The Americas Journal of Plant Science and Biotechnology *©2011 Global Science Books*

Genetically-Modified Crop Production in Canada: Agronomic, Ecological and Environmental Considerations

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ABSTRACT

Since the commercial introduction of genetically modified (GM) crops for field cultivation in 1996, the area of GM crop production in Canada has increased from 0.14 million ha to the current 8.2 million ha. Inserting genes tolerant to herbicides and resistant to insect pests into the crops allows GM crops to be grown with fewer pesticide applications, thus reducing production cost. With the rapid adoption of GM crops, modern agricultural systems offer new crop management strategies so that both production efficiency and crop yield on a per-hectare basis are increased. As a result, there is a potential to offer better agricultural productivity than that conventional crops can provide. Despite the potential economic benefits, commercial production of GM crops at large has also raised some concerns about potential adverse effects on the environment. Over the past decade, there have been many research projects conducted to assess the risks posed by GM crops in the environment, especially for gene flow and non-target effects. Using a balanced approach to appraise agronomic benefits and environmental issues, this review summarizes the results obtained in numerous studies associated with GM crop production in Canada with special reference on the major GM crops of canola (*Brassica* spp.), maize or corn (*Zea mays*), and soybean (*Glycine max*), and the two main GM traits of herbicide tolerance (HT) and insecticidal toxins from a bacterium, *Bacillus thuringiensis* (*Bt*).

Keywords: *Bacillus thuringiensis* (*Bt*), canola, corn or maize, gene flow, herbicide-tolerance, soybean
Abbreviations: Bt, *Bacillus thuringiensis* (*Bt*); **ECB**, European corn borer, **GM**, genetically modified; **HT**, herbicide tolerant

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ADOPTION OF GENETICALLY MODIFIED CROPS IN CANADA

Since it was first introduced in 1996, genetically-modified (GM) crop area has now reached 134 million ha globally (James 2009). As one of the six founding countries of biotech crop, Canada is now the fifth major GM crop producer in the world, with total GM crop area of 8.2 million ha and steady 9% year-over-year growth. The following review focuses on the three major GM crops - canola, soybean and corn grown in Canada.

Canola

Canola or rapeseed (*Brassica* spp.) is successfully grown as a spring-seeded crop in the cooler agricultural regions, and also as a winter crop in temperate climates of Europe and Asia. Oil obtained from *Brassica* seeds (rapeseed, mustard or canola) constitutes 40% of the total seed weight on average, and the seed meal remaining after extraction can be used as animal feed or as a crop nutrient source when returned to the field. Beginning in the 1960's, rapeseed with low erucic acid and low glucosinolates was released in Canada with the trademark name "canola" (derived from Canadian oil, low acid), which stimulated rapid expansion of rapeseed production throughout the world. Canola oil is

Received: 3 December, 2009. Accepted: 10 December, 2010.

Invited Review

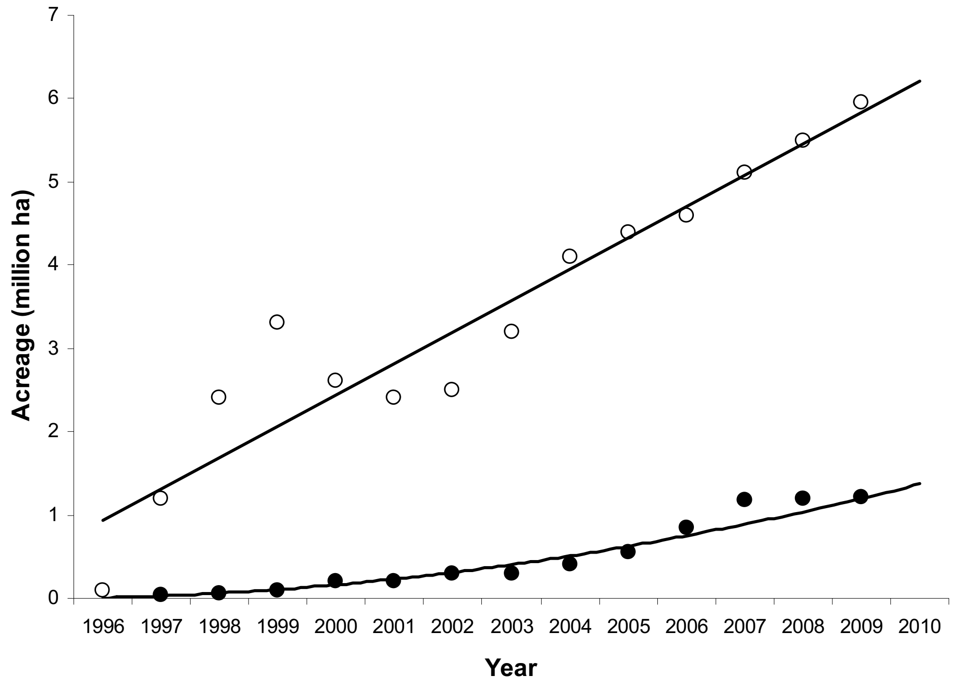

Fig. 1 Acreage (in million ha) of genetically modified (GM) canola (○) and GM-maize (●) crop production in Canada from 1996 to 2009 (adapted from James 2009).

