# Pollen-Mediated Gene Flow in Wheat Fields in Western Canada

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Crop intraspecific pollen-mediated gene flow (PMGF) can be scale-dependent. A study was conducted in western Canada to examine PMGF in commercial spring wheat (*Triticum aestivum* L.) fields. At each of two sites located in the semiarid Grassland region of Saskatchewan, PMGF was measured from a 16-ha (400 × 400 m) field of imidazolinone (IMI)-resistant wheat (cultivar CDC Imagine) to an adjacent field of conventional (non-IMI-resistant) wheat (cultivar AC Barrie) with the same dimensions. Wheat grain samples of AC Barrie were collected at varying distances up to 400 m along five equally-spaced transects oriented perpendicular to the common border with CDC Imagine. A total of 2,000 seedlings per sample were screened for resistance to imazamox at 200 µM in a 7-d soil-less bioassay. Putative-resistant hybrids were confirmed by two allele-specific analyses: cleaved amplified polymorphic sequence (CAPS) marker detection of the IMI-resistance allele, TaAhasL-D1, and polymerase chain reaction detection of heterozygotes. Averaged across transects and sites, PMGF was 0.20% at the common border and declined exponentially with increasing distance; the maximum distance that PMGF was detected was 80 m from the donor field. Within 5 m of the common border, hybridization frequency averaged 0.14%. Averaged across the entire field, hybridization frequency averaged 0.006%; when grain from a 5-m harvested strip adjacent to the donor field was excluded from the harvest-blended seedlot, hybridization frequency was reduced by 33% to 0.004%. The largest contributor to adventitious presence in wheat is likely not PMGF, but seed-mediated gene flow. Frequency and distance of PMGF can guide grower stewardship practices for identity preservation in wheat cultivars possessing different traits.

***Key words:*** adventitious presence, gene flow, GM crops, identity preservation, outcrossing, plants with novel traits, pollen flow, threshold, *Triticum aestivum*.

## Introduction

Numerous biological, environmental, and crop-management factors and their interactions influence the frequency and distance of pollen-mediated gene flow (PMGF) between donor and receptor fields, including type of vector (wind and/or insect), genotype or cultivar, fertility (e.g., male-fertile or -sterile receptor plants), pollen viability and longevity, synchrony of flowering or pollen production, wind speed and direction, air turbulence/convective air currents, temperature, humidity, and area and plant density of donor and receptor populations (Beckie & Hall, 2008). At a landscape or regional scale, additional factors influencing PMGF are topography; vegetation; distribution and abundance of volunteer and feral populations; and number, shape, and spatial arrangement of pollen donor and receptor fields.

The frequency of PMGF generally declines rapidly with increasing distance from the donor field, often described by a leptokurtic curve. The length and shape of the asymptote tail depends on the species, environment, and limits of transgene or phenotypic detection, and can be the result of wind currents and/or insects. Wheat, *Triticum aestivum* L., is highly selfing (< 1-2% outcrossing), therefore the frequency and distance of PMGF are markedly less than that of canola (*Brassica napus* L.) or corn (*Zea mays* L.). However, long-distance (hundreds of meters) PMGF is possible (Matus-Cádiz, Hucl, Horak, & Blomquist, 2004). In a study in western Canada, PMGF was measured from a 20- to 33-ha spring wheat donor field to numerous receptor fields within a 10-km radius (closest distance of receptor field from edge of donor field was 0.15 km); PMGF was detected at distances as great as 2.75 km, though at a very low level of 0.01% (Matus-Cádiz, Hucl, & Dupuis, 2007). In general, levels of outcrossing in wheat vary widely among studies (Hanson, Mallory-Smith, Shafii,

Thill, & Zemetra, 2005; Willenborg & Van Acker, 2008), similar to that of other crops.

Because of the complex interactions among biological, environmental, and agronomic factors, only empirical models have been employed to predict PMGF in this crop (Gustafson et al., 2005; Hanson et al., 2005; Loureiro, Escorial, González-Andujar, García-Baudin, & Chueca, 2007; Willenborg, Brûlé-Babel, & Van Acker, 2009). These authors all used a negative exponential function to model PMGF; in those studies, the pollen donor size was small (< 1 ha), and outcrossing was usually measured only at short distances (Gustafson et al., 2006; Willenborg & Van Acker, 2006). At most, a 7-m isolation distance was recommended to achieve outcrossing levels at the closest field edge below the European Union adventitious presence (AP) threshold of 0.9% (Loureiro et al., 2007). However, it is clear from reviewing wheat PMGF studies that no isolation distance would be required in realistic cropping systems, based on field-average or harvest-blended AP, even if AP attributed to PMGF was limited to one-third of total AP.

