

**EVALUATING CANOLA GENOTYPES AND HARVEST METHODS TO REDUCE
SEEDBANK ADDITION AND LONGEVITY**

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By

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ABSTRACT

Seed loss in canola (*Brassica napus*, *Brassica rapa* and *Brassica juncea*) leads to considerable loss of revenue and dispersal of canola seeds into the soil seedbank. Once canola seeds enter the soil seedbank a portion can become secondarily dormant and persist for many years creating volunteer weed problems in following crops. Reducing canola seed loss and seedbank persistence can be an important strategy to reduce the incidence of volunteer weeds. The primary hypothesis of this research was that canola seedbank addition and dormancy induction in the seed are affected by genotype and harvest method. To test this hypothesis, three studies were conducted with the following objectives: i) to determine canola seedbank addition from windrowing and direct-harvesting operations on commercial farms in western Canada, ii) to determine agronomic- and harvest-related factors that may increase seed loss in canola, iii) to determine the effect of stage of crop maturity at harvest on potential to develop seed dormancy in canola, iv) to evaluate canola genotypes and harvest methods to reduce canola seedbank addition. A total of 66 canola fields were surveyed across Saskatchewan in 2010 and 2011. Shattered seeds from these fields were sampled within 3 weeks of harvest by using a vacuum cleaner. Agronomic- and harvest-related data were collected for each field using questionnaires. In a separate small plot study the effects of harvest methods (windrowing and direct-harvesting) and pod sealant products (Pod-Stik[®] and Pod Ceal DC[®]) on seed loss in five canola genotypes (InVigor5440, RR45H26, InVigor5020, RR4362, and CL8571) were evaluated in 2010 and 2011. In both years, 6 harvest samples were collected weekly from InVigor5440 and InVigor5020 genotypes starting at early stage of crop maturity until harvest to assess the effect of seed maturity on dormancy induction. On commercial farms, the average seedbank addition was 5,821 viable seeds m⁻², which was equivalent to 7.3% of the total seed yield. There was no difference in the reported yield and seedbank addition between windrowed and direct-harvested canola on commercial farms. But in the small plot study, windrowing resulted in higher seedbank addition. Higher seedbank addition was observed when the yield of canola was higher and when producers had a larger area seeded to canola. The observed seedbank addition was also higher in Roundup Ready genotypes and when a conventional combine harvester was used to harvest canola. Little primary dormancy and low potential to secondary dormancy induction was

observed in InVigor5440 and InVigor5020 seeds at an early stage of crop maturity. But at full maturity seeds of both genotypes had no primary dormancy but showed high potential for secondary dormancy induction. This indicates that windrowing the evaluated genotypes at early stage of crop maturity lowered the potential for secondary dormancy induction. There were appreciable differences in seedbank addition among the evaluated canola genotypes but pod sealant products did not affect seed yield and seed shatter in canola. The results of this study suggest that canola seedbank addition can be minimized by growing genotypes having reduced seed loss and with the adoption of direct-harvesting operations.

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
AIC	Akaike information criterion
ANOVA	Analysis of variance
CL	Clearfield
DAF	Days after flowering
DDFM	Degrees of freedom method
DH	Direct-harvested
G	Genotype
GA	Gibberellic acid
GM	Genetically modified
H	Harvest
LL	Liberty Link
LSD	Least significant difference
PEG	Polyethylene glycol
MPa	Megapascal
PG	Polygalacturonase
RCBD	Randomized complete block design
REML	Restricted maximum likelihood
RR	Roundup Ready
SAS	Statistical analysis system
SCC	Seed color change
TSW	Thousand seed weight
VIF	Variance inflation factor

1. INTRODUCTION

Canola (*Brassica napus*, *Brassica rapa* and *Brassica juncea*), also known as oilseed rape, is a member of *Brassicaceae* family, and is a major economic oilseed crop. Canola was developed from rapeseed through Canadian crop breeding programs in the mid-1970s (Stefansson and Downey, 1995). Canola is widely grown throughout the world for the purpose of vegetable oil, animal feed and biodiesel. Canada is the largest producer and exporter of canola with over 8.6 million harvested hectares and 15.2 million tonnes of production in 2012 (Statistics Canada, 2012).

Canola has a number of weedy characteristics including silique dehiscence at maturity. Silique dehiscence results in considerable yield loss and dispersal of seeds to the soil seedbank. Thus, volunteer canola (plants growing from seeds left in the field after crop harvest) can create weed problems in following crops. Volunteer canola was ranked as the 12th most abundant weed in western Canada (Leeson et al., 2005). The increase in area seeded to canola and the introduction of herbicide resistant traits are some of the reasons for the increased incidence of volunteer canola in western Canada (Gulden et al., 2003a). Volunteer canola can also cause genetic contamination of the following canola genotypes through pollen transfer and seed dispersal (Morgan et al., 1998; Gruber et al., 2004). The risk of gene flow between canola genotypes, seed contamination and the potential persistence of herbicide resistant volunteer canola have severe consequences for weed management programs and for the marketability of the crop (Légère, 2005).

Little variation has been reported for natural resistance to shattering within the earlier *B. napus* genotypes (Brown et al., 1997; Morgan et al., 2000). However, increased resistance to shattering has been reported within the other *Brassica* lines as well as *B. napus* genotypes derived from interspecific hybridizations with *B. rapa*, *B. juncea*, *Brassica oleraceae* and *Brassica carinata* (Morgan et al., 1998; Child et al., 2003; Summers et al., 2003). In western Canada, Wang et al. (2007) screened 22 *B. napus* genotypes including commercially available open pollinated

cultivars and hybrids and found some variation for pod shatter within the tested genotypes. Selection of genotypes with reduced seed shatter can be an important strategy to reduce canola seedbank addition and the resulting volunteer canola.

Seed loss in canola can be influenced by the method and time of harvest. Canola can be direct-harvested or windrowed (swathed) followed by picking up the windrow (swath) when it dries. In western Canada, it is recommended to windrow canola to reduce seed loss, to avoid adverse climatic conditions and to promote more even ripening (Government of Alberta, 2009). However, windrowing canola increases the cost of production and can reduce seed quality due to higher chlorophyll content in the seed (Wang et al., 2007). On the other hand, direct-harvesting has been observed to work well when the crop has a heavy stand and for shorter, spring planted canola genotypes (Price et al., 1996; Boyles et al., 2010). In the UK, Price et al. (1996) reported 11% yield loss when winter canola was direct-harvested but when it was windrowed yield loss ranged from 11 to 25%. The overall reported yield loss was lower in spring than the winter genotypes but yield loss did not differ when spring canola was windrowed and direct-harvested. In western Canada, where only spring canola genotype is commercially grown, there has been no field scale evaluation of yield loss and seedbank addition between windrowing and direct-harvesting operations.

The long term seedbank persistence of volunteer canola in western Canada is related to its potential to develop secondary seed dormancy (Gulden et al., 2004a). The absence of certain environmental signals such as water, oxygen and light induces secondary dormancy in canola seeds (Pekrun et al., 1997a; Momoh et al., 2002). Canola seedbank persistence for over 11 years has been reported in the UK (Lutman et al., 2003). In western Canada, persistence of volunteer canola for 7 years has been reported in the field (Beckie and Warwick, 2010). In France, persistence of volunteer canola up to 9 years has been reported even outside of cultivated fields (Pessel et al., 2001). Genotype is the major factor controlling secondary dormancy potential in western Canadian spring canola genotypes (Gulden et al., 2004a). Other factors such as seed size, pre- and post-harvest environment also have smaller influence on the development of secondary dormancy in canola (Gulden et al., 2004a). Due to the difference in the time and method of harvest, canola seeds can enter the soil seedbank at different stages of maturity.

However, the potential to secondary dormancy induction in the seed at different stages of crop maturity has not been clearly evaluated in canola.

This research examined the effect of genotype and harvest methods on canola seedbank addition and development of dormancy in the seed. The primary hypothesis was that canola seedbank addition and dormancy induction in the seed are affected by the method of harvest and genotypic differences. To test this hypothesis, three studies were conducted with the following objectives:

1. To determine canola seedbank addition from windrowing and direct-harvesting operations on commercial farms in western Canada.
2. To determine agronomic and harvest-related factors that may increase seed loss in canola.
3. To determine the effect of stage of crop maturity at harvest on potential to develop seed dormancy in canola.
4. To evaluate canola genotypes and harvest methods to reduce canola seedbank addition.

The knowledge gained from this research will help producers improve canola harvest management to reduce yield loss, seedbank addition and longevity that may result in the loss of revenue and the long term volunteer weed problems. This research assessed differences in seed shatter among canola genotypes and the results will enable growers to select genotypes with reduced seed loss and seedbank addition. Agronomic- and harvest-related factors were also examined to identify the possible causes of seed loss in canola. The results obtained from this research will help producers design agronomic and harvest management accordingly.

2. LITERATURE REVIEW

2.1 The origin and history of canola

Canola is the name applied to edible oilseed rape or rapeseed that is low in erucic acid and glucosinolates (Stefansson and Downey, 1995). It belongs to the *Cruciferae* (*Brassicaceae* or mustard) family. Canola is comprised of three species namely *B. rapa* (Polish canola), *B. napus* (Argentine canola) and *B. juncea* (brown mustard) (Canola Council of Canada, 2012). *B. napus* (n=19), *B. juncea* (n=18), and *B. carinata* (n=17) are amphidiploids derived from three diploid species *Brassica nigra* (n=8), *B. oleraceae* (n=9) and *B. rapa* (n=10) (U, 1935, Cited in Kimber and McGregor, 1995). *B. rapa* is an annual, native from western Europe to central Asia (Prakash and Hinata, 1980, Cited in Hall et al., 2005) whereas *B. oleraceae* is a perennial that is found distributed along the coasts of Spain, France, the British Isles and Helgoland (Snogerup et al., 1990). *B. napus* is believed to have evolved in the Mediterranean area, where these two wild forms of its ancestral species cohabited (Prakash and Hinata, 1980, Cited in Hall et al., 2005).

Prior to 1960, rapeseed oil contained large amounts (about 50%) of long-chain fatty acids (erucic and eicosenoic) which were believed to have a health hazard to animals (Stefansson and Downey, 1995). The nutritional value of rapeseed meal was also limited due to the presence of glucosinolates (Stefansson and Downey, 1995). The first strains of rapeseed with seed oil free from erucic acid were isolated at Winnipeg and Saskatoon in early 1960s (Stefansson et al., 1961). Similarly the first strain of *B. rapa* with zero erucic acid in the seed was isolated at Saskatoon (Downey, 1964). The first double low (00) *B. napus* genotype (low erucic acid and low glucosinolate) was released in Canada in 1974 and the name “canola” was adopted for the 00 cultivar of rapeseed to differentiate it from the traditional rapeseed (Stefansson and Downey, 1995). To prevent misuse of the name it was later copyrighted in several countries by the Canola Council of Canada (Stefansson and Downey, 1995). The conversion of the Canadian rapeseed crop to canola quality was accomplished in 1980; however, it took another decade to changeover to canola quality rapeseed in most European countries (Stefansson and Downey, 1995).

2.2 Production trends of canola

Rapeseed is an ancient crop which was cultivated in Asia and along the Mediterranean as a source of lamp oil and later cooking oil (Shahidi, 1990). The history of rapeseed growth in Asia is somewhat contradicting. Shahidi (1990) indicated that there were records of rapeseed cultivation in India over 3,000 years ago. Rapeseed was believed to have been introduced to China and Japan around the time of Christ (Hougen and Stefansson, 1982). However, Khachatourians et al. (2001) indicated that the earliest records of rapeseed growth in Asia belong to the Sanskrit writings of 2000-1500 BC. The Japanese literature; on the other hand, shows the introduction of rapeseed through Korea and China around 1000 BC (Khachatourians et al., 2001). Rapeseed was grown in Europe in the 13th century as a source of lamp oil (Appelqvist, 1972; Hougen and Stefansson, 1982; Shahidi, 1990). However, its use for industrial purposes was not widespread in Europe until its superior qualities as lubricant oil were realized (Hougen and Stefansson, 1982; Shahidi, 1990). In western countries, the use of rapeseed as an edible vegetable oil is recent (Shahidi, 1990).

The earliest record of rapeseed in Canada began at Shellbrook, Saskatchewan in 1936, where a farmer called Mr. Fred Solvoniuk introduced rapeseed from Poland from where he had emigrated in 1927 (Khachatourians et al., 2001). Mr. Solvoniuk planted this seed in his garden and found it well adapted to the Canadian prairie. This rapeseed was later found to be the *B. rapa* species (Khachatourians et al., 2001). The commercial production of rapeseed in Canada began in 1942, mainly in response to demand for industrial lubricating oil, as the European and Asian supplies were stopped during the Second World War (Craig, 1971; Shahidi, 1990). However, its production almost ceased after the Second World War since the requirement for rapeseed oil as a lubricant decreased (Hougen and Stefansson, 1982).

The world canola market is mainly supplied by *B. napus* and *B. rapa* and to a lesser extent by *B. juncea* (Raymer, 2002). Both *B. napus* and *B. rapa* have winter and spring genotypes. The winter genotypes are more productive than the spring genotypes in favorable growing conditions (Booth and Gunstone, 2004; Khachatourians et al., 2001). Winter canola is widely grown in parts of Europe and Asia but it is not grown commercially in western Canada because of low hardiness in the extreme winter conditions (Khachatourians et al., 2001). Only spring genotypes are

suitable in western Canada (Khachatourians et al., 2001). *B. juncea* is more heat and drought tolerant than the other canola species and is widely grown in northern India and China (Mendham and Salisbury, 1995; Booth and Gunstone, 2004). This species has good adaptation to the semi-arid conditions on the Canadian prairies (Woods et al., 1991; Stefansson and Downey, 1995). *B. juncea* lines that meet the characteristics of canola quality have been developed in western Canada (Woods et al., 1991).

Brassica napus is commonly grown in Europe, Canada, and China while *B. rapa* was formerly grown in some parts of western Canada because of its early maturity and cold hardiness (Kimber and McGregor, 1995). Similar areas were seeded to *B. napus* and *B. rapa* in western Canada in late 1980's but in 1990's the proportion of an area seeded to *B. rapa* diminished to about 15 to 20% (Khachatourians et al., 2001). The proportion of *B. napus* in harvest samples has increased from 89% in 1998 to 99.8% in 2008 in western Canada (Canadian Grain Commission, 2008), mainly because of its greater seed yield and presence of herbicide resistant traits. The development of short season genotypes and implementation of early seeding are also reasons for the increased proportion of *B. napus*.

Currently canola is one of the most important oilseed crops in the world. Global canola production has grown rapidly over the past 40 years, rising from a rank of the sixth largest oil crop to the second largest with its production being 10-15% of world oil crop production for the last decade (Ash, 2012). Canola has been referred to as the economic engine of the farming system in the sub-humid regions of western Canada (Clayton et al., 2000). The total area seeded to canola in Canada reached 8.6 million hectares in 2012 (Statistics Canada, 2012). Almost 99% of the canola production in Canada is concentrated in the prairies (Saskatchewan, Alberta, Manitoba and the Peace River region of British Columbia); however, canola is also grown in all provinces except Newfoundland and Labrador (Casseus, 2009).

2.3 Canola harvest methods

Optimum time and appropriate method of harvest are crucial for optimum yield and quality of canola. Canola can be direct-harvested or alternatively, windrowed followed by combine pickup

when it dries. Canola is usually direct-harvested in the Southern Great Plains of the United States and Europe (Boyles et al., 2010). In the UK, over 50% of canola is direct-harvested usually after chemical desiccation, although around 20% of the crop is direct-harvested after a longer period of natural ripening (Price et al., 1996; Hobson and Bruce, 2002). Direct-harvesting can be successful when the crop matures evenly and crop density is uniform (Booth and Gunstone, 2004; Boyles et al., 2010). Direct-harvesting has been observed to work well when the crop has a relatively heavy stand, partial lodging or siliques laced together as these conditions reduce shattering and silique drop due to strong winds (Boyles et al., 2010). In general, direct-harvesting is appropriate for shorter, spring planted canola and for seasons or situations without strong winds or hail (Price et al., 1996). In western Canada, direct-harvesting is practiced as it reduces the cost of production and the chlorophyll content in the seed. The use of pre-harvest desiccants can facilitate direct-harvesting of canola by allowing more uniform ripening of the crop. Desiccation of canola using different chemical desiccants such as glyphosate, diquat or glufosinate allows the seed to mature on the standing crop (Booth and Gunstone, 2004). *B. rapa* can be directly harvested as it is more resistant to shattering than *B. napus* (Khachatourians et al., 2001). However, for more shatter-susceptible *B. napus* cultivars, the use of desiccants allows direct-harvesting as it reduces shattering (Khachatourians et al., 2001).

Windrowing, also known as swathing, is another option to harvest canola. It involves cutting the crop at early stage of maturity using a windrower and leaving it in windrows (swaths) on the cut stubble to hasten drying. Following drying the crop is then harvested with a combine harvester equipped with a pickup header. It is a preferred method of harvesting canola in western Canada as it reduces uneven seed ripening and seed shatter due to adverse weather conditions (Vera et al., 2007). Windrowing has been observed to reduce canola maturation time and seed loss due to pod shatter (Irvine and Lafond, 2010). However, the stage of maturity at which a crop is windrowed can affect both seed yield and quality (Vera et al., 2007). Windrowing too early results in higher green seed, reduced yield, low oil, and protein content since once the crop is windrowed the seed does not continue to fill (Vera et al., 2007; Boyles et al., 2010). Also in periods of prolonged rain, windrows can become wet and be slow to dry leading to sprouting of seeds (Price et al., 1996).

A relatively new method for harvesting canola involves pushing the crop followed by direct combining. Ag Shield Manufacturing in Manitoba manufactures Yield Shield™, a device which pushes the crop making it artificially lodged at a height of 10 to 20 cm above the soil surface (Irvine and Lafond, 2010). At maturity, the lodged crop is directly combined in an opposite direction to the direction the crop was pushed (Irvine and Lafond, 2010). Pushing has been suggested as a faster and less expensive alternative to windrowing where the crop is mechanically lodged before maturation to limit the movement of canola siliques by wind (Irvine and Lafond, 2010). In an experiment conducted in eastern Oregon, Wysocki et al. (2007) reported that winter canola yields were similar for forced lodging and windrowing but were significantly lower when direct-harvested. However, for spring canola direct-harvesting produced the highest yields compared to forced lodging and windrowing (Wysocki et al., 2007). Irvine and Lafond (2010) also indicated that canola seed yield, oil concentration and green seed counts were generally similar between windrowing and pushing. However, the pushed crop had higher yield when the windrows were damaged by wind and had lower yield when the crop did not remain lodged (Irvine and Lafond, 2010). Although pushing and windrowing canola had similar yields, windrowing is slower and more costly than pushing (Wysocki et al., 2007).

2.4 The prevalence of volunteer canola

Weeds can originate either directly from a crop or from hybrids between a crop and a wild taxon (Gressel, 2005). Volunteer weeds are off-springs of crop seed or propagules from the previous crop (Lutman et al., 2003; Gressel, 2005). Before domesticated crops become totally wild or feral they remain in agricultural fields as volunteers which is the first step to ferality (Gressel, 2005). In the volunteer population there will be further selection for shattering and enhanced secondary dormancy, which leads to non-uniform germination during the following years (Gressel, 2005).

Canola has the weedy characteristics of seed loss due to shattering and secondary seed dormancy (Morgan et al., 2000; Gulden et al., 2003b; Gruber et al., 2004; Hall et al., 2005). The absence of the right environmental signals such as temperature, moisture and oxygen that promote germination of imbibed seeds induces secondary dormancy in canola (Momoh et al., 2002). Once

canola seeds reach the soil seedbank a portion can persist for many years due to induction of secondary dormancy (Pekrun et al., 1998; Gulden et al., 2003b). These seeds can then germinate to create volunteer weeds in following crops.

