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Estimating Gene Flow from Wheat to Wheat and Wheat to Jointed Goatgrass (*Aegilops cylindrica*)

Estimating Gene Flow from Wheat to Wheat and Wheat to Jointed Goatgrass (*Aegilops cylindrica*)

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A Cooperative Project

of

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Summary

The transfer of genes between plant populations, known as gene flow, can occur through the dispersal of pollen or seed. The introduction of herbicide-tolerant wheat (*Triticum aestivum* L.) cultivars and the potential release of cultivars with transgenic traits have generated increased interest in gene flow, both between wheat cultivars and between wheat and jointed goatgrass (*Aegilops cylindrica* Host). Information on gene flow is also relevant to maintaining varietal purity in seed production operations. For these reasons, we undertook a series of studies in eastern Colorado with the following objectives:

(1) To evaluate landscape-level gene flow from CLEARFIELD® herbicide-tolerant wheat to adjacent fields of non-CLEARFIELD wheat.

(2) To compare estimates of gene flow from large-scale commercial field sampling to those obtained from smaller experimental plots.

(3) To evaluate seed-mediated gene flow in certified and farmer-saved seed lots.

(4) To evaluate landscape-level gene flow from CLEARFIELD wheat to jointed goatgrass.

Landscape-level gene flow from wheat to wheat. The objectives of this study were to estimate pollen-mediated gene flow (PMGF) between commercial wheat fields in multiple years and locations. Grain was sampled from a total of 56 commercial fields in eastern Colorado in 2003, 2004, and 2005. We estimated PMGF by tracking the movement of the CLEARFIELD tolerance gene, which provides tolerance to imazamox (an imidazolinone herbicide), from tolerant to susceptible cultivars, sampled at distances of 0.5 to 200 feet. All 18 evaluated cultivars and all sampled fields showed detectable levels of PMGF, as high as 5.3% in one sample collected at 0.75 feet. The farthest distance at which we detected gene flow was 180 feet, with one sample at that distance showing 0.25% cross-pollination. Our data analysis revealed that relative heading dates and distance were both important factors in determining the frequency of gene flow. We constructed a statistical model with a random location effect that predicted that the distance required to ensure 95% confidence that 95% of locations have PMGF less than 0.9% is 135 feet for cultivars heading earlier than the pollen source and 2.3 feet for cultivars heading later than the pollen source. These confidence limits should represent the highest levels of PMGF expected to occur in winter wheat in the west-central Great Plains and will be useful for wheat biotechnology regulation.

Gene flow estimates from large commercial fields versus small experimental plots. Because most gene flow information has come from small research plots, we wanted to compare more realistic estimates from commercial-scale fields to results from smaller plots to see if the estimates were similar. We planted a square central block (33 feet on each side) of the CLEARFIELD wheat cultivar ‘Above’, surrounded by alternating strips of the herbicide-susceptible cultivars ‘Prairie Red’ and ‘Halt’. Samples were collected at distances up to 128 feet along transects radiating in all directions from the edge of the ‘Above’ plot. Results from this experiment were compared to data from commercial fields with ‘Prairie Red’ and ‘Halt’ bordering fields of ‘Above’. Higher levels and greater distances of PMGF were detected in commercial fields than in experimental plots. For example, the maximum amount of cross-pollination detected in ‘Prairie Red’ was 0.74% in experimental plots and 1.15% in commercial fields; the maximum distance at which PMGF was detected was 36 feet in experimental plots versus 200 feet in commercial fields. Our results indicate that PMGF estimated in small research plots may seriously underestimate the amount observed in large fields.

Seed-mediated gene flow. Varietal purity in wheat seed production is necessary for agronomic uniformity and to enable potential market segregation. We conducted a survey of certified and farm-saved seed samples using a CLEARFIELD herbicide-tolerant wheat variety in 2004 and 2005 in eastern Colorado. The objective was to compare varietal purity based on type of seed producer and the producer's previous history of growing CLEARFIELD varieties. Ninety-two samples of herbicide-susceptible varieties were taken from certified and farm-saved seed growers, who either produced or had never produced CLEARFIELD wheat. Herbicide-tolerant seeds were detected using a seed soaking technique in samples from each producer type and each production history. Levels of herbicide-tolerant seed ranged from 0% to 11.28%. One certified sample and three farm-saved samples exceeded the 0.1% threshold for off-types in certified wheat seed. Using a two-factor analysis, farm-saved production class and positive CLEARFIELD history increased the estimated proportion of off-type herbicide-tolerant seed. Based on grower interviews, higher levels of herbicide-tolerant seed presence were associated with volunteer plants from previous crops of the tolerant variety and with mechanical mixture during harvesting. Production practices for certified seed address these factors and may need to be strengthened if more stringent purity criteria are adopted. This information is important for risk assessment and policy development for potential commercial release of transgenic wheat varieties.

Gene flow between wheat and jointed goatgrass. Gene flow between jointed goatgrass and winter wheat is a current concern because transfer of herbicide tolerance genes from CLEARFIELD winter wheat cultivars to jointed goatgrass could restrict weed management options in winter wheat cropping systems. In the future, potential release of wheat cultivars with transgenic traits such as drought tolerance could have significant environmental effects if the genes are incorporated into goatgrass populations. Our objectives in this study were (1) to investigate the frequency of interspecific hybridization between CLEARFIELD wheat and jointed goatgrass in eastern Colorado, and (2) determine the activity of the herbicide-tolerant acetolactate synthase (*Als1*) allele in hybrids of CLEARFIELD wheat × jointed goatgrass and in hybrids of CLEARFIELD wheat × herbicide-susceptible wheat. Jointed goatgrass was sampled side-by-side with CLEARFIELD wheat and at distances up to 175 feet away both in experimental plots and at commercial field study sites in 2003, 2004, and 2005. A greenhouse screening method was used to identify herbicide-tolerant hybrids in collected jointed goatgrass seed. The average percent hybridization across sites and years when CLEARFIELD wheat and jointed goatgrass were grown side-by-side was 0.1% and the maximum was 1.6%. The greatest distance over which hybridization was documented was 50 feet. The CLEARFIELD *Als1* allele contributed 40% of untreated acetolactate synthase (ALS) activity in CLEARFIELD wheat × jointed goatgrass F_1 plants, as measured by an *in vitro* ALS assay. The hybridization rate between wheat and jointed goatgrass and expression of the CLEARFIELD wheat *Als1* allele in hybrid plants will both influence trait introgression into jointed goatgrass.

Introduction

Gene flow is the transfer of genes between populations, and occurs in plants through the dispersal of pollen or seeds. Gene flow through cross-pollination occurs to some degree in all crops, even those like wheat that are regarded as predominantly self-pollinated (Ellstrand et al., 1999; Committee on Genetically Modified Pest-Protected Plants, 2000). In conventionally bred crop varieties, this is usually not a problem, either because cross-pollination is minimized by distance between fields or because reasonable tolerances exist for off-types in marketing channels. However, two situations in which gene flow has much greater significance are seed production and the growing of transgenic[1] crops.

Seed production requires high standards of purity in order to maintain the genetic integrity of crop cultivars and breeding materials. State seed programs mandate that a set of practices be followed for the production of certified seed (e.g., the Colorado Seed Certification Standards handbook available at http://seeds.colostate.edu/CSGA/documents/standards/2008StandardsUpdateFinal.pdf). These include minimum isolation distances, field inspections, harvest practices, and purity analysis by a certified seed lab. Isolation distances recommended by USDA are very general and do not take into account environmental and varietal differences that may influence the frequency of gene flow. For example, USDA does not require any isolation distance for foundation wheat seed in order to keep cross-pollination below 0.5% (Committee on Genetically Modified Pest-Protected Plants, 2000), even though several studies have documented gene flow well above that level. For example, maximum cross-pollination rates for wheat reported in the literature include 6.7% by Hucl (1996), 5.6% by Martin (1990), and 3.95% by Griffin (1987). Other studies have found lower maximum frequencies of outcrossing: 0.45% in Hanson et al. (2005b) and 0.44% in Matus-Cadiz et al. (2004). The greatest distance at which confirmed gene flow in wheat has occurred is 1.7 miles, even though the level of cross-pollination was a very low 0.01% (Matus-Cadiz et al., 2007). Many of these previous reports have utilized relatively small pollen sources, ranging from single rows to 530-square-foot blocks. This led Matus-Cadiz et al. (2004) to suggest that more research at the commercial field scale was needed, because small pollen sources may underestimate gene flow.

With transgenic crops, the concern over gene flow takes on new dimensions because cross-pollination can lead to undesirable consequences in some situations. Growers wishing to market their products as organic or non-transgenic, for example, may be unable to do so if detectable levels of cross-pollination have occurred from nearby transgenic cultivars. Crops engineered to produce pharmaceuticals present a sensitive issue, because even minor amounts of out-crossing will be unacceptable. In addition to its capacity for crop-to-crop gene flow, wheat has the ability to hybridize at a low frequency with jointed goatgrass (*Aegilops cylindrica* Host), an invasive weedy species in the Great Plains and Pacific Northwest. The environmental significance of this crop-to-weed gene flow will vary with the nature of the transgene, but at least in some cases may increase the weediness of jointed goatgrass. Although transgenic wheat cultivars have not been released to date, they may appear on the market in the future. Knowledge of landscape-level gene movement from transgenic wheat

[1]We use the term "transgenic" to describe plants containing DNA transferred using recombinant DNA technology. It is synonymous with the terms "genetically engineered", "genetically modified", and "GM".

will be important information for federal regulatory agencies in their evaluation of applications to commercialize such cultivars.

The ability to estimate gene flow from commercial-scale wheat fields was greatly improved with the first-time commercial planting in 2002 of the cultivar ‘Above’, a CLEARFIELD (imazamox herbicide-tolerant) winter wheat cultivar developed jointly by Colorado State University (CSU) and Texas A&M University (Haley et al., 2003). The CLEARFIELD trait was developed by BASF Corp. (Research Triangle Park, NC; Newhouse et al., 1992), and was derived from an induced mutation rather than transgenic methods (Tan et al., 2005). However, it is controlled by a single gene and is expected to be transferred in pollen in the same way as a transgenic trait.

Most importantly for our studies, the trait can be easily tracked by evaluating herbicide tolerance in seeds produced in fields of non-CLEARFIELD cultivars adjoining those planted to CLEARFIELD wheat. Any of those seeds whose pollen parent was the CLEARFIELD cultivar will produce seedlings that are tolerant to the herbicide imazamox. We took advantage of the CLEARFIELD herbicide tolerance trait by undertaking a series of studies in eastern Colorado wheat growing regions with the following objectives:

(1) to evaluate landscape-level crop-to-crop gene flow from CLEARFIELD wheat to adjacent fields of non-CLEARFIELD wheat.

(2) to compare estimates of gene flow from large-scale commercial field sampling to estimates obtained from smaller experimental plots.

(3) to evaluate seed-mediated gene flow in certified and farmer-saved seed lots.

(4) to evaluate landscape-level gene flow from CLEARFIELD wheat to jointed goatgrass.

(5) to determine the activity of the herbicide-tolerant acetolactate synthase (*Als1*) allele in hybrids of CLEARFIELD wheat × jointed goatgrass and in hybrids of CLEARFIELD wheat × herbicide-susceptible wheat.

This report is organized into three sections corresponding to these objectives. More formal descriptions of these studies are contained in the articles by Gaines et al. (2007a, 2007b, 2008).

I. Pollen-Mediated Gene Flow from Wheat to Wheat

Objectives

- To evaluate landscape-level crop-to-crop gene flow from CLEARFIELD wheat to adjacent fields of non-CLEARFIELD wheat.
- To compare estimates of gene flow from large-scale commercial field sampling to estimates obtained from smaller experimental plots.

