EVIDENCE OF CONTAMINATION OF PEDIGREED CANOLA (*BRASSICA NAPUS*) SEEDLOTS IN WESTERN CANADA WITH GENETICALLY ENGINEERED HERBICIDE RESISTANCE TRAITS

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Abstract

The objective of this study was to survey pedigreed canola (*Brassica napus* L.) seedlots for contaminating herbicide resistance traits because of complaints from farmers regarding glyphosate [*N*-(phosphonomethyl)glycine]-resistant canola volunteers occurring unexpectedly in their fields at densities and in patterns that suggested that pollen-mediated gene flow from neighboring fields in previous years was not the source of contamination. Twenty-seven unique, commercial certified canola seedlot samples were collected. Glyphosate-resistant seedlot samples were not collected. Canola samples were planted in the field, and when the canola had two to four true leaves, glyphosate, glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid], and thifensulfuron {methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate} herbicides were applied. Surviving canola plants were counted. Of the 27 seedlots, 14 had contamination levels above 0.25% and therefore failed the 99.75% cultivar purity guideline for certified canola seed. Three seedlots had glyphosate resistance contamination levels in excess of 2.0%. Unexpected contamination (even at 0.25%) can cause problems for producers that practice direct seeding and depend on glyphosate for nonselective, broad-spectrum weed control. To avoid unexpected problems and costs, it is important that farmers are cognizant of the high probability that pedigreed canola seedlots are cross-contaminated with the various herbicide resistance traits.

CANOLA (*Brassica napus* L. and *B. rapa* syn. *B. campestris* L.) is the second most widely grown and the second most valuable crop in western Canada [after wheat (*Triticum aestivum* L.)], with annual plantings over the past decade of 3.0 to 5.7 million ha (Statistics Canada, 1992–2001). In recent years, more than 90% of the canola grown has been *B. napus* cultivars (Canadian Grain Commission, 2002) for reasons that include greater yields and availability of cultivars with novel-trait herbicide resistance. There currently are three novel-trait, herbicide-resistant *B. napus* types commercially available in western Canada, namely, glyphosate resistant, glufosinate resistant, and imidazolinone resistant (IR). Two of these herbicide-resistant types, glyphosate and glufosinate, are transgenic with the genes conferring resistance derived from bacteria (Canadian Food Inspection Agency, 1995a, 1995b). The IR trait in canola, which also confers resistance to certain other acetolactate synthase inhibitor herbicides such as thifensulfuron, was derived by in vitro microspore mutagenesis and selection (Swanson et al., 1989).

Since its commercial introduction in 1996 (IR canola in 1995), herbicide-resistant *B. napus* canola technology has been rapidly and widely adopted by Canadian farmers. In 1998, it was estimated that nearly 60% of a total of 4.9 million ha of canola was planted to herbicide-resistant *B. napus* cultivars (Anonymous, 1998, p. 47). For the year 2000, it was estimated that approximately 1.8 to 2 million ha of glyphosate-resistant canola were planted in Canada by 20 000 farmers (40% of the total canola area) (Sharlow, 2002).

The agronomic practice of direct seeding, where the soil is not disturbed in spring before planting the crop, has become common in western Canada. This practice is beneficial in terms of minimizing soil erosion and conserving soil moisture as well as reducing wear on tillage implements and tractors (Lafond et al., 1992). With direct seeding, weeds that have emerged before planting must be controlled to minimize subsequent competition and crop yield loss. These weeds—which can be large, established plants if they germinated late in the fall or early in the spring—are normally controlled in one of two ways. Some producers practicing direct seeding use a planting implement that provides complete disturbance of the soil surface (e.g., discer seeder or large sweep shovels on an air seeder), which kills most annual weeds present at the time of seeding. Other producers spray a broad-spectrum, nonselective, nonresidual herbicide (most commonly glyphosate) before planting the crop, or after planting but before crop emergence, to control emerged weeds. These producers generally plant the crop with an implement that does not cause complete soil disturbance (e.g., narrow openers on an air seeder). The herbicide application before crop emergence is often referred to as spring *burn-off* or *burn-down*. A direct account of the area treated with glyphosate as a spring burn-off is not publicly available. However, it is estimated that 13, 39, and 27% of the total area prepared for seeding in Manitoba, Saskatchewan, and Alberta, respectively, is seeded following no-till or zero-till practices (a total of 8.1 million ha) (Statistics Canada, 2002). Glyphosate as a spring burn-off treatment would be applied to the majority of this land.

