The Effect of Seedling Root Length on Seed Yield in *Brassica napus* L.

BY

CHADWICK BRUCE KOSCIELNY

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

Department of Plant Science

University of Manitoba

Winnipeg, MB

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

****

COPYRIGHT PERMISSION

The Effect of Seedling Root Length on Seed Yield in *Brassica napus* L.

By

Chadwick Bruce Koscielny

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

Master of Science

ABSTRACT

Koscielny, Chadwick Bruce. M. Sc., The University of Manitoba, October, 2011. The Effect of Seedling Root Length on Seed Yield in *Brassica napus* L. Professor, Dr. Rob Gulden.

The objective of this research was to determine the relationship between seedling root length and seed yield in spring canola. Field and growth room experiments were conducted using the same eight genotypes. In the field experiment, root length and root area had a strong positive relationship to seed yield with R^2 values at the 1-2 leaf stage of 0.90 and 0.93, respectively. Shoot dry weight had a strong positive relationship to seed yield at the cotyledon stage, with an R^2 of 0.99. In the growth room, root length was compared to short- and long-term seed yield. The R^2 values when root length was compared to short- and long-term seed yield were 0.95 and 0.96, respectively.

Acknowledgements

This research and thesis would not have been possible without the support of the following people:

Dr. Rob Gulden, who had a tremendous amount of patience and diligence working with me on the research concepts, statistics and most of all writing.

Dr. Peter McVetty and Dr. Don Flaten for providing guidance and serving on my advisory committee.

Dr. Martin Entz for suggesting roots as a topic of research. I was initially hesitant, but seeing all the exciting advancements in root research I am truly grateful for being directed toward this area.

The Pioneer Hi-Bred Carman canola research team Winnie McNabb, Tyler Huck, Jason Peters, Ian Junkin and Jay Sprott for all their help with the field plots.

Derek Lewis for his help with all the supplies and equipment around the lab and university.

All the summer staff that helped with field plots and root washing including but not limited to JoAnne Rex, Yirgalem Kidane, Jaclyn Rampton, Lori Reimer, Norm Chabbert, Katie Stakylo and Brenden Rex.

Pioneer Hi-Bred Production LP canola research for supporting me with the resources and time necessary to complete my research.

Most importantly my wife Michelle and 4 children Lincoln, Max, Isablle and Zoe who made tremendous sacrifices so I could further my education.

Foreword

This thesis was written manuscript style following the Canadian Journal of Plant Science style. There are two manuscripts presented, each with an abstract, introduction, methods and materials, results, discussion and conclusion. The thesis also contains a general abstract, general introduction and literature review prior to the manuscripts and a synopsis and literature cited following the manuscripts.

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

In 2008, canola (*Brassica napus* L.) was the primary source of crop cash receipts in Canada (Anonymous 2008). This demonstrates the importance of this crop to Canadian farmers and the need for research and development efforts to continually provide growers with better cultivars and agronomic information.

Breeders are continually looking for new traits to help them select genotypes with increased yield potential. A trait that can be measured early in a plant's life cycle with a strong relationship to seed yield can help a breeder make more accurate selections during a breeding cycle potentially increasing the rate of genetic gain of seed yield per cycle. Campbell and Kondra (1978) and Thurling and Vijendra Das (1979) demonstrated that vegetative shoot biomass measured at maturity and anthesis respectively, had a strong relationship with seed yield. This was an important finding, but due to vegetative biomass being measured at later development stages this measure is not ideal as seed yield is obtained shortly thereafter.

The radicle is first to emerge from a seed and if early root parameters can be used to predict seed yield this may be a trait that would be very useful in plant breeding. Corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) root length at the vegetative stages has been shown to have a positive relationship with seed yield (MacKay and Barber 1986, Brown and Scott 1984). In *B. napus,*

differences in root biomass accumulation and its effects on nutrient acquisition have been documented, but none of this research linked these differences to seed yield (Duan et al. 2009; Rose et al. 2008; Solaiman et al. 2007).

The purpose of this research was to determine if differences in seedling root parameters were related to seed yield in *B. napus* with the following specific objectives:

1. To determine if root length, root area and root weight were indicative of seed yield in the field and the growth chamber.

2. To determine at what growth stage *B. napus* can be sampled to provide the most consistent data with a strong relationship to seed yield.

Chapter 2

Literature Review

2.1 *Brassica napus*

Brassica napus L. (*B. napus*) is an alleotetraploid known as argentine canola, canola, rapeseed or oilseed rape. There are winter and spring types of *B.* napus. In this thesis *B. napus* will refer to the spring types only unless otherwise stated. *B. napus* originated when *Brassica rapa* (AA genome) and *Brassica oleracea* (CC genome), both diploid species, crossed resulting in the alleotetraploid B. *napus* (AACC). In Canada, brassicacea crops were grown for industrial oil production and in the 1970s the first low erucic acid (less than 2%) and low glucosinolate (less than 30 µm g^{-1} of air dried seed meal) genotype was produced and branded canola which is an acronym for Canadian oil and low acid (Anonymous 2006). By lowering the levels of erucic acid and glucosinolate content, the resulting oil and meal became healthier for human consumption and more palatable to livestock, respectively. With the bitter taste, caused by glucosinolates, removed from the meal a new high value human consumption market for *B. napus* was created.

2.2 *B. napus* Production

In 2009, *B. napus* was grown on 6.1 million hectares of the 23.8 seeded hectares in Western Canada. Over 90% of the *B. napus* heactares in Canada are

concentrated in Manitoba, Saskatchewan and Alberta (Anonymous 2010a). In 2008, canola was the primary source of crop cash receipts for farmers in Canada demonstrating the importance of *B. napus* to Canadian agriculture (Anonymous 2008). *B. napus* production has changed significantly since the crop was initially introduced to Canadian farmers in the 1970s. The development of herbicide-resistant hybrid cultivars and intensive management has contributed to increasing canola yields (Karamanos et al. 2006) as growers are better able to manage weeds and maximize applied fertilizers. Average yield, in 2008 and 2009, was 1.9 tonnes ha^{-1} which was the greatest average Canadian canola yield ever recorded. In addition, the area planted to canola was at an all time high which also contributed to the record total production of *B. napus* in Western Canada (Anonymous 2010b).

2.2.1 Hybrid vs. Open Pollinated *B. napus* genotypes

From the 1970s until 1989, the only *B. napus* grown in Western Canada had an open-pollinated (OP) fertility system. In 1989, the first hybrid cultivars were sold in Western Canada and since this introduction there has been a dramatic shift in the breeding and production of *B. napus* hybrid genotypes to where hybrid cultivars now account for over 90% of the canola seed sales and acreage seeded in Canada (Charne 2010). A number of studies have documented a yield advantage of 17-33% for hybrid genotypes compared to OP genotypes (Clayton et al. 2009; Van Deynze et al. 1992; Karamanos et al. 2005; Karamanos et al.

2006). High yielding hybrids require more nutrients to reach maximum yield potential and Karamanos et al. (2005) reported that hybrids require approximately 40 kg N ha^{-1} more than open pollinated cultivars to reach their maximum yield potential. In the same study, hybrid genotypes yielded 24% more than open pollinated cultivars when supplied with the same amount of nitogen fertilizer. Karamanos et al. (2005) went on to demonstrate that residual soil nitrate levels were lower after hybrid canola cultivars than after open-pollinated cultivars were grown, especially at increased nitrogen application rates. It was also speculated that because the genotypes were grown in the same soil with the same nutrients that the roots of the hybrid genotypes were exploring a greater volume of soil compared to the OP genotypes.

2.2.2 *B. napus* germination and emergence

B. napus is a cool season crop that typically is planted into cool soils that can be less than ideal for germination and emergence. The Canola Council of Canada recommends planting *B. napus* into soil with a temperature of at least 10 C to a depth of 12-25 mm because temperatures below this can significantly reduce seedling emergence (Nykiforuk and Johnson-Flanagan 1994; Vigil et al. 1997). Blackshaw (1991) noted a decrease of soil temperature from 25 C to 10 C decreased the emergence of winter *B. napus* by 20-25% and decreasing the soil temperature from 30 C to 5 C resulted in a delay in emergence of 11-15 days. At temperatures of 5 C and 10 C soil moisture levels play an increasingly important

role in emergence with cool dry soil decreasing winter *B. napus* emergence by 60% compared to 90% emergence in moist soils at 10–25 C (Blackshaw 1991). However, there can be advantages to seeding earlier as long as the crop establishes well. Kirkland and Johnson (2000) and Kondra (1977) show that seeding earlier (late April or early May) compared to late seeding (mid May to late May) of *B. napus* can increase seed yield. However, planting earlier in spring increases the risk of damage from frost. *B. napus* seedlings are sensitive to frost as the growing point is above the ground and temperatures below 0 C can result in reduced biomass accumulation or death if the growing point is completely frozen.

Speed of emergence has been considered advantageous for competitiveness with weeds and the ability to maximize resource acquisitions in a short season. It is possible to select fast emerging *B. napus* and Acharya (1983) found differences in time to emergence within a selected group of genotypes. However, King et al. (1985) found that fast emergence did not lead to increased above ground biomass measured at 12 days after emergence which contradicts the idea that early emerging *B. napus* will increase seed yield. King et al. (1985) did not collect seed yield.

Kondra et al. (1983) found *B. napus* had significant differences in emergence depending on the temperatures at which it was tested. Research on corn emergence and corn roots contradicts this and concluded that soil temperature did not affect root growth among corn hybrids and all hybrids exhibited similar root growth at each temperature studied (Cutforth et al. 1985; Hund et al. 2007).

2.2.3 *B. napus* base temperature for germination and growth

The base temperature for *B. napus* is between 0.44 to 1.2 C (Vigil et al. 1997; Kondra et al. 1983) and 5 C (Morrison et al. 1989). The variation of base temperature in the literature indicates that there are differences in base temperatures among *B. napus* genotypes. Marshall and Squire (1996) demonstrated that four cultivars do indeed differ in base temperature and that using a linear model to predict base temperature is not accurate when there are genetic differences within a crop species.

Research into genetic differences in base temperature for *B. napus* has shown that there is indeed a genetic element controlling the base temperature for *B. napus* germination. When plants that emerged under cool conditions were crossed, an increase in the propensity to emerge under cool conditions was observed (Marshall and Squire 1996; Squire 1999). More research is needed to establish whether current hybrid *B. napus* genotypes have similar base temperatures for germination and growth as OP genotypes as most research on *B. napus* base temperatures for germination and growth has been conducted on the latter.

Seed size also has an effect on emergence, seedling vigour and a plant's ability to accumulate shoot biomass. A positive relationship between these traits and final seed yield has been demonstrated in *B. napus* (Elliot et al. 2008).

2.2.4 Biomass accumulation & seed yield

B. napus requires between 80 - 120 days to fully mature in Western Canadian environments (Anonymous 2003). *B. napus* planted in late-April or early-May will maximize yields when compared to *B. napus* planted in mid- to late-May (Clayton et al. 2004; Degenhardt and Kondra 1981; Kirkland and Johnson 2000).

Above ground biomass accumulation is closely related with seed yield in *B. napus* (Campbell and Kondra 1978). Malhi et al. (2007a) demonstrated that there is a close relationship for both biomass accumulation and nutrient uptake in *B. napus* both of which occur at relatively greater rates at earlier stages of crop development. This was not only demonstrated in *B. napus*, but also in pea *(Pisum sativum* L.), lentil *(Lens culinaris* L.), mustard *(Brassica rapa* L.), flax (*Linum usitatissimum* L.) and barley *(Hordeum vulgare* L.) (Malhi et al. 2006; Malhi et al. 2007b). This suggests that early-season above-ground biomass might be useful in predicting late-season biomass and consequently seed yield.

2.3 Roots

Roots are defined as multicellular vascular organs without leaves or other multicellular appendages that function in the anchorage and the acquisition of solutes and nutrients for plant growth (Fitter 2002). In comparison to shoots,

roots are poorly understood due to their high plasticity and the challenges in the ability to them *in situ*.

2.3.1 *B. napus* root morphology

There are two basic types of root systems in plants; namely, fibrous root systems which includes crops such as wheat (*Triticum aestivum* L.), barley, oats *(Avena sativa L*.) and corn, and tap root systems as found in *B. napus*, sunflower *(Helianthus annus L.)*, alfalfa *(Medicago sativa L*.), chickpea *(Cicer arietinum* L.), and flax. The fibrous root systems do not have a recognizable primary root as all the roots are similar in size, whereas tap root systems have a primary root that is markedly larger in diameter than the secondary roots. A tap root improves plant anchorage, but is more costly for a plant to develop (Ennos and Fitter 1992). In winter *B. napus,* lateral roots develop approximately 3 cm below the soil surface and appears not to contribute significantly to plant anchorage in the soil (Goodman et al. 2001). Numerous studies demonstrate the root biomass in the top 20 cm of the soil profile accounts for >60% of the total root in *B. napus* (Gan et al. 2009; Pietola and Alakukku 2005; Rose et al. 2008). Maximum rooting depth in *B. napus* has been documented at 100-150 cm (Angadi et al. 2003; Gan et al. 2009; Kjellstrom and Kirchamnn 1994; Nielsen 1997). The rigid tap root in *B. napus* does not significantly add to water and nutrient uptake, but has developed for anchorage and may have been involved in nutrient storage at some point during the evolution of the species (Goodman et al. 2001). Because

the *B. napus* tap root does not contribute significantly to water and nutrient uptake it may be viewed as less efficient compared to a fibrous root system, but likely continues to contribute significantly to plant anchorage. Even though *B. napus* has a tap root, Lui et al. (2010) demonstrated that up to 85% of the root length is comprised of fine roots defined as roots that are less than 0.4 mm in diameter. This is very important information when studying *B. napus* roots as samples must be handled with extreme care to ensure that these fine roots are not lost during sampling or recovery of the roots from the soil (Figure 2.1).

2.3.2 Root hairs

Root hairs also known as trichoblasts are single wall epidermal cells that grow between the tip of a root and the zone of lignification (Segal et al. 2008) (Peterson and Farquhar 1996). Nutrient status, namely nitrogen and phosphorus, within the plant can strongly influence the number and length of root hairs in *B. oleracea* (Foehse and Jungk 1983). Segal et al. (1983) used a barley genotype capable of producing roots hairs and one genotype incapable of root hair production to demonstrate that root hairs help increase water uptake within the specific area of the root in which the root hairs developed.

Corn inbreds with differing root hair phenotypes were used to show that plants with plastic root hair development or longer root hairs accumulated significantly greater above-ground biomass than the inbreds with the capacity to only produce short root hairs in low phosphorus environments (Zhu et al. 2010). This

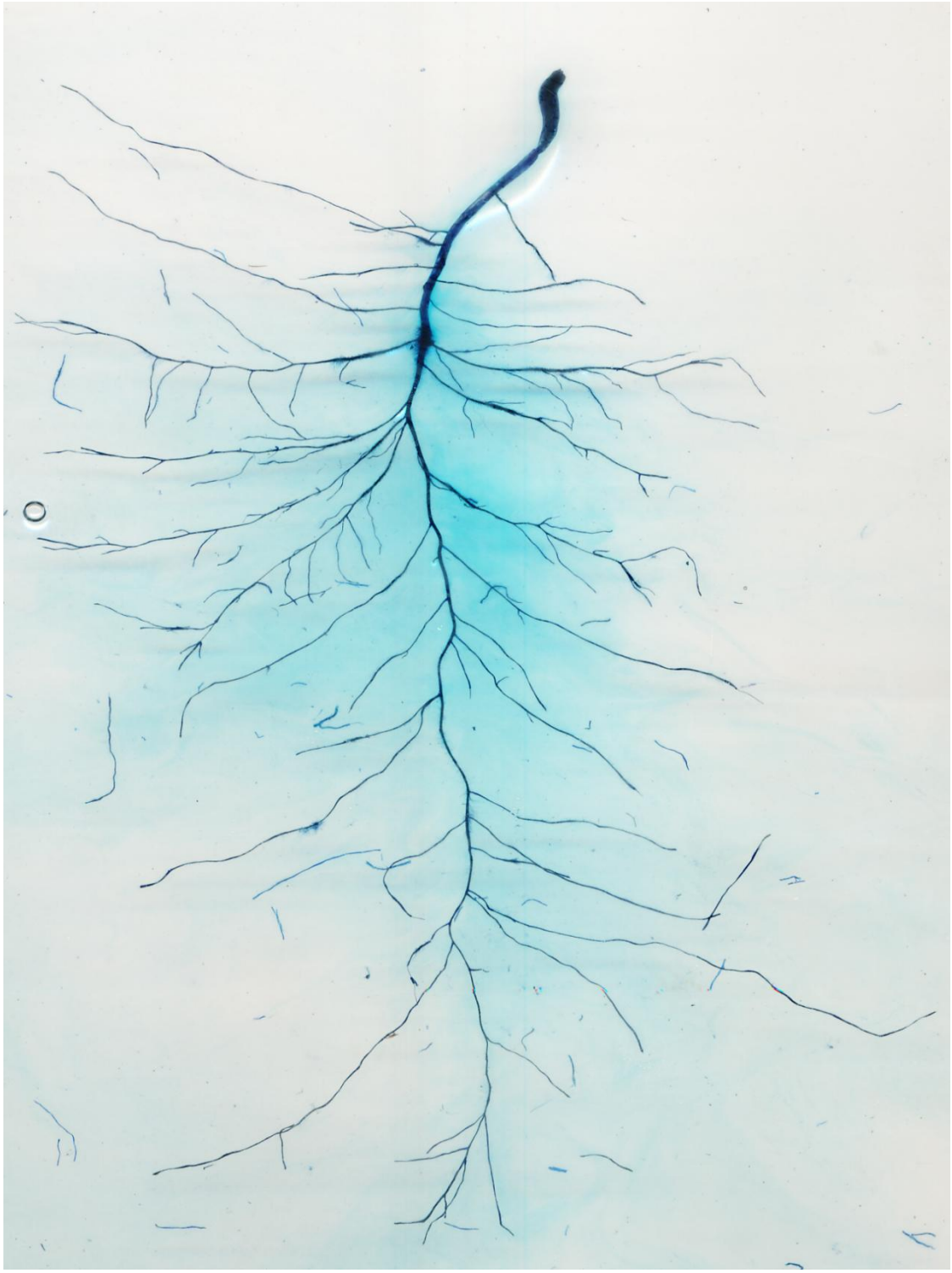

Figure 2.1 Digitized *B. napus* root from a 3 leaf plant after elutriation, staining, plating and scanning.

demonstrates the importance of root hairs in the uptake of phosphorus given its limited mobility in the soil.

