POST-RELEASE MONITORING OF GENETICALLY MODIFIED CANOLA (*BRASSICA NAPUS* L.) IN WESTERN CANADA:

ESCAPE, PERSISTENCE AND SPREAD OF NOVEL TRAITS

by

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To think… that our actions are without effect is surely one of the great deceptions of our culture

Norman Wirzba,
The Essential Agrarian Reader

ABSTRACT

Genetically modified (GM) canola (*Brassica napus* L.) has been widely adopted in Canada since its commercial release in 1995 and now represents over 85% of the canola grown in western Canada. Concurrently, GM canola volunteers have become an increasing management problem in cultivated fields and are ubiquitous in adjacent ruderal (non-cropped disturbed) habitats. However, systematic post-release monitoring is lacking and the ecological and agronomic impacts of escaped GM canola are poorly understood. In this dissertation, I characterize the escape, demography and distribution of GM canola in ruderal habitats in southern Manitoba, at multiple spatial and temporal scales. I characterized GM herbicide tolerance traits in 16 escaped canola populations. The progeny of 129 plants were tested in herbicide trials; 74% of plants produced glyphosate-tolerant progeny, 63% produced glufosinate-tolerant progeny, and 34% produced multiple herbicide-tolerant progeny as a result of gene flow between escaped plants. At the population-scale, four escaped GM canola populations were monitored and periodic matrix models were constructed to describe the dynamics and persistence of flowering plants. Escaped populations were observed to flower in synchrony with adjacent crops and were projected to persist for 2 to 5 years, confirming the potential for gene flow between escaped and cultivated canola populations. At the landscape-scale, the distribution of escaped canola was surveyed in three agricultural regions. Regional factors were important determinants of distribution; escaped canola density was positively correlated with canola cropping intensity and with traffic intensity, and was negatively correlated with distance to grain distribution centres. Local seed dispersal had negligible impact on distribution compared to landscape-scale anthropogenic seed inputs resulting from agricultural transport. These findings suggest that escaped canola persists as a metapopulation, where long-distance dispersal and colonization compensate for frequent extinction of local populations. Escaped populations play an important role in the persistence and spread of GM traits at large spatial scales, with substantial implications for the coexistence of GM and non-GM crops, and especially for organic and reduced-tillage farming operations. Landscape-scale management approaches, designed and implemented collaboratively by multiple stakeholders, are necessary to mitigate the risks of contamination resulting from GM trait escape. Regulation and ongoing monitoring of GM crops must acknowledge and address the dynamic regional nature of seed- and pollen-mediated gene flow.

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To my parents,
Ray and Deb,
who taught me that I can do anything

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CHAPTER 1

DISSERTATION INTRODUCTION

General Introduction: Herbicide-tolerant canola in western Canada

Recombinant DNA technology, variously referred to as genetic modification, genetic engineering or transgensis, enables the insertion of genetic material from organisms of interest into the genome of unrelated species. The transformation of crop plants using this new technology produces novel varieties that express marketable traits purported to benefit producers, consumers and/or the environment. Many such novel varieties are under development, while others are currently cultivated commercially. Among the first genetically modified (GM) crops to be developed, herbicide-tolerant (HT) canola (*Brassica napus* L.; refers to Argentine canola or spring oilseed rape) was approved for large-scale release in 1995 and has been cultivated in Canada since 1996 (Duke 2005). Over 99% of Canadian canola is grown in the Prairie Provinces of Manitoba, Saskatchewan and Alberta (Beckie *et al.* 2006). In this region, two transgenic canola types, resistant to the non-selective herbicides glyphosate or glufosinate, currently represent over 80% of the canola cultivated (Beckie *et al.* 2006). For both these transgenic types, herbicide tolerance is inherited as a dominant nuclear allele, and both homozygous dominant and hemizygous plants exhibit resistance (Hall *et al.* 2000). A third novel HT canola type was developed through induced chemical mutagenesis (i.e. non-transgenic), resulting in tolerance to selective imidazolinone herbicides expressed by an additive two gene complex (Tan *et al.* 2005). These mutagenic varieties represent approximately 10% of the Canadian canola crop (Beckie *et al.* 2006). The rapid adoption of HT canola varieties has resulted in the concurrent decline in the number of non-HT cultivars available to producers (Beckie *et al.* 2006). To date, no commercial varieties of canola have been released that express multiple tolerances to herbicides with differing modes of action.

Despite the rapid adoption and perceived operational benefits of HT canola, the cultivation of these new varieties remains controversial in Canada (Mauro & McLachlan 2008), in part due to the agronomic challenges associated with the crop itself. As a recently domesticated crop, canola retains a number of weedy characteristics (Hall *et al.* 2005), including phenotypic plasticity, high seed production, seed shattering, and the potential for secondary seed dormancy (Warwick & Stewart 2005; Gulden, Warwick & Thomas 2008). Over the last 30 years, weedy canola volunteers have increased in prevalence in western Canadian cropping systems (Leeson *et al.* 2005). Seed losses at harvest can reach over 50 times the average seeding rate (Gulden, Shirtliffe & Thomas 2003), and canola seeds can persist in arable fields for up to three years without seed bank replenishment (Harker *et al.* 2006). Clearly, where volunteers are able to set seed in subsequent crops, considerable populations may become established.

Though primarily self-fertilized, canola shows high potential for outcrossing as a result of both wind and insect pollination (Gulden, Warwick & Thomas 2008); outcrossing rates range from 6% to 81%, and average 20% for closely spaced plants (Cuthbert & McVetty 2001). Additionally, canola pollen is widely dispersed within agricultural landscapes where the crop is grown, with long distance pollination events observed from 3 km (Rieger *et al.* 2002) to 26 km (Ramsay, Thompson & Squire 2003) from pollen sources. Given the adoption of three unique HT canola types in western Canada, pollen-mediated gene flow has resulted in genetic contamination and trait-stacking in both canola volunteers (Hall *et al.* 2000) and cultivated crops (Beckie *et al.*

2003). Domestic canola may also hybridize with compatible wild relatives including weedy field mustard (*Brassica rapa* L.) and, to a lesser extent, wild radish (*Raphanus raphanistrum* L.) (Warwick *et al.* 2003). Stable introgression of HT traits from commercially-cultivated canola into weedy *B. rapa* has been reported in Quebec, resulting in the persistence of the HT transgene for six years in a wild non-crop population (Warwick *et al.* 2008).

Despite the agronomic challenges arising from inherent characteristics of the crop, stewardship plans for HT canola varieties were not provided when these novel crops were deregulated in Canada. Such plans have only been required from crop developers since 2004 (Beckie *et al.* 2006) and risk-reduction legislation is still lacking in Canada (Van Acker, Brule-Babel & Friesen 2004). In the absence of systemic means to manage the persistence and spread of GM canola varieties, producers in western Canada are advised to assume that as many as three adventitious herbicide tolerance traits may be present in their cropping systems (Hall *et al.* 2005) and generally have to adjust their herbicide regimes and agronomic practices accordingly. In this management context, farmers are increasingly concerned over the uncontrolled spread of GM HT canola, which they identify as originating from both within and outside of their farming operations (Mauro & McLachlan 2008). Such concerns point to the importance of considering both cropped and non-cropped landscape elements within the agroecosystem when developing strategies to mitigate seed- and pollen-mediated gene flow from novel canola varieties. Indeed, escaped populations of canola volunteers are ubiquitous in ruderal and wayside habitats throughout western Canada (Yoshimura, Beckie & Matsuo 2006; Gulden, Warwick & Thomas 2008), and researchers increasingly recognize that feral (self-sustaining) and escaped populations in non-crop habitats represent a “barrier” to the coexistence of GM and non-GM crops (Bagavathiannan & Van Acker 2009). Unfortunately, lack of consideration of the challenges posed by GM crop escape on the part of policy-makers and regulators underscores the limitations of the North American approach to post-release management and monitoring of GM crops. Despite assured reviews of the North American situation provided by some GM proponents (e.g. Sanvido, Romeis & Bigler 2007), our understanding of the full extent of long-term, multi-scale risks from commercial GM crop production remains uncertain at best.

Scope of the thesis

The Canadian regulatory process for novel crops is predicated on private, proprietary research, with a conspicuous absence of public research and input into decision-making. As such, crop developers and industry groups continue to promote the agronomic and operational benefits of GM varieties, including simplified herbicide management and improved weed control (CCC 2001). In contrast, farmers are increasingly concerned with risks, including management of adventitious HT traits, market harm, and legal liabilities (Mauro & McLachlan 2008; Mauro, McLachlan & Van Acker 2009). Unfortunately, this perspective remains largely unheard in the debate surrounding agricultural biotechnology.

The research presented in this thesis emerges from a multi-disciplinary public research program, initiated to identify and document the important contribution of local farmer knowledge in the comprehensive assessment of GM crops around the globe (Hwang 2008; Mauro 2008). In the Canadian context, this research is directed by and responsive to Prairie farmers' decade-long experience with GM crop production on a commercial scale (Mauro, McLachlan & Sanders 2005; Mauro 2008). Farmers' attitudes to the new technology were greatly influenced by their experiences with HT canola volunteers; indeed, this was the most important factor contributing to perceptions of risk associated with GM crop cultivation (Mauro & McLachlan 2008). Almost half of farmers surveyed in Manitoba reported HT volunteers on their farm and for many these volunteers compromised their operations and their livelihoods. Additionally, the majority of farmers surveyed felt that it was not possible to prevent the spread of HT traits and subsequent crop contamination (Mauro & McLachlan 2008). Indeed, farmers indicated that HT volunteers often originated from outside of their farming operations, leaving them with little ability to effectively manage the problem:

> *These volunteers are showing up in fields that have never been planted to these crops. Farmers that have never seeded genetically modified crops are finding volunteers on their farm and that the volunteer picture is much broader than we had expected to see.* (Manitoba farmer, interview, cited in Mauro & McLachlan 2008).

Despite the experiences reported by farmers, existing GM crop management plans focus on field- or farm-scale practices and ignore the larger landscape context. There exists a considerable gap in our understanding of and our ability to manage the spread of HT traits between different human-mediated agricultural production systems. This is compounded by a lack of formal post-release monitoring of GM crops in Canada, particularly at spatial and temporal scales beyond experimental field trials (Clark 2006; Beckie *et al.* 2010).

In response to the concerns raised by farmers, and given the important but understudied role of HT canola volunteers in the Canadian context, an ecological and agronomic research program was initiated with a particular focus on canola volunteers occurring outside of cultivated fields (i.e. escaped plants). Little is known of the population dynamics of these plants, or of the extent to which escaped populations contribute to genetic contamination and trait-stacking. There is a need to characterize the presence, demography and dispersal of both escaped plants and escaped transgenes in agricultural landscapes where GM varieties have been widely cultivated over the long-term. In this thesis, ecological concepts, models and methodologies are integrated with the discipline of weed science to aid in addressing these issues (Ervin *et al.* 2003). As such, this research acknowledges the limitations of pre-release risk assessments that arbitrarily segregate the potential risks of GM crop cultivation, ignoring the interactions between environmental and agronomic effects (Levidow & Carr 2000). Thus, despite the high levels of farmer concern for agronomic impacts, these have not been adequately addressed in North America.

Thesis objectives

The overall aim of this research is to characterize escaped GM HT canola populations in agricultural landscapes in western Canada at multiple spatial and temporal scales, and to consider escaped canola demography and dispersal in light of relevant ecological theory and methodology.

Specific objectives relating to the chapters that follow include:

I. To document the presence of single and multiple HT traits and assess the extent of gene flow within escaped canola populations.

- **i.** To characterize the frequency of glyphosate, glufosinate, and imidazolinone tolerance in escaped canola populations in roadside habitats in southern Manitoba over multiple years;
- **ii.** To determine whether intraspecific gene flow contributes to the spread and stacking of HT traits within these escaped populations.

II. To better understand the demography and phenology of escaped canola and any implications for population persistence, particularly in relation to gene flow from GM crops.

- **i.** To characterize spatial and temporal variation in escaped population demography in agricultural landscapes;
- **ii.** To examine the relative importance of different life stages to population persistence;
- **iii.** To explore the underlying role of disturbance and agricultural activities in affecting population dynamics.

III. To review the current literature on weed patch dynamics and distribution at multiple spatial scales and to consider the relevance of metapopulation theory in addressing questions of weed patch persistence and spread, with particular attention to the containment of novel GM traits.

- **i.** To review approaches commonly used in the study of weed patches and consider how these have informed emerging management recommendations;
- **ii.** To consider the utility of the metapopulation concept for improved understanding of weed patch persistence and spread in human-dominated agricultural landscapes;
- **iii.** To examine the implications of regional metapopulation structure for the persistence of escaped populations of GM crop plants, and describe the contributions of these populations to gene flow at the landscape scale.

IV. To examine the long-term dynamics of escaped canola at large spatial scales.

- **i.** To characterize landscape-scale spatial and temporal variations of canola distribution in roadside and field-edge habitats in southern Manitoba;
- **ii.** To contrast the relative importance of landscape and local-scale factors in determining the distribution of escaped canola;
- **iii.** To assess the potential for natural seed dispersal between escaped populations of canola in roadside and field-edge habitats.

Structure of the thesis

Each chapter of this thesis is structured as a self-contained, publishable manuscript. The chapters are arranged to examine the ecology of escaped canola plants at increasing levels of organization, from the genetic to the landscape scale. Taken together, these individual papers present the results of an integrated multi-scale research program, examining the ecological and agronomic impacts of escaped GM canola in Manitoba, Canada. I begin with a comprehensive review of the literature, describing the role of biotechnology in agriculture and the current state of risk assessment, regulation and post-release monitoring (Chapter Two). Subsequently, I characterize the presence of three HT canola types in roadside habitats and present evidence of pollen-mediated gene flow within these escaped populations, resulting in transgenic trait-stacking (Chapter Three; Knispel *et al.* 2008). At the population scale, I describe the demography and phenology of escaped canola, assessing the role of local processes in population persistence (Chapter Four). I then present a critical evaluation of the impacts of changing spatial scales on the outcomes of weed studies, and explore the utility of metapopulation theory and landscape ecology to our understanding of GM crops at large spatial scales (Chapter Five). With reference to these ecological disciplines, I present the results of a landscape-scale assessment of the persistence and spread of escaped canola (Chapter Six; Knispel & McLachlan 2010). Finally, I outline the conclusions and recommendations arising from this work, providing insight into fruitful future directions for GM risk assessment, regulation and management within human-mediated agroecosystems (Chapter Seven).

References

Bagavathiannan, M.V. & Van Acker, R.C. (2009) The biology and ecology of feral alfalfa (*Medicago sativa* L.) and its implications for novel trait confinement in North America. *Critical Reviews in Plant Science*, **28**, 69-87.

Beckie, H.J., Hall, L.M., Simard, M.-J., Leeson, J.Y. & Willenborg, C.J. (2010) A framework for postrelease environmental monitoring of second-generation cropswith novel traits. *Crop Science*, **50**, in press.

Beckie, H.J., Harker, K.N., Hall, L.M., Warwick, S.I., Légère, A., Sikkema, P.H., Clayton, G.W., Thomas, A.G., Leeson, J.Y., Séguin-Swartz, G. & Simard, M.J. (2006) A decade of herbicide resistant crops in Canada. *Canadian Journal of Plant Science*, **86**, 1243-1264.

Beckie, H.J., Warwick, S.I., Nair, H. & Séguin-Swartz, G. (2003) Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). *Ecological Applications*, **13**, 1276-1294.

CCC (Canola Council of Canada) (2001) *An Agronomic and Economic Assessment of Transgenic Canola*. Prepared by Serecon Consulting and Koch Paul Associates. Available at http://www.canola-council.org/report_gmo.html

Clark, E.A. (2006) Environmental risks of genetic engineering. *Euphytica*, **148**, 47-60.

Cuthbert, J.L. & McVetty, P.B.E. (2001) Plot-to-plot, row-to-row, and plant-to-plant outcrossing studies in oilseed rape. *Canadian Journal of Plant Science*, **81**, 657-664.

Duke, S.O. (2005) Taking stock of herbicide-resistant crops ten years after introduction. *Pest Management Science*, **61**, 211-218.

Ervin, D.E., Welsh, R., Batie, S.S. & Line Carpentier, C. (2003) Towards an ecological systems approach in public research for environmental regulation of transgenic crops. *Agriculture, Ecosystems and Environment*, **99**, 1-14.

Gulden, R.H., Shirtliffe, S.J. & Thomas, A.G. (2003) Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. *Weed Science*, **51**, 83-86.

Gulden, R.H., Warwick, S.I. & Thomas, A.G. (2008) The Biology of Canadian Weeds. 137. *Brassica napus* L. and *Brassica rapa* L. *Canadian Journal of Plant Science*, **88**, 951-996.

Hall, L.M., Rahman, M.H., Gulden, R.H. & Thomas, A.G. (2005) Volunteer oilseed rape – will herbicide-resistance traits assist ferality? *Crop Ferality and Volunteerism* (ed J. Gressel), pp. 59-79. Taylor & Francis Group, Boca Raton, Florida, USA.

Hall, L., Topinka, K., Huffman, J., Davis, L. & Good, A. (2000) Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Science*, **48**, 688-694.

Harker, K.N., Clayton, G.W., Blackshaw, R.E., O'Donovan, J.T., Johnson, E.N., Gan, Y., Holm, F.A., Sapsford, K.L., Irvine, R.B. & Van Acker, R.C. (2006) Persistence of glyphosate-resistant canola in western Canadian cropping systems. *Agronomy Journal*, **98**, 107-119.

Hwang, S. (2008) *Farmer and Consumer Attitudes toward GM Foods in South Korea*. MEnv Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Knispel, A.L. & McLachlan, S.M. (2010) Landscape-scale distribution and persistence of genetically modified oilseed rape (*Brassica napus*) in Manitoba, Canada. *Environmental Science and Pollution Research*, **17**, 13-25.

Knispel, A.L., McLachlan, S.M., Van Acker, R.C. & Friesen, L.F. (2008) Gene flow and multiple herbicide resistance in escaped canola populations. *Weed Science*, **56**, 72-80.

Leeson, J.Y., Thomas, A.G., Hall, L.M., Brenzil, C.A., Andrews, T., Brown, K.R & Van Acker, R.C. (2005) *Prairie Weeds Survey: Cereal, Oilseed and Pulse Crops 1970s to 2000s.* Weed Survey Series Publication 05-1. Agriculture and Agri-Food Canada, Saskatoon, SK.

Levidow, L. & Carr, S. (2000) Unsound science? Transatlantic regulatory disputes over GM crops. *International Journal of Biotechnology*, **2**, 257-273.

Mauro, I.J. (2008) *Riding the Risk Wave: Farmer Knowledge and Experience with GM Crops in the Canadian Prairies.* PhD Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Mauro, I.J. & McLachlan, S.M. (2008) Farmer knowledge and risk analysis: Postrelease evaluation of herbicide-tolerant canola in western Canada. *Risk Analysis*, **28**, 463-476.

Mauro, I.J., McLachlan, S.M. & Sanders, J. (2005) *Seeds of Change: Farmers, Biotechnology and the New Face of Agriculture.* Dead Crow Productions and Dada World Data, Winnipeg, Manitoba, Canada. Documentary film available at: www.seedsofchangefilm.org

Mauro, I.J., McLachlan, S.M. & Van Acker, R.C. (2009) Farmer knowledge and a priori risk analysis: A pre-release evaluation of genetically modified Roundup Ready wheat across the Canadian prairies. *Environmental Science and Pollution Research*, **16**, 689-701.

Ramsay, G., Thompson, C. & Squire, G. (2003) *Quantifying landscape-scale gene flow in oilseed rape.* Department of Environment, Food and Rural Affairs, London, UK.

Rieger, M.A., Lamond, M., Preston, C., Powles, S.B. & Roush, R.T. (2002) Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science*, **296**, 2386-2388.

Sanvido, O., Romeis, J. & Bigler, F. (2007) Ecological impacts of genetically modified crops: Ten years of field research and commercial cultivation. *Advances in Biochemical Engineering/Biotechnology*, **107**, 235-278.

Tan, S., Evans, R.R., Dahmer, M.L., Singh, B. K. & Shaner, D. L. (2005) Imidazolinone-tolerant crops: history, current status and future. *Pest Management Science*, **61**, 246-257.

Van Acker, R.C., Brule-Babel, A.L. & Friesen, L.F. (2004) Intraspecific gene movement can create environmental risk: the example of Roundup Ready wheat in western Canada. *Schriftenreihe für Landschaftspflege und Naturschutz,* **79**, 35-48.

Warwick, S.I., Légère, A., Simard, M.-J. & James, T. (2008) Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population. *Molecular Ecology*, **17**, 1387-1395.

Warwick, S.I., Simard, M.-J., Légère, A., Beckie, H.J., Braun, L., Zhu, B., Mason, P., Séguin-Swartz, G. & Stewart, C.N. (2003) Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O.E. Schulz. *Theoretical and Applied Genetics*, **107**, 528-539.

Warwick, S.I. & Stewart, C.N. (2005) Crops come from wild plants – how domestication, transgenes, and linkage together shape ferality. *Crop Ferality and Volunteerism* (ed J. Gressel), pp. 9-30. Taylor & Francis Group, Boca Raton, Florida, USA.

Yoshimura, Y., Beckie, H.J. & Matsuo, K. (2006) Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. *Environmental Biosafety Research*, **5**, 67-75.

CHAPTER 2

LITERATURE REVIEW

AGRICULTURAL BIOTECHNOLOGY: RISK, REGULATION AND THE CHALLENGES OF FERALITY

Agriculture and biotechnology

In 1996, the first novel agricultural crops derived from recombinant DNA technology were released commercially in the United States (USA), China, Canada, Argentina, Australia and Mexico (Nap *et al*. 2003). This first generation of genetically modified (GM) crops consisted of varieties with enhanced ‘input-traits’, designed to provide agronomic benefits to farmers by reducing the need for additional farm inputs and/or increasing efficiency and ease of management (Castle, Wu & McElroy 2006). Predominant among first generation GM crops were herbicide-tolerant (HT) varieties of canola (*Brassica napus* L.), soybean (*Glycine max* (L.) Merr.), cotton (*Gossypium hirsutum* L.) and corn (*Zea mays* L.). Insect resistant (IR) cotton and corn, expressing insecticidal genes from *Bacillus thuringiensis* (*Bt*), were also widely commercialized (Castle, Wu & McElroy 2006).

Proponents of GM crops claimed agronomic, economic and environmental benefits associated with the new varieties, which were expected to improve weed and pest management, reduce chemical herbicide and insecticide usage, and thereby reduce farm input costs (Castle, Wu & McElroy 2006; Schwember 2008). Proponents have also argued that the use of HT crops has facilitated the adoption of reduced-tillage production methods among farmers, by providing effective in-crop chemical weed control (Duke 2005; Sanvido, Romeis & Bigler 2007). However, new risks associated with the release of GM crops were also expected. Some researchers expressed concerns regarding the evolution of weed and insect resistance to chemical controls, as well as the potential for gene flow from GM crops to wild and weedy relatives, exacerbating weed problems (Schwember 2008).

Despite these potential risks, adoption of GM crops was rapid and widespread in the decade following their commercial introduction. Indeed, GM technology is argued to be among the most rapidly adopted agricultural technologies in recent years (James 2009). Currently, four major GM crops are grown globally: soybean, cotton, corn and canola (James 2009). Additional approved crops include sugar beet (*Beta vulgaris* L.), squash (*Cucurbita* spp.) and papaya (*Carica papaya* L.) (James 2009). Combined, GM crops were cultivated on 134 million ha in 2009, with first generation input-trait varieties, particularly HT crops, remaining predominant (Castle, Wu & McElroy 2006; James 2009). Commercial cultivation of GM crops is widespread in North America, where the USA grows 64 million ha of these novel varieties, and Canada 8.2 million ha. Adoption of GM varieties is also increasing rapidly in South America (Brazil: 21.4 million ha; Argentina: 21.3 million ha) and in Asia (India: 8.4 million ha; China: 3.7 million ha) (James 2009). However, adoption of GM crops remains low in the European Union, where only six of 27 countries planted commercial GM corn in 2009, with the majority of the crop grown in Spain (James 2009).

Second-generation GM varieties will soon be introduced, and consist of crops with stacked input-traits, expressing multiple HT or IR transgenes with differing modes of action, and/or expressing both HT and IR traits (Castle, Wu & McElroy 2006). New traits for viral and fungal resistance are being developed, as are competitive traits such as drought tolerance and increased nitrogen efficiency (Schwember 2008). Third-generation GM crops with ‘output-traits’ designed to benefit the consumer are the focus of increasing research interest. These include nutraceutical crop varieties with enhanced nutrition; most famously, Golden Rice (*Oryza sativa* L.) varieties engineered to express carotenoids in the grain are promoted as a means to combat vitamin A deficiency in children (Potrykus 2001). Additionally, regulatory approvals are currently being sought for confined field trials of new GM crops designed to produce pharmaceutical and/or industrial compounds in their tissues (Smyth, Khachatourians & Phillips 2002). However, despite continued increases in the number of regulatory approvals for experimental GM field trials in the USA, approval rates remain low in Europe (Nap *et al.* 2003).

Importantly, any environmental and agronomic benefits of agricultural biotechnology remain contested for both current and future crops. While proponents have claimed environmental benefits resulting from reduction in the application of commercial herbicides and insecticides, opponents argue that rates of chemical usage have actually increased (Benbrook 2003). Additionally, predicted risks arising from the release of HT crops have been realized more rapidly than anticipated. Weed resistance to herbicides associated with GM crops, particularly glyphosate (Round Up), is being observed around the globe (Service 2007; Heap 2010), and the spread of HT traits into weed populations has also been documented (Warwick *et al.* 2008). Development and spread of weed resistance, either by evolution through selection or by introgression, raises agronomic concerns for the future utility of common chemical herbicides (Duke & Powles 2009) and has important implications for natural ecosystems. However, after over a decade of cultivation, the impacts of GM crop cultivation on both natural and anthropogenic systems remain difficult to anticipate and measure.

Risk assessment for environmental release

Environmental risk assessment is required to receive regulatory approval for the release of new GM varieties in confined field trials or for unconfined environmental release. Risk assessment aims to provide a structured, repeatable framework enabling the quantification of both the probability and the consequences of a negative event (Conner, Glare & Nap 2003; Pirondini & Marmiroli 2008). New GM varieties are assessed on a case-by-case basis, examining risks associated with each crop and transgene (Andow & Zwahlen 2006). However, formal risk assessment for the environmental release of GM crops is limited to consideration of pre-defined ecological risks (Clark 2006), while new and emergent impacts and long-term, cumulative effects are difficult to predict and characterize prior to release. Currently, recognized environmental concerns associated with GM HT and IR crops include negative effects on non-target organisms and biodiversity, development of resistance in target organisms, invasiveness or enhanced weediness, and gene flow to wild relatives (Sanvido, Romeis & Bigler 2007; Pirondini & Marmiroli 2008). To aid in describing, quantifying, and managing these potential risks, GM crop developers provide information to regulators on parental organisms used in the transformation, GM construct and insertions, characteristics of release sites, spatial and temporal extent of planned releases, and factors which may contribute to recognized environmental impacts (Nap *et al.* 2003).

Assessment of the effects of GM crops on non-target organisms has focused largely on the impacts of IR varieties on non-pest insects, which may consume IR plant material either directly or indirectly by ingesting prey insects that have consumed IR plant material. Of particular concern are potential increases in mortality among beneficial insects (insect predators and pollinators) and among species of conservation concern (Conner, Glare & Nap 2003; Sanvido, Romeis & Bigler 2007; Pirondini & Marmiroli 2008). Increasingly, potential non-target effects of HT varieties are also recognized: while HT crops are designed to provide herbicidal control of unwanted weeds, there is concern regarding the effectiveness of weed control in HT crops. At the field scale, HT crop management has been associated with decreases in weed density and shifts in weed communities, with potential implications for insect and wildlife populations and for biodiversity in agricultural landscapes (Heard *et al*. 2005).

Another important concern associated with the widespread use of both IR and HT GM varieties is the development of resistance within target populations. Increased selection pressure for *Bt* resistance among insect populations associated with IR crops has resulted in the widespread implementation of mandatory resistance management practices (Andow & Zwahlen 2006). However, no formal resistance management strategies are in place to prevent the development of herbicide resistant weeds in HT crops (Andow & Zwahlen 2006), though increasing numbers of resistant weed biotypes are expected as HT crop cultivation becomes even more widespread and farmers become reliant on a smaller number of herbicides (Cerdeira & Duke 2006; Service 2007).

While domestication is generally believed to result in the loss of weedy characteristics among crop plants, researchers expressed early concern that new GM varieties might become invasive in natural habitats if transgenic traits conferred a selective advantage (Conner, Glare, & Nap 2003). Studies assessing the population growth rates of HT crops in natural habitats show little evidence of invasiveness (Crawley *et al.* 1993; 2001), though concerns remain for future GM crops with ecologically adaptive traits (Andow & Zwahlen 2006). More recently, researchers have examined the potential for GM crops to become weeds in agricultural fields. Unwanted HT varieties may be disseminated in agricultural fields through seeding of contaminated seed (Friesen, Nelson & Van Acker 2003) and by seed lost at harvest (Gulden, Shirtliffe & Thomas 2003), creating a need for additional herbicides to achieve effective weed control.

Contrary to early concerns about the invasiveness of GM crops, it was widely believed that gene flow from these new varieties would have negligible impact, presenting little environmental risk (Andow & Zwahlen 2006). Yet reports of hybridization between transgenic crops and wild relatives are becoming increasingly common (Quist & Chapela 2001; Wilkinson *et al*. 2003; Warwick *et al.* 2003; Warwick *et al*. 2008). Crop-to-wild gene flow may exacerbate existing weed problems and may also have important implications for biodiversity, if small rare plant populations become 'swamped' with novel transgenes (Andow & Zwahlen 2006; Sanvido, Romeis & Bigler 2007). Researchers have also begun to consider the risks associated with crop-to-crop gene flow (Ellstrand 2001) and the potential for the transgenic contamination of non-GM crops (Reiger *et al.* 2002; Van Acker, Brule-Babel & Friesen 2004; Demeke, Perry & Scowcroft 2006), though some argue that this is not explicitly an environmental concern and is thus beyond the scope of environmental risk assessment (Sanvido, Romeis & Bigler 2007).

In formal assessments, environmental risks associated with GM crops are generally examined relative to the risks associated with 'conventional' crops: parental non-GM varieties grown in input-intensive monocultures (Conner, Glare & Nap 2003; Sanvido, Romeis & Bigler 2007). This comparative regulatory approach is not without controversy: some proponents of GM crops feel that this creates an undue regulatory burden for the developers of GM technology, compared to traditional plant breeders (Schwember 2008). Conversely, others have questioned the validity of using crops grown under chemically-intensive industrial farming systems as a baseline for assessing GM crops (Levidow & Carr 2000), particularly in light of increasing public interest in, and market share for, organic and natural production (Ervin *et al*. 2003). However, conventional agriculture remains the accepted baseline for risk assessment, and comparative approaches inform GM crop regulation in many countries.

Regulation and controversy

Despite similarities in the information required from applicants and an emphasis on ostensibly ‘objective’ scientific information as the basis for risk assessment, differences exist between the regulatory frameworks developed for GM crops around the globe (Levidow & Carr 2000). In particular, the principles guiding regulatory decisions vary greatly between North American and European countries. These differences create market challenges and are largely responsible for disparity in the extent of GM cultivation between the two continents (Pirondini & Marmiroli 2008).

North American countries have adopted a regulatory approach based on the concepts of substantial equivalence and familiarity in assessing potential health-related risks and environmental risks. When GM varieties can be deemed substantially equivalent to familiar conventional crop plants, they are believed to represent little additional risk and may be approved for unconfined environmental release (Levidow, Murphy & Carr 2007). The concept of substantial equivalence is based on the assumption that genetic modification is a precise method with anticipated single-gene effects that can be easily quantified (Ervin *et al.* 2003). As such, the regulation of GM plants in Canada is ‘product-based’ (Nap *et al.* 2003), and falls under previously-existing regulatory frameworks for new crop varieties. Canadian regulators are thus tasked with a mandate to assess all plants with novel traits, regardless of the process of development. This regulatory approach reflects international guidelines proposed in the early 1990s by the Organization for Economic Cooperation and Development (OECD), and endorsed by the United Nations Food and Agriculture Organization and the World Health Organization (Millstone, Brunner & Mayer 1999; Levidow, Murphy & Carr 2007). These early regulatory guidelines were aimed at global harmonization, to facilitate widespread GM crop adoption and market acceptance.

Initially, to facilitate regulatory harmonization, the European Union (EU) also accepted and adopted the concept of substantial equivalence, as put forth by the OECD, as a suitable framework for GM safety regulation (Levidow, Murphy & Carr 2007). However, this approach was met with widespread consumer protest, fueled by public distrust of the regulatory process and governments as a whole (Deblonde & Du Jardin 2005). Critics denounced the “frontier research” backing GM safety claims (Levidow, Murphy & Carr 2007) and substantial equivalence was deemed a “pseudo-scientific concept” used to rationalize economic and political decisions (Millstone, Brunner & Mayer 1999). These concerns prompted the establishment in 1999 of a *de facto* moratorium on new GM crop approvals in the EU, until a revised regulatory system could be put in place (L’heureux & Menrad 2004). Subsequently, EU legislation for risk assessment of GM crops was adapted with the goal of increasing consumer confidence and ensuring consumer choice. The concept of substantial equivalence was replaced in EU regulatory guidelines by an explicitly precautionary approach, which has guided environmental risk assessment of GM crops in the EU since 2002 (Rogers 2004; Levidow, Murphy & Carr 2007). As defined in EU regulations, the precautionary principle stipulates that scientific uncertainty regarding the environmental or health risks of GM crops does not preclude preventative action; appropriate measures should be taken to avert risk even if conclusive scientific evidence regarding the risk is lacking (Levidow & Carr 2000; Rogers 2004). The precautionary principle represents an explicit recognition that society's technological developments outstrip our scientific understanding of any attendant effects (DeBlonde & Du Jardin 2005). Thus, EU regulations of GM crops are 'process-based' (Nap *et al.* 2003), and entirely new regulatory systems were developed, which assess not only the risk associated with the GM product, but also risks that may arise as a result of the process of genetic modification itself.

Currently, European regulators are facing increasing pressure to reduce regulatory oversight of GM crops. This pressure comes from USA and Canadian trade sectors, which are again seeking to harmonize transatlantic GM regulation in order to liberalize trade (Deblonde & Du Jardin 2005; Levidow, Murphy & Carr 2007). Proponents of GM technology argue that the EU precautionary regulation and associated delays in GM crop approvals have been introduced to disguise anti-GM political decisions that lack scientific basis (e.g. De Greef 2004). In order to support their own calls for deregulation, proponents cite the ten-year history of GM crop cultivation as scientific evidence of its safety (Drobník 2008; Sanvido, Romeis & Bigler 2007). However, such arguments ignore the social, economic and ethical assumptions inherent in and normalized by all regulatory systems. Thus, despite rhetorical claims of ‘sound science’, both the European precautionary principle and the North American concept of substantial equivalence are inherently value-based and politically charged (Levidow & Carr 2000), often reflecting the perspectives of industry and government stakeholders. Futhermore, GM safety claims based solely on the length of time for which a GM variety has been in cultivation cannot be scientifically supported without appropriate data from systematic post-release monitoring studies (CBCGEO 2004, Beckie *et al.* 2010). Such data are, in most cases, conspicuously absent.

Post-release monitoring and management

In North America, there has been no mandatory post-release monitoring of the impacts of GM crops (Sanvido *et al.* 2005), though arguably such an approach is needed to verify and refine regulatory decisions based on pre-release assessments (Clark 2006). Only recently have small steps been taken to promote appropriate post-release management of GM crops; since 2004, Canadian regulators have required GM crop developers to submit farm-based stewardship plans as part of the regulatory process (Beckie *et al.* 2006). These stewardship plans, which are voluntarily implemented, are aimed at reducing adverse impacts for both GM and non-GM farmers. More specifically, requisite stewardship plans and other extension documents outline farm-scale management practices designed to control and contain GM volunteers in order to prevent contamination of adjacent crops (Beckie *et al.* 2006).

Despite these efforts, the presence of unwanted GM traits is currently “inevitable” and “unavoidable” in western Canada (Demeke, Perry & Scowcroft 2006), where seed production, commercial cultivation, and supply chains have not been adequately segregated and attempts at containing GM traits have not been systematic. This has resulted in considerable market harm for Canadian producers, coupled with reduced choice in production systems. Notably, uncontained GM traits have contaminated certified seedlots (Friesen, Nelson & Van Acker 2003), threatened non-GM mustard production (Demeke, Perry & Scowcroft 2006), resulted in the loss of organic canola production in western Canada (Smyth, Khachatourians & Phillips 2002; Mauro, McLachlan & Sanders 2005), and present a future threat to non-GM wheat production and marketing in Canada’s breadbasket (Van Acker, Brule-Babel & Friesen 2004). Left to manage these risks at the farm-scale, Canadian farmers fear that the full “burden of responsibility” for GM trait confinement has been unfairly shifted to producers (Mauro & McLachlan 2008), while systemic monitoring of potential environmental and agronomic impacts by GM crop developers and regulators is still lacking.

In the EU, the revised GM regulations implemented in 2002 mandated ongoing monitoring of GM crops after their approval for market or environmental release (Rogers 2004). Additionally, approvals are granted for a limited time, pending the evaluation of future monitoring data and any necessary mitigation actions (Rogers 2004; Sanvido *et al.* 2005). Conceptually, the framework for monitoring includes two components: ‘case-specific monitoring’, which focuses as required on potential risks identified in pre-market assessments; and mandatory ‘general surveillance’, which characterizes unanticipated environmental changes associated with commercial cultivation (Sanvido *et al.* 2005).

However, no standardized GM monitoring protocols are currently in place for commercially-approved GM crops in the EU (Züghart *et al.* 2008). As such, GM crop developers who have been granted approval for the release of new GM varieties are solely responsible for the design and implementation of monitoring programs that comply with the directives established by the EU (Sanvido *et al.* 2005; Züghart *et al.* 2008).

In the EU, post-release management of GM crops was implemented with an eye to the challenges resulting from unsegregated production systems in North America. In particular, concerns arose regarding the inadvertent mixing of GM and non-GM seed, in both imported commodities and in crops cultivated in Europe. The European Commission adopted the term 'adventitious' to describe the unwanted and unavoidable presence of GM material in non-GM crops (Levidow & Boschert 2008). While a zero-tolerance approach to adventitious presence of EU approved GM events was not considered feasible, the EU argued that effective agricultural management would limit the presence of adventitious GM material, and a 0.9% labeling threshold was established for non-GM agricultural products containing approved GM material (Leivdow and Boschert 2008). Effectively, however, the EU exercises a zero-tolerance approach to GM events that have not been approved for cultivation in Europe.

To manage the cultivation of GM crops in Europe and reduce the spread of GM traits and consequent genetic contamination, the EU introduced a coexistence policy, aiming to ensure the economically-viable production of both GM and non-GM crops. Drawing from the communist slogan of 'peaceful coexistence', this policy was designed to guarantee farmers' choice of cropping system, with the aim of enabling GM, non-GM and organic producers to coexist in the agricultural landscape (Levidow & Boschert

2008). European Union directives stipulate that coexistence measures must be economically proportionate, both to potential benefits gained by GM producers and to potential losses resulting from contamination (Devos *et al.* 2009). As such, management practices are effectively limited to the spatial isolation of GM and non-GM production fields (Devos *et al.* 2009). However, such measures become infeasible as the proportion of GM crops increases in agricultural landscapes, as in North America (Beckie *et al.* 2006). Additionally, co-existence policy does not explicitly address the management of GM plants occurring outside of cultivated fields, despite growing recognition of the potential impacts of such crop escapes.

Crop escape and ferality

In the global context of GM crop regulation and post-release management, there is increasing interest in the processes by which GM traits escape cultivation and the emergent risks arising from such escape. Despite the relevance of such processes for questions of adventitious GM presence, the multi-faceted nature of GM trait escape has yet to be fully characterized, particularly in North America where GM varieties have been widely cultivated for over a decade.

Crop plants growing in non-crop habitats, as distinguished from volunteers in arable fields, have been variously described and defined in both agronomic and ecological contexts. Feral plants are defined by Gressel (2005) as derived fully or in part from crop plants that have lost some or all of their domestic traits. Such a view posits the evolution of genetic differences between feral and cultivated forms of a given crop, largely believed to result from the loss of recessive domestic traits and the expression of dominant wild-type alleles (Warwick & Stewart 2005). In contrast, Bagavathiannan and Van Acker (2008) suggest that even undifferentiated, domesticated crop plants may become feral, if such plants are able to establish self-perpetuating populations in non-crop habitats or landscapes. Indeed, as the concept of ferality is commonly applied in the scientific literature, it generally describes crop plants that are able to reproduce successfully in non-crop habitats and are not necessarily genetically distinct from their cultivated conspecifics.

Regardless of the processes by which crop plants become feral, ferality greatly complicates the confinement of novel transgenes (Bagavathiannan & Van Acker 2008), and the risks of transgene spread and persistence need to be assessed with respect to both the nature of the GM trait and the crop in question. Ecologically-adaptive traits, including viral and fungal resistance, increased nitrogen use efficiency, and drought and salinity tolerance, are not dependent on anthropogenic inputs for their beneficial effects. Such traits are expected to increase plant performance in non-crop habitats and may have strong implications for ferality (Bagavathiannan & Van Acker 2008), meriting increased prudence in both pre-release assessment and post-release monitoring (Clark 2006; Beckie *et al.* 2010). Beyond the nature of the novel trait, innate characteristics of the crop itself may also increase the potential for ferality. As such, these risks are particularly pronounced for grass and pasture species (Warwick & Stewart 2005) and other forage crops such as alfalfa (*Medicago sativa* L.) (Bagavathiannan 2010), due to their perennial growth habit and rapid vegetative spread in relatively competitive habitat conditions. In other cases, risks of escape and spread may arise even among short-lived species, including recently domesticated crops like canola that retain the weedy characteristics of their wild ancestors (Hall *et al.* 2005). Additionally, wind- and insect-pollinated crops, as well as obligate outcrossing species, are likely to contribute greatly to the spread of transgenes beyond arable fields and at the landscape-scale (Bagavathiannan & Van Acker 2009).

While the defining characteristic of ferality appears to be the ability to reproduce successfully outside of cultivation, most definitions also assume that such reproduction leads to local persistence of feral populations over the medium- to long-term. Thus, there is some confusion regarding plants that reproduce successfully in non-cultivated habitats, but that exhibit transient local dynamics, including canola. Indeed, canola in non-crop habitats has been alternately described as “feral” (e.g. Crawley & Brown 1995; 2004; Pessel *et al.* 2001), “temporary feral” (Warwick & Stewart 2005), “escaped” (Bagavathiannan & Van Acker 2008), and “weedy” or “naturalized” (Gulden, Warwick & Thomas 2008), while other authors seem to dismiss the risk of canola ferality altogether (Hall *et al.* 2005). These disparate classifications point to the ‘paradox’ of canola presence in non-crop habitats (Crawley & Brown 1995); despite the transience of small populations, canola remains a permanent feature of ruderal habitats in landscapes where this crop is widely grown (Knispel & McLachlan 2010). As such, canola is of increasing interest in the study of escaped crop populations and gene flow, and the processes of canola establishment, spread and persistence are widely debated. In France, Pivard *et al.* (2008) found that a large proportion of populations of escaped canola originated from adjacent cultivated canola crops, as seed was dispersed into roadside habitats by harvest and sowing activities. Conversely, landscape-scale studies in England (Crawley & Brown 1995), New Zealand (Peltzer, Ferriss & Fitzjohn 2008) and North

America (Yoshimura, Beckie & Matsuo 2006) suggest that most escaped canola populations in roadside habitats are founded by seed lost from agricultural transport vehicles. This ongoing debate points to the need for further study of the dynamics of escaped and feral crop plants, particularly with regard to the apparent importance of human-mediated processes in determining population establishment and persistence in non-crop habitats. Indeed, it may be useful to expand the definition of ferality to include not only the potential for local persistence in self-sustaining populations, but also the contribution of ongoing agricultural activities and anthropogenic seed dispersal to persistence at larger spatial scales and to potential metapopulation dynamics. Included in formal GM risk assessment and post-release monitoring, such an expanded definition would increase the practical relevance of assessment outcomes, by realistically addressing both the ecological and agronomic factors contributing to GM crop escape and spread at the landscape-scale.

Using ecological concepts to inform agronomic problems

Pre-release risk assessment of novel GM crops remains limited in scope, able to address only pre-identified risks (Clark 2006) and constrained by the need to ensure rapid and cost-effective decision-making (Raybould 2005). Unfortunately, it seems that once such decisions are made, there is little incentive to re-examine the methods, assumptions, and data on which they were based; revisiting the issue, as it were, seems only to delay the rate at which new decisions can be made regarding the deregulation of even newer GM crops (e.g. Raybould 2005).

Despite claims of sound science, a fast-paced, frantic and industry-directed approach to GM risk assessment and regulation (e.g. Drobník 2008) overlooks the underlying tenets of any scientific venture, namely that experiments and observations should be systematic, repeatable and subject to independent review. Regulatory decisions made based on small-scale field trials should not preclude the pursuit of further information on the genetics, population biology, landscape ecology and extent of risk presented by the commercial cultivation of these new varieties. If predicted outcomes are not borne out following the release of new GM varieties, decisions need to be reexamined and assessments reviewed. In this context, risk assessment could gain much from a fuller integration of other applied scientific disciplines, including weed science (Van Acker 2009) and invasion ecology (Buckley 2008), which also focus on the development of practical decision-making tools and the generation of accurate predictions. Importantly, researchers are working to strengthen the linkages between these applied sciences and the underlying biology and ecology of the species of concern (Clements & Catling 2007; Van Acker 2009).

Knowledge of the post-release biology and ecology of novel GM crops is still lacking, especially for escaped plants that have become established outside of cultivated fields. To address this gap, relevant ecological theory and methodologies are useful in furthering our understanding of the dynamics of escaped GM crop plants. Indeed, the escape of transgenes is now recognized as a “hot” new topic in invasion ecology (Buckley 2008) and conceptual models describing the establishment, spread and impact of invasive species can also be used to structure post-release monitoring of GM crop escape (Warwick, Beckie & Hall 2009). Furthermore, integrated approaches to invasion ecology combine knowledge of population dynamics with information on ecosystem functioning. Such an approach recognizes that both intrinsic and extrinsic factors contribute to the impacts of novel species and need to be considered in relation to management goals (McMahon, Cadotte & Fukami 2006; Buckley 2008). Importantly, understanding of environmental factors should extend beyond simple measurements of habitat susceptibility to invasion; it is necessary to identify particular ecosystem functions, including dispersal vectors and disturbance regimes, that may themselves actively facilitate the establishment, spread and impact of novel species (McMahon, Cadotte & Fukami 2006).

