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Quantification and risk assessment of seed-mediated gene flow with flax as a platform crop for bioproducts

by

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For my brother David Dexter,

I am my beloved's and my beloved is mine.

Abstract

Flax (*Linum usitatissimum* L.) is being considered as a platform crop for the development of bioproducts. Potential benefits of bioindustrial farming include the provison of bioenergy and biomaterials, and opportunities for biorefining. Prior to the commercialization of crops intended for bioproducts however, the safety of the food/feed system and the environment must be assured.

As part of a preliminary biosafety assessment I conducted a literature review and experiments to quantify seed-mediated gene flow from flax to the environment and food/feed system. Flax seed losses at harvest, seed persistence in soil, efficacy of herbicides to control volunteer survival and fecundity in subsequent crops, volunteerism (density and occurrence) and volunteer emergence periodicity in follow crops in commercial fields were examined. Total seed losses at harvest in commercial fields were variable (2.7 to 44.2 kg ha^{-1}). Flax has a short longevity in the seed bank (2 to 3 years). Flax has been selected for reduced seed dormancy and volunteer flax seed persistence may be hastened by burial. Compared to other domesticated crops, flax has a prolonged period of emergence and calculated EM_{50} values (the growing degree days required for 50% emergence) ranged from 227 to 340 growing degree days (GDD). Flax volunteers reached their period of peak emergence earlier in conventional tillage than in reduced tillage fields. Volunteer flax densities were highest prior to herbicide applications (10.4 to 570.2 plants m^{-2}) in all fields the year following flax production (2005) and diminished over time. Volunteers that emerge in the spring may be controlled with registered herbicides. Glyphosate and fluroxypyr tank-mixed with either monohydrate sodium salt of 2,4-dicholorophenoxy acetic acid (2,4-D) or monochlorophenoxyacetic acid Ester 500 (MCPA) were most effective in reducing volunteer flax density, biomass, and fecundity.

These herbicides also reduced the adventitious presence of volunteer flax seed in spring wheat (from over 8.5% to 0.16%). Best management practices could be adopted to mitigate seed-mediated gene flow from flax in agricultural productions systems, but thresholds of zero are not biologically realistic. The agronomic baseline data generated in this thesis however, suggests that flax may be an appropriate crop platform for bioindustrial products.

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List of Abbreviations

Adventitous presence	AP
After harvest	POSTHARV
After seeding and before in-crop herbicide application	PREHERB
After in-crop herbicide application	POSTHERB
alpha-linolenic acid	ALA
Analysis of variance	ANOVA
Before harvest	PREHARV
Before seeding	PREPLA
Best management practices	BMPs
Canadian Food Inspection Agency	CFIA
Conventional tillage	CT
Commission of the European Communities	EC
Days after planting	DAP
Docosahexaenoic acid	DHA
Edmonton Research Station	ERS
Eicosapentaenoic acid	EPA
Ellerslie Research Station	Ellerslie
European Union	EU
Fischer's Protected Least Significant Difference	LSD
Gibberellic acid	GA_3
Growing degree days	GDD
Genetically engineered	GE
Mixed model	PROC MIXED

No-till	NT
Plant with Novel Trait	PNT
Plants with Novel Traits	PNTs
Poly unsaturated fatty acids	PUFA
Reduced tillage	RT
Statisical Analysis Software	SAS
Vegreville Research Station	VRS
Very long polyunsaturated fatty acids	VLCPUFA
Windrow	WR
Inter-windrow	IWR
Direct combine	DC
Windrow/combine	WC

Chapter 1: Biosafety assessment of flax (*Linum usitatissimum* L.) as a platform crop for bioproducts

INTRODUCTION

Flax *(Linum usitatissimum L.*) is being considered as a platform crop for the development of novel bioindustrial products. Research on the use of flax for bioproduct production is currently being conducted in North America, Europe, Australia, and Asia (Anonymous 2005). Future development of the crop is predicated on the use of genetic transformation and flax varieties that contain an industrial trait will be recognized as a Plant with a Novel Trait (PNT) by the Canadian Food Inspection Agency (CFIA) (CFIA 2004a). The primary market for Canadian flax seed is the non-food European linoleum/industrial oil market (Anonymous 2002); flax meal however, is a valued bioproduct, routinely fed to livestock for feed. Consumer and political concerns about genetically engineered (GE) crops for food and feed continue to be persistent in the European Union (EU) and the Commission of the European Communities (EC) has established a 0.9% labelling threshold for adventitious or technically unavoidable presence of authorized GE material in non-GE (conventional and organic) food and feed (Commission of the European Communities 2003). As a crop, flax has been extensively studied, but the population biology of volunteer flax is not well characterized. Crop volunteers may contribute to trait movement in the environment via pollen- and seed-mediated gene flow. Gene flow via seed has the potential to influence agriculture on a large temporal and spatial scale (Hall et al. 2003). Flax volunteers arising from seed lost at harvest may serve as a pollen source for the dispersal of transgenes to nearby flax fields or to wild or weedy relatives. Uncontrolled transgenic flax volunteers may replenish the

flax seed bank during harvest operations through seed shed and/or seed boll losses. Finally, if volunteer flax is harvested with other crops, the adventitious presence (AP) of transgenic flax seed in commodity products could jeopardize market access to major export markets, including the EU. If flax is to be developed for bioproducts, the relative contribution of flax volunteers to transgene flow on a spatial and temporal scale must be quantified as part of an environmental risk assessment.

RESEARCH OBJECTIVES

This research project is a component of a larger program to develop flax as a bioindustrial crop. Bio-based products are a rapidly emerging opportunity in the agricultural sector and there is a strong need to create novel germplasm and molecular markers for the Canadian flax industry customized to meet the demands requirements of new bioproduct and nutraceutical markets. Novel flax varieties that contain an industrial trait developed through non-transgenic or recombinant DNA techniques will be recognized as a PNT by the CFIA. Plants with Novel Traits (PNTs) are subject to an environmental safety assessment prior to their release into the environment.

A significant barrier to the development of flax as a platform for novel bioproducts is the ability to demonstrate that transgenic oilseed and fiber flax varieties have the appropriate environmental and biosafety profile, and that conventional flax varieties can be effectively segregated from industrial flax varieties that contain a novel trait. This thesis primarily quantified seed-mediated gene flow from volunteer flax in agroecosystems. The following key questions were addressed:

1. What is the frequency of occurrence and persistence of volunteer flax under contrasting tillage systems and crop types on commercial farms in western Canada?

2. How does the application of pre-emergent and post-emergent herbicides influence the fecundity of volunteer flax in hard red spring wheat (*Triticum aestivum* L.)?

3. What is the annual pattern of volunteer flax seedling emergence in conventional and reduced tillage cereal fields?

4. How large are seed bank inputs due to flax harvest losses on commercial farms in central Alberta?

5. How long do different yellow and brown seeded flax genotypes persist in the soil seed bank in western Canada?

It is hypothesized that seed-mediated gene flow from transgenic flax to the environment and food/feed system could be effectively restricted if flax were to be developed as a platform crop for bioproducts. This research project will provide the flax industry and the CFIA with data to help facilitate a decision on flax biosafety and will aid in the development of best management practices to reduce the adventitious presence of volunteer flax in subsequent grain and oilseed crops.

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Chapter 2: Literature review

This literature review will briefly review flax production in Canada; its biology and ecology, its potential for future use as a bioindustrial crop and current market and regulatory constraints that limit its development as a transgenic crop. Since GE flax varieties will be recognized as PNTs by the CFIA, Canada's regulatory system is outlined. The potential pathways for seed-mediated gene flow, the primary topic of the thesis, are reviewed in detail.

FLAX PRODUCTION IN CANADA

Flax as a minor crop

Flax is an annual broadleaf plant cultivated for the production of oil and fiber (Vaisey-Genser and Morris 2003). Currently, Canada is the world's leader in the production and export of oilseed flax (Anonymous 2002). In Canada, flax is primarily grown in Saskatchewan (81%), Manitoba (17%) and Alberta (2%) (Statistics Canada 2007). Oilseed varieties of flax are well adapted to the Canadian prairies, where the cool climate results in production of flax seed with high oil content and quality. The annual acreage seeded to oilseed flax in western Canada has remained relatively stable over the last 30 years. In 2008, Canada produced 767, 900 metric tonnes of flax seed from an area of approximately 611, 100 hectares (Statistics Canada 2008a). The 2007 flax seed yield estimate of 1200 kg ha^{-1} was less than the 1300 kg ha^{-1} reported in 2006, but slightly above the 10 year average of 1182 kg ha^{-1} (DeClercq 2006, DeClercq 2007). While flax production in Canada is important to world production, flax is a minor crop in Canada compared to wheat (10, 341, 000 ha) or canola (6, 395, 000 ha) (Statistics Canada 2008b). The flax industry has not benefited from intensive breeding efforts by the public or private sector because of its minor crop status. While a variety of cultivars have been

released (> 20) in the last 20 years, resources have been relatively small compared to those expended on canola (Kenaschuck and Rowland 1995).

Potential future use of flax as a platform for production for high value bioproducts

An evaluation of the market opportunities for flax is beyond the scope of this thesis. Along with other minor oilseed crops such including *Camelina sativa* and *Brassica oleracea*, flax is being evaluated as a crop platform for bioproducts. Due its unique oil profile, flax also may be a model plant species for the production of high-value bioproducts, including specialized nutraceutical and industrial products, edible oils, and fiber products. Molecular and gene expression experiments are not widely conducted in flax, and this currently limits the future development of the crop. Funds (approximately 15 million dollars) have recently become available however, for the sequencing of the flax genome and this should facilitate transgenic development of the crop (Anonymous 2009).

Conventional flax seed oil is extensively used for industrial purposes due to its high level (45-65%) of alpha-linolenic acid (ALA) (Vrinten et al. 1995). The seed oil of flax has a high drying quality as the double bonds of ALA react with oxygen when exposed to air, resulting in a relatively durable film (Rowland et al. 1995). Flax oil is traditionally used as an industrial drying oil for manufacturing paints, stains, inks, varnishes and for linoleum (Rowland et al. 1995).

Due to the high level of ALA in the seed oil of conventional flax varieties, it is not suitable as an edible oil (Saeidi and Rowland 1999). Conventional flax seed oil is highly susceptible to auto-oxidation of ALA (the spontaneous reaction of oxygen with unsaturated fatty acids on exposure to air), resulting in an undesirable odour and flavour

reversion (Ralph 1992). Plant breeding efforts have resulted in the development of low-linolenic acid flax lines (Green 1986, Rowland 1991) known as Solin. Oil derived from Solin contains less than 5% ALA (Anonymous 2002). Two recessive genes at independent loci control the low-linolenic acid trait in flax (Rowland 1991). The fatty acid composition (high palmitic/low ALA) of Solin oil provides an opportunity to produce a cocoa-butter replacement. A domestic source of a vegetable oil high in palmitic acid for the manufacture of margarines has attracted attention in Canada but its utilization is very dependent upon global value and supply of other plant oils (Rowland et al. 1995).

Cultivar development of flax is currently focused on enhancing the oil content and nutritional value to meet the demand of nutraceutical market supply as an alternative source to fish and fish oils. Fish and fish oils are rich sources of omega-3 (ω-3) very long chain polyunsaturated fatty acids (VLCPUFA), particularly eicosapentaenoic acid (EPA, C20:5 $\Delta^{5, 8, 11, 14, 17}$) and docosahexaenoic acid (DHA, C22:6 $\Delta^{4, 7, 10, 13, 16, 19}$) (Qi et al. 2004). EPA and DHA are the subject of much interest, because of their important roles in human health and nutrition. These important roles include neonatal retinal and brain development, as well as cardiovascular health and disease prevention (Carlson et al. 1993, Gill and Valivety 1997, Crawford 2000, Lauritzen et al. 2001, Thies et al. 2003). Obtaining these ω-3 fatty acids from higher plants in commercial and sustainable quantities is highly desirable as global fish stocks are declining, and the oils derived from fish are sometimes contaminated with a range of pollutants. In some fish, heavy metals have been detected such as cadmium, and these compounds are known to affect neuropsychological function in adults (Drexler et al. 2003, Yokoo et al. 2003). Flax is the richest plant source of ALA (C18:3 $\Delta^{9,12,15}$) (ω-3), a precursor of VLCPUFA, EPA and

DHA. Because flax is naturally high in polyunsaturated fatty acids (PUFA), particularly ALA, flax may be the choice platform species for producing VLCPUFA in higher plants.

Fiber flax was an economically important crop in eastern Canada until the end of World War II (Hammond and Miller 1994, Stephens 1997, Roberts 1998). The loss of government subsidies, competition from newly developed synthetic fibers and technological advancements promoting the adoption of other natural fibers during the post war years reduced and eventually ended fiber flax production in North America (Roseberg 1996, Stephens 1997). Fiber flax production is mainly concentrated in western Europe and in China, where breeding programs and technological improvements have led to highly efficient production systems (Roseberg 1996).

Renewed interest from a variety of industrial and manufacturing sectors in flax fibers has led to the potential large scale reintroduction of fiber flax production into Canada (Smeder and Liljedahl 1996). The fibers of flax that are extracted from bast fibers (located in the outer regions of the plant stem between the outermost cuticle-epidermis layer and the innermost woody tissues) through retting are well suited for the textile industry as these fibers are soft, lustrous and flexible (Sharma 1992). Compared to cotton or wool, the yarns spun from flax are twice as strong and are more durable; as the individual fibers lack elasticity (Vaisey-Genser and Morris 2003). Long-line and short staple (i.e. tow, a by product of long line production) fibers of flax have specialized uses in the textile industry (Sharma and Van Sumere 1992). Long-line fiber is used for manufacturing high value linen products whereas short fiber flax is used in the production of lower value textile products like blankets, mats, mattresses and carpets. There is also interest in the use of flax fiber for nontextile purposes in the paper industry for printed banknotes and cigarette paper (Lay and Dybing 1989). Flax fibers are also used in

sustainable building materials such as particleboard and are also utilized in new composite materials in the automobile and construction industry. Green building materials made up from flax fibers based on the polymer polyhydroxybutrate (PHB) could potentially serve as an environmentally friendly and biodegradable alternative to conventional plastics (Yamazaki et al. 1992).

Flax seed mucilage (occurs in the epidermis layer of the seed coat) has potential industrial uses as it has emulsifying properties better than gum Arabic and Tween 80 (Minker et al. 1973). Mucilage removal is either by mechanical means through dry dehulling (Smith et al. 1946) or with a wet process of demucilaging followed by dehulling (Schlab et al. 1955, Mandokhot and Singh 1979, Singh 1979). The discarded hull fraction may be used as a raw material in the extraction of phytochemicals (Oomah and Mazza 1998).

Market constraints

Flax, as a minor crop, has had limited conventional breeding and molecular biotechnology resources. While flax has been transformed with several novel traits including resistance to glyphosate (Jordan and McHughen 1998), glufosinate-ammonium (McHughen and Holm 1995) and the acetolactate synthase (ALS) inhibitors chlorsulfuron and metsulfuron (McSheffrey et al. 1992), only one transgenic flax cultivar (CDC Triffid) has been released in Canada (McHughen et al. 1997). At the time of the unconfined release of CDC Triffid, Canada's main export market for flax seed was the EU. Although this transgenic flax cultivar posed no unacceptable risk to food, feed, or the environment (CFIA 2004b), CDC Triffid was deregulated shortly after its release in 1998 at the request of the Canadian flax seed industry due to market concerns regarding the EU's opposing stance to the importation GE crops. A clear regulatory framework did not exist for

transgenic crops or feed products in Europe at the time of release of CDC Triffid, products derived from GE flax were considered to be unacceptable to the European market (McHughen 2002). The EU is now moving towards being more open to bioproducts and transgenic crops (Hricova 2002, Breithaupt 2004, Millam et al. 2005).

GE crops are grown worldwide, and the number of species and the area under production continues to increase (James 2007). Most countries, including Canada and the United States, have implemented the principle of substantial equivalence as the basis of the approval process for the unconfined release of GE crops. In Canada specifically, the regulatory framework takes into account the need to exercise due diligence and caution. In Canada, foods derived from GE crops do not require mandatory labelling under the condition that the products are substantially equivalent to their non-GE (conventional) counterparts (Demeke et al. 2006). In contrast, the EU is proposing and implementing measures to achieve coexistence between GE and non-GE (conventional and organic) agricultural production systems (Beckie and Hall 2008). Regulation (EC) No 1829/2003 (implemented in April of 2004) provides a legal basis for the national and/or regional implementation of co-existence frames in the EU (Devos et al. 2005). The EC has established a 0.9% labeling threshold for adventitious or technically unavoidable presence of authorized GE material in non-GE food and feed (Commission of the European Communities 2003). For EU unapproved events, a zero tolerance is applied, but international thresholds have not been established for other special products, such as industrial or plant-made pharmaceutical compounds (Beckie and Hall 2008).

Several barriers must be overcome to facilitate the development of flax as a platform crop for novel bioproducts. Crop development is mostly predicated on the use of genetic transformations and this raises new marketing and food safety concerns. Unlike

insecticide and herbicide resistant GE crops, whose products are considered substantially equivalent to their conventional counterparts, the products of bioindustrial crops may substantially differ, requiring segregation from conventional crops. Currently, the primary market for Canadian flax seed is the EU (Anonymous 2002). Consumer and political concerns about GE crops for food and feed continue to be important in Europe and these concerns could effectively block future transgenic crop development (Demeke et al. 2006). If flax is to be cultivated for bioproducts in western Canada, effective management practices to mitigate gene flow and to segregate GE flax from conventional flax cultivars is necessary to preserve conventional and organic flax market value.

Regulatory framework in Canada

PNTs play a significant role in Canada's crop industry. A PNT is defined 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 2004a). These traits may be introduced using biotechnology, mutagenesis, or conventional breeding techniques. PNTs are subject to an environmental safety assessment prior to their release into the environment. Determinations of associated risk to the environment include the potential to impact weediness, gene flow, plant pest potential, non-target organisms, and biodiversity (CFIA 2004a).

In Canada, the CFIA shares responsibility with Health Canada for regulating the unconfined environmental release of PNTs. The unconfined environmental release of PNTs is handled by the Plant Biosafety Office of the CFIA through the Seeds Act while approval of PNTs as a livestock feed is handled by the CFIA's Feed Division under the Feeds Act. Health Canada is exclusively responsible for regulatory approval of PNTs for food through the Novel Foods Regulation of the Food and Drugs Act (CFIA 2004a).

Since 1995, the CFIA has released 77 cultivars with novel traits for cultivation and/or importation (CFIA, 2008).

Novel flax varieties that contain an industrial trait developed through non-transgenic or recombinant DNA techniques will be recognized as a PNT by the CFIA (CFIA 2004a). Since all PNTs are assessed on a case-by-case basis, the agency does want to predefine data package requirements for PNTs as a new trait or crop may require additional tests which were not appropriate for previous submissions. Theoretically, Canada's regulatory framework thus provides flexibility, time and cost efficiencies to both industry and to the CFIA. In recent years, Canada's regulatory framework has been heavily criticized however, as its flexible scheme can lead to gathering excessive and sometimes superfluous data for biosafety assessments, especially when the trait is non-GE (derived from conventional plant breeding or mutagenesis techniques), which can add to timely and monetary costs to both industry and regulators (eg. Low Phytate Barley) (Manalo and Ramon, 2007).

The choice of plant species platform for bioproduct production influences its regulation by Canadian government agencies. Domesticated food or feed crops such as corn (*Zea mays* L.) or canola (*Brassica napus* L.) are often desirable choices for plant platforms as they often produce high yields, have refined cultural methods and are well characterized for transformation and protein expression. Weedy or undomesticated plant species may not be appropriate platforms for bioproduct production due to potential difficulties containing novel trait movement in the environment (Sparrow et al. 2007). In addition, these weedy or undomesticated plant species may not be amenable to genetic engineering or protein production. It is recommended that platform plant species have no,

or limited use, for food and/or feed. Flax may be suitable for PNT production as it is predominantly grown as a non-food crop for industrial purposes on low acreage in Canada.

FLAX BIOLOGY AND AGRONOMY

To determine whether GE flax can be segregated from conventional flax requires an understanding of flax biology, gene movement, flax volunteers and agronomy.

Description of the plant and pollination mechanisms

Linum usitatissimum L. (cultivated flax) belongs to the family *Linaceae* and order *Linacles* (Vromans 2006). Flax has a long, branched taproot which may extend to a depth of more than 1 m with side branches stretching approximately 30 cm (Diederichsen and Richards 2003). The leading shoot is long, slender and erect and lateral branching occurs at the stem base. The plant will develop several secondary basal prostrate-ascending shoots if the leading shoot of the young plant is injured (Diederichsen and Richards 2003). A high level of soil fertility also contributes to a high degree of lateral branching at the stem base (Dillman and Brinsmade 1938). In contrast, dense planting suppresses the formation of secondary stems at the stem base and branching in the apical parts of the stem (Diederichsen and Richards 2003). Flax plant height varies between cultivars and ranges from 20 to 150 cm (Hegi 1925). The leaves vary in size and range from 3 to 13 mm in width and 15 to 55 mm in length. The three-veined leaves are alternate. Smaller leaves are linear and larger leaves are linear-lanceolate. During seed ripening, the leaves senesce and fall off the plant.

A single flax flower is complete, perfect and pedicellate (Dybing and Lay 1981) and measures 2-3 cm in diameter (Dillman 1938). Individual flax flowers are borne terminally on the pedicle in a multiflowered panicle and have five sepals, five petals, five stamens, and a compound pistil of five carpels (Dillman 1938, Dybing and Lay 1981).

The five sepals are acuminate (terminating in a sharp point), carinate (ridged) and ovate. The petals are relatively inversely oval. Some genotypes have flower petals with a longitudal fold whereas other genotypes have inward folded petal margins (Diederichsen and Richards 2003). Petal color varies from violet to red violet, blue, dark blue, light blue, white and pink. The most frequent petal color is blue, followed by white (Tammes 1928, Dillman 1936). The stamens are inserted alternately with the petals into a fleshy ring at the base of the flower which secretes nectar from five small, flat pits on its outer side. The anthers are introrse (the stamens open and shed their pollen inwards). The stamens and anthers vary in color and have the same color range as the petals. The ovary is composed of five united cells; each cell is divided into two chambers by a false septum and each chamber contains an ovule. The ovary is surmounted by an erect style (Williams 1988).

Flax is a highly self-pollinating species (Beard and Comstock 1965), but cross-pollination rates have been reported in the range of 1 to 5% (Eyre and Smith 1916, Robinson 1937, Dillman 1938). Wind is not considered to be an important pollinating agent of flax, as flax pollen grains are heavy and are not readily transported by wind (Gubin 1945). Important pollinators of flax include honeybees (*Apis mellifera*), bumble bees (*Bombus spp*.) and butterflies (*Lepidoptera*) (Dillman 1938, Gill 1987). Records of the insects visiting flax flowers have invariably shown the honeybee to be the most abundant species, comprising up to 93% of all insects captured (Gubin 1945, Kozin 1954, Smirnov 1954). The amount of self- and cross-pollination that occurs is influenced by the position of the anthers relative to the stigmas. In most cultivars, the anthers are above and entirely surround the stigma, favouring self-pollination, whereas, in some flowers, the tip of the stigma extends above the anthers, increasing the chance of cross-pollination (Williams 1988).

The fruit is a globular boll of five joined carpels. Each carpel forms two septa and each flax boll has 10 lodicules which may fill with seed (one seed per lodicule). Ripe bolls are either completely closed or open slightly along the septa, depending on the genotype. The seeds are flattened, rounded at base, acute at the apex, ovoid or oblong elliptic and are 3-3.5 mm long (Diederichsen and Richards 2003).

The anatomical parts of flax seed include the testa, the endosperm and the embryo. The outer surface of the testa is slightly wavy and is shiny. The pigment cells of the testa are square, and are contained in the innermost layer in a pericline section. The pigment cells of the testa influence the outer appearance of seed color and they often contain tannic pigments, which are yellow-brown in color. In yellow-seeded flax varieties, the pigment cells are often absent, but if present, the cells do not contain any pigments. In the absence of pigments, the main factor that influences the outer color of the seed is the color of the cotyledons, typically white or yellowish in color (Diederichsen and Richards 2003). The endosperm contains oil and protein and occupies 1/3 or less of the seed volume. The endosperm surrounds the embryo, which contains 2 large cotyledons and fills more than 2/3 of the inner seed volume (Diederichsen and Richards 2003).

A single layer of epidermal cells covers the flax seed. Below the epidermal cells are one to five layers of parenchyma cells. The ring-cells of the parenchyma cells may contain dark, tannin-like substances and occasionally chlorophyll. The presence of these substances also contributes to seed color. The ring cells and the epidermis originate from the outer integument of the ovule. Below the ring cells of the parenchyma is a single layer of sclerenchyma cells, 16-25 μm thick. The transversal cells have an irregular orientation and are more or less collapsed in mature seeds (Diederichsen and Richards 2003).

Unlike the seeds of other oilseed crops such as canola (*Brassica napus* L.) and sunflower (*Helianthus annus* L.), flax seeds contain mucilage (a polar glycoprotein). The mucilage is contained in the outer layer of the seed hull (Pyrde 1983). The muclilage accounts for approximately 8% of the seed weight, and is known to be primarily composed of polysaccharides (BeMiller 1973). The mucilaginous content of the seed may aid in flax seed germination as the outer layer of the hull easily absorbs soil moisture. Flax bolls are resistant however, to degradation and may delay seed release and thus contribute to prolonged seedling emergence.

Growth habit

There are twelve distinct growth stages in the development of the flax plant (Table 2-1). The lifecycle of the flax plant consists of a 45 to 60 day vegetative period, a 15 to 25 day flowering period and a fruit maturation period of 30 to 40 days. Flax is indeterminate. A small number of flowers may continue to appear right up to the end of fruit maturation and under conditions of high soil moisture and fertility, new growth may occur, leading to a second period of intense flowering (Anonymous 2002).

There are several environmental factors that affect flax, and are mainly associated with an imbalance of nutrients in the plant during periods of environmental stress. Under high soil moisture conditions, plant stems and leaves often become chlorotic and symptoms are often associated with terminal bud death and extensive basal branching. Very high or freezing air temperatures may result in the formation of heat or frost cankers respectively, on the stem when the crop is in early stages of growth. Frost cankers are commonly inconspicuous, but flax seedlings that are severely damaged may reduce plant stands by as much as 50%. Canker damage is usually most severe in thin stands on light

sandy soils, while leaf chlorosis is usually on heavier saturated soils that contain a high percentage of lime (Anonymous 2002).

Flax cultivars are well adapted to most growing regions of the Canadian prairies, where the cool climate results in production of flax seed with high oil content and quality (high iodine value) (Saeidi and Rowland 1999). Air temperatures below 10°C in the spring however, inhibit both growth and development which can delay flowering. A delay in flowering can result in reduced seed set, seed weight, oil quantity and quality (lower iodine value) (Dillman and Hopper 1943, Kraft et al. 1963, Dybing and Zimmerman 1965, Kenaschuk 1975, Gusta et al. 1997). It has also been reported that flax at flowering and seed set is sensitive to heat, particularly to temperatures exceeding 30°C (Dillman and Hopper 1943, Painter et al. 1944, Ford and Zimmerman 1964). Kraft et al. (1963) reported that flax plants exposed to a continuous 3-5 day heat stress of 31°C produced up to 64% malformed seed. Kraft et al. (1963) further reported that air temperatures of 31°C resulted in partial and complete necrosis of the ovule after 1 and 5 days respectively. Gusta et al. (1997) reported that a heat stress of 40°C for 3 days reduced the seed yield of Norman flax by 31%, whereas a 7 day stress reduced flax seed yield by 58%.

Flax fiber yield and its associated physical characteristics such as length and fineness, depend on climatic conditions and soil nutrients (Sizov 1970, Les 1977). High quality flax fiber and high fiber yields per plant are favored by a mild, humid climate and long day lengths (Anonymous 2002). If the daily temperature is high (maximum > 28°C), the flax plants will remain short, which affects fiber yield and quality (Sultana 1992). Mikhailova (1975) reported that high soil nitrogen improved the quality of flax fiber by reducing the lignin content and increasing the cellulose content. Tarent'ev et al. (1976) reported that soil phosphorous improves fiber quality by increasing the percentage of long

fibers (fibers measuring > 50 cm). In contrast, excess calcium has been shown to lower flax fiber quality by inducing more lignin, pectin and ash at the expense of cellulose (Elhaak et al. 1999).

Compared to other oilseed crops such as canola (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.), flax is a poor competitor with weeds (Friesen and Shebeski 1960, Friesen et al. 1990, Friesen et al. 1992). Friesen et al. (1990) reported that volunteer wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) reduced flax yields by up to 53 and 67% respectively at an average density of 30 plants m^{-2}. Bell and Nalewaja (1968) reported that a density of 49 wild oat plants m^{-2} reduced flax stand densities by up to 11 plants m^{-2} and also reported that an average 24 and 65 wild oat plants m^{-2} reduced flax seed yields by 263 and 803 kg ha^{-1} respectively. In Manitoba, Bowden and Friesen (1967) reported average flax yield reductions of 24 and 50% when wild oat (*Avena fatua* L.) occurred at an average density of 12 and 48 plants m^{-2} respectively. In field trails in Manitoba, Friesen et al. (1992) reported that round-leaved mallow (*Malva pusilla*) at an average of 20 plants m^{-2} caused flax seed yield losses up to 33%.

Crop rotation

Although flax has been grown for more than 30 years on the Canadian Prairies, few crop rotational studies have included flax (Lafond et al. 1992). In western Canada, flax is rarely grown on the same field more than once in a four year crop rotation (Anonymous 2002). Flax should not be seeded into its own stubble, as it has been reported that yield and quality are greatly reduced, primarily due to the presence of foliar plant pathogens such as Pasmo (*Septoria linicola*) (Johnston et al. 2005).

In Saskatchewan, yield losses were observed when flax was grown in rotation after canola (*Brassica napus* L.) (Vera et al. 1987, Beckie and Brandt 1997). Vera et al. (1987) reported that flax yields were reduced when young volunteer canola seedlings were tilled just before seeding the flax crop. Similarly in Manitoba, flax yields were reduced by up 5% when flax was seeded directly into canola stubble (Gubbels and Kenaschuk 1989). It has been previously reported that a natural growth inhibitor, indole glucosinolate is readily leached from all parts of the canola plant and its residues (Brown and Morra 1996). This alleopathic compound may have phytotoxic detrimental effects on flax seed germination, resulting in reduced flax plant stands and yield (Vera et al. 1987, Gubbels and Kenaschuk 1989). Unlike canola, flax is a mycorrhizal plant. Growing non-mycorrhizal plants prior to flax may negatively influence arbuscular mycorrhizae fungi populations in the soil and consequently, lower flax yields (Johnston et al. 2005, Krupinsky et al. 2006). Flax is generally followed in rotation by a cereal crop, usually spring wheat (*Triticum aestivum* L.) or barley (*Hordeum vulgare* L.) (Wall and Smith 1999).

