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Volunteer wheat (*Triticum aestivum* L.) biological parameters for the development of a mechanistic agronomic model

by

Ryan Louis Nielson

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Abstract

Genetically modified wheat resistant to the herbicide glyphosate (GR) was being evaluated for commercial use in Canada. Gene flow, seed and pollen mediated, by volunteer GR wheat was a major concern. Volunteer GR wheat control and fecundity was measured in glufosinate-resistant canola and peas. Imazamox + imazethapyr in peas were more effective in-crop than glufosinate in canola. The combination of pre-seeding and in-crop herbicides was the most effective at reducing volunteer wheat fecundity. A dose response study measuring volunteer wheat fecundity was conducted in glufosinate-resistant canola and imidazolinone-resistant canola. Imazamox + imazethapyr more consistently controlled volunteer wheat. Cereal crop competition was measured on native volunteer wheat populations. Barley crops seeded earlier relative to the time of the volunteer emergence had the greatest effect volunteer wheat fecundity. Volunteer wheat emerging prior to the crop was the most fecund but was the most affected by agronomic treatments.

Dedication

This thesis is dedicated to my loving wife and children. Beth, who never fails to provide support, guidance and understanding, you are a constant inspiration. Isabel and Finley, you provide me with the strength and conviction to keep moving forward. Special thanks to both my parents Gerry and Linda Nielson and my wife's parents Al and Melanie Rudd, they are always there to help and have taught me the importance of hard work and ethics.

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List of Acronyms:

AP – Adventitious presence

DAT - Days after treatment

GM – Genetically modified

GR – Glyphosate resistant

HR – Herbicide resistant

HSD – Honestly significant difference

HT – Herbicide tolerant

IP – Identity preservation

LSM – Least squared means

OC - Outcrossing

PMGF - Pollen mediated gene flow

PNT – Plant with a novel trait

SEM – Standard error of the mean

SMGF – Seed mediated gene flow

WAT - Weeks after treatment

List of Abbreviations:

BBCH – Scale to outline the physiological growth stage and development of crop and weed species.

ED_{50} – Effective dose of a herbicide to cause 50% reduction over the control

ED_{85} – Effective dose of a herbicide to cause 85% reduction over the control

PREP- Volunteer wheat emergence prior to the pre-seeding herbicide application

PRES- Volunteer wheat emergence prior to the in-crop herbicide application

POSTSP- Volunteer wheat emergence after the in-crop herbicide application

Chapter 1

1.0 Background and Literature Review

1.1. Introduction

The first commercial release of genetically modified (GM) crops was in 1996 and adoption has increased 50-fold to over 102 million hectares planted in 2006. GM crops are currently grown in 22 countries by 10.3 million farmers, with numerous measurable benefits to both producers and consumers (James 2006). Crops such as herbicide-resistant (HR) canola and soybean have been commercially grown in Canada since 1996, and have almost completely displaced conventional varieties (Duke 2005; Cerdeira and Duke 2006; Beckie et al. 2006). There have been measurable benefits, both economic and environmental, including improved weed management, economic returns, yields and lower volumes of less toxic herbicides are being applied (Brooks and Barfoot 2005; James 2006; James 2006; Beckie et al. 2006). The benefit to Canada's farm income in profit and cost savings from GM soybean, canola and maize between 1996 and 2004 totaled U.S. $ 829 million. Environmental benefits include a decrease in the global pesticide usage by 172.5 million kilograms and a 13.8 percent decrease in the environmental impact quotient for the same years (Allen et al. 2001). Notwithstanding these benefits, the introduction of GM crops has become a polarizing issue within the agricultural community and beyond.

Not all HR crops have been viewed as having the same potential risks or benefits. Genetically modified spring wheat, tolerant to the herbicide glyphosate, was evaluated for commercial release in Canada. Significant weed control benefits from this new technology were identified. However there was a high degree of concern raised, including the persistence and fecundity of GM volunteer wheat and the potential market response to GM wheat. These concerns led to the withdrawal of the application for unconfined release by Monsanto Inc. in 2004.

Whether in support or opposition of the introduction of GM wheat, most participants agreed that there was a need for further study of the biology and ecology of wheat. There was a need to investigate gene flow to non-GM crops leading to adventitious presence (AP), volunteer wheat control and persistence of wheat in the agro-

ecological system. The long term consequences of gene flow from GM crops are difficult to study in small, short term experiments. Simulation modeling may provide greater insights into the potential outcome of gene flow events, thus improving risk mitigation, and assist in the development of best management practices. The purpose of this research was to measure key biological and management parameters that influence the persistence of volunteer spring wheat. This data will address gaps in the literature to improve the accuracy of a mechanistic gene flow model.

1.2. Issues surrounding glyphosate-resistant GM crops

Since the introduction of GM crops in 1996 in North America, there has been considerable controversy over the use of GM crops. The first of these concerns was food safety. Most of the traits used have either been herbicide or insect resistance traits that have not significantly affected food quality. The food and feed safety of GM crops have been the most studied crops in history and to date; there have been no significant food safety consequences associated with the use of released GM crops. There were no indications that glyphosate-resistant crops differed in food safety from conventional wheat (CFIA 1997; Health Canada 1997; Health Canada 2001).

Environmental safety has also been closely scrutinized, including both non-target effects and indirect effects. Indirect effects include changes to agronomic practices that may have and influence on the environment. With relation to glyphosate-resistant crops, such as wheat, these include altered (enhanced) selection of glyphosate-resistant weeds, changes in tillage practices, and changes in weed biodiversity (see below).

Finally, for the adopters and non-adopters of GM crops, a key consideration has been the market acceptance of these crops (see below).

1.2.1. Indirect effects on conservation tillage practices

The benefits of conservation tillage have been recognized and include time saving, reduced fuel costs and improved soil quality. Conservation tillage relies heavily on glyphosate to control weeds prior to seeding the crop. This pre-seeding weed control is relatively inexpensive and highly effective, primarily due to the many favorable benefits of glyphosate. Volunteer GR crops can not be controlled by a glyphosate pre-seeding

herbicide application, therefore, requiring the addition of another herbicide. There is a fear that returning to conventional tillage as a low cost and effective method to control volunteers will be required. Herbicide-resistant canola facilitated the use of primarily foliar applied herbicides and thus increased the use of reduced tillage practices in western Canada (Canola Council of Canada 2001). Although there has been much concern that GM wheat would necessitate the return to conventional tillage practices (Saskatchewan Soil Conservation Association 2001; Van Acker et al. 2003), there is little evidence to suggest that the introduction of glyphosate-resistant wheat would alter this trend to reduced tillage (Harker et al. 2004; Harker et al. 2005a).

1.2.2. Glyphosate-resistant weeds

Resistant weeds can result from the selection of rare resistant weeds after the repetitive use of glyphosate and by gene flow from GR wheat to sexually compatible species. The increased adoption of GR crops has inevitably led to more frequent usage of glyphosate in contexts previously not possible. Resistance to glyphosate has been relatively slow to develop, even with a high frequency the glyphosate use, but have been reported. Prior to 1996 there were no discovered cases of GR weeds (Bradshaw et al. 1997), there are now 12 confirmed species in 12 countries. No weeds with resistance to glyphosate have been reported in Canada (WeedScience.com 2007). Glyphosate-resistant weeds occurred first in Australia and more recently in Africa, South America and the United States (Lee and Ngim 2000; Powles et al. 2000; VanGessel 2001). Horseweed (*Conyza Canadensis* (L.) Cronq.) developed after only 3 years of continuously cropped GR soybeans in Delaware (VanGessel 2001). The increased prevalence of GR weeds appears to be correlated in the U.S. with the release of GR crops. Rotations with multiple GR crops such as canola, soybean and corn, and potentially wheat, would eventually lead to GR weeds. Effective stewardship of this technology is important to ensure that GR resistance weeds do not negate its benefits.

The potential of wheat to cross with wild relatives may also lead to resistant weeds. Limited sexually compatible weeds occur in Canada. In the U.S. jointed goatgrass commonly inhabits fields and comingles with winter wheat. Hexaploid wheat and jointed

goatgrass share a common D genome and, therefore, the potential for gene flow is high if the GR gene were to be located on this genome (Morrison et al. 2002a).

1.2.3. Biodiversity of weeds

Weeds are important ecologically, and the reduction of weed biodiversity will impact species that rely on them for food (Watkinson et al. 2000). The adoption of GR wheat grown in conjunction with GR canola would lead to more frequent applications of glyphosate. In addition to selecting for herbicide resistance, this increased use pattern of glyphosate may have an effect on the biodiversity of weeds selecting for weeds poorly controlled by glyphosate. Experiments were conducted across western Canada investigating the impact of multiple in-crop glyphosate application applied in GR wheat and canola on weed communities. At individual sites various weeds were associated with three consecutive years of in-crop glyphosate applications. Canada thistle (*Cirsium arvense*), henbit (*Lamium amplexicaule*), volunteer wheat (*Triticum aestivum*), volunteer canola (*Brassica napus*) and round-leaved mallow (*Malva pusilla*) were associated with specific locations throughout the study area (Harker et al. 2005). The use of GR wheat did not lead to short-term weed management risks, but the importance of a more integrated weed management approach was emphasized. Conventional wheat production also favored the success of specific weed communities.

Weed populations are dynamic and will respond to different levels of selection pressures (Harker et al. 2005). Although effective weed control is a common goal of agricultural production, the total eradication of weeds is not ecologically desirable (Heard et al. 2003).

1.2.4. Admixture in seed

Seed sources accessed by growers are either certified, farm saved, or brown bagged. Certified seed in Canada is the first generation of open pollinated crops and the second generation of self pollinating crop to be grown from foundation seed that will be grown for commercial production. All certified seed in Canada is grown under the direction of the Canadian Seed Growers Association (CSGA) and must meet strict purity guidelines. Farm-saved seed is grown by the producer and a portion of the crop is retained for

replanting. Brown-bagged seeds are grown and sold by producers, and may or may not be cleaned prior to planting. There are no established limits for AP in these seed sources. A survey conducted by Le Buanec (2005) found that 17, 76 and 7 % of wheat planted in Canada was certified, farm-saved and brown-bagged, respectively. Canola was dramatically different, with 92, 6 and 2 % of seed being certified, farm-saved and brown-bagged, respectively.

Seed lots, both certified and farm-saved, were surveyed for the presence of admixed imidazolinone-resistant (IR) wheat in the United States (Gaines et al. 2007). Producer that had grown IR wheat in the same field prior to the sampled seed lots had higher incidences of AP. The level of AP ranged from zero to 11.28% for certified seed lots were IR wheat had not been grown to farm-saved seed where IR wheat had previously been produced, respectively. The majority of IR seeds recovered from seed lots were homozygotes, indicating that seed mediated gene flow was the source of the AP rather than pollen mediated gene flow (Gaines et al. 2007).

Although generally consisting of a single variety, certified seed can be a source of off-type contamination. The CSGA oversees commercial seed production and provides certification after visual inspections for crop varietal purity. Seed samples from these fields must be submitted to the Canadian Food Inspection Agency (CFIA) to ensure purity of variety (POV). Impurities in certified seed may be due to either pollen or seed movement. Plots from POV trials were used to determine the source of impurities in certified wheat seed. The contamination levels were generally low (< 0.02%). Using awns as a marker, outcrossing (OC) resulted in < 0.002% contamination, and mechanical mixing added < 0.01% (Hucl et al. 2004). The CSGA standards for current agronomic and operational procedures for spring wheat production can achieve high purity levels.

While generally the outcrossing rates in canola are much higher than for wheat, 30% vs. 2% on average, HR traits have facilitated accurate estimations of gene flow in seed. Of 33 conventional canola samples from 27 CSGA-numbered certified seed lots, 26 contained detectable levels of HR seeds. The seed lots with detectable levels had 14 in excess of 0.25 %, therefore exceeding the 99.75 % cultivar purity threshold. Glyphosate resistance was detected more frequently than glufosinate in these 14 seed lots, (9 and 5, respectively) corresponding to the more frequent usage of GR canola in western Canada.

Three seed lots contained the GR trait in excess of 2 % (Friesen et al. 2003). These findings indicate the certification of seed and testing of varietal purity should be considered essential for GM crops where identity preservation (IP) is required.

1.2.5. Market concerns

Prior to the introduction of GM canola, the major markets for Canadian canola also approved the release and market acceptability. This included Japan, Korea, USA and Mexico, but did not include the European Union. Because Canada relies heavily on export markets to sell wheat, our international consumers have an important role in shaping the technologies for the production of this commodity. In Canada, 10% of wheat exports are to Europe, where a virtual moratorium on GM crop imports is in effect. GM wheat may provide production and economic advantages to producers, but without an end market for the product it is futile to proceed with such technologies.

One way to address market concerns is to segregate or channel products. Grain channeling is a method of moving commodities that are specific to a certain market while maintaining segregation or keeping unregistered commodities from entering a specific market (Demeke et al. 2006). Channeling is essential to the identity preservation (IP) of a novel crop. Cost sensitivities of three proposed supply chains for conventional wheat were examined by Huygen (2003) for different levels of AP thresholds. A non-GM supply chain (#1) handling both GM and non-GM wheat was identified as the most cost sensitive to lower tolerance levels followed by grain handlers that only accepted non-GM wheat (#2) . The least sensitive supply chain to lower GM tolerances was to use containers to store and ship all non-GM wheat prior to movement off farm (#3). Supply chain #2 was more cost effective than supply chain #1 due to lower sensitivity to changes in admixed tolerance levels. Supply chain #3 is the most costly because the tolerance for GM content became tighter. The introduction of GM wheat would increase the cost to maintain IP but 6 of 7 grain handlers surveyed in the previous study indicate that 1% or higher tolerances could be achieved with current elevator systems (Huygen 2003).

1.3. Environmental safety assessment criteria

Canada conducts a risk assessment for food, feed and environmental safety on all transgenic and novel crops. The regulatory system is science-based and conducted on a case by case assessment, like other Organization for Economic Cooperation and Development (OECD) countries, and the process is transparent and iterative. Over 70 plants with novel traits (PNTs) have met with regulatory approval in Canada, but many are not currently cultivated in Canada.

The CFIA is responsible for the registration of plants with a novel trait (PNT). The Plant Biosafety Office in Canada (PBO) defines a PNT as "a plant that contains a trait which is both new to the Canadian environment and has the potential to affect the specific use and safety of the plant with respect to the environment and human health" (CFIA 2007b). The risks of novel plants are compared to those of conventional crops because no activity is risk free. Plant species considered novel can be derived through genetic engineering or other methods, including mutagenesis or wide outcrossing.

The purpose of the environmental biosafety assessment is to ensure that the new crop will not have any adverse environmental impacts, and is on based on 5 key criteria (CFIA 2004a; CFIA 2005):

- the potential to become more weedy or invasive;
- the potential for gene flow to related species and the potential consequences;
- altered pest potential;
- impact on non-target organisms (including humans);
- impact on biodiversity (CFIA 2004a).

Information from this thesis will contribute to an understanding of the potential for GR wheat to become weedy or invasive.

1.4. Gene flow

Gene flow influences two factors associated with the acceptance of GR wheat. First it influences whether the GR wheat will be a weed in agronomic systems. This is a specific regulatory concern identified by CFIA (see Section 1.3). Volunteer wheat can emerge in

subsequent crops, flower, exchange pollen, produce seed and possibly perpetuate in the seed bank. Secondly, gene flow influences the admixture of glyphosate-resistant wheat in conventional wheat and in follow crops. While this is not specifically addressed by CFIA, admixture influences the market acceptability of the glyphosate-resistant wheat and conventional wheat from Canada (see Section 1.2.4). The following is a review of literature on gene flow in wheat, including pollen and seed mediated gene flow and the importance of volunteer wheat populations.

1.4.1. Pollen mediated gene flow

Pollen-mediated gene flow (PMGF) is an important mechanism used by plants to maintain genetic diversity by exchanging genes between populations. Plant species that rely almost exclusively upon PMGF are considered obligate outcrossers, and include crops such as corn. Although primarily self-pollinating, many selfing crops may partially outcross, ranging from canola that can average 30% and wheat averaging < 2%. As the outcrossing frequency increases, the relative importance of PMGF to gene movement also increases. Much of the literature to date has focused on PMGF, however, pollen is short lived and travels relatively short distances (Hall et al. 2000; Hucl and Matus-Cádiz 2001; Beckie et al. 2003; Hanson et al. 2005).

Wheat florets can behave both cleistogamously (closed flowers) or chasmogamously (open flowers) during anthesis. The degree of flower opening is environmentally, morphologically, and genetically influenced (De Vries 1974). The potential to outcross is directly correlated with the degree of flower opening in the wheat flowers (Veldhuis et al. 2000). De Vries (1971) reviewed the literature and reported a greater proportion of chasmogamous flowers in the first florets of the spikelet. Wheat is predominantly a selfing species, low levels of outcrossing (OC) occurs depending on variety, crop planting date and environmental factors. Hucl (1996) quantified wheat outcrossing levels by variety in Canada, and found values ranging from 0.2 through 2.4, although rates as high as 6.7 percent.

Wheat outcrossing using a direct spike contact method with four seeding dates to extend the flowering period found outcrossing rates commonly below 2.8 with some cultivars exceeding 10 % (Lawrie et al. 2006). Outcrossing has been quantified in small

plot studies between adjacent plants (30 cm or less) in Canada, New Zealand and the United States. Low outcrossing rates were measured (<2 %) with the exception of cv. Oslo 5.2 % in Canada (Hucl 1996), cv. Rongotea 2.84 % in New Zealand (Griffin 1987), and cv. KS75210 3.1 %, and Newton 2.1 % in the United States (Martin 1990). Outcrossing rates at the field scale, measuring gene flow, reported a rapid decrease as the distance increased between the pollen sources. An average OC rate of 0.003 % at 100 m was measured for CDC Teal. Beyond 100 m, a single gene flow event (0.005 %) was confirmed at 300 m (Matus-Cadiz et al. 2004). Wheat pollen is primarily transported by wind and influenced by relative humidity. Field scale OC studies found maximum outcrossing rates and distances correlating with the prevailing wind direction (Matus-Cadiz et al. 2004; Hanson et al. 2005).

The frequency of long distance pollen flow for wheat is reflected in the CSGA standards for pedigree and certified seed isolation distances, and the CFIA isolation zones for confined field trials. The CSGA requires 10 m isolation for select seed, and 3 m for other classifications (Hucl et al. 2004; CSGA 2005). Hucl and Matus-Cádiz (2001) recommended a 30 meter isolation distance to minimize outcrossing between wheat cultivars, but this is viewed as excessive by a meta-analysis conducted by Gustafson et al. (2005).

1.4.2. Seed mediated gene flow

Agricultural commodities are traded worldwide and therefore, seed and the genes contained therein have the potential to move very long distances. Seed mediated gene flow (SMGF) can occur when admixed seed is planted or when seed is lost at harvest or during transportation. Volunteers may produce pollen and seed, perpetuating gene flow. For smaller seeded crops, the potential for SMGF may be higher than PMGF due to the many non-biological factors that influence the movement of genes. The nature of grain handling systems in Canada is not conducive to segregating GM from non-GM crops, and therefore, maintaining purity is difficult. SMGF is difficult to study due to the complexities of commodity movement, but may be a more important factor in the wider gene flow debate, particularly in crops such as wheat with very low outcrossing rates (< 2%) (Gaines et al. 2007; Hall et al. 2007).

1.4.3. Harvest losses

Harvest loss can result in large volunteer crop seed bank populations, if uncontrolled, can contribute to both pollen- and seed-mediated gene flow. Anderson and Soper (2003) reported harvest losses in wheat can vary between 240 to 700 seeds m^{-2}, which can be 2.5 times the recommended seeding rate of a commercial wheat field. In western Canada swathing at higher moisture reduced harvest losses (Clarke 1985). Harvest losses were highly variable and not correlated to wheat cultivars. Shatter resistance, and therefore harvest loss, was highly dependant on the kernel size as previously reported by Vogel (1938). Volunteer seeds may be smaller than seeds grown as a crop and therefore may have increased harvest losses. Harvest losses can also be dependent of the harvest machinery and can consist of both naked seed and unthreshed seed heads. A four-fold increase in harvest losses were recovered between two different harvesting systems in Japan (Komatsuzaki and Endo 1996). A higher number of unthreshed heads were recovered with both harvesting systems. Less seeds from unthreshed heads had germinated 3 months after seed dispersal.

In a large rotational cropping study across western Canada, Harker et al. (2004) recorded large harvest losses in wheat due to weather and pests. Hail storms and wheat stem sawfly (*Cephus cinctu)* resulted in very high volunteer densities (>300 plants m^{-2}). These densities would require additional effort to control volunteers.

1.4.4. Seed banks

Seed dormancy is a trait that favors weediness because it allows growth to be delayed until conditions are favorable. Because seed banks determine the subsequent weed population, replenishment and practices that reduce seed banks are of interest. Seed predation may be increased by allowing seed to be exposed on the soil surface (Hulme 1994; Westerman et al. 2006). Seed germination in the fall is also an important factor, reducing the wheat seed bank density (Harker et al. 2004). Intrinsic factors, including seed size, maturity and dormancy, influence persistence (Figure 1.1).

Wheat has been described as short-lived in the soil seed bank. Based on a review of the literature, Anderson and Soper (2003) indicate that cereals generally persist less than one year based on classical burial studies. In the same review, the authors describe a

study that showed volunteer wheat emergence 14 months after wheat harvest and observations were reported of volunteer wheat emergence 2 years after wheat harvest. Volunteer spring wheat recruitment was studied in southern Manitoba where wheat did not persist past 12 months (De Corby et al. 2007). In the same study, significant differences were observed between different genotypes and site locations for emerged volunteer wheat. De Corby (2007) reported a wheat seed recruitment of 1-5% for the majority of the site years studied. This was similar to the 1.4% recruitment of wheat seeds measured by Harker et al. (2005a) across western Canada. The persistence of volunteer GR wheat in cropping systems was studied extensively at 8 sites across western Canada (Harker et al. 2005a). GR wheat seed was spread on the soil surface in the fall of 2000 and volunteer emergence was evaluated through 2003. GR wheat volunteers were observed until the third year after seed dispersal. Seed bank sampling in the fall of the third year confirmed GR wheat seed bank exhaustion. When seed bank contributions by volunteers are prevented, volunteer wheat seed banks extinguished within three years Harker et al. (2005a). The average density of volunteers measured by Harker et al. (2005a) was 2.6 plants m^{-2}. Weed survey data in western Canada suggest volunteer wheat can emerge 5 years after wheat was grown in commercial fields in western Canada (Thomas and Leeson 1999). Unsubstantiated reports reviewed by Pickett (1993) indicate wheat seed banks persisting up to 5 years after harvest.

Many seed bank experiments are conducted using artificial seed banks and there have been numerous questions raised as to their congruence with naturally occurring seed banks (Leon and Owen 2004). These studies indicate that wheat seed persistence is variable ranging from one to five years (Anderson and Neilsen 1996; Thomas and Leeson 1999; Harker et al. 2004; Harker et al. 2005a; De Corby et al. 2007).

1.4.5. Volunteer wheat in the cropping system

Field crops are grown in specific environments because they are successful within the agro ecosystem; therefore, it is understandable that volunteer crops are also successful. Volunteer crops are domesticated commercial crops growing inadvertently in fields after the original crop was harvested. Volunteers are self sown by natural seed shatter before harvest or seed losses through harvest operations. In western Canada, volunteer wheat has

increased in relative abundance, from 31st in the 1980's to 12th in the 2000's. This increase in volunteers was not due to an increase in spring wheat acreage, wheat production declined in this time period (Table 1.1). Volunteer wheat is under-reported as it cannot be identified in wheat crops. Average volunteer wheat densities recorded in fields after weed control operations were 5.9 plants m^{-2}, the highest density recorded was 280 plants m^{-2} (Leeson et al. 2005).

Volunteer wheat perpetuates temporal gene flow, including both pollen and seed. Volunteer emergence is greatest in the year following wheat production and decreases rapidly with time. In the U.S., Anderson and Soper (2003) reviewed volunteer wheat persistence and indicted that volunteer wheat emergence had been recorded up to two years. An extensive examination of GR wheat volunteers by Agriculture Canada was conducted at eight locations across western Canada. The highest probability of volunteer wheat occurrence was prior to the in-crop herbicide application. Volunteer wheat was present at low densities (< 10 plants m^{-2}) in the cropping system at all locations in the second and third year following seed dispersal. In the fall of the third year following wheat seed dispersal, no GR wheat seeds were detected in the soil seed bank at any of the eight sites. As indicated by the authors, when volunteer seed production is not permitted the seed bank is exhausted rapidly (Harker et al. 2005a).

The emergence of volunteer wheat occurs early in the spring, but can continue throughout the cropping season (Anderson and Neilsen 1996). A minimal number of growing degree days were required for volunteer emergence in the spring, and only a small number of growing degree days (GDD) were required to increase emergence from 25% to 75% (De Corby et al. 2007). The findings of De Corby et al. (2007) indicate that the majority of volunteers emerge prior to seeding the crop, and therefore, pre-seeding herbicide applications would control the majority of the volunteer wheat populations. Harker et al. observed the highest proportion of volunteers prior to the in-crop herbicide application, and would require an effective in-crop application for control. Across all sites studied by Harker et al. (2005a) the total recruitment of volunteers in the year following distribution was 1.4% of seeds prior to the in-crop herbicide application. Both studies used artificial seed banks and reinforced the variability and environmental dependence of weed emergence.

Volunteer wheat, if uncontrolled can reduce the crop yield of canola (O'Donovan et al. 1989; Marshall et al. 1989). Because volunteer wheat can emerge early in the growing season (De Corby et al. 2007), the relative time of emergence of the volunteer wheat and crop the can influence the fecundity of both. O'Donovan (1992) studied the seed production of volunteer barley relative to canola time of emergence and found the seed yield of volunteer barley was greater the earlier it emerged relative to the canola.

The prairie weed survey (Leeson 2005) indicated that volunteer wheat, counted after in-crop herbicide applications, was present in 10.8% of the fields sampled at field densities averaging 6 and as high as 281 plants m^{-2}.

1.4.5.1. Herbicide control of GR volunteer wheat

Crop rotations incorporating species and lifecycle diversity provide the greatest opportunities for herbicidal control of volunteer wheat, and therefore, reducing gene flow via pollen and seed (Harker et al. 2002; Tingle and Chandler 2004). Herbicide control of GR volunteer wheat can be achieved at both the pre-seeding and in-crop herbicide applications. Pre-seeding herbicide applications most commonly consist of glyphosate alone but GR wheat can be controlled using alternative herbicides mixed with glyphosate prior to seeding for most crops (Rainbolt et al. 2004). Rainbolt et al. (2004) studied the efficacy of herbicides applied to GR wheat in the absence of a crop. Clethodim (0.104 kg ai $^{-ha}$) applied alone and tank-mixed with glyphosate plus ammonium sulfate (AMS) provided 95 and 96 % control 21 days after treatment (DAT). Likewise, quizalofop-P (0.062 kg ai ha^{-1}) alone and in tank-mixes with glyphosate plus AMS provided control (93 and 97 %, respectively) at the same interval. Paraquat (0.49 kg ai ha^{-1}) and glufosinate (0.56 kg ai ha^{-1}) both contact herbicides (groups 22 and 10, respectively), alone or with glyphosate plus AMS did not provide adequate control (< 70 %) of GR volunteer wheat 21 DAT. When paraquat was mixed with diuron, control was increased to 93 % at 21 DAT. These applications applied pre-seeding would add additional cost, and according to a survey conducted by (Ogg and Isakson 2001) this may limit the adoption of GR wheat. Paraquat plus diuron provided the most rapid and consistent control of GR wheat, and was recommended by Rainbolt et al. (2004) as the most likely alternative to glyphosate for pre-seeding GR wheat control.

In-crop herbicide options for controlling GR volunteer wheat are strongly tied to crop rotation strategies. Canola, primarily GM, commonly follows wheat in crop rotations of western Canada (Beckie et al. 2006). The use of GR canola would not provide control of volunteer GR wheat unless tank mixtures included a group 1 herbicide. Group 1 herbicides are commonly used to control volunteer wheat in conventional canola and flax. Glufosinate- and imidazolinone-resistant canola are both registered in Canada for the control of volunteer wheat (Brooks 2006).

Although, glufosinate is registered in Canada for control of volunteer wheat, for high volunteer population densities, the inclusion of a group 1 herbicide in tank mixtures is recommended (Brooks 2006). Fifty five percent of glufosinate-resistant canola growers included a group 1 tank mix partner, primarily clethodim, with glufosinate to increase volunteer cereal control (Woycheshin 2007).

Group 1 (Mallory Smith and Retzinger 2003) herbicides are commonly mixed with a broadleaf tank mix partner to expand weed control spectrums. Volunteer wheat control was investigated using clethodim and quizalofop-P in tank mix combinations with broadleaf herbicides (Blackshaw et al. 2006). Bromoxynil, bromoxynil plus MCPA and 2, 4-D ester when tank mixed with clethodim and quizalofop-P did not reduce volunteer wheat control. Quizalofop-P and clethodim efficacy was reduced (antagonism) when tank mixed with 2, 4-D amine. Thifensulfuron plus tribenuron was antagonistic with clethodim but not with quizalofop-P. Volunteer wheat control was reduced by > 90% at 50% of the recommended rate (36 g ai ha^{-1}) at 68% of the site years. Quizalofop-P was recommended as the more effective group 1 herbicide for controlling volunteer wheat (Blackshaw et al. 2006). In this, and most volunteer wheat studies, seed yields (fecundity) of surviving volunteers were not quantified.

1.4.5.2. Cultural control of GR volunteer wheat

Mechanical weed control has traditionally been used to control weeds and volunteer crops (Thill 1996; Timmons 2005). Mechanical tillage is non-selective and therefore, it does not impose the same selective pressure on weed or volunteer populations. Because tillage is primarily utilized prior to seeding the crop, later emerging weeds may be selected. The adoption of GR crops has made it possible for producers to decrease the use

of mechanical tillage; therefore it has become less utilized in recent cropping systems (Duke 1999; Brooks and Barfoot 2005; Duke 2005). By adopting GR wheat there is a concern that zero or reduced tillage management systems would be jeopardized (Saskatchewan Soil Conservation Association 2001; Van Acker et al. 2003). However, this seems unlikely given the substantial benefits of direct seeding and the investments growers have made adopting this system.

1.4.5.3. Volunteer wheat fecundity

Although weed biomass reduction is a frequently measured variable in weed control experiments, weed seed production is not as commonly measured but is an important factor in weed proliferation, persistence and gene flow. Volunteer seed production can contribute to AP in harvested crops, and to the repopulation of the soil seed bank. Currently very little is known on the fecundity of volunteer wheat and how it may differ from wheat grown as a crop. Due to a lack of volunteer fecundity data, literature on spring wheat as a crop was used a baseline for initial model development. Yield components influencing wheat fecundity include spikes plant $^{-1}$, kernels spike $^{-1}$, and kernel weight (Spaner et al. 2000; Guitard et al. 1961; Wang et al. 2002; Zhang et al. 2007). These components are also highly influenced by biotic and abiotic conditions such as temperature (Gibson and Paulsen 1999), plant nutrition and (Dawson and Wardlaw 1984) fungal infection (Simón M.R. et al. 2002). Yield components for 4 Canadian western red spring wheat varieties averaged 3.1 spikes (ears) plant $^{-1}$, 33.8 kernels spike $^{-1}$, and 31.1 mg kernel $^{-1}$ (Wang et al. 2002). Of the yield components studied by Guitard et al. (1961), the number of spikes produced may be the most influential yield component, while the number of seeds spike^{-1} and the kernel weight remained the most constant. The number of spikes plant^{-1} is also most frequently influenced by agronomic factors such as plant competition. Grain yields were significantly related to the number of spikes/area and the dry matter production at anthesis, but not significantly related to grain spike^{-1} or kernel weight (Zhang et al. 2007). Yield components and grain yield were measured for transgenic wheat in Spain, and it was determined that the addition of the transgene studied did not significantly affect the yield components measured or the overall grain yield (Barro et al. 2002).

Volunteer wheat that escaped herbicide applications and set viable seed was observed in flax. The number of surviving volunteers ranged from 2 to 6 plants m^{-2}, spikes m^{-2} ranged from 0.1 to 3.6, and the number of seeds produced ranged from 0.2 to 53.6 m^{-2} (De Corby et al. 2007).

1.4.6. Adventitious presence (AP)

High quality agricultural commodities are important for maintaining market access and commanding price premiums for countries such as Canada. Achieving absolute purity of agricultural commodities is statistically and realistically not possible; therefore, threshold harmonization of GM material in non-GM commodities is important to maintain international trade partnerships (Conner et al. 2003; Demeke et al. 2006). AP is the inadvertent mixing of unwanted materials such as seed, dirt, insects etc. in agricultural commodities at levels that can reasonably be expected. With respect to transgenic crops, AP is the inadvertent presence of transgenic seeds in conventional or organic crops (Drew and McHughen 2005). AP can be broken down into three categories: (1) admixture of approved commodity crops (2) admixed unapproved GM crops with commercial commodities and (3) admixed plant made pharmaceuticals (PMP) or industrial proteins. The first is not a safety issue but an economic problem stemming from market and consumer restrictions. The last two are potential safety issues and require more monitoring and direct management to prevent contamination of the food and feed system.

Coexistence is the production of crops intended for different markets or different uses (streaming) that are being grown in the same locality without becoming admixed at levels that would decrease the market values of both crops. Because markets exist for both GM and organic crops, it is necessary to have both tolerance and mutual respect between growers. For coexistence to be possible it is necessary to have AP allowable threshold levels that will facilitate management strategies to reduce off-type or approved GM admixed seeds. Thresholds are the maximum allowable level of GM crops that can be commingled with conventional crops.

Numerous countries have developed, or are developing, thresholds for adventitious presence of GM crops. These thresholds range from 0.9% for the European

Union, Russia, and Switzerland, to 5% for Indonesia, Japan, Mexico, Taiwan and Thailand. Canada has set a 5% voluntary labeling standard for the AP of GM (Demeke et al. 2006). Some countries, such as Turkey, have not set a tolerance limit for AP, requiring GM-free certification before the commodity can be imported. A standardized detection method for GM adventitious presence has not been adopted. Currently, GM content is detectable by using genotypic and phenotypic methods (Demeke et al. 2006).

The admixing of unapproved GM material in commercial commodities poses a potential human health risk, and decreased consumer confidence in the food supply. In 1999 Starlink® corn, a GM variety sold by Aventis CropScience, containing the *cry*9C *Bacillus thuringiensis* protein, received an initial feed only registration with a human food registration pending. Genetic testing of food products containing corn revealed low levels of the *cry*9C protein. No AP thresholds were in place as guidelines to deal with this incident; therefore mass recalls were implemented by manufactures and food retailers for products containing corn. Minimum limits for foreign proteins such as *cry*9C must be established when commodities receive split registration for either feed alone or human consumption alone (Dorey 2000). Whether this AP incident was a result of pollen or seed mediated gene flow, there is a need to have a greater understanding of the movement of genes within the agricultural commodity stream.

The coexistence of both GM and non-GM wheat will require diligent practices by producers wishing to achieve proposed thresholds required for market exports. Of the producers surveyed by Huygen (2003), 71% indicated that they were confident they could meet the most stringent tolerance levels of the survey of 0.1%.

Harker et al. (2004) reported levels of GR wheat admixture in conventional wheat as high as 14%. This was a result of poor control of volunteer GR wheat in the year following GR wheat production. Effective control with herbicides in the year following GR wheat production is critical to prevent high levels of AP.

1.5. GM wheat

1.5.1. Glyphosate

Glyphosate is the most successful herbicide in the world, it has been in production since 1974 and its use and applications are still increasing (Franz et al. 1997; Woodburn 2000). Glyphosate inhibits the enzyme EPSP synthase in the shikimic acid pathway, present only in micro-organisms and plants, making this pathway a desirable site of action for herbicidal activity. Glyphosate is a non-selective systemic herbicide that has many applications, including agricultural, industrial and silvicultural weed control. Monsanto discovered the herbicidal properties of glyphosate in 1970 and has held the patent from 1974 until 1999; currently there are many companies around the world producing glyphosate (Woodburn 2000). Glyphosate has very low acute mammalian toxicity, a desirable environmental profile, is translocated readily in plants, and has a very low probability of developing resistant weeds; these factors have made this herbicide very successful (Baylis 2000; Caseley and Copping 2000). The lack of selectivity previously limited the frequency of glyphosate applications (Bradshaw et al. 1997; Dill 2005).

1.5.2. GR traits – Origins

Multiple strategies were employed to generate GR crops. Whole plant selection to develop GR crops has proven largely unsuccessful for commercial tolerance levels. Transgenic technologies have made commercial GR possible. Three methods have been used to achieve gene transfer, *Agrobacterium tumefaciens*-mediated transformation, particle bombardment, and protoplast transformation (Dyer 1996). Particle bombardment involves projecting metallic fragments containing foreign DNA into cells, then using a selection pressure to culture recombinants that are desirable. Protoplasts are used for tissue culture regeneration after uptake of the foreign DNA. The most common method used to derive current GR crops is, a gram-negative soil bacterium that causes tumor growth (crown galls). The bacterium transfers a segment of DNA from a Ti plasmid to the plant through wounded plant tissue. The tumor inducing gene is replaced with the gene of interest to create transgenic plants (Dyer 1996). The 35S non-specific promoter from the cauliflower mosaic virus is used to induce transcription of the transgene

(Clemente et al. 2000). The first *A. tumefaciens*-mediated transformation has only recently been reported for wheat (Cheng et al. 1997). The efficiency of *A. tumefaciens*-mediated transformation for monocots is only 1-4% (Hu et al. 2003).

1.5.3. Roundup Ready™ (GR) wheat

Roundup Ready wheat, resistant to the herbicide glyphosate, was developed by Monsanto Inc. to improve weed control and increase yields. The product concept for Roundup Ready™ (GR) wheat was to provide non-selective weed control in wheat using the herbicide glyphosate. The hard red spring wheat cv. Bobwhite was used as the recipient genotype due to its high transformation and regeneration efficiencies (Zhou et al. 2003). In Canada, cv. AC Superb was later transformed for the Canadian market (Kidnie 2004).

1.5.4. Canadian GR wheat decision

The GR wheat case in Canada illustrates the importance of producer and consumer acceptance of novel GM technology. The proposed, unconfined release of transgenic GR wheat engaged many opinions within the agriculture community and the public alike. The GR wheat debate led to the intense scrutiny of this technology by the scientific community. Independent research was published documenting improved weed control (Lyon et al. 2002;Blackshaw and Harker 2002), GR volunteer wheat control (Rainbolt and Thill 2003; Rainbolt et al. 2004; Blackshaw et al. 2006), PMGF in wheat (Hucl and Matus-Cádiz 2001; Matus-Cadiz et al. 2004; Matus-Cádiz et al. 2007), volunteer wheat emergence and persistence (Harker et al. 2005a; De Corby et al. 2007) and the effect of increased glyphosate use on weed communities (Harker et al. 2005b). Studies concluded GR wheat improves in-crop weed control, volunteer GR wheat is not more invasive and can be controlled using available herbicides, wheat seed banks are short lived, PMGF is limited, and effects of increased glyphosate use on weed biodiversity was minimal.

Concerns surrounding seed mediated gene flow and the ability to meet undefined AP thresholds leading to potential market harm was expressed by the Canadian Wheat Board (CWB 2003). Due to the movement of wheat between U.S and Canada, Monsanto agreement to release GR wheat was based on regulatory approval in both the United States and Canada or not at all. In May 2004 the registration for commercial release of

GR wheat in Canada was voluntarily withdrawn by Monsanto. The CWB's strong opposition to GR wheat played a significant roll in shaping the outcome of this technology in Canada (Berwald et al. 2006).

The lack of producer and market acceptance, despite regulatory approval, may ultimately decide the fate of new transgenic technologies. This regulatory disconnect between market acceptance and scientific research will continue to play a significant role in the development of new GM crops. The uncertainty that exists within this framework will prevent further investment and ultimately hinder the development of beneficial technologies.

1.6. Canadian cropping systems

1.6.1. Wheat background

Common bread wheat has been cultivated for approximately 8000 years and its progenitors as far back as 12,000 years (Stallknecht et al. 1996). The fertile crescent of Near East, near the Tigris-Euphrates regions, was the origin of domesticated Einkorn (*Triticum monococcum*) wheat. Two proposed centers for the origin for spelt (*Triticum spelta*) wheat are the geographical region of present day Iran and two independent centers, Iranian and European, with the Iranian being the most widely accepted center (Zohary and Hopf 1993).

