Is GM and Non-GM Crop Co-existence Possible in a Perennial, Outcrossing Species with High Ferality Potential? An Investigation with Alfalfa (*Medicago sativa*, L.) in North America

Rene Van Acker
Department of Plant Agriculture, University of Guelph, Canada
vanacker@uoguelph.ca

Muthukumar Bagavathiannan
Department of Plant Science, University of Manitoba, Canada
umbagava@cc.umanitoba.ca

Abstract

Genetically modified (GM) alfalfa is the first perennial, highly outcrossing, insect pollinated species to receive authorization for deregulation. It is not yet clear whether co-existence is possible between GM and non-GM alfalfa. Our research in Western Canada indicates that roadside feral alfalfa populations are common and fully dynamic. These populations can serve as reservoirs for trait or transgene movement and as such are potential barriers for achieving co-existence. Mowing can reduce the dynamics of feral populations but not eliminate them in the short term. Strict feral alfalfa stewardship would greatly reduce gene flow potential and would in part aid the co-existence of GM and non-GM alfalfa.

Introduction

Most of the GM crops authorized and commercially cultivated to-date are annuals with limited persistence in nature. Perennial crops with high outcrossing potential, on the other hand, may pose great challenges for transgene confinement.

Genetically modified glyphosate resistant (GM-GR) alfalfa was approved for unconfined release in Canada (CFIA, 2005) and the United States (US) (USDA-APHIS, 2005) in 2005. In 2005, GM-GR alfalfa was grown on over 260,000 acres on more than 5400 farms in the US. In 2007, a permanent injunction order was issued by a US district court on further sales and cultivation of GM-GR alfalfa upon the grounds that they could have contaminated organic and conventional alfalfa fields in the production system (USDC, 2007). The US court recommended a thorough investigation on the biosafety of GM-GR alfalfa before it is deregulated again.

Among the GM crops developed to-date, alfalfa is a high risk crop in terms of transgene movement (Table 1). The biology and ecology of alfalfa is favorable for gene flow (Bagavathiannan and Van Acker, 2009). In particular, alfalfa's pollination biology favors outcrossing. The flowers of alfalfa need to be tripped for pollination (Bolton, 1962). Pollination in alfalfa is therefore primarily insect mediated and insects such as leaf-cutter bees (*Megachile rotundata*) (Richards, 1991), honey bees (*Apis mellifera*) (Bohart, 1957), bumble bees (*Bombus spp.*) (Holm, 1966; Osborne et al., 2008) and alkali bees (*Nomia melanderi*) (Stephen, 1959) are efficient in tripping and pollinating alfalfa. Moreover, the existence of self-incompatibility supports cross pollination in alfalfa (Allard, 1988).

Alfalfa is also capable of escaping cultivation and establishing in unmanaged natural and semi-natural habitats (Kendrick et al., 2005). A deep tap root system, cold and drought tolerance and symbiotic nitrogen fixation are traits of alfalfa that aids its ferality potential. Feral populations of cultivated crops may act as sources and sinks of transgenes and aid in gene flow at landscape scale (Mueller, 2004; Bagavathiannan and Van Acker, 2008). In nature, the intraspecific movement of traits among sub-populations occurs in the context of a metapopulation (Crawley and Brown, 1995). In agricultural landscapes, and for crop species, the metapopulation includes subpopulations of cultivated crops, volunteers and feral plants (Van Acker, 2007). The presence of feral populations may hinder transgene confinement under field conditions.

Our research in Western Canada was aimed at characterizing roadside feral alfalfa populations and to determine their role in transgene movement across the landscape. We worked to answer a few basic questions including: a) What is the extent of occurrence of escaped alfalfa populations in roadside habitats? b) Are escaped populations capable of establishing self-sustaining feral populations? c) What is the level of outcrossing between cultivated and escaped alfalfa populations?

Table 1 Comparison of the ecology and biology of major GM crops.