widely used as cooking oil and salad oil, and can be processed into margarine, generating around 80% of the cash value of the crop, and is now considered an important feedstock for renewable energy products, mainly biodiesel. As one of the leading production countries, Canada produced 9% of the total world production (20.33 million tonnes) of canola/rapeseed oil during 2008/09 (USDA 2009).

There are two plant species (*Brassica napus* and *Brassica rapa*) of canola grown in Canada. *Brassica napus* (Argentine type), which constitutes over 90% of the canola area in Canada, was GM to be tolerant of herbicides. Since the commercial release, the adoption of herbicide tolerant (HT) canola has been rapid in Canada (**Fig. 1**). Currently three types of HT canola are commercially available, including GM varieties resistant to glyphosate (Roundup Ready$^{\mathrm{TM}}$) or glufosinate (Liberty Link$^{\mathrm{TM}}$), and mutagenic varieties resistant to certain imidazolinone herbicides (Clearfield$^{\mathrm{TM}}$). These three HT canola types constitute 50, 32, and 14%, respectively of the canola cultivation area (Beckie *et al.* 2006). In 2009, HT canola occupied 6.0 million ha (93% of the total 6.4 million ha national canola area) in Canada. The most widely grown varieties are Roundup Ready$^{\mathrm{TM}}$ canola due to the effectiveness of weed control and a lower cost of the herbicide (Canola Council of Canada 2001). The yield of hybrids, irrespective of HT type, has consistently been higher than conventional types. Meanwhile, higher yields are also achieved because HT canola can often be sown earlier in the spring when it is still too cold for conventional herbicides to work effectively (Canola Council of Canada 2001).

Soybean and maize

Soybean (*Glycine max* L.), originated from East Asia, is a relatively new crop in Canada. Before the mid-1970s, soybean production was restricted to southern Ontario due to the climate. Genetic improvement and intensive breeding programs have since opened up more widespread growing possibilities across Canada for this incredibly versatile crop. The 1.2 million ha of soybean crop in 2006 marked a near eight-fold increase in area since 1976, during which the ground-breaking varieties that perform well in Canada's shorter growing season were introduced (Statistics Canada 2008). In 2009, total plantings of soybean reached 1.4 million ha, a sharp 17% increase from the previous years (James 2009). In Canada, the commercial cultivation of HT soybean started in 1997. All registered HT soybean cultivars are the Roundup Ready$^{\mathrm{TM}}$ type which is primarily grown in eastern Canada (Beckie *et al.* 2006), with 80% of the nation's soybean area in Ontario and Quebec (Statistics Canada 2005). HT soybean constituted about 85% (1.2 million ha) of the total crop area planted in 2009 (James 2009).

Maize or corn (*Zea mays* L.), orginated in Mexico and from there became a major food crop all over the world. In North America, corn is grown as a food, feed and industrial crop, and covers over 25% of the nations' cropland and consumes more than 40% of the commercial fertilizer in the USA. In Canada, corn production has an annual acreage of approximately 1.5 million ha, and is concentrated in Ontario and Quebec (Ma *et al.* 2006). Since the commercial introduction of GM crops for field production, many corn producers have begun planting GM corn hybrids instead of conventional hybrids. The first transgenic hybrids introduced consisted of *Bt* genes that express the insecticidal 1 epidopteran-active crystalline protein (Cry1Ab) endotoxin for the control of European corn borer (ECB, *Ostrinia nubilalis* Hubner). The adoption of corn hybrids with HT and *Bt* (or a combination of HT and *Bt*) traits reached 1.19 million ha in 2008, of which 68% had single GM genes and 32% had double or triple stacked genes (James 2009).

Both glyphosate and glufosinate provide broad-spectrum control of annual grass and broadleaf weeds. Like HT canola, HT corn and HT soybean varieties were developed through genetic modification. The cultivation of HT soybean in eastern Canada has provided effective control of biennial and perennial weed species such as wirestem muhly (*Muhlenbergia frondosa* (Poir.) Fern.), perennial sowthistle, Canada thistle and horsenettle (*olanum carolinense* L.) (Sikkema and Soltani 2007).

