Does the presence of the gene for glabrous hull in annual canarygrass affect the response to chloride fertilizer?

W. E. May[1], C. B. Holzapfel[2], G. P. Lafond[1], and J. J. Schoenau[3]

[1]Agriculture and Agri-Food Canada, Indian Head Research Farm, RR#1 Gov Rd, P.O. Box 760, Indian Head, Saskatchewan, Canada S0G 2K0 (e-mail: william.may@agr.gc.ca); [2]Indian Head Agricultural Research Foundation, RR#1 Gov Rd, Box 156, Indian Head, Saskatchewan, Canada S0G 2K0; and [3]University of Saskatchewan, Department of Plant Sciences, 51 Campus Drive, Saskatoon, Saskatchewan, Canada S7N 5A8.
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May, W. E., Holzapfel, C. B., Lafond, G. P. and Schoenau, J. J. 2013. **Does the presence of the gene for glabrous hull in annual canarygrass affect the response to chloride fertilizer?** Can. J. Plant Sci. **93**: 109–118. Annual canarygrass is an important cereal crop in western Canada with a unique niche market as feed for caged birds. Chloride (Cl) fertilizer has been shown to increase seed yield in annual canarygrass; however, the response was only tested in one glabrous cultivar. Currently, glabrous cultivars created through mutagenesis, are lower yielding than cultivars with trichomes on their lemma, palea and glumes. The objective of this study was to determine if the mutagenic process which created cultivars that lack trichomes on their lemma, palea and glumes also affected the response of annual canarygrass to chloride fertilizer. A two-way factorial study was conducted across 7 site-years. The first factor was Cl applied at two rates (0 and 18.2 kg Cl ha^{-1}) and the second factor was four cultivars (Keet, Cantate, CDC Togo (glabrous) and CDC Bastia (glabrous). The application of Cl increased the seed yield of annual canarygrass by 25% and most of this increase was due to a 21% increase in seeds per panicle. Kernel weight also contributed to increased seed yield. Chloride did not interact with the presence or absence of trichomes and therefore growers can expect to receive a yield increase from the application of Cl regardless of the annual canarygrass cultivar grown. Growers should apply 9 kg ha^{-1} of Cl when growing annual canarygrass. In conclusion, Cl is not involved in the physiology of the lower yield in glabrous cultivars compared with cultivars with trichomes, and Cl could not explain the seed yield differences between the two types of annual canarygrass.

Key words: Canaryseed, annual canarygrass, environmental conditions, seed yield, yield stability

May, W. E., Holzapfel, C. B., Lafond, G. P. et Schoenau, J. J. 2013. **La présence du gène de la cosse glabre chez l'alpiste roseau affecte-t-elle la réaction aux engrais contenant du chlore?** Can. J. Plant Sci. **93**: 109–118. L'alpiste roseau est une importante céréale annuelle dans l'ouest du Canada. Cette culture occupe un créneau unique, qui est celui de la nourriture pour les oiseaux en cage. On sait que les engrais à base de chlore (Cl) accroissent le rendement grainier de l'alpiste, cependant, cette réaction n'a été testée que sur un cultivar glabre. Or, à l'heure actuelle, les variétés glabres, qui résultent de la mutagénèse, s'avèrent moins productifs que les cultivars présentant des trichomes sur leur lemme, leur paléole et leurs glumelles. L'étude devait établir si le processus de mutagénèse à l'origine des cultivars sans trichomes sur le lemme, le paléole et les glumelles modifie aussi la réaction de l'alpiste roseau annuel aux engrais renfermant du chlore. À cette fin, les auteurs ont procédé à une expérience bifactorielle pendant 7 années-sites. Le premier facteur consistait en deux taux d'application de l'engrais Cl (0 et 18,2 kg de Cl par hectare) et le second, en quatre cultivars [Keet, Cantate, CDC Togo (glabre) et CDC Bastia (glabres)]. L'application d'engrais Cl augmente le rendement grainier de l'alpiste roseau annuel de 25 % et la majeure partie de cette hausse résulte d'une hausse de 21 % du nombre de graines par panicule. Le poids des graines concourt aussi à accroître le rendement grainier. Le chlorure n'interagit pas avec la présence ou l'absence de trichomes, de sorte que les producteurs peuvent s'attendre à une hausse de rendement s'ils appliquent de l'engrais Cl, quelle que soit la variété d'alpiste cultivée. Les producteurs devraient appliquer 9 kg de Cl par hectare aux cultures d'alpiste roseau annuel. En conclusion, le Cl n'intervient pas dans les mécanismes physiologiques responsables du rendement plus faible des cultivars glabres, comparativement à celui des cultivars pourvus de trichomes, et le Cl n'explique pas l'écart du rendement grainier entre ces deux types d'alpiste roseau.