Currently, there are no suitable substitutes for glyphosate as a spring burn-off herbicide considering spectrum of activity, efficacy, absence of soil residue, and cost. Consequently, those farmers that expect glyphosate-resistant canola volunteers (from previous crops) still use glyphosate as a spring burn-off but usually add an auxin-type herbicide such as 2,4-D [(2,4-dichlorophenoxy)acetic acid] or MCPA [(4-chloro-2-methylphenoxy)acetic acid] to the spray tank. In addition to extra cost, there are other concerns with tank-mixing glypho-

Dep. of Plant Sci., Univ. of Manitoba, Winnipeg, MB, Canada R3T 2N2. Received 16 Dec. 2002. *Corresponding author (Lyle_Friesen@umanitoba.ca).

Published in Agron. J. 95:1342–1347 (2003).

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Abbreviations: CSGA, Canadian Seed Growers' Association; IR, imidazolinone resistant.

sate and an auxin-type herbicide. The auxin-type herbicides have some soil residual activity, and this soil residue can seriously injure certain sensitive broadleaf crops as they emerge, such as field pea (*Pisum sativum* L.), field bean (*Phaseolus vulgaris* L.), lentil (*Lens culinaris* Medic.), chickpea (*Cicer arietinum* L.), and sunflower (*Helianthus annuus* L.) (Saskatchewan Pulse Growers, 2000, p. 5.8–5.18). Furthermore, volunteer canola plants that emerge early in the spring are generally large, hardy, and robust at the time of spring burn-off; therefore, complete control may be difficult with alternative herbicides such as 2,4-D, MCPA, or thifensulfuron/tribenuron {tribenuron, methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]amino]sulfonyl] benzoate}. Thifensulfuron/tribenuron will not control IR canola volunteers, even if these volunteers are small. If there are unexpected glyphosate-resistant canola volunteers, due to pollen-mediated gene flow from a neighboring field or from a contaminated seed source, these become very obvious 5 to 7 d after application of the spring glyphosate burn-off treatment. Depending on the crop planted (e.g., field bean, lentil, chickpea, sunflower), applying auxin-type herbicides in-crop to the escaping glyphosate-resistant canola volunteers may not be an option. Also, glyphosate-resistant volunteers escaping the spring burn-off treatment may be relatively large and difficult to control by the time alternative herbicides can be applied in-crop. Depending on surviving volunteer canola density and the crop that was sown, the resulting problem may be cosmetic, or the competitive growth habit of volunteer canola may actually reduce crop yield and contribute to the glyphosate-resistant canola seedbank in the soil.

Currently, for Canadian pedigreed canola seed, there are no specific standards regarding the adventitious presence of genetically engineered herbicide resistance traits in seedlots. However, if novel-trait herbicide resistance is considered an integral component of a herbicide-resistant canola cultivar, then cultivar purity standards would apply. The Association of Official Seed Certifying Agencies allows 0.25% maximum for the presence of other canola cultivars in a certified canola seedlot (Association of Official Seed Certifying Agencies, 1999; Downey and Beckie, 2002). For Breeder and Foundation canola seedlots, the tolerance level for other canola cultivars (genetic purity limit) is 0.05%. Before the introduction of novel-trait herbicide resistance in canola, there were no definitive genetic markers in canola to easily, quickly, and precisely quantify the levels of genetic impurity in a canola cultivar/seedlot (Downey and Beckie, 2002).

Contamination of pedigreed canola seedlots or commercial crops with herbicide resistance traits can occur in two ways: via either pollen-mediated gene flow or whole seed. Whole-seed contaminants may be homozygous for the herbicide resistance trait while contaminants resulting from pollen-mediated gene flow will be heterozygous for the resistance trait in the initial progeny generation. Canola seedlings heterozygous for the herbicide resistance traits (glyphosate, glufosinate, or imidazolinone) can survive and thrive following recommended commercial dosages of these herbicides in the field (Hall et al., 2000; Rieger et al., 2002).

