

Landscape-scale distribution and persistence of genetically modified oilseed rape (*Brassica napus*) in Manitoba, Canada

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Abstract

Background, aim and scope Genetically modified herbicide-tolerant (GMHT) oilseed rape (OSR; *Brassica napus* L.) was approved for commercial cultivation in Canada in 1995 and currently represents over 95% of the OSR 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 OSR 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 OSR remains poorly understood. We conducted a multi-year survey of the landscape-scale distribution of escaped OSR plants adjacent to roads and cultivated fields. Our objective was to examine the long-term dynamics of escaped OSR at large spatial scales and to assess the relative importance of landscape and localised factors to the persistence and spread of these plants outside of cultivation.

Materials and methods From 2005 to 2007, we surveyed escaped OSR plants along roadsides and field edges at 12 locations in three agricultural landscapes in southern

Manitoba where GMHT OSR is widely grown. Data were analysed to examine temporal changes at large spatial scales and to determine factors affecting the distribution of escaped OSR 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 OSR.

Results Densities of escaped OSR fluctuated over space and time in both roadside and field edge habitats, though the proportion of GMHT plants was high (93–100%). Escaped OSR was positively affected by agricultural landscape (indicative of cropping intensity) and by the presence of an adjacent field planted to OSR. Within roadside habitats, escaped OSR 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, OSR density was affected by localised 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 OSR populations in these two habitats, especially at very fine spatial scales (i.e. <100 m), suggesting that natural propagule exchange is infrequent.

Discussion Escaped OSR 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 OSR crops, escaped GMHT traits will likely remain predominant in agricultural landscapes. While escaped OSR 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

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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 OSR populations.

Conclusions 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 OSR 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.

Recommendations and perspectives Given the large-scale factors driving the spread of escaped transgenes, localised 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 organisation. 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.

Keywords *Brassica napus* · Dispersal · Gene flow · Genetically modified (GM) · Herbicide-tolerant (HT) · Landscape · Metapopulation · Oilseed rape (OSR)

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1 Background, aim and scope

Genetically modified herbicide-tolerant (GMHT) oilseed rape (OSR; *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 OSR grown in western Canada (Beckie et al. 2006). Farmers have rapidly adopted these new varieties in part due to their operational benefits. However, OSR 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 OSR 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 OSR plants, and consequently GMHT transgenes, escape from cultivation and spread in adjacent non-field habitats. Early studies of escaped OSR 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 OSR 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 OSR-importing countries (Aono et al. 2006; Kawata et al. 2009). Theoretical studies suggest that these escaped OSR 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 OSR plants, which are often located in close proximity to cultivated OSR fields (Knispel et al. 2008). Additionally, hybridisation between escaped OSR and sympatric wild relatives, particularly *Brassica rapa*, may also contribute to the spread of unwanted GM traits (Warwick et al. 2003).

It is increasingly recognised that landscape-scale assessments are required to accurately characterise the extent of transgene escape and spread in areas where GM crops are cultivated. Local processes that underlie the dynamics of OSR populations have been widely studied and include population demographics (Crawley et al. 1993; Pessel et al. 2001; Pivard et al. 2008) as well as localised 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 OSR populations at the landscape scale are poorly understood. Notably, the importance of human-mediated dispersal of OSR 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 OSR at large spatial scales. In particular, our objectives were to (1) characterise landscape-scale spatial and temporal variations of OSR 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 OSR; and (3) determine the extent to which natural seed dispersal occurs between escaped populations of OSR in roadside and field edge habitats.

2 Materials and methods

2.1 Study area, site selection and field sampling

The large-scale distribution of escaped OSR 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 1, Fig. 1). All three landscapes are located in the Lake Manitoba Plain Ecoregion, characterised 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 OSR seed and other commodities (Canadian Grain Commission (CGC) 2007; Table 1, Fig. 1). The MacDonald and Carman landscapes are important OSR-growing areas in Manitoba, where OSR is generally grown once every 4 years in rotation. Conversely, OSR 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 OSR plants in all three agricultural landscapes, through both protein analysis of maternal plant tissue and herbicide screening of progeny from escaped plants (Table 1; see Knispel et al. 2008 for full description of methodology).

