

# Risks and consequences of gene flow from herbicide-resistant crops: canola (*Brassica napus* L) as a case study<sup>†</sup>

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**Abstract:** Data from the literature and recent experiments with herbicide-resistant (HR) canola (*Brassica napus* L) repeatedly confirm that genes and transgenes will flow and hybrids will form if certain conditions are met. These include sympatry with a compatible relative (weedy, wild or crop), synchrony of flowering, successful fertilization and viable offspring. The chance of these events occurring is real; however, it is generally low and varies with species and circumstances. Plants of the same species (non-transgenic or with a different HR transgene) in neighbouring fields may inherit the new HR gene, potentially generating plants with single and multiple HR. For canola, seed losses at harvest and secondary dormancy ensures the persistence over time of the HR trait(s) in the seed bank, and the potential presence of crop volunteers in subsequent crops. Although canola has many wild/weedy relatives, the risk of gene flow is quite low for most of these species, except with *Brassica rapa* L. Introgression of genes and transgenes in *B rapa* populations occurs with apparently little or no fitness costs. Consequences of HR canola gene flow for the agro-ecosystem include contamination of seed lots, potentially more complex and costly control strategy, and limitations in cropping system design. Consequences for non-agricultural habitats may be minor but appear largely undocumented.

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**Keywords:** gene flow; transgenic crop; herbicide resistance; canola; oilseed rape; *Brassica napus*

## 1 INTRODUCTION

Agricultural crops that have been modified for herbicide resistance (HR) include: alfalfa (*Medicago sativa* L), canola (oilseed rape, *Brassica napus* L and *B rapa* L), corn (maize, *Zea mays* L), cotton (*Gossypium hirsutum* L), flax (linseed, *Linum usitatissimum* L), rice (*Oryza sativa* L), soybean (*Glycine max* (L) Merr), sunflower (*Helianthus annuus* L), sugarbeet (*Beta vulgaris* L), tobacco (*Nicotiana tabacum* L) and wheat (*Triticum aestivum* L). Many of these crops have resistance to the broad-spectrum herbicides glyphosate (alfalfa, canola, corn, cotton, soybean, sugar beet) and glufosinate (canola, corn, cotton, soybean, rice, sugar beet). Resistance to other herbicides include the ALS inhibitors (imidazolinones and sulfonyl ureas) (canola, cotton, flax, rice, wheat: all non-transgenic), bromoxynil (canola, cotton, tobacco) and sethoxydim (wheat). Some of these HR crops are currently grown on large areas whereas others have not yet been commercialized. Globally, in 2003, crops with an HR

trait were grown on nearly 50 M ha. In comparison, crops with insect resistance (Bt) were grown on 12 M ha, and crops with combined HR and Bt traits on approximately 6 M ha.<sup>1</sup> Transgenic crops (all traits combined) are currently grown mostly in USA (42.8 M ha), Argentina (13.9 M ha), Canada (4.4 M ha), Brazil (3.0 M ha) and China (2.8 M ha).<sup>1</sup> Of the 68 M ha of transgenic crops grown world-wide in 2003, soybean, corn, cotton and canola respectively captured 61, 23, 11 and 5% of the total area.

As they are being introduced, HR crops are readily adopted by producers. This success has been associated with improved weed control, ease of management, higher yields, lower input costs and greater net returns.<sup>2,3</sup> However, in spite of having been approved by regulators in many countries, questions remain as to the environmental impact of transgenic (GM) crops in general.<sup>4–6</sup> Issues include effects on non-target organisms, weediness of the GM crop within and outside the cropping system, and

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the likelihood and consequences of gene transfer by pollination to crops or feral species.<sup>7</sup> The nature and significance of gene flow from crops to wild relatives has been extensively reviewed.<sup>8–11</sup> Gene flow can be defined as the exchange of genes between different, usually related, populations through pollen transfer. Pollen flow is a subset of gene flow. I will use here a broad definition of gene flow which includes the notion of migration, ie the movement of an organism or group from one habitat or location to another, generally through seed dispersal.

