

ROOT SYSTEMS OF OILSEED AND PULSE CROPS
--MORPHOLOGY, DISTRIBUTION AND GROWTH PATTERNS

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By

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ABSTRACT

This study determined the key characteristics of temporal patterns of root growth during the crop development period, as well as the vertical patterns of root distribution in the soil profile for important oilseed and pulse crops grown on the semiarid Canadian Prairie. Rooting characteristics greatly influence the nutrient acquisition and water-use patterns for any plants. However, crop root systems have not been studied intensively due to time, labor and costs constraints. In the literature, root studies mostly focus on cereal crops and very limited information is available for oilseeds and pulses even though these broadleaf crops are critical in the diversification of cropping systems. Thus the objectives of this study were to 1) examine the root morphological characteristics, root distribution patterns in the soil profile, and the fine root distributions of oilseeds and pulses in comparison with wheat; 2) to determine the rhizospheric properties of pulse crops. In 2006 and 2007, canola (*Brassica napus* L.), flax (*Linum usitatissimum* L.), mustard (*Brassica juncea* L.), chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.), lentil (*Lens culinaris*), and spring wheat (*Triticum aestivum* L.) were grown under low- (natural rainfall) and high-water (rainfall+irrigation) conditions in southwest Saskatchewan. Roots were sampled at the seedling, early-flower, late-flower, late-pod, and physiological maturity growth stages, and root parameters determined using image analysis. The growth of roots progressed markedly from seedling to late-flowering and then declined to maturity. Root growth of pulse crops was not significantly affected by water conditions, but canola had 70% greater root length, 67% more root surface area, and 79% more root tips under high-water than under low-water conditions. At the late-flower stage, over 70% of the roots in oilseeds and pulses were distributed within the 0-60 cm soil profile and the largest proportion (around 50%) were found in the top 20-cm of the soil depth. About 85% of the roots in oilseeds and pulses were

classified as “extra fine” (diameter <0.4mm). The rhizosphere fungi were significantly different among tested pulses and also pulse rhizosphere fungi were significantly affected by soil depth but not by water conditions. Inoculation of *Penicillium bilaiae* (product -JumpStart®) to the pulse crops increased the amount of the fungi in their rhizospheres by as much as 42% compared to the pulses not inoculated. Results from this study are novel and provide the baseline for model-related studies on water use and nutrient uptake by root systems of oilseed and pulse crops in semiarid environments.

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TABLE OF CONTENTS

PERMISSION TO USE	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
1.0 INTRODUCTION	1
2.0 REVIEW OF LITERATURE.....	4
2.1 Alternative crops in the Canadian Semiarid Prairie	4
2.1.1 Oilseed crops in the Canadian Prairie	5
2.1.2 Pulse crops in the Canadian Prairie	7
2.2 Plant root system	8
2.2.1 Root morphology	9
2.2.2 Root distribution in the soil profile.....	11
2.3 Rhizosphere	13
2.3.1 Rhizosphere microbial community	15
2.3.2 Factors affecting rhizosphere microbial community	16
2.3.3 Rhizosphere of pulse crops	18
3.0 ROOT SYSTEMS OF OILSEEDS AND PULSE CROPS: GROWTH PATTERNS AND ITS RELATION TO WATER AVAILABILITY	20
3.1 Introduction	20
3.2 Material and methods	22
3.2.1 Experiment design	22
3.2.2 Seedling, root sampling and data collection	26
3.2.3 Statistical analysis	27
3.3 Results and discussion.....	30
3.3.1 Year, crop species effects and their interactions	30
3.3.2 Water effects.....	35
3.3.3. Root growth patterns of oilseed crops	38

3.3.4. Root growth patterns of pulse crops	41
3.4. Conclusions	43
4.0 ROOT SYSTEMS OF OILSEED AND PULSE CROPS: DISTRIBUTIONS IN THE SOIL PROFILE	44
4.1 Introduction	44
4.2 Material and methods	46
4.2.1 Experiment design	46
4.2.2 Seedling, root sampling and data collection	47
4.2.3 Statistical analysis	51
4.3. Results	51
4.3.1 Root distribution patterns of oilseeds and pulses in the soil profile	51
4.3.2 Water effects on root distributions of oilseeds and pulses	56
4.3.3 Root distributions of oilseeds and pulses in different years	61
4.4 Discussion	63
4.4.1 Root distribution patterns of oilseeds and pulses in the soil profile	63
4.4.2 Water availability and root distributions of oilseeds and pulses	64
4.4.3 Root distributions and year effect	65
4.5. Conclusions	66
5.0 FINE ROOT DISTRIBUTIONS OF OILSEED AND PULSE CROPS	68
5.1. Introduction	68
5.2. Material and methods	69
5.2.1 Site description and experiment design	69
5.2.2 Root sampling and data collection	70
5.2.3 Statistical analysis	71
5.3. Results and discussion	71
5.4 Conclusions	75
6.0 MICROBIAL COMMUNITIES IN THE RHIZOSPHERE OF PULSE CROPS IN A SEMIARID ENVIRONMENT	77
6.1. Introduction	77
6.2. Material and methods	78
6.2.1 Experiment design	78

6.2.2 Soil sampling	81
6.2.3. Determination of bacterial and fungal population	81
6.2.4. Statistical analysis	82
6.3. Results	82
6.4. Discussion	86
6.5. Conclusions	88
7.0 GENERAL DISCUSSION AND CONCLUSION	90
8.0 REFERENCES	94
APPENDIX A	122
APPENDIX B	123
APPENDIX C	124
APPENDIX D	125

LIST OF TABLES

Table 3.1. Crop cultivars and agronomy information for oilseed, pulse and cereal crops grown in the lysimeter experiment at Swift Current, Saskatchewan, 2006-2007	24
Table 3.2. The amounts of water (mm) that crop received during the different growth stages under low and high-water conditions at Swift Current, Saskatchewan, 2006-2007	25
Table 3.3. Overall analysis of variance of root parameters for oilseed and pulse crops at 5 crop growth stages, Swift Current, Saskatchewan, 2006-2007.....	29
Table 3.4. Root morphological parameters influenced by crop year at five crop growth stages, in Swift Current, Saskatchewan. The data shown are averaged across all tested crops	31
Table 3.5. Root parameters of oilseeds, pulses, and wheat as affected by year at the late-pod stage (soft-dough for wheat), in Swift Current, Saskatchewan, Canada	34
Table 3.6. Root length density, surface area and tips for oilseeds, pulses, and wheat at the late-flower stage under low- and high-water conditions, in Swift Current, Saskatchewan, 2006-2007	37
Table 4.1. Crop cultivars and agronomy information for oilseed, pulse and cereal crops grown in the lysimeter experiment at Swift Current, Saskatchewan, 2006-2007	49
Table 4.2. The amounts of water (mm) that crop received during the different growth stages under low- and high-water conditions at Swift Current, Saskatchewan, 2006-2007.....	50
Table 4.3. Distribution of root length density (RLD), root surface area (RSA) and root tips (RT) of oilseeds, pulses and wheat in different soil depths under low-and high-water conditions. The data shown are averaged across stages and years. For each crop, the values of RLD, RSA and RT, their proportion of the total (%).and ratios (%) between oilseeds and wheat and ratios (%) between pulses and wheat are indicated. The units of RLD, RSA and RT are mm cm^{-3} , $\text{mm}^2 \text{cm}^{-3}$ and $10^3 \# \text{cm}^{-3}$ respectively	60
Table 6.1. Crop cultivars and agronomy information for pulses grown in the lysimeter experiment at Swift Current, Saskatchewan, 2006	80
Table 6.2. The amounts of water (mm) that crop received during the different growth stages under low- and high-water conditions at Swift Current, Saskatchewan, 2006.....	80

LIST OF FIGURES

Figure 3.1 The progress of root growth during the growing season for oilseed crops in comparison with wheat control with (A) root length density, (B) root surface area, (C) root diameter and (D) root tips measured in Swift Current, Saskatchewan, Canada, 2006-2007. For wheat, the corresponding stages are: seedling, boot, anthesis, soft dough and maturity. The data shown were averaged over the two water conditions. Vertical bars represent the LSD (0.05) for mean comparison between crops at a given stage. 40

Figure 3.2 The progress of root growth during the growing season for pulse crops in comparison with wheat control with (A) root length density, (B) root surface area, (C) root diameter and (D) root tips measured in Swift Current, Saskatchewan, Canada, 2006-2007. For wheat, the corresponding stages are: seedling, boot, anthesis, soft dough and maturity. The data shown were averaged over the two water conditions. Vertical bars represent the LSD (0.05) for mean comparison between crops at a given stage. 42

Figure 4.1 Distributions of (A) root length, (B) root surface area, (C) root diameter and (D) number of root tips for oilseed crops at late-flower stage, Swift Current, Saskatchewan, 2006-2007. Horizontal bars represented the LSD at level of 0.05..... 53

Figure 4.2 Distributions of (A) root length, (B) root surface area, (C) root diameter and (D) number of root tips for pulse crops at late-flower stage, Swift Current, Saskatchewan, 2006-2007. Horizontal bars represented the LSD at level of 0.05..... 55

Figure 4.3 Root distributions of oilseed crops under low- and high-water conditions at late flower, Swift Current, Saskatchewan, 2006-2007. Horizontal bars represented the LSD at level of 0.05. A-C are root parameters of canola, D-F are root parameters of flax and X-Z are root parameters of mustard..... 57

Figure 4.4 Root distributions of pulse crops under low- and high-water conditions at late flower, Swift Current, Saskatchewan, 2006-2007. Horizontal bars represented the LSD at level of 0.05. A-C are root parameters of chickpea, D-F are root parameters of field pea and X-Z are root parameters of lentil 58

Figure 4.5 Root distributions of oilseeds, pulses and wheat in 2006 and 2007. * indicates significance at $P < 0.05$ probability level; blank is not significant. A-C are root parameters of oilseeds, D-F are root parameters of pulses and X-Z are root parameters of wheat..... 62

Figure 5.1 Fine root distribution of oilseed crops, Swift Current, Saskatchewan, 2006-2007. Vertical bars represent the LSD at level of 0.05 73

Figure 5.2 Fine root distribution of pulse crops, Swift Current, Saskatchewan, 2006-2007. Vertical bars represent the LSD at level of 0.05. 74

Figure 6.1 Fungal population in the rhizosphere of pulses, Swift Current, Saskatchewan, 2006. 84

Figure 6.2 Rhizosphere fungal population of pulses at different soil depths at different crop stages, Swift Current, Saskatchewan, 2006. 84

Figure 6.3 Rhizosphere fungi of 3 pulses at different soil depths Swift Current, Saskatchewan, 2006..... 85

Figure 6.4 Effects of *Penicillium bilaiae* on the rhizosphere fungal population of pulses Swift Current, Saskatchewan, 2006..... 85

1.0 INTRODUCTION

Fundamental changes are occurring globally in the utilization of agricultural commodities. The general public is searching for new and novel food products for their dietary needs, nutrition improvement and life style changes, meanwhile, crop producers are seeking new opportunities to increase production, reduce risk and improve their economic returns. This has led to diversification by Canadian prairie grain farmers into crop rotation containing cereals, grain legumes and oilseeds. Various factors have stimulated interest in crop diversification to stabilize returns, accelerating the adaptation of alternative crops to cereals in current agricultural systems in Canada. Alternative crops including pulses, oilseeds and fiber are often used in crop rotations with traditional cereal crops to break pest cycles, reduce weeds and diseases, and improve crop productivity at a system level. The inclusion of annual legumes in cropping systems in the North Great Plains improves nutrient and water use efficiency (Miller et al. 2003b), increases grain yield and quality of subsequent crops (Gan et al. 2003), and improves economic sustainability of production systems (Zentner et al. 2001). Long-term rotation of annual legumes with cereals increases the soil's nutrient supply capacity (Campbell et al. 1992), improves soil physical and chemical properties (Campbell et al. 2000), and enhances the soil's biological attributes (Biederbeck et al. 2005). However, farm diversification using alternative crops still requires considerable research and appropriate planning from assessing available resources to selecting potentially feasible crops and exploring the crop market (Sauer and Sullivan 2000).

In the scientific literature, information regarding alternative crops is limited especially as related to their roots, even though these crops account for about 25% of the total seeded area annually in western Canada. Root information on alternative crops may help producers to develop crop rotation systems with improved resource use efficiency because the inclusion of

crops with different rooting patterns in a cropping system may improve water and nutrient use efficiency (Black et al. 1981). In the semiarid Canadian Prairie, water is the most limiting factor for crop production especially during crop growing season when the short and warm summer together with the frequent strong winds (Cutforth et al. 2007; Knights et al. 2007) may accelerate soil water loss. However, alternative crops have been widely adopted in such region; therefore their rooting patterns would be one of the determinants for the success in the final yield.

Being highly associated with plant root system, soil is an essential part of the entire terrestrial ecosystems, necessary for maintaining most life processes due to its unique ecological composition. Also, soil is a critical natural source for agricultural production in which there are diverse organisms involve in nutrient cycling, regulating soil organic matter, soil structure modification, and enhancing plant health. As the most dynamic environment in the soil, the rhizosphere can influence plant growth and health via intensive biological and biochemical processes conducted by various microorganisms. Therefore a greater understanding of rhizosphere microbial diversity and optimizing the management of this natural resource would help develop the more sustainable agriculture system by minimizing the application of synthetic fertilizers and pesticides, which can lower production costs and be beneficial for the entire environment. Rhizosphere microorganisms contributing to the improvement of nutrients uptake for plants have been studied frequently for many crops, however, those on alternative crops grown in a semiarid condition are rare. Thus, the experiment was conducted to explore rhizosphere microbial community of pulses crops in this study.

This thesis aimed at characterizing rooting patterns and characteristics of alternative crops in a semiarid Canadian Prairie and the objectives were to 1) examine the root growth characteristics, determine root distribution patterns in the soil profile and examine the fine root

distributions of oilseeds and pulses in comparison to wheat; 2) to determine the rhizospheric properties of pulse crops. Root morphological traits were determined for oilseeds (canola, flax and mustard) and pulses (chickpea, field pea and lentil) under low- (natural rainfall) and high- (rainfall + irrigation) water conditions at different crop growth stages using image analysis. Comparisons on the root distribution patterns of these alternative crops were made under the same conditions. Furthermore, bacterial and fungal populations in the rhizosphere of chickpea, field pea and lentil were determined for soil sampled three times during the season at early-flower, late-flower and crop maturity stages to examine how water availability, soil depths and the inoculation of JumpStart® (*Penicillium bilaiae*) affect them in a semiarid Canadian Prairie.

2.0 REVIEW OF LITERATURE

2.1 Alternative crops in the Canadian Semiarid Prairie

There are about 32 Mha of arable land in the Canadian prairies suited to the production of annual grain crops. The prairies account for 85% of Canada's arable land, making it the most important agricultural region of the country, with over 40% of the cultivated land located in the semiarid Brown and Dark Brown soil zones (Gan et al. 2002). Historically, the Canadian semiarid prairie has been dominated by cereal grain production. In 1991, 89% of the seeded area was in spring wheat production, and 42% of the arable area in the Brown soil zone of Saskatchewan was in fallow (Saskatchewan Agriculture and Food 1991). Low market prices for cereal grains coupled with increasing production problems in wheat monoculture systems (Hume et al. 1991; Fernandez et al. 1998) stimulated producers to seek information on alternative oilseed and pulse crops. Cropping systems that are not flexible to change become unsustainable. Therefore crop producers have shifted to more optimal crop sequences that can take advantage of soil nutrients and soil water, while also capitalizing on weather, markets, government programs, and new technology (Tanaka et al. 2007).

Monoculture is the practice of continuous production of the same crop. Monoculture is always inferior to production systems or crop rotations where a variety of crops are grown (Johnston et al. 1999). In addition, crop diversification can be critical to breaking pest infestations that are common with monoculture (Bailey et al. 2000; Elliot and Lynch 1995; Holtzer et al. 1996; Krupinsky et al. 2002). Alternative crops to cereals are of great importance in contributing to the diversification and intensification of what was formerly a wheat-dominated monoculture cropping of western Canada (Zentner et al. 2002). Many studies on crop rotations in the North Great Plains indicated the inclusion of oilseeds in rotation with cereal crops could

increase net returns and reduce risk through improved production stability (Lafond et al. 1993; Dhuyvetter et al. 1996; Zentner et al. 2002). The yield of wheat has also increased when following oilseeds in rotation, confirming that monoculture systems are the least effective as means of optimizing wheat production (Lafond et al. 1992; Brandt and Zentner 1995; Anderson et al. 1999). In contrast, higher and more intensive production often requires greater use of nitrogen fertilizer that is the largest consumer of fossil fuel energy in agriculture and a major source of greenhouse gas emissions. Conservation farming that uses crop rotations with pulse crops improve energy output per unit input ratios compared to conventional farming systems (Lindwall and McConkey 2001). Alternative pulse crops also have an associated N effect which impacts yield effects associated with crop sequence in semiarid regions (Miller et al. 2002).

2.1.1 Oilseed crops in the Canadian Prairie

The oilseed crops canola, mustard, and flax are well adapted to cool, short-season conditions found on the Canadian prairies and northern States of the USA. The oilseed genus *Brassica*, including canola and mustard, is part of the cruciferous family of crops. Cruciferous crops are widely adapted and cultivated around the world for human consumption and livestock feed (Downey et al. 1974). Historically, rapeseed was first grown in North America for use on marine oil during World War II, after which plant-breeding efforts in Canada contributed to the improvement in the fatty acid composition of the oil (reduction of erucic acid) and a marked reduction in the level of glucosinolates in the meal (Johnston et al. 2002). Therefore, after soybean (*Glycine max* L.) and palm (*Elaeis guineensis* Jacq.), rapeseed has become the world's third most important vegetable oil due to these two significant improvements (Downey and Rimmer 1993). These modifications to the oil and meal of rapeseed led to the development of the

name *canola* as a means of distinguishing the edible oil quality rapeseed from industrial quality oil (Johnston et al. 2002).

Low water availability is the most limiting factor to crop production in the Canadian semiarid prairie (Cutforth et al. 2006). Potential evaporative demand in the Brown soil zone is the highest among the regions on the Prairies (Cutforth et al. 1993). From the long-term weather data, average daily precipitation begins to decline 3–4 weeks before average daily maximum temperatures have reached their highest values in late July (Angadi et al. 2004). Therefore, the water deficit stress on the Canadian prairies typically increases as the growing season progresses. However, *Brassica* spp. have taproot systems facilitating crop access to water and nutrients deep in the soil profile (Downey et al. 1974). Therefore when grown in semiarid regions, the rooting characteristics of canola require adequate subsoil moisture to sustain the crop growth during flowering and seed filling (Johnston et al. 2002). Root growth rates for canola were greater than many other spring crops in a multi-year study at Swift Current, SK, more quickly depleting reserved soil moisture, and thus sooner becoming dependent on rainfall to sustain growth (Angadi et al. 1999). Nielsen (1997) reported canola was able to extract water to a depth of 165 cm and up to 95% of the growing season water use of the crop came from the upper 119 cm of the soil profile.

Being a cool season crop, canola is sensitive to high temperature stress (Morrison 1993; Brandt and McGregor 1997; Angadi et al. 2000; Morrison and Stewart 2002). The early-maturing cultivars of *B. napus*, and most *B. rapa* cultivars are better suited to the short growing season regions of the semiarid prairie than other oilseed crops like sunflower and safflower (Miller et al. 2001). As a result, in the driest regions of the semiarid region, canola should be grown on summer fallow rather than continuous crop due to the high water deficit during the growing

seasons (Johnston et al. 2002).

2.1.2 Pulse crops in the Canadian Prairie

Pulse crops have many on-farm benefits from the agronomic point of view; therefore have been increasingly incorporated into crop rotations on farms of the semiarid prairie. Pea is prominent in Canadian prairie cropping systems, while chickpea production is more recent and located exclusively in the drier areas of the prairies (Zentner et al. 2002). Seeded areas of pea increased from 600 000 ha in 1998 to 1.2 million ha in 2008, chickpea increased from 10 000 ha in 1998 to 44 000 ha in 2008 and that of lentil increased from 32 0000 in 1998 to 65 0000 in 2008 (Saskatchewan Agriculture and Food 2008). When the higher market value of chickpea and lentil are considered, these crops have an equally strong fit in diversifying wheat production (Miller et al. 2001).

The advantages of including pulse crops in cropping systems are their ability to fix atmospheric N (Beckie et al. 1997), improve soil physical and chemical properties (Biederbeck et al. 2005), and enhance the yield and quality of subsequent crops (Gan et al. 2003). Spring wheat following pulse crops has averaged higher yields and protein content of grains than spring wheat following spring wheat in rotations even when equal amounts of nitrogen were made available for each crop (Miller et al. 1998; McVicar et al. 2000). This is because pea and lentil do not extract soil water to as great a depth as spring wheat (McVicar et al. 2000, 2001; Zentner et al. 2001). Therefore spring wheat following pea and lentil in rotation is able to extract the water at depth not used by these pulse crops (Carlyle 2004). A deeper rooting crop grown after pea may receive more benefits from water conservation in the soil profile than when grown after chickpea under semiarid environmental conditions (Gan et al. 2007). In addition, pea conferred stronger

rotational benefits to wheat than mustard by conserving more soil water and contributing greater soil N, especially when growth was terminated at midseason as green manure crop (Miller et al. 2006).