Establishment

The first step in post-release monitoring of GM crop escape necessarily involves an inventory of the presence of both crop plants and novel transgenes in non-crop habitats. As with the search for other non-indigenous species, surveys of escaped GM crop plants generally need to cover large areas, often in the early stages of introduction, when the species of concern may be relatively rare and before population growth precludes effective management. Frequently, limited time and funding create further challenges in the design of appropriate sampling methodologies. Good research is designed to balance these factors to ensure that survey results are representative and encompasses appropriate levels of spatial and temporal environmental variation (Rew *et al.* 2006). In such situations, Rew *et al.* (2006) found that targeted stratified sampling protocols, designed using even minimal prior knowledge of species-environment interactions, most accurately described species presence and distribution.

Once escaped GM crops have been identified in non-crop habitats, characterization of the population biology of these species is important when assessing potential for population establishment and persistence (McMahon, Cadotte & Fukami 2006). Some of the earliest studies of escaped GM crops were undertaken in experimentally seeded populations of GM canola in natural habitats (Crawley *et al.* 1993). Measurement of GM canola population growth rates in this context suggested that local population dynamics were transient as a result of competition from native perennial vegetation (Crawley *et al.* 1993; 2001). Indeed, many empirical risk assessment studies have examined intrinsic population growth rates in natural habitats as a means of evaluating potential invasion impacts (Godfree *et al.* 2004; Eastick & Hearnden 2006); populations with declining local dynamics in natural habitats are considered non-invasive and low risk. However, ecologists increasingly stress that biological invasions and attendant impacts result as much from the biotic and abiotic characteristics of the host community as from the biology of the species themselves (Warren, Law & Weatherby 2006; Buckley 2008). As such, site-specific population growth rates of novel species are determined both by the habitat and the species in question. Thus, realistic assessment of the impacts of escaped GM crops necessitates consideration of their dynamics in appropriate (agro)ecosystem contexts.

To complement existing empirical data, modeling approaches may be developed to address questions of escaped GM crop population establishment and persistence. Matrix population models are now routinely used in the study of biological invasions to predict the behaviour of invasive plant populations and to assess the effectiveness of various control options (Davis *et al.* 2006; Jongejans, Sheppard & Shea 2006; Pardini *et* *al*. 2009). However, inherent challenges exist in creating meaningful population models for invasive and weedy species. The human-mediated environments where such species are found are subject to ongoing disturbance and management, resulting in high levels of spatial and temporal variation that are generally not adequately represented in population models (Freckleton & Stephens 2009). Additionally, standard matrix population models normally focus on year-to-year changes in population number and may often obscure important periodic and seasonal variation affecting the life cycle of many short-lived weedy species (e.g. Borger *et al.* 2009), including crop volunteers and crop escapes. To address these challenges and increase the utility of population models for the assessment of escaped transgenic crops, multi-site and multi-year models (e.g Kalisz & McPeek 1992) can be developed to characterize population behaviour in variable environments and account for site-specific environmental interactions affecting establishment success. Such an approach is complemented by the use of periodic matrix models (Caswell 2001) that explicitly consider seasonal- or phase-specific variation in annual life cycles (e.g. Vavrek, McGraw & Yang 1997; Mertens, van den Bosch & Heesterbeek 2002). Periodic models may be fine-tuned to describe not only overall population growth, but also seasonal demographic processes of particular concern: in the context of transgene escape, concerns are likely to focus on the dynamics of flowering and reproductive plants and attendant gene flow risks. Despite their potential utility, to my knowledge no such models have been parameterized with empirical data from escaped populations of GM crops. Indeed, to date, most population-level studies of crop escapes, whether empirical or theoretical, have focused on the detection, mapping and characterization of escaped conventional crop plants in regions where GM crops have yet to be deregulated (e.g.

Pessel *et al.* 2001; Pivard *et al.* 2008). Such proactive assessment of potential risks is commendable, but it also points to the conspicuous lack of either pre- or post-release surveys of escaped crop plants in regions where GM varieties have been widely commercialized (but see Yoshimura, Beckie & Matsuo 2006).

Spread

The spread of escaped crop plants is likely to occur at multiple spatial and temporal scales, within natural and ruderal elements of heterogeneous agricultural landscapes. Research on plant distribution and spread in such landscapes is informed by metapopulation ecology, which posits that groups of small disjunct populations can persist regionally as a result of ongoing dispersal, despite frequent local extinctions (Hanski 1997, 1999). This ecological approach is often appropriate in describing the invasion of novel species into new habitats and focuses our attention on two distinct spatial scales: the scale of the patch and the scale at which patches are connected by dispersal processes (Harding, McNamara & Holt 2006). Successful invasions occur when the rates of dispersal and colonization of new sites exceed the rate of local extinction of established populations (Harding, McNamara & Holt 2006). Thus, ecologically, the processes of local patch establishment and persistence cannot be thoroughly assessed without reference to dispersal dynamics, which requires ongoing monitoring of novel plant distribution at large spatial scales (Rew, Lehnhoff & Maxwell 2007). Indeed, recent expansions in the regional distribution of invasive prickly lettuce (*Lactuca serriola* L.), for example, have been attributed in part to metapopulation dynamics, as large source populations provide high numbers of propagules for the establishment of new sink populations in less suitable habitat (Hooftman, Oostermeijer & den Nijs 2006). Interestingly, the rapid spread of weedy *L. serriola* presents increased opportunity for genetic exchange with cultivated lettuce (*Lactuca sativa* L.) and associated genetic displacement of either the crop or its wild relatives (Hooftman *et al.* 2008). These challenges should serve as an important reminder of the potential impacts of gene flow from GM crops, particularly in a metapopulation context. Within agroecosystems, escaped crop plants in ruderal habitats interact with larger conspecific populations in cultivated fields. Indeed, the deliberate annual reintroduction of a given (transgenic) crop in a particular landscape constitutes a major propagule source contributing to the dispersal potential of that species, with impacts on distribution both within and outside of fields.

Outside of arable fields, at large spatial scales, specific landscape elements are widely associated with high numbers of weedy and invasive species. Roadsides have been widely identified as highly susceptible habitats for invasion (Christen & Matlack 2006; Rew *et al.* 2006). These habitats provide ample colonization opportunities for new species, as a result of increased levels of disturbance and reduced competition (Gelbard & Belnap 2003) and high light availability (Parendes & Jones 2000; Christen & Matlack 2009). Such factors undoubtedly provide suitable habitat conditions for crop plants that have escaped from adjacent cultivated fields. Beyond the simple physical suitability of roads as habitat, the suite of human activities accompanying these landscape elements also contributes to their effects, and facilitates invasion. Indeed, in human-mediated environments, ‘ecosystem’ functions necessarily include both natural and anthropogenic processes. Both road maintenance activities and regular vehicle traffic have been identified as contributing to the dispersal and expansion of invasive species (Jodoin *et al.* 2008; Christen & Matlack 2009; Vitalos & Karrer 2009) and crop escapes (Crawley & Brown 2004; von der Lippe & Kowarik 2007; Garnier, Pivard & Lecomte 2008). For crop species in agricultural landscapes, commercial agricultural transport serves as a particularly important dispersal vector, as large numbers of seeds are spilled from transport vehicles (Crawley & Brown 1995; 2004). Roads thus serve both as suitable habitat and important dispersal corridors for the spread of unwanted plants (Christen & Matlack 2006; 2009), and should thus be explicitly considered in the risk assessment of GM crop escape.

Impact

While invasive species are defined in large part by their impact on invaded communities (McMahon, Cadotte & Fukami 2006), the severity of the impact is not entirely dependent on a species' intrinsic invasiveness *sensu stricto* (i.e. annual growth rate $\lambda >> 1$). Rather, impact will vary depending on the community in question, and invasiveness and impact should be assessed simultaneously (Rew, Lehnhoff & Maxwell 2007), as even non-invasive populations may represent meaningful risks over multiple spatial and temporal scales. Importantly, in the consideration of cultivated crops, which are largely dependent on human agency for their dispersal and propagation, detrimental impacts are likely to occur at any scale of human activity. Within agricultural production regions, ecological studies at the landscape scale suggest that the spread of deregulated (Zapiola *et al.* 2008) and anticipated (Peltzer, Ferriss & Fitzjohn 2008; Pivard *et al.* 2008) transgenic crops will compromise plans for the coexistence of GM and non-GM varieties, reducing cropping system choice and market opportunities for farmers and communities. At even larger spatial scales, invasion rates are correlated with the growth of national economies; international trade and transport have been implicated in the rapid spread of invasive species around the globe (Weber & Li 2008; Hulme 2009). This invasive pathway is also important in the spread of transgenic crops, which are increasingly detected at points of import in countries where such crops are not yet cultivated (Aono *et al.* 2006; Kawata, Murakami & Ishikawa 2009).

Despite this recognition of the large-scale role of human agency in the spread of both invasive and transgenic plants, many ecologists continue to compartmentalize the risks associated with the spread of novel GM crops (e.g. Ceddia, Bartlett & Perrings 2007; Zapiola *et al.* 2008), separating those impacts that are explicitly ecological from those that are anthropogenic (i.e. agronomic, socioeconomic), the latter of which are, by implication, viewed as less important. However, given the overlap of human and natural patterns and processes in agricultural landscapes, impacts from GM crop cultivation are likely to be multi-faceted and iterative. Comprehensive risk assessments must cross disciplinary boundaries in order to adequately characterize all potential impacts. Indeed, savvy researchers recognize that the compartmentalization of risks associated with GM crops is arbitrary and artificial; ecological and agronomic risks are interdependent and inextricable (Van Acker, Brule-Babel & Friesen 2004; Graef *et al.* 2007; Mauro & McLachlan 2008). Though often not considered under the pre-identified ecological risk categories, intra-specific gene flow has now been identified as an important process linking the ecological and agronomic impacts of GM crops. These issues have come to the fore as a result of the long-term cultivation of GM canola in western Canada, and have also been highlighted in recent debates surrounding the deregulation of HT wheat in Canada and the deregulation of HT alfalfa in the USA. In these cases, the agricultural practices of both adopters and non-adopters of the new technology have changed (or are expected to change) in response to increases in HT volunteers and risks of genetic contamination. These agronomic changes result in ecological risks, including increased herbicide loads in the environment (Graef *et al.* 2007; Van Acker, Brule-Babel & Friesen 2004), decreased agroecosystem biodiversity (Graef *et al.* 2007) and reduction in the environmental benefits of reduced-tillage practices (i.e. improved soil moisture, increased organic matter, reduced soil erosion) (Friesen, Nelson & Van Acker 2003; Van Acker, Brule-Babel & Friesen 2004; Mauro & McLachlan 2008). At the level of whole production systems, the spread of GM canola has had a crippling effect on the organic canola industry (Smyth, Khachatourians & Phillips 2002), precluding a more widespread adoption of the environmentally beneficial practices of organic agriculture and undermining the livelihoods of these and other like-minded farmers (Mauro, McLachlan & Sanders 2005).

These conclusions point to the need for multi-scale and trans-disciplinary studies of GM crops, designed to examine the agroecosystem-level effects of these novel varieties (Schröder & Schmidt 2008). While it is crucial that such assessments are guided at least in part by a deep understanding of ecological concepts (Ervin *et al.* 2003), researchers must also recognize that scientific ventures contribute only part of the necessary information. Regardless of geographic or political context, the impacts of GM crop cultivation are inevitably identified and addressed in reference to established regulatory protocols, which are themselves defined by perceptions of acceptable risk.

Thus, regulatory decisions should integrate all scientific, social, economic and political information at hand (Ervin *et al.* 2003; Mauro & McLachlan 2008), as well as the meaningful input of all stakeholders, including farmers. Such decisions will be further strengthened by continuous re-evaluation of underlying assumptions. This approach to comprehensive, responsive risk assessment will only be possible with ongoing post-release monitoring of deregulated GM crops.

References

Andow, D.A. & Zwahlen, C. (2006) Assessing environmental risks of transgenic plants. *Ecology Letters*, **9**, 196-214.

Aono, M., Wakiyama, A., Nagatsu, M., Nakajima, N., Tamaoki, M., Kubo, A. & Saji, H. (2006) Detection of feral transgenic oilseed rape with multiple-herbicide resistance in Japan. *Environmental Biosafety Research*, **5**, 77-87.

Bagavathiannan, M.V. (2010) *Feral Nature of Alfalfa (*Medicago sativa *L.): Implications for Novel Trait Confinement.* PhD Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Bagavathiannan, M.V. & Van Acker, R.C. (2008) Crop ferality: Implications for novel trait confinement. *Agriculture, Ecosystems and Environment*, **127**, 1-6.

Bagavathiannan, M.V. & Van Acker, R.C. (2009) The biology and ecology of feral alfalfa (*Medicago sativa* L.) and its implications for novel trait confinement in North America. *Critical Reviews in Plant Science*, **28**, 69-87.

Beckie, H.J., Hall, L.M., Simard, M.-J., Leeson, J.Y. & Willenborg, C.J. (2010) A framework for postrelease environmental monitoring of second-generation cropswith novel traits. *Crop Science*, **50**, in press.

Beckie, H.J., Harker, K.N., Hall, L.M., Warwick, S.I., Légère, A., Sikkema, P.H., Clayton, G.W., Thomas, A.G., Leeson, J.Y., Séguin-Swartz, G. & Simard, M.J. (2006) A decade of herbicide resistant crops in Canada. *Canadian Journal of Plant Science*, **86**, 1243-1264.

Benbrook, C.M. (2003) Impacts of genetically engineered crops on pesticide use in the United States: The first eight years. *Biotech InfoNet*, Technical Paper No. 6, November 2003.

Borger, C.P.D., Scott, J.K., Renton, M., Walsh, M. & Powles, S.B. (2009) Assessment of management options for *Salsola australis* in south-west Australia by transition matrix modelling. *Weed Research*, **49**, 400-408.

Buckley, Y.M. (2008) The role of research for integrated management of invasive species, invaded landscapes and communities. *Journal of Applied Ecology*, **45**, 397-402.

Castle, L.A., Wu, G. & McElroy, D. (2006) Agricultural input traits: past, present and future. *Current Opinion in Biotechnology*, **17**, 105-112.

Caswell, H. (2001) *Matrix Population Models: Construction, Analysis, and Interpretation*, 2nd edn. Sinauer Associates Inc., Sunderland, MA, USA.

CBCGEO (Committee on Biological Confinement of Genetically Engineered Organisms) (2004) *Biological Confinement of Genetically Engineered Organisms*, National Academies Press, Washington, DC, USA.

Ceddia, M.G., Bartlett, M. & Perrings, C. (2007) Landscape gene flow, coexistence and threshold effect: the case of genetically modified herbicide tolerant oilseed rape (*Brassica napus*). *Ecological Modelling*, **205**, 169-180.

Cerdeira, A.L. & Duke, S.O. (2006) The current status and environmental impacts of glyphosate-resistant crops: a review. *Journal of Environmental Quality*, **35**, 1633-1658.

Christen, D. & Matlack, G. (2006) The role of roadsides in plant invasions: a demographic approach. *Conservation Biology*, **20**, 385-391.

Christen, D.C. & Matlack, G.R. (2009) The habitat and conduit functions of roads in the spread of three invasive plant species. *Biological Invasions*, **11**, 453-465.

Clark, E.A. (2006) Environmental risks of genetic engineering. *Euphytica*, **148**, 47-60.

Clements, D.R. & Catling, P.M. (2007) Invasive species issues in Canada – How can ecology help? *Canadian Journal of Plant Science*, **87**, 989-992.

Conner, A.J., Glare, T.R. & Nap, J.-P. (2003) The release of genetically modified crops into the environment Part II. Overview of ecological risk assessment. *The Plant Journal*, **33**, 19-46.

Crawley, M.J. & Brown, S.L. (1995) Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. *Proceedings of the Royal Society of London B.*, **259**, 49-54.

Crawley, M.J. & Brown, S.L. (2004) Spatially structured population dynamics in feral oilseed rape. *Proceedings of the Royal Society of London B*, **271**, 1909-1916.

Crawley, M.J., Hails, R.S., Rees, M., Kohn, D. & Buxton, J. (1993) Ecology of transgenic oilseed rape in natural habitats. *Nature*, **363**, 620-623.

Crawley, M.J., Brown, S.L., Hails, R.S., Kohn, D.D. & Rees, M. (2001) Transgenic crops in natural habitats. *Nature*, **409**, 682-683.

Davis, A.S., Landis, D.A., Nuzzo, V., Blossey, B., Gerber, E. & Hinz, H.L. (2006) Demographic models inform selection of biocontrol agents for garlic mustard (*Alliaria petiolata*). *Ecological Applications*, **16**, 2399-2410.

De Greef, W. (2004) The Cartagena Protocol and the future of agbiotech. *Nature Biotechnology*, **22**, 811-812.

Deblonde, M. & Du Jardin, P. (2005) Deepening a precautionary European policy. *Journal of Agricultural and Environmental Ethics*, **18**, 319-343.

Demeke, T., Perry, D.J. & Scowcroft, W.R. (2006) Adventitious presence of GMOs: scientific overview for Canadian grains. *Canadian Journal of Plant Science*, **86**, 1-23.

Devos, Y., Demont, M., Dillen, K., Reheul, D., Kaiser, M. & Sanvido, O. (2009) Coexistence of genetically modified (GM) and non-GM crops in the European Union. A review. *Agronomy for Sustainable Development*, **29**, 11-30.

Drobník, J. (2008) Time to relax GMO regulation in Europe. *Plant Cell, Tissue, and Organ Culture*, **94**, 235-238.

Duke, S.O. (2005) Taking stock of herbicide-resistant crops ten years after introduction. *Pest Management Science*, **61**, 211-218.

Duke, S.O. & Powles, S.B. (2009) Glyphosate-resistant crops and weeds: Now and in the future. *AgBioForum*, **12**, 346-357.

Eastick, R.J. & Hearnden, M.N. (2006) Potential for weediness of Bt cotton in northern Australia. *Weed Science*, **54**, 1142-1151.

Ellstrand, N.C. (2001) When transgenes wander, should we worry? *Plant Physiology*, **125**, 1543-1545.

Ervin, D.E., Welsh, R., Batie, S.S. & Line Carpentier, C. (2003) Towards an ecological systems approach in public research for environmental regulation of transgenic crops. *Agriculture, Ecosystems and Environment*, **99**, 1-14.

Freckleton, R.P. & Stephens, P.A. (2009) Predictive models of weed population dynamics. *Weed Research*, **49**, 225-232.

Friesen, L.F., Nelson, A.G. & Van Acker, R.C. (2003) Evidence of contamination of pedigreed canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. *Agronomy Journal*, **95**, 1342-1347.

Garnier, A., Pivard, S. & Lecomte, J. (2008) Measuring and modelling anthropogenic secondary seed dispersal along roadverges for feral oilseed rape. *Basic and Applied Ecology*, **9**, 533-541.

Gelbard, J.L. & Belnap, J. (2003) Roads as conduits for exotic plant invasions in a semiarid landscape. *Conservation Biology*, **17**, 420-432.

Godfree, R.C., Young, A.G., Lonsdale, W.M., Woods, M.J. & Burdon, J.J. (2004) Ecological risk assessment of transgenic pasture plants: a community gradient modelling approach. *Ecology Letters*, **7**, 1077-1089.

Graef, F., Stachow, U., Werner, A. & Schütte, G. (2007) Agricultural practice changes with cultivating genetically modified herbicide-tolerant oilseed rape. *Agricultural Systems*, **94**, 111-118.

Gressel, J. (2005) Introduction – the challenges of ferality. *Crop Ferality and Volunteerism* (ed J. Gressel), pp. 1-7. Taylor & Francis Group, Boca Raton, Florida, USA.

Gulden, R.H., Shirtliffe, S.J. & Thomas, A.G. (2003) Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. *Weed Science*, **51**, 83-86.

Gulden, R.H., Warwick, S.I. & Thomas, A.G. (2008) The Biology of Canadian Weeds. 137. *Brassica napus* L. and *Brassica rapa* L. *Canadian Journal of Plant Science*, **88**, 951-996.

Hall, L.M., Rahman, M.H., Gulden, R.H. & Thomas, A.G. (2005) Volunteer oilseed rape – will herbicide-resistance traits assist ferality? *Crop Ferality and Volunteerism* (ed J. Gressel), pp. 59-79. Taylor & Francis Group, Boca Raton, Florida, USA.

Hanski, I.A. (1997) Metapopulation dynamics: from concepts and observations to predictive models. *Metapopulation Biology: Ecology, Genetics, and Evolution* (eds I.A. Hanski & M.E. Gilpin), pp. 69-91. Academic Press, San Diego, California, USA.

Hanski, I.A. (1999) *Metapopulation Ecology*. Oxford University Press, Oxford.

Harding, K.C., McNamara, J.M. & Holt, R.D. (2006) Understanding invasions in patchy habitats through metapopulation theory. *Conceptual Ecology and Invasion Biology: Reciprocal Approaches to Nature* (eds M.W. Cadotte, S.M. McMahon & T. Fukami), pp. 371-403. Springer, Netherlands.

Heap, I. (2010) The International Survey of Herbicide Resistant Weeds. http://www.weedscience.org. Accessed: June 30, 2010.

Heard, M.S., Rothery, P., Perry, J.N. & Firbank, L.G. (2005) Predicting longer-term changes in weed populations under GMHT crop management. *Weed Research*, **45**, 331-338.

Hooftman, D.A.P., Oostermeijer, J.G.B. & den Nijs, H.J.C.M. (2006) Invasive behaviour of *Lactuca serriola* (Asteraceae) in the Netherlands: Spatial distribution and ecological amplitude. *Basic and Applied Ecology*, **7**, 507-519.

Hooftman, D.A.P., Oostermeijer, J.G.B., Marquard, E. & den Nijs, H.J.C.M. (2008) Modelling the consequences of crop-wild relative gene flow: a sensitivity analysis of the effects of outcrossing rates and hybrid vigour breakdown in *Lactuca*. *Journal of Applied Ecology*, **45**, 1094-1103.

Hulme, P.E. (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, **46**, 10-18.

James, C. (2009) *Global Status of Commercialized Biotech/GM Crops: 2009*. ISAAA Brief No. 41-2009. International Service for the Acquisition of Agri-biotech Applications: Ithaca, NY, USA.

Jodoin, Y., Lavoie, C., Villeneuve, P., Theriault, M., Beaulieu, J. & Belzile, F. (2008) Highways as corridors and habitats for the invasive common reed *Phragmites australis* in Quebec, Canada. *Journal of Applied Ecology*, **45**, 459-466.

Jongejans, E., Sheppard, A.W. & Shea, K. (2006) What controls the population dynamics of the invasive thistle *Carduus nutans* in its native range? *Journal of Applied Ecology*, **43**, 877-886.

Kalisz, S. & McPeek, M.A. (1992) Demography of an age-structured annual: resampled projection matrices, elasticity analyses, and seed bank effects. *Ecology*, **73**, 1082-1093.

Kawata, M., Murakami, K. & Ishikawa, T. (2009) Dispersal and persistence of genetically modified oilseed rape around Japanese harbours. *Environmental Science and Pollution Research*, **16**, 120-126.

Knispel, A.L. & McLachlan, S.M. (2010) Landscape-scale distribution and persistence of genetically modified oilseed rape (*Brassica napus*) in Manitoba, Canada. *Environmental Science and Pollution Research*, **17**, 13-25.

Levidow, L. & Boschert, K. (2008) Coexistence or contradiction? GM crops versus alternative agricultures in Europe. *Geoforum*, **39,** 174-190

Levidow, L. & Carr, S. (2000) Unsound science? Transatlantic regulatory disputes over GM crops. *International Journal of Biotechnology*, **2**, 257-273.

Levidow, L., Murphy, J. & Carr, S. (2007) Recasting “Substantial Equivalence”. *Science, Technology and Human Values*, **32**, 26-64.

L’heureux, K. & Menrad, K. (2004) A decade of European field trials with genetically modified plants. *Environmental Biosafety Research*, **3**, 99-107.

Mauro, I.J. & McLachlan, S.M. (2008) Farmer knowledge and risk analysis: Postrelease evaluation of herbicide-tolerant canola in western Canada. *Risk Analysis*, **28**, 463-476.

Mauro, I.J., McLachlan, S.M. & Sanders, J. (2005) *Seeds of Change: Farmers, Biotechnology and the New Face of Agriculture.* Dead Crow Productions and Dada World Data, Winnipeg, Manitoba, Canada. Documentary film available at: www.seedsofchangefilm.org

McMahon, S.M., Cadotte, M.W. & Fukami, T. (2006) Tracking the tractable: using invasions to guide the exploration of conceptual ecology. *Conceptual Ecology and Invasion Biology: Reciprocal Approaches to Nature* (eds M.W. Cadotte, S.M. McMahon & T. Fukami), pp. 3-14. Springer, Netherlands.

Mertens, S.K., van den Bosch, F. & Heesterbeek, J.A.P. (2002) Weed populations and crop rotations: exploring dynamics of a structured periodic system. *Ecological Applications*, **12**, 1125-1141.

Millstone, E., Brunner, E. & Mayer, S. (1999) Beyond "Substantial Equivalence". *Nature*, **401**, 525-526.

Nap, J.-P., Metz, P.L.J., Escaler, M. & Conner, A.J. (2003) The release of genetically modified crops into the environment. *The Plant Journal*, **33**, 1-18.

Pardini, E.A., Drake, J.M., Chase, J.M. & Knight, T.M. (2009) Complex population dynamics and control of the invasive biennial *Alliaria petiolata* (garlic mustard). *Ecological Applications*, **19**, 387-397.

Parendes, L.A. & Jones, J.A. (2000) Role of light availability and dispersal in exotic plant invasion along roads and streams in the H.J. Andrews Experimental Forest, Oregon. *Conservation Biology*, **14**, 64-75.

Peltzer, D.A., Ferriss, S. & FitzJohn, R.G. (2008) Predicting weed distribution at the landscape-scale: using naturalized *Brassica* as a model system. *Journal of Applied Ecology*, **45**, 467-475.

Pessel, F.D., Lecomte, J., Emeriau, V., Krouti, M., Messean, A. & Gouyon, P.H. (2001) Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. *Theoretical and Applied Genetics*, **102**, 841-846.

Pirondini, A. & Marmiroli, N. (2008) Environmental risk assessment in GMO analysis. *Rivista di Biologia – Biology Forum*, **101**, 215-246.

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., Gouyon, P.H. & Huet, S. (2008) Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. *Journal of Applied Ecology*, **45**, 476-485.

Potrykus, I. (2001) Golden rice and beyond. *Plant Physiology*, **125**, 1157-1161.

Quist, D., Chapela, I.H. (2001) Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature*, **414**, 541-543.

Raybould, A. (2005) Assessing the environmental risks of transgenic volunteer weeds. *Crop Ferality and Volunteerism* (ed J. Gressel), pp. 389-401. Taylor & Francis Group, Boca Raton, Florida, USA.

Rew, L.J., Lehnhoff, E.A. & Maxwell, B.D. (2007) Non-indigenous species management using a population prioritization framework. *Canadian Journal of Plant Science,* **87**, 1029-1036.

Rew, L.J., Maxwell, B.D., Dougher, F.L. & Aspinall, R. (2006) Searching for a needle in a haystack: evaluating survey methods for non-indigenous plant species. *Biological Invasions*, **8**, 523-539.

Rieger, M.A., Lamond, M., Preston, C., Powles, S.B. & Roush, R.T. (2002) Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science*, **296**, 2386-2388.

Rogers, M.D. (2004) Genetically modified plants and the precautionary principle. *Journal of Risk Research,* **7**, 675-688.

Sanvido, O., Romeis, J. & Bigler, F. (2007) Ecological impacts of genetically modified crops: Ten years of field research and commercial cultivation. *Advances in biochemical engineering/biotechnology,* **107**, 235-278.

Sanvido, O., Widmer, F., Winzeler, M. & Bigler, F. (2005) A conceptual framework for the design of environmental post-market monitoring of genetically modified plants. *Environmental Biosafety Research,* **4**, 13-27.

Schröder, W. & Schmidt, G. (2008) Implications of GM-crop cultivation – Series. *Environmental Science and Pollution Research,* **15**, 527-528.

Schwember, A.R. (2008) An update on genetically modified crops. *Ciencia e Investigación Agraria,* **35**, 185-204.

Service, R.F. (2007) A growing threat down on the farm. *Science*, **316**, 1114-1117.

Smyth, S., Khachatourians, G.G. & Phillips, P.W.B. (2002) Liabilities and economics of transgenic crops. *Nature Biotechnology,* **20**, 537-541.

Van Acker, R.C. (2009) Weed biology serves practical weed management. *Weed Research*, **49**, 1-5.

Van Acker, R.C., Brule-Babel, A.L. & Friesen, L.F. (2004) Intraspecific gene movement can create environmental risk: the example of Roundup Ready wheat in western Canada. *Schriftenreihe für Landschaftspflege und Naturschutz*, **79**, 35-48.

Vavrek, M.C., McGraw, J.B. & Yang, H.S. (1997) Within-population variation in demography of *Taraxacum officinale*: season- and size-dependent survival, growth and reproduction. *Journal of Ecology*, **85**, 277-287.

Vitalos, M. & Karrer, G. (2009) Dispersal of *Ambrosia artemisiifolia* seeds along roads: the contribution of traffic and mowing machines. *Biological Invasions: Towards a Synthesis, Proceedings*, **8**, 53-60.

von der Lippe, M. & Kowarik, I. (2007) Crop seed spillage along roads: a factor of uncertainty in the containment of GMO. *Ecography*, **30**, 483-490.

Warren, P.H., Law, R. & Weatherby, A.J. (2006) Invasion biology as a community process: messages from microbial microcosms. *Conceptual Ecology and Invasion Biology: Reciprocal Approaches to Nature* (eds M.W. Cadotte, S.M. McMahon & T. Fukami), pp. 343-367. Springer, Netherlands.

Warwick, S.I., Beckie, H.J. & Hall, L.M. (2009) Gene flow, invasiveness, and ecological impact of genetically modified crops. *Annals of the New York Academy of Sciences*, **1168**, 72-99.

Warwick, S.I., Légère, A., Simard, M.-J. & James, T. (2008) Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population. *Molecular Ecology*, **17**, 1387-1395.

Warwick, S.I., Simard, M.-J., Légère, A., Beckie, H.J., Braun, L., Zhu, B., Mason, P., Séguin-Swartz, G. & Stewart, C.N. (2003) Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O.E. Schulz. *Theoretical and Applied Genetics*, **107**, 528-539.

Warwick, S.I. & Stewart, C.N. (2005) Crops come from wild plants – how domestication, transgenes, and linkage together shape ferality. *Crop Ferality and Volunteerism* (ed J. Gressel), pp. 9-30. Taylor & Francis Group, Boca Raton, Florida, USA.

Weber, E. & Li, B. (2008) Plant invasions in China: what is to be expected in the wake of economic development? *Bioscience*, **58**, 437-444.

Wilkinson, M.J., Elliott, L.J., Allainguillaume, J., Shaw, M.W., Norris, C., Welters, R., Alexander, M., Sweet, J. & Mason, D.C. (2003) Hybridization between *Brassica* *napus* and *B. rapa* on a national scale in the United Kingdom. *Science*, **302**, 457-459.

Yoshimura, Y., Beckie, H.J. & Matsuo, K. (2006) Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. *Environmental Biosafety Research*, **5**, 67-75.

Zapiola, M.L., Campbell, C.K., Butler, M.D. & Mallory-Smith, C.A. (2008) Escape and establishment of transgenic glyphosate-resistant creeping bentgrass *Agrostic stolonifera* in Oregon, USA: a 4-year study. *Journal of Applied Ecology*, **45**, 486-494.

Züghart, W., Benzler, A., Berhorn, F., Sukopp, U. & Graef, F. (2008) Determining indicators, methods and sites for monitoring potential adverse effects of genetically modified plants to the environment: the legal and conceptual framework for implementation. *Euphytica*, **164**, 845-852.

CHAPTER 3

GENE FLOW AND MULTIPLE HERBICIDE TOLERANCE IN ESCAPED CANOLA POPULATIONS

This chapter has been published in the journal *Weed Science* in co-authorship with my primary advisor, committee member and a research associate. I was responsible for data collection, analysis and much of the writing, while my co-authors provided insight into overall study design, design of the herbicide trial, and reviewed multiple drafts. The paper is reproduced here with kind permission of the authors.

Knispel, A.L., McLachlan, S.M., Van Acker, R.C. & Friesen, L.F. (2008) Gene flow and multiple herbicide resistance in escaped canola populations. *Weed Science*, **56**, 72-80.

Chapter Summary

Gene flow among herbicide-tolerant (HT) canola varieties can lead to the development of multiple HT canola plants, creating volunteer canola management challenges for producers. In western Canada, escaped populations of HT canola are ubiquitous outside of cultivated fields, yet the extent of gene flow resulting in HT trait-stacking in individuals within these populations remains unknown. The objectives of this study were to document the presence of single and multiple HT traits and assess the extent of gene flow within escaped canola populations. Seed was collected from 16 escaped canola populations along the verges of fields and roadways in four agricultural regions in southern Manitoba from 2004 to 2006. Glyphosate tolerance was found in 14 (88%) of these populations, glufosinate tolerance in 13 (81%) populations, and imidazolinone tolerance in five (31%) populations. Multiple herbicide tolerance was observed at levels consistent with previously published canola outcrossing rates in 10 (62%) of the tested populations. In 2005 and 2006, maternal plants from two escaped populations were tested using trait indicator test strips for glyphosate and glufosinate tolerance to confirm outcrossing events. In 2005, two of 13 tested maternal plants with single HT traits produced progeny with both glyphosate and glufosinate tolerance. In 2006, of 21 tested plants, 10 single tolerant maternal plants produced multiple tolerant progeny, and five non-tolerant maternal plants produced tolerant offspring. This is the first report indicating that intraspecific gene flow results in stacking of HT traits in individuals within escaped canola populations, confirming that multiple HT canola volunteers are not confined to agricultural fields. Results of this study suggest that escaped populations of crop plants can contribute to the spread of genetically engineered

novel traits, which has important implications for containment, especially for highly controversial pharmaceutical and industrial traits in crop plants.

Introduction

Canola varieties genetically engineered for herbicide tolerance were approved for large scale commercial release in the United States and Canada in 1995 (for the purposes of this paper, *canola* refers exclusively to *Brassica napus* L.). Currently, three herbicide-tolerant (HT) canola types are widely grown in western Canada, including transgenic varieties tolerant to glyphosate or glufosinate, and mutagenic varieties tolerant to certain imidazolinone herbicides. In 2005, glyphosate and glufosinate tolerant canola varieties were grown on 42% and 47%, respectively, and imidazolinone tolerant varieties on 9%, of the 902,000 hectares of canola grown in Manitoba, Canada (2006 data not yet available) (MASC 2006a). Conventional non-HT canola represented only 2% of the area planted to canola. High adoption rates of HT canola varieties are largely associated with operational benefits, including improved weed control and ease of management (Beckie et al. 2006; Mauro and McLachlan 2008).

Despite these benefits, risks to producers associated with the cultivation of genetically engineered/modified (GM) crops are increasingly recognized. These risks include compromised weed control in zero till systems (Friesen et al. 2003), increased herbicide costs (Van Acker et al. 2003), crop contamination with adventitious HT traits, and even legal liability (Mauro and McLachlan 2008; Smyth et al. 2002). Yet post-release monitoring of HT crops has focused primarily on their impacts in natural ecosystems (Crawley et al. 2001; Crawley et al. 1993) and on gene flow to wild relatives

(Scott and Wilkinson 1998; Warwick et al. 2003; Wilkinson et al. 2003). However, the importance of intraspecific gene flow within and between arable fields is increasingly recognized (Ellstrand 2001; Légère 2005; Smyth et al. 2002; Waines and Hegde 2003), raising concerns about the containment of HT traits (Beckie et al. 2006) and implications for environmentally beneficial practices such as direct seeding in western Canada (Friesen et al. 2003). Among canola plants, where individual outcrossing rates range from 6 to 81% (Cuthbert and McVetty 2001), pollen-mediated gene flow may result in the relatively rapid movement of HT genes. Outcrossing between adjacent canola fields with differing HT traits has resulted in multiple HT canola volunteers in western Canada (Beckie et al. 2003; Hall et al. 2000). Multiple HT canola plants appear not to be subject to fitness costs (Simard et al. 2005), and HT traits may potentially accumulate and spread in volunteers growing both in cultivated fields and adjacent habitats.

Canola is a weedy plant that frequently grows in disturbed habitats (Crawley and Brown 1995; Pessel et al. 2001), and escaped canola populations have become ubiquitous in rural landscapes throughout western Canada. Escaped populations most likely arise from seed dispersed by agricultural activities and transport (Crawley and Brown 1995), and commonly contain glyphosate and glufosinate tolerance traits in areas where these HT varieties are widely grown (Yoshimura et al. 2006). While localized escaped canola populations are believed to be transient (Crawley and Brown 2004), and thus of little management significance (Hall et al. 2005), such populations may contribute to the spread of HT traits through intraspecific gene flow within a single generation. Where differing HT canola types are locally grown, gene flow within escaped canola populations has the potential to result in stacking of tolerance traits in individuals in disturbed, non-crop habitats. Moreover, survival of canola plants over winter (Lawson et al. 2006) may contribute to increased persistence of HT traits. The movement of stacked traits from disturbed habitats into non-HT canola crops, or to canola crops possessing different HT traits, has important implications for subsequent management of volunteer canola and choice of cropping system. Although escaped populations of HT canola appear to be widespread, the extent to which outcrossing within these populations contributes to the spread of HT traits remains unknown.

The objectives of this study were to: (1) characterize the frequency of glyphosate, glufosinate, and imidazolinone tolerance in roadside escaped canola populations in southern Manitoba over multiple years, and (2) determine whether intraspecific gene flow contributes to the spread and stacking of HT traits within these escaped populations. Multiple tolerant progeny produced by outcrossing events were confirmed though phenotypic testing of maternal plants combined with herbicide screening of progeny.

Methods

SITE SELECTION

To determine the herbicide tolerance characteristics of escaped canola volunteers over several years, roadside canola populations were identified in the rural municipalities (RMs) of MacDonald (lat. 49º 40'N, long. 97º 30'W), Rhineland (lat. 49º 9'N, long. 97º 39'W), Roland (lat. 49º 20'N, long. 97º 54'W), and Dufferin (lat. 49º 31'N, long. 98º 4'W) in southern Manitoba. MacDonald and Dufferin, which cover 1106 km^2 and 882 km^2, respectively, are among the most important canola-growing regions in the province, and canola is generally planted once every three or four years in rotation. In Rhineland

and Roland, which cover 923 km^2 and 466 km^2, respectively, canola is grown less frequently and in some fields has not been planted for over 30 years (Table 3.1). However, one of the province’s two canola crushing facilities is located in the Rhineland region, resulting in much transport of canola seed in the region.

Escaped canola populations were initially identified in the RMs of MacDonald and Rhineland in July 2004, and subsequently in the RMs of Roland and Dufferin in July 2006, at the time of canola flowering. Populations were located along paved highways or gravel grid roads, adjacent to cropped land. Current and past crop cultivation in adjacent fields were identified through interviews with landowners. All populations had an initial size of at least 30 flowering plants, and occupied 50 to 200 linear metres of roadside habitat. Density of each escaped population was determined in August of each year in 30 randomly located permanent 1 x 1 m quadrats.

SEED COLLECTION AND FIELD TESTING

In August and September of 2004, 2005, and 2006, seed was collected from mature escaped canola plants in the roadside populations. All plants were collected from outside the permanent quadrats, to minimize impact on population dynamics within the quadrats. In 2004, six of eight monitored populations produced reproductive plants, and seed was collected from each of 45 escaped canola plants. In 2005, seed from 37 plants was collected from five of eight monitored populations. In 2006, seed from 47 plants was collected from five of 12 monitored populations. In all cases, all pods were collected from each plant. Three populations (Rhineland4, MacDonald2, and MacDonald4) produced viable seed in both 2004 and 2005. Only one population (MacDonald1) produced viable seed in all three years of the study, attesting to the transient nature of these populations.

To assess gene flow in escaped canola populations, the maternal phenotypes of escaped canola plants growing in the MacDonald2 and Dufferin1 populations were tested in 2005 and 2006, respectively. In each of these two populations, a relatively large number of plants produced enough vegetative tissue to permit maternal phenotype testing, and sufficient seed for subsequent herbicide trials. In 2005, leaf tissue samples were collected from 21 marked plants from the MacDonald2 population in July, and tested for glyphosate and glufosinate tolerance using trait indicator test strips.[1] Trait indicator test strips detect the presence in leaf tissue of proteins associated with glyphosate or glufosinate tolerance. In September 2005 when seed had matured, seed of 13 of the tested maternal plants was collected. The remaining eight tested plants had died or had been mowed over the course of the summer. In 2006, leaf tissue samples from 27 marked plants from the Dufferin1 population were tested for glyphosate and glufosinate tolerance using test strips in July, and seed from 21 surviving plants was collected in September. In both years, collected seed from tested plants was included in herbicide screening trials (as described below) to determine the herbicide tolerance characteristics of the progeny. Results of herbicide screening of plants from these two populations are shown in a separate figure, permitting examination of the herbicide tolerance characteristics of progeny.

Sub-samples of between 25-100 seeds from canola plants collected in 2004 and 2006 were tested for germinability on filter paper in Petri dishes, while ensuring sufficient seeds remained for herbicide screening. Extremely wet weather in 2005

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resulted in low seed production and all seeds were required for herbicide screening tests, precluding any germination tests that year. Average germination of seeds from escaped plants was 98% (S.E. 0.6) in 2004, and 96% (S.E. 1.1) in 2006. Percent germination per individual sample ranged from 71 to 100% in both years. One-thousand seed weights were determined for seed collected from escaped plants in each of the three years.

HERBICIDE TOLERANCE TRIALS

Herbicide trials were conducted in 2004, 2005, and 2006 to determine the proportion of escaped canola progeny exhibiting single and multiple herbicide tolerance. A subset of 28 seeds from each maternal canola plant was tested in two separate herbicide trials. Seedlings were grown indoors in a growth chamber with a 16-h photoperiod and 21/17˚C day/night temperature to the three to four-leaf stage (≈ 14 d). Numbers of seedlings were counted prior to herbicide application, and only those plants with at least 20 progeny (seedlings) were included in herbicide trials. Herbicide screening of between 18 to 24 progeny results in a 0.995 to 0.999 probability of detecting segregation from selfed heterozygous maternal plants (Rédei 1982), where the trait of interest is controlled by a single dominant nuclear gene. In the case of glyphosate and glufosinate tolerance, segregation will occur among the progeny of hemizygous maternal plants which carry one HT allele, but lack a second allele on the homologous chromosome (Hall et al. 2000).

In the first herbicide trial, seedlings were sprayed with glyphosate at 700 g ae ha^{-1} and assessed for survival after 7 d. Surviving seedlings were subsequently sprayed with glufosinate at 600 g ai ha^{-1} and further survival assessed after an additional 4 d. In the second herbicide trial, the order of herbicide application was reversed, and seedlings were first sprayed with glufosinate at 400 g ai ha^{-1} and assessed for survival after 4 d. Surviving seedlings were then sprayed with glyphosate at 700 g ae ha^{-1} and further survival assessed after 7 d. Growth response of seedlings in both trials was compared to that of 12 seedlings each of known glyphosate tolerant (LG 3235), glufosinate tolerant (SW Flare), and susceptible (46A65) cultivars. As glyphosate and glufosinate tolerance are conferred by dominant nuclear alleles from two different genes (Hall et al. 2000), both homozygous dominant and hemizygous seedlings will exhibit tolerance, whereas homozygous recessive plants will be susceptible. Seedlings sprayed with these herbicides were scored as either tolerant or susceptible to glyphosate and glufosinate, and tolerance to the first herbicide was assessed prior to application of the second. The results of glyphosate and glufosinate herbicide trials were verified by testing at least 50% of surviving, putatively double-tolerant seedlings with trait indicator test strips for both glyphosate and glufosinate tolerance.

A third herbicide trial was conducted to detect imidazolinone tolerance for those escaped canola plants that produced sufficient seed ($n = 42$ plants in 2004, $n = 24$ in 2005, and $n = 46$ in 2006). Twenty-eight seeds from each maternal plant were planted and seedlings were sprayed at the three- to four-leaf stage with thifensulfuron at 10 g ai ha^{-1} + non-ionic surfactant at 0.2% v/v. The imidazolinone tolerance trait also confers tolerance to thifensulfuron, a sulfonylurea herbicide, which was registered and commercially available for use on mutagenic imidazolinone tolerant canola varieties until 2002 (Friesen et al. 2003) and which provides a more predictable plant response than imazethapyr or imazamox in controlled environments (A.L. Knispel, personal observation). Tolerance to acetolactate synthase inhibitors (imidazolinones and sulfonylureas) is determined by an additive two-gene system in canola (Tan et al. 2005), and, depending on their genetics, outcrossed second and third generation plants may exhibit intermediate tolerance. Canola seedlings sprayed indoors with thifensulfuron are visually distinguishable as either tolerant, intermediate, or susceptible. Growth response of seedlings was compared to that of known imidazolinone tolerant (45A77) and susceptible (46A65) cultivars, and seedlings were scored as tolerant, intermediate, or susceptible. Generally the time required for satisfactory development of plant injury response after the application of thifensulfuron (10 d) precludes any sequential herbicide application, as surviving seedlings are then too advanced for normal herbicide activity. In 2006, because of the large number of thifensulfuron survivors, these plants were subsequently sprayed with glyphosate. Surviving seedlings in all instances were tested for glyphosate and glufosinate tolerance using test strips.

Results and Discussion

POPULATION PERSISTENCE AND REPRODUCTIVE OUTPUT OF ESCAPED CANOLA VOLUNTEERS

In the roadside canola populations observed in this study, many canola plants were mowed or died over the course of each growing season (data not shown), and relatively few plants survived to produce seed. Additionally, based on soil sampling at each of the roadside canola sites and subsequent cycles of emergence monitoring of these soil samples in the greenhouse (following the protocol described in De Corby et al. 2007), it appears that roadside canola seedbanks are small (less than 5 germinable seeds

m^{-2}) and lack substantive dormancy (A.L. Knispel, unpublished data). Low numbers of reproductive plants and lack of seed dormancy likely contribute to the transience of the escaped canola populations observed in this study. Indeed, very few of the populations observed in this study produced reproductive plants in consecutive years at the same location; only 25% of populations produced seed over two years and only one produced seed in all three years. Escaped rapeseed (*B. napus*) populations observed over multiple years in England also were found to be transient (Crawley and Brown 1995; Crawley and Brown 2004). However, this apparent transience is likely offset at a landscape scale by yearly seed addition from agricultural sources.

