

# Pollen-mediated gene flow in wheat: How low is too high and what can $R^*$ say about it?

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## Abstract

*Field experiments and empirical modeling conducted between 2005 and 2006 found varying rates of pollen-mediated gene flow (PMGF) between volunteer wheat and spring wheat crops that were dependent on density, emergence timing, and height. Although maximum observed PMGF was always less than 0.6%, the question remains, 'Is this low enough?' Mechanistic modeling demonstrated that these seemingly low rates could indeed be large enough to become problematic. Given the looming introduction of second-generation abiotic stress traits, we propose that future models coupling resource-ratio theory ( $R^*$ ) with population genetics are needed and could prove useful in predicting the spread of second-generation transgenes.*

## Introduction

Transgenic crops have been widely adopted over the past decade, and their cultivation continues to grow at a sustained pace (James, 2007). The acreage devoted to transgenic crops increased 12% between 2006 and 2007, with an estimated 114.3 million ha currently sown globally (James, 2007). However, there are ecological and agricultural concerns associated with the cultivation of transgenic crops. Of particular importance with regard to risk assessment is the migration of transgenes to the same species (intraspecific gene flow) including neighbouring non-transgenic crops and volunteer crop plants (Ellstrand, 2003; Willenborg and Van Acker, 2008).

The pressing need to feed a rapidly increasing global population necessitates wheat varieties with enhanced yield, pest resistance, and tolerance to an array of environmental stresses. It is very likely that these varieties will be produced by biotechnology. The development of wheat genotypes with novel traits has raised concerns regarding the presence of volunteer wheat populations and the role they may play in facilitating transgene movement (Brûlé-Babel et al., 2006). Because some markets may require segregation between GE and non-GE varieties, legitimate concern exists regarding the possible contamination of non-GE wheat crops by volunteers, either through seed contamination or PMGF. Volunteer wheat is the 12<sup>th</sup> most abundant weed in western Canada, persisting on at least 11% of fields an average density of 6 plants m<sup>-2</sup>, but infestations as high as 281 plants m<sup>-2</sup> have been reported (Leeson et al., 2005).

Because of the predominantly inbreeding nature of wheat species (Poehlman and sleeper, 1995; Waines and Hegde, 2003), genetic purity should be relatively easy to maintain in theory. In practice, however, intraspecific PMGF in wheat can be as high as 10% (Lawrie et al., 2006), and PMGF has been identified as far as 2.8 km from the pollen source (Matus-Cádiz et al., 2007). Furthermore, off-types are frequently identified in pedigreed seed production in Canada (Hucl et al., 2004). A study in the United States found that approximately one third of the growers whose seed was sampled contained imazamox-resistant (IR) wheat varieties that were adventitiously present (AP) in non-IR wheat samples, with four samples actually exceeding the 0.01% threshold for off-types in certified wheat seed (Gaines et al., 2007). Thus, volunteer wheat populations represent a significant challenge to meeting and maintaining genetic purity and AP thresholds in non-GE wheat crops.

Given that transgenic wheats are currently being field tested (Finkel, 2008), the issue of PMGF in wheat is receiving renewed attention. A number of recent Canadian studies have investigated natural PMGF in wheat. Hucl (1996) measured outcrossing among 10 Canadian spring wheat cultivars and found outcrossing to vary between 0 and 6.7%, with a mean of 0.88%. A more extensive study, which measured outcrossing from a 0.25 ha pollen source, found the frequency of PMGF declined from 0.44% 1 m from the source to negligible levels beyond 60 m (Matus-Cádiz et al., 2004). However, PMGF was confirmed at 300 m from the source by Matus-Cádiz et al. (2004), and a subsequent study has detected PMGF as far as 2.75 km from a 33 ha pollen source (Matus-Cádiz et al., 2007).

Although the above studies demonstrate a relatively low propensity for PMGF in wheat, none of the aforementioned studies have investigated the level of PMGF

between volunteer wheat populations and wheat crops. Spatial isolation is not possible where volunteers arise within wheat crops and therefore, other mitigation measures will have to be employed to minimise gene flow between these conspecifics in areas where they coexist. In this paper, we report the results of two field experiments designed to quantify PMGF between volunteer wheat populations and spring wheat crops. We then use population genetics models to simulate a worst-case scenario regarding the fate of transgenes in a spring wheat crop. Finally, we discuss the limitations of population genetics and propose integrating resource-ratio theory with population genetics for predicting the spread of second-generation GE crops with abiotic stress-tolerance traits.

