The Feral Nature of Alfalfa and Implications for The Co-Existence of Genetically Modified (GM) and Non-GM Alfalfa

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Glyphosate resistant (GR) alfalfa has been approved for unconfined release in Canada allowing for its commercialization. GR alfalfa is genetically modified (GM), therefore organic and conventional alfalfa growers in Canada have concerns regarding adventitious presence (AP) of GM alfalfa (or GM traits) in their crops and the agronomic issues and potential market loss associated with such GM AP. Co-existence strategies are proposed by some stake holders as the solution for these concerns however feral (persisting outside of cultivation) alfalfa populations occur in natural and semi-natural habitats and will act as barriers to co-existence. This report summarizes the potential role of feral populations in novel trait movement and the implications for the establishment of co-existence among GM and non-GM alfalfa production fields.

Understanding the nature of gene flow and contamination

Management of gene escape requires recognition of the various routes of gene escape from cultivated fields. Potential routes include: a) pollen escape through outcrossing including insect mediated pollination [pollen mediated gene flow (PMGF)]; b) seed escape during transport, harvesting and other farming activities; and c) seed escape via rodents, arthropods, birds and other seed predators. Pollen mediated gene escape is the major concern for hay producers while both pollen and seed mediated gene escape are concerns for seed producers.

Pollen mediated gene flow (PMGF) from alfalfa fields

Alfalfa is a cross-pollinated crop where pollination is facilitated by insects including leaf cutter, alkali, honey and bumble bees. In Canada, producers typically place leaf cutter bees in seed production fields to increase pollination and seed set, however wild populations of both leaf cutter and bumble bees are common. Honey bee mediated long distance dispersal of pollen from alfalfa seed and hay production fields has been confirmed for distances up to 1000m (St. Amand et al., 2000). The proportion of outcrossed seeds was about 22% for seed production fields and 15% for hay fields at 1 km from the pollen source. In a similar study, Teuber et al. (2004) found outcrossing levels of 1.5% at 270m and 0.2% at 1.5 km and were able to detect very low levels of outcrossing (<0.03%) at 4 km. Fitzpatrick et al. (2003) observed outcrossing levels of 1.4% at 152m and only 0.28% at 274m with no outcrossing at 610m under leaf cutter bee pollination. In the same study, a single outcrossing event was detected at 804m, although at a very low frequency. However, it is vital to note that the sizes of the pollen source fields employed in this particular study were only between 1 and 1.6 acres. However, in reality, the production fields are much larger and that practical levels of long distance gene flow could be much higher, as discovered by Watrud et al. (2004) in creeping bentgrass.

Feral alfalfa and PMGF

Feral alfalfa plants are commonly observed in road sides, field shoulders and other natural and semi-natural habitats in Canada. We have been conducting experiments since 2006 in selected rural municipalities in Southern Manitoba (Hanover, MacDonald and Springfield) designed to investigate the nature and dynamics of feral alfalfa populations and their role in long distance PMGF. A detailed road side survey was initially carried out to record the extent of occurrence of feral alfalfa populations in these municipalities. The survey revealed widespread occurrence of feral populations in alfalfa growing regions. The mean number of populations recorded per km respectively in Hanover, MacDonald and Springfield were 1.68, 1.32 and 0.21. On average, feral alfalfa populations were located within 87m (MacDonald), 210m (Hanover) and 328m (Springfield) of cultivated alfalfa fields, a distance sufficient to effect cross pollination in alfalfa. St. Amand et al. (2000) investigated GM trait movement among widely dispersed, individual feral plants on road sides and confirmed PMGF at a distance of 230m with an outcrossing frequency of 92%. In our study in southern Manitoba, greater levels of outcrossing were detected between feral and cultivated alfalfa populations. Estimated levels of outcrossing varied between 62% and 85% within a maximum distance of 15m. Feral alfalfa plants therefore can act as bridges for GM trait movement out of cultivated fields.

Unlike other GM crops, alfalfa is a perennial, highly outcrossing crop where pollination is facilitated by insects. It is a very hardy species that is highly adapted to resource poor environment such as road verges. The biology and ecology of alfalfa is favorable for their persistence and long-distance gene flow (Bagavathiannan and Van Acker, 2009). In Manitoba, some roadside alfalfa populations have been shown to be self-sustaining and therefore truly feral (Bagavathiannan and Van Acker, 2008; Figures 1-4). Road verge management including mowing and herbicide application has considerable influence on the dynamics of these populations. Mowing can substantially reduce seed output and plants that escape mowing in June continue can grow to produce a large mature seed crop. Applications of 2, 4-D herbicide kills alfalfa seedlings and established alfalfa plants, but dormant seeds in the soil seed bank may slowly re-establish the population, if the conditions are favorable.

Feral alfalfa and seed mediated gene escape

Seed escape from transgenic varieties followed by successful establishment outside of cultivation will directly result in the adventitious presence of transgenes in the environment. Farming activities serve as the main source of seed escape from cultivated fields. Additionally alfalfa seeds are rich in protein and serve as a nutritious food source for the seed predators. Seed predator mediated seed dispersal is possible both from alfalfa seed production fields and from feral populations.