We surveyed the distribution of escaped OSR plants occurring along rural grid roads and highways at 12 locations in the three landscapes (see Fig. 1), originating from the sites of high-density escaped OSR 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 (1×1 mile) were surveyed for escaped OSR. Plants were

Table 1 Characteristics of the Rhineland, MacDonald and Carman agricultural landscapes in southern Manitoba, Canada

| Landscape | Area (km ²) | Average OSR crop (ha) ^a and proportion of area, 2005–2007 | Number of grain storage facilities and capacity (tonnes) ^b | Relative abundance of in-field OSR volunteers ^c | Proportion of escaped OSR 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 (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)

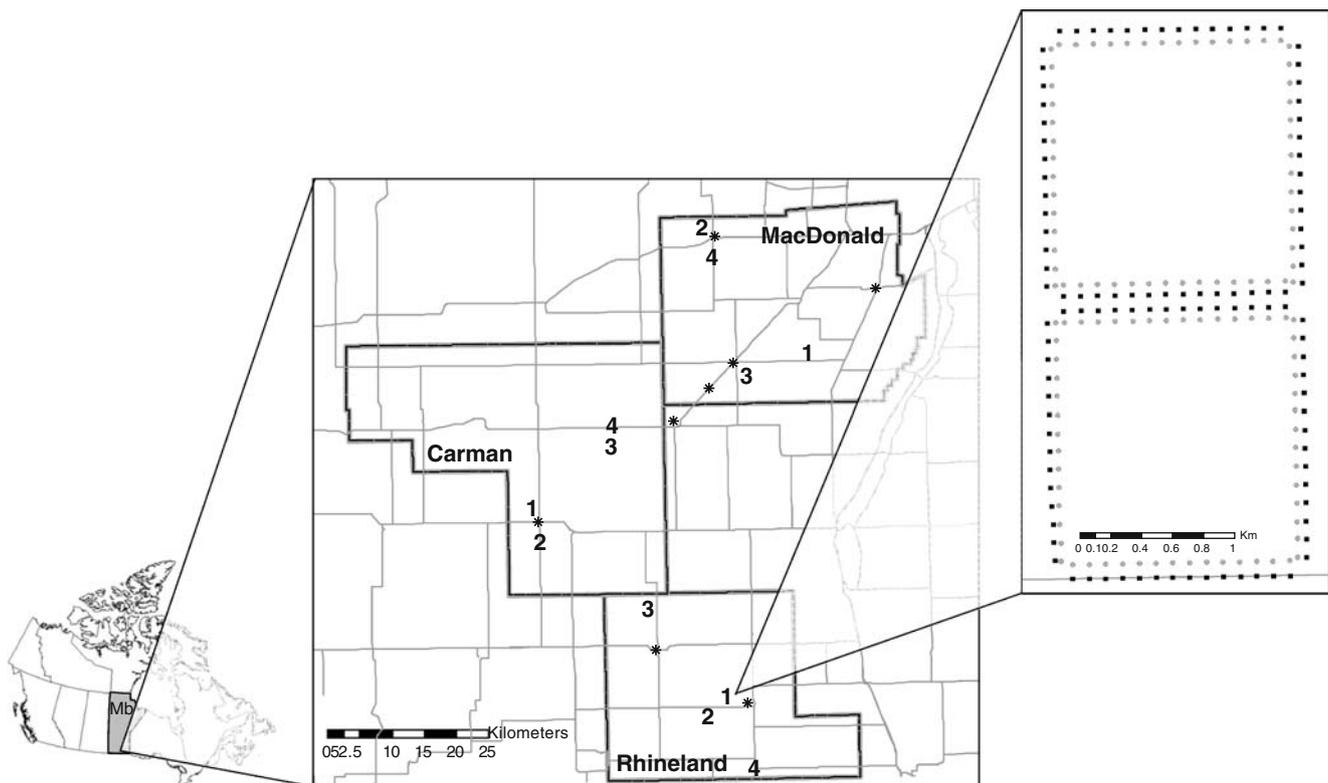


Fig. 1 Landscape-scale distribution of escaped OSR was assessed in southern Manitoba (*Mb*), Canada (*left*). Four sampling locations were surveyed in the Rhineland (*Rhine 1–4*), MacDonald (*MacD 1–4*) and Carman (*Car 1–4*) agricultural landscapes (*middle*). Locations of grain

elevators (*asterisks*) and major roads (*grey lines*) are indicated. At each location, escaped OSR was sampled in roadside (*black squares*) and field edge (*grey circles*) habitats along eight one-mile roads (*right, inset*)

