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Adventitious Presence: Volunteer Flax (*Linum usitatissimum*) in Herbicide-Resistant Canola (*Brassica napus*)

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Flax is in the process of development as a crop for bio-industrial and nutraceutical products predicated on the use of genetic modification. Before genetically modified (GM) flax is commercially released, effective management practices should be developed to minimize adventitious presence (AP) of GM volunteer flax in subsequent crops. Field research was conducted at four locations during 2007 and 2008 in central Alberta to quantify and mitigate AP of volunteer flax in glufosinate-resistant (GR) and imidazolinone-resistant (IR) canola. A single preplant application of glyphosate at 1,250 g ae ha^{-1} in GR canola reduced volunteer flax density from 54 to 3 plants m^{-2} and seed production from 5,963 to 233 seeds m^{-2}. Similarly, the recommended rate of POST glufosinate (600 g ai ha^{-1}) alone effectively controlled volunteer flax and reduced flax seed viability to < 8% and AP to 0.2%. A combination of preplant (glyphosate) and POST (glufosinate) at recommended rates reduced volunteer flax seed production, yield, and AP to near zero in GR canola. Glyphosate applied preplant was equally effective in IR canola, reducing volunteer flax density from 56 to 2 plants m^{-2}, and seed production from 5,571 to 472 seeds m^{-2}. Imazamox + imazethapyr applied POST at all the rates poorly controlled volunteer flax and, even in combination with preplant glyphosate, cannot be recommended for control of flax volunteers in IR canola.

Nomenclature: Glufosinate; glyphosate; imazamox; imazethapyr; Canola, *Brassica napus* L. 'Invigor 5030', '45H73-CL'; flax, *Linum usitatissimum* L. 'CDC Bethune'.

Key words: Comingling, herbicide resistance, mitigation, seed-mediated gene flow, weed.

El *Linum usitatissimum* (linaza) está en proceso de desarrollo para ser considerado como cultivo bio-industrial y nutricional con base a modificaciones genéticas para su uso. Antes de que el genéticamente modificado (GM) *Linum usitatissimum* sea liberado comercialmente, se deberían desarrollar prácticas de manejo efectivas que minimicen la presencia adventicia (AP) que pueda afectar posteriormente a otros cultivos. Durante 2007 y 2008, en cuatro localidades de la región central de Alberta se realizó una investigación de campo para cuantificar y mitigar la presencia adventicia (AP) del *Linum usitatissimum* en cultivos de canola resistente a glufosinato (GR) y a imidazolinonas (IR). Una sola aplicación de glifosato antes de sembrar (PRE-SIEMBRA) en dosis de 1,250 g ae/ha en canola GR, redujo la densidad de *Linum usitatissimum* de 54 a 3 plantas/m^2 y la producción de semilla de 5,963 a 233 semillas/m^2. De manera similar, la dosis recomendada de glufosinato (600 g ia/ha) aplicado POST, controló efectivamente a *Linum usitatissimum* y redujo la viabilidad de la semilla de linaza a < 8% y AP a 0.2%. Una combinación de glifosato (PRE-SIEMBRA) y glufosinato (POST), en las dosis recomendadas, redujo en *Linum usitatissimum* la producción de semilla, su rendimiento y la AP a casi cero, en cultivos de canola GR. El glifosato aplicado en PRE-SIEMBRA, fue igualmente efectivo en canola IR, y redujo la densidad de *Linum usitatissimum* de 56 a 2 plantas/m^2 y la producción de semilla de 5,571 a 472 semillas/m^2. El imazamox más el imazethapyr aplicados POST en todas las dosis, y aún combinados con glifosato en PRE-SIEMBRA, presentaron un pobre control de *Linum usitatissimum*, por lo que no pueden ser recomendados para controlar a esta planta, en cultivos de canola resistente a imidazolinonas.

Since the commercialization of genetically modified (GM) crops in 1996, the total area and number of countries growing GM crops has increased rapidly (Brookes and Barfoot 2008). In 2008, 25 countries cultivated GM crops on approximately 125 million ha (James 2008). Many GM crops have been developed and commercialized, but the four most extensively grown GM crops include canola, cotton (*Gossypium hirsutum* L.), maize (*Zea mays* L.), and soybean (*Glycine max* L.). Several other crops, including flax, are being considered for the development of novel bio-industrial products using genetic transformation technologies (Kymalainen and Sjoberg 2008; Moryganov et al. 2008).

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Flax, also known as linseed, is an annual dicotyledonous oilseed crop. In temperate and subtropical countries, flax has been grown either for oil extracted from the seed or for fiber extracted from stems. Flax is a well-adapted crop in western Canada. In 2008, Canadian growers produced ~ 861,000 tonnes of flax seed from approximately 631,000 ha (Statistics Canada 2009) and exported about 675,000 tonnes (AAFC 2009). Current research on medicinal applications, especially reducing risk factors contributing to cardiovascular diseases (Bloedon and Szapary 2004) and cancer (Thompson et al. 2005), has opened an opportunity to use this oilseed crop for functional food applications (Fitzpatrick 2007). Using its unique oil biosynthesis pathways, transgenic cultivars of flax are under development to supply the market demand for novel bioproducts (Jhala et al. 2009; Sorensen et al. 2005; Wrobel-Kwiatkowska et al. 2007).