2.2 Plant root system

Root systems are fundamental components of terrestrial ecosystems which are important for absorbing water and nutrients as well as providing mechanical support for growing plants. In addition, roots are able to respire carbon from shoot photosynthesis and maintain a balance of plant biomass between below-ground and above-ground (Smucker 1993). In agro ecosystems, the main sources of organic inputs to soil are soil amendments and crop roots; however, this role of roots is often neglected (Spedding et al. 2004). In addition, crop roots affect the microbial growth negatively or positively by reducing soil available N or soil moisture, or by providing carbon substrates for microbial growth (Fogel 1985; Jackson et al. 1989). Seasonal crop growth can regulate the temporal and spatial distribution of organic inputs in the form of crop roots, rhizodeposition, and residues, which both influence the dynamics of soil microbial biomass (Franzluebbers et al. 1995). Roots are also known to synthesize hormones such as cytokinins, which are important regulatory agent in numerous shoot processes, cell division and in grain development (Evans et al. 1976). In addition, roots of legume plants also function as hosts to *Rhizobium leguminosarum*, which greatly contributes to N fixation in soil.

Being an important organ for growing plants, roots are the only connection between plants and soil, thus their morphological traits and their distribution patterns in soil profiles are particularly important affecting water use and nutrient uptake patterns of plants.

2.2.1 Root morphology

Root morphology and root branching patterns are important determinants of water and nutrient uptake by plants (Fitter 1985; Hutchings and De Kroon 1994; Jungk 1996; Fageria 2004). Crop establishment requires a well-developed root system at an early growth stage in order to exploit limited soil resources. The capacity of root systems to support crop growth largely depends on the morphology and uptake ability (RAO and ITO 1998). A well-developed root system may prevent plants from lodging especially under adverse environmental conditions. The initial root system development is particularly important for crop establishment especially in semiarid regions in which crops grown in soils with limited water and nutrient resources would frequently experience stresses during their life cycles. Under more humid environmental conditions limited root systems can adequately support plant growth (Lee et al. 1996, Fageria 2004). Among morphological traits, the maximum root length, root diameter and root:shoot dry weight ratio were associated with drought resistance in dry-land conditions (O'Toole and Soemartono 1981; Yoshida and Hasegawa 1982). Root thickness conferred drought resistance, as roots are capable of increasing root length density and water uptake by producing more and larger root branches (Ingram et al. 1994).

Soil may become harder as it dries (Bengough et al. 2006), which has an impact on root morphology and consequently water and nutrient uptake. In general, root elongation in soils is possible only when root pressure exceeds the mechanical impedance of the soil (Bennie 1991). Mechanical impedance affected root morphology (Taylor and Ratliff 1969), and root length decreased nonlinearly with increasing mechanical impedance (Bennie and Burger 1981). Greater soil compaction and greater soil strength mean greater mechanical impedance. Generally, soil compaction can cause unfavorable changes in soil bulk density, porosity and penetration

resistance (Soane et al. 1981), resulting in limited water and nutrient availability to plants that are major constraints to plant growth and yields in many soils. Ishaq et al. (2001a) reported that an increase in bulk density and penetration resistance due to subsoil compaction decreased the nutrient uptake by wheat and the reduction in nutrient concentration in wheat was 12–35% for N, 17–27% for P and up to 24% for K. Consequently, water use efficiency and nutrient use efficiency by wheat was significantly decreased by increased soil strength (Ishaq et al. 2001b). Root diameter can also be an indicator of the effects of soil strength on root growth (Qin et al. 2004). Large diameter roots represent most of the root system biomass and form long-distance transport pathways that conduct water and nutrients. In addition they can contribute to resource storage, anchorage and supporting lateral roots, while smaller-diameter roots or fine roots make up most of the surface area of the root system for water and nutrient exchange (Eissenstat and Yanai 2002; Waisel and Eshel 2002). During growth, roots contribute to soil porosity (Goss 1991), with root diameter controlling the size of pores. These pores, which have specific physical and chemical properties (Blanchart et al. 1999; Jegou et al. 2001; Read et al. 2003), are used as microhabitats by the non-burrowing fauna, as well as by specific microbial communities (Lavelle et al. 2004; Loranger et al. 1998).

Enhancing nitrogen use efficiency in agriculture systems is urgent since excess nutrient applied to cultivated soils can result in nitrate leaching to the groundwater, and increasing concentrations of volatile NH_3 produced in soils (Eichner 1990). However, nitrogen use efficiency is composed of nitrogen uptake efficiency and utilization efficiency, which is associated with the amount of roots. Being a source of nitrogen, nitrate is an easily mobile nutrient in soil, and the uptake of which is dependent on root morphological characteristics (Sullivan et al. 2000). Root length density is of great importance in nitrate uptake in soil

(Robinson et al. 1983). Differences in root morphology, root age, allocation of assimilate and distribution of nitrate reductase (Siebrecht et al. 1995) may lead to variations in the functionality of root morphological characteristics in affecting nitrate uptake. For wheat, root length density was of minor importance for nitrate uptake from soil (Robinson et al. 1994), whereas maize root length was closely related to the depletion of nitrate in the subsoil (Wiesler and Horst 1994). Moreover, root morphology has been shown to influence accumulation of mineral elements, and increased phosphorus supply was associated with longer root hairs or different root length/ shoot weight ratios of plants (Föhse et al. 1988).

Root morphological characteristics can adapt to the changes in nutrient status in soil (Clements et al. 1993). Maize has greater root branching with increasing levels of applied fertilizer N (Maizlish et al. 1980), while N stress in maize reduced root branching (Eghball et al. 1993). With increased N supply, there were increases in the root length, the number of primary roots, and the elongation rate of first order laterals in maize (Maizlish et al. 1980). Similarly in wheat, greater root length occurred with increased levels of applied fertilizer N (Tennant 1976).

2.2.2 Root distribution in the soil profile

A dynamic root system is related to soil moisture status and regulates the amount of water available to the plant depending on its distribution in the soil (Toorchi et al. 2002). The spatial distribution of roots and their density in the soil are the major determinants of the ability of crops to acquire the nutrients and water necessary for growth (Li et al. 2006). The spatial distribution of roots can reflect the crop's potential to take up nutrients and water (Qin et al. 2004). Usually, a shallower and less proliferated root system will affect the volume of soil available to plants for extracting water and nutrients; moreover, water uptake may be limited by clumping of roots

(Passioura, 1983). The optimum distribution of roots is dependent on the distribution of water and nutrients in the soil especially in dry seasons; plants may require long main root axes to access water stored deep in the soil profile (Bengough et al. 2006).

Roots of individual plants may experience a wide range of soil conditions (Jackson and Caldwell 1993). However, root systems are able to adapt to soil depth and to changes in the availability of water and nutrients and the chemical properties in the soil (Feddes and Raats 2004). Root distribution varies widely according to plant species, soil properties and water regimes (Lehmann et al. 1998). Snake weed (*Polygonum bistorta*) altered its root distribution patterns due to variations in seasonal precipitation (Wan et al. 2002). Spring wheat had shallower root system with decreased available water (Merrill and Rawlins 1979). Crops can respond in various ways to soil water deficits by varying root distribution, which may be largely dependent upon timing, duration and severity of soil water (Hsiao 1990). Wheat crops declined in root growth in the upper 40 cm of the soil profile during a drought without a compensatory root growth in the sub-soil (Weir and Barraclough 1986). However, cotton (*Gossypium* spp.) crops attained substantial increases in root density between 70 and 180 cm in a drying profile compared to a well watered soil profile (Klepper et al. 1973). In general, the ability of plants to change root distribution to exploit water deeper in soil profiles can be an important mechanism to avoid drought stress. Under dry conditions peanut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L.) were able to change root distribution to extract water deeper in the soil profile (Pandey et al. 1984). As available water decreased in soil, soybean (*Glycine max* L.) and sorghum (*Sorghum bicolor* L.) could develop deeper root systems (Hoogenboom et al. 1987; Merrill and Rawlins 1979).

Tillage is another factor affecting crop root distribution patterns by inducing variations in

the soil nutrient status and the its impact on root distribution was evident in the layer affected by plowing (Gerik et al. 1987; Rasmussen 1991). However, tillage intensity can influence the patterns of root distribution (Ehlers et al. 1983; Cornish 1987). Zero tillage often resulted in the stratification of soil nutrients, especially the immobile elements such as phosphorus (Logan et al. 1991; Holanda et al. 1998; Crozier et al. 1999), thus inducing a higher root length density in the topmost layer under zero tillage (Gregory, 1994; Cannell and Hawes 1994). Roots in the zero tillage system accumulated to a greater extent from 0 to 5 cm compared with the roots in the conventional tillage system (Chan and Mead 1992; Rasmussen 1991).

2.3 Rhizosphere

The rhizosphere is the narrow soil zone adjacent to plant roots which is an area with intense biological and chemical activities. This term was first introduced in 1904 by Lorenz Hiltner, a professor of Agronomy at the Technical College of Munich, Germany, who defined it as the specific region of soil affected by plant roots. The word, "rhizosphere" comes from "rhizo" or "rhiza" which is a Greek word for root, and sphere which means an environment or area of influence. At very first, Hiltner used "rhizosphere" to describe the interaction between microorganisms and legume plant roots, but now the term covers all plants and is a topic of fundamental importance in crop production. Plants influence the composition and dynamics of microbial communities present in the rhizosphere, and in turn microbial communities in the soil or rhizosphere contribute to plant growth by recycling nutrients and making them available (Lynch 1990), increasing root health through competition with root pathogens (Weller et al. 2002) or enhancing nutrient uptake (Smith and Read 1997).

Root systems play important roles in releasing organic and inorganic compounds into the

rhizosphere, which may induce chemical changes in the root environment thus affecting the microbial population and availability of nutrients (Neumann and Romheld 2001). Root excretion of H^+ in the rhizosphere is an effective mechanism for improving uptake of micronutrients (Fageria et al. 2002). Phosphorous is strongly retained by soil due to its strong reactions with iron and aluminium ions exposed at the surfaces of soil particles. Therefore in most cases, the mobility of P is extremely low in soils leading to their low availability for plants (Holford 1997; Barrow 1999). However, in some crop species, plant-induced changes in the rhizosphere can increase the availability of unavailable soil P, and the mechanisms include the manipulation of root morphology (hair length/density), the provision of extra carbon for mycorrhizal exploitation of non-rhizosphere soil, and the release of phosphatases and organic acids (Jones 1998). Enhanced secretion of acid phosphatases and phytases by plant roots and also by rhizosphere microorganisms under P deficient conditions may contribute to P acquisition by hydrolysis of organic P esters in the rhizosphere (Neumann and Romheld 2001).

Soil physical properties strongly influence nutrients uptake, and in addition they largely determine rhizosphere extension due to their influence on root growth and transfer of ionic and molecular compounds (Nye 1981; Hinsinger 1998). Usually soil temperature affects physical, chemical, and biological processes in the rhizosphere and nutrient availability. Extreme temperatures are detrimental to the rhizosphere environment affecting root growth and microbial processes adversely. The size, arrangement and stability of soil aggregates have a great influence on soil physical and chemical processes (Fageria and Stone 2006), thus affecting the rhizosphere; while in turn the rhizosphere can affect soil structure. Grassland soils generally have a very stable soil structure (Reid and Gross 1980) due to the greater root biomass around which a higher rhizosphere microbial biomass is present (Lynch 1981). Soil pH is one of the most important

chemical properties that can influence nutrient solubility and consequently the nutrients availability to plants. Acidification of the rhizosphere can solubilize several less soluble macronutrients (Riley and Barber 1971) and micronutrients (Sarkar and Wyn Jones 1982).

2.3.1 Rhizosphere microbial community

All organisms in the biosphere are dependent on microbial activity (Pace 1997). Soil microorganisms play important roles in nutrient cycling in ecosystems (van der Heijden et al. 1998; Cairney 2000; Klironomos et al. 2000; Ovreas 2000). Soil microorganisms influence above-ground ecosystems by contributing to plant nutrition (George et al. 1995; Timonen et al. 1996), plant health (Srivastava et al. 1996; Fillion et al. 1999; Smith and Goodman 1999), and soil structure (Wright and Upadhyaya 1998; Dodd et al. 2000) and soil fertility (Yao et al. 2000; O'Donnell et al. 2001).

The diverse microorganisms are the most important component of the rhizosphere. Plant growth-promoting rhizobacteria (PGPR) are beneficial microorganisms which colonize the rhizosphere resulting in the improvement of plant growth and development. Members of PGPR can be divided into two classes according to whether they can affect plant growth either directly or indirectly (Bashan and Holguin 1998). Direct influence is related to the increased solubilization, nutrients uptake and production of phytohormones, whereas indirect effect is associated with pathogen suppression, production of Fe-chelating siderophores, and antibiotics, and the induction of plant resistance mechanisms (Persello-Cartieaux et al. 2003). Mycorrhizal fungi are the other important group of beneficial soil microorganisms and they vary widely in structure and function. About 80% of all terrestrial plants can form an association with arbuscular mycorrhizal fungi (AMF) in which the fungi receive carbon from the host meanwhile

supplying minerals to the host (Harrier and Watson 2003). The AMF can improve nutrient availability by enhancing acquisition of low-mobile micronutrients such as Zn, Fe, and Cu (Marschner 1995). The AMF are especially effective for P acquisition due to their hyphae, which greatly increase the volume of bulk soil that the plant roots can explore. Quantitatively, P is the most important nutrient taken up by the extra-radical hyphae and influx of P in roots colonized by AMF can be three to five times higher than in non-mycorrhizal roots (Harrier and Watson 2003). Generally, microbial communities in the rhizosphere can be affected by a wide range of factors. Any changes of microbial community may affect plant growth either negatively or positively.

2.3.2 Factors affecting rhizosphere microbial community

In most cases, a variety of abiotic and biotic factors greatly influence the composition and activities of the microbial community in the rhizosphere.

2.3.2.1 Plant and soil

Plants have a strong influence on the composition and dynamics of microbial communities present in the rhizosphere (Germida and Siciliano 2001). The structural and functional diversity of rhizosphere populations is affected by plant species due to differences in root exudation and rhizodeposition in different root zones (Jaeger et al. 1999; Sørensen 1997). In many cases the rhizosphere communities of different plant species growing in the same soil are distinct (Ibekwe and Kennedy 1998), whereas plants may have similar microbial community structures in different soils. Mutually the composition of the rhizosphere community can also significantly influence the development of phytopathogens (Nehl et al. 1997), nutrient

acquisition (Lynch 1990), heavy metal resistance (Bradley et al. 1981) and ecological fitness of plants (Bever et al. 1997; Parker 1995).

Soil has a great effect on the microbial community in the rhizosphere and many of the soil properties including soil type (Buyer et al. 1998), soil texture (van Elsas et al. 1989) soil bulk density and soil temperature (Ratray et al. 1993). Soil water is important for both plants and soil microorganisms. Generally, roots can maintain relatively higher water content in the rhizosphere in the upper, drier soil horizons by pumping water from roots in contact with available water in the lower soil horizons (Dawson 1997). Mucilage on the root surface could protect rhizosphere microbial community from desiccation by stabilizing the soil structure and maintaining hydraulic conductivity (Read and Gregory 1997; Czarnes et al. 2000).

2.3.2.2 Agricultural practices

To make agriculture more sustainable, management practices have been shifting to reduction in tillage, input of organic materials, and nutrient strategies based on crop rotations (Pankhurst et al. 1996) which may affect soil microbial diversity. Reduced tillage enhances soil microbial diversity (Hassink et al. 1991; Wander et al. 1995) while the no-tilled phase of a crop rotation reduces microbial diversity (Zelles et al. 1992). The impact of different crop species that are used in various combinations is likely to be an important factor in determining the structure of plant beneficial microbial communities that function in nutrient cycling, the production of plant growth hormones, and suppression of root diseases (Alvey et al. 2003). As a sequence of plant species are grown on a soil, the predominant bacteria associated with the previous crop species will exert at least some temporary influence on bacterial communities in the rhizosphere of the subsequent species, particularly during early growth of crops (Alvey et al. 2003). The

substrate utilization patterns of microbial communities can be changed by crop rotations (Lupwayi et al. 1998). However, effects on rhizosphere microbial community of crop rotations are different from that of intercropping. Root contact can change rhizosphere microbial community structure and all crops had similar microbial community structure when their roots intermingled in intercropping system (Wang et al. 2007).

2.3.2.3. Introduced microorganisms

The requirements of sustainable agriculture have stimulated the investigation of bio-source alternatives to reduce inorganic fertilizer inputs and also the use of bio-control or plant growth promoting agents. Biofertilizer and bioinoculants containing living micororganisms can contribute to the improvement in the biochemical and physical composition of soil organic matter, increase bio-availability of soil nutrients and control of pathogen or pest populations (Abrol 1999; Ladha et al. 2000; Timsina and Connor 2001) due to the effects on the rhizosphere. The bio-inoculation by PGPR or AMF, in the form of seed coating or in the soil near the seed, may cause shifts in the community composition of either small or high magnitude (Nacamulli et al. 1997; Marschner et al. 2001; Bankhead et al. 2004). In addition, PGPR and AMF bio-inoculation induced a significant modification in the bacterial community of wheat, and the type of PGPR consortium had more impact on the bacterial community structure than the presence of AMF (Roesti et al. 2006).

2.3.3 Rhizosphere of pulse crops

It is well known that pulse crops can add N to the soil, break disease cycles, improve the quality of the soil, and increase the yield of the following non-legume crops when grown in

rotation (Wouterlood et al. 2004). Pulse crops can also induce rhizosphere acidification (Tang et al. 1997), and root-induced rhizosphere pH is known to influence availability of soil inorganic P (Gahoonia and Nielsen 1992) and micronutrients to plants (Marschner and Romheld 1996). Chickpea roots can exude large amounts of low-molecular-weight organic carboxylates in the rhizosphere (Ae et al. 1990; Gerke et al. 1994; Hocking et al. 1997; Ohwaki and Hirata 1992). Plant phosphorus uptake was positively correlated with the concentration of carboxylates in the rhizosphere (Veneklaas et al. 2003). The main carboxylate released from chickpea roots is malonate (Wouterlood et al. 2005) which can defend against pathogens and inhibit microbial activities in the rhizosphere (Li and Copeland 2000).

3.0 ROOT SYSTEMS OF OILSEEDS AND PULSE CROPS: GROWTH PATTERNS AND ITS RELATION TO WATER AVAILABILITY

3.1 Introduction

Plant roots are the primary organ for water and nutrient uptake and thus play an essential role in the soil-plant continuum (Lynch et al. 2007). Also, plant roots are an important sink of photosynthates and the decomposition of roots contributes carbon to the soil, thus increasing soil organic matter (Pietola and Alakukku 2005). The morphological traits of roots greatly influence the capacity of nutrient uptake and water extraction in crop plants (Fageria 2004) which ultimately influences the aboveground growth and biomass yield (Jia et al. 2008). Root morphological traits usually include the root length, surface area, diameter, and the number of tips. In general, root morphology varies widely between plant species ranging from a dominant taproot system with few lateral roots to highly developed fibrous root systems (Clement et al. 1993).

The characteristics of morphological traits can directly influence the functionality of the roots. For example, roots with a larger surface area can absorb a greater amount of phosphorus and ammonium (Marschner 1998) and nitrate (Sullivan et al. 2000). However, large variations exist in terms of the association of root morphological traits and their functionality. For example, in spring wheat (*Triticum aestivum* L.), root length density was of minor importance for nitrate uptake from soil (Robinson et al. 1994), whereas root length in corn (*Zea mays* L.) was closely related to the depletion of nitrate in the subsoil (Wiesler and Horst 1994). Additionally, root morphology influences the accumulation of mineral elements in plant tissues (Föhse et al. 1988). Roots with either greater length or longer root hairs increased nutrient supply to the plant. In wheat, certain genotypes with longer and thinner roots tended to be more efficient in Zn uptake (Dong et al. 1995).

The morphology of root systems in field crops is important for the acquisition of soil available water especially under water-limited conditions. In areas where drought occurs frequently such as in the northern Great Plains of North America, improved root morphological traits such as increased root length, root diameter and root-to-shoot ratio help reduce drought stress in crops (O'Toole and Soemartono 1981; Yoshida and Hasegawa 1982). The size of root systems confers drought resistance, because roots are capable of increasing their length density and water uptake ability by producing more lateral roots and larger root volume (Ingram et al. 1994). The development of a vigorous and deeper root system is particularly important for a crop to be better adapted to water stress environments (Fageria 2004).

Oilseed and pulse crops have been increasingly adapted in cereal-based cropping systems in the semiarid areas of the northern Great Plains. In western Canada, for example, the area seeded to oilseed crops has increased from 3.46 million hectares in 1990 to 6.57 million hectares in 2006 (or 90% increase); the area seeded to pulse crops has increased 8 fold from 257,000 hectares in 1990 to 2.33 million hectares in 2006 (Statistics Canada 2007). These crops are playing a significant role in the development of sustainable agricultural systems (Zentner et al. 2001) where the use of these broadleaf crop species to replace conventional summer fallow for significant environmental benefits (Gan and Goddard 2008), and to mitigate greenhouse gas emissions from crop production (Lemke et al. 2007). Unfortunately, knowledge on the root systems of these crops is limited and most of the published studies have focused on cereals (Bolinder et al. 1997). There is a paucity of relevant root data for oilseed and pulse crops, so information on the roots of these alternative crops is urgently needed. For crop producers, root information may help develop the crop rotation systems with improved water and nutrient use efficiency by selecting the optimum crop sequences. The inclusion of crops with different rooting

patterns in a cropping system can improve water and nutrient use efficiency (Black et al. 1981). For scientific modelers, specific information on crop roots may help validate the models used to examine nutrients and water use patterns in soil by crops. The objectives of this study were to (i) determine root morphological characteristics of selected oilseed and pulse crops at different growth stages in comparison with spring wheat, and (ii) examine the influence of water availability on the root growth patterns under semiarid environmental conditions.

