

## Pollen-Mediated Gene Flow from Blue Aleurone Wheat to Other Wheat Cultivars

B. D. Hanson,\* C. A. Mallory-Smith, B. Shafii, D. C. Thill, and R. S. Zemetra

### ABSTRACT

Cross-pollination among wheat (*Triticum aestivum* L.) cultivars historically has not been a major concern for wheat producers. However, with the introduction of genetically engineered wheat, there may be a need to ensure genetic purity, which will require knowledge of the frequency and distance that pollen-mediated gene flow can occur in wheat. This information will be necessary to develop (i) isolation distances that maintain predictable degrees of genetic purity in seed wheat production and (ii) practices to manage pollen-mediated gene flow in commercial wheat production. Field experiments were conducted from 2000 to 2003 at five locations in Oregon, Idaho, and Washington to determine the potential for pollen-mediated gene flow among winter wheat cultivars. Each experiment was designed as a Nelder wheel with 16 equally spaced rays extending away from a central pollen source of blue aleurone wheat. Each ray was 46 m long and contained two rows each of an early- and late-flowering soft white wheat cultivar. Seed samples were collected at 1.8-m intervals along each ray and examined for blue seed, which indicated successful hybridization with the pollen source. Although blue seed was found in some samples at all five locations, most samples (98%) contained no blue seed. Pollen-mediated gene flow generally was in the direction of the prevailing wind and tended to occur more often at the sites with lower temperature and higher humidity during pollination. The maximum distance that gene flow was detected was 42 m from the pollen source and the maximum outcrossing in an individual sample was 0.45%. At four locations, no outcrossing was detected beyond 30 m from the pollen source; however, at one site, 13% of the blue seed was found between 30 and 42 m. Pollen-mediated gene flow potential may differ for other wheat cultivars and environmental conditions. Depending on the required genetic purity standards for wheat seed, an isolation distance of 45 m or more may be required.

IN THE PAST, gene flow among cultivars of major field crops was generally a concern only in cultivar development and limited generation seed production programs because of stringent purity requirements associated with seed production. Low levels of gene flow among commercial fields of crops such as wheat, corn (*Zea mays* L.), and rice (*Oryza sativa* L.) have not been a major concern because absolute genetic purity was not required to market most commodity crops. However, cultivars with special quality traits or end use products or with specific pest or herbicide resistance traits may require higher purity standards thus making gene flow

among fields a more important issue. Recent concern over market acceptance of genetically engineered crops and the need for protection of trademarked plant cultivars brings further attention to the issue of crop-to-crop gene flow (Conner et al., 2003; Kershner, 2004).

Gene flow can occur by pollen movement and hybridization or by direct movement of seed or vegetative propagules (Ennos, 1994; Slatkin, 1987). Pollen dispersal is the main mode of gene flow in flowering plants and can provide a mechanism of gene flow into populations of the same species or sexually compatible relatives (Garcia et al., 1998; Levin and Kerster, 1974). Pollen-mediated gene flow rates can vary greatly among crops due to different modes of pollination, which range from highly autogamous [soybean, *Glycine max* (L.) Merr.] to completely allogamous (hops, *Humulus lupulus* L.) (Frankel and Galun, 1977; Poehlman and Sleper, 1995). Interspecific or intergeneric gene flow commonly is utilized by plant geneticists to develop and improve crop cultivars; however, minimizing gene flow from outside pollen sources is critical to maintaining genetic purity of breeding lines and seed multiplication fields (Hucl and Matus-Cadiz, 2001). Genetic purity in breeding lines currently is maintained at acceptable levels using isolation buffers, the size of which depends on the breeding strategy of the crop. For example, USDA-APHIS foundation seed production guidelines suggest an isolation distance of 800 m for nonhybrid sunflower (*Helianthus annuus* L.) (a primarily outcrossing species pollinated by insects), but only a "distance to prevent mechanical mixing" for soybean (a primarily inbreeding species) (USDA Animal and Plant Health Inspection Service, 2004).

Wheat is classified as an inbreeding species; however, low rates of outcrossing (usually less than 2%) can occur via wind-borne pollen (Lersten, 1987; Poehlman and Sleper, 1995). Dispersal of wind-borne pollen depends on both flowering biology of the species or cultivar and environmental conditions before or during flowering (Bitzer and Patterson, 1967; De Vries, 1971; Delph et al., 1997). Potential for pollen-mediated gene flow in wheat as affected by environmental conditions at flowering recently has been reviewed (De Vries, 1972; Treu and Emberlin, 2000; Waines and Hegde, 2003). Factors such as air temperature, relative humidity, rainfall, light intensity, and other stress factors can influence gene flow by affecting the degree of floret opening, length of stigma receptivity, number of anthers extruded, amount of pollen released, and length of pollen viability.