Common name	Scientific name	Life cycle	Type of pollination	Mode of pollination	Level of self-incompatibility	Issue of volunteers	Ferality potential	Other remarks	Risk of gene flow
Alfalfa	*Medicago sativa*	Perennial	Predominantly outcrossing	Insect	High	Low – medium	High	Can be weedy/invasive[†]	High
Canola	*Brassica napus*	Annual	Selfing/ outcrossing	Insect, wind	Low	High	Medium – high	Seed shattering/ huge seed bank	Medium - high
Cotton	*Gossypium hirsutum*	Annual/ perennial	Predominantly selfing	Insect	Negligible	Low	Medium	Can be weedy/invasive[†]	Low - medium
Creeping bentgrass	*Agrostis stolonifera*	Perennial	Predominantly outcrossing	Wind	High	N/A	High	Both vegetative propagules/seeds	High
Flax	*Linum usitatissimum*	Annual	Predominantly selfing	Insect	Low	Medium	Medium	Hermaphroditic flowers	Low - medium
Lentil	*Lens culinaris*	Annual	Predominantly selfing	Insect	Low	Medium	Negligible	Pollination occurs before flower opens	Low
Maize	*Zea mays*	Annual	Selfing/ outcrossing	Wind	Low	Low – medium	Low	Staminate and pistillate flowers	Medium
Potato	*Solanum tuberosum*	Annual	Selfing/ outcrossing	Insect	Low	Low – medium	Low	True seeds and tubers	Low
Rice	*Oryza sativa*	Annual	Predominantly selfing	Wind	Low	Medium	Low – medium	Can be weedy/invasive[†]	Medium
Soybean	*Glycine max*	Annual	Predominantly selfing	Insect	Negligible	Low	Negligible	Flowers attract few bees	Low
Sugar beet	*Beta vulgaris*	Biennial	Predominantly outcrossing	Wind, insect	High	Medium	High	High levels of self-sterility	High
Sunflower	*Helianthus annuus*	Annual	Predominantly outcrossing	Insect	Medium - high	Medium	Low – medium	Recent varieties are self compatible	Low - medium
Wheat	*Triticum aestivum*	Annual	Predominantly selfing	Wind	Low	Medium – high	Low-medium	Florets remain open only for short period	Medium

Sources: Agbios (2008), CFIA (2008)
[†]SWSS (1998)

Materials and Methods

Roadside survey

A survey was conducted in August 2006 along roadsides in three rural municipalities in Southern Manitoba (Springfield, Hanover and MacDonald). The survey methodology was adapted from Kendrick et al. (2005) with some modifications. In each location, 30 observation sites were identified. Data pertaining to the population size (number of patches population^{-1}) and patch size (number of individuals patch^{-1}) were documented at each of these sites (~500 m^2 per site). Alfalfa plants that occurred within 1m of each other were considered to be part of a single patch and each patch consisted of at least one plant. Data pertaining to the number of escaped populations, existence of cultivated alfalfa fields, type of production (hay/seed) and distance as well as flowering synchrony between cultivated and escaped alfalfa populations were documented while traveling between each of the observation sites. Flowering in hay production fields were confirmed if there was at least one fully opened flower in a minimum of 10% of the branches. The degree of roadside mowing along the survey route was also noted.

Demography of escaped populations

Research sites were identified in the municipalities of Springfield, Hanover and MacDonald in Southern Manitoba in 2006. In each location, four sites 150 to 200m long and 10 to 15m wide were chosen and at each site 30 escaped alfalfa plants of different age and size were randomly selected for further investigation. A total of 360 plants were studied for a period of more than two years. The plants were harvested at the end of the season and seed output was estimated. Plant survival was documented during May (winter survival) and August (summer survival) for three consecutive years. Three soil cores were taken around each plant (10cm diameter and 7cm deep) in early May and the seed bank was estimated by growing out the samples in a greenhouse. Soil seedbank samples were subjected to freeze-thaw cycles to break dormancy. In addition, sub-samples were drawn from collected soil and were washed out to find the seeds that failed to germinate. Seedling recruitment around each plant was noted during early May prior to the collection of seedbank samples. Roadsides were partially mowed by rural municipalities twice each year (June-July and August-September). Plants in the mown zone were mown once but harvested before the second mowing.

Gene flow

We determined the gene flow between cultivated and escaped alfalfa populations using white flower color as a scoreable marker (Kehr, 1973; Pedersen, 1974; Pedersen and Barnes, 1973; Barnes, 1972). A white flowering alfalfa plant was clonally multiplied and used as the female pollen recipient population. The gene flow experiment consisted of four treatments and three replications in a completely randomized design. The treatments were designed to test levels of gene flow in the following situations: a) among individuals within an escaped alfalfa population, b) from hay production fields to escaped populations, c) from seed production fields to escaped populations, and d) from escaped to cultivated populations.

In each treatment, 10 white flowering female clones were randomly planted at intervals of about 5m. The surrounding alfalfa populations were controlled such that the pollen would only come from the male population under investigation. Leaf-cutter bees were released in the seed production fields and in other treatments the seed set was based on natural pollinators. The clones were harvested at the end of the season and planted in small trays in the greenhouse and allowed to grow until flowering. Positive scoring for outcrossing was carried out in the progenies based on the presence of pigmented petals and roots (other than white).

