Isolation Distances for Minimizing Out-crossing in Spring Wheat

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Abstract

Currently recommended isolation distances of three or 10 m for pedigreed seed production of spring wheat (*Triticum aestivum* L.) may not be sufficient for cultivars with high out-crossing (OC) rates. The detection of higher than expected OC rates in wheat has directed this research to reassess currently recommended minimum isolation distances. The objective of this study was to determine if increased isolation distances are needed for cultivars that exhibit higher than normal levels of out-crossing. In each of two years, OC rates were determined for four Canadian spring wheat cultivars at each of 15 distances (0-33 m) from a blue aleurone pollen source. Cultivars were grown in rows perpendicular to the pollinator block to the north, south, west, & east. Target rows were replicated four times within each direction. Out-crossing in 'Katepwa' & 'Biggar' was not detected beyond three meters. Cultivars 'Roblin' & 'Oslo' exhibited higher than normal OC at distances of up to 27m. For Roblin & Oslo, an isolation distance of 30m is recommended to mitigate OC-derived off-types in the subsequent generation of pedigreed seed.

Introduction

The Canadian pedigreed seed production system endeavors to provide crop producers with a guaranteed product. Classes of seed including Select, Foundation, & Registered seed are usually involved in the ancestry of certified seed (Anonymous, 1994). Certified seed, the terminal pedigree class, is recommended for use by the crop producer. In Canada, a maximum impurity tolerance of 0.01% (one off-type per 10,000 plants x 100) in Foundation or Registered seed & 0.05% in Certified seed has been deemed acceptable in wheat production. The Canadian Prairie Spring class is the only exception, 0.03% in Registered seed & 0.08% in Certified seed are acceptable in the production of this class of wheat. The appearance of morphological off-types indicates that cultivar purity has been compromised. This can result from intra- or inter-specific out-crossing (OC). Minimum isolation requirements, from another genotype of the same crop species, of 10 m for the production of Select seed & three meters for the production of Foundation, Registered, or Certified seed are currently recommended in wheat. A number of currently registered Canadian spring wheat cultivars have OC rates that are 10 to 15 times higher than the assumed level of approximately 0.3% (Hucl, 1996). For example, highest OC rates were detected for Oslo (6.1%) followed by Roblin (1.4%), Biggar (1.1%), & Katepwa (0.4%). The detection of these higher than expected OC rates in wheat has directed our research to reassess currently recommended minimum isolation distances in the production of pedigreed seed.

Wheat is a self-pollinated species with OC rates that are usually less than 1%; however, higher OC rates have been reported. For example, OC rates ranging from 0.1 to 4.0% were reported for bread wheat cultivars (Griffin, 1987), 0.1 to 5.6% for 12 winter wheat cultivars (Martin, 1990), & 2.4 to 10.1% for six experimental wheat populations (Enjalbert et al., 1998). Out-crossing rates ranging from 0.3 to 6.1% have been reported for ten Canadian spring wheat cultivars (Hucl, 1996). High OC rates are of little concern in large commercial fields because the majority of OC will be intra-varietal. In contrast, problems may arise in the case of small plot increases where a number of genotypes are grown in close proximity. High OC rates can result in obvious off-types if morphologically diverse cultivars are grown in adjacent pedigreed seed plots.
Pollen dispersal also contributes to the appearance of off-types in adjacent pedigreed seed plots. Wheat pollen dispersal distances range from five to a minimum of 48 m (Khan et al., 1973). A number of studies have reported seed set on target male sterile lines at five (Zeven, 1968), six (Miller et al., 1975), eight (Bitzer & Patterson, 1967), & 48 m (Khan et al., 1973) from their pollen source. Consequently, high OC rates & optimum conditions for pollen dispersal (e.g., strong winds) may lead to an unacceptable level of off-types in the production of pedigreed seed at isolation distances of three or 10 m. The objective of this study was to determine if increased isolation distances are required in the production of pedigreed seed for wheat cultivars that exhibit higher than normal levels of OC.

