Herbicide-Resistant (HR) Crops in Canada: HR Gene Effects on Yield Performance
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Summary
Herbicide-resistant (HR) crops have been genetically modified through gene insertion or mutation to resist a specific herbicide or herbicide class. Since the first HR crop, triazine-HR canola, was introduced in Canada in 1981, several HR crops are now commonly grown. In this paper, the effect of the HR gene(s) on cultivar performance, primarily yield, is reviewed. Although some of the earliest-introduced HR crop cultivars suffered a yield penalty, currently grown cultivars often have similar or better yield performance than non-HR cultivars because of intensive breeding efforts by the private sector.

Introduction
The major field crops grown in Canada are tolerant to many registered herbicides due to natural tolerance, for example, lack of wheat injury to 2,4-D. The primary mechanism for this tolerance is the ability of the crop to rapidly metabolize the herbicidal active ingredient. Additionally, herbicide tolerance by a crop may be enhanced by the addition of a safener to the herbicide formulation. This tolerance is in contrast to herbicide-resistant (HR) crops, which have been genetically modified through gene insertion or mutation to resist a specific herbicide or herbicide chemical class. The first HR crop available to farmers in Canada was triazine-HR canola in 1981. The gene responsible for triazine resistance was incorporated into canola lines through conventional breeding, i.e., crosses with the HR weed biotype, triazine-resistant bird’s rape (B. rapa L.)23. The cultivation of triazine-HR canola was cost-effective in areas where competition was high from cruciferous weeds, such as wild mustard (Sinapis arvensis L.) and stinkweed (Thlaspi arvense L.). However, the trait imposed a fitness penalty that caused lower productivity. Yields of triazine-HR cultivars were 20 to 30% less than conventional canola because of reduced photosynthetic efficiency4. Cultivation of triazine-HR canola peaked at 4% of the total canola area in 1988, and decreased to less than 1% by 19951. In contrast, triazine-HR canola is still widely grown in Australia despite the fitness penalty (reportedly lower than that indicated above) to control problem weeds such as wild radish (Raphanus raphanistrum L.).

Beginning in 1995, HR crops commonly grown today were introduced commercially in Canada5. These crops were resistant to bromoxynil (2000 and 2001 only), glyphosate (Roundup Ready® system), glufosinate (Liberty Link® system), or imidazolinone herbicides (Clearfield® system). Only the latter system is non-transgenic (non-GM). The adoption rate of many of these HR crops has been rapid, especially glyphosate-HR canola, soybean, and corn5.
Table 1. Major herbicide-resistant field crops grown in Canada

<table>
<thead>
<tr>
<th>Species resistance</th>
<th>Herbicide registration</th>
<th>Variety approval</th>
<th>Regulatory system</th>
<th>Breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canola, <em>Brassica napus</em> Imidazolinone</td>
<td>Glyphosate</td>
<td>Yes</td>
<td>Yes</td>
<td>ca. 30% outcrossing</td>
</tr>
<tr>
<td>Soybean, <em>Glycine max</em></td>
<td>Glyphosate</td>
<td>Yes</td>
<td>Yes</td>
<td>Highly selfing</td>
</tr>
<tr>
<td>Corn, <em>Zea mays</em></td>
<td>Glyphosate</td>
<td>NA</td>
<td>Yes</td>
<td>Highly outcrossing</td>
</tr>
<tr>
<td>Wheat, <em>Triticum aestivum</em></td>
<td>Imidazolinone</td>
<td>Yes</td>
<td>Yes</td>
<td>Highly selfing</td>
</tr>
<tr>
<td>Lentil, <em>Lens culinaris</em></td>
<td>Imidazolinone</td>
<td>Yes</td>
<td>Yes</td>
<td>Highly selfing</td>
</tr>
<tr>
<td>Sunflower, <em>Helianthus annuus</em></td>
<td>Imidazolinone</td>
<td>Yes</td>
<td>Yes</td>
<td>Highly outcrossing</td>
</tr>
</tbody>
</table>


NA: not required.

In 2011 in Canada, glyphosate-HR canola, soybean, and corn comprised 47, 72, and 90% of the respective crop area (R. Ripley, B. Senft, M. Reidy, personal communication). However, market share of glufosinate- and glyphosate-HR canola is similar. Western Canada accounts for 99% (8.5 million ha) of the nation’s canola area, 20% of soybean area (344,000 ha), and 9% of grain corn area (122,000 ha). Amongst Clearfield crops, lentil has the highest adoption rate because of the weed control benefits (F. Holm, personal communication).

In the following section, I will briefly summarize the effect of the inserted or mutated HR gene(s) on cultivar performance, primarily yield. This analysis, however, is a moving target as breeding efforts are continually improving the agronomic performance of HR crop cultivars. Therefore, the analysis is very much retrospective in nature. Some of the information listed below is derived from an excellent review by Darmency.