We used the following formula to determine the sample size required for detecting gene flow at a given level:

$N = \ln(1-P)/\ln(1-p)$ (Alibert et al. 2005)

Where, P-probability of detection of one individual in least frequent class; p-probability of the least frequent class

Based on this calculation, 458 individuals were screened in each replication in order to allow us to test gene flow at 1% detection level (p=0.01) with 99% probability (P=0.99).

Data analyses

The survey data were analyzed using a Mixed Procedure analysis (PROC MIXED) using the Statistical Analysis Software (SAS) version 9.1 (SAS Institute, 2003). Dependent variables were examined using analysis of variance (ANOVA). The locations were considered as fixed effects while the detailed observation sites were regarded as random effects. The demography data was also analyzed using PROC MIXED, with the locations as fixed effects and the replications within locations as random effects. Gene flow data were analyzed following a Generalized Linear Model procedure (PROC GLM) of SAS. In each analysis, outliers were removed based on the studentized residual values and normality was confirmed using Kolmogorov-Smirnov test. Mean separation was performed using Fisher's protected Least Significant Difference (LSD) at $P \leq 0.05$. Letter groupings were carried out using the PDMIX800 macro in SAS (Saxton 1998).

Results

Roadside survey

Escaped alfalfa populations were common along the roadsides in the rural municipalities we surveyed. Frequency of occurrence and population size varied among municipalities. In MacDonald, frequency was only 20% of frequency in Hanover or Springfield (Table 2). Frequency showed some relationship to the extent of alfalfa cultivation within a given municipality (Table 3).

Table 2 Prevalence of escaped alfalfa populations in road side habitats

Parameter	Hanover		MacDonald		Springfield	
	Mean	SE	Mean	SE	Mean	SE
Number of populations km^{-1}	1.68 a	0.16	0.21 b	0.04	1.32 a	0.15
Number of patches population^{-1}	11.52 b	1.11	12.57 b	1.33	17.54 a	1.66
Number of individuals patch^{-1}	3.04	0.38	4.06	0.67	3.68	0.37

Values within each row followed by different letters are significantly different (α=0.05)

Table 3 Alfalfa production data of the rural municipalities included in the survey[†]

Particulars	Hanover	MacDonald	Springfield
Number of farms cultivated to alfalfa	51	27	40
Production area (acres)	3,807	1849	2,320
Total production (tones)	7,655.59	2150.32	4,511.13

[†]Production data from 2006, Source: MMPP (2008)

Across all municipalities, patches were generally small (about 3.5 individuals patch^{-1}) (Table 2) and there were no significant differences in patch size among the municipalities. Roadside alfalfa populations were often observed close to the cultivated alfalfa fields. In 68% of cases, the roadside alfalfa populations were found within 250m of a cultivated alfalfa field (fig.1).

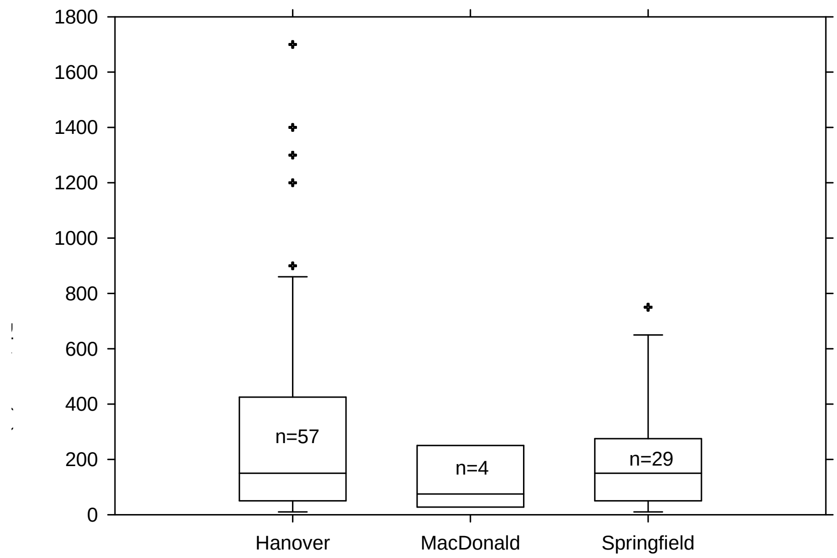

Fig.1 Distance of escaped alfalfa populations to the alfalfa production fields. The center line of the boxes represents sample median. Lower and upper hinges are the estimates respectively of the first and third quartiles representing the lower and upper halves of the samples respectively.