Materials and Methods

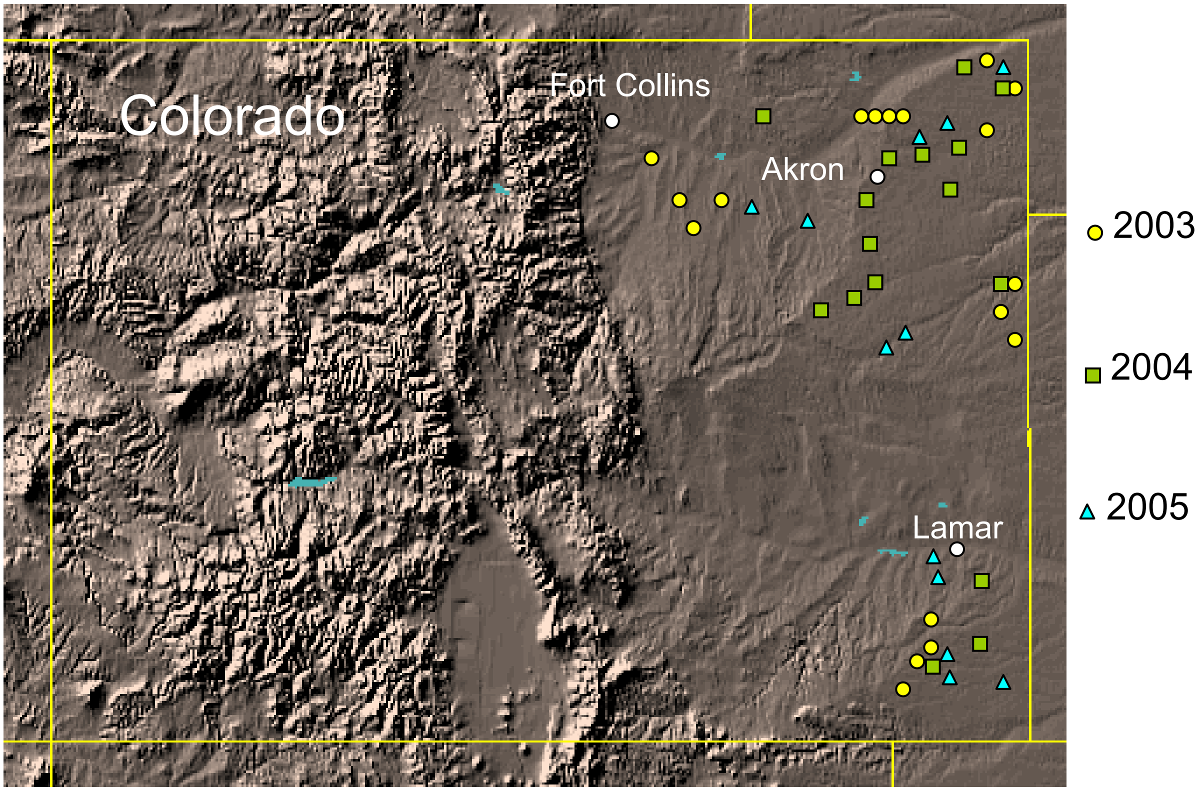
Commercial fields

Appropriate sampling sites, where a CLEARFIELD wheat cultivar was planted adjacent to a non-CLEARFIELD cultivar, were identified in eastern Colorado in 2003, 2004, and 2005. The CLEARFIELD cultivar was 'Above' in all locations except one, where 'Bond CL' (Haley et al., 2006) was the herbicide-tolerant cultivar. We sampled from two types of site. The first type was a single large field of a non-CLEARFIELD cultivar planted next to a large field of a CLEARFIELD cultivar; these fields ranged in size from 80 to 325 acres. The second type of site consisted of a strip trial, where multiple herbicide-susceptible cultivars were planted in strips parallel to a strip of CLEARFIELD wheat. Plots in the strip trials were 20 to 80 feet wide, with a border from 660 to 1,320 feet long. For all trial sites, heading dates (when approximately 50% of heads in a field had emerged above the flag leaf) were recorded for pollen source plots and for recipient plots as an indication of relative flowering time.

Seed samples of the non-CLEARFIELD cultivars were harvested by hand at distances from 0.75 to 200 feet from the CLEARFIELD cultivar. Each sample was bulked from a minimum of 20 subsamples of all the wheat heads in a 3-foot length of row at the same distance and direction from the CLEARFIELD pollen source. We collected a total of 455 samples representing 18 non-CLEARFIELD cultivars from 56 locations in the northeast, southeast, and east central regions of Colorado (Figure 1). The 18 cultivars plus 'Above' and 'Bond CL' were assigned a relative heading class (RHC), an indicator of flowering maturity ranging from 1 to 9, developed by CSU wheat breeder Dr. Scott Haley (Table 1).

To quantify pollen-mediated gene flow (PMGF), we used the method described in detail by Gaines et al. (2007a). The bulked seed samples were first threshed individually with a stationary thresher. Portions of each sample were then planted in the fall following harvest in replicated field trials at CSU's Agricultural Research, Development, and Education Center (ARDEC) in Fort Collins. The number of seeds sown per sample was approximately 15,000 in 2003 and 11,250 in 2004 and 2005. Check plots of 'Above' were also included in the field design. The following April, when plants were at the 3- to 5-leaf stage, plots were sprayed with imazamox (Beyond® herbicide, BASF Corp.) and a surfactant at a rate of 5 ounces per acre. A second imazamox treatment was applied in early May at a rate of 4 ounces per acre to ensure adequate differentiation among herbicide-susceptible and herbicide-tolerant plants. In this system, we expect most plants derived from non-CLEARFIELD seed to be killed by the herbicide treatment, and only plants derived from cross-pollinated seed to survive.

Figure 1. Field sampling locations for pollen-mediated gene flow during this study.



Map © Ray Sterner and JHUAPL. http://fermi.jhuapl.edu/states/

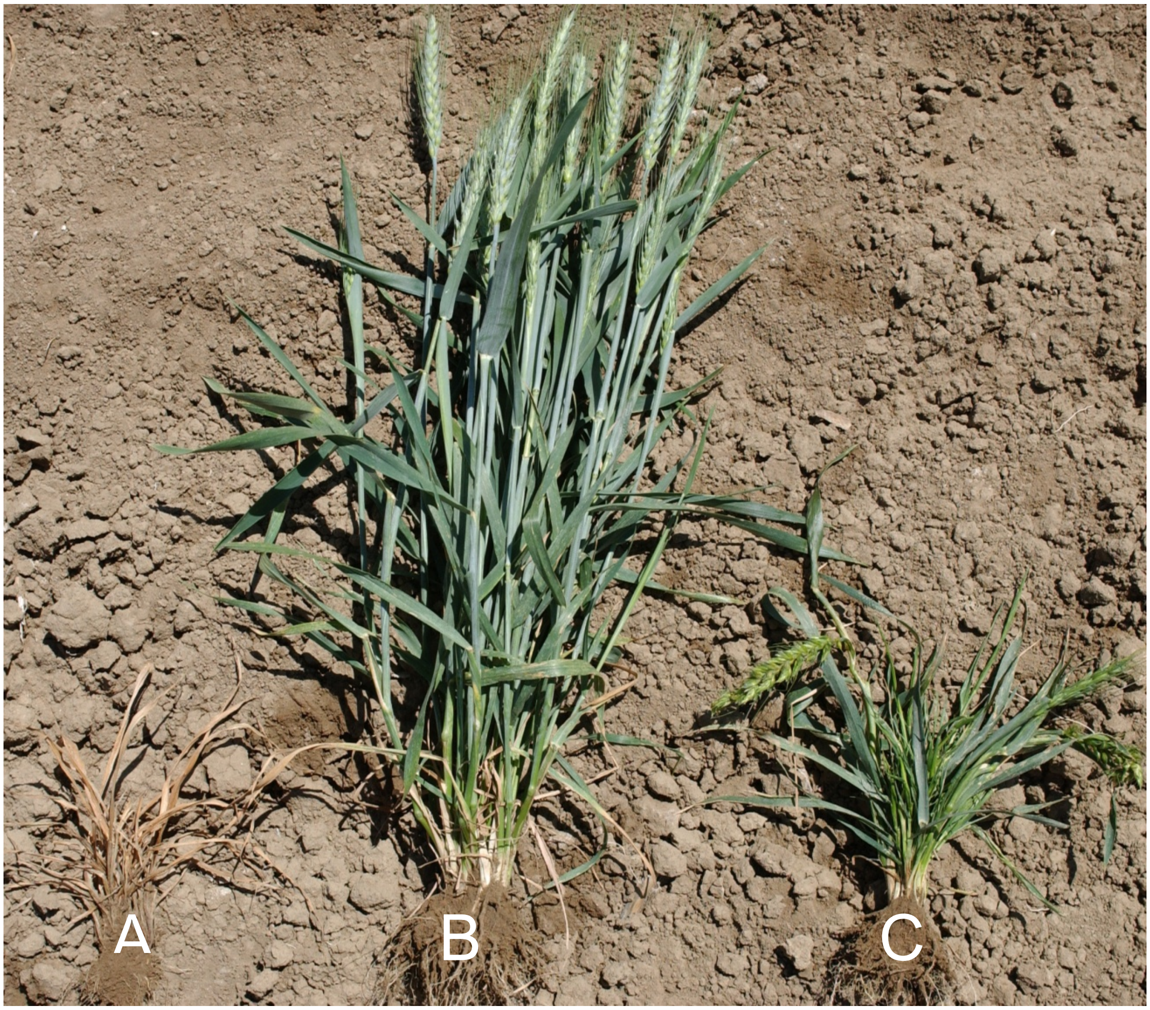
Table 1. Relative heading class (RHC) for cultivars used in this study.

Cultivar	RHC	Number of locations	Number of samples
Above	3	55	*
Akron	5	11	45
Alliance	5	3	16
Ankor	5	21	50
Avalanche	5	16	29
Bond CL	5	1	*
Enhancer	5	8	18
Halt	3	2	7
Hatcher	5	2	14
Ike	6	1	8
Jagalene	5	13	41
Jagger	2	8	57
Millennium	6	1	1
Platte	6	1	5
Prairie Red	1	11	72
Prowers 99	8	3	20
TAM 107	1	1	7
Trego	6	19	52
Yuma	5	2	9
Yumar	5	2	4

* This cultivar was a pollen source for the CLEARFIELD trait and therefore seed samples were not collected.

We counted the number of surviving plants in each plot in late May. A heterozygous (derived from cross-pollination between two pure-breeding cultivars) plant type was identified by a distinct set of symptoms that included mild foliar chlorosis, stunting, increased tillering, and twisted spikes (Figure 2). The total number of emerged plants per plot was estimated by counting subsamples. Percent cross-pollination per plot was then calculated as the number of heterozygous plants divided by the total number of plants, multiplied by 100. To verify that suspected heterozygous plants were in fact derived from cross-pollination, we extracted DNA from a subsample of those plants and conducted a polymerase chain reaction assay, with protocols provided by BASF Corp., to detect the presence of the CLEARFIELD gene.

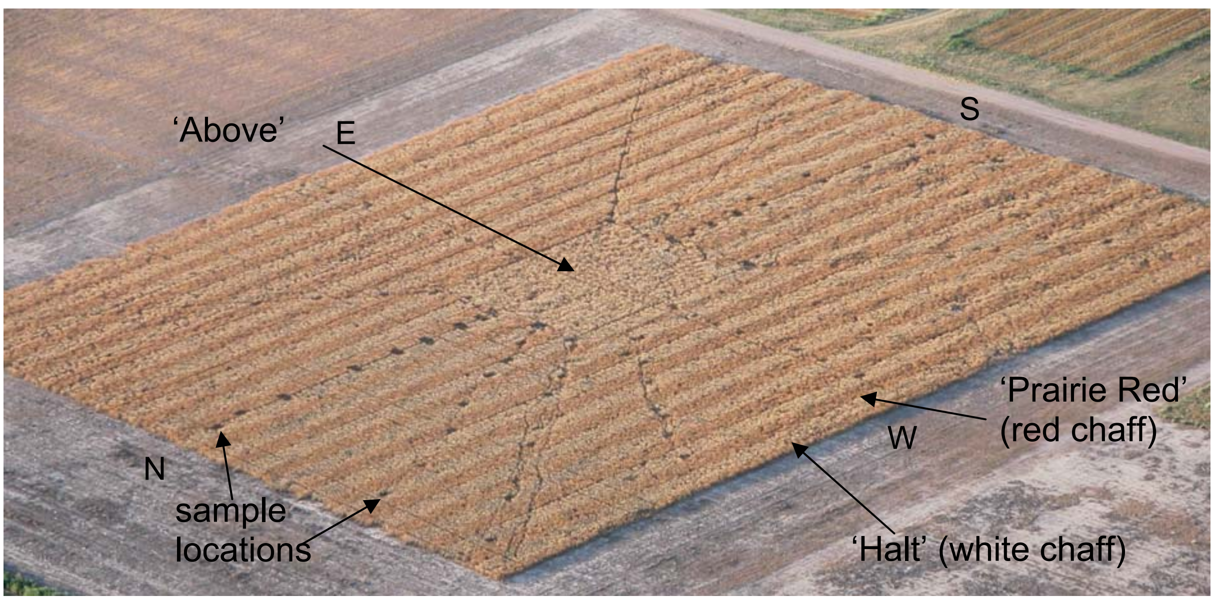
Figure 2. Three sets of symptoms observed in the field following application of imazamox. A, homozygous susceptible plant severely affected; B, homozygous tolerant plant (‘Above’) unaffected; C, heterozygous tolerant plant partially affected, indicating gene flow resulting from cross-pollination.



Experimental plots

Most published work on gene flow has been done in relatively small experiment station plots, rather than in “real world” commercial-scale fields. To compare our results from large fields (80 acres or greater) with results from much smaller experimental plots in a similar environment, we conducted a study at the USDA Central Great Plains Research Station in Akron, CO. We used a Nelder wheel design (Nelder, 1962) for trials planted in fall of 2003 and 2004 and harvested the following summer. A central 33-x-33-foot block of ‘Above’ was surrounded by alternating strips of the non-CLEARFIELD cultivars ‘Prairie Red’ and ‘Halt’. Heading dates, wind speed, and wind direction were recorded. Samples consisted of all wheat heads within a 3-foot-x-3-foot area at approximately 3, 10, 25, 35, 50, 75, 100, and 125 feet from the edge of the pollen source along eight transects radiating from the central plot in wagon wheel fashion (Figure 3). In 2004, two additional transects were added north-northwest and west-northwest directions. In some cases, samples were collected up to 230 feet from the pollen source.

Figure 3. The Nelder wheel plot at Akron, Colorado.