In 2007 PMGF in commercial winter wheat fields (32-130 ha) in eastern Colorado was reported (Gaines et al., 2007). Maximum PMGF determined using the imidazolinone (IMI) herbicide resistance marker in 56 commercial winter wheat field locations involving 18 cultivars from 2003 to 2005 was 5.3% at 0.23 m; maximum PMGF at the furthest distance measured (61 m) was 0.25%. Greater outcrossing frequencies and distances were determined in commercial fields than experimental plots, highlighting the impact of field size on pollen flow as documented previously (Beckie, Warwick, Nair, & Seguin-Swartz, 2003; Hüsken & Dietz-Pfeilstetter, 2007; Salisbury, 2002). The empirical model of Gustafson et al. (2005) was refined by adding a term for cultivar maturity (relative heading) class (Gaines et al., 2007); pollen flow tends to be greater when donor plants shed pollen later (i.e., later heading) than receptor plants (Matus-Cádiz et al., 2004). They estimated that the distance at which gene flow was less than 0.9% was 41 m for cultivars heading earlier than the pollen donor, but only 0.7 m for cultivars heading later than the pollen donor.

In 2004, CDC Imagine (Canada Western Red Spring class) was the first IMI-resistant (Clearfield®) wheat cultivar grown commercially in Canada (Beckie et al., 2006). The main agronomic advantage of IMI-resistant wheat over conventional (non-IMI-resistant) wheat is control of volunteer barley (*Hordeum vulgare* L.) by imazamox, which increases crop rotation options. Yield, maturity, and disease resistance of CDC Imagine are generally similar to that of AC Barrie (McCaig et al., 1996), which was the standard/check hard red spring cultivar in variety trials and the most widely grown in 2004 (Canadian Wheat Board, 2004).

Cultivar CDC Imagine has a single semi-dominant nuclear gene (Ta*AhasL-D1*, previously *Imi1*; GenBank accession No. AY210407) encoding for resistance to IMI herbicides on the chromosome arm 6DL (Anderson, Matthiesen, & Hegstad, 2004; Pozniak et al., 2004; Pozniak & Hucl, 2004). A single base pair change in the Ta*AhasL-D1* gene results in an amino acid change ($Ser_{653}$ to $Asn_{653}$) of the acetohydroxyacid synthase (AHAS) protein, creating the IMI-resistance trait.

Although PMGF has been extensively examined in wheat, further research is still required to determine levels occurring between neighboring commercial fields of spring cultivars. In a previous study, receptor fields were no closer than 0.15 km from the pollen donor field (Matus-Cádiz et al., 2007). The IMI-resistance trait has proven an ideal phenotypic marker for PMGF studies (Willenborg et al., 2009). Therefore, a study detailed in Beckie et al. (2011) was conducted in western Canada in 2004 (the first year IMI-resistant wheat was commercially available) to examine PMGF from commercial fields of the IMI-resistant cultivar CDC Imagine to the conventional wheat cultivar AC Barrie.

## Materials and Methods

### *Site Description*

Two field sites 4 km apart (Sites 1 and 2) were located in central Saskatchewan, Canada (latitude 51.4° N; longitude 106.0° W and 106.2° W for Sites 1 and 2, respectively) in the semi-arid Moist Mixed Grassland ecoregion. The IMI-resistant cultivar CDC Imagine was not planted by neighbouring growers within at least a 10-km radius of the study sites. At each site, a 16-ha (400 m × 400 m) field planted to CDC Imagine was located immediately adjacent to a field of AC Barrie, also measuring 400 m × 400 m (Figure 1). The IMI-resistant wheat field was located north (Site 1) or east (Site 2) of the conventional wheat field. Conventional wheat was not planted within an 800-m radius of the two sites. The soil at both sites is a Weyburn clay loam (Typic Boroll) with 4.5% organic matter and pH 6.5. Topography at both sites is gently sloping (< 5%).
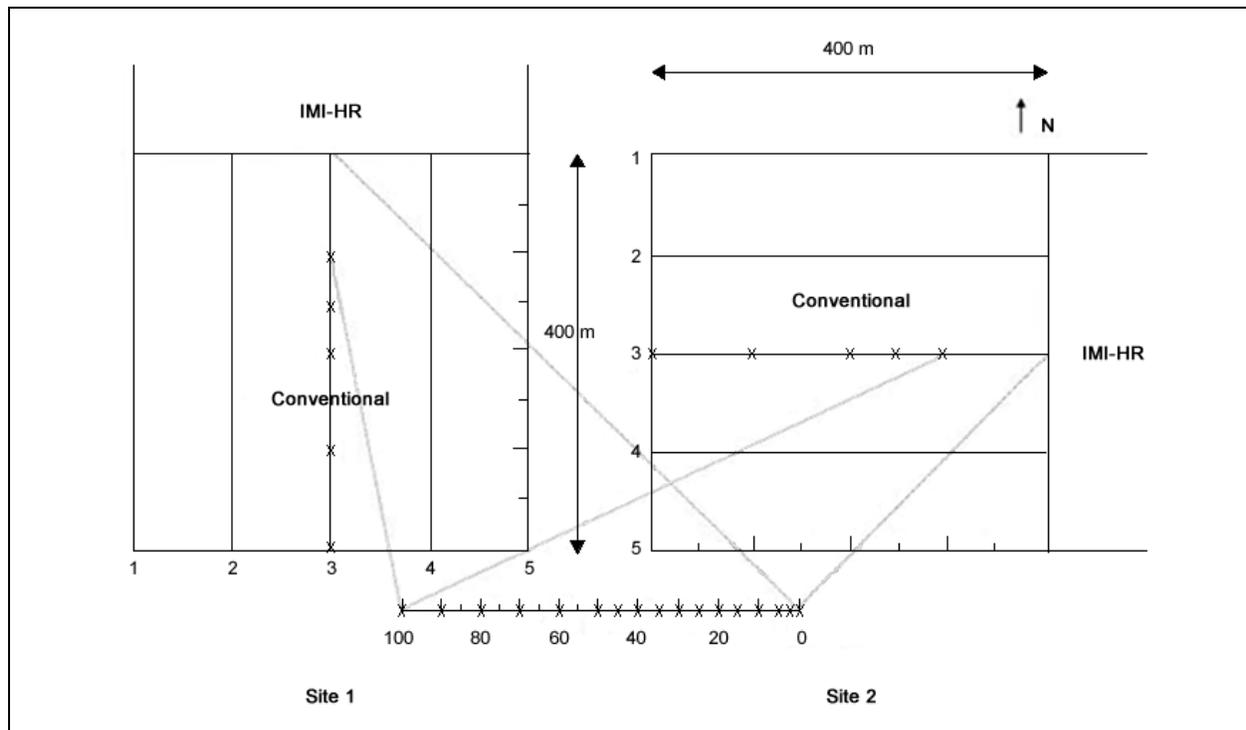
**Figure 1. Orientation of imidazolinone-resistant (IMI-HR; cultivar CDC Imagine) and conventional (non-IMI-HR; cultivar AC Barrie) spring wheat fields at two sites (Sites 1 and 2, located 4 km apart) in central Saskatchewan, Canada, in 2004.**
*Orientation of transects and sampling distances (x) in the conventional wheat field are also shown (adapted from Beckie et al., 2011).*