Materials & Methods

Breeder seed of four spring wheat cultivars, Katepwa (Canada Western Red Spring [CWRS]), Roblin (CWRS), Biggar (Canada Prairie Spring [CPS]), & Oslo (CPS), possessing different OC levels (Hucl, 1996) were used in this study. Out-crossing rates for these cultivars were determined at distances ranging from 0 to 33 m from the pollen source. Blue aleurone wheat (Abdel-Aal & Hucl, 1999) was used as the pollen source in each of two years (1995 & 1996) at Saskatoon to quantify OC rates. A field protocol similar to that described by Khan et al. (1973) was used to determine desirable isolation distances. The blue-seeded pollinator was seeded in a 5 x 5 m block on 03 May 1995 & 03 May 1996 at a low rate (60 seeds/m^2) to promote tillering & thus extend the period of pollen shedding. The pollinator block was at the one leaf stage when the target rows were seeded. Fertilizer (11-51-0, N-P-K) was drilled in with the seed at a rate of 50 kg ha^{-1}. The experiments were established on fallow land. The soil type was a Vertic Haploborall silty clay.
In 1995 (May 10) & 1996 (May 24), four target rows of each cultivar were seeded perpendicular to the sides of the pollinator block to the north, south, west, & east. Target rows were 35 m in length (250 seeds/m^2) & spaced 30 cm apart. The four cultivars were randomized within four blocks (replications) in each direction. The experiment was bordered by 12 rows of 'Sceptre', a Canada Western Amber Durum wheat (*T. turgidum* L.) cultivar. The area surrounding the experiment was filled with spring wheat increase plots, none of which carried the blue aleurone gene.

At maturity, within each target row, 10 cm lengths of row were cut & threshed from the following distances from the pollen source: 0, 0.5, 1, 2, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, & 33m. Total seeds per sample were counted using an ESC-1 electronic seed counter (Agriculex Inc). The number of seeds counted per sample averaged 538 (standard error ±35) in 1995 & 644 (±9) in 1996. The blue aleurone trait is expressed in the hybrid seed (F_1). Seeds with light-blue aleurone were visually identified using a fluorescent light box source. Light blue seeds were grown out the following year

to confirm visual identifications. Wind speed & direction data were collected within 5 km of the trail site by Environment Canada. Out-crossing rates were calculated as follows:
OC (%) = # blue seeds observed in c_i at d_j/total # seeds collected of c_i at d_j x 100
where c_i is the *i* th cultivar & d_j is the *j* th distance. For each year, the averaged OC rate of a cultivar at any particular distance from the pollen source is based on four replications. Average percent out-crossing values were tested to be significantly different from zero (P=0.05) based on one-tailed t-tests.

Results & Discussion

Average OC rates were higher in 1995 than in 1996 (Table 1). The magnitude of OC was greatest for the cultivar Oslo followed by Roblin, Biggar, & Katepwa. The highest OC rates detected in this present study (3.8%, Oslo; 2.6%, Roblin; 0.4%, Biggar; 0.2%, Katepwa) were generally not as high as in our previous study (6.1%, Oslo; 1.4%, Roblin; 1.1%, Biggar; 0.4%, Katepwa; Hucl, 1996); however, the tendencies of these cultivars towards high/low OC rates are consistent across studies. The single seeding date for target rows in our current study, unlike the three seeding dates in our previous study, probably reduced the level of "nicking" between the pollen source & target cultivars. The estimated pollination period in both years was approximately 11 days. In 1995, averaged heading dates were as follows: pollen source (06-28), Roblin (06-22), Oslo (06-23), Biggar (06-27), & Katepwa (06-28). High levels of nicking were expected between the pollen source & Biggar/Katepwa. Low levels of nicking, if any, were expected between the pollen source & Roblin/Oslo. Detectable levels of OC for Roblin & Oslo suggest that nicking did in fact occur between the pollen source & Roblin/Oslo (Table 1). This successful level of nicking is probably explained by the observation that stigmas of male-fertile plants are known to be receptive for a period of four to 13 days (DeVries, 1971). In 1996, averaged heading dates were as follows: pollen source (07-06), Roblin (07-08), Oslo (07-10), Biggar (07-15), & Katepwa (07-14). Thus, higher levels of nicking were expected between the pollen source & Roblin/Olso compared to the pollen source & Biggar/Katepwa. The higher level of nicking between the pollinator source & Roblin/Oslo in 1996, compared with 1995, did not translate into increased OC rates in 1996. Thus, the differential heading of target cultivars does not appear to be associated with differential cultivar OC rates.

