Out-crossing rates for 10 Canadian spring wheat cultivars

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Hucl, P. 1996. **Out-crossing rates for 10 Canadian spring wheat cultivars**. Can. J. Plant Sci. **76**: 423–427. The genetic purity of pedigreed wheat seed can be compromised by inter-cultivar cross-pollination. Common wheat (*Triticum aestivum* L.) is assumed to be highly self-pollinated, an assumption that has not been verified for more recent western Canadian spring wheat cultivars. This field study was designed to estimate the outcrossing (OC) rate in three market classes of spring wheat in each of 2 yr and to determine whether OC rates were associated with specific spike characteristics. Highest OC rates were detected for the cultivar Oslo (6.05%) followed by Rongotea (2.30%), Roblin (1.43%), Wildcat (1.35%), Biggar (1.05%) and Glenlea (0.95%). In contrast, Katepwa and CDC Makwa had OC rates of 0.38 and 0.30%, respectively. The cultivars Oslo, Columbus, Roblin and Glenlea tended to have lower iodine pollen viabilities. Of those four cultivars, three had higher-than-average OC rates. No single spike characteristic was correlated with OC rate. However, the cultivars Oslo, Wildcat and Glenlea had high OC rates, low pollen staining and spikes which tapered at the extremeties. Furthermore, cultivars with higher OC rates tended to have a greater degree of spikelet opening at anthesis. The high levels of OC observed in this study could result in obvious off-types if morphologically diverse cultivars were grown in adjacent pedigreed seed fields. The current 3 to 10 m isolation distance for pedigreed wheat seed production may not be enough to ensure that OC is minimized.

Key words: Cross-pollination, spring wheat, market class

Hucl, P. 1996. **Taux de croisement hétérogène spontané chez dix cultivars canadiens de blé de printemps.** Can. J. Plant Sci. **76**: 423–427. La pureté génétique des semences de blé contrôlées peut être compromise par la pollinisation croisée entre divers cultivars. Bien que le blé tendre (*Triticum aestivum* L.) soit censé être fortement autogame, cette supposition n'a pas été vérifiée chez les cultivars récents de blé de printemps de l'Ouest canadien. Une étude au champ avait pour objet d'estimer pendant deux saisons de végétation, le taux de croisement spontané (CS) dans trois classes commerciales de blé de printemps et de déterminer si ces taux sont liés à des caractères particuliers de l'épi. Les taux de CS les plus élevés étaient observés chez la variété Oslo (6,05%). Venaient ensuite Rongotea (2,30%), Roblin (1,43%), Wildcat (1,35%), Biggar (1,05%) et Glenlea (0,95%). En revanche, les taux n'étaient que de 0,38 et de 0,30%, respectivement, pour Katepwa et pour CDC Makwa. Oslo, Columbus, Roblin et Glenlea manifestaient une plus faible viabilité du pollen et sur ces quatre variétés, trois manifestaient des taux de CS supérieurs à la moyenne. Aucun caractère particulier de l'épi n'était corrélé avec le taux de CS. Toutefois, Oslo, Wildcat et Glenlea avaient à la fois un taux de CS élevé, une faible coloration du pollen à l'iode et des épis fusiformes. En outre, ces cultivars manifestaient des taux de CS élevés avec, en général, une ouverture plus grande des épillets à l'anthèse. Les niveaux élevés de CS observés dans cette étude pourraient donner lieu à des hors-types évidents si des cultivars de morphologie différente étaient cultivés dans des champs de culture de semences contrôlées adjacents. La distance d'isolation de 3 à 10 mètres, fixée pour la production des semences contrôlées de blé, ne serait peut-être pas suffisante pour réduire au minimum les risques de CS.

Mots clés: Pollinisation croisée, blé de printemps, classe commerciale

Since the introduction of short-statured spring wheat cultivars in the mid-1980s western Canadian pedigreed seed growers have occasionally made the observation that a number of cultivars, particularly those carrying *Rht* genes, appear to produce a higher than accustomed-to frequency of off-types. To date there have been no data available to either substantiate or refute this observation.

