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

Contents

Acknowledgements
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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

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.

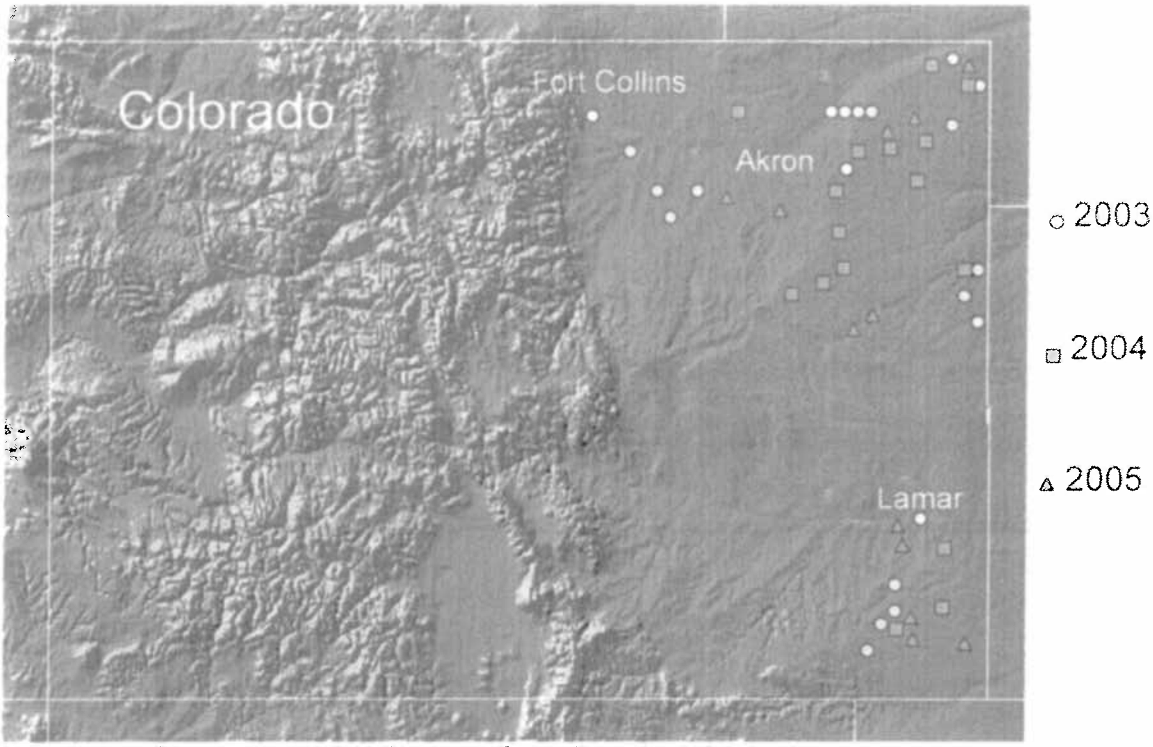
(3) to evaluate seed-mediated gene flow in certified and farmer-saved seed lots.

(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).

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.

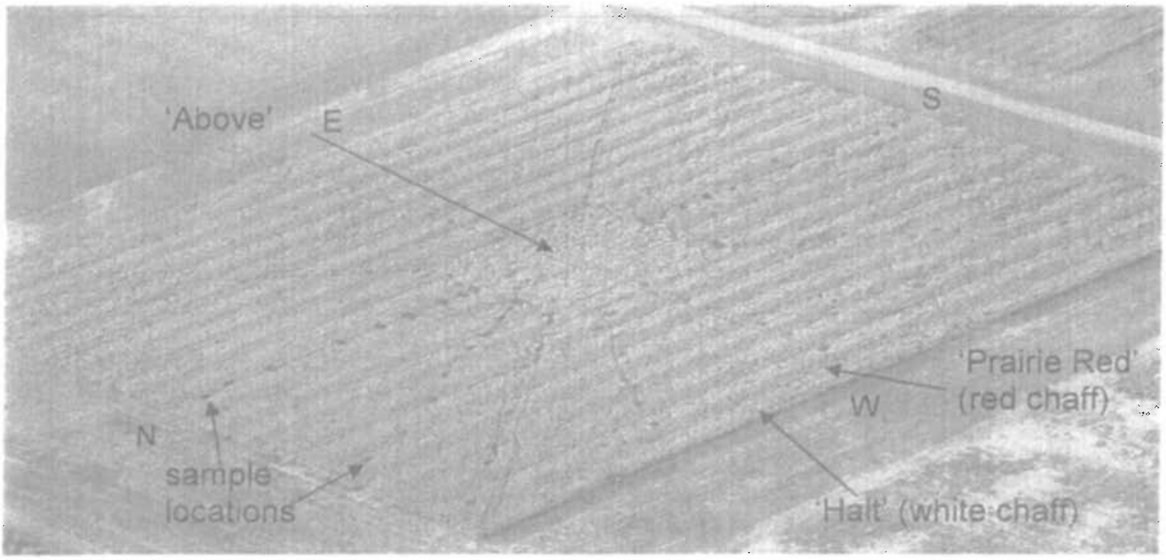
Cultivar	RHC	Number of locations	Number of samples
Above	3	55	*
Akron	5	11	45
Alliance	5	3	16
Ankor	5	21	50
Avalanche	5	16	29
Bond CL	5	1	*
Enhancer	5	8	18
Halt	3	2	7
Hatcher	5	2	14
Ike	6	1	8
Jagalene	5	13	41
Jagger	2	8	57
Millennium	6	1	1
Platte	6	1	5
Prairie Red	1	11	72
Prowers 99	8	3	20
TAM 107	1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