Interestingly, although populations are highly transient, seed yields from individual escaped canola plants were comparable to those of cropped canola plants in southern Manitoba. In our study in 2004, escaped canola plants produced an average of 1667 seeds $plant^{-1}$ (S.E. 297), with an average 1000-seed weight of 3.6 g (S.E. 0.1). In 2005 due to very wet and cold conditions, escaped canola plants produced substantially less seed, averaging 355 seeds $plant^{-1}$ (S.E. 111), with an average 1000-seed weight of 3.0 g (S.E. 0.1). In 2006, escaped canola plants produced an average of 883 seeds $plant^{-1}$ (S.E. 121), with an average 1000-seed weight of 3.1 g (S.E. 0.1). Based on the ten-year provincial average canola yield of 1677 kg ha^{-1} (MASC 2007) and given typical crop stand densities, in-field canola plants produce approximately 700 seeds $plant^{-1}$ with an average 1000-seed weight of 3.0 g (Gulden et al. 2003). The comparable reproductive output of escaped canola volunteers and cropped canola plants in western Canada, as well as the high viability of seed from escaped plants in this study (98% in 2004 and 96% in 2006), suggests that seed inputs from mature plants should contribute to population persistence in roadside habitats. Additionally, in the spring of 2006, overwintered canola rosettes were observed in many arable fields in Manitoba (Lawson et al. 2006) and HT canola plants also survived the winter in roadside habitats (pers. obs.). These plants may have further contributed to population persistence. However, to date, little work has focused on the persistence of escaped canola populations at either the population or landscape scale in western Canada.

GLYPHOSATE AND GLUFOSINATE TOLERANCE

In all three years of this study, all escaped canola populations exhibited the presence of glyphosate and/or glufosinate herbicide tolerance traits, and in all cases test strip results confirmed the results of the herbicide screening trials of progeny. In 2004, glyphosate tolerance was identified in all 6 populations and in 39 (87%) of the 45 tested maternal plants, and glufosinate tolerance was found in 4 of 6 populations and in 56% of tested maternal plants (Table 3.2). In 2005 samples, glyphosate tolerance was found in only 3 of the 5 populations and 15 (41%) of the 37 tested maternal plants. However, glufosinate tolerance was found in all populations and in 68% of maternal plants. In 2006, glyphosate tolerance was again found in all populations and in 41 of the 47 maternal plants tested (87%). In that same year, glufosinate tolerance was found in 4 of the 5 populations and in 66% of the tested maternal plants. These results indicate that transgenic herbicide tolerance is widespread in roadside canola populations, reflecting the popularity of glyphosate and glufosinate tolerant canola varieties with farmers in southern Manitoba. Similar correspondence between frequency of transgenic HT traits in escaped canola plants and cultivated acreage was also observed in the neighbouring province of Saskatchewan in 2005 (Yoshimura et al. 2006). These results suggest that HT canola plants will remain common in non-crop, ruderal environments as long as HT canola varieties continue to be widely cultivated in western Canada, especially as HT canola plants seem not to be subject to large fitness costs (Simard et al. 2005).

Varying frequencies of HT traits in escaped canola populations suggests that non-crop habitats receive substantial seed input from agricultural sources. Indeed, canola plants from one escaped population (MacDonald1) exhibited only glyphosate tolerance in 2004, only glufosinate tolerance in 2005, and both glyphosate and glufosinate tolerance in 2006 (Table 3.2), indicating that ongoing addition of new seed represents an important source of HT traits in roadside populations. In this study, escaped canola plants producing 100% tolerant progeny (Table 3.2), inferred to be homozygous for the dominant glyphosate or glufosinate alleles conferring tolerance, likely arose from whole seed contamination of roadside habitats. Canola seed may be lost through seeding and/or harvest operations, and in this study 9 (56%) of the 16 escaped canola populations were adjacent to a canola field or adjacent to a field planted to canola in the previous year (Table 3.1). However, seed losses during agricultural transport are the most likely cause of escaped HT canola plants. Indeed, producers identified agricultural transport intensity as being high on the roads adjacent to 14 (88%) of the 16 escaped populations, and the majority (75%) of the escaped populations examined over three years were located along provincial roads and highways. Transport has also been identified as the likely source of escaped canola plants both in western Canada (Yoshimura et al. 2006) and in countries such as Japan where whole HT canola seed is imported for processing, but where HT varieties are not currently cultivated (Aono et al. 2006).

Within escaped populations, intraspecific gene flow may frequently occur within a single generation, thereby facilitating the spread of HT traits within a relatively short time frame (Hall et al. 2000) and potentially accelerating the development and spread of multiple HT canola volunteers. Escaped maternal canola plants tested in this study also produced progeny that segregated for the tolerance trait, with tolerance ranging between 4 and 96%, for both glyphosate and glufosinate tolerance traits (Table 3.2). These proportions may be used to infer maternal genotypes, according to segregation ratios of offspring (Rédei 1982). Plants that produced high proportions (i.e. 50 to 99%) of tolerant offspring were assumed to have been hemizygous for either the glyphosate or the glufosinate tolerance allele, as wholly self-fertilized hemizygous plants are expected to produce offspring in a 3:1 tolerant:susceptible ratio. Seed giving rise to plants hemizygous for HT traits is present in commercial HT pedigreed seedlots (Beckie et al. 2003), and may be introduced into roadside habitats by nearby agricultural activities. Alternatively, these hemizygous maternal plants may have been the second generation of escaped canola resulting from the movement of tolerance traits via pollen-mediated gene flow (PMGF).

Low proportions (i.e. 4 to 30%) of glyphosate tolerance were observed in the progeny of 14 (31%) of 45 tested maternal plants in 2004, 3 (8%) of 37 tested maternal plants in 2005, and 6 (13%) of 47 tested maternal plants in 2006 (Table 3.2). Similarly low levels of glufosinate tolerance were observed in the progeny of 9 (20%) of 45 tested maternal plants in 2004, 6 (16%) of 37 tested maternal plants in 2005, and 23 (49%) of 47 tested maternal plants in 2006. These low levels of tolerant progeny are consistent with reported rates of plant-to-plant outcrossing in canola, which average approximately

20% for closely spaced plants (Cuthbert and McVetty 2001). These levels of HT are thus likely the result of intraspecific PMGF, occurring either between escaped plants and adjacent crops, or among escaped canola plants. Escaped canola populations containing HT traits such as those examined here are ubiquitous in canola growing regions of western Canada (Yoshimura et al. 2006), and may thereby serve as pollen sources or pollen sinks, contributing to the spread of HT traits across the landscape and complicating volunteer control.

IMIDAZOLINONE TOLERANCE

Imidazolinone tolerance was less frequent among escaped canola populations in this study than glyphosate and glufosinate tolerance. In 2004, imidazolinone tolerance was observed in four of the six tested populations and in progeny from 12 (29%) of 42 tested maternal plants (Table 3.3). In 2005, imidazolinone tolerance was not observed in any of the tested populations; in 2006, imidazolinone tolerance was observed in only one of the five tested populations, and in progeny from 4 (9%) of 46 tested maternal plants. Progeny exhibiting full tolerance (as visually assessed) were rare, occurring only from three maternal plants (7%) in 2004 and two maternal plants (4%) in 2006. Most imidazolinone tolerant plants produced progeny exhibiting only partial tolerance to the herbicide, and therefore probably were not homozygous for the two genes conferring the commercial tolerance trait (Tan et al. 2005). Plants with partial imidazolinone tolerance are likely the result of outcrossing events giving rise to heterozygous plants, containing some combination of the four tolerance alleles. Also, there would be further segregation of these alleles with each subsequent generation of escaped canola plants. The low observed frequency of imidazolinone tolerance in escaped canola populations reflects the lower hectarage planted to imidazolinone tolerant canola varieties in Manitoba. In 2004, only 14% of canola grown in Manitoba was imidazolinone tolerant and in 2005 this proportion had further dropped to 9% (2006 data not yet available) (MASC 2006a). These patterns contrast with those of the more prevalent glyphosate and glufosinate traits, and again suggest that the prevalence of traits in escaped canola populations reflects the presence of the same types in surrounding fields.

MULTIPLE HERBICIDE TOLERANCE

Pollen-mediated gene flow has resulted in the stacking of HT traits in canola volunteers in commercial fields (Beckie et al. 2003; Hall et al. 2000), creating management problems for producers. Such trait-stacking may also occur in non-crop habitats where plants with differing HT traits are present. In this study, one-third of the total tested maternal plants (43 of 129), from 10 populations over three years, produced progeny tolerant to both glyphosate and glufosinate at levels consistent with outcrossing rates in canola (i.e. < 30%) (Table 3.2). Although imidazolinone tolerance was relatively rare in the escaped canola populations, low levels of stacked imidazolinone-glyphosate tolerance were observed in the progeny of seven maternal plants in 2004 and of two plants in 2006; and imidazolinone-glufosinate tolerance was observed in the progeny of four plants in 2004 (data not shown). A single plant from the Rhineland1 population in 2004 produced a seedling with tolerance to all three herbicides. This triple tolerant seedling would had to have been produced through two distinct outcrossing events (Ellstrand 2001), with one of those events involving at least one double tolerant parent plant. Such outcrossing events may occur either within commercial agricultural fields or during the production of pedigreed seed (Beckie et al. 2003; Hall et al. 2000), or within escaped canola populations containing multiple tolerance traits. In this study, conditions necessary for transgene stacking existed within escaped canola populations, as the majority of populations tested contained multiple HT traits, and three populations in 2004 and one in 2006 contained all three HT traits (Tables 3.2 and 3.3). Indeed, all plants producing double or triple tolerant offspring were detected within populations containing multiple tolerance traits. Correspondingly, none of the plants from populations containing only one HT trait produced multiple tolerant progeny (Table 3.2). Rates of outcrossing are reported to increase with increasing proximity of canola plants (Cuthbert and McVetty 2001), indicating that adjacent plants within escaped populations would serve as the most likely source of gene flow to create multiple tolerant progeny.

Whereas low levels of double tolerant progeny likely arose through outcrossing events in the immediate preceding (maternal) generation, the higher proportions of multiple tolerance (i.e. 60-99%) observed are well above those expected for single-season outcrossing events and are unlikely to be produced through PMGF within one generation in canola. In 2004, two escaped plants produced high levels of double tolerant offspring, one producing 65% glyphosate-glufosinate tolerant progeny (Table 3.2), and the other producing 68% glyphosate-imidazolinone tolerant offspring (data not shown). These levels of double tolerant progeny are close to those expected for multiple tolerant maternal plants hemizygous/heterozygous for two herbicide tolerance traits, which would produce approximately 56% (9:7) double tolerant progeny according to Mendelian segregation ratios for self-fertilized maternal plants heterozygous for two independent nuclear dominant genes (Rédei 1982). Pedigreed HT canola seedlots containing adventitious HT traits (Friesen et al. 2003) may serve as a source of seed producing multiple tolerant plants, which can be introduced into non-crop habitats by agricultural activities. Alternatively, given the apparent frequency of outcrossing events occurring among canola plants in escaped populations in this study, these putative double tolerant maternal plants might be the progeny of escaped plants from the previous year.

OUTCROSSING WITHIN ESCAPED CANOLA POPULATIONS

To confirm the production of multiple HT progeny through outcrossing events within escaped canola populations, both the leaf tissue and the progeny of certain escaped plants in two populations were tested for glyphosate and glufosinate tolerance. A comparison of the HT traits of maternal plants with those of their progeny allows identification of outcrossing events within escaped canola populations or with adjacent canola crops. Without such testing, it is impossible to conclusively determine if the multiple HT progeny of escaped canola plants are the result of outcrossing events, or introduction of multiple HT seed into roadside habitats (Aono et al. 2006). However, this method may underestimate actual outcrossing rates, as gene flow between canola plants with the same HT traits will not be detected. The objective of this study was to detect the occurrence of outcrossing events in escaped canola populations, rather than to precisely quantify the rate at which this occurs. Additionally, given that levels of outcrossing are expected to be low, and that seed and space limitations precluded the testing of larger numbers of progeny from each maternal plant, outcrossing events were not always similarly detected among the progeny of the same maternal plants in the two herbicide trials. However, the use of two parallel glyphosate/glufosinate herbicide trials doubled the sample size used to detect double tolerant progeny.

In 2005, among the 21 tested maternal canola plants from the escaped MacDonald2 population, six plants were identified as glyphosate tolerant, 13 plants as glufosinate tolerant, and only two plants as neither glyphosate nor glufosinate tolerant. In 2004, fields adjacent to the MacDonald2 population were seeded to glufosinate tolerant canola, and seed losses during seeding or harvest may have contributed to the escaped population in 2005. Additionally, producers that were interviewed identified the adjacent road as having high agricultural traffic intensity, which may also have served as a source of seed for the escaped population. In September 2005, 13 tested maternal plants were harvested from the MacDonald2 population for herbicide screening of the progeny. Maternal phenotypes inferred from herbicide trial results were consistent with test strip results, though the production of a small proportion of susceptible offspring by certain HT maternal plants (Y_4 and $U_{1, 2, 4, 6}$; Figure 3.1a, c) indicates that these plants were hemizygous for the glyphosate and glufosinate tolerance traits. Low levels of double tolerance were identified among the progeny of two maternal plants (Y_3 and U_7) that expressed only single tolerance traits in their leaf tissue (Figure 3.1a, c), confirming that double tolerant progeny in the escaped population resulted from outcrossing events. As fields adjacent to the MacDonald2 population were seeded to wheat and oats in 2005, the most likely source of pollen contributing to outcrossing events were canola plants within the escaped population itself, which contained both glyphosate and glufosinate tolerant plants.

Outcrossing events were much more frequent in the Dufferin1 escaped population in 2006, introducing adventitious tolerance traits into the progeny of single tolerant and susceptible maternal plants. Among the 27 tested maternal plants, 17 were identified as glyphosate tolerant, two as glufosinate tolerant, and eight maternal plants as neither glyphosate nor glufosinate tolerant. In 2005, the field adjacent to this escaped population was seeded to glyphosate tolerant canola, and seeding or harvest activities may have contributed seed to the escaped population. As before, producers also indicated that agricultural traffic intensity was high on the adjacent road, and this may also have served as a seed source. In September 2006, 21 tested maternal plants were harvested from the Dufferin1 population for herbicide screening of the progeny. Again, maternal phenotypes inferred from herbicide trials were consistent with test strip results. However, all HT maternal plants from the Dufferin1 population produced some susceptible offspring (Figure 3.1b, d), indicating that these plants were hemizygous for the HT traits. Progeny from 15 of the tested plants expressed HT traits acquired through outcrossing events. These included 10 of the 15 glyphosate tolerant maternal plants ($Y_{1, 3\text{-}6, 8, 12\text{-}15}$) which produced double tolerant and/or glufosinate tolerant progeny, and five susceptible plants ($S_{1\text{-}5}$), which produced glyphosate tolerant progeny (Figure 3.1b, d). Surprisingly, two susceptible maternal plants ($S_{2, 5}$) produced high levels (i.e. 71-79%) of glyphosate tolerant progeny, well above the average rate of outcrossing in canola (Figure 3.1b). In 2006, fields adjacent to the Dufferin1 population were seeded to corn and glufosinate tolerant canola, and would not have provided a source of canola pollen containing the glyphosate tolerance trait. The large number of glyphosate tolerant plants present within the escaped population itself likely provided a considerable pollen source, and the proximity of escaped canola plants may have contributed to high levels of outcrossing. Similarly, very high individual plant outcrossing rates (i.e. 82%) have been reported for closely spaced (i.e. 10 cm) canola plants in field plots (Cuthbert and McVetty 2001). That outcrossing can also occur at such high levels in escaped canola populations suggests that these populations may contribute considerably to the spread and stacking of HT traits in agricultural landscapes. Because of the widespread planting of hybrid canola varieties (MASC 2006b), there may also be male sterility traits present in escaped canola populations; however, this was not assessed in this study.

The progeny of four susceptible maternal canola plants ($S_{1, 2, 4, 5}$) acquired glufosinate tolerance traits also through outcrossing events, and one maternal plant (S_2) produced a double tolerant seedling (Figure 3.1d), which necessarily would have arisen through PMGF from a double tolerant paternal plant. Movement of multiple HT traits among escaped canola plants or between escaped populations and neighbouring canola crops has important implications for farmers. Adventitious HT traits may potentially contaminate canola crops of other varieties, complicating volunteer control (Friesen et al. 2003), compromising purity standards (Smyth et al. 2002), and putting producers at legal risk (Mauro 2008).

IMPLICATIONS OF THIS STUDY

Escaped canola populations are ubiquitous in agricultural landscapes in western Canada. Although transient in nature, these populations appear to receive substantial seed- and pollen-mediated gene flow from neighbouring fields and other agricultural sources, which contributes to their persistence. Localized escaped populations may also facilitate gene flow between separate agricultural fields, and, indeed, may comprise larger metapopulations that persist at higher scales of organization (A. Knispel, unpublished data). The results of our study confirm that HT traits are common in escaped canola populations in agricultural regions where these tolerant varieties are widely grown. Additionally, intraspecific gene flow occurs frequently and at high levels in escaped canola populations, resulting in the development of multiple tolerant progeny within these non-crop habitats. The rapid spread and stacking of HT genes in escaped populations may increase chances of contamination of adjacent fields and may compromise agricultural production systems, especially non-GM, zero-till, and organic systems. The presence and movement of HT traits in non-crop environments confirms the difficulty of retracting novel plant traits once approved for unconfined release (Marvier and Van Acker 2005), and has important implications for the contamination of non-GM and organic crops (Friesen et al. 2003; Mauro and McLachlan 2008; Smyth et al. 2002). Given the widespread cultivation of GM-HT canola in western Canada, and the common presence of escaped HT plants in roadside habitats, satisfactory isolation distances for non-HT crops are increasingly difficult to maintain (Beckie et al. 2006). This study also provides insight into the potentially detrimental spread and persistence of future novel plant traits once approved for unconfined release, and has important implications for the introduction of even more controversial pharmaceutical and industrial traits into crop plants (Ellstrand 2001). Such traits represent substantial potential liabilities for producers (Smyth et al. 2002), which would be compounded by dispersal, persistence, and outcrossing in non-crop habitats and with neighbouring fields. Given the current concern over GM trait confinement (Demeke et al. 2006), the observed prevalence of escaped canola populations with HT traits and the frequency of outcrossing events within these escaped populations warrants increased caution when regulating and releasing other GM plant traits in the future.

Sources of Materials

[1] TraitChek™ test strips, Strategic Diagnostics Inc., 111 Pencader Drive, Newark, Delaware 19702.

References

Aono, M., S. Wakiyama, M. Nagatsu, N. Nakajima, M. Tamaoki, A. Kubo, and H. Saji. 2006. Detection of feral transgenic oilseed rape with multiple-herbicide resistance in Japan. Environ. Biosafety Res. 5:77-87.

Beckie, H. J., K. N. Harker, L. M. Hall, S. I. Warwick, A. Légère, P. H. Sikkema, G. W. Clayton, A. G. Thomas, J. Y. Leeson, G. Séguin-Swartz, and M.-J. Simard. 2006. A decade of herbicide-resistant crops in Canada. Can. J. Plant Sci. 86:1243-1264.

Beckie, H. J., S. I. Warwick, H. Nair, and G. Séguin-Swartz. 2003. Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). Ecol. Appl. 13:1276-1294.

Crawley, M. J. and S. L. Brown. 1995. Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. Proc. R. Soc. Lond. B. 259:49-54.

Crawley, M. J. and S. L. Brown. 2004. Spatially structured population dynamics in feral oilseed rape. Proc. R. Soc. Lond. B. 271:1909-1916.

Crawley, M. J., S. L. Brown, R. S. Hails, D. D. Kohn, and M. Rees. 2001. Transgenic crops in natural habitats. Nature 409:682-683.

Crawley, M. J., R. S. Hails, M. Rees, D. H. Kohn, and J. Buxton. 1993. Ecology of transgenic oilseed rape in natural habitats. Nature 363:620-623.

Cuthbert, J. L. and P. B. E. McVetty. 2001. Plot-to-plot, row-to-row, and plant-to-plant outcrossing studies in oilseed rape. Can. J. Plant Sci. 81:657-664.

De Corby, K. A., R. C. Van Acker, A. L. Brûlé-Babel, and L. F. Friesen. 2007. Emergence timing and recruitment of volunteer spring wheat. Weed Sci. 55:60-69.

Demeke, T., D. J. Perry, and W. R. Scowcroft. 2006. Adventitious presence of GMOs: Scientific overview for Canadian grains. Can. J. Plant Sci. 86:1-23.

Ellstrand, N. C. 2001. When transgenes wander, should we worry? Plant Physiol. 125:1543-1545.

Friesen, L. F., A. G. Nelson, and R. C. Van Acker. 2003. Evidence of contamination of pedigreed canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. Agron. J. 95:1342-1347.

Gulden, R. H., S. J. Shirtliffe, and A. G. Thomas. 2003. Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. Weed Sci. 51:83-86.

Hall, L., K. Topinka, J. Huffman, L. Davis, and A. Good. 2000. Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. Weed Sci. 48:688-694.

Hall, L. M., M. H. Rahman, R. H. Gulden, and A. G. Thomas. 2005. Volunteer oilseed rape: will herbicide-resistance traits assist ferality? Pages 59-79 *in* J. Gressel (ed.) Crop Ferality and Volunteerism. Boca Raton, FL: Taylor and Francis Books.

Lawson, A. N., R. C. Van Acker, and L. F. Friesen. 2006. Emergence timing of volunteer canola in spring wheat fields in Manitoba. Weed Sci. 54:873-882.

Légère, A. 2005. Risks and consequences of gene flow from herbicide-resistant crops: canola (*Brassica napus*) as a case study. Pest Manag. Sci. 61:292-300.

Marvier, M. and R. C. Van Acker. 2005. Can crop transgenes be kept on a leash? Front. Ecol. Environ. 3:99-106.

MASC (Manitoba Agricultural Services Corporation). 2006a. Manitoba Management Plus Program. http://www.masc.mb.ca/mmpp.nsf/Home_Page.html. Accessed: May 15, 2007.

MASC (Manitoba Agricultural Services Corporation). 2006b. Yield Manitoba 2006. Published by Farmers' Independent Weekly Ltd., Box 1846, Station Main, Winnipeg, MB R3C 3R1. 52 pp.

MASC (Manitoba Agricultural Services Corporation). 2007. Yield Manitoba 2007. Published by Farmers' Independent Weekly Ltd., Box 1846, Station Main, Winnipeg, MB R3C 3R1. 52 pp.

Mauro, I.J. 2008. *Riding the Risk Wave: Farmer Knowledge and Experience with GM Crops in the Canadian Prairies.* PhD Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Mauro, I. J. and S. M. McLachlan. 2008. Farmer knowledge and risk analysis: postrelease evaluation of herbicide-tolerant canola in western Canada. Risk Anal. 28:463-476.

Pessel, D., J. Lecomte, V. Emeriau, K. Krouti, A. Messean, and P. H. Gouyon. 2001. Persistence of oilseed rape (*Brassica napus*) outside of cultivated fields. Theor. Appl. Genet. 102:841-846.

Rakow, G. and D.L. Woods. 1987. Outcrossing in rape and mustard under Saskatchewan prairie conditions. Can. J. Plant Sci. 67: 147-151.

Rédei, G. P. 1982. Genetics. Macmillan: New York. pp. 98-99.

Scott, S. E. and M. J. Wilkinson. 1998. Transgene risk is low. Nature 393:320.

Simard, M.-J., A. Légère, D. Pageau, J. LaJeunesse, and S. Warwick. 2002. The frequency and persistence of volunteer canola (*Brassica napus*) in Quebec. Weed Technol. 16: 433-439.

Simard, M.-J., A. Légère, G. Séguin-Swartz, H. Nair, and S. Warwick. 2005. Fitness of double vs. single herbicide-resistant canola. Weed Sci. 53:489-498.

Smyth, S., G. C. Khachatourians, and P. W. B. Phillips. 2002. Liabilities and economics of transgenic crops. Nature Biotech. 20:537-541.

Tan, S., R. R. Evans, M. L. Dahmer, B. K. Singh, and D. L. Shaner. 2005. Imidazolinone-tolerant crops: history, current status and future. Pest Manag. Sci. 61:246-257.

Van Acker, R. C., A. L. Brûlé-Babel, L. F. Friesen, and M. H. Entz. 2003. GM - Non-GM crops coexistence in western Canada: Can it work? Pages 189-196 *in* Manitoba Agronomists Conference. Winnipeg, Manitoba: University of Manitoba. http://www.umanitoba.ca/afs/agronomists_conf/2003/pdf/vanacker_GM_nonGM_crops.pdf. Accessed: May 23, 2007.

Waines, J. G. and S. G. Hegde. 2003. Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. Crop Sci. 43:451-463.

Warwick, S. I., M.-J. Simard, A. Légère, H. J. Beckie, L. Braun, B. Zhu, P. Mason, G. Séguin-Swartz, and C. N. Stewart. 2003. Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O.E. Schulz. Theor. Appl. Genet. 107:528-539.

Wilkinson, M. J., L. J. Elliott, J. Allainguillaume, M. W. Shaw, C. Norris, R. Welters, M. Alexander, J. Sweet, and D. C. Mason. 2003. Hybridization between *Brassica napus* and *B. rapa* on a national scale in the United Kingdom. Science 302:457-459.

Yoshimura, Y., H. J. Beckie, and K. Matsuo. 2006. Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. Environ. Biosafety Res. 5:67-75.

Table 3.1. Characteristics of escaped canola populations in the rural municipalities of Rhineland, MacDonald, Roland, and Dufferin, in southern Manitoba, Canada.

Site / year	Density [a]	Adjacent crop	Adjacent crop in previous year	Time since last canola crop in adjacent field
	plants/m^{-2}			yr
Rhine1[b] 2004	2.8 (0.45)	corn	beans	10
Rhine2 2004	1.5 (0.23)	corn	beans	>10
Rhine4 2004	5.9 (0.66)	GLU canola	n/a	0
MacD1 2004	1.0 (0.25)	GLY canola	barley	0
MacD2 2004	n/a	GLU canola	wheat	0
MacD4 2004	3.4 (0.74)	alfalfa	alfalfa	>10
Rhine3 2005	n/a	pinto beans	corn	>6
Rhine4 2005	1.9 (0.60)	wheat	GLU canola	1
MacD1 2005	1.2 (0.29)	barley	GLY canola	1
MacD2 2005	1.3 (0.34)	wheat	GLU canola	1
MacD4 2005	0.3 (0.10)	alfalfa	alfalfa	>10
MacD1 2006	0.5 (0.13)	IMI canola	barley	0
Rol1 2006	10.4 (1.56)	corn	soybeans	4
Rol2 2006	15.5 (1.29)	corn	barley	2
Duff1 2006	11.2 (0.77)	corn	GLY canola	1
Duff2 2006	3.1 (0.41)	wheat	GLY canola	1

[a] Density of escaped canola plants was counted in 30 1 x 1 m quadrats in the roadside ditches in August 2004, 2005, and 2006. Mean density is presented with standard errors in parentheses.

[b] Abbreviations: Rhine, Rhineland; MacD, MacDonald; Rol, Roland; Duff, Dufferin; GLY, glyphosate tolerant; GLU, glufosinate tolerant; IMI, imidazolinone tolerant; n/a, not available.

Table 3.2. Number of escaped canola plants growing in roadside populations producing glyphosate and glufosinate tolerant progeny in 2004, 2005, and 2006 in southern Manitoba, Canada.

Site / year	Maternal plants tested	Plants with GLY[a] tolerant progeny	Plants with GLU tolerant progeny	Plants with double tolerant progeny
	no.	----------------- no. (percent range) [b] -----------------		
Rhine1 2004	5	5 (18-75%)	5 (38-100%)	5 (4-65%)
Rhine2 2004	4	4 (63-96%)	0	0
Rhine4 2004	10	7 (4-79%)	8 (4-100%)	3 (4-21%)
MacD1 2004	10	8 (4-100%)	0	0
MacD2 2004	10	9 (4-100%)	9 (4-100%)	9 (4-25%)
MacD4 2004	6	6 (4-100%)	3 (4-10%)	2 (4-10%)
Rhine3 2005	7	7 (68-84%)	1 (4%)	1 (7%)
Rhine4 2005	2	0	2 (25-100%)	0
MacD1 2005	7	0	7 (4-100%)	0
MacD2 2005	13	5 (7-100%)	9 (7-100%)	2 (7%)
MacD4 2005	8	3 (4-100%)	6 (4-100%)	0
MacD1 2006	4	4 (86-100%)	1 (7%)	2 (4-7%)
Rol1 2006	10	7 (4-100%)	9 (4-100%)	5 (4-25%)
Rol2 2006	8	6 (13-100%)	7 (4-96%)	5 (4-11%)
Duff1 2006	21	20 (5-82%)	14 (4-52%)	10 (4-7%)
Duff2 2006	4	4 (59-86%)	0	0

[a] Abbreviations: Rhine, Rhineland; MacD, MacDonald; Rol, Roland; Duff, Dufferin; GLY, glyphosate; GLU, glufosinate; HT , herbicide-tolerant.

[b] The percent range of the proportion of tolerant progeny per maternal plant is given in parentheses; values ranged from 0 to 100%.

Table 3.3. Number of escaped canola plants growing in roadside populations producing intermediate and fully imidazolinone tolerant progeny in 2004, 2005, and 2006 in southern Manitoba, Canada.

Site / year[a]	Maternal plants tested	Plants with IMI[b] intermediate progeny	Plants with IMI tolerant progeny
	no.	----- no. (percent range)[c] -----	
Rhine1 2004	5	3 (4-8%)	0
Rhine2 2004	4	0	0
Rhine4 2004	10	0	0
MacD1 2004	9	2 (4-11%)	2 (100%)
MacD2 2004	10	1 (68%)	0
MacD4 2004	4	3 (8-100%)	1 (100%)
Rhine3 2005	6	0	0
MacD1 2005	5	0	0
MacD2 2005	11	0	0
MacD4 2005	2	0	0
MacD1 2006	3	0	0
Rol1 2006	10	0	0
Rol2 2006	8	0	0
Duff1 2006	21	2 (48-100%)	2 (52-100%)
Duff2 2006	4	0	0

[a] Note: There was insufficient seed available from the Rhine 4 2005 population for imidazolinone tolerance testing.

[b] Abbreviations: Rhine, Rhineland; MacD, MacDonald; Rol, Roland; Duff, Dufferin; IMI, imidazolinone.

[c] Percentage of tolerant progeny per maternal plant varied from 0 to 100%, and ranges are included in parentheses.

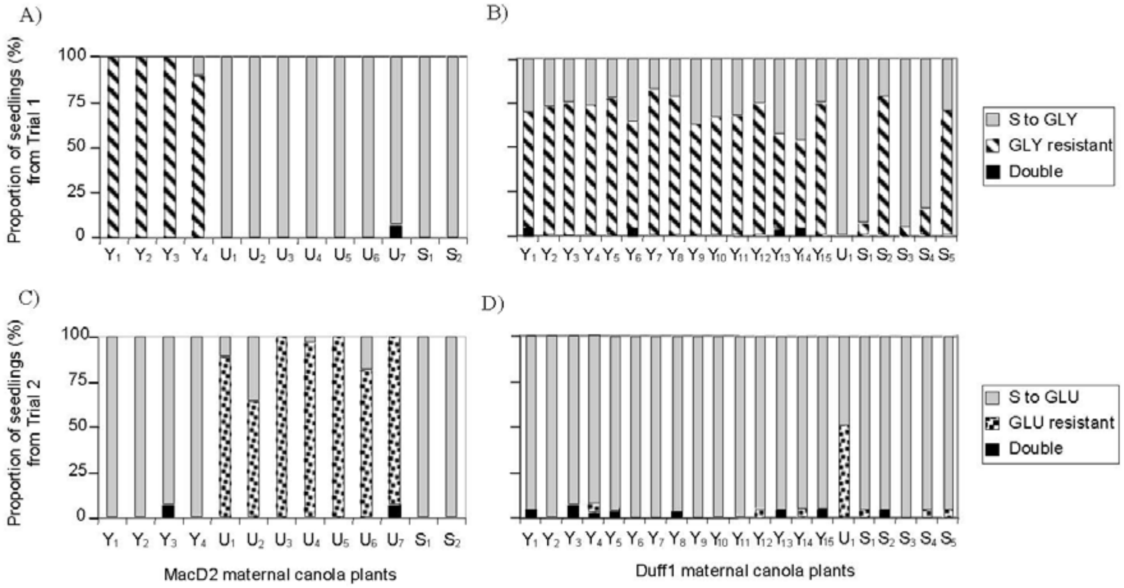

Figure 3.1. Proportions of herbicide-tolerant progeny from 13 escaped canola plants collected from the MacDonald2 population in 2005 (a, c) and from 21 escaped canola plants collected from the Dufferin1 population in 2006 (b, d). Seedlings (progeny) were sprayed with glyphosate and then glufosinate in Trial 1 (a, b), and sprayed with glufosinate and then glyphosate in Trial 2 (c, d). Proportions of tested seedlings (20 to 28 seedlings per maternal plant in each trial) exhibiting single and double tolerance to glyphosate and glufosinate are depicted by the different shades in each stacked bar. Phenotypes of maternal canola plants were determined using test strips. *Abbreviations: S, susceptible; GLY, glyphosate; GLU, glufosinate; Y, glyphosate tolerant; U, glufosinate tolerant.

CHAPTER 4

DEMOGRAPHIC MODELS IN POST-RELEASE MONITORING OF GENETICALLY MODIFIED CANOLA IN WESTERN CANADA: IMPLICATIONS FOR GENE FLOW AND MANAGEMENT

Chapter Summary

Matrix population modeling is an important tool for risk assessment of novel genetically modified (GM) crops. However, few such models exist for GM crops in North America, where transgenic canola has been grown commercially for over a decade and where it is increasingly recognized as a prevalent weed. Matrix models may be particularly useful for better understanding the post-release dynamics of GM canola, which escapes easily from cultivation and is ubiquitous in ruderal habitats. This study represents the first time that the multi-year population dynamics of transgenic herbicide-tolerant canola varieties have been monitored outside of cultivated fields. Dynamics of four escaped populations of GM canola were examined within roadside habitats over two years; all plants tested (n = 44) in the four populations expressed transgenic herbicide tolerance traits in maternal plant tissue or among progeny. Seasonally-structured periodic matrix models were used to describe the dynamics of flowering plants in escaped populations of GM canola and to examine projected population fates. Additionally, a replicated seed addition experiment was conducted to quantify canola seedling establishment and to compare experimental and background rates of seed input in roadside habitats. Escaped populations of GM canola in roadside habitats flowered in synchrony with cultivated canola crops, confirming the potential for escaped plants to contribute to gene flow within agricultural landscapes. Even where models projected overall population decline, flowering plants were projected to persist for 2 to 5 years. As crops are generally rotated every 3 years in Manitoba, flowering plants in escaped GM canola populations will co-occur with at least one canola crop in adjacent fields. Our demographic models reflected high levels of spatial and temporal variation in roadside habitats, distinguishing two patterns of population dynamics for GM canola. Along less-traveled gravel roads, escaped canola populations will develop if crop management activities introduce seed into ideal habitat. Persistence of such populations relies on ongoing disturbance, enabling recruitment and establishment of large reproductive plants. Conversely, along high-traffic highways, escaped canola population growth is dependent on the seed life stage and is supplemented by anthropogenic seed inputs, while ongoing roadside management opens recruitment sites and prevents competitive exclusion by perennial vegetation. Our findings point to the importance of systematic post-release monitoring of GM crops, even for varieties normally considered non-invasive. While targeted control of escaped populations arising from sowing or harvest activities is feasible, management of escaped canola seed banks and prevention of seed inputs from agricultural transport is largely impossible. Effective control of gene flow from escaped GM populations necessitates collaborative regional approaches to management and, in some cases, will require the development of segregated production areas.

Introduction

Matrix population models allow for the quantitative projection of population demographics over time, enabling improved understanding of population viability and more effective management of both threatened (e.g. Morris & Doak 2002) and invasive populations (e.g. Jongejans, Sheppard & Shea 2006). Such models have also been used to assess the potential invasiveness of plants with novel traits. Indeed, Bullock (1999) issued an early call for the use of matrix models in proactive risk assessments of the environmental impacts of new transgenic crop varieties. Advocated as a means of identifying crucial demographic parameters associated with increased invasiveness in transgenic plants, this approach has been used in the pre-release assessment of genetically modified (GM) canola (*Brassica napus* L.) in Europe, with a particular focus on the movement of canola plants beyond arable fields. Matrix models at both the population (e.g. Claessen *et al.* 2005a; Begg *et al.* 2006; Garnier, Deville & Lecomte 2006) and metapopulation (e.g. Claessen *et al.* 2005b) levels identified the importance of seed parameters in contributing to persistence of escaped canola populations in ruderal non-crop habitats. Generally, these models predict persistence times of 5 to 10 years for escaped canola populations; persistence increases with higher levels of disturbance (Claessen *et al.* 2005a) and seed dispersal (Claessen *et al.* 2005b; Garnier & Lecomte 2006), factors that reduce competition from perennial vegetation and enable colonization of suitable habitat, respectively. These models further suggest the potential contribution of escaped canola populations to gene flow at large spatial scales (Garnier, Deville & Lecomte 2006). However, to our knowledge, such models have not yet been parameterized using data from escaped populations containing commercially-released transgenic traits. Furthermore, demographic models are generally parameterized using data from a wide range of literature sources (e.g. Claessen *et al.* 2005 a, b; Begg *et al.* 2006), rather than from empirical surveys of escaped plants in non-crop habitats. Without such empirical data, spatial and temporal variation in habitat conditions is often overlooked, reducing the relevance of the models, particularly with regard to determination of effective management practices (Garnier, Deville & Lecomte 2006).

In western Canada, canola is widely cultivated as a spring annual crop; second only to wheat, canola represents 25% of the annual field crop cover in any given year

(Statistics Canada 2009). Herbicide-tolerant varieties represent over 95% of the canola grown, having been rapidly adopted since their commercial release in 1996 (Beckie *et al.* 2006). Concurrently, canola has become increasingly problematic as a volunteer in arable fields (Leeson *et al.* 2005; Mauro & McLachlan 2008) and as a prominent weed of ruderal habitats in the agricultural province of Manitoba and across the Canadian prairies (Yoshimura, Beckie & Matsuo 2006; Knispel & McLachlan, 2010).

Though canola is not normally considered to be invasive (Gulden, Warwick & Thomas 2008), risks associated with GM crops are not limited to invasiveness (Claessen *et al.* 2005a). Quantification of long-term population growth rates and simple measures of extinction probability are not sufficient for comprehensive risk assessment of GM crops. Consideration of even transient population dynamics is necessary where ecological or agronomic consequences may arise over shorter time frames and where metapopulation dynamics are observed at larger scales of organization (Knispel & McLachlan 2010). In this context, knowledge of the population biology of volunteer GM canola in western Canada remains limited (Gulden, Warwick & Thomas 2008), and still less is known regarding escaped GM canola in ruderal habitats. Dynamics of escaped GM crop populations need to be fully characterized to accurately describe the risks emerging following commercial release of these crops (Bagavathiannan & Van Acker 2008).

In this study, we undertook multi-site and multi-year demographic surveys of escaped canola populations in Manitoba, Canada, and developed periodic matrix population models to describe the seasonal and annual dynamics of canola in roadside habitats. The overall aim of this research is to better understand the demography and phenology of escaped canola and any implications for population persistence, particularly in relation to gene flow from GM crops. More specifically, our objectives were to: (1) characterize spatial and temporal variation in escaped population demography in agricultural landscapes; (2) examine the relative importance of different life stages to population persistence; and (3) explore the underlying role of disturbance and agricultural activities in affecting population dynamics.

Methods

STUDY SYSTEM

In roadside habitats in Manitoba, Canada, canola population dynamics are seasonal. Most seeds germinate in early spring and seedlings require open, disturbed habitats to become established and to develop into adult plants (Crawley *et al.* 1993). Active growth of canola plants from seedling to vegetative, flowering and reproductive stages occurs in late spring and summer. Plant growth form is highly plastic in response to habitat conditions and disturbance (Gulden, Warwick & Thomas 2008), and new growth may be initiated from cut or damaged stems. Flowers are produced in racemes and are primarily self-fertilized, though outcrossing rates ranging between 50 and 80% have been reported (Cuthbert & McVetty 2001; Gulden, Warwick & Thomas 2008). Seed production in roadside habitats is highly variable, but can be comparable to seed production by cultivated canola (Knispel *et al.* 2008). Seeds are produced in August in pods (siliques) that shatter easily when dry, releasing seeds in proximity to the parent plant (Gulden, Warwick & Thomas 2008). Seeds lack primary dormancy and may germinate in response to rainfall at the end of the growing season. Though some plants may persist over winter in Manitoba if conditions are ideal (Lawson *et al.* 2006; Knispel *et al.* 2008), above-ground plant stages are generally killed by extreme winter temperatures and the population persists solely as seeds at or near the soil surface.

The Lake Manitoba Plain Ecoregion of southern Manitoba is characterized by high quality arable land suitable to cultivation of dryland grain and oilseed crops (Smith *et al.* 1998). Within this ecoregion, our study was located in the Carman agricultural landscape, an important production area for canola where the crop is generally cultivated every 3 to 4 years in rotation and where escaped plants are common. Roadside verge habitats in this agricultural landscape receive anthropogenic seed inputs from the commercial transport of grain and these inputs are generally proportional to the intensity of agricultural traffic (Knispel & McLachlan 2010).

Four populations of escaped canola were identified along roadside verges in the Carman landscape when plants were in flower in early July 2006. Two populations, C1 and C2, were located along a primary paved provincial highway in proximity (i.e. ≤ 4 km) to a grain distribution elevator. In contrast, populations C3 and C4 were located on a secondary rural gravel road and were farther (i.e. 11 – 12 km) from grain distribution centres (Table 4.1). Most, if not all, escaped canola plants in these four populations exhibited transgenic herbicide tolerance traits; all plants tested (*n* = 44) in associated herbicide trials (Knispel *et al.* 2008) expressed herbicide tolerance or produced herbicide-tolerant progeny (Table 4.1). At all sites, escaped canola plants were generally growing in the open, disturbed gravel verge adjacent to perennial ditch vegetation. This habitat is subject to periodic management, and vegetation at all four sites was mowed repeatedly over the course of the study. In addition, highway sites were repeatedly graded as part of regular highway maintenance (Table 4.1) and gravel verges at these sites were also frequently disturbed by vehicles pulling to the side of these high-traffic roads (A.L. Knispel, personal observation).

DEMOGRAPHIC CENSUS

Escaped canola plants were monitored in the four study populations from July to October 2006 and from May to October 2007. In 2006, 30 randomly-located permanent 1 x 1 m quadrats were established along a 100 m transect in the open gravel verge at each site. In both years, biweekly surveys were conducted in all quadrats. Emerging canola seedlings were marked and mapped, and their survival and transition to vegetative, flowering and podding (reproductive) stages was subsequently recorded. In 2006, 1789 plants were monitored from July to August (i.e. four biweekly surveys) in the four populations (Table 4.1). In 2007, 4309 plants were monitored from May to August (i.e. eight biweekly surveys; Table 4.1). Additionally, in both years, newly established seedlings were counted throughout September and October in all populations, to account for seed lost to lethal fall germination.

To quantify fecundity of escaped canola, mature podding plants were collected from outside the permanent quadrats in August of both years. In 2006, all seeds were counted from ten plants collected in population C1, eight plants in C2, 26 plants in C3, and four plants in C4. In 2007, fewer mature reproductive plants were present in the populations and seed were counted from 22 racemes from 15 plants in populations C1, C2 and C3. For all reproductive plants surveyed in 2007 ($n = 156$) we recorded number of podding racemes per plant. Seed production was calculated by multiplying the average number of racemes per plant in each site by the overall average number of seeds per raceme. Seed viability was tested in indoor germination experiments in 2006 (see Knispel *et al.* 2008), and non-germinating seeds were considered non-viable.

SEED-ADDITION EXPERIMENT

In spring 2007, seed-addition experiments were initiated to quantify canola seedling establishment in roadside habitats. On April 30th and May 1st, commercial canola seed from the 'Clearfield' variety 45A77 (imidazolinone tolerant) was spread on the soil surface of six permanent quadrats in each population. Three 1 x 1 m quadrats received 600 seeds, three quadrats received 100 seeds, and three additional quadrats were designated as untreated controls. Seedling emergence was monitored in May and June in experimental quadrats, and the number of newly established seedlings observed in each biweekly survey was recorded for all experimental quadrats in all populations. Average biweekly seedling establishment was log+1-transformed and compared using factorial analysis of variance (ANOVA) with two between-groups factors: treatment level (i.e. 600 seeds, 100 seeds, control) and road type (i.e. paved highway [C1,C2] and gravel surface [C3, C4]). Given a significant ($p < 0.0001$) interaction, we tested the simple effect of treatment level within each road type. Where overall model effects were significant, the Ryan-Einot-Gabriel-Welsch multiple range test was used to separate means (SAS 9.1, SAS Institute Inc., USA). Untransformed means are presented.

MATRIX MODEL CONSTRUCTION

We modeled the dynamics of escaped canola in roadside habitats using five life stages: seeds, seedlings, vegetative plants, flowering plants and podding plants (stages 1 to 5, respectively). Four unique phases were identified to describe seasonal variation in the annual canola life cycle (Caswell 2001). Life cycle diagrams corresponding to these phases are graphical representations of seasonal transition matrices (Figure 4.1). Arrows (i.e. stage transitions) in each diagram correspond to matrix elements (e.g. g_{ij}) describing the contribution of stage j individuals at time t to stage i individuals at time $t + 1$. For annual organisms such as canola, not all life stages and stage transitions are observed in all seasons; correspondingly, seasonal transition matrices may vary in dimension (Caswell 2001). Additionally, seasonal matrices may project the population over time-steps of varying duration. However, when combined in sequence, the seasonal transition matrices describe population dynamics over a complete annual cycle (Caswell 2001).

For each of the study populations, we constructed seasonal transition matrices describing population demographics in the germination (Figure 4.1a), spring (Figure 4.1b), summer (Figure 4.1c), and winter (Figure 4.1d) phases of the annual cycle. Matrix elements were calculated from seasonal- and stage-specific vital rates derived from biweekly demographic surveys. For seedling, vegetative and flowering stages, we calculated survival (σ_2, σ_3, and σ_4, respectively) as the probability that a plant in a given life stage survived from one survey to the next, regardless of transitions between stages. Given the annual habit of canola, podding plants were assumed to die after producing seed (i.e. $\sigma_5 = 0$).

Stage transition probabilities between seedling, vegetative, flowering and podding plants were calculated as the probability that surviving individuals from stage j in the previous survey transitioned to stage *i* in the current survey (i.e. τ_{ij}) or, conversely, remained in the same stage (i.e. τ_{jj}). Based on life stage transitions observed in the study populations in spring and summer, surviving seedlings could transition to vegetative (τ_{32}) or flowering (τ_{42}) stages, or remain as seedlings (τ_{22}). Vegetative plants could transition to flowering (τ_{43}) or podding (τ_{53}) stages, or remain as vegetative plants (τ_{33}). Finally, flowering plants could transition back to vegetative plants if cut (τ_{34}), could remain as flowering plants (τ_{44}), or could develop into mature podding plants (τ_{54}). The contribution of podding plants to the seed stage was calculated as the average seed production per plant (π) corrected for average seed viability (ϖ). Survival (σ_j), transition (τ_{ij}) and seed production (π) vital rates for seedling, vegetative, flowering and podding plants were calculated separately from spring (May – June 2007) and summer (July – August 2006, 2007) survey data.