The establishment, presence and persistence of feral alfalfa populations will have implications for the release of GM alfalfa as these populations will serve as reservoirs for novel traits and act as bridges for long-distance gene flow. As such, feral alfalfa is a barrier for the successful co-existence of GM and non-GM alfalfa. Strict adherence to purpose designed stewardship practices can help minimize the potential of GM trait escape into feral and non-GM alfalfa and increase the chances of successfully achieving coexistence between GM and non-GM alfalfa.

Stewardship approaches for reducing adventitious presence of GM traits in non-GM alfalfa

Minimizing pollen mediated gene flow

i) Seed production fields: Because feral alfalfa plants growing in road verges and other unmanaged areas will facilitate GM trait movement, management of these populations is necessary. In Canada, current isolation distance required for certified alfalfa seed production is 50 meters and for foundation seed it is 200 meters (for fields exceeding 5 acres) or 300 meters (for fields that are 5 acres or less) (CSGA, 2003). These isolation distances are designed to achieve variety purity (within limits) but not necessarily genetic purity (or the prevention of GM trait entry). As such, and given the evidence of long distance PMGF in alfalfa, the commercial production of GM alfalfa will require non-GM alfalfa growers to greatly increase these isolation distances if they want to assure GM-free seed sources. Frequent testing of seed sources will also be required to provide assurance of the effectiveness of isolation approaches.

ii) Hay production fields: Hay fields are required to be managed properly and cut regularly before flowering. However, bad weather conditions can delay haying operations, resulting in flowering within hay crops and opportunities for PMGF and GM trait escape. This may mean that producers who have neighbors growing GM alfalfa may also need to consider isolation distances in relation to their hay fields.

b) Minimizing seed mediated gene flow

Seeding, spraying and harvesting equipment must be cleaned prior to and after use in any GM alfalfa fields (preferably at the field's edge). Alfalfa seed (especially GM alfalfa seed) should be transported in spill proof containers to avoid seed escape and reduce the establishment of feral GM alfalfa populations in road verges. The effective control of feral alfalfa populations can also help to prevent predator mediated seed movement.

c) Sustained stewardship practices

Producers who wish to maintain crops GM-free will need to make conscientious efforts to do so and need an understanding of the routes and mechanisms of GM trait movement. Establishing region-wide stewardship practices will be necessary to reduce the potential for gene flow between GM and non-GM alfalfa (Van Acker et al., 2007). Co-operative efforts from GM growers would greatly facilitate the coexistence of GM and non-GM crops, with the neighbourly declaration of GM crop cultivation by GM crop growers being particularly helpful. Currently such cooperation is voluntary. In addition, all alfalfa growers (both GM and non-GM) should work to identify and control feral alfalfa populations both on their farm sites and along roadsides. Special collaborative programs with municipalities, including weed supervisors would be required in order to facilitate the management of these populations. Further, individual growers may want to monitor volunteer alfalfa plants from previous GM alfalfa fields to ensure that populations from one stand do not persist to flower in subsequent stands.

References

Bagavathiannan, M.V. and Van Acker, R.C. 2008. The ability of *Medicago sativa* (alfalfa) to establish feral populations in natural and semi-natural environments in Western Canada. In: Proceedings of the Fifth International Weed Science Society Congress, Vancouver, BC, Canada.

Bagavathiannan, M.V. and 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. Cri. Rev. Pl. Sci. 28: 69-87.

CSGA (Canadian Seed Growers' Association). 2003. Regulations and procedures for pedigreed seed production. Circular 6-94. Section 7. www.seedgrowers.ca/ regulations.

Fitzpatrick, S., Reisen, P. and McCaslin, M. 2003. Pollen-mediated gene flow in alfalfa: a three year summary of field research. In: Proceedings of 2003 Central Alfalfa Improvement Conference. Available at: http:www.naaic.org/meetings/central 2003.

St. Amand, P.C., Skenner, D.Z. and Peaden, R.N. 2000. Risk of alfalfa transgene dissemination and scale-dependent effects. Theor. Appl. Genet. 101: 107-114.

Teuber, L.R., Van Deynze, A., Mueller, S., McCaslin, M., Fitzpatrick, S. and 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.

Van Acker R.C, McLean, N. and Martin, R.C. 2007. Development of quality assurance protocols to prevent GM-contamination of organic crops. Pages 466-489. In: Handbook of Organic Food Safety and Quality, J. Cooper, U. Niggli and C. Leifert (Eds), CRC Press, Boca Raton, FLA, USA.

Watrud, L., Lee, E., Fairbrother, A., Burdick, C., Reichman, J.R., Bollman, M., Storm, M., King, G. and Van de Water, P.K. 2004. Evidence for landscape level pollen mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. PNAS 101: 14533-14538.

Fig.1. Feral alfalfa plants occurring at road verges

Fig.2. Reproductive success of feral alfalfa plants in roadside habitats

Fig.3. Alfalfa seeds in the soil seedbank (greenhouse grow outs)

Fig.4. Successful alfalfa seedling recruitment in roadside habitats