Mots clés: Alpiste roseau, alpiste roseau annuel, conditions environnementales, rendement grainier, stabilité du rendement

The major use of canaryseed or annual canarygrass (*Phalaris canariensis* L.) has been as a feed for caged birds. Approximately 69 to 79% of the world's annual canarygrass is produced in Canada, centered in the province of Saskatchewan (FAOSTAT 2008). The first recorded test of annual canarygrass as a grain crop in Saskatchewan was at Indian Head, SK, in 1906 (MacKay 1907). In Canada, 800 ha of annual canarygrass was first grown commercially in 1971. Seeded area has ranged from 95 000 to 350 000 ha over the past 20 yr, with 89 to 98% of the production in Saskatchewan (Saskatchewan Ministry of Agriculture 2009). Annual canarygrass growers reported in an informal survey that their greatest concern was spatial and temporal

Abbreviation: **NDVI**, normalized difference vegetation index variability in seed yield, especially the production of very large amounts of biomass accompanied by very low seed yield (May 1998). Research into this problem has identified several factors that can contribute to yield variability but do not entirely account for the problem. May et al. (2012a) and Miller (2000) reported that seeding date affected seed yield and could account for some but not all of the annual variability reported by growers. Control of septoria leaf mottle, a major disease in annual canarygrass, also appears to account for some of this yield variability (May et al. 2002).

Recently, chloride has been identified as an important nutrient for reducing yield variability in annual canarygrass. The application of chloride increased seeds per panicle thereby directly impacting seed yield, while potassium had little effect on canaryseed in this study (May et al. 2012b). There was no measurable effect of the chloride on annual canarygrass before seed filling. The exact mechanism of how chloride increases the number of seeds that develop on each annual canarygrass panicle is not known. When researchers observed an effect of chloride in other crops they have attributed the response to several factors including, osmoregulatory functions, interactions with other nutrients, interaction with diseases and alteration of crop development (Fixen 1993). Many studies could not identify how chloride actually increased seed yield (Fixen et al. 1986; Engel 1994; Gaspar et al. 1994; Diaz-Zorita et al. 2004). Several studies did identify that in wheat and barley, chloride response varied across cultivars (Mohr et al. 1995a, 1995b; Grant et al. 2001; Evans and Riedell 2006). Research on other crops rarely report a strong response to chloride as observed for annual canarygrass, especially during seed filling.

Previous testing of annual canarygrass' response to chloride was conducted using one cultivar, CDC Togo, a glabrous (hairless) cultivar free of trichomes on the palea, lemma and glumes (May et al. 2012b). Cultivars free of trichomes were developed from a single cultivar, Keet, through mutagenesis using sodium azide (Hucl et al. 2001). The reasons for the development of glabrous cultivars was to reduce the skin irritation that producers and handlers experience during harvest and processing and to address concerns surrounding the effects of trichome hairs, consisting mainly of silica, on human health. The inheritance of the glabrous trait in annual canarygrass was identified as a single recessive gene (Matus-Cadiz et al. 2003). Unfortunately, current cultivars developed with this mutation have lower seed yield than non-mutated cultivars (Saskatchewan Ministry of Agriculture 2012). The reason for a lower seed yield is not understood at this time. It is also not known if the mutagenic event used to produce glabrous annual canarygrass altered the sensitivity of annual canarygrass to chloride. Therefore, the objective of this study was to determine if the mutagenic process which created glabrous cultivars altered the response of annual canarygrass to chloride fertilizer.

MATERIALS AND METHODS

A field experiment was conducted at Indian Head (lat. 50°33′08.37″N, long. 103°38′39.82″W, elevation 579 m) and Carry The Kettle (two sites: lat. 50°24′56.59″N, long. 103°34′47.13″W, elevation 642 m and lat. 50°24′ 46.98″N, long. 103°35′39.29″W, elevation 616 m) in 2008 and 2009 and Riceton (lat. 50°08′13.17″N, long. 104°21′41.51″W, elevation 580 m) in 2009. The soil series were Indian Head heavy clay (Orthic Vertisol or Haplocryert) at Indian Head, an Oxbow loam (Orthic Black Chernozem or Udic Boroll) and Ellisboro (Rego Black Chernozem or Udic Boroll) at Carry The Kettle, and Regina heavy clay (Orthic Vertisol or Haplocryert) at Riceton. To differentiate between the two sites near Carry The Kettle they will be referred to as CTK ellisboro and CTK loam.

The experiment consisted of a two-way factorial randomized complete block design with four replications. The first factor involved two rates of chloride, 0 and 18.2 kg Cl ha^{-1}, in the form of KCl fertilizer applied at the time of seeding in a side-band, 2.5 cm to the side and 3 to 5 cm below the seed. In a related study, the annual canarygrass was proven to be responding to Cl and not K at all the sites where this current study was carried out (May et al. 2012b). The second factor consisted of four cultivars, Keet, Cantate, CDC Togo, and CDC Bastia. Keet and Cantate have trichome hairs on the lemma palea and glumes while CDC Togo and CDC Bastia are glabrous (hairless).