Although pollen-mediated gene flow can occur at great distances in wind-pollinated species (Dowding, 1987), wheat pollen has been reported to travel relatively short distances. Jensen (1968) reported that 90% of wheat pollen fell within 6 m of its source, but small amounts traveled as far as 60 m. Other researchers have reported low levels of viable wheat pollen at distances

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of 24, 60, and even as far as 1000 m from the source (De Vries, 1971; Khan et al., 1973; Virmani and Edwards, 1983). The presence of viable pollen at a given distance, however, does not ensure that successful pollination and gene flow will occur (Levin and Kerster, 1974). Successful outcrossing and seed set on wheat from foreign pollen provides more direct evidence of gene flow.

Several research studies on outcrossing in wheat have been conducted to examine outcrossing among adjacent breeder plots. Results indicate that outcrossing rates can vary among wheat cultivars. For example, Griffen (1987) found that 10 winter wheat cultivars in New Zealand had outcrossing rates between 0.14 and 3.95% at a distance of 15 cm, while Martin (1990) in Kansas found that 12 winter wheat varieties outcrossed 0.1 to 5.6% over 30 cm. Outcrossing in spring wheat is reported to be similar to winter wheat with values ranging from 0.2 to 6.4% over 20 cm (Hucl, 1996) and 0 to 2.16% over 30 cm (Harrington, 1931). Less research has been conducted on outcrossing over longer distances in wheat. Hucl and Matus-Cadiz (2001) examined outcrossing from blue aleurone wheat to hard red spring wheat and found 0 to 3% hybrid seed in samples collected adjacent to the pollen source and 0 to 0.09% hybrid seed at a distance of 27 m from the pollen source. In a review on seed set in male-sterile wheat, De Vries (1974) reported seed set of up to 15.3% at 29 m, 4.8 to 5.4% at 40 m, and 3 to 10% at 48 m from the pollen source in various experiments. While the frequency of seed set on male sterile wheat from outside pollen was undoubtedly higher than would be expected for male-fertile varieties due to the lack of pollen competition, the possibility of gene flow occurring at these distances was confirmed.

Standards for wheat seed production vary among Idaho, Oregon, and Washington (Anonymous, 2004). The allowable number of "off-type" wheat kernels in foundation seed lots is zero in Oregon and Washington, and one seed per 4.5 kg in Idaho. Number of "off-type" seed allowed in 454 g of registered and certified seed varies among states and ranges from 1 to 2 for registered seed and from 2 to 4 in certified seed. Because of the relatively low levels of outcrossing in wheat, these levels of purity are maintained through very minimal isolation requirements. Currently, Idaho and Washington require a 27-m isolation for foundation seed production, but Oregon requires only a distance "to prevent mechanical mixing" of seed. Registered and certified seed fields in the Pacific Northwest require at most a 90-cm separation between varieties. There currently are no isolation requirements for commercial fields of wheat grown in Idaho, Washington, and Oregon.

Recent interest in ensuring genetic purity of conventional and specialized wheat cultivars and concern over potential movement of genetically engineered traits into non-genetically engineered seed lots have illustrated the shortage of information on the frequency of outcrossing among winter wheat cultivars at intermediate distances. Therefore, the objective of this study was to determine the potential frequency and distance of outcrossing among winter wheat cultivars in the inland Pacific Northwest using blue aleurone wheat as a screenable marker.

## MATERIALS AND METHODS

Field experiments were conducted from 2000 to 2003 at five locations near Athena, OR, Clyde, WA, and Moscow and Lewiston, ID. Soil types varied among experimental sites and were typical silt loam soils found in dryland wheat production areas of the Pacific Northwest (Table 1). Management practices were those used by each cooperating grower and varied among fields.

The experimental design at each location was a Nelder (1962) wheel plot. The Nelder wheels consisted of a 45.7-m-diameter (0.16 ha) central plot surrounded by 16 equally spaced rays. The rays, oriented to true north and spaced 22.5° apart, began at the edge of the central plot and were 46.3 m long. Each ray was labeled with a letter beginning with ray "A" for the true north ray and continuing alphabetically, clockwise to ray "P." The central plot served as the pollen source in the experiment and was seeded with a blue aleurone winter wheat (provided by Dr. Robert Metzger, USDA-ARS, retired, Corvallis, OR) at 112 kg/ha in 20-cm spaced rows. The blue aleurone wheat contained a mixture of maturity types to maximize the length of time that pollen was shed. Each ray contained two rows each of 'Brundage 96' (Zemetra et al., 2003) and 'Madsen' (Allan et al., 1989) soft white winter wheat seeded at 112 kg/ha in 20-cm spaced rows. These cultivars are commonly grown in the Pacific Northwest and were chosen to represent early- and late-flowering types to maximize the overlap in flowering (nicking) between blue aleurone and white wheat plants.

