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Harvest Loss and Seed Bank Longevity of Flax (*Linum usitatissimum*) Implications for Seed-Mediated Gene Flow

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Flax is a minor oilseed crop in Canada largely exported to the European Union for use as a source of industrial oil and feed ingredient. While flax could be genetically engineered (GE) to enhance nutritional value, the adoption of transgenic technologies threatens conventional flax market acceptability. Harvest seed loss of GE crops and the persistence of GE crop volunteers in the seed bank are major factors influencing transgene persistence. Ten commercial fields in Alberta, Canada, were sampled after harvesting conventional flax in 2006 and 2007, and flax seed density and viability were determined. Additionally, artificial seed banks were established at two locations in Alberta in 2005 and 2006 to quantify persistence of five conventional flax cultivars with variability in seed coat color (yellow or brown) and α-linolenic acid (ALA, 18:3$^{cis\Delta 9,13,15}$) content (3 to 55%) at three soil depths (0, 3, or 10 cm). Harvest methods influenced seed loss and distribution, > 10-fold more seed was distributed beneath windrows than between them. Direct harvested fields had more uniform seed distribution but generally higher seed losses. The maximum yield loss was 44 kg ha^{-1} or 2.3% of the estimated crop yield. Seed loss and the viability of flax seed were significantly influenced by year, presumably because weather conditions prior to harvest influenced the timing and type of harvest operations. In artificial seed bank studies, seed coat color or ALA content did not influence persistence. Flax seed viability rapidly declined in the year following burial with < 1% remaining midsummer in the year following burial but there were significant differences between years. In three of four locations, there was a trend of longer seed persistence at the deepest burial depth (10 cm). The current study predicts that seed-mediated gene flow may be a significant factor in transgene persistence and a source of adventitious presence.

Nomenclature: Flax, *Linum usitatissimum* L.

Key words: Harvest loss, persistence, soil seed bank, volunteer flax.

Flax oil is used principally as an industrial drying oil for manufacturing paints, stains, inks, varnishes, and linoleum due to its high level (45 to 65%) of α linolenic acid (ALA, 18:3 $^{cis\Delta 9,13,15}$; Green et al. 2008). The meal remaining after the seed is pressed is used as animal feed. Flax was among the first crops to be genetically engineered (GE) with herbicide resistance (McHughen 1989, 2002). In 1998, the sulfonylurea-resistant GE flax cultivar CDC Triffid was approved for unconfined released in Canada and the United States (CFIA 2001). Approval for importation to Europe, the primary export market for Canadian flax, was not pursued, in part because of the absence of a clear regulatory framework for GE crops. European concerns led to the voluntary deregistration of CDC Triffid prior to commercial release, at the request of the Flax Council of Canada. Approximately 50 t of CDC Triffid seed was recovered from seed growers and crushed. In 2009, a low-level presence of CDC Triffid was identified in shipments of Canadian flax in Germany (Bedard 2009) 9 yr after its withdrawal. Because there is zero tolerance for unapproved GE events in the European Union, Canadian flax was quarantined and international trade in flax disrupted (Anonymous 2010a).

Flax, like other minor crops, has not benefited recently from investments in crop breeding. Enhancements of flax oil and fiber quality to increase the value of the products are under development but are predicated on the use of genetic engineering. The protection of existing conventional markets is a significant concern that influences crop development. Before another GE flax variety can be released, an understanding of the gene flow within crops and between crops and volunteers is required to determine if GE flax can coexist with conventional production without market harm.

Transgenes may move within agronomic systems via pollen- and seed-mediated gene flow (PMGF and SMGF). Flax has relatively low levels of outcrossing at short distances (Dillman 1938). In a recent study using a high α-linolenic acid trait as a marker, PMGF in flax was estimated to be < 2.0% immediately adjacent to source plots (10 cm apart) and declined rapidly with distance (Jhala et al. 2009). At 35 m from the pollen source plot, gene flow to adjacent source plots was estimated to be < 0.003%. While PMGF could contribute to adventitious presence (the unintentional and incidental commingling of trace amounts of one type of seed, grain, or food product with another), isolation distances between fields are usually sufficient to reduce adventitious presence from PMGF below the 0.9% labeling threshold for adventitious presence of approved traits by the European Union (Beckie and Hall 2008; Devos et al. 2009). However, once gene flow has occurred, the use of farm-saved seed perpetuates adventitious presence at similar frequencies.