Although herbicide-resistant flax was one of the first GM crops to receive regulatory approval in Canada (McHughen et al. 1997), currently no transgenic flax cultivars are available on a commercial scale in Canada or elsewhere (Jhala et al. 2008).

Consumer and political concerns about GM crops for food and animal feed continue to be pervasive in Europe, and these concerns could effectively block future transgenic crop development (Demeke et al. 2006; Devos et al. 2005). Although currently released GM crops and their products are considered substantially equivalent to conventional crops in the United States and Canada, if GM flax is to be cultivated in western Canada, it must be segregated from conventional and organic flax to preserve these valuable markets in the European Union (EU).

Trust in GM crops has eroded in part because transgenes from GM crops have been detected in non-GM feed and food products and have been widely reported in the media and in the scientific literature (Demeke et al. 2006; USDA/APHIS 2008). Nonapproved transgenes are illegal in food or feed, and detection has led to serious economic consequences (Holst-Jensen 2008; Krueger and Le Buanec 2008; Ramessar et al. 2008). Adventitious presence (AP), the unintentional comingling of trace amounts of genetically engineered crop seeds (in addition to other unwanted materials) in conventional or organic crop seeds, is a significant world trade constraint. For example, GM maize intended to produce a specialty pharmaceutical protein was found as crop volunteers in a soybean field grown in rotation in Nebraska (USDA/APHIS 2008). These GM maize volunteers were subsequently harvested with soybean and were transported to storage and mixed with a half-million bushels of stored soybeans (Ellstrand 2003). Because Prodigene Inc. failed to confine the GM maize, the company received a fine of US$250,000 by the U.S. Department of Agriculture (USDA) and was required to buy and destroy the contaminated soybean at an approximate cost of US$3.5 million (USDA/APHIS 2008). This has led to changes in government regulatory policies and resulted in more stringent confinement procedures for field experiments and commercialization of GM crops.

The presence of GM seed can occur through pollen-mediated gene flow between conventional and GM crops and seed handling in the commodity system (Ellstrand et al. 1999; Hall et al. 2000). For crops that have low rates of out-crossing and thus limited pollen-mediated gene flow, volunteer seed production might be the most likely source of AP (Devos et al. 2009). Information about gene dissemination by seeds and volunteers has been compiled for some important GM crops (Gressel 2005). A study of postharvest gene movement for GM canola, maize, sugar beet (*Beta vulgaris* L.), and wheat (*Triticum aestivum* L.) suggest that all species can disseminate transgenes via crop volunteers (Gruber et al. 2008).

Genetically engineered crop volunteers that emerge in subsequent crops can also be a significant agronomic concern (Beckie 2001; Beckie and Owen 2007). They are considered weeds because they compete with crops for nutrients, moisture, space, and light (Blackshaw et al. 2005; O'Donovan et al. 2007), thereby reducing crop yield and quality, and could also interfere in harvest operations (O'Donovan et al. 2005, 2007; Williams and Boydston 2006). Herbicide-resistant volunteers could become more problematic and difficult to control if a crop with the same trait is planted in rotation. For example, soybean is grown in rotation with cotton in the southeast United States. Volunteer soybean in cotton became a problem with the commercialization of glyphosate-resistant soybean and cotton because glyphosate was ineffective for the control of glyphosate-resistant soybean volunteers (York et al. 2005). Similar problems have been observed in Ontario, Canada, for controlling glyphosate-resistant volunteer maize in maize–soybean cropping systems after the commercialization of glyphosate-resistant traits in both crops (Deen et al. 2006).

Where flax is grown, it volunteers in subsequent crops (Leeson et al. 2005). Volunteer flax initially arises from seed and capsule losses incurred during flax harvest. The relative abundance of volunteer flax has increased across the Canadian Prairie over the last 30 yr. Averaged across Manitoba, Saskatchewan, and Alberta, volunteer flax ranked as the 32nd most abundant weed in the 1970s and as the 26th most abundant weed in the 1990s and early 2000s (Leeson et al. 2005). A survey conducted in Manitoba suggested that relative abundance of volunteer flax has increased from 2.0 to 15.3 in last two decades (Thomas et al. 1997). An understanding of the biology of volunteer flax and the agronomic practices that mitigate its occurrence in agroecosystems is essential to the reduction of seed-mediated gene flow.

Flax is usually grown in rotation with cereals to interrupt cereal disease cycles (Wall and Smith 1999). However, farmers might also grow canola after flax in western Canada (Dexter et al. 2006). Few PRE and POST herbicides have been registered for control of volunteer flax in cereals in Canada (Brook 2008). Glyphosate-, glufosinate-, and imidazolinone-resistant canola systems have been rapidly adopted by Canadian growers and now comprise $> 90\%$ of canola grown in Canada (Buth 2007). Volunteer flax could be a significant weed problem in canola if not controlled.

Best management practices have been introduced to reduce dissemination of transgenes by seed and pollen (Devos et al. 2004). Non-controlled GM crop volunteers are major routes of seed-mediated gene flow. An integrated weed management strategy is required to control volunteer flax effectively and reduce AP in the crops grown in rotation with flax. In addition to other cultural practices and tillage, chemical control of volunteer flax could be an effective method of reducing the risk of seed-mediated gene flow. Currently, no information is available on control of volunteer flax in canola, volunteer seed production, or potential AP. Therefore, the objectives of this study were (1) to determine the potential of preplant or POST herbicides used alone or in combination for mitigating AP of volunteer flax in glufosinate-resistant (GR) and imidazolinone-resistant (IR) canola; (2) to evaluate the viability of noncontrolled volunteer flax seed affected by crop competition or weed management practices, and (3) to quantify the amount of AP (wt/wt) of volunteer flax in GR and IR canola under herbicide-treated and -nontreated conditions.