The literature on the origin of crop off-types has recently been reviewed (Laverack and Turner 1995). Briefly, cultivar purity can be compromised by mechanical admixture or the appearance of genetic off-types. Genetic off-types can arise in an initially homogeneous cultivar due to mutations or chromosomal instability. The pedigreed seed grower can do little about controlling these processes beyond roguing the off-types. A second source of variants in self-pollinated crops can arise from OC. The OC frequency will vary with floral modification and/or environmental conditions. Cultivars which are predisposed to OC have been identified in a number of economically important self-pollinated crop species.

Wheat (*Triticum aestivum* L.) is a self-pollinated species with OC rates that are usually less than 1%. Under certain growing conditions individual genotypes may have OC rates of up to 4–5% (Griffin 1987; Martin 1990). Harrington (1932) measured OC at Saskatoon over a 5-yr period and reported levels ranging from 0 to 1.1% for four red spring wheat cultivars. Morphological traits such as spike laxness and lemma flatness appear to result in a greater degree of floret opening in wheat (DeVries 1971). The magnitude of floret opening is considered a varietal trait, but environmental conditions at anthesis may over-ride genotypic differ-

Abbreviations: **CPS**, Canada Prairie Spring; **CWES**, Canada Western Extra Strong; **CWRS**, Canada Western Red Spring; **OC**, outcrossing ences. Stigma exposure may occur in certain genotypes, increasing the risk of OC. Awns may trap pollen, reducing OC (DeVries 1971).

The objective of this project was to determine outcrossing rates for selected cultivars and to relate the OC rates to specific floral characteristics. The resulting information could eventually be used by pedigreed seed growers to establish new isolation distances for cultivars prone to OC. If there were to be a clear-cut relationship between OC and floral or spike morphology, wheat breeders could make use of this information in their programs in order to avoid the release of cultivars prone to out-crossing.

MATERIALS AND METHODS

Eleven spring wheat (*Triticum aestivum* L.) cultivars representing three classes of wheat were evaluated for OC rate in each of two years (1992 and 1993) at Saskatoon. The cultivars evaluated were Biggar, Genesis, and Oslo (Canada Prairie Spring class) carrying *Rht* genes; Wildcat and Glenlea (Canada Western Extra Strong class) and Katepwa, Laura, CDC Makwa, Columbus, Roblin (Canada Western Red Spring class). The semidwarf cultivar Rongotea from New Zealand (Griffin 1987) was used a control. For data presentation purposes, Rongotea has been grouped with the CPS cultivars as it has a similar agronomic and quality profile. Breeder seed was used for all Canadian cultivars with the exception of Glenlea, where Foundation seed was used. Seed of Rongotea was one generation removed from the equivalent of Certified seed and was obtained from a rogued plot.

The cultivars were subjected to a seedling giberrellic acid response assay (Gale et al. 1975) to confirm whether they carry *Rht* alleles. Biggar, Genesis, Oslo, Wildcat and Rongotea were giberrellic acid-insensitive, indicating that these cultivars are semidwarf.

Paired pollinator rows of a blend of two purple-seeded cultivars (75% Konini:25% line 3496.3) were seeded on 6 May in 1992 and 1993. Konini flowers 3 to 4 d later than the cultivar Katepwa at Saskatoon and appears to be a prolific pollen shedder under western Canadian growing conditions. Line 3496.3 is 2–3 d later heading than Konini. Purple pericarp color has been used as a morphological marker to measure OC rates in wheat. Purple pericarp colour in the cultivar Konini is controlled by dominant duplicate genes (Griffin 1987).

Single rows of the target cultivars were seeded at three dates: 14 May, 22 May and 29 May 1992 and 17 May, 25 May and 3 June, 1993. Three seeding dates were used in order to synchronize the flowering of the target cultivars with that of the pollinator rows. Each target row was planted with a single-row push-type seeder equipped with disc openers. Each target cultivar plot consisted of a 6-m row with each plot being sandwiched between a pair of pollinator rows. The row spacing was 20 cm. The target cultivars were seeded at a rate of 250 seeds m^{-2} but the pollinator rows were seeded at one-quarter that rate to promote tillering and hence spread out the flowering period. Fertilizer (11-51-0, N-P-K) was drilled in with the seed at a rate of approximately 50 kg ha^{-1}. The experiments were established on fallow land. The soil type was a Bradwell clay loam.