Wheat is the 2nd largest food crop grown in the world, second only to rice. Wheat is a major dietary component of many counties because of its agronomic adaptability. Wheat can be grown from within the Arctic Circle to higher elevations near the equator (Curtis 2002). Wheat is grown around the world and is used for food and feed as well as starch production for ethanol for biofuels.

Modern wheat is a product of interspecific hybridization between three diploid species to produce an allopolyploid. Modern bread wheat (hexaploid, 2n=6x=42) is derived from three species contributing three genomes (AABBDD). The hybridization of wild Einkorn (*Triticum urartu*) (AA) and *Aegilops speltoides* (BB) produced wild Emmer (*Triticum dicoccoides*) and cultivated Emmer (*Triticum dicoccon*) (AABB). The introgression of *Aegilops tauschaii* (DD) resulted in spelt wheat (*Triticum spelta*), which

is the precursor to modern hexaploid wheat (Hegde and Waines 2004). The D genome is shared with many weedy relatives that occur in North America such as jointed goatgrass (*Aegilops cylindrica* Host.) and has the potential to outcross with cultivated wheat (Morrison et al. 2002b).

1.6.2. Wheat in the cropping system

Spring wheat (*Triticum aestivum* L.) is a major crop in Canada. In 2001, spring wheat (excluding durum *Triticum durum*) was grown on more acres in Canada than any other crop, followed by barley (*Hordeum vulgare*), alfalfa (*Medicago sativa*), canola (*Brassica napus*) and soybeans (*Glycine max*) (Statistics Canada 2001). The Prairie Provinces, Alberta, Saskatchewan and Manitoba, account for 99 percent of the spring wheat acres produced in 2001 (Table 1.1). Wheat production has declined 35 percent between 1991 and 2001, possibly due to the increased adoption of herbicide-resistant (HR) canola in western Canada and lower market value for this commodity.

Canada is the 2nd largest wheat producing and exporting nation in the world, accounting for 18 percent of the world exports. All western Canadian wheat is exclusively marketed and sold through the Canadian Wheat Board (CWB), a marketing agency representing over 85,000 producers. Canada exports to over 70 countries annually over 20 million tonnes of wheat and barley. The largest overseas consumer of Canadian wheat was China in 2005, purchasing over 1.6 million tonnes (Canadian Wheat Board 2005).

1.6.3. Frequency of wheat in rotation

Although the spring wheat seeded area has been on the decline, it is still a significant portion of cropping rotations in western Canada. Ultimately driven by market prices, the frequency of wheat in rotation can very diverse and can be as frequent as continuous cereals crops that are grown for animal feed. In Alberta, wheat is commonly grown one in every three cropping seasons (Hall et al. 2007).

1.6.4. Weed control in wheat

Herbicides may be applied to spring wheat production systems at several timings within the cropping year, and the time of application will influence the chemical, rate, and combinations applied. Application intervals fall into three main categories: pre-seeding, in-crop and pre-harvest. Pre-seeding applications generally are composed of a non-selective alone or combined with a selective herbicide for specific weed spectrums (Retzinger and Mallory Smith 1997). The most commonly used herbicides are glyphosate (group 9) applied alone or in a mixture with group 2 or 4 herbicides; group 3 herbicides may be soil applied but are less commonly used. In-crop applications include groups: 1, 2, 4, 5 or 6, applied alone or in mixtures. Pre-harvest herbicides are applied prior to harvest to control weeds and uniformly lower the moisture level of the crop for harvest efficiency; these herbicides include groups 9, 10 and 22.

1.6.5. Use of glyphosate in cropping systems

Glyphosate is the most commonly used and most successful herbicide in the world. Prior to GR crops, the primary use was for non-crop (industrial uses), pre-seeding and post harvest applications. The introduction of GR crops has made in-crop applications of glyphosate possible, increasing the annual usage six fold between 1992 and 2002 (Cerdeira and Duke 2006).

1.6.6. Herbicide-resistant wheat in crop rotations

GR wheat may allow additional in-crop glyphosate to be used in rotations, therefore reducing the impact of current herbicide-resistant weeds. Wild oat (*Avena fatua*) has been identified in Canada with both cross and multiple resistance to both groups 1 and 2 (Friesen 2000). GR crops would provide herbicide rotations in cropping systems that would decrease selection of group 1 and 2 herbicide-resistant weeds, but increase selection for glyphosate-resistant weeds.

Crop rotations in dry environments are limited to herbicides with few re-cropping restrictions. Spring wheat herbicides from some sulfonylurea and imidazolinone chemical families may have soil residues that require re-cropping restrictions in subsequent years.

Due to environment and soil properties, soil residues can be difficult to predict and may reduce the yield of rotational crops. In-crop control of wild oat was limited to group 1 herbicides (Mallory Smith and Retzinger 2003) which has increased the risk of herbicide resistance. Glyphosate has no soil activity or rotational cropping restrictions, which may increase crop rotation options where crops sensitive to group 2 herbicides are commonly grown.

1.6.7. Imidazolinone-resistant (IMI) wheat

Clearfield® wheat (Teal 11A) resistant to the imidazolinone (IMI) herbicides, the first and only HR wheat in Canada, was registered in 2004. This tool provides producers with the opportunity to control cereal volunteers, therefore, reducing dockage and reducing adventitious presence (admixture) of off-type wheat varieties. This HR wheat variety was derived through point mutation of a single nucleotide in one of the three acetohydroxyacid synthase (AHAS) genes, thereby altering the binding site of the imidazolinone herbicide (CFIA 2004b). Developed using the process of mutagenesis, rather than genetic modification, Clearfield® wheat has sidestepped many of the controversial issues relating to GM crops (Tan et al. 2005). With regards to gene flow, the biological potential is similar to GR wheat, but the marketing and international trade barriers do not exist. This technology was evaluated by the CFIA as a novel crop and was approved for unconfined release in 2004. The addition of Clearfield® wheat, and other IMI crops such as canola, lentils, alfalfa and sunflowers, can lead to the increased use of group 2 herbicides (Mallory Smith and Retzinger 2003) and greater selection for group 2-resistant weeds.

1.7. Modeling

Science based regulatory decisions on the potential environmental and agronomic impacts from the deregulation of new GM crops are costly and data limited. It is not possible to conduct field research to mimic all possible outcomes, therefore, modeling may be a valuable tool for predicting gene flow and aiding regulatory decision making. Understanding critical parameters required to develop gene flow models may help focus research efforts and provide congruent datasets between crop species. A complete review

of population dynamics is beyond the scope of this thesis, for a complete review of the subject readers are referred to Groenendael (1988) and France (1988). Here, the various types of models that exist to predict biological events and their relevancy will be discussed.

1.7.1. Model types

Rouch (2006) has categorized models into four general groups including: verbal, statistical, simulation and analytical. Verbal models are intended to simplify complex relationships by breaking these relationships into smaller components. These include visual descriptions to illustrate concepts and ideas, commonly with pictures, diagrams or graphs (Figure 2). These models have a limited ability to calculate risks and potential outcomes, but are very effective at illustrating concepts. The last three models are mathematically based and can be utilized to analyze processes and potential outcomes from model simulations.

Statistical models are based on data sets that have been developed and a mathematical equation is used to describe data. These models are very useful because they are based on real datasets. Statistical models incorporate empirical and stochastic model types. Stochastic models are based on assigning probabilities to certain outcomes. Pollen mediated gene flow has been modeled empirically by developing a general regression equation for wheat pollen flow data. This model examined pollen flow movement and the effect of harvest blending of GM wheat with non-GM wheat (Gustafson et al. 2005). Statistical and empirical models are developed to describe an event that has occurred, simulation models are based on describing and understanding the causation of the mechanisms that contribute to the collective outcome (France 1988).

Simulation (mechanistic) models mimic the mechanisms of an organism or an event, and can include empirical and stochastic components to describe an event. Mechanistic models are commonly used in agricultural and biological sciences including the aforementioned development of a volunteer wheat gene flow model; therefore the majority of the discussion will be devoted to mechanistic models.

An example of a mechanistic model is lifecycle or demographic modeling. Mechanistic lifecycle modeling involves the movement between lifecycle stages,

regulated by model transitions (fluxes), based on datasets or assumptions from literature, and also input and output variables. An example of a transition in lifecycle mechanistic modeling would be a herbicide application that would select a volunteer genotype that will survive and progress to the next stage of the lifecycle (Figure 1.2). A mechanistic model describing lifecycle transitions was developed to describe the effects of agronomic practices on HR wheat and jointed goatgrass (*Aegilops cylindrical*) (Hanson et al. 2002). This model describes transitions in the lifecycle of jointed goatgrass. It includes input (published literature) and output variables (transitions matrices) within different rotation strategies to minimize gene flow. This is a simple mechanistic model that was effective in illustrating the effect of specific agronomic factors on population dynamics of jointed goatgrass (Hanson et al. 2002). Lifecycle models have been used to investigate gene flow between HR crops and volunteers. This model (GeneSys) was developed using rapeseed (*Brassica napus*) and includes the effects of cropping systems on the movement of transgenes (Colbach et al. 1999), evolution of volunteers in fields (Colbach et al. 2001b; Fargue et al. 2005), the exchange of transgenes among volunteers and the crop (Colbach et al. 2001a) and the effect of rapeseed genotypes on gene flow (Colbach et al. 1999).

Mechanistic gene flow models have been developed to predict the selection of herbicide resistance (Cavan et al. 2000; Diggle et al. 2003; Neve et al. 2003; Neve et al. 2003b; Vidotto and Ferrero 2005), the effects of HR crops on biodiversity (Watkinson et al. 2000) and to investigate ferality in domesticated crops. A mechanistic gene flow model was developed by Brûlé-Babel et al. (2006) to predict the selection pressure of GR wheat in non-GR wheat. Using weed population dynamics principals the number of years was predicted for GR volunteers to reach a frequency of 1.0 in the soil seed bank. Based on 95% selection pressure at various levels of pollen mediated gene flow, it was predicted that after only two to four generations of herbicide treatments, 50% of the volunteer population would be of the GR resistant. This conclusion does not include other important mechanisms that would reduce volunteer GR wheat frequencies in a population such as seeding a conventional wheat crops or crop rotations that provide in-crop control of GR wheat volunteers. Mechanisms that are critical to the movement and persistence of volunteer wheat in the agro-ecosystem must be incorporated into models to increase confidence in the predictions.

1.7.2. Data Gaps

There is a wealth of knowledge on plant species grown as commercial crops and also the weeds that occupy the agricultural ecosystem in which these crops are grown. With the introduction of HR crops and concerns about gene flow and admixture there is an increasing need to understand the biology of volunteer crops. This includes persistence of seed banks, fecundity, harvest losses and admixture. As previously discussed, there has been a greater research focus on the movement of genes via pollen. There is little information on the fecundity of crop species in the volunteer context. This thesis research was developed to fill some of the data gaps required for modeling purposes.

1.7.3. Biological parameters

Model parameterization is an important aspect of model development. Key parameters that describe model transitions are necessary to improve model accuracy. The contribution of specific parameters to the model can be tested using sensitivity analyses and will aid the focus of data generation to support the model development (Vidotto et al. 2001; Colbach et al. 2004; Karsten et al. 2005; Vidotto and Ferrero 2005). Initial model sensitivity analyses, using the aforementioned volunteer wheat model, determined that volunteer fecundity and seed bank longevity were key to the persistence of volunteer wheat (data not shown).

This thesis discusses the relevant biological parameters used to develop a volunteer wheat gene flow model. The data provides relevant biological parameters that are the basis for many transition assumptions in the lifecycle of wheat as a volunteer and as a seeded crop.

1.8. Research Objectives

The continued research and development of new GM crops is ongoing, with more crops and traits to be added in the future. It is important to have a sound understanding of the potential agronomic, environmental and socioeconomic impacts of these technologies to manage them effectively. This thesis presents the results of field experiments to quantify biological parameters of volunteer wheat in cropping systems. The increased

understanding of volunteer wheat will aid both regulators and early adopters of new technologies and will contribute to the development of a mechanistic gene flow model. Because the primary objective of this research was to develop data for model development, all possible herbicide options were not included. The importance of herbicide rates and application timings were explored to provide data ranges for model inclusion. The thesis is divided into two sections presenting field experiments, first in crops where herbicide options exist for controlling volunteer wheat, and secondly in cereal crops where in-crop herbicide options are currently not available for volunteer wheat. The final chapter will summarize the results and their applications and future research needs.

1.8.1. The effects of herbicides on volunteer wheat fecundity

Field experiments evaluating the interaction of pre-seed and in-crop herbicide control of volunteer GR volunteer wheat in both glufosinate-resistant canola and peas are presented. Increasing rates of quizalofop-P were applied pre-seed and increasing rates of glufosinate and glufosinate + sethoxydim were applied in-crop in glufosinate-resistant canola. The same treatments were applied pre-seeding in peas but with imazamox + imazethapyr applications in-crop.

The objectives of this study were to:

- Document the control and survival of GR volunteer wheat after herbicide applications
- Determine which application timing, pre-seeding or in-crop herbicides are more effective at reducing volunteer density and volunteer fecundity in canola and peas
- Quantify the admixture of volunteer GR wheat with increasing herbicide rates
- Quantify the effect of herbicides on volunteer wheat kernel size and viability
- Develop regression curves describing volunteer wheat control and fecundity
- Determine the effects of herbicide treatments on volunteer wheat AP in two HR canola varieties where control of GR wheat is possible

Study hypotheses:

- Volunteer wheat survivability, biomass and fecundity are reduced with increasing rates of both pre-seeding and in-crop herbicides

- Crop yields will increase with one or more application of herbicides
- The admixture of GR volunteer wheat seeds will be less with one or more applications of pre-seeding or in-crop herbicides and their combinations
- GR volunteer wheat seeds will be reduced in size and or viability with increased rates of pre-seeding or in-crop herbicides and their combinations

1.8.2. Volunteer wheat fecundity in cereal crops

Field experiments were conducted to investigate the effects of cereal crop competition in the absence of herbicides on volunteer wheat fecundity. Naturally occurring volunteer populations were marked within both wheat and barley crops seeded at 2 planting times and at 4 seeding rates. Individual volunteer fecundity was measured along with the timing of anthesis for both the seeded crop and the volunteer. The admixture of volunteer wheat in the absence of herbicide control was measured in barley.

The objectives of this study were to:

- Quantify the effect of crop competition on volunteer wheat fecundity
- Quantify volunteer wheat mortality in the absence of herbicides
- Investigate the most effective crop competition tool to reduce volunteer wheat gene flow.

Study hypotheses:

- The effect of crop competition will reduce volunteer wheat fecundity
- Volunteer wheat fecundity will be most affected by spikes plant^{-1}
- The yield component most affected by competition will be spikes plant^{-1}
- Volunteer mortality will increase with the competitive nature of the crop
- Volunteer wheat anthesis will flower synchronously with the seeded crop
- Crop competition will not affect the AP of volunteer wheat in harvested samples of barley

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Table 1.1 Spring wheat acres seeded in Canada and major wheat producing provinces (> 100 000 acres in 2001).

Location	1991[a]	1996[a]	2001[c]	2005	Change (1991-2005)
	seeded acres				%
Canada	29 463 938	24 634 614	20 513 265	19 158 000[d]	- 35.0
Alberta	6 885 763	6 448 110	5 809 275	5 737 000[d]	- 16.7
Saskatchewan	17 253 151	13 898 926	10 695 013	9 800 000[e]	- 43.1
Manitoba	5 083 636	4 022 128	3 693 662	2 805 000*[f]	- 44.8
Ontario	40 071[b]	59 149[b]	125 477	155 000*[g]	+ 286.8

* Estimates of production

[a] (Statistics Canada 1991)

[b] (Ontario Ministry of Agriculture, Food and Rural Affairs 2006a)

[c] (Statistics Canada 2001)

[d] (Alberta Agriculture, Food and Rural Development 2006)

[e](Personal Comm 2006)

[f](Manitoba Agriculture, Food and Rural Initiatives 2006)

[g](Ontario Ministry of Agriculture, Food and Rural Affairs 2006b)

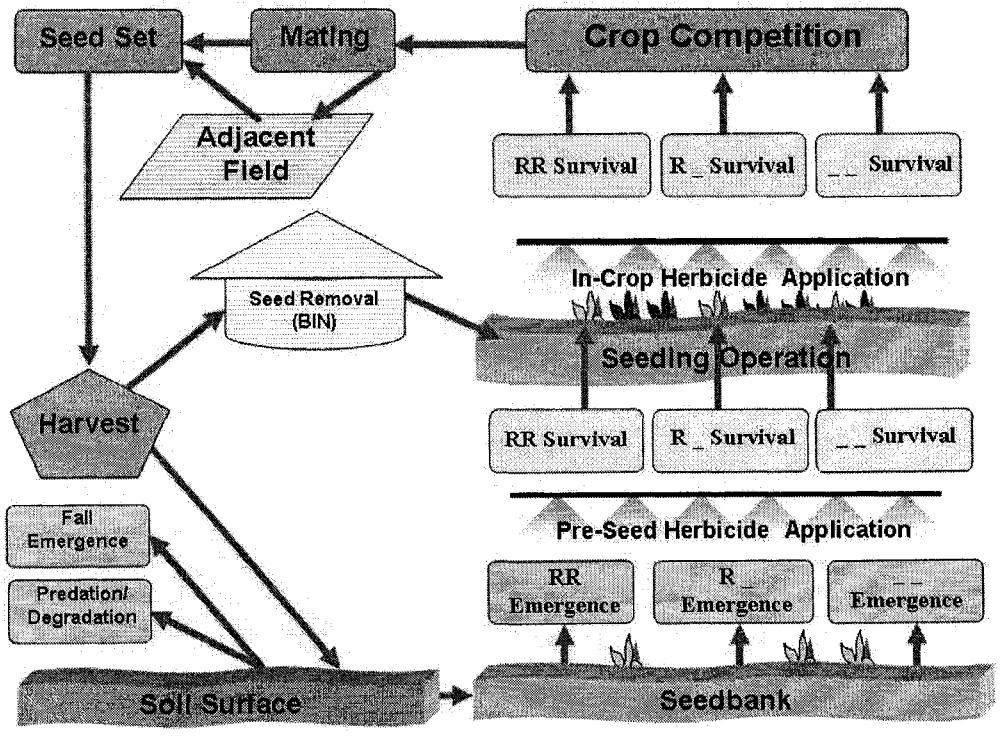

Figure 1.1. Flowchart illustrating the annual lifecycle of wheat using Roundup Ready® (RR) wheat as a model crop. RR, R _ indicates homozygous dominant and hemizygous, respectively, for the Roundup Ready® gene; and _ _ represents the absence of the transgene, susceptible to glyphosate. Differential herbicide selection occurs at the pre-seeding and in-crop herbicide applications for seeded crops and volunteers (Hall et al. 2007)

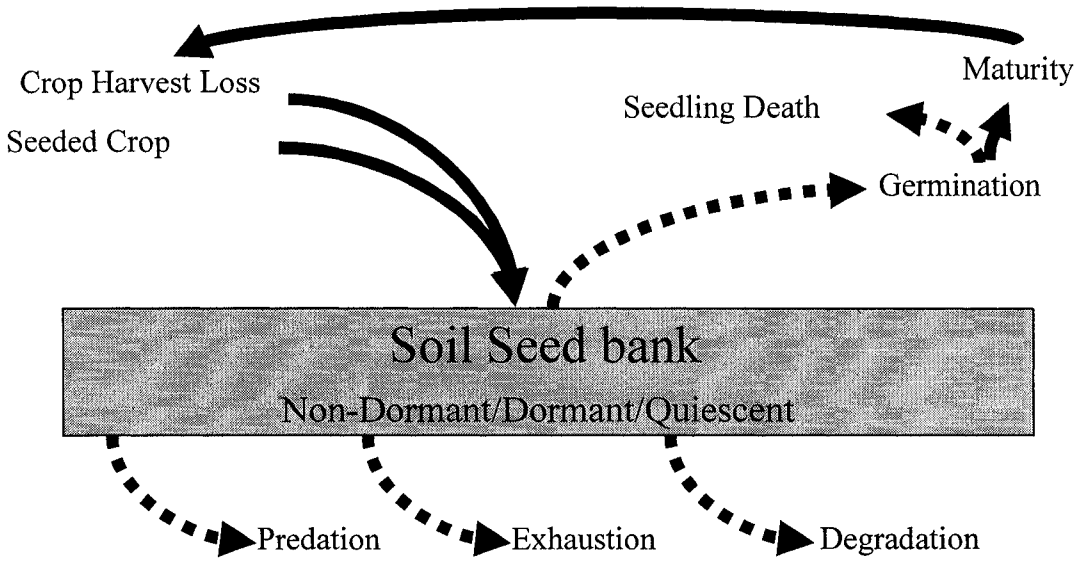

Figure 1.2 Soil seed bank flowchart representing the movement of seed cohorts and their possible fates. The shaded box indicates the state of the seeds within the seed band, the dotted arrows indicate the withdrawal processes and solid arrows indicate seed input (Adapted from Baskin and Baskin 1985 and Booth et al. 2003).

Chapter 2

2.0 Effect of herbicides on volunteer wheat (*Tritium aestivum* L.) fecundity

2.1 Introduction

The proposed commercialization of genetically modified (GM) glyphosate-resistant (GR) wheat polarized the interests and opinions within the agriculture community. GR wheat would provide Canadian wheat growers with improved in-crop weed control (Blackshaw and Harker 2002). However, GR wheat may pose a risk to export markets due to adventitious presence (AP) of undefined thresholds for admixture of GR wheat in conventional wheat. Market acceptance of GM crops by importing countries can also limit the development of new technologies (Wilson et al. 2003; Berwald et al. 2006). Canadian growers were also concerned with the increased frequency of use of glyphosate, increasing the rate of selection for GR weeds (Lyon et al. 2002; Gurian-Sherman 2003). Volunteers may become more difficult to control with pre-seeding herbicides, therefore, reducing the utility of glyphosate for conservation tillage (Ogg and Isakson 2001; Saskatchewan Soil Conservation Association 2001: Van Acker et al. 2003). While the opponents and proponents had many divergent opinions, there was a consensus that more research was required to determine potential impacts of new technologies on the cropping system (Ogg and Isakson 2001; Grain industry working group on genetically modified wheat 2003; Van Acker et al. 2003).

Spring wheat was grown on 7.7 million hectares in Canada in 2005 with 99% grown in the three Prairie Provinces (Statistics Canada 2005). The number of hectares dedicated to growing spring wheat has decreased by 35 percent between 1991 and 2001 with the greatest decrease occurring in Saskatchewan. Although spring wheat production has been decreasing, the relative abundance of volunteer wheat found in other crops has been rising over the last four decades (Leeson et al. 2005).

The Canadian prairies have traditionally been dominated by cereal crops grown in rotation with summer fallow. Within the last 20 years, there has been a shift from a fallow/cereal rotation to continuous cropping (Gan and Stobbe 1995), leading to changes in weed demographics and control methods. Cereal crops in Alberta, predominantly

wheat and barley, are grown in rotation with canola, flax, dry peas and lentils (Zentner et al. 2002). In Alberta, cropping rotations commonly include wheat being grown once every three years (Hall et al. 2007). Producers make cropping rotation decisions based on agronomic, economic, environmental and social conditions, and these decisions vary significantly by agroecosystem and market fluctuations.

In the years following wheat production, volunteers can be controlled with herbicides applied before seeding (pre-seeding) and early in a follow crop (in-crop). Glyphosate-resistant wheat may be difficult to control because glyphosate is frequently used alone in the Canadian cropping system as a pre-seed weed control in reduced tillage systems and in-crop with canola in the west and both corn and soybean in the east. Uncontrolled volunteers could replenish the seed bank and extend the need for control in future years.

Adventitious presence is the mixing of foreign objects within a harvested commodity, with respect to GM crops, AP is the inadvertent presence of GM seeds in conventional or organic crops (Drew and McHughen 2005). Due to the scale of modern agricultural practices AP is a common phenomenon and thresholds are in place to deal with acceptable levels of mixed foreign objects. At the time of testing GM wheat, the European Union had a virtual moratorium on GM imports, with no established thresholds for AP in conventional wheat.

With respect to GM wheat, the European Union and other major Canadian wheat importers threatened to reject all Canadian wheat exports, both GM and non-GM if GR wheat was commercialized. The potential for market loss to Canadian producers raised many concerns and objections to GR wheat, predominantly by the Canadian Wheat Board (Canadian Wheat Board 2003).

Commingling leading to AP may occur if GR wheat volunteers reach maturity in subsequent crops and are mixed during harvest. With the exception of cereal crops such as wheat and barley, GR volunteer wheat can be controlled in subsequent crops, but the fecundity (seeds produced plant^{-1}) of wheat after herbicide treatment has not been quantified (Rainbolt and Thill 2003; Rainbolt et al. 2004; Harker et al. 2005). Volunteer wheat fecundity is an important model parameter that will contribute to the increased accuracy of a mechanistic gene flow model currently being developed. Initial model

development has been based on parameters using wheat grown as a crop, but volunteers may be greater or less fecund is a less competitive crop or micro-sites requiring competition for resources, respectively. Pure stands of four spring wheat cultivars in commercial fields in Canada averaged 104 seeds plant^{-1} with a seed kernel weight of 31 mg (Campbell et al. 2002). We hypothesize that wheat volunteer survival and fecundity is influenced by the time of emergence, crop competition and herbicides but the influence of these parameters have not been quantified. Herbicides may prevent volunteer wheat survival or have sub-lethal effects that reduce seed set or viability.

Two studies (A and B) were conducted to measure the effects of crop competition and herbicides on volunteer wheat survivorship and fecundity. Study A was conducted under confined release with GR volunteer wheat, in two follow crops, peas and glufosinate resistant canola, to measure the impact of pre-seeding and in-crop herbicides on the volunteers. Study B was conducted with conventional wheat volunteers to determine their response to different doses of in-crop herbicides when growing in glufosinate and imidazolinone-resistant canola. The herbicides used were expected to have a similar effect on GR and conventional volunteers (Blackshaw et al. 2006). The use of conventional wheat for the dose response experiments eliminated the requirement for post-trial monitoring for transgenic wheat volunteers and decreased the total trial area. The results of these studies will quantify volunteer wheat survivorship and fecundity under different herbicide regimes in the broad leaf crops, canola and peas.

Data will be used to paramatize a model to predict gene flow and volunteer wheat population dynamics for GR wheat. These findings may also be used, in part, to develop stewardship or best management practices for the production of GR wheat production in the future.

2.2 Materials and Methods

2.2.1 Study A: Interaction of pre-seed and in-crop herbicides on the survivorship and fecundity of GR wheat volunteers.

Research trials were conducted at Alberta Agriculture and Food, Crop Diversification Center North (CDCN) and the University of Alberta Ellerslie Research station (Ellerslie)

in 2004, and near Calmar Alberta and Ellerslie in 2005 (Table A.3). Soil properties and fertility were analyzed[1] from 10 bulked soil samples taken from each site in the spring. All research sites were black chernozemic soils. Ellerslie was a silty clay loam soil with a pH of 5.7-5.9 and 26-27 % sand, 49 % silt and 23 % clay content. Calmar had a pH of 6.5 with 12, 19, and 51 % sand, silt and clay content, respectively (Table A.3). Soil at CDCN had a pH of 6.0 and an organic matter content of 6.0 %, and 42, 38, and 20 % sand, silt and clay content respectively. Meteorological data was collected on location by the respective organization or from the nearest Environment Canada weather station (Fig. 2.1-2.4).

Separate experiments for glufosinate[2] resistant canola cv. 'InVigor 5030' and peas cv. 'Toledo' were established in a randomized complete block with four replicates in a factorial arrangement including pre-seed and in-crop herbicides. Plots were seeded 2 x 8.5 m and later trimmed to 2 x 6.5 m after in-crop herbicide application.

Glyphosate-resistant wheat volunteers were seeded, to facilitate faster emergence over broadcasted seeds, in early spring at a target rate of 75 plants m^{-2} using a research scale zero-till seeder at a depth of 1 to 1.5 cm perpendicular to the direction of the crop to be seeded and both pre-seed and in-crop herbicide applications. Volunteers were established without fertilization or seed treatments to simulate a population established from a previous years harvest loss. After volunteer emergence, one randomly placed 2 m^2 permanent quadrat was established in each plot. Volunteers in the quadrats were counted prior to herbicide applications and at crop maturity. Canola and peas were seeded at a target plant density of 150 and 75 plants m^{-2} respectively. A double disk research seeder was used to seed both crops to reduce volunteer disturbance, therefore, attributing volunteer mortality to herbicide applications rather than seeding mortality. Both canola and peas were fertilized according to soil test recommendations. To facilitate N_2 fixation, the peas were inoculated with the appropriate rhizobium innoculum.

Quizalofop-p-ethyl[3] was applied at 4 rates 0, 12, 18 and 24 g ai ha^{-1} with 0.5 % v/v Sure-Mix® surfactant tank mixed with glyphosate[4] at 444 g ai ha^{-1} as recommended

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[1] Soil analysis performed by Norwest Labs, 7217 Roper Rd. Edmonton, AB. Canada. T6B 3J4
[2] InVigor Liberty Link Bayer CropScience Canada Inc. #100, 3131-114 Ave. S.E. Calgary, AB. T2Z 3X2
[3] Assure II DuPont Canada Inc. 4444 – 72 Ave. S.E. Calgary, AB. T2C 2C1
[4] Roundup Transorb Monsanto Canada Inc. 67 Scurfield Blvd. Winnipeg, MB. R3Y 1G4.

for control of volunteer GR wheat volunteers (Rainbolt et al. 2004; Blackshaw et al. 2006). The untreated control (UTC) received a pre-seed glyphosate application to control weeds and mimic uncontrolled volunteer GR wheat (Rainbolt and Thill 2003). All herbicide treatments were applied using a CO_2 propelled herbicide applicator delivering a water volume of 100 L ha^{-1}. Each replicate included an untreated control as a reference for the treatments. Both glufosinate-resistant canola and peas received the same pre-seed treatments, applied when GR wheat volunteers were at BBCH 12 to 13, 2 to 3 leaf, (BBCH Monograph 2001) (Table A.5). The volunteer wheat in the check was at BBCH 22-23 at the in-crop herbicide timing (Table A.5).

In-crop treatments for glufosinate-resistant canola included 300 and 500 g ai ha^{-1} of glufosinate ammonium alone and 300 g ai ha^{-1} glufosinate + 211 g ai ha -1 of sethoxydim applied when the canola was at the 2 to 3 leaf stage (BBCH scale 22 to 23). An untreated weed-free control was maintained by hand weeding as a reference. In-crop herbicide treatments in peas were 14.7, 22.5, and 29 g ai ha^{-1} of imazamox + imazethapyr[5] with 0.5 % v/v Merge surfactant applied at the 1 to 2 node stage (BBCH 11 to 12). A glyphosate-only treated control was included as a reference. Volunteer wheat developmental stages varied between the treatments due to differential response to the different pre-seeding herbicide treatments.

Volunteer wheat phytotoxicity was assessed using visual ratings of the whole plot at 7, 14, and 21 days after treatment (DAT) for both the pre-seeding and in-crop herbicide applications. Visual control ratings were on a scale from 0% (no control) to 100% (total control), based on comparisons to the untreated controls. A benchmark control rating of 80% at 21 DAT for the in-crop application was deemed to be acceptable control, meaning no reproductive spikes were present at the time of observation.

At crop maturity and prior to volunteer seed shatter, crop and volunteers were hand harvested within the 2 m^2 quadrats. The crop was cut at soil height. The volunteer wheat plants were removed, counted and the roots removed and biomass determined. Plots were harvested with a research scale combine using the appropriate settings to remove chaff from harvested samples. Above ground biomass of the crop and volunteers were dried at 37 °C for at least 5 days and weighed, volunteer wheat spikes were counted

[5] Odyssey BASF Canada Inc. 345 Carlingview Dr., Toronto, ON. M9W 6N9

and hand threshed to determine individual plant fecundity. Harvested crop samples were dried for 5 days at 52 °C. Wheat seeds were recovered from the harvested crop grain samples to determine volunteer wheat AP. Harvested and cleaned grain (crop) was weighed and yields (tons ha^{-1}) calculated. Wheat seed recovered from the harvested grain during cleaning was counted, weighed and 1000 kernel weight calculated. The wheat seed recovered from the harvested samples (AP) was reported as the number of volunteer wheat seeds recovered m^{-2} and also as a percent weight of the volunteer seed to the weight of the harvested crop.

2.2.2 Study B: Herbicide dose response of volunteer wheat.

A dose response field experiment was conducted in both glufosinate and IMI resistant canola production systems at the Edmonton and Ellerslie research stations in 2005 and 2006. Soils at the Edmonton research station were black Chernozemic characterized by high organic matter (11-13 %), with a pH of 5.7-6.0 and a sand silt and clay content of 24-26, 39-45, and 32-34 %, respectively (Table A.4). Ellerslie research station, also a black chernozemic soil, had a pH of 5.8-6.1 % and an organic matter content of 10-11 % and 23-29 % sand, 41-53 % silt and 30-24 % clay content. Soil was sampled and analyzed as described in Study A.

Field trials were established on barley stubble that had been harvested prior to seed maturity for silage to reduce barley volunteers. Separate split plot experiments, for each canola system, were established as a randomized complete block with four replications. Herbicide rates were established as the main plot with a split at seeding rates as the subplot. Plots were seeded 4 x 8.5 m and trimmed to 4 by 6.5m after herbicide application, subplots were 2 by 6.5 m after trimming. To control weeds prior to seeding, glyphosate (1 L ha^{-1}) was applied. Hard red spring wheat cv. 'AC Superb' was hand broadcast over the plot area at a target rate of 75 plants m^{-2} to establish a volunteer wheat population. Canola was seeded at a rate of 75 and 150 plants m^{-2} using a research scale low disturbance air seeder using 20 cm row spacing. Each plot was six rows of canola with two border rows of winter wheat to reduce weed establishment between plots.

Herbicide treatments were applied using a CO_2 propelled sprayer delivering 100 L ha^{-1}. Herbicide rates of 0.25, 0.5, 0.75, 0.88, 1.0 and 1.25 times the recommended rates were applied. In imidazolinone-resistant canola 7.35, 14.7, 22.05, 25.9, 29.4, and 36.75 g ai^{-1} of imazamox + imazethapyr with 0.5 % v/v of merge surfactant and in glufosinate resistant canola 0, 100, 200, 300, 350, 400 and 500 g ai ha^{-1} of glufosinate ammonium. The range of rates, which correspond to 1.25 times the recommended rate at the top of the range (Brooks 2006), were intended to provide the a range of volunteer wheat responses to the herbicide. For both canola systems herbicides were applied at BBCH 13-15 and BBCH 14-16 in 2005 and 2006, respectively. The volunteer wheat was at BBCH 13-14 in 2005 and at BBCH 21-22 in 2006 (Table A.6). An untreated control (UTC) was included for both seeding rates for comparison.

Wheat volunteer phytotoxicity was evaluated visually at 7, 14 and 21 DAT. Volunteer wheat dry weight were assessed 28 DAT by cutting two random 1/4 m^2 samples that were dried at 52 °C for 5 days. At maturity, volunteer wheat with reproductive spikes within pre-established 0.25 m2 quadrats were removed by hand with their roots intact, counted and roots removed. Volunteers and crop biomass collected at maturity were dried at 37 °C and 52 °C for 5 days, respectively, and then weighed. The volunteer wheat plants were dried at a lower temperature to prevent seed mortality to enable later viability testing (see seed viability testing below). Dry weights were measured, reproductive spikes were counted and hand threshed. The volunteer wheat seeds were weighed and counted to determine the average volunteer fecundity.

Crop biomass was also removed from the three pre-established quadrats, dried at 52 °C for 5 days and weighed. The remainder of the plots was harvested with a research scale combine set to clean chaff from canola samples. Harvested canola seed was cleaned and AP volunteer wheat seeds separated.

Harvested volunteer wheat seeds from each 1/4 m^2 quadrats were counted and weighed to calculate a kernel weight (mg). Adventitious presence of volunteer wheat seed recovered from the harvested crop was counted, when samples were too large to be individually counted (> 5000 seeds), three sub samples were collected, counted and weighed to determine the 1000 kernel weight and applied to the whole sample to estimate seed numbers.

2.2.3 Seed Viability Analysis

Viability of hand-harvested volunteer wheat seeds were assessed by germinating three replications of 100 seeds from each quadrat. If there were less than 300 seeds total, the number of seeds were divided evenly among the three replications. Seeds were germinated in a 24 x 16 x 4 cm square germination container containing one 23 x 15cm Hoffman #601 blotter paper[6] with 14 ml of 0.2% v/v Helix Xtra (thiamethoxam + difenoconazole + metalaxyl-M + fludioxonil). Seeds were placed in the dark at room temperature for 5 days. Germinated seeds were then counted and ungerminated seeds were cut and placed in a Petri dish with a Whatman No. 1 filter paper and 2 mL of a 0.1% tetrazolium chloride solution (2, 3, 5-triphenyltetrazolium chloride) and placed in the dark at 60 °C for 2 hours. The seeds were designated as viable or non-viable based on staining and the distribution of stain on the embryo as recommended by the Association of Official Seed Analysts of North America (1970). Tetrazolium-positive seeds may artificially inflate the positive seed viability calculation. It is unlikely that the tetrazolium-positive seeds may never produce a viable seedling from the soil seed bank.

2.1 Statistical analysis

All data was checked for normality prior to analysis by using PROC UNIVARITE in SAS prior to analysis. Volunteer fecundity parameters, volunteer density at harvest and recovered admixture (seed numbers and percent of the crop) for study A were square root transformed $(x+1)^{0.5}$ prior to ANOVA. For study B the admixture data, both seed numbers and percent of the crop, were square root transformed $(x+1)^{0.5}$ prior to ANOVA using the PROC MIXED procedure in SAS (SAS Statistical Analysis Systems 2007) with year, location and block considered random. The denominator degrees of freedom used to calculate the significance of the fixed effects were adjusted using the Kenward Rogers method (Kenward and Roger 1997). For this model, location and years were considered random effects, and all other effects were considered fixed. When year and seeding rates were significant ($P < 0.05$), these were analyzed separately. To minimize the potential of

[6] Hoffman Manufacturing Inc. 16541 Green Bridge Rd Jefferson, Oregon. USA. 97352

type 1 error associated with pairwise comparisons, the Tukey-Kramer honestly significant difference (HSD) was used to determine levels of significance ($P < 0.05$) between pairwise comparisons as suggested by Steel et al. (1997). Mean separation was conducted with the PDIFF option in SAS, and letters were assigned to means using the PDMIXED 800 macro in SAS (Saxton 1998).

Dose response curves were derived for volunteer wheat biomass 4WAT and the fecundity of volunteer wheat at harvest. Volunteer wheat biomass and fecundity data were subject to non-linear mixed model regression using the NLMIXED procedure of SAS (Nielsen et al. 2004; SAS Statistical Analysis Systems 2007). The relationship between herbicide dosage and volunteer wheat biomass 4 weeks after herbicide treatments (WAT) and the fecundity of the hand-harvested volunteers at harvest were described with an exponential decay curve (Equation 1) (Belles et al. 2000). The independent variables were fit to approximate the normal distribution:

$$y = a^{(-b\{x\})} + e \qquad (1)$$

where y is the estimated biomass and volunteer wheat seed production relative to the herbicide dosage of glufosinate in glufosinate resistant canola and imazamox + imazethapyr in imidazolinone-resistant canola, a is the intercept, b is the slope, x is the herbicide rate and the error (e) was assumed to approximate a normal distribution (~normal (0, σ2)). This model was chosen based on the fit of the predicted curve and the residual structures. The data is expressed as means including standard errors (+/-). PROC NLMIXED calculates 95% confidence intervals that were used to determine significance between the measurements. Effective dosage rates for 50, 85 and 90% reduction of the untreated control were derived with the following equations:

$$ED_{50} = \frac{[\ln(0.5 * a) - (\ln a)]}{-b} \qquad (2)$$

$$ED_{85} = \frac{[\ln(0.15 * a) - (\ln a)]}{-b} \qquad (3)$$

$$ED_{90} = \frac{[\ln(0.10 * a) - (\ln a)]}{-b} \quad (4)$$

where a is the intercept and b is the slope of the line. SigmaPlot was used to fit the regression line for the estimated parameters derived from SAS PROC NLMIXED.