**Herbicide-Resistant Genes: Effect on Crop Yield**

**Clearfield Crops**

Imidazolinone (IMI) herbicides, such as imazamox (Solo®), are inhibitors of acetolactate synthase (ALS; Group 2). Most of the IMI-HR crop cultivars have single or multiple dominant or semi-dominant mutations of *ALS* genes. These include the Ser653Asn amino acid substitution (i.e., mutation) in canola, wheat, corn, and rice; Ala205Val mutation in sunflower; and Trp574Leu mutation in canola and corn. In studies in the United States and Brazil, there was no difference in seed production between IMI-HR and non-HR (susceptible) sunflower cultivars. Similarly, field trials showed that yield of IMI-HR canola was equal to or even greater than their isogenic (i.e., same genetic background) parents. A study by Pozniak et al. did not find any effect of the Ser653Asn mutation on yield of IMI-HR wheat; lines of this HR crop possess two genes needed to confer...
resistance to IMI herbicides. IMI-HR wheat may be grown after barley to control volunteers; HR cultivars have similar development and agronomic characteristics as non-HR cultivars within the wheat class (Figure 1).

The lack of a yield penalty in IMI-HR crops parallels the general lack of a fitness penalty in ALS-HR weeds. There are over 20 amino acid substitutions at eight sites of the ALS gene conferring resistance to Group 2 herbicides in weedy species. It is unfortunate that all mutations found in weeds can potentially confer resistance to IMI herbicides. Group 2-HR weeds are widespread across the prairies. Therefore, it is important that Group 2 herbicides are applied in a mixture, sequence, or rotation with a herbicide(s) with a different mode of action (herbicide group) to delay the evolution of Group 2 resistance in weeds – which can directly reduce crop yield.

**Figure 1.** A field of imidazoline (IMI) herbicide-resistant (HR) (Clearfield®) and non-HR spring wheat in central Saskatchewan (photo by H. Beckie).

**Liberty-Link Crops**

Glufosinate-HR cultivars have been developed by genetic engineering with insertion of the *pat* (or *bar*) gene derived from *Streptomyces* sp. that encodes an acetyl transferase that metabolizes the herbicide into non-toxic metabolites. There was no significant yield difference between experimental lines of HR and non-HR flax in Saskatchewan. Similarly, no yield difference was observed between glufosinate-HR and check/control cultivars of canola in a fitness study conducted on non-arable land in the United Kingdom. Another study found no difference in biomass production between glufosinate-HR and control canola lines under varying light and plant competition conditions. Paired comparisons of glufosinate-HR and non-HR lines in a field study in Canada determined that one-third of the glufosinate-HR lines had the same characteristics as their non-transformed counterpart, but several lines had 20 to 30% reduced yield and modified lipid and protein contents. However, plant breeders routinely examine lines from a number of transformation events, and then advance the most promising ones. Today, glufosinate-HR canola cultivars are amongst the highest yielding due to intensive breeding efforts in the past two decades. In contrast to the dominance of glyphosate- vs. glufosinate-HR cultivar hectarage of corn and soybean in Canada, glufosinate-HR canola area is similar to that of glyphosate-HR canola (Figure 2).
Glufosinate is usually tank-mixed with a Group 1 herbicide, such as clethodim, to enhance grass weed control, particularly that of wild oat (*Avena fatua* L.)\(^{16}\). Therefore, those growers presumably find that glufosinate applied alone does not consistently provide acceptable wild oat control and crop yield protection. Applied postemergence in glufosinate-HR crops, glufosinate is considered a key herbicide in proactively and reactively managing glyphosate-HR weed populations (reviewed in Beckie\(^{21}\)). Therefore, the potential for glufosinate-HR crop cultivars to manage HR weed biotypes, especially those that are glyphosate-HR, will depend upon the future incidence of weed resistance to this herbicide group. To date, only two weed species worldwide have been confirmed resistant to glufosinate\(^{22}\).

Figure 2. A field of glufosinate herbicide-resistant (HR) (Liberty Link®) canola adjacent to a field of glyphosate-HR resistant (Roundup Ready®) canola in Saskatchewan (photo by H. Beckie).

**Roundup-Ready Crops**

Glyphosate inhibits enolpyruvylshikimate-3-phosphate synthase (EPSPS). Most of the glyphosate-HR crops possess the *CP4* gene derived from an *Agrobacterium*. Early studies with soybean showed a yield loss associated with glyphosate-HR lines\(^{5,23,24}\). Gertz et al.\(^{25}\) compared several non-isogenic cultivars and noted reduced growth and rate of lignin production at high temperatures. Field trials in the United States found 5% lower yield in glyphosate-HR vs. non-HR lines\(^{23}\). Shaw et al.\(^{26}\) found a 17 to 58% yield reduction of HR cultivars vs. non-isogenic control cultivars when plants were grown under weed competition without herbicide application; however, there was no yield difference observed under herbicide application. In addition to excellent weed control, crop injury to glyphosate may be less than some other herbicides\(^{27}\). More recent studies show little yield difference between glyphosate-HR and reference soybean lines or cultivars, although subtle differences in quality-related traits may be observed\(^{28-30}\).