Volunteer canola is the 12th most abundant weed in western Canada following in-crop herbicide application (Leeson et al., 2005). In the main canola growing areas of eastern Canada, Simard et al. (2002) reported an average density of 4.9 and 3.9 volunteer canola m⁻² in fields and field margins respectively one year after canola production. Volunteer canola plants were also present in low densities after 4 and 5 years of canola production (Simard et al., 2002). In a more recent report, Beckie and Warwick (2010) found the presence of bromoxynil-resistant spring canola (BX Armour) 7 years after its commercial production. This transgenic cultivar was produced for only two seasons (2000 and 2001) suggesting that transgene canola can persist in the environment for a number of years even after all cultivars with the conferred trait have been removed from the market (Beckie and Warwick, 2010).

There are variable reports of canola seedbank persistence in agricultural fields. In the UK, Lutman et al. (2003) reported that considerable numbers of canola seeds persisted in the soil up to 4 years in normal cropping conditions but in the absence of cultivation persistence for over 11 years has been observed. But in western Canada, Gulden et al. (2003b) reported a greater proportion of persisting seeds under conventional tillage than under zero tillage. There are also variable reports on the proportion of canola seedbank persistence. In the UK, Hails et al. (1997) reported survival of only 1.5% and 0.2% of canola seeds in the soil after the first and second year of burial, respectively. But in western Canada, 0.2% of spring canola seeds survived after three winters (Gulden et al., 2003b). Lutman et al. (2005) developed a regression model that predicted seedbank persistence of 5% of seeds after 9 years. These differing reports may indicate varietal or environmental differences in mortality factors controlling canola seedbank persistence.

The persistence of canola seeds in the soil may increase when unopened siliques enter the soil seedbank. *B. napus* seeds remained dormant for more than 2 years when preserved in fruits but

they lost their dormancy completely in 3 months when separated from fruits (Tokumasu, 1975). However, in *Brassica japonica*, dormancy of seeds disappeared one month after harvest, whether they were in fruits or without fruits (Tokumasu, 1975). There was no explanation given on the prolongation of dormancy of *B. napus* seeds preserved in fruits but this could be linked to slow degradation of germination inhibitors inside the silique or siliques may deprive the right environment for germination of seeds.

There has been an increasing concern of canola seedbank persistence and temporal gene flow following the introduction of genetically modified (GM) herbicide resistant canola genotypes. In the UK, Lutman et al. (2003) reported that there were no clear differences between the conventional and GM herbicide resistant genotypes in either the numbers of seeds shed at harvest or in their subsequent persistence. Other reports from the UK indicated that there was no indication of increased invasive potential of canola due to genetic engineering for herbicide resistance but in cases where there were significant differences, transgenic lines were less invasive and less persistent than their conventional counterparts (Crawley et al., 1993). This has been confirmed by Hails et al. (1997), who reported greater survival (2%) of non-transgenic canola than two transgenic lines which showed 0.3% and 0.25% survival after 2 years of burial in the soil. This indicates that canola seedbank persistence is not linked to the presence of GM herbicide-resistant traits, rather it is due to induction of secondary dormancy which is influenced mainly by genotypic differences (Gulden et al., 2004a). However, there has been no report showing any relationship in dormancy attributes between GM herbicide resistant and conventional canola cultivars in western Canada.

The prevalence of herbicide resistant canola genotypes and an increase in the annual area seeded to canola are thought to be some of the reasons for increased incidence of volunteer canola in western Canada over the last two decades (Gulden et al., 2003a). Introduction of herbicide resistant traits to crops is an advantage for the subsequent volunteer population as these traits may reduce the damage from herbicides and improve the chances of replenishing the seedbank. An average density of 5.4 volunteer canola plants m⁻² was reported in fields even after post emergence herbicide application in western Canada (Simard et al., 2002). Hall et al. (2000)

identified the presence of multiple herbicide resistant volunteer canola (resistant to glyphosate, glufosinate and imazethapyr) in the Canadian agricultural fields. Multiple herbicide resistance has also been identified in volunteer canola grown outside of cultivated fields in western Canada (Knispel et al., 2008). This restricts the option of using herbicides to control volunteer canola especially when they grow in broad leaf crops.

Feral canola populations can also be found outside cultivated fields along road verges and field margins. In France, Pessel et al. (2001) confirmed that old cultivars of canola persisted outside of cultivated fields for at least 8 years after they were last cultivated. This gave evidence that the genetic background of feral plants on road verges did not reflect directly the genetic composition of recently cultivated cultivars (Pessel et al., 2001). In another report, Pivard et al. (2008) indicated that up to 40% of the feral canola populations in a typical open-field area of France originated from seed immigration from neighboring fields in the previous year at harvest or during sowing, while the other 40% originated mostly from persistent seedbanks. A small proportion of feral canola population was attributed to long distance immigration through seed transport or from seeds produced within the feral population the previous year (Pivard et al., 2008). The long term persistence of feral canola outside cultivated fields is a great concern especially when growing transgenic canola as it might lead to escape of genetically engineered novel traits to wild relatives. Transgenic cultivars could spread and persist in natural or semi-natural habitats even if the transgene does not increase the fitness of the crop (Pessel et al., 2001). In western Canada, a study conducted on 16 escaped canola populations along field edges and roadways in southern Manitoba indicated that glyphosate resistance was found in 14 (88%) of these populations, glufosinate resistance in 13 (81%) of the populations, and imidazolinone resistance in five (31%) of the populations (Knispel et al., 2008). Multiple herbicide resistance, resistance to glyphosate and glufosinate, was also observed in 10 (62%) of the tested feral population (Knispel et al., 2008).

2.5 Seed loss in canola

Silique shatter is of benefit for wild species but is an economically significant problem with *Brassica* crops and has still to be overcome (Spence et al., 1996). Canola siliques have two carpels (valves) separated into 2 loculi by a false septum, and each locule contains a single row

of seeds (Hougen and Stefansson, 1982). The two carpels join at the dehiscence zone, which is made up of few thin-walled cells that are morphologically distinct from the valve edges prior to wall degradation (Ferrándiz, 2002; Child et al., 2003). During silique maturation weakening of valve attachment occurs as a result of cell wall breakdown which occurs in the dehiscence zone (Meakin and Roberts, 1990a). Mechanical weakening of the dehiscence zone cells leads to silique opening and releasing the seed in a process known as dehiscence or silique shatter (Davies and Bruce, 1997). Meakin and Roberts (1990a) suggested that silique shatter in oilseed rape may be mediated by a senescence-related autolysis of cells within the dehiscence zone. However, actual dehiscence does not necessarily succeed zone weakening and that some external factors coupled with desiccation are necessary for silique shatter (Meakin and Roberts, 1990a). In addition to internal stress caused by thermal effects and drying, canola siliques shatter when they come into contact with other plant parts and harvest machinery, or when gravitational and aerodynamic forces are exerted on them (Kadkol et al., 1984). Other factors such as frost, hail, disease and insect damage may also reduce the strength of canola siliques.

During canola silique maturation structural and biochemical changes are apparent. Silique maturation in canola is accompanied by an increase in the activity of cellulase and degradation of pectin-rich middle lamella along a discrete layer of cells in the dehiscence zone (Meakin and Roberts, 1990b; Jenkins et al., 1996; Petersen et al., 1996; Child et al., 1998). The strong correlation between cellulase activity and pericarp degradation can be an indication that the hydrolase may be involved in cell wall degradation leading to silique shatter (Meakin and Roberts, 1990b). There is a transitory increase in ethylene production which precedes pericarp rupture indicating a possibility that ethylene also plays an important role in the process of silique shatter (Meakin and Roberts, 1990b; Child et al., 1998). The majority of ethylene production in *B. napus* silique is attributed to the seeds and it precedes the elevation of cellulase activity suggesting that there is a relationship between ethylene and cellulase activity in the dehiscence zone (Meakin and Roberts, 1990b; Child et al., 1998).

Other cell wall degrading enzymes such as polygalacturonases (PGs) were also reported to be involved in *B. napus* silique dehiscence. Petersen et al. (1996) suggested that there are temporal and spatial correlations between the breakdown of the middle lamella of the dehiscence zone

cells and the pattern of synthesis of polygalacturonase (PG35-8) transcripts which may indicate a role for this particular PG in *B. napus* silique dehiscence. Silique dehiscence in *Arabidopsis thaliana* is also accompanied by an increase in the expression of a polygalacturonase (Jenkins et al., 1999). However, in an earlier study Meakin and Roberts (1990b) did not find any correlation between the activity of polygalacturonase and *B. napus* silique dehiscence.

Auxins have also been suggested to play a role in the regulation and timing of silique dehiscence. Chauvaux et al. (1997) reported a decrease in auxin content in the dehiscence zone prior to moisture loss in *B. napus* siliques and this was correlated with a tissue specific increase in β -1,4-glucanase activity. Furthermore, treatment of the siliques with the auxin mimic 2-methyl-4-chlorophenoxyacetic acid resulted in 10 days delay of β -1,4-glucanase activity and its associated cell separation in the dehiscence zone (Chauvaux et al., 1997). This indicates that there may be a relationship between auxin activity and cell separation in the dehiscence zone. A low level of auxin in the dehiscence zone is necessary for dehiscence to take place but other factors may also be important (Chauvaux et al., 1997).

Silique drop or silique shatter prior to and during harvesting can result in significant seed loss in canola. It has been observed that the level of seed loss can be influenced by the time and technique of harvest but it also depends on the weather before and during harvest (Pekrun et al., 1997a). In a small plot study conducted in the UK, Price et al. (1996) determined that seed loss in winter canola was around 11% when direct-harvested but when windrowed canola yield loss ranged from 10.7% to 24.8%. In spring canola yield loss was much lower and ranged from 1.7% to 4.9% when direct-harvested and 2.6% to 4.6% when windrowed (Price et al., 1996). Under ideal harvest conditions yield losses of 2 to 5% has been reported but it can reach up to 50% under unfavorable weather conditions (Devos et al., 2004). Given a potential seed yield of 2,000-4,000 kg ha⁻¹ and a thousand seed weight (TSW) of 4-5 g in canola, a yield loss of 2 to 5% means a loss of 1000-5000 seeds m⁻², which is 10 to 35 times more than the sowing rates of 80-150 seeds m⁻² (Devos et al., 2004). Lutman (1993) also reported canola seedbank addition as high as 10,000 seeds m⁻² after harvest. In western Canada, Gulden et al. (2003a) reported an average seed loss of 107 kg ha⁻¹ or 5.9% of the crop seed yield and resulted in seedbank addition of approximately 3,000 viable seeds m⁻².

Seed loss in canola can also be influenced by the combine harvester header type. Hobson and Bruce (2002) indicated that it is advantageous to use a wider header to reduce seed loss when combining a standing canola. Hobson and Bruce (2002) reported similar losses from the side knife of a conveyor-assisted and a standard header, but because of the differing header widths, losses were translated into 18.4 and 34.6 kg ha⁻¹, respectively. Without the natural shedding and side knife losses, seed loss was 59 kg ha⁻¹ and 104 kg ha⁻¹ from the conveyor-assisted header and the standard header, respectively (Hobson and Bruce, 2002). Canola windrows can be picked up using a rubberized draper belt, an aluminium draper fitted with fingers or direct cut open front headers (Alberta Agriculture and Rural Development, 2012). The rubber belt type pick-up with rubber or synthetic fingers has been observed to reduce shattering losses due to its gentle action while the aluminium pick-up is more suited to bunched windrows (Alberta Agriculture and Rural Development, 2012). This indicates that it is important to use the appropriate harvest equipment in order to minimize harvest loss in canola.

2.6 Breeding canola for shatter resistance

Several attempts have been made to develop shatter resistant *B. napus* genotypes through plant breeding. However, it has been indicated that introgression of shatter resistance traits from related species into *B. napus* were complicated due to the presence of unwanted characters (Child et al., 2003). Increased shatter resistance will avoid the need to windrow canola at early stage of maturity. This avoids the additional cost of windrowing as the crop can be directly combined with minimal seed loss. Moreover, it will allow more time for canola seeds to mature while on the plant for improved yield and quality. An increase in harvestable seed yield arising from reduced silique shattering will result in significant economic benefits. The total canola production in Canada was estimated to reach 15.2 million tons in 2012 and with the current canola market price this is worth \$10 billion (Statistics Canada, 2012); thus, 0.5% change in loss results in a saving of \$50 million a year. Increased shatter resistance also reduces the cost of controlling volunteer canola in subsequent crops and minimizes seed loss outside the field boundary providing an added ecological benefit (Summers et al., 2003).

There have been variable reports on the relationship between plant morphological characters with silique shattering in canola. Morgan et al. (2000) indicated that plant height and silique wall thickness had correlations with silique shatter resistance. There was a tendency for shatter resistant plants to be tall and wide with thick stems and to have short, thick walled siliques with short beaks (Morgan et al., 1998). Siliques that are held erect in the canopy are thought to be more protected from shattering than siliques that are horizontally orientated (Summers et al., 2003). Moreover, plant height and silique characters such as the thickness of the valves were positively correlated with resistance, as taller, more vigorous plants produced thicker and more shatter resistant siliques (Summers et al., 2003). Child et al. (2003) also reported that increased shatter resistance was related to silique wall dimensions, the dehiscence zone characters and silique vascular tissue in *B. napus*. The presence of thicker silique wall and large sized main vascular bundle in the silique were correlated with increased shatter resistance in *B. napus* (Child et al., 2003). However, Kadkol et al. (1984) indicated that silique strength is a major component of shatter resistance than morphological characters of the plant such as the angle between siliques and the axis of the infructescence. Wang et al. (2007) also reported that there were no significant correlations of seed loss with plant height, plant width, the ratio of plant height to plant width, number of branches and stem diameter. Most silique morphological characteristics were also not correlated with the average seed loss, except silique length where silique shatter resistance was significantly correlated with shorter siliques (Wang et al., 2007).

Difference in dry matter partitioning to different tissues of the silique has been reported between *B. napus* lines that are resistance and susceptible to shattering (Summers et al., 2003). In shatter resistant cultivar (DK142) more dry matter stored in the receptacle while in shatter susceptible cultivar (Apex) dry matter was preferentially stored in the seed (Summers et al., 2003). Reducing the number of siliques per plant by removing the whole racemes; however, increased the weight of the remaining siliques and their resistance to shattering in both *B. napus* genotypes (Summers et al., 2003). Reducing silique number increased the weight of the silique receptacle more than the weight of the seed (Summers et al., 2003). This indicates that dry matter accumulation in the silique can reduce shattering but this might have a negative impact on seed yield and quality of canola.

There was little reported variation for shatter resistance within the existing *B. napus* genotypes but resistant lines have been found within the other *Brassica* lines such as *B. oleraceae*, *B. rapa*, *B. juncea*, *B. carinata* and *B. nigra* (Kirk and Hurlstone, 1983, Cited in Morgan et al., 1998). *Sinapis alba* (yellow mustard), which is grown as condiment use, is much more shatter resistant than *B. napus* (Brown et al., 1997). From a recent study in western Canada, Gan et al. (2008) also reported lower seed loss in *B. rapa*, *B. juncea* and *S. alba* genotypes compared to a *B. napus* genotype. But introgression of this character to *B. napus* has so far proved difficult since the other *Brassica* lines also have other undesirable characteristics (Morgan et al., 1998; Child et al., 2003). Increased shatter resistance has been reported within *B. napus* lines developed through interspecific hybridization with *B. oleraceae* and *B. rapa* (Morgan et al., 1998; Morgan et al., 2000; Summers et al., 2003; Child et al., 2003). The synthetic *B. napus* lines; however, contained many undesirable traits including poor seed set and disease susceptibility that made them unsuitable as cultivars (Morgan et al., 2000). This indicates that it is possible to utilize the genetic variation for shatter resistance that exists within the other *Brassica* species; however, modern plant breeding techniques such as marker assisted selection have to be used to incorporate this trait to *B. napus* without affecting the current yield potential and quality of the crop.

In *Arabidopsis*, which is a close relative of canola, the closely related MADS-box genes *SHATTERPROOF1* (*SHP1*) and *SHATTERPROOF2* (*SHP2*) are required for fruit dehiscence (Liljegren et al., 2000; Kadkol, 2009). These *SHP1* and *SHP2* genes control dehiscence zone differentiation and promote the lignification of adjacent cells (Liljegren et al., 2000). The *FRUITFULL* MADS-box gene, which is necessary for fruit valve differentiation, is a negative regulator of *SHATTERPROOF* and the expression of *FRUITFUL* is sufficient to prevent formation of dehiscence zone in *A. thaliana* fruit (Ferrándiz et al., 2000). Ferrándiz et al. (2000) suggested that the expression of *FRUITFULL* may allow the control of silique shatter in canola by preventing formation of the dehiscence zone. Østergaard et al. (2006) also indicated that ectopic expression of the *FRUITFULL* gene was sufficient to produce shatter resistant *B. juncea*. The knowledge gained by modifying traits from *Arabidopsis* can be successfully transferred to oilseed rape (Girin et al., 2010). Chandler et al. (2005) indicated that in both winter and spring canola plants, the constitutive expression of 35S:MADS-box gene prevented the formation of

dehiscence zone without any negative effect on seed yield or viability and this produced siliques that remained closed after-ripening. However, a total block of shattering is not desirable as it will lead to significant seed damage during threshing and careful fine-tuning is required to optimize this trait (Girin et al., 2010).

2.7 Seed dormancy in canola

Seeds are essential part of a plant which ensure perpetuation of the population by allowing the establishment of new seedlings. For a seed to germinate in a wide range of physical environment it should be completely non-dormant. Seed dormancy can be simply defined as a temporary block to the completion of germination of an intact viable seed under favorable conditions (Kucera et al., 2005; Finch-Savage and Leubner-Metzger, 2006). Most seeds are well equipped to survive extended periods of unfavorable conditions before germination and to establish plants under the most favorable conditions (Cadman et al., 2006). This is mainly due to the presence of various dormancy mechanisms. A dormant seed is one that does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors (Baskin and Baskin, 2004). Seed dormancy is determined by genetics with a substantial environmental influence which is mediated, at least in part, by the plant hormones such as abscisic acid (ABA) and gibberellic acid (GA) (Finch-Savage and Leubner-Metzger, 2006). The net result of the dormant state is characterized by increased ABA biosynthesis and GA degradation (Finch-Savage and Leubner-Metzger, 2006).

Seed dormancy and germination are complex adaptive traits of higher plants that are influenced by many genes and environmental factors (Koornneef et al., 2002). Besides the basic requirement for water, oxygen and appropriate temperature, seeds may also be sensitive to other factors such as light and nitrates for successful germination (Finch-Savage and Leubner-Metzger, 2006). Seed dormancy is generally undesirable in agricultural crops where rapid germination and growth are required; however, some degree of dormancy is advantageous during seed development to prevent pre-harvest sprouting particularly for cereal crops (Bewley, 1997). Extensive domestication and breeding of crop species have removed most dormancy

mechanisms that were present in the seeds of their wild ancestors, although under adverse environmental conditions seeds may become dormant (Bewley, 1997).

Based on the timing of induction, dormancy can be divided into primary and secondary. Primary dormancy is exhibited during the late stages of seed development by the interactions between the phyto-hormones, especially ABA and environmental conditions (Fei et al., 2007). After-ripening, which is a period of usually several months of dry storage of freshly harvested mature seeds, is a common method to release primary dormancy (Finch-Savage and Leubner-Metzger, 2006). Secondary dormancy is exhibited when seeds that are non-dormant at maturity or dormant seeds that have after-ripened are induced back into dormancy under certain conditions (Baskin and Baskin, 1985).