Because of the smaller number of seeds available from the experimental plots, we evaluated them with a greenhouse screening method rather than the field screening method described for commercial-scale samples. Samples from the 2004 Nelder wheel were planted in soil mix in rectangular flats, with two replications of 360 seeds each. At the 2- to 3-leaf stage plants were sprayed with imazamox at a rate of 4 ounces per acre in a calibrated spray chamber. Two days later, plants were clipped to approximately one-half inch above the newest leaf. Plants that re-grew and showed an injured, multi-tillered appearance were identified as heterozygous survivors. Percent PMGF was calculated as the number of survivors divided by the number of emerged plants, multiplied by 100. For the 2005 Nelder wheel trial, samples were screened with a more efficient method (Gaines et al, 2007b) that involved soaking seeds in an imazamox solution (25 micromolar, 8 parts per million), planting the seeds in flats, and spraying emerged plants with imazamox at a rate of 4 ounces per acre 10 to 14 days after emergence. Percent PMGF was calculated as the number of surviving plants divided by the expected number of emerged plants, multiplied by 100. To compare the results from the two greenhouse screening methods we used both methods to evaluate a set of 28 commercial samples collected in 2003. The correlation between the two sets of results was high (r = 0.90, P<0.0001), indicating that the two methods produced highly similar results. We verified a subsample of survivors from both methods with a polymerase chain reaction assay, as described for the commercial field samples.

Data analysis

Commercial fields. Analysis of variance for PMGF was conducted with the GLM procedure of SAS software (SAS, 2004), using samples taken within 20 feet of the pollen source. Means for each cultivar in each year were separated with Fisher's Least Significant Difference (LSD) at $\alpha = 0.05$.

Experimental plots. The GLM procedure of SAS (SAS, 2004) was used to conduct analysis of variance and to calculate overall means and means of samples in the range of 3 to 20 feet for each variety in each year.

Modeling gene flow. To better understand the factors that affect PMGF and to predict the frequency of PMGF at a given distance, we used the commercial field data to construct an empirical model. We started from the "General Wheat Model" (GWM) proposed by Gustafson et al. (2005), and developed the model through a series of steps described in detail in Gaines et al. (2007a). The final model includes equations for three lines that represent the median location response, the response for a location at the upper 95th percentile of locations, and the 95% confidence limit (indicating that we have 95% confidence that 95% of locations in a given RHC have gene flow less than this amount at a given distance).

Results and Discussion

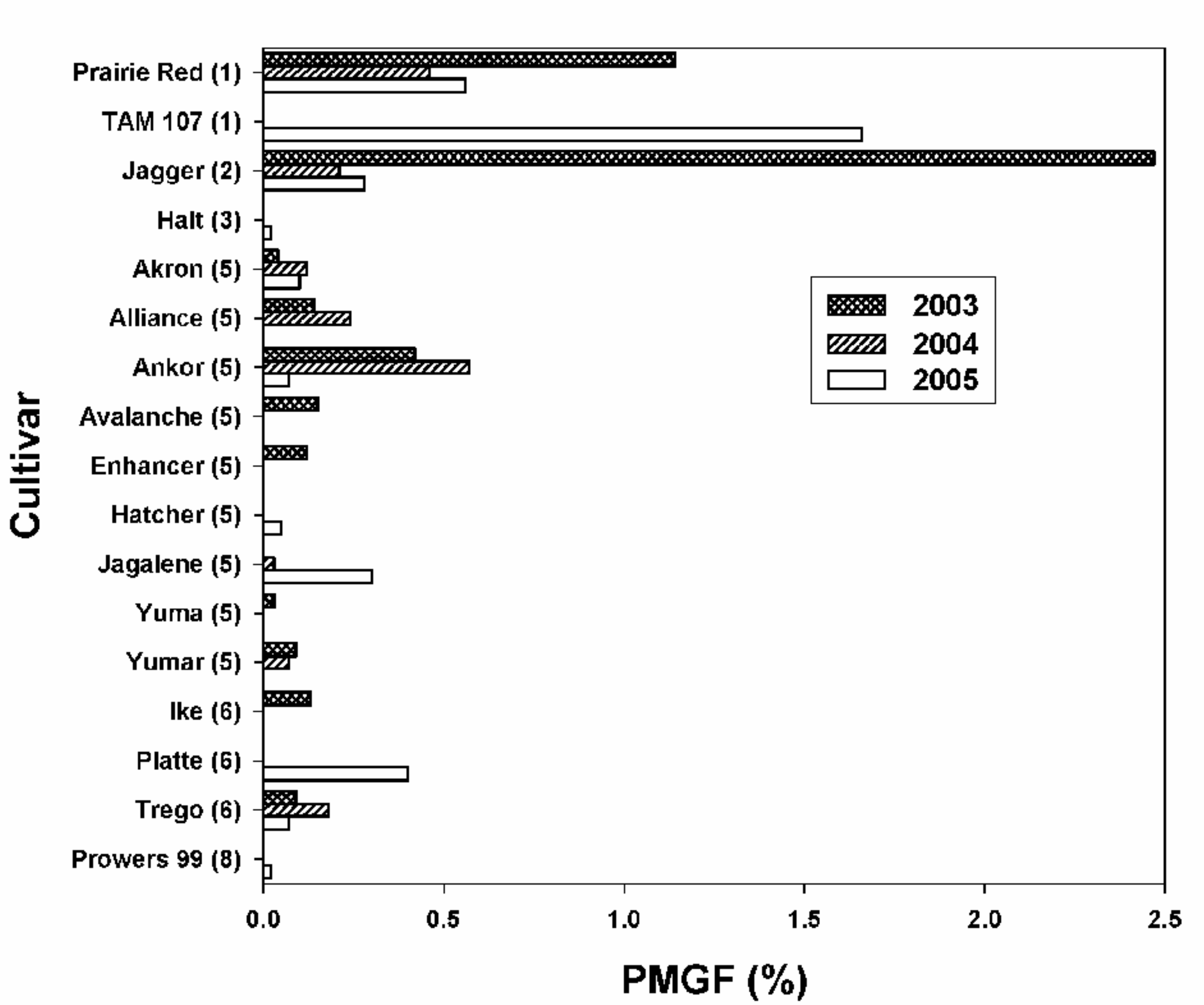
Commercial fields

Tolerance to the herbicide imazamox proved to be a reliable, high-throughput trait for the estimation and analysis of gene flow. Based on the average number of plants evaluated per sample, we were able to screen a total of 4 million plants in the three years of the study. DNA of 92 suspected heterozygous plants was analyzed by the PCR method, and all were confirmed as heterozygotes.

We detected PMGF in samples from all 56 locations and in all evaluated cultivars. The highest observed PMGF was 5.3% in a field of 'Jagger' sampled at 0.75 feet. The farthest distance at which PMGF was detected was 200 feet, our most distant sampling point, and the highest PMGF at that distance was 0.25% in a field of 'Prairie Red'. No PMGF was detected in 27% of the samples (Figure 4). Combined over all three years, 67% of observed gene flow occurred within 20 feet of the pollen source and 92% of observed gene flow occurred within 100 feet. Data on the location, distance, cultivar, and PMGF of all samples are compiled in the Appendix.

Analysis of variance of samples collected within 20 feet of the CLEARFIELD cultivar revealed that the recipient cultivar, year, and the cultivar x year interaction were all significant factors (P<0.01) influencing PMGF. 'Jagger', 'Prairie Red', and 'TAM 107', all early-heading cultivars in RHC 1 or 2, had mean PMGF >1% in at least one year (Figure 4). 'Ankor', in RHC 5, also had relatively high PMGF in two of three years.

Figure 4. Mean percent pollen-mediated gene flow (PMGF) in samples collected within 20 feet of CLEARFIELD wheat.



Experimental plots

In the smaller-scale experimental plots, we evaluated a total of 191 samples from the Akron site in 2004 and 2005. Samples from 2004 consisted of an average of 675 plants, while 2005 samples had an average of 5700 plants due to a higher throughput screening system. Only 7 of 98 samples (7%) had detectable gene flow in 2004, whereas 17 of 93 samples (18%) showed evidence of gene flow in 2005. Mean and maximum gene flow values and the maximum distance at which gene flow was detected were all higher in 'Prairie Red' than 'Halt'.

Comparison of results between experimental plots and commercial fields showed higher levels and farther distances of PMGF in commercial field samples. For example, for the cultivar 'Prairie Red', we detected a mean PMGF of 0.28% and a mean maximum distance of 177 feet in commercial fields vs. 0.03% mean PMGF and a mean maximum distance of 70 feet in the experimental plots. The differences, which are most likely due to the much larger size of the pollen source and a longer border between pollen source and recipient in the commercial-scale trials, will be important information for regulators and seed producers to keep in mind when reviewing results of small-scale trials.

Modeling gene flow

We developed a generalized linear mixed model with a random location effect to explain and predict PMGF (Figure 5). As seen in many other gene flow studies, the level of cross-pollination starts out at a relatively high level close to the pollen source, declines quickly with distance, then levels into a long "tail", where low frequencies of cross-pollination can occur at considerable distances. Based on estimates from the model, the distance required to ensure 95% confidence that 95% of locations would have PMGF less than 0.9% is 135 feet for the earliest-heading cultivars, 20 feet for early-intermediate cultivars, and 2.3 feet for intermediate- to late-heading cultivars (Table 2).

If a higher degree of purity is required, then the isolation distances become increasingly greater. For example, to keep PMGF below 0.1% would require an isolation distance of 997 feet for cultivars like 'Prairie Red' in the earliest heading class and 126 feet for cultivars like 'Hatcher' and 'Akron' in RHC 5 (Table 2).

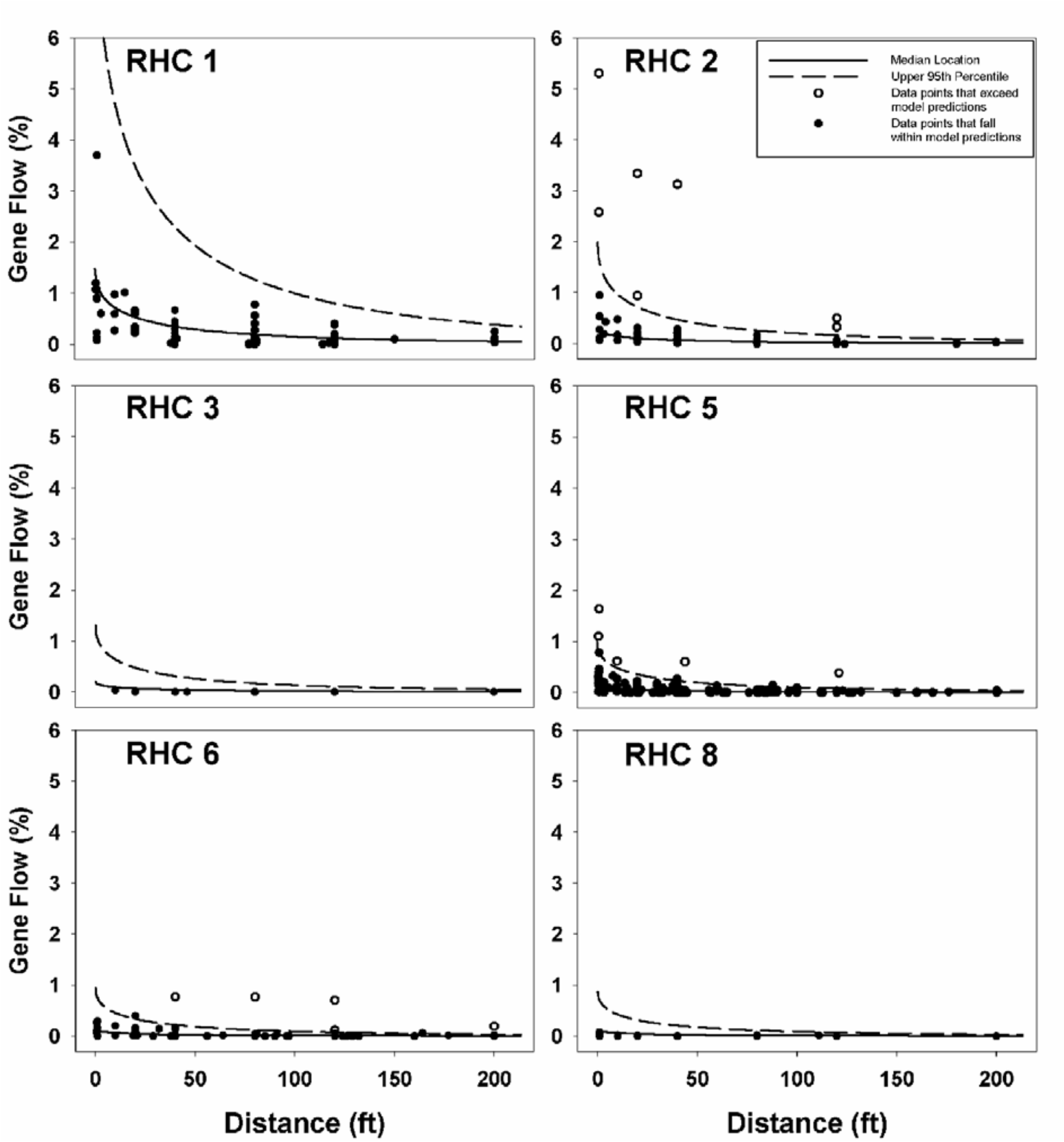
Table 2. Isolation distances (feet) required to be within the 95% confidence limit for the indicated maximum level of gene flow.