Despite an efficacious weed control option or convenient control of ECB, the adoption of HT or *Bt* corn and HT soybean has not always led to an increased crop yield and net return. Compared to that of conventional corn hybrids, glyphosate tolerant and glufosinate tolerant corn systems have shown no differences in yield. Further, glyphosate tolerant soybean in eastern Canada has even shown an average of 4% lower yield than conventional soybean (Bohner 2003; Beckie *et al.* 2006). Better weed control in glyphosate tolerant soybean may overcome the lower average yield potential and result in an equivalent or a net increase in the yield compared to conventional soybean (Beckie *et al.* 2006).

The yield of *Bt* and non-*Bt* corn near-isolines grown side-by-side was compared in several studies at experimental field sites in Canada. Soil textures ranged from loamy sand to clayey loam, and a variety of cultivation practices (plant populations, different rates of N fertilizer, tillage, herbicides, etc.) were used. It is generally accepted that yields are greater with *Bt* corn than non-*Bt* corn in years with high ECB infestation (more than 2 larvae per plant) (Ma and Subedi 2005). Under natural infestations of low to medium ECB pressure, the first generation of *Bt* corn often produced similar grain yields to those of their non-*Bt* counterparts (Ma and Subedi 2005; Subedi and Ma 2007). Hybrids with the *Bt* trait appeared to be greener at harvest. The later maturing and higher moisture concentration at maturity in *Bt* hybrids may have been caused by their stay-green characteristic, which maintains leaves and stalk greenness at physiological maturity (Subedi and Ma 2005). For the next generation of *Bt* or other GM hybrids against ECB or other pests, it would be desirable to combine GM traits with superiority in yield, N use and agronomic performance to justify the higher technology fee (Ma *et al.* 2009). Presently, while *Bt* corn is still popular, the "stacked" GM hybrids that provide resistance to insect attack and tolerance of herbicide damage are favoured in some regions.

HERBICIDE TOLERANCE

Approximately 99% of the 6.4 million ha of canola in Canada is cultivated in western Canada (Manitoba, Saskatchewan, and Alberta), with only a small area of *B. napus* grown in Ontario (22, 000 ha; Canola Council of Canada 2009) and Quebec (12, 000 ha; Simard *et al.* 2006). After the three major types of HT canola were approved for commercial release in Canada, HT canola systems have become dominant due to: 1) fewer restrictions on the range of weed and crop growth stages for herbicide application, 2) better control of previously difficult weed species, 3) increased flexibility in timing of weed control, 4) fewer herbicide applications and lower herbicide costs, and 5) reduced crop injury that may be caused by herbicide mixtures (Harker *et al.* 2000, 2004).

Benefits

1. Broad-spectrum weed control

The adoption of HT canola allowed farmers to manage previously difficult weed species including false cleavers (*Galium spurium* L.), stork's-bill (*Erodium cicutarium* (L.) L'Hér. Ex Aiton), cow cockle (*Vaccaria hispanica* (Mill.) Rauschert), Canada thistle (*Cirsium arvense* (L.) Scoop.), and several sowthistle species (*Sonchus* spp.) (Harker *et al.* 2000; Devine and Buth 2001; Stringham *et al.* 2003; Beckie *et al.* 2006; Upadhyay *et al.* 2006). The availability and cost of glyphosate has been a major driver for some changes of agronomic practices over the last ten years. It is now estimated that 30% of the crop area is directly seeded without any tillage and 60% of the area is tilled to 5-10 cm, mainly in the spring. HT canola has also enabled farmers to grow them on weed abundant fields and also allowed the control of weeds that had become resistant to conventional selective herbicides used in this crop. Research data showed that glyphosate-tolerant canola systems often provide a higher level of weed management than glufosinate-canola systems (Harker *et al.* 2000; Clayton *et al.* 2002; Harker *et al.* 2004).

2. Higher net return

HT canola resulted in generally higher net returns, compared to conventional varieties. Survey results showed that HT canola yielded approximately 200 kg ha^{-1} or three bu ac^{-1} (> 10%) more than conventional canola in 2000 (Canola Council of Canada 2001). From a field study, O'Donovan *et al.* (2006) reported a very similar yield advantage for HT canola. The net return advantage for HT canola resulted from better weed control, from lower labour and fuel costs, etc. Meanwhile, dockage was significantly lower in the HT canola practice, largely attributed to more effective weed control.

3. Fewer tillage passes

The majority of the HT vs. conventional canola comparisons in both the survey and the case studies was performed with minimum or zero tillage systems (Canola Council of Canada 2001). Conventional growers are more likely to utilize summer fallow in their rotations; 36% of the conventional sample had summer fallow acreages as compared to 18% of the transgenic sample in 1999 (Canola Council of Canada 2001).