2.7.1 Primary dormancy in canola

There is ambiguous information in the literature concerning primary dormancy in canola. Japanese researchers have shown that *B. napus* seeds lost their primary dormancy in two and half months when they were separated from fruits immediately after harvest (Tokumasu, 1975; Kato, 1987). In contrast, it has been observed that freshly harvested *B. napus* seeds have little or no primary dormancy (Lutman, 1993; Pekrun et al., 1997a; López-Granados and Lutman, 1998; Pekrun et al., 1998; Momoh et al., 2002; Lutman et al., 2003). These contrasting results suggest that there could be genotypic or environmental differences in the development of primary dormancy in *B. napus*.

2.7.2 Secondary dormancy in canola

The long term persistence of volunteer canola in western Canada is related to its potential to develop secondary seed dormancy (Gulden et al., 2004a). The presence of dormant seeds in the soil seedbank provides an opportunity for germination to occur over several seasons maximizing the chance of long-term success (Gubler et al., 2005). Secondary dormancy is imposed in non-dormant seeds after seed dissemination by abiotic stresses such as light, moisture, oxygen, and temperature (Fei et al., 2007). The induction of secondary dormancy in canola is influenced by

the time of exposure to light and darkness, temperature regime and genotype (Pekrun et al., 1998). Light inhibits secondary dormancy induction but darkness or far red light associated with water or oxygen stress induces secondary dormancy in canola (Pekrun et al., 1997a; Pekrun et al., 1998; López-Granados and Lutman, 1998). Thus, buried canola seeds can become secondarily dormant and remain ungerminated until exposed to light by a subsequent cultivation (Lutman et al., 2003). Canola seeds that were left on the soil surface for 4 weeks prior to cultivation showed a much lower potential to persist than seeds that were immediately incorporated into the soil (Pekrun et al., 1998). The presence of crop residue in the field, which is characteristic of no-till systems, could also provide some of the conditions favoring the induction of secondary dormancy in canola seeds (Simard et al., 2002).

The potential for secondary dormancy induction in canola is influenced by genetic differences. Through evaluation of 16 commercially available *B. napus* genotypes in western Canada, Gulden et al. (2004a) reported that genotype contributed up to 82% to the total variation in secondary seed dormancy. Differences in the potential to develop secondary dormancy have also been reported in the European and Chinese *B. napus* genotypes (Pekrun et al., 1997a; Momoh et al., 2002). Other factors such as seed size, pre- and post-harvest environment were also reported to have influence on secondary dormancy expression in western Canadian *B. napus* genotypes (Gulden et al., 2004a). Seed size contributed 21% while pre-harvest temperature, precipitation and frost contributed up to 4.5% to the total variation in secondary dormancy (Gulden et al., 2004a). The potential for secondary dormancy decreased during storage of seeds (Gulden et al., 2004a).

Prolonged imbibition under conditions of water stress or oxygen deficiency in darkness can lead to the development of light sensitivity in canola (Pekrun et al., 1997a). The presence of light sensitivity in seeds provides a significant advantage in disturbed habitats as it enhances the probability of successful seedling establishment by avoiding ineffective depletion of the soil seedbank (Pekrun et al., 1997a). However, imbibition under oxygen deficiency was not as effective in inducing secondary dormancy as was imbibition under water stress (Pekrun et al., 1997a). Germination was reduced when seeds were exposed to water stress and far-red light or darkness but in the absence of water stress by far-red light alone indicating that the phytochrome

system is present in canola seeds (López-Granados and Lutman, 1998). Treatment with far-red light was more effective than darkness at inducing secondary dormancy in canola (López-Granados and Lutman, 1998).

2.7.3 Role of abscisic acid in seed dormancy

Abscisic acid and GA are the major phyto-hormones that regulate seed dormancy and germination (Zhang et al., 2010; Gulden et al., 2004b). ABA is a positive regulator of dormancy induction and maintenance but it is a negative regulator of germination whereas GA releases dormancy, promotes germination and counteracts the effects of ABA (Kucera et al., 2005). During seed development there is a change in both ABA content and sensitivity in response to internal and external signals (Nambara et al., 2010). Control of seed physiological processes by ABA depends on active hormone levels, which can be modulated through the rate of synthesis, catabolism or translocation from or to other sites (Nambara and Marion-Poll, 2003; Nambara and Marion-Poll, 2005). The primary function of ABA in developing seeds is inhibition of precocious germination and induction of primary dormancy (Nambara et al., 2010).

Abscisic acid regulates dormancy in other plants of the mustard family which are close relatives of canola. Karssen et al. (1983) indicated that the mutant lines of *A. thaliana*, which were characterized by the absence of seed dormancy, showed much lower levels of endogenous ABA in developing seeds and siliques. The initiation of primary dormancy in *A. thaliana* involved ABA (Karssen et al., 1983; Hilhorst and Karssen, 1992). Seeds of *S. alba*, which were kept dormant by ABA for several days rapidly absorbed water and continued the germination process after removal of the hormone (Schopfer et al., 1979). During seed development, the inability of the immature embryo to germinate might result from the inhibitory effect of endogenous ABA and restricted water uptake because of high external osmotic concentration (Schopfer et al., 1979; Schopfer and Plachy, 1984; Corbineau and Come, 2000). The inhibition of water uptake by ABA is a rapid and fully reversible process which appears to be the cause rather than the result of changes of the energy metabolism (Schopfer and Plachy, 1984).

The developing seeds and fruits of *A. thaliana* showed dual origin of ABA (Karssen et al., 1983). The first fraction is regulated by the genome of the mother plant (maternal ABA) which showed a sharp rise in ABA content half-way seed development (Karssen et al., 1983). The embryo was responsible for a second ABA fraction (embryonic ABA) which reached a much lower levels but persisted for some time after the maximum maternal ABA (Karssen et al., 1983). The onset of dormancy in *A. thaliana* correlated well with the presence of the embryonic ABA fraction and not with the maternal ABA or external ABA application (resembling maternal ABA) (Karssen et al., 1983; Kucera et al., 2005). However, in *B. napus* external application of ABA (0.1 millimoles per liter) has been shown to prevent the embryo from entering its growth phase (Schopfer and Plachy, 1984).

There is association among ABA sensitivity, biosynthesis and accumulation and secondary dormancy potential in canola (Gulden et al., 2004b). It has been indicated that ABA regulates many important aspects of plant development such as the synthesis of seed storage proteins and lipids, and the promotion of seed desiccation tolerance (Leung and Giraudat, 1998; Finkelstein et al., 2002; Kermodé, 2005). The tendency of *Brassica* seeds to manifest secondary dormancy may be determined by changes in gene expression related to carbohydrate metabolism, lipid biosynthesis, and storage protein accumulation that occur during late seed development (Fei et al., 2007). The expression of these genes during seed maturation is dependent on both genotype and environmental conditions and the extent of secondary dormancy may vary from year to year (Fei et al., 2007).

The action of ABA in the seed is not only from its accumulation rather it has been shown to result from its synthesis, catabolism, transport and sensing (Nambara and Marion-Poll, 2003). The ABA content of wild-type mature seed of *A. thaliana* is only slightly higher than the peak ABA level in an ABA-deficient mutant, suggesting that dormancy maintenance in mature seeds relies on signals other than residual endogenous ABA (Finkelstein, 2010). Seeds of *S. alba*, which have been imbibed in water, lost their sensitivity to be arrested by ABA after sowing and this escape from ABA mediated dormancy was not due to an inactivation of the hormone but to a loss of competence to respond to ABA during the course of germination (Schopfer et al., 1979).

3. EFFECT OF HARVEST METHODS ON CANOLA SEEDBANK ADDITION IN WESTERN CANADA

3.1 Introduction

Seed shatter is a common seed dispersal mechanism of weeds and is also significant problem in some crops such as canola. Mature canola siliques can easily split open and release their seed to the ground when they become in contact with harvest machinery, during strong wind or hail damages. It has been observed that seed loss during harvest is the main source of canola seed to the soil seedbank (Zhu et al., 2012; Gulden et al., 2003a). Volunteer canola can then create weed problems for the following crops. In weed surveys conducted on the Canadian prairies, volunteer canola was ranked as the 12th most abundant weed (Leeson et al., 2005). It has been indicated that the prevalence of herbicide resistant canola genotypes and an increase in the annual area seeded to canola are responsible for the increased incidence of volunteer canola in western Canada over the last two decades (Gulden et al., 2003a).

Seed loss in canola may be influenced by the method of harvest. Canola can be direct-harvested or windrowed and then threshed. Direct-harvesting refers to directly combining the standing canola with a combine harvester equipped with a direct cut header after natural ripening. Windrowing, on the other hand, refers to cutting the crop at early stage of maturity using a windrower and leaving it in windrows (swaths) on the cut stubble to hasten drying. Following drying the crop is then harvested with a combine harvester equipped with a pickup header. Windrowing canola is recommended when there is uneven maturity as it reduces maturation time and seed losses caused by shattering (Irvine and Lafond, 2010). The recommended practice to harvest canola in western Canada is windrowing the crop at 60% seed color change (SCC) on the main stem (Canola Council of Canada, 2012). Canola should be windrowed at the correct time to maximize yield and quality. Windrowing too early results in higher chlorophyll content in the seed and it may also reduce the yield, oil, and protein content of the seed (Vera et al., 2007; Boyles et al., 2010) and windrowing late can result in significant seed loss. In periods of prolonged rain, windrows can become very wet and slow to dry out which may lead to sprouting

of seeds (Price et al., 1996). In case of strong winds, windrows may also be blown resulting in higher seed loss.

Direct-harvesting is the common method of harvesting canola in Europe and the Southern Great Plains of the United States (Boyles et al., 2010). In western Canada, direct-harvesting is also practiced as it reduces the cost of windrowing and the chlorophyll content in the seed (Irvine and Lafond, 2010). However, seed loss can be high if strong winds hit the ripe standing canola. Direct-harvesting can be successful when the crop matures evenly, crop density is uniform, the crops is relatively heavy, partially lodged or with siliques laced together as these conditions reduce shattering and silique drop due to strong wind (Boyles et al., 2010).

Seed loss in canola is highly variable and may also depend on the genotype and the weather condition at or prior to harvest. In a small plot study conducted in the UK, Price et al. (1996) reported similar losses when spring canola was windrowed and direct-harvested but in winter canola windrowing resulted in higher seed loss than direct-harvesting. The overall reported seed loss in winter canola was also much higher compared to the spring canola (Price et al., 1996). High seed loss in canola can result in significant seedbank addition. Seedbank addition of up to 10,000 seeds m^{-2} was reported in the UK after harvesting of windrowed canola (Lutman, 1993). In western Canada, Gulden et al. (2003a) reported canola seedbank addition of 3,000 viable seeds m^{-2} which was equivalent to 5.9% of the seed yield. However, Gulden's study only examined seed loss in windrowed canola and did not investigate seed loss from direct-harvesting operation. Furthermore, since the first report of canola seed loss in western Canada, new canola genotypes have been added to the market. However, seedbank addition of the current canola genotypes has not been determined on commercial farms in western Canada. Thus, the primary objective of this study was to determine canola seedbank addition from windrowing and direct-harvesting operations on commercial farms in western Canada. The secondary objective was to determine agronomic and harvest-related factors that may increase seed loss in canola.

3.2 Materials and methods

3.2.1 Study area and sampling method

This study was part of a larger three-year (2010-2012) study that examined seed loss in canola across the three western Canadian provinces (Alberta, Saskatchewan and Manitoba). But the results reported here were from the first two years of the study in Saskatchewan. Over the two years a total of 66 fields were surveyed from 16 producers within 300 km radius from Saskatoon, Saskatchewan. The producers were randomly selected with the help of regional agronomists. Then fields were identified in an initial telephone contact. The canola in 15 of the surveyed fields was direct-harvested while the rest was windrowed. Up to a maximum of three direct-harvested and three windrowed fields were sampled from a single producer and where possible fields were sampled from the same producer over the two years. Seven producers were able to provide fields in both years while others were only able to provide fields for one year.

Samples were taken from 3 random transects which were laid in each field perpendicular to where two windrows had laid before harvest. All fields were sampled within 3 weeks of harvesting. In windrowed fields, transects were oriented from the center of an area where one windrow had laid to the center of an area where the adjacent windrow had laid before combining. In the direct-harvested fields, transects were laid from the center of one combine pass to the center of the adjacent combine pass. It was possible to identify the area where the windrow had been laid as the stubble underneath was less weathered than the exposed stubble. In direct-harvested fields, the combine pass was recognizable by the combine harvester tire tracks. A total of six to seven 0.25 m² quadrats were located along each transect at 1m intervals. Using a Shop Vacuum Cleaner, all the remaining crop residue, shattered seeds, and some surface soil were removed from each quadrant. Samples from the quadrats were combined for each transect, kept in a cloth bag, air dried at room temperature, and stored for further cleaning.

Fields where canola was last grown at least two years prior to the sampled canola crop were included in the survey (Table A2.4). This reduces the chance of sampling seeds from the previous canola crop since only 0.2% of spring canola seeds were reported to survive after three

winters in western Canada (Gulden et al., 2003b). Moreover, only the top 1-2 cm of soil was removed to minimize seed sampling from persistent seedbanks. Germinated seeds were counted in each quadrat before taking the sample and the population was included in the total seed loss. In 2010, where precipitation received was 115 to 150% of the normal average in large parts of Saskatchewan (Saskatchewan Ministry of Agriculture, 2011), there was considerable germination of canola seeds in the fields but 2011 was relatively dry during harvest and there was no germination in most of the sampled fields. In 2011, canola plant density at harvest was determined by counting the main stems of the canola stubble in 4 of the quadrats. For each field, data concerning agronomic- and harvest-related information were collected from the producer using a survey questionnaire (Appendix 1).

3.2.2 Seed separation

Air dried samples were passed through a dockage tester (CEA. Simon-Day Ltd.). Each sample was separated using a 3.6 x 17.5 mm oblong sieve on top and a 3.2 mm round sieve in the middle position which removed the large soil clods and chaff thereby allowing the passage of canola seeds, fine chaff and soil. This combination of sieve size was selected after testing that seeds passed through while separating the soil clods and larger chaff. In 2010, the larger soil clods were crushed using homemade belt thresher and again passed through the dockage tester in order to separate seeds which might have been stuck in the soil clods, whereas in 2011 there were no large sized soil clods as the fields were relatively dry. The remaining samples were again hand sieved using 1.3 x 8 mm oblong and 1 mm round sieves to remove the fine soil from the remaining sample. At this point, 50 seeds were hand-picked from each sample for seed viability test and the remaining samples were wet sieved to remove smaller soil aggregates. Following this, the washed samples were dried for 24 hours at 40 C. After drying, the remaining crop residue was wind-blown from the samples which were then rolled down a smooth inclined surface to separate seeds from stones and some weed seeds. Finally, cleaning of smaller stones and remaining weed seeds was done by hand before measuring the weight of pure seed. The total number of shattered seeds per unit area was then calculated by dividing the weight of pure seed by TSW and multiplying it by 1000.

3.2.3 Seed viability test

From each sample, 50 seeds were placed in 9 cm plastic petri dish on 2 layers of filter paper (Reeve Angel, WHATMAN INC., NJ, U.S.A) and 6 ml of distilled water to examine their germinability. The seeds were allowed to germinate in a germination cabinet at 20 C for two weeks with germinated seeds counted every other day. Following this, the few remaining ungerminated seeds were stratified at 2 to 4 C for 5 days (Gulden et al., 2004a). They were then returned to 20 C and allowed to germinate for 1 week. Exposing seeds to temperature alterations (2 to 4 C followed by germination at 20 C) were reported to break dormancy in *B. napus* (Pekrun et al. 1997b). Seeds which did not germinate after 1 week were examined for viability using 2,3,5-triphenyl tetrazolium chloride (Sigma Chemical Company, MO U.S.A) (Gulden et al., 2004a). The imbibed seeds were laterally dissected using a sharp razor blade near the center of the seed without damaging the embryo. Then the dissected seeds were soaked in 1% triphenyl tetrazolium chloride solution and kept in a germination cabinet at 20 C overnight (Grabe, 1970). The next day the seed coats were removed and individual seeds were examined under a light microscope (10x/21) for color change of the embryo. Seeds with the embryo stained red were considered viable while those seeds with non-stained embryo were considered non-viable (Grabe, 1970). All germinated seeds and those seeds that did not germinate but proved to be viable from the tetrazolium chloride test were considered viable.

3.2.4 Statistical analysis

The average seed loss from the three transects was taken to calculate the mean seed loss per unit area for each field. The mixed procedure of SAS statistical software (SAS 9.2, SAS Institute Inc., Cary, NC, U.S.A) was used to perform Analysis of Variance (ANOVA) to determine the variability of seed loss between the years, producers and harvest methods. This study had a multi-stage, nested sampling design with producers nested in years and fields nested in producers. Producers nested in years and fields nested in producers were considered random variable. To determine differences in seed loss, percent yield loss, and seedbank addition among producers, a subset of data (composed of 40 fields) were chosen, where two field-years of the same producer were available. To compare yield, yield loss and seedbank addition between direct-harvested and windrowed fields, another subset of data (composed of 15 windrowed and 12 direct-harvested fields) were chosen, where the same producer used both operations to harvest

canola. Means were separated using Fisher's protected Least Significant Difference (LSD) at $P < 0.05$.

Stepwise multiple linear regression analysis was also conducted to model the relationship of seed loss with agronomic- and harvest-related data. The PROC REG procedure of SAS was used with stepwise and AIC options to conduct the stepwise multiple regression and to select the best model producing the lowest Akaike Information Criterion (AIC). AIC is a model fitting criteria in which the best-fitting model is represented by the lowest AIC (Littell et al., 2006). The variance inflation factor (VIF) option was used in PROC REG procedure of SAS to check for multicollinearity among the independent variables. Those variables whose VIF was greater than 10 were considered a linear combination of other independent variables and were inspected and when necessary omitted from the model. The agronomic- and harvest-related data included in the analysis were total canola area, area of sampled field, genotypes grown, seeding rate, rate of nitrogen fertilizer, time of windrowing, time of combining, percent seed color change on the main stem at the time of windrowing, speed of windrowing, width of the windrower cutter bar, combine separator type, combine model, combine speed, days from windrowing to combining, combine fan speed, total yield and TSW (Appendix 2). Total yield was the sum of seed yield and yield loss. A total of 14 different canola genotypes were reported in this study and these genotypes were grouped based on herbicide resistant trait as Liberty Link (LL) and Roundup Ready (RR) for convenience in the regression analysis (Table A2.5). Then all categorical explanatory variables were coded as dummy variables for the regression analysis.

3.3 Results and discussion

3.3.1 Canola seedbank addition

The results of ANOVA indicate that total reported yield, yield loss, seed shatter and seedbank addition of canola differed by year (Table 3.1). However, there was no difference in yield loss and seedbank addition among producers and between the harvest methods (Table 3.1). This indicated that seed loss in canola was influenced more by the weather at or prior to harvest than the agronomy or harvest method. There was no difference in percent yield loss and TSW between the years but canola TSW differed between windrowing and direct-harvesting operations (Table 3.1).

Table 3.1 *P*-values from mixed model ANOVA F-test for Total reported yield, yield loss, TSW, shattered seeds, seed viability and seedbank addition as influenced by year, producer and harvest method assessed on commercial canola farms across Saskatchewan in 2010 and 2011.

	Total yield	Yield loss		TSW	Shattered seeds	Seed viability	Seedbank addition
	(kg ha ⁻¹)	(kg ha ⁻¹)	(%)	(g)	(seeds m ⁻²)	(%)	(viable seeds m ⁻²)
Year	0.0362*	0.007**	0.075	0.2543	0.0104*	0.0957	0.0187*
Producers	0.3161	0.9818	0.217	0.0572	0.9085	0.5058	0.9325
Harvest	0.9913	0.9121	0.639	0.0042**	0.9011	0.3301	0.864

*, **, denote significance at the 0.05 and 0.01 probability levels, respectively.

