994; Ryan and Angus, 2003; Ryan et al., 2008), mycorrhizal species/strain, soil and environmental conditions (Weissenhorn et al., 1995; Liu et al., 2000; Ryan and Angus, 2003; Gohre and Paszkow, 2006; Ryan et al., 2008) and soil trace element concentration (Heggo et al., 1990; Chen et al., 2004; Wong et al., 2007).

However, mycorrhizae could increase plant concentration of Cd via delivery of Cd through fungal hyphae (Guo et al., 1996; Smith and Read, 1997; Chen et al., 2004). Alternatively, AMF can also reduce plant uptake of Cd, especially at elevated soil Cd concentrations (Galli et al., 1993; Galli et al., 1994; Weissenhorn et al., 1995; Guo et al., 1996; Smith and Read, 1997; Chen et al., 2004). Also, some studies have not reported any impact of AMF on plant Cd uptake (Weissenhorn et al., 1995; Gao et al., 2010). However, if P fertilizer application causes a decrease in AMF colonization as mentioned by previous authors, it could either decrease crop Cd uptake with the decrease in AMF root colonization or increase crop Cd uptake due to the lack protection from AMF.

Rhizosphere is considered as the volume of soil around the root which is influenced by the activity of living plant roots (Darrah, 1993*;* Hinsinger, 2003; Gregory, 2006). The rhizosphere is actively involved in the process of trace element uptake

including Cd (Marschner, 1995) but the influence of the rhizosphere is often neglected in many studies. Agricultural management practices have been shown to affect rhizosphere soil in a way that is different from bulk soil (Wenzel et al., 2003; Gunes et al., 2007) and the application of MAP may also have a different effect on rhizosphere soil compared to bulk soil. In that case, it is important to understand the changes in rhizosphere soil along with bulk soil in order to enhance the understanding of MAP effect on plant Cd uptake.

The rhizosphere may have higher plant available Cd concentration than bulk soil. For example, the rhizosphere receives large quantities of root exudates (rhizodeposits). Root exudation of protons and organic acids can reduce rhizosphere soil pH and may increase trace element availability in rhizosphere soil and plant uptake (Marschner, 1998; Clemens et al., 2002; Dakora and Phillips, 2002; Gunes et al., 2007). Plants secrete organic ligands into the rhizosphere for metal chelation. Chelated metals have high affinity for plants and can increase plant uptake (Mench and Martin, 1991; Marschner, 1995; Krishnamurti et al., 1997). For example, phytosiderophores have the ability to increase availability of several trace elements such as Fe, Mn, Cu and Zn (Dakora and Phillips, 2002). The phytosiderophores may have a potential to increase Cd availability, as well (Grant et al., 1998). In contrast, certain other phytochelatins secreted by plants bind with trace elements and reduce phytoavailability through formation of organic-metal complexes, serving as a mechanism of plant protection at elevated soil trace element concentrations (Schmoger et al., 2000; Clemens et al., 2002).

The definition of rhizosphere boundary is still a matter of debate and therefore rhizosphere soil sampling is a challenge. One to two millimetre thick soil layers surrounding the root are often identified as rhizosphere (Hojberg et al., 1996). However, Gregory (2006) stated that the rhizosphere boundary varies with the processes that

researcher is interested. He suggested that most of the soil in upper 10 cm in managed production systems can be considered as rhizosphere soil during a considerable part of growing season when considering the dynamics of mobile nutrients, water and volatile compounds because distances between roots are small enough to consider the entire area as rhizosphere.

It has been reported that the effect of MAP on plant Cd uptake is not only due to Cd impurity but may also be due to fertilizer induced changes in soil-plant system; yet, the mechanism is not fully known. The main objective of this study was to investigate the short term effect of MAP itself (MAP-RG) on plant Cd uptake via fertilizer induced changes in soil and soil solution pH, EC, Zn and Cd, as well as AMF root colonization in three agricultural crops commonly grown in Manitoba.

2.3 Materials and Methods

The study was conducted in growth chambers at the Dept. of Soil Science, University of Manitoba. Soil was collected from the 0-15 cm depth of a Newdale clay loam soil (Orthic Black Chernozem) on Agriculture Agri-Food Canada's Brandon Research Station (Philips research farm, 50" 01' 17.04 N 49" 53' 2.88 W). Extractions were made with KCl for NH_4^+-N and NO_3^--N, with $NaHCO_3$ (Olsen-P) for P and with DTPA at pH 7 for Cd and Zn. Soil was digested with $HNO_3/HClO_4$ for total soil Cd and Zn. Soil pH and EC were measured with a soil:deionized water ratio of 1:2 w:w (Carter et al., 1993). Extractable NO_3^--N and NH_4^+-N were quantified with a Technicon II Auto analyzer by automated cadmium reduction and automated phenate method, respectively.

Extractable P was quantified colorimetrically by molybdate blue colour method at 880 nm (Carter et al., 1993) with a spectrophotometer (Ultrospec 2100 pro). Extractable Cd and Zn were quantified with inductively coupled plasma (Perkin Elmer 5300 DV). Soil pH and EC were measured using an Accumet AB 15 pH meter with a Ross "Sure Flow" electrode and an Accumet AB 30 conductivity meter, respectively. Soil chemical characteristics are reported in Table 2.1. Container moisture capacity was determined by the pill bottle method (Eastley, 2008). A known weight of soil was packed into a series of transparent pill bottles containing drainage holes at the bottom mimicking the soil bulk density in larger containers and watered with a range of water volumes covering a range of moisture capacities. The minimum soil moisture content that allowed the wetting front to move to the bottom of the bottle after 24 hours was considered as the container moisture capacity for the study soil.

Table 2.1 The physical and chemical characteristics of the experimental soil

Characteristic	
Soil texture	Clay loam
Soil organic matter (%)	4.3
Soil pH	7.52
CEC (cmol+ kg^{-1})	31
EC (mS cm^{-1})	0.18
KCl extractable NH_4^+-N (mg kg^{-1})	2.9
KCl extractable NO_3^--N (mg kg^{-1})	4.7
$NaHCO_3$ extractable P (mg kg^{-1})	22
DTPA extractable Cd (mg kg^{-1})	0.11
DTPA extractable Zn (mg kg^{-1})	2.2
Total Cd (mg kg^{-1})	0.56
Total Zn (mg kg^{-1})	66

Soil was air dried and sieved with a 1 cm mesh screen and filled into pots. Each pot contained 3 kg of soil and was sealed at the bottom to prevent drainage. Each pot was 15.5 cm in diameter and 20.5 cm in height. Rhizon soil moisture samplers were buried into each pot to collect soil solution samples. This device consists of a 10 cm long hydrophilic polymer head and a PVC tube attached to the head on one end and with an access cap at the free end. Soil solution enters through the hydrophilic polymer head once buried in soil and it is collected at the free end of the PVC tube. One Rhizon sampler was installed in each pot during filling, placing the hydrophilic polymer head in the middle of the soil column and allowing the attached PVC tube to extend out of the soil.

Prior to planting, two rates of P (0 and 80 mg kg^{-1} soil) were added as mono-ammonium phosphate-reagent grade (MAP-RG). Nitrogen, as urea, was added to apply a total of 150 mg kg^{-1} soil (i.e., after accounting for any N that came from MAP-RG). Both MAP-RG and urea were dissolved in deionized water and added to each pot as a solution. Six seeds of canola (*Brassica napus* L. variety Invigor 5440), ten seeds of durum wheat (*Triticum turgidum* L. variety AC Avonlea) or twelve seeds of flax (*Linum usitatissimum* L. variety Bethune) were seeded in each pot. An unplanted control treatment was also included. All seeds were pre-germinated prior to planting. In planted plots, excess plants were thinned after emergence, leaving three plants of canola, five plants of durum wheat or six plants of flax per pot. Experimental design was a complete randomized design with a fully factorial arrangement of treatments. Each treatment was replicated four times. Treatments were:

Canola with no MAP-RG addition

Canola with MAP-RG at 80 mg P kg^{-1} soil

Durum wheat with no MAP-RG addition

Durum wheat with MAP-RG at 80 mg P kg^{-1} soil

Flax with no MAP-RG addition

Flax with MAP-RG at 80 mg P kg^{-1}soil

Unplanted soil with no MAP-RG addition

Unplanted soil with MAP-RG at 80 mg P kg^{-1} soil

Sixteen hours light at a temperature of 22 ºC and 8 hours dark at a temperature of 15 ºC were provided to represent day and night cycle, respectively. Radiation intensity at the top of the plant canopy was 762 µmol S^{-1} m^{-2}. Humidity was maintained at 50%. Pots were watered with deionized water up to the estimated container moisture capacity when the soil moisture level dropped to 60% of this capacity. Pots were randomized inside the growth chamber weekly. Four weeks after planting, additional nitrogen was applied at 50 mg kg^{-1} soil to all treatments to avoid any N limitation. Urea was dissolved in deionized water and added soon after soil solution collection.

Soil solution was collected from each pot at weekly intervals, starting from the second week of planting, using the Rhizon soil moisture samplers installed. Pots were watered in the evening up to the container moisture capacity and soil solution was collected the next morning, two hours after the growth chamber lights were turned on. A syringe was used to draw approximately 10 mL of soil solution out from soil. Soil solution pH and EC were measured. Concentrations of Cd, Zn and P in soil solution were determined using inductively coupled plasma mass spectroscopy (Varian 820).

Fifty days after planting, plants were held by the base of the stem and uprooted. Soil aggregates trapped in between roots were removed and soil attached to roots was sampled for rhizosphere soil (Hojberg et al., 1996; Gunes et al., 2007; Wenzel et al.,

2003). Soil remaining in the pot was sampled for bulk soil after mixing. Soil was air dried and ground. Soil DTPA extractable Cd and Zn, $NaHCO_3$ extractable P, soil pH and soil EC were measured. Analytical procedures were similar to those reported earlier. A hand auger was driven through the soil column in each planted pot and a small portion of fresh root was collected with soil immediately before the plant was uprooted. Fresh roots were washed with water, cleared with 10% KOH and stained with 0.05% chlorazol black E. The percentage of arbuscular mycorrhizal fungi root colonization was assessed by the magnified intersections method (McGonigle et al., 1990). Plant shoots were rinsed with deionized water. Roots were washed with tap water to remove soil and rinsed with deionized water. Both shoot and root samples were oven dried at 60^oC, until moisture was constant. Shoot and root biomass was recorded. Plant samples were ground and digested with $HNO_3/HClO_4$ mixture. Concentrations of Cd, Zn and P were determined by inductively coupled plasma (Perkin Elmer 5300 DV).

The entire experiment was repeated to create two separate runs. The accuracy of all plant and soil analyses was assessed by the inclusion of standard reference materials and analytical values matched the stated ranges of standards. Data analysis was conducted using SAS 9.1 statistical package (SAS Institute 2004). Proc mixed ANOVA was performed after satisfying the assumptions underlying ANOVA (i.e., residuals were normally distributed; residuals had similar variance across the range of data and the residuals had means close to zero and they were uncorrelated). Significant differences in means were assessed using the Tukey mean comparisons test. Data from the two runs were combined when each run was not significantly different from the other.

2.4 Results and Discussion

2.4.1 The soil solution concentration of P, Cd and Zn, soil solution pH and soil solution EC during plant growth in canola, durum wheat, flax and unplanted control

The addition of MAP-RG significantly increased the soil solution concentration of P compared to control MAP-RG in canola, durum wheat, flax and unplanted control (Table 2.2). Soil solution P concentration declined over time, most probably due to the soil retention of P since the solution P trend for the unplanted control was not significantly different from the planted treatments.

Contrary to expectations, the soil solution concentration of Cd was not significantly different between MAP-RG added and control MAP-RG for canola, durum wheat, flax and an unplanted-control for all six weeks of growth. This lack of effect of MAP-RG occurred even though the soil solution concentration of P was significantly increased with MAP-RG addition and soil solution P concentration was positively but weakly correlated with soil solution Cd concentration ($r = 0.20$, $P = 0.0001$) (Table 2.2).

