Life Cycle Management of Volunteer Canola (*Brassica napus* L.) in Western Canada

BY

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

ABSTRACT

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Volunteer canola (*Brassica napus*) is among the top five most abundant weed species in western Canada. Yet, several knowledge gaps in volunteer canola management remain, particularly in crops with herbicide-resistance traits similar to canola [i.e., soybean (*Glycine max*)]. Large seed losses at harvest, seedbank persistence, phenotypic plasticity, rapid growth and development, efficient resource uptake and prolific plant fecundity all contribute to volunteerism in this crop species. This thesis research consisted of five field studies conducted in Manitoba, Canada, from 2013 to 2016 and aimed to discover effective tools for management of volunteer canola with particular focus on effective management for soybean production. Soil disturbance in early autumn, shortly after canola harvest, halved the persistence of volunteer canola over winter compared with zero tillage. Early autumn soil disturbance doubled autumn seedling recruitment, regardless of tillage implement, and winterkill effectively terminated the volunteer canola seedlings. Soil texture contributed to opposing seasonal dynamics of the volunteer canola seedbank. Soils with fine texture (clays) were associated with greater survival of volunteer canola seed over winter, but reduced seed survival over summer compared with coarse textured soils (loamy sands). Volunteer canola was a competitive weed in soybean due to rapid growth and development during soybean establishment. Individual cultural or physical weed management tools in soybean had minimal effect on volunteer canola seed production or biomass accumulation when implemented alone. Spring-seeded inter-row living cereal mulches in soybean, however, interfered with volunteer canola early during the growing-season and reduced volunteer canola seed production by about one-third (by up to 9,000 seeds m^{-2}). In another study, volunteer canola interference with soybean increased as soil mineral N increased. This research showed that volunteer canola is a competitive weed that is difficult to manage in soybean production. Using timely post-harvest soil disturbance for volunteer canola seedbank depletion, planting soybean on fields with limited soil mineral N and growing inter-row living cereal mulches with soybean for early-season interference with volunteer canola should be effective components of an integrated program for management of volunteer canola in western Canada.

FOREWARD

The thesis includes an introduction, literature review and five research chapters followed by general discussion and conclusions. The research chapters contain work conducted at the University of Manitoba from January 2013 to July 2017. The research chapters are written in the format of Weed Science and follow the style defined by the Department of Plant Science, University of Manitoba, Winnipeg, MB, CA.

1.0 INTRODUCTION

Canola (*Brassica napus* L.) is the most abundant oilseed crop grown in Canada (Statistics Canada 2017). Volunteer canola is currently among the top five most abundant weeds in western Canada and has steadily increased in relative abundance since the commercial release of canola in the late 1970s (Leeson et al. 2005, 2015; Leeson 2016). Volunteer canola can appear as a weed in years following canola production in part due to pod drop and pod shatter (Cavalieri et al. 2014; Gulden et al. 2017) which contribute to large harvest seed losses (Cavalieri et al. 2016; Gulden et al. 2003a). Absolute harvest losses average about 4,300 seeds m^{-2} in western Canada (Cavalieri et al. 2016), but can reach up to 14,000 seeds m^{-2} in some instances (RH Gulden, personal communication). Canola seed can enter secondary dormancy and persist for greater than three years in the soil seedbank (Gulden et al. 2003b). Contemporary conventional crop rotations implemented in western Canada generally last only two or three years. Once established, volunteer canola populations could persist throughout these crop rotations, especially if volunteer canola seed production is not limited. Non-transgenic imidazolinone-resistant or transgenic glyphosate- or glufosinate-resistant canola varieties are currently available for commercial production in western Canada (Beckie et al. 2006). Adventitious presence of unsolicited herbicide-resistance (HR) traits in pedigreed canola seedlots (Downey and Beckie 2002; Friesen et al. 2003) also may create problems when adopting other HR crops in canola-dominant crop rotations.

Soybean [*Glycine max* (L.) Merr.] is a warm-season oilseed crop with transgenic HR traits similar to those of canola. In Manitoba, Canada, the seeded hectarage of soybean has almost tripled in the last half decade (MMPP 2016; Statistics Canada 2017). Based on seeded hectarage, soybean is now the third most grown crop in Manitoba, following canola and wheat. Little is known about the competitive ability of soybean with weeds in general and, particularly, cool-season weed species in western Canada. Volunteer canola can be a problematic weed in soybean (Gregoire 2017) and growers in western Canada are looking for solutions to manage this weed. Research on both proactive and reactive management of volunteer canola is warranted to facilitate soybean production in this growing-region.

A wide range of research on management of summer-annual volunteer canola has been conducted in western Canada. Yet, few reports have summarized the effective management tools for inclusion in a comprehensive integrated weed management strategy (e.g., Gulden et al. 2008). The overall objectives of this thesis were to (a) summarize the existing weed management tools, (b) identify current knowledge gaps and (c) fill some of the most pertinent knowledge gaps for management of volunteer canola in western Canada. To facilitate this process, the summer-annual weed life cycle was used as a template for summary of the available knowledge on volunteer canola management. The pertinent research gaps were addressed through the design and implementation of five specific field research experiments conducted in Manitoba, Canada from 2013 to 2016. The main objectives of these research experiments were:

1. To determine how timing and implement of post-harvest tillage influence the volunteer summer-annual canola life cycle in western Canada (Chapter 3).
2. To determine whether soil texture and associated edaphic factors influence survival of volunteer canola seed in the soil seedbank (Chapter 4).

3. To identify effective non-chemical weed management tools for the development of an integrated volunteer canola management strategy in soybean (Chapter 5).
4. To evaluate the utility of using spring-seeded inter-row living cereal mulches in soybean to interfere with volunteer canola during soybean establishment (Chapter 6).
5. To determine the influence of soil mineral nitrogen on volunteer canola interference in soybean (Chapter 7).

2.0 LITERATURE REVIEW

2.1 Abundance and Distribution of Volunteer Canola in Western Canada

Canola (*Brassica napus* L.) is a cool-season oilseed crop. Based on seeded hectarage, canola is one of the most common crops grown in Canada, second only to wheat (*Triticum aestivum* L.) (Statistics Canada 2017). More than 99% of canola in Canada is grown in western Canada [in Manitoba (MB), Saskatchewan (SK) and Alberta (AB)] (Beckie et al. 2006, 2011a). After the release of herbicide-resistant (HR) canola in 1995 (Beckie et al. 2006), the total annual seeded hectarage of canola in western Canada increased rapidly and is now similar to the total annual hectarage of all wheat classes combined (Statistics Canada 2017).

In western Canada, harsh winter conditions [e.g.,30-year (1980-2010) climatic normal average daily air temperatures ranging from -20.5 to -10.3°C in January at Carman, MB (Environment Canada 2017)] limit production of canola to summer-annual (SA) varieties only (Gulden et al. 2008). Currently, canola production in Canada is comprised of three species, *B. napus*, *B. rapa* L. and *B. juncea* L., although about 99% of canola hectarage is seeded to *B. napus* (CGC 2008). *Brassica napus* has been the focus of the majority of literature on volunteerism. For this reason and for the purpose of the literature review, canola refers to *B. napus* unless identified otherwise.

In western Canada, surveys following in-crop weed management have shown a consistent increase in relative abundance of volunteer canola (*B. napus* or *B. rapa*) over the last 3 to 4 decades (from the 22nd most abundant weed species in the 1980s to the 4th or 5th most abundant weed species currently) (Leeson et al. 2005, 2016; Leeson 2015). In the spring of 1994, volunteer canola (*B. napus* or *B. rapa*) was the most abundant weed species in MB prior to in-crop weed management (Thomas et al. 1997). This survey found volunteer canola in 39% of farm fields with an average occurrence density of 102 plants m^{-2} and a density range of < 1 to 812 plants m^{-2} (Thomas et al. 1997). Recent mid-season surveys following in-crop weed management have ranked volunteer canola (*B. napus* or *B. rapa*) as the 4th most abundant weed species in SK (Leeson 2015) and 5th most abundant weed species in MB (Leeson et al. 2016).

Several changes in arable agriculture may have contributed to the increase in relative abundance of volunteer canola since the 1970s. In this time period, most of western Canada experienced a transfer from conventional tillage to reduced or zero tillage systems. This change in tillage system has been associated with shifts in weed communities (Derksen et al. 1993; Thomas et al. 2004) and emergence periodicity (Bullied et al. 2003). Pulse crop production also has increased in the canola growing-regions of western Canada. Pulse crops have few options for herbicide management of volunteer canola (Anonymous 2015). Increased relative abundance of volunteer canola in western Canada in part may have been caused by a greater frequency of pulse production in canola growing-regions, or an increase in frequency of canola grown in crop rotations (Leeson et al. 2005; Beckie et al. 2011a; Gulden 2003). The release of HR canola (Hall et al. 2005) and the use of glyphosate for pre-emergence weed management or chemical fallow in reduced tillage systems (Friesen et al. 2003) also may have contributed to volunteerism of domesticated canola within the cropping systems of this region. A recent, survey has shown that volunteer canola is the most abundant weed in soybean in western Canada (Leeson et al. 2017). The more recent increase in seeded hectarage of other HR crops in western Canada [i.e., soybean [*Glycine max* (L.) Merr.] and corn (*Zea mays* L.)] (Statistics Canada 2017) may contribute to increased relative abundance of volunteer canola.

2.2 Traits Assisting in Volunteerism

Current canola cultivars have several biological characteristics that influence species fitness and facilitate volunteerism within cropping systems. Phenotypic plasticity (e.g., Angadi et al. 2003; Morrison 1990a), prolific seed production (e.g., Harker et al. 2015a; Simard and Légère 2001), pod drop and pod shatter (e.g., Cavalieri et al. 2014; Gulden et al. 2017), secondary seed dormancy (e.g., Gulden et al, 2003b, 2004b) and rapid growth and resource uptake (e.g., Blackshaw et al. 2003, 2004a) are among some of the most important traits that assist in the development of volunteer canola populations. Transgenic or non-transgenic HR traits also may facilitate the development of volunteer canola populations in cases where selection pressure is imposed by herbicide application (Hall et al. 2005).

2.2.1 Phenotypic Plasticity. Canola plants have high phenotypic plasticity and readily adapt to their immediate environment (Hall et al. 2005; Gulden et al. 2008). For example, canola can compensate for reduced plant densities by altering its yield components (Morrison et al. 1990a, 1990b). Angadi et al. (2003) reported complete canola yield compensation between uniform stands of 40 and 80 plants m^{-2}. Complete canola yield compensation also was observed at densities as low as 20 plants m^{-2} in favorable conditions. In their study, altered canola stand uniformity by removing 1 m lengths of adjacent canola rows did not affect yield when population density remained constant. However, yield reductions were observed when spatial arrangement of canola was non-uniform and population densities were reduced. Yield compensation by canola was primarily due to increased branching, number of pods per plant and pod retention (Angadi et al. 2003). The ability for canola to alter yield components based on environmental cues may allow for ample seed production and seedbank contributions even under conditions of adversity.

2.2.2 Fecundity. Volunteer canola can be a prolific weed species. For example, overwintered volunteer canola in eastern Canada produced up to 3,000 seeds plant^{-1} (Simard and Légère 2001). Similar to observations in Canada (e.g., Gregoire 2017; Harker et al. 2015a; Morrison et al. 1990a), crop plants of winter-annual (WA) canola produced 1,100 to 3,480 seeds plant^{-1} in Germany [(Cramer 1990; Geisler 1983; Geisler and Dipenbrock 1985; Lütke Entrup and Oehmichen 2000) summarized by Gruber and Claupein (2007)], and volunteer plants produced about 1,577 seeds plant^{-1} in canola crops and < 320 seeds plant^{-1} in cereal crops (Gruber and Claupein 2007). Little information is available on plant fecundity of volunteer canola in western Canada.

2.2.3 Pod Drop and Pod Shatter. Sequential rather than synchronous pod (silique) maturation contributes to the inherent potential for pod drop or pod shatter in canola (Morgan et al. 1998). Pod drop is caused by complete abscission of the silique at the pedicle-rachis junction (Gulden et al. 2017). Pod shatter is caused by the dehiscence of seeds from the silique, while the repulm remains intact (Gulden et al. 2017). Both pod drop and pod shatter contribute to harvest seed losses in canola (Cavalieri et al. 2014; Gulden et al. 2017). Genotype, environment and crop management all play a role in canola harvest losses (Cavalieri et al. 2016). However, pod shatter is primarily influenced by genotype, while pod drop is primarily influenced by environment (Cavalieri et al. 2014; Gulden et al. 2017).

In western Canada, seedbank additions before and during harvest amount to an average of about 6% of canola yield (about 4,300 seeds m^{-2}) (Cavalieri et al. 2016; Gulden et al. 2003a). The average percentage of canola yield losses in western Canada is similar to those reported in other regions [2-5% in the United Kingdom (Pekrun et al. 1998b), 3-8% in China (Zhu et al. 2012); 7% in Italy (Pari et al. 2012) and 6% in Finland (Peltonen-Sainio et al. 2014)]. Unfavorable harvest conditions in the United Kingdom have resulted in seed losses as large as 50% of canola yield, however (Price et al. 1996). Harvest losses of canola cause large seedbank inputs and are the primary source for volunteer recruitment in subsequent years (Gulden et al. 2003a).

2.2.4 Seed Dormancy. When a viable seed has met all of the requirements for germination, yet fails to germinate, it is considered dormant (Baskin and Baskin 2014). The categorization of seed dormancy has been disputed in the literature and several dormancy classification systems have been developed (Baskin and Baskin 2014). Baskin and Baskin (1985) categorized seed dormancy by time of initiation/induction into two broad categories, namely primary and secondary seed dormancy. Throughout this literature review, these two broad categories are used to describe the dormancy status of canola seed.

2.2.4.1 Primary Seed Dormancy. Primary seed dormancy is imposed during seed maturation on the mother plant (Baskin and Baskin 1985, 2014). Although induced while the seed is still on the mother plant, primary dormancy may extend beyond seed dissemination. Primary seed dormancy is a mechanism that prevents precocious germination (vivipory) during seed maturation on the mother plant or germination immediately after the seed has been disseminated (Baskin and Baskin 2014; Fenner and Thompson 2005).

In general, primary dormancy in canola seed is negligible (Gulden et al. 2004a, 2004b, 2008; Pekrun et al. 1998b; Schlink 1994, 1995). The level of primary seed dormancy decreases as seeds mature in the siliques (Haile and Shirtliffe 2014; Huang et al. 2016; Schlink 1994). For example, 13% to 16% primary dormancy in canola seed harvested 32-33 days after flowering (DAF) decreased with increasing harvest date, and was absent at 78 DAF (fully mature seeds) (Haile and Shirtliffe 2014).

2.2.4.2 Secondary Seed Dormancy. After seed dispersal from the mother plant, secondary seed dormancy may be imposed by environmental conditions unfavorable for germination (Baskin and Baskin 1985, 2014). Seeds that are non-dormant, yet have not met all of the requirements for germination, are considered quiescent (Fenner and Thompson 2005). Secondary dormancy is commonly induced in quiescent seeds. However, secondary dormancy also may be induced in seeds that have not yet been alleviated entirely of primary dormancy. Secondary seed dormancy serves two principal purposes (a) temporal dispersal of a cohort of progeny by preventing germination of all seeds at the same time and (b) limiting germination and seedling recruitment to a time of year when reproduction is likely to be successful (Baskin and Baskin 1985, 2014).

Secondary seed dormancy prolongs seedbank persistence in canola (Gulden et al. 2003b). Several environmental factors govern the induction and release of secondary dormancy in canola seed. A combination of warm temperatures (about 20°C), dryness (near -1.5 MPa), darkness, hypoxia and time [> 2 weeks (Weber et al. 2010)] contribute the induction of secondary dormancy in this species (Boyd and Van Acker 2004b, 2004c; Gulden et al. 2004a; Lopez-Granados and Lutman 1998; Momoh et al. 2002; Pekrun et al., 1997a, 1998a; Schlink 1994).

The potential for canola induction into secondary seed dormancy is influenced also by plant maturity at harvest (Haile and Shirtliffe, 2014; Huang et al. 2016), maternal environment (Gruber et al. 2009; Gulden et al. 2004a; Huang 2016), the filial generation of a hybrid genotype (Seerey 2010), after-ripening, seed size (Gulden et al. 2004a) and burial depth (Gruber et al. 2010) (see Chapter 2.4.1.1.5). Predisposition of canola seed to secondary dormancy, however, is highly variable among genotype (Gruber et al. 2004a; 2009; 2012; Gulden et al. 2003b; Momoh et al. 2002; Pekrun et al. 1997d; Schatzki et al. 2013; Weber et al. 2013). Alternating temperatures (Pekrun et al. 1997b), cold stratification (Pekrun et al. 1998a), gibberellic acid (Pekrun et al. 1998a) and a flash of white light (Pekrun et al. 1997c; Schlink 1994) release secondary dormancy in canola seed.

2.2.5 Rapid Growth, Resource Uptake and Hybrid Vigor. Canola grows rapidly in western Canada and is both a competitive crop and a competitive weed (Seerey et al. 2010; Zand and Beckie 2002). In this region, volunteer canola (*B. napus* or *B. rapa*) can cause yield losses in wheat that range from 0.24% to 2.4% per volunteer plant per m^2 (O'Donovan et al. 2008; Seerey et al. 2010). In a replacement series study, canola was more tolerant to interference than lambsquarters (*Chenopodium album* L.) but less tolerant than wild mustard (*Sinapis arvensis* L.) (Blackshaw and Dekker 1989). The relative competitive ability of these species was related to leaf area development and light interception (Blackshaw and Dekker 1989).

Hybrid canola can be more competitive than open-pollinated canola due to positive heterosis (i.e., hybrid vigor) (Harker et al. 2003; Zand and Beckie 2002). Positive heterosis in canola can manifest as greater dry matter and seed yield (Van Deynze et al. 1992). However, hybrid breakdown may result in reduced competitive ability of second and third filial volunteer canola generations compared with the F1 hybrid generation (Seerey and Shirtliffe 2010).

Efficient resource uptake and rapid growth of canola may contribute to its competitive ability as a weed in other crops. In greenhouse studies evaluating the response of over 20 agricultural weed species to added nitrogen (N) and phosphorus (P) fertilizer, canola was ranked among the species with the greatest responsiveness to N but least responsiveness to P fertilizer (Blackshaw et al. 2003, 2004a). Canola also was ranked among the species with the greatest percentage uptake (extraction) of available N and P in these studies.

2.2.6 Herbicide-Resistance. In Canada, canola varieties resistant to three distinct herbicide modes-of-action (MOA) are currently available for commercial production (Beckie et al. 2006, 2011a, 2011b). Non-transgenic imidazolinone- and transgenic glyphosate- or glufosinate-resistant canola varieties were released for commercial production in 1995 and 1996, respectively (Beckie et al. 2006). In the first decade after release of HR canola, adoption of HR canola varieties reached 95% of the hectarage seeded to canola (Beckie et al. 2006). In 2008, 86% of canola grown on the Canadian prairies was genetically-engineered (GE) HR (45% glyphosate- and 41% glufosinate-resistant) [(CCC, personal communication) cited in Beckie and Warwick (2010)]. The remaining 14% of canola grown in 2008 consisted of 13% non-transgenic imidazolinone-resistant and 1% non-HR varieties.

2.2.6.1 Intraspecific Gene Flow. Two years after the introduction of HR canola in Canada, double- and triple-HR volunteers were discovered (Hall et al. 2000). Multiple-HR in this species was attributed to pollen-mediated gene flow from fields with different HR traits grown in close proximity (Hall et al. 2000). Multiple-HR in canola has not been associated with a fitness penalty (Simard et al. 2005) and HR canola biotypes have no competitive advantage over non-HR biotypes unless selection pressure is imposed by herbicide application (Hall et al. 2005; Warwick et al. 2009).

Canola is primarily self-pollinated, although intraspecific plant-to-plant outcrossing can occur at rates of about 21% (Cuthbert and McVetty 2001). Beckie et al. (2003) documented outcrossing of HR genes at rates of 1.4% at the edge of neighboring canola fields. In this study, outcrossing of canola extended beyond 400 m from the pollen source (0.04%). Similar rates of pollen-mediated gene flow have been observed elsewhere in Canada, the United States, the United Kingdom, France, and Germany [(Brown et al. 1996; Champolivier et al. 1999; Dietz-Pfeilstetter and Zwerger 2004; Downey 1999; Feldmann 2000; Hommel and Pallut 2003; Morris et al. 1994; Scheffler et al. 1993, 1995; Simpson 1999; Staniland et al. 2000) summarized by Hüsken and Dietz-Pfeilstetter (2007)]. In Australia, low frequency of outcrossing in canola was found up to 3 km from the pollen source indicating potential for long-distance gene transfer (Rieger et al. 2002). Cultivar, isolation distance, pollinators, flowering synchrony, field size, topography and environment all influence intraspecific gene flow in canola (Hüsken and Dietz-Pfeilstetter 2007).

2.2.6.2 Adventitious Presence. In Canada, commercially available pedigreed canola seed is not guaranteed to be free from contamination with unsolicited HR traits (Downey and Beckie 2002; Friesen et al. 2003). Out of 27 Canadian Seed Growers Association certified canola seedlots tested in western Canada, almost all showed adventitious presence of HR traits (Friesen et al.

2003). Over half of the canola seedlots tested were contaminated at levels greater than the 0.25% varietal impurity threshold (Friesen et al. 2003). In two studies, HR contamination of pedigreed canola seedlots was observed up to 4.9% (Friesen et al. 2003) and 6.8% (Downey and Beckie 2002) with the greatest HR trait contamination being glyphosate-resistance. Almost all glufosinate-resistant seedlots tested contained biotypes resistant to both glyphosate and glufosinate (up to 1.45%) which indicated adventitious presence of HR traits due to gene transfer (Friesen et al. 2003). The frequency at which canola is grown in a crop rotation and persistence of the volunteer seedbank may influence HR, varietal, and/or oil profile contamination levels in harvested seed of future canola crops (Andersen et al. 2010; Begg et al. 2008; Friesen et al. 2003; Jørgensen et al. 2007; Messéan et al. 2007; Thöle et al. 2012).

2.2.6.4 Implications for Production of Other Herbicide-Resistant Crops. Other HR crops grown in rotation with HR canola may have limited options for chemical management and increased risk of yield loss due to interference from canola volunteers. Volunteer HR canola is particularly challenging to manage in soybean (CM Geddes, personal observation). Soybean is a warm-season N-fixing legume with HR traits similar to canola. In 2015, transgenic glyphosate-resistant soybean biotypes occupied about 97% of the soybean hectarage in MB (MMPP 2016). In MB, seeded hectarage of soybean has almost tripled in the last half decade (232,700 ha in 2011 to 661,700 ha in 2016) (Statistics Canada 2017). Based on seeded hectarage, soybean was the third most grown crop in MB in 2016 (MMPP 2016). There are limited options for effective and consistent chemical management of HR volunteer canola in HR soybean (Tozzi and Willenborg 2015, 2016). A lack of herbicide options for management of volunteer canola in soybean likely contributes to seedbank inputs when soybean is grown in rotation with canola. As widespread soybean production is relatively new to this region, little regional information is available on the interference of cool-season weeds in warm-season soybean. The evaluation of techniques that can increase the interference of soybean with volunteer canola is necessary to limit volunteer canola seedbank inputs in this phase of the crop rotation.

2.3 A Life Cycle Approach to Integrated Weed Management

Integrated weed management consists of multiple biological, chemical, cultural and physical weed management tools used in combination to diversify weed management strategies. Liebman and Gallandt (1997) discussed the benefits of using "many little hammers" (integrated approach) rather than "one large hammer" (herbicides) for weed management. For example, this approach to weed management may help mitigate or manage HR weed populations and contribute to cropping system sustainability (Liebman and Gallandt 1997). Utilization of "many little hammers" for weed management (e.g., Beckie 2006; Beckie and Harker 2017; Blackshaw et al. 2008; O'Donovan et al. 2007) can result in additive or synergistic effects on reduction of weed populations (e.g., Harker et al. 2009). Furthermore, implementation of "many little hammers" throughout a weeds life cycle may be more effective than focusing on management of weeds at a single life-stage (Davis et al. 2004, 2005a).

Previous studies have used a systematic model of population demography to evaluate the effect of weed management on a weed population at multiple life-stages (e.g., Davis et al. 2004). In Chapter 2.4, a systematic life cycle of volunteer SA canola is used as a template to organize the findings of past research on this species. The current knowledge gaps identified using this research summary will be the basis and focus of the five research studies that follow (Chapters 3, 4, 5, 6 and 7).

2.3.1 Models of Population Demography and Spatial Dynamics. Several models explaining the population demography and spatial dynamics of volunteer canola have been developed. An abundance of previous research on volunteer canola has focused on WA varieties alone, and these reports have been the basis of previous modeling efforts (Begg et al. 2006; Colbach et al. 2001; 2008; Crawley and Brown 2004; Gruber and Claupein, 2007; Pekrun et al. 2005; Soltani et al. 2013; Squire et al. 1997). From a global perspective, SA canola varieties are primarily grown in Canada, while WA canola varieties have dominated in European countries (Gulden et al. 2008). Even though similarities exist between the biology of SA and WA canola varieties, the life cycle of these weeds and the climatic conditions in which they are grown differ. For this reason, there is a need for future research focusing on population demography of volunteer SA canola. In western Canada, canola production is limited to SA varieties. The majority of research on volunteer SA canola has been conducted in this region. Yet, a comprehensive quantitative description of the volunteer SA canola life cycle has not yet been conducted and warrants investigation. Using the life cycle of a SA weed as an organizational tool for previous research on SA canola may be beneficial to identify research gaps, guide future research efforts and develop a comprehensive strategy to manage this weed effectively.

2.3.2 Using the Life Cycle as a Template for Weed Management. In Canada, volunteer canola is an indeterminate cool-season facultative (near obligate) SA weed (see Chapter 2.4.2.2). Figure 2.1 depicts a life cycle diagram outlining the life-stages (demographic states) and life-stage transition rates (demographic rates) of a SA weed [Adapted from Davis et al. (2004)]. The annual weed life cycle is composed of an aboveground and a belowground pathway (Fig 2.1). The aboveground pathway describes the successful reproduction and addition of new individuals into the population. These individuals then re-enter the belowground pathway upon life cycle completion (Fig 2.1). The aboveground pathway includes the demographic rates of autumn seedling recruitment ($\gamma_{(a)}$), plant survival over winter ($\sigma_{p(w)}$), spring seedling recruitment ($\gamma_{(s)}$), plant survival to maturity ($\sigma_{p(s)}$), plant fecundity (Φ) and seed dispersal ($\sigma_{s(disp)}$) (Fig 2.1). The belowground pathway describes seed persistence in the soil seedbank. The belowground pathway is composed of the demographic rates of seed survival over winter ($\sigma_{s(w)}$) and over summer ($\sigma_{s(s)}$) (Fig 2.1).

Weed population dynamics under different management scenarios can be modeled by combining the demographic life-stage transition rates in the annual weed life cycle using a series of linear equations (e.g., Davis et al. 2004). This approach is known as matrix population modeling (Caswell 2001). Matrix population modeling also may be used to identify life-stage transitions (or demographic rates) in the weed life cycle where weed management may be more effective (e.g., Davis et al. 2003). The approaches used in demographic matrix population modeling are beyond the scope of this literature review and have been discussed in detail by Caswell (2001). In brief, a demographic approach to matrix population modeling can be used to analyze the growth of a population from one time point to the next. For an annual weed, the annual population growth rate is calculated using a linear equation of the demographic rates in the aboveground and belowground life cycle pathways (e.g., Caswell 2001; Davis et al. 2004). The number of individuals at each life-stage (in Fig 2.1) is quantitative and ideally is determined using field research. Then, the demographic transition rates are the quotient of the number of

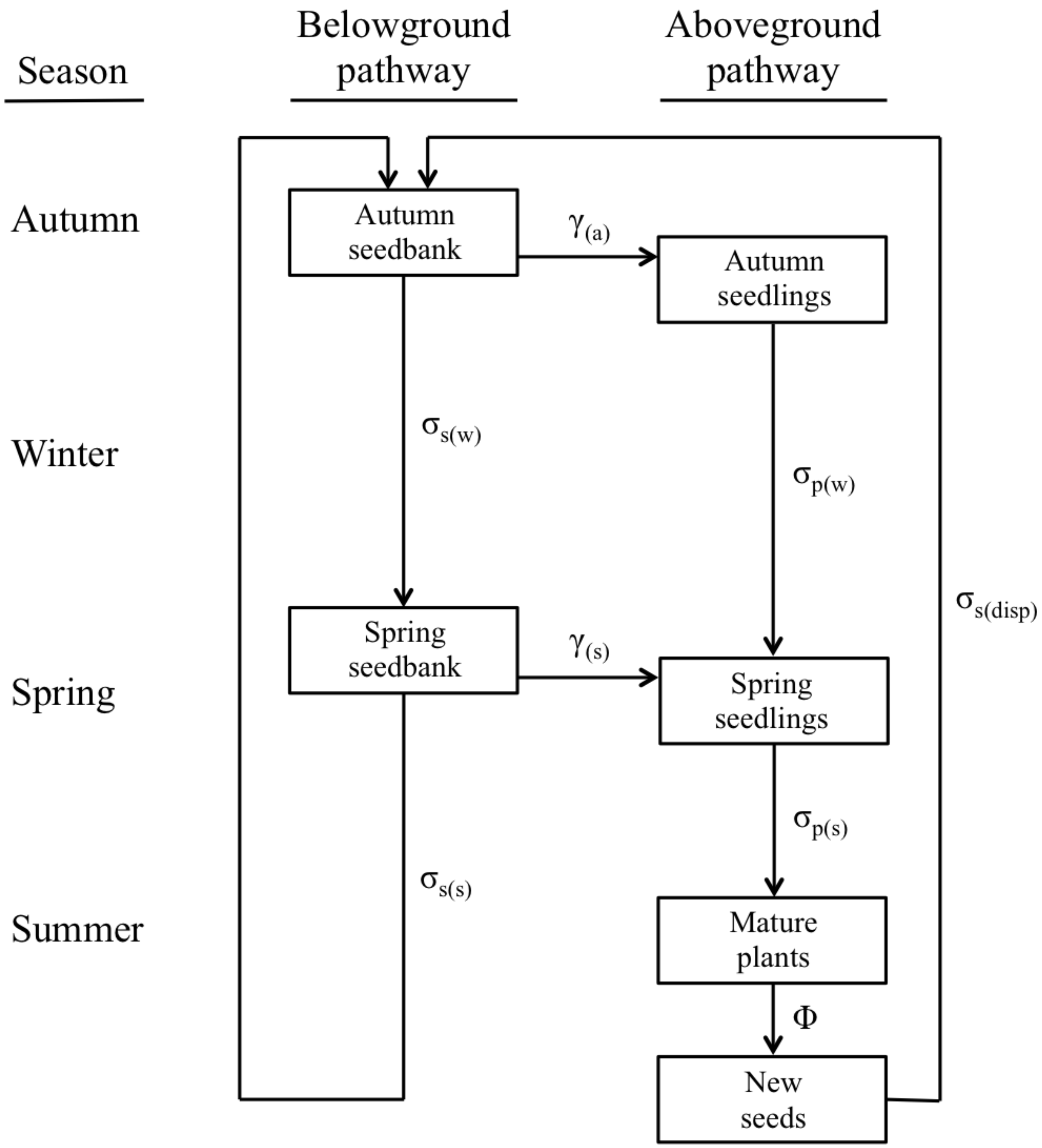

Figure 2.1. Life cycle diagram outlining the life-stage transition rates of a summer-annual weed in an annual cropping system. Boxes represent life-stages (demographic states), while arrows indicate life-stage transition rates (demographic rates). Demographic rates include: autumn seedling recruitment ($\gamma_{(a)}$), plant survival over winter ($\sigma_{p(w)}$), spring seedling recruitment ($\gamma_{(s)}$), plant survival to maturity ($\sigma_{p(s)}$), fecundity (Φ), seed dispersal ($\sigma_{s(disp)}$), and seed survival in the soil seedbank over summer ($\sigma_{s(s)}$) or winter ($\sigma_{s(w)}$). Abbreviations, outside brackets: Aut, autumn; Spr, spring; s, seed; p, plant; inside brackets: a, autumn; w, winter; s, summer; disp, dispersal.

individuals observed at these life-stages (between successive life-stages) (Davis et al. 2004). Weed management tactics can decrease the proportion of individuals that survive from one life-stage to the next (i.e., a demographic rate). In general, a reduction in a demographic rate will reduce the overall growth rate of the weed population.

2.4 Management Options that Impact Each Life-Stage Transition

In this chapter, the SA weed life cycle is used as a template to summarize the available information for management of volunteer SA canola. This systematic summary of canola management strategies may be used to provide a basis from which knowledge gaps can be identified and new hypotheses can be generated. Previous research on volunteer canola management will be summarized based on the life-stage transition impacted.

2.4.1 The Belowground Pathway. In autumn of each year, the entire SA weed population is returned to the belowground pathway (i.e., the soil seedbank) (Fig 2.1). Mature SA weeds disperse their seeds in autumn. The majority of seeds dispersed from terrestrial weeds enter the soil seedbank. Management tools that influence seed survival in the belowground pathway are imposed on the entire population at this time of year (Davis 2006; Davis et al. 2004). These management tools may therefore have improved efficacy for management of a SA weed population when implemented in autumn (Davis 2006; Davis et al. 2004).

Seedbank research is commonly conducted on an annual basis. Almost no previous studies have separated canola seed survival in the soil seedbank over seasonal time periods. For this reason, the demographic transition rates of seed survival in the soil seedbank over winter and summer are discussed together.

2.4.1.1 Seed Survival Over Winter ($\sigma_{s(w)}$) and Over Summer ($\sigma_{s(s)}$). In western Canada, a single cohort of canola seed can survive for at least 3 years in the soil seedbank (Gulden et al. 2003b, 2004b) and at least 7 years when seed return is not prevented (Beckie and Warwick 2010). Seedbank survival of volunteer SA canola in western Canada tends to be less than that of WA canola in Europe. In Europe, single cohorts of canola have persisted for more than 11 years (Lutman et al. 2003; Schlink 1998) and established populations of volunteer canola have persisted for even longer (Belter 2016; D'Hertefeldt et al. 2008; Jørgensen et al. 2007). In western Canada, as in other countries (Baker and Preseton 2008), most of the canola seedbank is transient and tends to decline rapidly (Lutman et al. 2002), although small proportions of the seedbank can survive for longer periods of time (Gulden et al. 2003b).

2.4.1.1.1 Rates of Seed Survival in the Soil Seedbank. Studies on survival of canola seed in the soil seedbank have generally not presented winter and summer seed survival as separate demographic rates. However, a limited number of studies have determined volunteer canola seedbank persistence using a monthly time series (e.g., Gulden et al. 2004b). Extrapolation of data from a previous study in western Canada indicated that percentage survival of canola seed in the soil seedbank ranged from 29% to 47% over winter and from 17% to 38% over summer (Gulden et al. 2004b). This agrees with other research that found about 44% of the canola seed in the soil seedbank remained viable in spring following establishment of an artificial seedbank in autumn (Gulden et al. 2003b).

2.4.1.1.2 Processes that Influence Seed Survival in the Soil Seedbank. Seed survival in the soil seedbank is related to a seeds inherent ability to resist succumbing to seedbank processes that lead to seed demise (Long et al. 2015). These processes include seed aging, microbial decay, biological predation or lethal germination (Fenner and Thompson 2005). Little information is available on the fate of canola seed in the soil seedbank in response to each of these processes, in part due to the lack of suitable methods to study these processes *in situ*. Recent laboratory and field research in western Canada found that omnivorous carabid species (Coleoptera: Carabidae) preferentially consumed canola seeds compared with wild mustard or field pennycress (*Thalaspi arvense* L.) (Kulkarni et al. 2016). Among experimental locations in western Canada, weekly seed removal rates due to omnivorous ground beetle predation ranged from 42% to 77% of canola seed (Kulkarni et al. 2016). These results suggest that biological predation can be a significant source of seed morality in this species. Canola seed consumption by ground beetles was greatest near the soil surface and decreased with increasing soil depth (to almost absent at 4 cm depth) (Kulkarni et al. 2015). Thus, predation of canola may be greater if seeds remain on or near the soil surface following canola harvest.

2.4.1.1.3 Seed Burial. The canola seedbank tends to be more persistent when seeds are buried. Following seedbank establishment in autumn, Gulden et al. (2004b) found that the shallow canola seedbank (1 cm depth) was transient and declined to negligible levels of viable canola seed in early spring (April to June). In the deep seedbank (10 cm depth), however, seeds tended to shift from a germinable to an ungerminable state as spring progressed into summer. This study reported greater overall survival of canola seed in the deep seedbank. These results suggest that post-harvest burial of canola seed may contribute to seed survival in the soil seedbank in western Canada. Other reports from Europe found that leaving seed losses on the soil surface following WA canola harvest reduced seed survival over winter (Gruber et al. 2004b, 2005; Lutman et al. 2003; Pekrun and Lutman 1998; Pekrun et al. 1998a, 2006). The European studies concluded that zero or delayed tillage after WA canola harvest were the best options for post-harvest seedbank management. No research on timing of tillage on survival of the SA canola seedbank has been conducted.

2.4.1.1.4 Soil Texture. Fine soil textures were associated with greater winter survival of canola seed in the soil seedbank in Germany (Gruber et al. 2012; 2014; Thöle et al. 2011; Weber et al. 2011). In a common garden study, Gruber et al. (2014) found greater seed survival of WA canola after six months of burial in a clay and a silty clay loam soil compared with a sandy loam soil. Similar observations of greater seed survival at study locations with fine compared with coarse textured soils have been reported in western Canada (Gulden et al. 2003b, 2004b), the United Kingdom (Lutman et al. 2003, 2005) and across soil climatic regions in Germany (Gruber et al. 2012; Thöle et al. 2011). On the other hand, coarse textured soils also have resulted in greater seedbank persistence in some instances (Pekrun et al. 1998a). Canadian observations have indicated that the amount of precipitation received in autumn may influence survival of canola seed in the soil seedbank (Gulden et al. 2004b; Légère et al. 2001). These reports suggested a negative association of soil moisture in autumn and volunteer canola seedling densities the subsequent spring. Yet, proportional survival of canola seed in the soil seedbank is generally greater in Europe (e.g., Lutman et al. 2003) compared with Canada (e.g., Gulden et al. 2003b), even though Europe commonly experiences wet autumn conditions. Perhaps, environmental differences such as the extent of soil freeze over winter contribute to canola seedbank dynamics. The influence of precipitation, soil texture and associated edaphic factors on survival of SA volunteer canola seeds in the soil seedbank has not been evaluated under Canadian conditions and warrants future investigation.

2.4.1.1.5 Factors that Influence Secondary Seed Dormancy. Factors that influence secondary seed dormancy in canola may impact seed survival in the soil seedbank (Gulden et al. 2003b; see Chapter 2.2.4.2). Timing of harvest, seed size and after-ripening contribute to the potential for canola seed to enter secondary dormancy (Gulden et al. 2004a; Haile and Shirtliffe 2014). For example, the potential for canola seeds to enter secondary dormancy increased as harvest date progressed from early developmental stages to physiological maturity (Haile and Shirtliffe 2014). A similar trend was observed by Gulden et al. (2004a), however, the contribution of time of windrowing to the total variation in induction of secondary dormancy in 16 canola genotypes was minimal (1.5%) compared with a series of other factors including genotype, seed size and pre- and post-harvest environment.

Temperature, light quality, day length, moisture stress, nutrient availability, and position of the seed on the mother plant all have maternal effects on dormancy predisposition of progeny seed in other species (Fenner and Thompson 2005). The specific influence of each of these individual factors on canola predisposition to secondary seed dormancy is largely unknown. However, Gulden et al. (2004a) reported that maternal pre-harvest environment explained only up to 4.5% of the variation in expression of secondary seed dormancy among 16 canola genotypes grown in four different environments. These results suggest that maternal environment has only a minor influence on predisposition of canola seed to secondary dormancy.

2.4.2 The Aboveground Pathway. The aboveground life cycle pathway is comprised of all the post-emergence life-stages (Fig 2.1). The transitions between life-stages of the aboveground pathway are the direct target of almost all weed management strategies in contemporary conventional cropping systems. Review of management tactics influencing the aboveground pathway will begin with the primary source of the volunteer canola population, seed losses at harvest.

2.4.2.1 Seed Dispersal ($\sigma_{s(disp)}$).

2.4.2.1.1 Short-Distance Seed Dispersal. Seed losses during canola harvest contribute a large cohort of viable seed to the soil seedbank in autumn each time a canola crop is grown (Cavalieri et al. 2016; Gulden et al. 2003a). A regional survey near Saskatoon, SK found consistent differences in harvest losses among producers ranging from 3% to 10% of canola yield (Gulden et al. 2003a). In this survey, the absolute number of seeds lost during canola harvest was about 9 to 56 times the average canola seeding density. In a larger survey of 310 canola fields across western Canada, combine ground speed, windrower width, windrow date, harvest date and number of days in the windrow were associated positively with proportional harvest losses (Cavalieri et al. 2016). On the other hand, nitrogen fertilizer application rates, average maximum wind speed between windrowing and harvest and crop yield were associated negatively with proportional harvest losses. Canola variety, maturation, seeding density, stand density, windrower ground speed, time of day at harvest, combine type and combine manufacturer were not related to on-farm canola harvest losses in this survey.

Harvest method and maturity at harvest can influence canola harvest losses (Haile et al. 2014a, 2014b; Price et al. 1996; Thomas et al. 1991; Vera et al. 2007). Windrowing can be effective at hastening pod maturation (Brown et al. 1999). In Canada, canola harvest recommendations have traditionally suggested windrowing at 60% seed color change (from green to brown) on the main rachis (CCC 2016). More recently in western Canada, interest in direct-harvest operations for canola has grown primarily due to reduced time and input costs. In this region, direct harvest operations have resulted in reduced harvest losses compared with windrowed canola in small plot research (Haile et al. 2014a), but not in a complimentary field scale survey (Haile et al. 2014b). Direct harvesting may, however, increase potential for weather-induced pod drop or pod shatter (Gulden et al. 2017; Vera et al. 2007). Pod sealant products have been developed to limit seed lost due to pod shatter (CCC 2016). These products may allow for reduced seed losses while the crop matures prior to direct-harvest operations. However, two commercially available pod sealant products did not influence harvest losses in a small plot field study near Saskatoon, SK (Haile et al. 2014a), suggesting limited efficacy of these products. Desiccating the canola crop to hasten maturation may reduce potential seed losses by decreasing maturation time. Alternatively, pushing canola prior to 40% seed color change – where the crop is mechanically lodged while remaining attached to the root system – also may potentially be an effective alternative to reduce seed losses while allowing the crop to fully mature prior to harvest (Irvine and Lafond 2010).

Differences in the predisposition to pod shatter among genotypes (Cavalieri et al. 2014; Haile et al. 2014a; Raman et al. 2014; Wang et al. 2007) indicate that breeding efforts can contribute to a reduction in canola harvest losses. Pod shatter-reduction varieties are now commercially available in western Canada (Anonymous 2016). However, little peer-reviewed information exists documenting the extent to which seed losses may be reduced using these varieties. High throughput methods to assist with breeding for reduced pod drop are being developed (Gulden et al. 2017).

Volunteer canola seed production and dispersal in crops grown subsequent to canola can prolong volunteer canola population persistence (Beckie and Warwick 2010). Like canola harvest losses (Gulden et al. 2003a), volunteer canola seed production may be a large source of seedbank inputs in other crops. Little information is available on volunteer canola seed production and dispersal in crops grown in western Canada.

2.4.2.1.2 Long-Distance Seed Dispersal. Pod drop and pod shatter are the only specialized autochorous dispersal mechanisms implemented by canola (Gulden et al. 2008). Canola seed may be dispersed via endozoochory, however, the extent to which this mechanism facilitates dispersal is unknown. Stanton et al. (2003) found viable canola seed in sheep excrement 5 days following ingestion. This report suggests that ruminant mammals may be vectors for canola seed transport over long-distances. Long distance dispersal of canola is principally caused by anthropochory, however. As a result, feral canola populations with diverse HR traits are ubiquitous along railways and roadways used for canola transportation (Knispel et al. 2008; Yoshimura et al. 2006). These feral populations provide a source for pollen-mediated gene flow and may contribute to multiple-HR volunteer canola populations in cropped fields (Knispel et al. 2008).

***2.4.2.2 Autumn Seedling Recruitment* ($\gamma_{(s)}$).** Previous Canadian reports have alluded to volunteer canola as having a facultative SA life cycle. However, autumn seedling recruitment of SA canola has been observed on several occasions in Canada (Gulden et al. 2008; Harker et al. 2006; Légère et al. 2001; Seerey 2010; Simard et al. 2002). In eastern Canada, canola plants have survived over winter in some instances (Simard and Légère 2001). Together, these reports imply that the SA life cycle of this weed may be obligate rather than facultative. However, autumn recruitment of volunteer canola may be minimal or absent during uncharacteristically dry autumn conditions (e.g., Gulden et al. 2003b, 2004b). Aside from personal observations, a paucity of quantitative data is available on autumn seedling recruitment of volunteer SA canola in Canada.

***2.4.2.3 Plant Survival Over Winter* ($\sigma_{p(w)}$).** Volunteer canola plant survival over winter has been observed in zero tillage fields in eastern Canada (Simard et al. 2002). In a survey of 131 fields in the canola growing-regions of Québec (QC), 1.4% of volunteer SA canola plants survived over winter in zero tillage fields (Simard et al. 2002). Interestingly, overwintering was isolated to zero tillage conditions and may have been a result of mild winter temperatures. In this survey, most of the overwintered volunteer canola plants were derived from seedlings that emerged in autumn and overwintered at the rosette stage. Some of the overwintered canola plants also were derived from regrowth of previously harvested canola plants (Simard and Légère 2001). Evidence of volunteer SA canola plants overwintering in western Canada is non-existent (Gulden et al. 2003b; Hall et al. 2005), feasibly due to colder temperatures over winter (Environment Canada 2017). Since volunteer canola plants are subject to winterkill in western Canada – and to a large degree in eastern Canada (Simard et al. 2002) – promotion of autumn recruitment of harvest seedbank inputs may be a viable strategy to decrease volunteer canola population densities in subsequent years.

2.4.2.4 Spring Seedling Recruitment ($\gamma_{(s)}$). Successful spring recruitment of volunteer canola ranges from 0.1% to 10% of the autumn seedbank (Gulden et al. 2003b, 2004b; Lawson et al. 2006). In the first, second and third spring following an autumn seedbank input of 2000 seeds m^{-2}, spring seedling densities in the absence of further seedbank additions were 100-210, 0-3, and < 1 plant m^{-2}, respectively (Gulden et al. 2003b). Seedling recruitment of volunteer canola is highly variable even among locations with similar autumn seedbank densities (Harker et al. 2006).

2.4.2.4.1 Tillage Regime. The base temperature for germination of SA canola ranges from 0.4 to 4.8°C (Kondra et al. 1983; Lawson et al. 2006; Morrison et al. 1989; Vigil et al. 1997). In western Canada, volunteer canola seedling recruitment occurs principally in May and June (Boyd and Van Acker 2004a; Gulden et al. 2003b, 2004b; Lawson et al. 2006). Unlike some other weed species (e.g., Bullied et al. 2003), tillage regime does not influence the emergence periodicity of volunteer canola (Lawson et al. 2006).

Tillage at depths below 10 cm may decrease volunteer canola seedling recruitment in the field. Under greenhouse conditions, successful canola seedling recruitment decreased with increasing burial depth (Gruber et al. 2010; Vigil et al. 1997). Recruitment of canola seed buried at > 10 cm depth has not been observed (Gruber et al. 2010; Gulden et al. 2004a; Lutman 1993). Farm field surveys in Canada have shown a greater density of canola seedlings in zero compared with conventional tillage fields (Lawson et al. 2006; Simard et al. 2002; Thomas et al. 1997). Field studies in western Canada also showed a negative association of volunteer canola seedling densities with the intensity of tillage systems (Derksen et al. 1993; Gulden et al. 2003b; Légère et al. 2001; Simard and Légère 2003). These reports suggest that post-harvest burial of canola seed may reduce the rate of spring seedling recruitment.