The area between each of the rays was seeded with 66 kg/ha of 'Boyer' (Muir et al., 1977) winter barley (*Hordeum vulgare* L.) to maintain a canopy structure similar to wheat without producing pollen that could confound the experiment. To further isolate the experimental plot from competing pollen, the experiments were seeded at least 60 m from the nearest winter wheat field. The border area was seeded to Boyer winter barley except at Clyde, WA, in 2000–2001 where the border area was maintained under chemical fallow conditions. Blue aleurone wheat, white wheat in the rays, and winter barley were seeded with either a 1.1-m plot seeder or a 3-m seeder with double disk openers except at Lewiston, ID, in 2002–2003 where a 2-m no-till seeder was used.

A weather station located in the center of each experiment was configured to measure air temperature, relative humidity, wind speed, and wind direction throughout the flowering period. Average air temperature and relative humidity 1 m above

**Table 1. Crop management and production procedures in 2000–2003 winter wheat gene flow experiments in the Pacific Northwest.**

Year	Location	Soil type	Previous crop	Tillage system	Seeding date	Harvest date
2000–2001	Athena, OR	Athena silt loam	spring pea	conventional	4 Oct. 2000	18 July 2001
2000–2001	Clyde, WA	Ritzville silt loam	summer fallow	conventional	26 Sept. 2000	25 July 2001
2001–2002	Moscow, ID	Latahco silt loam	winter wheat–spring barley	conventional	28 Sept. 2001	17 July 2002
2002–2003	Moscow, ID	Palouse silt loam	spring pea	conventional	26 Sept. 2002	13 Aug. 2003
2002–2003	Lewiston, ID	Endicott silt loam	spring mustard	direct seed	22 Oct. 2002	29 July 2003

the soil surface were recorded at 3-h intervals during flowering. Wind speed in 16 vectors (relative to rays of the Nelder plots) was measured once per minute, averaged, and recorded at 3-h intervals. An equipment malfunction resulted in the loss of weather data from the 2001–2002 Moscow, ID, experiment; however, temperature and humidity data were available from a weather station located 1 km from the experimental site.

The cooperating growers used standard fertilization and broadleaf weed control practices for wheat and barley in all experiments. Additionally, the blue aleurone plot and rays at Clyde, WA, in 2000–2001 were treated with 45 g a.i./ha BAY MKH 6561 (proposed name = proproxycarbazone) and the pollen source and rays at Lewiston, ID, in 2002–2003 were treated with 30 g a.i./ha flucarbazone to suppress jointed goatgrass (*Aegilops cylindrica* Host.) and downy brome (*Bromus tectorum* L.), respectively. In spring 2002, the area between the rays and in the border area at Moscow, ID, was mowed before flowering to minimize pollen competition from a large population of volunteer non-blue aleurone winter wheat plants among the winter barley plants in a portion of the experimental area.

### Data Collection

At maturity, wheat spikes were harvested by hand from 0.6 m of two rows (1.2 m total) for each cultivar at 1.8-m intervals beginning at the edge of the pollen source (0, 1.8, 3.7, 5.5, 7.3, 9.1, 11.0, 12.8, 14.6, 16.5, 18.3, 20.1, 21.9, 23.8, 25.6, 27.4, 29.3, 31.1, 32.9, 34.8, 36.6, 38.4, 40.2, 42.1, 43.9, and 45.7 m = 26 samples per cultivar per ray). Spikes from each sample were placed in labeled paper bags and transported to a central location for processing with a stationary threshing machine. Individual grain samples were weighed and the total weight was divided by the average weight of 1000 seeds for each variety at each location to estimate total seed number in each sample.

Each grain sample was evaluated visually for the presence of blue wheat kernels among the white kernels, which would indicate hybridization with the blue aleurone pollen source. The hybrid nature of the seeds was confirmed by planting, vernalizing, and growing to maturity the putative hybrid plants and verifying the 3:1 ratio (blue to non-blue) expected for this single-gene, dominant trait (Keppenne and Baenziger, 1990) in the F<sub>2</sub> population.

### Data Analysis

Outcrossing data from each wheat cultivar were expressed as percent blue seeds in a sample for simple observation of distance and rate of pollen-mediated gene flow in all directions. Percent outcrossing was determined using the following equation (Huel and Matus-Cadiz, 2001):

$$\text{OC \%} = (\text{number of blue seeds in a sample} / \text{total number of seeds in a sample}) \times 100 \quad [1]$$

Air temperature and relative humidity during pollination were averaged from the data recorded at 3-h intervals. Wind run in each vector was calculated by multiplying the percentage of the time that the wind blew in a given vector by the average wind speed in that vector. Wind runs from each 3-h period were summed for the duration of pollination to get total wind run in each vector. The wind run for all vectors was summed and used to convert wind run in each vector to a percentage of total wind run in each vector during pollination. Most wheat pollen is shed during daylight hours; thus only environmental conditions from 0600 to 1800 h were used in the analysis (De Vries, 1972). Outcrossing data from six rays

downwind of the predominant wind at each location were then aggregated for further analysis.

