Secondary seed dormancy and the seedbank ecology of *Brassica napus* L. in western Canada

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Abstract

The release of genetically modified, herbicide tolerant canola (*Brassica napus* L.) genotypes in western Canada has increased interest in the persistence of volunteer canola. *B. napus* seed may be induced into secondary dormancy in the laboratory, however, little is known of the seedbank ecology and the role of secondary dormancy as a persistence mechanism in this species in the region. The objectives of this research were i) to determine seedbank additions at the time of harvest, ii) determine the role of secondary seed dormancy in seedbank persistence under different management systems, iii) determine the relative importance of factors contributing to secondary dormancy potential and iv) evaluate the role of abscisic acid (ABA) during secondary dormancy induction in *B. napus*. On farms, average seedbank additions during harvest were approximately 20 times the normal seeding rate of canola. High secondary seed dormancy potential prolonged seedbank persistence in fields, irrespective of tillage system. *B. napus* exhibited seedling recruitment of a typical summer annual weed where seedling recruitment was only observed in the spring. Seasonal seedling recruitment was the result of two fates: seed death in the shallow seedbank, irrespective of dormancy potential, and increased ungerminability in buried seeds which was related to secondary seed dormancy potential. Among the factors that contribute to secondary seed dormancy potential, genotype was of greatest significance. Seed size was of lesser importance, while the contributions of pre-harvest factors including seed maturity, year, and location were negligible in comparison. Differences in ABA synthesis and the response to ABA application were related to secondary seed dormancy potential and correlated well previous reports linking ABA to seed dormancy. Conclusions that emerge from this research are i) that on some farms, seedbank additions may be lowered by more diligent harvest practices, ii) seedbank persistence of *B. napus* may be reduced by growing low dormancy genotypes and avoiding seed burial for one year after seedbank establishment and iii) ABA + ABA-glucose ester (ABA-GE) and the ability of seeds to respond to ABA application after seed dormancy induction may potentially be used to identify seed dormancy potential in this species.

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7'OH-ABA	7'-hydroxy-abscisic acid
ABA	Abscisic acid
ABA-GE	Abscisic acid glucose ester
CT	Conventional tillage
ddi	Double distilled
DPA	Dihydrophaseic acid
DTV	Diurnal temperature variation
GA	Gibberellic acid
GM	Genetically modified
HDP	High dormancy potential
HT	Herbicide tolerant
LDP	Low dormancy potential
MDP	Medium dormancy potential
PA	Phaseic acid
PEG	Polyethylene glycol
RCBD	Randomized complete block design
RP-HPLC ESI-MS/MS	Reversed phase high-performance liquid chromatography electrospray ionization-tandem mass spectrometry
SB	Seedbank
SS	Sums squares
ZT	Zero tillage

1.0 Introduction

Canola (*Brassica napus* ssp. *oleifera* (DC.) Metzger and *B. rapa* L.) is one of the more recently domesticated crops grown in western Canada and has been used as an oilseed for less than a century. Volunteer canola populations (i.e. unwanted populations in arable fields) have been observed in subsequent crops, but have generally not been considered difficult to control. In the past two decades, however, the annual acreage seeded to canola has increased substantially (Statistics Canada 2002) as has the occurrence of volunteer canola (Thomas and Wise 1983, 1987; Thomas et al. 1996). Economic and agronomic factors have contributed to the increase in both crop acreage and subsequent volunteer populations. Recently, successful breeding efforts, including genetic modification (GM), in addition to other factors have allowed for a dramatic shift in the proportions of the two canola species grown in western Canada. While traditionally a greater portion of the total annual acreage was seeded to *B. rapa*, currently the higher yielding and later maturing *B. napus* genotypes are grown almost exclusively (Canola Council of Canada 2001). The release and extensive adoption (> 80% in 2000) (Canola Council of Canada 2001) of *B. napus* genotypes tolerant to herbicides has strongly contributed to this shift in species preference. Although herbicide tolerance enhances weed control options within the canola crop, a series of agronomic, economic as well as ecological concerns are associated with subsequent volunteer populations.

One of the primary concerns associated with the introduction of transgenic crops is the potential for transgene escape. High rates of interplant outcrossing (21.8%) have been documented in *B. napus* (Rakow and Woods 1987) and shortly after the release of herbicide tolerant genotypes, *B. napus* volunteers resistant to herbicides with three of a possible four different modes of action were reported (Hall et al. 2000). These volunteers limit the subsequent cropping and herbicide options and producers must now be cognisant of the type of canola grown in adjacent fields as this and other cases (Rieger et al. 2002) have illustrated significant rates of pollen-flow between fields. Fortunately, the number of wild species related to *B. napus* is small in western Canada and their distribution is

limited; therefore, the likelihood of transgene escape to wild relatives is limited. In addition, there are no reports of persisting feral populations of *B. napus* outside agricultural fields in the region.

In fields, volunteer *B. napus* plants may influence germination of other crops as well as compete with other crops if not controlled. Incorporation of high densities of canola seedlings by tillage in spring prior seeding may reduce germination and emergence in many other crop species planted into this residue (Mason-Sedun et al. 1986; Vera et al. 1987). In Europe, low densities of uncontrolled volunteer winter *B. napus* may be highly competitive in subsequent crops (Lutman et al. 1996). Volunteer canola populations have been shown to persist for at least four years in rotation in western Canada (Légère et al. 2001). Therefore, inadequate separation in time between subsequent canola crops may result in direct seed contamination as well as pollen-mediated gene flow from persisting volunteer plants in the current canola crop in the same field. Contamination could exceed the stringent export standards with respect to content of GM material (Phillips and McNeil 2000) if a non-GM genotype is grown in close succession following a GM genotype. Contamination from persisting volunteers also may exceed national standards in specialty trait genotypes which are becoming more prominent. For example, contamination of a genotype intended for human consumption through volunteers from a previously grown genotype with industrial quality oil is extremely undesirable. Thus, if successive canola crops are not adequately separated in time and space, producers as well as seed growers may be limited to growing only canola genotypes of similar quality on a particular field.

Characteristics that result in agronomically successful annual weeds include prolific seed production and a persisting seedbank. In *B. napus*, high shatter potential and small seeds can result in high seedbank additions at the time of harvest (Thomas et al. 1991; Brown et al. 1995). The amount of seed added to the soil (seedbank additions) at the time of harvest, however, is currently not known in western Canada. Furthermore, winter and spring *B. napus* genotypes may develop a stress-induced secondary seed dormancy (Pekrun 1994) that may prolong persistence in the field. Due to cold, harsh winters, the seedbank is the only known source for volunteer populations in the region. Therefore, understanding the seedbank ecology of *B. napus* is of great importance for facilitating integrated control measures that minimize the above concerns by reducing the seedbank persistence of this species. Little is known of the seedbank ecology of spring *B. napus* under different management systems in western Canada. Furthermore, the relative

importance of factors contributing to secondary seed dormancy potential in *B. napus* are still unclear. A genetic component has been suggested (Pekrun et al. 1997a; Momoh et al. 2002); however, its contribution to secondary seed dormancy potential in relation to other factors such as environment and harvest technique warrants investigation. The current method of determining seed dormancy potential in *B. napus* is time consuming (Pekrun et al. 1997a). Therefore, metabolic markers that identify secondary seed dormancy status in this species would be useful. The identification of metabolic markers that are linked to stress-induced seed dormancy may aid in more rapid identification of seed dormancy status and/or potential. Although genetic markers are more ideal for marker selected breeding programs, the complex genetics controlling seed dormancy present challenges for the identification of genetic markers for this trait (Foley and Fennimore 1998).

The focus of my thesis research was the study of the seedbank ecology and seedling recruitment behaviour of *B. napus*. The primary hypothesis of this research was that secondary seed dormancy potential prolongs seedbank persistence in *B. napus* populations in western Canada. The following key questions were addressed:

(1) How large are seedbank inputs at the time of harvest on farms in western Canada?
(2) How long does a single cohort of *B. napus* genotypes persist in the seedbank in contrasting tillage systems?
(3) What is the annual pattern of seedling recruitment of volunteer *B. napus* in western Canada?
(4) What factors control secondary seed dormancy potential in *B. napus*?
(5) How are the seasonal seedbank dynamics in *B. napus* with contrasting secondary seed dormancy potential influenced by tillage system, burial depth and soil type?
(6) Are differences in seed dormancy potential in *B. napus* related to differences in the abscisic acid (ABA) metabolism and/or responsiveness to ABA?

Answers to these questions will provide information to formulate recommendations for producers, seed growers, as well as the canola breeding industry to minimize the concerns associated with volunteer populations of this important crop species. Harvest methods, tillage practices and genotype selection that reduce seedbank additions and persistence of this species are of interest to producers and seed growers and are addressed by questions one through five. The relative importance of genotype to secondary seed dormancy potential and the identification of possible metabolic markers linked to secondary seed dormancy, may be of interest to the canola breeding industry and are addressed by

questions four and six. Results from this research will enable a more integrated approach to controlling volunteer *B. napus* populations.

2.0 Literature Review

2.1 The occurrence of volunteer canola in western Canada

The annual acreage seeded to canola (*B. napus* L. and *B. rapa* L.) in western Canada has increased substantially over the past 25 years. The canola production acreage for the 1999 growing season reached an all time high at 5,564,400 ha. Since then, the total canola acreage has decreased by approximately 10% (Statistics Canada 2002). Nonetheless, the total acreage grown on the Canadian prairies in the late-1990s represents an approximate 2.5- to 3-fold increase relative to that of 1975, with the vast majority being currently planted to *B. napus* (Statistics Canada 2002). While in the 1970s and 1980s few *B. napus* genotypes were available to producers, currently more than 150 spring genotypes are licensed for commercial production in western Canada (Canadian Food Inspection Agency 2003). Of the total canola acreage, 45% has consistently been grown in Saskatchewan (Statistics Canada 2002).

In concert with increases in total seeded acreage, the abundance of volunteer canola also has increased notably during this time. The relative abundance ranking of volunteer canola has increased from a rank of 26 in the mid-1970s to 22 in the mid-1980s to 12 in the mid-1990s in surveys conducted after in-crop weed control (Thomas and Wise 1983, 1987; Thomas et al. 1996). Interestingly, the relative abundance of volunteer canola was higher than that of any other weed, in a pre-weed control survey conducted in Manitoba in 1994 (Thomas et al. 1997). The relative abundance of a weed species is a synthetic index calculated from the relative frequency, relative uniformity, and relative density of that species (Thomas 1985). In volunteer canola, all three parameters have increased substantially over the past two decades (Thomas and Wise 1983, 1987; Thomas 1991; Thomas and Donaghy 1991; Thomas et al. 1997). The increase in relative abundance of volunteer canola since the mid-1970s has been 4.7-fold on average (Thomas and Wise 1983; Thomas et al. 1996). Thus, the increase in the abundance of volunteer canola cannot be explained by the 2.5-fold increase in acreage alone. Many changes in cropping practices have occurred during this time, that also may have contributed to an increase in

volunteer canola. These include changes in tillage practices (Gray et al. 1996) as well as increases in oilseed and pulse crops (Thomas and Wise 1983; Thomas et al. 1996) which may have increased the difficulty of controlling volunteer canola using in-crop herbicides. The average yield of canola, however, has not increased substantially across the Canadian prairies over the past two decades (Statistics Canada 2002) and harvesting methods of canola also have not changed significantly. Thus, it seems unlikely that the observed increase in volunteer canola is simply the result of increased harvest losses.

2.2 Life cycle, weedy and feral populations

B. napus is a summer- or winter-annual member of the *Brassicaceae* family native to Eurasia. Winter annual genotypes are primarily grown in Europe and eastern Canada because they are higher yielding and able to survive moderate winters (Lutman 1993; Simard et al. 2002). Volunteers may originate from either root stocks that survive the winter (Simard et al. 2002) as well as seed lost at the time of harvest (Pekrun et al. 1998a). Only spring canola is grown in western Canada where cold, harsh winters tend to kill all seedlings and plants. Any subsequent volunteers must originate from seed in western Canada and therefore, the seedbank ecology is of great importance in understanding the persistence of *B. napus* in this region.

A flowchart can aid in the visualization of the life cycle of volunteer *B. napus* (Figure 2.1). Any *B. napus* seed that is not removed from the field at the time of harvest or enters a field through seed contamination becomes part of the seedbank. In the seedbank, seeds may have several fates, which include: immediate germination, a period of quiescence followed by germination, predation, attack by microbes, or seed death. *B. napus* seeds that enter a period of quiescence and/or seed dormancy, followed by successful recruitment at a later time are of greatest concern. Secondary seed dormancy has been documented in European *B. napus* genotypes and may aid seedbank persistence (Pekrun 1994, Schlink 1994). When seeds emerge at a time that permits the completion of the life cycle, the seedbank may be replenished by a seed rain from mature volunteer plants (Figure 2.1). In eastern Canada, individual plants that survived the winter and remain uncontrolled may add up to 3,000 seed to the seedbank (Simard et al. 2002) while seedbank return by volunteers in western Canada is not known. The addition of a new cohort to the seedbank completes the life cycle. This extends the period of time required to deplete the seedbank and sustains volunteer populations. If the life cycle is completed

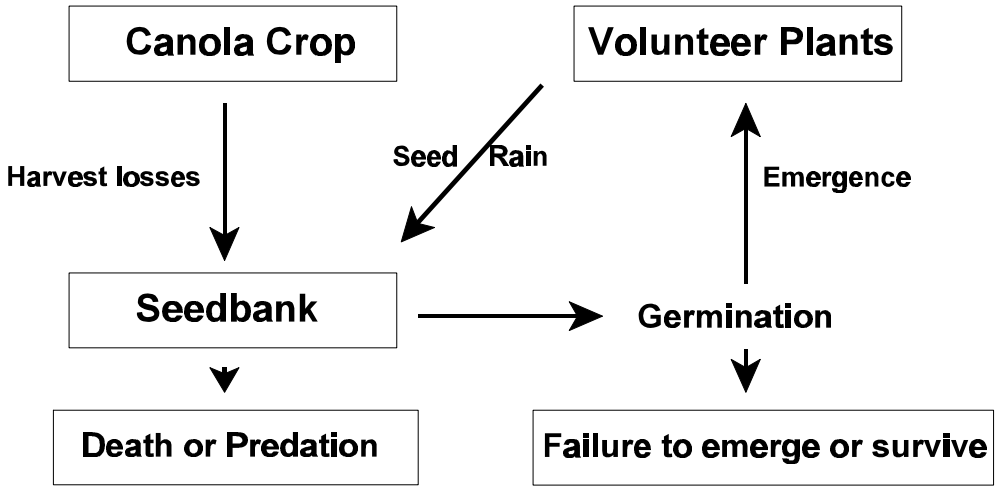

FIGURE 2.1 Flow chart of the life cycle of volunteer *B. napus*.

before the seedbank is depleted entirely, feral (i.e. self sustaining) populations such as those observed in rye (*Secale cereale* L.) in the mid-western USA may arise (Stump and Westra 2000).

Persistence of feral winter *B. napus* populations, i.e. those at non-agricultural sites, have been studied in Europe. In the U.K., feral winter *B. napus* persistence was low and populations tend to go extinct after as few as three years (Crawley and Brown 1995). When significantly different, feral populations of transgenic lines of winter *B. napus* populations were less persistent than non-transgenic lines in the U.K. (Crawley et al. 1993). In France, feral winter *B. napus* populations persisted for as long as eight years on road verges (Pessel et al. 2001). There are reports of feral *B. rapa* populations in western Canada (Warwick et al. 2000); however, there are no reports of long-term persistence in feral *B. napus* populations in western Canada.

2.2.1 Seedbank additions

The initial routes for seeds to enter the seedbank where none exists is through seed contamination and via seed losses during canola harvest. Several studies examining seed losses at harvest in *B. napus* may be found in the literature. Unfortunately, these studies focussed mainly on very specific aspects of the harvest procedure and as a result, total harvest losses (natural seed shatter + equipment losses) are rarely reported. Nevertheless, these studies give an indication of the range of harvest seed losses. In the U.K., mean harvest seed losses of winter *B. napus* during direct harvest range from 2 to 5% under ideal conditions, but may be as high as 50% under unfavourable harvest conditions (Price et al. 1996). A more recent report indicates harvest seed losses between 200 to 300 kg ha^{-1} (Pekrun et al. 1998a), which amounts to approximately 5,000 to 7,000 seeds m^{-2} in the U.K. (Pekrun et al. 1998a). In the southeastern USA, average winter *B. napus* harvest seed losses of 34.5% were reported by Thomas et al. (1991) and were determined in three cultivars over three years comparing mechanized harvest techniques to hand harvest. Spring *B. napus* harvest seed losses of 350 kg ha^{-1} or approximately 15% of the attainable yield have been reported in the Pacific Northwest of the USA (Brown et al. 1995). In western Canada, canola harvest losses of 9 kg ha^{-1} have been reported when the crop was windrowed at 37% seed moisture content (Bowren and Pittman 1975). These increased to 14.5 kg ha^{-1} when the crop was windrowed at 12% seed moisture content. Nevertheless, due to the small seed size of *B. napus* relative to other crops (1,000 kernel

weight < 4 g based on dry matter), even small seed losses amount to large additions to the seedbank.

Some evidence suggests that the timing of the harvest operations contributes more to seed losses than harvest technique. In the Pacific Northwest, *B. napus* seed losses as high as 28.5% have been attributed to delayed harvesting (Brown et al. 1995) with proportionally similar seed losses (25%) observed in Europe when windrowing of the crop was delayed (Price et al. 1996).

2.2.2 Seedbank persistence in relation to burial and soil type

Seed burial increases seedbank persistence in both canola species. This is similar to many other small seeded weeds (Baskin and Baskin 1998). Studies on winter *B. napus* in Europe have demonstrated greater seedbank persistence when seeds were buried immediately after seed shed compared to delayed or no tillage (Pekrun and Lutman 1998). In their study, 30% of the seedbank survived one winter when plots were tilled immediately after establishing a winter *B. napus* seedbank, whereas only 0.1% of the seeds survived one winter when left on the soil surface (Pekrun and Lutman 1998). Even delayed tillage (ploughing) resulted in a significantly lower winter *B. napus* seedbank the following spring compared to tillage immediately after seed shed (Pekrun and Lutman 1998), suggesting increased seed mortality or predation when seeds were left on the soil surface. The highest levels of seedbank persistence occurred when the seedbank was not disturbed after burial (Schlink 1995). Under these conditions, a small proportion of *B. napus* seeds readily persist for at least five years or longer in Europe (Sauerman 1993; Schlink 1994, 1995).

The depth of seed burial also influences the persistence of the canola seedbank. An increase in seedbank persistence with increasing burial depth of up to 27 cm have been reported in winter *B. napus* in Europe (Schlink 1995). Similarly, burial of *B. rapa* (cv. Tobin) up to depths of 12 cm also increased the proportion of persisting seed over one winter in Alaska, in a climate more similar to that of western Canada than Europe (Sparrow et al. 1990). This study also demonstrated the importance of snow cover to seedbank persistence. *B. rapa* seed survival was enhanced significantly when covered with snow (62% seed survival) compared to without snow cover (39% seed survival) (Sparrow et al. 1990). High levels of seedbank mortality were found between March and April, the timing of which varied between 1987 and 1988, although similar final

persistence (approximately 20%) was observed in May of each year (Sparrow et al. 1990). An explanation for the change in the period of seed mortality was not provided.

Soil texture also may affect the persistence of the *B. napus* seedbank. Seedbank persistence of winter *B. napus* genotypes tended to be higher in a silty clay soil than in a sandy soil in U.K. (López-Granados and Lutman 1998).

2.2.3 Seedling recruitment

2.2.3.1 Densities, recruitment pattern and longevity

Little is known about the persistence of the *B. napus* seedbank under the environmental and soil conditions experienced in western Canada; however, indirect measures through seedling recruitment exist. In a spring weed survey conducted prior to weed control in Manitoba in 1994, fewer than one and as many as 812.4 volunteer canola plants m^{-2} were counted in farmer's fields (Thomas et al. 1997). In those fields where volunteer canola was present, a mean density of 85.7 volunteer canola plants m^{-2} was observed in the spring, while this density decreased to 13.7 plants m^{-2} by autumn (Thomas et al. 1997). Autumn counts were conducted shortly after harvest and before freeze up. There are three possible explanations for the presence of autumn volunteers. Some of these plants may have escaped weed control, some may have emerged after in-crop weed control, or some may be seedlings from canola harvest losses incurred during that season. Emergence throughout the growing season would be consistent with observations in winter *B. napus* in Europe (Pekrun et al. 1997b); however, the source of these volunteer plants in western Canada is currently not clear. In western Canada, seedling recruitment of volunteer canola has been observed as long as four years after the last canola crop in rotation (Légère et al. 2001). The inability to control seedbank replenishment in these surveys, however, reveals little of the long-term persistence of individual seeds.

2.2.3.2 Influence of tillage system

Management practices such as tillage system directly influence the weed community as well as the abundance of species within the community (Froud-Williams et al. 1981; Thomas et al. 1997). The bulk of the seedbank remains closer to the soil surface under zero tillage (ZT), while under conventional tillage (CT), the seedbank tends to be

distributed more evenly throughout the cultivation zone (Clements et al. 1996; Swanton et al. 2000). In Europe, winter *B. napus* seedling recruitment in autumn after seed shed was approximately 20 times greater in undisturbed soil compared to any form of tillage treatment (Pekrun et al. 1998a). In western Canada, the relative abundance of volunteer canola was 1.6 and 2 times greater in ZT compared to neighbouring CT fields in spring and autumn, respectively (Thomas et al. 1997). The relative frequency and relative uniformity of volunteer canola were similar in ZT and CT (Thomas et al. 1997) and the observed increase in the relative abundance of volunteer canola was primarily due to differences in mean density in ZT (spring = 144 plants m^{-2}, autumn = 18 plants m^{-2}) compared to CT (spring = 73.4 plants m^{-2}, autumn = 9 plants m^{-2}) (Thomas et al. 1997). A more recent report, however, suggests that tillage system does not influence the persistence of volunteer canola in western Canada (Légère et al. 2001). This report was based on volunteer canola counts conducted after in-crop weed control which may have masked the effects of tillage system.

Differences in physical soil properties as well as the soil microclimate may influence seedbank persistence among contrasting tillage systems. The remaining residue on the soil surface under ZT reduces wind and water erosion of the soil. Residue on the soil surface also increases the albedo or reflective ability of the surface. This results in lower maximum and mean soil temperatures throughout the season. In western Canada, mean temperature reductions of 1 to 5 C have been reported in ZT compared to CT during the first month of crop growth (Gauer et al. 1982; Carter and Rennie 1985; Carefoot et al. 1990; Arshad and Azooz 1996). During winter, the thicker layer of snow trapped by residue on the soil surface in ZT also results in mean soil temperatures that may on occasion be as much as 10 C higher in ZT compared to CT (Hay 1977). During a soil temperature and soil water content characterization experiment conducted in Manitoba, soil temperatures did not fall below -8 C in a Sperling clay loam soil in ZT whereas temperatures as low as -18 C were observed in the cultivation zone in CT (Gauer et al. 1982). Thus, soil temperatures are more moderate in ZT compared to CT throughout the entire growing season. In ZT, increased snow trapping and reduced evaporation caused by the residue on the soil surface generally resulted in higher soil water contents compared to CT when significant differences were observed (Gauer et al. 1982; Carefoot et al. 1990; Arshad and Azooz 1996). Temperature differences of this magnitude may influence seed dormancy characteristics (Probert 2000). Tillage also has been shown to increase organic matter breakdown which results in changes in the compositon of the

gaseous components in the soil. Higher CO_2 emissions observed in tilled soil (Rochette and Angers 1999) may directly or indirectly, through reductions in the partial pressure of O_2, influence the environment immediately surrounding the seeds in the seedbank.

2.3 Germination characteristics of *B. napus*

Seed germination characteristics of *B. napus* may directly influence volunteer seedling recruitment in the field. Seed longevity and germination of *B. napus* and other *Brassicaceae* stored in an imbibed state in the laboratory have been investigated. A long-term, bench-top study conducted in petri dishes has shown that a small proportion of *B. napus* seeds may remain dormant in an imbibed state for at least 5 years under ambient light and temperature (Tokumasu and Kakihara 1990). Most of the seeds did not germinate until the fourth and fifth year after initiation of the study and germination peaks correlated closely with annual peak temperatures (Tokumasu and Kakihara 1990).

Temperature requirements for germination of non-dormant *B. napus* are relatively well understood. In European spring and winter *B. napus* genotypes, base temperatures of 3 C and lower have been reported (Marshall and Squire 1996; Squire 1999). In Canadian spring *B. napus* genotypes, base temperatures ranging between 0.44 and 2 C (Kondra et al. 1983; Vigil et al. 1997) have been documented and suggest a moderate degree of variability among genotypes and/or seedlots. Breeding for lower base temperatures in *B. napus* has been attempted with limited success (Acharya et al. 1983; King et al. 1986).

In the field, low temperatures tend to delay seedling emergence and also may decrease total emergence percentage. Reductions in germination rate have been observed in several studies in *Brassica spp.* (Acharya et al. 1983; King et al. 1986) and emergence may take up to 18 days at 5 C (Blackshaw 1991). Emergence of winter *B. napus* genotypes tends to take longer than that of spring genotypes under identical conditions (Vigil et al. 1997). In some cases, greater reductions in final germination percentage are observed in some genotypes than others at low temperatures (Wilson et al. 1992; Marshall and Squire 1996). In addition, germination at low temperature often results in poor seedling vigour in *B. napus* (Elias and Copeland 1997; Larsen et al. 1998). Poor germination at low temperatures may in part be overcome by seed priming (Zheng et al. 1994).

Freezing temperatures may prevent emergence of germinated seedlings without irreversible damage. Germinated seeds of *B. napus* with radicles protruding by up to 1.5 mm may be stored at -20 C for 336 days and still exhibit 96% viability when air dried to an equilibrium water content of 11% prior to storage (Finch-Savage and McKee 1989). However, at higher storage temperatures (1 and 15 C), the loss of viability was significantly greater (Finch-Savage and McKee, 1989). These temperatures are within the range that may be experienced in fields throughout the winter in western Canada.

As would be expected, more negative water potentials adversely affect the germination characteristics of *B. napus*. In the laboratory, Schopfer and Plachy (1984) demonstrated that below -0.6 MPa, the germination rate of winter *B. napus* diminished rapidly. In soil, water uptake by spring *B. napus* seed is inhibited to a greater extent than in polyethylene glycol (PEG-8000) at equal water potentials (Shaykewich and Williams 1971). The authors suggested the observed difference between PEG and soil was due to the differences in the hydraulic conductivity of these systems. In the soil system, less contact between the seed and imbibing solution would be expected due to an increase in air volume as water potential decreases. A similar change in contact area between the seed and the imbibing solution does not occur in a petri dish using PEG. Similarly, germination of forage *B. napus* and *B. rapa* was not affected by soil water potentials of -0.01 to -0.10 MPa, but did decrease at soil water potentials of -0.25 and -0.50 MPa at 7.5 C (Rao and Dao 1987). Livingston and de Jong (1990) observed an interaction between matric and osmotic water potentials. Matric potential is the reduction of free energy of water due to adsorption to hydrophilic surfaces, whereas osmotic potential is the reduction of free energy due to solutes in solution. At osmotic potentials of -1.2 MPa, the germination rate of *B. napus* was slower in soils with higher matric potential (-0.02 MPa) than soils with lower matric potential (-0.2 MPa). A lack of sufficient aeration was discounted as a possible explanation and increased pathogen pressure was also not observed in the soil with higher matric potential, but a clear explanation was not provided. Nevertheless, these findings indicate a possible interaction between soil salinity and total soil water potential that may influence germination and thus, seedling recruitment. The degree to which temperature and low water potentials affect germination and seedling recruitment of volunteer *B. napus* populations arising from currently grown genotypes in western Canada remains unclear.

2.4 Seed Dormancy

Seed dormancy is the failure of viable seeds to germinate under otherwise optimal conditions. Over the past 5 decades, several seed dormancy classifications systems have been suggested (Harper 1957; Nikolaeva 1977; Baskin and Baskin 1985). The primary/secondary seed dormancy classification system by Karssen (1980/81) which was further clarified by Baskin and Baskin (1985) is among the most popular seed dormancy classification systems at this time and will be used throughout this thesis. The basis of this system is the time at which seed dormancy occurs. Primary and secondary seed dormancy are further subdivided according to the degree of dormancy.

Primary dormancy has been defined as a state where germination of the progeny is prevented while maturing on the mother plant and for some time after the seed has been separated from its parent (Karssen 1980/81; Hilhorst and Toorop 1997). A period of 'after-ripening' is generally required to alleviate primary dormancy. After-ripening is the period of time between seed dissemination and the time when maximum germination percentage can be achieved under optimal germination conditions. Several different mechanisms may cause this response (Baskin and Baskin 1998). Secondary dormancy is usually defined as a reduction in seed germinability which develops at any time after seed dissemination and may, in some instances, be induced prior to the complete alleviation of primary dormancy.