Table 2.2 The concentration of P, Cd and Zn, pH and EC in soil solution

Treatment			P	Cd	Zn	pH	EC
				μg L^{-1}			mS cm^{-1}
P	0		115b†	0.033	2.87	7.14	1.98
	80 (mg kg^{-1})		1693a	0.038	3.27	7.18	1.86
Plant	Canola		1005	0.040	4.95	7.18	1.41
	Durum wheat		913	0.030	2.94	7.23	1.61
	Flax		948	0.038	2.54	7.14	2.14
	Unplanted		749	0.035	1.84	7.08	2.53
Week	Week 2		1135a	0.047	1.87	7.02	2.61
	Week 3		1085a	0.036	1.88	7.03	2.22
	Week 4		1068a	0.033	2.32	7.18	1.98
	Week 5		741b	0.039	3.80	7.22	1.97
	Week 6		715b	0.033	4.42	7.25	1.49
	Week 7		678b	0.027	4.13	7.26	1.28
P×Plant	0	Canola		0.037ab	6.02a	7.10c	
	80	Canola		0.042a	3.87ab	7.23ab	
	0	D. wheat		0.029ab	2.17bc	7.22ab	
	80	D. wheat		0.031b	3.72abc	7.25a	
	0	Flax		0.034ab	2.05bc	7.12bc	
	80	Flax		0.042ab	3.03bc	7.15abc	
	0	Unplanted		0.032ab	2.44bc	7.09c	
	80	Unplanted		0.038ab	1.24c	7.08c	
P×Week	0	Week 2		0.047acd	2.19d	7.03e	
	80	Week 2		0.047ab	1.55d	7.01e	
	0	Week 3		0.035befg	2.18d	7.02e	
	80	Week 3		0.037cdefg	1.58d	7.05de	
	0	Week 4		0.032efgh	2.75bcd	7.18abc	
	80	Week 4		0.034dfgh	1.89d	7.17bc	
	0	Week 5		0.033efgh	2.45cd	7.16cd	
	80	Week 5		0.045abcde	5.15ab	7.26ab	
	0	Week 6		0.027fg	2.95bcd	7.19bc	
	80	Week 6		0.039abcdef	5.89a	7.31a	
	0	Week 7		0.026fg	4.70abc	7.23abc	
	80	Week 7		0.028g	3.55bcd	7.28ab	
	ANOVA		df		P>F		
	P		1	0.018	NS	0.0079	NS
	Plant		3	NS	0.0024	<0.0001	<.0001	<0.0001
	Week		5	<0.0001	<0.0001	<0.0001	<.0001	<0.0001
	P*Plant		3	NS	NS	0.0029	0.013	NS
	Plant*Week		15	NS	0.0198	NS	<.0001	<0.0001
	P*Week		5	NS	0.0049	<0.0001	0.0045	NS
	P*Plant*Week		15	NS	0.0153	NS	NS	NS
	MSE			321817	0.00014	8.40	0.0098	0.3648

† Within columns, means followed by the same letter are not significantly different according to the Tukey multiple comparison test ($p > 0.05$)

‡ NS, not significantly different ($p > 0.05$)

Soil solution Cd concentration often decreased with plant growth but this decrease was not consistent. For example, for canola, when MAP-RG was not added, soil solution Cd concentration decreased over time (Appendix A); whereas soil solution Cd was similar over time when MAP-RG was added. Conversely, for durum wheat, when MAP-RG was added, the soil solution concentration of Cd decreased over time, but there was no decrease over time when MAP-RG was not added. For flax and unplanted pots, soil solution Cd was similar over time for all P treatments. The decrease in soil solution Cd concentration with plant growth may have been due to plant uptake of Cd (Lorenz et al., 1997).

The soil solution concentration of Zn averaged over canola, durum wheat, flax and unplanted control was significantly greater when MAP-RG was added than when not added in the fifth and sixth weeks of growth but there were no significant differences in the second, third, fourth and seventh weeks, resulting in a significant P rate by week interaction (Table 2.2). An increase of Zn concentration in soil solution may promote the desorption of Cd due to competition from Zn for Cd adsorption sites, increasing soil solution Cd concentration (Christensen, 1984; Moraghan, 1993; Norvell et al., 2000; Lambert et al., 2007); however, there was no evidence for such an interaction in this study.

In contrast to expectations, soil solution pH was significantly greater when MAP-RG was added than when not added for canola (Table 2.2). However, there was no significant difference between MAP-RG added and control MAP-RG for durum wheat, flax and unplanted control, resulting in a significant P rate by plant treatment interaction. Furthermore, soil solution pH generally rose over time. However, the soil solution pH

when MAP-RG was added was significantly greater than the control in the week 5 and week 6 treatments but not in the week 2, 3, 4 and 7 treatments, resulting in a significant P rate by week interaction.

Application of MAP had been expected to reduce soil solution pH (Lambert et al., 2007) and subsequently increase soil solution Cd concentration (Levi-Minzi and Petruzzelli, 1984; Fotovat and Naidu, 1998; Grant et al., 1998; Grant et al., 2002; Grant and Sheppard, 2008). However, in this study, the application of MAP-RG did not reduce soil solution pH; instead, MAP-RG increased soil solution pH in certain situations. Also, although soil solution pH of planted soil increased with plant growth, it remained constant in the unplanted control, creating a significant interaction between week and plant treatments (Appendix A). Similarly, in a growth chamber study by Lorenz et al. (1997), the growth of radish increased soil solution pH compared to unplanted soil. Ammoniacal nitrogen (as urea) was applied in this study. However, nitrates can be formed quickly from soil applied urea under oxic soil conditions. Plant uptake of nitrates and orthophosphates may lead to the root excretion of OH^- in order to maintain electrostatic balance inside the plant and that may increase soil solution pH with plant growth (Haynes, 1990; Gahoonia et al., 1992; Marschner, 1995). Steady to increasing soil solution pH as plants grew may have prevented an increase in soil solution Cd concentration because Cd availability decreases with increasing soil pH (Christensen, 1984; Levi-Minzi and Petruzzelli, 1984; Grant et al., 1998; Helmke, 1999; Sauve et al., 2000; Grant and Sheppard, 2008).

Soil solution EC was not affected by MAP-RG addition in this study, perhaps because application of N fertilizer overwhelmed any effect of added MAP. Application of MAP had been expected to increase soil solution EC via addition of soluble salts

(Lorenz et al., 1994; Mitchell et al., 2000) and subsequently increase soil solution Cd concentration (Grant et al., 1998; Grant et al., 2002; Grant and Sheppard, 2008). Accordingly, the soil solution EC was significantly correlated with soil solution Cd concentration ($r = 0.23$, $P < 0.0001$). However, the variability in EC within the experiment was associated with plant species and week but not with P treatment.

The soil solution EC of planted treatments gradually decreased with plant growth while it remained stable over time in the unplanted control, resulting in a significant week by plant treatment interaction (Appendix A). This indicates a decrease in ionic strength of soil solution due to the plant uptake of ions from soil solution as the plants grew. The decrease in ionic strength of the soil solution may have allowed soluble Cd to redistribute onto soil exchange sites reducing soil solution Cd concentration (Lorenz et al., 1997). A gradual decrease in soil solution Cd concentration may decrease the plant uptake of Cd over the life of the plant (Jarvis et al., 1976; Lorenz et al., 1997).

2.4.2 The plant dry weight and AMF root colonization of canola, durum wheat and flax

Durum wheat and canola had significantly greater shoot dry weight when MAP-RG was added than when MAP-RG was not added (Table 2.3). However, flax shoot dry weight was not affected by MAP-RG addition, resulting in a significant P rate by crop interaction. There was no significant difference in root dry weight between MAP-RG addition and the control for all three crops.

Table 2.3 The shoot and root dry weight and arbuscular mycorrhizal fungi (AMF) root colonization of canola, durum wheat and flax

Treatment			Dry weights		AMF colonization†	
			Shoot	Root	AC‡	TC§
			g pot^{-1}		%	
P	0		23.10	7.79	8.01a¶	34.46
	80 mg kg^{-1}		27.19	7.94	2.68b	10.13
Plant	Canola		23.32	7.24b		na#
	Durum wheat		31.96	8.43a		24.21
	Flax		20.16	7.93ab		20.37
P×Plant	0	Canola	19.13c			na
	80	Canola	27.52b			na
	0	D. wheat	29.57b			40.30a
	80	D. wheat	34.34a			8.12bc
	0	Flax	20.62c			28.62b
	80	Flax	19.70c			12.13c

ANOVA	df	P>F		df	P>F	
P	1	<0.0001	NS††	1	0.0006	0.0001
Plant	2	<0.0001	0.0181	1	NS	NS
P*Plant	1	<0.0001	NS	1	NS	0.0103
MSE		0.85	0.89		11.4	137

† Due to the lack of AMF colonization on canola roots, colonization data and statistical analysis are reported for durum wheat and flax, only

‡ Arbuscular colonization is the percentage of root length colonized by arbuscules

§ Total colonization is the sum of arbuscular colonization and hyphal colonization (where hyphal colonization is the percentage of root length colonized by AMF hyphae)

¶ Within columns, means followed by the same letter are not significantly different according to the Tukey multiple comparison test ($p > 0.05$)

Canola was confirmed as non-mycorrhizal by analysis of random root samples

†† NS, not significantly different ($p > 0.05$)

The arbuscular colonization and total colonization of AMF in durum wheat and flax were significantly lower when MAP-RG was added than when MAP-RG was not added (Table 2.3). Our observations are in accordance with many researchers who have reported a reduction of AMF root colonization with an increase in P availability for plants (Smith and Read, 1997; Liu et al., 2000; Wong et al., 2007; Ryan and Angus, 2003; Ryan et al., 2008). Arbuscular mycorrhizal fungi root colonization costs photosynthetic energy for the plant. Benefits derived via the supply of P may outweigh the cost for plants when P availability is high and therefore root colonization may be limited (Smith and Read, 1997). However, AMF colonization is not always affected by P addition (Chen et al., 2004; Gao et al., 2010).

2.4.3 The concentration and accumulation of P, Cd and Zn for canola, durum wheat and flax

Shoot and root P concentrations were significantly greater when MAP-RG was added than when MAP-RG was not added for all three crops (Table 2.4). The shoot P concentrations in canola and durum wheat were significantly correlated with bicarbonate extractable P concentrations in rhizosphere soil ($r = 0.87$, $P < 0.0001$ and $r = 0.75$, $P = 0.0014$, respectively) and bulk soil ($r = 0.62$, $P = 0.0104$ and $r = 0.70$, $P = 0.0033$, respectively), indicating an increase in shoot P concentration with increasing soil P concentration.

Table 2.4 The P, Cd and Zn concentration of canola, durum wheat and flax

Treatment			Shoot P	Root P	Shoot Cd	Root Cd	Shoot Zn	Root Zn
			mg kg^{-1}					
P	0		1272	1015b†	0.40	0.81	13.8	24.1
	80 mg kg^{-1}		2811	1662a	0.39	0.77	9.50	26.2
Plant	Canola		1941	1463	0.34	0.73	12.9	22.7
	Durum wheat		1752	1222	0.30	0.83	12.5	24.7
	Flax		2431	1330	0.56	0.79	9.5	28.1
P×Plant	0	Canola	1464c		0.37b	0.82a	15.0a	24.6ab
	80	Canola	2418b		0.30c	0.64b	10.9b	20.7b
	0	D. wheat	1230c		0.27c	0.84a	15.8a	23.4b
	80	D. wheat	2275b		0.32bc	0.83a	9.2bc	25.9ab
	0	Flax	1122c		0.52a	0.76ab	10.5bc	24.3ab
	80	Flax	3741a		0.59a	0.83a	8.5c	31.9a

ANOVA	df	P>F					
P	1	<0.0001	<0.0001	NS	NS	<0.0001	NS‡
Plant	2	<0.0001	NS	<0.0001	0.0041	<0.0001	0.0286
P*Plant	2	<0.0001	NS	0.0003	0.0003	0.0004	0.0195
MSE		109255	246642	0.0021	0.0064	0.65	28.7

† Within columns, means followed by the same letter are not significantly different according to the Tukey multiple comparison test ($p > 0.05$)
‡ NS, not significantly different ($p > 0.05$)

Accumulation refers to the total mass of P or Cd or Zn collected by plants in a pot (i.e., accumulation = total dry weight in a pot × concentration of Cd or Zn or P). Shoot and root P accumulations were significantly greater when MAP-RG was added than when MAP-RG was not added (Table 2.5). In contrast to the P rate by plant interaction for dry matter yield and shoot P concentration, there were no differences among plant species in the effect of P rate on shoot P accumulation.

Table 2.5 The P, Cd and Zn accumulation† of canola, durum wheat and flax

Treatment			Shoot P	Root P	Shoot Cd	Root Cd	Shoot Zn	Root Zn
			—— mg pot^{-1}——		—— µg pot^{-1}——		—— mg pot^{-1}——	
P	0		29.2b‡	7.91b	8.62	6.33	0.32	0.19
	80 mg kg^{-1}		71.7a	14.2a	10.54	6.08	0.25	0.21
Plant	Canola		46.5b		7.73	5.32	0.28	0.16
	Durum wheat		57.1a		9.81	7.03	0.39	0.21
	Flax		47.8b		11.2	6.27	0.19	0.22
P×Plant	0	Canola			7.08b	6.13ab	0.29b	0.18bc
	80	Canola			8.38b	4.51b	0.28b	0.15c
	0	D. wheat			8.01b	6.80a	0.47a	0.19abc
	80	D. wheat			11.6a	7.27a	0.31b	0.22ab
	0	Flax			10.8a	6.09ab	0.22c	0.19abc
	80	Flax			11.6a	6.47a	0.17d	0.25a

ANOVA	df	P>F					
P	1	<0.0001	0.0001	<0.0001	NS§	<0.0001	NS
Plant	2	0.0067	NS	<0.0001	0.0021	<0.0001	0.0015
P*Plant	2	NS	NS	0.0126	0.0055	<0.0001	0.0075
MSE		55	17	1.76	0.97	0.0009	0.0016

† Accumulation = concentration * dry weight per pot
‡ Within columns, means followed by the same letter are not significantly different according to the Tukey multiple comparison test ($p > 0.05$)
§ NS, not significantly different ($p > 0.05$)

There were no significant differences in shoot and root Cd concentration between MAP-RG addition and the control (no MAP-RG addition) for durum wheat and flax. However, for canola, shoot and root Cd concentrations were significantly lower when MAP-RG was added than when MAP-RG was not added, resulting in a significant P rate by crop interaction (Table 2.4). The shoot Cd concentration of canola correlated negatively with shoot dry weight ($r= 0.62$, $P = 0.0299$), indicating that the reduction of shoot Cd concentration in canola with MAP-RG application in this study was probably due to the increase of shoot dry weight and the subsequent dilution effect of Cd inside the

plant (Choudhary et al., 1994). However, the soil solution pH of canola was significantly lower when MAP-RG was not added than when MAP-RG was added (Table 2.2). Canola can decrease soil pH via root exudation of protons and organic acids (Brennan and Bolland, 2005) when P availability is not ample and this decrease in soil solution pH may have increased the Cd uptake of canola in the control MAP-RG treatment, creating a greater shoot Cd concentration in control MAP-RG treatment than in the MAP-RG treatment. However, this process is not likely a major cause since the difference in pH was small, relative to that required for a significant change in Cd availability (Lambert et al. 2007).