Materials and Methods

Two separate field experiments were conducted for GR (cultivar [cv.] 'Invigor 5030') and IR (cv. '45H73-CL') canola systems in 2007 at one location in Edmonton, Alberta,

Table 1. Dates of preplant and POST herbicides applications and agronomic operations conducted at various locations in 2007 and 2008.

	2007	2008		
Operations	Edmonton	Ellerslie	Edmonton	St. Albert
Flax cv. 'CDC Bethune' seeded	April 29	May 5	May 16	May 8
Preplant herbicide applied	May 22	May 29	June 2	June 5
Glufosinate-resistant (GR) and imidazolinone-resistant (IR) canola seeded	May 24	May 29	June 2	June 5
POST herbicides applied	June 26	June 23	June 24	June 27
Volunteer flax counts after preplant herbicide application	June 2	June 10	June 13	June 17
Volunteer flax counts at harvest	August 28	September 25	September 27	September 28
Volunteer flax and canola biomass cut	August 28	September 25	September 27	September 28
Canola harvest	September 27	October 9	October 9	October 15
Sand (%)	34.2	28.2	34.1	23
Silt (%)	37.5	41.1	37.6	40.5
Clay (%)	28.3	30.7	28.2	36.5
pH	5.6	6.5	5.6	7.6
Organic matter (%)	12.9	11.2	12.7	13
Expected maturity time for GR canola (d)	120	120	120	115
Expected maturity time for IR canola (d)	115	118	115	118

Canada; and in 2008 at three locations in Edmonton, Ellerslie, and St. Albert, Alberta, Canada. Soil texture at various locations is given in Table 1. The experiments at all sites and in all years were established in areas that had not been seeded to flax for at least 5 yr.

To simulate volunteer flax infestations, flax cv. 'CDC Bethune' was seeded at a rate of 12.2 kg ha^{-1} with the target population of 150 plants m^{-2} and row spacing of 20 cm using a low-disturbance air seeder[1] at a depth of 2 to 3 cm (Table 1). A light tillage operation followed immediately after to incorporate the seeds. Flax plants were allowed to emerge and preplant herbicide (glyphosate was considered preplant because it was applied before seeding canola) was applied when flax plants were at least at the three-leaf stage (Table 1). After herbicide application, either canola cv. Invigor 5030 (GR[2] canola) or 45H73-CL (IR canola) were seeded perpendicular to the flax seeding direction with the use of a low-disturbance air seeder at a target population of 160 plants m^{-2} and with a row spacing of 20 cm. Canola seeding dates were delayed (May 24 onward) at all locations compared with recommended timings (first or second week of May) for this region because of the need to establish volunteer flax populations and apply preplant herbicide treatments (Table 1). Fertilizer rates for canola were based on soil test recommendations for each site-year (data not shown).

Plots were 2.0 m wide by 8.5 m long and were arranged in a randomized complete block design (RCBD) with nine treatments (nine for GR and IR canola each) and four replications at all the locations and years (Table 1). In GR canola, treatments consisted of preplant application of glyphosate[3] at the recommended rate (1,250 g ae ha^{-1}); the sole application of glufosinate was applied POST at three application doses (150, 300, or 600 g ai ha^{-1}); a combination of glyphosate was applied preplant at the recommended dose (1,250 g ha^{-1}) followed by glufosinate (POST) at three application doses (150, 300, or 600 g ha^{-1}). Weed-free plots were maintained by removing all weeds (including volunteer flax) by hand weeding at each location, and nontreated control plots were left uncontrolled. Similarly, nine treatments were applied in IR canola, except POST treatments, where imazamox + imazethapyr[4] (35% + 35%) was applied POST at three application doses (10.5, 21, or 42 g ai ha^{-1}) alone, and as POST at three application doses (10.5, 21, or 42 g ha^{-1}) in combination after preplant application of glyphosate (1,250 g ha^{-1}). A surfactant[5] was mixed with imazamox + imazethapyr 0.5% (v/v). Glyphosate was applied when flax plants were 6 to 8 cm in height and the third pair of leaves was unfolded in GR and IR canola. Glufosinate (in GR canola) and imazamox + imazethapyr (in IR canola) were applied POST when canola plants were at the three- to five-leaf stage and volunteer flax plants were about 20 to 30 cm in height with $>$ 20 leaves. Herbicides were applied with a self-propelled, high-clearance sprayer[6] equipped with flat fan nozzles[7] delivering 100 L ha^{-1} at 214 kPa at a speed of 5.3 to 5.5 km per hr.