Subdivisions of this classification system are defined by the degree of dormancy, where either primary or secondary dormancy may be conditional or innate (Baskin and Baskin 1985). Relative to non-dormant seeds, conditionally dormant seeds germinate under a more limited range of conditions, while seeds in innate dormancy fail to germinate under any condition. In this model, seeds are presumed to pass through conditional dormancy when going from an innate dormant to a non-dormant state and vice versa. Seeds may go from non-dormant to a conditionally secondary dormant to an innately secondary dormant state and back to a non-dormant state in the reverse order within the span of one year (Baskin and Baskin 1985). This cycle may repeat itself for several years in any one seed in the seedbank. It serves as a buffer to rapid genetic adaptation as well as genetic bottlenecks that may result from adverse conditions experienced in the short-term in annual species. The timing of this cycle is strongly regulated by temperature (Probert 2000) and is defined by the life cycle of a species (summer- vs. winter-annual). Non-dormant seeds that fail to germinate as a result of unfavourable external conditions rather than factors within the seed are categorized as quiescent (Baskin and Baskin 1985). This

classification system has no provisions for the cause of seed dormancy.

2.4.1 Primary dormancy in *B. napus*

Reports describing primary dormancy in *B. napus* are few and ambiguous. Low germinability in winter and spring *B. napus* exists exclusively during seed maturation and declines with increasing seed maturity (Finkelstein et al. 1985; Schlink 1994). Primary dormancy levels in winter *B. napus* seed range between 10 to 20% (Pekrun et al. 1998a), but may be as high as 60% in some genotypes between 8 and 12 weeks after flowering (Schlink, 1994). By harvest, primary seed dormancy was no longer present.

In contrast, researchers in Japan have shown that most cruciferous crops, including *B. napus*, required a period of after-ripening before high germination percentages were achieved (Tokumasu et al. 1981; Tokumasu and Kato 1987). Storage of cruciferous seeds within the siliques decreased the maximum germination percentage as well as the rate at which primary dormancy was released (Tokumasu et al. 1981). This may have implications for seedbank persistence if undehissed siliques enter the seedbank. Furthermore, the optimum storage conditions for the release of primary dormancy were found to be at a relative humidity below 35%, while optimum germination temperatures ranged between 15 to 35 C (Tokumasu et al. 1981). Research in the 1950s on *B. napus* in Japan showed that seed dormancy (presumably primary) was released by low temperatures in conjunction with washing and pricking the seeds (Sugiyama 1949, cited by Takahashi and Suzuki 1980) or by treatment with either thiourea or urea (Hori and Sugiyama 1954, cited by Takahashi and Suzuki 1980).

2.4.2 Secondary dormancy in *B. napus*

Although primary dormancy is negligible in fully mature *B. napus*, seed of this species may develop secondary seed dormancy (Schlink 1994; Pekrun 1994; Pekrun et al. 1997a). In the laboratory, incubation in darkness for up to 4 weeks in conjunction with the use of polyethylene glycol (PEG-8000) solutions with an initial water potential of -1.5 MPa were most successful in inducing secondary dormancy (Pekrun 1994; Schlink 1994). Seed dormancy expression tends to be lower at higher initial osmotic potentials of the imbibing solution given equal exposure intervals (Pekrun et al. 1998b). Low O_2 concentrations (3% O_2 : 97% N_2), simulating wet soil conditions, in combination with

darkness also have been reported to induce secondary dormancy in *B. napus*, albeit to a far lesser extend than osmotic stress in combination with darkness (Pekrun 1994; Pekrun et al. 1997c; Momoh et al. 2002).

The importance of temperature in seed dormancy development in *B. napus* is less clear than that of osmotic potential. It has been established that increasing the diurnal temperature variations during dormancy induction tend to decrease secondary seed dormancy development (Pekrun et al. 1997b). This behaviour is analogous to dormancy release in other species and has been suggested as a depth and gap sensing mechanism (Thompson and Grime 1983; Goedert and Roberts 1986). The effect of static temperatures on dormancy induction is less clear. Recent investigations have suggested higher mean seed dormancy induction among a group of genotypes induced at 20 C compared to 12 C, although the observed differences were not statistically significant (Momoh et al. 2002). Moreover, the temperature difference between the induction temperature and the subsequent germination test temperature appears to influence secondary dormancy development (Pekrun et al. 1997c; Momoh et al. 2002). When the absolute difference between these temperatures is increased, secondary seed dormancy tends to decrease. These observations suggest a high sensitivity of *B. napus* seed dormancy to temperature although empirical data confirming this is still sparse. A genetic component to secondary seed dormancy potential in *B. napus* has been suggested. Among 25 spring and 21 winter *B. napus* cultivars tested in Europe, the mean proportion of seeds induced into secondary dormancy ranged from 0.7 to 76.1% (Pekrun et al. 1997a). Among the two groups of cultivars tested, the average potential for secondary dormancy was similar in spring and winter *B. napus* cultivars. In a different study, however, higher maximum levels of secondary seed dormancy were observed in spring compared to winter *B. napus* genotypes (Momoh et al. 2002).

In *B. napus*, secondary seed dormancy is readily reversed by several factors. Schlink (1994) demonstrated that a single exposure to a camera flash with a duration of 0.002 s was sufficient to increase mean germination in dormant seeds of two *B. napus* cultivars from 13.3 to 63.0%. A final germination percentage of 98.1% was reached when dormant seeds were exposed to continuous light. The observed phenomenon is similar to the well documented light requirement for germination of lettuce seed (*Lactuca sativa* L.) (Brothwick et al. 1954) and various small seeded weeds (Wesson and Wareing 1969a, b). Consequently, the involvement of the phytochrome system has been implicated in *B. napus* seed (López-Granados and Lutman 1998). A seasonal response of germination to

light exposure in *B. napus* seed has been observed in winter *B. napus* where the readily germinable proportion of the viable seedbank was lower under light from seed exhumed during summer compared to seed exhumed at any other time of the year (Schlink 1995). In contrast, Bazanska and Lewak (1986) found that germination of *B. napus* may be inhibited by the continuous exposure to white light at lower temperatures in combination with moisture stress. A similar light inhibition of germination also has been shown in mustard (*Sinapis alba* L.) seeds (MacDonald and Hart 1981).

A stratification treatment at 2 to 4 C for three days also has proven effective in releasing secondary seed dormancy in *B. napus* (Pekrun et al. 1998b). In addition, exogenous applications of gibberellic acid (0.2 mg l^{-1}) have been used to reverse secondary seed dormancy (Pekrun et al. 1998b). The effectiveness of these methods in relieving seed dormancy in other species is well established in the literature (Bewley and Black 1994; Baskin and Baskin 1998).

The factors affecting secondary seed dormancy expression are still unclear in *B. napus*. Although a genetic component has been implicated, its importance relative to other factors such as environment during seed maturation (Gutterman 1980/81) remain unclear. Furthermore, the influence of seed storage on secondary seed dormancy characteristics have not yet been directly investigated in this species (Pekrun et al. 1997a; Momoh et al. 2002) and the role of constant temperatures on seed dormancy induction rates is also unclear (Momoh et al. 2002).

2.5 Abscisic acid (ABA) and seed dormancy

2.5.1 Hormone content correlation with seed dormancy and other markers

Abscisic acid has been implicated in the regulation of a number of plant responses, including dormancy. Experiments examining the effects of exogenous applications of ABA and gibberellins on seed have shown antagonism between these two growth regulating substances. High ratios of ABA:gibberellins have been suggested to promote dormancy, while a low ratio of these hormones tends to lead to the completion of germination (Wareing and Saunders 1971).

More recently, a role of ABA in seed dormancy regulation has been indicated in several species. In *Nicotiana plumbaginifolia* Viv., an ABA-deficient mutant displayed a non-dormant phenotype and endogenous ABA concentrations decreased during afterripening

(Grappin et al. 2000). Fluridone, an ABA biosynthesis inhibitor (Gamble and Mullet 1986; Xu and Bewley 1995), was equally effective at breaking seed dormancy as exogenous applications of gibberellic acid (GA_3) (Grappin et al. 2000). Fluridone inhibits phytoene desaturase, a key enzyme in the carotenoid synthesis pathway which provides the precursors to ABA. Similarly, ABA deficient and insensitive mutants of *Arabidopsis thaliana* L. Heynh., a species closely related to *B. napus*, have implicated ABA in the regulation of seed dormancy in this species (Schwartz et al. 1997; Finkelstein et al. 2002; Brocard-Clifford et al. 2003). In *B. napus*, endogenous ABA and GA levels were manipulated via exogenous hormone applications (Fu and Lu 1991). These authors found that endogenous ABA levels correlated negatively with seed germination rate ($r = -0.9486$), while endogenous gibberellin levels correlated positively with seed germination rate ($r = 0.9666$). Whether such a correlation exists during secondary seed dormancy induction and release in *B. napus* remains to be investigated. In other species, however, endogenous levels of ABA and gibberellins have not adequately explained seed germination behaviour (Wareing and Saunders 1971; Bewley and Black 1994; Ramagosa et al. 2001), indicating that a low ratio between these two hormones does not ubiquitously release seed dormancy. Cytokinins and ethylene also may promote germination in some species (Wareing and Saunders 1971) and may be more effective than gibberellins at antagonizing ABA to promote germination (Wareing and Saunders 1971).

Most investigations on the role of ABA on seed dormancy have been with respect to primary seed dormancy in crop species (Gosling et al. 1981; Walker-Simmons 1987; Wang et al. 1995; Benech-Arnold et al. 1999). Nevertheless, ABA also has been implicated in the maintenance of thermodormancy, a secondary dormancy induced by exposure to high temperatures during imbibition. When seeds of these species are imbibed at supraoptimal temperatures, re-exposure to temperatures that would have resulted in germination prior to the high temperature treatment, result in seed dormancy. This phenomenon is well documented in lettuce seed. In this species, thermodormancy is readily released by 30 ∶M fluridone (Yoshioka et al. 1998), suggesting a role of ABA in thermodormancy maintenance. Further investigations have shown a different route of ABA metabolism in thermodormant lettuce seed compared to non-dormant seed (Chiwocha et al. 2003). In cereals, thermodormancy correlates well with a release of ABA from the seed coat (Corbineau and Côme 2000). In other cases, thermodormancy may be regulated by the embryo or a combination of the two (Bewley and Black 1994). An increase in ABA levels during high temperature exposure, however, is not ubiquitous among all seeds as decreases in ABA levels also have been observed during warm as well

as cold stratification (Subbaiah and Powell 1992; Ren et al. 1997; Chien et al. 1998).

Correlating other metabolic indicators to seed dormancy also has been attempted. Indicators that have been correlated to seed dormancy with some success include carbohydrate status (Foley et al. 1992; Nichols et al. 1993), red/ox charge (Gallais et al. 1998), as well as changes in membrane properties (Di Nola et al. 1990; Faust et al. 1997; Hilhorst 1998). Whether these indicators are a cause, consequence or merely correlate with seed dormancy and its release is less clear.

2.5.2 ABA synthesis and metabolism

Recent investigations have revealed that ABA levels alone may not suffice in revealing its role in seed dormancy. Abscisic acid content may be similar in dormant and non-dormant seed (Yoshioka et al. 1998), but the rate and route of ABA inactivation may differ between dormant and non-dormant seed (Chiwocha et al. 2003). Whether this is of importance in secondary seed dormancy in *B. napus* has yet to be investigated. The biochemical pathway(s) of ABA synthesis and metabolism have been difficult to establish due to the low *in vivo* concentration of the molecules involved. Recently it has been shown that the precursors of ABA are pyruvate and glyceraldehyde-3-phosphate (Lichtentahler 1999). Prior to these findings, it was generally accepted that ABA was primarily synthesized from mevalonic acid (Cutler and Krochko 1999). The early steps of ABA synthesis up to and including xanthoxin occur in plastids, while the remaining steps occur in the cytosol. There, only the (+)-stereoisomer of ABA may be formed from one of three immediate precursors (Cutler and Krochko 1999), while (-)-ABA is not synthesized in plants (Yamamoto and Oritani 1996). Alternative biosynthesis pathways of ABA also have been suggested, but appear to play only a minor role (Cutler and Krochko 1999).

ABA may be degraded via a number of pathways. The primary route of ABA degradation appears to be via hydroxylation in the 8' position, followed by rapid conversion to (-)-Phaseic acid (PA). A further reduction may occur, forming dihydro-phaseic acid (DPA) (Cutler and Krochko 1999). Other metabolites also have been observed in relatively small amounts, including (+)-7'-hydroxy-ABA (7'OH-ABA) (Zeevaart and Creelman 1988). The hydroxylated metabolites of ABA may still play an active role in regulation (Walker-Simmons et al. 1997), but PA and DPA appear to be

inactive relative to ABA and its hydroxylated catabolites. Abscisic acid, PA, DPA and 7'OH-ABA also may be inactivated via conjugation, thus forming glucose esters, glucosides or other conjugates. All conjugates are believed to accumulate in the vacuole (Zeevaart and Creelman 1988) where they exhibit no biological activity.

Abscisic acid is readily translocated within plants (Sauter et al. 2001) and therefore a cell's ABA content may be a function of *de novo* synthesis as well as import/export. This, in conjunction with the continuous degradation of this hormone results in ABA content dynamics that are difficult to decipher from single measurements of the hormone and its metabolites (Cutler and Krochko 1999; Schmitz et al 2002; Chiwocha et al. 2003).

2.5.3 ABA and seed maturation in *B. napus*

Changes in endogenous ABA levels during seed maturation in *B. napus* are well documented. Similar to many other species (Bewley and Black 1994), endogenous ABA levels increase during seed maturation and are followed by a decrease during dessication (Juricic et al. 1995). Depending on the genotype, one or two peaks in ABA content may be observed during seed maturation. The first peak is maternally derived, while a second peak, when present, is the result of *de novo* ABA synthesis in the embryo (Karssen et al. 1983). In developing *B. napus* seed, a spike in endogenous ABA levels also has been observed one day after a mild freezing stress, after which ABA levels declined again to control levels (Green et al. 1998). The influence of the freezing stress on seed germinability and sensitivity to ABA were not investigated.

In *B. napus*, endogenous ABA levels correlated well with germination rates of excised embryos early during seed maturation (Finkelstein et al. 1985). As seed ABA levels decline during desiccation, however, low seed water content appears to be the primary factor preventing germination (Finkelstein et al. 1985).

2.5.4 Sensitivity to ABA

In addition to endogenous hormone levels, the ability of tissues to respond to plant hormones (tissue sensitivity) must be considered (Trewavas 1982). Indeed, this is also of importance in seed dormancy. Lower sensitivity to ABA has been shown in non-dormant wheat (*Triticum aestivum* L.) seed relative to dormant seed (Walker-Simmons 1987;

Morris et al. 1989), as well as barley (*Hordeum vulgare* L.) (Wang et al. 1995) and sunflower (*Helianthus annuus* L.) (Bianco et al. 1994). In seeds of some species, sensitivity to ABA may be influenced by temperature, as decreases in the sensitivity to ABA have been reported during cold stratification (Singh and Browning 1991; Jarvis et al. 1997).

During seed maturation, differences in embryo sensitivity to ABA also have been observed among three genotypes of *B. napus* (Juricic et al. 1995). Changes in sensitivity, however, were not correlated to changes in endogenous ABA levels. Interestingly, manipulating GA levels during maturation also altered seed sensitivity to ABA (Juricic et al. 1995).

2.5.5 Molecular approaches

Some promise lies in the application of molecular techniques in elucidating the mechanisms and regulation of seed dormancy (Chen et al. 2002; Foley 2002; Horvath and Anderson 2002). The generation of ABA synthesis mutants (under and over-production relative to the wild type) (Schwartz et al. 1997) and those insensitive to ABA (selected by germination at normally inhibiting concentrations of ABA) are beginning to reveal the complexities of the signalling cascades involving this hormone. At least two of the six *A. thaliana* mutants that are insensitive to ABA (*ABI1 and ABI2*) have been shown to express lower levels of seed dormancy (Finkelstein et al. 2002; Brocard-Clifford et al. 2003). Furthermore, lower levels of seed dormancy have been observed in *Arabidopsis* mutants that produce decreased levels of ABA relative to the wild type (Finkelstein et al. 2002). These findings also strongly suggest a link between ABA synthesis and seed dormancy in this species.

Mutant analysis has indicated a considerable degree of communication between signalling cascades influenced by ABA and other signalling cascades, indicating a high degree of complexity in the regulation of plant responses involving hormones. For example, in *Arabidopsis*, ABA-induced seed dormancy may be alleviated by sucrose, glucose, and only partially by fructose, while seed dormancy expression was unaffected by mannitol and sorbitol (Finkelstein and Lynch 2000; Brocard-Gifford et al. 2003). Interactions between ABA and ethylene as well as other plant hormones also may influence the expression of seed dormancy (Small and Gutterman 1992; Ghassemian et al.

2000; Rock 2000; Chiwocha et al. 2003). These ongoing discoveries are revealing complexities to seed dormancy mechanisms and regulation that were difficult, if not impossible to discern using traditional techniques and may aid in explaining results obtained from hormone studies that investigate seed dormancy. An understanding of the metabolic events that result in secondary seed dormancy in *B. napus* may ultimately be used to minimize 'weedy' attributes of this crop.

3.0 Harvest losses of canola (*B. napus*) cause large seedbank inputs

3.1 Introduction

Volunteer canola initially arises from seed losses incurred during harvest. In the U.K., where winter *B. napus* is usually direct harvested, seed losses range from 2 to 5% under ideal conditions to as high as 50% under unfavourable harvest conditions (Price et al. 1996). These harvest losses equate to average seedbank additions of approximately 5,000 to 7,000 seeds m^{-2} (Pekrun et al. 1998a). In the USA, similar harvest seed losses have been reported in the southeast (Thomas et al. 1991) and the Pacific Northwest (Brown et al. 1995). *B. napus* seed losses resulting from delayed harvesting tend to be greater than seed losses incurred by different harvest methods (Thomas et al. 1991; Brown et al. 1995; Price et al. 1996).

In western Canada, little is known about the total harvest seed losses incurred during canola production. Short growing seasons and the potential for pod shatter generally do not allow Canadian producers to direct harvest canola. Prewindrowing harvest losses of 9 kg ha^{-1} have been documented when seed moisture content was 37% at the time of windrowing (Bowren and Pittman 1975). These prewindrowing seed losses increased to 14.5 kg ha^{-1} when seed moisture content was allowed to reach 12% before windrowing (Bowren and Pittman 1975). These authors did not report seed losses incurred during and after windrowing.

A comprehensive determination of *B. napus* yield losses associated with harvest procedures on commercial farms in western Canada has not yet been documented. Because of the low seed size of *B. napus* (typically < 4 g based on weight of 1,000 seeds in western Canada), even small harvest losses can result in large additions to the seedbank. The objective of this research was to determine the average seedbank additions of *B. napus* from harvest losses incurred by producers in the semi-arid prairies.

3.2 Materials and Methods

3.2.1 Sample collection

In 1999 and 2000, fields were selected from producers located within a 30-km radius of Saskatoon, Saskatchewan. In the study conducted in 2000, fields from two producers were located approximately 150 km from Saskatoon. Where possible, two fields per producer were selected in each year. Some producers were included in both years while others were only able to provide field(s) in 1 year. A total of 16 and 19 fields were surveyed in 1999 and 2000, respectively. The sampled fields ranged from 24 to 130 ha. Canola was last grown on all sampled fields 3 or more years before the *B. napus* crop that was sampled, minimizing the potential for confounding effects from an established seedbank. All fields were sampled within 3 weeks of harvest. Autumn precipitation before freezing temperatures was low in both years (September to October: 20 mm in 1999, 24 mm in 2000) which resulted in negligible germination of *B. napus* seeds before and after sampling. Precipitation data were obtained from the University of Saskatchewan weather station located near Saskatoon, Saskatchewan, Canada, the centre of the sampling area and were representative of the region sampled. For each field, data collected from producers using a survey questionnaire included: crop seed yield, cultivar grown, years since the last canola crop, and perceived time of windrowing relative to crop maturity.

Samples were collected at three random locations in each field. A measuring tape was laid perpendicular to where two windrows had laid before harvest, from the centre of one windrow to the centre of the next windrow. The location of windrows was readily determined as the stubble that was under the windrow was distinctly less weathered than stubble not covered by windrows. Using a wet-dry vacuum cleaner, all remaining crop residue, non-harvested seeds, and some soil were removed from 25 cm by 25 cm quadrats at 1 m intervals along the transect. The number of quadrats from each transect (ranging from 6 to 9) was noted and used to determine the number of seeds lost per unit area. The bulked sample of each transect was placed in a paper bag, air dried at approximately 25 C and stored prior to further analysis.

3.2.2 Seed separation

Samples were passed through a round 7.14 mm hand sieve (Can-Seed Equipment Ltd., Winnipeg, MB, Canada) to remove large crop residue particles. A dockage tester (Carter-Day XT3 dockage tester, Carter-Day Company, Minneapolis, MN, USA) was used to separate the remaining samples into fractions larger and smaller than the *B. napus* seeds. The first separating steps were conducted with a No. 6 riddler sieve and a wind speed setting of 7.5. The main separating sieves consisted of a No. 20 (2.38 mm) round sieve that was used in the top position and a No. 1 (0.99 mm) round sieve that was placed in the middle position. A pan was located in the bottom position of the dockage tester. This sieve combination was chosen after repeated trials with *B. napus* seeds from various seedlots to ensure that all *B. napus* seeds passed through the top sieve, but were retained by the bottom sieve. The fraction retained by the middle sieve was then subjected to a wet sieving through a No. 16 (1.18mm) square-holed brass sieve (Endecotts Ltd., London, U.K.). The gentle stream of water was used to dissolve soil aggregates of a size similar to *B. napus* seeds, thus retaining only *B. napus* seeds, small stones and crop residue. Upon oven drying, the jagged stones were separated from *B. napus* seeds by rolling the seeds down a flat surface at a slight angle, followed by final hand sorting where necessary. The 1,000-seed weight of each sample also was determined after drying and adjusted to 8.5% moisture content. To determine seed viability in 2000, a sub-sample of 100 seeds was hand picked from each sample prior to wet-sieving and oven drying. Viability was determined by placing the 100-seed samples into petri dishes containing two layers of filter paper and 8 ml of distilled water. Dark germination was monitored over two weeks in a germination cabinet at 20 C and final germination percentages were tabulated. The remaining yield loss samples were adjusted, using the 1,000-seed weights of each sample.

3.2.3 Data Analysis

All data were tested for normality prior to analysis. One producer, who provided one field each season had harvest seed losses that were more than two standard deviations greater than the grand mean. Therefore, these field years were deemed outliers and were omitted. Total yield was determined by summing the yield and harvest losses. To determine differences among years, the remaining data were analysed using the MIXED procedure of SAS (Littell et al. 1996) as mean separation of unbalanced data is possible

using this procedure. Where significant differences among years could not be detected, the data were pooled. To determine differences in harvest seed loss, percent harvest loss and seed loss per square meter among producers, a subset of data was chosen, where at least two field-years of the same producer were available. In both analyses, a three-level nested design structure was used where fields were nested within producers as well as years. Least significant differences were determined when the model showed significance ($Pr > F = 0.05$).

The linear regression analysis was conducted on harvest loss vs. total yield, harvest loss vs. 1,000-seed weight and percent harvest loss vs. relative time of windrowing using the REG procedure of SAS (SAS Institute Inc., Cary, NC, USA). In all cases, the residuals did not indicate a more complex relationship.

3.3 Results and Discussion

Differences in yield, harvest loss and seedbank additions could not be detected between 1999 and 2000, whereas the 1,000-seed weights of *B. napus* were significantly different between the two study years (Table 3.1). The higher 1,000-seed weights in 1999 were probably a function of the higher precipitation that occurred during late summer that year (Table 4.2). The 1,000-seed weights measured in this study compared well with those reported in other experiments conducted in the area (Kirkland and Johnson 2000), suggesting that the harvesting process did not bias seed losses towards a particular seed size fraction.

The high standard errors observed in the seed loss and seedbank addition data (Table 3.1) indicates a large range in the observed values which contributed, in part, to the lack of significant differences. Over the 2 years, the mean seed loss was similar and found to be 107 kg ha^{-1} or the equivalent of approximately 3,600 seeds m^{-2} (Table 3.1). This amounted to approximately 5.5% (s.e. 0.8) of the total yield (Table 3.1). The germination test conducted in 2000 indicated that 82% (s.e. 6.6) of the seeds were viable. Thus, an average of 3,000 viable seed m^{-2} were added to the *B. napus* seedbank in 2000. Although seed viability was relatively consistent within year, seed viability may be more variable from year to year. As the typical seeding rate for *B. napus* is 4 to 5 kg ha^{-1}, seed loss observed in this experiment is 18 to 22 times the normal seeding rate. Quantitatively, these losses represent approximately 50% of those typically observed under ideal harvest conditions in the U.K. (Price et al. 1996). The generally lower 1,000-seed weight

TABLE 3.1 Total crop seed yield, harvest loss, percent harvest loss, thousand-seed weight, and seedbank addition of *B. napus* during on-farm harvest as influenced by year[a].

Year	Total yield	Harvest loss		1000-seed weight	Seedbank add.
	kg ha^{-1}	kg ha^{-1}	%	g	seeds m^{-2}
1999	1,980 (131)	110 (16)	5.6 (0.8)	3.15 (0.07)	3,570 (518)
2000	1,890 (115)	106 (14)	5.3 (0.7)	2.83 (0.07)	3,610 (473)
$LSD_{0.05}$	N.S.[b]	N.S.	N.S.	0.20	N.S.
Mean	1,930 (123)	107 (15)	5.5 (0.8)		3,590 (496)

[a] Standard errors are indicated in parentheses.

[b] N.S., not significant.

observed in *B. napus* grown in the semi-arid prairies compared to the U.K. account for the higher number of seeds per unit weight that was added to the seedbank. The harvest seed losses observed here (Table 3.1) also were lower than those reported for spring and winter *B. napus* in the United States using direct and windrowing harvest techniques (Thomas et al. 1991; Brown et al. 1995). Harvest losses observed here were proportionally similar to those observed under good harvesting conditions (2 to 5%) in the U.K. (Price et al. 1996) and were, on average, substantially less than those observed in winter *B. napus* (34.5%) (Thomas et al. 1991) and spring *B. napus* (15%) (Brown et al. 1995) in the United States. Nevertheless, the means reported in Table 3.1 do not include the two fields from one producer who incurred harvest losses of 25.0% of the total yield, which equated to seedbank additions of 13,900 seeds m^{-2}.

A relationship between harvest loss and 1,000-seed weight could not be detected ($Pr > F = 0.168$). A significant relationship between harvest loss and total yield also was not observed ($Pr > F = 0.432$). The lack of significant relationships indicates that seed size and total yield contributed little to the observed seedbank additions, which suggests that one or more other factors also must contribute to the *B. napus* seedbank additions.

Differences were observed in the quantity and percent harvest loss as well as seedbank additions among producers from whom two or more field years of data was collected (Table 3.2). Among these producers, average harvest seed loss percentages ranged from 3.3 to 9.9% (Table 3.2). These losses equated to seedbank additions of 1,530 to 7,130 seeds m^{-2} (Table 3.2), or a fourfold variation in the size of the seedbank additions generated by harvest losses.

Barring adverse environmental conditions, the two critical events that contribute to harvest losses in western Canada are the time of windrowing in relation to crop maturity as well as harvester setting and operation. In an attempt to isolate one of these components, producers were asked to estimate the time of windrowing of each field relative to the recommended time of windrowing (30 to 40% of seeds in pods on the main raceme have changed colour). These data were categorized into five distinct classes: early, early recommended, recommended, late recommended, and late, and were then compared to the observed percentage of harvest losses. No clear relationship was found ($Pr > F = 0.705$), indicating that one or more other factors also must contribute to the harvest losses incurred. The lack of a relationship was surprising as a number of producers indicated that they do examine the losses caused by the harvester and felt that

TABLE 3.2 Harvest loss and seedbank additions summarized by producers for which more than one field-year of data was available[ab].

Producer	Harvest loss		Seedbank addition
	kg ha^{-1}	%	seeds m^{-2}
1	146 (29) bc	9.6 (1.7) b	5,250 (994) bc
2	107 (29) ab	4.9 (1.7) a	3,760 (994) ab
3	44 (23) a	3.5 (1.4) a	1,530 (811) a
4	83 (20) ab	3.3 (1.2) a	2,660 (703) ab
5	97 (28) ab	4.3 (1.7) a	3,110 (994) ab
6	125 (23) b	6.8 (1.4) ab	4,300 (811) b
7	134 (28) b	6.9 (1.7) ab	4,480 (994) bc
8	78 (20) ab	5.4 (1.2) a	2,580 (703) ab
9	73 (20) ab	3.3 (1.2) a	2,430 (703) ab
10	226 (30) c	9.9 (1.7) b	7,130 (994) c

[a] Standard errors are indicated in parentheses.