***2.4.2.5 Plant Survival to Maturity* ($\sigma_{p(s)}$).** Plant survival to maturity is arguably the most easily manipulated life-stage transition rate in an annual weed life cycle (Fig 2.1). Weed management tools commonly implemented during this life-stage transition comprise both chemical and non-chemical weed management options. In a canola crop, plant survival to maturity has ranged from 55% to 100% and tends to be associated negatively with seedling density [81-85% (Harker et al. 2015a); 75-100% (Morrison et al. 1990a); 55-75% (Van Deynze et al. 1992)]. In wheat, approximately 60%, 54%, and 48% of volunteer canola seedlings survived to maturity in first, second and third filial generation hybrid volunteer canola populations (Seerey and Shirtliffe 2010). Aside from this study, there is a paucity of information describing volunteer canola plant survival to maturity in the absence of herbicide management.

2.3.2.5.1 Chemical Management. Herbicides have been used extensively for management of volunteer canola. In western Canada alone, there are about five herbicide MOA (> 30 registered herbicides) available for in-crop management of volunteer canola in cereal crops (Anonymous 2015; Beckie et a. 2011b). However, in-crop herbicide management may be limited to only one or two MOA in broad-leaved crops such as field pea (*Pisum sativum* L.), field bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medikus), chickpea (*Cicer arietinum* L.) sunflower

(*Helianthus annuus* L.) or soybean (Anonymous 2015).

Effective herbicide management of volunteer canola tends to be inconsistent and dependent on the developmental stage of canola at the time of application. Common examples of herbicide MOA to which all known canola biotypes are susceptible include auxinic- (e.g., 2,4-D or MCPA) or photosystem (PS) II-inhibitors (e.g., metribuzin) (Beckie et al. 2004; Retzinger and Mallory-Smith 1997). However, auxinic herbicides applied to volunteer canola at late stages of development have reduced efficacy of management (Légère et al. 2006). When applied at the correct stage, 6% to 12% of volunteer canola plants survived application of auxinic herbicides (2,4-D or MCPA) in wheat (Légère et al. 2006; Simard et al. 2002). In the Pacific Northwest (USA), Rainbolt et al. (2004) applied herbicides (including 2,4-D, dicamba, diuron, glufosinate, glyphosate or paraquat) alone or in-mixture for pre-plant management of glyphosate-, glufosinate- or imidazolinone-resistant volunteer canola. Of the herbicide options applicable for management of volunteer canola in each HR system, 2% to 63% of the glyphosate-, 2% to 27% of the glufosinate- and 2% to 52% of the imidazolinone-resistant volunteer canola was not visually controlled at 21 days after treatment. A study evaluating herbicide options for management of glyphosate-resistant volunteer canola in glyphosate-resistant soybean also found inconsistent efficacy of management (Tozzi and Willenborg 2015, 2016). In this study, an acetolactate synthase (ALS)-inhibitor applied pre-emergence, and/or an ALS-inhibitor, PS II-inhibitor or protoporphyrinogen oxidase (PPO)-inhibitor applied post-emergence were the best options for providing acceptable control of glyphosate-resistant volunteer canola in soybean. The recent (2017) Canadian release of Roundup Ready 2 Xtend® soybean, conferring HR traits to glyphosate and dicamba (Monsanto Canada Inc., Winnipeg, MB), or Enlist™ soybean, conferring HR traits to glyphosate and 2,4-D (Dow AgroSciences Canada Inc., Calgary, AB), may provide effective options for chemical management of HR volunteer canola. These reports show that herbicide options currently exist for both pre- and post-emergence management of volunteer canola, however, the efficacy of volunteer canola management using herbicide is often inconsistent and rarely complete.

2.3.2.5.2 Non-Chemical Management. The integration of physical, cultural or biological tools with herbicides may provide increased efficacy and/or consistency of volunteer canola management. Identification of management tools that increase the competitive ability of crops grown in western Canada may therefore assist in reduction of volunteer canola population persistence in western Canadian cropping systems. There are several potential non-chemical weed management tools that may help reduce volunteer canola plant survival to maturity. For example, crop/weed interference can be manipulated effectively using narrow row widths (e.g., Chandler et al. 2001; Rich and Renner 2007), increased population densities (e.g., Harker et al. 2003, 2009), competitive cultivars (e.g., Harker et al. 2003; O'Donovan et al. 2005), fertilizer management (e.g., Blackshaw et al. 2004b; Kirkland and Beckie 1998) or cover crops (e.g., Blackshaw et al. 2001). Little information on the efficacy of these management techniques is currently available for volunteer canola.

***2.4.2.6 Plant Fecundity* (Φ).** Plant fecundity, measured as the number of new seeds produced per plant, is the only demographic rate that leads to an increase in number of individuals in the population. Almost no information is available on the effect of weed management practices on volunteer canola plant fecundity. In a canola crop, greater plant stand density can reduce canola plant fecundity. Extrapolation of data reported by Harker et al. (2015a) showed that plant fecundity of canola grown in monoculture was 1,095 and 2,088 seeds plant^{-1} at stand densities of 67 and 35 plants m^{-2}, respectively. Likewise, extrapolation of data from Morrison et al. (1990a) showed that canola plant fecundity was about 494 and 2,592 seeds plant^{-1} at average stand densities of 164 and 35 plants m^{-2}, respectively. A similar response in plant fecundity to increasing volunteer canola density has been observed when interfering with a soybean crop (PMJ Gregoire, unpublished research). Together, these data indicate that weed management tools used to manipulate interference of crop species with volunteer canola may facilitate a reduction of volunteer canola plant fecundity. The influence of weed management tools on volunteer canola plant fecundity warrants particular focus of future research efforts in crops with HR traits similar to canola (such as soybean).

2.5 Remaining Questions

In summary, a large body of research on volunteer SA canola has been developed with particular focus on Canadian cropping systems. Use of the SA weed life cycle as an organizational tool for summarizing the results of previous research, has facilitated the identification of several knowledge gaps in volunteer canola management. The overall goal of this Ph.D. thesis is to fill some of the remaining knowledge gaps and provide estimates of the demographic life-stage transitions that remain unevaluated under Canadian conditions. In recent years, volunteer canola has been particularly problematic in western Canadian soybean production (Leeson et al. 2017). Widespread soybean production is relatively new to western Canada and little is known about management of volunteer canola in soybean. With this in mind, some of the most pertinent remaining questions in volunteer SA canola management include:

- Does timing or implement used for post-harvest tillage influence persistence of the volunteer canola seedbank in western Canada? (Chapter 3)
- Does soil texture influence canola seed survival in the soil seedbank under Canadian conditions? (Chapter 4)
- How can volunteer canola be managed in crops that have similar HR traits to canola? (Chapters 5, 6 and 7)
- What non-chemical weed management tools can be used to increase the interference from crop species with volunteer canola? (Chapters 5, 6 and 7)
- Quantification of the demographic rates of:
    - Post-harvest autumn seedling recruitment of volunteer SA canola
    - Volunteer SA canola plant survival over winter in western Canada
    - Survival of SA canola seed in the soil seedbank over winter and over summer
    - Plant fecundity of volunteer SA canola in crops other than canola
    - Seedbank inputs from volunteer canola in crops other than canola

These questions were addressed in the five research studies included in this Ph.D. research project.

3.0 Early Autumn Soil Disturbance Decreases Persistence of Volunteer Summer-Annual Canola (*Brassica napus*)

This chapter was published in June 2017. Early autumn soil disturbance decreases persistence of volunteer summer-annual oilseed rape (*Brassica napus*). Charles M. Geddes, Robert H. Gulden. Weed Research 57:182-192 © 2017 European Weed Research Society. John Wiley & Sons Inc. DOI: 10.1111/wre.12248

3.1 Abstract

Volunteer summer-annual (SA) canola is an on-going concern in Canadian crop production. Large harvest seed losses and secondary dormancy in this species generate a persistent volunteer seedbank. Yield loss in subsequent crops, potential canola oil profile contamination and herbicide-resistance trait introgression create a need for effective volunteer canola seedbank management. This field study evaluated the effects of timing and type of implement of post-harvest soil disturbance and seeding a winter cereal on volunteer SA canola population persistence and demographic life-stage transition rates at five sites in Manitoba, Canada. Following SA canola harvest and supplemental seedbank additions, seedbank densities ranged from 6,770 to 15,360 and 50 to 2,610 seeds m^{-2} among sites in autumn and spring, respectively. In contrast to European research on winter-annual canola, early autumn soil disturbance, shortly after SA canola harvest, was the best strategy to decrease volunteer SA canola persistence (3% population persistence from autumn to spring, compared with 6% in zero tillage). Substantial autumn seedling recruitment (38% of the autumn seedbank) and subsequent winterkill contributed to lower population persistence. Soil disturbance in spring stimulated spring

seedling recruitment compared with the other disturbance timings (11% and 3% of the spring seedbank, respectively). The implement used for soil disturbance and seeding winter wheat had minimal effect on population persistence. This research showed that timing of post-harvest soil disturbance should be utilized as an effective tool to decrease population persistence of volunteer SA canola via stimulation of autumn seedling recruitment and concomitant winterkill.

3.2 Introduction

Volunteer summer-annual (SA) canola (*Brassica napus* L.) is among the top five most abundant weed species in western Canada (Leeson 2015). Canola continues to retain weedy characteristics such as pod drop, pod shatter (Cavalieri et al. 2016) and secondary seed dormancy (Gulden et al. 2003b), which contribute to large on-farm harvest losses (6% of yield or 4,300 seeds m^{-2} regional average) (Cavalieri et al. 2016) and seedbank persistence (Gulden et al. 2003b), allowing volunteer SA canola to perpetuate for several years in rotation (Beckie and Warwick 2010). In western Canada, contemporary conventional crop rotations often only span two or three years, enabling a continuous presence of volunteer SA canola (Gulden et al. 2003b). This not only causes concern for yield loss from interference in subsequent crops, but potentially also oil profile contamination in canola due to the adventitious presence of volunteers, hybrid breakdown and/or variation in canola varieties grown (Jørgensen et al. 2007). The prospective release of herbicide-resistant (HR) winter-annual (WA) canola varieties in Europe has prompted similar concerns (Gruber et al. 2004b).

In Canada, non-transgenic imidazolinone-resistant, or transgenic glyphosate- or glufosinate-resistant traits have been commercially available for canola production since 1995 and 1996, respectively. Adventitious presence of unwanted HR traits in pedigreed canola seedlots (Friesen et al. 2003), in combination with seedbank persistence (Gulden et al. 2003b), can create management issues in other HR crops (Beckie et al. 2006). Volunteer SA canola is particularly difficult to manage in soybean [*Glycine max* (L.) Merr.], due to limited options for chemical management. As a result, volunteer SA canola is abundant in western Canadian soybean production and frequently present at densities above the economic threshold (Gregoire 2017; Leeson et al. 2017). Unmanaged volunteers in other HR crops can create large seedbank additions, similar to that of canola harvest seed losses (Chapters 5 and 6), further exacerbating the problem. Due to the recent rise in HR crop production in western Canada (99% HR canola and 97% HR soybean) (Beckie and Warwick 2010; Beckie et al. 2006; MMPP 2016), effective management practices that decrease volunteer canola population persistence are warranted.

An annual weed must pass through the soil seedbank to allow reproductive success of future generations (Davis 2006). The soil seedbank is therefore an effective, yet underutilized, management target for annual weeds, especially those with enduring seedbank persistence (Davis 2006). Secondary seed dormancy, induced by environmental conditions following seed shed from the mother plant (Baskin and Baskin 2014), is related to seedbank persistence in canola (Gulden et al. 2003b). Much research has been conducted on the development of secondary dormancy in this species (e.g., Lopez-Granados and Lutman 1998; Momoh et al. 2002; Pekrun et al. 1997a), however, applied research on preventing seedbank persistence in Canadian cropping systems is lacking. Research conducted in England (Lutman et al. 2003; Pekrun et al. 1998a), Austria (Pekrun et al. 2006) and Germany (Gruber et al. 2004b; 2005) revealed that zero tillage or delayed tillage one month following WA canola harvest caused the largest depletion of viable volunteer WA canola seed from the soil seedbank the following spring. It is suspected that tillage also will affect seedbank persistence of SA canola in Canada. However, due to the temporal differences between SA and WA life cycles, climatic differences between Canada and western/central Europe, and depth of tillage practices therein, the optimal timing and type of implement used for soil disturbance may differ for management of volunteer SA canola in Canada.

This field study was designed to assess the timing and type of implement of post-harvest soil disturbance used for management of volunteer SA canola. The first objective was to evaluate soil disturbance timing and tillage implement and the second objective was to evaluate the effect of relay-seeding a winter-annual cereal following SA canola harvest. The hypotheses tested were (a) early autumn soil disturbance would result in lower persistence of the volunteer SA canola population the spring following canola production compared with late autumn, spring, or no-disturbance, (b) soil disturbance would decrease volunteer SA canola population persistence compared with zero tillage, where tine harrow would be more effective than tandem disc, and (c) relay-seeding a WA cereal following SA canola harvest would result in lower volunteer persistence compared with not seeding a WA crop, regardless of the presence or absence of pre-seed soil disturbance.

3.3 Materials and Methods

3.3.1 Experimental Site Locations and Duration. Field experiments were established on SA canola stubble following harvest at five sites in southern Manitoba, Canada. Three sites near Carman, MB (49°29’27”N, 98°02’42”W), Howden, MB (49°41’15”N, 97°08’22”W) and Melita, MB (49° 15’ 02”N, 101° 03’ 06”W) were initiated in autumn 2013 and two sites near Carman, MB (49°29’42”N, 98°02’14”W) and Pilot Mound, MB (49° 12’ 17”N, 98° 56’ 10”W) were initiated in autumn 2015. The duration of each experiment was from SA canola harvest in September until the following June, the end of the volunteer canola spring seedling recruitment period. Average monthly temperatures and total monthly precipitation recorded from the weather station nearest each site can found in Table 3.1, while information on soil texture can be found in Table 3.2.

Table 3.1. Mean daily air temperature (°C) and total precipitation (mm) for each month from experimental initiation (September) to experimental termination (June) at each site individually.[a,b]

	Mean temperature					Total precipitation				
	Experimental site					Experimental site				
Month	MTA 2013	HDN 2013	CMN 2013	CMN 2015	PMD 2015	MTA 2013	HDN 2013	CMN 2013	CMN 2015	PMD 2015
	°C					mm				
September	16	17	15	16	15	79	34	33	42	42
October	4	7	5	7	7	45	11	14	37	35
November	-5	-4	-5	-1	-2	8	20	12	16	12
December	-18	-19	-19	-8	-9	12	28	11	27	17
January	-16	-18	-18	-14	-13	11	34	15	7	6
February	-19	-18	-19	-10	-10	3	18	4	10	10
March	-9	-10	-11	-1	-1	8	21	6	15	18
April	2	1	1	2	3	67	46	40	55	55
May	12	13	11	14	13	105	40	31	108	101
June	17	18	17	17	16	153	170	117	95	86

[a] Adapted from: Environment Canada (2017)

[b] Abbreviations: MTA, Melita; HDN, Howden; CMN, Carman; PMD, Pilot Mound.

3.3.2 Seedbank Establishment. Wheat (*Triticum aestivum* L.) was cultivated the year prior to canola at all experimental sites. In the year of study initiation, SA canola was grown and harvested (see Table 3.2 for variety). Seed losses from the SA canola crop (forming the *in situ* seedbank) were supplemented with 7,000 seeds m^{-2} of freshly harvested F2 generation hybrid ‘DKL 73-45 RR’ (glyphosate-resistant) SA canola seed (Monsanto Canada Inc., Winnipeg, MB, CA) broadcast evenly over the experimental area. Prior to supplemental seedbank additions, SA

Table 3.2. Soil texture, variety of canola crop, over-winter population persistence (r), and absolute densities of seeds in the autumn seedbank (*in situ* seedbank + supplemental seedbank additions; $N_{\mathrm{sd(Aut)}}$), autumn seedlings ($N_{\mathrm{plt(Aut)}}$), seeds in the spring seedbank ($N_{\mathrm{sd(Spr)}}$) and spring seedlings ($N_{\mathrm{plt(Spr)}}$) for the zero tillage control at each site individually. [a,b]

Experimental site [c]	Soil texture [d]	Preceeding crop variety [e]	r	Absolute densities			
				$N_{\mathrm{sd(Aut)}}$	$N_{\mathrm{plt(Aut)}}$	$N_{\mathrm{sd(Spr)}}$	$N_{\mathrm{plt(Spr)}}$
			proportion	no. m^{-2}			
MTA 2013	LS	InVigor L130	0.29 (0.54) a	8,970 (95) c	533 (23) c	2,610 (51) a	254 (8.0) a
HDN 2013	C	InVigor L130	0.07 (0.27) b	13,050 (114) ab	3,708 (61) ab	950 (31) ab	2 (0.8) b
CMN 2013	SL	InVigor L130	0.01 (0.08) b	6,770 (82) c	698 (26) c	50 (7) c	0 (-4.9) b
CMN 2015	SL	InVigor L252	0.03 (0.17) b	9,570 (98) bc	5,505 (74) a	260 (16) bc	1 (0.1) b
PMD 2015	SCL	InVigor L252	0.03 (0.18) b	15,360 (124) a	2,391 (49) b	470 (22) bc	28 (4.8) a
			(± 0.060) [f]	(± 5.9)	(± 5.6)	(± 6.2)	(± 1.29)

[a] $N_{\mathrm{sd(Aut)}}$, $N_{\mathrm{sd(Spr)}}$, and r values are back-transformed means with square root-transformed means in parentheses, while $N_{\mathrm{plt(Spr)}}$ values are back-transformed means with natural logarithm transformed means in parentheses.

[b] Within columns, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

[c] Abbreviations: MTA, Melita; HDN, Howden; CMN, Carman; PMD, Pilot Mound.

[d] Abbreviations: LS, loamy sand; C, clay; SL, sandy loam; SCL, sandy clay loam.

[e] The preceeding InVigor canola crop variety (Bayer CropScience Inc. Canada, Calgary, AB, CA).

[f] Within columns, parenthetical values preceded by ± indicate the standard error of the difference of the square root- or natural logarithm-transformed means above.

canola seedlots were air dried at 22°C, cleaned, and stored for a maximum of one month at 4°C. Seed viability was quantified immediately prior to seedbank establishment via Petri dish germination assay after seven days of imbibition (Fisherbrand 9 mm Petri dish; P8 filter paper; 6 mL di H_2O). Seedlot predisposition to secondary dormancy was determined using a standard assay (Gulden et al. 2004a), with a 4 week dark imbibition in -1.5 MPa polyethylene glycol (PEG-8000) solution at 20°C.

3.3.3 Experimental Design and Treatment Structure. The experiment used a randomized complete block design with nine treatments and four replications (blocks). The individual experimental units were 4 x 6 m in size. Soil disturbance treatments were conducted at three separate times: (a) early autumn (September, shortly after SA canola harvest and supplemental seedbank additions/immediately after experiment initiation), (b) late autumn (October, one month after harvest), or (c) early spring (May, prior to the spring seeding period). Each time point included soil disturbance using either spring-tooth tine harrow (1 cm depth) or tandem disc (12 cm depth) implements with two passes, each in opposite directions. A zero tillage control treatment also was included and remained undisturbed throughout the duration of the experiment. In the remaining two treatments, winter wheat 'Flourish' (19 cm row spacing, 1-2 cm seeding depth, 89 kg seed ha^{-1}) was established in September using a double disc seeder into disturbed (tine harrow) or undisturbed (zero tillage) soil (208 and 127 average seedlings m^{-2} in autumn and spring, respectively).

3.3.4 Sampling Intervals and Measurements.

3.3.4.1 Seedbank Density. Following SA canola harvest, *in situ* volunteer SA canola seedbank densities (seeds; sd) were quantified ($N_{\mathrm{sd(Sep)}}$) and the supplemental seedbank addition was added to create the initial seedbank ($N_{\mathrm{sd(Aut)}}$). Seedbank densities were determined again the following spring (April), as early as soil thaw would allow and prior to spring seedling recruitment ($N_{\mathrm{sd(Apr)}}$). To quantify seedbank density, twenty soil core samples were removed from each experimental unit (6.4 cm diameter; 15 cm depth) and homogenized in plastic trays (28 x 54 x 6 cm, Kord Products, Brampton, ON, CA). Trays were placed in the greenhouse [temperature: 24°C (± 6°C); relative humidity: 22% (± 11%); light: ambient and supplemental 600 W high pressure sodium, 16 hour photoperiod] immediately after sampling and watered daily. The soil was stirred biweekly and cold stratified (4°C for 72 hours) every four weeks to alleviate seed dormancy (Schlink 1994), until seedbank exhaustion (Gulden et al. 2003b). Recruited canola seedlings were counted and removed weekly.

3.3.4.2 Seedling Density. Emerged volunteer SA canola seedling densities (plants; plt) were quantified before ($N_{\mathrm{plt(Sep)_{pre}}}$, $N_{\mathrm{plt(Oct)_{pre}}}$ and $N_{\mathrm{plt(May)_{pre}}}$) and after ($N_{\mathrm{plt(Sep)_{post}}}$, $N_{\mathrm{plt(Oct)_{post}}}$ and $N_{\mathrm{plt(May)_{post}}}$) soil disturbance in early autumn, late autumn, and the following spring, in addition to four weeks after the last disturbance of the season in autumn ($N_{\mathrm{plt(Nov)}}$), and spring ($N_{\mathrm{plt(Jun)}}$). Seedling densities were quantified by counting the number of plants in four randomly placed quadrats in each experimental unit. Quadrats ranged from 10 x 10 cm in autumn to 50 x 50 cm or whole experimental unit counts in spring, depending on seedling densities.

3.3.5 Calculation of Demographic Parameters.

3.3.5.1 Demographic States. The above life-stage measurements were used to calculate the demographic states of seedbank density ($N_{\mathrm{sd(Aut)}}$ and $N_{\mathrm{sd(Spr)}}$) and cumulative seedling density ($N_{\mathrm{plt(Aut)}}$ and $N_{\mathrm{plt(Spr)}}$) in autumn and spring, respectively (Fig 3.1), using the equations in Table 3.3. It was assumed that no seedling death occurred between recruitment measurements within the autumn and spring seedling recruitment periods. Therefore, in calculation of $N_{\mathrm{plt(Aut)}}$ and $N_{\mathrm{plt(Spr)}}$, differences within brackets (in Table 3.3) were equated to seedling death due to physical soil disturbance.

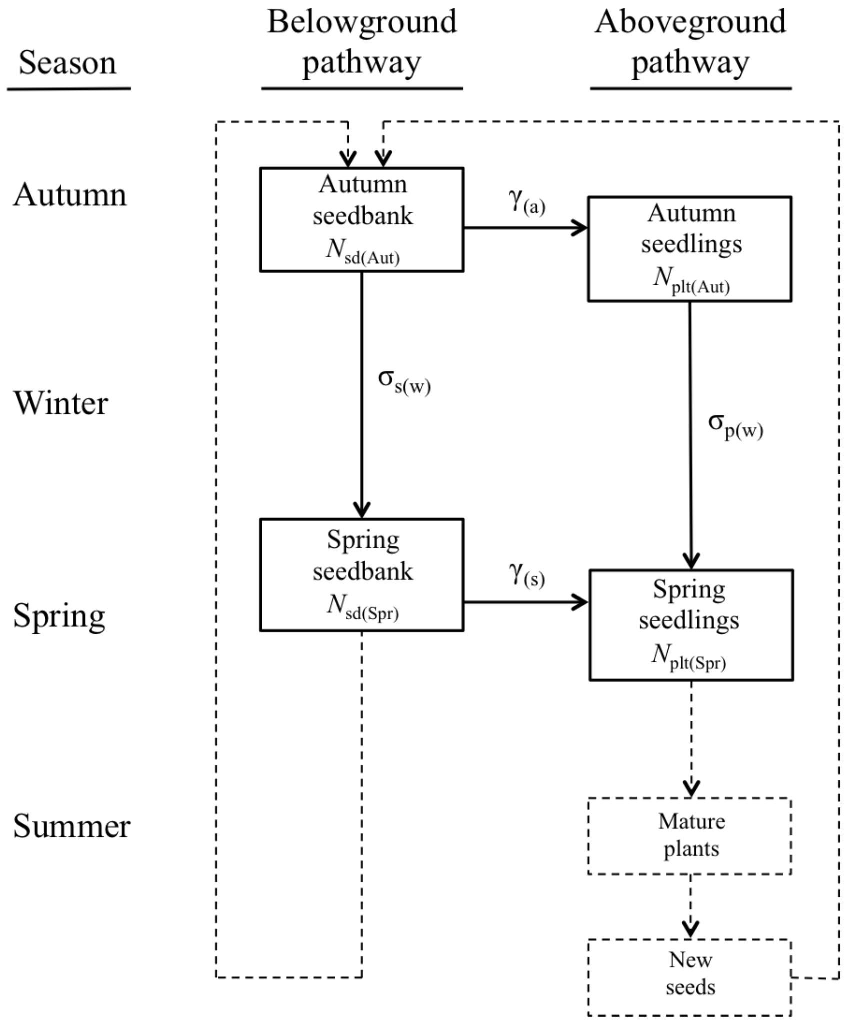

Figure 3.1. Life cycle diagram of volunteer-summer annual canola. Boxes represent life-stages (demographic states) and arrows indicate life-stage transition rates (demographic rates). Solid boxes and arrows indicate the states and rates evaluated, while dashed boxes and arrows were excluded from the study.

Table 3.3. Abbreviation and calculation of demographic states and rates for volunteer summer-annual canola. [a]

Parameter	Abbreviation	Equations/Units
Demographic state		
Seedbank density$_{\text{Autumn}}$	$N_{\text{sd(Aut)}}$	$N_{\text{sd(Sep)}} + 7000\,(\text{proportional seedlot viability})$
Seedbank density$_{\text{Spring}}$	$N_{\text{sd(Spr)}}$	$N_{\text{sd(Apr)}}$
Seedling density$_{\text{Autumn}}$	$N_{\text{plt(Aut)}}$	$\left(N_{\text{plt(Sep)}_{\text{pre}}} - N_{\text{plt(Sep)}_{\text{post}}}\right) + \left(N_{\text{plt(Oct)}_{\text{pre}}} - N_{\text{plt(Oct)}_{\text{post}}}\right) + N_{\text{plt(Nov)}}$
Seedling density$_{\text{Spring}}$	$N_{\text{plt(Spr)}}$	$\left(N_{\text{plt(May)}_{\text{pre}}} - N_{\text{plt(May)}_{\text{post}}}\right) + N_{\text{plt(Jun)}}$
Demographic rate		
Recruitment$_{\text{Autumn}}$	$\gamma_{\text{(a)}}$	$N_{\text{plt(Aut)}}\ N_{\text{sd(Aut)}}{}^{-1}$
Plant survival$_{\text{Nov-Apr}}$	$\sigma_{\text{p(w)}}$	$N_{\text{plt(Apr)}}\ N_{\text{plt(Nov)}}{}^{-1}$
Seed survival$_{\text{Sep-Apr}}$	$\sigma_{\text{s(w)}}$	$N_{\text{sd(Spr)}}\ \left(N_{\text{sd(Aut)}} - N_{\text{plt(Aut)}}\right)^{-1}$
Recruitment$_{\text{Spring}}$	$\gamma_{\text{(s)}}$	$N_{\text{plt(Spr)}}\ N_{\text{sd(Spr)}}{}^{-1}$
Over-winter persistence$_{\text{Sep-Apr}}$	r	$\left(N_{\text{sd(Spr)}} + N_{\text{plt(Apr)}}\right)\ N_{\text{sd(Aut)}}{}^{-1}$

[a] Abbreviations: N_{sd}, number of seeds; N_{plt}, number of plants; Aut, autumn; Spr, spring; Sep, September; Oct, October; Nov, November; Apr, April; May, May; Jun, June; pre, density previous to soil disturbance; post, density remaining after soil disturbance.

3.3.5.2 Demographic Rates. The demographic states and life-stage measurements were used to determine the demographic rates of autumn seedling recruitment ($\gamma_{(a)}$), plant survival over winter ($\sigma_{p(w)}$), seed survival in the soil seedbank over winter ($\sigma_{s(w)}$), spring seedling recruitment ($\gamma_{(s)}$) (Fig 3.1), and over-winter population persistence (r), using the equations in Table 3.3. Over-winter population persistence was the proportional change in number of individuals in the population from September to April and was therefore a combination of the effects of autumn seedling recruitment, plant survival over winter and seed survival over winter.

3.3.6 Statistical Analysis. To address the specific hypotheses, each experiment was subdivided into a two-way augmented factorial (3 times x 2 implements + 1 control) (substructure-A) and a two-way full factorial (2 crops x 2 implements) treatment substructure (substructure-B) for statistical analyses (Piepho et al. 2006). Substructure-A was used to test the effects of timing and implement used for soil disturbance on volunteer SA canola demographic parameters. In this analysis, tine harrow (shallow) or tandem disc (deep) in early autumn, late autumn or spring were compared to a zero tillage control. Substructure-B was used to test the effects of seeding winter wheat in early autumn with and without pre-seed soil disturbance on volunteer SA canola population dynamics. In early autumn treatments only, seeding winter wheat following tine harrow or zero tillage was compared with the same tillage treatments without seeding winter wheat. For each treatment substructure, all treatments were used to estimate experimental error (Piepho et al. 2006).

The mixed model procedure (Littell et al. 2006) in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) was used for ANOVA of the demographic states ($N_{\mathrm{sd(Aut)}}$, $N_{\mathrm{sd(Spr)}}$, $N_{\mathrm{plt(Aut)}}$ and $N_{\mathrm{plt(Spr)}}$) and life-stage transition rates ($\gamma_{(\mathrm{a})}$, $\sigma_{\mathrm{s(w)}}$, $\gamma_{(\mathrm{s})}$ and r) (Table 3.2; Fig 3.1). Data were square root-transformed (or natural logarithm-transformed; $N_{\mathrm{plt(Spr)}}$) to meet the assumptions of normality and homoscedasticity, and Lund's test (Lund 1975) was used to remove extreme outliers when justified. In substructure-A, experimental site, timing and implement were considered fixed effects, while experimental block nested within site was considered a random effect. In substructure-B, experimental site, implement and crop were considered fixed effects, while experimental block nested within site was considered a random effect. Initial seedbank density ($N_{\mathrm{sd(Aut)}}$) data were based on whole block measurements prior to study establishment. For this analysis, site was considered a fixed effect while experimental block was considered a random effect.

The covariance structure of residuals was adjusted by selecting the R matrix repeated effect based on minimization of Akaike's Information Criterion when necessary to meet the assumption of homoscedasticity (Onofri et al. 2010). To control the family-wise error rate, Tukey's honest significant difference (HSD) ($\alpha = 0.05$) was used for *post hoc* multiple comparison of means (Onofri et al. 2010) and letter separation was generated using the %MULT macro (Piepho 2012).

The correlation procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) was used to examine the association among transformed site means of demographic states, rates, and autumn- (Sept-Nov), winter- (Dec-Mar), and spring-precipitation (Apr-Jun) from the weather station nearest each site (Environment Canada 2017; Table 3.1).

3.4 Results

3.4.1 Experimental Site. Over-winter persistence of volunteer SA canola was influenced by site and ranged from less than 1% of the autumn seedbank in Carman 2013 (6,770 to 50 seeds m^{-2} in autumn and spring, respectively) to 29% in Melita 2013 (8,970 to 2,610 seeds m^{-2} in autumn and spring, respectively) under zero tillage (Table 3.2). Larger variability in population persistence was observed among sites initiated in 2013 compared with 2015 (Table 3.2). Interestingly, supplemental seedlot predisposition to secondary dormancy also was greater in 2013 than 2015 [36% (± 5.1%) and 5% (± 1.0%) dormancy potential and 96% (± 1.3%) and 94% (± 1.8%) viability in 2013 and 2015, respectively]. As experimental sites differed in absolute density of the autumn seedbank ($N_{\mathrm{sd(Aut)}}$; 6,773 to 15,361 seeds m^{-2}; Table 3.2), and no correlation with demographic parameters was found, the results and discussion focus on demographic rates for ease of interpretation.

Although the global F-test indicated significant differences in winter seed survival in the soil seedbank among soil disturbance timing, implement type and seeding winter wheat (Table 3.4), lack of mean separation (Table A1) suggested that the below-ground pathway is not as readily manipulated by soil disturbance as the above-ground pathway. Nevertheless, volunteer SA canola population persistence was correlated positively with winter seed survival (Pearson r = 0.93, P = 0.028), but not autumn recruitment (Pearson r = -0.45, P = 0.444). Therefore, differences in persistence among sites were better explained by variation in winter seed survival ($\sigma_{\mathrm{s(w)}}$) compared with survival of autumn recruited seedlings ($\gamma_{\mathrm{(a)}}$ and $\sigma_{\mathrm{p(w)}}$).

The amount of precipitation received at each site in spring, from the beginning of April (soil thaw) to the end of recruitment in June (Table 3.1), was correlated negatively with spring seedling recruitment (Pearson r = -0.98, $P = 0.003$) only. Autumn- (Sep-Nov, primarily rainfall) or winter-precipitation (Dec-Mar, primarily snowfall), however, did not correlate with any response variables.

Table 3.4. ANOVA results (*P*-values) for the main and interaction effects of site, time and implement (substructure-A) or site, crop and implement (substructure-B) on volunteer summer-annual canola autumn seedling recruitment ($\gamma_{(a)}$), seed survival over winter ($\sigma_{s(w)}$), spring seedling recruitment ($\gamma_{(s)}$) and population persistence from autumn to spring (r) (see life cycle diagram in Fig 3.1).

Source	Demographic rates [a,b]			
	$\gamma_{(a)}$	$\sigma_{s(w)}$	$\gamma_{(s)}$	r
	P-values			
Augmented factorial treatment substructure-A				
Experimental site (E)	**< 0.001**	**< 0.001**	**0.001**	**< 0.001**
Time (T)	**< 0.001**	**0.023**	**< 0.001**	**< 0.001**
Implement (I)	ns	**0.038**	**0.005**	ns
E * T	**< 0.001**	**0.024**	**0.001**	ns
E * I	**0.039**	ns	**0.015**	ns
T * I	ns	ns	ns	ns
E * T * I	**0.020**	ns	ns	ns
Full factorial treatment substructure-B				
Experimental site (E)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Crop (C)	**< 0.001**	**0.043**	ns	**0.019**
Implement (I)	**< 0.001**	ns	ns	**0.007**
E * C	**< 0.001**	**0.005**	ns	**0.011**
E * I	**< 0.001**	**0.017**	ns	ns
C * I	**0.001**	ns	ns	ns
E * C * I	ns	ns	ns	ns

[a] Bold values indicate significant effects at $P < 0.05$.
[b] ns indicates lack of a significant effect at $P \geq 0.05$.

3.4.2 Timing of Soil Disturbance. Early autumn soil disturbance was most effective at reducing over-winter persistence of volunteer SA canola compared with the other soil disturbance treatments (Fig 3.2). Irrespective of tillage implement, SA canola persistence under early autumn disturbance was half that compared with the absence of tillage or spring soil disturbance only (Fig 3.2). Volunteer SA canola persistence under late autumn tillage was intermediate, but more similar to early autumn tillage than zero tillage or spring soil disturbance (Fig 3.2).

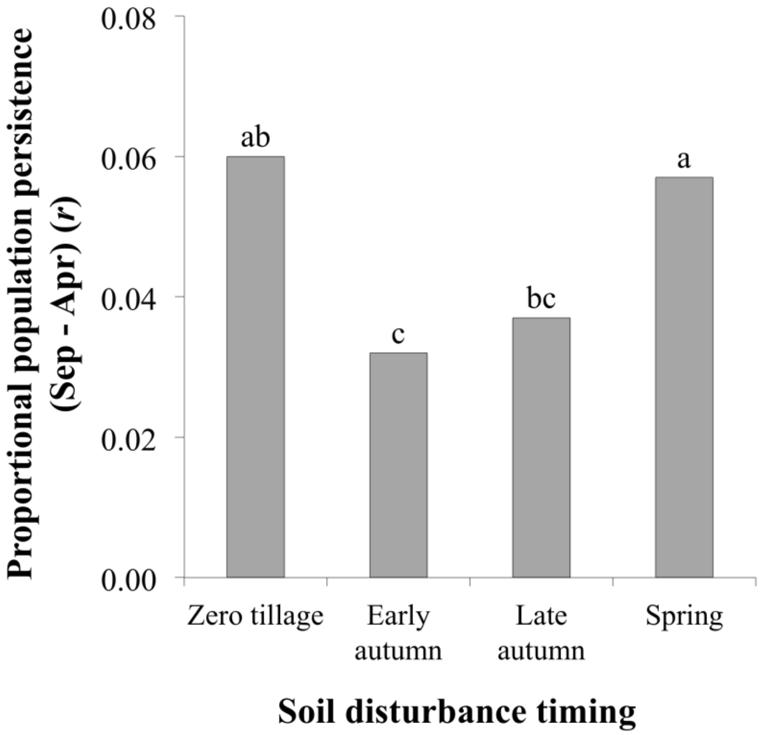

Figure 3.2. Persitence of the volunteer summer-annual canola population from September to April (*r*; proportional) in response to the timing of post-harvest soil disturbance in a combined analysis among implements and sites. Values are back-transformed square root means. Different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

Early autumn was the only time of soil disturbance that resulted in greater autumn recruitment than zero tillage (Table 3.5). Overall, soil disturbance in early autumn resulted in approximately double the autumn seedling recruitment (38%) compared with zero tillage (20%), late autumn (23%) or spring (23%) soil disturbance. Volunteer SA canola plant survival over winter was only observed at Carman 2015 at rates less than 2% (Table A2; Fig A1). Winterkill of autumn-recruited seedlings was complete at all other sites (data not shown). Thus, nearly all autumn-

Table 3.5. Proportional autumn seedling recruitment ($\gamma_{(a)}$) of volunteer summer-annual canola at each site in response to the timing and implement of soil disturbance in the augmented factorial (substructure-A) and presence (tine harrow) or absence (zero tillage) of autumn pre-seed soil disturbance or presence (winter wheat) or absence (unseeded) of winter wheat in the full factorial (substructure-B) treatment substructure.

			Experimental site [a,b]				
Timing	Implement	Crop	Melita 2013	Howden 2013	Carman 2013	Carman 2015	Pilot Mound 2015
					proportions		
Augmented factorial treatment substructure-A							
Control	Zero tillage		0.06 (0.24) c	0.28 (0.53) b	0.10 (0.32) b	0.58 (0.76)	0.16 (0.40) cd
Early autumn	Tine harrow		0.12 (0.35) ab	0.97 (0.99) a	0.27 (0.52) a	0.62 (0.79)	0.31 (0.56) bc
Early autumn	Tandem disc		0.11 (0.33) a	0.65 (0.80) a	0.28 (0.53) a	0.44 (0.67)	0.45 (0.67) ab
Late autumn	Tine harrow		0.06 (0.24) c	0.28 (0.52) b	0.12 (0.35) b	0.69 (0.83)	0.21 (0.46) cd
Late autumn	Tandem disc		0.04 (0.20) bc	0.31 (0.56) b	0.11 (0.33) b	0.66 (0.81)	0.25 (0.50) d
Spring	Tine harrow		0.06 (0.25) c	0.30 (0.55) b	0.11 (0.34) b	0.63 (0.79)	0.22 (0.47) ad
Spring	Tandem disc		0.04 (0.21) c	0.33 (0.57) b	0.12 (0.35) b	0.67 (0.82)	0.20 (0.45) bd
			(± 0.019) [c]	(± 0.053)	(± 0.029)	(± 0.051)	(± 0.039)
Full factorial treatment substructure-B							
	Zero tillage		0.08 (0.28) b	0.35 (0.59) b	0.17 (0.41) b	0.56 (0.75)	0.26 (0.51)
	Tine harrow		0.13 (0.36) a	0.87 (0.93) a	0.35 (0.59) a	0.57 (0.76)	0.35 (0.59)
		Unseeded	0.09 (0.30)	0.58 (0.76)	0.18 (0.42) b	0.60 (0.77)	0.23 (0.48) b
		Winter wheat	0.12 (0.34)	0.59 (0.77)	0.33 (0.58) a	0.54 (0.74)	0.40 (0.63) a
			(± 0.013)	(± 0.039)	(± 0.021)	(± 0.036)	(± 0.028)

[a] Values are back-transformed means with square root-transformed means in parentheses.

[b] Within columns and effect groupings, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

[c] Within columns and treatment substructures, parenthetical values preceded by ± indicate the standard error of the difference of the square root-transformed means above.

Table 3.6. Proportional spring seedling recruitment ($\gamma_{(s)}$) of volunteer summer-annual canola at each site in response to the timing or implement of soil disturbance in the augmented factorial (substructure-A) treatment substructure.

		Experimental site [a,b]				
Timing	Implement	Melita 2013	Howden 2013	Carman 2013 [c]	Carman 2015	Pilot Mound 2015
				proportions		
Augmented factorial treatment substructure-A						
Zero tillage		0.13 (0.35) b	0.00 (0.05) b	-	0.01 (0.07) b	0.06 (0.25) ab
Early autumn		0.12 (0.35) b	0.00 (0.05) b	-	0.01 (0.09) b	0.06 (0.25) ab
Late autumn		0.16 (0.40) ab	0.00 (0.05) b	-	0.01 (0.08) b	0.05 (0.22) b
Spring		0.31 (0.56) a	0.01 (0.12) a	-	0.07 (0.26) a	0.17 (0.42) a
		(± 0.047) [d]	(± 0.014)		(± 0.033)	(± 0.054)
	Zero tillage	0.13 (0.35)	0.00 (0.05)	-	0.01 (0.07)	0.06 (0.25)
	Tine harrow	0.20 (0.45)	0.00 (0.07)	-	0.03 (0.17)	0.12 (0.35)
	Tandem disc	0.18 (0.42)	0.01 (0.08)	-	0.01 (0.11)	0.06 (0.24)
		(± 0.044)	(± 0.013)		(± 0.031)	(± 0.050)

[a] Values are back-transformed means with square root-transformed means in parentheses.

[b] Within columns and effect groupings, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

[c] A dash (-) indicates data excluded from the analysis.

[d] Within columns and effect groupings, parenthetical values preceded by ± indicate the standard error of the difference of the square root-transformed means above.

recruited seedlings contributed to SA canola mortality and led to decreased population persistence following early autumn disturbance (Fig 3.2). Spring soil disturbance resulted in the largest spring seedling recruitment among sites (Table 3.6); averaging 11% of the viable seed in the spring seedbank compared with 3% at all other timings. Carman 2013 was removed from this analysis, as low spring seedbank densities (Table 3.2) confounded recruitment proportions. Nevertheless, the stimulation of spring recruitment by spring soil disturbance remained consistent among sites (Table 3.6).

3.4.3 Implement used for Soil Disturbance. The implement used for soil disturbance resulted in minor effects on volunteer SA canola demographic parameters. Although demographic rates differed among tillage implement based on the F-test (Table 3.4), differences did not manifest into significant mean separation using Tukey's HSD (Tables 3.5 and 3.6).

3.4.4 Seeding Winter Wheat and Pre-Seed Soil Disturbance. In early autumn, soil disturbance via tine harrow prior to seeding winter wheat decreased population persistence of volunteer SA canola by 35% compared with no tillage prior to winter wheat (data not shown), while differences in the presence and absence of seeding winter wheat (Table 3.4) did not result in separation of means using Tukey's HSD (Table A3). Soil disturbance during seeding of winter wheat (double-disc seed-row openers) occurred only near the seed row, while tine harrow caused more uniform surface disturbance. This explained the higher proportional autumn recruitment following early autumn tine harrow compared to seeding winter wheat alone (Fig 3.3). Moreover, the effect of seeding winter wheat was only significant when the soil was undisturbed prior to seeding (59% increase in autumn recruitment under zero tillage) (Fig 3.3).

Although the effects of seeding winter wheat and pre-seed soil disturbance on autumn recruitment were dependent on site (Table 3.6), it was clear that any type of soil disturbance in early autumn increased recruitment before winter (Table 3.5; Fig 3.3). Nevertheless, presence or absence of soil disturbance prior to seeding winter wheat resulted in differences in population persistence of volunteer SA canola (3% and 5%, respectively), whereas seeding winter wheat alone did not influence persistence (Table A3), seed mortality (Table A1), or spring seedling recruitment (Table 3.4).

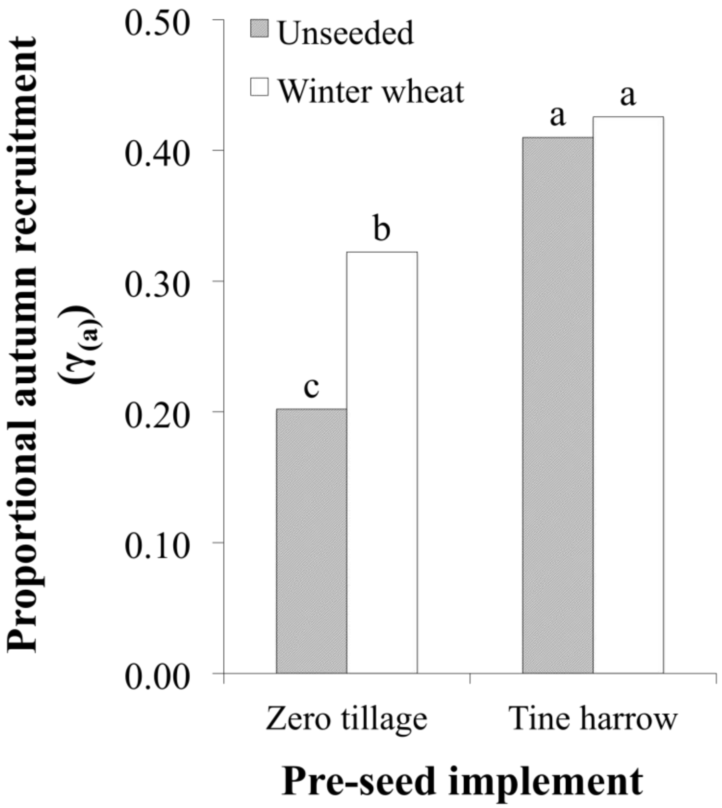

Figure 3.3. Proportional autumn seedling recruitment ($\gamma_{(a)}$) of volunteer summer-annual canola in response to the presence (winter wheat) or absence (unseeded) of winter wheat seeded in early autumn with the presence (tine harrow) or absence (zero tillage) of pre-seed soil disturbance in a combined analysis among sites. Values are back-transformed square root means. Different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

3.5 Discussion

In the current study, early autumn soil disturbance, shortly after SA canola harvest, was the most effective option for reducing volunteer SA canola population persistence, while zero tillage was the least effective. Reduced volunteer SA canola persistence was principally caused by large proportional autumn seedling recruitment triggered by early autumn soil disturbance. In western Canada, harsh winter conditions result in winterkill, which limits SA canola plant survival over winter. Therefore, autumn recruitment caused SA canola mortality and depleted the soil seedbank. Previous surveys also have observed larger volunteer SA canola population densities (and therefore persistence) under zero tillage compared with conventional tillage in Canada (Légère et al. 2001; Simard et al. 2002; Thomas et al. 1997). As anticipated, this result is in contrast to those found in England (Pekrun et al. 1998a; Lutman et al. 2003), Austria (Pekrun et al. 2006), and Germany (Gruber et al. 2004b, 2005), where delayed or zero tillage caused the greatest reduction in volunteer WA canola seedbank persistence, while immediate tillage (post-harvest) resulted in the largest seedling recruitment the following spring.

The difference in SA and WA canola life cycles (in the current study and European studies, respectively) was expected to be the cause of contrasting results among soil disturbance timing. Harvest dates of WA canola in western/central Europe (Gruber et al. 2004b, 2005) are four to six weeks before harvest of SA canola in western Canada. Hence, the duration in which post-harvest tillage can occur is much shorter following SA canola than WA canola. It is therefore suggested that the optimal timing for post-harvest soil disturbance may differ temporally between SA and WA canola production. Winter-annual canola is not currently grown in Canada due to concerns with winter-hardiness (H Rahman, personal communication). Moreover, greater than 99% of Canadian SA canola is grown in western Canada (Beckie et al. 2006) under similar continental conditions to those of southern Manitoba. Thus, results observed in the current study are representative of the northern Great Plains region and 99% of canola grown in Canada.

Contrary to observations of WA canola in Europe (Pekrun et al. 1998a; Lopez-Granados and Lutman 2002), where conditions following early post-harvest seed burial are conducive to the induction of secondary seed dormancy (Gruber et al. 2005, 2010), it appears (due to lack of differences) that dormancy may play a limited role in initial post-harvest SA canola seedbank survival over winter in Canada. Indeed, conditions following SA canola harvest in Canada are tantamount to those that release secondary dormancy in this species (Lopez-Granados and Lutman 1998; Momoh et al. 2002; Pekrun et al. 1997b). Moreover, intense light during cold temperatures (Bazanska and Lewak 1986) and variable moisture potential, due to inadequate contact with the soil matrix (Gruber et al. 2010) under zero tillage conditions, suggest that quiescence may govern volunteer seed persistence over the first winter following SA canola harvest in Canada. This premise is consistent with the observed effects of early autumn post-harvest soil disturbance on autumn recruitment and concomitant persistence of SA canola in the current study.