SMGF is more complex, occurs over a longer time, and is more difficult to mitigate. It may occur through the inadvertent admixture of planted seed or via seed lost during transport or harvest. Volunteer flax is a common weed where flax crops are grown in western Canada (Leeson et al. 2005). In the recent Prairie Weed Survey, volunteer flax was ranked just after volunteer barley (*Hordeum vulgare* L.) as the 26th most abundant weed in Western Canada, although barley was grown on an average of 3,719,000 ha while flax was grown on only 658,000 ha from 2003 to 2008 (Anonymous 2010b). Volunteer flax emerges over a prolonged period during the growing season, with 50% emergence occurring prior to in-crop herbicide application (Dexter et al. 2010a). Volunteer flax population density surveyed in 20 commercial fields varied widely among

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fields from 31 to 4,597 plants m^{-2} were recorded in the year following the crop. In direct seeded fields, fewer volunteers emerged and emergence tended to be delayed compared to conventionally tilled fields. Volunteers continued to emerge in some fields three growing seasons following the flax crop.

Flax, like other crops, has been selected for reduced seed dormancy. The outer layer of the seed hull of flax seed contains hydrophilic mucilage and is generally considered to facilitate germination under dry conditions. Flax seeds are produced in a boll (Lay and Dybing 1989), which may provide a protective barrier, buffering seeds from microsite conditions that favor rapid germination and emergence of volunteer flax from the soil seed bank. Both naked seeds and flax seed bolls can be lost to the seed bank, both prior to and during harvest. Flax is more difficult to harvest than other small grain and oilseed crops due to its indeterminate growth habit and the presence of bast fibers in the stem. It is recommended to directly harvest the standing crop after 75% or more of the seed bolls have turned brown and the crop appears uniform in maturity (Anonymous 2006). However, swathing before combining is the most common harvest procedure as windrow-drying allows the seed to reach moisture levels safe (< 10%) for long-term storage (Anonymous 2006). The quantity of flax seed losses at harvest and the factors that influence these losses have not been quantified.

Flax seeds and seed bolls, lost before or at harvest, are the primary input into the seed bank and are the reason for perpetuation of volunteer crop populations through time. Seed predation, mechanical damage, disease, and germination or extinction account for losses of seeds from the seed bank. In crops without seed dormancy, the depth of the seed within the seed bank and environmental factors including available moisture and temperature appear to be the primary influence on seed bank persistence (Gulden et al. 2004; McPherson et al. 2009; Nielson et al. 2009).

Two conventional types of flax seeds are grown in Canada, the common brown-seeded flax with high ALA and yellow-seeded flax with low ALA (< 5%) Solin flax cultivars (Dribnenki et al. 2003; Green 1986; Rowland 1991). Flax seed vigor and germination capacity may be affected by seed color and fatty acid composition (Culbertson and Kommedahl 1956), but evidence regarding differences in seed vigor and germination frequencies between yellow- and brown-seeded flax have been contradictory (Comstock et al. 1963; Culbertson et al. 1960). Persistence of flax seeds in the seed bank and the relationship of persistence to seed color and ALA content are essential crop biology information should GE flax be commercially produced.

The objectives of this study were to (1) quantify flax harvest seed losses at different sites and years; (2) assess the effect of harvest method (windrow/harvest and direct harvest) on flax seed bank additions; (3) evaluate and compare seed bank persistence of yellow- and brown-seeded flax cultivars; (4) assess the effect of burial depth on seed persistence and viability. The results of this study, along with previous publications on volunteer emergence, population persistence, and control and fecundity of flax volunteers were used to quantify SMGF of flax under western Canadian climatic conditions.

