Topics in Canadian Weed Science Volume 4

The first decade of herbicide-resistant crops in Canada

Edited by
Robert H. Gulden and Clarence J. Swanton

Canadian Weed Science Society
Société canadienne de malherbologie

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Edited by

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Foreword

The discipline of weed science in Canada has come a long way since the first formal Canadian weed committee, the Associate Committee on Weed Control, held its inaugural meeting in Edmonton, Alberta in 1929. Eighteen committee members discussed the ever increasing problem of weeds on Canadian farms. Since then, similar committees including the Canada Weed Committee, the National Weed Committee and the Expert Committee on weeds, have met regularly to address the challenges associated with weed management in Canada. Weed science as a scientific discipline blossomed after the introduction of 2, 4-D in the 1940s. The numerous synthetic herbicides became the dominant control strategy for the next forty years. In the 1980s, however, it became apparent that more integrated approaches to weed management were required. The prolonged use of some herbicide classes resulted in the selection of resistant weed populations while other herbicides had a propensity to persist in soil and groundwater, resulting in both production and environmental problems. These issues and others stimulated a renewed interest in topics such as integrated weed management, weed biology and ecology, biological weed control, application technology, and the environmental impact of herbicides. In response to these challenges, a vibrant, new weed science society emerged in Canada in 2002.

Today, the Canadian Weed Science Society – Société canadienne de malherbologie, includes a rich mixture of members involving federal, provincial and municipal government employees, multinational herbicide industry researchers and managers, university professors and graduate students, and contract research, consultants and industry agronomists. Our goals are (1) to establish and maintain a process for sharing and disseminating weed science knowledge in Canada; (2) to provide a forum for discussion of weed management issues in Canada; and (3) to take a proactive stand on behalf of all stakeholders on issues related to weed management at provincial and federal levels.

I am pleased to introduce the fourth volume in the series – "Topics in Canadian Weed Science". It is our intention to utilize this publication format to more consistently publish and distribute the relevant proceedings of our annual workshops and symposia. I encourage you to visit our website for further information regarding our society (www.cwss-scm.ca).

Denise Maurice
President, 2004-2005
CWSS-SCM

Preface

Welcome to the fourth volume of *Topics in Canadian Weed Science*, which is published periodically by the Canadian Weed Science Society – Société canadienne de malherbologie (CWSS-SCM). The series provides current information, reviews, research results and viewpoints on weed-related topics and issues. It is intended to advance the knowledge of weed science and increase awareness of the consequences of weeds in agroecosystems, forestry, and natural habitats. The topics addressed are diverse and exemplify the challenges facing the various stakeholder groups that make up CWSS-SCM.

This volume is a compilation of peer-reviewed papers based on oral presentations made at the plenary session of the 2005 CWSS-SCM annual meeting held in Niagara Falls. Ontario. The Local Arrangements Committee for the Annual Meeting chose the timely theme of '*Transgenic herbicide-resistant crops: agronomy, environment and beyond*'. The topic was addressed in a balanced manner, with both proponents and opponents of transgenic technology presenting scientific results.

The CWSS-SCM Board of Directors expresses their gratitude to Clarence Swanton and the Niagara Falls Local Arrangements Committee, the contributing authors, reviewers, and the editors who have made this publication possible. We also ask the readers of this volume to publicize this series to a more global audience. Other volumes include *Field boundary habitats: implications for weed, insect, and disease management*; *Weed management in transition*; and *Soil residual herbicides: science and management*.

Eric Johnson
Publications Director
CWSS-SCM

Acknowledgements

Cover

Top photograph: A clean canola field in full bloom. This image provided by the Canola Council of Canada, Winnipeg, Manitoba, Canada.

Middle photograph: Herbicide application to a corn crop. This image provided by Kevin Chandler, Department of Plant Agriculture, OAC, University of Guelph, Guelph, Ontario, Canada.

Bottom photograph: A glyphosate-resistant soybean crop. This image provided by Clarence Swanton, Department of Plant Agriculture, OAC, University of Guelph, Guelph, Ontario, Canada.

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Contents

SYMPOSIUM

Transgenic herbicide-resistant crops: agronomy, environment and beyond

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Introduction

This fourth volume of Topics in Canadian Weed Science 'The first decade of herbicide-resistant crops in Canada' originated from a one-day symposium entitled 'Transgenic herbicide-resistant crops: agronomy, environment and beyond' which was held at the annual meeting of the Canadian Weed Science Society – Société canadienne de malherbologie in Niagara, ON, on November 28, 2005. Ten years ago, herbicide-resistant (HR) crops were commercialized in Canada which was one of the first countries to do so. This technology was a new echelon for weed management and presented new opportunities for weed science. However, in many HR crops, this trait was introduced through genetic engineering which made these crops the first organisms of their kind to be released into the environment at a large scale. This association led to unprecedented challenges to weed science and other disciplines. Since their release, HR production systems have been subjected to greater scrutiny. They have received more media attention than previous weed management technologies and remain a contentious topic among many sectors of society. The intent of this symposium was to include many different voices in this debate. Members of the scientific community, industry, and government in Canada shared their expertise on a broad range of topics associated with HR and genetically-engineered HR crops. This monograph is a reflection of these broad issues covered by this divergent group of presenters, making it different from previous volumes in the series.

At the time of their introduction, weed control, agronomic, and some environmental attributes of HR crops were known, but other impacts of this technology could hardly have been predicted. In crops where this technology

provided an advantage over conventional herbicides, acceptance and adoption of this technology was rapid, resulting in more than 80 % of the annual canola and soybean acreage in Canada being sown to HR genotypes. To date, one dozen HR crops have been approved for unconfined release in Canada, although several are not available commercially. Weed control, agronomics, and economics of the major HR crops in eastern and western Canada were reviewed by Sikkema et al., Harker et al., and Buth.

Some segments of society and entire nations have been less accepting of this technology and the food produced by it. Dobson reviewed the major political, social, and market related reasons for the reluctance to accept this technology by some. The release of this technology also resulted in the identification of communication gaps between the scientific community and other sectors of society. Mowling discussed the current efforts to bridge this gap in Canada. Aside from socio-political reasons, food safety and environmental concerns top the list for rejection of this technology. MacAllister (see Alexander et al.) provided an excellent review on the science behind the safety of HR and genetically-engineered crops to livestock. Environmental concerns associated with HR and genetically-engineered HR technology include shifts in the weed community and the development of herbicide-resistant weed biotypes as well as persistence and ferality of crop volunteers. These topics were reviewed by Tardif and by Hall. Warwick, Gulden and Powell covered other environmental issues including the escape and persistence of HR transgenes in other species and the effects of HR technology on non-target species. In Canada, the commercial release of HR crops has allowed the scientific community to examine these concerns at the appropriate scale, thereby providing meaningful data on environmental issues regarding this technology.

For any new technology, including HR and genetically-engineered HR crops, there will be producers who adopt and those that do not adopt a technology. Issues regarding the side-by-side coexistence of conventional and HR production systems remain only partially resolved. Containment of this technology and the costs to non-adopters was discussed by Clark, while Van Acker investigated the potential for coexistence of separate production streams in Canada. The unprecedented success of weed management in HR crop production systems has been accompanied also by a number of concerns and issues that present new challenges for regulators. Corbett et al. reviewed these challenges from the regulator's perspective and outlined the current status of regulation of HR and other crops with novel traits in Canada.

The editors wish to thank the authors and reviewers for their contributions to the symposium and this monograph. Our goal was to present a balanced and holistic view of the current status and knowledge of HR crops in Canada. We hope to have accomplished this goal, and that the reader enjoys this monograph and appreciates the complexities of these production systems that extend beyond the discipline of weed science.

Herbicide-resistant crops in Eastern Canada

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Glyphosate-resistant corn and soybean and glufosinate-resistant corn provide crop producers in Eastern Canada with additional efficacious, cost-effective weed management options that do not result in unacceptable risks to the environment. Herbicide-resistant (HR) crops offer many benefits including excellent crop tolerance, a wide window of herbicide application, broad-spectrum weed control, improved control of difficult to control species, more consistent weed control under a range of environmental conditions, flexible crop rotation options, lower cost of weed control in some situations, greater yields and/or net returns, and reduced environmental impact. However, there are concerns with the use of this technology including adventitious presence of genetically modified (GM) seed in non-GM produce, injury to crops in adjacent fields due to glyphosate drift, misapplication of either glyphosate or glufosinate to non-transformed hybrids or cultivars, delayed herbicide application resulting in losses in crop yield and net returns due to early weed interference, and overuse of glyphosate which results in increased selection for glyphosate-resistant weed biotypes and weed species naturally tolerant to glyphosate. Since HR crops provide so many benefits in respect to weed management in eastern Canada, it is the responsibility of all weed management practitioners to steward this technology properly. Guidelines need to be implemented to minimize the adventitious presence of GM seeds in non-GM and organic crops. In addition, all personnel involved in weed management need to implement long-term glyphosate stewardship programs so that this valuable weed management tool will still be effective for weed management many years in the future. Cropping system diversity, including diverse weed management approaches, is the pillar of sustainable agriculture and stewardship of HR crops must adhere to this fundamental principle.

Introduction

A number of herbicide-resistant (HR) crops have been registered in eastern Canada over the past decade. They include glyphosate-resistant, glufosinate-resistant and sulfonylurea-resistant soybean [*Glycine max* (L.) Merr.]; glyphosate-resistant, glufosinate-resistant, imidazoline-resistant and sethoxydim-resistant corn (*Zea mays* L.); and glyphosate-resistant canola (*Brassica napus* L.). Glufosinate-resistant soybean, although registered, have never been marketed in Eastern Canada. Sulfonylurea-resistant soybean, imidazoline-resistant corn and sethoxydim-resistant

corn were all marketed for a number of years but for various reasons have been withdrawn from the marketplace. The primary market for glyphosate-resistant canola is in western Canada. This manuscript will focus on glyphosate-resistant soybean and glyphosate-resistant and glufosinate-resistant corn in eastern Canada.

Glyphosate-resistant soybean was introduced in eastern Canada in 1997 (Figure 1). This technology has been rapidly adopted by soybean producers in eastern Canada with a 61 % market share in 2005, nine years after introduction (G. McGregor, Monsanto Canada Inc., personal communication, Nov. 2005). Glyphosate-resistant corn was first available in 2001 with a 4 % market share (Figure 2). Each year there was an increase in the number of hectares planted to glyphosate-resistant corn and by 2005, 21 % of the corn hectarage in eastern Canada was planted to glyphosate-resistant hybrids (G. McGregor, Monsanto Canada Inc., personal communication, Nov. 2005). Glufosinate-resistant corn was first sold in eastern Canada in 2002 and the market share has hovered around 15 % each year since its introduction (R. Chyc, Bayer Inc., personal communication, Nov. 2005). The rapid and widespread adoption of this technology by corn and soybean producers in eastern Canada strongly suggests a net economic benefit to farmers (Beckie et al. 2006). However, there are concerns with the use of this technology that need to be addressed.

Benefits of herbicide-resistant crops

Excellent crop tolerance

Glyphosate-resistant corn and soybean and glufosinate-resistant corn have excellent tolerance to the postemergence (POST) application of glyphosate and glufosinate, respectively. Very little injury has been observed with the application of glyphosate or glufosinate in HR crops. In contrast, crop injury occurs with many herbicides in conventional corn and soybean under specific field and environmental conditions (Knezevic and Cassman 2003). Crop injury with conventional herbicides may be due to incorrect application timing, extremes in weather conditions (i.e. too hot and humid, too cold and wet), soil crusting, light soil texture, low or high soil pH, low soil organic matter content, low cation exchange capacity or sensitive crop hybrids or cultivars (OMAF 2006). Generally, injury in HR crops is not as severe and does not occur as frequently as in conventional corn and soybean. This is especially evident under stressed conditions (Knezevic and Cassman 2003). Although, injury has been observed after the application of glyphosate in both glyphosate-resistant soybean and corn, this injury has been transient with little to no impact on yield (Owen 2005).

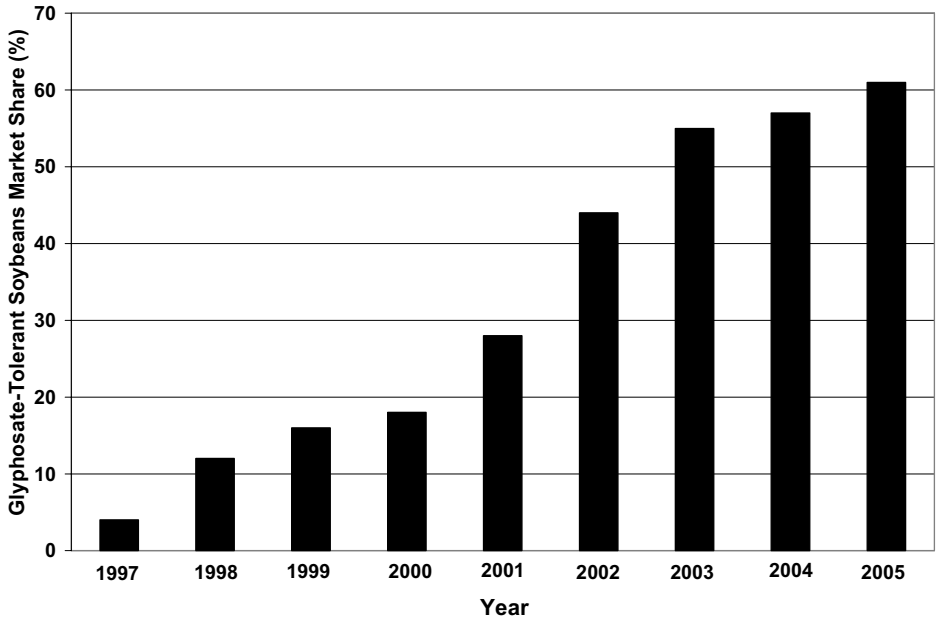

Figure 1. Percent market share of glyphosate-resistant soybean in Canada during 1997-2005.

Excellent broad-spectrum weed control

The use of HR crops has resulted in improved control of some weed species. Both glufosinate and glyphosate provide broad-spectrum control of annual grass and broadleaf weeds. Glyphosate, the most widely used herbicide in the world, is a non-selective herbicide that provides broad-spectrum control of annual, biennial and perennial weeds (Bohner 2003; Franz et al. 1997; Knezevic and Cassman 2003; Woodburn 2000). Glyphosate, with its systemic activity, effectively controls perennial weeds such as quackgrass [*Elytrigia repens* (L.) Beauv], orchardgrass (*Dactylis glomerat*a L.), johnsongrass [*Sorghum halepense* (L.) Pers.], smooth brome (*Bromus inermis* L.), and foxtail barley (*Hordeum jubatum* L.) (Knezevic and Cassman 2003; OMAF 2006; Vencill 2002). The application of glyphosate in glyphosate-resistant corn and soybean in eastern Canada has provided control of biennial and perennial weed species such as wirestem muhly [*Muhlenbergia frondosa* (Poir.) Fern.], perennial sowthistle (*Sonchus arvensis* L.), Canada thistle [*Cirsium arvense* (L.) Scop.] and horsenettle (*Solanum carolinense* L.) (OMAF 2006).

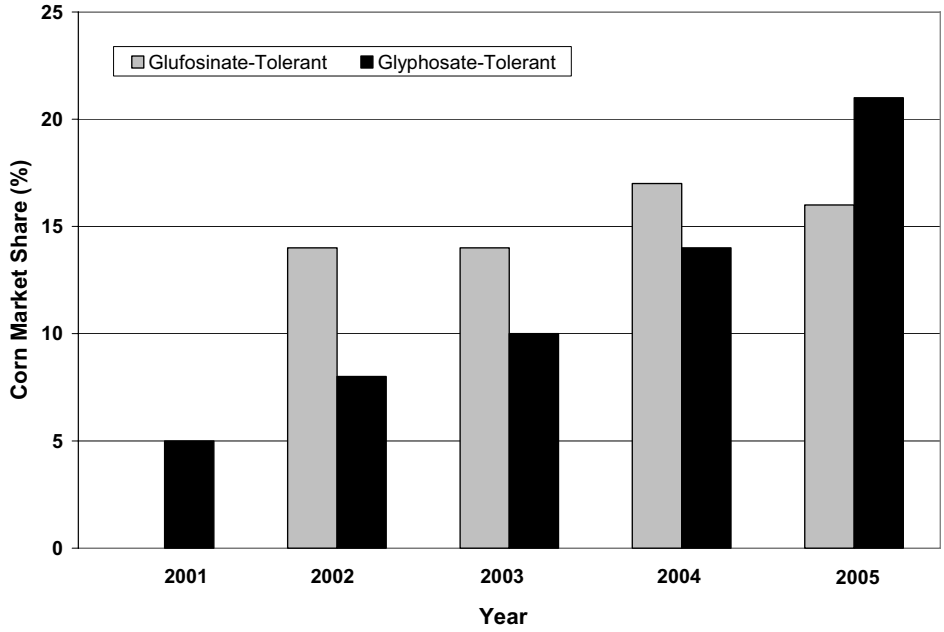

Figure 2. Percent market share of glufosinate-resistant and glyphosate-resistant corn in Canada during 2001 to 2005.

Improved consistency of weed control

Excellent weed control with herbicides in conventional corn and soybean occurs annually on most farms in eastern Canada. However in some environments, weed control with some conventional herbicides is disappointing. In contrast, weed control with glyphosate is more consistent in stressed environments resulting in improved weed control and increased crop yields (Owen 2005; Peterson et al. 2002).

Wide application window

Glyphosate in glyphosate-resistant soybean and corn as well as glufosinate in glufosinate-resistant corn have a wide window of application. The application timing for glyphosate in glyphosate-resistant soybean is from the cotyledon stage to first flower, while glyphosate and glufosinate can be applied from the 1 to 8-leaf stage in corn (Knezevic et al. 2003; OMAF 2006; Peterson et al. 2002). This is far more flexible than some conventional herbicides. For example, fomesafen is only registered from the first to second trifoliate in soybean and rimsulfuron must be applied before the three leaf stage in corn (OMAF 2006). In contrast, glyphosate and glufosinate have a wide window of application which is very important in years with frequent rain events during early crop growth.

Increased crop-rotation flexibility

Since glyphosate is strongly adsorbed to soil colloids and glufosinate is rapidly degraded by microorganisms in the soil they both allow for complete crop rotation flexibility the year after either glyphosate-resistant or glufosinate-resistant crops are grown (OMAF 2006; Vencill 2002). This is advantageous relative to some conventional herbicides. For example, there are severe crop-rotation restrictions after the application of imazethapyr and flumetsulam (OMAF 2006). In contrast, there are no crop-rotation restrictions after the application of either glyphosate or glufosinate (OMAF 2006).

Lower cost of weed control

Brookes and Barfoot (2005) concluded that the global use (including Canada) of GM crops resulted in substantial net economic benefits at the farm level nine years after their introduction. In Eastern Canada, the overall cost of weed control with HR crops is equivalent or lower than weed control with herbicides in conventional crops. In addition, crop yields are equivalent and may be higher in stressed environments resulting in greater net returns to the grower (Brethour et al. 2002; Bohner 2003; Swanton 2004).

Reduced environmental impact

Although this is more difficult to assess it is generally accepted that both glyphosate and glufosinate have little to no impact on soil microorganisms. Glyphosate and glufosinate have low toxicity to humans and animals. Since glyphosate and glufosinate decompose readily in the soil and are adsorbed to organic colloids in the soil there is reduced potential for leaching and subsequent contamination of ground water (Vencill 2002). The increased adoption of no-till and reduced-till crop production and the resultant savings in soil erosion, fossil fuel, and time can also be considered indirect environmental benefits ascribed to glyphosate-resistant crops (Owen 2005). Brookes and Barfoot (2005) concluded that the introduction of GM crops resulted in a reduction in the total kilograms of pesticides used and a 14 % reduction in the environmental footprint associated with pesticide use.

Concerns with the use of herbicide-resistant crops

Yield potential

Although the availability of HR corn and soybean in eastern Canada has provided producers with an efficacious weed management option, this has not always resulted in an increase in crop yield and net return. Unlike glyphosate-resistant and glufosinate-resistant corn that have shown no differences in yield compared to conventional corn hybrids, glyphosate-resistant soybean in variety

trials in eastern Canada have shown an average of 4 % lower yield potential than non-glyphosate-resistant soybean (Beckie et al. 2006; Bohner 2003; OOPSCC 2005). This is consistent with findings in the United States that have shown 5-10 % yield drag with some varieties of glyphosate-resistant soybean compared to non-glyphosate-resistant soybean (Elmore et al. 2001; King et al. 2001). Increased yield with non-glyphosate-resistant soybean compared to glyphosate-resistant soybean has been associated with differences in variety genetics, breeding line germplasm not being adapted for the region, and environmental conditions (Beckie et al. 2006; Bohner 2003; Elmore et al. 2001; King et al. 2001). It is expected that with the increase in use of HR soybean, plant breeders will overcome this yield drag developing newer glyphosate-resistant varieties with improved traits and higher yield potential (Bohner 2003; Knezevic and Cassman 2003). Better weed control in glyphosate-resistant soybean can overcome the lower mean yield potential and result in equivalent or a net increase in the yield compared to non-glyphosate-resistant soybean (Beckie et al. 2006; Bohner 2003).

Adventitious presence

Due to pollen drift, impure seed, volunteer HR plants and human error during seeding, harvesting, handling, transporting, storing and processing the adventitious and technically unavoidable presence of GM material in non-GMO produce occurs in areas where a substantial portion of the corn and soybean grown are HR hybrids or varieties. This negatively affects identity preserved (IP) and organic crop producers who seek a premium in the market place (Beckie et al. 2006; Brethour et al. 2002; Swanton 2004). In reality coexistence is a complex issue since most of the crops are not grown under confined conditions, and the supply chains are rarely segregated. As a result, adventitious mixing of GM material with non-GM produce can occur at all steps of production and grain handling (Devos et al. 2005). Continued vigilance on the part of HR crop growers and crop handlers is imperative. Realistic tolerance thresholds need to be established to protect this segment of crop producers.

Misapplication to non-transformed hybrids/varieties and drift to adjacent crops

The use of HR crops requires careful record keeping. The application of glyphosate or glufosinate to non-transformed hybrids/varieties results in severe crop injury and corresponding yield losses (Knezevic and Cassman 2003; OMAF 2006).

Although the potential for physical drift with glyphosate is similar to any other herbicide the consequences are dramatically different. Historically, glyphosate was primarily applied as a pre-plant burndown before the emergence of crops in adjacent fields. Consequently, there was no detrimental effect of herbicide movement since there was no crop foliage to intercept and absorb the herbicide. With the introduction of HR crops, glyphosate is now applied POST, later in the season, when crops in adjacent fields have emerged. Glyphosate is such a

biologically active herbicide that the effect of drift on adjacent crops can be very serious and result in yield losses hundreds of metres from the source of the drift (Knezevic and Cassman 2003).

Delayed application of postemergence herbicides

Since glyphosate is strongly bound by soil colloids and glufosinate is readily degraded by soil microorganisms neither of these herbicides provides any residual weed control (Vencill 2002). Consequently, many weed management practitioners delay the application of these herbicides until the majority of the weeds have emerged. If the application of the herbicide is delayed too long this can result in yield and profit losses for the grower due to early weed interference. The optimum application timing of glyphosate and glufosinate in HR crops is dependent on the relative time of weed and crop emergence, weed species composition and density, environmental conditions, fertility levels, herbicide cost (including the technology fee) and crop value. VanGessel et al. (2000) reported that the optimum glyphosate application timing in glyphosate-resistant soybean was 18 to 28 days after planting when the soybeans were in the one- to three-trifoliate leaf stage. The POST glyphosate application timing could be delayed if a preemergence residual herbicide was applied.

The delay in herbicide application results in increased weed size at the time of application. As weed size increases there is a decrease in weed control with both glyphosate (Knezevic et al. 2003; Knezevic and Cassman 2003) and glufosinate. To address the reduced efficacy on larger weeds the rate of both glyphosate and glufosinate must be increased.

Increased pressure for the selection of herbicide-resistant weed biotypes or weeds adapted to this management program

Glyphosate is an extremely efficacious broad-spectrum herbicide. Consequently, some growers have chosen to grow glyphosate-resistant crops multiple times over the past ten years. This creates an environment with an intense selective force for the selection of glyphosate-resistant weed biotypes (Holt 1992). Repeated use of glyphosate has contributed to the selection of resistant biotypes of rigid ryegrass (*Lolium rigidum* Gaudin) in S. Africa and Australia, goosegrass [*Eleusine indica* (L.) Gaertn.] in Malaysia, ryegrass in California, and horseweed (*Conyza canadensis* L.) in Delaware and Tennessee (Culpepper et al. 2001; Knezevic and Cassman 2003; Powles et al. 1998; VanGessel 2001; Vencill 2002). In addition, the frequent use of glyphosate selects for weeds with delayed emergence such as waterhemp (*Amaranthus tuberculatus* var. *rudis*), eastern black nightshade (*Solanum ptycanthum* Dunal) and fall panicum (*Panicum dichotomiflorum* Michx.) or weeds for which glyphosate is not as efficacious such as the polygonum species and Asiatic dayflower (*Commelina communis* L.) which is naturally tolerant to glyphosate, almost irrespective of the application rate (Owen 2005). There is much discussion in the literature in respect to the effect of

glyphosate rate and its impact on selection for glyphosate-resistant weeds. Neve and Powles (2005) concluded that exposure to low herbicide rates selects for individuals of rigid ryegrass with initially low-level resistance to herbicides. In an outcrossing species, such as rigid ryegrass, all minor resistance mechanisms will be selected and enriched, and will accumulate in subsequent generations leading to polygenically endowed herbicide resistance. In contrast, Kniss (2006) reported that common lamb's-quarters (*Chenopodium album* L.) plants with elevated resistance to glyphosate were selected with full- rather than half-rates of glyphosate.

Stewardship of herbicide-resistant crops

HR crops have been beneficial for weed management in corn and soybean production in Eastern Canada. It is the responsibility of all weed management practitioners to ensure that these valuable weed management tools are still effective for generations to come. History has shown that cropping systems that over-rely on the same herbicidal mode of action or the same type of crop will cause an increase in a few dominant weed species and eventually lead to weed resistance to the herbicide in question (Beckie et al. 2006; Heap 2001; Knezevic and Cassman 2003). Frequent use of glyphosate-resistant crops with in-crop glyphosate applications can lead to the selection of herbicide-resistant biotypes and the corresponding increase in additional herbicide use to control these herbicide-resistant biotypes (Beckie et al. 2006). Guidelines need to be established to protect all non-GM crop producers including IP and organic corn and soybean producers. In addition, guidelines should be established so the selection for herbicide-resistant weed biotypes is maintained at a reasonable level. Crop producers should limit their purchases of glyphosate-resistant hybrids or varieties to not more than fifty percent of their acreage in any crop year (or over a number of years). This will ensure that glyphosate-resistant crops will be grown not more than one year in two. This will reduce the selection of glyphosate-resistant weed biotypes as well as delay the shift to late-emerging weed species and those species which are naturally more tolerant to glyphosate. Long term weed management programs that consider selective forces for the selection of herbicide-resistant weed biotypes and weed shifts should be an integral component of any HR cropping system.

Summary

The use of HR soybean and corn has been a benefit to crop producers in Eastern Canada. Their use has resulted in improved control of some weed species. In addition, the use of glyphosate in glyphosate-resistant crops provides improved control of larger weeds and provides more consistent control across a range of environmental conditions. Due to the efficaciousness of glyphosate across a range

of weed species and environmental conditions there is the potential for growers to use this technology too frequently. This will result in intense pressure for the selection of glyphosate-resistant weed biotypes, weeds that emerge after the last glyphosate application and weeds that are naturally more tolerant to glyphosate. To avoid this tragedy, it is the responsibility of all people involved in weed science to ensure that this technology is used responsibly so that it will still be useful to crop producers, decades into the future.

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Weed management with herbicide-resistant crops in Western Canada

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Several herbicide-resistant (HR) crops are available in western Canada, but canola (*Brassica napus* L.) currently dominates. Imidazolinone-resistant wheat (*Triticum aestivum* L.) was introduced in western Canada in 2004 and will likely play a larger role in the future. HR soybean (*Glycine max* L.) and HR corn (*Zea mays* L.) are relatively minor western Canadian crops. The three currently available HR canolas have novel traits that enable them to tolerate glyphosate, glufosinate or imidazolinone herbicides. The primary weed-related issues associated with HR crops are: 1) weed shifts, 2) herbicide resistance, and 3) HR volunteers. Weed control, yields and net returns in HR crops are generally greater than in non- HR crops; as a result they can be overused. However, current HR crop frequency issues in western Canada are probably minor when compared to the United States. From a weed resistance and herbicide residue point of view, imidazolinone-resistant wheat and canola, and the extensive use of imidazolinones and sulfonylureas in several other crops, are potentially more problematic than glyphosate- or glufosinate-resistant canola. The volunteer canola management issue has often been over-stated. When necessary, the extra cost of controlling volunteer glyphosate-resistant canola in pre-plant, burn-off applications has not been a deterrent for low-disturbance, direct-seeders. Other issues such as: potential for lower species diversity from unprecedented levels of weed control in HR crops; the recent rapid reduction in the number of herbicide manufacturers; or reduced investment in herbicide discovery, may be more important in the future.

Introduction

Canola is the dominant herbicide-resistant (HR) crop in western Canada. In 2005 HR canola occupied approximately 95 % of the western Canadian canola market (Beckie et al. 2006). HR canola has been primarily glyphosate-resistant (GLYR), but glufosinate-resistant (GLUR) and imidazolinone-resistant (IMIR) canola are also important. GLYR and GLUR are transgenic while IMIR was developed using mutagenesis, a conventional breeding technique.

In addition to HR canola, there are several other HR crops marketed in western Canada, but these are relatively minor players. IMIR wheat was introduced in 2004 and approximately 100,000 ha were grown in 2005. HR soybean and HR corn also occupy rather small areas in western Canada. IMIR lentil will be available in western Canada in 2006. Given the lesser role of these "other" HR crops in western Canadian cropping systems, the major focus of this chapter will be on HR canola.

In canola, advances in herbicidal weed management have been relatively rapid. By the time the first canola-quality cultivars were developed in the mid- to late-1970s (CCC 2006, Downey and Rakow 1987), dinitroaniline herbicides developed for rapeseed and some other crops were already available to control a variety of important weeds (Friesen and Bowren 1973). In the 1980s, selective postemergence graminicides were introduced to control annual (Chow et al. 1983) and perennial grasses (Harker and O'Sullivan 1993). These herbicides raised monocot weed management expectations in canola to a relatively high level. Specialty herbicides such as clopyralid for Canada thistle (*Cirsium arvense* (L.) Scop.) (O'Sullivan and Kossatz 1984), and ethametsulfuron for *Brassica* weeds closely related to canola (Blackshaw 1989; Swanton and Chandler 1989) increased the spectrum of weeds that could be effectively managed in canola.

Herbicide combinations were useful, but in some cases costly (Blackshaw and Harker 1992); some combinations resulted in canola injury and yield loss (Harker et al. 1995). However, even the best conventional herbicide combinations had suboptimal activity on species such as false cleavers (*Galium spurium* L.), cow cockle (*Vaccaria hispanica* (Mill.) Rauschert), and stork's-bill (*Erodium cicutarium* (L.) L'Hér. ex Aiton).

The first HR canolas were triazine-resistant (Beversdorf and Hume 1984). Triazine-resistant varieties improved weed management levels in canola, but the yield penalty associated with those varieties (Beversdorf et al. 1988) severely restricted their adoption. After the three major HR canolas (GLUR, GLYR, IMIR) were approved for unconfined release into the Canadian environment (CFIA 1995a, 1995b, 1995c), they and their respective postemergence herbicides were rapidly adopted. Individual HR canola herbicides were broad-spectrum and led to little or no concern for crop injury.

HR versus non-HR crop comparisons

Weed management

It is not surprising that weed control levels in HR crops have been greater than in non HR crops; improved weed management was the major goal of HR crop development. In the United States, GLYR- corn, cotton (*Gossypium hirsutum* L.) and soybeans have increased weed management levels in all three crops (Askew and Wilcut 1999; Ateh and Harvey 1999; Faircloth et al. 2001; Johnson et al. 2000; Reddy and Whiting 2000). In Canada, HR canola allowed producers to manage previously difficult weed species (Beckie et al. 2006, Devine and Buth 2001; Harker et al. 2000; Stringham et al. 2003). Species such as false cleavers, stork's-bill, cow cockle, Canada thistle, and several sowthistle species (*Sonchus spp.*) are less challenging problems since the adoption of HR canolas.

GLYR canola systems often provide a higher level of weed management than either GLUR or IMIR canola systems (Harker et al. 2000, Harker et al. 2004). Prior to HR canola introduction, canola was reserved for fields where weeds were not a major challenge. Soon after GLYR introduction, canola became the crop to grow in fields where weed management challenges were greatest.

Economics and environment

Transgenic canola production (GLYR and GLUR) led to higher net returns than conventional herbicide systems (CCC 2001; O'Donovan et al. 2006). Less tillage, lower fuel costs and higher yields were all more common in GLYR and GLUR canolas than in conventional herbicide systems (CCC 2001). Over the long-term, it is possible that the soil conservation benefits more commonly associated with HR crops will be the most important advantage over conventional crops. However, O'Donovan et al. (2006) also determined that total herbicide active ingredient entering the western Canadian environment was lower with the GLYR system than with most of the traditional regimes, especially when glyphosate was applied only once in-crop.

High weed management levels in IMIR canola systems versus conventional canola systems (Harker et al. 2000) may have similar economic benefits as GLYR and GLUR systems. However, there are no current studies in canola to confirm economic advantages of IMIR over conventional canola systems. It is notable that the highest yielding canola cultivars available today are usually HR hybrids (CCC 2005b). The combination of superior genetics and superior weed management will help ensure the economic advantage of the three major HR canola systems.

From an economic risk point of view, not all HR canolas and their associated management practices are equal. For example, GLYR canola was more commonly profitable that IMIR or GLUR canola (Upadhyay et al. 2006). In another study, GLUR canola hybrids led to a higher mean net return than open-pollinated cultivars, and spring seeding was more risk efficient than fall (dormant) seeding

(Upadhyay et al. 2005). Thus, it is reasonable to assume that poorly managed HR canola could lead to lower economic returns than well managed non-HR canola; the domain of high economic returns is not the exclusive territory of HR canola.

From 1995 to 2000, Brimner et al. (2005) found that production of all HR canolas versus conventional canola reduced herbicide active ingredient applied ha^{-1} by 42.8 %. Reduction in herbicide-use intensity combined with the lower environmental impact quotient of herbicides used in HR canola led to a 36.8 % reduction in environmental impact ha^{-1} for HR versus conventional canola production. HR canola systems increased use of low application rate herbicides, reduced total herbicide applications, and decreased the need for herbicide combinations (Brimner et al. 2005). In general, the high adoption level of HR canola systems by Western Canadian canola growers has led to environmental as well as economic benefits.

HR crops and weed shifts

Weed populations shift when a particular management practice does not control all species in a population equally. When weed management practices such as tillage, crop rotation or herbicide are changed, weeds populations shift over time (Ball 1992; Buhler et al. 1997) depending on the relative effectiveness of individual practices. High efficacy herbicides can cause rapid weed shifts.