Volunteer flax densities were assessed during the growing season within pre-established 0.25-m^2 quadrats (three quadrats per plot), after preplant herbicide treatments and at harvest (Table 1). Volunteer flax that survived herbicide treatments in pre-established quadrats were cut at the stem base close to the soil surface, placed in paper bags in August or September (Table 1), dried at room temperature for 2 wk and dry weight of volunteer flax recorded. Flax seed bolls were threshed by hand, and seeds were tested for viability (see below) after counting. Canola biomass was also determined in pre-established 0.25-m^2 quadrats by cutting the plants near the soil surface and by drying for 72 h at 60 C, after which, biomass weight was recorded. Plots were harvested at maturity (Table 1) and seeds were dried to uniform moisture content for 72 h at 62 C. The seeds of volunteer flax and canola were separated from the harvested admixture by passing through sieve number 8 to remove large pieces of chaff. To remove canola seeds from admixture, a 5/64 sieve (sieve no. 8) was used, followed by separation of remaining seeds by hand. The recovered seeds were weighed and used to determine the seed yields of volunteer flax and canola. AP of volunteer flax seed in harvested canola seed was determined by according to the following formula and expressed as the percentage (wt/wt) of

Table 2. Volunteer flax density and dry weight in GR and IR canola as influenced by herbicide treatments.[a,b,c]

Treatment	Application timing	Flax density after preplant[d]	Flax density at harvest[d]	Flax dry weight[d]
		plants m^{-2}		g m^{-2}
Glufosinate-resistant canola				
Weed free	—	0 (1.0) d	0 (1.0) d	0 (1.0) e
Nontreated	—	1.737 (54.6) a	1.64 (44.3) a	2.18 (150) a
Glyphosate (1,250 g ae ha^{-1})	Preplant	0.5 (3.2) b	0.25 (1.8) cd	0.52 (3.3) d
Glufosinate (150 g ai ha^{-1})	POST	1.786 (61.1) a	1.50 (31.7) a	1.76 (57.3) b
Glufosinate (300 g ai ha^{-1})	POST	1.816 (65.5) a	1.04 (11.0) b	1.2 (15.7) c
Glufosinate (600 g ai ha^{-1})	POST	1.658 (45.5) a	0.39 (2.5) c	0.6 (4.0) d
Glyphosate (1,250 g ae ha^{-1}) fb glufosinate (150 g ai ha^{-1})	Preplant/POST	0.2 (1.6) cd	0.17 (1.5) cd	0.32 (2.1) de
Glyphosate (1,250 g ae ha^{-1}) fb glufosinate (300 g ai ha^{-1})	Preplant/POST	0.28 (1.9) c	0 (1.0) d	0 (1.0) e
Glyphosate (1,250 g ae ha^{-1}) fb glufosinate (600 g ai ha^{-1})	Preplant/POST	0.32 (2.1) bc	0.07 (1.2) d	0 (1.0) e
Imidazolinone-resistant canola				
Weed free	—	0 (1.0) c	0 (1.0) b	0 (1.0) c
Nontreated	—	1.746 (55.7) a	1.68 (48.3) a	2.24 (175) a
Glyphosate (1,250 g ae ha^{-1})	Preplant	0.32 (2.1) b	0.30 (2.0) b	0.43 (2.7) b
Imazamox + imazethapyr (10.5 g ai ha^{-1})	POST	1.83 (67.1) a	1.79 (61.7) a	2.31 (205) a
Imazamox + imazethapyr (21 g ai ha^{-1})	POST	1.75 (56.8) a	1.71 (51.4) a	2.23 (169) a
Imazamox + imazethapyr (42 g ai ha^{-1})	POST	1.82 (66.2) a	1.64 (43.9) a	2.17 (149) a
Glyphosate (1,250 g ae ha^{-1}) fb imazamox + imazethapyr (10.5 g ha^{-1})	Preplant/POST	0.28 (1.9) b	0.30 (2.0) b	0.46 (2.9) b
Glyphosate (1,250 g ha^{-1}) fb imazamox + imazethapyr (21 g ha^{-1})	Preplant/POST	0.2 (1.6) b	0.23 (1.7) b	0.34 (2.2) bc
Glyphosate (1,250 g ha^{-1}) fb imazamox + imazethapyr (42 g ha^{-1})	Preplant/POST	0.32 (2.1) b	0.14 (1.4) b	0.28 (1.9) bc

[a] Abbreviations: GR, glufosinate-resistant; fb, followed by; IR, imidazolinone-resistant.

[b] Each value represents pooled data over locations and years.

[c] The data were log transformed for homogeneous variance before analysis; back-transformed means have been presented in parentheses. A value of 1 was added to data before transformation, which is reflected in the values of the weed-free treatment.

[d] Least square means within columns with no common letters are significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.

volunteer flax AP in harvested canola.

$$\text{Volunteer flax AP (\%)} = W_f / W_c \times 100 \quad [1]$$

where AP is the adventitious presence of volunteer flax in canola expressed as a percentage, W_f is the weight of flaxseed, and W_c is the weight of canola seed.