2.3.3 Root biomass and root length in relation to seed yield

Little work has been done to relate root parameters to seed yield in *B. napus*. Much of the research has focused on the direct influences of roots on plant water uptake, nutrient acquisition or anchorage. All these factors can lead to increased seed yield although most studies did not measure final seed yield, because the studies used destructive sampling techniques to measure the roots.

There is a strong positive relationship between increased above-ground biomass and seed yield in *B. napus* (Johnson 2008; Taylor and Smith 1992; Thurling and Vijendra Das 1979). Because increased root biomass can lead to increased above ground biomass in *B. napus* (Akhtar et al. 2008) and above ground biomass has been correlated to final seed yield it can be hypothesized that root biomass is positively linked to final seed yield in *B. napus*.

In soybean, for example, root length and longevity are positively related to seed yield (Brown and Scott 1984). In corn the impact of root length, rooting depth and biomass on seed yield has been studied extensively. Increased rooting depth and root branching in corn is positively related to seed yield and increased above ground biomass (MacKay and Barber 1986; Wan et al. 2000).

One issue that has been raised in regards to studies that have demonstrated a positive relationship between root biomass accumulation, root length, above ground biomass, and seed yield is that they often do not take ontogenetic drift into consideration (McConnaughay and Coleman 1999). Ontogenetic drift occurs when plants that have a larger root and shoot may simply be further advanced in their development and this may be the factor involved in increasing shoot biomass and seed yield. Much of the root research conducted on field crops use sampling methodology in which all genotypes are sampled at the same point in time. Therefore ontogeny may account for many of the observed differences in root growth of field crops. Because most field crops in Western Canada are annuals, ontogenetic drift among genotypes and their components have the potential to impact overall seed yield. For example, if a given genotype matures much later than other genotypes, this has the potential to impact yield due to the effect of environment on seed set at this particular stage of development. However, if the length of time to seed maturity is known and does not influence final seed yield ontogenetic drift during the growing season between genotypes may not be apparent at physiological maturity. Throughout the growing season, ontogeny may vary, but if final seed maturity may not be related directly to seed yield. Collecting data at a single point in time can provide an accurate comparison between two genotypes regardless of the ontogenetic drift.

2.3.4 Abiotic factors that affect root growth

2.3.4.1 Water uptake

A primary function of roots is to take up water and provide the shoot with adequate water throughout the life of the plant. Nielsen (1997) recorded *B. napus* extracting water from a depth of 150 cm, but 92-95% of the seasonal total uptake was removed from the top 105 cm of the soil profile. Research on *B. napus* has indicated that the roots proliferate in areas with increased soil moisture (Wang et al. 2005) thereby increasing access to water with selective root proliferation in resource rich areas. However, roots do not grow in a specific direction seeking out water and nutrients, but once an area of increased moisture and/or nutrients is discovered the roots will proliferate to fully utilize this resource. This plasticity, combined with the variation in resource concentrations within the soil, makes sampling or modeling root proliferation challenging. A plant's ability to acquire a resource before its competitor is expected to help it achieve greater seed yield potential. Robinson (1995) studied this and determined that if a plant can access water or mobile nutrients sooner than a neighboring plant it will have an advantage over that plant. This supports the theory that speed of resource capture exploitation from the soil is as important as the physiological efficiency with which resource acquisition is achieved.

Lamba (1949) studied root growth of alfalfa, another tap rooted species and discovered that tap rooted plants require greater soil aeration and drainage when compared to fibrous rooted species. Roots have difficulty growing into soil

devoid of moisture (Cutforth et al. 2009) or into soil that is completely saturated and low in oxygen. In contrast, Merrill et al. (2002) found that rooting depth in 6 of 8 crops was the greatest in the wettest year. This is somewhat counterintuitive, but the authors suggest the roots were able to continue to grow to greater depths due to the available moisture in the subsoil. Greater rooting depth, however, did not lead to a longer total root length. In the drier year, safflower (*Carthamus tinctoris* L.), crambe (*Crambe abysinnica* L.), mustard (*Brassica rapa* L.), soybean (*Glycine max* [L.] Merr.) and common bean (*Phaseolus vulgaris* L.) produced greater root length than in the wet years despite not rooting to the same depth.

MacKay and Barber (1986) and Wan et al. (2000) suggest there are differences among corn hybrids in root biomass production and their ability to access water. This differentiation demonstrates that corn hybrids that were selected based on their ability to produce more root biomass, were able to avoid drought conditions. A great deal of research has and continues to be conducted on corn roots, with comparatively little ongoing root research in *B. napus*. A greater understanding on *B. napus* roots could facilitate the development of *B. napus* genotypes that maintain productivity under drought conditions.

2.3.4.2 Nutrient acquisition

2.3.4.2.1 Preferential root growth

Efficient fertilizer acquisition by crops is becoming increasingly important as fertilizer costs and environmental concerns over fertilizer runoff and leaching increase (Sharpley et al. 2001). Research on roots has often involved nutrient uptake with the focus on nitrogen and phosphorus. Typically, when roots intercept a region of increased nitrogen concentration in the soil, they will proliferate in that region. In barley, Drew (1975) demonstrated that nitrogen and phosphorus can cause preferential root proliferation in soil zones rich in these nutrients, whereas the same was not observed in soil supplemented with potassium. Strong and Soper (1974) found similar effects in *B. napus* and its ability to proliferate in areas of high phosphorus concentrations. Roots system architecture and biomass accumulation in other crop species can also be affected dramatically by soil environment and distribution of nutrients with proliferation of roots in areas of higher nutrient concentration (Akhtar et al. 2008; Drew 1975; Hammond et al. 2009; Kamh et al. 2005; Laine et al. 1994; Robinson 1995; Wang et al. 2007; Williamson et al. 2001). Differences exist among *B. napus* genotypes in their ability for phosphorus and nitrogen uptake, although the studies did not relate these differences in nutrient acquisition to final seed yield (Rose et al. 2008; Akhtar et al. 2008; Kamh et al. 2005; Ye et al. 2010).

Access to nutrients during the vegetative stage of plant growth is essential to maximize seed yield potential. Rose et al. (2007) reported that access to

phosphorus and potassium during the vegetative stage was more critical than access during the reproductive stage in *B. napus*. Skinner et al. (1998) demonstrated the importance of nutrient uptake in corn at early plant growth stages because uptake later in the season may be limited by soil moisture status. *B. napus* has been shown to absorb more nutrients than it requires during vegetative growth suggesting that these nutrients may be stored for later use during flowering or pod fill (Wang et al. 2007).

2.3.4.2.2 Nitrogen acquisition

In *B. napus*, Nitrogen uptake has been linked to total root biomass and not higher uptake per unit of length in a study by Kamh et al. (2005). This contradicted findings by Laine et al. (1994) which demonstrate that if half of a *B. napus* root is starved of nitrogen the other half can still supply the shoot with sufficient nitrogen through increased uptake per unit of root length. The ability of a plant to increase its nitrogen uptake per unit length may be dependent on sufficient nitrogen availability which may be variable under field conditions.

2.3.4.2.3 Phosphorus acquisition

Increased lateral root length and rate of biomass accumulation has been positively correlated with phosphorus uptake and yield in *B. oleracea (*Hammond et al. 2009) and *B. napus* (Duan et al. 2009), while root angle of secondary roots

from the primary root was not related to phosphorus uptake or yield (Hammond et al. 2009). Solaiman et al. (2007) found a strong positive relationship between the phosphorus removed from the soil and root length in *B. napus*. The explanation of this was related not only to root length, but also to the ability of *B. napus* roots to reduce the pH in the rhizosphere with the release of organic acids. This allows for the insoluble phosphorus to become more readily available to the plant roots through, acidification of the soil near the rhizosphere (Hedley et al.1982). This is important in *B. napus* because it does not form a symbiotic relationship with arbuscular mycorrhizae. In other field crops, mycorrhiza help the plant access phosphorus that is otherwise inaccessible.

B. napus genotypes that have the ability to produce increased root length and biomass under low phosphorus environments have been identified (Rose et al. 2007). The ability to produce more root length and biomass in this type of environment improved the total above ground biomass of the genotypes (Akhtar et al. 2008).

2.3.4.3 Root/Shoot communication

Communication between the shoot and the root is important to ensure the shoot is adequately supplied with nutrients and water required for growth. It is also important to avoid inefficient allocation of assimilates such as producing more roots than required for shoot growth which could potentially negatively affect seed yield. Root proliferation and biomass accumulation are highly plastic and

thought to be controlled by the plant throughout its life. This ability allows the plant to react to its environment by increasing its ability to survive or thrive depending the conditions. Laine and Boucaud (1994) demonstrated a strong negative relationship between shoot nitrate content and nitrate uptake in *B. napus* roots suggesting that shoot nitrate content drives the uptake of nitrate in the roots. Although shoots signal the need for nutrients within the plant, there is evidence that root tips also produce signals that alter growth of lateral roots when areas of higher nutrient concentration are discovered (Williamson et al. 2001).

2.3.5 Biotic factors affecting root growth

2.3.5.1 *B. napus* root pathogens

The three major root pathogens that affect *B. napus* roots are phytophthora (*Phytophthora megasperma* Drechs. and *Phytophthora sojae* L.), rhizoctonia (*Rhizoctonia solani* L.) and clubroot (*Plasmodiophora brassicae* L.*)*. Root diseases can have a major impact on *B. napus* yield by decreasing plant population early in the season or limiting individual plants access to water and nutrients.

Phytophthora are oomycetes that reside in the soil and can infect *B. napus* beginning at the seedling stage. The infection is facilitated by warm, wet and waterlogged soils. Infected plants often become stunted and may develop a purple canker on the stem. These plants are easily pulled from the soil as a large portion of the root system is dead. There are few options to manage this

disease, the most effective being, improved drainage. Phytophthora is not a major concern in Western Canada in most years as it prefers warms soils and most of Western Canada has relatively cool soils compared to areas of the United States where phytophthora is more prevalent (Rimmer et al. 2007).

Rhizoctonia diseases, also known as damping-off, wirestem and brown girdling root rot, can infect *B. napus* at various stages of development. The growth stage at which the infection occurs dictates the common name of the disease. Damping-off occurs when the seedling dies before emergence. Damping-off and wirestem can both occur post-emergence. Infections at this stage will constrict the root and the shoot can become purple in color and then break at the base and senesce. Brown girdling root rot symptoms develop during flowering. The tap root and lateral roots will become girdled and turn brown or black in color causing premature ripening or death. The shoot will wither and either break at the base or be easily pulled from the soil due to lack of anchorage by the roots. Plants with girdled roots have shown 17% yield loss; however, if roots die at this stage of development yield loss can be as high as 65% (Klein-Gebbinck and Woods (2002). *B*. *napus* is not affected by brown girdling root rot, but *Brassica rapa* can be severely impacted, as shown by Klein-Gebbinck and Woods (2002). Seed treatments are used to help manage damping-off and wirestem in *B. napus* in Western Canada (Rimmer et al. 2007).

In 2003 near Edmonton, Alberta, a clubroot outbreak was discovered in *B. napus* fields. Clubroot has the potential to greatly reduce *B. napus* yields (Alberta Clubroot Management Committee, 2008). Clubroot is a soil borne

disease caused by *Plasmodiophora brassicae* that infects the roots of *B. napus* and other brassica species. It causes swelling of the root and the resulting club like galls greatly reduce root length and biomass (Rimmer et al. 2007). These galls limit a plant's ability to acquire water and nutrients which leads to stunted shoot development and subsequently lower seed yields. Currently, the only proven method of control of clubroot in *B. napus* is genetic resistance. However, research on manipulating seeding date to seed early and avoid the warm conditions under which clubroot thrives is being conducted. The preliminary results (Gossn et al. 2009) are promising, as symptom severity is reduced by 10-50% when planted earlier but more research is needed to confirm these results. If early seeding of *B. napus* is indeed an effective method in reducing severity of clubroot, *B. napus* genotypes with lower base temperatures for root accumulation and development may be at an advantage by developing larger root systems before being infected by clubroot.

2.3.5.2 *B. napus* root insects

The major recurring insect pest for *B. napus* roots are *Delia* spp., *Diptera*: *Anthomyiidae* (root maggots). There is currently no method of control for root maggots in canola which can cause 9-20% yield loss depending on infestation levels (Griffiths 1991). Soroka and Elliott (2006) established a positive relationship between *B. napus* basal stem diameter and infestation of root maggots. A larger tap root will attract more root maggot feeding. The research

noted that an increased seeding rate lowered the amount of root maggot feeding as high plant population result in smaller individual stem sizes due to intraspecific competition.

2.3.6 Root sampling techniques

The high spatial variability and challenges in recovering roots from soil make plant roots a difficult subject to study. Depending on the question to be answered there are different methods by which to study plant roots and with the difficultly in collecting accurate root data, using an appropriate method is an important consideration.

Polomski and Kuhn (2002) list various methods for studying roots, including: *Excavation Methods* (excavation, soil block, soil core sampling, in-growth core), *Direct Monitoring of roots in situ* (profile wall technique, root window, rhizotron, minirhizotron), studying roots in *Root Containers* (hydroponics, root tubes), and root *Labeling Methods* (e.g. radioisotopes, stable isotopes, dye methods).

Excavation allows researchers to view roots in their entirety, but this method is destructive to the plant and does not allow for further investigation of that root. Root excavation is also very labour intensive. For example, Pavlychenko (1937) excavated roots of mature plants to study the effects of weed competition on a variety of cereal crops and the root system of an entire mature wild oat (*Avena fatua* L.) plant yielded 86.9 km of root length. This root was elutriated from soil and measured by hand.

Direct monitoring allows for multiple measurements of the same root, but only allows for a portion of the root to be measured. Spatial variability of roots in soil can induce unwanted variation into the results obtained from this sampling method. *In situ* observations do not allow the researcher to distinguish root origin when multiple plants are present. When sampling tap rooted plants, large measurement differences occur when the taproot is included in the sample. Gentile et al. (2003) observed a 250% difference in alfalfa root biomass samples depended on whether the tap root was included in the measurement.

Root containers allow researchers to grow plants in a controlled environment and in a medium of their choice. The intent of root containers is to minimize field variation, but root containers can introduce their own variation. If plants become too large for the container, roots can become root bound and this may not be representative of a plant's rooting habit under field environments. Research would be required to investigate the effects of container type and size to minimize any effects the container may have on the root growth, taking into consideration the length of time the plant will be growing prior to sampling.

Labeling methods are used to study the distribution of roots and their ability to source water and solutes. Limitations of these methods include the need for equilibrium of isotopes within the plant to be reached prior to data collection and the differential water and solute uptake of roots. Also, older root tissue takes up less water and solutes than younger root tissue (Michunas, 2009). When using the labeling method to study roots it would be difficult to differentiate an area of few new roots with an area with many older roots, for example.

Gentile et al. (2003) stress that within treatment variation generated when sampling partial root systems make it necessary to maximize the number of replications sampled for each treatment. Much of the literature on roots cites these errors as difficult to minimize and many replications are needed especially when the measurements are conducted in the field, where soil conditions can vary substantially (Gentile 2003).