	Maximum level of pollen-mediated gene flow		
Relative heading class	**0.9%**	**0.5%**	**0.1%**
1	135	202	997
2	21	51	204
3	7.5	28	155
5	2.3	17	126
6	1.6	15	120
8	0.9	13	113

These confidence limits are very conservative estimates that represent the highest levels of PMGF expected to occur in winter wheat in eastern Colorado. The average level of PMGF in a seed lot will be reduced if seed is bulk-harvested and blended from sections of the field closest to and more distant from a pollen source.

Besides distance, one of the most significant factors in explaining PMGF was the flowering time of the recipient cultivar relative to the pollen source. The earliest-heading cultivars ('Prairie Red' and 'TAM 107' in RHC 1 and 'Jagger' in RHC 2) experienced the highest levels of gene flow from the pollen source ('Above' in RHC 3). Cultivars that headed later than 'Above' (RHC 5, 6, and 8) all had lower levels of PMGF. This trend suggests that bordering

Figure 5. Modeling of gene flow versus distance. Relative Heading Class 1 is the earliest ('Prairie Red', 'TAM 107') and 8 is the latest ('Prowers 99'). The open circles for RHC 2, 5, and 6 exceed the 95% curve. They represent cultivars that may have been affected by a late freeze.



fields are more susceptible to cross-pollination if environmental stresses cause some level of male sterility in recipient plants. If the pollen source sheds pollen somewhat later than the recipient cultivar, then pollen will be available for male sterile flowers of the recipient plants. In eastern Colorado, a fairly common scenario is a late frost in May while anthers are susceptible to freeze damage. Freezing or near-freezing temperatures that occur during the boot, heading, or anthesis stages can damage anthers and kill pollen, leaving the surviving stigmas more receptive to foreign pollen (Figure 6; Shroyer et al., 1995). At 3 of our 56 sampling locations the observed cross-pollination exceeded the model predictions; these samples are seen as the points above the curves in Figure 5 in the panels labeled RHC 2, RHC 5, and RHC 6. Weather stations near those three locations recorded near-freezing or freezing temperatures at some point during boot and heading stages, possibly cold enough to cause low levels of male sterility in the recipient cultivars, thus increasing the rate of outcrossing.

In addition to an early heading habit, other factors may predispose a cultivar to higher levels of gene flow. ‘Prairie Red’ and ‘Jagger’, for example, open their florets more widely than other cultivars (Gaines, 2006), making them more receptive to foreign pollen. Because of the limited number of cultivars in our study, it was not possible to determine whether earliness or other factors were the most important causes of higher levels of cross-pollination.

Figure 6. Damage caused by freezing temperatures. A. Healthy, undamaged flower. B. Freeze-damaged flower. The anthers become twisted and shriveled within 48 hours after a freeze. C. Freeze-damaged flower. Anthers turn white 3 to 5 days after a freeze. Photos by Jim Shroyer, Kansas State University.

Conclusions

Our study showed that significant levels of gene flow occur in wheat at the commercial field-scale, with some degree of cross-pollination detected in all 18 tested cultivars. Earlier-heading cultivars like ‘Prairie Red’ and ‘Jagger’ had higher levels of PMGF, possibly due to late frosts that caused some level of male sterility in recipient plants. There may also be flowering traits of certain cultivars, such as a wide glume opening, that make flowers more receptive to foreign pollen, or prolific pollen production, which makes plants more likely to cross-pollinate other plants. Higher levels and greater distances of PMGF occurred in large commercial fields than in smaller experimental plots, indicating that gene flow estimates from small wheat plots should be interpreted cautiously. We constructed a general linear mixed model to fit a median estimate of PMGF based on relative heading date and an upper estimate to account for unpredictable environmental variables, such as wind speed and direction. The calculated confidence limits are very conservative and should represent the highest levels of gene flow expected to occur in wheat in Colorado. These results should be useful to biotechnology regulatory agencies, seed production organizations, wheat growers, and others seeking to minimize gene flow in wheat.

II. Seed-Mediated Gene Flow from Wheat to Wheat

Objective

- To evaluate seed-mediated gene flow in certified and farmer-saved seed lots.

Materials and Methods

Sample collection

Certified and farm-saved seed samples were collected from eastern Colorado wheat producers. Producers were classified as to whether or not they had produced the imazamox-tolerant (IT) wheat cultivar ‘Above’ during the 2005 growing season or any previous season. Producers supplied information about crop rotations, field histories, and seed cleaning. Fifty non-IT seed samples produced in 2004 or 2005 were collected from five certified seed producers who had grown ‘Above’ both years. Seventeen non-IT seed samples produced in 2005 were collected from five certified seed producers who had never grown ‘Above’. Twelve non-IT seed samples produced in 2005 were collected from eight producers who saved seed of non-IT cultivars grown on their farms and also grew ‘Above’. Finally, 13 non-IT seed samples produced in 2005 were collected from 10 producers who saved seed of non-IT cultivars grown on their farms and had never grown any IT wheat.

Sample screening

The imazamox tolerance trait of ‘Above’ wheat provided a selectable marker that was used to screen large samples. A seed soaking method was used to determine the frequency of herbicide-tolerant seed in each sample. Seeds from each sample were soaked in imazamox herbicide (25 micromolar, 8 parts per million) for 24 hours (Figure 7).

Figure 7. Wheat seeds soaking in imazamox solution.

Each seed sample was then planted in potting soil and grown in the greenhouse (Figure 8).

A foliar imazamox application at 4 ounces per acre with 0.25% (v/v) non-ionic surfactant and 1.0% (v/v) urea ammonium nitrate was applied to samples 10 to 14 days after emergence to eliminate any susceptible plants that escaped imazamox treatment during seed soaking.

A total count of tolerant plants (Figure 9) from each sample was taken to calculate percent tolerant plants and survivors were transplanted.

Figure 8. Injured and stunted wheat seedlings emerging after soaking in imazamox solution.

Figure 9. A tolerant wheat plant that survived the imazamox treatment.

Leaf tissue samples were taken from surviving plants for genetic analysis. Using proprietary polymerase chain reaction (PCR) protocols and primers from BASF Corporation (Research Park Triangle, NC), survivors were tested for the presence of the tolerance gene found in the cultivar ‘Above’. The PCR protocol allowed determination of whether the surviving plants were homozygous or heterozygous for the trait.

Results and Discussion

Sample screening

Between 4,000 and 7,000 seeds were evaluated for each sample. Imazamox-tolerant seeds were detected in samples from certified and farm-saved growers (Figure 10A-10D). Observed levels of IT seed ranged from 0% to a high of 11.28%. One sample from a certified IT grower had 4.2% IT wheat in a non-IT seed lot (Figure 10A), which considerably exceeded the 0.1% certified seed threshold for presence of other cultivars. This sample was grown in a field that was in tilled fallow the previous season and planted to 'Above' (IT) wheat two seasons previously. One certified non-IT producer had detectable IT seed in one sample (Figure 10B). This producer commercially cleans wheat seed and cleaned seed for a grower who did produce IT wheat in 2005, which may explain the presence of IT wheat seed in one sample.

Three farm-saved samples from producers with a history of growing IT wheat were higher than 0.1% tolerant (Figure 10C). One grower produced the tested seed in a field that had been planted to 'Above' wheat two seasons previously. Another grower had not previously produced 'Above' in the same field as the sampled seed lots, but did harvest 'Above' before harvesting the non-IT varieties with the same equipment. The high IT seed levels in these samples may be due to mechanical mixture during harvest, or may reflect mixing during previous years in which 'Above' was grown and non-IT seed was saved.

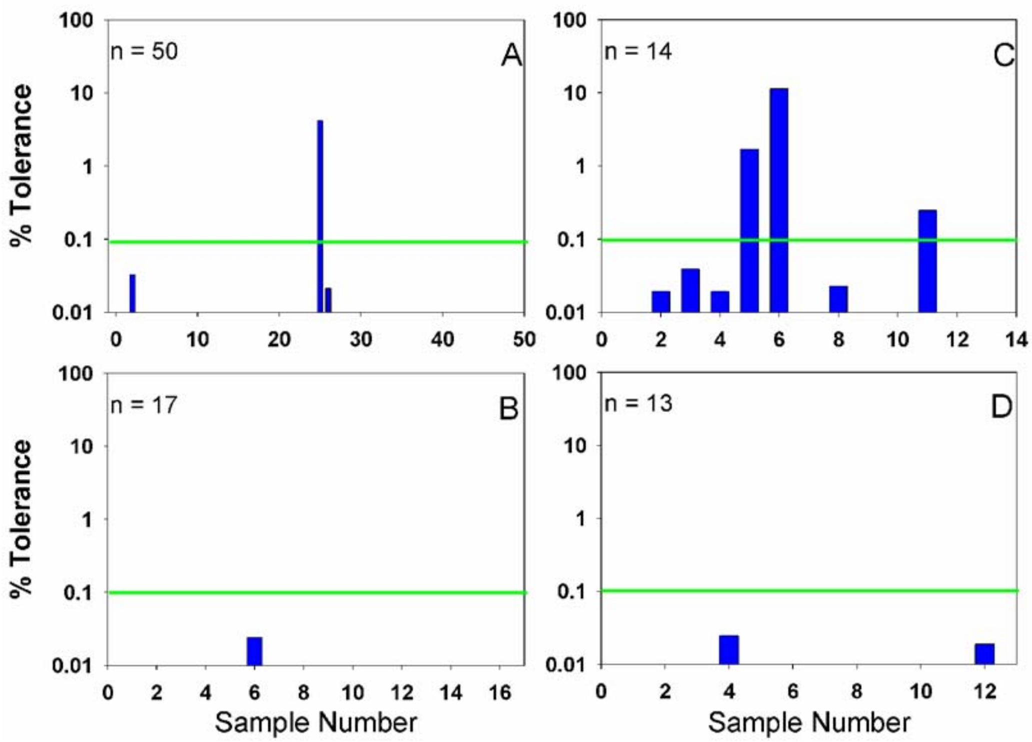
Statistical analysis showed that certified seed samples had a lower proportion of IT seed than farm-saved samples, and that IT seed was more likely to be found when a grower had a history of producing IT wheat. On average, certified and farm-saved producers had considerably lower IT presence than the 0.1% threshold for certified seed. However, that estimate does not indicate that levels of unwanted seed substantially above the 0.1% threshold will not occur, as some samples did exceed the threshold (Figures 10A and 10C).

Based on the PCR results from the tests on plants that survived the imazamox treatment, 93% of the plants tested were homozygous for the tolerance gene; the remaining 7% were heterozygous. All plants tested carried the tolerance gene found in 'Above'. Plants heterozygous for the tolerance gene, as determined by the PCR test, were found in one certified IT producer sample and in one farm-saved IT producer sample, with the majority of plants in both samples being homozygous. The sample containing a heterozygous plant from the certified producer (Figure 10A) was produced in a field that bordered 'Above' wheat, so pollen-mediated gene flow during the growing season was the most likely explanation. All tolerant plants from non-IT producers, whether certified or farm-saved, were homozygous.

These results indicate that seed-mediated gene flow is occurring in both certified and farm-saved seed production. Because most of the tolerant plants observed in non-IT seed lots were homozygous, seed mixture is a more likely explanation for the source of gene flow. If pollen drift during the growing season had occurred, heterozygous plants would have been observed. Producers of certified seed are less likely than farm-saved seed growers to have the presence of unwanted cultivars in a seed lot.

Our results suggest that a particular cultivar is more likely to be found in a different seed lot when a grower has a history of producing the cultivar. This implies that a grower with farm-

Figure 10. Percent imazamox-tolerant (IT) wheat seed detected in non-IT wheat seed lots from growers of certified seed with a history of IT seed production (A), growers of certified seed who had never produced IT wheat (B), farm-saved seed growers who had a history of IT seed production (C), and farm-saved seed growers who had never produced IT wheat (D). The green line represents the 0.1% standard for presence of off-types in certified seed.



saved seed and a history of producing a given cultivar has the highest probability for unintentional presence of that cultivar in other seed lots.

These results were obtained using a non-transgenic wheat cultivar with no marketing restrictions, but they have implications for policies regarding production of wheat seed with genetic traits that may be unacceptable in certain markets. A similar study in Canada using certified canola found that herbicide tolerance traits could be detected in seed of non-tolerant cultivars (Friesen et al., 2003) and concluded that varietal purity could not be maintained at a 99.75% level. Canola is a cross-pollinated crop and is therefore more likely to outcross than wheat, which is predominately self-pollinated. Nevertheless, the results of our study are consistent with the canola study, indicating that a “zero-tolerance” policy for seed lot purity is unachievable under current seed production practices.

Volunteer wheat plants from previous crops may be a potential source of seed-mediated gene flow. Volunteer wheat seed can survive at least 16 months in soil (Anderson and Soper, 2003). The Colorado seed certification standards establish land requirements for small grains (Anonymous, 2003) and recognize the importance of minimizing volunteer wheat to produce a pure seed lot. Certified seed cannot be produced on land where the same crop species but different variety was grown the previous cropping season. Registered seed cannot be produced on land where the same crop species but different variety was grown in either of the last two cropping seasons. Two growers had levels of IR wheat in some samples that were higher than 0.1% and were produced in fields where IR wheat had been grown two years previously, providing a one-year restriction. These examples demonstrate that volunteers can persist over a fallow period and that a one-year restriction may not always be sufficient to ensure less than 0.1% unintentional presence.