4. Other benefits

The other benefits of adopting GM crop production include (1) a wide herbicide application window, which allows glyphosate to be applied up to the six-leaf stage of canola with repeat applications if necessary (Clayton *et al.* 2002), (2) lower cost of weed control resulting from fewer herbicide applications (Harker *et al.* 2003; Blackshaw *et al.* 2008), and (3) more flexible rotations enabling growers to seed earlier in the spring, or in the fall, thus benefiting from soil moisture conservation (Blackshaw *et al.* 2005).

HERBICIDE-TOLERANT CROP-WEED RELATED ISSUES

Approximately 80% of GM crops planted worldwide are HT (James 2008). Increased reliance on a small number of herbicides for weed control may lead to 1) the occurrence of weed population shifts, 2) evolution of herbicide-resistant weed populations, 3) HT crops becoming volunteer weeds, and 4) transfer of the HT genes from the HT crop to its wild relatives.

Weed population shifts

There are concerns that the management of weeds in HT crops using broad-spectrum herbicides might lead to weed shift and the decline of farmland biodiversity. A western Canada study showed that a high frequency of in-crop glyphosate in a wheat-canola-wheat rotation was associated with greater henbit (*Lamium amplexicaule* L.) populations at Lacombe and volunteer wheat populations at Lethbridge (Harker *et al.* 2005). However, there are no other reports on significant shifts in weed populations or major difficulties in managing weeds in agricultural settings due to the widespread cultivation of HT canola in Canada.

Due to the extensive cultivation of HT canola and the cross pollination potential of *Brassica* crops, it is conceivable that gene flow between HT canola varieties through pollen dispersal may result in canola volunteers being resistant to two or more herbicides. Consequently, HT gene stacking may pose agronomic problems such as volunteer plant control. At a field site in western Canada, two volunteer plants with triple-herbicide resistance were found only after three years of commercial cultivation of HT canola (Hall *et al.* 2000). Beckie *et al.* (2003) also observed double-resistant canola volunteers at 11 field sites in Saskatchewan, Canada. The results of these studies suggest that HT gene stacking in canola volunteers can occur through gene flow. This is not surprising, given the cross pollination nature of canola, the large acreage of canola with different types of HT traits in western Canada, and the potential seed bank life leading to the incidence of canola volunteers (Hall *et al.* 2000; Beckie *et al.* 2003; Légère 2005). Moreover, there are limited regulations for farmers on where (distance separation) and which variety (HT or conventional) they can grow on their farmlands (Beckie *et al.* 2001). This has resulted in one HT variety being commonly grown next to other HT or conventional varieties. One consequence from this scenario is that rotations including many HT crops having the same trait (e.g. glyphosate tolerance) may result in various crop volunteers being resistant to the same herbicide (Légère 2005). Although pollen drift among different HT canola has led to multiple tolerant volunteers at some field sites (Hall *et al.* 2000; Beckie *et al.* 2003), all canola volunteers were readily managed with relatively low-cost alternative herbicides (Johnson *et al.* 2005). In Canada, over 30 registered herbicides are available to control single- or multiple-resistant HT canola volunteers in cereal fields, the most frequent crop to follow canola in a typical 4-year rotation in western Canada (Beckie *et al.* 2006). Although not all canola volunteers are controlled by herbicide application, most survivors are affected by the combination of crop competition and partial herbicide control that reduces seed set (Blackshaw *et al.* 2005a, 2005b). Furthermore, there is a multitude of agronomic practices that are recommended to growers to manage multiple-HT canola volunteers (Beckie *et al.* 2004; Légère *et al.* 2006). Currently, there is no evidence that the extensive cultivation of HT canola in western Canada has resulted in an increase of HT canola volunteers that would have been caused by the herbicide-tolerant traits (Hall *et al.* 2005). However, in a recent study, Knispel *et al.* (2008) demonstrated gene flow between HT canola varieties, resulting in stacking of herbicide tolerant traits in individuals within escaped canola populations. The field study suggests that multiple HT canola volunteers are not confined to agricultural fields and can contribute to the spread of HT traits in wild habitats (Knispel *et al.* 2008).

Herbicide resistance development

The wide cultivation of HT crops (especially Roundup Ready^{TM} with the herbicide, glyphosate) raised concerns that the intensive applications of one herbicide could pose high selection pressure against weeds, and thus, rapidly enhance the evolution of HT weed populations (Clayton *et al.* 2002). However, despite the extensive cultivation of HT canola in Canada during the last decade, no weed species has yet been reported being resistant to the herbicides glyphosate or glufosinate. Nonetheless, it is predictable that the occurrence of weed populations being resistant to glyphosate or glufosinate could just take time (Beckie 2009). At present, 15 weed species (including giant ragweed) have been confirmed elsewhere in the World as being resistant to glyphosate. Among the 15 species, nine were documented in the United States in 2008 (Johnson *et al.* 2009).