[b] Means followed by different letters are significantly different, as determined by $LSD_{0.05}$ means separation.

the majority of their harvest losses were the result of delayed windrowing due to uneven crop maturity. Individual producer's perception of the recommended time to windrow, however, may not have been the same. Harvester operation may have been a contributing factor to high seedbank additions in at least two cases where sampling of one field occurred at the time of harvest. For the producer with seedbank additions that were deemed outliers, consistent improper combine settings appeared to be a contributing factor, while excessive combine operating speed of producer 10 (Table 3.2) may have been a contributing factor.

In summary, *B. napus* seedbank additions resulting from harvest losses varied greatly among producers. The average seedbank additions equated to approximately 20 times the normal seeding rate of 4 to 5 kg ha^{-1}, but ranged from 9 to 56 times the normal seeding rate among producers. Even with high mortality rates in the seedbank, additions of this magnitude could readily result in a significant volunteer *B. napus* weed problem for several years without the further contribution of escaped volunteers replenishing the seedbank. Alternatively, seedbank persistence may be exacerbated if a portion of seeds develop secondary dormancy (Pekrun 1994). *B. napus* harvest losses were neither related to total yield nor related to the relative time of windrowing. The results, however, do suggest that in some cases, the possibility exists to reduce *B. napus* seedbank additions at the time of harvest through improved harvest management. Further research is required to determine the contribution of these management factors to the harvest losses of *B. napus*. The lack of clear relationships between harvest loss and total yield as well as 1,000-seed weight suggests that similar harvest losses may be expected throughout the *B. napus* growing area of the Canadian prairies.

4.0 Secondary seed dormancy prolongs persistence of volunteer canola (*B. napus*) in western Canada

4.1 Introduction

In western Canada, spring canola is produced using tillage systems ranging from zero tillage (ZT) (soil disturbance at seeding only) to conventional tillage (CT) where soil disturbance generally occurs in autumn as well as prior to seeding. Nevertheless, soil inversion in CT is minimized through the use of field cultivators (chisel plows) to retain crop residue on the surface. This reduces soil erosion and conserves soil moisture. The harsh winters only allow for the production of spring canola in western Canada which contrasts with *B. napus* production in Europe where due to the milder climate, primarily winter genotypes are grown (Lutman 1993).

The *B. napus* seedbank is initially generated through harvest losses or seed contamination. The fate of the seedbank, however, is currently unclear. Each seed in the seedbank may suffer one of three possible fates: persistence, successful germination and emergence, or death (Figure 4.1). In contrast to eastern Canada (Simard et al. 2002) and Europe (Pekrun et al. 1997b), no evidence of winter survival of volunteer seedlings and volunteer plants exists in western Canada. Thus, for successful reproduction, all post-germination phases of the life cycle must be completed in the growing season during which germination and emergence occurred. As a result, a clear understanding of the seedbank ecology of this species is essential for effective management of volunteer canola.

Volunteer canola has been shown to persist for at least four years in rotation in western Canada (Légère et al. 2001). Re-seeding of the seedbank by volunteers was neither measured, nor controlled in these studies and as a result, these experiments gave little indication of the persistence potential of any one cohort of seeds in the seedbank. A pre-weed control survey conducted in Manitoba showed higher volunteer canola densities in ZT compared to CT (Thomas et al. 1997). A more recent study, however, has suggested

that tillage system does not influence the persistence of volunteer canola in western Canada (Légère et al. 2001). This contradicts earlier findings in the U.K., where management practices that extend the period of time seeds remain at the soil surface tend to be most effective at minimizing seedbank persistence of winter *B. napus* (Schlink 1994; Pekrun and Lutman 1998). In Alaska, burial of *B. rapa* at different depths resulted in observations similar to those found in Europe (Sparrow et al. 1990) as persistence increased with increasing burial depth. This study also demonstrated that over one winter, *B. rapa* seed survival was significantly enhanced when covered by snow.

Investigations in European winter and spring *B. napus* genotypes have shown differential potential for induction into secondary dormancy in *B. napus* (Pekrun et al. 1997a). Field experiments, have indicated that persistence of volunteer *B. napus* may be linked to the secondary seed dormancy potential of a cultivar, although other factors affecting seedbank persistence also play a role (Pekrun et al. 1997b and c; Pekrun and Lutman 1998).

Little is currently known with respect to the seedbank ecology of *B. napus* and *B. rapa* in western Canada and as a result, recommendations for integrated management of volunteer canola are limited. The objectives of this study were to determine the seedbank persistence and seedling recruitment of one cohort of *B. napus* genotype groups with varying potential for development of secondary seed dormancy under contrasting tillage systems. Further objectives included the determination of seedbank persistence and seedling recruitment of one cohort of two *B. rapa* genotypes.

4.2 Materials and Methods

4.2.1 Site location and experimental design

An experiment was established on stubble of a *B. napus* crop seeded in the spring of 1999 at two sites located in the Moist Mixed Grassland ecoregion (Acton et al. 1998) near Saskatoon, Saskatchewan. At both locations, the Kernen research farm (lat 52^{o} 09', long 106^{o} 33') and a farmer's field near Dundurn (lat 51^{o} 51', long 106^{o} 23'), the *B. napus* crop was removed from the experimental site in early August prior to seed shatter to avoid potential seedbank contamination. The soil at Kernen (Bradwell dark-brown Chernozem; 26% sand, 34% silt, 40% clay; 2.9% organic C; pH = 6.7) was heavier textured than that at Dundurn (Bradwell dark-brown Chernozem; 40% sand, 37% silt, 23% clay; 2.1%

organic C; pH = 7.1). The experiment was designed as a two factor [genotype (8) x tillage (2)], RCBD with four replicates for a total of 64, 3 by 7 m experimental units per location. At the time of experiment establishment, several soil cores were removed from guard plots at the ends of each replicate to confirm no seedbank contamination by *Brassica* spp. These were elutriated and no *Brassica* spp. seeds were found. In addition, no seedling recruitment of volunteer *B. napus* and *B. rapa* was observed in the control experimental units at the ends of each block throughout the duration of this experiment.

4.2.2 Genotype selection

Six *B. napus* and two *B. rapa* genotypes were chosen for this experiment. The *B. napus* seed was obtained at the time of harvest from the Canola Production Centre located near North Battleford (Aspen Parkland ecoregion) in Saskatchewan. This ensured that these genotypes were exposed to the same environment during maturation, a developmental stage that has been shown to influence seed dormancy characteristics (Gutterman 1980/81). The genotypes had a broad range of breeding systems, oil quality, and herbicide tolerance characteristics and included: Quantum; 2273; 46A73; Nex 500; LG3235, and IMC106.

These six randomly chosen *B. napus* genotypes were then classified according to their potential for development of secondary dormancy using a laboratory assay described by Pekrun et al. (1997b). This assay was repeated four times. Four of the *B. napus* genotypes had high (HDP) potential for development of secondary dormancy (ranging from 72 to 88%) and included 2273, 46A73, LG3235, Nex 500, while the remaining two genotypes (Quantum and IMC106) had medium (MDP) potential for the development of secondary dormancy (46 and 49%) (Chapter 5). Genotypes were grouped according to this seed dormancy classification system for data analysis.

B. rapa seed of AC Parkland and Hysyn 111 was obtained from one of the Regional Variety Trial locations (Canora, SK) in the same ecoregion. Dormancy induction in *B. rapa* seed could not be achieved using the assay conditions that result in dormancy induction in seed of *B. napus* and as a result *B. rapa* genotypes could not be classified according to seed dormancy potential.

4.2.3 Tillage systems

Two commonly used tillage systems in western Canada were chosen for this experiment. For conventional tillage (CT), two tillage passes using a light field cultivator were conducted each autumn after harvest. Each spring, two more tillage passes using a light field cultivator were conducted immediately prior to seeding in this system. At each time, the two successive tillage passes were conducted in opposite directions and the direction of the initial tillage pass was reversed between spring and autumn to reduce the potential of the seedbank being moved outside of the experimental unit. The soil profile was tilled to a depth of 15 cm during each tillage operation. The other tillage system chosen was zero tillage (ZT), where soil disturbance only occurred during seeding.

4.2.4 Study establishment and maintenance

Plots were established on October 20, 1999, and the seedbank and volunteer seedling emergence was evaluated until August 2002. The seedbank was established by distributing 2,000 viable seeds m^{-2} (Figure 4.1 - phase A) on each experimental unit using a small plot seed drill. The tubes delivering the seeds were removed from the openers and held in place approximately 30 cm above the soil surface. The openers were not allowed to disturb the residue and soil surface. Threshed *B. napus* residue equal to the density of the surrounding field was manually returned to each experimental unit prior to the first tillage event. Spring wheat was planted at 100 kg ha^{-1} using a double-disc, minimum disturbance seeder between May 10th and 18th of 2000, 2001 and 2002. At the time of seeding, fertilizer was applied according to soil test recommendations. In late April of 2001, a pre-seeding glyphosate burn-off was applied to all treatments (CT and ZT) at both sites to control the winter-annual weeds that were present. The wheat crop was harvested at maturity and the crop residue was returned to the experimental units.

Soil temperatures were measured for the duration of the experiment with the exception of May 2000 to Oct 2000, as the equipment was not available during this time. Daily mean, minimum and maximum temperatures at 1 and 10 cm were recorded in one replication of each tillage system using a CR10 data logger (Campbell Scientific Canada Corp., Edmonton, AB, Canada) with four thermocouples per treatment connected in series. At each location, cumulative monthly precipitation was calculated from measurements using a rain gauge.

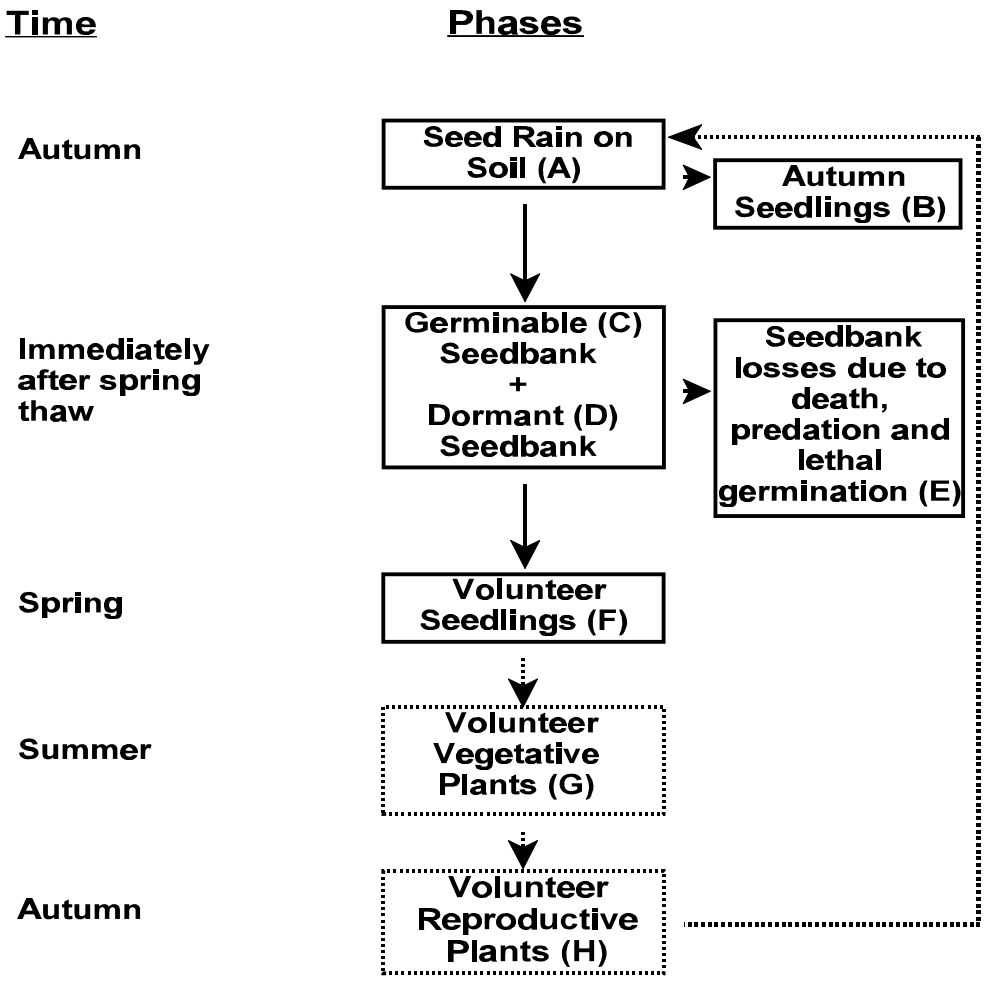

FIGURE 4.1 Flow diagram of the life cycle phases of volunteer canola and the temporal scale of their evaluation. Solid boxes and arrows indicate phases that were evaluated in this study, while finely dashed lines indicate phases that were not allowed. In the text, individual phases are referred to by their respective letters (A to F).

4.2.5 Spring seedbank determination

As early as the receding frost in the soil profile would allow (mid to late April of each year), the soil seedbank was sampled prior to any field operations. Ten soil cores per experimental unit were removed to a depth of 15 cm using a hydraulic soil probe, equipped with a 7.5 cm diameter coring tube. The cores were loosely broken and arranged in flats that were placed on shelves in a shed, immediately following removal from the field. Germination was conducted at ambient temperature (10 to 15 C) and artificial light conditions. The readily germinable seeds (Figure 4.1 - phase C) were quantified by irrigating the flats as necessary and recording seedling recruitment on a weekly basis. Immediately after quantification, the emerged seedlings were removed and each sample was stirred. This method ensured the germination of readily germinable as well as conditionally dormant seeds. After three weeks, the soil was allowed to air dry and stored for seed dormancy determination.

After one to six months, the remaining seeds were elutriated from the soil. The soil samples were coarsely broken and placed into 20 mesh sprayer strainers (Westward Parts Services Ltd., Red Deer, AB, Canada) that were capped at each end. Prior tests with various canola seedlots ensured that the seed would not pass through these strainers. The soil was washed from the strainers using an elutriator similar to that described by Wiles et al. (1996) with the exception that the cylinder to which the strainers were mounted was rotated by water pressure. Each strainer was submerged in water for approximately one half of each revolution. The total elutriation time for each sample was approximately 20 min. Immediately after elutriation, any canola seed was removed manually from the remaining sample and tested for viability by pinching with forceps. Firm seeds were presumed viable and dormant throughout the germination phase of sample analysis (Figure 4.1 - phase D). The validity of this test for *B. napus* was confirmed in one of the other field experiments. Spring seedbank persistence was determined from these measurements by summing the germinable and dormant seeds for each experimental unit (Figure 4.1 - phases C + D).

The minimum seedbank detection limit in this experiment was 22 seeds m^{-2} per experimental unit or 6 seeds m^{-2} per treatment if only one viable seed or emerged seedling was found among all soil cores taken from one experimental unit or treatment, respectively.

4.2.6 Volunteer seedling recruitment

Field emergence of volunteer seedlings (Figure 4.1 - phase B) was monitored and quantified prior to freeze-up in 1999 and at monthly intervals throughout April to October in 2000, 2001 and 2002 (Figure 4.1 - phase F). Seedling recruitment counts in May were conducted immediately prior to seeding, while counts in early June were conducted just prior to the typical period of in-crop weed control. Counts were conducted in six randomly placed 0.25 m^2 quadrats in each experimental unit. In 2002, whole plot counts were conducted as seedling recruitment densities were too low to quantify using quadrats. The first quantification of volunteer canola seedling recruitment was conducted immediately prior to seeding each year. When present, volunteer canola seedlings were controlled using low rates of MCPA immediately following each count (up to two applications per year). Total annual seedling recruitment is the sum of monthly seedling recruitment measurements per unit area.

4.2.7 Statistical analysis

All seedling and seed counts were pooled for each experimental unit. To meet the assumptions of ANOVA, all data were square root transformed. Prior to transformation, 0.001 was added to each value, to enable transformation of zero values. Means and standard errors of the means were calculated for genotype groups.

The GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA) was used to analyse the data for each location separately due to the strong influence of location on seedbank persistence. To determine differences among the genotype groups (HDP, MDP and *B. rapa*) four single-degree-of-freedom estimates were conducted within each ANOVA. This enabled the estimation of differences between HDP and MDP *B. napus* genotypes without the influence of the unclassified *B. rapa* genotypes. When the two-way interaction was significant ($Pr > F = 0.05$), differences between genotype groups were estimated within tillage systems.

The relationship between spring seed dormancy in 2000 was regressed against seedbank persistence of individual canola genotypes using the GLM procedure of SAS. In addition, total seedling recruitment in 2000 and 2001 was regressed against spring seedbank persistence of that year. These comparisons were conducted at both locations within tillage systems and on the combined data. Regression analysis was conducted on

transformed treatment means.

4.3 Results and Discussion

4.3.1 Spring seedbank

4.3.1.1 Seedbank persistence

Seed dormancy characteristics of *B. napus* genotype groups affected their seedbank persistence. The estimated differences between *B. napus* genotype groups indicated that spring seedbank persistence was higher in HDP *B. napus* genotypes compared to MDP *B. napus* in 2001, irrespective of tillage system and location (Table 4.1). A similar trend occurred in 2000, however, high variability obscured statistical significance. These results confirm trends observed in Europe where high dormancy genotypes also tend to exhibit greater seedbank persistence (Pekrun et al. 1997b; Pekrun and Lutman 1998). Mean persistence of HDP *B. napus* seed (back-transformed seeds m^{-2}) was about 6- and 12-fold higher than that of MDP *B. napus* genotypes in the clay (Kernen) and loam soil (Dundurn) in 2001, respectively (Table 4.1), indicating that fewer of the HDP seeds died and/or emerged as seedlings at this time. In 2002, few persisting seeds were detected in the seedbank, which was primarily due to persistence levels that were lower than the level of seedbank detection. Nevertheless, more persisting seeds of HDP *B. napus* genotypes were found than those of other genotype groups at both locations. In 2002, the highest seedbank persistence was found in LG3235 (HDP) in CT (11 seeds m^{-2}), with the equivalent of 6 seeds m^{-2} in 2273 (HDP) in ZT and Quantum (MDP) in CT in the clay soil. At the loam location, 11 seeds m^{-2} were found in 2273 (HDP) in CT, while 6 seeds m^{-2} were recorded in AC Parkland (*B. rapa*) in CT. As a result, the genotype group means ranged from 0 to 4 seeds m^{-2}. These results indicate that in western Canada, long-term seedbank persistence of *B. napus* can be reduced by growing genotypes with low potential for the development of secondary seed dormancy. Significant differences in seedbank persistence between other genotype groups were not observed and may in part be attributable to high variation among genotypes within these groups (Table 4.1).

In the spring of 2000, mean seedbank persistence was lower in conventional tillage (CT) compared to zero tillage (ZT) in the clay soil (Table 4.1). A similar trend was observed at the loam location, although the difference was not significant (Table 4.1). This suggests

TABLE 4.1 Seedbank persistence of two groups of *B. napus* (MDP, HDP) and *B. rapa* genotypes in conventional (CT) and zero tillage (ZT) at two locations in 2000 and 2001.

	2000			2001		
Genotype	**CT**	**ZT**	**Mean**	**CT**	**ZT**	**Mean**
	----------(Seeds $m^{-2})^{0.5}$----------					
Clay soil						
HDP B. napus[a]	23.3 (2.9)	27.3 (3.5)	25.3 (3.3)	5.2 (2.2)	2.6 (1.4)	3.9 (1.9)
MDP *B. napus*	19.5 (3.1)	29.7 (7.6)	24.6 (4.8)	2.3 (2.2)	0.9 (1.7)	1.6 (1.4)
B. rapa	20.3 (4.8)	29.2 (5.2)	24.8 (4.0)	4.6 (2.9)	0.6 (1.2)	2.6 (1.8)
Tillage mean[b]	21.6 b	28.4 a		4.3 a	1.7 b	
Estimates						
napus vs *rapa*[c]			0.3			0.5
HDP vs MDP			0.7			2.4 **
HDP vs *rapa*			0.5			1.3
MDP vs *rapa*			-0.2			-1.1
Loam soil						
HDP *B. napus*	27.2 (4.0)	27.2 (4.2)	27.2 (4.0)	1.2 (1.0)	3.1 (1.9)	2.1 (1.6)
MDP *B. napus*	20.1 (2.7)	28.3 (3.9)	24.2 (3.1)	0.6 (1.2)	0.6 (1.2)	0.6 (0.8)
B. rapa	25.6 (3.7)	23.2 (6.0)	24.4 (3.5)	2.9 (2.2)	0.6 (1.2)	1.7 (1.3)
Tillage mean	25.0 a	26.4 a		1.5 a	1.8 a	
Estimates						
napus vs *rapa*			1.8			-0.1
HDP vs MDP			3.0			1.5 *
HDP vs *rapa*			2.8			0.4
MDP vs *rapa*			-0.2			-1.1

[a] Data are square root transformed seeds m^{-2}. Standard errors of the means are indicated in parentheses.

[b] Means followed by different letters are significantly different and were separated using Fisher's protected LSD where appropriate.

[c] Estimated differences and significance of estimated differences are indicated where appropriate (** $p<0.01$ to <0.05, * $p<0.05$ to <0.10).

that short-term seedbank persistence may be greater under ZT compared to CT and that a greater portion of the seedbank in CT succumbed to those fates described in Figure 4.1 - phase E. No successful seedling recruitment was observed prior to freeze-up as well as prior to spring seedbank sampling in 2000 and therefore, all seeds lost from the seedbank in the spring must have been lost to death, predation, and/or lethal germination. These findings are in contrast to the observations in the U.K. where seed burial by tillage strongly contributes to seedbank persistence in winter *B. napus* (Pekrun et al. 1998b). Higher persistence in ZT after one winter may in part have contributed to observations in a Manitoba weed survey where higher volunteer canola seedling recruitment was found in ZT compared to CT (Thomas et al. 1997). In Alaska, greater seed survival of *B. rapa* was attributed to increased snow cover in ZT (Sparrow et al. 1990). Low autumn precipitation (Table 4.2) and higher mean soil temperatures in ZT between November and April (clay = -1.63 C, loam = -1.97 C) at a depth of 1 cm compared to CT (clay = -2.75 C, loam = -3.61 C) may have contributed to the higher levels of seedbank persistence in ZT after one winter in this study. In western Canada, low autumn precipitation has recently been associated with higher volunteer canola seedling recruitment during the following spring (Légère et al. 2001). Low autumn precipitation reduces the possibility of autumn germination as well as the loss of desiccation-tolerance in seed, thereby increasing seedbank persistence.

In the spring of 2001, the tillage system effect on seedbank persistence at the clay location was opposite to that observed in 2000. Although low in comparison to 2000, seedbank persistence was greater in CT compared to ZT (0.9 and 0.1% of back-transformed means, respectively) (Table 4.1). Trends were similar in all genotype groups (Table 4.1). These results suggest greater seed losses from the seedbank in *Brassica* spp. in ZT throughout the first year than in CT at this location. Nevertheless, similar results were not observed in the loam soil in 2001 where persistence in both tillage systems was similar to that observed in ZT after two winters in the clay soil (Table 4.1). The low persistence recorded in the loam soil in 2001 may be related to the sandy soil texture at this location. Similar observations were reported in one experiment conducted on winter *B. napus* in the U.K. (Lutman and López-Granados 1998).

In 2001, the seedbank persistence of *B. rapa* genotypes was substantially higher in CT than ZT, at both locations (Table 4.1). The significance of seed burial on overwintering of this species has been reported by Sparrow et al. (1990), but has not been documented over two seasons.

TABLE 4.2 Monthly norm and received precipitation during the growing season at the clay (Kernen) and loam (Dundurn) locations during this study.

Month	Norm[a]	1999	2000	2001	2002
	----------mm----------				
Clay soil location					
April	11	9	32	6	12
May	42	53	15	26	0
June	63	66	53	36	62
July	58	94	74	54	48
August	37	28	38	11	94
September	30	12	19	11	29
October	8	4	1	8	37
Total	254	266	232	152	282
Loam soil location					
April	13	13	20	0	0
May	37	43	18	18	0
June	64	102	58	18	99
July	63	119	0	43	97
August	40	31	15	0	91
September	34	3	33	0	61
October	14	0	0	3	8
Total	265	311	144	82	356

[a] 30-year norm obtained from Environment Canada (2002).

Although few persisting seeds were found in 2002, the tillage system trends were similar to those observed in 2001. Given maximum persistence of 0.2% of original seedbank additions in HDP *B. napus* in CT in the clay soil in 2002 and mean on-farm seedbank additions of 3,000 viable seeds m^{-2} using current production systems (Chapter 3), up to 6 seeds m^{-2} could be expected in fine textured CT fields three years after the last canola crop.

4.3.1.2 Seed dormancy

Differences in seed dormancy levels were found between canola genotype groups. The differences were highly significant in the clay soil in 2000 and showed weak levels of significance at the loam location in both years. Seed dormancy levels were greater in HDP than MDP genotypes in all cases (Table 4.3). In 2001, dormant seeds were only detected in HDP *B. napus* genotypes, at both locations (Table 4.3), indicating that spring seed dormancy is a more important component for seedbank persistence in HDP *B. napus* than in MDP and *B. rapa* genotypes. No dormant *Brassica* spp. seeds were detected in the spring of 2002 (data not shown). Statistical differences in spring dormancy levels were not detected between the tillage systems (Table 4.3). Little correlation between seed dormancy levels and tillage system has previously been observed in other weed species such as common lambsquarters (*Chenopodium album* L.) and redroot pigweed (*Amaranthus retroflexus* L.) (Mulugeta and Stoltenberg 1997).

On average, the dormant portion of the spring seedbank was low in all years (< 10% of back-transformed means) (Table 4.3). Nevertheless, a high proportion of the spring seedbank of the HDP *B. napus* genotypes was found to be dormant in CT in the loam soil in 2001. This observation may be related to low precipitation during 2000 at this location, particularly during the later portion of the growing season (Table 4.2). Moisture stress is an effective method for seed dormancy induction in *B. napus* (Pekrun 1994). Generally low levels of seed dormancy were not unexpected in the early spring because the laboratory experiments (Chapter 5) as well as those conducted on European genotypes have indicated that primary seed dormancy is negligible in *B. napus* (Schlink 1994; Pekrun 1994) and that secondary dormancy is readily released at low temperatures such as those experienced during late autumn and early spring. Higher persistence of HDP *B. napus* compared to MDP *B. napus* observed in 2001, but not 2000, may suggest that the

TABLE 4.3 Dormant seed of two groups of *B. napus* and *B. rapa* genotypes in conventional (CT) and zero tillage (ZT) at two locations in 2000 and 2001.

	2000			2001		
Genotype	**CT**	**ZT**	**Mean**	**CT**	**ZT**	**Mean**
	----------(Seeds $m^{-2})^{0.5}$----------					
Clay soil						
HDP *B. napus*[a]	7.0 (1.7)	7.0 (2.2)	7.0 (1.9)	0 (0)	0.4 (0.7)	0.2 (0.5)
MDP *B. napus*	3.0 (2.3)	3.8 (2.9)	3.4 (1.8)	0 (0)	0 (0)	0 (0)
B. rapa	4.3 (2.0)	5.3 (3.9)	4.8 (2.1)	0 (0)	0 (0)	0 (0)
Tillage mean[b]	5.3 a	5.8 a		0 a	0.2 a	
Estimates						
napus vs *rapa*[c]			1.0			0.1
HDP vs MDP			3.6 ***			0.2
HDP vs *rapa*			2.2 *			0.2
MDP vs *rapa*			-1.4			0
Loam soil						
HDP *B. napus*	4.8 (2.4)	6.4 (2.4)	5.6 (2.4)	0.8 (0.9)	1.0 (1.1)	0.9 (1.0)
MDP *B. napus*	2.5 (3.3)	3.4 (3.8)	3.0 (2.4)	0 (0)	0 (0)	0 (0)
B. rapa	6.1 (2.8)	5.9 (5.1)	6.0 (2.8)	0 (0)	0 (0)	0 (0)
Tillage mean	4.5 a	5.5 a		0.42 a	0.50 a	
Estimates						
napus vs *rapa*			-1.3			0.6
HDP vs MDP			2.6 *			0.9 *
HDP vs *rapa*			-0.4			0.9 *
MDP vs *rapa*			-3.0 *			0

[a] Data are square root transformed seeds m^{-2}. Standard errors of the means are indicated in parentheses.

[b] Means followed by different letters are significantly different and were separated using Fisher's protected LSD where appropriate.

[c] Estimated differences and significance of estimated differences are indicated where appropriate (*** $p<0.01$, * $p<0.05$ to <0.10).

influence of secondary seed dormancy on persistence is not completely realized until an entire year has passed and would concur with the laboratory findings where secondary seed dormancy is induced and maintained most effectively by higher temperatures (Chapter 6).

