Issues Related to Release of GM Wheat: Gene Flow and Selection

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Abstract
Release of genetically modified (GM) wheat will require segregation of GM and non-GM wheat to satisfy international markets. Before GM wheat is released, it will be important to understand the fate of a GM trait within the agronomic production system. The objective of this study was to evaluate the effect of gene flow and selection pressure on the frequency of GM traits in non-GM wheat and wheat volunteers. Gene flow of GM traits to non-GM wheat will occur through pollen or seed movement. Gene flow is inevitable. When a GM trait does not confer a selective advantage in the production system, the frequency of the GM trait within non-GM wheat will be a function of the rate of gene flow. Low rates of gene flow will lead to low levels of GM contamination in the non-GM crop. With repeated gene flow events, the frequency of the GM trait will slowly increase in the non-GM crop. When the GM trait has a selective advantage, the frequency of the GM trait will increase rapidly in volunteer wheat populations. Herbicide tolerance is an example of a GM trait that provides a high selective advantage when the herbicide is applied in the production system. Predictive models show that even with very low rates of gene flow, frequent application of a highly effective herbicide will quickly increase the frequency of the herbicide tolerant GM trait in volunteer populations. This has negative implications for control of volunteers and the ability to maintain tolerance levels of GM traits in non-GM wheat crops.

Introduction
Development of genetically modified (GM) wheat through recombinant DNA technologies is a reality in a number of wheat breeding programs. The Canadian Food Inspection Agency (CFIA) has established an extensive protocol for testing and evaluation of GM crops through regulation of Plants with Novel Traits (PNT’s). Environmental safety assessments focus primarily on the impact of the new trait on weediness characteristics of the crop, the effect of gene-flow to wild relatives, the potential for the crop to become a pest, the potential impact of the trait on non-target organisms and biodiversity. Comparisons are made relative to a known non-GM counterpart. In most cases the environmental safety assessments focus on impacts outside of the agronomic production system. However, some GM traits can have significant impacts on crop production practices, pedigreed seed purity, ability to manage volunteers, and the ability to produce non-GM wheat crops.

Surveys conducted by the Canadian Wheat Board show that there is significant customer resistance to GM wheat (Canadian Wheat Board, 2001). As a result, initial release of GM wheat will require segregation of GM and non-GM wheat to satisfy different customer demands. Under these circumstances, it will be important to understand the fate of GM traits in wheat within the production system.

The objectives of this paper were to: 1) briefly review the pollination biology and out-crossing rates of wheat and 2) assess the potential effect of gene flow and selection on the frequency of GM traits in non-GM wheat and wheat volunteers using basic population genetic principles.

Gene flow in wheat
In plants, genes move between populations either through pollen or seed movement. Pollen movement in wheat is facilitated by wind and gravity. Anthers normally dehisce within the floret, followed by filament elongation and extrusion of the anthers outside of the floret. A small amount of pollen is shed on the stigma within the floret, while 80% of the pollen is shed outside of the floret. Florets that have not been successfully self-pollinated will remain open and be receptive to pollen from other sources for up to
13 days after anthesis (de Vries, 1971). Estimates of out-crossing rates in wheat are dependent on synchrony of flowering between males and females, the presence of receptive females and the availability of single dominant nuclear genes to facilitate detection of out-crossing. Seed movement may occur in a number of ways such as movement with farm or transport equipment, animals, or wind and water. Seed that has previously been contaminated with a GM trait through prior pollen or seed movement can contribute to the introduction of GM traits into fields that were not previously planted to a GM crop or adjacent to a GM crop. Frequency of seed movement is expected to by highly variable and difficult to predict. However, extrapolations from weed seed studies and crop mixtures may be helpful in establishing a range of values.

**Methods**

Potential gene-flow rates were estimated using published estimates of out-crossing rates in wheat. Hucl and Matus-Cadiz (2001) compared out-crossing rates among four wheat cultivars (Katepwa, Roblin, Oslo and Biggar) using a dominant blue aleurone trait in the pollen source to quantify out-crossing rates. Out-crossing rates varied considerably among the different cultivars (Figure 1). Katepwa showed the lowest level of out-crossing and Oslo showed the highest level of out-crossing. Out-crossing was reported up to 27 m from the pollen source. The pollen source plot size in this study was small (5m²) and sample sizes evaluated were low (less than 700 seeds/sample). Others studies have shown that out-crossing rates in wheat range fall within the range 0.1% to 10.1% (Griffin, 1987; Martin, 1990; Hucl, 1996 and Enjalbert et al., 1998).

