

## ENVIRONMENTAL IMPACT OF HERBICIDE TOLERANT (HT) CROPS – THE CANADIAN EXPERIENCE

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**Abstract:** Herbicide tolerant crops have been available commercially for ten years in Canada. To date, these crops have been well adopted and more herbicide tolerant crops continue to become commercialized. Intense use of this technology may have environmental impacts such as intra- and inter-specific gene flow, require alternative control of volunteers, and result in changes to the weed community and non-target soil organisms. The detection of herbicide gene stacking in crop volunteers shortly after the commercial release of herbicide tolerant crops has provided the impetus for further investigation.

**Key words:** Canada, canola, corn, herbicide tolerant, non-target species, outcrossing, soybean, weed community

### Current status of herbicide tolerant cropping systems in Canada

In Canada, herbicide tolerant (HT) crops have been available commercially for ten years now. This technology, particularly crops tolerant to the non-selective herbicide glyphosate, has increased weed control options (e.g. management of resistant weeds), increased the duration in which a herbicide can be applied safely to the crop, and facilitated reduced-tillage production systems. These benefits have led to high rates of adoption of this technology in canola (*Brassica napus* L.) and soybean (*Glycine max* L. Merr.) with moderate, but increasing adoption in corn (*Zea mais* L.). Other herbicide tolerant crops have been commercialized recently including genetically-engineered glyphosate tolerant alfalfa (*Medicago sativa* L.) and imidazolinone tolerant wheat (*Triticum aestivum* L.) and lentils (*Lens esculentum* L.). Nevertheless, the most significant herbicide tolerant crops by acreage and proportion are canola in western Canada and soybean and corn in eastern Canada. In 2002, greater than 80% of the annual acreage of canola (about 6 million ha) was planted to herbicide tolerant genotypes with about 50% of the total acreage tolerant to glyphosate, 15% tolerant to glufosinate, and 20% tolerant to imidazolinones (non-genetically engineered) (Warwick et al. 2003). Of the total soybean acreage about 80% was tolerant to glyphosate, the only commercially available herbicide-tolerance system in soybean in Canada (Anonymous 2004). In corn, about 12% of the total annual acreage was seeded to glyphosate tolerant and about 8% of the total annual acreage was seeded to glufosinate tolerant genotypes (Anonymous 2004).

With the advent and commercialization of these crops have come a series of concerns from the public in Canada and abroad regarding the impacts of these cropping systems. The detection of pollen-mediated gene flow in canola shortly after the introduction of herbicide tolerant genotypes provided the impetus to study some of the potential impacts of transgenic crops on the environment. Areas that have and continue to receive attention in Canada include: intra- and inter-specific outcrossing, introgression of genes, effects on target weed species, and the effects on non-target soil organisms. This manuscript summarizes the key findings and currently ongoing research in these areas in Canada.

### Movement and introgression of HT genes

#### *Intra-specific gene movement*

Intra-specific stacking of herbicide tolerant genes through pollen mediated gene flow was first observed in commercial fields in 1998, three years after the commercial release (Hall et al. 2000). In this instance, imidazolinone tolerant and glufosinate tolerant genotypes were grown side-by-side in a field (# 1) with a glyphosate tolerant genotype growing in an adjacent field (# 2). Interplant outcrossing is typically high in canola and averages around 30% (Rakow and Woods 1987). Lack of control of volunteers by glyphosate in field # 1 indicated pollen mediated gene flow, as a glyphosate tolerant genotype had never been grown in that field. In 1998, further pollen mediated gene-flow occurred from an