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 OSR 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 OSR plants was recorded every 1.5 min, equivalent to a distance along the road of approximately 110 m. Oilseed rape plants were counted up to 1 m into the ditch vegetation bordering both the road and the field. Escaped OSR 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 m² 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.

2.2 Data analysis

For Rhineland and MacDonald from 2005 to 2007, differences in escaped OSR count within 110 m² 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 OSR counts in a given year with those from the previous year. For Carman, mean OSR 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 OSR. These included agricultural landscape (Rhineland, MacDonald, Carman); road surface (dirt, gravel, paved); adjacent OSR 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, OSR counts from the 2006 survey were also used to model counts of escaped OSR 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 ($n=1,337$) and field edge ($n=1,172$) data sets, the distribution of escaped OSR in 2007 was modelled using negative binomial regression (SAS 9.1, SAS Institute Inc.), 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 localised spatial scales along segments where OSR density was sparse (i.e. along unpaved roads and adjacent to fields planted to crops other than OSR). However, along 50% or more of paved roads and segments adjacent to OSR 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 OSR 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. OSR 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 OSR 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 localised OSR seed dispersal between roadsides and field edges, we compared the distribution of OSR 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 OSR 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 OSR 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).

3 Results

3.1 Spatial and temporal variations

Average OSR counts within 110 m² sampling units varied greatly over the course of this study, ranging from 0.7 to 60.6 plants in roadside habitats (Table 2) and from 1.0 to 49.5 plants in field edge habitats (Table 3). Generally, roadside OSR counts were highest in Carman and lowest in Rhineland (see Table 2). Conversely, field edge OSR counts showed less consistent trends and were highest in Rhineland

Table 2 Average roadside OSR counts for ~110 m² sampling units in Rhineland, MacDonald and Carman landscapes in 2005, 2006 and 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* (1,220) |
| Carman | – | 48.8 a (850) | 60.6 a (1,169) |

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

in 2005, highest in Carman in 2006, and highest in MacDonald in 2007 (see Table 3).

Significant differences in OSR count were detected between landscapes and years using factorial ANOVA. Within roadside habitats, mean OSR counts displayed a significant landscape \times year interaction ($p < 0.0001$), as well as significant simple effects for both factors. Within Rhineland ($n = 447$), OSR counts decreased significantly from 2005 to 2006 ($p < 0.0001$) and remained largely unchanged between 2006 and 2007. Within MacDonald ($n = 446$), roadside OSR counts also decreased significantly from 2005 to 2006 ($p < 0.0001$) but then increased significantly from 2006 to 2007 ($p < 0.0001$; see Table 2). There was a significant effect of landscape on roadside OSR populations in 2005 ($n = 895$) and 2007 ($n = 896$); in both years, OSR counts were significantly higher in MacDonald than in Rhineland ($p < 0.0001$ and $p < 0.0001$, respectively; see Table 2).

Similar trends were observed in the field edge data from Rhineland and MacDonald, where a significant landscape \times year interaction ($p < 0.0001$) was also observed. In Rhineland ($n = 401$), field edge OSR counts showed no significant effect of year at $\alpha = 0.025$ ($p = 0.0364$). However, in

Table 3 Average field edge OSR counts for ~110 m² sampling units in Rhineland, MacDonald and Carman landscapes in 2005, 2006 and 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 (3,050) | 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

MacDonald ($n = 361$), field edge OSR counts fluctuated, decreasing significantly from 2005 to 2006 ($p < 0.0001$) and again increasing significantly from 2006 to 2007 ($p < 0.0001$; see Table 3). There was also a significant effect of landscape on field edge OSR 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 3).