Herbicide-tolerant volunteers

During harvesting, seed shattering of canola in fields can be a very severe problem. The small size and the round shape of canola seeds help them fall easily into the soil, which facilitates their survival over winter (Hall *et al.* 2005). HT canola like other crop varieties may establish HT volunteer populations in the fields due to seed shattering and secondary seed dormancy (Gulden *et al.* 2003a, 2003b). Studies of canola fields showed that the density of canola volunteers was on average 4.3 plants m^{-2} in western Canada (Harker *et al.* 2007), and in Quebec, was about 5 plants m^{-2} in the year following canola cultivation (Simard *et al.* 2002). Harker *et al.* (2006) reported that the vast majority of glyphosate-tolerant canola volunteers were recruited in the year following canola production. Results from a number of studies demonstrated that low- or no-till seeding systems led to reduced number of HT tolerant canola volunteers, due to increased seed mortality and the prevention of secondary dormancy induction (Gulden *et al.* 2003b). Despite the fact that HT canola dominates the market, post-management surveys showed that the abundance of canola volunteers across western Canada has actually decreased over the last 10 years (Harker *et al.* 2007). The adoption of HT canola has not significantly changed canola volunteer management practices that are involved in pre- and post-applied herbicide options to manage volunteer populations in most crops.

Gene flow from herbicide-tolerant crops to related plants

Commercial cultivation of HT canola could possibly lead to the transfer and introgression of herbicide tolerance genes from the HT crop into its wild relatives. Spontaneous hybrids between HT canola and wild *B. rapa* are known to occur under field conditions (Halfhill *et al.* 2004). In Quebec, Canada, mean hybridization rates in wild populations of *B. rapa* were found to be 13.6% when sampled in or near a commercial field and 7% when sampled in two experimental fields (Warwick *et al.* 2003). The distance separating individual *B. rapa* plants in commercial fields might result in higher pollen competition with HT canola pollen, thus contributing to a higher hybridization frequency. By genetic analysis, Zhu *et al.* (2004a) demonstrated that the transgene (a *Bt* toxin gene) carried by a *Bt B. napus* crop could be transferred and integrated into the genome of wild *B. rapa* through interspecific hybridization. The integration of the *Bt* gene into the wild *B. rapa* genome took only two generations in some cases due to high chromosomal homology shared by the two species. Meanwhile, the expression (*Bt* toxin content) of the *Bt* gene in wild *B. rapa* plants was stable in all backcross generations, regardless of the integration of the *Bt* gene (Zhu *et al.* 2004b). Furthermore, Mason *et al.* (2003) reported that *B. rapa* containing the *Bt* gene had a fecundity advantage under high insect herbivore pressure, compared to wild-type *B. rapa* plants. However, hybrid populations of wild *B. rapa* containing the *Bt* gene performed worse or equivalent to wild-type *B. rapa* when competing against wheat (*Triticum aestivum* L.) in a field trial (Halfhill *et al.* 2005). It was suggested that the lower competitiveness of the hybrids carrying the transgene could be due to the retention of crop-specific genes in the hybrids that were obtained from *Bt* canola through chromosomal recombination in the initial hybridization. In a recent study, Warwick *et al.* (2008) reported the persistence of HT traits in wild *B. rapa* over a 6-year period, even without herbicide selection pressure and potential fitness cost.

Bt CROPS

Bt crop is a GM crop variety containing a toxin gene transformed from the naturally occurring gram-positive bacterium *Bacillus thuringiensis*, to express various insecticidal proteins. *Bt* is the most common insecticidal trait in GM crop plants such as corn, cotton, and potato (James 2008). However, at present, *Bt* corn is the only crop widely cultivated in Canada. *Bt* corn expressing Cry1Ab was initially developed to control a lepidopteran pest, the European corn borer (*Ostrinia nubilalis*; ECB). In general, ECB larvae bore into the stalks of corn, which reduces yield and grain quality of conventional corn hybrids. In GM corn fields, ECB larvae die within two days of ingesting the *Bt* corn tissue.

In an eight-site-yr study, it was found that corn ears can have up to 82% out-cross fertilization between yellow-kernelled *Bt* and white-kernelled non-*Bt* hybrids in adjacent rows (Ma *et al.* 2004). Does the 82% out-crossed seeds carrying the same amount of transgenic DNA as the original transgenic *Bt* seeds? The ELISA semi-quantitative test indicates that the out-crossed white corn by the pollen donor yellow *Bt* hybrids carried less amount of the cry proteins than the original *Bt* yellow-kernelled seeds (Ma *et al.* 2005). Because of its large size, corn pollen does not travel very far, for example, out-cross rate of the white-kernelled corn was dropped to < 1% at the 28 m bordering the yellow-kernelled *Bt* hybrids (Ma *et al.* 2004). Presently, *Bt* corn accounts for about 53% of the corn grown in Canada (Canadian Corn Pest Coalition 2007).