imidazolinone tolerant genotype planted in field # 2 to the uncontrolled volunteers growing in field # 1. In the autumn of 1998, triple resistance was confirmed in seeds from the volunteer plants in field # 2 that survived treatment with glyphosate. Using herbicide tolerance as an easily identifiable marker, further investigations into intraspecific pollen mediated gene flow in commercial fields indicated that the patterns of gene flow among adjacent fields varied in distance and space (Beckie et al. 2003). Average outcrossing rates between adjacent fields were 1.4% at the common border and 0.04% at 400m distance, although gene flow up to the study limit of 800m was detected in some cases. The spatial pattern of pollen mediated gene flow also varied among study sites. At some locations double resistant volunteers were concentrated near the common border, while at other locations, double resistant volunteer plants were distributed in a more uniform pattern throughout one or both of the adjacent fields. Molecular analysis of plants from this study indicated the adventitious presence of double resistant seeds in the certified seedlots that were planted in these fields. This prompted further studies into seedlot contamination in canola. A number of pedigreed seedlots of guaranteed quality were examined for adventitious presence of seeds containing tolerance to commercially available herbicide tolerance traits other than those specified. Of 27 separate seedlots, 52% exhibited adventitious presence of herbicide tolerance traits above the 0.25% contamination limit (Friesen et al. 2003), while many others showed lower levels of contamination. Thus, producers should be aware that unwanted traits can also be introduced at the time of planting, even when using high quality seed.

In addition to pollen mediated gene dispersal over great distances, genes from canola can also disperse over time through a persistent seedbank. In western Canada, canola typically is windrowed to accelerate and homogenize seed maturation and minimize seed losses due to seed shatter. Despite this practice, average canola seed losses at harvest were 3,000 seeds m<sup>-2</sup> (about 5% of total yield), but can be greater than 10% of total yield (Gulden et al. 2003a). This estimated seed loss value is considered high for a domesticated species. Seed persistence (up to 3 or more years) was influenced by seed burial through cultivation (Gulden et al. 2003b, 2004a) and by a genotype's potential for the development of secondary seed dormancy under unfavourable conditions (Gulden et al. 2003b). Many commercially available genotypes, including those tolerant to herbicides have a high potential for the development of secondary seed dormancy (Gulden et al. 2004b). Cold winters in western Canada preclude the survival of individual canola plants for more than one season. In contrast, autumn germinated seedlings and intact root stocks from harvested plants that were allowed to grow new leaves in the autumn survived the more mild winters in eastern Canada and gave rise to volunteer populations the following season (Simard et al. 2002). Intraspecific gene flow by any of these mechanisms may influence control options of volunteer populations expressing unexpected herbicide tolerance traits. Fitness studies in canola have shown that there was no distinct fitness penalty associated with multiple herbicide tolerance compared to tolerance to single herbicides (Simard et al. 2005). In addition, alternative herbicides provided excellent control of volunteer canola plants with tolerance to multiple herbicides (Beckie et al. 2004).

#### ***Interspecific gene movement***

Four common agricultural weeds are closely related to canola in Canada. They are wild mustard (*Brassica kaber* (DC.) L.C. Wheeler), dog mustard (*Erucastrum gallicum* (Willd.) O.E. Schulz), birdrape mustard (*Brassica rapa* L.), and wild radish (*Raphanus raphanistrum* L.). Among these, wild mustard is the most closely related to canola and as a result outcrossing frequencies may be as high as 7 to 14% between these two species (Warwick et al. 2003). The other three species are more distantly related to canola and outcrossing frequencies in the field have been estimated to be lower than  $5 \times 10^{-5}$ . Wild radish is only present in eastern Canada where canola is only a minor crop. However, flowering periodicity of canola and wild radish was sufficiently long that overlap of flowering cannot be avoided by altering the planting dates of the canola crop (Simard and Legere 2004). Research examining the introgression and stability of traits moving from canola to weedy relatives is currently underway.