2.3 Results

2.3.1 Herbicide interactions

Precipitation in 2004 at both Ellerslie and CDCN was below the 30 year average for the area, with the exception of July when the Ellerlsie site received 133.4 mm of rain (Environment Canada 2006). The temperature in 2004 at both locations was similar to the 30 year average (Fig. 2.1 and 2.4). Precipitation at Ellerslie in 2005 was below the 30 year average in all months except August. In 2006 the mean monthly temperate in the April through June was above the 30 year average, leading to flowering stress and reduced yields in the canola (Fig. 2.1) (Environment Canada 2006). Volunteer wheat emergence was not significantly affected by year or location. Volunteers emerged and were competitive at all locations, with the exception of CDCN peas. Volunteer GR wheat in the checks was treated with glyphosate for weed control purposes. Volunteer GR wheat is not controlled with glyphosate (Rainbolt et al. 2004), and will hereafter be referred to as the untreated control for comparison to the treatments.

Canola yield in 2004 and 2005 averaged 1.28 and 2.3 ton h^{-1}, respectively, in the absence of herbicides (Table A.7). Both herbicide applications were significant in both years for canola. The interaction of pre-seeding and in-crop herbicides increased crop yields in 2005. Pea yields in the untreated check were very similar, 1.6 and 1.5 t ha^{-1} in 2004 and 2005, respectively (Table A.8).

2.3.1.1 Efficacy of herbicides in glufosinate-resistant canola

The mean volunteer wheat density prior to herbicide applications for 2004 and 2005 in canola experiments were not significantly different by year or location, averaging 75.3 plants m^{-2}. The dependent variables measured were significantly different between years

but not experimental locations ($P < 0.05$), therefore data was pooled over location and presented by year.

Glufosinate applied alone in-crop at both rates (300 and 500 g ai ha^{-1}) did not provide acceptable control of volunteer GR wheat as indicated by the 21 DAT visual ratings of 24.3 and 61.3% for 300 g ai ha^{-1} in 2004 and 2005, respectively and 58.5 and 72.5% for 500 g ai ha-1 in 2004 and 2005, respectively (Tables 2.1 and 2.2).

Herbicides were not consistent when measured between years for both application timings (Figure 2.5). A pre-seeding rate of quizalofop-P at 24 g ai ha^{-1} was required to significantly reduce volunteer GR wheat biomass at harvest in 2004. The pre-seeding treatment was more effective in 2005, requiring only 12 g ai ha^{-1} to significantly reduce volunteer biomass. Quizalofop rates above 12 g ai ha^{-1} did not significantly reduce volunteer biomass further (Table 2.3). A significant interaction was observed in 2004 ($P=0.0465$). The in-crop herbicide applied alone did not significantly reduce volunteer biomass. The combination of both applications was more effective that either application alone. Both the pre-seeding and the in-crop herbicide applications were more effective in 2005. Quizalofop-P significantly reduced volunteer wheat biomass at 12 g ai ha^{-1} and 300 g ai ha^{-1} of glufosinate alone reduced the biomass greater that the check. An interaction was observed that significantly reduced volunteer biomass greater than either application alone (Table 2.3).

The density of volunteers recovered at harvest was significantly reduced in both years by pre-seeding and in-crop herbicides, and a significant interaction ($P=0.0465$) with the two applications was observed in 2004 (Table 2.5). No significant decrease in density was observed as the rate quizalofop-P increased from 12 g ai ha^{-1} (Table 2.5). In 2004, no significant rate effect between 300 and 500 g ai ha^{-1} was observed with the activity of glufosinate applied in-crop alone, although both treatments were significantly lower than the control. The addition of 211 g ai ha^{-1} of sethoxydim tank mixed with 300 g ai ha^{-1} of glufosinate significantly decreased volunteer density compared to glufosinate at 300 g ai ha^{-1} alone, but not 500 g ai ha^{-1}. In 2005, significant differences in volunteer density were observed between 300 and 500 g ai ha^{-1} of glufosinate from 54.7 to 40.6 plants m^{-2}, respectively (Table 2.5). Applied alone, glufosinate tank mixed with sethoxydim was the most effective at reducing volunteer density in 2005 (22 plants m^{-2}).

Volunteer GR wheat fecundity (seeds plant^{-1}) was higher in 2004 than in 2005 (Table 2.3). To significantly reduce the individual fecundity of surviving volunteer wheat over the control in 2004, 24 g ai ha^{-1} of quizalofop-P was required. In 2005, a significant reduction in fecundity was observed with 12 g ai ha^{-1}, and did not significantly decrease further with increasing rates of quizalofop-P (18, 24 g ai ha^{-1}) (Table 2.3). In the absence of pre-seeding herbicides in 2004, 300 g ai ha^{-1} did not reduce volunteer fecundity less than the control. Glufosinate at 500 g ai ha^{-1} and 300 + 211 g ai ha^{-1} of glufosinate + sethoxydim were not significantly different at reducing volunteer fecundity in 2004, and were both significantly more effective that both the control and 300 g ai ha^{-1} (Table 2.3). Volunteer fecundity was highly variable in 2004, no significant differences were observed between 0 and 57.7 seeds plant^{-1} (Table 2.3). Glufosinate applied alone was more effective in 2005, significantly reducing volunteer fecundity over the control at 300 g ai ha^{-1}, with no significant decreases in fecundity as the herbicide rates increased. Significant interactions were observed in both years. Plotting the main effect means from both factors indicate that the pre-seeding application was most effective at reducing volunteer fecundity (Figure A.3).

The yield components contributing to individual volunteer fecundity are the spikes plant^{-1} and the seeds spike^{-1}. No consistent trends were apparent with the response of the yield components to herbicide applications in glufosinate resistant canola. In the absence of herbicides, individual volunteers produced 5.6 and 4.9 spikes plant^{-1} in 2004 and 2005, respectively (Table 2.4). Higher rates of quizalofop-P were required in 2004 than in 2005 to significantly reduce the spikes plant^{-1}. In-crop herbicides alone in 2004 did not significantly reduce spikes plant^{-1}. Glufosinate applied at 500 g ai ha^{-1} and 300 + 211 g ai ha^{-1} of glufosinate + sethoxydim reduced the spikes plant-1 significantly below the check and 300 g ai ha^{-1}. The combined effect of the two herbicide applications significantly lowered spikes plant^{-1} in 2005, but was not significant ($P < 0.05$) in 2004 (Table 2.4).

Average seeds produced spike^{-1} in the untreated control was 25.9 and 18.3 in 2004 and 2005, respectively (Table 2.4). In 2004, pre-seeding quizalofop-P alone at all rates tested (12, 18, 24 g ai ha^{-1}) did not affect seeds spike^{-1}. Only the tank mix of glufosinate + sethoxydim (300 + 211 g ai ha^{-1}) significant reduced seeds spike^{-1}

compared to the check. The interaction of both herbicide applications was not significant in 2004. In 2005, the pre-seeding and in-crop herbicides significantly reduced seed spike^{-1}. The combined effect of the two factors contributed to lowering individual volunteer fecundity (seeds plant^{-1}).

Total seed density, the fecundity of all volunteer GR wheat m^{-2}, from uncontrolled GR volunteers in 2004 and 2005 recovered from the harvest quadrates averaged 8752.9 and 6717.9 seed m^{-2}, respectively (Table 2.5). Pre-seeding and in-crop herbicides used alone significantly reduced the total seeds produced.

Total seed density in both years was not reduced significantly with the addition of sethoxydim with glufosinate. The interaction between both herbicide timings was significant. Plotting the main effect means from both factors indicate that the pre-seeding herbicide has a greater influence on the reduction of the total seeds produced (Figure A.4.) The lowest, statistically significant, volunteer wheat fecundity m^{-2} was 614.4 and 299.5 in 2004 and 2005, respectively (Table 2.5).

Viability and seed size of recovered volunteer wheat seeds were significantly reduced with the effectiveness of the herbicide treatments in 2004 and 2005 (Table 2.6). More seeds were recovered from the surviving volunteers in 2004. Viable seeds from all treatments were recovered in 2004. The combination of a pre-seeding herbicide application with 300 +211 g ai ha^{-1} of glufosinate + sethoxydim did not result in any volunteer seeds being recovered in 2005 (Table 2.6).

The admixed volunteer GR wheat seeds recovered were reported as seeds m^{-2} and as the percent of the weight of the volunteer seeds recovered to weight of the crop m^{-2}. Volunteer GR wheat admixture varied significantly between locations; therefore the results were presented by location. At all site year pre-seeding herbicides alone had a greater affect than in-crop herbicide rates alone (Table 2.7, Figures A3 and A4). In response to both pre-seed and in-crop herbicides, admixed GR wheat seeds decreased from 5706.7 in the untreated control to $\leq$ 19.3 seeds m^{-2} at Calmar. This was the lowest statistically significant number of seeds recovered from any site in this experiment. The lowest statistically significant percent admixture recovered from this site was 2.4% which suggests that the two measures can be variable (Tables 2.7 and 2.8). The interaction of pre-seeding and in-crop herbicides was significant for all site years. Main effect means

suggest that pre-seeding herbicides are more effective at reducing admixture (Figures A.5 and A.6).

2.3.1.2 Efficacy of herbicides in peas

Poor crop and volunteer emergence at the crop diversification center north (CDCN) in 2004 invalidated the data for this location, therefore, only one location (Ellerslie) was analyzed. Each year was significantly different for volunteer GR wheat emergence, averaging 89 and 77 plants m^{-2} in 2004 and 2005, respectively.

The pre-seeding herbicide application was more effective in 2004 than in 2005. In 2004, a rate of 18 g ai ha^{-1} was required to significantly reduce volunteer biomass below the untreated control. At the 24 g ai ha^{-1} rate of quizalofop volunteer GR wheat biomass was still present, but in 2005 treatments significantly reduce the biomass below the untreated control (Table 2.11). The imazamox + imazethapyr at 22.5 and 29.4 g ai ha^{-1} resulted in no volunteer biomass recovered in 2004. In 2005, the in-crop application was more effective than the pre-seeding. The interaction of the two herbicide applications was not significant in 2004 or 2005.

The individual fecundity of volunteer wheat was 164 and 134 seeds $plant^{-1}$ in 2004 and 2005, respectively (Table 2.11). Pre-seeding and in-crop herbicides used alone significantly reduced seeds $plant^{-1}$ in both years. Used in combination, a significant interaction was observed.

Spikes $plant^{-1}$ averaged 9.1 and 5.4 in the absence of herbicides in 2004 and 2005, respectively (Table 2.12). Pre-seeding and in-crop herbicides used alone significantly reduced spikes $plant^{-1}$ in both years. Used in combination they were more effective. Seeds $spike^{-1}$ averaged 26.9 and 25.4 in 2004 and 2005, respectively (Table 2.12). Pre-seeding and in-crop herbicides used alone significantly reduced seeds $spike^{-1}$ in both years. In peas the in-crop herbicide is more effective than canola.

The total seeds produced m^{-2} in 2004 was 10745 and 10299 in 2005. Pre-seeding and in-crop herbicides used alone significantly reduced total seeds m^{-2} in both years. And in combination they were most effective. Similar responses were observed in volunteer wheat density at harvest and individual volunteer biomass.

The harvest yield for peas in 2004 in the absence of herbicides was 156 and 152 ton ha^{-1}. Pre-seeding and in-crop herbicides used alone significantly reduced seeds $plant^{-1}$ in both years. The use of both pre-seeding and in-crop herbicides maximized crop yields. Viability and seed size of recovered volunteer wheat seeds were significantly reduced with the effectiveness of the herbicide treatments in 2004 and 2005. This may be due to the delayed seed set and maturity of injured herbicide effected volunteer. In 2004, both pre-seeding and in-crop herbicides used alone both decreased viability and seed size (Table 2.14). In 2005, the combination of both herbicide timings reduced both the kernel weight and viability.

Year but not the locations were significantly different ($P < 0.05$) for the number of volunteer GR wheat seeds recovered from the combine harvested samples (Table (2.15). When the admixture was calculated and expressed as a percent w/w of the crop, the locations were significantly ($P<0.05$) different and will be presented by location (Table 2.16). The number of seeds recovered m^{-2} from the combine harvested untreated checks were 9588.4 and 14006.0 in 2004 and 2005, respectively. The statistically lowest number of volunteer GR wheat seeds was in 2005, which has a larger density than in the 2005 sample (Table 2.15). This would indicate the herbicide imazamox + imazethapyr was more effective in 2005. The percent admixture level was not statistically below the proposed 0.9% EU threshold at any of the three sites (Table 2.16).

2.3.2 Dose response

Dose response studies were conducted to investigate the influence of herbicide dose on volunteer wheat fecundity and admixture in glufosinate-resistant and imidazolinone-resistant canola. Excessive heat in June and July of 2006 severely heat stressed both varieties of canola (Figure 2.2). The crop biomass and grain yields were less than 50% of those in 2005 (Tables A.9 and A.10). This reduction of biomass and yield greatly affected the ability of the crop to compete with volunteer wheat, notably in glufosinate resistant canola. Volunteer wheat average densities in the glufosinate-resistant canola were 73.9 and 106.8 plants m^{-2} in 2005 and 2006, respectively. Mean volunteer wheat densities for the imidazolinone-resistant canola were 77 and 94 plants in 2005 and 2006, respectively.

2.3.2.1 Dose response in glufosinate resistant canola

Years, not location, significantly ($P < 0.05$) influenced the results. Therefore the data were pooled over location and presented by year (Figure 2.7). The glufosinate herbicide treatments were more effective in 2005 than in 2006.

Seeding rate did not have a significant affect on volunteer wheat fecundity or admixture, only visual ratings were significantly different and were presented by seeding rate in both years (Table A. 7). In 2005, the effective dosage (ED_x) of glufosinate to reduce volunteer wheat biomass 4 WAT to 50, 85 and 90% of the untreated control was 66.5, 201.0 and 248.7 g ai ha^{-1} respectively (Table 2.17). In 2006, the same biomass reduction required 134.4, 307.4 and 360.2 g ai ha^{-1} of glufosinate (Table 2.18). The ED rates for glufosinate on volunteer wheat at harvest indicate that there can be considerable re-growth following the 4WAT biomass sampling (Tables 2.19 and 2.20). In 2005, the ED values were within 10 g ai ha^{-1} for both sampling dates, with is within the 95% confidence interval indicating no significant different. For 2006 the rate of recovery was much greater, the rate required for ED_{90} was 126 g ai ha^{-1} greater at harvest, which is significantly higher, based on the 95% confidence interval (Tables 2.18 and 2.20). The estimated ED_{90} in 2006 exceeded the highest glufosinate dosage applied.

The admixture data for both the seeds m^{-2} and the percent of the harvested material indicated a significant year and location effect and data are presented separately by location. This is most likely due to various efficiencies of the combine harvester at each location. The number of volunteer wheat seeds recovered from either site in 2005 ranged from 635.5 to 593.8 seeds m^{-2}. When compared to the crop yield, the percent admixture was 5.3 to 5.5% (Table 2.21). In 2006, the number of seeds recovered was 60 to 71% higher than 2005, ranging from 1580.6 to 2035.5 seeds m^{-2} for Ellerslie and Edmonton, respectively (Table 2.22). With volunteer wheat at the densities recorded in this study, producers would be encouraged and commonly would include a group 1 herbicide with the glufosinate application (Brooks 2006; Woycheshin 2007).

The percent admixture was also markedly higher than in 2005, ranging from 76% for Ellerslie to 130.6% at the Edmonton site (Table 2.22). Using the proposed 0.9% for the EU as a threshold, it would have required 200 g ai ha^{-1} of glufosinate in 2005 and would not have been achieved in 2006 (Table 2.22).

2.3.2.2 *Dose Response in imidazolinone-resistant canola*

The effect of seeding rate did not significantly affect any of the variables measured ($P < 0.05$). The biomass sampled 4 WAT and the volunteer wheat fecundity was not significantly different ($P < 0.05$) for year or locations, therefore data was pooled for analysis (Figure 2.8). The imazamox + imazethapyr ED required reducing volunteer wheat biomass and fecundity to 50, 85 and 90% of the untreated control was 1.5, 6.2, 9.0 and 1.0, 6.6 and 9.7 g ai ha^{-1}, respectively (Table 2.23). All the effective dosages were below the recommend rate of 29.4 g ai ha^{-1} (Brooks 2006). The 95% confidence limits suggest that there was not a significant recovery of the volunteers after the herbicide treatments (Tables 2.23 and 2.24).

For volunteer GR wheat admixture, there was a significant year and location interaction for the percent and the number of seeds recovered from the harvested grain, therefore the years and locations were analyzed separately. The number of seeds produced in the untreated control in 2005 ranged from 400.5 seeds at Edmonton to 2983.0 at Ellerlsie (Tables 2.25 and 2.26). Increasing the rate of imazamox + imazethapyr greater than 7.35 g ai ha^{-1} did not provide a significantly greater reduction of volunteer seeds in 2005, ranging from 32.6 at Ellerlsie to 105.1 at the Edmonton site (Table 2.25). The corresponding percent admixture for these sites at all herbicide rates was $\leq 0.9\%$. In 2006, a rate of 22 g ai ha^{-1} at Ellerlsie reduced the percent admixture below 0.9% which is equivalent to < 55.0 seeds m^{-2}. The Edmonton site in 2006 did not achieve an admixture below 0.9%, presumably because of the poor crop yields at this location. Seeds recovered from this site were ≤ 49.4 seeds, resulting in an admixture % of 1.45 at 14.7 g ai ha^{-1} of imazamox + imazethapyr. More seeds recovered (53.7) at the same herbicide rate resulted in an admixture % or 0.39 (Table 2.26). Although the herbicide preformed similarly at both sites, increased grain yield lowered the % admixture.

2.4 Discussion

In the absence of herbicides, volunteers were more fecund in peas than in canola. Individual volunteer fecundity in canola, in the absence of herbicides, averaged 137.1 and 90.4 seeds $plant^{-1}$ in 2004 and 2005, respectively (Table 2.3). Uncontrolled volunteer GR

wheat in the pea trial yielded 163.9 and 134.5 seeds plant^{-1} in 2004 and 2005, respectively (Table 2.11). This was expected due to the limited competitiveness of peas compared to hybrid canola (Harker 2001). Glufosinate was less consistent than imazamox + imazethapyr between years at controlling volunteer wheat. Glufosinate, a contact herbicide, is more influenced by application coverage and environmental fluctuations.

Previous studies indicate that volunteer wheat, both conventional and GR can be controlled with herbicides (Rainbolt et al. 2004; Blackshaw et al. 2006). In study A, experiments were conducted under conditions where volunteer control was difficult. Uniformly planted volunteers, seeded 20 to 30 days prior to the crop, represent a worse case scenario for herbicidal control. In-crop herbicides were effective at control of volunteer wheat, but in many instances, control was increased by the use of a pre-seeding herbicide application. Herbicides were effective at reducing biomass, seed set and admixture. However, even when herbicides are affective, some volunteers can survive (Tables 2.7 and 2.15). More advanced volunteers were difficult to control with pre-seeding herbicides, and volunteer GR wheat that escapes a pre-seeding herbicide treatment will similarly be more difficult to control with an in-crop herbicide. Rainbolt and Thill (2003) also reported lower volunteer GR wheat control as the maturity of the volunteers increased. A more effective herbicide at the in-crop herbicide application, such as imazamox +imazethapyr, will reduce volunteer density and fecundity (Tables 2.11 and 2.13).

In study B, conditions were established to allow the volunteers to emerge at the same time as the crop. Glufosinate and imazamox + imazethapyr resistant canola varieties provided inconsistent control of volunteer wheat from year to year. Significant volunteer recovery was observed for glufosinate in 2006. Herbicide treatments were applied when volunteers were larger (BBCH 21-22) with a poorly established canola crop and low competition (Tables A4 and A9). Where growing conditions were poor, even high herbicides rates were ineffective at reducing admixture to acceptable levels.

Control of GR volunteers in the first year following production is critical to reducing the seed bank. Volunteer populations can be high and will produce abundant seeds in the absence of control. Harker et al. (2005) found volunteer wheat emerging throughout the growing season and effective in-crop herbicides the year after GR wheat

production would be important to prevent seed set by volunteers. This study illustrates that effective in-crop volunteer wheat control combined with a competitive crop can significantly reduce the potential for volunteer GR wheat admixture. These findings are congruent with previous studies concluding that volunteer GR wheat in the year following production can be controlled and is important to prevent further seed bank inputs and admixture (Harker et al. 2004).

Volunteers that remain uncontrolled can produce pollen, spreading transgenes to other volunteers or crops, produce seed that can perpetuate the seed bank, or be harvested with the following crop and be detectable as adventitious presence. Data produced in these studies will be used as parameters in a mechanistic demographic model. Models can be very useful to test both intensive (biological) and extrinsic (agronomic) factors effecting volunteer persistence. While GR wheat may not be commercialized in the immediate future, this information will be useful for other transgenic wheat, for example *Fusarium graminearum* tolerant wheat being proposed by Syngenta.

The method used to detect the level of GM material in harvested crops will be an important consideration when developing management strategies for GM coexistence. In the current studies two methods were used to illustrate differences. The number of seeds recovered per unit area is a very visual method that can be easily conceptualized. Adventitious presence expressed as a percent is highly dependent on the yield of the crop that can either under or over emphasize the volume of seed admixed. This harvest blending effect has been empirically modeled using pollen mediated gene flow as the vector for GM introduction into non-GM wheat fields (Gustafson et al. 2005). Pollen mediated gene flow at field margins is higher, but after harvesting the entire field the potential admixture was lowered. This may also be applicable to herbicide spray misses that would allow GR volunteers to survive and be harvested with the crop. Currently the European Union has proposed a 0.9% threshold limit for GM material in non-GM imports (European Commission 2003). This threshold is still under discussion with little information proposed on how it will be detected (European Commission 2006).

Under good growing conditions, crops will be competitive, herbicides more effective and volunteers produce less seed and adventitious presence. However, under conditions of poor weather, or inadequate crop management, admixture could exceed

thresholds and threaten the purity of products. It is advisable that best management practices, possibly including crop inspection for follow crops, be a part of the contractual arrangements between seed distributors and growers to ensure the integrity of markets.

The use of artificial volunteer populations commonly represents worst case scenarios. This is especially true for study A where uniformly seeded volunteer populations were established and were required for quick emergence. Many biological factors that commonly influence volunteer populations such as fall emergence and subsequent winter kill are not represented. This data was collected with the intention and will be used for modeling purposes; therefore, extrapolation to large scale scenarios should be done with caution.

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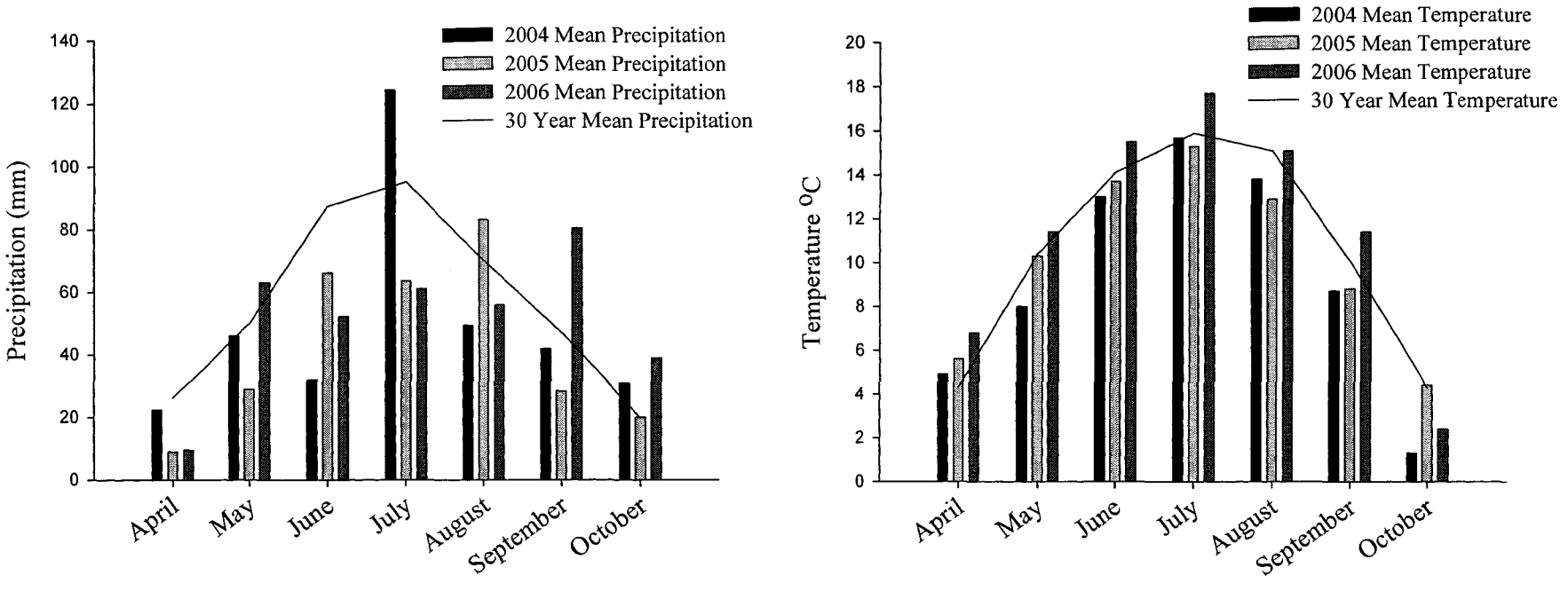

Figure 2.1 Precipitation and temperature for trials located at Ellerslie Research Station.

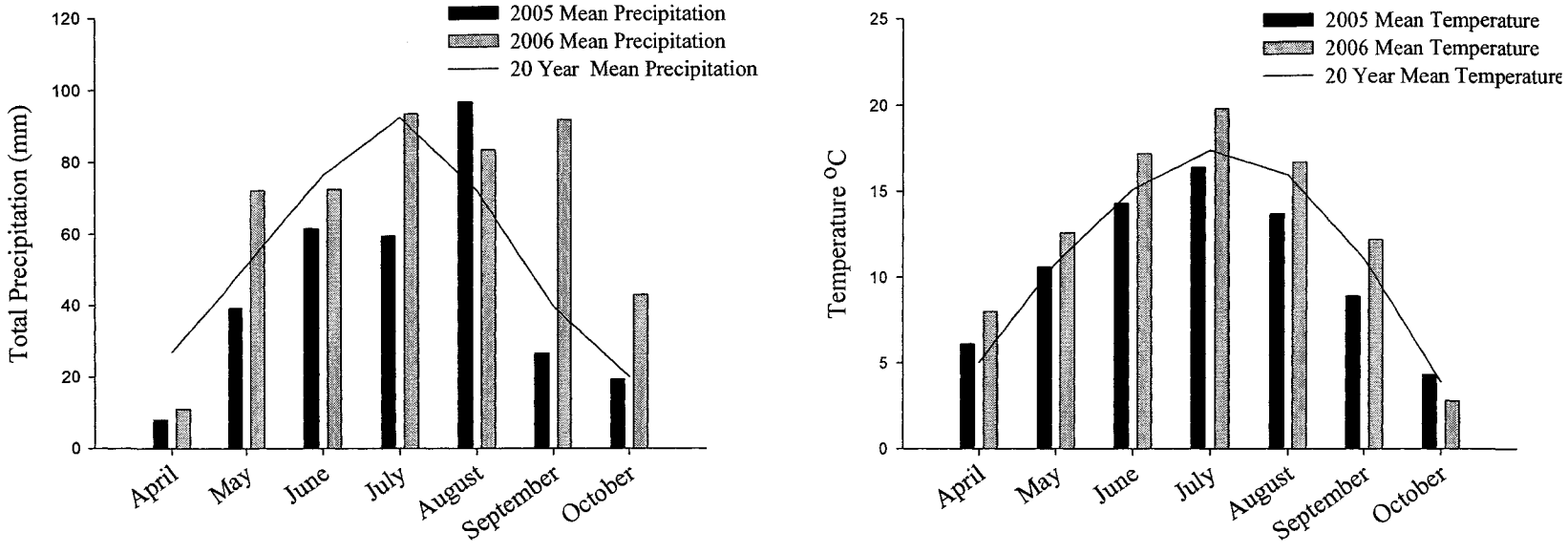

Figure 2.2 Precipitation and temperature for trials located at the Edmonton Research Station.

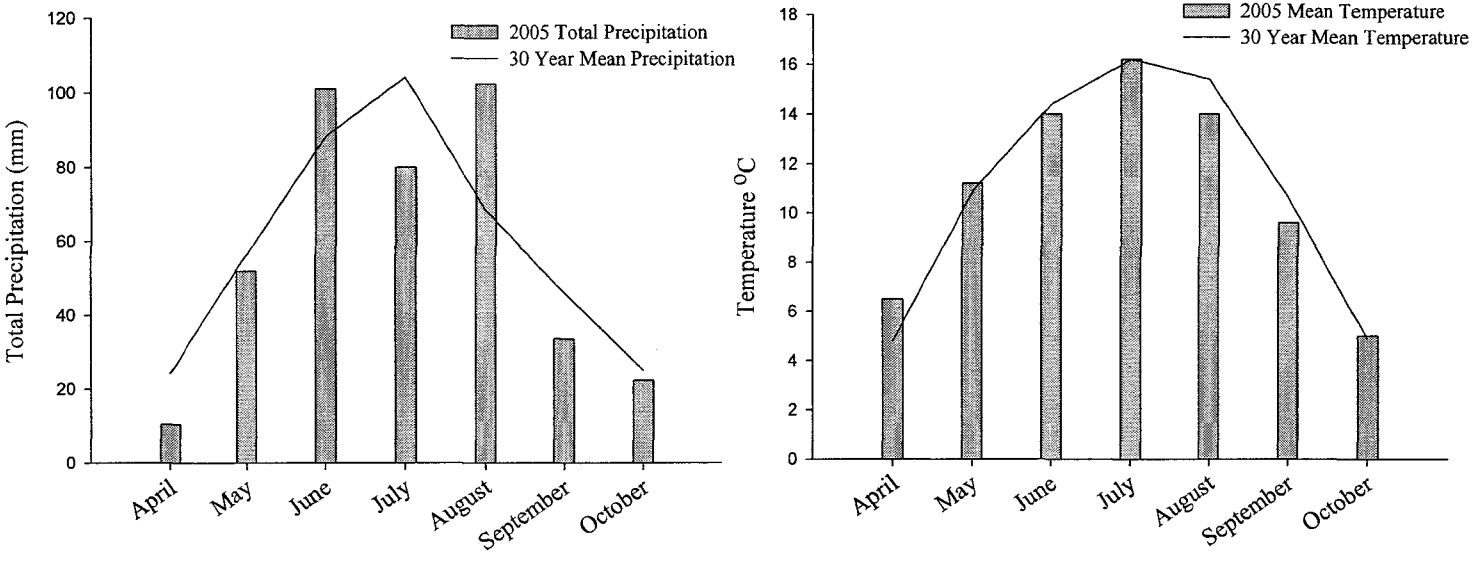

Figure 2.3 Precipitation and temperature for trials located near Calmar.

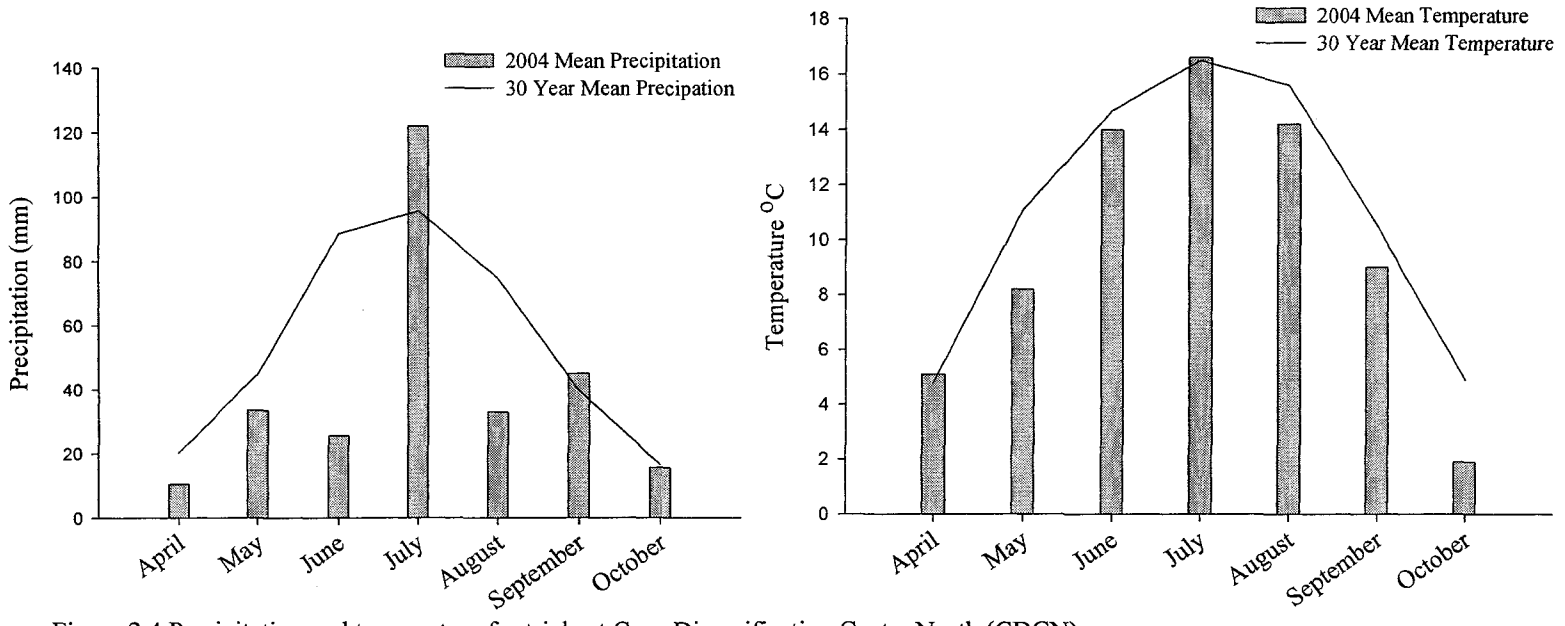

Figure 2.4 Precipitation and temperature for trials at Crop Diversification Center North (CDCN).

Table 2.1. Visual phytotoxicity ratings for herbicide treatments applied in glufosinate-resistant canola in 2004. Study A.

Treatment		Herbicide Application							
Pre	In	Pre-seeding				In-crop			
Glyphosate + Quizalofop-p-ethyl	Glufosinate + Sethoxydim	14 DAT		21 DAT		14 DAT		21 DAT	
g ai ha^{-1}		%							
444 + 0	0 + 0	0.0[1]	(1.6)	0.0[1]	(5.1)	0.0[2]	(4.6)	0.0	(5.5)
444 + 12	0 + 0	72.5	(1.6)	78.1	(5.1)	0.0	(4.6)	0.0	(5.5)
444 + 18	0 + 0	77.3	(1.6)	83.6	(5.1)	0.0	(4.6)	0.0	(5.5)
444 + 24	0 + 0	75.6	(1.6)	85.0	(5.1)	0.0	(4.6)	0.0	(5.5)
444 + 0	300 + 0	0.0	(1.6)	0.0	(5.1)	19.4	(4.6)	24.4	(5.5)
444 + 12	300 + 0	71.3	(1.6)	80.6	(5.1)	49.8	(4.6)	63.4	(5.5)
444 + 18	300 + 0	75.4	(1.6)	85.3	(5.1)	51.6	(4.6)	56.9	(5.5)
444 + 24	300 + 0	77.4	(1.6)	89.4	(5.1)	36.4	(4.6)	54.0	(5.5)
444 + 0	500 + 0	0.0	(1.6)	0.0	(5.1)	55.6	(4.6)	58.5	(5.5)
444 + 12	500 + 0	72.1	(1.6)	81.1	(5.1)	72.3	(4.6)	86.3	(5.5)
444 + 18	500 + 0	76.5	(1.6)	84.5	(5.1)	76.4	(4.6)	81.0	(5.5)
444 + 24	500 + 0	78.3	(1.6)	82.4	(5.1)	80.1	(4.6)	84.0	(5.5)
444 + 0	300 + 211	0.0	(1.6)	0.0	(5.1)	87.5	(4.6)	90.4	(5.5)
444 + 12	300 + 211	72.5	(1.6)	78.1	(5.1)	89.0	(4.6)	93.1	(5.5)
444 + 18	300 + 211	77.8	(1.6)	84.3	(5.1)	92.8	(4.6)	95.9	(5.5)
444 + 24	300 + 211	76.3	(1.6)	86.4	(5.1)	94.8	(4.6)	99.1	(5.5)

Control ratings are: 0 - no control, 80 - commercially acceptable, 90 - excellent and 100% - total control.
Least squared means and corresponding standard error in parenthesis
[1]No control of GR wheat with glyphosate alone application
[2]Did not receive an in-crop herbicide application

Table 2.2. Visual phytotoxicity ratings for herbicide treatments applied in glufosinate-resistant canola in 2005. Study A.

Treatment		Herbicide Application							
Pre	In	Pre-seeding				In-crop			
Glyphosate + Quizalofop-p-ethyl	Glufosinate + Sethoxydim	14 DAT		21 DAT		14 DAT		21 DAT	
g ai ha^{-1}		%							
444 + 0	0 + 0	0.0[1]	(1.3)	0.0	(1.2)	0.0[2]	(6.8)	0.0	(7.1)
444 + 12	0 + 0	85.0	(1.3)	90.3	(1.2)	0.0	(6.8)	0.0	(7.1)
444 + 18	0 + 0	89.8	(1.3)	95.3	(1.2)	0.0	(6.8)	0.0	(7.1)
444 + 24	0 + 0	92.5	(1.3)	95.9	(1.2)	0.0	(6.8)	0.0	(7.1)
444 + 0	300 + 0	0.0	(1.3)	0.0	(1.2)	58.8	(6.8)	61.3	(7.1)
444 + 12	300 + 0	85.6	(1.3)	92.1	(1.2)	82.6	(6.8)	85.1	(7.1)
444 + 18	300 + 0	89.8	(1.3)	94.0	(1.2)	82.6	(6.8)	84.3	(7.1)
444 + 24	300 + 0	93.6	(1.3)	96.5	(1.2)	81.4	(6.8)	82.4	(7.1)
444 + 0	500 + 0	0.0	(1.3)	0.0	(1.2)	73.8	(6.8)	72.5	(7.1)
444 + 12	500 + 0	85.6	(1.3)	90.9	(1.2)	87.0	(6.8)	88.8	(7.1)
444 + 18	500 + 0	91.6	(1.3)	95.3	(1.2)	90.6	(6.8)	90.1	(7.1)
444 + 24	500 + 0	92.8	(1.3)	97.0	(1.2)	92.5	(6.8)	91.6	(7.1)
444 + 0	300 + 211	0.0	(1.3)	0.0	(1.2)	88.8	(6.8)	85.6	(7.1)
444 + 12	300 + 211	86.3	(1.3)	92.1	(1.2)	90.1	(6.8)	90.1	(7.1)
444 + 18	300 + 211	91.0	(1.3)	95.3	(1.2)	92.0	(6.8)	90.6	(7.1)
444 + 24	300 + 211	92.6	(1.3)	96.5	(1.2)	94.3	(6.8)	93.4	(7.1)

Control ratings are: 0 - no control, 80 - commercially acceptable, 90 - excellent and 100% - total control.
Least squared means and corresponding standard error in parenthesis
[1]No control of GR wheat with glyphosate alone application
[2] Did not receive an in-crop herbicide application

Table 2.3. GR volunteer wheat biomass at harvest and fecundity expressed as seeds plant^{-1} as influenced by pre-seeding and in-crop herbicides in glufosinate-resistant canola. Study A.

Treatment		Volunteer Biomass				Seeds			
Pre-Seed	In-Crop	2004		2005		2004		2005	
Glyphosate + Quizalofop-p-ethyl	Glufosinate + Sethoxydim								
g ai ha^{-1}		g plant^{-1}				plant^{-1}			
444 + 0	0 + 0	9.2	a	7.6	a	137.1	a	90.4	a
444 + 12	0 + 0	7.5	ab	2.0	bcde	94.1	ab	11.2	bcd
444 + 18	0 + 0	5.8	abc	2.2	bcd	79.3	abc	7.6	bcde
444 + 24	0 + 0	4.1	bcef	1.5	cdef	38.3	bcd	7.9	cde
444 + 0	300 + 0	8.4	ab	3.5	b	127.2	a	25.0	b
444 + 12	300 + 0	1.8	cdef	0.4	def	20.5	bcd	4.2	cde
444 + 18	300 + 0	1.3	def	0.2	ef	21.4	bcd	3.8	cde
444 + 24	300 + 0	1.5	cdef	0.0	f	16.3	cd	0.0	e
444 + 0	500 + 0	5.3	abcd	3.2	bc	57.7	bcd	12.8	bc
444 + 12	500 + 0	2.1	cdef	0.1	f	24.4	bcd	0.3	de
444 + 18	500 + 0	1.0	def	0.0	f	12.6	bcd	0.0	e
444 + 24	500 + 0	0.8	ef	0.0	f	6.9	d	0.0	e
444 + 0	300 + 211	5.1	abcde	2.1	bcd	49.9	bcd	7.8	bcd
444 + 12	300 + 211	1.1	cdef	0.0	f	15.6	bcd	0.0	e
444 + 18	300 + 211	0.9	def	0.0	f	12.0	bcd	0.0	e
444 + 24	300 + 211	0.0	f	0.0	f	0.0	d	0.0	e
Preseed		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Incrop		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Preseed*Incrop		**0.0465**		**<0.0001**		**0.0375**		**<0.0001**	

Volunteer wheat biomass and seeds least-squared means were transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different (P<0.05). Untransformed least-squared means are presented.

