

## A Canadian Ethanol Feedstock Study to Benchmark the Relative Performance of Triticale: II. Grain Quality and Ethanol Production

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### ABSTRACT

Cereal grain ethanol production may need to supplement biomass ethanol production to meet the increasing long-term demand for ethanol. A study was initiated to benchmark the relative performance of triticale (*×Triticosecale* ssp.) to wheat (*Triticum aestivum* L.) classes utilized for ethanol production. Ten cultivars: three triticale, two Canada prairie spring (CPS) wheat, three Canada western soft white spring (CWSWS) wheat, one Canada western red spring (CWRS) wheat, and one Canada western general purpose (CWGP) wheat cultivars were grown at 45 locations across Canada from 2006 to 2009. The locations were subgrouped by agroecological zone for western Canada, by province for Ontario and Quebec, and Charlottetown, PEI, for the Maritimes. The greatest grain yield was usually observed for Hoffman (red spring wheat) followed by triticale cultivars and CWSWS cultivars. Ethanol yield varied by region as a reflection of grain yield, and differences among cultivars generally were: triticale (excluding Tyndal) = Hoffman = CWSWS > CPS > CWRS. Ethanol concentration was least for Tyndal triticale and AC Superb CWRS. Stability assessments indicated that Pronghorn and AC Ultima triticales and Bhisaj CWSWS wheat provide consistent and high ethanol yields. The other CWSWS cultivars, AC Sadash and AC Andrew, had similarly high ethanol yields but were variable, indicating that utilization outside the Parkland and Western Prairies agroecological zones could pose greater risk for ethanol plants over Pronghorn and AC Ultima. Ethanol fermentation plants could therefore increase efficiency by replacing CPS wheat feedstocks with select triticales and potentially improve the consistency of production by using select triticales in regions where CWSWS wheats are less stable.

GLOBALY, FUEL ETHANOL production has now reached 75 billion L yr<sup>-1</sup>, and Canada's contribution is approximately 2 billion L yr<sup>-1</sup> (Canadian Renewable Fuels Association, 2010; Klein et al., 2004). Some economists have argued that ethanol fuel production from grain feedstocks relies too heavily on government programs to offset what is considered to be an inefficient system incapable of adequately reducing greenhouse gas emission targets (Freeze and Peters, 1999; Klein et al., 2004). Others have argued that ethanol should only be produced using crop biomass and residues (Canadian Renewable Fuels Association, 2010), considered by many as agricultural waste (Freeze and Peters, 1999). However, the dramatic rise in plant construction and output, including seven ethanol plants operating in western Canada with a collective annual output of 0.5 billion L (Canadian Renewable Fuels Association, 2010), suggests that the economics may not be as important as provincial and federal policies targeting energy diversity, agricultural benefits, and rural renewal (Coad and Bristow, 2011; Klein et al., 2004). Studies also report that >60% of crop residues must be retained to adequately maintain proper C cycling in the soil in wheat production systems (Freeze and Peters, 1999). Therefore, if crop residue exports are limited to 40% to maintain soil quality,

ethanol production from grain is probably needed at some level to meet the increasing long-term demand for ethanol. Furthermore, grain-based ethanol production provides grain growers the opportunity to sell their grain into dual markets, which enhances marketing options for cereal production. Today, a producer of CWSWS and CPS wheat, which are preferred for wheat ethanol feedstocks, can choose to sell into a milling market or contract the production to an ethanol plant.

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**Abbreviations:** CPS, Canada prairie spring; CWGP, Canada western general purpose; CWRS, Canada western red spring; CWSWS, Canada western soft white spring.

If cereal grains remain an important feedstock for ethanol production, does wheat possess superior attributes for fermentation over alternative cereals? Barley (*Hordeum vulgare* L.) was once considered inferior to wheat for fermentation efficiency and economic feasibility considerations (Klein et al., 2004). Newer studies using updated fermentation techniques report similar ethanol concentrations among some barley (*Hordeum vulgare* L.) and CPS wheat cultivars (Vigil et al., 2012). Triticale is a cereal crop first created in the late 19th century by crossing common wheat with rye (*Secale cereale* L.) (Oettler, 2005). It generally possesses a low grain protein concentration and high grain yield and biomass potential, which are more desirable traits in biorefinery processes than currently used wheat classes (Beres et al., 2010; Goyal et al., 2011). Moreover, the greater yield potential for triticale relative to Canadian wheat classes affords greater competitiveness with weeds (Beres et al., 2010; Oettler, 2005), and it also displays better tolerance to drought and pests than wheat (Darvey et al., 2000; Erekul and Köhn, 2006). Preliminary studies conducted in the western prairies of Canada indicated that triticale does have potential as an ethanol feedstock (McLeod et al., 2010).

A crop that does not occur naturally in the ecosystem, has low presence in human consumption markets, and is compatible with all on-farm and industrial equipment and infrastructure may be more attractive as a cereal platform technology and better received by society overall. The case for triticale may strengthen further if gene transformation is used to enhance a plant trait to be exploited in a bioindustrial process. Reports to date have not assessed triticale grain yield and ethanol production across an array of environments, nor have these reports assessed the crop using modern fermentation technologies. The Canadian Triticale Biorefinery Initiative (CTBI) is a consortium of stakeholders representing the triticale value chain with a focus on positioning triticale as a cereal platform technology for the bioindustry. One of the short-term goals for the CTBI is to benchmark the relative performance of triticale to Canadian wheat classes currently utilized for ethanol production. To address this goal, our objective was to compare grain ethanol fermentation and production results and other grain quality characteristics for 10 triticale and spring wheat cultivars.