Soil disturbance in spring caused greater spring seedling recruitment, either by redistribution of quiescent volunteer SA canola seeds to ‘safe sites’ for successful recruitment (Harper et al. 1961) or by releasing secondary seed dormancy (Lopez-Granados and Lutman 1998; Schlink 1994). Spring precipitation, however, was negatively correlated with spring seedling recruitment, indicating that an abundance of soil moisture in spring caused lower seedling recruitment. This relationship concurs with previous research in China, where waterlogged soils decreased germination potential of WA canola (Zhu et al. 2012).

This study is one of few studies on canola persistence conducted on freshly harvested canola stubble (Gruber et al. 2005) rather than cereal stubble with artificial seedbank inputs (e.g., Gruber et al. 2004b, 2010; Gulden et al. 2003b; Pekrun et al. 2006). Differences in stubble architecture and decay influence temperature and moisture fluctuation, shading, as well as soil freeze and thaw (Brady and Weil 2007), in addition to habitat for biological predators (Kulkarni et al. 2016), all of which may affect the fate of seeds in the soil seedbank (Baskin and Baskin 2014). Indeed, delayed or zero tillage may allow for more biological seed predation than immediate tillage, by leaving canola seed on the soil surface following harvest (Gruber et al. 2004b; Kulkarni et al. 2016). Seedbank persistence in the current study, however, did not correspond with length of time prior to seed burial. Hence, the effects of lethal germination, seed decay, seedling recruitment and winterkill likely confounded any differences in biological seed predation.

The current study assumed that no seedling death other than physical management occurred during the seedling recruitment periods in autumn or spring. Although biotic or abiotic stresses may have resulted in seedling death during these time periods, seedling mortality due to external factors can be difficult to quantify, was believed to be minimal, and was generally observed outside the seedling recruitment periods. Regardless of the different combinations of *in situ* and supplemental volunteer SA canola varieties among sites, and a clear genetic link to seedbank persistence among genotypes in this species (e.g., Gulden et al. 2003b), the observed effects of soil disturbance on volunteer SA canola persistence remained relatively consistent. Even though the current study did not determine long-term persistence of volunteer SA canola, previous research suggests that treatment differences beyond the year subsequent to canola production would be minimal (Gruber et al. 2010; Gulden et al. 2003b; Lutman et al. 2003).

3.5.1 Management Implications. Early autumn soil disturbance, shortly after SA canola harvest, may be used as a management tool to decrease volunteer SA canola persistence over winter. Although zero tillage systems have numerous benefits regarding soil health (Brady and Weil 2007), tine harrow may be effective at stimulating SA canola seedling recruitment, while limiting the negative impacts of tillage on soil health. This approach would be optimal for management in areas where winterkill can cause termination of recruited volunteers. Due to widespread winterkill of SA canola in both eastern (Simard et al. 2002) and western Canada, practical implementation of this management strategy is expected to be effective in, but not limited to, Canada and the northern Great Plains. However, spring pre-seed herbicide application also may be utilized in areas that do experience plant survival over winter. Spring soil disturbance may be used as an additional management tool to stimulate volunteer SA canola recruitment in spring, prior to pre-seed herbicide application. However, due to the widespread utilization of HR SA canola in Canada, additional modes-of-action to broad-spectrum herbicides are imperative. Nevertheless, on-farm seed losses at harvest are so large that 3% population persistence (averaged among sites) achieved under early autumn soil disturbance would result in spring seedbank densities of approximately 128 viable seeds m^{-2} in the most effective management scenario. Therefore, timing of soil disturbance is an effective tool that should be used in addition to other management tactics as part of a comprehensive integrated program to manage this weed.

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4.0 Soil Texture Contributes to Divergent Winter and Summer Seedbank Dynamics in *Brassica napus*

4.1 Abstract

Volunteer canola is an abundant weed in western Canada derived from large seedbank inputs at canola harvest and potential induction into secondary seed dormancy. Due to seedbank replenishment each time canola is grown, rapid seed demise in the soil seedbank is imperative to prevent adventitious presence of volunteer canola in subsequent crops. The effect of soil texture and associated edaphic factors on survival of canola seed was evaluated over winter (Nov-Apr) and over summer (May-Sep). A common garden experiment was established at two locations in Manitoba, Canada in autumn 2015 and consisted of canola seed burial in nine soils covering a broad texture gradient. Fine textured soils (clays) were associated with the greatest canola seed survival over winter (15% of total seedbank additions) and the lowest seed survival over summer (4% of the total seedbank remaining in spring). The opposite was observed in coarse textured soils [loamy sands (6% and 15% seed survival over winter and summer, respectively)]. Medium textured soils (sandy loams) were associated with intermediate seed survival both over winter and over summer. Opposing seasonal seed survival in fine and coarse textured soils resulted in a lack of difference in seed survival among textural classes ten months after seed burial. Canola seed survival over winter was associated positively with soil characteristics related to fine soil textures, while seed survival over summer was associated negatively with the same soil characteristics. Canola seed survival over winter had a strong negative association with soil bulk density and a strong positive association with the mean diurnal range in soil temperature over winter. The significant influence of soil texture on canola seed survival and the discovery of

opposing seasonal seedbank dynamics are steps forward in understanding the *in situ* processes that affect the canola seedbank and potential strategies for seedbank management.

4.2 Introduction

Volunteer summer-annual (SA) canola (*Brassica napus* L.) is a problematic weed in western Canada, where it is now among the top five most abundant mid-season weed species after in-crop weed management (Leeson 2015; Leeson et al. 2016). The prevalence of this weed is due to a combination of high potential for pod drop and pod shatter (Cavalieri et al. 2014; Gulden et al. 2017) resulting in large seed losses at harvest (about 4,300 seeds m^{-2} regional average) (Cavalieri et al. 2016; Gulden et al. 2003a), and genetic predisposition to secondary seed dormancy related to seedbank persistence (Gulden et al. 2003b). The frequency at which SA canola is grown in this region (Beckie et al. 2011a; Harker et al. 2015b) also may contribute to population persistence that could span the duration of full crop rotation cycles (Beckie and Warwick 2010). Genetically-engineered herbicide-resistance (HR) and adventitious presence of unsolicited HR traits in pedigreed canola seedlots (Friesen et al. 2003) also may contribute to population persistence, particularly in other crops with similar HR traits that are grown in rotation with HR canola (Chapters 5, 6 and 7). Concerns have been raised not only about volunteer canola interference in subsequent crops (Seerey and Shirtliffe 2010; Chapters 5, 6 and 7), but potentially also seed or oil profile contamination via pollen-mediated gene flow (Beckie et al. 2003; Hall et al. 2000) or coexistence of volunteer canola in subsequent canola crops (Andersen et al. 2010; Jørgensen et al. 2007) resulting in seed admixtures (Messéan et al. 2007). Intraspecific pollen-mediated gene flow has resulted in the development of double- or triple-HR off-types in volunteer populations (Beckie et al. 2003; Hall et al. 2000).

Following seed shed from the mother plant, a seed is destined for one of two fates: (a) germination or (b) death (Long et al. 2015). Several different processes including seed aging, decay, predation, or lethal germination may impose the latter seed fate (Fenner and Thompson 2005). A seed's inherent resistance to these processes governs its potential for successful recruitment (Long et al. 2015). Seed dormancy or seed quiescence can alter the temporal dynamics of fates a or b (Baskin and Baskin 2014), therefore affecting seedbank persistence. Upon seed entry into the soil seedbank, edaphic factors stimulate ecophysiological responses that influence a seed's resistance to these seedbank processes (Fenner and Thompson 2005; Long et al. 2015).

In Canada, Geddes and Gulden (Chapter 3) recently found that soil disturbance shortly after SA canola harvest (early autumn) halved volunteer canola seedbank persistence over winter compared with no disturbance. More importantly, volunteer canola seedbank persistence over winter ranged from 1% to 29% of the autumn seedbank among locations (Chapter 3). A 29-fold difference in persistence among locations clearly indicated a strong contribution of environmental and/or edaphic factors to seedbank processes governing canola seed demise. Field research also has suggested that conditions causing seedbank persistence may differ for winter-annual (WA) canola in Europe compared with SA canola in Canada (Chapter 3; Légère et al. 2001; Lutman et al. 2003; Simard et al. 2002). The conditions contributing to such dissimilarity in seedbank persistence have not yet been determined.

A combination of environmental conditions including warm temperatures (around 20°C), dryness (near the permanent wilting point, -1.5 MPa), darkness, hypoxia, and time [> 2 weeks

(Weber et al. 2010)] contribute to the induction of secondary dormancy in canola seed (Gulden et al. 2004a; Lopez-Granados and Lutman 1998; Momoh et al. 2002; Pekrun et al. 1997a, 1998a; Schlink 1994). Genotype (Gruber et al. 2004a, 2009, 2012; Gulden et al. 2003b; Momoh et al. 2002), plant maturity (Haile and Shirtliffe 2014), maternal environment (Huang 2016), after-ripening, seed size (Gulden et al. 2004a) and also burial depth (Gruber et al. 2010) contribute to the induction of secondary dormancy in canola seed under controlled laboratory conditions. However, factors that induce secondary dormancy in canola seed in the laboratory are difficult to evaluate *in situ*. A recent regression tree analysis of 103 surveyed fields across landscapes in Germany found that the frequency at which WA canola was grown, and soil climatic region, were the main factors associated with volunteer canola plant abundance (Gruber et al. 2012; Thöle et al. 2011). A common garden field study evaluating several WA canola accessions in three soils found greater seed survival after six months in a clay soil and a silty clay loam soil compared with a silty loam soil (Gruber et al. 2014; Weber et al. 2011). These studies from Germany indicate that volunteer canola seeds may survive longer in fine compared with coarse textured soils. However, canola seed survival in the soil seedbank has not yet been investigated using multiple soils with similar texture in a common garden experiment that eliminates confounding environmental effects and allows for the isolation of the soil texture effect by testing multiple soils in the same environment. In addition, the influence of soil texture on canola seed survival over summer remains untested.

The objective of this study was to evaluate the potential influence of soil texture and other edaphic factors on SA canola seed survival in the soil seedbank over winter and over summer. In this study, differences among soils, soil textural classes and the association of physical and/or chemical soil properties with SA canola seed survival in the soil seedbank were evaluated using a common garden design.

4.3 Materials and Methods

4.3.1 Field Experiment.

4.3.1.1 Experimental Locations and Duration. Field experiments were established on fallow soil in late autumn at two locations in Manitoba, Canada. The first location, near Carman, MB (CMN; 49°29’42” N, 98°02’14” W; Denham soil series, Orthic Black Chernozem), and the second location, near Winnipeg, MB (WPG; 49 °48’06” N, 97°10’10” W; Osborne soil series, Rego Humic Gleysol), were situated in the Lake Manitoba Plain ecoregion within the Prairie ecozone (CanSIS 2013). The duration of each experiment lasted from November 2015, shortly before snowfall/soil freeze, to September 2016, the usual time for harvest of SA crops in this region.

4.3.1.2 Experimental Design and Treatment Structure. A randomized complete block design (RCBD) with a split-plot treatment structure and four replications (blocks) was used at each location to evaluate SA canola seed survival in the soil seedbank in mesocosms over winter ($\sigma_{s(w)}$; Nov-Apr), summer ($\sigma_{s(s)}$; Apr-Sep) and the entire duration of the experiment (winter and summer; $\sigma_{s(ws)}$; Nov-Sep). Main plots consisted of the different soils tested (9 soils), while sub-plots consisted of mesocosm retrieval date for seed recovery (2 dates).

4.3.2 Soil Burial Mesocosms.

4.3.2.1 Seedlot Production and Preparation. In the year of study initiation, 'DKL 73-45 RR' (glyphosate-resistant) hybrid SA canola seed (Monsanto Canada Inc., Winnipeg, MB, CA) was grown at the Ian N Morrison Research station, near Carman, MB (49°29'27" N, 98°02'42" W) according to recommended agronomic practices for this region. Following harvest, collected F2 generation hybrid seed was air dried at ambient temperature, cleaned, and stored at 4°C prior to use. The seedlot was subjected to standard tests for viability and predisposition to secondary seed dormancy (Weber et al. 2010).

4.3.2.2 Soil Collection and Preparation. In autumn 2015, topsoil (0-15 cm) was collected from nine different fields in southern Manitoba (Table 4.1), dried at ambient temperature, and sieved (1 cm mesh). For each soil textural category used in the experiment (loamy sand, sandy loam and clay), three soils were collected from separate fields within similar spatial proximity to obtain soils with similar texture, yet varying cropping histories and soil properties (Table 4.1). Soil physical [organic matter (%; OM; loss on ignition method) and mineral particle density (% sand/silt/clay; hydrometer method)] and chemical [pH (1:1 soil:water method), calcium carbonate (% CCE; modified Williams method using a pressure transducer), soluble salts (mmhos cm^{-1}; 1:1 soil:water method), cation exchange capacity (meq; CEC; summation method) and macronutrients (nitrate-nitrogen (kg ha^{-1}; N; 0.2 M KCl extraction method determined using Cd reduction), Olsen-phosphorus (ppm; P; Olsen P method), potassium (ppm; K; $NH_4CH_3CO_2$ extraction method with cations determined using inductively coupled plasma))] properties were evaluated prior to study initiation (Table 4.1) (NCR-13 1998) (Agvise Laboratories Inc., Northwood, ND, USA).

Table 4.1. Collection location, preceding crop and soil characteristics of the nine soils used in analyses of canola seed survival in the soil seedbank. [a]

Soil ID	Collection location		Preceding crop [b]	Soil texture [c]	Mineral particles			OM	BD [d,e,f]		pH	CEC	Soluble salts	Carbonate	Macronutrients		
	latitude	longitude			Sand	Silt	Clay		CMN	WPG					N	P	K
	N	W			%				g cm^{-3}			meq	mmhos cm^{-1}	% CCE	kg ha^{-1}	ppm	
1	49°55’53”	99°23’13”	Wheat	LS	86	10	4	2.1	1.31 a	1.43 ab	5.5	7.4	0.07	0.1	6	26	166
2	49°55’54”	99°23’31”	Potato	LS	80	13	7	2.5	1.32 a	1.50 a	5.3	8.9	0.09	0.1	9	34	229
3	49°55’52”	99°23’44”	Canola	LS	82	10	8	2.4	1.36 a	1.48 a	5.0	7.4	0.09	0.1	13	36	270
4	49°29’53”	98°02’26”	Fallow	SL	76	14	10	3.0	1.24 b	1.43 ab	5.6	11.4	0.15	0.0	20	25	230
5	49°29’44”	98°02’47”	Soybean	SL	66	20	14	4.4	1.13 c	1.34 b	6.2	14.8	0.15	0.0	7	16	262
6	49°29’45”	98°02’33”	Wheat	SL	60	20	20	5.0	1.17 c	1.37 b	5.5	15.1	0.33	0.3	73	14	281
7	49°48’45”	97°07’18”	Oats	CL[g]	38	24	38	5.7	0.99 d	1.15 c	6.3	25.7	0.46	0.2	40	31	442
8	49°38’30”	97°08’26”	Wheat	C	23	24	53	6.2	0.91 e	1.05 d	7.0	35.2	0.68	0.7	19	30	652
9	49°48’05”	97°10’09”	Fallow	C	26	19	55	6.1	0.97 de	1.18 c	7.6	37.8	0.57	1.6	24	45	651

[a] Abbreviations: OM, organic matter; BD, bulk density; CEC, cation exchange capacity; CCE, calcium carbonate equivalents; N, nitrate-nitrogen; P, Olsen-phosphorus; K, potassium.

[b] Latin names: wheat (*Triticum aestivum* L.); potato (*Solanum tuberosum* L.); canola (*Brassica napus* L.); soybean [*Glycine max* (L.) Merr.]; oats (*Avena sativa* L.)

[c] Abbreviations: LS, loamy sand; SL, sandy loam; CL, clay loam; C, clay.

[d] Measured in September 2016 at each location.

[e] Abbreviations: CMN, Carman; WPG, Winnipeg.

[f] Within columns, different letters indicate significant differences based on Tukey’s HSD ($\alpha = 0.05$).

[g] Indicates that the soil texture was grouped with clay in all analyses.

4.3.2.3 Mesocosm Preparation and Burial. Seed burial mesocosms were developed using 10 x 10 x 12 cm (length x width x height) plastic pots (Kord Products Inc., Brampton, ON, CA) with bottoms removed and replaced with a 1 mm fiberglass mesh (Gulden et al. 2004b). The mesh was added to facilitate rapid drainage, exclude seed predators and maintain adequate isolation of test soils from that of the native soil at each location. Each mesocosm was filled with one of the dry sieved test soils, into which 150 canola seeds (adjusted for viability) were buried at a depth of 10 cm. The test soils were allowed to settle in each mesocosm prior to burial in the field. Settling of test soils would have had minimal influence on seed burial depth because the buried seed was located 2 cm above the bottom of each mesocosm. Immediately prior to mesocosm burial in the field, mesocosms were filled completely with the respective test soils and buried level with the soil surface (to a depth of 12 cm) at each location. Seed burial mesocosms were retrieved five (Apr 2016; retrieval date 1) or ten (Sep 2016; retrieval date 2) months after the initial burial in November 2015.

4.3.3 Data Collection.

4.3.3.1 Seed Survival. Following mesocosm retrieval, the remaining viable seeds in the soil seedbank were quantified following the methods of Gulden et al. (2004b). In brief, the top 7 cm of soil were removed from each mesocosm immediately after recovery without disturbing the remaining soil. This left seed buried about 3 cm below the soil surface, which is within the recruitment range of this species (Gruber et al. 2010). Mesocosms were then placed in a growth chamber under alternating light and temperature (16:8 hr day/night, 22:18°C) regimes and watered daily for two weeks. Seedlings that emerged from each mesocosm were removed and considered the viable, germinable portion of the remaining canola seedbank. After two weeks in the growth chamber, remaining seeds in each mesocosm were elutriated (Gulden et al. 2004b; Wiles et al. 1996) from the soil and firm seeds were tested for viability using the forceps pressure test (e.g., Lutman et al. 2003). All elutriated viable seeds were considered the viable, ungerminable portion of the remaining seedbank. The absolute density of viable canola seeds in the soil seedbank (N_{sd}) at each time point was considered the sum of viable, germinable and ungerminable portions of seed remaining in the seedbank. The percentage of canola seeds that survived in the soil seedbank over winter ($\sigma_{\mathrm{s(w)}}$), over summer ($\sigma_{\mathrm{s(s)}}$), and over winter and summer combined ($\sigma_{\mathrm{s(ws)}}$) were determined using equations 1, 2 and 3, respectively. These equations followed the notation of Geddes and Gulden (Chapter 3), where $N_{\mathrm{sd(Nov\,15)}}$, $N_{\mathrm{sd(Apr\,16)}}$ and $N_{\mathrm{sd(Sep\,16)}}$ are the absolute densities of viable seed in the soil seedbank upon study initiation (Nov 2015), and on retrieval dates 1 (Apr 2016) and 2 (Sep 2016), respectively.

$$\sigma_{\mathrm{s(w)}} = \left(N_{\mathrm{sd(Apr\,16)}} \,/\, N_{\mathrm{sd(Nov\,15)}}\right) \text{x}\ 100 \qquad [1]$$

$$\sigma_{\mathrm{s(s)}} = \left(N_{\mathrm{sd(Sep\,16)}} \,/\, N_{\mathrm{sd(Apr\,16)}}\right) \text{x}\ 100 \qquad [2]$$

$$\sigma_{\mathrm{s(ws)}} = \left(N_{\mathrm{sd(Sep\,16)}} \,/\, N_{\mathrm{sd(Nov\,15)}}\right) \text{x}\ 100 \qquad [3]$$

4.3.3.2 Microclimate. Soil temperature (°C) and volumetric soil moisture ($\mathrm{m^3}$ water $\mathrm{m^{-3}}$ soil) were measured at a depth of 10 cm in each soil type. Temperature was measured using Thermochron DS1921G iButtons (Embedded Data Systems, LLC, Lawrenceburg, KU, USA) and volumetric soil moisture content was measured using EC5 soil moisture smart sensors attached to an H21-001 HOBO Weather Station data logger (Onset Computer Corporation, Bourne, MA, USA). The temperature and moisture sensors were placed in representative mesocosms of each soil at each location and remained undisturbed throughout the duration of the experiment. These sensors were read and the data were logged at two hours intervals for the duration of the experiment. Air temperature (°C) and precipitation (mm) for the duration of the experiment and the 30-year (1980-2010) climatic normal temperature and precipitation for each location were collected from the Government of Canada weather station nearest each location (Environment Canada 2017).

4.3.3.3 Bulk Density. In September 2016 (retrieval date 2), the bulk density of soil in each retrieved mesocosm was determined by measuring the weight and volume of soil in each mesocosm. These measurements were adjusted for soil moisture content using the gravimetric method. Gravimetric soil moisture was determined by weighing subsamples of moist soil from each experimental unit. Then, the soil subsamples were dried in an oven at 105°C until equilibrium. The weights of dry soils were determined and used to calculate the gravimetric soil moisture content of each soil. The gravimetric soil moisture content of each soil was used to determine the weight of dry soil per unit volume (bulk density) in each mesocosm.

4.3.4 Data Analysis.

4.3.4.1 Statistical Analysis. Univariate ANOVA ($\alpha = 0.05$) was conducted on the percentage of canola seeds that survived in each soil over winter ($\sigma_{s(w)}$), summer ($\sigma_{s(s)}$), and winter/summer combined ($\sigma_{s(ws)}$) using the MIXED procedure (Littell et al. 2006) in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). Extreme outliers were removed according to Lund's test (Lund 1975) and the square root-transformation was used to adjust for normality according to the Shapiro-Wilk test and/or homogeneity of variance according to visual inspection of residual vs. predicted values (Littell et al. 2006). In each analysis, soil and location were considered fixed effects, while experimental block nested within location was considered a random effect. To improve the model fit and further adjust for homoscedasticity, the covariance structure of residuals was adjusted by selecting the R matrix group effect based on minimization of Akaike's Information Criterion (Onofri et al. 2010).

To address the differences among soil textural classes, single-degree-freedom estimated mean differences (Littell et al. 2006) were used to test for lack of difference ($\alpha = 0.05$) among soils grouped into loamy sand (soils 1-3), sandy loam (soils 4-6) and clay (including the clay loam soil; soils 7-9) textural classes (Table 4.1). Due to the low number of hypotheses tested for each response variable (3), a correction for family-wise Type 1 error was not employed (Onofri et al. 2010).

4.3.4.2 Multivariate Analysis. Partial least squares (PLS) analysis was used to assess the contribution of edaphic factors to seed survival in the soil seedbank. Due to the collinear nature of these soil parameters, a method that was robust to collinearity among explanatory variables was needed. The PLS method is robust to collinearity and has the ability to analyze datasets that have a large number of explanatory variables relative to response variable data points (Sawatsky et al. 2015). A single PLS procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) was used to analyze the contribution of edaphic factors to the percentage of canola seeds that survived in the soil seedbank over winter ($\sigma_{s(w)}$) and over summer ($\sigma_{s(s)}$). The square root-transformation was used to adjust for normality and homogeneity of variance of response and explanatory variables prior to the analysis (Sawatsky et al. 2015). The original model contained transformed, centered and scaled data of 14 continuous explanatory variables, including those outlined in Table 4.1 in addition to the mean diurnal soil temperature range over winter and summer (the difference between daily maximum and minimum soil temperatures at 10 cm depth averaged over winter or summer periods). For each soil, the mean diurnal soil temperature range was used as a single measure of variation in soil temperature. This measure was chosen because soil texture has a greater effect on the magnitude of diurnal heating and cooling (thermal conductivity) of soils than on daily mean temperature (Brady and Weil 2007). A measurement of soil moisture was not included in the PLS model due to lack of a single metric of biological significance to an imbibing seed (i.e., soil water potential). The model was pruned iteratively to contain only variables that contributed to explaining variation in seed survival based on the variable importance values and loading coefficients (Sawatsky et al. 2015; Wold 1975). To avoid under- or over-fitting the model, split-sample cross-validation was used to determine the optimal number of extracted factors based on the absolute minimum predicted residual sum of squares (PRESS) and van der Voet's statistics (Sawatsky et al. 2015; van der Voet 1994). Using these statistics, consecutive iterations of the model were compared to their predecessor to ensure that the integrity of the extracted factors was not compromised (Sawatsky et al. 2015; Wold 1975).

4.4 Results

4.4.1 Soil Texture. In both experimental locations, fine textured soils (clays) resulted in greater survival of canola seed over winter (seed survival over winter averaged 15.1% in clays and 5.9% in loamy sands) but reduced survival over summer compared with coarse textured soils (loamy sands) (seed survival over summer averaged 3.9% in clays and 14.7% in loamy sands) (Table 4.2). Medium textured soils (sandy loams) resulted in intermediate seed survival over each time period (Table 4.2). The contrasting seed survival among winter and summer time periods resulted in lack of differences in seed survival among soil textural classes over the duration of the study (winter/summer combined) (Table 4.2). Differences in canola seed survival among soils within each textural class also were detected (Fig 4.1) and were consistent in both experimental locations (Table 4.3). These differences among soils were likely related to variation in edaphic factors unique to each individual soil (Table 4.1; Fig 4.2).

4.4.2 Edaphic Factors. The reduced PLS model contained 8 of the original 14 explanatory variables which contributed to variation in canola seed survival over winter and/or summer, including: percent soil mineral particle densities of sand, silt and clay, BD, OM, CEC, soluble salts and the mean diurnal soil temperature range over winter (Fig 4.2). Soil macronutrient supply (N, P and K), pH, carbonates and the mean diurnal soil temperature range over summer were not associated with canola seed survival over winter or over summer and were therefore removed from the PLS model. Two factors were extracted which when combined explained a total of 95% of the variability in explanatory (model effects) variables and 23% of the variability in response variables (seed survival) (Table B1). Factor 1 explained 79% of the variability in explanatory variables (Table B1) and was positively loaded by silt (0.354), clay (0.386), OM (0.382) and CEC (0.385) and negatively loaded by sand (-0.385) and BD (-0.367), all with similar loading coefficients (Table B2). This factor explained 15% of the variability in the response variables (Table B1) and was positively weighted on canola seed survival over winter (0.857) and negatively weighted on seed survival over summer (-0.515) (Table B3). Factor 2 explained an additional 15% of the variability in explanatory variables (Table B1) and was positively loaded by the mean diurnal soil temperature range over the winter (0.891), and negatively loaded by BD (-0.291) (Table B2). This factor explained an additional 8% of the

Table 4.2. Single-degree-freedom estimated mean differences between the percentages of volunteer canola seed that survived in the soil seedbank in soils grouped within each textural class in a combined analysis among locations. Estimated mean differences for all soil texture comparisons are shown for each time period individually [over winter (Nov-Apr), over summer (Apr-Sep) and over winter and summer combined (Nov-Sep)].

Seed survival period	Soil texture comparison[a]	Estimated mean difference[b]	Level of significance[c,d]
		%	*P*-values
Winter	C vs. LS	9.1 (1.5 ± 0.32)	**< 0.001**
|[e]	C vs. SL	4.9 (0.7 ± 0.32)	**0.027**
|	SL vs. LS	4.2 (0.8 ± 0.32)	**0.018**
Summer	C vs. LS	-10.8 (-2.0 ± 0.86)	**0.028**
|	C vs. SL	-5.8 (-1.3 ± 0.65)	ns
|	SL vs. LS	-5.0 (-0.7 ± 0.89)	ns
Winter/summer combined	C vs. LS	-0.4 (-0.3 ± 0.14)	ns
|	C vs. SL	-0.2 (-0.2 ± 0.14)	ns
|	SL vs. LS	-0.2 (-0.1 ± 0.14)	ns

[a] Abbreviations: LS, loamy sand; SL, sandy loam; C, clay.

[b] Values are approximate back-transformed square root estimated mean differences, while parenthetical values are square root-transformed mean estimated differences ± one standard error of the square root-transformed mean difference.

[c] Bold values indicate significant effects at $P < 0.05$.

[d] ns indicates lack of a significant effect at $P \geq 0.05$.

[e] A bar (|) indicates continuation of the entry above.

Table 4.3. ANOVA results (*P*-values) for the main and interaction effects of soil and location on the percentage of volunteer canola seed that survived at 10 cm depth in the soil seedbank over winter (Nov-Apr), over summer (Apr-Sep) and over winter and summer combined (Nov-Sep) following seed burial in autumn.

	Seed survival period[a,b]		
Source	Winter	Summer	Combined[c]
		P-values	
Soil	**< 0.001**	**0.011**	**0.049**
Location	**0.002**	ns	ns
Soil * location	ns	ns	ns

[a] Bold values indicate significant effects at $P < 0.05$.

[b] ns indicates lack of a significant effect at $P \geq 0.05$.

[c] Indicates winter and summer seasons combined.

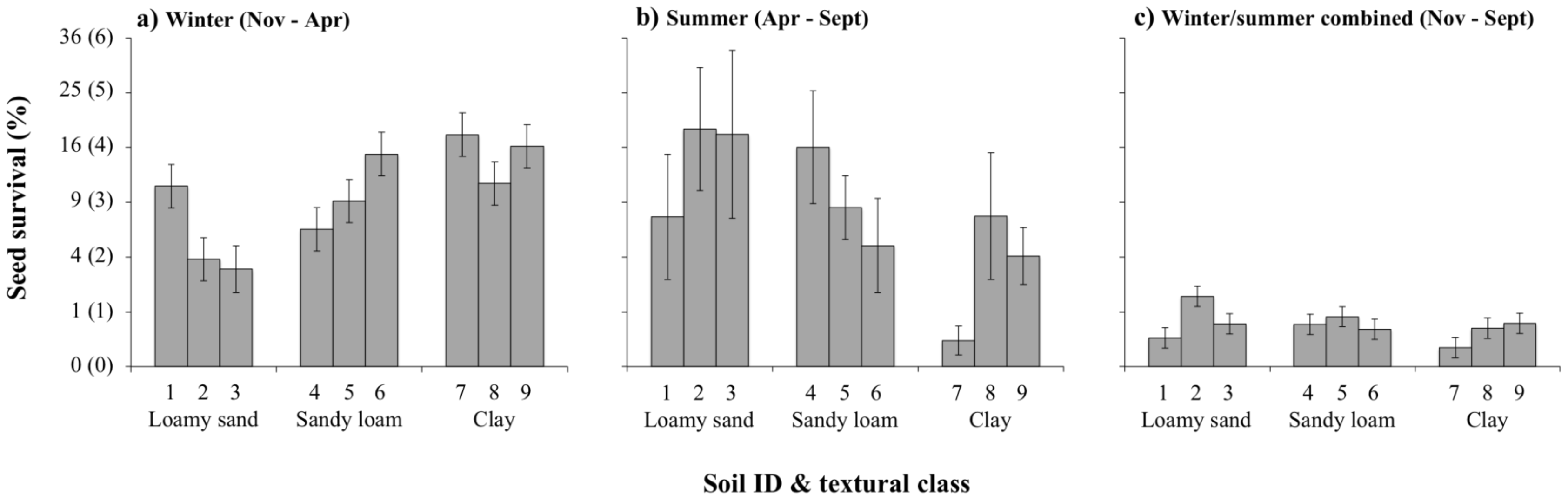

Figure 4.1. The mean percentage of volunteer canola seeds that survived at 10 cm depth in the soil seedbank in nine different soils following seed burial in autumn and organized by textural class. Each sub-figure depicts the percentages of seed that survived in each soil in separate analyses over (a) winter (Nov-Apr), (b) summer (Apr-Sep) and (c) winter/summer combined (Nov-Sep) time periods that were combined among locations. Values on the y-axis are the back-transformed square root scale with the square root-transformed scale in parentheses. Error bars indicate ± one standard error of the square root-transformed means. Within sub-figures, soils on the x-axis are arranged in order from least to greatest clay particle content.

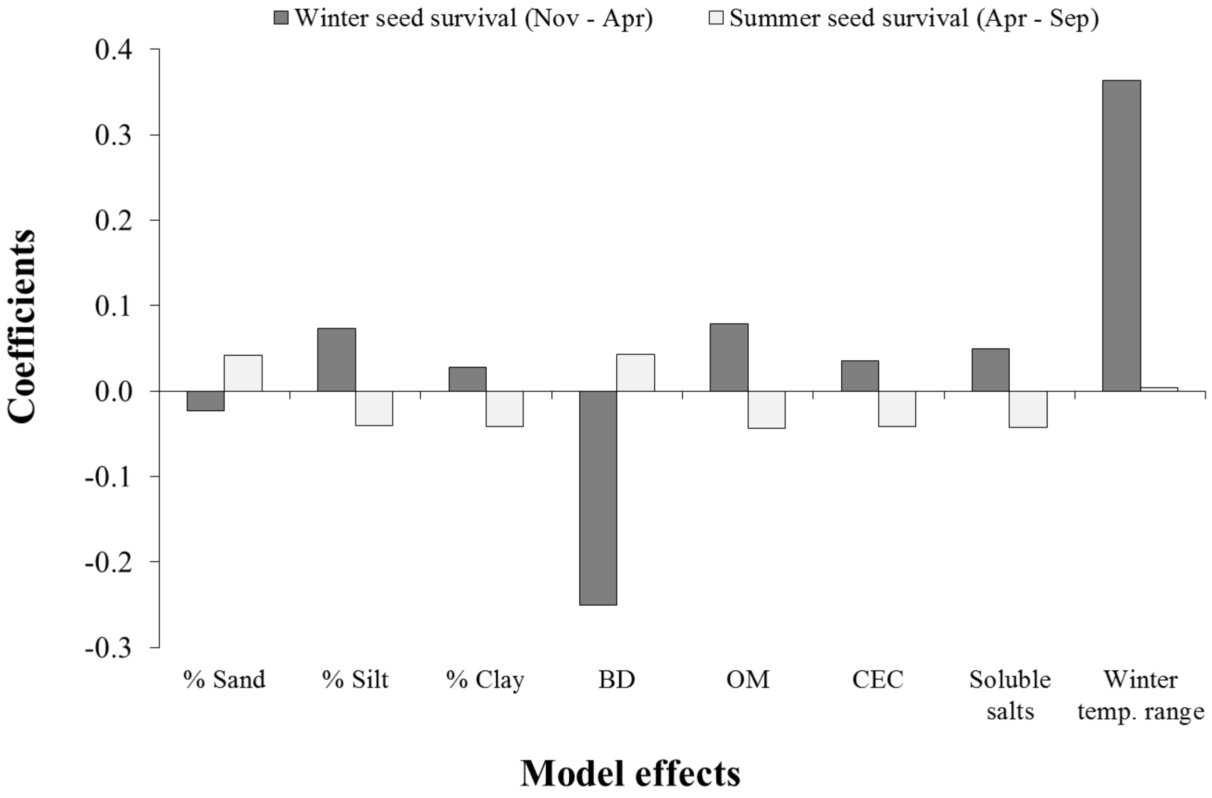

Figure 4.2. Parameter estimates of explanatory variables contributing to volunteer canola seed survival in the soil seedbank using centered and scaled data in partial least squares analysis. The coefficients describe the strength of association of edaphic factors (model effects) with volunteer canola seed survival over winter and over summer at the Carman and Winnipeg locations combined. Abbreviations: BD, bulk density; OM, organic matter; CEC, cation exchange capacity; Winter temp. range, the mean diurnal soil temperature range at 10 cm depth averaged over the winter time period.

variability in response variables (Table B1) and was almost entirely weighted on canola seed survival over winter (0.997) (Table B3).

4.4.2.2 Soil Properties. The parameter estimates for centered and scaled data from the PLS analysis revealed that the edaphic factors either directly or indirectly related to soil physical characteristics, were associated oppositely with seed survival over winter and over summer (with the exception of the mean diurnal soil temperature range over winter) (Fig 4.2). Weighting of factor 1 was in the opposite direction for winter and summer seed survival (Table B3). This factor was defined by soil physical properties and other edaphic factors related to soil texture (Table B2). The PLS analysis revealed that canola seed survival over winter was strongly positively associated with the mean diurnal soil temperature range over winter and strongly negatively associated with soil BD (Fig 4.2; see Table 4.1 for the range of BD among soils). The associations between canola seed survival over summer and all edaphic factors included in the model were similar in magnitude, with the exception of an absence of association with mean diurnal soil temperature range over winter (Fig 4.2).

4.4.2.1 Microclimate. Following mesocosm burial, soils remained frozen for about three to four months prior to spring thaw (Fig 4.3). Mesocosm retrieval date 1 separated mean daily soil temperature at 10 cm depth into consistently lower (about -5 to 5°C) and consistently higher (about 10 to 20°C) temperature periods (Fig 4.3). Overall, mean daily soil temperatures did not vary greatly among the soils tested (Fig 4.3). Despite large variation in mean daily air temperature over winter, mean daily soil temperature at 10 cm depth did not appear to vary any more among days in winter than in summer (Fig 4.3). In fact, the standard deviation of mean daily soil temperatures at 10 cm depth was 2.4°C among days in winter and 2.9°C among days in summer when averaged among soils (data not shown). The diurnal soil temperature range was greatest in the spring (Apr-Jun), where the largest variation in diurnal soil temperature among days was observed as well (Fig 4.4). Although a strong positive correlation with canola seed survival over winter was found (Fig 4.2), the diurnal soil temperature range over the winter months (Dec-Mar) was minor relative to the spring (Apr-Jun) or summer (Jul-Sep) time periods (Fig 4.4).

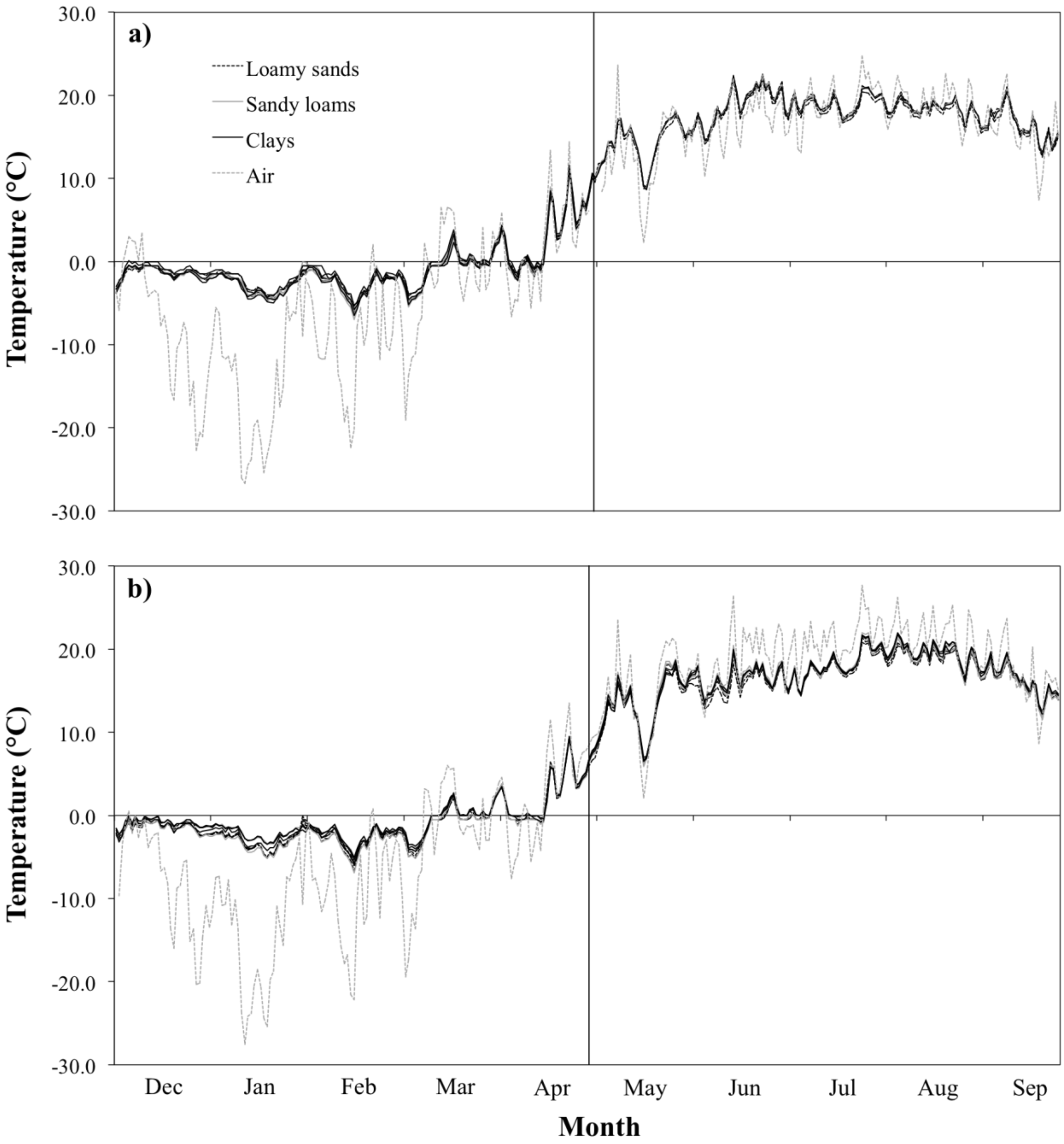

Figure 4.3. Mean daily air temperature (°C) and soil temperature (°C) at 10 cm depth in the loamy sand (soils 1-3), sandy loam (soils 4-6) and clay (soils 7-9) soils from December 2015 (shortly after study initiation) to September 2016 (retrieval date 2) at the (a) Carman and (b) Winnipeg locations. Discontinuity in lines indicates missing data. The vertical dissecting line indicates retrieval date 1.

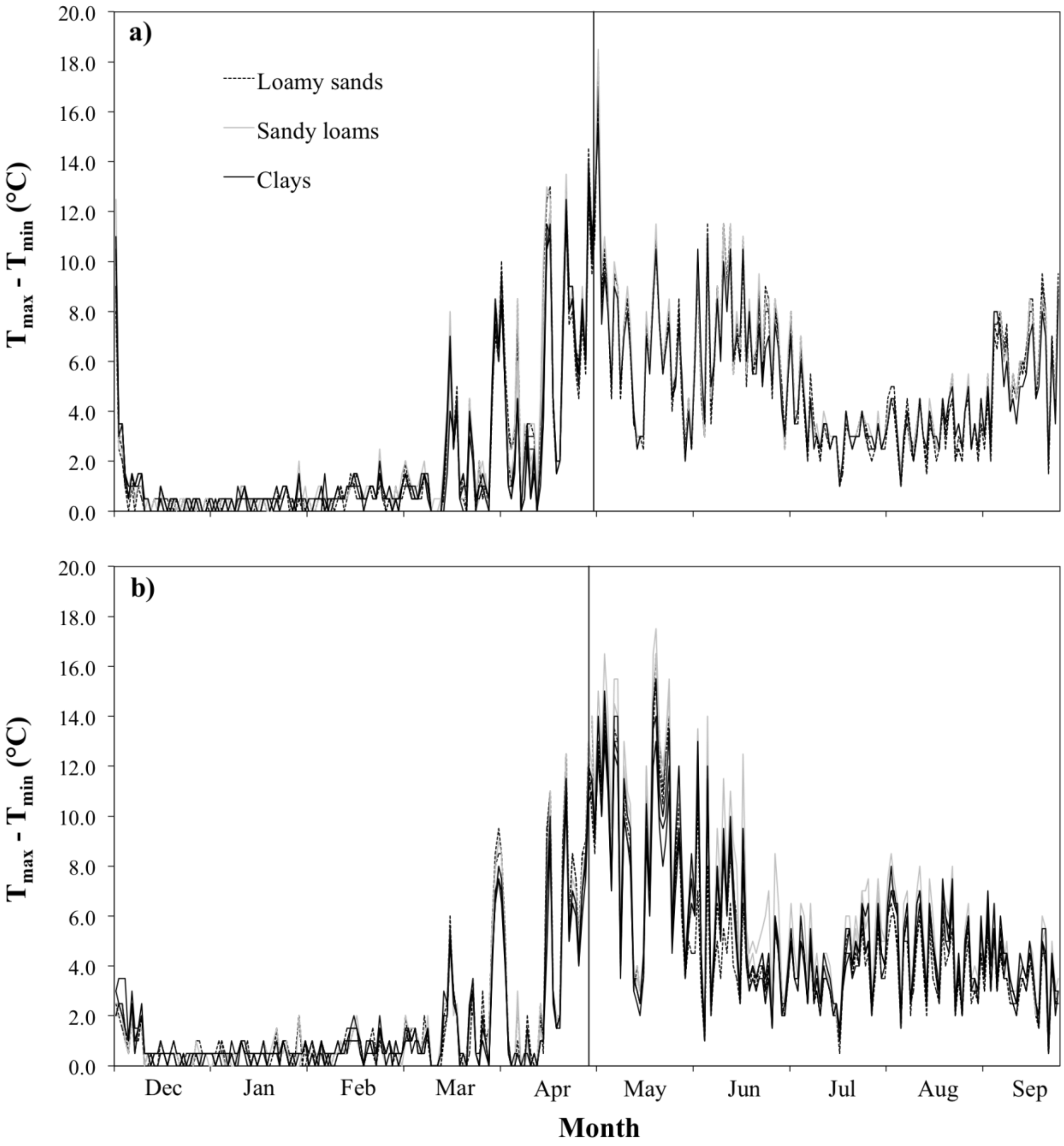

Figure 4.4. The mean range in diurnal soil temperature (°C) at 10 cm depth in the loamy sand (soils 1-3), sandy loam (soils 4-6) and clay (soils 7-9) soils from December 2015 (shortly after study initiation) to September 2016 (retrieval date 2) at the (a) Carman and (b) Winnipeg locations. Discontinuity in lines indicates missing data. The vertical dissecting line indicates retrieval date 1.

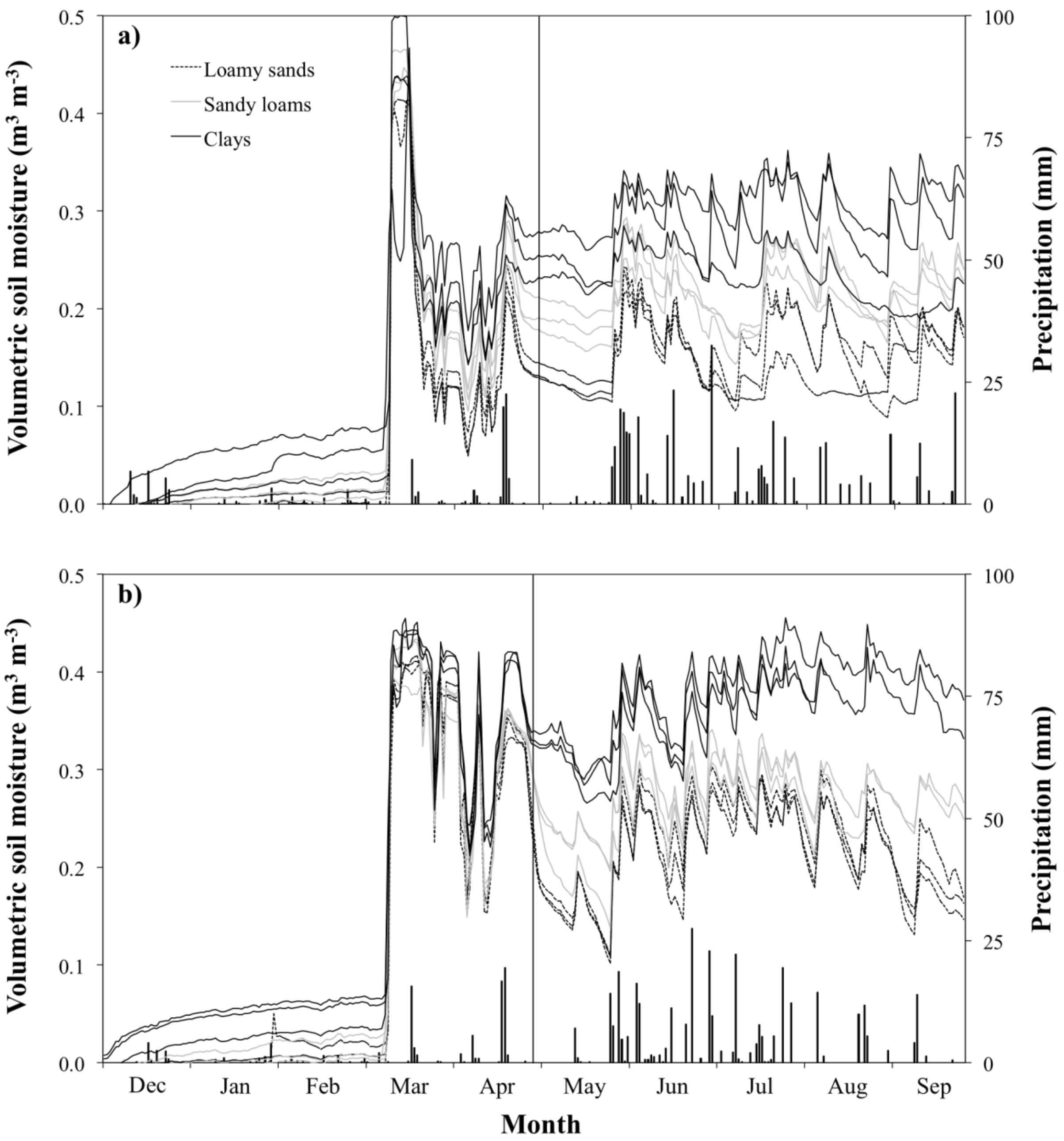

Figure 4.5. Daily precipitation (black bars; mm) and mean daily volumetric soil moisture (m^3 water m^{-3} soil) at 10 cm depth in the loamy sand (soils 1-3), sandy loam (soils 4-6) and clay (soils 7-9) soils from December 2015 (shortly following study initiation) to September 2016 (retrieval date 2) at the (a) Carman and (b) Winnipeg locations. Discontinuity in lines indicates missing data. The vertical dissecting line indicates retrieval date 1. Precipitation data adapted from: Environment Canada (2017).