Other rotational studies have indicated that grain yields of cereal and broadleaf crops are reduced when flax is planted as the preceding crop. Beckie and Brandt (1997) reported that the yield of wheat following field pea rather than flax was 14% greater. In Saskatchewan, Johnston et al. (2005) reported field pea yields were reduced 50-60% when seeded on flax stubble relative to wheat, barley and canola stubble. Blackshaw et al. (2007) reported that when zero-till dry bean (*Phaseolus vulgaris* L.) followed flax in rotation, dry bean yields were negatively affected. Dry bean yields however, were not consistently negatively influenced by flax stubble both site-years and yield reductions were mainly attributed to poor post-emergent control of volunteer flax with bentazon

(Blackshaw et al. 2007). Under drought conditions, canola, barley and wheat yields were reduced up to five-fold when seeded on flax stubble in Saskatchewan (Johnston et al. 2005). Risks associated with re-cropping these crops on flax stubble were mainly attributed to soil water shortages as flax stubble is relatively short and thin and has a reduced capacity to catch snow and minimize evaporative water losses in the spring (Lafond et al. 1992, Johnston et al. 2005). Should more flax be grown in western Canada more research on rotational effects on flax may be required.

Susceptibility to plant disease

The occurrence, severity and importance of flax diseases vary by region, but historically in western Canada, the fungal pathogens of flax rust (*Melampsora lini*), fusarium wilt (*Fusarium oxysporum*) and Pasmo (*Septoria linicola*) have caused both numerous and wide spread epidemics. Flax diseases may also be caused by viruses and phytoplasma, but infections are of minor economic importance in western Canada (Rashid 2003).

Unlike many other rusts that require alternate hosts, flax rust is an autoecious rust as the fungus can infect the flax plant at all stages of its lifecycle (Rashid 2003). Flax rust develops most severely on young seedlings. Early infection may cause the leaves to dry and wither resulting in heavy defoliation. Severe rust epidemics can cause major losses in quality and yield of both seed and fiber (Flor 1944, Hora et al. 1962, Hoes and Dorrell 1979, Acosta 1986, Shukla 1992). Infection of young flax plants by uredopores is favoured by dewpoint conditions lasting eight to ten hours and cool temperatures (15-18 $^{\circ}$C) (Flor 1954). Symptoms of flax rust are characterized by bright orange and powdery pustules, which are developed on the leaves, stems, bolls and other aerial plant parts (Rashid 2003).

Flax rust epidemics are influenced by the frequency of the pathogenic races occurring in the field, weather conditions and by the acreage of susceptible varieties (Rashid 2003). The most adequate and economic way to control flax rust is to use resistant cultivars. In areas where flax rust is abundant however, inoculum pressure may be reduced by destroying plant debris and volunteer plants and by following a three year crop rotation (Gill 1987).

Flax wilt is one of the most widespread diseases affecting flax and severe epidemics can result in 80-100% yield reduction (Kommedahl et al. 1970, Sharma and Mathur 1971, Kroes et al. 1999). The fungal pathogen is primarily soil-borne. The pathogen invades the roots, mainly through the root hairs, develops in the xylem vessels and over time, becomes systematic and interferes with water uptake from the soil environment (Nair and Kommedahl 1957, Kroes et al. 1998). Warm soil temperatures (21°C) and low soil moisture favour flax wilt development (Vanterpool 1949). The fungal pathogen can kill flax seedlings before and after emergence and may infect young flax plants up to the pre-flowering stage. Seedlings infected with flax will cease to grow, wilt from the top downwards and the leaves turn yellowish brown. The fungus, mycelia, and spores may persist in the soil for five to ten years in the crop debris (Rashid 2003).

Resistant cultivars may be used to control flax wilt. In order to maintain low levels of inoculum in the soil, crop residues in infected fields should be minimized or destroyed and a crop rotation of at least three years between flax crops is recommended. Also, seed treatment with fungicides may prevent the introduction of the disease and reduce the incidence of early wilt in seedlings (Rashid and Kenaschuk 1996).

Pasmo, also known as spasm or septoriosis, is a foliar pathogen that infects the leaves, stems and bolls of flax (Sackston and Gordan 1945, Rashid and Kenaschuk 1998).

This fungus is seed-, soil- and stubble-borne. Infection of flax plants by the pathogen occurs at the seedling stage, but the severity of the infection is generally not recognized until boll setting and seed ripening. Early symptoms of the disease include brown lesions on cotyledons and leaves of seedlings due to the formation of pycnidia. Pasmo disease can cause defoliation, stem weakening, premature ripening and seed boll losses which results in reduced yields and poor seed and fiber quality (Millikan 1951, Sackston and Carson 1951, Frederiksen and Culbertson 1962, Rashid and Kenaschuk 1998, Rashid 2003). High moisture, warm temperatures, dense crop canopies and heavy flax lodging favour infection by the pasmo pathogen (Rashid 2003).

To date, flax cultivars resistant to pasmo are not available. Registered fungicides and cultural control methods such as seedling early to avoid infections, use of certified seed, and use of a three-year crop rotation are currently used to control pasmo disease (Rashid and Kenaschuk 1998).

Susceptibility to insects

Flax may be affected from the time of emergence to maturity by various insect pests. Only a small number of insects damage flax and most insects feeding on flax are polyphagous. Major insect pests of flax include potato aphids (*Macrosiphum eurphorbiae* Thomas), cutworms (*Lepidoptera spp*.), and grasshoppers (*Melanoplus bivittatus* Say) (Wise and Soroka 2003).

The potato aphid is the most serious insect pest of flax in western Canada (Wise and Soroka 2003). Adults and nymphs damage flax seedlings by extracting plant fluid (phloem) from leaves and developing bolls, often resulting in large yield losses (Wise et al. 1995). A single insecticide application at full bloom or at the earlybloom stage is the most effective method to control potato aphids in flax (Wise et al. 1995).

The two most common species of cutworms (*Lepidoptera spp*.) that damage flax in western Canada are pale western cutworm (*Agrotis orthogonia* Morrison) and the red-backed cutworm (*Euxoa ochrogaster* Guenee) (Parker et al. 1921, King 1926, Philip 1977). Cutworms usually remain below ground and damage flax plants by either fully or partially by severing the stems of seedlings at the soil surface, leaving them completely destroyed or severely weakened and susceptible to further damage by wind or disease. Relatively low average densities of cutworm may result in large yield losses of flax. In field trails in Manitoba, Ayre (1990) reported that redbacked cutworms at a density of 32 larvae m^{-2} destroyed all flax plants that had been seeded an average seeding rate of 45 kg ha^{-1}. The most effective way to control cutworms is to apply an insecticide to the base of damage flax plants. It is recommended that the insecticide be applied using high water volumes to improve the coverage and penetration of the insecticide into the soil (Malik et al. 1998).

Grasshoppers are migratory and tend to feed on flax only after other food sources have been depleted. Young grasshoppers can cause damage to young seedlings by feeding on vegetative tissue. With heavy grasshopper infestations however, severe leaf defoliation can occur to the extent that all or nearly all leaf material is consumed, in which case replanting may be necessary. Compared to young grasshoppers, adult grasshoppers are more damaging to flax in terms of yield loss. Adult grasshoppers chew through the more succulent portions of the stem below the seed bolls. Due to the feeding habits of the adult grasshopper, a large percentage of flax seed bolls are removed from the plant and are not harvested with the flax crop (Anonymous 2002). Grasshoppers are best controlled in flax through the use of insecticides. The insecticide should be applied just after the

grasshopper eggs have hatched and while the nymphs are still concentrated in their breeding areas (Hardman and Mukerji 1982).

Herbicides registered for use in Canada for flax production

Few pre-emergent and post-emergent herbicides are registered for annual and broadleaf weed control in flax in western Canada. Depending on the diversity of the weeds present, time of seeding and tillage system, growers may use a combination of pre-emergent and post-emergent herbicides or use a single or split post-emergent herbicide application(s) to control weeds. The Group 9 herbicide, glyphosate (N-(phosphonomethyl) glycine) is the most commonly used herbicide for pre-emergent weed control in the Northern Great Plains (Cerdeira and Duke 2006). Glyphosate is widely used in no-till and/or reduced tillage flax production systems as it is non-selective and has little or no soil residual activity. Prior to seeding the flax crop, glyphosate may be applied alone or in combination with other registered post-emergent herbicides to control weeds. Trifluralin, a Group 3 herbicide, may also be used for pre-emergent weed control in flax. Trifluralin is best suited for flax production systems under conventional tillage as it is most effective when incorporated into the soil prior to planting (Anonymous 2007). Unlike glyphosate, trifluralin has residual soil activity and soil persistence is strongly influenced by soil properties such as texture, organic matter, iron content and anoxic conditions within the soil profile (Solbakken 1982).

Registered post-emergent herbicide options for weed control in flax are limited. The Group 1 herbicides quizalofop-p-ethyl, fluazifop-p-butyl, sethoxydim and/or clethodim may be used to control grass species in flax. MCPA, a Group 4 herbicide may be used alone or in combination with bromoxynil or clopyralid to control broadleaf weed species. Bentazon, a Group 6 herbicide may be also used to control broadleaf weeds in

flax (Anonymous 2007). In western Canada, most producers elect to use a tank mix of Group 1 and Group 4 herbicides to control weeds post-emergence in flax such as FlaxMax Ultra (sethoxydim + clopyralid + MCPA). The limited number of broadleaf herbicides available for use in flax along with its poor competitive ability will limit the use of flax in fields with high or diverse weed populations. Flax tolerates foliar applications of cholorsulfuron (Hutchinson et al. 1984), and flax producers generally apply this herbicide at low herbicide doses to ensure crop safety (Wall and Kenaschuk 1996). Other ALS inhibitor herbicides such as thifensulfuron and tribenuron can cause severe injury to flax when applied post-emergence. Thifensulfuron applied post-emergence caused reductions in flax dry weight, height and yield, especially under cool, wet growing conditions (Derksen and Wall 1996). Similarly, Wall and Kenaschuk (1996) reported that thifensulfuron caused chlorosis, stunting, delayed flowering and maturity in Norlin flax. In the same study, tribenuron was reported to cause severe yield losses in flax (11%) at doses as low 1.3 g a.i. ha^{-1}.

THE OCCURRENCE OF VOLUNTEER FLAX IN WESTERN CANADA

Volunteer flax distribution in weed surveys

Volunteer flax is an annual weed (Lay and Dybing 1989) that volunteers from seed losses incurred during flax harvest. Over the past 30 years, the relative abundance of volunteer flax has increased across western Canada (Leeson et al. 2005). The relative abundance ranking of volunteer flax has increased from a rank of 32 in the 1970s to a rank of 26 in the 1990s and 2000s in weed surveys following in-crop herbicide control (Leeson et al. 2005). The relative abundance index is a synthetic index calculated from the relative frequency, relative uniformity, and relative density of that species (Thomas 1985). The increase in the relative abundance of volunteer flax since the 1970s has been

2-fold on average (Leeson et al. 2005) and this increase may be explained based on changes in cropping practices, particularly the reduction in the use of tillage for weed control (Gray et al. 1996). It is unlikely that the observed increase in volunteer flax across the Canadian prairies is the result of greater flax seed harvest losses as average yields of flax have not increased substantially and harvest methods (direct combine or windrow/combine) of flax have not changed over the past three decades (Anonymous 2002).

Weed control of volunteer flax in rotational crops

Traditionally, summerfallow and tillage have been integrated with herbicide use as a means of controlling volunteer flax in rotational crops. The widespread adoption of diverse and continuous cropping systems and reduced and/or zero tillage practices however, has reduced the frequency and intensity of mechanical tillage for weed control in western Canada (McConkey et al. 2002, Schlegel et al. 2005). As a result, growers have increased their reliance on pre-emergent and post-emergent herbicides in conservation tillage systems for weed management of volunteer flax.

Volunteer flax is readily controlled post-emergence in rotational cereal crops such as spring wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). Post-emergent herbicide options for the control of volunteer flax in cereal crops include the Group 4 auxin inhibitors, fluroxypyr + 2,4-D, fluroxypyr + MCPA or quinclorac and Group 2 (acetolactate synthase, ALS) inhibitor, tribenuron-methyl used independently or in combination with 2,4-D or quinclorac (Anonymous 2007). Quinclorac applied alone at a rate of 100 or 200 g a.i. ha^{-1} was reported to provide consistent volunteer flax control without yield loss in spring wheat (Wall and Smith 1999). In Manitoba, Wall and Smith (1999) reported that fluroxypyr plus 2,4-D (105 + 560 g a.i. ha^{-1}) and fluroxpyr plus

clopyralid plus MCPA (144 + 100+ 560 g a.i. ha^{-1}) provided almost complete control of volunteer flax in spring wheat, reducing volunteer flax densities from 105 to 3 plants m^{-2} and from 105 to 4 plants m^{-2}, respectively. In the same study, Wall and Smith (1999) reported that tank mixes of the ALS inhibitor herbicides tribenuron and thifensulfuron plus tribenuron with herbicide 2,4-D, reduced the dry weight of volunteer flax relative to the untreated weedy control, but results were inconsistent among site-years. It should be noted that flax volunteers effected but not controlled by a herbicide have been observed to produce less seed, but fecundity has not been quantified.

Currently, there are no post-emergent herbicides registered for the control of volunteer flax in rotational broadleaf crops such as field pea (*Pisium sativa* L.) (Anonymous 2007). Volunteer flax may be controlled post-emergence however, in some herbicide resistant canola crops. In 1995, transgenic herbicide resistant canola cultivars were commercially introduced in Canada (Yoshimura et al. 2006). Currently, over 90% of the oilseed rape (*Brassica napus* L.) area in western Canada is seeded to cultivars resistant to glyphosate (50%) or glufosinate (30%) (Beckie et al. 2006). Although both herbicides are registered for volunteer flax control in canola, the phytotoxicity of glufosinate on volunteer flax is highly variable and its efficacy is strongly influenced by environmental conditions, especially low relative humidity and light intensity (Peterson and Hurle 2000).

Ability to become weedy or invasive

Feral plants are individuals of a cultivated crop which escape a managed area, reproduce successfully and establish a self-perpetuating population in natural or semi-natural habitats (Bagavathiannan and Van Acker 2008). The most important characteristic of feral crop populations is that they are able to successfully reproduce without

management intervention (White et al. 2006). Escaped populations are common for many cropped species (Crawley and Brown 1995, Bond et al. 2004, Claessen et al. 2005), but not many become feral (Crawley et al. 1993, Pessel et al. 2001). Thus the occurrence of cultivated species as feral populations has been studied to a limited extent, particularly in the context of novel trait confinement (Gressel 2005, Garnier and Lecomte 2006a, Garnier and Lecomte 2006b, Devaux et al. 2007).

Traits associated with ferality are generally associated with wild type or weediness traits. Key traits associated with the most successful feral species include indeterminant seed production, variety of pollinators, high seed production, habitat plasticity, seed dispersal over short and long distances, seed dormancy (ability to form a seed bank), broad germination requirements, discontinuous germination, rapid vegetative growth, tolerance of competition, tolerance to unfavourable biotic and abiotic conditions and rapid flowering (Baker 1965). Seed dormancy and the ability to form a seed bank appear to be a key feral trait for most crops including oilseed rape (*Brassica napus* L.) and alfalfa (*Medicago sativa* L.) (Warwick and Stewart 2005). Models developed to predict the persistence of feral populations demonstrate that seed persistence is a key trait driving population persistence (Bullock 1999, Garnier and Lecomte 2006a).

Specific traits were selected by humans to facilitate crop domestication and thus agricultural production. Crop domestication generally results in a loss of genetic diversity, with cultivated plants diverging from their wild relatives (Warwick and Stewart 2005). Domesticated crops feature traits that include retention of the seed or fruit on the plant at maturity, loss of germination inhibitors, synchronous germination (loss of secondary dormancy), narrow germination requirements, short-lived seeds (limited seed persistence), synchrony of flowering and fruit development, determinate growth, smaller

number of larger fruits or inflorescences, increase in seed or fruit size, reduction in seed dispersal (shattering), increase in vegetative vigor and apical dominance, reduced competitive ability, self-pollinated or self-incompatible, unspecialized pollinators and adaptation to disturbed habitats (Baker 1965, Baker 1974, Doebley 1992, Harlan 1992). Since domestication traits were subject to co-selection, many of these traits may be closely genetically linked (Warwick and Stewart 2005).

Crops vary in their degree of domestication (Warwick and Stewart 2005). For example, cranberry (*Vaccinium macrocarpon* L.) is essentially an almost completely undomesticated crop plant whereas maize (*Zea mays* L.) is highly domesticated. Other crops such as oilseed rape (*Brassica napus* L.), rice (*Oryza sativa*), oat (*Avena sativa*), sorghum (*Sorghum bicolour*) and sugarcane (Saccharum *officinarum*) are intermediate in domestication. For crops such as cranberry, there is little room for dedomestication whereas for highly domesticated crops such as maize, the switch from domesticated to feral may be too steep, limiting its potential to successfully establish a self-perpetuating population in natural or semi-natural habitats (Warwick and Stewart 2005).

In addition to being cultivated, *L. usitatissimum* is found as an escapee in waste areas, along roadsides (Richharia 1962), in disturbed land habitats, and in unmanaged ecosystems (CFIA 1994, Thomas et al. 1997). As a crop, flax possesses both domesticated and feral traits, with a greater proportion of domesticated traits than wild type traits. However, weedy characteristics such as plasticity in branching pattern, indeterminate flowering, and seed boll shatter may favor ferality in flax. There is no evidence to support the occurrence of seed dormancy. Feral populations of flax in naturalized areas could act as a genetic bridge allowing novel traits to spread within the environment. Feral flax could act as repositories for engineered genes where the pollen

source and pollen recipient are sexually compatible (i.e. with other feral flax populations, with cultivated flax crops and with wild or weedy relatives). However, the potential for escaped populations of cultivated flax to self-perpetuate in natural or semi-natural habitats has neither been investigated nor reported.

GENE FLOW

Gene flow is defined as the movement of genetic information from one population to another and takes place spatially and/or temporally (Hedrick 2005). Gene flow can occur by pollen movement or by direct movement of seed or vegetative propagules, but within an agronomic system these pathways are often linked (Slatkin 1987, Ennos 1994, Nielson et al. 2009). Although gene flow is not unique to GE crops, it has been suggested consistently and repeatedly as an environmental, food and feed biosafety concern (Mallory-Smith and Zapiola 2008). However, the presence of gene flow itself is not a hazard. Hazards occur when gene flow causes changes in food safety, market harm, and changes in biodiversity of agricultural or natural areas.

The global expansion in the development and cultivation of GE crops has increased concerns regarding the AP of GE material in non-GE (conventional or organic) crops (Demeke et al. 2006, James 2007). However, variation in the tolerance and traceability requirements for AP differs greatly among countries and has economic consequences for international trade (Demeke et al. 2006). There are many sources of AP, including extraneous pollen flow, impurities in planted seed, crop volunteers and commingling of seed during planting, harvesting, transportation and storage (Beckie and Hall 2008). While commingling of GE and non-GE grains and oilseeds in an unsegregated crop transport and production system is unavoidable, best management practices may mitigate the potential for pollen-and seed-mediated gene flow in the

environment and thus reduce the potential of exceeding international thresholds regarding AP.

Pollen-mediated gene flow

Pollen-mediated gene flow is the transfer and incorporation of genetic information between plant populations resulting from cross-pollination (Gustafson et al. 2005). Pollen dispersal is the main mode of gene flow in most flowering plants, including most major crops and can provide a mechanism of gene flow into populations of the same species (intraspecific) or sexually compatible wild relatives (interspecific) (Levin and Kerter 1974, Garcia et al. 1998).

A number of factors determine the likelihood and extent of pollen-mediated gene flow among plant populations. These include the reproductive biology of a species or cultivar within a species, flowering phenology, sexual compatibility and crossability, pollen load, environmental conditions at specific vegetative or reproductive stages of plant development, spatial and temporal distribution of pollen donors and recipient plants (Suneson and Cox 1964, Khan et al. 1973, Levin and Kerster 1974, Hamrick et al. 1979, Farris and Mitton 1984, Manasse 1992, Linder et al. 1998, Hucl and Matus-Cadiz 2001).

A widely acknowledged risk associated with the use of GE crops is that the transgenes could be transferred to wild or weedy relatives by hybridization (Raybould and Gray 1993, Hails 2000, Pilson et al. 2004). Pollen-mediated gene flow from a crop to a wild/native plant has been documented in 12 species of 13 major food crops including wheat (*Triticum aestivum* L. and *T.turgidum* ssp. *turgidum* L.), rice (*Oryza sativa* L. and *O. Glaberrima* Steud.), sugarbeet (*Beta vulgaris* L.), soybean (*Glycine max* L.), maize (*Zea mays* ssp. mays L.), barley (*Hordeum vulgare* L.), and canola (*Brassica napus* L. and *B. rapa* L.) (Ellstrand et al. 1999). In several instances, substantial levels of gene flow

to wild relatives of rice (Oka and Chang 1961), sugarbeet (Santoni and Berville 1992) and canola (Crawley et al. 1993) were observed under conditions commonly encountered in agricultural settings. Gene flow may alter the genetic diversity of a plant population(s) and change the ability to respond to changing stressors in the environment (insects, herbicide, drought, heat etc.) provided that the acquired genetic trait(s) result in a substantial fitness advantage (Gustafson et al. 2005). A better understanding of crop-to-crop and crop-to-wild gene flow is essential for ecological risk assessment of PNTs (Dale 1993, Conner et al. 2003).

Pollen movement in the environment

Pollen may be dispersed by wind and/or by insects. Pollen-mediated gene flow has been extensively studied and modeled in major crops species such as oilseed rape, wheat and maize. Canola is both wind- and insect-pollinated whereas wheat and maize are wind-pollinated only. For both moderately or highly outcrossing species (maize and canola) and for highly selfing species (wheat), the frequency of pollen-mediated gene flow generally declines rapidly with increasing distance from the donor field, often described by a leptokurtic curve (i.e. higher probability distribution in the tail than predicted by a normal distribution) (reviewed in Beckie and Hall 2008).

Reports of pollen-mediated gene flow in canola are highly variable and the relative contribution of wind and/or insects to pollen movement in the environment remains unclear (Beckie et al. 2003, Ramsay et al. 2003). Studies in Canada on canola (*Brassica napus* L.) found evidence of pollen flow at 336 m and at a distance of 800 m (Stringham and Downey 1982, Beckie et al. 2003), in the United Kingdom at 2.5 km (Timmons et al. 1995) and close to 3 km in Australia (Rieger et al. 2002). Interplant outcrossing in oilseed rape ranges 12 to 55% and the majority of cross-fertilization occurs

within 10 m of the receptor field (Beckie et al. 2003, Husken and Pfeilstetter 2007). While there is a general consensus that insects, particularly honey bees (*Apis mellifera* L.) and bumble bees (*Bombus* spp.) are the primary contributors to short-distance (a few metres) pollination, there is some evidence to suggest that insects also readily mediate long-distance (hundreds of meters) pollen dispersal (Ramsay et al. 2003, Funk et al. 2006). In Germany, Funk et al. (2006) found that wind direction did not have an effect on the distribution of short-distance (11 m maximum) cross-pollination in oilseed rape (*Brassica napus* L.), thereby suggesting that bees were the primary pollinator. In contrast, Cresswell et al. (2002) reported that insects were likely the main long-distance pollinator of oilseed rape as the flower of *B. napus* does not readily concentrate airborne pollen onto its stigma when facing upward and the flower is an ineffectual collector of airborne pollen when facing downwards. Ramsay et al. (2003) and Walklate et al. (2004) have similarly reported that insects are more likely to pollinate oilseed rape at long-distance than wind. However, other researchers maintain that the concentration and dispersal of pollen clouds enables wind pollination alone to be a sufficient explanation for medium to long-distance pollen dispersal (Timmons et al. 1996, Wilkinson et al. 2003, Hoyle et al. 2007). The abundance and foraging behaviour of bees and other insect pollinators varies among fields both seasonally and spatially and is thus likely to account for the observed differences among studies (Reboud 2003, Hayter and Cresswell 2006).

Considerable research has been conducted on pollen dispersal and cross-fertilization between maize genotypes (Jemison and Vayda 2001, Devos et al. 2005, Bannert and Stamp 2007, Sanvido et al. 2008). Maize is a monoecious plant with female (pistillate inflorescence) and male (staminate inflorescence) flowers formed in separate parts of the plant, leading to a high degree of cross-pollination (95%) between plants (Ma

et al. 2004, Weekes et al. 2007). Compared to the pollen of other wind pollinated species, the maize pollen grains are relatively heavy (0.25 μg) and large (70-100 μm) (Raynor et al. 1972, Di-Giovanni et al. 1995, Aylor et al. 2003). Due to its pollen characteristics, maize pollen has a high settling speed and most is deposited within 30-50 m of the source (Devos et al. 2005). For example, Bateman (1947) reported outcrossing rates up to 40% at a distance of 2.5 m and that cross-pollination dropped by 99% over a distance of 12 to 15 m. In a more recent study, Weekes (2007) reported cross-pollination rates up to a maximum of 60% within 2 m of the pollen source and at a distance of 100 m, rates of gene flow were less than 10%. Burris (2001) reported that only 1.11% of pollen grains at source were found at 200 m, but lower rates (0.06%) of cross-fertilization have been reported by Matsou et al. (2004) at the same distance. Under very arid and calm conditions, out-crossing between maize cultivars was not detected beyond 200 m by Baltazar and Schoper (2002).

In wheat, pollen-mediated gene flow depends on genotype, the receptivity of the stigmas, the viability and availability of pollen during the receptive period, and environmental conditions (relative humidity and temperature) (Waines and Hedge 2003). Wheat is a self-pollinated species with out-crossing rates usually less than 1-2% (Hucl and Matus-Cadiz 2001). However when plants are grown in close proximity, higher out-crossing rates (up to 10%) have been reported (Heyne and Smith 1967, Martin 1990, Lawrie et al. 2006). Gene flow in wheat has mostly been studied at distances of less than 50 m (Hucl and Matus-Cadiz 2001, Hanson et al. 2005, Wilson 1968, Miller et al. 1975). However, long-distance pollen flow in wheat is possible and has been detected at distances up to 2.75 km (Matus-Cadiz et al. 2007).

Transgene movement from transgenic flax to weedy relatives via pollen flow is currently being evaluated in western Canada under agricultural field conditions (Hall et al. 2006). Like wheat, flax is predominantly a self-pollinating crop and thus, seed-mediated gene flow may be more likely to contribute to transgene movement in the environment than pollen-mediated gene flow.

Seed-mediated gene flow

Seed-mediated gene flow primarily occurs through various forms of seed dispersal within and among agricultural fields but may also be facilitated by seed spill during transport, admixture of seed used for planting and by co-mingling of seed within the seed handling system. Although seed-mediated gene flow is a large source of gene flow in the environment and within the agricultural supply chain, it has received relatively little attention in the scientific literature.

Pre-dispersal and post-dispersal seed predation

Pre-dispersal and post-dispersal seed predation collectively limit seed-mediated gene flow in the environment as they are important forms of seed mortality (Crawley 1992, Cromar et al. 1999). Pre-dispersal seed predators can influence plant community dynamics by reducing the fecundity of host plant populations (Louda 1982) whereas post-dispersal seed predators influence the density and distribution of seeds in the soil seed bank (Harper 1977, Andersen and Ashton 1985, Louda et al. 1990).

Animals preying on undispersed seeds from trees or herbaceous plants are typically frugivorous birds (Jordano 2000, Willson and Traveset 2000, Herrera 2002, Hulme and Benkman 2002), but granivorous rodents and invertebrates have been reported to cause considerable damage to both maturing seeds and fruits (Forget et al. 1999, Reichman and Price 1993, Price and Joyner 1997, Herrera et al. 2002, Mezquida and

Benkman 2005). The most pervasive post-dispersal seed predators include birds, rodents, and ants (Brown and Davidson 1976, Brown and Heske 1990, Price and Joyner 1997, Hulme and Benkman 2002, Gomez 2004). Unlike pre-dispersal seed predation which occurs in localized areas for relatively short periods of time, post-dispersal seed predation exhibits spatial and temporal variability (Crawley 1992, Harrison et al. 2003) due to the seasonal influence of predator foraging, microhabitat effects on predator activity and seed redistribution (Barry 1976, Marino et al. 1997, Cromar et al. 1999). Furthermore, post-dispersal seed predation may occur shortly after seed dispersal, when seeds are released onto the ground surface, and after burial, when seeds have been incorporated into the soil profile (Hulme 1997, Price and Joyner 1997, Rey et al. 2002).

Large numbers of weed seeds are consumed by predators in agronomic fields, but the full impact of seed predation on weed population dynamics in agriculture has received insufficient attention (Hartzler et al. 2007). In experimental field trials in eastern Kansas, Cummings et al. (1999) reported that pre-dispersal seed predation by insects can result in large differences in fecundity in F_1 hybrid and wild sunflower plants. The average sunflower hybrid had 45 seeds per plant consumed by head-infesting insect larvae (36.5% of its seeds) while the average wild plant lost 95 seeds (only 1.8% of its seeds) to seed predators. Cummings et al. (1999) suggested that the larger seed size of the F_1 hybrid plants relative to the wild plants may have increased the survivorship of the insect larvae resulting in higher damage levels in hybrid flower heads than wild sunflower heads. Compared to their wild relatives, crop-wild hybrid sunflower plants are also more prone to post-dispersal seed predation (Alexander et al. 2001). Alexander et al. (2001) observed that post-dispersal seed predators including quail, cotton rats and foxes preferred the larger hybrid sunflower seed (47 mg) to wild sunflower seed (37 mg).

Other studies have examined the effects of tillage practices and crop residue management on the quantity of post-dispersal seed predation in agroecosystems (Burst and House 1988, Cromar et al. 1999). In general, reduced tillage systems tend to be associated with greater levels of seed predation than conventional tillage systems because the presence of high amounts of residue provides protection and a stable environment for seed predators (Mittelbach and Gross 1984, Burst and House 1988, Reader 1991). Burst and House (1988) reported that fall seed predation was 2.3 times lower in conventional-till soybean than in no-till soybean. In contrast, Cardina et al. (1996) found no differences in seed predation on velvetleaf (*Abutilon theophrasti* Medikus) seeds between no-till and conventional-till treatments as seed losses averaged 11.2% day^{-1} in both cropping systems. Similarly, Cromar et al. (1999) reported that post-dispersal seed predation of common lambsquarters (*Chenopodium album* L.) and barnyard grass (*Echinochloa crus-galli* L.) did not differ between mouldboard-plowed and no-tillage crop fields as seed losses averaged 32% for both. Cromar et al. (1999) concluded that the relationship between the level of disturbance in an agricultural field and post-dispersal seed predation is non-linear and other factors, such as the seasonality of seed predation, food availability and invertebrate mobility play important and collective roles in determining the quantity of seed predation. In western Canada, seed predation has not been quantified in agroecosystems but if data can be extrapolated from other regions, seed predation can be an important limiting factor of volunteer crop populations.

Animal attachment or ingestion

The spatial distributions of dispersed seeds play a crucial role in determining the structure and dynamics of some plant populations. The capacity for long-distance seed dispersal may be a key factor in the survival of local populations, especially in

fragmented landscapes (Ozinga et al. 2004). Epizoochory (dispersed by attachment to the surface of animals of visid (sticky), barbed, or hooked seeds or fruits) and endozoochory (the dispersal of plant seeds or spores within the body of an animal, as passing through the animal's digestive system) results in weed seed movement in agricultural fields into different microhabitats (which modulates seed germination and seedling survival) and also aids in seed dispersal into naturalized or ruderal areas (Benvenuti 2007). While large sized animals are frequently involved in epizoochory in agroecosystems, small mammals such as mice and voles are also common dispersers of diaspores (Kiviniemi and Telenuis 1998, Boedeltje et al. 2004). Birds are recognized as the main dispersal endozoochorous agent of seeds and fruits. While it is suggested that seed movement by animals is an important mechanism of seed dispersal for some plant species, flax seed morphology limits its transport by animals.