## MATERIALS AND METHODS

### Experimental Design and Management

The experiment was conducted at 45 locations across Canada from 2006 to 2009 (Table 1; Fig. 1). Data generated from each location varied from a single year to 4 yr. Therefore, depending on the variable, data were collected at 71 to 94 sites (location × year combinations).

Three triticale and two CPS, three CWSWS, one CWRS, and one CWGP wheat candidate cultivars (10 in total) were grown at the study sites (Table 2). Cultivars were chosen using the following criteria: (i) used as mid- to long-term checks used in either cultivar trials or cooperative registration trials or both; and (ii) provide a spectrum of yield and quality characteristics and disease resistance. Superb, for example, represented the CWRS class for most regional cultivar trials as well as cooperative registration trials and was popular with western Canadian producers. Hoffman, for example, was chosen for its high yield potential and its agronomic and disease attributes, particularly in the context of cultivar suitability to general purpose end uses such as feed or

ethanol. Cultivars were randomly assigned to each of three replicates at each location × year combination according to a randomized complete block design.

The locations in Ontario, Quebec, and Prince Edward Island were grouped together to represent eastern Canada (Table 1). All other locations represented western Canada. Locations in western Canada were subdivided into three agroecological zones of the Canadian prairie: Western Prairies, Eastern Prairies, and the Parkland (Table 1). The Western Prairies region has soil types that are generally Orthic Dark Brown Chernozem clay loam soils (Typic Borolls), with approximately 30 g kg<sup>-1</sup> organic matter content, or Brown Chernozem loam soils (Aridic Borolls), with approximately 20 g kg<sup>-1</sup> organic matter. The Western Prairies region is considered semiarid, with growing season precipitation ranging from 152 mm (Swift Current, SK) to 380 mm (Lethbridge, AB).

The Eastern Prairies region site soils were predominantly Orthic Black Chernozem clay loam soils (Udic Borolls), with 60 to 80 g kg<sup>-1</sup> organic matter, but also included Dark Grey Gleisolic soils. From an area standpoint, this region is smallest relative to other regions in the Western Prairies. Growing season precipitation ranges from 210 mm (Watrous, SK) to 462 mm (Minto, MB), and has relatively high yield and disease potential.

The Parkland region soils were typically Grey Wooded Luvisols in northern regions (Boralfs and Udalfs) and Orthic Black Chernozem clay loam soils in southern and transitional regions. The Parkland region is the largest, extending from Neepawa, MB (49°16' N) northwest to Fort St. John, BC (56°17' N, 120°50' W), and east to Arborg, MB (97°13' W) (Table 1). A shorter, humid growing season is typical for this region, but severe drought can occur; e.g., growing season rainfall was as low as 107 mm reported in Falher, AB, in 2009, but as high as 562 mm in Fort St. John, BC, in 2007.

The eastern Canadian sites in Ontario and Quebec were represented by Dark Grey Gleisolic (aquic suborders) or Podzolic (Spodosols) soils. The sites in Ontario were similar in terms of precipitation accumulation and did not vary greatly along latitudinal lines; these sites generally received approximately 350 mm of precipitation with extremes as high as 488 mm in Ottawa in 2009. The Ontario region included an area extending from the southwestern point of Elora (43°41' N, 80°25' W), north to Renfrew County (45°39' N, 77°11' W), and as far east as St. Isidore (45°23' N, 74°54' W) (Table 1). The Quebec sites were as far south and west as St. Hyacinthe (45°37' N, 72°56' W) and as far north as Normandin (48°50' N, 72°31' W) (Table 1). Normandin is in a region similar to the Parkland of western Canada and is typified by a short growing season and cooler climate. The Normandin area was relatively dry and received the least amount of precipitation of all locations in Quebec during the study period (average 305 mm). St. Foy was the wettest site during the study period and frequently experienced severe fusarium head blight (FHB) outbreaks. The Maritimes site of Charlottetown, PEI, had an Orthic Humo-Ferric Podzol soil characterized by fine sandy loam texture. The coastal climate of the Maritimes region is characterized by relatively high precipitation as well as high disease pressure from FHB (caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch]) (Table 1).