This survey of the purity of pedigreed canola seedlots with respect to herbicide resistance traits was prompted by complaints from several farmers regarding glyphosate-resistant canola volunteers occurring unexpectedly in their fields at densities and in patterns that suggested that pollen-mediated gene flow from neighboring fields in previous years was not the source of contamination. The authors are aware of only one other study investigating the purity of pedigreed canola seedlots with respect to genetically engineered herbicide resistance traits. Downey and Beckie (2002) tested a total of 70 certified canola seedlots drawn from 14 different conventional, open-pollinated *B. napus* cultivars for glyphosate and glufosinate resistance trait contamination. They screened 2000 seeds from each seedlot per herbicide in a Petri dish seed bioassay. They reported that 41 of the 70 seedlot samples had detectable levels of herbicide resistance trait contamination and that 18 of the 70 samples failed the 99.75% cultivar purity guideline. One of the samples tested had glyphosate resistance trait contamination levels of 6.8%. Downey and Beckie (2002) obtained their seedlot samples directly from pedigreed seed producers before seed treatment and packaging of the seed into bags for commercial sale. Their results may not directly reflect what farmers actually plant in their fields because seed treatment and packaging involves additional handling of the seedlot and opportunities for contamination due to inadvertent seed admixtures.

The objective of this study was not to determine the absolute level of herbicide resistance trait contamination in a given canola cultivar/seedlot, but rather to determine whether pedigreed canola seedlots in western Canada are contaminated with unwanted/unexpected herbicide resistance traits. Samples were drawn from commercially packaged, seed-treated, certified seedlots that were sown by farmers into their fields in May of 2002.

Materials and Methods

Thirty-three commercial certified canola (*B. napus*) seedlot samples were collected in the spring of 2002 from local cooperating farmers, representing 27 unique Canadian Seed Growers' Association (CSGA) seedlot numbers (i.e., some seedlots were sampled from different bags purchased by different farmers but had identical CSGA lot numbers). All canola samples were commercially treated with a combination fungicide/insecticide seed treatment. Eighteen conventional canola samples, eight glufosinate-resistant samples, and seven IR samples were collected. Glyphosate-resistant samples were not collected because farmers are contractually prohibited from providing seed to third parties for any reason, including research and testing. Furthermore, low levels of contamination (5% or less) of glyphosate-resistant seedlots by conventional, glufosinate-resistant, or IR canola is of minimal agronomic importance because neither glufosinate nor acetolactate synthase inhibitors normally are used as broad-spectrum spring burn-off treatments. The seedlots were collected without bias or foreknowledge of contamination levels. To minimize inconvenience to the farmer, the collection procedure consisted of

opening one bag and scooping out several cups of seed. Bags were not probed in multiple places, nor were multiple bags sampled of a specific cultivar held by a given farmer. It was assumed that the seedlot samples would be relatively homogeneous after harvest, handling, and commercial seed treatment. The results presented in Table 1 on seedlots with identical CSGA lot numbers generally support this assumption.

The canola seedlot samples were planted in the field at the University of Manitoba research station at Carman, Manitoba, on 18 June 2002 using a small-plot cone seeder with 12 double-disk openers spaced 15 cm apart. The plots were located in an area where a canola crop had not been grown for at least 8 yr. This area was limited in size, which limited the number of replicates that were planted. Strips 2 m wide were left unseeded between each replicate to function as a check for volunteer canola emergence from the soil seedbank and to separate the various herbicide treatments. The plot area was cultivated just before seeding the canola, and the soil was moist. Individual plot size was 1.8 by 6 m (11 m^2). Seven replicates were planted, with each replicate including all 33 seedlots. Because the canola was not being grown to maturity, seeding rate was higher than that normally used. An average thousand-seed weight of 3.5 g was assumed for all samples, and the target seeding rate was 3500 seeds per plot. Canola seedling density before herbicide application was determined on 2 July in three of the seven replicates by counting the number of canola seedlings occurring per 50-cm row length in three adjacent rows in each plot.