After ECB, corn rootworm (*Diabrotica* spp.; CRW) has become the most concern and widespread insect pest of corn in North America. Yield, grain quality losses, harvest delays and insecticide costs approximate $1 billion annually in the USA and Canada (Metcalf 1986; Ostlie 2001; Ulrich and Stefan 2002). In 2003, a new GM corn with rootworm resistance (MON 863), the gene encompasses the coleopteran specific insecticidal delta-endotoxin (Cry3Bb) from *B. thuringiensis*, was commercially released in Canada (Ma *et al*. 2009). In a three-year field study under continuous corn for 5 to 6 years, machine-harvestable yield was up to 66% greater for the *Bt* rootworm resistant hybrids than for the non-*Bt* corn hybrids on a clay loam soil, but was similar on a sandy loam soil (Ma *et al*. 2009).

Since Cry1Ab is selectively toxic to Lepidoptera (moths and butterflies), pollen dispersal from *Bt* maize fields might potentially have adverse effects on Lepidopteran species if their larvae feed on host plants covered with *Bt* crop pollen. Losey *et al*. (1999) reported that when pollen from *Bt* maize (event Bt11) was spread on milkweed (*Asclepias curassavica*) leaves in the laboratory and fed to monarch butterfly (*Danaus plexippus*) larvae, the larvae from these leaves consumed significantly less compared to leaves coated with non-GM pollen. However, other laboratory experiments showed that when small larvae were fed high doses of pollen (over 1,000 pollen grains cm^{-2} of milkweed leaf surface) for 4 or 5 days there were no measurable effects in terms of weight gain or mortality (Hellmich *et al*. 2001). Further, it was suggested that larval exposure to pollen on a population-wide basis is low, given the proportion of larvae in maize fields during pollen shed, the proportion of *Bt* maize fields, and the levels of pollen dispersal within and around maize fields (Oberhauser *et al*. 2001). Sears *et al*. (2001) demonstrated that the proportion of monarch butterfly population exposed to *Bt* pollen was estimated to be less than 0.8% of the total population. The number of Milkweed plants is usually higher in non-agricultural areas, particularly along field edges, compared with corn fields (Oberhauser *et al*. 2001). Meanwhile, pollen density on the upper leaves, where the monarch egg masses are laid, was only 30–35% of that on middle leaves, and pollen densities were significantly higher around the leaf midrib, an area avoided by younger larvae (Pleasants *et al*. 2001). Laboratory bioassays also showed that the only GM *Bt* maize pollen that consistently affected monarch larvae was from Event 176, an event that was withdrawn from the market. Pollen from the most widely planted *Bt* maize events (MON810 and Bt11) showed no acute effects on larvae in field studies (Hellmich *et al*. 2001; Stanley-Horn *et al*. 2001) since their pollen expresses 80-times less toxin than Event 176 (Stanley-Horn *et al*. 2001). Excessive pollen densities of the currently commercialized events (Bt11 and MON810) would be required to obtain relevant adverse effects on larval development (Hellmich *et al*. 2001). In conclusion, continuous exposure of monarch butterfly larvae to natural deposits of *Bt* crop pollen on milkweed plants within maize fields can affect individual larvae (Sears *et al*. 2001; Dively *et al*. 2004). However, long-term exposure of monarch butterfly larvae throughout their development to *Bt* maize pollen is detrimental to only a fraction of the breeding population because the risk of exposure is low. It is unlikely that *Bt* maize will affect the sustainability of monarch butterfly populations in North America (Sears *et al*. 2001; Dively *et al*. 2004). Further, toxic effects of *Bt* maize on monarch butterfly should be compared to mortality caused by other factors (e.g. traditional insecticides), which is very high in natural monarch butterfly populations (Oberhauser *et al*. 2001; Dively *et al*. 2004). Floate *et al*. (2007) showed that cultivation of Lepidoptera-specific *Bt* maize in southern Alberta did not significantly affect ground beetle populations, and year-to-year variation was greater than that between *Bt* and non-*Bt* maize lines.