Mechanical mixture during harvesting and seed cleaning was associated with unintended presence of IT wheat. Colorado seed certification standards establish equipment cleaning requirements for harvesting and seed cleaning that would not apply to farm-saved seed production (Anonymous, 2003). Our results indicate that equipment cleaning requirements appear to be critical for reducing the frequency of unintended seed. The level of unwanted seed attributed solely to seed cleaning was less than 0.05%, which is likely acceptable.

Conclusions

Herbicide-tolerant wheat seed was detected in seed lots of herbicide-susceptible varieties from both certified and farm-saved seed growers. Based on interviews, higher levels of unwanted seed were associated with volunteer plants from previous crops and mechanical mixture during harvesting. Seed cleaning was associated with detectable but much lower levels of tolerant seed presence. Production guidelines for certified seed address these issues but may need to be strengthened if more restrictive purity regulations are adopted in the future.

III. Gene Flow from Wheat to Jointed Goatgrass

Objectives

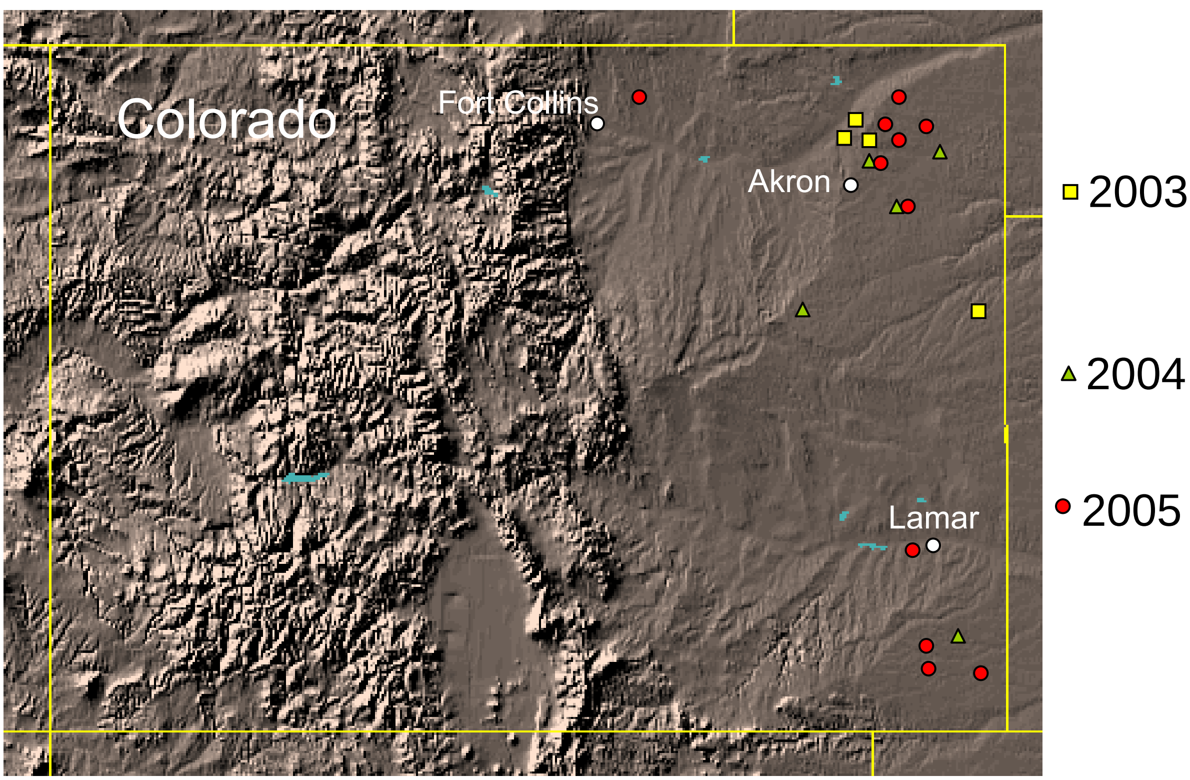
- To evaluate landscape-level gene flow from CLEARFIELD wheat to jointed goatgrass.
- To determine the activity of the herbicide-tolerant acetolactate synthase (*Als1*) allele in weed-wheat hybrids and in hybrids of herbicide-tolerant and -susceptible wheat.

Materials and Methods

Hybridization between CLEARFIELD imazamox-tolerant (IT) cultivars of winter wheat and jointed goatgrass was tested at commercial field sites in Colorado where jointed goatgrass was present as a naturally occurring weed and also in several experimental plots where jointed goatgrass was seeded near two cultivars of IT wheat.

At commercial sites where the IT cultivars 'Above' and 'Bond CL' were being grown, fields were scouted and the distance between naturally occurring jointed goatgrass plants and IT winter wheat plants was measured. Jointed goatgrass spikes were harvested by hand in June or July of 2003, 2004, and 2005 from maturing wheat fields (Figure 11).

Figure 11. Field sampling locations for gene flow from wheat to jointed goatgrass during the three years of this study. Map © Ray Sterner and JHUAPL. http://fermi.jhuapl.edu/states/



One experimental plot was planted at the USDA Central Great Plains Research Station in Akron, Colorado, in the fall of 2003. A Nelder wheel design (Nelder, 1962; Figure 3 on page 9 of this report) was used to test gene flow via pollen at multiple distances in all directions from a central plot of ‘Above’ IT winter wheat that measured 33 feet by 33 feet. The ‘Above’ cultivar was the source of IT pollen in this experiment. Two winter wheat cultivars, ‘Prairie Red’ (Quick et al., 2001) and ‘Halt’ (Quick et al., 1996), that are susceptible to imazamox herbicide, were planted in alternating strips 3 feet wide that ran from north to south across the field surrounding the central block of ‘Above’. In 2004, eight rays of jointed goatgrass, each 3 feet wide and 123 to 144 feet long, were planted extending outward like the spokes of a wheel in eight compass headings from the central plot of IT wheat. The imazamox-susceptible (IS) wheat simulated competition for IS wheat pollen that would normally be found in a field consisting mostly of wheat with some jointed goatgrass weeds. Wind speed and wind direction were recorded by a USDA weather station located about 1,095 feet from the Nelder wheel site. In 2005, naturally occurring jointed goatgrass plants were used to estimate gene flow, so rays of jointed goatgrass were not seeded in the field. In the summer of 2004 and 2005, jointed goatgrass spikes were collected at multiple distances from the central block of ‘Above’.

Additional experimental plots were planted in the fall of 2004, consisting of 33-foot-square blocks of ‘Above’ at Akron, Colorado, and Platner, Colorado, and 33-foot-by-66-foot blocks of ‘Above’ and ‘Bond CL’ at Fort Collins, Colorado. Jointed goatgrass was seeded into these plots along with the wheat. Spikes were collected in the summer of 2005.

Jointed goatgrass seeds were germinated in flats and were sprayed at the 2-leaf or 3-leaf stage with imazamox at a rate of 5 ounces per acre. The seedlings were clipped back 2 days later. Plants that survived and re-grew were identified as tolerant to the herbicide. Flats of ‘Above’ (IT), ‘Prairie Red’ (IS), and hybrids made by crossing ‘Above’ and ‘Prairie Red’ were used as comparisons when assessing the performance of the jointed goatgrass seedlings.

Plants that survived the herbicide treatment were examined to verify that they were hybrids, because tolerance to imazamox herbicides occurs naturally at a low rate in weeds. Verification was done by growing the plant to maturity and examining spikes for characteristic morphology associated with hybrids or by counting chromosomes in root tip spreads (Tsuchiya, 1971). Hybrids between wheat and jointed goatgrass have 35 chromosomes per cell (Figure 12), while jointed goatgrass has 28 chromosomes per cell.

Figure 12. Chromosome spread of a cell from a hybrid between wheat and jointed goatgrass.

After the hybrid status of surviving plants was established, the percentage hybridization was calculated as the number of hybrid survivors divided by the total number of plants subjected to the herbicide treatment, multiplied by 100.

The acetolactate synthase (ALS) function in known jointed goatgrass-wheat hybrids was measured using a modification of the method developed by Hanson et al. (2006). The results were compared to ALS function by ‘Above’ (IT), ‘Prairie Red’ (IS), and jointed goatgrass, evaluated in laboratory *in vitro* tests.

Results and Discussion

Heading dates for IT wheat and jointed goatgrass were similar, with 50%-heading dates occurring within three days of each other. Hybridization varied among sites and years, with the following results.

- The average percentage of hybridization between wheat and jointed goatgrass was 0.1% (Table 3).
- The highest percentage of hybridization in any single sample was 1.6% (Table 3).
- The greatest distance over which hybridization was observed to occur was 50 feet (Table 4).
- Acetolactate synthase function in pure jointed goatgrass was greatly reduced by the application of imazamox herbicide (Table 5).
- Hybrids between jointed goatgrass and IT 'Above' wheat were nearly as tolerant to herbicide applications as pure IT 'Above' wheat, and were more tolerant than hybrids of 'Above' (IT) and 'Prairie Red' (IS) wheat (Table 5).

Table 3. Hybridization rates in jointed goatgrass growing side-by-side with imazamox-tolerant (IT) winter wheat.

Collection Year	Site	Number of Samples	Number of Plants Screened	Number of Hybrids	Percent Hybridization
2004	Commercial fields	1	753	12	1.60
	Akron, CO	15	8,432	13	0.15
2005	Commercial fields	10	5,783	39	0.03
	Fort Collins, CO – 'Above'	10	9,531	1	0.01
	Fort Collins, CO – 'Bond CL'	10	7,277	0	0.00
	Platner, CO	3	2,154	0	0.00
	Akron, CO	18	4,842	11	0.23
Total		67	38,772	39	0.10[a]

[a] Mean hybridization (%) of all samples.

In this study, hybridization rates were relatively low and hybridization did not occur over long distances. Other studies have found hybridization at higher rates and over longer distances. For example, Morrison (2002) found up to 8% hybridization in Oregon and Hanson et al. (2005a) found hybridization at distances up to 132 feet in Nelder wheel experiments located in Washington and Idaho. Different environmental conditions may explain the different results found in this Colorado study.

The highest hybridization rate in a single sample in this study was 1.6%. At the site where this sample was taken, the farmer had planted IT wheat in a field infested with jointed goatgrass, intending to spray imazamox to kill the weed. The farmer later decided not to spray because the wheat plants were not expected to yield well. The dense population of jointed goatgrass in the field increased the likelihood that IT pollen would be captured by jointed goatgrass flowers instead of wheat flowers, leading to the higher than average hybridization in this field.

Resistance management plans emphasize that jointed goatgrass should be removed from IT wheat fields before heading, to reduce the chance of hybridization and subsequent transfer of the IT trait into weed populations (Seefeldt et al., 1998; Johnson et al., 2002; Tan, 2006). A required practice for CLEARFIELD stewardship is that certified seed must be planted every year, rather than using farm-saved seed. This reduces the chances that jointed goatgrass seed that obtained the herbicide tolerance gene through outcrossing with CLEARFIELD wheat will be planted together with the saved wheat seed. Recommendations for weed control to prevent outcrossing are outlined in a CSU fact sheet available at http://www.ext.colostate.edu/pubs/crops/03116.html and at http://www.ext.colostate.edu/pubs/crops/03116.pdf.

Table 4. Hybridization rates in jointed goatgrass growing at various distances from imazamox-tolerant (IT) winter wheat.

Collection Year	Site	Distance (feet)	Number of Samples	Number of Plants Screened	Number of Hybrids	Percent Hybrid-ization
2003	Commercial fields	1-40	3	2,034	0	0.00
2004	Commercial fields	1-3	5	2,629	0	0.00
	Akron	1-160	47	10,435	0	0.00
		1[a]	1	342	1	0.29
		4[b]	1	193	1	0.20
2005	Commercial fields	1-7	5	2,609	0	0.00
	Akron	1-70	11	4,163	0	0.00
		50[c]	1	173	1	0.60

[a], [b], [c]-sampled northwest, east, and south, respectively, from IT wheat.

The variant *Als1* allele that confers tolerance to imazamox herbicide appears to be highly effective in hybrids between jointed goatgrass and IT wheat. Weed-IT crop hybrids were nearly as tolerant as pure IT crop plants, and were more tolerant than hybrids between IT and IS cultivars of the crop. This high effectiveness may be due to the relatively larger contribution made by a single allele introduced into the smaller total genetic complement of jointed goatgrass. Jointed goatgrass is a tetraploid, with a total of four copies of wheat-like chromosomes, while bread wheat is a hexaploid, with a total of six copies of wheat chromosomes. Hybrids between jointed goatgrass and bread wheat are pentaploid, receiving two copies of wheat-like chromosomes from the weed parent and three copies of wheat chromosomes from the wheat parent. The variant *Als1* allele received from the IT wheat parent thus constitutes one-fifth of the *Als* alleles contained in the weed-wheat hybrid. An IT-IS wheat-wheat hybrid has six *Als* alleles, one of which is the variant allele received from the IT parent, so this variant allele constitutes one-sixth of the *Als* alleles, less than in the weed-wheat hybrid. The greater proportion of variant *Als1* alleles in weed-wheat hybrids translates into greater ALS activity and more effective tolerance to the herbicide (Table 5).

Table 5. Acetolactate synthase (ALS) activity under inhibition with 50 μM imazamox.