**Table 1. Description of locations for ethanol feedstocks study.**

Location	Agroecological zone	Soil zone	Years	Growing season precipitation				Latitude	Longitude
				2006	2007	2008	2009		
				mm					
Western Canada									
Dawson Creek, BC	Parklands	Grey Wooded	2007, 2009	-†	438	-	240	55°48' N	120°14' W
Fort St John, BC	Parklands	Grey Wooded	2007, 2009	-	562	-	222	56°17' N	120°50' W
Donnelly, AB	Parklands	Grey Wooded	2007	-	270	-	-	55°43' N	117°6' W
Edmonton, AB	Parklands	Black	2007, 2008, 2009	-	181	159	147	53°33' N	113°29' W
Falher, AB	Parklands	Grey Wooded	2007, 2008, 2009	-	270	234	107	55°46' N	117°10' W
Killam, AB	Parklands	Black	2006, 2007	416	370	-	-	52°47' N	111°51' W
Kitscoty, AB	Parklands	Black	2006	469	-	-	-	53°20' N	110°20' W
Lacombe, AB	Parklands	Black	2007, 2008, 2009	-	357	230	279	52°29' N	113°43' W
Lethbridge, AB (dry)	Western Prairies	Dark Brown	2007, 2008, 2009	-	164	380	241	49°41' N	112°50' W
Lethbridge, AB (irrigated)	Western Prairies	Dark Brown	2007, 2008, 2009	-	291	456	343	49°41' N	112°50' W
Neapolis, AB	Parklands	Black	2007, 2009	-	472	-	114	51°40' N	113°52' W
Sexsmith, AB	Parklands	Grey Wooded	2007	-	537	-	-	55°21' N	118°46' W
Vermilion, AB	Parklands	Black	2007	-	397	-	-	53°21' N	110°51' W
Westlock, AB	Parklands	Grey Wooded	2007	-	278	-	-	54°9' N	113°51' W
Canora, SK	Parklands	Grey Wooded	2007	-	365	-	-	51°38' N	102°26' W
Indian Head, SK	Eastern Prairies	Black	2007, 2008, 2009	-	275	217	210	50°32' N	103°39' W
Lake Lenore, SK	Parklands	Black	2007, 2008	-	369	178	-	52°25' N	104°58' W
Lashburn, SK	Parklands	Black	2006	339	-	-	-	53°7' N	109°36' W
Melfort, SK	Parklands	Black	2007, 2008, 2009	-	351	190	243	52°52' N	104°36' W
Outlook, SK	Western Prairies	Dark Brown	2007	-	291	-	-	51°29' N	107°3' W
Redvers, SK	Eastern Prairies	Black	2007	-	283	-	-	49°34' N	101°41' W
Regina, SK	Western Prairies	Dark Brown	2007, 2008	-	267	228	-	50°26' N	104°35' W
Saskatoon, SK	Western Prairies	Dark Brown	2006, 2007, 2008, 2009	489	278	180	215	52°8' N	106°38' W
Scott, SK	Western Prairies	Dark Brown	2007, 2008, 2009	-	313	207	173	52°21' N	108°49' W
Swift Current, SK	Western Prairies	Brown	2007, 2008, 2009	-	152	337	199	50°18' N	107°46' W
Valparaiso, SK	Parklands	Grey Wooded	2006	489	-	-	-	52°51' N	104°10' W
Watrous, SK	W. Prairies	Dark Brown	2007, 2008, 2009	-	210	238	256	51°40' N	105°27' W
Arborg, MB	Parklands	Grey Wooded	2008	-	-	466	387	50°54' N	97°13' W
Brandon, MB	Eastern Prairies	Black	2007, 2008, 2009	-	257	367	241	49°50' N	99°56' W
Carberry, MB	Eastern Prairies	Black	2007, 2009	-	389	-	235	49°51' N	99°21' W
Melita, MB	Eastern Prairies	Black	2006, 2007, 2008, 2009	378	283	258	213	49°16' N	100°59' W
Minto, MB	Eastern Prairies	Black	2006	462	-	-	-	49°24' N	100°1' W
Neepawa, MB	Parklands	Grey Wooded	2006, 2007	462	477	-	-	49°24' N	100°1' W
Portage, MB	Eastern Prairies	Black	2007, 2009	-	395	-	209	49°58' N	98°17' W
Roblin, MB	Parklands	Grey Wooded	2007, 2008, 2009	-	445	308	287	51°13' N	101°21' W
Rosebank, MB	Eastern Prairies	Black	2007, 2008, 2009	-	388	315	274	49°22' N	98°6' W
Eastern Canada									
Elora, ON			2007	-	252	-	-	43°41' N	80°25' W
Ottawa, ON		Dark Grey Gleisolic	2007, 2008, 2009	-	346	337	488	45°24' N	75°41' W
Renfrew County, ON		Dark Grey Gleisolic	2007, 2008, 2009	-	442	354	339	45°39' N	77°11' W
St. Isidore, ON		Dark Grey Gleisolic	2008	-	-	354	351	45°23' N	74°54' W
Normandin, QC			2007, 2008, 2009	-	253	339	322	48°50' N	72°31' W
St. Foy, QC		Dark Grey Gleisolic	2007, 2008	-	499	559	465	46°47' N	71°14' W
St. Hyacinthe, QC		Dark Grey Gleisolic	2007, 2008, 2009	-	351	487	409	45°37' N	72°56' W
Charlottetown, PEI		Orthic Humo-Ferric Podzol	2007, 2008, 2009	-	388	464	531	46°15' N	63°7' W

† Precipitation data not collected.