Materials and Methods

Harvest Loss of Flax Seeds. *Sample Collection.* In 2006 and 2007, commercial flax fields (producers) were selected for sampling within a 300 km radius of Edmonton, Alberta, Canada (53°34′N, 113°31′W). Fields ranged in size from 150 to 450 ha. A total of five fields were sampled from three sites (Viking, Minburn, and Wainwright) within 1 wk after harvest in 2006. In 2007, a total of five fields at four sites (Viking, Vegreville, Wainwright, and Vermillion) were sampled within 1 wk after harvest. Flax was last grown on all selected fields ≥ 4 yr before the time of sampling. In 2006, four fields were swathed and windrow-dried and one field was direct harvested. In 2007, one field was swathed and windrow-dried and four fields were direct harvested.

In swathed fields, flax seeds were collected from 0.25-m^2 quadrats in 10 selected pairs of windrows and adjacent interwindrows randomly located at 25-m intervals in an inverted W pattern (Thomas 1985). In direct harvested fields, flax seeds were collected from twenty 0.25-m^2 quadrats every 25 m in an inverted W pattern (Thomas 1985). Crop residue, nonharvested flax seeds and flax seed bolls were collected from each quadrat using a wet–dry vacuum cleaner.[1] Samples were placed in labeled cloth bags, dried for 48 h at 25 C and stored before further analysis.

To compare flax seed losses from harvesters among sampled commercial fields (windrow/harvest or direct harvest) 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 (kg ha^{-1}). Average seed loss density (D_F) in windrow harvested fields was calculated as

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

where D_W is the density of seeds measured in the windrow, W_W and W_I is the width of the windrow and interwindrow respectively, and D_I is the density of seeds measured in the interwindrow. Flax yield information was provided by the producer following harvest.

Sample Processing and Seed Viability Testing. Samples were hand threshed to extract seeds from seed bolls and sieved with a 7.14-mm screen to separate and remove inorganic materials from the flax seeds. Seeds were counted using a seed counter.[2] To determine seed viability, random subsamples of 100 seeds from each sample were placed in acrylic germination boxes[3] (24 by 16 by 3.8 cm) lined with 15 by 23 cm filter papers[4] equivalent to Whatman no. 1 (Dexter et al. 2010b). To reduce fungal growth, 14 ml of a 0.2% solution of the insecticide with fungicides (thiamethoxam [20.7%], difenoconazole [1.25%] , mefenoxam [0.39%], fludioxonil [0.13%])[5] was added. After 72 h in the dark at room temperature, germinated seeds (radicle emerged) were counted. Nongerminated seeds were transferred into acrylic germination boxes[3] moistened with 8 ml of 0.005 M giberrellic acid (GA_3)[6] solution. After 72 h, germinated and nongerminated seeds were recorded. Seeds that were soft and/or degraded were considered nonviable. In preliminary experiments nongerminated seeds were tested for viability using 0.15% of 2,3,5-triphenyltetrazolium chloride[7] and incubated for 2 h at room temperature. Treatment with 8 ml of 0.005 M GA_3 resulted in germination of 98.5% of viable seed (data not shown) and, therefore, GA_3 rather than 0.15% 2,3,5-triphenyltetrazolium chloride[7] was used in this study.

Statistical Analysis. Data were tested for normality before analysis to ensure that the residuals were random, homoge-

Table 1. Flax cultivars, seed coat color, α-linolenic acid (ALA) content and percent germination used in artificial seed bank experiments in 2005 and 2006.

			Year	
			2005	2006
Flax cultivar	Seed coat color	ALA[a] content	Initial germination	
		%		
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] Abbreviations: ALA, α-linolenic acid ($18:3^{cis\Delta\ 9,13,15}$).

nous with a normal distribution about a mean of zero. The analysis of variance (ANOVA) was carried out using the PROC MIXED procedure of SAS[8] (SAS Institute 2007) to determine differences in flax seed bank additions (seed loss from harvesters in seeds m^{-2}), total seed loss (average kg ha^{-1} seed loss), and percent viable seed (proportion of sampled seed that germinated in water and GA_3 solution) among sampled fields under a two-level nested design structure, where year was considered a random effect and fields were nested in sites and were considered a fixed effect. A two-level nested design structure was selected because too few flax fields were available to allow for random selection. The means of a fixed-effect factor were separated by least significant differences (LSD) at the significance level of $P < 0.05$.