	Drop in ALS activity after application of imazamox	ALS activity remaining after application of imazamox
JGG/'Above' hybrid	60%	40%
'Prairie Red'/'Above' hybrid	74%	26%

Conclusions

Wheat and jointed goatgrass in eastern Colorado hybridize at a relatively low rate. In this study, the average percent hybridization when wheat and jointed goatgrass were grown side-by-side was 0.1%. The highest percentage of hybridization in a single sample was 1.6% in a field where the density of jointed goatgrass was abnormally high due to a management decision not to control the weeds. Hybridization occurred at a distance of up to 50 feet, suggesting that weed management plans should include the control of weeds in the immediate vicinity of a wheat field as well as within the field. In order for the tolerance gene to be integrated into jointed goatgrass populations, a hybrid plant must successfully cross with jointed goatgrass, an event believed to happen at very low rates due to the near total sterility of hybrid plants. However, even low-frequency events do occasionally occur and in this case would represent an environmental risk.

The IT trait is highly effective in jointed goatgrass once transferred, probably because the variant *Als1* allele that confers tolerance to the herbicide constitutes a larger percentage of the genome in weed-wheat hybrids than it does in wheat-wheat hybrids. Weed-wheat hybrids retained 40% of ALS activity after exposure to the herbicide, while hybrids between herbicide-susceptible wheat and herbicide-tolerant wheat retained 26% of ALS activity.

Transference of the IT trait from wheat to jointed goatgrass represents a risk to the future effectiveness of imazamox herbicides in winter wheat fields. The initial hybridization rate in eastern Colorado is relatively low, and the subsequent backcrossing rate of the hybrids to goatgrass is even lower, but the high effectiveness of the trait, once transferred, suggests that tolerance management is important if farmers wish to preserve the usefulness of this weed management tool.

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Appendix

Data for samples taken for the section Pollen-Mediated Gene Flow from Wheat to Wheat.

Sample ID	Year	Loc. ID	County	Wheat variety	RHC[1]	Dist. (ft)	Dir.[2]	No. of survivors	Total plants	PMGF
1	2003	4	Weld	Yuma	5	3	N	9	11391	0.081
2	2003	4	Weld	Yuma	5	20	N	2	8341	0.024
3	2003	4	Weld	Yuma	5	40	N	2	11177	0.015
4	2003	4	Weld	Yuma	5	60	N	2	9097	0.023
5	2003	4	Weld	Yuma	5	90	N	1	9936	0.009
6	2003	4	Weld	Yuma	5	120	N	1	8451	0.012
7	2003	5	Baca	Enhancer	5	0.75	N	30	9232	0.322
8	2003	5	Baca	Trego	6	0.75	S	4	3968	0.101
9	2003	5	Baca	Enhancer	5	32	N	2	8748	0.023
10	2003	5	Baca	Enhancer	5	64	N	1	9288	0.010
11	2003	5	Baca	Trego	6	64	S	1	8181	0.013
12	2003	6	Baca	Ankor	5	0.75	W	105	9502	1.101
13	2003	6	Baca	Avalanche	5	0.75	E	33	11688	0.294
14	2003	6	Baca	Ankor	5	20	W	21	9232	0.230
15	2003	6	Baca	Avalanche	5	20	E	0	8696	0.000
16	2003	3	Baca	Prairie Red	1	0.5	S	102	9448	1.080
17	2003	3	Baca	Prairie Red	1	0.5	N	112	9367	1.198
18	2003	3	Baca	Prairie Red	1	40	S	5	8638	0.059
19	2003	3	Baca	Prairie Red	1	40	N	12	8125	0.150
20	2003	3	Baca	Avalanche	5	41	S	0	8696	0.000
21	2003	3	Baca	Avalanche	5	80	S	0	8696	0.000
22	2003	3	Baca	Enhancer	5	80	N	0	8696	0.000
23	2003	3	Baca	Prairie Red	1	81	S	4	9097	0.041
24	2003	3	Baca	Prairie Red	1	81	N	6	7747	0.078
25	2003	3	Baca	Prairie Red	1	120	N	0	8696	0.000
26	2003	7	Weld	Yumar	5	0.75	S	16	8667	0.185
27	2003	7	Weld	Yumar	5	20	S	0	8696	0.000
28	2003	7	Weld	Trego	6	40	N	2	7477	0.013
29	2003	7	Weld	Avalanche	5	41	N	0	8696	0.000
30	2003	8	Sedgwick	Alliance	5	0.8	N	14	10581	0.141
31	2003	8	Sedgwick	Trego	6	0.8	S	14	8505	0.168
32	2003	8	Sedgwick	Alliance	5	32	N	8	11283	0.076
33	2003	8	Sedgwick	Trego	6	32	S	10	7423	0.140
34	2003	8	Sedgwick	Akron	5	33	N	7	7396	0.091
35	2003	8	Sedgwick	Enhancer	5	33	S	5	9529	0.053
36	2003	8	Sedgwick	Akron	5	64	N	0	8696	0.000
37	2003	8	Sedgwick	Enhancer	5	64	S	0	8696	0.000
38	2003	8	Sedgwick	Ankor	5	65	S	1	8532	0.012
39	2003	8	Sedgwick	Avalanche	5	65	N	0	8696	0.000
40	2003	8	Sedgwick	Ankor	5	96	S	1	8613	0.012
41	2003	8	Sedgwick	Avalanche	5	96	N	2	10017	0.018
42	2003	1	Kit Carson	Ike	6	0.75	W	5	7909	0.065
43	2003	1	Kit Carson	Ike	6	0.75	N	28	10851	0.262
44	2003	1	Kit Carson	Ike	6	20	W	8	9016	0.088
45	2003	1	Kit Carson	Ike	6	20	N	12	10581	0.110
46	2003	1	Kit Carson	Ike	6	40	W	1	9396	0.010
47	2003	1	Kit Carson	Ike	6	40	N	17	10122	0.167

Sample ID	Year	Loc. ID	County	Wheat variety	RHC[1]	Dist. (ft)	Dir.[2]	No. of survivors	Total plants	PMGF
48	2003	1	Kit Carson	Ike	6	120	W	3	8748	0.034
49	2003	1	Kit Carson	Ike	6	120	N	10	9232	0.113
50	2003	9	Logan	Prairie Red	1	40	N	0	8696	0.000
51	2003	9	Logan	Prairie Red	1	80	N	0	8696	0.000
52	2003	9	Logan	Prairie Red	1	120	N	1	8181	0.013
53	2003	34	Logan	Jagger	2	40	N	1	8559	0.012
54	2003	34	Logan	Jagger	2	80	N	4	5264	0.080
55	2003	34	Logan	Jagger	2	120	N	2	6318	0.051
56	2003	2	Kit Carson	Jagger	2	0.75	W	497	9340	5.308
57	2003	2	Kit Carson	Jagger	2	0.75	NE	145	5884	2.579
58	2003	2	Kit Carson	Jagger	2	20	W	253	7612	3.344
59	2003	2	Kit Carson	Jagger	2	20	NE	34	4130	0.940
60	2003	2	Kit Carson	Jagger	2	40	W	247	7855	3.129
61	2003	2	Kit Carson	Jagger	2	40	NE	13	3482	0.286
62	2003	2	Kit Carson	Jagger	2	120	W	30	5911	0.504
63	2003	2	Kit Carson	Jagger	2	120	NE	15	4616	0.325
64	2003	10	Weld	Yuma	5	0.75	E	2	9367	0.022
65	2003	10	Weld	Yuma	5	20	E	0	8696	0.000
66	2003	10	Weld	Ankor	5	40	W	1	7344	0.017
67	2003	10	Weld	Yuma	5	40	E	0	8696	0.000
68	2003	10	Weld	Ankor	5	80	W	1	7695	0.015
69	2003	11	Baca	Akron	5	3.5	N	8	3968	0.198
70	2003	11	Baca	Enhancer	5	3.5	S	1	9558	0.010
71	2003	11	Baca	Akron	5	16.8	N	0	8696	0.000
72	2003	11	Baca	Enhancer	5	16.8	S	0	8696	0.000
73	2003	11	Baca	Akron	5	32	N	0	8696	0.000
74	2003	11	Baca	Enhancer	5	32	S	6	7477	0.080
75	2003	11	Baca	Ankor	5	36	N	3	11580	0.025
76	2003	11	Baca	Trego	6	38	N	0	8696	0.000
77	2003	11	Baca	Ankor	5	64	N	1	9045	0.011
78	2003	11	Baca	Trego	6	96	N	0	8696	0.000
79	2003	11	Baca	Avalanche	5	100	N	0	8696	0.000
80	2003	11	Baca	Avalanche	5	128	N	0	8696	0.000
81	2003	12	Logan	Akron	5	1	E	3	7936	0.037
82	2003	12	Logan	Akron	5	1	W	3	7047	0.056
83	2003	12	Logan	Akron	5	14	E	1	9909	0.009
84	2003	12	Logan	Akron	5	14	W	0	8696	0.000
85	2003	12	Logan	Akron	5	28	E	0	8696	0.000
86	2003	12	Logan	Akron	5	56	W	1	8262	0.013
87	2003	12	Logan	Enhancer	5	57	W	0	8696	0.000
88	2003	12	Logan	Enhancer	5	112	W	0	8696	0.000
89	2003	12	Logan	Ankor	5	113	W	0	6858	0.000
90	2003	13	Adams	Trego	6	0.75	W	8	9016	0.094
91	2003	13	Adams	Akron	5	20	E	1	9720	0.009
92	2003	13	Adams	Trego	6	20	W	1	8397	0.012
93	2003	13	Adams	Akron	5	40	E	0	8696	0.000
94	2003	13	Adams	Trego	6	40	W	1	8181	0.013
95	2003	13	Adams	Enhancer	5	41	E	0	8696	0.000
96	2003	13	Adams	Enhancer	5	80	E	0	8696	0.000
97	2003	13	Adams	Avalanche	5	81	E	0	8696	0.000
98	2003	13	Adams	Avalanche	5	120	E	3	9018	0.032
99	2003	13	Adams	Ankor	5	121	E	31	8017	0.386

Appendix

Sample ID	Year	Loc. ID	County	Wheat variety	RHC[1]	Dist. (ft)	Dir.[2]	No. of survivors	Total plants	PMGF
100	2003	14	Sedgwick	Ankor	5	1	W	21	8044	0.265
101	2003	14	Sedgwick	Akron	5	20	E	2	10851	0.019
102	2003	14	Sedgwick	Ankor	5	20	W	5	6802	0.066
103	2003	14	Sedgwick	Akron	5	40	E	10	9178	0.110
104	2003	14	Sedgwick	Ankor	5	40	W	1	8505	0.012
105	2003	14	Sedgwick	Avalanche	5	41	W	1	8154	0.013
106	2003	14	Sedgwick	Enhancer	5	41	E	9	9801	0.083
107	2003	14	Sedgwick	Enhancer	5	76	E	0	8696	0.000
108	2003	14	Sedgwick	Avalanche	5	80	W	2	7776	0.029
109	2003	14	Sedgwick	Trego	6	81	E	3	7722	0.044
110	2003	14	Sedgwick	Trego	6	120	E	4	7992	0.055
111	2003	15	Kit Carson	Enhancer	5	0.75	N	15	8557	0.182
112	2003	15	Kit Carson	Enhancer	5	15	N	6	7693	0.099
113	2003	15	Kit Carson	Enhancer	5	30	N	5	8584	0.058
114	2003	15	Kit Carson	Ankor	5	31	N	0	8696	0.000
115	2003	15	Kit Carson	Ankor	5	60	N	4	7992	0.055
116	2003	15	Kit Carson	Akron	5	61	N	4	10743	0.039
117	2003	15	Kit Carson	Akron	5	90	N	6	11769	0.050
118	2003	15	Kit Carson	Trego	6	91	N	4	9639	0.038
119	2003	15	Kit Carson	Avalanche	5	120	N	0	8696	0.000
120	2003	15	Kit Carson	Trego	6	120	N	2	8073	0.027
121	2003	16	Morgan	Jagger	2	20	N	23	11607	0.199
122	2003	16	Morgan	Jagger	2	40	N	19	9313	0.203
123	2003	16	Morgan	Jagger	2	120	N	8	11688	0.071
124	2004	17	Kit Carson	Ankor	5	1	N	131	8201	1.639
125	2004	17	Kit Carson	Ankor	5	44	N	54	8947	0.604
126	2004	17	Kit Carson	Ankor	5	88	N	12	8201	0.145
127	2004	17	Kit Carson	Jagalene	5	89	N	1	8201	0.015
128	2004	17	Kit Carson	Jagalene	5	132	N	1	7348	0.016
129	2004	17	Kit Carson	Jagalene	5	176	N	1	8947	0.011
130	2004	17	Kit Carson	Trego	6	177	N	1	8201	0.011
131	2004	18	Baca	Prairie Red	1	1	N	77	8177	0.915
132	2004	18	Baca	Prairie Red	1	1	N	77	8676	0.895
133	2004	18	Baca	Prairie Red	1	1	N	7	8947	0.078
134	2004	18	Baca	Prairie Red	1	20	N	27	8201	0.339
135	2004	18	Baca	Prairie Red	1	20	N	28	9964	0.276
136	2004	18	Baca	Avalanche	5	28	S	0	8201	0.000
137	2004	18	Baca	Trego	6	29	S	0	11294	0.000
138	2004	18	Baca	Prairie Red	1	40	N	19	8201	0.220
139	2004	18	Baca	Prairie Red	1	40	N	23	7456	0.320
140	2004	18	Baca	Prairie Red	1	40	N	49	7456	0.667
141	2004	18	Baca	Trego	6	56	S	0	8947	0.000
142	2004	18	Baca	Jagalene	5	57	S	0	8039	0.000
143	2004	18	Baca	Prairie Red	1	80	N	12	8429	0.139
144	2004	18	Baca	Prairie Red	1	80	N	8	8676	0.089
145	2004	18	Baca	Jagalene	5	84	S	0	8039	0.000
146	2004	18	Baca	Ankor	5	85	S	0	8676	0.000
147	2004	18	Baca	Ankor	5	112	S	1	8676	0.011
148	2004	18	Baca	Prairie Red	1	114	S	1	8947	0.011
149	2004	18	Baca	Prairie Red	1	120	N	11	8947	0.123
150	2004	18	Baca	Prairie Red	1	120	N	3	6372	0.050
151	2004	19	Baca	Prairie Red	1	1	W	7	6083	0.122