Generalized nonlinear regression analyses assuming a binomial distribution were employed to estimate outcrossing rate at increasing distance from the pollen source in the six downwind rays (PROC NL MIXED; SAS Institute, 2001). Various linear and nonlinear models and data transformation were considered in describing these data. The selected nonlinear model took the form:

$$p = \beta_0 \times \exp[-\beta_1(\text{distance})] + \epsilon \quad [2]$$

where  $p$  is the percent outcrossing,  $\beta_0$  and  $\beta_1$  are regression coefficients, distance is the distance from the pollen source (m), and  $\epsilon$  is a random error term assumed to be normally distributed. Adequacy of model fit was determined by testing for the significance of regression coefficients, evaluating correlation between the parameters, and examining the underlying residual structure.

Observational data such as the outcrossing data in these experiments can be subject to spatially dependent correlations (Cressie, 1991). Therefore, spatial variability between samples within the downwind rays was observed by plotting semivariance between pairs of samples separated by increasing distance. A nonlinear regression model was used to predict the spatial variability using the form:

$$\gamma_i = C + b \times h_i^L \quad [3]$$

where  $\gamma_i$  is the predicted semivariance,  $h_i$  is the distance between sampling points along a ray at lag  $i$ , lag distance is the proportion of the length of the ray (46.3 m),  $C$  is the sill or underlying variance when  $h = 0$ , and  $b$  and  $L$  are parameters related to shape and rate. If spatial variability is present, this equation describes a line that curves upward as lag distance increases whereas the line is flat if spatially dependent variability is absent. All parameters in Eq. [3] were estimated using PROC NLIN in SAS (SAS Institute, 2001).

## RESULTS

Average daytime temperature and relative humidity during pollination ranged from 14.6 to 20.1°C and 51 to 63%, respectively, at the five experimental sites (Table 2). Wind during pollination was predominately from the south and south-southeast at Athena, OR, and Clyde, WA, in 2001. A weather station malfunction resulted in a loss of wind data during pollination at Moscow, ID, in 2002. Wind during pollination in 2003 was predominately from the west at Moscow, ID, but was variable at Lewiston with slightly more wind from the southwest.

Individual wheat samples averaged 2689 kernels and ranged from 995 to 4745 among locations and wheat varieties (data not shown). Of 4062 samples examined (98 planned samples produced no grain) over three growing seasons, 3965 contained no blue aleurone seed; those containing at least one hybrid seed usually had less than 0.1% blue kernels (Table 3). Frequency of outcrossing varied among locations but was always less than 0.45% with the greatest outcrossing observed in plants grown closest to the blue aleurone wheat plot. Pollen from the blue aleurone wheat fertilized both Madsen and Brundage 96 white wheat in three locations, while two locations had blue kernels only in Madsen samples. The average amount of blue wheat in samples with outcrossing over all years was 0.07%.

**Table 2. Summary of daytime weather (0600–1800 h) during pollination at five winter wheat gene flow experiments from 2000 to 2003 in the Pacific Northwest.**

Year	Location	Pollination duration	Average temperature	Average relative humidity	Total wind run‡	Wind run from each vector†															
						A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
		d	°C	%	km	%															
2000–2001	Athena, OR	23	15.8	57	3206	3	2	1	1	1	1	4	12	21	23	16	8	4	2	1	1
2000–2001	Clyde, WA	17	14.6	63	1900	1	0	0	2	7	21	30	22	11	3	1	0	0	0	1	1
2001–2002	Moscow, ID§	14	20.0	51	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2002–2003	Moscow, ID	14	16.4	59	1816	1	1	2	2	5	3	2	1	3	7	14	21	23	11	4	1
2002–2003	Lewiston, ID	14	20.1	51	1700	7	5	6	5	4	5	7	10	12	11	8	4	3	2	3	6

† Vectors correspond to the 16 rays with Rays A, E, I, and M oriented to north, east, south, and west, respectively.

‡ Wind run was determined by multiplying the percent of time in a 3-h period that the wind blew from a vector by the average wind speed during the same period and summed over the flowering period. The wind run from all vectors was summed and used to calculate the percent of total wind run that occurred in each vector.

§ An equipment malfunction at the 2001–2002 Moscow, ID, location caused a loss of weather data during pollination. Temperature and relative humidity data were obtained from a weather station located 1 km from the experimental site.