The relative importance of issues related to gene flow will vary according to geographical location, presence of compatible wild or weedy relatives, biological characteristics of the crop and its relatives, and management practices. Most HR crops currently commercialized have wild or weedy relatives in all or parts of their range, suggesting the potential for gene flow.<sup>8</sup> The teosinte taxa, including wild or weedy relatives of corn, are found in Mexico and Guatemala.<sup>12</sup> Corn is compatible and can produce fertile hybrids with all teosinte species except *Zea perennis* (AS Hitchcock) Reeves & Manglesdorf.<sup>12</sup> Frequency of hybridization varies greatly with species, with *Z mays* ssp *mexicana* frequently forming hybrids with corn. However, introgression would only occur at low levels.<sup>12,13</sup> Soybean is predominantly selfing, with an outcrossing rate usually of less than 1%.<sup>14</sup> Soybean has wild relatives only in Asia (*Glycine soja* Steb & Zucc and *G gracilis* Skvortsov) with which it can produce fertile hybrids.<sup>14</sup> Cotton is normally self-pollinating. Natural crossing among cotton plants is generally low but may reach 50%.<sup>15</sup> Pollen grain dispersion in cotton depends nearly exclusively on insects, and thus will be affected by insecticide use. Canola (mainly *B napus* but also *B rapa*) has many wild and weedy relatives throughout its distribution range with which it can form fertile hybrids.<sup>16</sup>

This paper discusses HR gene flow using canola (*B napus*) as a case study. Canola is an ideal candidate, given that it satisfies many of the conditions essential to successful gene flow. Also, HR canola has been grown commercially for a number of years (since 1995 in Canada) such that certain consequences of gene flow have materialized or can be realistically estimated. I will first describe HR gene flow as it may occur with canola and then consider the consequences of HR gene flow, potential and realized, mainly in the context of the agro-ecosystem.

## 2 GENE FLOW THROUGH POLLEN

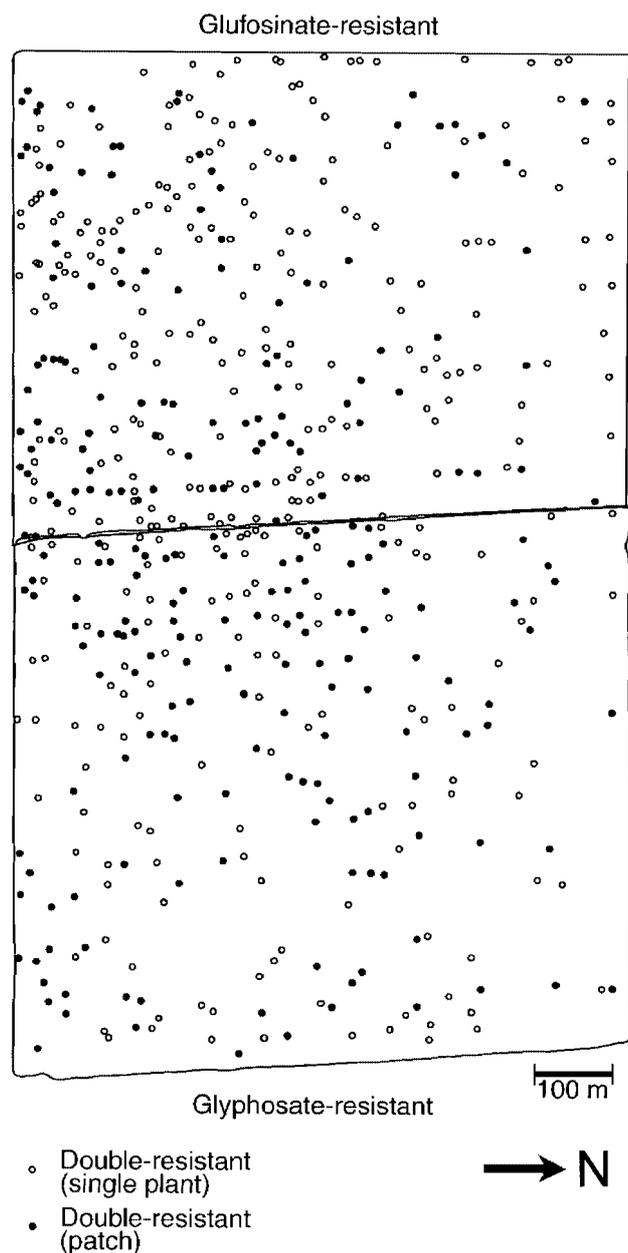
Gene flow and successful introgression of a transgene depend on sympatry of the HR crop with its relative(s), on hybridization rate, on the fitness of hybrids, on the advantages conferred by the HR gene(s) and other gene(s), and possibly on pleiotropic effects associated with these genes.<sup>10,17</sup> In turn, pollen flow is determined by relative plant density and synchrony

of flowering between donor and recipient populations, the quality, amount and viability of pollen produced, the means of pollen transport (generally wind or insect) and the compatibility of the species.