In corn, the *CP4* gene and a mutant *EPSPS* both confer glyphosate resistance. Studies showed both differences\(^{27,31}\) and lack of differences\(^{5}\) among different cultivars, with the contradictory results explained by genotype by environment interactions. Cox et al.\(^{32}\) concluded that the *EPSPS* gene was not primarily responsible for any yield differences among stacked-trait corn cultivars.
Glyphosate resistance in canola is conferred by the \textit{CP4} gene as well as the glyphosate oxidoreductase (\textit{GOX}) gene, which increases the metabolism of glyphosate. Studies involving glyphosate-HR canola under weed competition showed a slight yield penalty of transformed (glyphosate-HR) vs. nontransformed isogenic lines (reviewed in Stanton et al.\textsuperscript{33}), but no yield difference has been noted in commercially-released cultivars\textsuperscript{5} (Figure 2). However, in field trials, some differences in seed quality were sometimes noted\textsuperscript{34}. From 1974 to 1995 in Canada, glyphosate was commonly applied preseeding (burndown treatment), preharvest (primarily in cereals and pulses), or to a lesser extent, postharvest. With the introduction of glyphosate-HR crops beginning in 1996, glyphosate usage increased markedly\textsuperscript{3}. A majority of glyphosate-HR canola growers apply glyphosate twice in-crop\textsuperscript{16}. During the 7-yr period from 2005 to 2011, glyphosate usage tripled from 30.2 to 89.7 million L (standardized to 360 g ae L\textsuperscript{-1}) in western Canada, and from 3.8 to 12.3 million L in eastern Canada\textsuperscript{35}. In Canada, the first report of a glyphosate-HR weed was giant ragweed (\textit{Ambrosia trifida} L.) in 2008 in glyphosate-HR soybean in eastern Canada (southwestern Ontario); a survey conducted in 2009 and 2010 documented the glyphosate-HR weed in 47 new locations in three counties in the province\textsuperscript{36}. In southwestern Ontario, glyphosate-HR Canada fleabane (horseweed) and glyphosate-HR common ragweed (\textit{Ambrosia artemisiifolia} L.) were first documented in 2010 and 2011, respectively\textsuperscript{22}. To date, there are 71 sites (populations) with confirmed glyphosate-HR giant ragweed in six southwestern Ontario counties, 84 sites with glyphosate-HR Canada fleabane in five counties, and one site with glyphosate-HR common ragweed\textsuperscript{37}. In the area where glyphosate-HR giant ragweed or Canada fleabane are found, there tends to be a very high percentage of glyphosate-HR soybean fields. For example, some growers will have one to four crops of soybean, followed by winter wheat, then back to soybean. However, some of the confirmed glyphosate-HR Canada fleabane sites had a very diverse crop and herbicide rotation, e.g., corn-soybean-wheat-processing tomato-sweet corn, suggesting weed seed dispersal as a contributing factor in occurrence of glyphosate-HR weed populations. In western Canada, glyphosate-HR kochia (\textit{Kochia scoparia} (L.) Schrad.) was confirmed in a number of sites in Alberta and Saskatchewan\textsuperscript{35}. These sites were chem-fallowed or cereal-cropped fields, although glyphosate-HR canola was previously grown at some of the Saskatchewan sites.

**Conclusions**

To level the playing field, field crop varietal trials today compare HR cultivars with their respective registered herbicide(s), e.g., glyphosate-HR cultivars sprayed with glyphosate, glufosinate-HR cultivars sprayed with glufosinate, etc. – in contrast to the same herbicide regime applied across all cultivars\textsuperscript{38,39}. ‘Technology lag’ is a term used to describe a potential short-term yield drag as a result of foreign gene insertion into a genome (transgenic cultivars; e.g., pleiotropic effects) or as a result of gene mutation through chemical mutagenesis (non-transgenic cultivars). That phenomenon was observed in some early-released soybean cultivars. Nevertheless, breeders were able to rapidly develop better performing lines of this and other HR crops for yield and other agronomic traits, as well as better adapted lines for cultivation in different geographical regions having different climates\textsuperscript{40}. Today, HR crop cultivars are often higher yielding than non-HR cultivars because of intensive breeding efforts by the private sector. For some HR crops such as canola, there are few to nil commercially available non-HR cultivars. The introduction of cultivars with stacked pesticide-resistance traits, such as many corn hybrids, makes it increasingly difficult to assess the performance impact of a single gene or trait. The effect of the HR gene may depend upon unrelated traits\textsuperscript{41}. In any event, farmers do not consider HR traits in isolation from their weed control programs and cropping systems. Farmers will preferentially grow an HR cultivar rather than a non-HR cultivar of a crop if the profit margin (net revenue) is significantly greater, with higher yields due to greater yield potential, better weed control, or both. It will be interesting to track the future adoption of crops with stacked HR traits, such as glyphosate + dicamba-HR soybean, or glyphosate + glufosinate-HR corn. Adoption will likely be driven by the need to effectively and efficiently manage HR weeds. Although the HR gene(s) in today’s HR crop cultivars may have no inherent adverse effect on yield, the increasing incidence of HR weeds selected in these crops will likely substantially impact yield and profitability through increased weed competition and herbicide use.
References