## Field Experiment Protocol

Both cultivars were planted on canola stubble on May 18, 2004, using a hoe drill with 20-cm row spacing. One tillage operation, using a cultivar with mounted tine harrows, immediately preceded planting. Certified seed of each cultivar was planted 2 to 3 cm deep at a rate of 90 kg ha$^{-1}$. The cultivar AC Barrie was seeded before CDC Imagine to avoid potential seed admixture. Fertilizer nitrogen, phosphorus, and sulfur were mid-row banded at rates recommended by soil tests. On June 22, imazamox (20 g L$^{-1}$) plus 2,4-D ester (560 g L$^{-1}$) formulated mixture was applied to CDC Imagine at the four-leaf stage at 20 g ai ha$^{-1}$; a tank-mix of clodinofop (240 g L$^{-1}$) at 55 g ai ha$^{-1}$ plus bromoxynil (225 g L$^{-1}$)/MCPA ester (225 g L$^{-1}$) formulated mixture at 560 g ai ha$^{-1}$ was applied to AC Barrie on the same day. A non-ionic surfactant (Agral 90 at 0.25% v/v; Norac Concepts Inc., Ottawa, ON) was applied with imazamox/2,4-D, whereas an oil-based adjuvant (Score at 0.5% v/v; Syngenta, Guelph, ON) was applied with the clodinofop plus bromoxynil/MCPA tank-mix.

The estimated pollination period of both cultivars was similar, from July 8 to 16. Weather data during the growing season was compiled from a weather station located within 100 m of Site 2. In mid-August, plant density of AC Barrie and CDC Imagine was measured in 20, 1-m$^2$ quadrats randomly located in each field. On September 7, AC Barrie was hand-harvested along five equally-spaced transects, oriented perpendicular to the common border with CDC Imagine, at 21 sampling distances: 0.5, 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 300, and 400 m (Figure 1). At each sampling distance, wheat was harvested in three adjacent 1-m$^2$ quadrats oriented perpendicular to the transect direction. For example, at the 0.5-m sampling distance, quadrats were placed in a row from 0- (common border) to 1-m distance. Plants were cut at ground level and shoot biomass from each of the three quadrats was combined and collected in a cotton bag. Samples were subsequently dried in a forced-air drying room. Thereafter, plants were manually threshed. Seed samples were subsequently stored in a freezer at -80°C until herbicide resistance testing.

## IMI Wheat Bioassay

Grain samples were screened for imazamox resistance from 2006 to 2008. For each grain sample, 2,000 seed-

lings were screened. A soil-less bioassay was adapted from a proprietary protocol provided by BASF Canada (Missisauga, ON). Two steel blue blotter papers (Anchor Paper Co., St. Paul, MN)—10.25 × 10.25 cm—were placed in the bottom of a plexiglass box measuring 11 × 11 × 3.5 cm. A 22-ml aliquot of a 200 µM solution of imazamox was added to the blotter paper and allowed to equilibrate for 30 minutes. Wheat seeds were soaked in 5% bleach (sodium hypochlorite) solution for 2 minutes to control fungal growth. After seeds were air-dried for 1 hour, 40 seeds were applied to each dish by a vacuum seed head. Two boxes each of positive (CDC Imagine) and negative controls (AC Barrie) were included, as well as an untreated (water) control.