Additional nutrients were side banded to all plots at a rate of 60 kg N ha^{-1}, 11 kg P ha^{-1} and 12 kg S ha^{-1} to all treatments in the form of urea (46–0–0), monoammonium phosphate (11–51–0) and ammonium sulfate (21–0–0–24). All the cultivars were seeded at a rate of 35 kg ha^{-1} using 2.5-m-wide knife opener, a row width of 30.5 cm, and a plot size of 10.7 $\times$ 4.0 m. The seeder was custom built by Vale farms, Indian Head, SK, using independent lift openers. Seed and fertilizer were delivered to the openers using a Valmar metering and air delivery system (Valmar Airflo Inc., Elie, MB). The plots were managed as a no-till, continuously cropped production system. Glyphosate was applied before seeding and all in-crop broadleaf herbicide applications were determined separately for each site-year depending on the weed species and density encountered. Only recommended herbicides and rates were applied (Saskatchewan Ministry of Agriculture 2010).

Data Collection

Soil tests were carried out at each site for N, P, K, S and Cl. Spring soil test levels of NO_3-N, SO_4-S and Cl were measured to a depth of 60 cm; soil residual phosphate (PO_4-P) and potassium (K) were measured to a depth of 15 cm. A $NaHCO_3$ extraction procedure (Hamm et al. 1970) was used to estimate residual soil N (NO_3), P, and K. Available Cl was determined by extraction of 5 g of soil with 50 mL of water followed by filtration and determination of Cl in the filtered extract using a Technicon™ Auto-analyzer II. (Inland Waters Directorate 1979). Available S was determined by extraction of 10 g of soil with 50 mL of 0.001 M $CaCl_2$ followed by filtration and determination of S in the filtered extract using a Technicon™ Auto-analyzer II (Hamm et al. 1973).

The bioavailable K supply rates were determined using a Plant Root Simulator (PRS™) probe (ion exchange resin membranes) according to the procedures outlined in Qian and Schoenau (2002) and Qian et al. (2008). This measurement of K availability provides an indication of the supply of readily available K to an adsorbing surface, taking into account replenishment of soil solution K by the solid phase as well as movement by diffusion and competition with other cations (Qian et al. 1996). Cation PRS™ probes (Western Ag Innovations, Saskatoon, SK) were charged by soaking in 0.5 M HCl for 2–4 h to saturate the exchange sites with H^+ ions. The cation probes were then inserted directly into a sample of soil at field capacity moisture content for 24 h. After 24 h, the probes were removed, washed free of all soil particles and placed into a clean Ziplock™ plastic bag and treated with 20 mL of 0.5 M HCl for 1 h to elute the sorbed K ions from the membrane surface. The eluent was then analyzed using flame emission spectroscopy to determine K concentration. Using the concentration of K in the eluent and the surface area of the membrane, K supply rate was calculated as micrograms of K sorbed per square centimeter of membrane surface over 24 h.

Plant density was determined 3 to 5 wk after seeding and annual canarygrass panicles were counted after the completion of panicle emergence. Both plants and panicles were determined from two separate 1-m sections of crop row within each plot. Physiological maturity was reached when kernel moisture was approximately 30–35%. Lodging was rated in each plot at physiological maturity using a scale of 1 to 10 (1 = standing, 10 = completely lodged). At each location, the mean normalized difference vegetation index (NDVI) from each plot was measured between stem elongation and flag leaf using a handheld GreenSeeker™ optical sensor (NTech Industries 2009). Normalized difference vegetation index provides a measure of above-ground biomass (Moges et al. 2004; Freeman et al. 2007; Osborne 2007). Significant correlations between NDVI and grain yield have been obtained in spring wheat (Osborne 2007), corn (Zea Mays L.; Teal et al. 2006), and grain sorghum (Sorghum bicolor L.) Moench ssp. bicolour; Moges et al. 2007; Tucker and Mengel 2008) and canola (Brassica napus L.; Holzapfel et al. 2010). The GreenSeeker™ optical sensor determines NDVI by actively emitting radiation in the visible red (~660 nm) and near infrared (~770 nm) bandwidths and measuring the proportion of emitted radiation that is reflected from the canopy. The sensor's field of view is approximately 60 cm wide and NDVI is calculated using the following relationship, NDVI = (NIR − red) / (NIR + red), where red and NIR are the spectral reflectance measurements for the visible red and near-infrared regions, respectively. Approximately 30–70 individual NDVI values were logged for each plot and the average of these values for each plot was used in the data analysis.

The middle 5 of the 12 rows seeded were mechanically harvested and seed yield was determined on a clean seed basis corrected to a uniform kernel moisture content of 13%. Kernel weight, expressed per 1000 seeds (g), was calculated by weighing 700–1000 seeds with the number of seeds determined using an automated seed counter. Seeds per panicle were calculated using panicles per square meter, seed yield and kernel weight. Seeds per square meter were calculated from seed yield and kernel weight. Test weight was measured as specified by the Canadian Grain Commission's Official Grain Grading Guide (2011).