Observations in this study are in accordance with studies that reported no effect or a decrease in tissue Cd with MAP application under both growth chamber and field conditions (Choudhary et al., 1994; McLaughlin et al., 1995; Grant et al., 1998; Gao et al., 2010). In contrast, some other researchers have reported that the application of MAP increased the tissue Cd concentrations of durum wheat and flax due to the factors induced by MAP application (Choudhary et al., 1994; Choudhary et al., 1995; Grant et al., 2002; Jiao et al., 2004). However, similar to the above-mentioned studies, the shoot Cd concentrations of durum wheat and flax in our study had a tendency to increase with MAP-RG addition even though the magnitude of the increases was not large enough to be significant.

Plant concentration of Cd has been reported to relate positively to the total concentration of Cd in soil solution (Jarvis et al., 1976; Lorenz et al., 1997). The lack of increase in soil solution Cd concentrations with MAP-RG addition could be the foremost reason for the lack of increase in canola, durum wheat and flax shoot Cd concentrations with MAP-RG addition. In turn, the lack of decrease in soil solution pH or increase in soil

solution EC with MAP-RG addition could be the main reason for the lack of increase in soil solution Cd concentration in canola, durum wheat and flax with MAP-RG addition.

Overall, the observations in this study indicate that the application of MAP at rates within the typical range of agricultural application may not significantly increase plant available Cd concentration and plant Cd uptake due to the soil and plant characteristics induced by MAP fertilization when soil solution pH and EC remain relatively stable.

Arbuscular mycorrhizal fungi are able to increase (Guo et al., 1996; Smith and Read, 1997; Chen et al., 2004) or decrease plant Cd concentration (Galli et al., 1993; Galli et al., 1994; Weissenhorn et al., 1995; Guo et al., 1996; Smith and Read, 1997; Chen et al., 2004). However, even though AMF root colonization intensity was reduced with MAP-RG addition, the shoot concentration of Cd in this study was not affected by MAP-RG addition. Furthermore, the shoot Cd concentrations of durum wheat and flax were not significantly correlated to arbuscular colonization or total colonization. These observations indicate that AMF root colonization intensity did not influence shoot Cd concentration of durum wheat and flax during this study. Similar observations were made by Weissenhorn et al. (1995a) in maize, in a growth chamber study with an EDTA-NH_4OAc extractable soil Cd concentration of 13 mg kg^{-1}. In subsequent studies, the Cd concentration of field grown maize shoot was not related to AMF root colonization in a soil with EDTA-NH_4OAc extractable soil Cd concentrations starting from 0.2 to 56 mg kg^{-1} (Weissenhorn et al., 1995b,c). In addition, mycorrhizal root colonization did not explain durum wheat grain Cd concentration under field conditions in a recent study conducted at Brandon, Manitoba (Gao et al., 2010). However, it should also be noted that the net effect of AMF root colonization on plant Cd uptake has wide inconsistencies based on soil, plant and environmental conditions.

Accumulation of shoot Cd was significantly greater in durum wheat when MAP-RG was added than when MAP-RG was not added (Table 2.5). Shoot Cd accumulations of canola and flax also tended to increase with MAP-RG addition; however, the magnitude of increases in shoot Cd accumulation in these crops was not significant, resulting in a significant P rate by crop interaction. Even though the concentration of shoot Cd in durum wheat was not significantly influenced by MAP-RG addition, shoot dry weight per pot was significantly increased in durum wheat with MAP-RG addition compared to control MAP-RG addition (Table 2.2). Therefore, the increase in shoot Cd accumulation in durum wheat appears to be driven by the dry matter response to additional P, although the non-significant increase in shoot Cd concentration also contributed to the increase in Cd accumulation. The increased accumulation of Cd may also be due to the improved root absorption capacity of Cd as plant growth was improved with P nutrition (Grant and Sheppard, 2008; Gao et al., 2010). Our results are similar to those of Choudhary et al. (1994), who observed a greater accumulation of Cd when MAP + NH_4NO_3 was added compared to when NH_4NO_3 was added at equivalent N rates even though adding MAP resulted in a reduction in durum wheat Cd concentration in shoot tissue. However, there was no significant difference in root Cd accumulation between MAP-RG addition and control MAP-RG for any crop species.

This study also confirmed differences among plant species in their ability to concentrate Cd in shoot and root, even though species effects were not the main objective of the study. Shoot Cd concentration in flax was greater than in durum wheat and canola (Table 2.4), confirming that flax is a greater accumulator of Cd than durum wheat (Grant and Bailey, 1997; Jiao et al., 2004; Eastley, 2008). Further, canola had greater shoot Cd concentration than durum wheat when MAP-RG was not added but there was no

significant difference when MAP-RG was added, resulting in a significant P rate by crop interaction. Accordingly, in an Australian field study, the concentration of Cd in canola seed was approximately three times greater than that in spring wheat (Brennan and Bolland, 2005). Greater Cd concentration in canola than in durum wheat may be due to canola's ability to make Cd plant available in soil via root exudation processes (Brennan and Bolland, 2005). The root Cd concentrations of durum wheat and flax were significantly greater than the root Cd concentration of canola when MAP-RG was added but there were no significant differences among three crops when MAP-RG was not added, resulting in a significant P rate by crop interaction (Table 2.4). Shoot Cd concentration averaged over all three crops was approximately half of root Cd concentration. Similar results were observed by Choudhary et al. (1994) in durum wheat and Jiao et al. (2004) in durum wheat and flax. Restricted Cd translocation towards shoot reduces Cd accumulation in edible plant parts and is a mechanism of protecting the plant from Cd toxicity. Cadmium may form complexes with Cd binding peptides in roots and that may make root an effective barrier for Cd movement (Grant et al., 1998).

Flax had significantly greater shoot Cd accumulation than durum wheat or canola when MAP-RG was not added (Table 2.5). However, when MAP-RG was added, flax or durum wheat had significantly greater shoot Cd accumulation than canola, resulting in a significant P rate by crop interaction. The root Cd accumulation of flax or durum wheat was greater than that of canola when MAP-RG was added but there were no significant differences among all three crops when MAP-RG was not added, resulting in a significant P rate by crop interaction (Table 2.5).

Shoot Zn concentrations of canola and durum wheat were significantly lower when MAP-RG was added than when MAP-RG was not added (Table 2.4). However, the

shoot Zn concentration of flax remained similar for both P treatments, resulting in a significant P rate by crop interaction. Root Zn concentration was not affected by MAP-RG addition for canola, durum wheat and flax.

Similar observations in durum wheat were made by Jiao et al. (2004) in a growth chamber study, Grant et al. (2002) in field studies conducted in Alberta and Manitoba and Ryan et al. (2008) in field studies conducted in Australia. In contrast to these observations and our study, the concentration of Zn in flax seed and shoot was reported to be lower when P fertilizer was applied than when P fertilizer was not applied in other field and laboratory studies conducted in Manitoba (Grant and Bailey, 1997; Jiao et al., 2004).

Cadmium and Zn compete with each other for root uptake and translocation inside the plant (Grant et al., 1998). As a result, it is repeatedly reported that the concentration of Zn in durum wheat and flax is inversely associated with the concentration of Cd irrespective of the factor caused the change in Zn concentration (Moraghan, 1993; Choudhary et al., 1994; Grant and Bailey, 1997; Jiao et al., 2004). Therefore, the decrease in shoot concentration of Zn in durum wheat and canola may have increased the shoot concentration of Cd; however, there was no evidence for such interaction in this study.

Shoot Zn accumulation was significantly lower in durum wheat and flax when MAP-RG was added than when MAP-RG was not added but the shoot Zn accumulation of canola was not influenced by MAP-RG addition, resulting in a significant P rate by crop interaction (Table 2.5). For durum wheat, the shoot Zn accumulation was not significantly correlated to shoot Cd accumulation, indicating that the increase in shoot Cd accumulation with MAP-RG addition was not due to the decrease in shoot Zn accumulation with MAP-RG addition. Root Zn accumulations were similar for MAP-RG added and control MAP-RG for durum wheat, flax and canola (Table 2.5).

The reduction of Zn concentration and accumulation in durum wheat and the reduction of Zn accumulation in flax with MAP-RG addition in this study may be due to the direct interference of P with Zn translocation inside the plant because the root concentration and accumulation of Zn remained unchanged with MAP-RG addition even though the shoot concentration and accumulation of Zn decreased with MAP-RG addition. (Singh et al., 1988; Grant and Bailey, 1989; Grant et al., 1998; Grant et al., 2002). Mycorrhizae are known to facilitate plant Zn uptake. Durum wheat shoot Zn concentration in this study positively correlated with arbuscular colonization ($r = 0.64$, $P = 0.0081$) and total colonization ($r = 0.74$, $P = 0.0011$) in accordance to the previous research (Smith and Read, 1997). Therefore, the reduction of Zn concentration and accumulation in durum wheat and the reduction of Zn accumulation in flax with MAP-RG addition in this study may also be due to reduced AMF root colonization with MAP-RG addition (Smith and Read, 1997; Liu et al., 2000; Ryan et al., 2008). In addition, for durum wheat and canola, the reduction in shoot Zn concentration may also be due to the dilution effect of Zn inside the plant since shoot dry weight increased with MAP-RG addition (Singh et al., 1988; Choudhary et al., 1994).

2.4.4 The soil extractable concentration of P, Cd and Zn, soil pH and soil EC at harvest

At harvest, sodium bicarbonate extractable P concentration in rhizosphere soil and bulk soil were significantly greater when MAP-RG was added than when not added for canola, durum wheat and flax (Table 2.6). The increase of P concentration was greater for bulk soil than for rhizosphere soil, probably due to the root uptake of P in rhizosphere soil, resulting in a significant P rate by soil interaction.

In accordance with soil solution Cd concentration, the DTPA extractable concentration of Cd was also not significantly different between MAP-RG addition and control MAP-RG for both rhizosphere soil and bulk soil and for all three test crops (Table 2.6). The plant concentration of Cd has been reported to relate positively to soil extractable Cd concentration (Smilde et al., 1992; Norvell et al., 2000). As discussed earlier, the lack of increase in canola, durum wheat or flax shoot Cd concentration with MAP-RG addition could be due to the lack of increase in plant available Cd fraction in soil with MAP-RG addition.

Table 2.6 The extractable concentration of P, Cd and Zn, soil pH and soil EC in the rhizosphere soil and bulk soil of planted treatments at the end of the growth chamber experiment

Treatment			P	Cd	Zn	pH	EC
			mg kg^{-1}	µg kg^{-1}	mg kg^{-1}		mS cm^{-1}
P	0		18.1	109	1.35	7.47	0.38a†
	80 mg kg^{-1}		43.8	107	1.39	7.46	0.35b
Soil	Rhizosphere soil (RS)		30.4	116a	1.54	7.41	0.35
	Bulk soil (BS)		32.9	100b	1.20	7.52	0.37
Plant	Canola		30.9	110a	1.53a	7.43	0.29
	Durum wheat		31.6	108ab	1.30b	7.52	0.37
	Flax		30.4	107b	1.28b	7.44	0.43
P×Plant	0	Canola				7.41d	
	80	Canola				7.45cd	
	0	Durum wheat				7.53a	
	80	Durum wheat				7.51ab	
	0	Flax				7.47bc	
	80	Flax				7.42d	
P×Soil	0	RS	19.0b		1.44ab	7.41b	
	80	RS	40.9a		1.65a	7.42b	
	0	BS	17.3b		1.26b	7.53a	
	80	BS	46.6a		1.14b	7.50a	
Plant×Soi	RS	Canola				7.39d	0.28c
	RS	Durum wheat				7.45b	0.34bc
	RS	Flax				7.40cd	0.45a
	BS	Canola				7.48bc	0.31c
	BS	Durum wheat				7.59a	0.40ab
	BS	Flax				7.49b	0.41a
ANOVA		df			P>F		
P		1	<0.0001	NS	NS	NS	0.0387
Plant .		2	NS	0.0292	0.0011	<0.0001	<0.0001
Soil		1	NS	0.0003	0.0015	0.0004	NS
P×Plant		2	NS	NS	NS	0.0006	NS
P×Soil		1	0.0255	NS	0.0080	0.0465	NS
Plant×Soil		2	NS	NS	NS	0.0329	0.0064
P×Plant×Soil		2	NS	NS	NS	NS	NS
MSE			29	14.6	0.032	0.0012	0.0015

† Within columns, means followed by the same letter are not significantly different according to the Tukey multiple comparison test ($p > 0.05$)

‡ NS, not significantly different ($p > 0.05$)

Rhizosphere had 16% greater DTPA extractable soil Cd concentration than bulk soil averaged across all three crops (Table 2.6). For durum wheat only, the rhizosphere concentration of Cd was significantly correlated with shoot Cd concentration ($r = 0.50$, $P = 0.0461$), indicating an increase in shoot Cd concentration with increasing rhizosphere concentration. Even though the response to MAP application was not different in rhizosphere soil compared to bulk soil, the greater concentration of Cd in rhizosphere soil and correlation between rhizosphere Cd and plant Cd concentration in durum wheat illustrate the importance of considering rhizosphere soil Cd concentration in future studies for a better understanding of the plant Cd uptake process.