3.2 Material and methods

3.2.1 Experiment design

A study was conducted at the Semiarid Prairie Agricultural Research Centre of Agriculture and Agri-Food Canada, Swift Current (50°15'N, 107°44'W), Saskatchewan, in 2006 and 2007. The experiment was established on an Orthic Brown Chernozem (Aridic Haploboroll) soil with silt loam texture; the content of sand, silt, and clay was 28%, 49%, and 23%, respectively, organic matter of 3.0%, and pH (water paste) 7.3. The experiment field was on wheat stubble. Three oilseeds (canola, flax, mustard), three pulses (chickpea, field pea, lentil), and spring wheat were studied under low- (natural rainfall) and high-water (rainfall+irrigation) conditions in each year. A representative cultivar was chosen for each crop species based on their production popularity (Table 3.1). All crops were hand-planted in lysimeters of 15 cm in diameter and 100 cm in length that were installed in the field using a hydraulic system (Gan et al. 2009b). The crops under low-water treatments received natural rainfall only (Table 3.2), whereas crops under high-water condition received natural rainfall plus irrigation at the amount of 150 mm (which was about 2/3 of the long-term rainfall at the experimental site). Irrigation was applied using a hand-sprayer with 75 mm of the irrigation being applied between seedling and

flowering, split into 3 applications, and the remaining 75 mm applied from flowering to maturity in 2-3 applications. Seven crops with two water regimes were arranged in a factorial, randomized complete block design with two replicates. Each treatment contained 5 sampling times in each replicate. Thus, the experiment had a total of 140 lysimeters (7 crops \times 2 water conditions \times 5 sampling times \times 2 replicates) in each year.

Table 3.1. Crop cultivars and agronomy information for oilseed, pulse and cereal crops grown in the lysimeter experiment at Swift Current, Saskatchewan, 2006-2007.

Crop	Cultivar	Fungicide			Initial seeds lysimeter ⁻¹	Final plants lysimeter ⁻¹
		Trade name	Active ingredient	Rate (ml 100kg ⁻¹ seed)		
<u>Oilseed crop</u>						
Canola	45H21	Helix	Thiamethoxam	1500	11	3
Flax	Vimy	Vitaflo 280	Carbathiin + Thiram	525	12	4
Mustard	Cutlass	Helix	Thiamethoxam	1500	11	3
<u>Pulse crop</u>						
Chickpea	CDC Anna	Crown	Carbathiin + Thiabendazole	600	5	2
Field pea	Eclipse	Apron FL	Metalaxyl	16	5	2
Lentil	CDC Glamis	Crown	Carbathiin + Thiabendazole	600	7	3
<u>Cereal crop</u>						
Wheat	Lillian	Vitaflo 280	Carbathiin + Thiram	330	7	3

Table 3.2. The amounts of water (mm) that crop received during the different growth stages under low- and high-water conditions at Swift Current, Saskatchewan, 2006-2007.

Crop stage ^a	2006		2007	
	Low ^b	High ^b	Low ^b	High ^b
Seedling	131	156	98	136
Early-flower	14	41	17	40
Late-flower	22	45	5	22
Late-pod	1	14	0	28
Maturity	12	28	10	52
Total	180	284	130	278

^a For oilseed and pulse crops, root samples were taken in 2006 on DOY (day of year) of 178 (Seedling), 187-199 (Early-flower), 207 (Late-flower), 214 (Late-pod), and 223-233 (Physiological maturity); in 2007 the corresponding sampling days were 173-177, 184-193, 193-201, 204-207, and 214-219, respectively; for wheat the corresponding stages were: seedling, boot, anthesis, soft dough, maturity.

^b Low-water means crop received natural rainfall only, and high-water means crops received natural rainfall plus irrigation to amount of 150 mm.

3.2.2 Seedling, root sampling and data collection

All crops were planted in the first week of May in both years. Oilseed crops and wheat received fertilizer (46-0-0) at the rate of 80 kg N ha⁻¹ and superphosphate (0-45-0) at 27 kg P ha⁻¹ at seeding and all fertilizers were placed directly on crop seeds. After inoculated with *Rhizobium*, pulse crops received P only. Seeds were treated with an effective fungicide before planting to minimize seed- and soil-borne diseases (Table 3.1). The lysimeters were surrounded by a 2×6 m area of the crops that were same to the plants in the lysimeters. A fine-wired cage was installed around each lysimeter to prevent the potential damage from wild animals. The cages were removed at the time of lysimeters removal when sampling took place or when plants such as field pea started to attach tendrils to the cages. Two weeks after emergence, crop seedlings in the lysimeters were thinned to the desired plant population (Table 3.1).

Lysimeters of oilseed and pulse crops were withdrawn from field positions at crop growth stages of seedling, early-flower, late-flower, late-pod, and physiological maturity. For spring wheat, the corresponding stages were seedling, boot, anthesis, soft-dough and maturity, respectively. Crop plants were cut off at the ground level and the number of plants in each lysimeter was counted just before the lysimeters were withdrawn from field positions. Lysimeters were then transported to the laboratory, and stored at 2°C until processing. For the analysis, intact soil cores within the lysimeters were sectioned into 10 cm increments for the first 60 cm of the soil core and 20 cm intervals for the remaining core to a depth of 100 cm. Each soil-root matrix was soaked overnight in containers using 15 ml of calgon water softener for every 4 liter of water. Roots were washed out of soil manually using 4-mm hole size pan sieves, and then placed in containers full of water with a 0.8-mm screen mounted 3 cm below the water level. Debris and other extraneous materials from the root samples were removed using tweezers.

The entire cleaned root samples were sub-sampled; one part was used for dry weights (Gan et al. 2009a) and the other portion was used for the analysis of root morphology. Roots were stained with 0.08% Toliudine Blue O (Sigma, USA) for 5 min, and then rinsed thoroughly with distilled water. Rinsed root samples were placed on a tray and scanned with a “WinRHIZO” scanner (Regent Instruments Inc. Quebec, Canada) at a resolution of 400 dpi. Scanned root images were analyzed using the WinRHIZO program (Version 2003b) for root length, surface area, diameter, and the number of tips. For each lysimeter, the output of root length, root surface area and number of root tips were summed over all soil segments into the total root length (mm), total root surface area (mm²) and total number of root tips, while root diameters were averaged across these soil segments. Total root length density (mm cm⁻³), total root surface area density (mm² cm⁻³) and the number of root tips were calculated by dividing the total root length (mm), total root surface area (mm²) and the total number of root tips by the volume (cm⁻³) of the entire soil core.

3.2.3 Statistical analysis

The data on root morphological traits at each crop growth stage were subjected to analysis of variance using the MIXED procedure of SAS (Littell et al. 1996). Crop, water condition, and year were considered as fixed effects and replicates as random effects (Table 3.3). A combined analysis of variance was first performed on all variables, and the mean effects across years were presented if year by treatment interaction was either not significant or there was a similar trend of treatment effects (Hoshmand 2006). For variables where there was a significant year by treatment interaction and the interaction was large relative to the average effect, the treatment effect was determined for each year. For most variables, the mean of three pulse crops

or three oilseeds were compared to wheat using a single degree-of-freedom contrast. The means of differences were considered significant if the probability level was at $P \leq 0.05$, and Fisher's protected LSD was used to determine treatment effects.

Table 3.3. Overall analysis of variance of root parameters for oilseed and pulse crops at 5 crop growth stages, Swift Current, Saskatchewan, 2006-2007.

	Seedling ^a	Early-flower	Late-flower	Late-pod	Maturity
<u>Root length density (mm cm⁻³)</u>					
Year (Y)	**	NS	NS	NS	NS
Water (W)	NS	NS	* ^b	*	NS
Crop (C)	***	***	***	***	***
Y × W	NS	NS	NS	NS	NS
Y × C	*	NS	NS	**	**
W × C	NS	NS	*	NS	NS
Y × W × C	NS	NS	NS	NS	NS
<u>Root surface area (mm² cm⁻³)</u>					
Year (Y)	*	NS	**	NS	*
Water (W)	NS	NS	*	NS	NS
Crop (C)	***	***	***	***	***
Y × W	NS	NS	NS	NS	NS
Y × C	*	NS	NS	**	NS
W × C	NS	NS	*	NS	NS
Y × W × C	NS	NS	*	NS	NS
<u>Root diameter (mm)</u>					
Year (Y)	**	NS	NS	*	NS
Water (W)	NS	NS	NS	NS	NS
Crop (C)	***	***	***	***	***
Y × W	NS	NS	NS	NS	NS
Y × C	NS	NS	**	**	**
W × C	NS	NS	NS	NS	NS
Y × W × C	NS	NS	NS	NS	NS
<u>Root Tips (10³ cm⁻³)</u>					
Year (Y)	***	**	NS	NS	NS
Water (W)	NS	NS	*	NS	NS
Crop (C)	***	***	***	***	***
Y × W	NS	NS	NS	NS	NS
Y × C	**	NS	NS	*	NS
W × C	NS	NS	*	NS	NS
Y × W × C	NS	NS	NS	NS	NS

^a Corresponding stages for wheat are: seedling, boot, anthesis, soft-dough and maturity.

^b Levels of significance indicated: NS= not significant, * significant at the $P \leq 0.05$; ** significant at the $P \leq 0.01$, *** significant at $P \leq 0.001$.

3.3 Results and discussion

3.3.1 Year, crop species effects and their interactions

Year had a significant effect on all the measured root parameters of oilseeds and pulses at the seedling stage (Table 3.4). On average, the total root length density, total root surface area, the average root diameter, and the total number of root tips in 2007 were, respectively, 41, 25, 14, and 110% greater than the corresponding values of 2006. Crops in 2006 received 131 mm of water during the seedling stage or 34% more than that received by crops in 2007 (Table 3.2). The limited water availability during the seedling period in 2007 stimulated root growth which was greater than that in 2006. The results were in agreement with the findings of previous studies by Hoogenboom et al. (1987) and Merrill et al. (2002) where the roots of soybean (*Glycine max* L.) and dry bean (*Phaseolus* spp.) attained the greatest growth in driest years and the least growth in wettest years.

Crops had a larger root surface area in 2006 than that in 2007 at the late-flower stage (for oilseeds and pulses; for wheat the corresponding stage was anthesis) and maturity stages (Table 3.4). This was again largely due to rainfall during the period of late-flower and maturity when crops received more in 2006 compared to that in 2007. Other factors might also contribute to the large differences in root surface area between the two years such as soil environmental conditions under which the roots grew. Douglas et al. (2006) reported that root surface area was the most sensitive to environmental factors and varied greatly depending on crop season, rooting depth, and soil water content.

All crops in 2007 produced significantly more root tips than those in 2006 at the early flower stage (for oilseeds and pulses; for wheat the corresponding stage was boot). The greater number of root tips produced by the crop in the drier year was probably due to the tendency of

the root system to sense more soil volume for water. Root tips consist of the meristem and root cap; the meristem is responsible for detecting water and nutrients from soil, and the root cap senses water,

Table 3.4. Root morphological parameters influenced by crop year at five crop growth stages in regardless of water conditions, in Swift Current, Saskatchewan. The data shown are averaged across all tested crops.

Year effect	Growth stage				
	Seedling ^a	Early-flower	Late-flower	Late-pod	Maturity
Root length density (mm cm ⁻³ soil)					
2006	3.24 b	7.92 a ^b	11.55 a	10.79 a	9.77 a
2007	4.58 a	8.90 a	10.46 a	10.65 a	9.64 a
LSD _{0.05}	0.97	1.59	1.71	1.37	0.91
Root surface area (mm ² cm ⁻³ soil)					
2006	4.11 b	9.22 a	13.33 a	10.52 a	9.88a
2007	5.12 a	9.02 a	10.75 b	10.23 a	8.86 b
LSD _{0.05}	1.01	1.51	1.62	1.29	0.84
Root diameter (mm)					
2006	0.23 b	0.32 a	0.36 a	0.31 b	0.32 a
2007	0.26 a	0.34 a	0.36 a	0.33 a	0.32 a
LSD _{0.05}	0.02	0.03	0.02	0.02	0.02
Root tips (10 ³ cm ⁻³ soil)					
2006	651 b	1814 b	2985 a	2799 a	2704 a
2007	1370 a	2646 a	3030 a	3046 a	2579 a
LSD _{0.05}	274	453	531	410	304

^a Corresponding stages for wheat are: seedling, boot, anthesis, soft dough and maturity.

^b Followed by the same letter are not significantly different between the two years at $P \leq 0.05$, according to LSD.

gravity, touch, and other signals (Feldman 1984). The root cap can also control the direction of root growth towards positive stimuli such as nutrients and away from deleterious stimuli such as

toxins (Aiken and Smucker 1996). In addition, the growth activity of root tips is critical to dynamic root function since spatial and temporal changes in root distribution are defined by the growth path of root tips (Aiken and Smucker 1996).

Similar to root tip response, crops in 2007 had roots with a larger diameter at the late pod stage (the corresponding stage for wheat was soft dough) (Table 3.4). Again, this was largely due to the drier weather in 2007. These results were in accordance with the findings from a previous study where corn root system had a larger root diameter under the conditions of high water stress (Duruoha et al. 2007). Decreasing soil water content often increases soil strength (Whiteley and Dexter 1982), which influences root penetration into soil pore spaces. Soils with high penetration resistance result in thicker, larger diameter roots (Barley 1962; Materechera et al. 1992) with a slower rate of elongation (Taylor and Ratliff 1969).

Crop species differed significantly in their rooting traits, and these differences showed interactions with year as well as with water regime (Table 3.3). The crop by year interactions occurred mostly at the late pod stage for all root parameters; therefore, separate ANOVAs were performed to analyze the interactions between years and crop species at the late pod stage (Table 3.5). Among the oilseed crops, canola had larger root length density and surface area with more root tips in 2007 than those in 2006. However, flax had less root tips in 2007 than that in 2006. There was no difference in any root parameter for mustard between the two years. In the drier 2007, canola had greater values of root length density, surface area and root tips than mustard, suggesting that the root growth of canola responded to drier growing conditions better than mustard. Johnston et al. (2002) reported that despite belonging to the same family, canola has been adapted to semiarid regions better than mustard. In general, *Brassica* species have a taproot system facilitating roots to uptake water and nutrients deeper in the soil profile (Downey et al.

1974). These taproot systems may explain why the root growth of canola and mustard in the drier 2007 performed better than flax which has fibrous root system.

Table 3.5. Root parameters of oilseeds, pulses, and wheat as affected by year at the late-pod stage (soft-dough for wheat), in Swift Current, Saskatchewan, Canada.

	Root length density (mm cm ⁻³ soil)		Root surface area (mm ² cm ⁻³ soil)		Root diameter (mm)		Root tips (10 ³ cm ⁻³ soil)	
	2006	2007	2006	2007	2006	2007	2006	2007
<u>Oilseed crop</u>								
Canola	13.21cd*	20.80a	11.33bc	16.19a	0.29cd	0.25d	3925bc	5646a
Flax	8.87efgh	6.47gh	7.41de	5.01e	0.27cd	0.25d	2656defg	1244h
Mustard	9.87defg	11.48de	8.60cd	8.54cd	0.27cd	0.23d	3045cde	3435cd
<u>Pulse crop</u>								
Chickpea	8.44efgh	7.00fgh	10.62bcd	11.77bc	0.39b	0.49a	2245efgh	2727def
Field pea	7.99efgh	6.67fgh	8.69cd	8.63cd	0.31c	0.43b	1816fgh	2038efgh
Lentil	10.23def	5.43h	13.52ab	7.76de	0.38b	0.42b	1960fgh	1611gh
<u>Cereal crop</u>								
Wheat	15.94bc	17.67ab	13.48ab	13.74ab	0.28cd	0.26d	4007bc	4622ab
LSD _{0.05}	3.63		3.41		0.05		1085	

* For a given variable, means followed by the same letter were not significantly different at $P \leq 0.05$, according to LSD.

For pulse crops, there were also significant year by crop interactions for most of the root parameters measured (Table 3.5). Root length density and surface area of lentil were larger in 2006 than that in 2007; however, root diameter of chickpea and of field pea in 2006 were smaller than that in 2007, and there were no differences in root tips between the two years. In 2006, lentil had larger root diameters than field pea, but in 2007 there were no differences. In both years, chickpea had the largest root diameter among the three pulses. Differences in root tips only occurred between chickpea and lentil in 2007. In general, the changes in root growth patterns for pulse crops were inconsistent between years and these results indicate that the root growth patterns of pulse crops differ substantially depending on environmental conditions and that the root growth patterns of wheat and oilseeds respond to environmental conditions more consistently than pulse crops.

3.3.2 Water effects

Water availability as manipulated through irrigation had significant effects on root length density, root surface area and root tips at the late flower stage for oilseeds and pulses, while for wheat it occurred at anthesis. The intensity of the effect varied with crop species (Table 3.3). Among oilseed crops, canola had greater values in total root length density, total root surface area and total number of root tips under high-water (i.e., rainfall + irrigation) compared to low-water (rainfall only) conditions (Table 3.6). However, irrigation did not affect any of these root parameters for flax or mustard. The canola cultivar used in the experiment was a hybrid which is sensitive to water and nutrient availability (Gan et al. 2008). In a study conducted in Australia, Kirkegaard et al. (1997) demonstrated that root length density and rooting depth for canola were highly related to soil water availability.

In pulse crops, irrigation had little impact on root length density, surface area or the number of tips (Table 3.6). In contrast, wheat plants had larger root length density, surface area and more tips under high-water than those under low-water conditions. Our results differed from those reported by Xue et al. (2003) who found that wheat under rainfed conditions had greater root length density than under irrigation at booting stage. The current experiment was conducted in semiarid environments with limited soil moisture while Xue's study was under more humid conditions. Some other researchers found that the effect of water availability on root length density in wheat was not significant around flowering stage (Mishra et al. 1999). These inconsistent results among experiments indicate that root length density may not be an optimum indicator of root growth when examining irrigation effects for wheat although root length density was one of the most studied root parameters in various root publications. Moreover, this root parameter can be affected considerably by genetic makeup, plant development, soil physical and chemical properties, and foliar and root diseases (Sharratt and Gesch 2004).

Table 3.6. Root length density, surface area and tips for oilseeds, pulses, and wheat at the late-flower stage under low- and high-water conditions, in Swift Current, Saskatchewan, 2006-2007.

	Root length density (mm cm ⁻³ soil)		Root surface area (mm ² cm ⁻³ soil)		Root tips (10 ³ cm ⁻³ soil)	
	Low ^a	High ^a	Low	High	Low	High
<u>Oilseed crop</u>						
Canola	13.52bc	23.05a	12.32bc	20.58a	3337bc	5965a
Flax	7.43fg	6.45g	6.38d	5.50d	1677de	1231e
Mustard	11.23cdef	12.04cde	11.21c	10.99c	3452bc	3542bc
<u>Pulse crop</u>						
Chickpea	6.55g	7.91efg	10.74c	13.20bc	2270cde	2856cd
Field pea	9.93cdefg	10.11cdefg	12.86bc	13.29bc	2532cde	3021cd
Lentil	8.63defg	6.95fg	13.26bc	10.82c	2775cd	1882de
<u>Cereal crop</u>						
Wheat	12.77cd	17.80ab	11.81c	16.08ab	2958cd	4692ab
LSD _{0.05}	4.53		4.29		1403	

^a Low-water means crop received natural rainfall only, and high-water means crops received natural rainfall plus irrigation with a amount of 150 mm.

^b For a given variable, means followed by the same letter were not significantly different at $P \leq 0.05$, according to LSD.

3.3.3. Root growth patterns of oilseed crops

The growth of roots in oilseed crops progressed considerably from seedling to the late-flower stage, reached a maximum at late-flower or late-pod stages in the total root length density (Fig. 3.1A), total root surface area (Fig. 3.1B), the average root diameter (Fig. 3.1C) and the total number of root tips (Fig. 3.1D), and then declined to maturity. Canola and mustard had similar root growth patterns; both progressed faster than flax and reached their maximum values earlier than flax. For the wheat control, root length density, surface area, and root tips sharply increased from seedling to boot stage (equivalent to early-flower in oilseeds), and then levelled off to maturity rather than declining. Growth progress of root diameter in wheat followed a similar pattern as oilseed crops and reached the maximum at the soft dough stage. Other studies have also shown that wheat root biomass and root length peaked before anthesis (Barraclough and Leigh 1984; Robertson et al. 1993). In the study, the patterns of progression of root growth during the growing season was similar between the two water treatment regimes (data not shown); thus the results were averaged across the two water treatments (Fig. 3.1). The results suggest that the measurement of the maximum root length density, surface area, and number of root tips can be best made at the late-flower to late-pod stages for oilseeds and during the boot to anthesis period for spring wheat.

At any growth stage, canola always had the higher values for all root parameters, except root tips at seedling stage, than flax and mustard (Fig. 3.1). Compared with the two *Brassica* species, flax always had the lowest values in root length density, root surface area and root tips. Wheat had greater root length density and root surface area, and more root tips than flax at all growth stages.