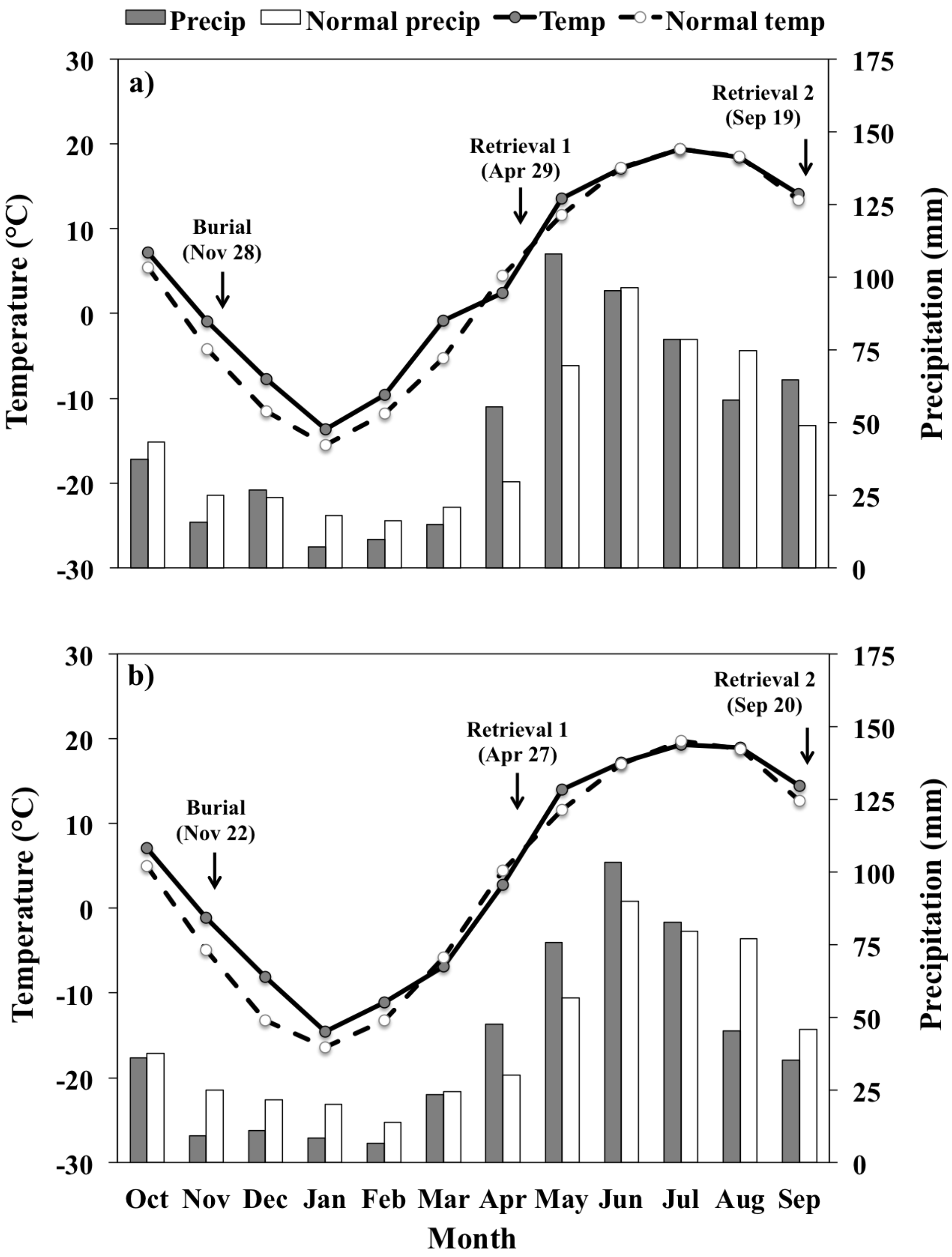

Figure 4.6. Mean daily air temperature (Temp; °C) and total precipitation (Precip; mm) for each month of the experiment and the regional climatic normal (1980-2010) mean daily air temperature (Normal temp) and total precipitation (Normal precip) for each month at the (a) Carman and (b) Winnipeg locations. Mesocosm burial and retrieval dates are indicated for each location. The x-axis spans October 2015 to September 2016. Adapted from: Environment Canada (2017).

Soil moisture at 10 cm depth tended to be grouped by soil texture, where volumetric moisture content went from least to greatest in loamy sand, sandy loam, and clay textural classes, respectively (Fig 4.5). Soils thawed near the end of the first week of March and both locations experienced a period of minimal precipitation for about one month following retrieval date 1 (Fig 4.5). Throughout the duration of the experiment, coarse textured soils (loamy sands) were observed to dry more rapidly than medium (sandy loams) or fine (clays) textured soils (Fig 4.5).

4.4.3 Experimental Locations. The magnitude of canola seeds that survived in the soil seedbank over winter ($\sigma_{s(w)}$) was about three times greater among soils at the Carman location than at Winnipeg (15.1% and 5.5% seed survival over winter in Carman and Winnipeg, respectively) (Fig B1). Seed survival over summer, however, was consistent among locations (Table 4.3). The Carman location received (slightly) greater snowfall in December compared with Winnipeg (Figs 4.5 and 4.6). Due to soil freeze (Fig 4.5), differences in volumetric soil moisture content between locations were observed to be minimal over the winter months (Dec-Feb). After soil thaw, volumetric soil moisture content among soils peaked at a greater initial level at Carman (reaching about 0.5 m^3 water m^{-3} soil) than at Winnipeg (reaching about 0.45 m^3 water m^{-3} soil), but soils dried quickly following the initial snowmelt at Carman compared with a more gradual decline in soil moisture over spring at Winnipeg (Fig 4.5).

Overall, winter air temperatures tended to be mild and precipitation tended to be low compared with the 30-year climatic normal for each location (Fig 4.6). A greater amount of precipitation than normal was received in the spring months at each location prior to and after retrieval date 1 (Fig 4.6). This precipitation was either received in mid-April or late-May (Fig 4.5). This resulted in a period of greater than one month with little precipitation, which may have caused soils to dry from mid-April to late-May (Fig 4.5). Temperatures in the summer period, following retrieval date 1, closely followed that of the climactic normal (Fig 4.6).

4.5 Discussion

The current study revealed that fine textured soils resulted in greater canola seed survival in the soil seedbank over winter, but lower seed survival over summer compared with coarse textured soils. Despite the hybrid seedlot being classified as having a generally low predisposition to secondary seed dormancy [5% (± 1.0%)], differences in seed survival among soil textures over winter and summer were obvious (Table 4.2). A low dormancy seedlot was chosen for this research because secondary seed dormancy in canola is related to seedbank persistence (Gulden et al. 2003b). Using a low dormancy seedlot was expected to provide rapid insight into the effects of soil texture on seed mortality. The results observed over the winter period agree with a previous study from Germany (Gruber et al. 2014), where comparison of multiple seed accessions in three different soils showed greater WA canola seed survival in a clay soil and silty clay loam soil, compared with a sandy loam soil after a six month burial period over winter. In the current study, reduced canola seed survival in fine textured soils over summer caused a lack of difference in overall seed survival among textural classes after ten months of burial. The opposite influence of soil texture on seed survival over winter and over summer may have been due to an interaction of soil physical properties with prevalent climatic conditions during these seasons. Even though a certain proportion of canola seed remained in each soil after ten months of burial (0.12% to 1.64% of the original seedbank among soils), it is possible that the lower number but different proportion of seeds remaining in each soil may have contributed to the opposing contribution of soil texture to canola seed survival observed over each time period.

A regression tree analysis among landscapes in Germany (Gruber et al. 2012; Thöle et al. 2011) found that volunteer WA canola plant densities in cropped fields were grouped by soil climatic region. After ten months of burial in the current common garden study, however, seed survival did not differ among a wide range of soil textures when the effects of confounding environmental conditions were excluded. Taken together, these discoveries suggest that regionally specific environmental conditions, rather than soil textures, largely govern absolute canola seed survival in the soil seedbank. However, regionally specific environmental conditions would conceivably be collinear with soil characteristics as they are a driving factor in soil formation. Other field studies conducted at multiple locations have shown a trend of greater canola seed survival in fine textured soils (Gulden et al. 2003b, 2004b; Lutman et al. 2003, 2005), although under certain conditions – which remain unclear – canola seeds also have survived longer in sandy soil (Chapter 3; Pekrun et al. 1998a). Thus, weather conditions divergent from climatic normals may be a significant contributor to the lack of consistency in observed trends among canola seed survival and soil texture in previous literature.

Soil texture has had contrasting effects on seedbank dynamics of other species (Benvenuti 2003; Narwal et al. 2008). Burial depth-mediated inhibition of germination in jimsonweed (*Datura stramonium* L.) was directly proportional to soil clay particle fraction (Benvenuti 2003). Soils with greater clay particle fraction were more prone to the development of a persistent jimsonweed seedbank (Benvenuti 2003). In contrast, dormancy of annual ryegrass (*Lolium rigidum* Gaudin) seed at 4 and 8 months after burial was greater in a sandy loam soil compared with a clay soil (Narwal et al. 2008). Viability of annual ryegrass seed also was greater and seed morality was reduced at 4 and 8 months after burial in the sandy loam soil compared with the clay soil. Thus, the influence of soil texture on seedbank dynamics is not consistent among species and is likely related to species-specific dormancy characteristics and germination requirements.

The edaphic factors shown to be associated with canola seed survival among soils were primarily physical in nature and consisted of sand, silt and clay mineral particle fractions, as well as BD and OM. Cation exchange capacity, soluble salt concentration, and the rate of heating and cooling of soil are closely associated with soil texture also (Brady and Weil 2007). It is therefore likely that the physical properties of soils primarily govern the short-term relationship between soil and survival of canola seed in the soil seedbank. Perhaps this relationship is governed through interaction with environmental conditions resulting in differences in soil moisture, temperature and aeration among soil textures (Brady and Weil 2007).

Warm temperatures over spring/summer, in combination with dry conditions for four weeks in early spring and darkness caused by seed burial, could have contributed to the induction of secondary dormancy in canola seed at the beginning of the summer burial period (Gulden et al. 2004a; Lopez-Granados and Lutman 1998; Momoh et al. 2002; Pekrun et al. 1997a, 1998a; Schlink 1994). Even though fine textured soils had increased volumetric soil moisture content (Fig 4.5), they likely had lower soil water potential than coarse textured soils due to the effect of soil particle and pore size on soil water retention (Brady and Weil 2007). Furthermore, fine textured soils also would have had lower oxygen concentrations under wet soil conditions due to reduced rates of gas exchange with the soil surface. Since dry soil conditions (e.g., Gulden et al. 2004a; Lopez Granados and Lutman 1998; Momoh et al. 2002; Pekrun et al. 1998a) and/or (to a lesser extent) low oxygen concentrations (e.g., Boyd and Van Acker 2004b; Pekrun et al. 1997a) in combination with darkness contribute to secondary dormancy induction in canola seed, and secondary dormancy is related to seedbank persistence in this species (Gulden et al. 2003b), canola seeds would be expected to persist for longer in fine compared with coarse textured soils under both wet and dry conditions. These predictions contrast the observation of greater canola seed survival over summer in coarse textured soils in the current study. Perhaps, greater fluctuation in soil temperatures during the spring/summer period mitigated induction of secondary dormancy in canola seed (Pekrun et al. 1998a; Weber et al. 2010). These hypotheses, however, are strictly speculative as it is not appropriate to compare volumetric moisture content among soil textures when determined using capacitance probes (Evett et al. 2002).

The more likely explanation for reduced survival of canola seed in fine textured soils over summer would be a greater propensity for microbial seed decay due to greater microbial activity. As microbial activity (generally) is associated positively with soil moisture, it is plausible that fine textured soils would have resulted in greater microbial-mediated seed decay over the summer time period (Schafer and Kotanen 2003; Schutte et al. 2008; Wagner and Mitschunas 2008). Mickelson and Grey (2006) reported that wild oat (*Avena fatua* L.) seed mortality over summer increased linearly with increased soil moisture content, while seed mortality over winter remained unaffected. These results correspond directly with the discovery of increased canola seed mortality over summer in fine textured soils.

Survival of canola seed over the winter period was about three times greater at the Carman location than at Winnipeg (Fig B1). Despite the difference in overall magnitude of the canola seedbank that survived at each location, the effects of different soils on seedbank persistence were consistent at each site (Table 4.3). Greater snowfall was received at the Carman location in the month of December compared with the Winnipeg location (Fig 4.6) and this could have contributed to greater persistence of the canola seedbank at Carman. Under winter conditions similar to those experienced in Canada (Alaska, United States), about double the amount of canola (*Brassica rapa* L.; formerly *Brassica campesitris* L.) seed survived under snow cover compared to the absence of snow (Sparrow et al. 1990).

It is possible that differences in cropping history may have influenced canola seed survival in the different soils tested in the current study. Cropping history can influence seed mortality in the soil seedbank (Davis et al. 2006), and an association of cropping history and seedbank community composition has been noted also (Davis et al. 2005b; Gulden et al. 2011). Despite different preceding crops at the time of soil collection, however, clear differences in canola seed survival over winter and summer were evident among the soil textural classes.

In correspondence with previous studies (Gruber et al. 2010; Gulden et al. 2004b), burial of canola seed at 10 cm depth prevented seedling recruitment. Lack of seed survival in the current study would therefore have been imposed by an inability to resist seedbank processes – such as aging, predation, microbial decay or lethal germination – that ultimately lead to seed demise. Yet, seed aging is not influenced by soil texture when the confounding effects of moisture potential and temperature are controlled (Long et al. 2009). The results of Long et al. (2009)

suggest that it is these factors related to soil texture that influence seed fate and not soil texture alone. The influence of microbial seed decay, seed predation or lethal germination in different soils under similar controlled temperature and moisture conditions, however, remains uncertain.

4.6 Conclusion

In this common garden study, soil texture and associated physical edaphic factors clearly influenced survival of canola seed in the soil seedbank over one season when the potential confounding environmental effects across landscapes were excluded. Fine textured soils resulted in greater seedbank survival of canola seed over winter, but lower seedbank survival over summer compared with coarse textured soils. A lack of difference in survival of canola seed among soil textures after ten months of burial suggested that regionally-specific environmental conditions, rather than soil textures, primarily govern absolute survival of canola seed in the soil seedbank over timescales longer than a single season. This study was the first to directly compare canola seed survival in multiple soils within each textural class, evaluate the association of canola seed survival with edaphic factors other than soil texture, and quantify canola seed survival over summer in different textured soils in the same environment. In the future, growers may be able to predict the risk and density of volunteer canola populations in the spring subsequent to canola production and determine what level of management is warranted. These results may be used in forecasting models to better calculate the density of volunteer canola populations among environments. The utilization of this information in demographic models may be beneficial to assess the potential for HR gene transfer via coexistence of genetically-engineered canola with that of conventional canola or canola genotypes with different HR systems. Though many questions remain regarding the impact of *in situ* environmental factors on ecophysiological responses in canola, understanding the association of different soil textures with canola seed survival may help elucidate potential opportunities for preventative seedbank management of this persistent weed species.

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5.0 Candidate Tools for Integrated Weed Management in Soybean (*Glycine max*) at the Northern Frontier of Production

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5.1 Abstract

Large canola harvest losses and seedbank persistence contribute to volunteer canola populations that can persist throughout short crop rotations in western Canada. Canola and soybean have similar genetically-engineered herbicide-resistance (HR) traits. When both of these crops are grown in a crop rotation, HR volunteer canola can be difficult to manage in HR soybean using herbicides alone. In 2013 and 2014, four experiments were conducted to identify candidate non-chemical weed management tools to facilitate management of volunteer canola in soybean in the short growing-season environment of Manitoba, Canada. Soybean row spacing (19 vs. 38 vs. 76 cm), seeding density (455,000 vs. 682,500 seeds ha^{-1}), nitrogen (N) fertilization (residual vs. residual + 23 kg N ha^{-1}) and inter-row tillage (presence vs. absence) were evaluated as potential candidate weed management tools. Volunteer canola was a competitive weed in soybean and produced an average of 20,900 and 42,100 seeds m^{-2} among management treatments at sites with volunteer canola densities of 39 and 89 plants m^{-2}, respectively. This led to volunteer canola seedbank inputs of about 3 to 6 times the regional average seedbank inputs that are associated with harvest losses when growing a canola crop. The direction of effects of weed management tools were consistent among sites with higher and lower volunteer canola densities. However, these effects generally did not manifest into significant differences at sites with lower volunteer canola densities. Overall, an elevated seeding density (1.5 times recommended) resulted in greater soybean yield (44% greater compared with the recommended seeding density) at sites with higher volunteer canola densities, but did not affect volunteer canola seed production or

biomass accumulation. Also at sites with higher volunteer canola densities, N fertilization increased canola seed production (77% greater), aboveground biomass (69% greater), and seedbank inputs as a percentage of total volunteer canola seed production (about double) compared with residual N levels. Inter-row tillage in wide-row soybean decreased volunteer canola plant survival (by about half at sites with higher canola densities and a similar trend was observed at lower density sites) and increased volunteer canola seed weight (by 8% to 11%), but not the number of seeds produced compared with the absence of inter-row tillage. Furthermore, inter-row tillage resulted in greater soybean yield (36% greater) and biomass (55% greater) at sites with higher volunteer canola densities compared with the absence of inter-row tillage. Seeding soybean at elevated seeding densities in fields with limited soil mineral N, forgoing N fertilization in soybean, or using inter-row tillage in wide-row production systems may be effective tools for inclusion in an integrated program for management of volunteer canola in soybean at the northern frontier of North American soybean production.

5.2 Introduction

Western Canada is located at the northern frontier of the North American soybean [*Glycine max* (L.) Merr.] growing-region, where the longest frost-free period generally ranges between 110 to 130 days (Nadler 2007). The development of soybean varieties with reduced time to maturity has lessened soybean production risks in short-season growing environments. These varieties have facilitated a rapid increase in soybean production in western Canada in the last half decade (Statistics Canada 2017). In Manitoba alone, seeded hectarage of soybean has almost tripled during this timeframe (Statistics Canada 2017), making soybean now the third most grown crop by hectarage following canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) (MMPP

2016). Volunteer canola, originating from large canola seed losses at harvest (Gulden et al. 2003a; Cavalieri et al. 2016), survives in a persistent seedbank (Gulden et al. 2004b) that could span short crop rotations in western Canada (Gulden et al. 2003b). Genetically-engineered herbicide-resistance (HR) in both soybean and canola makes post-emergence (POST) herbicide management of volunteer canola particularly difficult in soybean. Growers in this region have expressed concern over populations of glyphosate-resistant (GR) volunteer canola found in GR soybean fields that have never before been seeded to GR canola (CM Geddes, personal observation). This could potentially be caused by adventitious presence of unsolicited HR traits in pedigreed canola seedlots (Friesen et al. 2003), or pollen-mediated-gene flow in canola resulting in multiple-HR gene stacking (Beckie et al. 2003; Hall et al. 2000). In either case, the effectiveness of alternating HR systems between these two crops in rotation may be limited.

Until recently, GR canola was the only major GR crop grown in western Canada (Beckie et al. 2006). Crop rotations in this area predominantly focused on rotation among HR canola and non-HR cereals, wheat and barley (*Hordeum vulgare* L.) (Beckie et al. 2006). In addition, the availability of glufosinate- or imidazolinone-resistant canola varieties further allowed for diversification of herbicide modes-of-action (MOA) in canola production systems (Powles 2008); and may therefore be used to prolong the duration between GR canola production. The rotational herbicide diversity among major crops grown in this region likely has contributed to slowed development of GR weed species. For this reason, herbicide rotation in western Canada has remained an example of relatively sustainable utilization of the GR system for other parts of the world (Powles 2008). However, since the recent increase in GR soybean production in Manitoba, populations of GR kochia [*Kochia scoparia* (L.) Schrad.] also have been found in fields of GR soybean and corn (*Zea mays* L.) (Beckie et al. 2015).

Integrated weed management (IWM) is a fundamental component to managing HR weeds effectively (e.g., Liebman and Gallandt 1997; Owen et al. 2015; Vencill et al. 2012). The utilization of IWM in soybean production may help increase the competitive ability of this crop and therefore mitigate the development and spread of GR weeds in western Canada. One IWM tool used to hinder HR weeds is to limit the number of weed seeds returned to the soil seedbank (Norsworthy et al. 2012). Reduced seedbank inputs may facilitate management of species such as volunteer canola because the seedbank declines by roughly 90% per year (Gulden et al. 2003b). Barring volunteer canola seedbank replenishment, near-complete seedbank depletion may be possible over the course of a crop rotation in western Canada (Gulden et al. 2003b). Minimization of canola harvest losses (e.g., Cavalieri et al. 2016; Haile et al. 2014a), and preventative seedbank management (e.g., Chapter 3; Gulden et al. 2004b) are useful tools for limiting additions to and depleting the existing volunteer canola seedbank, respectively. However, even low densities of volunteer canola in soybean can cause economic yield loss (Gregoire 2017) and potentially large seedbank contributions.

Traditionally, inter-row tillage in wide-row soybean was the primary method for late-season weed management (Harder et al. 2007; Wax and Pendleton 1968). The release of GR soybean varieties has since provided the option of growing soybean in narrow rows and using glyphosate for broad-spectrum POST weed management (Harder et al. 2007; Reddy 2001). Inter-row tillage, however, may be an effective tool for difficult to manage weeds such as volunteer canola.

Soybean grown in narrow-rows can have increased competitive ability for weed suppression compared with wide-row production. Increased soybean population densities have generally resulted in improved weed management also. A large body of research has focused on the use of narrow soybean row widths and/or increased seeding densities for inhibition or reduction of late-season weed recruitment (Harder et al. 2007), biomass accumulation (e.g., Arce et al. 2009; Rich and Renner 2007), seed production (Nice et al. 2001) and seedbank inputs (Chandler et al. 2001). Narrow-row soybean production can result in more uniform spatial arrangement compared with wide-row systems. Uniformity of spatial arrangement in soybean can cause decreased intraspecific competition and improved resource capture (Burnside and Colville 1964a; Shibles and Webber 1966), rapid canopy closure (e.g., Légère and Schreiber 1989; Wax and Pendleton 1968; Willcott et al. 1984), more complete light interception throughout the growing-season (e.g., Dalley et al. 2004; Taylor et al. 1982) and reduced photosynthetic photon flux density beneath the soybean canopy (e.g., Green-Tracewicz et al. 2012; Swanton et al. 1998). These all affect weed interference and/or recruitment of late-season weeds (Yelverton and Coble 1991). Rich and Renner (2007) found that reducing soybean row widths from 76 to 19 cm resulted in a consistent decrease in dry weight of eastern black nightshade (*Solanum ptycanthum* Dun.). A reduction in soybean row width also has resulted in reduced seed production of pitted morningglory (*Ipomoea lacunosa* L.) (Howe and Oliver 1987) and *Amaranthus* spp. [palmer amaranth (*A. palmeri* S. Wats.), common waterhemp (*A. rudis* Sauer), powell amaranth (*A. powellii* S. Wats.) and redroot pigweed (*A. retroflexus* L.)] (Butts et al. 2016) and reduced seedbank inputs from a combination of late-emerging weeds [predominantly common lambsquarters (*Chenopodium album* L.) and witchweed (*Panicum capilare* L.)] (Chandler et al. 2001). Arce et al. (2009) found that increased soybean seeding densities resulted in a linear decrease in weed biomass, but had little effect on weed densities. Higher soybean populations (~676,000 plants ha^{-1}) combined with narrow (19 cm) soybean row widths reduced sicklepod [*Senna obtusifolia* (L.) Irwin and Barneby] population densities by 80% compared with low soybean populations (~245,000 plants ha^{-1}) in wide-rows (76 cm) (Nice et al. 2001). The impact of reduced row widths or increased seeding densities on management of cool-season weed species in soybean has not yet been tested at more northern latitudes (such as western Canada) and may differ from previous research conducted at lower latitudes.

The response of plants to soil nutrient supply is species dependent (Blackshaw and Brandt 2008; Blackshaw et al. 2003, 2004a). Fertilization management can be utilized to shift the competitive balance between certain species in an IWM program (e.g., Di Tomaso 1995). Soybean can acquire between 50% and 85% of its total nitrogen (N) requirements from symbiotic N-fixation (Rao and Reddy 2010; Salvagiotti et al. 2008). For this reason, soybean response to additional N fertilizer is often minimal (Salvagiotti et al. 2008). Canola, however, is highly responsive to mineral N supply. In response to greater mineral N supply, canola exhibits increased growth (Grant and Bailey 1993), shoot and root biomass (Blackshaw et al. 2003), leaf area index (Wright et al. 1988), branches per plant, fecundity (Allen and Morgan 1972), seed weight (Taylor et al. 1991) and seed production (e.g., Grant and Bailey 1993; Jackson 2000). Mineral N supply may therefore have a marked influence on the competitive balance among volunteer canola and soybean.

Several studies have been conducted on the use of cultural, chemical, or physical tools for weed management in soybean. However, no research has been conducted to-date on candidate tools for the development of an IWM strategy for volunteer canola in soybean. In western Canada, IWM research in soybean is in its infancy because wide-spread production of this crop has only recently been established at these northern latitudes (Statistics Canada 2017). These northern regions are dominated by cool-season C3 weed species (Leeson et al. 2016), such as volunteer canola (Gulden et al. 2008). The response of these weed species to integrated management practices in soybean may differ from previous research conducted at lower latitudes. This study was thereby designed to evaluate the effects of soybean row spacing, seeding density, N fertilization and inter-row tillage on volunteer canola interference in soybean. These non-chemical weed management tools were evaluated to identify effective candidate tools for utilization in an IWM program in western Canadian soybean production. It was hypothesized that soybean established using narrow-row widths would inhibit volunteer canola growth and development and increase soybean yield compared with soybean production in wide rows. An increase in soybean seeding density was expected to increase soybean interference with volunteer canola and inter-row tillage in wide-rows was expected to reduce volunteer canola interference with soybean. On the contrary, N fertilization was expected to increase volunteer canola interference with soybean and decrease soybean yield.

5.3 Materials and Methods

5.3.1 Field Experiment.

5.3.1.1 Experimental Sites. Field experiments were established on cultivated wheat stubble at four sites in Manitoba, Canada. In 2013, one site was established at the Ian N. Morrison Research Station near Carman, MB (49°29’35”N, 98°02’25”W). In 2014, sites were established at the Ian N. Morrison Research Station near Carman, MB (49°29’22”N, 98°02’26”W), the

Richardson Kelburn Farm near Howden, MB (49°41'33"N, 97°07'15"W) and the Westman Agricultural Diversification Organization near Melita, MB (49°14'47"N, 101°01'01"W). The sites were chosen based on low levels of residual nitrate-N in the soil prior to study establishment. Soil characteristics for each site can be found in Table 5.1.

Table 5.1. Soil characteristics and nutrient status in spring prior to seeding soybean at each experimental site.

Experimental site	Sample depth	Soil characteristics [a]						
		N	S	P	K	Soil series [b]	Texture [c]	Drainage
	cm	— kg ha^{-1} —		— ppm —				
Carman 2013	0-15	7	16	8	170	DHO	CL	Imperfect
	15-60	30	47	- [d]	-			
Melita 2013	0-15	9	6	4	324	NWS	L	Well
	15-60	24	60	-	-			
Carman 2014	0-15	4	16	27	296	RGD	SCL	Imperfect
	15-60	7	175	-	-			
Kelburn 2014	0-15	15	16	20	438	SOR	C	Well
	15-30 [e]	19	16	-	-			

[a] Abbreviations: N, nitrate-nitrogen; S, sulfate-sulfur; P, Olsen-phosphorus; K, potassium.
[b] Abbreviations: DHO, Deadhorse series; NWS, Newstead series; RGD, Rignold series; SOR, St. Norbert series.
[c] Abbreviations: CL, clay loam; L, loam; SCL, sandy clay loam; C, clay.
[d] A dash (-) indicates lack of measurement.
[e] Indicates a different sample depth range than the other sites.

5.3.1.2 Experimental Design and Treatment Structure. In the field experiment, a split-block randomized complete block design with four replications (blocks) per site was used to study the non-chemical weed management treatment by herbicide combinations. Non-chemical weed management treatments were considered main plots and herbicide MOA was considered the sub-plot. Each plot was 2.5 x 8 m and consisted of two 2.5 x 4 m sub-plots. Soybean management treatments consisted of cultural or physical weed management tools. Cultural management tools included 19, 38 and 76 cm soybean row widths, 19 cm row width with elevated (1.5 times recommended) seeding density, and 76 cm row width with elevated mineral N supply (23 kg N ha^{-1}, broadcast urea prior to planting). The physical management treatment consisted of a single inter-row cultivation (5 cm depth) at soybean stage BBCH 14, once in each direction. In the same field study, inter-row living cereal mulches were evaluated also. However, the living inter-row mulch treatments comprised a full-factorial treatment substructure used to address specific hypothesis related to living mulch production. For this reason, the effects of inter-row living cereal mulches on management of volunteer canola in soybean are addressed separately in Chapter 6.

Prior to establishing the soybean treatments, GR canola 'DKL 73-45 RR' (F1 generation hybrid treated with Acceleron® containing: difenoconaxole, metalaxyl, fludioxonil and thiamethoxam; Monsanto Canada Inc., Winnipeg, MB, CA) was seeded along each experimental block at 1 cm depth using a rate of 80 seeds m^{-2} (19 cm row width). All soybean 'DKL 23-10 RY' (treated with Acceleron® containing: fluxapyroxad, pyraclostrobin, metalaxyl, imidacloprid; Monsanto Canada Inc., Winnipeg, MB, CA) treatments were seeded perpendicular to canola rows at a depth of 2 cm. A soybean seeding density of 455,000 seeds ha^{-1} (432,000 target plants ha^{-1}) was used for all treatments except for the elevated seeding density treatment. This seeding density was chosen because it spanned recommendations for 19 to 76 cm soybean row widths. The elevated seeding density treatment was seeded at 682,500 seeds ha^{-1} (648,500 target plants ha^{-1}), 1.5 times the seeding density of all other treatments. TagTeam® MultiAction® granular soybean inoculant (minimum of 1×10^5 cfu *Penicillium bilaii* g^{-1} and 1×10^8 viable *Bradyrhizobium japonicum* cells g^{-1}; Monsanto BioAg and Design™, Monsanto Company, St.

Louis, MO, USA) was added in the soybean row at the recommended rate for each row width (6.5, 4.0 and 1.6 kg ha^{-1} in 19, 38 and 76 cm row widths, respectively). Experimental blocks were split by herbicide MOA, where glyphosate (Roundup WeatherMax®, 360 g a.e. ha^{-1} glyphosate, Monsanto Canada Inc., Winnipeg, MB, CA) was used for broad-spectrum weed management on one half of each block and pinoxaden (in 2013; Axial® BIA, 59 g a.i. ha^{-1} pinoxaden, Syngenta Canada Inc., Guelph, ON, CA) or clodinafop-propargyl (in 2014; Horizon® 240EC, 54 g a.i. ha^{-1} clodinafop-propargyl, Score® Adjuvant, 0.80 L ha^{-1}, Syngenta Canada Inc., Guelph, ON, CA) were used for management of monocotyledonous weed species only on the other half of each block at soybean stage BBCH 13. All of the herbicides were applied using AIXR 110015 TeeJet nozzles (TeeJet Technologies, Wheaton, IL, USA) at 276 kPa and with 100 L ha^{-1} water carrier. The different herbicide regimes were included in the overall field experiment to test the effect of mid-season termination of inter-row living cereal mulches only. For this reason, analyses of weed management tools in the current study focus on the glyphosate herbicide regime alone.

5.3.2 Data Collection. Soybean seedling emergence was determined at stage BBCH 10-12 by counting the number of seedlings in 1 m of two adjacent soybean rows in each experimental unit. Volunteer canola seedling emergence was determined at stage BBCH 12-13 by counting the number of seedlings in two randomly placed 50 x 50 cm quadrats oriented along the edge of a soybean row. The height of three randomly selected plants of volunteer canola and soybean was determined at 15, 30, 45 and 60 days after soybean emergence (DAE). Plant height was measured from the soil surface to the distal end of the fully extended shoot. At canola stage BBCH 82, aboveground biomass of volunteer canola and soybean (BBCH 77) was collected from one 50 x 50 cm quadrat placed along the edge of a soybean row. Collected plants were separated by species and canola plants were counted to determine late-season plant densities. Late season canola plant density was used to determine plant survival to maturity as a percentage of seedling emergence. Following species separation, aboveground plant biomass was dried at 60°C until equilibrium and dry weights were recorded for each species. Dry volunteer canola biomass samples were hand threshed and cleaned using a hand sieve (2.7 mm round hole, Can-Seed Equipment Ltd., Winnipeg, MB, CA) and column seed cleaner (Agriculex, Model CB-1, Guelph, ON, CA). The total amount of clean volunteer canola seed was weighed and individual seed weight (g thousand seeds^{-1}) was determined. Total volunteer canola seed production per unit area (no. seeds m^{-2}) was then determined from seed weight per unit area (g m^{-2}) and individual seed weight (g thousand seeds^{-1}). Volunteer canola plant fecundity (no. seeds plant^{-1}) was determined using seed production per unit area (no. seeds m^{-2}) and late-season plant densities (no. plants m^{-2}) collected from the same biomass sample area.

At soybean maturity, sub-plots were divided and harvested using a Kincaid 8-XP single plot combine (concave clearance 18 mm, cylinder speed 625 rpm, fan speed 900 rpm, sieve 18 mm; Kincaid Equipment Manufacturing, St. Haven, KS, USA). Yield samples were cleaned using a Clipper M2BC seed cleaner (Blount/Ferrell-Ross Bluffton, IN, USA) and separated into soybean and canola portions using a hand sieve (2.7 mm round hole, Can-Seed Equipment Ltd., Winnipeg, MB, CA). Cleaned soybean and canola fractions were weighed and adjusted to 13.0% and 8.5% moisture, respectively. The total number of volunteer canola seeds collected with soybean at harvest (i.e. dockage) was expressed as no. seeds m^{-2} using total seed weight per unit area (g m^{-2}) and individual seed weight (g thousand seeds^{-1}) data. Then volunteer canola seedbank inputs were determined using equation 1 and expressed as a percentage of the total number of seeds produced.

$$[((\text{no. seeds m}^{-2}\text{ at biomass}) - (\text{no. seeds m}^{-2}\text{ in dockage}))/(\text{no. seeds m}^{-2}\text{ at biomass})]\text{ x }100 \qquad [1]$$

5.3.3 Statistical Analysis. The mixed model procedure (Littell et al. 2006) in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) was used for ANOVA of all response variables. The soybean response variables included yield, aboveground biomass, plant height and seedling emergence. The volunteer canola response variables included seed production per unit area, seedbank inputs, plant fecundity, individual mature seed weight, aboveground biomass, plant height, seedling emergence and plant survival to maturity. The Shapiro-Wilk test was used to assess the assumption of normality, while visual inspection of residual vs. predicted values was used to assess homogeneity of variance (Littell et al. 2006). Lund's test (Lund 1975) was used to remove extreme outliers and the square root-transformation was used when necessary to meet the assumptions of normality and/or homoscedasticity (Littell et al. 2006).

A treatment substructure design (Piepho et al. 2006) was used for ANOVA of the row spacing, seeding density, N fertilization and inter-row tillage treatments within the glyphosate split-block only. The treatment substructure allowed focus on the effects of soybean row spacing, seeding density, soil mineral N and inter-row tillage. However, all treatments were included in the ANOVA to retain the overall experimental error (Piepho et al. 2006). In all analyses excluding plant height, candidate weed management treatment (excluding mulch treatments; 6 levels), experimental site (4 levels) and their interaction were considered fixed effects. Experimental block nested within site, the interaction of all candidate weed management treatments (including mulches; 10 levels) and experimental block nested within site, and the interaction of herbicide (2 levels) and experimental block nested within site were considered random effects. The covariance structure of residuals was fit by selecting the R-matrix repeated within-group effect based on minimization of Akaike's Information Criterion (AIC) to further adjust the model for homoscedasticity (Onofri et al. 2010). Plant height data, were analyzed within site using a repeated measures model (Littell et al. 2006) with the same treatment substructure as the other response variables (Piepho et al. 2006). For the analysis of plant height only, the main and interaction effects of management treatment (6 levels), measurement date (4 levels), and response species (soybean and canola) were considered fixed effects. Experimental block, the interaction of management treatment and experimental block, and the interaction of herbicide and experimental block were considered random effects. Volunteer canola and soybean (response species) were included as a factor in the analysis of plant height to estimate shade avoidance response to interference in each species at each sampling time. Measurements of the individual experimental units (herbicide by management treatment by response species) were repeated in time and a toeplitz covariance structure was fit based on minimization of AIC (Littell et al. 2006).

In the ANOVA, the family-wise Type 1 errors ($\alpha = 0.05$) were conserved using simulation-based multiple comparisons (Edward and Berry 1987; Onofri et al. 2010) and the pdmix800 macro was used for mean separation (Saxton 1998). To address each specific hypothesis, single-degree-freedom estimated mean differences (outlined in Table C1) were designed to test for lack of difference among treatments (Littell et al. 2006) using the simulation-based procedure also (Edward and Berry 1987; Schaarschmidt and Vaas 2009).

5.4 Results and Discussion

Initial combined site analyses revealed large differences in volunteer canola seedling emergence among sites. Carman 2013 and Melita 2014 (39 and 38 plants m^{-2}, respectively) resulted in about half the density of volunteer canola compared with Howden 2014 and Carman 2014 (80 and 99 plants m^{-2}, respectively) (Table 5.2). Coincidentally, these sites had lower levels of soil test Olsen-phosphorus (P), below the recommended level for canola production in this region (Manitoba Agriculture 2007). However, canola is efficient at taking up available P in the soil (Blackshaw et al. 2004a; Grant and Bailey 1993) and it was unlikely that lower soil P had an effect on canola at such early stages of development. The reason for differences in volunteer canola emergence among sites remains unknown. Even though volunteer canola tended to emerge near the same time as soybean at each site, the experimental sites with higher volunteer canola densities resulted in a greater magnitude of effects observed on both soybean and volunteer canola response variables compared with the sites that had lower densities. Due to differences in magnitude of effects among sites with higher and lower volunteer canola emergence densities, all analyses except for the plant height data (which were separated by site) were conducted within higher (Carman 2014 and Howden 2014; 89 ± 4 plants m^{-2}) and lower (Carman 2013 and Melita 2014; 39 ± 4 plants m^{-2}) volunteer canola emergence density classes (Table 5.2). Separation of sites by canola recruitment densities revealed that volunteer canola was less responsive to the weed management tools at sites with lower (Table 5.3) – as opposed to higher (Table 5.4) – volunteer canola densities.

5.4.1 Row Width. Yield differences in soybean (under volunteer canola interference) were not detected among narrow (19 cm), medium (38 cm) or wide (76 cm) row widths (Table 5.5).

Table 5.2. The main effect of experimental site on soybean and volunteer canola response variables separated by sites with higher (Carman 2014 and Howden 2014) and lower (Carman 2013 and Melita 2014) densities of volunteer canola. [a]

Canola density class	Experimental site	Soybean			Volunteer canola						
		Yield	Aboveground biomass	Seedling emergence [b]	Seed production	Seed return	Individual seed weight	Plant fecundity [c]	Aboveground biomass	Seedling emergence	Plant survival [c]
		—— kg ha^{-1} ——		no. plants m^{-2}	thousand seeds m^{-2}	%	g thousand seeds^{-1}	no. seeds plant^{-1}	kg ha^{-1}	no. plants m^{-2}	%
Lower	Carman 2013	2,210	5,360	47	18,600	60	3.58	450 (22.1)	2,370	39	104 (10.2)
| [d]	Melita 2014	1,360	2,920	23	23,200	67	2.84	1,060 (32.6)	1,686	38	67 (8.2)
	P-values [e,f]	***	***	***	ns	ns	***	***	ns	ns	ns
Higher	Carman 2014	960	1,860	28	41,600	79	3.27	960 (31.0)	3,920	99	46 (6.8)
|	Howden 2014	1,150	2,660	23	42,200	51	3.54	850 (29.1)	4,360	80	66 (8.1)
	P-values	***	***	***	***	***	***	**	***	ns	***

[a] Values are means.

[b] Indicates significant site by treatment interaction at sites with higher volunteer canola recruitment densities.

[c] Values followed by parenthetical values are back-transformed means with square root-transformed means in parentheses.

[d] A bar (|) indicates continuation of the entry above.

[e] Within volunteer canola density site-classes, differences between sites at $P < 0.05$, 0.01, and 0.001 are indicated by *, **, and ***, respectively.

[f] ns indicates lack of a significant difference $P \geq 0.05$.

Table 5.3. The effect of soybean row width, seeding density, nitrogen fertilization and inter-row tillage on volunteer canola response variables in a combined analysis of the sites with lower densities of volunteer canola (Carman 2013 and Melita 2014).

		Response variables						
Means [a,b] Row width [c]	Manage [d]	Seed Production	Seedbank inputs	Individual seed weight	Plant fecundity [e]	Aboveground biomass	Seedling emergence	Plant survival [e]
		no. seeds m^{-2}	%	g thousand seeds^{-1}	no. seeds plant^{-1}	kg ha^{-1}	no. plants m^{-2}	%
NR	Absent	25,000	63	3.35	680 (26.1)	2,520 a	47	83 (9.1)
| [f]	ESD	16,600	68	3.22	470 (21.6)	1,660 ab	40	121 (11.0)
MR	Absent	30,500	77	3.43	780 (27.8)	2,930 a	43	103 (10.2)
WR	Absent	19,300	61	3.26	1,030 (32.2)	2,020 ab	40	65 (8.1)
|	EN	19,700	41	3.41	950 (30.9)	1,680 ab	28	89 (9.4)
|	IRT	14,400	57	3.62	650 (25.5)	1,340 b	33	54 (7.4)
Estimated mean differences [g,h]								
Row width								
NR vs. WR		5,600	2	0.09	(-6.0)	500	7	(1.0)
NR vs. MR		-5,500	-14	-0.08	(-1.7)	-408	4	(-1.0)
MR vs. WR		11,200	15	0.17	(-4.3)	908	3	(2.1)
Seeding density								
NR ESD vs. NR absent		-8,200	5	-0.13	(-4.5)	-867	-8	(1.9)
Soil nitrogen								
WR EN vs. WR absent		400	-20	0.15	(-1.3)	-341	-13	(1.3)
Inter-row tillage								
WR IRT vs. WR absent		-4,900	-5	0.36 *	(-6.7)	-684	-7	(-0.7)

[a] Values are means.

[b] Within columns in the means section, different letters indicate significant differences using simulation-based multiple comparisons ($\alpha = 0.05$).

[c] Abbreviations: NR, narrow-row (19 cm); MR, medium-row (38 cm); WR, wide-row (76 cm).

[d] Abbreviations: Manage, additional management treatment; Absent, absent of additional management; ESD, elevated seeding density (1.5 times recommended); EN, elevated nitrogen (+23 kg N ha^{-1}, urea); IRT, inter-row tillage.

[e] Values followed by parenthetical values are back-transformed means with square root-transformed means in parentheses.

[f] A bar (|) indicates continuation of the entry above.

[g] Values are estimated differences between means.

[h] A significant estimated difference between means at $P < 0.05$ is indicated by *.

Table 5.4. The effect of soybean row width, seeding density, nitrogen fertilization and inter-row tillage on volunteer canola response variables in a combined analysis of the sites with higher densities of volunteer canola (Carman 2014 and Howden 2014).

Means [a,b]		Response variables						
Row width [c]	Manage [d]	Seed Production	Seedbank inputs	Individual seed weight	Plant fecundity [e]	Aboveground biomass	Seedling emergence	Plant survival [e]
		no. seeds m^{-2}	%	g thousand $seeds^{-1}$	no. seeds $plant^{-1}$	kg ha^{-1}	no. plants m^{-2}	%
NR	Absent	39,400 b	64	3.62 ab	805 (28.4)	3,890 b	91	59 (7.7) ab
| [f]	ESD	36,200 b	71	3.44 b	654 (25.6)	3,630 b	78	71 (8.4) a
MR	Absent	43,100 ab	72	3.57 ab	815 (28.6)	4,200 ab	81	67 (8.2) a
WR	Absent	37,000 b	50	3.59 ab	870 (29.5)	3,760 b	93	53 (7.3) ab
|	EN	65,500 a	73	3.63 ab	935 (30.6)	6,370 a	107	67 (8.2) ab
|	IRT	31,300 b	61	3.88 a	1,443 (38.0)	2,980 b	89	25 (5.0) b
Estimated mean differences [g,h]								
Row width								
NR vs. WR		2,400	14	0.03	(-1.1)	130	-2	(0.4)
NR vs. MR		-2,700	-7	0.05	(-0.2)	-310	11	(-0.5)
MR vs. WR		5,100	22	-0.02	(-0.9)	440	-12	(0.9)
Seeding density								
NR ESD vs. NR absent		-3,100	6	-0.18	(-2.8)	-260	-14	(0.8)
Soil nitrogen								
WR EN vs. WR absent		28,500 ***	23 *	0.04	(1.1)	2,610 ***	14	(0.9)
Inter-row tillage								
WR IRT vs. WR absent		-5,700	11	0.29 *	(8.5)	-780	-4	(-2.2) *

[a] Values are means.

[b] Within columns in the means section, different letters indicate significant differences using simulation-based multiple comparisons ($\alpha = 0.05$).

[c] Abbreviations: NR, narrow-row (19 cm); MR, medium-row (38 cm); WR, wide-row (76 cm).

[d] Abbreviations: Manage, additional management treatment; Absent, absent of additional management; ESD, elevated seeding density (1.5 times recommended); EN, elevated nitrogen (+23 kg N ha^{-1}, urea); IRT, inter-row tillage.

[e] Values followed by parenthetical values are back-transformed means with square root-transformed means in parentheses.

[f] A bar (|) indicates continuation of the entry above.

[g] Values are estimated differences between means.

[h] Significant estimated differences between means at $P < 0.05$ and $P < 0.001$ are indicated by * and ***, respectively.

Table 5.5. The effect of soybean row width, seeding density, nitrogen fertilization and inter-row tillage on soybean response variables under volunteer canola interference in combined analyses of the sites within each of the higher (Carman 2014 and Howden 2014) and lower (Carman 2013 and Melita 2014) volunteer canola density site-classes.