A strip plot design with four replications was used. Cultivars were assigned to main plots and randomized within a block (replication). Seeding dates were un-randomized subplots within each mainplot.

The experiment was flood irrigated three times from late May to late June in 1992 to ensure a good stand establishment in spite of the extremely dry conditions in June (15% of normal rainfall) of that year. The plots were rogued prior to anthesis.

The following data were collected from all plots:

1. Spike thickness at Zadoks Growth Stage (ZGS) 58 (Zadoks et al. 1974).
2. Spikelet width at ZGS 58 (measured across the glume shoulders of the spikelet at the median rachis node position).
3. Spikelet width at ZGS 60 (measured across the glume shoulders of first spikelet to extrude anthers).
4. Days to 5% anthesis (ZGS 60).
5. Days to 95% anthesis (ZGS 68).
6. Degree of stigma extrusion (1992 only).
7. Pollen viability estimated by potassium iodine (KI) staining.
8. Spikelet fertility (kernels $spikelet^{-1}$)
9. Spike shape on a scale of 1 to 4 (see Table 2 footnote).
10. Spike laxness at maturity (spikelets per cm of spike)

Measurements for items 1, 2, 3, 6 and 7 were conducted on five spikes per plot while those for items 8, 9 and 10 were based on a 10-spike sample per plot. Items 4 and 5 were estimated on a plot basis. Items 1 through 3 were measured using a calliper. For item 7 spikes were harvested at ZGS 60 and fixed in 450 mL of a 3:1 solution of 95% ethanol:acetic acid in 500 mL Mason jars. Anthers were stained with Newton’s stain. Three positions (basal, median and apical) on a spike were assayed for pollen staining. Spikelets containing sterile anthers were not assayed. Each determination was based on a count of 100 pollen grains. Anthers were placed on a microscope slide with three drops of stain, gently crushed to extract the pollen and then removed. A cover slip was placed over the pollen grains. Unstained or partially stained but shrivelled pollen grains were rated as unviable.

At maturity, each plot was mechanically sickled and bound with a Suzue binder and subsequently threshed with a Vogel thresher.

Seed from the 1992 and 1993 target plots was space-planted during the first week of May in 1993 and 1994, respectively. Each seed lot was inspected for possible mechanical contamination (i.e. presence of purple seed) prior to planting. Approximately 1000 seeds from each plot were planted 20 cm apart within rows spaced 30 cm apart. At maturity 500 spikes (one per plant) were harvested. The number of purple seeded plants out of 500 were used to estimate the rate of outcrossing.

With the exception of spike shape scores, data were subjected to analyses of variance (ANOVA) for a strip plot

Table 1. Percent outcrossing for 11 spring wheat cultivars planted at three dates

	1992				1993			
	Seeding date				Seeding date			
	1	2	3	Average	1	2	3	Average
Biggar	0.95	0.15	0.00	0.36	1.15	0.15	0.00	0.43
Genesis	0.65	0.30	0.05	0.33	0.30	0.15	0.00	0.15
Oslo	5.15	5.05	5.40	5.20	5.15	6.70	0.40	4.08
Rongotea	2.20	1.85	0.45	1.50	2.40	1.55	0.30	1.42
Wildcat	0.90	1.65	0.70	1.08	1.05	1.03	0.25	0.78
Glenlea	0.70	1.10	0.10	0.63	0.80	0.15	0.20	0.38
Katepwa	0.55	0.55	0.00	0.36	0.20	0.00	0.05	0.08
Laura	0.50	0.80	0.25	0.51	0.95	0.05	0.10	0.37
CDC Makwa	0.30	0.25	0.05	0.20	0.30	0.00	0.10	0.13
Columbus	0.35	0.20	0.00	0.18	0.10	0.05	0.05	0.07
Roblin	0.30	1.20	0.30	0.60	1.65	0.73	0.00	0.80
Average	1.14	1.19	0.66	0.99	1.28	0.96	0.13	0.79
Pooled SE = (df = 60)		0.23				0.18		