Root length density gradually increased from seedling stage for all three oilseeds, and the

increment was greatest for canola and smallest for flax (Fig. 3.1A). However, after reaching the maximum value at the late-flower stage, root length densities of canola and mustard declined but the decrease occurred somewhat later for flax at the late-pod stage (Fig. 3.1A). Root surface area of canola was greater than mustard and flax, with the fastest rate of growth occurring during the period from seedling to late flowering (Fig. 3.1B). Canola produces large numbers of root hairs (Johnston 2003) which represent about 70% of the total root surface area (Pez-Bucio et al. 2003). The increased root surface area through large numbers of root hairs is critical for the absorption of soil nutrients, especially P by plants (Johnston 2003). These results probably explain the reason that canola had greater nutrient uptake ability and nutrient use efficiency than mustard (Gan et al. 2008). In terms of root diameters, they had a narrow range of values and were similar among oilseed crops throughout the entire growth period (Fig. 3.1C). Roots with larger diameters tend to have greater root biomass, while smaller diameter roots result in greater surface area of the root system (Eissenstat and Yanai 2002; Waisel and Eshel 2002). The results demonstrated that canola had much finer roots with larger surface areas than mustard and flax, suggesting that canola may have higher efficiency in taking up nutrients and water than mustard and flax.

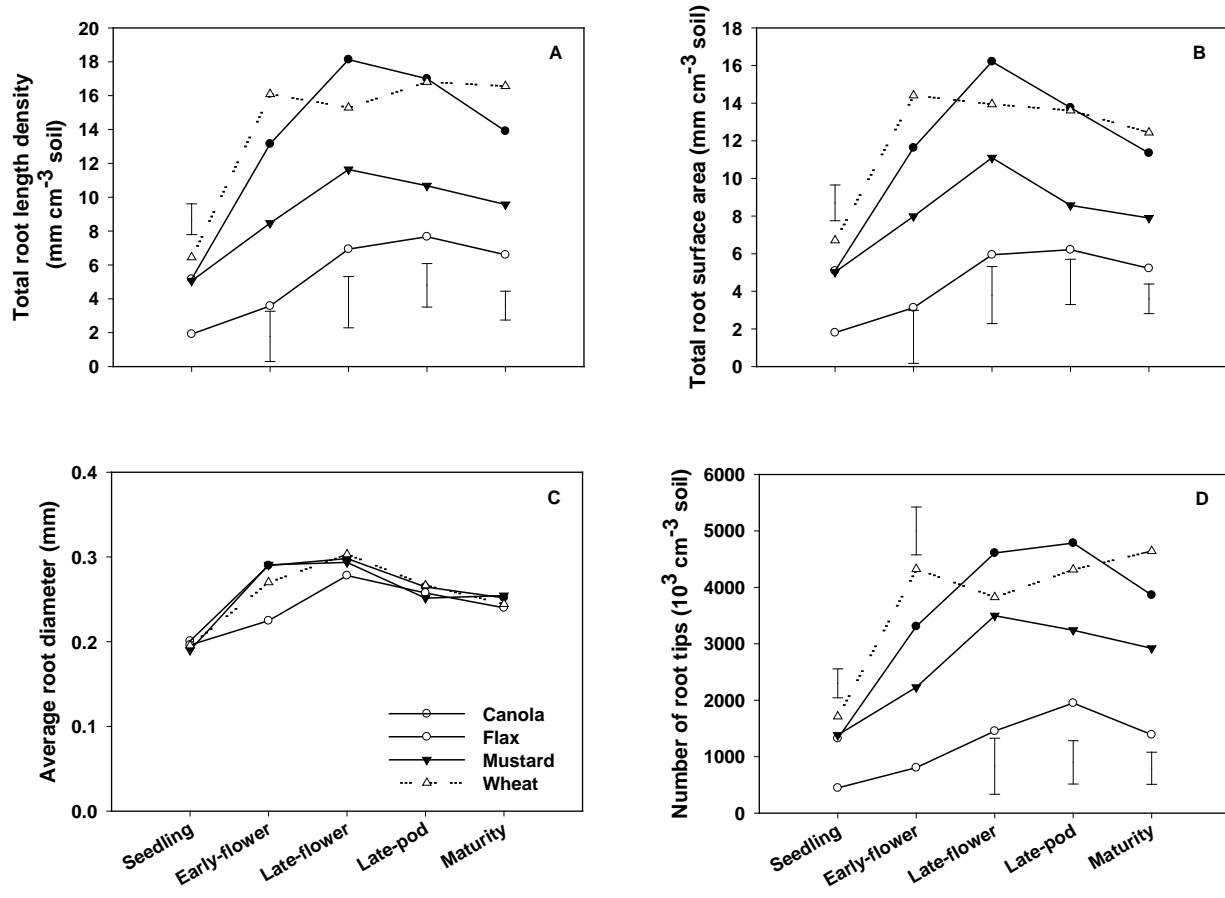


Figure 3.1 The progress of root growth during the growing season for oilseed crops in comparison with wheat control with (A) root length density, (B) root surface area, (C) root diameter and (D) root tips measured in Swift Current, Saskatchewan, Canada, 2006-2007. For wheat, the corresponding stages are: seedling, boot, anthesis, soft dough and maturity. The data shown were averaged over the two water conditions. Vertical bars represent the LSD (0.05) for mean comparison between crops at a given stage.

3.3.4. Root growth patterns of pulse crops

All three pulse crops tested in the experiment had similar patterns of root growth during the growing season; they reached the maximum in root length density (Fig. 3.2A), root surface area (Fig. 3.2B), root diameter (Fig. 3.2C), and the number of root tips (Fig. 3.2D) at the late-flower stage. In general, root length density and surface area increased rapidly from seedling to late-flower, and then declined to maturity. Among the three pulses, field pea had the greater root length density, larger surface area, and more root tips than chickpea and lentil at a given stage before late flowering. However, from late-flower to maturity, these advantages with field pea diminished because of a sharp decline thereafter. In a previous study, Benjamin and Nielsen (2006) demonstrated that root surface area in chickpea and field pea increased from late bloom to mid-pod stages under dryland conditions.

Root diameter is another important variable in morphological trait assessment, since root diameter has a significant influence on the ability of roots to penetrate soil (Materechera et al. 1992), especially a soil with increased strength as it dries (Bengough et al. 2006). Roots with large diameter can exert more force and penetrate deeper into compacted soil areas (Materechera et al. 1992). In the present study, chickpea had larger root diameter than field pea and lentil after flower, and this trend persisted to maturity (Fig. 3.2C). Field pea had consistently smaller root diameters than lentil during the period between late-flower and maturity stage, but the differences were rarely significant. The greater root diameter of chickpea suggests that chickpea plants may have a great ability to cope with water deficit by penetrating roots deeper in the soil profile. Bejamine and Nielsen (2006) also found that chickpea roots had better ability to penetrate deep in the soil profile under water deficit environment than field pea.

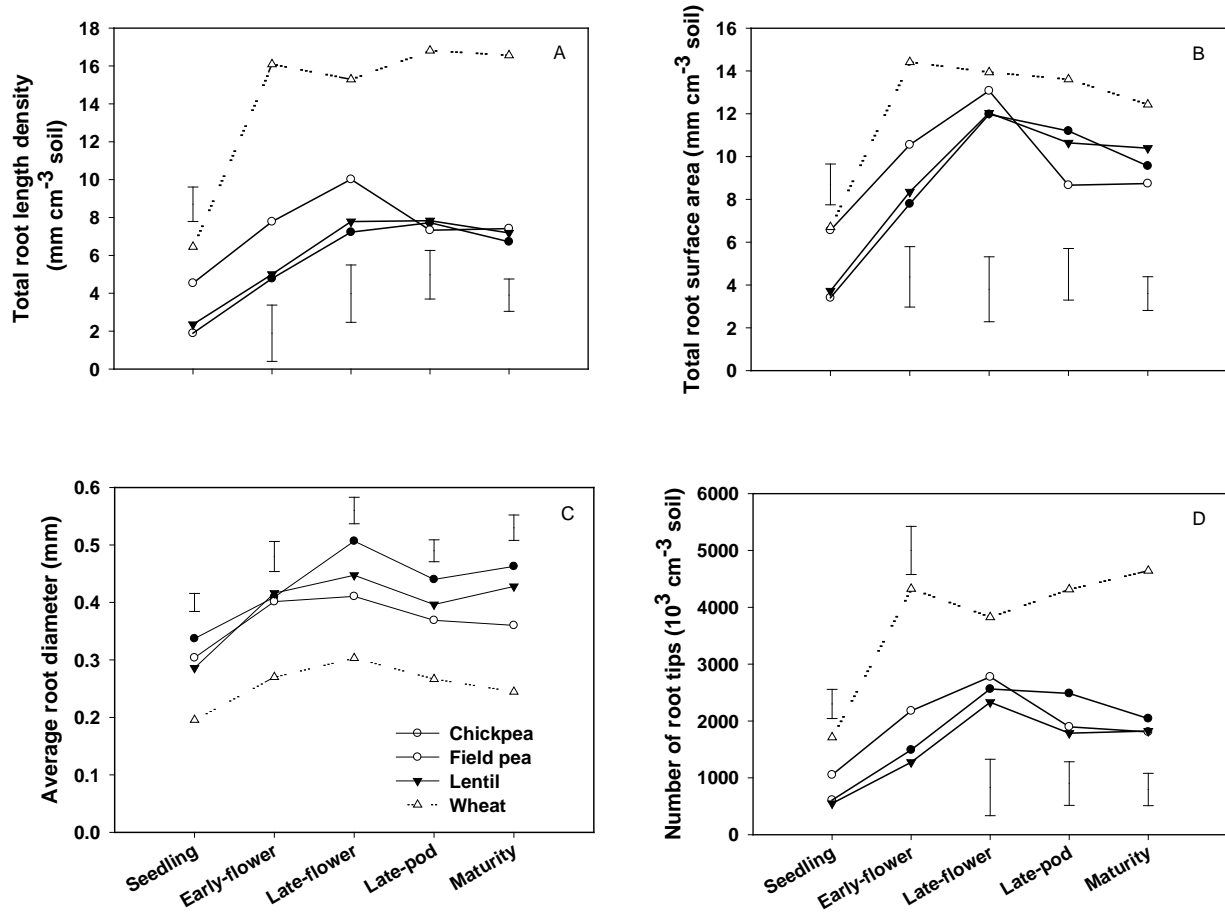


Figure 3.2 The progress of root growth during the growing season for pulse crops in comparison with wheat control with (A) root length density, (B) root surface area, (C) root diameter and (D) root tips measured in Swift Current, Saskatchewan, Canada, 2006-2007. For wheat, the corresponding stages are: seedling, boot, anthesis, soft dough and maturity. The data shown were averaged over the two water conditions. Vertical bars represent the LSD (0.05) for mean comparison between crops at a given stage.

The number of root tips can be a critical indicator to root function from water uptake to regulating whole plant growth (Aiken and Smuker 1996). Thus, the number of root tips is an important determinant of the plants in absorbing water and nutrients from the soil. In our study, field pea always had the greatest number of root tips and lentil the least (Fig. 3.2D), suggesting that field pea has a greater potential of producing biomass than lentil and chickpea. After late flower, the number of root tips of field pea and lentil declined rapidly to maturity, reaching a

similar level to chickpea. Compared to pulses, the wheat control always had greater root length density, higher surface areas, and more root tips than pulses, but the roots of wheat plants were always smaller in diameter than pulses.

3.4. Conclusions

Root growth progressed steadily from seedling to the late-flower or late-pod stages and then declined to maturity; this trend was consistent for all oilseeds and pulses tested. This suggests that scientific measurements of root growth traits and root biomass should be taken at these growth stages in order to achieve accurate assessments. Responses of root growth patterns to water availability varied among crop species. Canola root growth was the most sensitive to water availability and responded positively to irrigation. Increased water availability did not have an effect on the root growth of pulse crops, suggesting that pulses can be best adapted to semiarid environments, whereas canola appeared best adapted to high-water environments. Differences in root morphology were small among the three pulse crops, but field pea had a greater number of root tips from seedling to late-flower stage and presumably the greater capacity to exploit water than lentil and chickpea, demonstrating that field pea has greater potential of producing more biomass and yield than the two other pulses. The two *Brassica* species had similar root length and surface areas as spring wheat, both being greater than pulses and flax, suggesting that the two *Brassica* species are suitable to areas where spring wheat has been dominant. The root information generated from this study may serve as a scientific base for the development of the much steadier cropping systems with oilseed and pulse crops.

4.0 ROOT SYSTEMS OF OILSEED AND PULSE CROPS: DISTRIBUTIONS IN THE SOIL PROFILE

4.1 Introduction

Plant roots are of great importance in plant-soil systems (Lynch et al. 2007) because they carry out essential functions in providing mechanical anchorage to plants, taking up water and nutrients from soil, and regulating plant growth by producing diverse biological compounds (Groff and Kaplan 1988). Root distribution patterns in the soil profile are important determinants of the ability of a crop to acquire nutrients and the water necessary for the growth (Li et al. 2006). The configuration of a root system is a reflection of the ecologically optimized responses of the genetic property of plant roots to environmental factors (Yu et al. 2007). Plant root systems have the morphological and physiological plasticity that makes root distribution vary widely across soil depths in response to the availability of soil water (Smucker and Aiken 1992), distribution of nutrients (Drew et al. 1973), and the physical (Zhuang et al. 2001) and chemical properties of the soil (Feddes and Raats 2004). For instance, when soil is dry on the surface, roots may penetrate deeper in the lower soil layers where water may be available (Huang 2006). A shallow and less proliferated root system may interact with a limited volume of soil from where plants can extract water and nutrients, whereas a deep and more laterally clumped root system may improve water uptake (Passioura 1991). The ability of plants to change root distribution to exploit water deeper in the soil may be an important mechanism to reduce drought stress (Benjamine and Nielsen 2006).

Crop species differ in their root growth and distribution patterns in the soil profile, especially under stress conditions. The root growth of wheat plants, for example, tends to decline in the upper 40 cm of the soil during a drought without compensatory root growth in the sub-soil (Weir and Barraclough 1986). In contrast, cotton plants tend to attain substantial increases in root

density between 70 and 180 cm soil depth in a drought situation (Klepper et al. 1973). Peanut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata* L.) are able to alter root distribution to extract water in deeper soil layers under dry conditions (Pandey et al. 1984). As available water is low in soil, soybean (*Glycine max* L.) and sorghum (*Sorghum bicolor* L.) can extend their roots systems further to deeper soil layers (Merrill and Rawlins 1979; Hoogenboom et al. 1987), whereas spring wheat is able to develop more lateral roots in the top soil layers (Merrill and Rawlins 1979). In the scientific literature, there is a lack of information on root distributions of oilseed and pulse crops in response to growing conditions such as soil water availability. In semiarid regions, precipitation varies from season to season and is often unpredictable even during a growing season (Padbury et al. 2002). Water is the main factor limiting crop production (Campbell et al. 2007). Field crops grown in these areas frequently experience water deficit during their life cycle. One of the approaches to minimize the negative impact of water deficit on crop growth is to rotate crops that have shallow rooting systems with crops that have deep rooting systems; such an approach may increase water use efficiency (Gan et al. 2003; Miller et al. 2003a) and decrease soil nutrient loss by leaching (Gathumbi et al. 2002). Therefore, the inclusion of diverse crop species with varying root distribution patterns can be considered an important crop management strategy to maximize water use efficiency over period of years.

Most studies in rooting patterns have concentrated on cereals (Bland and Dugas 1988; Box et al. 1989; Box and Ramseur 1993), and there is little information available regarding rooting patterns of oilseeds and pulses. In the past two decades, the production of these broadleaf crops has been steadily increased in the semiarid northern Great Plains of North America, and these broadleaf crops play a critical role in cropping systems where they are rotated with cereals in a planned sequence in a rotation system. The objectives of this study were i) to characterize

root distribution patterns in the soil profile for selected oilseed and pulse crops; ii) to examine the effect of water stress on root growth and distribution patterns of these crops under semiarid environments.

4.2 Material and methods

4.2.1 Experiment design

A field study was carried out at the Semiarid Prairie Agricultural Research Centre of Agriculture and Agri-Food Canada, Swift Current (50°15'N, 107°44'W), Saskatchewan, in 2006 and 2007. The field soil was an Aridic Haploboroll soil that is a Swinton loam Orthic Brown Chernozem in Canadian soil classification (Ayers et al. 1985). With silt loam texture, the soil has the content of sand, silt, and clay of 28%, 49%, and 23%, respectively, organic matter of 3.0%, and pH (water paste) 7.3. The experiment field was on wheat stubble. Oilseed crops including canola, flax, and mustard along with three pulses that included chickpea, field pea and lentil, and one spring wheat were hand-planted in lysimeters of 15 cm in diameter and 100 cm in length that were installed in the field using a hydraulic system (Gan et al. 2009b). A representative cultivar was chosen for each crop species based on their production popularity (Table 4.1). All crops were tested under low- (natural rainfall) and high-water (rainfall + irrigation) conditions in each year. The crops under low-water treatments received natural rainfall only (Table 4.2), whereas crops under high-water received natural rainfall plus irrigation at the amount of 150 mm (which was about 2/3 of the long-term growing season rainfall at the experimental site). The irrigation was applied twice during crop growing season, with 75 mm of the water applied between seedling and flowering (2-3 applications) and the other 75 mm applied from flowering to physiological maturity (2-3 applications). Seven crops with two water regimes were arranged in

a factorial, randomized complete block design with two replicates. Each treatment contained 5 sampling times (seedling, early-flower, late-flower, late-pod and maturity) in each replicate. Thus, the experiment had a total of 140 lysimeters (7 crops \times 2 water regimes \times 5 sampling times \times 2 replicates) in each year.

4.2.2 Seedling, root sampling and data collection

All crops were planted in the first week of May in both years. Crop seeds were treated with an effective fungicide before planting to minimize seed- and soil-borne diseases (Table 4.1). At seeding, oilseed crops and wheat received fertilizer (46-0-0) at the rate of 80 kg N ha⁻¹ and superphosphate (0-45-0) at 27 kg P ha⁻¹, and all fertilizers were placed directly on crop seeds. *Rhizobium* was inoculated to pulse crops that received P only. All lysimeters were surrounded by a 2 \times 6 m area of the crop plants that were planted in the lysimeters. A fine-wired cage was installed around each lysimeter to prevent the potential damage from wild animals. The cages were removed at the time of lysimeters removal when sampling took place or when plants such as field pea started to attach tendrils to the cages. Two weeks after emergence, crop seedlings in the lysimeters were thinned to the desired plant population (Table 4.1).

Lysimeters of oilseed and pulse crops were withdrawn from field positions at crop growth stages of seedling, early-flower, late-flower, late-pod, and physiological maturity; for spring wheat, the corresponding stages were seedling, boot, anthesis, soft-dough and maturity, respectively. Crop plants were cut off at the ground level and the number of plants in each lysimeter was counted just before the lysimeters were withdrawn from field positions. Lysimeters were then transported to the laboratory, and stored at 2°C until processing. For the analysis, intact soil cores within the lysimeters were sectioned into 10 cm increments for the first

60 cm of the soil core and 20 cm intervals for the remaining core to a depth of 100 cm. Each soil-root matrix was soaked overnight in containers using 15 ml of calgon water softner for every gallon of water. Roots were washed out of soil manually using 4-mm hole size pan sieves, and then placed in containers full of water with a 0.8-mm screen mounted 3 cm below the water level. Debris and other extraneous materials from the root samples were removed using tweezers.

Table 4.1. Crop cultivars and agronomy information for oilseed, pulse and cereal crops grown in the lysimeter experiment at Swift Current, Saskatchewan, 2006-2007.

Crop	Cultivar	Fungicide			Initial seeds lysimeter ⁻¹	Final plants lysimeter ⁻¹
		Trade name	Active ingredient	Rate (ml 100kg ⁻¹ seed)		
<u>Oilseed crop</u>						
Canola	45H21	Helix	Thiamethoxam	1500	11	3
Flax	Vimy	Vitaflo 280	Carbathiin + Thiram	525	12	4
Mustard	Cutlass	Helix	Thiamethoxam	1500	11	3
<u>Pulse crop</u>						
Chickpea	CDC Anna	Crown	Carbathiin + Thiabendazole	600	5	2
Field pea	Eclipse	Apron FL	Metalaxyl	16	5	2
lentil	CDC Glamis	Crown	Carbathiin + Thiabendazole	600	7	3
<u>Cereal crop</u>						
Wheat	Lillian	Vitaflo 280	Carbathiin + Thiram	330	7	3

Table 4.2. The amounts of water (mm) that crop received during the different growth stages under low- and high-water conditions at Swift Current, Saskatchewan, 2006-2007.

Crop stage ^a	2006		2007	
	Low ^b	High ^b	Low ^b	High ^b
Seedling	131	156	98	136
Early-flower	14	41	17	40
Late-flower	22	45	5	22
Late-pod	1	14	0	28
Maturity	12	28	10	52
Total	180	284	130	278

50

^a For oilseed and pulse crops, root samples were taken in 2006 on DOY (day of year) of 178 (Seedling), 187-199 (Early-flower), 207 (Late-flower), 214 (Late-pod), and 223-233 (Physiological maturity); in 2007 the corresponding sampling days were 173-177, 184-193, 193-201, 204-207, and 214-219, respectively; for wheat the corresponding stages were: seedling, boot, anthesis, soft dough, maturity.

^b Low-water means crop received natural rainfall only, and high-water means crops received natural rainfall plus irrigation with a amount of 150 mm.