Another possible, but less well understood mechanism for gene escape from crops with novel traits (e.g. HT) is through transfer of genetic material to soil micro organisms. It is well recognized that certain soil bacteria are able to take up and incorporate foreign DNA into their own genome under certain circumstances (de Vries and Wackernagel 2004). When this involves naked DNA from plants or other organisms, the process is known as natural transformation. Plant DNA, including transgenes responsible for herbicide tolerance and antibiotic resistance which are often part of gene cassettes inserted into plants, may enter the soil throughout the life cycle of a plant. Root cells contribute DNA during the vegetative portion of their life cycle, pollen enters the soil during anthesis, and residue and volunteer seeds may also contribute DNA to the soil DNA pool. Recently, Gulden et al. (2005) showed that DNA enters the soil during the vegetative and early reproductive phases of corn and soybean growth and readily moves with leachate water. The majority of plant DNA in the soil (unpublished data) and water (Gulden et al. 2005) tended to degrade relatively quickly (weeks) through enzymatic degradation by nucleases released from bacteria (Blum et al. 1997). Nevertheless, free DNA may persist in soil for some time by binding to the soil matrix where this DNA is protected from degradation, but not from natural transformation (Romanowski et al. 1993). In eastern Canada, we are currently investigating the movement and persistence of plant DNA in soil and water, the factors affecting persistence of plant DNA in fields, and rates of natural transformation of soil bacteria with recombinant genes conferring tolerance to glyphosate in corn and soybean cropping systems.

### **Effect of technology on target species**

Weeds adapt quickly to changes in cropping systems and it is known that repeated use of the same technology for weed control influences weed community structure by selecting for and against certain species. Thomas et al. (2004) compared the weed community composition in western Canada from large scale on-farm weed surveys before and about 10 years after the introduction of herbicide tolerant canola, the only available herbicide tolerant crop at the time. In western Canada, canola is generally grown once in a 3 or 4 year rotation. Residual weed analysis compared to wheat over this time frame showed that climatic and other factors more strongly affected weed community composition than did the use of HT canola during the same period. Long-term studies examining similar effects in HT corn / HT soybean rotations in eastern Canada are being conducted to examine the effects of this technology on target species composition.

### **Effect of technology on non-target soil organisms**

Unique soil microbial communities are associated with the rhizosphere of crop plants. In western Canada, Dunfield and Germida (2001) investigated the soil microbial communities associated with the rhizosphere of several transgenic herbicide tolerant and conventional canola cultivars. The research showed unique rhizosphere microbial communities associated with genotypes that were different among transgenic HT and conventional genotypes. Moreover, microbial community structure varied between field sites and interacted among field sites and genotypes. A different study by the same authors found that the microbial community changed throughout the growing season (Dunfield and Germida 2003). Similar studies are currently also underway in eastern Canada in glyphosate tolerant corn and soybean cropping systems. These studies are investigating the effects of herbicide tolerant cropping systems on the soil food web, including bacteria, fungi, collembolans, protists, nematodes, and mites. Results from these studies will be forthcoming in the near future. These organisms perform many important soil functions including litter degradation, soil stabilization, and nutrient cycling. The biological and ecological significance of these differences in microbial community structure are not known and are currently under investigation.

In conclusion, the high efficacy and flexibility of herbicide tolerant cropping systems has led to high adoption rates of this technology in Canada over the past decade. However, these cropping systems may have impacts on target and non-target species within a

cropping system. Unexpected volunteer plants may require the use of different herbicides for effective control. In addition, genes may outcross into related weedy species or be taken up by bacteria in soil. More research investigating the long-term environmental effects of this technology is required, particularly as the adoption of HT cropping systems continues to increase.