Sample ID	Year	Loc. ID	County	Wheat variety	RHC[1]	Dist. (ft)	Dir.[2]	No. of survivors	Total plants	PMGF
152	2004	19	Baca	Prairie Red	1	1	E	19	8201	0.224
153	2004	19	Baca	Prairie Red	1	38	W	2	8201	0.022
154	2004	19	Baca	Ankor	5	39	E	2	7984	0.026
155	2004	19	Baca	Jagalene	5	39	W	1	8201	0.011
156	2004	19	Baca	Ankor	5	76	E	0	8947	0.000
157	2004	19	Baca	Jagalene	5	76	W	0	8201	0.000
158	2004	19	Baca	Prairie Red	1	77	W	0	8730	0.000
159	2004	19	Baca	Prairie Red	1	77	E	1	5566	0.020
160	2004	19	Baca	Prairie Red	1	114	W	0	8201	0.000
161	2004	19	Baca	Prairie Red	1	117	E	2	8947	0.022
162	2004	20	Phillips	Alliance	5	10	SW	9	7930	0.109
163	2004	20	Phillips	Alliance	5	10	NW	24	8947	0.268
164	2004	20	Phillips	Alliance	5	14	W	15	8459	0.181
165	2004	20	Phillips	Alliance	5	30	W	16	8947	0.179
166	2004	20	Phillips	Alliance	5	30	SW	14	7583	0.187
167	2004	20	Phillips	Alliance	5	30	NW	13	8947	0.145
168	2004	20	Phillips	Alliance	5	60	W	10	7456	0.134
169	2004	20	Phillips	Alliance	5	60	SW	8	7456	0.104
170	2004	20	Phillips	Alliance	5	60	NW	6	8201	0.071
171	2004	20	Phillips	Alliance	5	100	W	8	8947	0.089
172	2004	20	Phillips	Alliance	5	100	SW	2	8947	0.022
173	2004	20	Phillips	Alliance	5	100	NW	6	8201	0.075
174	2004	21	Phillips	Alliance	5	1	E	30	7507	0.408
175	2004	21	Phillips	Ankor	5	1	W	24	8947	0.268
176	2004	21	Phillips	Alliance	5	40	E	2	8201	0.026
177	2004	21	Phillips	Ankor	5	42	W	4	8201	0.056
178	2004	21	Phillips	Avalanche	5	43	W	3	8201	0.034
179	2004	21	Phillips	Avalanche	5	84	W	0	8730	0.000
180	2004	21	Phillips	Jagalene	5	85	W	0	7930	0.000
181	2004	21	Phillips	Jagalene	5	126	W	0	8947	0.000
182	2004	21	Phillips	Trego	6	127	W	2	10316	0.020
183	2004	22	Sedgwick	Jagalene	5	1	N	3	8304	0.041
184	2004	22	Sedgwick	Jagalene	5	20	N	1	8201	0.011
185	2004	22	Sedgwick	Ankor	5	32	S	2	7456	0.026
186	2004	22	Sedgwick	Avalanche	5	33	S	0	8947	0.000
187	2004	22	Sedgwick	Avalanche	5	64	S	1	8201	0.010
188	2004	22	Sedgwick	Jagalene	5	65	S	0	8091	0.000
189	2004	22	Sedgwick	Jagalene	5	96	S	0	8947	0.000
190	2004	22	Sedgwick	Trego	6	97	S	0	7456	0.000
191	2004	22	Sedgwick	Trego	6	128	S	1	8947	0.011
192	2004	22	Sedgwick	Millenium	6	129	S	0	7456	0.000
193	2004	23	Baca	Ankor	5	1	N	19	8505	0.222
194	2004	23	Baca	Jagalene	5	1	S	5	10980	0.042
195	2004	23	Baca	Ankor	5	44	N	4	8784	0.045
196	2004	23	Baca	Jagalene	5	44	S	0	7456	0.000
197	2004	23	Baca	Avalanche	5	45	N	0	8947	0.000
198	2004	23	Baca	Halt	3	46	S	0	8947	0.000
199	2004	23	Baca	Avalanche	5	87	N	2	11929	0.017
200	2004	23	Baca	Trego	6	90	N	0	8947	0.000
201	2004	23	Baca	Trego	6	132	N	0	7456	0.000
202	2004	24	Lincoln	Jagalene	5	1	S	1	7998	0.011
203	2004	24	Lincoln	Trego	6	1	N	14	8730	0.161

Sample ID	Year	Loc. ID	County	Wheat variety	RHC[1]	Dist. (ft)	Dir.[2]	No. of survivors	Total plants	PMGF
204	2004	24	Lincoln	Jagalene	5	21	S	0	8947	0.000
205	2004	24	Lincoln	Trego	6	21	N	1	7457	0.015
206	2004	24	Lincoln	Ankor	5	22	N	5	8201	0.056
207	2004	24	Lincoln	Ankor	5	42	N	0	8947	0.000
208	2004	24	Lincoln	Avalanche	5	43	N	0	8947	0.000
209	2004	24	Lincoln	Avalanche	5	63	N	0	7456	0.000
210	2004	25	Logan	Akron	5	1	E	24	10438	0.235
211	2004	25	Logan	Ankor	5	1	W	40	9693	0.414
212	2004	25	Logan	Akron	5	20	E	0	7290	0.000
213	2004	25	Logan	Ankor	5	56	W	4	8201	0.048
214	2004	25	Logan	Avalanche	5	57	W	0	7374	0.000
215	2004	25	Logan	Avalanche	5	112	W	0	11184	0.000
216	2004	25	Logan	Jagalene	5	113	W	0	8947	0.000
217	2004	25	Logan	Jagalene	5	168	W	0	6710	0.000
218	2004	26	Logan	Akron	5	3	S	8	8676	0.091
219	2004	26	Logan	Akron	5	20	S	4	10506	0.036
220	2004	26	Logan	Akron	5	40	S	2	8947	0.022
221	2004	26	Logan	Akron	5	80	S	2	8947	0.022
222	2004	26	Logan	Akron	5	120	S	1	7456	0.015
223	2004	26	Logan	Akron	5	150	S	0	7957	0.000
224	2004	27	Washington	Akron	5	8	S	27	8201	0.328
225	2004	27	Washington	Akron	5	20	S	10	6548	0.151
226	2004	27	Washington	Akron	5	40	S	13	8201	0.156
227	2004	27	Washington	Akron	5	80	S	3	8201	0.034
228	2004	27	Washington	Akron	5	120	S	3	8201	0.037
229	2004	27	Washington	Akron	5	160	S	1	8201	0.011
230	2004	27	Washington	Akron	5	200	S	0	8676	0.000
231	2004	28	Yuma	Jagger	2	1	S	60	10980	0.538
232	2004	28	Yuma	Prairie Red	1	1	E	91	8580	1.059
233	2004	28	Yuma	Jagger	2	4	S	38	8947	0.425
234	2004	28	Yuma	Jagger	2	20	S	6	7930	0.075
235	2004	28	Yuma	Jagger	2	20	S	19	8201	0.216
236	2004	28	Yuma	Prairie Red	1	20	E	16	7185	0.225
237	2004	28	Yuma	Jagger	2	40	S	12	8039	0.149
238	2004	28	Yuma	Jagger	2	40	S	8	7456	0.104
239	2004	28	Yuma	Prairie Red	1	40	E	22	8947	0.246
240	2004	28	Yuma	Jagger	2	80	S	6	8201	0.078
241	2004	28	Yuma	Jagger	2	80	S	13	8201	0.160
242	2004	28	Yuma	Prairie Red	1	80	E	10	8947	0.112
243	2004	28	Yuma	Jagger	2	120	S	2	8039	0.022
244	2004	28	Yuma	Jagger	2	120	S	2	8089	0.022
245	2004	28	Yuma	Prairie Red	1	120	E	4	8201	0.045
246	2004	28	Yuma	Prairie Red	1	150	E	9	8730	0.101
247	2004	28	Yuma	Jagger	2	180	S	2	8201	0.022
248	2004	28	Yuma	Jagger	2	180	S	1	8201	0.011
249	2004	29	Sedgwick	Jagger	2	1	N	10	8947	0.112
250	2004	29	Sedgwick	Ankor	5	1	S	36	8947	0.402
251	2004	29	Sedgwick	Jagger	2	20	N	3	8947	0.034
252	2004	29	Sedgwick	Jagger	2	40	N	2	8201	0.026
253	2004	29	Sedgwick	Ankor	5	41	S	7	8201	0.082
254	2004	29	Sedgwick	Avalanche	5	42	S	2	7243	0.045
255	2004	29	Sedgwick	Jagger	2	80	N	0	8060	0.000

Sample ID	Year	Loc. ID	County	Wheat variety	RHC[1]	Dist. (ft)	Dir.[2]	No. of survivors	Total plants	PMGF
256	2004	29	Sedgwick	Avalanche	5	82	S	4	8947	0.028
257	2004	29	Sedgwick	Jagalene	5	83	S	1	8676	0.012
258	2004	29	Sedgwick	Jagger	2	120	N	1	8947	0.011
259	2004	29	Sedgwick	Jagalene	5	123	S	3	7456	0.037
260	2004	29	Sedgwick	Trego	6	124	S	0	7456	0.000
261	2004	29	Sedgwick	Trego	6	164	S	5	8387	0.057
262	2004	30	Weld	Ankor	5	1	E	41	8947	0.458
263	2004	30	Weld	Yumar	5	1	W	12	8947	0.134
264	2004	30	Weld	Yumar	5	20	W	0	8093	0.000
265	2004	30	Weld	Ankor	5	42	E	5	7930	0.059
266	2004	30	Weld	Avalanche	5	43	E	2	8201	0.022
267	2004	30	Weld	Avalanche	5	84	E	0	8201	0.000
268	2004	30	Weld	Trego	6	85	E	0	7456	0.000
269	2004	30	Weld	Trego	6	126	E	0	8947	0.000
270	2004	30	Weld	Jagalene	5	127	E	0	8201	0.000
271	2004	31	Yuma	Jagger	2	3	N	17	8676	0.196
272	2004	31	Yuma	Jagger	2	20	N	8	8947	0.089
273	2004	31	Yuma	Ankor	5	38	S	11	8201	0.142
274	2004	31	Yuma	Jagger	2	40	N	7	8201	0.093
275	2004	31	Yuma	Jagger	2	80	N	1	8947	0.011
276	2004	31	Yuma	Jagger	2	124	N	0	8201	0.000
277	2004	31	Yuma	Jagger	2	180	N	0	8201	0.000
278	2004	32	Washington	Akron	5	20	W	0	8947	0.000
279	2004	32	Washington	Akron	5	40	W	3	8201	0.037
280	2004	32	Washington	Akron	5	80	W	2	11184	0.020
281	2004	32	Washington	Akron	5	120	W	0	8947	0.000
282	2004	32	Washington	Akron	5	160	W	0	8201	0.000
283	2004	32	Washington	Akron	5	200	W	0	7252	0.000
284	2004	33	Lincoln	Trego	6	1	E	34	11184	0.293
285	2004	33	Lincoln	Trego	6	20	E	7	7754	0.086
286	2004	33	Lincoln	Trego	6	40	E	12	8947	0.134
287	2004	33	Lincoln	Trego	6	80	E	4	8947	0.045
288	2004	33	Lincoln	Trego	6	120	E	0	7876	0.000
289	2004	33	Lincoln	Trego	6	160	E	0	7049	0.000
290	2004	33	Lincoln	Trego	6	200	E	1	8947	0.011
291	2005	34	.	.	.	1	W	0	9384	0.000
292	2005	34	.	.	.	10	W	0	9384	0.000
293	2005	34	.	.	.	20	W	1	9384	0.011
294	2005	34	.	.	.	40	W	0	6256	0.000
295	2005	34	.	.	.	80	W	0	9384	0.000
296	2005	34	.	.	.	120	W	1	9384	0.011
297	2005	34	.	.	.	200	W	0	9384	0.000
298	2005	35	Adams	Ankor	5	2	N	19	9384	0.202
299	2005	35	Adams	Ankor	5	10	N	11	9384	0.117
300	2005	35	Adams	Ankor	5	20	N	4	9384	0.043
301	2005	35	Adams	Ankor	5	40	N	19	9384	0.202
302	2005	35	Adams	Ankor	5	80	N	0	9384	0.000
303	2005	35	Adams	Ankor	5	120	N	0	9384	0.000
304	2005	35	Adams	Ankor	5	200	N	0	8602	0.000
305	2005	36	Phillips	Hatcher	5	6	E	8	9384	0.085
306	2005	36	Phillips	Hatcher	5	10	E	3	9384	0.032
307	2005	36	Phillips	Hatcher	5	20	E	1	9384	0.011