Over the 2 years, the average seed loss was approximately 6,200 seeds m^{-2} , which is equivalent to 184 kg ha^{-1} (Table 3.2). This amount represents 7.3% of the total yield (Table 3.2). With an average of 94% viability of seeds, yield loss of this magnitude resulted in seedbank addition of approximately 5,800 viable seeds m^{-2} (Table 3.2). The observed seedbank addition was almost twice of the previously reported amount of 3,000 viable seeds m^{-2} in western Canada (Gulden et al., 2003a). However, seedbank addition observed in the present study was much lower than the one reported by Lutman (1993) in the UK. But the average yield loss found in the present study was higher than the 2 to 5% range reported in spring genotypes in the UK in both windrowed and direct-harvested operations (Price et al., 1996). Much of these differences may be attributed to the difference in the canola genotypes and the weather conditions. Seed loss found in the present study was approximately 37 times the normal seeding rate of 4 to 6 kg ha^{-1} and this may create volunteer weed problems for many years.

The reported yield, yield loss, seed shatter and seedbank addition were higher in 2011 (Table 3.2). However, in an earlier study, Gulden et al. (2003a) did not detect differences in any of these variables between years. Lack of difference between the years at that time may be attributed to the smaller number of surveyed fields and the shorter radius of the study area from Saskatoon compared to the present study. The weather in 2011 was relatively favorable for canola production. Normally higher yield loss is expected in canola under adverse weather conditions. The higher yield loss in 2011 may be attributed to the reported high yield of the canola genotypes. Lack of difference in TSW between the years might indicate that seed number per plant rather than seed size varied between the years. This has been indicated in other crops such as wheat and soybean where agronomic selection led to narrow variability of seed size while cultivated crops retained high plasticity for seed number (Sadras, 2007). Percent yield loss is the proportion of seed loss out of the total yield and lack of difference in percent yield loss between the years was due to the observed variability in both seed loss and total yield.

Table 3.2 Total reported yield, yield loss, TSW, shattered seeds, seed viability and seedbank addition of canola as influenced by year.^a

Year	Total yield	Yield loss		TSW	Shattered seeds	Seed viability	Seedbank addition
	(kg ha ⁻¹)	(kg ha ⁻¹)	(%)				
2010	2,290 (129.6)	134.9 (22.4)	6.02 (0.9)	2.87 (0.1)	4,619 (756.2)	95 (1.3)	4,404 (742.3)
2011	2,670 (110.4)	220.7 (18.4)	8.3 (0.8)	3.01 (0.1)	7,374 (619)	92 (1.1)	6,859 (610)
LSD _{0.05}	*	**	NS	NS	*	NS	*
Mean	2,510 (91.5)	184.1 (16.5)	7.3 (0.6)	2.95 (0.1)	6,208 (555.7)	94 (0.9)	5,821 (534.3)

^a Standard errors are indicated in parentheses.

*, **, denote significance at the 0.05 and 0.01 probability levels, respectively.

NS - denotes not significant.

Total reported yield, yield loss and seedbank addition were not different among producers for whom two years of data were available (Table 3.1). This may imply that similar magnitude of canola seedbank addition is expected among Saskatchewan producers. Yield loss among producers ranged from 4.9 to 9% of the total yield and this was close to the 3.3 to 9.9% range that was reported by Gulden et al. (2003a). However, Gulden et al. (2003a) reported that there were differences in seedbank addition among producers. Despite the wide range in yield loss and seedbank addition among producers in the present study, lack of statistical difference in these variables could be due to the observed large standard errors.

There was no difference in yield loss as well as seedbank addition between the windrowed and direct-harvested canola (Table 3.1; Table 3.3). This indicates that direct-harvesting can be a viable option to harvest canola in western Canada. Price et al. (1996) also reported no difference in seed loss between windrowing and direct-harvesting operations of spring canola in the UK. However, in the present study there was a difference in TSW between the two harvest methods (Table 3.1; Table 3.3). Seeds of the direct-harvested canola were larger than seeds of the windrowed canola.

Table 3.3 Total reported yield, yield loss, TSW, and seedbank addition of canola as influenced by harvest methods.^a

Harvest	Total yield (kg ha ⁻¹)	Yield loss		TSW (g)	Seedbank addition (viable seeds m ⁻²)
		(kg ha ⁻¹)	(%)		
Windrowed	2,631 (169)	251 (49)	9.5 (1.7)	2.9 (0.1)	8,210 (1,628)
Direct-harvested	2,629 (176)	257 (51)	10.4 (1.8)	3.1 (0.1)	7,905 (1,680)
LSD _{0.05}	NS	NS	NS	**	NS

^a Standard errors are indicated in parentheses.

** , denote significance at the 0.01 probability level.

NS - denotes not significant.

3.3.2 Regression analysis

Multiple linear regression was used to determine the effect of agronomic and harvest data on seed loss in canola. To avoid multicollinearity problems, only independent variables that were not correlated with each other were analyzed in the regression analysis. The total farm size was omitted from the model during model simplification as it was collinear with the total canola farm area ($r = 0.98$). Producers who had large total farm size had more area seeded to canola. A model with the parameters of total canola farm area of a producer, the herbicide and seed system used, combine separator type and total yield resulted in a model fit with the smallest AIC and adjusted r^2 of 0.50 (Table 3.4; Figure A3.1). An adjusted r^2 of 0.50 indicates that the model has accounted for 50% of the variation in seed loss.

There was correlation of seed loss with the total canola farm area a producer had, herbicide and seed system used, combine separator type and total yield (Table 3.4). All the other independent variables (Appendix 2) did not have effects on seed loss. This correlation indicated that when the total canola farm area of a producer increased by 1 hectare, seed loss also increased by 0.06 kg ha^{-1} (Table 3.4; Figure 3.1). This could be because a producer who has large area seeded to canola may need longer time to windrow and combine the crop than a producer who has small area seeded to canola and this may delay the work from the optimum recommended time leading to higher seed loss.

Seed loss in canola was also influenced by the herbicide and seed system used. Producers who grew Roundup Ready canola experienced 58 kg ha^{-1} (2.3% of the total yield) higher seed loss than producers who grew Liberty Link canola (Table 3.4). InVigor5440 constituted 58% of the Liberty Link genotypes sampled in this study and the observed lower seed loss for this group may be due to lower seed loss from this particular genotype (Table A2.5; Chapter 5). Other Liberty Link genotypes sampled in this study include InVigor5030, InVigor5770, InVigor9590, L130 and L150 (Table A2.5). The Roundup Ready genotypes grown were Victory1037, D3150, Dekalb 72-65, Victory 1040, Pioneer 45H28, Viterra VT 500 and VT Barrier (Table A2.5).

Seed loss in canola was also correlated with combine separator type (Table 3.4). Producers who used conventional combines experienced 122 kg ha⁻¹ (4.9% of the total yield) higher seed loss than producers who used rotary combines (Table 3.4). The number of conventional combines used to harvest canola was small compared to the number of rotary combines used in this study (Table A2.13).

The observed positive correlation of seed loss with total yield in the present study showed that when total yield of canola increased by 1 kg ha⁻¹, seed loss also increased by 0.07 kg ha⁻¹ (Table 3.4; Figure 3.2). This may indicate that higher seed loss is expected when the yield of canola is high (Table 3.4; Figure 3.2). However, Gulden et al. (2003a) did not find a relationship between seed loss and total yield. Lack of significant relationship at that time could be due to the smaller number of sampled fields compared to the present study.

Absence of correlation of seed loss in canola with other independent variables may indicate that these variables have no effect on seed loss in canola. Unlike the analysis of Thomas et al. (1991) in the United States, in the present study canola seed loss was neither related to seed color change at the time of windrowing nor to the time of windrowing within a day. Gulden et al. (2003a) also reported the absence of a relationship between seed loss in canola and time of windrowing in relation to crop maturity. We did not find any relationship of canola seed loss with the speed and model of the combine, the time of combining, seeding rate, rate of nitrogen fertilizer, the number of days from windrowing until combining, TSW, area of the sampled field and the combine fan speed. Furthermore, in 2011 the plant density at harvest was not correlated to seed loss in canola.

Table 3.4 Model parameters of agronomic and harvest specific variables predicting seed loss in canola. The adjusted r^2 for the model is 0.50.

Variable	Parameter estimate	Standard error	Partial r^2	Pr > t
Intercept	-9.55	48.29	-	0.845
Canola farm area	0.06	0.02	0.16	0.019
Herbicide and seed system	58.44	25.5	0.08	0.03
Combine separator type	122.27	44.2	0.21	0.01
Total yield	0.07	0.02	0.11	0.002

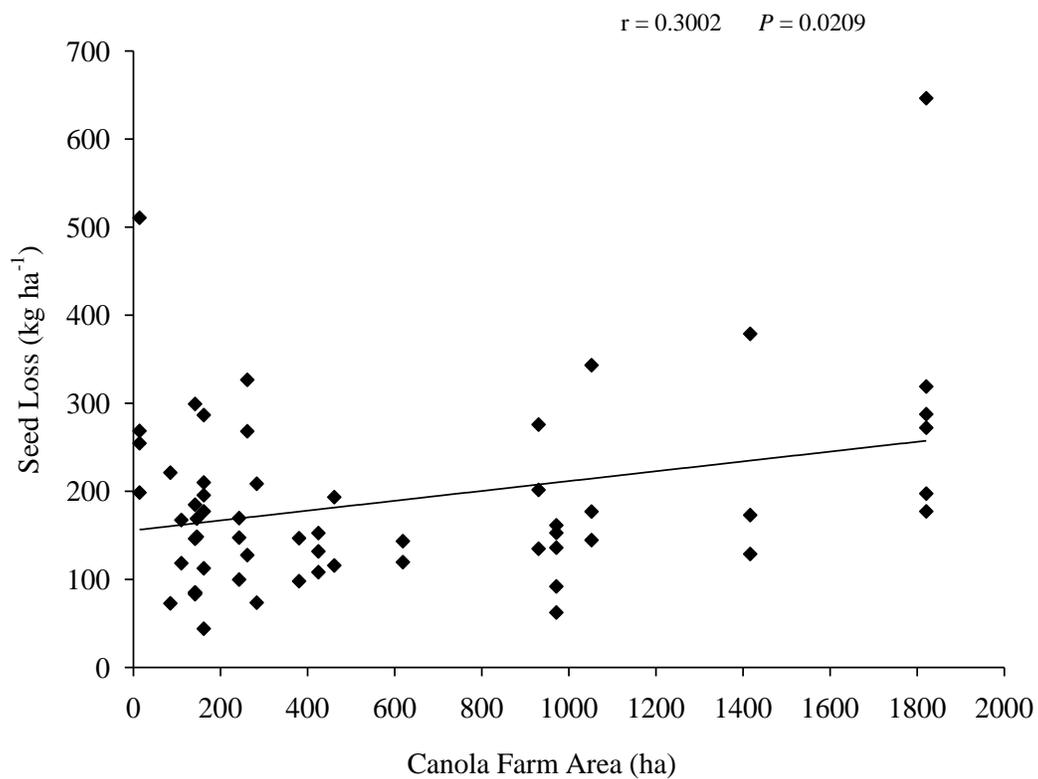


Figure 3.1 Correlation of seed loss with total canola farm area of a producer on 59 sampled fields.

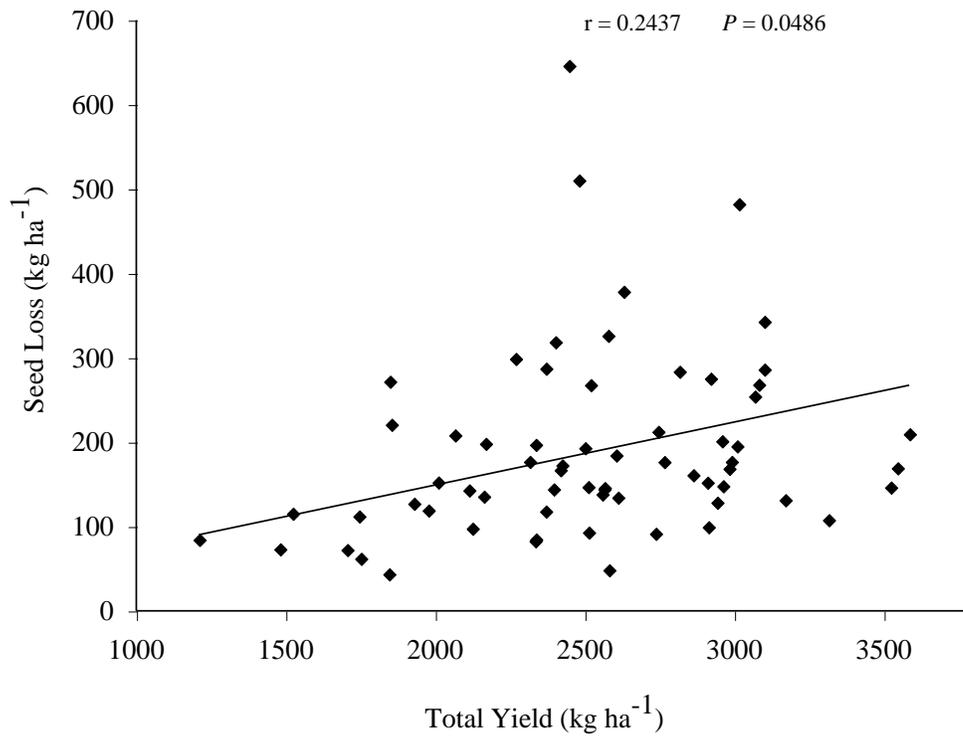


Figure 3.2 Correlation of seed loss with total yield on 66 sampled fields.

3.4 Conclusion

High seed loss in canola fields results in significant amount of yield loss and dispersal of seeds into the soil seedbank. Seed loss in canola was not statistically different among producers and between the harvest methods. This indicates that similar seed loss is expected among Saskatchewan canola producers and between the harvest methods. The difference in seed loss between the years, however, indicated that seed loss may be influenced by weather conditions. The observed correlation of seed loss with some of the agronomic- and harvest-related variables indicated that when seed yield of canola is high and when a producer has large canola farm area, then seed loss and seedbank addition can be higher. Similarly, growing Liberty Link genotypes and harvesting with a rotary combine harvester may result in lower seedbank addition than growing Roundup Ready canola and harvesting with a conventional combine harvester.

The lack of significant difference in the reported yield as well as seed loss between windrowed and direct-harvested canola indicates that windrowing canola has no advantage over direct-harvesting to reduce canola seedbank addition. Therefore, direct-harvesting can be considered as a feasible option to harvest canola in western Canada. However, significant seed shatter can occur if strong winds hit the ripe standing crop. The present study compared seed loss between 15 windrowed and 12 direct-harvested canola fields which were obtained from five producers who harvested their canola using both harvest methods. This sample size may not capture the total variance of seed losses between these harvest methods. Therefore, further research is required to evaluate seed loss between the two harvest methods in different agro-ecosystems in western Canada.

3.5 Prologue to chapter 4

The results reported in chapter 3 showed that there was seed loss of 7.3% of total yield in canola. Seed loss of this magnitude can lead to the problem of volunteer canola for many years due to induction of secondary dormancy in the seed. There was no significant difference in seed loss among producers and between the harvest methods. Some of the agronomic- and harvest-related activities, however, had effects on seed loss in canola. It is therefore possible to reduce seed loss by changing agronomic- and harvest-related activities. The incidence of volunteer canola can also be minimized by reducing secondary dormancy potential in the seed. Secondary dormancy can be influenced by different factors, one of which could be the developmental stage of the seed. Due to its indeterminate growth habit and differences in the method and time of harvest, canola seeds can enter the soil seedbank at different stages of development. However, little is known about the effect of seed maturity at harvest on secondary dormancy development in canola. A study was therefore conducted to determine the effect of crop maturity at harvest on potential to develop seed dormancy in canola. This study is presented in manuscript format in Chapter 4.

4. EFFECT OF CROP MATURITY AT HARVEST ON POTENTIAL TO DEVELOP DORMANCY IN CANOLA (*Brassica napus* L.)

4.1 Introduction

The long term persistence of canola in the soil seedbank is related to its potential to develop secondary dormancy (Pekrun et al., 1998; Gulden et al., 2004a). Canola genotypes with high potential to develop secondary dormancy have been reported to decline more slowly within the soil seedbank than genotypes with low potential for secondary dormancy (Gruber et al., 2010; Thöle and Dietz-Pfeilstetter, 2012). This leads to the problem of volunteer canola many years after canola production. Volunteer canola has been reported to persist for 10 years in Sweden (D'Hertefeldt et al., 2008), while in the UK, seedbank persistence of winter canola up to 11 years has been reported (Lutman et al., 2003). In eastern Canada, volunteer canola was present at low densities 5 years after canola production (Simard et al., 2002) whereas in western Canada volunteer canola has been found 7 years after canola production (Beckie and Warwick, 2010). The persistence of volunteer canola up to 9 years has been reported even outside of cultivated fields in France (Pessel et al., 2001). The long term persistence of volunteer canola is a major concern especially when growing genotypes with different quality traits as it may lead to genetic contamination and reduce the market value of the produce.

Certain environmental conditions may lead to the development of secondary dormancy in canola. Secondary dormancy is induced in non-dormant seeds by abiotic stresses such as light, moisture, anoxia, and temperature (Fei et al., 2007). Prolonged imbibition under conditions of water stress or oxygen deficiency in darkness can induce secondary dormancy in canola (Pekrun et al., 1997a). Exposure of dormant canola seeds to light and temperature changes were reported to break dormancy (Pekrun et al., 1997b). Dormancy induction in canola is mainly genetically controlled but other factors such as seed size, pre-harvest environment (precipitation, temperature and frost) and post-harvest storage conditions also have some influence on dormancy (Gulden et al., 2004a). Momoh et al. (2002) reported variation in the development of

secondary dormancy ranging from 0 to 85% among 25 Chinese and European canola genotypes. In Germany, Gruber et al. (2009) screened 44 *B. napus* genotypes for secondary dormancy under laboratory conditions and reported that secondary dormancy was influenced by the genotype. In western Canada, Gulden et al. (2004a) indicated that genotype contributed between 44 and 82% to the total variation in secondary dormancy among 16 commercial *B. napus* genotypes, seed size contributed 21% to the variation and the effect of different harvest regimes was 0.1 to 4.5%.

Dormancy is usually initiated during seed maturation and its maintenance in the mature seed is dependent on both environmental and genetic factors (Gubler et al., 2005). It has been indicated that several processes essential for seed viability and germination occur during seed development (Parcy et al., 1994). These processes include the accumulation of protein and lipid reserves, the acquisition of desiccation tolerance and induction of dormancy (Parcy et al., 1994). ABA has been reported to regulate the synthesis of seed storage proteins and lipids, and the promotion of seed desiccation tolerance during the plant development (Leung and Giraudat, 1998; Finkelstein et al., 2002; Kermode, 2005). ABA also plays an important role in controlling primary dormancy induction during seed maturation (Fei et al., 2007). An association among ABA sensitivity, biosynthesis and accumulation, and secondary dormancy potential has also been reported in canola (Gulden et al., 2004b).

In many crop species, the concentration of ABA in the seed is normally low during the early stages of development, increases and reaches the highest concentration at mid-development stages and declines as the seed progresses towards maturation (McWha, 1975; King, 1976; Prevost and Page-Degivry, 1985; Walker-Simmons, 1987; Bewley, 1997; Finkelstein, 2010). In *B. napus*, endogenous ABA level rose 3 to 4 fold during the storage protein accumulation phase (25 to 39 days after flowering), reaching peaks at 35 and 38 days after flowering (Finkelstein et al., 1985). Following the second peak; however, endogenous ABA declined rapidly to the pre-peak level and remained at this level until seed maturity (Finkelstein et al., 1985). Suppression of germination followed the same time course as endogenous ABA, and the longest suppression coincided with the highest ABA levels in *B. napus* (Finkelstein et al., 1985). An inverse correlation between ABA content and seed germination has also been reported throughout maturation of *Phaseolus vulgaris* embryos (Prevost and Page-Degivry, 1985). This may indicate

that there could be variation in potential for dormancy induction in seeds at different stages of crop maturity.