Table 2.4. GR volunteer yield components, seeds spike^{-1}and spikes plant^{-1}, as influenced by pre-seeding and in-crop herbicides in glufosinate-resistant canola. Study A.

Treatment		Seed				Spikes			
Pre-Seed	In-Crop	2004		2005		2004		2005	
Glyphosate + Quizalofop-p-ethyl	Glufosinate + Sethoxydim	Spikes^{-1}				plant^{-1}			
g ai ha^{-1}									
444 + 0	0 + 0	25.9	a	18.3	a	5.6	a	4.9	a
444 + 12	0 + 0	14.8	abcd	4.0	bc	4.0	abcd	1.7	cde
444 + 18	0 + 0	16.1	abc	4.1	bc	4.2	abcd	1.6	cde
444 + 24	0 + 0	19.7	abc	2.3	cd	2.6	bcd	1.4	cde
444 + 0	300 + 0	20.9	ab	6.1	b	6.1	a	3.7	ab
444 + 12	300 + 0	10.7	bcde	1.7	cd	1.5	cde	1.0	de
444 + 18	300 + 0	5.5	def	1.8	cd	2.0	cde	0.5	de
444 + 24	300 + 0	4.5	ef	0.0	d	1.7	cde	0.0	e
444 + 0	500 + 0	15.2	abc	4.3	bc	3.9	abcd	3.1	bc
444 + 12	500 + 0	6.4	def	0.3	d	1.7	cde	0.3	e
444 + 18	500 + 0	3.7	ef	0.0	d	1.1	cde	0.0	e
444 + 24	500 + 0	2.8	ef	0.0	d	1.0	de	0.0	e
444 + 0	300 + 211	8.0	cde	3.6	bc	4.6	abc	2.1	bcd
444 + 12	300 + 211	4.0	ef	0.0	d	1.4	cde	0.0	e
444 + 18	300 + 211	4.0	ef	0.0	d	1.2	cde	0.0	e
444 + 24	300 + 211	0.0	f	0.0	d	0.0	e	0.0	e
Preseed		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Incrop		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Preseed*Incrop		ns		**0.0081**		ns		**<0.0001**	

Volunteer wheat seeds and spikes least-squared means were transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different (P<0.05). Untransformed least-squared means are presented.

Table 2.5. GR volunteer wheat density assessed prior to harvest and volunteer wheat seeds recovered from all plants in quadrats as influenced by pre-seeding and in-crop herbicides in glufosinate-resistant canola. Study A.

Treatment		Volunteer Density				Seeds			
Pre-Seed	In-Crop	2004		2005		2004		2005	
Glyphosate + Quizalofop-p-ethyl	Glufosinate + Sethoxydim	plants m^{-2}				m^{-2}			
g ai ha^{-1}									
444 + 0	0 + 0	63.6	a	73.4	a	8752.9	a	6717.9	a
444 + 12	0 + 0	5.0	cd	4.9	de	614.4	cd	105.6	cd
444 + 18	0 + 0	3.31	cd	2.0	e	517.8	cd	39.7	cd
444 + 24	0 + 0	2.0	d	3.3	de	453.3	cd	37.4	d
444 + 0	300 + 0	27.4	b	54.7	b	2718.7	b	1767.9	b
444 + 12	300 + 0	1.1	d	0.6	e	73.6	d	85.5	d
444 + 18	300 + 0	2.5	cd	0.1	e	152.6	d	35.8	d
444 + 24	300 + 0	0.1	d	0.0	e	5.5	d	0.0	d
444 + 0	500 + 0	20.2	bc	40.6	c	1358.7	c	649.7	c
444 + 12	500 + 0	0.6	d	0.2	e	48.3	d	0.1	d
444 + 18	500 + 0	0.2	d	0.0	e	5.3	d	0.0	d
444 + 24	500 + 0	0.3	d	0.0	e	3.0	d	0.0	d
444 + 0	300 + 211	9.1	cd	21.6	d	1021.2	c	299.6	cd
444 + 12	300 + 211	0.1	d	0.0	e	2.1	d	0.0	d
444 + 18	300 + 211	0.1	d	0.0	e	1.5	d	0.0	d
444 + 24	300 + 211	0.0	d	0.0	e	0.0	d	0.0	d
Preseed		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Incrop		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Preseed*Incrop		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	

Volunteer wheat seeds and densities least-squared means were transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different ($P<0.05$). Untransformed least-squared means are presented.

Table 2.6. GR volunteer wheat seed size and viability as influenced by pre-seeding and in-crop herbicides in glufosinate-resistant canola. Study A.

Treatment		Kernel Wt.				Viable Seeds			
Pre-Seed	In-Crop	2004		2005		2004		2005	
Glyphosate + Quizalofop-p-ethyl	Glufosinate + Sethoxydim								
g ai ha^{-1}		mg				%			
444 + 0	0 + 0	24.8	a	27.1	a	91.3	a	99.5	a
444 + 12	0 + 0	16.9	ab	18.2	ab	65.1	ab	98.9	a
444 + 18	0 + 0	13.6	b	16.7	bcd	69.6	ab	49.8	b
444 + 24	0 + 0	10.2	bc	17.8	bcd	63.9	ab	43.4	bc
444 + 0	300 + 0	17.5	ab	9.8	b	81.5	a	97.2	a
444 + 12	300 + 0	8.7	bc	5.5	bcd	57.1	ab	29.2	bcd
444 + 18	300 + 0	8.1	bc	1.2	bc	44.4	b	23.5	bcd
444 + 24	300 + 0	9.2	bc	-		35.5	bc	-	
444 + 0	500 + 0	16.1	ab	21.5	ab	67.6	ab	96.5	a
444 + 12	500 + 0	8.1	bc	10.0	cd	34.7	bc	32.1	bcd
444 + 18	500 + 0	7.8	bc	-		29.7	bc	-	
444 + 24	500 + 0	9.3	bc	-		31.1	bc	-	
444 + 0	300 + 211	11.3	bc	7.2	b	35.9	bc	92.8	a
444 + 12	300 + 211	11.1	bc	-		31.4	bc	-	
444 + 18	300 + 211	11.7	bc	-		29.6	bc	-	
444 + 24	300 + 211	-		-		-		-	
Preseed		**<0.0001**		**<0.0001**		**0.0003**		**<0.0001**	
Incrop		**0.0028**		**0.0002**		**<0.0001**		**<0.0001**	
Preseed*Incrop		**0.0291**		**0.0272**		ns		**<0.0001**	

Volunteer wheat kernel weight and seeds least-squared means were transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different (P<0.05). Untransformed least-squared means are presented.

Table 2.7. Admixture of GR volunteer wheat seeds as influenced by pre-seeding and in-crop herbicides in glufosinate-resistant canola. Study A.

Treatment		Admixed Volunteer Wheat Seed[1]							
		2004				2005			
Pre-Seed	In-Crop	Ellerlsie		CDCN		Ellerlsie		Calmer	
Glyphosate + Quizalofop-p-ethyl	Glufosinate + Sethoxydim								
g ai ha^{-1}		m^{-2}							
444 + 0	0 + 0	2980.9	a	4000.7	a	10404.0	a	5706.7	a
444 + 12	0 + 0	39.6	c	1271.2	bcd	481.7	c	19.3	cd
444 + 18	0 + 0	70.4	c	589.0	cd	237.7	cde	12.8	cd
444 + 24	0 + 0	21.5	c	237.8	d	275.8	cde	2.3	cd
444 + 0	300 + 0	723.3	b	2446.8	ab	4245.8	b	623.2	b
444 + 12	300 + 0	10.3	c	183.5	e	71.0	de	1.0	cd
444 + 18	300 + 0	20.7	c	161.0	e	39.1	de	4.3	cd
444 + 24	300 + 0	5.4	c	55.3	e	30.7	de	9.6	cd
444 + 0	500 + 0	86.5	c	1448.1	bc	2328.4	b	175.1	c
444 + 12	500 + 0	22.5	c	89.2	e	10.0	e	0.5	d
444 + 18	500 + 0	27.0	c	37.8	e	56.6	de	0.6	d
444 + 24	500 + 0	23.7	c	42.4	e	44.2	de	2.1	d
444 + 0	300 + 211	12.5	c	610.2	cd	942.2	c	18.9	cd
444 + 12	300 + 211	4.8	c	21.1	e	6.0	e	5.0	cd
444 + 18	300 + 211	10.2	c	46.5	e	24.9	de	0.7	d
444 + 24	300 + 211	2.9	c	12.7	e	16.3	de	0.4	d
Preseed		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Incrop		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Preseed*Incrop		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	

Admixed volunteer wheat seeds least-squared means were transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different ($P<0.05$). Untransformed least-squared means are presented.

[1]The total number of GR wheat seeds recovered m^{-2} from combined harvested samples.

Table 2.8. Percent admixture of GR volunteer wheat seeds as influenced by pre-seeding and in-crop herbicides in glufosinate-resistant canola. Study A.

Treatment		Admixed Volunteer Wheat Seed[1]							
		2004				2005			
Pre-Seed	In-Crop	Ellerlsie		CDCN		Ellerlsie		Calmer	
Glyphosate + Quizalofop-p-ethyl	Glufosinate + Sethoxydim								
g ai ha^{-1}		%							
444 + 0	0 + 0	29.11	a	102.56	a	81.78	a	67.50	a
444 + 12	0 + 0	0.15	bcd	18.74	bcd	1.77	d	0.09	b
444 + 18	0 + 0	0.22	bcd	5.46	bcde	0.68	d	0.07	b
444 + 24	0 + 0	0.07	c	2.50	de	1.02	d	0.02	b
444 + 0	300 + 0	4.02	b	38.13	b	17.02	b	2.44	b
444 + 12	300 + 0	0.03	bcd	1.65	de	0.24	d	0.01	b
444 + 18	300 + 0	0.19	bcd	3.02	cde	0.14	d	0.02	b
444 + 24	300 + 0	0.02	bcd	0.39	de	0.07	d	0.03	b
444 + 0	500 + 0	0.68	bcd	29.11	bc	9.78	bc	0.47	b
444 + 12	500 + 0	0.07	cd	0.58	e	0.05	d	0.01	b
444 + 18	500 + 0	0.02	cd	0.17	de	0.25	d	0.01	b
444 + 24	500 + 0	0.11	cd	0.30	de	0.25	d	0.01	b
444 + 0	300 + 211	0.07	bc	15.49	bcde	3.47	cd	0.08	b
444 + 12	300 + 211	0.02	bcd	0.21	e	0.04	d	0.01	b
444 + 18	300 + 211	0.04	d	0.34	de	0.17	d	0.01	b
444 + 24	300 + 211	0.01	e	0.07	e	0.07	d	0.00	b
Preseed		**0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Incrop		**0.0002**		**0.0001**		**<0.0001**		**<0.0001**	
Preseed*Incrop		**<0.0001**		**0.042**		**<0.0001**		**<0.0001**	

Admixed volunteer wheat seeds least-squared means were transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different ($P<0.05$). Untransformed least-squared means are presented.

[1]The percent weight of volunteer wheat seeds recovered from the harvested canola sample w/w.

Table 2.9. Visual phytotoxicity ratings for herbicide treatments applied in peas in 2004. Study A.

Treatment		Herbicide Application							
Pre-Seed	In-Crop	Pre-seeding				In-crop			
Glyphosate + Quizalofop-p-ethyl	Imazamox + Imazethapyr	14 DAT		21 DAT		14 DAT		21 DAT	
g ai ha^{-1}		%							
444 + 0	0	0.0[1]	(1.5)	0.0	(3.2)	0.0[2]	(3.8)	0.0[2]	(1.3)
444 + 12	0	73.3	(1.5)	84.0	(3.2)	0.0	(3.8)	0.0	(1.3)
444 + 18	0	71.8	(1.5)	80.5	(3.2)	0.0	(3.8)	0.0	(1.3)
444 + 24	0	75.8	(1.5)	87.0	(3.2)	0.0	(3.8)	0.0	(1.3)
444 + 0	14.7	0.0	(1.5)	0.0	(3.2)	67.5	(3.8)	84.0	(1.3)
444 + 12	14.7	68.8	(1.5)	86.0	(3.2)	71.3	(3.8)	90.0	(1.3)
444 + 18	14.7	72.5	(1.5)	83.0	(3.2)	77.3	(3.8)	87.0	(1.3)
444 + 24	14.7	75.0	(1.5)	95.3	(3.2)	78.5	(3.8)	93.5	(1.3)
444 + 0	22.5	0.0	(1.5)	0.0	(3.2)	76.3	(3.8)	90.0	(1.3)
444 + 12	22.5	67.5	(1.5)	80.5	(3.2)	80.0	(3.8)	95.3	(1.3)
444 + 18	22.5	74.5	(1.5)	89.8	(3.2)	83.5	(3.8)	93.0	(1.3)
444 + 24	22.5	74.8	(1.5)	96.3	(3.2)	88.5	(3.8)	94.5	(1.3)
444 + 0	29.4	0.0	(1.5)	0.0	(3.2)	83.8	(3.8)	97.0	(1.3)
444 + 12	29.4	69.3	(1.5)	83.3	(3.2)	94.5	(3.8)	98.5	(1.3)
444 + 18	29.4	75.8	(1.5)	89.3	(3.2)	85.0	(3.8)	95.8	(1.3)
444 + 24	29.4	75.8	(1.5)	91.0	(3.2)	89.8	(3.8)	98.0	(1.3)

Control ratings are: 0 - no control, 80 - commercially acceptable, 90 - excellent and 100% - total control.
Least squared means and corresponding standard error in parenthesis
[1]No control of GR wheat with glyphosate alone application
[2] Did not receive an in-crop herbicide application

Table 2.10. Visual phytotoxicity ratings for herbicide treatments applied in peas in 2005. Study A.

Treatment		Herbicide Application							
Pre-Seed	In-Crop	Pre-seeding				In-crop			
Glyphosate + Quizalofop-p-ethyl	Imazamox + Imazethapyr	14 DAT		21 DAT		14 DAT		21 DAT	
g ai ha^{-1}		%							
444 + 0	0	0.0[1]	(1.4)	0.0	(1.6)	0.0[2]	(6.9)	0.0[2]	(4.1)
444 + 12	0	81.9	(1.4)	90.3	(1.6)	0.0	(6.9)	0.0	(4.1)
444 + 18	0	89.4	(1.4)	94.0	(1.6)	0.0	(6.9)	0.0	(4.1)
444 + 24	0	89.8	(1.4)	93.3	(1.6)	0.0	(6.9)	0.0	(4.1)
444 + 0	14.7	0.0	(1.4)	0.0	(1.6)	66.3	(6.9)	73.8	(4.1)
444 + 12	14.7	81.3	(1.4)	89.0	(1.6)	74.5	(6.9)	84.5	(4.1)
444 + 18	14.7	88.8	(1.4)	92.8	(1.6)	74.5	(6.9)	83.3	(4.1)
444 + 24	14.7	90.8	(1.4)	95.6	(1.6)	75.1	(6.9)	82.6	(4.1)
444 + 0	22.5	0.0	(1.4)	0.0	(1.6)	73.1	(6.9)	79.4	(4.1)
444 + 12	22.5	80.6	(1.4)	89.0	(1.6)	79.5	(6.9)	89.5	(4.1)
444 + 18	22.5	88.1	(1.4)	92.8	(1.6)	78.9	(6.9)	88.9	(4.1)
444 + 24	22.5	91.0	(1.4)	94.6	(1.6)	83.8	(6.9)	93.1	(4.1)
444 + 0	29.4	0.0	(1.4)	0.0	(1.6)	78.8	(6.9)	85.0	(4.1)
444 + 12	29.4	82.5	(1.4)	90.3	(1.6)	82.0	(6.9)	92.0	(4.1)
444 + 18	29.4	89.4	(1.4)	95.4	(1.6)	82.0	(6.9)	92.0	(4.1)
444 + 24	29.4	92.5	(1.4)	96.5	(1.6)	90.5	(6.9)	96.8	(4.1)

Control ratings are: 0 - no control, 80 - commercially acceptable, 90 - excellent and 100% - total control.
Least squared means and corresponding standard error in parenthesis
[1]No control of GR wheat with glyphosate alone application
[2] Did not receive an in-crop herbicide application

Table 2.11 GR volunteer wheat biomass at harvest and fecundity expressed as seeds plant^{-1} as influenced by pre-seeding and in-crop herbicides in peas. Study A.

Treatment		Volunteer Biomass		Seeds	
Pre-Seed	In-Crop	2004[1]	2005	2004[1]	2005
Glyphosate + Quizalofop-p-ethyl	Imazamox + Imazethapyr	g plant^{-1}		plant^{-1}	
g ai ha^{-1}					
444 + 0	0	10.7 a	10.4 a	163.9 a	134.5 a
444 + 12	0	8.7 ab	6.5 ab	100.8 b	66.2 b
444 + 18	0	7.0 b	6.7 ab	95.7 c	49.9 b
444 + 24	0	0.0 d	5.2 b	0.0 e	70.1 b
444 + 0	14.7	3.4 c	3.1 bcd	4.1 d	14.8 c
444 + 12	14.7	0.0 d	1.1 cd	0.0 e	4.8 c
444 + 18	14.7	0.0 d	1.3 cd	0.0 e	10.5 c
444 + 24	14.7	0.0 d	1.2 cd	0.0 e	5.5 c
444 + 0	22.5	0.0 d	2.7 bcd	0.0 e	2.8 c
444 + 12	22.5	0.0 d	0.8 cd	0.0 e	1.8 c
444 + 18	22.5	0.0 d	0.8 d	0.0 e	5.1 c
444 + 24	22.5	0.0 d	0.6 cd	0.0 e	1.5 c
444 + 0	29.4	0.0 d	2.4 bcd	0.0 e	2.4 c
444 + 12	29.4	0.0 d	0.9 cd	0.0 e	1.5 c
444 + 18	29.4	0.0 d	2.1 bcd	0.0 e	4.4 c
444 + 24	29.4	0.0 d	0.4 d	0.0 e	1.1 c
Preseed		**<0.0001**	**0.0031**	**<0.0001**	**<0.0001**
Incrop		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Preseed*Incrop		**ns**	ns	**<0.0001**	ns

Volunteer wheat biomass weight and seeds least-squared means were square root transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different (P<0.05). Untransformed least-squared means are presented.
[1]When no volunteers were found (0.0), prior to square root transformation, a value of 0.01 was added to aid statistical convergence.

Table 2.12. GR volunteer yield components, seeds spike-1and spikes plant^{-1}, as influenced by pre-seeding and in-crop herbicides in peas. Study A.

Treatment		Seed				Spikes			
Pre-Seed	In-Crop	2004[1]		2005		2004[1]		2005	
Glyphosate + Quizalofop-p-ethyl	Imazamox + Imazethapyr	Spikes^{-1}				plant^{-1}			
g ai ha^{-1}									
444 + 0	0	26.9	a	25.4	a	9.1	a	5.4	a
444 + 12	0	16.6	b	13.1	bc	6.1	a	4.1	ab
444 + 18	0	21.0	b	10.8	bcd	5.0	ab	3.1	abc
444 + 24	0	0.0	c	15.9	ab	0.0	c	3.8	abc
444 + 0	14.7	0.5	c	4.7	cde	3.2	b	3.1	abc
444 + 12	14.7	0.0	c	1.8	def	0.0	c	1.9	bc
444 + 18	14.7	0.0	c	1.9	ef	0.0	c	1.8	bc
444 + 24	14.7	0.0	c	2.6	cdef	0.0	c	1.9	bc
444 + 0	22.5	0.0	c	1.3	def	0.0	c	2.1	bc
444 + 12	22.5	0.0	c	0.5	ef	0.0	c	1.8	bc
444 + 18	22.5	0.0	c	1.5	ef	0.0	c	1.6	bc
444 + 24	22.5	0.0	c	0.6	ef	0.0	c	1.2	bc
444 + 0	29.4	0.0	c	1.9	def	0.0	c	1.5	bc
444 + 12	29.4	0.0	c	0.9	ef	0.0	c	1.9	abc
444 + 18	29.4	0.0	c	1.6	def	0.0	c	1.9	abc
444 + 24	29.4	0.0	c	0.7	f	0.0	c	0.8	c
Preseed		**<0.0001**		**0.0077**		**<0.0001**		**0.0081**	
Incrop		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Preseed*Incrop		**<0.0001**		ns		**<0.0001**		**0.0008**	

Volunteer wheat seeds and spikes least-squared means were square root transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different (P<0.05). Untransformed least-squared means are presented.
[1]When no volunteers were found (0.0), prior to square root transformation, a value of 0.01 was added to aid statistical convergence.

Table 2.13. GR volunteer wheat density assessed prior to harvest and volunteer wheat seeds recovered from all plants in quadrats as influenced by pre-seeding and in-crop herbicides in peas. Study A.

Treatment		Volunteer Density				Seeds			
Pre-Seed	In-Crop	2004[1]		2005		2004[1]		2005	
Glyphosate + Quizalofop-p-ethyl	Imazamox + Imazethapyr								
g ai ha^{-1}		plants m^{-2}				m^{-2}			
444 + 0	0	66.3	a	79.6	a	10745.3	a	10298.9	a
444 + 12	0	5.6	b	3.3	de	980.3	b	271.7	b
444 + 18	0	5.3	b	3.3	de	735.6	b	315.0	b
444 + 24	0	1.3	b	1.7	de	0.0	c	57.9	c
444 + 0	14.7	0.0	b	41.7	b	8.3	c	687.6	b
444 + 12	14.7	0.0	b	3.6	de	0.0	c	41.0	c
444 + 18	14.7	0.0	b	1.0	de	0.0	c	21.9	c
444 + 24	14.7	0.0	b	1.1	de	0.0	c	25.9	c
444 + 0	22.5	0.0	b	24.0	c	0.0	c	107.7	c
444 + 12	22.5	0.0	b	1.6	de	0.0	c	29.5	c
444 + 18	22.5	0.0	b	1.1	de	0.0	c	12.2	c
444 + 24	22.5	0.0	b	0.1	e	0.0	c	0.5	c
444 + 0	29.4	0.0	b	6.4	d	0.0	c	11.0	c
444 + 12	29.4	0.0	b	0.7	de	0.0	c	6.0	c
444 + 18	29.4	0.0	b	0.4	de	0.0	c	1.3	c
444 + 24	29.4	0.0	b	0.2	e	0.0	c	0.8	c
Preseed		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Incrop		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Preseed*Incrop		**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	

Volunteer wheat seeds and density least-squared means were square root transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different ($P<0.05$). Untransformed least-squared means are presented.

[1]When no volunteers were found (0.0), prior to square root transformation, a value of 0.01 was added to aid statistical convergence.

Table 2.14. GR volunteer wheat seed size and viability as influenced by pre-seeding and in-crop herbicides in peas. Study A.

Treatment		Kernel Wt.[1]				Viable Seeds			
Pre-Seed	In-Crop	2004		2005		2004		2005	
Glyphosate + Quizalofop-p-ethyl	Imazamox + Imazethapyr								
g ai ha^{-1}		mg				%			
444 + 0	0	29.8	a	31.5	a	96.7	a	98.1	a
444 + 12	0	26.9	ab	27.1	ab	94.0	a	87.1	abcd
444 + 18	0	24.8	b	31.0	abc	89.5	b	70.4	abcd
444 + 24	0	-[2]		31.1	ab	-		81.9	abc
444 + 0	14.7	10.6	c	20.3	abc	34.4	c	82.1	ab
444 + 12	14.7	-		19.3	abc	-		55.2	abcd
444 + 18	14.7	-		14.9	bc	-		34.5	bcd
444 + 24	14.7	-		18.6	abc	-		60.0	abcd
444 + 0	22.5	-		23.6	ab	-		70.3	abcd
444 + 12	22.5	-		17.0	abc	-		49.9	abcd
444 + 18	22.5	-		14.7	bc	-		32.8	cd
444 + 24	22.5	-		7.3	c	-		30.9	d
444 + 0	29.4	-		33.0	a	-		71.8	abcd
444 + 12	29.4	-		35.6	abc	-		73.0	abcd
444 + 18	29.4	-		25.7	abc	-		77.3	abcd
444 + 24	29.4	-		13.0	bc	-		33.0	d
Preseed		**<0.0001**		**0.0062**		**<0.0001**		**0.0243**	
Incrop		**<0.0001**		**0.0017**		**<0.0001**		**0.0005**	
Preseed*Incrop		ns		ns		ns		ns	

Volunteer wheat seeds least-squared means were square root transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different (P<0.05). Untransformed least-squared means are presented.

[1]When no seeds were recovered, prior to square root transformation (-) a value of 0.01 was added to aid statistical convergence.

[2] Indicates that no seeds were recovered, and therefore, were not tested for kernel weight or viability

Table 2.15. Admixture of GR volunteer wheat seeds as influenced by pre-seeding and in-crop herbicides in peas. Study A.

Treatment		Admixed volunteer wheat seed			
Pre-Seed	In-Crop				
Glyphosate + Quizalofop-p-ethyl	Imazamox + Imazethapyr	2004		2005	
g ai ha^{-1}		m^{-2}			
444 + 0	0	9588.4	a	14006.0	a
444 + 12	0	357.5	b	165.6	c
444 + 18	0	267.4	bc	86.6	c
444 + 24	0	67.1	bc	55.3	c
444 + 0	14.7	257.3	bc	1324.3	b
444 + 12	14.7	65.4	bc	61.3	c
444 + 18	14.7	40.1	bc	42.7	c
444 + 24	14.7	6.8	bc	16.4	c
444 + 0	22.5	324.2	bc	267.7	c
444 + 12	22.5	59.5	bc	19.4	c
444 + 18	22.5	46.3	bc	20.7	c
444 + 24	22.5	23.5	bc	29.8	c
444 + 0	29.4	16.9	bc	47.8	c
444 + 12	29.4	12.8	bc	14.1	c
444 + 18	29.4	8.1	bc	22.3	c
444 + 24	29.4	1.9	c	9.0	c
Preseed		**<0.0001**		**<0.0001**	
Incrop		**<0.0001**		**<0.0001**	
Preseed*Incrop		**<0.0001**		**<0.0001**	

Admixed volunteer wheat seeds least-squared means were transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different ($P<0.05$). Untransformed least-squared means are presented.
[1]The total number of GR wheat seeds recovered m^{-2} from combined harvested samples.

Table 2.16. Percent admixture of GR volunteer wheat seeds as influenced by pre-seeding and in-crop herbicides in peas. Study A.

Treatment		Admixed Volunteer Wheat Seed[1]					
Pre-Seed	In-Crop	2004		2005			
Glyphosate + Quizalofop-p-ethyl	Imazamox + Imazethapyr	Ellerslie		Ellerlsie		Calmer	
g ai ha^{-1}		%					
444 + 0	0	186.22	a	481.82	a	196.68	a
444 + 12	0	2.78	b	2.4	b	0.26	c
444 + 18	0	2.17	b	1.25	b	0.06	c
444 + 24	0	0.53	b	1.36	b	0.25	c
444 + 0	14.7	2.1	b	2.43	b	10.6	b
444 + 12	14.7	0.35	b	0.36	b	0.02	c
444 + 18	14.7	0.29	b	0.12	b	0.09	c
444 + 24	14.7	0.04	b	0.13	b	0.02	c
444 + 0	22.5	2.21	b	0.32	b	1.47	c
444 + 12	22.5	0.35	b	0.09	b	0.05	c
444 + 18	22.5	0.19	b	0.11	b	0.07	c
444 + 24	22.5	0.02	b	0.09	b	0.02	c
444 + 0	29.4	0.05	b	0.76	b	0.17	c
444 + 12	29.4	0.01	b	0.15	b	0.09	c
444 + 18	29.4	0.02	b	0.06	b	0.02	c
444 + 24	29.4	0.51	b	0.02	b	0.04	c
Preseed		**<0.0001**		**<0.0001**		**<0.0001**	
Incrop		**<0.0001**		**<0.0001**		**<0.0001**	
Preseed*Incrop		**ns**		**ns**		**<0.0001**	

Admixed volunteer wheat seeds least-squared means were transformed prior to mean separation using tukey's HSD test, least-squared means followed by the same letter are not significantly different ($P<0.05$). Untransformed least-squared means are presented.

[1]The percent weight of volunteer wheat seeds recovered from the harvested canola sample w/w.

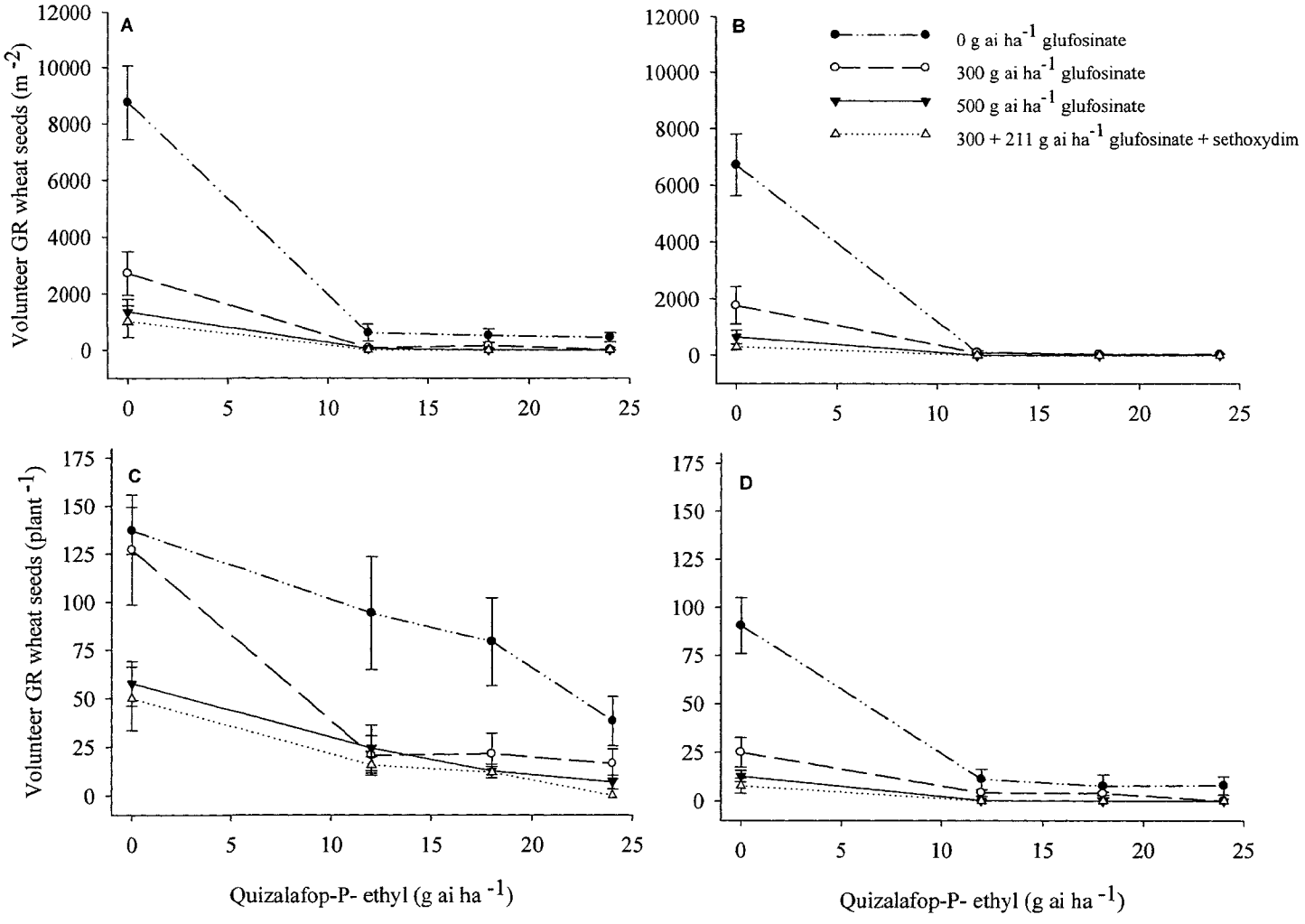

Figure 2.5. Total volunteer GR wheat seeds produced from established quadrates for study A: A) 2004 and B) 2005, and individual GR volunteer wheat fecundity in C) 2004 and D) 2005 in response to pre-seeding in in-crop herbicides with glufosinate-resistant canola.

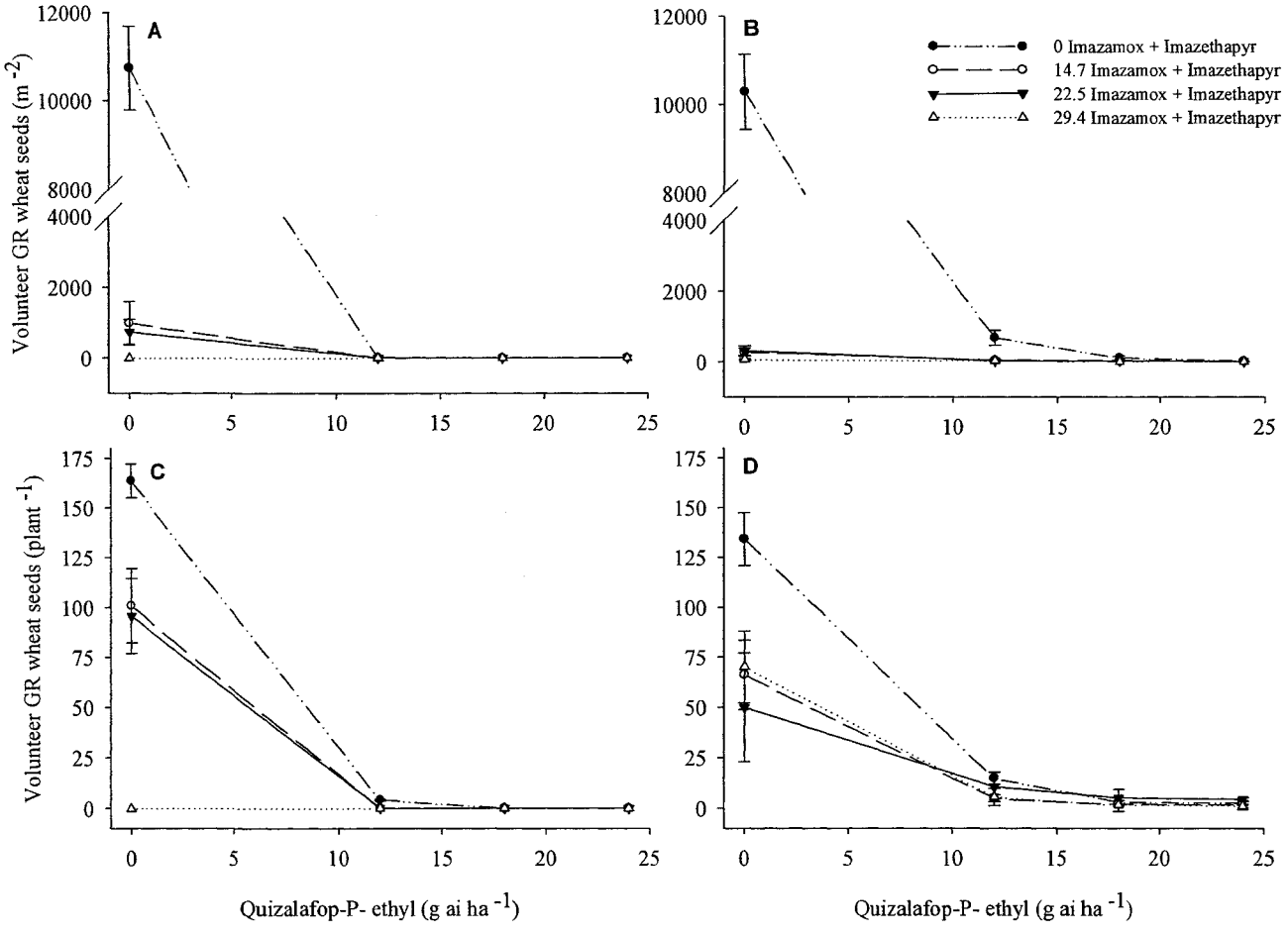

Figure 2.6 Total volunteer GR wheat seeds produced from established quadrates for study A: A) 2004 and B) 2005, and individual GR volunteer wheat fecundity in C) 2004 and D) 2005 in response to pre-seeding in in-crop herbicides with peas.

Table 2.17. Volunteer wheat biomass (4WAT) regression parameters and effective dose (EDx) estimates in glufosinate-resistant canola in 2005. Study B.

Parameter	Estimate	Lower CI	Upper CI	SE	DF	t Value	Pr > |t|	Alpha
a	99.9	93.9	106.1	3.1	111	32.5	<.0001	0.05
b	0.006	0.008	0.010	0.001	111	15.4	<.0001	0.05
s	154.2	113.2	195.2	20.7	111	7.5	<.0001	0.05
		——— g ai ha^{-1} ———						
ED_{50}	71.3	62.3	80.2	4.5	111	15.8	<.0001	0.05
ED_{85}	195.1	171.6	218.6	11.9	111	16.5	<.0001	0.05
ED_{90}	236.8	208.1	265.6	14.5	111	16.3	<.0001	0.05

Table 2.18. Volunteer wheat biomass (4WAT) regression parameters and effective dose (EDx) estimates in glufosinate-resistant canola in 2006. Study B.

Parameter	Estimate	Lower CI	Upper CI	SE	DF	t Value	Pr > |t|	Alpha
a	101.5	94.9	108.2	3.4	110	30.2	<.0001	0.05
b	0.006	0.005	0.007	0.000	110	17.9	<.0001	0.05
s	206.8	151.5	262.0	27.9	110	7.4	<.0001	0.05
		——— g ai ha^{-1} ———						
ED_{50}	120.5	107.9	133.0	6.3	110	19.0	<.0001	0.05
ED_{85}	325.2	293.2	357.3	16.2	110	20.1	<.0001	0.05
ED_{90}	394.2	354.8	433.5	19.9	110	19.9	<.0001	0.05

Table 2.19. In-crop volunteer wheat fecundity regression parameters and effective dose (EDx) estimates in glufosinate-resistant canola sampled at harvest in 2005. Study B.

Parameter	Estimate	Lower CI	Upper CI	SE	DF	t Value	Pr > |t|	Alpha
a	97.1	90.1	104.0	3.5	11	27.6	<.0001	0.05
b	0.009	0.008	0.011	0.000	109	13.1	<.0001	0.05
s	199.2	145.8	252.7	26.99	109	7.4	<.0001	0.05
		——— g ai ha^{-1} ———						
ED_{50}	71.3	60.9	81.7	5.26	109	13.6	<.0001	0.05
ED_{85}	200.7	172.6	228.8	14.18	109	14.2	<.0001	0.05
ED_{90}	244.2	209.7	278.7	17.41	109	14.0	<.0001	0.05

Table 2.20. In-crop volunteer wheat fecundity regression parameters and effective dose (EDx) estimates in glufosinate-resistant canola sampled at harvest in 2006. Study B.

Parameter	Estimate	Lower CI	Upper CI	SE	DF	t Value	Pr > |t|	Alpha
a	100.4	94.8	105.9	2.8	111	35.6	<.0001	0.05
b	0.004	0.004	0.005	0.000	111	20.9	<.0001	0.05
s	150.8	110.7	190.9	20.2	111	7.5	<.0001	0.05
		——— g ai ha^{-1} ———						
ED_{50}	157.2	144.4	144.4	6.5	111	24.4	<.0001	0.05
ED_{85}	428.7	393.7	393.7	17.7	111	24.3	<.0001	0.05
ED_{90}	520.1	476.8	563.5	21.9	111	23.8	<.0001	0.05

Table 2.21. Admixture of volunteer wheat seeds, pooled over seeding rate, that were recovered from combined harvested samples of glufosinate-resistant canola in 2005. Study B.