Volunteer Flax Seed Viability Test. To determine flax seed viability, seeds from volunteer flax were collected from plants that survived herbicide treatments. These plants were hand harvested from the fixed quadrats (three 0.25-m^2 quadrats per plot) and seed capsules were threshed by hand. A subsample of 300 seeds from each quadrat of harvested flax volunteers (if available) were randomly selected and further divided into three replications of 100 seeds each, to be replicated in time over three consecutive days. Seeds were placed in acrylic germination boxes[8] (24 by 16 by 3.8 cm) and lined with 15- by 23-cm absorbent blue filter paper[9] to prevent the seeds from drying out. To reduce fungal growth, an insecticide with fungicides (thiamethoxam, difenoconazole, mefenoxam, fludioxonil)[10] was added to each germination box at a concentration of 0.2% (40 ml box^{-1}) (Dexter et al. 2010). The germination trays were stored in the dark at ambient temperatures for 72 h to induce germination. Seeds were considered to have germinated when the radicle emerged through the seed coat. Weathered and moldy seeds were considered dead, and they were counted and removed from the germination boxes. Nongerminated seeds were transferred to petri dishes[11] lined with white filter paper[12] and moistened with 5.0 ml of 0.005 M gibberellic acid[13] (GA_3) solution (Dexter et al. 2010). After 72 h in the GA_3 solution, the number of seeds that did and did not germinate were recorded. Germinated seeds were considered to be viable and nongerminated seeds were considered to be nonviable.

Statistical Analysis. Data were analyzed separately for GR and IR canola systems. All data were subjected to ANOVA using the general linear models procedure of statistical analysis software (SAS 2007). Normality, homogeneity of variance, and interactions of treatment, year, and locations were tested. In this experiment, year by treatment and treatment by location interactions were nonsignificant; therefore, the data of all four locations were pooled, and combined data were presented. Volunteer flax density at various stages, flax and canola biomass, flax seed production, canola and volunteer flax yield and AP (%) of volunteer flax were analyzed as a randomized complete block design in SAS (2007). Volunteer flax density and dry weight data were log-transformed before analysis to meet assumptions of variance analysis. Because of zero values in the data, the value of 1 was added to each datum before transformation (Little and Hills 1978). Where the ANOVA indicated that treatment effects were significant, means were separated at $P \leq 0.05$ with Fisher's Protected LSD test.

Results and Discussion

Volunteer Flax Control. In GR canola, preplant glyphosate (1,250 g ae ha^{-1}) reduced volunteer flax densities from 55 to 3 plants m^{-2} 12 d after application (Table 2). Densities of

Table 3. GR and IR canola biomass and yield as influenced by herbicide treatments.[a,b]

Treatment	Application timing	Canola biomass[c]	Canola seed yield[c]
		g m^{-2}	kg ha^{-1}
Glufosinate-resistant canola			
Weed free	—	784 bc	2,830 ab
Nontreated	—	517 d	1,430 e
Glyphosate (1,250 g ae ha^{-1})	Preplant	871 ab	2,770 ab
Glufosinate (150 g ai ha^{-1})	POST	712 c	2,360 d
Glufosinate (300 g ai ha^{-1})	POST	827 abc	2,500 cd
Glufosinate (600 g ai ha^{-1})	POST	842 abc	2,650 bc
Glyphosate (1,250 g ae ha^{-1}) fb Glufosinate (150 g ai ha^{-1})	Preplant/POST	934 a	2,790 ab
Glyphosate (1,250 g ae ha^{-1}) fb Glufosinate (300 g ai ha^{-1})	Preplant/POST	896 ab	2,860 ab
Glyphosate (1,250 g ae ha^{-1}) fb Glufosinate (600 g ai ha^{-1})	Preplant/POST	960 a	2,930 a
Imidazolinone-resistant canola			
Weed free	—	941 a	2,650 a
Nontreated	—	416 d	1,330 d
Glyphosate (1,250 g ae ha^{-1})	Preplant	887 a	2,350 b
Imazamox+imazethapyr (10.5 g ai ha^{-1})	POST	602 c	1,680 c
Imazamox+imazethapyr (21 g ai ha^{-1})	POST	656 c	1,570 cd
Imazamox+imazethapyr (42 g ai ha^{-1})	POST	546 cd	1,780 c
Glyphosate (1,250 g ae ha^{-1}) fb Imazamox+imazethapyr (10.5 g ai ha^{-1})	Preplant/POST	865 ab	2,340 b
Glyphosate (1,250 g ae ha^{-1}) fb Imazamox+imazethapyr (21 g ai ha^{-1})	Preplant/POST	702 bc	2,380 ab
Glyphosate (1,250 g ae ha^{-1}) fb Imazamox+imazethapyr (42 g ai ha^{-1})	Preplant/POST	825 ab	2,280 b

[a] Abbreviations: GR, glufosinate-resistant; fb, followed by; IR, imidazolinone-resistant.

[b] Each value represents pooled data over locations and years.

[c] Least square means within columns with no common letters are significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.

volunteer flax at harvest were reduced by glufosinate applied POST from 11 to 2.5 plants m^{-2} for 300 g ai ha^{-1} and 600 g ha^{-1} rates, respectively, compared with the nontreated control (44 plants m^{-2}) (Table 2). Both a preplant application of glyphosate at 1,250 g ha^{-1} followed by a POST application of glufosinate at 150, 300, or 600 g ha^{-1} reduced volunteer flax densities to < 2 plants m^{-2} in GR canola. In this experiment, a preplant application of glyphosate was equally effective for controlling volunteer flax as the same preplant application followed by POST. However, for reducing seed-mediated gene flow by crop volunteers in other GM crops including canola, maize, sugar beet, and wheat, Gruber et al. (2008) reported that preplant herbicide applications followed by POST-applied herbicide treatments were most effective for reducing densities of GM crop volunteers.