Two harvest techniques are currently in use to harvest canola. Windrowing canola at approximately 60% SCC on the main stem and combining it following drying is the recommended method in western Canada (Canola Council of Canada, 2012). Direct-harvesting of the standing canola after natural ripening in the field is another method. Direct-harvesting is also practiced in western Canada as it reduces the cost of windrowing and the chlorophyll content in the seed (Irvine and Lafond, 2010). However, the stage of crop maturity at harvest can be variable in different places and seasons. Due to differences in crop maturity at harvest, canola seeds may enter into the soil seedbank at different stages of development. However, the potential for dormancy induction in the seed at different stages of development has not been clearly evaluated in canola. Thus, the objective of this study was to determine the effect of crop maturity at harvest on potential to develop seed dormancy in canola.

4.2 Materials and methods

4.2.1 Study site, seed source and sampling

The canola seed used in this study was obtained from a replicated canola trials at Kernen Crop Research Farm, Saskatoon, SK (lat 59°09', long 106°33') in 2010 and 2011. In both years, seed was obtained from plants that were collected from an unharvested portion of a plot (6m x 4m) established to study seedbank addition of different canola genotypes and harvest methods at Kernen (Chapter 5). Precipitation and temperature data for this site are indicated in Table 5.2. In 2010, sequential harvests of the InVigor5440 genotype were conducted beginning at 50 to 60% SCC on the main stem. In 2011, two *B. napus* genotypes were included in the study and harvesting started at an earlier crop maturity stage. InVigor5440 and InVigor5020 plants were harvested starting from 20 to 30% and 10 to 20% SCC on the main stem, respectively. Each harvest sample was taken from the three replications and blocking structure was retained for dormancy assay. A total of six harvest samples were taken in each year for both genotypes on a weekly interval until the *B. napus* was fully ripened and ready to be harvested. The stage of crop maturity at harvest was reported in days after flowering (DAF). Days to flowering was taken

when approximately 50% of the plants were in bloom (Chapter 5). Up to ten plants were harvested randomly from each of the three replications and the freshly harvested plants were allowed to dry at room temperature for one week. The pods from the middle portion of the main stem and branches were then removed and threshed by a belt thresher and the clean seed was separated manually.

4.2.2 Primary dormancy assay

Immediately after threshing and separating seeds, 100 seeds were taken from each sample and placed into a 9 cm diameter plastic petri dish (VWRTM, VWR international) with 2 layers of filter paper (Reeve Angel, Whatman Inc., NJ, U.S.A) and 6 ml of distilled water to determine germination of the seed lot and the presence of primary dormancy in freshly harvested seeds. These were replicated three times and allowed to germinate in a germination cabinet (Hotpack Phila., PA., U.S.A) at 20 C. Germinated seeds were counted every other day for 3 weeks. The remaining ungerminated seeds were stratified at 2 to 4 C for 5 days (Gulden et al., 2004a), then were returned to the 20 C germination cabinet for further germination. After 1 week firm ungerminated seeds were tested for viability using 1% tetrazolium solution as described previously (Chapter 3). Seeds that germinated after the stratification treatment and those seeds that did not germinate but proved to be viable from the tetrazolium test were considered to have been dormant during the preceding test (Pekrun et al., 1997a). Non-viable seeds were discarded from the total seed counts. After the primary dormancy assay, further samples were retained in a plastic bag and stored at -80 C for the secondary dormancy assay.

4.2.3 Secondary dormancy assay

Polyethylene glycol (PEG-8000) (Alfa Aesar, Ward Hill, MA., U.S.A) was used to lower the water potential of the imbibing solution to -1.5 MPa at which germination in non-dormant *B. napus* seed was prevented so that secondary seed dormancy could be induced (Pekrun et al., 1997a; Pekrun et al., 1998; Gulden et al., 2004a). To conduct the secondary dormancy assay, 100 seeds were placed in 9 cm diameter plastic petri dish as previously described. Each treatment was replicated three times and to induce secondary dormancy, the seeds were treated with 8 ml

of PEG-8000 (Fisher Scientific, NJ, U.S.A) solution and kept in a germination cabinet at 20 C (Gulden et al., 2004a).

All petri dishes were wrapped with double layers of light impervious black plastic bags to prevent exposure of seeds to light during the experiment. After 4 weeks, the seeds were rinsed with distilled water in a darkroom under a green safe light to remove the PEG and then transferred to new petri dishes containing 2 layers of filter paper and 6 ml of distilled water to determine their germination under non-limiting water and permanent darkness conditions afterwards. The new petri dishes were again wrapped with light impervious plastic bags and returned to the germination cabinet at 20 C. Germinated seeds were counted every other day in a darkroom under green safe light. After 2 weeks all firm ungerminated seeds were counted on a lab bench under normal fluorescent light and stratified at 2 to 4 C for five days (Gulden et al., 2004a). Then they were retested for germination at 20 C in a germination cabinet. After 1 week the firm, ungerminated seeds were tested for viability using 1% tetrazolium solution as described previously (Chapter 3). Non-viable seeds were discarded from the total seed counts. Each dormancy assay was conducted once in each year and the replications were completely randomized in the germination cabinet.

4.2.4 Statistical analysis

Data for the secondary dormancy assay were tested for significance between the years using nonlinear regression analysis of curves using the *multdrc* extension package in R Statistical Software (Version 2.6.1) (R Development Core Team, 2007; Ritz and Streibig, 2005). To compare dormancy induction potential in InVigor5440 genotype between the years, a global regression model fitted to the two years combined data, was compared with a regression model fitted to individual years. Similarly, differences in dormancy induction potential between InVigor5440 and InVigor5020 genotypes were determined by comparing a global regression model fitted to both genotypes combined data with a model fitted to the individual genotypes. Regression models and parameters were compared using an extra sum of squares F-test (Lindquist et al., 1996). In case where there was no difference between the models, years were combined. A common curve was then fitted using parameters from the global model that predicted the values in both years of data. The relationship between percent seed germination

and crop maturity at harvest was described using two parameters power relationship shown below:

$$y = a * x ^ b \quad (4.1)$$

In this equation, y is the dependent variable (percent seed germination), a is the y-intercept, x is the independent variable (crop maturity at harvest), and b is the slope of the line.

4.3 Results and discussion

4.3.1 Primary dormancy in *B. napus*

Freshly harvested seeds of InVigor5440 and InVigor5020 had 87% and 84% germination at 32 and 33 DAF, respectively (Figure 4.1). Germination of InVigor5440 seeds increased with harvest timings and reached 100% when seeds were harvested after maturing on the plant (78 DAF). However, InVigor5020 seeds showed slightly lower germination only during the first harvest timing (33 DAF) but all seeds germinated when harvested at the later stages of crop maturity. The observed lower seed germination at early stage of crop maturity was due to the presence of dormancy in a portion of the seeds. The presence of primary dormancy was confirmed as most ungerminated seeds later germinated after the stratification treatment. The few remaining ungerminated seeds after the stratification treatment were found to be viable from the tetrazolium chloride test which also indicated the presence of dormancy. Lack of germination of a viable seed could be due to the presence of underdeveloped embryo, which is also a type of morphological dormancy (Baskin and Baskin, 2004). These results agree with the earlier findings by Gulden et al. (2004a), who reported primary dormancy range of 0 to 4% in mature seeds of 16 commercially available *B. napus* genotypes in western Canada. Other reports from the UK and China also indicated that there is little or no primary dormancy in freshly harvested mature *B. napus* seeds (Lutman, 1993; Pekrun et al., 1997a; López-Granados and Lutman, 1998; Pekrun et al., 1998; Momoh et al., 2002; Lutman et al., 2003). However, there has been no report on the presence of primary dormancy at early stages of crop maturity in western Canadian *B. napus* genotypes.

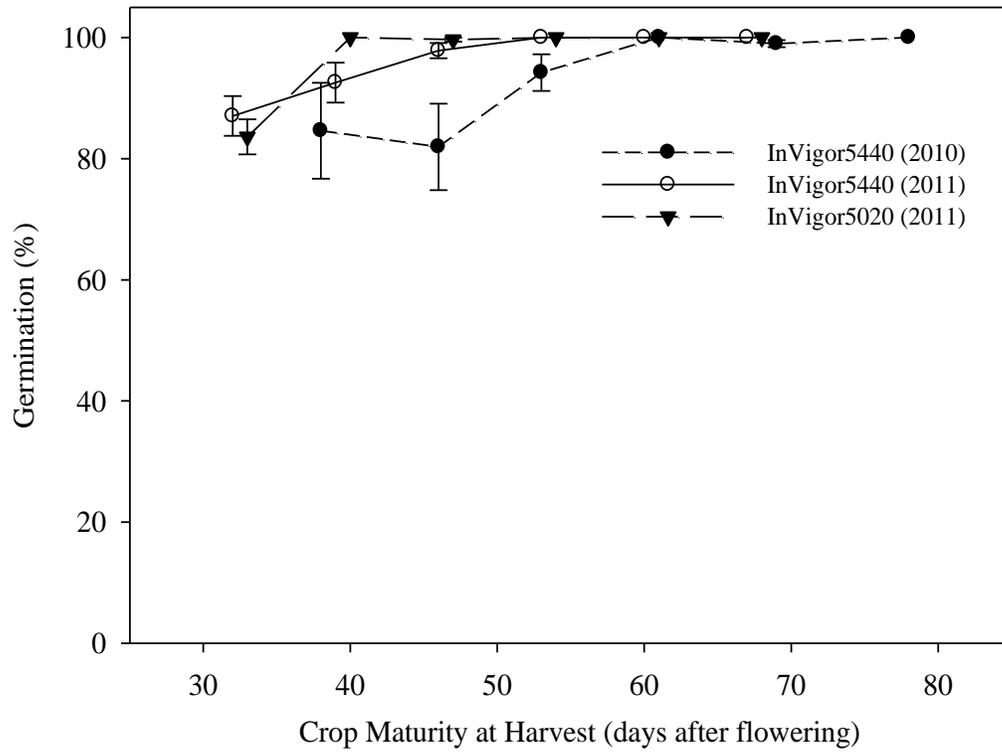


Figure 4.1 Germination of InVigor5440 and InVigor5020 seeds at different stages of crop maturity immediately after harvest. Each point is the mean of three replicates with bars indicating the mean standard error.

The presence of little primary dormancy in the seed at early stage of crop maturity in the evaluated *B. napus* genotypes may be linked to the action of ABA or simply due to the presence of underdeveloped embryo in a portion of seeds. The latter refers to morphological dormancy in which the embryo requires some time to fully mature and resume germination (Baskin and Baskin, 2004). During seed development there are changes in both ABA levels and sensitivity of the embryo to ABA in response to internal and external factors (Nambara et al., 2010). The effect of ABA in suppressing germination in *B. napus* seeds during the phase of rapid embryo growth preceding desiccation has been reported (Finkelstein et al., 1985). Juricic et al. (1995) also indicated a transient increase in endogenous ABA concentration around 30 days after pollination in three *B. napus* genotypes. Up to four-fold increase in endogenous ABA levels has also been reported in another *B. napus* genotype during the storage protein accumulation phase, which was 25 to 39 DAF, reaching peaks at 35 and 38 DAF (Finkelstein et al., 1985). In the present study, the first and second harvest timings (32 and 40 DAF) approximately coincide with this phase and that could be the reason for the observed little primary dormancy in a portion of seeds. The concentration of endogenous ABA and the sensitivity of the embryo to ABA have been shown to progressively decrease in *B. napus* up on seed development and maturation (Finkelstein et al., 1985; Juricic et al., 1995). Absence of primary dormancy in fully ripened seeds of the evaluated genotypes in the present study might also be due to reduced endogenous ABA or reduced sensitivity of the embryo to ABA.

4.3.2 Secondary dormancy potential in *B. napus*

The ability of seeds to be induced into secondary dormancy increased with later harvest timings in the evaluated *B. napus* genotypes. After dormancy induction, up to 90% of InVigor5440 seeds germinated at 32 DAF but only 6% of seeds germinated at 78 DAF (Figure 4.2). Similarly, 30% of InVigor5020 seeds germinated at 33 DAF but germination of seeds was reduced to 10% at 68 DAF (Figure 4.2). This indicates that seeds had lower potential to secondary dormancy at early stage of crop maturity but when seeds were harvested after maturing on the plant, they tended to have a higher potential to secondary dormancy induction (Figure 4.2). There was no difference between the regression model fitted to the individual years and the model fitted to the two years combined data for InVigor5440 genotype ($P = 0.2688$). Therefore, the model fitted to the two

years combined data was reported. A single line of best fit was then calculated using parameters from the combined model that predicted the parameters in both years (Figure 4.2). However, differences were observed between models fitted for InVigor5020 and InVigor5440 genotypes ($P = 0.0003$). The y-intercept (parameter a) was higher for InVigor5440 genotype ($P = 0.0106$). But the slope of decline in the germination of seeds (parameter b) was not different between the genotypes ($P = 0.0798$). This indicates that the potential for secondary dormancy induction differed between the evaluated *B. napus* genotypes. Compared to InVigor5440, the potential to secondary dormancy induction was lower in InVigor5020 at early stage of crop maturity (Figure 4.2). Genotypic differences in the potential for secondary dormancy induction were reported in *B. napus* in the UK (Pekrun et al., 1997a; Pekrun et al., 1998). A broad range in secondary seed dormancy expression was also observed among 16 commercially available western Canadian *B. napus* genotypes (Gulden et al., 2004a).

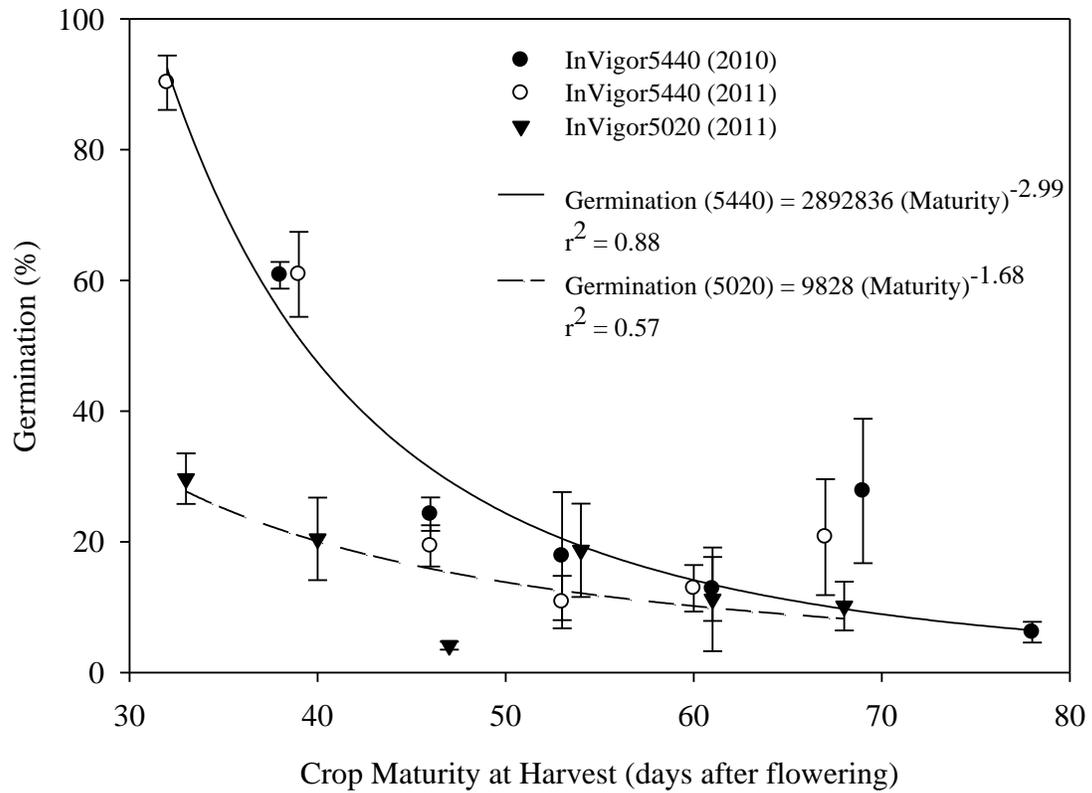


Figure 4.2 Germination of InVigor5440 and InVigor5020 seeds at different stages of crop maturity following dormancy induction. Each point is the mean of three replicates with bars indicating the mean standard error.

There is an association between the degree of secondary dormancy with ABA sensitivity, biosynthesis and accumulation in *B. napus* (Gulden et al., 2004b). Fei et al. (2009) indicated that the tendency of *Brassica* seeds to be induced into secondary dormancy may be determined by changes in gene expression related to carbohydrate metabolism, lipid biosynthesis, and storage protein accumulation that occur during late seed development. ABA has been indicated to regulate many of these important aspects of plant development such as the synthesis of seed storage proteins and lipids, and the promotion of seed desiccation tolerance (Leung and Giraudat, 1998; Finkelstein et al., 2002; Kermode, 2005).

Although not evaluated in this study, it has been indicated that ABA is involved in inducing secondary dormancy in Canola. The effect of PEG treatment on induction of secondary dormancy appeared to be due to ABA-related mechanism in *B. napus* (Gulden et al., 2004b; Fei et al., 2009). Gulden et al (2004b) indicated that there were similar levels of ABA in dry, untreated seed of high (LG3295) and low (Option 501) secondary dormancy potential *B. napus* genotypes. However, during PEG treatment endogenous levels of ABA remained the same in LG seeds but declined by 50% in Option 501 seeds (Gulden et al., 2004b). Moreover, incubating seeds of low and high dormancy genotypes in ABA inhibited germination to a similar degree but after PEG treatment seeds of Option became less sensitive to external application of ABA while LG seeds became more sensitive (Gulden et al., 2004b). In another study of high (AC Excel) and low (DH12075) secondary dormancy potential *B. napus* genotypes, Fei et al. (2009) indicated that mature seeds of AC Excel contained almost three-fold more ABA than DH12075 seeds. However, after 4 weeks of PEG treatment the level of ABA in AC Excel seeds further increased about three-fold but ABA level decreased in DH12075 seeds (Fei et al., 2009). This indicates that the mechanism of inhibition of germination following PEG treatment in *B. napus* seeds that have high potential for dormancy induction is due to increased ABA levels and increased sensitivity of the embryo to ABA.

The recommended time to windrow *B. napus* in western Canada is at 60% SCC on the main stem (Canola Council of Canada, 2012). In the present study, this time approximately coincided with the second stage of crop maturity at harvest (40 DAF) for InVigor5440 genotype and it was

associated with low potential for secondary dormancy induction in the seeds (Figure 4.2). Windrowing this genotype at the recommended time may reduce the persistence of seeds in the soil seedbank. Windrowing earlier than the recommended time further resulted in higher germination of seeds after dormancy induction (Figure 4.2). This indicates that seeds had lower potential to secondary dormancy induction and windrowing at this time may further reduce the persistence of seeds that enter the soil seedbank at early stage of maturity. However, windrowing *B. napus* earlier than the recommended time to minimize seedbank persistence may have negative effect on the yield and quality of the seed. Windrowing later than the recommended time or direct-harvesting was associated with high potential for secondary dormancy in mature InVigor5440 seeds. This may increase the persistence of seeds that enter the soil seedbank at the later stages of development. This study was conducted on seeds collected from the middle portion of the main stem and branches. However, canola has an indeterminate growth habit (Price et al., 1996; Gan et al., 2004) and seeds from different parts of the same plant can have different stages of maturity.

4.4. Conclusion

There was little primary dormancy in a portion of freshly harvested seed at early stage of crop maturity in the evaluated *B. napus* genotypes but at this stage the seeds had low potential for secondary dormancy induction. However, freshly harvested seeds from fully matured plants had no primary dormancy and could germinate immediately after harvest in favorable conditions but in case of unfavorable weather the seeds can become secondarily dormant and persist in the soil seedbank. The presence of variable potential for dormancy induction at different stages of crop maturity may be a mechanism that allows the evaluated genotypes to survive the severe winter in western Canada.