Seed shattering and seed loss during harvest operations

Sources of crop seed bank inputs include the initial seeding of the crop and, more importantly, the loss of seed prior to and during harvest. Seed dehiscence prior to harvest is a weedy trait that is selected against during crop domestication. However, shedding of grain occurs naturally in wheat at maturation and like the fully mature pods of oilseed rape and pea; the bolls of flax also are sensitive to opening, resulting in seed loss (Anonymous 2002, Andersen and Soper 2003, Child et al. 2003, Von Stackelberg et al. 2003). In general, seed losses prior to harvest may be attributed to disturbance of the crop canopy by wind as drying seed pods become fragile and split with only small energy input (Child et al. 2003, Von Stackelberg et al. 2003). A reduction in the sensitivity of pods to opening would increase the proportion of the yield recovered by the combine harvester. Plant organ abscission and the molecular mechanisms underlying the process is

an active area of research (Child et al. 1998, Roberts et al. 2000, Patterson 2001, Child et al. 2003, Von Stackelberg et al. 2003).

Harvest losses of crop seed are highly variable and can be sizeable. In commercial fields in southern Alberta, average safflower seed losses from combine harvesters ranged from 231 to 1069 seeds m^{-2} (McPherson 2008). Wheat harvest losses can range from 35 to 800 seeds m^{-2} with an appropriate average of 300 seeds m^{-2} (Clarke 1985, Anderson and Soper 2003, Decorby et al. 2007). Canola yield losses and seed bank additions associated with harvest procedures on commercial farms in Northern Saskatchewan averaged 107 kg ha^{-1} or the equivalent of 3,600 seed m^{-2} (Gulden et al. 2003). The 1000-seed weight of flax (6-7 g) is considerable lower than safflower (35-40 g) and spring wheat (30-40 g), and heavier than canola (3-4 g). In general, a lower 1,000-seed weight typically results in a higher number of seeds per unit weight added to the seedbank (Gulden et al. 2003).

Grain loss at harvest varies with timing of the harvest operations and with type of combine harvester. In the Pacific Northwest, canola seed losses as high as 28.5% have been attributed to delayed harvesting (Brown et al. 1995) with proportionally similar yield losses in Europe (25%) when windrowing of the crop was delayed (Price et al. 1996). Loss of grain from combine harvesters occurs both with grain entry at the cutter bar and with screenings discarded after threshing (Hughes 1974). Komatsuzaki and Endo (1996) summarizing field surveys in Japan reported that grain loss at harvest was greater with head-feeding (also known as stripper header) compared with combines with conventional grain headers. In western Canada, Gulden et al. (2003) reported that improper combine settings and excessive combine operating speeds resulted in above average canola seed bank additions. In California, harvest losses for safflower have been

estimated at 3 to 4% for yields of 2200 to 3400 kg ha^{-1} when safflower is at ca. 9% and combine adjustments are optimal (Knowles and Miller 1965). Information is currently lacking on the harvest yield losses of flax seed and flax seed bolls in western Canada.

Seed dispersal among agricultural fields and into non-agricultural areas

Weed and crop seed dispersal by farm machinery in and among agricultural fields is well documented (Blanco-Moreno et al. 2004, Shirtliffe and Entz 2005, Barroso et al. 2006). Combine harvesters have the potential to disperse seeds the farthest of any dispersal vectors within an arable farming system and are responsible for seed movement both within and between fields (Cousens and Mortimer 1995). Tall weeds, especially those which retain seed until harvest are more likely to be subjected to movement by combine harvesters than short weeds that readily dehisce their fruit prior to harvest (Barroso et al. 2006). McCanny and Cavers (2006) evaluated the seed dispersal of black (large seed size) and yellow (small seed size) proso millet (*Panicum miliaceum* L.) by combine harvesters in Ontario, Canada. An average of 0.9% of the yellow-seeded biotype was carried more than 50 m by combines, while 3.3% of the black seeds were carried the same distance. In Concabella, Spain, Blanco-Moreno et al. (2004) reported that seeds of *Lolium rigidum* were dispersed > 18 m from established stands by combine harvesters in cereal fields. Howard et al. (1991) introduced painted seeds of interrupted brome (*Bromus interruptus* L.) and *Bromus sterilis* L. during harvesting of a barley crop. Within the field, the majority of seeds were moved an average of 1.9 m and no seeds were moved more than 20 m from the point of introduction. Shirtliffe and Entz (2005) reported that seeds of *Avena fatua* L. were dispersed up to 145 m by combine harvesters in Saskatchewan, Canada. In contrast, Barroso et al. (2006) reported that combine harvesting does not move

A. fatua long distances in agricultural fields. Short dispersal distances (2 m) of *A. fatua* in cereal fields were attributed to early seed shed (> 90%) (Barroso et al. 2006).

The design of the combine harvesters may also influence the dispersal distance that seeds are moved (Barroso et al. 2006). Ballare et al. (1987) reported that the dispersal distance of oakleaf datura (*Datura ferox* L.) seeds harvested with a soybean crop differed among combine harvesters. Ballare et al. (1987) found that two of the combines dispersed oakleaf datura seeds up to 20 m from the source while the third combine moved the seed up to 98 m.

Weed and crop seed can be dispersed into neighbouring non-agricultural areas along roadside verges, around storage facilities and along railway lines through seed spillage during transport and through transportation of farm machinery. Herbicide-tolerant (glyphosate and glufosinate) canola (*Brassica napus* L.) volunteers have been found along railways and roads in the province of Saskatchewan; and near the port of Vancouver, British Columbia (where most of the canola seed destined for export is transported by rail) (Yoshimura 2006). In the United Kingdom, Crawley and Brown (1995) studied feral oilseed rape (*Brassica napus* L.) populations on the verges of the M25 motorway. Crawley and Brown (1995) reported that verges next to the carriageway carrying traffic towards the main oilseed rape (*Brassica napus* L.) crushing plant at Erith in Kent had more plants than the opposite verge carrying traffic away from Erith. Mean oilseed rape densities were also higher in the vicinity of exit and entry slip roads than on sections of verge between motorway junctions (Crawley and Brown 1995). An Australian weed survey encompassing a total of 400 km of road and 400 observations, cited incidences of ruderal canola plants growing within 5 km of the roadside in major canola (*Brassica napus* L.) growing districts in Tasmania (14%) and Victoria (13%) (Agrisearch

2001). The occurrence of predominantly isolated plants suggests that they had not originated from seed dropped from plants the previous season, but resulted from individual seeds being dropped during transportation (Anonymous 2002).

Admixture of seed used for planting and co-mingling of seed within the seed handling system

The term "adventitious presence" refers to the unintentional and incidental commingling of trace amounts of one type of seed, grain or food product with another (Demeke et al. 2006). When used in relation to GE crops, the term describes the inadvertent presence of transgenic seeds or other material in non-GE (conventional or organic) crops (Kershen and McHughen 2005). Low levels of impurities are inherent in commodity crops due to the nature of the supply chain; crops are grown in close proximity to other crops and common equipment is frequently used to plant, harvest, transport and store grain (Devos et al. 2005). Seed-mediated gene flow may be a greater source of AP than pollen-mediated gene flow, especially for small seeded crops like oilseed rape in which seed loss and volunteerism is common and frequent in a diversity of agricultural systems (Beckie and Hall 2008). The risk for gene flow via seed through natural dispersal mechanisms or human actions is generally greater for small seeded crops than for larger seeded crops (Mallory-Smith and Zapiola 2008).

Volunteer plants can emerge in large numbers and plants that are uncontrolled may contribute to AP via gene flow. Natural pollen flow from flax volunteers containing a transgene may contribute to AP by cross-pollination with conventional flax crops in adjacent fields and with sexually compatible wild or weedy relatives. Uncontrolled transgenic flax volunteers may also produce seed which may be admixed (co-mingled) during harvest with food or feed crops thereby contributing to AP. Although seed

admixture in the same field and co-mingling of seed within the seed handling system are important sources of AP, seed-mediated gene flow within the seed handling system has rarely been investigated and/or quantified (Messan et al. 2007).

GENE FLOW SUMMARY

Gene flow can occur via pollen and seed. Gene flow is influenced by a number of factors including the biology of the plants species, the environment, agricultural production practices and by the supply chain (Figure 2-1). Outcrossing (allogamous) crops such as canola and maize have a higher potential for gene flow via pollen than self-pollinating crops such as wheat and flax. The distance at which pollen-mediated gene flow can occur is variable. Most gene flow via pollen will occur at relatively short distances because pollen is viable only for a short time as it is subject to desiccation. With distance, the pollen cloud is diluted by pollen from adjacent plants and there is a decrease probability of landing on stigmatal surfaces with distance from the pollen source. Gene flow via seed may occur by natural dispersal mechanisms via animals, wind or water or by human actions via tillage and transport. Unlike natural seed dispersal mechanisms, seed movement by humans results in a limitless dispersal capability. Due to the 'permeable' nature of the supply chain, seed loss and thus seed-mediated gene flow can occur at most stages of production. When gene flow via pollen or seed from a GE crop occurs, it results in AP of the transgene. If seeds of a GE cultivar are mixed with seed of a non-GE cultivar, commingling of seed can occur at planting. It can also occur if volunteer plants (plants that emerge from seed from a previous crop from the seed bank) pollinate a sexually compatible plant or population or produce seed that is subsequently harvested with the crop, or during post harvest operations such as cleaning, transport or storage. Although more emphasis has been placed on gene flow via pollen in the scientific

literature, gene flow via seed may be of greater importance for long-distance dispersal of transgenes (Mallory-Smith and Zapiola 2008).

THE USE OF GE CROPS FOR BIOINDUSTRIAL FARMING

Gene flow from regulated and unregulated GE crops is well documented and is often mediated by pollen or seed. Transgenes from GE crops have been detected in non-GE feed and food products and have been widely reported in the media and in the scientific literature. The AP of an unregulated transgene is illegal and unregulated transgenic material in food or feed has lead to economic consequences. Instances such as transgenic Starlink™ corn (Carter 2006, EPA 2007), glyphosate resistant creeping bentgrass (*Agrostis stolonifera* L.) and GE corn intended for Plant Molecular Farming (a variety of corn developed by Prodigene Inc. which expressed a seed specific antigen for a swine vaccine) (Ellstrand 2003, Arcand and Arnison 2004, Elbehri 2005) has lead to changes in government regulatory policies and resulted in more stringent confinement procedures for field experiments. These instances also heightened biosafety awareness in the biotechnology industry and major seed companies which develop GE crops have become more conscientious in terms of safety compliance (McPherson 2008).

Both the public's opinions and perceptions and government policy on biotechnology are factors that could influence the rate of commercialization of GE crops. Bio-industrial farming opportunities encompass existing and emerging markets including: bioenergy, bio-refining, biomaterials and functional foods. Unlike insecticide and herbicide resistant GE crops, whose products are considered substantially equivalent to their conventional counterparts, the products of bioindustrial crops may differ, raising new marketing and safety concerns, and requiring segregation from conventional crops. Effective management practices will be required to minimize the potential for AP in conventional

products as a result of gene flow. A strong understanding of the biology of volunteer flax and the agronomic practices which mitigate its occurrence in agroecosystems is essential to the reduction of seed-mediated gene flow pathways and to lessening the persistence of transgenes in the environment.

Table 2-1. The twelve growth stages of flax (*Linum usitatissimum* L.).

Growth Stage	Characteristics
1	Cotyledon
2	Growing point emerged
3	First pair of true leaves unfolded
4	Third pair of true leaves unfolded; start of leaf spiral
5	Stem extension
6	Buds visible
7	First flower; early branching
8	Full flower; bolls start forming
9	Later flower; most branches and bolls formed
10	Green boll; seed white and lower leaves are yellow
11	Brown boll; seeds light brown and branches/stem yellow
12	Seed ripe; seeds maturity and branches/upper leaves senescent

Source: Anonymous 2002

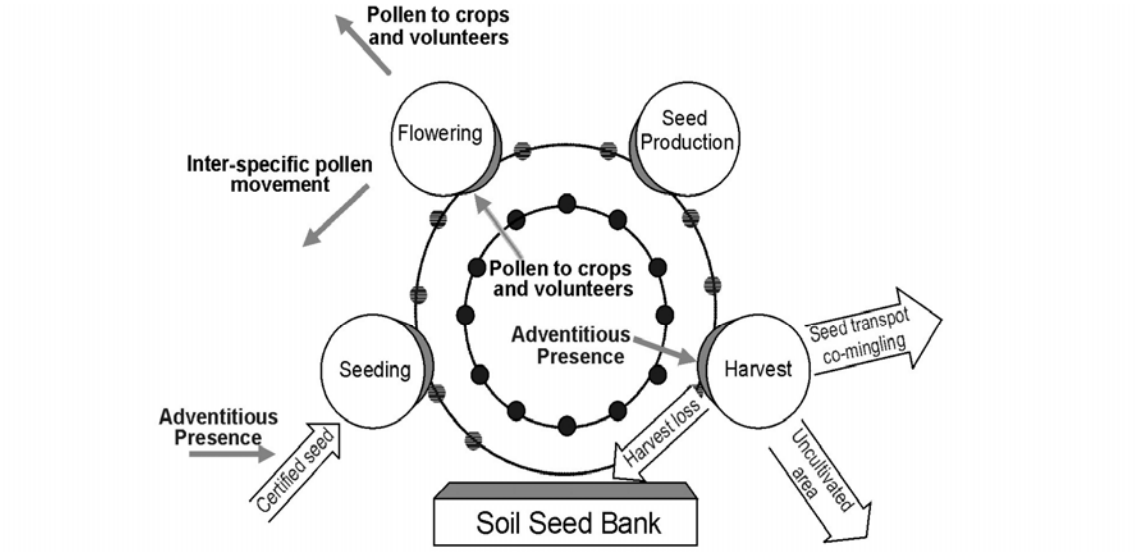

Figure 2-1. Pollen- and seed-mediated gene flow from flax as a platform crop for bioproducts (Warwick et al. 2008). The inner circle in the center of the diagram represents the transgenic crop while the larger outer circle represents transgene dispersal to nearby conventional crops and crop volunteers. The large square box and the smaller circles on the outside of the outer center circle represent potential sources of gene flow in the environment while arrows represent pathways of transgene dispersal.

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Chapter 3: The occurrence and persistence of volunteer flax (*Linum usitatissimum* L.*)* in twenty Alberta fields

INTRODUCTION

The oilseed crop flax (*Linum usitatissimum*) is being evaluated as a potential platform for the production of bioindustrial products (Anonymous 2005, Flax Council of Canada 2009a). Development of transgenic flax varieties for bioproducts raises concerns about the movement and persistence of transgenes in agroecosystems. The cultivation of genetically engineered (GE) crops has also increased concern about the adventitious presence (AP) of GE seeds or other material in non-GE (conventional and organic) commodity crops (Kershen and HcHugen 2005). Market disruptions have occurred due to the AP of GE material in non-GE products (Segarra and Rawson 2001, Harl et al. 2003, Demeke et al. 2006). The presence of GE flax in conventional flax products may limit access of Canadian flax to important export markets abroad such as the European Union (EU). In 2008, Canada produced 767.9 thousand metric tonnes of flax seed (includes conventional and organic production) (Statistics Canada 2008) and exported > 60% of the total production to Europe (Flax Council of Canada 2009b). The importance of the European market and its sensitivity to the importation of GE crops has resulted previously in the deregulation of transgenic flax (CDC Triffid) (McHughen et al. 1997, Flax Council of Canada 2009c). Consumer and political opposition to GE crops for food and feed continue to be strong in the EU and these concerns may block future transgenic development of the crop. Gene flow is a process that may initiate or contribute to an AP concern. If flax is to be cultivated for bioproducts, an understanding of gene flow in flax is required to effectively segregate transgenic flax from conventional crops.

Flax has been grown for more than 30 years on the Canadian prairies, primarily as an oilseed for non-food uses (Lay and Dybing 1989, Lafond et al. 1992). As a minor crop, cropping area varies, ranging from 1.30 to 2.14 million acres between 1998 and 2008 (Anonymous 2009). In western Canada, flax is usually followed in rotation with cereals and is rarely grown on the same field more than once in a four year crop rotation (Wall and Smith 1999, Anonymous 2002). Flax is grown in tillage systems ranging from conventional tillage (CT), where soil is cultivated in fall and prior to seeding, to no-till (NT), where soil disturbance occurs only when the crop is sown (Anonymous 2002). Reduced tillage (RT) practices are any farming practice which involve a fewer number of cultivations than those used in CT.

Like other crops, volunteer flax is also a component of the weed population (Leeson et al. 2005). Seed and seed boll losses occur prior to and during harvest and this seed enters the soil seed bank. The relative abundance of volunteer flax, a composite index of species frequency, field uniformity and field density, has increased relative to other species across western Canada over the last past 30 years (Leeson et al. 2005). Averaged across Alberta, Saskatchewan and Manitoba, volunteer flax ranked as the 32nd most abundant weed in the 1970s and as the 26th most abundant in the early 2000s (Leeson et al. 2005). This reflects changes in crop management practices including, reduction in the use of tillage and an increase in using pulse crops in rotation (Thomas et al. 1997, Wall and Smith 1999, Blackshaw et al. 2006). Tillage regime changes the distribution of seeds in the seed bank, environmental conditions of the seed microsite, including light, moisture, thermal fluctuations, and gas diffusion, but all annual weeds do not respond similarly to changes in tillage (Thompson and Grime 1983, Boyd and Van Acker 2004). There are conflicting reports on the response of volunteer flax to tillage

systems (RT and CT). Using data derived from Manitoba field surveys, volunteer flax has previously been reported to be more abundant in RT than in CT fields (Thomas et al. 1997). In contrast, Blackshaw et al. (2006) reported that in a summary of 56 site-years of data, volunteer flax was not consistently associated with a specific tillage regime. The relevence of these reports are difficult to assess because affects of tillage may be confounded by herbicide usage and their interactions.

Volunteer flax is poorly controlled in pulses, such as peas and lentils, which lack effective herbicides for control (Anonymous 2007) while in cereals it can be controlled by a number of registered herbicides (Derksen and Wall 1996, Wall and Kenaschuk 1996, Wall and Smith 1999). Uncontrolled GE flax volunteers can serve as a pollen source for the dispersal of transgenes to neighboring flax crops and may hybridize and form viable F_1 plants with at least nine wild species of *Linum* (Reviewed in Jhala et al. 2008). Risk assessment of transgene movement via pollen from transgenic flax to its weedy relatives is in progress (Hall et al. 2006). Transgenic flax volunteers that survive to set seed may replenish the seed bank or be harvested with the subsequent crop resulting in AP. The contribution of flax volunteers to replenishment of the seed bank has not yet been documented.

Crop seed bank inputs occur during the initial seeding of the crop and crop seed losses incurred prior to and during harvest. The soil seed bank is an essential component of the population dynamics of crop volunteers, buffering populations through time. In general, annual crop seeds do not persist for very long periods of time in the soil seed bank (Cavers and Benoit 1989). In field surveys where volunteer seed replenishment was not controlled, Beckie (2001) reported that volunteer wheat may continue to emerge for up to five years in rotation with subsequent crops. However, when seed production is

suppressed, volunteer wheat has a seed longevity of only up to two years in the soil seed bank (Harker et al. 2005) illustrating that seed replenishment may extend the persistence of weed populations over time and indicating the importance of effective volunteer control in reducing the potential for seed-mediated gene flow. In Saskatchewan, Gulden et al. (2003) reported that canola seed may persist in the seed bank for three years. However, using data from weed surveys, Légère et al. (2001) reported that volunteer canola (*Brassica napus* L.) continued to emerge in western Canadian cropping systems for up to four years after production. The contribution of seed replenishment to continuing weed seed banks has not been quantified. It is likely that both seed persistence and seed replenishment from volunteers play important roles in population persistence. The seed bank dynamics of volunteer flax are likely to play a key role in the potential for seed-mediated gene flow, but the persistence of flax seed on commercial farms in western Canada has not yet been documented.

The presence of volunteer flax has not been extensively documented in Alberta, Canada. Our objective was to monitor the occurrence and persistence of volunteer flax in twenty commercial fields in central Alberta over a three year period under a diversity of cropping systems (tillage and crop type). Tillage regimes included CT and RT and crop types included a number of different cereal crops (wheat, barley, oats) as well as canola and peas.

MATERIALS AND METHODS

Data collection

In the fall of 2004, twenty flax fields were selected from fourteen flax producers within a 300 km radius of Edmonton, Alberta, Canada. Surveyed fields were chosen to provide a diversity of environments and cropping systems. Producers provided

information on tillage practices (timing and intensity) and crop rotation pertaining to the field being surveyed. Surveyed fields ranged in size from 20 to > 200 hectares (data not shown). On each surveyed field, flax had not been grown four or more years prior to 2004, thereby minimizing the potential for confounding effects from a pre-established seed bank.

Fields were surveyed over a three year period, from the spring of 2005 to the fall of 2007 and five times throughout each growing season: before seeding (PREPLA), after seeding and before in-crop herbicide application (PREHERB), after in-crop herbicide application (POSTHERB), before harvest (PREHARV) and after harvest (POSTHARV) each year (Table 3-1). Fields were surveyed at least two weeks before or after each crop management practice. In 2005, the year following flax production, sixteen fields were seeded to cereals, either wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) or oats (*Avena sativa* L.), three were seeded to canola (*Brassica napus* L.) and one field was chemically fallowed (cropland was not seeded and treated with herbicide to control weeds) (Table 3-2). In 2006, eight of the surveyed fields were seeded to cereals and two to peas (*Pisium sativum* L.), one field was chemically fallowed and the remaining nine fields were seeded to canola (*Brassica napus* L.). In 2007, eleven fields were sown to cereals and two of the fields sown to peas and six to canola (Table 3-2). For all three years, five fields were in CT and fifteen were in RT (Table 3-2).

Volunteer flax was enumerated using a set pattern based on the methodology of Thomas (1985) using a modified W pattern. To begin the survey, the surveyor walked 100 paces along the edge of the field, turned at right angles, and walked 100 paces into the field. At this point, the sampling began. Five locations were counted along each arm of the W pattern, resulting in twenty locations, 25 m apart. The number of individuals and

their associated growth stage was determined in a 0.25 m^{-2} quadrat at each of the twenty locations.

Data analysis

Data were subject to analysis of variance (ANOVA) with a mixed model (PROC MIXED) using statistical analysis software (SAS) (SAS Institute Inc. 2007). The assumptions of the variance test were tested ensuring that residuals were random, homogenous with a normal distribution about a mean of zero. The test confirmed that the data met the assumptions of analysis of variance (ANOVA). The density of volunteer flax was analyzed within a mixed model using ANOVA within a completely randomized design in SAS. To determine differences in flax densities among sites, a three-level nested design structure was used where year and sample period effects were considered random, and fields which were nested in sites and cropping system (tillage and crop type) were considered fixed. Years were analyzed separately due to differences in the number of elapsed days between survey sample times. Lsmeans generated by the mixed model ANOVA are presented. Orthogonal contrasts statement statements were performed as part of the ANOVA procedure. Differences were considered significant when $P \leq 0.05$.

For each survey period (PREPLA, PREHERB, POSTHERB, PREHARV, POSTHARV) each year, data were summarized using standard format used in Weed Survey Series Reports (Leeson et al. 2005). The frequency (number of surveyed fields in which volunteer flax occurred), field uniformity in all surveyed fields (the number of quadrats in which volunteer flax occurred), field uniformity in individual fields (field uniformity in all surveyed fields expressed as a percentage of the number of quadrats for occurrence fields only), field density in all surveyed fields (a measure of the number of volunteer flax plants counted in a square meter), field density in individual fields (field

density values for volunteer flax in all surveyed fields averaged over only the fields in which volunteer flax occurred), high density (the highest field density of volunteer flax recorded in all surveyed fields), and highest typical growth stage (the highest growth stage of volunteer flax recorded in all surveyed fields) of volunteer flax calculated.

RESULTS AND DISCUSSION

Influence of cropping system (tillage and crop type) on volunteer flax densities

Influence of tillage regime on volunteer flax density

Although we predicted that cropping system (tillage and crop type) would affect volunteer flax densities, in only a few instances were differences significant. Cropping system may have been confounded by herbicide regime (timing and intensity), and this may have influenced volunteer flax densities within and amoung survey years. Tillage intensity did not influence volunteer flax densities in cereals fields in all site-years (Tables 3-3, 3-4, and 3-5). The effect of RT systems versus CT systems on volunteer abundance in other crops appears to be species specific (Derksen et al. 1993). For example, canola, fall rye and winter wheat generally occur at higher densities were more strongly associated with RT than CT systems (Derksen et al. 1993); whereas lentil, pea, barley and sunflower are more abundant with CT than with RT systems (Blackshaw et al. 2006). In addition, in field trials conducted in Saskatchewan, Derksen et al. (1993) reported that volunteer flax was associated with direct seeding and/or RT systems more than CT systems. In contrast to these reports, Blackshaw et al. (2006) reported that in western Canada volunteer flax was not associated with a specific tillage system based on 56 site-years of data. Our results are in agreement with Blackshaw et al. (2006) as volunteer flax does not appear to be strongly responsive to tillage (RT or CT) as densities of flax were relatively similar in RT and CT fields. However, our ability to detect

differences in volunteer flax densities among tillage regimes in surveyed commercial fields was limited, both by the number of fields sampled and by the diversity of the cropping systems sampled.

Influence of crop type on volunteer flax density

Crop type influenced the density of volunteer flax in surveyed commercial fields in only a few survey periods within and among years (2005-2007). In 2005, 16 of the 20 fields surveyed contained a cereal crop (Table 3-3). In practice, producers typically follow flax in rotation by wheat, barley or oats. Under RT in 2005, densities of volunteer flax were higher in canola fields (0 to 25 plants m^{-2}) than in cereal fields (0 to 11.6 plants m^{-2}) during two survey periods (PREHARV and POSTHARV) (Table 3-3). In contrast under RT in 2006, volunteer flax densities were lower in canola (0 to 2.4 plants m-2) than cereal (0 to 78.2 plants m^{-2}) fields (PREHERB and POSTHARV) (Table 3-4). The effect of crop type (cereals and canola) on volunteer flax densities in CT fields could not be compared in 2006 due to the limited number of fields sampled. Under RT in 2006, volunteer flax densities were lower in canola fields (0 to 2.4 plants m^{-2}) than in pea fields (1 to 9.6 plants m^{-2}) when measured PREHERB and POSTHERB (Table 3-4). Under RT in 2007, volunteer flax densities were again lower in canola fields (0 plants m^{-2}) than in pea fields (1.4 plants m^{-2}), but only during one survey period (POSTHERB) (Table 3-5). Historically, cereals have been found to be more competitive than canola and peas, and peas less competitive than canola (Dew 1972, Swinton et al. 1994, Blackshaw 1994, O'Donovan et al. 2000). In addition, weed control in canola is likely to be greater than in peas where the primary herbicides used are imidazolinones, offering limited control of flax. In this study, volunteer flax was more abundant in pea fields than canola and cereal fields and will require more intensive management in crop sequences that include a pulse

crop than rotations without a pulse crop. This result was expected given the relative competitive ability and weed control options of these crops.

Volunteer flax populations are influence by both tillage system, crop type and herbicide usage and these effects are confounded within cropping systems and environments. Survey data does show the potential range of responses within grower's fields under these cropping systems and can provide guidance for best management practices.

Occurrence of volunteer flax in surveyed commercial fields in 2005, 2006 and 2007

Volunteer flax persistence

Volunteer flax emerged in commercial fields up to 3 years after flax production (Table 3-6). Flax volunteer densities were highest in all fields in the year following flax production (2005) and then diminished over time. This may be attributed to high initial quantities of volunteer flax seed and seed bolls on or near the soil surface following flax harvest and the subsequent depletion of the seed bank due to germination and loss of seed viability. Average volunteer flax densities were highest at the PREHERB sampling period in all survey years (2005-2007) (Table 3-6). In 2005 PREHERB, volunteer flax was present in 95% of surveyed fields, was uniform where it occurred (79.5%), and appeared with average densities ranging from 10.2 to 570.2 plants m^{-2} (Tables 3-3 and 3-6). In 2006, volunteer flax was present in only 50% of fields at an average density of 12.2 plants m^{-2} (Table 3-6). In 2007, volunteer flax was present in only 15.8% of surveyed commercial fields with average densities ranging from 0.4 to 1.8 plants m^{-2} (Table 3-5 and 3-6). This suggests the seed bank is effemeral, diminishing over time. Additionally, the densities of volunteer flax observed each survey year PREHERB emphasizes the

importance of chemical weed control in subsequent crops. Volunteer flax densities were reduced in all surveyed commercial fields POSTHERB (Table 3-3, 3-4 and 3-5), most likely due to the application of in-crop herbicides and crop competition. The density (23 plants m^{-2}) and field uniformity (42.3%) of volunteer flax in occurrence fields POSTHERB in 2005 was similar to previous densities and field uniformities reported in 1997 and 2001 Alberta weed surveys (Thomas et al. 1997, Leeson et al. 2002). In surveyed commercial fields in 1997, Thomas et al. (1997) reported that volunteer flax was present at an average density of 18.0 plants m^{-2} and occurred at a field uniformity of 29%. In 2001 in fields in which volunteer flax occurred, Leeson et al. (2002) reported that volunteer flax was highly uniform (65.2%) and was present at an average density of 19.6 plants m^{-2}. In our fields in 2006, volunteer flax densities POSTHERB were reduced below 6 plants m^{-2} in occurrence fields and the field uniformity of volunteer flax in all fields surveyed decreased to 10% (Table 3-7). In 2007 POSTHERB volunteer flax occurred only in one field and at low densities (< 2 plants m^{-2}). These volunteer flax seedlings emerged in clusters from decayed flax seed bolls located within the first 3 cm of the soil surface, rather than from single seeds (data not shown), suggesting that the quantity of seed bolls in the soil seed bank and their ability to resist degradation may influence the density and persistence of volunteer flax in the environment. Although chemical weed control measures may mitigate the occurrence of volunteer flax under a diversity of cropping regimes, seed persistence may be influenced by other factors which are difficult to predict.

At the end of the growing season (PREHARV and POSTHARV), volunteer flax was observed at low densities in all site-years. In 2005, volunteer flax was present in only three of fourteen surveyed sites PREHARV with the highest density being 25 plants m^{-2}

(Tables 3-3 and 3-6). In 2005 POSTHARV volunteer flax densities ranged from 0 to 11 plants m^{-2} and occurred in 20% of fields surveyed (Table 3-3 and 3-6). In 2006 PREHARV, volunteer flax occurred in a greater proportion of surveyed fields (65%) compared to 2005 PREHARV (Table 3-6), but the densities recorded in 2006 in all surveyed sites were considerably lower (0 to 5.2 plants m^{-2}) (Tables 3-4 and 3-6). Densities of volunteer flax were observed to have further declined when measured POSTHARV in 2006 as flax was present in only 15% of surveyed fields at an average density of 3.6 plants m^{-2} in occurrence fields (Table 3-6). Flax is not tolerant to frost (Anonymous 2002) and seedlings observed in 2005 and 2006 POSTHARV were unlikely to have survived the Canadian winter. In 2007, volunteer flax was not present PREHARV or POSTHARV in surveyed commercial fields suggesting exhaustion of the flax seed bank (Table 3-6).

The high densities of volunteer flax observed each survey year PREHERB and the subsequent reduction in the density of volunteer flax at sampling times following herbicide application emphasizes the importance of chemical weed control in subsequent crops. Weed control measures should be aimed to reduce fecundity and seed return to the seed bank to limit the longevity of volunteer survival.