The highest OC rates were obtained to the west of the pollen source in 1995 & 1996 (Table 1). In 1995, averaged over distances & cultivars, OC rates were highest to the west of the pollen source (0.36%) followed by the north (0.17%), south (0.14%), & east (0.12%). In 1996, the highest OC rate was obtained to the west (0.11%) of the pollen source followed by the north (0.07%), east (0.05%), & south (0.04%). Contamination was increased in years with winds favorable to OC. Average wind speed during the estimated pollination period (11 days) was very similar in 1995 (13.9 km h^{-1}) & 1996 (12.7 km h^{-1}). The prevailing wind direction differed during the pollination period in 1995 & 1996. In 1995, the prevailing winds during the pollination period were northeastward or southeastward. Thus, the prevailing wind direction was associated with elevated OC rates in the westward direction of the experiment in 1995. In 1996, the prevailing winds during the same period were northeastward on four days with the remaining days being divided evenly among the other three directions. Thus, the prevailing wind direction did not appear to be associated with OC distribution in 1996. Differences in prevailing wind direction over years

suggest that isolation distances should not be based on experiments oriented in only one direction from the pollen source.

The distances at which OC was detected for a given cultivar x direction x year combination are presented in Table 1. The OC rate decreased with distance from the pollen source. For Katepwa & Biggar the maximum distance at which OC was detected was three meters. Thus, current isolation distances are satisfactory for cultivars Katepwa & Biggar, both low OC cultivars. The maximum distance at which OC was detected for both Roblin & Oslo was 27 m. No OC was detected for any of the four cultivars at a distance of 30 or 33 m to the north, east, south, or west of the pollen source in either year. The average distance at which 95% of the cumulative OC occurred for a given cultivar x direction combination is presented in Table 2. Roblin exhibited higher than normal OC at distances in the range from 0 to 12 m followed by Oslo, ranging from 0 to six meters. Oslo had the highest OC rate; however, OC generally occurred within seven meters of the pollen source. Averaged over years, OC at six meters to the west of the pollen source decreased by a factor of 10 for Roblin & five Oslo. This decrease in OC, for a self-fertile wheat crop, is substantially greater than that reported in the literature for male-sterile wheat (Khan et al., 1973). It appears that the high OC cultivars, Roblin & Oslo, are subject to contamination at isolation distances beyond those currently recommended by the Canadian Seed Growers' Association (CSGA). Contamination using 27m of isolation suggests that a requirement of 30 m should become the minimum isolation requirement for Roblin & Oslo in order to minimize OC off-types in the subsequent generation of seed. This distance is approximately 10 times the current isolation requirement for Foundation, Registered, or Certified seed production & three times the requirement for Select seed production.

Table 1. Average percent out-crossing for four wheat cultivars at various distances to the north, east, south, & west of the pollen source. Data are the average of four replications.