4.5 Prologue to chapter 5

The results from chapter 4 indicate that windrowing the evaluated *B. napus* genotypes at early stage of crop maturity reduced the potential for secondary dormancy induction in the seed (chapter 4). This might be important to reduce the long term persistence of seeds in the soil seedbank. However, windrowing canola increases the cost of production and may reduce the quality of the seed. Moreover, during strong winds the windrows may be blown resulting in significant amount of seed loss. Recent studies have shown that there are some variations for shatter resistance within canola genotypes. Other technologies such as pod sealant products may also reduce seed shatter when leaving canola in the field for an extended period. Growing genotypes having reduced seed shatter and use of pod sealant products might avoid the need to windrow canola. So, chapter 5 of this thesis presents the results of a study conducted to evaluate canola genotypes, harvest methods and pod sealant products to minimize seed loss and seedbank addition.

5. EVALUATING CANOLA HARVEST METHODS AND GENOTYPES TO REDUCE SEEDBANK ADDITION

5.1 Introduction

Silique shatter in canola (*B. napus*, *B. rapa* and *B. juncea*) results in significant yield loss and dispersal of seeds into the soil seedbank. Once the seeds enter the soil seedbank a portion can persist for many years creating volunteer weed problems for the following crops. Canola has an indeterminate growth habit and the upper siliques can still be immature when the lower siliques are mature and ready to be harvested (Price et al., 1996; Gan et al., 2004). The lack of developmental synchrony in canola leads to seed loss from the first formed siliques while later formed siliques still contain immature seeds (Morgan et al., 1998). Early harvesting to reduce seed loss can result in higher chlorophyll content in the seed whereas late harvesting can lead to higher seed loss.

There was little variation for silique shatter within the earlier *B. napus* lines (Brown et al., 1997; Morgan et al., 2000). However, it has been observed that *B. napus* lines derived from interspecific hybridization with other members of the *Brassica* have a wide variation in shatter resistance (Morgan et al., 1998; Summers et al., 2003; Child et al., 2003). In western Canada, Wang et al. (2007) evaluated 22 *B. napus* genotypes including commercially available open pollinated cultivars and hybrids and found variations in resistance for silique shatter within the tested genotypes. More than 99% of canola grown in western Canada is *B. napus* genotype because of its greater seed yield and presence of herbicide resistant traits (Canadian Grain Commission, 2008). *B. napus* and *B. rapa* have spring and winter cultivars. Winter cultivars are less prone to shattering than spring cultivars and are therefore better suited to direct-harvesting (Boyles et al., 2010). In the UK, Price et al. (1996) reported lower seed losses when winter canola was direct-harvested than when it is windrowed. Winter canola is widely grown in parts of Europe and Asia but it is not commercially grown in western Canada because of low cold hardiness and poor winter survival conditions (Canola Council of Canada, 2012).

Seed loss in canola can be influenced by the method and time of harvest (Pekrun et al., 1997a; Gan et al., 2008; Wang et al., 2007). Windrowing canola is the preferred harvest operation in western Canada as it is believed to hasten maturity and reduce seed loss due to shattering (Vera et al., 2007). However, in strong winds seed loss can be substantial in windrowed canola. Canola is usually direct-harvested in Europe and the Southern Great Plains of the United States (Boyles et al., 2010). Direct-harvesting is also practiced in western Canada as it reduces the cost of production and the chlorophyll content in the seed. It has been suggested that direct-harvesting works well when the crop matures evenly and crop density is uniform (Booth and Gunstone, 2004; Boyles et al., 2010). However, seed loss can be high when canola is direct-harvested, especially in adverse weather conditions.

Crop desiccants and pod sealant products may reduce seed loss when leaving canola in the field for direct-harvesting. The use of desiccants has been observed to hasten crop maturity and leads to more uniform ripening which in turn leads to the timely harvesting of canola especially in adverse weather conditions (Morgan et al., 1998). Pod sealant products are new technologies that may reduce seed shatter in the standing canola. Pod sealant products such as Pod-Stik[®] (United Agri-Products) and Pod Ceal DC[®] (Brett Young) have been available to Canadian growers since 2008. But the effectiveness of these polymers to reduce seed shatter in direct-harvested canola has not been evaluated in the Canadian prairies. Thus, the objective of this study was to evaluate canola genotypes, harvest methods and pod sealant products to reduce canola seedbank addition.

5.2 Materials and methods

5.2.1 Site and experimental description

This study was part of a larger trial that was conducted at five locations across Saskatchewan (Indian Head, Melfort, Swift Current, Scott and Saskatoon) for two years. The results reported here were from a study that was conducted at Kernen Crop Research Farm, Saskatoon, SK, (lat 59°09', long 106°33') in 2010 and 2011. In both years, the field experiment was set out in 60 plots, each plot having a double wide 6m X 2m area in a Randomized Complete Block Design (RCBD) with a split-plot arrangement. Genotypes were considered as whole-plots and harvest methods as sub-plots.

5.2.2 Design and treatments

The treatments were a factorial combination of 4 harvest methods and 5 canola genotypes for a total of 20 treatments. Each treatment was replicated 3 times. The harvest treatments were untreated direct-harvested, windrowed, Pod Ceal treated direct-harvested and Pod-Stik treated direct-harvested. The evaluated genotypes were InVigor5440 LL, RR4362, RR45H26, InVigor5020 LL and CL8571. InVigor5440 (Bayer Crop Science), RR4362 (Brett Young) and RR45H26 (Pioneer Hi-Bred) were chosen by the respective seed companies as their top recommended genotype for direct-harvesting. InVigor5020 was included because during the initiation of the study this hybrid was one of the standard genotypes which others are compared to in variety performance trials. EXCEED CL8571 (Viterra) is an imidazolinone resistant *B. juncea* genotype that has canola quality seed and is considered well suited to direct-harvesting.

5.2.3 Methodology

Seeding was done at a rate of 135 viable seeds m⁻² targeting 100 plants m⁻² for all genotypes. Canola was seeded in standing cereal stubble in both years. Plots were sown with a small plot seed drill at 20 cm row spacing. The seeds were buried in the soil at 2 cm depth. At the time of seeding fertilizer was band applied according to soil test recommendations (102 kg N ha⁻¹, 35 kg P₂O₅ ha⁻¹, 35 kg S ha⁻¹ in 2010 and 69 kg N ha⁻¹, 33 kg P₂O₅ ha⁻¹, 33 kg S ha⁻¹ in 2011).

Pests were controlled with the recommended practices in both years. Weeds were controlled by applying the respective herbicide to each canola genotype (i.e. Roundup for Roundup Ready genotypes, Liberty for InVigor genotypes and Odyssey for Clearfield genotype) at the recommended rate and growth stages by using a small plot sprayer. Volunteer canola plants were regularly hand weeded from the plots. Due to the presence of excessive moisture in 2010 (Table 5.2), the canola genotypes were infected with Sclerotinia Stem Rot (*Sclerotinia sclerotiorum*) and Alternaria black spot (*Alternaria spp.*). This infection was severe on InVigor5020 and RR4362 genotypes but no fungicide was applied to control these diseases as they were not identified until the late silique filling stage. In 2011, Decis (deltamethrin) was applied at a rate of 50 g a.i. ha⁻¹ at the three to four leaf stages to control flea beetles (*Phyllotreta spp.*).

Plots were windrowed at 50 to 60% SCC on the main stem. Pod Ceal DC[®] and Pod Stik[®] were applied at 30 to 40% SCC on the main stem. A solution volume of 225 L ha⁻¹ was applied using a small plot sprayer. At this stage the lower siliques were turning yellow but still pliable. The direct-harvested canola was desiccated in both years prior to harvest using Reglone at a rate of 415 g a.i. ha⁻¹ applied when approximately 80-90% of seeds on the main stem had turned brown. Dates of seeding and other agronomic operations are indicated in Table 5.1.

Table 5.1 Dates of selected agronomic practices for canola seedbank addition evaluation at Saskatoon.

Activity	Year	
	2010	2011
Seeding	May-18	May-11
Plant counts	June-26	June-6
Pod sealant application	Aug-16 (<i>B. napus</i>); Aug-31 (<i>B. juncea</i>)	Aug-7 (Inv5020, RR4362); Aug-11 (Inv5440, RR45H26, CL8571)
Plant and canopy height measurement	Aug-15	Aug-9 (Inv5020, RR4362); Aug-15 (Inv5440, RR45H26, CL8571)
Windrowing	Aug-20 (<i>B. napus</i>); Sep-3 (<i>B. juncea</i>)	Aug-9 (Inv5020, RR4362); Aug-15 (Inv5440, RR45H26, CL8571)
Seed loss measurement 1	Aug-23	Aug-16
Seed loss measurement 2	Sep-3	Aug-23
Seed loss measurement 3	Sep-23	Aug-27 (Direct-harvested plots)
Harvest	Sep-23	Aug-29 (Windrowed plots); Sep-7 (Direct-harvested plots)

5.2.4 Data collection

Plant density was calculated by counting a total of 2 m of crop rows from the front and back of each plot. Days to flowering was taken when approximately 50% of the plants were in bloom. Days to 60% seed color change was taken when approximately 60% of seeds from the siliques on the main stem changed their color to brown. Plant height and canopy height were measured at two random locations in each plot. Lodging index was calculated by dividing canopy height by the plant height. Grain yields were measured by taking the mass of clean seed harvested from each plot after the seeds were allowed to dry to constant moisture. TSW was calculated by taking the mass of 200 seeds and multiplying it by 5. Seed loss was measured using four mesh-lined catch trays in each plot. Each tray had a length and width of 1.2 m and 0.11 m respectively. Trays were inserted in between plant rows in the standing crop and beneath the ripening windrows from the front and back of each plot at early silique filling stage. Seed shatter and pod drop was believed to be similar between and within rows as the canopy closure was uniform after flowering. Trays were emptied three times starting from 50 to 60% SCC on the main stem until harvest. Seed loss measurements were conducted at weekly intervals except when it was too wet to complete the task in the field. The sample collected from the four trays was combined for each plot and then separated into dropped siliques and shattered seeds. Finally, shattered seeds and seeds from dropped siliques were combined for each treatment to determine the total seedbank addition per square meter.

5.2.5 Statistical analysis

Statistical analyses were performed using PROC MIXED procedure of SAS (SAS 9.2, SAS Institute Inc., Cary, NC, U.S.A) for a two way factorial RCBD design with split-plot arrangement. Natural log (\log_e) transformations were performed on all response variables except the grain yield to meet the assumptions of ANOVA. Data are presented in graphs using back transformed estimates and standard errors. Genotype, harvest method and the interaction between genotype and harvest method were considered fixed effects while year, block nested in year, the interaction of block with the main plot factor (genotype) and the interaction of year with genotype and harvest methods were considered random effects. The mixed model likelihood ratio test was used to estimate the variance components as it is more accurate than the default variance component estimation (Restricted Maximum Likelihood method (REML)) for

small sample size (Littell et al., 1996; Littell et al., 2002). The DDFM=Kr option was used for approximating the degrees of freedom for means. Treatments were compared using Fisher's Protected Least Significant Difference (LSD) method. A 5% significance level was used to determine the role of the fixed effects and their interaction.

5.3 Results and discussion

The rainfall amount in 2010 was higher than the 30 year average except in July where it was lower than the 30 year average (Table 5.2). The total rainfall in 2010 was 134 and 112% higher than the rainfall in 2011 and the 30 year average, respectively (Table 5.2). The presence of high moisture during August and September in 2010 led to the incidence of disease in late silique filling stage. The temperature in both years was more or less similar to the 30 years average except in September 2011 where it was warmer (Table 5.2). The dry and hot weather in September 2011 hastened drying of the canola crop.

The analysis of variance for the two years combined data indicated that there was no effect of genotype on the seed yield, yield loss and seedbank addition in canola (Table 5.3). However, there was an interaction of year by genotype for all response variables indicating that seed yield, seedbank addition and yield loss of the genotypes differed by year. There was no effect of harvest method on seed yield but seedbank addition and yield loss differed by harvest method for the combined analysis of data (Table 5.3). There was also genotype by harvest interaction for seedbank addition from shattered seeds in both 2010 and 2011. There was no interaction of year by harvest method indicating that the effect of harvest method on seed yield, yield loss and seedbank addition did not differ by year (Table 5.3).

Table 5.2 Monthly rainfall (mm) and mean daily temperature (C) for Saskatoon, Saskatchewan from May until September in 2010 and 2011 and the climate normals (30-yr average).

Location	Month	Rainfall			Temperature		
		2010	2011	Normal†	2010	2011	Normal†
		(mm)			(C)		
Kernen	May	128.5	17.5	41.5	9.7	10.9	11.8
	June	169.0	94.4	60.5	15.3	15.5	16.0
	July	46.0	68.6	57.3	17.6	18.4	18.3
	August	43.7	16.5	35.4	16.2	17.2	17.6
	September	87.9	6.0	28.9	10.5	14.7	11.5
	Total		475.1	203.0	223.6	-	-

† 1970-2000 Canadian Climate Normal's obtained from Environment Canada, 2012.

Table 5.3 *P*-values from mixed model ANOVA F-test for seed yield, seedbank addition from dropped siliques, seedbank addition from shattered seeds, total seedbank addition, and yield loss as affected by genotype and harvest method assessed at Kernan in 2010 and 2011.

	Seed yield	Seedbank addition from dropped siliques	Seedbank addition from shattered seed	Total seedbank addition	Yield loss
	(kg ha ⁻¹)	-----	(number m ⁻²)	-----	(%)
Genotype (G)	0.1856	0.6871	0.4788	0.5636	0.2282
Harvest (H)	0.065	0.0023**	<0.0001***	0.0002***	<0.0001***
G*H	0.2	0.5995	0.2115	0.0741	0.1721
Block (Year)	0.2659	0.1773	0.3411	0.1103	0.3134
Year	0.0305*	0.2467	0.4133	0.2362	0.2893
Block*G	0.4093	0.1115	0.1586	0.224	0.2287
Year*G	0.00005***	<0.0001***	0.00002***	0.00001***	<0.0001***
Year*H	0.0824	0.1958	0.3032	0.3411	0.2033
Year*G*H	0.5	0.1029	0.0113*	0.2314	0.0961

*, **, *** denote significance at the 0.05, 0.01 and 0.001 probability levels, respectively.

5.3.1 Plant density, days to flowering, days to 60% seed color change and lodging index

There was no statistically significant difference in plant density ($P = 0.324$), days to flowering ($P = 0.837$), days to 60% SCC on the main stem ($P = 0.463$) and lodging index ($P = 0.274$) among the genotypes. Lack of difference in these factors indicates that the observed differences in seed yield and seedbank addition among the genotypes were not affected by these traits.

5.3.2 Seed yield

There was no effect of harvest method on canola seed yield (Table 5.3). A lack of difference in seed yield among direct-harvested canola indicates that there was no effect of applying pod sealant products on the yield of canola. However, seed yield of canola was variable among the genotypes in each year (Figure 5.1). The excessive moisture and observed disease incidence contributed to the reduced yield of canola genotypes in 2010. RR4362 was the least yielding genotype in both years while InVigor5440 was the highest yielding genotype in 2011 (Figure 5.1). The lowest yield of RR4362 in 2010 could be due to the severe disease incidence on this genotype. The results of this study showed that the *B. juncea* genotype (CL8571) had higher or similar seed yield to the *B. napus* genotypes indicating that there is a potential for the production of this genotype in western Canada.

5.3.3 Seedbank addition from dropped siliques

There was considerable seedbank addition of canola from dropped siliques (Figure 5.2). Windrowing canola resulted in lower seedbank addition from dropped siliques than direct-harvesting. This indicates that windrowing canola reduced exposure of siliques to strong winds that might lead to silique drop. There was no difference in seedbank addition from dropped siliques among the direct-harvested treatments (Figure 5.2A). Seedbank addition from dropped siliques, however, varied among the genotypes in both 2010 and 2011 (Figure 5.2B). InVigor5440 consistently had lower seedbank addition in both years (Figure 5.2B). Seedbank addition was not consistent for RR4362 and CL8571 genotypes between the years. In 2011, CL8571 had the lowest seedbank addition from dropped siliques (Figure 5.2B).

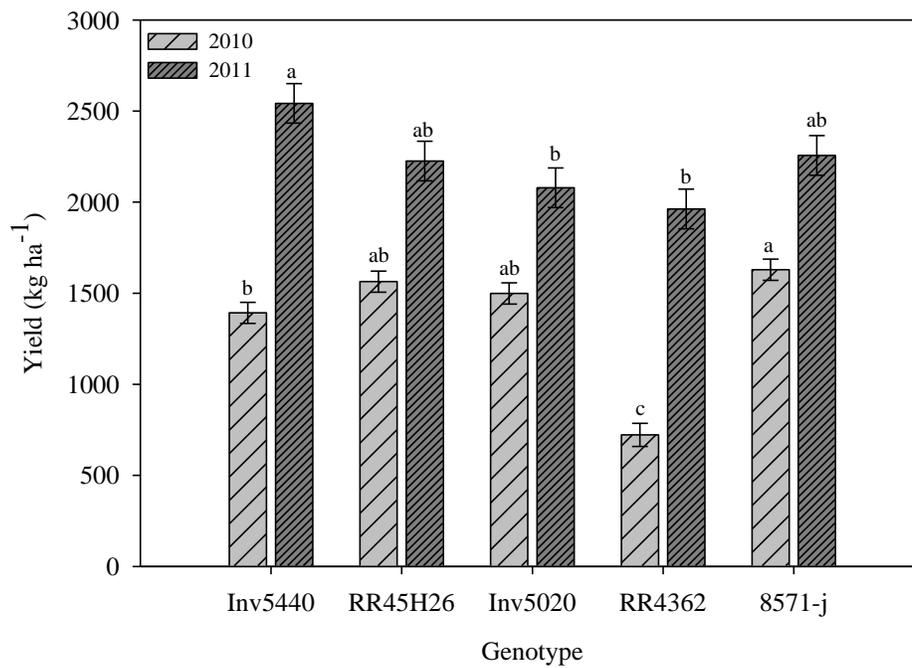


Figure 5.1 Seed yield of canola genotypes. Error bars represent the mean standard error and comparisons were made within the same year. Genotypes with similar letters were not significantly different at $P < 0.05$ significance levels.