Further evidence that spring seed dormancy may aid persistence could be found using regression analysis between seedbank persistence in 2001 and spring dormancy levels in 2000. Significant linear relationships were found in the clay soil between spring dormancy levels in 2000 and seedbank persistence in 2001 in mean ($y = 0.28 + 0.49x$, where x = spring dormancy levels in 2000, and y = seedbank persistence in 2001, $Pr > F = 0.014$, $r^2 = 0.66$) and in CT ($y = 0.91 + 0.64x$, $Pr > F = 0.034$, $r^2 = 0.56$), but not in ZT ($Pr > F = 0.102$). At the loam location, these relationships were not significant ($Pr > F = 0.169$ to 0.299). Seedbank persistence in 2002 and seed dormancy levels in 2001 were too low to regress and support these observations.

Where significantly different, spring seed dormancy levels in HDP *B. napus* tended to be higher than in *B. rapa* in this experiment, suggesting that the role of spring seed dormancy as a persistence mechanism was of less importance in *B. rapa* than in HDP *B. napus* (Table 4.3). In 2000, seed dormancy levels in *B. rapa* were somewhat lower than those observed by Sparrow et al. (1990) in Alaska in the same species. This may be due to differences in climate, snow cover, soil properties, genotype, or seed burial method used.

Seedbank persistence in 2001 was lower than spring seed dormancy in 2000 at both locations and in both tillage systems, indicating the loss of seeds that were dormant in the spring of 2000 (Tables 4.1 and 4.3). At what point during the season these seeds either germinated and emerged or lost viability could not be determined in this experiment.

4.3.2 Seedling recruitment in the field

In this study, seedling recruitment of all *Brassica* spp. genotypes was seasonal in nature. Seedling recruitment was only observed during the months of May (pre-seeding) and/or June (prior to in-crop weed control) in all years (Tables 4.4 and 4.5). Seedbank losses due to autumn seedling recruitment did not occur in 1999. Low soil moisture conditions resulting from low precipitation (Table 4.2) likely contributed to the lack of autumn seedling recruitment in 1999. It is currently not known whether a lack of autumn seedling

TABLE 4.4 Field recruitment of two groups of *B. napus* and *B. rapa* genotypes in conventional (CT) and zero tillage (ZT) at the clay location in 2000, 2001 and 2002.

	Prior to seeding		After seeding		Total	
Genotype	**CT**	**ZT**	**CT**	**ZT**	**CT**	**ZT**
	----------(Plants m^{-2})$^{0.5}$----------					
2000						
HDP *B. napus*[a]	14.4 (1.8)	2.6 (0.7)	3.4 (0.3)	14.3 (1.3)	14.8 (1.8)	14.7 (1.2)
MDP *B. napus*	14.4 (2.7)	3.6 (1.9)	2.8 (0.4)	13.7 (2.0)	14.7 (2.7)	14.5 (1.5)
B. rapa	13.0 (2.4)	2.4 (0.8)	5.8 (1.3)	13.6 (2.6)	14.3 (2.6)	13.9 (2.6)
Tillage mean[b]	14.0 a	2.8 b	3.8 b	14.0 a	14.6 a	14.4 a
Estimates						
napus vs *rapa*[c]	1.0		-2.6 ***	0.5	0.6	
HDP vs MDP	-0.5		0.6 ***	0.6	0.1	
HDP vs *rapa*	0.8		-2.4 ***	0.7	0.6	
MDP vs *rapa*	1.4		-3.0 ***	0.1	0.5	
2001						
HDP *B. napus*	0.8 (0.4)	0.1 (0.2)	1.4 (0.4)	1.0 (0.4)	1.7 (0.4)	1.0 (0.4)
MDP *B. napus*	0.4 (0.4)	0.1 (0.2)	1.0 (0.6)	0.4 (0.3)	1.2 (0.5)	0.5 (0.3)
B. rapa	1.1 (0.6)	0 (0)	1.3 (0.9)	0.8 (0.6)	1.9 (0.8)	0.8 (0.6)
Tillage mean	0.8 a	0.1 b	1.2 a	0.8 b	1.6 a	0.8 b
Estimates						
napus vs *rapa*	-0.2		0		-0.2	
HDP vs MDP	0.2		0.5 *		0.5 *	
HDP vs *rapa*	-0.1		0.1		0	
MDP vs *rapa*	-0.3		-0.3		-0.5 *	
2002						
HDP *B. napus*	-[d]		0.4 (0.1)	0.4 (0.1)		
MDP *B. napus*	-		0.2 (0.1)	0.2 (0.1)		
B. rapa	-		0.8 (0.3)	0.5 (0.1)		
Tillage mean			0.4 a	0.4 a		
Estimates						
napus vs *rapa*	-		-0.5 ***	-0.2		
HDP vs MDP	-		0.2 *	0.2 *		
HDP vs *rapa*	-		-0.5 ***	-0.1		
MDP vs *rapa*	-		-0.6 ***	-0.3 ***		

[a] Data are square root transformed recruitment (plants m^{-2}). Standard errors of the means are indicated in parentheses.

[b] Means followed by different letters are significantly different and were separated using Fisher's protected LSD where appropriate.

[c] Estimated differences and significance of estimated differences are indicated where appropriate (*** $p<0.01$, * $p<0.05$ to <0.10).

[d] No recruitment was observed at that time.

TABLE 4.5 Field recruitment of two groups of *B. napus* and *B. rapa* genotypes in conventional (CT) and zero tillage (ZT) at the loam location in 2000 and 2001.

	Prior to seeding		After seeding		Total	
Genotype	**CT**	**ZT**	**CT**	**ZT**	**CT**	**ZT**
	----------(Plants m^{-2})$^{0.5}$----------					
2000						
HDP *B. napus*[a]	9.7 (1.0)	4.1 (0.7)	1.7 (0.4)	11.0 (1.8)	9.7 (1.0)	11.9 (1.7)
MDP *B. napus*	8.5 (1.7)	4.3 (2.3)	2.0 (0.6)	8.8 (1.9)	8.8 (1.6)	10.3 (1.8)
B. rapa	10.9 (1.4)	4.7 (1.2)	2.7 (1.1)	6.1 (1.9)	11.3 (1.6)	7.8 (2.0)
Tillage mean[b]	9.7 a	4.3 b	2.0 b	9.2 a	10.0 a	10.5 a
Estimates						
napus vs *rapa*[c]	-1.1 **		-0.9 ***	4.2 ***	-1.8 ***	3.5 ***
HDP vs MDP	0.4		-0.2	2.2 **	1.0 *	1.6
HDP vs *rapa*	-1.0 *		-1.0 ***	4.9 ***	-1.4 **	4.1 ***
MDP vs *rapa*	-1.4 **		-0.8 **	2.7 **	-2.5 ***	2.5 **
2001						
HDP *B. napus*	0.1 (0.1)	0.1 (0.1)	0.5 (0.3)	0.1 (0.1)	0.6 (0.3)	0.2 (0.2)
MDP *B. napus*	0 (0)	0 (0)	0.2 (0.3)	0.3 (0.3)	0.2 (0.3)	0.2 (0.3)
B. rapa	0 (0)	0 (0)	0.9 (0.7)	0.4 (0.3)	0.9 (0.7)	0.4 (0.3)
Tillage mean	0.1 a	0.1 a	0.6 a	0.2 a	0.6 a	0.3 a
Estimates						
napus vs *rapa*	-[d]		-0.5 **	-0.3 *	-0.5 **	-0.2
HDP vs MDP	-		0.3	-0.1	0.3	-0.1
HDP vs *rapa*	-		-0.4 *	-0.3 *	-0.4 *	-0.2
MDP vs *rapa*	-		-0.7 **	-0.2	-0.7 **	-0.2
2002						
HDP *B. napus*	-[e]		0.2 (0.1)	0.1 (0.1)		
MDP *B. napus*	-		0.1 (0)	0.1 (0.1)		
B. rapa	-		0.4 (0.2)	0.2 (0.1)		
Tillage mean			0.2 a	0.1 a		
Estimates						
napus vs *rapa*	-		-0.2 ***			
HDP vs MDP	-		0.1			
HDP vs *rapa*	-		-0.2 ***			
MDP vs *rapa*	-		-0.3 ***			

[a] Data are square root transformed recruitment (plants m^{-2}). Standard errors of the means are indicated in parentheses.

[b] Means followed by different letters are significantly different and were separated using Fisher's protected LSD where appropriate.

[c] Estimated differences and significance of estimated differences are indicated where appropriate (*** $p<0.01$, ** $p<0.01$ to <0.05, * $p<0.05$ to <0.10).

[d] Too many zero values for valid comparisons.

[e] No recruitment was observed at that time.

recruitment was due to secondary seed dormancy or low autumn precipitation in all other years. These results are in contrast to results from volunteer winter *B. napus* in Europe, where seedling recruitment may occur throughout the entire growing season (Lutman 1993). To my knowledge, this periodicity of seedling recruitment has not been previously documented in these species. No volunteer *Brassica* ssp. had emerged prior to the pre-seeding glyphosate burn-off that was applied to all treatments in 2001.

Seedling recruitment of HDP *B. napus* was higher than MDP *B. napus* at all times when statistically significant differences were observed between these groups (Tables 4.4 and 4.5). These results were primarily a function of the greater seedbank persistence observed in the HDP *B. napus* genotype group (Table 4.1) at these times. In 2001 and 2002, however, these differences were statistically weak and observed only in the clay soil (Table 4.1). Nevertheless, the results suggest greater seedbank losses of MDP *B. napus* due to greater levels of seed mortality.

In CT, seedling recruitment of *B. rapa* tended to be greater than seedling recruitment of *B. napus* (individually and combined) when differences were observed (Tables 4.4 and 4.5). Given similar persistence between these two species throughout the experiment (Table 4.1), this suggests a greater proportion of successful seedling recruitment and concomitantly lower seed mortality in *B. rapa* compared to *B. napus* in CT. The opposite was observed in ZT at the loam location in 2000 only, indicating greater seed mortality in *B. rapa* after one winter in ZT relative to *B. napus*. An explanation for this difference is not known.

Seedling recruitment differences (Tables 4.4 and 4.5) observed in 2002 were strongly influenced by Hysyn 111 at both locations. This *B. rapa* genotype displayed greater seedling recruitment (approximately 1 plant m^{-2} in the clay soil) (data not shown) than all other genotypes (Table 4.4). These observations may give credence to testimonials that suggest that persistence of *B. rapa* is greater than *B. napus*; however, these observations were not detectable in the seedbank (Tables 4.1 and 4.3).

In general, total annual seedling recruitment in the field was poorly related to seedbank persistence. Significant linear relationships were only observed in mean seedling recruitment in 2000 ($y = 2.74 + 0.47x$, where x = seedbank persistence, and y = total seedling recruitment for that year, $Pr > F = 0.007$, $r^2 = 0.73$) and 2001 ($y = 0.75 + 0.16x$, $Pr > F = 0.025$, $r^2 = 0.60$) in the clay soil and in ZT in the loam soil in 2000 ($y = -1.80 + 0.46x$, $Pr > F = 0.043$, $r^2 = 0.52$). No highly significant relationship was found in the

remaining combinations of tillage systems within locations (Pr > F = 0.08 to 0.79). In all cases, the residuals did not indicate a more complex relationship. The method used here for seedbank evaluation (stirring the samples rather than observing germination from undisturbed cores), however, has previously been shown to result in poor predictions of seedling recruitment in the field (Cardina and Sparrow 1996). Nevertheless, high variation in seedling recruitment relative to the seedbank has been observed previously in other annual weeds and was attributed to variations in microclimate variables and induction into secondary dormancy (Forcella et al. 1997).

Although total seedling recruitment in 2000 was similar between the tillage systems, distinct differences in the pattern of volunteer seedling recruitment were observed between CT and ZT at both locations. In CT, the majority of total annual seedling recruitment (clay soil approximately 92%, loam soil approximately 95% of back-transformed means) occurred in early May prior to seeding, while only low seedling recruitment levels (clay soil approximately 3.8%, loam soil approximately 16% of back-transformed means) were observed in ZT during this period (Tables 4.4 and 4.5). In contrast, high levels of seedling recruitment were observed in ZT in June, the period after seeding but prior to in-crop weed control (Tables 4.4 and 4.5). This cohort of seedling recruitment in ZT was likely the result of a series of rains during that time (Table 4.2). Little residue left after canola harvest resulted in dry soil surface conditions in ZT that likely prevented germination of seeds until these rains. The findings suggest that after the first winter, pre-planting seedling recruitment of volunteer canola may be more reliable in CT tillage. This would facilitate volunteer canola management as a tillage pass effectively controls all volunteer seedlings, thereby avoiding the potential of incomplete seedling control through herbicide escapes. Post-planting seedling recruitment in CT in 2000 amounted to fewer than 15 and 4 plants m^{-2} in the clay and loam soil, respectively (Tables 4.4 and 4.5). Nevertheless, volunteer canola densities of this magnitude may still warrant in-crop weed control (Lutman et al. 1996). In contrast to 2000, maximum seedling recruitment occurred during the post-seeding, pre-weed control period in 2001 and 2002 at both locations (Tables 4.4 and 4.5). This change in seedling recruitment pattern may have been strongly influenced by the lack of precipitation earlier during these years (Table 4.2). In 2002, some seedling recruitment was still observed in most experimental units in the clay soil (Table 4.4), while seedling recruitment was less in the loam soil in either tillage system (Table 4.5). This supports the seedbank observations where persistence of canola tended to be greater in the fine-textured soil (Table 4.1).

Some evidence has indicated that residual 2,4-D, may decrease canola seedling emergence for some time after application (Kirkland 1997). The herbicide used to control successive flushes of volunteer seedlings, MCPA, is a phenoxycarboxylic acid similar to 2,4-D. It does not appear that the herbicide treatments (up to two applications per year, one in May and/or June) influenced the results substantially as the same patterns of seedling recruitment were observed in a different study at these locations, where emerged seedlings were controlled by hand.

4.3.3 Management implications

In this study, seedbank persistence differences between tillage systems were less pronounced compared to results previously reported in winter *B. napus* in the U.K. (Pekrun and Lutman 1998). This may be due to climatic differences, as well as the more shallow tillage operations in western Canada, which leave the bulk of the seedbank closer to the soil surface relative to the U.K. (Clements et al. 1996). The proportion of persisting seeds tended to be higher in CT than ZT due to lower seedbank mortality (Tables 4.1, 4.4 and 4.5), but no clear distinction in seedbank persistence in terms of absolute time could be made between these two tillage systems. Thus, the results agree with previous observations in western Canada that indicated no clear advantage of ZT over CT in reducing the longevity of the volunteer canola seedbank (Légère et al. 2001). In *B. napus*, secondary seed dormancy potential contributed to higher seedbank persistence and volunteer seedling recruitment over time. Both observations were a result of lower seedbank mortality in HDP genotypes. These results suggest that low dormancy genotypes be grown if the potential for volunteer populations is to be minimized. Unfortunately, information with respect to the seed dormancy potential of individual genotypes is currently not readily available to producers. In this study, initial seedbank additions were lower than on-farm harvest losses (Chapter 3). Nevertheless, some persistent seeds as well as volunteer seedling recruitment were observed 3 years after seedbank initiation, even at low initial seedbank levels and when seedbank replenishment was not allowed. Below normal precipitation during late summer and autumn (Table 4.2) may have contributed to the observations on canola seedbank longevity. Nonetheless, adequate temporal separation is recommended between subsequent canola crops of different quality and herbicide tolerance characteristics in the same field where pollen-mediated gene flow and direct seed contamination from volunteers are to be minimized. Volunteer canola seedling recruitment one and two years after canola production may

result in high levels of interference with subsequent crops. Therefore, crops in which volunteer canola is easily controlled using in-crop herbicides, should be grown immediately following a canola crop. In this study, all volunteer canola seedling recruitment occurred prior to the typical time of in-crop weed control. Therefore, the control of volunteer canola populations using in-crop herbicides was possible. The mechanisms regulating the seasonal seedling recruitment behaviour in *B. napus* remain unknown.

5.0 Genotype, seed size and environment contribute to secondary seed dormancy potential in Canadian spring *B. napus*

5.1 Introduction

B. napus is one of the most recently domesticated oilseed crops grown in western Canada. Recent investigations in Europe and western Canada have indicated that the persistence of volunteer *B. napus* may be linked to the potential for the development of secondary seed dormancy (Pekrun et al. 1997a, 1998a; Chapter 4). Nevertheless, the relative importance of factors that influence secondary seed dormancy potential in this species are unclear.

Seed dormancy is a complex phenomenon and is an important persistence mechanism for many annual weeds in temperate regions (Baskin and Baskin 1998). Seed dormancy expression is well characterized in some species and is a function of genotype (Garbutt and Witcombe 1986; Foley and Fennimore 1998), location of the progeny on the mother plant (Nimer at al. 1983), as well as environmental conditions during seed maturation (Gutterman 1980/81; Baskin and Baskin 1998). In addition, post-dissemination environmental conditions may influence seed dormancy (Baskin and Baskin 1976; Standifer and Wilson 1988; Hazebroek and Metzger 1990). A genetic component to secondary seed dormancy potential has been suggested in European and Chinese *B. napus* genotypes and dormancy potential ranged between zero and 85% among the genotypes investigated (Pekrun et al. 1997a; Momoh et al. 2002). However, the significance of this contribution relative to other factors remains unclear.

Seed size may also influence seed dormancy expression. In celery (*Apium graveolens* L.), for example, the smallest seeds tend to exhibit highest levels of primary seed dormancy (Thomas et al. 1979), while in India ricegrass (*Achnatherum hymenoides* [Roem. & Schult.] Barkw.), the largest seeds exhibit the greatest degree of seed dormancy (Jones and Nielson 1999). In *B. juncea* L., a species closely related to *B. napus*, germination tends to be lower in small seed (Dubey et al. 1989; Svaton 1993; Kant and

Tomar 1995), while this is generally not observed in *B. napus* (Svaton 1993). Dormancy status of the ungerminated seeds was not reported in the studies. The objectives of these experiments were to determine the relative contribution of genotype and other factors including location, year, seed size, seed maturity, and storage conditions on the potential for secondary seed dormancy expression in Canadian spring *B. napus*.

5.2 Materials and Methods

5.2.1 Dormancy assay

An efficient assay for assessing secondary seed dormancy in *B. napus* was described by Pekrun et al. (1997a). In this assay, polyethylene glycol (PEG) is used to lower the water potential of the imbibing solution (-1.5 MPa) to levels at which germination in non-dormant *B. napus* seed is prevented and secondary seed dormancy is induced. Persistence of *B. napus* seed in the field correlates well with secondary dormancy potential determined using this laboratory assay (Chapter 4). To conduct the assay, 100 seeds were placed in each 9 cm plastic petri dish (Phoenix Biomedical, ON, Canada) on two layers of filter paper (VWR Grade 413, VWR International, Inc.). Each treatment was replicated four times. To induce secondary dormancy, the seeds were incubated in the dark at 20 C in PEG-8000 (Sigma Chemical Co., MO, USA) solution with an initial osmotic potential of -1.5 MPa (Michel 1983). Plastic bags impervious to light were used to ensure no inadvertent exposure to light during these experiments. After 4 weeks in a germination cabinet, the seeds were transferred to new petri dishes containing distilled H_2O in a darkroom under a green (Strand Century Ltd., ON, Canada) safe light and then returned to the germination cabinet. One week later, the remaining ungerminated seeds were transferred again to new petri dishes under green light. After an additional week, all firm, non-germinated seeds were counted on the lab bench (approximately 20 C) under fluorescent lighting. Following this assessment, the firm, non-germinated seeds were stratified for 5 days at 2 to 4 C (Pekrun, 1994) and returned to the 20 C germination cabinet. After one week, any seed that did not germinate was examined for viability by exposure to tetrazolium (2,3,5 triphenyl tetrazolium chloride, 1% w/v) (Sigma Chemical Co., MO, USA) for 3 days at 20 C. To determine seedlot viability and primary dormancy, if present, germination of non-osmotically treated seed was conducted at 20 C in darkness, using two to four replicates of 100 seeds.

5.2.2 Seed source, genotype and environment comparison (Exp. 1)

In 1999, seed from 16 commercially available *B. napus* genotypes grown in large-scale plot evaluation trials were obtained at the time of harvest from two Canola Production Centre locations in Saskatchewan (Canola Council of Canada, 1999) (Table 2). The two sites from which the genotypes were obtained, North Battleford (lat 52^{o} 49', long 108^{o} 15') and Grenfell (lat 50^{o} 26', long 102^{o} 54'), are both located in the Aspen Parkland ecoregion (Acton et al. 1998), but were separated by approximately 550 km. In 1999, this study included 16 genotypes with a wide range of breeding systems, quality traits, and tolerance to herbicides (Table 2). Two of these genotypes, MilleniUM 01 and Nex 500, were of specialty oil quality. Only six of these 16 genotypes were common in the 2000 variety evaluations at these same locations. The dormancy assay was repeated four times in 1999 and two times in 2000 and replicates were blocked within the germination cabinet. In both years, the initiation of individual repetitions of the experiment occurred between mid-September and the following February. Unless otherwise indicated, seeds were stored at -15 C.

Precipitation varied between years at the two locations. At Grenfell, precipitation was 458 and 339 mm throughout the entire growing season (May to Sept.) and 104 and 100 mm during the seed maturation period (Aug. to Sept.) in 1999 and 2000, respectively (Canola Council, 1999, 2000). In North Battleford, accumulated precipitation throughout the growing season was 285 and 196 mm in 2000. Throughout the seed maturation period, total precipitation was 62 and 113 in 1999 and 2000, respectively (Canola Council of Canada, 1999, 2000). The locations, environmental parameters and agronomic data are described in more detail in Canola Council of Canada (1999, 2000).

5.2.3 Seed maturity (Exp. 2)

Seed from two high dormancy (HDP) (AC Excel and LG 3295) and two low dormancy (LDP) genotypes (LG Dawn and Option 501) obtained from North Battleford in 1999 was used for the remaining experiments. In the spring of 2000, 2001, and 2002, seed from these seedlots was planted at 5 kg ha^{-1} in 8m x 8m areas isolated by at least 20 m at the Kernen research farm (lat 52^{o} 09', long 106^{o} 33'). The plots were fertilized according to soil tests. Seed maturity was manipulated by varying the harvest regime. One-third of each plot was windrowed at the recommended time (30 to 40% of seed in pods on the main rachis have turned colour), one-third was windrowed later than recommended, once

95 to 100% of the seeds in pods on the main rachis had turned colour, while the remaining third was not windrowed prior to harvest. All treatments were harvested at the same time (Oct 3, 2000; Sept 13, 2001; Oct 22, 2002). In 2000, the recommended time of windrowing occurred during the last week of August in all genotypes. In 2001, the first windrowing date occurred approximately one week earlier than 2000, while in 2002, the first windrowing date occurred in early September. The late windrowing was conducted approximately two weeks after the first date of windrowing in all years. Due to excessively dry conditions in the field, approximately 75 mm of supplemental irrigation was applied during August 2001 to ensure some progeny and also in May 2002 to aid germination and establishment. Seed from each treatment was subjected to the dormancy assay immediately following harvest. The dormancy assay was conducted only one time each year and treatments were completely randomized in the germination cabinet.

5.2.4 Seed size (Exp. 3)

In 2000, 2001, and 2002, seeds from all four genotypes from the recommended windrowing date of the seed maturity study (Exp. 2) were passed through a stack of round-holed sieves (Can-Seed Equipment Ltd., SK, Canada) and sorted according to three arbitrary size classes (large, 1.98 to 2.38 mm; medium, 1.59 to 1.98 mm; small < 1.59 mm). The proportion of each fraction relative to the total seedlot was determined gravimetrically and the dormancy assay was used to determine secondary seed dormancy potential among the different seed size fractions. The dormancy assay was conducted only one time each year immediately following harvest and treatments were completely randomized in the germination cabinet.

5.2.5 Seed storage (Exp. 4)

Immediately following harvest in 2000, samples from the recommended time of windrowing from the four genotypes were stored dry in freezers at -15 C and -70 C, as well as at ambient conditions in an uninsulated building where the annual temperature variations ranged between -12 and +35 C. Every two months, seed from each treatment was subjected to the dormancy assay. This experiment was continued until October 2002.

5.2.6 Data analysis

All data were expressed as percent secondary seed dormancy in relation to the mean viable, non-dormant portion of seed from the non-osmotically treated germination controls. Bartlett's test was used to test for heterogeneity of variances among treatments, years and locations. This test was significant in several instances. To meet the assumptions of ANOVA, all data were transformed using the square root method. In experiments that contained zero values, 0.001 was added to all percentage values prior to square root transformation.

The genotype comparison experiment (Exp. 1) was analysed as a nested three-way factorial (genotype * location * year) using the MIXED procedure of SAS (SAS Institute Inc., NC, USA), where the treatments and replicates were nested within each repetition of the experiment. The MIXED procedure is more accurate when dealing with unbalanced (year) and nested designs than the GLM procedure (Littell et al. 1996). Repetitions of the experiment were considered a random factor and therefore any interactions containing this term could not be determined. Significant interactions between cultivars, years, and locations resulted in the need for analysis within year and location. Standard errors of the difference between cultivar means were calculated within location and year.

Data from the seed maturity (Exp. 2) and seed size experiments (Exp. 3) were analysed using the GLM procedure. Both experiments were analysed as three-way factorials (treatment * genotype * year) and the data were sliced as significant interactions demanded.

The seed storage experiment (Exp. 4) data were analysed as a repeated measures two-way factorial (storage treatment * time) within each genotype using the MIXED procedure of SAS. After choosing the most accurate covariance structure, time was modelled as the regression variable with linear and quadratic components and a solution for the most appropriate regression equation for each storage treatment within each genotype was determined (Littell et al. 1996). To determine differences among the storage treatments, three single-numerator-degree-of-freedom contrasts were conducted.

To determine the contribution of each factor to total variance in each experiment, the proportion of the Type III marginal sums squares (SS) of each factor in relation to the corrected total SS of the experiment was determined using the GLM procedure of SAS. To compare between the genotype experiment (16 genotypes) (Exp. 1) and all other experiments (Exp. 2 to 4), a subset of data from Exp. 1 containing only the genotypes

used in Exp. 2 through 4 was subjected to analysis of variance components (Exp. 1sub). The variation among treatments in the seed maturity study (Exp. 2) was considered to be primarily due to differences in seed maturity at the time of treatment implementation. Although there may have been an environmental component contributing to these observations, it was assumed that its contribution was negligible. There were no possible means of testing this assumption. In the seed storage experiment (Exp. 4), variation among successive repetitions of the dormancy assay was considered as the time factor, while the variation between storage treatments was considered to be primarily the result of temperature differences among these treatments.

5.3 Results

5.3.1 Genotype and environment study

A broad range in secondary seed dormancy potential was observed among the genotypes examined in this experiment (Table 5.1). Nonetheless, the 16 genotypes examined in 1999 could be consistently classified into three groups relative to each other. LG Dawn and Option 501 exhibited low secondary seed dormancy potential (LDP) (back-transformed means of 16 and 31% at North Battleford and 24 and 27% at Grenfell in 1999, respectively), while Quantum and IMC107 had medium (MDP) secondary seed dormancy potential (back-transformed means of 45 and 60% at North Battleford and 36 and 42% at Grenfell, respectively). Secondary seed dormancy potential in the remaining 12 genotypes was classified as high (HDP) and back-transformed means ranged from 74 to 90% at North Battleford and 49 to 77% at Grenfell in 1999. These observations were consistent among the two locations (Table 5.1). In 1999, secondary seed dormancy expression was lower in all genotypes at Grenfell compared to North Battleford, except in Option 501 (Table 5.1). In the six genotypes examined in 2000, secondary seed dormancy expression was similar to that observed in 1999 (Table 5.1). In all genotypes, germination of the non-osmotically treated seed in darkness was greater than 94% except for the seed size experiment (Exp. 3). No differences in primary seed dormancy were observed in any experiment (Exp. 1 to 4) and ranged from 0 to 4% among all replicates.