Overall, the DTPA extractable concentrations of Zn was not significantly different between MAP-RG added and control MAP-RG for both rhizosphere soil and bulk soil and for all three test crops (Table 2.6). However, DTPA extractable Zn concentration increased with MAP-RG addition in rhizosphere but not in bulk, resulting in a significant P rate by soil interaction. That indicates the rhizosphere behaves differently from bulk soil with MAP-RG addition. An increase in soil Zn concentration may result in a decrease in soil Cd concentration (Christensen, 1984; Moraghan, 1993; Norvell et al., 2000; Lambert et al., 2007); however, there was no evidence for a Cd-Zn interaction in this study.

At the end of the experiment, there was no significant difference in soil pH (D) (measured in a soil: deionized water suspension) between MAP-RG added and control MAP-RG for canola and durum wheat. However, for flax, the soil pH was significantly lower when MAP-RG was added than when not added, resulting in a significant P rate by crop interaction (Table 2.6).

Lambert et al. (2007) reported a lowering of soil pH when MAP was added (20, 40 and 80 kg ha^{-1}) in the range of insignificant to 0.64 units for field trials and insignificant to 0.47 units for laboratory trials. Further, the authors claimed that a pH reduction of approximately 0.2 units was required to significantly increase Cd solubility in soil. In our study, the reduction in soil pH for flax was only 0.05 units when MAP-RG was added, so the magnitude of pH reduction was not substantial enough to significantly increase soil Cd availability.

After harvest, soil pH was significantly lower in rhizosphere than in bulk soil. The decrease in soil pH was slightly more pronounced when MAP-RG was not added than when MAP-RG was added, resulting in a significant P rate by soil interaction (Table 2.6). The root exudation of protons and organic acids are two major processes involved in rhizosphere pH reduction (Marschner, 1998; Clemens et al., 2002; Dakora and Phillips, 2002; Gunes et al., 2007). Lower pH in rhizosphere may be a reason for higher concentration of Cd and Zn in rhizosphere soil than in bulk soil (Marschner, 1995; Dakora and Philips, 2002; Dong et al., 2007). However, since the pH difference was less than 0.2 units, the effect of pH, alone, on the increase in rhizosphere Cd concentration was probably not significant. Plant root exudation of phytometallophores may also have contributed to the higher concentrations of Cd and Zn in rhizosphere soil to that of bulk soil (Mench and Martin, 1991; Marschner, 1995; Krishnamurti et al., 1997; Grant et al., 1998; Dakora and Phillips, 2002).

In planted soils, soil EC was significantly lower when MAP-RG was added than when not added (Table 2.6), contrary to expectations. Since soil EC and available Cd in soil are positively correlated, the addition of MAP-RG did not encourage release of Cd

through its effect on soil EC (Garcia-Miragaya and Page, 1976; Petruzzelli et al., 1985; Lorenz et al., 1994; Fotovat and Naidu, 1998).

As in the planted soils, the application of MAP-RG significantly increased sodium bicarbonate extractable P in unplanted soils (Table 2.7). However, in unplanted soil, contrary to the observations in planted soils, MAP-RG also significantly increased DTPA extractable soil Cd concentration, compared to control MAP-RG. Extractable soil Zn concentration was also greater when MAP-RG was added than when MAP-RG was not added. Increase in the concentration of Zn may have contributed to the increase in extractable concentration of Cd since Zn competes with Cd for cation retention sites in soil and the increase of Zn concentration may increase desorption of Cd, increasing its availability (Christensen, 1984; Grant et al., 1998).

Table 2.7 The extractable concentration of P, Cd and Zn, soil pH and soil EC in unplanted treatments at the end of the growth chamber experiment

Treatment			P	Cd	Zn	pH	EC
			mg kg^{-1}	µg kg^{-1}	mg kg^{-1}		mS cm^{-1}
P	0		22.4	102	1.19	7.17	1.11
	80 mg kg^{-1}		59.1	109	1.33	7.00	1.09
ANOVA		df			P>F		
P		1	0.0041	0.0009	0.0123	0.0260	NS
MSE			56	11.1	0.004	0.003	0.014

Soil pH of the unplanted control was significantly lower when MAP-RG was added than when MAP-RG was not added, similar to observations made by Levi-Minzi and Petruzzelli (1984) and Lambert et al. (2007). Soil pH was negatively correlated with DTPA extractable soil Cd concentration ($r = 0.60$, $P = 0.0141$) indicating a potential

increase in extractable soil Cd concentration with the reduction of soil pH in the unplanted treatments (Grant et al., 1998; Grant et al., 2002; Grant and Sheppard, 2008). MAP-RG reduced soil pH more substantially in unplanted soil (i.e., 0.17 units) than in any of the planted treatments and that could be a reason for soil DTPA extractable Cd concentration to increase with MAP-RG addition compared to control MAP-RG addition in the unplanted treatments, but not in the planted treatments. Soil EC in unplanted soil was not affected by MAP-RG addition and therefore the contribution of soil EC to the increase in soil Cd concentration with MAP-RG addition can be considered negligible.

2.5 Summary and Conclusions

Contrary to expectations, addition of MAP-RG did not significantly increase shoot or root Cd concentration in canola, durum wheat or flax. In fact, shoot and root Cd concentrations in canola were significantly lower when MAP-RG was added than when not added. The reduction of shoot Cd concentration with MAP-RG application in canola in this study may be due to the increase of plant dry matter and subsequent dilution effect of Cd inside the plant. The lack of a significant increase in plant Cd concentration in response to MAP-RG addition was probably due to the lack of effect of MAP-RG on soil solution Cd concentration and DTPA extractable soil Cd concentration. In turn, the lack of marked decrease in soil pH and an increase in soil EC with MAP-RG addition could be the main reason for the lack of increase in soil solution and soil extractable Cd concentration. Mycorrhizal root colonization percentage decreased with MAP-RG

addition. However, the changes in AMF colonization percentage did not affect Cd concentrations in durum wheat or flax.

Application of MAP-RG significantly decreased shoot Zn concentration in canola and durum wheat and for durum wheat, the decrease may be due to the interference of P for Zn translocation from root to shoot. However, the decrease in shoot Zn concentration did not affect shoot Cd concentration.

Application of MAP-RG significantly increased shoot Cd accumulation in durum wheat but not in flax or canola. The increase in shoot Cd accumulation in durum wheat appeared be driven primarily by increased dry matter production because MAP-RG addition substantially increased shoot dry weight in durum wheat.

Overall, the observations in this study indicate that the application of MAP at rates within the typical range of agricultural application may not significantly increase plant available soil Cd concentration and plant Cd uptake due to fertilizer induced changes in soil and plant, especially in situations where soil pH and EC remain relatively stable.

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3. PRECEDING CROP EFFECT ON SUBSEQUENT CROP CADMIUM UPTAKE

3.1 ABSTRACT

Cadmium is a potentially toxic trace element and food is one of the main ways of exposing humans to Cd. Several types of preceding crops in a crop rotation have been shown to increase Cd uptake in the subsequent crop. Therefore, a growth chamber study was conducted to understand how: i) canola or barley grown soil; ii) addition of canola residue or barley residue or no crop residue affect Cd uptake in the subsequent crops of durum wheat and flax in a crop rotation.

Shoot Cd concentrations of durum wheat and flax and shoot Cd accumulation of durum wheat were 12% and 27% greater, respectively for canola grown soil than for barley grown soil regardless of what type, if any, crop residue was added. Root Cd concentration of durum wheat and flax was also generally greater for canola grown soil than for barley grown soil. The increased uptake of Cd for canola grown soil was probably due to greater plant availability of Cd in soil. For durum wheat, the increased uptake of Cd could also be due to the decrease in shoot Zn concentration when grown on canola soil. However, the increased uptake of Cd for canola grown soil was not due to the differences in colonization by arbuscular mycorrhizal fungi. Conversely, crop residue addition, by itself did not influence Cd uptake in durum wheat or flax.

3.2 Introduction

Cadmium (Cd) is a potentially toxic trace element. Cadmium is present naturally in soil and also added by anthropogenic activities (Alloway and Steinnes, 1999). Food crops accumulate cadmium from soil (Kuboi et al., 1986). Long term exposure to food containing high Cd concentration accumulates Cd inside the human body and leads to a chronic Cd toxicity, causing renal failures and osteoporosis (Ryan et al., 1982; Alloway and Steinnes, 1999; Nordberg et al., 2002; Dong et al., 2007). The maximum permitted concentration of Cd in wheat is 0.2 mg kg^{-1} in international trade (Codex Alimentarius Commission of FAO/WHO, 2009).

Several types of preceding crops in a crop rotation can increase Cd uptake in the subsequent crop. Wheat grown after lupin had significantly greater grain Cd concentration than wheat grown after wheat and wheat grown after wheat had greater grain Cd concentration than wheat grown after barley in a study conducted in Australia (Oliver et al., 1993). Tobacco grown after a fallow period produced greater shoot Cd concentration than tobacco grown after maize or tobacco in an experiment conducted in France (Mench, 1998). In Manitoba, greater Cd concentration was observed in flax seed grown after canola than after durum wheat (Grant, 2003). In Iran, wheat grown after cotton contained significantly more Cd concentration in the shoot and grain than wheat grown after sunflower (Khoshgoftarmanesh and Chaney, 2007). In a growth chamber study conducted in Manitoba, durum wheat had a greater shoot Cd concentration with canola grown soil than with barley grown soil even though there was no difference in flax shoot Cd concentration between canola grown soil and barley grown soil (Eastley, 2008). A field study conducted at Brandon, Manitoba, however, did not show a difference in

durum wheat grain Cd concentration between preceding crops of canola and flax (Gao et al., 2010)

A preceding crop's influence on subsequent crop Cd uptake may be due to lasting alterations in soil chemical properties (Oliver et al., 1993; Khoshgoftarmanesh and Chaney, 2007). Soil receives large quantities of root exudates (rhizodeposits) during plant growth. The root exudation of protons and organic acids can reduce soil pH and can subsequently increase trace element availability and plant uptake (Marschner, 1998; Clemens et al., 2002; Dakora and Phillips, 2002; Gunes et al., 2007). Plants also secrete organic ligands into the rhizosphere for metal chelation. These phytometallophores have a high affinity for plant uptake. For example, the phytosiderophores are a well known group of phytometallophores which are secreted for iron chelation under iron deficiency conditions. Phytosiderophores have an ability to increase availability of several other trace elements such as Mn, Cu and Zn (Marschner 1995; Dakora and Phillips, 2002). The phytosiderophores may have an ability to increase Cd plant availability, as well (Grant et al., 1998). For example, greater grain Cd concentration in subsequent wheat after lupin than after a cereal crop as a preceding crop may be due to the soil acidification effect of lupin. It may also be due to the enhanced root exudation of organic chelates by lupin which form soluble complexes with Cd, increasing its availability (Oliver et al., 1993).

The influence of a preceding crop on Cd uptake by the subsequent crop may also be due to depletion of plant available soil Cd pool by the preceding crop's growth. For example, Khoshgoftarmanesh and Chaney (2007) reported that Cd uptake by sunflower is greater than by cotton. As a result, the plant available pool of Cd in soil was smaller after sunflower was grown than after cotton was grown. Grain Cd concentration in wheat following sunflower was lower than in wheat following cotton, probably due to the

reduced plant available pool of Cd in sunflower grown soil compared to cotton grown soil.

Mycorrhizae form a symbiotic association with living plant roots, providing essential mineral nutrients required by the plant and receiving photosynthates, in return (Smith and Read, 1997). Therefore, the preceding crop's influence on subsequent crop Cd uptake may also be due to changes in subsequent crop AMF root colonization.

Durum wheat and flax are mycorrhizal host crops and, in contrast, canola is a non-mycorrhizal host crop (Gao et al., 2010). A mycorrhizal preceding crop may produce greater mycorrhizal root colonization in the subsequent crop than a non-mycorrhizal preceding crop (Harinikumar and Bagyaraj, 1988; Gavito and Miller, 1998; Arihara and Karasawa, 2000; Gao et al., 2010). The inoculum potential of AMF fungi (i.e., hyphal density and spore count in soil) increases when a mycorrhizal host crop is growing and that may increase AMF colonization percentage in the subsequent crop. Conversely, the inoculum potential of AMF decreases when a non-mycorrhizal host crop is growing and that may reduce AMF colonization percentage in the subsequent crop (Lu and Miller, 1989; Kabir et al., 1998). In a field study conducted in India, AMF root colonization and AMF spore counts in soil in a subsequent crop of cowpea (a mycorrhizal crop) were significantly smaller when the preceding crop was fallow or mustard (a non-mycorrhizal crop) than when it was cowpea (Harinikumar and Bagyaray, 1988). The AMF root colonization of maize was greater when preceding crop was mycorrhizal (sunflower, maize, soybean, potato and wheat) than when non-mycorrhizal (sugar beet and rape) in a field study in Japan (Arihara and Karasawa, 2000). Similarly, in Ontario, the AMF colonization of maize was delayed when canola, instead of maize, was the preceding crop (Gavito and Miller, 1998). In Manitoba, the arbuscular colonization of durum wheat was

61% and 56% greater when the preceding crop was flax, instead of canola in the second and third years of a field study, respectively (Gao et al., 2010).