The repeated use of GLYR crop systems exerts strong selection pressure on weed populations. In the United States, weed shifts have been documented in GLYR as well as other HR crops (Hilgenfeld et al. 2004; Marshall et al. 2000; Reddy 2004). In a western Canada study, 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. 2005b). Conversely, rotations which excluded in-crop glyphosate were associated with greater populations of green foxtail (*Setaria viridis* (L.) P. Beauv.), redroot pigweed (*Amaranthus retroflexus* L.), sowthistle (*Sonchus spp*.), wild buckwheat (*Polygonum convolvulus* L.) and wild oats (*Avena fatua* L.) at several locations. Diversified weed management practices will reduce the tendency for weed populations to shift in response to a single repeated strategy. Diversified practices may include cultural practices such as varied seeding dates, as well as using herbicides other than the one the specific HR crop is designed to tolerate.

Glyphosate-resistant spring wheat development in North America was suspended in May 2004. Therefore, it is not currently possible to utilize in-crop glyphosate continuously in wheat-canola rotations. However, in areas where corn, cotton, or soybean are more dominant crops, it is possible and sometimes common, to utilize in-crop glyphosate year after year. Moreover, sequential glyphosate applications in HR crops are also common.

Similar weed shift concerns are just as valid in rotations that overuse IMIR systems. In Canada, GLUR systems are only available in canola and corn, and unlike glyphosate, can only be applied in-crop. Therefore, the risk of enhancing weed population shifts or weed resistance is probably lower for GLUR than either IMIR or GLYR systems.

It should be pointed out that the consequences of weeds shifts are not necessarily negative. In agroecosystems, weed shifts are problematic only when they favour "difficult-to-control" weeds. The impacts of weed shifts in natural ecosystems are difficult to determine, but should be viewed in a context which acknowledges that cropping practices *per se* impose a high level of disruption in natural ecosystems (Froud-Williams 1988).

HR crops and weed resistance

Perhaps, just as important as improved weed management, GLYR and GLUR provide opportunities for growers with weed resistance concerns or problems. In-crop glyphosate or glufosinate provide growers with a reprieve from the extensive use of acetyl-CoA carboxylase (ACCase) or acetolactate synthase (ALS) inhibitor herbicides; the latter herbicides pose a much higher weed resistance risk than the former (Beckie 2006). Currently, there are confirmed reports of 18 weed species (most at multiple sites) resistant to ACCase or ALS inhibitors in Canada (Heap 2006).

Glyphosate-resistant weeds have not been confirmed in Canada, although eight weed species have been confirmed to be resistant to glyphosate elsewhere (Heap 2006). Worldwide, there are no reports of weed resistance to glufosinate.

On the other hand, IMIR systems may only exacerbate the ALS herbicide-resistant problem. In addition to IMIR canola, IMIR wheat (2004) and IMIR lentil (*Lens culinaris* Medik.) (2006) are approved for cropping in Canada. Given the fact that imidazolinones are also commonly applied in naturally tolerant field pea (*Pisum sativa* L.) and bean (*Phaseolus vulgaris* L.) crops, and that ALS inhibitor sulfonylureas are widely applied in small-grain cereal production, there can be a very high level of selection pressure for weed resistance to ALS herbicides in some rotations (Table 1). For example, in a wheat–canola–barley–pea rotation, it is possible, however unlikely, to apply ALS herbicides for monocot and dicot weed management in all crops. Western Canada survey respondents reported "back-to-back" applications of ALS inhibitors on 25 to 37 % of their cropped acres; imidazolinone herbicides constituted the most common repeated sequence (Johnson et al. 2005).

In addition to enhancing selection pressure by controlling successive flushes of weeds, ALS herbicide residues can also cause injury in following susceptible crops when environmental conditions and soil characteristics restrict herbicide degradation (Shaner and Hornford 2005). Johnson et al. (2005) have also

shown that crop-damaging residues of two or more different ALS herbicides applied in successive years can accumulate over time, especially at low soil organic matter sites which have received lower than average precipitation. With several IMIR crops on the western Canadian market, it will be important to follow procedures to reduce weed resistance and herbicide residue problems. Stewardship guidelines have been developed for IMIR lentils (CFIA 2004a) and IMIR wheat (CFIA 2004b), which, if followed, will mitigate problems associated with IMIR crop production.

Table 1. Opportunities for specific in-crop herbicide use in some western Canada crop rotations involving HR and conventional crops.

Glufosinate or Glyphosate (Group 9 or Group 10)		**Imidazolinone or Sulfonylurea (Group 2)**	
Crop Sequence	Use ratio*	Crop Sequence	Use ratio*
Wheat (W)–Fallow	0:1	***Wheat (W)***–Fallow	1:1
W-W	0:2	***W-W***	2:2
Wheat–**Canola** *(C)*	1:2	***W–Canola (C)***	2:2
C-C	2:2	***C-C***	2:2
Barley–***C***–W–Peas	1:4	***Barley–C–W–Peas***	4:4
W–Lentils–W–***C***	1:4	***W–Lentils–W–C***	4:4

*Maximum use ratio for possible herbicide applications in conventional or HR crops. Crops resistant (HR) or naturally tolerant to the respective herbicide groups are shown in bold italics.

The overuse of HR crops

Western Canada: The superior weed control and corresponding yield benefits associated with HR crops tend to encourage over-use of specific HR crop herbicides and to weed resistance. However, with the exception of IMIR systems (Table 1), current western Canadian crop rotations have relatively minor HR crop frequency concerns. Less than 15 % of western Canada prairies acres that are treated with herbicide receive in-crop glyphosate (Beckie et al. 2006).

Eastern Canada: Significant portions of cropland in southern Ontario and Quebec can be in corn-soybean rotations. These rotations afford the opportunity to apply in-crop glyphosate continuously, although GLYR corn does not currently have a large market share in eastern Canada. Perhaps more worrisome is the increasing prevalence of continuous GLYR soybean in southern Ontario (Beckie et al. 2006).

United States – soybeans and cotton: Neither western nor eastern Canada has the HR crop frequency issues that are commonplace in GLYR crops in the southern United States. Relatively recent confirmations of glyphosate resistance in Canada fleabane (*Conyza canadensis* (L.) Cronquist) (VanGessel 2001), common ragweed (*Ambrosia artemisiifolia* L.) (Sellers et al. 2005), and Palmer amaranth (*Amaranthus palmeri* S. Wats.) (Heap 2006) in the United States, are probably due to the lack of weed management diversity in GLYR cropping systems.

Young (2004) suggested that the rapid, large-scale adoption of glyphosate-resistant soybean and cotton in the United States has led to major shifts in herbicide-use patterns. In the last several years, there has been "over a 3-fold increase in kg of glyphosate applied per year in cotton and soybean" (Young 2004). Recently, GLYR cotton tolerance was improved to allow in-crop glyphosate application beyond the four-leaf stage of cotton (Keeling et al. 2006).

In Delaware, where horseweed was first confirmed to be resistant to glyphosate, VanGessel (2001) stated that "many no-tillage fields have been treated only with glyphosate". The latter author also noted that because soybeans are more drought-tolerant than corn, growers have planted soybeans continuously.

In Ohio, giant ragweed was not adequately controlled after two glyphosate applications (Stachler and Loux 2006) due to the following weed management regime: "The field had been planted with glyphosate-resistant soybeans for at least 4 continuous years, and treated with glyphosate exclusively during this period." Other glyphosate-resistant weeds are on the horizon (Zelaya and Owen 2005). Therefore, given current glyphosate usage patterns, it is highly probable that more glyphosate-resistant weeds will soon be added to the confirmed glyphosate-resistant list (Dotray and Wilcut 2004; Heap 2004).

The dilemma: In terms of weed resistance, the high level of in-crop weed control with new modes of action associated with HR crops is a "double-edged sword". On the one hand, high efficacy reduces seed production, and therefore, the number of possible weed genotypes that are subjected to subsequent herbicidal selection pressure is minimized (Beckie 2006). On the other hand, high efficacy equates with high selection pressure on the weedy genotypes that are available for selection (Gressel and Segel 1990). It is difficult to determine which of the latter issues is more important for weed resistance development. It is likely that the importance of weed numbers versus selection pressure levels would vary among weed species and depend on inherent biological traits such as seed production potential and seed dormancy. Given the high efficacy of herbicides used in HR canola, relative frequency of IMIR (high), GLYR and GLUR alleles (low) in weed

populations will also influence how fast resistance develops. In any case, HR crops have improved economics and simplified weed management to such an extent that even with confirmed resistance, and more resistance looming, many growers resist switching herbicides or employing other weed management solutions. Such growers often add a tank-mix partner to curb resistance development or to control the resistant weed.

Weed volunteers from HR crops

Volunteer crops pose particular threats when they occur in crops where control measures are limited, expensive or nonexistent, or when problematic densities emerge before the crop in direct-seeding systems. In the latter case, because glyphosate is the almost exclusive choice for pre-seed herbicide application in direct-seeding systems, GLYR volunteers can be much more troublesome than other HR crop volunteers.

Glyphosate-resistant wheat volunteers

The possible introduction of glyphosate-resistant wheat raised concerns regarding pre-seeding control costs for glyphosate-resistant volunteers, especially in direct-seeding systems (Anderson and Soper 2003; Lyon et al. 2002; Saskatchewan Soil Conservation Association 2001). A survey of no-till growers indicated that without an inexpensive and effective alternative to glyphosate for volunteer wheat control, few if any of them would be willing to grow glyphosate-resistant wheat (Ogg and Isakson 2001). In a multi-site western Canadian study, almost all wheat volunteers emerged in the year following wheat production (Harker et al. 2005a). At only one of eight sites were volunteer wheat densities in direct-seeding canola plots great enough (4 plants m^{-2}) to justify an additional herbicide in the pre-seed glyphosate application. The most important volunteer management consideration was to prevent newly recruited volunteers from producing seed in the year following wheat production. A separate western Canada study indicated that poorly controlled volunteers in the year following glyphosate-resistant wheat production could lead to problematic wheat densities for an additional year (Harker et al. 2005b).

Glyphosate-resistant canola volunteers

Considering the fact that canola harvest losses in Saskatchewan fields averaged 3,000 viable seeds m^{-2} (Gulden et al. 2003a), it is surprising that volunteer canola is not one of the most serious weed threats in Canada. In Quebec, the average density of volunteer canola in the year following canola crops was 5 plants m^{-2} (Simard et al., 2002). In western Canada post-management weed surveys, in fields where volunteer canola was detected, average canola density across the

Prairie Provinces was 4.3 plants m^{-2}, (Leeson et al., 2001; 2002; 2003). Across all surveyed fields, volunteer canola density averaged 0.5 plants m^{-2}.

The introduction of HR canolas has not significantly changed volunteer canola management issues. The vast majority of glyphosate-resistant canola volunteers are recruited in the year following canola production (Harker et al. 2006). The latter study also determined that preventing seed production of canola volunteer plants in the year following canola reduced volunteer densities in subsequent years to levels that would not require herbicidal intervention in those crops. Low-disturbance or no-till seeding systems that increase canola seed mortality and restrict secondary dormancy induction (Gulden et al. 2003b; Mohler 1993; Pekrun et al. 1998) are also likely to reduce glyphosate-resistant canola volunteers.

Despite the fact that HR canolas dominate the market, post-management surveys indicate that volunteer canola across western Canada have actually decreased in relative abundance over the last ten years (Leeson et al. 2005). Over the same time period, the relative abundance of non-HR volunteer wheat in western Canada has increased. Therefore, it is difficult to associate any recent volunteer canola weed management challenges with HR crop production.

Although pollen flow among different HR canolas has led to multiple-resistant volunteers at some sites (Hall et al. 2000), all canola volunteers are easily managed with relatively low-cost alternative herbicides (Johnson et al. 2004a). Indeed, the extra cost of controlling volunteer GLYR canola in pre-plant, burn-off applications has not generally been a deterrent for low-disturbance, direct-seeders (CCC 2005a). Without ignoring the validity of some concerns, the GLYR volunteer management issue has often been overstated.

HR crops and integrated weed management

Opportunities to practice integrated weed management are dependent upon the state of the weed community in a given field. When weed population densities are relatively low, cultural weed management practices without herbicides are more likely to be successful (Harker et al. 2003; Liebman and Davis 2000). Because HR crops are generally associated with high levels of weed management, they make it possible to more effectively practice integrated weed management in future rotational crops. Therefore, HR crops can play an indirect role in reducing herbicide dependence.

Integrated weed management opportunities in canola have broadened since HR canolas were introduced. For example, seeding canola in the fall immediately before soil freeze-up (fall- or dormant-seeded canola), helps canola to avoid the hot, dry period during flowering with subsequent improvements in canola yield and quality (Kirkland and Johnson 2000). The latter authors noted that prior to HR canola introduction, the lack of effective weed control options made fall-seeding

impractical. Fall-seeded canola facilitates integrated weed management by introducing operational (seeding date) diversity into canola production which serves to vary seeding date selection pressure on weed communities (Harker and Clayton 2003). Currently, other factors constrain the wide-spread adoption of fall-seeded canola (Clayton et al. 2004a; 2004b; Johnson et al. 2004b), but these constraints may be removed in the future.

Other HR crop issues

At first glance, high levels of weed control are desirable; they optimize crop yields and economic returns, and minimize weed management challenges in subsequent years. However, there are concerns that high levels of weed management associated with HR crops may reduce biodiversity (Freckleton et al. 2004; Watkinson et al. 2000). In a non-HR conventional herbicide study, Taylor and Maxwell (2001) found that untreated plots were weedier and supported a greater biomass of vegetation-dwelling arthropods and a higher number of ground-dwelling beneficial arthropods. They concluded that birds may suffer negative impacts from conventional herbicide weed management. Similar effects are likely in HR crops since herbicide efficacy is usually greater than in non-HR crops.

Weed management levels can also influence insect pests. In GLYR canola it is common to apply glyphosate relatively early to avoid losses due to weed competition (Clayton et al. 2002, Martin et al. 2001). In addition, GLYR canola growers often unnecessarily apply in-crop glyphosate sequentially (O'Donovan et al. 2006). Dosdall et al. (2003) hypothesize that early and complete weed removal fails to discourage root maggot (Diptera: Anthomyiidae) female egg-laying habits in canola due to the fact that gravid females lay more eggs in canola canopies without weeds. In other words, high efficacy weed management that is commonly associated with HR crops is not a desirable outcome for all pests.

Given the fact that we know very little about overall ecosystem interactions, it is likely that high levels of weed management in HR crops will have many other unforeseen impacts (positive or negative) on non-target organisms. Long-term cropping system studies which determine ecological impacts of HR crops in agroecosystems will be valuable (FAO 2003; Heard et al. 2003).

Ironically, the success of HR crops has negatively impacted the herbicide industry itself. Dotray and Wilcut (2004) suggested that "if glyphosate usage continues to increase, the industry incentive to support existing and older active ingredients may decrease." Duke (2005) acknowledged that the widespread adoption of HR crops has already reduced the value of the remaining herbicide market and led to dramatic reductions in the world-wide herbicide industry. As a result, there are fewer companies to invest in herbicide discovery solutions for current and future weed management problems. The latter problem is a major

concern in lower acreage “minor” crops where herbicide options are limited and dependent upon new herbicide registrations in major crops.

Summary

HR crops in western Canada have increased rapidly over the last 10 years and will probably continue to do so. HR crop production has improved weed management and increased net returns to farmers. Western Canadian farmers have managed HR canola volunteers that some have suggested were unmanageable. Although there are many detractors suggesting serious possible consequences arising from HR crops, the already documented soil conservation benefits and reduced fossil fuel usage associated with HR crop production should not be lightly dismissed. The challenge with HR crops is to utilize them moderately so we can continue to exploit their benefits for many years.

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Herbicide-resistant canola – the first ten years

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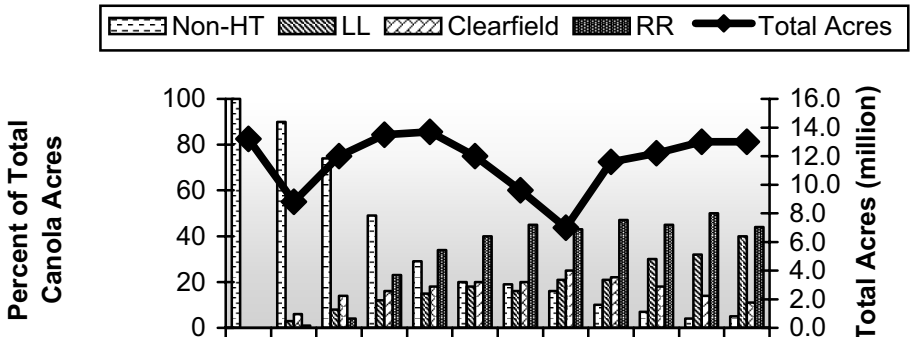
Herbicide-resistant (HR) canola systems, Roundup Ready® (glyphosate-resistant), Liberty Link®/InVigor® (glufosinate-resistant hybrid canola) and CLEARFIELD™ (imidazolinone-resistant), have been rapidly adopted by canola growers and now comprise over 90 % of the canola acres in western Canada. Studies commissioned by the Canola Council of Canada showed that growers choose HR systems primarily because of more effective weed control, increased profit and more flexible rotations. Growers reported that they rarely targeted volunteer canola as their primary weed in subsequent crops and that there were few differences in volunteer management amongst the different systems. Growers reported an increased yield and profit from Roundup Ready and Liberty Link/InVigor systems of $5.80 acre. They also reported other benefits such as reduced dockage, less tillage, reduced herbicide costs and less herbicide used. Total economic impact of transgenic HR canola was estimated at Can$ 464 million from 1997-2000.

Introduction

In western Canada there are three commercially available systems of herbicide-resistant (HR) canola. Roundup Ready® (glyphosate-resistant) and Liberty Link®/InVigor® (glufosinate-resistant hybrid canola) are considered genetically modified (GM) and were developed through transgenic methods. CLEARFIELD™ (imidazolinone-resistant) canola is not GM as it was developed through mutagenesis. Since the approval and introduction of these systems in 1995 and 1996, western Canadian canola growers have rapidly adopted this technology. In 2006 growers harvested 13 million acres of canola of which 44 % was Roundup Ready (RR), 40 % was Liberty Link (LL) and 11 % was CLEARFIELD™ (CL) canola (Figure 1). Non-HR canola was grown on only 5 % of the acres.

Growers have adopted HR technology for a variety of reasons, but primarily due to more effective weed control. Acceptable weed control is a weakness in conventional canola production and as canola is less competitive than cereals, significant yield losses due to weeds can occur. This adoption has occurred in spite of market uncertainties regarding genetically modified organisms, technology use agreements and higher seed costs. Not all HR canola that has been introduced into Canada has been readily adopted. Triazine- and bromoxynil-resistant systems were less effective, not adopted by growers and are no longer available.



	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Non-HT	10	90	74	49	29	20	19	16	10	7	4	5
LL	0	3	8	12	15	18	16	21	21	30	32	40
Clearfield	0	6	14	16	18	20	20	25	22	18	14	11
RR	0	1	4	23	34	40	45	43	47	45	50	44
Total Acres	13.	8.8	12.	13.	13.	12.	9.6	7.0	11.	12.	13.	13.

Figure 1. Acres of herbicide-resistant canola grown in western Canada, 1996-2006 (information compiled from Statistics Canada and seed developers' estimates by the Canola Council of Canada; LL = Liberty; RR = Roundup Ready.)

As the technology became more widely adopted questions arose about why growers chose or did not choose the technology, the economics of the systems and the impact on volunteer canola management. In response to these questions, two studies were commissioned by the Canola Council of Canada to determine the impacts of herbicide-resistant canola on agronomics, including volunteer canola management, and economics. This paper summarizes these detailed studies. The "Agronomic and Economic Assessment of Transgenic Canola" (Serecon Consulting Group 2000) was conducted to determine the impact of the Roundup Ready and Liberty Link/InVigor systems on agronomic practices and economics. This first study did not include the management practices and costs of managing the canola volunteers in following crops, so in 2005 the "Herbicide Tolerant Volunteer Management in Subsequent Crops" (Serecon Consulting Group 2005) study was conducted. This study included all three HR systems, Roundup Ready, Liberty Link/InVigor, and CLEARFIELD™.

Methods

The objective of the "Agronomic and Economic Assessment of Transgenic Canola" survey (Serecon Consulting Group 2000) was to qualify and quantify the agronomic and economic benefits associated with transgenic canola to better understand the impact it has had on agriculture in western Canada. The study included an analysis of an extensive producer survey, thirteen case studies in various production areas of western Canada, and an integrated industry economic model. It looked only at the transgenic HR systems (RoundUp Ready and Liberty Link/InVigor) and did not include the CLEARFIELD™ HR system. Conventional varieties were all those that are not transgenic and are not part of a herbicide-resistant system. Six hundred and fifty growers that grew over 80 acres of canola in western Canada were surveyed by telephone on their attitudes, production practices and production costs. Quotas were set so the survey sample would reflect the distribution of Polish (*B. rapa),* Argentine *(B. napus hybrid)* and Argentine *(B. napus open-pollinated)* for the conventional sample, and the distribution of Roundup Ready and Liberty Link for the transgenic sample. The geographic distribution of the sample was also controlled to ensure that the sample represented the distribution of canola farms by province and ecozone as determined by Statistics Canada. The refusal to complete ratio was 0.87:1, which is considered very good for a telephone survey of this type, particularly given that no incentives to respond were provided. One half of the growers answered questions on their transgenic canola fields, while the other half answered questions on their conventional canola fields. In addition, 13 case studies were conducted with growers who grew both transgenic and conventional varieties and could provide detailed information on their production and costs from 1997 to 2000.

The second survey, "Herbicide Tolerant Volunteer Management in Subsequent Crops" (Serecon Consulting Group 2005), included two information sources. The first was the 2001, 2002 and 2003 Prairie Weed Survey conducted by Agriculture and Agri-Food Canada in cooperation with the three prairie provinces (Thomas and Leeson, unpublished data). Data included summer weed counts (Thomas 1985) as well as the results of a grower-completed management survey for 316 fields previously planted to canola. The second included a telephone survey of 335 western Canadian canola growers regarding their 2004 volunteer canola weed management practices. The survey sample was managed to approximate the distribution of canola acres by province, according to estimates provided by the respective provincial canola organizations. Minimum quotas of 100 growers were established for each of the three-herbicide-resistant (HR) systems (Roundup Ready, Liberty/InVigor and CLEARFIELD™ as well as non-HR conventional canola. Because some growers grew more than one system, the total sample size was less than 400. The refusal to complete ratio was 2.3:1 which was considered good considering the survey was conducted in mid-summer and no incentives were provided. An additional component of the study was a series of simulations to

determine herbicide options and costs for controlling volunteer canola given different crop and target weed scenarios.

Results

The results presented here are subsets of the results of the complete studies as outlined above. They provide a snapshot of the key findings of the grower responses and the impact of HR canola on agronomic practices and economics.

Growers' reasons for growing or not growing transgenic HR canola

The majority of growers surveyed believed that there are significant advantages to transgenic canola. Participants in the survey and in the case studies stated that their primary reason for adopting transgenic canola was not economic, but agronomic (Serecon Consulting Group 2000). The transgenic system is simple, the weed control is early and effective, and the system fits well into a reduced or no-till operation. The key benefit and motivator to adopting transgenics was more efficient weed control (50 % of growers overall; 18 % for grassy weed control; 15 % for broadleaf weed control) and ease of herbicide management in preventing weed resistance. Other reasons, related to weed management, included cleaning up their fields (7 %), reducing the number of passes to control weeds and perennial weed control. Transgenic growers reported that, due to the ability to control weeds in fields where they would not have grown canola, their rotations were more flexible. Some producers reported better yields, higher returns, and the ability to reduce costs and generate more profit (19 % of growers). Other reasons for choosing transgenic varieties were to reduce tillage, seed earlier, conserve moisture and to compare transgenic varieties to conventional canola on a trial basis.

Growers mentioned a variety of reasons for not choosing transgenics. The most common were cost related, including the Technology Use Agreement (TUA) for RoundUp Ready varieties (19 % of growers) and the overall costs of the transgenic systems (18 % of growers). Growers were also concerned with market access for their crop (16 %), weed resistance (9 %) and unknown "health concerns" related to GMOs (9 %).

Weed control and volunteer canola management

Of those growers planting transgenic HR varieties, over 80 % of transgenic growers said that weed control was more effective and 59 % said herbicide management to delay weed resistance was easier. The study on volunteer management showed that management practices are unique to grower, field conditions and weed spectrum and are confounded by the fact that growers often plant multiple systems and rotate systems from year to year. For example, just one in five of the 2003 conventional growers surveyed in 2004 had grown only conventional varieties over the past five years. There are also many combinations of

subsequent crops (although spring wheat is the most common crop planted following canola), target weeds, available herbicides and tillage options. Weed management appears to have more to do with the subsequent crop and the full spectrum of weeds to be controlled, rather than the previous canola system. The percentage of fields with volunteer canola observed during the summer weed count was very similar across all systems (range 24 to 35 % of fields), and rarely exceeded economic thresholds for treatment as defined by the growers at 10 plants per square metre (Serecon Consulting Group 2005).

There were more similarities than differences in volunteer canola management between the systems, including conventional management. Overall, 82 % of the growers targeted volunteer canola with weed control measures. However, volunteer canola was rarely targeted as the primary broadleaf weed to be controlled with a herbicide application, regardless of system, as only 16 % mentioned volunteer canola as one of the top five weeds that they most often target with weed control measures in an average year. Overall, more serious weed problems were cited, including wild oats, wild buckwheat, Canada thistle, and other broadleaf annuals. Those respondents that targeted volunteer canola were asked if they were targeting this weed with control measures more so, less so or about the same as they did five years ago. Sixty per cent had made no change, 30 % were targeting it more, and 3 % less (Serecon Consulting Group 2005). The reported increase in targeting volunteer canola was not exclusive to herbicide-resistant varieties planted in 2003; those with a history of planting conventional canola also reported a change in targeting volunteers.

As few growers target herbicide treatments or tillage operations specifically for volunteer canola, the ability to compare costs with a good degree of confidence was limited. However, the study looked at three different sources of information and the results from the three lines of investigation were highly consistent. The incidence of herbicide use and tillage to control canola volunteers was similar across systems and was not exclusive to HR varieties. Slightly more Roundup Ready than conventional growers said they targeted volunteer canola with a herbicide application, although the percentage of acres treated (fewer than 20 % of the acres following canola) was similar across systems. There were no significant differences between systems in the costs of volunteer canola control, as estimated by the growers themselves; at $10-13 per acre treated with herbicides, and $5 per acre for tillage/harrowing operations.

It is somewhat difficult for growers to identify particular treatments for volunteer canola in isolation from their total weed management program. However, in the total weed management program, the herbicide options and timing of application (most often in-crop) were similar across canola systems. Group 2 and 4 herbicides used most often for in-crop volunteer canola control. The exception was CLEARFIELD™ volunteers that are resistant to specific Group 2 herbicides and therefore Group 4 is most commonly used.

Volunteer canola control two years after the canola crop was infrequent, but was conducted in both HR and conventional systems. Volunteer canola two years after the canola crop was comparable to volunteer grains two years after the grain crop, suggesting volunteer canola was no more persistent than grain volunteers.

Yield, dockage and grade

On average, transgenic systems resulted in a 3 bu per acre or 10 % yield advantage over conventional varieties in 2000 (Serecon Consulting Group 2000). Several factors that affect yield could be responsible for this increase, including: higher yielding varieties, early seeding and better weed control. Earlier seeding conserves soil moisture, produces more competitive plants and allows the crop to avoid high summer temperatures which are detrimental to flower and pod development. Dockage was significantly reduced in the transgenic samples. Transgenic growers reported 3.87 % dockage compared to conventional growers at 5.14 %. This difference is largely attributed to more effective weed control. There was no difference in grade between the two systems.

Less tillage and summerfallow

Growers use tillage to control weeds and prepare the soil for planting. However, excessive tillage can be detrimental to soil quality. Since the early 1990s, growers have been reducing their tillage operations for soil conservation benefits and the number of growers practicing direct-seeding or zero tillage has increased. Prior to the introduction of transgenic canola varieties, canola growers used tillage for weed control or incorporating herbicides prior to seeding the crop. With transgenic herbicide-resistant varieties, weed control can be done “in crop” allowing producers to direct-seed without pre-seeding tillage and thereby reaping the benefits of soil conservation. Transgenic growers are able to seed earlier in the spring, or in the fall, therefore realizing benefits from soil moisture.

The study showed that transgenic growers reduced the number of tillage operations compared to conventional growers. Half of transgenic growers practiced direct-seeding (50 % transgenic compared to 35 % conventional) and 26 % said their use of conservation or no-till practices has increased due to planting transgenics. This equates to an additional 2.6 million acres of canola with fewer tillage operations. Summerfallow is used by growers to conserve soil moisture. This can leave the soil exposed to erosion and cultivation for weed control can damage soil texture and reduce organic matter. Conventional growers are more likely to use summerfallow in their rotations (36 % had summerfallow in 2000 compared to 18 % of the transgenic growers).

Fuel savings of 31.2 million litres in one year

A determination of the difference in fuel consumption was made between transgenic and conventional canola production based on operating costs. Overall, there were added operating costs for conventional canola production due to the

greater emphasis on tillage and herbicide applications. From the per acre unit analysis, the net difference in operating costs for all tillage, harrowing, fertilizer, and chemical herbicide applications was determined. This information was then used to determine differences in fuel consumption. From the added operating costs of conventional production systems, the proportion of fuel cost was estimated, and from this, the number of litres this cost represented. The estimate of fuel savings was determined by the product of the fuel saving per acre used by transgenic canola production system, and the number of acres under transgenic production in each of the four analysis years.

Fuel saved by transgenic growers varied from 9.5 million litres in 1997 to 31.2 million litres in 2000. This equates to $13.1 million saved based on a June 2000 average farm fuel price of 42 cents per litre. Today that amount would double to $26 million.

Slight increase in fertilizer usage

Growers reported using slightly more fertilizer for transgenic canola. This translated into a higher cost ($1.72 per acre) compared to conventional. However, twice as many conventional growers used summerfallow in the year before their canola crop (18 % of transgenics used summerfallow compared to 36 % of conventional). As would be expected, fertilizer inputs for canola seeded on stubble were substantially higher for both systems, as compared to those areas that were previously in summerfallow and subsequently planted to canola.

Less herbicide used

Transgenic growers used less herbicide than conventional growers. The total amount of herbicide used (formulated product) from 1997 to 2000 was calculated using the grower reported herbicide applications and the acres of transgenic varieties grown. The total amount of herbicide reduction varies from 1500 tonnes in 1997 to 6000 tonnes in each of 1999 and 2000. Herbicide costs for transgenic growers were 40 % lower than for conventional growers, even though the average number of herbicide applications for the transgenic growers was slightly higher (2.13 applications) than the conventional growers (1.78 applications). This difference is largely due to more frequent glyphosate applications by the transgenic growers and increased cultivation to control weeds by the conventional growers. Conventional growers used more soil incorporated herbicides. This trend was confirmed by Brimner et al. (2005) in their study on the influence of HR canola on the environmental impact of weed management.

Economic impact on growers

Growers reported an average $5.80 per acre increase in net return on their transgenic acres (revenue less all input costs, labour, etc.) compared to conventional acres in 2000 while the economic model developed for the study calculated a $10.62 profit advantage per acre (gross revenue less specific input costs considered in the

analysis). Revenue was higher for transgenic growers due to a higher yield and less dockage. In addition, herbicide and tillage costs were lower while seed, fertilizer and the cost of the Technology Use Agreement was higher for transgenics. While conventional canola production had lower seed and fertilizer costs, the cost for herbicides, field operations, scouting and other services were higher.

The direct economic impact to growers of the adoption of transgenic canola from 1997 to 2000 was calculated to be within the range of $144 million and $249 million, varying between the farmer-based estimate and the value determined by the economic model.

Industry value

When a technology like transgenic canola is adopted, it can impact the whole community (e.g., added investment in canola crushing capacity, impacts on local seed, herbicide and equipment industry investments and development, added shipping, handling, marketing) The total indirect impact for the 1997 to 2000 period was estimated to range between $58 million and $215 million. The total value to the industry, including both direct revenue to the growers and the indirect value, was up to $464 million, cumulative from 1997-2000.

Summary

Over 90 % of producers now grow HR canola. Benefits include increased yield and profit ($5.80 per acre) and reduced dockage, less tillage, less herbicide used and less fuel used. In terms of weed control benefits, growers clearly identified better weed control as the primary reason for choosing HR canola. In addition, nearly 60 % of the growers who planted a HR variety in 2003 reported a perceived carry-over benefit to the 2004 crop in terms of improved weed control. Even though this perception was not supported by the Prairie Weed Survey where no difference in herbicide use for all weeds or summer weed counts were found between the HR group as a whole and the conventional group, about half of these growers put a dollar value on this perceived benefit of $11.80 per acre, roughly the cost of product and application of, for example, a typical glyphosate treatment. This cost benefit would offset any incremental cost of volunteer canola control as derived from the grower survey. All things considered, including volunteer canola control, the majority of HR growers continue to support the premise that the benefits of growing HR varieties are greater than the benefits of growing conventional varieties.

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Selection of herbicide resistance in weeds: the influence of herbicide-resistant crops

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The widespread adoption of herbicide-resistant crops has lead to concerns about their role in selecting for resistance in weeds. This is especially true for Roundup-Ready crops because of the prevalence glyphosate now has in many cropping systems. Glyphosate resistance has now been documented in eight species in seven countries. While the earlier cases were all linked to glyphosate usage in non crop situations, more recent occurrences have been linked to glyphosate use in Roundup-Ready cotton and soybeans.

Herbicide resistance in weeds and crops

Many definitions of herbicide resistance in weeds exist, but one of the most appropriate, albeit long and wordy, was proposed by Ian Heap and Homer LeBaron:

> *The heritable capacity of a previously herbicide-susceptible weed population to withstand a herbicide and complete its life cycle when the herbicide is used at its normal rate in an agricultural situation* (Heap and LeBaron 2001).

This definition explains the phenomenon of resistance very well and excludes cases of "innate resistance" (which is also called "tolerance"). It also excludes cases of resistance that might have occurred in the laboratory on crop plants or non-weedy species. One important process leading to resistance is the selection pressure that comes with repeated herbicide usage. One can assume that this is implied in the above definition although it is not explicitly stated.

Resistance in weeds can be selected through different biochemical or physiological alterations. Ultimately these changes reduce the amount of herbicide that reaches and/or binds to the target enzyme. This allows the weed to bypass the lethal action of the herbicide and survive, showing little or no herbicide injury. Alterations conferring resistance can be as follows: reduction in herbicide absorption through the cuticle, alteration of translocation, sequestration in the vacuole or outside the plasmalemma, increased metabolism, alteration of the target site reducing binding and overproduction of the target enzyme (Preston and Mallory-Smith 2001). While any of these mechanisms can render a weed resistant, in reality, the great majority of resistance cases are due to target site alteration, although increased metabolism or altered translocation have been documented in a few cases. This is probably due to target site alterations affording a very high level

of protection while requiring only a small change in the genetic make-up of the plants: target site alterations are encoded by single nucleotide changes in the target site genes.