Statistical Analysis

The student panel option of PROC GLIMMIX (SAS Institute, Inc. 2008) was used to investigate the behavior of residuals (heterogeneity) and distributional characteristics of the data prior to doing the main mixed model analysis. Data analysis was conducted using the MIXED procedure in SAS software (Littell et al. 2006). The effect of replicate and site (location by year combinations) were considered random, and the effects of cultivar and KCl application were considered fixed. A combination of variance estimates and P values were used to determine the importance of the random sites by fixed effects interaction. Site by treatment interactions were examined using deviations from overall mean. These deviations were best linear unbiased predictor (BLUP) estimates for the difference between mean for a given site by treatment combination from the overall fixed effect mean for that treatment combination. A t-test determined whether each deviation was significantly different from zero. A significant negative or positive deviation means that the treatment combination was lower or higher at a particular site than the mean of the treatment combination averaged over all the sites.

A grouping methodology, as previously described by Francis and Kannenberg (1978), was used to further explore treatment responses among sites. The mean and CV were estimated for each level of cultivar by Cl across sites. Means were plotted against CV for each treatment combination, and overall mean of the means and CVs was included in the plot to categorize the data into four categories: Group I: High mean, low variability (optimal); Group II: High mean, high variability; Group III: Low mean, high variability (poor); and Group IV: Low mean, low variability.

Table 1. Soil residual levels of chloride (Cl), potassium (K), nitrogen (N), phosphorus (P) and sulphur (S) at the start of the each field experiment

Year	Location	Cl (kg ha^{-1}) 0–15 cm	Cl (kg ha^{-1}) 0–60 cm	K (kg ha^{-1}) 0–15 cm	K (μg cm^{-2}) Supply rate 0–15 cm	K (μg cm^{-2}) Supply rate 15–30 cm	NO_3-N (kg ha^{-1}) 0–60 cm	PO_4-P (kg ha^{-1}) 0–15 cm	SO_4-S (kg ha^{-1}) 0–15 cm
2008	Indian Head	81.4	227.4	587	4.14	1.58	48.1	10.6	25.5
	CTK ellisboro	40	131.5	229.9	5.73	9.14	34.9	31.7	5.1
	CTK loam	38.3	187.9	245.6	6.68	2.32	39.2	20.3	7
2009	Indian Head	64.2	220.1	508.8	3.24	1.68	38.4	7.6	12.1
	CTK ellisboro	62.9	203.6	211.6	7.53	2.37	24.8	16.5	2.7
	CTK loam	63.7	251	380.9	6.79	2.26	34.1	19.6	6.5
	Riceton	73.4	208.2	599.3	3.35	1.66	30.6	5.1	4.6

RESULTS AND DISCUSSION

Soil and Environmental Conditions

The residual levels of Cl, K, NO_3, PO_4 and S in the soil before seeding at each location are presented in Table 2. A wide range of Cl levels was observed, with values ranging from 38 to 81 kg ha^{-1} for a soil depth of 15 cm or 132 to 251 kg Cl ha^{-1} for a soil depth of 60 cm. The ranking of sites for Cl level was similar at the 15 and 60-cm depths, but not exactly the same.

Growing season precipitation and average temperatures for each site year are presented and compared to the 30-yr averages for the sites (Table 3). Precipitation was at least 10% below average at Indian Head in 2008 and 2009, and Riceton in 2009. The temperature was less than 90% of the 30-yr average at all site years except at Indian Head in 2008, which had a temperature that was 92% of the 30-yr average. Therefore, temperature was below average and precipitation was at or below average at the sites when the experiment was conducted.

Crop Development and Seed Yield

Plant density was affected by cultivar but not by Cl or the interaction of Cl and cultivar (Table 3). The plant density of Keet, 298 plants m^{-2}, was significantly higher than the plant density of CDC Bastia, 274 plants m^{-2}, and CDC Togo, 226 plants m^{-2}, while Cantate, 294 plants m^{-2}, and CDC Bastia, had a higher plant density than CDC Togo (Table 4). The germination percentages of the seedlots used in this study were similar. The difference in plant density among the cultivars may have been due to differences in kernel weight amongst the seed lots used in this study since the seeding rate was 35 kg ha^{-1} of seed regardless of kernel weight. Research on seeding rates in annual canarygrass indicates that these differences in plant density should not result in differences in seed yield (May et al. 2012a).

CDC Togo compensated for the low plant density by having a higher number of panicles per plant than the other three cultivars (Table 4). Chloride and the interaction between Cl and cultivar did not affect panicles per plant or panicles per square meter (Table 3). In addition, cultivars did not differ for panicles per square meter. Therefore, the glabrous cultivars and cultivars with trichomes did not differ in tillering.

The addition of Cl increased seeds per panicle by 21% (Tables 3 and 4). Since the form of chloride applied in this study was KCl, one could argue that the effect was due to K and not Cl; however, a related study, conducted at the same sites and time as this study, compared the effects of Cl vs. K on yield components of annual canarygrass (May et al. 2012b). May et al.