Mycorrhizae can reduce the plant uptake of Cd (Galli et al., 1993; Galli et al., 1994; Weissenhorn et al., 1995; Guo et al., 1996; Smith and Read, 1997; Chen et al., 2004) and as a result, a preceding non-mycorrhizal crop may increase Cd uptake in a subsequent mycorrhizal crop, by decreasing the percentage of AMF root colonization in the subsequent crop. However, it should also be noted that AMF can also increase the plant uptake of Cd (Guo et al., 1996; Smith and Read, 1997; Chen et al., 2004). Therefore, the effect of AMF root colonization on the process of plant Cd uptake is specific to soil, plant and environmental conditions (Hetrick et al., 1994; Weissenhorn et al., 1995; Smith and Read, 1997; Ryan and Angus, 2003; Chen et al., 2004; Gohre and Paszkow, 2006; Wong et al., 2007; Ryan et al., 2008; Gao et al., 2010).

Crop residue management is another important factor affecting Cd availability for the subsequent crop in a crop rotation. The recycling of crop residue adds Cd back into agricultural soils. The amount of Cd addition increases with increasing Cd concentration in residue and the quantity of residue. The decomposition of crop residue releases Cd into soil, making Cd plant available (Grant et al., 1999). Crop residue decomposition also releases organic acids and protons and that have the potential to reduce soil pH (He and Singh, 1993b) and it is well known that soil pH is often inversely related to plant available soil Cd concentration and plant Cd uptake (Christensen, 1984; Levi-Minzi and Petruzzelli, 1984; Grant et al., 1998; Sauve et al., 2000; Grant and Sheppard, 2008). Over the long term, the soil organic matter formed from crop residue has the ability to form organic-metal complexes with Cd. Some of these complexes can increase Cd availability

for plants, while some others reduce it (Hinesly et al, 1982; Korcak and Fanning, 1985; He and Singh, 1993a; Grant et al., 1999; Grant and Sheppard, 2008).

Researchers have measured soil Cd concentration and plant Cd uptake in response to addition of various types of organic amendments; yet, there are few studies on crop residue addition. Also, the effects of organic matter incorporation on soil and plant Cd concentration are not consistent. In a study conducted in Norway, the incorporation of cow manure, hog manure and peat into an agricultural soil naturally high in Cd significantly decreased DTPA extractable soil Cd concentration, irrespective of its source (Narwal and Singh, 1997). Similarly, the incorporation of peat decreased NH_4OAc extractable Cd concentration in a clay and a loamy sand soil in Uppsala, Sweden (Eriksson, 1988). In contrast, the incorporation of peat into sand, sandy loam and clay soil increased NH_4NO_3 and DTPA extractable soil Cd concentration in a study conducted in Norway (He and Singh, 1993a). In another study, He and Singh (1993b) did not find a relationship between Cd extracted (by NH_4NO_3 and DTPA) and organic matter added to soil. In terms of plant Cd uptake, a reduction in tissue Cd concentration was observed with farm yard manure incorporation in long term trials at Rothamsted, UK (Jones and Johnston, 1989). Soil incorporation of peat decreased rye grass Cd concentration in a study in Norway (He and Singh, 1993a). Shoot Cd concentration of maize was decreased with sludge application in a greenhouse study conducted with Maryland soils in USA (Korcak and Fanning, 1985). In a growth chamber study conducted in Manitoba, flax shoot Cd concentration was greater when canola residue and barley residue were incorporated than when no residue was incorporated (Eastley, 2008). However, there were no differences in durum wheat shoot Cd concentration among addition of barley residue, canola residue and no residue.

The Cd concentrations of durum wheat and flax have the potential to be influenced by the preceding crop of rotation; however, the mechanism is not certain. Therefore, the objective of the current study was to understand the mechanisms responsible for differences in Cd uptake of durum wheat and flax when grown after canola and barley. Consequently, crop rotation management practices can be developed in order to reduce Cd concentration in durum wheat and flax. Two soils from preceding crops (a non-mycorrhizal canola and mycorrhizal barley grown soil) and three types of crop residue additions (canola residue addition, barley residue addition and no residue addition) were assessed for their effect on the uptake of Cd by subsequent durum wheat and flax test crops.

3.3 Materials and Methods

The study was conducted in growth chambers at the Dept. of Soil Science, University of Manitoba. Surface soil was collected from the Agriculture Agri-Food Canada Brandon Research Station (Philip's research farm, 50" 01' 17.04 N 49" 53' 2.88 W). Soil was collected from 0-15 cm depth of a Newdale clay loam soil (Orthic Black Chernozem) that had grown barley as the previous crop. Prior to the experiment, soil was extracted with KCl for NH_4^+-N and NO_3^--N, with $NaHCO_3$ (Olsen-P) for P and with DTPA at pH 7 for Cd and Zn. Soil was digested with $HNO_3/HClO_4$ to determine total soil Cd and Zn. Soil pH and EC were measured with a soil: deionized water ratio of 1:2 w: w (Carter et al., 1993). Extractable NO_3^--N and NH_4^+-N were quantified with a Technicon II Auto analyzer by automated cadmium reduction and automated phenate method, respectively. Extractable P was quantified colorimetrically by molybdate blue colour

method at 880 nm (Carter et al., 1993) with a spectrophotometer (Ultrospec 2100 pro). Extractable Cd and Zn were quantified with Inductively Coupled Plasma (Perkin Elmer 5300 DV). Soil pH and EC were measured using an Accumet AB 15 pH meter with a Ross "Sure Flow" electrode and an Accumet AB 30 conductivity meter, respectively. Soil physical and chemical characteristics are described in Table 3.1. Container moisture capacity was determined by the pill bottle method (Eastley, 2008). A known weight of soil was packed into a series of transparent pill bottles containing drainage holes at bottom mimicking the soil bulk density in larger containers and watered with a range of water volumes covering a range of moisture capacities. The minimum soil moisture content that allowed the wetting front to move to the bottom of the bottle after 24 hours was considered as the container moisture capacity for the study soil.

Table 3.1 Physical and chemical characteristics of experimental soil

Characteristic	
Soil texture	Clay loam
Soil organic matter (%)	4.3
CEC (cmol+ kg^{-1})	31
pH	7.5
EC (mS cm^{-1})	0.42
KCl extractable NH_4^+-N (mg kg^{-1})	2.9
KCl extractable NO_3^--N (mg kg^{-1})	4.7
$NaHCO_3$ extractable P (mg kg^{-1})	17
DTPA extractable Cd (mg kg^{-1})	0.13
DTPA extractable Zn (mg kg^{-1})	1.9
Total Cd (mg kg^{-1})	0.39
Total Zn (mg kg^{-1})	74

3.3.1 Preceding crop

Soil was air dried and sieved with a 1 cm mesh screen and filled into pots. Each pot contained 5 kg of soil and was sealed at the bottom to prevent drainage. One Rhizon soil moisture sampler was buried into each pot to collect soil solution samples. This device consists of a 10 cm long hydrophilic polymer head and a PVC tube attached to the head on one end and with a cap at free end. Soil solution enters through the hydrophilic polymer head once buried in soil and it is collected at the free end of the PVC tube. Each Rhizon sampler was installed, placing the hydrophilic polymer head in the middle of the soil column, allowing the attached PVC tube and cap to extend out of the soil.

Nitrogen (150 mg kg^{-1} soil as urea), phosphorus (5 mg kg^{-1} soil as reagent grade mono-ammonium phosphate), potassium and sulphur (50 mg kg^{-1} as potassium sulphate) were added to the soil. Fertilizer was dissolved in deionized water and added to each pot as a solution. Pots were watered up to container moisture capacity. Six seeds of canola (*Brassica napus* L, variety Invigor 5440) or ten seeds of barley (*Hordeum vulgare,* variety AC Ranger) were planted in each pot. Excess plants were thinned after emergence, leaving three plants of canola and five plants of barley per pot. Sixteen hours light at the temperature of 22 °C and 8 hours dark at the temperature of 15 °C were provided to represent day and night cycles, respectively. Light intensity at the top of the canopy was approximately 762 µmol S^{-1} m^{-2}. Humidity was maintained at 50%. Pots were watered with deionized water to container moisture capacity when the soil moisture dropped below 60% of the estimated container moisture capacity. Pots were re-randomized inside the growth chamber weekly.

Soil solution was collected from each pot at weekly intervals, starting from the second week of emergence using the Rhizon soil moisture samplers installed. Pots were watered in evening up to container moisture capacity and the soil solution was collected the next morning, two hours after growth chamber lights were turned on. A syringe was used to draw approximately 10 mL of soil solution out from soil. Soil solution pH and EC were measured. The concentrations of Cd, Zn and P in soil solution were determined using Inductively Coupled Plasma Mass Spectroscopy (Varian 820). Nitrogen was applied at 50 mg kg^{-1} soil as urea at the fourth week after planting, as a solution dissolved in deionized water, soon after soil solution sampling. Nitrogen was added to avoid any N limitation.

Plant shoots were clipped at the soil level 50 days after emergence. Root material was left with the soil in each pot. Shoot material was oven dried at 60^{o}C, until moisture was constant. Shoot biomass was measured, recorded, ground and Cd, Zn and P concentrations were determined with Inductively Coupled Plasma (Perkin Elmer 5300 DV) after a nitric-perchloric acid digestion. After shoots were clipped, subsamples of soil were collected immediately, air dried, ground and analyzed for $NaHCO_3$-P, DTPA-Cd and Zn, pH and EC. Analytical procedures were similar to those reported earlier. A hand auger was driven through the soil column in each pot and a small portion of fresh root was collected with soil immediately before the plants were clipped. Fresh roots were washed with water, cleared with 10% KOH and stained with 0.05 % chlorazol black E. The percentage of AMF root colonization was assessed by the magnified intersections method (McGonigle et al., 1990).

3.3.2 Test crop

Canola and barley grown pots of soil were kept separate and the soil in each pot was mixed. Roots were cut using stainless steel scissors, to allow uniform mixing when needed. Dried shoot material of canola and barley was cut into 2 cm pieces. Crop residue from each pot was mixed with soil that had grown either canola or barley as the preceding crop. In addition, each type of soil included a control where no residue was added. Total shoot biomass produced in a pot was mixed into the soil of a pot to maintain a coherent system as possible. As result, preceding crop grown soil and crop residue combinations were:

Barley residue into barley grown soil

Barley residue into canola grown soil

No residue addition to barley grown soil

Canola residue into canola grown soil

Canola residue into barley grown soil

No residue addition to canola grown soil

Pots were incubated for seven weeks in darkness at 15°C to stimulate crop residue decomposition. Humidity was maintained at 50%. We assumed that this period of crop residue decomposition was equivalent to approximately about 4 weeks after harvest in fall before soil freeze up and about 3 weeks in spring before cropping is started. Pots were watered with deionised water up to estimated container moisture capacity, when soil moisture level dropped below 60% of the estimated container moisture capacity. At the end of the seven week incubation, soil in each pot was taken out separately, mixed

thoroughly and refilled. A Rhizon soil moisture sampler was installed in the middle of each pot. Nitrogen (150 mg kg^{-1} soil as urea) and P (5 mg kg^{-1} soil as reagent grade mono-ammonium phosphate) were added into each pot as a solution dissolved in deionised water. Pots were watered up to container moisture capacity. Two test crops, durum wheat and flax were grown on the combination of preceding crop grown soil and crop residue treatments mentioned above. Each treatment was replicated four times. Ten seeds of durum wheat (*Triticum turgidum* L. variety AC Avonlea) or twelve seeds of flax (*Linum usitatissimum* L. variety Bethune) were planted in each pot. Excess plants were thinned after emergence, leaving five plants of durum wheat or six plants of flax per pot. Sixteen hours light at the temperature of 22 ºC was provided to represent day and 8 hours darkness at the temperature of 15 ºC was provided to represent night. Light intensity at the top of the canopy was approximately 762 µmol S^{-1} m^{-2}. Humidity was maintained at 50%. The soil solution was collected at a weekly interval starting from the second week of plant emergence. Method of soil solution collection and analytical procedures were similar to that reported for the preceding crop. Additional nitrogen was added at the fourth week of growth (50 mg kg^{-1} soil as urea) to prevent nitrogen deficiencies.

Test crop plants were uprooted after 50 days of growth. Soil was analyzed for DTPA-Cd and Zn, $NaHCO_3$-P, pH and EC. Prior to uprooting the plants, fresh root samples were collected and assessed for AMF root colonization. Plant shoot and root biomass per pot were recorded and Cd, Zn and P concentrations were determined. Sampling and analytical procedures were similar to that reported for the preceding crop.

The entire experiment was repeated to create two separate runs. The accuracy of all plant and soil analyses was assessed by the inclusion of standard reference materials and analytical values matched the stated ranges of standard materials. Data analysis was

conducted using SAS 9.1 statistical package. Proc mixed ANOVA was performed after satisfying the assumptions underlying ANOVA (i.e., residuals were normally distributed; residuals had similar variance across the range of data and the residuals had means close to zero and they were uncorrelated). Significant differences between means were assessed using Tukey mean comparisons. Data from two runs were combined when each run was not significantly different from the other.

3.4 Results

3.4.1 Preceding crop

Shoot dry weight was significantly greater in barley than canola (Table 3.2). Barley was mycorrhizal and canola was non-mycorrhizal (Table 3.2). However, the degree of mycorrhizal colonization in barley was lower than expected, perhaps due to intensive disturbance of the soil during the set up for the experiment. Shoot Cd concentration and accumulation were significantly greater in canola than barley (Table 3.3). In contrast, the shoot concentration and accumulation of P were significantly greater in barley than canola. Although shoot Zn concentration was greater in canola than in barley, accumulation of Zn was not significantly different between the two test crops. In contrast, extractable soil Cd concentration and soil pH were not significantly different between canola grown soil and barley grown soil (Table 3.4). However, the soil Cd concentration after canola showed a strong tendency ($P = 0.0554$) to be greater than after barley. Soil Zn concentration and soil P concentration were significantly greater and soil EC was significantly lower in canola grown soil than barley grown soil.