**Fig. 1. Geographical distribution of study sites at locations in agroecological zones and provinces used to assess grain quality and ethanol production assessment from 2006 to 2009.**

**Table 2. Summary of cultivars evaluated and corresponding market class description.**

Cultivar	Classification	Reference
AC Ultima	spring triticale	McLeod et al. (2001)
Pronghorn	spring triticale	Salmon et al. (1997)
Tyndal	spring triticale	Salmon et al. (2007)
AC Andrew	Canada western soft white spring wheat	Sadasivaiah et al. (2004)
AC Sadash	Canada western soft white spring wheat	Sadasivaiah et al. (2009)
Bhishaj	Canada western soft white spring wheat	Randhawa et al. (2011)
AC Superb	Canada western red spring wheat	Townley-Smith et al. (2010)
5700PR	Canada prairie spring red wheat	AgriPro/Syngenta (unpublished data, 2000)
AC Crystal	Canada prairie spring red wheat	Fernandez et al. (1998)
Hoffman	Canada eastern red spring wheat; Canada western general purpose candidate	H.Voldeng (unpublished data, 2004)

The plots were seeded at a rate of 300 seeds  $m^{-2}$  using a plot seeder equipped with a cone splitter and zero-tillage double disk openers. Seeding dates were typical for the respective regions within western and eastern Canada. Soil macronutrients were amended to levels that optimized wheat production for the region based on soil test recommendations. Plots were

scouted for incidence of weed, disease, and insect pressure, and pesticides were applied as needed based on product labels and field crop protection guides.

## Experimental Measurements

### Yield and Grain Quality

Each plot was harvested using a Wintersteiger plot combine (Wintersteiger AG) equipped with a straight-cut header, pickup reel, and crop lifters. Grain yield was calculated from the entire plot area, and a subsample was retained to characterize test weight and whole grain protein, starch, and pentosan concentrations. Grain yield and protein concentration were calculated at a moisture content of 135  $g\ kg^{-1}$ . Protein concentration was estimated by means of near-infrared reflectance spectroscopy (Foss Decater GrainSpec, Foss Food Technology). Composite samples of grain for each cultivar at each location were analyzed for starch, pentosan, and residual starch content. Starch concentration was measured using AACC International (1976). Total pentosan concentration was estimated from flour by the orcinol-HCl method (Hashimoto et al., 1987).

### Ethanol Fermentation

Grain from the composite samples was milled with a Perten 3100 Laboratory Mill (Perten Instruments) until the flour could pass through a 0.5-mm mesh screen and then mixed with water to obtain a mash with 32% (w/w) solids. The pH of the mash was adjusted to 4.0 using 12  $mol\ L^{-1}$  HCl for viscosity and raw

starch hydrolysis analyses. An enzymatic treatment with Opti-mash TGB (317  $\mu\text{L kg}^{-1}$  of grain) and Fermgen (952 mL  $\text{kg}^{-1}$  of grain) (Genencor International) was performed at 52 to 55°C in a water bath for 1 h with frequent stirring. At the end of the enzymatic treatment, the pH and total solids were adjusted to original conditions to compensate for any change. The mash was transferred aseptically to a sterile 500-mL flask. Diethyl pyrocarbonate (Sigma-Aldrich, 97%) was added (779 mL  $\text{kg}^{-1}$  of mash) to each flask as a sterilizing agent and kept at 4°C for 72 h.

Before starting fermentation, the mash was warmed to 53 to 55°C in a shaking incubator (Model Innova 44, New Brunswick Scientific). Stargen 002 (1.071 mL  $\text{kg}^{-1}$  of mash) (Genencor International) was then added to each flask for a 1-h incubation period. At this point, the temperature of the mash was reduced to 30°C and the stirrer speed was maintained at 200 rpm for the rest of the fermentation.

Urea (Fisher Scientific, 98%), Superstar yeast (Lallemand Ethanol Technology), and water were added to the mash in each flask to adjust to 30% (w/w) solids. Urea was used as an external N source at an initial concentration of 16 mmol  $\text{L}^{-1}$ . The yeast was prepared by rehydration with 0.2 g yeast  $\text{mL}^{-1}$  water in a 250-mL flask followed by incubation at 30°C for 30 min with shaking at 200 rpm. Each flask was individually inoculated with yeast to have an approximate concentration of  $2 \times 10^7$  colony-forming units  $\text{mL}^{-1}$ .

After inoculation, each flask was capped with a rubber stopper containing a gas trap (American Brewmaster) to allow  $\text{CO}_2$  venting. To prevent ethanol evaporation from the flasks during fermentation, 2 mL of sterile water was added to each gas trap before starting fermentation. The inoculated flask was then incubated at 30°C in a shaking incubator at 200 rpm for 72 h. At the end of the fermentation, a sample of the mash was subjected to gas chromatography analysis to determine the ethanol concentration. All remaining mash was freeze-dried for residual starch concentration determination.

### Statistical Analysis

Data were analyzed with the PROC GLIMMIX procedure of SAS version 9.2 (SAS Institute). The effects of replicate and site (location  $\times$  year combinations) were considered random, and the cultivar effect was considered fixed. A Gaussian error distribution was used for the analysis. Pairwise comparisons were assessed using the SAS pdmix800 macro, developed by Saxton (1998), which accounts for pairwise probabilities and converts them into letter groupings, and a Bonferroni adjustment was used to provide some protection against Type I errors. Variability for the cultivar effect among sites was assessed with a statistical test to determine if the variance estimates were significantly different from zero and also by comparing the relative size of the site  $\times$  cultivar variance estimate to the total variance associated with the site (main effect of site plus site  $\times$  cultivar interaction). Treatment effects were declared significant at  $P < 0.05$ .