ENVIRONMENTAL IMPACTS OF TRANSGENE DNA AND PROTEIN DERIVED FROM GM CROPS

Persistence of transgene DNA and related proteins in soil and water

Cultivation of GM crops may lead to the persistence of transgene DNA in soil and nearby water. Consequently, transgene DNA released from plant tissues may be accessible to indigenous bacteria. Gulden *et al*. (2005) showed that plant DNA (GM maize and soybean) can be released and moved with leachate water in agricultural soils throughout the vegetative phase. Temperature and microbial density of water may contribute to the rate of DNA degradation in the environment. Zhu (2006) used a real-time PCR technique to monitor the degradation dynamics of the transgene DNA of a *Bt* maize line in water microcosms. He showed that the concentration of plant DNA was reduced by two orders of magnitude (from 0.8 to 0.008 μg/ml) within 96 hrs in the intact and filter-sterilized treatments of groundwater and river water samples, in contrast to its persistence in the autoclaved treatment. Douville *et al*. (2008) reported the *cry1Ab* gene from GM maize persisted for more than 21 days in surface water and more than 40 days in sediment. Field surveys revealed that the *cry1Ab* gene from *Bt* maize and from naturally occurring *Bt* was more abundant in the sediment than in the surface water. Transgene DNA from *Bt* maize was found to persistent in aquatic environments and was detected in rivers receiving drainage from farming areas. Furthermore, transgene DNA of *Bt* maize was detected in feral freshwater mussels collected from sites located in proximity of maize fields (Douville *et al*. 2009), suggesting the transportation of transgene DNA from terrestrial to aquatic environments.

Lerat *et al*. (2007) reported that transgene DNA of GM maize and soybean was detected 7 months after the crop had been harvested, and transgene DNA was not detectable after one growing season with conventional soybean in the same plots. Our data also demonstrated that under continuous cultivation of the *Bt* maize line (event MON863), cv. 'DKC42-23', its transgene DNA can be detected in soil all year round. However, after the subsequent growing season with a soybean line at the same field site, the persistence of transgene DNA was reduced to undetectable levels (Zhu *et al*. 2010).

Effects of GM crops on microbial activity and diversity in soil

Microorganisms are the dominant organisms both in terms of biomass and activity in the soil. Soil microbial communities carry out complex processes that are of major ecological and agricultural significance. The soil microfauna is involved in a number of important processes including decomposition of organic matter, nutrient mineralization, regulation of plant pathogens, decomposition of agricultural chemicals, and improvement of soil structure. The close interaction between crop cultivation and soil processes inadvertently leads to contacts of soil organisms with *Bt*-toxins released from GM crops. In Canada, studies were conducted to investigate the effect of HT crops on the diversity of rhizosphere and endophytic microorganisms (Dunfield and Germida 2003, 2004). Sicilliano *et al*. (1998) reported that carbon utilization patterns and fatty acid methyl ester profiles of the microbial community associated with the roots of HT canola varieties were different from the profiles of two conventional canola varieties. The composition of the cultivable microbial community associated with an HT canola variety (Quest) was also significantly different from that of conventional canola varieties (Siciliano and Germida 1999). This difference was also detected by Dunfield and Germida (2001), who investigated the total microbial communities (both cultivable and non-cultivable) using molecular techniques. However, these observed effects were attributable to different varieties, regardless of GM or conventional, field site (Dunfield and Germida 2001), seasonal variation (e.g. plant growth stage; Dunfield and Germida 2003), and method of analysis (Dunfield and Germida 2004). Further, Lupwayi *et al.* (2007) indicated that effects of HT (glyphosate) crop frequency on soil microorganisms were minor and inconsistent over a wide range of growing conditions and crop management. However, in Saskatchewan, Fernandez *et al.* (2007a, 2007b) observed an increased incidence of Fusarium head blight in barley (important crop in rotation) with continuous use of glyphosate in HT canola, especially under no-tillage. Concerns have also been raised that *Bt* maize may influence soil nutrient cycling by increasing the amount *Bt* toxin contained in plant residues returned to the soil. Plant residue decomposition has therefore most often been chosen as an indicator of soil ecosystems functions. However, Hopkins and Gregorich (2003) found no detectable difference between the decomposition rates of *Bt* and non-*Bt* maize, as determined by CO_2 production. Crop residue of *Bt* maize may contain higher lignin or may have a lower decomposition rate compared to those of its non-*Bt* counterpart. For an up-to-date progress on this topic, readers are referred to a recent review article (Yanni *et al.* 2010).