Canola is predominantly self-fertile but out-crossing can range from 12 to 55%.<sup>18,19</sup> Canola pollen flow can occur from one HR canola field to an adjacent canola field (HR or non-HR), and to canola volunteers and weedy relatives located in the same field or in field margins. Canola pollen was detected on traps 1.5 km from source fields in Scotland.<sup>20</sup> Pollen was still viable at distances of 1.5 and 2.5 km, since emasculated pollen receptor canola plants were able to produce normal seeds. Although pollen concentrations decreased with distance from the source, concentrations at 360 m were still 10% of that at the field margin. In Canada, Beckie *et al*<sup>21</sup> examined gene flow between paired glyphosate- and glufosinate-resistant canola fields over two years and found evidence of gene flow to the limits of the study areas (Fig 1). In the first year, outcrossing ranged from 1.4% at the common border to 0.04% at the field edge (400 m), and showed the expected leptokurtic distribution (Fig 2). In the following year, canola volunteers were assessed for evidence of gene flow, which was confirmed up to a distance of 800 m. Rieger *et al*<sup>22</sup> reported pollen flow up to 3 km from a field source in Australia. Although some fields did show a decline in gene flow with distance from the source, most showed similar levels of gene flow, as evidenced by the presence of ALS HR individuals, regardless of location in the field. An exhaustive summary of outcrossing distances and frequency values from canola gene flow studies is provided in Beckie *et al*.<sup>21</sup>

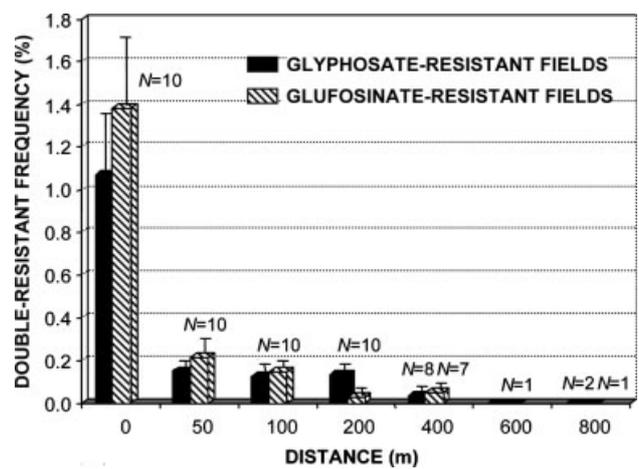
Canola may hybridize with many of its relatives, including *B rapa*, *Sinapis arvensis* L, *Erucastrum gallicum* (Willd) OE Schulz and *Raphanus raphanistrum* L.<sup>23</sup> Many of these species are found in sympatry with canola throughout the world. *Brassica rapa*, *S arvensis*, and *R raphanistrum* are obligate outcrossing species, whereas *E gallicum* is predominantly selfing. Recently, gene flow from canola to these relatives was assessed in greenhouse and/or field experiments and actual rates measured in commercial Canadian fields.<sup>16</sup> Overall, the probability of gene flow from canola to *R raphanistrum*, *S arvensis* or *E gallicum* was very low ( $<2-5 \times 10^{-5}$ ), and generally similar to that reported previously.<sup>24-30</sup> The one *R raphanistrum*  $\times$  *B napus* hybrid obtained was similar to that described in the study by Chèvre *et al*.<sup>16,24</sup> The F<sub>1</sub> hybrid was morphologically similar to *R raphanistrum*, was almost male-sterile (pollen viability: 0.12%), and did not set seed when selfed.<sup>16</sup> Hybridization rates between *R raphanistrum* and *B napus* reported in Australian ( $<4 \times 10^{-8}$ ) and French ( $10^{-7}-10^{-5}$ ) field studies were lower or similar to that reported in this Canadian study ( $3 \times 10^{-5}$ ).<sup>16,24,25</sup>