Canola seedlings were sprayed on 3 July when they had two to four true leaves. Herbicides were applied using an all-terrain vehicle mounted sprayer equipped with Teejet 11001 flat-fan nozzles (Spraying Syst. Co., Wheaton, IL) calibrated to deliver 55 L ha^{-1} of spray solution at 275 kPa at 8 km h^{-1}. Commercial formulations of the herbicides were applied. Glyphosate was applied at 750 g a.e. ha^{-1} (445 g a.e. ha^{-1} is the recommended dosage for glyphosate-resistant canola) to three replicates. Glufosinate was applied at 500 g a.i. ha^{-1} to one replicate. Thifensulfuron was applied at 10 g a.i. ha^{-1} + 0.2% v/v nonionic surfactant to one replicate. Glyphosate + glufosinate tank mix (at the above dosages) was applied to one replicate, and glyphosate + glufosinate + thifensulfuron tank mix (at the above dosages) was applied to one replicate. In the three-way tank mix, the nonionic surfactant was omitted. (The glyphosate and glufosinate commercial formulations include surfactants.) Thifensulfuron was used to identify canola seedlings with the IR trait because of the very short persistence of thifensulfuron in soil compared with an imidazolinone herbicide—herbicide soil residues are a concern on the University of Manitoba Carman research station. Thifensulfuron is registered for commercial use on IR canola in western Canada (Manitoba Agriculture and Food, 2002).

Surviving canola plants in all plots, except those plots

Table 1. Summary of canola seedling survival percentages for certified seedlots trated with various herbicides. Values based on three replicates (number of seedlings screened per plot and glyphosate-treated survivors) are presented as means followed by standard deviation in parentheses.

Seedlot	Cultivar†	Type‡	Glyphosate (three reps)§	Glufosinate	Glyphosate + glufosinate	Thifensulfuron	Mean no. of seedlings screened per plot
			%				
1	A_1	Conv	0.06 (0.08)	0.15	0.00	0.00	2640 (416)
2	A_1	Conv	0.02 (0.04)	0.07	0.00	0.03	3070 (333)
3	B	Conv	0.09 (0.13)	0.04	0.00	0.04	2480 (587)
4	B_1	Conv	0.29 (0.06)	0.04	0.00	0.00	2550 (520)
5	B_1	Conv	0.39 (0.13)	0.04	0.00	0.00	2390 (73)
6	C_1	Conv	0.19 (0.08)	0.10	0.00	0.05	1970 (393)
7	C_1	Conv	0.19 (0.07)	0.21	0.00	0.00	2800 (194)
8	D	Conv	0.24 (0.16)	0.00	0.00	0.00	1920 (173)
9	E	Conv	0.02 (0.03)	0.09	0.00	0.00	2130 (618)
10	F	Conv Hybrid	0.24 (0.11)	0.60	0.00	0.00	1840 (391)
11	G	Conv Hybrid	0.67 (0.10)	0.09	0.00	0.05	2180 (481)
12	H_1	Conv	4.89 (0.69)	0.50	0.00	0.05	2190 (290)
13	H_1	Conv	3.00 (0.64)	0.32	0.00	0.05	1860 (409)
14	I	Conv	0.05 (0.06)	0.31	0.00	0.00	2550 (520)
15	I_1	Conv	0.04 (0.06)	0.43	0.00	0.05	1870 (48)
16	I_1	Conv	0.02 (0.03)	0.19	0.00	0.05	2070 (375)
17	J	Conv HEAR	0.31 (0.08)	0.11	0.00	0.00	1830 (127)
18	J	Conv HEAR	0.03 (0.05)	0.20	0.00	0.00	2530 (100)
19	K	GluR Hybrid	0.27 (0.07)	NA¶	0.04	0.00	2340 (182)
20	K	GluR Hybrid	0.23 (0.12)	NA	0.28	0.00	2160 (267)
21	L	GluR Hybrid	0.08 (0.06)	NA	0.00	0.00	2000 (246)
22	L	GluR Hybrid	2.67 (0.18)	NA	1.45	0.00	2270 (431)
23	L	GluR Hybrid	0.44 (0.34)	NA	0.20	0.00	1520 (111)
24	M	GluR Hybrid	2.13 (0.42)	NA	1.20	0.00	2160 (300)
25	M_1	GluR Hybrid	0.32 (0.11)	NA	0.07	0.00	1440 (267)
26	M_1	GluR Hybrid	0.38 (0.17)	NA	0.15	0.00	3310 (660)
27	N	IR	0.05 (0.05)	0.82	0.00	NA	2190 (581)
28	N	IR	0.00 (0.00)	0.25	0.00	NA	2820 (680)
29	N	IR	0.00 (0.00)	0.15	0.00	NA	2630 (782)
30	O	IR	0.00 (0.00)	0.00	0.00	NA	2370 (227)
31	O	IR	0.00 (0.00)	0.12	0.00	NA	1600 (242)
32	O	IR	0.00 (0.00)	0.04	0.00	NA	2450 (646)
33	O	IR	0.00 (0.00)	0.19	0.00	NA	2100 (529)
Mean							2250 (423)

† The subscript 1 following the alphabetic seedlot designation indicates that the two seedlots had identical Candian Seed Growers' Association (CSGA) lot numbers. These seedlots were sampled from different 25-kg bags purchased by different farmers, but the CSGA lot number was identical. For the seedlots, CSGA crop certificate and lot numbers are available from the author based on a justifiable request.