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.

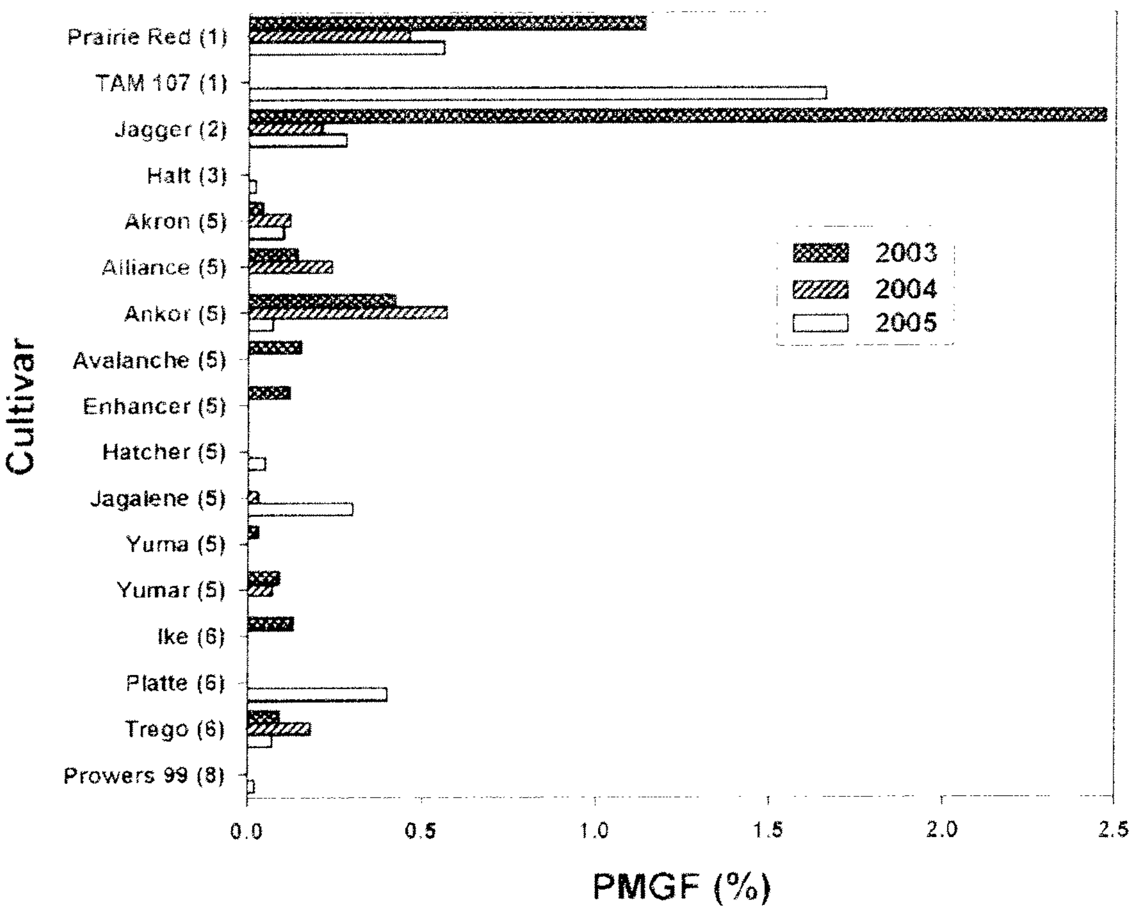
Figure 3. The Nelder wheel plot at Akron, Colorado.