Chapter 3

Relationship between seedling root growth and seed yield among Brassica *napus* L. genotypes with different sampling intervals

3.1 Abstract

A study was designed to discover whether there is a relationship between seedling root length, root area or root weight and seed yield in *Brassica napus* L. Determining the optimal plant development stage at which to measure these root metrics was also an important aspect of this study. The experiment was conducted at two locations over two years. Eight *B. napus* genotypes were tested in a randomized complete block design with 4 replicates. Roots were recovered from the field at the cotyledon, the 1-2 and the 3-4 stages and root length and root area were determined using digital image analysis. Shoot dry weight and leaf area were also collected at the time of sampling. The plots were maintained to physiological maturity and harvested to acquire final seed yield. Root length and root area at the 1-2 leaf stage had a strong positive linear relationship with seed yield with R^2 values of 0.90 and 0.93, respectively. Shoot dry weight at the cotyledon stage had a strong positive quadratic relationship with seed yield with a (R^2= 0.99). This study demonstrated that seedling root length and root area at the 1-2 leaf stage can be used to predict seed yield potential.

3.2 Introduction

Canola (*Brassica napus L.*) is an important crop in Canada. Based on statistics from the 2009-2010 crop year, *B. napus* generated over $14 billion in economic activity to the Canadian economy (Anonymous 2010a). One area of *B. napus* research that has received little attention is the root system. This, in part, is due to the challenges of root research due to the variability and amount of labor involved in studying roots in a field environment.

Rose et al. (2008) discovered two *B. napus* genotypes with significantly different root biomass when conducting research on phosphorus use efficiency. The genotype with greater root biomass production as was able to access more phosphorus from the soil under low phosphorus conditions. This research did not look at differences in seed yield. However, the same study also found that over 70% of the total root biomass of *B. napus* was located in the top 15 cm of the soil profile and that most of the nutrient uptake in *B. napus* occurs during the vegetative stage. Auf'm Erley et al. (2007) studied nitrogen uptake in winter *B. napus* and root biomass and did not find a relationship between root biomass and seed yield. However, the plants in which the root biomass was measured were grown in a nutrient solution and none were sampled from the field from which the seed yield data were collected.

Mackay and Barber (1986) found that corn genotypes with different root lengths had significantly different nutrient uptake with the longer roots systems being able to capture more nutrients and thus lead to increased seed yield. Wan et al.

(1999) studied the impact of root length and biomass on corn hybrids with respect to hydraulic lift. Hybrids with greater root length were able to capture more water, but no seed yield information was provided. Research in soybeans, in which seed yield was determined, found a positive relationship between root length and seed yield (Brown and Scott 1984).

Root sampling methods vary greatly and there are many different reasons a particular study may choose any given sampling method. Whole versus partial sampling of plant roots can be determined by the objectives of the research. Riekman (2005) found no difference in root length between two different *B. napus* genotypes; however, this also may have been influenced by sampling methods and time of sampling. The sampling date was at anthesis and at this growth stage it is very difficult to obtain accurate root measurements due to the size of the root system especially when only partial root systems are sampled. Once the tap root has developed, estimation of root size can become challenging. Gentile et al. (2003) demonstrated that including the tap root in alfalfa increases root biomass by up to 2.5 fold compared to not including the tap root. Removing entire root systems of at anthesis or maturity can also present challenges. Pavlychenko (1937) excavated a mature wild oat plant from a field and measured the entire root system which totaled 86.9 km in length. The amount of labour required to remove, wash and measure an entire mature root system is extensive. Taking representative measurements roots at the late vegetative stage are challenging as a core break or a root wall may only contain 20-30% of the root (Bengough et al. 1992) and as Gentile et al. (2003)

demonstrated in alfalfa, if the tap root was included or excluded there will be significant changes in root size estimation. Excavating and elutriating the root system can be an accurate method of measuring roots. Pearson and Jacobs (1985) found when roots of subterranean clover (*Trifolium subterraneum* L.) were washed the true root length density may be underestimated by 30% or more as many of the fine roots can be lost; therefore great care must be taken to minimize losses for accuracy to be maintained. Adequate replication is essential to minimize the impact of the variation within root measurements (Gentile et al. 2003) and if the amount of labour required per sample is less, the number of replicates should be increased to improving the overall accuracy of the estimates.

In Western Canada, farmers have transitioned from open pollinated (OP) *B. napus* to hybrid *B. napus* with close to 90% of the seed sales in 2010 being hybrid genotypes (Charne 2010). The reason for the transition was the increased seed yield of 17-33% in hybrid genotypes compared to OP genotypes (Clayton et al. 2009; Van Deynze et al. 1992; Karamanos et al. 2005; Karamanos et al. 2006). Besides choosing a hybrid genotype, early season planting is another important factor to maximizing seed yield potential in *B. napus* (Chen et al. 2005; Clayton et al. 2004; Degenhardt and Kondra 1981; Kirkland and Johnson 2000). If there are differences in *B. napus* roots with some genotypes with increased root length early in the season, it can be hypothesized that this will lead to increased seed yield, because the plant will be able to access the

necessary water and nutrients required to maximize its genetic potential for seed yield.

There has been contradictory research on *B. napus* root biomass and the relationship between root accumulation and seed yield. This experiment attempts to resolve the contradictions and provide insight into the appropriate plant stage to sample *B. napus* to provide accurate seedling root measurements. The objectives of this study were to determine whether there is a positive relationship between root length, root area, root weight or shoot weight and seed yield and if developmental stage of the plant affects the robustness of these relationships.

3.3 Materials and Methods

3.3.1 Field Experiments

To determine the relationship between seedling root accumulation and seed yield in *B. napus*, a field experiment was conducted at four site-years (two locations and two years). The field experiment was located at Rosebank, MB (49°20’43.9”N, 98°07’06.3”W degrees/minutes/seconds) and Carman, MB (49°31’15.5”N, 97°58’05.2”W) during the 2009 and 2010 growing seasons. The Rosebank soil was a Gleyed Rego Black Chernozem (Michalyna et al. 1988) with a sandy loam texture (48% sand, 26% silt, 26% clay), organic matter content of 2.9% and a pH of 7.1. The Carman soil was a Gleyed Rego Black Chernozem (Michalyna et al. 1988) with sandy loam texture (58% sand, 20% silt, 22% clay),

organic matter content of 2.4% and a pH of 7.9. *B. napus* was not grown at these locations within the previous 4 years to minimize possible confounding effects of *B. napus* volunteers and *B. napus* diseases.

The field experiment was a randomized complete block design. The eight *B. napus* genotypes consisted of four hybrid genotypes and four OP genotypes with different known seed yield potential. The second factor of the experiment consisted of 3 or 5 sampling dates. The sample dates corresponded to the cotyledon, 1-2 leaf, 2 leaf, 3-4 leaf, and 4 leaf developmental stages and are listed in Table 3.1. During the 2009 field experiment, it was noted that the OP genotypes were approximately 1 leaf stage behind the development of the hybrid genotypes when they had reached the 1-2 leaf stage. Due to the delayed development of the OP genotypes, they were also sampled at the 2 and 4 leaf stage to allow for a comparison between all genotypes at a similar leaf stage. Therefore, the roots of the OP genotypes were sampled 5 times whereas the roots of the hybrid genotypes were only sampled 3 times. To minimize the effect of inherently different seed size among genotypes, in 2010 only, the seed lots were hand sieved to contain only seed with thousand kernel weight (TKW) between 4.1 and 4.5 g per thousand seeds. In 2009, the TKW of the seed lots were not adjusted and had a range from 3.0 to 4.8 g.

Prior to seeding all sites were tilled and fertilized at recommended rates as recommended by a soil test conducted each spring prior to planting. The pre-seed applied fertilizer varied by site and year with the amount applied provided in Table 3.2. The in-furrow application of fertilizer at planting was the same for all 4

Table 3.1. Seeding dates, sampling dates and cumulative post planting GDD_{base2} at each location and year.

		Cotyledon Interval		1-2 Leaf Interval		2 Leaf Interval [z]		3-4 Leaf Interval		4 Leaf Interval[z]	
Sites	Seeding Dates	Sample Date	GDD	Sample Date	GDD	Sample Date	GDD	Sample Date	GDD	Sample Date	GDD
Carman, 2009	21-May-09	4-Jun-11	154	15-Jun-09	265	NA	NA	18-Jun-09	327	22-Jun-09	410
Carman, 2010	15-May-10	27-May-10	206	2-Jun-10	319	4-Jun-10	347	7-Jun-10	393	9-Jun-10	420
Rosebank, 2009	20-May-09	4-Jun-09	155	15-Jun-09	266	NA	NA	NA	NA	NA	NA
Rosebank, 2010	17-May-10	3-Jun-10	266	8-Jun-10	344	11-Jun-10	374	14-Jun-10	417	16-Jun-10	452

[z] Open pollinated cultivars only
NA – Not applicable

Table 3.2. Pre-seed fertilizer application rates.

Location	N	P_2O_5	K_2O	S
	kg ha^{-1}			
Carman, 2009	101	34	34	17
Carman, 2010	101	34	34	17
Rosebank, 2009	134	39	0	17
Rosebank, 2010	101	45	34	25

site years at a rate of 112 kg ha^{-1} of product with the actual nutrient supplied at 15 kg ha^{-1} of nitrogen, 37 kg ha^{-1} of phosphorus and 17 kg ha^{-1} of sulphur (Micro Essentials S15, Cargill AgHorizons Canada). In 2010, precipitation was 115-150% above normal between April 1st and June 30th (Anonymous 2011) and therefore both experiments were top dressed with granular ammonium sulphate at a rate of 112 kg ha^{-1} providing an extra 24 kg ha^{-1} of nitrogen and 27 kg ha^{-1} of sulphur at the 3-5 leaf stage to compensate for any nitrogen and sulphur that may have been lost to leaching.

The studies were planted using a double disk Hege 1000 planter (Wintersteiger, Salt Lake City, Utah) with plot dimensions of 1.5 m X 7.5 m, trimmed to 1.5 m X 6 m at the 5 leaf stage. All eight *B. napus* genotypes were seeded at a rate of 140 seeds m^{-2}. Planting occurred once the soil had reached a temperature of 10 C between a depth of 12-25 mm. The specific seeding dates are shown in Table 3.1. All sites were seeded into soil with adequate moisture for imbibition. All genotypes were treated with a liquid mixture of pesticides consisting of 20.7% thiamethoxam, 1.25% difenoconazole, 0.39% metalaxyl-M and 0.13% fludioxonil applied at a rate of 15 mL kg^{-1} of seed to minimize the effect of disease and insect damage. Border plots were utilized to minimize any border effect on seed yield. An herbicide mixture of sethoxidim (445 g ai ha^{-1}), ethametsulfuron-methyl (22 g ai ha^{-1}) and clopyralid (83 g ai ha^{-1}) was applied to the experiment at the 2-3 leaf stage to control all grassy and broadleaf weeds. The experiments were also sprayed with boscalid (99 g ai ha^{-1}) at the 30% bloom stage to minimize the impact of sclerotinia stem rot on seed yield. Days to physiological maturity were

recorded for all genotypes with the genotype considered mature once the seeds in pods 1/3 from the proximal end of the main raceme started to turn from green to brown on all plants within the plot. The *B. napus* genotypes were desiccated with diquat (168 g ai ha^{-1}) when 70% of the seeds on the main raceme had changed from green to black. Seed yield was collected by straight cutting the genotypes with a Kincaid XP8 combine (Kincaid Equipment Manufacturing, Haven, Kansas). Weight and moisture was collected for each genotype with seed yields adjusted to 8% moisture content.

Immediately after planting, Watchdog mini weather stations (Spectrum Technologies Incorporated, Plainfield, IL) were installed at a height of 60 cm to collect ambient air temperatures. Growing degree days (GDD) were calculated using a 2 C base temperature, the accumulated GDD for each sample date are indicated on Table 3.1.

3.3.2 Sampling and root measurements

At each sampling date, five 7.6 cm diameter X 15 cm deep root cores with one *B. napus* plant in the centre were removed from each plot using a piece of modified PVC pipe. The pipe was placed over a *B. napus* plant and pressed into the ground then removed from the soil, this removed the soil and *B. napus* roots intact. Each core was then bagged and frozen (-20 C) so the root could be recovered and measured at a later date. At the time the root cores were sampled, the shoot of the plant in the core was removed at ground level and

dried at 65 C for 3 days and the shoot dry weight was determined. In 2010 only, the leaf area of the same plants was also determined using a LI-COR LI-3000 portable leaf area metre (Lambda Instrument Corp., Blacksburg, VA).

To measure the roots, the frozen cores were thawed slowly for one day at 4 C in a refrigerator after which the roots were elutriated by hand to remove all soil and plant residue. The roots were stained with 84% toluidine blue mixed to 1% w/v, plated in glycerol on a clear glass plate and manipulated with tweezers to ensure all roots and root fragments were clearly separated. Root samples collected after the 2 leaf stage were cut into pieces approximately 5 cm in length to facilitate separation and minimize overlap. The roots were then scanned against a white background using a flatbed scanner (Canon 5600F, Canon, Tokyo, Japan) at 400 dpi. Assess 2.0 imaging software (American Phytopathological Society, St. Paul, MN) was then used to determine root length and root area. The software was calibrated by scanning a ruler which ensured that the lengths calculated by Assess 2.0 were accurate. Once the root was scanned, it was washed in distilled water to remove the glycerol, dried at 65 C for 3 days and root dry weight was determined.

3.3.3 Statistical analysis

Proc Univariate (SAS Institute, 2008) was used to test for the normality of the residuals for root length, root area, root weight, shoot weight, leaf area and shoot:root ratio. Outliers were removed based on studentized residuals using

Lund's test (Type 1 error rate = 0.05) (Lund, 1975). Root length, root area, root weight, shoot weight, leaf area and shoot:root ratio data were then analyzed using Proc Mixed (SAS Institute, 2008) to test the fixed effects and their interactions with genotype. Year and site were considered fixed effects and replication was considered a random effect. Homogeneity of variance was examined using the group statement and the correct model was used to determine whether site-years should be combined for subsequent regression analysis. Proc Mixed was utilized to ensure the correct model was used and means of the seed yield and maturity were separated using Fisher's protected LSD (<0.05) with the pdmix800 macro (Saxton 1998). Correlations between maturity and seed yield were conducted in Microsoft Excel® (Microsoft Office, 2007) and Pearson's r correlation coefficients were determined.

Proc GLM (SAS Institute, 2008) was used to separate linear and quadratic components of seed yield versus the independent variables. Where there were no fixed effect interactions the data for root length, root area, root dry weight, shoot dry weight, leaf area and shoot:root ratio were combined and the slope and intercept were calculated using seed yield as the independent variable. When either location or year effects were significant, the regression analysis was conducted as dictated by the Proc Mixed analysis.

3.4 Results

3.4.1 Yield & Maturity

Seed yield of *B. napus* was influenced by genotype and site year (Tables 3.3 and 3.4) and no significant interactions were observed among these factors. The hybrid genotypes on average produced 382 kg ha^{-1} more seed than the OP genotypes. This translated to a 14% increase in seed yield for the hybrid genotypes when compared to the OP genotypes. The seed yield of the lowest yielding hybrid was similar to that of the highest yielding OP genotype. No statistically significant differences were detected among the hybrid genotypes or within the OP genotypes. Among the site years, average seed yield at Carman, 2009 was greater than at all other site years, among which the average seed yield did not differ (Table 3.4).

Maturity among all eight genotypes was inconsistent with no apparent differences between the group of OP and hybrid genotypes (Table 3.5). The Pearson's r correlation between maturity and final seed yield was -0.26 indicating a weak, but significant relationship ($P < 0.01$).

3.4.2 Potential predictors of seed yield at various developmental stages

3.4.2.1 Cotyledon stage

Table 3.6 shows the coefficients of determination (R^2) for root length, root area, root weight, shoot weight, leaf area and shoot:root ratios. The root length to

Table 3.3. Average canola seed yield across 4 site years.

Cultivar	Yield (kg ha^{-1})
45H73	2744*a*
HYB1	2724*a*
HYB2	2633*a*
HYB3	2598*ab*
46A65	2355*bc*
OP 1	2315*c*
OP2	2277*c*
OP3	2220*c*

OP – Open Pollinated

HYB – Hybrid

a-c Means followed by the same letter do not differ at $P < 0.05$

Table 3.4. Average canola seed yield per site.

Year	Sites	Yield (kg ha^{-1})
2009	Carman	3114*a*
2010	Rosebank	2330*b*
2010	Carman	2250*b*
2009	Rosebank	2238*b*

a-b Means followed by the same letter do not differ at $P < 0.05$

Table 3.5. Average days to physiological maturity across 4 sites.

Cultivar	Days to Maturity
OP1	88*f*
46A65	93*cd*
OP2	95*bc*
OP3	96*a*
HYB1	94*b*
45H73	92*d*
HYB2	91*e*
HYB3	90*e*

OP - Open Pollinated

HYB - Hybrid

a-f Means followed by the same letter do not differ at $P < 0.05$

Table 3.6. Coefficients of determination (R^2) of seed yield regressed against root length, root area, root weight, shoot weight, leaf area and shoot:root ratio for the cotyledon stage. Data for regressions are combined as dictated by univariate statistics.