TABLE 5.1 The effect of location and year on secondary dormancy potential among *Brassica napus* genotypes. Tolerance to herbicides (HT), breeding system (BS), and seed distributer are also indicated.[ab]

				Secondary seed dormancy potential			
				North Battleford		Grenfell	
Genotype	**HT**[c]	**BS**[d]	**Distributer**	**1999**	**2000**	**1999**	**2000**
				----------(%)$^{0.5}$----------			
AC Excel	-	OP	SeCan Association	9.5 (0.1)	9.3 (0.2)	8.0 (0.7)	8.5 (0.2)
Exceed	glu	OP	Agricore	8.9 (0.1)		7.2 (1.0)	
IMC107	gly	OP	Inter-Mountain Canola	7.8 (0.3)		6.5 (1.0)	
2273	glu	HY	AgrEvo Canada Inc.	9.4 (0.1)		7.7 (0.7)	
2463	glu	HY	AgrEvo Canada Inc.	9.3 (0.1)		7.7 (0.8)	
2473	glu	HY	AgrEvo Canada Inc.	9.4 (0.1)		8.3 (0.6)	
LG3235	gly	OP	Limagrain Can. Seeds Inc.	9.1 (0.1)	8.0 (0.2)	7.3 (0.9)	8.5 (0.2)
LG3295	gly	OP	Limagrain Can. Seeds Inc.	9.5 (0.1)		8.8 (0.3)	
LG Dawn	gly	OP	Limagrain Can. Seeds Inc.	5.6 (0.3)	3.9 (0.3)	4.9 (1.0)	4.7 (0.5)
Magnum	-	OP	Value Added Seeds	9.0 (0.1)		8.3 (0.5)	
MilleniUM 01	-	OP	Can-Amera Foods	8.2 (0.3)		7.0 (0.9)	
Nex 500	-	OP	Dow AgroSciences Can. Inc.	8.6 (0.1)	7.3 (0.2)	8.2 (0.7)	7.4 (0.4)
Option 501	-	OP	Advanta Seeds	4.0 (0.3)		5.2 (0.6)	
Quantum	-	OP	University of Alberta	6.7 (0.4)	7.1 (0.3)	6.0 (1.1)	7.0 (0.7)
SW RideR	gly	OP	Agricore	9.3 (0.0)	8.2 (0.2)	7.6 (0.6)	8.4 (0.3)
Sentry	-	OP	Value Added Seeds	8.8 (0.1)		8.1 (0.6)	
$LSD_{0.05}$				0.5	0.7	0.8	0.9

[a] Means are square root transformed percentages.

[b] Standard errors of the means are indicated in parentheses.

[c] Herbicide tolerance, gly = glyphosate, glu = glufosinate, - = no tolerance.

[d] Breeding system, OP = open pollinated, HY = hybrid.

5.3.2 Seed storage

During storage, secondary seed dormancy potential decreased at significantly different rates among treatments. In AC Excel, LG3295 and LG Dawn, seed dormancy potential decreased most over time when stored at ambient temperatures and decreased least when stored at -70 C (Figure 5.1). In the HDP genotypes, the observed decrease in secondary seed dormancy potential was curvilinear over the two year duration of the experiment and differences among all storage treatments were significant ($Pr > F = <0.0001$ to 0.011). In the LDP genotypes the relationship was linear over time and significant differences were only observed in LG Dawn between the -70 C and ambient treatment ($Pr > F = 0.002$) as well as the -15 C and ambient treatments ($Pr > F = 0.028$). In Option 501, a significant treatment effect was observed between the -70 C and the ambient storage treatments ($Pr > F = 0.027$).

5.3.3 Seed size and maturity

In all genotypes, a greater portion of the large seeds developed secondary seed dormancy than small seeds (Figure 5.2). Observations indicated that the majority (range = 73 to 97 seeds replicate^{-1}) of the small seeds had lost viability by the end of osmotic stress treatment. This was not observed in the medium and large seeds (range = 0 to 12 seeds replicate^{-1}). In AC Excel, LG3295, and LG Dawn, a more rapid decline in seed dormancy potential as seed size decreased was detected in 2001, compared to 2000 and 2002 (Figure 5.2). This was not observed in Option 501.

Differences in seed viability among the three seed size classes were consistent, irrespective of genotype. In large seed (97.3%), seed viability was greater than in medium seed (95.5%) (Table 5.2), while seed viability in small seed (92.1%) was lowest (Table 5.2). The relative proportions of the large and medium seed size fractions of each genotype varied greatly among the three years (Table 5.2). In 2000, the large:medium seed size ratio was smallest among all genotypes, while in 2001, this ratio was greatest. The smallest seed size fraction consistently contributed very little to total seedlot composition (Table 5.2).

The effect of seed maturity as influenced by harvest regime on the secondary seed dormancy potential was statistically significant in only five of twelve instances (Figure 5.3). When statistically significant, secondary seed dormancy potential was greatest at

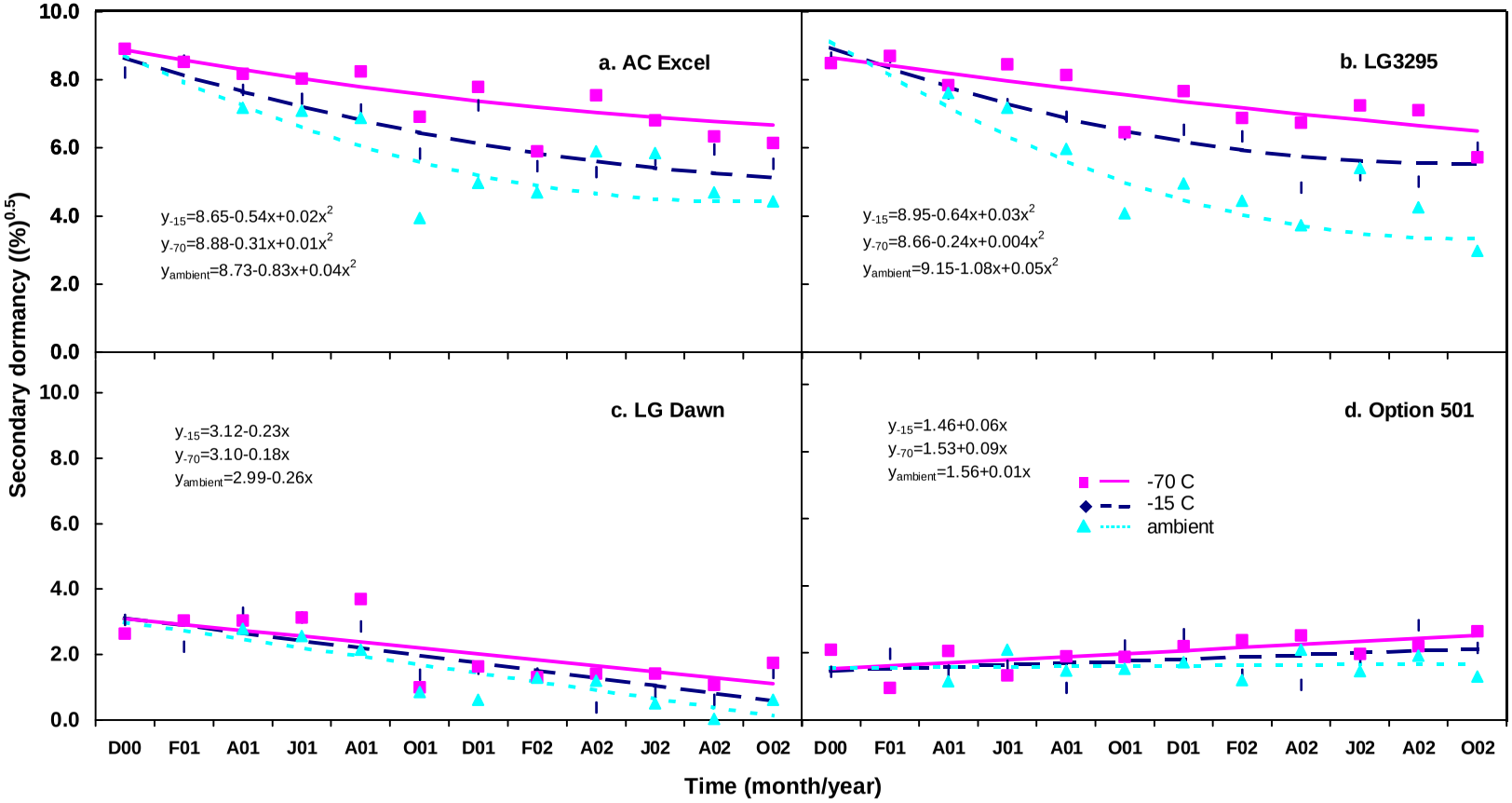

FIGURE 5.1 The influence of storage time and conditions (-15 C, -70 C, and ambient temperature) on *B. napus* secondary seed dormancy potential in two genotypes with high (a. AC Excel, b. LG3295) and two genotypes with low (c. LG Dawn, d. Option 501) secondary seed dormancy potential. The regression equations for each treatment are indicated.

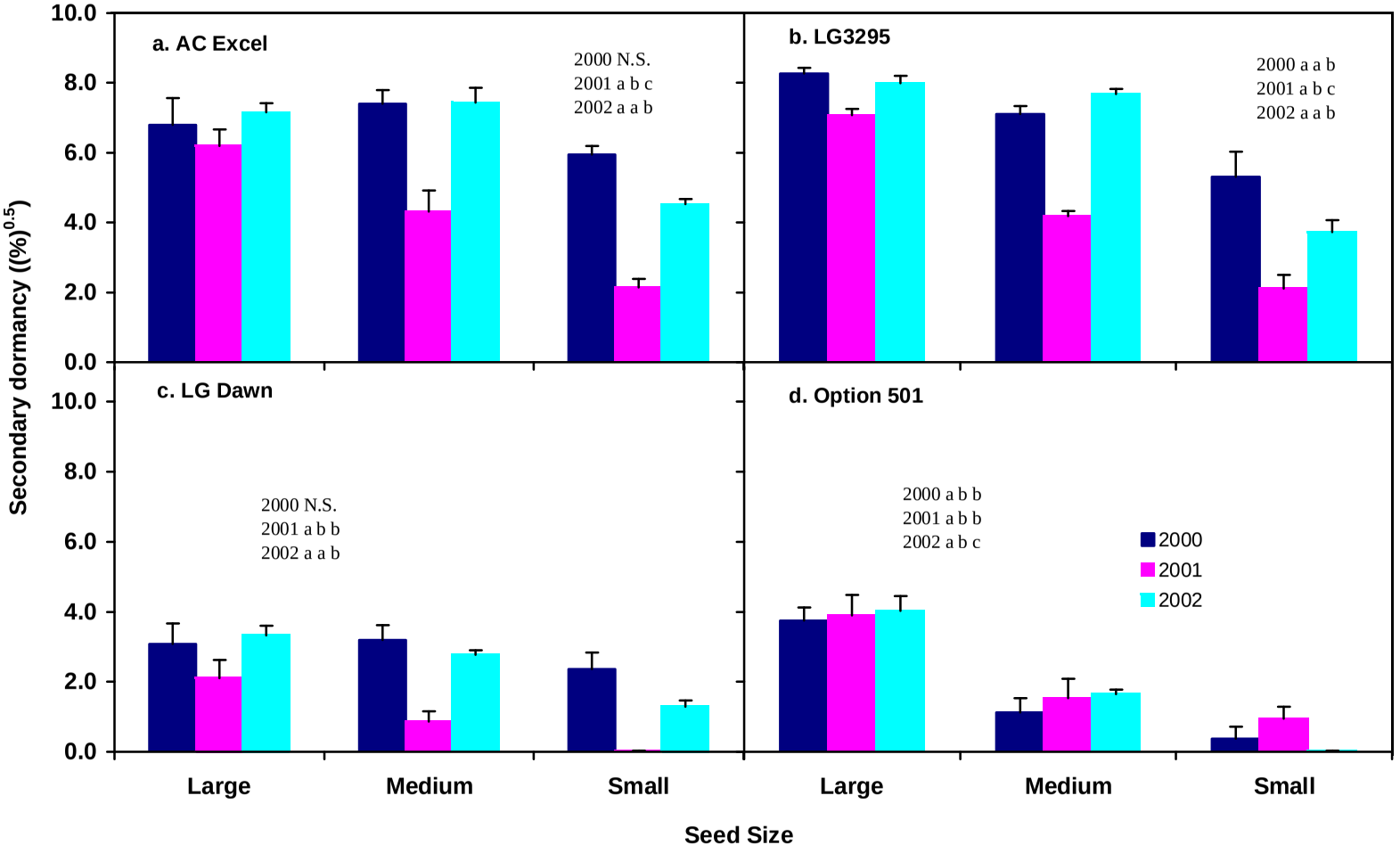

FIGURE 5.2 The influence of seed size on *B. napus* secondary seed dormancy potential in two genotypes with high (a. AC Excel, b. LG3295) and two genotypes with low (c. LG Dawn, d. Option 501) secondary seed dormancy potential. Standard errors of the means and means separation based on Fisher's protected LSD for each year are indicated (N.S., not significant).

TABLE 5.2 The proportion and viability of large, medium and small seeds of four *B. napus* genotypes grown from the same original seedlot in 2000, 2001, and 2002.[a]

	Seed Size					
	Large		**Medium**		**Small**	
Genotype/Year	prop.	viability (%)	prop.	viability (%)	prop.	viability (%)
AC Excel						
2000	0.08	97.0 (1.1)	0.81	97.0 (1.5)	0.10	91.8 (0.9)
2001	0.43	98.2 (0.6)	0.47	97.2 (1.3)	0.09	93.5 (0.6)
2002	0.26	98.0 (0.8)	0.71	96.0 (0.8)	0.04	90.0 (1.8)
LG3295						
2000	0.17	97.8 (1.9)	0.79	96.8 (1.7)	0.04	91.5 (1.7)
2001	0.70	98.2 (0.9)	0.29	95.0 (1.1)	0.02	90.8 (0.9)
2002	0.48	98.0 (1.7)	0.51	96.0 (0.9)	0.01	90.3 (1.0)
LG Dawn						
2000	0.17	97.0 (1.5)	0.72	94.0 (1.5)	0.11	94.8 (1.3)
2001	0.62	95.5 (1.8)	0.33	92.3 (1.1)	0.05	89.5 (1.7)
2002	0.38	95.3 (1.0)	0.59	94.8 (1.3)	0.04	92.3 (1.3)
Option 501						
2000	0.05	97.5 (1.3)	0.84	96.8 (1.4)	0.11	94.5 (1.2)
2001	0.48	98.3 (1.0)	0.44	95.5 (1.2)	0.08	94.2 (1.5)
2002	0.18	96.5 (1.9)	0.76	94.8 (1.5)	0.07	91.2 (1.4)
Grand Mean		97.3 (0.4)		95.5 (0.4)		92.1 (0.4)

[a] Standard errors of the means are indicated in parentheses.

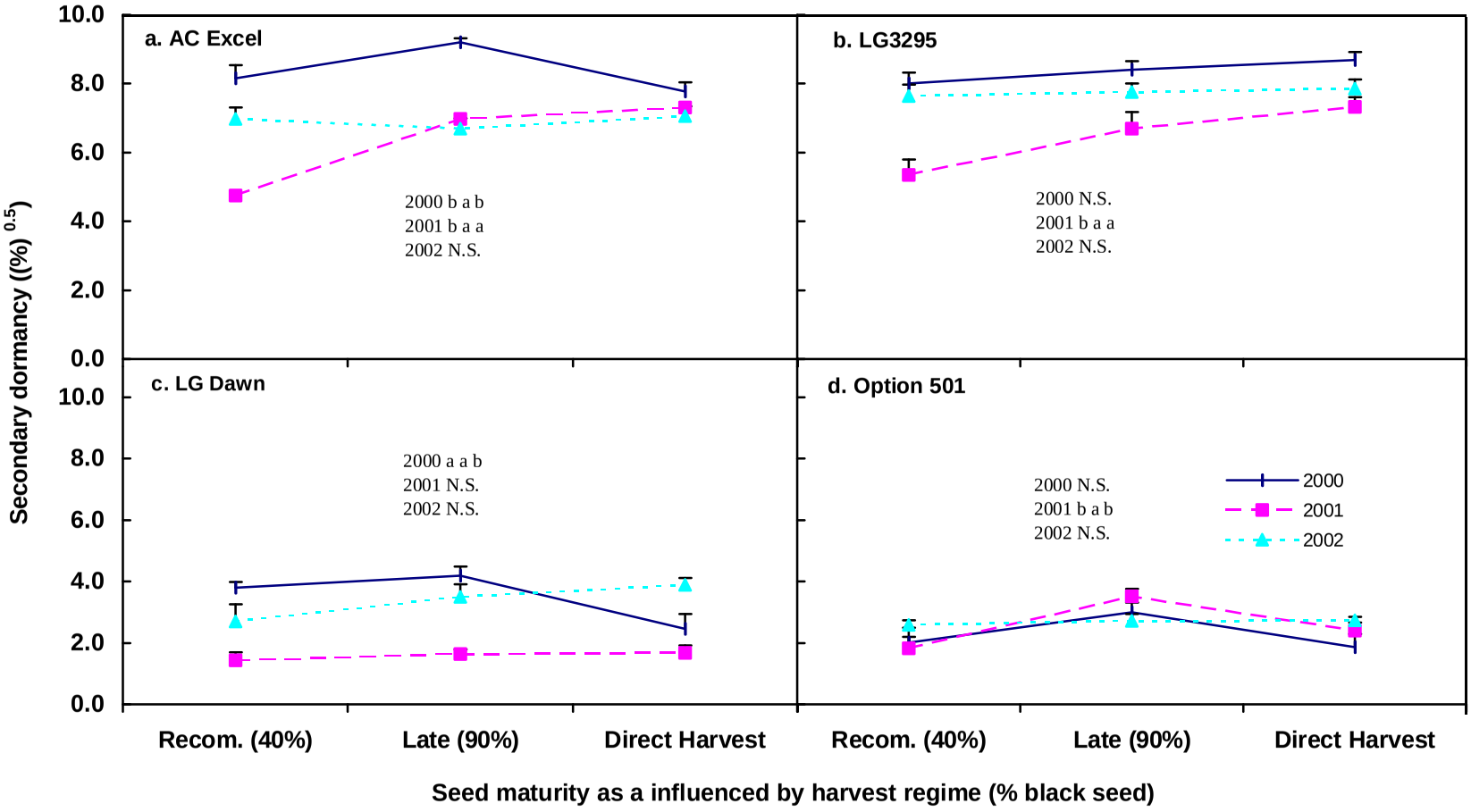

FIGURE 5.3 The influence of seed maturity (harvest regime) on *B. napus* secondary seed dormancy potential in two genotypes with high (a. AC Excel, b. LG3295) and two genotypes with low (c. LG Dawn, d. Option 501) secondary seed dormancy potential. Standard errors of the means and mean separation based on Fisher=s protected LSD for each year are indicated (N.S., not significant).

the late windrowing date, when 90% of the seed on the main rachis had turned colour (Figure 5.3). With the exception of LG Dawn, secondary seed dormancy potential was lower at the recommended time of windrowing when differences were observed (Figure 5.3). When plants were not windrowed prior to harvest, secondary seed dormancy potential was similar to that observed at the late windrowing date or lower (Figure 5.3).

5.3.4 Relative contribution of factors

Genotype contributed most prominently to the variation observed in secondary seed dormancy potential in all experiments (Table 5.3). Among non-environmental factors influencing dormancy potential, the contribution of seed size was 14 times greater than seed maturity. Nevertheless, seed size only contributed approximately one-third to the total variation in secondary seed dormancy potential relative to genotype (Table 5.3). In the experiments examining only four genotypes (Exp. 1sub to 4), the mean effect of year was 16 times less than genotype, while the mean influence of location was negligible (Exp. 1sub). Among post-harvest factors (Exp. 4), the contribution of time to the variation in seed dormancy potential was two times that of temperature in the storage experiment (Table 5.3). Repetition of the experiment contributed 16 and 7% to the total variation in seed dormancy potential in Exp. 1 and 1sub, respectively.

5.4 Discussion

5.4.1 Genotype

These experiments clearly indicated that genotype is the principal factor controlling secondary seed dormancy potential in western Canadian spring *B. napus* (Tables 5.1 and 5.3). The importance of genotype had previously been suggested in European and Chinese *B. napus* genotypes (Pekrun et al. 1997a; Momoh et al. 2002); however, the relative contribution of other factors was not examined. In *Triticum spp.*, genotype contributed 44 to 90% to the variation in primary seed dormancy (DePauw and McCaig 1991), while in *Avena fatua*, genotype contributed approximately 50% to the variability in the expression of primary seed dormancy in western Canada (Jana and Naylor 1980). These values are similar to results on the potential for secondary seed dormancy expression in *B. napus* (Table 5.3). Similar to findings by Pekrun et al. (1997a), no clear

TABLE 5.3 The contribution of genotype and factors affecting phenotype, expressed as a percentage of the total variation (Type III SS) in the cultivar comparison (Exp. 1, 1sub), seed size (Exp. 3), seed maturity (Exp. 2) and seed storage experiments (Exp. 4). The contribution of experimental repetition (Rpt) and replication within experiment (Rpl) is given for Exp. 1 and 1sub. The experimental coefficient of variation (c.v.) is indicated.

	Genotype	**Size**	**Maturity**	**Environment**			**Time**	**Rpt**	**Rpl**	**c.v. (%)**
Experiment				Year	Loc.	Temp				
Exp. 1	44.0			0.4	2.5			15.9	0.3	16.0
Exp. 1sub	67.0			1.2	0.1			7.3	0.4	18.2
Exp. 2	82.4		1.5	4.8						18.0
Exp. 3	54.6	21.1		7.4						27.9
Exp. 4	75.8					5.9	3.0			25.2
Mean (1sub - 3)	68.8	21.1	1.5	4.5	0.1					

links between secondary seed dormancy potential and the breeder and/or seed distributer were observed in this study (Table 5.1).

The broad range of secondary seed dormancy potential observed here was not unexpected as seed dormancy is a genetically complex phenomenon in dicots where nuclear as well as maternal components contribute to seed dormancy (Foley and Fennimore 1998). Seed dormancy in *Sinapis arvensis* L., an annual weed closely related to *B. napus*, is regulated by a single maternal locus, while the genetics of the nuclear component are more complex and remain unknown (Garbutt and Witcombe 1986). Despite the high range in seed dormancy potential, few genotypes exhibited medium and low expression of secondary seed dormancy relative to the remaining genotypes (Table 5.1). This suggests that although the potential for breeding low dormancy genotypes exists, the majority of genotypes available to producers in the late 1990s did not fit this category and may inadvertently be prolonging seedbank persistence in western Canada (Chapter 4). A broad range of secondary seed dormancy potential also was observed in previous studies (Pekrun et al. 1997a; Momoh et al. 2002). In these studies, however, most genotypes exhibited low secondary seed dormancy potential in comparison to these observations. Moreover, variation within genotypes among successive repetitions of the experiment and among different seedlots of the same genotype was high (Pekrun et al. 1997a; Momoh et al. 2002). Similarly, experimental replication contributed to total variation in Exp. 1 and 1sub (Table 5.3), although the extent of this variation appeared to be less than that reported by Pekrun et al. (1997a) and Momoh et al. (2002) (Table 5.1). Nevertheless, the grouping of genotypes into the proposed categories (HDP, MDP, and LDP) did not change among successive repetitions of the experiment (data not shown). Reasons for differences in variation in secondary seed dormancy potential within and among genotypes between successive repetitions of the experiment remain unknown. Seed storage conditions (Figure 5.1) and time between harvest and the dormancy assay may have played a role.

5.4.2 Other factors affecting dormancy potential

5.4.2.1 Seed size and maturity

These results showed that the importance of seed size to secondary seed dormancy potential in *B. napus* was second only to genotype (Table 5.3). The effect of seed size on

seed germinability is relatively well understood in some species (Baskin and Baskin 1998); however, little is known of the effects of seed size on secondary seed dormancy expression. The lower seed germinability observed in smaller seed (Table 5.2) was primarily due to differences in seedlot viability as primary dormancy was small and not significantly different among seed size classes. Secondary seed dormancy expression in *B. napus* was similar to that previously reported in *A. hymenoides* where the largest seeds (mean = 8.7 mg seed^{-1}) exhibit the greatest degree of primary seed dormancy (Jones and Nielson 1999).

The high rate of seed death observed in the small seed fraction during the induction phase of the experiment suggests either a limited metabolic capacity to endure the imposed stresses, an inability to switch the metabolism to a dormant state, or both. Little is known about the metabolic changes conferring secondary seed dormancy expression in *B. napus*. Small seed, however, only accounted for 1.1 to 11.2% by weight to the unsorted seedlot (Table 5.2) and therefore, contributed relatively little to seed dormancy observed in unsorted seedlots.

The relative contribution of seed maturity was negligible in comparison to other factors examined (Table 5.3). Nevertheless, the trend that early windrowing exhibited among the lowest potential for secondary seed dormancy expression relative to the remaining treatments in this experiment indicates that the recommended time of windrowing does not compromise the potential of secondary seed dormancy in *B. napus* in western Canada. The inconsistent results observed in the windrowing study were likely a result of varying environmental conditions experienced during seed maturation in the three years. In 2001, environmental conditions during seed maturation were uncharacteristically dry and hot in comparison to 2000 and 2002.

5.4.2.2 Environment

5.4.2.2.1 Pre-harvest

In *B. napus*, environmental conditions during seed maturation influenced secondary seed dormancy expression in the progeny (Table 5.3). There was an almost two-fold difference in precipitation between the two locations in the genotype comparison study in 1999, but the influence of this on seed dormancy expression was relatively small (Tables 5.1 and 5.2). More extreme differences in precipitation during seed maturation occurred

in the maturity experiment (Exp. 2) at Kernen in 2000, 2001, and 2002 which may explain the increase in the relative contribution of variation in dormancy expression caused by year when the four genotypes were examined, compared to the more extensive genotype comparison in 1999 and 2000 (Table 5.3). The general decrease in seed dormancy potential in 2001 (Figure 5.3) as well as differences in seed dormancy expression between large and medium seed in AC Excel, LG3295 and LG Dawn only found in 2001 (Figure 5.2), were most likely the result of uncharacteristically hot and dry conditions during seed maturation. Natural precipitation in August and September of 2001 was only 22 mm which is approximately one-third of normal, while only 70% of normal precipitation occurred between April and July, 2001. Although the plants were irrigated in 2001, it was not possible to avoid the effects of drought and heat stress during seed maturation. Average maximum temperatures were also higher in 2001, particularly in August (28.1 C), when average maximum temperatures were approximately 5 C higher than during August of 2000 and 2002 (23.9 and 22.3 C, respectively). The early harvest date relative to other years illustrates the accelerated seed maturation phase in 2001. The influence of temperature (Peters 1982; Sawhney 1989; Hume 1994; Miura and Hitoshi 1999) as well as moisture stress (Peters 1982; Sawhney and Naylor 1982; Meyer and Allan 1999) on seed dormancy expression of progeny is well established. Both decreases and increases in seed dormancy in response to high temperatures and low soil moisture have been reported; however, in most species these conditions result in decreased seed dormancy expression (Fenner 1991; Baskin and Baskin 1998; Wright et al. 1999). The results in *B. napus* agree well with these reports.

In 2002, autumn frosts may have influenced these observations, particularly in the late windrowing and direct harvest treatments which had not been applied at the time of the first killing frost (Sept. 26, -7.7 C). In 2000, the first killing frost occurred 10 days prior to harvest, on Sept. 24 (-6.3 C), while in 2001 no frost was recorded prior to harvest. Despite the high variation in climatic conditions among the growing seasons, this only contributed 4.5% to the total variation observed in seed dormancy expression (Table 5.3).

In 2000, AC Excel exhibited a high degree of seed shatter when left standing prior to harvest. It is not known whether this influenced secondary seed dormancy expression in the harvested sample (Figure 5.3) by preferential shatter of seeds with high dormancy expression. Differences in seed dormancy expression depending on location on the mother plant have previously been reported (Thomas et al. 1979; Nimer et al. 1983). Nevertheless, the influence of pod shatter on seed dormancy expression, if any, appears to

be small in relation to other factors investigated here.

5.4.2.2.2 Seed storage conditions and time

Seed storage temperature and duration also influence secondary seed dormancy potential (Table 5.3). Rate of loss in secondary seed dormancy potential was linked to storage temperature and increased as mean storage temperature increased (Figure 5.1). In the HD genotypes significant differences in secondary seed dormancy rate loss were observed between the -70 and -15 C storage treatments, indicating colder storage is better, if secondary seed dormancy expression is to be preserved (Figure 5.1). These results did not indicate an internal dormancy/non-dormancy cycle previously reported in other species during seed storage (Froud-Williams et al. 1986), even under ambient storage conditions. Similarly, Pekrun et al. (1997b) also reported no clear evidence of an annual dormancy cycle in *B. napus*. Whether the high mean temperature or the annual temperature fluctuations in the ambient storage treatment contributed more to the high rate of loss of secondary seed dormancy potential in the HDP genotypes is not known. Alternating temperatures have been shown to decrease secondary seed dormancy expression in this species (Pekrun et al. 1997b) and are a well known mechanism for increasing seed germinability (Thompson et al. 1977; Ellis et al. 1982; Thompson and Grime 1983). A decrease in secondary seed dormancy potential during storage has been previously reported in *B. napus* (Schlink 1995), although not at the low storage temperatures examined here. In contrast, Momoh et al. (2002) reported increases in secondary seed dormancy potential during seed storage in a number of the *B. napus* genotypes. However, these conclusion were based on the analysis of seed harvested in different years and may therefore have been confounded by the influence of environmental conditions during seed maturation. It is not clear whether Momoh et al. (2002) used the same seed source to grow progeny in different years which may further have confounded their observations (Baskin and Baskin 1998). The decrease in seed dormancy potential observed in *B. napus* is similar to an after-ripening response associated with the release of primary seed dormancy (Baskin and Baskin 1976; Bewley and Black 1994), however, it is not known if the mechanisms regulating primary and secondary seed dormancy are similar (Bewley and Black 1994).