In Carman, no significant differences were observed between roadside ($p = 0.1171$) and field edge ($p = 0.1787$) OSR counts in 2006 and 2007, despite considerable changes in OSR densities in both habitats (see Tables 2 and 3). This may be the result of the high variability of escaped OSR, which is also reflected in the maximum counts within 110 m² sampling units observed for each landscape and year. These ranged from 35 to 1,220 plants in roadsides (see Table 2) and from 35 to 3,050 plants in field edge habitats (see Table 3). Yet, despite extreme spatial and temporal fluctuations, escaped OSR plants commonly occurred at very high densities outside of planted OSR fields in all three agricultural landscapes. Additionally, given the widespread cultivation of GMHT varieties in these areas, the great majority (93–100%) of escaped OSR plants either exhibited GMHT traits or produced GMHT progeny as a result of pollen flow from neighbouring plants (see Table 1).

3.2 Factors affecting the distribution of escaped oilseed rape

Factors associated with the presence of escaped OSR differed considerably between roadsides and field edges, suggesting that OSR distribution is likely to vary independently within these two habitats. Negative binomial regression analysis of roadside data identified five significant predictive variables affecting OSR plants in this habitat, many of these operating at landscape scales (Table 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 OSR in roadsides. Presence of an adjacent OSR field in both the current (AdjCan07) and previous year (AdjCan06) also had a positive impact on roadside OSR density. Conversely, distance to the nearest elevator (DistElev) had a negative effect on OSR in roadsides, indicating that OSR density increases in proximity to grain storage and processing facilities (see Table 4, left side).

Compared to roadsides, escaped OSR plants in field edge habitats were significantly associated with much more localised features and disturbances (see Table 4, right side). Most importantly, field edge OSR density increased with the presence of an adjacent OSR field in

Table 4 Coefficients and *p* values for negative binomial regression models of all roadside (left) and all field edge (right) observations in 2007

| Parameter | All roadside samples | | | All field edge samples | | |
|------------|----------------------|------------|----------|------------------------|------------|----------|
| | Estimate | Chi-square | <i>p</i> | Estimate | Chi-square | <i>p</i> |
| 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 | | |

Landscape agricultural landscape, *RoadSurf* road surface, *DistElev* distance to nearest grain elevator, *AdjCan07* adjacent OSR crop in 2007, *AdjCan06* adjacent OSR crop in 2006, *Count06* OSR 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

the current year. Management variables were also important, and OSR 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 OSR field in the previous year had small positive effects on the presence of escaped OSR plants in field edges (see Table 4, right side).

Though neither roadside nor field edge samples of escaped OSR exhibited spatial autocorrelation when the data were considered as a whole, OSR counts were often spatially structured along individual road segments (see section 2.2). This was particularly evident for roadside samples adjacent to paved roads and for field edge samples adjacent to OSR fields, likely reflecting the higher densities of OSR in these locations. Additional negative binomial and auto-negative binomial regressions were compared for both paved road and OSR 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 OSR distribution (Table 5, left side). The density of escaped OSR plants increased significantly with the presence of an adjacent OSR crop in the previous year and, to a lesser extent, with the presence of an adjacent OSR crop in the current year. Escaped OSR density increased only slightly with the presence of escaped plants at the sample location in the previous year (Count06). Along paved roads, escaped OSR density decreased with increasing distance from an elevator (see Table 5, left side).

In the auto-negative binomial regression model, inclusion of the autocovariate reduced the significance of other parameter estimates (see Table 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 5, right side).

Along field edges adjacent to planted OSR fields, basic negative binomial regression identified five significant predictive variables (Table 6, left side). Local management variables again had important impacts on escaped OSR, which increased significantly in mowed sites and decreased significantly with soil disturbance and herbicide application. To a lesser extent, escaped OSR was also positively affected by landscape and negatively affected by distance to elevator (see Table 6, left side). In the auto-negative binomial 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, right side).