Flax Seed Bank Longevity. *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 ERS loam soil consisted of 28.6% sand, 46.4% silt, and 25% clay with a pH of 6.3 and 11.5% soil organic matter content. The VRS loam soil 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, 2.5 and 11.6 km, for VRS and ERS, respectively, and 30-yr (1971 to 2000) climatic means were calculated.

Five diverse flax cultivars, differing in ALA content and seed color were grown in adjacent rows at the Agriculture and Agri-Food Canada (AAFC) Research Center in Morden, Manitoba, Canada, to produce seed for the trial (Table 1). Seeds of each cultivar (1,000 seeds) were tested for germination. Two hundred seeds of each of the five flax cultivars were sewn into strips of 50 μm polypropylene mesh packets, each strip consisting of five 8 by 15 cm packets, with one cultivar per packet. Cultivars were labeled and randomly ordered in each strip. Mesh bags were permeable to water and allowed direct seed to soil contact. Soil was excavated to create three 11-cm-deep plots that were 80 by 90 cm wide per replicate, with 1 m separating each plot.

An 80 by 90 by 15 cm high metal cage of 6 by 6 mm wire mesh lined each plot, which was then filled with soil from the excavation until the plot soil surface was 0, 3, and 10 cm deeper than the surrounding (surface) soil. Nine strips of mesh packets with flax seed were placed on the top of the soil of each plot, and more soil was placed on the packets until the soil surface was even, resulting in burial depths of 0, 3, and 10 cm. Flax seeds were buried on October 12, 2005, and October 5, 2006, at the ERS and on October 13, 2005, and October 6, 2006, at the VRS at 0-, 3-, and 10-cm depth with soil that had been excavated to create the plots. A lid of metal mesh was secured by cable ties[9] to each cage to prevent rodents from accessing the seed. Each burial depth was replicated three times at each site-year.

Packets of seeds were extracted three times throughout the growing season: early spring, midsummer, and fall at approximately 9-wk intervals. They were washed to remove soil, and the seeds were immediately removed, counted, and germination tested. Air temperature and precipitation data for both research locations are summarized in Figure 1.

Experimental Design and Statistical Analysis. Field experiments were conducted in a split-plot design with three replicates in which seed depths were main plots and flax cultivars were the subplots. Viable flax seeds were expressed 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 ANOVA using the PROC MIXED procedure of SAS (SAS Institute 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 cultivars were fixed and block nested within site was random. Where the ANOVA indicated that treatment effects were significant, treatment means were separated at $P < 0.05$ by the 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 were presented.

Results and Discussion

Flax Seed Losses during Harvest. Flax seed losses during harvest were highly variable (Table 2). In 2006 in windrow/harvest fields, seed bank additions were higher ($P = 0.0001$) beneath windrows (696 to 1,986 seeds m^{-2}) than interwindrows (53 to 117 seeds m^{-2}). Similarly, in 2007 in the fields that were windrow/harvested, average flax seed bank additions were 11 times higher in windrows (246 seeds m^{-2}) than interwindrows (22 seeds m^{-2}). These results were expected as a higher number of flax seeds are generally returned to the soil surface below windrows due to flax seed boll shatter during swathing and additional processing during harvest pick-up and following the harvest track. Variable seed loss within fields partially explains variability in flax volunteer populations.

Total seed losses differed between years ($P = 0.0001$). In general, seed losses were higher in 2006 than in 2007 in both windrow/harvest and direct harvest fields (Table 2). 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 than normal in 2006; whereas in 2007, conditions were warmer and drier (Figure 1). Flax seed maturity may be delayed by cool and wet weather conditions and higher seed losses may be incurred at harvest due to difficulty in cutting and processing green or damp flax straw (Anonymous 2006).