We observed flowering synchrony between roadside and cultivated alfalfa populations but it varied depending on whether the cultivated alfalfa was a hay or seed field. Flowering synchrony between roadside and cultivated seed fields was absolute in our study. For hay fields, flowering was observed in only one third of the fields at the time of the survey. More than 90% of the alfalfa fields along the survey route were hay fields. More than 80% of the roadside ditches along the survey route were mown which impacted the amount of flowering in the roadside populations.

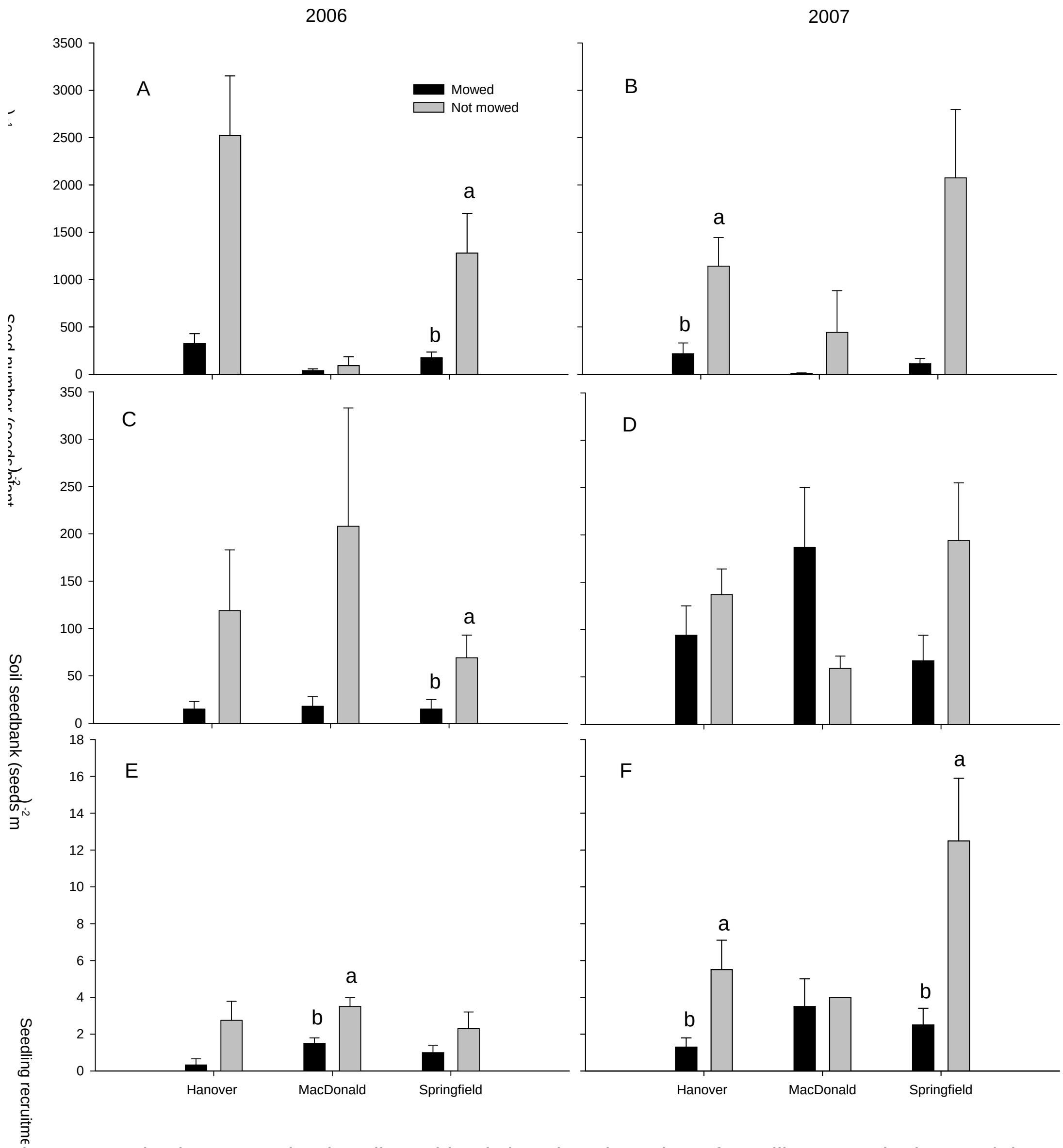

Fig. 2 Reproductive output (A,B), soil seed bank (C, D) and number of seedlings recruited around the study plants (E, F) both under mowed and not mowed conditions among different locations in 2006 and 2007. Statistical significance was determined at $P \leq 0.05$.