Treatment	2005			
Glufosinate	Ellerlsie	Edmonton	Ellerslie	Edmonton
— g ai ha^{-1} —	Seeds m^{-2}		%[1]	
0	635.5 a	593.8 a	5.48 a	5.30 a
100	174.8 b	267.1 b	1.22 b	2.13 b
200	92.3 bc	139.1 c	0.60 bc	0.93 c
300	22.3 c	81.3 cc	0.07 c	0.50 c
350	18.9 c	43.0 c	0.08 c	0.30 c
400	12.5 c	50.1 c	0.12 c	0.31 c
500	11.4 c	25.5 c	0.14 c	0.16 c
Herbicide Rate	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Seeding Rate	ns	ns	ns	ns

[1]The percent weight of volunteer wheat seeds recovered from the harvested canola sample w/w.

Table 2.22. Admixture of volunteer wheat seeds, pooled over seeding rate, that were recovered from combined harvested samples of glufosinate-resistant canola in 2006. Study B.

Treatment	2006			
Glufosinate	Ellerlsie	Edmonton	Ellerslie	Edmonton
— g ai ha^{-1} —	Seeds m^{-2}		%[1]	
0	1580.6 a	2035.5 a	76.0 a	130.55 a
100	902.3 b	1536.8 b	32.2 b	68.11 b
200	668.1 bc	948.5 c	15.7 c	34.90 c
300	453.4 bc	427.1 c	9.8 cd	13.88 c
350	574.9 bc	391.6 c	14.9 cd	11.96 c
400	335.0 d	252.6 ce	6.9 d	7.41 ce
500	320.1 d	128.6 e	6.2 d	3.81 e
Herbicide Rate	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Seeding Rate	ns	ns	ns	ns

[1]The percent weight of volunteer wheat seeds recovered from the harvested canola sample w/w.

Table 2.23. Volunteer wheat biomass (4WAT) regression parameters and effective dose (ED_x) estimates in imidazolinone-resistant canola pooled over years and location. Study B.

Parameter	Estimate	Lower CI	Upper CI	SE	DF	t Value	Pr > |t|	Alpha
a	114.8	105.8	123.8	4.6	108	25.2	<.0001	0.05
b	0.28	0.20	0.35	0.04	108	7.8	<.0001	0.05
s	163.5	119.4	207.7	22.3	108	7.4	<.0001	0.05
		——— g ai ha^{-1} ———						
ED_{50}	2.9	2.4	3.6	0.3	108	9.2	<.0001	0.05
ED_{85}	7.3	5.6	9.0	0.9	108	8.5	<.0001	0.05
ED_{90}	8.8	6.7	10.9	1.1	108	8.4	<.0001	0.05

Table 2.24. In-crop volunteer wheat fecundity regression parameters and effective dose (ED_x) estimates in imidazolinone-resistant canola sampled at harvest pooled over years and location. Study B.

Parameter	Estimate	Lower CI	Upper CI	SE	DF	t Value	Pr > |t|	Alpha
a	100.4	95.5	104.4	2.0	207	50.5	<.0001	0.05
b	0.26	0.22	0.30	0.02	207	13.6	<.0001	0.05
s	124.4	100.3	148.5	12.2	207	10.2	<.0001	0.05
		——— g ai ha^{-1} ———						
ED_{50}	2.7	2.3	3.1	0.2	207	13.4	<.0001	0.05
ED_{85}	7.4	6.3	8.4	0.5	207	13.7	<.0001	0.05
ED_{90}	8.9	7.7	10.2	0.7	207	13.7	<.0001	0.05

Table 2.25. Admixture of volunteer wheat seeds, pooled over seeding rate, that were recovered from combined harvested samples of imidazolinone-resistant canola in 2005. Study B.

Treatment	2005			
Imazamox+Imazethapyr	Ellerlsie	Edmonton	Ellerslie	Edmonton
g ai ha^{-1}	Seeds m^{-2}		%[1]	
0	816.8 a	1149.2 a	9.59 a	18.43 a
7.35	32.6 b	105.1 b	0.03 b	0.90 b
14.7	9.2 b	34.9 b	0.23 b	0.29 b
22	9.9 b	35.7 b	0.06 b	0.27 b
25	3.9 b	20.0 b	0.06 b	0.15 b
29.4	4.0 b	15.2 b	0.02 b	0.12 b
36.75	3.6 b	4.7 b	0.02 b	0.10 b
Herbicide Rate	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Seeding Rate	ns	ns	ns	ns

[1]The percent weight of volunteer wheat seeds recovered from the harvested canola sample w/w.

Table 2.26. Admixture of volunteer wheat seeds, pooled over seeding rate, that were recovered from combined harvested samples of imidazolinone-resistant canola in 2006. Study B.

Treatment	2006			
Imazamox+Imazethapyr	Ellerlsie	Edmonton	Ellerslie	Edmonton
g ai ha^{-1}	Seeds m^{-2}		%[1]	
0	2983.0 a	400.5 a	87.53 a	313.56 a
7.35	359.8 b	115.8 b	6.58 b	23.95 b
14.7	53.7 c	49.4 c	0.39 c	2.44 c
22	35.1 c	41.0 c	0.72 c	3.42 c
25	30.9 c	30.3 c	0.37 c	1.45 c
29.4	1.6 c	19.7 c	0.02 c	1.09 c
36.75	6.9 c	18.1 c	0.53 c	0.47 c
Herbicide Rate	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Seeding Rate	ns	ns	ns	ns

[1]The percent weight of volunteer wheat seeds recovered from the harvested canola sample w/w

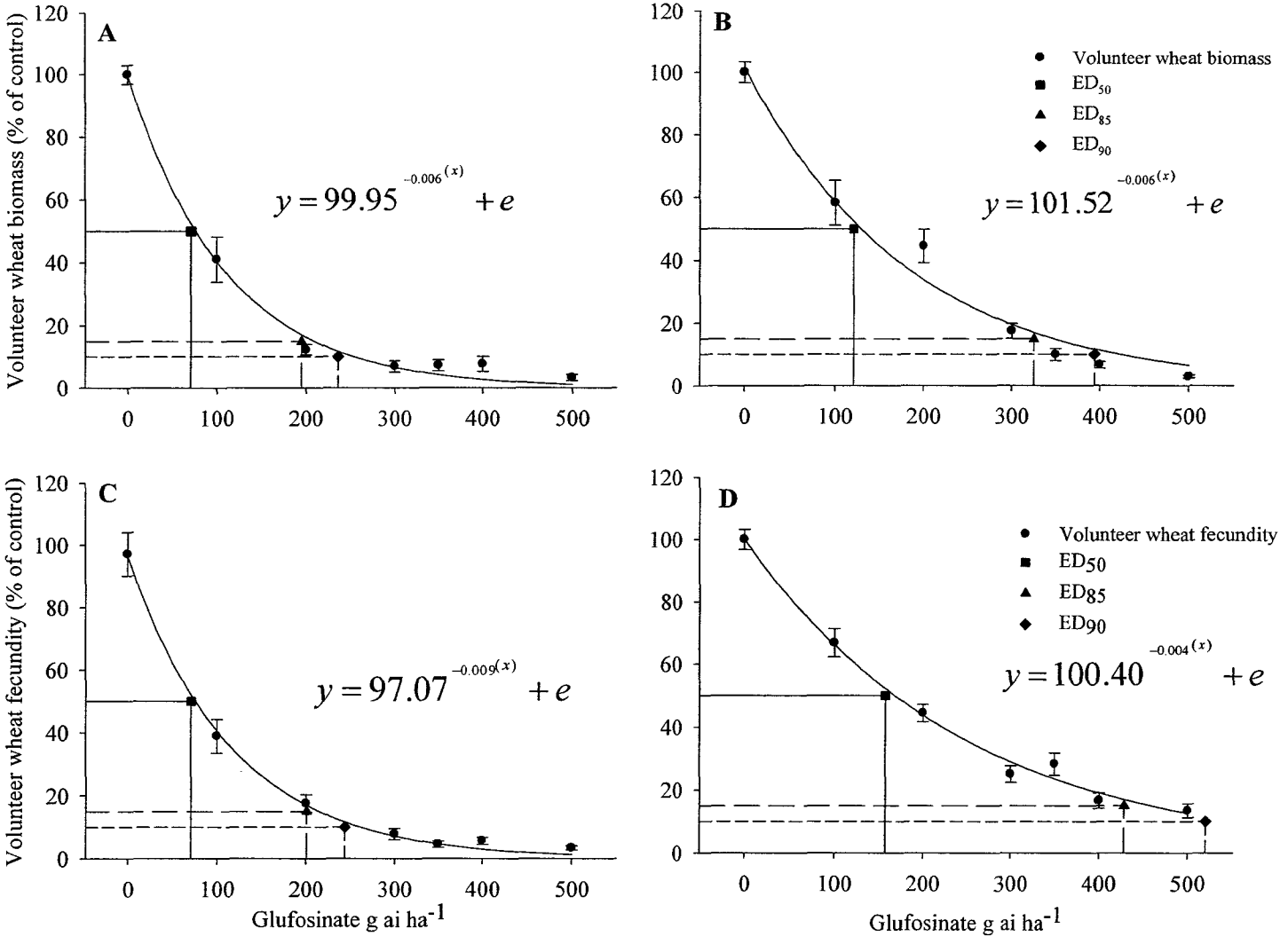

Figure 2.7. Volunteer wheat dose response curves in glufosinate-resistant canola for study B: A) biomass 4 WAT in 2005 B) biomass 4 WAT in 2006 C) volunteer wheat fecundity in 2005 D) volunteer wheat fecundity in 2006.

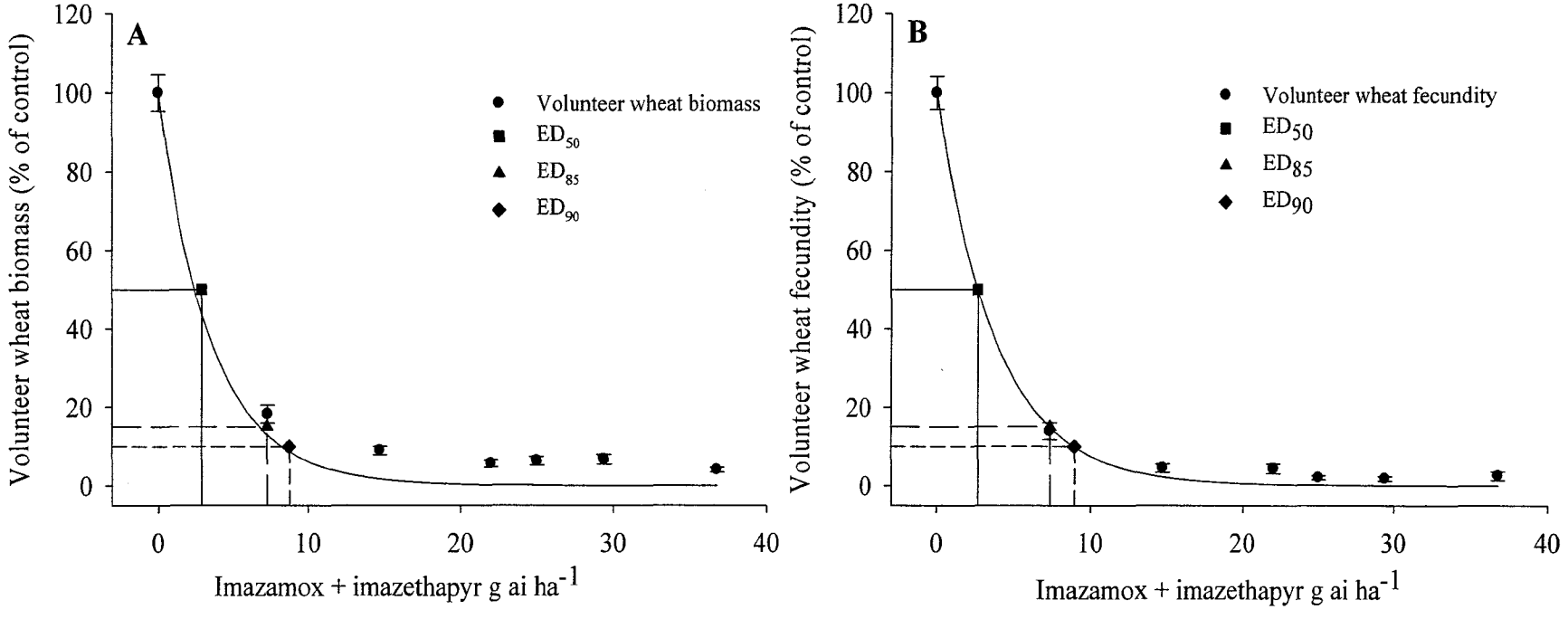

Figure 2.8. Volunteer wheat dose response curves for imidazolinone-resistant canola pooled over location and year for study B: A) biomass 4WAT B) volunteer wheat fecundity

Chapter 3

3.0 Influence of cereal crop competition on volunteer wheat (*Triticum aestivum* L.) fecundity.

3.1 Introduction

Genetically modified (GM) crops have been widely adopted (James 2005; Brookes and Barfoot 2005) with no significant environmental disadvantages and many measurable benefits for the producer and the environment (Blackshaw and Harker 2002; Brooks and Barfoot 2005; Beckie et al. 2006). GM crops and their products are not universally acceptable in all markets. In particular, the European Union (EU) has put forward a series of concerns regarding the use of GM crops and has established a 0.9% threshold for GM admixture in conventional crops (European Commission 2003).

Glyphosate-resistant (GR) wheat (*Triticum aestivum* L.) was evaluated for commercial release until a voluntary registration withdrawal by Monsanto in 2004, precipitated primarily by concerns for potential market harm from adventitious presence (AP) or commingling of GM and conventional wheat seeds (Monsanto 2004).

Uncontrolled herbicide-resistant (HR) volunteers are a potential mechanism contributing to adventitious presence (AP) and seed and pollen mediated gene flow in subsequent crops. Volunteer wheat in the absence of herbicides can also compete aggressively with the crop and reduce crop yields (O'Donovan et al. 1989; Friesen et al. 1990). Herbicide-resistant volunteers may confound herbicide choices for growers, either prior to seeding or in-crop (Anderson and Neilsen 1996; Harker et al. 2005; De Corby et al. 2007). Additionally the contribution of volunteers to weed seed bank replenishment and temporal gene flow are not well established. Transgenes can be introduced at seeding, deliberately or inadvertently (Friesen et al. 2003).

Although wheat is primarily a self-pollinating species, outcrossing has been recorded up to 10%, with an average of < 2% (Hucl 1996; Hucl and Matus-Cádiz 2001; Matus-Cadiz et al. 2004; Lawrie et al. 2006). Wheat is wind pollinated and has been confirmed to outcross at field scale up to 300 m (Matus-Cadiz et al. 2004). As would be the situation for volunteer wheat growing in a wheat crop, outcrossing between wheat is

maximized when plants are in direct contact, reaching as high as 10.63% for some varieties (Lawrie et al. 2006). Distance between crops, wind speed and direction and synchronicity of anthesis are significant factors influencing the frequency and distance of cross pollination between wheat crops and volunteers and the production of hemizygous seeds that carry the transgene.

Before crops are harvested, mature plants may shatter and shed seed. This effect is rarely quantified, and is commonly included with harvest losses and expressed as total seeds on the soil surface. Harvest efficiency from mechanical harvesters is never 100 %, leading to seed losses to the soil surface. Combines can either lose seed while cutting the crop before they enter the combine or seeds can be lost with the chaff that is returned from the rear of the harvester to the soil surface (Komatsuzaki and Endo 1996; Anderson and Soper 2003). Wheat seeds remaining on the soil surface after harvest can be from 240 to 700 seed m^{-2} and contribute to the soil seed bank, 1 to 3 times the normal seeding rate of 250 seed m^{-2} (Anderson and Soper 2003). In western Canada, harvest loss was investigated and was found to vary by wheat cultivar, environment and the harvesting date. Density of seeds on the soil surface was approximately 300 seeds m^{-2} (Clarke 1985). Seeds on the soil surface may become part of the soil seed bank and germinate in subsequent years. If uncontrolled, volunteers may replenish the seed bank, or be harvested with the crop, leading to seed mediated gene flow.

Spring wheat accounts for the largest number of seeded hectares in Canada. In 2005, 7.75 million hectares of spring wheat were seeded with 98.5% of this area in the Alberta, Saskatchewan and Manitoba (Statistics Canada 2001). Herbicide-resistant spring wheat, both GM and non-GM, have been developed with resistance to glyphosate and imidazolinone herbicides, respectively. Volunteer wheat is an increasingly common weed in western Canada, the most recent prairie weed survey ranked volunteer wheat 12^{th} of all weeds species surveyed; the highest of all volunteer crops. The relative abundance of volunteer wheat increased in western Canada from 1.4, 1.9, 2.6, to 6.7 from the 1970's, 1980's, 1990's to 2000's, respectively (Leeson et al. 2005). Volunteer wheat is a competitive weed in successive crops, leading to lower harvest yields and quality (Marshall et al. 1989; O'Donovan et al. 1989). Volunteer wheat also facilitates the temporal movement of pests, such as wheat curl mite and wheat streak mosaic, that can

reduce crop yields (Jiang and Garrett 2005). Controlling volunteer wheat in crop rotations, including dicotyledonous crops, has traditionally not been challenging, but with the introduction of HR wheat, greater thought is required in planning herbicide choices and rotations. However, in cereal crops, less emphasis has been placed on controlling volunteer cereals due to the lack of in-crop herbicide control options and their potential use as feed crops which would have a higher tolerance limit for AP in cereal crops.

Where wheat crops are followed by broadleaf crops such as canola, cultural control as part of an integrated weed management (IWM) strategy may reduce volunteer wheat fecundity. Cultural management techniques that improve the competitiveness of crops are an important aspect of IWM, and when integrated with herbicides, are an effective tool for improving crop health (Harker et al. 2004; Appleby 2005). Herbicides are effective tools to control volunteer wheat and reduce seed return to the seed bank (Rainbolt et al. 2004; Blackshaw et al. 2006). However, crop rotations in western Canada have traditionally been dominated by cereal crops, primarily hard red spring wheat and barley (Campbell et al. 2002). It is not uncommon in western Canada for crop rotations to include consecutive years of cereal crop production. These primarily cereal rotations create a challenge for controlling volunteer cereals. IWM practices without the benefit of selective herbicides may reduce volunteer wheat fecundity and reduce the AP of volunteer cereals in harvested cereal crops.

Crop choice is a key integrated weed management strategy. Barley is a more competitive crop than wheat (Dew 1972). Peas, particularly semi-leafless, have a low competitive ability. However, new hybrid canola varieties may exhibit competitive ability similar to barley (Harker 2001). Herbicides applied pre-seeding can provide acceptable control of volunteer wheat, these options include herbicide groups such as ACCase inhibitors, EPSP inhibitors, glutamine synthetase inhibitors and bipyridylium salts (Mallory Smith and Retzinger 2003; Rainbolt et al. 2004; Blackshaw et al. 2006). In-crop volunteer wheat control options in cereals are very limited. Until 2004, herbicidal control of volunteer wheat was not possible, but with the introduction of imidazolinone-resistant wheat cv. CDC Imagine, it is now possible to control susceptible wheat and volunteer barley (Pozniak et al. 2004).

Crop cultural control methods, such as planting date and seeding rates, influence the ability of a crop to compete with weeds and reduce their biomass and fecundity (O'Donovan 1992; O'Donovan et al. 2007). The effect of cereal cultural practices on volunteer wheat fecundity, admixture and crop-anthesis synchronicity is currently unknown.

With the introduction of HR wheat and the possibility of other GM wheat varieties in the future, understanding the biology and population dynamics of volunteer wheat in cereal crops is important for the development of best management practices and regulatory decisions. The purpose of this research was to quantify the effects of cultural cereal production practices that increase the competitive ability of the crop and reduce the fecundity and admixture of volunteer wheat in cereal crops. The synchronicity of crop-volunteer flowering in wheat and barley crop rotations was also investigated to determine the potential for pollen mediated gene flow.

An experiment was conducted to investigate the effect of cultural cropping practices on volunteer wheat fecundity and flowering synchronicity between volunteers and cereal crops. Crop competition, influenced by the cultural practices of increased seeding rate, seeding date, and crop species may be important tools that will help manage the introduction of new technologies such as HR wheat. Circumstances such as growing a herbicide-tolerant cereal variety followed by a conventional variety or breeder seed, which requires low levels of varietal contamination, has promotes the question: how much will the crop compete with the volunteer cereal and reduce fecundity, therefore reducing seed bank inputs from wheat volunteers and admixture?

3.2 Materials and Methods

Field experiments to quantify the fecundity of volunteer wheat in cereal crops were conducted in two production fields near Calmar, Alberta, hereafter referred to as Calmar Home and Calmar East, in 2005 and 2006. Calmar, in the Aspen Parkland region of central Alberta, is characteristic of the central Parkland black Chernozemic soil zone. The Calmar Home soil was composed of 19 % sand, 51 % silt, and 29 % clay with a pH of 6.5 and 11.6% organic matter (OM) content. Calmar East was composed of 25 % sand, 51 % silt, and 24 % clay with a pH of 6.2 and 9.6% OM content. Precipitation in this region is

rarely a limitation to germination, with the 30 year average between May 1 and August 31 being >300 mm. Precipitation was adequate for spring germination in both 2005 and 2006 (Figure 3.1) (Environment Canada 2006).

In the years preceding the experiments, both fields were commercially seeded to Canadian prairie spring (CPS) wheat in 2004 and to hard red spring wheat cv. CDC Imagine in 2005. A conventional tillage management regime was employed. Fields were tilled in the fall with a sweep cultivator and harrowed prior to planting in the spring. Wheat was swathed prior to harvesting in both 2004 and 2005; therefore wheat volunteers were visibly denser in swathed rows. Experiments were located at least 12 m from field boundaries and were established to include the most uniform and representative populations of naturally occurring volunteer wheat densities. All research sites were managed in the spring as a minimum tillage management regime, which includes no spring tillage and a pre-seeding herbicide application to control weeds. To simulate the effect of HR volunteer wheat populations, the trial was sprayed with 2,4-D prior to seeding to control broadleaf weeds and to leave the volunteer wheat uncontrolled.

Plots were positioned in a factorial split plot treatment arrangement with the main plot as crop species and seeding date with seeding rate as subplots. All experiments included 4 replicates arranged side by side to maintain uniformity of volunteer populations. Plot size at establishment was 2 m by 8.5 m and later trimmed to 2 m by 6.5 m.

Hard red spring wheat cv. 'AC Superb' and two row barley cv. 'AC Metcalf' were seeded at four target seeding rates: 0, 150, 250, and 350 plants m^{-2} ; the 0 seeding rate was included to simulate an unseeded control. The soil openers on the seeder were pulled through the plots for the zero seeding rate treatments, respective of the seeding date, to simulate a seeding miss. All plots were seeded with a research scale seed drill with 20 cm row spacing. Both early and late planting dates approximately two weeks apart were typical for the growing region (Tables B.1 and B.2). Volunteer wheat was at the 1 to 3 leaf stage when the crop was seeded. Three randomly placed permanent ¼ m quadrats were established prior to crop emergence. Volunteer wheat emergence periodicity was studied by counting and marking volunteers with coloured avian leg

bands[7] around the base of the plants within the established quadrats (Figure B.9). In 2005, volunteer plants were marked at the time of seeding the crop (Table B1). In 2006, the sampling dates were extended to include three emergence timings relative to commonly applied herbicide treatments: prior to planting (PREP), pre in-crop herbicides (PRES), and after in-crop (POSTSP) treatments (Harker et al. 2006) (Table B.2).

All plots were treated with 200 g ai ha^{-1} of tralkoxydim tankmixed with bromoxynil + MCPA at 560 g ai ha^{-1} in 2005 and thifensulfuron-methyl + tribenuron-methyl at 15 g ai ha^{-1} in 2006 to control wild oats and broadleaf weeds. All maintenance herbicide treatments were applied with a tractor mounted applicator delivering 125 L ha^{-1} with air induction low drift nozzles.

Crop growth stages were recorded using the extended BBCH scale (BBCH Monograph 2001) in all quadrats beginning prior to volunteer flowering (BBCH 60 to 70) and continuing weekly until crop harvest.

At crop maturity, marked volunteers within quadrats were counted and removed by hand including roots. Crop above-ground biomass was hand harvested from two 0.5 m rows from the three previously established quadrats, dried for five days at 52 °C, and dry weight recorded. Volunteer fecundity, including individual volunteer biomass, reproductive spikes $plant^{-1}$, seeds $spike^{-1}$ and seeds $plant^{-1}$ were determined for emerged wheat cohorts at each emergence date (Table 3.1). Seeds from each plant were counted and weighed to calculate the mean number of seeds $spike^{-1}$ and their 1000 kernel weight. The entire plot was harvested with a small plot combine, seed was dried for 5 days at 52 °C, and chaff and debris removed using a seed cleaner. Plots were harvested based on crop maturity, thus two harvest dates were required due to different planting dates (Table B.1). Because seed cleaning equipment could not remove volunteer wheat seeds form wheat and barley samples, the harvested seed weight includes both the crop and the admixed volunteer wheat. Three 50 g random sub-samples of the harvested barley seed were taken with replacement and hand separated to quantify the amount of admixed volunteer wheat seeds. The three sub-samples were averaged and admixture values were calculated as a percent weight of the volunteer seeds to the weight of the crop.

[7] QC Supply, 574 Rd 11, PO Box 581, Schuyler, NE. 68661

Hand harvested volunteer wheat seeds were stored at 4 °C for a minimum of 3, and no longer than 12 months. Viability of hand harvested volunteer wheat seeds were assessed by germinating three replications of 100 seeds each from each plot. If the number of wheat seeds was insufficient to facilitate 100 seeds per replication, the total seed lot was divided into three replications. Seeds were germinated in a 24 x 16 x 4 cm germination container lined with one 23 X 15 cm Hoffman #601 blotter paper and 14 ml of Helix Xtra 0.2% v/v (thiamethoxam + difenoconazole + metalaxyl-M + fludioxonil). Seeds were placed in the dark at room temperature for 5 days. Germinated seeds were then counted and ungerminated seeds were cut and placed in a Petri dish with a Whatman No. 1 filter paper and 0.1% tetrazolium chloride (2, 3, 5-triphenyltetrazolium chloride). Petri plates were incubated for two hours in the dark at room temperature and the seeds were determined to be viable or non-viable based on the intensity of staining and the distribution of staining on the embryo (Association of Official Seed Analysts of North America 1970). Volunteer seed viability reported includes both germinated and tetrazolium positive seeds together. Although this may artificially inflate a positive seed viability calculation, it is important to adhere to the precautionary principal. It is unlikely that the tetrazolium-positive seeds may never produce a viable seedling from the soil seed bank.

3.2.1 Statistical analysis

All data was checked for homogeneity of variances and normality using PROC UNIVARITE in SAS prior to analysis. Data was square root transformed when homogeneity of variances and normality was improved. Mixed model ANOVA using the MIXED procedure in SAS was preformed on all data ([SAT] Statistical Analysis Systems 2007). All analyses were conducted separately by years because additional sampling was done in 2006. A repeated measure ANOVA approach was initially used for the 2006 wheat volunteer fecundity data for the three emergence timings, but a positive correlation was not evident between sampling times, therefore, each emergence date was analyzed separately. All mixed models designated location and block as random effects with all other dependent variables as fixed. All denominator degrees of freedom were adjusted using the Kenward Rogers method (Kenward and Roger 1997).

Due to the highly variable nature of natural volunteer populations, volunteer emergence was included as a covariate along with their respective interaction for all fecundity analyses and tested for significance. When the volunteer emergence covariate was not significant ($P > 0.05$), it was removed from the analysis for the respective dependant variable. Mean separations were conducted using square root transformed $(x+1)^{0.5}$ data to improve the normality and the heterogeneity of the variances. To minimize the potential of type 1 error associated with pairwise comparisons, the Tukey-Kramer honestly significant difference (HSD) was used to determine levels of significance ($P < 0.05$) as suggested by (Steel et al. 1997). Significant P values were converted to letters by using the PDMIX800 macro in conjunction with the pdiff method in PROC MIXED (Saxton 1998). To simplify interpretation of results, untransformed LSMeans are presented. The differences between the LSMeans for the seeding rates within the main effects were illustrated using lower case letters and the main effect means are separated using upper case letters.

Volunteer wheat and crop synchronicities were analyzed using a MIXED Model repeated measures analysis in SAS with a compound symmetry covariance structure. For this model, location and block were considered random, with all other factors considered fixed. All denominator degrees of freedom were adjusted using the Kenward Rogers method (Kenward and Roger 1997). Due to the high mortality and the highly variable nature of the POSTSP volunteers, they were not subject to ANOVA. Because these data are of biological importance, the means and the standard error of the means were included for the sampling dates that anthesis was observed.

3.3 Results

In 2005, mean crop yields were 5.8 and 5.7 tons ha^{-1} for the early and late seeded wheat, respectively and 6.4 and 6.3 for the early and late seeded barley, respectively. Grain yields in 2006 were 3.6 and 2.7 tons ha^{-1} for early and late seeded wheat, respectively, and 4.8 and 3.9 tons ha^{-1} for early and late seeded wheat, respectively (Table B3, B4). The average growing season temperatures were warmer in 2006, averaging 12.1 compared to 10.9 in 2005 (Figure 3.1) and this area received 401.9 and 399.1 mm of precipitation in 2005 and 2006, respectively, equivalent to the 30 year average for the

same time period (Figure 3.1) (Environment Canada 2006). Target wheat seeding rates of 150, 250 and 350 plants m^{-2} resulted in crop stands with 134.0, 187.6 and 244.2 plants m^{-2} in 2005 and 139.3, 188.8, 247.6 and plants m^{-2} in 2006. Similarly barley seeding rates resulted in 129.1, 176.6 and 227.2 plants m^{-2} in 2005 and 121.2, 173.7, 225.9 plants m^{-2} in 2006. Volunteer wheat densities were high and uniformly distributed throughout the trial area, although, PREP volunteer wheat densities were higher in 2005 than in 2006, averaging 98.3 and 37.5 plants m^{-2}, respectively.

To differentiate the fecundity of wheat volunteers emerging at different times, sampling intervals were increased in 2006 and therefore, results were presented by year. No significant differences were observed between locations in 2005 and 2006, therefore data were pooled by location for analysis.

3.3.1 2005

The average fecundity (seeds $plant^{-1}$) of early emerging (PREP) volunteers where no crop was planted ranged from 115.7 to 139.9. Fecundity of individual early emerging volunteer wheat plants were affected by the crop, seeding rates and by seeding dates. Volunteer wheat (PREP) produced significantly (P=0.0330) fewer seeds $plants^{-1}$ in the early seeded barley than in the late seeded wheat, averaging 71.0 compared to 139.5 seeds $plant^{-1}$ (Table 3.1). All early seeded barley reduced the fecundity of the volunteers and ranged from a 47-70% reduction. Increasing the seeding rate reduced seed fecundity compared to the unseeded check in the earlier crops. For early planted wheat a seeding rate of 350 plants m^{-2} was required to reduce volunteer fecundity compared to the unseeded check. In the late seeded crop, there were no significant differences in seeds produced per plant between unseeded plots and those of the highest seeding rates (Table 3.1). The yield components that make up individual plant fecundity (seeds $plant^{-1}$) are the number of seeds $spike^{-1}$ and spikes $plant^{-1}$. Of these two components, the seeds $spike^{-1}$ was not affected by the treatments and averaged 33.2 seeds (Table 3.1). This is consistent with (Wang et al. 2002), who found that spring wheat averaged 33.8 seeds $spike^{-1}$ in western Canada.

Volunteer biomass (PREP) was similarly affected by crop choice, with barley and wheat reducing volunteer biomass by 50 and 57%, respectively. The mortality of PREP

volunteers was not affected by the agronomic treatments, and averaged 6.2 % across all treatments (Table 3.2).

The most important measure that determines potential seed mediated gene flow of volunteer wheat is how many seeds are produced per given area, and the factors studied were effective in reducing the PREP volunteer total seeds produced (Figure 3.4). Total seeds was significantly reduced (P = 0.0211) in early planted barley compared to late planted wheat, averaging 6139.2 and 10265.2 seeds m^{-2}, representing a reduction of 40 %. A significant interaction was observed between seeding dates and rates (P=0.0128), and their combined effect reduced the total seed production. The highest seeding rate (350 plants m^{-2}) in the earlier planted crops reduced the total seeds (Figure 3.4). The greatest factor affecting volunteer fecundity in 2005 was the number of reproductive spikes $plant^{-1}$, and earlier seeded competitive crops were effective at reducing this yield component (Table 3.1).

The viability of the recovered volunteer wheat seeds from the harvested samples were not significantly affected by any of the treatments and ranged from 98.2 to 98.8 % (Table 3.2).

The percentage of volunteer wheat seeds recovered from the harvested sample was significantly affected by the planting date and the seeding rate (Figure 3.6). The effect of crop could not be determined because the volunteer wheat could only be visually separated form the barley and therefore, was not separated. Planting barley earlier in 2005 significantly reduced admixture up to 60 % at the lowest seeding rate. Seeding rate was only significant in the late planting date, reducing the percent admixture from 66.3 to 49.8%. These data do not include the fecundity of later emerging (PRES and POSTSP) volunteers.

3.3.2 2006

In 2005, it was observed that high densities of volunteer wheat continued to emerge during the cropping season, therefore, in 2006 volunteer wheat emergence in established quadrates was quantified at three times during the growing season: prior to seeding (PREP), prior to in-crop herbicide application (PRES) and following in-crop herbicide

application (POSTSP). Total PREP volunteer emergence was 62% lower in 2006 compared to 2005 (Table 3.4).

Individual fecundity of early emerging volunteers in the unseeded check averaged 119.2 to 141.4 seeds plant $^{-1}$, similar to 2005. The fecundity of the volunteer wheat decreased with the later emergence timings. In the absence of the crop, PREP, PRES and POSTSP volunteers had a mean individual fecundity of 130.3, 81.5 and 17.2 seeds plant^{-1} (Tables 3.3, 3.5, 3. 7). The type of crop significantly influenced PREP and PRES volunteer fecundity (P = 0.0491 and 0.0091). The fecundity of all volunteers were affected by the time of planting wheat and barley crops (P = 0.005 and > 0.0001, respectively) but not affected by seeding rate (Table 3.6). Volunteer fecundity parameters in 2006, seeds spike^{-1} and spike plant^{-1} and biomass were significantly affected by crop type, seeding date and rate.

Increase in mortality of volunteers was directly proportional to the time of emergence in the cropping season. Mortality of PREP volunteers ranged from 15 to 32 % and was not affected by any of the agronomic factors. Later emerging volunteers were more influence by the agronomic treatments. PRES mortality was similar to the PREP mortality, ranging from 8 % in the unseeded check to 56 % for early planted barley at 350 plants m^{-2}. The early planted barley crop significantly increased volunteer mortality (P=0.0322). Mortality of the POSTSP volunteers increased from 33 to 65 % (Table 3.8). Decreased volunteer biomass was apparent for later emerging volunteers, the mean ranging from 11.8 for PREP volunteers to 1.5 grams for POSTSP volunteers in the absence of a crop. The PREP volunteer biomass was reduced by the crop, planting date and the seeding rate, with the early planted barley at the highest seeding rate reducing the biomass by 86%. PRESP and POSTSP volunteer biomass was less consistently affected by agronomic practices than early volunteers.

In 2006, PREP volunteer total seed production averaged 2792.0 to 3088.9 seed m^{-2} in the unseeded check, which is approximately one third of that recorded in 2005 (Figure 3.5, Table 3.7) . The emergence of PREP volunteers in 2006 was 62% less than in 2005, which would account for the greater seed production considering individual volunteer fecundity was similar in 2005 and 2006. Total seed produced per unit area in the absence of the crop for PREP, PRESP and POSTSP volunteers was 2947.7, 2420.1

and 369.4 plant m^{-2}, respectively (Tables 3.4, 3.6 and 3.8). Total seeds of PREP volunteers were significantly affected by crop choice (P = 0.0119), early planting (P = 0.0005) and seeding rate (P <0.0001), with the most competitive treatment reducing total seed production to 326.9 seeds m^{-2}, representing an 89% reduction over the unseeded check. This reduction was a result of lower number of seeds produced $spike^{-1}$ and spikes $plant^{-1}$ (Table 3.5). PRESP and POSTSP volunteers were inconsistently influenced by both the crop and planting date, the presence of a seeded crop was consistently different than the unseeded check. PREP and PRESP volunteers contributed the most to seed production. Volunteers that emerged later were much smaller, less fecund and contributed little to the total volunteer seeds produced (Figure 3.5).

Admixture levels of volunteer wheat in barley crops was derived by harvesting the whole plots and separating the volunteer wheat seeds from the barley samples. All volunteers, regardless of emergence timing, contributed to volunteer admixture. Because volunteer populations were higher in 2005, admixture was greater. In 2006, planting barley earlier reduced volunteer wheat admixture by 71% when averaged across seeding rates. The effect of seeding rates was not significant at either planting time. Late planting admixture ranged from 30.5 to 19.5% and earlier planting ranged from 9.7 to 5.3% (Figure 3.6).

3.3.3 Anthesis synchronicity

Wheat volunteers and wheat crop plants that are flowering synchronously have the potential to exchange genes and pollen. For both 2005 and 2006, the location, seeding rate and crop were not significantly different; therefore, the data was pooled accordingly. In 2005, early emerging volunteers flowered (BBCH 60-70) between July 8 through 20, synchronously with early seeded, but not late seeded wheat in both locations (Fig 3.3). In 2006 at both locations, PREP volunteers flowered in July 1 through 17; PRES flowered July 17th through Aug 1. POSTSP volunteers were observed flowering on Aug 9 and Aug 16. These late emerging volunteers were predominantly observed flowering in the unseeded checks. Crops planted early flowered from July 3 to 10th and late seeded crop from July 13 to 25 (Figure 3.4). Pollen movement to the early seed crop would have occurred readily with PREP emerging volunteers and only partially or not at all with

PRES and POSTSP emerging volunteers. Pollen flow to early seeded crops would have been minimized if early volunteers were controlled. While the late seeded crop flower at the same time as PRES volunteers only. Although wheat is primarily self pollinated, there is a small amount of outcrossing, averaging 2% depending on variety and environmental conditions (Hucl 1996). Results suggest that pollen mediated gene flow mayoccur between crops planted at both timings and PREP and PRES volunteers. By controlling volunteer wheat at these emergence intervals the greatest pollen mediated gene flow potential would be reduced.

3.4 Discussion

Crops and weeds compete for limited resources and resource capture depends on both time of emergence and plant density. Earlier emergence of the wheat volunteers, relative to the seeded crop, increased the fecundity of the volunteers. This is congruent with the findings of O'Donovan (1992), who reported that barley that emerged prior to the canola crops had higher seed production than volunteers that emerged after the crop.

Although crop seeding density was similar for both years, the crop biomass and grain yield was greater in 2005 than 2006. The density of volunteer wheat was greater in 2005 than in 2006, and may have directly resulted in the greater volunteer fecundity, lower crop yields and higher admixture of wheat within the harvested barley samples. These results are similar to O'Donovan et al. (2007) who quantified the effect of volunteer barley on wheat yield. They reported increased densities of volunteer barley resulted in decreased wheat yields while a higher wheat seeding rate resulted in a more competitive crop and decreased volunteer barley fecundity. As volunteer barley increased in density, barley fecundity was consistently lower at the higher wheat seeding rate. In the current study, volunteer wheat fecundity was similarly reduced by higher seeding rates that resulted in a more competitive crop. Target crop densities were not achieved using experimental seeding rates in either year of the current study, which may have reduced the competitive potential of the seeded crop. Barley is more competitive than wheat (Dew 1972), suggesting that if target seeding densities of barley had been achieved in the current study, the results may have been more dramatic. Considerable research on seeding densities has been conducted to determine the economic threshold of weeds in

crops. O'Donovan et al. (2007), in a study of volunteer barley in wheat crops, report that at one of the study locations with wheat densities of 461 plants m^{-2}, herbicide control of volunteer barley would not have been economically justified. Economic thresholds developed for herbicide applications do not account for the potential economic impact of GM volunteers on adventitious presence and may have to be readjusted should GM wheat be approved.