Hybridization between *B napus* and *B rapa* averaged 7% (range: 0–36%) in field experiments to 13.6% in commercial fields.<sup>16</sup> *Brassica napus*  $\times$  *B rapa*



**Figure 1.** The occurrence of confirmed double herbicide resistant volunteers as a result of gene flow at site 1 in Saskatchewan in 2000. The y (east–west) dimension is 1.6 times actual scale. Reproduced from Beckie *et al* (2003), by permission of the Ecological Society of America.<sup>21</sup>

F<sub>1</sub> hybrids were morphologically similar to *B rapa*, had reduced pollen viability (about 55%), and could be separated into self-incompatible and self-compatible individuals. Frequency of hybridization of *B napus* × *B rapa* in a Danish study ranged between 9 and 93%, according to the type of experimental design.<sup>31</sup> In another Danish study, estimation of hybridization frequency was apparently influenced by the less dormant hybrid seeds, the spatial structure of the parent populations, and the amount of overlap in flowering time.<sup>32</sup> Dormancy of hybrid seed was generally more like that of *B napus* (non-dormant), regardless of the direction of the interspecific cross,<sup>33</sup> but was also more strongly



**Figure 2.** Outcrossing ( $\pm 1$  SE) between adjacent glyphosate- and glufosinate-resistant *Brassica napus* canola fields in Saskatchewan in 1999, based on frequency of occurrence of confirmed double herbicide-resistant plants (glyphosate and glufosinate) as a function of distance from the common border. *N* = number of fields; when one value is shown per distance, it denotes the number of glyphosate- and glufosinate-resistant fields. Reproduced from Beckie *et al* (2003), by permission of the Ecological Society of America.<sup>21</sup>

responsive to maternal influence.<sup>34</sup> Introgression between *B rapa* and *B napus* populations, probably in co-existence for 12 years, was documented.<sup>35</sup> The distribution of AFLP markers in wild *B rapa* populations was similar to that of the control second backcross generation. Backcrossing would be more frequent than spontaneous hybridization, suggesting that interspecific hybridization could be the bottleneck in the introgression process. Fitness (estimate obtained from the multiplication of fitness component variables measured in pots grown in the field) of F<sub>1</sub> hybrids between *B rapa* and *B napus* would be intermediate to their parents but more fit than *B rapa*.<sup>36</sup> The lower fitness of F<sub>2</sub> and backcross offspring would probably slow down but not prevent introgression of genes/transgenes from canola into wild *B rapa* populations.<sup>37</sup> Introgression of glufosinate resistance from *B napus* into *B rapa* was shown to be of little fitness cost, as survival and seed production per plant were similar for HR and non-HR BC<sub>3</sub> plants.<sup>38</sup>

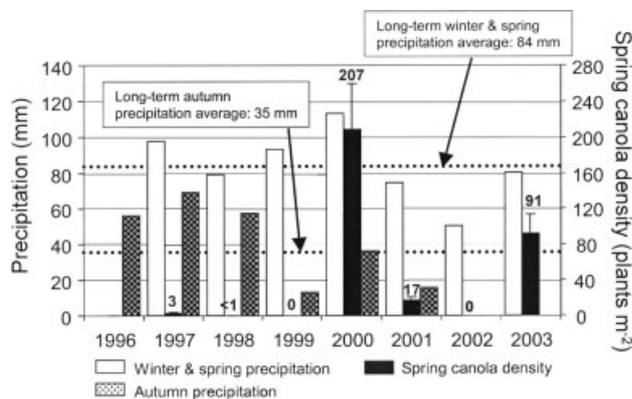
### 3 GENE FLOW THROUGH SEED

Canola gene flow can occur through the shattering of seed pods and dispersal of seeds which can either be shed locally or dispersed during harvest operations and subsequent seed transportation. The presence of a canola seed bank allows for the production of volunteers in subsequent years, which can serve as a source or bridge for pollen flow. Canola possesses characteristics that favour the formation and persistence of a seed bank, ie pod shattering and inducible secondary dormancy.