Implications of this research

Similar to other volunteer crops, mean flax population densities decline over years in rotation, persisting at least three growing seasons after seed production. Seedlings observed in 2006 and 2007 may have arisen from the original cohort from 2004 or may have been the result of volunteer seed production in the previous year. The uniformity of occurrence and the frequency of fields where volunteer flax occurred declined over the

rotation. The highest density of volunteer flax counted in any quadrat in the third year after a flax crop was very low at < 2 plants m^{-2}.

Control of volunteer flax was adequate as average densities ranged from 0 to 25 plants m^{-2} (0 to 25,000 plants ha^{-1}) PREHARV and at these densities, volunteer flax is of little agronomic concern as volunteer flax is considered an uncompetitive weed (Wall and Smith 1999). However, uncontrolled flax volunteers with novel traits that are adjacent to a subsequent flax crop may complicate weed management. Although flax is a self-pollinated crop (Lay and Dybing 1989), due to the construction and mechanism of the flower, cross-pollination does occur (up to 5%) (Dillman 1938, Rubis 1970). Flax also has weedy relatives in Canada with which it could potentially form hybrids (reviewed in Jhala et al. 2008). These hybrids could serve as a bridge for gene transfer to subsequent flax crops as well as providing the opportunity for introgression of transgenic material into wild and weedy populations. Uncontrolled flax volunteers were observed in reproductive stages of growth (first flower to boll formed) in some site-years and these volunteers may have contributed to pollen- and seed-mediated gene flow (Table 3-6). However, the contribution of volunteer flax to gene flow may have been limited as flax volunteers were only observed in reproductive stages of growth in the second and third year following flax production (Table 3-6).

Growing transgenic flax for bioproducts may necessitate an incremental cost for controlling volunteers, but the stringency of the mitigation procedures would be dependent on the acceptable thresholds for AP in commodity products and presence of volunteer flax in the environment. Information on presence, size (growth stage) and density of volunteer flax infestations is critical for planning control efforts. While this weed survey documented the persistence of volunteer flax in commercial fields, it did not

quantify the role of seed replenishment from uncontrolled flax volunteers to continuing weed seed banks. If transgenic flax is to be effectively segregated from conventional flax, the seed bank dynamics of volunteer flax should be considered as it plays a pivotal role in perpetuating volunteer survival and seed-mediated gene flow.

Table 3-1. Dates of volunteer flax survey periods for 2005, 2006 and 2007.

	Year		
Survey Period	2005	2006	2007
PREPLA[a]	May 6, 7	May 7, 8	May 14, 15
PREHERB[b]	June 14, 15	June 14, 15	June 14, 15
POSTHERB[c]	August 1, 2	July 15, 16	July 5, 6
PREHARV[d]	September 18, 19	August 14, 15	August 23, 24
POSTHARV[e]	October 15, 16	October 6, 7	October 19, 20

[a]Before seeding
[b]After seeding and before in-crop spray
[c]After in-crop spray
[d]Before harvest
[e]After harvest

Table 3-2. Crop type and tillage system in twenty surveyed commercial fields in 2005, 2006 and 2007.

				Year		
	Tillage			2005	2006	2007
Field	System	Timing	Total no. of operations	Crop type		
1	CT[a]	fall, spring	2	wheat	canola	barley
2	RT[b]	n/a[c]	0	barley	barley	barley
3	CT	spring	1	barley	pea	canola
4	RT	n/a	0	canola	barley	—[d]
5	CT	fall, spring	2	oats	canola	barley
6	CT	fall, spring	2	barley	oats	canola
7	RT	n/a	0	barley	canola	barley
8	RT	n/a	0	wheat	pea	canola
9	RT	n/a	0	wheat	canola	pea
10	RT	n/a	0	barley	canola	pea
11	RT	n/a	0	barley	canola	wheat
12	RT	n/a	0	fallow	wheat	barley
13	RT	n/a	0	barley	fallow	canola
14	CT	fall, spring	2	barley	canola	wheat
15	RT	n/a	0	wheat	barley	barley
16	RT	n/a	0	canola	canola	wheat
17	RT	n/a	0	barley	oat	canola
18	RT	n/a	0	barley	barley	barley
19	RT	n/a	0	barley	barley	canola
20	RT	n/a	0	canola	canola	barley

[a]Conventional tillage
[b]Reduced tillage
[c]Not applicable
[d]Field was not surveyed

Table 3-3. Effect of tillage and crop type on mean volunteer flax density at five different survey periods in twenty commercial fields in central Alberta in 2005.

Field	Tillage	Crop type	Survey Period				
			PREPLA[1a]	PREHERB[2a]	POSTHERB[3a]	PREHARV[4a]	POSTHARV[5a]
			plants m^{-2}				
1	CT[b]	wheat	40.6	510.4	22.8	0.0	0.0
2	RT[c]	barley	0.0	57.6	0.6	0.0	0.0
3	CT	barley	5.4	207.8	0.0	0.0	0.0
4	RT	canola	102.6	174.6	61.0	23.4	11.0
5	CT	oats	1.0	30.6	28.8	11.6	7.6
6	CT	barley	0.0	25.2	43.0	10.8	6.2
7	RT	barley	1.4	38.2	5.6	0.0	0.0
8	RT	wheat	1.4	139.6	2.2	0.0	0.0
9	RT	wheat	5.0	44.2	67.0	0.0	0.0
10	RT	barley	14.2	2.8	0.0	0.0	0.0
11	RT	barley	1.4	10.2	0.0	0.0	0.0
12	RT	fallow	0.2	0.0	1.0	0.0	0.0
13	RT	barley	0.0	31.2	0.0	0.0	0.0
14	CT	barley	0.0	36.8	1.4	0.0	0.0
15	RT	wheat	7.2	570.2	83.6	0.0	0.0
16	RT	canola	28.0	166.4	15.0	25.0	10.6
17	RT	barley	0.0	160.6	5.6	0.0	0.0
18	RT	barley	0.0	229.6	6.8	0.0	0.0
19	RT	barley	0.0	365.2	0.0	0.0	0.0
20	RT	canola	2.2	88.0	0.2	0.0	0.0
			Contrast statements[e]				
Cereal RT vs. cereal CT			**NS**	**NS**	**NS**	**NS**	**NS**
Canola RT vs. canola CT			na[d]	na	na	na	na
Cereal RT vs. pea RT			na	na	na	na	na
Cereal CT vs. pea CT			na	na	na	na	na
Canola RT vs. pea RT			na	na	na	na	na
Cereal CT vs. canola CT			na	na	na	na	na
Cereal RT vs. canola RT			*	**NS**	**NS**	*	*

[1]Before seeding
[2]Post seeding and before in-crop herbicide application
[3]Post in-crop herbicide application
[4]Before harvest
[5]After harvest
[a]Lsmeans from the mixed model ANOVA
[b]Conventional tillage
[c]Reduced tillage
[d]Not applicable
[e]Orthogonal contrasts denoted by asterisks (*) are significant at $P \leq 0.05$ and those denoted by NS are not significant

Table 3-4. Effect of tillage and crop type on mean volunteer flax density at five different survey periods in twenty commercial fields in central Alberta in 2006.

			Survey period				
Field	Tillage	Crop type	PREPLA[1a]	PREHERB[2a]	POSTHERB[3a]	PREHARV[4a]	POSTHARV[5a]
			plants m^{-2}				
1	CT[b]	canola	0.0	0.0	0.0	0.0	0.0
2	RT[c]	barley	0.0	1.4	1.4	1.0	1.4
3	CT	pea	0.0	1.2	1.0	0.8	0.0
4	RT	barley	0.0	2.0	7.4	2.4	6.6
5	CT	canola	0.0	0.4	0.0	0.4	0.0
6	CT	oats	0.0	0.0	0.0	0.4	0.0
7	RT	canola	0.0	0.0	0.0	0.0	0.0
8	RT	pea	0.0	9.6	5.6	0.0	2.8
9	RT	canola	0.0	0.0	0.0	0.6	0.0
10	RT	canola	0.0	2.4	0.6	1.2	0.0
11	RT	canola	0.0	1.4	0.0	0.0	0.0
12	RT	wheat	0.0	0.0	0.0	2.0	0.0
13	RT	fallow	0.0	0.2	0.4	0.0	0.0
14	CT	canola	0.0	0.0	1.0	0.2	0.0
15	RT	barley	0.0	78.2	23.8	0.0	0.0
16	RT	canola	0.0	0.0	0.0	5.2	0.0
17	RT	oat	0.0	0.0	0.4	0.0	0.0
18	RT	barley	0.0	0.0	0.0	0.4	0.0
19	RT	barley	0.0	0.0	0.0	0.4	0.0
20	RT	canola	0.0	0.0	0.0	0.2	0.0
			Contrast statements[e]				
Cereal RT vs. cereal CT			na[d]	na	na	na	na
Canola RT vs. canola CT			**NS**	**NS**	**NS**	*****	**NS**
Cereal RT vs. pea RT			**NS**	*****	**NS**	**NS**	**NS**
Cereal CT vs. pea CT			na	na	na	na	na
Canola RT vs. pea RT			**NS**	*****	*****	**NS**	*****
Cereal CT vs. canola CT			na	na	na	na	na
Cereal RT vs. canola RT			**NS**	*****	*****	**NS**	*****

[1]Before seeding
[2]Post seeding and before in-crop herbicide application
[3]Post in-crop herbicide application
[4]Before harvest
[5]After harvest
[a]Lsmeans from the mixed model ANOVA
[b]Conventional tillage
[c]Reduced tillage
[d]Not applicable
[e]Orthogonal contrasts denoted by asterisks (*****) are significant at $P \leq 0.05$ and those denoted by NS are not significant

Table 3-5. Effect of tillage and crop type on mean volunteer flax density at five different survey periods in twenty commercial fields in central Alberta in 2007.

			Survey period				
Field	Tillage	Crop type	PREPLA[1a]	PREHERB[2a]	POSTHERB[3a]	PREHARV[4a]	POSTHARV[5a]
			plants m^{-2}				
1	CT[b]	barley	0	0	0	0	0
2	RT[c]	barley	0	0.4	0	0	0
3	CT	canola	0	0	0	0	0
4	RT	—[d]	—	—	—	—	—
5	CT	barley	0	0	0	0	0
6	CT	canola	0	0	0	0	0
7	RT	barley	0	0	0	0	0
8	RT	canola	0	0	0	0	0
9	RT	pea	0	0	0	0	0
10	RT	pea	0	0.4	1.4	0	0
11	RT	wheat	0	0	0	0	0
12	RT	barley	0	0	0	0	0
13	RT	canola	0	0	0	0	0
14	CT	wheat	0	0	0	0	0
15	RT	barley	0	1.8	0	0	0
16	RT	wheat	0	0	0	0	0
17	RT	canola	0	0	0	0	0
18	RT	barley	0	0	0	0	0
19	RT	canola	0	0	0	0	0
20	RT	barley	0	0	0	0	0
			Contrast statements[f]				
Cereal RT vs. cereal CT			**NS**	**NS**	**NS**	**NS**	**NS**
Canola RT vs. canola CT			**NS**	**NS**	**NS**	**NS**	**NS**
Cereal RT vs. pea RT			**NS**	**NS**	*****	**NS**	**NS**
Cereal CT vs. pea CT			na[e]	na	na	na	na
Canola RT vs. pea RT			**NS**	**NS**	**NS**	**NS**	**NS**
Cereal CT vs. canola CT			**NS**	**NS**	**NS**	**NS**	**NS**
Cereal RT vs. canola RT			**NS**	**NS**	**NS**	**NS**	**NS**

[1]Before seeding
[2]Post seeding and before in-crop herbicide application
[3]Post in-crop herbicide application
[4]Before harvest
[5]After harvest
[a]Lsmeans from the mixed model ANOVA
[b]Conventional tillage
[c]Reduced tillage
[d]Field not surveyed
[e]Not applicable
[f]Orthogonal contrasts denoted by asterisks (*****) are significant at $P \leq 0.05$ and those denoted by NS are not significant

Table 3-6. Mean volunteer flax (*Linum usitatissimum* L.*)* frequency, field uniformity, density and growth stage at five different survey periods in twenty commercial fields in central Alberta in 2005, 2006 and 2007.

			Field uniformity		Field density			
Year	Survey period	Frequency	All	Occurrence	All	Occurence	High	High growth stage[f]
		%				plants m^{-2}		
2005	PREPLA[a]	65.0	21.8	33.5	10.4	16.0	102.6	growing point emerged
	PREHERB[b]	95.0	75.5	79.5	142.6	150.1	570.2	stem extension
	POSTHERB[c]	75.0	31.8	42.3	17.2	23.0	83.6	stem extension
	PREHARV[d]	20.0	12.0	60.0	3.5	17.7	24.4	stem extension
	POSTHARV[e]	20.0	7.0	5.0	1.8	8.9	11.0	growing point emerged
2006	PREPLA	0.0	0.0	0.0	0.0	0.0	0.0	na[g]
	PREHERB	50.0	15.5	31.0	6.1	12.2	74.2	stem extension
	POSTHERB	40.0	10.0	25.0	2.3	5.7	17.8	first flower
	PREHARV	65.0	7.3	11.2	0.8	1.2	4.6	first flower
	POSTHARV	15.0	3.8	25.0	0.5	3.6	6.6	stem extension
2007	PREPLA	0.0	0.0	0.0	0.0	0.0	0.0	na
	PREHERB	15.8	2.1	13.3	0.1	0.9	1.8	first true leaf
	POSTHERB	5.3	1.8	35.0	0.1	1.4	1.4	late flower, boll formed
	PREHARV	0.0	0.0	0.0	0.0	0.0	0.0	na
	POSTHARV	0.0	0.0	0.0	0.0	0.0	0.0	na

[a]Before seeding
[b]After seeding and before in-crop spray
[c]After in-crop spray
[d]Before harvest
[e]After harvest
[f]Typical growth stage of volunteer flax
[g]Not applicable

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Chapter 4: Influence of pre-emergent and post-emergent herbicides on the fecundity of volunteer flax (*Linum usitatissimum* L.) in wheat (*Triticum aestivum* L.).

INTRODUCTION

Volunteer crops are important weeds in western Canadian cropping systems (Leeson et al. 2005). Although there is generally excellent research on the response of crop species to management and environment, relatively little is known about their biology as volunteers. There has been renewed interest in the biology of volunteer crops since the introduction of herbicide resistant crops (for example see Beckie et al. 2006, Harker et al. 2007, Beckie and Owen 2007, Warwick et al. 2008). The use of genetically engineered (GE) crops for these products may raise concerns about the potential for gene flow to conventional crops and wild and weedy relatives. GE volunteers may flower and pollinate adjacent crops, contributing to pollen-mediated gene flow. GE volunteers may also survive to produce seed that can recharge the weed seed bank or be harvested along with the crop, resulting in adventitious presence (AP) of GE seed (Kershen and McHughen 2005). Seed dispersal of crops has the potential to contribute to large scale gene flow, both temporally and spatially (Hall et al. 2003).

GE oilseed crops, including flax, could be used for the production of a wide range of bioproducts including biofuels and lubricants, plastics, healthy oils, green building materials and pharmaceuticals (Hills et al. 2007, McPherson et al. 2008a, McPherson et al. 2008b). Conventional flax grown in Canada is marketed primarily to the European Union (EU) and the residual meal is used as a co-product in animal feed. A transgenic flax cultivar (CDC Triffid) has been released previously in Canada; this herbicide resistant cultivar was intended for use in fields with persistent sulfonylurea herbicides

residues. However, CDC Triffid was deregulated shortly after its release in 1998 at the request of the Canadian flax seed industry due to the negative market response of the EU to the import of GE crops (Anonymous 2002). Further transgenic development of the crop was halted as a clear regulatory framework did not exist for transgenic crops or feed products in Europe at the time of its release. At present, the Commission of the European Communities (EC) is proposing and implementing measures to achieve coexistence between GE and non-GE (conventional and organic) crops (Beckie and Hall 2008). The EC currently accepts an AP of authorized transgenes in organic and conventional non-GE products used for food or feed up to a 0.9% level (Commission of the European Communities 2003). Other countries such as Canada and the United States have not established regulatory guidelines for AP of GE crops. GE crops in Canada, upon obtaining regulatory approval for environmental, food and feed safety, are considered safe and food or feed products that contain GE material are not required to be labeled. It is not known whether GE and conventional flax could coexist in Canada without risk to the conventional and organic flax market.

The contribution of flax volunteers to AP has not yet been identified. While AP can be minimized through best management practices in crop production systems, it cannot be eliminated entirely. Effective seed separation during harvesting will minimize AP but is dependent upon environmental conditions at harvest and mechanical efficiency and crop type. The AP of flax seed in commodity crops is also affected by volunteer density, crop competiveness, predispersal predation, the fecundity of volunteers, boll shatter, days to maturity as well as by the effectiveness of herbicidal control. Herbicidal control of volunteers is an important component of risk reduction in the co-existence of GE and conventional crops. In Canada, few pre-emergent and post-emergent herbicides

are registered for volunteer flax control in cereals. The pre-emergent herbicide, glyphosate applied either alone, or tank-mixed with with the acteolactate synthsae (ALS) inhibitor tribenuron-methyl or the protoporphyrinogen oxidase (PPO) inhibitor carfentrazone-ethyl, are registered in Canada for the control of volunteer flax. Post-emergent herbicide options for the control of volunteer flax in spring wheat include the auxin inhibitors, fluroxypyr + 2,4-D, fluroxypyr + MCPA, or quinclorac, and the ALS inhibitor, tribenuron-methyl used independently or in combination with 2,4-D or quinclorac (Anonymous 2008). Quinclorac applied alone at a rate of 100 or 200 g a.i. ha^{-1} was reported to provide consistent volunteer flax control without yield loss in spring wheat (Wall and Smith 1999). Fluroxypyr + 2,4-D has been previously reported to be as effective as quinclorac in its ability to control volunteer flax, but flax control by fluroxypyr + MCPA has not been evaluated in spring wheat (Wall and Smith 1999). Flax crops are quite tolerant to some ALS inhibitors. Thifensulfuron was examined as a herbicide for weed control in flax, but caused injury to flax when applied post-emergent and reductions in flax dry weight, height and yield, especially under cool, wet growing conditions were reported (Derksen and Wall 1996). If the risk of seed-mediated gene flow from transgenic flax volunteers in rotational cereals crops is to be mitigated, an effective combination of either a pre-emergent herbicide and/or a post-emergent herbicide applied as a single or split application must be identified.

In western Canada, flax is typically followed in rotation by a cereal crop, usually spring wheat (Wall and Smith 1999, Anonymous 2002,). The objectives of this study were to (1) determine the ability of pre-emergent and post-emergent herbicides used alone or in combination to control volunteer flax in spring wheat, (2) to evaluate fecundity of volunteer flax plants and (3) to determine the ability of herbicides to reduce the AP of

flax seed in wheat. Data from these experiments will help inform the environmental risk assessment of GE flax and contribute to an understanding of flax seed-mediated gene flow.

MATERIALS AND METHODS

Site selection and experimental design

Field experiments were conducted in 2005 and 2006 at two locations, the University of Alberta Edmonton Research Station (ERS) and at the Ellerslie Research Station (Ellerslie). At the ERS the soil was a clay loam and consisted of 31.8% sand, 40.8% silt and 27.4% clay with a pH of 6.0 and an organic matter content of 12.2%. The soil at Ellerslie was a loam soil and consisted of 28.6% sand, 46.4% silt and 25% clay with a pH of 6.3 and 11.5% soil organic matter content. In the prior year to the 2005 and 2006 research experiments, the research sites were planted to barley (AC Metcalfe) and the excess straw removed by performing two light harrow operations before the wheat crop (AC Barrie) was planted. Prior to seeding the wheat crop, a 2 m^2 quadrat was permanently established and was randomly placed in each plot. The experiments at both sites were established in areas that had not been seeded to flax and had not been conventionally tilled for at least 5 years.

To simulate volunteer flax infestations, flax (*Linum usitatissimum* L.) cv. CDC Bethune was broadcast on the soil surface in spring at a rate of 12.22 kg ha^{-1} with target populations of 150 seeds m^{-2} with a low disturbance airseeder. Seed was immediately incorporated into the soil with a light tillage operation to a depth of 3 to 5 cm to ensure soil to seed contact (Table 4-1). Flax volunteers were allowed to emerge and pre-emergent herbicides applied. After herbicide application, spring wheat cv. AC Barrie was seeded using a double disc press drill at a rate of 114 kg ha^{-1} at a depth of 3 cm and with a

row spacing of 20 cm at both research locations (Table 4-1). Spring wheat seeding dates were delayed compared to those typical for the area because of the need to establish volunteer flax.

Fertilizer rates for wheat were based on soil test recommendations for each site-year. In 2005 and 2006 at Ellerslie, 170.24 kg ha^{-1} of urea (46-0-0) was broadcast on the soil surface and 44.8 kg ha^{-1} of phosphate (0-45-0) was placed with seed. At the ERS in 2005, 16.0 kg ha^{-1} of potassium sulfate (0-0-52-17) and 37.0 kg ha^{-1} of urea (46-0-0) was broadcast on the soil surface and in 2006, 170.24 kg ha^{-1} of urea (46-0-0) and 44.8 kg ha^{-1} of phosphate (0-45-0) was broadcast.

Plots (2 x 8.5 m^2) were arranged in a 2 x 5 factorial, completely randomized block design with 18 treatments and four replications. The experimental treatments consisted of two pre-emergent herbicide treatments and one of seven post-emergent herbicide treatments plus an untreated weedy control (Table 4-2). Prior to seeding the wheat crop, a 2 m^2 quadrat was randomly placed in each plot and was permanently established by marking each corner with colored plastic pegs. Pre-emergent and post-emergent herbicides were applied at recommended rates and stages of crop development (Table 4-2). Pre-emergent herbicides were applied when volunteer flax was 6-8 cm tall with the third pair of leaves unfolded and post-premergent herbicides were applied when the wheat crop was at the 5-6 leaf stage and volunteer flax was 15 cm tall. Herbicides were applied with a small plot-sprayer equipped with shrouded multiple 2 m booms equipped with Teejet XR 110015 nozzles delivering 100L ha^{-1} at 214 kPa.

Volunteer flax density within pre-established 2 m^2 quadrats was assessed during the growing season: 1) prior to herbicide treatment; 2) two weeks after pre-emergent herbicide application; 3) two weeks after post-emergent herbicide application and 4) at

the time of harvest (Table 4-1). Volunteer flax that survived herbicide treatment was cut at the stem base close to the soil surface, dried for 48 hours at room temperature (25°C) and the dry weights (g) recorded. Flax seed bolls were threshed by hand and seed tested for viability (see below). Wheat biomass and yield was also determined (Table 4-1) in established 1 x 2 m^2 quadrats by cutting the plants off at the stem base near the soil surface and by drying for 72 hours at 62ºC. Wheat heads were threshed by hand and the grain dried for 72 hours at 62ºC, weighed (g) and seed yield was determined (kg ha^{-1}). Plots were harvested at maturity (Table 4-1) and seed dried to a uniform moisture content for 72 hours at 62ºC, cleaned, and the seed yield (kg ha^{-1}) determined. AP of volunteer flax was determined by recovering volunteer flax seed from wheat samples. The recovered seed was weighed (g) and used to determine the AP of volunteer flax in spring wheat (kg ha^{-1}) and expressed as the percentage (w/w) of volunteer flax seed in harvested wheat.

To determine volunteer flax seed viability, a subsample of 100 seeds from each sample of harvested flax volunteers was randomly chosen after sample processing. Seeds were placed in acrylic germination boxes (24 x 16 x 3.8 cm) (Hoffman Manufacturing Inc.) lined with 15 x 23 cm non-toxic white filter paper equivalent to Whatman No. 1 (Hoffman Manufacturing Inc.). To reduce fungal growth, 14 mL of a 0.2% solution of the seed treatment Helix Xtra® (thiamethoxam + difenoconazle + metalaxyl-M + fludioxonil) was added to each germination box. The germination trays were stored in the dark at ambient temperatures for 72 hours to induce germination. Seeds were considered to have germinated when the radicle emerged through the seed coat.

Non-germinated seeds were transferred to an acrylic germination boxes (24 x 16 x 3.8 cm) (Hoffman Manufacturing Inc.) lined with 15 x 23 cm non-toxic white filter paper

equivalent to Whatman No. 1 (Hoffman Manufacturing Inc.) and moistened with 8 ml of 0.005 M giberellic acid (GA_3) solution. After 72 hours on the 0.005M GA_3 solution, the number of flax seeds that did and did not germinate were counted and recorded. Seed that were soft and/or degraded were considered to be dead. Alive seeds included seeds that germinated on water and GA_3.

Data analysis

Data were subject to analysis of variance (ANOVA) with a mixed model (PROC MIXED) using statistical analysis software (SAS) (SAS Institute Inc. 2007). The assumptions of ANOVA were tested to ensure that residuals were random, homogenous with a normal distribution about a mean of zero. Volunteer flax emergence, density after herbicide treatment, dry weight, fecundity (seed yield and seed number per m^2), AP as well as wheat dry weight (biomass) and yield were analyzed in a 2 x 5 factorial in a mixed model using ANOVA in SAS. Site and treatment effects were considered to be fixed and years and blocks considered random. Where the ANOVA indicated that treatment effects where significant, means were separated at $P \leq 0.05$ by lsmeans and adjusted with Fischer's Protected Least Significant Difference (LSD) test. When the effect of site, year and their interactions with treatments were not significant, data were pooled by site and/or year. Orthogonal contrasts statement statements were performed as part of the ANOVA procedure. Differences were considered to be significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

During 2005 and 2006 precipitation and moisture were within the expected range for the two sites (data not shown). Ellerslie had a mean annual precipitation of 482.7 mm (30 year normal) and a mean long-term temperature of 10.4, 15.9 and 4.3°C for May, July

and October, respectively, while the ERS site was slightly drier and warmer, having a precipitation of 476.9 mm (30 year normal) and mean long-term temperature of 11.7, 17.5 and 5.6°C for May, July and October, respectively. Site quality and soil fertility was higher at Ellerslie than at ERS both years (data not shown).

Volunteer flax density and biomass did not differ among sites ($0.0891 < p < 0.3125$) or years ($0.2275 < p < 0.4036$) (Table 4-3). Compared to the untreated weedy control, both pre-emergent herbicide treatments of glyphosate or glyphosate plus tribenuron reduced volunteer flax densities from 41 plants m^{-2} to 7 and 5 plants m^{-2}, respectively, two weeks following the treatment. Similarly at harvest, average densities of volunteer flax were lower in plots that received a pre-emergent herbicide treatment of glyphosate or glyphosate plus tribenuron (4 and 6 plants m^{-2} respectively) compared to plots which remained untreated (39 plants m^{-2}). Volunteer flax biomass was similarly reduced by both pre-emergent herbicide treatments and at harvest, dry weights of volunteer flax plants treated with glyphosate or glyphosate plus tribenuron averaged 9 and 19 g m^{-2} respectively whereas the untreated weedy control averaged 109 g m^{-2}. Based on these results, there were no differences in volunteer flax densities between the two pre-emergent herbicide treatments suggesting that a pre-emergent treatment of either glyphosate or glyphosate plus tribenuron was effective at controlling volunteer flax in wheat.

In this study, the addition of either 2,4-D or quinclorac to thifensulfuron plus tribenuron (applied post-emergent) reduced volunteer flax density and biomass at harvest (Table 4-3). Compared to the untreated weedy control, post-emergent thifensulfuron plus tribenuron with either 2,4-D or quinclorac reduced volunteer flax densities at harvest from 39 plants m^{-2} to 28 plants m^{-2} and 21 plants m^{-2} respectively. Volunteer flax dry

weight at harvest was also reduced by post-emergent thifensulfuron plus tribenuron with 2,4-D (69 g m^{-2}) or quinclorac (54 g m^{-2}) compared to the untreated weedy control (109 g m^{-2}). These results contrasted with those of Wall and Smith (1999) who reported that the addition of 2,4-D to thifensulfuron plus tribenuron provided ineffective control of volunteer flax (density and biomass) compared to the untreated weedy control. The post-emergent herbicide treatment of thifensulfuron plus tribenuron alone was ineffective at reducing the density of volunteer flax at harvest and was also ineffective in reducing volunteer flax biomass compared to the untreated weedy control (Table 4-3). These results were consistent with those of Wall and Smith (1999), who reported that post-emergent thifensulfuron plus tribenuron provided poor control of volunteer flax in spring wheat. These authors reported that volunteer flax densities in untreated weedy plots (344 to 476 plants m^{-2}) were similar to densities of volunteer flax in post-emergent thifensulfuron plus tribenuron plots (325 to 476 plants m^{-2}). These authors also reported that post-emergent thifensulfuron plus tribenuron did not consistently reduce volunteer flax biomass as dry weights were highly variable between site-years. In summary, post-emergent treatment of thifensulfuron plus tribenuron was ineffective in the control of volunteer flax unless 2,4-D or quinclorac were added.

The post-emergent herbicides fluroxypyr plus MCPA and fluroxypyr plus 2,4-D were most effective of the post-emergent treatments evaluated in reducing the density and biomass of volunteer flax in spring wheat (Table 4-3). Compared to the untreated weedy control, these post-emergent herbicide treatments reduced volunteer flax densities at harvest from 39 plants m^{-2} to 4 and 2 plants m^{-2} respectively. Similarly, volunteer flax biomass at harvest was lower in plots treated post-emergent with either fluroxypyr plus MCPA or fluroxypyr plus 2,4-D (5 and 3 g m^{-2} respectively) compared to plots which

remained untreated (109 g m^{-2}). Fluroxypyr plus 2,4-D applied post-emergent in spring wheat was previously reported to reduce volunteer flax density and biomass up to 3 and 80 times respectively in comparison to the untreated weedy control in Manitoba, Canada (Wall and Smith 1999).

In this study, both pre-emergent herbicide treatments (glyphosate or glyphosate plus tribenuron) were as effective as post-emergent fluroxypyr plus MCPA and fluroxypyr plus 2,4-D in reducing the density and biomass of volunteer flax at harvest. In addition, there were no differences in the density and biomass of volunteer flax when plots were treated pre-emergent with glyphosate or glyphosate plus tribenuron, post-emergent with either fluroxypyr plus MCPA or fluroxypyr plus 2,4-D or when plots were treated in combination with either of these pre-emergent and post-emergent herbicides. These results suggested that a combination of both treatments is not necessary. However, these results must be viewed with caution. To ensure high populations of volunteer flax, it was seeded prior to pre-seeding treatment and before the wheat crop. Under agronomic conditions, flax may continue to emerge after pre-seeding herbicides and thus these products would have reduced effectiveness.

Wheat biomass differed between sites ($p=0.0111$) but wheat yield did not differ among sites ($p=0.3449$) during experimental years (Table 4-4). Wheat biomass was increased by all herbicide treatments at ERS, but not at Ellerslie. Differences in wheat yield among treatments generally reflected the level of volunteer flax control. All herbicide treatments increased wheat yields in all site-years. This included the thifensulfuron plus tribenuron treatment which was ineffective at controlling flax volunteers suggesting that the yield increases were due, at least in part, to the control of other weeds present in the trials. In pre-emergent and post-emergent herbicide treated

plots wheat yields ranged from 1309 kg ha^{-1} to 1807 kg ha^{-1} whereas in untreated plots, wheat yield averaged 1079 kg ha^{-1}. Although volunteer flax seed yield was higher at Ellerslie than at ERS (Table 4-5), there were no differences in wheat yield between pre-emergent, post-emergent or additive herbicide treatments ($0.1876 < p < 0.9320$) between the two sites. Previous research has indicated that when volunteer flax is left uncontrolled by herbicides and present at high average densities (< 105 plants m^{-2}), spring wheat yields may be reduced by up to 27% in western Canada (Wall and Smith 1999). Although the densities of volunteer flax did not exceed 41 plants m^{-2} in this study (Table 4-3), wheat yields were reduced by up to 56% in untreated plots when volunteer flax was present and other abundant weed species (*Chenopodium album* L., *Amaranthus retroflexus* L. and *Hordeum vulgare* L.) were left uncontrolled by herbicides.