Demography of escaped alfalfa

We found dormant alfalfa seeds in our roadside soil seedbank samples as well as successful alfalfa seedling recruitment and reproductive success. Mowing substantially (and often significantly) reduced the reproductive success of alfalfa and subsequently soil seedbank population size as well as the number of seedlings recruited in the roadside habitats (fig. 2). Averaged across municipality and year, reproductive output was 146 and 1259 seeds plant^{-1} in mowed and not-mowed areas respectively. Most mowed plants were not reproductively successful.

The alfalfa seedbank around the study plants contained only a fraction of the seeds that the adult roadside plants could produce (fig. 2). Nevertheless there was a relationship between adult seed production level and seedbank size and mowing affected the size of the seedbank likely because it affected the reproductive output of adult plants. Seed extraction and tetrazolium testing revealed an average of 78 viable but ungerminated alfalfa seeds m^{-2}, in our roadside seedbanks. On average, recruitment levels represented only 3% of the germinable seedbank.

Cumulative adult alfalfa plant mortality in the roadside populations ranged between 13% and 22% over our three year monitoring period (fig. 3). Mowing had no significant effect on mortality of adult alfalfa plants in our study.

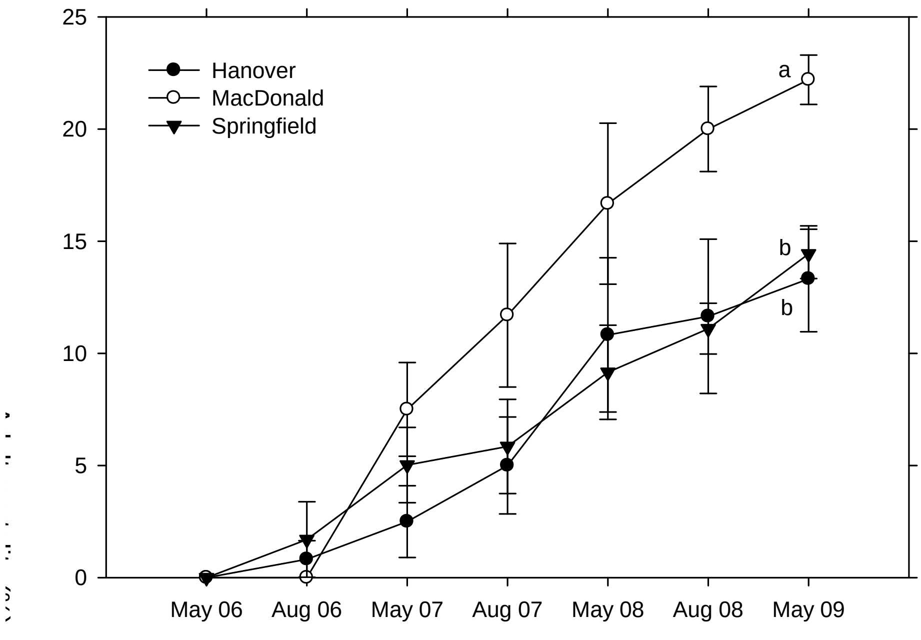

Fig. 3 Mortality over a three year monitoring period for adult alfalfa plants occurring along roadsides in three rural municipalities in Manitoba, Canada. Values followed by different letters show significant differences in means among municipalities for a given date (α=0.05). Bars around markers represent standard error of the mean.

Gene flow in escaped alfalfa populations

The gene flow study revealed greater levels of outcrossing in escaped alfalfa populations (fig.4). Estimated levels of outcrossing varied between 62% and 85%

across an average distance of 15m. Lower levels of outcrossing (62%) were observed when the pollen recipients were planted adjacent to the seed production fields (with leaf cutter bee pollination) when compared to other treatments.