Prior to initiation of testing, the certified seedlots of AC Barrie and CDC Imagine were checked for AP. For each seedlot, 2,000 seedlings at the two- to three-leaf stage were sprayed with imazamox at 20 g ai ha$^{-1}$ plus an oil-based adjuvant (Merge$^®$ at 0.5% v/v; BASF Canada, Missisauga, ON). The herbicide was applied using a moving-nozzle cabinet sprayer equipped with a flat-fan nozzle tip (TeeJet$^®$ 8002VS; Spraying Systems Co., Wheaton, IL) calibrated to deliver 200 L ha$^{-1}$ of spray solution at 210 kPa in a single pass over the foliage. No IMI-resistant individuals were detected in AC Barrie; in CDC Imagine, 2 individuals (0.1%) were found to be IMI-susceptible.

Covered boxes were placed in an incubator with an 8-hour light period at 20°C and a 16-hour dark period at 15°C for 7 days. Seedlings with short, stunted roots and short coleoptiles were classified as non-resistant. Seedlings that exhibited long roots and/or long shoots (coleoptile with first leaf extended) were classified as putatively-resistant. Each suspected resistant seedling was transplanted into potting mixture consisting of soil, peat, vermiculite, and sand (3:2:2:2 by volume) plus a slow-release fertilizer (150 g of 26-13-0 per 75 L potting mixture) in a 15-cm diameter pot and placed in the greenhouse. Environmental conditions were 20/16°C day/night temperature regime with a 16-hour photoperiod supplemented with 230 µmol m$^{-2}$ s$^{-1}$ illumination. No-Damp$^®$ fungicide (3.2% oxine benzoate; Plant Products Co. Ltd., Brampton, ON) was applied to soil at 10 ml L$^{-1}$ water to prevent damping-off of seedlings. Seedlings at the two- to three-leaf stage were tissue-sampled and samples were stored at -80°C. Seedlings were then sprayed with imazamox as described previously, and assessed 14 days after treatment as resistant or susceptible based on survival.

## Confirmation of IMI-Resistance Trait

After completion of herbicide resistance testing in 2008, freezer-stored tissue samples were freeze-dried for 3 days and DNA was extracted (Fast DNA Spin Kit; QBioGene, MP Biomedicals, Solon, OH). Allele-specific CAPS marker detection of the IMI-resistance allele, Ta*AhasL-D1*, as described by Neudorf and Matus-Cádiz (2009), was utilized to determine the presence of the resistant allele—Ser653 mutation on chromosome 6D—in leaf tissue samples (Beckie et al., 2011). The protocol involves polymerase chain reaction (PCR) amplification of the gene, with subsequent TaaI restriction enzyme digestion of the amplified PCR product. The presence of a 368-bp band after digestion indicates the presence of a mutation at Ser653 (i.e., IMI-resistant), but does not distinguish between heterozygote-resistant (as expected for hybrids in this study) and homozygote-resistant genotypes. CDC Imagine carries a mutation in the D genome (creating the CAPS marker), but there are also two wild-type genes in the A and B genomes that are also amplified with these primers.

The protocol described by Li, Barclay, Jose, Stefanova, and Appels (2008) was used for confirmation of the PCR/TaaI digestion results, and to determine if the resistant samples were indeed heterozygous, that is, contained both the wild type (Ser$_{653}$) and mutant (Asn$_{653}$) alleles, and to ensure that the mutant allele was absent and the wild type allele present in samples classified as IMI-susceptible. Two separate PCR reactions were used to detect the two alleles. The gene-specific primer ImiD-For (TTACAGTTGTTGACATTGACGGT) was paired with either the mutant allele ImiMu-Rev (TCCTTGAAAGCACCATCGT) or the wild type allele primer ImiWt-Rev (TCCTTGAAAGCACCATCGC) to amplify 390-bp fragments (Li et al., 2008). The presence of a 390-bp band in both PCR reactions confirms the presence of both alleles. Each PCR reaction contained 1X PCR buffer (1.5mM MgCl$_2$), 200 µM dNTPs, 1.0 units Hotstart Taq DNA Polymerase, 250 nM of each primer, and approximately 25 ng of genomic DNA. PCR reactions were performed in a total volume of 25µl with a 15-minute initial denaturation step at 95°C, followed by a touchdown PCR of 94°C for 45 seconds, 57-52°C (decreasing 0.5°C per cycle) for 45 seconds, 72°C for 1 minute, 36 cycles at the fixed-annealing temperature of 52°C, and a final elongation step of 72°C for 5 minutes. Reactions were separated on a 1.5% agarose gel (3:1 HRB; AMRESCO, Solon, OH).