Sites	Root Length (cm)	Root Area (cm^2)	Root Weight (g)	Shoot Weight (g)	Leaf Area (cm^2)	Shoot:Root Ratio
			R^2			
Carman 2009	0.52*[z]				NA	
Carman 2010	0.75**[z]				0.54[y]	
Carman, 2009&2010		NS				
Rosebank 2009	NS				NA	
Rosebank 2010	NS				NS[y]	
Rosebank 2009&2010		NS				
All Sites			NS	0.99***[x]		0.78**[z]

[z] Linear regression
[y] 2010 Sites only
[x] Quadratic regression
NS (not significant)
NA (not applicable)
*, **, *** F value at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

seed yield regressions were conducted between root length, site and year due to significant interactions. At Carman, the relationship between root length at the cotylendon stage and seed yield was significant in both years with a R^2 of 0.53 (2009) and 0.75 (2010). At Rosebank, the relationship of root length to seed yield was not significant in either year. An important consideration among the dependent variables regressed against seed yield was the interaction of the dependent variable among sites and years and therefore all significant interactions ($P < 0.05$) were reported.

An interaction between sites, but not years was observed in root area and therefore root area data were combined based on sites, but when regressed against seed yield, the relationship was not significant (Table 3.6).

Root dry weight was influenced by genotype only, with no interactions with sites or years. Therefore, data were pooled, but no significant relationship with seed yield was observed (Table 3.6).

Shoot weight was influenced only by genotype and the shoot weight was significantly related to seed yield. Shoot weight at the cotyledon stage explained 99% (R^2=0.99) of the variation in seed yield and shoot weight at this stage had the strongest relationship to seed yield compared to all the other traits measured. Figure 3.1 illustrates the regression showing the slope and the distribution of the shoot weight. The OP and hybrid genotypes was grouped separately, but the distribution of the data were uniform along the relationship and reached a peak

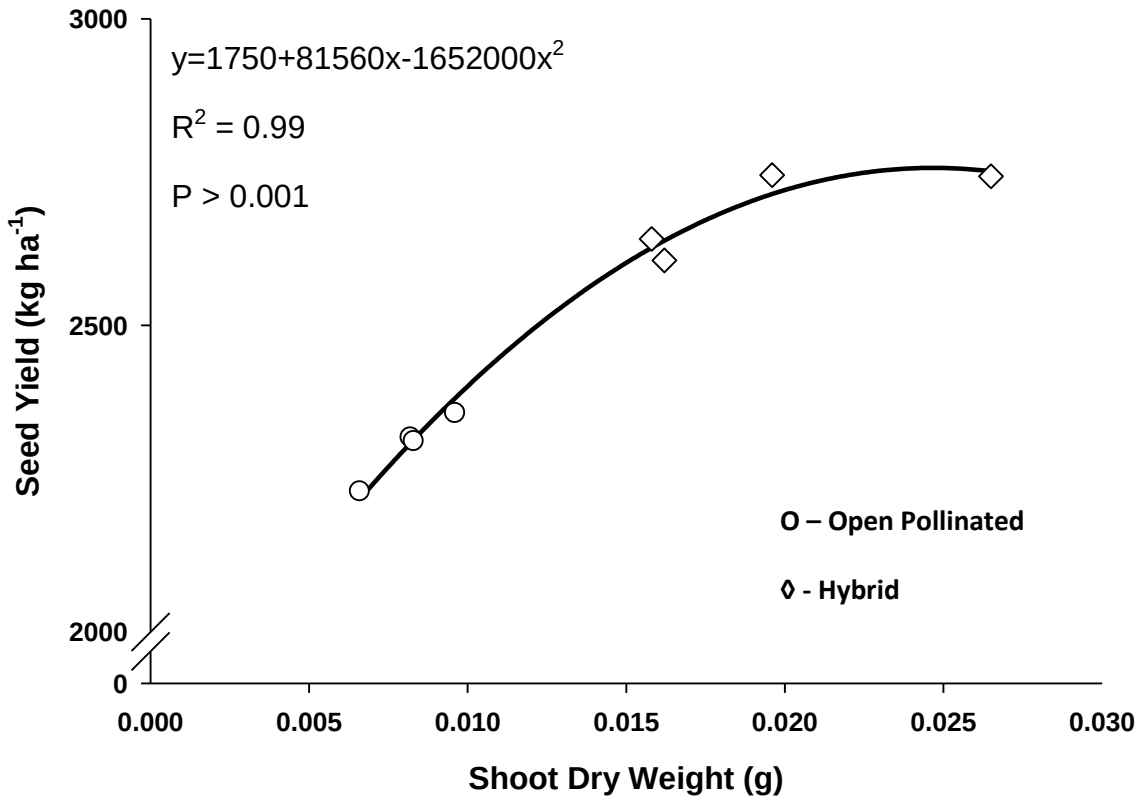

Figure 3.1. Shoot dry weight at the cotyledon stage vs. seed yield. Regression equations and coefficients of determination (R^2) are indicated.

where increases in shoot dry weight did not result in further increases in seed yield.

The shoot:root ratio data were influenced only by genotype and therefore the data for the sites and years were combined. The R^2 for the relationship between seed yield and shoot:root ratio was 0.78 which is a relatively strong relationship, but it was not as strong as the relationship between shoot weight and seed yield.

3.4.2.2 One to two leaf stage

Table 3.7 shows the coefficients of determination (R^2) for root length, root area, root weight, shoot weight, leaf area and shoot:root ratio at the 1-2 leaf stage. For root length, no site or year interactions were observed among genotypes and therefore all the data were combined when root length was regressed against final seed yield to produce an R^2 of 0.90. Figure 3.2 shows the distribution of the root length data points along the linear regression. The OP genotypes were clearly separated from the hybrid genotypes with an apparently curvilinear relationship with OP genotypes. The hybrid genotypes appeared to have a linear relationship. However, with only four data points within each group the dataset was too small to confirm these sub trends.

For root area data at the 1-2 leaf stage, no site or year interactions were observed among genotypes and therefore the root area data from the four site years were combined (Table 3.7). Once combined, there was a strong relationship between root area and seed yield with root area explaining 93%

Table 3.7. Coefficients of determination (R^2) of seed yield regressed against root length, root area, root weight, shoot weight, leaf area and shoot:root ratio are listed below for the 1-2 leaf stage. Data for regressions are combined as dictated by univariate statistics.

Sites	Root Length (cm)	Root Area (cm^2)	Root Weight (g)	Shoot Weight (g)	Leaf Area (cm^2)	Shoot:Root ratio
			R^2			
Carman, 2009			NS			NS
Carman, 2010			NS			NS
Rosebank, 2009			0.39*			NS
Rosebank, 2010			0.43*			NS
All Sites	0.90***	0.93***		0.84***	0.72**[z]	

[z] 2010 Sites only
NS (not significant)
*, **, *** F value at P $\leq$ 0.05, P $\leq$ 0.01 and P $\leq$ 0.001, respectively

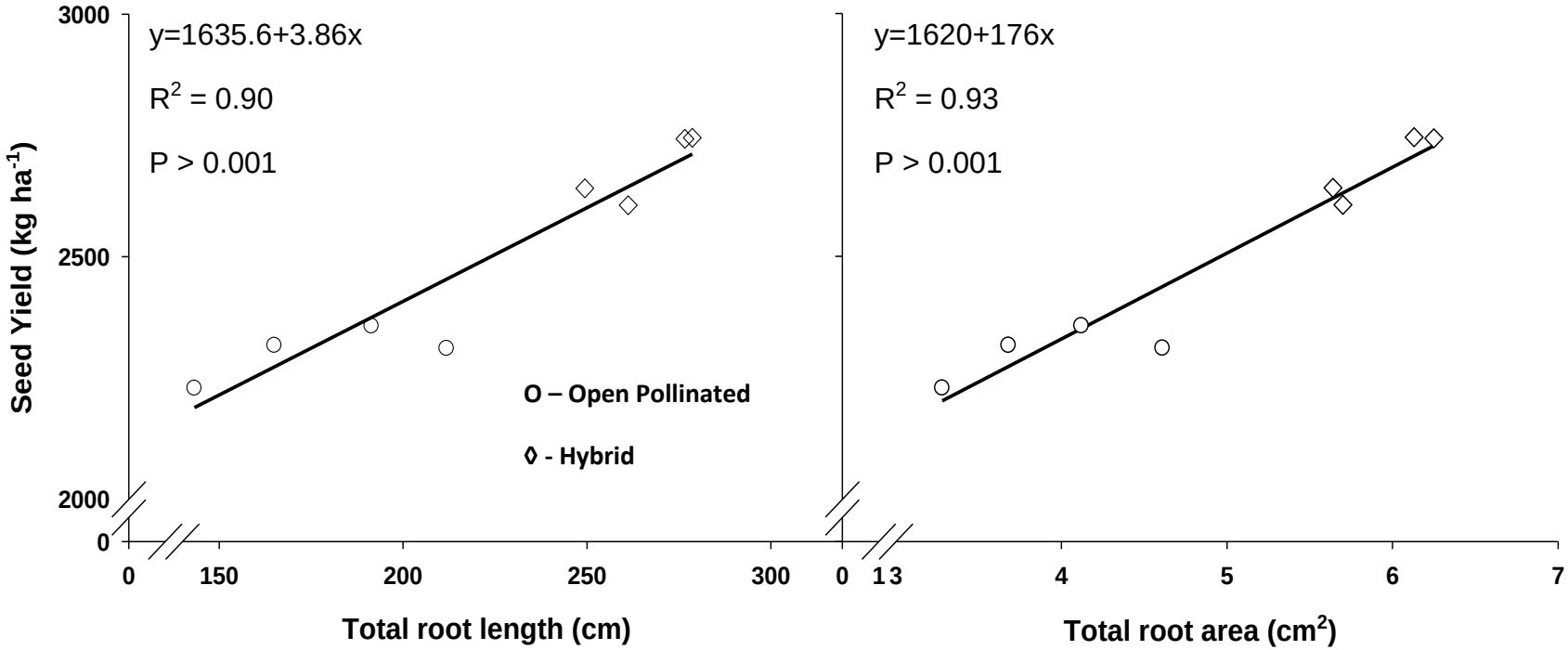

Figure 3.2. Root length (right) and root area (left) at the 1-2 leaf stage vs. seed yield. The regression equations and coefficients of determination (R^2) are indicated.

(R^2=0.93) of the total variation observed among the genotypes. In Figure 3.2, the linear regression is similar to that of root length for the 1-2 leaf stage. There appeared to be a unique relationship between root length and the yield within the OP and hybrid genotypes.

For root dry weight, interactions were observed for both sites and years (Table 3.7). There were no significant relationships observed between root weight and seed yield at any of the four site years.

No site or year interactions were observed for shoot dry weight data. The linear regression (Fig. 3.3) produced demonstrated that there was a strong relationship (R^2=0.84) between shoot weight at this stage and seed yield, but this relationship was not as strong as for root length or root area. When root area was plotted against seed yield, there was a clear separation between hybrid and OP genotypes with a cluster of each group of genotypes defining the relationship (Fig. 3.3).

No site interaction was observed between site and leaf area. Leaf area was sampled only in 2010; therefore year was not a factor in this metric. Both sites were combined and when regressed against seed yield the R^2 was 0.72. There was a strong positive linear relationship (Fig. 3.4) between leaf area and seed yield, but not as strong as for root length and root area. Open pollinated and hybrid genotypes behaved as two distinct groups and within each group, there appeared to be no clear relationship between leaf area and seed yield.

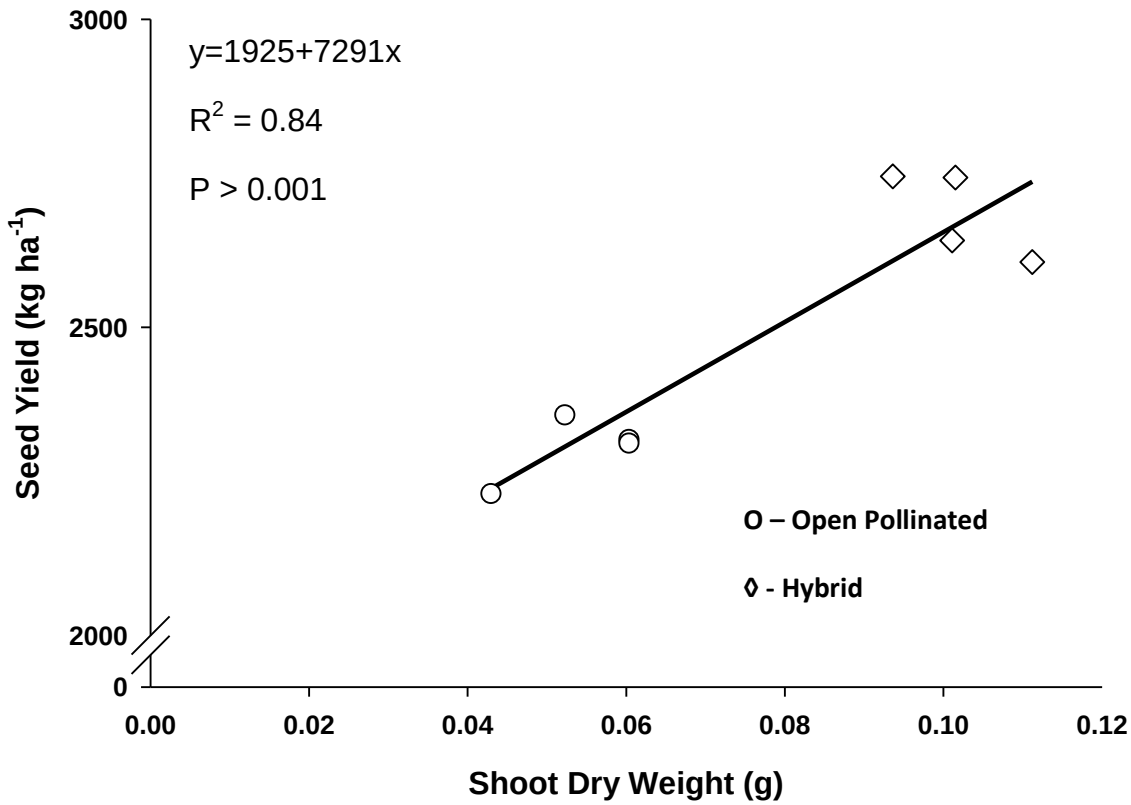

Figure 3.3. Shoot weight at the 1-2 leaf stage vs. seed yield. Regression equations and coefficients of determination (R^2) are indicated.

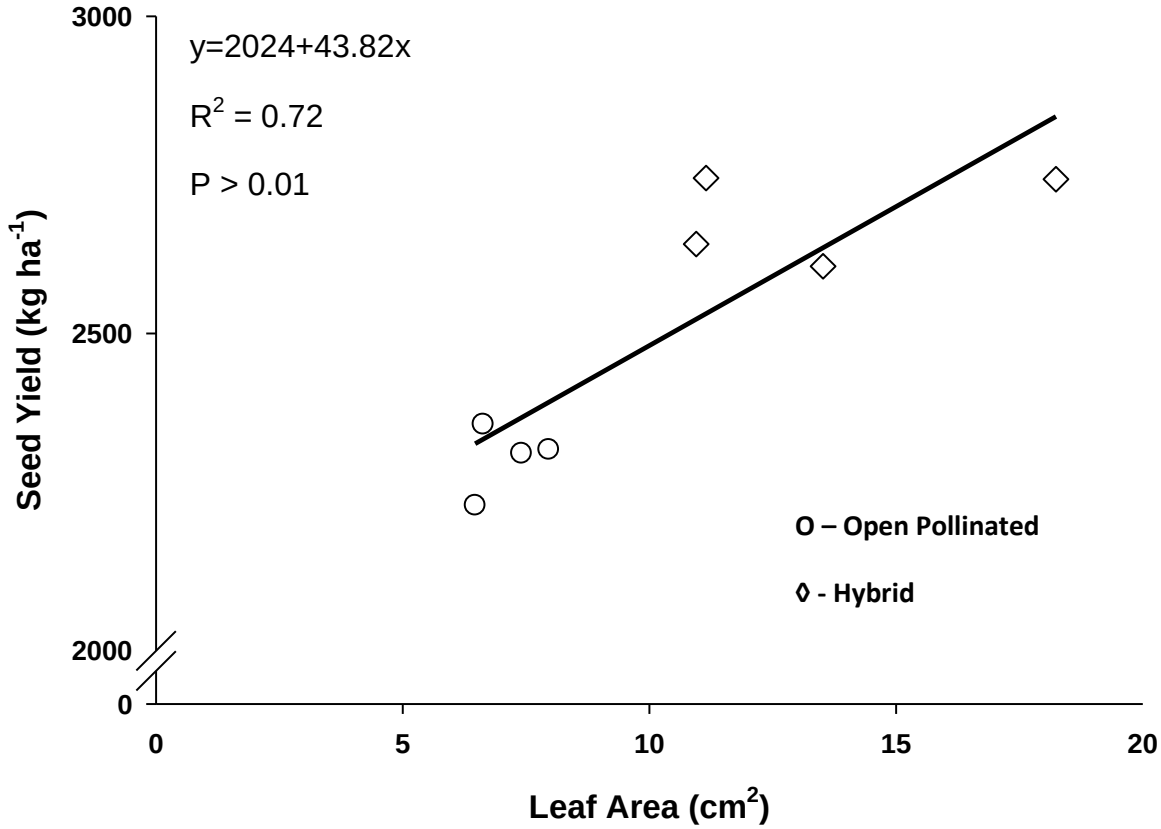

Figure 3.4. Leaf area at the 1-2 leaf stage vs. seed yield. Regression equations and coefficients of determination (R^2) are indicated.