Total flax seed losses in windrow/harvest and direct harvest fields were variable between fields within years (Table 2). In 2006, in fields that were windrow/harvested, total flax seed losses ranged from 8 to 24 kg ha^{-1} (0.3 to 1.4% of yield loss) and in one field that was direct harvested, total seed losses of

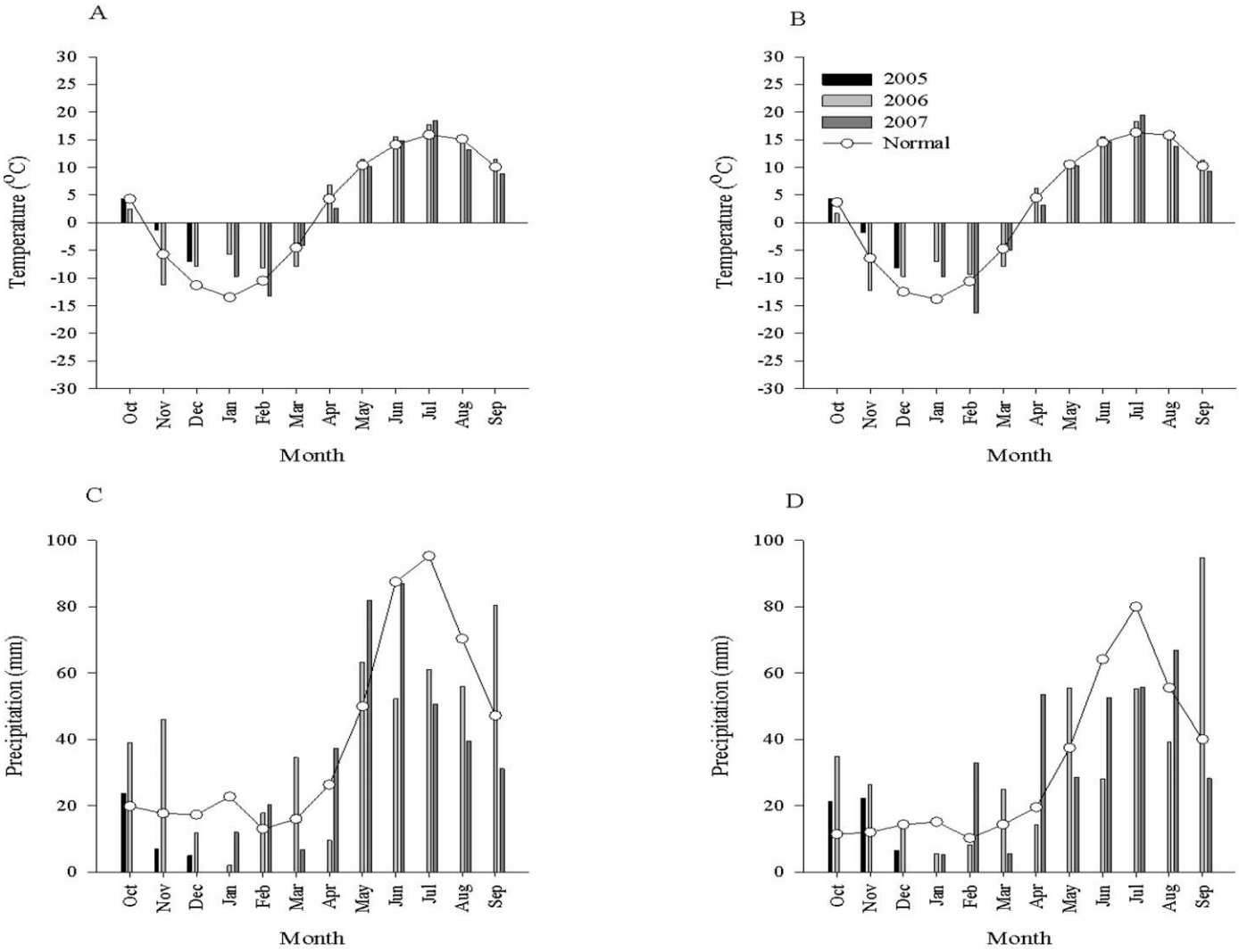

Figure 1. Mean monthly temperature and total precipitation compared to long-term normal values (1971 to 2000) recorded at weather stations closest to the Ellerslie and Vegreville research sites in 2005 to 2007. (A) Ellerslie mean temperature, (B) Vegreville mean temperature, (C) Ellerslie total precipitation, and (D) Vegreville total precipitation.