The probability of seedling establishment in spring and summer (ε) was calculated for each population by expressing the biweekly average number of emerging seedlings in the 600 seed treatment quadrats as a proportion of the seed added. Seedling emergence rates were corrected for background germination observed in control quadrats, as well as for viability (86.3%) of the experimental seed. Seed survival parameters used in our matrix models were derived from studies conducted in cultivated fields in the adjacent province of Saskatchewan (Gulden, Thomas & Shirtliffe 2004). There, annual seed loss from shallow canola seed banks (i.e. 1 cm below the soil surface) was 90%, including losses due to germination and death. As no information is available on seasonal variation of seed survival, the rate of seed loss from the seed bank was assumed to be constant throughout the year, and biweekly seed survival in spring and summer (σ_1)

can be calculated as $(1 - 0.9)^{1/26}$ (Morris & Doak 2002). Similarly, seed survival over the eight-month winter season ($\sigma_{1(\mathbf{W})}$) is calculated as $(1 - 0.9)^{8/12}$. Contrary to our study, lethal fall germination of canola seed was not observed in the Saskatchewan study, and is therefore not accounted for in the rate of seed loss reported by Gulden and colleagues (2004). We calculated the proportion of seed lost to fall germination (ε_f) as the number of seedlings established in September and October divided by the total annual seed production (i.e. π * number of podding plants) in each population.

Seasonal vital rates were combined to form the elements of the germination (**G**), spring (**P**), summer (**U**), and winter (**W**) transition matrices for each population (g_{ij}, p_{ij}, u_{ij}, and w_{ij}, respectively). The 5 x 1 **G** matrix describes the probability with which seeds in early May emerge as seedlings over a biweekly time-step and accounts for delays in population development associated with germination (i.e. adult plant stages could not develop until seedlings were established):

$$\mathbf{G} = \left| \begin{array}{c} \sigma_1 \\ \varepsilon \\ 0 \\ 0 \\ 0 \end{array} \right| \qquad \text{eqn 1}$$

The 5 x 5 **P** matrix describes biweekly transitions occurring in spring. Three **P** matrices are multiplied to project the population from mid-May to the end of June, though not all possible transitions were observed in every roadside population:

$$\mathbf{P} = \left| \begin{array}{ccccc} \sigma_1 & 0 & 0 & 0 & \pi_{(\mathbf{P})}\varpi \\ \varepsilon & \sigma_{2(\mathbf{P})}\tau_{22(\mathbf{P})} & 0 & 0 & 0 \\ 0 & \sigma_{2(\mathbf{P})}\tau_{32(\mathbf{P})} & \sigma_{3(\mathbf{P})}\tau_{33(\mathbf{P})} & \sigma_{4(\mathbf{P})}\tau_{34(\mathbf{P})} & 0 \\ 0 & \sigma_{2(\mathbf{P})}\tau_{42(\mathbf{P})} & \sigma_{3(\mathbf{P})}\tau_{43(\mathbf{P})} & \sigma_{4(\mathbf{P})}\tau_{44(\mathbf{P})} & 0 \\ 0 & 0 & \sigma_{3(\mathbf{P})}\tau_{53(\mathbf{P})} & \sigma_{4(\mathbf{P})}\tau_{54(\mathbf{P})} & 0 \end{array} \right| \qquad \text{eqn 2}$$

Similarly, the 5 x 5 **U** matrix describes biweekly transitions occurring in summer. Four **U** matrices are multiplied to project the population from the beginning of July to the end of August:

$$\mathbf{U} = \begin{vmatrix} \sigma_1 & 0 & 0 & 0 & \pi_{(\mathbf{U})}\varpi \\ \varepsilon & \sigma_{2(\mathbf{U})}\tau_{22(\mathbf{U})} & 0 & 0 & 0 \\ 0 & \sigma_{2(\mathbf{U})}\tau_{32(\mathbf{U})} & \sigma_{3(\mathbf{U})}\tau_{33(\mathbf{U})} & \sigma_{4(\mathbf{U})}\tau_{34(\mathbf{U})} & 0 \\ 0 & \sigma_{2(\mathbf{U})}\tau_{42(\mathbf{U})} & \sigma_{3(\mathbf{U})}\tau_{43(\mathbf{U})} & \sigma_{4(\mathbf{U})}\tau_{44(\mathbf{U})} & 0 \\ 0 & 0 & \sigma_{3(\mathbf{U})}\tau_{53(\mathbf{U})} & \sigma_{4(\mathbf{U})}\tau_{54(\mathbf{U})} & 0 \end{vmatrix} \qquad \text{eqn 3}$$

Finally, completing the annual cycle, the 1 x 5 **W** matrix describes the collapse of the population into the seed stage over the eight-month winter time-step from September to April:

$$\mathbf{W} = \begin{vmatrix} \sigma_{1(\mathbf{W})}(1-\varepsilon_f) & 0 & 0 & 0 & \pi_{(\mathbf{U})}\varpi\,\sigma_{1(\mathbf{W})}(1-\varepsilon_f) \end{vmatrix} \qquad \text{eqn 4}$$

DEMOGRAPHIC ANALYSIS

To describe the dynamics of escaped canola populations over a complete annual cycle, the seasonal transition matrices were multiplied in sequence to produce periodic matrix products (Caswell 2001). The constituent seasonal matrices can be cyclically permuted to produce matrix products that project the population from different points in the annual cycle. The resulting product matrix $\mathbf{A_G}$ projects the population from May to April of the following year:

$$\mathbf{A_G = W \cdot U_4 \cdot U_3 \cdot U_2 \cdot U_1 \cdot P_3 \cdot P_2 \cdot P_1 \cdot G} \qquad \text{eqn 5}$$

Similarly, the product matrix $\mathbf{A_{U1}}$ projects population dynamics from July to June of the following year:

$$\mathbf{A_{U1} = P_3 \cdot P_2 \cdot P_1 \cdot G \cdot W \cdot U_4 \cdot U_3 \cdot U_2 \cdot U_1} \qquad \text{eqn 6}$$

Periodic matrix products $\mathbf{A_G}$ and $\mathbf{A_{U1}}$ were constructed for all four study populations, using combined demographic data from 2006 and 2007 to increase the sample size for each life stage (MATLAB R2009a, Mathworks, USA). Given the dimensions of the seasonal matrices, the product matrix $\mathbf{A_G}$ reduces to a scalar corresponding to the annual population growth rate, λ.

To model the fate of escaped canola populations and to examine spatial variation between populations, the matrix product $\mathbf{A_{U1}}$ was used to project population dynamics annually from the time of peak flowering (i.e. from the beginning of the summer phase in year t to the end of the spring phase in year $t+1$):

$$\mathbf{n}(t+1) = \mathbf{A_{U1}} \cdot \mathbf{n}(t) \qquad \text{eqn 7}$$

For each population, the initial vector $\mathbf{n}(0)$ described observed population numbers in late June 2006, at the end of spring. As seeds were not observed in the study populations, we started all projections with n_1 = 1000 seeds. Iteration of the matrix model (eqn 7) yielded time-series projections of population dynamics at peak flowering (MATLAB R2009a, Mathworks, USA). While model projections cannot be used to predict absolute population numbers, they are useful in understanding possible population behaviour resulting from current environmental conditions (Caswell 2001). As a point of comparison with model projections, we examined changes in observed population structure from June 2006 to June 2007.

In order to examine temporal variation in population dynamics, we constructed separate summer matrices ($\mathbf{U_{06}}$ and $\mathbf{U_{07}}$) from the 2006 and 2007 demographic data, respectively. Summer population growth rates ($\lambda_{\mathbf{U06}}$ and $\lambda_{\mathbf{U07}}$) were calculated as the dominant eigenvalue of the respective $\mathbf{U_{06}}$ and $\mathbf{U_{07}}$ matrices (Caswell 2001; Morris &

Doak 2002), and were compared across the four populations. Elasticity analysis of matrices $\mathbf{U_{06}}$ and $\mathbf{U_{07}}$ was used to examine the relative importance of all individual life stage transitions in determining population growth (MATLAB R2009a, Mathworks, USA). The elasticity e_{ij} describes the proportional response of $\lambda_{\mathbf{U}}$ to a small proportional change in the associated matrix element u_{ij}. As true proportions, the elements of the elasticity matrix sum to one, enabling comparison of elasticity values across populations and years.

Results

ESCAPED CANOLA PHENOLOGY AND DEMOGRAPHY

Seasonal changes in escaped canola population structure were observed for all four populations between May and October 2007 (Figure 4.2) and phenological observations were used to determine the seasonal division of the matrix models developed. While seedlings emerged throughout the growing season, the highest seedling numbers were generally observed in late May/early June. Peak numbers ranged from 1200 to 1400 sampled seedlings in populations located along highways (Figure 4.2a, b), but were considerably lower in the gravel sites, peaking at approximately 400 sampled seedlings (Figure 4.2c, d). Comparatively, adult plant numbers were lower throughout the growing season in all populations, reflecting high seedling mortality. Vegetative plants developed in the study populations in late May and flowering generally occurred between early June and mid-August, though flowering continued in site C2 until mid-October (Figure 4.2b). The highest numbers of flowering plants were observed in late June and early July, and escaped plants in populations C1, C2 and C4 flowered synchronously with canola crops in adjacent fields (personal observation; Table 4.1).

Generally, all plants that reproduced had reached maturity by late August, completing the period of active growth within roadside habitats. Based on samples collected from all four populations in 2006, escaped canola plants produced between 6 and 2841 seeds/plant, averaging 779 seeds/plant. Non-reproducing adult plants that survived beyond the end of August did not develop further in September and October. However, seedling numbers increased again in October. This late fall emergence peak was generally small relative to spring emergence (Figure 4.2b, c, d), although for highway site C1 fall seedling emergence was almost double that observed in the spring (Figure 4.2a). Winter temperatures killed all vegetative plants and fall-emerged seedlings, as no surviving over-wintered plants were observed in the study populations in spring 2006 or 2007.

The seed addition experiments provided combined estimates of seed survival and seedling emergence in escaped populations. The number of seedlings emerging from experimental quadrats was affected by both adjacent road type (i.e. highway, gravel) and by seed treatment level (i.e. 600 seeds, 100 seeds, control). A significant interaction ($p < 0.0001$) between treatment level and road type was detected using factorial ANOVA. Subsequent tests for simple effects showed a significant effect of treatment level within both highway ($p < 0.0001$) and gravel ($p < 0.0001$) road types; significant differences (i.e. $p \leq 0.05$) between treatment level means were identified using post-hoc multiple range tests. Along highways, the number of emerging seedlings was significantly higher in 600-seed quadrats than in all other quadrats. There was no significant difference in emergence between 100-seed quadrats and control quadrats (Figure 4.3). In contrast, along gravel roads, the number of emerging seedlings differed significantly among all three treatments. Significantly more seedlings emerged from 600-seed quadrats than from either the 100-seed quadrats or the control quadrats. Additionally, significantly higher numbers of seedlings emerged from the 100-seed quadrats than from the control quadrats (Figure 4.3).

MATRIX POPULATION MODELS

Annual growth rates derived from combined 2006 and 2007 demographic data project considerable differences in the fates of the four study populations. Annual growth rates for highway populations C1 ($\lambda_{C1} = 0.2379$) and C2 ($\lambda_{C2} = 0.4164$) were below unity, and model projections of the $\mathbf{A}_{\mathbf{U1}}$ matrices for these populations described rapid decreases in vegetative and flowering plants over time (Figure 4.4a, b). Seedling numbers also decreased over time, after peaking initially in year $\mathbf{n}(1)$. These results suggest that populations C1 and C2 will not persist over the long term if environmental conditions remain constant. However, despite the projected extinction of these populations, seedlings persisted for 5 to 6 years, vegetative plants for 3 to 5 years, and flowering plants for 2 to 5 years (Figure 4.4a, b). Additionally, in these populations, the seed life stage was projected to persist for 2 to 4 years after the disappearance of above-ground plants (data not shown).

Annual growth rates for gravel road populations C3 ($\lambda_{C3} = 1.5076$) and C4 ($\lambda_{C4} = 1.1751$) were above unity, projecting population growth at these two sites under current environmental conditions. Projected growth was relatively rapid in both sites, with population C3 increasing in size by approximately 50% every year (Figure 4.4c). In population C4 seedling and vegetative plant numbers increased steadily, while flowering plants first declined before stabilizing and increasing by year $\mathbf{n}(6)$ (Figure 4.4d). Differences in annual growth rates and projected population fates among the four study populations reflect differences in roadside habitat conditions at the time of survey, suggesting that spatial variation plays an important role in the dynamics of escaped canola populations.

Projected changes in population structure from year $\mathbf{n}(0)$ to year $\mathbf{n}(1)$ were compared with observed changes from June 2006 to June 2007 to consider the predictive power of the models. Interestingly, trends were again apparent that distinguished highway sites from gravel road sites. For populations C1 and C2, annual models $\mathbf{A}_{\mathbf{U1}}$ tended to project lower numbers of vegetative and flowering plants in year $\mathbf{n}(1)$ than were actually observed in 2007 (Figure 4.4a, b). Additionally, despite changes in the observed numbers of seedlings, vegetative and flowering plants in populations C1 and C2 from 2006 to 2007, observed population structure (i.e. the proportion of plants in each life stage) tended to remain relatively stable from year to year (Figure 4.4a, b). Generally, in both 2006 and 2007, seedlings were most abundant, followed by vegetative and flowering plants.

Conversely, in gravel road populations C3 and C4, annual models $\mathbf{A}_{\mathbf{U1}}$ tended to overpredict plant numbers in year $\mathbf{n}(1)$ in comparison with observed population numbers in 2007 (Figure 4.4c, d). Additionally, observed population structure in C3 and C4 changed from 2006 to 2007. While vegetative plants dominated both sites in June 2006, seedlings were the dominant life stage observed in 2006 at the same point in the annual cycle (Figure 4.4c, d). These finding suggest that the overall annual projection models obscured important temporal variation in the four escaped canola populations studied here.

To further explore the temporal variation apparent in these populations, we compared summer growth rates λ_{U06} and λ_{U07} for each population. Summer growth rates were generally above unity, projecting population increases during this period of active growth and reproductive maturation (Table 4.2). In all four populations, however, λ_{U07} values were lower than λ_{U06} values, indicating a decline in population viability between the two years of the study. While this inter-annual decrease was relatively small in populations C1 and C2 (Table 4.2), the trend was particularly pronounced in population C4. In the latter population, extremely rapid summer growth in 2006 was followed by decline in 2007 (Table 4.2), reflecting the observed shift in population structure towards younger, immature life stages (Figure 4.4d).

Elasticity analysis enabled us to quantify the relative contributions of the five canola life stages to growth rates λ_{U06} and λ_{U07} (Figure 4.5). In 2006, transitions associated with the seed stage were important determinants of λ_{U06} in highway populations C1 and C2; seed survival (u_{11}) had the highest elasticity value, followed by seedling establishment (u_{21}) and seed production (u_{15}). Interestingly, in these populations, the lower elasticities associated with adult plant stages (Figure 4.5a, c) suggest that mature plants were less important in ensuring population persistence. Conversely, in populations C3 and C4 in 2006, the development and maturation of vegetative plants, particularly seedling to vegetative (u_{32}) and vegetative to podding (u_{53}) transitions, had large effects on λ_{U06} and seed survival was less important for population persistence (Figure 4.5e, g). However, in 2007, elasticity patterns changed: in all four populations, seed survival had considerable effect on λ_{U07}, with elasticity values over 50% (Figure 4.5b, d, f, h). This change was particularly pronounced in sites C3 and C4, where elasticity values for this transition were three- and six-times higher, respectively, than in the previous year. The increase in the relative importance of seed survival corresponded to decreasing importance of transitions associated with the vegetative stage. Additionally, these changes in elasticity values were concurrent with the decrease in summer growth rates from 2006 to 2007 (Table 4.2).

Discussion

Matrix models parameterized with empirical data from transgenic populations allow us to better understand the short-term dynamics and long-term fates of transgenic crops in agricultural landscapes. However, the utility of these models remains largely under-realized in post-release monitoring of transgenic crops. Indeed, to our knowledge, no such models have been developed in North America, where transgenic crops have been grown for over a decade and have widely escaped from cultivation. As such, our study is the first to enable explicit consideration of the unique demography of escaped transgenic crops in North America, where both large-scale cultivation and local management practices (e.g. herbicide application) contribute to the spread of novel herbicide-tolerant varieties (Knispel & McLachlan 2010). Thus, the empirical data generated through the present study complements and extends the findings of pre-release European assessments of conventional canola, which remain limited in their ability to incorporate both habitat variability and trait-specific plant behaviour (e.g. Begg *et al.* 2006; Garnier, Deville & Lecomte 2006).

Particularly interesting in the consideration of escaped transgenic populations is the potential synchronous development of plants in roadside habitats and those in adjacent cultivated fields, with attendant implications for transgene spread between the two habitats. Seedling emergence in the four roadside populations examined here peaked in late May and early June, corresponding with the emergence timing of volunteer GM canola plants in cultivated fields in Manitoba (Lawson, Van Acker & Friesen 2006). Similarly, plants in the escaped canola populations were observed to flower in synchrony with canola crops in fields adjacent to our study sites, and indeed, peak flowering in roadside habitats was concurrent with the flowering of cultivated canola throughout Manitoba (Canola Council of Canada 2007). While gene flow and trait-stacking have been reported separately within ruderal (Knispel *et al.* 2008) and cultivated (Hall *et al.* 2000; Beckie *et al.* 2003) habitats, the flowering synchrony observed here between escaped and cultivated plants indicates that the potential for genetic exchange between the two habitats cannot be overlooked. Thus, where new GM crops are being introduced into agricultural landscapes, consideration of crop plants growing in non-crop habitats is imperative when mitigating the spread of transgenes and the potential contamination of non-GM crops (Colbach, Clermont-Dauphin & Meynard 2001; Bagavathiannan & Van Acker 2008; Pivard *et al.* 2008).

Despite their considerable importance, the origins of escaped transgenic canola populations are still debated, given the range of potential biological and anthropogenic seed inputs in agroecosystems. In all likelihood, escaped populations of canola originate as a result of both local- and landscape-scale dispersal processes occurring in heterogeneous agricultural landscapes; such a view is supported by our seed additiona experiment. Interestingly, in highway populations C1 and C2, seedling emergence did not differ between 100-seed and control quadrats, suggesting that highway sites were receiving seed inputs beyond our experimental treatments. Seed inputs from commercial agricultural transport are widely recognized as contributing to the establishment and persistence of escaped canola populations (Crawley & Brown 1995; Yoshimura, Beckie & Matsuo 2006; Peltzer, Ferriss & FitzJohn 2008; Knispel & McLachlan 2010). Similarly, the 100-seed addition rate in this study appears to match 'background' rates of seed inputs along high-traffic highways.

Conversely, in gravel road sites C3 and C4, recruitment in the control quadrats of the seed addition experiment was negligible and significantly different from quadrats with added seeds. That no appreciable seed inputs were occurring beyond our experimental treatment indicates that seed, either locally produced or introduced from anthropogenic sources, was limited in populations C3 and C4. These findings suggest that the large robust canola populations observed in sites C3 and C4 in 2006 originated from a single seed addition event, likely associated with canola seeding and/or harvesting activities in the adjacent fields in 2005 (Table 4.1). Seed spillage during transport to and from cultivated fields has been identified as an important contributor to the establishment of escaped canola populations in France (Pivard *et al.* 2008). While such 'spill-event' populations may be large and viable, as in our study in 2006, they are also likely to be relatively rare at the landscape scale, requiring the co-occurrence of both a chance seed-spill event and high habitat suitability.

Notably, our matrix model projections distinguished two trends in population development and persistence, these differences again associated with escaped populations located along highways and with those along gravel roads. However, as matrix model projections rest on the assumption that present habitat conditions will remain constant (Caswell 2001), discrepancies between projected and observed population fates emerged as a result of spatial and temporal habitat variation in our study populations. Such discrepancies provide important insight into the effect of habitat variation on escaped canola demography.

The rapid growth projected for gravel road populations C3 and C4 most likely reflects highly suitable habitat conditions in 2006, when these sites supported many large reproductive plants, producing an average of 1283 seeds/plant (compared to 276 seeds/plant on average in highway populations; data not shown). In human-mediated agricultural landscapes, the suitability of roadside habitats for escaped canola is largely dependent on management and disturbance that opens recruitment sites and reduces competition from perennial vegetation (Crawley & Brown 1995; A.L. Knispel & S.M. McLachlan, unpublished data). As our models for gravel road populations C3 and C4 over-predicted actual population numbers in 2007, they suggest that the habitat conditions that supported rapid population growth in 2006 had already changed considerably in 2007. Though sites C3 and C4 were mowed in both years of this study, disturbance of perennial vegetation was limited as mowing generally occurred at the end of the growing season. Additionally, roadside verges at these sites were not graded over the course of the study (Table 4.1), a disturbance that would normally remove both leaves and rhizomes of perennial vegetation. As such, the relatively open gravel road verges observed in early 2006 in populations C3 and C4 were rapidly colonized in 2007 by rhizomatous grasses from adjacent ditches (A.L. Knispel, personal observation). Given the phenotypic plasticity of canola (Gulden, Warwick & Thomas 2008), seedlings that recruit under perennial vegetation may survive, but are unlikely to develop into large robust adult plants capable of high reproductive output (A.L. Knispel & S.M. McLachlan, unpublished data). In 2007, those seedlings that were able to emerge in populations C3 and C4 thus exhibited slow development, as evidenced by the shift in observed population structure over time; vegetative plants, predominant in June 2006, were largely replaced by seedlings in June 2007. Our elasticity analyses support this shift in the relative importance of vegetative plants towards a reliance on the seed life stage in 2007.

Escaped canola populations along paved highways exhibited different demographic patterns from those observed in gravel road sites. Generally, model projections of rapid decline for highway populations C1 and C2 under-predicted actual population numbers, and summer growth rates in these sites showed only small decreases over the two years of the study. Additionally, the observed numbers of seedlings, vegetative and flowering plants remained relatively stable from 2006 to 2007, suggesting that highway populations may actually be more likely to persist than the largely undisturbed gravel road populations. This apparent viability results in part from the high levels of management along paved highways in southern Manitoba, particularly from the soil disturbance associated with repeated grading (Table 4.1), which enables the ongoing establishment and development of reproductive canola plants.

Our matrix models for highway populations C1 and C2 generated similar outcomes to models of escaped canola in Europe, projecting the persistence of escaped populations over 5 to 10 years, despite negative population growth rates (Claessen *et al.* 2005b; Garnier, Deville & Lecomte 2006). In such models, the persistence of escaped canola plants resulted from both seed survival in the local seed bank and anthropogenic seed dispersal. Similarly, in populations C1 and C2, high elasticity values were associated with seed-related life stage transitions in both 2006 and 2007, and particularly with seed survival. The large elasticity effect of seed survival on population growth rate may point as much to the importance of 'background' seed sources in these highway populations as to the importance of *in situ* seed banks. Indeed, both anthropogenic seed inputs at the landscape-scale and seed survival at the population-scale represent effective means of overcoming seed limitation, contributing to population growth in roadside habitats. As such, the use of intrinsic population growth rates as simple "invasion criteria" (e.g. Crawley *et al.* 1993; Bullock 1999) is insufficient in describing the risks represented by transgenic crops, as such an approach ignores both landscape-scale contributions to population growth as well as the potential for transgene spread even from transient populations. Indeed, the persistence of flowering plants for 2 to 5 years, as projected in this study even for declining populations, will result in synchronous flowering with at least one, and likely two (Table 4.1, population C4), adjacent canola crops grown in the conventional three-year rotation in southern Manitoba. Thus, our study indicates that the risk of gene flow from escaped to cropped populations is considerable, and that the impacts of GM crop escape need to be considered even for intrinsically 'non-invasive' species. To a large degree, human-mediated processes within agro-ecosystems are likely to facilitate and exacerbate the risks of novel transgenic crops.

In such a context, there is an increased need for further research on the role of seeds in escaped populations of GM crops, with particular consideration of the ways in which interacting ecological and agronomic factors contribute to seed dispersal and seed survival in escaped populations. Few empirical data exist for transgenic seeds in non-crop habitats (Gulden, Warwick & Thomas 2008), even in North America where > 95% of the canola grown is herbicide-tolerant. Indeed, though the importance of anthropogenic seed sources has been widely recognized for canola, to our knowledge dispersal and seed input parameters have not been quantified for any crop species, much less transgenic varieties, in roadside habitats in North America. While empirical studies are necessary to evaluate such processes, further development of modelling approaches will also strengthen our understanding of escaped crop dynamics. To this end, extension of the matrix models developed here to consider seed and pollen dispersal at multiple scales will provide greater insight into feasible control options, as well as identifying any potential limits to the post-release management of transgenic crops.

MANAGEMENT IMPLICATIONS

The importance of conducting GM risk assessment at multiple spatial and temporal scales is increasingly recognized (Schröder & Schmidt 2008 and references therein; Knispel & McLachlan 2010). While landscape-scale models are very useful in predicting distribution 'hot-spots' of escaped population presence, such an approach is limited in detecting rare events arising from chance processes operating at local scales (Peltzer, Ferriss & FitzJohn 2008). Complementing such large-scale studies, our multi-site and multi-year population-level models allowed us to better understand factors affecting GM canola presence in different habitat types, in turn helping to direct subsequent surveys and management strategies that are responsive to highly variable agro-ecosystems.

For relatively rare 'spill event' populations resulting from sowing and harvest activities, physical control of large vegetative plants prior to flowering may assist in mitigating gene flow risks. Scouting and management activities should focus on field and road verges adjacent to the crop in question; such monitoring will be necessary in both the year of crop cultivation and in subsequent years, at least until flowering of escaped plants is negligible. In addition to targeted control of vegetative and flowering plants, reduced levels of mowing and soil disturbance are likely to result in the competitive exclusion of escaped plants by perennial vegetation in roadside verges.

Conversely, management of 'background' populations of escaped plants supplemented by ongoing seed inputs is more problematic. Prevention of seed addition from anthropogenic sources is practically impossible along roads that support a high volume of agricultural traffic. Further complicating management of escaped transgenic crops, founding seed for 'background' populations originates from pooled agricultural traffic and cannot be traced to individual fields or local cropping activities. As such, management is not the responsibility of individual farmers growing GM varieties; rather, it is a regional concern requiring multiple-scale approaches to ensure effective management and responsible transport of transgenic crops throughout agricultural landscapes. To date, the development of co-operative regional networks, encompassing farmers, researchers and novel plant developers, to manage widely dispersing weed species is limited (Colbach, Clermont-Dauphin & Meynard 2001; Dauer, Mortensen &

Vangessel 2007; Borger *et al.* 2009). However, such networks will be necessary to effectively control the spread of transgenic crops, including the many varieties already cultivated on a commercial scale in North America (Knispel & McLachlan 2010). These networks and the designation of segregated production areas will become even more important for new transgenic traits (Mauro, McLachlan & Van Acker 2009), especially when considering the anticipated environmental, health, and safety risks of transgenic varieties expressing pharmaceutical and industrial traits (Begg *et al.* 2006; Bagavathiannan & Van Acker 2008).

References

Bagavathiannan, M.V. & Van Acker, R.C. (2008) Crop ferality: Implications for novel trait confinement. *Agriculture, Ecosystems and Environment*, **127**, 1-6.

Beckie, H.J., Harker, K.N., Hall, L.M., Warwick, S.I., Légère, A., Sikkema, P.H., Clayton, G.W., Thomas, A.G., Leeson, J.Y., Séguin-Swartz, G. & Simard, M.J. (2006) A decade of herbicide resistant crops in Canada. *Canadian Journal of Plant Science*, **86**, 1243-1264.

Beckie, H.J., Warwick, S.I., Nair, H. & Séguin-Swartz, G. (2003) Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). *Ecological Applications*, **13**, 1276-1294.

Begg, G.S., Hockaday, S., McNicol, J.W., Askew, M. & Squire, G.R. (2006) Modelling the persistence of volunteer oilseed rape (*Brassica napus*). *Ecological Modelling*, **198**, 195-207.

Borger, C.P.D., Scott, J.K., Renton, M., Walsh, M. & Powles, S.B. (2009) Assessment of management options for *Salsola australis* in south-west Australia by transition matrix modelling. *Weed Research*, **49**, 400-408.

Bullock, J.M. (1999) Using population matrix models to target GMO risk assessment. *Aspects of Applied Biology*, **53**, 205-212.

Canola Council of Canada (2007) *Canola Watch Reports, 2007*. Canola Council of Canada, Winnipeg, Manitoba.

Caswell, H. (2001) *Matrix Population Models: Construction, Analysis, and Interpretation*, 2nd edn. Sinauer Associates Inc., Sunderland, MA, USA.

Claessen, D., Gilligan, C.A., Lutman, P.J.W. & van den Bosch, F. (2005a) Which traits promote persistence of feral GM crops? Part 1: implications of environmental stochasticity. *Oikos*, **110**, 20-29.

Claessen, D., Gilligan, C.A. & van den Bosch, F. (2005b) Which traits promote persistence of feral GM crops? Part 2: implications of metapopulation structure. *Oikos*, **110**, 30-42.

Colbach, N., Clermont-Dauphin, C. & Meynard, J.M. (2001) GENESYS: a model of the influence of cropping system on gene escape from herbicide tolerant rapeseed crops to rape volunteers II. Genetic exchanges among volunteer and cropped populations in a small region. *Agriculture, Ecosystems and Environment*, **83**, 255-270.

Crawley, M.J. & Brown, S.L. (1995) Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. *Proceedings of the Royal Society of London B.,* **259**, 49-54.

Crawley, M.J., Hails, R.S., Rees, M., Kohn, D. & Buxton, J. (1993) Ecology of transgenic oilseed rape in natural habitats. *Nature*, **363**, 620-623.

Cuthbert, J.L. & McVetty, P.B.E. (2001) Plot-to-plot, row-to-row, and plant-to-plant outcrossing studies in oilseed rape. *Canadian Journal of Plant Science,* **81**, 657-664.

Dauer, J.T., Mortensen, D.A. & Vangessel M.J. (2007) Temporal and spatial dynamics of long-distance *Conyza canadensis* seed dispersal. *Journal of Applied Ecology,* **44**, 105-114.

Garnier, A., Deville, A. & Lecomte, J. (2006) Stochastic modelling of feral plant populations with seed immigration and road verge management. *Ecological Modelling*, **197**, 373-382.

Garnier, A. & Lecomte, J. (2006) Using a spatial and stage-structured invasion models to assess the spread of feral populations of transgenic oilseed rape. *Ecological Modelling*, **194**, 141-149.

Gulden, R.H., Thomas, A.G. & Shirtliffe, S.J. (2004) Secondary dormancy, temperature, and burial depth regulate seedbank dynamics in canola. *Weed Science,* **52**, 382-388.

Gulden, R.H., Warwick, S.I. & Thomas, A.G. (2008) The biology of Canadian weeds. 137. *Brassica napus* L. and *Brassica rapa* L. *Canadian Journal of Plant Science,* **88**, 951-996.

Hall, L.M., Topinka, K., Huffman, J., Davis, L. & Good, A. (2000) Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Science,* **48**, 688-694.

Jongejans, E., Sheppard, A.W. & Shea, K. (2006) What controls the population dynamics of the invasive thistle *Carduus nutans* in its native range? *Journal of Applied Ecology*, **43**, 877-886.

Knispel, A.L. & McLachlan, S.M. (2010) Landscape-scale distribution and persistence of genetically modified oilseed rape (*Brassica napus*) in Manitoba, Canada. *Environmental Science and Pollution Research,* **17**, 13-25.

Knispel, A.L., McLachlan, S.M., Van Acker, R.C. & Friesen, L.F. (2008) Gene flow and multiple herbicide resistance in escaped canola populations. *Weed Science,* **56**, 72-80.

Lawson, A.N., Van Acker, R.C. & Friesen, L.F. (2006) Emergence timing of volunteer canola in spring wheat fields in Manitoba. *Weed Science*, **54**, 873-882.

Leeson, J.Y., Thomas, A.G., Hall, L.M., Brenzil, C.A., Andrews, T., Brown, K.R & Van Acker, R.C. (2005) *Prairie Weeds Survey: Cereal, Oilseed and Pulse Crops 1970s to 2000s.* Weed Survey Series Publication 05-1. Agriculture and Agri-Food Canada, Saskatoon, SK.

Mauro, I.J. & McLachlan, S.M. (2008) Farmer knowledge and risk analysis: Postrelease evaluation of herbicide-tolerant canola in western Canada. *Risk Analysis*, **28**, 463-476.

Mauro, I.J., McLachlan, S.M. & Van Acker, R.C. (2009) Farmer knowledge and a priori risk analysis: A pre-release evaluation of genetically modified Roundup Ready wheat across the Canadian prairies. *Environmental Science and Pollution Research*, **16**, 689-701.

Morris, W.F. & Doak, D.F. (2002) *Quantitative Conservation Biology: Theory and Practice of Population Viability Analysis.* Sinauer Associates Inc., Sunderland, MA, USA.

Peltzer, D.A., Ferriss, S. & FitzJohn, R.G. (2008) Predicting weed distribution at the landscape-scale: using naturalized *Brassica* as a model system. *Journal of Applied Ecology*, **45**, 467-475.

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., Gouyon, P.H. & Huet, S. (2008) Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. *Journal of Applied Ecology*, **45**, 476-485.

Schröder, W. & Schmidt, G. (2008) Implications of GM-crop cultivation – Series. *Environmental Science and Pollution Research*, **15**, 527-528.

Smith, R.E., Veldhuis, H., Mills, G.F., Eilers, R.G., Fraser, W.R. & Lelyk, G.W. (1998) *Terrestrial Ecozones, Ecoregions, and Ecodistricts of Manitoba: An Ecological Stratification of Manitoba's Natural Landscapes.* Technical Bulletin 98-9E. Agriculture and Agri-Food Canada, Winnipeg.

Statistics Canada (2009) *Field Crop Reporting Series: Preliminary Estimates of Principal Field Crop Areas, Canada.* Catlogue no. 22-022-X, **88** (4). Ottawa, Canada.

Yoshimura, Y., Beckie, H.J. & Matsuo, K. (2006) Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. *Environmental Biosafety Research*, **5**, 67-75.

Table 4.1. Site characteristics and management history of four escaped canola populations in southern Manitoba, Canada.

	Escaped canola population			
	C1	**C2**	**C3**	**C4**
Location	49º22'33'N, 98º0'7"W	49º20'8"N, 98º0'6"W	49º28'28"N, 97º51'57"W	49º29'31"N, 97º51'58"W
Road Type	Paved highway	Paved highway	Gravel	Gravel
Distance to Elevator (km)	0.5	4	12	11.5
Adjacent Crop (2005; 2006; 2007)	soybean; corn; canola	barley; corn; canola	canola; corn; oats	canola; oats; canola
Number of Plants Sampled	2006: 779 2007: 1593	2006: 548 2007: 1677	2006: 362 2007: 566	2006: 100 2007: 473
Proportion (%) of escaped plants with glyphosate (GLY), glufosinate (GLU) and multiple (M) herbicide tolerance traits*	GLY: 70 GLU: 90 M: 50	GLY: 75 GLU: 88 M: 62	GLY: 91 GLU: 68 M: 45	GLY: 100 GLU: 0 M: 0
Management: Mowing events	3 (9/20/06; 7/11/07; 10/30/07)	3 (9/20/06; 7/11/07; 10/3/07)	2 (9/20/06; 9/18/07)	3 (9/20/06; 7/24/07; 9/18/07)
Management: Grading and soil disturbance events	2 (10/18/06; 5/1/07)	4 (8/24/06; 5/1/07; 6/14/07; 9/18/07)	0	0

*Includes both maternal plants exhibiting transgenic traits and plants producing transgenic progeny, from among ten plants (C1), eight plants (C2), 22 plants (C3) and four plants (C4) tested (Knispel *et al.* 2008).

Table 4.2. Summer population growth rates (λ_U) for four escaped canola populations in 2006 and 2007 in southern Manitoba, Canada.

	λ_U	
Population	2006	2007
C1	1.1526	1.0303
C2	1.2163	1.0417
C3	1.7604	1.0638
C4	2.0783	0.9705

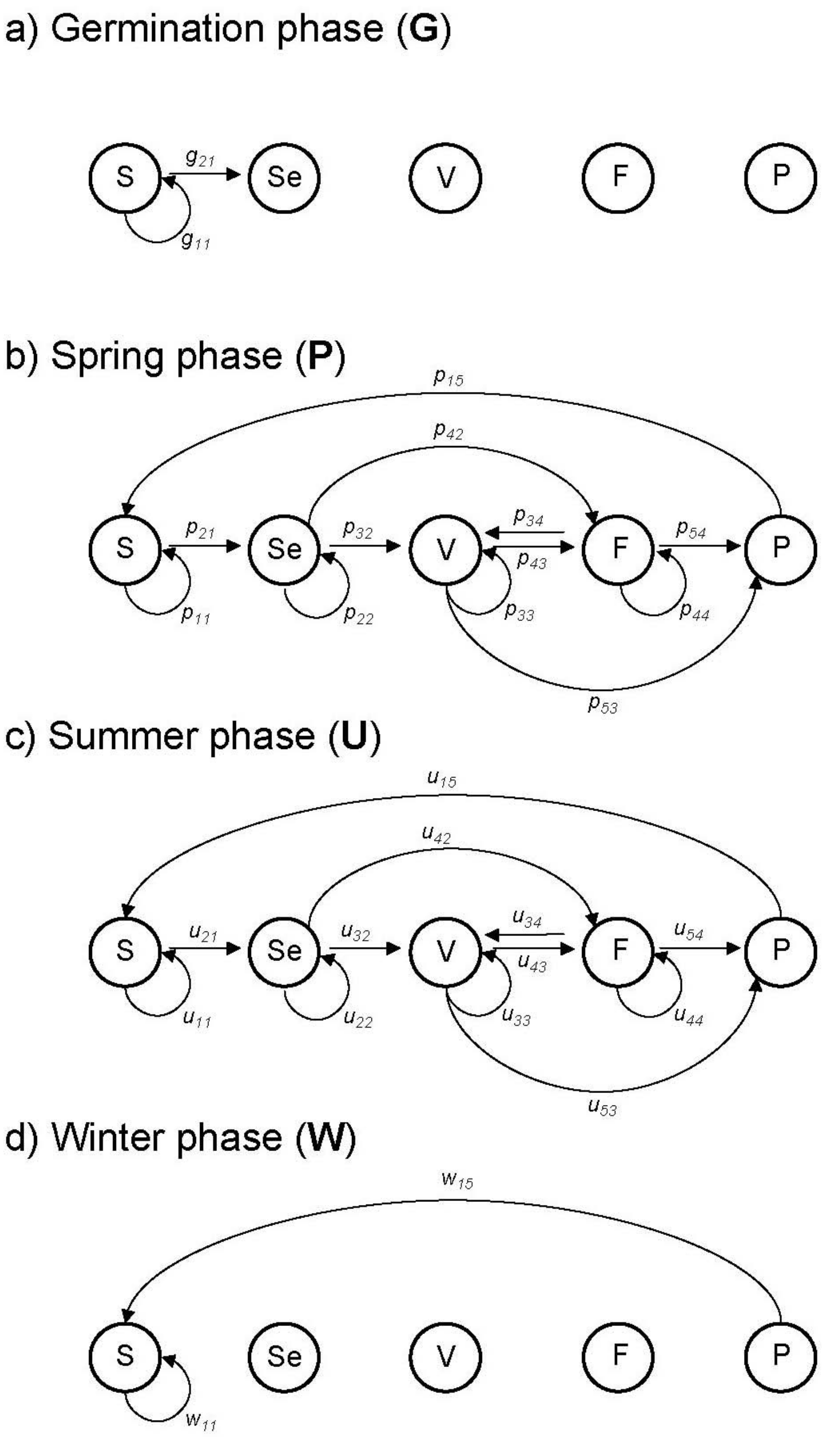

Figure 4.1. Life cycle graphs depicting seasonal phases in the annual life cycle of escaped populations of canola. Populations are structured in five life stages: seeds (S), seedlings (Se), vegetative plants (V), flowering plants (F) and podding plants (P). Arrows represent transitions (i.e. g_{ij}) between life stages.

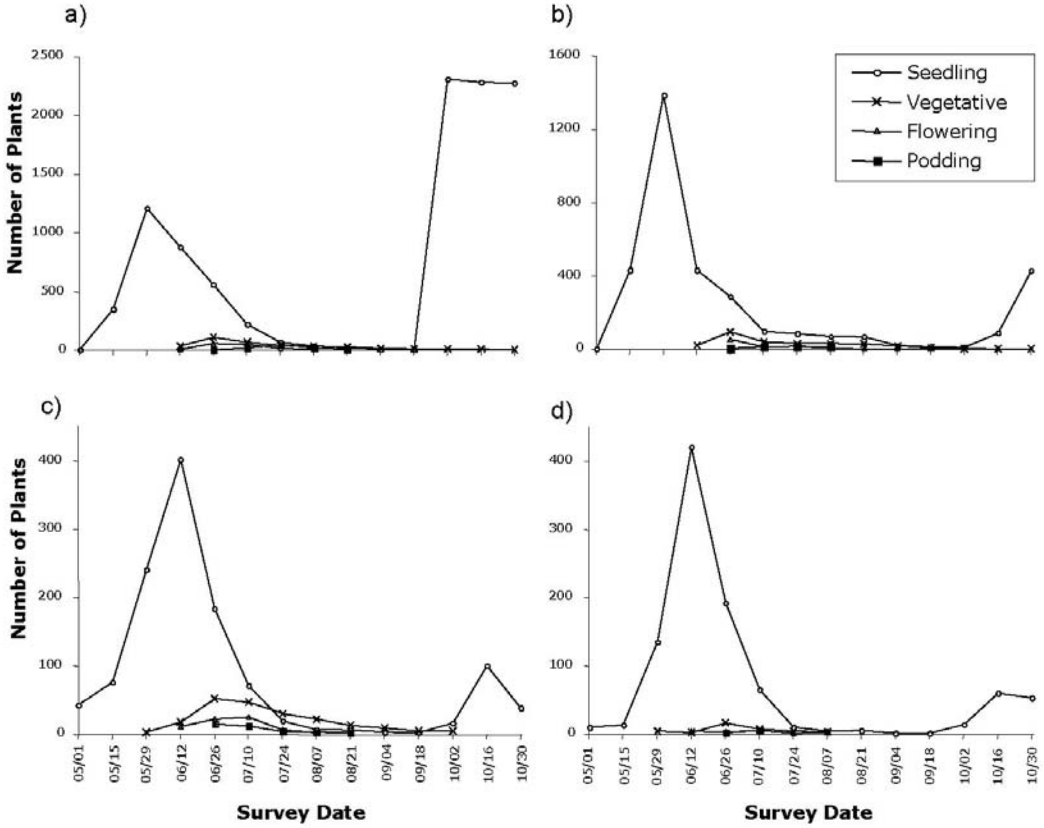

Figure 4.2. Phenology of escaped canola in populations C1 (a), C2 (b), C3 (c) and C4 (d). Observed changes in numbers of seedlings, vegetative, flowering and podding plants are shown from May to October 2007.

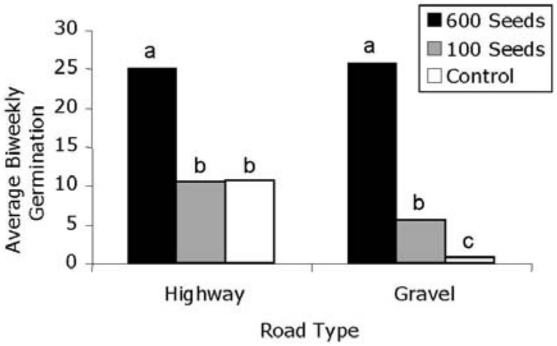

Figure 4.3. Differences in average seed germination in escaped canola populations for three seed addition treatment levels (i.e. 600 seeds added, 100 seeds added, control treatment with no seed addition) within two road types (i.e. paved highways and gravel roads). Within each road type, means identified by different letters are significantly different at $p < 0.0001$.

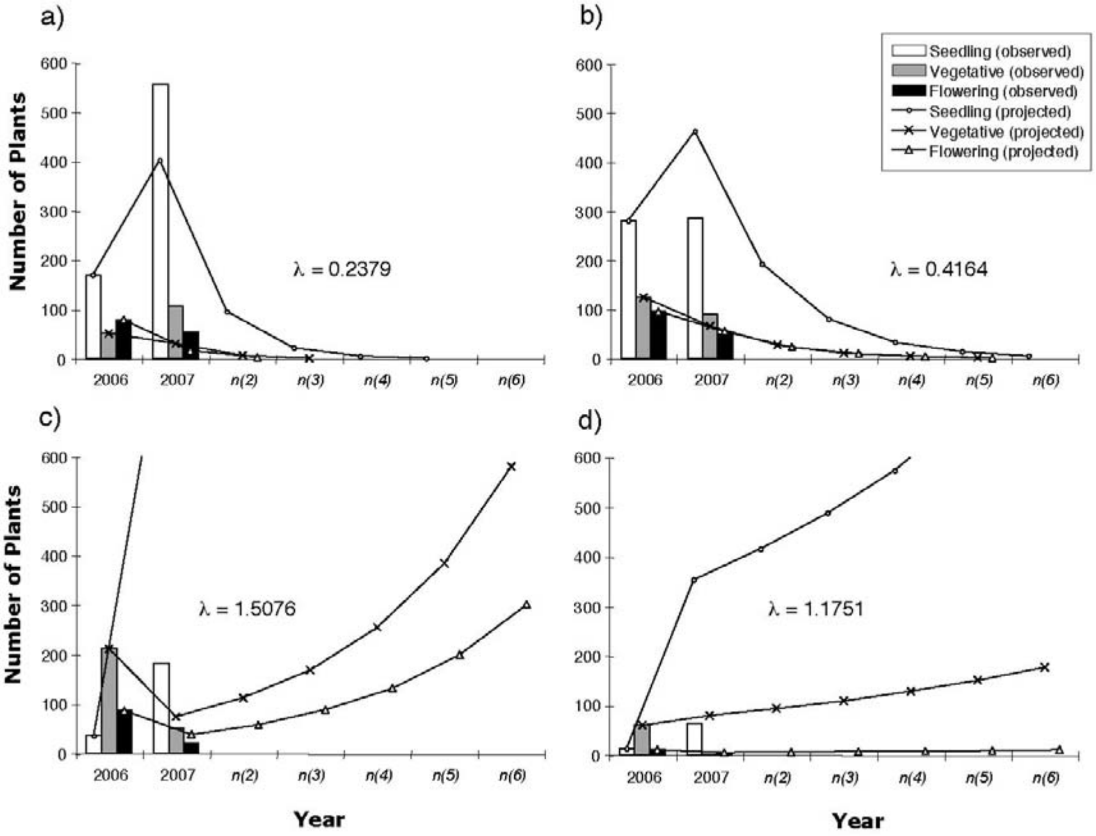

Figure 4.4. Periodic matrix model projections for escaped canola populations C1 (a), C2 (b), C3 (c) and C4 (d) in southern Manitoba, Canada. Models project canola dynamics from June to June of the subsequent year, describing population structure at peak flowering. Models are initiated from actual population numbers in 2006 and compared with observed population structure in 2007.