5.4.3 Management implications

These results showed that the primary factor influencing secondary seed dormancy potential was genotype. The small influence of pre- and post-harvest environment, in comparison to genotype, suggests that these findings are applicable over a wide geographic area. Therefore, if volunteer *B. napus* persistence is to be minimized (Chapter 4), a low dormancy genotype should be grown. Producers should windrow their *B. napus* crop as close to the recommended time as possible, as late windrowing may increase the potential for secondary seed dormancy expression (Figure 5.3) in addition to increasing the likelihood of seed shatter.

6.0 Secondary dormancy, temperature, and seed burial depth regulate seedbank dynamics in *B. napus*

6.1 Introduction

In western Canada, large additions to the canola seedbank appear to occur at the time of harvest (Chapter 3) and a small portion of the canola seedbank from a single cohort may persist for at least three years (Chapter 4). Seedbank persistence was found to be related to a genotype's potential for secondary seed dormancy development and seasonal seedling recruitment was observed where volunteer *B. napus* recruitment was limited to the spring (Chapter 4). These observations differ from studies on winter *B. napus* in Europe. In Europe, seedling recruitment was observed to occur in multiple flushes throughout the entire growing season (Lutman 1993). Furthermore, differences in seedbank persistence of volunteer *B. napus* populations under contrasting tillage systems were more pronounced in the U.K. (Pekrun et al. 1998b) than in similar experiments in western Canada (Chapter 4). Nevertheless, *B. napus* seedbank persistence tended to be enhanced in tillage systems with higher soil inversion in the U.K. as well as western Canada. The role of seed burial on seedbank persistence of *B. napus* has not been investigated in western Canada. Therefore, it remains unclear whether seedbank persistence in the zero-till system was a function of seed persistence near the soil surface or was due to seed burial via cracks in the soil at the time of seedbank establishment and/or via soil disturbance at the time of seeding.

In *B. napus*, secondary seed dormancy may be induced by darkness and osmotic stress (Pekrun et al. 1997a; Momoh et al. 2002). Hypoxia also may induce secondary seed dormancy to a lesser degree (Pekrun et al. 1997c; Momoh et al. 2002). Despite trends of higher seed dormancy induction at higher temperatures, a clear role of temperature on secondary seed dormancy induction in *B. napus* has not been established (Momoh et al. 2002). It is known, however, that secondary dormancy is readily released by cold stratification and that alternating temperatures reduce secondary seed dormancy induction (Pekrun 1994; Pekrun et al. 1997a). The objectives of the following experiments were to

(i) investigate the influence of temperature and osmotic potential on secondary seed dormancy induction in *B. napus*, (ii) relate these results to seedbank dynamics and seedling recruitment of two *B. napus* genotypes with different seed dormancy potential in the field, and (iii) investigate the influence of tillage system, burial depth and soil type on the seedbank dynamics and seedling recruitment in the field.

6.2 Materials and Methods

6.2.1 Laboratory experiment

The influence of constant temperature (5, 10, 15 and 20 C) and initial osmotic potential of the imbibing solution (-0.5, -1.0, -1.5 and -2.0 MPa) on the rate of secondary dormancy development in seed of two *B. napus* genotypes with high secondary seed dormancy potential (LG3295, AC Excel) (Chapter 5) was investigated. This range of temperatures and solution osmotic potentials approximates the range of temperatures and water potentials that might occur in the field during the growing season in western Canada. Primary seed dormancy and seedlot viability were evaluated at each temperature via germination tests in darkness on seeds not exposed to Polyethylene glycol (PEG). Each treatment was replicated four times with 100 seeds per 9 cm petri dish. The experiment was comprised of 272 experimental units per genotype, blocked by replication, each time it was conducted and was repeated two times. The temperatures were re-randomized among the germination cabinets between successive repetitions of the experiment. In addition to seed, each petri dish contained two layers of filter paper and 8 ml of the appropriate osmotic solution. Solutions of initial osmotic potentials were generated using Polyethylene glycol (PEG-8000) and adjusted for temperature (Michel 1983). All treatments were incubated in darkness. In the high initial osmotic potential treatments (-0.5 and -1.0 MPa), all germinated seeds were removed from the petri dishes under a green safe light as soon as radicle protrusion was observed. After 1, 2, 3, or 4 weeks of osmotic treatment, all remaining ungerminated seeds were transferred under a green safe light to new petri dishes containing distilled H_2O and returned to the 15 C germination cabinet. After one week, all remaining ungerminated seeds were again transferred to new petri dishes containing distilled H_2O and placed back into the 15 C cabinet. After one additional week of germination in darkness, seed dormancy was quantified. Ungerminated seeds were pinched with forceps and all firm seeds were considered dormant.

6.2.2 Field experiment

6.2.2.1 Study site, experimental design and establishment and genotype selection

This experiment was conducted at the two locations described in Chapter 4. At these locations, the influence of tillage system (main plot) and burial depth on *B. napus* seed persistence and volunteer seedling recruitment were examined in one *B. napus* genotype with high (LG3295 = HDP) and one genotype with low (Option 501 = LDP) secondary seed dormancy potential over time. The experiment was a split-plot three-way factorial randomized complete block design within each sampling date. Prior to establishment of the experiment, both locations had been under zero tillage management. Conventional tillage main plots were established beside the zero tillage main plots in the fall of 1999. The conventional tillage plots were tilled two times each fall and spring using a light field cultivator and spring wheat was grown under both tillage systems. At each location, the tillage system main plots were replicated four times and seedbank burial depth (1 and 10 cm) and genotype (HDP and LDP) were randomized within the tillage main plots.

The two *B. napus* genotypes, represent the highest and lowest potential for secondary seed dormancy among a group of 16 *B. napus* genotypes screened in a laboratory experiment (Chapter 5). Only recently harvested seed was buried in these experiments since secondary seed dormancy potential decreases during seed storage (Chapter 5). Fresh seed was obtained each year by planting seed from the original seedlot of each genotype in isolation in the field in 2000 and 2001. The progeny was also subjected to the laboratory seed dormancy assay immediately following harvest.

In the experiment established in 2000, eight 14 x 14 x 14 cm plastic pots (Kord Products Inc., Brampton, ON, Canada) per treatment were buried in four rows in each tillage plot on October 8 to 10, with the top of the pots level with the soil surface. In the zero tillage system, the pots were buried between rows of standing stubble. The standing stubble maintained the snow trapping ability inherent to zero tillage. Residue was placed on the buried pots at a density equal to the surrounding field. In the conventional tillage plots, the pots were also buried in four rows with similar spacing than those in the zero tillage plots. No residue was placed on the pots buried in the conventional tillage system. Prior to burial, the bottoms of the pots were removed and replaced with flexible nylon mesh to facilitate drainage. The pots were filled with the soil that was removed to bury the pots at each location and at this time, 200 viable seeds were sown into the pots at a depth of either 1 or 10 cm below the soil surface. Ten nylon mesh pouches per treatment

also were buried in rows at these depths. The mesh was impervious to *B. napus* seed. Each pouch contained 600 viable seeds as well as air dried soil from the respective location.

In a more comprehensive study established on September 25 and 26, 2001, 12 pots per treatment were buried as previously described. These pots also contained 200 viable, non-dormant seeds of the same two genotypes buried at either 1 or 10 cm. No nylon pouches were buried in 2001. After establishment, the seeds were not disturbed in either experiment. In mid-May of 2001 and 2002, a wheat crop was hand seeded (100 kg ha^{-1}) between the rows of buried pots to establish a crop canopy. The wheat crop was hand harvested at maturity.

6.2.2.2 Sampling intervals and measurements

In the field, volunteer *B. napus* seedling recruitment was quantified in all pots from establishment until the soil was frozen as well as at monthly intervals throughout the subsequent growing season(s) (mid-April to late October). In both experiments, 32 plastic pots were exhumed from each location at each time of sampling. In the 2000 experiment, sampling intervals included autumn 2000 (4 weeks after establishment); April, May, June, and September 2001; and April, June and September 2002. Sampling was conducted in the middle of each month and nylon pouches were only sampled in the autumn of 2000 and April 2001. In the 2001 experiment, sampling intervals were autumn 2001 (4 weeks after establishment); April, May, June, July, August, September and October, 2002; and April 2003 (deep buried seedbank only). Frozen soil throughout the winter months inhibited retrieval of pots.

Immediately following retrieval from the field, the pots were placed in darkness in a growth cabinet at 15 C and irrigated as necessary. Seedling recruitment was monitored for two weeks. Immediately following this portion of sample analysis (germinable seedbank), the remaining seeds were elutriated as described in Chapter 4. All firm *B. napus* seeds were picked from the remaining residue using forceps and transferred to petri dishes. This was conducted in a shed at ambient temperature under fluorescent lighting. Each petri dish was then moistened with approximately 8 ml of water and was placed in a germination cabinet in darkness at 15 C. After one week, germinated and ungerminated seeds were counted and the viability of all ungerminated seeds was determined by stratification for one week at 2 to 4 C (Pekrun 1994), followed by another

week of germination in darkness. All viable seeds that were elutriated from the soil constituted the viable, ungerminated portion of the remaining seedbank. In the experiment established in the autumn of 2001, the top 7 to 8 cm of the soil was removed from two repetitions of the samples of the 10 cm burial depth immediately prior to placing the pots into the growth cabinet. This was done in lieu of the nylon pouches used in the 2000 experiment to determine the readily germinable portion of the seedbank. The validity of this procedure was determined by exhuming two sets of pots at three sample dates and comparing the viable, ungerminated portion of the 10 cm seedbank in pots with and without soil removal. No differences in elutriated seeds were found among genotypes and locations ($Pr > F = 0.122$ to 0.733).

Soil temperatures were measured for the duration of the experiment. Daily mean, minimum and maximum temperatures at 1 and 10 cm were recorded in one replication of each tillage system using a CR10 data logger with four thermocouples per treatment connected in series. Gravimetric soil water contents were determined at each sampling date in each tillage system at each seed burial depth. Mean soil water potential was determined by generating standard curves for each soil ranging from 6 to 30% gravimetric soil water content (5 moisture contents, 3 replications each) using a W4P PotentiaMeter (Decagon devices Inc., Pullman, WA 99163, USA).

6.2.3 Statistical analysis

6.2.3.1 Laboratory experiment

Seed dormancy induction rates were determined by linear regression of square root transformed seed dormancy percentages of each replicate for each temperature and initial osmotic potential combination over time (3 df). Prior to regression analysis, secondary dormancy was corrected for viability and primary dormancy of seed. To enable the transformation of zero values, 0.001 was added to all percentages prior to data transformation. The residuals and regression coefficients did not suggest a more complex relationship. Bartlett's test for homogeneity of variances was used to determine if slopes from different repetitions of each experiment could be combined and standard errors of the means were calculated (n=8). The variance components of temperature and osmotic potential were determined as a proportion of the total Type III sums squares (SS) obtained using the GLM procedure in SAS.

6.2.3.2 Field experiment

All seedling and seed counts were converted to percent of original seedbank prior to analysis of variance. Total seedbank remaining was the sum of the germinable percentage of seed (i.e. successful seedling recruitment during the 2 week germination phase following pot retrieval from the field), and the viable, ungerminated percentage of seeds that were elutriated from the soil. A three-way split-plot ANOVA was conducted for individual sampling dates within each location using the MIXED procedure of SAS. Following this analysis, data were combined where possible within location and standard errors of the means were calculated. The standard errors of the means for the germinable portion of the seedbank at the 10 cm burial depth in the experiment established in 2001 were tabulated from half as many observations (n=4) as the remaining standard errors of the means (n=8).

The gravimetric soil water data were analysed within sample date as a split plot two-way factorial randomized complete block design (depth x tillage) using the MIXED procedure of SAS. Although differences in gravimetric soil water content were observed among the tillage systems at the shallow burial depth at Dundurn (Jun01, Apr02, Jul02, Aug02, Sep02), the means were combined as no differences in the seedbank were observed among the tillage systems at this depth.

Both axes of the standard curves developed to estimate soil water potential from gravimetric soil water contents were converted to absolute values and $\log_{10}$ transformed. Linear regression analysis was conducted and mean soil water potentials were determined from mean gravimetric soil water contents using the following equations:

$$\Psi_{Clay} = -10^{(3.78-3.22*\log(\%S.M.))},\ r^2 = 0.97, 3df_{(6.1)}$$

$$\Psi_{Loam} = -10^{(5.17-4.84*\log(\%S.M.))},\ r^2 = 0.94, 3df_{(6.2)}$$

where Ψ is water potential in MPa and *S.M.* is gravimetric soil moisture content expressed as %.

6.3 Results and Discussion

6.3.1 Laboratory Experiment

Temperature plays an important role in regulating secondary dormancy in *B. napus* seed (Figure 6.1). When examining the variance components in this study, temperature (48%) contributed 2.7 times more to total variation in rate of seed dormancy expression than did osmotic potential (18%). This is not unexpected since temperature has been shown to influence many aspects of primary and secondary seed dormancy (Probert 2000). Moreover, temperature principally controls seed dormancy in species that exhibit annual dormancy cycles (Baskin and Baskin 1977, 1985, 1988).

As the treatment temperature increased, an increase in seed dormancy induction rate was observed in both genotypes (Figure 6.1). Higher dormancy expression had previously been suggested when *B. napus* seeds were exposed to 20 C (4 weeks at -1.5 MPa) compared to 12 C (Pekrun et al. 1997a; Momoh et al. 2002), but this was not confirmed statistically. In addition, previous reports have indicated that a greater absolute temperature difference between induction and germination test temperatures reduces seed dormancy expression in *B. napus*. The results did not indicate such a trend between the 10 and 20 C treatments (Figure 6.1). The lowest dormancy induction rates, however, were observed at 5 C (Figure 6.1). We believe that these observations likely were due to 5 C being close to the ideal stratification temperature for dormancy release, rather than a function of the 10 C difference in absolute temperature between induction and germination temperatures. As previously reported (Pekrun et al. 1997b, c), decreasing osmotic potential to values approaching the permanent wilting point in soils (app. –1.5 MPa) also increased seed dormancy induction rates. In both genotypes, high levels of one factor, in part, compensated for low levels of the other factor, while high levels of both factors resulted in the highest dormancy induction rates (Figure 6.1).

Despite similar maximum seed dormancy induction rates in both genotypes (Figure 6.1), a clear interaction between treatment temperature and initial osmotic potential of the imbibing solution on rate of dormancy induction was observed between the two genotypes (Figure 6.1). While dormancy induction rates in both genotypes were similar at 20 C, they were higher in AC Excel than in LG3295 at all other temperatures examined (Figure 6.1). Whether this is primarily a function of genotype and of any significance in the field remains unknown.

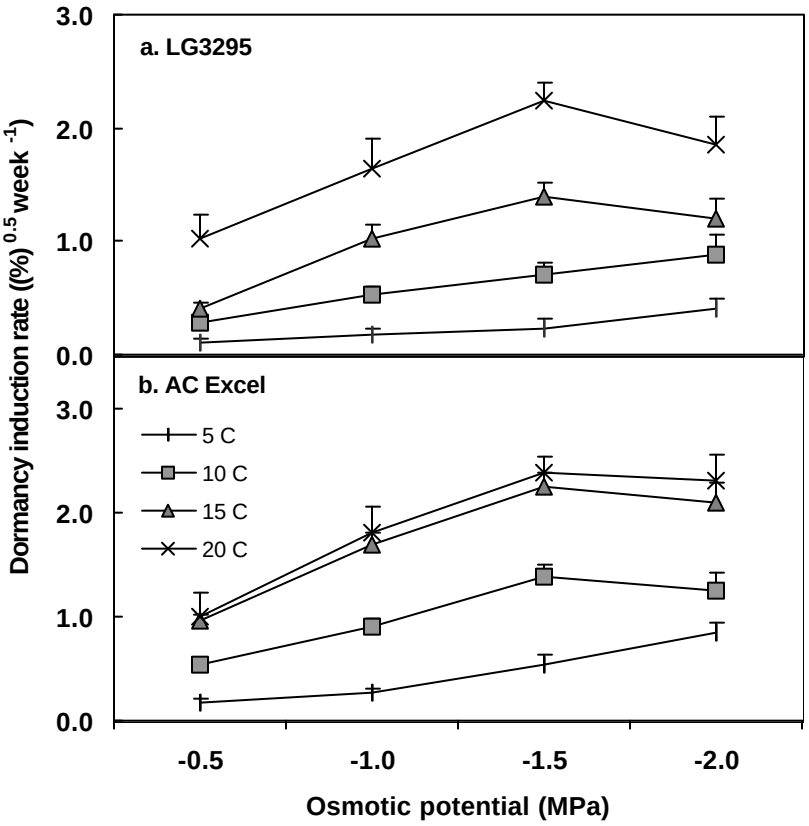

FIGURE 6.1 Rate of secondary dormancy induction as influenced by temperature and osmotic potential in two *B. napus* genotypes with high secondary seed dormancy potential (a. LG3295, b. AC Excel). Bars = 1 s.e.

6.3.2 Field Experiment

Seed dormancy potential of seed used in the experiment established in 2000 was 64.5% (s.e. 5.6) for the HDP genotype and 4.8% (s.e. 2.4) for the LDP genotype. In the 2001 progeny, seed dormancy potential of these genotypes was lower (29.3% (s.e. 5.5) - HDP and 3.8% (s.e. 1.6) - LDP). A late season drought during seed maturation may have been responsible for the lower values observed in 2001 since dormancy characteristics of progeny may be influence by environmental conditions during seed maturation (Gutterman 1980/81).

Tillage system did not significantly influence the *B. napus* seedbank at any time and therefore, the data were combined. Differences in soil water content between tillage systems were few and were only observed at the shallow burial depth when sampling occurred shortly after a rain. Few differences in gravimetric soil water content between tillage systems likely contributed to the lack of differences in the *B. napus* seedbank between tillage systems. Differences in soil temperature between the tillage systems were also small (max. 3 C) (data not shown). Lack of differences in *B. napus* seed persistence between zero and conventional tillage also suggests no differences in seed predation between these tillage systems. Uncharacteristically dry conditions (Table 4.2) and the relatively small size of the main plots (3 x 7 m) likely contributed to the lack of differences in seedling recruitment and seed persistence between tillage systems.

In autumn, four weeks after establishment, the viable, ungerminated portion of the seedbank was low in all treatments in both years (0.1 (s.e. 0.1) to 6.3% (s.e. 0.8)). Primary seed dormancy is low in *B. napus* (Pekrun 1994) and was not significantly different between these seedlots and genotypes (Chapter 5). Nevertheless, the HDP genotype tended to display higher levels of viable, ungeminated seed in the autumn (data not shown).

6.3.3 Deep seedbank

In the deep seedbank, higher levels of viable, ungerminated seed were observed in the HDP genotype than the LDP genotype throughout the growing season (Figures 6.2 and 6.3). In 3 of 4 site-years, we observed an increase in the viable, ungerminated portion of the deep seedbank of the HDP genotype as soil temperatures increased to those ideal for dormancy induction in the laboratory (Figure 6.1). This did not occur in the LDP

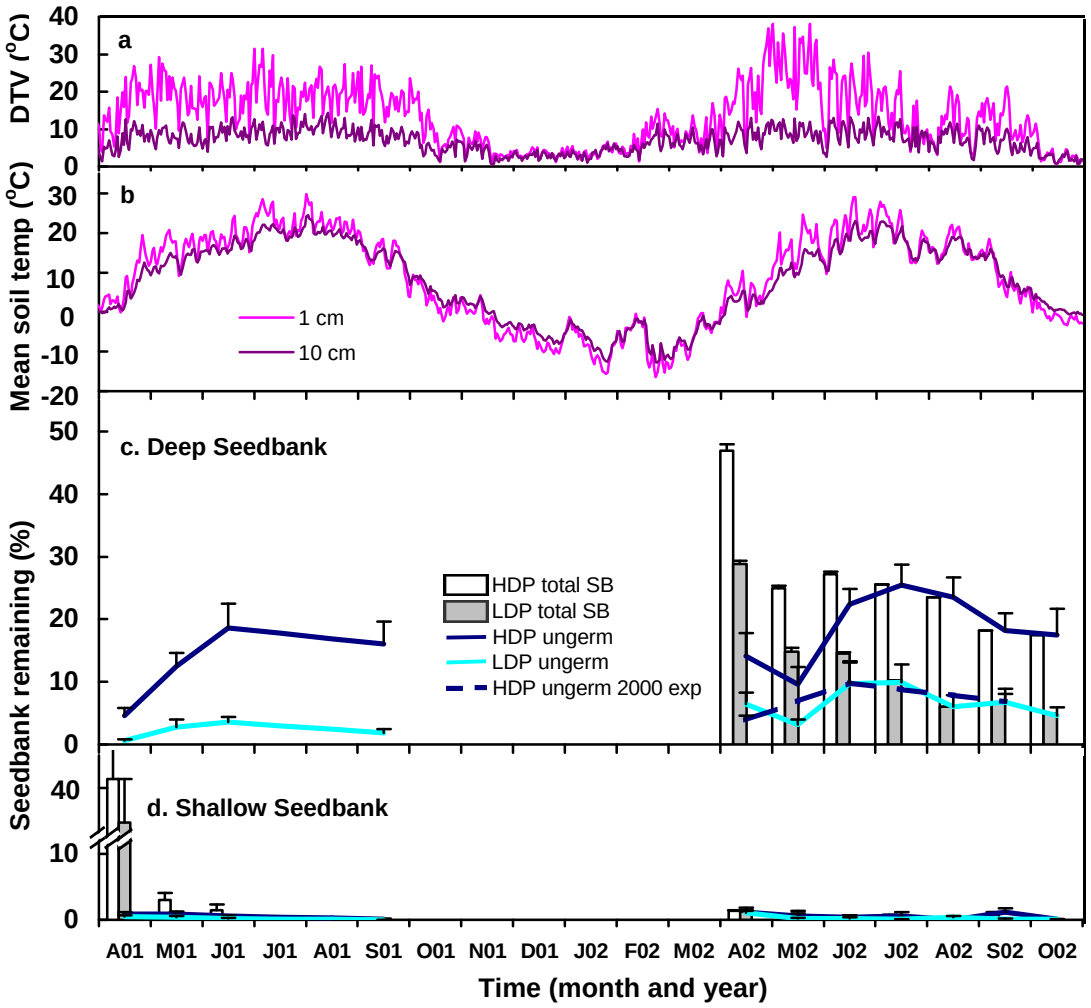

FIGURE 6.2 Diurnal temperature variations (DTV) (max-min) (a) and mean temperatures (b) at 1 and 10 cm soil depth at the clay soil location from autumn 1999 to autumn 2002. Total and ungerminated portion of the deep seedbank (SB) (10 cm) (c) and shallow seedbank (1 cm) (d) of one *B. napus* genotype with high (HDP) and one with low (LDP) secondary seed dormancy potential in 2001 and 2002. Bars = 1 s.e.

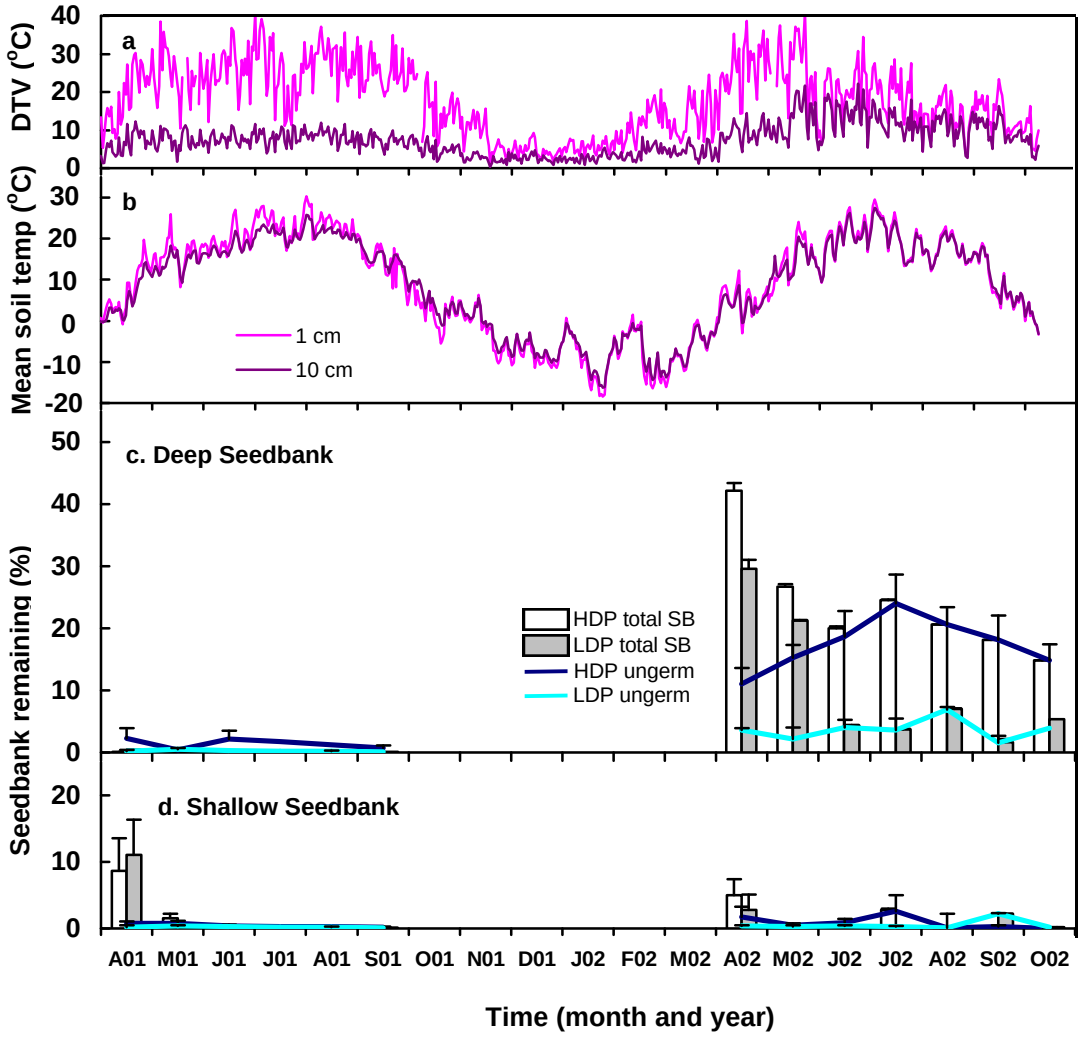

FIGURE 6.3 Diurnal temperature variations (DTV) (max-min) (a) and mean temperatures (b) at 1 and 10 cm soil depth at the loam soil location from autumn 1999 to autumn 2002. Total and ungerminated portion of the deep seedbank (SB) (10 cm) (c) and shallow seedbank (1 cm) (d) of one *B. napus* genotype with high (HDP) and one with low (LDP) secondary seed dormancy potential in 2001 and 2002. Bars = 1 s.e.

genotype (Figures 6.2 and 6.3) in which fewer than 5% of the seeds could be induced into secondary dormancy under ideal conditions in the lab. Between June and July 2002, the total deep seedbank population shifted entirely to an ungerminable state in both genotypes (Figures 6.2 and 6.3). This portion, however, was up to 6 times greater in the HDP genotype than in the LDP genotype at this time, and it remained higher throughout the rest of the growing season. In 2001, the total deep seedbank could not be determined since predatory insects, destroyed all samples. We found no correlation between the viable, ungerminated seedbank and the temperature difference between mean soil temperature at the time of sampling and the germination test temperature (15 C) (Figures 6.2 and 6.3), as suggested by Pekrun et al. (1997c).

Similar deep seedbank dynamics were not observed in the HDP genotype in the loam soil in 2001 (Figure 6.3). Despite similar gravimetric soil water contents (Table 6.1), the soil water potential at the 10 cm depth was substantially higher in the loam soil (-0.5 MPa) than in the clay soil (-1.2 MPa) in the autumn of 2000 (Table 6.1). This increased the potential for germination in autumn and seed death via desiccation throughout the winter. High survivorship over winter following seedbank establishment (Légère et al. 2001) and the subsequent increase in the viable, ungerminated portion of the *B. napus* seedbank as soil temperatures increased was observed only in the HDP genotype when soil water potentials were lower than –1.2 MPa in the autumn (Figures 6.2 and 6.3, Table 6.1). In the 2000 experiment in the clay soil, low autumn soil water potentials may have contributed to preconditioning of HDP seeds so that germination in the spring of 2001 was largely inhibited, despite soil water potentials that would otherwise have been adequate for *B. napus* germination (>-0.5 MPa) (Rao and Dao 1987). Limited oxygen availability also may have contributed to the increase in viable, ungerminated portion of the deep *B. napus* seedbank in the spring (Pekrun et al. 1997a; Momoh et al. 2002). Despite high soil water potentials throughout autumn and winter of 2002/2003 (Table 6.1), 12.8% (s.e. 2.6) and 4.3% (s.e. 2.0) of the total HDP seedbank remained in the clay and loam soil in April 2003, respectively. At this time, the total deep LDP seedbank contained fewer than 0.8% of the original seedbank. This suggests greater dessication tolerance in HDP seeds that had been in the seedbank for at least one full year. Between 70 and 75% of viable HDP seeds did not germinate in soil immediately following pot retrieval in April 2003 at both sites. These proportions were substantially higher than those observed during the previous spring (26 to 30%) and suggest that one complete season in the seedbank may increase persistence potential in seed (Figures 6.2 and 6.3).