Volunteer flax dry weight was also influenced significantly by applications of preplant and POST herbicides and their combinations compared with the nontreated control in GR canola. Highest dry weight of volunteer flax was recorded in the nontreated control (150 g m^{-2}) followed by a POST application of glufosinate at 150 g ha^{-1} (57.3 g m^{-2}), suggesting that glufosinate alone at this dose was not effective for reducing volunteer flax density and dry weight (Table 2). Dry weight of volunteer flax was reduced by a preplant application of glyphosate alone (3.3 g m^{-2}) and the POST application of glufosinate alone at 600 g ha^{-1} (4 g m^{-2}). Glyphosate applied preplant at 1,250 g ha^{-1} followed by POST-applied glufosinate at all the doses were effective for reducing volunteer flax dry weight to ~ 2 g m^{-2} in GR canola.

Volunteer flax was not controlled well within the IR canola system. Imazamox + imazethapyr applied at any dose (10.5, 21, or 42 g ai ha^{-1}) did not reduce the density or dry weight of volunteer flax in IR canola, suggesting that flax has natural tolerance to POST-applied imazamox + imazethapyr at these doses. Flax has also been reported to tolerate foliar applications of some POST-applied sulfonylurea herbicides (Wall and Kenaschuk 1996), although soil residues might injure juvenile flax plants (Friesen and Wall 1991; McHughen and Holm 1995). When glyphosate was applied at 1,250 g ha^{-1}, the density of volunteer flax at harvest was reduced to 2 plants m^{-2} compared with nontreated plots (48 plants m^{-2}), regardless of imazamox + imazethapyr treatment.

In summary, preplant glyphosate treatment was the most effective for reducing volunteer flax densities in GR and IR canola. POST-applied imazamox + imazethapyr at any dose was not an effective herbicide option for reducing volunteer flax densities in IR canola.

Crop Response. All herbicide treatments were effective for achieving greater GR canola biomass compared with the nontreated control (Table 3). Interestingly, the weed-free treatment was not different from glyphosate applied preplant. However, canola biomass was similar for all herbicide treatments except for glufosinate applied POST at 150 g ha^{-1}, which was lower (712 g m^{-2}) than the other treatments. The lowest GR canola biomass was recorded in the nontreated control (517 g m^{-2}) (Table 3).

Greater GR canola yields were observed when glyphosate was applied preplant alone or in combination with POST-applied glufosinate at all doses (Table 3). When glufosinate was applied alone at 150 g ha^{-1}, lower canola yields were recorded (Table 3). Field experiments conducted in western Canada have previously shown that, to maximize canola yield,

Table 4. Volunteer flax seed production, seed viability, and AP in GR and IR canola as influenced by herbicide treatments.[a,b]

Treatment	Application timing	Volunteer flax[c]	Seed viability[c]	Yield[c]	AP[c,d]
		seeds m^{-2}	%	kg ha^{-1}	%
Glufosinate-resistant canola					
Weed free	—	0 b	0.0 e	0 b	0.0 b
Nontreated	—	5,963 a	69.6 a	321 a	26.2 a
Glyphosate (1,250 g ae ha^{-1})	Preplant	233 b	10.9 cd	17 b	0.5 b
Glufosinate (150 g ai ha^{-1})	POST	590 b	25.8 b	30 b	1.5 b
Glufosinate (300 g ai ha^{-1})	POST	261 b	14.6 c	18 b	0.5 b
Glufosinate (600 g ai ha^{-1})	POST	92 b	7.4 cde	7 b	0.2 b
Glyphosate (1,250 g ae ha^{-1}) fb glufosinate (150 g ai ha^{-1})	Preplant/POST	37 b	2.1 de	4 b	0.1 b
Glyphosate (1,250 g ae ha^{-1}) fb glufosinate (300 g ai ha^{-1})	Preplant/POST	8 b	0.5 e	1 b	0.1 b
Glyphosate (1,250 g ae ha^{-1}) fb glufosinate (600 g ai ha^{-1})	Preplant/POST	0 b	NA	0 b	0.0 b
Imidazolinone-resistant canola					
Weed free	—	0 c	0.0 d	0 c	0.0 c
Nontreated	—	5,571 a	74.8 a	301 a	25.5 a
Glyphosate (1,250 g ae ha^{-1})	Preplant	472 c	20.2 c	27 c	1.1 c
Imazamox + imazethapyr(10.5g ai ha^{-1})	POST	3,594 b	58.0 b	194 b	13.0 b
Imazamox + imazethapyr (21 g ai ha^{-1})	POST	3,393 b	64.2 ab	179 b	14.8 b
Imazamox + imazethapyr (42 g ai ha^{-1})	POST	3,613 b	56.7 b	195 b	14.7 b
Glyphosate (1,250 g ae ha^{-1}) fb imazamox + imazethapyr (10.5g ai ha^{-1})	Preplant/POST	431 c	18.7 c	26 c	1.0 c
Glyphosate (1,250 g ae ha^{-1}) fb imazamox + imazethapyr (21 g ai ha^{-1})	Preplant/POST	283 c	17.0 c	13 c	0.7 c
Glyphosate (1,250 g ae ha^{-1}) fb imazamox + imazethapyr (42 g ai ha^{-1})	Preplant/POST	457 c	25.4 c	27 c	1.2 c

[a] Abbreviations: AP, adventitious presence; GR, glufosinate-resistant; fb, followed by; IR, imidazolinone-resistant; NA, not applicable.