**Table 1. Growing season (May to August) monthly precipitation and mean air temperatures at the field sites in central Saskatchewan, Canada, in 2004.**

| | Precipitation | | Temperature | |
|---|---|---|---|---|
| | **mm** | **%[a]** | **C** | **%[a]** |
| **May** | 48.2 | 191 | 8.0 | 110 |
| **June** | 81.0 | 98 | 13.5 | 111 |
| **July** | 27.4 | 36 | 17.5 | 106 |
| **August** | 96.4 | 108 | 14.6 | 106 |
| **Total** | 253 | 92 | | |
| **Avg.** | | | 13.4 | 108 |

[a] *Percentage of 30-year mean from 1971 to 2000 at a weather station 50 km from the two study sites.*

**Table 2. Daily weather (from 0600 to 1800 h except precipitation) during the estimated pollination period of the spring wheat cultivars at field sites in central Saskatchewan, Canada, in July 2004.**

| Days | Mean temperature C | Precipitation mm | Relative humidity % | Wind speed km h^{-1} | Prevailing direction |
|---|---|---|---|---|---|
| **8** | 16.2 | 0 | 70 | 23 | SE |
| **9** | 19.6 | 0 | 62 | 17 | SW |
| **10** | 19.9 | 0 | 65 | 16 | NW |
| **11** | 21.4 | 1.8 | 71 | 21 | NE |
| **12** | 16.9 | 0.4 | 80 | 13 | SE |
| **13** | 21.5 | 0 | 71 | 11 | NW |
| **14** | 23.0 | 0.6 | 71 | 8 | SE |
| **15** | 21.4 | 0 | 73 | 12 | SW |
| **16** | 21.9 | 0.4 | 69 | 9 | NE |
| **Avg./total** | 20.2 | 3.2 | 70 | 15 | |

## Data Analysis

For each grain sample analyzed, PMGF (%) was calculated as the number of confirmed heterozygotes divided by the total number of seedlings tested in a sample (2,000), multiplied by 100. Plant density and PMGF data were square-root transformed prior to an analysis of variance. Data were subjected to an analysis of variance using the Proc MIXED procedure in SAS software (SAS, 2008). Replicate (transect) and site were considered random effects, and sampling distance from the common field border as a fixed effect.

Because PMGF was not significantly affected ($P > 0.05$) by site or transect, data were averaged across transects and sites for each sampling distance. Analysis of variance confirmed the significant effect of distance from the pollen donor on PMGF. PMGF ($y$) was regressed against distance ($x$) from the common border using the double-exponential decay Model 1 (Yoshimura, Beckie, & Matsuo, 2006):

$$y = a\,e^{-bx} + c\,e^{-dx}, \qquad (1)$$

where $a + c$ is the intercept and $b, d$ quantify the slope. Data were fitted to the model using a derivative-free nonlinear regression procedure, provided with the NLIN procedure (SAS, 2008). The coefficient of determination ($R^2$) that quantifies the goodness of fit was calculated as described by Kvalseth (1985) using the residual sum of squares value from the analysis output. Standard errors of the parameter estimates were calculated; parameter estimates are considered significant at the 0.05 level if the standard error is less than one-half the value of the estimate (Koutsoyiannis, 1977).

## Results and Discussion

The weather during the 2004 growing season was conducive to excellent wheat seedling emergence, growth, and yields. Growing season (May to August) total precipitation and mean air temperature in 2004 were near-normal compared with 30-year long-term norms (Table 1). Growing season precipitation was 92% and temperature was 108% of long-term means. Monthly mean temperatures were 6-11% above normal. Precipitation was greater than normal in May, less than normal in July, and near-normal in June and August.
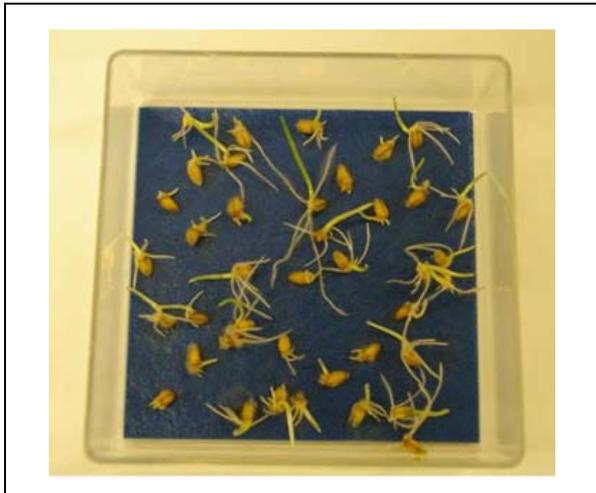
**Figure 2. Imidazolinone (IMI) wheat bioassay, with a single suspected resistant seedling later confirmed as IMI-resistant by PCR analysis.**

*Reproduced with permission from Beckie et al. (2011).*

The estimated pollination period of CDC Imagine and AC Barrie were similar, from July 8 to 16 (Table 2). The diurnal period during which pollen shed is greatest in wheat is between 0600 and 1800 hours (de Vries, 1972). During this diurnal period over the 9-day pollination interval, mean air temperature averaged 20.2°C, relative humidity 70%, and wind speed 15 km h$^{-1}$. Total precipitation during pollination was only 3.2 mm. The daily prevailing wind direction changed considerably over the pollination period. Temperatures of 16-20°C and relative humidity levels of 70-75% promote PMGF in wheat (de Vries, 1972). Thus, weather conditions during the estimated pollination period at the two sites were generally conducive to promoting PMGF.