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

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.

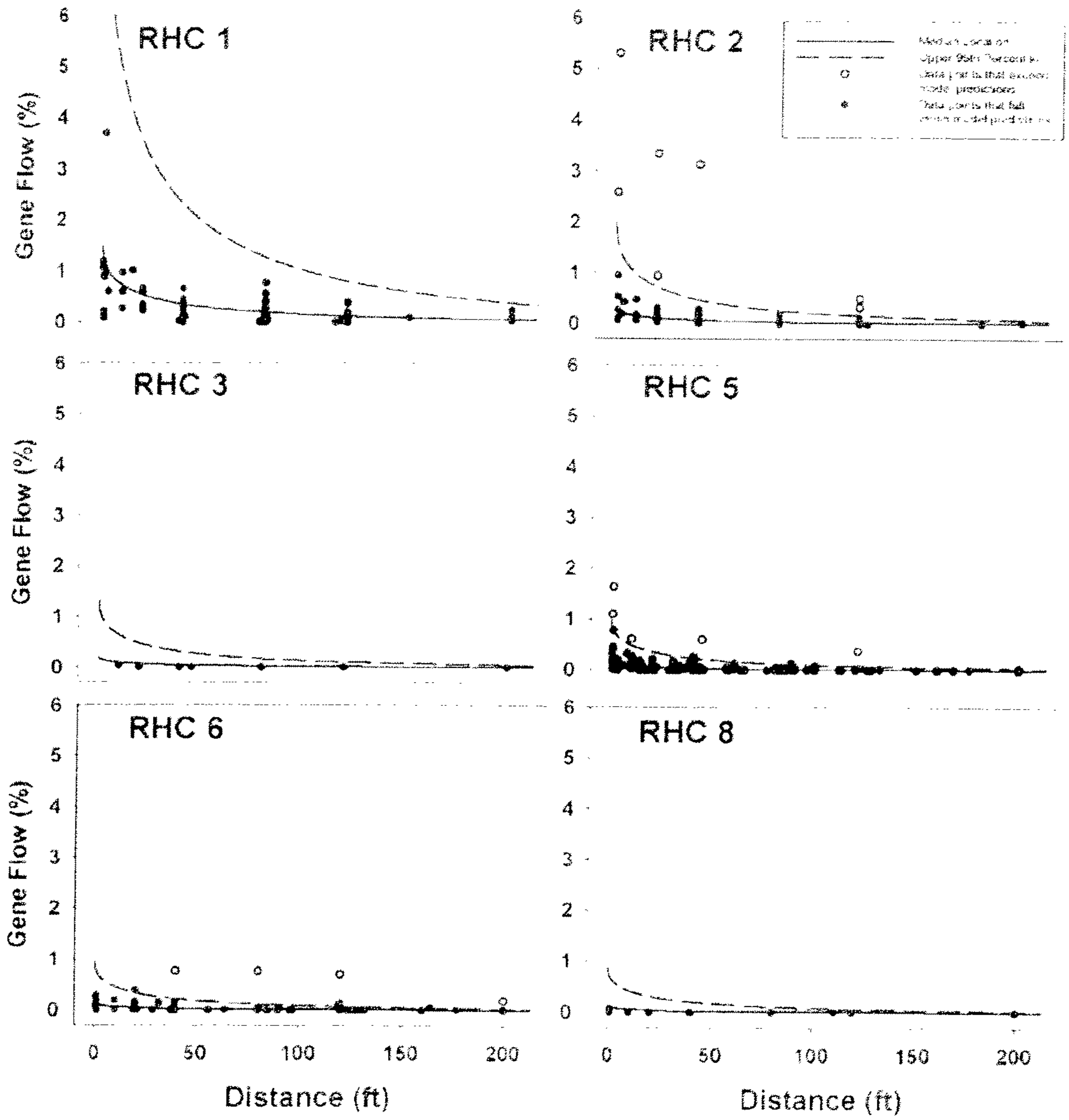
Figure 4. Mean percent pollen-mediated gene flow (PMGF) in samples collected within 20 feet of CLEARFIELD wheat.