Sample ID	Year	Loc. ID	County	Wheat variety	RHC[1]	Dist. (ft)	Dir.[2]	No. of survivors	Total plants	PMGF
308	2005	36	Phillips	Hatcher	5	40	E	2	9384	0.021
309	2005	36	Phillips	Hatcher	5	80	E	0	9384	0.000
310	2005	36	Phillips	Hatcher	5	120	E	0	9384	0.000
311	2005	36	Phillips	Hatcher	5	200	E	0	9384	0.000
312	2005	36	Phillips	Platte	6	20	E	27	6706	0.403
313	2005	36	Phillips	Platte	6	40	E	65	8430	0.771
314	2005	36	Phillips	Platte	6	80	E	56	7277	0.770
315	2005	36	Phillips	Platte	6	120	E	26	3703	0.702
316	2005	36	Phillips	Platte	6	200	E	10	5228	0.191
317	2005	37	Morgan	Akron	5	1	E	18	9384	0.192
318	2005	37	Morgan	Akron	5	10	E	3	9384	0.032
319	2005	37	Morgan	Akron	5	20	E	6	9384	0.064
320	2005	37	Morgan	Akron	5	40	E	4	9384	0.043
321	2005	37	Morgan	Akron	5	80	E	5	9384	0.053
322	2005	37	Morgan	Akron	5	120	E	1	9384	0.011
323	2005	37	Morgan	Akron	5	200	E	4	9384	0.043
324	2005	38	Washington	Trego	6	1	N	0	454	0.000
325	2005	38	Washington	Trego	6	10	N	4	1973	0.203
326	2005	38	Washington	Trego	6	20	N	9	5656	0.159
327	2005	38	Washington	Trego	6	40	N	13	9384	0.139
328	2005	38	Washington	Trego	6	80	N	2	9384	0.021
329	2005	38	Washington	Trego	6	200	N	2	9384	0.021
330	2005	39	Washington	Trego	6	19	N	1	9384	0.011
331	2005	39	Washington	Trego	6	40	N	2	9384	0.021
332	2005	39	Washington	Trego	6	80	N	1	9384	0.011
333	2005	39	Washington	Trego	6	120	N	0	9384	0.000
334	2005	39	Washington	Trego	6	200	W	0	9384	0.000
335	2005	40	Sedgwick	Jagger	2	1	W	89	9384	0.948
336	2005	40	Sedgwick	Jagger	2	10	W	45	9384	0.480
337	2005	40	Sedgwick	Jagger	2	20	W	29	9384	0.309
338	2005	40	Sedgwick	Jagger	2	40	W	21	9384	0.224
339	2005	40	Sedgwick	Jagger	2	80	W	16	9384	0.170
340	2005	40	Sedgwick	Jagger	2	200	W	4	9384	0.043
341	2005	40	Sedgwick	Jagger	2	1	W	26	9384	0.277
342	2005	40	Sedgwick	Jagger	2	10	W	16	9384	0.170
343	2005	40	Sedgwick	Jagger	2	20	W	12	9384	0.128
344	2005	40	Sedgwick	Jagger	2	40	W	24	9384	0.256
345	2005	40	Sedgwick	Jagger	2	80	W	5	9384	0.053
346	2005	40	Sedgwick	Jagger	2	120	W	0	9384	0.000
347	2005	40	Sedgwick	Jagger	2	200	W	3	9384	0.032
348	2005	41	Prowers	Prowers 99	8	1	N	0	6503	0.000
349	2005	41	Prowers	Prowers 99	8	10	N	2	9384	0.021
350	2005	41	Prowers	Prowers 99	8	20	N	0	9384	0.000
351	2005	41	Prowers	Prowers 99	8	40	N	0	9384	0.000
352	2005	41	Prowers	Prowers 99	8	80	N	0	9384	0.000
353	2005	41	Prowers	Prowers 99	8	111	N	1	9384	0.011
354	2005	42	Prowers	Prowers 99	8	1	N	2	9384	0.021
355	2005	42	Prowers	Prowers 99	8	10	N	0	6256	0.000
356	2005	42	Prowers	Prowers 99	8	20	N	0	9384	0.000
357	2005	42	Prowers	Prowers 99	8	40	N	0	9384	0.000
358	2005	42	Prowers	Prowers 99	8	80	N	0	9384	0.000
359	2005	42	Prowers	Prowers 99	8	120	N	0	9384	0.000

Sample ID	Year	Loc. ID	County	Wheat variety	RHC[1]	Dist. (ft)	Dir.[2]	No. of survivors	Total plants	PMGF
360	2005	42	Prowers	Prowers 99	8	200	N	0	9384	0.000
361	2005	43	Prowers	Ankor	5	1	S	3	9384	0.032
362	2005	43	Prowers	Ankor	5	10	S	2	9384	0.021
363	2005	43	Prowers	Ankor	5	20	S	1	9384	0.011
364	2005	43	Prowers	Ankor	5	40	S	0	9384	0.000
365	2005	43	Prowers	Ankor	5	80	S	0	9384	0.000
366	2005	43	Prowers	Ankor	5	120	S	1	9384	0.011
367	2005	43	Prowers	Ankor	5	200	S	0	9384	0.000
368	2005	43	Prowers	PR	1	41	N	10	9384	0.107
369	2005	43	Prowers	PR	1	80	N	73	9384	0.778
370	2005	43	Prowers	PR	1	120	N	38	9384	0.405
371	2005	43	Prowers	PR	1	200	N	11	9384	0.117
372	2005	44	Prowers	Halt	3	10	W	3	9384	0.032
373	2005	44	Prowers	Halt	3	20	W	0	9384	0.000
374	2005	44	Prowers	Halt	3	40	W	0	9384	0.000
375	2005	44	Prowers	Halt	3	80	W	0	9384	0.000
376	2005	44	Prowers	Halt	3	120	W	0	8117	0.000
377	2005	44	Prowers	Halt	3	200	W	0	9384	0.000
378	2005	45	Baca	PR	1	10	S	92	9384	0.980
379	2005	45	Baca	PR	1	20	S	56	9384	0.597
380	2005	45	Baca	PR	1	40	S	42	9384	0.448
381	2005	45	Baca	PR	1	80	S	53	9384	0.565
382	2005	45	Baca	PR	1	120	S	19	9384	0.202
383	2005	45	Baca	PR	1	200	S	12	9384	0.128
384	2005	45	Baca	Trego	6	10	S	1	9384	0.011
385	2005	45	Baca	Trego	6	20	S	1	9384	0.011
386	2005	45	Baca	Trego	6	40	S	0	9384	0.000
387	2005	45	Baca	Trego	6	80	S	0	9384	0.000
388	2005	45	Baca	Trego	6	120	S	0	9384	0.000
389	2005	45	Baca	Trego	6	200	S	0	9384	0.000
390	2005	46	Baca	TAM 107	1	1	E	282	7608	3.706
391	2005	46	Baca	TAM 107	1	10	E	56	9384	0.597
392	2005	46	Baca	TAM 107	1	20	E	63	9384	0.671
393	2005	46	Baca	TAM 107	1	40	E	34	9384	0.362
394	2005	46	Baca	TAM 107	1	80	E	26	6392	0.407
395	2005	46	Baca	TAM 107	1	120	E	9	9241	0.097
396	2005	46	Baca	TAM 107	1	200	E	9	9384	0.096
397	2005	47	Baca	Jagalene	5	10	E	58	9384	0.618
398	2005	47	Baca	Jagalene	5	20	E	20	9384	0.213
399	2005	47	Baca	Jagalene	5	40	E	5	9075	0.055
400	2005	47	Baca	Jagalene	5	80	E	3	9384	0.032
401	2005	47	Baca	Jagalene	5	120	E	2	9384	0.021
402	2005	47	Baca	Jagalene	5	200	E	1	9384	0.011
403	2005	48	Kit Carson	PR	1	1	N	74	6853	1.080
404	2005	48	Kit Carson	PR	1	10	N	26	9384	0.277
405	2005	48	Kit Carson	PR	1	20	N	20	8412	0.238
406	2005	48	Kit Carson	PR	1	40	N	17	9384	0.181
407	2005	48	Kit Carson	PR	1	80	N	7	9384	0.075
408	2005	48	Kit Carson	PR	1	120	N	8	9384	0.085
409	2005	48	Kit Carson	PR	1	200	N	9	9384	0.096
410	2005	49	Kit Carson	Jagger	2	1	S	7	9384	0.075
411	2005	49	Kit Carson	Jagger	2	10	S	6	9384	0.064

Appendix

Sample ID	Year	Loc. ID	County	Wheat variety	RHC[1]	Dist. (ft)	Dir.[2]	No. of survivors	Total plants	PMGF
412	2005	49	Kit Carson	Jagger	2	20	S	5	6256	0.080
413	2005	49	Kit Carson	Jagger	2	40	S	6	9384	0.064
414	2005	49	Kit Carson	Jagger	2	80	S	7	9384	0.075
415	2005	49	Kit Carson	Jagger	2	120	S	2	9384	0.021
416	2005	49	Kit Carson	Jagger	2	200	S	2	9384	0.021
417	2005	50	Kit Carson	PR	1	15	N	96	9384	1.023
418	2005	50	Kit Carson	PR	1	40	N	29	9384	0.309
419	2005	50	Kit Carson	PR	1	80	N	26	9384	0.277
420	2005	50	Kit Carson	PR	1	120	N	18	4716	0.382
421	2005	50	Kit Carson	PR	1	200	N	20	7940	0.252
422	2005	51	Kit Carson	Jagalene	5	1	N	61	7820	0.780
423	2005	51	Kit Carson	Jagalene	5	10	N	25	9384	0.266
424	2005	51	Kit Carson	Jagalene	5	20	N	17	9384	0.181
425	2005	51	Kit Carson	Jagalene	5	40	N	25	9384	0.266
426	2005	51	Kit Carson	Jagalene	5	80	N	4	9384	0.043
427	2005	51	Kit Carson	Jagalene	5	200	N	2	9384	0.021
428	2005	52	Kit Carson	Jagalene	5	1	N	15	9384	0.160
429	2005	52	Kit Carson	Jagalene	5	10	N	8	9384	0.085
430	2005	52	Kit Carson	Jagalene	5	20	N	9	9384	0.096
431	2005	52	Kit Carson	Jagalene	5	40	N	8	9384	0.085
432	2005	52	Kit Carson	Jagalene	5	80	N	3	9384	0.032
433	2005	52	Kit Carson	Jagalene	5	120	N	1	9384	0.011
434	2005	52	Kit Carson	Jagalene	5	200	N	1	9384	0.011
435	2005	53	Logan	PR	1	3	N	38	6256	0.607
436	2005	53	Logan	PR	1	10	N	25	9384	0.266
437	2005	53	Logan	PR	1	20	N	20	9384	0.213
438	2005	53	Logan	PR	1	40	N	4	6256	0.064
439	2005	53	Logan	PR	1	80	N	8	9384	0.085
440	2005	53	Logan	PR	1	120	N	7	9384	0.075
441	2005	53	Logan	PR	1	200	N	3	9384	0.032
442	2005	54	Baca	Hatcher	5	3	W	12	9384	0.128
443	2005	54	Baca	Hatcher	5	10	W	4	9384	0.043
444	2005	54	Baca	Hatcher	5	20	W	0	9384	0.000
445	2005	54	Baca	Hatcher	5	40	W	2	9384	0.021
446	2005	54	Baca	Hatcher	5	80	W	0	9384	0.000
447	2005	54	Baca	Hatcher	5	120	W	1	9384	0.011
448	2005	54	Baca	Hatcher	5	200	W	0	9384	0.000
449	2005	55	Morgan	PR	1	1	W	86	9384	0.916
450	2005	55	Morgan	PR	1	10	W	26	9384	0.277
451	2005	55	Morgan	PR	1	20	W	21	9384	0.224
452	2005	55	Morgan	PR	1	40	W	11	9384	0.117
453	2005	55	Morgan	PR	1	80	W	2	9384	0.021
454	2005	55	Morgan	PR	1	120	W	10	9384	0.107
455	2005	55	Morgan	PR	1	200	W	11	9384	0.117
456	2005	56	Morgan	Prowers 99	8	1	E	7	9384	0.075
457	2005	56	Morgan	Prowers 99	8	10	E	1	9384	0.011
458	2005	56	Morgan	Prowers 99	8	20	E	1	9384	0.011
459	2005	56	Morgan	Prowers 99	8	40	E	0	9384	0.000
460	2005	56	Morgan	Prowers 99	8	80	E	2	9384	0.021
461	2005	56	Morgan	Prowers 99	8	120	E	0	9384	0.000
462	2005	56	Morgan	Prowers 99	8	200	E	0	9384	0.000

[1] RHC, Relative heading class. [2] Direction of sample from the CLEARFIELD variety, 'Above' or 'Bond CL'.