The analysis of variance indicated that pre-harvest plant density did not differ by site or cultivar; plant density ($\pm$SE) averaged 186 $\pm$ 4 plants m$^{-2}$. In a small-plot study in a sub-humid ecoregion in Manitoba, Willenborg et al. (2009) identified a critical range of 175 to 200 plants m$^{-2}$, below which PMGF frequencies increased exponentially with decreasing plant density.

Seedlings in the soil-less bioassay that were classified as imazamox-resistant (Figure 2) later survived postemergence-applied imazamox (transplanted seedlings at the two- to three-leaf stage). However, those seedlings in the bioassay whose resistance classification was uncertain usually did not survive imazamox treatment at the two- to three-leaf stage. The third and final level of confirmation of the IMI-resistance trait in heterozygotes was obtained by PCR analysis.
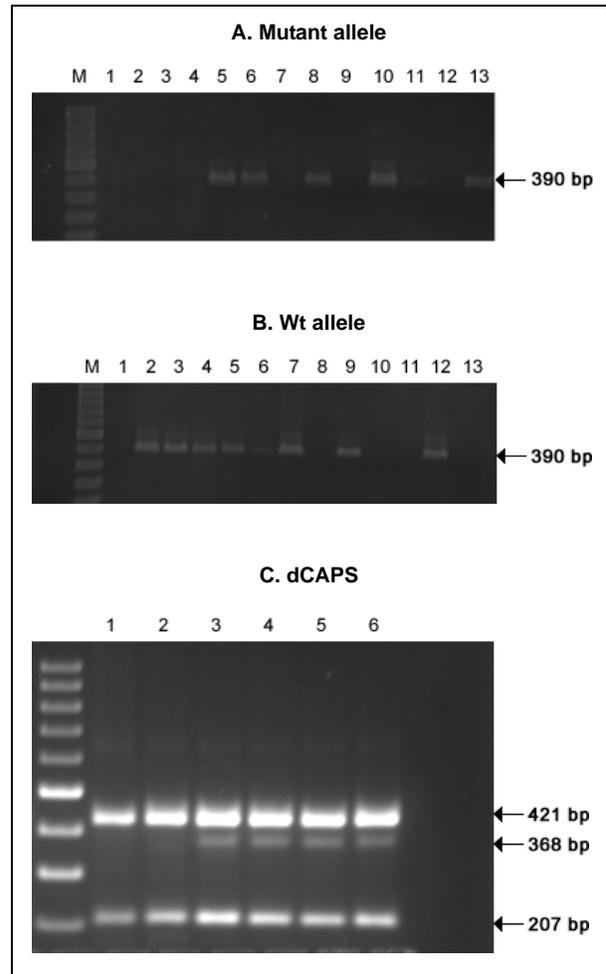


**Figure 3. Agarose gel electrophoresis of:**

**(A) allele-specific PCR with Li et al. (2008) Mu-Rev primer; band at 390 indicates presence of mutant allele**

**(B) allele-specific PCR with Li et al. (2008) Wt-Rev primer; band at 390 indicates presence of wild type (Wt) allele [gels A and B: M=100-bp marker; lane 1=water; lanes 2-4=AC Barrie; lanes 5-6=heterozygote-resistant (Rt-Het); lanes 7, 9, and 12=homozygote-susceptible; lanes 8,10, and 11=homozygote-resistant (Rt-Homo)]**

**(C) dCAPS analysis of individual samples. CDC Imagine (Ta*AhasL-D1* resistance gene) has two TaaI cut sites (421, 368, 207, and 54-bp bands (latter not shown); AC Barrie (Ta*ahasL-D1* susceptible gene) has only one cut site (only 421- and 207-bp bands present after TaaI digestion). After digestion, the 368-bp band is notably absent in non-resistant samples (gel C: lane M=100-bp marker; lane 1=AC Barrie; lane 2=susceptible; lanes 3-5=resistant; lane 6=CDC Imagine)**

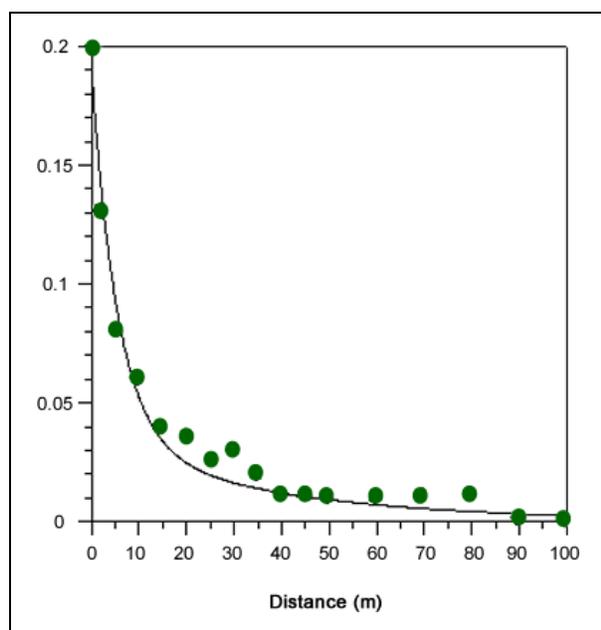*Reproduced with permission from Beckie et al. (2011).*