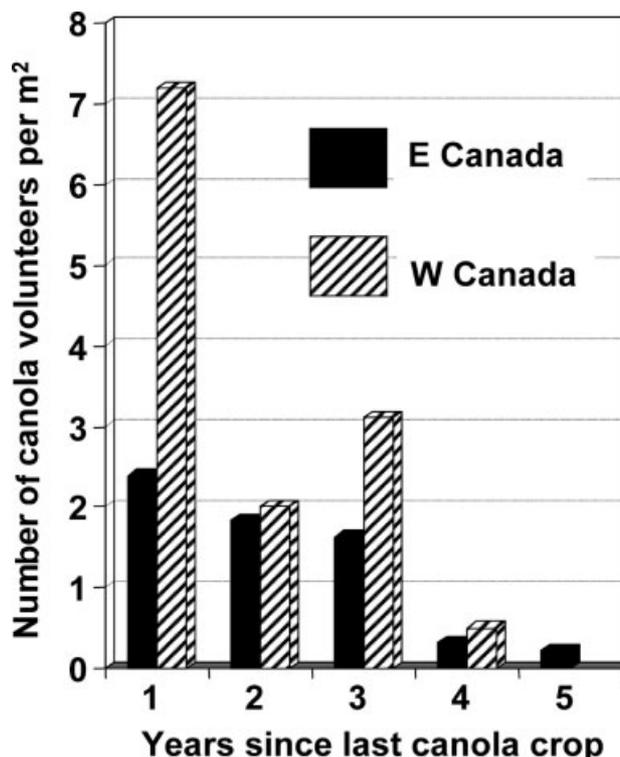
In a study of 35 fields, conducted over two years in Saskatchewan, Canada, additions to the seed bank from yield losses were estimated in the range of 3000 viable seeds m<sup>-2</sup>.<sup>39</sup> Yield losses

ranged from 9 to 56 times (average: 20) the normal canola seeding rate for the region (4–5 kg ha<sup>-1</sup>). Similar canola yield losses have been reported for the UK<sup>40</sup> and the USA.<sup>41,42</sup> The seeds being shed at harvest are not dormant but can be induced into secondary dormancy if germination conditions are sub-optimal.<sup>43</sup> Canola varieties differ in the ability of their seed to acquire secondary dormancy, which will affect their persistence in the seed bank.<sup>44</sup> High-dormancy genotypes exhibit greater seed bank persistence.<sup>39,44,45</sup> Still, total annual seedling recruitment (canola volunteers) seems less related to seed bank persistence than to environmental conditions.<sup>39</sup> Data from a cropping system field experiment in Saskatchewan, including treatments with various input and crop diversity levels, suggest that precipitation patterns in the period between cropping seasons would explain variability in volunteer canola seedling emergence (Thomas AG and Leeson JY, unpublished data).<sup>46</sup> When preceding autumn precipitation was high, few volunteer canola emerged in the spring (1997–1999), presumably because of high germination the previous autumn (Fig 3). When preceding autumn precipitation was low, volunteer canola densities in spring were high if sufficient moisture was available from winter and spring precipitation (2000, 2003), and low if insufficient moisture was present (2002). The year 2001 represents an intermediate situation, having average autumn precipitation and slightly less than average winter and spring soil moisture.

Persistence of canola volunteers was confirmed across agro-ecological regions of Canada.<sup>46</sup> Canola volunteers mainly originated from seeds germinating in the spring. A few canola volunteers (1.4%) overwintered in zero tillage cropping systems in eastern Canada.<sup>47</sup> The density of canola volunteers decreased with time since the last canola crop, but were still observed five and four years after canola production in eastern and western Canada, respectively (Fig 4). Volunteers initially originate from seed loss at harvest,



**Figure 3.** Autumn (September and October), and winter and spring precipitation and volunteer canola density (actual numbers shown above black bars) in early spring, prior to secondary tillage and all management inputs, in Scott, Saskatchewan. From Thomas AG and Leeson JY, unpublished data.