Table 2. Summary of climatic conditions and the 30-yr average for selected experimental sites in Saskatchewan in 2008–2009

Location/Year	Precipitation May	Precipitation Jun.	Precipitation Jul.	Precipitation Aug.	% of 30-yr average	Temperature May	Temperature Jun.	Temperature Jul.	Temperature Aug.	% of 30-yr average
	(mm)					(°C)				
Indian Head										
2008	21	60	90	47	86	9	14	17	18	92
2009	15	60	59	77	83	8	14	14	15	81
Long-term average	56	79	67	53		11	16	18	18	
Carry the Kettle										
2008	7	81	96	39	99	9	14	17	13	81
2009	20	57	42	105	100	8	14	14	15	81
Long-term average	43	87	49	45		11	16	18	18	
Riceton[z]										
2009	38	50	45	78	90	9	16	16	16	88
Long-term average	53	75	64	43		12	16	19	18	

[z]Bratts Lake has closes environmental station to this location.

Table 3. The statistical analysis of the effect of cultivar and chloride on annual canarygrass development and yield

	Plant density	Panicle density		Seed density		Kernel weight	Seed yield	Test weight	Height	NDVI
	(plants m^{-2})	(panicles plant^{-1})	(panicles m^{-2})	(seeds panicle^{-1})	(seeds m^{-2})	(g 1000 seeds^{-1})	(kg ha^{-1})	(g 0.5 L^{-1})	(cm)	(0–1)
					(*P* value)					
Cultivar (C)	**0.000**	**0.000**	0.763	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.010**
Chloride (Cl)	0.507	0.766	0.826	**0.000**	**0.000**	**0.029**	**0.000**	0.051	0.563	0.639
C × Cl	0.514	0.827	0.828	0.882	0.777	0.739	0.753	0.17	0.581	0.745
Keet[z]	0.124	0.655	0.650	0.090	0.040	0.430	0.018	0.789	0.469	0.765
Cantata	0.609	0.717	0.882	0.037	0.007	0.575	0.010	0.738	0.226	0.423
CDC Bastia	0.941	0.449	0.507	0.010	0.011	0.048	0.008	0.174	0.866	0.745
CDC Togo	0.835	0.804	0.616	0.009	0.001	0.265	0.000	0.012	0.621	0.455
					(Variance estimate *P* value)					
Site × C × Cl	0.059	**0.025**	**0.019**	**0.002**	**0.001**	0.281	**0.001**	0.192	**0.036**	0.751
					(Percentage of total variance estimate)[y]					
Site × C × Cl	36	38	33	6	6	6	6	49	3	1

[z]Contrasts comparing effect of KCl for each cultivar.
[y]Percentage of the variance associated with the effect of site by cultivar by KCl interaction divided by the sum of the total variance associated with the effect of site (sum of variance estimates including site).

Table 4. The effect of cultivar and chloride on annual canarygrass development and yield

	Plant density	Panicle density		Seed density		Kernel weight	Seed yield	Test weight	Height	NDVI
	(plants m^{-2})	(panicles plant^{-1})	(panicles m^{-2})	(seeds panicle^{-2})	(seeds m^{-2})	(g 1000 seeds)	(kg ha^{-1})	(g 0.5 L^{-1})	(cm)	(0–1)
Chloride (kg ha^{-1})										
0	270	2.61	666	25.9	16728	7.34	1235	314	85.8	0.548
18.2	275	2.58	671	31.3	20618	7.48	1543	326	86.3	0.544
LSD0.05	16	0.23	38	**2.3**	**1400**	**0.12**	**105**	12	1.7	0.017
Cultivar										
Keet	298	2.30	652	33.6	21517	7.34	1578	307	88.3	0.564
Cantate	294	2.39	667	30.2	20188	7.15	1450	303	83.3	0.521
CDC Bastia	274	2.55	676	25.5	16940	7.35	1261	339	86.1	0.555
CDC Togo	226	3.14	679	25.1	16047	7.8	1267	329	86.7	0.545
LSD0.05	**23**	**0.32**	54	**3.3**	**1980**	**0.18**	**148**	**17**	**2.4**	**0.023**
SED[z]										
Cultivar (C)	11	0.16	27	1.6	981	0.09	73	8	1.2	0.011
Chloride (Cl)	8	0.11	19	1.2	694	0.06	52	6	0.8	0.008
C × Cl	16	0.23	38	2.3	1388	0.12	104	12	1.7	0.015
DDF	42	42	42	42	42	42	42	41	42	14

[z]SED represents standard error of difference and DDF represents denominator degrees of freedom.

(2012b) reported that changes observed in annual canarygrass were due to Cl and not K; the application of Cl increased seeds per panicle from 21.7 to 25.7 or 18%. In the present study, the cultivars differed in seeds per panicle, with Keet having the largest number of seeds per panicle followed by Cantate, which, in turn, had more seeds per panicle than both CDC Togo and CDC Bastia. More importantly, the interaction between Cl and cultivar was not significant, indicating that the effect of Cl was consistent across all cultivars, both glabrous cultivars and cultivars with trichomes. When mean seeds per panicle are plotted against the coefficient of variation it becomes clear that addition of Cl increased the number of seeds per panicle and also reduced variability in seeds per panicle (Fig. 1). This trend was also observed by May et al. (2012b). Seeds per square meter followed the same trend as seeds per panicle with a positive response to the addition of Cl. The cultivars with trichomes, Keet and Cantate, had more seeds per square meter than either glabrous cultivar, CDC Togo or CDC Bastia.