## Methods

Two field experiments were conducted at two sites in Manitoba (Carman and Winnipeg), Canada, in 2005 and 2006 (Willenborg et al., 2009; 200X). The first experiment quantified PMGF between volunteer and cropped spring wheat as a function of volunteer density and emergence timing (temporal isolation) while the second quantified PMGF as a function of crop density and height (when both populations emerged at the same time). Both experiments utilised a paired pollinator row methodology (alternating rows of pollen source and receptor plants) in a split-plot design. Main plots in the first experiment were volunteer wheat densities ( $10 - 80 \text{ plants m}^{-2}$ ) with sub-plots composed volunteer plants emerging at various timings (growing degree days, GDD) relative to the spring wheat crop (50 GDD before to 50 GDD after crop emergence  $T_{\text{base}} 0^{\circ}\text{C}$ ). In the second experiment main plots were comprised of crop densities ( $75 - 600 \text{ plants m}^{-2}$ ) and sub-plots were crop cultivars of different heights (two tall, two semi-dwarf). The herbicide-resistant (HR) cultivar used to establish the volunteer population (pollen donor) in both studies was cv. CDC Imagine, a cultivar that contains a single, semi-dominant nuclear gene (*Imi1*) coding for imazamox-resistance located on the long arm of chromosome 6D (Anderson et al., 2004; Pozniak et al., 2004). Previous research has demonstrated that the IR trait provides an ideal phenotypic marker for gene flow studies in wheat (Willenborg et al., 2008).

Pollen-mediated gene flow was analysed by testing for imazamox herbicide resistance in the crop seedling progeny from each field trial plot (Willenborg et al., 200X). Approximately 2000 seedlings per plot (harvested the year prior) from each grain sample (one per plot), along with control plots for each of the resistant and susceptible genotypes were screened for imazamox resistance. A foliar application of imazamox was applied at a rate of  $0.020 \text{ kg a.i. ha}^{-1}$  to wheat seedlings at the 2- to 3-leaf stage and all surviving wheat seedlings were counted 16 d after herbicide application based on surviving plants exhibiting an injured phenotype, which included coleoptilar tillering, minor chlorosis, deep greening of developed leaves, and stunting (Pozniak et al., 2004). All surviving plants (where logistically possible) were then grown to maturity, hand-harvested, and transported back to the greenhouse for a second confirmatory screening. Plants were grown in trays, placed in a growth room and sprayed at the 2- to 3-leaf stage with imazamox at  $0.012 \text{ kg a.i.}$  Hybridisation was confirmed by classifying segregation among  $F_{1:2}$  seedlings as segregating (3 alive: 1 dead ratio) or nonsegregating (either all alive or all dead) for the single gene, semi-dominant imazamox-resistant trait (Pozniak and Hucl, 2004). Nonlinear regression analysis was used to fit predictive equations to PMGF data in both experiments.

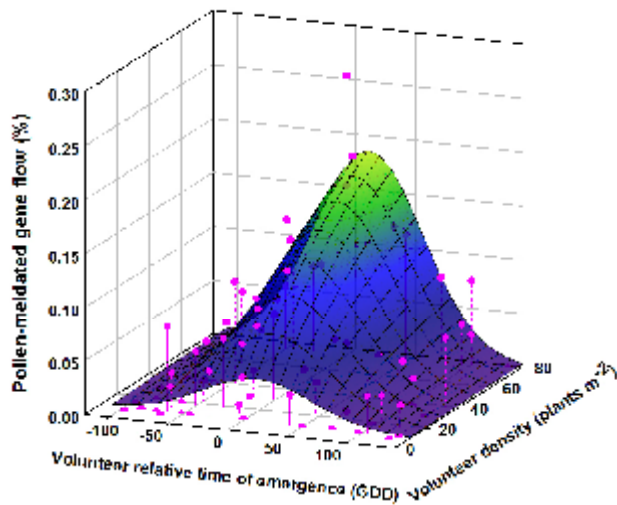
Mechanistic modeling was then used to model the effects of continued temporal isolation and various plant population densities over time as potential management strategies to minimise PMGF between populations of volunteer and cropped wheat. To do this, the pollen migration model developed in Brûlé-Babel et al. (2006) was employed to examine the effect of repeated generations of gene flow via pollen only at various rates from a migrant GE volunteer wheat population on the frequency of the GE trait in a non-GE wheat resident population. Pollen migration was modeled assuming unidirectional migration to the population under consideration (non-GE wheat crop) from the source population (GE crop), constant pollen migration rates and genetic composition over time in the source population, and continued planting of seed from the non-GE population (eg. farm-saved seed). This represents a worst-case scenario whereby a non-GE grower continually sows a wheat crop on the same land and is oblivious to the presence of the GE trait in the wheat populations. The rates of PMGF used in model simulations were based on those obtained empirically in the field studies for specific treatments. It was further assumed that resistance in this model is inherited in a completely dominant manner; the model cannot account for the effects of selection, mutation, and genetic drift nor can it account for rare, stochastic events.