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'.

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 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

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

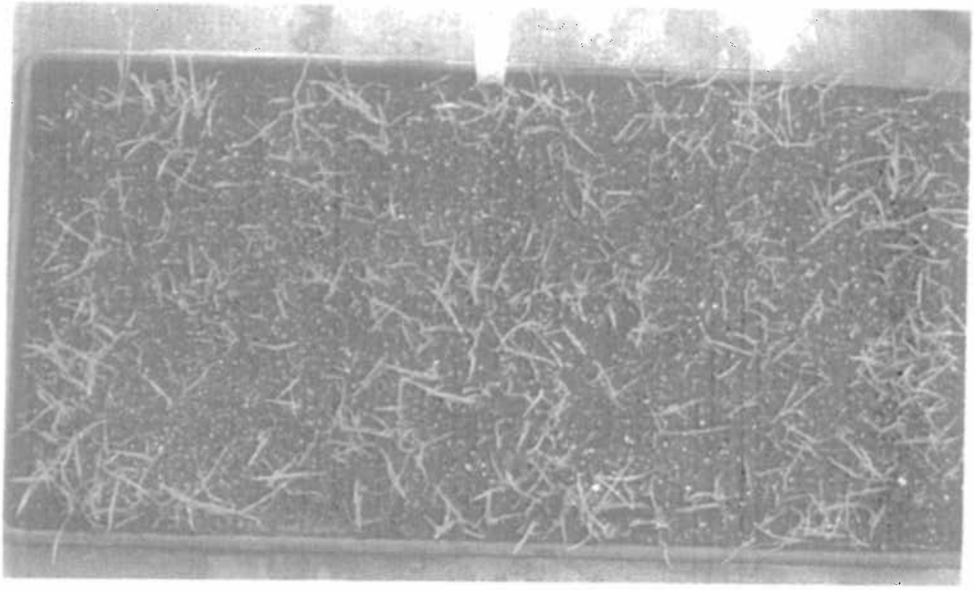
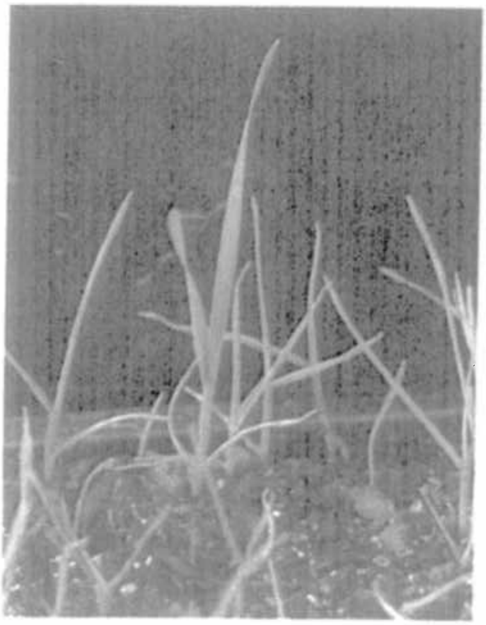
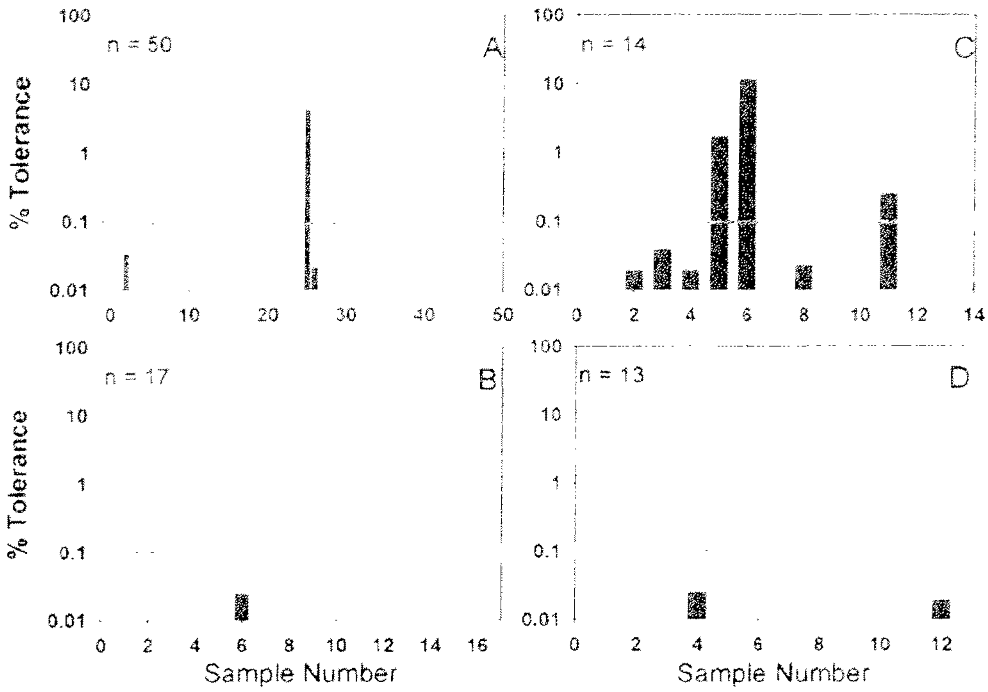