![Figure 1. Mean out-crossing rates reported for Katepwa, Roblin and Oslo in a two year study conducted by Hucl and Matus-Cadiz (2001). The highest out-crossing rate (Oslo 95W) occurred for the cultivar Oslo in 1995 for samples collected west of the pollen source.](image)

For the purposes of this study out-crossing rates similar to Katepwa and Oslo were used to provide comparisons of high and low out-crossing rates. Although out-crossing has been reported as far as 48 m (Khan et al., 1973) and 80 m (Hucl, personal communication) from the pollen source, levels of out-crossing are highest in the first 10 m from the pollen source. Beyond 10 m out-crossing rates tend to be quite low but can occur at low levels for a considerable distance from the pollen source. Measuring exact
out-crossing rates beyond 10 m from the pollen source becomes difficult because the area from which samples must be drawn increases exponentially with distance from the pollen source. Therefore, to simplify modeling, out-crossing was assumed to occur within 10 m of the pollen source at either a level of 0.01% (similar to Katepwa in the Hucl and Matuz-Cádiz, 2001 study) or 3% (similar to Oslo in the Hucl and Matuz-Cádiz, 2001 study).

Basic population genetics models were used to evaluate the effect of gene flow either on its own or followed by the application of selection pressure (Hartl and Clark, 1989). Since most GM traits are inherited as single nuclear dominant genes, this form of inheritance was modeled. The general selection equation was modified to accommodate the primarily self-pollinating nature of wheat such that following the initial gene flow event an out-crossing rate of 1% within the resulting population was used. The selection pressure used in the general selection model was set at 95% to simulate a typical herbicide efficacy rate.

Results and Discussion

Even though gene-flow rates in wheat may be relatively low when compared to crops that are primarily cross-pollinating, the levels of gene flow are sufficiently high that it will not be possible to guarantee 0% GM trait in non-GM wheat. Statistically, it is neither practical nor possible to prove a tolerance level of 0% GM. Therefore, prior to release of GM wheat it will be important to have established standards for tolerance levels of GM traits in non-GM wheat. This will be important for both conventionally and organically produced crops. Once tolerance levels are established, sampling and testing procedures can be established to guarantee that non-GM wheat crops do not exceed the tolerance levels.

The vast acreage of wheat in western Canada suggests that some wheat fields will be grown adjacent to each other with very little distance separating them. Similarly, the minimum isolation distance for production of pedigreed Breeder and Select seed is 10 m and for Foundation, Registered and Certified seed is only 3 m (Anonymous, 1994). Based on the out-crossing rates and distances reported above, gene flow between GM and non-GM wheat will be of concern in a production system that requires segregation of non-GM wheat from GM wheat. In the short term there is little concern of gene flow of non-GM traits to GM wheat. As a result, the main focus will be on the fate of GM traits in non-GM wheat crops and volunteers.

Fate of single gene flow events

When a field of GM wheat is grown adjacent to a non-GM wheat field some out-crossing may occur. The level of out-crossing will depend on the synchrony of flowering between the two fields, the level of male sterility in the non-GM wheat (i.e. degree to which receptive females are available), the non-GM cultivar, distance between the crops, and wind direction. The frequency of the GM trait in the harvested seed from the non-GM crop will be influenced by the rate of out-crossing experienced and size of the field being harvested. Since the highest level of out-crossing will occur on the field edge closest to the GM crop, it is expected that the frequency of the GM trait will be highest on the field edge of the non-GM crop and will diminish with distance from the GM crop. As the non-GM field is harvested, it is expected that the GM trait will be mixed with and diluted with the non-GM wheat from the remainder of the field. Depending on how the field is harvested the frequency of the GM trait may vary significantly from sample to sample with the highest frequency occurring in samples harvested from the areas closest to the GM wheat crop. If the harvested grain is used for seed, the GM trait may be introduced into a field that has never been near a GM wheat crop. Similarly, wheat volunteers that remain after harvest will contain the GM trait at a frequency equivalent to the out-crossing rate. The highest frequency of the GM trait in volunteers will occur in the field in areas close to the GM crop. Under situations that do not provide a selective advantage or disadvantage to the GM trait, the frequency of the trait will remain constant within the population. If volunteer population sizes are very low, the frequency of the GM trait may increase or decrease due to random genetic drift.
Effect of repeated gene flow events