In contrast, natural herbicide resistance in crops (which is the basis of selectivity) is almost always due to increased herbicide metabolism. The crop is able to degrade the herbicide more rapidly than the weeds, allowing it to survive. The most notable exception to this rule is with the postemergence grass herbicide belonging to the cyclohexanediones (CHDs) and aryloxyphenoxypropanoates (APPs) (also referred to as the DIMs and FOPs, respectively). These herbicides exert their lethal action to grasses through inhibition of acetyl-coenzyme-A carboxylase (ACCase) while the same enzyme in dicots and non-grass monocots is not affected (Burton 1997).

Resistance in bio-engineered crops can be conferred through enhanced herbicide deactivation. Such is the case with resistance to glufosinate, which is conferred by deactivation through phosphinothricine acetyltransferase (Lydon and Duke 1999), or resistance to bromoxynil which is endowed by a nitrilase that degrades the herbicide into a non-toxic molecule (Stalker et al. 1996). However, in the other cases, resistance has been engineered through target site modifications as is the case with resistance to glyphosate, imidazolinones, sulfonylureas and triazines.

Resistance in weeds: the selection process

The likelihood of resistance developing generally grows with over-usage of a particular herbicide or herbicide group. History tells us this is the case with the most prominent resistant cases. Resistance to triazine herbicides in Eastern Canada in the 1970s and 1980s was correlated with corn monoculture (Stephenson et al. 1990). The areas of Ontario which had the highest proportion of the cropping land devoted to corn had the highest incidence of resistance due to the dominance of atrazine in that crop. In Australia, a shift to no-till cereal production increased the reliance to CHD and APP graminicides and this selected for resistance to those herbicides in the grass weed annual ryegrass (*Lolium rigidum*) (Thill and Lemerle, 2001). A similar phenomenon occurred in Western Canada with wild oats (*Avena fatua*) developing resistance to ACCase inhibitors (Beckie et al., 2000). In Ontario, we have observed high incidence of resistance to acetolactate synthase (ALS) inhibitors in populations of ragweed (*Ambrosia artemisiifolia*) from counties where soybeans dominated the rotation (about 75 % percent of the cropping area planted to this crop for each year from 1995 to 2000). Because of the dominance of the herbicide imazethapyr in that crop over that period, resistance was quickly selected (Tardif and Smith, unpublished).

Herbicide-resistant crops were introduced in the mid 1990s and their usage has constantly increased since. Early on, concerns about how they would contribute

to select resistance in weeds have been expressed; however those concerns never were directed equally at all crops. For example, at that time, resistance to ALS inhibitors was already quite widespread in weeds, so the question as to whether imidazolinone- or sulfonylurea-resistant crops would select for resistance in weeds was basically a non-issue: there were already many ALS-inhibitor-resistant weeds. Sethoxydim- and bromoxynil-resistant crops could have selected for resistance, but they are no longer used (Duke 2005). Glufosinate-resistant crops are still used, but they do not have the same rate of adoption as glyphosate-resistant crops. In addition, glufosinate, because of its non-systemic and non-residual nature is most often used with other herbicides, which would slow down resistance evolution. This leaves concerns about resistance selection in weeds to be directed mostly at glyphosate-resistant (Roundup-Ready) crops. While glyphosate, like glufosinate, is non-residual, it differs by being systemic and highly efficacious on almost all weed species. Roundup-Ready has quickly become the dominant herbicide-resistant crop technology because of its simplicity (may require only one application and does not need tank mix partners), efficacy and low cost. In addition, because of its importance as a herbicide outside Roundup-Ready crops (pre and post harvest application, non field crop applications, etc), the potential loss of this herbicide through resistance would be perceived as more significant than resistance to other products.

The increased adoption of the Roundup-Ready system has resulted in an increased use of glyphosate. In some systems, Roundup-Ready has become the dominant weed control technology. It is ironic that, at about the same time Roundup-Ready canola was introduced in Western Canada, reports of glyphosate-resistant ryegrass started to emanate from Australia. Part of the irony is that it had been proposed by some that resistance to glyphosate in weeds was next to impossible. The target site enzyme, enol-pyruvyl-shikimate-phosphate synthase (EPSPS), did not support alterations endowing resistance as it disabled the enzyme's catalytic activity (Padgette et al. 1996). Metabolic degradation of glyphosate in plants is very limited and was not thought to be able to contribute to resistance. So, compared with the triazines, ALS inhibitors or ACCase inhibitors, resistance to glyphosate, from the perspective of a weed, appeared to be a more difficult "proposition" (Jasieniuk 1995).

Glyphosate resistance in weeds was first confirmed in 1996 in a rigid ryegrass population from Australia (Heap 2006). This population had been exposed to at least 11 applications of glyphosate in pre-seeding in the previous eight years. In the following years, more accounts of glyphosate-resistant weeds were reported in ryegrass and in other weeds species. To date, eight species with glyphosate-resistant biotypes have been reported in Ian Heap's Herbicide Resistance Web Site (Heap 2006). These reports are from seven different countries (Table 1). In the earlier reports, selection occurred mostly in orchard and vineyards where glyphosate might have been used more than once in a growing season to provide general weed control. There were also cases, mostly with rigid ryegrass, where glyphosate was

used for weed control prior to seeding cereals or oilseed crops. More recent cases have been linked to glyphosate usage in Roundup-Ready crops, mostly soybeans and cotton.

Table 1. A chronology of glyphosate resistance in weeds: for each species, the year of first confirmation is indicated as well as the country and cropping systems.

Year	Weed Species	Country	System
1996	*Lolium rigidum*	Australia,	Orchards and burndown in cereals
		USA,	Orchards
		South Africa	Orchards
1997	*Eleusine indica*	Malaysia	Orchards
2000	*Conyza canadensis*	USA (11 states)	RR soybeans (burndown and in-crop), RR cotton and roadsides
2001	*Lolium multiflorum*	Chile,	Orchards
		Brazil,	
		Oregon	
2003	*Plantago lanceolata*	South Africa	Orchards, vineyards
2003	*Conyza bonariensis*	South Africa	Orchards, vineyards
2004	*Ambrosia artemisiifolia*	USA (Missouri,	RR soybeans
		Arkansas)	
2005	*Amaranthus palmeri*	USA (Georgia)	RR cotton

How many years or how many successive applications of glyphosate are necessary before resistance becomes a problem in a field? This question is difficult to answer precisely, mostly because of lack of accurate records. However, observations from a few different cases suggest resistance to glyphosate will typically “appear” after many applications. For example, one annual ryegrass

population from Australia was discovered after glyphosate had been applied for 15 years with two to three applications per year (Lorraine-Colwill et al. 1999). One goosegrass population in Malaysia had received glyphosate for only three years, but the herbicide was applied six to seven times yearly (Lee and Ngim 2000). The first case of glyphosate-resistant Canada fleabane in Delaware was reported after only three years in Roundup-Ready soybeans; however, application of glyphosate in preemergence as a burndown in previous years was thought to have contributed to the selection pressure (VanGessel 2000).

Investigations into determining the mechanism of resistance have been conducted with some of the species. In goosegrass, it appears that resistance is conferred by alteration in the target enzyme EPSPS (Baerson et al. 2002). In fleabane and annual ryegrass, recent evidence suggests altered movement of the herbicide in the vascular system is implicated, likely due to differential cellular entry between resistant and susceptible plants (Feng et al. 2004, Wakelin et al. 2004). For a weed to have resistance to glyphosate does not preclude the possibility of resistance to other herbicides. There are for example, some multiple resistant ryegrass populations that are resistant to ACCase or ALS inhibitors and that are also glyphosate-resistant (Neve at al. 2004). There are glyphosate-resistant fleabane populations in Ohio that are also resistant to the ALS inhibitor cloransulam (Heap 2006) and glyphosate-resistant goosegrass in Malaysia is multiple resistant to the ACCase inhibitor fluazifop (Heap 2006).

Prevention and management of glyphosate resistance

Prevention of resistance before it appears and its management once it has become a problem essentially employ the same tools and similar approach. As resistance is the consequence of using a single tool repeatedly, any proactive or reactive approach should take an opposite view: use a diversity of methods to avoid repetition as much as possible. Unfortunately this goes against human nature: preventative measures will not be adopted, especially if they represent a disruption of normally used practice. Similarly, reactive measures (management) will generally mean the search of an effective alternative herbicide.

Because of the importance of glyphosate in many cropping systems and also because there is a realization that the number of potential new herbicides to be introduced in the future is dwindling, there has been relatively high interest in promoting resistance prevention measures. The use of crop rotation is frequently advocated as it generally means that different herbicides will be used in each crop. One has to be mindful though that this is not necessarily the case and, as Roundup Ready technology is available in a range of different crops, it is possible to have glyphosate as the main herbicide in all phases of a rotation (e.g. glyphosate-resistant corn and soybeans). Rotation in and out of glyphosate-resistant crops would at least slows down the potential development of glyphosate-resistant weeds. Computer

simulation work has shown that herbicide mixtures are superior to rotation of modes of action for preventing resistance (Diggle et al. 2003). Because of seed dormancy, individuals bearing resistance allele do not all germinate at the same time and are therefore not eliminated by the herbicide used in rotation. Herbicide mixtures allow those resistant individuals to be eliminated by the additional herbicide. Interestingly, some glyphosate manufacturers have been recommending the use of glyphosate along with residual herbicides in Roundup-Ready soybean, cotton and corn as a resistance prevention measure (see for example: www.weedresistancemanagement .com). It is clear however that, when glyphosate resistance has occurred, alternatives will be necessary. These can be residual herbicides applied along with glyphosate or postemergence rescue treatments.

Changes at the community level

While resistance can be explained as a change at the population level (resistant individuals arise in a weed population normally susceptible), herbicide usage can also cause changes at the community level: the emergence of species that were previously absent or occurring at unnoticeable levels. These changes are often referred to as weed shifts and have been documented in response to many crop management practices changes (new herbicides, tillage, etc). Weed species that are naturally-resistant (tolerant) to glyphosate exist. These become especially apparent at the lower rates of glyphosate that are used in crops (compared to the higher rates that have traditionally been used to target perennial weeds in non-crop situations) and it would be logical to assume that under continuous glyphosate use, these species would be favoured. Whether such species would actually become dominant depends also on the interaction with other agronomical practices such as planting density, row width, and cultivation.

Summary

Glyphosate-resistant crops are now widely available and planted. This is pushing the use of glyphosate to levels that did not exist when this herbicide could only be used before or after the crop was growing and therefore more annual weeds are targeted. Glyphosate resistance can develop in weeds and this is now a reality in seven countries. Resistance preventative measures are being advocated and may include the adoption of rotation or herbicide mixtures. Management mostly relies on alternative mode-of-action herbicides.

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Ten years of biotechnology – a historical perspective of science, politics and trade

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Canada, with the introduction of Liberty Link and Roundup Ready herbicide-resistant canola in 1995, was one of the first countries in the world to introduce transgenic crops on a large commercial scale. At the time little notice was taken beyond the biotech industry, scientists and growers. However, ten years hence, the introduction of transgenic crops has generated an intense and divisive international debate around science, politics and trade, against a backdrop of rapid adoption in the Americas to rejection in Europe. The introduction of transgenic crops is not just about the risks and benefits from utilizing a new technology in agriculture, but has evolved into a proxy for a broader debate that encompasses issues ranging from sustainable agriculture, biodiversity, food security and consumer choice, to international treaties, trade wars, corporate control and north-south socioeconomics. Canadian growers have benefited from the introduction of transgenic crops. However, this has not been without our own internal controversy about regulatory oversight, consumer choice and market access. As a major producer of transgenic crops, Canada has a large stake in the outcome of the international debate due to our dependence on trade and export markets.

Introduction

First commercial introductions of genetically modified (GM) crops globally occurred in Canada and the United States in 1995. The first five years saw exponential growth in acreages of canola, corn and soybean. In the last five years, significant adoption has occurred in developing countries. In 2004 global acreage of biotech crops reached 81M ha, a 20 % increase over 2003. The dominant producers of GM crops in 2004 were the United States and Argentina, with 59 % and 20 %, respectively, followed by Canada and Brazil at 6 % each (James 2004).

The major GM crops grown in 2004 were the commodity crops soybean, corn, cotton and canola, with acreages of rice emerging in countries such as China and Iran. The dominant traits are “input traits” such as herbicide resistance and insect resistance to enhance agronomic production. In Canada, the major GM crop is canola, with greater than 80 % of the acreage planted to herbicide-resistant GM varieties (Canola Council of Canada 2001).

Global acceptance and adoption of GM crops is highest in North America (NA) and Argentina, with acceptance and adoption gaining in Brazil and Asia. The

European Union (EU), however, continues to reject the growing of GM crops, the exception being small acreages of insect-resistant GM corn produced in Spain. This paper will articulate a personal and historical view of the socio-political, scientific, food, agriculture, environment and trade parameters that created such a divide in acceptance between NA and the EU (Gaskell et al. 1999).

Socio-political landscape

Both NA (Canada/US) and the EU developed their respective regulatory systems for the safety assessment and authorization of GM crops in the early to mid 1990s when the first approvals were granted. The first major crop to receive approval in both the US and the EU was soybean and soon the US was producing and exporting commodity soybean to the EU.

In NA, growers adopted GM crops rapidly and the public were neither concerned nor interested despite issues emerging in the scientific community on Bt corn's impact on the Monarch butterfly, ethical issues raised by Terminator technology, regulatory lapses with StarLink (Starlink Logistics Inc. 2006), and pressure from NGOs on food safety and the environment. In the EU, things were different as public confidence in food safety was shaken by the BSE crisis. This event created great public suspicion of the scientific and political institutions that regulate food and the integrity of modern industrial agriculture. This was at about the same time when the first GM soybeans were arriving at EU import terminals from the US. Moreover, the dioxin contaminated chicken feed (ESF 2000) and the Coca-Cola contamination scandals of 1999 (Lauwers 1999) in Belgium did little to lower public concerns regarding food safety in the EU. The perfect storm was beginning.

Opponents of modern agriculture and of GM technology were quick to link the arrival of a new and unknown technology to the uncertainty in food safety created by the Bovine Spongiform Encephalopathy (BSE) crisis (BBC 2006). The media headlines, most notably in the UK, jumped on the GM-BSE food safety story and GM crops were directly linked to even more uncertainty as to the safety of the food supply. This made the food industry nervous and retailers started distancing themselves from GM soybean by reformulating brand name products and claiming to be GM free. With all this publicity, the greater public could only believe that GM foods were risky. Add to this the notion that GM crops are a US technology owned by large multinational companies; the media stories became even more spectacular.

In response to the headlines, NGO pressure and public concern, the final blow came in the summer of 1999 when a critical mass of EU Environment Ministers joined together to declare a moratorium on any further approval of GM crops until more stringent rules and regulations were adopted (Foreign Affairs and International Trade Canada 2003). This generated a flurry of new legislation for risk assessment, labelling and traceability of genetically modified organisms (GMOs).

Political support for a moratorium not only had its roots in public opinion but in the prevalence of the Green Party having the balance of power in key EU Member States. Approvals have begun again in 2004 despite the fact that Member States remain divided as far as political support for GM crops.

Science

Public trust in regulatory institutions that regulate both environmental and food safety of GMOs has remained firm in both Canada and the US. These institutions practice a science based process for decision making and this has served them well when food safety issues have emerged. Throughout the debates on GMOs, politicians in NA have supported and relied on their science based regulatory instructions to make the decisions.

The BSE crisis broke the EU citizen's trust in science and regulatory institutions (BBC 2006). Multiple regulatory institutions and often competing regulatory bodies amongst Member States only added to the confusion and mistrust. Negative public opinion fuelled by the media and effective NGO campaigns caused many politicians to choose the "political" route on GMOs. This often meant undermining the decisions and advice of their scientists and regulatory institutions.

In the EU, much of the focus was on the uncertainty of GMOs driven by fluid interpretations of the Precautionary Principle. This approach resulted in a reactionary regulatory policy process that stopped and started each time new scientific information emerged, validated or not. By contrast, in NA much more was said about the scientific certainties of GM crops safety and new scientific information was carefully scrutinized before considering changes to policy. One consistency between NA and EU was that scientific opinion on both sides generally agreed as to the safety of the current generation of GM crops.

Food

Although most of the food consumed in the EU is from modern industrial scale farming, there remains a very strong attachment to the origin, culture and traditions associated with food. This relationship to food was in conflict with the image of mass produced GM food coming from foreign counties, most notably the US. In contrast, one can generalize that North Americans' view of food is more related to quantity, sustenance and convenience, which is much more akin to efficient low cost production.

Agriculture and environment

Typically agriculture in NA is separate from the "wild" or natural environment. For example, in a region such as agriculturally intensive southern Ontario, one can be in the "wild" within a few hours drive. Agriculture and nature are not so much in conflict and exist in separate and distinctive geographical areas fit for distinct purposes.

In the EU agriculture and nature are closely linked. In most European countries, the rural environment is the "natural" environment as "wild" environments are scarce. This quickly puts agriculture and nature in conflict and the notion of a new and uncertain technology invading agriculture raises environmental concerns, let alone concerns about traditional food production.

Trade

Trade was not an obvious issue early on in the relationship between NA and the EU on the GMO file. However, as the 1999 EU moratorium took hold and regulatory approvals stopped in the EU, GM crop regulatory approvals and commercialization continued in NA (CFIA 2006). This resulted in an imbalance and lack of synchronization of regulatory approvals that quickly created legal barriers for GM crops entering the EU. These new trade barriers created a convenient competitive advantage for European canola and corn producers, as much of the canola and corn produced in Canada and the US no longer had access to EU markets.

The gloves finally came off on this trade issue in May 2003, when Canada, the US and Argentina took a case to the World Trade Organization on the EU moratorium (Foreign Affairs and International Trade Canada 2003). The basis of the case was that the EU had no scientific justification to block the authorization of a number of GM crops that were submitted for approval before and during the moratorium.

Summary

Considering the differences in the culture of food, agriculture and the environment between NA and the EU, one could have predicted ten years ago some differences in acceptance and adoption of GM crops. However, it was a mix of circumstances and socio-political differences that led to very different outcomes. In the end, the single most significant trigger that set the stage for the rejection of GM crops in Europe was the BSE crisis, which seeded doubt in the credibility of science, government institutions and politicians.

There are a number of lessons learned from GM story in Europe.

1. Do not expect "science only" to be the basis of making decisions or policy on new technology. New technology developers must take a broader view on the potential impacts of new technologies when making development decisions.
2. Food value chain, down stream customers (food industry and consumers) will drive what is grown on farms. Technology developers in agriculture must consult with down stream customers early in product development.
3. Benefits to agricultural production at the farm level are of little interest to the public if there is a perceived risk.
4. New technology will be challenged as to the need, who benefits, who bears risk and availability of alternatives.

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Sowing the seeds of acceptance

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Public awareness and acceptance are key to the growth and success of agricultural biotechnology. Continuous science-based communication with Canadians is essential for creating positive discussions and interest. The industry has taken on the responsibility to encourage such conditions by relying on non-governmental organizations to interact with the public. While media often sheds negative light on biotechnology, it has great potential to help inform the public of scientific innovations and benefits. Although communication challenges are still ahead, it has been realized that trustworthy third-party spokespeople make the best connections with the Canadian public. Out of these efforts, individuals may better understand the topic through the broader context of food, science and innovation, and bio-products.

Public acceptance

In 1995, herbicide-resistant canola was the first genetically engineered (GE) food crop to be approved in Canada (CFIA 2006). To the industry's surprise, this scientific achievement was met with some public opposition. The Council for Biotechnology Information (CBI) is a stand-alone organization that was formed by the combined efforts of several industry leaders in 1999. This non-profit organization remains dedicated to delivering accurate science-based information to interested Canadians regarding benefits and risks of the technology.

Public acceptance of agricultural biotechnology is important on many levels of society. The economics is certainly an issue, but perhaps more importantly, the level of public acceptance plays a large role in future developments of biotechnology. To date, we have only scratched the surface of the technology's potential. While the first benefits of biotechnology were directed at growers and had limited perceived personal benefits, we are beginning to see developments in the area of consumer traits. However, negative public attitudes can create an uncertain climate for research and progress as they will affect decisions made by various regulators. Therefore, CBI has been working toward creating better opportunities to engage Canadians.

In planning information programs, CBI has carried out attitude surveys over the years. Pollara, a public opinion and marketing research firm in Canada, conducted a survey in September of 2005 regarding public opinion concerning the Canadian biotechnology sector. The results are encouraging; 29 % of Canadians have a positive reaction to the term biotechnology, while only 8 % reacted negatively (Pollara Inc. 2005). The majority of Canadians (56 %) had no reaction at

all. Despite this result, Pollara found that there is low awareness and depth of knowledge of the technology. Even though eight out of ten Canadians support further research in biotechnology in the broad sense of the word, Pollara concluded that the "industry needs to do more to educate Canadians about the risks in a creditable manner". John Olsthoorn, the Canadian Biotechnology Secretariat, presented some general trends in the fall of 2005 (Decima Research Inc. 2005) that were encouraging in this respect. He indicated that the knowledge and understanding of the technology has risen in the past few years with a correlation between familiarity and support. Therefore, there is a continual need for work in communication.

Engaging the population is no easy task. Not everyone is interested. Many just want a food safety assurance. CBI has conducted surveys and focus groups to identify what people what to hear about, and who they want to hear it from. For example, work was done in 1998 to better understand how Canadians make decisions about food. The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) and the Food and Consumer Products Manufacturers of Canada (FCPMC) funded the research. We, at CBI, have recognized the difference in attitudes towards biotechnology between different regions of Canada. English Canada and Quebec have clear differences in their views of the human body and eating. While Quebec sees the body and eating as a means to pleasure, English Canada has a more practical notion. We have to be able to cater to those views to increase interest and acceptance of plant biotechnology. In recent years we have been able to expand the context of plant biotechnology. Biotechnology is no longer merely a discussion about food. It can be in the broader context of science and innovation including bio-products.

At CBI, we aim to deliver clear, simple and consistent messages through spokespeople of different walks of life. Third party spokespeople who have credibility with Canadians, such as dietitians, farmers, teachers, and scientists, are trusted individuals who are able to provide a balanced discussion on the benefits and risks of biotechnology in a context to which the general public can relate. We continue to expand our work with key media groups and other non-government organizations (NGOs) to improve the image of agricultural biotechnology. Through third-party spokespeople we are able to make a personal connections with our target audience. Positive media attention to our "Green Kitchen" (BioProducts Canada Inc. 2005) – a kitchen that was built with mostly materials derived from plants, our Young Scientist scholarship program (CBI 2006), and our provincial networks provide exposure at a national level. Our support and relationship with Africa Harvest Biotech Foundation offers an international perspective to our organization. Nevertheless, earning the trust of Canadians is central to our role. If we continue to provide access to information to the public regarding biotechnology and the role of the government, we believe we will be able to create a positive environment for the technology to continue to grow.

Our most recent communication platform has been the Green Kitchen (BioProducts Canada Inc. 2005). It is an example of how CBI, together with BioProducts Canada, have been able to successfully intrigue the media and general consumers by employing all methods that we have found to be of value. First, it provides a setting with interesting visuals that encourage a personal connection for interaction between third-party spokespeople and the public. Second, it makes a good media story that further promotes bio-products and foods of biotechnology. Third, it is a channel to distribute collateral materials that provide additional information to those sufficiently captivated.

In the future, we are expecting to face some difficult communication challenges in Canada. Quebecers have adopted European attitudes toward GE foods and the opponents of biotechnology have attracted media limelight. In the breadbasket of Canada, the case of Percy Schmeiser versus Monsanto continues in the media. The story of small organic farmers versus a multinational corporation still draws sympathy with Schmeiser, despite a Supreme Court ruling against him (Supreme Court of Canada 2004). Moreover, some Organic and natural food product providers try to increase market share by criticizing the use of AgBiotech – meanwhile many parts of the world continue to adopt biotechnology.

There are several developments that will help in our communication work. First, CBI will focus our work on some success stories. The ISAAA report (James 2005) shows that 8.5 million farmers in 21 countries now use seed containing biotechnology. Partnerships with the developing world are being created. For example, the Gates Foundation has funded a five-year project to develop an improved sorghum (Vitamin A, C) using local scientists in Africa and Dupont intellectual property (ABS Consortium 2005). Second, consumer traits are starting to emerge including higher quality oils. The backlash on biotechnology was in part due to the limited benefits to consumers. The first generation of GE crops only possessed direct benefits for growers but no direct benefits to the consumers. In the second generation, we will see more focused consumer benefits such as increased nutrition and improved taste. Increase in consumer traits will likely increase the general public acceptance of biotechnology. Third, the context of biotechnology must expand. Broadening the context of biotechnology has helped increase public interest and awareness. In CBI's own Green Kitchen concept, we have expanded agricultural biotechnology beyond food and into environmentally friendly consumer products that come partly from agriculture – both the biomass, the use of enzymes and finally GE seed. This has been an excellent approach toward raising awareness and interest that has lead to increased confidence in the future of biotechnology.

Although, as a science-based community we understand the potential of biotechnology, we have to be more aware of how the general public perceives these technologies. We must maintain public trust with accurate research on the risks and benefits of existing and emerging technologies and communication must remain open to address concerns and interests. Most importantly, we have to keep conversations in a language that is easy to grasp. The success of future progress will

be rooted in public acceptance so let's create a positive condition in which the seeds may be properly sown.

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The feeding value of genetically modified feeds and the fate of ingested transgenic DNA

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The amount of arable land dedicated to the production of genetically modified (GM) feed has increased significantly since the commercialization of GM crops in 1996. Despite widespread adoption of GM foods and feeds, public perception of their safety remains mixed. Livestock studies have provided important information on the nutritional equivalence of GM feeds compared to non-GM feeds. To date, the commercially available GM crops that are used as feeds have not been shown to impact the safety or economic profitability of livestock production. Concern in the consumption of transgenic DNA has prompted investigations of transgene fate within the gastrointestinal tract of livestock and the potential to which transgenes may be incorporated into animal products used for food. Fragments of plant DNA from endogenous, high-copy number genes have been detected in poultry, swine, and ruminant tissues. Detection of low-copy endogenous and transgenic DNA in animal tissues have been reported but to a lesser extent than high-copy genes. Current research suggests that the passage of dietary DNA fragments across the intestinal wall is a natural physiological event, the likelihood of which is dependent on the size of fragment and its concentration in the feed. At this point there is no evidence that these events pose any risk to livestock health.

Introduction

Advances in molecular biology and recombinant DNA techniques have made it possible to engineer plant genomes by the selective inclusion of single or multiple genes. The majority of genetically modified (GM) plants currently produced have been engineered to enhance agronomic performance by transformation with genes encoding herbicide resistance, insect resistance, or a combination of both (ISAAA 2005). In 1996, the first GM crops that were used as feed for livestock entered the market in North America. These included herbicide-resistant (HR) soybeans (*Glycine max*) and canola (*Brassica napus*), and insect-resistant (IR) corn (*Zea mays*) and cotton (*Gossypium hirsutum*). During the ten-year period of 1996 to 2005, the global area of GM crops increased more than 50 fold. In 2005, GM crops were grown on a total of 90 million hectares world wide (ISAAA, 2005).

Regulations concerning GM plants were established by major international organizations prior to their commercialization. The policy of substantial equivalence was first introduced by the Organisation for Economic Co-operation and Development (OECD 1993) and was adopted by both the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as the most appropriate method to ensure the safety of GM plants (FAO/WHO, 2000). Despite regulations in place to assure GM food/feed safety, public and scientific interest in GM plants entering the food-chain still exist. Between 1996 and 1999, Europeans became increasingly opposed to GM foods (Gaskell et al. 2000) and a more recent study showed that European consumers place greater value on beef from cattle (*Bos taurus)* fed conventional corn grain as compared to those fed GM corn (Lusk et al. 2003).

In particular, there has been interest in transgenic plant DNA entering the food chain. The principal GM crops grown in 2005 were soybean, corn, cotton, and canola (ISAAA 2005), each of which is extensively used in livestock diets. Given that livestock consume large amounts of plant material and that high-protein feeds are among the most common GM crops, there is considerable opportunity for livestock to consume significant quantities of transgenic DNA. The objective of the present paper is to review digestibility and performance of GM feeds consumed by livestock. Additionally, the fate of ingested transgenic DNA is discussed.

Animal studies

The policy of substantial equivalence provides a framework for safety assessment by comparing similarities and differences between a biotechnology-derived plant and an appropriate counterpart such as the parental line of the GM plant. Once certain factors of the plants have been determined to be equivalent, the differences, which relate to the novel transgenic trait, are the focus of the safety assessment. While there are no formally defined parameters to be measured, minimal analyses performed after transformation should determine whether the major nutritional components (i.e., lipids, carbohydrates, proteins, vitamins, minerals, trace elements) and known anti-nutrients have changed in concentration to adversely affect the nutrition of the plant. For livestock nutrition, important measurements include crude protein, fat (ether extract), fiber, starch, amino acids, fatty acids, ash and sugar (Aumaitre et al. 2002). Alteration of the components may not only affect animal health and performance, but may also alter the composition and quality of animal products for consumers. For example, certain fats present in feeds can affect the composition of fat in animal tissues (Aumaitre et al. 2002). Glucosinolates in rapeseed, lectins in soybeans, and protease-inhibitors in soybeans and corn are examples of known anti-nutrients that can also adversely affect animal production (Novak and Haslberger 2000). Future transformations are more likely to alter the chemical composition of plants and as a result guidelines for second

generation GM crops that will lack substantial equivalence have been proposed (Flachowsky and Bohme 2005).

If a transgenic feed is deemed to be substantially equivalent to its near isogenic counterpart based on the factors described above, then animal studies may not be required for approval of the transgenic feed by authoritative bodies in some countries such as Canada and the United States. However, numerous studies over the last ten years have compared animal performance, health, and the end-products between animals consuming transgenic and non-transgenic feeds. Such investigations are useful for the assessment of the risk of consumption of novel GM feeds and foods by livestock and humans (*Homo sapiens*). Adverse effects of a plant in livestock could serve as a warning device for their potential effects on humans. In addition, economic concerns regarding animal performance are also addressed. To date, there have been no studies showing adverse effects on animal performance, health or end products when the currently registered GM plants or products derived from them have been used as feeds (Flachowsky et al. 2005). Information on most of the GM plants assessed in the animal studies outlined below has been described by AGBIOS (2005).

Swine digestibility and performance

Digestibility studies for swine (*Sus scrofa*) have included the feeding of HR corn and sugar beets, IR corn, and the stacked traits of IR plus HR corn (Table 1). Aulrich et al. (2001) compared the digestibility between mixed rations containing 50 % IR Bt176 corn or its parental line fed to five castrated male pigs. Digestibility of organic matter (OM), crude protein, (CP), and nitrogen free extract (NFE) were not different between the corn types. Similar results were reported in another study comparing Bt176 corn and its parental line (Reuter et al. 2002a), which measured digestibility throughout the grower and finisher phases. When digestibility of OM, NFE, CP, ether extract (EE), and crude fibre (CF) were analyzed, the values did not differ significantly among Bt176 and parental varieties (Reuter et al. 2002a). The calculated metabolizable energy (ME) values were also comparable.

For HR plants, comparing digestibility after application of the herbicide presents a more realistic situation since the plants are likely to have been sprayed at some point. In a study by Bohme and colleagues (2001), digestibility measurements were compared between glufosinate-resistant corn grains and sugar beet (*Beta vulgaris*) roots, the parental lines of each, and the glufosinate-resistant varieties sprayed with an herbicide containing glufosinate. Five castrated male pigs per treatment were fed diets containing 30 % corn grain or sugar beet root on a dry matter basis. Analysis of OM, CP, EE, CF, NFE, and sugars were similar between the three corn and sugar beet root treatments. Additionally, amino acid, fatty acid, and cell wall constituents were measured in corn and no differences between the parental, untreated and glufosinate-treated HR plants were found. Digestibility of OM, CP, and NFE between corn treatments also did not differ. For the sugar beet-containing diets, digestibility of OM for both glufosinate-resistant treatments (with

and without glufosinate application) were significantly greater (approximately 3.8 %) compared to the parental treatment, while digestion of CP and NFE were similar between all three sugar beet diets. Calculated ME and digestible energy (DE) values were also similar for all corn and sugar beet diets.

Performance studies for swine have included the feeding of HR corn and soybean meal, IR corn, and the stacked traits of IR plus HR corn. Piva et al. (2001a) compared performance between swine fed IR MON810 corn and its isogenic control. Diets containing 33 % (DM basis) of each corn were fed for 35 days. Nutritional analysis of the two GM and non-GM corns showed no differences. Throughout the study, feed intake did not differ among the experimental diets and the feed to gain ratio (F:G) was similar between 0-14, 15-35, and 0-35 days. Overall, average daily gain (ADG) was 5.6 % higher for animals fed MON810 corn compared to the parental corn (396 g/d versus 375 g/d). This resulted in the GM-fed pigs having a 2.8 % heavier final live weight (22.6kg versus 22.0 kg). The authors have suggested that the improved performance from the IR corn diet may have been due to lower levels of mycotoxin contamination. The IR corn had 69 % lower levels of fumonisin B1 and 14.4 % lower deoxynivalenol concentrations as compared to non-GM corn.

In contrast to the findings by Piva et al. (2001a) which suggested improved performance due to lower mycotoxin levels in IR corn, a long-term feeding study lasting 91 days found no differences in performance when swine were fed diets containing IR Bt176 corn or the parental hybrid (Reuter et al. 2002b). The mycotoxins zearalenone and deoxynivalenol were present at a higher concentrations in the parental diets compared to the IR diets however, the concentrations were less than the maximum allowable limits for both diet types. Daily weight gain, feed consumption, and energy efficiency were similar between the two treatments. Fattening performance did not differ between the GM and non-GM corn diets. Additional performance studies using swine are listed in Table 1, none of which showed adverse effects resulting from GM feeds.

Poultry digestibility and performance

Broilers (*Gallus gallus*) are useful for studying any unintended or pleiotropic effects in GM plants because of their rapid growth rate, in which body weight can increase 50-fold over 21 days. Consequently, their growth performance is highly sensitive to changes in nutrient quality (Sidhu et al. 2000). Studies have tested the effect of feeding IR corn and soybean, and combined HR and IR corn (Table 2). Piva et al. (2001b) compared growth in 432 male broilers fed either IR MON810 or isogenic control corn. Feed intake, ADG, and the feed efficiency of broilers did not differ regardless of the corn source. The final live weight at 42 days of age was 2.7 % greater for those broilers fed Bt corn and the difference was suggested to have resulted from a 72 % lower level of fumonisin B_1 in the Bt corn.