**Figure 4.** Average density of volunteer canola in producers' fields, in eastern and western Canada, according to time since the last canola crop. Density calculations include only fields where canola is present. Adapted from Légère *et al* (2001).<sup>46</sup>

but uncontrolled volunteers in subsequent crops may also contribute to the replenishment of the seed bank over the years. Canola also spreads outside fields during transport, spreading seed on roadsides, rights-of-way and railway verges.<sup>48</sup> Populations of volunteer canola have persisted five to eight years in semi-natural habitats such as road verges.<sup>47,49</sup>

#### 4 GENE FLOW IN HR CANOLA: RISKS AND POTENTIAL CONSEQUENCES

##### 4.1 Risks: if it can happen, it will

Data from the literature and recent experiments repeatedly confirm that genes and transgenes will flow and hybrids will form when the conditions described above are met. Overall, the risk of pollen flow from HR canola to wild/weedy relatives such as *S arvensis* L, *E gallicum* and *R raphanistrum* is quite low (probabilities nearing zero), whereas pollen flow across canola crop types, to canola volunteers and to *B rapa* would be inevitable. Canola seed loss at harvest is also a near-certainty. Canola seed dispersal ensures spatial gene flow whereas the persistence of a canola seed bank allows gene flow over time. Gene flow from HR canola has likely occurred where the crop is grown.<sup>21,22</sup> Gene flow between canola fields will be a matter of pollen competition and flowering synchrony, more than relative density, whereas gene flow to a wild relative will be affected by all three factors.<sup>50</sup> Gene flow from canola to wild relatives (particularly obligate outcrossers) will be favoured when the wild relative is

rare, ie when weed control was successful.<sup>29</sup> Inefficient weed control and patchy distribution of the wild relative could result in a lower chance of inter-specific hybridization as local weed densities will be higher and will favour cross-fertilization with conspecific pollen.<sup>29</sup> For example, hybridization rates of 0.023% versus 13.6% were observed in two field sites in Québec that differed greatly in the size of the *B rapa* population.<sup>16</sup>

#### 4.2 Consequences of gene flow: still to be fully understood

The potential for gene flow is quite well documented compared to the impact of such events. In particular, as outlined by Ellstrand,<sup>51</sup> there seems to have been little concern over the possible 'downsides of within-crop gene flow involving transgenic plants'. Consequences seem to have been considered more from a theoretical rather than an experimental point of view.<sup>9,52,53</sup> Still, some consequences of gene flow, mostly for the crop itself, are now being reported, as a result of increasing acreage in many areas of the world.

Consequences of gene flow for canola now include the presence of volunteers with stacked HR traits, and contamination (off-types or adventitious presence) of pedigreed seed lots. Only four years after the introduction of HR canola in western Canada, Hall *et al*<sup>54</sup> reported the presence of double HR (glyphosate–glufosinate, glyphosate–imazethapyr) and triple HR (glyphosate–imazethapyr–glufosinate) canola volunteers in a commercial field in Northern Alberta. Friesen *et al*<sup>55</sup> examined 27 unique commercial certified canola seed lots (33 samples: 18 conventional, 8 glufosinate-R and 7 imidazolinone-R cultivars) and confirmed HR contamination levels above 0.25% in 14 seed lots, and levels in excess of 2.0% in three seed lots (Canadian cultivar purity level: 99.75). Downey and Beckie (quoted in Beckie *et al*<sup>21</sup>) examined 70 seed lots from 14 herbicide susceptible open-pollinated cultivars and found the glyphosate-R gene in half of the lots, and either the glyphosate-R or glufosinate-R genes in 59% of the lots.<sup>21</sup> Only two lots were free of both genes. In addition, in an experiment examining gene flow between paired glufosinate-R and glyphosate-R fields, Beckie *et al*<sup>21</sup> uncovered the adventitious presence of double HR seed in the original glyphosate-R pedigreed seed lots. Clearly, producers often were not getting the quality of seeds that should be expected from pedigreed canola seed lots.