Volunteer flax, if left uncontrolled, can produce large amounts of seed (Table 4-5). Seed production of flax volunteers differed within sites ($p=0.0084$), ranging from 3.1 to 30.9 g m^{-2} and from 483 to 4755 seeds m^{-2} at ERS and Ellerslie respectively (Table 4-5). There were no differences in precipitation among site-years that would justify greater seed production of volunteer flax at Ellerslie compared to ERS. Higher volunteer flax seed yields at Ellerslie than at ERS reflect site quality differences. ERS had been in barley monoculture for at least 10 years and due to poor crop rotation, the soil had a lower fertility (data not shown). Experimental conditions, in which volunteer flax was allowed to emerge before the wheat was sown, may have increased flax seed production compared to normal field conditions in which the wheat would have been seeded earlier and would have been more competitive.

Both pre-emergent and most post-emergent herbicide treatments reduced seed production of volunteer flax at harvest ($0.0004 < p < 0.0071$) (Table 4-5). Compared to

the untreated weedy control, both pre-emergent herbicide treatments of glyphosate or glyphosate plus tribenuron reduced the seed yield of volunteer flax at both sites from < 4700 seeds m^{-2} to approximately 1188 seeds m^{-2}. There were no differences in seed yield between the two pre-emergent herbicide treatments. In addition, there were no differences in seed yield when pre-emergent glyphosate was used alone compared to the addition of a post-emergent herbicide treatment. Volunteer flax seed yield was decreased by post-emergent thifensulfuron plus tribenuron with 2,4-D (1.2 to 8.7 g m^{-2}) or quinclorac (1.2 to 4.4 g m^{-2}) compared to the untreated weedy control (3.1 to 30.9 g m^{-2}) (Table 4-5). Post-emergent herbicide treatments of fluroxypyr plus 2,4-D or fluroxypyr plus MCPA were the most effective in reducing volunteer flax seed production of the post-emergent herbicide treatments. Compared to the untreated weedy control, these post-emergent herbicide treatments reduced the seed yield of volunteer flax from 4755 seeds m^{-2} to approximately 11 seeds m^{-2}. Differences in seed yield among post-emergent herbicide treatments were detected at ERS, but not at Ellerslie. At ERS, post-emergent herbicide treatments of thifensulfuron plus tribenuron and thifensulfuron plus tribenuron with 2,4-D or quinclorac were not as effective as post-emergent fluroxypyr plus 2,4-D or fluroxypyr plus MCPA in reducing the seed production of volunteer flax. The variance observed in volunteer flax seed yield was higher at Ellerslie than ERS (data not shown), and our ability to make comparisons and to detect differences among post-emergent herbicide treatments at Ellerslie may have been limited by the variance in relation to the mean. In addition, there were no differences in the seed production of volunteer flax when plots were treated pre-emergent with glyphosate or glyphosate plus tribenuron or in combination with either of these pre-emergent and a post-emergent herbicide. These

results suggested that the fecundity of volunteer flax was reduced with a single post-emergent herbicide treatment of either fluroxypyr plus 2,4-D or fluroxypyr plus MCPA.

The AP of volunteer flax seed in spring wheat differed between sites ($p=0.0002$) (Table 4-6). In untreated plots, AP of volunteer flax seed averaged 4.4 and 135.0 kg ha^{-2} in ERS and Ellerslie, respectively (Table 4-6). Compared to the untreated control, both pre-emergent herbicide treatments (glyphosate or glyphosate plus tribenuron) and fluroxypyr plus MCPA or fluroxypyr plus 2,4-D applied post-emergent reduced AP below 0.05% (w/w) at both sites. The addition of a post-emergent herbicide treatment after a pre-emergent herbicide treatment of either glyphosate or glyphosate plus tribenuron did not reduce AP, but lower levels of AP were recorded when post-emergent fluroxypyr plus 2, 4-D was applied after a pre-emergent glyphosate. In conclusion, a combination of both herbicide treatments is not necessary, but does ensure that the contribution of flax volunteers to AP is minimized.

Plants that survive and set seed after herbicide treatment may produce seeds with decreased viability, either through a delay in seed maturity or directly (Azlin and McWhorter 1981, Cathey and Barry 1997). In untreated weedy plots, the viability of volunteer flax seed averaged 55% (Table 4-5). Compared to the untreated weedy control, the percentage of viable seeds was not reduced by either pre-emergent herbicide treatment (glyphosate or glyphosate plus tribenuron). However, the viability of volunteer flax seed was reduced by all post-emergent herbicide treatments and was reduced to as low as zero when glyphosate or glyphosate plus tribenuron was applied pre-emergent and followed post-emergent with fluroxypyr plus MCPA or 2,4-D. While this data suggested that seeds treated by herbicides are not as likely to produce viable seedlings and are therefore unlikely to contribute to gene flow in the environment, some of these seeds may be

harvested along with the crop and contribute to AP. Non-viable GE flax seeds may contain genetic information, including transgenes, that could be detected and quantified in grain shipments. At the genotypic level, there are several methods to identify transgene DNA sequences of which the polymerase chain reaction is most commonly used (Demeke et al. 2006). While AP is a difficult value to predict from small trials, the results of this study indicated that EU AP thresholds of 0.9% could be met (Table 4-6). It should be noted, however, that the methods to quantify GE AP have not been standardized world wide and can vary when processed products, crop seed within the same or different crops are considered. Should AP be determined on the weight rather than frequency basis, results may vary.

Effective herbicides for the control of volunteer flax were identified for both pre-emergent and post-emergent treatments. Glyphosate applied pre-emergent and fluroxypyr applied either with MCPA or 2,4-D post-emergent were effective. These herbicides reduced volunteer flax density, biomass and fecundity. They also reduced volunteer flax seed AP in spring wheat (from over 8.5% to > 0.16%). Herbicide control of GE volunteer flax will reduce the potential for pollen-mediated gene flow to adjacent crops and seed-mediated gene flow. However, some volunteers will escape control leading to AP and the replenishment of the seed bank. Our results indicated that AP of flax in wheat with the recommended herbicide control will be below the 0.9% threshold currently considered acceptable by the EC.

Experimental conditions in which flax was seeded prior to wheat represent a worst-case scenario, in which flax competitive ability and fecundity would be enhanced and control on larger flax plants by post-emergent treatment is likely to be reduced. Although our data does not suggest that both pre-emergent and post-emergent treatments

contribute to flax control and reduction of fecundity, under field conditions where volunteer flax emergence is less uniform, sequential herbicide treatments may be beneficial. In most wheat crops in the western Canada, application of post-emergent herbicides is routine and we suggest that flax density prior to post-emergent application be assessed to guide the choice of post-emergent herbicides.

Table 4-1. Dates of agronomic operations at ERS and Ellerslie in 2005 and 2006.

Operation	ERS		Ellerslie	
	2005	2006	2005	2006
Flax cv. CDC Bethune seeded	May 6	May 10	May 4	May 11
Flax incorporation by tillage	May 6	May 10	May 4	May 11
Pre-emergent herbicide treatment applied	June 7	May 31	June 2	June 4
Spring wheat cv. AC Barrie seeded	June 3	May 29	June 3	June 5
Volunteer flax density assessments before pre-emergent herbicide application	May 29	May 30	May 29	May 30
Volunteer flax density assessments after pre-emergent herbicide application	June 14	June 10	June 14	June 10
Volunteer flax density assessments before post-emergent herbicide application	June 27	June 20	June 27	June 20
Volunteer flax density assessments at the time of harvest	Oct 6	Sept 11	Oct 6	Sept 25
Post-emergent herbicide treatment	June 28	June 20	June 28	June 26
Volunteer flax and wheat biomass assessments	Oct 6	Sept 11	Oct 6	Sept 25
Wheat harvest	Oct 10	Sept 28	Oct 10	Sept 28

Table 4-2. Pre-emergent and post-emergent herbicides, adjuvants and application rates.

Treatment	Pre-emergent	Post-emergent	Pre-emergent rate	Post-emergent rate
1	—	—	0	0
2	Glyphosate	—	1.25 l ha^{-1}	0
3	Tribenuron-methyl Glyphosate AgSurf	—	7.41 g a.i. ha^{-1} 0.98 l ha^{-1} 0.2% vol/vol	0
4	—	Thifensulfuron-methyl + tribenuron-methyl AgSurf	0	14.82 g a.i. ha^{-1} 0.2% vol/vol
5	—	Fluroxypyr MCPA	0	0.59 l ha^{-1} 0.98 l ha^{-1}
6	—	Fluroxypyr 2,4-D	0	0.59 l ha^{-1} 1.11 l ha^{-1}
7	—	Thifensulfuron-methyl + tribenuron-methyl 2,4-D AgSurf	0	14.82 g a.i ha^{-1} 0.90 l ha^{-1} 0.2% vol/vol
8	—	Thifensulfuron-methyl + tribenuron-methyl Quinclorac Merge	0	14.82 g a.i ha^{-1} 124.12 g a.i ha^{-1} 0.2% vol/vol
9	Glyphosate	Thifensulfuron-methyl + tribenuron-methyl AgSurf	1.25 l ha^{-1}	14.82 g a.i. ha^{-1} 0.2% vol/vol
10	Glyphosate	Fluroxypyr MCPA	1.25 l ha^{-1}	0.59 l ha^{-1} 0.98 l ha^{-1}
11	Glyphosate	Fluroxypyr 2,4-D	1.25 l ha^{-1}	0.59 l ha^{-1} 1.11 l ha^{-1}
12	Glyphosate	Thifensulfuron-methyl + tribenuron-methyl 2,4-D AgSurf	1.25 l ha^{-1}	14.82 g a.i ha^{-1} 0.90 l ha^{-1} 0.2% vol/vol
13	Glyphosate	Thifensulfuron-methyl + tribenuron-methyl Quinclorac Merge	1.25 l ha^{-1}	14.82 g a.i ha^{-1} 124.12 g a.i ha^{-1} 0.2% vol/vol
14	Tribenuron-methyl + Glyphosate AgSurf	Thifensulfuron-methyl + tribenuron-methyl AgSurf	7.41 g a.i. ha^{-1} 0.98 l ha^{-1} 0.2% vol/vol	14.82 g a.i. ha^{-1} 0.2% vol/vol
15	Tribenuron-methyl Glyphosate AgSurf	Fluroxypyr MCPA	7.41 g a.i. ha^{-1} 0.98 l ha^{-1} 0.2% vol/vol	0.59 l ha^{-1} 0.98 l ha^{-1}
16	Tribenuron-methyl Glyphosate AgSurf	Fluroxypyr 2,4-D	7.41 g a.i. ha^{-1} 0.98 l ha^{-1} 0.2% vol/vol	0.59 l ha^{-1} 1.11 l ha^{-1}
17	Tribenuron-methyl Glyphosate AgSurf	Thifensulfuron-methyl + tribenuron-methyl 2,4-D AgSurf	7.41 g a.i. ha^{-1} 0.98 l ha^{-1} 0.2% vol/vol	14.82 g a.i ha^{-1} 0.90 l ha^{-1} 0.2% vol/vol
18	Tribenuron-methyl Glyphosate AgSurf	Thifensulfuron-methyl + tribenuron-methyl Quinclorac Merge	7.41 g a.i. ha^{-1} 0.98 l ha^{-1} 0.2% vol/vol	14.82 g a.i ha^{-1} 124.12 g a.i ha^{-1} 0.2% vol/vol

Table 4-3. Volunteer flax density and dry weight from fixed quadrats as influenced by herbicide treatments at ERS and Ellerslie, Alberta, in 2005 and 2006.

Treatment	Application timing		Density after pre-emergent herbicide application[a]	Density at harvest[a]	Dry weight[a]
			plants m^{-2}	plants m^{-2}	g m^{-2}
Untreated	---	---	41 *ab*	39 *a*	109 *a*
Glyphosate	PRE	---	7 *cd*	4 *d*	9 *d*
Glyphosate + tribenuron	PRE	---	5 *d*	6 *d*	19 *cd*
Thifensulfuron + tribenuron	---	POST	38 *b*	36 *ab*	82 *ab*
Fluroxypyr + MCPA	---	POST	36 *b*	4 *d*	5 *d*
Fluroxypyr + 2,4-D	---	POST	36 *b*	2 *d*	3 *d*
Thifensulfuron + tribenuron + 2,4-D	---	POST	45 *a*	27 *bc*	69 *b*
Thifensulfuron + tribenuron + quinclorac	---	POST	43 *ab*	21 *c*	54 *bc*
Glyphosate *fb* thifensulfuron + tribenuron	PRE	POST	14 *c*	7 *d*	10 *d*
Glyphosate *fb* fluroxypyr + MCPA	PRE	POST	8 *cd*	1 *d*	3 *d*
Glyphosate *fb* fluroxypyr + 2,4-D	PRE	POST	6 *d*	0 *d*	1 *d*
Glyphosate *fb* thifensulfuron + tribenuron + 2,4-D	PRE	POST	6 *cd*	2 *d*	5 *d*
Glyphosate *fb* thifensulfuron + tribenuron + quinclorac	PRE	POST	6 *cd*	1 *d*	2 *d*
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron	PRE	POST	7 *cd*	5 *d*	7 *d*
Glyphosate + tribenuron *fb* fluroxypyr	PRE	POST	5 *d*	0 *d*	0 *d*
Glyphosate + tribenuron *fb* fluroxypyr + 2,4-D	PRE	POST	7 *cd*	1 *d*	1 *d*
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron + 2,4-D	PRE	POST	7 *cd*	2 *d*	3 *d*
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron + quinclorac	PRE	POST	6 *d*	6 *d*	12 *cd*
Sources of variation					
Pre-emergent herbicide			*	*	*
Post-emergent herbicide			**NA**[b]	*	*
Pre-emergent herbicide*Post-emergent herbicide			**NA**	*	*

Abbreviations: fb, followed-by

[a]Least square means within columns followed by a common letter are not significantly different according to the mixed model ANOVA at $P \leq 0.05$

*Significant according to the mixed model ANOVA at $P \leq 0.05$ level

[b]Not applicable

Table 4-4. Wheat biomass and yield as influenced by herbicide treatments for ERS and Ellerslie, Alberta in 2005 and 2006.

Treatment	Application timing		Wheat dry weight[a] ERS	Wheat dry weight[a] Ellerslie	Wheat seed yield[a]
			g m^{-2}		kg ha^{-1}
Untreated	---	---	311	744	1079
Glyphosate	PRE	---	751	838	1807
Glyphosate + tribenuron	PRE	---	663	873	1666
Thifensulfuron + tribenuron	---	POST	465	749	1364
Fluroxypyr + MCPA	---	POST	614	697	1515
Fluroxypyr + 2,4-D	---	POST	523	636	1390
Thifensulfuron + tribenuron + 2,4-D	---	POST	506	594	1309
Thifensulfuron + tribenuron + quinclorac	---	POST	521	705	1469
Glyphosate *fb* thifensulfuron + tribenuron	PRE	POST	594	863	1695
Glyphosate *fb* fluroxypyr + MCPA	PRE	POST	666	886	1831
Glyphosate *fb* fluroxypyr + 2,4-D	PRE	POST	690	974	1815
Glyphosate *fb* thifensulfuron + tribenuron + 2,4-D	PRE	POST	691	940	1852
Glyphosate *fb* thifensulfuron + tribenuron + quinclorac	PRE	POST	675	961	1793
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron	PRE	POST	648	904	1816
Glyphosate + tribenuron *fb* fluroxypyr	PRE	POST	678	885	1899
Glyphosate + tribenuron *fb* fluroxypyr + 2,4-D	PRE	POST	655	926	1771
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron + 2,4-D	PRE	POST	686	870	1749
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron + quinclorac	PRE	POST	744	975	1847
Contrast statements					
Untreated vs Pre-emergent			*	NS	*
Untreated vs Post-emergent			*	NS	*
Glyphosate vs glyphosate + tribenuron			NS	NS	NS
Thifensulfuron + tribenuron vs. thifensulfuron + tribenuron + 2,4-D			NS	NS	NS
Thifensulfuron + tribenuron vs. thifensulfuron + tribenuron + quinclorac			NS	NS	NS
Thifensulfuron + tribenuron vs. fluroxypyr + MCPA			NS	NS	NS
Thifensulfuron + tribenuron vs. fluroxypyr +2,4-D			NS	NS	NS
Fluroxypyr + MCPA vs. fluroxypyr + 2,4-D			NS	NS	NS
Glyphosate vs. Pre-emergent * Post-emergent			NS	NS	NS
Glyphosate+ tribenuron vs. Pre-emergent * Post-emergent			NS	NS	NS

Abbreviations: fb, followed-by

[a]Least square means from the mixed model ANOVA

Non-orthogonal contrasts denoted by an asterisk (*) are significant at $P \leq 0.05$ and those denoted by NS are not significant at $P \leq 0.05$

Table 4-5. Volunteer flax seed yield from fixed quadrats as influenced by herbicide treatments at ERS and Ellerslie, Alberta in 2005 and 2006.

Treatment	Application timing		Seed yield[a]				
			ERS	Ellerslie	ERS	Ellerslie	Viable seed
			g m^{-2}		seeds m^{-2}		%
Untreated	---	---	3.1	30.9	483.2	4755.3	55.5
Glyphosate	PRE	---	0.4	0.8	55.6	121.8	39.9
Glyphosate + tribenuron	PRE	---	0.4	7.7	57.0	1187.4	36.9
Thifensulfuron + tribenuron	---	POST	4.1	4.2	626.1	650.5	46.8
Fluroxypyr + MCPA	---	POST	0.0	0.1	0.6	11.4	10.0
Fluroxypyr + 2,4-D	---	POST	0.0	0.0	2.1	0.0	3.8
Thifensulfuron + tribenuron + 2,4-D	---	POST	1.2	8.7	184.4	1342.7	45.2
Thifensulfuron + tribenuron + quinclorac	---	POST	1.2	4.4	177.0	680.0	33.2
Glyphosate *fb* thifensulfuron + tribenuron	PRE	POST	0.4	0.1	65.5	17.3	17.3
Glyphosate *fb* fluroxypyr + MCPA	PRE	POST	0.0	0.0	0.0	5.3	4.0
Glyphosate *fb* fluroxypyr + 2,4-D	PRE	POST	0.0	0.0	0.0	0.0	0.0
Glyphosate *fb* thifensulfuron + tribenuron + 2,4-D	PRE	POST	0.1	0.0	15.7	6.1	5.0
Glyphosate *fb* thifensulfuron + tribenuron + quinclorac	PRE	POST	0.1	0.0	7.1	0.0	5.0
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron	PRE	POST	0.2	0.8	29.8	115.0	12.4
Glyphosate + tribenuron *fb* fluroxypyr	PRE	POST	0.0	0.0	0.0	0.0	0.0
Glyphosate + tribenuron *fb* fluroxypyr + 2,4-D	PRE	POST	0.0	0.0	0.0	3.1	1.4
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron + 2,4-D	PRE	POST	0.1	0.1	11.0	15.7	9.0
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron + quinclorac	PRE	POST	0.5	0.0	78.3	3.0	10.0
Contrast statements							
Untreated vs Pre-emergent			*	*	*	*	NS
Untreated vs Post-emergent			*	*	*	*	*
Glyphosate vs glyphosate + tribenuron			NS	NS	NS	NS	NS
Thifensulfuron + tribenuron vs. thifensulfuron + tribenuron + 2,4-D			*	NS	*	NS	*
Thifensulfuron + tribenuron vs. thifensulfuron + tribenuron + quinclorac			*	NS	*	NS	*
Fluroxypyr + MCPA vs. fluroxypyr + 2,4-D			NS	NS	NS	NS	NS
Glyphosate vs. Pre-emergent * Post-emergent			NS	NS	NS	NS	*
Glyphosate+ tribenuron vs. Pre-emergent * Post-emergent			NS	NS	NS	NS	*

Abbreviations: fb, followed-by

[a]Least square means from the mixed model ANOVA

Non-orthogonal contrasts denoted by an asterisk (*) are significant at $P \leq 0.05$ and those denoted by NS are not significant at $P \leq 0.05$

Table 4-6. Volunteer flax adventitious presence in spring wheat from harvested plots as influenced by herbicide treatments at ERS and Ellerslie, Alberta in 2005 and 2006.

Treatment	Application timing		Adventitious presence[a]			
			ERS	Ellerslie	ERS	Ellerslie
			kg ha^{-1}		%	
Untreated	---	---	4.4	134.9	0.6	8.6
Glyphosate	PRE	---	0.3	4.7	0.0	0.2
Glyphosate + tribenuron	PRE	---	0.6	25.0	0.1	0.1
Thifensulfuron + tribenuron	---	POST	4.8	11.8	0.5	0.6
Fluroxypyr + MCPA	---	POST	0.1	0.5	0.0	0.0
Fluroxypyr + 2,4-D	---	POST	0.4	0.4	0.0	0.1
Thifensulfuron + tribenuron + 2,4-D	---	POST	1.5	24.8	0.2	1.5
Thifensulfuron + tribenuron + quinclorac	---	POST	1.7	7.7	0.1	0.4
Glyphosate *fb* thifensulfuron + tribenuron	PRE	POST	0.3	0.2	0.1	0.0
Glyphosate *fb* fluroxypyr + MCPA	PRE	POST	0.1	0.3	0.0	0.0
Glyphosate *fb* fluroxypyr + 2,4-D	PRE	POST	0.0	0.1	0.0	0.0
Glyphosate *fb* thifensulfuron + tribenuron + 2,4-D	PRE	POST	0.2	0.1	0.0	0.0
Glyphosate *fb* thifensulfuron + tribenuron + quinclorac	PRE	POST	0.1	0.0	0.0	0.0
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron	PRE	POST	0.3	0.5	0.0	0.0
Glyphosate + tribenuron *fb* fluroxypyr	PRE	POST	0.0	0.4	0.0	0.0
Glyphosate + tribenuron *fb* fluroxypyr + 2,4-D	PRE	POST	0.0	0.2	0.0	0.0
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron + 2,4-D	PRE	POST	0.1	1.6	0.0	0.1
Glyphosate + tribenuron *fb* thifensulfuron + tribenuron + quinclorac	PRE	POST	0.1	0.2	0.0	0.0
Contrast statements						
Untreated vs Pre-emergent			*	*	*	*
Untreated vs Post-emergent			*	*	*	*
Glyphosate vs glyphosate + tribenuron			**NS**	**NS**	**NS**	**NS**
Thifensulfuron + tribenuron vs. thifensulfuron + tribenuron + 2,4-D			*	**NS**	*	**NS**
Thifensulfuron + tribenuron vs. thifensulfuron + tribenuron + quinclorac			*	**NS**	*	**NS**
Thifensulfuron + tribenuron vs. fluroxypyr + MCPA			*	**NS**	*	**NS**
Thifensulfuron + tribenuron vs. fluroxypyr +2,4-D			*	**NS**	*	**NS**
Fluroxypyr + MCPA vs. fluroxypyr + 2,4-D			**NS**	**NS**	**NS**	**NS**
Glyphosate vs. Pre-emergent * Post-emergent			**NS**	**NS**	**NS**	**NS**
Glyphosate+ tribenuron vs. Pre-emergent * Post-emergent			**NS**	**NS**	**NS**	**NS**

Abbreviations: fb, followed-by

[a]Least square means from the mixed model ANOVA

Non-orthogonal contrasts denoted by an asterisk (*) are significant at $P \leq 0.05$ and those denoted by NS are not significant at $P \leq 0.05$

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Chapter 5: Emergence periodicity of volunteer flax (*Linum usitatissimum* L.) in conventional and reduced tillage systems

INTRODUCTION

Flax is being investigated as a crop for the production of bioproducts in western Canada. Crop volunteers may perpetuate transgene movement via pollen to adjacent conventional crops (Hall et al. 2000, Beckie et al. 2003) and seed could be harvested, and contribute to AP in subsequent crops (Downey and Beckie 2002, Friesen et al. 2003), or enter the seed bank and serve as secondary sources of gene flow in the ruderal environment (Yoshimura et al. 2006). Understanding volunteer flax biology and developing effective management strategies to minimize gene flow is critical if flax is to be developed as a bioindustrial crop.

Several transgenic herbicide resistance traits have been previously incorporated into cultivars of flax including resistance to chlorsulfuron and metsulfuron (McSheffrey et al. 1992), glufosinate ammonium (McHughen and Holm 1995), and glyphosate (Jordan and McHughen 1988). In 1998, the chlorsulfuron- and metsulfuron-resistant transgenic flax cultivar 'CDC Triffid' was released in Canada (CFIA 2001). Although this transgenic flax cultivar posed no unacceptable risk to food, feed, or the environment (CFIA 2001), CDC Triffid was deregulated shortly after its release at the request of the Canadian flax seed industry due to concerns about potential market harm (Anonymous 2002). A clear regulatory framework did not exist for transgenic crops or feed products in Europe at the time of its release and products derived from GE flax were unacceptable to the European market (McHughen 2002). Consumer concerns about GE crops for food and feed continue to be important in Europe. If flax is to be cultivated for bioproduct

applications in western Canada, effective management practices to mitigate gene flow and to segregate GE flax from conventional flax cultivars are necessary to preserve the value of the conventional flax market.

Similar to other crops, seed movement from transgenic flax may be a major pathway for gene flow (Hall et al. 2003). Flax seeds or seed bolls are lost prior to, during and after harvest and these seeds may enter the seed bank, forming a subsequent volunteer population. Flax volunteers may survive to produce seed which may be admixed (co-mingled) during harvest with food or feed crops, thereby contributing to AP (Kershen and McHughen 2005), or this seed may replenish the flax seed bank. The contribution of volunteer flax to seed-mediated gene flow under western Canadian agricultural conditions has not been quantified.

Information on the emergence periodicity of volunteer crops, the time when seedlings typically emerge during the year from the soil seed bank, is critical for the development of effective management practices that minimize weed interference during critical periods of crop development (Norsworthy and Oliveira 2004). Germination and emergence from the seed bank is governed by both release from seed dormancy and by environmental conditions governing germination including soil temperature and soil moisture (Forcella et al. 1993, King and Oliver 1994, Weaver et al. 1998, Roman et al. 1999). Domestic crops, including flax, have been selected for reduced dormancy to facilitate a high level of synchronous germination and emergence of the crop (Harlan 1992). The time of weed emergence in relation to the crop and to control practices such as tillage operations and herbicide treatments, are important factors that determine the outcome of crop and weed competition (O'Donovan et al. 2007, Chikoye et al. 1995, Hall et al. 1992, Swanton and Murphy 1996) and the persistence of volunteer populations in

subsequent years. Uncontrolled seedlings that emerge early are more competitive with crops for resources and produce more seeds than those that emerge, following the crop (Peters 1984, Peterson and Nalewaja 1992). However, weed seedlings that emerge with the crop, or shortly thereafter, may escape early control efforts (tillage and pre-seeding herbicide applications) (Sattin et al. 1992) and may survive to produce seed if not effectively controlled by a post-emergent herbicide. Late emergence may therefore be more likely to result in contributions to the soil seed bank and to AP unless an effective post-planting management strategy is implemented (Page et al. 2006, Page et al. 2007).

In temperate climates, soil temperature, expressed as thermal time or growing degree days (GDD), is commonly used to model and predict cumulative emergence. The addition of hydrothermal time may improve accuracy. With notable exceptions (Bullied and Van Acker 2003, Lawson et al. 2006, De Corby et al. 2007), few studies have characterized the emergence periodicity of annual crop volunteers in relation to environmental conditions within the seedling recruitment zone.

Tillage, including the timing (date), number and intensity of disturbances, influences soil temperature and moisture, vertical seed distribution, seed losses by predation and unsuccessful seed germination, and the biotic community within agroecosystems (Johnson and Lowery 1985, Addae et al. 1991, Buhler 1995, Buhler et al. 1997, Cromar et al. 1999). No-till or direct seeded fields tend to have a higher proportion of seed located on the soil surface where they are subject to seed predation, whereas conventional tillage fields have seeds more uniformly distributed throughout the soil profile (Hoffman et al. 1998). The presence of straw and chaff decreases the mean surface temperature and temperature variance in direct seeded fields while increasing the available moisture at the soil surface. Tillage may affect the emergence timing of

volunteer flax and higher numbers of residual crop volunteers have been reported in fields under conventional tillage (CT) than reduced tillage (RT) (Thomas et al. 1997). For weed seeds, soil microclimates can influence the proportion of seeds that are dormant; but, this may not be a significant factor for volunteer flax that, as a domestic crop, has reduced seed dormancy (Anonymous 2009). For small seeds, such as flax, deep burial may reduce successful seedling emergence. All of these factors influence the density and periodicity of seedling emergence (Buhler et al. 1997). The effect of tillage system (conventional or reduced) on the time of emergence and density of flax seedlings has not been determined.

Where flax is grown, volunteer flax is commonly observed in western Canadian cropping systems (Leeson et al. 2005). The purpose of this study was to (1) characterize the emergence periodicity of volunteer flax as a function of environmental conditions in central Alberta and (2) to determine the influence of tillage system (conventional vs. reduced) on emergence periodicity. In each of two years, flax emergence was quantified over the growing season in four commercial flax fields in eastern Alberta in the year following flax production. Patterns of emergence may be used to develop best management practices for volunteer flax to limit pollen- and seed-mediated gene flow.

MATERIALS AND METHODS

Site selection and data collection

Volunteer flax emergence was measured at two locations in commercial production fields in RT and CT wheat fields in Armena and Holden NW respectively in 2005 and in RT barley and CT wheat fields in Viking and Holden W respectively in 2006. Fields selected for this study had been sown to flax the previous cropping year (Table 5-1) and flax had not been grown four or more years prior (as indicated by the surveyed

producer), minimizing the potential for confounding effects from a pre-established seed bank.

Ten fixed $1m^{-2}$ quadrats were established at each location, located at least 20 m from the field perimeter. Each quadrat was randomly selected from the center meter of several adjacent 2 × 34 m rows. Following quadrat selection, fields were sown by the grower to either spring wheat or spring barley (Table 5-1). Fertilizer was applied uniformly to the field by the grower, either in the spring prior to seeding or in the fall following flax harvest (Table 5-2). Within the quadrats, weeds were removed by rouging. Newly emerged volunteer flax plants in each pre-established quadrat were quantified weekly and were removed from each established quadrat (May 18, 2005 and May 24, 2006) until pre-harvest desiccation (August 22, 2005 and August 16, 2006).

Precipitation (mm), soil moisture (m^3/m^3) and soil temperature ($^{\circ}C$) data were recorded hourly using on-site data loggers (HOBO Micro Station) equipped with rain gauge and sensory probes. The data logger along with rain gauge was located approximately 1 meter above the soil surface. Soil moisture and soil temperature sensory probes were placed at two soil depths, 2.5 cm and 10 cm, to monitor soil moisture and soil temperature. Data collection began on May 26, 2005 at RT Armena and CT Holden NW and on May 22, 2006 at RT Viking and at CT Holden W.