When a non-GM wheat crop is grown adjacent to a GM wheat crop over multiple generations, the frequency of the GM trait is expected to increase in the non-GM wheat crop. If no other forces are acting on the population, the rate of increase will be directly related to the level of gene flow between the two crops (Table 1). If tolerances for GM trait in non-GM wheat are as low as 0.25%, it will be difficult to maintain this standard if out-crossing rates are relatively high (between 1% and 0.1%) but it will be fairly easy to maintain under lower levels of out-crossing. The results from Table 1 stress the importance of ensuring that seed supply is produced under conditions that limit the potential for gene flow between GM and non-GM wheat. Producers that use “farm-saved” seed may need to rethink this strategy if there is a potential for gene flow from a GM wheat crop into their non-GM wheat crop. Alternatively, they may wish to examine their harvesting and seed handling procedures to limit the potential for introduction of GM wheat into their non-GM wheat seed crop.

Table 1. The effect of repeated generations of out-crossing from GM wheat to non-GM wheat on the frequency of the GM trait in a non-GM wheat population.

<table>
<thead>
<tr>
<th>Generation</th>
<th>% Out-crossing</th>
<th>1.0</th>
<th>0.1</th>
<th>0.01</th>
<th>0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.000</td>
<td>0.100</td>
<td>0.010</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.990</td>
<td>0.199</td>
<td>0.020</td>
<td>0.002</td>
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</tr>
<tr>
<td>3</td>
<td>2.970</td>
<td>0.299</td>
<td>0.030</td>
<td>0.003</td>
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</tr>
<tr>
<td>4</td>
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<td>0.399</td>
<td>0.040</td>
<td>0.004</td>
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</tr>
<tr>
<td>5</td>
<td>4.901</td>
<td>0.499</td>
<td>0.050</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>

Generations to exceed 0.25% GM trait in non-GM wheat

Effect of selection pressure

The fate of GM traits within production systems will depend on the selective advantage or disadvantage conferred by that trait within the production system. Many GM traits will not confer a significant selective advantage either within or outside of the production system. For these traits, their frequency in the non-GM wheat population will be maintained at fairly low levels related to the rate of gene flow. However, GM traits that confer a selective advantage either within the production system or outside of the production system will increase in the population with each generation in which the selection pressure is present. Herbicide tolerance traits are an example of the type of trait that confers a selective advantage within the production system. The more effective the herbicide and the more frequently it is applied, the more rapid the increase in the frequency of the GM trait in the population.

Figure 2 illustrates the effect of selection pressure on the frequency of a GM trait in a non-GM wheat volunteer population. In this situation the GM trait is assumed to be a herbicide resistance trait. To simulate a field situation, gene flow rates similar to those reported by Huel and Matus-Cadiz (2001) for Oslo (Figure 2a) or Katepwa (Figure 2b) were used as the upper limit. The remaining gene flow rates simulate a situation in which gene flow occurs in the first 10 m of the field, but then a larger field area is harvested that dilutes the frequency of the GM trait by 10, 50 or 100 times. In these scenarios, it is assumed that gene flow has occurred only in the initial generation. Figure 2a indicates that 50% of the volunteer wheat population will be resistant to the herbicide after only 2 to 4 generations of treatment. Even at low initial gene flow rates, 50% of the volunteer population will be resistant after only 4 to 6 generations of herbicide treatment. Therefore, even with relatively low gene flow rates, the frequency of
traits that have a high selective advantage in the production system will increase rapidly with the application of the selective agent. For herbicide resistance traits, this will have a significant impact on volunteer management, crop rotation, herbicide management, and ability to maintain minimum tolerance levels of GM traits in non-GM crops.