Table 2. Spike traits for 11 spring wheat cultivars averaged over two years and three seeding dates

Cultivar	spike laxness (spikelets cm^{-1})	spike shape[z] (1–4)	spike thickness (mm)	spikelet width[y] (mm)	relative spikelet opening[x] (%)	FLOWDUR[w] (d)	Kernels $spikelet^{-1}$ (no.)
Biggar	1.90	1.5	8.5	6.8	19.5	3.7	2.9
Genesis	1.61	1.0	7.9	6.5	27.4	3.7	3.1
Oslo	1.66	3.4	7.2	6.5	16.9	3.1	2.4
Rongotea	1.87	1.0	7.6	6.3	14.6	3.5	2.2
Wildcat	1.64	3.4	7.7	6.8	11.4	3.5	2.9
Glenlea	1.48	3.5	7.9	6.5	16.0	3.3	2.6
Katepwa	1.87	2.5	7.5	6.1	9.2	3.1	2.6
Laura	2.08	1.0	7.4	6.2	12.9	3.7	2.4
CDC Makwa	1.80	2.5	7.6	6.2	8.8	3.1	2.6
Columbus	1.93	1.8	7.8	6.1	9.8	3.4	2.1
Roblin	1.82	1.0	7.7	6.5	10.4	3.1	2.4
Pooled SE (df =60)	0.01	0.1	0.02	0.02	13.9	0.2	0.01

[z]1 = strap; 2 = basal tapering; 3 = apical tapering; 4 = fusiform.
[y]At ZGS 58.
[x][(width at ZGS 60 – width at ZGS 58)/width at ZGS 58] × 100.
[w]Duration of flowering period calculated as the time from 5 to 95% anthesis.

design, using a mixed model where replications were considered random effects and the remaining factors, fixed effects. Data collected on 5 or 10 spikes per plot were averaged prior to statistical analysis. OC data were subjected to a $\sqrt{X} + 0.5$ transformation prior to ANOVA. Untransformed data are presented in Table 1. The spike shape data were not averaged but analyzed using the Mood median test (MINITAB 1989). The median shape score was calculated for each plot. The plot median was then used to determine the median score for each cultivar. Pollen data was collected from three replications.

RESULTS AND DISCUSSION

Significant cultivar and seeding date effects were detected for all the traits measured except degree of stigma extrusion. No stigma extrusion was observed during flowering for any of the genotypes, regardless of date of seeding. This would indicate that stigma extrusion is not likely a cause of OC in this set of cultivars.

Maximum OC rates for the 11 cultivars ranged from 0.3 (CDC Makwa) to 5.4% (Oslo) in 1992 and from 0.2 (Katepwa) to 6.4% (Oslo) in 1993 (Table 1). The OC rate varied with seeding date. The earliest and latest cultivars

Table 3. Pollen staining (%) at three spike positions for 11 spring wheat cultivars averaged over three seeding dates

	1992				1993			
	Spike position				Spike position			
Cultivar	Basal	Median	Apical	Avg.	Basal	Median	Apical	Avg.
Biggar	99.5	98.4	96.4	98.0	97.3	96.8	92.9	95.7
Genesis	99.1	99.7	97.9	98.9	97.7	97.9	96.9	97.5
Oslo	94.1	93.5	57.9	81.8	94.8	92.9	79.7	89.1
Rongotea	94.6	96.4	93.5	94.8	95.2	93.8	92.2	93.7
Wildcat	94.0	95.5	92.7	94.1	94.1	92.9	89.1	92.1
Glenlea	92.9	96.2	91.9	93.6	87.6	90.8	86.4	88.3
Katepwa	99.5	99.0	98.3	98.9	97.1	96.8	95.6	96.5
Laura	99.2	98.9	98.4	98.8	96.6	95.3	92.2	94.7
CDC Makwa	98.8	98.7	98.1	98.5	95.8	95.8	91.5	94.4
Columbus	91.6	87.2	90.3	89.7	91.7	85.7	83.0	86.8
Roblin	98.1	96.4	92.2	95.6	88.7	90.2	84.0	87.7
Average	96.5	96.4	91.5	94.8	94.2	93.5	89.4	92.4
Pooled SE (df = 176)		4.5				7.4		