The entire cleaned root samples of each soil segment were sub-sampled; one part was used for dry weights (Gan et al. 2009a) and the other portion was used for root scanning. Roots were stained with 0.08% Toliudine Blue O (Sigma USA) for 5 min, and then rinsed thoroughly with distilled water. Rinsed root samples were placed on a tray and scanned with a “WinRHIZO” scanner (Regent Instruments Inc. Quebec, Canada) at a resolution of 400 dpi. Scanned root images from each depth increment sample were analyzed using the WinRHIZO program (Version 2003b) for root length, root surface area, root diameter, and number of root tips. Root length density (mm cm^{-3}), root surface area density ($\text{mm}^2 \text{cm}^{-3}$) and root tips (10^3cm^{-3}) of each soil segment were calculated by dividing the total root length (mm), root surface area (mm^2) and number of root tips in each soil segment by the volume (cm^3) of the corresponding soil core.

4.2.3 Statistical analysis

The data on distribution patterns of root length density, root surface area, root diameter and number of root tips in the soil profile were subjected to analysis of variance using the MIXED procedure of SAS (Littell et al. 1996). The means of difference were considered significantly different if the probability level was at $P \leq 0.05$.

4.3. Results

4.3.1 Root distribution patterns of oilseeds and pulses in the soil profile

The growth of roots progressed rapidly with the growth of the plants, but the distribution of root length, root surface area, and the number of root tips in the soil profile followed a similar pattern from one growth stage to the other (data not shown). By the late-flower stage, the values of root growth reached a maximum when large differences in the distribution pattern were shown

among crop species. Therefore, only the results of crop roots at the late-flower stage are presented and the data shown were the average of the two water conditions.

Across the rooting depth from 0 to 100 cm, the root growth of all crop species decreased substantially with increased soil depth. Among the three oilseeds, canola always had the greatest root length density and flax the smallest across the soil profile (Fig. 4.1A). The highest root length density was observed in the top 10 cm soil for the two *Brassica* species but that for flax was in 10-20 cm soil layer. Root surface area of the three oilseeds followed the same distribution patterns as root length density (Fig. 4.1B). The three oilseeds had similar root diameter across the soil profile and this diameter was essentially the same in the soil depth from 40 cm to 80 cm (Fig. 4.1C). The number of root tips of the *Brassica* species was greater than that of flax across the soil profile (Fig. 4.1D). It was also observed that within the soil profile, wheat had similar root surface area and number of root tips as those of *Brassica* species (Fig. 4.1B and Fig 4.1 D).

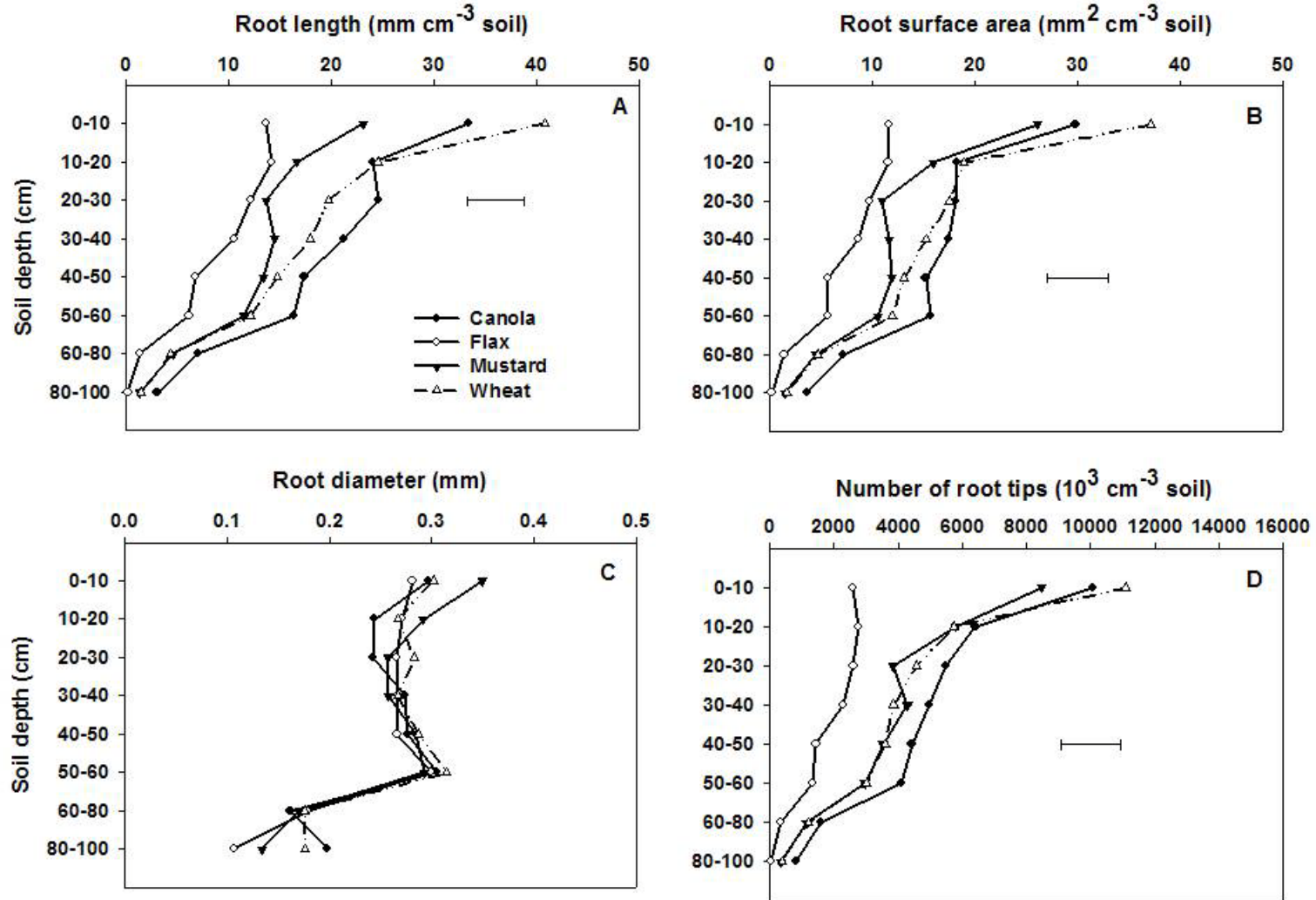


Figure 4.1 Distributions of (A) root length, (B) root surface area, (C) root diameter and (D) number of root tips for oilseed crops at late-flower stage, Swift Current, Saskatchewan, 2006-2007. Horizontal bars represented the LSD at level of 0.05.

For pulse crops, root length density was smaller than those of wheat throughout the entire soil profile (Fig 4.2A). However, field pea and lentil attained a similar root surface area as wheat plants throughout the soil profile (Fig. 4.2B). The average root diameters of pulses were significantly larger than the root diameters of wheat in the 0-60 cm soil depth (Fig. 4.2C), while they did not differ below the 60-cm depth. The number of root tips in wheat plants was significantly greater than those of the pulse plants in the top 10 cm soil, but field pea and chickpea had a similar number of root tips as wheat plants below 40-cm soil depth (Fig. 4.2D).

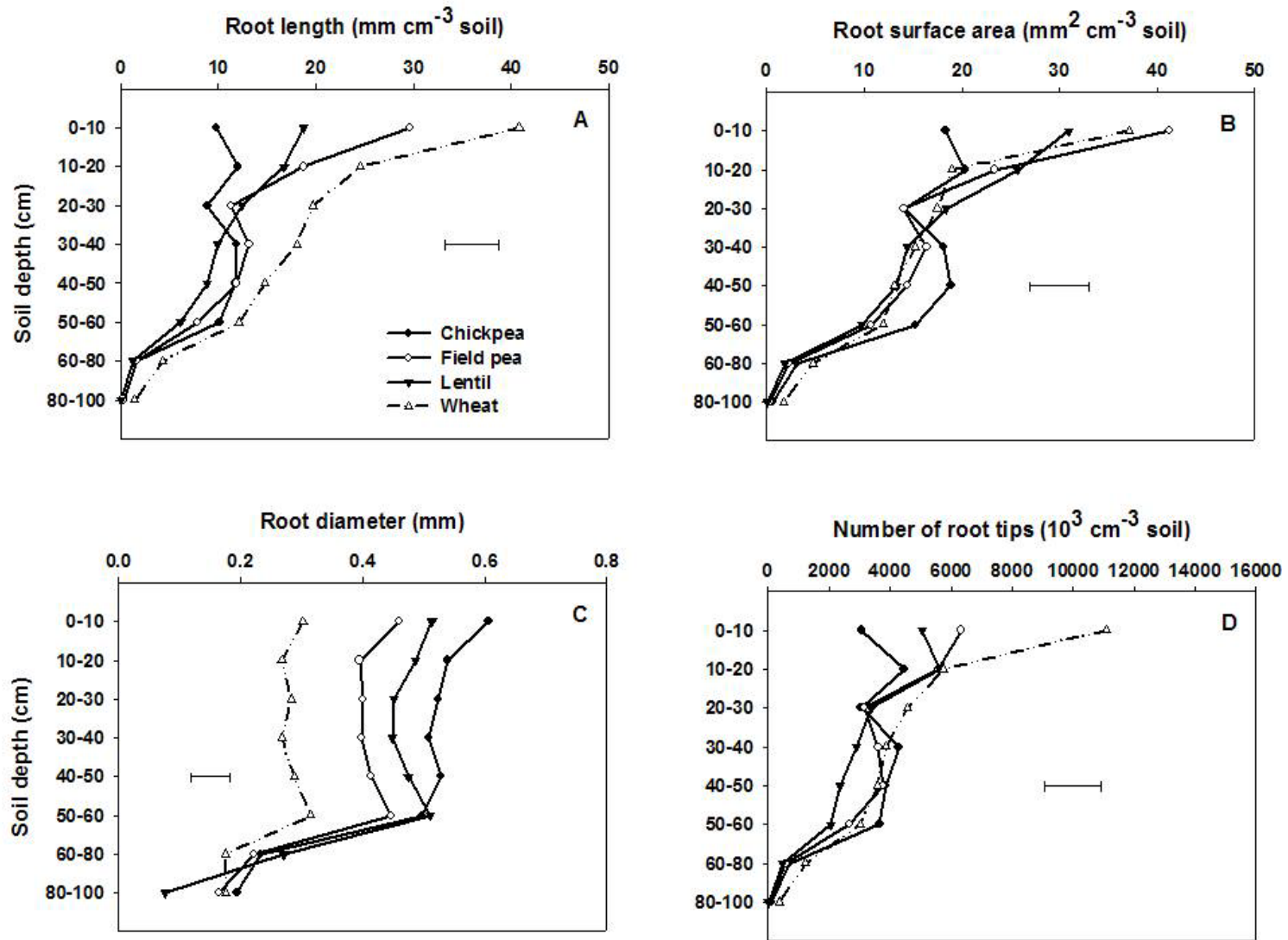


Figure 4.2 Distributions of (A) root length, (B) root surface area, (C) root diameter and (D) number of root tips for pulse crops at late-flower stage, Swift Current, Saskatchewan, 2006-2007. Horizontal bars represented the LSD at level of 0.05.

4.3.2 Water effects on root distributions of oilseeds and pulses

Root length, root surface area, and the number of root tips were significantly affected by water availability at the late-flower stage, but the magnitude of the effect varied with crop species (Fig. 4.3 and Fig 4.4). Canola had significantly greater root length (Fig. 4.3A), larger root surface area (Fig. 4.3B), and more root tips (Fig. 4.3C) from the top to the soil depth of 50 cm under high-water compared to those under low-water conditions. In contrast, water availability had a marginal effect on those root variables in flax and mustard.

Chickpea plants had greater root length (Fig. 4.4A), root surface area (Fig. 4.4B), and the number of root tips (Fig. 4.4C) under the high-water conditions compared to low-water, but the differences were not statistically significant. In contrast, under low-water, field pea had 75% greater ($P<0.01$) root length (Fig. 4.4D) and 74% larger root surface area (Fig. 4.4E) than those under high-water conditions in the 0-10 cm soil. In the 10-20 cm soil, the opposite was observed: field pea had 96% more root length and 104% greater root surface area under high-water conditions. Below 20 cm soil depth, none of these root variables differed between the two water conditions. Lentil plants had longer roots (Fig. 4.4X) with greater root surface area (Fig. 4.4Y) under low-water conditions only in the 30-60 cm soil depth than the plants grown under high-water conditions. A greater number of root tips (Fig. 4.4Z) in lentil plants were also observed when the crop was grown under high-water conditions.

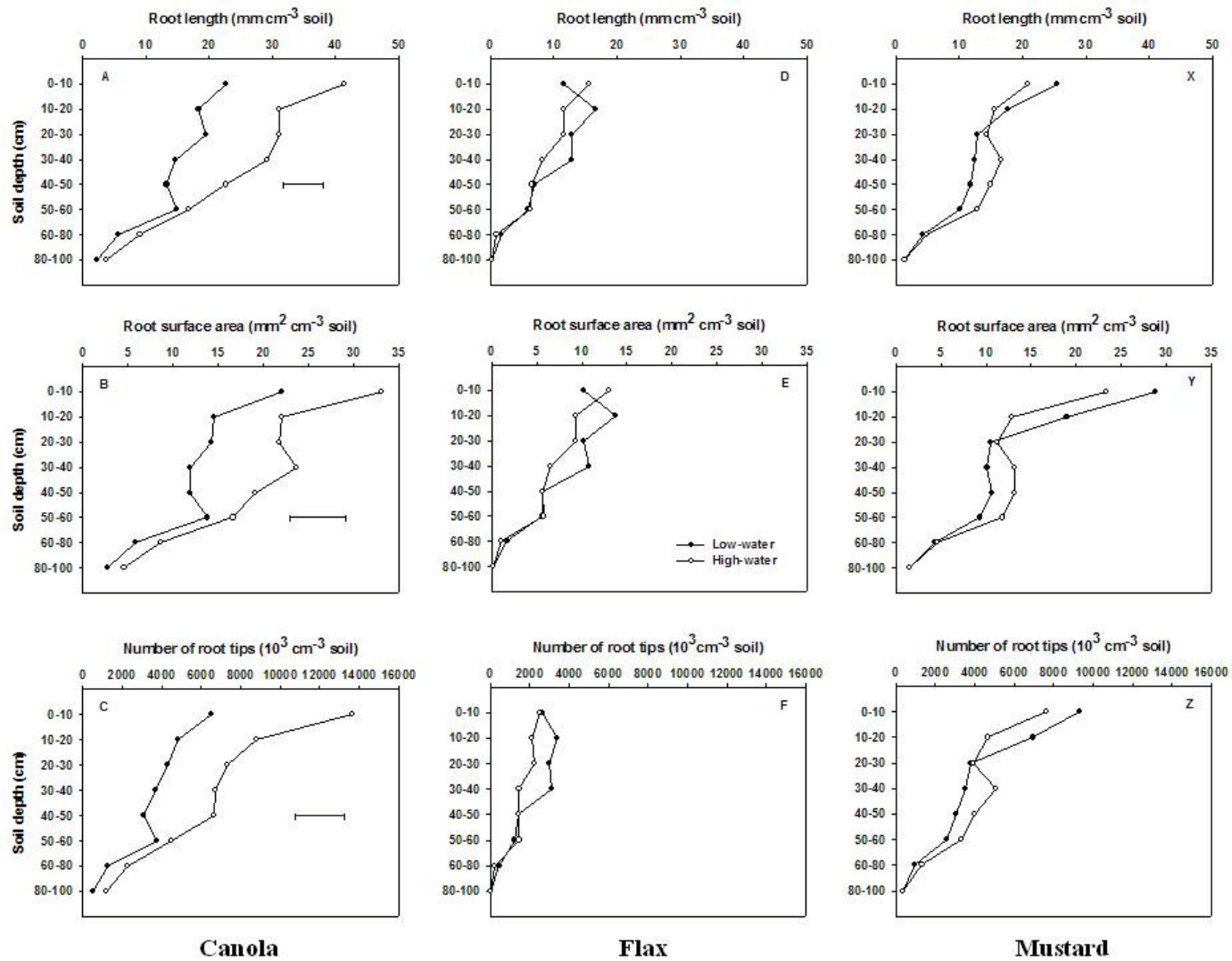


Figure 4.3 Root distributions of oilseed crops under low- and high-water conditions at late flower, Swift Current, Saskatchewan, 2006-2007. Horizontal bars represented the LSD at level of 0.05. A-C are root parameters of canola, D-F are root parameters of flax and X-Z are root parameters of mustard.

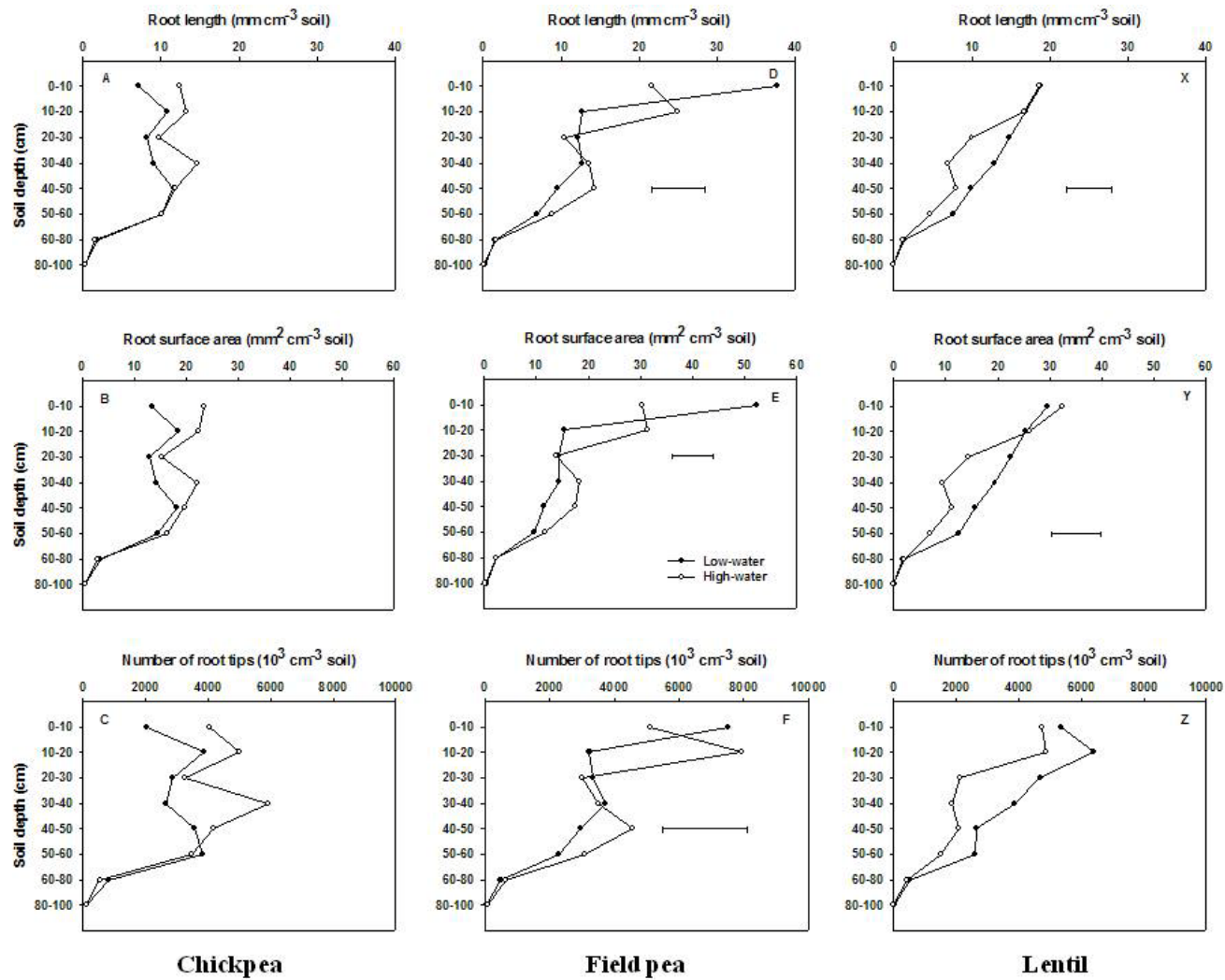


Figure 4.4 Root distributions of pulse crops under low- and high-water conditions at late flower, Swift Current, Saskatchewan, 2006-2007. Horizontal bars represented the LSD at level of 0.05. A-C are root parameters of chickpea, D-F are root parameters of field pea and X-Z are root parameters of lentil.

Overall, root length density, surface area, and the number of root tips decreased with the increase of soil depth, and the trend was true for all crop species tested (Table 4.3). The largest proportion of roots was consistently concentrated in the 0-20 cm soil depth for all crop species, regardless of water conditions, and wheat plants always had roots with greater length density, larger surface area, and more tips than oilseeds and pulses in a given depth across the entire soil profile.

Table 4.3. Distribution of root length density (RLD), root surface area (RSA) and root tips (RT) of oilseeds, pulses and wheat in different soil depths under low-and high-water conditions. The data shown are averaged across all stages and years. For each crop, the values of RLD, RSA and RT, their proportion of the total (%) and ratios (%) between oilseeds and wheat and ratios (%) between pulses and wheat are indicated. The units of RLD, RSA and RT are mm cm^{-3} , $\text{mm}^2 \text{cm}^{-3}$ and $10^3 \# \text{cm}^{-3}$ respectively.