3.4.2.3 Two leaf stage for OP and hybrid genotypes

During the 1-2 leaf stage it was noted that there were differences between the OP and hybrid genotypes. The hybrid genotypes were approximately 1 leaf stage ahead of the OP genotypes. Therefore, in 2010, the OP genotypes were sampled 3-5 days after the 1-2 leaf sampling of the hybrid genotypes when they had reached the same leaf stage as the hybrid genotypes at the 1-2 leaf sample date. No significant relationships were observed between root length, root area, root dry weight, shoot dry weight, leaf area or shoot:root ratio and seed yield when the hybrids and OP genotypes were at the 2 leaf developmental stage (data not shown).

3.4.2.4 Three to four leaf stage

Table 3.8 shows the coefficients of determination (R^2) for root length, root area, root weight, shoot weight, leaf area and shoot:root ratio at the 3-4 leaf sample date. The 3-4 leaf stage was collected only in 2010; therefore, year was not a factor in the metric. Due to no interactions among root length, sites and data were combined for regression against seed yield (R^2=0.71). Figure 3.5 shows that the root length data of all genotypes were distributed more uniformly along the slope, with less clustering than at the 1-2 leaf stage.

Interactions among sites and years were observed in the response variables root area, root weight, shoot weight, leaf area and shoot:root ratio and therefore these data were not combined. When root area at the 3-4 leaf stage was regressed

Table 3.8. Coefficients of determination (R^2) of seed yield regressed against root length, root area, root weight, shoot weight, leaf area and shoot:root ratio are listed below for the 3-4 leaf stage. Data for regressions are combined as dictated by univariate statistics.

Sites	Root Length (cm)	Root Area (cm^2)	Root Weight (g)	Shoot Weight (g)	Leaf Area (cm^2)	Shoot:Root ratio
			R^2			
Carman 2010		0.49*	NS	NS	NS	NS
Rosebank 2010		0.57*	0.63**	0.61*	0.62*[z]	NS
All Sites	0.71*					

[z] 2010 Sites only
NS (non-significant)
*, **, *** F value at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively

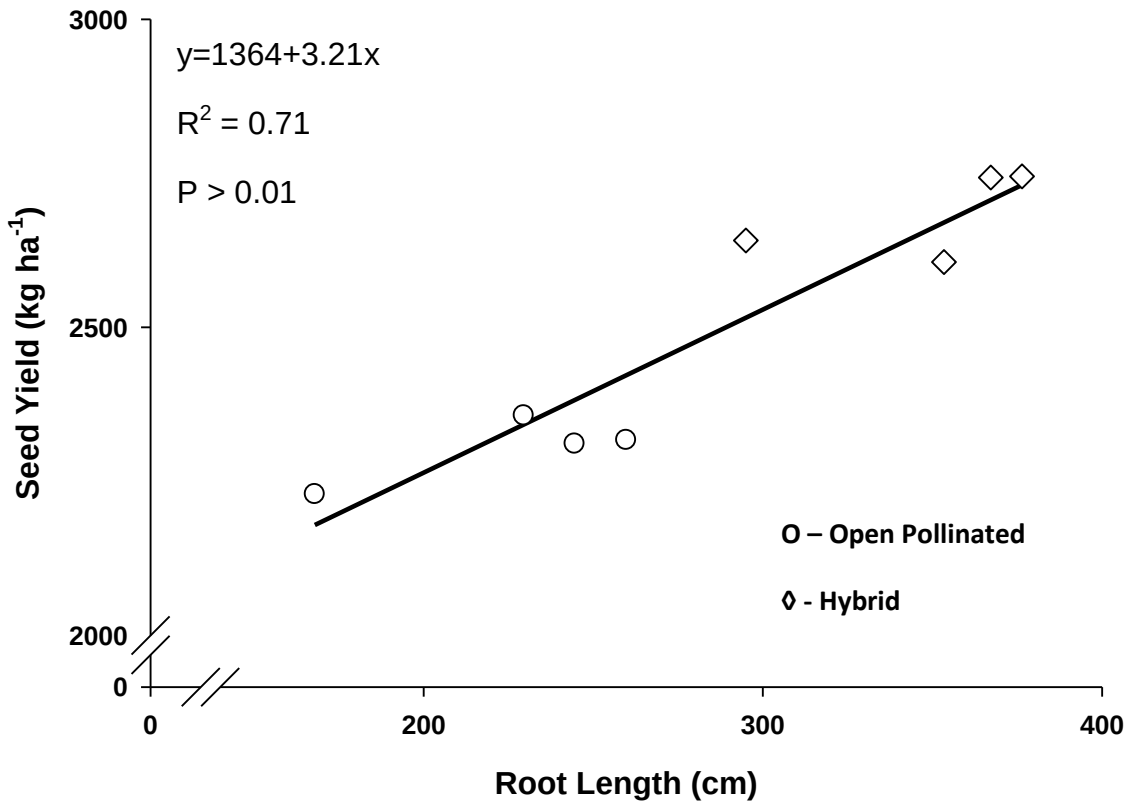

Figure 3.5. Root length at the 3-4 leaf stage vs. seed yield. Regression equations and coefficients of determination (R^2) are indicated.

against final seed yield, a weak positive relationship was observed at both locations (Carman R^2=0.49, Rosebank R^2=0.57). The mean comparisons of root weight, shoot weight, leaf area and shoot:root ratio were all not significant at Rosebank (data not shown). At Carman, the R^2 values for the relationship between root dry weight, shoot dry weight and leaf area and final seed yield were 0.63, 0.61 and 0.62, respectively. The relationships for root area, root weight, shoot weight and leaf area were significant, but explained less of the total variation in seed yield than root length at this leaf stage and in addition, unlike root length data, interactions among sites and years did not allow the data to be combined for these parameters before regression with seed yield. No relationship between shoot:root ratio and seed yield was observed.

3.4.2.5 Four leaf stage for OP and hybrid genotypes

At the 4 leaf stage, only the OP genotypes were sampled so they could be compared to the hybrid genotypes at a similar growth stage only. Only three of the four site years were available at this development stage because too much time had elapsed between the 3-4 leaf sample date and the 4 leaf sample date at Rosebank, 2009. The OP genotypes were more advanced than the hybrids at the 3-4 leaf sample date making a comparison at a similar growth stage not possible at this site.

Root length, root area and shoot:root ratio at this sample stage did not contribute significantly to explaining any of the variation in seed yield at the sites and the

data from the sites could not be combined due significant interactions between sites, years and these parameters (Table 3.9). At this stage, root dry weights and shoot dry weights were consistent across sites and these data were combined. After the data for the root dry weight and shoot dry weight were combined, the R^2 values were 0.56 and 0.53 for root and shoot dry weight, respectively, indicating a weak, but significant relationship with seed yield. Figure 3.6 shows the linear regressions of root dry weights and shoot dry weights, illustrating the weak positive relationship these two metrics had with seed yield. Open pollinated and hybrid genotypes were clearly separated with no apparent relationship within the OP and hybrid genotypes when viewed as distinct groups. In 2010, leaf area data were not consistent across the two sites and therefore could not be combined. At Carman 2010, the relationship between leaf area and seed yield was not significant and at the Rosebank 2010, site the R^2 was 0.53 indicating a weak relationship between the dependent and independent variables.

3.5 Discussion

The two sites in this study were within 20 km and the soils belong to the same soil classification. Nevertheless, the relatively small differences in texture influenced the ability to recover *B. napus* seedling roots from these soils. Recovering roots from the Carman site, which had a higher sand content than the Rosebank site, was much easier. Another factor that impacted root recovery

Table 3.9. Coefficients of determination (R^2) of seed yield regressed against root length, root area, root weight, shoot weight, leaf area and shoot:root ratio are listed below for the 4 leaf stage. Data for regressions are combined as dictated by univariate statistics.

Sites	Root Length (cm)	Root Area (cm^2)	Root Weight (g)	Shoot Weight (g)	Leaf Area (cm^2)	Shoot:Root Ratio
			R^2			
Carman, 2009	NS	NS			NA	NS
Carman, 2010	NS	NS			NS	NS
Rosebank, 2010	NS	NS			0.53*[z]	NS
All Sites			0.56*	0.53*		

[z] 2010 Sites only
NS (not significant)
NA (not applicable)
*, **, *** F value at P $\leq$ 0.05, P $\leq$ 0.01 and P $\leq$ 0.001, respectively

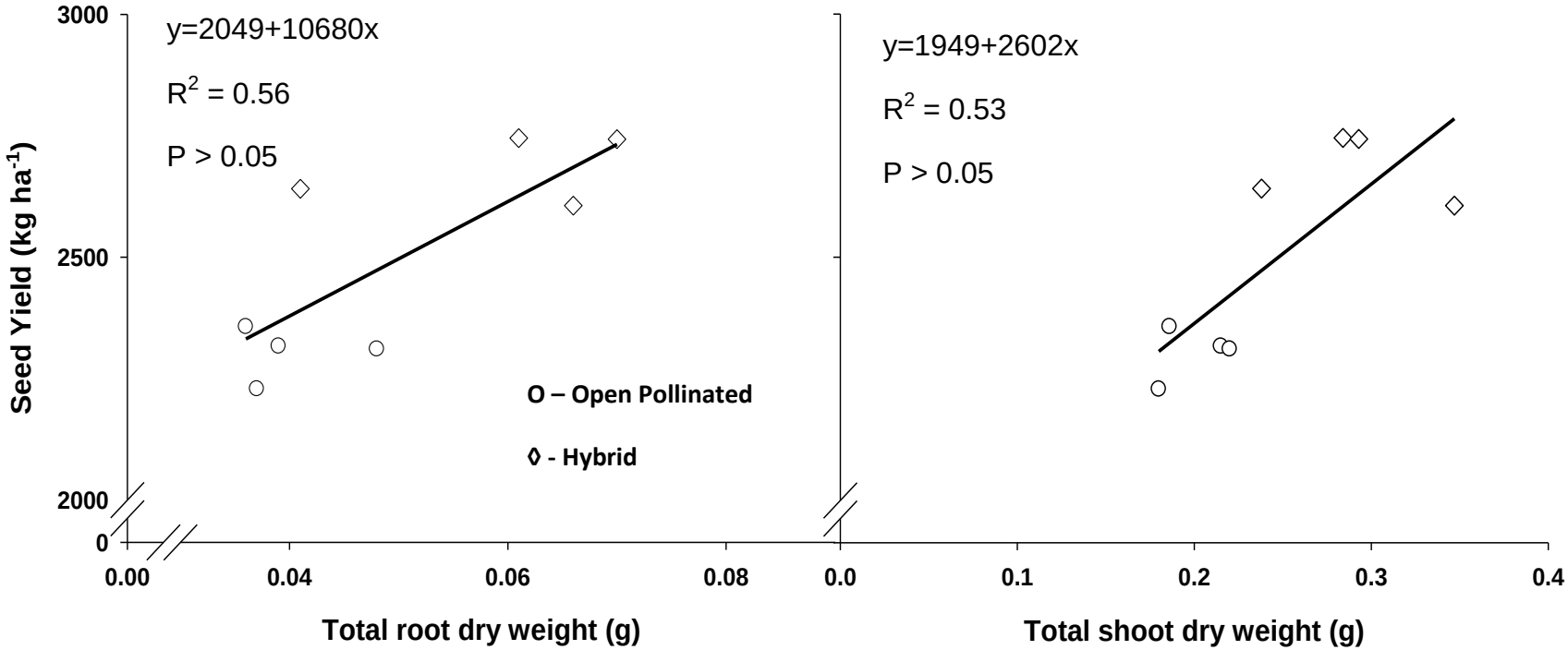

Figure 3.6. Root dry weight (left) and shoot weight (right) at the 4 leaf stage vs. seed yield. Regression equations and coefficients of determination (R^2) are indicated.

was the remnant residue from previous crops. Cereal crop residue such as corn and wheat made it much more time consuming to recover roots from the 2009 sites.

Root length and root area in *B. napus* showed the strongest positive relationship with seed yield when the plants were sampled at the 1-2 leaf stage. The 1-2 leaf stage also provided the most consistent relationship of root length and root area to seed yield among the sites and years.

The consistency of the root length and root area at the 1-2 leaf stage over site years may, in part, have been attributable to the overall size of the root systems. The roots were still small enough that much of the recoverable part of the entire root system was contained within the volume of the core. At the 3-4 leaf and 4 leaf sample dates, *B. napus* roots had grown to a depth beyond the bottom of the core. Removing larger volume samples may have improved root system recovery of the total root of a single plant at the later developmental stages; however, recovery of the roots of individual plants may have been confounded by encroaching roots from neighbouring plants in larger volume cores. At the 4 leaf stage, *B. napus* roots were still small enough to be contained within the core and the roots were large enough so that they could be handled more easily during elutriation. If some of the very fine roots were lost at this stage, the impact on the overall length and area was likely less significant compared to the cotyledon stage where even small losses during recovery may have had a significant impact on the measured parameters. At the cotyledon stage, root length and root area were more variable than at the 1-2 leaf stage likely because at the

cotyledon stage the root systems were so fine that a greater proportion may have been lost during recovery. This may have contributed to the substantially better relationship between shoot dry weight and seed yield compared to root length and root area and seed yield at this growth stage.

At the 3-4 leaf stage, root area may have been influenced more by the dominance of the developing tap root. If at the 3-4 leaf stage, the tap root was slightly larger for a given genotype it could have had a substantial influence on the overall root area. Given that the taproot does not contribute significantly to water and nutrient uptake, this increase in root area may have little effect on overall seed yield thereby diminishing the relationship of root area to seed yield.

Root dry weight did not show a strong relationship with final seed yield at any sample stage and was often inconsistent between sites and years. This suggests that root biomass which is strongly influenced by the size of the tap root in *B. napus,* was not as consistent as root length or root area in explaining final seed yield. Increased biomass is not always related to increased root length or root area (Svejcar 1990; Russelle and Lamb 2011).

Of all the metrics investigated at the cotyledon stage, shoot dry weight had the strongest relationship with seed yield. The genotypes that had the greatest shoot dry weight at the cotyledon stage may have emerged earlier and this may have contributed to increased seed yield. Clayton et al. (2004) demonstrated the link between early emergence of *B. napus* and seed yield. The ability of shoot dry weight and leaf area to predict final seed yield concurs with research by

Campbell and Kondra (1978) where they found above ground biomass of *B. napus* at physiological maturity could be used to predict seed yield potential. The strength of this relationship diminished at other developmental stages in this study where root length was more predictive of seed yield than shoot parameters, indicating that the strength of this relationship was highly dependent on development stage of the plants. The relationship of shoot dry weight at the 1-2 leaf stage was significant with a R^2 of 0.84; however due to the barbell distribution of the OP and hybrid genotypes the biological value of this relationship was not as strong as root length and root area,

The lack of differences among OP and hybrid genotypes when sampling the OP genotypes a few days after the hybrid genotypes when they had reached the same developmental stage, indicated that the differences observed between OP and hybrid genotypes were primarily due to the more rapid increase in root length, root area, shoot weight and leaf area in the hybrid genotypes. Studies that have shown earlier planted *B. napus* will achieve increased seed yield often suggest increased moisture availability and cooler environments during flowering as reasons for the increased yield (Clayton et al. 2004; Degenhardt and Kondra 1980; Kirkland and Johnson 2000). This study confirms that genotypes with the ability to increase the amount of vegetative biomass more rapidly during the growing season are associated with increased seed yield potential when planted at the same time. More rapid development at the seedling stages did not translate to earlier physiological maturity, allowing these plants more time at the reproductive stages.