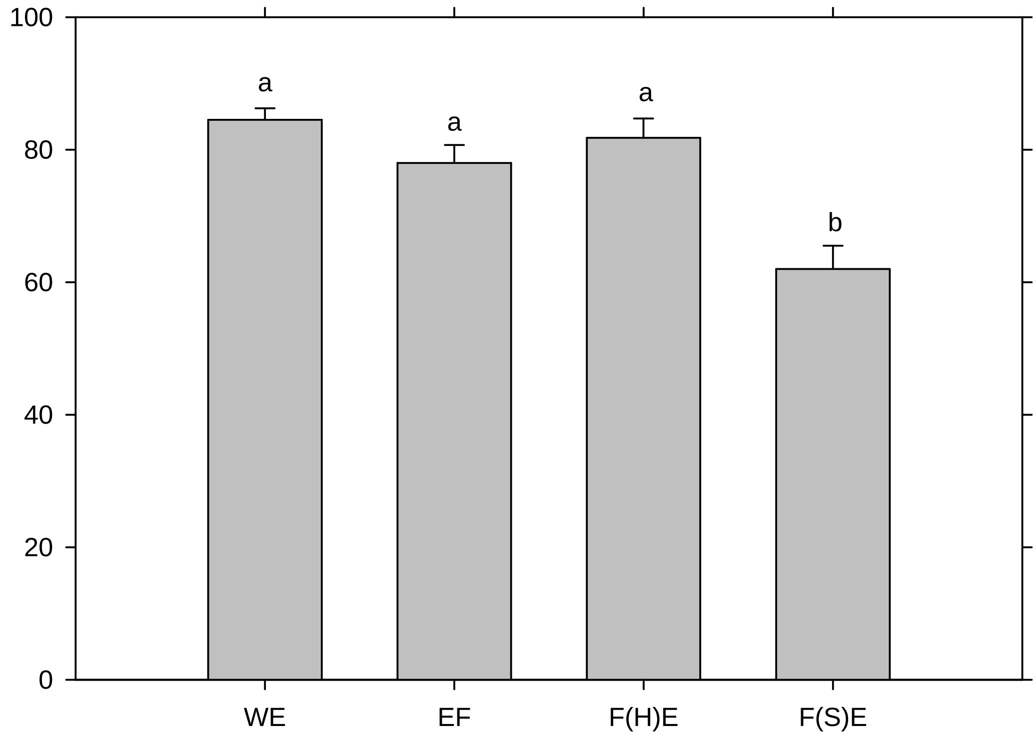

Fig.4 Outcrossing levels estimated by white flowering clones as influenced by outcrossing within escaped populations (WE); outcrossing from escaped alfalfa to field alfalfa (EF); outcrossing from hay fields to escaped populations [F(H)E]; outcrossing from seed production fields to escaped populations [F(S)E]. Columns followed by different letters are significantly different ($\alpha=0.05$). Bars above columns represent standard error of the mean.

Discussion

The widespread occurrence of escaped alfalfa populations along roadsides demonstrates alfalfa's feral capability. A greater frequency of roadside populations in areas with widespread alfalfa cultivation suggests that farming activities play a role in the escape of alfalfa to roadside habitats and this result is similar to that reported by Kendrick et al. (2005) for surveys in the US. The size of individual populations, however, showed no relationship to the extent of alfalfa cultivation in the region. Population size may be related to habitat conditions which impact recruitment and establishment. Larger patches may also represent a single large founding while smaller patches may represent the effects of auto-allelopathy as per Jennings and Nelson (2002).

The reproductive success of escaped plants, dormant seeds in the soil seedbank and seedling recruitment in roadside habitats indicate that alfalfa is capable of establishing self-perpetuating feral populations in unmanaged environments. The detrimental effect of mowing on reproductive success shows that mowing is one means of limiting roadside population growth. The length of the period between mowings determines the likelihood of the reproductive success. The extent and frequency of mowing varies greatly among routes within rural municipalities and even among rural municipalities in Manitoba (Moffat, B. Springfield Municipality manager, Personal Communication) and this can lead to tremendous variation in the nature, growth and success of roadside alfalfa populations. Roadside alfalfa plants that are not mowed can form a more dynamic population.

In our experiment, a substantial portion of the seeds we retrieved from soil seedbank had a very hard seed coat and were viable but not germinable. This is evidence that alfalfa can form a persistent seedbank in roadside habitats. Multi-year alfalfa seed dormancy has been demonstrated by other authors (Wilton et al. 1978; Rincker 1983) but not for seed extracted from roadside seedbanks.

Many of the roadside alfalfa populations we observed were 250m or less from cultivated alfalfa fields, a distance sufficient for outcrossing at a level of 0.28% and 1.5% respectively under leaf cutter bee (Fitzpatrick et al. 2003) and honey bee (Teuber et al. 2004) mediated pollination. Both cultivated and escaped populations exhibit cross compatibility and flowering synchrony and the indeterminate growth habit of alfalfa further extends the period of flowering synchrony improving the chances for outcrossing (Bolton 1962).