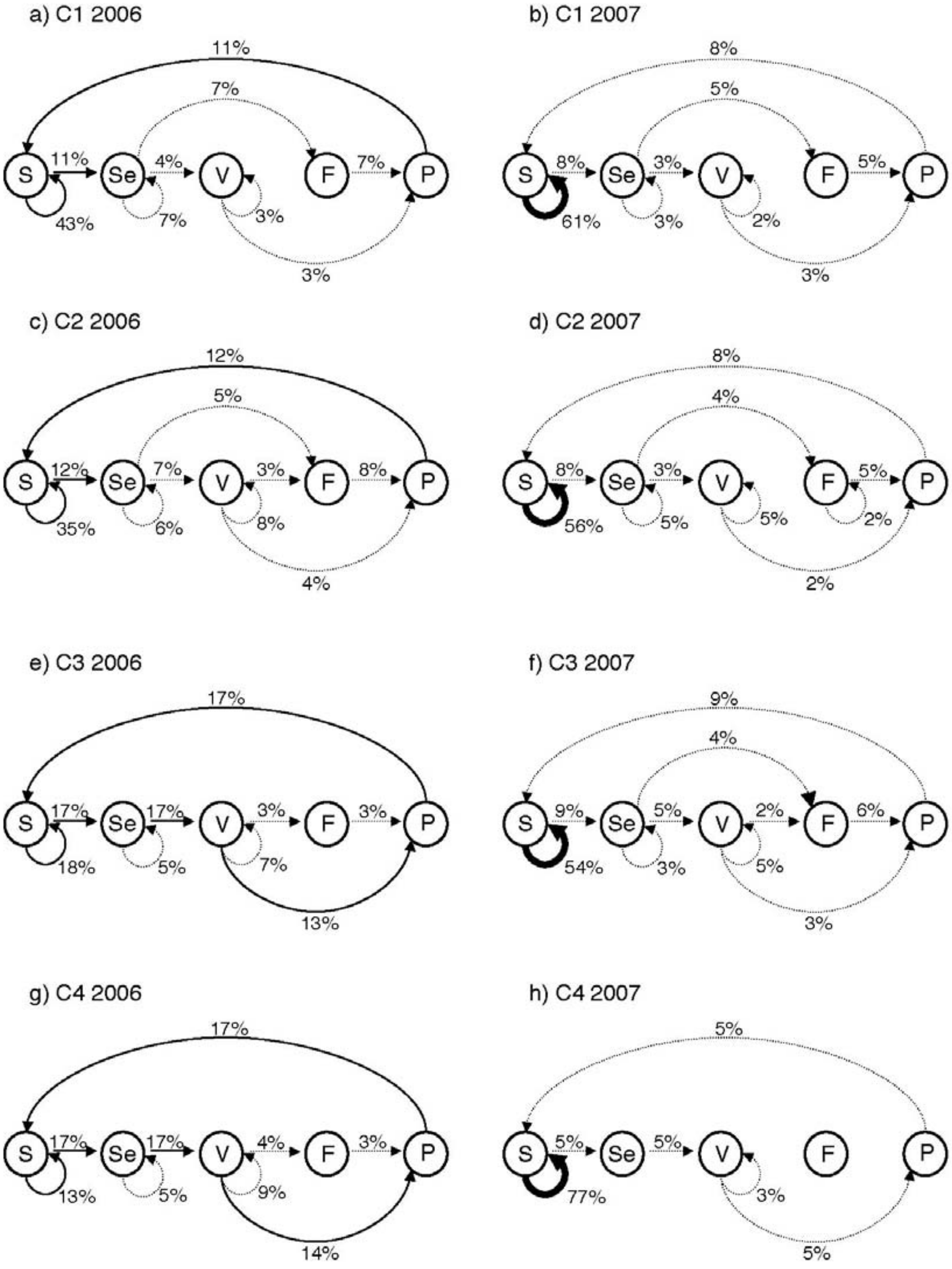

Figure 4.5. Summer life cycle graphs for four populations of escaped canola in 2006 and 2007. Populations are structured in five life stages: seeds (S), seedlings (Se), vegetative plants (V), flowering plants (F) and podding plants (P). Elasticity values > 1% are represented on their respective life stage transitions.

CHAPTER 5

THE IMPORTANCE OF SCALE IN CONSIDERATIONS OF WEED PATCH PERSISTENCE AND SPREAD: IMPLICATIONS OF METAPOPULATION STRUCTURE FOR GENE FLOW AND NOVEL TRAIT CONFINEMENT

Introduction

In current agronomic research, weed patches are widely studied at the local scale, and persistence is assessed within individual arable fields. Recognition of the patchiness of weed populations results in more accurate predictions of crop yield loss compared to predictions generated under the assumption of uniform weed density (Cousens & Mortimer, 1995). Additionally, studies examining the stability of weed patches in space and time have frequently pointed to the possibility of herbicide use reduction through site-specific management, which targets only specific weed patches for control. However, comparisons of weed distributions at multiple scales suggest that weed patch patterns change with increasing spatial scale (Clay *et al.*, 1999). As such, conclusions and subsequent management recommendations are likely to vary depending on the scale at which weed patches are considered.

In the past decade, the distribution patterns of weed patches have increasingly been considered in ecological contexts (Cousens & Mortimer, 1995; Cardina *et al.*, 1997; Rew & Cousens, 2001). Seemingly stable, isolated weed patches may in fact be dynamic, interacting with other populations within the field or within field margins. Weed patches may exchange propagules through dispersal, enabling spread into adjacent crops or non-crop habitats and potentially increasing weed persistence at larger spatial scales. Because weed persistence informs decisions regarding control, consideration of the multi-scale processes determining weed spatial distribution and persistence is required to ensure the proper allocation of management resources and the development of effective long-term control strategies. To this end, appropriate ecological frameworks may increase our understanding of the dynamics of weed patches at both local and landscape scales.

Metapopulation theory has been suggested as a useful conceptual model for the examination of weed persistence and spread at regional scales in agricultural landscapes (Cousens & Mortimer, 1995; Cardina *et al.*, 1997). Classical metapopulation models describe groups of spatially isolated sub-populations interconnected through dispersal (Levins, 1969, 1970). Though individual sub-populations may become extinct, survival at the regional scale is ensured by recolonization of suitable habitat through dispersal. As such, metapopulation dynamics may have important implications for persistence and gene flow, both for newly invading weed species and for the spread of herbicide tolerance and other novel traits in crops and weedy plants (Claessen *et al.*, 2005b; Willenborg & Van Acker, 2006).

The introduction of new genetically modified (GM) crop varieties necessitates multi-scale risk assessments to determine the probability and consequences of the escape of GM crops from cultivation (Conner *et al.*, 2003). Following commercial release of new GM varieties, widespread cultivation and the development of field volunteer populations often lead to the escape of GM plants into ruderal, non-crop habitats (Knispel *et al.*, 2008). The extent to which these broadly-distributed populations interact as metapopulations must be considered in light of the need to confine these traits in order to meet regulatory and food safety standards (Smyth *et al*, 2002) and to ensure marketability (Demeke *et al.*, 2006). While responsibility for confinement is generally perceived to lie with individual land managers, the potential for metapopulation interactions points to the need for a broader regional management approach in order to effectively address weed control and GM trait confinement.

The overall objective of this paper is to review the current literature on weed patch dynamics and distribution at multiple spatial scales and to consider the relevance of metapopulation theory in addressing questions of weed patch persistence and spread, with particular attention to the containment of novel GM traits. Specifically, I will: (1) review approaches commonly used in the study of weed patches and consider how these have informed emerging management recommendations; (2) consider the utility of the metapopulation concept for improved understanding of weed patch persistence and spread in human-dominated agricultural landscapes; and (3) examine the implications of regional metapopulation structure for the persistence of escaped populations of GM crop plants, and describe the contributions of these populations to gene flow at the landscape scale.

Weed patches: current approaches

Increasing interest in weed spatial distribution has resulted in the widespread recognition that weed density is spatially heterogeneous and many weeds occur in distinct patches (Wiles *et al.*, 1992; Gerhards *et al.*, 1997; Dieleman & Mortensen, 1999; Gonzalez-Andujar & Saavedra, 2003). Weed patches are described by weed scientists as aggregated populations of weed species located in areas of favourable habitat. Patches are generally characterized by a high density patch centre, with weed densities decreasing towards fragmented and variable patch edges; these patterns are attributed to interactions between environmental heterogeneity and local demographic processes (Dieleman &

Mortensen, 1999; Burton *et al.*, 2005). At spatial scales above the individual patch, weed distribution patterns have generally been assessed using discrete sampling methods (Rew & Cousens, 2001), which examine weed density within quadrats spaced in a regular grid over the field (Wiles *et al.*, 1992; Gerhards *et al.*, 1997; Colbach *et al.*, 2000; Barroso *et al.*, 2004a; Blanco-Moreno *et al.*, 2006). Both spatially-implicit (Wiles *et al.*, 1992; Gonzalez-Andujar & Saavedra, 2003) and spatially-explicit (Colbach *et al.*, 2000; Blanco-Moreno *et al.*, 2006) analyses of data collected using this method have demonstrated aggregated patterns for both individual weed species and weed communities. Patchy distributions within fields are frequently explained in relation to mechanisms of species dispersal and management factors.

Although weed distribution patterns are highly variable, weed density has often been assumed to be uniform within arable fields. As such, the entire field is generally managed homogenously, as has been the convention with herbicide applications. However, where weed distribution is actually patchy, conventional field-scale management may be inefficient (Cardina *et al.*, 1997). Thus, descriptions of discrete weed patches led to the early recognition of the potential for managing weeds on a patch-by-patch basis (Wiles *et al.*, 1992; Rew *et al.*, 1996). In recent years, interest in site-specific weed control, particularly herbicide-based methods (Swinton 2005), has grown substantially due to the potential reduction in herbicide costs as well as environmental benefits derived from reduced herbicide applications (Lindquist *et al.*, 1998; Goudy *et al.*, 2001; Nordmeyer, 2006). Attempts at minimizing the scale of herbicide applications in weed management have recognized the importance of assessing patch stability, defined as consistency of weed patch location and density across years (Gerhards *et al.*, 1997).

Weed patches that are stable from year to year are more easily controlled by site-specific practices, as maps of patch distribution may be used over multiple years, reducing the cost and labour associated with detection and mapping (Colbach *et al.* 2000; Goudy *et al.*, 2001; Heijting *et al.*, 2007).

Although patterns of weed patches vary greatly among species, sites, and years (Wiles *et al.*, 1992; Rew *et al.*, 1996), weed patches have been widely described as stable over time at the field- or plot-scale (Gerhards *et al.*, 1997; Dieleman & Mortensen, 1999; Goudy *et al.*, 2001; Barroso *et al.*, 2004a; Beckie *et al.*, 2005; Heijting *et al.*, 2007). Patch stability is often strongest for species whose seeds are dispersed prior to harvesting, for perennial species with vegetative reproduction, and for species with persistent seed banks (Gerhards *et al.*, 1997; Colbach *et al.*, 2000; Blanco-Moreno *et al.*, 2006; Heijting *et al.*, 2007). However, density of patches can vary substantially from year to year and is not accurately predicted from previous years' distribution (Clay *et al.*, 1999; Colbach *et al.*, 2000; Blanco-Moreno *et al.*, 2006). Additionally, many spatial distribution studies have detected anisotropy, a directional trend in the shape, arrangement, and spread of weed patches, resulting from field management practices undertaken in repeated patterns (Gerhards *et al.* 1997; Dieleman & Mortensen, 1999; Colbach *et al.*, 2000; Beckie *et al.*, 2005; Humston *et al.*, 2005; Blanco-Moreno *et al.*, 2006; Heijting *et al.*, 2007). These directional trends tend to be more pronounced for annual species whose seeds are released at the time of harvest activities and are thereby dispersed along crop rows by harvest machinery (Colbach *et al.*, 2000; Blanco-Moreno *et al.*, 2006). Species with highly developed natural dispersal mechanisms (e.g. wind-dispersed composites) often show no structured spatial pattern or stability at all (Heitjing *et al.*, 2007). Understanding of the mechanisms underlying spatial pattern in arable fields, particularly anisotropic patterns affecting weed patch movement and expansion, is necessary to fully characterize patch stability. Importantly, changes in the stability (density or location) of weed patches are most likely, and least understood, in fields with ongoing, spatially-targeted (i.e. patch-based) management activities (Goudy *et al.*, 2001; Barroso *et al.*, 2004a).

Most spatial distribution studies of weed patches are now undertaken explicitly in the context of site-specific herbicide-based weed control, focusing on the persistence and stability of weed patches at small localized scales approximating the scale of proposed site-specific management activities. This has led to the rapid development of intensive, technology-based, fine-scale approaches to weed mapping and herbicide application (Goudy *et al.*, 2001; Nordmeyer 2006). However, the mapping and technology costs associated with site-specific management are high (Swinton 2005), and estimations of the economic returns gained from site-specific weed control are misleading if these additional expenses are not considered (e.g. Lindquist *et al.*, 1998). Indeed, these expenses may make site-specific management cost-prohibitive (Clay *et al.*, 1999; Barroso *et al.*, 2004b; Ruiz *et al.*, 2006). Furthermore, not all weed infestations in arable fields may be effectively managed using site-specific approaches (Goudy *et al.*, 2001; Wallinga *et al.*, 2002; Barroso *et al.*, 2004b; Heijting *et al.*, 2007). Despite this, innovations in weed control and management are increasingly limited by the localized resolution of spatial studies, and are now largely elaborated at the scale of the field or at even smaller scales.

Some authors argue that site-specific technological developments have outstripped our knowledge of the variable dynamics of weed patches (Rew & Cousens, 2001). While localized studies provide insight into mechanisms determining weed patch stability at the field scale (e.g. Gerhards *et al.*, 1997; Colbach *et al.*, 2000; Blanco-Moreno *et al.*, 2006; Heijting *et al.*, 2007), different mechanisms, resulting in different spatial distributions and stability, are likely to operate at larger scales (Levin 1992; Blanco-Moreno *et al.*, 2006). Indeed, researchers acknowledge that perceived patterns of weed patch distribution and stability can vary even with relatively small changes in the resolution of the sampling grid (Clay *et al.*, 1999; Cousens *et al.*, 2002; Blanco-Moreno *et al.*, 2006). Distribution patterns dictated by small-scale processes, such as natural dispersal from the maternal plant, will only be detected when sampling with fine grid resolution (Gerhards *et al.*, 1997; Colbach *et al.*, 2000). Conversely, long-distance dispersal events and the establishment of new patches generally cannot be considered in localized spatial studies (e.g. Dieleman & Mortensen, 1999; Goudy *et al.*, 2001; Beckie *et al.*, 2005; Burton *et al.*, 2005), although these processes are expected to have a large impact on weed invasions and weed control within fields (Ghersa & Roush, 1993; Wallinga *et al.*, 2002).

While there is no ‘correct’ spatial scale at which to examine population structure and dynamics (Levin, 1992), restriction to a single scale of study can result in misunderstanding of patch persistence and inappropriate management decisions (Cardina *et al.*, 1997). Studies undertaken at fine spatial resolutions are limited in their ability to identify large-scale processes and consequently to inform practical management decisions at commercial field and landscape scales (Firbank, 1993; Cardina *et al.*, 1997; Swinton 2005). Though it is recognized that a localized patch-based approach does not adequately describe all weed infestations or predict all potential weed problems, very little attention has been given to implications of varying spatial scale beyond the level of the individual plot or field.

Weed patches in agricultural landscapes

Despite early calls for broader ecological approaches to weed patch study and management (Cousens & Mortimer, 1995; Cardina *et al.*, 1997), comparatively little work has focused on weed distribution outside of agricultural fields. Indeed, in the context of weed control and management, interest in the potential interactions between arable fields and other habitats in the agricultural landscape seems to have decreased in recent decades (e.g. Froud-Williams & Chancellor, 1982; Marshall, 1985; Hume & Archibold, 1986; Marshall, 1989; but see Blumenthal & Jordan, 2001; and Sosnoskie *et al.*, 2007 for recent theoretical and empirical examples, respectively). As a result, the agronomic risks associated with the spread of weeds into fields from margin habitats remain poorly understood (Devlaeminck *et al.*, 2005; Boutin, 2006; Sosnoskie *et al.*, 2007). Conversely, movement of species between arable and adjacent non-arable habitats is increasingly examined from the perspective of rare species conservation (Wilson & Aebischer, 1995; Smith *et al.*, 1999; Bischoff, 2005; Boutin, 2006). These conservation studies are largely undertaken on the same spatial scale as previous agronomic studies, and tend to report similar distribution patterns for weeds and weed seeds in edge habitat and arable fields. As such, both control- and conservation-focused field margin studies are useful in describing weed spread from adjacent habitats into arable fields.

Field margin studies have generally examined whole weed communities rather than individual species of interest, considering both standing vegetation and seed bank composition. Species composition often varies considerably between field interior and margin habitats (Hume & Archibold, 1986; Boutin, 2006), and these differences are attributed to differences in the suitability of the two habitat types (Marshall, 1989). Indeed, many agricultural species have been classified as either field interior or field margin species (Marshall, 1985; Devlaeminck *et al.*, 2005). Additionally, species diversity and/or density tends to be higher at the edge of fields, with a substantial decrease towards the interior (Hume & Archibold, 1986; Marshall, 1989; Wilson & Aebischer, 1995; Devlaeminck *et al*, 2005). These general findings have led many authors to conclude that most species exhibit limited dispersal, preventing their spread between margin and field interior habitats and between adjacent fields (e.g. Hume & Archibold, 1986; Marshall, 1989; Devlaeminck *et al.*, 2005; Bischoff, 2005). At most, dispersal of weeds from adjacent habitats is expected to impact a narrow border at the edge of the field (Hume & Archibold, 1986; Wilson & Aebischer, 1995; Devlaeminck *et al.*, 2005), where regular management practices are likely to adequately control the low levels of weed influx (Hume & Archibold, 1986).

Although field margin studies consider weed distribution outside individual fields and recognize the importance of habitat interactions in the agricultural landscape, their predominant focus on overall community composition potentially obscures the agronomic implications of the spread of a few important weed species. Indeed, margin habitats may contain a small number of agronomically- and economically-detrimental species, providing a potential seed source for adjacent fields (Marshall, 1989; Smith *et* *al.*, 1999), although this remains poorly understood. Additionally, field margin studies undertaken to date generally are not spatially explicit, and the majority of these studies remain limited in scale, examining species composition in replicated experimental plots located in paired fields and field margins (Hume & Archibold, 1986; Marshall, 1989; Wilson & Aebischer, 1995; Bischoff, 2005; Devlaeminck *et al.*, 2005; Boutin, 2006). Indeed, at the extreme, some studies have exclusively examined the narrow linear interface between field and margin habitats, limiting their sampling efforts to within less than a metre of the crop edge (e.g. Smith *et al.*, 1999; Sosnoskie *et al.*, 2007).

Given the spatial limitations of most field margin studies, it is not surprising that many authors have concluded that plant species in agricultural landscapes are dispersal limited. This general conclusion is supported by the fact that the vast majority of seeds of a given plant or species are dispersed over very short distances (Cain *et al.*, 2000). However, a small proportion of seeds may be dispersed over long distances relative to the average dispersal distance, and these long distance dispersal events are known to have a disproportionately important effect on the distribution and spread of plant species (Higgins & Richardson, 1999; Nehrbass *et al.*, 2007). As such, relatively rare dispersal events may contribute substantially to the severity of weed problems, and a better understanding of weed dispersal processes, particularly over long distances, is needed for improved weed control (Ghersa & Roush, 1993; Wallinga *et al.*, 2002).

Despite the expected importance of long distance dispersal to the spread of plant species, it is inherently difficult to measure and predict long distance dispersal events, i.e. the tail of the dispersal curve, describing the probability of dispersal distances longer than the modal dispersal distance (Cain *et al.*, 2000; Bullock & Clarke, 2000; Wallinga *et al.*, 2002). The lack of empirical data on long distance seed dispersal (Cain *et al.*, 2000; Greene & Calogeropoulos, 2002) means that our understanding of the importance of these events comes largely from modeling studies (Higgins & Richardson, 1999; Soons & Ozinga, 2005; Nehrbass *et al.*, 2007). The shape of the tail of the dispersal curve has been shown to determine the pattern by which plant populations expand. A plant population with limited dispersal (i.e. dispersal curve with a short, narrow tail) spreads predominantly through local dispersal, invading new habitat as an advancing closed front (Wallinga *et al.*, 2002). Conversely, simulations that include long distance dispersal events (i.e. using dispersal curves with longer, fatter tails) indicate that small numbers of seeds dispersing over long distance may increase invasion rates by several orders of magnitude (Higgins & Richardson, 1999; Nehrbass *et al.*, 2007). This substantial increase in rates of spread has been attributed to the establishment of multiple satellite populations from the initial source population (Ghersa & Roush, 1993; Wallinga *et al.*, 2002). The continually growing numbers of satellite populations also expand by local dispersal, rapidly increasing the area invaded (Nehrbass *et al.*, 2007).

Empirical studies examining seed dispersal have focused mainly on wind dispersal (anemochory), as the long distance dispersal potential of many weed species has been attributed to morphological adaptations for wind dispersal (Benvenuti, 2007). Given the difficulty of recording long distance dispersal, measurements of dispersal are often supplemented by empirical or mechanistic models to permit extrapolation from the data (Bullock & Clarke, 2000; Skarpaas *et al.*, 2004; Dauer *et al.*, 2007). Definitions of long distance dispersal events vary between species and studies, and may be described relative to the proportion of seed dispersed (i.e. 99th percentile of dispersal distances), or be specified as absolute distances (Cain *et al.*, 2000; Higgins *et al.*, 2003). For wind-dispersed seeds of herbaceous and shrubby plants, maximum dispersal distances of over 30 m (Skarpaas *et al.*, 2004), 80 m (Bullock & Clarke, 2000), and 500 m (Dauer *et al.*, 2007) have been recorded in arable field and grassland habitats in Norway, England and the USA, respectively. In all cases, seeds were observed dispersing to the end of the sampling transect, indicating that actual dispersal likely occurred at distances greater than those measured in the studies (Skarpaas *et al.*, 2004; Dauer *et al.*, 2007). Thus, the scale at which sampling occurs will have an obvious impact on the measurement of maximum dispersal distances, and consequently on our understanding of weed distribution processes. Dispersal distance is also shown to increase with increasing seed source strength in simulations (Dauer *et al.*, 2007) and larger source strength may explain the high dispersal distances observed by Dauer *et al.* (2007).

Individual empirical models fitted to wind dispersal data often underestimate the tail of the dispersal curve (e.g. Bullock & Clarke, 2000; Dauer *et al.*, 2007). As such, mixed empirical models, combining features from two or more statistical distributions, best fit dispersal data, and this likely reflects the fact that many seeds may be dispersed by multiple mechanisms (Higgins & Richardson, 1999; Bullock & Clarke, 2000; Higgins *et al.*, 2003). Although standard dispersal mechanisms have traditionally been assigned to species based on the morphological adaptations of dispersing seeds, it is increasingly recognized that non-standard dispersal mechanisms also play a routine and important role in seed dispersal, often at larger spatial scales (Higgins *et al.*, 2003). As such, multiple dispersal vectors must be considered for accurate characterization of species' dispersal curves at a range of distances. Additionally, both the dispersal potential of the seed itself, as well as the disper*sing* potential of the environment must be considered, particularly when describing weed dispersal in agricultural landscapes and other human-manipulated environments (Ghersa & Roush, 1993). In these landscapes, the potential for human-mediated seed dispersal (anthropochory) is considerable (Hodkinson & Thompson, 1997; Benvenuti, 2007). Additionally, anthropogenic dispersal processes frequently operate at spatial scales that are orders of magnitude larger than natural dispersal processes, potentially removing the barriers imposed by the isolation of suitable habitat areas in fragmented landscapes (Ghersa & Roush, 1993).

Although not recognized as a standard dispersal mechanism, human vectors facilitate the spread of weeds and invasive species in fragmented landscapes (Hodkinson & Thompson, 1997; Benvenuti 2007; von der Lippe & Kowarik, 2007a). The most widely described mechanisms of anthropogenic dispersal within arable fields include weed seed movement within the soil profile caused by tillage and displacement of ripe seed by harvesting equipment (e.g. Beckie *et al.*, 2005; Humston *et al.*, 2005; Shirtliffe & Entz, 2005). Agricultural machinery may also spread weed seeds between individual fields if the same machinery is used in multiple fields without cleaning (Benvenuti, 2007). At even larger spatial scales, weed seeds are dispersed by a variety of less well-studied anthropogenic vectors. Human-mediated movement of soil containing weed seeds may result in substantial seed dispersal, particularly where large volumes of soil are moved, as in the transport of commercial topsoil (Hodkinson & Thompson, 1997). Roads and other human-influenced linear features of agricultural landscapes serve as dispersal corridors for invasive and weedy species, as a result of reduced vegetative cover and increased levels of disturbance associated with construction, maintenance, and frequent use (Parendes & Jones, 2000; Gelbard & Belnap, 2003; Lu & Ma, 2006). Additionally, seeds may be dispersed widely by adherence to vehicles (Hodkinson & Thompson, 1997; von der Lippe & Kowarik, 2007a). Seeds dispersed in this manner are often from highly fecund weedy plants with small, persistent seeds (Hodkinson & Thompson, 1997; Zwaenepoel *et al.*, 2006), and dispersal by motor vehicles frequently results in the long distance dispersal of these seeds (von der Lippe & Kowarik, 2007a). At even larger spatial scales, human vectors are extremely effective in dispersing weed seed at regional, national, and global scales, through the transport of grain and the commercial grain trade (Benvenuti, 2007; Shimono & Konuma, 2008). This vector not only drastically increases the dispersal distance of seeds, but also substantially increases the proportion of seeds dispersed over long distances, compared to other human dispersal vectors (von der Lippe & Kowarik, 2007b; Shimono & Konuma, 2008).

Anthropogenic seed dispersal may substantially accelerate the invasion rates and increase the range of weed species. Indeed, human-mediated long distance dispersal of weed seeds enables colonization of a wider variety of habitat types and landscape elements, particularly in highly fragmented agricultural landscapes (Gelbard & Belnap, 2003; Lelong *et al.*, 2007). Thus, at regional scales, both natural and anthropogenic dispersal processes interact with physical landscape features to create and modify the spatial pattern of species distribution. As such, understanding of large-scale patterns of weed distribution in fragmented landscapes must be informed by the spatial connections created by overlying anthropogenic mechanisms, including cropping practices, traffic and transport routes, destination points, and disturbance and management effects.

Regional weed distributions: the utility of the metapopulation concept

At regional scales, both long distance dispersal processes and fragmented landscapes contribute to the patchy distribution of species. Even in homogenous habitats, long distance dispersal events result in the spread of weeds by the establishment of isolated satellite populations (Wallinga *et al.*, 2002). In complex, human-altered landscapes, variation in the habitat suitability of diverse landscape elements may further enhance the disjunct distribution of weed patches at large spatial scales. Analysis of the patchy patterns of weed distribution at the regional scale can be used to infer the mechanisms underlying the spread of weedy and invasive species. Understanding these processes is essential to the design of effective control strategies at large spatial scales (Laffan, 2006).

A number of studies have examined distribution patterns of weeds and invasive species at regional scales, using aerial photographs (Müllerová *et al.*, 2005; Maheu-Giroux & de Blois, 2007), herbarium records (Lavoie *et al.*, 2007; Lelong *et al.*, 2007), or broad-scale population surveys (Deckers *et al.*, 2005; Laffan, 2006; Lelong *et al.*, 2007). These studies range in scale from 60 ha (Müllerová *et al.*, 2005) to 840,000 km^2 (Lavoie *et al.*, 2007), and use geostatistical analyses to interpret distribution patterns. Changes in spatial distribution over time indicate that population expansion is frequently facilitated along linear habitat elements in both semi-natural ecosystems and highly disturbed agricultural landscapes. These patterns may result directly from the suitability of linear landscape features for species establishment and survival; alternatively, distribution along linear habitats may be indirectly influenced by the effects of landscape structure on dispersal vectors (With, 2004). In dry landscapes in Australia, invasive species are present in riparian habitats along streams and rivers where moisture conditions are adequate (Lawes & Grice, 2007). Additionally, dispersal vectors, such as flooding (Lawes and Grice, 2007), are confined to these riparian landscape elements. The distribution of the invasive wind-dispersed species serrated tussock (*Nassella trichotoma* (Nees) Hack.) is also patterned along deep river valleys in Australia, suggesting that the effects of topography on prevailing wind direction and speed determine this species' distribution in the landscape (Laffan, 2006).

In agricultural landscapes, linear anthropogenic habitats, such as hedgerows, roads, and drainage ditches, also play an important role in the regional distribution and spread of weed species. The invasive tree black cherry (*Prunus serotina* Ehrh.) is spreading through agricultural hedgerow networks in Belgium, where its distribution is largely determined by the effects of landscape structure on avian dispersal vectors (Deckers *et al.*, 2005). While younger individuals tend to be spatially clustered around older reproductive trees, the patchy distribution of *P. serotina* at the landscape scale results from long distance dispersal by frugivorous birds living in the hedgerows. Additionally, patches of *P. serotina* tend to be located close to hedgerow intersections and tall roosting trees, landscape features used by the avian dispersers (Deckers *et al.*, 2005). Road networks and associated drainage ditches also facilitate the spread of weedy species, influencing both survival and dispersal processes. Highly disturbed roadside habitats are generally suitable for the establishment of ruderal weedy species (Gelbard & Belnap, 2003) and vehicles serve as important dispersal vectors for the spread of weedy and invasive species in these habitats (von der Lippe & Kowarik, 2007a). In Quebec, Canada, the rapid spread of common ragweed (*Ambrosia artemisiifolia* L.) in the last eight decades has been attributed to the expansion of the road network in that province (Lavoie *et al.*, 2007). While *A. artemisiifolia* is believed to have spread along riparian habitats in the early 20th century and may have first been introduced into arable lands by a seed contaminant, these initial dispersal processes are now considerably less important than the facilitated expansion and persistence of this weed within anthropogenic roadside habitats (Lavoie *et al.*, 2007). In the same Canadian province, rate of invasion by the weedy exotic genotype of common reed (*Phragmites australis* (Cav.) Trin. ex Steud.) has increased in recent years, again coinciding with expansion of the road network and associated ditches, which facilitated inland spread from the St. Lawrence River (Lelong *et al.*, 2007). The spread of this weed was faster in anthropogenic wetlands (road and agricultural ditches) than in natural wetlands, and the latter tended to be invaded only where they intersected anthropogenic wetlands (Maheu-Giroux & de Blois, 2007). Formation of new, distinct *P. australis* patches within linear anthropogenic wetlands likely resulted from seed dispersal by wind or water, as newly colonized patches were spatially aggregated at the regional scale with older weed patches that served as seed sources (Maheu-Giroux & de Blois, 2007).

Although increasing focus on conservation issues within disturbed agricultural landscapes has highlighted the detrimental impacts of fragmentation for many native or rare species, human activities within these managed landscapes may also prove beneficial for some species. In particular, the spread of weeds is facilitated in landscapes structured by wide-ranging, highly connected linear networks, including hedgerow, road and ditch habitats (Deckers *et al.*, 2005; Lavoie *et al.*, 2007; Lelong *et al.*, 2007; Maheu-Giroux & de Blois, 2007). As well as providing suitable habitat and potential dispersal corridors for weedy species, linear habitat networks also prescribe human-use patterns in agricultural landscapes, including seeding, harvest, drainage, and transport. As such, linear habitat networks have substantial direct and indirect impacts on weed seed dispersal, increasing connectivity for these species in otherwise fragmented agricultural landscapes. Additionally, road and ditch networks increase the accessibility of adjacent habitat areas, including both arable fields and semi-natural habitats. Indeed, habitats adjacent to invaded linear road or waterway networks are increasingly susceptible to invasion (Müllerová *et al.*, 2005; Maheu-Giroux & de Blois, 2007), proportional to the intensity of use and disturbance along the linear network (Gelbard & Belnap, 2003). Therefore, despite effective control in adjacent habitats, these may be re-infested from source populations persisting in nearby road or ditch networks (Lavoie *et al.*, 2007). Survival in both roadside habitats and adjacent cultivated land may be common for ruderal species adapted to highly disturbed, resource-rich environments; such species may include both traditional annual agricultural weeds, as well as 'weedy' crop plants capable of becoming established in disturbed habitat outside of arable fields (Leeson *et al.*, 2005). Thus, in agricultural landscapes structured by linear habitat networks, multiple habitat types must be considered in devising effective management strategies. Although 'snapshot' perspectives of static spatial patterns may suggest that weed patches are isolated, it is more likely that in dynamic landscapes, weed patches are connected by inter-patch dispersal between linear networks and adjacent habitats.

The frequent clustering of distinct, newly colonized satellite weed patches around older source populations at various spatial scales (Deckers *et al.*, 2005; Laffan, 2006; Maheu-Giroux & de Blois, 2007), suggests that dispersal events resulting in colonization of new habitat areas play an important role in the expansion of weed species distributions. Species that expand both by local growth of established populations and by colonization of new habitat are expected to spread more rapidly as new satellite populations begin to expand locally as well (Nehrbass *et al.*, 2007). Simulations have shown that the combined area of expanding satellite populations often surpasses the area covered by the initially larger, spreading source population (Moody & Mack, 1988). Although weed control has traditionally focused on large core populations (Moody & Mack, 1988), debate continues regarding appropriate management of satellite populations (Lawes & Grice, 2007), and consideration should be given to these small populations, though they seem less critical than larger source populations. In recent studies, the importance of explicitly managing isolated satellite populations is frequently recognized, both due to the efficiency with which this approach may slow weed spread (Maheu-Giroux & de Blois, 2007; Nehrbass *et al.*, 2007), as well as the relative ease with which newly formed satellites, rather than source populations, may be controlled (Laffan, 2006). Indeed, if left uncontrolled, satellite populations may converge (Moody & Mack, 1988), eventually becoming large source populations capable of forming more new satellites. Thus, interactions and dispersal between initially isolated populations may eventually lead to persistence and spread at the regional scale, necessitating new approaches to management and control.

In the last 25 years, recognizing the importance of regional dispersal between isolated local populations, population biologists have developed the metapopulation model to describe these spatially-structured species distributions (Hanski & Simberloff, 1997). Despite early calls for integration of ecological concepts in the study of weed patches (Firbank, 1993; Cousens & Mortimer, 1995), and the general use of the metapopulation approach to conceptually model weed invasions (Cousens & Mortimer, 1995; Radosevich *et al.*, 2003), this theory has not been widely applied in understanding real weed distribution problems and strategies for effective control.

First proposed by Levins (1969, 1970) to characterize insect populations, the metapopulation model describes a 'population of populations': a set of small isolated sub-populations, each of which may be subject to local extinction but remains connected with other sub-populations through dispersal processes, enabling recolonization following extinction. Thus, the balance of extinction and recolonization events enables the dynamic set of sub-populations to persist at the regional scale despite high levels of local population turnover (Hanski & Simberloff, 1997). Hanski (1997, 1999) outlines four criteria for regional persistence within a classical metapopulation structure. Firstly, sub-populations are arranged in discrete areas of suitable habitat, embedded in a uniformly hostile matrix. Suitable habitat areas may support standing populations (e.g. 'weed patches'); alternatively habitat areas may be suitable but currently unoccupied and available for colonization (hereafter referred to as 'empty patches'). To ensure metapopulation persistence, some empty patches must always be available, i.e. not all suitable habitat will be occupied at a given time. Secondly, all sub-populations are subject to extinction, which results in the disappearance of a sub-population from a previously occupied habitat area, creating an empty patch. Thirdly, all suitable discrete habitat areas, whether occupied or empty, are interconnected by the potential of dispersal from other habitat areas. Finally, population dynamics are asynchronous across the set of sub-populations comprising the metapopulation. Therefore, survival, extinction, and recolonization events do not occur simultaneously across the entire regional distribution of the species. The above criteria collectively translate into an inability to predict the regional dynamics of metapopulations simply from small-scale examination of local populations (Freckleton & Watkinson, 2002). Rather, where populations interact regionally as a metapopulation, appropriate assessment of spatial distribution and persistence must be calibrated to detect differences between dynamics at landscape and local scales (Levin, 1992; Cardina *et al.*, 1997).

The application of the classical metapopulation framework to plant species distributions has been both widespread and controversial (Bullock *et al.*, 2002; Freckleton & Watkinson, 2002; Ehrlén & Eriksson, 2003; Freckleton & Watkinson, 2003; Pannell & Obbard, 2003). For plant populations sustained predominantly by local processes of reproduction, rather than by regional dispersal, metapopulation theory is unlikely to aid in elucidating population dynamics (Freckleton & Watkinson, 2003). However, where regional spatial distributions conform plausibly to Hanski's (1997, 1999) four criteria, the classical metapopulation framework will be useful in providing improved understanding of species dynamics and persistence. In a conservation context, metapopulation dynamics have been used to describe the distribution of the endangered endemic plant Furbish's lousewort (*Pedicularis furbishiae* S. Watson), found along the St. John River in Maine, USA (Menges, 1990). Isolated colonies of this plant grow only in wet or mesic sites with low vegetative cover, located in the mid-level transition zone of the riverbank. All populations may be destroyed due to bank slumping or catastrophic ice scour events. Seeds of *P. furbishiae* may be transported over long distances by water, potentially enabling colonization of new suitable habitat located along the riverbank.

Finally, disturbance dynamics are not synchronous across the range of *P. furbishiae*; while ice scour and bank slumping may destroy multiple populations (especially those located close together), a single disturbance event does not impact all sub-populations within the regional distribution (Menges, 1990). The classical metapopulation model has also been used to characterize the dynamics of water hyacinth (*Eichhornia paniculata* (Spreng.) Solms) in north-eastern Brazil (Husband & Barrett, 1998). This emergent aquatic plant grows in ephemeral pools and ditches, which are easily distinguishable from the surrounding dry caatinga habitat. Populations appear to be subject to extinction regardless of population size, such that even large populations may disappear from habitat patches as a result of human disturbance or drought. While direct measures of colonization rates are not available, correlation between the density of suitable habitat areas in a region and the proportion of those areas occupied by *E. paniculata* suggests that populations are connected by seed dispersal. Thus, where suitable habitat is abundant, these areas are more easily accessed and colonized by dispersing propagules. Finally, size of *E. paniculata* populations varied asynchronously for multiple populations sampled from year to year, implying that local environmental conditions are independent across the region (Husband & Barrett, 1998). The findings of Menges (1990) and Husband and Barrett (1998) suggest that persistence of *P. furbishiae* and *E. paniculata* is determined more by regional than local processes. Furthermore, these results agree with the four criteria for classical metapopulation structure (Husband & Barrett, 1998; Freckleton & Watkinson, 2002), demonstrating the potential utility of this approach in large-scale plant population biology.

Although strictly defined metapopulation concepts are useful and frequently applied, recent definitions of metapopulation dynamics have been expanded to include a larger range of spatially-structured populations (Hanski & Simberloff, 1997; Harrison & Taylor, 1997). Lest these new approaches become too prolific to remain useful (Freckleton & Watkinson, 2002), all new definitions are still characterized by the occurrence of isolated local sub-populations interconnected by dispersal processes (Hanksi & Simberloff, 1997; Harrison & Taylor, 1997). The most frequent variations on the classical metapopulation model are the closely related mainland-island and source-sink structures (Harrison & Taylor, 1997), which are both characterized by asymmetrical dispersal (Vuilleumier & Possingham, 2006) and differential survival of sub-populations. In mainland-island metapopulations, sub-populations vary in size, with large populations (i.e. 'mainlands') generally providing a source of propagules for smaller populations (i.e. 'islands'), which are prone to higher rates of extinction (Harrison & Taylor; 1997). As such, regional metapopulation persistence is determined by the persistence time of mainland sub-populations, as island sub-populations will quickly become extinct unless supported by dispersal of propagules from the mainland (Hanski & Simberloff, 1997). The source-sink metapopulation structure is similar to the mainland-island structure. However, in the source-sink model, differential persistence of source and sink sub-populations is determined by differences in local environmental conditions affecting population growth rates, which are positive in source and negative in sink sub-populations (Hanski & Simberloff, 1997). Again, overall regional persistence is determined by source sub-populations, which disperse propagules to declining sink sub-populations. The mainland-island and source-sink metapopulation models are both essentially in violation of Hanski's (1997, 1999) second criterion, as certain sub-populations are not subject to the high risk of extinction experienced by other sub-populations. Thus, unlike the classical model, these two variations focus attention not on regional *persistence*, but on the ways in which regional *distribution* may increase the expected persistence time and potential recolonization of small or declining local populations (Harrison & Taylor, 1997). Increased persistence of local populations growing in suboptimal conditions, associated with dispersal from source populations, has been implicated in the invasive spread of weedy plant species (Hooftman *et al.*, 2006).

Recently, a further requirement for the classification of regional plant distributions as metapopulations has been proposed; namely that landscapes are structured by an unvarying number of suitable habitat sites in fixed spatial arrangement (Bullock *et al.*, 2002), and that these suitable habitat sites are easily defined by the researcher (Freckleton & Watkinson, 2002). However, the importance of this condition to the metapopulation concept is debatable. Firstly, it is argued that undue attention to the identification and description of suitable habitat results in an unbalanced focus on the cause of patchy species distribution, when it is often the effects of such a distribution that are of greater interest (Pannell & Obbard, 2003). Furthermore, most metapopulation species may in fact be found in successional habitats (Hanski & Simberloff, 1997), where the successional development of suitable sites eventually makes them unsuitable for the species in question, while new suitable habitat is created through disturbance (e.g. Menges, 1990). Importantly, relaxing the requirement for a fixed arrangement of suitable habitat areas recognizes the dynamic nature of fragmented landscapes, and allows for consideration of the effects of the landscape itself on the persistence of regional plant distributions (Geertsema *et al.*, 2002). A wide variety of agricultural landscapes, including both traditional and modern farming systems, may be characterized by cyclical changes in landscape structure and habitat suitability, generally resulting from repeated disturbance and successional development driven by human activity (Kleyer *et al.*, 2007). Most obviously, both cultivation and crop rotation result in recurring changes in habitat suitability (e.g. removal of vegetation and changes in competitive pressure) for weeds in arable fields (Kleyer *et al.*, 2007). Habitat suitability also varies greatly in non-crop habitats in agricultural landscapes, where temporal changes may be either cyclical or stochastic. Linear networks of agricultural ditches are highly dynamic, impacted both by disturbance from agricultural activities in adjacent fields (Geertsema, 2005), and by use and maintenance of adjacent roads (Gelbard & Belnap, 2003). Successional development of ditch communities in the absence of disturbance also changes habitat suitability. Thus, suitable habitat sites within these linear networks are often ephemeral, and consequently difficult to detect (Loehle, 2007). Despite this, ephemeral habitat sites may act as ‘stepping stones’ for species movement, effectively enhancing dispersal between ideal habitat areas and increasing the persistence of plant metapopulations in fragmented landscapes (Loehle, 2007). This effect of ephemeral habitat sites is often dependent upon high propagule pressure arising from populations in ideal habitat sites, enabling the temporary establishment of reproducing populations in transient sites (i.e. source-sink dynamics: Hooftman *et al.*, 2006; Kleyer *et al.*, 2007). While agricultural landscapes are highly dynamic over short temporal scales, landscape structure and habitat suitability may also be impacted more permanently by changing farming systems (Baudry *et al.*, 2003). Changes associated with agricultural intensification tend to reduce non-crop habitat area across the landscape and often also increase the rate at which habitat changes occur. Generally, the impacts of these temporal changes are species-specific and related to the rate at which habitat turnover occurs (Geertsema *et al.*, 2002; Matlack & Monde, 2004; Kleyer *et al.*, 2007). For species with low dispersal ability, stochastic disturbances in suitable habitat areas may occur too frequently to enable persistence (Matlack & Monde, 2004). Conversely, lack of disturbance may reduce the survival probabilities of ruderal species, as successional development of suitable habitat areas eventually results in competitive exclusion (Kleyer *et al.*, 2007). Indeed, the well-developed dispersal and dormancy characteristics of many weedy and ruderal species enable persistence in landscapes characterized by high spatial and temporal variability in habitat suitability (Geertsema *et al.*, 2002).

At the landscape scale, consideration of cyclical habitat changes and environmental stochasticity (i.e. variability) leads to a re-examination of the perceived binary nature of suitable habitat and hostile matrix (Murphy & Lovett-Doust, 2004); what is suitable habitat at one point in time may eventually become unsuitable and new suitable habitat may simultaneously be created (Loehle, 2007). This view of dynamic landscapes promotes a more realistic understanding of the spatial habitat patterns underlying metapopulation structure. Additionally, it serves to integrate the approaches of metapopulation biology and landscape ecology (Wiens, 1997; Murphy & Lovett-Doust, 2004). While the former discipline has traditionally viewed the ‘matrix’ as uninhabitable and unchanging, landscape ecologists recognize that the surrounding landscape may impact both ecological processes within suitable habitat areas and species’ movement patterns (Wiens, 1997). As a result, all components of the mosaic landscape impact the regional persistence of metapopulations. Thus, this dynamic view of metapopulations in changing environments suggests that regional persistence may be partly dependent on sufficient levels of *habitat* turnover creating suitable areas for colonization; this contrasts from earlier models, in which persistence is dependent only on sufficient levels of *population* turnover (Harrison & Taylor, 1997; Bullock *et al.*, 2002).

The large-scale dynamics of plant populations are best considered as a mix of the regional structures described by metapopulation biology and landscape ecology, and these variant forms are expected to be more common than the strictly defined classical model (Harrison & Taylor, 1997; Vuilleumier & Possingham, 2006). Dynamic models of metapopulation persistence are especially suited to anthropogenic landscapes and may serve as a useful framework for examining weed patch dynamics at large spatial scales. Weed spatial distributions are greatly impacted by anthropogenic forces, resulting in long distance dispersal events, high rates of population turnover, and interactions with mosaic landscape structures. While important to local survival, demographic processes in individual weed patches are likely to be less influential in determining regional persistence and spread. As such, the importance of calibrating weed patch studies to capture ecological processes occurring at multiple spatial scales is increasingly recognized (Cousens & Mortimer, 1995; Cardina *et al.*, 1997; Rew & Cousens, 2001). Concurrently, weed management strategies must be extended to larger spatial scales, to increase the efficiency with which regionally-distributed weeds are controlled. Indeed, in some cases, conclusions regarding persistence may change drastically when multiple spatial scales are considered, potentially leading to different management recommendations. In a multi-scale study of the distribution of patches of Canada thistle (*Cirsium arvense* (L.) Scop.) in an agricultural landscape in Bavaria, findings differed at the local and regional levels (Eber & Brandl, 2003). At the local scale, management of individual thistle patches decreased patch size by more than half over the course of the study, increasing vulnerability to extinction. Conversely, and simultaneously, the number of individual weed patches in the landscape increased by approximately 50%, through colonization of recently disturbed sites. While local observations point to declining weed populations, investigations at the regional scale suggest that the weed may be persisting as a metapopulation (Eber & Brandl, 2003), if long-distance dispersal and recolonization compensate for decreases in patch size. Theoretical approaches have also demonstrated potential metapopulation structure in weeds distributed across different landscape elements. In a spatial model of *C. arvense* patches at the field edge, the presence of *C. arvense* in the field margin increased invasion rates by enabling the establishment of new patches in previously unoccupied fields (Blumenthal & Jordan, 2001). Margin weed patches, which are generally not explicitly controlled, may therefore serve as source populations for controlled sink populations within managed fields (Blumenthal & Jordan, 2001; Lavoie *et al.,* 2007). Thus, field weed patches can only be understood with reference to adjacent habitats, and control activities may need to be extended beyond individual fields to remain effective. The metapopulation framework can improve our understanding of the mechanisms of weed patch persistence at the regional scale, enabling the development of new strategies for weed control that are responsive to the ecological processes governing weed spatial distribution. Such an approach may be especially useful in the consideration of newly developing weed problems, where patterns of range expansion and spread can be directly linked to human activities in agricultural landscapes.