## Results

Summed across both experiments, nearly 939,000 wheat seedlings were screened for hybridization across all locations and years. Segregation ratios of suspected hybrids consistently fit the expected 3:1 phenotypic ratio for a single, semi-dominant gene in the F<sub>1:2</sub> generation at both sites in 2005 and 2006, confirming hybridisation had occurred. The maximum PMGF frequency in samples where IR hybrids were identified was 0.55%.

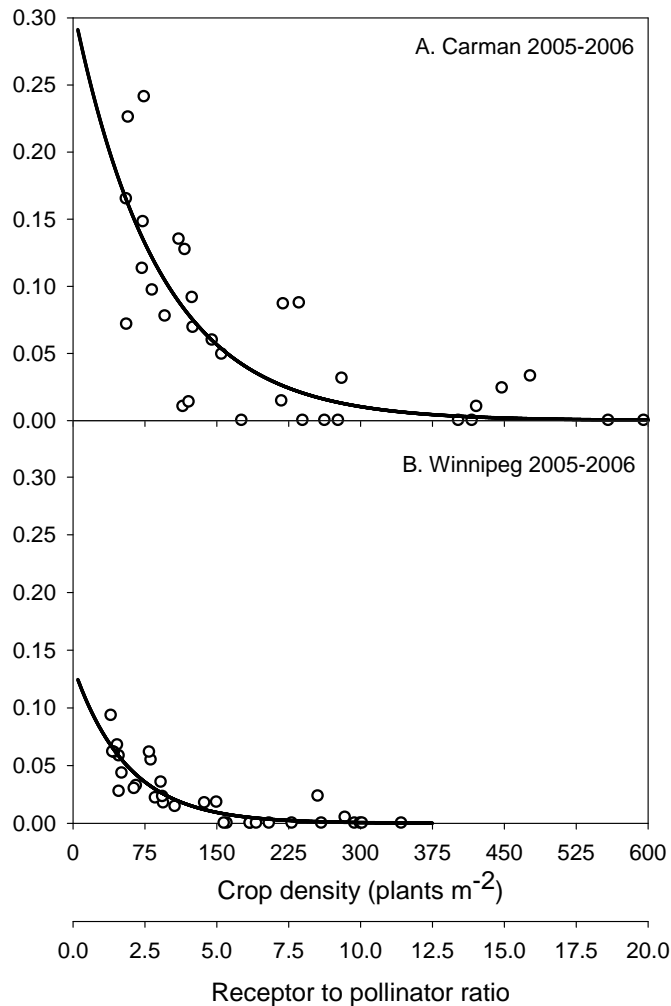
We observed relatively low frequencies of PMGF regardless of volunteer wheat emergence timing or density (Figure 1). The maximum estimated PMGF predicted by nonlinear regression was just 0.396% and occurred as a well-defined peak when volunteers emerged just after the crop (11 GDD). As the emergence timing of volunteers became less synchronous with the crop, PMGF frequencies declined dramatically such that the model predicted very low frequencies of PMGF where volunteers emerged greater than 50 GDD before or 75 GDD after the spring wheat crop (Figure 1). Based on the observed data, nonlinear regression identified a hybridisation window of 125 GDD in the spring wheat crop, as a function of volunteer emergence timing. Indeed, nearly 80% of samples with confirmed hybrids occurred within this interval.