differed by 7 to 8 d with respect to flowering date. Better synchronization with the pollen source would have been expected with an early planting for the later-maturing cultivars (Biggar, Genesis, Rongotea, Glenlea) as opposed to a later planting for early maturing cultivars (Oslo, Wildcat, Roblin). Indeed, that is what happened in 1992 (Table 1). In 1993, however, the third seeding date resulted in low OC rates for even the early flowering cultivars. The purple-seeded cultivar Konini is a facultative wheat (Sparks et al. 1987), its vernalization requirement was better met by the very cool growing conditions encountered during the spring of 1993. Both Konini and 3496.3 ceased flowering prior to anthesis of the later-seeded material in 1993.

The current Canada Western Red Spring (CWRS) agronomic standard, Katepwa had, on average, a maximum OC rate of 0.38%. Canada Prairie Spring (CPS) cultivars tended to have the highest (2.53%) maximum OC rates followed by the Canada Western Extra Strong (CWES) (1.15%) and CWRS wheat cultivars (0.64%). The CPS cultivar average was inflated by the high OC rate of the cultivar Oslo. However, Biggar had a maximum OC rate nearly twice that of the cultivar Katepwa while Genesis had an OC rate similar to that of Katepwa. Of the CWRS cultivars, CDC Makwa and Columbus had the lowest OC levels while Laura and Roblin had the highest OC rates. The cultivar Rongotea had an average maximum OC rate of 2.30% (Table 1) compared to the 2.84% reported by Griffin (1987) under New Zealand growing conditions. The similar OC rates obtained for Rongotea in the present study and that of Griffin (1987) suggest that OC is a repeatable trait. The inter-year correlation for maximum OC rate in this study was very high (r = 0.98**, n = 11), providing further evidence of repeatability across environments.

Based on wheat class averages the maximum OC rates reported here are approximately 13 to 31 times higher than the Canadian Seed Growers Association (CSGA) allowable off-type frequency of 5 (CWRS, CWES) or 8 (CPS) in 10 000 plants for wheat at the Certified seed level (CSGA 1994) and 64 to 115 times the tolerance at the Foundation and Registered levels. Since the pollen contamination source was only 0.2 m from the seed sources in this study, one would expect lower contamination levels with the required (CSGA 1994) isolation distance of 3 m. Furthermore, contaminant levels will be diluted as a function of field size and shape.

Cultivar rankings for spike traits and flowering period were similar across seeding dates and years. Thus, the data are presented averaged over years and seeding dates (Table 2). Cultivars differed for each of the seven spike traits measured. Although statistically significant (P = 0.01) the cultivar differences for spike thickness, spikelet width and duration of flowering period were relatively small (Table 2). The cultivars Glenlea and Genesis had the laxest spikes while Laura and Columbus had the densest spikes. Glenlea, Wildcat and Oslo had the most tapered spikes while Genesis, Rongotea, Laura and Roblin had the squarest spikes. Genesis and Biggar exhibited the greatest degree of spikelet opening; CDC Makwa and Katepwa, the least. The cultivars Genesis and Biggar produced the most kernels spikelet^{-1}; Columbus and Rongotea the least. Based on cultivar means (n = 11), the correlations between spike traits and OC rate were low and statistically non-significant. This suggests no single morphological trait can be used for predicting cultivar OC rate.