‡ Conv, conventional; Conv HEAR, conventional high-erucic-acid rapeseed; GluR, glufosinate resistant; IR, imidazolinone resistant.

§ For glyphosate, the total number of seedlings screened per seedlot is three times the mean number of seedlings screened per plot.

¶ NA, not assessed.

treated with thifensulfuron alone, were counted on 9 July. Survivors were very obvious and response very definitive, particularly for plots treated with glyphosate and tank mixtures containing glyphosate (i.e., plants were either dead or alive with no stunted green seedlings). For glufosinate, seedlings exhibiting obvious crisping of leaf edges were classified as susceptible (i.e., not carrying the glufosinate resistance trait). Plots treated with thifensulfuron were assessed 15 July. In the non-IR plots, the majority of canola seedlings treated with thifensulfuron were not dead and desiccated but were green and very stunted. However, canola plants with the IR trait were bolting at this time, and this was the basis for discrimination. For each plot, percentage resistance was calculated by dividing the number of surviving or uninjured canola plants by the total number of seedlings before herbicide application and then multiplying by 100.

Results and Discussion

Canola emerged uniformly and visually grew normally in all plots. A relatively high percentage of the seeds sown successfully established seedlings (approximately 2250/3500 = 64%) (Table 1, refer to overall mean density per plot). Flea beetle (*Phyllotreta* spp.) damage to the canola seedlings was minimal, probably because all seedlots had a seed treatment. There was no emergence of canola outside of the seeded plots, indicating no viable canola seed in the soil seedbank.

There was some variability between seedlots in the mean number of seedlings screened per plot (Table 1). This variability may have been due to differences in thousand-seed weight between seedlots and cultivars. (When calculating the seeding rate, a thousand-seed weight of 3.5 g was assumed for all seedlots to facilitate packaging of the seed for the small-plot cone seeder.) Furthermore, an occasional seedlot sample had either fertilizer- or insecticide-impregnated granules mixed with the seed. For example, Seedlot 26 had some fertilizer mixed with the seed, and this was overcompensated for when packaging the amount of seed per plot (compare seedlings screened to Seedlot 25). Regardless, variable seedling density did not compromise either early growth or spray coverage in any of the plots.

Of the 33 seedlots, only one seedlot (Seedlot 30) had no detectable herbicide resistance trait contamination based on the numbers of seedlings screened (Table 1). Of the 27 unique CSGA-numbered seedlots, 14 had contamination levels above 0.25% and therefore failed to meet the 99.75% cultivar purity guideline for certified seed. Of the 14 unique seedlots that had contamination levels in excess of 0.25%, nine failed due to glyphosate resistance trait contamination while five failed due to glufosinate resistance trait contamination. One of the unique CSGA-numbered seedlots (Seedlots 12 and 13) failed to meet the purity guideline because both glyphosate and glufosinate resistance trait contamination exceeded 0.25%. However, double-resistant individuals, resistant to both glyphosate and glufosinate, were not detected in Seedlots 12 and 13. Three of the unique seedlots had very high levels of glyphosate resistance trait contamination, that is, greater than 2%.

Glufosinate resistance trait contamination (in the non-glufosinate-resistant cultivars) occurred at lower levels compared with glyphosate resistance trait contamination, with no seedlots exceeding 1% glufosinate resistance trait contamination (Table 1). As might be expected, double-resistant individuals (glyphosate and glufosinate) were detected only in the glufosinate-resistant cultivars. Of the seven unique CSGA-numbered glufosinate-resistant seedlots, six of the seven had lower levels of double-resistant individuals compared with the levels of glyphosate-resistant individuals. These results indicate that some of the glyphosate-resistant individuals in the glufosinate-resistant seedlots were, in fact, susceptible to glufosinate. This may be a result of whole-seed contamination as opposed to pollen-mediated gene flow.