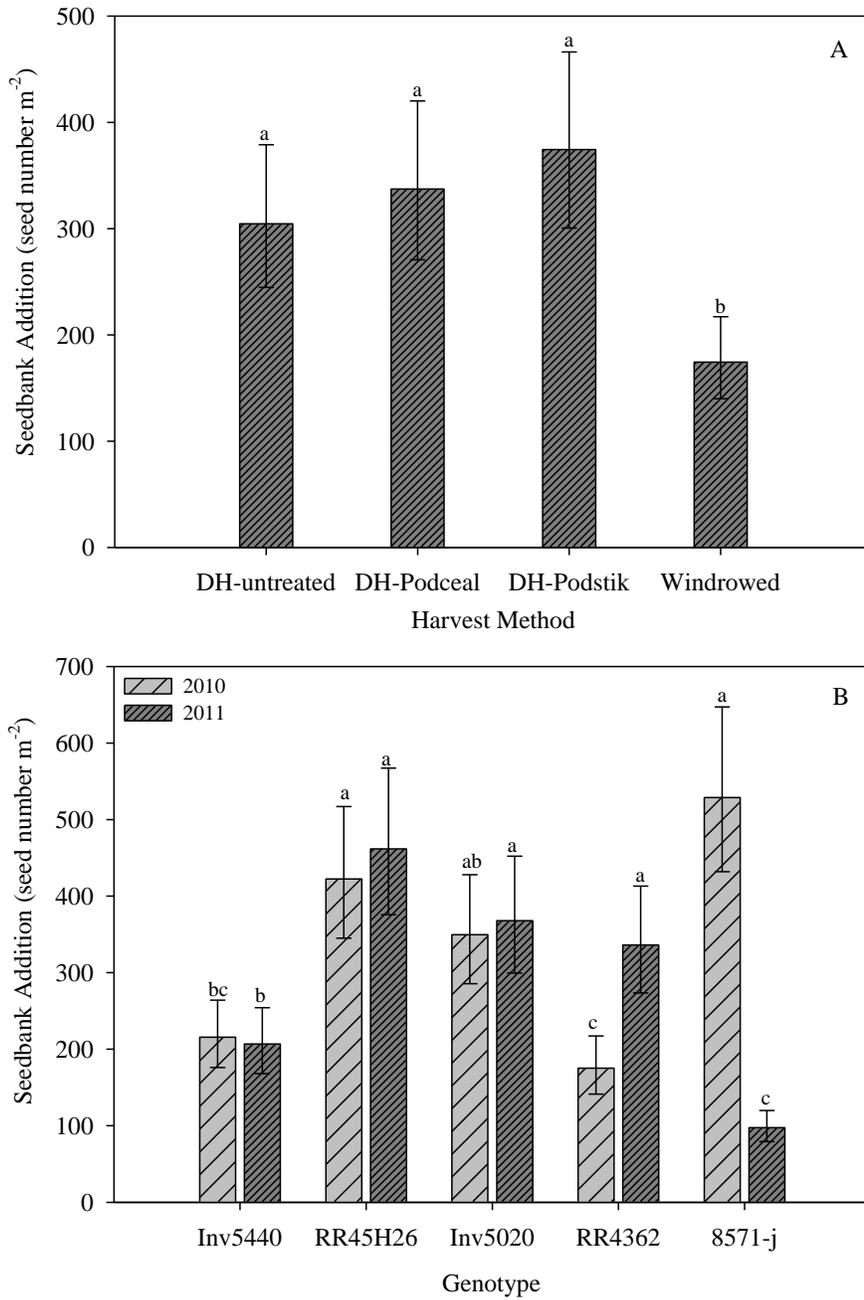


Figure 5.2 Seedbank addition from dropped siliques for canola harvest methods (A) and genotypes (B). Error bars represent the mean standard error and comparisons were made within the same year. Genotypes and harvest methods with similar letters were not significantly different at $P < 0.05$ significance level. DH stands for direct-harvested treatments.

5.3.4 Seedbank addition from shattered seeds

There was significant three way interaction of year with genotype and harvest method for canola seedbank addition from shattered seeds ($P = 0.0113$) (Table 5.3). InVigor5020 had higher seedbank addition from shattered seeds in all harvest methods in both 2010 and 2011 (Figure 5.3A and B). In 2010, CL8571 had higher seedbank addition from shattered seeds when directly harvested but windrowing reduced seed shatter (Figure 5.3A). Whereas in 2011, RR4362 had the highest seedbank addition in all harvest methods (Figure 5.3B). Seedbank addition of InVigor5020 and RR4362 genotypes was also higher when windrowed compared to the direct-harvested treatments in both years indicating that they are more suited for direct-harvesting (Figure 5.3A and B). Lack of difference in seed shatter of the canola genotypes among the direct-harvested treatments indicates that there was no effect of applying Pod-Stik and Pod Ceal DC to minimize seedbank addition in canola (Figure 5.3A).

5.3.5 Total seedbank addition

There was significant effect of harvest method on total seedbank addition (Table 5.3). Total seedbank addition was the sum of shattered seeds and seeds from dropped siliques. Total seedbank addition was high when the canola was windrowed but it was significantly low in direct-harvested canola (Figure 5.4A). But there was no difference in total seedbank addition among the direct-harvested treatments. Although seedbank addition from dropped siliques was higher in direct-harvested canola, the observed low total seedbank addition could be due to the reduced seed shatter of the canola genotypes in the direct-harvested methods (Figure 5.2A, 5.3 and 5.4A). There was also an interaction of year by genotype for total seedbank addition indicating that total seedbank addition differed by year for the genotypes (Table 5.3). InVigor5440 and RR45H26 consistently had lower total seedbank addition than InVigor5020 genotype (Figure 5.4B). However, total seedbank addition was not consistent between years for RR4362 and CL8571 genotypes. In 2011 when it was relatively hot and dry towards maturity, CL8571 genotype had the least total seedbank addition (Figure 5.4B).

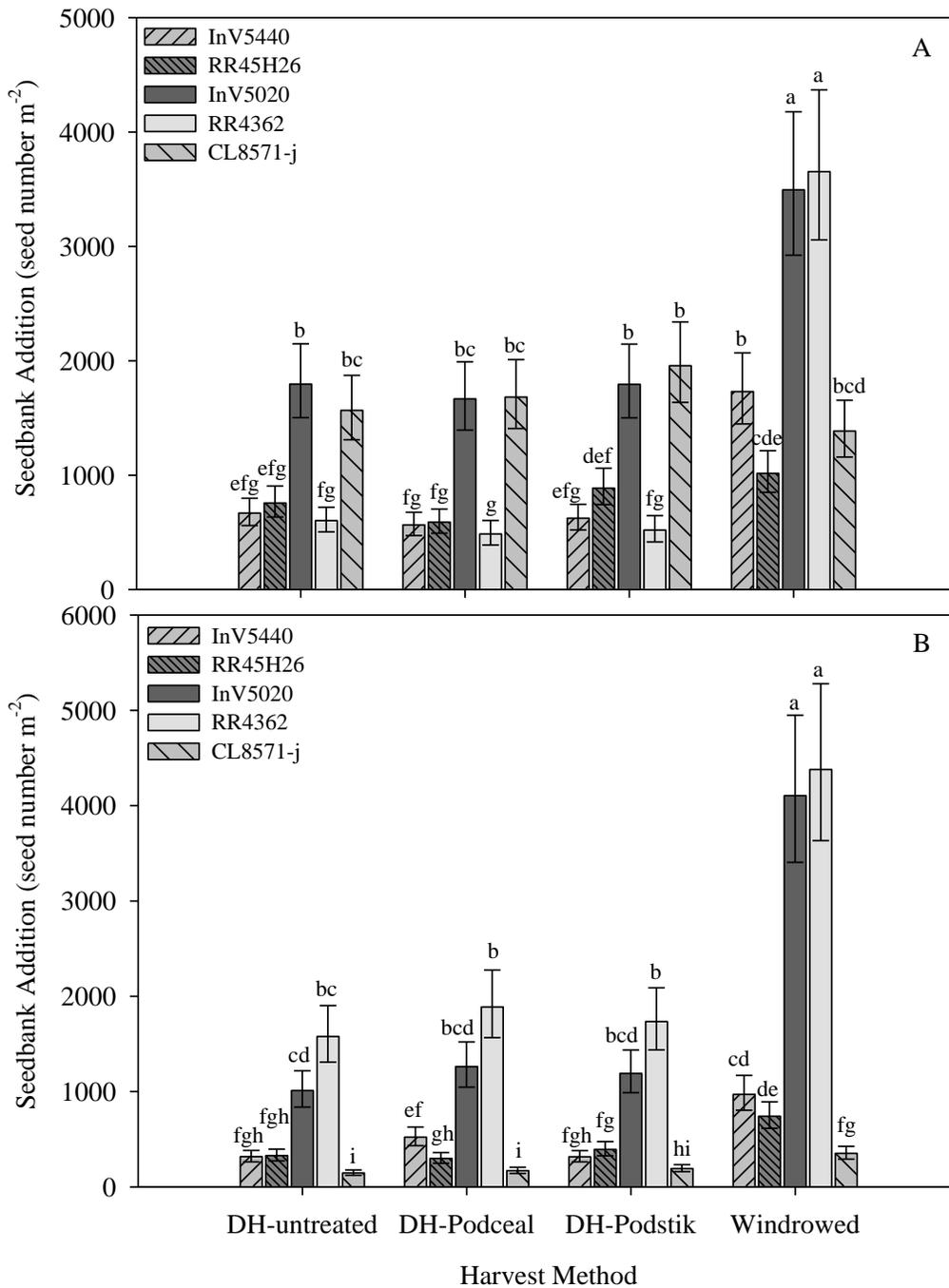


Figure 5.3 Seedbank addition from shattered seeds for the interactions of canola genotype by harvest method in 2010 (A) and 2011 (B). Error bars represent the mean standard error and comparisons were made within the same year. Genotypes with similar letters were not significantly different at $P < 0.05$ significance level.

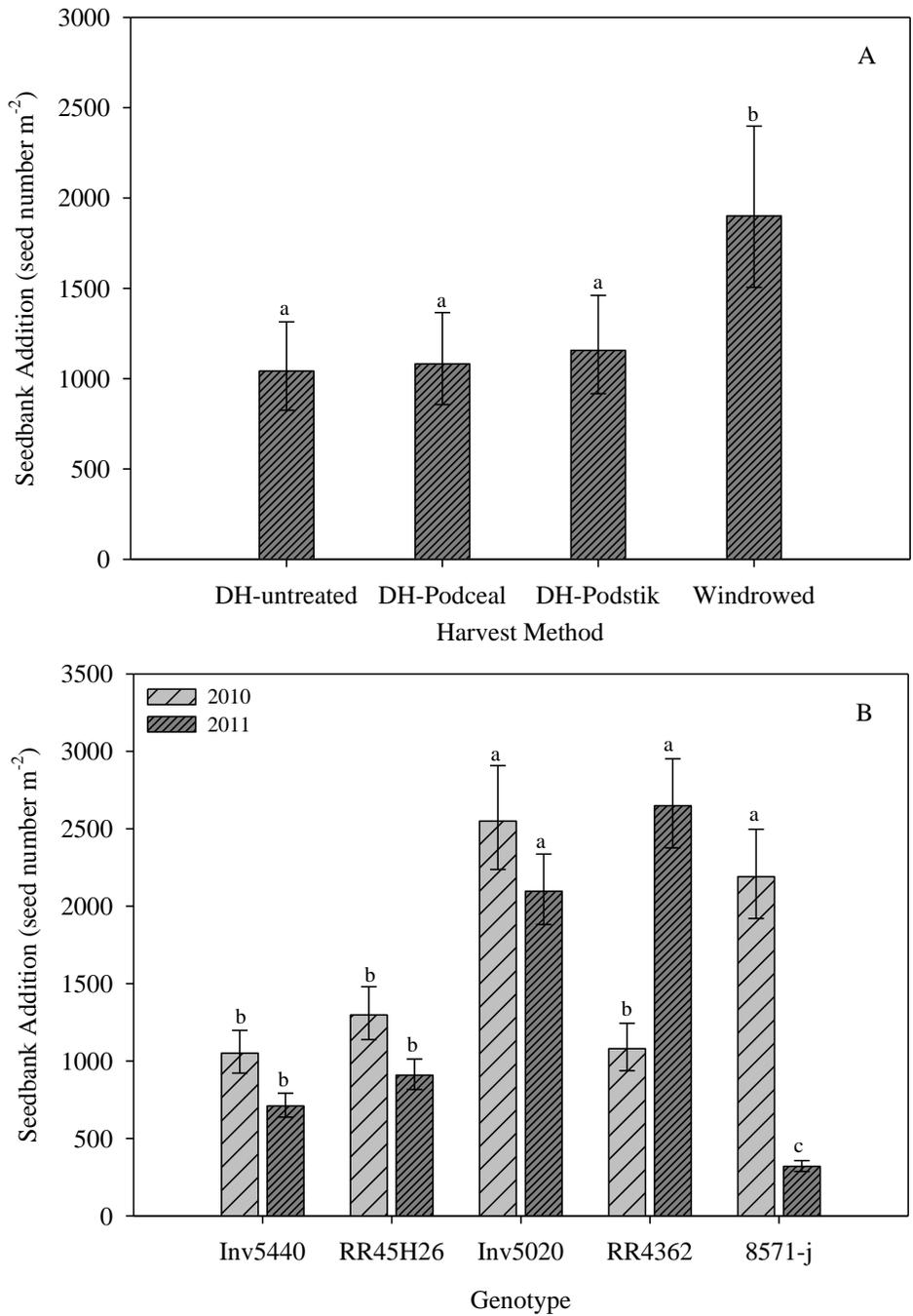


Figure 5.4 Total seedbank addition for canola harvest method (A) and genotypes (B). Error bars represent the mean standard error and comparisons were made within the same year. Genotypes and harvest methods with similar letters were not significantly different at $P < 0.05$ significance level.

5.3.6 Percent yield loss

Percent yield loss is the proportion of seed loss out of the total seed yield. Percent yield loss for canola harvest methods and genotypes followed similar trend to the total seedbank addition. Windrowing canola resulted in higher yield loss than direct-harvesting (Figure 5.5A). This result is similar to those reported by Gan et al. (2008) who indicated that windrowing crucifer species resulted in greater yield loss than direct-harvesting. Lack of difference in seed yield and seed loss among direct-harvested treatments resulted in the absence of any difference in percent yield loss among these harvest methods (Figure 5.5A). Percent yield loss also differed among canola genotypes in both 2010 and 2011. Yield loss was significantly lower for InVigor5440 and RR45H26 genotypes while it was higher for InVigor5020 and RR4362 genotypes in both years (Figure 5.5B). Similar to the total seedbank addition, the CL8571 genotype had the lowest percent yield loss in 2011 where relatively hot and dry weather conditions occurred (Figure 5.5B).

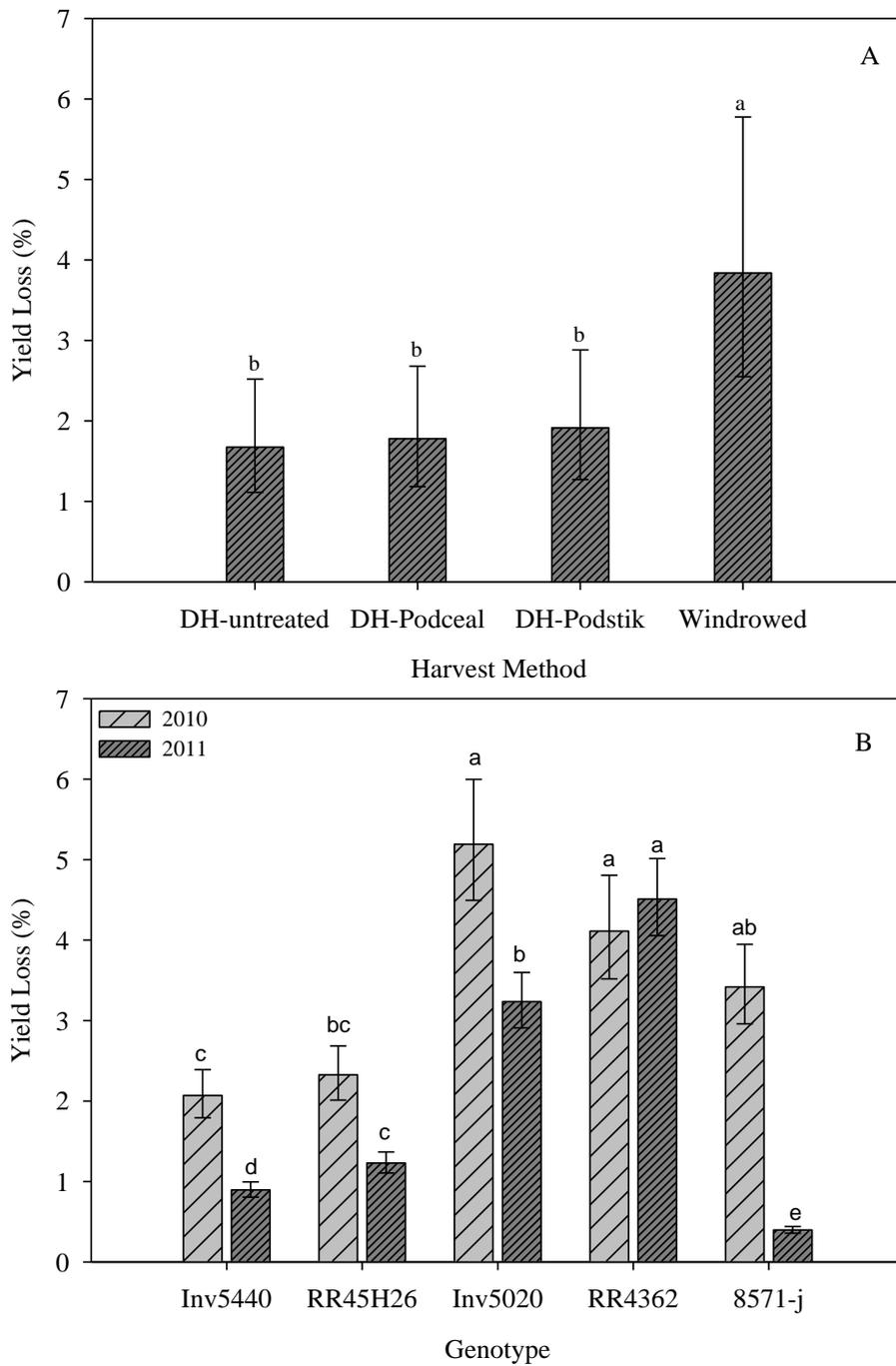


Figure 5.5 Percent yield loss for canola harvest methods (A) and genotypes (B). Error bars represent the mean standard error and comparisons were made within the same year. Genotypes and harvest methods with similar letters were not significantly different at $P < 0.05$ significance level.

5.4 Conclusion

In summary, there were differences in seedbank addition among the evaluated western Canadian canola genotypes. InVigor5440 and RR45H26 genotypes relatively had lower seedbank addition. InVigor5020 consistently had higher seedbank addition in all harvest methods indicating that it is the most susceptible genotype for seed loss. RR4362 genotype is least suitable for windrowing while CL8571 had the lowest seedbank addition in 2011 where the weather was relatively hot and dry. The results of this study showed that there were differences in seed loss among the evaluated genotypes. Growing genotypes with lower seed loss can be a strategy to minimize seedbank addition and the resulting volunteer canola. However, in adverse weather conditions and in the presence of severe disease incidence, both the yield and seedbank addition of canola can be highly affected and appreciable differences could not be detected.

This study showed that direct-harvesting canola resulted in lower seedbank addition than windrowing. The evaluated pod sealant products, however, did not have effect on the yield as well as seed shatter when canola was direct-harvested. The results of this study suggest that canola seedbank addition can be minimized by growing genotypes having increased shatter resistance and with the adoption of direct-harvesting operations.

6. GENERAL DISCUSSION

6.1 Canola seedbank addition

The primary objective of the study conducted on commercial canola farms across Saskatchewan was to estimate seedbank addition of canola between windrowing and direct-harvesting operations. The results suggest that seed loss in canola is substantial in both harvest methods. Seed loss of this magnitude will result in considerable yield loss and the problem of volunteer canola in following crops. Compared to the previously reported value by Gulden et al. (2003a) seedbank addition observed in this study has increased by 94%. Large proportion of these seeds may germinate in the fall if retained on the soil surface when there is sufficient moisture (López-Granados and Lutman, 1998). This can reduce seedbank addition as the germinated plants will die in the winter. Moreover, seed predation by invertebrates such as ground beetles (Honek and Martinkova, 2001; Honek et al., 2003) or seed mortality due to other biotic or abiotic factors can reduce canola seedbank addition. However, a significant proportion of viable seeds can enter into the soil seedbank. Once seeds reach the soil seedbank they may germinate and emerge, may germinate and die, remain dormant or may die as a result of fungal attack or other causes (Lutman, 1993). Those buried dormant seeds can persist for many years resulting in volunteer weed problems even without further replenishment of seed from escaped volunteers.