		Response variables					
		Higher canola density sites			Lower canola density sites		
Means [a,b] Row width [c]	Manage [d]	Yield	Aboveground Biomass	Seedling emergence [e]	Yield	Aboveground Biomass	Seedling emergence
		kg ha^{-1}		no. plants m^{-2}	kg ha^{-1}		no. plants m^{-2}
NR	Absent	1,020 bc	2,560 ab	25 bc	1,750	5,490 a	34 b
| [f]	ESD	1,470 a	3,070 a	43 a	1,950	4,820 ab	54 a
MR	Absent	1,140 ab	2,210 abc	29 b	1,790	4,920 ab	29 b
WR	Absent	890 bc	1,700 bc	19 bc	1,690	3,010 b	31 b
|	EN	590 c	1,390 c	19 bc	1,650	3,460 ab	28 b
|	IRT	1,210 ab	2,630 ab	19 c	1,890	3,940 ab	32 b
Estimated mean differences [g,h]							
Row width							
NR vs. WR		140	860 *	6	70	2,490 ***	3
NR vs. MR		-120	360	-4	-30	580	5
MR vs. WR		260	510	10 **	100	1,910 *	-2
Seeding density							
NR ESD vs. NR absent		450 **	510	18 ***	200	-670	20 ***
Soil nitrogen							
WR EN vs. WR absent		-290	-310	1	-40	460	-3
Inter-row tillage							
WR IRT vs. WR absent		330 *	930 *	0	200	930	1

[a] Values are means

[b] Within columns in the means section, different letters indicate significant differences using simulation-based multiple comparisons ($\alpha = 0.05$).

[c] Abbreviations: NR, narrow-row (19 cm); MR, medium-row (38 cm); WR, wide-row (76 cm).

[d] Abbreviations: Manage, additional management treatment; Absent, absent of additional management; ESD, elevated seeding density (1.5 times recommended); EN, elevated nitrogen (+23 kg N ha^{-1}, urea); IRT, inter-row tillage.

[e] indicates treatment main effect when a treatment by site interaction was present

[f] A bar (|) indicates continuation of the entry above.

[g] Values are estimated differences between means.

[h] Significant estimated differences between means at $P < 0.05$, $P < 0.01$ and $P < 0.001$ are indicated by *, ** and ***, respectively.

Differences in soybean row width also did not influence any volunteer canola response variables (Tables 5.3 and 5.4). The difference in cool-season canola and warm-season soybean developmental patterns resulted in rapid volunteer canola growth prior to soybean canopy closure (CM Geddes, personal observation). Perhaps rapid growth of volunteer canola early during the growing-season negated any potential impact of more rapid soybean canopy closure using narrow-row widths (e.g., Willcott et al. 1984). Measurements of the date of canopy closure would facilitate with testing this hypothesis. Unfortunately, these data were not collected in the current study. Volunteer canola plant height did, however, surpass that of soybean shortly after 15 DAE (Fig 5.1). Volunteer canola was taller than soybean well before the typical time of soybean canopy closure. As a result, any potential competitive advantage of soybean over volunteer canola provided by soybean canopy closure, regardless of row width, was likely reduced. The difference in early-season growth and development between soybean and canola may explain the lack of difference in soybean yield among row widths (Table 5.5). Moreover, soybean canopy development and closure, even at narrow soybean row widths may not be rapid enough for competition with volunteer canola emerging shortly after soybean planting.

In contrast with previous research on other weed species conducted at lower latitudes, volunteer canola was not affected by soybean row width, perhaps due to its rapid growth and development relative to soybean (Fig 5.1) in the cool, short-season growing conditions of western Canada. Other studies evaluating the effect of soybean row width on weed interference have generally concluded that reduced row widths result in increased interference with weed species (e.g., Chandler et al. 2001; Rich and Renner 2007). Previous research also has demonstrated a soybean yield benefit at narrow/medium row spacing (≤ 38cm) compared with wide row widths (≥ 76 cm) under weed-free conditions even at lower latitudes (e.g., Cox and Cherney 2011; De Bruin and Pedersen 2008). In the current study, interference of volunteer canola with soybean may have eliminated any potential yield benefits of reduced soybean row widths.

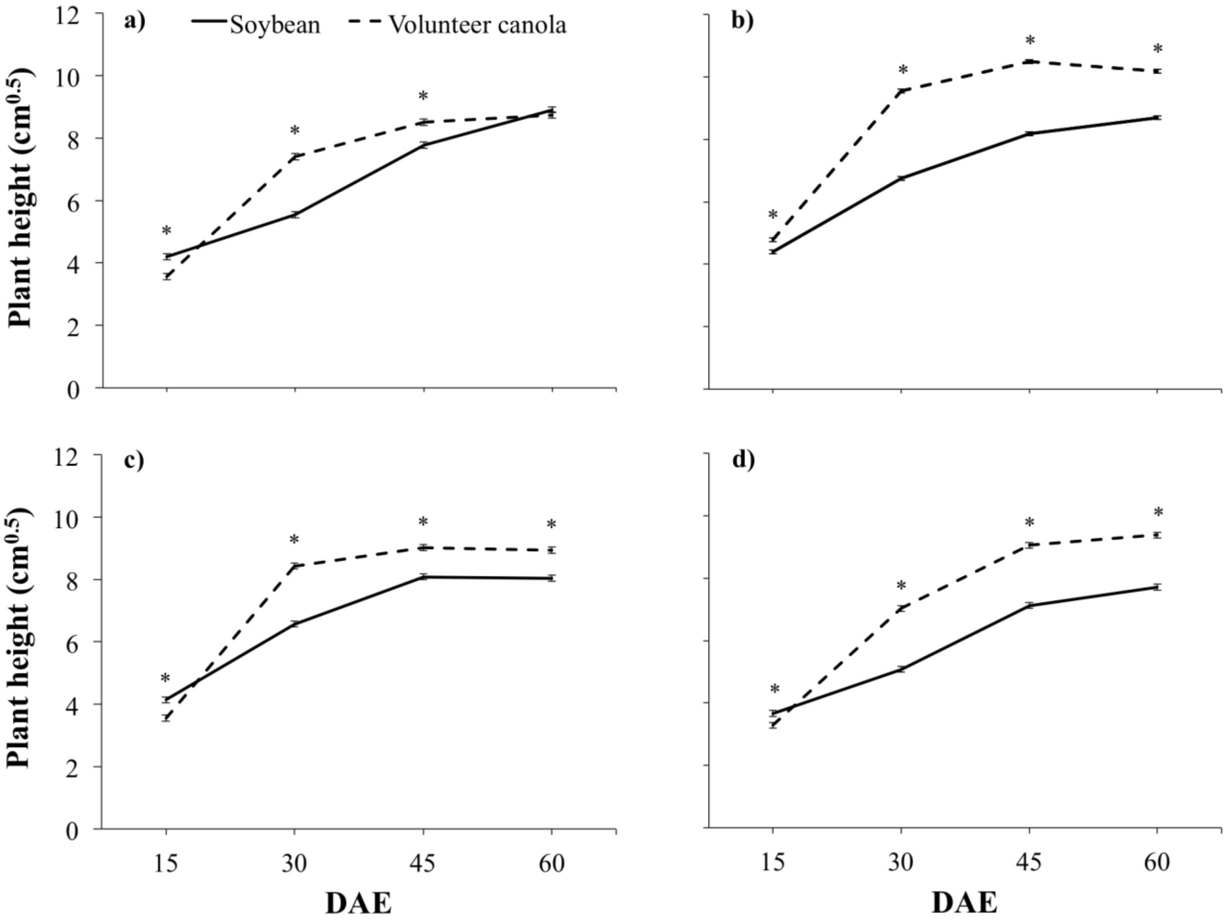

Figure 5.1. Mean plant heights of soybean or volunteer canola at 15, 30, 45 and 60 days after soybean emergence (DAE) at the (a) Carman 2013, (b) Carman 2014, (c) Melita 2014 and (d) Howden 2014 sites. Data points indicate response species means at each measurement date in each site in a combined analysis among management treatments. Error bars indicate ± one standard error of the mean. An asterisk (*) indicates a significant difference among species within site and measurement date using simulation-based multiple comparisons ($\alpha = 0.05$).

Based on estimated mean differences, narrow-row soybean resulted in greater aboveground soybean biomass than wide-row soybean (51% and 83% more biomass at sites with higher and lower volunteer canola densities, respectively), while medium-row widths resulted in intermediate, yet greater biomass (63% greater) than wide-row widths at sites with lower volunteer canola densities only (Table 5.5). The effect of soybean row width on aboveground soybean biomass accumulation was diminished using simulation-based multiple comparisons, with the exception of greater biomass in narrow-row compared with wide-row soybean at sites with lower volunteer canola densities only. Inconsistent results in the literature have indicated that total dry weight of soybean can be either a good indicator (e.g., Carkner and Entz 2017; Egli 2010) or a poor indicator (e.g., Shibles and Weber 1966; Taylor et al. 1982) of soybean yield. In the current study, differences in soybean dry weight did not manifest into yield differences among soybean row widths (Table 5.5). Greater soybean biomass observed in narrow- and medium- compared with wide-row widths was likely due to more uniform spatial arrangement (Weber et al. 1966), leading to increased resource capture (e.g., Dalley et al. 2004; Willcott et al. 1984) and reduced intraspecific competition (e.g., Duncan 1986; Egli 1988). Cox and Cherney (2011) also found greater soybean biomass and increased leaf area indices as soybean row widths decreased from 76 to 38 to 19 cm. Soybean pod density, seed density and yield also were associated negatively with soybean row width in their study. In the current study, differences in aboveground biomass were greater between soybean in medium- and wide-row widths (63% greater biomass at lower volunteer canola density sites) compared with narrow- and medium-row widths (not significantly different) (Table 5.5). These results suggest that, soybean grown in 19 or 38 cm rows may be less vulnerable to interference from volunteer canola than 76 cm row widths.

5.4.2 Seeding Density. Soybean seeded in narrow rows at 648,500 seeds ha^{-1} (1.5 times recommended) resulted in a 44% yield increase compared with a seeding density of 455,000 seeds ha^{-1} (recommended) at sites with higher volunteer canola recruitment densities (Table 5.5). A similar trend was observed at the lower canola density sites also. Greater soybean yield in the elevated seeding density treatment was likely due to more rapid canopy closure (e.g., Willcott et al. 1984) and/or enhanced resource capture (e.g., De Bruin and Pedersen 2009; Norsworthy and Oliver 2001) at the population level. Previous studies also have found a positive association of soybean population density with soybean yield under weedy conditions (e.g., Arce et al. 2009; Butts et al. 2016).

Like row width, seeding density of soybean did not influence any volunteer canola response variables (Tables 5.3 and 5.4), further demonstrating the competitive ability of this weed species. Previous studies evaluating the effect of soybean population density on weed interference have shown reduced weed population densities (Nice et al. 2001), biomass (Arce et al. 2009) and seed production (Butts et al. 2016) at higher soybean population densities. In the current study, estimated mean differences comparing elevated vs. recommended seeding densities showed an obvious trend where the total number of seeds produced per unit area, seed weight, fecundity, aboveground biomass, and early-season density of volunteer canola tended to be lower under the elevated soybean seeding density, although differences were not significant (Tables 5.3 and 5.4).

High seed costs have spurred interest in the reduction of soybean seeding density recommendations as a possible strategy to reduce crop-input costs and potentially increase net profit margins (e.g., Kratochvil et al. 2004; Norsworthy and Frederick 2002). Rudimentary partial budgeting analysis using the same seed cost (\$0.36 thousand seeds^{-1}) and grain prices (\$0.37 kg seed^{-1}) as Tkachuck (2017), indicated that the elevated seeding densities used in the current study resulted in increased profit by \$4 ha^{-1} at sites with lower volunteer canola densities and by \$96 ha^{-1} at sites with higher volunteer canola densities (data not shown). These results indicate that elevated soybean seeding densities from current recommendations could provide greater financial benefit to farmers growing soybean in this region. In Manitoba, Canada, the recommended target population density for soybean is 395,000 plants ha^{-1} (Mohr et al. 2014). Recent research has reported maximum soybean yield at population densities of 452,000 and 324,200 plants ha^{-1} in early-mid and late-very late planting date groups under weed-free conditions in the northern Great Plains (Tkachuk 2017). However, the economic optimum seeding densities for these planting date groups under weed-free conditions were 492,000 and 314,000 seeds ha^{-1}, respectively. If seeding densities were decreased from current recommendations, results from the current study indicate that the competitive response of the soybean crop likely also would decrease (Arce et al. 2009; Nice et al. 2001) and the antagonistic effects of competitive weeds – like volunteer canola – on soybean yield would be enhanced. The current study only evaluated the effect of increasing the seeding density of soybean from current recommendations. The effect of reduced seeding densities below current recommendations has yet to be investigated under weedy conditions in this region.

5.4.3 Soil Nitrogen. Mineral N fertilizer application prior to seeding soybean resulted in a large increase in volunteer canola seed production and biomass accumulation (and seedbank inputs using estimated mean differences only) at sites with higher volunteer canola densities (Table 5.4). At these sites, elevated levels of soil mineral N resulted in an increase in absolute volunteer canola seed production from about 37,000 to 65,500 seeds m^{-2}. A 69% increase in volunteer canola biomass accumulation (and about double the volunteer canola seedbank inputs using estimated mean differences) also was observed in the enhanced soil mineral N treatment compared with the unfertilized control at these sites (Table 5.4). Canola is highly responsive to mineral N fertilizer (e.g., Blackshaw et al. 2003; Jackson 2000). In a controlled environment study evaluating the response of 23 agricultural weed species to N fertilizer, Blackshaw et al. (2003) found that canola was grouped among the weeds with the greatest response in shoot biomass accumulation and greatest N uptake with added N fertilizer. However, under lower-N conditions, canola may be rather inefficient at taking up N relative to other weed species (Blackshaw et al. 2003). In the current study, it was hypothesized that N fertilization would increase interference from volunteer canola compared with forgoing N fertilization. Results from the current study suggest that volunteer canola interference with soybean was greater following application of mineral N fertilizer.

The supplementation of 23 kg N ha^{-1} as pre-seed broadcast urea, simulating a starter N fertilizer application and a greater amount of soil mineral N, did not affect any soybean response variables compared with the unfertilized control (Table 5.5). In the northern Great Plains, starter N fertilization increases soybean yield and early-season plant growth (Osborne and Riedell 2006). However, Salvagiotti et al. (2008) found that generally, N fertilization did not affect soybean yield among a wide range of environments, and preliminary research conducted in Manitoba, Canada, showed the same (Brar and Lawley 2016). Results from the current study indicate that N fertilization in soybean could increase interference from unmanaged weeds and result in additional inputs into the weed seedbank (Table 5.4). Carkner and Entz (2017) reported that the level of residual soil inorganic N in ten field experiments in southern Manitoba was negatively associated with organic soybean yield. This was potentially due to enhanced weed interference at sites with greater levels of soil inorganic N (Carkner and Entz 2017). In the current study, a 23 kg N ha^{-1} difference in soil mineral N perhaps was not large enough to cause a difference in soybean yield. However, under volunteer canola interference, soybean yield tended to be lower in the presence compared with the absence of mineral N fertilization (Table 5.5). The differential response of soybean and volunteer canola to N fertilization suggests that growing soybean under lower mineral N conditions may provide soybean with a competitive advantage over weed species not capable of N-fixation (Chapter 7).

Soil tests prior to study establishment showed low levels of nitrate-N in the soil profile at each experimental site (Table 5.1). These levels of nitrate-N, however, were representative of the lower spectrum or residual soil N commonly observed in fields under annual crop production in western Canada (e.g., AGVISE Laboratories 2016; Drury et al. 2007). In the current study, the residual level of soil mineral N was similar among sites (Table 5.1). It is therefore unlikely that residual levels of soil N prior to fertilization confounded results among these sites. The levels of soil test P at Carman 2013 and Melita 2014 were considered low compared with the recommended rates for soybean production in this region (Manitoba Agriculture 2007). However, Bardella (2016) showed that additional P fertilization rarely manifests into increased soybean yield in Manitoba, Canada. For this reason, the level of soil P was not amended.

5.4.4 Inter-row Tillage. Inter-row tillage in wide-row soybean resulted in 36% greater soybean yield and 55% greater soybean biomass (using estimated mean differences) at the sites with higher volunteer canola recruitment densities compared with the absence of inter-row tillage in the same production system (Table 5.5). Although differences in soybean yield among all treatments were not detected at sites with lower volunteer canola recruitment densities, directional responses to inter-row tillage (evident in estimated mean differences) remained consistent and tended to result in greater soybean yield and aboveground biomass under volunteer canola interference (Table 5.5). Greater soybean yield and biomass at sites with higher volunteer canola densities was likely due to decreased volunteer canola plant survival to maturity (by about half) caused by inter-row cultivation (Table 5.4). A similar trend was observed at the sites with lower volunteer densities also (Table 5.3). Nevertheless, reduced impact of inter-row tillage on volunteer canola plant survival to maturity at sites with lower volunteer canola densities explained the lack of difference in soybean yield and biomass accumulation between wide-row soybean with and without inter-row tillage (Table 5.5).

Tillage between 76 cm soybean rows resulted in increased individual volunteer canola seed weight (by 8% and 11% using estimated mean differences at sites with higher and lower volunteer canola densities, respectively) compared with the absence of inter-row tillage (Tables 5.3 and 5.4). Inter-row tillage also tended to reduce volunteer canola plant survival to maturity. A reduction in volunteer canola population densities following inter-row tillage could have created niche space and a potentially greater supply of resources to the remaining volunteer canola plants. Greater availability of resources on a per plant basis in the later-season may have contributed to increased mature seed weight. Cultivation and plant residue incorporation also could have induced shifts in soil N dynamics (Brady and Weil 2007). Greater N supply can cause increased volunteer canola seed size (Taylor et al. 1991). In the current study, however, volunteer canola seed weight did not differ between the fertilized treatment and the unfertilized control (Tables 5.3 and 5.4). Together, these data indicate that mineral N was likely not the only resource that contributed to increased volunteer canola seed weight following inter-row tillage in soybean.

5.4.5 Experimental Site. Separation of sites into higher and lower volunteer canola density site-classes eliminated all significant treatment by site interactions, with the exception of soybean emergence in the higher volunteer canola density site class only (Table 5.2). Heterogeneous soybean emergence was observed in the wide-row inter-row tillage treatment (data not shown). This treatment resulted in the lowest soybean emergence in Carman 2014, but not Howden 2014 (data not shown). The cause of differential soybean emergence in the inter-row tillage treatment was unclear because early-season density counts were conducted prior to inter-row cultivation. An ANCOVA of soybean emergence density with the other response variables was not performed due to the inclusion of multiple soybean seeding density treatments in the experiment.

5.4.6 Management Implications. This study tested the effects of individual candidate cultural or physical weed management tools implemented alone as a prerequisite for development of an effective IWM strategy for volunteer canola in soybean in the short-season environment of western Canada. Results from the current study revealed that volunteer canola was a competitive weed in soybean production. Volunteer canola also was relatively insensitive to many singular weed management tools, especially at lower plant densities. It is therefore suggested that weed densities be considered in recommendation and implementation of volunteer canola management strategies in soybean.

Forgoing N fertilization was the only management tool that reduced volunteer canola seed production. Elevated soybean seeding densities in narrow-row, or inter-row tillage in wide-row production systems resulted in increased soybean yield at sites with higher volunteer canola population densities. Together, these results indicate that planting soybean on fields with limited soil mineral N, and avoiding N fertilizer application in soybean, may provide a competitive advantage over volunteer canola. Inter-row tillage in wide-row soybean could be used to decrease volunteer canola populations and greater soybean seeding densities may result in greater soybean yield under volunteer canola interference, but have little effect on volunteer canola management. The effect of soybean row width was minimal, as increased aboveground biomass at reduced row widths did not manifest into increased yields or a detectable competitive effect on volunteer canola.

Volunteer canola seedbank inputs in soybean averaged about 12,700 and 27,200 seeds m^{-2} among management treatments at sites with volunteer canola population densities of about 39 and 89 plants m^{-2}, respectively (i.e., about 100 and 200 times the recommended canola seeding density for this region). These seedbank inputs represent about 3 to 6 times the regional average seedbank inputs that occur when harvesting a canola crop (Cavalieri et al. 2016). Volunteer canola seed return in soybean therefore caused significant seedbank replenishment at these population densities and was clearly difficult to control via singular non-chemical weed management tools. Since the integration of multiple management tools can result in additive or synergistic effects on weed management, tools deemed ineffective when implemented alone in the current study may contribute to volunteer canola management when used in combination. Based on evaluation of singular candidate weed management tools, a few recommendations can be made. It is proposed that optimal soybean yield and volunteer canola management may be achieved when limitation of N fertility in soybean, elevated seeding densities in narrow-row soybean or inter-row tillage in wide-row soybean are integrated with appropriate crop and herbicide rotation.

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6.0 Living Cereal Mulch Systems for Early-Season Weed Interference in Soybean (*Glycine max*)

6.1 Abstract

Similar herbicide-resistance traits in soybean and canola leave limited options for reactive management of canola volunteers in soybean production. Volunteer canola grows rapidly early during the growing-season (early-season) relative to soybean. In this study, the utility of using living cereal mulch systems in soybean for early-season weed interference is proposed and tested. In 2013 and 2014, field experiments were conducted at four sites in the short growing-season (short-season) environment of Manitoba, Canada. The presence or absence of spring-established spring wheat or spring-established winter cereal rye living mulches (mulch species) seeded between 38 or 76 cm soybean rows (soybean row width) and the presence or absence of herbicides used for mid-season mulch termination (herbicide regime) were evaluated based on management of volunteer canola in soybean. Inter-row mulches, living or terminated mid-season, reduced volunteer canola seed production by about one-third (up to 9,000 seeds m^{-2}) relative to the respective mulch-free controls. In the presence of volunteer canola, soybean yield was similar with or without interference from living mulches. Mulches that were terminated mid-season resulted in effective management of volunteer canola in soybean even though they were only present during early soybean growth and development. Terminated mulches also tended to result in greater soybean yield compared with mulches that remained live throughout the growing-season. Overall, living inter-row cereal mulches successfully provided early-season interference with volunteer canola in soybean. Spring-established living cereal mulch systems also may be effective for early-season weed interference when other warm-season crops are

grown in short-season environments dominated by cool-season weeds.

6.2 Introduction

Soybean [*Glycine max* (L.) Merr.] is the third most commonly grown crop in Manitoba, Canada, following canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) (MMPP 2016). Volunteer canola, derived from canola harvest losses (Gulden et al. 2003a), can enter secondary seed dormancy (Gulden et al. 2003b) and persist throughout crop rotations (Beckie and Warwick 2010). Volunteer canola can be difficult to manage in soybean due to similar transgenic herbicide-resistance (HR) traits. Rapid growth and development early during the growing-season (early-season) relative to soybean, and lack of other effective herbicide options, makes development of an integrated approach to volunteer canola management imperative for soybean production in this region (Chapter 5). Proactive management, including minimization of canola harvest losses (Cavalieri et al. 2016; Haile et al. 2014a) and increased seedbank decline with timely tillage (Chapter 3) can reduce volunteer canola population densities. However, the large quantity of volunteer seedbank inputs at canola harvest (Cavalieri et al. 2016) warrants intervention throughout the volunteer canola life cycle to limit further seedbank additions.

A companion study showed limited response of volunteer canola to individual cultural or physical weed management tools in soybean (Chapter 5). Forgoing nitrogen (N) fertilization in soybean was the only strategy that reduced volunteer canola biomass and/or seed production (Chapter 5). Lack of volunteer canola response to a range of in-crop weed management tools was attributed to rapid early-season growth relative to soybean (Chapter 5). Management tools that target early-season interference in soybean may aid in reduction of growth, development and seed production of this weed species.

Cover crops have multiple benefits in agroecosystems (e.g., Hartwig and Ammon 2002). Recent interest has postulated the utility of cover crops for tailored interference with HR weeds in HR cotton (*Gossypium hirsutum* L.) (e.g., Norsworthy et al. 2011; Price et al. 2012; Wiggins et al. 2016). One such strategy may be the utilization of spring-established living mulches (Liebman and Dyck 1993; Teasdale 1996). Living mulches are planted prior to or with a grain crop and are maintained as living soil cover throughout the growth cycle of the grain crop (Hartwig and Ammon 2002; Liebman and Dyck 1993). In these systems, living mulches may be tailored to reduce detrimental impacts on grain crop yield, and at the same time facilitate interference with weeds.

Plant competition is comprised of both resource-limiting (direct) and non-resource-limiting (indirect) competition (Harper 1977). Plant interference, however, also includes indirect effects that do not always result in a negative interaction for both plants involved (e.g., allelopathy). Living mulches can interfere with weeds directly via resource consumption (e.g., Liebman and Dyck 1993; Teasdale and Mohler 2000) and potentially also via root exudates (e.g., Bais et al. 2006), volatile organic compounds (e.g., Barney et al. 2009), tissue leachate (e.g., Krogh et al. 2006), or microbial transformation of plant-derived compounds (e.g., Inderjit 2005) (i.e., allelopathy). Yet, current knowledge gaps have prevented adoption of allelopathy in many integrated weed management (IWM) programs (Duke 2010). Aqueous extracts of rye (*Secale cereale* L.) and wheat tissues chemically inhibit canola radicle elongation, and vegetative tissues of these species have greater allelopathic potential than reproductive tissues (Geddes et al. 2015).

Simultaneously seeding a winter cereal with a grain crop in spring prevents the winter cereal from reaching anthesis during the growing-season (e.g., Ateh and Doll 1996) and could facilitate greater alleopathic activity during this timeframe.

In cover crop-based systems (e.g., Halde et al. 2014; Mirsky et al. 2012), cereal rye is often paired with soybean (Mirsky et al. 2013). In these systems, cereal rye is commonly established in autumn, and physically or chemically terminated in the reproductive stage just before or after planting soybean the following spring (e.g., Bernstein et al. 2011; Smith et al. 2011). Seeding living rye mulch into or with soybean (inter-seeding) can facilitate interference with weeds also (Ateh and Doll 1996; Uchino et al. 2009). Inter-seeded rye in soybean, however, can use additional soil moisture and may have a detrimental impact on soybean yield under moisture limiting conditions (Ateh and Doll 1996; Thelen et al. 2004). Mid-season mulch termination during soybean canopy development may be necessary to minimize mulch-induced soybean yield loss and to maximize early-season weed interference (Ateh and Doll 1996; Thelen et al 2004). In HR soybean, broad-spectrum post-emergence (POST) herbicide application could be used to effectively terminate living mulches during the growing-season.

Several factors influence interference from inter-seeded living mulches, including: planting dates (Uchino et al. 2009), plant stand densities (Ateh and Doll 1996), plant spatial arrangement, resource niches and soil fertility (Liebman and Dyck 1993). For example, soybean yield loss was reduced with delayed rye inter-seeding (Uchino et al. 2009). However, if early-season interference with cool-season weeds is sought in warmer-season crops – like volunteer canola in soybean – simultaneous seeding of cover and grain crops may facilitate early-season interference and optimal crop yields.

Manitoba, Canada is located at the northern frontier of North American soybean production. The limited duration of frost-free days in this region (Nadler 2007) provides a narrow window for soybean production. Cool-season weed species are abundant in this region (Leeson et al. 2016). Many of these cool-season weed species grow rapidly in the early-season. In other regions, living rye mulch has been effective for weed interference, but the impact of living rye mulch on soybean yield is dependent on the environment (Ateh and Doll 1996; Thelen et al. 2004). Spring wheat is a cool-season crop that can be competitive in the early-season (Mason and Spaner 2006) and tolerant to volunteer canola interference at low densities (Seerey and Shirtliffe 2010). Inter-seeded spring wheat or winter cereal rye with short-season soybean varieties may provide early-season interference with volunteer canola in this short-season environment.

This study was designed to determine whether spring-established living inter-row cereal mulches could be used to increase early-season interference with volunteer canola in soybean. The objectives of this study were to evaluate the effects of (a) presence or absence of spring-established spring wheat or winter cereal rye living mulches growing between (b) 38 or 76 cm soybean row widths and (c) presence or absence of mid-season mulch termination on management of volunteer canola in soybean. It was hypothesized that spring-established living cereal mulches would reduce seed production of volunteer canola, but also reduce soybean yield relative to mulch absence. Soybean was expected to have greater yield and biomass accumulation when grown in 38 cm rows compared with 76 cm rows. It also was hypothesized that mid-season mulch termination would result in volunteer canola management and lessen the negative impact of mulch interference on soybean yield relative to mulches that remained live past soybean anthesis.

6.3 Materials and Methods

6.3.1 Field Experiment.

6.3.1.1 Experimental Sites. Cultural or physical tools for management of volunteer canola in soybean were evaluated at four experimental sites in 2013 and 2014 in Manitoba, Canada. Field research locations included the Ian N Morrison Research Station near Carman, MB in 2013 (49°29’35”N, 98°02’25”W) and 2014 (49°29’22”N, 98°02’26” W), the Richardson Kelburn Farm near Howden, MB in 2014 (49°41’33”N, 97°07’15”W) and the Westman Agricultural Diversification Organization near Melita, MB in 2014 (49°14’47”N, 101°01’01”W). These locations included both the Lake Manitoba Plain (Carman and Howden) and Aspen Parkland (Melita) ecoregions within the Prairie ecozone (CanSIS 2013). The previous crop in each year was wheat and field preparation consisted of autumn tillage via tandem disc followed by spring cultivation prior to study establishment. Data for mean daily temperature (°C) and total precipitation (mm) for each month were collected from the weather station nearest each site (Fig 6.1) (Environment Canada 2017).

6.3.1.2 Experimental Design and Treatment Structure. A split-block randomized complete block design (RCBD) with four experimental replications (blocks) per site was used to evaluate the effect of spring-established wheat or rye inter-row mulches for management of volunteer canola in soybean. Other cultural or physical weed management tools including soybean row widths, seeding densities, N fertilization, and inter-row tillage also were evaluated in the same

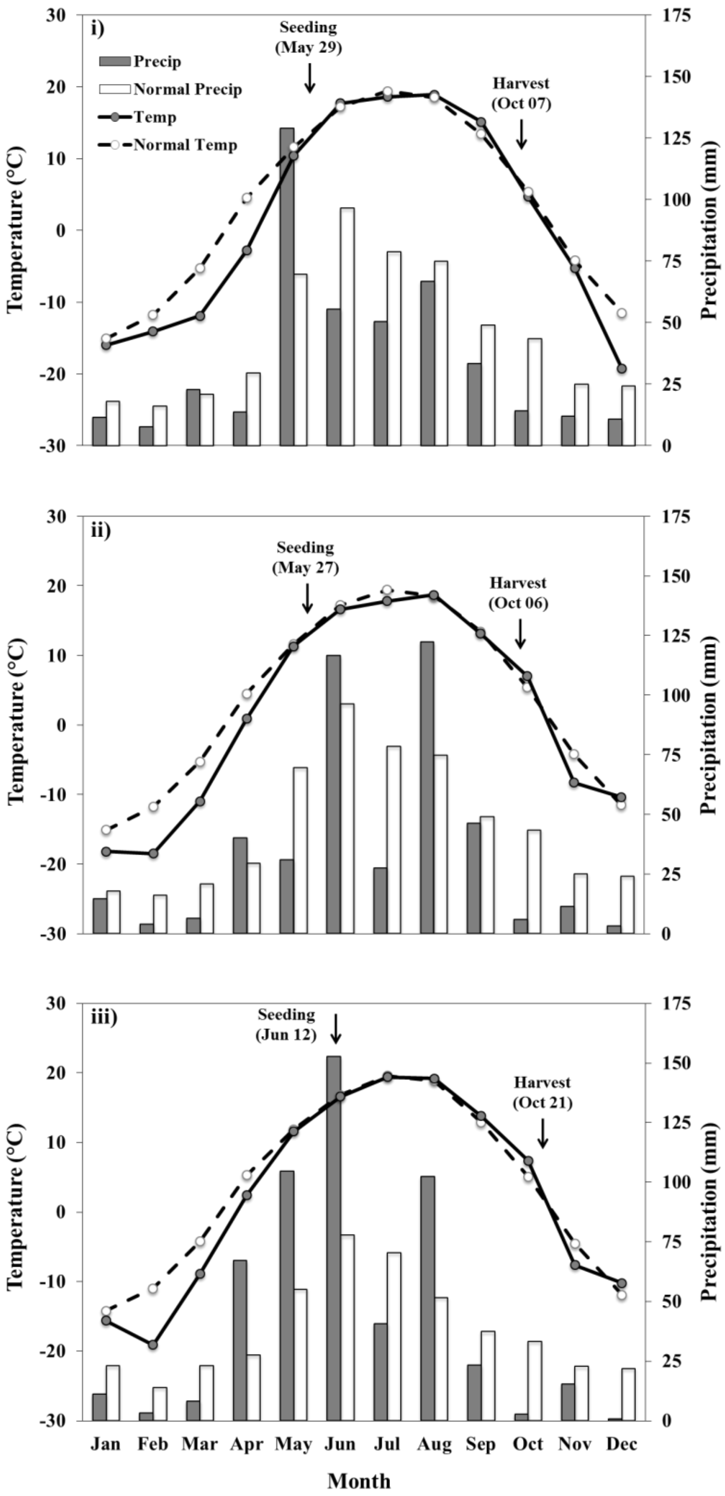

Figure 6.1. Mean daily air temperature (Temp; °C) and total precipitation (Precip; mm) for each month of the experiment and the regional climatic normal (1980-2010) mean daily air temperature (Normal Temp) and total precipitation (Normal Precip) for each month at the (a) Carman 2013, (b) Carman 2014 and (c) Melita 2014 sites. Seeding and harvest dates are indicated for each experiment. Adapted from: Environment Canada (2017).

field experiment. Data from these weed management tools were analyzed separately and are presented and discussed in Chapter 5. In the overall field experiment, main plots (2.5 x 8 m) consisted of cultural or physical weed management tools, while sub-plots (2.5 x 4 m) resulted from the application of herbicides with different modes of action (herbicide regime) across the front- or back-half of each block.

The treatments used in this study include soybean planted at 38 or 76 cm row widths alone or with spring-established spring wheat 'Kane' (71 kg ha^{-1} within-row seeding density) or spring-established winter cereal rye 'Hazlet' (78 kg ha^{-1} within-row seeding density) inter-row mulches. These mulches were seeded simultaneously between soybean rows at 19 cm row intervals [resulting in either alternating mulch and soybean rows (38 cm soybean row width) or three mulch rows between each soybean row (76 cm soybean row width)]. The same within-row mulch-seeding density was used between the two different soybean row widths. Soybean 'DKL 23-10 RY' (treated with Acceleron® containing: fluxapyroxad, pyraclostrobin, metalaxyl and imidacloprid; Monsanto Canada Inc., Winnipeg, MB, CA) was seeded at 2 cm depth using the recommended rate of 455,000 seeds ha^{-1} (432,000 target plants ha^{-1}) in all treatments. TagTeam® MultiAction® granular soybean inoculant (minimum of 1 x 10^{5} cfu *Penicillium bilaii* g^{-1} and 1 x 10^{8} viable *Bradyrhizobium japonicum* cells g^{-1}; Monsanto BioAg and Design™, Monsanto Company, St. Louis, MO, USA) was added in each soybean row at the recommended rate for each soybean row width (4.0 kg ha^{-1} in 38 cm and 1.6 kg ha^{-1} in 76 cm soybean row widths). Prior to seeding soybean, canola 'DKL 73-45 RR' (F1 generation hybrid treated with Acceleron® containing: difenoconaxole, metalaxyl, fludioxonil and thiamethoxam; Monsanto Canada Inc., Winnipeg, MB, CA) was seeded at 1 cm depth (19 cm row width) along each experimental block (perpendicular to soybean rows) using a seeding density of 80 seeds m^{-2}. At soybean stage BBCH 13, glyphosate (Roundup WeatherMax®, 360 g a.e. ha^{-1} glyphosate, Monsanto Canada Inc., Winnipeg, MB, CA) was used for POST broad-spectrum weed management in one half of each block and pinoxaden (in 2013; Axial® BIA, 59 g a.i. ha^{-1} pinoxaden, Syngenta Canada Inc., Guelph, ON, CA) or clodinafop-propargyl (in 2014; Horizon® 240EC, 54 g a.i. ha^{-1} clodinafop-propargyl, Score® Adjuvant, 0.80 L product ha^{-1}, Syngenta Canada Inc., Guelph, ON, CA) were used for selective monocotyledonous weed management in the other half of each block. In essence, glyphosate (the EPSPS herbicide regime) was used to terminate wheat and rye living mulches at stage BBCH 21 and pinoxaden or clodinafop-propargyl (the ACCase herbicide regime) were used for selective management of monocotyledonous weeds while allowing mulches to remain live (hereafter referred to as terminated and living mulches, respectively). All herbicides were applied at 276 kPa using AIXR 110015 TeeJet nozzles (TeeJet Technologies, Wheaton, IL, USA) and with 100 L ha^{-1} water carrier.

6.3.2 Data Collection. The densities of emerged soybean (BBCH 10-12), inter-row mulch (BBCH 13-14), and volunteer canola seedlings (BBCH 12-13) were determined by counting all soybean and mulch seedlings in 1 m of two adjacent rows and all volunteer canola seedlings within two 50 x 50 cm quadrats aligned with the edge of a soybean row. To determine shade avoidance, the height of three randomly selected plants of soybean, inter-row mulch and volunteer canola were measured from the soil surface to the distal end of the fully-extended shoot tissue. The plant height measurements were collected at 15, 30, 45 and 60 days after soybean emergence (DAE), and averaged for each species.

At canola stage BBCH 82 and soybean stage BBCH 77, the late-season densities of volunteer canola were determined and aboveground biomass of soybean, mulch and volunteer canola were collected from one 50 x 50 cm quadrat aligned with the edge of a soybean row in each experimental unit. Volunteer canola, soybean, and inter-row mulch plants were separated by species, biomass for each species was dried at 60°C until equilibrium, and dry weights were determined for each species. Aboveground canola biomass from each experimental unit was hand threshed and the collected seed was cleaned using a hand sieve (2.7 mm round hole, Can-Seed Equipment Ltd., Winnipeg, MB, CA) and seed blower (Agriculex, Model CB-1, Guelph, ON, CA). The total weight of canola seed produced per unit area (g seed m^{-2}) and individual seed weight (g thousand seeds^{-1}) were determined. These total (g seed m^{-2}) and individual (g thousand seeds^{-1}) seed weights were used to determine the total number of volunteer canola seeds produced per unit area (thousand seeds m^{-2}). Volunteer canola plant fecundity (seeds plant^{-1}) was determined by dividing the total number of seeds produced per unit area (no. seeds m^{-2}) by late-season canola plant densities (no. plants m^{-2}). The percentage of emerged volunteer canola seedlings that survived to maturity (%) was determined for each experimental unit by dividing the late-season plant density by the respective density of emerged volunteer canola seedlings.

At soybean maturity, each experimental unit was harvested using a Kincaid 8-XP single plot combine (Kincaid Equipment Manufacturing, Haven, KS, USA; concave clearance 18 mm, cylinder speed 625 rpm, fan speed 900 rpm, sieve 18 mm) and harvest samples were air-dried, cleaned using a clipper M2BC seed cleaner (Blount/Ferrell-Ross Bluffton, IN, USA), and soybean, wheat and volunteer canola were separated using hand sieves (4.7 mm and 2.7 mm round hole, Can-Seed Equipment Ltd., Winnipeg, MB, CA). Cleaned and separated soybean yield (kg ha^{-1}), wheat intercrop yield (kg ha^{-1}), and volunteer canola dockage (g m^{-2}) from harvest samples were weighed and adjusted to 13.0%, 13.5% and 8.5% moisture, respectively. Individual volunteer canola seed weight (g thousand seeds^{-1}) at maturity (harvest samples) was determined and also used to determine the number of volunteer canola seeds collected per unit area (no. seeds m^{-2}) (i.e., dockage) during harvest of soybean and wheat. Seedbank inputs, as a percentage of the total number of volunteer canola seeds (collected at biomass sampling) that were returned to the soil seedbank in each experimental unit was determined using equation 1.

$$[((\text{no. seeds m}^{-2}\text{ at biomass}) - (\text{no. seeds m}^{-2}\text{ in dockage}))/(\text{no. seeds m}^{-2}\text{ at biomass})]\text{ x }100 \qquad [1]$$

6.3.2 Statistical Analysis. The inter-row mulch objectives encompassed unique and detailed hypotheses that warranted separate analyses from the other weed management treatments (in Chapter 5). For this reason, a treatment substructure design (Piepho et al. 2006) was used to separate and analyze the 12 treatment full factorial treatment substructure composed of two soybean row widths (38 or 76 cm), three mulch species (spring wheat, winter cereal rye or no mulch) and two herbicide regimes (EPSPS or ACCase). For each response variable, the Shapiro-Wilk test was used to test the assumption of normality (Littell et al. 2006) and visual inspection of residual vs. predicted values was used to examine homogeneity of variance (Kozak and Piepho 2017). Lund's test (Lund 1975) was used to remove extreme outliers and the square root-transformation was used when necessary to meet the assumptions of normality and/or homoscedasticity.

The MIXED procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) was used to analyze all response variable data. The soybean response variables included seedling emergence, plant height, aboveground biomass and yield. The volunteer canola response variables included seedling emergence, seedling survival to maturity, plant height, aboveground biomass, plant fecundity, seed production per unit area and seedbank inputs. Seedling emergence, plant height, aboveground biomass and seed yield (wheat) of spring-established inter-row mulches also were analyzed using the MIXED procedure. With the exception of plant height data, all volunteer canola and soybean response variables were analyzed using the main and interaction effects of soybean row width (2 levels), herbicide regime (2 levels), mulch species (3 levels) and experimental site [3 levels; Howden 2014 was removed from all analyses (see Chapter 6.4.5)] as fixed effects. To conserve the experimental error of the entire experiment, all management treatments – including those not in the inter-row mulch factorial treatment substructure – were included in the ANOVA (Piepho et al. 2006). With this in mind, the random effects included experimental block nested within site, the interaction effect of management treatment (from the entire experiment; 10 levels) and experimental block nested within site and the interaction effect of herbicide regime and experimental block nested within site. The analyses of mulch emergence and aboveground biomass focused on the subset of treatments containing living mulches only. This subset of mulch treatments formed a RCBD. The model for analyses of mulch emergence and biomass used the main and interaction effects of soybean row width (2 levels), response species (2 levels; wheat or rye) and experimental site (3 levels) as fixed effects, while experimental block nested within site was considered a random effect. The analysis of wheat intercrop yield focused only on the experimental units containing living wheat and formed a RCBD also. In this analysis, the main and interaction effects of soybean row width (2 levels) and experimental site (3 levels) were considered fixed effects and experimental block nested within site was considered a random effect.

A hierarchical approach to mixed model ANOVA was used to trim the number of parameters (factors) in the factorial models to achieve the model of best fit based on minimization of Akaike's Information Criterion (AIC) and the likelihood ratio test (Luschei and Jackson 2005). To further adjust for homoscedasticity, the repeated within-group covariance structure of residuals was selected based on minimization of AIC (Onofri et al. 2010) when justified using the likelihood ratio test (Luschei and Jackson 2005). *Post hoc* multiple mean comparisons were conducted using Tukey's honest significant difference (HSD) ($\alpha = 0.05$) and mean letter separations were generated using the pdmix800 macro (Saxton 1998). Additional ANCOVA was used to test the influence of any unequal soybean, canola and mulch emergence densities (when applicable) on the significance of effects on the other response variables (Littell et al. 2006).

Within each site, plant heights of volunteer canola and soybean were analyzed together using a repeated measures split-block RCBD (Littell et al. 2006) with the same treatment substructure (Piepho et al. 2006) and hierarchical model reduction approach (Luschei and Jackson 2005) described above. Due to presence of living mulch in one subset of treatments only, soybean and volunteer canola plant heights were analyzed separately from the heights of the mulch species. The analysis of soybean and volunteer canola plant height used the main and interaction effects of soybean row width (2 levels), herbicide regime (2 levels), mulch species (3 levels), response species (2 levels; volunteer canola or soybean) and measurement date (4 levels) as fixed effects.

Experimental block, the interaction effect of management treatment (10 levels) and experimental block and the interaction effect of herbicide regime and experimental block were considered random effects. For the analysis of living mulch plant height, the fixed effects included soybean row width (2 levels), response species (2 levels; wheat or rye) and measurement date (4 levels), while experimental block was considered a random effect. In each analysis, measurements of the individual experimental units (sub-plots) were repeated in time and the toeplitz covariance structure was fit based on minimization of AIC (Littell et al. 2006). *Post hoc* multiple mean comparisons were conducted using Tukey's HSD ($\alpha = 0.05$) and mean letter separations were generated using the pdmix800 macro (Saxton 1998).

6.4 Results and Discussion

6.4.1 Mulch Species.

6.4.1.1 Mulch Presence or Absence. The presence of inter-row mulches in soybean decreased volunteer canola seed production by about one-third (by about 7,200 to 9,000 seeds m^{-2}) among sites, regardless of whether mulches were terminated or living (Fig 6.2). Similar to Chapter 5, about 51% to 67% of the volunteer canola seeds were returned to the soil seedbank in soybean (among sites) (Table 6.1). Presence or absence of inter-row mulches in soybean did not affect the percentage of volunteer canola seeds returned to the seedbank (Table 6.2). Taken together, these data indicate that inclusion of inter-row mulches in soybean reduced absolute volunteer canola seedbank inputs by about 3,700 to 6,000 seeds m^{-2}. This reduction in absolute seedbank inputs was about the same as the average seedbank inputs that occur during harvest of a canola crop in this region (Cavalieri et al. 2016). In western Canada, a single cohort of volunteer canola seed can survive for more than three years in the soil seedbank (Gulden et al 2003b). Following

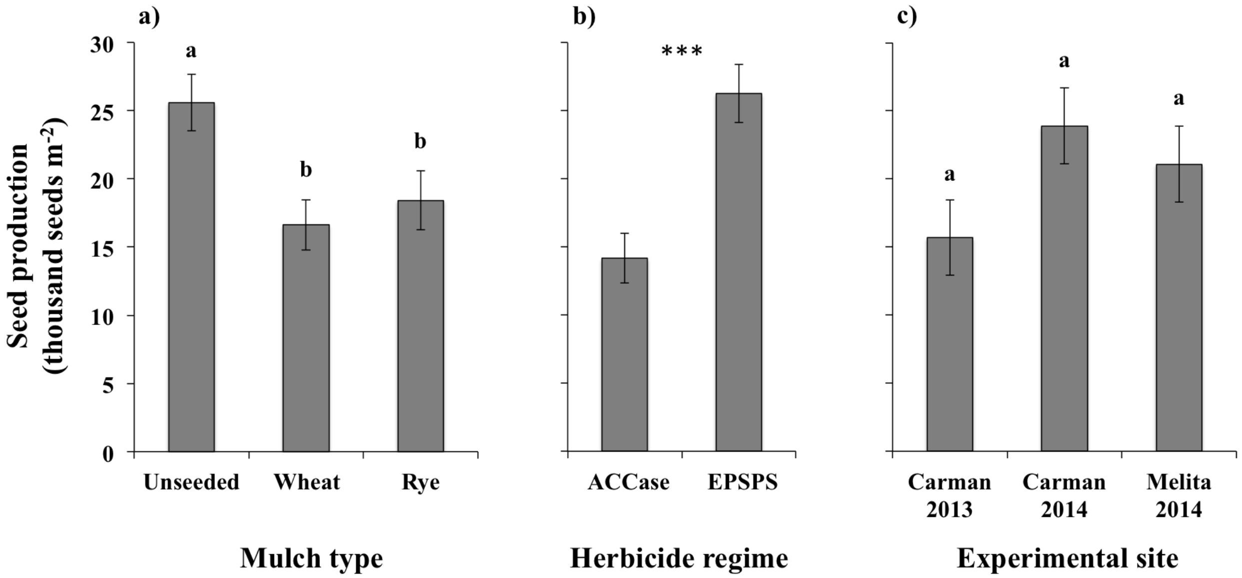

Figure 6.2. The total number of volunteer canola seeds produced per unit area in soybean in (a) the presence or absence of spring-established wheat or rye inter-row mulches (mulch species) in a combined site analysis, (b) two different herbicide regimes in a combined site analysis and (c) at each experimental site individually. Error bars indicate ± one standard error of the mean. Within sub-figures where multiple comparisons are made, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$). Within sub-figures where a single mean comparison is made, *** indicates a significant F-test effect at $P < 0.001$.

Table 6.1. The percentage of volunteer canola seeds that were returned to the soil seedbank in soybean in two different herbicide regimes at three experimental sites individually and in a combined analysis. [a,b]

Herbicide regime	Experimental site			
	Carman 2013	Carman 2014	Melita 2014	Combined
		%		
ACCase	59 ± 4.9	36 ± 5.7 b	59 ± 5.1	51 ± 3.0 b
EPSPS	58 ± 5.1	75 ± 2.2 a	67 ± 6.1	67 ± 2.7 a

[a] Values are means ± one standard error of the mean.