**Figure 4. Frequency of PMGF (%) as a function of distance from the pollen-donor field of imidazolinone-resistant spring wheat (cultivar CDC Imagine).**

$y = 0.16e^{-0.38x} + 0.075e^{-0.038x}$

$R^2 = 0.99**$

*Standard errors of parameter estimates: a=0.01; b=0.05; c=0.01; d=0.005 (adapted from Beckie et al., 2011).*

The PCR protocol of Li et al. (2008) confirmed the PCR/TaaI digestion results from the Neudorf and Matus-Cádiz (2009) protocol (Figure 3). Susceptible samples amplified a PCR band of 390 bp when using the reverse primer specific to the wild type allele (ImiWt-Rev), but did not amplify a band with the reverse primer specific to the mutant allele (ImiMu-Rev). The presence of a 390-bp band for all resistant samples when using the ImiMu-Rev primer confirmed the presence of the resistant allele. However, not all of the resistant samples showed a PCR band for the wild type allele as would be expected in hybrids resulting from gene flow, indicating a homozygous mutant genotype. All homozygous-resistant samples originated from one perimeter transect on the north side of Site 2.

The observed maximum PMGF was 0.2% at the common border (0.5 m distance) but declined exponentially with increasing distance (Figure 4). For example, average PMGF was 0.13% at 2.5 m, 0.08% at 5 m, and 0.06% at 10 m. The observed PMGF data fit the double-negative exponential model with a goodness of fit ($R^2$) of 0.99 ($P < 0.01$), and each parameter estimated was significant at the probability level of $P < 0.05$. At distances beyond 80 m, PMGF was not detected. Because

the detection limit in this study was 0.05% (i.e., 1 in 2,000 individuals per sample), it is possible that PMGF occurred beyond 80 m at frequencies below that threshold.

Generally, higher levels of PMGF were measured in this study than that determined in Saskatchewan in the early 2000s with a pollen donor measuring 50 m × 50 m (Matus-Cádiz et al., 2004). The rate of decline of PMGF with distance (Figure 4) was less than that observed in the previous study. In Colorado, Gaines et al. (2007) similarly found higher levels of PMGF in commercial winter wheat fields than experimental plots. Together, these results suggest that it will be important after commercial release of crops with novel traits to compare PMGF rates in commercial fields with that obtained in small-plot studies.

In agreement with previous studies, PMGF in wheat declines rapidly with distance, by more than 50% at a 5-m distance from the pollen donor. However, PMGF in wheat can persist at low levels over considerable distance, as similarly documented in highly-outcrossing crops such as corn and canola. Within 5 m of the common border, hybridization frequency averaged 0.14%. Averaged across the entire field, hybridization frequency averaged 0.006%. Based on the regression equation (Figure 4), exclusion of grain from a 5-m harvested strip adjacent to the donor field from the harvest-blended seedlot reduced hybridization frequency by 33% to 0.004%.

Thus, the contribution of pollen flow to AP in wheat is likely markedly less than that from seed admixture. With numerous studies conducted at various spatial scales in different agroecoregions of Canada and elsewhere over the past 10 years, we now have a comprehensive dataset of PMGF in wheat. When wheat cultivars with different traits are commercialized, the collective results of these studies can guide recommendations of grower stewardship practices for identity preservation.

## References

Anderson, J.A., Matthiesen, L., & Hegstad, J. (2004). Resistance to an imidazolinone herbicide is conferred by a gene on chromosome 6DL in the wheat line cv. 9804. *Weed Science*, *52*, 83-90.

Beckie, H.J., & Hall, L.M. (2008). Simple to complex: Modelling crop pollen-mediated gene flow. *Plant Science*, *175*, 615-628.

Beckie, H.J., Harker, K.N., Hall, L.M., Warwick, S.I., Légère, A., Sikkema, P.H., et al. (2006). A decade of herbicide-resistant crops in Canada. *Canadian Journal of Plant Science*, *86*, 1243-1264.

Beckie, H.J., Warwick, S.I., Nair, H., & Seguin-Swartz, G. (2003). Gene flow in commercial fields of herbicide-resistant canola (*Brassica napus*). *Journal of Ecological Applications*, *13*, 1276-1294.

Beckie, H.J., Warwick, S.I., Sauder, C.A., Hall, L.M., Harker, K.N., & Lozinski, C. (2011). Pollen-mediated gene flow in commercial fields of spring wheat in western Canada. *Crop Science*, *51*, 306-313.