The genotype  $\times$  environment interaction was assessed with a grouping methodology biplot, as described by Francis and Kannenberg (1978). The mean and CV were estimated across sites and replicates for each cultivar. These means were plotted against CV to explore average responses relative to variability for all cultivars. The mean of the cultivar values and CVs was used in the plot to divide the ordination space into four quadrants

or 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.

A general form of principal component analysis, otherwise known as *multidimensional preference analysis*, was performed to further explore the relationships among mean responses for the different crop traits (multivariate analysis of means). The data matrix for the analysis included cultivar means as rows and means for selected response variables as columns. The analysis was conducted with the PRINQUAL procedure of SAS version 9.2 (SAS Institute), using an identity transformation. The results were summarized in a biplot, which is a plot of the mean principal component scores for treatments for the first two principal components. Eigenvectors (correlation between the transformed and original data) for the crop responses were plotted as points at the end of vectors projecting from the origin into various positions in the ordination space. The coincidence of response variable vectors and cultivars across the ordination space suggested crop response variable associations with the cultivars. The lack of coincidence for response variable vectors and cultivars indicates cultivars for which the associated responses were lesser than other cultivars. The relative lengths of the vectors indicated the strength of these associations.

## RESULTS

### Entry and Class Differences

The analysis of variance indicated that the overall effect of cultivar always was highly significant ( $P < 0.001$ ). The statistical test for the interaction effect of cultivar with agroecological zone or province was often important ( $P < 0.041$ ), with a few exceptions. The cultivar  $\times$  agroecological zone interaction effect for starch and ethanol concentration was not statistically significant.

Hoffman usually was the highest yielding cultivar, and the triticale and CWSWS cultivars almost always were part of the highest yielding group with Hoffman in and across all Canadian agroecological zones and provinces (Table 3). The CPS and CWRS cultivars consistently yielded least across most agroecological zones and provinces. The average yields of Hoffman, Pronghorn, and AC Ultima exceeded those for the CWRS cultivar AC Superb by an average of 24% and CPS red cultivars 5700PR and AC Crystal by 19% across western Canada. The overall yield potential was reduced in eastern Canada, but the magnitude of the difference over the CPS and CWRS cultivars was usually greater (the difference was 46% greater relative to AC Superb and 52% greater relative to CPS cultivars). Responses for Ontario were unique relative to the other eastern Canada zones because of increased variability and lower yield potential, which made statistically significant differences difficult to detect. The average yield for the triticale class was similar to that for CWSWS wheat in western Canada, whereas the triticales yielded 23% more than CWSWS cultivars in eastern Canada; however, specific within- and across-class comparisons for the triticale and CWSWS cultivars by agroecological zone in western Canada deviated from the preceding overall differences.

The superior milling and baking qualities of a CWRS class cultivar were evident in the study: AC Superb was always in a group of cultivars with the highest test weights and protein concentration in or across all agroecological zones and provinces

**Table 3. Grain yield means for data collected in six agroecological zones and provinces across Canada from 2006 to 2009.**

Cultivar	Class†	Western	Eastern	Parkland	Western	Ontario	Quebec	Maritimes	Eastern	Overall
		Prairies	Prairies		Canada				Canada	
Mg ha <sup>-1</sup>										
AC Ultima	TRIT	5.48 a‡	5.12 a	5.71 ab	5.44 a	2.95 a	3.56 bc	3.51 ab	3.34 ab	4.39 ab
Pronghorn	TRIT	5.66 a	5.24 a	5.84 ab	5.58 a	2.97 a	4.24 ab	3.74 a	3.65 a	4.61 a
Tyndal	TRIT	5.10 abc	4.66 abc	5.39 bc	5.05 b	2.89 a	3.59 bc	2.99 ab	3.16 abc	4.10 bc
AC Andrew	CWSWS	5.49 a	4.78 abc	5.78 ab	5.35 ab	2.75 a	3.87 abc	1.63 b	2.75 bc	4.05 bc
AC Sadash	CWSWS	5.51 a	4.74 abc	6.14 a	5.46 a	2.70 a	3.82 abc	2.24 ab	2.92 bc	4.19 bc
Bhishaj	CWSWS	5.46 a	4.84 abc	5.81 ab	5.37 ab	2.61 a	3.37 bc	1.64 b	2.54 c	3.95 cd
AC Superb	CWRS	4.39 c	4.19 c	4.75 c	4.44 c	2.49 a	3.52 bc	1.94 ab	2.65 c	3.55 e
Hoffman	CWGP	5.27 ab	5.05 ab	6.06 a	5.46 a	3.31 a	4.87 a	2.89 ab	3.69 a	4.58 a
5700PR	CPS-R	4.51 c	4.35 bc	4.89 c	4.58 c	2.60 a	3.06 c	1.96 ab	2.54 c	3.56 e
AC Crystal	CPS-R	4.66bc	4.28 c	5.10 c	4.68 c	2.34 a	3.51 bc	1.72 b	2.52 c	3.60 de
LSD(0.05)§		0.55	0.63	0.46	0.32	0.92	0.92	1.50	0.66	0.37
Red¶		4.71	4.47	5.20	4.79	2.68	3.74	2.13	2.85	3.82
CWSWS		5.49	4.79	5.91	5.39	2.69	3.69	1.84	2.74	4.07
Triticale		5.41	5.01	5.65	5.35	2.93	3.80	3.41	3.38	4.37
P value		<0.001	<0.001	<0.001	<0.001	0.041	<0.001	<0.001	<0.001	<0.001

† TRIT, spring triticale; CWSWS, Canada western soft white spring wheat; CWRS, Canada western red spring wheat; CWGP, Canada western general purpose candidate; CPS-W, Canada prairie spring white wheat; CPS-R, Canada prairie spring red wheat.