[b] Within columns, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

Table 6.2. ANOVA results (*P*-values) for the main and interaction effects of soybean row width, herbicide regime, mulch species and experimental site on each soybean and volunteer canola response variable.

	Response variables[a,b]									
	Soybean			Volunteer canola						
Source	Seedling emergence	Aboveground biomass	Yield	Seedling emergence	Seedling survival	Aboveground biomass	Individual seed weight	Plant fecundity	Seed production	Seedbank inputs
	P-values									
Row width (R)	**0.003**	**< 0.001**	**0.007**	ns	ns	ns	ns	ns	ns	ns
Herbicide regime (H)	**< 0.001**	**< 0.001**	**< 0.001**	ns	ns	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.013**
Mulch species (M)	ns	ns	ns	ns	ns	**< 0.001**	**0.024**	**0.001**	**< 0.001**	ns
Experimental site (E)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**[c]	**< 0.001**	**< 0.001**	**< 0.001**	**0.021**[d]	ns
R x H	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R x M	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R x E	**< 0.001**	ns	**< 0.001**	ns	ns	ns	ns	ns	ns	ns
H x M	ns	ns	**0.001**	ns	ns	**0.025**	ns	ns	ns	ns
H x E	**< 0.001**	**< 0.001**	**< 0.001**	ns	ns	ns	**< 0.001**	**0.013**	ns	**< 0.001**
M x E	ns	ns	ns	ns	**0.025**	ns	ns	ns	ns	ns
R x H x M	**0.006**	ns	ns	ns	ns	ns	ns	ns	ns	ns
R x H x E	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R x M x E	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
H x M x E	ns	ns	**0.045**	ns	ns	ns	**0.010**	ns	ns	ns
R x M x H x E	**0.003**	ns	ns	ns	ns	ns	ns	ns	ns	ns

[a] Bold values indicate significant effects ($P < 0.05$).

[b] ns indicates lack of a significant effect ($P \geq 0.05$).

[c] Indicates loss of statistical significance ($P \geq 0.05$) after including volunteer canola seedling emergence as a covariate.

[d] Indicates loss of statistical significance ($P \geq 0.05$) after including soybean seedling emergence as a covariate.

Table 6.3. The response of volunteer canola plant fecundity in soybean to the presence or absence of spring-established wheat or rye inter-row mulches (mulch species) or to two different herbicide regimes at three experimental sites individually and in a combined analysis. [a,b]

Herbicide regime	Mulch species	Experimental site			Combined
		Carman 2013	Carman 2014	Melita 2014	
		no. seeds plant^{-1}			
ACCase		270 ± 33 b	330 ± 61 b	550 ± 57	380 ± 30 b
EPSPS		590 ± 64 a	870 ± 74 a	890 ± 84	780 ± 43 a
	Unseeded	-[c]	-	-	700 ± 42 a
	Wheat	-	-	-	530 ± 42 b
	Rye	-	-	-	520 ± 42 b

[a] Values are means ± one standard error of the mean.

[b] Within columns and effect groupings, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

[c] A dash (-) indicates lack of significant F-test effect ($P \geq 0.05$).

Table 6.4. The percentage of volunteer canola seedlings that survived to maturity in soybean in the presence or absence of spring-established wheat or rye inter-row mulches (mulch species) at three experimental sites individually and in a combined analysis. [a,b]

Mulch species	Experimental site			Combined
	Carman 2013	Carman 2014	Melita 2014	
	%			
Unseeded	138 (11.8 ± 0.63)	49 (7.0 ± 0.35)	62 (7.9 ± 0.82)	76 (8.7 ± 0.23)
Wheat	89 (9.4 ± 0.50)	42 (6.5 ± 0.40)	68 (8.2 ± 0.51)	|[c]
Rye	90 (9.5 ± 0.55)	57 (7.6 ± 0.94)	112 (10.6 ± 1.07)	|

[a] Values are back-transformed square root means, while parenthetical values are square root-transformed means ± one standard error of the square root-transformed mean.

[b] Within columns, significant differences were not observed based on Tukey's HSD ($\alpha = 0.05$).

[c] A bar (|) indicates continuation of the entry above due to lack of significant difference ($P \geq 0.05$) among mulch species.

canola seedbank additions in autumn, up to 44% of the seedbank can survive the winter (Gulden et al. 2003b) and if the seedbank is disturbed in spring, volunteer canola seedling recruitment in this region averages 11% of viable seed in the seedbank (Chapter 3). These assumptions indicate that the inclusion of inter-row mulches to manage volunteer canola in soybean – at the densities observed in the current study (39 to 92 volunteer canola plants m^{-2}) – could reduce the density of volunteer canola seedlings that emerge during the following spring by up to 290 seedlings m^{-2}. Clearly, the contribution of inter-row mulches to reduced absolute volunteer canola seedbank inputs in soybean could reduce densities of this weed in subsequent crops.

The reduction in absolute number of volunteer canola seeds produced per unit area in the presence compared with the absence of inter-row mulches in soybean was due to a reduction in volunteer canola plant fecundity and not a reduction in plant survival to maturity (Tables 6.3 and 6.4; Fig 6.2). The presence of inter-row mulches in soybean reduced volunteer canola plant fecundity by about one-third compared with the absence of inter-row mulches (Table 6.3). Volunteer canola plant survivorship was unaffected by mulch treatments (Table 6.4). All species were seeded on the same day. Perhaps differences in stand thinning of volunteer canola were not observed because all species emerged near the same point in time, thereby resulting in seedling establishment prior to the onset of direct competition.

The presence of inter-row cereal mulches for management of volunteer canola resulted in the same soybean yield as the absence of inter-row mulches (Table 6.5). The presence of inter-row mulches also did not influence soybean biomass accumulation relative to the respective without-mulch controls (Table 6.2). It is likely that spring-established cereal mulches alone would have interfered with soybean to a certain extent. This could have been determined by including treatments with soybean and inter-row mulches in the absence of volunteer canola. These treatments, however, were not included in the current study. Nevertheless, the lack of difference in soybean yield and biomass accumulation between the presence and absence of inter-row mulches in the current study suggested that mulches and volunteer canola occupied the same niche space and had no additional overlap with soybean.

6.4.1.2 Differences Between Mulch Species. Living rye mulch reduced later-season volunteer canola and soybean plant heights at Carman in 2013 and 2014 (Table 6.6). A similar trend was observed at Melita 2014 (Table D1). The presence of living wheat mulch had no effect on plant heights of volunteer canola or soybean. At 45 and 60 DAE, the height reductions in volunteer canola and soybean attributed to living rye mulch at Carman 2013 and 2014 were about 13% compared with living wheat mulch or the absence of mulch altogether (Table 6.6). Double the aboveground biomass accumulation (Fig 6.3) and consistently greater plant height (Fig 6.4) in spring-established wheat compared with rye mulches suggested a greater capability of living wheat mulches to compete with weeds. However, living rye mulches caused a reduction of volunteer canola and soybean plant heights, while living wheat mulches did not (Table 6.6). Shade avoidance response in soybean commonly manifests as increased plant height (e.g., Green-Tracewicz et al. 2011). Canopy closure is not needed to induce a shade avoidance response in soybean (Green-Tracewicz et al. 2011). Even light reflected from neighboring plants may induce morphological changes related to shade avoidance as early as the hypocotyl arch stage in soybean (McKenzie-Gopsill et al. 2016), well before the onset of direct plant competition. These reports imply that shade avoidance response in soybean was likely present in all treatments in the current study due to the presence of mulch or weed neighbors, regardless of plant height. Winter cereal rye has high allelopathic potential (e.g., Geddes et al. 2015; Schulz et al. 2013). Perhaps allelopathy in rye reduced or offset the shade avoidance response in volunteer canola and soybean causing reduced plant height. Similar to previous work (e.g., Barnes and

Table 6.5. The response of soybean yield in the presence of volunteer canola to soybean row width or to the presence or absence of spring-established wheat or rye inter-row mulches (mulch species) with (EPSPS) or without (ACCase) mid-season mulch termination using two different herbicide regimes at three experimental sites individually and in a combined analysis. [a,b]

Soybean row width	Herbicide regime	Mulch species [c]	Experimental site			Combined
			Carman 2013	Carman 2014	Melita 2014	
			kg ha^{-1}			
38 cm			2,080 ± 116	1,010 ± 116 a	1,320 ± 116	1,410 ± 67
76 cm			1,800 ± 116	650 ± 116 b	1,130 ± 116	1,260 ± 67
	ACCase	Absent	1,920 ± 127 ab	680 ± 127	1,170 ± 127	1,260 ± 73 bc
	| [d]	Wheat (L)	1,490 ± 127 b	680 ± 127	1,160 ± 127	1,110 ± 73 c
	|	Rye (L)	1,480 ± 127 b	580 ± 127	1,050 ± 127	1,040 ± 73 c
	EPSPS	Absent	2,140 ± 144 a	920 ± 144	1,320 ± 144	1,460 ± 83 ab
	|	Wheat (T)	2,330 ± 144 a	1,120 ± 150	1,510 ± 144	1,650 ± 84 a
	|	Rye (T)	2,270 ± 144 a	990 ± 144	1,120 ± 144	1,460 ± 83 ab

[a] Values are means ± one standard error of the mean.

[b] Within columns and effect groupings, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

[c] Abbreviations: L, living; T, terminated mid-season.

[d] A bar (|) indicates continuation of the entry above.

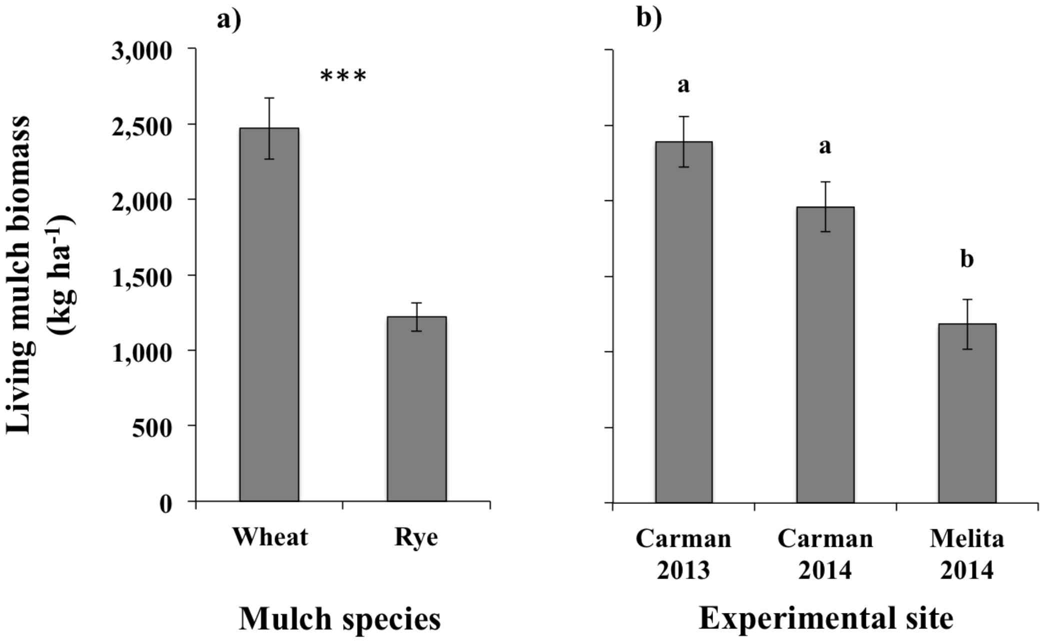

Figure 6.3. Spring-established inter-row cereal mulch aboveground biomass accumulation under volunteer canola interference in soybean for (a) each mulch species in a combined site analysis and (b) each experimental site individually. Error bars indicate ± one standard error of the mean. Within sub-figures where multiple comparisons are made, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$). Within sub-figures where a single mean comparison is made, *** indicates a significant F-test effect at $P < 0.001$.

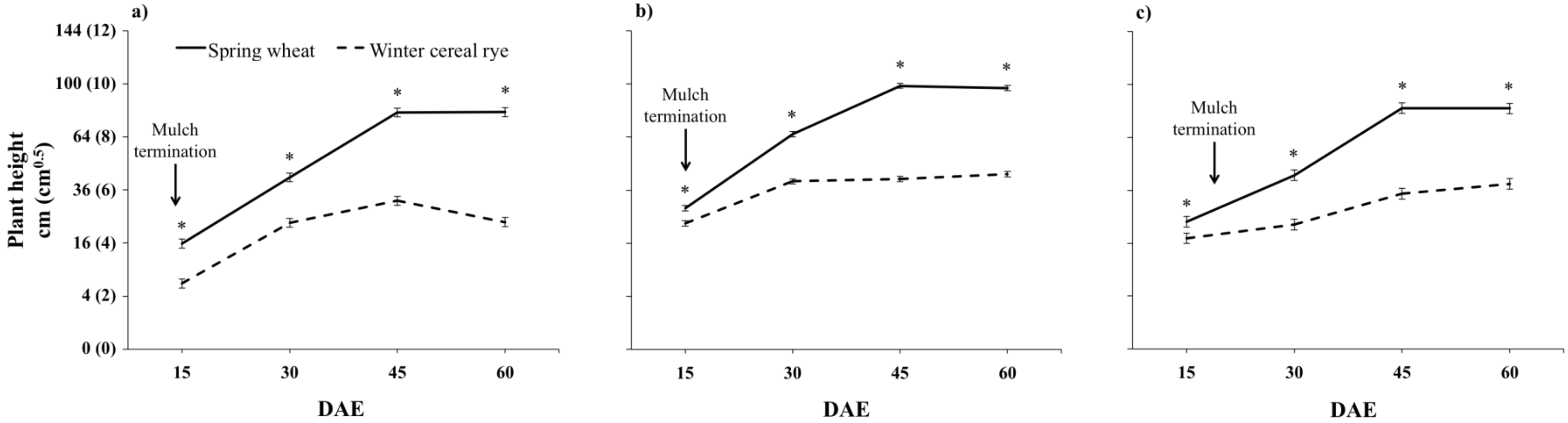

Figure 6.4. Mean plant heights of spring-established inter-row living wheat or rye mulches used to manage volunteer canola in soybean. Plant heights were measured at 15, 30, 45 and 60 days after soybean emergence (DAE) and are shown separately for the (a) Carman 2013, (b) Carman 2014 and (c) Melita 2014 experimental sites. The date of mulch termination is indicated for each site, beyond which, minimal growth of terminated mulches was observed. Values on the y-axis are the back-transformed square root scale with the square root-transformed scale in parentheses. Error bars indicate ± one standard error of the square root-transformed mean. An asterisk (*) indicates a significant difference between mulch species based on Tukey's HSD ($\alpha = 0.05$).

Table 6.6. The response of soybean and volunteer canola mean plant heights at 15, 30, 45 and 60 days after soybean emergence (DAE) to the interaction effect of herbicide regime, mulch species and measurement date in individual analyses conducted within the Carman 2013 and Carman 2014 sites.

Experimental site	Herbicide regime	Mulch species[a]	Measurement date[b,c,d]			
			15 DAE	30 DAE	45 DAE	60 DAE
			cm			
Carman 2013	ACCase	Absent	15 (3.9)	45 (6.7)	67 (8.2) a	71 (8.4) bc
│[e]	│	Wheat (L)	16 (4.0)	46 (6.7)	66 (8.1) a	73 (8.6) bc
│	│	Rye (L)	15 (3.9)	45 (6.7)	58 (7.6) b	68 (8.2) c
│	EPSPS	Absent	16 (4.0)	44 (6.6)	68 (8.3) a	79 (8.9) ab
│	│	Wheat (T)	16 (4.1)	41 (6.4)	67 (8.2) a	78 (8.8) ab
│	│	Rye (T)	16 (4.0)	42 (6.4)	67 (8.2) a	82 (9.0) a
			(± 0.10)[f]	(± 0.10)	(± 0.10)	(± 0.10)
Carman 2014	ACCase	Absent	22 (4.6)	69 (8.3) a	90 (9.5) a	93 (9.7) a
│	│	Wheat (L)	22 (4.6)	66 (8.2) a	88 (9.4) ab	91 (9.6) a
│	│	Rye (L)	20 (4.5)	59 (7.7) b	73 (8.5) c	79 (8.9) b
│	EPSPS	Absent	22 (4.6)	66 (8.1) a	87 (9.3) ab	90 (9.5) a
│	│	Wheat (T)	22 (4.6)	59 (7.7) b	82 (9.1) ab	87 (9.4) a
│	│	Rye (T)	20 (4.5)	57 (7.6) b	82 (9.0) b	86 (9.3) a
			(± 0.08)	(± 0.08)	(± 0.08)	(± 0.08)

[a] Abbreviations: L, living; T, terminated mid-season.
[b] Values are back-transformed square root means with square root-transformed means in parentheses.
[c] Within columns and effect groupings, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).
[d] Abbreviation: DAE, days after soybean emergence.
[e] A bar (|) indicates continuation of the entry above.
[f] Within columns, parenthetical values preceded by ± indicate the standard error of the square root-transformed means above.

Putman 1983), these results suggest the contribution of allelopathy to plant interference by living rye.

6.4.2 Mulch Termination. At Carman 2013, soybean yields were about one-third lower in the presence of living mulches compared with mulches terminated mid-season (Table 6.5). A similar trend was observed at Carman and Melita in 2014. Living mulches can use additional soil moisture, which may reduce soybean yield if precipitation throughout the growing-season is insufficient (e.g., Ateh and Doll 1996; Thelen et al. 2004). Unfortunately, plant available soil moisture was not measured in the current study. However, a potentially greater moisture deficit due to below normal precipitation at Carman in 2013 may have contributed to reduced yield in the presence of living compared with terminated mulch systems (Table 6.5; Fig 6.1).

Greater mulch density (about 2- to 4-fold) at Carman in 2013 compared with Carman or Melita in 2014 also may have contributed to reduced soybean yield when mulches remained live compared with mid-season mulch termination (Tables 6.5 and D2). Ateh and Doll (1996) reported reduced soybean yield at higher seeding densities of inter-seeded living rye mulch (seeding densities of 56 vs. 112 vs. 168 kg ha^{-1}). In their study, soybean yield reductions at increased rye mulch seeding densities only were observed at the site that had notably lower than average precipitation. This was explained by a reduction in soil moisture as the density of living rye mulch increased. Furthermore, greater soybean yield was observed in the presence compared with the absence of mid-season mulch termination at high mulch seeding densities only (seeding densities of 112 vs. 56 kg ha^{-1}). The results from the current study correspond with those of Ateh and Doll (1996) and showed that mid-season termination of spring-established cereal mulches may alleviate soybean yield reductions due to mulch interference at higher densities. Increased mulch densities at Carman 2013, however, did not result in improved management of volunteer canola compared with the other sites (Figs 6.2 and 6.5). Together, these data suggest that low mulch seeding densities may be possible to optimize weed management while mitigating potential mulch-induced soybean yield loss.

Nitrogen assimilation by living mulches likely contributed to the observed reductions in volunteer canola biomass and seed production (Figs 6.2 and 6.5), as reductions in biomass and seed production were not observed in soybean to the same degree (Table 6.5). Canola growth

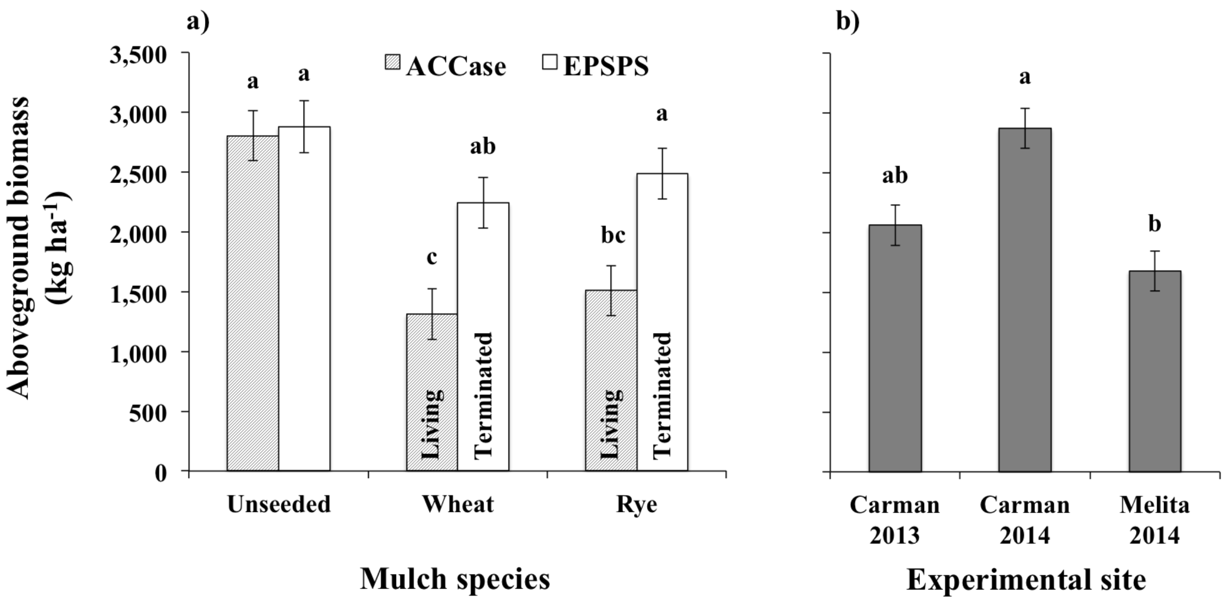

Figure 6.5. Aboveground volunteer canola biomass accumulation in soybean in (a) the presence or absence of spring-established wheat or rye inter-row mulches (mulch species) with (EPSPS) or without (ACCase) mid-season mulch termination using two different herbicide regimes in a combined site analysis and (b) each experimental site individually. Error bars indicate ± one standard error of the mean. Within sub-figures, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

and development is dependent on soil mineral N (Blackshaw et al. 2003). Soybean growth and development, however, does not require mineral N to the same extent because soybean has the capacity to enter symbioses with diazotrophic organisms that fix atmospheric N, with maximum symbiotic N-fixation occurring between soybean stages BBCH 71-80 (Salvagiotti et al. 2008). The assimilation of mineral N was not measured in the current study. Nevertheless, mulches that were allowed to remain live would have continued to assimilate soil mineral N. Potentially greater total soil mineral N assimilation by living mulches likely contributed to the reduction in volunteer canola biomass compared with the mulches that were terminated mid-season. Perhaps in future, living mulches could be used before or in a soybean crop to temporarily assimilate soil mineral N and thereby reduce interference from non-N-fixing weeds like volunteer canola. This hypothesis is complemented by a report of N-starvation of redroot pigweed in soybean grown subsequent to termination of a winter cereal rye cover crop (Wells et al. 2013).

In the ACCase herbicide regime, unmanaged broadleaf weeds may have contributed to reduced soybean biomass, reduced volunteer canola plant fecundity, seed production per unit area, seedbank inputs (Tables 6.1, 6.3 and 6.7; Fig 6.2) and increased individual volunteer canola seed weight at maturity (Appendix D, Table D3). Lack of a herbicide regime by mulch species interaction effect, but presence of a herbicide regime main effect in these response variables infers that the effects observed in the presence of inter-row mulches also were observed in the without-mulch controls in each herbicide regime (Table 6.2). Unmanaged weeds other than volunteer canola were minimal and did not warrant biomass collection. However, these results suggest that unmanaged broadleaf weeds may have been influential under the ACCase-only herbicide regime and contributed to similar effects observed in the treatments with living inter-row mulches and the respective without-mulch controls.

Table 6.7. Aboveground soybean biomass accumulation in the presence of volunteer canola in response to soybean row width or to two different herbicide regimes at three experimental sites individually and in a combined analysis. [a,b]

Soybean row width	Herbicide regime	Experimental site			Combined
		Carman 2013	Carman 2014	Melita 2014	
		kg ha^{-1}			
38 cm		-[c]	-	-	3,080 ± 150 a
76 cm		-	-	-	2,530 ± 150 b
	ACCase	3,490 ± 279 b	1,200 ± 216	2,470 ± 286	2,380 ± 152 b
	EPSPS	5,450 ± 365 a	1,690 ± 206	2,520 ± 271	3,220 ± 166 a

[a] Values are means ± one standard error of the mean.

[b] Within columns and effect groupings, different letters indicate significant differences based on Tukey's HSD (α = 0.05).

[c] A dash (-) indicates lack of significant F-test effect ($P \geq 0.05$).

6.4.3 Soybean Row Width. Aboveground soybean biomass was on average 18% lower in the 76 cm compared with the 38 cm soybean row width regardless of the presence or absence of inter-row mulches (Table 6.7). Reduced soybean biomass using the 76 cm row width corresponded to similar trends in soybean yield and resulted in a significant yield difference at Carman 2014 only (Table 6.5). Reduced soybean yield in the 76 cm compared with the 38 cm row width may have been due to delayed canopy closure (Willcott et al. 1984) or greater in-row intraspecific soybean competition. These effects were not evaluated directly in the current study. The observed reductions in soybean biomass accumulation at wide-row widths were similar to those reported in Chapter 5. In Chapter 5, a negative association of soybean biomass accumulation and soybean row width was observed in the presence of volunteer canola. Like the current study, Chapter 5 reported a similar trend in soybean yield where yield tended to be reduced when soybean was grown using a wide-row width. Together, these results suggest that the soybean yield reductions observed when soybean was grown using wide- compared with medium-row widths were variable and not large enough to consistently result in significant yield differences in the presence of volunteer canola.

6.4.4 Mulch Growth and Development. Living inter-row wheat mulches produced about twice as much late-season biomass compared with rye and greater overall biomass accumulation was observed in Carman 2013 and 2014 compared with Melita 2014 (Fig 6.3). Unlike living rye mulch, wheat mulch entered reproductive development, resulted in greater plant height than rye (Fig 6.4) and consistently yielded 720 ± 60 kg seed ha^{-1} when collected with soybean at harvest (data not shown). This additional harvestable product could be used as an additional source of income for growers and may contribute to alleviation of the additional seed cost required for establishment of inter-row cereal mulches.

6.4.5 Experimental Site. Howden 2014 was omitted from all inter-row mulch analyses because glyphosate drift terminated all inter-row mulch treatments. Of the remaining sites, about double the density of volunteer canola seedlings emerged at Carman 2014 compared with Carman 2013 or Melita 2014 (92, 39 and 48 ± 4.8 plants m^{-2}, respectively). In Chapter 5, significance of weed management treatment effects on soybean and volunteer canola response variables was found at sites with higher volunteer canola densities, while similar trends at lower density sites generally did not manifest into significant differences. In the current study, the presence or absence of significant effects of inter-row mulches on soybean and volunteer canola response variables did not correspond to differences in volunteer canola seedling emergence among sites. For this reason, analyses were not separated into volunteer canola density classes.

The living rye mulch between 38 cm soybean rows resulted in about one-third lower soybean seedling emergence than the living wheat mulch or the respective without-mulch control at Carman 2014 only (Table D4). The reason for differential soybean emergence was unclear, however, ANCOVA revealed that differences in soybean, volunteer canola or mulch emergence densities had minimal influence on the statistical significance of the effects observed on each response variable (Tables 6.2 and D5) and therefore were not corrected for in these data.

6.5 Conclusion

Spring-established inter-row cereal mulches were a viable option for interference with volunteer canola in soybean grown at the northern frontier of North American soybean production.

Soybean yield under volunteer canola interference remained the same in the presence and absence of inter-row mulches. Spring wheat or winter cereal rye mulches reduced volunteer canola seed production by about one-third compared with the without-mulch controls. This resulted in a reduction of absolute volunteer canola seedbank inputs in soybean by up to 6,000 seeds m^{-2}. Soybean yield loss in the presence of volunteer canola and inter-row mulches tended to be reduced when mulches were terminated mid-season. Volunteer canola seed production was the same regardless of termination regime of the inter-row mulches. These results suggest that early-season interference from inter-row cereal mulches, prior to mulch termination, was likely responsible for the observed reductions in volunteer canola seed production. Spring-established inter-row cereal mulches in soybean can increase early-season interference with problematic weeds like volunteer canola and may therefore be an effective part of a comprehensive IWM strategy for soybean production in short-season growing environments.

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7.0 Exploiting Tilman's Resource-Ratio Theory for Weed Management in Legume Crops – Soybean vs. Volunteer Canola

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7.1 Abstract

Poor competitive ability and sensitivity to many herbicides create challenges for weed management in legume production. The exploitation of Tilman's resource-ratio theory may provide nitrogen (N)-fixing legumes with a competitive advantage over non-leguminous weed species. A field study was conducted to test whether the level of soil mineral nitrogen (N) affected yield loss of an annual legume crop (soybean; *Glycine max*) in the presence of four different populations of an interfering non-leguminous weed (volunteer canola; *Brassica napus*). The experiment consisted of banding five rates of urea fertilizer (0-180 kg N ha^{-1}) prior to seeding volunteer canola and soybean and was repeated thrice in Manitoba, Canada. Soybean yield remained unaffected in the absence of volunteer canola. Interference from the volunteer canola populations consistently caused a linear decrease in soybean yield by 2.6 kg ha^{-1} for every kg ha^{-1} increase in soil mineral N. In the presence of volunteer canola, soybean yield decreased by 17% from the lowest to the highest soil mineral N additions among environments (residual nitrate-N plus fertilizer N; 30-251 kg N ha^{-1}). In the lowest N conditions (30 kg N ha^{-1}), soybean yield was the greatest (3,350 kg ha^{-1} among environments) and volunteer canola seed production and aboveground biomass were the lowest (about one-quarter of the maximum volunteer canola seed production and aboveground biomass in 2016). Therefore, growing legume crops on fields with lower soil mineral N may reduce interference from unmanaged weeds. As N fertilization intensifies interference of many other weed species, tailoring weed management in legume crops around their capacity for N-fixation may facilitate inclusion of legume species in crop rotations.

7.2 Introduction

Legumes grown for seed production can benefit cropping systems and contribute to the diversity of crop rotations. Most notably, legumes can form symbioses with nitrogen (N)-fixing bacteria and access pools of atmospheric N (dinitrogen gas) unavailable to most other plant species (Walley et al. 2007). However, legumes are sensitive to many herbicide modes of action and generally have reduced competitive ability compared with other crops. These qualities make weed management particularly challenging in legumes. Unmanaged weeds in legume crops can result in yield losses and weed seedbank additions. The development of integrated weed management (IWM) strategies based on the unique characteristics of legume crops may facilitate their inclusion in crop rotations and contribute to cropping system sustainability.

Nitrogen (N) is the most yield-limiting macronutrient in cropping systems (Jarrell 1990). Nitrogen fertilizer application is frequently studied with focus on crop response only, while potential impacts on non-target organisms are often ignored (Di Tomaso 1995). In fact, certain weed species common in cropping systems can respond to soil mineral N more than (or similar to) crop species (e.g., Blackshaw et al. 2003). Greater N assimilation by weeds results in increased weed biomass and seed production (e.g., Blackshaw et al. 2003; Tungate et al. 2006). In this context, fertilizer application may increase interference from unmanaged weeds with present and potentially future crops (e.g., Blackshaw et al. 2004b).

Timing, placement and source of N fertility all have been studied with respect to their potential impact as cultural weed management tools in field crop production (e.g., Blackshaw et al. 2004b; Di Tomaso 1995). For example, placing N fertilizer in bands beneath the soil surface rather than surface broadcasting reduced the competitive ability of wild oat (*Avena fatua* L.) (Kirkland and Beckie 1998), jointed goatgrass (*Aegilops cylindrical* Host.) (Mesbah and Miller 1999), foxtail barley (*Hordeum jubatum* L.) (Blackshaw et al. 2000) and downy brome (*Bromus tectorum* L.) (Rasmussen 1995). Nitrogen use efficiency (Liebman 1989; Liebman and Robichaux 1990) and the effect of spatial dynamics of crop species on nutrient utilization (Johri et al. 1992) also may influence the competitive balance among crop and weed species (Di Tomaso 1995). However, a paucity of information is available on the impact of soil mineral N on weed interference in legume crops. Crop species with the capacity to fix atmospheric N (i.e., legumes) may have a competitive advantage over weeds in lower-N conditions.

Soybean [*Glycine max* (L.) Merr.] forms symbioses with *Bradyrhizobium japonicum* and acquires 50-85% of its total N requirements from N-fixation (Salvagiotti et al. 2008; Rao and Reddy 2010). Biological N-fixation in soybean conforms to a negative exponential relationship with soil mineral N (Salvagiotti et al. 2008). This allows a soybean plant to adapt to lower-N environments and thrive under these conditions relative to non-leguminous species. Due to N-fixation, soybean yield commonly does not respond to N fertilization under weed-free conditions (Salvagiotti et al. 2008). A field study testing the performance of soybean cultivars in organic production found that growing environment (primarily soil N status) greatly contributes to soybean yield under organic conditions (Carkner and Entz 2017). A negative association of residual soil N and soybean yield at ten sites, suggested that yield differences may in part have been due to increased weed interference at sites with higher soil inorganic N (Carkner and Entz 2017). In a different study, a preceding roller-crimped cereal rye (*Secale cereale* L.) green manure immobilized soil mineral N prior to termination (Wells et al. 2013). Lower soil mineral

N following green manure termination then caused N-deprivation of redroot pigweed (*Amaranthus retroflexus* L.) and reduced weed interference in soybean (Wells et al. 2013).

The exploitation of Tilman's resource-ratio theory may reduce the competitive ability of unmanaged weeds when legume crops are grown in lower-N environments. The most common postulate of Tilman's resource-ratio theory predicts that when neighbouring species compete for the same resource, the species that can survive at the lowest level of the resource will out-compete its neighbouring species when the resource is limited (Miller et al. 2005; Tilman 1980, 1982, 1985). In this context, lower-N conditions may reduce the competitive ability of weed species that rely on soil mineral N for growth, development and reproduction. Legume crops may then have a competitive advantage over unmanaged weeds due to N-fixation.

To test the utility of the resource-ratio theory as a cultural tool for weed management in annual legume production, the current study examined an economically damaging herbicide-resistant (HR) weed in western Canadian soybean production (Gregoire 2017). Volunteer canola is primarily derived from large canola harvest losses (Gulden et al. 2003a). Following seed dispersal, volunteer canola can enter secondary seed dormancy (Gulden et al. 2003b) and persist throughout crop rotations (Beckie and Warwick 2010). Limitation of seed production in crops grown subsequent to canola is therefore imperative to reduce volunteer canola population persistence (Chapters 5 and 6). Canola and soybean have similar HR traits. In western Canada, volunteer canola is the most abundant weed species in soybean after in-crop herbicide-management (Leeson et al. 2017). Chapter 5 revealed that pre-seed urea fertilizer application (23 kg N ha^{-1}) doubled seed production of volunteer canola in soybean. Likewise, living inter-row cereal mulches decreased volunteer canola seed production, but not soybean yield (Chapter 6). Perhaps this reduction in weed seed production in part was due to immobilization of soil mineral N by living mulches, thereby leaving volunteer canola N-deprived. Based on these observations, it is likely that soil N dynamics influence volunteer canola interference in soybean. The extent to which N dynamics play a role in soybean/volunteer canola interference, however, is unknown. Evaluation of the effect of soil mineral N on interference between these species may help elucidate the potential for exploitation of the resource-ratio theory as a cultural tool for weed management in legume production.

Novel candidate tools for weed management must meet two criteria to be considered effective. These criteria include (a) differential impact on the crop and weed species, and (b) consistency of efficacy (Li et al. 2016). This study was designed to test the influence of soil mineral N on weed interference in legume production. Volunteer canola and soybean were used as exemplar (non-leguminous) weed and (legume) crop species. The objectives were to test whether soil mineral N affects these crop and weed species (a) differently and (b) consistently among weed populations and environments. Specifically, soybean performance was tested across a soil mineral N gradient in the presence or absence of interference from four different volunteer canola populations in three environments. It was hypothesized that all populations of volunteer canola would result in greater interference with soybean as soil mineral N was increased from lower- to higher-N conditions. It also was hypothesized that increased soil mineral N would result in decreased soybean yield in the presence, but not the absence, of all volunteer canola populations.

7.3 Materials and Methods

7.3.1 Field Experiment.

7.3.1.1 Environments. The impact of soil mineral N on volunteer canola interference in soybean was evaluated in three temporal environments at the Ian N Morrison Research Station near Carman, MB, Canada. In 2015, two experiments were conducted adjacent to one another, one seeded earlier (May 12, hereafter referred to as 2015i) and one seeded later (May 29; hereafter referred to as 2015ii) (49°29'42"N, 98°02'15"W; Sandy loam, Hisbin Series Orthic Black Chernozem). The experiment also was repeated once in 2016 (June 8; later seeding date; hereafter referred to as 2016) in a different field (49°29'44"N, 98°02'33"W; Sandy clay loam, Denham Series Orthic Black Chernozem). The preceding crop in each year was wheat (*Triticum aestivum* L.) and environments were selected based on lower levels of nitrate-N in the top 60 cm of the soil profile (Table 7.1). Mean daily temperature (°C) and total precipitation (mm) for each month and the 30-year (1980-2010) climactic normal temperature and precipitation for this location were collected from the on-farm weather station (Fig 7.1) (Environment Canada 2017).

Table 7.1. Soil nutrient status, organic matter and pH separated by measurement depth for each year of the study.

		Soil characteristics [a]					
Year	Depth	N	S	P	K	OM	pH
	cm	kg ha^{-1}		ppm		%	
2015 [b]	0-15	10	13	26	288	3.3	5.8
	15-60	61	> 400	- [c]	-	-	7.1
2016	0-15	7	16	12	235	4.4	5.5
	15-60	23	33	-	-	-	6.5

[a] Abbreviations: N, nitrate-nitrogen; S, sulfate-sulfur; P, Olsen-phosphorus; K, potassium; OM, organic matter.

[b] 2015i and 2015ii environments were adjacent to one another and grouped under the same soil test.

[c] A dash (-) indicates lack of measurement.

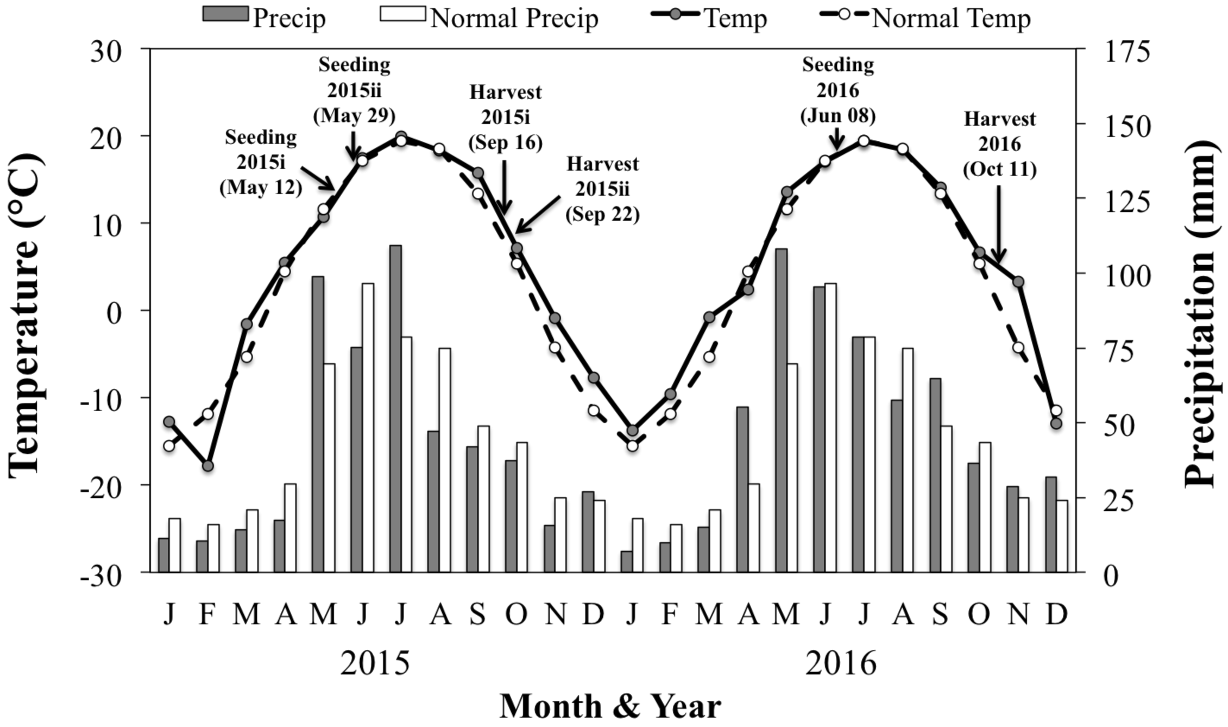

Figure 7.1. Mean daily air temperature (Temp; °C) and total precipitation (Precip; mm) for each month in each year of the study and the regional climatic normal (1980-2010) mean daily air temperature (Normal temp) and total precipitation (Normal precip) for each month. Seeding and harvest dates are indicated for each experiment. Adapted from: Environment Canada (2017).

7.3.1.2 Experimental Design and Treatment Structure. In each environment, a split-block randomized complete block design was used to test the main and interaction effects of volunteer canola population and level of soil mineral N on volunteer canola interference in soybean. Main plots (2.3 x 13.5 m) consisted of four different glyphosate-resistant (GR) volunteer canola populations in soybean and one control absent of volunteer canola. These populations included F1 and F2 generation hybrid 'DKL 73-45 RR' (Monsanto Canada Inc., Winnipeg, MB, CA) and two open-pollinated varieties 'Café' and 'Rugby' (SeCan, Kanata, ON, CA). Seed from the F1 73-45 population was treated with Acceleron® (containing: difenoconaxole, metalaxyl, fludioxonil and thiamethoxam; Monsanto Canada Inc., Winnipeg, MB, CA). Seed from all other canola populations remained untreated. Experimental blocks were split (13.5 x 2.3 m) by the rate of urea fertilizer applied (0, 22.5, 45, 90 and 180 kg N ha^{-1}). Fertilizer was applied parallel to the soybean rows in bands spaced 19 cm apart at a depth of 8 cm prior to seeding. Soil mineral N was considered the amount of nitrate-N in the top 60 cm of the soil profile in each environment (Table 7.1) plus the amount of urea fertilizer N applied to each treatment. A distance of 0.5 m of soybean only was left between adjacent canola populations (main plots) to ensure isolation and limit outcrossing among canola populations (Staniland et al. 2000). A distance of 0.5 m also was left between fertilizer treatments (split blocks) to prevent potential overlap among the relatively small sub-plots (2.3 x 2.3 m). Each experiment was replicated (blocked) four times in each environment.

Following spring cultivation and before seeding soybean, canola populations were seeded at a density of 80 viable seeds m^{-2} at 1 cm depth and using 19 cm row spacing. Glyphosate-resistant soybean 'DKL 23-10 RY' (treated with Acceleron® containing: fluxapyroxad, pyraclostrobin, metalaxyl, imidacloprid; Monsanto Canada Inc., Winnipeg, MB, CA) was seeded over the entire experiment perpendicular to the direction of canola rows. The recommended soybean density of 455,000 seeds ha^{-1} (432,000 target plants ha^{-1}) was seeded at 2 cm depth using 38 cm row widths. To facilitate symbiotic N-fixation, Cell-Tech™ granular single-action soybean inoculant (1 x 10^8 viable cfu g^{-1} *B. japonicum*; Monsanto BioAg and Design™, Monsanto Company, St. Louis, MO, USA) was applied in the seed-row at a rate of 4.0 kg ha^{-1}. A single application of glyphosate (Roundup WeatherMax®, 270 g a.e. ha^{-1} glyphosate, Monsanto Canada Inc., Winnipeg, MB, CA) was applied at soybean stage BBCH 13 to remove all weeds except GR volunteer canola. Glyphosate was applied at 276 kPa using AIXR 110015 TeeJet nozzles

(TeeJet Technologies, Wheaton, IL, USA) and with 100 L ha^{-1} water carrier.

7.3.2 Data Collection.

Soybean seedling emergence was determined at BBCH 12-13 by counting the number of soybean plants in two randomly selected 1 m lengths of two adjacent soybean rows in each subplot. Volunteer canola seedling emergence was determined at the same time (BBCH 14-15) by counting the number of canola plants in two randomly placed 1 x 1 m quadrats in each subplot. The average plant height of volunteer canola and soybean were determined at 30, 45 and 60 days after soybean emergence (DAE) (Appendix A). At canola stage BBCH 82 and soybean stage BBCH 77, aboveground biomass was collected from a 0.5 x 0.5 m quadrat placed along the edge of a soybean row in each subplot. Soybean and canola plants were separated and species-specific sub-samples were dried at 60°C for 48 hrs and dry weights were determined. Then the volunteer canola biomass samples were hand-threshed, cleaned using a hand sieve (2.7 mm round hole, Can-Seed Equipment Ltd., Winnipeg, MB, CA) and column seed cleaner (Agriculex, Model CB-1, Guelph, ON, CA), and the weight of seed from each biomass sample was determined (g seed m^{-2}). Individual seed weight (g thousand seeds^{-1}) of volunteer canola from each biomass sample was determined by weighing triplicate subsamples of 200 seeds. The total weight of volunteer canola seed collected from the biomass samples and the individual seed weight in each sample were used to determine the total number of volunteer canola seeds produced per unit area (thousand seeds m^{-2}). At canola maturity, samples of volunteer canola also were collected, hand-threshed and the weight of individual mature seed was determined (g thousand seeds^{-1}) and adjusted to 8.5% moisture. At soybean maturity, 1 m of two randomly selected soybean rows per subplot was hand-harvested and threshed using a stationary thresher. Soybean yield samples were cleaned using a Clipper M2BC seed cleaner (Blount/Ferrell-Ross Bluffton, IN, USA), weighed and adjusted to 13% moisture. All response variables were determined in each subplot within the main plots consisting of volunteer canola populations, while in the control treatments (no volunteer canola), only soybean yield was determined.

7.3.3 Statistical Analysis. The MIXED procedure (Littell et al. 2006) in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) was used for ANCOVA of the level of soil mineral N (kg ha^{-1}) with all response variable data (ANOVA was used to analyze plant heights; see Appendix A). The soybean response variables included seedling emergence, aboveground biomass and yield. The volunteer canola response variables included seedling emergence, aboveground biomass, individual mature seed weight, and seed production per unit area. Total aboveground biomass accumulation (the sum of soybean and volunteer canola aboveground biomass) also was included as a response variable in the analyses. The Shapiro-Wilk test was used to assess the assumption of normality and visual inspection of residual vs. predicted values was used to examine homogeneity of variance (Littell et al. 2006). Lund's test (Lund 1975) also was used to remove extreme outliers when necessary to meet the assumptions of normality and homoscedasticity.

In a preliminary ANOVA, linear and quadratic single-degree-freedom contrasts for unequally spaced N fertilization treatments were used to explore the relationship of each response variable with N fertilization rate (Littell et al. 2006). In this analysis, N fertilization rate was considered a categorical variable. The main and interaction effects of environment (3 levels), volunteer canola population (4 levels) and N fertilization (5 levels) were considered fixed effects. Experimental block nested within environment and the interaction of volunteer canola population with experimental block nested within environment were considered random effects.

Based on significance ($\alpha = 0.05$) of linear and quadratic contrasts in the preliminary categorical model, a respective continuous linear mixed regression model, saturated with all relevant main and simple fixed effects [i.e., environment, volunteer canola population and linear and/or quadratic terms for soil mineral N (residual N + fertilizer N)] was developed. An ANCOVA was conducted using soil mineral N (kg N ha^{-1}) as a continuous variable (i.e. covariate), while volunteer canola population and environment remained categorical (Littell et al. 2006). In this analysis, the effects of experimental block nested within environment and the interaction of volunteer canola population with experimental block nested within environment were considered random effects. After checking whether the slopes of each response variable regressed over soil mineral N were different from zero ($\alpha = 0.05$) for at least one of the population by environment interactions, the model was adjusted to test whether a difference existed among slopes within each level of main and interaction effect of population and environment (Littell et al. 2006). An iterative hierarchical reductionist approach was used to converge the saturated model to achieve the most parsimonious model structure by sequentially removing insignificant higher-order effects (Luschei and Jackson 2005). Model fit was assessed using Akaike's Information Criterion and the likelihood ratio test. To further adjust for homoscedasticity, the covariance structure of residuals was adjusted by selecting the R-matrix repeated group effect based on minimization of AIC (Onofri et al. 2010). This was done only when the increased number of model parameters was justified using the likelihood ratio test (Luschei and Jackson 2005). Marginal ($R^2_{(M)}$) and conditional ($R^2_{(C)}$) coefficients of determination (i.e., variance explained by fixed factors or both fixed and random factors, respectively) were calculated for each linear mixed regression model following the methods of Nakagawa and Schielzeth (2013).