3.3 Interactions between escaped oilseed rape 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 OSR 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

Table 5 Coefficients and *p* values for negative binomial (left) and auto-negative binomial (right) regression models of observations adjacent to paved roads

| Parameter | Paved Roads | | | Auto-regression | | |
|------------|-------------|------------|----------|-----------------|------------|----------|
| | Estimate | Chi-square | <i>p</i> | Estimate | Chi-square | <i>p</i> |
| 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 | | |

Landscape agricultural landscape, *DistElev* distance to nearest grain elevator, *AdjCan07* adjacent OSR crop in 2007, *AdjCan06* adjacent OSR crop in 2006, *Count06* OSR 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

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 (Figs. 2 and 3). At distances of 100 m, there was no evidence that escaped OSR populations in field edges were spatially dependent on proximal roadside populations from

the previous year (see Fig. 2). However, at sites Rhine 4 (2005–2006) and MacD 4 (2006–2007), clustering was apparent at spatial scales greater than 200 m.

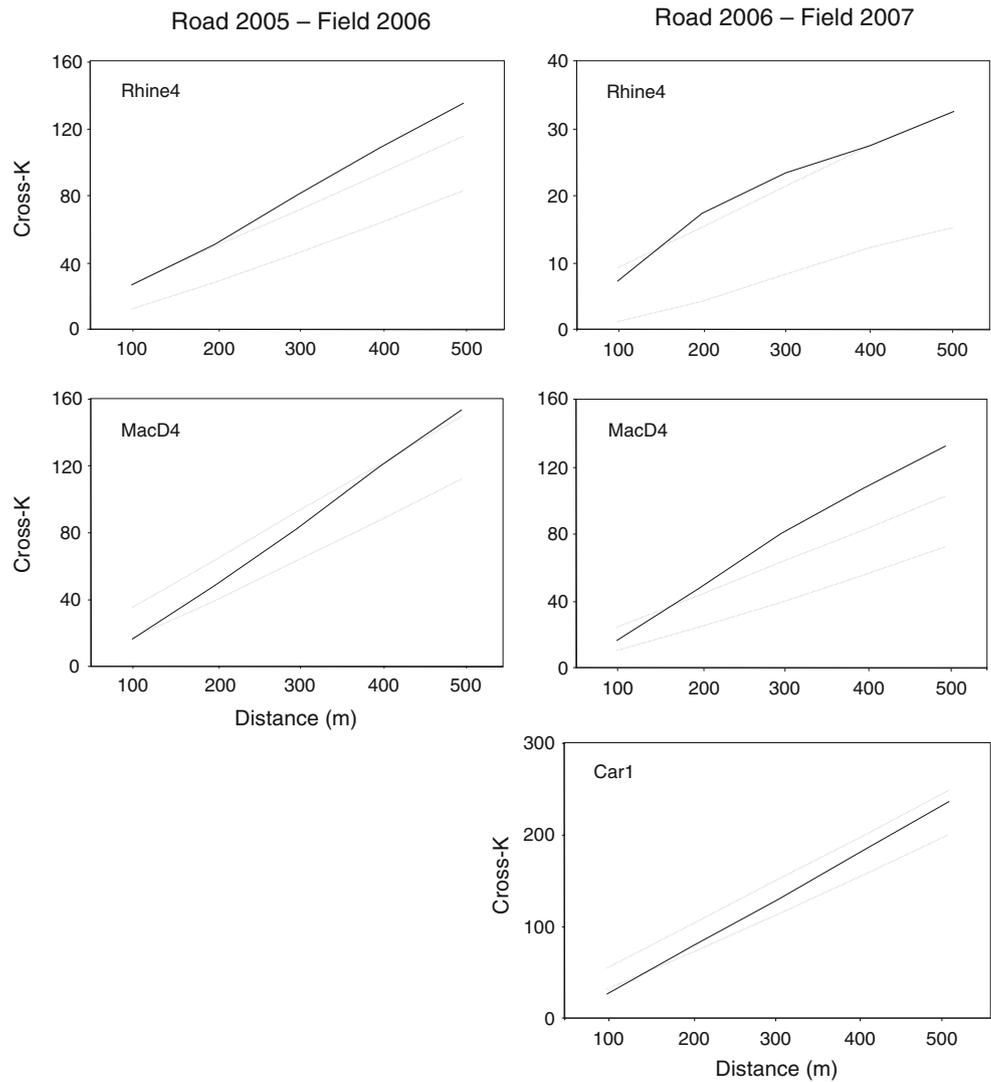
Escaped OSR populations in roadside habitats also showed little evidence of clustering around the locations of field edge populations from the previous year (see Fig. 3). In only one site, Rhine 4 (2005–2006), were roadside