## BIBLIOGRAPHY

- Anonymous. 2004. Ontario field crops research and service committee annual report. [Online] <http://www.uoquelfh.ca/research/omafra/forms/oascc.shtml>. Accessed March 31, 2006.
- Beckie, H.J., Seguin-Swartz, G., Nair, H., Warwick, S.I., Johnson, E. 2004. Multiple herbicide-resistant canola can be controlled by alternative herbicides. *Weed Sci.* 52:152-157.
- Beckie, H.J., Warwick, S.I., Harikumar, N., and Seguin-Swartz, G. 2003. Gene flow in commercial fields of herbicide resistant canola (*Brassica napus*). *Ecol. Appl.* 13:1276-1294.
- Blum, S.A.E., Lorenz, M.G., and Wackernagel, W. 1997. Mechanism of retarded DNA degradation and prokaryotic origin of DNases in nonsterile soils. *Appl. Microbiol.* 20:513-521.
- de Vries, J. and Wackernagel W. 2004. Microbial horizontal gene transfer and the DNA release from transgenic crop plants. *Plant Soil* 266:91-104.
- Dunfield K.E., and Germida J.J. 2001. Diversity of bacterial communities in the rhizosphere and root interior of field-grown genetically modified *Brassica napus*. *FEMS Microbiol. Ecol.* 38:1-9.
- Dunfield K.E., and Germida J.J. 2003. Seasonal changes in the rhizosphere microbial communities associated with field-grown genetically modified canola (*Brassica napus*). *Appl. Environ. Microbiol.* 69:7310-7318.
- Friesen, L.G., Nelsen, A.G., and Van Acker, R. 2003. Evidence of contamination of pedigreed canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. *Agron. J.* 95:1342-1347.
- Gulden, R.H., Lerat, S., Hart, M.M., Powell, J.R., Trevors, J.T., Pauls, K.P., Klironomos, J.N., and Swanton, C.J. 2005. Quantitation of transgenic plant DNA in leachate water: Real-time polymerase chain reaction analysis. *J. Agric. Food Chem.* 53:5858-5865.
- Gulden R.H., Shirliffe S.J., and Thomas A.G. 2003a. Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. *Weed Sci.* 51:83-86.
- Gulden R.H., Shirliffe S.J., and Thomas A.G. 2003b. Secondary seed dormancy prolongs persistence of volunteer canola in western Canada. *Weed Sci.* 51:904-913.
- Gulden R.H., Thomas A.G., and Shirliffe S.J. 2004a. Secondary dormancy, temperature, and burial depth regulate seedbank dynamics in canola. *Weed Sci.* 52:382-388.
- Gulden R.H., Thomas A.G., and Shirliffe S.J. 2004b. Relative contribution of genotype, seed size and environment to secondary seed dormancy potential in Canadian spring oilseed rape (*Brassica napus*). *Weed Sci.* 44:97-106.
- Hall, L., Topinka, K., Huffman, J., Davis, L., Good, A. 2000. Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Sci.* 48:688-694.
- Rakow G., and Woods D.L. 1987. Outcrossing in rape and mustard under Saskatchewan prairie conditions. *Can. J. Plant Sci.* 67:147-151.
- Romanowski G., Lorenz, M.G., and Wackernagel, W. 1993. Plasmid DNA in a groundwater aquifer microcosm: adsorption, DNase resistance and natural genetic transformation of *Bacillus subtilis*. *Mol. Ecol.* 2:171-181.
- Simard, M.J., Legere, A., Pageau, D., Lajeunesse, J., and Warwick, S. 2002. The frequency and persistence of volunteer canola (*Brassica napus*) in Quebec cropping systems. *Weed Tech.* 16:433-439.
- Simard, M.J., Legere, A., Seguin-Swartz, G., Nair, H. and Warwick, S. 2005. The fitness of double vs. single herbicide-resistant canola. *Weed Science.* 53:489-498.
- Simard, M.J., and Legere, A. 2004. Synchrony of flowering between canola and wild radish (*Raphanus raphanistrum*). *Weed Science.* 52:905-912.
- Thomas, A.G., Leeson, J.Y., Hall, L.M., Beckie, H.J., Van Acker, R., and Brenzil, C.A. 2004. Has herbicide-tolerant canola altered weed diversity in western Canada. Proceedings of the Canadian Weed Science Society Annual Meeting, Winnipeg, MB. Nov. 28-Dec.1.
- Warwick, S.I., Simard, M.J., Legere, A., Beckie, H.J., Braun, L., Zhu, B., Mason, P., Seguin-Swartz, G., and Stewart, C.N. 2003. Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) OE Schulz. *Theor. Appl. Genet.* 107:528-539.