‡ Means followed by the same letter in a column are not significantly different ( $P < 0.05$ ; Bonferroni adjustment).

§ Adjusted LSD(0.05) can be used only to compare cultivar means.

¶ Red includes CPS-R, CWRS, and CWGP (Hoffman).

**Table 4. Test weight means for data collected in six agroecological zones and provinces across Canada from 2006 to 2009.**

Cultivar	Class†	Western	Eastern	Parkland	Western	Ontario	Quebec	Maritimes	Eastern	Overall
		Prairies	Prairies		Canada				Canada	
g L <sup>-1</sup>										
AC Ultima	TRIT	712 b‡	693 d	699 d	701 f	642 e	642 d	611 d	632 f	666 d
Pronghorn	TRIT	705 b	683 d	689 d	692 f	639 e	678 cd	646 cd	655 e	673 d
Tyndal	TRIT	708 b	689 d	694 d	697 f	651 de	672 cd	613 d	645 ef	671 d
AC Andrew	CWSWS	770 a	722 c	757 c	749 e	679 cd	697 bc	652 cd	676 d	713 c
AC Sadash	CWSWS	779 a	733 bc	770 abc	760 cd	691 bc	704 bc	693 abc	696 cd	728 b
Bhishaj	CWSWS	770 a	727 c	759 bc	752 de	687 cd	697 bc	656 bcd	680 d	716 c
AC Superb	CWRS	786 a	752 ab	778 a	772 ab	727 ab	755 a	695 abc	726 ab	749 a
Hoffman	CWGP	785 a	762 a	781 a	776 a	750 a	769 a	713 ab	744 a	760 a
5700PR	CPS-R	786 a	755 ab	776 ab	772 ab	739 a	745 a	726 a	736 a	754 a
AC Crystal	CPS-R	780 a	744 abc	769 abc	764 bc	699 bc	732 ab	691 abc	708 bc	736 b
LSD(0.05)§		18	2.0	1.5	10	29	29	47	21	12
Red¶		784	75.3	77.6	771	729	750	706	728	750
CWSWS		773	72.7	76.2	754	686	700	667	684	719
Triticale		708	68.8	69.4	697	644	664	623	644	670
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

† TRIT, spring triticale; CWSWS, Canada western soft white spring wheat; CWRS, Canada western red spring wheat; CWGP, Canada western general purpose candidate; CPS-W, Canada prairie spring white wheat; CPS-R, Canada prairie spring red wheat.

‡ Means followed by the same letter in a column are not significantly different ( $P < 0.05$ ; Bonferroni adjustment).

§ Adjusted LSD(0.05) can be used only to compare cultivar means.

¶ Red includes CPS-R, CWRS, and CWGP (Hoffman).

(Tables 4 and 5). Yield components other than test weight must be responsible for the high grain yield potential of the triticales because they all displayed the lowest test weights (Table 4). Lower protein concentration also occurred for the triticales relative to the other cultivars (Table 5). The other hard red spring wheat cultivars often had moderate to high test weights, which indicates other yield components were unable to maintain the grain yield (Table 4). Also, moderate to high protein concentration was observed for this same group of cultivars (Table 5). The CWSWS class generally had intermediate test weight and protein concentration values (Tables 4 and 5). These trends

were similar for all regions, although test weights were generally higher in western Canada. Differences in pentosan concentration were sometimes detected (Table 6); however, the most notable and consistent difference was the elevated pentosan levels for Tyndal, especially in the Western Prairies and Quebec.

There were notable interactions for starch and ethanol yield, which are proxies for energy potential per unit area, although there was no interaction between cultivar and region for starch and ethanol concentration. The overall cultivar differences for starch and ethanol yields matched closely those for grain yield; starch and ethanol yields often were greater for the triticales

**Table 5. Protein concentration means for data collected in six agroecological zones and provinces across Canada from 2006 to 2009.**