Table 3.2 Shoot and root dry weight and arbuscular mycorrhizal fungi (AMF) root colonization of preceding crop

	Dry weight	AMF	
Preceding crop	Shoot	AC†	TC‡
	g pot^{-1}	——	% ——
Barley	29.6	3.08	7.92
Canola	22.5	n/a§	n/a

ANOVA	df	P>F
Treatment	1	<0.0001
MSE		11.3

† Arbuscular colonization is the percentage of root length colonized by arbuscules

‡ Total colonization is the sum of arbuscular colonization and hyphal colonization (where hyphal colonization is the percentage of root length colonized by AMF hyphae)

§ Canola was confirmed as non-mycorrhizal by analysis of random root samples

Table 3.3 Concentration and accumulation of Cd, Zn and P in preceding crop

		Concentration			Accumulation†		
Preceding crop		Shoot Cd	Shoot Zn	Shoot P	Shoot Cd	Shoot Zn	Shoot P
		——	mg kg^{-1}	——	µg pot^{-1}	——	mg kg^{-1}——
Barley		0.18	15.3	2340	5.13	0.42	62.2
Canola		0.43	16.8	2020	9.68	0.38	45.8

ANOVA	df	P>F					
Treatment	1	<0.0001	0.0276	0.0032	<0.0001	NS‡	<0.0001
MSE		0.002	3.47	51699	2.87	0.006	53.8

† Accumulation = concentration * dry weight per pot

‡ NS, not significantly different (P > 0.05)

Table 3.4 Concentration of DTPA extractable Cd, Zn and $NaHCO_3$ extractable P, pH and EC in soil after the preceding crop

Preceding crop	Cd	Zn	P	pH	EC
	$\mu g\ kg^{-1}$	—— $mg\ kg^{-1}$ ——			$\mu S\ cm^{-1}$
Barley	120	1.29	17.5	7.58	347
Canola	122	1.34	20.1	7.56	279

ANOVA	df	P>F				
Treatment	1	NS(0.055)	0.0279	<0.0001	NS†	<0.0001
MSE		28.1	0.02	12.14	0.011	3207

† NS, not significantly different (P > 0.05)

3.4.2 Dry matter yield and arbuscular mycorrhizal fungi (AMF) root colonization of durum wheat and flax test crops

Durum wheat produced greater shoot dry weight than flax on both soils. However, the dry matter yield advantage for durum wheat over flax was greater on canola grown soil than on barley grown soil, resulting in a significant test crop by preceding crop grown soil interaction (Table 3.5). When grown on barley grown soil, the root dry weight of flax was greater when either barley residue or canola residue was added than when no residue was added (Table 3.5). In contrast, when on canola grown soil, there were no significant differences in flax root dry weight among canola residue, barley residue and no residue addition. Conversely, the root dry weights of durum wheat were not significantly different among soils or residues, resulting in a significant test crop by preceding crop grown soil by crop residue interaction.

Table 3.5 Shoot and root dry weight and arbuscular mycorrhizal fungi (AMF) root colonization of durum wheat and flax test crops

Treatment				Dry weight		AMF	
				Shoot	Root	AC†	TC‡
				g pot^{-1}		%	
Test crop	Durum wheat (D)			23.5	6.53	2.75	9.83
	Flax (F)			15.5	5.80	3.06	10.6
Soil	Barley grown soil (BS)			19.6	6.16	2.81	10.4
	Canola grown soil (CS)			19.5	6.18	3.00	10.0
Residue	Barley residue (BR)			19.9	6.25	2.94	10.3
	Canola residue (CR)			19.0	6.18	2.97	10.2
	No residue (NR)			19.8	6.08	2.81	10.1
Test crop × Soil	D		BS	22.7a§	6.41		
	D		CS	24.3a	6.67		
	F		BS	16.5b	5.91		
	F		CS	14.6b	5.70		
Test crop× Residue	D		BR		6.50		
	D		CR		6.57		
	D		NR		6.54		
	F		BR		6.00		
	F		CR		5.79		
	F		NR		5.63		
Soil × Residue	BS		BR		6.25		
	BS		CR		6.26		
	BS		NR		5.97		
	CS		BR		6.25		
	CS		CR		6.10		
	CS		NR		6.20		
Test crop × Soil	D	BS	BR		6.28abc		
× Residue	D	BS	CR		6.49ab		
	D	BS	NR		6.45ab		
	F	BS	BR		6.22abc		
	F	BS	CR		6.02bc		
	F	BS	NR		5.49d		
	D	CS	BR		6.72a		
	D	CS	CR		6.65ab		
	D	CS	NR		6.64ab		
	F	CS	BR		5.79cd		
	F	CS	CR		5.55d		
	F	CS	NR		5.77cd		
ANOVA		df		P>F			
Test crop		1		<0.0001	0.0002	NS(0.08)	NS
Soil		1		NS¶	NS	NS	NS
Residue		2		NS	NS	NS	NS
Test crop × Soil		2		0.0123	<.0001	NS	NS
Test crop × Residue		2		NS	0.0092	NS	NS
Soil × Residue		2		NS	0.0212	NS	NS
Test crop × Soil × Residue		2		NS	0.0023	NS	NS
MSE				2.25	0.15	0.82	5.67

† Arbuscular colonization is the percentage of root length colonized by arbuscules

‡ Total colonization is the sum of arbuscular colonization and hyphal colonization (where hyphal colonization is the percentage of root length colonized by AMF hyphae)

§ Within columns, means followed by the same letter are not significantly different according to the Tukey multiple comparison test ($p > 0.05$)

¶ NS, not significantly different ($p > 0.05$)

The AMF root colonization of flax was not significantly different from the AMF root colonization of durum wheat (Table 3.5). Also, the root colonization of durum wheat and flax was not significantly different between barley grown soil and canola grown soil. Similarly, the root colonization of durum wheat and flax was not significantly different among barley residue addition, canola residue addition and no residue addition.

3.4.3 Concentrations of Cd, Zn and P in durum wheat and flax test crops

Flax had significantly greater shoot Cd concentration than durum wheat (Table 3.6). The shoot Cd concentrations of durum wheat and flax were 12% greater when on canola grown soil than when on barley grown soil, regardless of residue treatment. However, the shoot Cd concentrations of durum wheat and flax were not affected by crop residue addition.

In contrast to shoot Cd concentration, the root Cd concentration of durum wheat was greater than the root Cd concentration of flax (Table 3.6). Root Cd concentration was generally greater for test crops on canola grown soil than on barley grown soil. When canola residue was added, the root Cd concentrations of test crops were significantly greater with canola grown soil than with barley grown soil but when barley residue or no residue was added there were no significant differences in the root Cd concentrations of the test crops between the two soils, resulting in a significant preceding crop grown soil by crop residue interaction. Furthermore, when on barley grown soil, root Cd concentrations of test crops were significantly greater when barley residue was added than when canola residue was added. However, when on canola grown soil, the root Cd concentrations of test crops were not affected by crop residue addition.

Table 3.6 Concentrations of Cd, Zn and P in durum wheat and flax test crops

Treatment				Shoot Cd	Root Cd	Shoot Zn	Root Zn	Shoot P	Root P
				mg kg^{-1}					
Test crop	Durum wheat (D)			0.34b†	0.94a	21.0	43.0	2071	1418
	Flax (F)			0.55a	0.79b	14.6	50.1	2075	1433
Soil	Barley grown soil (BS)			0.42b	0.83	18.7	45.3b	2070	1447
	Canola grown soil (CS)			0.47a	0.88	16.9	48.2a	2076	1405
Residue	Barley residue (BR)			0.44	0.90	18.1	47.1	2118	1607
	Canola residue (CR)			0.44	0.81	18.6	47.2	2096	1409
	No residue (NR)			0.45	0.85	16.7	46.1	2005	1261
Test crop × Soil	D		BS			23.1a			1517a
	D		CS			18.9b			1318c
	F		BS			14.8c			1375bc
	F		CS			14.4c			1490ab
Test crop × Residue	D		BR				40.4c		1636a
	D		CR				44.9bc		1354cd
	D		NR				43.8bc		1263d
	F		BR				53.8a		1578ab
	F		CR				49.5ab		1463bc
	F		NR				48.3ab		1258d
Soil × Residue	BS		BR		0.88a				1681a
	BS		CR		0.77b				1414bc
	BS		NR		0.85ab				1244d
	CS		BR		0.92a				1533ab
	CS		CR		0.90a				1404bc
	CS		NR		0.86ab				1277cd
Test crop × Soil	D	BS	BR						1833a
× Residue	D	BS	CR						1450bcd
	D	BS	NR						1268d
	F	BS	BR						1528bc
	F	BS	CR						1379bcd
	F	BS	NR						1219d
	D	CS	BR						1438bcd
	D	CS	CR						1259d
	D	CS	NR						1257d
	F	CS	BR						1628ab
	F	CS	CR						1546bc
	F	CS	NR						1296cd
ANOVA		df		P>F					
Test crop		1		<0.0001	0.0051	<0.0001	0.0027	NS	NS
Soil		1		0.0409	0.0028	0.0062	0.0366	NS	NS
Residue		2		NS‡	0.0436	NS(0.07)	NS	NS	<0.0001
Test crop × Soil		2		NS	NS	<0.0001	NS(0.08)	NS	<0.0001
Test crop × Residue		2		NS	NS	NS	0.0153	NS	NS
Soil × Residue		2		NS	0.0353	NS	NS	NS	NS(0.06)
Test crop × Soil × Residue		2		NS	NS	NS	NS	NS	0.0303
MSE				0.003	0.007	0.896	22.4	51699	23652

† Within columns, means followed by the same letter are not significantly different according to the Tukey multiple comparison test ($p > 0.05$)

‡ NS, not significantly different ($p > 0.05$)

The shoot Zn concentration of durum wheat was significantly greater than the shoot Zn concentration of flax (Table 3.6). The shoot Zn concentration of durum wheat was significantly greater for barley grown soil than for canola grown soil. However, the shoot Zn concentration of flax was not significantly different between canola grown soil and barley grown soil, resulting in a significant test crop by preceding crop grown soil interaction. Shoot Zn concentrations were not affected by crop residue treatments.

The root Zn concentration of durum wheat was significantly greater than the root Zn concentration of flax when barley residue was added but there was no such difference between durum wheat and flax when canola residue or no residue was added, resulting in a significant test crop by crop residue interaction (Table 3.6). The root Zn concentrations of durum wheat and flax were significantly greater for canola grown soil than for barley grown soil. However, the root Zn concentrations of durum wheat and flax were not significantly different among the three types of crop residue additions.

Shoot P concentrations were not affected by preceding crop grown soil or crop residue addition. Conversely, the root P concentrations were generally highest for durum wheat and flax grown on soil where barley residue was added, followed by canola residue and no residue, in declining order of concentration (Table 3.6). However, the root P concentration of durum wheat was not affected by residue addition when on canola grown soil, resulting in a significant test crop by preceding crop grown soil by crop residue interaction.

3.4.4 Accumulations of Cd, Zn and P in durum wheat and flax test crops

The shoot Cd accumulation of durum wheat was significantly greater for canola grown soil than for barley grown soil (Table 3.7). However, the shoot Cd accumulation of flax was not significantly different between canola grown soil and barley grown soil, resulting in a significant test crop by soil interaction. In flax, root Cd accumulation was significantly greater when barley residue was added than when no crop residue was added, but in durum wheat, root Cd accumulation was not affected by crop residue treatments, resulting in a significant test crop by crop residue interaction (Table 3.7). Also, durum wheat had significantly greater root Cd accumulation than flax when canola residue or no residue was added, but not when barley residue was added.

Shoot Zn accumulation was greater in durum wheat than flax (Table 3.7). In both test crops, shoot Zn accumulations were significantly greater for barley grown soil than for canola grown soil and when canola residue was added than when no residue was added. Root Zn accumulation in flax was significantly greater when barley residue was added than when no residue was added (Table 3.7). However, the root Zn accumulation of durum wheat was not affected by crop residue addition, creating a significant interaction between test crop and crop residue.

Table 3.7 Accumulations† of Cd, Zn and P in durum wheat and flax test crops

Treatment			Shoot Cd	Root Cd	Shoot Zn	Root Zn	Shoot P	Root P
			—— µg pot^{-1} ——		———— mg pot^{-1} ————			
Test crop	Durum wheat (D)		7.86	6.15	0.49a‡	0.29	47.7	9.25
	Flax (F)		8.59	4.68	0.22b	0.29	32.0	8.35
Soil	Barley grown soil (BS)		7.89	5.26	0.38a	0.29	40.4	8.93
	Canola grown soil (CS)		8.55	5.57	0.33b	0.30	39.4	8.67
Residue	Barley residue (BR)		8.44	5.75	0.36ab	0.30	41.8	10.0
	Canola residue (CR)		7.97	5.19	0.37a	0.29	39.8	8.68
	No residue (NR)		8.26	5.31	0.33b	0.28	38.0	7.71
Test crop × Soil	D	BS	6.93b					9.70a
	D	CS	8.79a					8.80b
	F	BS	8.86ab					8.16b
	F	CS	8.31ab					8.54b
Test crop × Residue	D	BR		6.22a		0.29ab	49.8a	
	D	CR		5.88a		0.30ab	49.7a	
	D	NR		6.36a		0.29ab	43.7b	
	F	BR		5.28ab		0.32a	33.8c	
	F	CR		4.49bc		0.28ab	29.8c	
	F	NR		4.27c		0.27b	32.3c	
Soil × Residue	BS	BR						10.5a
	BS	CR						8.87bc
	BS	NR						7.43d
	CS	BR						9.53ab
	CS	CR						8.49bcd
	CS	NR						7.98cd

ANOVA	df				P>F		
Test crop	1	NS§	0.0056	<0.0001	NS	<0.0001	0.0026
Soil	1	0.0274	NS(0.08)	0.0034	NS	NS	NS
Residue	2	NS	0.0332	0.0156	NS	0.0049	<0.0001
Test crop × Soil	2	0.0004	NS	NS	NS	NS	0.0049
Test crop × Residue	2	NS	0.0108	NS	0.0256	0.0018	NS
Soil × Residue	2	NS	NS	NS	NS	NS	0.0226
Test crop × Soil × Residue	2	NS	NS	NS	NS	NS	NS
MSE		0.803	0.76	0.002	0.002	0.973	0.539

† Accumulation = concentration * dry weight per pot

‡ Within columns, means followed by the same letter are not significantly different according to the Tukey multiple comparison test ($p > 0.05$)

§ NS, not significantly different ($p > 0.05$)

Durum wheat had significantly greater shoot P accumulation than flax (Table 3.7). However, in durum wheat, shoot P accumulation was significantly greater when barley residue or canola residue was added than when no residue was added but the shoot P accumulation of flax was not affected by crop residue treatments, resulting in a significant test crop by crop residue interaction.