Implications for consideration of gene flow and trait confinement in GM crops

Crop volunteers have long been recognized as important weeds with the potential to reduce crop yield through competition and reduce crop quality through seed contamination, resulting in lower economic return for farmers (Orson, 1993). The advent of GM crops has underscored the need to control volunteers as weeds, particularly for herbicide-tolerant (HT) varieties, which are the most commonly cultivated GM crops (Beckie *et al.*, 2006). Widely grown in North America for over a decade, GM crops have been rapidly adopted by farmers due to perceived operational benefits; this is especially evident in the high adoption rates of GMHT canola (*Brassica napus* L.) in western Canada (Beckie *et al.*, 2006; Mauro & McLachlan, 2008). However, volunteers from GMHT crops are increasingly reported by farmers as representing a substantial risk associated with the cultivation of these crops (Mauro & McLachlan, 2008). For GMHT *B. napus*, as well as conventional canola lines, most volunteers recruited from seeds lost during harvest emerge in the year immediately after cultivation (Harker *et al.*, 2006). Control of these HT volunteers requires application of additional herbicides (e.g. 2,4-D [(2,4-dichlorophenoxy) acetic acid] or MCPA [(4-chloro-2-methylphenoxy) acetic acid]; Simard *et al.*, 2002; Friesen *et al.* 2003; Hall *et al.* 2005), especially where multiple GM varieties are grown in close proximity, potentially resulting in volunteers with multiple herbicide tolerances (Hall *et al.*, 2000; Beckie *et al.*, 2003). Because of the additional expense required, volunteers may not be directly controlled, especially if low densities are perceived to result in low levels of competitive interference with subsequent crops (Harker *et al.*, 2006). However, GM volunteers may persist at low densities for up to five years in Canadian cropping systems (Simard *et al.*, 2002). In France, seed of GM crops may contaminate succeeding conventional crops up to eight years after cultivation, reducing the ability of the farmer to market these conventional crops as GM-free (Messéan *et al.*, 2007). Indeed, farmers in western Canada have identified the loss of markets due to genetic contamination as the most important risk associated with the cultivation of GM crops (Mauro & McLachlan, 2008). This risk extends beyond individual farmers to a national scale, impacting global export markets for countries where GM cultivation is widespread (Demeke *et al.*, 2006). As well as limiting marketability and consequently economic returns, GM contamination may necessitate changes in farming operations to ensure effective control of HT or other GM volunteers. The contamination of pedigreed seedlots with adventitious GM traits (Friesen *et al.*, 2003) results in the widespread dissemination of these unwanted traits in arable fields. Producers practicing zero- or conservation-tillage deliberately avoid tillage to reduce soil erosion; as such, they are often dependent on the broad-spectrum herbicide glyphosate to control weeds and volunteers (Friesen *et al.*, 2003). The adventitious presence of GMHT volunteers tolerant to glyphosate in zero-till fields considerably reduces the effectiveness of herbicidal weed control, and in some cases has resulted in the resumption of tillage operations (Mauro & McLachlan, 2008) at the expense of the conservation benefits of zero-till practices. Organic farming operations are also at risk from GM contamination, and widespread cultivation of GM varieties has severely limited the development of an organic canola industry in western Canada (Smyth *et al.*, 2002). It is increasingly difficult to establish and maintain appropriate isolation distances to ensure GM confinement in areas where GM crops are widely grown (Beckie *et al.*, 2006). Even among producers who have willingly adopted GM varieties, the presence of GM volunteers may prohibit subsequent cultivation of conventional varieties (Messéan *et al.*, 2007), thereby further limiting cropping system choices for farmers. Although first-generation GM food crops were not segregated from conventional varieties (Smyth *et al.*, 2002; Marvier & Van Acker, 2005), it is anticipated that control and confinement of GM volunteers will become crucial with the pending commercial introduction of the next generation of GM crops, engineered for altered nutritive qualities, as well as pharmaceutical and industrial traits (Smyth *et al.*, 2002; Conner *et al.*, 2003). The escape of nutraceutical, pharmaceutical and industrial GM varieties from fields where they have been deliberately planted may result in substantial health risks for consumers, as well as potential environmental risks (Marvier & Van Acker, 2005). As such, failure to effectively contain these new GM varieties will represent important liabilities for the developers and regulators of crops with novel traits, and potentially for producers as well (Smyth *et al.*, 2002).

The perceived effectiveness of GM trait confinement strategies depends on the role anthropogenic forces are assumed to play (Marvier & Van Acker, 2005) and on the scale at which risk of transgene escape is assessed. Just as successful weed control strategies require assessment of weed persistence and distribution at multiple scales and by multiple mechanisms, effective confinement strategies for current and future GM crops must consider the potential persistence of GM volunteers at the landscape scale. Crop plants containing GM traits are most obviously present in large populations growing in optimal conditions, i.e. in arable fields where these crops are deliberately planted or where they occur as volunteers. However, smaller patches of suitable habitat may also exist in other landscape elements. Non-field habitats are frequently colonized by crop plants that are capable of dispersing and establishing beyond the boundaries of arable fields, effectively ‘escaping’ from cultivation. Both in-crop volunteers and escaped plants may be precursors to feral plants, which are derived from domestic plants that have evolved to sustain reproducing populations outside of human management and cultivation (Gressel, 2005).

The recognition that GM crops might escape from arable fields and subsequently develop feral traits informed early risk assessment of these new varieties. Studies assessing the persistence and invasiveness of new GM varieties have generally been undertaken at the individual patch scale and involve sowing and monitoring small artificial volunteer populations in natural (Crawley *et al.*, 1993; Crawley *et al.*, 2001; Godfree *et al.*, 2004) and semi-natural habitats (Eastick & Hearnden, 2006). Generally, experimental populations of GM crops are compared to conventional varieties, and invasiveness is assessed based on the intrinsic rate of population increase (λ) of individual volunteer weed patches (Conner *et al.*, 2003). Such experiments have been undertaken for a variety of GM crops, including HT *B. napus* (Crawley *et al.*, 1993; Crawley *et al.*, 2001); HT corn (*Zea mays* L.), HT sugar beet (*Beta vulgaris* L.), and insect tolerant potato (*Solanum tuberosum* L.) (Crawley *et al.*, 2001); subterranean clover (*Trifolium subterraneum* L.) modified for improved nutrition (Godfree *et al.*, 2004); and insect tolerant cotton (*Gossypium hirsutum* L.) (Eastick & Hearnden, 2006). The population growth rate (λ) of experimentally established crop populations is highly dependent on the level of competition from surrounding native vegetation (Crawley *et al.*, 1993). While persistence is possible for a short time in disturbed habitat, population growth rates decline as competitive pressure from native perennial vegetation increases through succession (Crawley *et al.*, 2001; Godfree *et al.*, 2004). Generally, patch-level studies report local population growth rates below replacement levels (i.e. $\lambda < 1$), indicating that experimental patches of GM volunteers are declining to extinction (Crawley *et al.*, 1993; Godfree *et al.*, 2004; Eastick & Hearnden, 2006). As such, GM varieties are considered to be no more persistent than their conventional counterparts in non-crop habitats. Thus, most authors conclude that the invasion risk of GM crops is negligible, particularly in habitats of conservation value that are generally dominated by native perennial vegetation (Godfree *et al.*, 2004).

Although GM volunteer patches are perceived to be transient and easily controlled (Hall *et al.*, 2005), often leading to the dismissal of risks associated with GM escape, these conclusions are generally based on assessment of persistence at a single spatial scale. Just as early weed patch studies emphasized local demographic processes rather than regional distributions, experimental studies focusing on individual volunteer patches ignore the larger landscape context. Consideration of the impacts of changing spatial scale, increasingly recognized in weed research, must similarly be extended to studies of GM volunteer persistence. Indeed, in agricultural landscapes, multiple escaped crop populations are likely to be present in a variety of different landscape elements, and regional inter-population processes may interact with local population growth rates to impact persistence at larger spatial scales. As such, multi-scale studies are necessary to ensure appropriate risk assessment of escaped GM crops, and to evaluate the effectiveness of control and confinement strategies designed to minimize that risk.

While empirical assessment of escaped crop plants at larger spatial scales remains limited, the research that has been conducted at the landscape level generally focuses on *B. napus*, due to early introduction of GM *B. napus* varieties, and the weedy tendencies of this species. Populations of *B. napus* are ubiquitous in roadsides and other ruderal habitats in a wide range of agroenvironments (Crawley & Brown, 1995; Pessel *et al.*, 2001; Crawley & Brown, 2004; Aono *et al.*, 2006; Yoshimura *et al.*, 2006; Knispel *et al.*, 2008). Where GM varieties are widely cultivated, these are proportionally represented in escaped populations (Yoshimura *et al.*, 2006; Knispel *et al.*, 2008). Additionally, in countries where GM varieties are not cultivated but are imported as food and feed grain, escaped GM volunteers are found at ports where grain shipments are received (Aono *et al.*, 2006), indicating that these novel varieties are widely dispersed by global commercial trade. Landscape scale surveys report varying levels of persistence for individual escaped *B. napus* populations, ranging from two years (Crawley & Brown, 1995) to nine years (Pessel *et al.*, 2001). However, these populations are generally not considered to be locally self-sustaining (Crawley & Brown, 2004), reflecting conclusions derived from small-scale, patch-based assessments. Despite this, *B. napus* remains a permanent feature of roadside habitats, in what has been termed the ‘paradox’ of escaped volunteer populations (Crawley & Brown, 1995).

It is precisely this paradox that points to the utility of the metapopulation concept as a framework for understanding the contribution of escaped volunteer populations to the persistence and spread of GM crops at the landscape scale. Indeed, the distribution and dynamics of escaped crop populations may be considered with reference to Hanski's (1997, 1999) four criteria for classical metapopulation structure. Metapopulations are structured by discrete areas of suitable habitat within a relatively hostile matrix, and within these habitat areas, all populations are subject to extinction (criteria 1 & 2). For escaped populations of crops plants, the highly disturbed nature of road and ditch networks contributes to both successful establishment and eventual extinction. Dynamic roadside habitats are subject to cycles of soil disturbance and succession, creating discrete areas of bare ground for volunteer establishment and growth that are subsequently recolonized by surrounding vegetation, resulting in competitive exclusion (Crawley & Brown, 1995, 2004; Yoshimura *et al.*, 2006). As such, escaped populations of *B. napus* in roadside habitats exhibit high rates of local population turnover (Crawley & Brown, 1995, 2004) and all volunteer populations are subject to extinction risk as habitat suitability decreases over time. Hanski (1997, 1999) also specifies that the dynamics of subpopulations within a metapopulation are asynchronous at large spatial scales (criteria 4). The shifting habitat mosaics represented by road and ditch networks in agricultural landscapes support many escaped volunteers populations that are subject to different environmental conditions due to their regional distribution. In a survey of escaped *B. napus* volunteers along 189 km of roadside habitat in England, Crawley & Brown (2004) found that population survival was determined primarily by local disturbance and succession, which synchronously affected only small areas of potential habitat (i.e. 100 – 2000 m). Additionally, local population levels were correlated with total mean regional population levels in only 23% of 3658 roadside sites sampled

(Crawley & Brown, 2004), indicating that individual populations frequently varied independently of the overall metapopulation dynamics.

Finally, metapopulation persistence is dependent on dispersal between suitable habitat areas, effectively connecting discrete sub-populations and enabling recolonization (Hanski 1997, 1999). Frequent recolonization of empty patches was observed in roadside *B. napus* populations (Crawley & Brown, 2004), and the establishment of new volunteer populations is largely attributed to continual seed input into roadside habitats from seed lost during agricultural transport (Crawley & Brown, 1995, 2004; Yoshimura *et al.*, 2006). Indeed, the seeds of many arable crop species are widely dispersed into road verges by vehicles (von der Lippe & Kowarik, 2007b). Although it is argued that populations dependent on anthropogenic seed inputs are not truly self-sustaining (Crawley & Brown, 2004), implying that concerns regarding their persistence are unwarranted, it is unrealistic to assume that agricultural transport of grain and associated seed spillage will cease, for either conventional or GM varieties. As such, the local dynamics of escaped volunteer populations in roadside habitats will continue to be supplemented by regional human-assisted dispersal processes. In addition to transport-mediated dispersal, other anthropogenic dispersal vectors operate at large spatial scales; importantly, seeding and cultivation of GM crops represent deliberate dispersal of these varieties into ideally suited habitats. Arable fields support highly reproductive crop and volunteer populations, and may serve as large source populations and/or refuges for GM cultivars, exchanging propagules with widely distributed sink populations in less suitable roadside habitats. Although ephemeral escaped volunteer populations are likely to be reliant on high propagule pressure, arising either from surrounding fields or from seed spilled in transport, they may nonetheless contribute importantly to metapopulation persistence by facilitating dispersal between larger sub-populations growing in more suitable conditions (Loehle, 2007). Additionally, escaped volunteer populations, once established in roadside habitats, are generally not explicitly controlled (Yoshimura *et al.*, 2006), and the extent to which they may themselves serve as a source of seed for the colonization of adjacent habitat remains unknown.

In human-managed agricultural landscapes, regionally distributed crop species likely persist in what may essentially be considered ‘culturally-mediated’ metapopulations, dependent as much on ‘artificial’ dispersal mechanisms as on ‘natural’ ecological dispersal mechanisms. As such, both artificial and natural dispersal processes must be considered in assessing the persistence and spread of escaped volunteer populations. However, large-scale ecological surveys are often limited in their ability to evaluate the relative importance of the various dispersal mechanisms contributing to population persistence (Crawley & Brown, 1995; Pessel *et al.*, 2001). It is inherently difficult to monitor dispersal and recolonization processes at the large spatial and temporal scales required for empirical assessment of metapopulation dynamics. Additionally, of necessity, large-scale ecological surveys generally evaluate persistence based solely on the presence of reproductive adult plants (Crawley & Brown, 1995, 2004; Pessel *et al.*, 2001), thereby underestimating the contribution of other growth stages to population survival. Thus, while empirical studies have been very important in describing the spatial distribution of escaped volunteer populations, they can be greatly complemented by theoretical approaches. Metapopulation models enable researchers to examine the contributions of specific dispersal mechanisms to the persistence of escaped populations, and to consider the impacts of changing landscape structure and the number and arrangement of suitable habitat sites. Additionally, these models can be designed to consider the entire plant life cycle, including seeds in the seed bank, when assessing population persistence. As such, models simultaneously enhance our understanding of the dynamics of regionally distributed populations, and point to important areas for future empirical study.

Simulating individual populations of escaped *B. napus* founded by seed spill events, Claessen *et al.* (2005a) show that higher initial numbers of spilled seeds result in increased persistence of established populations, reinforcing the importance of anthropogenic dispersal events. In the absence of repeated seed inputs, populations developed locally in a stochastic environment, incorporating both good and bad years for seed production and favourable and unfavourable sites for germination and seedling establishment. Although stochasticity decreased growth rates, populations were able to increase when the probability of favourable (disturbed) conditions was relatively high (i.e. > 28%). Local seed production contributed to population persistence when conditions were favourable and seed bank survival was crucially important during unfavourable years, suggesting that local population dynamics remain important despite initial anthropogenic seed inputs. The model predicted longer persistence times than those observed in ecological studies, largely due to the consideration of multiple growth stages. However, despite these predictions, escaped populations were still expected to behave as sinks, ultimately becoming extinct (Claessen *et al.*, 2005a). In a second paper, Claessen *et al.* (2005b) extended the previous model to consider the effects of metapopulation structure, including spatial variation in habitat suitability and seed dispersal between habitats. Complete spatial autocorrelation in habitat conditions (i.e. disturbance rates) created uniformly suitable or unsuitable conditions throughout the entire region, and thus produced similar outcomes to the initial, single-population model. However, even small levels of asynchrony in habitat dynamics increased metapopulation persistence, as populations were able to disperse seeds to nearby suitable habitat sites when the current site became unsuitable, thereby reducing extinction risks. Although ruderal populations of escaped crop species are largely dependent on disturbance, mowing and herbicide application in roadside habitats may also reduce survival and seed production. Simulations including the negative effects of roadside management showed that these control measures were the most important factors limiting population survival (Garnier *et al.*, 2006). However, the extent to which roadside populations of escaped volunteers are explicitly controlled varies considerably, being relatively high in Europe (Garnier *et al.*, 2006) but negligible in North America (Yoshimura *et al.*, 2006).

In their metapopulation model, Claessen *et al.* (2005b) also considered dispersal between suitable habitat sites, including both 'natural' seed dispersal processes between adjacent escaped populations and repeated anthropogenic inputs from spilled seed. When considered as a set of interconnected sub-populations, regional persistence of escaped *B. napus* was considerably greater than when these populations were studied in isolation (e.g. Claessen *et al.*, 2005a). Specifically, increasing rates of dispersal between neighbouring populations increased both local population growth rates and overall metapopulation growth rate, measured as change in the proportion of occupied habitat sites over time. Thus, local dispersal contributed importantly to the recolonization of sites following extinction, although positive metapopulation growth may require unrealistically large dispersal rates between neighbouring sites (i.e. > 0.25) (Claessen *et al.*, 2005b). Other simulations of escaped *B. napus* volunteer populations also emphasize the role of seed dispersal from local populations, stressing specifically the importance of long distance dispersal (Garnier & Lecomte, 2006). Indeed, even a small proportion (i.e. 1%) of seeds dispersing over long distances (i.e. 20 – 300 m) can have important effects on the speed at which escaped *B. napus* populations invade roadside habitats (Garnier & Lecomte, 2006), and both dispersal distance and invasion speed can be greatly augmented by adherence to vehicle tires and road verge management machinery (von der Lippe & Kowarik, 2007b). These results point to the importance of using realistic seed dispersal functions, which do not underestimate the tail of the dispersal curve. Additionally, these models suggest that escaped *B. napus* will be difficult to control in semi-natural habitats, as local management efforts will be counteracted by rare long distance dispersal events that drive the spread of this species, despite their low frequency (Garnier & Lecomte, 2006).

Generally, simulations of escaped *B. napus* volunteers which consider local population dynamics, including seed bank survival and successful recruitment, predict average persistence times of five years or more for individual populations (Claessen *et al.*, 2005a; Garnier *et al.*, 2006), corresponding to the observations of some empirical studies (e.g. Pessel *et al.*, 2001). However, the population growth rates calculated from these models are generally less than one, indicating that local population dynamics are not self-replacing (Claessen *et al.*, 2005a; Garnier *et al.*, 2006). Despite this, frequent seed inputs from both seed spills and seed dispersal from adjacent *B. napus* crops, may increase persistence of escaped populations up to ten years (Garnier *et al.*, 2006).

Additionally, although local extinction is certain, regional persistence is ensured by anthropogenic dispersal mechanisms when populations are considered in the context of dynamic agricultural landscapes; overall metapopulation extinction is ‘artificially’ prevented as long as the *B. napus* crop is cultivated and transported within the agricultural landscape (Claessen *et al.*, 2005b). Indeed, the movement of seeds between arable fields and non-crop populations is an important potential escape route for GM traits, as field populations are regularly replenished by the cultivation of *B. napus* crops. Nonetheless, in unfavourable conditions, when escaped populations of crop volunteers are dependent on repeated anthropogenic seed inputs, they are unlikely to evolve traits promoting ferality, as non-feral genotypes are introduced continually and remain more prevalent than locally recruited genotypes. However, negative impacts arising from the escape of GM crops from cultivation are not limited solely to the evolution of ferality. Although they may be transient, escaped crop plants capable of reproducing in non-crop habitats are still a potential source of concern, and effective risk assessment must identify all risks posed by the new technology as these become apparent (Conner *et al.*, 2003).

The focus in GM risk assessment on the environmental impacts of these new crops, although warranted, has often led to the neglect of agronomic impacts. As a result, ferality and gene flow to wild relatives have both been widely assessed, while the implications of crop-to-crop gene flow have only recently been considered (Ellstrand, 2001). However, levels of pollen-mediated gene flow (PMGF) between conspecific GM and non-GM crops may be considerable, even over a single growing season. This is particularly relevant for wind- and insect-pollinated crops such as *B. napus*, in which outcrossing events occur commonly and in a large proportion of plants. As with seed dispersal, intra-specific pollination may result in rapid contamination of non-GM crops, potentially reducing marketability, increasing control costs, and reducing cropping system choices for farmers (Smyth *et al.*, 2002). Furthermore, the observed and predicted impacts of PMGF are also scale-dependent.

As herbicide tolerance traits provide useful and unambiguous genetic markers, many studies assessing PMGF have examined pollen dispersal from HT varieties of *B. napus* to susceptible non-GM *B. napus* pollen receptor populations. These studies, conducted under field conditions, examine outcrossing rates between small experimental plots of HT *B. napus* and surrounding borders of synchronously-flowering susceptible cultivars (Staniland *et al.*, 2000; Klein *et al.*, 2006). Generally, pollen flow decreases with increasing distance, with reported outcrossing rates declining from 1.56% at 0 m to 0.03% at 30 m (Staniland *et al.*, 2000), and from 4.80% at 1.5 m to 0.0024% at 40 m (Klein *et al.*, 2006) from the pollen source. Although specific values of PMGF vary considerably between studies due to both environmental and methodological factors, consistent patterns are generally observed, showing relatively high outcrossing rates in close proximity to pollen sources and rapid decline over short to intermediate distances from the experimental plot. These patterns have been variously described with empirical models. While negative exponential curves are frequently used to describe pollen dispersal functions (Staniland *et al.*, 2000), dispersal curves with either thinner or fatter tails have also been fit to empirical data (Klein *et al.*, 2006). Choice of appropriate dispersal functions has considerable impacts on the extrapolation of experimental results to larger spatial scales; indeed, predictions of outcrossing rates at long distances are most sensitive to the empirical model used to describe the dispersal curve, and particularly, the tail of the curve (Klein *et al.*, 2006). However, when pollen flow is not experimentally assessed or observed at long distances, it is impossible to correctly characterize the tail of the pollen dispersal function and it is inaccurate to use this function to predict long-distance cross-pollination rates (Willenborg & Van Acker, 2006). Consequently, pollination studies undertaken under field *conditions* do not necessarily represent realistic descriptions of PMGF from field *scale* populations.

At the landscape scale, pollination events have been observed between large commercial fields of *B. napus*. In agroecosystems where HT varieties were recently introduced, pollen flow from commercial HT fields resulted in the contamination of conventional *B. napus* fields, at distances up to 2.5 km within a single growing season (Rieger *et al.*, 2002; though seed impurities may also have contributed to contamination). Similarly, where multiple GM crops are widely grown, outcrossing between different cultivars in adjacent fields has resulted in the production of individual volunteers with multiple herbicide tolerance traits (i.e. trait-stacking) in a single growing season (Hall *et al.*, 2000; Beckie *et al.*, 2003); such volunteers are effectively controlled only with additional herbicides and associated expenses (Friesen *et al.*, 2003). These findings emphasize the rapid rate at which intraspecific gene flow occurs (Smyth *et al.*, 2002; Marvier & Van Acker, 2005). Additionally, they reinforce that pollen dispersal at large scales is best characterized by fat-tailed dispersal curves (Devaux *et al.*, 2005) that adequately describe the relatively common occurrence of PMGF between distant sites. Furthermore, multiple pollen vectors are expected to contribute to cross-pollination rates at landscape scales (Devaux *et al.*, 2005), and each vector is likely to be characterized by a different pollen dispersal curve. Cross-pollination rates are also expected to vary with changes in the size and dimension of both the pollinator and receptor fields (Klein *et al.*, 2006; Willenborg & Van Acker, 2006); generally, both larger pollen sources and smaller receptor fields are expected to increase contamination rates. Thus, assessment of PMGF at the landscape scale ensures that the impacts of both long distance dispersal and large pollen sources are integrated into predictions of outcrossing rates, increasing the relevance of these predictions for risk assessment of GM crops approved for unconfined environmental release and commercial cultivation (Rieger *et al.*, 2002; Klein *et al.*, 2006).

Despite increasing recognition of the potential contribution of intra-specific gene flow to transgene escape, little work has examined the role of escaped populations of crop volunteers in facilitating gene flow. As escaped populations are generally small, they may have proportionally small contributions to PMGF at the landscape scale when compared to pollen flow from commercial fields (Devaux *et al.*, 2005). Conversely, these small populations may serve as effective stepping stones for gene flow, connecting larger field populations of GM and/or non-GM crops. The model GENESYS, developed by Colbach *et al.* (2001a; 2001b), simulates the spread of GMHT traits in *B. napus* by seed dispersal and pollen flow at the landscape level. The model considers both cropped and volunteer populations within arable fields, as well as escaped populations growing along field margins and road verges. In model simulations, the presence of escaped volunteers in border habitats greatly facilitated transgene spread from a single field cultivated with GM *B. napus* into an agricultural landscape where GM crops were not previously grown. The presence of these escaped plants increased both the frequency and proportion of the GM trait in neighbouring non-GM fields (Colbach *et al.*, 2001b). Over the course of an eight-year simulation, the spread of the GM trait resulted in considerable contamination of non-GM fields, at levels necessitating additional control practices and jeopardizing the non-GM status of the conventional crops. Thus, although escaped populations of GM *B. napus* are small and exhibit equivalent persistence times to corresponding conventional varieties, the agronomic risks posed by the escape of the GM trait remain considerable (Claessen *et al.*, 2005a). The survival of escaped GM crops over even a single growing season must be evaluated based on what is acceptable within a given cropping system, agricultural landscape, and social context.

In Europe, outcrossing events observed between cultivated fields and experimentally established escaped populations of *B. napus* indicate that isolated volunteer populations receive pollen from distant sources. Male-sterile plants are frequently used to quantify the distance at which PMGF can occur in agricultural landscapes, as any seed produced by these plants is unambiguously the result of cross-pollination events. In a *B. napus* production region in France, 13% of the seed produced by experimental male-sterile plants was attributed to pollen sources more than 1 km away, using genetic analyses to assign paternal cultivars from among those grown in the region (Devaux *et al.*, 2005). The pollen cloud produced by cultivars grown in the region was highly diverse, and, on average, the male-sterile plants were fertilized by pollen from seven different pollen sources (Devaux *et al.*, 2005), indicating that landscape-scale cultivation patterns greatly impact escaped populations. In Great Britain, pollination of male-sterile plants was observed up to 26 km away from any identified pollen source; these exceptionally long distance pollination events are attributed to insect pollination vectors (Ramsay *et al.*, 2003). Additionally, genetic analyses of seed produced in escaped populations suggest that small proportions of this seed arose from cross-pollination with adjacent cultivated fields of *B. napus* (Ramsay *et al.*, 2003). Contrary to trends observed for gene flow between arable field populations, landscape-level gene flow to small escaped populations of *B. napus* appears to be greater than gene flow to larger escaped populations, likely as a result of the lack of adjacent pollinating individuals in small populations (Ramsay *et al.*, 2003; Devaux *et al.* 2005; also observed for weed beet populations by Fénart *et al.*, 2007). Thus, even individual escaped plants may contribute to gene flow at large scales, although apparently insignificant and very difficult to effectively detect and control (Fénart *et al.*, 2007). In North American agricultural landscapes where GM crops are widely cultivated, herbicide tolerance traits are ubiquitous in escaped populations of *B. napus* (Yoshimura *et al.*, 2006; Knispel *et al.*, 2008). These escaped populations are found in close proximity to arable fields, having been established from seed spilled during seeding or harvest activities. Additionally, repeated anthropogenic seed inputs results in the accumulation of multiple GM traits within escaped populations. Consequently, as outcrossing events occur commonly within these relatively short-lived populations (Knispel *et al.*, 2008), escaped volunteers may serve as sources of both GM pollen and GM seed. Indeed, cross-pollination events within escaped populations result in trait-stacking and the production of multiple herbicide-tolerant offspring in ruderal roadside habitats (Knispel *et al.*, 2008). These findings suggest that geographical isolation alone will be ineffective in preventing seed contamination by adventitious cultivars (Ramsay *et al.*, 2003), and consequently other approaches must be undertaken to ensure cultivar purity.

Increasing recognition of the importance of PMGF in escaped volunteer populations, particularly over long distances (Willenborg & Van Acker, 2006), reinforces the role of these populations as potential refuges or stepping stones contributing to the persistence of transgenes at the regional scale. While metapopulation models traditionally focus on the role of propagule dispersal in ensuring persistence (Freckleton & Watkinson, 2002), this conceptual framework may also be applied to the spread and persistence of individual genes of interest. In agricultural landscapes, escaped populations, field volunteers and cultivated crops all serve as suitable ‘habitats’ supporting GM transgenes, which may be frequently dispersed between these ‘sites’ by PMGF. Although individual plant populations may periodically become ‘unsuitable’ for the transgene (i.e. through local extinction), the regional persistence of the gene may be possible within a set of patchy sub-populations. Additionally, fitness advantages conferred by the transgene may even increase its frequency. This ‘meta-persistence’ of transgenes may potentially occur without repeated introductions of the gene of interest, and may be likely even in situations where the widespread cultivation of GM varieties has been discontinued. As such, regulation of widespread environmental release for current and future GM varieties must consider the potential that these genes are irrevocable (Marvier & Van Acker, 2005).

Conclusions

Research on the spatial distribution of weed patches is increasing, and is currently undertaken on multiple spatial scales. At the scale of the arable field or experimental plot, this research has generated considerable interest in site-specific weed control practices. However, such fine scale management approaches are predicated on the stability of weed distribution patterns in space and time, although our growing understanding of the nature of weed distributions suggests that weed patch stability may often decrease with increasing spatial scale. Indeed, the multiple dispersal processes underlying weed patterns interact to change the spatial structure observed from the field to the landscape scale. Natural dispersal processes over short distances often result in the spread of weed patches as an advancing front. Conversely, human-mediated dispersal is orders of magnitude larger than natural processes, resulting in weed spread through the establishment of isolated satellite weed patches; consequently, the efficacy of site-specific management approaches is reduced (Wallinga *et al.*, 2002). Increasingly, authors examining regional weed distribution patterns and processes of spread have called for collaborative, broad-scale management plans that integrate multiple stakeholders to achieve effective weed control (Firbank, 1993; Colbach *et al.*, 2001b; Dauer *et al.*, 2007; Lavoie *et al.*, 2007). This differs greatly from the site-specific approaches currently promoted, and even from the tradition of managing single fields in isolation. However, frequent long distance dispersal events likely result in the movement of weed propagules between fields, as well as the colonization of new non-crop habitats (Dauer *et al.*, 2007). Management plans need to integrate these diverse landscape elements, and explicitly consider both the natural and anthropogenic dispersal processes connecting them.

In the context of the development and spread of herbicide-tolerant weeds and volunteers, regional management approaches, designed to mitigate anthropogenic seed dispersal and limit PMGF, would both necessitate and facilitate increased collaboration among farmers as they generate knowledge for effective management of tolerant weed populations through experience (Nazarko *et al.*, 2005; Dauer *et al.*, 2007). Additionally, these collaborative strategies should be extended to university and government weed researchers, who would then be able to partner with farmers to make more effective use of shared resources and knowledge in addressing realistic concerns identified in the field (Firbank, 1993; Nazarko *et al.*, 2005). Indeed, in the case of new weeds developing as a result of GM crop cultivation, it will likely be producers who are the first to identify these problems (Hall *et al.*, 2005). Thus, just as patch-based management is ineffective for regional weed problems, isolated patch-based assessments are inappropriate for examining the ecological and agronomic risks arising from broad-scale, commercial GM crop cultivation. Rather, the identification, documentation and evaluation of potential risks associated with this new agricultural technology must be undertaken collaboratively by the farm community, public researchers, and industry, and must address concerns arising from the local to the landscape scales.

To date, proprietary seed companies have been responsible for managing the risk and assuming the liabilities associated with the spread of GM varieties from field trials, including the escape of pharmaceutical and industrial traits (Marvier & Van Acker, 2005; Demeke *et al.*, 2006). Unfortunately, following commercial release, both the management (Colbach *et al.*, 2001b; Hall *et al.*, 2005; Yoshimura *et al.*, 2006) and legal (Koch, 2007; Mauro & McLachlan, 2008) responsibilities for GM crop escape have generally been relegated to individual farmers, whether or not they have grown GM varieties. Here again, changes in spatial scale result in changing implications for persistence and spread. While seed companies have been concerned almost exclusively with the relatively minor risk of GM trait escape from small experimental plots, farmers are required to manage seed dispersal and pollen flow from commercial fields on a landscape scale, as well as considering spread from transport and seeding losses. The potential increase in persistence and spread at larger spatial scales corresponds to larger risks assumed by all GM, non-GM, and organic farmers. Thus, consideration of multiple scales suggests that the risks of GM crop cultivation are unequally distributed with regards to the potential benefits.

Plants have been successfully escaping from human cultivation, both in space and time, since the crop domestication process began (Warwick & Stewart, 2005). With the advent of GM crop technology, it becomes increasingly apparent that it is not only seed escape, but also the spread of the novel genetic construct itself, which may pose environmental, agricultural, and health risks. Human actions, not the least of which is human error (Marvier & Van Acker, 2005), facilitate this potential gene escape from intended areas of cultivation. With this recognition, the addition of more novel genetic constructs must be carefully considered in light of their potential escape. Even those genetic modifications aimed at reducing transgene escape (genetic use restriction technologies; GURTS) cannot be expected to be foolproof, and may themselves escape from cultivation into neighbouring crop and/or non-crop populations. Indeed, the strong interest in technological developments for weed control is likely a consequence of our focus on the immediate and specific ‘tactics’ of weed control; this comes at the expense of the development of appropriate ‘strategies’ creating long term solutions in the context of ecological processes (Moody & Mack, 1988)

Although many farmers are indeed primary users of GM crops, farmers generally have been neither the developers nor the regulators of this new technology, and as such, cannot be held solely responsible for GM trait escape from varieties approved for environmental release and commercialization. Indeed, in future, the personal risks and public liabilities associated with the production and management of second and third generation GM crops will undoubtedly be too large for individual producers. Technological tactics aimed at limiting this risk must not take the place of integrated control strategies, designed to limit or contain GM seeds and pollen at a regional scale. As such, broad-scale collaborative management approaches must be developed for GM crops, integrating the responsibilities of seed developers, growers, suppliers, transporters and farmers over large spatial and temporal scales, from short-term field trials to long-term commercialization. Such partnerships will increase the effectiveness of strategies for GM trait confinement.

References

AONO M, WAKIYAMA A, NAGATSU M, NAKAJIMA N, TAMAOKI M, KUBO A & SAJI H (2006) Detection of feral transgenic oilseed rape with multiple-herbicide resistance in Japan. *Environmental Biosafety Research* **5,** 77-87.

BARROSO J, FERNÀNDEZ-QUINTANILLA C, RUIZ D, HERNAIZ P & REW LJ (2004a) Spatial stability of *Avena sterilis* ssp. *Ludoviciana* populations under annual applications of low rates of imazamethabenz. *Weed Research* **44,** 178-186.

BARROSO J, FERNÀNDEZ-QUINTANILLA C, MAXWELL BD & REW LJ (2004b) Simulating the effects of weed spatial pattern and resolution of mapping and spraying on economics of site-specific management. *Weed Research* **44,** 460-468.

BAUDRY J, BUREL F, AVIRON S, MARTIN M, OUIN A, PAIN G & THENAIL C (2003) Temporal variability of connectivity in agricultural landscapes: do farming activities help? *Landscape Ecology* **18,** 303-314.

BECKIE HJ, WARWICK SI, NAIR H & SÉGUIN-SWARTZ G (2003) Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). *Ecological Applications* **13,** 1276-1294.

BECKIE HJ, HALL LM & SCHUBA B (2005) Patch management of herbicide-resistant wild oat (*Avena fatua*). *Weed Technology* **19,** 697-705.

BECKIE HJ, HARKER KN, HALL LM, WARWICK SI, LÉGÈRE A, SIKKEMA PH, CLAYTON GW, THOMAS AG, LEESON JY, SÉGUIN-SWARTZ G & SIMARD MJ (2006) A decade of herbicide resistant crops in Canada. *Canadian Journal of Plant Science* **86,** 1243-1264.

BENVENUTI S (2007) Weed seed movement and dispersal strategies in the agricultural environment. *Weed Biology and Management* **7,** 141-157.

BISCHOFF A (2005) Analysis of weed dispersal to predict chances of re-colonisation. *Agriculture, Ecosystems and Environment* **106,** 377-387.

BLANCO-MORENO JM, CHAMORRO L & SANS FX (2006) Spatial and temporal patterns of *Lolium rigidum-Avena sterilis* mixed populations in a cereal field. *Weed Research* **46,** 207-218.

BLUMENTHAL D & JORDAN N (2001) Weeds in field margins: a spatially explicit simulation analysis of Canada thistle population dynamics. *Weed Science* **49,** 509-519.

BOUTIN C (2006) Comparison of the vegetation and seedbanks of soybean fields, adjacent boundaries, and hedgerows in Ontario. *Canadian Journal of Plant Science* **86,** 557-567.

BULLOCK JM & CLARKE RT (2000) Long distance seed dispersal by wind: measuring and modelling the tail of the curve. *Oecologia* **124,** 506-521.

BULLOCK JM, MOY IL, PYWELL RF, COULSON SJ, NOLAN AM & CASWELL H. (2002) Plant dispersal and colonization processes at local and landscape scales. In: *Dispersal Ecology* (eds JM Bullock, RE Kenward & RS Hails), 279-302. Blackwell Science Ltd, Oxford.

BURTON MG, MORTENSEN DA & MARX DB (2005) Environmental characteristics affecting *Helianthus annuus* distribution in a maize production system. *Agriculture, Ecosystems and Environment* **111,** 30-40.

CAIN ML, MILLIGAN BG & STRAND AE (2000) Long-distance seed dispersal in plant populations. *American Journal of Botany* **87,** 1217-1227.

CARDINA J, JOHNSON GA & SPARROW DH (1997) The nature and consequence of weed spatial distribution. *Weed Science* **45,** 364-373.

CLAESSEN D, GILLIGAN CA, LUTMAN PJW & VAN DEN BOSCH F (2005a) Which traits promote persistence of feral GM crops? Part 1: implications of environmental stochasticity. *Oikos* **110,** 20-29.

CLAESSEN D, GILLIGAN CA & VAN DEN BOSCH F (2005b) Which traits promote persistence of feral GM crops? Part 2: implications of metapopulation structure. *Oikos* **110,** 30-42.

CLAY SA, LEMS GJ, CLAY DE, FORCELLA F, ELLSBURY MM & CARLSON CG (1999) Sampling weed spatial variability on a fieldwide scale *Weed Science* **47,** 674-681.

COLBACH N, FORCELLA F & JOHNSON GA (2000) Spatial and temporal stability of weed populations over five years. *Weed Science* **48,** 366-377.

COLBACH N, CLERMONT-DAUPHIN C & MEYNARD JM (2001a) GENESYS: a model of the influence of cropping system on gene escape from herbicide tolerant rapeseed crops to rape volunteers I. Temporal evolution of a population of rapeseed volunteers in a field. *Agriculture, Ecosystems and Environment* **83,** 235-253.

COLBACH N, CLERMONT-DAUPHIN C & MEYNARD JM (2001b) GENESYS: a model of the influence of cropping system on gene escape from herbicide tolerant rapeseed crops to rape volunteers II. Genetic exchanges among volunteer and cropped populations in a small region. *Agriculture, Ecosystems and Environment* **83,** 255-270.

CONNER AJ, GLARE TR & NAP J-P (2003) The release of genetically modified crops into the environment Part II. Overview of ecological risk assessment. *The Plant Journal* **33,** 19-46.

COUSENS R & MORTIMER M (1995) *Dynamics of Weed Populations*. Cambridge University Press, Cambridge.

COUSENS RD, BROWN RW, MCBRATNEY AB, WHELAN B & MOERKERK M (2002) Sampling strategy is important for producing weed maps: a case study using kriging. *Weed Science* **50,** 542-546.

CRAWLEY MJ & BROWN SL (1995) Seed limitation and the dynamics of feral oilseed rape on the M25 motorway. *Proceedings of the Royal Society of London B* **259,** 49-54.

CRAWLEY MJ & BROWN SL (2004) Spatially structured population dynamics in feral oilseed rape. *Proceedings of the Royal Society of London B* **271,** 1909-1916.

CRAWLEY MJ, HAILS RS, REES M, KOHN D & BUXTON J (1993) Ecology of transgenic oilseed rape in natural habitats. *Nature* **363,** 620-623.

CRAWLEY MJ, BROWN SL, HAILS RS, KOHN DD & REES M (2001) Transgenic crops in natural habitats. *Nature* **409,** 682-683.

DAUER JT, MORTENSEN DA & VANGESSEL MJ (2007) Temporal and spatial dynamics of long-distance *Conyza canadensis* seed dispersal. *Journal of Applied Ecology* **44,** 105-114.

DECKERS B, VERHEYEN K, HERMY M & MUYS B (2005) Effects of landscape structure on the invasive spread of black cherry *Prunus serotina* in an agricultural landscape in Flanders, Belgium. *Ecography* **28,** 99-109.

DEMEKE T, PERRY DJ & SCOWCROFT WR (2006) Adventitious presence of GMOs: scientific overview for Canadian grains. *Canadian Journal of Plant Science* **86,** 1-23.

DEVAUX C, LAVIGNE C, FALENTIN-GUYOMARC'H H, VAUTRIN S, LECOMTE J & KLEIN EK (2005) High diversity of oilseed rape pollen clouds over an agro-ecosystem indicated long-distance dispersal. *Molecular Ecology* **14,** 2269-2280.

DEVLAEMINCK R, BOSSUYT B & HERMY M (2005) Seed dispersal from a forest into adjacent cropland. *Agriculture, Ecosystems and Environment* **107,** 57-64.

DIELEMAN JA & MORTENSEN DA (1999) Characterizing the spatial pattern of *Abutilon theophrasti* seedling patches. *Weed Research* **39,** 455-467.

EASTICK RJ & HEARNDEN MN (2006) Potential for weediness of Bt cotton in northern Australia. *Weed Science* **54,** 1142-1151.

EBER S & BRANDL R (2003) Regional patch dynamics of *Cirsium arvense* and possible implications for plant-animal interactions. *Journal of Vegetation Science* **14,** 259-266.

EHRLÉN J & ERIKSSON O (2003) Large-scale spatial dynamics of plants: a response to Freckleton & Watkinson. *Journal of Ecology* **91,** 316-320.

ELLSTRAND NC (2001) When transgenes wander, should we worry? *Plant Physiology* **125,** 1543-1545.

FÉNART S, AUSTERLITZ F, CUGUEN J & ARNAUD, J-F (2007) Long distance pollen-mediated gene flow at a landscape level: weed beet as a case study. *Molecular Ecology* **16,** 3801-3813.

FIRBANK LG (1993) The implications of scale on the ecology and management of weeds. In: *Landscape ecology and agroecosystems* (eds RGH Bunce, L Ryszkowski & MG Paoletti), 91-104. Lewis Publishers, Boca Raton, Florida.

FRECKLETON RP & WATKINSON AR (2002) Large-scale spatial dynamics of plants: metapopulations, regional ensembles and patchy populations. *Journal of Ecology* **90,** 419-434.

FRECKLETON RP & WATKINSON AR (2003) Are all plant populations metapopulations? . *Journal of Ecology* **91,** 321-324.

FRIESEN LF, NELSON AG & VAN ACKER RC (2003) Evidence of contamination of pedigreed canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. *Agronomy Journal* **95,** 1342-1347.

FROUD-WILLIAMS RJ & CHANCELLOR RJ (1982) A survey of grass weeds in cereals in central southern England. *Weed Research* **22,** 163-171.

GARNIER A & LECOMTE J (2006) Using a spatial and stage-structured invasion model to assess the spread of feral populations of transgenic oilseed rape. *Ecological Modelling* **194,** 141-149.

GARNIER A, DEVILLE A & LECOMTE J (2006) Stochastic modelling of feral plant populations with seed immigration and road verge management. *Ecological Modelling* **197,** 373-382.

GEERTSEMA W, OPDAM P & KROPFF MJ (2002) Plant strategies and agricultural landscapes: survival in spatially and temporally fragmented habitat. *Landscape Ecology* **17,** 263-279.

GEERTSEMA W (2005) Spatial dynamics of plant species in an agricultural landscape in the Netherlands. *Plant Ecology* **178,** 237-247.

GELBARD JL & BELNAP J (2003) Roads as conduits for exotic plant invasions in a semiarid landscape. *Conservation Biology* **17,** 420-432.

GERHARDS R, WYSE-PESTER DY, MORTENSEN D & JOHNSON GA (1997) Characterizing spatial stability of weed populations using interpolated maps. *Weed Science* **45,** 108-119.

GHERSA CM & ROUSH ML (1993) Searching for solutions to weed problems: Do we study competition or dispersion? *BioScience* **43,** 104-109.

GODFREE RC, YOUNG AG, LONSDALE WM, WOODS MJ & BURDON JJ (2004) Ecological risk assessment of transgenic pasture plants: a community gradient modelling approach. *Ecology Letters* **7,** 1077-1089.

GONZALEZ-ANDUJAR JL & SAAVEDRA M (2003) Spatial distribution of annual grass weed populations in winter cereals. *Crop Protection* **22,** 629-633.

GOUDY HJ, BENNETT KA, BROWN RB & TARDIF F (2001) Evaluation of site-specific weed management using a direct-injection sprayer. *Weed Science* **49,** 359-366.

GREENE DF & CALOGEROPOULOS C (2002) Measuring and modelling seed dispersal of terrestrial plants. In: *Dispersal Ecology* (eds JM Bullock, RE Kenward & RS Hails), 3-23. Blackwell Science Ltd, Oxford.

GRESSEL J (2005) Introduction – the challenges of ferality. In: *Crop Ferality and Volunteerism* (ed J Gressel), 1-7. Taylor & Francis Group, Boca Raton, Florida.

HALL L, TOPINKA K, HUFFMAN J, DAVIS L & GOOD A (2000) Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Science* **48,** 688-694.

HALL LM, RAHMAN MH, GULDEN RH & THOMAS AG (2005) Volunteer oilseed rape – will herbicide-resistance traits assist ferality? In: *Crop Ferality and Volunteerism* (ed J Gressel), 59-79. Taylor & Francis Group, Boca Raton, Florida.