TABLE 6.1 Gravimetric soil water content and soil water potential at the clay and loam soil location at 1 and 10 cm depth for at each sampling date.[a]

	Clay soil location				Loam soil location			
	1 cm		10 cm		1 cm		10 cm	
	%	MPa	%	MPa	%	MPa	%	MPa
2000								
Nov	8.4 (0.4)	<-5.0	14.2 (0.8)	-1.2	7.9 (0.4)	<-5.0	13.2 (0.8)	-0.5
2001								
April	4.2 (0.2)	<-5.0	21.1 (0.8)	-0.3	3.4 (0.3)	<-5.0	15.5 (0.7)	-0.3
May	4.6 (0.5)	<-5.0	21.0 (1.2)	-0.3	2.5 (0.2)	<-5.0	15.3 (1.0)	-0.3
June	12.0 (0.9)	-2.0	18.5 (0.8)	-0.5	9.6 (1.0)	-2.6	16.0 (0.8)	-0.2
September	4.0 (0.4)	<-5.0	11.9 (0.8)	-2.1	2.1 (0.1)	<-5.0	6.0 (0.3)	<-5.0
October	6.7 (0.5)	<-5.0	11.0 (1.3)	-2.7	3.5 (0.2)	<-5.0	5.0 (0.5)	<-5.0
2002								
April	9.3 (2.2)	-4.6	15.9 (1.6)	-0.8	5.9 (1.2)	<-5.0	9.6 (0.6)	-2.6
May	5.7 (0.8)	<-5.0	11.4 (1.0)	-2.4	2.1 (0.4)	<-5.0	7.7 (0.9)	<-5.0
June	4.9 (0.7)	<-5.0	18.3 (0.9)	-0.5	6.2 (0.9)	<-5.0	20.0 (3.6)	-0.1
July	13.3 (1.3)	-1.5	20.1 (1.4)	-0.4	10.0 (0.8)	-2.1	16.1 (0.4)	-0.2
August	26.4 (1.5)	-0.2	27.3 (1.1)	-0.1	21.5 (1.8)	-0.1	20.5 (0.6)	-0.1
September	19.7 (1.4)	-0.4	25.2 (1.0)	-0.2	10.2 (1.5)	-1.9	17.2 (0.7)	-0.2
October	24.3 (1.1)	-0.2	24.5 (1.0)	-0.2	20.2 (1.2)	-0.1	17.5 (0.3)	-0.1
2003								
April	N/A[b]		27.7 (1.1)	-0.1	N/A		19.1 (0.1)	-0.1

[a] The standard errors of the means are indicated in parentheses.

[b] N/A = not available. Seedbank was not sampled at this depth due to low persistence.

Germination may have been an important contributor to loss of seed from the deep seedbank. Nevertheless, no successful recruitment was observed from the deep seedbank, and therefore germination must have been lethal in all cases. The deep seedbank was buried below the biological maximum depth of seedling recruitment for some genotypes of this species (Lutman 1993).

Although the field experiment was not designed to provide evidence of an annual dormancy cycle *sensu stricto* (Baskin and Baskin 1985; Bouwmeester and Karssen 1992; Baskin et al. 1996), these seedbank data suggest that such a cycle may occur in HDP genotypes (Figures 6.2 and 6.3). The viable, ungerminated portion of the deep seedbank increased as soil temperatures increased (Figures 6.2 and 6.3), and the total, viable seedbank was in an ungerminable state throughout summer and autumn. This increase in the viable, ungerminated fraction of the seedbank occurred in the same cohort over two consecutive seasons in the clay soil (Figure 6.2) which is typical of a dormancy/non-dormancy cycle in summer annuals (Baskin and Baskin 1985). These observations are in contrast to those in winter *B. napus* in Europe where no evidence of cyclical dormancy behaviour was observed (Pekrun et al. 1997a).

6.3.2.2 Shallow Seedbank

Shallow seedbank dynamics differed from dynamics in the deep seedbank. Despite differences in winter survivorship among years (10 to 40% in 2001 vs. <5% in 2002) and inherent genetic differences in seed dormancy potential, total shallow seedbank levels were negligible at all times after June of the year following seedbank establishment (Figures 6.2 and 6.3). We believe these observations were primarily a function of high diurnal temperature variations near the soil surface, which caused seed death (Figures 6.2a and 6.3a). Lower seedbank persistence at shallow burial depths is typical in *B. napus* (Schlink 1995; Pekrun and Lutman 1998) and closely related species (Sparrow et al. 1990). Nevertheless, the effect of inherently different seed dormancy potential on seedbank dynamics of *B. napus* had not been investigated to date.

B. napus seedling recruitment was low (0.1 to 1.5% of original seedbank additions) and was observed only from the shallow seedbank in May and June. This was the result of low precipitation (Table 4.1) as reflected by low soil moisture contents and water potentials (Table 6.1). No autumn seedling recruitment was observed at either location in either 2001 or 2002.

6.3.2.3 Seed dormancy status

The dormancy status of the viable, ungerminated seedbank remains unclear. Virtually all firm seeds germinated in the petri dishes immediately following elutriation. This provided little evidence of secondary innate dormancy in which germination is inhibited under all conditions (Baskin and Baskin 1985). As a control, we also subjected seeds that were induced into, and confirmed to be dormant in the laboratory to the elutriation procedure. Secondary dormancy also was released in a similar proportion of seeds by the elutriation procedure (data not shown). In the laboratory, white fluorescent light does not readily release secondary seed dormancy in this species (data not shown). Rapid temperature fluctuation between ambient and water used during seed elutriation may have been contributed to the release of secondary seed dormancy. Rapid temperature fluctuations have been shown to release seed dormancy in other species (Takaki et al. 1981; Taylerson and DiNola 1989). Regardless, a clear link between *B. napus* secondary seed dormancy potential as determined in the laboratory and seedbank dynamics and persistence under field conditions was established.

In summary, these experiments have shown that seasonal seedling recruitment of volunteer *B. napus* in western Canada was the result of two fates. In the shallow seedbank, lack of seedling recruitment was the result of high seed mortality during the winter and the first spring following seedbank establishment (Figures 6.2 and 6.3). Thus, the shallow *B. napus* seedbank was transient. When buried, however, viable seeds shifted from a germinable to an ungerminable state. This was the primary mechanism preventing germination throughout summer and autumn which contributed to a more persistent seedbank when buried, particularly in HDP seed (Figure 6.1). The seedbank dynamics of *B. napus* seed documented here are characteristic of small seeded annual weeds in temperate regions (Baskin and Baskin 1988) and clearly indicate that *B. napus* does possess weedy characteristics that may influence management strategies for feral populations. These results also have shown that temperature appears to play a significant role in regulating secondary dormancy induction in *B. napus* seed in the laboratory and in the field.

7.0 Response to ABA application and hormone profiles in *B. napus* seed in relation to secondary seed dormancy

7.1 Introduction

In addition to being closely associated with plant responses to abiotic stresses, ABA has been implicated in the prevention of precocious germination during seed maturation, and in the maintenance of primary dormancy once the seed is mature. The role of ABA in preventing premature germination (vivipary) is well documented (Finkelstein et al. 1985; Morris et al. 1989; Walker-Simmons 1987; Juricic et al. 1995; Wang et al. 1995). However, during desiccation associated with maturation of *B. napus* seed, decreasing seed water content is more important in preventing vivipary than endogenous ABA content (Finkelstein et al. 1985) and than responsiveness to ABA (Juricic et al. 1995).

The role of ABA in modulating the acquisition of secondary dormancy after seed dissemination is less clear. *De novo* ABA synthesis within the seed is essential for the maintenance of seed dormancy (Yoshioka et al. 1998; Grappin et al. 2000). In lettuce (*Lactuca sativa* L.), thermodormancy (an induced secondary dormancy caused by exposure to high temperatures during imbibition) was released by fluridone, an ABA synthesis inhibitor (Gamble and Mullet 1986), despite no differences in endogenous ABA levels among non-dormant and thermodormant seeds (Yoshioka et al. 1998).

Physiological response and ABA content may not always be correlated. ABA levels in seed are believed to be the result of a dynamic balance between continuous synthesis and degradation (Cutler and Krochko 1999). Thus, changes in ABA levels may be due to changes in the rate of synthesis, degradation or both, and no change in level does not preclude altered ABA metabolism. Recently, a shift in ABA metabolism during thermodormancy has been reported in lettuce seed that could not be detected by measuring ABA content alone (Chiwocha et al. 2003). The lack of a correlation between physiological responses with hormone levels may reflect changes in hormone sensitivity (Trewavas 1982). For example, lower sensitivity to ABA has been shown in non-

dormant seed after dissemination (Walker-Simmons 1987; Morris et al. 1989; Bianco et al. 1994; Wang et al. 1995). Sensitivity to ABA also has been shown to be lost upon imbibition in water, but may be restored by re-drying of the seed (Schopfer et al. 1979).

While primary seed dormancy is generally not observed in *B. napus* (Schlink 1994), *B. napus* may develop secondary seed dormancy (Pekrun 1994). A broad range in stress-induced secondary seed dormancy potential exists among genotypes in this species (Pekrun et al. 1997a; Momoh et al. 2002; Chapter 5). In *B. napus*, endogenous ABA and gibberellin levels were shown to correlate negatively and positively with germinability, respectively (Fu and Lu 1991). Moreover, germination is inhibited by exogenous application of ABA in this species (Schopfer and Plachy 1984) and reduced water uptake through an inability for cell wall expansion has been suggested as a cause for this ABA mediated inhibition of germination (Schopfer and Plachy 1985). It is currently not clear whether ABA is involved in the expression of secondary seed dormancy in this species. Endogenous levels of ABA are not influenced by exposure to PEG (Iglesias and Babiano 1996, 1997) and PEG had no detrimental effect on germination in *B. napus* (Schopfer and Plachy 1984). A series of experiments on osmotic stress induced seed dormancy with PEG were designed to i) determine the effect of ABA and fluridone on germination and seed water content and, ii) profile endogenous levels of gibberellins, ABA, and ABA metabolites in two *B. napus* genotypes with contrasting secondary seed dormancy potential.

7.2 Materials and Methods

7.2.1 Seed source

B. napus seed of two genotypes, LG3295 and Option 501, were used in the following experiments. LG3295 and Option 501 were identified as the genotypes with the highest (HDP) and lowest secondary seed dormancy potentials (LDP) among a group of *B. napus* genotypes, respectively (Chapter 5). Seed from these genotypes was multiplied in isolation plots in the summer of 2000 and stored at -70 C immediately following harvest. This technique has proven most successful in limiting the loss of seed dormancy potential during seed storage (Chapter 5). All experiments were independently replicated two to four times and each treatment was replicated three times within each experiment.

7.2.2 Effect of ABA on germination of dry and osmotically treated seed

Seed of LG3295 (HDP) and Option 501 (LDP) were incubated in osmotic solution (-1.5 MPa using PEG-8000 (Michel 1983)) at 20 C in darkness for up to 4 weeks. After 2 or 4 weeks of imbibition in PEG, seeds were transferred to new petri dishes and secondary seed dormancy was determined as described in Chapter 5 by using control seed imbibed in double distilled (ddi) water in darkness for 2 or 4 weeks to correct for viability and primary dormancy. Under green light, 100 seeds per replicate of control were transferred to ddi water, while seeds of three other treatments were transferred to either 0.01, 0.1, or 1.0 mM (+)-cis,trans-ABA, 98% (Shanghai Mapji Import and Export Crop. Ltd., Shanghai, China) and incubated at 20 C in darkness. Germination was defined as the protrusion of the radicle through the testa and was evaluated after 10 days. In seeds exposed to ABA, relative germination was determined by subtracting the average number of dormant seed in the control treatment from each replicate prior to determining the percentage of germinated seed in the remaining portion of each sample. Relative germination also was corrected for seedlot viability and primary dormancy.

7.2.3 Effect of ABA and fluridone on secondary seed dormancy

To further investigate the effects of ABA, and to investigate the effects of fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl-4(1H)-pyridinone), an inhibitor of ABA synthesis, seed of both genotypes were osmotically stressed by incubating at 20 C in darkness for up to 4 weeks in -1.5 MPa PEG-8000 or -1.5 MPa PEG-8000 containing either 0.01 mM or 1.0 mM ABA or 0.03 mM fluoridone (Yoshioka et al. 1998). After either 2 or 4 weeks seed was rinsed three times in ddi water and transferred to new petri-dishes containing ddi water under green light. To determine seedlot viability and primary dormancy, dry seed was imbibed in ddi water. Secondary seed dormancy was determined in the osmotic treatments by correcting for viability and primary seed dormancy determined from the water imbibed control (Chapter 5). Seed dormancy was evaluated after 10 days at 20 C. In a second set of replicates, gravimetric seed water content was determined in all treatments after 2 and 4 weeks of exposure to osmotic stress. Seeds were dried at 105 C for 24 hrs.

In a further experiment, only dormant seeds (confirmed dormant), were transferred to new petri dishes (35 or 50 seeds per petri dish for LG3295, 15 or 20 seeds per petri dish

for Option 501, depending on experiment) under green light and were subjected to 0.03 mM fluridone or ddi water. Confirmed dormant seeds were those which had been induced into secondary dormancy using the above described assay, followed by 2 weeks of germination in ddi water in darkness. This ensured that all non-dormant seeds had been removed. After 10 days at 20 C in darkness, seed dormancy was recorded. Gravimetric seed water content also was determined in these treatments when enough dormant seed remained at the end of the experiment.

7.2.4 Hormone and metabolite analysis

Seeds of both genotypes were induced into secondary seed dormancy by incubating at 20 C in darkness for 1, 2, 3, or 4 weeks in -1.5 MPa PEG-8000. Following osmotic stress, the seed was removed, blotted dry and immediately frozen in liquid N_2, then stored at -70 C until analysed. Dry, untreated seed also were frozen in liquid N_2 as a control and stored at -70 C. With the exception of dry, untreated seed (non-dormant) and confirmed dormant seed (all dormant), samples used for hormone analysis contained genotype specific mixtures of dormant and non-dormant seed.

For plant hormone and metabolite analysis, the frozen sample was lyophilized for 48 h and ground for 15 s at a speed of 5 m s^{-1} using a FastPrep FP120 tissue grinder (Qbiogene, Carlsbad, CA, USA). Approximately 50 mg (exact weight was recorded) was extracted with 4 mL of extraction buffer (99:1 isopropanol:glacial acetic acid (v/v)) to which 20 ng of the following deuterium labelled internal standards were added: ABA, phaseic acid (PA), dihydrophaseic acid (DPA), 7-hydroxy-abscisic acid (7'OH-ABA), abscisic acid glucose ester (ABA-GE), and gibberellins (GA_1, GA_3, GA_4 and GA_7). Exposure to light was minimized by wrapping the flask in aluminium foil while shaken on an orbital shaker (300 rpm) at 4 C for 24 hrs. The sample was then centrifuged at 290 g for 10 min and the supernatant removed and saved. The pellets were resuspended in 0.5 mL of the extraction buffer, centrifuged once more and the supernatant added to the initial extracted volume. The extracted supernatant was then passed through Sep-Pak C 18 columns (Waters Ltd., Mississauga, ON, Canada) that had been first equilibrated with 100% methanol followed by extraction buffer. Then the column was rinsed with 0.5 mL of 80% methanol acidified with 1% glacial acetic acid (Chiwocha et al. 2003) and the eluate dried using a Centrivap concentrator (Labconco Corp., Kansak City, MO, USA). The residue was resuspended in 0.2 mL of 100% methanol and centrifuged at 1270 g for

10 min to remove any particulate matter prior to HPLC analysis.

Ten µL of sample were analysed by reversed phase high-performance liquid chromatography electrospray ionization-tandem mass spectrometry (RP-HPLC ESI-MS/MS) (2695 Waters HPLC, Waters Ltd., Mississauga, ON, Canada; Quattro Ultima, Micromass, Manchester, U.K.) and the endogenous levels of compounds quantified as described in Chiwocha et al. (2003). High compound recovery rates using this method have been documented (Chiwocha et al. 2003).

7.2.5 Data analysis

In all experiments, the data from the independent repetitions of each experiment were combined and the appropriate standard errors of the means were calculated. In the ABA and fluridone experiment, the data were combined and analysed as a one-way treatment structure using the GLM procedure in SAS (SAS Institute Inc., Cary, NC, USA). Fisher's protected LSD was used to determine differences among means in secondary dormancy and seed water content. Due to unbalanced replication in the confirmed dormant seeds, T-tests were used to compare seed water contents between the genotypes where possible.

7.3 Results

7.3.1 Effect of ABA on germination of dry and osmotically treated seed

Increasing concentrations of ABA inhibited germination in non-osmotically treated seed of the HDP and LDP genotypes to a similar degree (Figure 7.1). A dose response was observed, where the lowest ABA concentration resulted in little inhibition of germination, while at the highest ABA concentration (1.0 mM), germination in both genotypes was inhibited by 90 and 89% after 10 days of incubation at 20 C in darkness, respectively.

In contrast, after 2 and 4 weeks of exposure to osmotic stress, exposure to ABA resulted in distinct differences in relative germination between seed from the HDP and LDP genotypes. In HDP seed, the low ABA concentration (0.01 mM) inhibited germination by 45 and 64% at 2 and 4 weeks, respectively (Figure 7.1a). Increasing ABA concentration resulted in a further but relatively small reduction in germination. In contrast, in LDP seed, low ABA concentration (0.01 mM) had little effect on germination and increasing ABA concentration did not further reduce germination (Figure 7.1b).

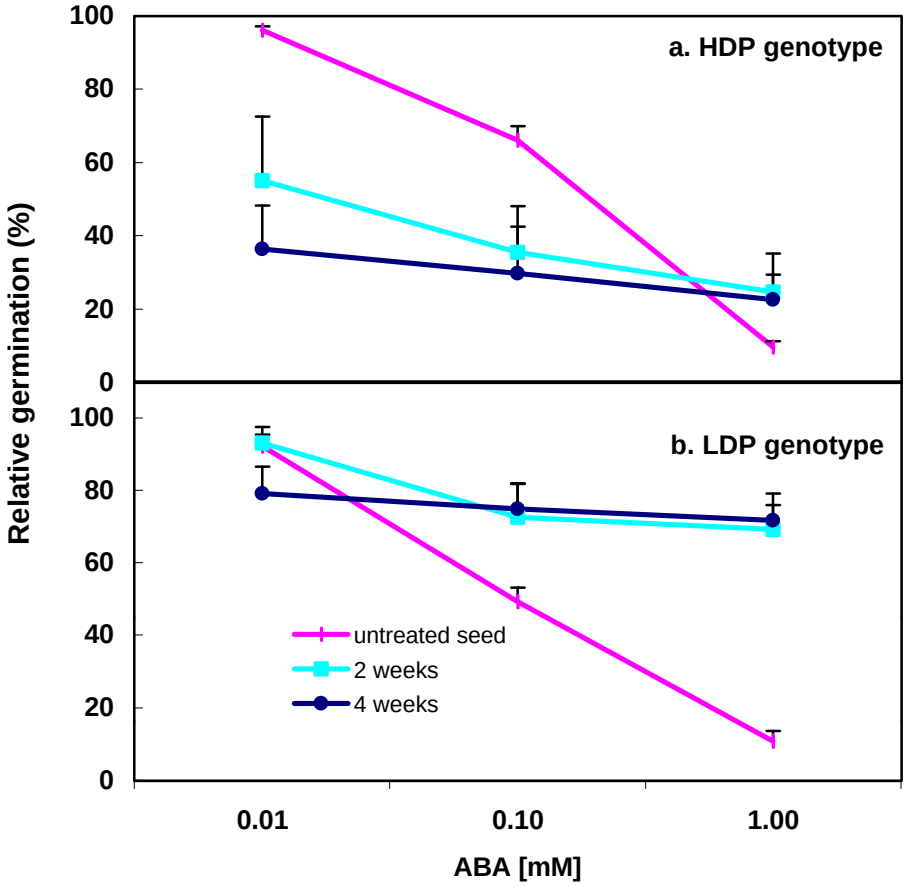

FIGURE 7.1 Effect of ABA on germination of dry or osmotically treated seed of one *B. napus* genotype with high (HDP (a)) and one genotype with low (LDP (b)) secondary seed dormancy potential relative to the control. Seed was exposed to dormancy-inducing osmotic stress treatment for 2 or 4 weeks, then treated with ABA for 10 days and germination recorded. Bars = 1 s.e.

Thus, the dose response to ABA application shown in non-osmotically treated seed was no longer observed in either genotype after exposure to osmotic stress. The germinable portion of seed on which relative germination is based was not the same in the two genotypes after exposure to dormancy-inducing conditions. In the LDP genotype, relative germination was based on 85% (s.e. 5.6) and 71% (s.e. 7.2) of the seeds that were still germinable at 2 and 4 weeks, respectively. In contrast, relative germination in the HDP genotype was based on 32% (s.e. 4.5) and 19% (s.e. 4.0) of the seeds that were still germinable at 2 and 4 weeks, respectively.

7.3.2 Effect of ABA and fluridone on secondary dormancy

Exposure of HDP seed to fluridone during 2 or 4 weeks of osmotic stress treatment reduced secondary dormancy by 54 and 75% compared to the control, respectively (Figure 7.2). Conversely, exposure to exogenous ABA had no effect on secondary seed dormancy in this genotype. Although differences were much smaller, exposure of LDP seed to fluridone reduced dormancy on average by 90% and 1.0 mM ABA increased dormancy significantly (approximately 2.4 times) relative to the control.

In contrast, fluridone did not release secondary dormancy in confirmed dormant seed of both genotypes. After two weeks of exposure to fluridone, secondary seed dormancy was 80% (s.e. 4.1) in HDP seed and 87% (s.e. 2.0) in LDP seed, while secondary seed dormancy in control treatments was 91% in seed of both genotypes (s.e. 1.9 in HDP and 2.1 in LDP seed) (data not shown).

No differences in seed water content were found between 2 and 4 weeks after exposure to dormancy-inducing conditions and therefore, the data were combined. Although the differences were small (# 6%), mean seed water content in the HDP seed was higher than in LDP seed in the control and ABA treatments, but not in the fluridone treatment during osmotic stress treatment (Table 7.1). Within genotype, exposure to ABA and fluridone did not affect seed water content in HDP seed significantly, while in the LDP genotype, seeds exposed to fluridone exhibited 4% higher seed water content than the control (Table 7.1). After exposure to ddi water for two weeks, seed water content in dormant seed increased by approximately 20% all treatments and no statistical differences in seed water content were observed between the genotypes nor among the different treatments within genotype that had been applied during osmotic stress treatment (Table 7.1).

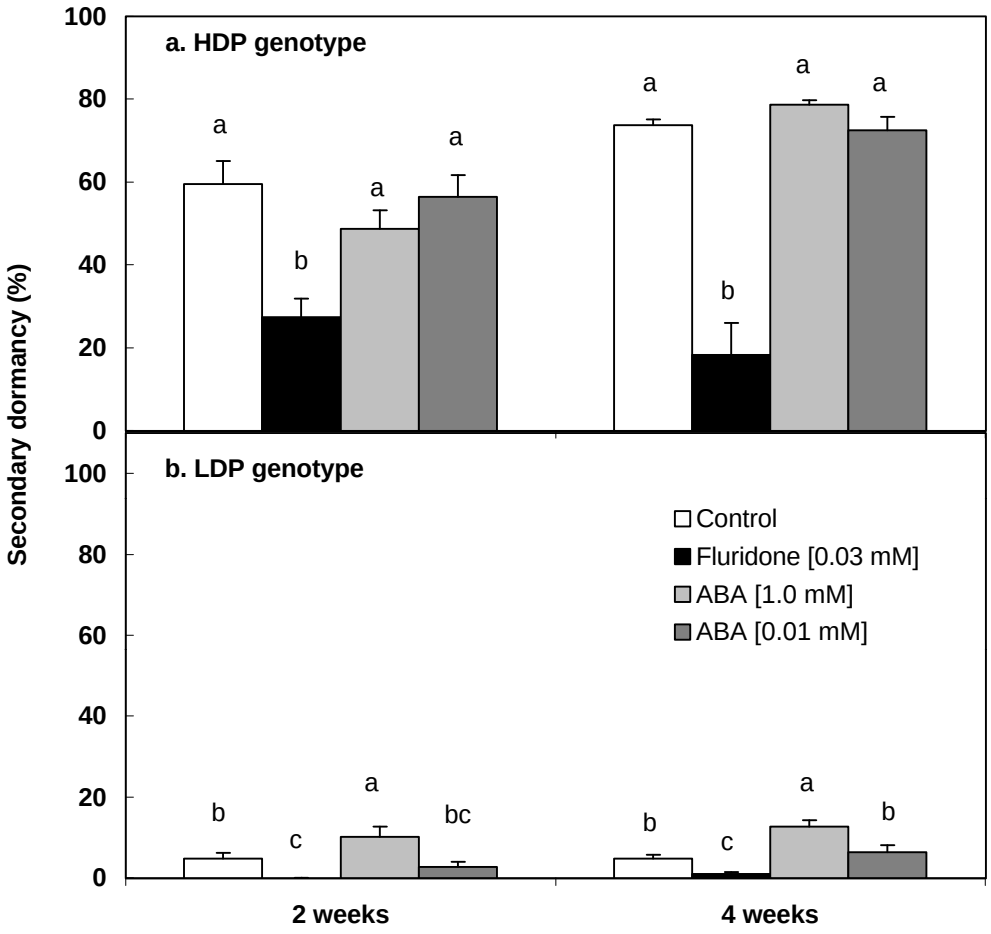

FIGURE 7.2 Effect of ABA and fluridone treatment on secondary seed dormancy of one *B. napus* genotype with high (HDP (a)) and one genotype with low (LDP (b)) secondary seed dormancy potential. Seed was exposed to ABA and fluridone during dormancy-inducing osmotic stress treatment for 2 or 4 weeks then transferred to ddi H_2O for 10 days and the number of seeds remaining dormant recorded. Bars = 1 s.e.

TABLE 7.1 Seed water content in one *B. napus* genotype with high (HDP) and one with low (LDP) secondary seed dormancy potential as influenced by fluridone and two different concentrations of exogenous ABA during and after dormancy-inducing osmotic stress treatment.

	Seed Water Content			
Genotype	**Control**	**Fluridone [0.03 mM]**	**ABA [1.0 mM]**	**ABA [0.01 mM]**
	----------% (w/w)----------			
during osmotic stress treatment (dormant and non-dormant seed)				
HDP	51.3 a[a]	49.0 a	49.4 a	48.7 a
LDP	45.2 b	49.2 a	47.3 ab	45.2 b
	0.0001[b]	N.S.	0.039	0.009
confirmed dormant seed				
HDP	69.6 a	73.0 a	69.2 a	68.5 a
LDP	67.3[c]	-	65.7	66.4
	N.S.		N.S.	N.S.

[a] Within genotype, means followed by different letters indicate significant differences using Fisher's protected $LSD_{0.05}$.

[b] Probabilities of significant differences between genotypes when less than 0.05.

[c] Not enough seeds for proper replication in some treatments in this genotype at this time.

7.3.3 Hormone and metabolite analysis

Similar levels of ABA and ABA metabolites were observed in dry seed of both genotypes (Figure 7.3). During osmotic stress treatment, endogenous levels of ABA remained unchanged in HDP seed while declining by 50% in LDP seed (Figure 7.3). Over the 4 week stress period ABA-GE increased in seed of both the HDP and LDP genotype but by almost twice as much in the former. In the HDP genotype this increase in seed ABA-GE content during osmotic stress treatment was approximately one order of magnitude relative to dry, untreated seed (Figure 7.3). Together, the total content of ABA + ABA-GE of HDP seed was between 1.6 and 2.1 times greater than that of LDP seed after 2 to 4 weeks of osmotic stress treatment. During the first week, levels of the ABA catabolite, DPA, decreased substantial but similarly in seed of both genotypes (i.e. by 93.3 and 102.2 ng Å gDW^{-1}, respectively) and remained low for the rest of the osmotic stress period. The ABA metabolite 7'OH-ABA also decreased in seed of both genotypes during the first week of osmotic stress treatment to unmeasurable levels. Phaseic acid (PA), was not detected at any time in either genotype.