[b] Each value represents pooled data over locations and years.

[c] Least square means within columns with no common letters are significantly different according to Fisher's Protected LSD test at $P \leq 0.05$.

[d] AP of volunteer flax was calculated from the formula given in the text.

early weed removal is critical (Blackshaw et al. 2008; Harker et al. 2008).

The greatest IR canola biomass (941 g m^{-2}) was observed in the weed-free treatment, followed by glyphosate applied preplant at 1,250 g ha^{-1} (887 g m^{-2}), preplant glyphosate followed by POST imazamox + imazethapyr at 10.5 g ha^{-1} (865 g m^{-2}), and preplant glyphosate followed by POST imazamox + imazethapyr at 42 g ha^{-1} (825 g m^{-2}) (Table 3). The IR canola yield increased with application of herbicides when compared with the nontreated control (1,326 kg ha^{-1}), except when imazamox + imazethapyr was applied alone at 42 g ha^{-1} (Table 3). Noncontrolled volunteer flax could reduce IR canola yields by 51%. In a similar experiment in wheat, Wall and Smith (1999) reported that noncontrolled volunteer flax could reduce wheat yields up to 27%. Increasing POST-applied imazamox + imazethapyr rates did not influence crop biomass or yield (Table 3).

Volunteer Flax Seed Production, Viability, Yield, and AP. Volunteer seed production determines the ability of the volunteer population to perpetuate within the field as well as the potential for AP in subsequent crops. Volunteer flax seed production in GR canola measured within established quadrats of nontreated controls was 5,963 seeds m^{-2} (Table 4). Volunteer seed production might have been influenced by the experimental conditions in which flax was allowed to emerge before seeding of the canola crop. Volunteer flax seed yield was dramatically reduced by herbicide applications from 320 kg ha^{-1} in nontreated controls to almost 0, but was variable. Flax yield did not differ among any herbicide treatments (Table 4). Unlike volunteer flax population density, volunteer flax seed production (seeds m^{-2}) and yield (kg ha^{-1}) were not significantly affected by increasing glufosinate doses in GR canola.

In IR canola, flax volunteers produced 5,571 seeds m^{-2} in the nontreated control, and all other treatments including imazamox + imazethapyr reduced volunteer flax seed production (Table 4). Preplant glyphosate alone was effective in reducing volunteer flax seed production to 472 seeds m^{-2} in IR canola, and any other glyphosate-containing treatments also received the same results and reduced volunteer flax seed production to as low as 283 seeds m^{-2} (Table 4).

Herbicides can cause mortality, reduce plant growth, and delay maturity of weeds and thus influence seed viability as well as fecundity (McPherson et al. 2009). In the nontreated control, viability of volunteer flax seed averaged 69.6% and reflects the longer maturation period of flax compared with GR canola, even when planted earlier. Viability was reduced to about 11% by preplant glyphosate alone (Table 4). Increasing POST-applied glufosinate rates significantly decreased seed viability from 26 to 7%. The use of both preplant followed by POST-applied herbicides reduced viability to $< 3\%$ (Table 4). Volunteer flax plants were stunted by herbicide treatments; thus, they produced smaller, malformed seeds. This was the probable reason for reduction in seed viability in herbicide-treated plots.

Seed viability in the nontreated controls was 74.8% in IR canola and when glyphosate was applied preplant alone, seed viability was reduced to 20.2%. Imazamox + imazethapyr applied POST also significantly reduced volunteer flax seed viability (Table 4). The combination of both treatments (preplant followed by POST) reduced seed viability from 17 to 25.4% in various treatments in IR canola, suggesting that a preplant application was required.

Escaped volunteer transgenic flax may be harvested with subsequent crops and could contribute to AP. GM-derived foods do not require mandatory labeling in Canada and the United States, but some countries have established tolerance and traceability requirements for AP of approved GM material in non-GM grain varying from 0.9 to 5% (Demeke et al. 2006). Volunteer flax that emerged before seeding canola was not well controlled by POST herbicides and produced 320 kg ha^{-1} flax seeds. That amount of flax seeds could contribute to 26.2% contamination in harvested GR canola seeds (Table 4). Flax seed harvested with GR canola, like flax seed production, was variable. All herbicide treatments reduced AP of volunteer flax from 0 to 32 kg ha^{-1} in GR canola. However, when glyphosate was applied preplant at 1,250 g ha^{-1}, the contamination of flax seeds (AP) decreased to 0.5%, suggesting that preplant glyphosate was very effective in reducing AP of flax seeds in GR canola.

Volunteer flax yield and AP were also reduced when herbicides were applied in IR canola. Glyphosate applied preplant alone reduced volunteer flax seed yield to 27 kg ha^{-1} and resulted in volunteer flax AP of 1.1% compared with 300 kg ha^{-1} flax yield and 25.5% AP in nontreated controls (Table 4). Imazamox + imazethapyr applied POST alone was not effective for reducing the density and dry weight of volunteer flax (Table 2). Similar results were also reflected in volunteer flax seed yields. Volunteer flax seed yields ranged from 179 to 195 kg ha^{-1} and AP of flax seeds ranged from 13 to 15% when imazamox + imazethapyr was applied POST alone, depending on rate (Table 4). However, the preplant treatment of glyphosate followed by POST imazamox + imazethapyr at 21 g ha^{-1} reduced flax yield and AP to 13 kg ha^{-1} and $< 1\%$, respectively, in IR canola. A single preplant application of glyphosate reduced volunteer flax AP in both of the canola systems (0.5 and 1.1% in GR and IR canola, respectively). Although the treatments in this experiment did not show increased performance from sequential applications, in conditions when more of the flax emerged later, the PRE–POST treatment would have facilitated the prevention of disease and insect problems associated with "green bridge."