Cultivar	Class†	Western Prairies	Eastern Prairies	Parkland	Western Canada	Ontario	Quebec	Maritimes	Eastern Canada	Overall
		g kg <sup>-1</sup>								
AC Ultima	TRIT	116 f‡	106 e	110 ef	111 f	109 d	105 d	116 b	110 e	111 f
Pronghorn	TRIT	112 f	105 e	101 f	106 f	112 cd	105 d	119 b	112 e	109 f
Tyndal	TRIT	115 f	112 de	107 ef	111 f	116 cd	108 cd	122 b	115 de	113 f
AC Andrew	CWSWS	131 cde	123 bcd	117 de	124 d	126 bcd	125 bc	134 ab	128 bc	126 de
AC Sadash	CWSWS	124 ef	119 cde	112 ef	118 e	124 bcd	123 bcd	127 b	125 cd	121 e
Bhishaj	CWSWS	129 de	123 bcd	117 de	123 de	126 bcd	127 b	130 ab	128 bc	126 de
AC Superb	CWRS	160 a	145 a	146 a	150 a	148 a	147 a	157 a	150 a	150 a
Hoffman	CWGP	140 bcd	126 bcd	123 cd	130 c	122 bcd	124 bc	129 ab	125 cd	127 cd
5700PR	CPS-R	145 b	136 ab	134 b	138 b	137 ab	140 ab	137 ab	138 b	138 b
AC Crystal	CPS-R	141 bc	132 abc	127 bc	133 bc	129 bc	132 ab	136 ab	132 bc	133 bc
LSD(0.05)§		9	11	8	5	14	14	23	10	6
Red¶		146	135	132	138	134	136	140	137	137
CWSWS		128	122	115	122	125	125	130	127	124
Triticale		114	108	106	109	112	106	119	112	111
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.013

† TRIT, spring triticale; CWSWS, Canada western soft white spring wheat; CWRS, Canada western red spring wheat; CWGP, Canada western general purpose candidate; CPS-W, Canada prairie spring white wheat; CPS-R, Canada prairie spring red wheat.

‡ Means followed by the same letter in a column are not significantly different ( $P < 0.05$ ; Bonferroni adjustment).

§ Adjusted LSD(0.05) can be used only to compare cultivar means.

¶ Red includes CPS-R, CWRS, and CWGP (Hoffman).

**Table 6. Pentosan concentration means for data collected in six agroecological zones and provinces across Canada from 2006 to 2009.**

Cultivar	Class†	Western Prairies	Eastern Prairies	Parkland	Western Canada	Ontario	Quebec	Maritimes	Eastern Canada	Overall
		g kg <sup>-1</sup>								
AC Ultima	TRIT	58.7 ab‡	68.1 a	61.0 a	62.6 ab	71.3 a	74.5 ab	58.7 a	68.2 a	65.4 ab
Pronghorn	TRIT	51.8 b	65.6 a	56.6 a	58.0 b	73.2 a	72.6 ab	54.7 a	66.8 a	62.4 b
Tyndal	TRIT	71.9 a	68.6 a	62.5 a	67.7 a	71.8 a	80.2 a	65.0 a	72.3 a	70.0 a
AC Andrew	CWSWS	56.0 b	62.2 a	62.4 a	60.2 b	68.7 a	69.3 ab	70.0 a	69.3 a	64.8 ab
AC Sadash	CWSWS	55.1 b	66.7 a	58.2 a	60.0 b	72.4 a	69.2 ab	66.7 a	69.4 a	64.7 ab
Bhishaj	CWSWS	55.6 b	63.2 a	55.0 a	57.9 b	72.4 a	58.2 b	66.4 a	65.6 a	61.8 b
AC Superb	CWRS	58.6 b	63.6 a	56.7 a	59.7 b	75.5 a	68.7 ab	66.9 a	70.4 a	65.0 ab
Hoffman	CWGP	57.3 b	68.4 a	58.6 a	61.4 ab	67.0 a	71.0 ab	69.0 a	69.0 a	65.2 ab
5700PR	CPS-R	58.7 ab	69.5 a	60.0 a	62.7 ab	65.5 a	67.3 ab	64.8 a	65.9 a	64.3 ab
AC Crystal	CPS-R	57.2 b	61.9 a	58.0 a	59.1 b	68.5 a	65.2 ab	53.6 a	62.4 a	60.7 b
LSD(0.05)§		9.8	12.3	8.9	6.0	14.9	14.1	24.4	10.6	6.1
Red¶		58.0	65.9	58.3	60.7	69.1	68.0	63.6	66.9	63.8
CWSWS		55.6	64.0	58.5	59.4	71.2	65.5	67.7	68.1	63.8
Triticale		60.8	67.4	60.0	62.8	72.1	75.7	59.5	69.1	65.9
P value		<0.001	0.302	0.080	<0.001	0.514	<0.001	0.275	0.138	<0.001

† TRIT, spring triticale; CWSWS, Canada western soft white spring wheat; CWRS, Canada western red spring wheat; CWGP, Canada western general purpose candidate; CPS-W, Canada prairie spring white wheat; CPS-R, Canada prairie spring red wheat.

‡ Means followed by the same letter in a column are not significantly different ( $P < 0.05$ ; Bonferroni adjustment).

§ Adjusted LSD(0.05) can be used only to compare cultivar means.

¶ Red includes CPS-R, CWRS, and CWGP (Hoffman).

Pronghorn and AC Ultima, the CWSWS class, and Hoffman than for the CPS and CWRS cultivars (Tables 7 and 8); however, the residual starch concentration, which is a reflection of resistance to starch digestibility, did reveal notable cultivar differences, with one exception (Table 9). The class mean for triticale displayed lower resistant starch values than the mean for the CWSWS class in two of three western Canada agroecological zones and Ontario; the CWSWS class produced 37% more residual starch than the triticales in these agroecological zones. These differences were not related to ethanol yield in western Canada and Ontario because the triticales (AC Ultima and Pronghorn)

and the CWSWS class produced similar ethanol yield in most regions of western Canada and Ontario. The triticale class, however, mainly Pronghorn and AC Ultima, did produce more ethanol at the Maritimes site (78% more) and when averaged across all of Canada (10% more; the percentage increase did not include Tyndal) (Table 8). When assessing starch and ethanol concentrations averaged across all regions, the values were relatively similar among cultivars with the exception of Tyndal and AC Superb, which were among a group of cultivars that tended to produce lower concentrations of starch and ethanol (Table 10).