flax averaged 44 kg ha^{-1} (2.8% of yield loss). In 2007, in the one field that was windrow/harvested, total flax seed losses averaged 3 kg ha^{-1} (0.2% of yield loss) and among four fields that were direct harvested, total seed losses of flax ranged from 8 to 19 kg ha^{-1} (0.4 to 1% of yield loss) (Table 2). These results indicated that flax harvest method (windrow/harvest or direct harvest) may influence the amount of flax seed loss from harvesters. The stochasticity of harvest seed loss have been previously reported in canola (*Brassica napus* L.) (Gulden et al. 2003a), wheat (*Triticum aestivum* L.) (Clarke 1985), oat (*Avena sativa* L.) (Clarke 1984), and safflower (*Carthamus tinctorius* L.) (McPherson et al. 2009). Flax seed losses quantified in this study were lower than reported for canola, another small oilseed crop. Gulden et al. (2003a) reported that in canola, seed losses during harvest ranged from 3.3 to 9.9% of the harvest yield, which equated to canola seed bank additions of 1,530 to 7,130 seeds m^{-2}.

The proportion of flax seeds that were viable ranged from 59 to 99% among surveyed fields, with seed viability being significantly lower ($P = 0.0001$) in 2006 (59 to 91%) than in 2007 (71 to 99%) (Table 2). 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 (Figure 1). Adverse weather conditions delayed maturation of the crop and may have resulted in a greater proportion of green and/or unripe seeds (viable seeds with an immature embryo) returned to the soil seed bank. Immature seeds generally have a lower ability to germinate and reduced seedling vigor.

Losses of viable seed at harvest are the largest inputs into the seed bank and are the source of subsequent volunteer populations. Dexter et al. (2010a) reported that a total of 484 to 4597 m^{-2} flax volunteers emerged in the first year following commercial flax production, with higher densities associated with direct seeded fields.

Flax Seed Bank Longevity. Flax varieties for seed bank studies were chosen to provide both high and low ALA content and yellow and brown seed color (Table 1). There was no indication that either seed color or ALA content of flax seed influenced the persistence of seed in the seed bank ($P = 0.0953$). A lack of dormancy allowed for rapid

Table 2. Flax seed losses at harvest in windrow (WR) areas and interwindrows (IWR) in windrow/harvested (WH) fields and in direct harvested fields (DH) expressed as flax seed density, on a per hectare basis, as a percentage of yield and seed viability in 2006 and 2007.[a,b]

		Flax seed density			Total seed loss		Total seed loss		Percent viable seeds	
Year	Field	WR	IWR	DH	WH	DH	WH	DH	WH	DH
		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	1,986 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

	Seed bank addition		Total seed loss		Total seed loss		Proportion of viable seeds	
	2006	2007	2006	2007	2006	2007	2006	2007
	seeds m^{-2}		kg ha^{-1}		%		%	
WR vs. IWR	*	*	NA	NA	NA	NA	NA	NA
WH vs. DH	NA	NA	*	*	*	*	NS	*

[a] Abbreviations: NA, not applicable, NS, nonsignificant.
[b] Least square means from the mixed model ANOVA. Mean separations were determined with Fisher's Least Protected Significant Difference (LSD) test at $P < 0.05$. Values with the same letter in a main column indicate they are not significantly different.
* Orthogonal contrast statements are significant at $P < 0.05$.

germination of all seeds where environmental conditions permitted, removing viable seeds from the seed bank. Variety data were pooled for subsequent analyses.

Viable seeds persisted in the soil seed bank for a longer period of time in 2005 than in 2006 ($P = 0.0001$). In 2005 persistence of viable flax seed in the soil seed bank was also influenced by site ($P = 0.0005$) (Tables 3 and 4). In 2006 there were no significant differences between sites and the data was pooled (Table 4). Variation in environmental conditions between years and sites may have influenced viable seed persistence. While average long-term temperatures at both the sites were similar, mean annual precipitation was higher at ERS than VRS (Figure 1).