The addition of Cl increased the kernel weight of annual canarygrass by 1.9% (Tables 3 and 4). May et al. (2012b), reported that the addition of Cl caused a numerical increase in kernel weight; however, the increase was not significant ($P = 0.079$). Relative to May et al. (2012b), smaller differences can be detected in the current study because there are more observations for each Cl treatment, due to the study design of four cultivars and no interaction between Cl and cultivars. Unlike seeds per panicle the addition of Cl did not lower the coefficient of variation for kernel weight (Fig. 2).

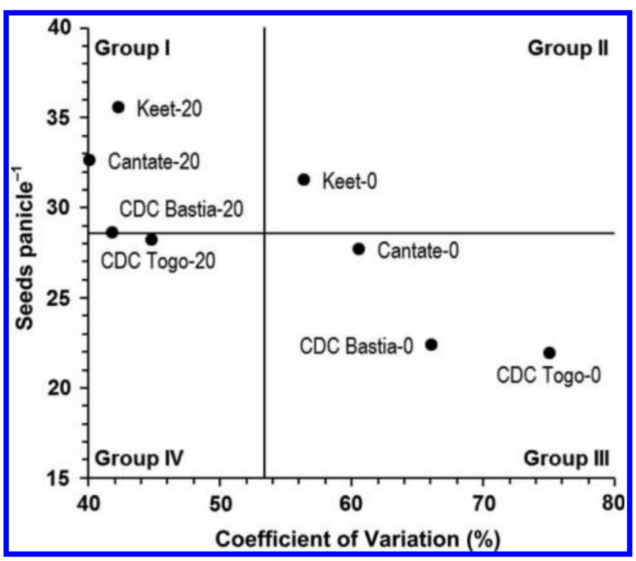

Fig. 1. Biplot (means vs. coefficient of variation) of the chloride by cultivar treatments for seeds per panicle data collected from 7 site years in Saskatchewan from 2008 to 2009. Group I: High mean, low variability (optimal); Group II: High mean, high variability; Group III: Low mean, high variability (poor); Group IV: Low mean, low variability.

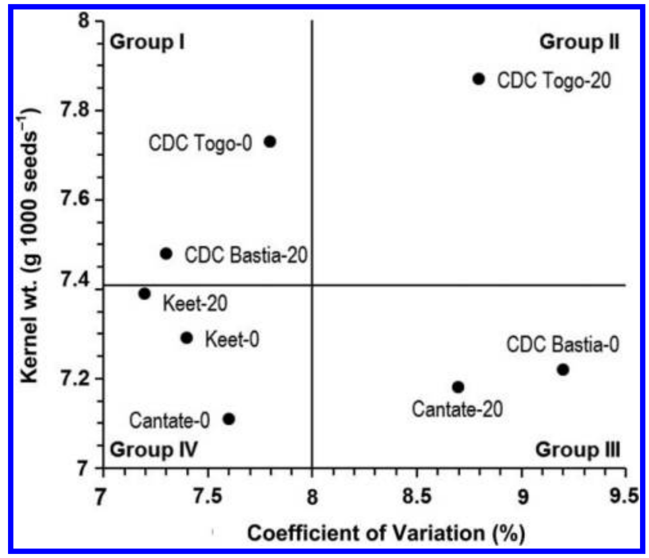

Fig. 2. Biplot (means vs. coefficient of variation) of the chloride by cultivar treatments for kernel weight data collected from 7 site years in Saskatchewan from 2008 to 2009. Group I: High mean, low variability (optimal); Group II: High mean, high variability; Group III: Low mean, high variability (poor); Group IV: Low mean, low variability.

The small increase in kernel weight compared with the large increase in seeds per panicle indicates that seeds per panicle is the yield component most affected by Cl.

The cultivars differed in their kernel weight with CDC Togo having the highest kernel weight. Both CDC Bastia and Keet had a higher kernel weight than Cantate. The lack of separation between glabrous cultivars and cultivars with trichomes indicates that seeds per panicle was the yield component most affected by the mutagenesis that created the glabrous cultivars. The interaction, chloride × cultivar, was not significant for kernel weight. In wheat, an increase in seed density and not kernel weight with the addition of Cl has been observed (Evans and Riedell 2006); however, this response was in a specific hard red spring wheat cultivar, Marshall, creating a significant cultivar × chloride interaction that was not observed in this study. The cultivar Marshall appears to take up less Cl than other wheat cultivars at a given Cl concentration in sand culture (Evans and Riedell 2006). On the other hand, Engel (1994) found that kernel weight in winter wheat was increased by 6% with the addition of Cl with no differences among six cultivars. Mohr et al. (1995b) reported that when chloride increased seed yield in spring wheat, kernel weight was often increased. Gaspar et al. (1994) found an increase in the kernel weight of oat at the locations where Cl increased seed yield.