Soil depth (cm)	Low-water					High-water										
	Oilseeds (O)		Pulses (P)		Wheat (W)	O/W Ratio	P/W Ratio	Oilseeds (O)		Pulses (P)		Wheat (W)	O/W Ratio	P/W Ratio		
	RLD	%	RLD	%	RLD	%	%	%	RLD	%	RLD	%	RLD	%	%	%
0-20	27.0	40	33.9	50	60.0	48	45	57	31.7	41	33.5	47	68.4	51	46	49
20-40	21.8	33	19.2	28	32.8	26	66	59	23.9	31	20.9	29	34.6	26	69	60
40-60	15.5	23	12.7	19	27.5	22	56	46	17.9	23	14.7	21	26.3	20	68	56
60-80	2.1	3	1.5	2	3.1	3	68	48	2.3	3	1.8	2	3.7	3	62	49
80-100	0.6	1	0.3	1	0.9	1	67	33	0.9	1	0.4	1	1.1	1	82	36
	RSA	%	RSA	%	RSA	%	%	%	RSA	%	RSA	%	RSA	%	%	%
0-20	28.7	42	43.4	53	50.9	48	56	85	31.8	42	41.8	50	55.7	50	57	75
20-40	21.0	31	21.7	26	26.4	25	80	82	22.7	30	22.8	27	29.2	26	78	78
40-60	16.1	23	14.9	18	23.6	22	68	63	18.5	24	16.2	20	22.7	20	81	71
60-80	2.3	3	1.7	2	3.2	3	72	53	2.5	3	1.9	2	3.6	3	69	53
80-100	0.7	1	0.3	1	1.0	1	70	30	1.0	1	0.4	1	1.2	1	83	33
	RT	%	RT	%	RT	%	%	%	RT	%	RT	%	RT	%	%	%
0-20	7753	42	8719	48	16163	51	48	54	8769	42	8989	46	19057	53	46	47
20-40	5760	31	5240	29	7869	25	73	67	6471	31	5524	28	8788	24	74	63
40-60	4232	23	3589	20	6671	21	63	54	4868	23	4262	22	6970	19	70	61
60-80	585	3	459	2	755	2	77	61	554	3	542	3	1089	3	51	50
80-100	168	1	84	1	304	1	55	28	229	1	122	1	293	1	78	42

4.3.3 Root distributions of oilseeds and pulses in different years

Year had a large influence on the root morphology in the study, but the magnitude of the influence varied with crop species and the soil depth. Root length density of oilseeds in 2007 was higher than that measured in 2006 but a significant difference was shown only in the 20-40 cm soil depth (Fig. 4.5A). In 2006, pulse plants attained greater root length density (Fig. 4.5D) and larger root surface area (Fig. 4.5E) within the 0-50 cm soil than those in 2007. In contrast, wheat plants had significantly greater root length density (Fig. 4.5X), larger root surface area (Fig. 4.5Y) and more root tips (Fig. 4.5Z) in 2007 than those in 2006 but the significant difference was only found in the 0-20 cm soil depth. Overall, the distribution of root surface area for oilseeds, pulses and wheat in the two years followed the same patterns as root length density (data not shown). In both years, the number of root tips in wheat plants appeared to be greater than those of oilseeds and pulses across the entire soil profile especially in the top 10 cm soil depth.

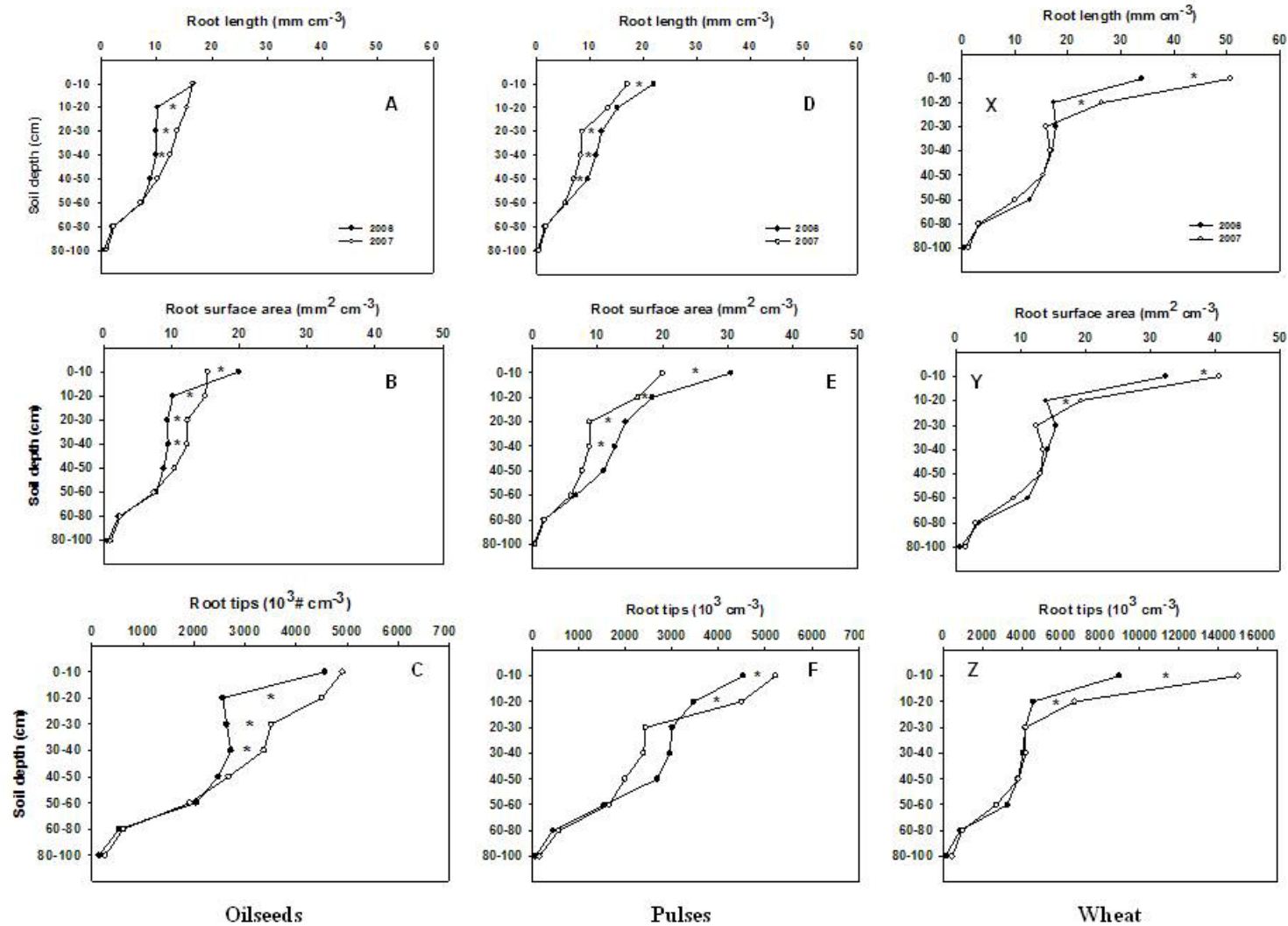


Figure 4.5 Root distributions of oilseeds, pulses and wheat in 2006 and 2007. * indicates significance at $P < 0.05$ probability level; blank is not significant. A-C are root parameters of oilseeds, D-F are root parameters of pulses and X-Z are root parameters of wheat.

4.4 Discussion

4.4.1 Root distribution patterns of oilseeds and pulses in the soil profile

Root growth in the soil profile decreased with increased soil depth; this trend was consistent for all crop species tested in the study. Results were in accordance with the results presented by Yu et al. (2007) who indicated that in most soil profiles root density decreases exponentially with soil depth. Crop species differed substantially in their root morphology under the same growing conditions. Among oilseeds, canola plants had the root system with greater root length, larger surface area and more root tips across the entire soil profile than mustard and flax. Large rooting systems may facilitate canola plants to take up more water and nutrients from the soil, inferring that canola crops should perform better than mustard and flax under conditions where water is the major limiting factor. In a recent study, Gan et al. (2008) demonstrated that canola plants take up more N than mustard resulting in greater productivity under semiarid growing conditions.

In the present study, roots were assessed in 10cm or 20cm increment across the soil profile of 100 cm. Most of the root length, root surface area and root tips seen for the whole profile were distributed in the top 0-20 cm soil depth. This distribution pattern was consistent for all crop species in both years and the pattern was similar under both water treatments. These results suggest that the root distribution patterns of field crops in a soil profile will be relatively consistent even though the growth can be influenced to a great extent by environmental conditions. In addition, the results strongly illustrated that the substantial portion of the crop root system was concentrated in the shallower soil layer rather than deeper soil layers. In most cases, crops have root growth that begins in the layer closest to the soil surface, and roots grow and develop for a longer time than those deeper layers (Adiku et al 1996). Soil nutrients are often

concentrated in the upper layers of the soil and plant roots tend to proliferate preferentially in the region with high nutrient content (Birch and Hutchings 1994; Van Vuuren et al. 1996). Additionally, soil water is readily available in the top soil layers in early spring and after rains during the season; this water distribution may provide crop roots with more favorable growing environments (Ben-Asher and Silberbush 1992; Gallardo et al. 1994). Therefore, the upper soil layers are the preferential location for crop root growth.

In this study root diameters of oilseed crops were of a similar size to wheat; however, pulse plants had thicker roots than oilseeds and wheat, indicating that pulse root penetration into the soil may help improve soil physical property by loosening micro pores of the soil in the vertical direction. Thomas and Hammer 1995 indicated that chickpea had much greater root diameter and roots were more resilient than those of cereal. Root diameter has often been considered one of the more important root properties to evaluate plant adaptability to varying growing environments (Xie et al. 2006) including soil aeration, temperature, nutrient status, physical impedance, and soil microorganisms (Fitter 1985; Macduff et al. 1986; Price et al. 1989).

4.4.2 Water availability and root distributions of oilseeds and pulses

Water availability had a large impact on root distribution patterns but the intensity of the influence varied, depending on crop species. Among all the crops studied, canola responded with significantly greater root length density, larger root surface area and more root tips present in the 0-50 cm depth under high-water availability. In a study conducted in Australia, Kirkegaard et al. (1997) indicated that root length density and rooting depth for canola were highly related to soil water availability. In the present study, chickpea plants had longer roots with larger surface area and greater number of tips under high-water conditions than under low-water, but the difference

was only observed in the 0-40 cm depth. Benjamin and Nielsen (2006) also found that irrigation increased root surface area density for chickpea only in the topmost soil layer. In contrast with canola and chickpea, field pea root growth within the top 20 cm soil presented the opposite trend of greater root length, larger root surface area and more root tips under low-water. This suggested that field pea root growth in the top soil layer might be stimulated by somewhat lower water availability. The responses of flax and lentil to water conditions were similar, both having more roots under low-water conditions. Among the factors affecting plant growth, water is most crucial that greatly influences the below-ground root distribution patterns. A drying soil surface with high water stress often 'forces' roots to grow in the lower, wetter soil layers (Adiku et al. 1996). However, frequent irrigation can help maintain the top soil wet for a longer period of time, thus most of the crop root systems are found in the upper part of the soil profile (Klepper 1991). Generally, a well-watered crop has more root length density in the surface soil and it decreases with soil depth (Klepper et al. 1973).

4.4.3 Root distributions and year effect

In semiarid environments, dry and hot weather depletes soil moisture rapidly and the plant growth largely depends on rainfall during the growing seasons. In 2007, the growing season rainfall was 130 mm, 28% less than that obtained in 2006. The drier weather in 2007 appeared to stimulate root growth for oilseeds and wheat but it decreased root growth for pulse crops. In 2007, root length density and root tips of oilseeds were significantly greater in the 20-40 cm soil depth than those in 2006; and this was also true for wheat although only in the 0-20 cm soil depth. In contrast, pulse roots presented an opposite tendency with larger root length and more root tips in the wetter year than those in dryer year, suggesting that root growth of pulse

crops is reduced in years of reduced water availability. In previous studies, Hoogenboom et al. (1987) and Merrill et al. (2002) found the greatest root growth of soybean (*Glycine max* L.) and dry bean (*Phaseolus* spp.) took place in driest years.

4.5. Conclusions

Root length density, surface area, and the number of root tips all decreased rapidly with the increase of soil depth from 0 to 100 cm; this trend was consistently similar for all crop species tested in the study. Substantial portions (>70%) of the roots recovered in oilseeds and pulses were distributed within the 0-60 cm soil profile and the largest proportion of roots was found in the top 20-cm soil depth. These results indicate that intensive water and nutrient uptake by those crops are mostly in the top soil layers under semiarid growing conditions. Among the seven crop species tested, canola root growth was most sensitive to soil water conditions; the higher the water availability in the soil, the greater the growth of the roots. Conversely, the root growth of pulse crops responded to soil water availability differently than canola; the lower the soil water availability, the greater the root growth. The root growth of wheat, flax, and mustard had marginal responses to soil water conditions. These results suggest that canola may be more suitable for high-water environments, and that pulses (pea, lentil and chickpea) exhibit greater ability to tolerate drought due to their strong tendency to grow roots under dry conditions. Also, pulse plants have the thickest root systems (i.e., roots with the largest diameters), among the species tested, which may facilitate root penetration into the soil profile under dry conditions. The knowledge of the root morphological characteristics of various crop species generated from this study can be used by breeders to develop cultivars with high drought tolerance, and for producers to optimize root growth and development to reduce risks associated with drought in

semiarid environments.

5.0 FINE ROOT DISTRIBUTIONS OF OILSEED AND PULSE CROPS

5.1. Introduction

The growth of a plant root system is hierarchical. Compared with higher order roots, lower order roots have smaller diameters, shorter lengths, less mass per length unit, and shorter life spans. Therefore, the substantial proportion of a plant's root is found in the finer and smaller classes of size within root systems (Merrill et al. 2002). In general, roots of larger diameter represent most of the biomass for a root system and they can also form the long-distance transport pathways delivering water and nutrient. Furthermore, this class of roots performs the functions of storing resources, anchoring the plant and supporting the development of lateral roots. However, roots of smaller diameter make up most of the surface area for the entire root system, and they are the location responsible for the exchange of water and nutrients (Eissenstat and Yanai 2002; Waisel and Eshel 2002).

Fine roots are stated as roots of less than 2mm in diameter by classic description (Böhm 1979); however, most plant fine roots are much smaller. In the literature, fine roots of hardwoods, crop species, forages, and weeds have been measured down to 0.06mm in diameter (Lyford 1975; Zobel 2005). Even of a diminutive size, fine roots are important physiological components for a plant, comprising the majority of the root system surface area and 90% or more of the total root length (Zobel et al. 2007). Fine roots are likely the prominent sink for carbon acquired in terrestrial net primary productivity (Nadelhoffer and Raich 1992; Vogt et al. 1986; Hendrick and Pregitzer 1994; Caldwell and Richards 1986), and are the primary component in carbon and nutrient cycling in an ecosystem (Matamala et al. 2003; Trumbore and Gaudinski 2003). In most cases, the primary production allocated below ground is greater than that above ground, and annual carbon and nutrient inputs to the soil from fine roots frequently equal or exceed those of

above-ground leaves (Nadelhoffer and Raich 1992; Vogt et al 1986; Hendrick and Pregitzer 1994; Caldwell and Richards 1986). Moreover, the turnover of fine roots is usually faster than other components of a plant so the total carbon returned to soil via fine roots exceeds the decomposition of litter fall (Vogt et al. 1996; Gill and Jackson 2000; Nadelhoffer 2000). For example, a study on short-grass steppe demonstrated that fine roots are the major contributors to soil organic matter and soil carbon pools due to their rapid turnover (Gill et al. 2002).

Being crucial in nutrient cycling and resource capture, fine roots have been studied and documented well, especially for forest trees and in some cases, vegetable (Pietola and Smucker 2006) and herbaceous species (Gill et al. 2002; Zobel et al. 2006). However, the documentation of fine roots for annual planting crops is very limited, especially for oilseeds and pulses. Therefore an experiment was conducted to assess the contribution of fine roots to the root morphological traits including total root length, surface area, root volume and number of root tips for oilseeds and pulses in a semiarid growing condition. This research could provide unique information on the fine root constitution of alternative crops that can serve as the science basis for the further study on root nutrient uptake processes, functionality and dynamics.

5.2. Material and methods

5.2.1 Site description and experiment design

A field study was conducted at the Semiarid Prairie Agricultural Research Centre of Agriculture and Agri-Food Canada, Swift Current (50°15'N, 107°44'W), Saskatchewan, 2006-2007, on an Orthic Brown Chernozem (Aridic Haploboroll) soil with silt loam texture; the content of sand, silt, and clay was 28%, 49%, and 23%, respectively, organic matter of 3.0%, and pH (water paste) 7.3. The experiment field was on wheat stubble. Three oilseeds (canola, flax,

mustard), three pulses (chickpea, field pea, lentil), and spring wheat (the control) were hand-planted in lysimeters of 150 mm in diameter and 1.0 m in length that were installed in the field using a hydraulic system (Gan et al. 2009b). Seven crops were arranged in a factorial, randomized complete block design with two replicates. Crops were planted in the first week of May of 2006 and 2007. Oilseed crops and wheat received fertilizer (46-0-0) at the rate of 80 kg N ha⁻¹ and superphosphate (0-45-0) at 27 kg P ha⁻¹ at seeding. Fertilizers were applied on crop seeds directly. Pulse crops were inoculated with *Rhizobium* and received P only. The lysimeters were surrounded by a 2×6 m area of the same crop plants as were planted in the lysimeters.

5.2.2 Root sampling and data collection

Roots were sampled at seedling, early-flower, late-flower, late-pod, and physiological maturity (for wheat, the corresponding stages were seedling, boot, anthesis, soft-dough and maturity, respectively). Soil cores within the lysimeters were sectioned into 10 cm increments for the first 60 cm and 20 cm intervals for the remaining depths to 100 cm. Each soil-root matrix was soaked overnight in water. Crop roots were washed out of soil manually using 4-mm hole size pan sieves, and then placed in containers full of water with a 0.8-mm screen mounted 3 cm below the water level. Debris and other extraneous materials from the root samples were removed using tweezers. The cleaned root samples were stained with 0.08% Toliudine Blue O (Sigma, USA), rinsed thoroughly with distilled water, and analysed with a “WinRHIZO” system (Regent Instruments Inc. Quebec, Canada). Mean root length, surface area, diameter, and number of tips of each segment of the root systems were determined, and then these variables were categorized into six diameter classes, i.e., the roots with the diameter 0-0.2 mm, 0.2-0.4 mm, 0.4-0.6 mm, 0.6-0.8 mm, 0.8-2.0 mm, and > 2.0 mm.

5.2.3 Statistical analysis

The proportion of roots in each of the six classes was calculated as percentage of the total roots present. The data were analyzed using the MIXED model of SAS (Littell et al. 1996). Two years of data were combined since there was no year by treatment interaction. Two water conditions did not have significant effects thus in data analysis, the two water conditions were treated as 2 replicates. Significant differences between treatments were declared at $P \leq 0.05$.

5.3. Results and discussion

Among the seven crops investigated, wheat had the greatest mean root length density totaling 14.2 mm cm^{-3} for the full 100-cm soil profile, followed by canola (13.5 mm cm^{-3}) and mustard (9.1 mm cm^{-3}), with flax and lentil the lowest (5.3 mm cm^{-3}). Root surface area for the entire soil profile followed the same order among the seven crops as root length density. However, the average root diameters were in the order of chickpea (0.43 mm), lentil (0.39 mm), field pea (0.37 mm), canola (0.26 mm), wheat (0.26 mm) and mustard (0.25 mm). Mean root volume in a cubic centimeter was in the order of chickpea with 1.14 mm^3 for the 100-cm soil profile, followed by lentil ($1.10 \text{ mm}^3 \text{ cm}^{-3}$), field pea ($1.05 \text{ mm}^3 \text{ cm}^{-3}$), canola ($0.83 \text{ mm}^3 \text{ cm}^{-3}$), mustard ($0.63 \text{ mm}^3 \text{ cm}^{-3}$) and flax ($0.63 \text{ mm}^3 \text{ cm}^{-3}$). These results indicated that wheat and canola plants had stronger rooting systems that were capable of extracting water and nutrients from deeper soils due to their longer roots, whereas pulse crops had larger root diameters and had greater potential to affect soil physical properties by vertical penetration into soil.

For the oilseed crops investigated, roots in the 0-0.2 mm diameter class comprised of about 60% of the total root length, and those in the 0.2-0.4 mm diameter class about 30% (Fig. 5.1A). The roots with larger ($>0.4 \text{ mm}$) diameters only contributed a small proportion of the total root length. Three oilseed crops exhibited similar root length distribution patterns as wheat. For

the root surface area (Fig. 5.1B), the greatest proportion was comprised of roots belonging to the 0.2-0.4 mm diameter class, followed by the 0-0.2 mm diameter category; together contributed over 60% of total root surface area. The rest of the root surface areas were equally divided into roots within 0.4-0.6, 0.6-0.8, and 0.8-2.0 classes. Similarly, the roots in the 0.2-0.4 mm diameter contributed the highest proportion to the total root volume compared with the roots in other diameter classes (Fig. 5.1C). The thicker roots belonging to the 0.4-0.6 mm, 0.6-0.8 mm, and 0.8-2.0 mm diameter classes each contributed an equivalent portion to the total root volume. However, it was noticeable that the roots with diameter > 2.0 mm contributed over 20% to the total root volume in the two *Brassica* species. Unlike the three variables described above, the majority of root tips of oilseeds and wheat were in the 0-0.2 mm diameter class (Fig. 5.1D), and the roots in the >0.2 mm diameter contributed only a small portion to the total number of root tips. The above results showed that wheat and oilseed crops differed substantially in terms of absolute values in root length, root surface area, root volume, and root tips, but their distribution patterns in the six diameter classes followed a similar trend. For both wheat and oilseed crops, 60-80% of root length and surface area were in the <0.4 mm diameter classes and $>80\%$ of the root tips were in the 0-0.2 mm class. These results suggest that roots <0.4 mm in diameter were the predominant contributors to the rooting system for both wheat and oilseed crops.