Our outcrossing study revealed a very high potential for pollen mediated gene flow between escaped and cultivated populations of alfalfa. The significantly lower outcrossing from the white flowering clones to the seed production fields (versus the hay production fields) seemed counter intuitive to us and it could have been due to preferential pollination by leaf-cutter bees with respect to flower color as has been demonstrated by Kehr (1973) and Steiner et al. (1992). If this is true then our results would represent an underestimation of the outcrossing potential to alfalfa seed fields given that flowers in the roadside populations were typically coloured. The results of our gene flow study also show that gene flow from hay fields to roadside alfalfa populations is possible and this corroborates the results of a study by Forage Genetics International (FGI 2008). Hay fields are typically harvested before flowering yet hay harvests are never absolute and flowering adult plants can commonly be observed in field corners and along field edges. In addition, unfavorable weather conditions often cause unavoidable harvest delays leading to flowering prior to harvest. We observed high levels of outcrossing among individuals within roadside populations and these results were similar to those of St. Amand et al. (2000). Our results support the idea that feral alfalfa populations can act as a genetic bridge for traits and transgenes. In this respect, the management of feral alfalfa populations will be important for managing the coexistence of GM and non-GM alfalfa.

The results of our studies confirm that alfalfa can commonly maintain fully dynamic roadside populations in rural municipalities in Manitoba, Canada. Our results show that mowing is effective at reducing the population dynamics of feral alfalfa populations but within a three year time span mowing will not eliminate feral populations. If there is a need and/or desire to confine specific alfalfa crops, perhaps GM alfalfa and/or alfalfa which produces a specific trait then roadside feral populations need to be considered in the confinement protocols and practices, and the elimination of feral alfalfa populations in the short term requires more than just mowing.

Acknowledgements

Funding sources include the Agri-Food Research and Development Initiative (ARDI) of the governments of Manitoba and Canada, the Natural Sciences and Engineering Research Council (NSERC) and a University of Manitoba Graduate Fellowship and Graduate Scholarship for M. Bagavathiannan.

References

Agbios 2008, 'Global status of approved genetically modified plants', viewed 27 July, 2009 <http://www.agbios.com/dbase.php?action=Synopsis>.

Bagavathiannan, MV, Gulden, RH, Begg, GS & Van Acker, RC 2009 'The demography of feral alfalfa (*Medicago sativa*, L.) populations occurring in Southern Manitoba, Canada' in *Proceedings of the Weed Science Society of America Annual Meeting*, Orlando, FL, USA.

Bagavathiannan, MV & Van Acker, RC 2008, 'Crop ferality: Implications for novel trait confinement', *Agriculture Ecosystems & Environment*, vol. 127, no.1-2, pp. 1-6.

Bagavathiannan, MV & Van Acker, RC 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 Sciences*, vol. 28, no.1, pp. 69-87.

Bohart, GE 1957, 'Pollination of alfalfa and red clover', *Annual Review of Entomology*, vol. 2, pp. 355-380.

Bolton, JL 1962, '*Alfalfa: Botany, cultivation and utilization*', Interscience Publishers, New York, USA.

Canadian Food Inspection Agency (CFIA) 2005, 'Decision document DD2005-53: Determination of the safety of Monsanto Canada Inc.'s Roundup Ready Alfalfa (*Medicago sativa* L.) events J101 and J163', viewed 19 June, 2009, <http://www.inspection.gc.ca/english/plaveg/bio/dd/dd0553e.shtml>.

Canadian Food Inspection Agency (CFIA) 2008, 'Biology documents: Companion documents for directive 94-08', Canadian Food Inspection Agency, viewed 19 June, 2009, <http://www.inspection.gc.ca/english/ plaveg /bio/dir/ biodoce.shtml>.

Canadian Seed Growers' Association (CSGA) 2003, 'Regulations and procedures for pedigreed seed production, Circular 6-94. Section 7, viewed 19 June, 2007, <http://www.seedgrowers.ca/cropcertification/ index.asp?lang=e>.

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*, vol. 259, pp. 49-54.

Forage Genetics International 2008, ‘Research brief: measurement of pollen-mediated alfalfa gene flow from Roundup Ready hay sources to conventional seed fields (2000-2006 data)’, viewed 19 June, 2009, <http://www.foragegenetics.com/pdf/ResearchBriefHAY-TO-SEEDSummary2000-2006.pdf>.

Fitzpatrick, S, Reisen, P & McCaslin, M 2003, ‘Pollen-mediated gene flow in alfalfa: a three year summary of field research’ in *Proceedings of the 2003 Central Alfalfa Improvement Conference*, viewed 19 June, 2009, <http://www.foragegenetics.com/pdf/>.

Holm, SN 1966, ‘The utilization and management of bumble bees for red clover and alfalfa seed production’, *Annual Review of Entomology*, vol. 11, pp. 155-182.