This study is the first to compare yield loss and seedbank addition of canola between windrowing and direct-harvesting operations on commercial farms in western Canada. The results suggest that there was no difference in canola seed loss and seedbank addition between the harvest methods and among the producers. This may suggest that similar magnitude of seed loss and seedbank additions are expected among Saskatchewan canola producers. Lack of difference in seed loss between windrowed and direct-harvested canola indicates that with the current harvest management practices windrowing may not be important to reduce seedbank addition in canola. The difference in seedbank addition between the years, however, indicates that seed loss in canola is influenced by the weather condition at or prior to harvest.

The second objective of the study on commercial canola farms was to determine agronomic and harvest-related factors that contribute to seed loss in canola. Results showed that total canola farm area, the herbicide and seed system used, combine separator type and total yield had an effect on canola seed loss. There was a positive correlation of seed loss with total canola farm area ($P = 0.019$). This indicates that producers having large canola farm area experience more seed loss than producers having small canola farm area. This may be due to delay in harvest operations as more time may be needed to windrow and combine the crop on large farms. The positive relationship of seed loss in canola with total yield suggests that increased canola seedbank addition is inevitable with the introduction of high yielding canola genotypes into the market. Crop improvement programs in canola that focus on increasing yield by increasing seed number and weight may confer a fitness advantage for the volunteer population as they might increase seedling numbers and vigor, respectively (Hall et al., 2005). Combining canola with a conventional combine harvester resulted in higher seed loss than combining with a rotary combine harvester. Higher seed loss was observed in Roundup Ready genotypes compared to the Liberty Link genotypes. Therefore, it is possible to minimize canola seedbank addition by growing genotypes with reduced seed loss and combining using a rotary combine harvester. The observed lower seedbank addition in Liberty Link genotypes may be due to InVigor5440 genotype which constituted 58% of the Liberty Link group (Table A2.5). This particular genotype was also found to have lower seedbank addition in the small plot study conducted at Kernen Crop Research Farm (Chapter 5). The results of this study suggest that the increased incidence of volunteer canola in western Canada may partly be due to the increase in seeded area and the increase in yield of the current canola genotypes.

The objective of the small plot study was to evaluate canola genotypes, harvest methods and pod sealant products to reduce canola seedbank addition. There were differences in seedbank addition among the evaluated canola genotypes with InVigor5440 and RR45H26 genotypes consistently having lower seedbank addition in all harvest methods. Growing these genotypes can minimize seedbank addition and the incidence of volunteer canola in western Canada. Growing genotypes with reduced seed loss can also avoid the need to windrow canola at early stage of maturity. This indirectly reduces the cost of production and the chlorophyll content in the seed. Pod sealant products did not have effects on either yield or seed shatter in canola under

Canadian Prairie conditions. Direct-harvesting canola resulted in lower seedbank addition than windrowing in both years. However, on commercial farms there was no difference in seedbank addition between the harvest methods. The observed differences of the results from the two studies could be due to the difference in the harvest equipment used or the sampling methods. It may also be due to the small sample size to detect the variability on commercial fields. The results from these studies suggest that direct-harvesting can be a viable option to harvest canola in western Canada. Adoption of direct-harvesting practices to minimize harvest loss in crucifer crops has also been suggested from a small plot study conducted at three sites in western Canada (Gan et al., 2008).

6.2 Seed dormancy in canola

The objective of the dormancy study was to determine the effect of crop maturity at harvest on potential to develop seed dormancy in canola. In the evaluated *B. napus* genotypes, little primary dormancy was observed in a portion of seeds when the crop was harvested at earlier stage of maturity but there was no primary dormancy in the seed when the crop was harvested at maturity (Figure 4.1). After dormancy induction; however, the *B. napus* seeds showed low potential for secondary dormancy at an early stage of crop maturity. But when seeds were allowed to mature on the plant they had high potential for secondary dormancy induction. There were differences in the potential to secondary dormancy induction between the evaluated genotypes. Earlier studies have also reported differences in the potential to secondary dormancy in the European, Chinese and western Canadian *B. napus* genotypes (Pekrun et al., 1997a; Momoh et al., 2002; Gulden et al., 2004a). For InVigor5440 genotype, the recommended time of windrowing (60% SCC on the main stem) was approximately at 40 DAF. At this time approximately 60% of seeds germinated after dormancy induction but only 6% of seeds germination at full maturity (78 DAF) (Figure 4.2). This suggests that windrowing this genotype at the recommended time may reduce the persistence of seeds in the soil seedbank compared to windrowing later or direct-harvesting. However, this may not always be the case as the seeds from the first formed pods at the bottom of the main stem may be ripe at the recommended time of windrowing and can have high potential to secondary dormancy induction. In InVigor5020, 30% of seeds germinated at early stage of maturity but only 10% of fully ripened seeds germinated following dormancy induction.

This indicates that the ability of InVigor5020 seeds to be induced to secondary dormancy also gradually increased with maturity.

During canola seed development, the change in secondary dormancy potential may be due to the change in ABA content and sensitivity (Nambara et al., 2010). Although not evaluated in this study, others have implicated ABA involvement in secondary dormancy in Canola. Zhang et al. (2010) found higher levels of ABA in ungerminated than germinated *B. napus* seeds indicating that endogenous ABA is associated with seed dormancy. The effect of PEG treatment on induction of secondary dormancy appeared to be due to ABA-related mechanism in *B. napus* (Gulden et al., 2004b; Fei et al., 2009). Studies have shown that following PEG treatment, both the ABA level and sensitivity of the embryo to ABA have either increased or remained the same in high dormancy potential *B. napus* genotypes (Gulden et al., 2004b; Fei et al., 2009). However, ABA level and sensitivity of the embryo to ABA gradually decreased in low dormancy potential *B. napus* genotypes (Gulden et al., 2004b; Fei et al., 2009). This indicates that the mechanism of inhibition of germination following PEG treatment in high dormancy potential *B. napus* seeds is either due to increased ABA levels or reduced sensitivity of the embryo to ABA.

Overall, these studies attempted to evaluate seedbank addition of canola genotypes and harvest methods in commercial canola fields (Chapter 3) and on the small plot study (Chapter 5). The dormancy study (Chapter 4) also evaluated the effect of harvest methods and genotypic differences on dormancy induction in *B. napus* genotypes. Results indicate that canola seedbank addition and dormancy induction in the seed are affected by the harvest methods and genotypic differences; hence, the main hypothesis of this thesis that canola seedbank addition and dormancy induction in the seed are affected by genotypes and harvest methods is accepted.

6.3 Management implications

Several recommendations can be extracted from this study with respect to managing volunteer canola. With some of the recently introduced canola genotypes having reduced seed loss, genotype selection can be a good strategy to be considered to minimize canola seedbank addition. Among the few canola genotypes evaluated in the small plot study, InVigor5440 and RR45H26 were found to have lower seed loss than the other genotypes. Similarly, InVigor5440

was the major component of the Liberty Link group which was found to have lower seed loss from the study on commercial canola farms. Therefore, growing these genotypes may reduce seedbank addition and the incidence of volunteer canola in western Canada. However, it is imperative to evaluate more canola genotypes for reduced seed shatter at different sites as this can help producers to choose genotypes with reduced seed loss.

Harvest methods were also found to have effects on seed loss of canola genotypes. The results of the small plot study showed that windrowing canola resulted in lower seed yield and higher yield loss than direct-harvesting. On commercial farms there was no difference in seed loss between windrowing and direct-harvesting operations. These results suggest that direct-harvesting can be a feasible strategy in reducing seed loss and seedbank addition of canola in western Canada. However, producers should be cautious when leaving canola in the field for direct-harvesting as large seed losses have been observed when high velocity winds hit a ripe standing crop. On the other hand, windrowing at a later stage of maturity than the recommended time or direct-harvesting canola may result in higher potential for secondary dormancy induction in the seed. There is no need to windrow canola if growing shatter resistant genotypes. But in case where growing shatter resistant genotypes is not practical, the choice between windrowing and direct-harvesting operations becomes a compromise between high yield loss and the long term persistence of seeds in the soil seedbank, respectively.

6.4 Future research

These studies have provided insights on the magnitude of seed loss in canola and the measures to be taken to minimize canola seedbank addition and longevity. The study conducted on commercial farms sampled small number of direct-harvested fields compared to windrowed fields. However, this study is part of a larger study that was conducted in Alberta Saskatchewan and Manitoba for three years. More canola fields were sampled in the last year of the study in Saskatchewan as well as from the other provinces and the combined analysis of data will provide a complete understanding on the magnitude of canola seedbank addition and agronomic- or harvest-related factors that may affect seed loss in canola. Moreover, combined analysis of data will provide insight on whether canola seed loss is affected by agro-ecological differences of the major canola growing regions of the Canadian prairies.

The small plot study that was conducted to evaluate canola genotypes and harvest methods to reduce seedbank addition only evaluated five genotypes. There are many canola genotypes available in the market in western Canada. Furthermore, there is a rapid turnover in canola genotypes. InVigor5440, which is one of the low shatter genotype identified in this study, has been withdrawn from the market shortly after the beginning of the study. Therefore, more genotypes need to be assessed for seed yield, seedbank addition and the potential for dormancy induction in the seed. Moreover, it is important to evaluate seedbank addition and yield difference between windrowing and direct-harvesting operations in multiple locations as these traits can be influenced by the weather conditions. The dormancy study was also conducted using two *B. napus* genotypes in two harvest seasons. More canola genotypes need to be evaluated in different site-years as dormancy induction is also influenced by the environment during seed development (Gulden et al., 2004a). However, the results of this study suggest that there are appreciable differences in seed loss and secondary dormancy potential among the evaluated canola genotypes. The western Canadian canola industry needs to focus on these problems and plant breeding programs need to incorporate objectives to breed shatter resistant and low dormancy genotypes. This will help to reduce loss of significant portion of revenue and the cost of controlling volunteer canola in the subsequent crops.

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APPENDICES

Appendix 1. Canola seed loss survey questionnaire

Canola seed loss survey questionnaire to be filled out for each field. The purpose of this survey is to gather information about agronomic- and harvest-related activities that may have effect on seed loss in canola. Please answer as many questions as possible and fax it to Teketel Haile, Department of Plant Sciences, University of Saskatchewan. Fax no: (306) 966 5015.

1. Identification and general info:

Producer Name: _____

Contact Information: _____

Producer Code (e.g. WPG 01): _____

Sampling year: _____

Farm size (total cropped acres): _____

Total canola farm area (acres): _____

2. Field and year specific information:

Field location (sec tsp range): _____

Field size (acres): _____

Canola variety _____

Seeding rate (lbs/acre): _____

Nitrogen fertility (lbs/acre): _____

Fungicide applications: _____

Year of last canola crop: _____

If important, last spring / first fall hard frost: _____

3. Harvest questions:

Harvest date: _____

Canola yield (bu/acre): _____

Harvest method (swathed / straight cut): _____

Pod sealing product applied (name): _____

3.1. Swathed:

Swathing date: _____

Crop maturity (% color change): _____

Time of day (morning / mid-day / evening): _____

Average swathing speed (mph): _____

Swather width (ft.): _____

3.2. Swathed and straight cut:

Combine type (conventional / rotary): _____

Combine model: _____

Combine speed (mph): _____

Time of harvest (morning / mid-day / evening): _____

Combine settings (pickup / reel speed, wind speed): _____

4. Other important remarks:

e.g. disease / insect infestations, noteworthy
 weather (hail) / harvest issues (note dates)

Appendix 2. Responses of the canola seed loss survey

Table A2.1 Identification and general information: total farm size of the producer.

Total farm size (ac)	All fields
100 - 2,000	23
2,100 - 4,000	16
4,100 - 6,000	-
6,100 - 8,000	8
8,100 - 10,000	6
> 10,000	6
No response	7

Table A2.2 Identification and general information: total canola farm area of the producer.

Total canola area (ac)	All fields
100 - 1,000	32
1,100 - 2,000	7
2,100 - 3,000	11
3,100 - 4,000	3
4,100 - 5,000	6
No response	7

Table A2.3 Field and year specific information: area of surveyed fields.

Area of sampled field (ac)	All fields
1 - 100	27
101 - 200	19
201 - 300	16
301 - 400	2
401 - 500	-
501 - 600	1
601 - 700	1

Table A2.4 Field and year specific information: year of last canola crop on surveyed fields.

Year of last canola crop in 2010 survey	All fields
2008	9
2007	12
2006	4
2005	1
Year of last canola crop in 2011 survey	All fields
2009	7
2008	7
2007	9
2006	1
Before 2005	7
Never	5
No response	4

Table A2.5 Field and year specific information: canola genotype grown.

Canola genotype grown	All fields
Dekalb 72-65 RR	3
InVigor 5030 LL	2
InVigor 5440 LL	26
InVigor5770 LL	6
InVigor 9590 LL	3
InVigor L130 LL	2
InVigor L150 LL	6
Pioneer 45H28 RR	3
Pioneer D3150 RR	2
Victory 1037 RR	5
Victory 1040 RR	4
Viterra VT 500 RR	1
Viterra VT Barrier RR	1
YN-429 (yellow seeded <i>B. napus</i>)	2

Table A2.6 Field and year specific information: seeding rate.

Seeding rate (lbs/ac)	All fields
3 - 4	7
4.1 - 5	47
5.1 - 6	10
6.1 - 7	2

Table A2.7 Field and year specific information: nitrogen rate.

Nitrogen applied (lbs/ac)	All fields
None	3
1 - 50	2
51 - 100	48
101 - 150	11
151 - 200	2

Table A2.8 Harvest questions: harvest method.

Harvest method	All fields
Windrowed	51
Direct-harvested	15

Table A2.9 Harvest questions: time of windrowing.

Time of windrowing	All fields
Morning	8
Mid-day	10
Afternoon	2
Evening	8
All-day	20
No response	18

Table A2.10 Harvest questions: percent seed color change at windrowing.

Seed color change on the main stem at the time of windrowing (%)	All fields
20 - 30	4
31 - 40	12
41 - 50	15
51 - 60	12
61 - 70	-
71 - 80	1
80 - 90	3
No response	19

Table A2.11 Harvest questions: average windrowing speed.

Windrowing speed (mph)	All fields
2 - 3	2
3.1 - 4	2
4.1 - 5	20
5.1 - 6	20
6.1 - 7	5
7.1 - 8	-
8.1 - 9	2
No response	15

Table A2.12 Harvest questions: windrower width.

Windrower width (ft.)	All fields
20 - 25	20
26 - 30	19
31 - 35	3
35 - 40	7
No response	17

Table A2.13 Harvest questions: combine separator type.

Combine separator type	All fields
Rotary	53
Conventional	8
Rotary and conventional	5

Table A2.14 Harvest questions: combine model.

Combine type	All fields
John Deere	24
New Holland	24
International Harvester	11
Gleaner	4
New Holland, Gleaner and Massey Ferguson	3

Table A2.15 Harvest questions: combine speed.

Combine speed (mph)	All fields
1 - 3	8
3.1 - 5	45
5.1 - 6	11
6.1 - 7	2

Table A2.16 Harvest questions: time of combining.

Time of combining	All fields
Mid-day	23
Afternoon	16
Evening	6
All-day	19
No response	2

Table A2.17 Harvest questions: days from windrowing to combining.

Days from windrowing to combining	All fields
5 - 10	4
11 - 15	6
16 - 20	6
21 - 25	13
26 - 30	5
31 - 35	6
36 - 40	4
No response	22

Table A2.18 Harvest questions: combine fan speed.

Combine fan speed (rpm)	All fields
400 - 500	6
501 - 600	4
601 - 700	34
701 - 800	11
801 - 900	2
No response	9

Table A2.19 Harvest questions: total yield.

Total yield (bu/ac)	All fields
20 - 30	4
31 - 40	14
41 - 50	29
51 - 60	16
61 - 70	3

Table A2.20 Thousand seed weight of shattered seeds.

Thousand seed weight (g)	All fields
2.0 - 2.5	8
2.6 - 3.0	35
3.1 - 3.5	20
3.6 - 4.0	3

Appendix 3. Multiple linear regression fit diagnostics

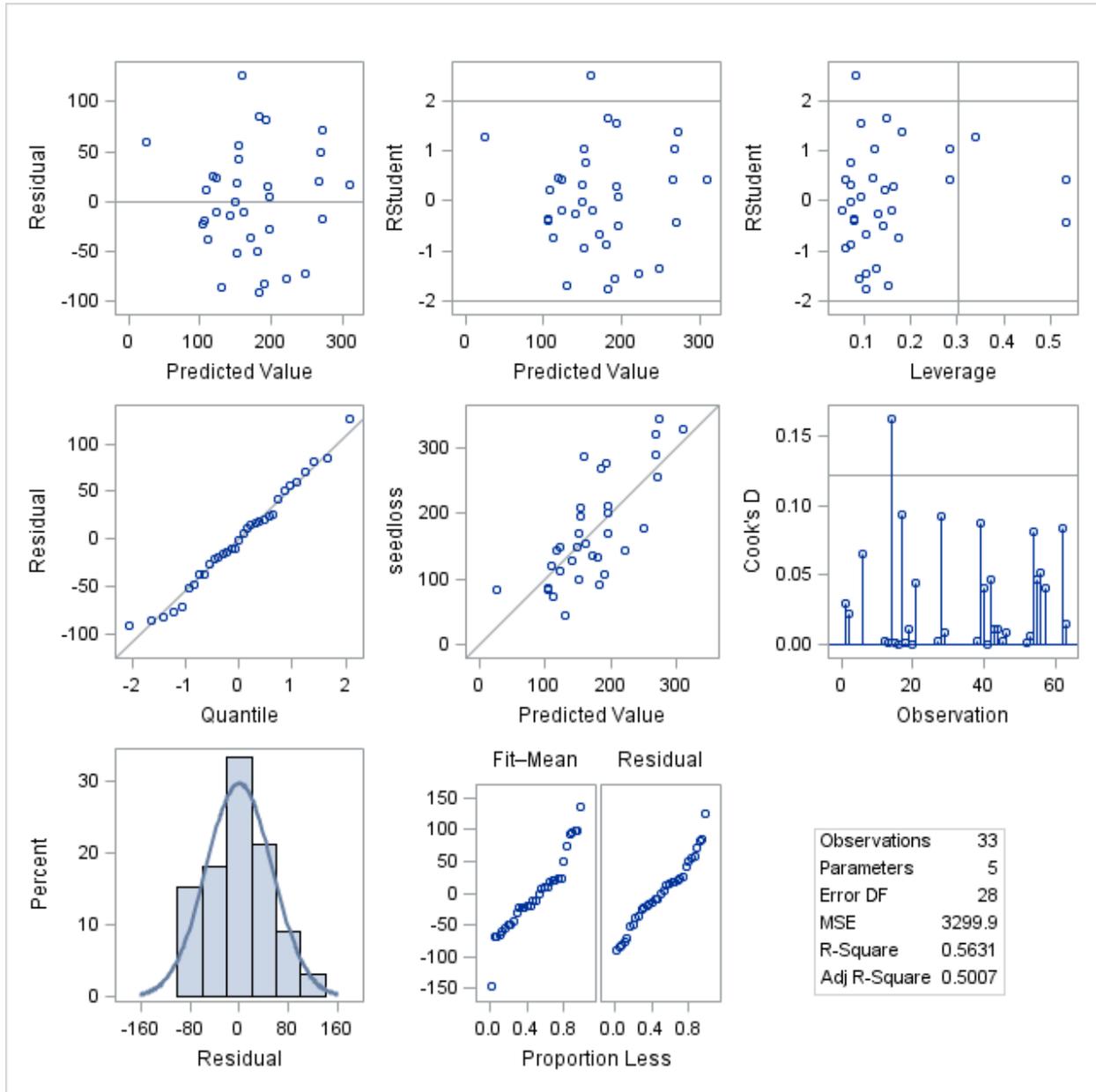


Figure A3.1 Multiple linear regression fit diagnostics for simplified model of agronomic and harvest specific variables predicting seed loss in canola. This panel shows the residuals and predicted values of the simplified model.