The differences between hybrid and OP genotypes were significant in this study which was not unexpected, as there is a wide body of research showing heterosis can positively impact vegetative biomass and seed yield of the hybrid genotypes in various crops (Grosse et al. 1992; Ojo et al. 2007; Stuber 1994). Grosse et al. (1992) demonstrated heterosis in winter *B. napus* not only impacts seed yield, but can also impact biomass at anthesis, harvest index and the number of siliques per unit area. In corn and rice (*Oryza sativa* L.) hybrids, root systems are longer and grow to greater depths than their respective inbreds. Corn hybrids had 22% greater root length than their respective inbreds 5 days after germination (Hoecker et al. 2006), and rice hybrids were shown to reach 4-11% greater depths than the respective inbreds at 25, 50 and 75 days after transplanting (Sh et al. 2009). This experiment did not compare the hybrids to their respective inbred lines as the corn and rice examples cited above; however the ability for heterosis to clearly impact seedling root length in other crops as well as the research indicating that heterosis impacts many aspects of *B. napus* plant growth and development supports the notion that the clear differences between OP and hybrid genotypes in this study were likely due to heterosis. The hybrid genotypes may have had the influence of hybrid vigour which not only affected the seed yield and shoot biomass as shown by Grosse et al. (1992), but also root length and root area.

Increased seed yield hybrid genotypes have been shown to require increased levels of nitrogen and sulphur to achieve maximum yield potential (Karamanos et al. 2006). The research also indicated that hybrid genotypes removed more

nitrogen and sulphur from the soil than the OP genotypes. The ability of a hybrid to acquire more nutrients under similar conditions as an OP genotype indicates that the hybrid genotypes may have increased root length to access more nutrients required to achieve greater seed yields. In areas of nutrient deficient soils, increased root length may also increase seed yield by allowing plants to access more nutrient rich areas in the soil profile than a similar genotype with shorter total root length (Lynch 2011). Two different *B. napus* genotypes were shown to exhibit different root length potential (Rose et al. 2008) and the *B. napus* genotype with greater root length was able to access more phosphorus in phosphorus limited soil.

3.6 Conclusion

Overall this study provides some valuable insights into seedling root growth in *B. napus* and its relationship to seed yield. It also highlights the best developmental stage for sampling of roots in the field when using these methods. Early development of *B. napus* was essential for it to reach maximum seed yield.

Further research into the differences in root length and root area among hybrid genotypes and their parental lines would provide insight into the potential of selecting genotypes with increased early root length and root area accumulation. Within this study the root length and root area of hybrids were more evenly distributed when regressed against seed yield, therefore it may be useful to do more research with a greater number of hybrids to more closely look into the

differences in root length and root area within this specific group. Studying hybrids and their parental lines could help researchers understand the inheritance of root length and root area to ascertain whether these are traits that can be used as selection criteria within breeding programs.

Chapter 4

Relationship between seedling root length and area to short- and long-term seed yield among *Brassica napus* L. genotypes

4.1 Abstract

Seedling root length may have the potential to predict seed yield. This study utilized short- and long-term yield data to analyze the relationship between seedling root length and seed yield. Eight different *Brassica napus* (*B. napus*) genotypes were grown in transparent plastic germination boxes on blotter paper in a growth room at 20/16 C day/night temperatures. After 7 days, the trays were removed and root length and area were measured using digital imaging software. The root lengths and areas were then regressed against short-term yield data (four sites years) collected in Chapter 3 (R^2=0.95) and long-term yield data (88 to 2469 pair wise comparisons) acquired from Pioneer Hi-Bred Production Limited Partnerships database (R^2=0.96). Root area data were not as reliable as root length due to differential root hair development among the plants which introduced errors during image analysis. Collectively, hybrid genotypes produced more seed yield and root length than OP genotypes and the relationship between root length and long-term yield was more meaningful than that of root length and short-term yield.

4.2 Introduction

The ability to predict *B. napus* seed yield potential shortly after the completion of germination could revolutionize plant breeding. Research has shown that canola shoot biomass at physiological maturity is a reliable indicator of seed yield (Campbell and Kondra 1978).

The shoot provides the plant with the energy and carbon required to produce seed and the root provides the plant with all other resources required to accumulate biomass, providing researchers with valuable information much earlier. The radicle emerges first from the seed before the cotyledons to form the plant root. Therefore, at the seedling stage the root measurements may be more informative and predictive of seed yield. The seedling root length may have the potential to be indicative of seed yield before late season shoot biomass. Because of the importance of the root it is surprising how little research has been conducted on the relationship between early root development and total seed yield in *B. napus*.

The local field experiment (Chapter 3) showed that *B. napus* seedling root length and root area at the 1-2 leaf stage were related to final seed yield over 4 site years. Chapter 3 also showed that at the cotyledon stage, the relationship between cotyledon shoot biomass and seed yield was stronger than that with root length and root area and seed yield, despite roots developing more rapidly immediately after germination. Difficulty recovering smaller root systems at the cotyledon stage (Chapter 3) may have confounded the results by introducing

increased variation and therefore, a strong relationship of root length and root area to seed yield was difficult to establish at this developmental stage in a field environment. In contrast, research on winter *B. napus* roots showed that there was no relationship between seed yield and root length at 7, 14 and 21 days after germination (auf'm Erley et al. 2007). The research was conducted on a limited number of cultivars without nutrient limitations; the roots were grown hydroponically; and the seed yield data were collected separately from only two field sites. Contradicting the work by auf'm Erley et al. (2007) is research in soybean which showed a strong relationship between root length, when measured at pod fill to maturity and seed yield (Brown and Scott 1984). In corn, a similar positive relationship exists between seed yield and root length when roots were measured several times between 31 and 109 days after planting (MacKay and Barber 1986).

During the short growing season in western Canada (110 days), a plant's ability to capture resources early is essential to maximize yield potential (Chen et al. 2005; Clayton et al. 2004; Degenhardt and Kondra 1981; Kirkland and Johnson 2000). It then follows that a *B. napus* genotype that has the ability to produce more root length prior to and shortly after emergence should be in a position to achieve greater seed yield than a genotype with an inherently shorter root under similar conditions.

The objectives of this experiment were to investigate seedling root length and area accumulation among four hybrid and four OP *B. napus* genotypes growing

on growth plates without supplemental nutrients and relate these too short- and long-term seed yields obtained from small plot field studies.

4.3 Methods and Materials

4.3.1 Experimental conduct

An indoor experiment was conducted using transparent plastic boxes in a growth cabinet (16hr/8hr, 20/16 C day/night). The boxes used to germinate the seeds and grow the seedlings were 13 x 13.5 x 3.5 cm in dimension. The bottom of each box was lined with K-24 Kimpak® (Anchor Paper Company, St. Paul, MN) material to retain water, and single layer of Anchor steel blue germination blotter paper (Anchor Paper Company, St. Paul, MN) was placed on the Kimpak®. No supplementary nutrients were added.

The experiment used four OP genotypes and four hybrid genotypes with differing genetic backgrounds. Nine *B. napus* seeds of the respective genotypes were evenly spaced on the germination blotter paper and 70 mL of distilled water were added to the boxes before placing them into the growth cabinet. The germination boxes were laid out in a completely randomized design with four replicates per genotype and the experiment was conducted twice.

4.3.2 Root measurements

After 7 days, the boxes were removed from the growth cabinet and the hypocotyls and cotyledons were removed. The boxes were then placed on a photo stand and a digital image was taken of the roots *in situ* with a Nikon D100 (Nikon Corporation, Tokyo, Japan). Each tray was placed in the same position on the stand with the camera in a fixed position to ensure the same field of view for each image. The images were then processed using Assess 2.0 (American Phytopathological Society, St. Paul, MN) imaging software and the total *B. napus* seedling root length and area for each box were measured. A ruler affixed to the stand was included in each image for, calibration and maintenance of accuracy. Individual root length was calculated from the total by dividing by the number of seedlings in each box. Seeds that did not germinate were excluded from the study.

4.3.3 Statistical analysis

The data were analyzed using Proc Mixed and tested for normality of residuals using Proc Univariate (SAS Institute, 2008). Outliers were removed using Lund's test (Lund, 1975). In the mixed procedure, genotype was designated as the fixed effect and experimental run designated as the random effect. SAS was also used to generate the means separations of genotypes for the response variables: seed yield, root length and root area using Fisher's protected least signifcant difference with the pdmix800 macro (Saxton 1998).

Two different yield data sets were used to assess the relationship between seedling root length and root area, and *B. napus* seed yield. The first yield data set referred to as short-term was that generated in the field experiments of this research project (Chapter 3) and provided four site-years (2 sites x 2 years) of yield data. The second set of yield data referred to as long-term were acquired from Pioneer Hi-Bred Production Limited Partnership's database. The long-term yields were expressed as a percentage of the control (46A65) as absolute yield values were not available for this data set. The long-term dataset was comprised of 88 pair-wise comparisons over 5 years to a maximum of 2469 pair-wise comparisons over 11 years depending on the genotype (Table 4.1). These pair-wise comparisons were derived from data from 10-20 sites per year. To compare the two datasets, a correlation analysis was conducted using Microsoft Excel® (Windows 2007). The SAS GLM procedure (SAS Institute, 2008) was used to regress root length or root area with each seed yield data set and linear and quadratic components were determined.

4.4 Results

Root length was the preferred root metric for estimating seed yield in this experiment. When using the imaging software the presence of root hairs made the ability to accurately threshold the images for primary and lateral root area difficult. Figure 4.1 shows an example of primary and secondary roots as well as the root hairs. The imagining software was able to accurately measure the

Figure 4.1. An image of *B. napus* (HYB2) roots 7 days after imbibition.

length of all roots, but the inability to separate root hairs from primary and secondary roots as well as separate spaces between root hairs introduced error. As a result, small threshold adjustments in the software substantially changed the root area calculations while having minimal impact on the root length. Root length and area are shown in Table 4.1. After 7 days, there was a clear separation in root length between the OP and hybrid genotypes with no overlap in the means separation between the two groups, while the separation in root area was not as clear between the genotype groups. The root area of the hybrid genotype with the smallest root area was similar to that of the two OP genotypes with the largest root area. Among the hybrid genotypes there were significant differences between three of the four hybrids while the OP genotypes were all grouped together and were not significantly different in root length or root area.

There were differences between the short- and long-term yield datasets, and the Pearson's r correlation coefficient between the yield data sets was 0.69. Table 4.2 lists the short- and long-term seed yields as well as the number of pair-wise comparisons and years from which the long-term data were collected. Means separation for the short-term yield data shows seed yields were more similar within breeding types with only the smallest yielding hybrid and the highest yielding OP genotypes producing similar seed yield (Table 4.2). One notable difference between the short- and long-term yield data were the differences between HYB4.

When root length was compared to long-term and short-term yield, the coefficients of determination (R^2) and slopes indicated strong positive

Table 4.1. Average root length and root area from four OP and four hybrid *B. napus* genotypes averaged over both experiments.

Cultivar	Root Length (cm)	Root Area (cm^2)
OP1	6.2*d*	0.634*cd*
46A65	6.1*d*	0.567*d*
OP2	6.4*d*	0.558*d*
OP3	6.2*d*	0.653*cd*
HYB1	10.2*b*	1.130*a*b
45H73	11.1*b*	0.926*b*
HYB2	8.6*c*	0.730*c*
HYB3	13.5*a*	1.217*a*

OP - Open Pollinated

HYB – Hybrid

a-d Means followed by the same letter do not differ at $P < 0.05$

Table 4.2. Canola yield data from the long-term field data with number of pair-wise comparisons for each genotype as well as the yield data collected from the field study discussed in Chapter 3 which included four site years.

Cultivar	Relative Yield (% 46A65) (Long-term)	Pair-wise comparisons (Long-term)	Years (Long-term)	Yield Short-term kg ha^{-1}	Relative Yield (% of 46A65) (Short-term)
OP1	106	153	9	2317*c*	98
46A65	100	2469	11	2357*bc*	100
OP2	101	198	9	2311*c*	98
OP3	100	2469	11	2229*c*	95
HYB1	119	88	5	2745*a*	116
45H73	119	288	8	2743*a*	116
HYB2	114	288	7	2641*a*	112
HYB3	122	478	7	2606*ab*	111

Source of long-term yield data Pioneer Hi-Bred Production Limited Partnership

a-c Means followed by the same letter do not differ at $P < 0.05$

relationships between root length and short- and long-term seed yield. The R^2 value of root length regressed against long-term yield data was 0.96 (Fig. 4.2 left panel) and was similar to the R^2 of 0.95 (Fig. 4.2 right panel) when comparing root length to the relative short-term seed yield. Seven days after imbibition, total root length was an accurate predictor of final seed yield among these genotypes. Although the difference between the R^2 value was only 0.01 and both relationships had significant linear and quadratic components, the interpretations of the relationship between root length and short- or long-term yield differs (Fig. 4.2). When root lengths were compared to long-term seed yield, the genotype with the longest seedling roots also produced the most seed yield in the field. However, when root length was compared to the short-term yield data the genotype with the longest roots and the genotype with the greatest seed yield were not the same and as a result, the relationship between root length and seed yield in genotypes with the highest seedling root length accumulation became negative.

The relationships between seedling root area and short- and long-term seed yield were not as strong as those for seedling root length (Fig. 4.3). The R^2 values were 0.79 for short-term yield and 0.90 for long-term seed yield and both relationships were curvilinear. For all relationships, the similar short root length, small root area and low final seed yield among the OP genotypes resulted in a clustering of these genotypes at the lower end of the relationship (Fig. 4.2, Fig. 4.3). Consequently, the differences among the hybrid genotypes defined most of the observed relationships.

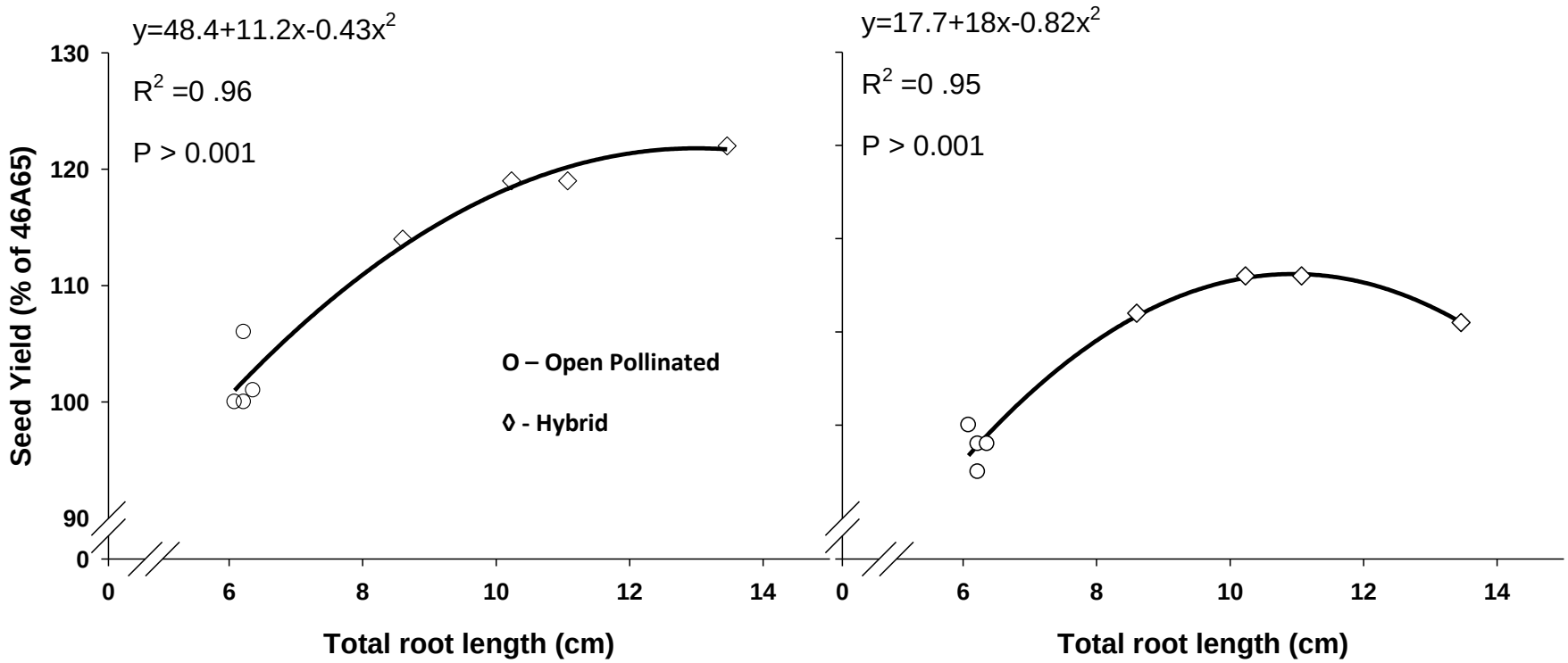

Figure 4.2. Seedling root length 7 days after imbibition vs. long-term (left) and short-term (right) seed yield expressed as a % of 46A65. The regression equations and coefficients of determination (R^2) are indicated.