Canada is the largest producer and exporter of flaxseed in the world. Canada exports $> 80\%$ of domestically produced flaxseed mainly to the European Union (EU), Japan, Korea, and the United States every year (Flax Council of Canada 2007). Concerns regarding volunteerism, ferality, and AP have been increasing with the expanding area and production of GM crops (Gressel 2005). Crops are grown in an open system in which complete isolation is not possible. To facilitate co-existence of GM, conventional, and organic crop production systems, threshold levels of contamination are required (Weber et al. 2007). Compliance with international standards is complicated because various countries have different threshold levels for AP of GM seeds in conventional crops/seeds. The EU has established a labeling threshold level of 0.9% (Devos et al. 2009). Japan and South Korea have a 5 and 3% tolerance limit, respectively (Demeke et al. 2006). In addition, international standard testing methods for AP of GM seeds have yet to be resolved. Nonviable seeds, although not able to propagate, may register as GM. To avoid market risks for flaxseed export, especially to the EU, Canadian growers will be required to adopt best management practices to allow GM flax production to co-exist with commodity or organic flax production and with other crops grown in rotation with flax.

These experiments represent a worst-case scenario for volunteer flax AP in herbicide-resistant canola. Flax populations were seeded before canola at a target population of 150 plants m^{-2}. The results of multisite–multiyear experiments to measure volunteer flax emergence after commercial flax production in conventional tillage (CT) and reduced tillage (RT) cereals grown in rotation suggest that volunteer flax emergence occurred throughout most of the growing season (Amit J. Jhala, personal observation). Canola is usually seeded early and a delay of seeding can reduce the relative competitive ability of canola. However, when assessing risk, worst-case scenarios provide valuable information to decision makers.

This is the first report quantifying and offering mitigation strategies for volunteer flax AP in two types of herbicide-resistant canola. With effective control of volunteer flax in GR canola, AP was reduced. However, the IR canola system did not reliably reduce AP; therefore, growers in western Canada are advised not to grow IR canola in the year after GM flax production. Other imidazolinone-resistant/tolerant crops (for example IR lentil [*Lens culinaris* L.], pea [*Pisum sativum* L.], wheat [*Triticum aestivum* L.]), in rotation with flax might have similar concerns. A preplant application of glyphosate at the recommended rate enhanced canola yields in both canola systems by reducing volunteer flax density and crop–weed competition. Effective preseeding control of volunteer populations is suggested for volunteer flax control and for reduced AP potential.

When proper mitigation strategies were adopted in this study, AP of volunteer flax in canola was reduced. Effective herbicides applied the year after transgenic flax production would control flax volunteers and reduce seed-mediated gene flow from transgenic volunteer flax. Herbicides are one component of best management systems. Other practices include reducing harvest loss by properly adjusting combine settings, adopting isolation distances between GM and organic flax fields, diversifying crop rotations, cleaning equipment, and separating supply chains for GM and organic flax. This information will be useful to the flax industry, growers, and regulators for policy development and risk assessment for potential commercial release of transgenic flax. Integrated management of currently available (herbicide- and insect-resistant GM crops) and future transgenic crop volunteers (abiotic stress-tolerant and biopharmaceutical crops) will be required to reduce AP in subsequent crops and minimize market and international trade impacts.

Sources of Materials

[1] Fabro Enterprises Ltd., 2545 North Service Road (W), Swift Current, Saskatchewan S9H 5L3, Canada.

[2] Glufosinate Liberty Link® herbicide, Bayer Canada, 77 Belfield Road, Toronto, Ontario M9W 1G6, Canada.

[3] Weathermax® herbicide, Monsanto Canada, 900 One Research Road, Winnipeg, Manitoba R3T 6E3, Canada.

[4] Imazamox + imazethapyr® herbicide Odyssey, BASF Canada, 100 Milverton Drive, 5th Floor, Mississauga, Ontario L5R 4H1, Canada.

[5] Merge® surfactant blend + solvent (petroleum hydrocarbons), BASF Canada, 100 Milverton Drive, Mississauga, Ontario L5R 4H1, Canada.

[6] Spider sprayer, Fabro Enterprises Ltd., 2545 North Service Road (W), Swift Current, Saskatchewan S9H 5L3, Canada.

[7] Flat-flan nozzle, Teejet XR 110015, Max-Quip, 11423-163 Street, Edmonton, Alberta T5M 3Y3, Canada.

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

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

[10] Helix XTra™, Syngenta Crop Protection Canada Inc., Suite 300, 6700 Macleod Trail South, Calgary, Alberta T2H 0L3, Canada.

[11] Petri dishes, Hoffman Manufacturing Inc., 16541 Green Bridge Road, Jefferson, OR 97352-9201.

[12] White filter paper, Hoffman Manufacturing Inc., 16541 Green Bridge Road, Jefferson, OR 97352-9201.

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

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