The frequency of PMGF decreased hyperbolically with decreasing volunteer wheat plant population density which, by holding the density of the crop constant, served to decrease the pollinator to source plant ratio (Figure 1). The predicted frequency of PMGF was always below 0.1% when volunteer wheat plant population density was below 40 plants m<sup>-2</sup>. By contrast, PMGF frequencies increased rapidly at densities above 40 plants m<sup>-2</sup> (a recipient to pollinator ratio of 6:1) to a maximum observed frequency of 0.26%. Little gene flow was both observed and predicted at volunteer wheat densities of 10 plants m<sup>-2</sup> or lower, especially outside of the hybridisation window.



**Figure 1.** Pollen-mediated gene flow in spring wheat predicted as a function of volunteer wheat density and time of emergence relative to the wheat crop (temporal isolation). Predicted values are represented by lines on the response surface and are the result of fitting a nonlinear regression model to data combined across years (2005-2006) and locations (Carman, Winnipeg).

Similarly, spring wheat crop plant population density had a profound influence on PMGF (Figure 2). Maximum predicted gene flow at Carman, MB, was 0.31% at very low plant population densities and declined exponentially to a low of 0.0003% at the highest density (600 plants  $m^{-2}$ ). Although maximum estimated gene flow at Winnipeg, MB, was only 0.14%, gene flow at this site also declined exponentially with increasing plant population density to a low of 0.0002% at 375 plants  $m^{-2}$ . Genotype and plant height of volunteer plants and the spring wheat crop had no effect on PMGF rates at either location. Nevertheless, higher frequencies of PMGF were observed when the receptor to donor plant ratio is low, with as much as a 400% reduction in gene flow as this ratio increases ten-fold from 2:1 to 20:1. Consequently, the importance of achieving a minimum plant population density of 175 to 200 wheat plants  $m^{-2}$  can be seen in Figure 2 since PMGF increases exponentially with each per plant decrease in population density of the pollen receptor below these densities.



**Figure 2.** Mean percent gene flow (and estimated regression curves) from imazamox-resistant spring wheat to four spring wheat genotypes as a function of crop density near (A) Carman and (B) Winnipeg, MB, in 2005-2006 (Willenborg et al., 200X).

Mechanistic (migration) modeling revealed that both temporal isolation and increased plant population densities provided excellent mitigation of gene flow in future generations (Figure 3). By ensuring that the emergence timing of volunteer populations and spring wheat crops is asynchronous (by at least 50 GDD), it would take 35 years of continuous wheat to reach the current EU threshold of 0.9% for labeling GE content. Similarly, ensuring that spring wheat crop plant population densities of at least 200 plants m<sup>-2</sup> are consistently achieved greatly reduces the frequency of GE plants in the cropped wheat population; in fact, at densities typical of western Canadian fields (300 plants m<sup>-2</sup>), it would take more than 50 years of continuous wheat to exceed 0.9% admixture (Figure 3). Given that these simulations are based on the unlikely worst-case scenario of a continuous wheat system, both temporal isolation and plant population density appear to represent excellent tools for mitigating PMGF over the long-term. Any volunteer plants growing within the crop will, however, also contribute to seed mediated gene flow (SMGF), which would greatly increase admixture. Moreover, caution must be exercised in interpreting these simulation results because they are based on a static density of the pollen source population (volunteer). This is problematic because population genetics provides a great tool for generating inferences within populations, but is less than