Flax seed viability rapidly declined in the year following burial in all sites and years. In the spring following burial (175 DAP), persistence was variable (7 to 62%), but by midsummer (271 DAP), < 1% of the seeds were viable as most had germinated (Table 3). No viable seeds remained by 574 DAP in 2005 (Table 4) and by 339 DAP in 2006 (Table 4). Compared to similar studies, flax seed persistence in artificial seed banks appears similar to safflower (McPherson et al. 2009), but less persistent than wheat (Nielson et al. 2009) and canola (Gulden et al. 2003b).

In the spring following burial, in three of four site-years, there was a trend to longer seed persistence at the deepest burial depth (10 cm) (Tables 3 and 4). This is opposite to the trend in wheat (Nielson et al. 2009) and safflower (McPherson et al. 2009) for which burial of seed decreased seed persistence. For many annual weed species and volunteer canola, secondary dormancy is induced at greater depths of burial, increasing seed persistence (Gulden et al. 2004; Lopez-Granados and Lutman 1998). However, for flax there are no reports of secondary seed dormancy and recovered viable seed germinated readily under laboratory conditions. Slight persistence of flax at greater burial depths may be due to the lack of a critical light or O_2/CO_2 germination trigger. Alternatively, it may reflect the mucilaginous nature of the

Table 3. Proportion of viable flax seeds in artificial seed banks as influenced by depth of seed burial and days after planting at Ellerslie Research Station (ERS) and Vegreville Research Station (VRS) in 2005.[a]

		DAP			
		175	271	328	574
Site	Depth	Proportion of viable seeds[b]			
	cm	%			
ERS	0	49.9 (8.9) a	0.0 (0.2) a	0.0 (0.0) a	0 (0.0)
	3	9.8 (8.9) b	0.3 (0.2) a	0.0 (0.0) a	0 (0.0)
	10	23.8 (9.0) ab	0.0 (0.2) a	0.0 (0.0) a	0 (0.0)
VRS	0	6.7 (16.6) a	0.1 (0.0) a	0.2 (0.1) a	0 (0.0)
	3	40.8 (16.6) a	0.0 (0.0) a	0.0 (0.1) a	0 (0.0)
	10	61.8 (16.6) a	0.0 (0.0) a	0.0 (0.1) a	0 (0.0)

[a] Abbreviations: DAP, days after planting.
[b] Viable seeds are expressed as a proportion of the initial number of viable seed. Least square means with associated standard error (in parentheses) within columns followed by a common letter are not significantly different according to Fisher's Least Protected Significant Difference (LSD) test at $P < 0.05$.

Table 4. Proportion of viable flax seeds as influenced by depth of seed burial and days after planting at Ellerslie Research Station (ERS) and Vegreville Research Station (VRS) in 2006.[ab]

	DAP[c]		
	216	277	339
Depth	Proportion of viable seeds		
cm	%		
0	3.2 (2.3) a	0.3 (0.8) a	0 (0.0)
3	6.5 (2.3) a	2.4 (0.8) a	0 (0.0)
10	8.4 (2.3) a	0.0 (0.8) a	0 (0.0)

[a] Abbreviations: DAP, days after planting.
[b] Data were pooled due to nonsignificant differences among sites (ERS and VRS).
[c] Least square means with associated standard error (in parentheses) within columns followed by a common letter are not significantly different according to Fisher's Least Protected Significant Difference (LSD) test at $P < 0.05$. Viable seeds are expressed as a proportion of the initial 200 seeds buried at ERS or VRS.

flax seeds that enhances the ability of seeds at the surface to retain moisture for germination.

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 (Conn et al. 2006; Schwartz et al. 2006). 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 overestimated. 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). Soil disturbances that occur during the placement of seed bags may alter soil bulk density, gas exchange, water infiltration, and soil temperature and cannot duplicate natural conditions of buried seed.