Figure 9. A tolerant wheat plant that survived the imazamox treatment.



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.

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

Objectives

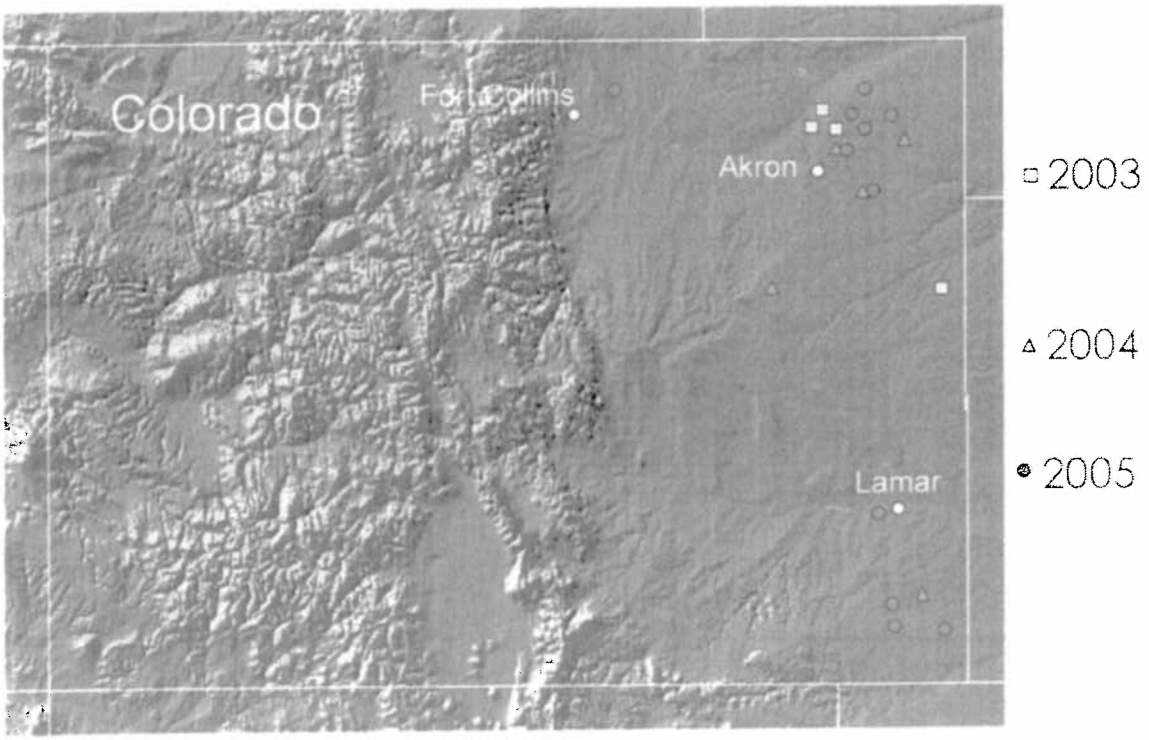
- To evaluate landscape-level gene flow from CLEARFIELD wheat to jointed goatgrass
- To determine the activity of the herbicide-tolerant acetolactate synthase (*Als1*) allele in weed-wheat hybrids and in hybrids of herbicide-tolerant and -susceptible wheat

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/



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 Hybrid-ization
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

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
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	0.102

Appendix

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	5	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
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

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.