Air temperature and precipitation (rainfall) data available from three Environment Canada weather stations were used to determine local weather conditions at established sites for the duration of the study. The Camrose and Vegreville weather stations were used to determine mean monthly and long-term (1971-2000) climate (air temperature and precipitation) normals at Armena and Vegreville respectively, whereas the Edmonton International Airport weather station was used to determine mean monthly and long-term

(1971-2000) climate (air temperature and precipitation) normals for both Holden NW and Holden W.

To calculate daily growing degree days (GDD) each site-year, air temperature data were compiled from the nearest available Environment Canada weather station; Camrose and Vegreville for Armena and Vegreville respectively, and Edmonton for Holden NW and W. Available climatic means (air temperature and precipitation) from the three Environment Canada weather stations were used to determine long-term (30 years, 1971-2000) climatic normals for each site.

Data analysis

Total volunteer flax emergence was quantified for each site-year and was calculated as the sum of the average number of seedlings that emerged per square meter each week during the entire period of observation. Average emergence (plants m^{-2}) among pre-established quadrats (blocks) at each site among years was used to calculate cumulative weekly emergence and expressed as a percentage of the total germination over the period of experimental observation (May to August) (De Corby et al. 2007).

Density of flax volunteers that emerged over time (weeks) in commercial cereal fields were subject to analysis of variance (ANOVA) and were analyzed as a repeated measures design using the proc mixed (PROC MIXED) procedure in statistical analysis software (SAS) (SAS Institute Inc. 2007). To determine the average density of volunteer flax plants per square meter over time, data were analyzed separately for each year due to an unbalanced number of sample periods (weeks) among years. In the mixed model, site was considered a fixed effect, and quadrat (nested in site) and week of emergence were considered random effects. Lsmeans generated by the mixed model ANOVA are presented.

GDD were calculated separately for each site-year throughout the observational period (May 26-August 22, 2005 and from May 22-August 16, 2006). The following equation was used to calculate GDD:

$$GDD = \sum[(T_{max} + T_{min}]/2\text{-}T_{base}] \quad [1]$$

where T_{max} is the maximum daily air temperature, T_{min} is the minimum daily air temperature, T_{base} is the base temperature at which plant growth and development does not occur (5°C in this study) and GDD is a non-negative value (daily GDD values that were negative were replaced by 0) as suggested by Lawson et al. (2006). GDD are dependent upon a base temperature at which biological activity is greatly reduced. Flax seed germination is inhibited at a minimum of 0°C (DeCandolle 1865, Haberlandt 1874, Haberlandt 1875) suggesting the minimal temperature for germination as measured by radicle emergence in cultivated varieties of flax is above 0°C (O'Connor and Gusta 1994). O'Connor and Gusta (1994) reported that temperatures between 5-15°C did not influence percent germination of flax seed. The time for 50% of the flax seeds to germinate at 15°C averaged 30 hours compared to 160 hours at 5°C, but at a temperature range of 1.4°C to 1.9°C, flax seed germination averaged 34 days. It has previously been reported that the optimum range of constant temperatures for the germination of flax seed ranges from 4 to 20°C (Harper and Obeid 1967, Trifonov 1980). O'Connor and Gusta (1994) suggested a T_{base} 5°C was appropriate for flax.

GDD were summed over a seven day period to provide an accumulated weekly GDD value. Density of flax volunteers were expressed as a cumulative percentage of total volunteer flax emergence on a weekly basis during the period of observation. Data was analyzed separately for each site-year by nonlinear regression analysis as a function of cumulative GDD using NLIN procedure in SAS.

The logistic model fitted was:

$$y = [C + (C + D)]/ (1 + (x/EM_{50})^{b}) \quad [2]$$

where y is cumulative percentage emergence of volunteer flax, x is cumulative GDD, C is the lower limit (asymptote) of the response curve, $C + D$ is the upper limit (asymptote) of the responsive curve i.e. maximum emergence, EM_{50} is the x value (GDD) at the midpoint of the inflection point of the curve (not necessarily the GDD value at the 50% emergence depending upon the values of the fitted C and D response curve parameter estimates and the shape of the curve) and b is the slope (Seefeldt et al. 1995, Burke et al. 2005). The notation used in Equation 2 is similar to notation used by Seefeldt et al. (1995) for the description of log-logistic models. Individual curves were statistically analyzed systematically for common C and D, common EM_{50} and common b using the lack of fit F test at the 0.05 level of significance (Seefeldt et al. 1995). A coefficient of determination (R^2) was calculated for the model using the residual sum of square values from the SAS output (Kvalseth 1985) as SAS provides only one residual sum of squares value for the model as a whole, even though parameters for several curves are estimated concurrently (Seefeldt et al. 1995). Standard errors of parameter estimates were calculated.

RESULTS AND DISCUSSION

Weather conditions

Weather patterns were variable during the experimental period (May-August) both site-years (data not shown). In western Canada, year-to-year variance in environmental conditions may be extreme and some weed species may respond more strongly to temperature and precipitation conditions than to agronomic conditions (Blackshaw et al., 2001; Derksen et al. 1993).

In 2005, below normal (1971-2000) precipitation was received every month from May to August at RT Armena (the more southern site) whereas at CT Holden NW (the more northern site), conditions were close to the long-term normal (1971-2000). In 2006 with the exception of the month of May, RT Viking (the more eastern site) received less precipitation than CT Holden W (the more northern site) throughout the growing season.

During most of the growing season, mean monthly temperatures were comparable to the long-term normal at all locations (data not shown). However, mean monthly air temperatures at RT Armena were cooler than CT Holden NW in 2005, and in 2006, cooler conditions were experienced at RT Viking than at CT Holden W.

Period of seedling emergence

Volunteer flax emergence occurred throughout most of the growing season both site-years (Figure 5-1). In 2005, volunteer flax emergence occurred in all but 4 of the weeks observed (n=15 weeks) at RT Armena, whereas at CT Holden NW, volunteer flax emerged in all weeks (n=15 weeks). In 2006, volunteer flax emerged in all but 7 weeks at RT Viking and in all but 4 weeks at CT Holden W (n=13 weeks). As expected, peak emergence was not predictable by calendar date, although most emergence occurred between weeks 1 and 9.

There was no clear relationship between volunteer flax emergence and soil temperature (Figure 5-1). In some cases slight changes in emergence did correspond to changes in temperature; but, these changes were too infrequent and inconsistent to indicate that a relationship between soil temperature and emergence existed in this experiment. For example, in 2005 emergence appeared to increase during weeks 6 and 7 in RT Armena as soil temperature decreased. However, in RT Viking in 2006 a slight peak in emergence in week 3 corresponded to elevated temperatures. In 2005, mean

temperatures were fairly consistent and no clear trends were apparent regarding temperature and emergence for CT Holden NW. In CT Holden W 2006 a drop in temperature in week 4 corresponds to a drop in emergence, while higher temperatures in weeks 8, 9 and 10 correspond to periods where no emergence was recorded. The absence of any clear trends suggested that soil temperature cannot be used alone to predict the emergence patterns of volunteer flax.

There was a response in emergence to increased soil moisture in some instances (Figure 5-2). In RT Armena 2005, spring emergence was highest during week 6 with an increase in soil moisture. In CT Holden NW 2005 there was a large increase in emergence in week 6 compared to week 5 which corresponded to an increase in soil moisture and emergence decreased following this as moisture also decreased. In RT Viking 2006 there was no clear response to soil moisture. In CT Holden W 2006, later season germination occurred after increased soil moisture. In all cases, low levels of soil moisture appeared to correlate to periods where low emergence was recorded.

GDD as a measure of thermal time, are a heuristic tool frequently used to predict plant phenology, including seedling emergence. Cumulative volunteer flax emergence was effectively modeled as a function of GDD using T_{base} 5°C for each site-year (R^2 = 98.9 to 99.6) (Figure 5-3, Table 5-3). The use of T_{base} 5°C was suitable because plant development expressed in thermal time was relatively consistent across different environments (Lawson et al. 2006). The slopes of the line (*b*) were similar between tillage types (CT and RT) between years but differed between tillage types in the same growing year (Figure 5-3, Table 5-3). In 2005, calculated EM_{50} values (the growing degree day required for 50% emergence) at RT Armena and CT Holden NW was 340 and 228,

respectively (Table 5-3) whereas in 2006, EM_{50} were at RT Viking and CT Holden W and averaged 297 and 236, respectively (Table 5-3).

Seed germination, and radicle and shoot elongation are three biological processes involved in seedling development and emergence. While seed germination is driven by temperature and water potential (Meyer et al. 2000), radicle and shoot elongation is strongly influenced by temperature (Gummerson 1986, Carberry and Campbell 1989, Fyfield and Gregory 1989, Angus et al. 1991, Wheeler and Ellis 1991, Dahal and Bradford 1994). Seeds that imbibed are capable of germination in accordance with accumulated thermal time, i.e. the temperature above a defined base temperature (T_{base}) (Kiniry et al. 1991, Ritchie and Ne Smith 1991). Although flax seedling emergence was accurately modeled using thermal time (expressed in units of GDD), estimates to describe emergence may be further improved through the use of hydrothermal time. The ability to model flax seed germination and seedling elongation through hydrothermal time would be advantageous as the model could be used as management decision making tool for weed control efforts (Forcella 1998). To develop an effective hydrothermal time seedling emergence model for volunteer flax, more research is required.

Compared to other western Canadian crops, flax has a prolonged period of emergence. In the southwestern region of Manitoba, Lawson et al. (2006) reported that volunteer canola (*Brassica napus* L.) required ≤132 soil GDD (T_{base} 5°C) to attain 50% emergence and that the majority of volunteer canola seedlings emerged either just prior to or within seeding the spring wheat crop. Although not expressed in thermal time, Harker et al. (2006) also reported that most volunteer canola emerged pre- or post-seeding. Similarly, volunteer wheat emergence occurred during the same pre- and post-seeding period (Harker et al. 2005). Volunteer canola has no primary seed dormancy, but does

have inducible secondary dormancy (Pekrun et al. 1997, Gulden et al. 2003, Gulden et al. 2004) while volunteer wheat been reported to require a short after ripening period (Pickett 1989, Benech-Arnold 2004). Studies evaluating dormancy in flax have not been published, and seed dormancy may be a key factor in the lag in emergence seen in flax. Harvest losses of flax include both flax seed and flax bolls. Flax seed contains mucilage in the outer layer of the seed hull (Mazza and Biliaderis 1989). Under dry conditions, the mucilaginous content of the seed may aid flax seed germination as it easily absorbs soil moisture (Anonymous 2002). However, flax bolls are resistant to degradation and may buffer seeds from microsite conditions that favor germination and emergence.

Effects of tillage system on time of emergence and cumulative emergence

The rate of volunteer flax emergence (*b*) was different between tillage systems in 2005 (p=0.0001) and in 2006 (p=0.0001) (Table 5-3). Volunteer flax emerged more rapidly and reached its period of peak emergence earlier (EM_{50} = 228 and 236) in CT fields than in RT fields (EM_{50} = 340 and 297) in 2005 and 2006 respectively (Table 5-3). In addition, in CT fields, volunteer flax continued to emerge late into the growing season, although at low average densities (> 10 plants m^{-2}). It is possible that in CT fields, tillage accelerated disruption of the seed bolls and thus increased seed to soil contact, resulting in earlier emergence.

CT fields had more volunteers emerge in both years than RT fields (p=0.0001). In 2005, CT Holden NW had > 9 fold more seedlings emerge than RT Armena (4597 and 484 plants m^{-2}, respectively) and in 2006, the total density of flax volunteers was 60 times higher in CT Holden compared to RT Viking (1883 and 31 plants m^{-2} respectively). There were also differences between years. Total volunteer flax densities in sampled commercial fields were greater in 2005 than in 2006. Many flax fields were harvested

after the first snow in 2004 suggesting that other crop management practices such as flax seed harvest losses may have also influenced volunteer flax seed bank additions in the subsequent year.

There are many factors that could be contributing to the differences in emergence in CT and RT fields, including harvest loss, seed bank persistence, mechanical disruption of flax bolls and available microsites for germination. Harvest losses may vary considerably between fields and were not measured in these sampled fields in the year of flax production. They can not be eliminated as a factor influencing seedling density. Seed bank persistence can be influenced by fatal germination of non-dormant seeds, seed predation, disease and abiotic stress. CT fields (Holden NW and Holden W) also received two spring tillage operations (approximately ≤5 cm deep) prior to plot establishment. Because tillage occurred in RT fields only in spring, seed bank size may not have varied greatly between CT and RT fields. Greater recruitment of volunteer flax under CT regimes may be partially explained by the larger number of microsites created by tillage, by differences in the vertical distribution patterns of seeds in the soil profile, and by enhanced mechanical breakup of the flax bolls contributing to greater soil-seed contact.

Shallow tillage has been shown to increase weed seed germination and emergence (Ogg and Dawson 1984, Warnes and Andersen 1984), through changes to the vertical distribution of weed seeds in the soil profile and through modified soil temperature and moisture regimes in seedling recruitment microsites (Johnson and Lowery 1985, Addae et al. 1991, Egley and Williams 1991). While crop residues on the soil surface may reduce water losses and provide a more favorable moisture environment for volunteer flax emergence under dry conditions (Teasdale and Mohler 1993), surface residues may also obstruct hypocotyl elongation and reduce light stimuli fluctuations required for

germination (Buhler et al. 1996). Slightly greater fluctuations in soil moisture, particularly during the early part of the growing season (weeks 1-5), may have contributed to a more rapid emergence rate in CT compared to RT fields both site-years. In addition, a greater proportion of seeds may have been recruited to germinate from greater soil depths in CT than RT fields. Differences in seedling emergence associated with tillage have been reported previously for many weed species including volunteer flax. In field trials conducted in Saskatchewan, Derksen et al. (1993) reported that volunteer flax was more greatly associated with direct seeding and/or RT systems than CT systems. Our data indicated that RT results in lower numbers of volunteers than CT. However, a consideration not addressed in the current study is the density of seed and seed bolls in the seed bank and their persistence in RT and CT fields. It is possible that seed that does not germinate in RT fields in the first year following a flax crop and has prolonged emergence in subsequent years.

Implications of emergence timing for volunteer flax control

At all site-years, 50% of volunteer flax seedlings emerged before in-crop herbicide application (Table 5-1 and 5-3, Figure 5-4). In 2005, volunteer flax attained 50% emergence on June 11th and May 31st at RT Armena and CT Holden NW respectively based on calculated GDD values (T_{base} 5°C). In 2006 at RT Viking and CT Holden W, volunteer flax attained 50% emergence based on calculated GDD values (T_{base} 5°C) on June 6th and May 24th respectively. In cereal fields in central Alberta, post-emergent herbicides are generally applied in the second week of June when the crop has reached the 3rd or 4th leaf stage (Table 5-1). Flax volunteers that emerge after in-crop herbicide application may escape control, mature and produce seed and contribute to the soil seed bank. However, late-emerging volunteers are less able to compete with the crop

as volunteer flax is an uncompetitive weed and therefore seed production may be limited (Wall and Smith 1999).

If flax is to be developed for bioproducts, volunteer seedlings which emerge in high densities and have a prolonged emergence may be a large source of gene flow, via pollen to adjacent fields and via seed, as AP in the following crop or by recharging the seed bank. Growers who practice tillage may be discouraged from production of flax for bioproducts to reduce the possibility of AP in subsequent crops. Additionally, post-emergent herbicide treatment(s) may need to be delayed to control a large portion of late emerging flax cohorts and in uncompetitive crops, yield losses may be severe. Information regarding the fecundity of flax, herbicide control and the amount of AP of flax in subsequent crops would aid in the development of best management practices to control volunteer flax.

Table 5-1. Dates for field operations in commercial fields in 2004, 2005 and 2006.

Site	Year	Crop	Variety	Tillage		Seeding date	Seeding rate	Date of in-crop herbicide application
				Timing	No. of operations			
							kg ha^{-1}	
Armena	2004	flax	CDC Bethune	n/a*	0	May 21	55	---*
	2005	wheat	AC Splendor	n/a	0	May 18	115	June 14
Holden NW	2004	flax	McGregor	Spring	1	May 20	40	---
	2005	wheat	Parkland	Spring	2	May 22	110	June 10
Viking	2005	flax	Hanley	Spring	0	May 19	80	---
	2006	barley	AC Metcalfe	Spring	0	May 30	115	June 12
Holden W	2005	flax	Normandy	Spring	1	May 21	40	---
	2006	wheat	Parkland	Spring	2	May 20	110	June 9

*Not applicable

Table 5-2. Fertilizer rates and application timing in commercial fields in 2005 and 2006.

Year	Site	Nitrogen	Phosphorous	Time of application
		kg ha^{-1}		
2005	Armena	115	25	prior to planting
	Holden NW	110	50	prior to planting
2006	Viking	140	65	after harvest
	Holden W[a]	110	50	prior to planting

[a]6 kg Cu ha^{-1} was applied prior to seeding

Table 5-3. Parameter estimates (standard errors in parentheses) for emergence period response of volunteer flax (T_{base} 5°C) in small plot experiments at RT Armena and CT Holden NW in 2005 and RT Viking and CT Holden W in 2006 (Figure 5-3)[1].

Site	Year	C[a]	D[b]	b[c]	EM_{50}[d]	EM_{50}	R^2
		%			GDD[e]	Date	
Armena	2005	0.0 (0.00)	101.5 (0.68)	-5.5 (0.20) *a*	340 (2.57)	June 11	99.5
Holden NW	2005	-7.9 (1.59)	108.2 (1.75)	-4.5 (0.15) *b*	228 (2.42)	May 31	99.6
Viking	2006	-2.7 (1.02)	103.0 (1.13)	-9.8 (0.36) *a*	297 (1.46)	June 6	99.6
Holden W	2006	0.8 (0.20)	100.5 (0.00)	-3.9 (0.05) *b*	236 (1.24)	May 24	98.9

[1]Percentage cumulative volunteer was expressed as a function of cumulative soil growing degree days (GDD). A logistic model was fitted to the data (refer to materials and methods for a description of the model fitted) as suggested by De Corby et al. (2007). Values within in a column with the same letter are not significantly different according to Fischer's Protected LSD test at $P \leq 0.05$.

[a]Lower limit (asymptote) of the response curve

[b]Upper limit (asymptote) of the responsive curve

[c]Slope

[d]The *x* value (GDD) at the midpoint of the inflection point of the curve

[e]Growing degree days

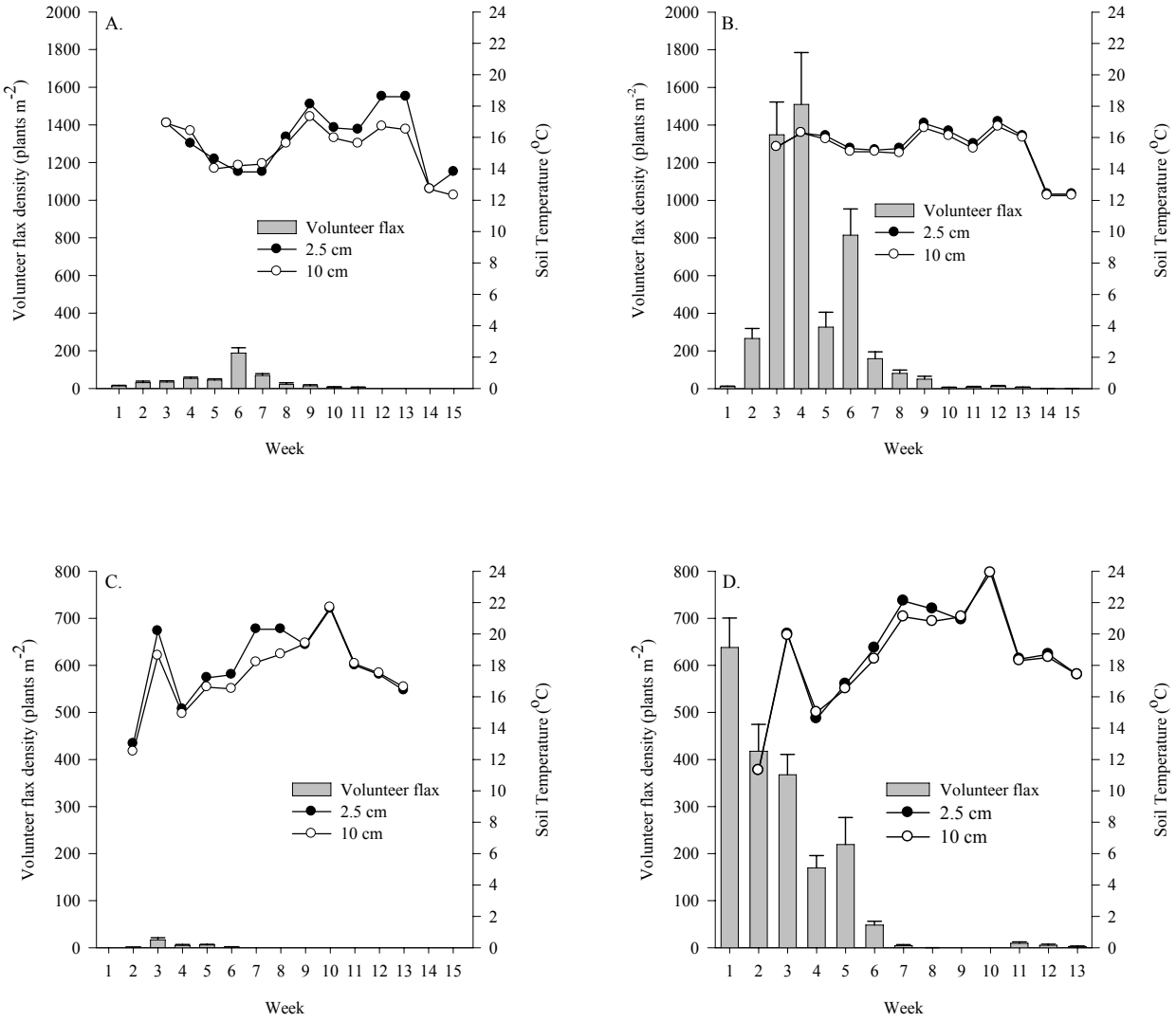

Figure 5-1. Volunteer flax density and average soil temperatures at 2.5 cm (upper) and 10 cm (lower) soil depths at (A) RT Armena and (B) CT Holden NW in 2005 and at (C) RT Viking and (D) CT Holden W in 2006 during the period of experimental observation. In 2005, week one corresponds to May 18 and week 15 corresponds to August 22. In 2006, week one corresponds to May 23 and week 13 corresponds to August 16.

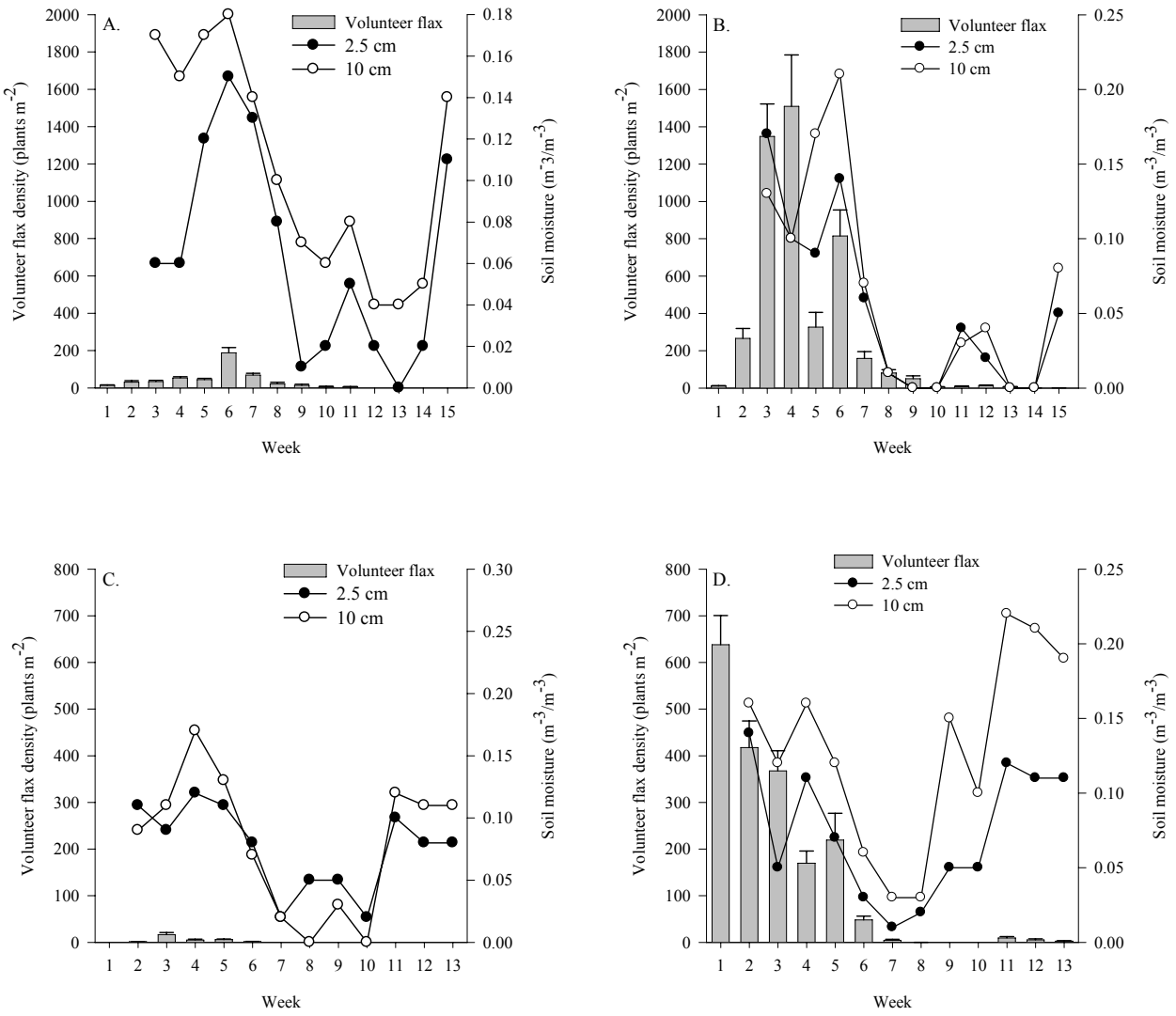

Figure 5-2. Volunteer flax density and average soil moisture at 2.5 cm (upper) and 10 cm (lower) soil depths at (A) RT Armena and (B) CT Holden NW in 2005 and at (C) RT Viking and (D) CT Holden W in 2006 during the period of experimental observation. In 2005, week one corresponds to May 18 and week 15 corresponds to August 22. In 2006, week one corresponds to May 23 and week 13 corresponds to August 16.

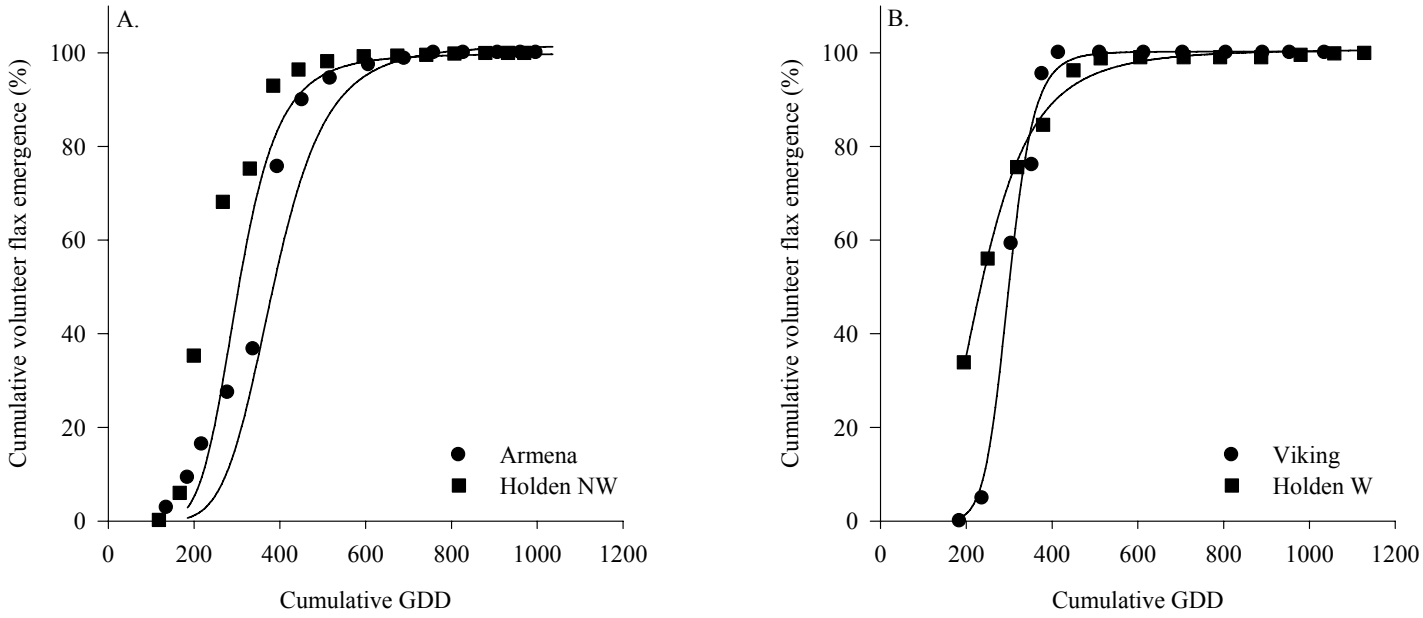

Figure 5-3. Volunteer flax cumulative emergence at (A) RT Armena and CT Holden NW, Alberta in 2005 and at (B) RT Viking and CT Holden W in 2006 as related to cumulative GDD T_{base} 5˚C. Symbols represent mean values for each assessment date and the line represents the fitted logistic regression equation. Refer to Table 5-3 for parameter estimates.

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Chapter 6: The contribution of volunteer flax (*Linum usitatissimum* L.) to seed-mediated gene flow in western Canadian cropping systems

INTRODUCTION

The quantity and persistence of weed and volunteer crop seed in the soil seed bank is influenced by many factors including harvest method, predation, seed dormancy, and environmental conditions such as moisture and temperature (Crawley 1992, Komatsuzaki and Endo 1996, Baskin and Baskin 1998, Cromar et al. 1999, Benech-Arnold et al. 2000, Gulden et al. 2003b, Hesse et al. 2007). The soil seed bank allows the perpetuation of crop volunteer populations through time and, for transgenic crops, may facilitate spatial and temporal transgene movement (Yoshimura et al. 2006). Volunteer flax (*Linum usitatissimum* L.) is a summer annual, dicotyledonous weed that initially arises from seed and seed boll losses incurred during harvest. If genetically engineered (GE) flax cultivars are developed, volunteers may be harvested with subsequent crops and contribute to seed-mediated gene flow. Adventitious presence (AP) of transgenic flax bioproducts in conventional crops jeopardizes market access to major export markets such as the European Union (EU) (McHughen 2002). Transgenic flax volunteers may also contribute to seed-mediated gene flow through the replenishment of the flax seed bank during harvest operations via seed and/or seed boll losses. Therefore, an understanding of what factors influence flax seed loss at harvest and the persistence of viable seed in the seed bank is essential.