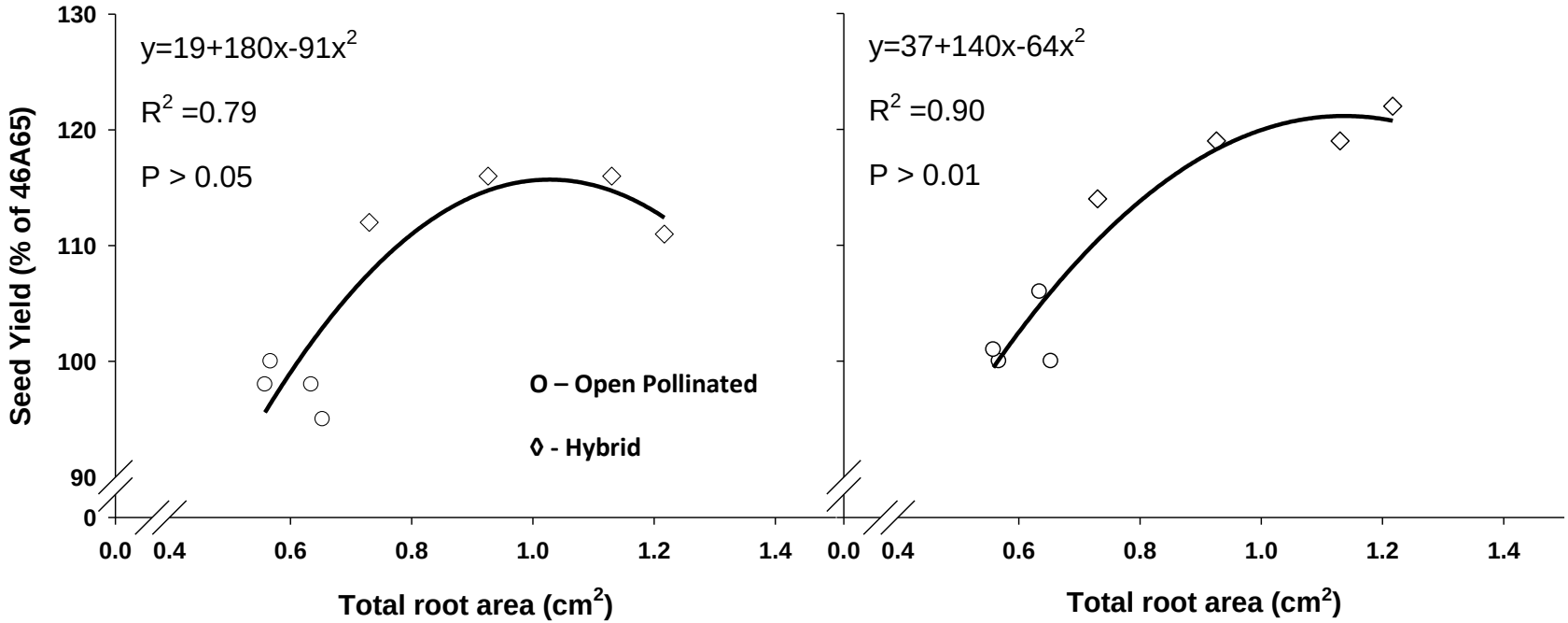

Figure 4.3. Seedling root area 7 days after imbibition vs. long-term (left) and short-term (right) seed yield expressed as a % of 46A65. The regression equations and coefficients of determination (R^2) are indicated.

4.5 Discussion

The long-term yield used in this study was probably more indicative of seed yield for the genotypes selected because the long-term yield results were compiled across many more sites and years in western Canada. Increasing the years and locations at which a genotype is tested most often minimizes the genotype x environment interaction and thereby give a more accurate representation of the genetic potential of seed yield of a genotype (Allard 1999). When the seedling root length was regressed against the long-term seed yield, the slope appeared to be reaching a peak, which suggested that there may be a trade-off or a limit to the contribution of seedling root length to seed yield. Efficient assimilate partitioning is essential for a plant to balance its use of energy and nutrients, and this balance must be maintained throughout the growing season (Bonifas and Lindquist 2006; Singleton and van Kessel 1987; Ericsson 1995). The short-term yield comparison with root length suggested that a trade-off between seedling root length and seed yield had been reached and that further increases in seedling root length might not lead to increases or may lead to a decrease in seed yield. This may be true if the plant does not encounter a need for the increased seedling root length or was unable to alter its biomass portioning during the growing season (Reich 2002).

One of the most interesting observations in this study was the differences between the OP and hybrid genotype groups in all measurements recorded. The lack of differences within OP genotypes may have been due to a lack of inherent genetic differences in seedling root length potential and final seed yield among

the OP genotypes. In the field experiment (Chapter 3), the OP genotypes required 3-4 more days to reach a similar leaf stage as the hybrid genotypes which support the latter observation. Similar observations were reported by Harker et al. (2002).

Losses of small roots are a source or error when recovering roots from soil cores through elutriation (Bonifas and Lindquist, 2006) and this was also suspected have been the case in the earlier conducted field experiment (Chapter 3). This study was able to eliminate that source or error as the roots were not disturbed prior to taking the digital images. Eliminating this source of variation greatly improved the relationship between early seedling root length and seed yield despite the fact that the estimates were derived from different experiments.

The ability to predict potential seed yield within 7 days after imbibition would be of great benefit to plant breeders. *B. napus* breeders are continuously searching for new morphological traits that will allow them to select high yielding genotypes more quickly. This study showed a clear link between early seedling root length and seed yield using a simple, cost-effective assay. A breeder could potentially conduct a study such as this on the seed prior to establishing a field experiment and use the information to implement more stringent selection criteria on the genotypes being evaluated. This could also allow the selection of parents (inbreds) with increased seedling root length accumulation for crossing thereby producing hybrids with increased early root length accumulation, or crosses between inbreds with robust early root length accumulation to inbreds with other favorable attributes such as early maturity or disease resistance.

Early vigor of *B. napus* has been attributed to improving weed competitiveness and overall seed yield (Harker et al. 2002). This experiment indicates that early vigor can potentially be quantified immediately after the completion of germination and before the accumulation of substantial shoot biomass. These findings could have implications with respect to studying seed production of *B. napus* and its competitiveness as a crop and as a weed. Much of the current plant competition research examines shoot biomass accumulation as an indicator of the competitive nature of a crop (e.g., Zand and Beckie 2001) and the results from this study indicate that seedling root length may be an important early indicator of competitive potential.

4.6 Conclusion

This study showed accumulation of root length in seedlings not supplemented with nutrients was closely related to the long-term seed yield of these *B. napus* genotypes determined in independent field experiments. Further experiments should investigate more genotypes, particularly hybrids to test the robustness of these findings. Also including the parental lines of the hybrid genotypes tested would allow for an analysis of the general combining ability of seedling root length growth.

Chapter 5

Synopsis

The strong positive relationship between root length at the seedling stage and seed yield in both field and growth chamber experiments indicates that this is an area of research that plant breeders, weed scientists, soil scientists and others may want to explore further. Investigating if this could be used to further advance the rate of genetic gain in seed yield as well as the specific reasons why increased root length at the seedling stages was indicative of increased seed yield would be of interest.

Canola breeding programs may be interested in applying the growth room methods within their programs to differentiate germplasm for seedling root length. The growth room methodology is a quick and cost effective method to screen a high number of samples with little investment required. This information would allow a wider range of hybrids to be characterized and the broad- and narrow-sense heritability of seedling root length should be determined. Even though shoot weight at the cotyledon stage had a strong relationship to seed yield in this study, the strength of the root length relationship to seed yield grown for only 7 days in germination boxes may allow researchers the ability to collect this information more rapidly and easily without having to plant seed in the field. This would save significant time and resources.

Our research studied *B. napus* root parameters up to the 4 leaf stage and there relationship to seed yield, but did not examine roots at later developmental stage.

To determine the cause of the increased seed yield when seedlings produce longer roots more field work could be conducted. Riekman's (2005) attempt to relate nitrogen and sulphur acquisition of a *B. napus* hybrid genotype compared to that of an OP genotype did not find any significant differences in root length. This contradiction should be examined more closely to determine if ontogenetic drift between the OP and hybrid genotype was no longer present at anthesis or perhaps there were insufficient inherent genetic differences between the genotypes chosen. At anthesis, when Riekman (2005) sampled the root systems, there may have been preferential root growth (Strong and Soper 1974) in nutrient rich zones and this may have contributed to variation in root length that made detecting differences difficult. The research outlined in this thesis was able to minimize the potential effects of preferential root growth in the field by sampling the entire root system.

Other research of interest that may contribute to elucidating the underlying mechanisms for my observations include the investigation into base temperatures for growth. Much of the past research on base temperature for the growth of *B. napus* was conducted prior to the introduction of hybrid genotypes (e.g. Morrision et al. 1989) and if the base temperature of the more recently commercialized hybrid genotypes is lower; this may affect a plant's ability to compete with weeds, and explore soil volume and access nutrients early in its life cycle. The growth room study could easily be modified to induce differing levels of cold stress to further investigate the impact of differing levels of colds stress on root elongation in a broad range of *B. napus* germplasm.

Brassica napus root dynamics in response to moisture stress is another area that could be explored further. A *B. napus* genotype with greater root length early in its development may be better able to withstand drought stress than a similar genotype with a shorter total root length. Cheema et al. (2004) demonstrated that through heterosis, *B. napus* accumulated significantly greater root length in hybrid genotypes vs. the two parental lines in 3 of the 6 hybrids they tested under normal and drought conditions. This increased root length may allow these genotypes to be more productive in more arid climates. Through personal observation, I have also noted that hybrid *B. napus* genotypes are better able to withstand excess moisture stress than OP genotypes before symptoms become visible in the shoot (Fig. 5.1). This may be related to the increased vigour and perhaps also a larger root system in the hybrids. As more research is conducted in the area of moisture stress researchers may have to utilize a combination of field characterization to phenotype germplasm based on *in situ* field conditions and then do follow-up studies on the roots to characterize what the differences are between the genotypes which are moisture stress tolerance versus the genotypes which are not. The ability to accurately phenotype B. napus root systems *in situ* while characterizing the shoot agronomics and seed yield has not been demonstrated, however, is pivotal to better understand the relationship between the two.

A number of current research efforts are exploring root biomass production and root length. For example, Penn State University is studying root phenotypes of various crops with respect to drought tolerance and, phosphorus acquisition.

Figure 5.1. The picture on the top is of OP4, 46A65 and a research coded hybrid (left to right) in a field that had received 75mm of rain 10days prior to this picture (2011). The picture on the bottom shows a research coded hybrid and 46A65 in a field under moisture stress (2010).

Kiran et al. (2011) recently looked at the genetic variation of root development in winter *B. napus* seedlings as it relates to root architecture. Duke University is investigating corn roots grown in a clear gel with the use of conical microscopy to build 2-D and 3-D images of the roots and explore how the roots then respond to various stresses and competition. These groups are exploring new methods to obtain more accurate root measurements while minimizing the resources required in collecting this information.

Overall this research provided useful insights into seedling root length of *B. napus* and its relation to seed yield. This work will be useful for others to utilize as a platform to delve deeper into the impact of root growth and development in *B. napus* and how the inherent differences that currently exist within germplasm may be used to drive further advancement in *B. napus* yield gains, tolerance to moisture stress and improved understanding of nutrient acquisition.

Literature Cited

Acharya, S. N., Dueck, J., Downey, R. K. 1983. Selection and heritability studies on canola/rapeseed for low temperature germination. Can. J. Plant Sci. **63**: 377-384.

Akhtar, M S., Oki, Y., Adachi, T. 2008. Genetic variability in phosphorus acquisition and utilization efficiency from sparingly soluble P-sources by Brassica cultivars under P-stress environment. J. Agron. & Crop Sci. **194**: 380-392.

Alberta Clubroot Management Committee. 2008. Alberta clubroot management plan. AGDEX 140/638-2. Alberta Agriculture and rural development. Page 8. [Online] Available: http://www.seed.ab.ca/pdf/Clubroot%20management%20plan.pdf http//www1.agric.gov.aB.ca/$Department/deptdocs.nsf/all/agdex11519

Allarad, R.W. 1999. Heredity and environment. Pages 71-83 in Principles of plant breeding. 2nd edn. John Wiley & Sons Inc.

Angadi, S., Entz, M. H., Bullock, P. 2003. Why crops produced the way they did in 2003 – Ability to handle drought and heat stress. Pages 6-13 in Proc. Manitoba Agronomist Conf. Dec. 2003, Winnipeg, MB [Online] Available: http://umanitoba.ca/afs/agronomists_conf/2003/pdf/angadi_why_crops_produced.pdf [2011 Jan. 11]

Anonymous, 2003. Canola Growers Manual. Canola Council of Canada. [Online] Available: http://www.canolacouncil.org/chapter3.aspx#ch3_sec9

Anonymous, 2006. Canola facts. Soyatech Growing opportunities. [Online] Available: http://www.soyatech.com/canola_facts.htm

Anonymous, 2008. Canada's Canola Industry. Canola Council of Canada. [Online] Available: http://www.canolacouncil.org/uploads/Canada's%20Canola%20Industry%20-%20Adding%20Billions%20to%20the%20Economy%20(Summary).pdf

Anonymous. 2009. Canola standards and regulations. Canola Council of Canada. http://www.canolacouncil.org/uploads/Standards1-2.pdf

Anonymous, 2010a. Canada: grain and oilseeds outlook. Statistics Canada. [Online] Available: http://www.agr.gc.ca/pol/mad-dam/index_e.php?s1=pubs&s2=go-co&s3=php&page=go-co_2010-08-31

Anonymous, 2010B. Provincial acreage and yield. Canola Council of Canada. [Online] Available: http://www.canolacouncil.org/acreageyields.aspx

Anonymous, 2011. Drought Watch - Map Archive. Agriculture and Agri-Food Canada. [Online] Available: http://www4.agr.gc.ca/DW-GS/historical-historiques.jspx?lang=eng&js Enabled=true

Bengough AG, Mackenzie CJ, Diggle AJ, 1992. Relations between root length densities and root intersections with horizontal and vertical planes using root growth modeling in 3-dimensions. Plant Soil **145**: 245-252.

Blackshaw, R. E. 1991. Soil temperature and moisture effects on downy brome vs. wintercanola, wheat, and rye emergence. Crop Sci. **31**: 1034-1040.

Bonari, E., Mazzoncini, M., Peruzzi, A. 1994. Effects of conventional and minimum tillage on winter oilseed rape (Brassica napus L.). Soil Tillage Research. **33**: 91-108.

Bonifas, K.D. and Lindquist, J.L. 2006. Prediciting biomass portioning to root versus shoot in corn and velvetleaf (Abutilon theophrasti). Weed Sci. **54**: 133-137.

Brown, D. A. and Scott, H. D. 1984. Dependence of crop growth and yield on root development and activity. Pages 101-136 *in* S.A. Barber, D. R. Bouldin, D. M. Kral, S. L. Hawkins, eds. Roots, nutrient and water influx, and plant growth. Soil Science Society of America, Madison, WI

Campbell, D. C. and Kondra, Z. P. 1978. Relationships among growth patterns, yield components and yield of rapeseed. Can. J. Plant Sci. **58**: 87-93.

Charne, D. G. 2010. Personal communication.

Cheema, K. L. and Sadaqat, H. A. 2004. Potential and genetic basis of drought tolerance in canola (Brassica *napus*) II. Heterosis manifestation in some morpho-physiological traits in canola. Int J. of Agric & Biol. **6**: 82-85.

Chen, C., Jackson, G., Neill, K., Wichman, D., Johnson, G., Johnson, D. 2005. Determining the feasibility of early seeding canola in the Northern Great Plains. Agron. J. **97**: 1252-1262.

Clayton, G. W., Harker, K. N., O' Donovan, J. T., Blackshaw, R. E., Dosdall, L. M., Stevenson, F. C., Ferguson, T. C. 2004. Fall and spring seeding date

effects on herbicide-tolerant canola (*Brassica napus* L.) cultivars. Can. J. Plant Sci. **84**: 419-430.

Clayton, G.W., Brandt, S., Johnson, E.N., O'Donovan, J.T., Harker, K.N., Blackshaw, R.E., Smith, E.G., Kutcher, H.R., Vera, C., Hartman, M. 2009. Comparasion of certified and farm saved seed on yield quality characteristics of canola. Agron. J. **101**: 1581-1588.

Cutforth, H. W., Shaykewich, C. F., Cho, C. M. 1985. Effect of soil water and temperature on corn (*Zea mays* L.) root growth during emergence. Can. J. Soil Sci. **66**: 51-58.

Cutforth, H.W., Angadi, S.V., McConkey, B.G., Entz, M.H., Volkmar, K.M., Miller, P., Brandt, S.A. 2009. Comparing plant water relations for wheat with alternative pulse and oilseed crops grown in the semiarid Canadian prairie. Can. J. Plant Sci. **89**: 823-835.

Degenhardt, D. F. and Kondra, Z. P. 1981. The influence of seeding date and seeding rate on seed yield and yield components of five genotypes of *Brassica napus*. Can. J. Plant Sci. **61**: 175-183.

Drew, M. C. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. New Phytol. **75**: 479-490.

Duan, H., Shi, L., Ye, X., Wang, Y., Xu, F. 2009. Identification of phosphorus efficient germplasm in oilseed rape. J. of Plant Nutri. **32**: 1148-1163.