Figure 2. Frequency of herbicide resistant GM volunteers following application of the herbicide. The selection pressure of the herbicide is 95%. a) Initial gene flow rates range from 0.003 to 3% to simulate a cultivar such as Oslo that has a high out-crossing rate. b) Initial gene flow rates range from 0.00001 to 0.01% to simulate a cultivar such as Katepwa that has a low out-crossing rate.

With the exception of Canada Prairie Spring wheat, current standards for pedigreed wheat seed production allow a maximum of 1 in 10,000 off-types in Breeder and Select seed and 5 in 10,000 in Foundation, Registered, and Certified seed (Anonymous, 1994). Therefore Certified seed could have a frequency of GM traits equivalent to a gene flow rate of 0.05% and still meet pedigreed seed standards. If the GM trait is resistance to a herbicide that is used frequently within the production system, it is possible
that producers could rapidly increase the frequency of GM traits within their volunteer populations through their normal production practices. The rate of increase of the GM trait would be similar to the gene flow rate of 0.06% shown in figure 2a.

**Ability to maintain minimum levels of GM traits in non-GM crops**

The potential for selection to increase the frequency of GM traits in volunteer populations must be considered when trying to meet standards for non-GM crops. Figure 3 indicates that when the frequency of a GM trait within a volunteer wheat population is relatively high, even a small number of volunteers could make it difficult to meet non-GM standards. For example, if the frequency of a GM trait in a volunteer population is 50% and a non-GM wheat is sown into a field at a standard seeding rate of 250 seeds/m², as few as 6 wheat volunteers/m² would lead to a GM contamination rate that exceeds 1%. Similarly, 16 and 27 volunteers/m² would lead to contamination rates that exceed 3% and 5%, respectively. Marginet (2001) reported that pre-treatment wheat volunteer densities ranged from 1-171/ m², and most frequently ranged between 20-40/ m². Therefore, the typical density of wheat volunteers could cause concern even if the frequency of the GM trait in the volunteer population was as low as 20-30%. In addition, wheat volunteers continue to persist after control measures have been applied. In the most recent post-control survey of Manitoba, volunteer wheat was found in 15.8% of fields at an average occurrence density of 2.1 plants/m² (Leeson et al., 2002)

Since pre-seed spring “burn-off” herbicides may be applied immediately before seeding and possibly before emergence of all volunteers, producers may not be aware that there may be a high frequency of resistant GM volunteers in their fields. If the problem is not noted prior to marketing of the grain, this could cause economic losses to the producer as well as grain buyers. GM wheat volunteers that are not controlled prior to seeding, or that cannot be controlled in crop, could also cause problems for producers of other non-GM crops such as barley or oat. Since wheat seed is similar in size to barley or oat, GM wheat could not be easily removed from these crops.

![Diagram](image)

**Figure 3.** Number of volunteers that would lead to 1%, 3%, or 5% GM contamination in non-GM wheat crops sown at 250 seeds/m² relative to the frequency of resistant GM plants in the volunteer wheat population.

**Conclusions**

Once GM wheat is released for commercial production, there will be the potential for gene flow of GM traits from GM wheat to non-GM wheat crops. This can occur through either seed movement or pollen movement. Customer resistance to GM traits will require segregation of GM and non-GM wheat in order
to ensure ongoing marketability of the Canadian wheat crop. Tolerance limits for GM traits in non-GM wheat will have to be set to meet customer demands. Since tolerance limits will vary among different customers, it will be important to set tolerance limits that will satisfy the majority of customers. However, it will not be possible to maintain tolerance levels of 0% GM trait.

The need to segregate GM and non-GM wheat, at least for the short-term, will require a clear understanding of the fate of the GM trait within the production system. GM traits that do not confer a selective advantage within the production system may increase slowly within seed populations of wheat, but may not present significant problems for segregation of GM and non-GM crops unless tolerance levels are very low. GM traits that confer a selective advantage within the production system are expected to increase in frequency within volunteer wheat populations. The highest rate of increase will occur for GM traits that confer a high selective advantage to a selective agent that is applied frequently within the production system. Resistance to glyphosate is an example of such a trait. As GM traits are released, it will be important to review standards for pedigreed seed production to ensure that problems are not generated for those who choose to grow non-GM crops. Crop production practices, herbicide management strategies, and environmental impacts of these practices will also need to be reviewed.

References