When hierarchical model reduction resulted in removal of the continuous effect of soil mineral N, the model collapsed to an ANOVA (Littell et al. 2006). In this case, *post hoc* multiple comparisons of means were conducted using Tukey's honest significant difference (HSD) ($\alpha = 0.05$) (Onofri et al. 2010) and mean letter separation was generated using the pdmix800 macro (Saxton 1998).

The same procedure described above was used to analyze the soybean yield data. In this analysis, an additional variable was used to create an augmented treatment structure and include the control measurements of soybean yield in the absence of volunteer canola at each level of soil mineral N (Piepho et al. 2006). Each main and interaction fixed effect was nested within the new categorical variable (presence vs. absence of volunteer canola) to assess whether a difference in response of soybean yield to level of soil mineral N existed between the presence and absence of volunteer canola at each level of each fixed effect (Piepho et al. 2006). In the case where regression models for soybean yield differed between the presence and absence of volunteer canola, single-degree-freedom contrasts were used to determine the level of soil mineral N at which a significant difference between models began (Littell et al. 2006).

7.4 Results and Discussion

7.4.1 Soybean Yield. In the presence of volunteer canola, soybean yield consistently declined by 2.6 kg ha^{-1} for every kg ha^{-1} increase in soil mineral N (Table 7.2; Fig 7.2). In the absence of volunteer canola, soybean yield was not affected by increasing soil mineral N (Table 7.2;

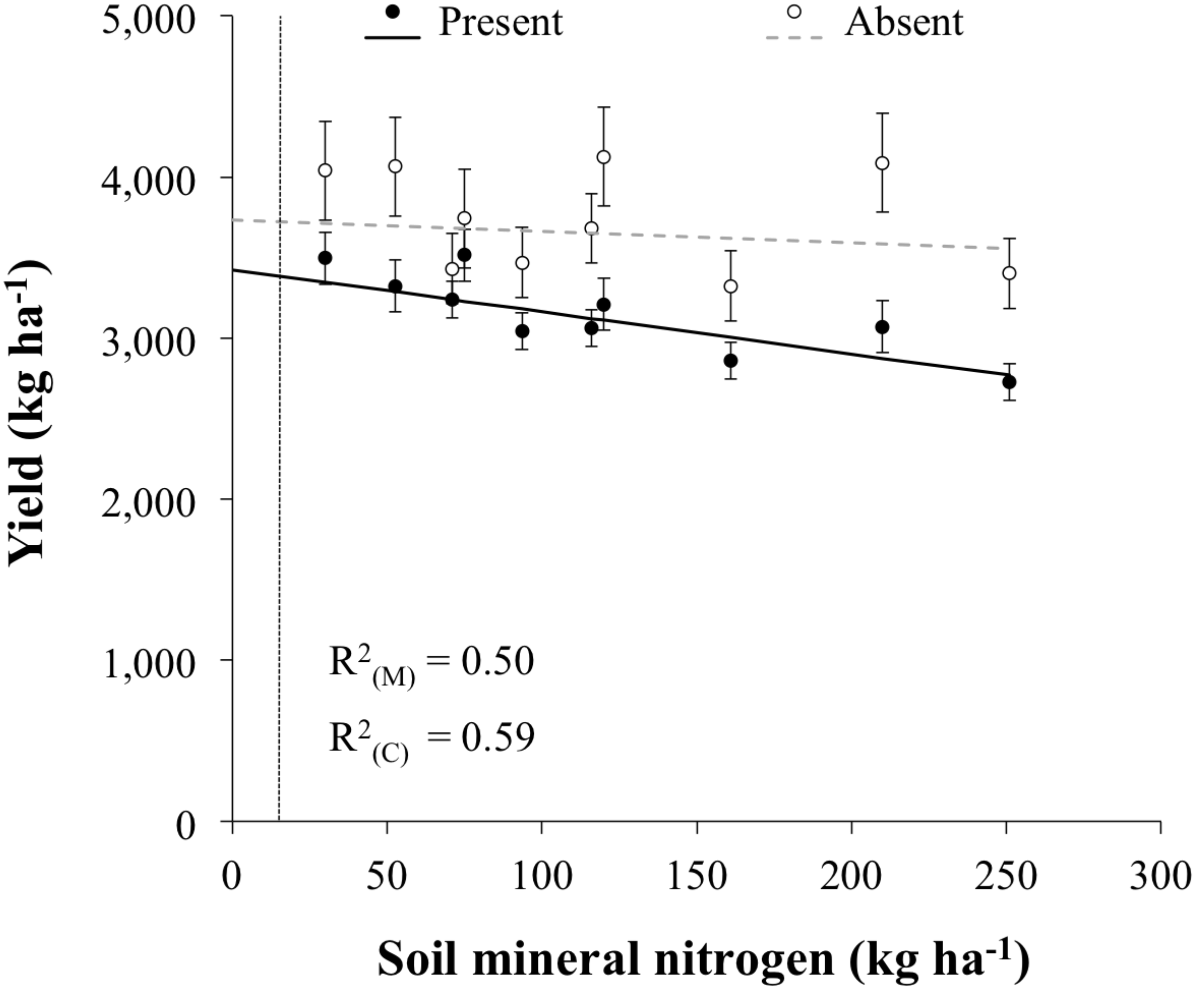

Figure 7.2. Soybean yield in response to the level of soil mineral nitrogen in the presence and absence of volunteer canola. A significant difference (α = 0.05) in soybean yield between the presence and absence of volunteer canola interference was found above the vertical dissecting line (15 kg N ha^{-1}). Dots indicate means of soybean yield for each level of soil mineral nitrogen in a combined analysis of volunteer canola populations and environments. Error bars indicate ± one standard error of the mean. The black line indicates a significant linear component, while the grey line indicates lack of significant difference of the linear component from zero (α = 0.05). Marginal ($R^2_{(M)}$) and conditional ($R^2_{(C)}$) coefficients of determination are indicated for the entire linear mixed effects model.

Table 7.2. Intercepts and slopes for linear (X) or quadratic (X^2) effects of each soybean and volunteer canola response variable regressed over soil mineral nitrogen (kg N ha^{-1}). Equations are given for each combination of volunteer canola population and/or environment when a significant difference in intercepts or slopes was detected. [a,b]

		Effects			Intercept	Slopes	
Species	Response variable	Main [c]	Environ [d]	Population	Y	X	X^2
Soybean	Yield				-------kg ha^{-1}-------		
| [e]	|	Absence			**3,733**	-0.7 ns	- [f]
|	|	Presence			**3,424**	**-2.6**	-
|							
|	Aboveground biomass				-------kg ha^{-1}-------		
|			2015i		**6,535**	**-30.3**	**0.0956**
|	|		2015ii		**6,229**	|	|
|	|		2016		**9,308**	|	|
|							
|	Seedling emergence				----no. plants m^{-2}----		
|			2015i		**56**	**-0.2**	-0.0004 ns
|	|		2015ii		**67**	-0.2 ns	**0.0006**
|	|		2016		**38**	-0.1 ns	0.0003 ns
Volunteer canola	Seed production				thousand seeds m^{-2}		
			2015i		**25,028**	**290.4**	**-0.7946**
|	|		2015ii		-6,756 ns	|	|
|	|		2016		-1,398 ns	|	|
|							
|	Aboveground biomass				-------kg ha^{-1}-------		
|			2015i		**1,757**	**36.2**	**-0.1038**
|	|		2015ii		-684 ns	|	|
|	|		2016		-120 ns	|	|
|							
|	Seedling emergence				----no. plants m^{-2}----		
|			2015i	F1 73-45	**21**	**-0.1**	**0.0002**
|	|		|	F2 73-45	**18**	0.0 ns	|
|	|		|	Cafe	**21**	**-0.1**	|
|	|		|	Rugby	**18**	0.0 ns	|
|	|		2015ii	F1 73-45	**24**	**-0.1**	|
|	|		|	F2 73-45	**30**	**-0.1**	|
|	|		|	Cafe	**25**	**-0.1**	|
|	|		|	Rugby	**26**	**-0.1**	|
|	|		2016	F1 73-45	**35**	**-0.1**	|
|	|		|	F2 73-45	**26**	**0.1**	|
|	|		|	Cafe	**23**	0.0 ns	|
|	|		|	Rugby	**16**	0.0 ns	|
Total [g]	Aboveground biomass				-------kg ha^{-1}-------		
|			2015i		**8,135**	**5.6**	-
|	|		2015ii		**5,468**	|	-
|	|		2016		**9,110**	|	-

[a] Bold values indicate a significant difference from zero at $P < 0.05$.

[b] ns indicates lack of a significance difference from zero at $P \geq 0.05$.

[c] The main effect of presence or absence of volunteer canola in soybean, tested for soybean yield only.

[d] Abbreviation: Environ, environment.

[e] A bar (|) indicates continuation of the entry above.

[f] A dash (-) indicates lack of significant quadratic component.

[g] Indicates the total aboveground biomass of soybean and volunteer canola.

Fig 7.2). This finding corroborates the observation by Carkner and Entz (2017), who found a negative association between soil inorganic N and organic soybean yield. In their case, soybean yield loss may have been caused by weed interference as a result of incomplete weed management (Carkner and Entz 2017). In the current study, a significant difference between the regression models for soybean yield in the presence and absence of volunteer canola was found above 15 kg N ha^{-1} (Fig 7.2). This indicated that volunteer canola interference would not impact soybean yield at very low levels of soil mineral N. It is important to note, however, that this observation is outside the tested range of N levels as the lowest measured level was 30 kg N ha^{-1}. Together, these results suggest that seeding soybean on fields with lower soil mineral N or strategies that may reduce soil mineral N in soybean – such as preceding cover crops (Wells et al. 2013) or inter-seeded living mulches (Chapter 6) – may reduce interference from difficult to manage weeds.

7.4.2 Volunteer Canola Seed Production. The number of volunteer canola seeds produced per unit area was positively related to soil mineral N at levels below 183 kg N ha^{-1} (Fig 7.3). Volunteer canola seed production began to decline as soil mineral N levels increased above 183 kg N ha^{-1} (Fig 7.3). Canola populations were consistently influenced by soil mineral N even though the magnitude of seed production differed among earlier- and later-seeded environments (Fig 7.3). The linear or quadratic relationships of volunteer canola seed production in response to soil mineral N were similar among canola populations and environments (Tables 7.2 and 7.3). This result agrees with past research where canola yield had a quadratic relationship with N fertilization that peaked at similar levels of N fertilization (Cutforth et al. 2009). Chapter 5 also found that pre-plant N fertilization doubled seed production of volunteer canola in soybean.

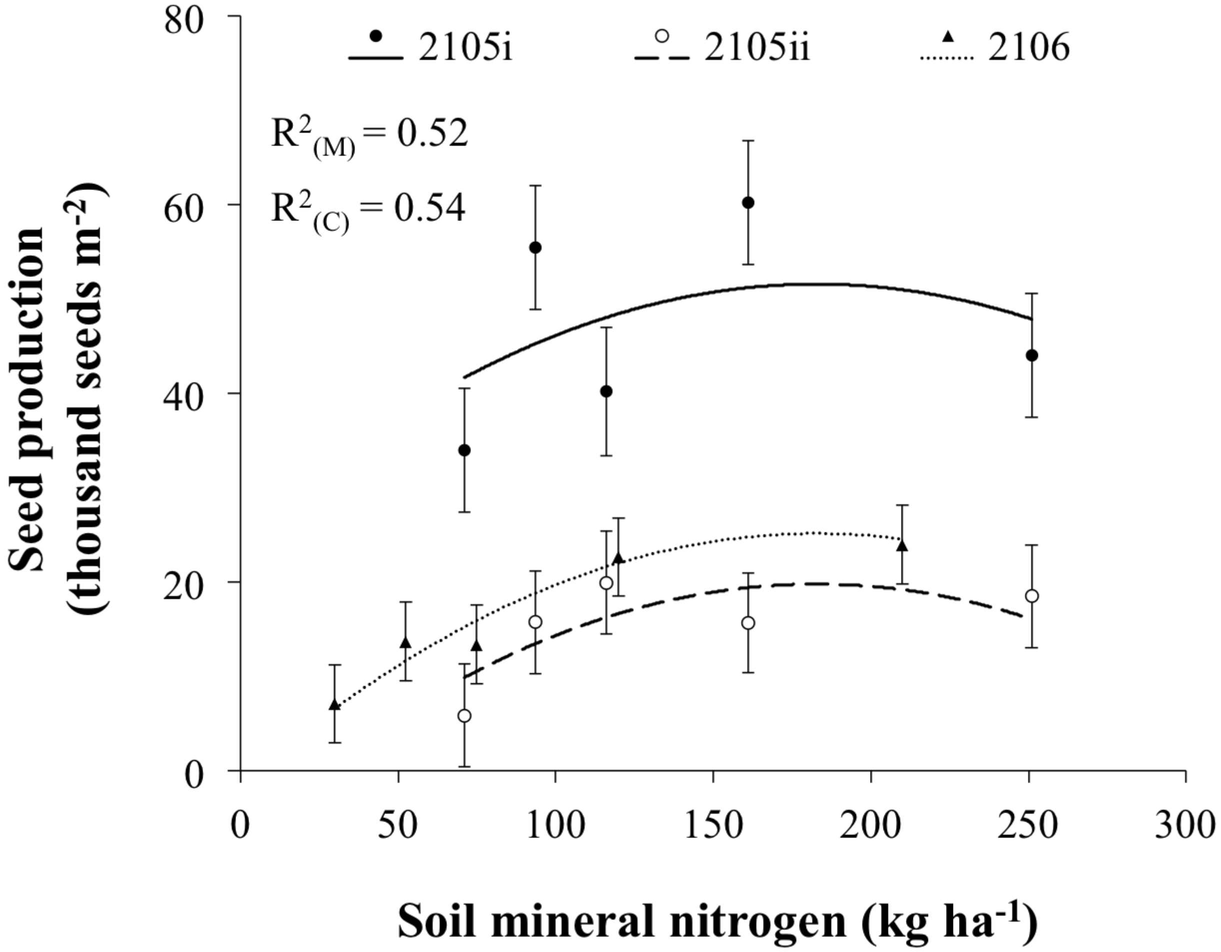

Figure 7.3. The number of volunteer canola seeds produced per unit area (seed production) in response to the level of soil mineral nitrogen in soybean in the 2015i, 2015ii and 2016 environments. Dots indicate means of volunteer canola seed production for each level of soil mineral nitrogen in each environment in a combined analysis of volunteer canola populations. Error bars indicate ± one standard error of the mean. All linear and quadratic components were significantly different from zero ($\alpha = 0.05$). Marginal ($R^2_{(M)}$) and conditional ($R^2_{(C)}$) coefficients of determination are indicated for the entire linear mixed effects model.

Table 7.3. ANCOVA results (*P*-values) showing significant differences in intercepts (volunteer canola population and/or environment) and slopes for linear or quadratic effects of each soybean and volunteer canola response variable regressed over soil mineral nitrogen (kg N ha^{-1}).

		Response species[a,b]							
		Soybean			Volunteer canola				Total[c]
Component	Source	Yield	Aboveground biomass	Seedling emergence	Seed production	Individual seed weight	Aboveground biomass	Seedling emergence	Aboveground biomass
					P-values				
Intercept	Environment (E)	ns	**<0.001**	**0.001**	**0.001**	ns	**0.008**	ns	**<0.001**
|[d]	Population (P)	ns	ns	ns	ns	**0.006**	ns	ns	ns
|	E x P	ns	ns	ns	ns	ns	ns	**0.002**	ns
Linear	Nitrogen (N)	**<0.001**	**0.001**	ns	**<0.001**	ns	**<0.001**	ns	**0.003**
|	N x E	ns	ns	**0.019**	ns	ns	ns	ns	ns
|	N x P	ns	ns	ns	ns	ns	ns	ns	ns
|	N x E x P	ns	ns	ns	ns	ns	ns	**<0.001**	ns
Quadratic	N x N	ns	**0.001**	ns	**0.001**	ns	**<0.001**	**0.039**	ns
|	N x N x E	ns	ns	**0.022**	ns	ns	ns	ns	ns
|	N x N x P	ns	ns	ns	ns	ns	ns	ns	ns
|	N x N x E x P	ns	ns	ns	ns	ns	ns	ns	ns

[a] Bold values indicate significant effects at $P < 0.05$.
[b] ns indicates lack of a significant effect at $P \geq 0.05$.
[c] Indicates the total aboveground biomass of soybean and volunteer canola.
[d] A bar (|) indicates continuation of the entry above.

In all environments, seed production of volunteer canola reached a maximum at about 183 kg N ha^{-1} and ranged from 19,800 to 51,600 seeds m^{-2} among environments (Fig 7.3). About two-thirds of the seed produced by volunteer canola in soybean contributes to seedbank additions (Chapters 5 and 6). Together, these data indicate that higher-N conditions (183 kg N ha^{-1}) in soybean likely resulted in seedbank inputs of about 13,200 to 34,400 seeds m^{-2} at volunteer canola densities ranging from 13 to 46 plants m^{-2} (Appendix E). Thus, higher-N conditions would have resulted in volunteer canola seedbank inputs that ranged from about 3 to 8 times the regional average amount of seed lost at canola harvest (Cavalieri et al. 2016). In western Canada, canola harvest losses result in large seedbank inputs (Gulden et al. 2003a). A single cohort of volunteer canola can persist for greater than three years in the soil seedbank (Gulden et al. 2003b). Evidently, seedbank inputs of volunteer canola in soybean could have a large impact on volunteer densities in crops grown subsequent to soybean in this region. However, these results show that lower-N conditions in soybean can reduce volunteer canola seed production to as low as one-quarter those of higher-N conditions (71 vs. 183 kg N ha^{-1} in 2016; Fig 7.3).

7.4.3 Aboveground Biomass. In the presence of volunteer canola, soybean biomass decreased as soil mineral N increased to 159 kg N ha^{-1} (Fig 7.4). At mineral N levels greater than 159 kg ha^{-1}, soybean biomass increased with increasing soil mineral N. The quadratic relationship of soybean biomass in response to soil mineral N was consistent among canola populations but differed in magnitude the 2015 and 2016 environments (Table 7.3; Fig 7.4). Insufficient mineral N supply for maximum soybean biomass accumulation and reduced N-fixation inhibited by the assimilation of mineral N (Salvagiotti et al. 2008) may have caused soybean biomass to be lowest at intermediate levels of mineral N. Tissue N concentration would assist with confirming

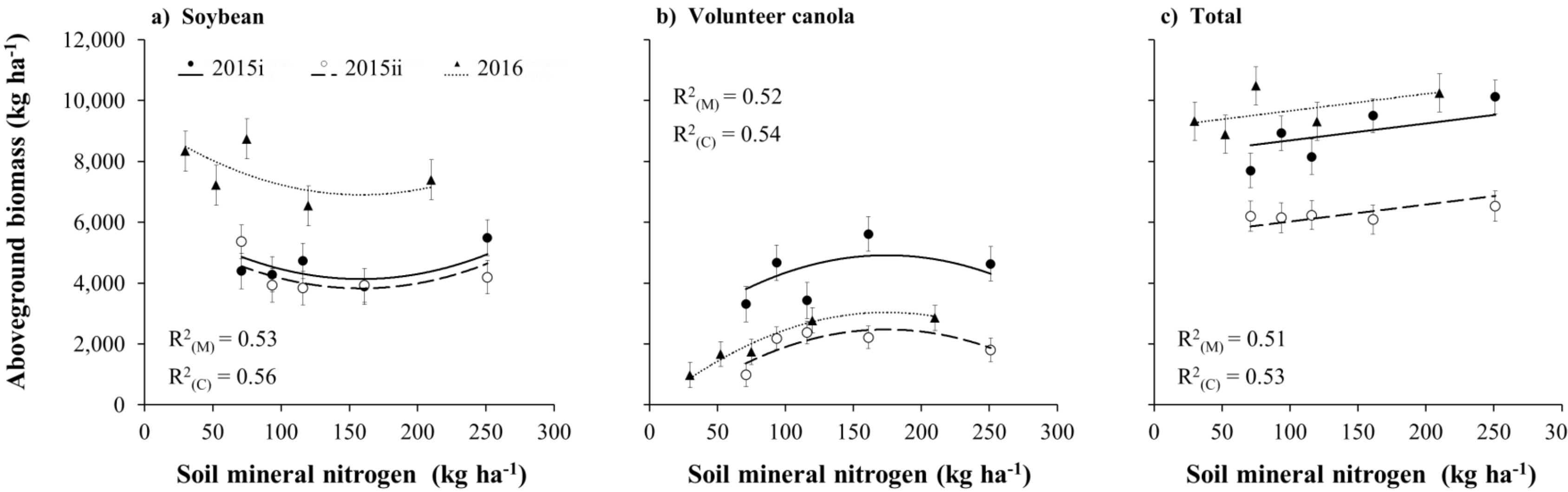

Figure 7.4. Aboveground biomass accumulation of (a) soybean, (b) volunteer canola and (c) soybean plus volunteer canola (total) in response to the level of soil mineral nitrogen in the 2015i, 2015ii and 2016 environments. Dots indicate means of aboveground biomass at each level of soil mineral nitrogen in each environment in a combined analysis of volunteer canola populations. Error bars indicate ± one standard error of the mean. All linear and/or quadratic components were significantly different from zero (α = 0.05). Each sub-figure indicates a separate analysis. Marginal ($R^2_{(M)}$) and conditional ($R^2_{(C)}$) coefficients of determination are indicated for each linear mixed effects model.

this hypothesis, however, these data were not collected. Alternatively, the level of soil mineral N that resulted in the lowest soybean biomass (159 kg N ha^{-1}) also was about the same level of soil mineral N that resulted in the greatest accumulation of volunteer canola biomass (174 kg N ha^{-1}) (Fig 7.4). Indeed, soybean and canola biomass showed opposing quadratic relationships in response to soil mineral N (Fig 7.4). Like seed production (Fig 7.3), biomass of all canola populations responded similarly to soil mineral N and differed among earlier- and later-seeded environments in magnitude only (Table 7.2; Fig 7.4). These biomass data showed that interference from volunteer canola in soybean peaked consistently at levels of soil mineral N between 159 to 174 kg N ha^{-1} (Fig 7.4). These results confirm the hypothesis that increased soil mineral N in soybean would cause more interference from volunteer canola. These results also agree with Chapter 5, which suggested forgoing N fertilization in soybean could limit interference from volunteer canola.

Total aboveground biomass (of soybean and volunteer canola) resulted in a positive linear relationship in response to soil mineral N (Fig 7.4). This relationship was similar among volunteer canola populations and differed among environments in magnitude only (Table 7.2; Fig 7.4). Greater overall biomass with increasing soil mineral N indicated the potential for coexistence of soybean and volunteer canola when soil mineral N was non-limiting. Furthermore, the opposing quadratic relationships of soybean and volunteer canola biomass individually suggest that the balance of coexistence was dependent on mineral N resource availability and agrees with Tilman's hypotheses (Tilman et al. 1980, 1982, 1985). However, at higher levels of soil mineral N (> 174 kg N ha^{-1}) – beyond levels commonly present in unmanaged systems – interference of volunteer canola with soybean was reduced.

Lodging of canola in soybean was observed in the current study, although visual differences among levels of N fertilization were not obvious and the extent of lodging was therefore not measured. Even though visual differences were not obvious, lodging of volunteer canola may have contributed to the decline in volunteer canola seed production and biomass accumulation at very high levels of soil mineral N. Like seed production, canola biomass increased as soil mineral N increased, but a decline in canola biomass began at levels above 174 kg N ha^{-1} (Fig 7.4). Canola biomass and seed production among populations and environments were closely correlated (Pearson r = 0.90, $P < 0.001$) and began to decline above similar levels of soil mineral N (Figs 7.3 and 7.4). These data indicate that potential differences in canola lodging among levels of soil mineral N may have contributed to the observed decrease in volunteer canola biomass accumulation and seed production. In Canada, total N assimilation of a canola crop yielding 1,960 kg seed ha^{-1} ranges between 89 and 109 kg N ha^{-1} (CFI 2001). Application of N fertilizer above the recommended rate for a canola crop can cause lodging (Grant and Bailey 1993; Scott et al. 1973). Other studies have observed canola lodging at N fertilization rates above of 100 kg N ha^{-1} (e.g., Sheppard and Bates 1980; Wright et al. 1988). Islam and Evans (1994) reported that lodging of canola caused a 16% yield reduction and was associated with a reduction in pod number, number of seeds per pod, seed weight, aboveground biomass and plant density. Greater shade avoidance response of canola and soybean at increased levels of soil mineral N manifested as increased plant heights (Appendix E; Figs E1 and E2). A positive linear relationship of canola and soybean plant heights in response to soil mineral N was significant for each measurement date in each environment (Table E1; Figs E1 and E2). A quadratic relationship of plant height in response to soil mineral N also was observed in both species at most measurement dates (Figs E1 and E2). This relationship indicated that the positive contribution of soil mineral N to plant height diminished as mineral N increased to levels near that of the observed reductions in canola biomass and seed production (Figs 7.3, 7.4, E1 and E2). Similar to Chapters 5 and 6, volunteer canola plant height was greater than that of soybean at 30, 45 and 60 DAE (Table E2). Shade avoidance in canola may have weakened stems and caused lodging, which contributed to reduced canola biomass and seed production at mineral N levels above 174 to 183 kg N ha^{-1}.

7.4.4 Seedling Emergence. The response of soybean seedling emergence to soil mineral N differed among environments (Table 7.3; Fig E3). The response of volunteer canola seedling emergence to soil mineral N, however, differed among environments and volunteer canola populations (Table 7.3; Fig E4). Since ANCOVA was used to evaluate soil mineral N as a continuous variable, additional ANCOVA was not employed for evaluation of the contribution of heterogeneous seedling emergence to significant differences in the other response variables. However, volunteer canola and soybean population densities were weakly correlated with the other response variables (Pearson r = -0.31 to 0.29, P = 0.001 to 0.952) and thus did not directly impact the main objectives of this study. Since heterogeneous seedling emergence among treatments was not strongly correlated with any other response variables, the possible causes of differential soybean and volunteer canola seedling emergence are discussed in Appendix E.

7.4.5 Environment. The number of volunteer canola seeds produced per unit area in the earlier-seeded environment (2015i) was consistently greater (by about 26,400 to 31,800 seeds m^{-2}) than the later-seeded environments (2015ii and 2016) (Table 7.2; Fig 7.3). Likewise, volunteer canola accumulated about 1,880 to 2,440 kg ha^{-1} more biomass in the earlier- compared with the later-seeded environments (Table 7.2; Fig 7.4). The difference in volunteer canola biomass and seed production between earlier- and later-seeded environments did not correspond to differences in soybean biomass (Figs 7.3 and 7.4). Soybean accumulated about 2,770 to 3,080 kg ha^{-1} more aboveground biomass in the experiment established in 2016 compared with the experiments in 2015 (Table 7.2; Fig 7.4). Differences in soybean biomass (Fig 7.4), however, did not manifest into differences in soybean yield among environments (Table 7.3). These results suggest that a later soybean seeding date may be used to facilitate volunteer canola management, however, more experiments are needed to validate this interpretation.

Soil tests prior to seeding revealed that the soil for the 2015 experiments contained 71 kg nitrate-N ha^{-1} and soil for the 2016 experiment contained 30 kg nitrate-N ha^{-1} in the top 60 cm of the profile (Table 7.1). These levels of residual nitrate-N were in the lower range of soil mineral N that is still representative of both conventional and organic cropping systems in this region (AGVISE Laboratories 2016; Entz et al. 2001). Soil phosphorus, potassium and sulfur were considered non-limiting and did not warrant application of additional fertilizer (Table 7.1). In each year, growing-season air temperatures closely followed the climatic normal for this region (Fig 1). The amount of spring precipitation received near seeding (in May) was greater than normal in both years and reached 99 mm in 2015 and 108 mm in 2016 compared to the 30-year climatic normal of 70 mm (Fig 7.1). The total amount of precipitation received between seeding and harvest was 340 mm, 260 mm and 292 mm in the 2015i, 2015ii and 2016 environments, respectively. Hence, greater growing-season precipitation in the earlier-seeded environment (2015i) in part may have contributed to greater volunteer canola biomass (Fig 7.4) and seed production (Fig 7.3) compared with the later-seeded environments (2015ii and 2016).

7.4.6 Ecological and Management Implications. Plant competition can be comprised of both resource-dependent (direct) and resource-independent (indirect) competition (Harper 1977). Resource-dependent competition describes rapid uptake of resources such as nutrients or water, while resource-independent competition describes morphological changes induced by the plant sensing environmental conditions prior to the onset of direct competition (e.g., shade avoidance response). Plant interference, however, also can include other plant relationships not necessarily resulting in a negative interaction for both plants involved (e.g., allelopathy resulting in amensalism). Tilman's resource ratio theory predicts the outcome of direct competition (e.g. Tilman 1982), but ignores the broader implications of plant interference as a whole. The current study measured the interference of volunteer canola and soybean as a whole, but did not measure direct competition alone because it is challenging to separate plant competition from plant interference even under controlled environmental conditions (Duke 2015). Nevertheless, the current study showed clearly that the resource ratio theory predicted the outcome of our field experiment, indicating that direct competition likely had a pivotal role in the interference between these species.

Rhizodeposition of N sources from soybean roots (Brophy and Heichel 1989) and subsequent assimilation by non-leguminous weed species may contribute to reduced efficacy of the resource-ratio theory as a tool for weed management. Crop/weed symbiosis with arbuscular mycorrhizal fungi (AMF) also may contribute to assimilation of fixed-N by non-leguminous species in association with a legume crop (e.g., Moyer-Henry et al. 2006; Smith and Smith 2011). In the current study, mineral- and fixed-N were not tracked throughout soil and plant systems. However, canola is non-mycotrophic (Medve 1983) and transfer of fixed-N from soybean to non-mycorrhizal-host weed species is minimal (Moyer-Henry et al. 2006). Therefore, rhizodeposition and AMF-mediated N-transfer likely had minimal influence on canola N assimilation in the current study.

To fully understand the utility of exploiting the resource-ratio theory as a cultural weed management tool in legume crops, investigation of other crop/weed species combinations is needed. The optimal level of N fertility differs among soybean and other legume species that meet less of their total N demand via N-fixation. Nevertheless, the present study agrees with other reports of reduced dry bean competitiveness with weeds following N fertilization (e.g., Saberali et al 2012; Ugen et al. 2002). These observations indicate that the utility of the resource-ratio theory as weed management tool is likely to extend beyond soybean and volunteer canola.

7.5 Conclusion

The current study showed the successful exploitation of Tilman's resource-ratio theory as a cultural tool for management of volunteer canola in soybean. This tool met both prerequisite criteria used to test novel tools for weed management, including (a) differential impact on the crop and weed species, and (b) consistency of efficacy (Li et al. 2016). Soil mineral N impacted soybean and volunteer canola differently under lower- and higher-N conditions. Under lower soil mineral N conditions, volunteer canola aboveground biomass and seed production were suppressed to as low as one-quarter those of higher soil N conditions (71 vs. 183 kg N ha^{-1} in 2016). Under lower soil mineral N conditions, soybean also produced the greatest seed yield under interference from volunteer canola (17% greater than higher N conditions). However, very low levels of soil mineral N (≤ 15 kg N ha^{-1}) were required to completely alleviate soybean yield loss under volunteer canola interference. These effects of soil mineral N on yield loss caused by weed interference were consistent among volunteer canola populations and environments. Crop and weed biomass and weed seed production had similar relationships in response to soil mineral N in all the weed populations and differed among environments in magnitude only. Therefore, in the presence of non-leguminous weed species, legume crops have the potential to benefit from (a) being grown in fields depleted of soil mineral N, (b) avoiding or limiting N fertilization, and (c) potentially using cover crops or inter-seeded living mulches to assimilate and immobilize soil mineral N and limit its availability to weeds. These results also suggest that N fertilization in legume crop species may result in increased severity of yield loss when weeds are not managed adequately. The present study indicates that exploitation of Tilman's resource-ratio theory can be an effective tool for weed management and should be considered as part of a comprehensive IWM program in legume production.

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8.0 GENERAL DISCUSSION AND CONCLUSIONS

This Ph.D. thesis research furthered knowledge of volunteer canola management in western Canada and provided a basis of information from which demographic models can be developed. The research filled some of the most pertinent knowledge gaps in volunteer summer-annual (SA) canola management and identified effective candidate tools for management of volunteer canola throughout its life cycle. The overall objectives of the research studies were:

1. To determine how timing and implement of post-harvest tillage influence the volunteer summer-annual canola life cycle in western Canada (Chapter 3).
2. To determine whether soil texture and associated edaphic factors influence survival of volunteer canola seed in the soil seedbank (Chapter 4).
3. To identify effective non-chemical weed management tools for the development of an integrated volunteer canola management strategy in soybean (Chapter 5).
4. To evaluate the utility of using spring-seeded inter-row living cereal mulches in soybean to interfere with volunteer canola during soybean establishment (Chapter 6).
5. To determine the influence of soil mineral nitrogen on volunteer canola interference in soybean (Chapter 7).

The general discussion and conclusions chapter will summarize the pertinent findings from this research along with other effective management tools that may be used in an integrated life cycle approach to volunteer canola management.

8.1 Effective Management Tools Throughout the Volunteer Canola Life Cycle

8.1.1 Where Does This Research Fit With Respect to Previous Findings? My research on volunteer canola seedbank dynamics corroborates previous observations of differential seedbank behavior in Canada (e.g., Gulden et al. 2003b; Légère et al. 2001; Simard et al. 2002) and Europe (e.g., Gruber et al. 2004b; Lutman et al. 2003; Pekrun et al. 1998a). Early autumn soil disturbance was the best option for post-harvest management of the volunteer SA canola seedbank (Chapter 3). This result contradicted those of research in Europe (Gruber et al. 2004b; 2005; Lutman et al. 2003; Pekrun 1998, 2006). This contradiction was in part due to the evaluation of WA canola in Europe and SA canola in Canada. However, WA canola in Europe tends to persist in the soil seedbank for longer periods of time (e.g., Lutman et al. 2003) compared with SA canola in Canada (e.g., Gulden et al. 2003b). Furthermore, Chapter 4 showed a strong seasonal component to volunteer canola seedbank dynamics in western Canada. In fact, an opposing effect of soil texture on canola seed survival was observed over winter and over summer. Fine soil textures were related to greater canola seed survival over winter, but reduced seed survival over summer compared with coarse textured soils. These results suggest that environmental conditions (perhaps temperature and moisture regimes or soil freezing) also have a strong influence on volunteer canola seed survival in the soil seedbank. Greater soil moisture and generally mild temperatures and deeper tillage in Europe (e.g., Gruber et al. 2004b, 2010), and lower levels of precipitation, greater temperature extremes, greater soil freezing over winter or shallow or zero tillage in Canada also may be driving factors causing the observed differences in volunteer canola seedbank dynamics between Europe and Canada.

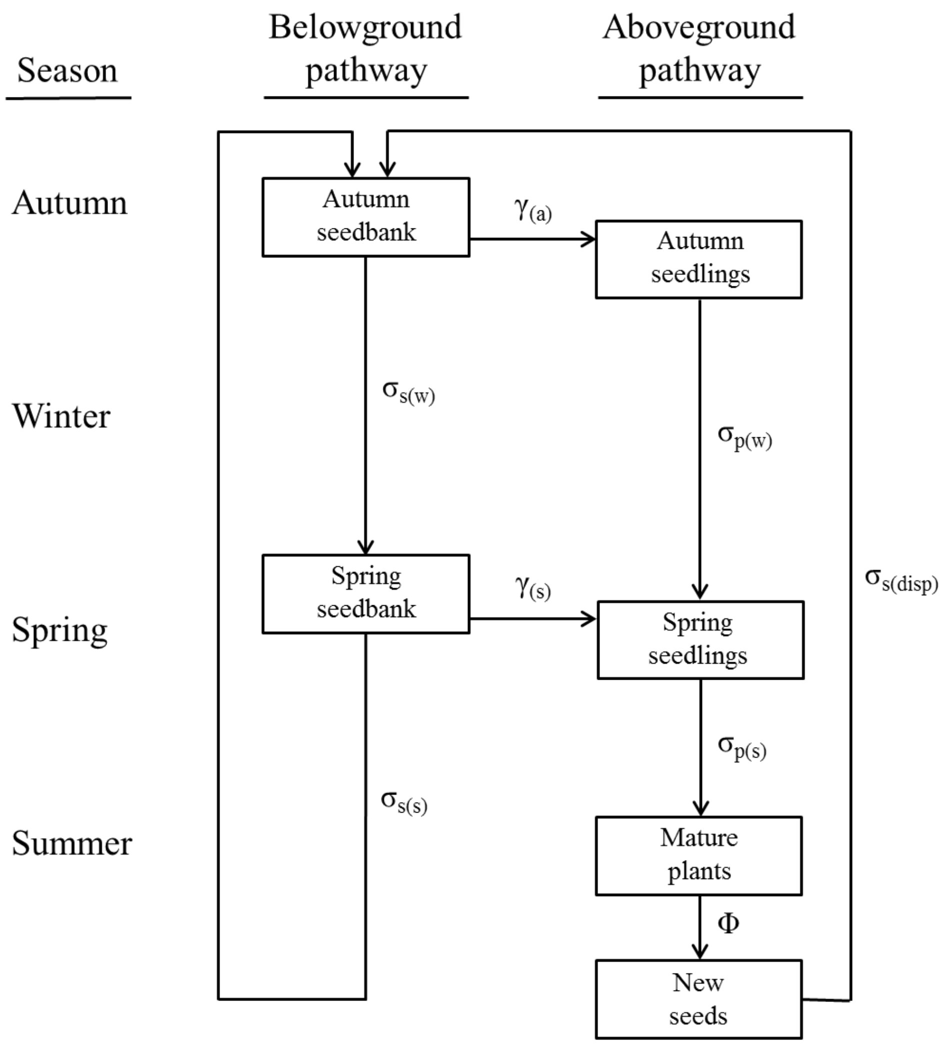
In western Canada, few options are currently available for management of volunteer canola in soybean crops. Previous research has showed inconsistency of volunteer canola management using herbicides (e.g., Légère et al. 2006; Tozzi and Willenborg 2015, 2016). Chapter 5 showed that many non-chemical weed management tools did not manage volunteer canola effectively when implemented alone. Rapid growth and development of volunteer canola during soybean establishment (when both these species emerged near the same point in time) was likely one of the principal causes of the lack of efficacy of the weed management tools tested. Inter-row living cereal mulches in soybean, however, were effective at reducing volunteer canola seed production in soybean (Chapter 6). The inter-row mulches consistently managed volunteer canola regardless of whether the mulches were or were not terminated mid-season. Together, these studies suggest that warm-season soybean was not competitive enough in the early-growing-season to effectively interfere with cool-season volunteer canola, even when cultural tools that would generally increase the competitive ability of the crop were implemented.

In Chapter 7, soil mineral N had a marked influence on soybean yield loss under volunteer canola interference. As soil mineral N increased, interference from volunteer canola also increased and caused greater soybean yield loss. To a certain extent, these effects were observed in each of my three studies evaluating volunteer canola management in soybean. In Chapter 5, even a 23 kg N ha^{-1} pre-seed fertilization resulted in increased seed production and biomass accumulation of volunteer canola. In Chapter 7, the gradient of N fertilization imposed increased volunteer canola seed production and biomass accumulation as soil mineral N increased. Even though soil mineral N was not evaluated in Chapter 6, the observed decrease in volunteer canola seed production and biomass accumulation in the presence of inter-row cereal mulches in soybean may have in part been due to N uptake by the living mulches. These results concur with previous research that showed a close positive association of volunteer canola growth and development with the level of soil mineral N supplied (Blackshaw et al. 2003). Overall, limiting the amount of soil mineral N available to volunteer canola was one of the most effective strategies for management of this problematic weed in soybean production.

Even though volunteer canola was difficult to manage in soybean using individual non-chemical weed management tools, the present research revealed some tools that could be used to manage volunteer canola effectively. Many of these weed management tools were implemented alone and were not integrated in the current research studies. It is expected that the efficacy of these weed management tools may be additive or even synergistic if they are implemented in combination (e.g., Blackshaw et al. 2008; Harker et al. 2003, 2009, 2016; O'Donovan et al. 2007). The next step is to develop an integrated weed management strategy using the effective management tools gleamed from previous studies in combination with the present research. The effective tools for management of volunteer canola in western Canada are briefly outlined in Chapter 8.1.2.

8.1.2 A Guide to Management of Volunteer Canola in Western Canada.

Based on the existing literature and the effective weed management tools described in the current Ph.D. research project, a strategy for management of volunteer canola in western Canada can be developed. The management tools listed in Figure 8.1 all have been shown to influence the volunteer canola life cycle in western Canada. The utility of these management tools is briefly reiterated in Chapters 8.1.2.1 and 8.1.2.2, however, further detail can be found in the literature



Potential management tools

Belowground pathway

Seed survival over winter $\sigma_{s(w)}$

a) Genotypes with low predisposition to secondary seed dormancy
b) Coarse soil textures
c) Ground beetle predation
d) Wet autumn conditions

Seed survival over summer $\sigma_{s(s)}$

a) Genotypes with low predisposition to secondary seed dormancy
b) Fine soil textures
c) Ground beetle predation
d) Shallow seed burial

Aboveground pathway

Seed dispersal $\sigma_{s(disp)}$

a) Agronomic practices associated with synchronous canola maturation
b) Direct harvesting canola
c) Reduced harvest speed
d) Windrow early
e) Reduced time in windrow
f) Clean equipment when transferring between fields

Autumn seedling recruitment $\gamma_{(a)}$

a) Early autumn disturbance with winterkill
b) Wet autumn conditions with winterkill

Plant survival over winter $\sigma_{p(w)}$

a) Avoid zero-tillage

Spring seedling recruitment $\gamma_{(s)}$

a) Deep seed burial (below 10 cm)
b) Avoid spring soil disturbance, OR…
c) Use spring soil disturbance to promote pre-seed recruitment then manage volunteers prior to crop seeding (stale seedbed technique)

Plant survival to maturity $\sigma_{p(s)}$

a) PRE & POST herbicides
b) Inter-row tillage

Plant fecundity Φ

a) Plant soybean in fields with low soil mineral N
b) Avoid N fertilization in soybean
c) Plant living cereal mulches with soybean
d) Plant crops that are competitive in the early growing-season (e.g., cereals)

Figure 8.1. Potential tools that may be effective for management of volunteer canola in western Canada and the volunteer canola life-stage transitions affected by each management tool. Boxes represent life-stages (demographic states) and arrows indicate life-stage transition rates (demographic rates).

review (Chapter 2) and thesis research chapters (Chapters 3, 4, 5, 6 and 7). The proposed tools should be used in combination (when possible) to limit the number of individuals in the volunteer canola population that progress from one life-stage to the next. This list of volunteer canola management tools is in no way complete. Many avenues for future research on volunteer canola management remain and a short list of potential research topics is presented in Chapter 8.2.

8.1.2.1 The Belowground Pathway. There are few effective options for management of the volunteer canola seedbank. In fact, Chapter 3 showed that early autumn soil disturbance decreased volunteer canola seedbank persistence via promotion of autumn seedling recruitment in the aboveground pathway and did not influence seed survival in the belowground pathway. A brief explanation of the management tools and environmental factors (identified in Figure 8.1) that have been shown to be associated with reduced volunteer canola seedbank persistence in western Canada are listed below.

8.1.2.1.1 Seed Survival Over Winter ($\sigma_{s(w)}$).

a) Secondary seed dormancy in canola is associated positively with seedbank persistence (Gruber et al. 2004a, 2009; Gulden et al. 2003b, 2004b; Pekrun et al. 1997d; Webber et al. 2013). Growing canola varieties that have low genetic predisposition to secondary seed dormancy can reduce volunteer canola seedbank persistence over winter.

b) Volunteer canola seed survival over winter is reduced in coarse textured soils compared with fine textured soils (Chapter 4; Gruber et al. 2014).

c) Carabid beetle populations common to canola agroecosystems in Canada may have greater rates of seed predation when seeds are left on the soil surface or buried at a depth less than 4 cm (Kulkarni et al. 2015). However, Chapter 3 showed that the decrease in volunteer canola population persistence following early autumn soil disturbance out-weighed any potential benefit of biological seed predation near the soil surface.

d) A greater amount of precipitation received in autumn may be associated with reduced volunteer canola seedbank persistence (Gulden et al. 2004b; Légère et al. 2001). However, Chapter 3 showed that autumn precipitation was not correlated with over-winter seedbank persistence among the five experimental sites tested.

8.1.2.1.2 Seed Survival Over Summer ($\sigma_{s(s)}$).

a) Canola genotypes with low predisposition to secondary seed dormancy also can have reduced seed survival over summer (Chapter 8.1.2.1.1.a; Gulden et al. 2004b).
b) Volunteer canola seed survival over summer is reduced in fine textured soils compared with coarse textured soils (Chapter 4).
c) Biological seed predation also may reduce volunteer canola seed survival over summer if seeds remain on or close to the soil surface (Chapter 8.1.2.1.1.c; Kulkarni et al. 2015).
d) Shallow seed burial results in transient canola seedbank dynamics (Gulden et al. 2004b). If canola seed is buried at 1 cm depth, seed survival over summer may be negligible.

8.1.2.2 The Aboveground Pathway. The aboveground pathway of the volunteer canola life cycle is more easily manipulated compared with the belowground life cycle pathway. There are several options that have been effective for management of volunteer canola in the aboveground pathway. The management tools and environmental factors (identified in Figure 8.1) that have been shown to be associated with reduced volunteer canola survival in the aboveground pathway in Canada are listed below.

8.1.2.2.1 Seed Dispersal ($\sigma_{s(disp)}$).

a) Homogeneous maturation of the canola crop is associated with reduced canola seed losses at harvest, likely because it can allow for optimal harvest timing. Agronomic practices that facilitate synchronous maturation of canola plants, such as high population densities and adequate nitrogen fertilization may allow for optimal timing of harvest operations and thereby reduce canola harvest losses (Cavalieri et al. 2016).

b) Direct harvesting of canola has been shown to result in reduced canola harvest losses compared with windrowing in small plot field research in western Canada (Haile et al. 2014a). However, differences between windrowing and direct harvest operations were not observed in a field scale survey (Haile et al. 2014b).

c) Reduced harvest speed was associated with reduced canola harvest losses in a survey of canola fields conducted across the northern Great Plains (Cavalieri et al. 2016).

d) In the case of heterogeneous canola maturation, early windowing may reduce volunteer canola harvest losses compared with late windrowing (Cavalieri et al. 2016).

e) Reduced time between windrowing and harvest has been associated with reduced canola harvest seed losses (Cavalieri et al. 2016).

f) Cleaning equipment prior to leaving a field may reduce the number of canola seeds dispersed among fields and reduce the potential for adventitious presence of canola volunteers with unwanted HR or oil profile traits in subsequent canola crops (Friesen et al. 2003).

8.1.2.2.2 Autumn Seedling Recruitment ($\gamma_{(a)}$).

a) Soil disturbance in early autumn, shortly after canola harvest, can result in increased autumn seedling recruitment compared with the absence of soil disturbance (Chapter 3). In western Canada, most of the seedlings that emerge in autumn will not survive the winter due to winterkill.

b) Previous Canadian studies noted reduced volunteer canola seedbank persistence over winter following greater autumn precipitation (Gulden et al. 2004b; Légère et al. 2001). Results from Chapter 3 infer that this may in part be due to increased autumn seed germination or seedling recruitment as a result of greater moisture availability. However, a correlation between autumn precipitation and over-winter seedbank persistence or autumn seedling recruitment was not observed among the five sites in Chapter 3. In western Canada, most of the seedlings that emerge in autumn will not survive the winter due to winterkill, thereby depleting the volunteer canola seedbank.

8.1.2.2.3 Plant Survival Over Winter ($\sigma_{p(w)}$).

a) Previous observations in eastern Canada have associated volunteer canola plant (rosette stage or stems of previously harvested plants) survival over winter with zero tillage systems (Simard and Légère 2001; Simard et al. 2002). Avoiding zero tillage may reduce the potential for volunteer canola plants to survive over winter under Canadian conditions.