In durum wheat, root P accumulation was significantly greater for barley grown soil than for canola grown soil but the root P accumulation of flax was unaffected by the soil's cropping history, resulting in a significant test crop by preceding crop grown soil interaction (Table 3.7). Also, for barley grown soil, durum wheat had significantly greater root P accumulation than flax. However, for canola grown soil, there was no difference in root P accumulation between durum wheat and flax.

Root P accumulations of durum wheat and flax were generally greatest for barley residue, followed by canola residue and no residue, respectively (Table 3.7). However, for canola grown soil, the effects of residue treatment were more subtle than for barley grown soil and the only significant difference in root P accumulation was between barley residue and no residue. Therefore, there was a significant test crop grown soil by crop residue interaction.

3.4.5 Extractable Cd, Zn and P concentrations in soil, soil pH and soil EC in test crop soil after 50 days of growth

Crop residue and the soil from the preceding crop had no effect on extractable Cd in soil after the test crops were harvested (Table 3.8). For canola grown soil, the soil Cd concentration after flax was significantly greater than the soil Cd concentration after durum wheat. However, there was no difference in soil Cd concentration between flax

and durum wheat, for barley grown soil, resulting in a significant test crop by preceding crop grown soil interaction.

The soil Zn concentrations after durum wheat and flax were significantly greater with barley residue addition than with canola residue addition or no residue addition (Table 3.8). Similar to soil Cd concentration, the soil Zn concentration of flax was significantly greater than the soil Zn concentration of durum wheat for canola grown soil. However, there was no difference in soil Zn concentration between flax and durum wheat, for barley grown soil, resulting in a significant test crop by preceding crop grown soil interaction.

Soil P concentration was significantly greater after flax than after durum wheat (Table 3.8). Soil P concentration was also significantly greater when barley residue was added than when canola residue or no residue was added.

Soil pH was generally lower after flax than after durum wheat (Table 3.8). However, the differences in soil pH were slightly greater for canola grown soil than for barley grown soil, resulting in a significant test crop by preceding crop grown soil interaction. Also, after durum wheat, the soil pH of barley and canola residue treatments was significantly lower than the soil pH where no residue was added, but in flax there were no significant differences in soil pH among crop residue treatments, resulting in a significant test crop by crop residue interaction.

After durum wheat, the soil EC where barley residue was added or where canola residue was added was significantly greater than the soil EC where no residue was added (Table 3.8). However, the soil EC after flax was not affected by residue treatment, resulting in a significant test crop by crop residue interaction.

Table 3.8 Concentrations of extractable Cd, Zn and P in test crop soil after 50 days of growth

Treatment			Cd	Zn	P	pH	EC
			$\mu g\ kg^{-1}$	—— $mg\ kg^{-1}$ ——			$\mu S\ cm^{-1}$
Test crop	Durum wheat (D)		141	1.09	14.4b†	7.69	631
	Flax (F)		141	1.13	17.2a	7.50	791
Soil	Barley grown soil (BS)		139	1.09	15.6	7.61	695
	Canola grown soil (CS)		143	1.13	16.0	7.58	726
Residue	Barley residue (BR)		144	1.16a	17.0a	7.55	766
	Canola residue (CR)		142	1.09b	15.5b	7.61	763
	No residue (NR)		137	1.07b	14.8b	7.63	604
Test crop × Soil	D	BS	142ab	1.09ab		7.69a	
	D	CS	139b	1.08b		7.70a	
	F	BS	137ab	1.09ab		7.53b	
	F	CS	146a	1.18a		7.47b	
Test crop × Residue	D	BR				7.62bc	717a
	D	CR				7.68b	731a
	D	NR				7.78a	443b
	F	BR				7.48d	815a
	F	CR				7.54cd	795a
	F	NR				7.48d	765a

ANOVA	df			P>F		
Test crop	1	NS‡	0.005	<0.0001	<.0001	0.0005
Soil	1	NS	NS	NS	NS	NS
Residue	2	NS	0.0021	<0.0001	0.0024	0.0002
Test crop × Soil	2	0.0013	0.0059	NS	0.0228	NS
Test crop × Residue	2	NS	NS	NS	<.0001	0.0035
Soil × Residue	2	NS	NS	NS	NS	NS
Test crop × Soil × Residue	2	NS	NS	NS	NS	NS
MSE		103	0.005	0.945	0.004	19429

† Within columns, means followed by the same letter are not significantly different according to the Tukey multiple comparison test ($p > 0.05$)

‡ NS, not significantly different ($p > 0.05$)

3.5 Discussion

3.5.1 Durum wheat and flax Cd uptake

Flax had significantly greater shoot Cd concentration than durum wheat, in agreement with previous studies (Jiao et al., 2004; Eastley, 2008). Conversely, durum wheat had significantly greater root Cd concentration than flax. Durum wheat's lower shoot Cd concentration and greater root Cd concentration than flax is evidence for the efficient Cd translocation barrier from root to shoot via xylem in durum wheat (Grant et al., 1998; Jiao et al., 2004). Genetic differences in Cd translocation pathways might be the reason for effective Cd movement control in durum wheat (a monocotyledonous species) than flax (a dicotyledonous species) (Jiao et al., 2004).

3.5.2 Preceding crop grown soil effect on the Cd uptake of durum wheat and flax

The shoot Cd concentrations of durum wheat and flax and the shoot Cd accumulation of durum wheat were significantly greater for canola grown soil than for barley grown soil, regardless of what type of crop residue, if any, was added. Similarly, root Cd concentration of test crops was generally greater for canola grown soil than for barley grown soil.

Similar increases in durum wheat shoot Cd concentrations after canola were observed in a growth chamber study conducted in Manitoba by Eastley (2008) and during the third year of a field study conducted near Brandon, Manitoba, by Gao et al. (2010) on Newdale clay loam soil (Orthic Black Chernozem), soil that is similar to ours. However, in contrast to the findings of this study, Eastley (2008) did not observe a difference in flax shoot Cd concentration between canola grown soil and barley grown soil. Furthermore,

Gao et al. (2010) did not observe a preceding crop (i.e., canola vs. flax) effect on the grain Cd concentration of subsequent durum wheat in the first two years of their field study.

Extractable soil Cd concentration of canola grown soil was not significantly different ($P > 0.05$) from that of barley grown soil at the end of the preceding crops or test crops. Therefore, the increase in shoot and root Cd concentration when test crops were grown on canola soil compared to barley soil cannot be attributed easily to differences in extractable soil Cd. However, at the end of the preceding crop, the extractable soil Cd concentration of canola grown soil was very close to being significantly greater than barley grown soil ($P = 0.0554$). Also, the soil Cd concentration at the end of preceding crop was modestly, but significantly correlated with shoot Cd concentration of durum wheat and flax ($r = 0.36$, $P = 0.0003$). Therefore, the greater concentration of shoot Cd in subsequent test crops on canola grown soil than on barley grown soil may be partly due to greater plant available Cd concentration soil. Although the DTPA soil test may not be a perfect predictor of plant available Cd, Norvell et al. (2000) observed a significant correlation between DTPA extractable soil Cd concentration and durum wheat grain Cd concentration ($r = 0.62$, $P < 0.01$) and Smilde et al. (1992) observed a linear positive relationship between $CaCl_2$-extractable soil Cd and spring wheat grain Cd concentration. Similarly, Khoshgoftarmanesh and Chaney (2007) hypothesized that the greater concentration of Cd in wheat grain after cotton than after sunflower was due to the greater concentration of extractable Cd in soil after cotton than after sunflower.

Durum wheat on canola grown soil had significantly lower shoot Zn concentration and accumulation and significantly greater shoot Cd concentration and accumulation than when it grew on barley grown soil. Furthermore, across all treatments, shoot Cd concentration was significantly and negatively correlated with shoot Zn concentration (r =

- 0.46, $P < 0.0001$). Therefore, higher shoot Cd concentration and accumulation in durum wheat for canola grown soil than for barley grown soil may also be due to decreases in shoot Zn concentration and accumulation. Cadmium and Zn compete with each other for root uptake and translocation inside plant and therefore a decrease in the concentration of Zn in plant can increase the concentration of Cd (Grant and Bailey, 1997; Grant et al., 1998; Grant et al., 2002; Jiao et al., 2004; Grant and Sheppard, 2008). Cadmium – zinc interactions were observed in durum wheat by Grant et al. (2002) in field studies conducted in Alberta and Manitoba. Accordingly, in a growth chamber study conducted at Brandon, Manitoba, Jiao et al. (2004) reported that 81% of variability in durum wheat grain Cd concentration could be explained by grain Zn concentration. However, our observations of lower uptake of Zn in durum wheat on canola grown soil are not explained by differences in residual extractable Zn in soil. After growing canola as a preceding crop, DTPA extractable Zn concentration in soil were higher, not lower, than after growing barley. Therefore, based on higher concentration of DTPA-Zn at the beginning of the test crop phase, we would have expected the canola grown soil to decrease, rather than increase shoot Cd concentration in the test crops, relative to barley grown soil.

Another complementary explanation for the greater plant uptake of Cd when on canola grown soil may be the greater secretion of organic ligands by canola than by barley for metal chelation. Residuals of such chelates may be preserved in canola grown soil and subsequently increase the Cd uptake of durum wheat and flax since chelated metals can substantially increase trace element uptake (Marschner, 1995; Dakora and Phillips, 2002). A similar hypothesis was proposed by Oliver et al. (1993) to explain the greater concentration of Cd in wheat grain after lupin than after a cereal crop. The greater

secretion of chelates by canola than by barley may be partly evidenced by the higher concentration and accumulation of Cd in canola shoot than in barley shoot at the end of preceding crop growth. Furthermore, the preceding crop of canola may also have had a greater root Cd concentration and accumulation than barley and the decomposition of roots in the canola grown soil may have gradually released plant available Cd to the subsequent durum wheat and flax test crops. Although there is no evidence of such a process in the measurements of DTPA-extractable soil Cd, this type of mineralizeable Cd may not have been extracted by DTPA.

The role of AMF in accounting for greater uptake of Cd from canola grown soil appears to be negligible in our study. The AMF root colonization of durum wheat and flax were not significantly different for barley grown soil (a mycorrhizal crop) compared to canola grown soil (a non-mycorrhizal crop) in this study. However, in contrast to the findings of this study, several other studies reported a greater AMF root colonization in subsequent crop when the preceding crop was a mycorrhizal host crop compared to a non-mycorrhizal host crop (Harinikumar and Bagyaray, 1988; Arihara and Karasawa, 2000; Gao et al., 2010). It should be noted, however, that the AMF root colonization percentages in the preceding barley crop and in the durum wheat and flax test crops were much lower than expected. For example, these AMF root colonization percentages were much lower than those reported in field studies (Harinikumar and Bagyaray, 1988; Gavito and Miller, 1998; Arihara and Karasawa, 2000; Gao et al., 2010). A large part of the reason for the low colonization percentage may be the high degree of soil disturbance in our study as result of soil collection, air drying and sieving. It is also possible that during the early stages of test crop growth AMF root colonization of durum wheat and flax was lower following canola than following barley but colonization became equal for both soils

by the time of sampling. For example, in a field study in Ontario, Gavito and Miller (1998) observed a reduced AMF root colonization in maize when canola was the preceding crop than that of maize during the early stages of plant growth, but there was no difference in AMF root colonization between maize after canola and maize after maize when the subsequent crop reached up to the stage of silking. However, due to the short, 50 day growing period in our growth chamber experiment, this process is not likely a major factor in our study.

Soil pH is a key factor governing soil Cd availability for plants. Decreasing soil pH favours Cd desorption from soil particles and increases the partitioning of Cd into soil solution, making more Cd available for plants (Christensen, 1984; Levi-Minzi and Petruzzelli, 1984; Grant et al. 1998; Sauve et al. 2000; Grant and Sheppard, 2008). In this study, however, soil pH was not different between canola grown soil and barley grown soil at the end of either the preceding crop or the subsequent test crop. Therefore, the effect of the preceding crop on the subsequent crop's Cd concentration does not appear to be due to residual pH effects in the soil after growing canola or barley. These observations are similar to Eastley (2008) who also found no influence of preceding crop on soil pH.