At the Athena, OR, location, outcrossing occurred in both Brundage 96 and Madsen samples, primarily in the northeast rays with a maximum distance of 42.1 m (Fig. 1). In the “B” ray at Athena, 39 seed were found in 24 samples, resulting in an estimated 0.02% (39/170 000 kernels) outcrossing rate over the entire ray (data not shown). Outcrossing at the Clyde, WA, location occurred three times more frequently in Brundage 96 than Madsen samples (Table 3). Blue kernels were found in 10 rays mostly on the north side of the Clyde, WA, experiment with a maximum distance of 21.9 m from the pollen source (Fig. 1). Only three Madsen samples contained a blue kernel in the Moscow, ID, location harvested in 2002 and the maximum distance was 29.3 m from the pollen source (0.04%) (Fig. 1). In the Moscow, ID, experiment harvested in 2003, outcrossing was split between Brundage 96 and Madsen samples and occurred primarily in the eastern rays to a maximum distance of 11 m (0.02%) (Fig. 1). At the Lewiston, ID, site, the only outcrossing event was in a north-northwest ray at a distance of 9 m (0.03%) (Fig. 1).

A meaningful prediction of potential outcrossing could be determined only for the locations having a substantial number of gene flow events (Fig. 2). Thus, data from the 2001–2002 Moscow location and the 2002–2003 Lewiston location were not used in this analysis. The exponential decay model used in the analysis provided a good description of pollen-mediated gene flow in these experiments with both the intercept ( $\beta_0$ ) and

rate of exponential decay ( $\beta_1$ ) estimates highly significant and not redundant (Table 4). Residual analysis indicated that the errors generally conformed to the underlying regression assumptions with the exception of several high outcrossing outliers near the pollen source plots (Hanson, 2004).

Predicted outcrossing at Athena, OR, ranged from 0.0414% adjacent to the pollen source to 0.000796% at 45.7 m (Fig. 2). The lower 95% prediction interval remained positive at 45.7 m (the limits of the experiment), indicating that outcrossing likely would have occurred at even greater distances (data not shown). Predicted outcrossing adjacent to the pollen source at Clyde, WA, and Moscow, ID, was 0.045 and 0.007%, respectively (Fig. 2). Although outcrossing was predicted at 45.7 m at Clyde, WA, and Moscow, ID, the lower 95% prediction bound fell below zero at 12.8 and 7.3 m, respectively (data not shown).

Spatial variability was evident at the Athena, OR, and Clyde, WA, locations in 2000–2001 where all parameter estimates were highly significant (Table 5). The between-sample variability increased with increasing distance from the pollen source indicating that samples more distant from one another (further from the pollen source) have significantly different predicted outcrossing (Fig. 3). However, at the 2002–2003 Moscow, ID, location, spatial variability was not observed indicating that the variability in outcrossing was relatively uniform across all samples.

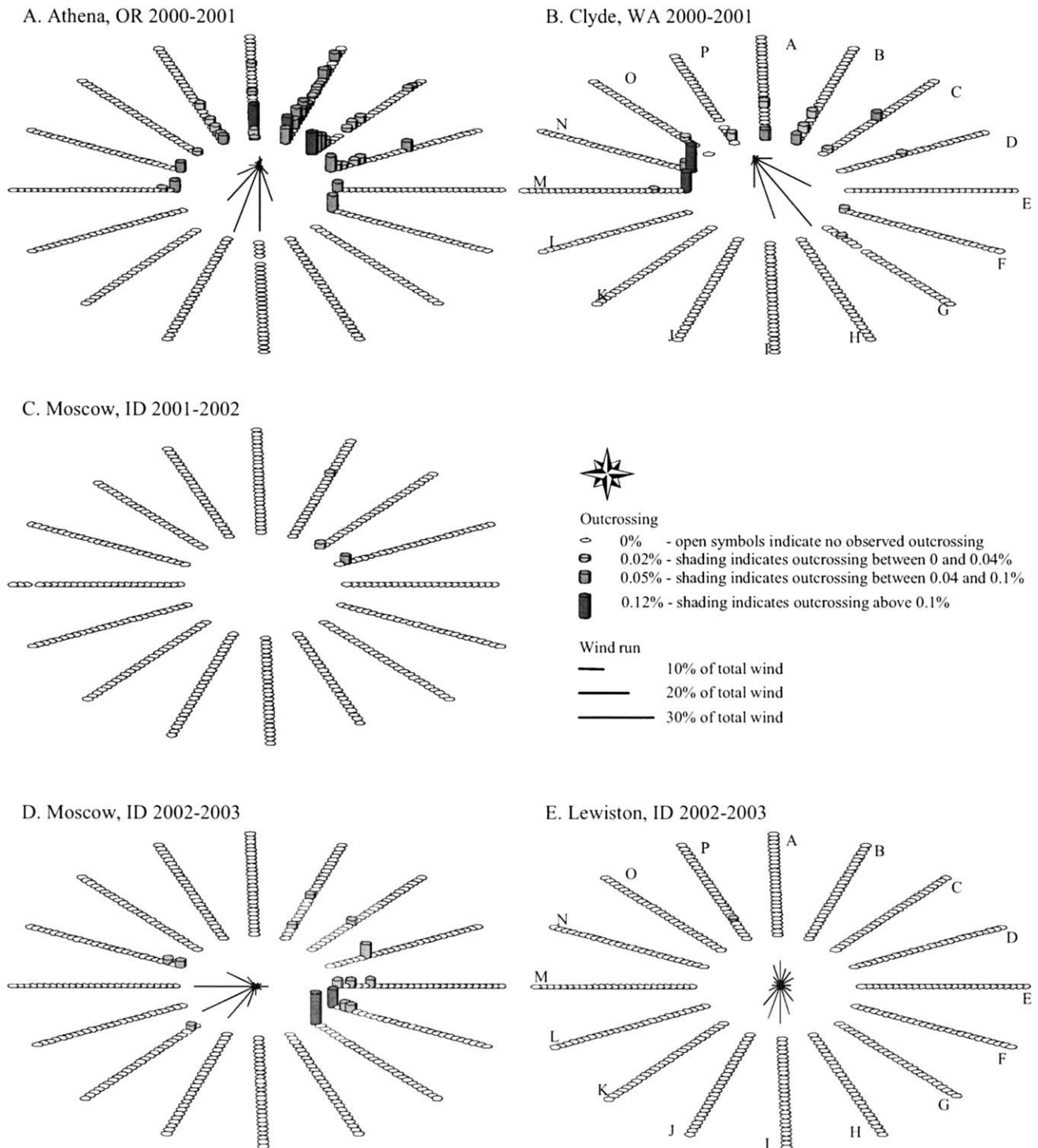
**Table 3. Descriptive statistics of wheat samples with at least one outcrossing event in 2000–2003 winter wheat gene flow experiments.**