Table 6 Coefficients and *p*-values for negative binomial (left) and auto-negative binomial (right) regression models of observations adjacent to OSR fields

| Parameter | OSR fields | | | Auto-regression | | |
|------------|------------|------------|----------|-----------------|------------|----------|
| | Estimate | Chi-square | <i>p</i> | Estimate | Chi-square | <i>p</i> |
| 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 | | |

Landscape agricultural landscape, *RoadSurf* road surface, *DistElev* distance to nearest grain elevator, *AdjCan06* adjacent OSR crop in 2006, *Count06* OSR 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

Fig. 2 Spatial aggregation of field edge OSR populations around roadside OSR 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



populations clustered around field edge populations at all spatial scales, though aggregation was still not pronounced at the 100 m distance (see Fig. 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 OSR plants growing in roadside and field edge habitats.

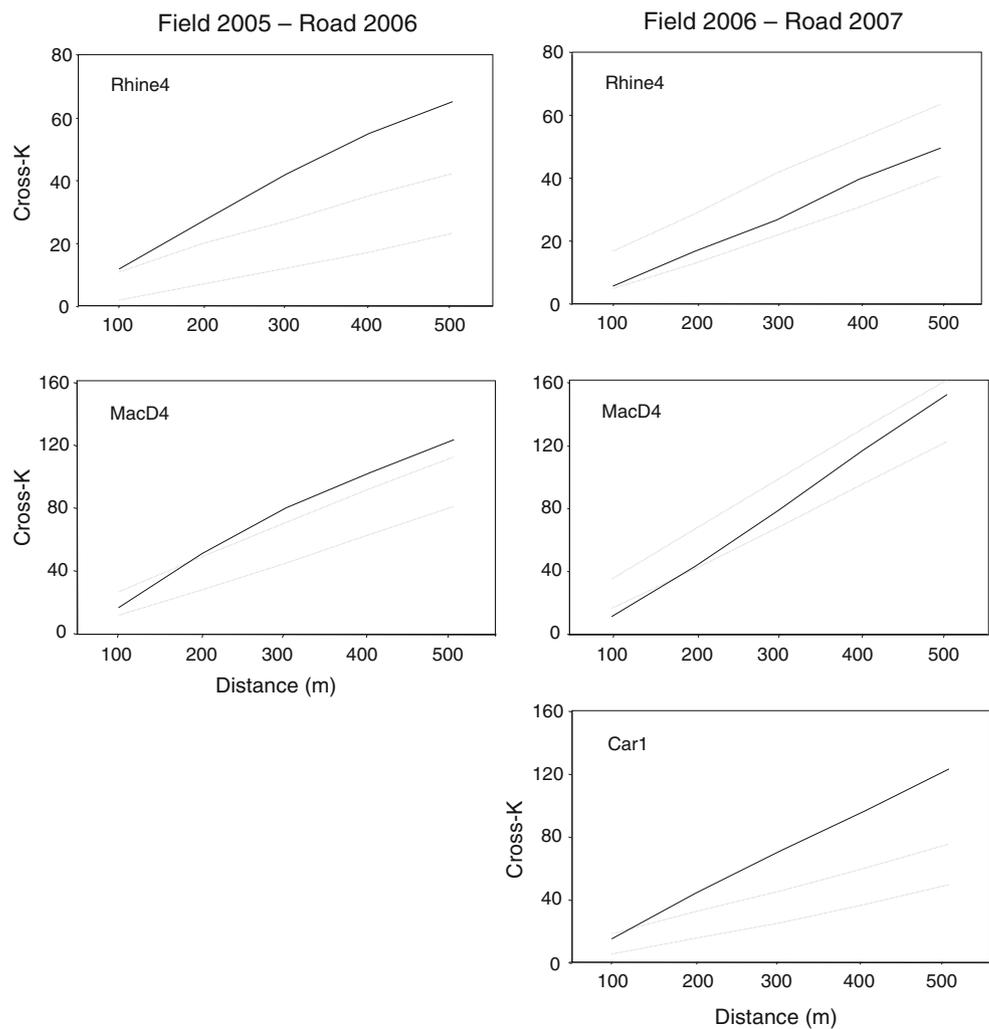
4 Discussion

To date, science-based risk assessment of novel GM crops has been largely reliant on findings from localised 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 OSR 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 OSR populations examined in our study were highly dynamic, and densities fluctuated widely in both space and time. Spatial variation in escaped OSR in part reflected landscape-level differences in the presence of OSR 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 OSR cultivation in MacDonald as well as the high abundance of in-field OSR volunteers in Carman (see Table 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 OSR in field edge habitats in Rhineland likely