Elliot, R.H., Franke, C., Rakow, G. G. W. 2008. Effects of seed size and seed weight on seedling establishment, vigour and tolerance of Argentine canola (Brassica *napus*) to flea beetles, *Phyllotreta* spp. Can. J. Plant Sci. **88**: 207-217.

Ennos, A. R., and Fitter, A. H. 1992. Comparative functional morphology of the anchorage systems of annual dicots. Func. Biol. **6**: 71-78.

Ericsson, T. 1995. Growth and shoot:root ratio of seedlings in relation to nutrient availability. Plant Soil. **168**: 205-214.

auf'm Erley, G.S., Wijaya, K., Ulas, A., Becker, H., Wiesler, F., Horst, W.J. 2007. Leaf senescence and N uptake parameters as selection traits for nitrogen efficiency of oilseed rape cultivars. Physiologia Plantarum. **130**: 519-531.

Fitter, A. 2002. Characteristics and functions of root systems. In Y. Waisel, A. Eshel, U. Kafkafi, eds. Plant roots the hidden half 3rd edn. Marcel Dekker. New York, USA. pp 15-32

Foehse, D. and Jungk, A. 1983. Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. Plant Soil. **74**: 359-368.

Gan, Y. T., Campbell, C. A., Janzen, H. H., Lemke, R., Lui, L. P., Basnyat, P., McDonald, C. L. 2009. Root mass for oilseed and pulse crops: Growth and distribution in the soil profile. Can. J. Plant Sci. **89**: 883-893.

Gentile, R. M., Martino, D. L., Entz, M. H. 2003. Root characterization of three forage species grown in southwestern Uruguay. Can. J. Plant Sci. **83**: 785-788.

Gentile, R. M., de Rocquigny, P. J., Entz, M. H. 2004. Soil Water depletion by tall and semidwarf oat and wheat cultivars. Can. J. Plant Sci. **85**: 385-388.

Goodman, A. M., Crook, M. J., Ennos, A. R. 2001. Anchorage mechanics of the tap root system of winter-sown oilseed rape (*Brassica napus* L.). Ann. Bot. **87**: 397-404.

Gossen, B. D., McDonald, M., Hwang, S., Kalpana, K. C. 2009. Manipulating seeding date to minimize clubroot (Plasmodiophora brassicae) damage in canola and vegetable brassics. Phytopathology. **99**: S45.

Griffiths, G. C. D. 1991. Economic assessment of cabbage maggot damage in canola in Alberta. Pages 528-535 in D.I. McGregor, ed. Prodeedings, GCIRC Eighth International Rapeseed Congress. Saskatoon, SK.

Grosse, F., Leon, J., Diepenbrock, W. 1992. Yield formation and yield structure of winter oilseed rape (Brassica *napus* L.). Abstract in English. J. Agron. & Crop Sci. **169**: 94-103.

Hammond, J. P., Broadley, M. R., White, P. J., King, G. J., Bowen, H. C., Hayden, R., Meacham, M. C., Mead, A., Overs, T., Spracklen, W. P., Greenwood, D. J. 2009. Shoot yield drives phosphorus use efficiency in *Brassica oleracea* and correlates with root architecture traits. J. Exp. Bot. **60**: 1953-1968.

Harker, K.N., Clayton, G.W., Blackshaw, R.E., O'Donovan, J.T., Stevenson, F.C. 2002. Seeding rate, herbicide timing and competitive hybrids contribute to

integrated weed management in canola (Brassica napus). Can. J. Plant Sci. **83**: 433-440.

Hedley, M.J., White, R.E, Nye, P.H. 1982. Plant induced changes in the rhizosphere of rape (*Brassica napus* var. Emerald) seedlings. New Phytol. **91**: 45-56.

Hoecker, N., Keller, B., Piepho, H., Hochholdinger, F. 2006. Manifestation of heterosis during early maize (Zea mays L.) root development. Theor. Appl. Genet. **112**: 421-429.

Hund, A., Fracheboud, Y., Soldati, A., Stamp, P. 2007. Cold tolerance of maize seedlings as determined by root morphology and photosynthetic traits. Eur. J. Agron. **28**: 178-185.

Johnson, E. N. 2008. Quantifying herbicide carryover injury in canola (Brassica *napus* L.) with hand-held sensors or digital imagery. Page 12. 48th Annual meeting of the Weed Science Society of America, Hilton Chicago, Chicago, IL, ISA, Feb 4-7, 2008,

Kamh, M., Wielser, F., Ulas, A., Horst, W. J. 2005. Root growth and N-uptake activity of oilseed rape (*Brassica napus* L.) cultivars differing in nitrogen efficiency. J. Plant Nutr. Soil Sci. **168**: 130-137.

Karamanos, R. E., Goh, T. B., Poisson, D. P. 2005. Nitrogen, phosphorus, and sulfur, fertility of hybrid canola. J. Plant Nutr. **28**: 1145-1161.

Karamanos, R.E., Goh, T. B., Flaten, D. N. 2006. Nitrogen and sulphur fertilizer management for growing canola on sulphur sufficient soils. Can. J. Plant Sci. **87**: 201-210.

King, J. R., Kondra, Z. P., Thiagarajah, M. R. 1985. Selection for fast germination in rapeseed (*Brassica napus* L. and *B. campestris* L.). Euphytica. **35**: 835-842.

Kiran, A., Bus, A., Stich, B., Snowdon, R. 2011. Genetic variation for root development traits in *Brassica napus* seedlings. Page 677 In the proceedings of the 13th International Rapeseed Congress.

Kirkland, K. J. and Johnson, E. N. 2000. Alternative seeding dates (fall and April) affect *Brassica napus* canola yield and quality. Can. J. Plant Sci. **80**: 713-719.

Kjellstrom, C.G. and Kirchmann, H. 1994. Dry matter production of oilseed rape (*Brassica napus*) with special reference to the root system. J. Agric. Sci. **123**: 327-332.

Klein-Gebbinck, H. W. and Woods, D. L. 2002. Yield loss assessment in canola: Effects of brown girdling root rot and maggot damage on single plant yield. Plant Dis. **86**: 1005-1010.

Kondra, Z.P. 1977. Effects of planting date on rapeseed. Can J. Plant Sci. 57: 607-609.

Kondra, Z. P., Campbell, D. C., King, J. R. 1983. Temperature effects on germination of rapeseed (*Brassica napus* L. and *B. campestris* L.). Can. J. Plant Sci. **63**: 1063-1065.

Laine, P., Ourry, A., Boucaud, J. 1994. Shoot control of nitrate uptake rates by roots of *Brassica napus* L.: Effects of localized nitrate supply. Planta. **196**: 77-83.

Lamba, P. S., Ahlgren, H. L., Muckenhirn, R. J. 1949. Root growth of alfalfa, medium red clover, bromegrass, and timothy under various soil conditions. Agron. J. **41**: 451- 458.

Liu, L., Gan, Y., Bueckert, R., Van Rees, K., Warkentin, T. 2010. Fine root distributions in oilseed and pulse crops. Crop Sci. **50**: 222-226.

Lund, R. E. 1975. Tables for an approximate test for outliers in linear models. Techometrics. **17**: 473-476.

Lynch, J.P. 2011. Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. Plant Physiol. **156**: 1041-1049.

Mackay AD, Barber SA, 1986. Effect of nitrogen on root growth of two corn genotypes in the field. Agron. J. **78**: 699-703.

Malhi, S. S., Johnston, A. M., Schoenau, J. J., Wang, Z. H., Vera, C. L. 2006. Seasonal biomass accumulation and nutrient uptake of wheat, barley and oat on a Black Chernozem soil in Saskatchewan. Can. J. Plant Sci. **86**: 1005-1014.

Malhi, S. S., Johnston, A. M., Schoenau, J. J., Wang, Z. H., Vera, C. L. 2007a. Seasonal biomass accumulation and nutrient uptake of canola, mustard, and flax on a Black Chernozem soil in Saskatchewan. J. Plant Nutri. **30**: 641-658.

Malhi, S. S., Johnston, A. M., Schoenau, J. J., Wang, Z. H., Vera, C. L. 2007b. Seasonal biomass accumulation and nutrient uptake of pea and lentil on a black chernozem soil in Saskatchewan. J. Plant Nutri. **30**: 721-737.

Marshall, S.D. and Squire, G.R. 1996. Non-linearity in rate-temperature relations of germination in oilseed rape. J. Exp. Bot. **47**:1369-1375.

McConnaughay, K. D. M. and Coleman, J. S. 1999. Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. Ecology. **80**: 2581-2593.

Merrill, S. D., Tanaka, D. L., Hanson, J. D. 2002. Root length growth of eight crop species in Haplustoll soils. Soil Sci. Soc. Am. J. **66**: 913-923.

Michalyna, W., Podlolsky, G., St. Jacques, E. 1988. Soils of the rural municipalities of Grey, Dufferin, Roland, Thompson and part of Stanley. Canada – Manitoba Soil Survey. Report D60.

Milchunas, D. G. 2009. Estimating root production: Comparison if 11 methods in shortgrass steppe and review of biases. Ecosystems. **12**: 1381-1402.

Morrison, M. J., McVetty, P. B. E., Shaykewich, C. F. 1989. The determination and verification of a baseline temperature for the growth of Westar summer rape. Can. J. Plant Sci. **69**: 455-464.

Nielsen, D. C. 1997. Water use and yield of canola under dryland conditions in the central Great Plains. J. Prod. Agric. **10**: 307-313.

Nykiforuk, C. L. and Johnson-Flanagan, A. M. 1994. Germination and early seed development under low temperature in canola. Crop Sci. **34**: 1047-1054.

Ojo, G.O.S., Adedzwa, D.K., Bello, L.L. 2007. Combining ability estimates and heterosis for grain yield and yield components in maize (*Zea mays* L.). J. Sust. Devel. Agric. and Environ. **3**: 49-57.

Pavlychenko, T. K. 1937. Quantitative study of the entire root systems of weed and crop plants under field conditions. Ecology. **18**: 62-79.

Pearson, C.J. and Jacobs, B.C. 1985. Root distribution in space and time. Austr. J. Agric. Res. **36**: 601-614.

Peterson, R. L. and Farquhar, M. L. 1996. Root Hairs: Specialized tubular cells extending root surfaces. Bot. Rev. **62**: 1-40.

Pietola, L. and Alakukka, L. 2005. Root growth dynamics and biomass input by Nordic annual field crops. Agric. Ecosyst. Environ. **34**: 261-267.

Polomski, J. and Kuhn, N. 2002. Root research methods. In Waisel, Y., Eshel, A.,Kafkafi, U., (eds.) Plant roots the hidden half, 3rd edn. Marcel Dekker, New York, USA. Pp. 295-321.

Reich, P. B. 2002. Root-Shoot relations: Optimality in acclimation and adaptation or the “emperor’s new clothes”? . In Waisel, Y., Eshel, A.,Kafkafi, U., (eds.) Plant roots the hidden half, 3rd edn. Marcel Dekker, New York, USA. Pages 205-220.

Riekman, M.R. 2005. The effect of canola cultivar on water extraction and nitrogen and sulphur uptake. M.Sc. thesis, University of Manitoba, Winnipeg, MB. 130pp.

Rimmer, S. R., Shattuck, V. I., Buchwaldt, L. 2007. Infectious diseases. Part II. Pages 15-81. Compendium of Brassica Diseases. The American Phytopathological Society. St. Paul, Minnesota

Robinson, D. 1995. Resource capture by localized root proliferation: Why do plants bother? Ann. Bot. **77**: 179-185.

Rose, T. J., Rengel, Z., Ma, Q., Bowden, J. W. 2007. Differential accumulation patterns of phosphorus and potassium by canola cultivars compared to wheat. J. Plant Nutr. Soil Sci. **170**: 404-411.

Rose, T. J., Rengel, Z., Ma, Q., Bowden, J.W. 2008. Hydraulic lift by canola plants aids P and K uptake from dry topsoil. Aust. J. of Agric. Res. **59**: 38-45.

Russelle, M.P. and Lamb, F.S. 2011. Divergent alfalfa root system architecture is maintained across environment and nutrient supply. Agron. J. **103**: 1115-1123.

Saxton, A. 1998. A macro for converting mean separation output to letter groupings in proc mixed. Pages 1243-1246 In the proceedings of the 23rd SAS users group international, Nashville, TN.

Segal, E., Kushnir, T., Mualem, Y., Shani, U. 2008. Water uptake and hydraulics of the root hair rhizoshphere. Vandose Zone J. **7**: 1027-1034.

Sh, M., Bhuiya, M.S.U., Gomosta, A.R., Sarkar, A.R., Hussain, M.M. 2009. Evaluation of growth and yield of selected hybrid and inbred rice varieties grown in net-house during tansplanted aman season. Bangladesh J. Agric. Res. **34**: 67-73.

Sharpley, A.N., Kleinman, P., McDowell, R. 2001. Innovative management of agricultural phosphorus to protect soil and water resources. Commun. Soil Sci. Plant Ann. **32**: 1071-1100.

Singleton, P.W. and van Kessel, C. 1987. Effect of localized nitrogen availability to soybean half-root systems on photosynthate portioning to roots and nodules. Plant Physiol. **83**: 552-556.

Skinner, R. H., Hanson, J. D., Benjamin, J. G. 1998. Root distribution following spatial separation of water and nitrogen supply in furrow irrigated corn. Plant Soil. **199**: 187-194.

Solaiman, Z., Marschner, P., Wang, D., Rengel, Z. 2007. Growth, P uptake and rhizosphere properties of wheat and canola genotypes in alkaline soil with low P availability. Biol. Fertil. Soils **44**: 143-153.

Soroka, J. J. and Elliot, R. H. 2006. Size doesn't matter: The effects of seed size and seedling rate on injury by root maggots (Delia spp., Diptera: Anthomyiidae) to canola (*Brassica rapa* L. and *B. napus* L.). Can. J. Plant Sci. **86**: 907-909.

Squire, G.R. 1999. Temperature and heterogeneity of emergence time in oilseed rape. Ann. Appl. Biol. **135**: 439-447.

Strong W.M. and Soper R.J. 1974. Phosphorus utilization by flax, wheat, rape, and buckwheat from a band or pellet-like application I. reaction zone root proliferation. Agron. J**. 66**: 597-601

Svejcar, T. 1990. Root length, leaf area, and biomass of crested wheatgrass and cheatgrass seedlings. J. of Range Mngmt. **43**: 446-448.

Taylor, A.J., Smith, C.J. 1992. Effect of sowing date and seeding rate on yield and yield components of irrigated canola (*Brassica napus* L.) grown on a red-brown earth in South-Eastern Australia. Aust. J. Agric. Res. **43**: 1629-1641.

Thaler, P. and Pages, L. 1998. Modelling the influence of assimilate availability on root growth and architecture. Plant Soil. **201**: 307-320.

Thurling, N., Vijendra Das, L. D. 1979. The relationship between pre-anthesis development and seed yield of spring rape (Brassica *napus* L.). Aust. J. Agric. Res. 31: 25-36.

Van Deynze, A. E., McVetty, P. B. E., Scarth, R., Rimmer, S. R. 1992. Effect of varying seeding rates on hybrid and conventional summer rape performance in Manitoba. Can. J. Plant Sci. **72**: 635-641.

Vigil, M. F., Anderson, R. L., Beard, W. E. 1997. Base temperature and growing-degree-hour requirements for the emergence of canola. Crop Sci. **37**: 844-849.

Wan, C., Xu, W., Sosebee, R.E., Machado, S., Archer, T., 2000. Hydraulic lift in drought-tolerant and susceptible maize hybrids. Plant Soil **219**: 117-126.

Wang, L., de Kroon, H., Boegemann, G. M., Smits, A. J. M. 2005. Partial root drying effects on biomass production in *Brassica napus* and the significance of root responses. Plant Soil. **276**: 313-326.

Wang, L., Kroon, H., Smits, A. J. M. 2007. Combined effects of partial root drying and patchy fertilizer placement on nutrient acquisition and growth of oilseed rape. Plant Soil. **295**: 207-216.

Williamson, L. C., Ribrioux, S. P. C. P., Fitter, A. H., Ottoline Leyser, H. M. 2001. Phosphate availability regulates root system architecture in Arabidopsis. Plant Physiol. **126**: 875-882.

Ye, X., Hong, J., Shi, L., Xu, F. 2010. Adaptability mechanism of nitrogen-efficient germplasm of natural variation to low nitrogen stress in *Brassica napus*. J. Plant Nutri. **33**: 2028-2040.

Zand, E., Beckie, H.J. 2001. Competitive ability of hybrid and open-pollinated canola (*Brassica napus*) with wild oat (*Avena fatua*). Can. J. Plant Sci. **82**: 473-480.

Zhu, J., Zhang, C., Lynch J. P. 2010. The utility of phenotypic plasticity of root hair length for phosphorus acquisition. Functional Plant Biol. **37**: 313-322.