PERSPECTIVE AND FUTURE RESEARCH NEEDS

Over the last 14 years, cultivation of GM crops has experienced rapid development worldwide, especially in the Americas where GM cultivation acreage has increased at an unprecedented pace (James 2009). GM crops could potentially provide many agronomic, ecological, and environmental benefits. Some benefits associated with the most widely used GM traits (HT and protection from insects - *Bt*) include better control of farmland weeds, increased net return due to the reduction in the frequency of pesticide applications, and the effective control of target insect pests. Compared to the conventional varieties, future GM crops may include improved genotypes with greater yield potential associated with better tolerance to drought, nutrient limitation, salinity, or drastic temperature changes (cold, heat, etc.). In addition, development of GM crops for biofuel production (e.g. increased biomass and altered composition), nutritionally enhanced products, and producing pharmaceutically active substances has progressed dramatically in recent years. GM plants with such new traits may be commercially released in the near future. Accordingly, the agronomic performance (benefits) of GM crops with these new GM traits needs to be assessed in comparison with that of their conventional varieties. Research should also be conducted to monitor the potential environmental effects of new GM crops on biodiversity dynamics on farmlands, weed population shifts, volunteer GM crops and seeds dormancy in soils, gene flow, non-target effects, etc. These new GM crops may also contain different components in their residues. Further research on the processes of GM crop residue decomposition and the long-term impact of continuous GM crop production on soil C transformation and turnover is warranted. Development of new detoxification or decomposition protocols (Guan *et al.* 2005, 2008) is also needed to treat the GM crop residues from pharmaceutical and biofuel feedstock production. Overall, case-by-case assessments of the potential benefits, ecological, and environmental risks of each GM crop will be the most appropriate way to ensure the agricultural benefits and environment sustainability.

ACKNOWLEDGEMENTS

We thank Dr. Yiu-Kwok Chan, Research Scientist of Agriculture and Agri-Food Canada, for his critical review of the drafted manuscript and constructive suggestions. Thanks are extended to Ms. Lucy X. Ma for proof reading. This review is a joint contrition of Environment Canada and Agriculture and Agri-Food Canada (AAFC). AAFC-ECORC contribution No. 10-092.

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Dedication: Dr. Bin Zhu

Dr. Bin Zhu, Research Scientist of Environment Canada (EC) passed away in May 2010 at the age 45.

Bin earned his B.Sc. (Plant Pathology) at the Huazhong Agricultural University in 1986, M.Sc. (Plant Pathology) in 1989 at the Nanjing Agricultural University in China, and Ph.D. (Plant Science) in 2000 at the University of Manitoba. Before coming to Canada, Bin was appointed as a lecturer at the prestigious Huazhong University of Science and Technology, where he taught plant pathology courses for six years. He joined the Government of Canada in August of 2000 after receiving his Ph.D., and established a genetics laboratory at the National Water Research Institute of Environment Canada in Saskatoon since 2002. Over the past years, Dr. Bin Zhu has been conducting research on investigating possible adverse ecological effects of products of biotechnology such as genetically modified organisms (e.g. corn genotypes that have a gene copied over from another living organism meant to protect the crop and add its commercial value) in support of government environmental policies. His research focused on the detection and persistence of novel genetic material in the environment and monitoring long-term ecological effects after commercial release of biotechnology products using genomic techniques. His research was supported by a number of funding sources including Canadian Regulatory System of Biotechnology, Canadian Environmental Protection Act, Strategic Technology Application of Genomics in the Environment Program, and Canadian Biotechnology Strategy. He published a number of key scientific articles, book chapters and reviews of his field.

Dr. Bin Zhu was an expert in the identification of genes and environmental toxicology. He was an international authority on the detection of changes in genetic coding in living organisms, and was very keen on finding new applications and learning new methods for making his research as relevant as possible to the mandate he was given. The field of ecosystem effects of genetically-modified-organisms is new for Environment Canada (EC), indeed worldwide, and Dr. Zhu has been forced to break new ground and be particularly innovative in his research program, attracting no fewer than 46 different collaborators from around the world. Through his accomplishments, he was able to demonstrate the persistence of certain transgenes (pieces of DNA) in natural waters and the rhizosphere of soils; he was also among the first to demonstrate uncontrolled gene transfers from herbicide-tolerant commercial plants to wild relatives, raising concerns over unpredictable changes in natural species. Dr. Zhu's research has had a significant impact on the development of EC's Ecosystem Effects program and governmental policies. As such, he was a valuable resource for the EC bioregulatory community. Since coming to EC, he has worked tirelessly to demonstrate the importance of understanding genetic contamination, and area in which very little is known, and has contributed to high-profile reports such as those of the Royal Society of Canada. Dr. Zhu's work was well recognized through invitations to serve on expert panels.

Bin was very active on the national and international scene, in his research and on expert committees as with his personal interests. He was widely published, had many collaborators, and took an active interest in everything that he did. He was influential in setting research directions in his difficult, developing field within Environment Canada and with other government departments. He was truly dedicated to his work, and he cared deeply about the people with whom he worked.

Bin was a good man, wise, sensitive, honest and decent. He was always ready to help, and always asked. Dr. Bin Zhu was a well respected and recognized scientist, and the way he lived his life to help others. He was also a good husband and father; he was very proud of his children and spoke of them. Bin’s family, relatives, friends and the research community shall miss his constant good humour, dedication, passion for his work, unique skills, and great contributions to science.

Bao-Luo Ma (December, 2010)