Fig. 3 Spatial aggregation of roadside OSR populations around field edge OSR 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



reflected wet spring conditions that delayed seeding and resulted in the replacement of long-season crops such as corn and soy with early-maturing OSR varieties (Canola Council of Canada (CCC) 2005). In turn, lower overall escaped OSR 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 OSR populations have been observed in the UK 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 OSR 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 OSR populations were particularly important when considering dispersal. Escaped OSR 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 OSR 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 OSR 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 OSR seed may be expected to occur at spatial scales much larger than the landscapes where OSR 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 OSR plants. Regular mowing of weedy perennial vegetation likely enables the establishment of annual weed species including escaped OSR (Crawley et al. 1993). Given the predominance of GMHT varieties in cultivation in western Canada, application of common broad-spectrum herbicides such as glyphosate is often ineffective in controlling GMHT OSR 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-dichlorophenoxyacetic acid would normally be required to control GMHT OSR volunteers, which may explain the apparent effectiveness of herbicide application observed adjacent to a small number of OSR fields in this study. Generally, however, the negative effect of soil disturbance on the distribution of escaped OSR in field edges suggests that tillage is likely to serve as the only reliable means to control escaped OSR 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 GMHT OSR volunteers thus creates additional management challenges. In some cases, producers are reverting to tillage in order to manage problematic GMHT OSR volunteers (Mauro and McLachlan 2008).

The lack of aggregation observed between roadside and field edge escaped OSR at very fine spatial scales indicates that natural seed dispersal from escaped populations was negligible relative to OSR seed inputs from landscape-scale anthropogenic vectors. As such, control efforts directed at individual escaped OSR populations are likely to be counteracted by the volume and spatial extent of OSR seed dispersal along agricultural distribution networks. Farmers will thus be unable to reduce or eliminate the long-distance dispersal of

OSR 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 OSR seed, compounded by the ubiquitous presence of escaped GMHT OSR 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 OSR 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.

5 Conclusions

As a result of widespread cultivation and large-scale dispersal, escaped GMHT OSR 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 OSR 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 self-recruitment. That these escaped OSR populations are relatively persistent at larger scales of organisation 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 OSR into cultivated fields will result in the potential contamination of OSR crops, while hybridisation 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.

6 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 OSR 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 localised approaches.

The importance of escaped GM plants and the role of agricultural transport in facilitating their dispersal suggest 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 OSR 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.

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References

- Allison PD (1999) Logistic regression using the SAS system: theory and application. SAS Institute, Cary
- 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, Warwick SI, Nair H, Séguin-Swartz G (2003) Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). *Ecol Appl* 13:1276–1294
- 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
- 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
- Canadian Grain Commission (CGC) (2007) Grain elevators in Canada, crop year 2007–2008. Canadian Grain Commission, Winnipeg
- 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 coexistence of GM and non-GM crops. *Environ Sci Pollut Res* 16:348–360
- Crawley MJ, Brown SL (2004) Spatially structured population dynamics in feral oilseed rape. *Proc R Soc Lond 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
- 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, Cary
- Kawata M, Murakami K, Ishikawa T (2009) Dispersal and persistence of genetically modified oilseed rape around Japanese harbors. *Environ Sci Pollut Res* 16:120–126

- 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, Washington, DC, pp 335–343
- 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
- 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
- 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 Mathematical Society, Providence, pp 75–107
- Magas OK, Gunter JT, Regens JL (2007) Ambient air pollution and daily pediatric hospitalizations for asthma. *Environ 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. *Environ Sci Pollut Res*. doi:10.1007/s11356-009-0177-6
- 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
- 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
- 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, New York
- 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
- 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, 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
- 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
- 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