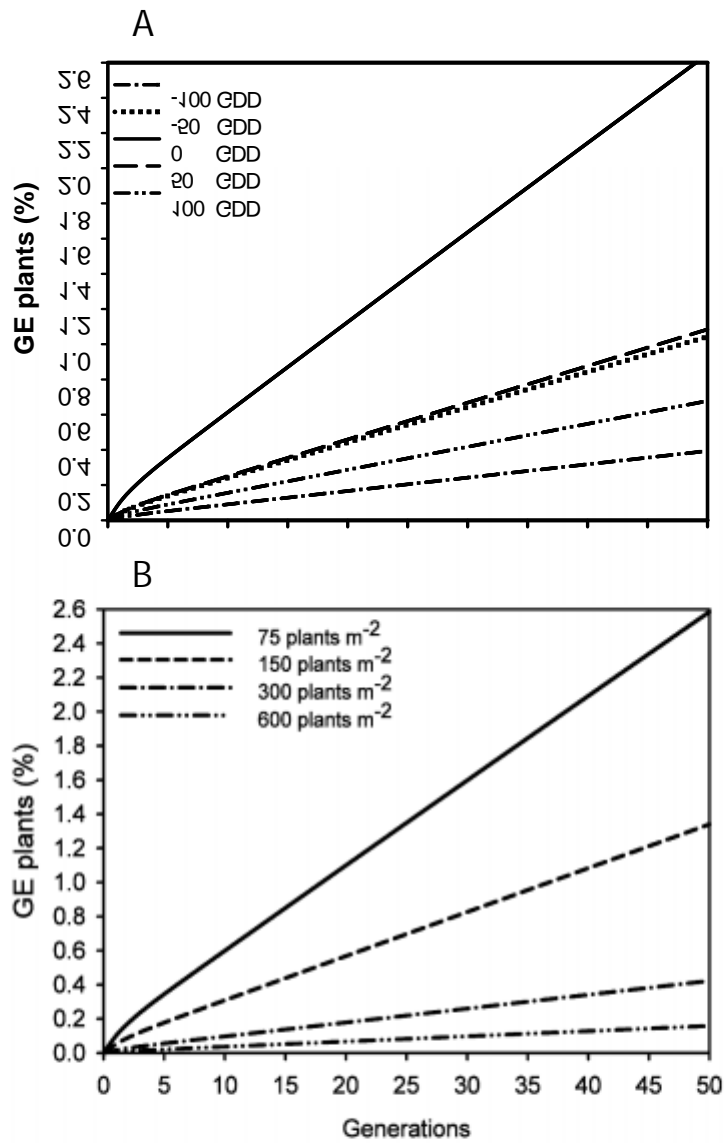
effective at the community level. Incidentally, it is at the level of the plant community where second-generation GE crops with abiotic stress tolerance could have significant impacts.

$R^*$  represents the environmental resource concentration required for the growth rate of a species to exactly balance its sources of loss (Tilman, 1997). By definition, a species will only be able to survive in a habitat if that habitat possesses a resource concentration of at least  $R^*$ . Resource concentrations lower than  $R^*$  result in growth rates which are lower than losses and thus, the species may go extinct locally (Tilman, 1997).  $R^*$  may be important to the study of crops with abiotic stress-tolerance traits because these species could possess traits that enhance their tolerance to drought and salinity, for example, as well as the potential for better use of nutrients such as nitrogen. Because resource-ratio theory predicts that the species with the lowest  $R^*$  should displace all other species from the habitat (Tilman, 1976, 1977), plants with abiotic stress-tolerance traits may be at a competitive advantage should a given trait enhance resource-use efficiency and hence, their ability to potentially withstand lower resource concentrations in a habitat. In effect, these plants would be more competitive with neighbouring species and 'win', as shown in Figure 4. The potential therefore exists for these types of traits to shift community dynamics and ultimately, community structure. To our example with volunteer wheat, this would represent an increase in the density of the volunteer wheat population as more and more plants acquire the stress-tolerance trait, which would then alter the pollen source to receptor plant ratio. This would create a density-dependent feedback loop that would modify any predictions made by the migration model. Although wheat may be of little concern with regard to its invasive potential, acquisition of such traits could, nevertheless, alter the structure and composition of the weed community. More concerning would be the transfer of an abiotic stress-tolerance trait to a wild relative outside of the agroecosystem, where resources and in particular, nitrogen, frequently limit plant growth and reproduction (Tilman, 1997). In either case, there could be much to gain in our ability to predict the movement of second-generation transgenes by developing models that integrate resource-ratio theory with population genetics. We propose that this approach be given consideration as we move towards the commercialisation of plants with abiotic stress-tolerance traits.