Canadian Wheat Board. (2004). *2004 Canadian Wheat Board variety survey*. Winnepeg, Manitoba: Author.

de Vries, A.P. (1972). Some aspects of cross pollination in wheat (*Triticum aestivum* L.). 1. Pollen concentration in the field as influenced by cultivar, diurnal pattern, weather conditions, and level as compared to the height of the pollen donor. *Euphytica*, *21*, 185-203.

Gaines, T.A., Byrne, P.F., Westra, P., Nissen, S.J., Henry, W.B., Shaner, D.L., & Chapman, P.L. (2007). An empirically derived model of field-scale gene flow in winter wheat. *Crop Science*, *47*, 2308-2316.

Gustafson, D.I., Horak, M.J., Rempel, C.B., Metz, S.G., Gigax, D.R., & Hucl, P. (2005). An empirical model for pollen-mediated gene flow in wheat. *Crop Science*, *45*, 1286-1294.

Gustafson, D.I., Horak, M.J., Rempel, C.B., Metz, S.G., Gigax, D.R., & Hucl, P. (2006). Letters to the editor. *Crop Science*, *46*, 1019.

Hanson, B.D., Mallory-Smith, C.A., Shafii, B., Thill, D.C., & Zemetra, R.S. (2005). Pollen-mediated gene flow from blue aleurone wheat to other wheat cultivars. *Crop Science*, *45*, 1610-1617.

Hüsken, A., & Dietz-Pfeilstetter, A. (2007). Pollen-mediated intraspecific gene flow from herbicide resistant oilseed rape (*Brassica napus* L.). *Transgenic Research*, *16*, 557-569.

Koutsoyiannis, A. (1977). *Theory of econometrics* (2nd ed.; pp. 81-91). London: MacMillan Education.

Kvalseth, T.O. (1985). Cautionary note about $R^2$. *American Statistician*, *39*, 279-285.

Li, D., Barclay, I., Jose, K., Stefanova, K., & Appels, R. (2008). A mutation at the Ala122 position of acetohydroxyacid synthase (AHAS) located on chromosome 6D of wheat: Improved resistance to imidazolinone and a faster assay for marker assisted selection. *Molecular Breeding*, *22*, 217-225.

Loureiro, L., Escorial, M.C., González-Andujar, J.L., García-Baudin, J.M., & Chueca, M.C. (2007). Wheat pollen dispersal under semiarid field conditions: Potential outcrossing with *Triticum aestivum* and *Triticum turgidum*. *Euphytica*, *156*, 25-37.

Matus-Cádiz, M.A., Hucl, P., & Dupuis, B. (2007). Pollen-mediated gene flow in wheat at a commercial scale. *Crop Science*, *47*, 573-581.

Matus-Cádiz, M.A., Hucl, P., Horak, M.J., & Blomquist, L.K. (2004). Gene flow in wheat at the field scale. *Crop Science*, *44*, 718-727.

McCaig, T.N., DePauw, R.M., Clarke, J.M., McLeod, J. G., Fernandez, M.R., & Knox, R.E. (1996). AC barrie hard red spring wheat. *Canadian Journal of Plant Science*, *76*, 337-339.

Neudorf, T., & Matus-Cádiz, M. (2009). *Allele-specific CAPS marker detection of imidazolinone resistance alleles TaA-hasL-D1, -B1, and -A1 (S653N) – CLEARFIELD® WHEAT* (RDIMS 1793531, Version 1.0). Saskatoon, SK: Canadian Food Inspection Agency, Saskatoon Laboratory.

Pozniak, C.J., Birk, I.T., O'Donoughue, L.S., Menard, C., Hucl, P.J., & Singh, B.K. (2004). Physiological and molecular characterization of mutation-derived imidazolinone resistance in spring wheat. *Crop Science*, *44*, 1434-1443.

Pozniak, C.J., & Hucl, P.J. (2004). Genetic analysis of imidazolinone resistance in mutation-derived lines of common wheat. *Crop Science*, *44*, 23-30.

Salisbury, P.A. (2002). Genetically modified canola in Australia: Agronomic and environmental considerations. Australia Square: Australian Oilseeds Federation.

SAS. (2008). *SAS Online Doc®, version 9.2*. Cary, NC: SAS Institute.

Willenborg, C.J., Brûlé-Babel, A.L., & Van Acker, R.C. (2009). Low crop plant population densities promote pollen-mediated gene flow in spring wheat (*Triticum aestivum* L.). *Transgenic Research*, *18*, 841-854.

Willenborg, C.J., & Van Acker, R.C. (2006). Comments on "An empirical model for pollen-mediated gene flow in wheat." *Crop Science*, *46*, 1018.

Willenborg, C.J., & Van Acker, R.C. (2008). The biology and ecology of hexaploid wheat (*Triticum aestivum* L.) and its implications for trait confinement. *Canadian Journal of Plant Science*, *88*, 997-1013.

Yoshimura, Y., Beckie, H.J., & Matsuo, K. (2006). Transgenic oilseed rape along transportation routes and port of Vancouver in western Canada. *Environmental Biosafety Research*, *5*, 67-75.

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