Volunteer wheat controlled at the PREP interval will result in removal of the most fecund volunteers. This can be accomplished either by tillage or by pre-seeding herbicidal control measures. The fecundity of these volunteers is also most readily influenced with early planting of competitive crops. Later emerging volunteers (PRESP and POSTSP) that were uncontrolled also contributed significantly to seed production and subsequent admixed with the harvested crop. Harker et al. (2005) reported that in-crop control of volunteer wheat is also required to reduce volunteer wheat persistence as volunteers are capable of emerging throughout the growing season. The proportion of volunteers emerging at the three intervals studied was highest at POSTSP, ranging from 27.8 to 55.4%. This finding is contrary to the study reported by De Corby et al. (2007), which reported 75% of volunteers emerged at the PRESP interval. Variance between natural and artificial seed banks and environmental conditions may be contributing factors between these findings (Leon et al. 2003). Harvest losses can consist of both naked seed and unthreshed spikes; the latter have been known to persist longer in the soil seed bank (Komatsuzaki and Endo 1996).

Volunteers flowered synchronously with seeded cereal crops. A study conducted with canola and wild radish reported that synchronous flowering between these sexually compatible species may increase potential hybridization events (Simard and Légére 2004). Although hybridization between these two relatives is very low (Warwick et al. 2003), the potential was increased due to synchronous flowering. Simard and Légére (2004) found that the seeding rate of wheat did not affect the mean flowering times of canola and wild radish. Similarly for wheat, primarily a selfing species, pollen mediated gene flow between volunteers and the crop would be maximized when plants are in direct contact (Lawrie et al. 2006). When GR volunteers grow synchronous with a conventional

wheat crop, pollen mediated gene flow between them will lead to the production of some hemizygote seeds which would be resistant to glyphosate.

Harvest seed loss can be high and if volunteers are uncontrolled, as is the case in this study, admixture in the subsequent crops can also be high (5.3 to 66.3%). Harker et al. (2004) reported that volunteer control in the first year following a wheat crop was critical to reduce volunteer wheat persistence and admixture. Admixture of GR wheat in conventional wheat was as high a 14% when volunteers were not controlled in previous year. However at most sites where volunteer control was successful in the first year, GR wheat admixture was < 0.9%, which would meet the proposed EU thresholds for GM content for import commodities.

The purpose of this study was to quantify volunteer wheat fecundity under worst case scenarios to facilitate the modeling of gene flow in wheat. This data may not reflect the amount of volunteer seed admixture that would result from large scale production, but provides information on anomalies that can occur such as herbicide application spray misses. Mixing and blending at harvest may then occur that can decrease AP in harvested crops (Gustafson et al. 2005).

In the absence of pre-seeding and selective herbicides, the cultural weed management practices resulted in a significant reduction in volunteer wheat seed production and admixture but were not sufficient in the absence of herbicide control to meet acceptable potential AP threshold requirements and varietal purities required by regulators and seed growers. Although it is not expected that cereal agronomic effects will control volunteer cereals, it will provide additive effects to meet adventitious presence thresholds that may be required in the future for market access. If and when GM cereals are released for commercial production, the effects of sound agronomic management will provide assistance in managing volunteers.

The use of artificial volunteer populations commonly represents worst case scenarios. This is especially true for study A where uniformly seeded volunteer populations were established and were required for quick emergence. Many biological factors that commonly influence volunteer populations such as fall emergence and subsequent winter kill are not represented. This data was collected with the intention and

will be used for modeling purposes; therefore, caution should be exercised in large scale extrapolation to field scale scenarios.

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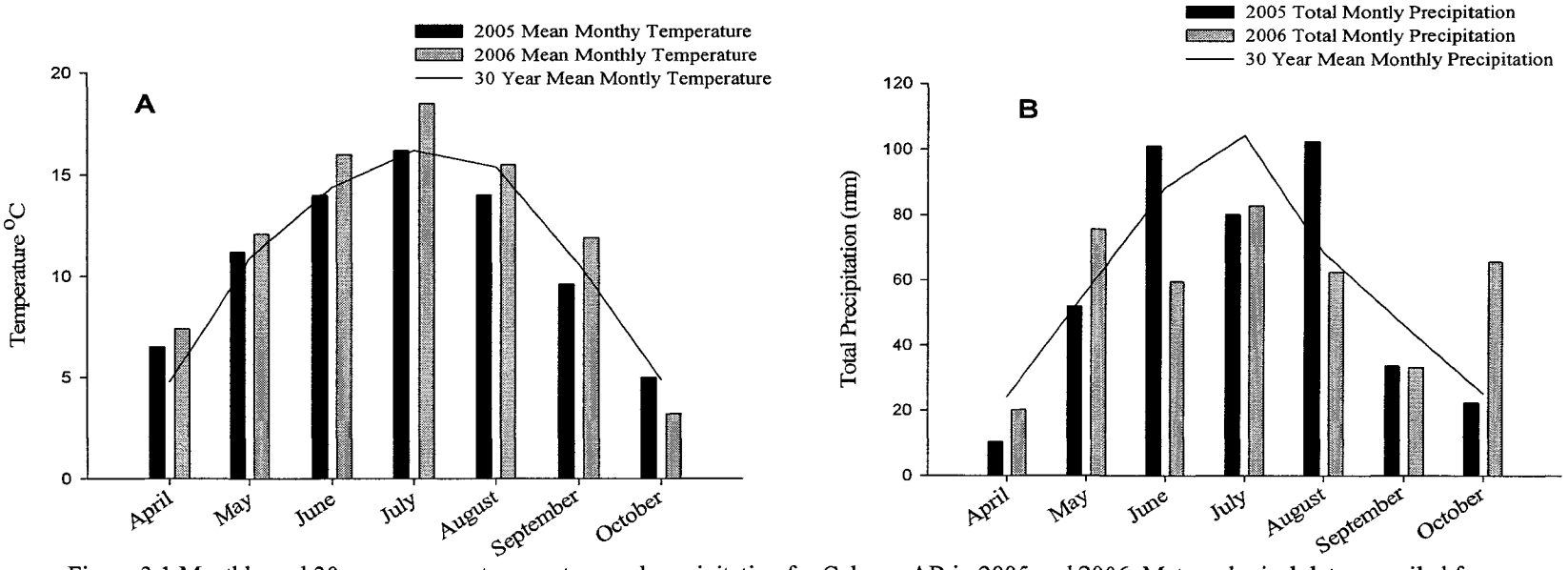

Figure 3.1 Monthly and 30 year average temperature and precipitation for Calmar, AB in 2005 and 2006. Meteorological data compiled from the nearest Environment Canada weather station.

Table 3.1. Volunteer wheat biomass and fecundity of plants that emerged prior to the crop (PREP) as influenced by agronomic factors at two locations in 2005.

Crop	Planting date	Seeding rate	Biomass plant^{-1}		Seeds spike^{-1}		Spike plant^{-1}		Seeds plant^{-1}	
		Plants m^{-2}	— g —		— c —		— m^{-2} —		— mg —	
Wheat	Early	0	9.3	a	37.9	a	3.6	a	136.9	a
Wheat	Early	150	7.4	b	32.9	a	3.0	a	107.1	a
Wheat	Early	250	6.0	b	36.4	a	2.6	a	98.2	a
Wheat	Early	350	4.1	c	34.1	a	1.9	b	73.1	b
[1]*Main Effect Means*			*6.7*	*C*	*35.3*	*A*	*2.8*	*C*	*103.8*	*AB*
Wheat	Late	0	13.3	a	30.5	a	4.9	a	122.7	a
Wheat	Late	150	11.4	b	34.3	a	4.6	a	152.3	a
Wheat	Late	250	10.6	b	32.1	a	4.0	a	133.0	a
Wheat	Late	350	10.9	b	34.2	a	4.4	a	149.9	a
[1]*Main Effect Means*			*11.6*	*A*	*32.8*	*A*	*4.5*	*A*	*139.5*	*A*
Barley	Early	0	9.3	a	35.5	a	3.6	a	126.1	a
Barley	Early	150	4.6	b	28.8	a	2.2	b	65.0	ab
Barley	Early	250	2.7	b	31.8	a	1.5	b	53.1	b
Barley	Early	350	2.1	c	31.9	a	1.3	b	39.9	b
[1]*Main Effect Means*			*4.7*	*D*	*32.0*	*A*	*2.2*	*D*	*71.0*	*B*
Barley	Late	0	13.1	a	27.4	a	4.9	a	115.7	a
Barley	Late	150	8.5	b	27.4	a	3.7	a	94.7	a
Barley	Late	250	7.7	b	29.0	a	3.2	a	92.8	a
Barley	Late	350	8.5	b	37.6	a	3.3	a	93.2	a
[1]*Main Effect Means*			*9.5*	*B*	*30.3*	*A*	*3.8*	*B*	*104.1*	*AB*
ANOVA F-values										
Crop			**0.0002**		ns		**<0.0001**		**0.0003**	
Planting date			**<0.0001**		ns		**<0.0001**		**0.0330**	
Crop* Planting date			ns		ns		ns		ns	
Seeding Rate			**<0.0001**		ns		**<0.0001**		**0.0042**	
Seeding rate * Crop			**0.0075**		ns		**0.0086**		ns	
Seeding rate * planting date			ns		ns		ns		ns	
[2]Emergence			**0.0015**		ns		**0.0032**		ns	

[1]LSMeans for main factors Crop*SeedTiming, LSMeans followed by the same lower case letter are not significantly different, upper case letters indicate significance between main effects (P< 0.05).
[2]Emergence density of the volunteer wheat was included in the ANOVA as a covariate when significant (P<0.05), when not significant it was removed.

Table 3.2. Volunteer wheat survivorship and viability as influenced by agronomic factors at two locations in 2005.

Crop	Planting date	Seeding rate	Emerged volunteers		Mortality		Total seeds		Kernel Wt.		Viable seeds	
		Plants m^{-2}	— m^{-2} —		— % —		— m^{-2} —		— mg —		— % —	
Wheat	Early	0	90.4	a	4.9	a	9650.1	a	38.7	a	98.5	a
Wheat	Early	150	84.2	a	7.5	a	6506.4	a	34.2	a	98.4	a
Wheat	Early	250	73.2	a	7.6	a	6594.5	a	32.6	a	97.9	a
Wheat	Early	350	90.7	a	15.5	a	5532.7	b	31.1	a	97.8	a
[1]Main Effect Means			***84.6***	***A***	***8.9***	***A***	***7070.9***	***BD***	***34.1***	***A***	***98.2***	***A***
Wheat	Late	0	116.2	a	1.8	a	10943.0	a	38.4	a	97.9	a
Wheat	Late	150	106.7	a	4.4	a	10628.0	a	37.2	a	98.1	a
Wheat	Late	250	91.3	a	5.3	a	8725.8	a	35.5	a	97.7	a
Wheat	Late	350	94.8	a	5.5	a	10764.0	a	34.9	a	98.2	a
[1]Main Effect Means			***102.3***	***A***	***4.3***	***A***	***10265.2***	***ABC***	***36.5***	***A***	***98.0***	***A***
Barley	Early	0	90.4	a	4.9	a	9125.3	a	37.5	a	98.5	a
Barley	Early	150	72.7	a	3.0	a	6045.8	a	29.1	a	98.6	a
Barley	Early	250	74.7	a	6.2	a	5228.3	a	31.3	a	98.6	a
Barley	Early	350	73.2	a	12.5	a	4157.2	b	32.0	a	97.0	a
[1]Main Effect Means			***77.7***	***A***	***6.6***	***A***	***6139.2***	***D***	***32.5***	***A***	***98.2***	***A***
Barley	Late	0	116.2	a	1.8	a	10487.0	a	39.5	a	97.7	a
Barley	Late	150	89.7	a	4.8	a	6966.8	a	31.4	a	97.8	a
Barley	Late	250	72.7	a	6.4	a	7251.1	a	32.6	a	98.1	a
Barley	Late	350	82.2	a	7.2	a	9258.2	a	32.1	a	98.1	a
[1]Main Effect Means			***90.2***	***A***	***5.0***	***A***	***8490.8***	***ABC***	***33.9***	***A***	***98.0***	***A***
ANOVA F-values												
Crop			ns		ns		**0.0211**		ns		ns	
Planting date			ns		ns		ns		ns		ns	
Crop* Planting date			ns		ns		ns		ns		ns	
Seeding rate			ns		ns		ns		ns		ns	
Seeding rate * Crop			ns		ns		ns		ns		ns	
Seeding rate * Planting date			ns		ns		**0.0128**		ns		ns	
[2]Emergence			ns		ns		**<0.0001**		ns		ns	

[1]LSMeans for main factors Crop*SeedTiming, LSMeans followed by the same lower case letter are not significantly different, upper case letters indicate significance between main effects ($P< 0.05$).

[2]Emergence density of the volunteer wheat was included in the ANOVA as a covariate when significant ($P>0.05$), when not significant it was removed.

Table 3.3. Volunteer wheat fecundity of plants that emerged prior to the crop (PREP) as influenced by agronomic factors at two locations in 2006.

Crop	Planting date	Seeding rate	Biomass plant^{-1}		Seeds spike^{-1}		Spike plant^{-1}		Seeds plant^{-1}	
		Plants m^{-2}	— g —		— c —		— m^{-2} —		— mg —	
Wheat	Early	0	12.9	a	26.8	a	2.9	a	141.42	a
Wheat	Early	150	6.0	b	20.2	a	2.2	a	44.6	b
Wheat	Early	250	4.2	b	20.4	a	1.9	a	46.2	b
Wheat	Early	350	2.7	b	17.6	b	1.3	b	24.1	b
[1]Main Effect Means			***6.5***	***BC***	***21.3***	***B***	***2.1***	***B***	***64.1***	***AB***
Wheat	Late	0	10.7	a	24.7	a	5.7	a	119.2	a
Wheat	Late	150	9.8	a	24.6	a	3.3	a	75.6	a
Wheat	Late	250	7.5	a	24.2	a	2.8	a	60.5	a
Wheat	Late	350	7.0	a	24.2	a	2.6	a	55.3	a
[1]Main Effect Means			***8.8***	***A***	***24.4***	***A***	***3.6***	***A***	***77.7***	***A***
Barley	Early	0	12.9	a	26.8	a	2.8	a	141.4	a
Barley	Early	150	2.8	b	15.1	b	1.1	a	17.8	b
Barley	Early	250	1.3	b	12.2	b	1.2	b	15.4	b
Barley	Early	350	1.8	b	12.4	b	1.5	b	18.2	b
[1]Main Effect Means			***4.7***	***C***	***16.6***	***C***	***1.7***	***B***	***48.2***	***B***
Barley	Late	0	10.7	a	24.7	a	5.7	a	119.2	a
Barley	Late	150	6.7	a	21.6	a	2.2	a	55.3	a
Barley	Late	250	6.9	a	17.7	a	1.7	a	29.8	b
Barley	Late	350	4.7	b	18.9	a	2.1	b	36.3	b
[1]Main Effect Means			***7.2***	***AB***	***20.7***	***B***	***2.9***	***AB***	***60.2***	***AB***
ANOVA F-values										
Crop			**0.0013**		**0.0032**		**0.0436**		**0.0491**	
Planting date			**<0.0001**		**<0.0001**		**0.0025**		**0.005**	
Crop* Planting date			ns		ns		ns		ns	
Seeding rate			**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Seeding rate * Crop			ns		ns		ns		ns	
Seeding rate * Planting date			ns		**0.0003**		ns		**<0.0001**	
[2]Emergence			ns		ns		**0.0480**		ns	

[1]LSMeans for main factors Crop*SeedTiming, LSMeans followed by the same lower case letter are not significantly different, upper case letters indicate significance between main effects (P< 0.05).

[2]Emergence density of the volunteer wheat was included in the ANOVA as a covariate when significant (P<0.05), when not significant it was removed.

Table 3.4. Volunteer wheat survivorship and viability of plants that emerged prior to the crop (PREP) as influenced by agronomic factors at two locations in 2006.

Crop	Planting date	Seeding rate	Emerged volunteers		Mortality	Total seeds	Kernel Wt.	Viable seeds
		Plants m^{-2}	— m^{-2} —	% total[3]	— % —	— m^{-2} —	— mg —	— % —
Wheat	Early	0	22.0 a	16.9	14.9 a	3075.9 a	32.1 a	99.4 a
Wheat	Early	150	35.6 a	26.8	35.2 a	841.1 b	30.3 a	99.5 a
Wheat	Early	250	31.3 a	24.4	35.0 a	897.0 b	31.0 a	99.5 a
Wheat	Early	350	22.4 a	21.2	44.7 a	295.6 b	30.1 a	99.3 a
[1]Main Effect Means			***27.8 A***	***22.3***	***32.4 A***	***1277.4 B***	***30.9 AB***	***99.4 A***
Wheat	Late	0	47.5 a	31.0	17.1 a	2792.0 a	31.6 a	98.7 a
Wheat	Late	150	44.0 a	36.3	15.3 a	2498.4 a	32.3 a	99.1 a
Wheat	Late	250	37.5 a	33.7	11.2 a	1846.0 a	32.3 a	98.5 a
Wheat	Late	350	51.5 a	36.1	17.1 a	2194.4 a	31.7 a	99.2 a
[1]Main Effect Means			**45.1 A**	***34.3***	***15.2 A***	***2332.7 A***	***31.9 A***	***98.9 A***
Barley	Early	0	22.0 a	16.9	14.9 a	3088.9 a	32.1 a	99.4 a
Barley	Early	150	28.4 a	23.4	30.1 a	731.9 b	27.2 a	100.0 a
Barley	Early	250	40.7 a	34.7	32.8 a	359.3 b	27.5 a	99.5 a
Barley	Early	350	46.7 a	31.5	36.2 a	327.0 b	24.6 b	99.7 a
[1]Main Effect Means			**34.4 A**	***26.6***	***28.5 A***	***1126.8 B***	***27.8 C***	***99.7 A***
Barley	Late	0	47.5 a	31.0	17.1 a	2833.8 a	31.6 a	98.7 a
Barley	Late	150	44.6 a	29.1	19.9 a	1564.3 a	28.6 a	98.5 a
Barley	Late	250	40.7 a	30.7	26.6 a	1088.4 b	27.7 a	99.4 a
Barley	Late	350	38.2 a	31.3	25.2 a	1086.4 b	26.5 a	99.0 a
[4]Main Effect Means			**42.7 A**	***30.5***	***22.2 A***	***1643.2 B***	***28.6 BC***	***98.9 A***
ANOVA F-values								
Crop			ns		ns	**0.0119**	**0.0011**	ns
Planting date			ns		ns	**0.0005**	**ns**	**0.0254**
Crop* Planting date			ns		ns	ns	ns	ns
Seeding rate			ns		ns	**0.0006**	**0.0154**	ns
Seeding rate * Crop			ns		ns	ns	ns	ns
Seeding rate * Planting date			ns		ns	ns	ns	ns
[2]Emergence			ns		ns	**<0.0001**	ns	-

[1]LSMeans for main factors, LSMeans followed by the same lower case letter are not significantly different, upper case letters indicate significance between main effects (P< 0.05)
[2]Emergence density of the volunteer wheat was included in the ANOVA as a covariate when significant (P<0.05), when not significant (ns) it was removed
[3]Represents the percent of the total volunteers within the treatment that emerged at the respective sampling timing

Table 3.5. Volunteer wheat fecundity of plants that emerged prior to in-crop herbicide (PRES) application as influenced by agronomic factors at two locations in 2006.

Crop	Planting date	Seeding rate	Biomass plant^{-1}		Seeds spike^{-1}		Spike plant^{-1}		Seeds plant^{-1}	
		Plants m^{-2}	— g —		— c —		— m^{-2} —		— mg —	
Wheat	Early	0	6.2	a	21.7	a	3.7	a	82.1	a
Wheat	Early	150	3.8	a	19.3	a	2.5	a	45.9	b
Wheat	Early	250	1.7	b	12.6	b	1.4	b	19.3	b
Wheat	Early	350	1.8	b	15.4	b	1.3	b	21.1	b
[1]*Main Effect Means*			*3.4*	*A*	*17.3*	*A*	*2.2*	*AB*	*42.1*	*A*
Wheat	Late	0	6.5	a	18.3	a	4.4	a	80.9	a
Wheat	Late	150	3.0	a	15.7	a	1.9	b	30.8	b
Wheat	Late	250	1.9	b	15.9	a	1.7	b	25.9	b
Wheat	Late	350	1.7	b	15.8	a	1.4	b	21.8	b
[1]*Main Effect Means*			*3.3*	*A*	*16.4*	*A*	*2.4*	*A*	*39.8*	*AB*
Barley	Early	0	6.2	a	21.7	a	3.7	a	82.1	a
Barley	Early	150	0.7	b	7.0	b	1.1	b	7.3	b
Barley	Early	250	0.7	b	6.2	b	1.1	b	7.1	b
Barley	Early	350	1.1	b	7.6	b	1.3	b	11.3	b
[1]*Main Effect Means*			*2.2*	*B*	*10.6*	*B*	*1.8*	*B*	*27.0*	*B*
Barley	Late	0	6.5	a	18.3	a	4.4	a	80.9	a
Barley	Late	150	1.1	b	13.2	a	1.3	b	16.6	b
Barley	Late	250	2.5	b	8.5	b	1.2	b	10.5	b
Barley	Late	350	1.0	b	10.0	b	1.2	b	11.7	b
[1]*Main Effect Means*			*2.8*	*AB*	*12.5*	*B*	*2.0*	*AB*	*29.9*	*AB*
ANOVA F-values										
Crop			**0.0002**		**<0.0001**		**0.0059**		**0.0091**	
Planting date			ns		**ns**		ns		ns	
Crop* Planting date			ns		**0.0088**		ns		ns	
Seeding rate			**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Seeding rate * Crop			**0.0026**		**0.0155**		**ns**		ns	
Seeding Rate * Planting date			ns		ns		ns		ns	
[2]Emergence			ns		ns		ns		ns	

[1]LSMeans for main factors Crop*SeedTiming, LSMeans followed by the same lower case letter are not significantly different, upper case letters indicate significance between main effects (P< 0.05)

[2]Emergence density of the volunteer wheat was included in the ANOVA as a covariate when significant (P<0.05), when not significant it was removed

Table 3.6. Volunteer wheat survivorship and viability of plants that emerged prior to the in-crop herbicide (PRES) application as influenced by agronomic factors in 2006.

Crop	Planting date	Seeding rate	Emerged volunteers		Mortality	Total seeds	Kernel Wt.	Viable seeds
		Plants m^{-2}	— m^{-2} —	% total[3]	— % —	— m^{-2} —	— mg —	— % —
Wheat	Early	0	63.8 a	39.2	7.5 a	3059.0 ab	32.0 a	99.6 a
Wheat	Early	150	36.8 a	23.8	26.0 a	666.4 de	34.5 a	99.1 a
Wheat	Early	250	30.8 a	20.2	19.1 a	372.3 e	30.7 a	98.7 a
Wheat	Early	350	50.1 a	33.6	26.5 a	481.9 e	33.3 a	98.6 a
[1]Main Effect Means			***45.4 A***	**29.2**	***19.8 A***	***1144.9 A***	***32.6 A***	***9.0 A***
Wheat	Late	0	51.2 a	33.0	13.4 a	1781.2 cd	30.4 a	98.9 a
Wheat	Late	150	29.5 a	21.7	8.7 a	445.9 e	31.8 a	98.6 a
Wheat	Late	250	34.1 a	26.2	12.8 a	452.7 e	32.2 a	99.1 a
Wheat	Late	350	36.0 a	24.1	30.7 a	283.8 e	32.4 a	97.8 a
[1]Main Effect Means			**37.7 *A***	**26.2**	***16.4 A***	***740.9 AB***	***31.7 AB***	***98.6 A***
Barley	Early	0	63.8 a	39.2	7.5 b	3059.0 ac	32.0 a	99.6 a
Barley	Early	150	51.9 a	36.5	28.1 b	187.4 e	27.7 a	99.7 a
Barley	Early	250	43.3 a	29.0	39.7 b	94.1 e	23.2 b	99.8 a
Barley	Early	350	60.0 a	40.7	55.8 a	163.5 e	26.0 b	99.6 a
[1]Main Effect Means			**54.8 *A***	**36.4**	***32.8 A***	***876.0 B***	***27.2 B***	***99.7 A***
Barley	Late	0	51.2 a	33.0	13.4 a	1781.2 bd	30.4 a	98.9 a
Barley	Late	150	34.8 a	20.9	17.5 a	241.9 e	30.1 a	98.1 a
Barley	Late	250	40.0 a	31.0	45.4 a	135.9 e	29.3 a	98.5 a
Barley	Late	350	36.3 a	26.0	35.8 a	160.4 e	30.0 a	99.3 a
[1]Main Effect Means			**40.6 *A***	***27.7***	***28.0 A***	***579.9 B***	***30.0 AB***	***99.5 A***
ANOVA F-values								
Crop			ns		ns	**0.0165**	ns	ns
Planting date			ns		ns	**ns**	**0.0083**	ns
Crop* Planting date			ns		ns	ns	ns	ns
Seeding rate			ns		**<0.0001**	ns	ns	ns
Seeding rate * Crop			ns		**0.0065**	**0.0459**	ns	ns
Seeding rate * Planting date			ns		ns	**0.0477**	ns	ns
[2]Emergence			-		ns	ns	-	-

[1]LSMeans for main factors Crop*SeedTiming, LSMeans followed by the same lower case letter are not significantly different, upper case letters indicate significance between main effects ($P< 0.05$)

[2]Emergence density of the volunteer wheat was included in the ANOVA as a covariate when significant ($P<0.05$), when not significant it was removed

[3]Represents the percent of the total volunteers within the treatment that emerged at the respective sampling timing

Table 3.7. Volunteer wheat fecundity of plants that emerged after the in-crop herbicide (POSTSP) application as influenced by agronomic factors at two locations in 2006.

Crop	Planting date	Seeding rate	Biomass plant^{-1}		Seeds spike^{-1}		Spike plant^{-1}		Seeds plant^{-1}	
		Plants m^{-2}	— g —		— c —		— m^{-2} —		— mg —	
Wheat	Early	0	1.1	a	8.0	a	1.5	a	13.1	a
Wheat	Early	150	0.3	b	2.3	b	1.0	a	2.3	b
Wheat	Early	250	0.3	b	2.1	b	1.0	a	2.4	b
Wheat	Early	350	0.2	b	1.5	b	1.0	a	1.3	b
[1]Main Effect Means			***0.5***	***C***	***3.5***	***B***	***1.1***	***A***	***4.8***	***B***
Wheat	Late	0	1.8	a	11.2	a	1.9	a	21.3	a
Wheat	Late	150	0.5	b	5.4	b	1.1	a	6.0	b
Wheat	Late	250	0.5	b	4.4	b	1.1	a	5.1	b
Wheat	Late	350	0.4	b	3.4	b	1.0	a	3.7	b
[1]Main Effect Means			***0.8***	***A***	***6.1***	***A***	***1.3***	***A***	***9.1***	***A***
Barley	Early	0	1.1	a	8.0	a	1.5	a	13.1	a
Barley	Early	150	0.3	b	1.7	b	1.1	a	2.0	b
Barley	Early	250	0.5	b	2.4	b	1.1	b	4.0	b
Barley	Early	350	0.2	b	0.8	b	0.9	b	0.5	b
[1]Main Effect Means			***0.5***	***BC***	***3.2***	***B***	***1.1***	***A***	***4.9***	***B***
Barley	Late	0	1.8	a	11.2	a	1.9	a	21.3	a
Barley	Late	150	0.5	b	4.1	b	1.1	b	5.0	b
Barley	Late	250	0.5	b	4.2	b	1.0	b	5.8	b
Barley	Late	350	0.3	b	4.3	b	1.0	b	4.0	b
[1]Main Effect Means			***0.8***	***AB***	***6.0***	***A***	***1.2***	***A***	***9.0***	***A***
ANOVA F-values										
Crop			ns		ns		**0.0312**		ns	
Planting date			**<0.0001**		**<0.0001**		**0.0347**		**<0.0001**	
Crop* Planting date			ns		ns		ns		ns	
Seeding rate			**0.0002**		**<0.0001**		**0.0010**		**0.0002**	
Seeding rate * Crop			ns		ns		ns		ns	
Seeding rate * Planting date			ns		ns		ns		**0.0466**	
[2]Emergence			ns		ns		ns		ns	

[1]LSMeans for main factors Crop*SeedTiming, LSMeans followed by the same lower case letter are not significantly different, upper case letters indicate significance between main effects (P< 0.05)

[2]Emergence density of the volunteer wheat was included in the ANOVA as a covariate when significant (P<0.05), when not significant it was remove

Table 3.8. Agronomic factor effects on survivorship and viability of volunteer wheat that emerged after the in-crop herbicide (POSTSP) in 2006.

Crop	Planting date	Seeding rate	Emerged volunteers		Mortality		Total seeds		Kernel Wt.		Viable seeds	
		Plants m^{-2}	— m^{-2} —	% total[3]	— % —		— m^{-2} —		— mg —		— % —	
Wheat	Early	0	88.3 a	43.9	21.0 a		311.1 a		23.1 a		98.1 a	
Wheat	Early	150	73.8 a	49.5	56.3 a		17.3 b		21.3 a		98.9 a	
Wheat	Early	250	94.5 a	55.4	55.6 a		28.9 b		18.7 a		99.4 a	
Wheat	Early	350	68.6 a	45.3	66.3 a		8.9 b		22.6 a		100.0 a	
[1]Main Effect Means			***81.3 A***	***48.5***	***49.8 AB***		***91.6 B***		***21.5 A***		***99.2 A***	
Wheat	Late	0	64.8 a	36.1	18.4 a		427.6 a		27.6 a		98.5 a	
Wheat	Late	150	55.0 a	41.9	40.6 a		93.3 b		23.7 a		97.4 a	
Wheat	Late	250	57.0 a	42.1	42.8 a		138.8 b		25.1 a		98.5 a	
Wheat	Late	350	63.8 a	39.9	33.8 a		57.3 b		20.0 a		96.9 a	
[1]Main Effect Means			**60.2 *A***	**40.0**	***33.9 B***		***179.3 A***		***24.1 A***		***97.8 A***	
Barley	Early	0	88.3 a	43.9	21.0 a		311.1 a		22.7 a		98.1 a	
Barley	Early	150	67.1 a	40.2	65.1 b		14.0 b		18.2 a		91.6 a	
Barley	Early	250	39.3 a	36.3	80.1 b		61.8 b		26.1 a		94.9 a	
Barley	Early	350	29.7 a	27.8	93.7 b		17.1 b		19.9 a		100.0 a	
[1]Main Effect Means			**56.1 *A***	**37.0**	***65.0 A***		***101.0 B***		***21.7 A***		***96.5 A***	
Barley	Late	0	64.8 a	36.1	18.4 a		427.6 a		23.0 a		98.5 a	
Barley	Late	150	88.1 a	50.0	51.6 a		71.7 b		21.2 a		94.1 a	
Barley	Late	250	55.7 a	38.3	79.2 ab		79.2 b		22.7 a		93.8 a	
Barley	Late	350	59.4 a	42.7	68.9 b		27.5 b		24.3 a		96.5 a	
[1]Main Effect Means			**67.0** A	**41.8**	***54.5 AB***		***151.5 A***		***22.8 A***		***95.7 A***	
ANOVA F-values												
Crop			ns		**0.0322**		ns		ns		ns	
Planting date			ns		ns		**<0.0001**		ns		ns	
Crop* Planting date			ns		ns		ns		ns		ns	
Seeding rate			ns		**0.0010**		**0.0004**		ns		ns	
Seeding rate * Crop			ns		ns		ns		ns		ns	
Seeding rate * Planting rate			ns		**0.0153**		ns		ns		ns	
[2]Emergence			-		**<0.0001**		ns		ns		-	

[1]LSMeans for main factors Crop*SeedTiming, LSMeans followed by the same lower case letter are not significantly different, upper case letters indicate significance between main effects (P<0.05)

[2]Emergence density of the volunteer wheat was included in the ANOVA as a covariate when significant (P<0.05), when not significant it was removed

[3]Represents the percent of the total volunteers within the treatment that emerged at the respective sampling timing

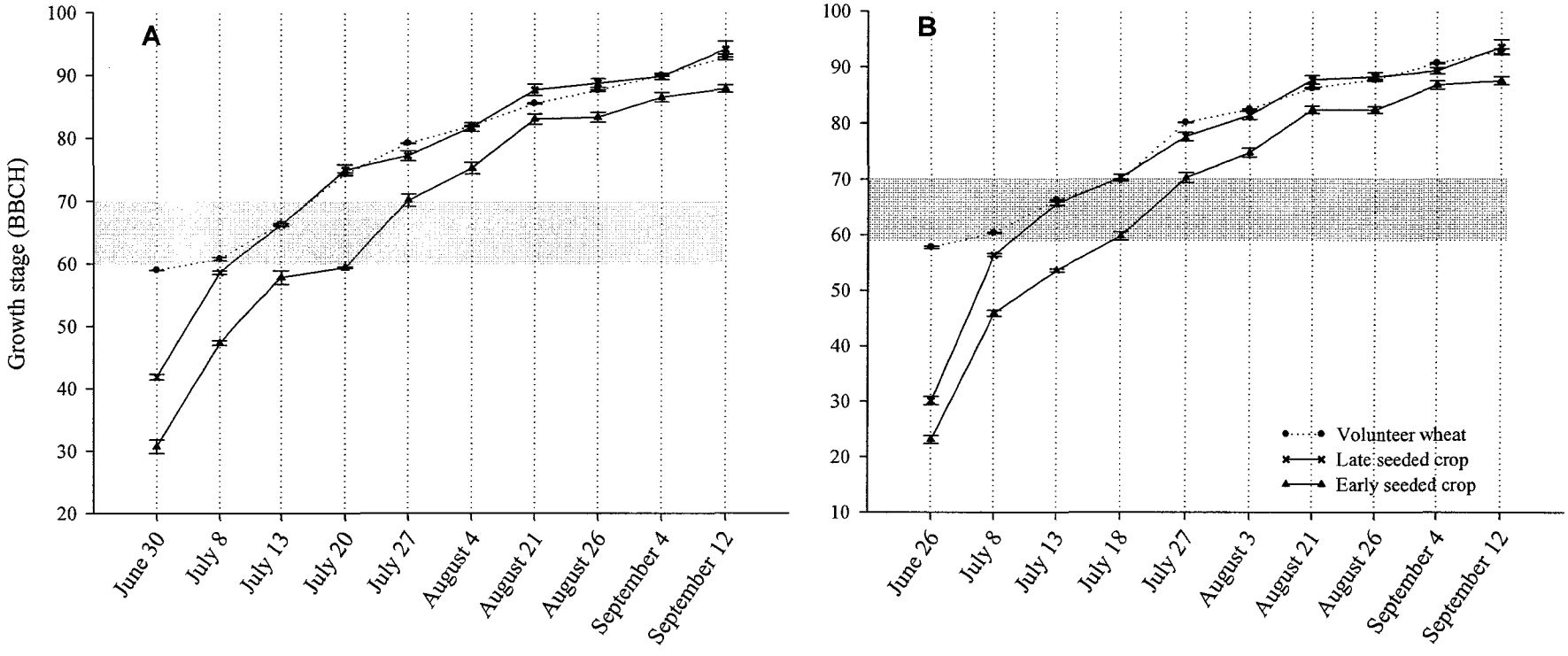

Figure 3.2. Growth stages (BBCH) of volunteers and seeded crops, both wheat and barley in 2005 at A) Home and B) East locations. Analysis was preformed by location because of different assessment days. The gray band indicates the period of anthesis

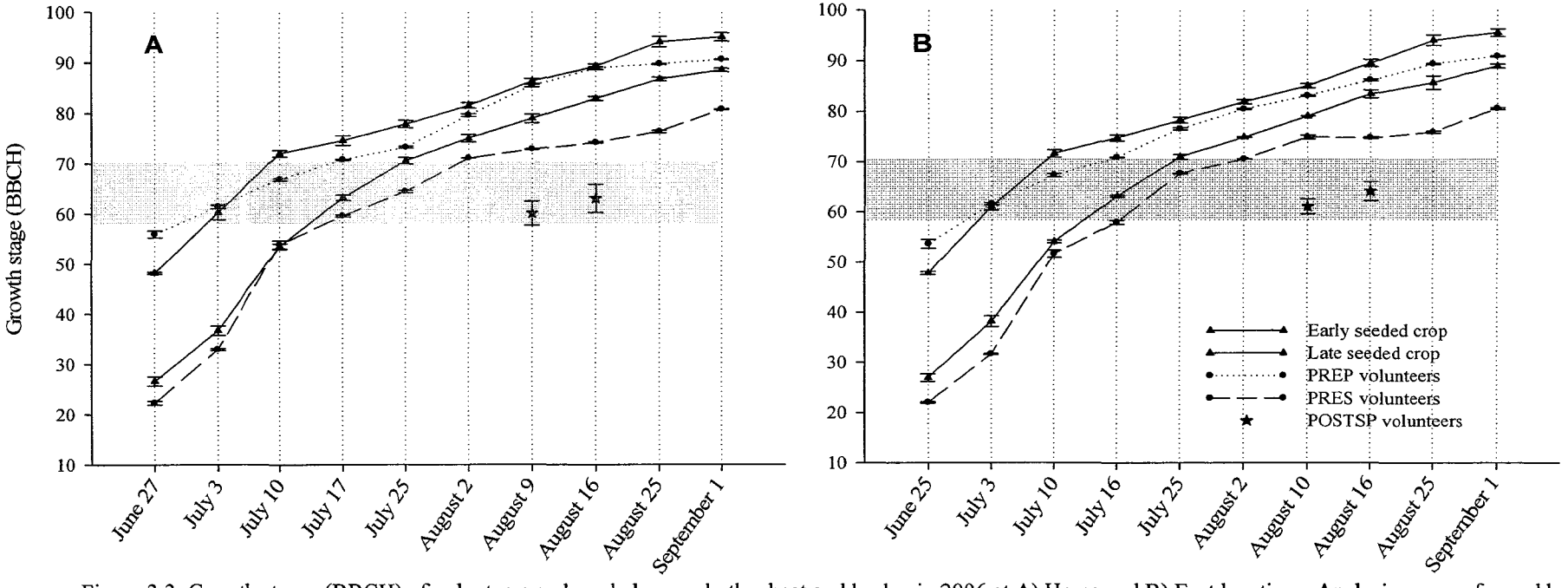

Figure 3.3. Growth stages (BBCH) of volunteers and seeded crops, both wheat and barley in 2006 at A) Home and B) East locations. Analysis was preformed by location because of different assessment days. The gray band indicates the period of anthesis

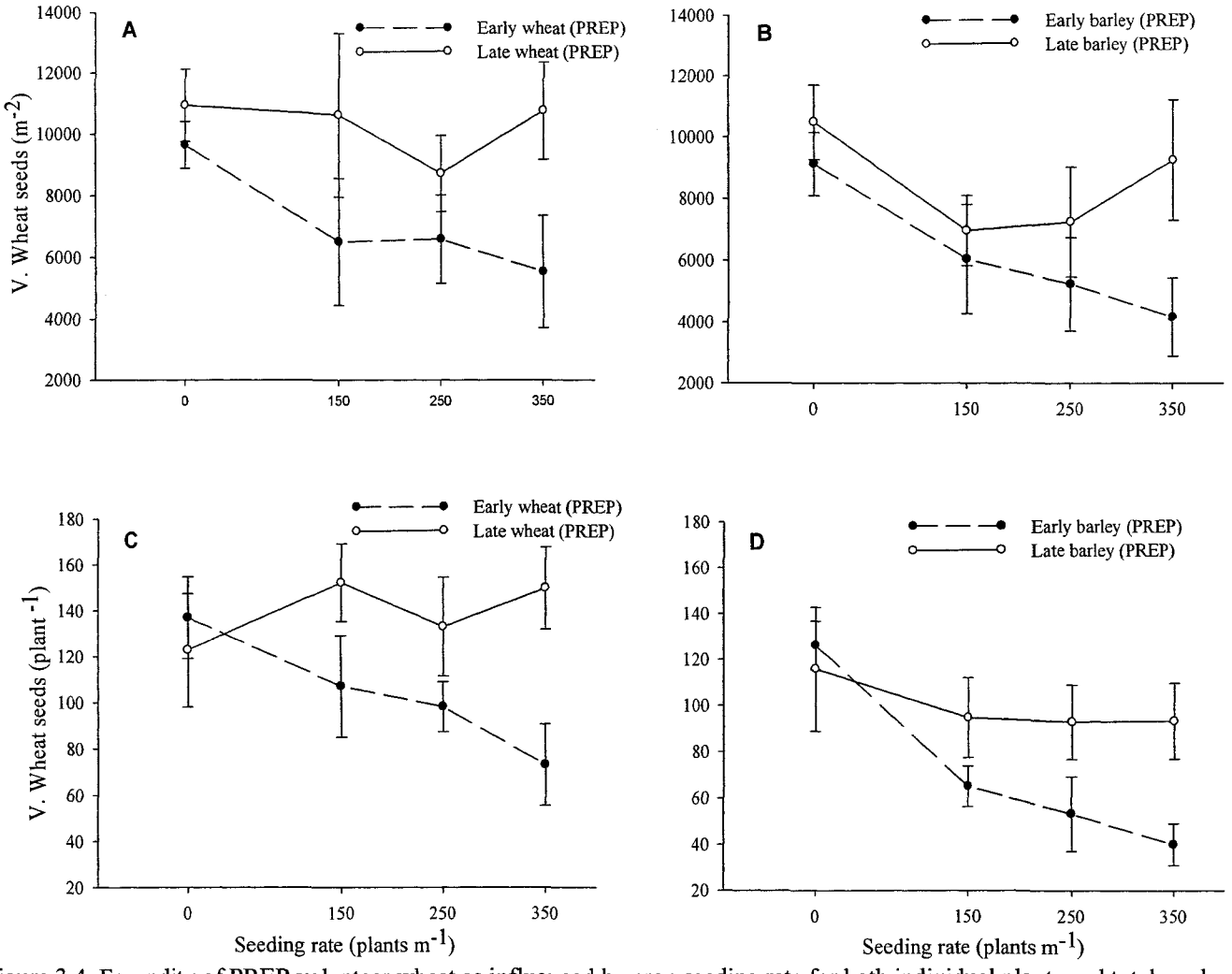

Figure 3.4. Fecundity of PREP volunteer wheat as influenced by crop seeding rate for both individual plants and total seed production m $^{-2}$, in 2005. A) Total seeds produced m $^{-2}$ in wheat B) total seeds produced m $^{-2}$ in barley C) seeds produced plant $^{-1}$ in wheat D) seeds produced plant^{-1} in barley

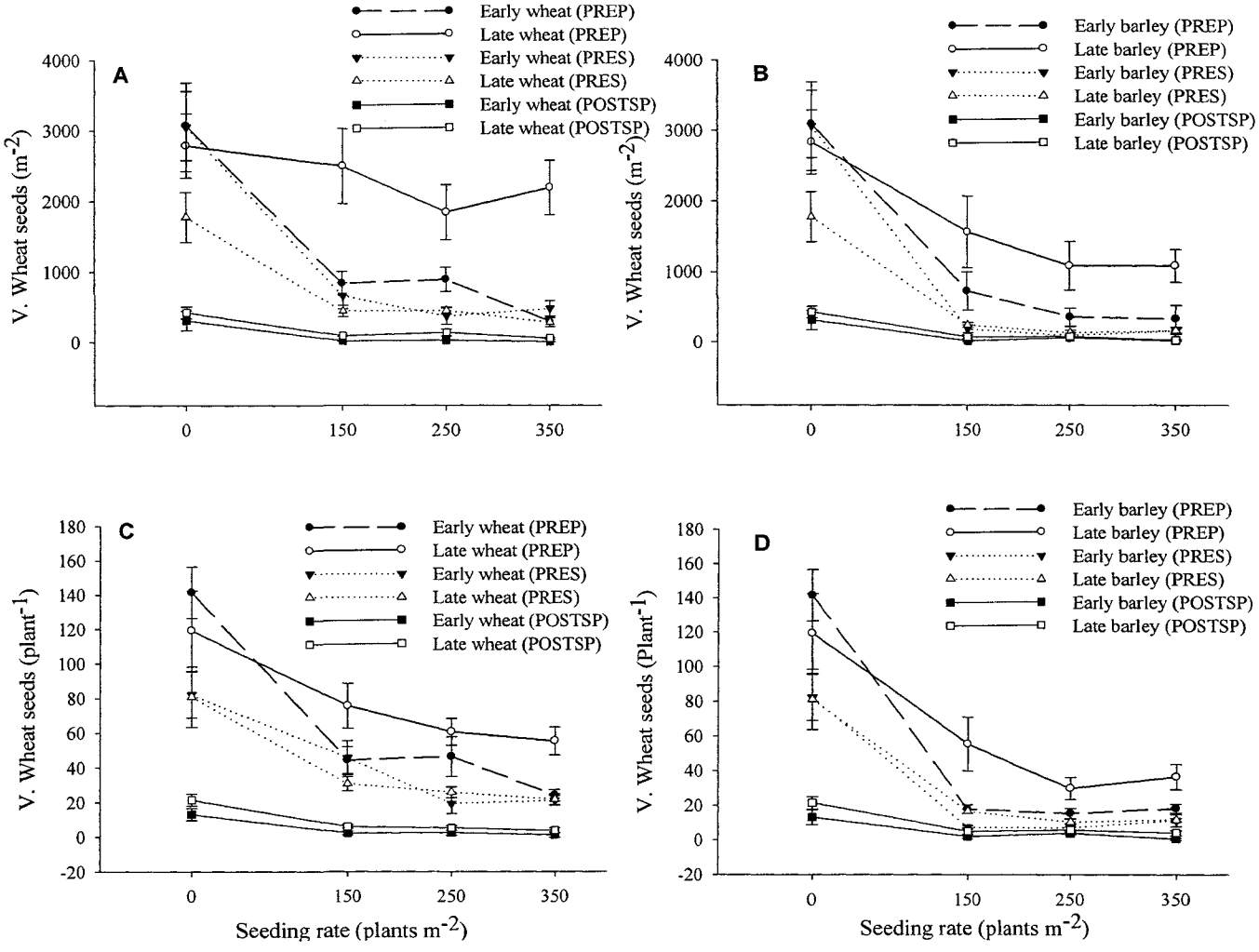

Figure 3.5. Fecundity of PREP, PRES and POSTSP volunteer wheat as influenced by crop seeding rate for individual plants and total seed production m^{-2} in 2006. A) Total seeds produced m^{-2} in wheat B) total seeds produced m^{-2} in barley C) seeds produced plant $^{-1}$ in wheat D) seeds produced plant $^{-1}$ in barley.