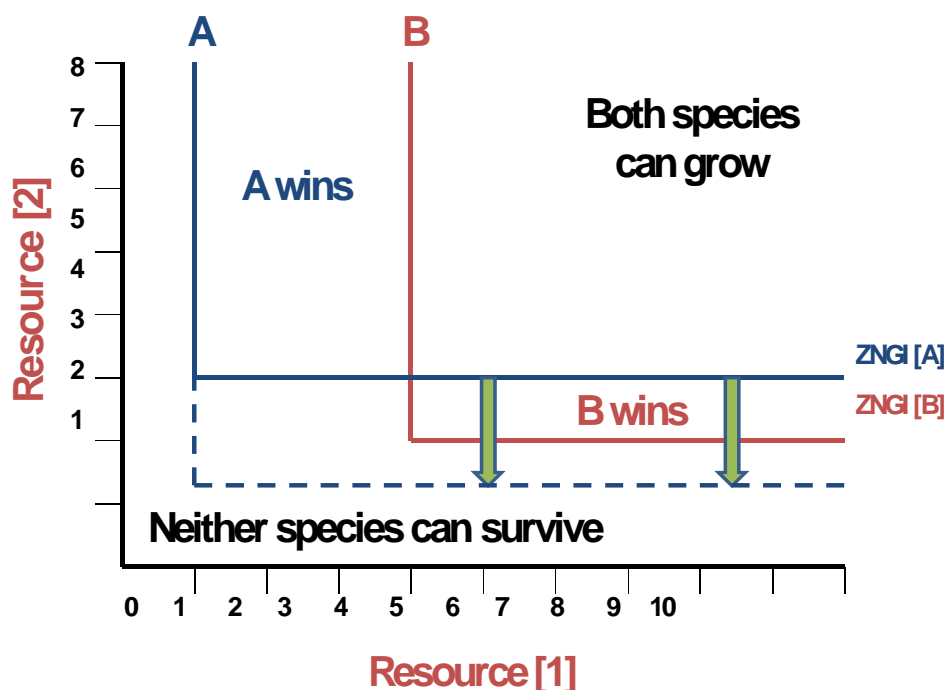
## **Conclusions**

Pollen-mediated gene flow between volunteer populations and spring wheat crops was low and did not exceed the 0.9% EU threshold for labeling. Both temporal isolation (isolation in time) and increased crop sowing densities dramatically reduced rates of PMGF between volunteer and cropped wheat populations. The results of the field studies suggest that ensuring volunteers emerge greater than 50 GDD on either side of the crop could be a viable method to reduce PMGF between conspecific wheat populations, provided an adequate plant stand has been achieved in the spring wheat crop. A migration model confirmed the viability of this strategy over the long term under a continuous wheat system in which a portion of the harvested seed is used to plant the crop in the next year.

Rates of PMGF where poor temporal isolation or low wheat crop plant stands were achieved were concerning with respect to the observed magnitude of PMGF



**Figure 3.** The accumulation of a GE trait in a non-GE wheat crop resulting from repeated generations of migration at rates which vary as a function of (A) emergence timing of volunteers relative to the crop and (B) wheat crop plant population density. Rates of PMGF for each treatment were determined empirically based on field studies carried out at Carman and Winnipeg, MB, Canada.



**Figure 4.** Theoretical predictions of resource-ratio ( $R^*$ ) theory applied to second-generation transgenes with abiotic-stress tolerance. If Resource 2 is nitrogen and a trait for enhanced tolerance to nitrogen stress is introduced into a population, it could lower its  $R^*$  and thus, zero net growth isocline (ZNGI). By this mechanism, a species endowed with abiotic stress-tolerance may displace resident species if nitrogen is one of the limiting factors in the habitat. Such dynamics may alter community structure and composition, thereby limiting the utility of population genetics in forecasting the fate of transgenes.

(upwards of 0.55%), the predictions of the migration model, and the potential for rapid increase of the trait in the population given adequate selection pressure (Brûlé-Babel et al., 2006). This model did not, however, account for seed-mediated gene flow, which would be expected to greatly increase admixture. Caution also must be exercised in interpreting these results given that migration and selection models are based on the principles of population genetics and thus, make population-level predictions. Second generation GE crops that contain abiotic stress-tolerance traits may elicit community-level responses via altered competitive dynamics among weed communities. Thus, even the relatively low PMGF rates that we observed in wheat could have an important influence on population and community composition, structure, and the long term fate of a transgene. Future models coupling resource-ratio theory ( $R^*$ ) with population genetics could prove useful in predicting the spread of second-generation transgenes.

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