Table 1. Studies comparing the feeding of genetically modified to conventional crop genotypes in swine

Reference	GM Feed[a]	Trait(s)[b]	Animals	Duration[c]	Parameters[d]
Aulrich et al. 2001	Corn grain (50%)	IR x HR	n=5	8 d	Digestibility of: OM, CP, NFE; ME
Bohme et al. 2001	Corn grain (30%)	HR	n=5	10 d	Digestibility of: OM, CP, NFE; DE and ME
"	Sugar beets (30%)	HR	n=5	10 d	Digestibility of: OM, CP, NFE; DE and ME
Cromwell et al. 2002	Soybean meal (14-24.3%)	HR	n=100	24-111 kg	ADG, FI, F:G, CM, sensory analyses
Gaines et al. 2001b	Corn grain (?)	IR	n=20	2- 5 d periods	DE
"	Corn grain (?)	HR	n=20	2- 5 d periods	DE
Hyun et al. 2004	Corn grain (68.07-81.79%)	HR	n=144	103 d	FI, ADG, ADFI, F:G, CM
Hyun et al. 2004	Corn grain (65-77%)	HR	n=160	29.9-120 kg	FI, ADG, ADFI, F:G, CM
Piva et al. 2001a	Corn grain (33%)	IR	n=128	35 d	FI, F:G, ADG, BW
Reuter et al. 2002a	Corn grain (70%)	IR x HR	n=12	39-89 kg	Digestibility of: OM, CP, EE, CF[17], NFE; ME
Reuter et al. 2002b	Corn grain (70%)	IR x HR	n=48	97-115 d	BW, FI, F:G, CM
Stanisieweski et al. 2001	Corn (?)	HR	n=160	72-117 kg	ADG, ADFI, F:G, CM
Weber et al. 2001	Corn (?)	IR	n=180	5.2-121 kg	ADG, ADFI, F:G, CM

[a] Percent of diet; ?, not reported
[b] IR, Insect-resistant; HR, Herbicide-resistant
[c] Duration in days or final weight
[d] ADFI, Average daily feed intake; ADG, Average daily gain; BW, Body weight; CF, Crude fibre; CM, Carcass measurements; CP, Crude protein; DE, Digestible energy; EE, Ether extract; F:G, Feed to gain; FI, Feed intake ; ME, Metabolizable energy; NFE, Nitrogen free extract; OM, organic matter

Table 2. Studies comparing the feeding of genetically modified to conventional crop genotypes in poultry

Reference	GM Feed[a]	Trait(s)[b]	Animals	Duration	Parameters[c]
Aulrich et al. 2001	Corn grain (50%)	IR x HR	n=12	5 d (collection)	F:G, egg mass, digestibility of OM and CP
Brake and Vlachos 1998	Corn grain (61.4-67.4%)	IR x HR	n=1280	38 d	BW, F:G, survival, CM
Brake et al. 2003	Corn grain (50-64%)	IR x HR	n=1600	42 d	BW, F:G, CM
Gaines et al. 2001a	Corn grain (?)	IR	n=300	14 d	ADG, ADFI, F:G, ME
"	Corn grain (?)	HR	n=300	14 d	ADG, ADFI, F:G, ME
Kan et al. 2001	Soybean meal (?)	IR	n=900	41 d	BW, F:G, CM
Kan and Hartnell 2004	Wheat (≈. 40%)	HR	n=1200	40 d	BW, F:G, CM
Piva et al. 2001b	Corn grain (?)	IR	n=432	42 d	ADG, ADFI, F:G
Sidhu et al. 2000	Corn grain (50-60%)	HR	n=560	38-40 d	BW, F:G, CM
Taylor et al. 2003a	Corn grain (55-60%)	IR	n=700	42 d	FI, F:G, adjusted F:G, CM
"	Corn grain (55-60%)	IR x HR	n=700	42 d	FI, F:G, CM
Taylor et al. 2003b	Corn grain (55-60%)	IR	n=800	42 d	FI, F:G, CM
"	Corn grain (55-60%)	IR x HR	n=800	42 d	FI, F:G, CM
Taylor et al. 2003c	Corn grain (55-60%)	IR	n=1000	42 d	BW, F:G, CM
"	Corn grain (55-60%)	IR x IR	n=1000	42 d	BW, F:G, CM
Taylor et al. 2004	Canola meal (20-25%)	HR	n=800	42 d	BW, F:G, adjusted F:G, CM

[a] Percent of diet; ?, not reported

[b] IR, Insect-resistant; HR, Herbicide-resistant

[c] ADFI, Average daily feed intake; ADG, Average daily gain; BW, Body weight; CF, Crude fibre; CM, Carcass measurements; CP, Crude protein; F:G, Feed to gain; FI, Feed intake; ME, Metabolizable energy; OM, organic matter

Another study using IR MON810 and a HR (glyphosate-resistant) corn, their isogenic counterparts, and three commercially-available conventional corn varieties also failed to find any differences in performance resulting from the insertion of transgenes (Gaines et al. 2001a). During a 14-day growth study, ADG was similar for broilers consuming either IR corn, its parental line or any of the conventional varieties. Birds fed the parental corn as compared to the IR line had lower daily feed intake and one of the conventional varieties resulted in a reduction in feed efficiency. However, ME coefficients were similar between all treatments. The same experiment was repeated with HR corn and none of the parameters measured (average daily feed intake, ADG, F:G, and ME) were different among treatments.

Performance and carcass measurements have been compared between IR Bt176 corn and its parental line in either mashed or pelleted diets fed to 1280 broilers (Brake and Vlachos 1998). Total pen weights were measured on days 1, 14, 28, and 38 and the animals were slaughtered on day 41. There were no differences in body weight at any time between birds receiving the transgenic corn and those that received the conventional corn. The birds fed IR corn exhibited improved feed conversion at d 28 and 38, but this may have been due to the higher levels of corn in the diets containing IR corn as compared to the control. The carcass data revealed that animals fed IR corn diets had higher breast skin and pectoralis minor yield, but this response was not clearly attributable to the GM trait. The authors designed the study so that potential deleterious effects of the transgenic corn would be more apparent in broilers fed the mash diets. However, because there were no statistical interactions between corn variety and diet, performance was similar for each corn. The effect of pelleting had equal improvements on performance for the transgenic and conventional corn, suggesting that the feeding value of the IR corn was equal to its parental line.

Bt11 corn is IR and HR by expressing the *cry1Ab* and *pat* transgenes. Brake et al. 2003, compared diets containing a Bt11 corn, Bt11 that had been sprayed with glufosinate, the isogenic control to Bt11, and a commercially-available conventional corn variety fed to 1600 broilers. The constituents of each corn genotype were analyzed so that diets could be adjusted to have equal ME contents. The commercial corn variety had slightly higher protein content so cardboard, fat and sand were added to that diet to produce isonitrogenous diets. The starter, grower, and finisher diets contained 50, 55, and 64 % corn, respectively. Body weight (BW) did not differ for chickens fed the Bt11, Bt11 sprayed with herbicide, and the isogenic control on days 21, 35 and 42. On each weigh day, birds fed the commercial corn diet had lower body weights compared to at least one or more of the other three treatments. Feed conversion ratios were also similar for the isogenic and both Bt11 treatments, but conversion of the commercial variety was significantly less. None of the carcass measurements, which included percentage of BW for dressed carcass, fat pad, drum, thigh, wing, pectoralis major, and pectoralis minor, were affected by

any of the corn genotypes. Overall, the Bt11 corn varieties performed equally well as the isogenic control.

In a series of three comprehensive studies by Taylor and colleagues (Taylor et al. 2003a; Taylor et al, 2003b; Taylor et al. 2003c), GM corn varieties with single traits and stacked traits, produced from conventionally breeding two transgenic-derived single trait plants, were compared against their isogenic lines and a variety of commercial corns for nutritional status in broilers. The study designs were all similar and entailed 100 broilers per treatment that were grouped by sex. There were 10 chickens per pen that were fed *ad libitum* amounts of diets containing 55 % corn for the first 20 days and diets containing 60 % corn thereafter. Pen weights were measured on days 1 and 42 and each individual animal's weight was measured at day 43 for males and 44 for females. Data for the average feed conversion, chill weights, and breast, thigh, wing, drum, and fat pad weights were collected. Moisture, protein, and fat analyses were also conducted on the breast and thigh meat from the first male and female selected from each pen. In all cases, diets containing GM corn (events MON810, NK603, MON810 x GA21, MON810 x NK603, and MON810 x MON863) were as nutritious as traditional corn. Deleterious effects from poultry fed commercially available GM feeds have not been reported to date.

Ruminant digestibility and performance

Studies involving ruminants have examined the effects of feeding GM cotton, corn, soybean, sugar beet, and canola (Table 3). The digestibility of transgenic whole beets and beet pulp resistant to glyphosate-containing herbicides has been compared against conventional varieties fed to sheep (*Ovis aries*) in three separate experiments (Hartnell et al., 2005). In the first two experiments, nutrient composition (dry matter, total ash, acid insoluble ash, nitrogen, EE, neutral detergent fibre, acid detergent fibre, and gross energy) of transgenic fodder and sugar beets were comparable to the conventional feeds. The apparent digestibilities of dry matter (DM), OM, CP, neutral detergent fibre (NDF), acid detergent fibre (ADF), and DE did not differ among whole beet or beet pulp treatments. In the third study, the composition of beet pulp varied and there were treatment effects ($P<0.001$) for all nutrient categories when both the mixed diets and beet substrates were analyzed. However, the apparent digestibility of the diet containing transgenic pulp did not differ from that of all commercial varieties for any measurement, with the exception of ADF. In addition, the apparent digestibility values fell within the range of measurements for sheep fed the commercial varieties.

For sheep, diets containing meal derived from HR GT73 canola had comparable nutritional value to parental and commercial hybrids (Stanford et al., 2003). Sixty early- weaned lambs were fed the diets until 45 kg body weight. Feed intake was similar between lambs fed the HR and parental meal diets and between the two commercial meals. However, intake was significantly greater for animals fed the commercial diets when compared to the HR diet. Average daily feed gain and F:G were not affected by diets. Carcass yield grade was similar between the

parental and HR diets however, both yields for commercial diets were greater in comparison to the parental and HR diets. Meat tenderness, drip loss, intramuscular fat content and color were similar between all diet treatments. In a digestion experiment using the same diets fed to lambs, the digestibility of DM, OM, ADF, NDF and nitrogen balance were not different among sheep fed transgenic or conventional canola meal.

Feedlot steer performance and carcass characteristics have been compared between two types of HR corns (events GA21 and NK603), their isogenic controls, and two commercial varieties (Erickson et al. 2003). The GA21 hybrids express a modified corn EPSPS protein while the NK603 line expresses the CP4 EPSPS protein from the CP4 strain of *Agrobacterium tumefaciens.* The GM corn hybrids were analyzed in a series of three experiments. In the first experiment, 175 steers were fed finishing diets containing 75 % GA21 (DM basis), parental control, or two reference varieties for 92 days. The second experiment compared performance between 196 steers fed the NK603 corn, its isogenic control, and two reference hybrids. The animals were fed mixed diets containing 73 % corn for 94 days. The third experiment also utilized the NK603 hybrid, but the corn was grown and tested in a different location. This last experiment was a blind study for feedlot personnel and entailed 200 steers being fed mixed diets containing 79.5 % dry rolled corn for 144 days. Animals in all of the experiments were marketed to commercial abattoirs for carcass evaluation. For the first two experiments, corn hybrid did cause significant variation in dry matter intake (DMI) but no differences were observed in the pre-planned contrasts between steers fed transgenic varieties and their controls. In contrast, no differences were present for DMI in the third experiment. Carcass weight, longissimus dorsi area, and marbling scores were similar between steers fed transgenic corns and their isogenic controls or reference hybrids. Some differences were observed for fat depth between animals fed the transgenic corn diets and the controls or reference diets however, the variation was not attributed to the presence of the transgene. For all of the experiments, meat composition (moisture, protein or fat content) was comparable irrespective of the diet fed.

The HR line of NK603 corn has also shown to provide similar nutrition to dairy cattle after application of glyphosate- containing herbicides, in comparison to non-GM corn (Ipharraguerre et al. 2003). Although not significant, DMI tended to be lower for the transgenic diet compared to the isogenic control (0.9 kg/d) and one commercial corn (1.5kg/d). As a result, some variation between intakes of CP, ADF, NDF, and non-forage carbohydrates (NFC) arose however, none of these measurements differed between the transgenic and isogenic control-fed animals. Differences in animal performance were not detected between any of the treatments. Milk production, 3.5 % fat-corrected milk (FCM) production, body condition score and BW change were similar among treatments. In addition, milk composition (concentration of fat, CP, true protein, milk urea nitrogen, lactose, and somatic cell count) was not altered by the presence of the transgene in corn hybrids. Similarly, HR GA21 corn, after being treated with glyphosate, resulted in comparable milk

production when compared to cows fed a control corn diet (Donkin et al. 2003). Additional publications testing performance and digestion of GM feeds in ruminants are listed in Table 3.

Table 3. Studies comparing the feeding of genetically modified to conventional crop genotypes in ruminants

Reference	GM Feed[a]	Trait(s)[b]	Animals	Duration[c]	Parameters[d]
Aulrich et al. 2001	Corn Silage (*ad libitum*)	IR x HR	n=40 bulls	246 d	DMI, F:G, BW, CM
"	Corn Silage (*ad libitum*)	IR x HR	n=4 sheep	?	Digestibility of: OM, EE, CF, NFE; ME
Barriere et al. 2001	Corn silage (100%)	IR x HR	n=12 sheep	5 d (collection)	Digestibility of OM, CF, NDF; NE and N
"	Corn silage (70%)	IR x HR	n=48 dairy[e]	91 d	BW, MYC
"	Corn silage (65%)	IR x HR	n=5 dairy	21 d	MYC, cheese properties
Bohme et al. 2001	Sugar beet top silage (60%)	HR	n=4 sheep	10 d (collection)	Digestibility of: OM, CP, CF, NFE; DE, ME, NE_L
Castillo et al. 2004	Cotton seed (11%)	IR	n=12 dairy	7 d (collection)	BW, BCS, DMI, MYC
"	Cotton seed (11%)	IR x IR	n=12 dairy	7 d (collection)	BW, BCS, DMI, MYC
"	Cotton seed (11%)	HR	n=12 dairy	7 d (collection)	BW, Body condition score, DMI, milk yield, composition
"	Cotton seed (11%)	IR x HR	n=12 dairy	7 d (collection)	BW, BCS, DMI, MYC
Donkin et al. 2003	Corn grain (34.1%) + corn silage (41.8%)	IR	n=12 dairy	7 d (collection)	FI, MYC
"	Corn grain (19.9%) + corn silage (59.6%)	IR	n=16 dairy	14 d (collection)	BW, BCS, DMI, MYC, ruminal digestibility
"	Corn grain (19.9%) + corn silage (59.6%)	HR	n=16 dairy	14 d (collection)	BW, BCS, DMI, MYC, ruminal digestibility
Erickson et al. 2003	Corn grain (75%)	HR	n=175 steers	92 d	BW, CM
"	Corn grain (73%)	HR	n=196 steers	94 d	BW, ADG, F:G, CM
"	Corn grain (79.5%)	HR	n=200 steers	144 d	BW, ADG, F:G, CM
Folmer et al. 2002	Corn grain (28%)+silage (40%)	IR x HR	n=12 dairy	7 d (collection)	BW, MYC, rumen pH and VFA, NDF digestion

Table 3. (*continued*)

Reference	GM Feed[a]	Trait(s)[b]	Animals	Duration[c]	Parameters[d]
"	Corn residue (?)	IR x HR	n=67 steers	70 d	BW, grazing preference between GM and non- GM residual corn
Grant et al. 2003	Corn grain (26.7%)	IR	n=16 dairy	14 d (collection)	BW, BCS, MYC
"	Corn grain (23.1%) + corn silage (40%)	HR	n=16 dairy	14 d (collection)	BW, BCS, MYC
Hartnell et al. 2005	Fodder beet (30%)	HR	n=35 sheep	7 d (collection)	Digestibility of: DM, OM, CP, NDF, ADF
"	Sugar beet (30%)	HR	n= 42 sheep	7 d (collection)	Digestibility of: DM, OM, CP, NDF, ADF,
"	Sugar beet pulp (20%)	HR	n=42 sheep	7 d (collection)	Digestibility of: DM, OM, CP, NDF, ADF,
Ipharraguerre et al. 2003	Corn grain (27.34%) + silage(30%)	HR	n=16 dairy	14 d (collection)	FI, BW, BCS, MYC
Stanford et al. 2003	Canola meal (6.5%)	HR	n=8 sheep	21 d (collection)	Digestibility of DM, OM, ADF, NDF; N balance
"	Canola meal (6.5%)	HR	n=60 lambs	21.5-45kg	Morphology of organs, CM, sensory evaluation

[a] Percent of diet; ?, not reported
[b] IR, Insect-resistant; HR, Herbicide-resistant
[c] Duration in days or final weight
[d] ADF, Acid detergent fibre; ADFI, Average daily feed intake; ADG, Average daily gain; BCS, Body count score; BW, Body weight; CF, Crude fibre; CM, Carcass measurements; CP, Crude protein; DE, Digestible energy; EE, Ether extract; F:G, Feed to gain; FI, Feed intake; ME, Metabolizable energy; MYC, Milk yield and nutrient concentrations; N, Nitrogen; NDF, Neutral detergent fibre; NFE, Nitrogen free extract; OM, organic matter; VFA, Volatile fatty acids
[e] Dairy cows

The fate of transgenic DNA

Concerns regarding recombinant DNA have been mainly based on indirect consequences resulting from possible transformation events. It has been suggested that the 35S CaMV (cauliflower mosaic virus) promoter, which is a regulatory sequence common to most registered GM plants, could cause cancer through over-expression of oncogenes should the promoter be integrated into tissue cells after absorption (Ho et al. 1999). However, the cauliflower mosaic virus is ubiquitous and its promoter has been detected in food that does not contain transgenic DNA from GM plants (Wolf et al. 2000). Both FAO and WHO have stated that there is no direct health risk to consumers ingesting transgenic DNA because the DNA from all organisms is structurally similar (WHO 1991). However, fate of transgenic DNA in GM feed consumed by livestock has received interest, mainly as a result of consumer concern. This can be related in part to questions regarding the possible appearance of transgenic DNA sequences in animal products entering the human food chain and the possible effect such events would have on commercial trade.

Another concern regarding transgenic plant DNA is the possibility of transfer of antibiotic-resistant markers (ARMs) to bacteria. Antibiotic-resistant markers in the currently registered GM plants are unlikely to result in the development of resistance to the therapeutic antibiotics presently used in animal and human health (FAO/WHO 2000). The antibiotics used as markers are either rarely used in human medicine or widespread resistance is already prevalent in nature. Consequently, ARMs in GM plants are unlikely to pose a significant threat to human health (Gay and Gillespie 2005).

For the stable transfer of plant DNA into a microbial or mammalian cell to occur, FAO/WHO (2000) have proposed that the transgene would have to be released from plant material, survive nucleases within the gastrointestinal tract (GIT), and then be inserted into host DNA by rare repair or recombination events in competent microbial or mammalian cells. It is important to note that the transgene would have to survive any feed processing events prior to intake by livestock as well. Aside from competing with other plant DNA, the transgene would be heavily diluted by microbial DNA of intestinal origin and would have to compete with that DNA for absorption across the GIT.

Prior to the marketing of GM plants, there was little interest in the fate of plant DNA after consumption because dietary nucleotides were not considered a requirement for efficient animal production (Beever and Kemp 2000). It has been documented that DNA is reduced to smaller fragments, nucleotides, and nucleosides resulting from digestive processes (Armstrong 1974; McAllan 1982; Beever and Kemp 2000). Mastication contributes to DNA digestion by physical cell rupture and by increasing the surface area of feed available for microbial or endogenous enzymatic digestion of plant cells. Once free, plant DNA is susceptible to degradation by nucleases. The endonuclease DNase I has optimal activity between pH 6.8-8.2 and is secreted by salivary glands and the pancreas (Armstrong and

Hutton 1974). Another class of endonuclease, DNase II, functions optimally at an acid pH between 4.5-5.5 (Evans and Aguilera 2003), however the role of DNase II in digestion is unclear. Theoretically, if present in fluid of the small intestine, DNase II would be most active in the jejunum while DNase I would most efficiently degrade DNA in the jejunum to ileum. Phosphodiesterases I and II, with optimal activities at pH 9.3 and 7, respectively, are also responsible for the degradation of nucleic acids in the distal small intestine (Armstrong and Hutton 1974). In ruminants, significant nuclease activity is present in rumen fluid and accounts for rapid degradation of free DNA (Flint and Thomson 1991; Ruiz et al. 2000). In addition to enzymatic digestion, free DNA exposed to low pH conditions of the stomach in monogastrics or the abomasum in ruminants are susceptible to depurination (Beever and Kemp 2000). Although DNA degradation throughout the digestive tract has been investigated, knowledge about the fate of ingested feed DNA fragments was previously limited. In recent years, studies have added significant insight into the fate of plant DNA fragments after consumption.

Transgene intake by livestock

The amount of transgenic DNA ingested depends on the number of transgene copies in the plant's genome, the percentage of GM feed in the diet, and feed intake. The quantity of DNA in most crops is less than 0.02 % on a DM basis (Beever and Kemp 2000). Beever and Phipps (2001) estimated that a dairy cow consuming 24 kg DM/d of a diet containing 40 % transgenic corn silage and 20 % corn grain, would have an intake of 57 g/day of total plant DNA. Of that, 54 μg would be recombinant DNA and account for only 0.000094 % of the total DNA intake. Actually, the total of transgenic DNA intake may be lower, considering that ensiling GM plants quickly leads to degradation of large plant DNA fragments (Hupfer et al. 1999). Other processes too, such as heat treatment, will also degrade plant DNA including transgenes (Alexander et al. 2002; Chiter et al. 2000; Gawienowski et al. 1999; Yoshimura et al. 2005).

Swine

Chowdhury et al. (2003a) analyzed transgenic and intrinsic DNA fragments by PCR throughout the intestinal tract of pigs fed diets containing 70 % IR corn. Both endogenous and transgenic primer sets detected their respective DNA fragments in cecal and rectal contents of the pigs (range of 25-50 % of the samples were positive), indicating that plant DNA is detectable towards the end of the swine digestive tract when such a diet is consumed. Another study detected three separate endogenous genes and two transgenic fragments of varying size from digestive contents of the stomach, duodenum, ileum, cecum, and rectum of 10 pigs fed diets containing 60 % insect-resistant corn (Chowdhury et al. 2003b). All of the endogenous fragments, which included sequences from the *rubisco* (1028 bp), *invertase* (226 bp), and *zein* (242 bp) genes were detected in 30-100 % of the samples, depending of the origin of the contents and primer set employed. The

relatively large amplicon size of the *rubisco* gene indicates that even substantially sizeable fragments of DNA survive the digestive process in swine. However, the authors noted that corn kernels were visible within gastrointestinal contents. It is therefore possible that DNA detected towards the end of the digestive tract was protected within undigested corn residue that was inaccessible to DNA degrading enzymes.

Klotz and colleagues (2002) described the time- dependent persistence of plant DNA in the upper part of the GIT of pigs. The animals were switched over from a non-transgenic diet and fed 1 kg of feed containing 50 % IR corn (event 176), after which they were slaughtered at sequential times up to 12 h. A 199 bp sequence of plant chloroplast DNA was amplifiable from contents of the stomach, duodenum, jejunum, and ileum for 12 h after feeding, although the intensity of each PCR product eventually diminished over time. In contrast, transgenic sequences of the *cry1Ab* gene (211 and 251 bp) were not detected from any digestive sample at any time point. The differences are likely due to the number of copies of each gene per genome. Chloroplast DNA genes can be present between 500-50000 copies (Bendich 1987) per genome compared to a single insert of most transgenes. In addition, neither the endogenous or transgenic DNA sequences could be detected in blood or lymph nodes from the animals at any time point. These results contrast those reported by Reuter and Aulrich (2003) who also performed gene persistence studies using Bt176 corn. Animals in this study were fed 2.6 kg of a ground diet (1 mm particle size) containing 70 % corn throughout the fattening phase. A 211 bp fragment of DNA amplified from the *cry1Ab* gene was detected in the stomach up to 24 h, the duodenum, jejunum and ileum up to 48 h, the cecum up to 12 h, colon up to 24 h, and rectum up to 48 h after feeding the diet. A 140 bp fragment from chloroplast DNA was detected in every type of sample taken from the GIT, even 72 h after feeding. While it might be expected that DNA digestibility would increase with the degree of grinding of the feed, as was the case in the study by Reuter and Aulrich (2003), the amount of transgenic DNA ingested will also affect the likelihood of gene persistence within the digestive tract. This may explain the differences in results between the above two studies. Klotz et al. (2002) fed the pigs 0.5 kg of insect-resistant corn whereas the animals in the study by Reuter and Aulrich (2003) were fed 1.82 kg of corn per day. Although detection of plant genes is possible in the GIT of swine, the relative stability of free DNA has not been reported.

The above two studies also attempted to detect plant DNA in animal tissues. Reuter and Aulrich (2003) were able to detect the 140 bp fragment for chloroplast DNA in blood, liver, lymphatic glands, spleen, kidney, *musculus gluteus maximus*, *musculus longissimus dorsi*, *musculus trapezius* and ovary samples in 16.7, 54, 16.7, 12.5, 27, 33.3, 54.2, 22.9, and 62.5 % of the samples tested, respectively. Again emphasizing the importance of transgene copy number in digesta detection, the transgenic DNA from the single copy *cry1Ab* gene was never detected in any tissue sample. The relatively high detection rate in the ovaries was suggested to

result from high blood flow to that organ. Klotz et al. (2002) were also unable to detect transgenic DNA in muscle, liver, spleen, lymph nodes and blood from pigs fed diets containing 20-25 % corn. In contrast with Reuter and Aulrich (2003), chloroplast DNA was also undetectable in any of the mentioned tissues. These differences between and within each study likely highlight the significance of the number of genes ingested, or perhaps the sensitivity of detection methods. The animals in the study by Klotz et al. (2002) consumed fewer copies of transgenic and endogenous corn genes, as compared to those in the study of Reuter and Aulrich (2003).

The presence of plant DNA in 118 samples from the *longissimus* muscles of pigs fed approximately 85 % insect-resistant corn (event MON810) or an isogenic control has also been explored (Nemeth et al. 2004). These researchers used highly sensitive primers with low limits of detection (LOD), that amplified two fragment lengths from a chloroplast *rubisco* gene (173 bp, LOD = 0.02 genome equivalents; 500 bp, LOD = 0.08 genome equivalents) and a fragment from the *p35S* gene (123 bp, LOD = 5 genome equivalents), the promoter of the transgenic construct. If any of the muscle samples tested positive for the *p35S* gene, then analysis for MON810 construct- specific sequence (149 bp, LOD = 10 genome equivalents) was carried out. For the 173 bp *rubisco* sequence, 53 % of the samples tested positive (both duplicates positive), 43 % negative (both duplicates negative) and 4 % indeterminate (duplicate samples were both positive and negative). The 500 bp *rubisco* fragment proved to be positive in 43 % of the samples, negative in 43 % and indeterminate in 14 %. One tissue sample out of the total 118 tested positive for the *p35S* sequence. To confirm the results, the analysis was repeated with new tissue subsamples, which again were positive. When tested with the MON810 primer set, the result was indeterminate, suggesting that the number of transgene copies was below the LOD. This study demonstrated that transgenic DNA acts similarly to endogenous DNA, which when present in high enough quantities, may cross the gastrointestinal barrier. Additionally, despite detection of the transgene in pork tissue, the study showed no effect on growth performance among pigs fed diets containing transgenic or conventional corn (Weber et al. 2000). Thus, neither transformation of the corn nor the transgene itself appeared to have any adverse affect on animal health.

Poultry

Chambers et al. (2002) investigated the fate of plant DNA throughout the digestive tract of chickens fed IR corn (event 176) present at 80 % of the diet. PCR-restriction fragment length polymorphism (RFLP) analysis indicated that the *beta lactamase* (*bla*) gene, present as part of the transgenic construct found in 176 corn, could be detected in the crops of each bird tested (n=5) and in the stomach of two of the birds. Results for the transgenic DNA were negative in the small intestine, large intestine, cecum, and rectum. In contrast, the corn mitochondrial gene, *nad5,* was detected in the crop and stomach of all birds. Mitochondrial genes are generally

present at a higher copy number, probably resulting in more frequent detection within digesta contents. The *nad5* gene was not detected in digestive contents collected from other regions on the intestine. A study testing diets containing 60 % of the same IR corn used by Chambers et al. (2002) reported similar gene detection results (Aeschbacher et al. 2005). The transgenic *bla* (479 bp) could be detected in the crop of broilers by PCR and not in the gizzard, small intestine, cecum, and excreta. The endogenous *invertase* gene was also limited mostly to the upper part of the digestive tract, being detected in the crop, gizzard, and to a lesser extent, the small intestine. Contradicting the above two studies was an experiment that detected DNA throughout the GIT of poultry fed diets with the same Bt176 corn when it accounted for 74 % of the diet (Tony et al. 2003). Using real time PCR, the authors showed that sequences of the corn- specific *hmg* gene (79 bp) and the transgenic *cry1Ab* gene (129 bp) were detected in the crop, proventriculus, gizzard, duodenum, jejunum, ileum and cecum and rectum. However, the differences in persistence between the studies may also reflect smaller PCR products and greater assay sensitivity in the latter experiment. Generally, real-time PCR provides lower LOD compared to conventional PCR.

Using conventional PCR, Tony and colleagues (2003) were unable to detect both a 211 bp sequence of transgenic DNA and 226 bp sequence of the endogenous *invertase* genes in blood, pectoral, thigh, liver, heart, spleen, kidney, bursa, or thymus tissues from broilers fed diets containing 73.6 % Bt176 corn. Like the transgene, the *invertase* gene is a low copy gene and is present at one copy per plant genome (Hernandez et al. 2004). However, a 199 bp sequence of high copy chloroplast DNA was detected in all these tissues except the heart, bursa, and kidney, up to 4 h after starvation. Aeschbacher et al. (2005) reported similar results for transgenic DNA detection in birds fed diets containing 60 % Bt176 corn. The transgenic *bla* gene (479 bp fragment), was not identified in the liver, spleen, muscle, blood, and eggs of hens or broilers. The authors were however, able to detect the same low copy 226 bp *invertase* sequence as described by Tony et al. (2003), in the liver, spleen, muscle, blood, crop, gizzard, and small intestine of broilers, but not in the cecum or excreta. Because all DNA behaves similarly, it would be expected that the absorption of one low copy gene fragment should signify the absorption of other low copy gene fragments, including those from the transgenic *bla* gene. However, fragment absorption may be size-dependent (Klotz et al. 2002) and the authors did note that the majority of DNA recovered from the digestive tracts of the birds was less than 180 bp in length which is smaller than the amplicon size of the *bla* primer set.

Nemeth et al. (2004) were unable to detect the 149 bp segment of the transgenic construct in breast muscle, however a 173 bp sequence of the high copy chloroplast *rubisco* gene was positive in 15 % of the samples, negative in 75 % of the samples, and indeterminate in 10 % of the samples, again emphasizing the importance of gene copy number. While detection of plant DNA fragments in

poultry muscle tissues appears possible, detection of high and low copy plant genes has not been reported in eggs (Einspanier et al. 2001; Klotz et al. 2002).

Ruminants

Deoxyribonuclease activity has been shown to be present in bovine rumen fluid (Duggan et al. 2000; Flint and Thomson 1990; Ruiz et al. 2000) and ovine intestinal fluid (Alexander et al., 2004). This likely explains why most plant DNA genes, at least those present at low copy numbers, have been shown to be mainly associated with feed residue. Phipps et al. (2003) analyzed gene stability throughout the digestive tracts of dairy cows fed 18.5 % IR corn (event MON810) and 13.0 % HR soybean meal (event GT 40-3-2). In the liquid phases of both ruminal and duodenal fluids, only a 167 bp sequence of the high copy chloroplast *rubisco* gene was detected. In contrast, none of the low copy amplicons from the endogenous soybean *lectin* (240 bp), corn *hmp* (209 bp) or recombinant (171 bp, soybean; 203 bp, corn) genes could be detected. All of the fragments were amplifiable from the solid phases of digestive fluid. In the feces, only the *rubisco* gene was detected. Similar results were reported for the 1363 bp *cp4 epsps* transgene found in herbicide-resistant canola (event Gt73) when canola substrates were incubated in ruminal batch cultures (Alexander et al. 2002). The same was also true for smaller fragments of the transgenic construct in Gt73 canola substrates, ranging in size between 300 to 527 bp, when incubated in ruminal batch cultures (Sharma et al. 2004). A 62 bp sequence of the transgenic construct however was detected in the aqueous phase of intestinal fluid *in vitro* but the copy number of this amplicon only reached a maximum number of 1600 copies when digestion was at its greatest (Alexander et al. 2004). The small amplicon size likely affected these results, as the entire 1363 bp transgene was not detected in the liquid phase of intestinal contents.

Einspanier et al. (2004) used real-time PCR to quantify transgenic and endogenous genes throughout the digestive tract of cattle fed diets containing 88.5 % Bt176 corn silage. After ensiling, the quantity of each gene decreased to less than 3 % of the starting quantity. Surprisingly, the amounts of both transgene and endogenous gene seemed to increase after passage from the rumen to the abomasum, before decreasing dramatically to unquantifiable levels in the jejunum and colon. It should be noted that the quantity of each gene was expressed per 90 ng of total DNA. Therefore, it is likely that the plant genes were diluted with microbial DNA to a greater extent in the rumen than the abomasum. Both the transgene and endogenous gene followed similar trends throughout the GIT.

Should plant DNA fragments be absorbed, the likely place for such an event would be the intestine, and more specifically, the Peyer's patches of the distal ileum or proximal large intestine (Schubbert et al. 1997). Because of the highly unstable nature of DNA in the ruminant digestive tract, it is probable that DNA released in the rumen, at least for low copy genes, does not persist to the proximal small intestine. Therefore, digestion of plant residue in the ileum may be necessary for plant genes to have a chance of crossing the intestinal barrier. There is evidence that

digestion does occur in the ileum (Alexander et al. 2004; Erfle et al., 1982) and that plant DNA is released into the aqueous intestinal phase. The first study to probe for plant DNA in ruminant tissues showed that transgenic DNA was not detectable in the muscle, liver, spleen, kidney, and blood lymphocytes of cattle fed Bt 176 silage *ad libitum* (Einspanier et al. 2001). However, given the reduction in transgene concentration during ensiling, these results are not surprising (Einspanier et al. 2004). A 199 bp chloroplast sequence of DNA was detected in the blood lymphocytes (Einspanier et al. 2001). Similarly, a *rubisco* gene fragment was detected in the blood of cattle fed GM corn and soybean meal, but transgenic sequences were never detected (Phipps et al., 2003). The same occurred for calves being fed rations containing 43.3 % Bt11 IR corn (Chowdhury et al. 2004). A 231 bp fragment of the *rubisco* gene was detected in the liver, spleen, kidney, mesenteric lymph nodes, and longissimus muscle samples. However, the c*ry1Ab* transgene tested negative in all of the tissue samples. Nemeth et al. (2004) were also able to detect a 173 bp sequence of the *rubisco* gene in the beef brisket muscle of cattle fed 75 % dry rolled corn and 15 % corn silage (event MON810) for 5 % of the samples, whereas transgenic DNA was not detected.