8.1.2.2.4 Spring Seedling Recruitment ($\gamma_{(s)}$).

a) Canola seedling recruitment has not been observed when seed was buried below 10 cm depth (e.g., Gruber et al. 2010; Gulden et al. 2004b). Autumn tillage may bury a portion of the seed below 10 cm and thereby reduce spring seedling recruitment. However, reduced seedling recruitment following autumn tillage was not observed in the current Ph.D. research (Chapter 3).

b) Spring soil disturbance can increase spring seedling recruitment of volunteer canola (Chapter 3). Spring soil disturbance should be avoided to limit the number of volunteer canola seedlings that emerge after crop establishment.

c) Alternatively, soil disturbance in early spring may promote volunteer canola seedling recruitment prior to crop establishment (Chapter 3). The volunteer canola seedlings that emerge prior to crop establishment may then be managed by pre-emergence herbicide application or subsequent tillage passes. This technique is known as the stale seedbed technique and may potentially be used to deplete the density of viable volunteer canola seeds in the soil seedbank prior to seeding.

8.1.2.2.5 Plant Survival to Maturity ($\sigma_{p(s)}$).

a) Pre-emergence and post-emergence herbicides are available for management of volunteer canola (e.g., Anonymous 2015; Légère et al. 2006; Rainbolt et al. 2004; Tozzi and Willenborg 2015, 2016). Even though herbicides are not always 100% effective when used as the only tool for weed management, herbicides do cause a significant reduction in plant survival to maturity and should be used as part of an integrated management strategy.

b) Inter-row tillage can remove volunteer canola plants between crop rows and reduce the number of plants that survive to maturity (Chapter 5).

8.1.2.2.6 Plant Fecundity (Φ).

a) Planting soybean on fields with lower soil mineral nitrogen (N) may reduce volunteer canola growth and development and result in reduced interference with soybean (Chapter 7).

b) Nitrogen fertilization in soybean results in increased volunteer canola seed production and biomass accumulation (Chapter 5). Avoiding N fertilization in soybean also may reduce interference from volunteer canola.

c) Spring-seeded living cereal mulches were effective for early-season interference with volunteer canola in soybean. When established with soybean, these mulches reduced volunteer canola seed production and biomass accumulation (Chapter 6).

d) Cereal crops are competitive in the early growing-season (Chapter 6). In Europe, volunteer canola has been shown to have reduced plant fecundity in cereal crops compared with canola monoculture (Gruber and Claupein 2007). Including competitive crops after growing canola and before growing soybean in a crop rotation may reduce volunteer canola population densities prior to soybean production.

8.2 Future Research

This Ph.D. research used a systematic life cycle approach to summarize effective tools for management of volunteer canola throughout its life cycle. This approach also identified several knowledge gaps, all of which could not be addressed in a single Ph.D. research project. Some of the remaining questions include:

- Does soil moisture in autumn influence autumn seedling recruitment of volunteer canola?
- Does soil freeze affect volunteer canola seed survival over winter?

- Does pushing canola instead of windrowing reduce canola harvest losses?
- Are pod sealant products effective for reducing canola pod shatter?
- Does desiccation of a canola stand with heterogeneous maturation facilitate a reduction in canola harvest losses?
- Do pod-shatter-reduction canola varieties result in reduced seedbank inputs at harvest?
- What is the magnitude of volunteer canola seed production and seedbank inputs in other crops grown in western Canada?
- How do the identified volunteer canola management tools affect volunteer canola population dynamics when implemented in combination?

8.3 Importance of the Ph.D. Work for the Advancement of Science

Volunteer canola is a problematic weed in western Canada and "conventional" weed management (i.e., herbicides) alone has been unsuccessful at managing this weed effectively. This research evaluated several cultural and physical tools for management of volunteer canola, with particular focus on management in soybean. The current research evaluated weed management tools for proactive or reactive weed management. The tools identified to be effective for management of volunteer canola when implemented alone also likely will be effective when integrated together as a comprehensive weed management strategy (e.g., Blackshaw et al. 2008; Harker et al. 2003, 2009, 2016; O'Donovan et al. 2007). The integration of these management tools may provide effective management of volunteer canola in western Canada and facilitate the inclusion of soybean in crop rotations.

This thesis research integrated weed biology, population ecology and management. Recently it has been suggested that agricultural weed research should branch out from purely descriptive efficacy studies for further development of the discipline (e.g., Ward et al. 2014). One possible solution to the stagnation in novelty of weed research could be the integration of other research disciplines with weed science. This Ph.D. thesis has provided some of the early work on integration of quantitative population ecology with weed management. The five research studies conducted determined critical life-stage transition rates in the volunteer canola life cycle which will be useful for future development of demographic models of volunteer summer-annual canola population demography. Using the life cycle as a basis for the development of a weed management strategy provided a systematic view of volunteer canola population dynamics and has identified additional knowledge gaps where further research is warranted.

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9.2 Appendices

9.2.1 Appendix A.

Table A1. Proportional winter survival of volunteer summer-annual canola seed in the soil seedbank ($\sigma_{s(w)}$) at each site in response to the timing of soil disturbance in the augmented factorial (substructure-A) and presence (tine harrow) or absence (zero tillage) of autumn pre-seed soil disturbance or presence (winter wheat) or absence (unseeded) of winter wheat in the full factorial (substructure-B) treatment substructure.

			Experimental site [a,b]				
Timing	Implement	Crop	Melita 2013	Howden 2013	Carman 2013	Carman 2015	Pilot Mound 2015
			proportions				
Augmented factorial treatment substructure-A							
Zero tillage			0.31 (0.56)	0.10 (0.32)	0.01 (0.08)	0.07 (0.27)	0.04 (0.20)
Early autumn			0.17 (0.42)	0.24 (0.49)	0.00 (0.00)	0.03 (0.17)	0.03 (0.19)
Late autumn			0.20 (0.45)	0.07 (0.27)	0.00 (0.01)	0.08 (0.28)	0.03 (0.18)
Spring			0.29 (0.54)	0.11 (0.34)	0.01 (0.08)	0.12 (0.35)	0.04 (0.20)
			(± 0.054) [c]	(± 0.066)	(± 0.033)	(± 0.095)	(± 0.039)
Full factorial treatment substructure-B							
	Zero tillage		0.25 (0.50)	0.10 (0.31)	0.00 (0.04)	0.07 (0.26)	0.05 (0.23)
	Tine harrow		0.20 (0.45)	0.27 (0.52)	0.00 (0.00)	0.04 (0.20)	0.04 (0.20)
		Unseeded	0.28 (0.53)	0.24 (0.49)	0.00 (0.04)	0.06 (0.25)	0.03 (0.17)
		Winter wheat	0.18 (0.42)	0.12 (0.34)	0.00 (0.00)	0.04 (0.20)	0.07 (0.26)
			(± 0.049)	(± 0.064)	(± 0.031)	(± 0.085)	(± 0.036)

[a] Values are back-transformed means with square root-transformed means in parentheses.

[b] Within columns and effect groupings, significant F-test differences did not manifest into significant mean separation based on Tukey's HSD ($\alpha = 0.05$).

[c] Within columns, parenthetical values preceded by ± indicate the standard error of the difference of the square root-transformed means above.

Table A2. Proportional volunteer summer-annual canola plant survival over winter ($\sigma_{p(w)}$) and absolute density of plants that survived the winter ($N_{plt(Apr)}$) in response to the timing and implement of soil disturbance and seeding winter wheat at Carman 2015. [a]

			Carman 2015	
Time of year	Implement	Crop	$N_{plt(Apr)}$	$\sigma_{p(w)}$
			no. plants m^{-2}	proportion
None	Zero tillage	Unseeded	0.10	4.7 x 10^{-5}
None	Zero tillage	Winter wheat	0.01	4.2 x 10^{-6}
Early autumn	Tine harrow	Unseeded	0.16	1.0 x 10^{-4}
Early autumn	Tine harrow	Winter wheat	0.13	7.7 x 10^{-5}
Early autumn	Tandem disc	Unseeded	0.08	1.5 x 10^{-4}
Late autumn	Tine harrow	Unseeded	0.01	6.6 x 10^{-5}
Late autumn	Tandem disc	Unseeded	0.08	1.6 x 10^{-2}
Spring	Tine harrow	Unseeded	0.00	0
Spring	Tandem disc	Unseeded	0.00	0

[a] Data did not meet the assumptions of ANOVA due to abundance of 0 values.

Table A3. Proportional persistence of the volunteer summer-annual canola population from September to April (r) at each site in response to the presence (winter wheat) or absence (unseeded) of winter wheat seeded in early autumn.

	Experimental site [a,b]				
Crop	Melita 2013	Howden 2013	Carman 2013	Carman 2015	Pilot Mound 2015
			proportions		
Full factorial treatment substructure-B					
Unseeded	0.25 (0.50)	0.07 (0.27)	0.00 (0.04)	0.02 (0.15)	0.02 (0.15)
Winter wheat	0.16 (0.40)	0.04 (0.19)	0.00 (0.00)	0.02 (0.14)	0.04 (0.20)
	(± 0.047) [c]	(± 0.030)	(± 0.029)	(± 0.041)	(± 0.026)

[a] Values are back-transformed means with square root-transformed means in parentheses.

[b] Within columns, significant F-test differences did not manifest into significant mean separation based on Tukey's HSD ($\alpha = 0.05$).

[c] Within columns, parenthetical values preceded by ± indicate the standard error of the difference of the square root-transformed means above.

Figure A1. A summer-annual volunteer canola plant grown from a seedling that emerged in autumn and survived over winter in western Canada. Picture captured on May 19, 2016 in Carman, MB, CA.

9.2.2 Appendix B.

Table B1. The percentage of variation in explanatory and response variables accounted for by the two significant factors extracted using partial least squares analysis.

Factor no.	Explanatory variables	Response variables
	%	
1	79.2	14.8
2	15.4	7.8

Table B2. Factor loadings for each explanatory variable (model effect) retained using partial least squares analysis.

	Explanatory variables [a]							
	Mineral particle density							
Factor no.	Sand	Silt	Clay	BD	OM	CEC	Soluble salts	Winter temp. range
1	-0.385	0.354	0.386	-0.466	0.382	0.385	0.385	-0.040
2	0.142	-0.136	-0.150	-0.291	-0.153	-0.135	-0.139	0.891

[a] Abbreviations: BD, bulk density; OM, organic matter; CEC, cation exchange capacity; Winter temp. range, the mean diurnal soil temperature range at 10 cm depth averaged over the winter time period.

Table B3. Response variable weights for each factor extracted using partial least squares analysis.

	Seed survival period	
Factor no.	Winter ($\sigma_{s(w)}$)	Summer ($\sigma_{s(s)}$)
1	0.857	-0.515
2	0.997	0.074

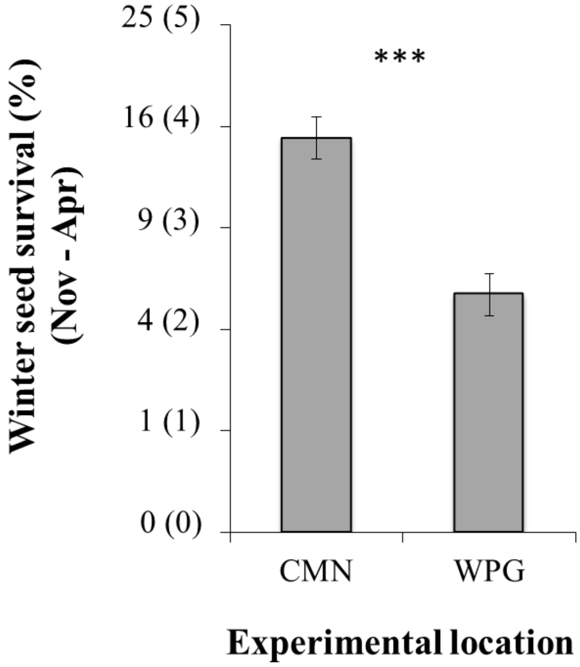

Figure B1. The percentage of volunteer canola seeds that survived at 10 cm depth in the soil seedbank over winter (Nov-Apr) at each experimental location. Values on the y-axis are the back-transformed square root scale with the square root-transformed scale in parentheses. Error bars indicate ± one standard error of the square root-transformed means. A significant F-test effect at $P < 0.001$ is indicated by ***. Abbreviations: CMN, Carman; WPG, Winnipeg.

9.2.3 Appendix C.

Table C1. Pre-formulated estimated mean differences between treatments. [a]

	Row width and additional management treatment					
	NR		MR	WR		
Estimated mean differences	Absent	ESD	Absent	Absent	EN	IRT
Row width						
NR vs. WR	1	0	0	-1	0	0
NR vs. MR	1	0	-1	0	0	0
MR vs. WR	0	0	1	-1	0	0
Seeding density						
NR ESD vs. NR absent	-1	1	0	0	0	0
Soil nitrogen						
WR EN vs. WR absent	0	0	0	-1	1	0
Inter-row tillage						
WR IRT vs. WR absent	0	0	0	-1	0	1

[a] Abbreviations: absent, absent of additional management; ESD, elevated seeding density (1.5 times recommended); EN, elevated nitrogen (+23 kg N ha^{-1}, urea); IRT, inter-row tillage; NR, narrow-row (19 cm); MR, medium-row (38 cm); WR, wide-row (76 cm).

9.2.4 Appendix D.

Table D1. The response of soybean and volunteer canola (response species) mean plant heights at 15, 30, 45 and 60 days after soybean emergence (DAE) to the significant higher-order main and interaction effects of soybean row width, herbicide regime, mulch species, response species and measurement date at each site individually. These reported data exclude the significant interaction effect of herbicide regime, mulch species and measurement date (shown in Table 6.6).[a,b]

Experimental site	Soybean row width	Herbicide regime	Mulch species	Response species	Measurement date				Main effect[c]
					15 DAE	30 DAE	45 DAE	60 DAE	
					cm				
Carman 2013		ACCase		Soybean	18 (4.2)	33 (5.7)	58 (7.6)	68 (8.2)	c
[d]				Canola	13 (3.7)	60 (7.7)	70 (8.3)	74 (9.6)	a
		EPSPS		Soybean	18 (4.3)	31 (5.6)	61 (7.8)	81 (9.0)	b
				Canola	14 (3.7)	55 (7.4)	74 (8.6)	78 (8.8)	a
					(± 0.09)[e]	(± 0.09)	(± 0.09)	(± 0.09)	
	38 cm				16 (4.0)	43 (6.5)	66 (8.1)	76 (8.7)	
	76 cm				16 (4.0)	45 (6.7)	65 (8.1)	74 (8.6)	
					(± 0.08)	(± 0.08)	(± 0.08)	(± 0.08)	
				Soybean	18 (4.2) a	32 (5.7) b	59 (7.7) b	74 (8.6)	
				Canola	14 (3.7) b	57 (7.6) a	72 (8.5) a	76 (8.7)	
					(± 0.08)	(± 0.08)	(± 0.08)	(± 0.08)	
Carman 2014	38 cm		Unseeded		21 (4.6)	66 (8.1)	88 (9.4)	90 (9.5)	ab
			Wheat		22 (4.6)	63 (7.9)	86 (9.3)	90 (9.5)	ab
			Rye		21 (4.6)	61 (7.8)	80 (9.0)	87 (9.3)	bc
	76 cm		Unseeded		22 (4.7)	69 (8.3)	90 (9.5)	93 (9.7)	a
			Wheat		21 (4.6)	62 (7.9)	84 (9.2)	89 (9.4)	ab
			Rye		20 (4.5)	56 (7.5)	74 (8.6)	78 (8.8)	c
					(± 0.09)	(± 0.09)	(± 0.09)	(± 0.09)	
				Soybean	20 (4.5) b	43 (6.6) b	65 (8.1) b	75 (8.7)	
				Canola	22 (4.7) a	86 (9.3) a	104 (10.2) a	101 (10.1)	
					(± 0.04)	(± 0.04)	(± 0.04)	(± 0.04)	

Table D1, continued.

Melita 2014	38 cm		Unseeded		15 (3.9)	55 (7.4)	75 (8.6)	76 (8.7)	
|	|		Wheat		14 (3.8)	53 (7.3)	70 (8.4)	74 (8.6)	
|	|		Rye		16 (3.9)	56 (7.5)	67 (8.2)	69 (8.3)	
|	76 cm		Unseeded		15 (3.8)	57 (7.5)	70 (8.4)	70 (8.4)	
|	|		Wheat		16 (4.0)	57 (7.5)	74 (8.6)	73 (8.5)	
|	|		Rye		15 (3.9)	56 (7.5)	72 (8.5)	73 (8.5)	
|					(± 0.11)	(± 0.11)	(± 0.11)	(± 0.11)	
|									
|	38 cm			Soybean	16 (4.0)	41 (6.4)	64 (8.0)	66 (8.1)	b
|	|			Canola	13 (3.7)	69 (8.3)	77 (8.8)	80 (8.9)	a
|	76 cm			Soybean	18 (4.2)	44 (6.6)	67 (8.2)	67 (8.2)	b
|	|			Canola	13 (3.6)	71 (8.4)	77 (8.8)	77 (8.8)	a
|					(± 0.09)	(± 0.09)	(± 0.09)	(± 0.09)	
|									
|		ACCase		Soybean	17 (4.1)	43 (6.6)	68 (8.2)	69 (8.3)	b
|		|		Canola	13 (3.6)	69 (8.3)	75 (8.6)	77 (8.8)	a
|		EPSPS		Soybean	17 (4.1)	42 (6.5)	64 (8.0)	65 (8.0)	b
|		|		Canola	13 (3.6)	71 (8.4)	79 (8.9)	79 (8.9)	a
|					(± 0.10)	(± 0.10)	(± 0.10)	(± 0.10)	
|									
|				Soybean	17 (4.1) a	43 (6.5) b	66 (8.1) b	67 (8.2) b	
|				Canola	13 (3.6) b	70 (8.4) a	77 (8.8) a	78 (8.8) a	
|					(± 0.08)	(± 0.08)	(± 0.08)	(± 0.08)	

[a] Values are back-transformed square root means with square root-transformed means in parentheses.

[b] Within columns and effect groupings, different letters indicate significant differences based on Tukey's HSD (α = 0.05).

[c] When a simple effect with measurement date was not significant but the main effect was significant among measurement dates, letter separation is given for the main effect only based on Tukey's HSD (α = 0.05).

[d] A bar (|) indicates continuation of the entry above.

[e] Within columns, parenthetical values preceded by ± indicate the standard error of the square root-transformed means above.

Table D2. Seedling emergence of spring-established wheat or rye inter-row mulches (mulch species) used to manage volunteer canola in soybean and the influence of soybean row width on mulch seedling emergence at each site individually and in a combined analysis.[a,b]

Soybean row width	Mulch species	Experimental site			Combined
		Carman 2013	Carman 2014	Melita 2014	
		no. plants m^{-2}			
38 cm		115 ± 8.5	69 ± 1.9 a	66 ± 5.0 a	-[c]
76 cm		158 ± 10.7	35 ± 1.5 b	39 ± 1.0 b	-
	Wheat	-	-	-	91 ± 3.5 a
	Rye	-	-	-	70 ± 3.5 b

[a] Values are means ± one standard error of the mean.

[b] Within columns and effect groupings, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

[c] A dash (-) indicates lack of significant F-test effect.

9.2.4.1 Individual Mature Seed Weight. At the Carman 2014 site only, the percentage of volunteer canola seeds returned to the soil seedbank in soybean was halved in the selective graminicide (ACCase) regime compared with the broad-spectrum herbicide (EPSPS) regime, regardless of presence or absence of inter-row mulches (Table 6.1). Also at this site, individual volunteer canola seed weight was 14% to 22% greater in the ACCase herbicide regime compared with the EPSPS herbicide regime (Table D3). Reduced individual volunteer canola seed weight at maturity (Table D3) was negatively correlated with increased proportional seedbank inputs among sites (Pearson $r = -0.26$, $P < 0.001$). Considering that the plot combine was set for larger seeded soybean, a large proportion of small canola seeds may have been expelled by the combine during threshing. Chapter 5 also showed greater volunteer canola seed weight in wide-row soybean in the presence compared with the absence of inter-row tillage. It was presumed that greater volunteer canola seed weight using inter-row tillage was related to an increase in available resources on a per-plant basis (more niche space) after a portion of the canola plants were removed using inter-row tillage (i.e., reduced canola densities) (Chapter 5). However, increased volunteer canola seed weight in plots absent of chemical management of broadleaf weeds (ACCase regime) in the current study indicates that either greater weed interference or potentially the herbicide regime imposed caused the volunteer canola plants to increase seed weight (Table D3). The reason for increased individual seed weight of volunteer canola in the ACCase herbicide regime remains unknown.

Table D3. The response of individual mature volunteer canola seed weight to the presence or absence of spring-established wheat or rye inter-row mulches in soybean (mulch species) with (EPSPS) or without (ACCase) mid-season mulch termination using two different herbicide regimes at three experimental sites individually and in a combined analysis. [a,b]

Herbicide regime	Mulch species [c]	Experimental site			Combined
		Carman 2013	Carman 2014	Melita 2014	
		g thousand seeds^{-1}			
ACCase	Absent	3.57 ± 0.085	3.90 ± 0.085 ab	3.05 ± 0.085	3.60 ± 0.034 a
| [d]	Wheat (L)	3.85 ± 0.085	4.06 ± 0.085 a	2.94 ± 0.085	|
|	Rye (L)	3.79 ± 0.085	4.24 ± 0.089 a	2.97 ± 0.085	|
EPSPS	Absent	3.67 ± 0.085	3.40 ± 0.085 c	3.02 ± 0.085	3.43 ± 0.034 b
|	Wheat (T)	3.68 ± 0.085	3.52 ± 0.085 bc	3.30 ± 0.085	|
|	Rye (T)	3.62 ± 0.085	3.47 ± 0.085 bc	3.23 ± 0.085	|

[a] Values are means ± one standard error of the mean.

[b] Within columns, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

[c] Abbreviations: L, living; T, terminated mid-season.

[d] A bar (|) indicates continuation of the entry above.

Table D4. The response of soybean seedling emergence in the presence of volunteer canola to the presence or absence of spring-established wheat or rye inter-row mulches (mulch species) with (EPSPS) or without (ACCase) mid-season mulch termination using two different herbicide regimes and between 38 or 76 cm soybean row widths at each experimental site individually.

Soybean row width	Herbicide regime	Mulch species [a]	Experimental site [b,c]		
			Carman 2013	Carman 2014	Melita 2014
			seedlings m^{-2}		
38 cm	ACCase	Absent	43	48 a	16
| [d]	|	Wheat (L)	48	42 a	18
|	|	Rye (L)	42	31 bc	13
|	EPSPS	Absent	43	33 ab	15
|	|	Wheat (T)	48	31 abcd	16
|	|	Rye (T)	42	40 ab	15
76 cm	ACCase	Absent	45	18 cd	19
|	|	Wheat (L)	45	14 d	13
|	|	Rye (L)	39	22 cd	16
|	EPSPS	Absent	45	19 cd	17
|	|	Wheat (T)	45	22 cd	13
|	|	Rye (T)	39	27 cd	16
			(± 2.9) [e]	(± 2.9)	(± 2.9)

[a] Abbreviations: L, living; T, terminated mid-season.

[b] Values are means.

[c] Within columns, different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).

[d] A bar (|) indicates continuation of the entry above.

[e] Parenthetical values preceded by ± indicate the standard error of the means above.

Table D5. ANOVA results (*P*-values) for the main and interaction effects of soybean row width, mulch species and experimental site on each inter-row mulch response variable.

	Inter-row mulch response variables [a,b]		
Source	Wheat yield [c]	Aboveground biomass	Emergence density
	P-values		
Row width (R)	ns	ns	ns
Mulch species (P)	N/A	**<0.001**	**0.001**
Experimental site (E)	ns	**<0.001** [d]	**<0.001**
R x P	N/A	ns	ns
R x E	ns	ns	**<0.001**
P x E	N/A	ns	ns
R x P x E	N/A	ns	ns

[a] Bold values indicate significant effects ($P < 0.05$).
[b] ns indicates lack of a significant effect ($P \geq 0.05$).
[c] N/A indicates effects that were not applicable to analysis of wheat yield.
[d] Indicates loss of statistical significance ($P \geq 0.05$) after including inter-row mulch seedling emergence density as a covariate.

9.2.5 Appendix E.

9.2.5.1 Plant Heights.

9.2.5.1.1 Materials and Methods. Plant heights were determined at 30, 45 and 60 days after soybean emergence (DAE). Plant heights were determined by measuring the distance from the soil surface to the most distal point of the shoot with the shoot fully extended. At each sampling date, the height of 3 randomly selected plants per species was determined using this approach in each sub-plot. The MIXED procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) was used for repeated-measures ANOVA of the mean plant height data in each sub-plot at each time point. The analyses of plant height were separated by environment. In each analysis the main and interaction effects of soil mineral N (5 levels), response species (2 levels), volunteer canola population (4 levels) and measurement date (3 levels) were considered categorical fixed effects. The main effect of experimental block and the interaction effect of volunteer canola population and experimental block were considered random effects. The determination of plant height in each combination of soil mineral N by canola population by plant species nested within each experimental block were repeated in time (date) and the autoregressive covariance structure was fit based on minimization of Akaike's Information Criterion (Littell et al. 2006; Onofri et al. 2010). The same model reduction approach used for the other response variables also was used for plant height (Luschei and Jackson 2005). Single-degree-freedom contrasts were used to assess the linear and quadratic relationship of plant height in response to soil mineral N (Littell et al. 2006). Significant main and interaction effects that excluded soil mineral N were analyzed using multiple mean comparisons (Littell et al. 2006) and mean separation was generated using the pdmix800 macro (Saxton 1998). The simulation-based procedure ($\alpha = 0.05$) was used to correct the family-wise error of single-degree-freedom contrasts and multiple comparisons of means (Edward and Berry 1987; Onofri et al. 2010; Schaarschmidt and Vaas 2009).

9.2.5.1.2 Results and Discussion. At each measurement date, mean plant height of both soybean and volunteer canola increased as soil mineral N increased (Table E1; Figs E1 and E2). These results suggest that shade avoidance response in both plant species was linked to soil mineral N and perhaps was related to increased intensity of interference from volunteer canola at higher levels of soil mineral N. Rapid internode elongation may have contributed to greater plant height. This change in plant morphology may have weakened plant stems also. Thus, shade avoidance response could have contributed to greater lodging of volunteer canola in higher-N conditions. In this case, shade avoidance would have indirectly caused the decline in volunteer canola biomass and seed production above 174 and 183 kg N ha^{-1}, respectively (Figs 7.3 and 7.4).

Among environments and levels of soil mineral N, soybean generally resulted in lower plant height than volunteer canola at 30, 45 and 60 DAE (Table E2). These results are similar to previous work on volunteer canola in soybean (Chapters 5 and 6). The rapid vertical growth of volunteer canola could provide this weed with a competitive advantage over soybean.

Interference of open-pollinated volunteer canola populations tended to result in (slightly) reduced plant heights of crop and weed species at 30 DAE (Table E2). Hybrid canola has greater competitive ability than open-pollinated canola (Zand and Beckie 2002). The difference in competitive ability between hybrid and open-pollinated canola may have influenced interference with soybean and contributed to reduced plant height of both species observed under interference from the open-pollenated volunteer canola populations at 30 DAE (Table E2).

Table E1. Statistical significance (P-values) of the single-degree-freedom contrasts for the linear (X) and quadratic (X^2) regression components of the relationship of soybean and volunteer canola mean plant heights in response to the level of soil mineral nitrogen in each environment individually. Categories include the simple effects of soil mineral nitrogen with plant species or measurement date [days after soybean emergence (DAE)] in each environment.

			Soil mineral nitrogen [a,b]	
Environment	Species	Measurement date	X	X^2
2015i			**< 0.001**	**< 0.001**
|[c]				
|	Soybean		**< 0.001**	**0.004**
|	Canola		**< 0.001**	**< 0.001**
|		30 DAE	**< 0.001**	**< 0.001**
|		45 DAE	**< 0.001**	**< 0.001**
|		60 DAE	**< 0.001**	ns
2015ii			**< 0.001**	**< 0.001**
|				
|	Soybean		**0.018**	**0.048**
|	Canola		**< 0.001**	**< 0.001**
|		30 DAE	**< 0.001**	**< 0.001**
|		45 DAE	**0.003**	**0.007**
|		60 DAE	**0.028**	**0.008**
2016			**< 0.001**	**< 0.001**
|				
|	Soybean		**< 0.008**	ns
|	Canola		**< 0.001**	**< 0.001**
|		30 DAE	**0.031**	ns
|		45 DAE	**< 0.001**	**0.003**
|		60 DAE	**< 0.001**	**< 0.001**

[a] Bold values indicate a significant effect at $P < 0.05$.

[b] ns indicates lack of a significant effect at $P \geq 0.05$.

[c] A bar (|) indicates continuation of the entry above.

Table E2. Mean plant heights of soybean or volunteer canola and the mean plant heights of soybean and volunteer canola in response to four different volunteer canola populations at 30, 45 and 60 days after soybean emergence (DAE) in each environment individually.

			Mean plant heights [a,b]		
			Measurement date		
Environment	Species	Population	30 DAE	45 DAE	60 DAE
			cm		
2015i	Soybean		46 b	65 b	97 b
[c]	Canola		85 a	108 a	115 a
			(± 2.7) [d]	(± 2.7)	(± 2.7)
		F1 73-45	68 a	88 a	105 a
		F2 73-45	64 a	86 a	108 a
		Café	65 a	86 a	107 a
		Rugby	66 a	86 a	105 a
			(± 2.8)	(± 2.8)	(± 2.8)
2015ii	Soybean		48 b	81 b	103 b
	Canola		71 a	105 a	107 a
			(± 2.0)	(± 2.0)	(± 2.0)
		F1 73-45	64 a	93 a	108 a
		F2 73-45	60 ab	93 a	107 a
		Café	57 b	93 a	104 a
		Rugby	57 b	92 a	100 a
			(± 2.1)	(± 2.1)	(± 2.1)
2016	Soybean		41 a	68 b	90 b
	Canola		58 a	89 a	96 a
			(± 1.3)	(± 1.3)	(± 1.3)
		F1 73-45	53 a	81 a	94 a
		F2 73-45	50 ab	78 a	93 a
		Café	50 ab	76 a	91 a
		Rugby	46 b	78 a	95 a
			(± 1.5)	(± 1.5)	(± 1.5)

[a] Values are means.

[b] Within columns and effect groupings, different letters indicate significant differences using simulation-based multiple comparisons of means ($\alpha = 0.05$).

[c] A bar (|) indicates continuation of the entry above.

[d] Within columns and effect groupings, parenthetical values preceded by ± indicate the standard error of the means above.

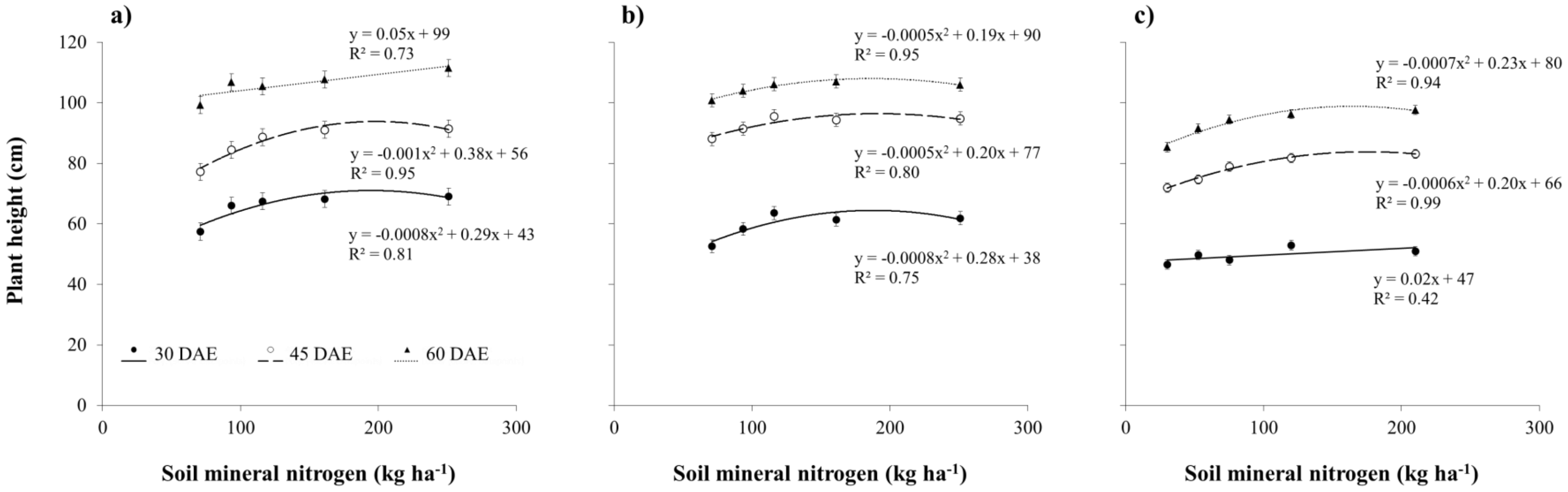

Figure E1. Mean plant heights of soybean and volunteer canola at 30, 45 and 60 days after soybean emergence (DAE) in response to the level of soil mineral nitrogen in the (a) 2015i, (b) 2015ii and (c) 2016 environments. Dots indicate means of plant height for each level of soil mineral nitrogen and each measurement date in a combined analysis of volunteer canola populations and response species. Regression equations and the coefficients of determination for the response of plant height to level of soil mineral nitrogen are indicated for each measurement date within each environment. The relationship of plant height in response to soil mineral nitrogen did not differ among measurement dates in the 2015ii environment.

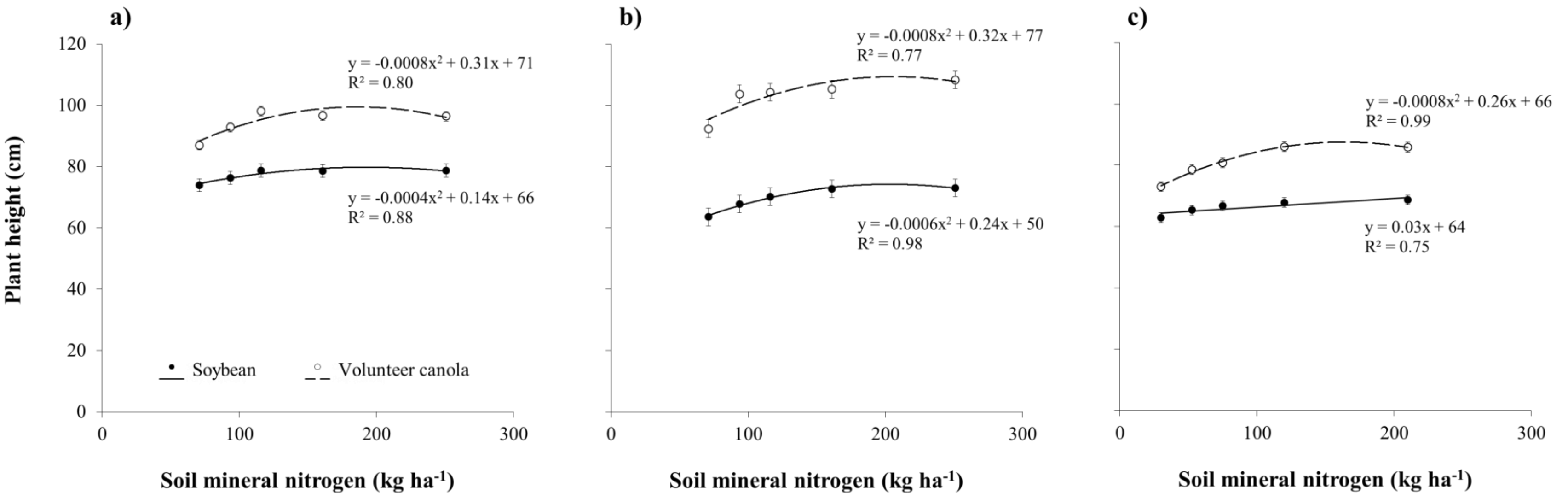

Figure E2. Mean plant heights of soybean or volunteer canola in response to the level of soil mineral nitrogen in the (a) 2015i, (b) 2015ii and (c) 2016 environments. Dots indicate means for each level of soil mineral nitrogen and each response species in a combined analysis of volunteer canola populations and measurement dates. Regression equations and the coefficients of determination for the response of plant height to level of soil mineral nitrogen are indicated for each species within each environment.

9.2.5.2 Seedling Emergence, Continued. The response of soybean seedling emergence to soil mineral N differed among environments (Table 7.3) and was significant in the 2015 experiments only (Tables 7.2 and 7.3; Fig E3). Likewise, the response of volunteer canola seedling emergence to soil mineral N differed among environments and was influenced by canola population (Table 7.3; Fig E4). Seedling emergence of soybean and volunteer canola were not closely correlated with one another (Pearson r = 0.14, $P = 0.027$). The weak correlation among these response variables implies that heterogeneous seedling emergence of these two species was not influenced by the same source of variation. The factor(s) causing differential recruitment of these species remain(s) unknown.

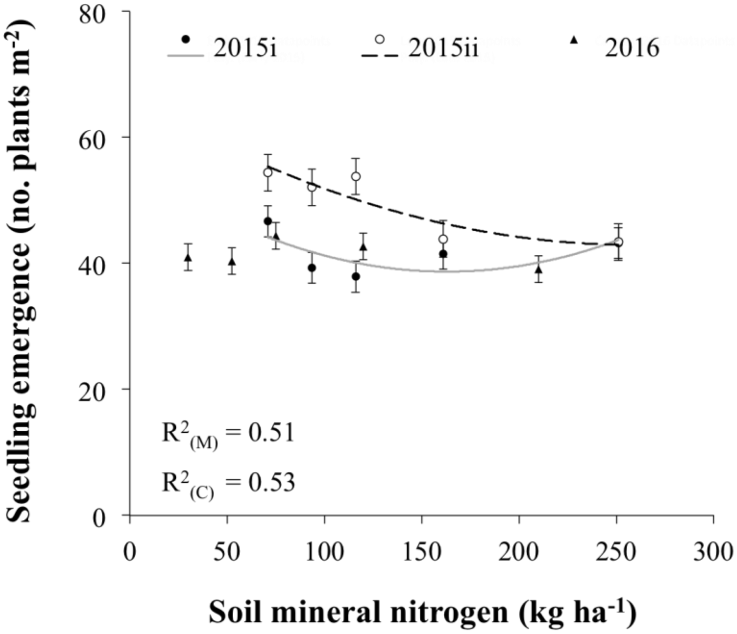

Figure E3. Soybean seedling emergence in response to the level of soil mineral nitrogen in the 2015i, 2015ii and 2016 environments. Dots indicate means of soybean seedling emergence for each level of soil mineral nitrogen in each environment in a combined analysis of volunteer canola populations. Error bars indicate ± one standard error of the mean. The black line indicates a significant linear component, the grey line indicates a significant quadratic component, and line absence indicates lack of difference of both linear and quadratic components from zero ($\alpha = 0.05$). Marginal ($R^2_{(M)}$) and conditional ($R^2_{(C)}$) coefficients of determination are indicated for the entire linear mixed effects model.

The relationship of canola seedling emergence in response to soil mineral N was consistent among environments in the F1 73-45 population only (Fig E4). However, a similar direction of relationship of canola seedling emergence in response to the level of soil mineral N was observed within each environment in all canola populations except for the F1 hybrid canola population (Fig E4). Canola seedling emergence had a positive relationship with soil mineral N in each population in 2016, with the exception of the F1 73-45 population, where an opposite (negative) relationship was observed. Brandt et al. (2007) found that canola plant density was reduced by 7% in lower- compared with higher-N fertilization rates (applied at 67% and 133% of the recommended rate). Another study found that N fertilization (up to 120 kg N ha^{-1}) had no effect on canola seedling emergence (Malhi and Gill 2004). In the current study, the F1 hybrid canola population was the only seedlot to receive seed treatment. These results suggest that the consistency in emergence response of the F1 hybrid canola population to soil mineral N may have been due to the presence of seed treatment resulting in protection against seedling disease or flea beetle (*Phyllotreta* spp.) infestation (Soroka et al. 2008). Such seedling disease or flea beetle infestation may have been more prevalent in 2016 than 2015. However, the presence of these pests was not detected. Genetic uniformity of the canola population, heterosis resulting in early season vigor in the F1 hybrid population (Sernyk and Stefansson 1983) and/or segregation in the F2 hybrid population (Clayton et al. 2009) also may have influenced consistency of the response of volunteer canola emergence to soil mineral N (Fig E4). Clayton et al. (2009) showed no difference in seedling emergence of certified (F1) and farm-saved (F2) hybrid canola seed. However, canola plant survival was higher for populations originating from certified compared with farm-saved seed. In the current study, perhaps loss of canola plants prior to density counts (at BBCH 14-15) contributed to the divergent behavior in canola density among environments.

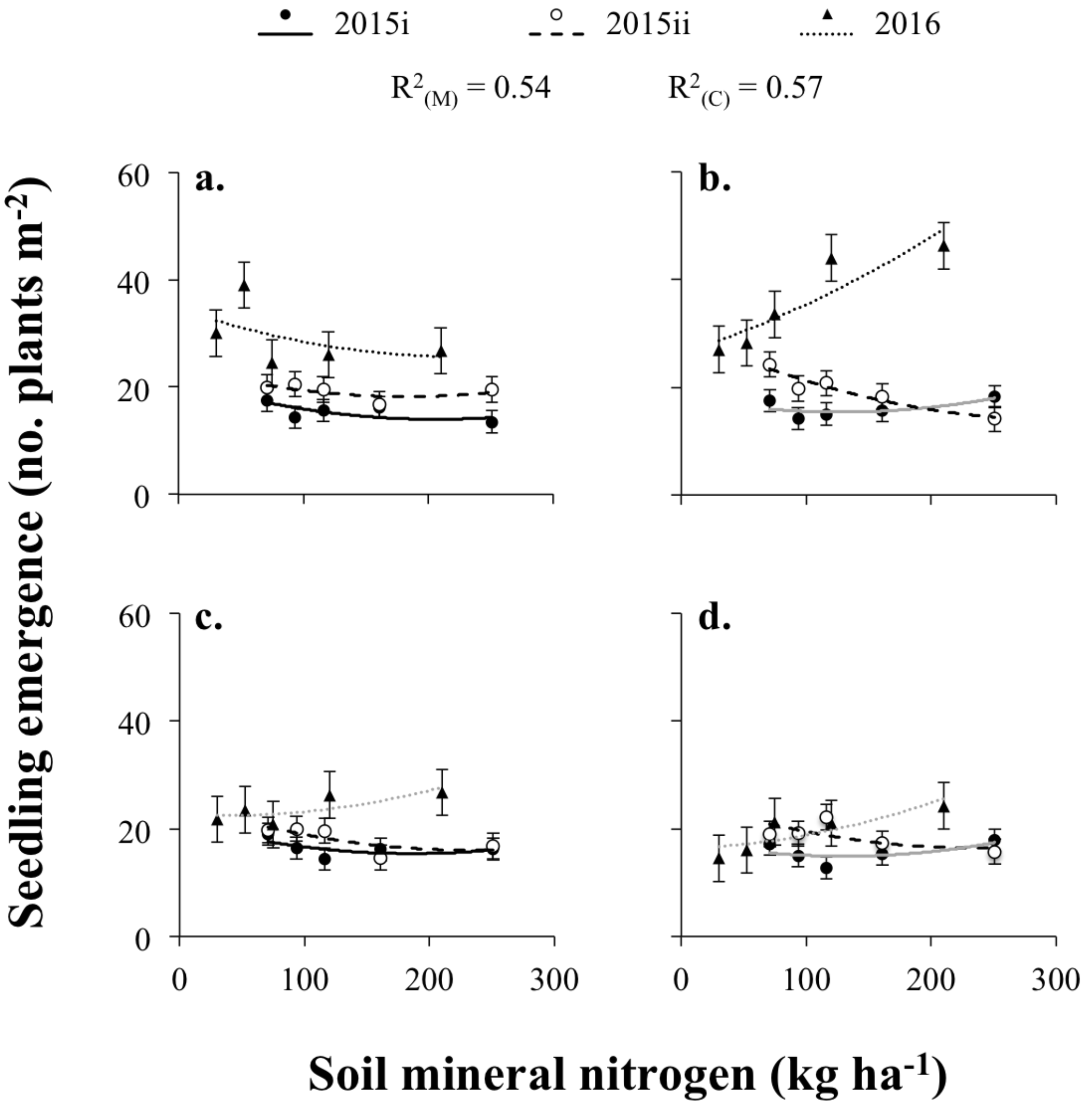

Figure E4. Volunteer canola seedling emergence in response to the level of soil mineral nitrogen for each volunteer canola population and environment combination. F1 73-45, F2 73-45, Café and Rugby canola populations are indicated by a, b, c and d, respectively. The 2015i, 2015ii and 2016 environments are shown for each volunteer canola population. Dots indicate means of volunteer canola seedling emergence for each level of soil mineral nitrogen in each canola population and environment combination. Error bars indicate ± one standard error of the mean. A black line indicates a significant linear component and a grey line indicates lack of a significant difference of the linear component from zero ($\alpha = 0.05$). The quadratic component was significant throughout. Marginal ($R^2_{(M)}$) and conditional ($R^2_{(C)}$) coefficients of determination are indicated for the entire linear mixed effects model.

9.2.5.3 Individual Mature Seed Weight. Hybrid canola populations did not result in greater interference with soybean compared with open-pollinated populations. In fact, the influence of soil mineral N on volunteer canola interference in soybean remained consistent among all volunteer canola populations tested (Table 7.3). The only response variable that differed among the volunteer canola populations was volunteer canola individual seed weight at maturity (Fig E5). Under soybean interference, the weight of individual canola seeds in the hybrid populations were greater compared with the open-pollinated population Café (Fig E5). This result was expected as hybrid canola commonly produces larger seed than open-pollinated canola under monoculture conditions (e.g., Brandt et al. 2007). Positive heterosis in hybrid canola varieties can provide a competitive advantage with weeds (e.g., Harker et al 2003; Zand and Beckie 2002) and heterosis varies depending on parental lines (Starmer et al. 1998). Hybrid breakdown in volunteer generations can cause reduced competitive ability of canola (Seerey and Shirtliffe 2010). Perhaps the hybrid canola population used in the current study did not confer positive heterosis for traits related to competitive ability, or phenotypic plasticity in soybean diminished any potential differences in competitive ability among canola populations. The lack of difference in interference imposed by the commercial and volunteer generation hybrid canola populations also suggests that heterosis did not confer increased competitive ability in the hybrid population used in the current study.

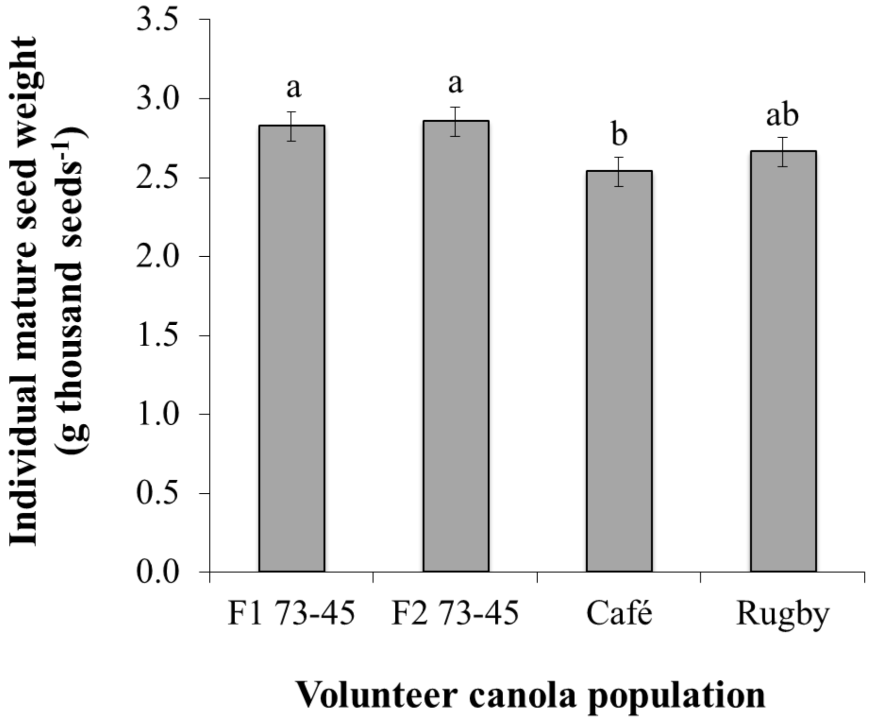

Figure E5. The weight of individual mature seed collected from four populations of volunteer canola in soybean. Values are means for each volunteer canola population in a combined analysis of levels of soil mineral nitrogen and environments. Error bars indicate ± one standard error of the mean. Different letters indicate significant differences based on Tukey's HSD ($\alpha = 0.05$).