The addition of Cl increased seed yield by 25% when averaged over all seven site years and all four cultivars (Tables 3 and 4). This is similar to the 24% seed yield response to Cl reported by May et al. (2012b). When the chloride response is examined at individual sites, the increase from chloride tended to increase as the residual level of chloride in the soil decreased (Table 5); however, even at the highest level of chloride found at these seven sites, 81 kg ha^{-1} for a 15-cm soil depth, there was still a response to Cl. In the previous study, a response could not be detected when the Cl level increased above 70 kg ha^{-1} for a 15-cm soil depth (May et al. 2012b). The numerical differences between the treatments which had received Cl relative to those which had not were similar between this study and the previous study (May et al. 2012b); however, with the larger number of observations per mean in this trial smaller differences can be detected. These results indicate that until a better response curve is developed we recommend that growers apply Cl when growing annual canarygrass, regardless of the Cl level in the soil.

In wheat, Fixen (1993) identified residual levels of Cl in the soil that specified when to apply chloride. Fixen (1993) suggested that once Cl level rose above 67 kg ha^{-1} to a depth of 60 cm, no chloride fertilizer was required. In this study, significant responses were observed when the Cl level was at 81 kg ha^{-1} and only to a depth of 15 cm. In addition, a study on the responsiveness of wheat to chloride at the most responsive site, CTK ellisboro, reported no response to Cl fertilizer for wheat (Lafond 2001). Taken together this indicates that annual canarygrass is more sensitive to chloride than common wheat. In addition to Cl increasing seed yield for each cultivar, the addition of Cl lowered the coefficient of variation indicating a lowered variability in seed yield (Fig. 3). Therefore, the application of chloride fertilizer reduced the risk farmers assume when growing annual canarygrass by increasing seed yield and reducing variability in seed yield.

Both Keet and Cantate had between 14 and 25% higher seed yield than either CDC Togo or CDC Bastia. Long-term results have reported an 11–15% yield advantage for Keet and Cantate over CDC Togo or CDC Bastia (Saskatchewan Ministry of Agriculture 2012). More importantly, the Cl $\times$ cultivar interaction was not significant (Table 3). This indicates that the effect of Cl on seed was consistent in both glabrous cultivars and cultivars with trichomes; therefore, the mutagenic process that annual canarygrass underwent to create glabrous cultivars did not alter its response to chloride fertilizer.

The variance estimate and statistical significance of the Site $\times$ C $\times$ Cl interaction for each variable is presented in Table 3. This three-way interaction was significant for panicles per plant, panicles per square meter^2, seeds per panicle, seeds per square meter, seed yield and plant height. However, only the deviations, where the deviation is the difference between the treatment mean at an individual site year and the overall treatment mean combined across all sites, for seeds per square meter and seed yield appeared to be large enough to be important and are reported in Table 6. A significant deviation indicates that the mean for a treatment at that site year was different from the overall mean averaged across the 7 site years of data. The CTK loam site in 2008 had the largest seed yield response to chloride of all the sites and significant deviations in both seeds per square meter and seed yield (Tables 5 and 6). Both glabrous cultivars, CDC Bastia and CDC Togo, deviated by having lower seeds per square meter and seed yield at the 0 kg Cl ha^{-1} rate than the overall mean averaged across site years and cultivars at CTK loam site in 2008. The seeds per square meter and seed yield of Keet at the CTK loam site in 2008 at the18.2 kg Cl ha^{-1} rate deviated by being larger than the overall mean averaged across site years and cultivars.

The other two locations with significant deviations were the 2 site years with the lowest yield increase

Table 5. The response of annual canarygrass to chloride at individual sites and years

Year	Site	Seed yield (kg ha^{-1}) Chloride (kg ha^{-1}) 0	18.2	Yield increase (kg ha^{-1})	Residual chloride in soil (kg ha^{-1}) 0–15 cm	0–60 cm
2008	CTK loam	1053	1697	644	38	188
2008	CTK ellisboro	131	574	443	40	132
2009	CTK ellisboro	439	793	354	63	204
2009	CTK loam	1601	1833	233	64	251
2009	Indian Head	2124	2353	228	64	220
2009	Riceton	1284	1417	133	73	208
2008	Indian Head	2010	2133	123	81	227
	SE[z]	64				
	SED[y]	57				
	Den. df	189				
	LSD0.05	113				

[z]SE for each mean.
[y]Standard error of difference (SED), denominator df (Den. df), and LSD0.05 to assess KCl effect at each site.