The majority of the IR cultivars had undetectable levels of glyphosate resistance trait contamination while still exhibiting glufosinate resistance trait contamination (Table 1). Because only one company is involved in the breeding and development of IR cultivars in Canada to date, it appears that IR seedlots were screened for glyphosate resistance trait contamination at all stages of pedigreed seed production (and contaminated seedlots were discarded). However, the same vigilance appears to have not been exercised for glufosinate resistance trait contamination. Glufosinate resistance trait contamination does not have the same agronomic implications for farmers practicing direct seeding as does glyphosate resistance trait contamination. The IR seedlot results indicate that it is possible to produce certified canola seedlots in western Canada with low levels of herbicide resistance trait contamination.

Only six unique CSGA-numbered seedlots had detectable levels of IR trait contamination, and contamination levels were 0.05% or less in all instances (Table 1). This may reflect the relative popularity of the various herbicide-resistant canola types (refer to the Introduction). Fewer acres would result in fewer opportunities for outcrossing and also reduced whole-seed contamination. No triple-resistant individuals (resistant to glyphosate, glufosinate, and thifensulfuron) were detected in this study.

The overall results of this study are comparable to those reported by Downey and Beckie (2002) although somewhat more contamination was identified in the current study [14 out of 27 unique CSGA-numbered seedlots failed the 99.75% cultivar purity guideline in the current study compared with 18 out of 70 seedlot samples that failed in the Downey and Beckie (2002) study].

Given current knowledge of pollen-mediated gene flow in *B. napus* (Staniland et al., 2000; Rieger et al., 2002), it is unlikely that pollen flow would cause greater than 0.1% contamination in a single generation of pedigreed seed production. Pedigreed seed crops are grown with mandatory isolation distances from sexually compatible species (CSGA, 2002), which limits pollen-mediated gene flow. Therefore, the contamination occurring in certified canola seedlots with contamination levels greater than 0.25% is either the result of inadvertent mechanical mixing of certified seedlots during harvest or handling or the result of contamination occurring in

earlier generations of pedigreed seed production (i.e., Breeder or Foundation seed) that was not tested for or detected (Downey and Beckie, 2002).

The planting of pedigreed canola seedlots that do not exceed the 0.25% contamination guideline for certified seed does not necessarily mean that there will be no agronomic concern the following year with regard to the unexpected presence of herbicide resistance traits in volunteer canola seedlings. Given some reasonable assumptions regarding canola seeding rates and thousand-seed weight (5.5 kg/ha, 4.0 g per thousand seeds), there are approximately 1.4 million seeds planted per hectare. At the 0.25% contamination level of a herbicide resistance trait in a seedlot, there will be 3500 resistant seeds planted per hectare. If one-half of these seeds result in mature canola plants, which is a typical establishment rate for a commercial canola crop in western Canada, then there will be 1750 resistant canola plants per hectare. Given a 2000 kg/ha crop yield and harvest losses of 6% (Gulden et al., 2003), there will be 120 kg/ha of seed remaining in the field. Resistant seeds will be 0.25% of this 120 kg/ha. [In the absence of selection and given equal fitness of susceptible and resistant individuals, a resistance trait will remain at the same frequency in a population over time (Jasieniuk et al., 1996).] Therefore, 300 g of resistant seed will shatter onto the soil per hectare, or 75 000 resistant seeds per hectare. If one-tenth of these seeds successfully establish a seedling the following year, there will be one herbicide-resistant volunteer canola plant every 1.3 m^2. If the resistance trait is glyphosate and the farmer practices direct seeding and sprays with glyphosate alone before crop emergence, one surviving canola plant every 1.3 m^2 will be a weed problem. Depending on the crop planted, there may not be in-crop herbicide options that will provide satisfactory control of relatively large volunteer canola plants (large because the canola volunteers would have survived the spring glyphosate burn-off applied before crop emergence). If the crop planted is not as competitive as cereals (e.g., flax, lentil, or field bean), one volunteer canola plant every 1.3 m^2 may be more than a cosmetic problem and probably will cause crop yield losses. The above scenario applies to pedigreed canola seedlots that meet the cultivar purity guideline of 99.75%. Downey and Beckie (2002) acknowledged this problem and noted that even when the genetic purity standards are met, the sowing of a conventional cultivar will almost certainly result in a significant population of herbicide-resistant plants within that field.