Gene stacking in canola volunteers in western Canada appears to be common, and is a direct consequence of pollen flow.<sup>21</sup> Canola plants with double-HR (glyphosate–glufosinate, glufosinate–imazethapyr) and triple-HR (glyphosate–imazethapyr–glufosinate) traits appear to be as fit as the single-HR cultivars.<sup>56,57</sup> However, canola with multiple-HR traits appear generally susceptible to the same herbicides that are used for the control of non-HR and single-HR canola.<sup>58,59</sup> The persistence of HR volunteers, whether with single and/or multiple traits, will influence cropping practices in

subsequent crops, particularly the choice of herbicides. Eventually, the presence of such volunteers may require weed-control methods other than herbicides, and may impose restrictions on the choice of crops in the rotation. Control of seedling and overwintering volunteers can be expected to be difficult, as the efficacy of herbicides will be curtailed by the early emergence and rapid growth of these plants, particularly in zero tillage systems.<sup>60</sup> Ultimately, contamination of a canola crop with multiple HR seeds may compromise its marketability.<sup>39</sup>

Rotations that include many HR crops with the same trait (eg glyphosate resistance) may result in dense stands of volunteers from various crops resistant to the same herbicide and thus jeopardize certain cropping systems. For example, elimination of glyphosate as a herbicide option for burndown (burn-off) could seriously compromise conservation tillage and thus the success of an environmentally beneficial crop-production system in the western Canadian Prairies.<sup>55</sup> By extension, the use of direct-seeding no-till practices could also be compromised in corn-soybean rotations using cultivars with the same HR trait, again contributing to the complexity of weed management over time. In addition to gene flow problems, the repeated use of crops with the same HR trait may also result in the selection of resistant weed biotypes and in the build-up of their populations. This is particularly critical for the ALS resistance trait, as resistance to ALS inhibitors occurs in 83 weed species globally.<sup>61</sup> This could be of some concern for western Canada, as 25% of HR canola area is imidazolinone-resistant. This issue may also become of concern for glyphosate, as resistance to this herbicide has now been found in six weed species.<sup>61</sup> The recent increasing number of reports of resistance to this herbicide and the issue of glyphosate-R volunteers should force reconsideration of the use patterns of this herbicide and its resistant crops.

Ultimately, the presence of an HR field and the impending gene flow may affect the crop management options of neighbouring fields. This may have economic and sociological impacts, particularly if the adjacent field belongs to an organic grower or to a producer wishing to produce a GM-free crop for specialized markets. He/she will be denied freedom of choice as crop selection will need to take into consideration the presence of the HR crop in the adjacent field. As a result, it may force the inclusion of physical barriers in the hope of curtailing gene flow.<sup>62</sup> Although a gene flow problem may in some ways be analogous to herbicide drift, it is not treated as such legally. However, we can expect liability aspects concerning gene flow to evolve as case problems are brought forward. As a case in point, the state legislature of Vermont has voted a bill that holds corporations accountable for contamination by genetically engineered crops.<sup>63</sup>

Contamination of seed lots, the presence of volunteers and seed banks with single or multiple

HR traits, of HR weed biotypes, and weed species shifts affect crop and weed management options, not to mention crop yield losses. The above constraints eventually have financial impact on crop production. Still, it seems so far that none of these constraints has been a major impediment to the adoption of the HR canola currently available on the market.

Beyond impacts on the agricultural system, HR gene flow could have ecological consequences in non-agricultural habitats,<sup>64</sup> which to my knowledge have still not been thoroughly assessed. The apparent lack of concern may be due to the nature of the HR trait, which, in theory, only confers an advantage to its carrier when the herbicide is being used. This advantage will be restricted to managed environments where herbicides are used, mostly crop fields. However, canola volunteers along with wild or weedy relatives can be found in other habitats, generally at the fringe of agricultural fields or along roadsides, where herbicides can be used occasionally. In a study of canola populations on roadside verges in the UK, Crawley and Brown<sup>48</sup> found no evidence that adjacent arable fields were a source of introduced canola, but that roadside populations originated from spillage from trucks transporting seed to the crushing plant. There was a substantial turnover in site occupancy, and only 22% of the quadrats supported canola in both years of the study. Populations would be extinct within three years in the absence of soil disturbance. Crawley *et al*<sup>65,66</sup> also examined the likelihood of HR canola becoming invasive in 10 different natural habitats over 10 years and found no evidence of increased potential of invasiveness, and noted that HR lines were at times less invasive and persistent than conventional lines. Gene flow, if it is to occur in natural habitats, must rely on the transient presence of HR volunteers and the presence of compatible relatives in the vicinity. In the UK, a national assessment of hybridization between canola and *B. rapa* ecotypes estimated that 32 000 hybrids would form annually in waterside *B. rapa* (populations occupying semi-natural communities alongside waterways: rivers and canals) compared with 17 000 hybrids with weedy *B. rapa* (populations infesting agricultural fields).<sup>67</sup> The authors cautioned that the hybrids with the riverside *B. rapa* ecotype could potentially cause complex ecological changes, although this appears to remain undocumented.