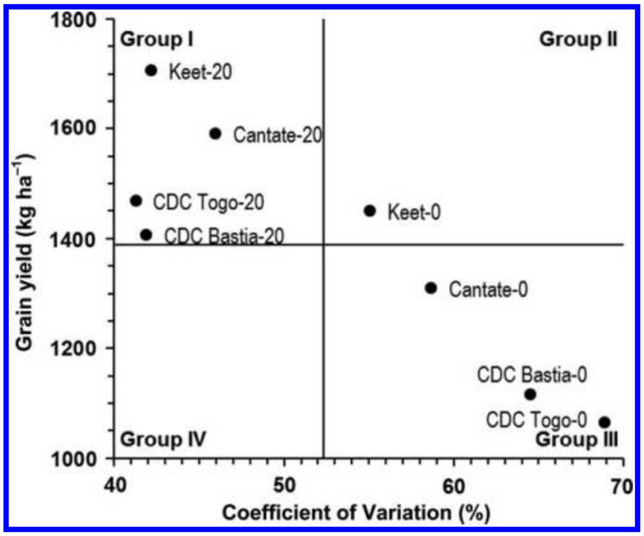

Fig. 3. Biplot (means vs. coefficient of variation) of the chloride by cultivar treatments for seed yield data collected from 7 site years in Saskatchewan from 2008 to 2009. Group I: High mean, low variability (optimal); Group II: High mean, high variability; Group III: Low mean, high variability (poor); Group IV: Low mean, low variability.

to chloride, Indian Head 2008 and Riceton in 2009 (Table 6). At Indian Head in 2008, CDC Togo deviated by having both lower seeds per square meter and seed yield at the 18.2 kg Cl ha^{-1} rate than the overall mean averaged across site years and cultivars. At the same site year Keet deviated by having both higher seeds per square meter and seed yield at the 0 kg Cl ha^{-1} rate than the overall mean averaged across site years and cultivars. The deviations in both of these cultivars at Indian Head in 2008 resulted in a lower response to chloride. At the Riceton site in 2009, CDC Togo deviated by having both a higher seeds per square meter and seed yield at the 0 kg Cl ha^{-1} rate than the overall mean averaged across site years and cultivars. Therefore, deviations in seeds per square meter and seed yield occurred at the site years that were the most or least responsive to chloride.

Test weight varied among cultivars and Cl did not increase test weight (P = 0.051; Table 3). The same trend was observed by May et al. (2012b) in the previous study with a P value of 0.052 for a linear increase in kernel weight as the level of Cl increased.

Chloride and the Cl × cultivar interaction had no effect on plant height or NDVI (Table 3). The cultivars differed in their height and NDVI (Table 4). Cantate was shorter and had a lower NDVI than the other three cultivars. Normalized difference vegetation index is associated with biomass. The lack of difference in NDVI between the chloride treatments is a further indication, along with panicles per plant, that the effect of chloride was not apparent before heading. This is in contrast with the reported effect of chloride in winter wheat where plant growth and development were accelerated by the application of Cl (Engel 1994).

CONCLUSIONS

The application of Cl increased the seed yield of annual canarygrass by 25% and most of this increase was due to a 21% increase in seeds per panicle. Seeds per panicle was the yield component that created the yield difference between the two types of annual canarygrass. Kernel

Table 6. Deviation of the site means from the overall mean for seed density and seed yield

		2008			2009			
Cultivar	Chloride	CTK loam	Indian Head	CTK ellisboro	CTK ellisboro	CTK loam	Indian Head	Riceton
				Deviations (seed m^{-2})				
Cantate	0	387	2000	−1870	−1302	−20	789	16
	18.2	1268	224	−793	−726	242	1930	−2145
CDC Bastia	0	−3220*[z]	117	−723	62	944	435	2384
	18.2	−501	−1342	2516	1086	−1144	−1059	444
CDC Togo	0	−3840*	−288	399	491	−620	845	3013*
	18.2	1357	−3465*	2590	1091	−121	−907	−544
Keet	0	−1710	3663*	−2805	−184	1763	740	−1467
	18.2	6218**	−287	−100	−1144	−883	−2129	−1675
SE		1481						
				Seed yield (kg ha^{-1})				
Cantate	0	37	135	−101	−90	−6	50	−25
	18.2	128	32	−44	−4	16	55	−182
CDC Bastia	0	−246*	−20	−35	−18	72	50	197
	18.2	−24	−138	165	80	−93	−31	40
CDC Togo	0	−347**	27	1	−22	−51	93	299**
	18.2	101	−255*	118	50	24	−11	−26
Keet	0	−115	248*	−165	16	146	−9	−121
	18.2	466**	12	0	−58	−88	−146	−184
SE		111						

[z]The statistical significance of the deviation is indicated as follows: * = 0.05 ≥ *P* value ≥ 0.01; and ***P* value < 0.01.

weight also contributed to increased seed yield when Cl was applied. Chloride did not interact with cultivar, which means that growers can expect to receive a yield increase from the application of Cl regardless of the type of annual canarygrass grown, glabrous cultivars or cultivars with trichomes on their lemma, palea and glumes. Chloride uptake did not account for the seed yield differences between the two types of annual canarygrass. Annual canarygrass responded to chloride at every site regardless of the residual Cl level in the soil, the current recommendation is changed to recommend that growers apply 9 kg ha^{-1} of Cl when growing annual canarygrass. Further research is needed to develop a calibration of the response of annual canarygrass Cl fertilizer based on the residual level of Cl in the soil. In conclusion, the mutagenic process which facilitated the development of glabrous cultivars did not affect the response of annual canarygrass to chloride fertilizer.

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