HANSKI IA & SIMBERLOFF D (1997) The metapopulation approach, its history, conceptual domain, and application to conservation. In: *Metapopulation Biology: Ecology, Genetics, and Evolution* (eds IA Hanski & ME Gilpin), 5-26. Academic Press, San Diego, California.

HANSKI IA (1997) Metapopulation dynamics: from concepts and observations to predictive models. In: *Metapopulation Biology: Ecology, Genetics, and Evolution* (eds IA Hanski & ME Gilpin), 69-91. Academic Press, San Diego, California.

HANSKI IA (1999) *Metapopulation Ecology.* Oxford University Press, Oxford.

HARKER KN, CLAYTON GW, BLACKSHAW RE, O'DONOVAN JT, JOHNSON EN, GAN Y, HOLM FA, SAPSFORD KL, IRVINE RB & VAN ACKER RC (2006) Persistence of glyphosate-resistant canola in western Canadian cropping systems. *Agronomy Journal* **98,** 107-119.

HARRISON S & TAYLOR AD (1997) Empirical evidence for metapopulation dynamics. In: *Metapopulation Biology: Ecology, Genetics, and Evolution* (eds IA Hanski & ME Gilpin), 27-42. Academic Press, San Diego, California.

HEIJTING S, VAN DER WERF W, STEIN A & KROPFF MJ (2007) Are weed patches stable in location? Application of an explicitly two-dimensional methodology. *Weed Research* **47,** 381-395.

HIGGINS SI & RICHARDSON DM (1999) Predicting plant migration rates in a changing world: the role of long-distance dispersal. *The American Naturalist* **153,** 464-475.

HIGGINS SI, NATHAN R & CAIN ML (2003) Are long-distance dispersal events in plant usually caused by nonstandard means of dispersal? *Ecology* **84,** 1945-1956.

HODKINSON DJ & THOMPSON K (1997) Plant dispersal: the role of man. *Journal of Applied Ecology* **34,** 1484-1496.

HOOFTMAN DAP, OOSTERMEIJER JGB & DEN NIJS JCM (2006) Invasive behaviour of *Lactuca serriola* (Asteraceae) in the Netherlands: Spatial distribution and ecological amplitude. *Basic and Applied Ecology* **7,** 507-519.

HUME L & ARCHIBOLD OW (1986) The influence of a weedy habitat on the seed bank of an adjacent cultivated field. *Canadian Journal of Botany* **64,** 1879-1883.

HUMSTON R, MORTENSEN DA & BJORNSTAD ON (2005) Anthropogenic forcing on the spatial dynamics of an agricultural weed: the case of the common sunflower. *Journal of Applied Ecology* **42,** 863-872.

HUSBAND BC & BARRETT SCH (1998) Spatial and temporal variation in population size of *Eichhornia paniculata* in ephemeral habitats: implication for metapopulation dynamics. *Journal of Ecology* **86,** 1021-1031.

KLEIN EK, LAVIGNE C, PICAULT H, RENARD M & GOUYON P-H (2006) Pollen dispersal of oilseed rape: estimation of the dispersal function and effects of field dimensions. *Journal of Applied Ecology* **43,** 141-151.

KLEYER M, BIEDERMANN R, HENLE K, OBERMAIER E, POETHKE H-J, POSCHLOD P, SCHRÖDER B, SETTELE J & VETTERLEIN D (2007) Mosaic cycles in agricultural landscapes of northwest Europe. *Basic and Applied Ecology* **8,** 295-309.

KNISPEL AL, MCLACHLAN SM, VAN ACKER RC & FRIESEN LF (2008) Gene flow and multiple herbicide resistance in escaped canola populations. *Weed Science* **56,** 72-80.

KOCH BA (2007) Liability and compensation schemes for economic losses caused by the adventitious presence of GM crops in non-GM crops – A European overview. In: *Proceedings of the 3rd International Conference on Coexistence between Genetically Modified (GM) and non-GM based Agricultural Supply Chains* (eds AJ Stein & E Rodríguez-Cerezo), 16-19, Seville, Spain.

LAFFAN SW (2006) Assessing regional scale weed distributions, with an Australian example using *Nassella trichotoma*. *Weed Research* **46,** 194-206.

LAVOIE C, JODOIN Y & GOURSAUD DE MERLIS A (2007) How did common ragweed (*Ambrosia artemisiifolia*) spread in Quebec? A historical analysis using herbarium records. *Journal of Biogeography* **34,** 1751-1761.

LAWES RA & GRICE AC (2007) Controlling infestation of *Parkinsonia aculeate* in a riparian zone at the landscape scale. *Austral Ecology* **32,** 287-293.

LEESON JY, THOMAS AG & SHEARD JW (2005) Weed distribution across field boundaries adjacent to roadsides. In: *Field Boundary Habitats: Implications for Weed Insect and Disease Management* (ed AG Thomas), Topics in Canadian Weed Science, Vol.1, 185-199. Canadian Weed Science Society, Sainte-Anne-de-Bellevue, Quebec.

LELONG B, LAVOIE C, JODOIN Y & BELZILE F (2007) Expansion pathways of the exotic common reed (*Phragmites australis*): a historical and genetic analysis. *Diversity and Distributions* **13,** 430-437.

LEVIN SA (1992) The problem of pattern and scale in ecology. *Ecology* **73,** 1943-1967.

LEVINS R (1969) Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin of the Entomological Society of America* **15,** 237-240.

LEVINS R (1970) Extinction. In: *Some Mathematical Problems in Biology* (ed M Gerstenhaber), 75-107. American Mathemetical Society, Providence, Rhode Island.

LINDQUIST JL, DIELEMAN A, MORTENSEN DA, JOHNSON GA & WYSE-PESTER DY (1998) Economic importance of managing spatially heterogeneous weed populations. *Weed Technology* **12,** 7-13.

LOEHLE C (2007) Effect of ephemeral stepping stones on metapopulations on fragmented landscapes. *Ecological Complexity* **4,** 42-47.

LU Z & MA K (2006) Spread of the exotic croftonweed (*Eupatorium adenophorum*) across southwest China along roads and streams. *Weed Science* **54,** 1068-1072.

MAHEU-GIROUX M & DE BLOIS S (2007) Landscape ecology of *Phragmites australis* invasion in networks of linear wetlands. *Landscape Ecology* **22,** 285-301.

MARSHALL EJP (1985) Weed distributions associated with cereal field edges – some preliminary observations. *Aspects of Applied Biology* **9,** 49-58.

MARSHALL EJP (1989) Distribution patterns of plants associated with arable field edges. *Journal of Applied Ecology* **26,** 247-257.

MARVIER M & VAN ACKER RC (2005) Can crop transgenes be kept on a leash? *Frontiers in Ecology and the Environment* **3,** 99-106.

MATLACK GR & MONDE J (2004) Consequences of low mobility in spatially and temporally heterogeneous ecosystems. *Journal of Ecology* **92,** 1025-1035.

MAURO IJ & MCLACHLAN SM (2008) Farmer knowledge and risk analysis: Postrelease evaluation of herbicide-tolerant canola in western Canada. *Risk Analysis* **28,** 463-476.

MENGES ES (1990) Population viability analysis for an endangered plant. *Conservation Biology* **4,** 52-62.

MESSÉAN A, SAUSSE C, GASQUEZ J & DARMENCY H (2007) Occurrence of genetically modified oilseed rape seeds in the harvest of conventional oilseed rape over time. *European Journal of Agronomy* **27,** 115-122.

MOODY ME & MACK RN (1988) Controlling the spread of plant invasions: the importance of nascent foci. *Journal of Applied Ecology* **25,** 1009-1021.

MÜLLEROVÁ J, PYSEK P, JAROSÍK V & PERGL J (2005) Aerial photographs as a tool for assessing the regional dynamics of invasive plant species *Heracleum mantegazzianum*. *Journal of Applied Ecology* **42,** 1042-1053.

MURPHY H & LOVETT-DOUST J (2004) Context and connectivity in plant metapopulations and landscape mosaics: does the matrix matter? *Oikos* **105,** 3-14.

NAZARKO OM, VAN ACKER RC & ENTZ MH (2005) Strategies and tactics for herbicide use reduction in field crops in Canada: a review. *Canadian Journal of Plant Science* **85,** 457-479.

NEHRBASS N, WINKLER E, MÜLLEROVÁ J, PERGL J, PYSEK P & PERGLOVÁ I (2007) A simulation model of plant invasion: long-distance dispersal determines the pattern of spread. *Biological Invasions* **9,** 383-395.

NORDMEYER H (2006) Patchy weed distribution and site-specific weed control in winter cereals. *Precision Agriculture* **7,** 219-231.

ORSON JH (1993) The penalties of volunteer crops as weeds. *Aspects of Applied Biology* **35,** 1-8.

PANNELL JR & OBBARD DJ (2003) Probing the primacy of the patch: what makes a metapopulation? *Journal of Ecology* **91,** 485-488.

PARENDES LA & JONES JA (2000) Role of light availability and dispersal in exotic plant invasion along roads and streams in the H.J. Andrews Experimental Forest, Oregon. *Conservation Biology* **14,** 64-75.

PESSEL FD, LECOMTE J, EMERIAU V, KROUTI M, MESSEAN A & GOUYON PH (2001) Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. *Theoretical and Applied Genetics* **102,** 841-846.

RADOSEVICH SR, STUBBS MM & GHERSA CM (2003) Plant invasions – process and patterns. *Weed Science* **51,** 254-259.

RAMSAY G, THOMPSON C & SQUIRE G (2003) *Quantifying landscape-scale gene flow in oilseed rape.* Report for the Department for Environment, Food and Rural Affairs, London.

REW LJ & COUSENS RD (2001) Spatial distribution of weeds in arable crops: are current sampling and analytical methods appropriate? *Weed Research* **41,** 1-18.

REW LJ, CUSSANS GW, MUGGLESTONE MA & MILLER PCH (1996) A technique for mapping the spatial distribution of *Elymus repens*, with estimates of the potential reduction in herbicide usage from patch spraying. *Weed Research* **36,** 283-292.

RIEGER MA, LAMOND M, PRESTON C, POWLES SB & ROUSH RT (2002) Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* **296,** 2386-2388.

RUIZ D, ESCRIBANO C & FERNÀNDEZ-QUINTANILLA C (2006) Assessing the opportunity for site-specific management of *Avena sterilis* in winter barley fields in Spain. *Weed Research* **46,** 379-387.

SHIMONO Y & KONUMA A (2008) Effects of human-mediated processes on weed species composition in internationally traded grain commodities. *Weed Research* **48,** 10-18.

SHIRTLIFFE SJ & ENTZ MH (2005) Chaff collection reduces seed dispersal of wild oat (*Avena fatua*) by a combine harvester. *Weed Science* **53,** 465-470.

SIMARD M-J, LÉGÈRE A, PAGEAU D, LAJEUNESSE J & WARWICK S (2002) The frequency and persistence of volunteer canola (*Brassica napus*) in Quebec cropping systems. *Weed Technology* **16,** 433-439.

SKARPAAS O, STABBETORP OE, RONNING I & SVENNUNGSEN TO (2004) How far can a hawk's beard fly? Measuring and modelling the dispersal of *Crepis praemorsa*. *Journal of Ecology* **92,** 747-757.

SMITH H, FIRBANK LG & MACDONALD DW (1999) Uncropped edges of arable fields managed for biodiversity do not increase weed occurrence in adjacent crops. *Biological Conservation* **89,** 107-111.

SMYTH S, KHACHATOURIANS GG & PHILLIPS PWB (2002) Liabilities and economics of transgenic crops. *Nature Biotechnology* **20,** 537-541.

SOONS MB & OZINGA WA (2005) How important is long-distance seed dispersal for the regional survival of plant species? *Diversity and Distributions* **11,** 165-172.

SOSNOSKIE LM, LUSCHEI EC & FANNING MA (2007) Field margin weed-species diversity in relation to landscape attributes and adjacent land use. *Weed Science,* **55,** 129-136.

STANILAND BK, MCVETTY PBE, FRIESEN LF, YARROW S, FREYSSINET G & FREYSSINET M (2000) Effectiveness of border areas in confining the spread of transgenic *Brassica napus* pollen. *Canadian Journal of Plant Science* **80,** 521-526.

SWINTON SM (2005) Economics of site-specific weed management. *Weed Science* **53,** 259-263.

VON DER LIPPE M & KOWARIK I (2007a) Long-distance dispersal of plants by vehicles as a driver of plant invasions. *Conservation Biology* **21,** 986-996.

VON DER LIPPE M & KOWARIK I (2007b) Crop seed spillage along roads: a factor of uncertainty in the containment of GMO. *Ecography* **30,** 483-490.

VUILLEUMIER S & POSSINGHAM HP (2006) Does colonization asymmetry matter in metapopulations? *Proceedings of the Royal Society of London B* **273,** 1637-1642.

WALLINGA J, KROPFF MJ & REW LJ (2002) Patterns of spread of annual weeds. *Basic and Applied Ecology* **3,** 31-38.

WARWICK SI & STEWART CN (2005) Crops come from wild plants – how domestication, transgenes, and linkage together shape ferality. In: *Crop Ferality and Volunteerism* (ed J Gressel), 9-30. Taylor & Francis Group, Boca Raton, Florida.

WIENS JA (1997) Metapopulation dynamics and landscape ecology. In: *Metapopulation Biology: Ecology, Genetics, and Evolution* (eds IA Hanski & ME Gilpin), 43-62. Academic Press, San Diego, California.

WILES LJ, OLIVER GW, YORK AC, GOLD HJ & WILKERSON GG (1992) Spatial distribution of broadleaf weeds in North Carolina soybean (*Glycine max*) fields. *Weed Science* **40,** 554-557.

WILLENBORG CJ & VAN ACKER RC (2006) Comments on "An empirical model for pollen-mediated gene flow in wheat" (Crop Sci. 45:1286-1295). *Crop Science* **46,** 1018-1019.

WILSON PJ & AEBISCHER NJ (1995) The distribution of dicotyledonous arable weeds in relation to distance from the field edge. *Journal of Applied Ecology* **32,** 295-310.

WITH K (2004) Assessing the risk of invasive spread in fragmented landscapes. *Risk Analysis* **24,** 803-815.

YOSHIMURA Y, BECKIE HJ & MATSUO K (2006) Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. *Environmental Biosafety Research* **5,** 67-75.

ZWAENEPOEL A, ROOVERS P & HERMY M (2006) Motor vehicles as vectors of plant species from road verges in a suburban environment. *Basic and Applied Ecology* **7,** 83-93.

CHAPTER 6

LANDSCAPE-SCALE DISTRIBUTION AND PERSISTENCE OF GENETICALLY MODIFIED CANOLA (*BRASSICA NAPUS*) IN MANITOBA, CANADA

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Chapter Summary

Genetically modified herbicide-tolerant (GMHT) canola (*Brassica napus* L.) was approved for commercial cultivation in Canada in 1995, and currently represents over 95% of the canola grown in western Canada. After a decade of widespread cultivation, GMHT volunteers represent an increasing management problem in cultivated fields and are ubiquitous in adjacent ruderal habitats, where they contribute to the spread of transgenes. However, few studies have considered escaped GMHT canola populations in North America, and even fewer have been conducted at large spatial scales (i.e. landscape scales). In particular, the contribution of landscape structure and large-scale anthropogenic dispersal processes to the persistence and spread of escaped GMHT canola remains poorly understood. We conducted a multi-year survey of the landscape-scale distribution of escaped canola plants adjacent to roads and cultivated fields. Our objective was to examine the long-term dynamics of escaped canola at large spatial scales, and to assess the relative importance of landscape and localized factors to the persistence and spread of these plants outside of cultivation. From 2005 to 2007, we surveyed escaped canola plants along roadsides and field edges at 12 locations in three agricultural landscapes in southern Manitoba where GMHT canola is widely grown. Data were analysed to examine temporal changes at large spatial scales, and to determine factors affecting the distribution of escaped canola plants in roadside and field-edge habitats within agricultural landscapes. Additionally, we assessed the potential for seed dispersal between escaped populations by comparing the relative spatial distribution of roadside and field-edge canola. Densities of escaped canola fluctuated over space and time in both roadside and field-edge habitats, though the proportion of GMHT plants was high (93–

100%). Escaped canola was positively affected by agricultural landscape (indicative of cropping intensity) and by the presence of an adjacent field planted to canola. Within roadside habitats, escaped canola was also strongly associated with large-scale variables, including road surface (indicative of traffic intensity) and distance to the nearest grain elevator. Conversely, within field edges, canola density was affected by localized crop management practices such as mowing, soil disturbance, and herbicide application. Despite the proximity of roadsides and field edges, there was little evidence of spatial aggregation among escaped canola populations in these two habitats, especially at very fine spatial scales (i.e. < 100 m), suggesting that natural propagule exchange is infrequent. Escaped canola populations were persistent at large spatial and temporal scales, and low density in a given landscape or year was not indicative of overall extinction. As a result of ongoing cultivation and transport of canola crops, escaped GMHT traits will likely remain predominant in agricultural landscapes. While escaped canola in field-edge habitats generally results from local seeding and management activities occurring at the field-scale, distribution patterns within roadside habitats are determined in large part by seed transport occurring at the landscape-scale, and at even larger regional scales. Our findings suggest that these large-scale anthropogenic dispersal processes are sufficient to enable persistence despite limited natural seed dispersal. This widespread dispersal is likely to undermine field-scale management practices aimed at eliminating escaped and in-field GMHT canola populations. Agricultural transport and landscape-scale cropping patterns are important determinants of the distribution of escaped GM crops. At the regional level, these factors ensure ongoing establishment and spread of escaped GMHT canola despite limited local seed dispersal. Escaped populations thus play an important role in the spread of transgenes, and have substantial implications for the coexistence of GM and non-GM production systems. Given the large-scale factors driving the spread of escaped transgenes, localized co-existence measures may be impracticable where they are not commensurate with regional dispersal mechanisms. To be effective, strategies aimed at reducing contamination from GM crops should be multi-scale in approach and be developed and implemented at both farm and landscape levels of organization. Multiple stakeholders should thus be consulted, including both GM and non-GM farmers, as well as seed developers, processors, transporters, and suppliers. Decisions to adopt GM crops require thoughtful and inclusive consideration of the risks and responsibilities inherent in this new technology.

Introduction

Genetically modified herbicide-tolerant (GMHT) canola (*Brassica napus* L.) was approved for commercial cultivation in Canada in 1995. Varieties tolerant to glyphosate, glufosinate, and imidazolinone herbicides now represent over 95% of the canola grown in western Canada (Beckie et al. 2006). Farmers have rapidly adopted these new varieties in part due to their operational benefits (Mauro & McLachlan 2008). However, canola volunteers are increasingly prevalent in cultivated fields (Leeson et al. 2002), where the presence of multiple GMHT traits can lead to transgene spread (Hall et al. 2000; Beckie et al. 2003). Management challenges associated with GMHT volunteers, as well as widespread GM crop cultivation in western Canada, have generated much doubt among farmers regarding GMHT trait containment (Mauro and McLachlan 2008; Mauro et al. 2009). Indeed, the presence of unwanted GMHT traits is inevitable in Canadian production systems and agricultural supply chains (Demeke et al. 2006). Consequently, there is increasing interest among both canola importers and GM regulators in ensuring proper prevention and management of adventitious GMHT traits (Levidow and Boschert 2008; Devos et al. 2009).

In light of these concerns, there is a need for greater understanding of the processes by which canola plants, and consequently GMHT transgenes, escape from cultivation and spread in adjacent non-field habitats. Early studies of escaped canola focused on small experimental populations; though easily established in disturbed habitats, these populations were not competitive with encroaching perennial vegetation and quickly became extinct (Crawley et al. 1993). Despite this putative transience, escaped GMHT canola volunteers are ubiquitous in roadsides and other ruderal habitats, both in areas where these crops are grown (Yoshimura et al. 2006; Knispel et al. 2008) and in canola-importing countries (Aono et al. 2006; Kawata et al. 2009). Theoretical studies suggest that these escaped canola populations could play a considerable role in the spread of unwanted GMHT traits (Claessen et al. 2005; Colbach 2009). Indeed, gene flow occurs frequently among escaped canola plants, which are often located in close proximity to cultivated canola fields (Knispel et al. 2008). Additionally, hybridization between escaped canola and sympatric wild relatives, particularly field mustard (*Brassica rapa* L.), may also contribute to the spread of unwanted GM traits (Warwick et al. 2003).

It is increasingly recognized that landscape-scale assessments are required to accurately characterize the extent of transgene escape and spread in areas where GM crops are cultivated. Local processes that underlie the dynamics of canola populations have been widely studied, and include population demographics (Crawley et al. 1993;

Pessel et al. 2001; Pivard et al. 2008) as well as localized dispersal by seeding and harvest machinery (Pivard et al. 2008) and other vehicular traffic (Garnier et al. 2008). In contrast, mechanisms contributing to the apparent permanence of escaped canola populations at the landscape-scale are poorly understood. Notably, the importance of human-mediated dispersal of canola seed over long distances, associated with agricultural transport networks, is still contested (Crawley and Brown 2004; von der Lippe and Kowarik 2007; Pivard et al. 2008). Consequently, the effectiveness of proposed management and mitigation strategies, and the spatial scales at which these should be implemented, remain unclear.

The overall aim of this study was to examine the long-term dynamics of escaped canola at large spatial scales. In particular, our objectives were to: (1) characterize landscape-scale spatial and temporal variations of canola populations in roadside and field-edge habitats in southern Manitoba; (2) contrast the relative importance of landscape and local-scale factors in determining the distribution of escaped canola; and (3) assess the potential for natural seed dispersal between escaped populations of canola in roadside and field-edge habitats.

Methods

STUDY AREA, SITE SELECTION AND FIELD SAMPLING

The large-scale distribution of escaped canola was examined from 2005 to 2007 in three agricultural landscapes in southern Manitoba (central Canada): the rural municipality (RM) of Rhineland, the RM of MacDonald, and the Carman landscape, consisting of the RMs of Dufferin and Roland (Table 6.1, Figure 6.1). All three landscapes are located in the Lake Manitoba Plain Ecoregion, characterized by a climate suitable to dryland agriculture and some of the highest quality arable land in Manitoba (Smith et al. 1998). Additionally, all three landscapes contain grain distribution and/or processing facilities associated with road and rail transport of canola seed and other commodities (CGC 2007; Table 6.1, Figure 6.1). The MacDonald and Carman landscapes are important canola-growing areas in Manitoba, where canola is generally grown once every four years in rotation. Conversely, canola is grown less frequently in Rhineland, where soil conditions and climate are better suited to long-season crops such as corn, soy and potatoes (Smith et al. 1998). Transgenic GMHT traits were widely detected in escaped canola plants in all three agricultural landscapes, through both protein analysis of maternal plant tissue and herbicide screening of progeny from escaped plants (Table 6.1; see Knispel et al. 2008 for full description of methodology).

We surveyed the distribution of escaped canola plants occurring along rural grid roads and highways at 12 locations in the three landscapes (see Figure 6.1), originating from the sites of high-density escaped canola populations (see Knispel et al. 2008). Four locations were sampled in both Rhineland and MacDonald in 2005, 2006 and 2007. An additional four locations were sampled in Carman in 2006 and 2007. At each sampling location, eight one-mile road segments surrounding two contiguous sections of land (1 mile x 1 mile) were surveyed for escaped canola. Plants were counted separately along both the roadside and the adjacent field edge by two researchers travelling parallel to each other at a constant walking speed. Preliminary sampling was undertaken to compare and calibrate canola counts between researchers. Additionally, to prevent observer bias in the counts, researchers alternated between roadside and field edge for each one-mile segment. Number of flowering canola plants was recorded every 1.5 minutes, equivalent to a distance along the road of approximately 110 m. Canola plants were counted up to 1 m into the ditch vegetation bordering both the road and the field. Escaped canola plants occurred most frequently in the sparse vegetation immediately bordering roads, and rarely occurred in denser ditch vegetation where they were less competitive (A. Knispel, unpublished data). For each ~110 m^2 sampling unit, we also recorded environmental and anthropogenic features including adjacent crop type, road surface, presence of intersecting roads, and evidence of common management practices.

DATA ANALYSIS

For Rhineland and MacDonald from 2005 to 2007, differences in escaped canola count within 110 m^2 sampling units were assessed using factorial analysis of variance (ANOVA) with one repeated measures factor (year) and one between-group factor (landscape) (SAS 9.1, SAS Institute Inc., USA). Separate analyses were performed for the roadside (n = 893) and field-edge (n = 762) data. When interactions were significant, the simple effects of landscape and year were also tested at $\alpha = 0.025$ to account for multiple tests (Hatcher and Stepanski 1994). Significant effects of year were assessed by contrasting mean canola counts in a given year with those from the previous year. For Carman, mean canola counts for 2006 and 2007 were compared using paired samples t-tests for both roadside (n = 447) and field-edge (n = 401) data. All data were log-transformed to meet assumptions of normality (Sokal and Rohlf 1981). Original untransformed data are presented.

We selected 11 independent variables expected to affect dispersal and persistence of escaped canola. These included agricultural landscape (Rhineland, MacDonald, Carman); road surface (dirt, gravel, paved); adjacent canola crop in 2007 or 2006 (yes, no); presence of a field access point (yes, no) or a cross-road (yes, no); herbicide damage to ditch vegetation (yes, no); mowing (yes, no); and soil disturbance (yes, no). Sampling locations were mapped in a geographical information system (GIS) and distance to the nearest grain elevator or processing facility was measured (ArcGIS 9.1, ESRI Inc., USA). Finally, canola counts from the 2006 survey were also used to model counts of escaped canola plants in the 2007 survey. Independent variables were assessed for multicollinearity using Spearman's rank-order correlation coefficient. No highly correlated variables (i.e. $r \geq 0.4$) were identified and all independent variables were included in subsequent analyses. For both the roadside (n = 1337) and field-edge (n = 1172) data sets, the distribution of escaped canola in 2007 was modelled using negative binomial regression (SAS 9.1, SAS Institute Inc., USA), which is suitable for overdispersed count data in which the variance exceeds the mean (Allison 1999; Magas et al. 2007; Ver Hoef and Boveng 2007). Negative binomial regression models include an additional variable, the dispersion parameter, to account for overdispersion within the data (Allison 1999). Preliminary residual analysis was used to remove any outlying observations from subsequent regression models.

In spatially-structured data sets, spatial dependence between adjacent observations is common and often violates assumptions of independence, leading to inflated parameter estimates and p-values in regression analyses (Klute et al. 2002). As such, alternative regression models that account for spatial structure are increasingly used to predict species distribution (Dormann et al. 2007). However, these spatial models should be carefully interpreted, with reference to corresponding non-spatial models, to ensure that correction for spatial dependence does not remove biologically important variation from the data set. Examination of both roadside and field-edge data provided no evidence of spatial structure at the landscape scale, suggesting that the basic negative binomial regression model was appropriate for these data. Similarly, spatial autocorrelation was not detected at localized spatial scales along segments where canola density was sparse (i.e. along unpaved roads and adjacent to fields planted to crops other than canola). However, along 50% or more of paved roads and segments adjacent to canola fields, we detected evidence of spatial autocorrelation using semivariograms, which describe the variance between pairs of data points as a function of distance (Dormann et al. 2007). At short distances, low variance indicates spatial autocorrelation between points. Generally, variance increases with distance, as data points become less similar. The range, or zone of influence, of a semivariogram describes the distance at which the influence of a point on neighbouring points becomes negligible (Haining 2003). For semivariograms of data points along paved road segments, the range varied between 400 and 1,000 m. Similarly, for semivariograms of data points adjacent to canola fields, the range varied between 200 and 800 m. To account for this spatial dependence, an additional explanatory variable, the autocovariate, was included in subsequent regression models. The autocovariate describes the effect on a given response (i.e. canola count) of the response at spatially adjacent sample locations (Maheu-Giroux and de Blois 2007) and is generally calculated at relatively small spatial scales (Dormann et al. 2007). Thus, for samples along paved roads (n = 260) and for samples adjacent to canola fields (*n* = 276), we performed additional negative binomial regressions, considering the ten remaining independent variables and the autocovariate. We calculated the autocovariate using neighbouring observations within 300 m of the given sample point, weighted by inverse distance (R 2.8.1, R Foundation for Statistical Computing, Austria). Regression models with and without the autocovariate were compared to assess the effect of the autocovariate, which is generally expected to reduce the significance of other predictive variables in the model (Dormann et al. 2007).

To examine the potential for localized canola seed dispersal between roadsides and field edges, we compared the distribution of canola populations in these two habitats using the network cross-K function available through ESRI ArcGIS extension SANET (Okabe et al. 2008). For two unique sets of points, the cross-K function detects aggregated, random, or regular patterns, based on the number of points of one set located within a certain distance of points of the other set (Spooner et al. 2004; Maheu-Giroux and de Blois 2007). Spatial aggregation of escaped populations, particularly at very fine spatial scales (i.e. ≤100 m), would suggest that natural propagule exchange occurs between these two habitats. To examine the role of roadside canola populations as seed sources for new field-edge populations, we compared the spatial distributions of roadside populations in 2005 and 2006 with the distributions of field-edge populations in 2006 and 2007, respectively. Likewise, to detect potential seed dispersal from field-edge canola populations into roadside habitats, we compared the spatial distributions of field-edge populations in 2005 and 2006 with the distributions of roadside populations in 2006 and 2007, respectively. We focused our analysis at spatial scales up to 500 m. Observed network cross-K functions were compared to 90% confidence envelopes for random and independent spatial distribution of points (Okabe et al. 2008).

Results

SPATIAL AND TEMPORAL VARIATIONS

Average canola counts within 110 m^2 sampling units varied greatly over the course of this study, ranging from 0.7 to 60.6 plants in roadside habitats (Table 6.2), and from 1.0 to 49.5 plants in field-edge habitats (Table 6.3). Generally, roadside canola counts were highest in Carman and lowest in Rhineland (see Table 6.2). Conversely, field-edge canola counts showed less consistent trends, and were highest in Rhineland in 2005, highest in Carman in 2006, and highest in MacDonald in 2007 (see Table 6.3).

Significant differences in canola count were detected between landscapes and years using factorial ANOVA. Within roadside habitats, mean canola counts displayed a significant landscape x year interaction ($p < 0.0001$), as well as significant simple effects for both factors. Within Rhineland ($n = 447$), canola counts decreased significantly from 2005 to 2006 ($p < 0.0001$), and remained largely unchanged between 2006 and 2007. Within MacDonald ($n = 446$), roadside canola counts also decreased significantly from 2005 to 2006 ($p < 0.0001$), but then increased significantly from 2006 to 2007 ($p < 0.0001$) (see Table 6.2). There was a significant effect of landscape on roadside canola populations in 2005 ($n = 895$) and 2007 ($n = 896$); in both years, canola counts were significantly higher in MacDonald than in Rhineland ($p < 0.0001$ and $p < 0.0001$, respectively) (see Table 6.2).

Similar trends were observed in the field-edge data from Rhineland and MacDonald, where a significant landscape x year interaction ($p < 0.0001$) was also observed. In Rhineland ($n = 401$), field-edge canola counts showed no significant effect of year at $\alpha = 0.025$ ($p = 0.0364$). However, in MacDonald ($n = 361$), field-edge canola counts fluctuated, decreasing significantly from 2005 to 2006 ($p < 0.0001$), and again increasing significantly from 2006 to 2007 ($p < 0.0001$) (see Table 6.3). There was also a significant effect of landscape on field-edge canola counts; in 2005 ($n = 809$), counts were significantly higher ($p = 0.0025$) in Rhineland, while in 2007 ($n = 788$) counts were significantly higher ($p < 0.0001$) in MacDonald (see Table 6.3).

In Carman, no significant differences were observed between roadside ($p = 0.1171$) and field-edge ($p = 0.1787$) canola counts in 2006 and 2007, despite considerable changes in canola densities in both habitats (see Tables 6.2 and 6.3). This may be the result of the high variability of escaped canola, which is also reflected in the maximum counts within 110 m^2 sampling units observed for each landscape and year. These ranged from 35 to 1220 plants in roadsides (see Table 6.2) and from 35 to 3050 plants in field-edge habitats (see Table 6.3). Yet, despite extreme spatial and temporal fluctuations, escaped canola plants commonly occurred at very high densities outside of planted canola fields in all three agricultural landscapes. Additionally, given the widespread cultivation of GMHT varieties in these areas, the great majority (93–100%) of escaped canola plants either exhibited GMHT traits or produced GMHT progeny as a result of pollen flow from neighbouring plants (see Table 6.1).

FACTORS AFFECTING THE DISTRIBUTION OF ESCAPED CANOLA

Factors associated with the presence of escaped canola differed considerably between roadsides and field edges, suggesting that canola distribution is likely to vary independently within these two habitats. Negative binomial regression analysis of roadside data identified five significant predictive variables affecting canola plants in this habitat, many of these operating at landscape scales (Table 6.4, left side). Most importantly, the number of escaped plants increased with changes in the surface of the adjacent road (RoadSurf) that correspond with increasing traffic intensity. Agricultural landscape (Landscape) also affected escaped canola in roadsides. Presence of an adjacent canola field in both the current (AdjCan07) and previous year (AdjCan06) also had a positive impact on roadside canola density. Conversely, distance to the nearest elevator (DistElev) had a negative effect on canola in roadsides, indicating that canola density increases in proximity to grain storage and processing facilities (see Table 6.4, left side).

Compared to roadsides, escaped canola plants in field-edge habitats were significantly associated with much more localized features and disturbances (see Table 6.4, right side). Most importantly, field-edge canola density increased with the presence of an adjacent canola field in the current year. Management variables were also important, and canola density in field edges increased with mowing (Mow) and herbicide application (HerbApp) and decreased with soil disturbance (SoilDist). As with roadside habitats, agricultural landscape and the presence of an adjacent canola field in the previous year had small positive effects on the presence of escaped canola plants in field edges (see Table 6.4, right side).

Though neither roadside nor field-edge samples of escaped canola exhibited spatial autocorrelation when the data were considered as a whole, canola counts were often spatially-structured along individual road segments (see *Data Analysis*, above). This was particularly evident for roadside samples adjacent to paved roads and for field-edge samples adjacent to canola fields, likely reflecting the higher densities of canola in these locations. Additional negative binomial and auto-negative binomial regressions were compared for both paved road and canola field samples, to consider the effects on parameter estimates of correcting for spatial dependence among the samples.

Within roadside habitats adjacent to paved roads, basic negative binomial regression identified four significant predictive variables affecting canola distribution (Table 6.5, left side). The density of escaped canola plants increased significantly with the presence of an adjacent canola crop in the previous year and, to a lesser extent, with the presence of an adjacent canola crop in the current year. Escaped canola density increased only slightly with the presence of escaped plants at the sample location in the previous year (Count06). Along paved roads, escaped canola density decreased with increasing distance from an elevator (see Table 6.5, left side). In the auto-negative binomial regression model, inclusion of the autocovariate reduced the significance of other parameter estimates (see Table 6.5, right side). Only the negative effect of distance to elevator and the small positive impact of the presence of escaped plants in 2006 were significant, as was the autocovariate itself (AutoC300; see Table 6.5, right side).

Along field edges adjacent to planted canola fields, basic negative binomial regression identified five significant predictive variables (Table 6.6, left side). Local management variables again had important impacts on escaped canola, which increased significantly in mowed sites and decreased significantly with soil disturbance and herbicide application. To a lesser extent, escaped canola was also positively affected by landscape and negatively affected by distance to elevator (see Table 6.6, left side). In the auto-negative bionomial regression, inclusion of the autocovariate again reduced the significance of other explanatory variables, and the auto-regressive model retained only the negative effect of herbicide application and a relatively small positive effect of the autocovariate itself (see Table 6.6, right side).

INTERACTIONS BETWEEN ESCAPED CANOLA PLANTS IN ROADSIDES AND FIELD EDGES

Despite the proximity of roadsides and field edges, network cross-K function analysis revealed little evidence of aggregation between escaped canola populations within these two habitats, suggesting that natural seed dispersal is infrequent. In many sites, the spatial distribution of roadside and field-edge populations was random, and the observed network cross-K function fell within the 90% confidence envelope (data not shown). Observed and expected cross-K function results are presented for three sites for which spatial aggregation was most apparent (Figure 6.2 and Figure 6.3). At distances of 100 m, there was no evidence that escaped canola populations in field edges were spatially dependent on proximal roadside populations from the previous year (see Figure 6.2). However, at sites Rhine 4 (2005–2006) and MacD 4 (2006–2007) clustering was apparent at spatial scales greater than 200 m.

Escaped canola populations in roadside habitats also showed little evidence of clustering around the locations of field-edge populations from the previous year (see Figure 6.3). In only one site, Rhine 4 (2005–2006), were roadside populations clustered around field-edge populations at all spatial scales, though aggregation was still not pronounced at the 100 m distance (see Figure 6.3). Sites MacD 4 (2005–2006) and Car 1 (2006–2007) also showed evidence of spatial aggregation, though only at spatial scales greater than 200 m. At these distances, which exceed natural seed dispersal distances, aggregated spatial patterns are likely not the result of direct propagule exchange between escaped canola plants growing in roadside and field-edge habitats.

Discussion

To date, science-based risk assessment of novel GM crops has been largely reliant on findings from localized trials occurring at the field-scale or smaller spatial scales. However, full understanding of the potential for transgene escape requires landscape-scale assessments that consider the environmental and agronomic implications of GM crops at multiple spatial scales. Our research examines the distribution and persistence of escaped canola at large spatial scales, in landscapes where GMHT varieties are widely cultivated and gene flow is frequent within escaped populations (Knispel et al. 2008).

The escaped GMHT canola populations examined in our study were highly dynamic, and densities fluctuated widely in both space and time. Spatial variation in escaped canola in part reflected landscape-level differences in the presence of canola in cultivated fields, as both crop and volunteer plants. Generally, escaped population densities were higher in MacDonald and Carman than in Rhineland, likely reflecting the high frequency of canola cultivation in MacDonald as well as the high abundance of in-field canola volunteers in Carman (see Table 6.1; Manitoba Agricultural Services Corporation (MASC) 2009; Leeson et al. 2002). These spatial differences are also temporally dynamic: in 2005, relatively high levels of escaped canola in field-edge habitats in Rhineland likely reflected wet spring conditions that delayed seeding and resulted in the replacement of long season crops such as corn and soy with early-maturing canola varieties (Canola Council of Canada (CCC) 2005). In turn, lower overall escaped canola densities in all landscapes in 2006 are likely associated with hot and dry summer weather conditions, which resulted in considerable heat and moisture stress (CCC 2006), especially for plants growing in dry roadside habitats. In contrast, favourable weather conditions in 2007 resulted in population increases in MacDonald and Carman. Similar spatial and temporal variation in escaped canola populations have been observed in the United Kingdom and Australia (Crawley and Brown 2004; Peltzer et al. 2008), and this stochasticity is often interpreted as evidence of the transience, and thus relative unimportance, of escaped canola populations. However, our results suggest that declines in density are likely to be temporary, and are asynchronous at large spatial scales. Ultimately, populations of escaped GMHT plants will remain a persistent feature in agricultural landscapes, especially if cultivation is widespread.

Landscape-scale factors that underlie the distribution of escaped canola populations were particularly important when considering dispersal. Escaped canola was more prevalent along high-traffic roads and in proximity to grain elevators and processing plants. Even when spatial dependence among adjacent roadside populations was considered, the impacts of these landscape-scale factors remained significant. These findings point to the importance of agricultural transportation networks in facilitating the spread of escaped crop plants in western Canada. Agricultural transport has also been implicated in canola seed dispersal in Europe (Crawley and Brown 2004; Von der Lippe and Kowarik 2007) and Japan (Aono et al. 2006; Kawata et al. 2009), though studies have often focused on (sub)urban areas where agronomic impacts are less important. In rural Canada, agricultural transportation networks link large cultivation areas in the central prairies, where over 5.8 million hectares of canola were harvested in 2007 (Statistics Canada 2007), with points of export thousands of kilometres away (Yoshimura et al. 2006). As such, the dispersal of GMHT canola seed may be expected to occur at spatial scales much larger than the landscapes where canola is intentionally cultivated, and ongoing cultivation and transport will help establish and maintain GMHT populations outside of cultivated fields. Once dispersed at regional scales, these GMHT traits are even more unlikely to be retracted.

The regional spread of GMHT traits poses challenges for farmers wishing to maintain non-GM production systems, and may compromise the ability of individual farmers to control volunteers within or outside of fields. Escaped populations along roadsides are not actively controlled by municipal or highway authorities (Yoshimura et al. 2006), and management efforts undertaken by farmers are generally limited to fields and immediately adjacent habitats. However, our results indicate that common management practices, including mowing and herbicide application, were actually associated with higher densities of escaped canola plants. Regular mowing of weedy perennial vegetation likely enables the establishment of annual weed species including escaped canola (Crawley et al. 1993). Given the predominance of glyphosate-tolerant varieties in cultivation in western Canada, application of common broad spectrum herbicides such as glyphosate is often ineffective in controlling the majority of GMHT canola volunteers, both in the field-edge habitats examined here, and within cultivated fields (Hall et al. 2000; Friesen et al. 2003). Alternative herbicides such as 2,4-

dichlorophenoxy acetic acid (2,4-D) would normally be required to control GMHT canola volunteers, which may explain the apparent effectiveness of herbicide application observed adjacent to a small number of canola fields in this study. Generally, however, the negative effect of soil disturbance on the distribution of escaped canola in field edges suggests that tillage is likely to serve as the only reliable means to control escaped canola plants. Unfortunately, the use of tillage is problematic for increasing numbers of farmers in western Canada who practice zero- or conservation-tillage in order to reduce erosion, conserve soil moisture, and reduce costs (Friesen et al. 2003). These farmers rely on glyphosate for weed control, and the spread of glyphosate-tolerant canola volunteers thus creates additional management challenges. In some cases, producers are reverting to tillage in order to manage problematic GMHT canola volunteers (Mauro and McLachlan 2008).

The lack of aggregation observed between roadside and field-edge escaped canola at very fine spatial scales indicates that natural seed dispersal from escaped populations was negligible relative to canola seed inputs from landscape-scale anthropogenic vectors. As such, control efforts directed at individual escaped canola populations are likely to be counteracted by the volume and spatial extent of canola seed dispersal along agricultural distribution networks. Farmers will thus be unable to reduce or eliminate the long-distance dispersal of canola seed into cultivated fields or adjacent ruderal habitats, and remain at risk of contamination by adventitious GMHT traits. In western Canada, the long-distance dispersal of canola seed, compounded by the ubiquitous presence of escaped GMHT canola and the high proportion of GMHT varieties in cultivation, all compromise the isolation of non-GM varieties (Beckie et al. 2006). This has resulted in the contamination of certified seed (Friesen et al. 2003), has created challenges for non-GM mustard production (Demeke et al. 2006), and has resulted in the loss of a promising organic canola industry in western Canada (Smyth et al. 2002). The regional spread of GMHT traits and attendant impacts on crop and production system choices thus raise questions regarding the feasibility of co-existence strategies that ignore large-scale anthropogenic dispersal vectors.

CONCLUSIONS

As a result of widespread cultivation and large-scale dispersal, escaped GMHT canola plants have become a permanent feature of agricultural landscapes in western Canada. Though small local populations may be prone to extinction, dispersal through agricultural transport enables the ongoing establishment of new populations. This balance of local extinction and landscape-scale dispersal suggests that escaped canola populations may be operating as a metapopulation. Metapopulation ecology stresses the importance of dispersal in sustaining the landscape-scale distribution of small isolated populations (Levins 1970; Hanski 1999) and may be useful in understanding the risks associated with the spread of GM crop varieties (Claessen et al. 2005). As large-scale dynamics cannot be understood simply by ‘scaling up’ local population processes (Freckleton and Watkinson 2002), assessment of new GM technologies are best undertaken at multiple spatial and temporal scales.

While populations dependent on seed immigration are often distinguished from those that are self-replacing (Crawley and Brown 2004; Pivard et al. 2008), the impacts of ongoing landscape-scale dispersal are no less pervasive than those of local selfrecruitment. That these escaped canola populations are relatively persistent at larger scales of organization suggests that they can contribute considerably to gene flow. Regionally, both intraspecific and interspecific gene flow have important agronomic consequences; the spread of transgenes from escaped canola into cultivated fields will result in the potential contamination of canola crops, while hybridization and stable incorporation of GMHT traits in weed (meta)populations is likely to exacerbate weed problems and further contribute to transgene spread (Warwick et al. 2008). Given the high proportion of GMHT traits in escaped populations and the high frequency of outcrossing events (Knispel et al. 2008), our results support concerns (e.g. Marvier and Van Acker 2005) that escaped transgenes cannot be retracted once released and may persist even if GM crop cultivation ceases.

RECOMMENDATIONS AND PERSPECTIVES

Importantly, our findings underscore the regional scope of GMHT transgene escape, with implications for the regulation and management of current and future GM crops. Currently, '*ex ante*' management strategies for the cultivation of GM crops are being developed and adopted with the goal of reducing the unwanted presence of GM traits in agricultural production systems. To date, stewardship plans in North America (Beckie et al. 2006) and coexistence measures in Europe (Devos et al. 2009) have focused on farm-scale management practices. Though coexistence strategies emphasise geographic isolation of GM crops as an effective means of containing GM traits, these are likely to be inadequate where canola volunteers and escaped plants are not effectively controlled (Colbach 2009). Our results indicate that landscape-scale factors contribute substantially to the spread of escaped GMHT traits, which will further confound local management efforts and the reliance of coexistence strategies on localized approaches.

The importance of escaped GM plants, and the role of agricultural transport in facilitating their dispersal, suggests that a broader view of stakeholder responsibility is important in the management of GM crops. New coexistence measures should extend to regional scales, thereby identifying the central role that seed developers, growers, suppliers, transporters, as well as farmers can play in managing GM trait escape. Management practices should also be commensurate with large-scale dispersal vectors. Though still controversial, interest in regional isolation of non-GM and organic production from GM crop cultivation has been growing (Levidow and Boschert 2008). Consideration of the North American experience reveals that a decade of GM crop cultivation has resulted in the ubiquitous presence, long-term landscape-scale persistence, and long-distance dispersal of escaped canola volunteers. Distinct regional production areas for GM and non-GM crops may thus be necessary to ensure that cropping system choice is maintained in accordance with the objectives of coexistence.

References

Allison PD (1999) Logistic Regression Using the SAS System: Theory and Application. SAS Institute Inc., Cary, NC.