## 5 GENE FLOW OF AN HR TRAIT: A CASE FOR CAUTION

In spite of the fact that the HR trait can be considered of little ecological significance in natural habitats,<sup>68</sup> the current situation with a crop like canola, that is commercialized on a large scale, illustrates a number of agro-ecological issues. Although the risk of gene flow to weedy relatives exists throughout the crop's range, it is the risk of gene flow between canola varieties, seed contamination and the potential persistence of

HR canola volunteers that is of immediate concern, as this has severe consequences for weed management programs and for the marketability of the crop.

From an environmental standpoint, the HR trait is only advantageous when and where the herbicide is used. However, other agronomic genes passed along with the HR transgene may determine the success or failure of the introgression of the HR transgene in a wild population. Characters such as lack of dormancy, reduced shattering, uniform maturation and high oil content (potentially linked to increased seed susceptibility to soil pathogens and seed predators)<sup>53</sup> would be detrimental to wild populations and the persistence of the HR gene. However, the deleterious crop gene would need to be linked to the transgene for it to have any major effect on its persistence. A deleterious gene not linked to the transgene could be purged from the weedy/wild population, with the transgene still being passed down generations. Conversely, a trait like selfing, as observed in a *B. napus* × *B. rapa* hybrid,<sup>16</sup> would likely favour the establishment and persistence of the transgene in the crop/weed hybrid swarm or population, and the successful introgression of a transgene, whether HR or other.

Studies of HR gene flow have provided background information that should be useful in the assessment of novel traits that may have more disruptive effects. In the absence of the herbicide, HR is quite a neutral trait when compared with salt or drought tolerance, insect or disease resistance.<sup>69</sup> Such fitness-enhancing traits may have more serious effects in terms of invasiveness and ecological impact. However, gene flow of an HR or any other GM trait may eventually become a non-issue as biotechnology provides new tools to limit transgene flow, for example by modifying proteins on the surface of pollen grains to prevent fertilization of weedy or crop relatives,<sup>70</sup> or by introducing seed lethal genes.<sup>71</sup>

In summary, gene/transgene flow between canola varieties (HR and non-HR), between the crop and its volunteers, and with its relative, *B. rapa*, is currently inevitable. Spatial proximity is a relative issue as evidence of hybridization has been found several kilometres from the pollen source throughout the crop's range. HR gene flow results in contamination of canola seed lots. Gene flow problems in canola are exacerbated by seed losses at harvest, the possible induction of secondary seed dormancy, and the formation of persistent seed banks. Volunteers with single and multiple HR traits, potentially having similar fitness, may create management problems in the current and subsequent crops. Consequences of HR gene flow beyond the agro-ecosystem still need to be fully documented. Although successful introgression has been shown in *B. rapa* populations, the impact on population and community dynamics in natural habitats is still largely unknown.

Issues related to HR gene flow in canola may apply to other crops that may satisfy similar conditions such as: outcrossing, seed shattering, seed dormancy,

persistent seed banks, the occurrence of volunteers and sympatric compatible relatives. Gene flow problems comparable with those observed with canola are likely for crops like sugar beet,<sup>72</sup> sunflower<sup>69</sup> and rice,<sup>73</sup> species which are found also in major weedy/wild forms. In addition, there is cause for concern for crops like wheat which have weedy relatives in parts of their range, and with which successful hybridization and introgression are possible.<sup>74</sup> The case has repeatedly been made that the evaluation of the agro-ecological impact of crops with novel traits should be specific to the trait (rather than the means of trait introduction into the crop), the crop and the environment(s) where it is likely to be found.<sup>6,75</sup> Risk evaluation cannot be definitive, given the rapidity at which new novel traits are being introduced, the consequent changes in cropping practices and, particularly, the unpredictable nature of biological systems.

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