Jenczewski, E, Prosperi, JM & Ronfort J 1999, ‘Evidence for gene flow between wild and cultivated *Medicago sativa* (Leguminosae) based on allozyme markers and quantitative traits’, *American Journal of Botany*, vol. 86, pp. 677-687.

Kehr, WR 1973, ‘Cross-fertilization of alfalfa as affected by genetic markers, planting methods, locations, and pollinator species’, *Crop Science*, vol. 13, pp. 296-298.

Kendrick, D, Pester, T, Horak, M, Rogan, G & Nickson, T 2005, ‘Biogeographic survey of feral alfalfa populations in the U.S. during 2001 and 2002 as a component of an ecological risk assessment of Roundup Ready Alfalfa®.p.124’, in *Proceedings of North Central Weed Science Society Meeting*, UT, USA.

Manitoba Community Profiles (MCP) 2007, ‘Municipal community Profiles’, viewed 19 June, 2009, <http://www.communityprofiles.mb.ca/csd/>.

Manitoba Management Plus Program (MMPP) 2008, ‘Variety and yield data, viewed 19 June 2009, <http://www.mmpp.com/mmpp.nsf/mmpp_publications.html>.

Mueller, S 2004, ‘Seed production issues for genetically enhanced alfalfa’ in *Proceedings of the national Alfalfa Symposium*, San Diego, CA, viewed 19 June, 2009, <http://alfalfa.ucdavis.edu/+symposium/proceedings/2004/04-369.pdf>.

Osborne, JL, Martin, AP, Carreck, NL, Swain, JL, Knight, ME, Goulson, D, Hale, RJ & Sanderson, RA 2008, ‘Bumblebee flight distances in relation to the forage landscape’, *Journal of Animal Ecology*, vol. 77, pp. 406-415.

Pascher, K, Narendja, F & Rau, D 2006, ‘Feral oilseed rape – investigations on its potential for hybridization’, *Final report in commission of the federal ministry of Health and Women (BMGF)*, Section IV, GZ: 70420/0116-IV/B/12/2004.

St. Amand, PC, Skenner, DZ & Peaden, RN 2000, 'Risk of alfalfa transgene dissemination and scale-dependent effects', *Theoretical and Applied Genetics,* vol. 101, pp. 107-114.

Statistics Canada 2006, 'Census of Agriculture, Farm Data and Farm Operator Data, catalogue no. 95-629-XWE', viewed 20 September, 2008 <http://www.statcan.ca/english/freepub/95-629XIE/2007000/crops.htm# hay>.

Steiner, JJ, Beuselinck, PR, Peaden, RN, Kojis, WP & Bingham, ET 1992, 'Pollinator effects on crossing and genetic shift in a three-flower-color alfalfa population', *Crop Science*, vol. 32, pp. 73-77.

Stephen, WP 1959, 'Maintaining alkali bees for alfalfa seed production', *Oregon Agricultural Experimental Station Bulletin*, no. 568, pp. 23.

Stewart, CN, Halfhill, MD & Warwick, SI 2003, 'Transgene introgression from genetically modified crops to their wild relatives', *Nature Reviews Genetics*, vol.4, pp. 806-817.

SWSS 1998, '*Weeds of the United States and Canada'*, Southern Weed Science Society Publications, Champaign, Illinois.

Teuber, LR, Van Deynze, A, Mueller, S, McCaslin, M, Fitzpatrick, S & Rogan, G 2004, 'Gene flow in alfalfa under honey bee (*Apis mellifera*) pollination', in *Proceedings of the Joint Conference of the 39th North American Alfalfa Improvement Conference and the 18th Trifolium Conference*, Quebec City, Quebec, Canada.

United States Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS) 2005, 'Monsanto Co. and forage genetics international; Availability determination of non regulated status for alfalfa genetically engineered for tolerance to the herbicide glyphosate', Docket number [04-085-3], *Federal Registrar*, vol. 70 pp. 36917-18, viewed 19 June, 2009 <http://www.aphis.usda.gov/brs/ fedregister/BRS_20050627a. pdf>.

United States District Court (USDC) 2007, 'Memorandum and order re: permanent injunction Report from the United States District Court for the Northern District of California', *Order No. C 06-01075 CRB*, pp. 1-15.

Van Acker, RC 2007, 'The potential for the coexistence of GM and non-GM crops in Canada' in RH Gulden & CJ Swanton (eds.), *'The first decade of herbicide-resistant crops in Canada'*, Topics in Canadian Weed Science, Volume 4, Canadian Weed Science Society, Sainte-Anne-de Bellevue, Québec.

Wong DK 2008, 'Alfalfa seed marketing in Canada', viewed 21 July, 2008 <http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/sis955>.