Aono M, Wakiyama A, Nagatsu M, Nakajima N, Tamaoki M, Kubo A, Saji H (2006) Detection of feral transgenic oilseed rape with multiple-herbicide resistance in Japan. Environ Biosafety Res 5:77–87

Beckie HJ, Harker KN, Hall LM, Warwick SI, Légère A, Sikkema PH, Clayton GW, Thomas AG, Leeson JY, Séguin-Swartz G, Simard MJ (2006) A decade of herbicide resistant crops in Canada. Can J Plant Sci 86:1243–1264

Beckie HJ, Warwick SI, Nair H, Séguin-Swartz G (2003) Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). Ecol Appl 13:1276–1294

Canadian Grain Commission (CGC) (2007) Grain elevators in Canada, Crop year 2007–2008. Canadian Grain Commission, Winnipeg, Manitoba

Canola Council of Canada (CCC) (2005) Canola Watch Reports, 2005. Canola Council of Canada, Winnipeg, Manitoba

Canola Council of Canada (CCC) (2006) Canola Watch Reports, 2006. Canola Council of Canada, Winnipeg, Manitoba

Claessen D, Gilligan CA, van den Bosch F (2005) Which traits promote persistence of feral GM crops? Part 2: implications of metapopulation structure. Oikos 110:30–42

Colbach N (2009) How to model and simulate the effects of cropping systems on population dynamics and gene flow at the landscape level: example of oilseed rape volunteers and their role for co-existence of GM and non-GM crops. Env Sci Pollut Res 16:348-360

Crawley MJ, Hails RS, Rees M, Kohn D, Buxton J (1993) Ecology of transgenic oilseed rape in natural habitats. Nature 363:620–623

Crawley MJ, Brown SL (2004) Spatially structured population dynamics in feral oilseed rape. Proc R Soc Lond B 271:1909–1916

Demeke T, Perry DJ, Scowcroft WR (2006) Adventitious presence of GMOs: scientific overview for Canadian grains. Can J Plant Sci 86:1–23

Devos Y, Demont M, Dillen K, Reheul D, Kaiser M, Sanvido O (2009) Coexistence of genetically modified (GM) and non-GM crops in the European Union. A review. Agron Sustain Dev 29:11–30

Dormann CF, McPherson JM, Araújo MB, Bivand R, Bolliger J, Carl G, Davies RG, Hirzel A, Jetz W, Kissling D, Kühn I, Ohlemüller R, Peres-Neto PR, Reineking B, Schröder B, Schurr FM, Wilson R (2007) Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. Ecography 30:609–628

Freckleton RP, Watkinson AR (2002) Large-scale spatial dynamics of plants: metapopulations, regional ensembles and patchy populations. J Ecol 90:419–434

Friesen LF, Nelson AG, Van Acker RC (2003) Evidence of contamination of pedigreed canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. Agron J 95:1342–1347

Garnier A, Pivard S, Lecomte J (2008) Measuring and modelling anthropogenic secondary seed dispersal along roadverges for feral oilseed rape. Basic Appl Ecol 9:533–541

Haining R (2003) Spatial Data Analysis: Theory and Practice. Cambridge University Press, New York.

Hall L, Topinka K, Huffman J, Davis L, Good A (2000) Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. Weed Sci 48: 688–694

Hanski IA (1999) Metapopulation Ecology. Oxford University Press, Oxford.

Hatcher L, Stepanski EJ (1994) A step-by-step approach to using the SAS system for univariate and multivariate statistics. SAS Institute Inc., Cary, NC

Kawata M, Murakami K, Ishikawa T (2009) Dispersal and persistence of genetically modified oilseed rape around Japanese harbors. Env Sci Pollut Res 16:120–126

Knispel AL, McLachlan SM, Van Acker RC, Friesen LF (2008) Gene flow and multiple herbicide resistance in escaped canola populations. Weed Sci 56:72–80

Klute DS, Lovallo MJ, Tzilkowski WM (2002) Autologistic regression modeling of American woodcock habitat use with spatially dependent data. In: Scott JM, Heglund PJ, Morrison ML et al. (eds) Predicting species occurrences: Issues of accuracy and scale. Island Press, Washington, DC, pp 335–343

Leeson JY, Thomas AG, Andrews T, Brown KR, Van Acker RC (2002) Manitoba weed survey of cereal and oilseed crops in 2002. Weed Survey Series Publication 02-2. Agriculture and Agri-food Canada, Saskatoon, Saskatchewan.

Levidow L, Boschert K (2008) Coexistence or contradiction? GM crops versus alternative agricultures in Europe. Geoforum 39:174–190

Levins R (1970) Extinction. In: Gerstenhaber M (ed) Some Mathematical Problems in Biology. American Mathemetical Society, Providence, Rhode Island, pp 75–107

Magas OK, Gunter JT, Regens JL (2007) Ambient air pollution and daily pediatric hospitalizations for asthma. Env Sci Pollut Res 14:19–23

Maheu-Giroux M, de Blois S (2007) Landscape ecology of *Phragmites australis* invasion in networks of linear wetlands. Landscape Ecol 22:285–301

Manitoba Agricultural Services Corporation (MASC) (2009) Manitoba Management Plus Program. http://www.mmpp.com, accessed: January 20, 2009

Marvier M, Van Acker RC (2005) Can crop transgenes be kept on a leash? Front Ecol Environ 3:99–106

Mauro IJ, McLachlan SM (2008) Farmer knowledge and risk analysis: Postrelease evaluation of herbicide-tolerant canola in western Canada. Risk Anal 28:463–476

Mauro IJ, McLachlan SM, Van Acker, RC (2009) Farmer knowledge and *a priori* risk analysis: pre-release evaluation of genetically modified Roundup Ready wheat across the Canadian prairies. Env Sci Pollut Res 16:689-701

Okabe A, Okunuki K, Shiode S (2008) SANET: A toolbox for spatial analysis on a network – Version 3.4. Centre for Spatial Information Science, University of Tokyo, Tokyo, Japan

Peltzer DA, Ferriss S, FitzJohn RG (2008) Predicting weed distribution at the landscape scale: using naturalized *Brassica* as a model system. J Appl Ecol 45:467–475

Pessel FD, Lecomte J, Emeriau V, Krouti M, Messean A, Gouyon PH (2001) Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. Theor Appl Genet 102:841–846

Pivard S, Adamczyk K, Lecomte J, Lavigne C, Bouvier A, Deville A, Gouyon PH, Huet S (2008) Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. J Appl Ecol 45:476–485

Smith RE, Veldhuis H, Mills GF, Eilers RG, Fraser WR, Lelyk GW (1998) Terrestrial ecozones, ecoregions, and ecodistricts of Manitoba: An ecological stratification of Manitoba's natural landscapes. Technical Bulletin 98-9E. Agriculture and Agri-Food Canada, Winnipeg, Manitoba

Smyth S, Khachatourians GG, Phillips PWB (2002) Liabilities and economics of transgenic crops. Nat Biotechnol 20:537–541

Sokal RR, Rohlf FJ (1981) Biometry: the principles and practice of statistics in biological research. WH Freeman, NY

Spooner PG, Lunt ID, Okabe A, Shiode S (2004) Spatial analysis of roadside *Acacia* populations on a road network using the network K-function. Landscape Ecol 19:491–499

Statistics Canada (2007) November estimate of production of principal field crops, Canada, 2007. Field Crop Reporting Series 86:8, Catalogue no 22-002-XIE. Statistics Canada, Ottawa, Ontario

Ver Hoef JM, Boveng PL (2007) Quasi-Poisson and negative binomial regression: How should we model overdispersed count data? Ecology 88:2766–2772

von der Lippe M, Kowarik I (2007) Crop seed spillage along roads: a factor of uncertainty in the containment of GMO. Ecography 30:483–490

Warwick SI, Simard M-J, Légère A, Beckie HJ, Braun L, Zhu B, Mason P, Séguin-Swartz G, Stewart CN (2003) Hybridization between transgenic *Brassica napus* L. and its wild relatives: *Brassica rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O.E. Schulz. Theor Appl Genet 107:528–539

Warwick SI, Légère A, Simard M-J, James T (2008) Do escaped transgenes persist in nature? The case of an herbicide resistance transgene in a weedy *Brassica rapa* population. Mol Ecol 17:1387–1395

Yoshimura Y, Beckie HJ, Matsuo K (2006) Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. Environ. Biosafety Res. 5:67–75

Table 6.1. Characteristics of the Rhineland, MacDonald and Carman agricultural landscapes in southern Manitoba, Canada.

Landscape	**Area (km^2)**	**Average canola crop (ha)[a] and proportion of area, 2005–2007**	**Number of grain storage facilities and capacity (tonnes)[b]**	**Relative abundance of in-field canola volunteers[c]**	**Proportion of escaped canola plants with GMHT traits(%)[d]**
Rhineland	923	12,385 (13%)	2 (42,570)	9.2	100
MacDonald	1,106	25,546 (23%)	4 (39,970)	1.1	93
Carman	1,348	17,513 (13%)	1 (4,170)	15.8	100

[a] MASC 2009

[b] Indicates the number of grain elevators and/or grain processing facilities, and their combined total capacity (Canadian Grain Commission (CGC) 2007).

[c] Relative abundance is a composite index of frequency, uniformity within fields, and density (Leeson et al. 2002).

[d] Includes both maternal plants exhibiting GMHT traits and plants producing GMHT progeny, from among 28 plants (Rhineland), 58 plants (MacDonald), and 43 plants (Carman) tested by Knispel et al. (2008).

Table 6.2. Average roadside canola counts for ~110m^2 sampling units in Rhineland, MacDonald and Carman landscapes in 2005, 2006, 2007.

Landscape	2005	2006	2007
Rhineland	1.6^{a*} (100)	0.8^{b} (80)	0.9^{b*} (55)
MacDonald	13.8^{a*} (260)	0.7^{b} (35)	45.8^{c*} (1220)
Carman	–	48.8^{a} (850)	60.6^{a} (1169)

Maximum counts for each landscape by year are given in brackets. Within each landscape, means followed by different letters are significantly different from the previous year at $p < 0.025$.

* Within each year, denotes significant differences between Rhineland and MacDonald landscapes at $p < 0.025$. Differences between the Carman landscape and other landscapes were not assessed.

Table 6.3. Average field-edge canola counts for ~110m^2 sampling units in Rhineland, MacDonald and Carman landscapes in 2005, 2006, 2007.

Landscape	**2005**	**2006**	**2007**
Rhineland	8.7^{a*} (600)	4.4^{a} (220)	1.3^{a*} (70)
MacDonald	4.4^{a*} (160)	1.0^{b} (35)	11.5^{c*} (530)
Carman	–	49.5^{a} (3050)	6.5^{a} (300)

Maximum counts for each landscape by year are given in brackets. Within each landscape, means followed by different letters are significantly different from the previous year at $p < 0.025$.
* Within each year, denotes significant differences between Rhineland and MacDonald landscapes at $p < 0.025$. Differences between the Carman landscape and other landscapes were not assessed.

Table 6.4. Coefficients and *p*-values for negative binomial regression models of all roadside (left) and all field-edge (right) observations in 2007.

	All Roadside Samples			**All Field-Edge Samples**		
Parameter[a]	**Estimate**	**Chi-Square**	***p***	**Estimate**	**Chi-Square**	***p***
Landscape	1.0953	63.06	< 0.0001	0.966	43.39	< 0.0001
RoadSurf	1.9017	307.47	< 0.0001	0.0811	0.4	0.5276
DistElev	–0.158	50.42	< 0.0001	0.0378	1.73	0.1883
AdjCan07	1.5689	72.43	< 0.0001	3.5688	212.1	< 0.0001
AdjCan06	1.4625	29.71	< 0.0001	0.6979	4.11	0.0426
Count06	0.0016	1.34	0.2472	0.0008	1.13	0.287
FieldAcc	0.15	0.33	0.5636	–0.4073	1.79	0.1809
Xroad	0.2436	1.39	0.239	0.1844	0.48	0.488
HerbApp	0.5091	2.29	0.1303	0.6104	5.38	0.0204
Mow	0.3444	3.67	0.0555	2.0849	51.13	< 0.0001
SoilDist	0.3805	0.98	0.3219	–1.9894	9.51	0.002
Dispersion	6.4699			7.5612		

[a] Parameter abbreviations: *Landscape*, agricultural landscape; *RoadSurf*, road surface; *DistElev*, distance to nearest grain elevator; *AdjCan07*, adjacent canola crop in 2007; *AdjCan06*, adjacent canola crop in 2006; *Count06*, canola count from 2006 survey; *FieldAcc*, presence of a field access point; *Xroad*, presence of a cross road; *HerbApp*, herbicide damage to ditch vegetation; *Mow*, mowing of ditch vegetation; *SoilDist*, soil disturbance; *Dispersion*, additional regression parameter accounting for overdispersion of the data.

Table 6.5. Coefficients and *p*-values for negative binomial (left) and auto-negative binomial (right) regression models of observations adjacent to paved roads.

	Paved Roads			Auto-regression		
Parameter[a]	**Estimate**	**Chi-Square**	***p***	**Estimate**	**Chi-Square**	***p***
Landscape	0.3218	3.12	0.0773	–0.0417	0.1	0.7484
DistElev	–0.5308	162.01	< 0.0001	–0.326	95.17	< 0.0001
AdjCan07	0.8546	11.07	0.0009	0.223	1.31	0.2524
AdjCan06	1.7476	18.87	< 0.0001	0.3989	1.52	0.217
Count06	0.0036	5.09	0.0241	0.003	7.81	0.0052
FieldAcc	0.3479	1.08	0.2984	0.0897	0.11	0.7401
Xroad	0.0955	0.14	0.7042	0.1567	0.63	0.4291
HerbApp	0.2905	0.36	0.5493	0.2434	0.38	0.5358
Mow	–0.0114	0	0.9674	–0.0905	0.21	0.6488
SoilDist	–0.9299	2.92	0.0873	–0.5961	2.15	0.1426
AutoC300	–	–	–	0.0067	127.3	< 0.0001
Dispersion	2.2037			1.4099		

[a] Parameter abbreviations: *Landscape*, agricultural landscape; *DistElev*, distance to nearest grain elevator*; AdjCan07*, adjacent canola crop in 2007; *AdjCan06*, adjacent canola crop in 2006; *Count06*, canola count from 2006 survey; *FieldAcc*, presence of a field access point; *Xroad*, presence of a cross road; *HerbApp*, herbicide damage to ditch vegetation; *Mow*, mowing of ditch vegetation; *SoilDist*, soil disturbance; *AutoC300*, autocovariate calculated over a neighbourhood distance of 300 m; *Dispersion*, additional regression parameter accounting for overdispersion of the data.

Table 6.6. Coefficients and *p*-values for negative binomial (left) and auto-negative binomial (right) regression models of observations adjacent to canola fields.

	Canola fields			**Auto-regression**		
Parameter[a]	**Estimate**	**Chi-Square**	***p***	**Estimate**	**Chi-Square**	***p***
Landscape	0.7121	12.74	0.0004	0.2262	1.69	0.1937
RoadSurf	–0.1443	0.86	0.3534	–0.2043	2.17	0.1409
DistElev	–0.0721	4.72	0.0297	–0.0228	0.59	0.4435
AdjCan06	–2.26	1.49	0.222	–1.6717	0.99	0.3187
Count06	0.0067	0.01	0.9106	0.0272	0.29	0.5908
FieldAcc	0.1811	0.24	0.623	0.4777	2.1	0.1476
Xroad	–0.3796	1.31	0.2529	–0.1423	0.24	0.6276
HerbApp	–3.5629	22.71	< 0.0001	–3.346	34.36	< 0.0001
Mow	1.3806	14.49	0.0001	–0.5984	2.79	0.0947
SoilDist	–4.0508	11.67	0.0006	–2.0206	3.47	0.0624
AutoC300	–	–	–	0.0239	38.56	< 0.0001
Dispersion	2.8873			2.2689		

[a] Parameter abbreviations: *Landscape*, agricultural landscape; *RoadSurf*, road surface; *DistElev*, distance to nearest grain elevator; *AdjCan06*, adjacent canola crop in 2006; *Count06*, canola count from 2006 survey; *FieldAcc*, presence of a field access point; *Xroad*, presence of a cross road; *HerbApp*, herbicide damage to ditch vegetation; *Mow*, mowing of ditch vegetation; *SoilDist*, soil disturbance; *AutoC300*, autocovariate calculated over a neighbourhood distance of 300 m; *Dispersion*, additional regression parameter accounting for overdispersion of the data

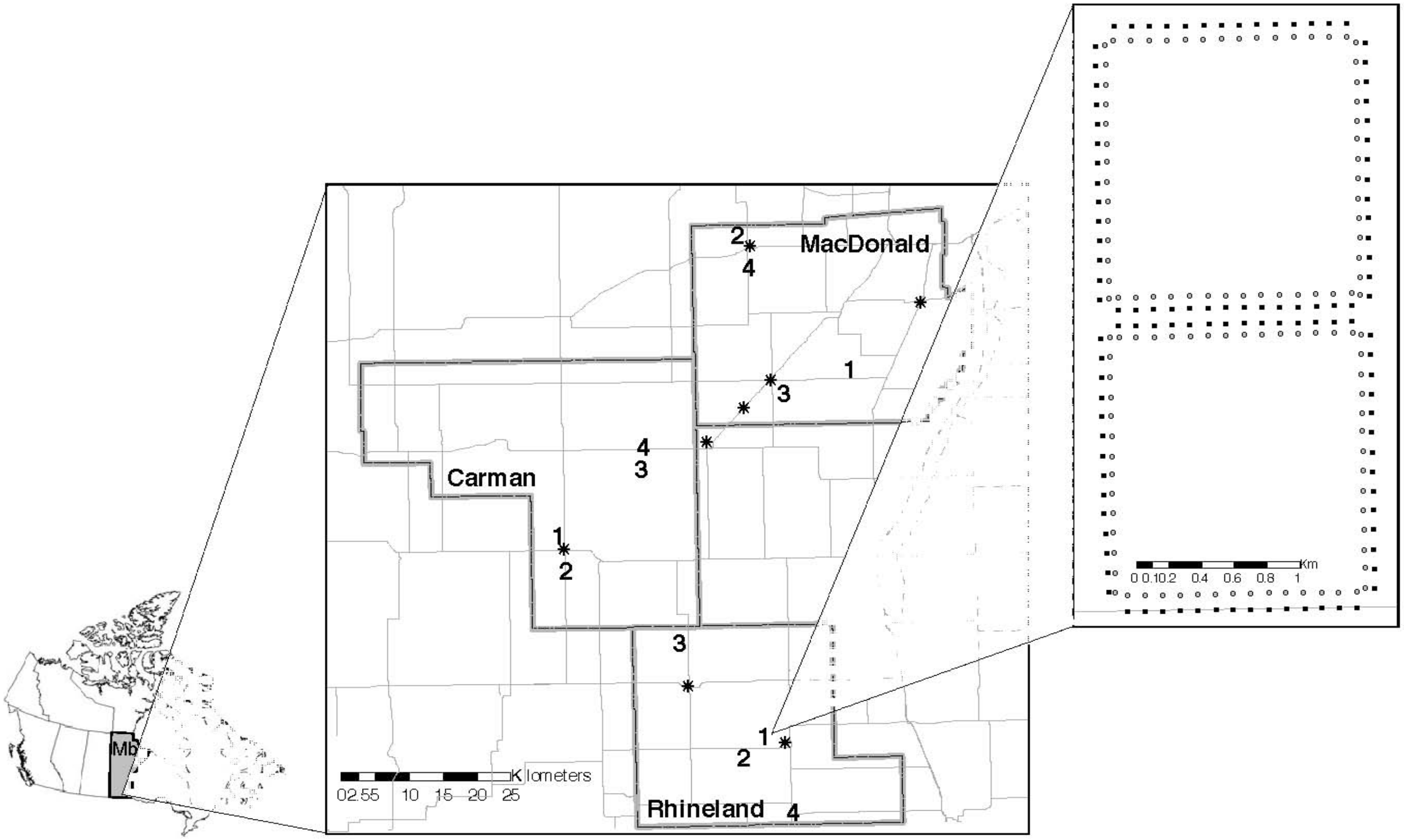

Figure 6.1. Landscape-scale distribution of escaped canola was assessed in southern Manitoba (*Mb*), Canada (*left*). Four sampling locations were surveyed in the Rhineland (*Rhine 1–4*), MacDonald (*MacD 1–4*), and Carman (*Car 1–4*) agricultural landscapes (*middle*). Locations of grain elevators (*asterisks*) and major roads (*grey lines*) are indicated. At each location, escaped canola was sampled in roadside (*black squares*) and field-edge (*grey circles*) habitats along 8 one-mile roads (*right, inset*).

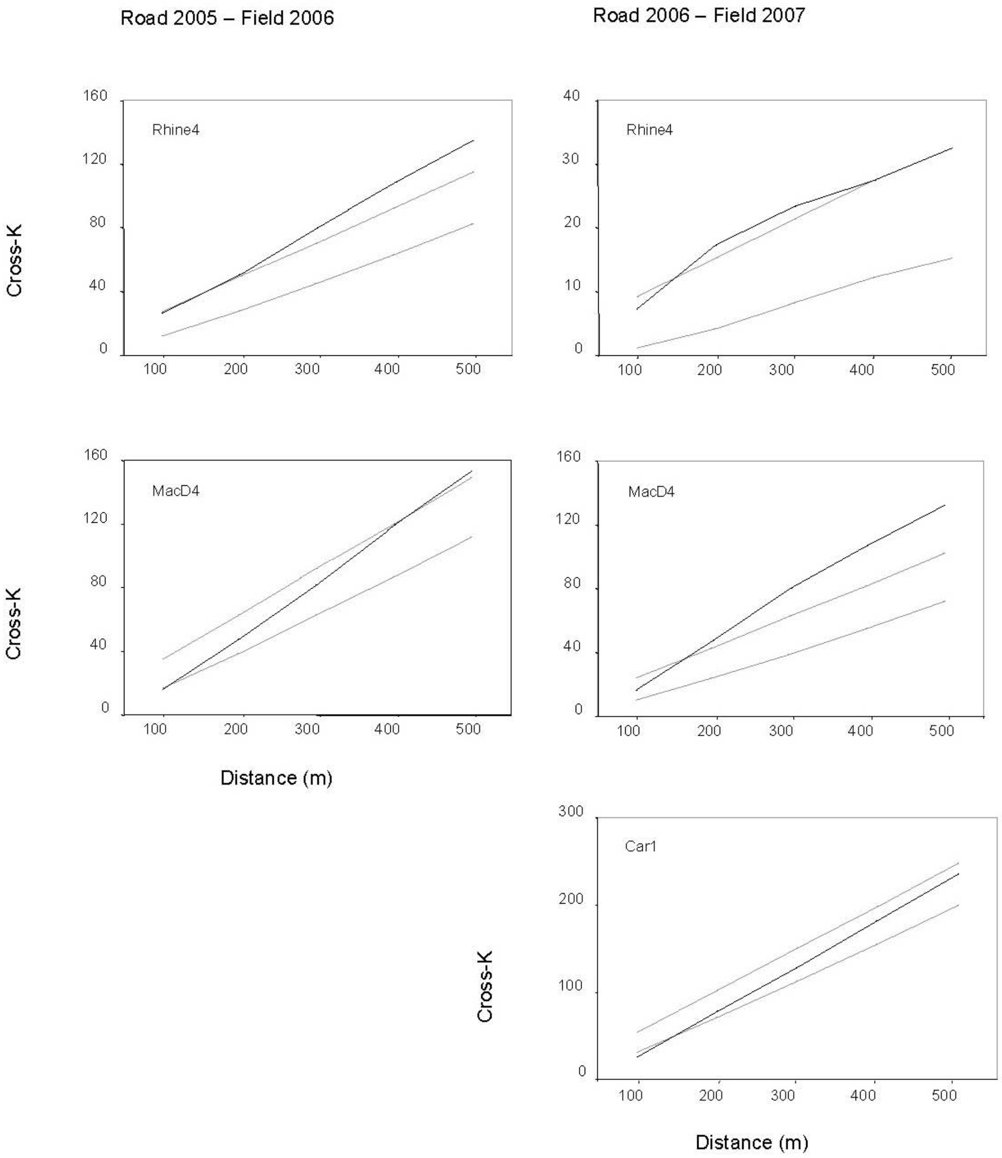

Figure 6.2. Spatial aggregation of field-edge canola populations around roadside canola populations from the previous year for sites in Rhineland (*Rhine4*), MacDonald (*MacD4*) and Carman (*Car1*) landscapes. When the observed cross-K function (*solid line*) is above the 90% confidence interval for random distribution (*dashed lines*), aggregation is observed.

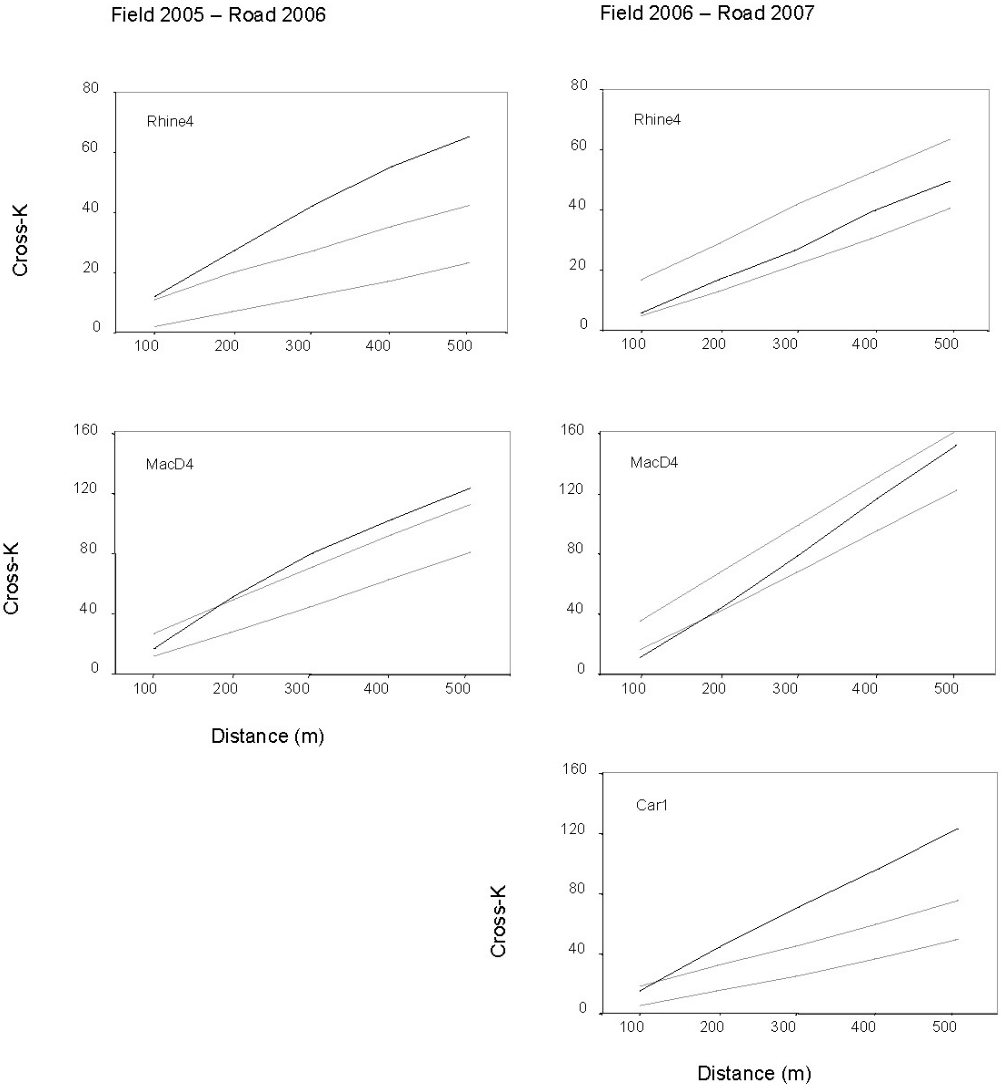

Figure 6.3. Spatial aggregation of roadside canola populations around field-edge canola populations from the previous year for sites in Rhineland (*Rhine4*), MacDonald (*MacD4*) and Carman (*Car1*) landscapes. When the observed cross-K function (*solid line*) is above the 90% confidence interval for random distribution (*dashed lines*), aggregation is observed.

CHAPTER 7

DISSERTATION SYNTHESIS

FROM PUBLIC RESEARCH TO PERSONAL LEARNING

Public Research: Primary Contribution and Recommendation

The research presented here represents the most comprehensive ecological study undertaken to date in North America examining the long-term, multi-scale impacts of escaped genetically modified (GM) canola. This research describes the unintended and inevitable escape, persistence and spread of novel herbicide tolerance traits in non-crop habitats. Within Canadian agroecosystems, GM canola populations outside of cultivated fields are not explicitly monitored or controlled, and thus serve as reservoirs for escaped transgenes at large spatial and temporal scales. As such, escaped populations contribute to GM trait-stacking and may be expected to play an important role in facilitating gene flow among agricultural fields, thereby compromising Canadian production systems, with particularly severe agronomic, economic and ecological impacts on non-GM, zero-till and organic operations.

Despite the apparent novelty of the research presented in this thesis, I am not the first to voice these findings; this study is both responsive and responsible to the concerns of farmers, who have predicted and understood the risks associated with GM crop cultivation across the Canadian Prairies. However, farmers' voices, and indeed any form of meaningful public input, is still lacking from Canadian regulation of GM crops (Mauro & McLachlan 2008; Mauro, McLachlan & Van Acker 2009). Furthermore, formal post-release monitoring of GM crops is not mandatory in Canada, where regulatory oversight is premised on voluntary input from private crop developers. Indeed, when undertaken at all, 'monitoring' generally takes the form of an investigation conducted by crop developers at the field scale (Mauro 2008), to detect escaped GM traits in non-GM crops with the sole aim of protecting intellectual property and attendant economic benefits through litigation. Such a narrow mandate does not contribute to a mechanistic understanding of GM trait escape, nor does it allow for evaluation of the impacts of GM crops at large spatial scales and over the long-term. Thus, as a result of corporate control in both the regulation and monitoring of GM crops, farmers remain at considerable legal and socioeconomic risk when GM traits escape, while causality and accountability are overlooked and understudied.

The findings of this research, and of the larger research program of which it is a part (e.g. Hwang 2008; Mauro 2008), indicate that farmers have valuable knowledge and experience to guide the risk assessment of GM crops. Indeed, the ecological research presented in this thesis confirms and quantifies the risks identified by Canadian Prairie farmers (Mauro & McLachlan 2008). These outcomes point to the shortcomings of the current Canadian regulatory framework, which systemically marginalizes the concerns of producers and other public stakeholders, and indeed, puts farmers at considerable legal and socioeconomic risk (Mauro 2008). To redress these shortcomings, I recommend the implementation of mandatory post-release monitoring for GM crops (e.g. Beckie *et al.*, 2010), conducted by public researchers and guided by public concerns and experience, with especial consideration of the experiences of farmers, including both adopters and non-adopters of new GM technologies. The application of ecological theory and methodology to characterize and confirm farmer-identified risks, as described throughout this thesis (see Chapter One), serves as a useful framework for the development of practical monitoring processes for GM crops, and indeed for any new agricultural technology. Monitoring protocols should be designed to enable the integration of new and emergent risks and benefits into iterative decision-making processes; post-release data should be used systematically to verify and validate pre-release regulatory decisions. Indeed, the implementation of mandatory monitoring protocols for current GM crops will increase our ability to make informed decisions about the release of future GM crops in the United States and Canada. The findings of this post-release study of GM canola are timely and will be useful in directing ongoing assessment and monitoring of herbicide-tolerant (HT) alfalfa, as attempts to commercialize this crop are now underway in North America (USDA 2009). In particular, the research presented here indicates that crop plants that are able to escape from cultivated fields represent high risks of gene flow; for competitive, outcrossing, perennial species such as alfalfa, the risks are likely to be even higher (Bagavathiannan & Van Acker 2009).

Additionally, these findings can provide valuable lessons to complement pre-release GM field trials undertaken in Europe and Australia. Faced with increasing pressures to introduce GM crops, these countries are looking to the North American experience with GM crop cultivation for improved understanding of the long-term effects of this new technology. Without appropriate monitoring protocols in place, however, rhetorical claims of GM crop safety and profitability in North America remain unsubstantiated.

Specific Research Outcomes: HT canola in Western Canada

Escaped populations: Contributions to gene flow

In Western Canada, escaped populations of canola are ubiquitous in non-crop habitats, reflecting the widespread and frequent cultivation of this crop in prairie agroecosystems. Additionally, given the current Canadian regulatory climate, commercial cultivation of HT canola types remains unsegregated across the Prairies. As such, novel GM traits are not effectively contained within individual agricultural production systems: the escape of these traits into natural and semi-natural elements of Canadian agroecosystems is inevitable and irretractable. This research documents and confirms the common occurrence of both transgenic and non-transgenic HT traits in escaped canola populations (Chapter Three). Indeed, in some populations, 100% of plants tested exhibited novel HT traits, in either maternal plant tissues or among progeny. Within escaped canola populations, intra-specific gene flow occurs frequently and at much higher levels (e.g. 79%) than initially anticipated in roadside habitats, resulting in escaped plants with stacked HT traits (Chapter Three). As such, plants are not easily controlled by common management practices. Even short-term persistence of escaped populations is likely to result in co-occurrence with cultivated canola, grown typically every three to four years in rotation. Additionally, in the agricultural regions studied here, escaped HT canola plants flowered in synchrony with canola plants in adjacent arable fields (Chapter Four). Given the high potential for outcrossing in canola, flowering synchrony would facilitate pollen-mediated gene flow between escaped and cultivated canola, leading to the adventitious presence of HT traits in GM, non-GM and organic production fields. Such contamination has consequences for ecological, agronomic and economic aspects of individual production systems, with demonstrated impacts on international markets; however, these consequences remain unaddressed more than a decade after GM crop release.

Patterns and processes of escape

Diverse processes, operating at multiple spatial and temporal scales, contribute to the escape of GM canola into non-crop habitats. As such, the scale at which GM risk assessment studies are undertaken will affect both observed research outcomes and associated implications and recommendations (Chapter Five). This points to the importance of continued monitoring and iterative assessment of GM crops after approval and deregulation; the findings of localized field trials are not likely to scale-up to provide an accurate understanding of the impacts of commercial cultivation, particularly with regards to gene flow and transgene escape.

Locally, farm-level management and production practices can introduce large numbers of seeds into habitats adjacent to cultivated fields; when habitat conditions are suitable, viable escaped populations develop rapidly (Chapter Four). While such populations remain vulnerable to competitive exclusion, their persistence may also increase in response to common localized management processes, including mowing and non-targeted herbicide application (Chapter Six).

At larger spatial scales, escaped populations of canola originate in roadside habitats from seeds lost from agricultural transport vehicles. Habitats along high-traffic roads and in close proximity to grain distribution centres support high numbers of escaped canola plants (Chapter Six); these plants exhibit multiple HT traits (Chapter Three), reflecting the unsegregated nature of agricultural transport in Canada and throughout North America. Continued seed inputs from these far-reaching agricultural transport networks will increase the persistence of escaped populations in roadside habitats (Chapter Four) and introduce new GM traits as these are released commercially. As such, contamination risk is high for canola production fields along highways and in the vicinity of grain elevators, where individual farmers are likely to have little ability to prevent the establishment and spread of escaped transgenes (Chapter Six).

Large spatial scales: risks from anthropogenic processes

These findings point to the important contribution of anthropogenic factors, including widespread cultivation and large-scale dispersal, to the establishment and persistence of escaped GM canola populations. Indeed, these landscape-level processes have been shown to be more important than local processes in determining the distribution of escaped canola (Chapter Six). The limitations of canola natural seed dispersal are negligible in comparison with the volume and distance of agricultural seed transport. These findings suggest that escaped populations of canola are likely to persist as a result of metapopulation dynamics, with frequent recolonization of suitable habitat compensating for local extinctions, as a result of asynchronous dynamics at the population- and landscape-scales (Chapter Five). As such, escaped canola populations remain a permanent feature of roadside habitats, and the risks associated with such populations will extend to scales commensurate with the scope of human activities in agroecosystems.

These findings further substantiate the need for large-scale assessments designed to examine the interactions between biological and anthropogenic processes (Chapters Four and Six). Furthermore, such interactions point to the fact that ecological and agronomic risks of GM crops are conflated. As farmers respond to the widespread and frequent occurrence of GM volunteers and escaped plants, they adapt and intensify management practices; in many cases farmers must revert to tillage practices or increase the toxicity and frequency of herbicide applications (Friesen *et al.* 2003; Van Acker *et al.* 2004; Mauro & McLachlan 2008). Consequently, the important ecological benefits of zero-till practices are eroded and low-input/organic canola production systems are almost completely eliminated across the Canadian Prairies.

Management and Policy Recommendations

These findings indicate that current regulations, requiring only small-scale, pre-release field trials for GM crops, do not guarantee sufficient data to characterize the long-term and large-scale behaviour of these new varieties. Indeed, a relatively small number of ecological post-release monitoring studies undertaken by public researchers in Canada (this dissertation; Yoshimura, Beckie & Matsuo 2006; Beckie *et al.* 2003; Hall *et al.* 2000) have greatly altered our understanding of the volume and distance of transgene spread from commercial agricultural production. Concurrently, innovative trans-disciplinary research approaches have revealed the complex of agronomic, economic and social impacts arising as a result of transgenic contamination (Mauro & McLachlan 2008; Mauro, McLachlan & Sanders 2005). Combined, these studies suggest that escaped transgenes are impossible to contain or retract, and that their effects are much more pervasive than anticipated by small pre-release field trials. Currently in Canada, however, responsibility for GM trait confinement at all spatial scales remains solely with individual farmers, even though local management practices are expected to be limited in their ability to mitigate landscape-level gene flow. Similarly, confinement strategies based on the spatial isolation of GM and non-GM crops, as advocated by European coexistence policies, are unlikely to be effective, given the permanent presence of escaped plants in non-crop habitats.

To accurately describe the far-reaching impacts of GM crops, risk assessment strategies must consider the complementary roles of both biological and anthropogenic processes in facilitating persistence and spread. Such an approach necessarily recognizes that intrinsic invasiveness alone is not an appropriate measure of the potential impact of escaped GM crops. As such, even transient escaped populations require proactive and comprehensive management and monitoring strategies that integrate ecological, agronomic, economic and social impacts. Furthermore, to be truly effective, these strategies must be responsive to spatial and temporal variation in habitat conditions, from the population to the landscape scale.

At the field- and farm-scales, targeted scouting and management of escaped GM canola plants along field edges can be used to decrease the potential for localized gene flow. Large reproductive plants can be managed directly, while habitat conditions can also be managed indirectly to facilitate the competitive exclusion of canola by perennial vegetation. Though time-consuming, expensive, and arguably ineffective given the landscape-scale factors at play, responsibility for this field- and farm-scale management is likely to rest solely with farmers, whether or not they are adopters of GM technology.

At larger spatial scales, escaped populations of GM canola cannot be traced to individual farm fields or specific production systems; rather, escaped populations are likely to originate from pooled regional seed sources, including agricultural transportation. As such, the development of co-operative risk-prevention and mitigation strategies is necessary. I recommend that management of the ecological, agronomic, economic and social risks of GM crops be regional in scope, including collaborative input from farmers, eaters, researchers and governments. Though advocated in the Canadian Prairies (Nazarko, Van Acker & Entz 2005), such networks have not been widely or systemically adopted. To facilitate a collaborative approach to management, governments should play an active role in redistributing the risks associated with agricultural biotechnology, to ensure that the benefits accrued to the agro-industrial developers, growers, suppliers and transporters of GM seed are balanced with appropriate responsibility for confinement of all novel traits. Legislative and litigative mechanisms must be put in place to ensure ongoing accountability from the field trial to the landscape-scale. Such mechanisms will require a revision of Canadian regulatory policy and a reversal of past judicial decisions as they relate to agricultural biotechnology. Science-based risk assessment and subsequent monitoring requirements must be strengthened and expanded for both current and future GM crops, in order to redress and prevent the impacts of GM crop contamination.

In light of the improved scientific understanding of transgene escape provided by this research and in consideration especially of the landscape-scale processes by which such escape occurs, I recommend the development of agricultural policy designed to maintain collective responsibility, foster increased choice for farmers and eaters, and respect the diversity of agricultural landscapes. To this end, Canadian agricultural policy would:

1) Explicitly acknowledge, respect, and adhere to farmer-generated knowledge, as well as to farmer and eater perspectives, throughout pre- and post-release assessments of GM crops;

2) Take immediate and tangible steps to redress the legal, economic, and cultural consequences that have befallen farmers as a result of the unintended and inevitable escape of transgenes, and ensure ongoing accountability; and

3) Implement a regional approach to GM crop cultivation and management, including segregated production areas and designated transport routes for GM and non-GM crops, as directed and determined at the community-level by collaborative groups of farmers and eaters.

Personal Learning: Revaluing Farmers and Farm Landscapes

Throughout the design, implementation, analysis and interpretation of this research, I have been fortunate to be exposed to multiple perspectives on the nature of farming and the limits and possibilities of agricultural innovation. Running alongside my own experience, these many currents and voices have both complemented and challenged my own perspectives on life, work, and the power of planting seeds. I have listened to the perspectives of thoughtful researchers and heard the wise and quiet words of octogenarian rural farmers. I have been inspired by landless peasants in Bolivia to find my own place in the solution *in my own home place*; I have worked side by side with landless farmers in Winnipeg, experiencing always the uncontrollable enthusiasm, cynicism, and hope of those who work to change our food systems for the better. Throughout, I have heard stories more compelling than statistics, and watched things grow that cannot be mapped or modeled.

Most importantly, these influences (as well as the space, freedom and encouragement provided by my advisor and committee) have helped me question the assumptions that underlie the agricultural paradigm currently in favour among governments, industries and a good many academic researchers. This paradigm represents an input-intensive, extractive model of agriculture, a simplified process that eschews the closed-cycle systems of responsible land stewardship, substituting instead purchased inputs and exported products. Such a model is heavily dependent on technological fixes to plug the leaks in the system: synthetic fertilizers are manufactured from petroleum, while new manure storage systems are developed for intensive livestock operations. Our current agricultural paradigm is unmatched in its ability, as Wendell Berry (1996) says, to "take a solution and divide it neatly into two problems", subsequently allowing corporate interests to capitalize on those problems. Today, most agricultural research is tied to corporate funding, and new knowledge creation is inherently limited and co-opted by attached corporate interests. The makers of new agricultural technologies have appropriated and privatized centuries of public knowledge, both scientific and experienced-based, and nowhere is this more apparent than among the developers of agricultural biotechnology; witness the development of GM canola, made possible by the public scholarship and plant breeding efforts of Baldur Stefansson at the University of Manitoba.

Hopefully, I have been able to break through the prescribed assumptions of the prevailing agricultural paradigm in my own learning, whether in the classroom, the field, or the farmhouse. I have learned that at the most basic level, current agricultural policy and economy continues to ignore the underlying value and contribution of ecological processes in renewing land and water. I have learned that this is possible because of the deep-seated cultural assumption that all human impact on natural systems is damaging;

an assumption that fails to distinguish the current extractive paradigm from the human care and culture of land that have enabled us to grow food and fibre for 10,000 years. Based on this assumption, in the context of agricultural biotechnology, we assess benefits and risks using our most devalued, damaged and degraded systems as a baseline for comparison. We never seek to ask *why* these systems are devalued, *who* is responsible for the damage, or *how* we might responsibly and ethically redress the degradation. By continuing to ignore these questions, we pave the way for ongoing exploitation of natural and human systems. Rapid technological advancements continue to outpace our scientific, economic and social understanding of their effects. Our current policies devalue land, culture and people, both on the Canadian landscape and throughout the many countries where our export-based agricultural colonialism takes hold.

And so, while industry measures the impacts of novel transgenes against the insidious effects of chemically-dependent extractive agribusiness, I begin to shape a new personal standard for the assessment of agricultural innovations. I have learned to measure their effects against the tireless generosity of local communities; the lived wisdom and knowledge of those who work the land; the spiritual and physical health imparted by the growing and eating of garden-fresh vegetables; and the sense of pride and accomplishment arising from a line of full sealer jars on a shelf, coloured red and orange and purple like jewels. None of these small and priceless things alone is the answer: but if enough people listen, feel and share these lessons within their own communities, a diverse and resilient patchwork of true innovation will coalesce. I hope to have a part in piecing it together.

I have spent the past six years studying the large-scale industrial problems that plague us; I hope now to devote my work to the small-scale community solutions that may save us. As I garden, market, teach, learn, cook, eat, write, participate and facilitate, I will continue to carry with me the perspectives and people that have taught me, challenged me, and allowed me to grow over the last six years.

References

Bagavathiannan, M.V. & Van Acker, R.C. (2009) The biology and ecology of feral alfalfa (*Medicago sativa* L.) and its implications for novel trait confinement in North America. *Critical Reviews in Plant Science*, **28**, 69-87.

Beckie, H.J., Warwick, S.I., Nair, H. & Séguin-Swartz, G. (2003) Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). *Ecological Applications*, **13**, 1276-1294.

Beckie, H.J., Hall, L.M., Simard, M.-J., Leeson, J.Y., & Willenborg, C.J. (2010) A framework for postrelease environmental monitoring of second-generation crops with novel traits. *Crop Science*, **50**, in press.

Berry, W. (1996) *The Unsettling of America: Culture and Agriculture*, 3rd edn. Sierra Club Books, San Francisco, CA, USA.

Friesen, L.F., Nelson, A.G. & Van Acker, R.C. (2003) Evidence of contamination of pedigreed canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. *Agronomy Journal*, **95**, 1342-1347.

Hall, L.M., Topinka, K., Huffman, J., Davis, L. & Good, A. (2000) Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Science*, **48**, 688-694.

Hwang, S. (2008) *Farmer and Consumer Attitudes toward GM Foods in South Korea.* MEnv Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Mauro, I.J. (2008) *Riding the Risk Wave: Farmer Knowledge and Experience with GM Crops in the Canadian Prairies.* PhD Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Mauro, I.J. & McLachlan, S.M. (2008) Farmer knowledge and risk analysis: Postrelease evaluation of herbicide-tolerant canola in western Canada. *Risk Analysis*, **28**, 463-476.

Mauro, I.J., McLachlan, S.M. & Sanders, J. (2005) *Seeds of Change: Farmers, Biotechnology and the New Face of Agriculture.* Dead Crow Productions and Dada World Data, Winnipeg, Manitoba, Canada. Documentary film available at: www.seedsofchangefilm.org

Mauro, I.J., McLachlan, S.M. & Van Acker, R.C. (2009) Farmer knowledge and a priori risk analysis: A pre-release evaluation of genetically modified Roundup Ready wheat across the Canadian prairies. *Environmental Science and Pollution Research*, **16**, 689-701.

Nazarko, O.M., Van Acker, R.C. & Entz, M.H. (2005) Strategies and tactics for herbicide use reduction in field crops in Canada: a review. *Canadian Journal of Plant Science*, **85**, 457-479.

USDA (2009) *Glyphosate-Tolerant Alfalfa Events J101 and J163: Request for Nonregulated Status. Draft Environmental Impact Statement*. United States Department of Agriculture, Riverdale, MD, USA.

Van Acker, R.C., Brule-Babel, A.L. & Friesen, L.F. (2004) Intraspecific gene movement can create environmental risk: the example of Roundup Ready wheat in western Canada. *Schriftenreihe für Landschaftspflege und Naturschutz*, **79**, 35-48.

Yoshimura, Y., Beckie, H.J. & Matsuo, K. (2006) Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. *Environmental Biosafety Research*, **5**, 67-75.