Gibberellin A_1 levels were low in dry seed of both genotypes and increased during the first week of osmotic stress treatment (Figure 7.3). After one week of osmotic stress treatment, gibberellin A_1 levels in LDP seed were 1.5 times those measured in seed of the HDP genotype. Throughout the remainder of the osmotic stress treatment, gibberellin A_1 decreased to lower, but measurable levels in seed of the LDP genotype while gibberellin A_1 levels declined to unmeasurable levels in HDP seed. No consistent trends were observed in GA_3 levels which were below 1.5 ng Å g DW^{-1} when detected (data not shown), while gibberellins A_4 and A_7 were not detected at any time.

Confirmed dormant seeds of the HDP genotype also were subjected to hormone profile analysis. These seeds contained approximately 14 ng Å g DW^{-1} (s.e. 1.6) ABA and approximately 29 ng Å g DW^{-1} (s.e. 1.2) ABA-GE. These levels were similar to those noted for mixtures of germinable and dormant seeds at the end of osmotic stress treatment. In confirmed dormant seeds, however, none of the other compounds could be detected (data not shown). Not enough seed of the LDP genotype could be collected to conduct a similar analysis.

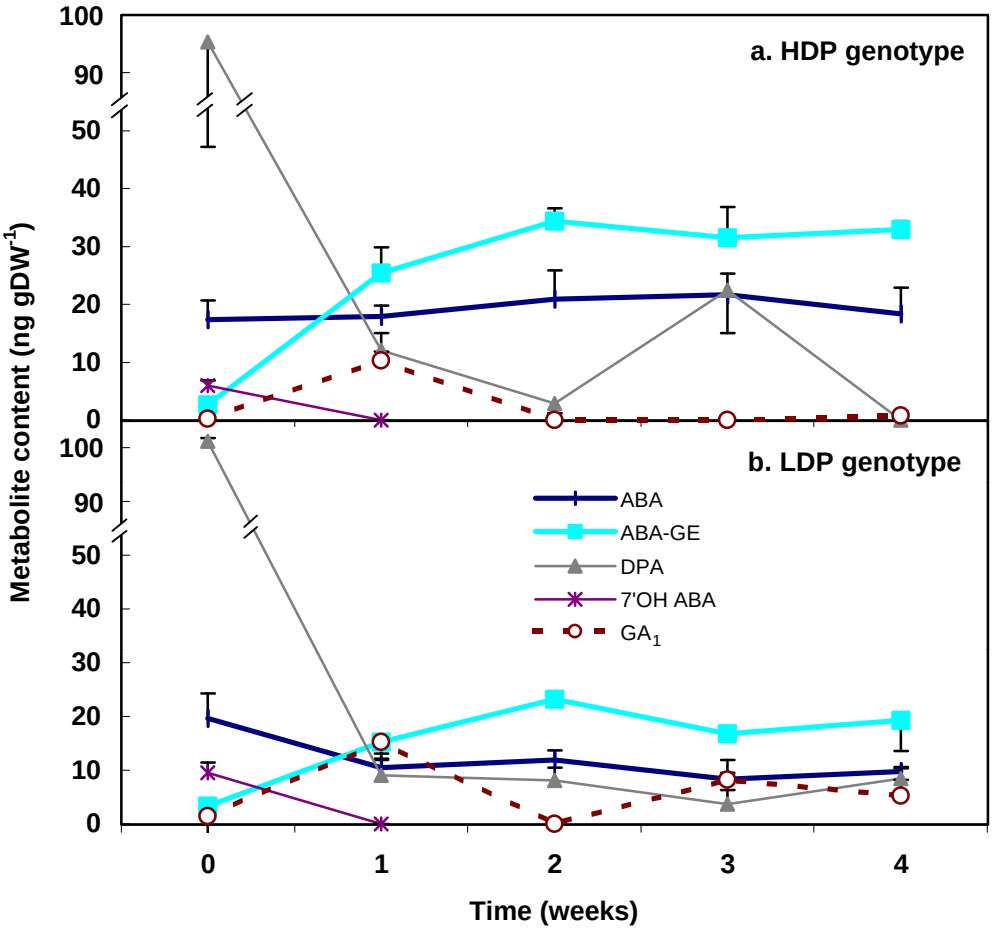

FIGURE 7.3 Hormone and hormone metabolite profiles for *B. napus* seed of one genotype with high (HDP (a)) and one genotype with low (LDP (b)) secondary seed dormancy potential during dormancy-inducing osmotic stress treatment. Bars = 1 s.e.

7.4 Discussion

Osmotic stress treatment resulted in subsequent differential seed germination responses (sensitivity) to ABA between LG3295 (HDP) and Option 501 (LDP) seed that are consistent with ABA having a role in the development and maintenance of seed dormancy in *B. napus* (Figure 7.1). Exogenous applications of ABA failed to inhibit germination in the non-dormant fraction of LDP seed while inhibiting germination in the non-dormant fraction of HDP seed. Thus, it appears that high secondary seed dormancy potential in *B. napus* is associated with the retention of an ability to respond to ABA application. In fact, seed of the HDP genotype became more responsive to low exogenous concentrations of ABA during osmotic stress treatment (Figure 7.1). Further support for this conclusion comes from the observed response to fluridone application (Figure 7.2). After both 2 and 4 weeks of osmotic stress treatment, dormancy induction in seed of the HDP genotype was substantially lower when exposed to fluridone. Similar results were observed in seed of the LDP genotype, although the response was less pronounced in comparison. These findings are consistent with reports that have implicated ABA in the development and maintenance of seed dormancy (Walker-Simmons 1987; Wang et al. 1995; Yoshioka et al. 1998; Grappin et al. 2000).

Analysis of ABA and ABA metabolite profiles during osmotic stress treatment suggest that high dormancy potential may be associated with accumulation of ABA in both the free and conjugated forms (Figure 7.3). This contrasts with the response of lettuce seed to thermodormancy as a result of exposure to high temperatures, where dormancy is accompanied by ABA degradation to DPA rather than accumulation of ABA-GE (Chiwocha et al. 2003). Nevertheless, the accumulation of ABA and ABA-GE in seed of the HDP genotype is consistent with the response of seed dormancy to fluridone application during osmotic stress treatment; notwithstanding that fluridone has been shown to interfere with ABA metabolism to PA (Zeevaart et al. 1990) and the action of tetcyclacis (Kahn 1994), an inhibitor of gibberellin synthesis. Hormone levels in this study were similar to those previously reported in *B. napus* seed (Finkelstein et al. 1985; Fu and Lu 1991; Juricic et al. 1995).

Interestingly, little response to fluridone was observed in confirmed dormant seed in both *B. napus* genotypes. These results contrast reports in thermodormant lettuce seed, where fluridone readily releases seed dormancy when applied after dormancy induction (Yoshioka et al. 1998). In *B. napus*, it seems the significance of ABA in maintaining seed dormancy declines sharply after the termination of exposure to osmotic stress. In the

HDP genotype, similar levels of ABA and ABA-GE were found in confirmed dormant seed and the mixture of dormant and non-dormant seed at the end of the osmotic stress treatment. It is not clear whether the lack of response to fluridone in confirmed dormant seed in this genotype was due to a change in the ability to respond to ABA, a change in ABA turnover, or both.

Although dormancy increased in LDP seed after osmotic treatment in combination with ABA, this increase was only significant for the higher ABA concentration (1.0 mM) and was relatively small compared to the dormancy achieved in seed of the HDP genotype (Figure 2). Therefore, low dormancy potential seemed to be associated with a lack of ABA response.

The increase in gibberellin A_1 observed in both genotypes one week after initiation of the experiment suggests metabolic changes correlating with germination, as increases in this hormone have been associated with the onset of germination in this and other species (Fu and Lu 1991; Chiwocha et al. 2003). Moreover, exposure to gibberellic acid (0.2 mg l^{-1}) effectively releases secondary seed dormancy in *B. napus* (Pekrun et al. 1998b). A mode of action has recently been elucidated where gibberellins have been shown to promote germination by repressing products from genes that inhibit germination (Peng and Harberd 2002). In seed of the HDP genotype, gibberellin A_1 accumulated only transiently during osmotic stress while in LDP seed gibberellin A_1 synthesis persisted (Figure 7.3). This suggests that higher gibberellin A_1 along with lower ABA may be responsible for low dormancy potential or, at least, is associated with low dormancy potential. Reduced gibberellin levels in HDP seed after the first week of osmotic stress may be indicative of a reversion to a more dormant state that is characteristic in this genotype after 4 weeks of osmotic stress treatment (Figure 7.2).

In both genotypes, rapid degradation of relatively large amounts of DPA remaining from seed maturation occurred during the first week of exposure to osmotic stress (Figure 7.3). Nevertheless, the fate of DPA remains unknown as internal standards for the quantification of DPA metabolites are not yet available. In *B. napus*, 7'OH-ABA and PA were not observed during dormancy induction. The lack of PA suggests either immediate conversion to DPA, or to another PA related metabolite, e.g. PA-glucose ester. ABA degradation via other minor pathways (Cutler and Krochko 1999) was not examined in this experiment.

Interestingly, mean seed water content of osmotically treated HDP seed tended to be reduced while mean seed water content of osmotically treated LDP seed tended to be increased by application of ABA, although the differences were not significant (Table 1). The response of seed water content in osmotically treated HDP seed to ABA application, and lack thereof in osmotically treated LDP seed, is consistent with the ability of these two genotypes to respond to ABA application after osmotic stress treatment (Figure 7.1). In the HDP genotype, the response in seed water content to exogenous application of ABA during osmotic stress treatment was similar to that previously reported in non-osmotically treated *B. napus* seed (Schopfer and Plachy 1984, 1985). The significant increase in mean seed water content by the application of fluridone during osmotic stress treatment only observed in LDP seed may be linked to higher germinability in this treatment. Cell osmolyte content increases early during the progression towards germination due to starch breakdown to soluble carbohydrates (Fischer et al. 1988). This, in turn, may have resulted in the increased seed water content in this treatment during exposure to osmotic stress.

In summary, these experiments demonstrated a link between the ability of seed to respond to ABA as well as hormone content (total ABA (ABA + ABA-GE) and gibberellin A_1), and secondary seed dormancy potential in *B. napus*. Increases in total ABA content and the ability to respond to ABA were associated with high secondary seed dormancy potential, while persistence of low gibberellin A_1 levels throughout osmotic stress treatment was associated with low secondary seed dormancy potential. A more rapid and less time consuming method for identifying secondary seed dormancy potential in *B. napus* would facilitate breeding programs that wish to select for low seed dormancy potential. However, it remains unknown whether the links to secondary seed dormancy potential described here may be used to clearly identify dormancy characteristics among *B. napus* genotypes with a broad range of secondary seed dormancy potentials.

8.0 General Discussion

8.1 Seedbank ecology of *B. napus*

Results presented in this thesis suggest that the seedbank ecology of *B. napus* is similar to many other small seeded annual weeds (Baskin and Baskin 1998), despite the lack of primary seed dormancy in *B. napus*. Secondary seed dormancy potential was consistently linked to increased seedbank persistence in *B. napus* in the field (Tables 4.1, 4.4 and 4.5; Figures 6.2 and 6.3). These findings led to the acceptance of the main hypothesis and thereby confirmed the importance of secondary seed dormancy as a persistence mechanism in this species in western Canada. Moreover, greater seedbank persistence increased with burial depth (Figures 6.2 and 6.3), which also is observed in many other species (Benech-Arnold et al. 2000). The seedbank ecology of *B. napus* contrasts with that of other escaped crops such as feral rye where greater burial depths increase the rate of seedbank decline (Stump and Westra 2000).

In *B. napus*, rates of seedbank decline were relatively rapid when the seedbank was disturbed (minimum of approximately one order of magnitude per year in HDP genotypes in CT) (Chapter 4). These rates of seedbank decline would conform well to the negative exponential models used to describe seedbank decline in other species (Donald 1993; Rees and Long 1993; Stump and Westra 2000). Despite rapid rates of seedbank decline, high levels of seedbank input occurred at the time of harvest (Table 3.1), which in addition to high secondary seed dormancy potential, may result in *B. napus* seedbank persistence for several years.

Thompson and Grime (1979) subdivided seedbanks into four categories depending on persistence. Transient seedbanks (Types I and II) were defined as those in which seeds germinate or lose viability prior to the addition of new progeny to the seedbank, while in the two persistent seedbank types (Types III and IV), a portion of the seeds persist for several generations. The long-term field study showed that a small portion of the *B. napus* seedbank persisted for several years (Table 4.1), indicating a Type III seedbank

(Thompson and Grime 1979). The results from the seasonal seedbank dynamics experiment (Chapter 6), however, indicate different seedbank types in *B. napus* as a function of seed burial depth. While the buried seedbank could be described as a Type III seedbank with a small portion of persisting seeds (Table 4.1, Figures 6.2 and 6.3), the shallow seedbank was consistently transient. All seeds had either germinated or lost viability before new progeny would be added to the shallow seedbank (Figures 6.2 and 6.3). Different seedbank types also have been observed in dwarf snapdragon (*Chaenorrhinum minus* (L.) Lange), an annual found in eastern Canada where soil water content has been linked to the transience or persistence of the seedbank (Cavers 1983). Similarly, my results indicated that the buried *B. napus* seedbank was more transient when the soil water potential was high prior to freeze-up in autumn (Figure 6.2 and 6.3) and thus, autumn soil water potential appears to substantially influence the dynamics of the buried seedbank (Table 6.1).

8.2 Management implications

Several management recommendations that enable a more integrated approach to controlling volunteer *B. napus* can be drawn from this research. Only low dormancy potential genotypes should be grown if concerns associated with volunteer canola are to be minimized. Factors other than genotype appear to have relatively little influence on secondary seed dormancy potential (Table 5.1, Figure 5.3). Most spring *B. napus* genotypes investigated in these studies, however, possessed high secondary seed dormancy potentials (Table 5.1). Therefore, integrated seedbank management strategies to minimize seedbank persistence should be adapted. In western Canada, mean seedbank additions during canola harvest were higher than expected given that canola is generally windrowed prior to harvest. One advantage of this harvest method is reduced seed shatter by wind. Nevertheless, more diligent canola harvest practices may have reduced additions to the *B. napus* seedbank on several of the surveyed farms (Table 3.2). Interestingly, many producers appear to be unaware of their canola harvest losses.

Seed burial increases seedbank persistence of *B. napus* in western Canada (Figures 6.2 and 6.3) and should be avoided. The dramatic seedbank decline that was observed in the shallow burial depth in Chapter 6, however, was not realized in ZT (Chapter 4). In ZT, the seedbank tends to be concentrated near the soil surface, while seed burial in CT results in a more even vertical distribution of the seedbank throughout the soil profile

(Clements et al. 1996). Given that the shallow seedbank was transient (Figures 6.2 and 6.3), seedbank persistence under ZT (Chapter 4) can only be explained by seed burial to a depth where persistence occurs. This may have occurred either via cracks in the soil at the time of seedbank establishment, perhaps through vertebrate and invertebrate assisted seed burial, or most likely due to soil disturbance at the time of seeding. In fact, no seeds remained on the soil surface in the ZT plots after the first winter following seedbank establishment, which was most likely due to wind and water assisted soil movement. Seeds were clearly visible on the soil surface in ZT at the time of seedbank establishment. Some seedbank burial to depths where persistence is prolonged seems unavoidable in ZT and therefore, it appears that exploiting the transient seedbank near the soil surface as part of an integrated control strategy cannot be fully realized in continuous cropping systems. In circumstances where minimum seedbank persistence is a primary goal, chemical fallow following a *B. napus* crop may be the best option. Rapid seedbank decline using this method may be of interest to the *B. napus* breeding industry and seed growers where any contamination by persisting volunteers is undesirable. Whether a shallow harrowing (1-2 cm) in autumn, which may facilitate seed imbibition and as a result germination and/or seed desiccation, is of benefit in western Canada has yet to be verified experimentally.

The seasonal seedling recruitment observations in the field (Table 4.4 and 4.5) suggest a high potential for overlap in flowering periods between volunteer plants and a seeded *B. napus* crop. As a consequence, pollen-mediated gene flow between volunteer and crop plants may be impossible to avoid in western Canada. This is in contrast to eastern Canada, where overwintering plants tend to reach anthesis before a spring seeded *B. napus* crop (Simard et al. 2002).

High volunteer *B. napus* seedling recruitment throughout spring in the year following seedbank establishment may be expected (Tables 4.4 and 4.5). Therefore, a competitive crop in which volunteer *B. napus* may readily be controlled using in-crop herbicides should be seeded if chemical fallow is not an option. Competitive crops not only limit yield reductions that may be incurred by uncontrolled volunteer *B. napus*, but also reduce potential seed return by any uncontrolled or escaped volunteer plants. The choices of competitive crops in which volunteer *B. napus* is readily controlled by herbicides, however, may become more limited if the previous canola crop was herbicide tolerant.

8.3 Seed dormancy

Results presented in this thesis indicate that secondary seed dormancy is an important seedbank persistence mechanism in *B. napus* in western Canada (Chapters 4 and 6). The seed dormancy status in the field (i.e. conditional vs. innate secondary seed dormancy), however, could not be discerned with the elutriation method used in these experiments. Nevertheless, in one unreplicated experiment with seed from an HDP and an LDP genotype that was retrieved from the deep seedbank, changes in the ability to respond to exogenous ABA application between spring (May) and summer (July) of 2002 were similar to those observed in the laboratory between non-dormant, non-osmotically treated seed and seed exposed to dormancy-inducing osmotic stress, respectively (Figures 7.1 and Appendix A.1). These findings suggest similar changes in dormancy state in seed of the HDP and LDP genotypes in the seedbank from spring to summer as those during dormancy-inducing osmotic stress in the laboratory (Figures 6.1, 6.2, and 6.3).

The seasonal seedbank dynamics experiment (Chapter 6) illustrated the dilemma with respect to seed dormancy evaluation in the field versus determining persistence rates. When seed was buried at high densities in nylon pouches to facilitate easy retrieval from the soil, predation by insects influenced seedbank persistence. In contrast, when seed was buried in soil at a lower density to simulate more realistic seedbank densities, seed predation seemed reduced, while seed retrieval from the soil became more complex. Seed dormancy characteristics, however, were influenced by seed retrieval through elutriation (Chapter 6). Hence, a metabolic marker linked to seed dormancy expression that remains unaffected by elutriation would be ideal for determining *in situ* seed dormancy status in addition to persistence in the field using the same samples. Whether the metabolic markers and the ability to respond to ABA identified in Chapter 7 remain unaffected by seed elutriation and are reliable for quantifying seed dormancy status in *B. napus* has yet to be determined.

Evidence of the importance of temperature in regulating secondary seed dormancy in *B. napus* during osmotic stress treatment is strong (Pekrun et al. 1997b and c; López-Granados and Lutman 1998; Momoh et al. 2002; Chapter 6), while hypoxia alone is relatively poor at inducing secondary seed dormancy in *B. napus* (Pekrun et al. 1997c; Momoh et al. 2002). Nevertheless, the effects of hypoxia in combination with osmotic stress may have influenced ungerminability in the deep seedbank (Chapter 6). Whether

hypoxia and osmotic stress interact with respect to seed dormancy induction alone or in combination with temperature has not been investigated in the laboratory.

Although not quantified, observations in the harvest regime (seed maturity) experiment (Chapter 5) indicated higher levels of vivipary in siliques of LDP genotypes (Option 501 and LG Dawn) compared to HDP genotypes (LG3295 and AC Excel). The role of ABA in preventing premature germination has been established in this species (Finkelstein et al. 1985; Juricic et al. 1995). These observations suggest a possible link between vivipary and secondary seed dormancy potential and may indicate common elements between preventing vivipary and the mechanisms regulating seed dormancy in *B. napus*.

8.4 Future research

8.4.1 Field studies

This research has provided important information on the seedbank ecology of *B. napus* in western Canada with respect to secondary seed dormancy, however, some questions regarding the seedbank ecology as well as dormancy characteristics of weedy populations remain unanswered. *B. napus* seedbank persistence of one cohort for up to three years is readily possible in western Canada (Chapter 4). Derksen and Watson (unpublished data) observed higher seedbank populations in this species after two and three years in rotation in western Canada than those recorded in my experiment. Assuming similar seedbank decline rates among the studies, their observations suggest significant seed return to the seedbank by volunteer plants. In eastern Canada, seed return of up to 3,000 seeds plant^{-1} (Simard et al. 2002) has been observed from overwintering volunteer *B. napus*, however, the significance of seed return by volunteer plants in rotation has not been quantified in western Canada. It also is not known how long weedy *B. napus* populations retain the seed dormancy characteristics of the source genotype or whether strong selection pressures towards greater or reduced seed dormancy potential exist in successive weedy generations. The influence, if any, of hybrid breeding systems and subsequent segregation on secondary seed dormancy potential in progeny of the first or higher weedy generations is also unclear. A field study in which seed return by uncontrolled volunteer *B. napus* is allowed and quantified for several years and in which secondary seed dormancy potential of the seed rain is determined annually would provide some of these answers.

8.4.2 Laboratory studies

The current test for secondary seed dormancy potential is labour and time intensive and is not practical for large scale genotype screening (5 to 6 weeks for each independent replication). A more rapid method for the identification of secondary seed dormancy potential is required if *B. napus* breeding programs were to consider incorporating selection for low dormancy genotypes. Therefore, the identification of metabolic or molecular makers linked to seed dormancy potential would be a great asset. Whether the ability to respond to ABA and/or ABA and gibberellin profiles identified in Chapter 7 are applicable for this purpose remains to be investigated. Marker assisted breeding programs prefer the use of molecular markers. The genetic complexity of seed dormancy in dicots with the influence of nuclear as well as maternal factors (Garbutt and Witcombe 1986; Foley and Fennimore 1998), however, complicates the task of identifying molecular markers for this trait. Nevertheless, the divergent secondary seed dormancy potentials among *B. napus* genotypes (Table 3.1) and significance of genotype to secondary seed dormancy potential (Table 5.1) suggests that identification of molecular markers associated with secondary seed dormancy potential may be possible. The metabolic markers identified in Chapter 7 may be more suitable for seedbank ecology studies where seed dormancy status rather than dormancy potential is of interest (e.g. Appendix A).

The mechanisms regulating seed dormancy are poorly understood. It is also not clear whether the regulation of primary and secondary seed dormancy are similar (Bewley 1997). As secondary seed dormancy is easily manipulated in *B. napus*, this species may be a good model for investigations into the mechanisms controlling secondary seed dormancy. The close genetic relationship between *B. napus* to *A. thaliana* also is advantageous as numerous genes and markers linked to quantitative trait loci for seed dormancy have been identified in *Arabidopsis* (Rock 2000; Finkelstein et al. 2002; Alonso-Blanco 2003). Using molecular techniques such a microarrays and/or similar techniques (Chao 2002; Foley 2002) it may be possible to identify genes and gene products that aid in establishing the events leading to secondary seed dormancy development.

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Appendix A - Response to ABA application in *B. napus* seed exhumed from the field

A.1 Introduction

Despite differences in ungerminability of the deep seedbank between spring and summer in two *B. napus* genotypes with contrasting secondary seed dormancy potential, it was not possible to discern seed dormancy status in *B. napus* seed exhumed from the field after elutriation from the soil (Chapter 6). However, differences in the ability to respond to exogenous applications of ABA were observed between these same two *B. napus* genotypes in the laboratory and these were linked to secondary seed dormancy (Chapter 7). The objectives of this experiment were to determine whether the response to ABA in seed exhumed from the field at different times during the growing season is related to changes in the response to ABA observed in seed during dormancy-inducing osmotic stress in the laboratory.

A.2 Materials and Methods

In the seasonal seedbank dynamics experiment established in 2001 (Chapter 6), 400 seeds from each genotype (LG3295 (HDP) and Option 501 (LDP)) were buried in nylon pouches at a depth of 10 cm within 2 modified plastic pots in each tillage treatment at the time of seedbank establishment. No soil was mixed with the seed to facilitate rapid seed retrieval. Immediately following exhumation in spring (May 2002) and summer (July 2002), seeds in pouches were air dried, whole seeds were removed under fluorescent lighting and stored at -70 C for further analysis.

At a later date, seeds were subjected to 0.01, 0.1, and 1.0 mM ABA in triplicate as described in 7.2.2 (25 seeds per plate for HDP genotype, 15 seeds per plate for LDP genotype). The control treatment (3 repetitions) was imbibed in ddi water in darkness to determine viability and seed dormancy. In the ABA treated seed, relative germination

was determined after 10 days as described in Chapter 7. From the two locations, enough seed for only one replication of the experiment was recovered from the soil using this burial method.

A.3 Results and Discussion

In both genotypes, relative germination of seed exhumed form soil in spring responded in a similar dose response manner to that observed in non-dormant, non-osmotically treated seed in the laboratory (Figures 7.1 and Appendix A.1). These results suggest little seed dormancy in seed of both genotypes at this time and correlate well with the ungerminable portion of the deep seedbank in the spring of 2002 (Figures 6.2 and 6.3). In contrast, relative germination in HDP seed retrieved in the summer was more responsive to ABA application than in spring, particularly at the low (0.01 mM) and medium (0.10 mM) ABA concentrations, while seed of the LDP genotype exhumed in summer was less responsive to exogenous applications of ABA than in spring (Figure Appendix A.1). These changes in the ability of seed to respond to ABA were similar to those observed in the laboratory after seeds of both genotypes had been exposed to dormancy-inducing osmotic stress (Figure 7.1) and may suggest similar dormancy status in the field, i.e. low dormancy in both genotypes in the spring and higher levels of secondary dormancy in HDP seed than in LDP seed in the summer. Nevertheless, it must be noted that these results have not been repeated in time and space and care must be taken with respect to their significance. Further replication of this experiment is required to verify these results.

The seeds used for this experiment were intended for hormone profile analysis and were air-dried prior to analysis. Schopfer et al. (1979) showed that the loss of sensitivity to exogenous ABA during the later stages of germination of non-dormant *Sinapis alba* L. seeds imbibed in water (no osmotic stress) may be restored by drying. At this time, it is not clear whether air-drying the seed in these experiment affected their ability to respond to exogenous ABA application.

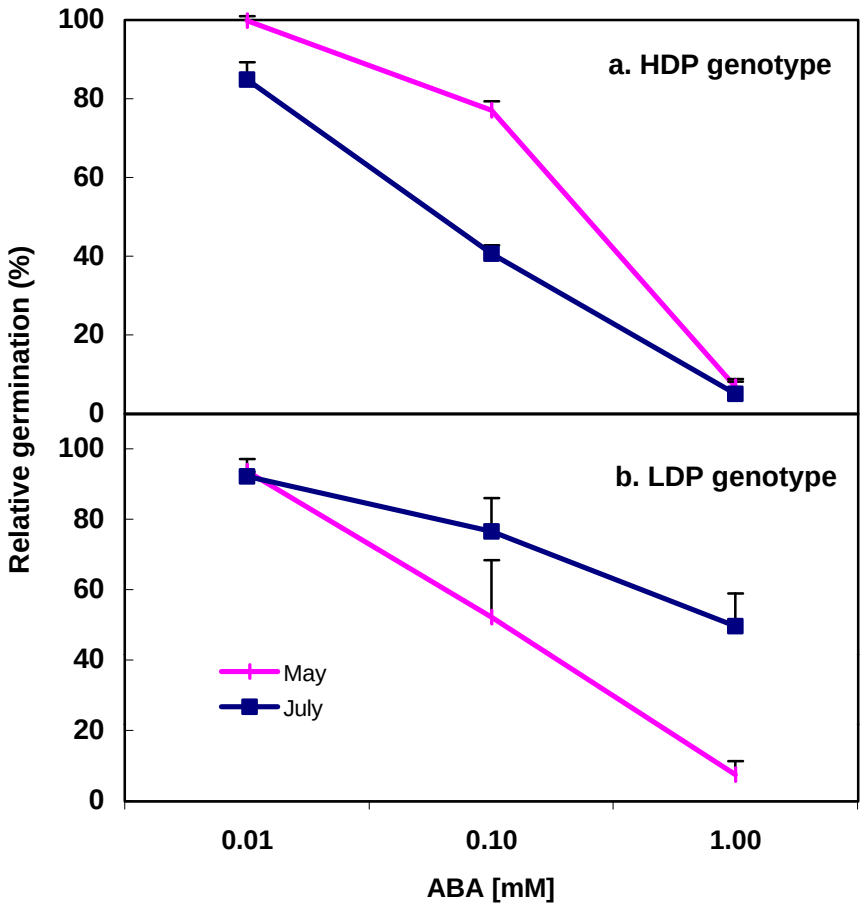

FIGURE A.1 Effect of ABA on germination of seed of one *B. napus* genotype with high (HDP (a)) and one genotype with low (LDP (b)) secondary seed dormancy potential exhumed from the field in May and July. Germination was recorded after 10 days of ABA treatment. Bars = 1 s.e.