Year	Location	Cultivar	Samples		Outcrossing			
			Analyzed†	With outcrossing‡	Minimum	Maximum	Mean	Standard deviation
					%§			
2000–2001	Athena, OR	Brundage 96	402	33	0.017	0.167	0.042	0.033
		Madsen	408	32	0.034	0.223	0.081	0.048
2000–2001	Clyde, WA	Brundage 96	405	13	0.029	0.288	0.089	0.077
		Madsen	401	4	0.044	0.105	0.080	0.027
2001–2002	Moscow, ID	Brundage 96	400	0	–	–	–	–
		Madsen	401	3	0.043	0.086	0.062	0.022
2002–2003	Moscow, ID	Brundage 96	412	5	0.019	0.128	0.051	0.045
		Madsen	415	6	0.018	0.451	0.102	0.172
2002–2003	Lewiston, ID	Brundage 96	407	0	–	–	–	–
		Madsen	411	1	0.027	0.027	0.027	–

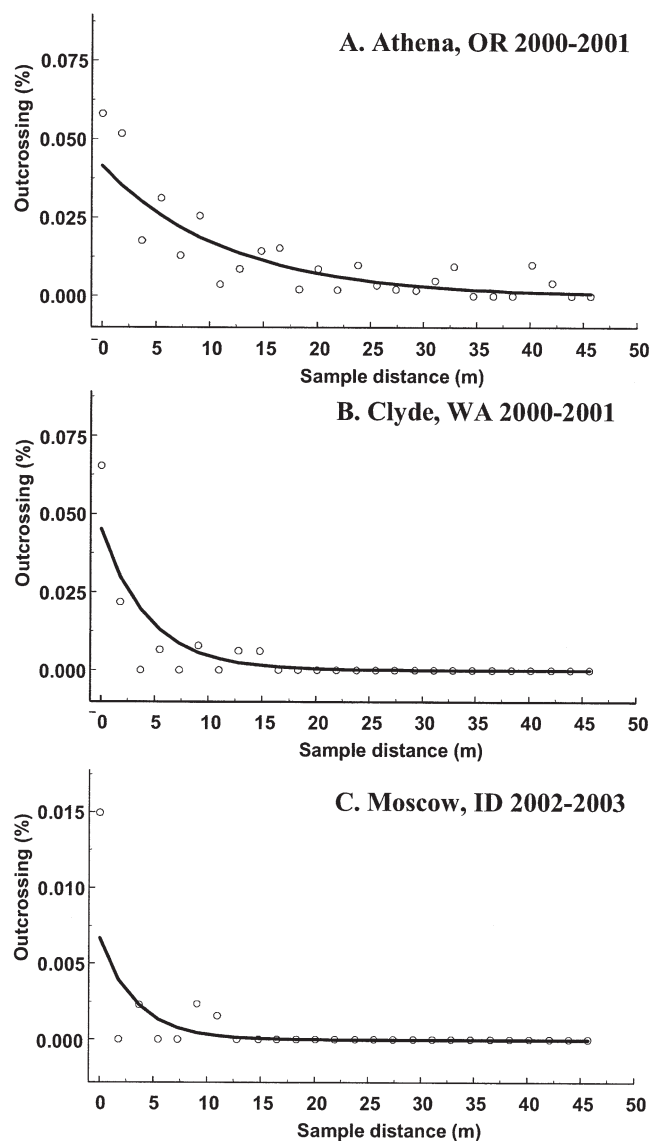
† Number of samples analyzed out of a possible 416 samples per cultivar at each location. Several samples at each location had no grain due to seeding skips or poor emergence.