Nemeth et al. (2004) additionally tested for plant DNA in milk from dairy cattle fed 20 % corn plus 60 % corn silage of the same MON810 event described above. A 173 bp sequence of the rubisco gene was amplifiable in 86 % of the samples, and not detected in the other 14 %. A larger 500 bp sequence of the same gene could be detected in 79 % of the samples, while the remaining 21 % were negative. None of the milk samples tested positive for low copy transgenes. Similar results have been reported for other low copy plant genes. Investigations attempting to detect transgenic and low copy endogenous DNA in milk from animals fed GM cotton (Castillo et al. 2004; Jennings et al. 2003), corn (Jennings et al. 2003; Phipps et al. 2003; Yonemochi et al. 2003), soybean (Phipps et al. 2002) or non-GM corn and soybean (Poms et al. 2003) have reported negative results. These studies suggest that while absorption of plant DNA is possible throughout the ruminant digestive tract, passage of foreign plant DNA into milk is related to the plant gene copy number.

Summary

To ensure the safety of GM plants as animal feed, regulatory bodies have adopted the policy of substantial equivalence. Though this policy does not prove nutritional equivalence, to date, there have been no adverse effects in animals consuming commercialized GM crops that have been approved under these guidelines. Differences in digestibility and performance of the currently marketed GM feeds are more likely to result from the genetic background of the plant and the conditions under which it is grown as opposed to the insertion of transgenic DNA. Studies undertaken to address concerns that transgenic DNA may enter the food

chain by means of animal products have shown that transgenes are unlikely to be incorporated into animal products, due to the low copy number inserted into plant genomes and the activity of nucleases throughout the digestive tract of livestock. Absorption of plant DNA across the intestinal barrier of livestock does seem to be a normal occurrence when fragments of DNA are present in digesta at high concentrations. Absorption of DNA fragments does not appear to have adverse effects on livestock, whether the DNA is transgenic or endogenous. Given the popularity of GM crops, which is expected to further increase over the next few years, GM plants in the food-chain should continually be monitored. Rigorous testing procedures for novel crops should remain in place, especially for traits that alter the nutritional composition of the plant. In these instances, nutritional equivalence should be determined through animal experiments in addition to substantial equivalence tests. However, the fate of recombinant molecules in the currently registered GM plants does not need to be included in feed safety assessments.

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Intraspecific gene flow in herbicide-resistant crops: influencing factors

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Gene flow, the movement of genes between two populations, can occur between varieties of the same crop (intraspecific) and sexually compatible relatives (interspecific). Intraspecific gene flow can occur at a high frequency and be vectored by both pollen and seed. Frequency and distance of intraspecific gene flow depend on crop biology, the environment, and management scenarios. Therefore, to mitigate gene flow, an understanding of the relative impact of these factors is crucial. Canola (*Brassica napus* L.) and wheat (*Triticum aestivum L.*) containing novel traits conferring herbicide resistance have been released in western Canada. Canola has a relatively high degree of outcrossing, 12 to 55 %, diminishing rapidly with distance but still measurable at 800 metres. Outcrossing between herbicide-resistant varieties has resulted in multiple herbicide-resistant individuals. Wheat is less likely to outcross, <10 %, limiting gene flow via pollen. Both crops occur commonly as volunteers in western Canada, but canola seeds persist longer in the seedbank. Volunteers, along with mixing during handling, transport and replanting of seed, can cause adventitious presence, the unintended, technically unavoidable presence of genetically engineered material in an agri-food commodity and allow gene flow via seed. Seed can be transported over long distances. In canola, both pollen and seed mediated gene flow are significant, while in wheat, seed may be the primary vector of gene flow. To integrate the biological, spatial and temporal parameters, an index ranking of the probability of gene flow is proposed for four Alberta crops.

Introduction

Movement of transgenes from genetically modified (GM) crops has attracted considerable interest around the world. Canada regulates plants with novel traits (PNT), encompassing both GM and other novel crops, and has approved PNT varieties of nine crop species for commercial release since 1995. Canola varieties resistant to either glyphosate (Roundup Ready®), glufosinate (Liberty Link®) or imazethapyr + imazamox (CLEARFIELD®) were granted unconfined release status in Canada in 1995 (Duke 2005). Roundup Ready® and Liberty Link® varieties are considered GM while CLEARFIELD® varieties were developed using mutagenesis. Herbicide-resistant canola varieties have been overwhelmingly adopted by producers in western Canada, encompassing 91-93 % of the canola acres in Alberta in 2005 (M. Hartman, personal communication 2006). Imidazolinone

(CLEARFIELD®) wheat was approved in Canada for unconfined release in 2004 and glyphosate-resistant (Roundup Ready®) wheat was evaluated in extensive confined release field trials prior to a voluntary registration withdrawal by Monsanto. A decade of experience with wide scale release of HR crops provides a substantive data base on the impact of their release (Canola Council of Canada 2001; Brookes and Barfoot 2005).

Gene flow is defined as the movement of genes, as gametes (pollen), zygotes (seeds), individuals or groups of individuals between two populations, and the incorporation into the gene pool of the new population (Slatkin 1987). Gene flow can occur spatially, with the movement of seeds or pollen or temporally, through residual seedbanks and volunteer crop plants. Gene flow can occur within varieties of the same crop (intraspecific) (Reiger et al. 2002) and also between sexually compatible relatives (interspecific) (Ellstrand et al. 1999) as discussed in the subsequent paper in this volume (Warwick). Movement of herbicide resistance genes presents new challenges and obstacles to Canadian producers. Herbicide-resistant (HR) volunteers may require producers to adopt additional or alternative weed control practices (Ogg and Isakson 2001; Beckie et al. 2004). Gene flow and identity preservation (IP) was a concern primarily for plant breeding and seed multiplication. However, with the introduction of PNT crops and the global market for commodities, genetic purity and IP is becoming more important to producers and consumers. Maintaining purity and segregation of GM from non-GM crops is also required for the coexistence of future niche markets providing premiums for GM or non-GM products. Segregation of first generation PNT crops with agronomic traits was not warranted because the products were substantially equivalent to products of conventional crops. With the potential introduction of second and third generation PNT crops (nutritional and industrial applications, respectively) gene flow regulation and mitigation will be critical to food safety and market access (Smyth et al. 2002). The ability of Canadian producers to contain these traits and meet international purity standards will be a challenge, resulting in a new paradigm for agricultural production. In this chapter, we will compare the relative importance of pollen and seed as vectors for intraspecific gene flow using examples from canola and wheat in western Canada.

Temporal and spatial gene flow in cropping systems

Within the cropping system, gene flow occurs in both space and time. When assessing cropping systems for potential vectors of gene flow, it is advantageous to separate individual components and the potential influencing mechanisms involved (Figure 1). Genes conferring novel traits are introduced to a field either by deliberate planting or in a contaminated seedlot (see certified seed purity below). In the case of herbicide-resistant crops, resistant plants are selected with herbicide applications, modifying the genetic composition of the population. Surviving

individuals flower and produce pollen. Pollen mediated gene flow occurs between deliberately planted individuals, and between crops and/or volunteers in adjacent fields. Seeds produced have a range of fates. They can be harvested and sold locally or internationally, mixed inadvertently with other crops or varieties, or replanted into the same or other fields. Some seeds fail to be harvested, and may be degraded or germinate without successful reproduction. They can be consumed or moved by birds or animals. Alternatively, they can enter the soil seedbank and reside for months to years. Volunteers can emerge, be selected, produce and receive pollen, and set seeds, thus initiating another cycle.

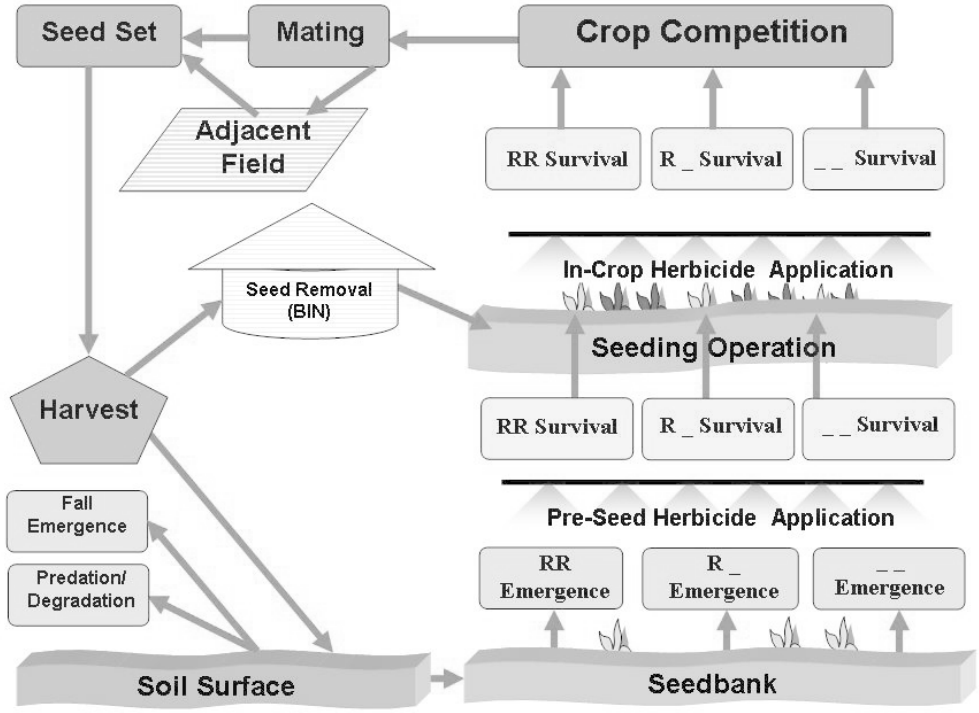

Figure 1. Flowchart showing gene flow through the annual lifecycle of wheat using Roundup Ready® (RR) wheat as a model crop. RR, R _ indicates homozygous dominant and hemizygous, respectfully, for the Roundup Ready® gene; and _ _ represents the absence of the transgene, susceptible to glyphosate. Differential herbicide selection occurs at the pre-seeding herbicide application for volunteers and at the in-crop herbicide application for seeded crops and volunteers.

Gene flow within a cropping system can be partially explained through a mechanistic model. However, model outcomes can be enhanced by taking a stochastic approach that accounts for parameter variability and generates a probability distribution of outcomes. Gene flow models may be adapted from similar models developed to predict the selection of herbicide resistance (Hanson et al. 2002; Monjardino et al. 2003; Diggle et al. 2003; Neve et al. 2003). For example, a simulation model was developed to assess both temporal and spatial intraspecific gene flow from HR rape to volunteer rape (*B. napus* to *B. napus*) (Colbach et al. 2001a; Colbach et al. 2001b). Based on the annual lifecycle of oilseed rape, this model assessed the risk of HR gene escape and ranked the associated cropping system accordingly.

Pollen mediated gene flow

Frequency of outcrossing is influenced by crop species and variety, environmental, temporal and spatial variables. Crops vary widely in outcrossing potential, pollen production, duration of pollen viability, and the extent of flower opening at anthesis (Waines and Hegde 2003). Pollination vectors, wind, animals or insect(s), affect outcrossing distance and pollination success. Environmental factors that affect outcrossing include temperature, wind speed and relative humidity (RH). Flowering synchrony of adjacent fields presumably affects the potential intraspecific pollen flow by increasing the probability of receiving pollen from an adjacent population. Pollen-mediated gene flow occurs at the highest frequency within the first few metres of a pollen source, and rapidly decreases over distance. The distance at which interspecific cross pollination is extinguished is difficult to determine. The maximum distances recorded for pollination are a function of the sensitivity of the detection method, the inherent sampling error in low frequency events, as well as the absolute distance which viable pollen can be carried by wind, insect or animal vectors.

Canola pollen flow

Canola is primarily self pollinated but outcrossing in adjacent plants has been reported from 12 to 55 % (Légère 2005). Outcrossing may occur between adjacent canola fields and canola volunteers. Gene flow between HR varieties results in gene stacking and multiple HR volunteers (Hall et al. 2000). Beckie et al. (2003) documented gene flow in 11 paired glyphosate/glufosinate-resistant commercial fields. As expected, pollen mediated gene flow diminished with distance; from an average of 1.4 % at the common border to 0.04 % at 400 m. Volunteers with multiple herbicide resistance were confirmed in following years at up to 800 m, the limit of the study area. While the presence of unexpected multiple HR volunteer canola has been widely reported, the economic and agronomic consequences of these volunteers to growers has been minimal.

Wheat pollen flow

Wheat is also primarily self pollinated, but the outcrossing frequency and the distance of pollen movement is lower than canola. Outcrossing frequency is variety specific, and can vary significantly with the crop planting date (Hucl 1996). Lawrie et al. (2006) investigated outcrossing of wheat using a direct spike contact method with four seeding dates to extend the flowering period, and found outcrossing rates commonly below 2.8 % with some cultivars exceeding 10 %. Results were variety specific, with Canada western extra strong cv. Glenlea (10.6 %) having the highest outcrossing rate in 2001. During anthesis, wheat florets can behave both cleistogamously (closed flowers) or chasmogamously (open flowers). The degree of flower opening is environmentally, morphologically, and genetically influenced (De Vries 1971). The potential to outcross is highly and directly correlated with the degree of flower opening in the wheat flowers (Hucl 1996). Outcrossing between adjacent plants (30 cm or less) was measured in small plot studies in Canada (Hucl 1996), New Zealand (Griffin 1987) and the United States (Martin 1990). All reported low outcrossing rates (<2 %) with the exception of cv. Oslo 5.2 % in Canada, cv. Rongotea 2.84 % in New Zealand, and cv. KS75210 3.1 % and Newton 2.1 % in the United States.

As the distance increases between the pollen source and recipient plants, outcrossing frequency declines rapidly. Matus-Cadiz et al. (2004) reported an average of 0.003 % outcrossing at 100 m in CDC Teal. Beyond 100 m, one gene flow event was confirmed at 300 m (0.005 %). Pollination frequency is influenced by wind direction and by relative humidity (Hansen et al. 2005).

Seed mediated gene flow

Much of the literature addressing intraspecific gene flow focuses on pollen movement (Hall et al. 2000; Hucl and Matus-Cadiz 2001; Beckie et al. 2003; Hanson et al. 2005), however, pollen is short lived and travels relatively short distances. Seed movement has the potential to move transgenes over greater distances, and seed can persist over long periods of time to serve as a genetic reservoir or bank. Seeds can be lost during transport and moved between fields by machinery, or inadvertently mixed with other crops or other varieties. Admixed seeds can be produced from seeds unintentionally sown with the crop or by volunteers growing with the crop. Seed is traded and transported internationally as bulk commodities, therefore seed contamination has the potential to influence cropping agriculture in countries around the world.

Volunteer crops are one avenue for spatial and temporal gene flow. Seeds may move with machinery, especially harvesting equipment. Volunteer crops in the seedbank may germinate in the years following crop production. The propensity of crops to occur as volunteers is species, environmental, and management specific. The predominant source for volunteer crops is harvest loss. Both seed shatter and

the seed expelled from the combine contribute to harvest loss, resulting in seeds returning to the soil surface. Harvest loss is generally expressed as a percentage of the harvested yield and may be highly variable, depending on weather conditions, pests, machinery setup, and crop condition.

Canola volunteers

Canola has significant dehiscence (seed shatter) prior to and during harvest, dispersing this small seeded crop. A study of 35 fields in western Canada showed an average seed loss of 107 kg ha^{-1}, equivalent to 6 % of the crop seed yield or 3,000 viable seeds m^{-2} (Gulden et al. 2003b), which is 20 times the normal seeding rate. However, seed losses ranged widely, from 1,530 to 7,130 seeds m^{-2} and were as high as 14,000 seeds m^{-2} in isolated cases. If even a portion of that seed survives, inputs to the seedbank can be significant.

Canola lacks primary dormancy, and seed lost at harvest may germinate in the fall under favourable conditions and be killed by frost. Subsequent seedling emergence occurs primarily in spring prior to the seeding or in-crop herbicide application (Gulden et al. 2003a). Seed burial immediately following harvest, either naturally or by cultivation, prolongs seedbank persistence in canola; particularly in genotypes with high secondary seed dormancy potential (Pekrun et al. 1997; Gulden et al. 2003a; Roller et al. 2003), while leaving seed on or near the soil surface reduces persistence of seed in the seedbank. Hence, the reduction of fall and spring tillage can significantly reduce seed persistence.

The volunteer canola seedbank tends to decline rapidly in agroecosystems. Canadian spring genotypes germinate in the first 2 to 3 years after a seedbank establishment (Gulden et al. 2003a). After approximately 3 years, however, rapid seedbank decline appears to slow with low, but stable levels of volunteer canola seeds remaining viable for up to 10 years (Lutman et al. 2003). Canola has been reported to occur in >10 % of fields surveyed with densities up to 143 plants m^{-2} (Table 1).

Table 1. Volunteer canola and wheat from western Canadian field surveys (2000s). Field frequency indicates the proportion of fields where the volunteer occurred, while the average and maximum field density refers only to fields in which the volunteer occurred.

Abundance Rank	Species	Field Frequency	Average Field Density	Max Field Density	Relative Abundance
		%	plants m^{-2}		
12	V. wheat	10.8	5.9	281	6.7
14	V. canola	10.2	4.5	143	5.5

Source: (Leeson et al. 2005)

Wheat volunteers

Anderson and Soper (2003) reported typical wheat harvest losses of 2 to 6 %, which can lead to 240 to 700 seeds m^{-2} on the soil surface. Minimizing harvest loss presents the greatest opportunity to decrease volunteer wheat densities in subsequent years. Harker et al. (2005) observed a seedling recruitment of 1.4 % prior to crop seeding the year following seedbank inputs, indicating that the overwintering mortality or predation of wheat seeds may be high.

Wheat has a short primary dormancy period during which germination will not occur even if conditions are favourable. This after-ripening period can be both environmentally (Pickett 1989) and genetically controlled (Komatsuzaki and Endo 1996). Harker et al. (2005) observed emergence of volunteer wheat and found the majority of volunteers emerged the year following wheat production. Volunteers predominantly emerge in the spring but can emerge throughout the growing season depending on the crop canopy (Anderson and Nielsen 1996).

The persistence of wheat in the seedbank has been described as short lived but can be highly variable. Anderson and Soper (2003) reviewed the literature and reported that in classical burial studies, wheat generally persists less than 1 year. However, volunteer wheat was observed emerging 16 months after harvest in field studies (Pickett 1993), and field survey data reported by Thomas and Leeson (1999) indicated volunteer wheat persisted in fields up to 5 years after harvest. Harker et al. (2005) investigated the persistence of volunteer glyphosate-resistant (GR) wheat in an eight location multi-year study. Three years after seed dispersal volunteer GR wheat was evident at very low levels at most locations. At the end of the third year, no viable seeds were found in the seedbank. In contrast to the reports of Derksen et al. (1994), the effect of continuous cropping rotations did not result in increased volunteer wheat persistence.

Conventional wheat volunteers are readily controlled by the available pre-seed and in-crop herbicide options in broadleaf crops but not in other cereals. Control of PNT wheat volunteers is necessary to prevent seed mediated gene flow and decrease adventitious mixing of off-types. As a pre-seeding application, quizalofop-p-ethyl provides control of volunteer wheat regardless of HR variety (Lyon et al. 2002; Rainbolt et al. 2004), but at additional cost to the producer (Ogg and Isakson 2001).

Volunteer wheat is a common weed in fields of western Canada. In fields where volunteer wheat occurred, the average density across all eco-regions was 5.9 plants m^{-2} and the highest density recorded was 281 plants m^{-2} after herbicide application (Table 1). Volunteer wheat increased in frequency and relative abundance in the Canadian Prairie Provinces between 1970 and 2000 and is now ranked 12th in abundance relative to other weeds (Leeson et al. 2005). Changes in farming practices have led to effective control of many weed species and decreased overall weed density, possibly leading to the increased relative abundance of volunteer wheat.

Certified seed purity

Certified seed can be a source of unknown or unwanted novel traits. Producers usually (80 %) purchase canola seed to obtain hybrid canola varieties, comply with technology use agreements, simplify seed treatment and/or ensure high germination. The presence and ease of detection of HR traits has facilitated more accurate estimation of contamination in canola seed. Friesen et al. (2003) examined 33 canola samples from 27 Canadian Seed Grower Association numbered certified seedlots and found that 26 contained detectable levels of HR seeds. Fourteen seedlots had contamination in excess of 0.25 %, therefore exceeding the 99.75 % cultivar purity threshold. Glyphosate resistance was detected more frequently than glufosinate in the contaminated seedlots (9 and 5 respectively), corresponding to the higher usage of glyphosate-resistant canola in western Canada. Three seedlots contained the glyphosate resistance trait in excess of 2 %.

Wheat is commonly replanted by growers from the previous crop unless new varieties are introduced. A direct comparison using a PNT marker in certified wheat seed has not been conducted, however Hucl et al. (2004) used awnedness as a visual marker to determine impurities in foundation and registered wheat seed. Contamination levels were reported to be less than 0.02 %. The sources of contamination were differentiated: outcrossing resulted in < 0.002 % contamination, and mechanical mixing was responsible for < 0.01 %. Hucl concluded that Canadian Seed Growers current agronomic and operational procedures for spring wheat achieve high purity levels.

The Canadian certified seed system is based primarily on visual markers. With the introduction of PNT crops with phenotypes that are not evident in seed but might affect variety performance or market acceptability, additional testing and stringency by the seed industry is essential.

Spatial isolation and gene flow

Spatial isolation and rotational intervals have been used as a principal mitigation tool to limit interspecific and intraspecific gene flow by seed growers. Isolation distances, derived by years of experience by seed growers are an indicator of the relative gene flow of a crop species (Table 2). As these crops are grown in a landscape, the frequency of cropping may also influence the propensity for gene flow. Using four crops grown in Alberta as an example, in counties and municipalities where these crops are most frequently grown, the highest proportion of canola fields per county is 1 in 5, while wheat can be as common as 1 in 3 fields. Flax (*Linum usitatissimum* L.) and corn (*Zea mays L.*) are grown much less frequently and presumably spatial isolation of fields would therefore increase.

Gene flow indices

Because of the numerous factors affecting gene flow, it is important to assess gene flow conduits and the associated risks at a regional scale. An interspecific gene flow index was presented by Ammann and Jacot (2003); based on several parameters, crops were assigned to risk categories indicating potential environmental effects. Adapting this concept, a preliminary intraspecific gene flow index was developed for four Alberta crops (Table 2). Based on pollen dispersal, spatial requirements and seedbank persistence, a relative gene flow index was assigned to each crop within a specific growing region. The relative gene flow index may direct initial or regional PNT production, selection of appropriate host crops for novel traits and suggest research to determine appropriate mitigation measures and best management practices.

Table 2. Preliminary gene flow index for Alberta crops.

Crop	Outcrossing Frequency [a]	Vector [a]	Isolation Distance [b]	Landscape Frequency [c]	Seedbank Persistence [d]	PFI [e]
	%		m	Field	Years	
Canola	30	Insects/wind	100	1 in 5	1-5	High
Corn	100	Wind	200	1 in 59	1	Moderate
Wheat	< 9	Wind	30	1 in 3	1-5	Moderate
Flax	0-5	Wind/insects	3	1 in 114	NA	Low

[a] Canadian Food Inspection Agency 2005a,b,c; Canadian Food Inspection Agency 2006

[b] Canadian Seed Growers Association 2005a,b,c

[c] Based on the frequency in the AB county where the crops are most frequently grown.

[d] Canadian Food Inspection Agency 2006b; Thomas and Leeson 1999

[e] Relative index based on parameters

NA- Not Available

Summary

Intraspecific gene flow has a higher probability of occurrence than interspecific gene flow and therefore may be more difficult to predict, manage or mitigate. Gene persistence will differ between traits as some, such as herbicide resistance, may confer a selective advantage and thus be enriched in the population. To date, PNTs have a selective advantage within agriculture but not roadsides or natural areas. The selective advantage of future traits on populations may not be confined to fields.

Seeds have the ability to move farther, and persist longer than pollen. Seed admixture has many potential causes; including volunteer seed production and seed mixing during harvest, storage, transport or handling. Seed admixture is difficult to predict and mitigate because of the scale of seed handling involving multiple processes, most of which are outside the control of the grower. Seeds are transported internationally and seed contamination has the potential to influence commodity value. The ability to sample seed lots and measure the frequency of inadvertent PNT is critical to our ability to minimize contamination in certified seed production and seed handling processes.

A range of mitigation measures could be used for reducing gene flow, from simple isolation distance and equipment cleaning requirements to controversial genetic use restriction technologies (GURT) to reduce pollen flow or reduce volunteer seed viability (Daniell 2002). For some crops, such as canola and corn, outcrossing potential is considerable and pollen will be a significant vector for gene flow. GURT to reduce gene movement via pollen and spatial isolation may be most effective. For other crops, including wheat and flax, where the outcrossing potential is lower, harvested seed and volunteers in subsequent crops may be the most important avenue for gene flow. In this instance, GURTs curtailing seed germination may be more effective, along with enhanced agronomic management and seed handling practices. Crop rotation and landscape frequency of the crop will also determine the degree of intraspecific gene flow because it influences effective isolation distance and the impact of volunteers on gene flow. Ultimately, to account for the variability in gene flow parameters such as outcrossing, volunteers, seed loss at harvest and seed source contamination, a stochastic modelling approach will be needed to determine the relative contribution of pollen and seed to gene flow in each crop and for each trait. The model must take into account the cropping system, including rotation and distance to adjacent crops in the landscape. By using a modelling approach, the influence of genetic and physical mitigation measures may be compared and appropriate crop choices for future novel traits made.

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Gene flow between GM crops and related species in Canada

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Transgenic crop varieties are increasingly grown in commercial agriculture. Concerns exist over potential transgene escape to related species, including crop, wild, and/or weedy relatives, via the production of transgenic hybrid populations. One possible negative consequence associated with the inadvertent production of transgenic hybrids is an increase in fitness and invasiveness of weedy species. Since most currently commercialized genetically modified (GM) crops worldwide have wild or weedy relatives in all or parts of their range, the potential for gene flow will vary according to geographical location. The following paper provides a review of interspecific gene flow from GM crops from a Canadian perspective. Although *Brassica napus* (Argentine canola) is currently the only commercial GM crop in Canada with both crop and wild relatives, GM *Helianthus annuus* (sunflower) and *Triticum aestivum* (wheat) may soon cause similar concerns. Data from recent Canadian studies on hybridization between GM herbicide-resistant *B. napus* and other *Brassica* crops, *B. rapa* (Polish canola) and *B. juncea* (Oriental mustard), have indicated low hybridization rates at distances of up to 200 m. GM *B. napus* can also potentially hybridize with four related weedy species in Canada (weedy *B. rapa* (bird rape), *Raphanus raphanistrum* (wild radish), *Erucastrum gallicum* (dog mustard), and *Sinapis arvensis* (wild mustard). Hybridization with weedy *B. rapa* populations has been observed at two Québec sites (first global report of transgene escape into a natural weed population). Monitoring studies, to date, indicate transgene persistence in F_1 hybrid, backcross, and introgressed plants in one of the two populations. The various factors affecting hybridization success will be reviewed, including fitness data for herbicide- and insect-resistant GM weedy *B. rapa* × *B. napus* hybrids. These studies indicate the importance of assessing the risks posed by transgenic hybrid weed populations under field conditions, and the need to evaluate hybrids on a trait by trait basis, especially when dealing with "fitness-enhancing" traits, such as tolerances to various stress factors.

Introduction

Transgenic crop varieties are increasingly grown in commercial agriculture (James 2005). Concerns still exist over potential transgene escape to wild relatives via the production of transgenic hybrids (Ellstrand 2001; Stewart et al. 2003; Warwick et al. 1999, 2004), likely leading to transgenic weedy populations. One

potential negative consequence associated with the inadvertent production of transgenic weeds is an increase in their fitness and invasiveness. Although crops and weeds have exchanged genes for centuries, genetic engineering raises additional concerns. It not only introduces into ecosystems genes that confer novel or enhanced fitness-related traits, but also allows novel genes to be introduced into many diverse types of crops, each with its own specific potential to outcross (Snow 2002). Since most currently commercialized genetically modified (GM) crops worldwide have wild or weedy relatives in all or parts of their range (Ellstrand et al. 1999), the potential for gene flow will vary according to geographical location.

There are currently three commercial GM crops grown in Canada. Of these, *Brassica napus* L. (Argentine canola) is the only one with both crop and wild relatives, whereas *Glycine max* L. (soybean) and *Zea mays* L. (corn) have no wild relatives in Canada (Warwick et al. 1999). GM *Triticum aestivum* L. (wheat), although not grown commercially yet in Canada, may be of future concern, as natural hybridization and introgression has been reported with the weedy relative *Aegilops cylindrica* Host. (jointed goatgrass) (reviewed in Hedge and Waines 2004). The latter is an important weed in the western United States, and although currently absent in Canada, its presence just across the international border suggests that routine field surveys monitoring for the occurrence of this weed in the southern Canadian prairies would be prudent. Also of future concern will be GM *Helianthus annuus* L. (sunflower), which does have weedy relatives in Canada, including *H. annuus* (wild sunflower), *H. tuberosus* L. (Jerusalem artichoke), and *H. petiolaris* Nutt. (prairie sunflower) (reviewed in Bervillé et al. 2005; Massinga et al. 2003; Reagon and Snow 2006). Hybrid formation between cultivated sunflower and weedy relatives has been documented for wild sunflower in a Manitoba population (Linder et al. 1998) and for prairie sunflower in the south-western United States (Rieseberg et al. 1999).

The potential environmental impact of transgenic herbicide-resistant (HR) *B. napus* is of particular concern, as the crop has high inter-plant outcrossing rates (averaging 30 %), and is both insect and wind pollinated with pollen-mediated geneflow recorded up to 1-3 km from the crop (reviewed in Warwick et al. 2004). Transgenes can escape by both pollen and seed as *B. napus* can form a persistent seed bank, producing volunteer weed populations in subsequent crops. Volunteers can emerge from the seed bank for at least 3-4 subsequent years and can serve as a pollen source or genetic bridge for dispersal of transgenes to wild relatives and *B. napus* crops that follow in rotation or are located in nearby fields (reviewed in Hall et al. 2005; Légère 2005). As indicated above, *B. napus* has several sexually-compatible crop and wild relatives present in cultivated areas in Canada.

Most Canadian research studies on interspecific gene flow from GM crops have been on transgenic HR *B. napus*, and it will therefore form the main focus of the remainder of this paper. Empirical evidence for gene flow from *B. napus* to its crop, wild, and weedy relatives will be reviewed. The likelihood of transgene

persistence and the ecological fitness of hybrids will be documented and discussed to illustrate the potential risks associated with interspecific gene flow.

Interspecific gene flow between *B. napus* and related species

Hybridization and introgression

Interspecific gene movement via pollen flow occurs in a step-wise fashion starting with the initial hybridization between the crop and the wild relative. Various factors will affect hybridization frequency (reviewed in Chèvre et al. 2004), including spatial isolation of crop and weed populations, relative density of the weed compared to that of the crop source, synchrony of flowering, direction of the cross, specific parental genotypes, and presence of pollen vectors. Introgression of genes into a wild species population, i.e. the incorporation of genes from one differentiated gene pool into another, is the ultimate step in the process and will only occur if barriers of incompatibility, genetic instability, and low hybrid pollen fertility are overcome. Stable introgression through the formation of backcross (BC) generations is also dependent on F_1 hybrid fitness, i.e. their growth vigour, fertility, ability to set viable seed, and persistence of this seed in the seed bank.

Gene flow to other *Brassica* crops

There are two other *Brassica* crops grown in Canada, both of which are sexually compatible with *B. napus*. Ongoing pollen flow studies from transgenic HR *B. napus* to *Brassica juncea* (L.) Czern. (Oriental mustard) and *B. rapa* L (Polish canola) have documented gene flow to both crops at distances up to 200 m (Table 1; Séguin-Swartz, Beckie, and Warwick, unpubl. data). The consequence of gene flow to *B. rapa* Polish canola, which occupies approx. 5 % of the total Canadian canola acreage, is considered to be negligible. However, at present there are no established thresholds for transgene presence in the oriental mustard crop. Adjustments may be needed to the current regulations for pedigreed mustard seed producers set by the Canadian Seed Growers Association which stipulates a 100 m buffer zone between *B. napus* and *B. juncea* crops.

Gene flow to related wild/weedy species

In Canada, there are four wild relatives that have the potential to cross with *B. napus*, including three outcrossing (i.e. self-incompatible) species: *Sinapis arvensis* L. (wild mustard), *Raphanus raphanistrum* L. (wild radish), and weedy *B. rapa* L. (bird rape), and one predominantly selfing species, *Erucastrum gallicum* (Willd.) O.E. Schulz (dog mustard).

***Sinapis arvensis*:** Gene flow from *B. napus* to *S. arvensis* has a low probability of occurrence. Moyes et al. (2002) is the only study to date to report fertile hybrids when *S. arvensis* was the maternal parent, under greenhouse

conditions. In the field, studies have not detected gene transfer from *B. napus* to *S. arvensis* in experiments conducted in Saskatchewan (Bing et al. 1996), France (Lefol et al. 1996), and the UK (Moyes et al. 2002). In Canada, *S. arvensis* is the most common of the four weeds listed above. In recent studies (Warwick et al. 2003), the absence of gene flow was inferred by screening seed collected from *S. arvensis* populations for the presence of the herbicide resistance trait found in adjacent commercial HR *B. napus* fields in Saskatchewan. No *S. arvensis* × HR *B. napus* hybrids were detected in 42,828 seedlings, suggesting that the probability of interspecific gene flow from *B. napus* to *S. arvensis* is very low ($<5 \times 10^{-5}$) under commercial field conditions.

Erucastrum gallicum: Gene flow from *B. napus* to *E. gallicum* has not been extensively studied. In one report, a single *B. napus* × *E. gallicum* hybrid was obtained under greenhouse conditions, but no hybrids were detected when *E. gallicum* served as the maternal parent (Lefol et al. 1997). *E. gallicum* occurrence in *B. napus* growing areas of Canada is limited and found primarily in Saskatchewan. In the same Canadian field study described above for *S. arvensis* (Warwick et al. 2003), no *E. gallicum* × *B. napus* hybrids were detected in 21,841 *E. gallicum* seedlings from commercial HR *B. napus* fields in Saskatchewan. These results again indicate a very low probability of interspecific gene flow ($<2 \times 10^{-5}$).

Raphanus raphanistrum: Studies in France and Australia have indicated that hybridization between *R. raphanistrum* and *B. napus* is very rare. Only three hybrids were detected in numerous field experiments in France when *R. raphanistrum* served as the maternal parent (Baranger et al. 1995; Chèvre et al. 2000; Darmency et al. 1998; Eber et al. 1994). The hybridization rate was estimated at between 10^{-7} and 10^{-5} (Chèvre et al. 2000). In Australia, gene flow studies between *R. raphanistrum* and imidazolinone-resistant *B. napus* growing in experimental field plots (Rieger et al. 2001) indicated even lower hybridization rates ($<4 \times 10^{-8}$), with no hybrids found when *R. raphanistrum* was the maternal parent. In Canada, *R. raphanistrum* coexists with *B. napus* only in Québec and Alberta. Canadian studies (Warwick et al. 2003) confirm that gene flow between *R. raphanistrum* and *B. napus* is also rare. A single *R. raphanistrum* × *B. napus* F_1 hybrid was obtained in an HR *B. napus* field plot experiment in ON, where *R. raphanistrum* plants were grown at a density of one plant per m^2 with HR *B. napus*. This hybrid had an unstable genomic structure consistent with the fusion of an unreduced gamete of *R. raphanistrum* and a reduced gamete of *B. napus* (RrRrAC, $2n = 37$ chromosomes) and <1 % pollen viability. No hybrids were detected in commercial HR *B. napus* fields in Québec and Alberta (22,114 seedlings screened, probability of $<2 \times 10^{-5}$).