Because of the value and popularity of direct seeding to farmers in western Canada and the dependence of direct-seeding systems on glyphosate, the adventitious presence of the glyphosate resistance trait in pedigreed canola seedlots has greater agronomic implications than either the glufosinate or IR traits. Neither glufosinate nor the various herbicides used in IR canola are registered for use as direct-seeding, spring burn-off treatments (Manitoba Agriculture and Food, 2002). However, it is possible that IR canola volunteers emerging with a subsequent crop could survive an in-crop herbicide application if an acetolactate synthase inhibitor herbicide is applied alone. In this study, the adventitious presence of the IR trait in canola seedlots was relatively rare.

Conclusions

The results of this study indicate that the pedigreed canola seed production system in western Canada is cross-contaminated with the various herbicide resistance traits at a high level and that purchasing and planting a pedigreed conventional canola seedlot does not guarantee the absence of genetically engineered traits. For those producers that grow canola and practice direct seeding, it means that glyphosate no longer is a nonselective, broad-spectrum herbicide that can be used alone as a spring burn-off treatment. Because other herbicides have to be tank-mixed with glyphosate to achieve broad-spectrum vegetation control in the spring burn-off treatment, additional costs will be incurred.

While this survey of pedigreed canola seedlots was not repeated in time or space, we believe that this study has merit despite the lack of repetition. The objectives of this study were not to determine the actual or absolute level of herbicide resistance trait contamination in a given canola cultivar/seedlot, but rather to determine whether pedigreed canola seedlots in western Canada are contaminated with unwanted/unexpected herbicide resistance traits. Of the 27 unique CSGA-numbered canola seedlots in this study, 26 had detectable levels of herbicide resistance trait contamination, even given the relatively low numbers of individual seedlings screened (compared with the number of individual seedlings that normally are present in 1 ha of a canola crop, for example). Results in all field plots were very clear and easy to assess. Furthermore, the similar results of the Downey and Beckie (2002) canola seedlot study (refer to the Introduction) confirm our results and indicate that our survey was and is representative of reality. The Downey and Beckie (2002) study is not published in a widely circulated scientific journal, though. The results of these canola seedlot surveys are extremely important, particularly to those farmers and organizations that are hoping to avoid or minimize the occurrence of genetically modified traits on their land or in their crops.

The pedigreed seed production system can be considered a stringent segregation/identity preservation system complete with mandatory isolation distances, crop rotation restrictions, and inspections (CSGA, 2002). The results of this study indicate that this stringent segregation system does not result in the genetic purity of pedigreed canola seedlots in western Canada. The results of this study can be considered as a model before the commercialization of other genetically engineered crops where segregation/identity preservation systems are being considered. Factors such as the inherent outcrossing rate, seedbank recruitment and longevity, and frequency of the crop in the rotation would influence subsequent contamination levels in conventional pedigreed seed and commercial grain lots. The specific genetically engineered trait also is important in considering the implications of commercializing a genetically engineered crop.

For example, the glyphosate resistance trait in plants directly affects the success and economics of a direct-seeding crop production system. Furthermore, a successful segregation/identity preservation system requires agreed-on tolerances for contaminants and enforcement of the standards through frequent testing and discarding of out-of-tolerance seed or grain lots, creating additional costs for the entire production system.

For example, the commercialization of glyphosate-resistant wheat in western Canada is currently being contemplated, possibly initially under an identity preservation protocol. Considering that wheat can outcross to nearly the same extent as *B. napus* (Staniland et al., 2000; Hucl and Matus-Cádiz, 2001), has similar seedbank longevity to *B. napus* (Beckie et al., 2001; Légère et al., 2001), and is grown more often in the rotation than canola (Thomas et al., 1999), it can be predicted that the extent of glyphosate resistance trait contamination in pedigreed conventional wheat seedlots and commercial grain lots will eventually be similar to or greater than the situation currently in canola. The presence of two widely grown commercial glyphosate-resistant crops will cause additional problems and increased costs for those farmers practicing direct seeding in western Canada, whether they choose to seed-resistant cultivars or not, and may threaten the viability of this widely adopted and beneficial farming practice (Van Acker and Entz, 2001).

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