		Distance from pollen source (m)												
Cultivar	Direction	0	0.5	1	2	3	6	9	12	15	18	21	24	27
1995														
Katepwa	N	0	0.09^{ns} †	0	0.03^{ns}	0.04^{ns}	0	0	0	0	0	0	0	0
	E	0	0	0	0.04^{ns}	0	0	0	0	0	0	0	0	0
	S	0	0	0.06^{ns}	0	0	0	0	0	0	0	0	0	0
	W	0	0.04^{ns}	0	0.03^{ns}	0	0	0	0	0	0	0	0	0
Biggar	N	0.12^{ns}	0	0.06^{ns}	0.04^{ns}	0.04^{ns}	0	0	0	0	0	0	0	0
	E	0.18^{ns}	0.09^{ns}	0.10^{ns}	0.04^{ns}	0	0	0	0	0	0	0	0	0
	S	0.26^{ns}	0.11^{ns}	0.08^{ns}	0	0	0	0	0	0	0	0	0	0
	W	0.24^{ns}	0.09^{ns}	0.06^{ns}	0	0	0	0	0	0	0	0	0	0
Roblin	N	0.74^{ns}	0.47^{ns}	0.34^{ns}	0.16^{ns}	0.11^{ns}	0.09^{ns}	0.04^{ns}	0.06^{ns}	0	0.04^{ns}	0.05^{ns}	0	0
	E	1.62	0.33	0.23	0	0	0	0	0	0	0	0	0	0
	S	2.09	0.86	0.18	0.36	0.08^{ns}	0.19^{ns}	0	0	0	0	0	0	0
	W	2.63	0.89^{ns}	0.58^{ns}	0.78	0.53	0.14^{ns}	0.44^{ns}	0.05^{ns}	0.06^{ns}	0.12^{ns}	0.13^{ns}	0	0
Oslo	N	3.23	1.42	0.97	0.85	0.44	0.49	0.16^{ns}	0.06^{ns}	0.07^{ns}	0.13^{ns}	0	0	0
	E	2.38	0.57	0.99	0.21	0.04^{ns}	0	0.08^{ns}	0	0	0	0	0	0
	S	1.52^{ns}	1.03	1.01	0.21	0.13^{ns}	0.16^{ns}	0.04^{ns}	0	0	0	0	0	0
	W	3.00	3.78	2.56	2.99	1.62	0.67^{ns}	0.11^{ns}	0.07^{ns}	0	0.05^{ns}	0	0	0.09^{ns}
1996														
Katepwa	N	0.15^{ns}	0	0	0.07^{ns}	0	0	0	0	0	0	0	0	0
	E	0.05^{ns}	0.05^{ns}	0.09^{ns}	0	0.09^{ns}	0	0	0	0	0	0	0	0
	S	0.08^{ns}	0	0	0	0	0	0	0	0	0	0	0	0
	W	0	0	0	0	0	0	0	0	0	0	0	0	0
Biggar	N	0.05^{ns}	0	0	0	0	0	0	0	0	0	0	0	0
	E	0.17^{ns}	0	0.10^{ns}	0.06^{ns}	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0	0
	W	0.43^{ns}	0	0.07^{ns}	0	0	0	0	0	0	0	0	0	0
Roblin	N	0.43	0.37	0.21	0.03^{ns}	0	0.02^{ns}	0	0	0	0	0	0	0
	E	0.59	0.23	0.36	0.03^{ns}	0.14^{ns}	0	0	0.04^{ns}	0.03^{ns}	0	0.03^{ns}	0.03^{ns}	0
	S	0.28	0.33^{ns}	0.04^{ns}	0.15^{ns}	0.03^{ns}	0.07^{ns}	0	0	0	0	0	0	0
	W	0.70	0.57	0.60	0.74	0.12	0.19	0.18^{ns}	0	0.07^{ns}	0.08^{ns}	0	0	0.03^{ns}
Oslo	N	2.00	0.37^{ns}	0.13^{ns}	0.14^{ns}	0.10^{ns}	0.26^{ns}	0.03^{ns}	0.03^{ns}	0.04^{ns}	0	0	0	0
	E	0.12^{ns}	0.35^{ns}	0.12^{ns}	0.19^{ns}	0.12^{ns}	0.04^{ns}	0.04^{ns}	0	0	0	0	0	0
	S	0.63^{ns}	0.42	0.08^{ns}	0.04^{ns}	0	0	0	0	0	0	0	0	0
	W	0.84^{ns}	0.78^{ns}	0.42	0	0.32^{ns}	0.06^{ns}	0	0.05^{ns}	0	0.04^{ns}	0	0	0

† Ns, Not significantly different from zero at P=0.05 based on one-tailed t-tests.