In western Canada, flax is grown primarily as an annual oilseed crop and in 2008, 611.1 thousand hectares were grown; predominantly in Saskatchewan (81%) and Manitoba (17%) (Statistics Canada 2007, Statistics Canada 2008). Flax seed oil contains a

high level of linolenic acid (ALA) (45-60%) (Johnston et al. 2002), which gives it an excellent drying quality useful for industrial purposes (Saeidi and Rowland 1999, Anonymous 2002). The development of low-ALA (< 5%) Solin flax lines (Green 1986, Rowland 1991, Dribnenki and Green 1995) expanded potential markets for flax seed oil. Low-ALA Solin or linola oil is less susceptible to rapid oxidation (Ralph 1992, Vrinten et al. 2005) and is thus more suitable for use in products such as margarine (Bhatty 1995). These Solin lines were developed using advanced crop breeding (conventional) techniques. In Canada, Solin seed is required to be yellow to differentiate it from conventional brown seeded varieties (Saeidi and Rowland 1999, Mittapalli and Rowland 2003). Seed vigor and germination capacity may be affected by seed color and fatty acid composition (Culbertson and Kommendahl 1956, Dogras et al. 1977); but, evidence regarding differences in seed vigor and germination frequencies between yellow and brown seeded flax have been contradictory (Culbertson and Kommedahl 1956, Culbertson et al. 1960, Comstock et al. 1963, Saeidi and Rowland 1999), so it is unclear if seed color and fatty acid composition influence seed vigor and germination.

Flax was among the first crop species to be genetically engineered to express traits of agronomic value such as herbicide resistance (McHughen 2002). In 1998, the chlorsulfuron and metsulfuron resistant transgenic flax cultivar ‘CDC Triffid’ was released in Canada (McSheffrey et al. 1992, CFIA 2004), but was deregulated shortly after its release at the request of the Canadian flax seed industry due to concerns about potential market harm (Anonymous 2002). The primary market for Canadian flaxseed is the non-food European linoleum/industrial oil market; but, flax meal is routinely fed to livestock for feed (Anonymous 2002). If GE flax is to be cultivated in Canada, effective

management practices to mitigate gene flow and segregate GE flax from non-GE flax cultivars is necessary to preserve conventional and organic flax market value.

Crop yield losses during harvest can be considerable (Clarke 1985, Anderson and Soper 2003, Gulden et al. 2003a, De Corby et al. 2007, McPherson 2008). Gulden et al. (2003a) reported that canola yield losses among producers ranged from 3.3 to 9.9% which equated to canola seed bank additions of 1,530 to 7,130 seeds m^{-2}. Flax is more difficult to thresh than other small grain and oilseed crops due its indeterminate growth habit and the presence of bast fibers in its stem. It is possible to directly combine the standing crop when 75% or more of the seed bolls have turned brown and the crop appears uniform in maturity (Anonymous 2002). However, swathing before combining is the most common harvest procedure as windrow-drying allows the seed to reach moisture levels safe for long-term storage (less than 10%) (Anonymous 2002). Harvest losses may be influenced by factors such as the harvest procedures employed, combine settings, the timing of harvest with respect to crop maturity and environmental conditions (Gulden et al. 2003a). The quantity of flax seed losses at harvest from combine harvesters and the factors which influence these losses have not been characterized.

Persistence of flax in the seed bank is influenced by endogenous and environmental factors. Flax seeds are produced in a boll (Lay and Dybing 1989) which may provide a protective barrier, buffering seeds from microsite conditions that favor germination and emergence, thus enhancing seed persistence. Seed dormancy (primary and secondary) is an important persistence mechanism for many annual weeds (and some crop volunteers) (Baskin and Baskin 1998). Loss of dormancy is critical in crop domestication as it facilitates the synchronous germination of sown crop seed (Harlan 1992). Flax, like other crops, has been selected for reduced dormancy, but the degree of

seed dormancy and the duration of seed persistence have not been reported. Seed bank losses result from germination, damage, disease, exhaustion and predation. The outer layer of the seed hull contains mucilage which may aid in flax seed germination as the outer layer of the hull easily absorbs soil moisture (Van Rheede van Oudtshoorn and Van Rooyen 1999, Penfield et al. 2001, Western et al. 2004). The percentage of seeds in the seed bank that germinate in a given year varies with crop management practices, environmental conditions and depth of seed burial (Cavers 1983). Germinated seeds may perish as seedlings, or may survive to set seed and replenish the seed bank. Physical damage to seed, which may be influenced by environmental conditions, can reduce seed persistence and disease may also affect the viability of seeds. Seed predation can result in large crop seed losses from the soil seed bank during or after harvest and buried seeds are less susceptible to predation than seeds on or near the soil surface (Hulme 1998, Hartzler 2007, Orrock and Damschen 2007). While it is understood that seed predation is a significant factor in seed bank deposits and losses, estimates of the influence of predation on seed bank dynamics have been reported for a only a few plant species (Louda 1989, Cummings et al. 1999, Alexander et al. 2001, Cummings et al. 2002), and have not been investigated for flax.

The potential development of GE flax for bioproducts has raised concerns about the persistence of transgenes in agroecosytems. Currently, little is known about the seed bank ecology of flax, and a comprehensive determination of flax seed losses associated with harvest procedures on commercial farms has not yet been documented. The objectives of this study were to: (1) quantify flax seed losses from combine harvesters during harvest at different sites and years; (2) assess the effects of harvest method (windrow/combine and direct combine) on flax seed bank additions; (3) evaluate and

compare seed bank persistence of yellow- and brown-seeded flax varieties; (4) assess the effect of burial depth on viable seed persistence. The results of this study can be used to quantify seed-mediated gene flow of flax under western Canadian agricultural conditions.

MATERIALS AND METHODS

Determination of flax seed viability with gibberellic acid

An experiment was conducted in the laboratory to determine if gibberellic acid (GA_3) could be used as an effective substitute for 2,3,5-triphenyltetrazolium chloride in flax seed viability experiments. Because of the difficulty examining large numbers of small seeds, a tetrazolium based test could not be used as a primary test to determine flax seed viability. Our methodology closely follows that of Zorner et al. (1984) and was developed with the flax cultivar CDC Bethune.

Eleven random sub-samples of 800 seeds were tested for germination by placing the seeds in acrylic germination boxes (24 x 16 x3.8 cm) (Hoffman Manufacturing Inc.) between 15 x 23 cm non-toxic white filter paper equivalent to Whatman #1 (Hoffman Manufacturing Inc.). To reduce fungal growth, 14 mL of a 0.2% solution of the seed treatment Helix Xtra® (thiamethoxan + difenoconazole + metalaxyl-M + fludioxonil) was added to each germination box. After 72 hours in the dark at ambient temperatures, germinated seeds were counted and recorded. Seeds were considered to have germinated when the radicle had broken the seed coat. The non-germinated seeds were transferred into acrylic germination boxes (24 x 16 x 3.8 cm) (Hoffman Manufacturing Inc.) lined with 15 x 23 cm non-toxic white filter paper equivalent to Whatman No. 1 (Hoffman Manufacturing Inc.) and moistened with 8 ml of 0.005 M GA_3 (MP Biomedicals Inc.) solution. After 72 hours on the 0.005M GA_3 solution, the number of flax seeds that did and did not germinate after GA_3 treatment were counted and recorded. Seeds that were

soft and/or degraded were considered to be dead. Turgid and fully imbibed non-germinated seeds were cut along the pericarp (seed coat) suture line and placed embryo side down in a Petri dish containing Whatman No. 1 filter paper and 0.15% of 2,3,5-triphenyltetrazolium chloride (Mallinckrodt Baker) and incubated for 2 hours at room temperature. Positive seeds turned pink and were considered viable and the balance considered dead.

Flax harvest losses from commercial fields

Sample collection

In 2006 and 2007, flax fields were selected from flax producers within a 300 km radius of Edmonton, Alberta, Canada (Appendix 1, Table A1). A total of five fields were sampled at three sites (Viking, Minburn and Wainwright) within one week of harvest in 2006. In 2007, a total of five fields at four sites (Viking, Vegreville, Wainwright and Vermillion) were sampled within one week of harvest. Sampled fields ranged in size from 10 to 375 hectares. Flax was last grown on all fields four or more years before the flax crop that was sampled. In 2006, four fields were swathed and windrow-dried and one field was directly combined. In 2007, one field was swathed and windrow-dried and four fields were directly combined. For each field surveyed, data were collected from producers using a survey questionnaire that included crop seed yield, cultivar grown, years since the last flax crop was sown, combine settings, perceived time of windrowing relative to crop maturity and harvest date.

In swathed fields, flax seeds were collected from ten randomly selected windrows and inter-windrows in a 0.25 m^2 quadrat located every 25 m in an inverted W pattern in each field (Thomas 1985). In direct combined fields, flax seeds were collected in twenty 0.25 m^2 quadrats every 25 m in an inverted W pattern (Thomas 1985). Crop residue, non-

harvested flax seeds and flax seed bolls were removed from each quadrat using a wet-dry vacuum cleaner. Samples were placed in labeled cloth bags, dried for 48 hours at 25°C and stored before further analysis.

To compare flax seed losses from combine harvesters among sampled commercial fields (windrow combine or direct combine) within years, total seed losses were calculated in proportion to the total field area sampled and expressed either as average density of seeds (seeds m^{-2}), or as percentage of yield or kg ha^{-1}. Average seed loss density (D_F) in windrow combined fields was calculated as

$$D_F = D_W /(W_W / W_w + W_I) + D_I /(W_W / W_w + W_I) \qquad [1]$$

where D_W is the density seeds measured in the windrow, W_W and W_I is the width of the window and inter-window respectively, and D_I is the density of seeds measured in the inter-windrow.

Sample processing and seed viability testing

Samples were initially sieved with a 7.14 mm screen to remove large crop residue particles, and all remaining inorganic materials were separated from the flax seeds by hand. To determine flax seed viability, random subsamples of 100 seeds from each sample were germinated (see above methodology). Non-germinated seeds were transferred to acrylic germination boxes (24 x 16 x 3.8 cm) (Hoffman Manufacturing Inc.) lined with 15 x 23 cm non-toxic white filter paper equivalent to Whatman No. 1 (Hoffman Manufacturing Inc.) and moistened with 8 ml of 0.005 M GA_3 solution. After 72 hours on the 0.005M GA_3 solution, the number of flax seeds that did and did not germinate were counted and recorded. Seeds that were soft and/or degraded were considered to be dead. Alive seeds included seeds that germinated on water and GA_3.

Data analysis

All data were tested for normality before the analysis to ensure that the residuals were random, homogenous with a normal distribution about a mean of zero. The data were subject to analysis of variance (ANOVA) and were analyzed as a completely randomized design using the proc mixed (PROC MIXED) procedure in statistical analysis software (SAS) (SAS Institute Inc. 2007). To determine differences in flax seed bank additions (seed loss from combine harvesters per square meter), total seed loss (seed loss from combine harvesters as a proportion of the total field area) and percent viable seed (the sum of germinated seed from combine harvesters as a proportion of the total field area) among sampled fields, a two-level nested design structure was used where year was considered a random effect and fields were nested in sites and were considered a fixed effect. Least significant differences were determined when the model showed significance ($P \leq 0.05$) and means were separated by Least Significant Difference (LSD).

Flax seed bank persistence

Field selection and seed sources

Artificial seed banks were established in the fall of 2005 and 2006 at the Ellerslie Research Station (ERS) and at the Vegreville Research Station (VRS). The soil at ERS was a loam soil and consisted of 28.6% sand, 46.4% silt and 25% clay with a pH of 6.3 and 11.5% soil organic matter content. At the VRS, the soil was a loam and consisted of 35% sand, 34% silt and 31% clay with a pH of 6.3 and an organic matter content of 7.2%. Weather data were compiled from the nearest available weather stations, Vegreville in the case of VRS and Edmonton Woodbend for ERS, and available climatic means were estimated at Vegreville (30 years, 1971-2000) and Edmonton (30 years, 1971-2000). Air

temperature and precipitation data for both research locations are summarized for Ellerslie and Vegreville in Figure 6-1.

Five genetically diverse cultivars of flax were chosen for this study (Appendix 1, Table A2). Cultivars differed in ALA content and seed coat color. Flax seed used in the 2005 and 2006 experiments were obtained from the Agriculture and Agri-Food Research Center in Morden, Manitoba within one week of harvest to ensure that the genotypes were exposed to the similar environments during growth, flowering and maturation. Seeds of each variety (1000) were tested for germination prior to burial (Appendix 1, Table A2). Prior to burial, 200 seeds of each variety were placed into a single 3 x 5 in^2, 50 μ mesh polypropylene bags. The mesh bags were permeable to water and allowed direct seed to soil contact. The bags were divided into five equal packets to separate the five flax varieties. Flax varieties were initially assigned a random packet order (1-5). After the order of the varieties had been recorded, the packets were filled. The seed bags were not disturbed following burial and were buried at the ERS on October 12th, 2005 and October 5th, 2006 and at the VRS on October 13th, 2005 and October 6th, 2006.

The field plots were established in an area with no previous history of flax production at both research locations. Before the establishment of the experiment at the ERS, the plot area had been chemically fallowed and undisturbed for the previous 2 years and at the VRS, the plot area had been seeded to canola (*Brassica napus* L.). The soil surface was kept free of weeds throughout the experimental period by applying glyphosate [*N*-(phosphonomethyl) glycine] at 100 L ha^{-1} at 214 KPa with a self-propelled plot sprayer equipped a 1m boom with Teejet XR 110015 nozzles and by additional hand weeding.

Experimental design and evaluation of seeds

All experiments were arranged in a split-plot design with four replicates. At each research location, soil was excavated to create three 8 x 34 in^2 plots spaced 1 m^2 apart per block. In all years, the main plot was burial depth (0, 3 and 10 cm) with flax variety as the sub-plot. Each soil depth (0, 3 and 10 cm) occurred only once in each of the three blocks at each research location. For each excavated plot, a single 6 x 32 in^2 metal cage constructed out of avian restriction mesh wire was placed into the ground at the time of trial establishment. The bottom of the cage was covered with 1 cm of excavated soil and a total of nine mesh bags were randomly placed on top. The seed bags were then fully covered with the soil that had been removed to create the excavated plots. A 6 x 32 in^2 lid constructed out of avian restriction mesh wire was placed on top of the cage after the bags had been covered with soil and the lid was secured with plastic cable ties.

Packets of seed were extracted from plots at both sites three times throughout each growing season: early spring, mid-summer and fall, at approximately nine week intervals. Seed packages were washed to remove soil and seeds were removed and counted. Seeds were stored at 4°C for two to five days prior to sample processing. Seeds were categorized as germinated or non-germinated. Any non-germinated seeds were then tested for viability with Helix Xtra® and GA_3 (methodology described above).

Data analysis

The number of viable flax seeds remaining in the seed bank was calculated as the sum of germinated and GA_3 germinated seeds. The proportion of viable seeds (y) at each extraction time, and days after planting (DAP) were subject to analysis of variance (ANOVA) and were analyzed as a split-plot design using the proc mixed (PROC MIXED) procedure in SAS (SAS Institute Inc. 2007). Years were analyzed separately due to differences in the number of elapsed days between extraction times. In the mixed

model, site, depth of seed burial and variety were fixed and block, which was nested in site, was random. Where the ANOVA indicated that treatment effects were significant, means were separated at $P \leq 0.05$ by Least Significant Difference (LSD) test. Where data sets were too sparse to meet the assumptions of ANOVA (i.e. data sets included too many zero values), but results were still of biological importance, means and associated standard errors are presented.

RESULTS AND DISCUSSION

Method to determine viability of flax seeds with gibberellic acid

Since tetrazolium tests to determine flax seed viability could not be accurately performed on flax seeds due to their small size, we evaluated the accuracy of an alternative method using GA_3 (Appendix 1, Table A3). In preliminary experiments (n = 8800), an average of 86.7% of seed germinated at room temperature in water without GA_3 and an additional 2.1% of seed germinated after the addition of GA_3. Of the remaining non-germinated seeds, an average of 1.5% were viable and 9.4% were dead, including soft/degrading seed and those that did not show metabolic activity according to the tetrazolium test. The error rate of 1.5% was deemed acceptable to continue use of the GA_3 method to test the viability of flax seeds. In subsequent experiments (flax harvest loss and flax seed burial), GA_3 rather than tetrazolium was used as the primary test to examine flax seed viability and the error rate (1.5%) was not accounted for in subsequent experiments.

Seed dormancy is the failure of a seed to germinate under favorable environmental conditions and includes primary dormancy, which is present upon seed shed or harvest, and secondary dormancy where dormancy is imposed later in response to unfavorable environmental conditions. Loss of seed dormancy is often a key step in crop

domestication (Harlan 1992). Unlike weed seeds, most crop seeds do not express significant primary seed dormancy. To our knowledge seed dormancy has not been reported in cultivated varieties of flax. In this study an average of 86.7% of seeds immediately germinated at room temperature when moistened, suggesting that flax does not have high levels of seed primary dormancy. An average of 2.1% of seeds that failed to germinate at room temperature when moistened did germinate when treated with GA_3 and an average of 1.5% of seeds that still failed to germinate on GA_3 were determined to be viable on tetrazolium suggesting the potential that 3.6% of the seeds may have been exhibiting primary dormancy. The low levels of potential dormancy suggest that flax seed persistence in the seed bank is primarily influenced by a number of abiotic and biotic stressors influencing germination and seed death (Batalla and Benech-Arnold 2007).

Flax seed losses during harvest

Flax seed losses during harvest were large and highly variable in direct combine fields and windrow/combined fields for all sites in both years (2006 and 2007) (Table 6-1). In 2006 in windrow/combine fields, seed bank additions were higher ($p=0.0001$) and more variable in windrows (845 to 1986 seeds m^{-2}) than inter-windrows (53 to 117 seeds m^{-2}). Similarly, in 2007 in the one field that was windrow/combined, average flax seed bank additions were 11 times higher in windrows (246 seeds m^{-2}) than inter-windrows (22 seeds m^{-2}). These results were expected as a higher number of flax seeds are generally returned to the soil surface in concentrated aggregates in windrows than in inter-windrows due to flax seed boll shatter during swathing and additional processing during combine pick-up. In 2006 and 2007, flax seed bank additions in direct combine fields ranged from 145 to 804 seeds m^{-2} and were generally lower than the seed bank additions observed in harvested windrows in windrow/combine fields (246 to 1986 seeds m^{-2}).

These results indicated that flax harvest method (windrow/combine or direct combine) may influence the amount of flax seed lost from combine harvesters.

Total seed losses were different ($p=0.0001$) among years (2006 and 2007) (Table 6-1). In general, seed losses were higher in 2006 than in 2007 in both windrow/combine and direct combine fields. The higher losses in 2006 may reflect adverse weather conditions around the time of flax harvest. Conditions prior to harvest were cooler with higher total precipitation then normal in 2006; whereas in 2007, conditions were warmer and drier (data not shown). The maturation of flax is delayed by cool and wet weather conditions and higher seed losses may be incurred at harvest due to difficulty in cutting and processing flax straw (Anonymous 2002). However, our ability to make flax harvest loss comparisons among years was highly restricted by the number of fields surveyed and by the variance in relation to the mean so the factors influencing total seed losses remain unclear.

Estimated total seed losses of flax in windrow/combine and direct combine fields were highly variable between fields within years (Table 6-1). In 2006, in fields that were windrow/combined total flax seed losses ranged from 8 to 24 kg ha^{-1} (0.3 to 1.4% of yield loss) and in the one field that was directly combined, total seed losses of flax averaged 44 kg ha^{-1} (2.8% of yield loss). In 2007, in the one field that was windrow/combined total flax seed losses averaged 3 kg ha^{-1} (0.2% of yield loss) and the four fields that were directly combined total seed losses of flax ranged from 8 to 19 kg ha^{-1} (0.4 to 0.1% of yield loss). The time of flax harvest in relation to crop maturity, combine settings and environmental conditions are three critical factors that could influence flax seed losses (Gulden et al. 2003a). Observed variability in total seed losses in 2006 and 2007 could not be explained by differences in combine settings as surveyed producers indicated

similar rotary, sieve and wind-speed settings (data not shown). However in 2006, surveyed producer's indicated that crop maturity was not highly uniform and that wet weather conditions and the high potential for frost damage influenced flax harvest date and resulted in a relatively early harvest. Therefore, differences in flax seed losses among surveyed fields in 2006 may be partially explained by differences in producer's perceptions of when to combine the standing flax crop in relation to crop maturity. Differences in flax seed losses among surveyed fields in 2007 could not be directly attributed to adverse weather patterns or to differences in crop management practices among surveyed producers. Other factors are known to influence harvest losses, such as seed moisture content (Bowren and Pittman 1975). The variability between fields suggested that seed losses may be influenced by factors which are difficult to predict and may not be solely attributed to harvest technique. The stochasticity of harvest loss studies have been previously reported in canola, wheat and safflower (Clarke 1985, Gulden et al. 2003a, De Corby et al. 2007, McPherson 2008).

Of the estimated total seed losses, the proportion of flax seeds which were viable ranged from 59 to 99% among surveyed fields, with seed viability being lower ($p=0.0001$) in 2006 (59 to 91%) than in 2007 (72 to 99%) (Table 6-1). In 2006, the lower seed viability observed may be partially attributed to the cool and wet weather conditions incurred during the growing season and post-harvest. These adverse weather conditions delayed the maturity of the flax crop, and this may have resulted in a greater proportion of green and/or unripe seeds (viable seeds with an immature embyro) returned to the soil seed bank as most producers harvested the crop early due to the high potential for frost. Immature seeds generally have lower ability to germinate and reduced seed vigor (Zimmerman and Zimmer 1978, TeKrony and Hunter 1995, Woltz et al. 2006).

Unfavorable weather conditions post-harvest may have further reduced the germination potential of flax seeds on the soil surface due to weathering and/or exposure to frost. It is likely that multiple factors influence flax seed viability, but the specific causes are not well understood.

Seed persistence in the seed bank

There was no indication that seed color or ALA content in flax seed influenced the persistence of seed in the seed bank ($p=0.0953$) (data not shown) so results were pooled for subsequent analyses. Flax seed viability rapidly declined in the year following burial. In the spring following burial (175 days after planting or DAP), persistence was variable, ranging from 62% to only 7%, but at 271 DAP, most seed was not viable, due presumably to germination. No viable seeds remained by 574 DAP in 2005 and by 339 DAP in 2006 (Tables 6-2 and 6-3). Viable flax seed persistence appears similar to that of safflower where less than 1% of seed remained viable after two years (McPherson 2008) and shorter than wheat and canola where less than 1% of seed remained viable after three years (Gulden et al. 2003b, Nielson et al. 2009).

Viable seeds persisted in the soil seed bank for a longer period of time in 2005 than in 2006 ($p=0.0001$) and in 2005 the quantity of viable flax seed in the soil seed bank was influenced by site ($p=0.0005$) (Table 6-2). However, there were no differences between sites in 2006 (data not shown) (Table 6-3). Variation in environmental conditions between years and sites may have influenced viable seed persistence. Mean long-term temperatures at both sites were similar, but mean annual precipitation was higher at Ellerslie (ERS) than Vegreville (VRS) (Figure 6-1). However, differences in weather patterns do not fully explain the variance in seed persistence between years and

between sites in 2005. The variables that influence flax seed viability and persistence require further investigantion.

In the spring following burial, there is a trend to longer seed persistence at the deepest (10 cm) burial depth, however this is not consistent in ERS in 2005 (Tables 6-2 and 5-3). Persistence of flax at greater burial depths may be due to secondary dormancy. However, because of the rapidity of decline of seed viability with burial depth, it may not make a practical difference. For many annual weed species, including volunteer canola, secondary dormancy is induced at greater depths of burial increasing seed persistence through the prohibition of seed germination (López-Granados and Lutman 1998, Gulden et al. 2003b).

In agroecosystems, artificial seed banks are practical for evaluating weed seed longevity over time because the number, species composition and depth of seed burial may be managed under a range of environmental conditions (Leon and Owen 2004, Conn et al. 2006). In seed longevity experiments, artificial seed banks may be used to assess viability of seeds within retrievable material (boxes, pots, nylon mesh bags) at specific intervals of time (Hartzler et al. 1999, Leon and Owen 2004). However, protected seeds in the artificial seed banks may respond and interact differently with biotic (predation, microbes) and abiotic (temperature, moisture) stressors, influencing rates of germination, emergence and exhaustion (Leon and Owen 2004) and as a result, seed longevity may be over estimated (Masin et al. 2006).

Implications of this study

This research indicates that seed loss during harvest and subsequent persistence of viable seed may contribute to gene flow. Flax seed loss during harvest was significant, but was not solely influenced by the method of harvest (direct combining or

windrow/combining). Seed losses and viable seed persistence did show some variation between sites and years; however, the factors influencing harvest loss and seed persistence are difficult to predict. There are some transient differences in seed persistence at different burial depth. It was observed that seed on the surface in three of four site-years appears to be less persistent than seed buried more deeply. This result suggests that flax seed may exhibit photodormancy (seed will not germinate unless exposed to light).

The results indicate that complete seed bank depletion is most likely to occur prior to the summer of the following harvest. Flax producers in Alberta usually practice reduced tillage or direct-seeding and therefore, few of the flax seeds lost at harvest would be buried. Thus, the longevity of these seeds should be similar to those in the surface treatments of the artificial seed bank studies. However, artificial seed banks studies must be interpreted with care because the packets used to contain and retrieve the seeds may limit seed to soil contact and the packets act to protect the seeds from natural processes, including predation (Masin et al. 2006). The brief persistence of viable seeds in the seed bank enhances the importance of volunteer control in the year following harvest to reduce seed- and pollen-mediated gene flow. The main factor which contributes to seed persistence is seedbank recharge (seed replishment of the seedbank via seed from weeds and/or crop volunteers). Although flax seeds lost during the crop harvest rapidly lose viability, recharging of the seed bank with seed produced by volunteer flax may result in depletion of the seed bank taking considerably longer. For example, Harker et al. (2005) reported that when volunteer wheat is prevented from producing seed, it has seed longevity of up to two years in western Canada. In contrast, Beckie (2001) reported that in Canadian weed surveys, volunteer wheat plants that produce seed and replenish the

seed bank may continue to emerge for up to five years in rotational subsequent crops. Simard et al. (2002) reported that volunteer canola (*Brassica napus* L.) continued to emerge in western Canadian cropping systems for up to five years after production, but canola seed banks can be rapidly depleted in 3 years when canola seed production is suppressed (Harker et al. 2006). Best management practices to should be aimed to limit flax fecundity and seed replenishment of the soil seed bank in follow crops. Seed-mediated gene flow from GE flax should be limited as the flax seed bank is rapidly depleted. However, control of flax volunteers will be critical to reduce seed-mediated gene flow from GE flax.

Table 6-1. Flax seed bank additions, total seed loss, percent total seed loss and percent viable seed loss in commercial fields as influenced by harvest method in 2006 and 2007.

		WR[b]	IWR[c]	DC[d]	WC[e]	DC[d]	WC[e]	DC[d]	WC[e]	DC[d]
Year	Field	Seed bank addition[a]			Total seed loss[a]		Total seed loss[a]		Percent viable seed loss[a]	
		seeds m^{-2}			kg ha^{-1}		%		%	
2006	06-1	845 *ab*	53 *b*		8 *b*		0.3 *b*		59 *ab*	
	06-2	696 *ab*	117 *b*		13 *b*		0.6 *b*		91 *a*	
	06-3	873 *ab*	81 *b*		10 *b*		0.4 *b*		59 *b*	
	06-4	1986 *a*	109 *b*		24 *ab*		1.4 *ab*		76 *ab*	
	06-5			804 *ab*		44 *a*		2.8 *a*		71 *ab*
2007	07-1	246 *ab*	22 *c*		3 *c*		0.2 *c*		72 *b*	
	07-2			218 *b*		12 *ab*		0.8 *ab*		98 *a*
	07-3			351 *a*		19 *a*		1.0 *a*		99 *a*
	07-4			182 *b*		10 *bc*		0.6 *bc*		99 *a*
	07-5			145 *bc*		8 *bc*		0.4 *bc*		99 *a*
		Contrast Statements[f]								
		2006	2007		2006	2007	2006	2007	2006	2007
		Seed bank addition			Total seed loss[f]		Total seed loss[f]		Proportion of viable seeds[f]	
		seeds m^{-2}			kg ha^{-1}		%		%	
WR[b] versus IWR[c]		*	*		n/a	n/a	n/a	n/a	n/a	n/a
WC[e] versus DC[d]		n/a[g]	n/a		*	*	*	*	**NS**	*

[a]Least square means from the mixed model ANOVA. Mean separations were determined with Fischer's Least Protected Significant Difference (LSD) test in SAS (α=0.05). Values with the same letter in a main column indicate they are not significantly different.
[b]Windrow
[c]Inter-windrow
[d]Direct combine
[e]Windrow/combine
[f]Orthogonal contrast statements denoted by * are significant at $P \leq 0.05$ and those denoted by NS are not significant at $P \leq 0.05$
[g]Not applicable

Table 6-2. Proportion of viable flax seed as influenced by depth of seed burial and days after planting for ERS and VRS in 2005.

		DAP[1]			
		175[a]	271[a]	328[a]	574[b]
Site	Depth	Proportion of viable seed			
	cm	%			
ERS	0	50 (8.9) *a*	0.0 (0.2) *a*	0.0 (0.0) *a*	0 (0.0)
	3	10 (8.9) *b*	0.3 (0.2) *a*	0.0 (0.0) *a*	0 (0.0)
	10	24 (9.0) *ab*	0.0 (0.2) *a*	0.0 (0.0) *a*	0 (0.0)
VRS	0	7 (16.6) *a*	0.1 (0.0) *a*	0.2 (0.1) *a*	0 (0.0)
	3	41 (16.6) *a*	0.0 (0.0) *a*	0.0 (0.1) *a*	0 (0.0)
	10	62 (16.6) *a*	0.0 (0.0) *a*	0.0 (0.1) *a*	0 (0.0)

[1]Days after planting

[a]Least square means with associated standard error (in parentheses) within columns followed by a common letter are not significantly different according to Fischer's Protected LSD test at $P \leq 0.05$

[b]Mean with associated standard error

Table 6-3. Proportion of viable flax seed as influenced by depth of seed burial and days after planting for ERS and VRS in 2006.