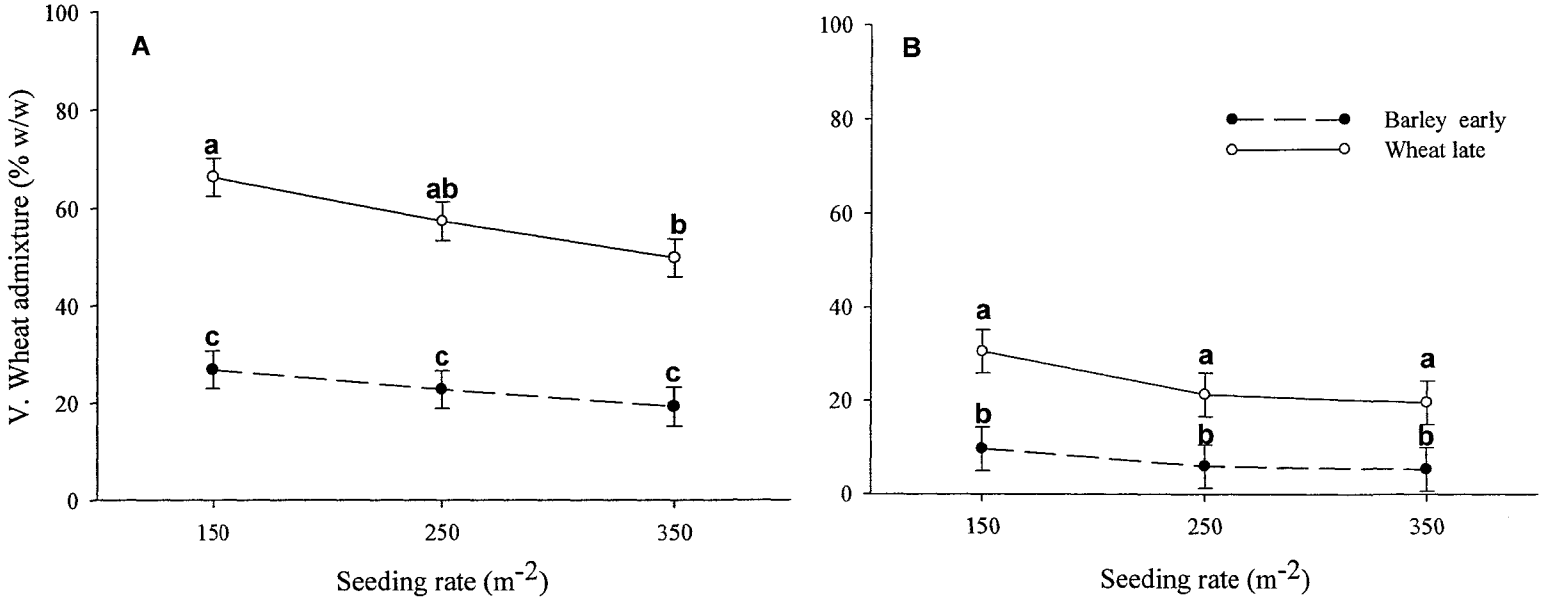

Figure 3.6. Admixed volunteer wheat seeds (%w/w) recovered from the harvested barley treatments in A) 2005 and B) 2006

Chapter 4

4.0 Summary

The Canadian Food Inspection Agency (CFIA), following a review of the environmental biosafety assessment, stated that glyphosate-resistant (GR) and imidazolinone-resistant (IMI) wheat would not present excessive risks compared to conventional varieties. Several biological and management aspects of wheat contributed to this decision. Wheat is predominantly a selfing species with pollen that is short lived and moves only short distances (Matus-Cádiz et al. 2007), seed banks commonly persist for < 3 years (Harker et al. 2005; De Corby et al. 2007), and volunteers can be controlled in crop rotations with herbicides (Rainbolt et al. 2004; Harker et al. 2005). The case of GR wheat in Canada is an example of where scientific evidence, market concerns and public opinions diverge. Regulation decisions based on the scientific risk assessment identified few concerns and did not withhold unconfined release of IMI herbicide-resistant (HR) wheat. Market acceptance and consumer confidence is not easy to predict because of the large number of factors that must be assessed. Potential market harm was a valid concern but was not answerable with an environmental risk assessment. Economic assessments of benefits and risk were required to address this concern. GM crops will continue to be controversial with consumers, particularly within the European Union, and consequently, commodity growers must be sensitive to these issues.

The purpose of this research was to generate data required for the development and increased sensitivity of a mechanistic demographic gene flow model. This thesis quantifies many aspects of the annual lifecycle of volunteer wheat (Figure 1.2); seeding emergence (Chapter 3); volunteer wheat control at pre-seeding and in-crop herbicide application timings (Chapter 2); volunteer wheat competition and fecundity (Chapter 3); pollen mediated gene flow (Chapter 3); admixture at harvest; and seed mediated gene flow (Chapter 2 and 3).

4.1 Objectives of the thesis research

The objective of this thesis was to create scenarios that would contribute key information on the intrinsic (biological) and extrinsic (management) factors affecting volunteer wheat. While much is understood about wheat as a crop, comparatively little is understood about the biology of wheat as a weed in subsequent crops. Management strategies in follow crops are also key data requirements. Controlling volunteer wheat the year after production is critical to preventing wheat seed bank inputs and adventitious presence in harvested crops contributions by volunteers (Harker et al. 2005). This is commonly achieved in canola or peas and includes the use of pre-seeding and in-crop herbicides. Therefore, experiments were conducted primarily in canola and peas crops using herbicides from widely used and effective herbicides in groups 1, 2 and 10 (Mallory Smith and Retzinger 2003).

The objectives of these studies were:

1. To quantify the key biological components of volunteer wheat:
    a. Fecundity in the presence and absence of herbicides within typical follow crops
    b. Seed bank persistence (not included)

2. To investigate integrated weed management practices include both culture and herbicidal controls. For cereal crops were volunteer wheat in-crop herbicide control is limited, the use of cultural cropping practices to improve crop competitiveness may be effective in reducing both seed and pollen mediated gene flow. The objectives were:
    a. Quantify the effect of crop competition on volunteer wheat fecundity
    b. Quantify volunteer wheat fecundity and mortality in the absence of herbicides
    c. Investigate the most effective crop competition tool to reduce volunteer wheat seed and pollen mediated gene flow.
3. To quantify herbicide control of GR volunteer wheat
    a. Document the control and survival of GR volunteer wheat after herbicide applications

b. Determine which application timing, pre-seeding or in-crop herbicides are more effective at reducing volunteer density and volunteer fecundity in canola and peas
c. Determine if the admixture potential of volunteer GR wheat decreases with increasing herbicide rates
d. Quantify the effect of herbicides on volunteer wheat kernel size and viability
e. Develop regression curves describing volunteer wheat control and fecundity
f. Determine the effects of herbicide treatments on volunteer wheat AP in two HR canola varieties were control options exist for GR wheat control.

4.2 Summary of experimental results

4.2.1 Effects of herbicide control on volunteer wheat fecundity

Study A

- Pre-seeding herbicide application is the critical and effective at reducing volunteer GR wheat densities. This was applicable for canola and peas (Figures 2.5 and 2.6). The greater in-crop efficacy of imazamox + imazethapyr was more effective at controlling GR wheat, but pre-seeding quizalofop-P was still important. Because the volunteer staging was earlier at the pre-seeding herbicide timing they are more susceptible to group 1 herbicides (Mallory Smith and Retzinger 2003).
- Volunteer wheat was more fecund in peas than in canola crops, presumably due to the relative competitive ability of the two crops.
- Volunteer GR wheat that survived pre-seeding quizalofop-P herbicide applications were still highly fecund when not controlled in-crop.
- Volunteer GR wheat that was not controlled with quizalofop-P pre-seeding was less likely to be controlled at the in-crop herbicide timing. In canola, 500 g ai ha^{-1} of glufosinate or 300 + 211 g ai ha^{-1} of glufosinate and sethoxydim was needed to reduce individual volunteer fecundity. The advanced growth stage of volunteers in this scenario resulted in poor control.

- For many parameters measured, the combination of pre-seeding and in-crop herbicide applications were the most effective at reducing volunteer densities and fecundity.
- Adventitious presence of GR wheat was variable and was only significantly reduced below 0.9% at one location for glufosinate resistant canola and not at any site for peas. AP was dependent on the yield of the crop and was presumably influenced by the crop competition.
- Seeded volunteers in this study were intended to represent a worst case scenario for modeling purposes; therefore, caution should be used in the interpretation and extrapolation of the data.

Study B

- Volunteer wheat control at the in-crop herbicide interval was less consistent with glufosinate that with imazamox + imazethapyr.
- The effective dose for 90% biomass reduction was achieved in 2005 and 2006 for glufosinate and imazamox + imazethapyr. Significant volunteer recovery was observed in 2006 for glufosinate as indicated with the effective dose required for a 90% reduction in volunteer seed production. This was not observed for imazamox + imazethapyr in either year.
- The adventitious presence of volunteer wheat was significantly ($P > 0.05$) $\leq 0.9\%$ the proposed European Union threshold at both sites in 2005 but at neither site in 2006 for glufosinate resistant canola. Imazamox + imazethapyr adventitious presence was significantly($P > 0.05$) $< 0.9\%$ at both sites in 2005 and 1 of 2 in 2006.

4.2.2 Effect of crop competition on volunteer wheat fecundity

- Volunteer wheat in the absence of herbicide control can be very competitive, reduce yields and cause high levels of adventitious presence in the harvested crop
- Cultural agronomic effects resulted in a significant reduction of volunteer wheat fecundity that emerged prior to the crop (PREP). In 2005, the yield component most significantly contributing to volunteer fecundity was the number of reproductive spikes plant^{-1}. The effect of competition reduced the number spikes plant^{-1} and therefore, the total fecundity of volunteers. Both spikes plant^{-1} and

seeds spike^{-1} was significantly reduced with increased crop competition for PREP volunteers, resulting in lower individual volunteer fecundity in 2006.

- Volunteer wheat fecundity decreased with later emerging volunteers.
- Viable seed was produced by volunteers at all emergence dates.
- Planting a more competitive crop such as barley, earlier and at high seeding rates was effective at reducing volunteer wheat fecundity.
- Volunteer wheat emergence was observed at all sampling intervals, with the highest emergence proportion occurring at the POSTSP interval.
- Mortality increased the later volunteer wheat emerged in the season. The agronomic treatments had a greater effect on mortality the later the volunteers emerged. Volunteer mortality was as high as 93.7% for POSTSP emerged volunteers in the early planted barley seeded at 350 plants m^{-2}.
- Only volunteer wheat emerging at the POSTSP interval would not flower synchronously with the seeded crops.

4.3 Research contributions

The research provides data that will contribute to the understanding of the biology and management of volunteer crops. It takes a novel approach to studying volunteer wheat fecundity. No studies to date have measured volunteer wheat in the absence of herbicide controls under a range of conditions. Data will be important as model parameters considering the biological potential of volunteer wheat under various levels of crop competition. The research has also quantifies the fecundity of volunteers following herbicide applications, an important modeling parameter previously unreported in the literature prior to this research.

Studies on gene flow in wheat and other crops have focused predominantly on pollen mediated gene flow. The research substantiates the importance of seed mediated gene flow, and the significance of management practices to limit seed production. Seed mediated gene flow is influenced by more factors than pollen mediated gene flow, is harder to confine, and has the potential to move transgenes over greater distances. The controversy and the ultimate decision to withdraw the registration of GR wheat in Canada

and the United States was a direct result of seed mediated gene flow that may have resulted in market access loss and economic harm to producers.

4.4 Future research

These data will be incorporated into a mechanistic gene flow model that will predict the outcomes of releasing GM crops. The release of these technologies into the environment is irreversible (Furtan et al. 2003); therefore, best management practices for coexistence of GM and non-GM crops will be investigated using a modeling approach. Further research using this model framework will reveal further data requirements to improve model sensitivity and validation; these may include:

1. Expand the volunteer wheat fecundity dose response experiment to include glufosinate tank mixes with more group 1 (Mallory Smith and Retzinger 2003) herbicides. The use patterns of these tank mixes has increased to over 50% with glufosinate-resistant canola growers (Woycheshin 2007). This includes sethoxydim, clethodim and quizalofop-P and can be used in both glufosinate- and glyphosate-resistant canola. A more expansive rate structure at the lower end of the recommended rates would provide a more complete data set that would more closely mimic spray misses and loss of volunteer control in the field. This would include more intervals below the 50% recommended rate. These data would provide a greater number of options and a more detailed understanding of the biological potential of volunteer wheat.
2. It has been reported in this thesis that volunteer wheat can have significantly smaller seed. The hypothesis that smaller seed size would result in shorter lived wheat seed banks has not been tested. There is a need to finish analyzing and publish the data collected on seed bank longevity between wheat varieties and seed size.
3. Collect an additional year of data for the volunteer wheat in cereals study to have a replicated year for the multiple sampling dates. Additional crops could be included to provide a greater range of crop competitiveness.

4. Conduct surveys of fields with high volunteer wheat densities and quantify the level of survival following herbicide applications. Grain sampling at harvest for volunteer wheat admixture would provide insights and validity to small plot research trials.
5. Conduct field competition studies between conventional and transgenic volunteer wheat to establish if differences exist. These studies, including a null segregant, would confirm the hypotheses that no difference exists between the two.

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Appendix A

A.1 Chapter 2 Abstract

Volunteer wheat fecundity: Contributions to a mechanistic agronomic model

Abstract

A mechanistic model is being developed to predict volunteer glyphosate-resistant (GR) wheat persistence, and potential admixture in western Canadian cropping rotations. Volunteer wheat fecundity (seed production plant^{-1}) is an important model parameter to accurately predict volunteer populations. Field trials were conducted in 2004 and 2005 near Edmonton, Alberta to investigate the effect of pre-seeding and in-crop herbicide applications and crop competition on volunteer wheat fecundity and density in Liberty Link canola and pea crops. GR volunteer wheat fecundity was greater than wheat grown as a crop in the absence of herbicides. GR volunteer wheat fecundity was reduced as herbicide rates increased. Pre-seeding herbicide application had a greater effect on volunteer densities, and in-crop herbicides had a greater effect on fecundity. Volunteer GR wheat seed admixture decreased as herbicide rates increased. Pre-seeding herbicide treatments alone had the greatest effect on volunteer admixture in Liberty Link canola. In peas, the in-crop herbicide alone had a greater effect on GR wheat admixture than the pre-seeding herbicide application alone. In both crops, the GR wheat admixture was the lowest when the high rates of pre-seeding and in-crop herbicides were combined. The data derived from these field trials will be used to develop a wheat fecundity sub-model to more accurately predict volunteer wheat persistence, seed bank longevity and the amount of admixture in crops.

A.2 Chapter 2 Tables

Table A.3. Analysis of soil properties at each research location for Study A.

Year	Location	pH	O M	Sand	Silt	Clay
				%		
2004	Ellerslie	5.9	11.8	27.0	49.9	23.1
2004	CDCN	6.0	9.6	42.1	38.4	19.5
2005	Ellerslie	5.7	11.5	26.4	49.7	23.9
2005	Calmar	6.5	11.6	19.4	51.4	29.2

Table A.4. Analysis of soil properties at each research location for study B.

Year	Location	pH	O M	Sand	Silt	Clay
				%		
2004	Ellerslie	6.1	11.2	22.8	52.7	24.4
2004	Edmonton	6.0	10.8	23.4	44.8	31.8
2005	Ellerslie	5.8	10	28.8	40.8	30.4
2005	Edmonton	5.7	12.5	26.2	39.4	34.4

Table A.5. Date and volunteer wheat grow stages for agronomic operations for Study A.

					Herbicide application		Vol. wheat stage at application[b]		Grain Harvest	
Location	Year	GPS location	Volunteer planting date	Crop planting date	Pre-seeding	In-crop	Pre-seeding	In-crop	Peas	Canola
Ellerslie, AB	2004	N 50° 38.836 W 113° 21.110	May 14	June 4	June 1	June 26	12 - 13	22-23	Oct 4	Oct 15
CDCN[c]	2004	N 53° 38.847 W113° 21.115	May 13	June 3	May 31	June 25	12 - 13	22-23	_[a]	Oct 15
Ellerslie, AB	2005	N 53° 25.018 W 113° 32.962	May 3	June 1	May 24	June 24	12	22-24	Oct 22	Oct 22
Calmar, AB	2005	N 53° 17.265 W 113° 52.831	May 3	June 1	May 24	June 24	12	22-24	Oct 18	Oct 18

[a] Data from peas at CDCN location was omitted
[b] Volunteer staged using the BBCH scale
[c] Crop Diversification Center North Alberta Agriculture, Food and Rural Development Edmonton, AB.

Table A.6. Date and volunteer wheat grow stages for agronomic operations for Study B.

Location	Year	Crop planting date	Herbicide application	Vol. wheat stage at application[a]	Grain harvest
Edmonton, AB	2005	May 12	June10*	13 - 14	Sept 23, 05
	2006	May 22	June 24	21 - 22	Sept 20, 06
Ellerslie, AB	2005	May 10	June 10	13 - 14	Sept 24, 05
	2006	May 22	June 22	21 - 22	Sept 20, 06

[a] Volunteer staged using the BBCH scale

Table A.7. Crop biomass and grain yield of glufosinate resistant canola in 2004 and 2005.

Treatment		Crop Biomass				Grain Yield			
Pre-Seed	In-Crop	2004		2005		2004		2005	
Glyphosate + Quizalofop-p-ethyl	Glufosinate + Sethoxydim	$g m^{-2}$				$t ha^{-1}$			
$g ai ha^{-1}$									
444 + 0	0 + 0	656.2	b	526.4	b	1.3	d	2.3	d
444 + 12	0 + 0	821.1	ab	1164.4	a	2.6	abc	4.2	abc
444 + 18	0 + 0	920.5	ab	1230.4	a	2.7	abc	4.8	a
444 + 24	0 + 0	900.3	ab	1205.5	a	2.5	abc	4.3	abc
444 + 0	300 + 0	732.4	ab	1023.0	a	2.0	bcd	3.6	c
444 + 12	300 + 0	1036.3	ab	1184.7	a	3.1	ab	4.7	a
444 + 18	300 + 0	979.9	ab	1136.7	a	2.5	abc	4.6	ab
444 + 24	300 + 0	1089.9	ab	1188.9	a	3.3	a	4.5	ab
444 + 0	500 + 0	727.3	ab	1029.2	a	2.0	cd	3.9	bc
444 + 12	500 + 0	1196.6	a	1219.0	a	3.3	a	4.6	ab
444 + 18	500 + 0	1077.8	ab	1139.1	a	3.4	a	4.5	ab
444 + 24	500 + 0	1094.2	ab	1205.9	a	2.9	abc	4.4	ab
444 + 0	300 + 211	754.0	ab	1125.6	a	2.1	bcd	4.1	abc
444 + 12	300 + 211	1074.1	ab	1219.6	a	2.9	abc	4.6	ab
444 + 18	300 + 211	971.2	ab	1206.0	a	3.0	ab	4.7	a
444 + 24	300 + 211	1040.1	ab	1260.8	a	3.1	ab	4.8	a
Preseed		**<0.0001**		**<0.0001**		**0.0003**		**<0.0001**	
Incrop		ns		**<0.0001**		**<0.0001**		**<0.0001**	
Preseed*Incrop		ns		**<0.0001**		ns		**<0.0001**	

Table A.8. Crop biomass and grain yield of peas in 2004 and 2005.

Treatment		Crop Biomass				Grain Yield			
Pre-Seed	In-Crop								
Glyphosate + Quizalofop-p-ethyl	Imazamox + Imazethapyr	2004		2005		2004		2005	
g ai ha^{-1}		$g\ m^{-2}$				$t\ ha^{-1}$			
444 + 0	0	162.3	b	380.1	d	1.6	c	1.5	d
444 + 12	0	468.1	a	1234.0	abc	3.4	ab	4.8	abc
444 + 18	0	493.8	a	1159.8	abc	3.5	ab	5.3	abc
444 + 24	0	633.5	a	1282.2	ab	3.4	ab	4.9	abc
444 + 0	14.7	497.6	a	881.9	c	2.9	bc	4.0	c
444 + 12	14.7	734.9	a	1300.3	ab	4.1	ab	5.6	ab
444 + 18	14.7	641.1	a	1419.6	ab	4.5	ab	5.7	a
444 + 24	14.7	648.6	a	1404.5	ab	4.4	ab	5.3	abc
444 + 0	22.5	554.0	a	1117.8	bc	3.6	ab	4.4	bc
444 + 12	22.5	762.1	a	1383.9	ab	4.2	ab	5.5	ab
444 + 18	22.5	733.6	a	1308.7	ab	4.4	ab	4.9	abc
444 + 24	22.5	660.3	a	1188.0	abc	3.4	a	5.7	a
444 + 0	29.4	678.9	a	1193.7	abc	3.8	ab	4.6	abc
444 + 12	29.4	599.5	a	1552.1	a	3.5	ab	5.1	abc
444 + 18	29.4	687.4	a	1330.5	ab	4.7	ab	5.3	abc
444 + 24	29.4	571.7	a	1499.4	ab	4.0	abc	5.0	abc
Preseed		**0.0002**		**<0.0001**		**<0.0001**		**<.0001**	
Incrop		**<0.0001**		**<0.0001**		**<0.0001**		**<.0001**	
Preseed*Incrop		**0.0023**		**<0.0001**		ns		**<.0001**	

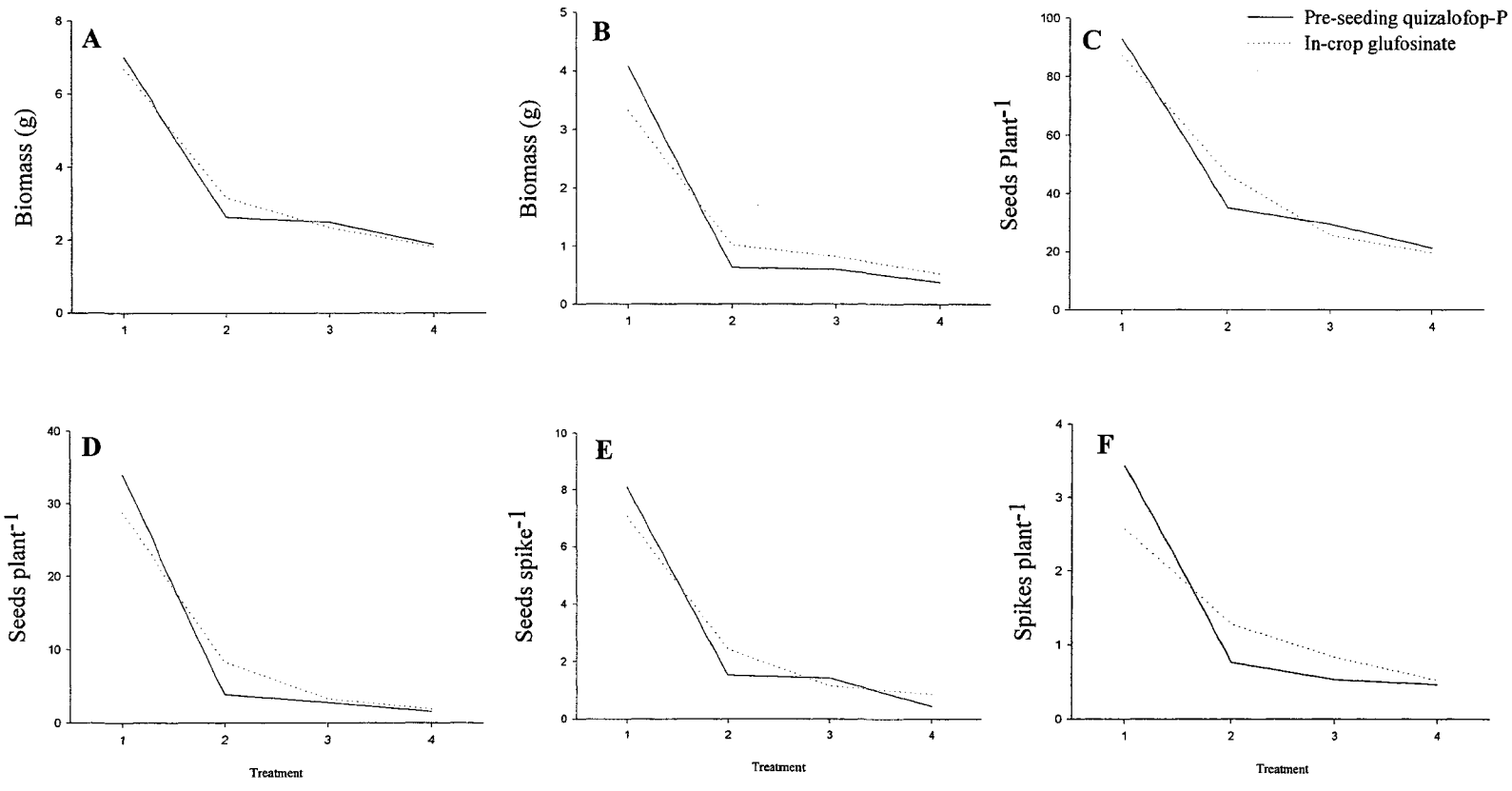

Figure A.2. Study A main effect means for pre-seeding and in-crop herbicide applications applied in glufosinate-resistant canola. Pre-seed treatments are 0, 12, 18 and 24 g ai ha^{-1} of quizalofop-P and in-crop treatments are 0 300, 500 g ai ha^{-1} of glufosinate and 300 + 211 g ai ha^{-1} of glufosinate + sethoxydim A) volunteer wheat biomass in 2004 B)) volunteer wheat biomass in 2005C) seeds $plant^{-1}$ in 2004 D) seeds $plant^{-1}$ in 2005 E) seeds $spike^{-1}$ in 2005 F) spikes $plant^{-1}$ in 2005

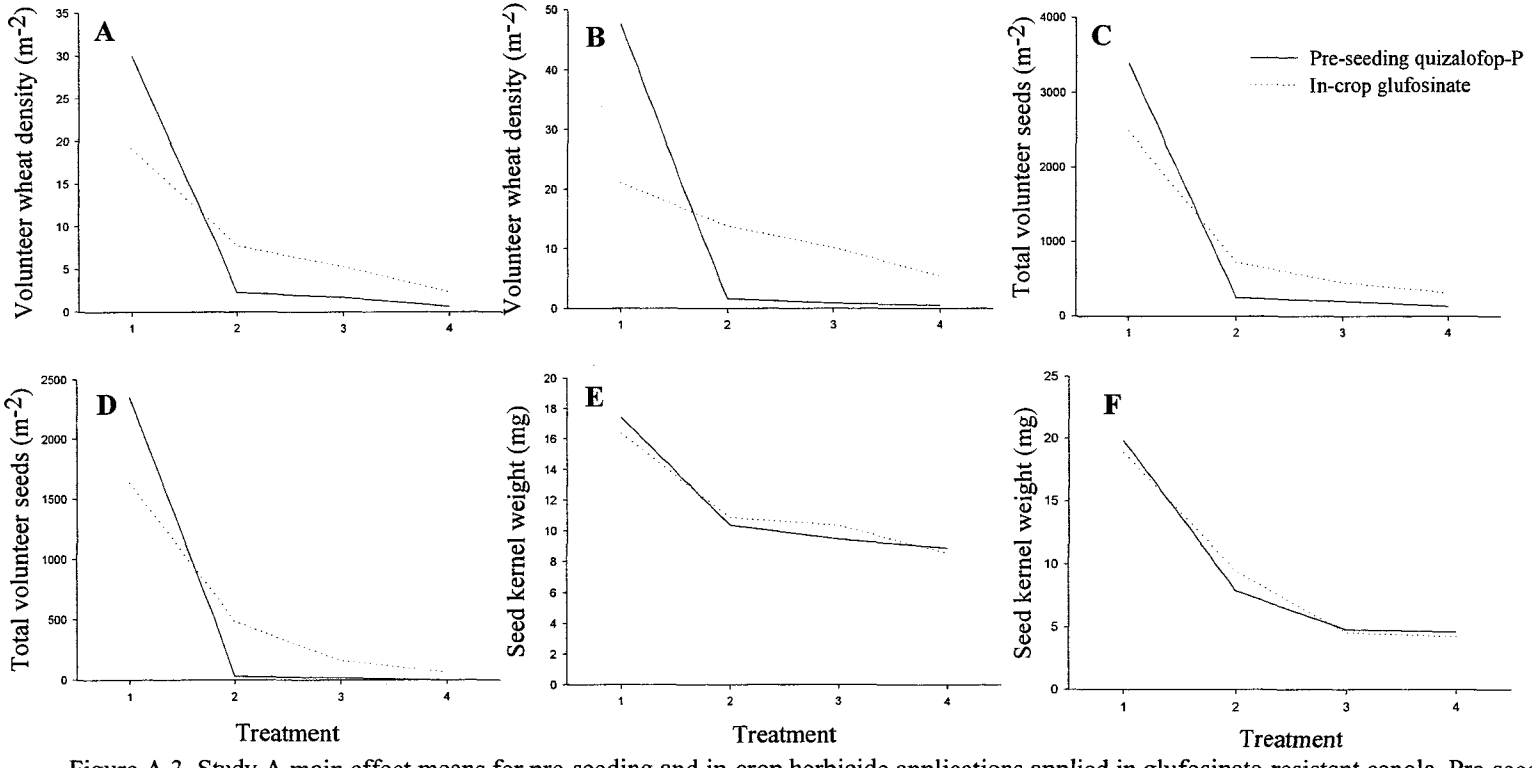

Figure A.3. Study A main effect means for pre-seeding and in-crop herbicide applications applied in glufosinate-resistant canola. Pre-seed treatments are 0, 12, 18 and 24 g ai ha^{-1} of quizalofop-P and in-crop treatments are 0 300, 500 g ai ha^{-1} of glufosinate and 300 + 211 g ai ha^{-1} of glufosinate + sethoxydim A) volunteer density at harvest in 2004 B)) volunteer density at harvest in 2005 C) total volunteer seed production in 2004 D) total volunteer seed production in 2005 E) volunteer kernel weight in 2004 F) volunteer kernel weight in 2005

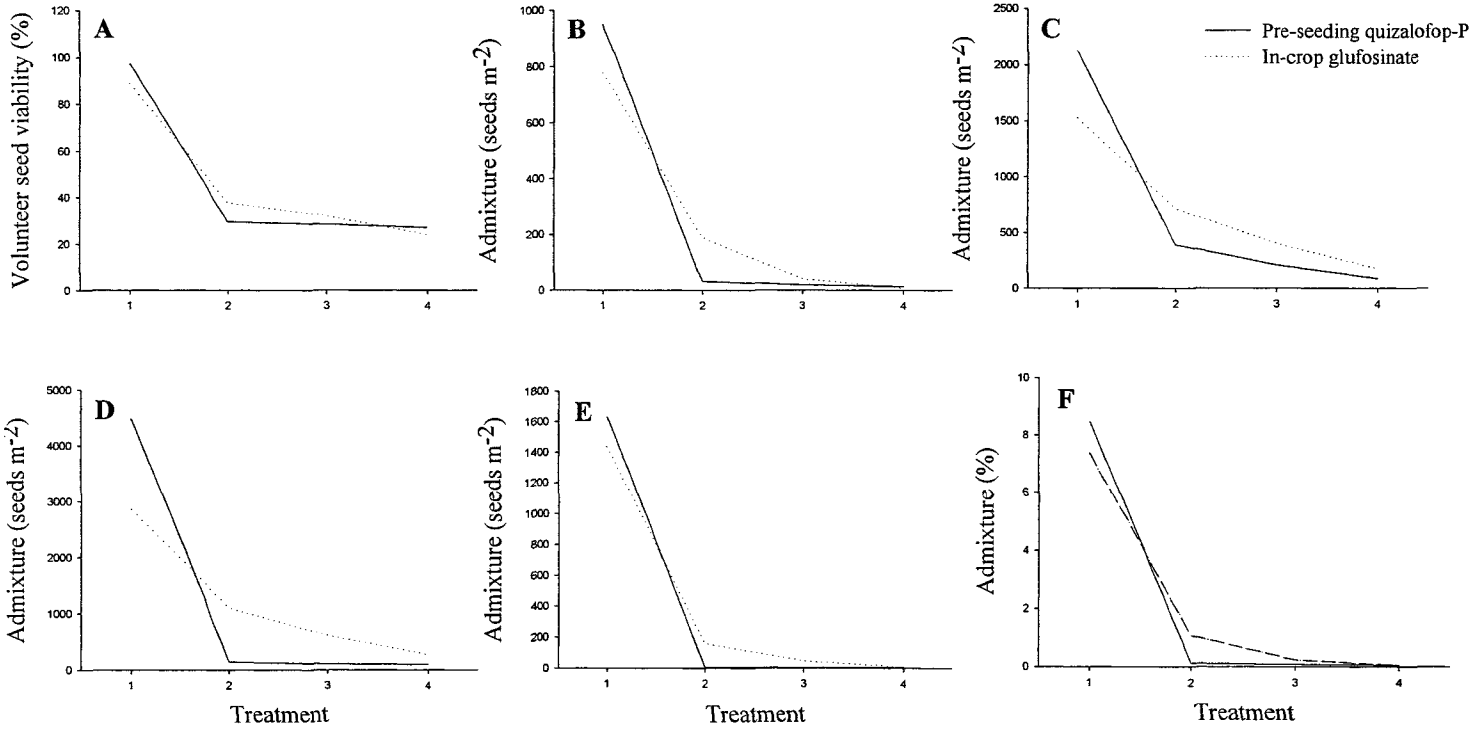

Figure A.4. Study A main effect for pre-seeding and in-crop herbicide applications applied in glufosinate-resistant canola. Pre-seed treatments are 0, 12, 18 and 24 g ai ha^{-1} of quizalofop-P and in-crop treatments are 0 300, 500 g ai ha^{-1} of glufosinate and 300 + 211 g ai ha^{-1} of glufosinate + sethoxydim A) viability of volunteer wheat seed in 2005 B) admixed volunteer wheat seeds recovered from combine harvested samples at Ellerslie in 2004 C) admixed volunteer wheat seeds recovered from combine harvested samples at CDCN in 2004 D) admixed volunteer wheat seeds recovered from combine harvested samples at Ellerslie in 2005 E) admixed volunteer wheat seeds recovered from combine harvested samples at Calmar in 2005 F) percent admixture of the recovered volunteer wheat seeds recovered from the harvested samples (w/w) at Ellerslie in 2004

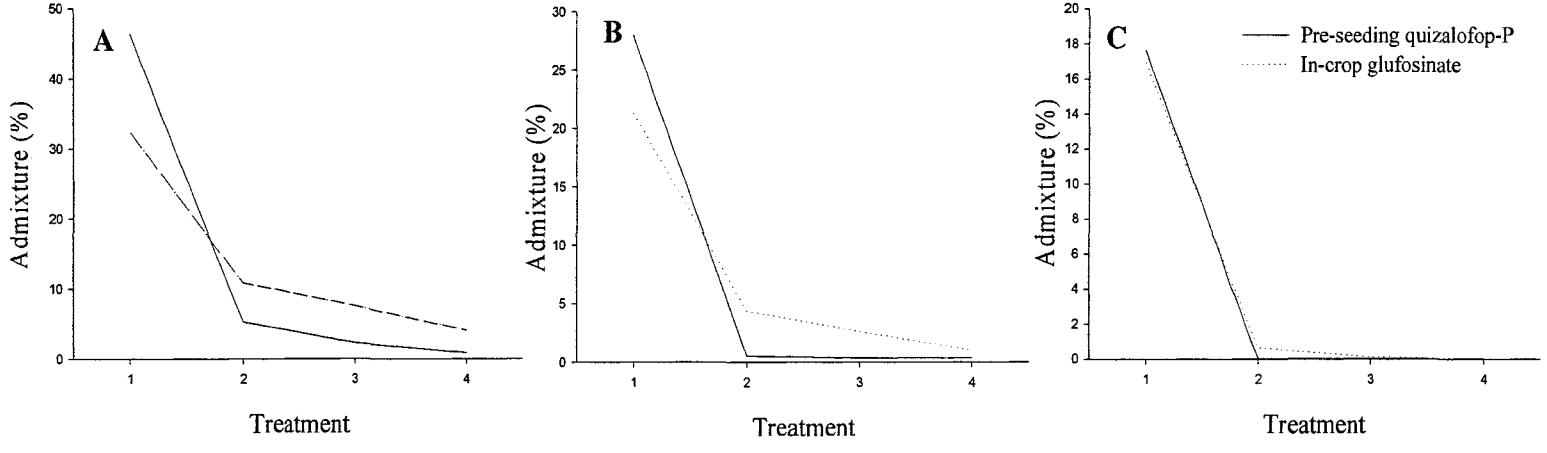

Figure A.5. Study A main effect means for pre-seeding and in-crop herbicide applications applied in glufosinate-resistant canola. Pre-seed treatments are 0, 12, 18 and 24 g ai ha^{-1} of quizalofop-P and in-crop treatments are 0 300, 500 g ai ha^{-1} of glufosinate and 300 + 211 g ai ha^{-1} of glufosinate + sethoxydim A) percent admixture of the recovered volunteer wheat seeds recovered from the harvested samples (w/w) at CDCN in 2004 B) percent admixture of the recovered volunteer wheat seeds recovered from the harvested samples (w/w) at Ellerslie in 2005 C) percent admixture of the recovered volunteer wheat seeds recovered from the harvested samples (w/w) at Calmar in 2005.