If the cultivars with the highest OC rates are examined, Oslo tended to have a laxer, fusiform spike, greater degree of spikelet opening at anthesis and a lower spikelet fertility (Table 2). Rongotea tended to have a lower spikelet fertility. Oslo and Rongotea had spikelet fertility levels approximately 30% lower than those of the other semidwarf cultivars. Biggar and Glenlea, which also tended to have higher OC rates, had a higher degree of spikelet opening at anthesis. CDC Makwa and Columbus, which had the lowest OC rates, tended to have denser spikes and reduced spikelet opening.

Pollen staining levels were, on average, similar for the two years of this study. The cultivars Oslo, Columbus, Glenlea and Roblin had the lowest estimated pollen viability levels while Katepwa and Genesis had the highest levels (Table 3). Although not as pronounced as in 1992, the pollen staining of Oslo dropped off in the apical region of the spike (Table 3). Owuoche et al. (1994) evaluated six of the cultivars used in the current study and found that Oslo and Roblin had the lowest pollen staining levels while Columbus had a satisfactory level. Furthermore, Oslo had the lowest floret fertility of the eight cultivars studied by Owuoche et al. (1994). Owuoche et al. (1994) ascribed the reduced fertility of Oslo and Roblin to sensitivity to soil copper deficiency. The plant-available copper levels in the soil from the 1992 and 1993 experiment sites measured at two depths (0 to 15 and 16 to 30 cm) were 0.90 ± 0.14 and 1.65 ± 0.35 μg g^{-1} ($n = 2$) for the 2 yr, respectively. These copper levels are considered to be adequate for wheat. Thus, it appears that copper deficiency was not the cause of elevated OC rates for the cultivars Oslo and Roblin in this study.

When cultivars are grouped according to their giberrellic acid response, semidwarfs averaged a maximum OC rate of 2.25% compared to 0.70% for the standard height cultivars. When the extreme cultivars Oslo and Rongotea are not included, the semidwarf group average is 0.95%. Thus, based on the cultivars used in this study, there appears to be, on average, a marginally higher OC rate for semidwarfs. Both Griffin (1987) and Martin (1990) concluded that semidwarf and standard height cultivars did not differ significantly in OC. However, specific aneuploids are responsible for tall variants in semidwarf cultivars (Storlie et al. 1993; Laverack and Turner 1995) providing a genetic basis for a higher frequency of variants in GA-insensitive cultivars.

In conclusion, outcrossing was observed in each of three spring wheat market classes. Highest OC rates were detected for the cultivar Oslo followed by Rongotea, Roblin, Wildcat, Biggar and Glenlea. As suggested by others (Griffin 1987; Martin 1990) high levels of OC are likely to result in obvious off-types if morphologically diverse cultivars are grown in adjacent pedigreed seed fields. The current 3 m isolation distance may not be enough to ensure that contamination levels are kept within current CSGA tolerances. A number of cultivars (Oslo, Columbus, Roblin and Glenlea) had low pollen viability levels, as estimated by KI staining, in 2 yr of testing. Of those four cultivars, three had higher-than-average OC rates. Over-all, spike characteristics did not appear to be correlated with OC level. However, Oslo, Wildcat and Glenlea had spikes which tapered at the extremeties. This tapering results from reduced spikelet fertility in the base and apex of the spike (Herzog and Stamp 1983). These three cultivars were among those with the lowest pollen stainability and highest OC rates. In addition, some of the cultivars with higher OC rates tended to have a greater degree of spikelet opening at anthesis.

ACKNOWLEDGEMENTS

Thanks are extended to Janet Fehr, Ken Jackle, Glen Trowell, Coralie Sorochuck and Wendy Schatz for their technical assistance and Dr. G. Scoles for the use of a microscope in his laboratory. Mr. D. Gehl (Agriculture and Agri-Food Canada), Mr. W. Farley (Farley Seeds), Dr. R.J. Graf (Saskatchewan Wheat Pool) and Mr. G. Ovenden (New Zealand Institute for Crop and Food Research, Lincoln) donated seed of the cultivars used in this study. This research was funded by a grant from the Canadian Seed Growers Association.

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