	DAP[1]		
	216[a]	277[a]	339[b]
Depth	Proportion of viable seed		
cm		%	
0	3 (2.3) *a*	0.3 (0.8) *a*	0 (0.0)
3	7 (2.3) *a*	2.4 (0.8) *a*	0 (0.0)
10	8 (2.3) *a*	0.0 (0.8) *a*	0 (0.0)

[1]Days after planting

[a]Least square means with associated standard error (in parentheses) within columns followed by a common letter are not significantly different according to Fischer's Protected LSD test at $P \leq 0.05$

[b]Mean with associated standard error

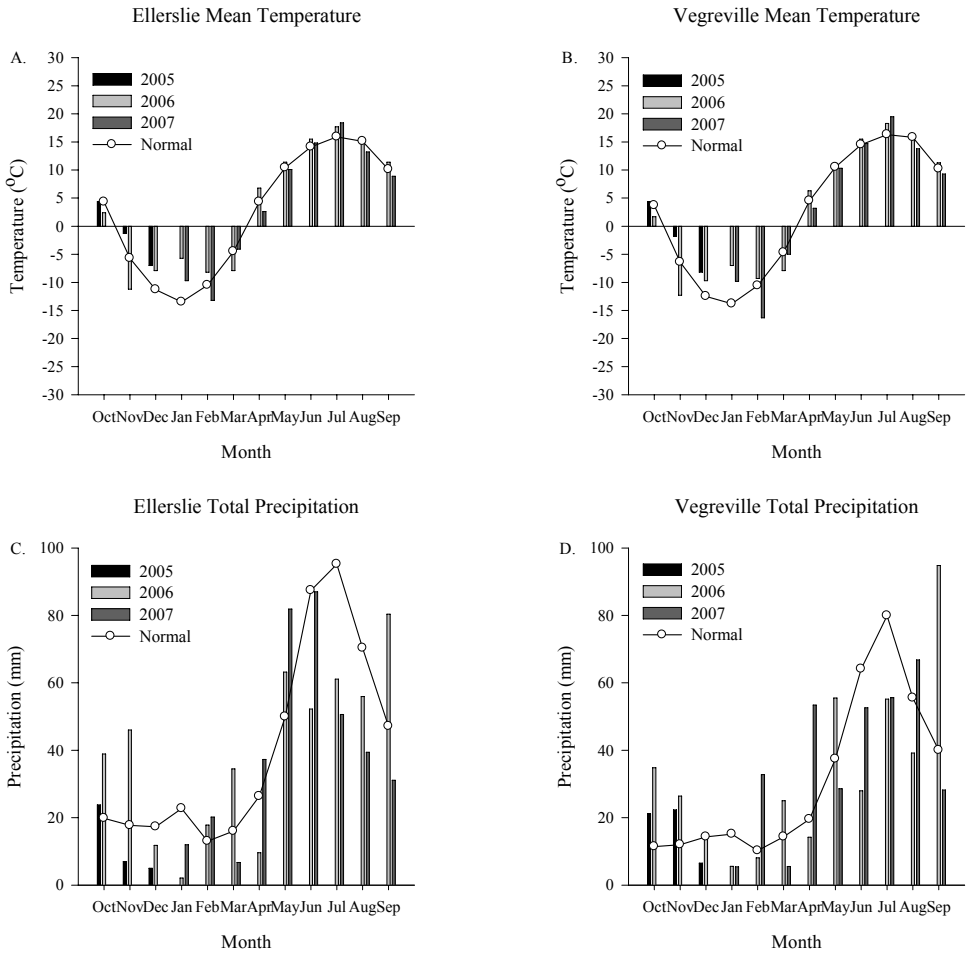

Figure 6-1. Mean monthly temperature and total precipitation compared to long-term normal values (1971-2000) at weather stations closest to the Ellerslie and Vegreville sites in 2005 to 2007.

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Chapter 7: Conclusions

SUMMARY OF RESULTS

The research presented in this thesis has provided baseline information on the biology and agronomy of flax to inform the environmental biosafety of flax as a platform crop for bioindustrial products. Routes of seed-mediated gene flow through the life history of volunteer flax were identified and quantified (Figures 7-1 and 7-2). Results of this research will contribute to the development of risk mitigation strategies to reduce seed-mediated gene flow from transgenic flax volunteers in agroecosystems.

Seed-mediated gene flow may occur at all stages of flax production and during flax transport, and temporal gene flow may be perpetuated in the environment via seed bank recharge by flax volunteers (Figure 7-1). Flax seed and flax seed bolls are primarily introduced into the soil seed bank prior to and during flax harvest operations. In the seed bank, seeds may persist or lose viability as a result of abiotic and biological processes such as weathering, disease or predation. Alternatively, seeds may undergo fatal germination under unfavourable conditions. Viable seed may germinate to produce volunteer flax which may either be controlled with herbicides or survive to produce seed. Seed from uncontrolled flax volunteers may replenish the seed bank, or be admixed (co-mingled) during harvest with food or feed crops that follow thereby contributing to adventitious presence (AP). The results of these studies indicate that seed-mediated gene flow from volunteer flax occurs up to three years following production (Figure 7-1). We have no evidence to show volunteer flax population persistence without seed bank recharge from subsequent flax crops.

The lifecycle of volunteer flax is illustrated in Figure 7-2. Flax seeds and flax seed bolls primarily enter the flax seed bank from pre- and post-harvest losses (reviewed in Chapter 5). Flax seed losses associated with harvest procedures are highly variable and can be substantial (21 to 1986 seed m^{-2}). In the seed bank, flax seeds may either persist or be removed by germination, predation, exhaustion, disease or by other abiotic factors, including frost, anoxia and desiccation. The persistence of flax seed in the soil at different burial depths was reported in Chapter 5. Burial of flax seeds hastened their decline and their viability was reduced by over 35% and 99% from the time of establishment (harvest, October) to early spring (follow year, April) and fall (follow year, October), respectively. Reduced seed dormancy in this crop means that seed bank persistence is primarily a function of factors influencing germination and seed death. While germinated seeds may perish as seedlings, some plants may survive to set seed and may replenish the seed bank. Flax volunteerism in commercial fields was reported in Chapter 2. Volunteer flax plant densities were highest (570 plants m^{-2}) the first year following flax production (2005) and were lower (< 78 plants m^{-2}) in years 2006 and 2007. Flax volunteers often did not survive to produce flowers or to set seed in cereal crops such as wheat and barley indicating that flax volunteers respond to competition and post-emergent herbicide application(s). Uncontrolled volunteers in rotational crops may produce a large amount of seed. At an average density of 570 plants m^{-2}, volunteer flax is capable of producing up to 69899 seeds m^{-2} (Figure 7-2). Excellent herbicide control of volunteer flax in rotational crops is critical to the reduction of flax fecundity and AP (reviewed in Chapter 3).

Although flax seed losses from combine harvesters were considerable (Figure 7-2), flax seed losses were below or equal to the recommended seeding rate (45 kg ha^{-1} or 692 seeds m^{-2}) of this crop. It is likely that some seeds adhered to the soil surface by

means of mucilage and were not collected. In addition, flax seeds were observed to lethally germinate in the fall prior to sampling. Therefore, it is likely that flax pre- and post-harvest losses were under estimated. Flax harvest losses were comparable to published harvest losses for canola (1530 to 7130 seeds m^{-2}) and safflower (231 to 1069 seeds m^{-2}) and much higher than wheat (35 to 800 seeds m^{-2}) (Clarke 1985, Anderson and Soper 2003, Gulden et al. 2003a, De Corby et al. 2007, McPherson 2008). Adjustments in flax harvest practices may reduce additions to the flax seed bank on sampled commercial farms. For example, harvest losses of flax seed can be minimized in windrow/combine and direct combine systems if the flax crop is harvested at maturity at the appropriate seed moisture content (30%) with suitable combine settings (Gubbels 1993, Anonymous 2002). However, flax seed bank inputs at harvest may be adversely influenced by cool and wet weather patterns and because environmental conditions are highly variable within and among years on the Canadian Prairies, it is difficult to predict total flax seed losses from combine harvesters (reviewed in Chapter 5).

Although flax seed losses were large at the time of harvest and 59 to 91% of seeds were viable, artificial seed bank studies using unprotected seed indicate that flax seeds do not persist beyond two years in the soil seed bank in western Canada (Figure 7-2). In these studies, seed viability rapidly diminished over the first winter and by the first spring, seed viability ranged from 7% to 62% (Figure 7-2). In our studies, surface and buried seed were subject to fall germination, which was generally lethal in flax as it is not frost tolerant (Anonymous 2002). There was no indication that brown-seeded flax cultivars persisted longer in the soil seed bank than yellow-seeded flax cultivars. Rapid depletion of the flax seed bank may be attributed to a number of different factors including germination, seed decay and disease. While seeds at all depths of burial did not

persist beyond two years, seed on the soil surface remained viable the longest. At the soil surface, the mesh bags used to contain the seeds may have limited seed contact with soil, increased intermittent seed drying (moisture loss), reduced predation, and prevented seed dispersal within the soil profile (Nielson et al. 2009). In contrast, seeds which were buried were likely exposed to more consistent temperature and moisture regimes, which may have facilitated their germination or promoted decay. The seed bank ecology of volunteer flax and the response of flax seeds to burial was similar to other volunteer crops such as safflower and wheat (McPherson 2008, Nielson et al. 2009), but different from canola (Gulden et al. 2003b).

Surveying commercial flax fields allowed data related to volunteer seedling density and seed persistence to be collected over a range of environmental conditions. In Alberta weed surveys, volunteer flax was shown to persist in commercial agricultural fields for up to 3 years (Figure 7-2). Volunteer flax densities were observed to vary over the season but were the highest prior to herbicide applications (PREHERB) (10.4 to 570.2 plants m^{-2}) in all fields the year following flax production (2005) and then diminished over time (Figure 7-2). The application of in-crop herbicides and crop competition effectively reduced volunteer flax densities within all site-years ($\leq$ 84 plants m^{-2}) indicating that crop management strategies strongly influence residual flax densities. Most flax volunteer plants in rotational crops under reduced tillage (RT) and conventional tillage (CT) did not survive to set seed, presumably due to crop competition and herbicide control. However, some volunteer flax plants were observed in reproductive stages of growth in the second and third year of the field survey and may have contributed to seed bank recharge. In western Canada, seed bank recharge has previously been reported to be a key factor driving volunteer persistence in other crops (Beckie 2001, Harker et al. 2005,

Harker et al. 2006). Suppression of seed bank recharge through the control of flax volunteers using herbicides or growing competitive follow crops is critical in reducing the potential for seed-mediated gene flow.

The seed burial studies and commercial field surveys provided different estimates of flax seed longevity in the soil seed bank under western Canadian agricultural conditions with reduced seed longevity observed in burial studies compared with observations under natural conditions (Figure 7-2). A comparison of the results suggested that the seed bank depletion data generated from artificial seed banks may underestimate flax seed viability relative to natural seed banks. For some annual weed species, artificial seed bank studies tend to overestimate seed viability as seeds are protected from natural processes such as predation and mechanical damage, and seeds and soil are disturbed upon planting (Leon and Owen 2004, Masin et al. 2006). However, the results presented in this thesis suggested that the rate of volunteer flax seed depletion was more rapid in the artificial seed bank than in natural seed banks. The disparity between the artificial seed bank studies and the field surveys may be due to differences in germination and recruitment of seed from flax seeds versus flax seed bolls. Artificial seed bank studies used only unprotected flax seed, whereas in the commercial field surveys, the flax seed bank consisted of both flax seed and flax seed bolls. Flax seed bolls appear to be resistant to degradation and may buffer seeds from conditions that favour germination and emergence. In the third year of the field survey, volunteer flax seedlings emerged in clusters from decayed flax seed bolls located within the first 3 cm of the soil surface, rather than from single seeds. This result suggested that flax seeds were less likely to persist than seeds protected by seed bolls. Therefore, flax seed longevity in natural seed banks, where seed contained in flax seed bolls, may be longer than in artifical seed banks.

Other factors may have also influenced seed persistence in the artificial seed bank studies compared to the field surveys. For example, microbial activity and pathogenic fungi may accelerate the rate of seed mortality in the seed burial studies due to the high density of seeds contained within the enclosed mesh bags (Van Mourik et al. 2005). Soil physical characteristics such as bulk density and soil compaction can also affect how seeds respond to their environment and these conditions may differ among in natural and artificial seed banks (Jurik and Zhang 1999, Leon and Owen 2004). We did not investigate the specific causes of seed nonviability in the artificial seed bank studies; therefore, the exact causes of seed mortality are unknown. While the results of artificial and natural seed bank studies should be interpreted with caution, when considered together they provide information for predicting flax seed longevity in the soil seed bank.

In temperate regions, the most important environmental factor limiting weed seed germination is soil temperature (Leon and Owen 2004). The simplest approach to compare plant development stages (germination and emergence) across different environments is to relate plant development (germination and emergence) and temperature by thermal time (GDD) (Prostko et al. 1998). Cumulative volunteer flax emergence was effectively modeled as a function of cumulative GDD using T_{base} 5°C for each site-year (R^2 = 98.9 to 99.6). An interesting characteristic of volunteer flax emergence in the field was its variability; calculated EM_{50} values (the growing degree day required for 50% emergence) ranged from 227 to 340 GDD. Compared to other western Canadian crops, flax has a prolonged period of emergence. In the southwestern region of Manitoba, Lawson et al. (2006) reported that volunteer canola (*Brassica napus* L.) required ≤132 soil GDD (T_{base} 5°C) to attain 50% emergence. Crop management practices such as tillage, which affects soil temperature and moisture and many other factors, may

affect the emergence timing of volunteer flax. The results of the emergence periodicity studies demonstrated that volunteer flax emerged more rapidly and reached its period of peak emergence earlier in CT fields than in RT fields the first year following flax production. In addition, more volunteers emerged in CT fields (0 to 1509 plants m^{-2}) than in RT fields (0 to 188 plants m^{-2}) and by comparison, volunteer flax continued to emerge later into the growing season under CT than RT (reviewed in Chapter 3). Soil-seed contact may be a key factor influencing flax seed germination as the mucilage content of the seed coat promotes adhesion to soil particles, facilitates seed hydration and assists germination processes. These results suggest that RT rather than CT tillage systems may be greater suited to genetically engineered (GE) flax production as RT systems are associated with lower volunteer flax densities throughout the growing season.

Commercial field surveys did not confirm the association of volunteer flax density with tillage system (CT or RT) on flax seedling density. Although emergence periodicity studies showed more volunteer emergence in CT than in RT fields, the results of the commercial field surveys indicate that volunteer flax densities were not generally affected by tillage regime (CT or RT) as differences were detected in only a few survey periods within and among years. The results of the emergence periodicity study may have been influenced by the method in which volunteer flax emergence was measured over time. In these experiments, newly emerged volunteer flax plants were removed by hand to accurately quantify the density of emerged flax plants in each pre-established quadrat. Therefore, volunteer flax emergence was not quantified in a density-dependent manner as the effects of other emerged crop volunteers and weeds were not observed. In addition, the effects of weed-crop competition were not observed as plants were removed from pre-established quadrats at the cotyledon stage of growth. These experiments also restricted

the use of both pre- and post-emergent herbicides and emerged flax seedlings were not exposed to pesticide control efforts for the duration of the study. Due to the artificial conditions imposed in the emergence periodicity experiments, the results of the commercial field surveys may have provided a better estimate of the influence of tillage system on volunteer flax densities. It is thus more likely that volunteer flax is not highly responsive to tillage regime (RT or CT). Quantification of germination timing and seedling recruitment from flax under different tillage systems (RT and CT) in commercial fields requires further investigation. The results of the commercial field surveys also indicated that volunteer flax densities were influenced by the crop type that followed flax in rotation as densities were lower in competitive crops such canola and wheat and higher in pea, a less competitive crop. However, our ability to detect differences among cropping systems (tillage and crop type) in surveyed fields was limited, both by the number of fields sampled and by the diversity of the cropping systems sampled. In addition, results of the commercial field studies were confounded by other crop management practices employed by the different producers such as variation in herbicide applications (timing, frequency of use and mode of action) and densities of volunteer flax were found to be highly variable among fields indicating that flax is highly responsive to implemented weed management strategies. Further analyses where a larger number of fields are surveyed and crop management practices are more consistent among surveyed fields may help clarify the influence of cropping system on the density of volunteer flax. However, the results of the emergence periodicity and commercial field surveys both confirm that the first year following flax production is critical to the reduction of seed-mediated gene flow and AP as volunteer flax was observed at the highest densities and consistently present.

The observed densities of volunteer flax in the CT fields in the emergence periodicity studies were higher than expected given the results of the seed burial experiments which indicated that that only 7 to 62% of seeds are still viable in spring (Figure 7-2). However, the proportion of flax seeds present in flax seed bolls on or near the soil surface and the influence this might have on seed persistence was not accounted for in this experiment. Since it is probable that flax seed bolls resist degradation and weathering, it is likely that the seeds contained within these bolls had greater seed viability than exposed seeds on or near the soil surface. In the spring in the CT fields, shallow (< 5 cm) spring tillage operations potentially released large quantities of seeds from intact seed bolls and may have further positioned them in favorable recruitment microsites for germination and emergence.

Under worst-case scenarios, volunteer flax if left uncontrolled, can produce large amounts of seed (483-4755 seeds m^{-2}) (Figure 7-2). Herbicide control of volunteer flax will be required the year following transgenic flax production to reduce AP and seed bank recharge. Volunteer flax may be effectively controlled with a number of registered pre- and post-emergent herbicides. Glyphosate applied pre-emergent and fluroxypyr applied with either 2,4-D or MCPA as a post-emergent herbicide are effective in reducing volunteer flax density, biomass, and fecundity. However the effectiveness of glyphosate and glyphosate plus tribenuron was influenced by experimental conditions. Volunteer flax was broadcast on the soil surface and seedlings emerged uniformly and prior to the crop, creating ideal conditions for pre-emergent herbicide control. Combinations of herbicides, and an effective single herbicide application reduced volunteer flax seed AP in spring wheat (from over 8.5% to < 0.05%) indicating that the Commission of European Communities AP thresholds of 0.9% could be met. Although data does not suggest that

both pre-emergent and post-emergent herbicide applications contribute to increased flax control and reduction of fecundity and AP, under field conditions where volunteer flax emergence is less uniform, sequential applications may be beneficial to mitigate the occurrence of seed-mediated gene flow.

These studies have used several experimental methods to quantified seed-mediated gene flow under western Canadian agricultural conditions (Figure 7-2). In flax, the low prevalence of pollen-mediated gene flow limits the potential for transgene movement from GE crops to conventional crops, volunteers and ruderal or wild species (Hall et al. 2006). The risk of seed-mediated gene flow from transgenic volunteer flax to the environment and to the food/feed system can be reduced through the implementation of best management practices (BMPs). Tools for managing transgenic volunteer flax are the same tools used for the management of conventional flax volunteers. However, the stringency of the mitigation procedures for managing transgenic volunteer flax is dependent upon reasonable thresholds for the AP of GE flax in commodity crops. A summary of potential of BMPs to mitigate the risks of seed-mediated gene flow over time from transgenic flax volunteers is outlined in Table 7-1. BMPs based on the findings of this research suggest that the selection of RT regimes, a competitive rotational cereal crop such as wheat or barley, and effective pre-emergent and/or post-emergent herbicide control should effectively reduce the potential for seed-mediated gene flow from transgenic flax volunteers under western Canadian agricultural conditions and may be used to implement coexistence practice on the same land base.

Flax is a minor crop in Canada and growers may experience economic benefits from the registration of a transgenic variety of flax for bioindustrial products. While flax seed and seed boll losses at harvest contribute to flax seed bank inputs and to seed-

mediated gene flow, it seems unlikely that transgenic flax volunteers will become a significant weed within agricultural fields unless the GE trait confers a fitness advantage. Volunteers appear to have a relatively short persistence in seed bank (2 to 3 years) and seed-mediated gene flow from GE flax should be limited as populations are depleted. While tillage tends to establish secondary dormancy in other volunteer crops such as canola and increases seed and volunteer persistence, flax expresses reduced seed dormancy. Seeds which germinate and emerge in the fall are subject to lethal germination as flax is not a frost tolerant crop and seedlings observed post-harvest are unlikely to survive the Canadian winter. Flax volunteers that emerge in spring may be effectively controlled both pre- and post-emergent in subsequent crops. Although volunteer flax is considered an uncompetitive weed, vigilant herbicide control of volunteers will be required in the year following GE crop production to prevent AP and reduce seed bank replenishment. Cultivation of GE flax requires the strict implementation of BMPs to lessen the risk of gene flow from GE flax volunteers, to protect our conventional flax seed markets from harm, and to ensure the biosafety of our food/feed system. While gene flow from GE flax can not be contained or eliminated entirely, the collective agronomic baseline data generated in this thesis suggests that flax is an appropriate crop platform for bioindustrial products.

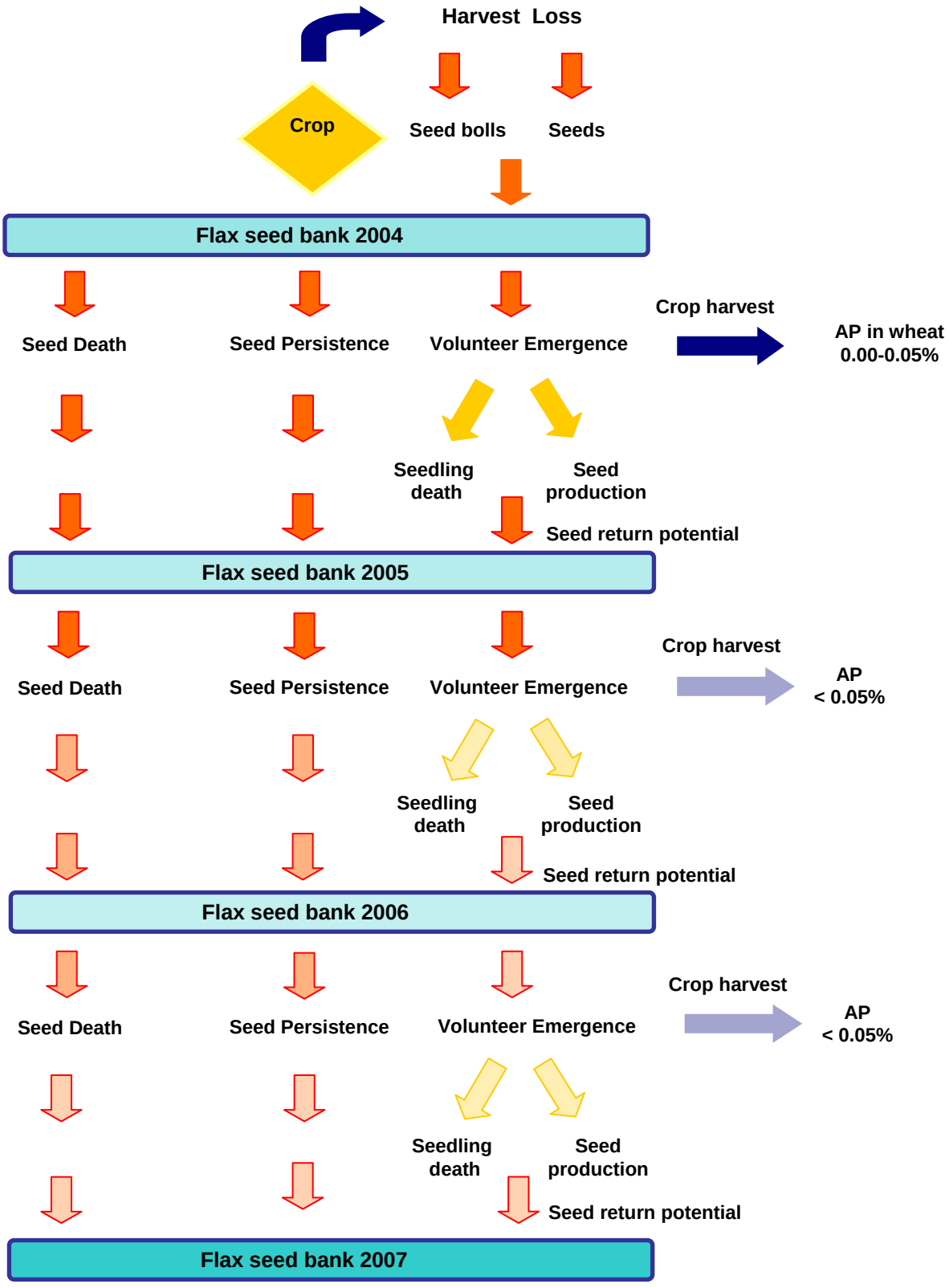

Figure 7-1. Potential pathways of seed-mediated gene flow in flax. Boxes and arrows indicate the phases and processes that were investigated in this thesis. The shades of the boxes and arrows indicate the temporal dissipation of volunteer flax and lighter colors are used over time (2004-2007) to emphasize the ephermeral nature of the species.

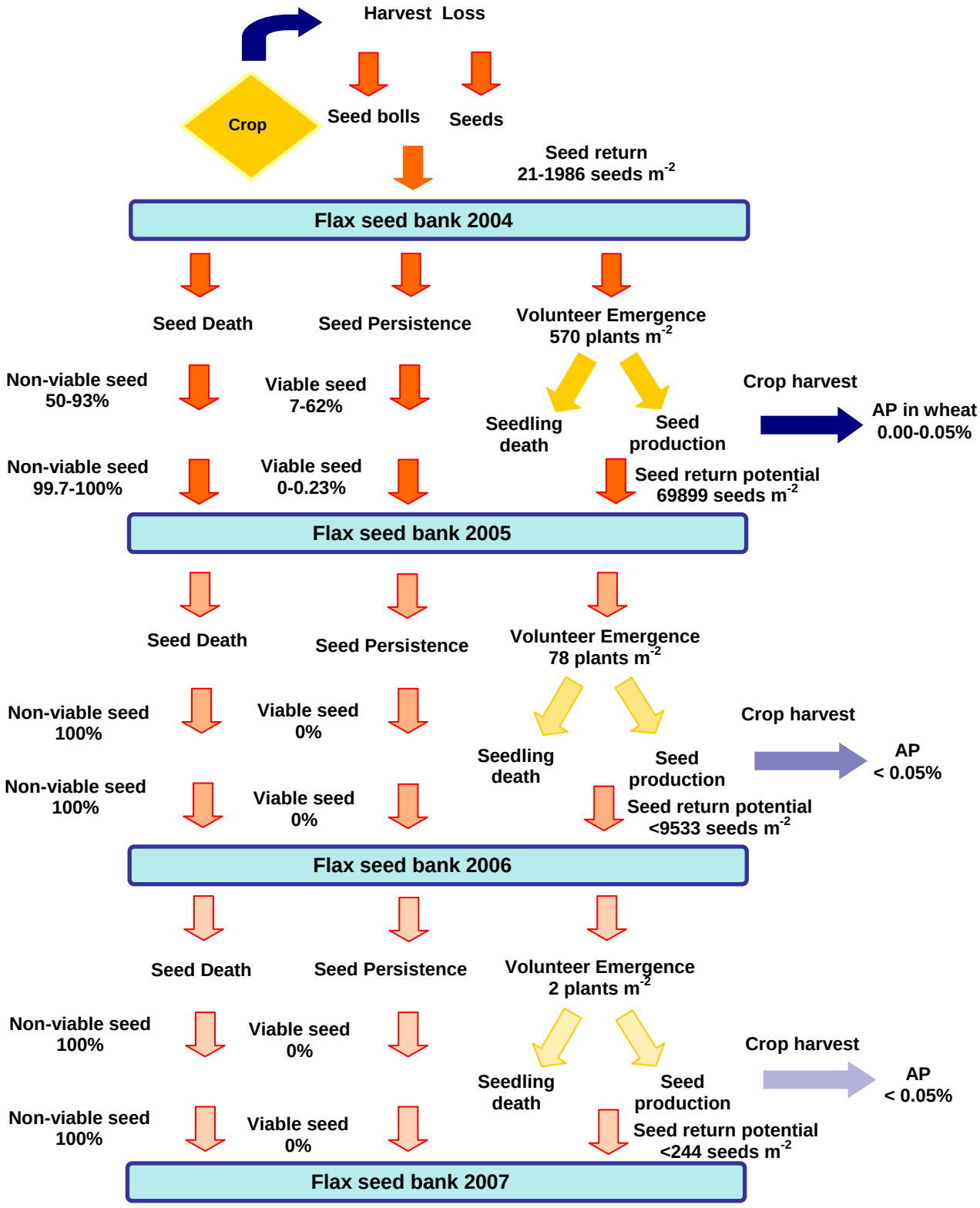

Figure 7-2. Quantification of seed-mediated gene flow in flax. Boxes and arrows indicate the processes investigated in this thesis. Boxes and arrows indicate the temporal and special dissipation of volunteer flax. Color intensity is used to suggest the reduction in population densisties over time (2004-2007).

Table 7.1. Best management practices to limit seed-mediated gene flow from transgenic flax production to the environment and food/feed system during specific crop.

Crop	Operation	Concern	Mitigation
GE flax	Planting	Seed movement off-site	Clean seeding equipment prior to movement off field Producers should not share seeding equipment
	Harvest	Seed bank additions	Desiccate flax with glyphosate to reduce seed viability Harvest flax at maturity Use appropriate combine settings and chaff wagon
		Seed movement off-site	Clean harvest equipment prior to movement off field Producers should not share harvest equipment
	Post-harvest	PNT flax seed	Tillage to shatter intact seed bolls, increase seed burial, minimize seed persistence and reduce predation
	Tillage	Seed movement off-site	Clean tillage equipment prior to movement off field Producers should not share tillage equipment
Follow crop[ab]	Pre-planting	GE flax volunteers	Soil should not be disturbed by tillage to prevent flax seed boll shatter and to lessens volunteer emergence from seed Clean seeding equipment prior to seeding (free from GE flax seed) Herbicide(s) to control volunteers
	Crop production	GE flax volunteers	Herbicide(s) to control volunteers
	Harvest	Adventitious presence	On-farm processing of harvested material prior to being transported off-site Extensive seed cleaning to remove GE volunteer flax seed and seed bolls
		Seed bank additions	Use appropriate combine settings and chaff wagon
	Post-harvest	PNT flax seed	Tillage to shatter intact seed bolls, increase seed burial, minimize seed persistence and reduce predation

[a]Chemical fallow is preferred over a follow crop in the first year after PNT flax production

[b]Under continuous cropping regimes, a competitive cereal crop is preferred over less competitive crops such as canola or field pea in the first year after PNT flax production

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APPENDIX 1

Table A1. Size of surveyed fields and flax harvest date in 2006 and 2007.

	Field		Date[a]	
Year	No.	Size	Windrowed	Harvested
		ha		
2006	06-1	300	Oct 9	Oct 9
	06-2	375	Oct 9	Oct 9
	06-3	300	Oct 9	Oct 9
	06-4	250	Sept 23	Oct 5
	06-5	300	n/a[b]	Oct 11
2007	07-1	300	n/a	Oct 3
	07-2	350	n/a	Oct 6
	07-3	150	Oct 3	Oct 3
	07-4	325	n/a	Oct 6
	07-5	450	n/a	Oct 3

[a]Date was estimated by the surveyed producer
[b]Not applicable

Table A2. Initial viability of five flax varieties in 2005 and 2006.

			Year	
			2005	2006
Variety	Seed coat color	ALA[a] content	Germinated seed[bc]	
		%	%	
Hanley	Brown	45-55	66.3	64.7
Brown Solin	Brown	3-5	74.7	75.7
Yellow Solin	Yellow	3-5	71.0	68.3
Nugget	Yellow	45-55	44.7	42.7
Liflax	Brown	45-55	61.3	58.7

[a]alpha-linolenic acid

[b]Alive with sufficient energy reserves to germinate

[c]0.2% Helix Xtra® (thiamethoxam + difenconazle + metalaxyl-M + fludioxonil)

Table A3. Preliminary seed germination tests to determine the number and percentage of germinated and non germinated flax seeds (cv. CDC Bethune) on Helix Xtra® and GA_3 and percentage of viable and non-viable flax seeds (cv. CDC Bethune) on tetrazolium chloride under laboratory conditions.

	Germinated		No germination	
Replicate	Water + Helix Xtra[a]	GA_3[b]	Viable seed[c]	Non-viable seed[d]
	%		%	
1	85.8	3.9	1.1	6.4
2	85.1	4.5	1.4	5.1
3	85.0	4.5	1.8	4.0
4	85.0	1.3	4.1	7.3
5	85.0	1.8	2.3	8.0
6	96.3	0.5	1.3	0.6
7	85.0	1.3	0.0	8.4
8	85.0	4.1	0.1	7.4
9	82.4	0.8	0.9	6.4
10	78.8	1.0	3.0	9.6
11	100.0	0.0	0.0	0.0
Average	86.7	2.1	1.5	5.7

[a]0.2% Helix Xtra® (thiamethoxam + difenconazle + metalaxyl-M + fludioxonil)
[b]0.005 M GA_3 solution
[c]Tetrazolium chloride positive (0.15% w/v)
[d]Tetrazolium chloride negative (0.15% w/v)