Weedy B. rapa: Numerous studies have indicated a high potential for hybridization between weedy *B. rapa* and *B. napus*. This is not surprising, as *B. rapa* (AA genome, $2n = 20$ chromosomes) is one of the progenitor species of *B. napus* (AACC genome, $2n = 38$ chromosomes). Spontaneous hybridization and introgression between weedy *B. rapa* and *B. napus* was reported in Danish studies

(Hansen et al. 2001, 2003; Jørgensen and Andersen 1994; Jørgensen et al. 1996; Landbo et al. 1996), US field studies (Halfhill et al. 2002, 2004), and UK studies (Wilkinson et al. 2003); and between cultivated lines of *B. rapa* and *B. napus* in field experiments in Canada (Bing et al. 1996). Based on the distribution of herbarium specimens, weedy *B. rapa* has a limited distribution as an agricultural and/or ruderal weed in *B. napus* growing areas in Québec (Simard et al. 2005). *Brassica rapa* Polish canola can also be a weedy volunteer in western Canada. In recent Canadian studies (Warwick et al. 2003), which include data from experimental field trials and commercial HR *B. napus* fields, hybridization between weedy *B. rapa* and *B. napus* occurred at a frequency of ca. 7 % in two field experiments where weedy *B. rapa* plants were grown at a density of one plant per m^2 with HR *B. napus*. *B. rapa* × *B. napus* F_1 hybrids were also detected in two weedy *B. rapa* populations growing in or near commercial HR *B. napus* fields in Québec. This represented the first reported case globally of transgene escape into a natural weed population. A high frequency of hybridization (13.6 %) was observed in one of the weedy *B. rapa* populations and was likely due to greater distance between *B. rapa* plants (i.e. a thin stand). All F_1 hybrids were morphologically similar to weedy *B. rapa*, but hybrids were confirmed by the presence of the herbicide resistance trait, the presence of species-specific AFLP molecular markers from both parental species, and a triploid ploidy level (AAC, $2n = 29$ chromosomes). The F_1 hybrids had reduced pollen viability (ca. 55 %) and segregated for both self-incompatible and self-compatible individuals, the latter being a *B. napus* trait. Other researchers have also showed that F_1 hybrids produced from the hybridization of weedy *B. rapa* and *B. napus* were triploid (Halfhill et al. 2002; Metz et al. 1997).

Transgene persistence

Previous studies have shown that a HR transgene can be passed from *B. napus* to weedy *B. rapa* and be active in successive generations (Mikkelsen et al. 1996; Metz et al. 1997). Genetic studies of transgenic hybrids have also indicated that after one backcross generation, the ploidy of the BC_1F_1 generation (as assessed by nuclear DNA content) shifted towards that of weedy *B. rapa* (Halfhill et al. 2002). In subsequent backcross generations (BC_2F_1 and BC_2F_2), the trend toward the loss of *B. napus* genetic material continued and ploidy level was indistinguishable from that of the diploid weedy *B. rapa* parental species. The diploid composition was stable after the inter-mating of BC_2F_1 individuals (Halfhill et al. 2003) demonstrating that a stable transgenic diploid weedy *B. rapa* population can be reached after hybridization and two generations of backcrossing.

The two natural weedy *B. rapa* populations [Ste-Agathe and St.-Henri, Québec], where the F_1 *B. rapa* × *B. napus* hybrids were found in 2001, were monitored in 2002 (St. Henri site only), 2003, and 2005 for persistence of the

glyphosate-resistance trait and for evidence of introgression of the HR transgene into the weedy *B. rapa* genome (Warwick et al. 2005; Warwick et al. manuscript in preparation). Hybrid detection was based on the presence of the HR trait, intermediate ploidy level, reduced male fertility (pollen viability), and/or both *B. napus*- and *B. rapa*-specific AFLP molecular markers. Hybrid individuals were detected in all 3 years at the St. Henri site, and in 2003 at the Ste-Agathe site (in 2005 the Ste-Agathe site, where the *B. rapa* and other weed populations observed on the field edge in 2003, had been destroyed). Numbers decreased dramatically over the 3-year period at the St. Henri Site, from 85 (34.4 %) out of ca. 247 plants surveyed in 2002, to 32 (20.4 %) out of 157 in 2003, to only 5 (2.5 %) out of 199 plants in 2005. Most hybrids had the HR trait, reduced male fertility (although some had as high as 98 % fertility), intermediate or unusual genome structure, and presence of both species-specific AFLP markers. Both F_1 and backcross hybrid generations were detected. At least one introgressed individual, i.e. with the HR trait and diploid ploidy level of weedy *B. rapa*, was observed in 2005. The latter had reduced fertility but did produce a large amount (ca. 1.6 g) of viable seed (progeny currently under study). These results indicate the persistence of the HR trait over time, as a result of seed bank longevity and/or continued F_1 hybrid production with *B. napus* volunteers. The consequence of such hybridization events on the weedy/invasive potential of weedy *B. rapa* populations remains a concern, particularly as transgenic *B. napus* lines with multiple HR and stress tolerance traits may become commercially available.

Overall, the actual consequences of hybridization and introgression will be trait-dependent, with some traits being more likely than others to increase weediness/invasiveness. It is clear that in the case of herbicide resistance, the positive selective value of the trait will be restricted to habitats in the agroecosystem where the herbicide is applied. However, even in absence of selection pressure, the persistence of a few fertile HR transgenic hybrids will ensure persistence of the transgenic trait over time, as was observed with weedy *B. rapa* in Québec (i.e. the GM trait, once released, is impossible to fully retract).

Ecological fitness of hybrids

Fitness of the various generations of hybrids is critical to the successful introgression of a transgene. Previous studies of transgenic glufosinate-resistant F_1 weedy *B. rapa* × *B. napus* hybrids (Snow et al. 1999) indicated no fitness effect in the F_1 hybrid. In similar studies, Hauser et al. (1998a) found that F_1 hybrids had intermediate fitness between the two parental species based on several combined characteristics, and they concluded that F_1 hybrids were significantly more fit than weedy *B. rapa*. In a subsequent study, Hauser et al. (1998b), found that a fitness penalty occurred in F_2 and backcrossed individuals, although a small percentage of hybrids were as fit as the weedy parent. The fitness of F_1 hybrids may also be

frequency dependent (based on hybrid versus parent ratio), and the experimental design in future research may need to include the appropriate ratio of hybrid to parental weedy *B. rapa* plants to simulate selection for the hybrids with the highest fitness (Hauser et al. 2003; Pertl et al. 2002). An experimental field study was conducted in Ottawa, ON in 2005 to determine the fitness of two Canadian glyphosate-resistant weedy *B. rapa* × *B. napus* BC_2F_2 backcross hybrid populations under competitive field conditions (with vs. without wheat; weedy vs. weed-free) (Warwick, studies in progress). Preliminary results from this study suggest that the hybrid is less fit than the parental weed population, regardless of the presence of the transgene (Table 2).

Weedy *B. rapa* × *B. napus* hybrids with the insect resistance transgene Bt-GFP [*Bacillus thuringiensis* (Bt)-green fluorescent protein (GFP)] also showed reduced fitness/competitiveness (Halfhill et al. 2005). In a non-competitive greenhouse experiment, both transgenic and non-transgenic hybrids showed reduced vegetative growth and seed production. In a field experiment conducted under two herbivory levels and high intraspecific competition, transgenic hybrids also produced less vegetative dry weight and fewer seeds than weedy *B. rapa*. In competition experiments with wheat, the hybrids were the least competitive as compared with parental *Brassica* competitors. Again, reduced hybrid fitness appeared to be independent of transgene introgression.

To date, only two other studies have assessed reproductive fitness of wild × crop hybrids for putative fitness-enhancing transgenes - both with common sunflower. Snow et al. (2003) reported that enhanced fitness, as measured by fecundity, was conferred by a Bt transgene in male-sterile BC_1 wild × crop sunflower hybrids in one of two field populations. Burke & Rieseberg (2003) examined the effect of a disease resistance transgene (coding for oxalate oxidase, OxOx) on the fitness of BC_3 wild sunflower hybrids. Under white mould (*Sclerotinia sclerotiorum* (Lib.) de Bary) pathogen pressure, the transgene protected BC_3 plants from disease, but did not increase their reproductive fitness.

Even though there are no compelling data to suggest that the presence of transgenes is inherently risky, the findings of studies to date might not fully describe the risks posed by transgenic weed hybrid populations under field conditions, since they used single transgenic events and a limited number of hybrid families. Future experiments should be performed under field conditions that incorporate selection pressure and competition among hybrids with different genetic backgrounds and examine how additional or different crop markers other than the transgene sort during introgression. Future risk assessment studies on transgenic hybrids should simulate natural selection under agricultural/ecological conditions or preferably test transgenic hybrid weed populations under realistic field conditions. There should be a focus on the impact of fitness-enhancing traits, such as disease and insect resistance and stress tolerance to cold, drought, and salt. These are less well understood ecologically and clearly could have more impact if they were to spread to plants in non-agricultural habitats.

Table 1. Interspecific gene flow from *Brassica napus* to other *Brassica* crops, *B. rapa* (Polish canola) and *B. juncea* (Oriental mustard) (Warwick et al. 2005; Séguin-Swartz, Beckie and Warwick, unpubl. data), relative to intraspecific gene flow distances between *B. napus* fields (second column from Beckie et al. 2003).

Distance (m)	*B. napus* (%) Argentine canola	*B. rapa* (%) Polish canola	*B. juncea* (%) Oriental mustard
0	1.25	-	0.245
50	0.19	0.11	0.030
100	0.14	0.01	0.021
200	0.08	0.01	0.005
400	0.04	-	-

Table 2. Preliminary results from a hybrid fitness field trial conducted in Ottawa 2005 for two experimental BC_2F_2 (2^{nd} generation backcross/selfed F_1) hybrid populations derived from weedy *B. rapa* populations 2974 and 9039 and *B. napus* investigating effect of wheat on mean total dry weight and seed weight per plant; R: resistant, S: susceptible to glyphosate. Within each column and population, means followed by the same letter are not significantly different (P= 0.05, n = 40).

Plant Type	With Wheat		Without Wheat	
	Plant Wt. (g)	Seed Wt. (g)	Plant Wt. (g)	Seed Wt. (g)
BC_2F_2-R 2974	1.73 a	0.14 a	39.0 a	3.01 a
BC_2F_2-S 2974	2.55 a	0.26 a	40.1 a	4.28 a
2974 *B. rapa*	4.30 b	1.14 b	57.3 b	16.11 b
BC_2F_2-R 9039	3.50 a	0.36 a	31.9 a	3.15 a
BC_2F_2-S 9039	2.71 a	0.30 a	40.9 a	3.52 a
9039 *B. rapa*	8.20 b	2.07 b	79.4 b	19.70 b

Summary

The frequency of gene flow from *B. napus* to four wild relatives, weedy *Brassica rapa*, *Sinapis arvensis*, *Erucastrum gallicum*, and *Raphanus raphanistrum* was assessed in greenhouse or field experiments, and actual rates were measured in commercial fields in Canada. Hybridization between weedy *B. rapa* and *B. napus* occurred in two field experiments (7 % frequency), and varied from <0.01 % up to 14 % in indigenous weed populations in commercial fields in eastern Canada. The higher frequency in commercial fields was attributed to the greater distance separating individual weedy *B. rapa* plants. Hybrids were morphologically similar to weedy *B. rapa* and had reduced pollen viability (about 55 %). Such hybridization was not unexpected as *B. rapa* is one of the parental progenitors of *B. napus*. In contrast, gene flow between *R. raphanistrum* and *B. napus* was rare. A single hybrid was detected in a field experiment. The hybrid was morphologically similar to *R. raphanistrum*, and had less than 1 % pollen viability. No hybrids were detected in commercial fields in Québec or Alberta. Similarly, no *S. arvensis* or *E. gallicum* × *B. napus* hybrids were detected from commercial fields in Saskatchewan. These findings suggest that the probability of gene flow from transgenic HR *B. napus* to *R. raphanistrum*, *S. arvensis*, or *E. gallicum* is very low (< 2-5 × 10^{-5}). However, transgenes can persist and disperse in the environment via weedy *B. rapa* in eastern Canada. Enrichment of these transgenic weedy plants in the population, however, will only occur when and where herbicide selection pressure is applied, particularly since the hybrid weedy *B. rapa* appears to be less fit than the parental plants. The risk of future fitness-enhancing transgenic traits, such as disease and insect resistance and stress tolerances, are, however, less well understood ecologically and clearly could have more impact if they were to spread to plants in non-agricultural habitats.

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Fate of plant DNA in soil and water – implications for the DNA cycle

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Since the introduction of genetically-modified herbicide-resistant crops ten years ago, researchers have investigated the movement of plant recombinant DNA (rDNA) above the soil surface. However, little remains known about its fate in the soil environment. From microcosm studies, it is known that free plant DNA in the soil enters the soil DNA cycle. This cycle has elements similar to many nutrient cycles and it also is primarily mediated by soil microbes. Soil bacteria excrete enzymes that degrade free DNA into its component (sugar, base, P-group) or elemental constituents which serve as building blocks for bacterial DNA or as a nutrient source, respectively. Alternatively, DNA may bind to the soil matrix and be protected from degradation. Foreign DNA can be incorporated into the bacterial genome under certain circumstances, although this has not yet been observed in agricultural fields. Recent developments in molecular tools and DNA recovery methods allow for the investigation of the role of plant DNA in the soil DNA cycle at the field scale. It is now possible to validate findings from microcosm studies at the larger field scale and ask important questions regarding the movement, fate, and the factors influencing the fate of plant DNA in the soil environment.

Why study plant DNA in the soil?

Each living cell contains DNA, yet little is known about the fate of DNA in the environment, particularly in agricultural systems. Transgenic technology has provided the molecular tools and the large scale adoption of transgenic crops has provided the impetus, to examine the fate of plant DNA in soil and water. Plant recombinant DNA (rDNA) is a convenient marker in that the DNA in inserted cassettes is unique, uniform and the time of introduction into the environment is known. Moreover, the transgenic era has led to the development of the tools required to study the DNA in the environment and it is only recently that these tools have become sensitive and affordable enough for large scale field experimentation. Since their introduction, transgenic herbicide-resistant crops have been adopted at high levels in western and eastern Canada (**see Chapters 2 and 3**) and the combination of glyphosate-resistant corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.), two crops often grown in rotation in eastern Canada, has made it possible to rely on a single herbicide mode of action for weed control in these cropping systems. Application of glyphosate has been shown to influence soil

microbes (Haney et al. 2002) and soil microbes are instrumental in DNA turnover in the soil environment. Whether such heavy reliance on a single herbicide system contributes to short- or long-term environmental impacts in the soil environment is not known.

What is known about the DNA cycle in soil and water?

The DNA cycle in soil has been largely pieced together from microcosm studies conducted under controlled conditions. Microbes, in particular bacteria, play a key role in mediating the DNA cycle and thus, factors that affect these organisms are likely to influence the DNA cycle. Figure 1 is a schematic outlining the key fates of free (extracellular) plant DNA in soil or water. Plants contribute free DNA to the soil throughout their life cycle. During the vegetative phase this is thought to primarily occur from the sloughing off root cap cells which protect and lubricate growing root tips (Gulden et al. 2005). At anthesis, another source of plant DNA is pollen. The amount of pollen production varies by breeding system and plant species. For example, corn, a wind-pollinated species with heavy pollen contributes about 10^6 to 10^7 pollen grains m^{-2} to the soil in the immediate vicinity of the plant (Uribelarrea et al. 2002; Westgate et al. 2003). Other species, in particular, insect pollinated plants, tend to be more conservative in their pollen production. Nevertheless, the pollen wall must rupture before DNA is able to enter the free soil DNA pool. Further points of plant DNA entry into the soil environment are during plant residue decomposition (Widmer et al. 1997; Paget et al. 1998; Gebhard and Samalla 1999) and from decaying volunteer seed. The amount of DNA added during residue decomposition to the free DNA pool appears to be limited. Recent evidence indicated that the majority of plant DNA is degraded within plant cells prior to entering the soil matrix (Poté et al. 2005). Free plant DNA joins free DNA from all organisms that inhabit the soil environment. In the soil DNA pool, free DNA is subject to one of three fates, i) degradation, ii) horizontal gene transfer, or iii) persistence.

DNA degradation in soil is mainly an enzymatic process that is mediated primarily by soil bacteria through their ubiquitous release of DNAse and nuclease enzymes into the environment (Blum et al. 1997). Bacteria and other organisms are able to take up deoxyribose and phosphate groups of DNA as nutrients and assimilate the free bases directly. Conversely, the bases may be further degraded to their elemental constituents and the resulting nitrogen can then be assimilated into amino acids. The elemental constituents of DNA degradation become parts of the soil carbon, nitrogen and phosphorous cycles. In soil, most free DNA appears to be degraded rapidly (Gebhard and Smalla 1999; Ceccherini et al. 2002; Poté et al. 2005).

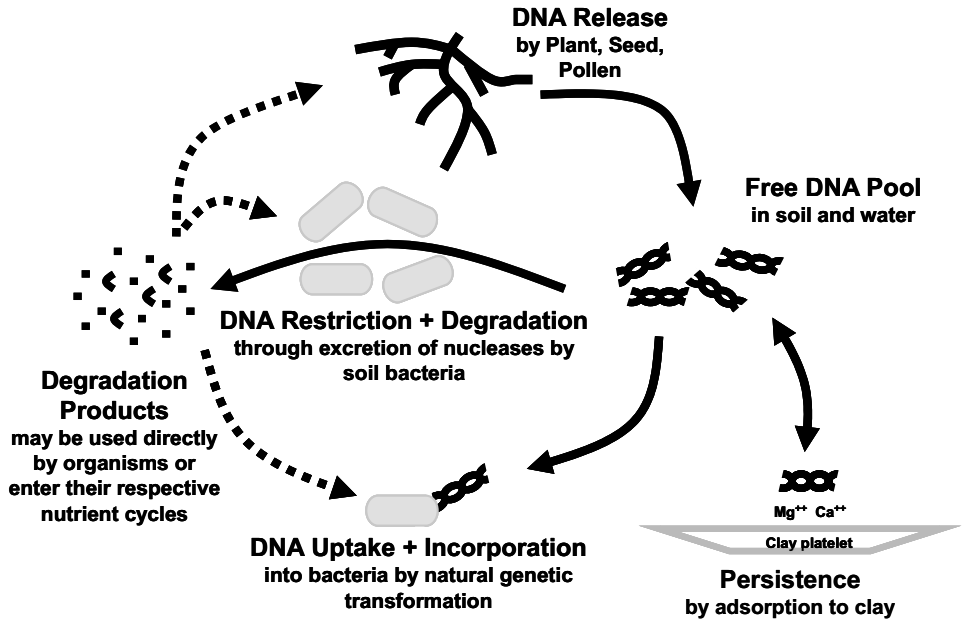

Figure 1. The DNA cycle in the soil environment. Bacteria play a key role in mediating the degradation of all free DNA in soil and water. DNA breakdown products are taken up and assimilated by living organisms (dashed arrows). Conversely, free DNA may persist by binding to clay platelets or may be incorporated into bacterial genomes through natural transformation.

Secondly, DNA may be protected from degradation by sorption to the soil matrix (Demanèche et al. 2001). Above a pH of about 5.0, each phosphate moiety on the DNA strand has a net negative charge and therefore DNA can be thought of as a poly-anion in soil (Greaves and Wilson 1969). Clay platelets and acid moieties on organic matter also carry a net negative charge. Despite similar charges, sorption of DNA to the soil matrix is possible. This is thought to occur via bridging of divalent cations between DNA and the soil matrix (Romanowski 1991). Sorption of DNA to the soil matrix has been shown to be influenced by the form of DNA (greater sorption of linear chromosomal DNA than supercoiled plasmid DNA), the type of clay, and calcium content of soil (Poly et al. 2000). Sorption of free DNA and sorption of free nucleases to clay offer protection from degradation. Sorption of nucleases seems to be more important in protecting DNA than sorption of the nucleotides (Demanèche et al. 2001). Previously, it was thought that sorption to clay protects DNA from degradation, but does not preclude protection from transformation into bacteria (Khanna and Stotsky 1992; Romanowski et al. 1993),

however, more recent evidence suggests that bound DNA is equally available for transformation and degradation (Demanèche et al. 2001).

A third fate of plant DNA is natural transformation. Natural transformation refers to the successful incorporation of foreign genes into microbial genomes. The rate at which this happens depends greatly on the sequence homology between foreign and indigenous DNA. For example, as few as 180 base pairs of homology were sufficient for detectable rates of incorporation of linear naked DNA into a bacterial plasmid (de Vries and Wackernagel 2002). Similar observations have been made in chromosomal DNA (Prudhomme et al. 2002). Transformation rates are also greater when foreign DNA contains two areas of homology. This can result in the successful incorporation of over 2 kb of non-homologous DNA between the areas of homology and may span several genes. To date, natural transformation has been shown to occur in at least 87 species of bacteria (de Vries and Wackernagel 2004). This process occurs in both gram-negative and gram-positive bacteria, although the mechanics of DNA uptake differ between these two types of bacteria (Thomas and Nielsen 2005). Competency for natural transformation within bacteria species is influenced by many factors, including nutritional status and the growth stage of the cells. In *Acinetobacter* sp., a common soil bacteria with high competence and low stringency for foreign DNA uptake, rates of natural transformation ranging from 0.007 to 3 % have been recorded under various conditions in microcosms (de Vries et al. 2003). Similar rates also have been reported in other studies (Nielsen et al. 2000; Nielsen and van Elsas 2001). De Vries et al. (2004) confirmed movement of plant DNA into *Acinetobacter* sp. through homology-facilitated illegitimate recombination. When the homologous anchor sequences were absent, transformation was not detected. For stable introgression of foreign DNA into soil microbes, successful natural transformation must be accompanied by a selection pressure that provides some level of selective advantage to the transformants. However, a number of barriers, outside and within competent bacterial cells, limit the success of natural transformation (Thomas and Nielsen 2005). This, in part, explains why successful natural transformation of recombinant plant DNA in soil bacteria has not yet been observed in natural environments (Nielsen and Townsend 2005).

DNA in leachate water

Free DNA in soil can move with soil water. This may have implications for the DNA cycle in that degradation or natural transformation could be spatially separated from the point of entry of free DNA into the soil environment. Supercoiled, open circular, and linearized DNA readily moves with bulk water flow in soil (Poté et al. 2003). In a greenhouse experiment, Gulden et al. (2005) showed that plant DNA (corn and soybean) readily enters and moves with leachate water in agricultural soils throughout the vegetative phase. In leachate water, DNA

degradation tended to be rapid and depended on temperature (Figure 2). DNA degradation followed a simple negative exponential decay curve and half-lives ranged from 2 to 30 hrs for the gene fragments that were monitored (Gulden et al. 2005). The Q_{10}, or change in the reaction rate for a 10 degree increase in temperature, clearly indicated that DNA degradation in leachate water was primarily an enzyme mediated process. In corn, no differences in degradation between indigenous and recombinant DNA were observed. In soybean, slower DNA degradation was likely due to lower bacterial densities present in soybean leachate water (Gulden et al. 2005). Given the rapid degradation of plant DNA in leachate water, it appears that sorption to the soil particles in leachate water was minimal. Little is known about natural transformation in soil water, although one study has shown that water flow rate has little effect on the rate of plasmid transfer between bacteria (i.e. conjugation) in the rhizosphere (Pearce 2001).

Technological advances that enable this research

DNA recovery

To study the DNA cycle in soil and water, suitable methods for DNA recovery are essential. A number of protocols are described in the literature (Zhou et al. 1996; Howeler et al. 2003; Reeleder et al. 2003; Robe et al. 2003). However, these are generally not suitable for high-throughput sample analysis. To investigate the DNA cycle in agricultural systems over multiple years, high spatial variability in combination with the technical constraint of meaningful DNA recovery from small soil samples (>1 g) only necessitate the use of high-throughput methods to accurately assess DNA persistence at the field scale. Lerat et al. (2005) developed such a method based on a commercially available soil DNA recovery kit adapted for plant DNA recovery. Typically, commercial kits are designed for total microbial DNA recovery and cell rupture by physical and/or chemical means is a key component of the protocol. The addition of aurintricarboxylic acid, a nuclease inhibitor (Blagodatskaya et al. 2003), and the additional removal of polymerase chain reaction inhibiting substances by flocculation with $AlNH_4(SO4)_2$ (Braid et al. 2003) improved plant DNA recovery of this soil DNA recovery kit (Lerat et al. 2005).

DNA recovery from leachate water is more simple. Gulden et al. (2005) described a low-cost, high throughput method based on chelation of divalent cations followed by DNA concentration using alcohol precipitation. Chelation of divalent cations is thought to release sorbed DNA from the soil matrix and prevent the action of DNAses and nucleases by removing essential cation cofactors from solution before these enzymes are subjected to heat inactivation.

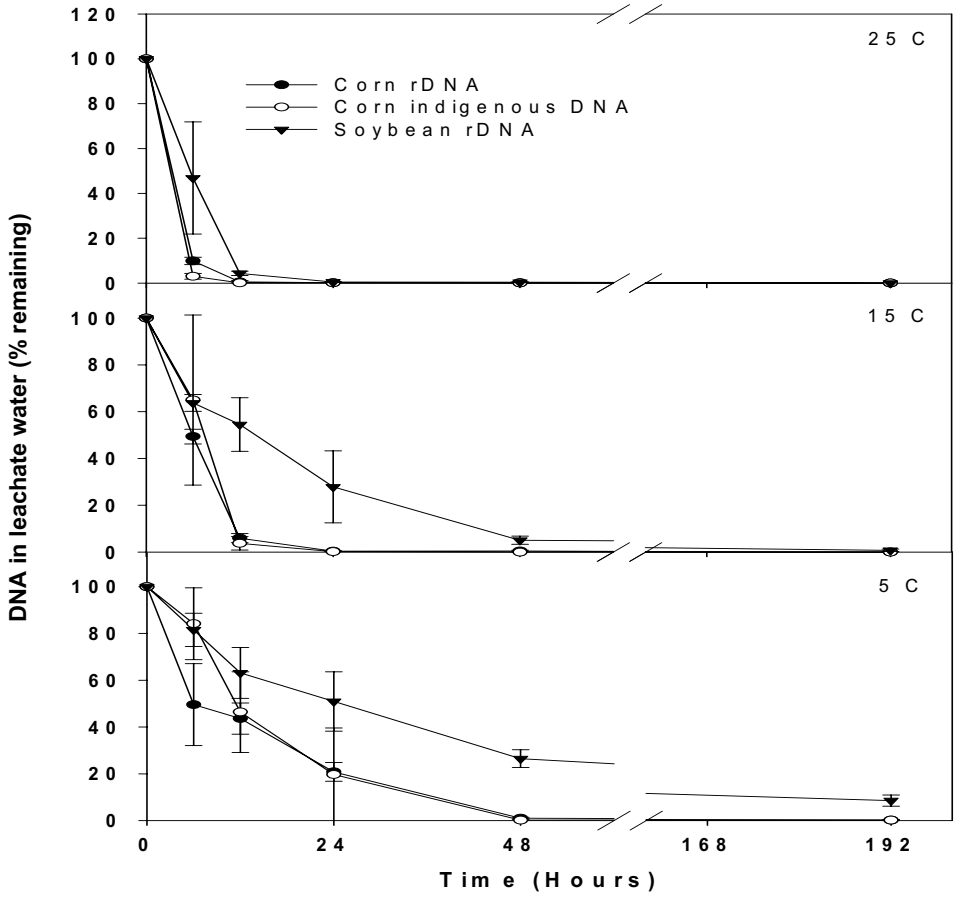

Figure 2. Proportion of free recombinant and indigenous DNA persisting in leachate water derived from conventional genotypes of the respective plant species at three different temperatures (5, 15, and 25 C) over time. Bars equal 1 SEM.

Detection of plant DNA

A number of qualitative and quantitative techniques for the detection of target DNA exist. Quantitative real-time PCR offers several advantages over alternative detection methods in that it is highly specific, sensitive, and responds linearly over a large range of initial target DNA concentrations (Ding and Cantor 2004). The use of fluorescent probes in real-time PCR adds an extra measure of specificity in that these probes (e.g. molecular beacons) are specifically designed for the target DNA and have very stringent binding requirements that are only met by a perfectly homologous target DNA sequence. One limitation of this technique is that

the size of the sequences that can be quantified is constrained, limiting the quantification to mainly gene fragments rather than entire genes. In the digestive tracts of animals, persistence of DNA appears to be influenced by DNA length in that shorter fragments tend to persist longer (Alexander et al. 2004). Therefore it is possible that real-time PCR may overestimate gene persistence depending on the difference in size between the assayed fragment and the entire gene.

Detection of the glyphosate-resistant recombinant *CP4 EPSPS* gene in corn (event NK603) and soybean (event 40-3-2) from soil extracts presents a challenge as this gene originated from *Agrobacterium*, a common soil bacteria. Most other soil bacteria contain an *EPSPS* gene and often, the nucleotide sequence of this gene is unknown. Moreover, in corn and soybean, the sequences of the recombinant *EPSPS* gene are identical. Therefore, to be able to discern between corn and soybean transgenic *EPSPS* and, at the same time, avoid the false positive detection of plant DNA though detecting soil microbial *EPSPS* with similar sequences, Lerat et al. (2005) targeted the junction between the beginning of the *EPSPS* gene and the adjacent chloroplast transit peptide (CTP) for PCR amplification. The CTP gene adjacent to the *CP4 EPSPS* is different in corn and soybean and therefore targeting this junction provided the desired species specificity. This approach required only a single probe (molecular beacon), one reverse primer and two distinct forward primers. The sensitivity of this real-time PCR protocol is high, i.e. a single copy of target DNA in the reaction mixture can routinely be detected. Development of probes for indigenous plant DNA detection from environmental samples has also been described (Gulden et al. 2005). The presence of DNA from many different organisms in soil and leachate water extracts increases the complexity of developing PCR targets and associated probes compared to the development of these for detecting transgenes in comparatively homogenous seed and food products.

These methods allow for large scale evaluation of plant DNA persistence in soil, providing an estimate of the rate of DNA degradation. To date, high-throughput methods for the examination of specific components of the DNA cycle (e.g. transformation assays, nuclease content and activity, nuclease sorption, DNA sorption) have not been developed, limiting the scale at which these processes can be examined. As a result, more traditional methods must be applied to investigate specific steps of the DNA cycle in soil which has not yet been attempted in the field over multiple locations and years.

What questions can be addressed?

Research on the diversity of soil microbial communities has shown changes in soil microbial diversity associated with transgenic crops (Dunfield and Germida 2004). Whether some function of the bacterial community was compromised by these changes in community diversity remains unknown. Studying the soil DNA cycle which is primarily mediated by soil bacteria is one way of examining the

gross effects of high use of herbicide-resistant technology on the function of the soil microbial community. We are currently monitoring plant DNA persistence in glyphosate-resistant and conventional corn/soybean rotations at two locations in Ontario and in glyphosate-resistant and conventional corn in Alberta. Two of these rotations are mature (years 4 and beyond), while the third has been established recently (years 1 to 4). Using a combination of high-throughput and traditional techniques, a number of questions regarding the DNA cycle are currently being addressed, including:

i) Duration of persistence of indigenous and recombinant plant DNA
ii) The effect of technology use intensity on the function of the DNA cycle in soil.
iii) Potential differences between short-term and long-term use of this technology.
iv) Relative importance of factors that affect the soil DNA cycle (DNA persistence) including location, environment, crop type, depth in soil profile
v) Effect of genotype (HT or conventional) and herbicide (glyphosate or conventional) on the DNA cycle at the field scale.
vi) Functionality of plant DNA in soil determined through monitoring the occurrence and frequency of natural transformation into selected soil bacteria

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Non-target impacts of genetically-modified, herbicide-resistant crops on soil microbial and faunal communities

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Genetically-modified, herbicide-resistant (GMHR) crops are widespread and being increasingly adopted, raising concerns over their impacts on non-target organisms. Soil organisms perform many important ecosystem functions that influence crop productivity, decomposition, and the formation of soil structure, yet our knowledge regarding how they are impacted by GMHR cropping systems is incomplete. There is ample evidence that the diversity of endophytic and rhizosphere bacterial communities can change in response to GMHR genotypes, which may be important because it is via these communities that most nutrient transformation occurs. Management practices associated with GMHR crops, including the use of herbicides associated with GMHR varieties and the timing of weed control, have been observed to affect the abundance and/or activity of soil organisms associated with decomposition processes. More research is necessary to understand the long-term effects of GMHR cropping systems on soil organisms and the consequences of these effects for ecosystem functioning.

Introduction

Genetically-modified, herbicide-resistant (GMHR) crops are among the most successful of the first generation of transgenic products: GMHR soybean, corn, canola and cotton represented 71 %, or 63.7 million hectares of the global 90 million hectares of genetically-modified (GM) crops grown in 2005 (James 2006). GM crops in general, and GMHR crops in particular, face considerable scrutiny from scientists, government officials, and public interest groups. One concern is the potential for impacts on non-target organisms within and interacting with agroecosystems (Kowalchuk et al. 2003, Dunfield and Germida 2004, Lynch et al. 2004, Snow et al. 2005). Non-target impacts may be associated with GMHR genotypes (e.g., gene flow, unintended phenotypes) and/or with corresponding cropping practices (e.g., herbicide toxicity, weed community dynamics, reduced tillage).

Studies to estimate the non-target impacts of GMHR genotypes and cropping practices, conducted under laboratory, greenhouse, and field conditions, have focused primarily on aboveground organisms, including pollinators, herbivores, and predators (e.g., Hawes et al. 2003, Morandin and Winston 2005). Studies of soil organisms are less common, despite the important roles that many soil organisms play in maintaining soil health, with their responsibility in key ecosystem processes, such as nutrient cycling, organic matter turnover, soil physical structure and plant growth promotion. To date, a major belowground focus has been to study the effects of GMHR genotypes on the diversity of rhizosphere and endophytic microorganisms (e.g., Dunfield and Germida 2003). Other researchers explored effects of GMHR cropping systems on microbial biomass and abundance of soil invertebrates (e.g., Liphadzi et al. 2005).

Although many studies document effects of employing GMHR genotypes and/or management on the structure of soil communities, many questions remain to be answered. For example, most studies are conducted over one or two years (e.g., Hawes et al. 2003; but see Liphadzi et al. 2005); therefore we currently have limited knowledge about how soil communities respond to GMHR cropping systems over the long term. In addition, Tilman and Downing (1994) suggested that the preservation of biodiversity is essential for the maintenance of stable productivity in ecosystems; however, the functional consequences of effects on the structure of soil communities have not been adequately addressed. Here we provide a brief overview of how soil communities respond to GMHR genotypes and management and draw attention to areas where we currently lack knowledge and where research should be directed.