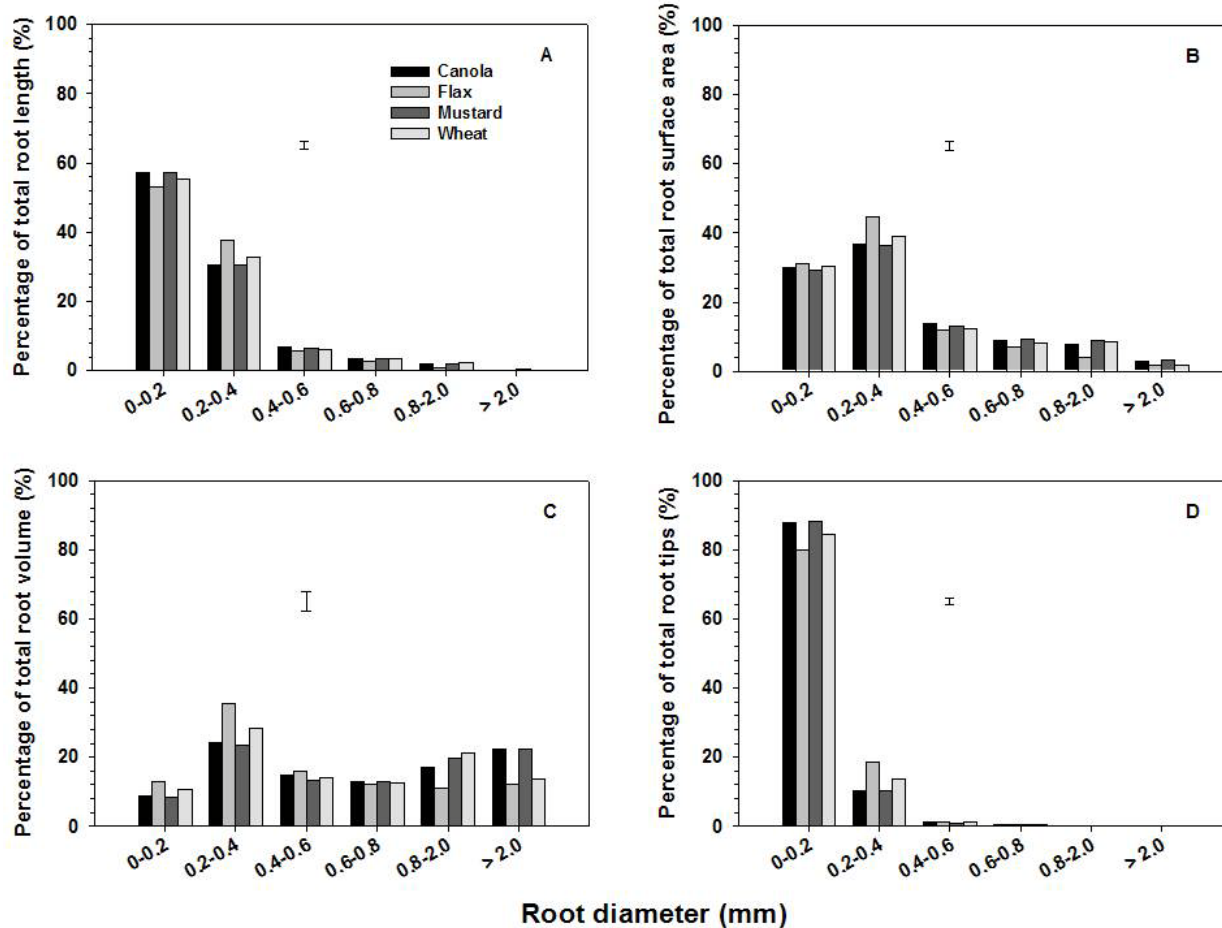


Figure 5.1 Fine root distribution of oilseed crops, Swift Current, Saskatchewan, 2006-2007. Vertical bars represent the LSD at level of 0.05.

In contrast, pulse crops had quite different root distribution patterns (Fig. 5.2) from oilseed crops (Fig. 5.1). The percentage of total root length (Fig. 5.2A) and root surface area (Fig. 5.2B) for pulses in the 0-0.2 mm diameter class were significantly smaller compared to that of wheat. The opposite was also true in that the proportion of roots measured by root length and surface areas in the thicker roots (in the 0.4-0.6, 0.6-0.8, and 0.8-2.0 diameter classes) for pulses were significantly greater than those for wheat. There was a trend that the proportion of root surface area increased within the thicker diameter classes for chickpea and lentil, and roots in the diameter of 0.8-2.0 mm contributed the greatest surface areas (Fig. 5.2B) for chickpea. Thicker

roots with the diameter in 0.8-2.0 mm contributed the greatest proportion to the total root volume for all three pulses (Fig. 5.2C).

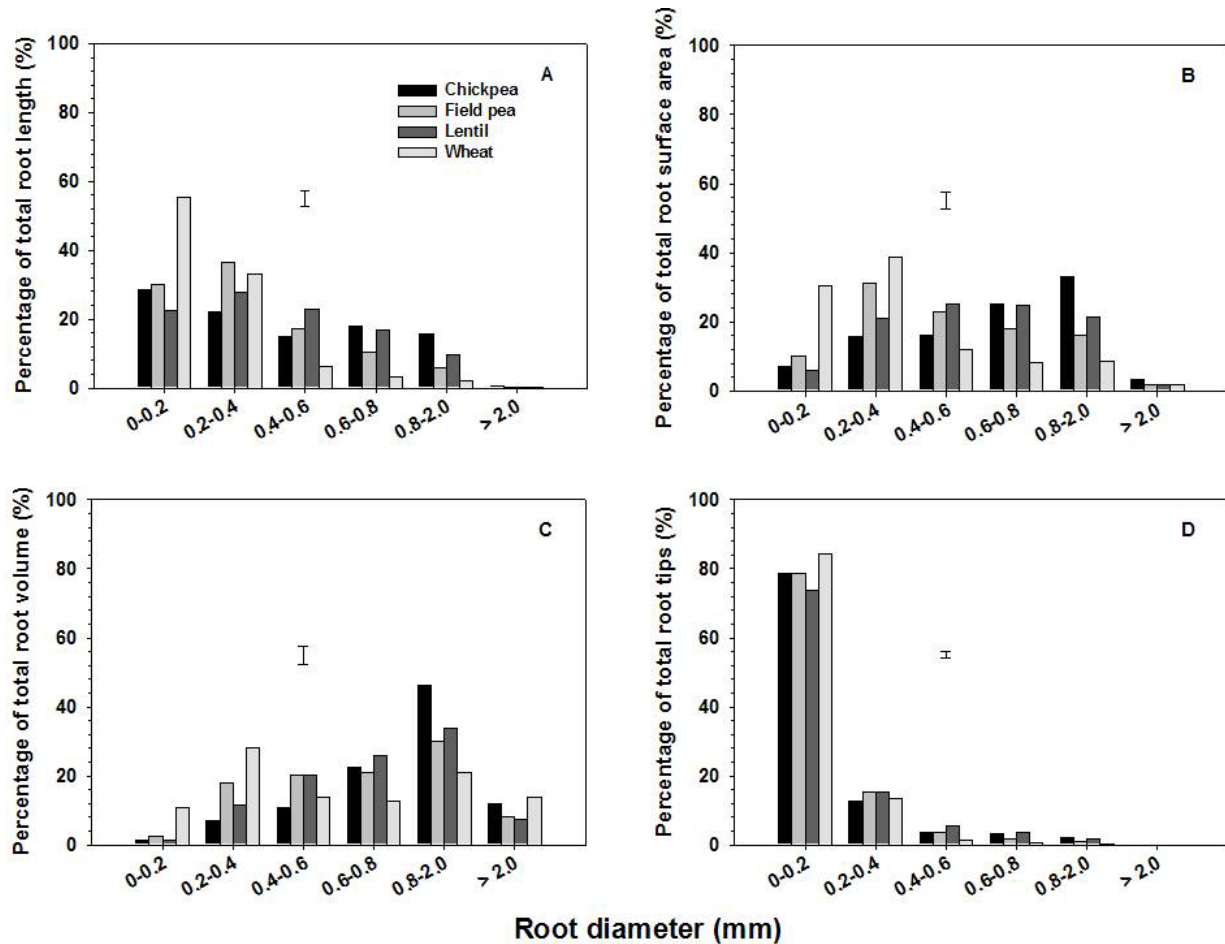


Figure 5.2 Fine root distribution of pulse crops, Swift Current, Saskatchewan, 2006-2007. Vertical bars represent the LSD at level of 0.05.

In the scientific literature, fine roots are defined as those with the diameter 2 mm or less and this definition is applicable to roots from trees, vegetables (Pietola and Smucker 2006) and forage crops (Zobel et al. 2006) because these species usually have large diameter roots in addition to fine roots. Our study indicated that this classification of “fine roots” needs to be further defined for oilseed and pulse crops. The majority of the roots (85% of total root length, and 63% of the total root surface area) in oilseed and pulse crops were distributed within the <0.4

mm diameter classes, and there was a small proportion of roots greater than 0.4 mm in diameter. Therefore the evaluation of roots for oilseeds and pulses could be made by using an “extra fine” roots category with roots <0.4 mm diameter along with “fine roots” (0.4-2.0mm) category. In the present study, for oilseeds and wheat, the greatest proportion (>85%) of total root length was comprised of the “extra fine” roots, while that comprised by “fine roots” (0.4-2.0mm) was only about 15%. For pulse crops, the contributions of extra fine roots to the total root length accounted for about 50%, with the remainder of the roots being equally distributed in the classes of 0.4-0.6, 0.6-0.8, and 0.8-2.0 mm diameters.

The results also suggest that oilseed species and wheat may have greater abilities for water uptake and nutrient acquisition than pulse crops. Oilseeds had more, longer, and finer roots than wheat. The mechanism responsible for the species differences is unknown, but it was speculated that the availability of nitrogen, the foremost important nutrient for crop growth, may play a role. Pulse crops rely heavily on symbiotic N-fixation as the main nitrogen source, whereas wheat and oilseeds rely on nitrogen supplied through inorganic fertilizers that are usually applied in the top soil layers. The greater number of fine roots in oilseeds and wheat may help in uptake of nutrients from the top soil layers. For number of root tips, roots in the <0.2 mm diameter class comprised >80% of the total tips and there was no difference between crop species, suggesting that root tips may function similarly among crops for root proliferation, sensory function, and synthesizing of plant growth regulators (Torrey 1976; Van Staden et al. 1988).

5.4 Conclusions

In summary, extra fine roots accounted for a substantial portion of the entire root system

in oilseed and pulse crops, as demonstrated by the observation that >80% of root length and >65% of root surface area were comprised of extra fine roots (<0.4 mm in diameter). For all the seven crop species investigated, the extra fine plus fine roots together formed the core exchange sites through which the plants exact water and nutrients from the soil. Roots with larger diameters (0.4-2.0 mm) may represent most of the biomass of the root system and they can form the long-distance transport pathways for water and nutrients, anchor plants, and support the development lateral roots (Eissenstat and Yanai 2002; Waisel and Eshel 2002). The proportion of extra fine and fine roots may change depending on bioclimatic zone, soil type and nutrients, and crop management practices, among others. Oilseed and pulse crops are widely used in cropping systems to break pest cycles, enhance subsequent crop yield, and improve soil water and nutrients use efficiency. Results generated from this study could be critical for understanding the functionality of root systems of various broadleaf crops for crop models.

6.0 MICROBIAL COMMUNITIES IN THE RHIZOSPHERE OF PULSE CROPS IN A SEMIARID ENVIRONMENT

6.1. Introduction

As a dynamic and complex system, soil contains diverse microorganisms that are involved in many biological activities (Young 1998; Newton et al. 2003). The rhizosphere is the zone adjacent to plant roots which is directly influenced by plant roots and their associated microorganisms (Hiltner 1904). Microbial communities in the rhizosphere are involved in various fundamental processes related to nutrient cycling, such as improving the ability of plants to acquire nutrients from soil by either increasing the extent of the root system (e.g. fungal hyphae) or solubilizing macronutrients such as phosphorus or sulfur (Smith and Read 1997; Lynch 1990). Further, the composition of rhizosphere microbial communities can influence plant health by influencing plant-pathogen interactions (Siciliano et al. 1998). However, microbial communities in the rhizosphere can be affected widely by plant and soil factors.

Roots are capable of releasing into the rhizosphere about 1–25% of the net photosynthesis originating from the plant shoot as soluble and insoluble compounds (Merbach et al. 1999); this process is highly affected by a wide range of factors including plant genotype (Rengel et al. 1997; Grayston et al. 1998), plant age (Van Veen et al. 1991; Marschner et al. 2001), nutritional status (Marschner and Crowley 1998; Fan et al. 2001), colonization of mycorrhizal fungi (Po and Cumming 1997; Marschner et al. 1997) and soil physical and chemical properties (Marschner et al. 2004). In most cases, the rhizosphere microbial communities are greatly affected by plant species because the amount and composition of root exudates of plants may result in differences in the ability of microbial communities to metabolize and compete for different carbon sources (Marschner et al. 2004).

Due to their great importance in plant-soil systems, a rhizosphere microbial community

has received increased research attention in recent years. The improvement of agricultural sustainability requires the optimal use of soil fertility, which greatly relies on soil microbial communities. Understanding of rhizosphere microbial diversity can help optimize nutrient management, and the use of this natural resource could help develop sustainable agricultural systems by minimizing the application of synthetic fertilizers and pesticides. As important rotation crops, pulses play significant roles in enhancing crop yield, breaking pest cycles and improving soil quality. However, the information on the rhizosphere microbial communities of pulse crops is limited, especially in semiarid environments. Thus, the objectives of this study were to determine bacterial and fungal populations in the rhizosphere of chickpea, field pea and lentil at early-flower, late-flower and maturity stages, and to assess whether different water regimes, soil depths and the inoculation with *Penicillium bilaiae* affect microbial populations in the semiarid Canadian prairie.

6.2. Material and methods

6.2.1 Experiment design

The study was conducted at the Semiarid Prairie Agricultural Research Centre (SPARC) of Agriculture and Agri-Food Canada, Swift Current (50°15' N, 107°44' W), Saskatchewan in 2006. The experiment was established on an Orthic Brown Chernozem (Aridic Haploboroll) soil with silt loam texture; the content of sand, silt, and clay was 28%, 49%, and 23%, respectively, organic matter of 3.0%, and pH (water paste) 7.3. The experiment field was on wheat stubble. Three pulses [chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.), and lentil (*Lens culinaris*)] were hand-planted in lysimeters of 15 cm in diameter and 100 cm in length that were installed in the soil using a hydraulic system (Gan et al. 2009b). Crop seeds were treated with

effective fungicides before planting to minimize seed- and soil-borne diseases (Table 6.1). Before the installation of the lysimeters, Treflan QR5 (DowAgro Science Canada Inc.) was applied at a rate of 11 kg ha⁻¹ to the entire experiment site for weed control. JumpStart is a phosphate inoculant containing the naturally occurring soil fungus *Penicillium bilaiae* that can colonize plant roots, releasing organic acids that improve the P availability to crops. In this study, JumpStart® solution was made by mixing 1g product with 1000ml distilled water and 1ml of such solution was then put into 419ml distilled water to make the final inoculant solution. Then each of the pulse seeds was treated with final solution of 1ml.

The crops under low-water treatments received natural rainfall only (Table 6.2), whereas crops under high-water received natural rainfall plus irrigation at the amount of 150 mm (which was about 2/3 of the long-term rainfall at the experimental site). Irrigation was applied using a hand-sprayer with 75 mm of the irrigation being applied between seedling and flowering (3 applications) and the remaining 75 mm applied from flowering to maturity (2-3 applications). Three pulses with two water regimes were arranged in a factorial, randomized complete block design with two replicates. Each treatment contained 3 sampling times in each replicate. Thus, the experiment had a total of 72 lysimeters (3 pulses × 2 water conditions × 3 sampling times × 2 treatments × 2 replicates).

Table 6.1. Crop cultivars and agronomy information for pulses grown in the lysimeter experiment at Swift Current, Saskatchewan, 2006.

Pulses	Cultivar	Fungicide			Initial seeds lysimeter ⁻¹	Final plants lysimeter ⁻¹
		Trade name	Active ingredient	Rate (ml 100kg ⁻¹ seed)		
Chickpea	CDC Anna	Crown	Carbathiin + Thiabendazole	600	5	2
Field pea	Eclipse	Apron FL	Metalaxyl	16	5	2
lentil	CDC Glamis	Crown	Carbathiin + Thiabendazole	600	7	3

Table 6.2. The amounts of water (mm) that crop received during the different growth stages under low- and high-water conditions at Swift Current, Saskatchewan, 2006.

Crop stage	Low ^a	High ^a
Early-flower	14	41
Late-flower	22	45
Maturity	12	28

^a Low-water means crop received natural rainfall only, and high-water means crops received natural rainfall plus irrigation with a amount of 150 mm.

6.2.2 Soil sampling

Lysimeters of pulses were withdrawn from the field at early-flower, late-flower and physiological maturity. Crop plants were cut off at ground level and the number of plants in each lysimeter was counted just before the lysimeters were withdrawn from field positions. For the analysis, intact soil cores were taken out from lysimeters, and sectioned into 10 cm in length for the first 60 cm of the soil core and 20 cm interval for the remaining 40 cm to the depth of 100 cm. The bulk soil of each segment was broken into pieces and pulse roots with attached soil were taken as samples. Rhizosphere soil samples were collected by shaking the roots with attached soil gently by hand and the soil samples were then bulked into “upper” (0-20 cm) and “lower” (20-100 cm) soil depth groups. The fresh rhizosphere soil samples were transported to the laboratory immediately, and stored at -4°C until processed.

6.2.3. Determination of bacterial and fungal population

A culture-based method was used to determine rhizosphere bacterial and fungal populations. From the results of pre-culture, the final determined dilute series for bacteria were 10^{-6} , 10^{-5} , 10^{-4} , and 10^{-3} and those for fungi were 10^{-5} , 10^{-4} , 10^{-3} and 10^{-2} . In brief, 3 g of soil sample was placed into 27 ml 0.1% Tween 80[®] to make a 10 fold serial dilution, and the dilution procedure was repeated to make a series of dilutions. Rose Bengal Potato Dextrose Agar (RBA) media were used for fungi, and Tryptic Soy Agar (TSA) was made for bacteria. After plating each dilution, the RBA plates were incubated at 22°C for 120 hrs in temperature-controlled room and TSA plates were incubated at 28°C for 72 hrs in the incubator, before colony counting was conducted. Fungi counting took place when the plates had 15 to 150 colonies/ plate, whereas bacterial counts were made when there were 25 and 250 colonies/ plate. Then the Standard Plate

Count (SPC) was calculated by using the mean of duplicates of the dilution.

6.2.4. Statistical analysis

The data on pulse rhizosphere bacterial and fungal populations were subjected to analysis of variance using the MIXED procedure of SAS (Littell et al. 1996). The means of difference were considered significant if the probability level was at $P \leq 0.05$.

6.3. Results

Significant differences were present in the rhizosphere fungal populations of the crops. Chickpea had significantly more fungi in its rhizosphere than field pea and lentil at early-flower and maturity stages (Fig. 6.1). Soil depths had a significant effect on the amount of rhizosphere fungi for pulses (Fig. 6.2). From early-flowering to maturity, significantly more rhizosphere fungi were present in the upper soil than the lower soil for pulses. In addition, the rhizosphere fungal population in the lower soil demonstrated a declining trend from early-flower to maturity stage. The same tendency of declining of fungal populations was seen in the upper soil from early-flowering to late-flowering. At maturity, there were significant interactions between crops and soil depths. Chickpea, field pea and lentil had more rhizosphere fungi in the upper soil than at the lower soil where chickpea always had the largest amount. However, there were no significant differences in rhizosphere fungal population in the lower soil among the three pulses (Fig. 6.3). The treatment of JumpStart (*Penicillium bilaiae*) significantly increased the rhizosphere fungi for pulse crops (Fig. 6.4). However, the total bacterial population in the rhizosphere of three pulses was not significantly affected by the given factors including crop species, crop growth stages, water regimes, soil depths and their interactions in the present study

(data were not shown).