‡ Number of samples with at least one blue kernel.

§ Percent outcrossing determined by dividing the number of blue kernels by the estimated total kernel number in each sample.



**Fig. 1.** Outcrossing among winter wheat cultivars in 2000–2003 gene flow experiments averaged over two wheat cultivars at each distance. Experiments were located near (A) Athena, OR, and (B) Clyde, WA, in 2000–2001, (C) Moscow, ID, in 2001–2002, and (D) Moscow and (E) Lewiston, ID, in 2002–2003. Column height in each figure represents the percentage of hybrid seed out of the total number of seeds in a sample at each distance from the pollen source. A polar plot in the center of each figure indicates the percentage of wind coming from each of 16 vectors during the pollination period except at Moscow, ID, in 2002 due to an equipment failure.



**Fig. 2.** Mean outcrossing and estimated regression curves from blue aleurone wheat to 'Brundage 96' and 'Madsen' winter wheat near (A) Athena, OR, in 2000–2001, (B) Clyde, WA, in 2000–2001, and (C) Moscow, ID, in 2000–2003. Data are an average of six downwind rays and two cultivars at each sample distance (12 samples), many of which had 0% outcrossing.

## DISCUSSION

The frequency and level of contamination in these experiments was lower than predicted (especially for samples adjacent to the pollen source) based on winter wheat outcrossing reports in the literature (Waines and Hegde, 2003). Brundage 96 and Madsen were used in the experiments because they are widely grown in the Pacific Northwest and have slightly different flowering periods. The propensity of Brundage 96 and Madsen wheat to outcross has not been previously characterized; therefore, it is not known whether these cultivars have higher or lower risk of gene flow than other cultivars commonly grown in the region.

Environmental conditions at flowering appeared to influence the amount of pollen-mediated gene flow in these five experiments conducted over three growing

**Table 4.** Model parameter estimates, standard errors, and *P* values for predicted outcrossing from blue aleurone wheat to 'Brundage 96' and 'Madsen' winter wheat in field gene flow experiments.

Year	Location	Parameter†	Estimate	Standard error	<i>P</i>
2000–2001	Athena, OR	$\beta_0$	0.041	0.006	<0.0001
		$\beta_1$	2.63	0.317	<0.0001
2000–2001	Clyde, WA	$\beta_0$	0.045	0.015	0.0028
		$\beta_1$	6.939	1.683	<0.0001
2002–2003	Moscow, ID	$\beta_0$	0.007	0.003	0.032
		$\beta_1$	8.871	3.059	0.004

† The regression parameters  $\beta_0$  and  $\beta_1$  are the intercept and the rate of exponential decay, respectively.

seasons. Air temperature during flowering at all sites was within the optimum range for seed set reported by Porter and Gawith (1999); however, the temperature optimum for pollen longevity may differ (De Vries, 1971). The three sites having the most outcrossing events (Athena, OR, and Clyde, WA, in 2000–2001 and Moscow, ID, in 2002–2003) had an average air temperature during flowering 5.4°C lower than the two low outcrossing sites, supporting the hypothesis that shorter duration of pollen viability may reduce outcrossing. Low relative humidity also can reduce duration of pollen viability and may have contributed to relatively low outcrossing in our studies compared with previously published results. The sites with little outcrossing had 9% lower relative humidity compared with the higher outcrossing sites (51 vs. 60%) although relative humidity at all sites was lower than the optimum reported by De Vries (1972). Average wind run per day was similar among locations (112–139 km/d) although more total wind was accumulated at Athena, OR, and Clyde, WA, due to longer pollination duration. Wind direction at the three higher outcrossing locations was primarily unidirectional, which may result in greater potential for longer distance pollen transport compared with the Lewiston, ID, location where the wind came almost equally from all directions (Levin and Kerster, 1974). This hypothesis, unfortunately, could not be tested at Moscow, ID, in 2001–2002 due to the loss of wind data. The temperature, relative humidity, and wind data from the three locations with greater pollen-mediated gene flow suggest that blue aleurone wheat pollen may have been viable longer and moved farther than at the low outcrossing locations.

Cultivar biology and the size, shape, and density of pollen donor populations and receptor populations can affect the pollen load, which is directly related to the frequency and distance of pollen-mediated gene flow (Bitzer and Patterson, 1967; Rognli et al., 2000). Additionally, Kwon and Kim (2001) speculated that pollen released from large fields could interact on a regional scale to increase the distance and frequency of pollen-mediated gene flow. While the size of the pollen source in our experiments (0.16 ha) was larger than many previous experiments, the pollen load likely was still much smaller than would be expected from commercial wheat production fields. Duration of pollination ranged from 14 to 23 d at all locations; however, peak pollination may have differed among donor and receptor plants causing a shortened period of overlapping flowering.