Table 2. Average distance (m) from pollinator source (± SE) at which 95% of total OC was observed within a target row.

Cultivar	Direction			
	North	East	South	West
1995				
Katepwa	1.0 ± 0.6	1.0 ± 0	0.5 ± 0	1.0 ± 0
Biggar	0.9 ± 0.4	0.7 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
Roblin	10.5 ± 3.5	0.5 ± 0	2.3 ± 0.5	12.0 ± 2.1
Oslo	6.8 ± 2.9	1.1 ± 0.3	1.6 ± 0.6	3.3 ± 1.0
1996				
Katepwa	1.0 ± 0	1.1 ± 0.5	0	--- †
Biggar	0	0.7 ± 0.2	---	0.5 ± 0
Roblin	1.1 ± 0.7	8.5 ± 5.0	8.3 ± 6.3	9.0 ± 2.7
Oslo	3.5 ± 1.9	3.3 ± 1.0	0.5 ± 0.2	6.6 ± 3.4

† No out-crossing observed at any of the distances sampled (0 to 33m) from the pollinator source.

Conclusions

In wheat, the CSGA currently recommends an isolation distance, from another genotype of the same crop species, of 10 m for the production of Select seed & three meters for the production of Foundation, Registered, & Certified seed. This study indicates that isolation requirements are satisfactory for Katepwa & Biggar, cultivars with low OC rates. In contrast, cultivars Roblin & Oslo, with high OC rates, exhibited contamination at distances of up to 27m. In the absence of other studies, we suggest that 30 m be used as the minimum isolation requirement for the production of pedigreed seed of cultivars suspected to exhibit higher than normal levels of OC (Hucl & Matus-Cadiz, *In Press*).

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References

Abdel-Aal, E.-S.M., & P. Hucl. 1999. A rapid method for quantifying total anthocyanins in blue aleurone & purple pericarp wheats. Cereal Chem. 76:350-354.

Anonymous. 1994. Regulations & procedures for pedigreed seed crop production. Circular 6-94. Canadian Seed Growers' Association. Ottawa, ON.

Bitzer, M.J., & F.L. Patterson. 1967. Pollen dispersal & cross-pollination of soft red winter wheat (*Triticum aestivum* L.). Crop Sci. 7:482-484.

DeVries, A.P. 1971. Flowering biology of wheat particularly in view of hybrid seed production: a review. Euphytica 20:152-170.

Enjalbert, J., I. Goldringer, G. Jacques, & P. Brabant. 1998. The relevance of out-crossing for the dynamic management of genetic resources in predominantly selfing *Triticum aestivum* L. (bread wheat). Gen. Sel. Evo. 30(Suppl. 1):S197-S211.

Griffin, W.B. 1987. Out-crossing in New Zealand wheats measured by occurrence of purple grain. New Zealand J. Agric. Res. 30:287-290.

Hucl, P. 1996. Out-crossing rates for 10 Canadian spring wheat cultivars. Can. J. Plant Sci. 76:423-427.

Hucl, P. & M. Matus-Cadiz (2001). Isolation Distances for Minimizing Out-crossing in Spring Wheat. Crop Sci. (*In Press*).

Khan, M.N., E.G. Heyne, & A.L. Arp. 1973. Pollen distribution & the seed set on *Triticum aestivum* L. Crop Sci. 13:223-226.

Martin, T.J. 1990. Out-crossing in twelve hard winter wheat cultivars. Crop Sci. 30:59-62.

Miller, J.F., K.J. Rogers, & K.A. Lucken. 1975. Effect of field production techniques on hybrid wheat seed quality. Crop Sci. 15:329-332.

Zeven, A.C. 1968. Cross pollination & sources of restorer genes in wheat & a semi-hybrid variety. Euphytica 17(Suppl.1):75-81.