3.5.2 Crop residue effect on the Cd uptake of durum wheat and flax

Crop residue addition, by itself, did not affect shoot Cd concentration or accumulation. Root Cd concentration and accumulation were also not generally affected by crop residue addition. There is little literature on the effect of crop residue addition under agricultural conditions on plant Cd availability. However, many researchers reported a potential for Cd immobilization in soil with various types of organic material

addition to soil (Korcak and Fanning, 1985; Jones and Johnston, 1986; Eriksson, 1988; He and Singh, 1993a; Narwal and Singh, 1997; Eastley, 2008). In contrast, potential release of Cd was also observed with organic matter incorporation in some other studies (He and Singh, 1993a; Eastley, 2008).

In our study, residue treatments that caused a decrease in soil pH or an increase in soil EC were expected to increase uptake of Cd in the test crops. However, this was generally not true. After the test crop was grown, addition of barley and canola shoot residue decreased soil pH in durum wheat compared to treatments where no residue was added, probably because of the release of protons and organic acids during crop residue decomposition (Jones and Darrah, 1994). Soil pH was negatively associated with shoot Cd concentration in durum wheat in this study ($r = -0.35$, $P = 0.0143$) as expected based on literature (Christensen, 1984; Levi-Minzi and Petruzzelli, 1984; Grant et al. 1998; Sauve et al. 2000; Grant and Sheppard, 2008). However, the relationship was not strong and not necessarily due to differences in residue treatment. For example, the treatment means for the soil Cd concentration and shoot Cd concentration were not increased in durum wheat with crop residue addition, even though soil pH was decreased.

In durum wheat, soil EC was greater when crop residue was added than when not added, probably because of the release of ions into soil solution during crop residue decomposition (Jones and Darrah, 1994). Greater ionic concentration in durum wheat when crop residue was added was expected to increase plant uptake of Cd (Garcia-Miragaya and Page, 1976; Petruzzelli et al. 1985; Lorenz et al. 1994). However, in this study, the soil Cd concentration and shoot Cd concentration of durum wheat were not significantly correlated with soil EC.

After growing the test crop, extractable soil Zn and P concentrations were greater for barley residue than for canola residue or the control. However, the increase in soil Zn and P concentration did not increase plant shoot concentrations of these elements. Therefore, there is no evidence for a Cd and Zn interaction or a Cd, Zn and P interaction with crop residue addition in this study.

The lack of effect of crop residue on Cd uptake and soil Cd concentration may be due to the small addition of Cd along with crop residues. The average addition of crop residue Cd in this study was equivalent to 2 and 4 g ha^{-1} for barley and canola, respectively. However, in a similar growth chamber study conducted in Manitoba with similar soil, Eastley (2008) observed an increase in shoot Cd concentration in flax when barley residue or canola residue was added at a rate of 1 g Cd ha^{-1}. Therefore, our experiment's low rate of residue Cd addition, on its own, does not explain the lack of response to crop residue addition.

Also, adding crop residues may have increased the soil's capacity to retain Cd to match the addition of Cd. For example, the added residue may have increased soil organic matter concentration. Increased soil organic matter concentration often reduces Cd availability to plants (Hinesly et al., 1982; Korcak and Fanning, 1985; He and Singh, 1993a; Grant et al., 1999; Grant and Sheppard, 2008). Formation of organic metal complexes between Cd and soil organic matter may also have reduced plant available soil Cd concentration and plant Cd uptake (Grant et al., 1999; Grant and Sheppard, 2008).

3.6 Summary and Conclusions

Durum wheat and flax had significantly greater shoot Cd concentration when grown on soil where the previous crop was canola compared to barley regardless of what type of crop residue, if any was added. This increase in shoot Cd concentration may have been due to the greater plant availability of Cd in canola grown soil than in barley grown soil and was not due to the differences in AMF root colonization between canola grown soil and barley grown soil. Also, for durum wheat, the increase in shoot Cd concentration may be due to decreased shoot Zn concentration. Conversely, addition of crop residue did not affect shoot Cd concentration in durum wheat and flax, perhaps due to the immobilization of Cd in soil by soil organic matter formed through the decomposition of the residue.

This study shows that canola can increase plant Cd uptake if a Cd sensitive crop is grown as the subsequent crop in rotation. However, the incorporation of canola and barley residue will not increase plant Cd uptake in the year of application under conditions similar to ours.

3.6 References

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4. OVERALL SYNTHESIS

Cadmium is a potentially toxic trace element contained in food in trace quantities. Phosphorus fertilization and crop rotation are two major agricultural management practices which can affect Cd uptake in food crops. The main purpose of our research was to understand the processes by which these crop management practices might reduce Cd concentration in food crops in an economical way. Accordingly, two growth chamber studies were conducted to understand how: i) mono-ammonium phosphate (MAP) affects Cd uptake in canola, durum wheat and flax due to fertilizer induced changes in soil and plant; ii) preceding crops of canola and barley affect Cd uptake in subsequent crops of durum wheat and flax.

Contrary to our expectations, the application of MAP-RG (reagent grade) at 80 mg P kg^{-1} soil did not significantly increase the shoot and root Cd concentrations of canola, durum wheat or flax. Furthermore, the application of MAP reduced Cd concentration in the shoot and root of canola. Observations confirmed that the application of MAP doesn't always increase canola, durum wheat or flax Cd uptake in the year of application due to soil and plant factors induced by fertilization. However, it is still too early to draw firm conclusions about the role of MAP fertilization programs in managing crop uptake of Cd, considering the trend for increasing shoot Cd concentration in durum wheat and flax with MAP-RG addition in this study and also considering the contrasting observations made under similar conditions by other researchers.

In this study, neither soil solution Cd concentrations nor soil extractable Cd concentrations were increased with MAP-RG application in planted soils and the lack of increase in plant available Cd in soil could have prevented the increase in plant Cd

concentration. The lack of increase in soil solution Cd concentration and soil extractable Cd concentration with MAP-RG application could be due to the lack of decrease in soil and soil solution pH and/or a lack of increase in soil and soil solution EC in planted soils with MAP-RG application. Future research could focus on repeating these studies for different soil types to investigate possible differences in the response of shoot Cd concentration to MAP application with the changes in soil characteristics. Also, future research could explore soil characteristics other than pH, EC, Cd, Zn and P and AMF which might affect plant Cd uptake.

Our studies confirmed that canola as a preceding crop can increase subsequent durum wheat shoot Cd concentration. In contrast to previous research under similar soil conditions, soil after a preceding crop of canola also increased subsequent flax shoot Cd concentration compared to soil after barley. The root Cd concentrations of durum wheat and flax were also greater with canola grown soil, but only when canola residue was added. Therefore, this study indicates a potential risk for increased Cd uptake if a Cd sensitive crop is planted following canola in a crop rotation. The increase in durum wheat and flax Cd uptake with canola grown soil was probably due to greater plant availability of Cd in soil. However, additional studies are required to identify the exact soil factor or factors responsible for higher plant uptake of Cd in crops after canola.

The addition of crop residue by itself did not affect durum wheat and flax Cd uptake, perhaps due to the immobilization of added Cd in soil. Therefore, recycling of crop residues will not always increase Cd uptake in the subsequent crop.

Determination of soil solution concentrations of Cd, Zn and P was used in these studies, helping to understand the impact of treatments on Cd availability and plant uptake. The Rhizon soil moisture sampler made the collection of soil solution easy and

fast. One challenge of using the Rhizon samplers is that the concentrations of Cd is very low in soil solution and therefore quantification is expensive, requiring inductively coupled plasma spectrometry technology that is not readily available in many labs.

Overall, these studies revealed that the effect of added MAP and preceding crop on plant uptake of Cd is complex and unpredictable. In several cases, the results of our studies were different from previous studies with similar soils and crops. Therefore, the practical relevance of these observations must be considered with caution. These studies were conducted under controlled conditions to reduce environmental variability, for a better understanding of processes governing plant Cd uptake in response to added MAP and preceding crop. Furthermore, only shoot Cd concentrations were measured in this study to assess the potential risk of each treatment on the Cd accumulation in seeds and grains of each crop because an increase in shoot Cd concentration can lead to an increase in seed or grain Cd concentration via translocation of Cd inside the plant. However, it is essential to repeat these studies under field conditions, growing each crop up to seed maturity to validate the implications for real production systems.

5. APPENDIX

Appendix A Concentrations of P, Cd and Zn, pH and EC in soil solution after MAP-RG addition, showing means for plant × week and P× plant × week interactions.

Treatment			P	Cd	Zn	pH	EC
				µg L^{-1}			mS cm^{-1}
Plant	Canola	Week 2		0.056a		7.03ij	2.67ab
×Week	Canola	Week 3		0.039bcde		7.06hij	1.65cd
	Canola	Week 4		0.034bcdef		7.19cdefg	1.40def
	Canola	Week 5		0.045abc		7.26abcdef	1.18def
	Canola	Week 6		0.035bcde		7.28abcdef	0.73ghi
	Canola	Week 7		0.030def		7.20cdefgh	0.59h
	D. wheat	Week 2		0.047ab		6.93j	2.75a
	D. wheat	Week 3		0.030cdef		7.02ij	2.09bc
	D. wheat	Week 4		0.028def		7.26bcde	1.47de
	D. wheat	Week 5		0.030cdef		7.40a	1.32deg
	D. wheat	Week 6		0.027def		7.40ab	1.15deg
	D. wheat	Week 7		0.018f		7.40ab	0.82fhi
	Flax	Week 2		0.047ab		7.00ij	2.65ab
	Flax	Week 3		0.040abcde		6.99ij	2.62ab
	Flax	Week 4		0.035bcde		7.11fghi	2.50ab
	Flax	Week 5		0.042abcd		7.12efghi	2.55ab
	Flax	Week 6		0.037bcde		7.25abcd	1.43d
	Flax	Week 7		0.027ef		7.35abc	1.02efgh
	Unplanted	Week 2		0.038bcde		7.12defghi	2.28abc
	Unplanted	Week 3		0.034bcdef		7.06ghij	2.38ab
	Unplanted	Week 4		0.035bcde		7.14defghi	2.56ab
	Unplanted	Week 5		0.037bcde		7.07ghij	2.62ab
	Unplanted	Week 6		0.033bcdef		7.06ghij	2.64ab
	Unplanted	Week 7		0.033bcdef		7.07ghij	2.67ab

P×Plant	0	Canola	Week 2	0.064a
×Week	80	Canola	Week 2	0.047abcdef
	0	Canola	Week 3	0.043abcdefg
	80	Canola	Week 3	0.036abcdefg
	0	Canola	Week 4	0.035bcdefg
	80	Canola	Week 4	0.033cdefg
	0	Canola	Week 5	0.032bcdefg
	80	Canola	Week 5	0.058ab
	0	Canola	Week 6	0.027cdefg
	80	Canola	Week 6	0.043abcdefg
	0	Canola	Week 7	0.022efgh
	80	Canola	Week 7	0.037abcdefg
	0	D. wheat	Week 2	0.045abcdef
	80	D. wheat	Week 2	0.049abcde
	0	D. wheat	Week 3	0.032bcdefg
	80	D. wheat	Week 3	0.028dfgh
	0	D. wheat	Week 4	0.031bcdefg
	80	D. wheat	Week 4	0.026cdefg
	0	D. wheat	Week 5	0.025cdefg
	80	D. wheat	Week 5	0.035bcdefg
	0	D. wheat	Week 6	0.023efgh
	80	D. wheat	Week 6	0.032cdefg
	0	D. wheat	Week 7	0.021fg
	80	D. wheat	Week 7	0.015g
	0	Flax	Week 2	0.044abcdef
	80	Flax	Week 2	0.050abcd
	0	Flax	Week 3	0.033bcdefg
	80	Flax	Week 3	0.047abcdef
	0	Flax	Week 4	0.033bcdefg
	80	Flax	Week 4	0.037abcdefg
	0	Flax	Week 5	0.037bcdefg
	80	Flax	Week 5	0.048abcdef
	0	Flax	Week 6	0.031bcdefg
	80	Flax	Week 6	0.043abcdef
	0	Flax	Week 7	0.028cdefg
	80	Flax	Week 7	0.026cdefg
	0	Unplanted	Week 2	0.034bcdefg
	80	Unplanted	Week 2	0.042abcdef
	0	Unplanted	Week 3	0.030bcdefg
	80	Unplanted	Week 3	0.037abcdefg
	0	Unplanted	Week 4	0.031bcdefg
	80	Unplanted	Week 4	0.038abcdefg
	0	Unplanted	Week 5	0.037bcdefg
	80	Unplanted	Week 5	0.038abcdefg
	0	Unplanted	Week 6	0.028cdefg
	80	Unplanted	Week 6	0.039abcdefg
	0	Unplanted	Week 7	0.032bcdefg
	80	Unplanted	Week 7	0.034bcdefg

ANOVA	df	P>F				
P	1	0.018	NS	NS‡	0.0079	NS
Plant	3	NS	0.0024	<0.0001	<.0001	<0.0001
Week	5	<0.0001	<0.0001	<0.0001	<.0001	<0.0001
P*Plant	3	NS	NS	0.0029	0.013	NS
Plant*Week	15	NS	0.0198	NS	<.0001	<0.0001
P*Week	5	NS	0.0049	<0.0001	0.0045	NS
P*Plant*Week	15	NS	0.0153	NS	NS	NS
MSE		321817	0.00014	8.40	0.0098	0.3648

† Within columns, means followed by the same letter are not significantly different according to the Tukey multiple comparison test ($p > 0.05$)

‡ NS, not significantly different ($p > 0.05$)