Effects of GMHR genotypes on soil organisms

There are two main areas of study concerning the effects of GMHR genotypes on soil organisms; the possibility of horizontal gene transfer from transgenic plant to the microbial community, and direct effects on the biodiversity of the microbial community through contact in the rhizosphere with novel genes or proteins. Examination of the potential for horizontal gene transfer in the rhizosphere of transgenic plants has been reviewed by Nielsen et al. (2001). In brief, it is possible for genetic material to transfer from a GM plant to a native soil microorganism; however, its occurrence within a natural soil environment has yet to be proven.

The potential for GMHR plants to impact soil organisms exists, because novel genes within these plants can be released directly into the soil through root exudation, sloughing of root cells, pollen, or through decomposition of plant residues (Paget and Simonet 1994; Widmer et al. 1997; Paget et al. 1998; Gebhard and Smalla 1999; Uribelarrea et al. 2002; de Vries et al. 2003; Poté et al. 2005). Incorporation of transgenes into the soil could alter soil microbial biodiversity due

to variable responses by microorganisms to the novel genes, and their respective novel proteins.

Research on how GMHR genotypes affect soil microorganisms has primarily focused on the potential that root exudates from GMHR canola or corn can influence rhizosphere or endophytic microbial communities. One of the first studies by Siciliano et al. (1998) assessed the root-interior and rhizosphere bacterial communities associated with a field-grown Roundup Ready® canola variety (containing the *epsps* and *gox* genes), and two conventional canola varieties. The carbon utilization patterns and fatty acid methyl ester profiles of the microbial community associated with the roots of the GMHR canola variety differed from the profiles of two conventional canola varieties. Furthermore, isolation and characterization of representative bacteria showed that the composition of the cultivable microbial community associated with a GMHR canola variety, Quest, was significantly different than the conventional canola varieties (Siciliano and Germida 1999). Follow-up work examining the total bacterial community has confirmed that the root-interior and rhizosphere bacterial community associated with the GMHR canola variety, Quest, was different from two conventional canola varieties tested; however, the finding was not generalized for other GMHR canola varieties tested, including three glufosinate-resistant varieties containing the *pat* gene (Dunfield and Germida 2001). More work examining GMHR canola also shows differences in the microbial communities associated with GM plants. Gyamfi et al. (2002) found minor differences in the denaturing gel gradient electrophoresis (DGGE) patterns of the eubacterial population associated with a glufosinate-resistant GMHR canola; however, this was subject to seasonal variation. Furthermore, the transgenic plants hosted different *Pseudomonas* populations than wild-type plants throughout the growing season. Similarly, different populations of *Rhizobium leguminosarum* bv. *viceae* were associated with transgenic glufosinate-resistant canola compared to their non-transgenic counterparts (Becker et al. 2001). In addition, Sessitsch et al. (2004) found that metabolically active rhizosphere bacteria associated with GMHR canola (glufosinate-resistant) were affected by the genetic modification. Furthermore, at plant senescence, selected soil enzyme activities were significantly enhanced in GMHR plants, likely due to altered root exudation compared to the conventional varieties. However, these effects were minimal as compared to the influence of plant growth stage.

In contrast, the microbial communities associated with glufosinate-resistant transgenic corn were not different in their single strand conformation polymorphism (PCR-SSCP) patterns compared to those communities associated with wild-type corn plants (Schmalenberger and Tebbe 2002). Similarly, in greenhouse and field studies, an examination of a Roundup Ready® GMHR corn variety, as well as its nontransgenic isogenic lines showed no differences between community-level physiological profiles (CLPP) and DGGE profiles of the microbial communities associated with GMHR and conventional corn rhizospheres (Fang et al. 2005).

It is clear from the current studies that GMHR plants can influence the composition of the plant-associated microbial communities. Moreover, these effects have been shown in a variety of plants with different transgenes. However, it has also been shown that these effects are dependent on field site, seasonal variation and method of analysis used to assess the community (Dunfield and Germida 2004). Dunfield and Germida (2001) demonstrated that field site influenced microbial community composition and interacted with plant varieties in their influence on the microbial community. The effect of plant variety on the microbial community at one field site was sometimes entirely different from that observed at another field site, suggesting that the environment can play a major role in determining the potential ecological significance of GM plants. A time-course study examining GMHR canola over an entire field season suggests that changes to the microbial community structure associated with GM plants are not permanent. CLPP, fatty acid methyl ester (FAME) and 16S ribosomal DNA analysis all showed that a variety of Roundup Ready® canola did significantly influence bacterial community structure over multiple field sites and years; however, by April, there were no differences between the microbial communities associated with canola plants after plants were harvested in the preceding September (Figure 1; Dunfield and Germida 2003).

Little attention has been paid to soil organisms outside the roots and rhizospheres of GMHR plants, even though soil food webs are important drivers of decomposition processes in agricultural systems (Wardle 1995). Recent work demonstrated that in the first year of a field experiment, an early season reduction in abundance of bacterial-feeding protozoans and a post-harvest increase in bacterial biomass were associated with Roundup Ready® corn relative to a conventional genotype (Figure 2; Powell and Klironomos, unpublished data).

Soil organisms can be affected by growing GMHR crops. However, often these effects are relatively transient and difficult to predict, due to the influence of environmental factors, such as sampling date and field site, on these communities.

Effects of GMHR management on soil organisms

The introduction of GMHR crops has led to more prevalent use of herbicides to which these crops are resistant (Carpenter et al. 2002). The majority of GMHR crop varieties express bacterial variants of the gene encoding an enzyme that is not inhibited by glyphosate [N-(phosphonomethyl) glycine], a systemic, foliar-applied, highly efficacious, and broad-spectrum herbicide that is commonly formulated as Roundup® by Monsanto. In sensitive plants, glyphosate inhibits the 5-enolpyruvylshikimic acid-3-phosphate synthase (EPSPS) enzyme, resulting in the inability of the plant to synthesize aromatic amino acids and the accumulation of shikimic acid, certain hydroxybenzoic acids, and other toxic intermediates of the shikimic acid pathway. Glyphosate is used, conventionally, as a burndown in which the herbicide is applied prior to the seeding of the crop in order to control any early

emerging weeds. The use of glyphosate-resistant (GR) genotypes allows for in-crop sprays for highly efficacious weed control with a minimum number of applications. As a result, glyphosate was applied to 79 % and 24 % of cotton and corn acreage, respectively, in 2003 and 89 % of soybean acreage in 2004 (US data; NASS 2005).

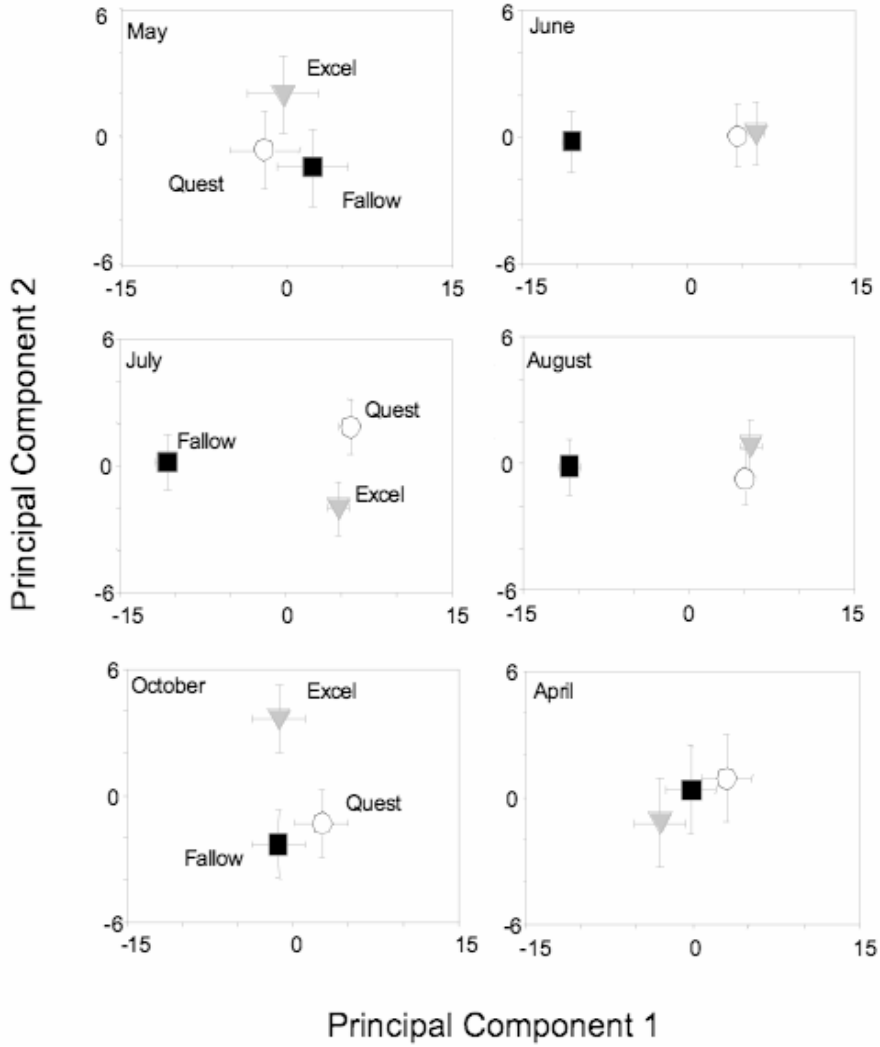

Figure 1. Principal component analysis (PCA) of community level physiological profiles (CLPP) obtained from fallow soil and rhizosphere microbial communities of canola varieties grown at Watson, Saskatchewan, sampled, May, June, July, August and October 1999 and April 2000. Each symbol is the average PC score of four replicates at one field site (n=4). Error bars represent the standard error of the mean. (Adapted from Dunfield and Germida (2003) with permission from the American Society for Microbiology).

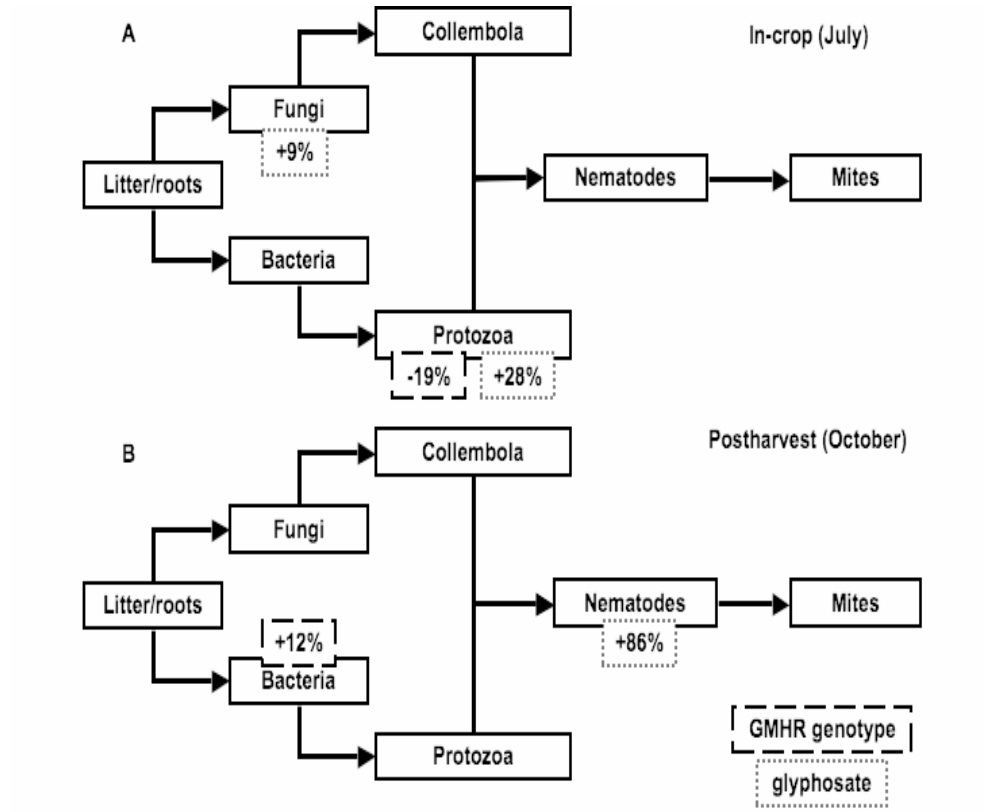

Figure 2. Simplified soil food web demonstrating direction and magnitude of effects of glyphosate-resistant corn (boxes bordered by coarse dashes) and GMHR management using glyphosate (boxes bordered by fine dashes), relative to conventional genotype and herbicide, respectively, at two time points: A) one month following first in-crop glyphosate application and B) within three days of corn harvest (Powell and Klironomos, unpublished data). Arrows represent flow of energy through trophic interactions.

Changing herbicide use patterns may influence agroecosystem biodiversity due to differences among herbicides in their toxicity to non-target organisms (Carpenter et al. 2002). Glyphosate is frequently cited as having low toxicity to non-target organisms and low persistence in soil, especially compared to several other herbicides commonly used in field crops, yet glyphosate use has been associated with changes in the structure and activity of soil microbial communities (e.g., Carlisle and Trevors 1986; Wardle and Parkinson 1990a, 1990b). Highly toxic effects of glyphosate are limited to organisms utilizing the shikimic acid pathway to produce aromatic amino acids, including bacteria, some fungi, some other microbial eukaryotes (including some parasites of humans), and plants. Other organisms either

utilize variants of the EPSPS enzyme that are less affected by glyphosate or are unable to synthesize aromatic amino acids in the first place. Toxic effects observed in these other organisms are more frequently linked to the formulated herbicide rather than the parent compound (Atkinson 1985).

Glyphosate can also affect non-target populations in ways unrelated to its toxicity. For instance, the chemical structure of glyphosate is analogous to that of an amino acid. The herbicide is metabolized by a variety of microorganisms, breaking it down into phosphoric acid, ammonia, and carbon dioxide (Franz 1985). In addition, the use of glyphosate in agroecosystems results in an abundance of plant litter entering the soil, especially when applied postemergence since more weed biomass has been allowed to accumulate prior to weed control. Therefore, any effect of glyphosate on non-target organisms may be indirect or unrelated to its toxicity, due to an increase in resources for glyphosate-degrading and saprotrophic microorganisms (Roslycky 1982) and/or the production of toxic allelochemicals during the decomposition of dead plant tissue (Lynch and Penn 1980).

Although relatively few field studies have been conducted, cropping systems associated with transgenic varieties and/or their herbicides affected the abundance of soil organisms in most cases. For example, GMHR weed management practices for GR-corn and soybean (one or two postemergent glyphosate applications) resulted in a transient increase in soil microbial biomass relative to a conventional program (preemergent acetochlor and atrazine for corn; preemergent cloransulam, S-metolachlor, and sulfentrazone for soybean) but had no detectable effect on substrate-induced respiration, the community physiological profile, or the nematode community profile (Liphadzi et al. 2005). GMHR cropping systems had greater abundances of detritus-feeding collembolans in beets, spring and winter oilseed rape, and corn than in conventional cropping systems using a variety of weed management tools (Brooks et al. 2003; Bohan et al. 2005). Weed seed-feeding carabid beetles were less abundant in GMHR beets and oilseed rape, but more abundant in GMHR corn, than in their conventional counterparts, effects that mirrored responses in weed communities (Brooks et al. 2003). Postemergent glyphosate application to GR-corn, in combination with a conventional herbicide program (preemergent application of isoxaflutole, atrazine, and nicosulfuron), resulted in transient increases in bacterial, fungal, protozoan, and nematode biomass when compared with the conventional herbicide program alone (Figure 2; Powell and Klironomos, unpublished data). Sessitsch et al. (2004) observed shifts in rhizosphere bacterial community structure and increased activities of four soil enzymes (invertase, phosphatase, urease, and arylsulfatase) associated with glufosinate application to glufosinate-resistant oilseed rape relative to untreated controls, although these effects were dependent on plant growth stage. In contrast, Schmalenberger and Tebbe (2002) were unable to detect differences in bacterial community structure in the rhizosphere of transgenic, glufosinate-resistant (Liberty Link®) corn, regardless of whether glufosinate or conventional herbicides (terbuthylazine, bentazon, and nicosulfuron) were used. These results appear to

suggest that the effects of weed management in GMHR cropping systems are more stimulatory of decomposer organisms than toxic toward members of the soil community.

More pronounced is our lack of knowledge regarding the effects of GMHR crop management on ecosystem functioning. In a conventional cropping system, glyphosate application to grass litter accelerated soluble nutrient loss but inhibited carbon decay in litter, possibly due to increased utilization of the herbicide by microorganisms followed by increased grazing by microarthropods (House et al. 1987). Glyphosate-treated soybean litter in GR-corn field plots decomposed at a slower rate than untreated soybean litter (Powell and Klironomos, unpublished data). Glyphosate application to GR-soybeans under field conditions can have negative impacts on rhizobial nodule formation and biomass, which may have consequences for nitrogen fixation (Motavelli et al. 2004). More research is needed to anticipate the functional consequences of structural changes to soil communities in GMHR cropping systems.

Summary

GMHR cropping systems are widespread in North America and are increasing in prevalence globally. There is evidence for both GMHR genotype and management to impact on non-target soil communities. It is important to consider both types of effects when making predictions as to the consequences of GMHR cropping systems. More research is necessary to address the interactive effects of GMHR genotype and management on non-target organisms in order to determine if these effects are additive, antagonistic, or synergistic. In addition, while we can detect short-term effects of GMHR cropping systems on non-target organisms, we are currently unaware as to the direction and magnitude of these effects over longer timescales and their consequences for ecosystem functioning.

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GM crops are uncontainable: so what?

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Evidence is presented from 10 years of commercial experience to challenge the premise that genetically modified (GM) field crops can coexist without agronomically significant gene flow to either weedy relatives or non-GM crops. It is further argued that agricultural biotechnology is commercially feasible only if costs of mitigation for or consequences of adventitious gene flow are externalized involuntarily and unavoidably to non-adopters and society at large.

Coexistence is futile

Gene flow from transgenic crops involves crop-to-weed as well as crop-to-crop concerns, each with the potential to externalize costs involuntarily.

Crop-to-weed

Many have discounted the risks of crop-to-weed transmission of transgenes. Both the CFIA and the USDA, for example, contend that because neither GM crops nor their weedy relatives evolved in Canada or the US, transmission to weeds is a non-issue. Alternatively, it is reasoned that the probability of outcrossing resulting in fertile hybrids is negligible, or at worst, controllable with standard agronomic practice.

In contrast, from both a global and a US perspective, Ellstrand (2003) argued that because weedy relatives have migrated alongside modern crop species, risk of crop-weed hybrids pertains well beyond their place of geographic origin. For example, spontaneous hybridization with wild/weedy relatives occurs in 90 % of the 25 most important global crops, the exceptions being peanut (*Arachis hypogaea* L.), chickpea (*Cicer arietinum* L.), and sweet potato (*Ipomoea batatas* (L.) Lam.). The 25 crops encompass 23 genera and 11 plant families, including annuals and perennials, wind and insect pollinated species, and highly outcrossing as well as largely selfing species, distributed within a single continent or globally.

He noted further that in the US, all but 2 of the 20 most important crops also hybridize spontaneously with wild/weedy relatives. Just over half (11) hybridize within the US itself, namely, wheat (*Triticum aestivum* L.), alfalfa (*Medicago sativa* L.), cotton (*Gossypium* sp.), sorghum (*Sorghum bicolor* L.), oats (*Avena sativa* L.), rice (*Oryza sativa* L.), sunflower (*Helianthus annuus* L.), canola (*Brassica napus* L.), beets (*Beta vulgaris* L.), rye (*Secale cereale* L.), and grape (*Vitis vinifera* L.).

Detection of gene flow into wild/weedy relatives is difficult, simply because frequency is typically very low. But according to Ellstrand (2003), very little gene flow is needed to maintain a gene within a weedy population. Just a few successful pollinations leading to adult plants in each generation is enough to maintain gene frequency. Particularly if the number of weed individuals is low, genetic swamping from sown cropland pre-empts weedy plant pollen, maintaining gene flow to wild populations each year.

Despite the often very low frequency of outcrossing leading to fertile progeny, introgression of cultivated genes into wild relatives has preceded the evolution of increased weediness and/or aggressiveness in a number of wild species, including weed beets, weedy rye, and johnsongrass (*Sorghum halepense* (L) Pers.) (Ellstrand, 2003). Indeed, the genes from cultivated crops appear disproportionately likely to promote increased weediness in wild species. Out of 28 examples of hybridization leading to increased invasiveness, 25 % involved crops. Given that crops account for a tiny fraction of all plants, Ellstrand (2003) concluded that "Chances of a domesticate-wild hybrid derivative becoming a problem plant are an order of magnitude greater than if both hybridizing parents are wild."

Are Transgenes Different? Is there any reason to think that transgenic crop hybridization potential might differ from that suggested by patterns based on conventionally bred crops? Ellstrand (2003) presented at least two reasons suggesting that the risks posed by transgenic crops may be different.

First, all transgenes in commerce to date are genetically dominant, in contrast to the recessive genes which more typically code for the traits that distinguish crop from weed species. Dominant traits are immediately acted upon by selection, while recessive genes are masked from natural selection and are acted upon much more slowly. Thus, when exposed to the relevant selection pressure, traits coded for by transgenes may be eliminated or amplified in weed populations at a much faster rate than conventional genes. While herbicide resistance (HR) - which accounts either solely or jointly for about 75 % of all transgenic hectarage - is unlikely to be adaptive in the absence of herbicides, a wide range of plant, animal, and microbial applications is in varying degrees of readiness for commercialization, as tabulated by the CBCGEO (2004) and others. Adaptiveness is trait-specific, with the benefit of tolerance to a given pest or pathogen, for example, depending on the selection pressure exerted by the given pest or pathogen. Many pending traits have never existed in the environment on a commercial scale, resulting in potentially novel ecological and evolutionary impacts.

Secondly, regardless of the intended traits, transgenic crops also express a range of unintended gene expression effects such as increased bolting in weedy beets (cited in Ellstrand, 2003), larger flowers, which may have contributed to the 20-fold higher rate of outcrossing in transgenic *Arabidopsis thaliana* (L.) Heynh.)(Bergelson et al., 1998), and changes to ecologically important fitness traits, as seedling survival and dormancy in subterranean clover (*Trifolium subterraneum* L.) fitted with a nutrition-enhancing transgene (Godfree et al., 2004). A range of

inadvertent outcomes specific to the mode of action of glyphosate-resistant crops is reviewed by Pline-Srnic (2005). To the extent that unintended gene expression is greater in transgenic than in conventionally bred crops, the ecological effects of transgene flow into wild/weedy relatives could be less predictable.

Thus, crop-to-weed gene flow is not the exception but the rule. Implications should be viewed as "when" rather than "if" gene flow occurs.

What is at stake? Current concerns of crop-to-weed flow concentrate on HR, a trait which would create or exacerbate weed problems. According to Duke (2005), it is highly likely that HR will move from canola to weedy relatives, and from cultivated rice to weedy or wild rice. For canola, Légère (2005) cited evidence that hybridization rates were highest between canola and *B. rapa* L.. Other acknowledged high risk combinations include sorghum and johnsongrass, sugar beets and wild beets (*Beta vulgaris* L.), and sunflower and its wild progenitor (*H. annuus* L.) (Messeguer, 2003). Excluding sugar beet, which is not widely sown, these 4 high risk crops account for 243 million ha or 25 % of the 997 million ha sown to the 25 most important global crops (calculated from Ellstrand, 2003). Threatening the viability of cost-effective weed control in globally important crops is an example of a cost necessarily externalized involuntarily to others to permit some to grow GM crops today (see below).

Not yet? That transgenic crop-weed hybrids have not yet become problematic may reflect the limited number of species - corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), canola, and cotton - commercialized to date, each of which is grown in just a few countries. The only acknowledged high risk crop - canola - is grown largely in Canada. Warwick and colleagues (unpublished) have reportedly detected transgene flow to weedy canola relatives in Canada. Regulators have abdicated responsibility for post-commercialization monitoring by vesting it entirely in the proprietor of each approved construct. The absence of systematic monitoring by an independent agency precludes detection and a pro-active response to incipient crop-weed hybrids - if any.

Crop-to-crop

Quantifying and attempting to mitigate crop-to-crop transgene flow have been the subject of numerous articles in recent years, as reviewed by Messeguer et al. (2004), Clark (2004), Légère (2005), and others. Virtually all studies report the same leptokurtic extinction curves - subject to variation induced by species-specific attributes, wind direction, and pollinator activity - for pollen movement and outcrossing.

The confirmed contaminating radius is crop-specific, measured in:

kilometres for creeping bentgrass (*Agrostis stolonifera* L.) (Watrud et al. 2004), alfalfa (St. Amand et al., 2000), sugar beet (various, cited in Ellstrand, 2003) and canola (Rieger et al., 2002; various in Légère, 2005),

hundreds of metres for corn (Ma et al., 2004; Stevens et al., 2004; Halsey et al., 2005),

tens of metres for wheat (Matus-Cadiz et al., 2004 but up to 300 m; Hanson et al., 2005) and cotton (Van Deynze et al., 2005 but up to 1.6 km), and

metres to tens of metres for rice (various in Gealy et al. 2003; Messeguer et al., 2004; Rong et al. 2005), tobacco (*Nicotiana tabacum* L.) (Paul et al., 1995), soybean (Ahrent and Caviness (1994) and barley (*Hordeum vulgare* L.) (Ritala et al., 2002).

The technical and logistical feasibility of genetic isolation to guard against inadvertent contamination of adjoining cropland remains to be shown for many major crop types.

What is at stake? The two issues which appear to have motivated interest in crop-to-crop gene flow are: growing problems with persistent volunteer HR plants in fields of adopters and non-adopters, and recognition of the inability of any existing measures to fully contain pharmaceutical crops, and hence, the need to develop alternative strategies.

The CBCGEO (2002) analyzed the efficacy of all possible methods of controlling transgene movement. They concluded that major gaps in information preclude rigorous assessment or design of bioconfinement strategies. They also found that no single method of bioconfinement is likely to be completely effective, suggesting that multiple approaches should be applied simultaneously to reduce risk. Potential ecological risks and consequences of control failure are not yet fully characterized. They also noted that bioconfinement, particularly for pharmaceutical crops, requires a regime that is rigorous, comprehensive, and subject to inspection and enforcement.

Failure to meaningfully resolve these issues will either shorten the lifespan or curtail further commercialization of agricultural biotechnology.

Human nature/human error? While most academic studies focus on plant reproductive behaviour, containment is most often compromised or facilitated by human error. Examples include the release of transgenes in impossible-to-contain outcrossing species which then contaminate non-GM crops, such as that which motivated the SOD (Saskatchewan Organic Directorate) lawsuit from canola. Other examples include the StarLink debacle from corn (Smyth et al., 2004), and the various ProdiGene biopharmaceutical scandals with corn. Authorization of commercial production of transgenic crops without regard to the infrastructure to accommodate societal demand for GM-free grain created a further suite of avoidable problems, whether from the StarLink debacle or GM encroachment into Mexican landraces despite a 1998 moratorium on growing GM corn in Mexico.

Synthesis.

According to Ellstrand (2003), risk of cultivated gene flow to weedy relatives is real, is higher than between wild parents, and is increased when the gene is a transgene. To date, transgene flow is most likely in 5 key food crops. Compromising chemical weed control, due to the difficulty of controlling HR weeds themselves or to the unacceptable expense of alternative herbicides - externalizes risks to global food security in order to sustain a proprietary technology today.

Complications induced by the uncontrollability of same-crop transgene flow today are currently limited to the growing problem of intractable HR volunteers, a problem which has been externalized involuntarily to non-adopters as well as to adopters. The many publications dealing with transgene flow in recent months may mirror concerns about similarly uncontrollable biopharmaceutical, industrial enzyme, and other pending 'next generation' traits.

The hidden costs of growing GM crops: to adopters and to non-adopters

The decision to authorize, market, and grow GM crops imposed a range of hidden "GM-plus" costs on those adopting - as well as those not adopting - the technology.

The lemon effect.

Furtan et al. (2003) referred to RR wheat as a 'lemon', in that inability to segregate GM to enable a GM-free channel would effectively sour the entire export market for wheat. They calculated that the proposed introduction of RR wheat in Canada would cause losses of $46 and $32 million annually to the adopters and non-adopters of the technology, respectively, while bringing in a positive $157 million to Monsanto. Wheat producers would lose whether they grew GM or not because 82 % of those who import Canadian wheat say they won't accept GM wheat, and it is not possible to segregate.

Wisner (2005) calculated that with 46 countries now labelling for GM ingredients, introduction of GM wheat in the US would reduce the wheat export market by 20 to 37 %. Market rejection figures were derived from a 2004 survey by the USDA FAS, as well as earlier surveys by US Wheat Associates and the Canadian Wheat Board. Wisner's (2005) estimates are reduced from the earlier loss estimates of one-third to one-half of the hard red spring wheat and even more of the durum market (Wisner, 2003). Increased US wheat exports from 8 to 12 % of sales to China in particular, a country which has not yet rejected GM soybean, moderated previous loss estimates. However, he also noted that as a food grain, wheat differs from corn or soybean, which are feed grains. People may be less accepting of GM in crops directly consumed. Market rejection of wheat would quickly depress wheat

from food to feed grade, depressing prices not simply of the wheat but also of competing feed grains - another externalized cost.

For growers of hard red spring wheat in the northern Great Plains, the 'optimistic' scenario of Benbrook (2005) found that non-adopters (70 % of farmers) would lose US$5.60/ac in income - owing to a 4 % decline in market price - while adopters (30 % of farmers) would lose US$11.03/ac in net cash receipts owing to the higher cost of seed as well as a 4 % lower market price. The whole industry (13 million ac in hard red spring wheat) would lose $94 million a year under the optimistic scenario.

Thus, particularly for crops grown for direct human consumption, introduction of GM traits disregarding the infrastructural changes needed to accommodate dual stream GM and non-GM grain will reduce marketability of export crops.

Farm management complications.

Consideration of increasingly evident "GM-plus" costs was forced by the prospect of RR wheat in Canada (CFIA, 2003; Furtan et al., 2003; Van Acker et al., 2003) and the US (Benbrook, 2005; Wisner, 2005). According to these sources, adopters of GM canola in western Canada have had to absorb the logistical costs of compensatory temporal and spatial shifts in crop rotations and herbicide combinations. Additional costs have been imposed to deal with HR volunteers or volunteers with stacked HR traits resulting from within-crop crossing (Hall et al., 2000) or HR weeds (Heap 2006).

Less acknowledged are the costs externalized to non-adopters of GM technology. For example, in a workshop on the management of HR crops, the CFIA (2003) reported that

> "Most workshop participants felt that control of HR volunteers, in-crop as well as in fallow, is a manageable agronomic issue for current HR crops (canola, corn, and soybean). Discussion centered around increasing need for development of **more elaborate, as well as site-specific**, weed management strategies, not only for controlling HR volunteers but also for minimizing the selection pressure for HR biotypes in weed species...also noted that adoption of these weed management strategies **will not be limited to adopters but also extend to non-adopters.."** (emphasis added)

The terms 'more elaborate' and 'site-specific strategies' suggest that adopters need to absorb the extra costs created by the increasingly intractable HR volunteers. But the presumption that non-adopters will necessarily *also* have to absorb these same "GM-plus" costs when they are under no contractual obligation to anyone implicitly acknowledges the literal uncontainability of this technology. Consequences are downloaded or externalized to all, to allow some to benefit.

While adopters reportedly do not experience problems with volunteers because they are expected and ready, non-adopters do not expect, and hence are ill-prepared to respond to problems not of their own making (CFIA, 2003). Response is complicated as the presence of adventitious volunteers is undetected until after the herbicide has been bought and applied. While the CFIA (2003) asserted that non-adopters had not experienced major difficulty to date, conspicuous by its absence was mention of the loss of the organic canola market from the prairies and the pending SOD lawsuit.

A partial listing of "GM-plus" costs imposed on all farmers would include:

- more expensive herbicides to cope with HR volunteers and weeds (controlling RR-wheat volunteers, for example, does not have a cheap alternative comparable to 2,4-D for RR- canola volunteers)
- company withdrawal from herbicide and HR crop development, due to sector domination by RR-crops, reducing herbicide rotation options and exacerbating selection for resistance
- research, testing, and redesign of seed production protocols and trade practices to avoid contamination of pedigreed seed
- costs of stewardship education and compliance monitoring for dealers, growers, applicators, and extension agents
- infrastructural costs to establish dual stream handling, storage, and shipping protocols for marketed crops
- delayed planting and realigned field plantings to mitigate against neighbour cropping patterns; changes to crop choice specifically to employ alternate herbicides, and other managerial practices to cope with HR volunteers
- coping with a persistent soil seedbank of HR canola (or wheat?) volunteers owing to shattering losses at harvest (Friesen et al., 2003)
- lost production and profit from refugia set aside to sustain the effectiveness of Bt crops
- costs for testing and monitoring for GM contamination, and lost premia when contamination is detected
- lost markets for IP products - including but not limited to organics
- lowered market value overall, due to the lemon effect
- threat of liability for inadvertent patent infringement.

These costs can only become more onerous if biopharmaceutical crop contamination becomes a reality. Smyth et al. (2004) reported that a global total of 134 biopharmaceutical field trials occurred between 1992 and 2002, of which 62 were in the US and 53 were in Canada. Of these, corn, tobacco, and canola accounted for 46, 25, and 17 trials, respectively. The contaminating radius for corn and canola ranges from hundreds to thousands of metres. To date, regulations governing commercialization of biopharmaceutical crops have not been formalized.

Spatial isolation is one of the few options available to a non-GM grower seeking to protect their crop from contamination. Ma et al. (2004) determined that 200 m was sufficient to reduce contamination of a non-GM corn field to <1 % from an adjacent Bt-corn field. Based on this estimation, if the non-GM crop was organic (Table 1), white food grade (Table 2), or high amylose (Table 3) – each of which is required to be non-GM to meet market demands - economic losses from employing a 200 m isolation zone to reduce contamination to <1 % could be calculated assuming:

- a square 40 ha (100 ac) field measures 636 m on a side
- white food grade and high-amylose corn yield the same as conventional (131.3 bu was the 2004 ON average), while organic corn yield is conservatively assumed to be 80 % of the provincial average (131.3 * 0.8 = 105 bu/ac)
- price of corn (mean of Minnesota and Detroit prices for Week of 15 Nov 05) is $5.50/bu for organic and $1.75/bu for conventional #2 yellow corn (The New Farm 2005)
- the premia over Chicago Board of Trade options for white food grade and high amylose were estimated as $0.25/bu (2002) (ISFP 2003a) and $1.00/bu (2003) (ISFP 2003b), respectively
- corn within the 200 m buffer separating the organic, white food grade, or high amylose corn from GM-corn is sold as conventional corn

Table 1. Gross receipts (US$) from 40 ha of organic corn with 200 m buffers to safeguard against GM contamination from adjoining fields.

Type of corn	Degree of GM corn in adjacent fields				
	None	1-side	2-sides	3-sides	4-sides
Organic ($5.50/bu)	57,772	39,863	27.140	14,696	7,915
Conventional ($1.75/bu)	-	5,696	9,739	13,781	15,857
Total from 40 ha	57,772	45,559	36,879	28,477	23,773
Percentage loss in receipts		21	36	51	59

Table 2. Gross receipts (US$) from 40 ha of non-GM white food grade corn with 200 m buffers to safeguard against GM contamination from adjoining fields.