Results from artificial seed bank studies indicate that seed bank depletion is rapid; however, this is not corroborated either by periodicity of volunteer emergence or volunteer field survey data (Dexter et al. 2010a). In this instance, artificial seed bank analysis may have underestimated flax seed persistence. Flax harvest losses are in the form of both naked seeds (as used in the artificial seed bank study) and seed retained in flax bolls. A comparison of germination between naked and flax seeds retained in seed bolls showed that germination was slowed by retention in the flax boll. Unprotected seeds exhibited 80% germination 4 DAP while seeds protected in flax bolls started to germinate only after 10 d and continued to germinate $>$ 50 DAP (Dexter et al. 2010a). Seed bank persistence plays a larger role in SMGF in flax than predicted by artificial seed banks studies.

Seeds that germinate successfully form volunteer populations. Volunteer flax emerged relatively slowly, with 50% of volunteers emerging before in-crop herbicide application (Dexter 2010a). Flax is usually followed in rotation with cereals so it can be controlled with herbicides (Dexter et al. 2010b; Wall and Smith, 1999). However, uncontrolled volunteers or those emerging after in-crop herbicide application can persist. In a recent survey of 20 Western Canadian fields following flax production, volunteers continued to emerge in some fields for three growing seasons at low densities (Dexter et al. 2010a).

SMGF in flax is initiated by seed loss at harvest. Seed losses are variable but may be large, depending on harvest methods and environmental factors beyond the control of the growers. The loss of seeds and flax bolls before and during harvest places seed in the seed bank. Germination does not appear to be influenced by seed dormancy, but may be prolonged by protection of seed bolls. Germination of naked seed during fall may rapidly deplete the seed bank, depending on post harvest environmental conditions. Dense volunteer populations of up to 570 plants m^{-2} were initiated following spring germination (Dexter et al. 2010a). Under usual cropping practices, few of these plants survive to perpetuate SMGF and data suggest that the seed bank contribution of flax volunteers is less than that from a harvested flax crop. In studies of volunteer flax fecundity in a wheat crop following PRE and POST herbicides, flax fecundity ranged from 0 to 7.1 seeds m^{-2} (Dexter et al. 2010b) and in canola 0 to 8 seeds m^{-2} (Jhala et al. 2010), suggesting that volunteer flax can be effectively controlled by proper selection of herbicides and the potential for SMGF can diminish over time.

SMGF also can occur via movement of crop seeds by farm equipment and by co-mingling during the transportation of crop seed. Compared to PMGF, the risk of SMGF contributing to adventitious presence is higher and occurs over a longer time frame. The use of farm-saved seed maintains the frequency of any adventitious presence. As the case of CDC Triffid flax illustrates, once a GE trait has been released into the production system, it may persist for years. GE flax may offer considerable benefit to growers and consumers (Jhala et al. 2009), but it cannot coexist with conventional flax without regulatory approval in key markets to provide thresholds for adventitious presence, routine seed testing, and careful production practices that consider both SMGF and PMGF.

Sources of Materials

[1] Vacuum cleaner, Vacuflo, 9331-63 Avenue, Argyll Road, Edmonton, Alberta, Canada.

[2] Seed counter, Model # 946, Key-Mat Equipment Company, St. Charles, IL.

[3] Germination boxes, Hoffman Manufacturing, Inc., 16541 Green Bridge Road, Jefferson, OR 97352-9201.

[4] Filter paper, Hoffman Manufacturing, Inc., 16541 Green Bridge Road, Jefferson, OR 97352-9201.

[5] Insecticide with fungicides (thiamethoxam, difenoconazole, mefenoxam, fludioxonil), Helix XTra™, Syngenta Crop Protection Canada, Inc., Suite 300, 6700 Macleod Trail South, Calgary, AB T2H 0L3, Canada.

[6] Gibberellic acid, Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178.

[7] 2,3,5-triphenyltetrazolium chloride, Sigma-Aldrich Corp., P.O. Box 14508, St. Louis, MO 63178.

[8] SAS, Statistical Analysis Systems, The SAS systems for windows, SAS Institute Inc., P.O. Box 8000, Cary, NC 27512.

[9] Cable ties, Avery Dennison Corporation, Fastener Division, 224 Industrial Road, Fitchburg, MA 01420.

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