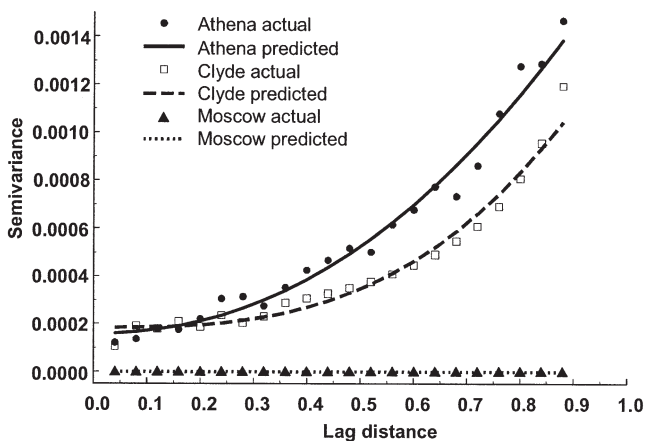
**Table 5. Model parameter estimates, standard errors, and *P* values for predicted spatial variability in observed outcrossing from blue aleurone wheat to 'Brundage 96' and 'Madsen' winter wheat in field gene flow experiments.**

Year	Location	Parameter†	Estimate	Standard error	<i>P</i>
2000–2001	Athena, OR	<i>c</i>	0.000160	0.000020	<0.0001
		<i>b</i>	0.00161	0.000082	<0.0001
		<i>l</i>	2.1462	0.1620	<0.0001
2000–2001	Clyde, WA	<i>c</i>	0.000184	0.000013	<0.0001
		<i>b</i>	0.00125	0.000092	<0.0001
		<i>l</i>	2.9396	0.2583	<0.0001
2002–2003	Moscow, ID	<i>c</i>	$1.514 \times 10^{-9}$	$1.223 \times 10^{-9}$	0.1115
		<i>b</i>	$3.737 \times 10^{-9}$	$1.334 \times 10^{-9}$	0.0057
		<i>l</i>	1.000	1.060	0.1795

† Regression parameter *c* is the sill or underlying variance, *b* represents rate, and *l* is the shape of the response curve.

The mixture of blue aleurone wheat genotypes heterogeneous for maturity used in the experiments successfully increased the length of time that some pollen was being shed thus increasing the likelihood of nicking with the white wheat in the rays. However, the longer flowering period likely resulted in decreased pollen load at any given time because only a portion of the blue aleurone plants shed pollen simultaneously.

Gene flow in these experiments varied among sites and years. Because wheat genotype and environmental factors have great impacts on the potential for gene flow, it is not known if the gene flow observed in these experiments is "normal" or if other wheat cultivars or environmental conditions could result in higher levels of outcrossing among wheat grown in the Pacific Northwest. However, the results of these experiments clearly indicate that pollen-mediated gene flow can occur in winter wheat production in the Pacific Northwest. The number of individual gene flow events and the proportion of hybrid seed in these experiments were relatively low. However, despite pollen competition, outcrossing events were detected at significant distances from the pollen source (42.1 m at one location and 9.1 to 29.3 m at the other four locations) and at potentially significant frequencies (0.017 to 0.451% in some samples).



**Fig. 3. Semivariance plots of spatial variability at Athena, OR, Clyde, WA, and Moscow, ID, in winter wheat gene flow experiments. Data are an aggregate of six downwind rays and two cultivars at each sample distance (12 samples) many of which had no outcrossing. Lag distance is the distance between samples expressed as a proportion of the length of the ray (46.3 m).**

The biological and economical significance of pollen-mediated gene flow observed in these experiments will depend on the purity requirements of the grain. Only 2.4% of the samples tested had any hybrid seed; however, when gene flow did occur the average contamination (0.067%) exceeded the tolerances for registered and certified seed in the Pacific Northwest. This level of contamination likely would have been diluted to acceptable levels in commercial wheat fields. However, there are currently no tolerance standards for genetically engineered wheat in non-genetically engineered seed lots in the United States and several major export markets have a zero-trace tolerance standard (Kershen, 2004). Zero tolerance levels cannot be met with isolation requirements alone; but appropriate isolation combined with a nonzero threshold for off-type wheat can result in predictable degrees of genetic purity in wheat seed lots. While Hucl and Matus-Cadiz (2001) suggested a 30-m minimum isolation requirement in spring wheat, our results at one location (Athena, OR) suggest that 45 m may be more appropriate for winter wheat in the Pacific Northwest. Isolation may reduce pollen-mediated gene flow to predictable levels; however, seed mixing during harvest, transport, or processing also may provide a significant risk of off-type wheat in seed lots and should be examined in future research.

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