Type of corn	Degree of GM corn in adjacent fields				
	None	1-side	2-sides	3-sides	4-sides
Non-GM White Food Grade ($2.00/bu)	26,260	17,962	12,342	6,675	3,613
Conventional ($1.75/bu)	-	7,261	12,178	17,233	19,819
Total from 40 ha	26,260	25,223	24,520	23,907	23,431
Percentage loss in receipts		4	7	9	11

Table 3. Gross receipts (US$) from 40 ha of non-GM high amylose corn with 200 m buffers to safeguard against GM contamination from adjoining fields.

Type of corn	Degree of GM corn in adjacent fields				
	None	1-side	2-sides	3-sides	4-sides
High Amylose ($2.75/bu)	36,107	24,698	16,970	9,171	4,965
Conventional ($1.75/bu)	-	7,261	12,178	17,233	19,819
Total from 40 ha	36,107	31,959	29,149	26,404	24,784
Percentage loss in receipts		12	19	27	31

Non-GM IP corn growers would lose from 21 to 59 % (organic), 4 to 11 % (white food grade), or 12 to 31 % (high amylose) of their gross receipts if obliged to sacrifice from 1 to 4 200 m wide buffers to safeguard a 40 ha crop from neighbouring GM corn fields. Alternatively, they could carve up their 40 ha field into smaller management blocks, strategically planting each block to forestall contamination from a neighbour, with ensuing complications for rotation integrity, machinery use efficiency, and other logistical considerations, each of which costs money.

With an estimated 6,650 ac of organic corn in ON (2003; Macey, 2004) and in the US:

- 42,000 ac of organic corn (http://www.ers.usda.gov/emphases/harmony/issues/organic/organic.html#data),
- 900,000 ac (2002) of white food grade corn, and
- 55,000 ac (2003) of high amylose corn (figures unknown for Canada),

annual mitigation costs (US$) for imposing 200 m buffers on from 1 to 4 sides of 100 ac IP corn fields would range from:

- $0.8 to 2.4 million for ON organic corn growers
- $5.1 to 14.3 million for US organic corn growers
- $9.4 to 25 million for white food grade growers, and
- $2.4 to 6 million for high amylose growers.

The same pattern of externalized loss would pertain to any IP grower attempting to isolate their crop from neighbouring GM crops, although the magnitude loss would vary with the crop-specific buffer and price spread.

Fractional and potentially absolute dollar losses would increase as field size decreases. With a mean Ontario farm size of 91 ha (McGee 2002), most corn fields are actually *smaller* than 40 ha - meaning greater losses. Although based on a

number of broad assumptions, this simple analysis reveals the economic hardship imposed involuntarily on IP producers, so that their neighbours can grow GM crops.

Broader societal complications

"GM-plus" costs which are currently being externalized to society at large, assessed through taxpayer dollars, include:

- the research and extension needed to create the "more elaborate as well as site-specific...strategies" referenced by the CFIA (2003)
- the monitoring and vigilance costs for policing compliance with Bt refugia, roguing HR volunteers and stacked volunteers on millions of hectares a year, forever, and potentially, even more intrusive requirements for staggered planting dates, regional restrictions on crop choices, and obligatory management practices (Bock et al., 2002)
- food cost implications – transport, processing, retailing, and monitoring - to enable consumer choice for GM v. non-GM containing foodstuffs
- the soil, water, and GHG implications of a return to tillage, should weed control on the prairies become too costly or complicated with HR or stacked HR volunteers

All of these hidden costs - whether imposed on adopters, non-adopters, or society at large - are excluded from standard cost:benefit analyses for GM crops, specifically because they are imposed involuntarily and unavoidably on everyone else. Unlike users of innovations such as precision farming or oxygen limiting silos, users of GM technology necessarily and unavoidably harm their non-adopter neighbours because of market rejection of GM crops (Rousu et al., 2004; Hu et al., 2005; Kiesel et al., 2005). Even in the absence of market rejection, harm is exerted through complications of controlled HR or stacked HR volunteers or other disadvantageous traits. In the absence of regulations ensuring liability for the lost income and other harms externalized by GM crops, agricultural biotech has become the tail wagging the dog of agriculture today.

Summary

Crop-weed hybridization is the norm, even in places where the crop did not originate, as human activity has inadvertently carried weedy relatives as well as crops around the globe. Very low rates of gene flow, particularly when sustained over years by commercial-scale plantings, are sufficient to convey crop genes to wild and weedy relatives, resulting in documented increases in weed severity in some species. The issues raised by transgenic crops may differ fundamentally from those raised by conventionally bred crops, specifically because all transgenes to date are dominant - resulting in more immediate exposure to natural selection - and

further, may code for novel and potentially adaptive traits. Pleiotropic effects further complicate prediction of the significance of transgene flow to wild/weedy relatives.

Crop-crop gene flow is creating increasingly intractable crop volunteer problems. While the issue now is HR, which directly affects only the farming community, the literal uncontainability of HR today clearly portends problems for other traits in the future. Significant gaps in information preclude rigorous review or development of containment methods. None of the existing methods have proven fully effective.

Specifically because it is uncontainable - and in the absence of regulatory constructs forcing GM users or purveyors to pay for harm - financial liability and logistical responsibility for mitigation failure have somehow been imposed on those who declined to grow GM crops in the first place. These costs are not small, and for crops such as canola and corn are wholly unavoidable, even by organic and other IP growers.

What is wrong with making the technology pay for itself - as does organic? It is irrational to argue that buyers of non-GM crops should foot the bill just so that GM-growers can market crops that the market has already rejected. Since when do people have to buy something just because someone wants to sell it?

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The potential for the coexistence of GM and non-GM crops in Canada

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Transgene escape has been happening in Canada and it can cause problems. Transgene escape occurs via a variety of routes within crop production systems, and it can sometimes occur because of human error. It may be relatively difficult to confine transgenes in commonly grown food and feed crop species, especially for species which produce large feral and volunteer populations. The Canadian government has initiated mechanisms for consultation on the issue of transgene confinement and its implications but it has not created new regulations or new laws to address either the recent Supreme Court ruling on the case of Percy Schmeiser or the Royal Society of Canada precautionary action recommendations (released in 2001). The apparent position of the Canadian government as a promoter of biotechnology and the lack of mandated thresholds in Canada for the adventitious presence of transgenes may be affecting governmental will to produce concrete action on transgene confinement. As pharmaceutical and industrial traits are introduced into crop plants there will be greater risk that Canadians will be directly affected by transgene escape. At the same time, the risks posed by these new traits may create the political will necessary to support a new regulatory category of 'confined commercial release' allowing for the coexistence of GM-crops with novel traits with non-GM crops in Canada.

Introduction

Genetic engineering (GE) is truly novel technology which allows for the inclusion of almost any trait imaginable into crop plants to serve all manner of desired functions and end-uses (Tolstrup et al. 2003). In Canada, farmer adoption levels of GM crops have been high with more than 75 % of the canola grown in 2004 being GM while GM soybean and corn crop acreages represent over 60 % of total acreage (James 2004). Although GM crops are registered for unconfined release in Canada they continue to be a concern in countries where GM crops are not yet registered for unconfined release. In addition, because GE allows for the realization of truly extraordinary traits in crop plants, it can also produce novel and unexpected risks (Demeke et al. 2006). Most risks related to the release of GM crops are related to transgene (trait) movement, which remains relatively poorly understood and has been studied to only a very limited extent (Marvier and Van Acker 2005). The exploitation of GM crops will require responsible introduction

which, in turn, requires the creation of effective and acceptable transgene confinement protocols. These protocols must be based on knowledge of the nature and interaction of those factors which contribute to transgene movement (Tolstrup et al. 2003). In order that they be effectively administered, the protocols must include the assignment of responsibilities for transgene confinement and these must be enforced through law. To-date Canada has taken a non-regulatory approach to novel trait confinement. This approach may become increasingly risky as pharmaceutical and industrial traits are introduced into crops.

GM trait (transgene) escape happens and it can cause problems

In North America where there have been many documented cases of transgene escape (Demeke et al. 2006; Marvier and Van Acker 2005). In Canada, intraspecific transgene movement in canola (*Brassica napus* L.) has been common. In western Canada, there has been so much intraspecific transgene escape in canola that farmers in this region have come to expect the unintended appearance of transgenes in their canola (Van Acker et al. 2004). Hall et al. (2000) found that the specific transgenes encoding for different herbicide resistance traits were stacking within individual volunteer canola plants, giving rise to multiple herbicide-resistant volunteer canola plants. Independent testing of certified canola seed lots from western Canada revealed that the majority of tested seed lots contained at least trace amounts of genetically engineered herbicide resistance traits (transgenes) (Friesen et al. 2003). For these seedlots, the source of the adventitious presence was never determined but the high level of adventitious presence of unintended transgenes in pedigreed certified seed lots shows that seed production segregation systems are not sufficient to prevent significant adventitious presence levels for unintended transgenes.

Extensive intraspecific transgene movement in canola in western Canada occurred because a number of conditions aligned to allow the transgenes to move relatively easily. In western Canada canola is commonly grown on many acres, canola is frequently grown in common crop rotations, there is a very large volunteer canola population both in fields and next to fields (roadways etc.), volunteer canola can flower in subsequent canola crops and canola can outcross relatively readily, these volunteer canola plants can easily pass their transgenes into the current canola crop (Van Acker et al. 2004). In addition, farmers of non-GM canola have been unwittingly seeding GM canola through contaminated seed and because all current GM canola varieties have unconfined release status in Canada there has been no effort made to limit intraspecific transgene movement in canola in Canada. Transgene escape can occur via seed or pollen and many escape routes are present within seed and crop production systems. Volunteer and feral populations can also play an important role in transgene movement and transgene confinement requires knowledge of whether or not a given crop species has an operational

metapopulation (including cropped, volunteer and feral sub-populations) within a farmed region (Crawley and Brown 1995). In this regard, transgene confinement may be relatively difficult depending upon whether a given crop species produces effective volunteer and feral sub-populations, the outcrossing nature of the species, its frequency in rotation, the size of farmers fields and whether or not there is effective selection pressure on the trait which raises its frequency within the metapopulation (Brûlé-Babel et al. 2006).

Transgene escape can also be facilitated by human error. An example is the 'StarLink' case where corn, engineered to express an insecticidal protein, was approved for animal feed but not human consumption. There was insufficient segregation oversight between food and feed streams in the US bulk commodity handling systems and the insecticidal protein was found in a number of processed foods (Demeke et al. 2006). Traces of the StarLink protein could still be commonly found with both food and feed handling streams in the US three years after the initial escape (USDA 2003). The StarLink case demonstrated that full retraction of transgenes (and their products) from complex and massive commercial food and feed systems is difficult, and perhaps impossible. In Canada there have also been cases of human error leading to transgene escape. A well documented case was the inadvertent release of the GT200 event for Roundup Ready canola in western Canada (Demeke et al. 2006). The company's response to the mistake was swift and effective, but the case demonstrated the possibility for these types of mistakes to occur even within the highly managed logistical systems of major multi-national companies.

GM trait escape can cause problems. The adventitious presence of the Roundup Ready (glyphosate resistance) trait in non-Roundup Ready canola has caused direct-seeding farmers in western Canada to have to add another herbicide to their glyphosate pre-seeding burn-off. This has added costs, and caused some re-cropping problems for farmers including non-adopters of Roundup Ready technology (Van Acker et al. 2004). The unmitigated movement of transgenes in canola populations within western Canada has prevented organic farmers in the region from growing certified organic canola (Cullet 2005). Because commercial GM canola varieties have unconfined release status in Canada the movement of transgenes is not regulated and there is no formal recourse for those who are affected by intraspecific transgene movement. Crops transformed to produce industrial or pharmaceutical proteins will pose greater risks to human health and the environment.

Coexistence requires a regulatory and legal framework

In North America, the registration and commercialization of GM crops has been approached in a non-regulatory fashion (Boisson de Chazournes and Mbengue 2005). This approach has expedited the commercialization of GM crops in North

America but it is also creating some potential problems. Even for those who consider GM to be a safe technology there are fears regarding the introduction of certain traits into food and feed crops. These fears are based first on the fact that genetic engineering allows for the introduction into cropped plants of novel traits which will definitely present risks to human health and the environment. The fears are also based on the fact that when unconfined release is granted to a GM crop in North America the liability associated with effects resulting from transgene movement does not rest with the technology developer (patent holder). In Canada it is currently not clear where such liability rests. The recent voluntary withdrawal of glyphosate-resistant spring wheat (Roundup Ready wheat) from the regulatory process in Canada may have been due in part to pressure from grain and food company executives who realized that their shareholders would hold the liability associated with intraspecific transgene escape in wheat.

In Canada, the case of Monsanto versus Percy Schmeiser, tried under patent law, was settled at the Canadian Supreme court level in May 2004. Mr. Schmeiser lost his case in final appeal to the Supreme Court of Canada, which ruled that Monsanto could retain the full rights and privileges of patent ownership, as well as the right to sue farmers for the possession of this transgene, regardless of how it came to be in their possession and regardless of whether or not they profited from possessing it (Supreme Court of Canada 2004). The ruling is problematic because it does not explicitly consider the case of innocent infringement (and proportion of patented product within an admixture of non-patented product) and because the Roundup Ready (GM) transgene is now present in the majority of certified non-Roundup Ready canola seed sold in western Canada. The outcome of this case points to a real need to assign liability and responsibility in regard to transgene ownership and the effects of transgene escape, and that in the current context, all burdens resulting from transgene escape are shifted to the users of GM-crops and those potentially affected by their unconfined cultivation (Cullet 2005). In the absence of legislation which clearly assigns liability and responsibility, redress for damage suffered by transgene escape will be difficult to achieve. For example, the Saskatchewan Organic Directorate (SOD) is suing in class action, Monsanto Canada Ltd and Bayer CropScience for the ubiquity of GM transgenes in canola in western Canada and the resultant inability of organic farmers in this region to produce GM-free organic canola. The case was denied class action status initially but the plaintiffs have since been allowed to appeal this ruling (Smith 2005). Some industry proponents suggest that transgene confinement can be effectively managed via voluntary guarantees on the part of the patent holder. Such guarantees are problematic because they are voluntary and even if they were enforced by *ad hoc* contracts it would be insufficient because they would only provide protection and recourse to signatories of the contracts.

In June, 2004, Denmark became the first nation-state to pass a coexistence law in order to allow for the regulated cultivation of GM crops (Danish Ministry of Food, Agriculture and Fisheries 2004). The legislation will be useful for enforcing

coexistence because it contains key elements including; confinement training requirements, an assignment of confinement responsibilities to GM crop growers, open information access on GM crop sites, compensation mechanism based on threshold of contamination, criminal liability for transgene escape resulting from negligence and search and seizure rights. However, the existence of such legislation does not provide absolute protection for those who might be affected by transgene escape. In this context it is important to remember that one of the critical control points for transgene confinement is the receptor crop, and in the case of transgene confinement the receptor crop is grown by the farmers not growing the GM crop (non-adopters). Therefore, even with legislation in place, non-adopters (organic farmers in Denmark for example) must test their crops to assure customers that they are meeting threshold requirements. The cost and responsibility for this testing would be borne by the non-adopters.

In Canada where there is a non-regulatory approach to the registration of GM crops there is no legislative protection for those who might be affected by transgene escape. There is, however, one effort to create a GM crop exclusion zone in Canada's smallest province. The Prince Edward Island Certified Organic Producers Co-op is assessing a market for agriculture products produced in an Island GMO free grow zone (PEI COPC 2005). There are political and legal efforts challenging the validity of the arguments being used to establish this zone. In Canada, GM crops are not regulated *per se* because Canada subscribes to the notion of substantial equivalence between GM and non-GM crops. In addition, where there are concerns about transgene (trait) movement the regulatory body in Canada is only allowed to regulate on the basis of human health or environmental risk and not economic risk. Whether such GM free regions can prevent adventitious presence or contamination has yet to be determined. However, such regions are changing the concept of coexistence from spatial differentiation at the farm level to larger more isolated regions including islands.

Thresholds for transgene presence are only useful to business entities within the agri-food industry (including farms and farm organizations) if the thresholds are set within law (Boisson de Chazournes and Mbengue 2005; Demeke et al. 2006). Threshold levels may be set by various organizations, including organic certification agencies, but they must be recognized in law within the political jurisdiction within which that agency is functioning if there is to be any enforcement of the threshold or recourse in the event that the threshold has been exceeded. A good example of this is the fact that the EU has established transgene thresholds in law while in Canada, the right of organizations such as the Saskatchewan Organic Directorate to no threshold ('zero threshold') for transgene contamination of organic crops has not yet been recognized in Canadian law (Cullet 2005). In the context of this issue it is worth noting, however, that the International Federation of Organic Agriculture Movements (IFOAM) adopted the position in 2002 that organic certification is a certification of a process of production and as such does not imply an end product guarantee (ISF 2004). In this sense, IFOAM

does not necessarily support *de minimis* threshold levels ('zero thresholds' or minimal testing level thresholds). This creates a challenge for organic farmers who are trying to keep their products 'GM-free' because it is not certain what GM-free means. In practice, although a single global threshold for transgene presence would expedite testing and trade, case-specific thresholds are more likely to be required (Demeke et al. 2006) and may make more sense, especially if one is regulating on a trait basis and not on a process basis [e.g. Canada's plant with novel traits (PNT) based regulatory system].

In Canada PNTs receive either full regulatory approval (unconfined commercial release) or they are not approved for release at all. If there are PNTs offered for registration which pose significant environmental or human health risk if they are not confined then the only option is to not grant registration. If there are PNTs which industry (and/or the Canadian government) are keen to introduce, but which truly pose human health or environmental risks there is currently no possibility of commercial release. If a third commercial release category were created, a category for confined commercial release, then these types of compelling PNTs could be released under regulated confinement conditions designed to ensure human health and environmental safety. Of course to provide effective safety the regulations for this third regulatory category would have to be supported by legislation which would clearly assign responsibility and liability relating to transgene escape. The existence of such a 'third regulatory category' would require that Canada create and pass a coexistence law. In Canada this law could be trait based (and not processed based) and could be considered a coexistence law for PNTs.

Actions of the Canadian government on the issue of coexistence

The government of Canada promotes biotechnology as an opportunity for Canadian industry and Canadians. It has positioned itself as a "catalyst, reasoned advocate, interlocutor and facilitator in advancing Canada's plant and animal molecular farming sector" (Industry Canada 2004). This sentiment affects how the Canadian government approaches the regulation of GM crops and helps to explain why Canada adopts a non-regulatory approach to GM crop commercialization. The Canadian government supports the notion of substantial equivalence with regard to GM and non-GM crops. The government of Canada uses the term "adventitious presence" in the context of GM crops and defines it as "the unintended, technically unavoidable presence of genetically engineered material in an agri-food commodity" (CFIA 2005). Without a formal recognition that transgene escape can lead to contamination situations there will be little or no progress towards Canadian regulation for transgene confinement. The Royal Society of Canada released a formal report on the future of food biotechnology in Canada (Royal Society of Canada 2001) which called for more direct regulatory oversight for biotechnology

in agriculture and food products as well as a significant intensification of research into the potential effects of consumption of GM crops and the potential effects (primarily environmental) of transgene escape. The government of Canada has responded by developing several initiatives related to the introduction of GM crops. Environment Canada has created an interdepartmental committee which has been charged with developing a research strategy, the purpose of which is to generate knowledge on long term ecosystem effects of novel living organisms (NLOs) (including GM crops), in order to strengthen the sound scientific basis for policies, decisions and management of NLOs (Environment Canada 2005). The government has also created an industry consultation initiative called "responsible introduction of novel agricultural products" (RIONAP) (Industry Canada 2004) but no formal commitment has been made to act on recommendations that may come from the RIONAP activities. As of the summer of 2005 the government of Canada is still submitting progress reports on their actions related to the Royal Society report (Health Canada 2005). The most common governmental action has been consultation but no new regulations or laws have been created yet and there has been no dedicated allocation of governmental funding to achieve the research recommendations. There has been no governmental response to the Supreme Court ruling on Percy Schmeiser nor has there been any response to the issue of adventitious presence of transgenes in certified seedlots. The Canadian Seed Growers Association (CSGA), along with the Canadian Seed Trade Association (CSTA), has initiated a review of the seed production regulations, with specific consideration of genetic purity issues (CSGA 2005). The CSGA along with international seed industry argues in support of an update of seed and varietal purity notions within the context of GM crops but they also express strong concern over the ability of the seed industry to meet absolute genetic purity standards and the tremendous cost of meeting such standards (ISF 2004). The Canadian government has maintained a consistent non-regulatory approach to the commercialization of GM crops and has done nothing to suggest it will act differently in the future.

Conclusion and future directions

Transgene escape has been happening in Canada and it has caused some problems. The apparent position of Canada as a promoter of biotechnology means that there is little governmental will to produce concrete action and there are no signs that regulations or laws specific to issues related to transgene escape will be created any time soon in Canada. To date the experience in Canada has been with GM crops engineered to be herbicide–resistant, a novel trait that posed little human health or environmental risk. However, as pharmaceutical and industrial traits are introduced into crop plants Canadians will face greater risks from transgene (trait) escape and cases will occur where adventitious presence will in fact be dangerous contamination. At that time the political will may exist to formally address the issue

of transgene confinement and the Canadian government may perhaps then create a coexistence law for PNTs in Canada.

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Incorporating rapidly evolving scientific knowledge into risk assessment for plants with novel traits

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The Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA) operates under a strong, science based framework for the regulation of the environmental release of plants with novel traits (PNTs) in Canada. The current regulatory framework has been sufficiently flexible to address the regulation of the current range of PNTs in Canada through the on-going development of clear and transparent criteria for environmental release and commercial production. Although the CFIA's regulatory oversight has performed acceptably to this point, the maturing biotechnology industry and advances in technology and science will continue to test the regulatory framework. A number of new types of products, such as plants with increased abiotic stress tolerance and plants intended for plant molecular farming, will pose significant challenges for the biotech industry, government, the farm community and agri-business. The confined field trial program is one mechanism that enables the PBO to forecast where these future regulatory pressures will lie, and the PBO has used this forecasting mechanism to sponsor research with its Contract Research Fund (CRF) into areas where knowledge gaps exist and to expand its database of knowledge for decision making. The Government of Canada's Ecosystem Effects of Novel Living Organisms (EENLO) initiative is another opportunity for the PBO to effectively access a wide pool of knowledge. Above all, regulatory oversight needs to keep pace with these evolving developments while maintaining high standards for environmental, human and livestock health.

Introduction

The Plant Biosafety Office (PBO) of the Canadian Food Inspection Agency (CFIA) regulates the import and environmental release of plants with novel traits (PNTs). PNTs are plants containing a trait that is not present in plants of the same species already existing in Canada, or is present at a level outside the range of that trait in stable, cultivated populations of that plant species in Canada.

Canada has a product-based regulatory system for PNTs. It is the presence of a novel trait in a plant, irrespective of the method used to introduce it, which triggers regulation (under the *Seeds Act)*. The method of introduction of the trait can be genetic engineering, mutagenesis, cell fusion, somaclonal variation, conventional

breeding, or other methods. For example, herbicide-resistant canola has been produced by genetic engineering (glyphosate resistance), mutagenesis (imidazolinone resistance), and conventional breeding (triazine tolerance).

The concept of substantial equivalence is used to assess the relative risk of a PNT in comparison to its unmodified or non-novel counterpart. A PNT that is substantially equivalent, in terms of its specific use and safety for the environment, as well as for human and animal health, to plants currently cultivated in Canada, having regards to its potential changes in weediness/invasiveness, gene flow, plant pest properties, impacts on other organisms and impact on biodiversity, should pose no greater risk to the Canadian environment compared with its counterpart. These assessment criteria are described in the CFIA *Directive 94-08: Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* (http://www.inspection.gc.ca/english/plaveg/bio/dir/dir9408e.shtml). If the PNT is assessed to pose no greater environmental, food, or feed risk than its counterpart, it will be approved for unconfined environmental release. Any actual commercial release may require variety registration and/or approval by the Pest Management Regulatory Agency (PMRA), and the actual commercialization is a business decision by the applicant.

The applicant is responsible for providing the PBO with appropriate data and relevant scientific information describing the environmental risk of the PNT relative to its counterpart(s) already present in the Canadian environment. The PBO compares this information to information on the relevant biology of the counterpart organism as described in a Biology Document (available at http://www.inspection.gc.ca/english/plaveg/bio/dir/biodoce.shtml). Biology Documents contain baseline information on agronomics, breeding history and methods, reproductive biology, interactions with related species, endogenous toxins, major interactions with other life forms within the Canadian production range, and other relevant information. The PBO relies on current scientific knowledge to help create new Biology Documents and to update existing Biology Documents.

The challenge for the PBO as regulators is to respond effectively to increasing scientific activity and knowledge with regard to agricultural ecosystems and the interactions that take place in them.

The confined field trial program – a forecasting mechanism for future regulatory pressures

The confined field trial program provides an indication of new products in the pipeline and can provide a useful means to forecast future regulatory information requirements. From 1988 to 1994, the PBO's activities focused almost exclusively on this program. Confined field trials provide for the environmental release of a PNT for research purposes, under CFIA-developed and -imposed species-specific terms and conditions of confinement designed to minimize

environmental impact. These releases allow applicants to perform experiments with a PNT in the Canadian environment, whether for research purposes or to collect data for an application for unconfined environmental release. Field trials are inspected by the CFIA to ensure that the applicant is in compliance with the terms and conditions of confinement.

In 1995, the PBO began to receive and assess applications for the approval of unconfined release of PNTs. These are often for PNTs that have been tested in confined field trials in Canada. Since the beginning of the confined field trial program, the types of products that the PBO has been asked to review have evolved. In the early years, approximately 80 % of the trials were for herbicide-resistant plants and the remainder was mainly for modified composition (i.e. a different oil profile). However, this ratio has changed with time. Herbicide-resistant plants now account for approximately 40 % of the program, with the other major categories being stress tolerance (25 %), plant molecular farming (15 %), insect resistance (10 %), and modified composition (10 %). As well, these traits have been developed in a wider variety of crop species. In some instances, more than one trait is being "stacked" in a single PNT, which can raise regulatory concerns regarding the stewardship of these PNTs. The stacking of two different insect-resistance traits into one plant has to be considered in light of the most up-to-date scientific information available on insect resistance management.

Using the insights derived from the confined field trial program, the CFIA is able to identify where knowledge gaps exist and where future regulatory concerns may lie. Information is also collected from scientific conferences and literature searches. The PBO relies heavily on published, peer-reviewed research for developing terms and conditions for confined field trials and in considering applications for unconfined release.

Contract research fund

Over the years, the PBO has become engaged in the application of biotechnology to a variety of uses, such as forestry trees, fruit trees, ornamental plants, and plant molecular farming (the use of plants in agriculture to produce novel compounds rather than for the production of food, livestock feed, or fibre), (quite often, these PNTs are tested in the confined field trial program). Other PBO concerns have included developing testing and monitoring policies and procedures for the presence and effects of PNTs and for adventitious presence of unapproved PNTs. Monitoring has highlighted the importance of effective deployment of PNTs, and the PBO has responded by strongly recommending stewardship plans for the sustainable deployment of herbicide-resistant and insect-resistant PNTs. Information in addition to that provided by applicants in submissions for unconfined environmental release is therefore required by the PBO to address the challenge of regulatory knowledge gaps.

The Contract Research Fund (CRF) is a small CFIA-based fund used by the PBO to support independent research to address regulatory knowledge gaps that affect the current or future ability of the PBO to regulate PNTs. The information gathered by CRF-based research allows the PBO to make informed decisions about PNTs that are currently regulated or likely to be regulated in the future. CRF funding is generally allocated on a yearly basis, and researchers cannot access the CRF to study their own products, but they could be asked to study a regulatory need that pertains to their area of expertise. Alternatively, researchers with ideas for proposals are encouraged to contact the PBO for a discussion about and/or a submission of a research proposal at any time of the year.

The CRF has helped support several valuable studies that have addressed or are addressing regulatory knowledge gaps with regards to potential changes in weediness/invasiveness, gene flow, plant pest properties, impacts on other organisms and impact on biodiversity. Some examples of studies that have been partially or wholly supported by the CRF are: outcrossing and hybrid fitness in canola (*Brassica napus)* and *B. juncea*; commercial-scale pollen flow from wheat (*Triticum aestivum)* to different varieties in adjacent fields; emergence periodicity (timing) of volunteer wheat in Prairie cropping systems; factors contributing to gene movement between volunteer and cropped wheat populations; frequency and occurrence of herbicide-resistant wheat volunteers; modelling pollen flow in wheat; outcrossing between cultivated apples and wild relatives in Canada; potential for outcrossing between triticale and related cereals; bee-mediated gene flow between safflower (*Carthamus tinctorius*) plants; European Corn Borer (*Ostrinia nubilalis*) resistance to Bt proteins; and global changes in gene expression in *Arabidopsis* (Miki and El Ouakfaoui, 2005).

Incorporating rapidly evolving scientific knowledge – herbicide-resistant Sunflower (*Helianthus annuus* L.) as a case study

A recent example of the importance of incorporating the most current scientific knowledge into the PBO's review process was the application received by the PBO in 2003 for the unconfined release of an herbicide-resistant sunflower (*Helianthus annuus* L.). This sunflower is resistant to the imidazolinone herbicides, which kill susceptible plants by binding to the acetohydroxyacid synthase (AHAS) enzyme (which is critical in the production of the branched chain amino acids (valine, leucine, and isoleucine)). The imidazolinone resistance trait originated from a wild population of *Helianthus annuus* and was introduced into domestic germplasm by conventional breeding. The herbicide resistance trait is conferred by a single point mutation in the AHAS gene, such that the enzyme is no longer affected by imidazolinones. Different point mutations in the AHAS gene can have different cross-resistant patterns with respect to the other AHAS-inhibiting

herbicides (i.e. sulfonylureas, triazolopyrimidines, etc.) (Reviewed in Tranel and Wright, 2002).

Canada is a Centre of Origin of sunflowers with numerous native wild relatives. The PBO used the CRF to contract a paper entitled '*Biosafety implications of the introduction of imidazolinone-resistant sunflower (*Helianthus annuus *L.) in Canada'* (J.E. Dexter *et al., in preparation*). The paper described the wild relatives present in Canada, barriers/pathways to outcrossing and/or introgression, and implications of gene flow. The paper concluded that although gene flow is very likely to occur, it would flow to wild relatives and volunteers that were unlikely to be subjected to a strong imidazolinone selection pressure. Resistance to imidazolinone herbicides does not provide a fitness advantage in the absence of imidazolinone herbicides. Without the imidazolinone herbicide selection pressure to remove plants that are not imidazolinone-resistant, one would not expect to see an increase in the frequency of the imidazolinone resistance trait (or any other domestic traits linked to the imidazolinone resistance trait in the sunflower genome) in the wild population. In effect, the consequences of gene flow from imidazolinone-resistant sunflowers would be similar to the consequences of gene flow from conventional sunflowers.

In addition to the information supplied by the applicant and the paper commissioned under the CRF, the PBO performed literature searches to ensure that information on the herbicide resistance trait itself was as accurate and up-to-date as possible. The information gathered during the course of the assessment indicated that there was not enough evidence available to determine whether the single point mutation in the AHAS gene that confers imidazolinone resistance could also confer resistance to other AHAS-inhibiting herbicides (i.e. sulfonylureas). The stewardship plan was amended to include a provision indicating that acceptable control of volunteers or wild sunflowers may not be achieved with a sulfonylurea or other AHAS-inhibiting herbicide (CFIA 2005). This case study is an excellent example of both the value of scientific information to the PBO and the value of the CRF in facilitating the development of that information.

Ecosystem effects of novel living organisms

Another potential source of research funding for the PBO is the Ecosystem Effects of Novel Living Organisms (EENLO) initiative (please note that at the time of publication, the EENLO initiative has not received long-term financial support from the Government of Canada). The PBO anticipates that this interdepartmental science initiative (led by Environment Canada) will allow the PBO to access a wider pool of knowledge than can be accessed with the CRF, since EENLO supports more long-term projects.

The EENLO initiative was created in response to the findings of the Royal Society of Canada's Expert Panel (charged by the Government of Canada to

examine the Future of Food Biotechnology (2001)), in addition to the Biotechnology Ministers' advisory board (Canadian Biotechnology Advisory Committee, 2002). Both the panel and the board recognized a need for increased research investment into both the short- and long-term impacts of PNTs on the environment. The purpose of EENLO is to support the generation of knowledge, through an effective and integrated approach, on long-term ecosystem effects of novel living organisms in order to strengthen the sound scientific basis for policies on, decisions about, and management of novel living organisms (NLOs). Neither EENLO nor the CRF will have any direct connection to industry, and EENLO and/or CRF funding will not be used to promote or discredit specific products.

EENLO is composed of a central secretariat and seven nodes: Baseline Data; Detection and Monitoring; Ecosystem Impacts of NLO's; Gene Flow and its Consequences; Risk Assessment Method Development; Containment and Mitigation; and Stewardship of Approved Products. A Community of Practice (CoP) website is also a central part of the EENLO network. The CoP facilitates interactive discussion through a website where members can discuss relevant articles and issues. The CoP also contains a document repository with EENLO-related journal articles. For more information on the Community of Practice, please email eenlo-enove@ec.gc.ca.

The PBO acts as the champion of the Stewardship of Approved Products node of EENLO. This node held a workshop in Ottawa on February 21st, 2005 ('*Technical Workshop on the Stewardship of Herbicide Tolerant and Insect Resistant Crops: What does Canada need now?*'), that focussed on identifying additional regulatory and research needs and identified potential projects with partner linkages (both fiscal and human resources). The outcomes of the workshop were used in the request for funding to Treasury Board.

Summary

PNTs and the issues surrounding their use are becoming more complex. Regulatory oversight needs to keep pace with this increasing complexity while maintaining high standards for environmental, human, and livestock health. Decision makers must have access to and incorporate rapidly evolving scientific knowledge in order to meet these goals. Information from published literature and generated from the CRF are invaluable to PBO. Participation in EENLO will broaden the pool of solid knowledge that PBO can access.

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Index

The papers in this volume of *Topics in Canadian Weed Science* were presented at a symposium during the Canadian Weed Science Society Société canadienne de malherbologie (CWSS SCM) meeting held in Niagara Falls, Ontario in November 2005. The topic of herbicide-resistant crops was chosen as the symposium theme because 2005 marked the 10th anniversary since the commercial release of the first herbicide-resistant crop cultivars in Canada. This technology opened a new paradigm for weed control with several immediate agronomic advantages including high efficacy, broad-spectrum weed control with a single herbicide, and an extended herbicide application period. In many of these crops, herbicide-resistance is conferred by transgenes, making these cultivars also the first widely released genetically-modified organisms. This resulted in unprecedented interest and scrutiny of this technology from various sectors of society. As a result, additional questions have been associated with these cropping systems including environmental concerns, such as the fate of transgenes in the environment and effects of these cropping systems on non-target organisms, and concerns that focus on food safety, contamination, and the costs to non-adopters. The symposium hosted a number of experts from universities, government, and industry who presented the current status of the science, politics, and regulation of herbicide-resistant and genetically-modified crops in Canada. The papers presented in this symposium provide an excellent basis in these areas and serve as a guide outlining the opportunities and challenges inherent in modern cropping systems that go well beyond the discipline of weed science.

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