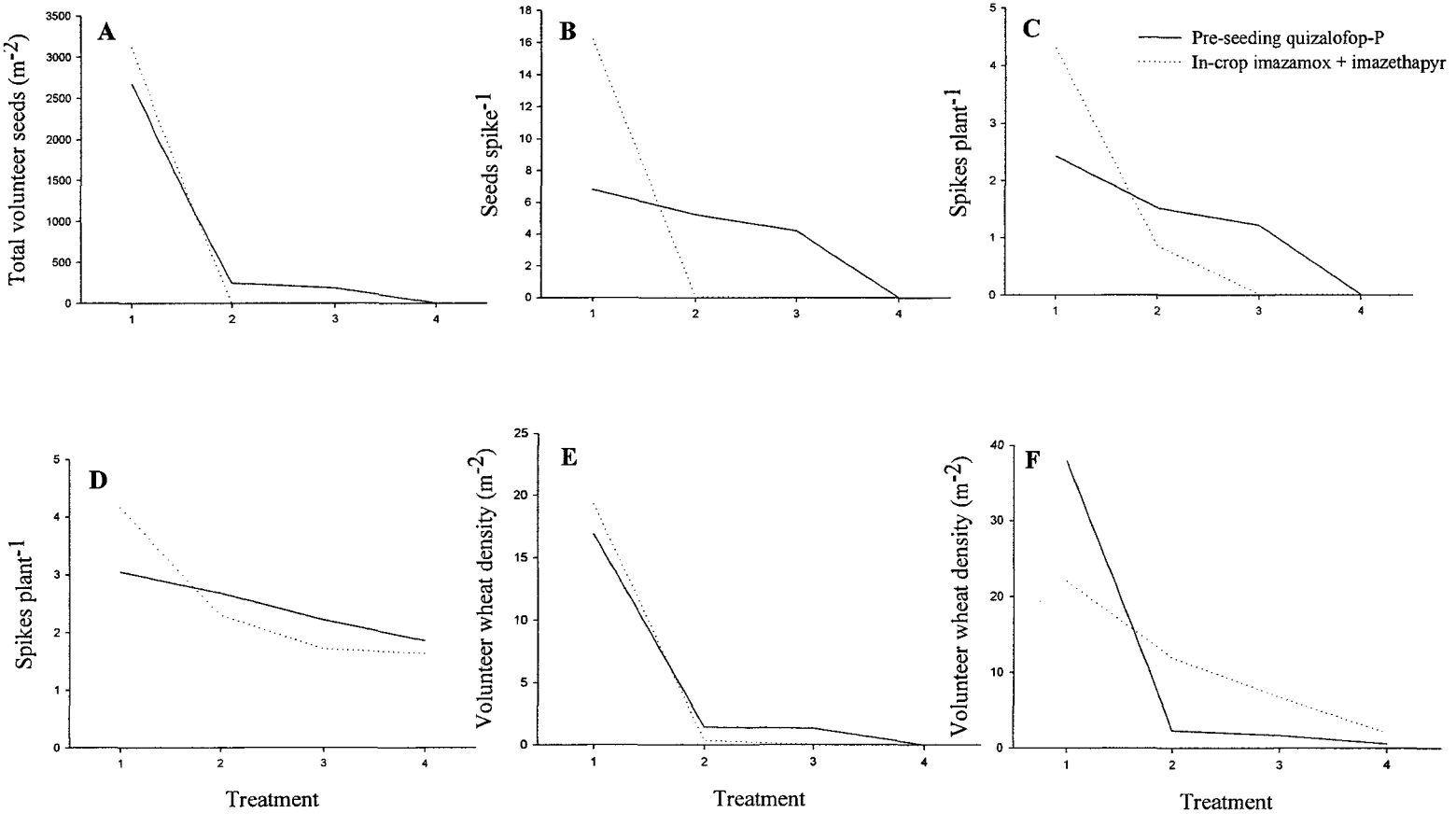

Figure A.6. Study A main effect means for pre-seeding and in-crop herbicide applications applied in peas. Pre-seed treatments are 0, 12, 18 and 24 g ai ha^{-1} of quizalofop-P and in-crop treatments are 0 14.7, 22.5, 29.4 g ai ha^{-1} of imazamox + imazethapyr. A) total volunteers seeds produced in 2004 B) seeds $spike^{-1}$ in 2004 C) spikes $plant^{-1}$ in 2004 D) spikes $plant^{-1}$ in 2005 E) volunteer wheat density in 2004 F) volunteer wheat density in 2005.

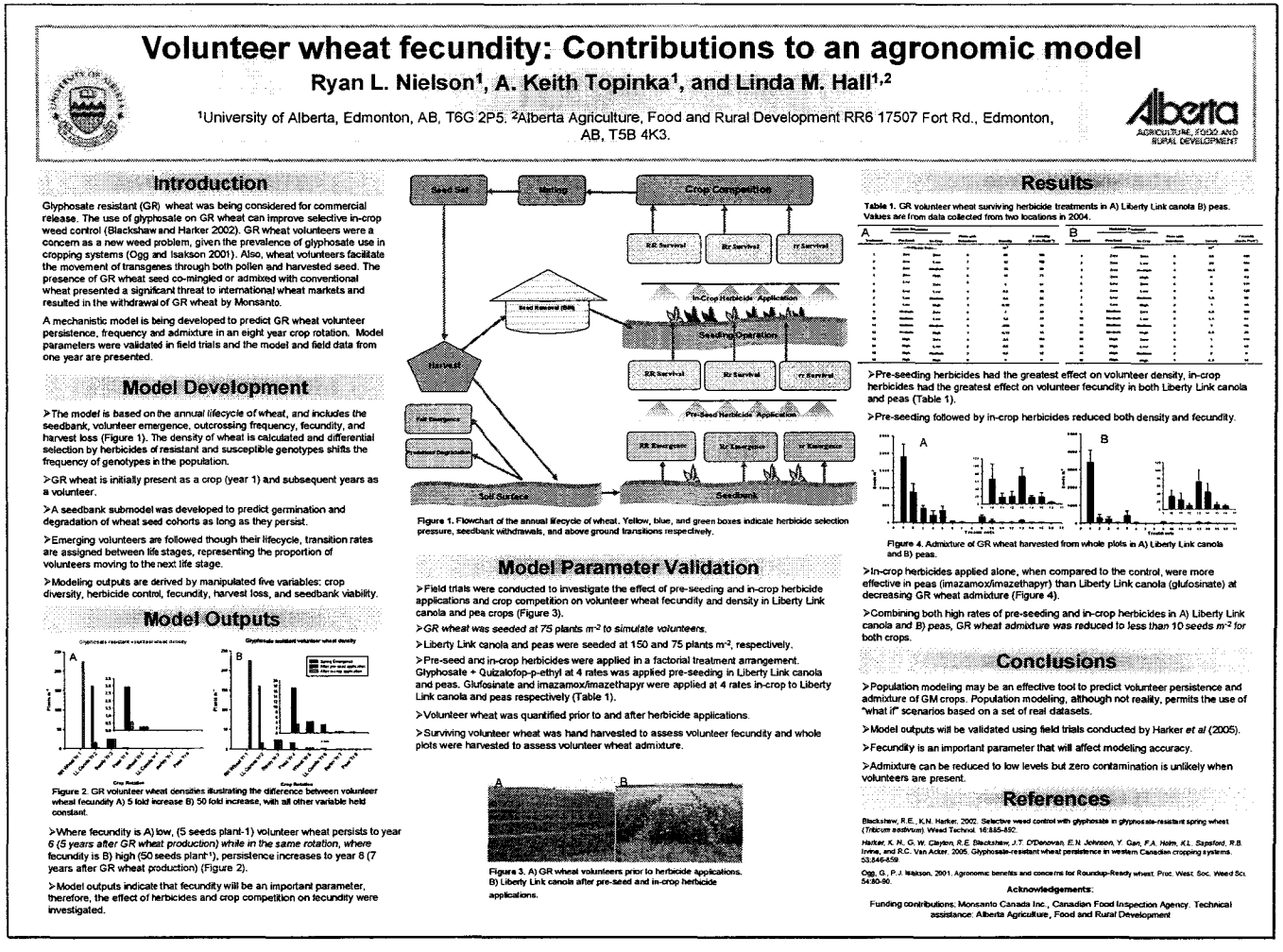

Figure A.9. Poster presented at Farmtech 2006 in Edmonton, AB.

Table A.7.Study B Volunteer wheat visual phytotoxicity ratings for glufosinate at Edmonton and Ellerslie research stations in 2005 and 2006.

Treatment	2005				2006			
	Low Seed Rate[1]		High Seed Rate[1]		Low Seed Rate[1]		High Seed Rate[1]	
Glufosinate	14 DAT	21 DAT	14 DAT	21 DAT	14 DAT	21 DAT	14 DAT	21 DAT
g ai ha^{-1}	%		%		%		%	
0	0.0 (4.7)	0.0 (5.4)	0.0 (3.6)	0.0 (3.3)	0.0 (3.4)	0.0 (2.2)	0.0 (3.1)	0.0 (2.5)
100	29.4 (4.7)	45.6 (5.4)	35.0 (3.6)	53.8 (3.3)	13.8 (3.4)	16.3 (2.2)	13.1 (3.1)	13.6 (2.5)
200	60.6 (4.7)	66.3 (5.4)	66.9 (3.6)	73.1 (3.3)	33.1 (3.4)	34.4 (2.2)	36.3 (3.1)	15.0 (2.5)
300	74.4 (4.7)	78.8 (5.4)	78.5 (3.6)	82.5 (3.3)	63.8 (3.4)	67.9 (2.2)	62.5 (3.1)	38.1 (2.5)
350	76.9 (4.7)	83.8 (5.4)	81.3 (3.6)	86.9 (3.3)	70.0 (3.4)	70.6 (2.2)	71.3 (3.1)	65.0 (2.5)
400	69.4 (4.7)	73.8 (5.4)	87.5 (3.6)	90.5 (3.3)	74.4 (3.4)	72.0 (2.2)	75.0 (3.1)	69.1 (2.5)
500	86.3 (4.7)	91.4 (5.4)	90.0 (3.6)	94.5 (3.3)	79.4 (3.4)	79.1 (2.2)	79.4 (3.1)	76.5 (2.5)

Control ratings are: 0-no control, 80-commercially acceptable, 90-excellent and 100%-total control.
[1]Low and High target seeding rates = 75 and 150 plants m^{-2} respectively.

Table A.8. Study B Volunteer wheat visual phytotoxicity ratings for imazamox + imazethapyr at Edmonton and Ellerslie research stations in 2005 and 2006.

Treatment	2005								2006							
	Low Seed Rate[1]				High Seed Rate[1]				Low Seed Rate[1]				High Seed Rate[1]			
Imazamox + Imazethapyr	14 DAT		21 DAT		14 DAT		21 DAT		14 DAT		21 DAT		14 DAT		21 DAT	
g ai ha^{-1}	%								%							
0	0.0	(1.3)	0.0	(1.5)	0.0	(1.4)	0.0	(1.2)	0.0	(2.1)	0.0	(1.5)	0.0	(1.4)	0.0	(1.1)
7.35	31.9	(1.3)	77.5	(1.5)	42.5	(1.4)	74.4	(1.2)	46.3	(2.1)	53.8	(1.5)	50.6	(1.4)	69.4	(1.1)
14.7	50.6	(1.3)	86.9	(1.5)	49.4	(1.4)	86.3	(1.2)	61.3	(2.1)	71.6	(1.5)	59.1	(1.4)	76.3	(1.1)
22	54.4	(1.3)	91.3	(1.5)	55.0	(1.4)	90.4	(1.2)	69.8	(2.1)	78.8	(1.5)	63.1	(1.4)	81.3	(1.1)
25	67.5	(1.3)	95.0	(1.5)	60.0	(1.4)	93.1	(1.2)	73.8	(2.1)	80.6	(1.5)	67.5	(1.4)	82.5	(1.1)
29.4	66.3	(1.3)	95.0	(1.5)	68.1	(1.4)	95.8	(1.2)	76.0	(2.1)	86.3	(1.5)	71.9	(1.4)	86.0	(1.1)
36.75	65.0	(1.3)	94.4	(1.5)	66.3	(1.4)	95.8	(1.2)	78.8	(2.1)	87.5	(1.5)	73.8	(1.4)	90.0	(1.1)

Control ratings are: 0-no control, 80-commercially acceptable, 90-excellent and 100%-total control.
[1]Low and High target seeding rates = 75 and 150 plants m^{-2} respectively.

Table A.9. Biomass and grain yields from the dose response experiments (study B) in glufosinate-resistant canola in 2005 and 2006

	Crop Biomass				Grain Yield			
Glufosinate	2005		2006		2005		2006	
— g ai ha^{-1} —	— $g\ m^{-2}$ —				— $t\ ha^{-1}$ —			
0	919.8	a	382.3	b	4.7	d	1.5	d
100	1001.9	a	457.0	ab	5.2	c	2.1	c
200	1046.6	a	549.0	ab	5.6	bc	2.6	b
300	1057.9	a	534.5	ab	5.9	ab	2.8	ab
350	1047.6	a	550.1	ab	6.1	a	2.6	b
400	1101.6	a	555.5	ab	6.3	a	3.2	a
500	1033.2	a	596.8	a	6.0	a	3.2	a
Herbicide Rate	**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Seeding Rate	ns		ns		ns		ns	

Table A.10 Biomass and grain yields from the dose response experiments (study B) in imidazolinone-resistant canola in 2005 and 2006

	Crop Biomass				Grain Yield			
Imazamox + Imazethapyr	2005		2006		2005		2006	
— g ai ha^{-1} —	— $g\ m^{-2}$ —				— $t\ ha^{-1}$ —			
0	651.1	a	236.0	b	3.1	b	1.0	d
7.35	894.6	a	418.0	ab	4.4	a	1.7	c
14.7	871.7	a	483.4	a	4.4	a	2.0	b
22	892.9	a	474.9	a	4.8	a	2.2	ab
25	780.9	a	439.2	a	4.7	a	2.2	ab
29.4	966.4	a	477.4	a	4.7	a	2.4	a
36.75	925.0	a	477.7	a	4.8	a	2.2	ab
Herbicide Rate	**<0.0001**		**<0.0001**		**<0.0001**		**<0.0001**	
Seeding Rate	ns		ns		ns		ns	

Appendix B

B.1 Chapter 3 Abstract

Influence of cereal crop competition on volunteer wheat (*Triticum aestivum* L.) fecundity.

Nielson, R.L[1] and L.M. Hall[1,2] [1]Department of Agriculture, Food and Nutritional Science, University of Alberta, Edmonton, AB. [2]Alberta Agriculture, Food and Rural Development (AAFRD) Edmonton, AB.

Wheat a major crop in western Canada and should genetically modified (GM) wheat be released, minimizing gene flow is important to reduce adventitious presence (AP) and maintain varietal purity. Controlling volunteer wheat with herbicides can minimize the movement of genes and AP. Currently, few herbicide options exist for the control of volunteer cereals in cereal crop rotations. Field studies were conducted to investigate the use of agronomic practices on the reduction of volunteer wheat fecundity in central Alberta in 2005 and 2006. In commercial fields containing volunteer wheat, barley and wheat crops were seeded at both early and late timings, with four seeding rates including a non-seeded check. Volunteer wheat plants were banded for later identification three times in the growing season and hand harvested at crop maturity to identify individual volunteer wheat survival, biomass and fecundity. Volunteer and crop anthesis synchronicity was recorded weekly throughout the growing season using the BBCH scale. Early emerging volunteer wheat fecundity (seeds plant $^{-1}$) and biomass was significantly reduced by seeding barley, a more competitive crop. For both wheat and barley crops the individual volunteer fecundity was significantly reduced by seeding the crop earlier. Later emerging volunteers had higher mortality and were less fecund. Increased seeding rates reduced volunteer fecundity over the unseeded checks and were most evident in barley. By controlling early emerging volunteers, volunteer seeds mediated gene flow would be reduced. Only late emerging volunteers did not flower synchronously with the seeded crops. The results from this study will make a significant contribution to gene flow modeling effort

B.2 Chapter 3 Tables

Table B.1. Agronomic operations and sampling dates in 2005.

Location	Crop	Seed timing	Seeding date	Crop emergence	Herbicide application	PREP Volunteers	Harvest
Calmar Home	Wheat	Early	May 10	May 22	June 15	May 19	Aug 29
	Barley		May 10	May 18	June 15	May 19	Aug 29
	Wheat	Late	May 26	June 3	June 15	June 6	Sept 5
	Barley		May 26	June 1	June 15	June 6	Sept 5
Calmar East	Wheat	Early	May 10	May 21	June 15	May 18	Aug 29
	Barley		May 10	May 18	June 15	May 18	Aug 29
	Wheat	Late	May 26	June 4	June 15	June 6	Sept 5
	Barley		May 26	June 1	June 15	June 6	Sept 5

Table B.2. Agronomic operations and sampling dates in 2006.

Location	Crop	Seed timing	Seeding date	Crop emergence	Herbicide application	PREP Volunteers	PRES Volunteers	POSTSP Volunteers	Harvest
Calmar Home	Wheat	Early	May 11	May 19	June 18	May 15	June 14	July 2	Aug 23
	Barley		May 11	May 17	June 18	May 15	June 14	July 2	Aug 23
	Wheat	Late	May 30	June 4	June 18	June 1	June 14	July 2	Sept 8
	Barley		May 30	June 5	June 18	June 1	June 14	July 2	Sept 8
Calmar East	Wheat	Early	May 11	May 20	June 18	May 15	June 14	July 2	Aug 23
	Barley		May 11	May 17	June 18	May 15	June 14	July 2	Aug 23
	Wheat	Late	May 30	June 7	June 18	June 1	June 14	July 2	Sept 8
	Barley		May 30	June 5	June 18	June 1	June 14	July 2	Sept 8

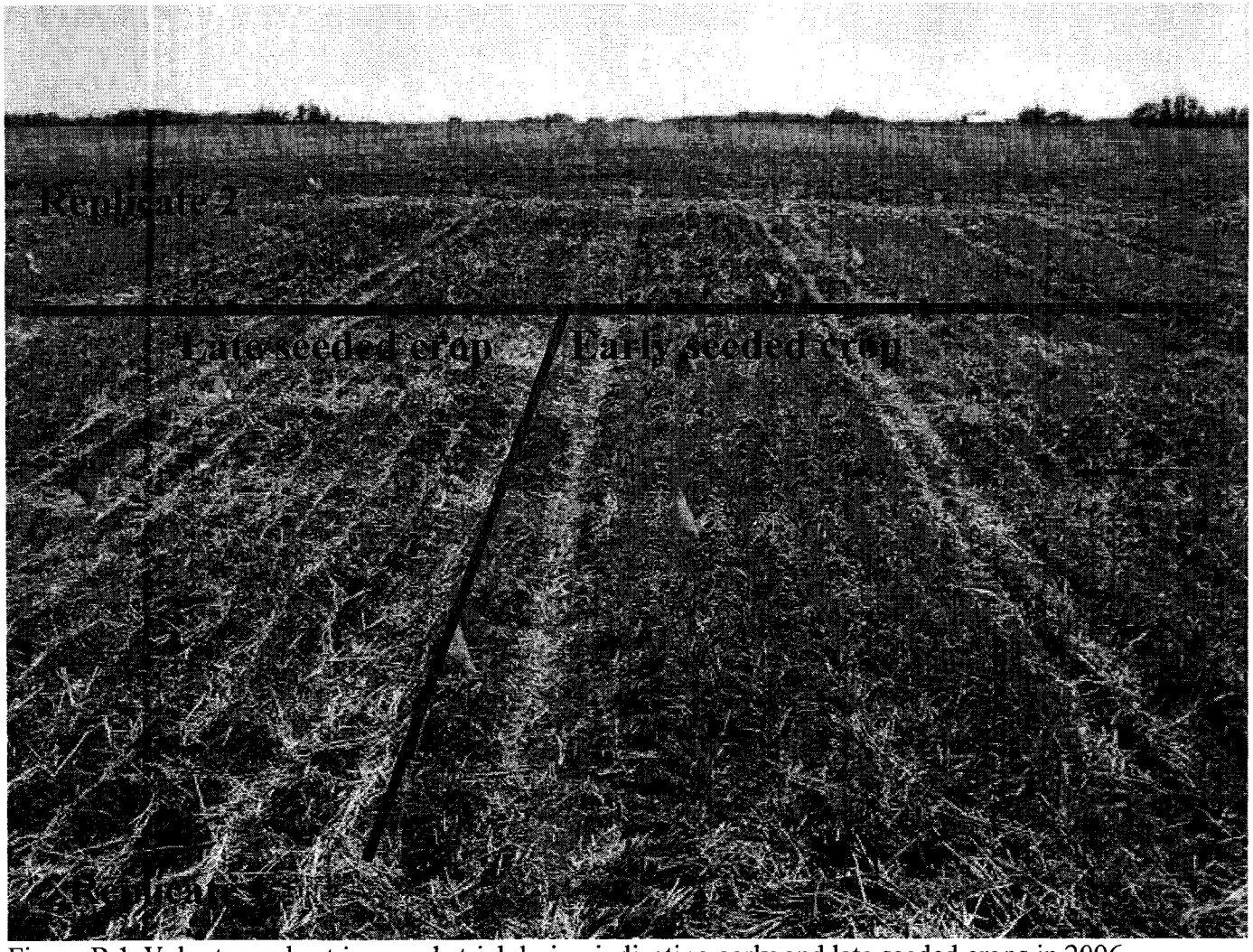

Figure B.1. Volunteer wheat in cereals trial design indicating early and late seeded crops in 2006.

Figure B.2 Volunteer wheat in cereals experiment with avian leg bands marking volunteer wheat

Table B.3. Crop biomass, grain yield and dockage of volunteer wheat in barley samples from two locations in 2005

Crop	Planting date[2]	Seeding Rate	Crop biomass		Crop yield	
		Plants m^{-2}	—— g m^{-2} ——		—— t ha^{-1} ——	
Wheat	Early	0	-[3]	-	-	-
Wheat	Early	150	619.1	a	6.0	a
Wheat	Early	250	623.0	a	6.0	a
Wheat	Early	350	640.3	a	5.7	a
[1]Main Effect Means			*627.5*	*B*	*5.9*	*A*
Wheat	Late	0	-	-	-	-
Wheat	Late	150	364.7	a	5.9	a
Wheat	Late	250	437.6	a	6.1	a
Wheat	Late	350	390.1	a	5.7	a
[1]Main Effect Means			***397.4***	***C***	***5.9***	***A***
Barley	Early	0	-	-	-	-
Barley	Early	150	695.6	a	6.4	a
Barley	Early	250	765.8	a	6.3	a
Barley	Early	350	800.7	a	6.1	a
[1]Main Effect Means			***754.0***	***A***	***6.3***	***A***
Barley	Late	0	-	-	-	-
Barley	Late	150	585.5	a	6.4	a
Barley	Late	250	544.7	a	6.5	a
Barley	Late	350	626.2	a	6.5	a
[1]Main Effect Means			***585.5***	***B***	***6.4***	***A***
ANOVA F-values						
Crop			**0.0343**		**0.0068**	
Seeding Date			ns		**0.0126**	
Crop* Seeding Date			ns		ns	
Seeding Rate			ns		**ns**	
Seeding Rate * Crop			ns		**ns**	
Seeding Rate * Seeding Date			ns		**ns**	
[1]Emergence			**<0.0001**		**ns**	

LSMeans for main factors Crop*SeedTiming, LSMeans within the main effects followed by the same letter are not significantly different (P> 0.05)

[1] Emergence density of the volunteer wheat was included in the ANOVA as a covariate when significant (P>0.005), when not significant it was removed

[2] Planting dates available in Tables B.1

[3] No yields were measured for the unseeded check

Table B.4. Crop biomass, grain yield and admixture of volunteer wheat in barley samples from two locations in 2006

Crop	Planting Date[2]	Seeding Rate	Crop biomass		Crop yield	
		Plants m^{-2}	—— g m^{-2} ——		—— t ha^{-1} ——	
Wheat	Early	0	-[3]	-	-	-
Wheat	Early	150	495.8	a	3.6	a
Wheat	Early	250	508.9	a	3.6	a
Wheat	Early	350	441.0	a	3.7	b
[1]Main Effect Means			***481.9***	***A***	***3.6***	***BC***
Wheat	Late	0	-	-	-	-
Wheat	Late	150	303.6	a	2.5	a
Wheat	Late	250	346.6	a	2.7	a
Wheat	Late	350	392.9	a	2.9	a
[1]Main Effect Means			***347.7***	***B***	***2.7***	***C***
Barley	Early	0	-	-	-	-
Barley	Early	150	534.9	a	4.6	a
Barley	Early	250	596.3	a	4.7	a
Barley	Early	350	625.6	a	5.0	a
[1]Main Effect Means			***585.6***	***A***	***4.8***	***A***
Barley	Late	0	-	-	-	-
Barley	Late	150	591.9	a	3.9	a
Barley	Late	250	573.7	a	3.9	a
Barley	Late	350	568.8	a	3.8	a
[1]Main Effect Means			***578.1***	***A***	***3.9***	***AB***
ANOVA F-values						
Crop			**0.0343**		**0.0068**	
Seeding Date			ns		**0.0126**	
Crop* Seeding Date			ns		ns	
Seeding Rate			ns		**ns**	
Seeding Rate * Crop			ns		**ns**	
Seeding Rate * Seeding Date			ns		**ns**	
[1]Emergence			**<0.0001**		**ns**	

LSMeans for main factors Crop*SeedTiming, LSMeans within the main effects followed by the same letter are not significantly different (P> 0.05)

[1]Emergence density of the volunteer wheat was included in the ANOVA as a covariate when significant (P>0.005), when not significant it was removed.

[2] Seeding dates available in Tables B2

[3] No yields were measured for the unseeded check

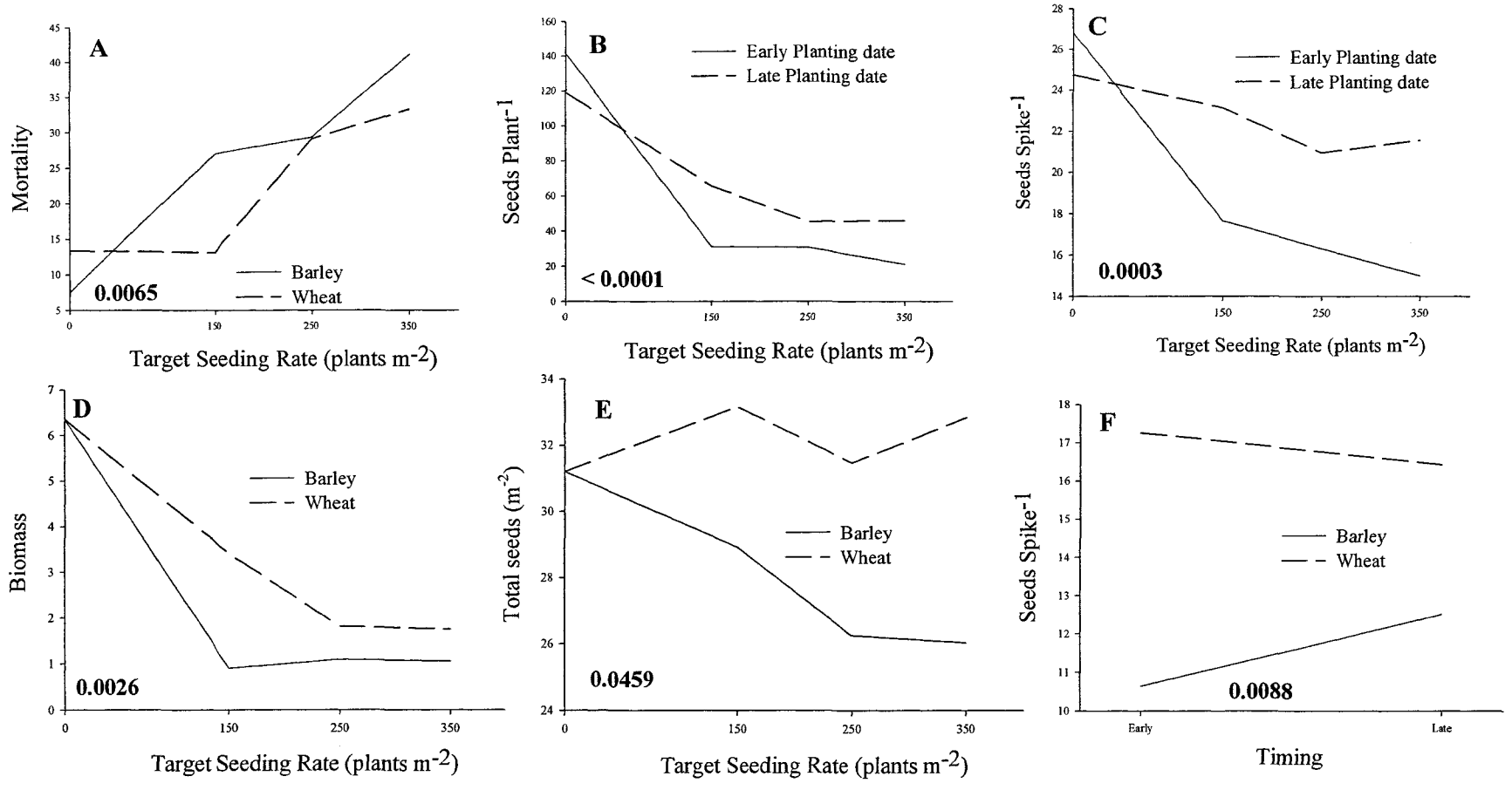

Figure B.3 Significant main effect means interactions for volunteer wheat in cereals trials in 2006 A) PRES mortality: Seeding rate*Crop B) PREP seeds plant^{-1}: Seeding rate*Planting date C) PREP seeds spike^{-1}: Seeding rate*Planting date D) PRES volunteer biomass: Seeding rate*Crop E) PRES total seed production: Seeding rate*Crop F) PRES seeds spike^{-1}: Crop*Planting date

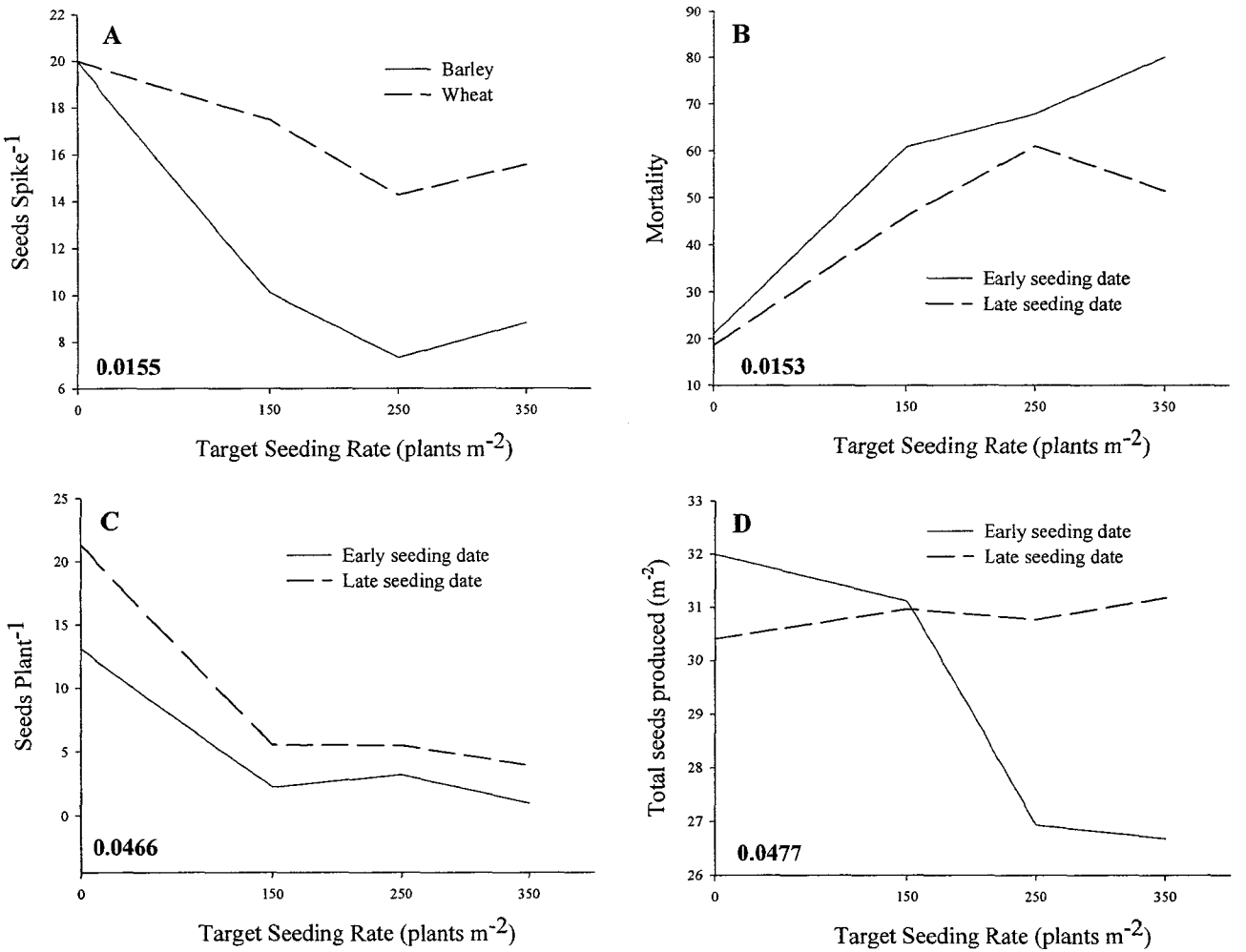

Figure B.4. Significant main effect means interactions for volunteer wheat in cereals trials in 2006 A) PRES seeds spike^{-1}: seed rate*crop B) POSTSP mortality: planting date*seeding rate C) POSTSP seeds plant^{-1}: planting date *seeding rate D) PRES total seed production: planting date*seeding rate

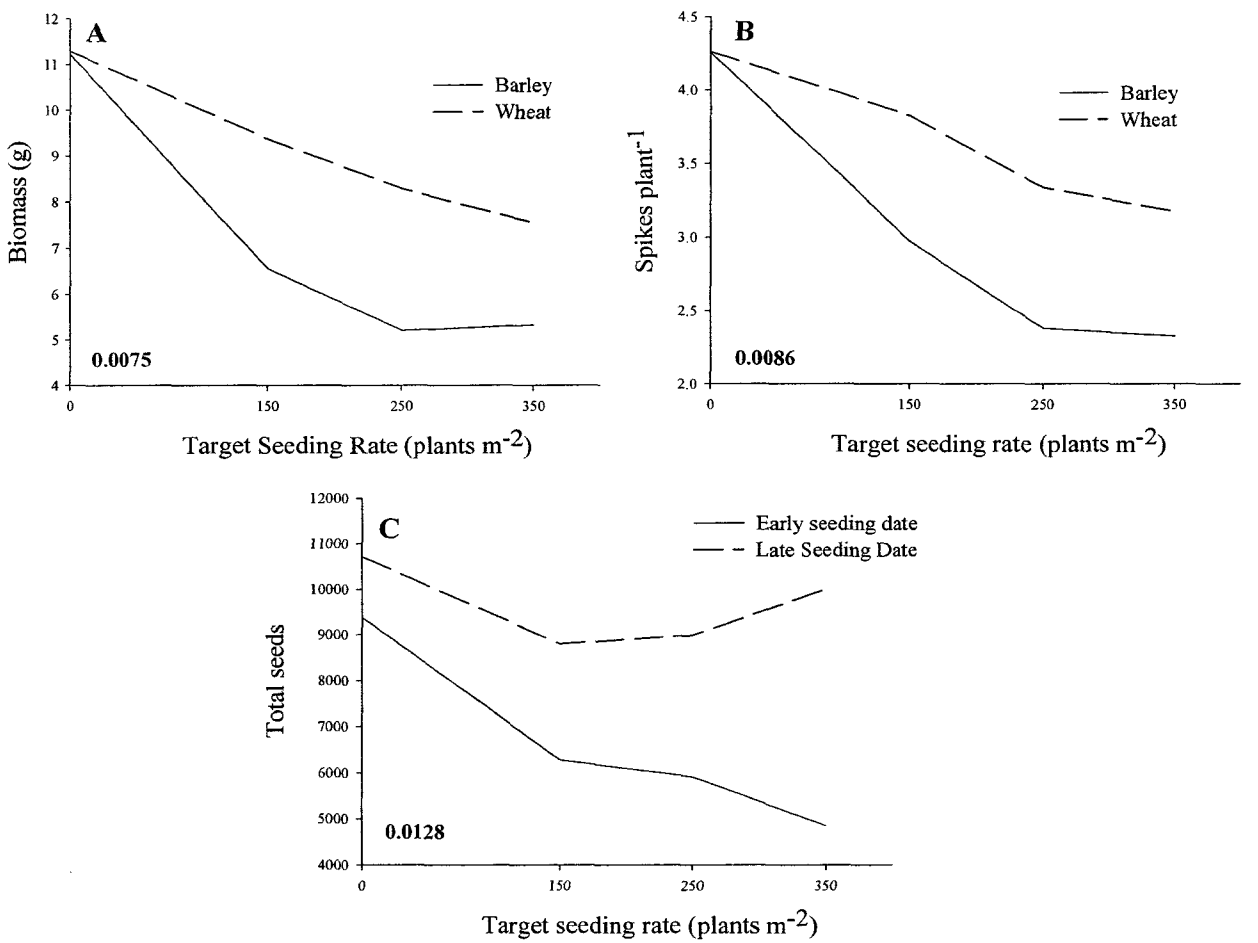

Figure B.5. Significant main effect means interactions for volunteer wheat in cereals trials in 2005 A) PREP volunteer biomass: seed rate*crop B) PREP spikes plant^{-1}: seed rate*crop C) PREP total volunteer seed production (m^{-2}): planting date *seeding rate