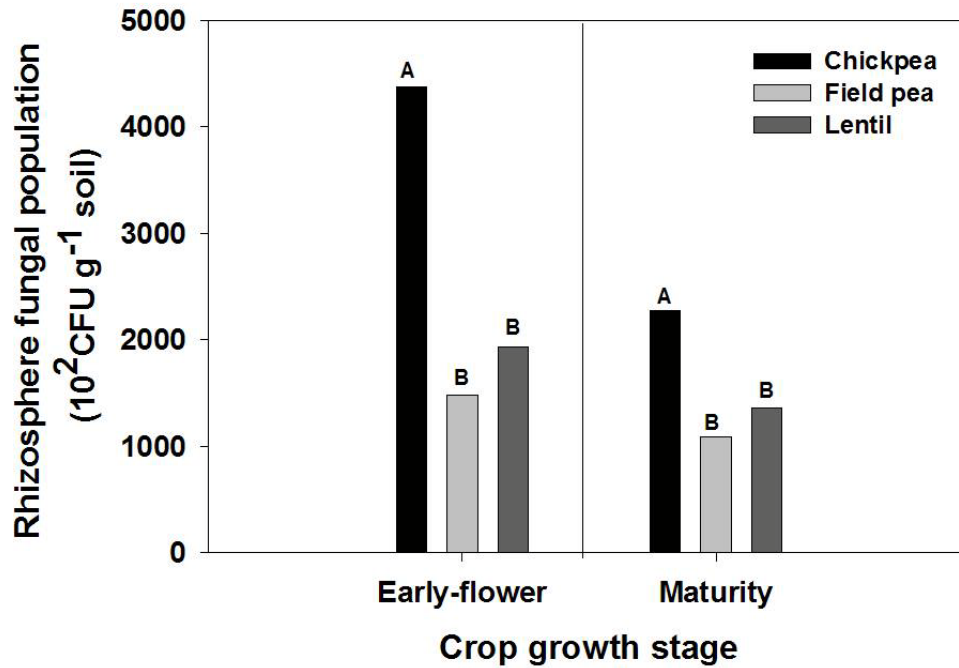


Figure 6.1 Fungal populations in the rhizosphere of pulses, Swift Current, Saskatchewan, 2006. Different letters indicate significance at $P < 0.05$ probability level.

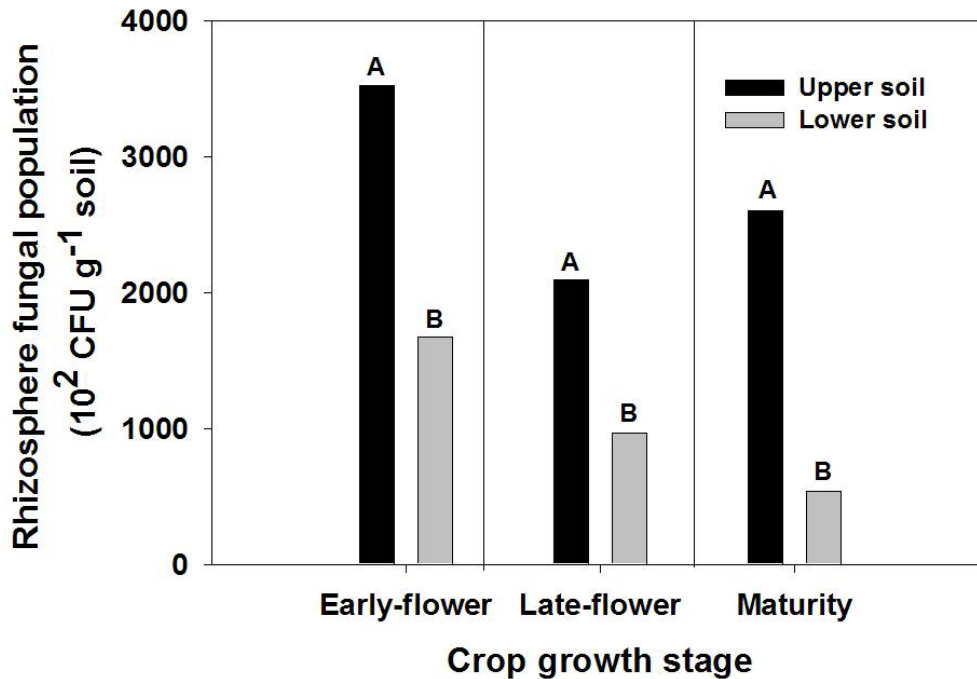


Figure 6.2 Rhizosphere fungal populations of pulses at different soil depths (upper vs. lower) at different crop stages, Swift Current, Saskatchewan, 2006. Different letters indicate significance at $P < 0.05$ probability level.

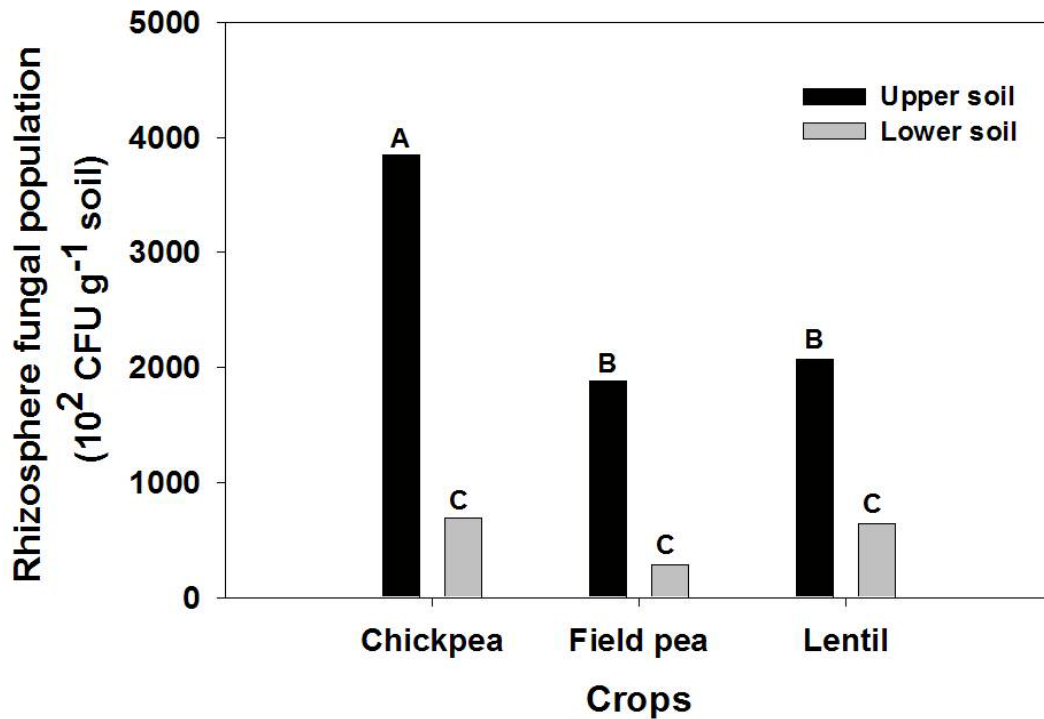


Figure 6.3 Rhizosphere fungi of pulses in upper and lower soil at maturity stage, Swift Current, Saskatchewan, 2006. Different letters indicate significance at $P < 0.05$ probability level.

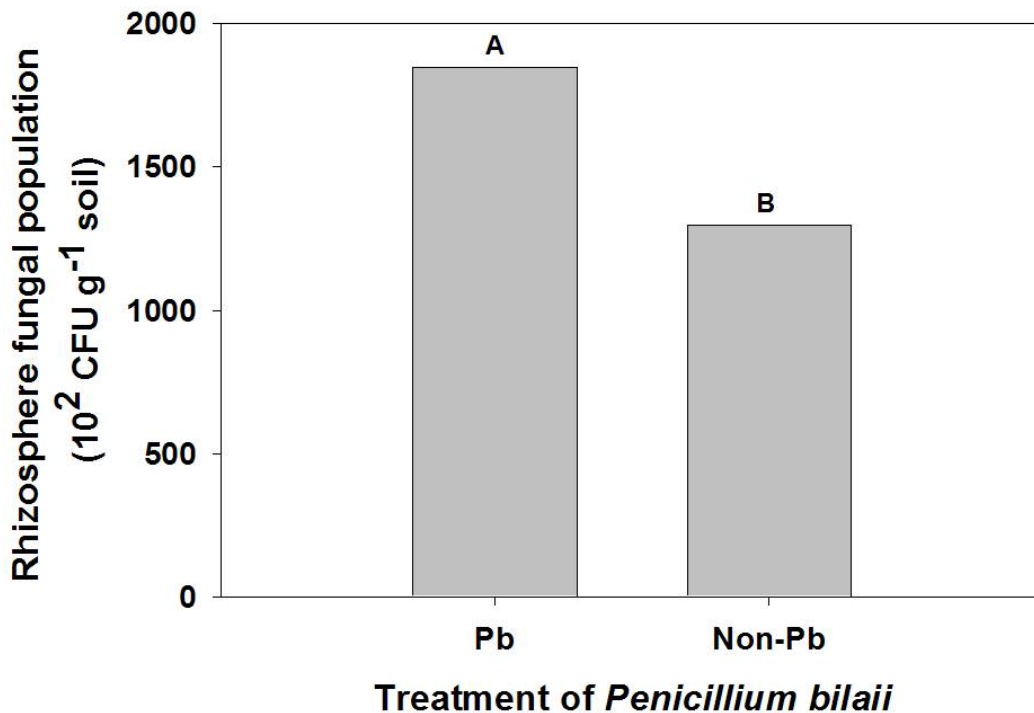


Figure 6.4 Effects of *Penicillium bilaiae* on the mean rhizosphere fungal populations of three pulses Swift Current, Saskatchewan, 2006. Different letters indicate significance at $P < 0.05$ probability level.

6.4. Discussion

The rhizosphere fungal population was significantly different among crop species. Chickpea had the most fungi in its rhizosphere while field pea had the smallest amount regardless of growth stage. Generally, the composition and amount of microorganisms present in the rhizosphere varied among different plants due to their different requirements in the quantity and quality of the compounds exuded by plant roots (Bowen and Rovira 1991; Curl and Truelove 1986). As a consequence, differences in rhizosphere microbial communities are associated with differences in plants (Miller et al. 1989; Kremer et al. 1990). The results in this thesis support these findings. Chickpea is known to exude large amounts of low-molecular-weight organic anions and carboxylate in the rhizosphere, which enhance the availability of phosphorous to the plant (Ae et al. 1990; Gerke et al. 1994; Hocking et al. 1997; Ohwaki and Hirata 1992). Assuming a sufficient phosphorus supply, the above-ground shoot of chickpea had better growth, resulting in better root growing conditions thus leading to a better rhizosphere environment with favorable rhizodeposition for supporting fungi.

Fungal populations of all pulses differed for the two soil depths, with more in the upper soil than the lower soil. This was likely due to soil nutrient and plant root distribution. Generally, the gradient of soil resources such as organic matter, nutrients, and moisture, are important drivers of soil microbial community composition (Bååth et al. 1995; Bossio and Scow 1998). On the other hand, differences in plant root distributions result in the variations of the distribution of the root-derived organic carbon inputs (Gill et al. 1999; Jackson et al. 1996). Typically crop roots are more concentrated in the upper soil layer than lower soil layer suggesting there would be less root-derived carbon in the deep soil. Therefore, with less carbon sources as food supply, microbial numbers would be lower than those in the shallow soil. The populations of rhizosphere

fungi in the lower soil were also found to decrease with crop growth stages (Fig. 6.2). El-Hissy et al. (1980) indicated that the composition of the rhizosphere fungi was significantly influenced by the plant type and age, and counts of total fungi reached maximum after 90 DAP and sharply declined by 150 DAP. A previous study on maize also demonstrated that more fungi were isolated from roots at seedling stages than from roots of plants at silking (Windham 1983). Crops by soil depths interactions were significant at crop maturity, and the rhizosphere fungi population in the upper soil of chickpea was significantly greater than that of field pea and lentil. However there was no significant difference in the rhizosphere fungi population found in the upper soil in field pea and lentil (Fig. 6.3). This might be because the belowground biomass production of lentil was similar to that of field pea (Brandt 1996) suggesting similarities between the two pulse crops in the turnover of decomposing root matter (Dylon et al. 2006) which directly affects the rhizosphere microbial community.

As a naturally occurring fungus, *Penicillium bilaiae* has been demonstrated to solubilize both precipitated and bound P into soil solution, which facilitates P uptake by plants (Kucey 1983). Inoculation with this microorganism has been shown to increase P uptake, vegetative growth, or seed yield of wheat (Kucey 1987, 1988; Asea et al. 1988; Goos et al. 1994), canola (Kucey and Leggett 1989), bean (Kucey 1987), field pea and lentil (Gleddie 1993). In the present study, rhizosphere fungi were found to be significantly increased by the treatment of *Penicillium bilaiae*. Bio-inoculation has caused shifts in the microbial community composition of either small or high magnitude (Nacamulli et al. 1997; Marschner et al. 2001; Bankhead et al. 2004), which is supportive to the results. However, more research is needed to better understand the mechanisms of how introduced microorganisms affect the amount and functionality of indigenous microorganisms in the soil.

The types of micro-organisms in the rhizosphere are diverse and their numbers are greatly dependent on the plant species, soil conditions, root growth and development and uptake and release of solutes from the roots (Garbeva et al. 2004). However, in the present study, many factors including crops species, soil depth, water conditions and treatment of *Penicillium bilaiae* as well as their interactions did not significantly affect rhizosphere bacterial community. Generally, rhizosphere microbial growth and activity can be affected by plant root exudates such as amino acids, sugars and growth factors (Rovira 1956a) and the differences in the composition of root exudates can influence the type of bacteria present in the rhizosphere (Rovira 1956b). In the present study, the culture-based method that was used to determine rhizosphere microbial population can only detect up to 10% of soil microorganisms (Bakken 1985). Perhaps the species of the culturable rhizosphere bacteria in the current study were not sensitive to the changes of root exudates caused by the factors in the experiment.

6.5. Conclusions

Rhizosphere fungal populations of chickpea, field pea and lentil were more sensitive to the factors including crop species, crop growth stages and soil depth than bacteria. Compared with field pea and lentil, chickpea had the largest amount of fungi in the rhizosphere regardless of crop stage and soil sampling depths. Different water regimes did not significantly affect rhizosphere fungal populations of pulses. Soil depth significantly affected the amount of rhizosphere fungi. More were located in the upper soil than in the lower soil layer. JumpStart (*Penicillium bilaiae*) significantly increased the amount of the fungi in the rhizosphere of pulse crops. This study presented a picture of microbial communities in the rhizosphere of pulse crops providing some information of rhizospheric properties of dominant rotation crops in a semiarid

environment.

7.0 GENERAL DISCUSSION AND CONCLUSION

Due to their benefits in cropping systems and market potential, oilseeds and pulses have been increasingly adopted in the agricultural production system on the Canadian Prairies during the last two decades (Campbell et al., 2002). These broadleaf crops play an important role in the development of sustainable agricultural systems (Zentner et al. 2001). A significant environment benefit can be gained by using these broadleaf crops to replace conventional summer fallow (Gan and Goddard, 2008). In addition, use of pulse crops could potentially reduce greenhouse gas emissions from agricultural systems due to the elimination of nitrogen fertilizer (Lemke et al., 2007). Previous crop rotation studies looking at replacement of crop monoculture with pulses and oilseeds in rotation have focused on crop adaptation and physiology. Limited research has been reported on their root systems. Studies on crop root systems are essential because rooting patterns are the determinant of root functionality especially under water-limited conditions. On the semiarid Canadian Prairie, water is the most important factor limiting crop production since the temperatures are usually high during the mid-summer, while growing season rainfall is limited and unpredictable. Effective use of available water, from soil reserves and rainfall, is the key for crop production. Therefore, the present study focused on the morphological traits and distribution characteristics of root systems for important oilseed and pulse crops under a semiarid environment. Detailed growth patterns of roots, both temporally and spatially, have been investigated.

Root parameters determined in this study were those highly related to the functionality of the root systems. Temporally, the root growth of oilseeds and pulses varied among crop growth stages, progressing steadily from seedling to the late-flower or late pod stages. The root growth of *Brassica* species and pulses reached the peak at the late-flower stage, while that of flax took

place at late-pod. This information suggests the optimal period of crop growth at which the accurate assessment of root morphological traits in oilseeds and pulses can be made; this may serve as a scientific guide for future studies on crop roots. Among the crops tested, canola root morphological traits (length, surface area, and number of tips) were most sensitive to water availability with the greater root growth under higher water conditions. In contrast, the root growth of pulses responded to different water conditions equally. These findings suggest that oilseed crops are more productive under high-water conditions. Pulse crops can be adapted to a wider range of environments because of their lower responsiveness to water availability.

Spatially, the largest proportion (>85%) of roots for oilseeds and pulses was concentrated in the top (0-40) cm soil layer, suggesting that the top soil conditions and micro-environment play critical roles in crop root growth and development in semiarid environments. Such information may help crop producers conduct the proper top-soil management to optimize crop production. Maintaining the optimum conditions (i.e. nutrients and water) of the seedbed may be the key for root growth and development.

The majority of the roots in oilseeds and pulses are fine roots, and fine roots are important contributor to the soil carbon pool. Understanding temporal or spatial production of fine roots is crucial to precisely evaluate the productivity of the entire terrestrial ecosystem. However, most previous studies on fine roots have concentrated on forest plantation and forage crops, while those on field crops are very limited especially for oilseed and pulse crops. Therefore, the findings on the fine root distributions of oilseeds and pulses from this study partly fill the research gap by providing the scientific information to modelers studying the functionality of root systems of various broadleaved crops.

In addition to the rooting characteristics, this study also investigated the rhizosphere

characteristics of pulse crops. Chickpea had the most fungi in its rhizosphere among the tested pulses. The inoculation of *Penicillium bilaiae* significantly increased the rhizosphere fungi in pulses that could lead to an increased microbial activities involved in nutrients cycling, a process highly beneficial to crop growth.

Being the most essential part in any root-related study, the methodology must be scientifically reliable and sound. In this study, the roots of oilseeds and pulses were sampled and studied under field conditions. All crops were grown in lysimeters of 15-cm in diameter and 100 cm in depth that were installed in the field using a hydraulic-pushing system. The lysimeters were large enough to allow crops to grow without restricting the roots relative to vertical distribution and were deep enough to accommodate the full root length. Therefore crops were grown in columns with soil structure intact, root growth and the resulting root mass was likely more accurately represented than most other studies where crops are usually grown in artificial mixtures of media. Hence, it is suggested that the hydraulic-pushing system using large-diameter lysimeters with undisturbed soil columns employed in this study, despite intensive labor requirement, is a reliable way to generate scientific data for root-related studies.

Crop roots are difficult to measure, in the scientific literature there is limited information available about root systems of oilseed and pulse crops. Therefore, the root information generated from this study can provide the scientific basis for further studies to explore the relationship between rooting patterns and functionality of the root system for oilseed and pulse crops. The information on the rooting depth and distribution characteristics in the soil profile of oilseeds and pulses from this study may serve as the basis of the development for more diversified cropping systems with cereals, oilseeds and pulses in semiarid environments. In addition, such root information can also be used by modelers to simulate and estimate water use

and nutrients uptake for these broadleaved crops. However, the methodology used in this study may be basic and further improvement may be required for more advanced root-related studies in the future such as root exudates, microbial biomass, and rhizodeposition, etc.

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APPENDIX A**INFORMATION ON THE INOCULATION OF STUDIED PULSE CROPS**

	Inoculant solution (Liquid inoculant + Distilled water)	Rate (ml seed ⁻¹)
Chickpea	1ml (4.0×10 ⁶ Rhizobia ml ⁻¹) + 999ml	1.0
Field pea	1ml (6.5×10 ⁶ Rhizobia ml ⁻¹) + 999ml	1.0
Lentil	1ml (6.5×10 ⁶ Rhizobia ml ⁻¹) + 999ml	1.0

APPENDIX B

AVERAGE TEMPERATURES OF EXPERIMENT SITE DURING CROP GROWING SEASON, 2006-2007

	2006 (°C)	2007 (°C)
May	12.5	11.6
June	16.2	15.9
July	21.2	22.9
August	19.2	17.7

APPENDIX C
SOIL CORING METHOD USED IN THE STUDY



Soil coring method



APPENDIX D
PROCEDURES OF ROOT SAMPLING AND ANALYSIS



1. Sampling



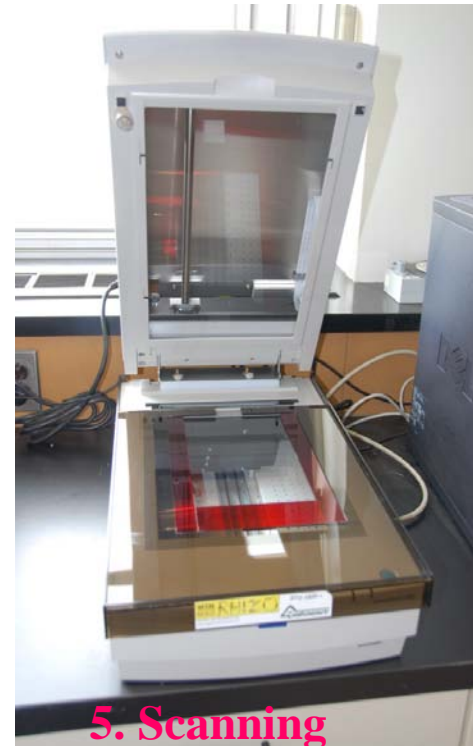
2. Soaking



3. Hand-washing



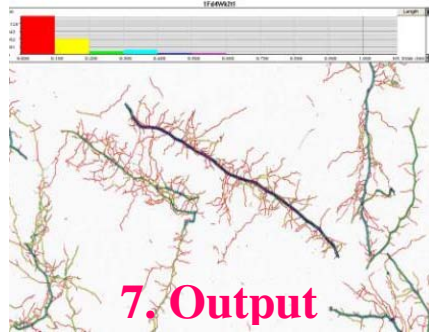
4. Dyed root



5. Scanning



6. Analysis



7. Output

Root sampling and analysis