**Table 7. Cultivar starch yield means for data collected in six agroecological zones across Canada from 2006–2009.**

Cultivar	Class†	Western Prairies	Eastern Prairies	Parkland	Western Canada	Mg ha <sup>-1</sup>					Overall
						Ontario	Quebec	Maritimes	Eastern Canada		
AC Ultima	TRIT	3.37 a‡	3.05 a	4.11 abc	3.51 a	2.02 a	2.36 ab	1.94 a	2.11 abc	2.81 bc	
Pronghorn	TRIT	3.41 a	3.06 a	4.17 abc	3.54 a	2.16 a	2.88 ab	2.14 a	2.39 a	2.97 a	
Tyndal	TRIT	2.92 ab	2.54 ab	3.75 cd	3.07 b	1.98 a	2.25 ab	1.51 a	1.91 abc	2.49 cde	
AC Andrew	CWSWS	3.29 ab	2.80 ab	4.23 abc	3.44 a	2.05 a	2.63 ab	0.71 a	1.80 bc	2.62 cd	
AC Sadash	CWSWS	3.34 a	2.80 ab	4.49 a	3.54 a	2.11 a	2.55 ab	0.99 a	1.88 abc	2.71 abcd	
Bhishaj	CWSWS	3.29 ab	2.79 ab	4.16 abc	3.41 a	1.91 a	2.09 b	0.74 a	1.58 c	2.50 cde	
AC Superb	CWRS	2.75 b	2.31 b	3.37 d	2.81 b	1.80 a	2.23 ab	0.94 a	1.66 c	2.23 e	
Hoffman	CWGP	3.25 ab	3.00 a	4.40 ab	3.55 a	2.25 a	3.30 a	1.41 a	2.32 ab	2.94 ab	
5700PR	CPS-R	2.74 b	2.43 ab	3.61 cd	2.93 b	1.84 a	2.02 b	0.96 a	1.61 c	2.27 e	
AC Crystal	CPS-R	2.86 ab	2.52 ab	3.80 bcd	3.06 b	1.84 a	2.36 ab	0.92 a	1.71 c	2.38 de	
LSD(0.05)§		0.57	0.67	0.50	0.34	0.84	0.83	1.33	0.60	0.34	
Red¶		2.90	2.57	2.80	3.09	1.93	2.48	1.06	1.82	2.45	
CWSWS		3.30	2.80	4.29	3.47	2.02	2.43	0.81	1.75	2.61	
Triticale		3.24	2.88	4.01	3.38	2.05	2.50	1.86	2.14	2.76	
P value		<0.001	<0.001	<0.001	<0.001	0.716	<0.001	0.001	<0.001	<0.001	

† TRIT, spring triticale; CWSWS, Canada western soft white spring wheat; CWRS, Canada western red spring wheat; CWGP, Canada western general purpose candidate; CPS-W, Canada prairie spring white wheat; CPS-R, Canada prairie spring red wheat.

‡ Means followed by the same letter in a column are not significantly different ( $P < 0.05$ ; Bonferroni adjustment).

§ Adjusted LSD(0.05) can be used only to compare cultivar means.

¶ Red includes CPS-R, CWRS, and CWGP (Hoffman).

**Table 8. Ethanol yield means for data collected in six agroecological zones and provinces across Canada from 2007 to 2009.**

Cultivar	Class†	Western Prairies	Eastern Prairies	Parkland	Western Canada	L ha <sup>-1</sup>					Overall
						Ontario	Quebec	Maritimes	Eastern Canada		
AC Ultima	TRIT	2279 a‡	2025 abc	2518 abcd	2274 a	1074 a	1326 b	1283 a	1228 abc	1751 abc	
Pronghorn	TRIT	2289 a	2122 a	2659 abc	2356 a	1094 a	1596 ab	1344 a	1345 ab	1851 a	
Tyndal	TRIT	2011 abcd	1717 abc	2361 cde	2030 b	1008 a	1260 b	1073 ac	1113 abcd	1571 def	
AC Andrew	CWSWS	2209 ab	1926 abc	2716 ab	2284 a	937 a	1458 ab	628 d	1008 cd	1646 cd	
AC Sadash	CWSWS	2139 abc	1950 abc	2752 a	2280 a	920 a	1461 ab	846 bcd	1075 bcd	1678 bcd	
Bhishaj	CWSWS	2215 ab	1873 abc	2620 abc	2236 a	976 a	1274 b	603 cd	951 cd	1594 cde	
AC Superb	CWRS	1681 d	1597 c	2123 e	1800 c	857 a	1267 b	716 cd	947 cd	1374 g	
Hoffman	CWGP	2030 abcd	2054 ab	2780 a	2288 a	1192 a	1863 a	1119 ab	1391 a	1840 ab	
5700PR	CPS-R	1819 cd	1629 bc	2276 de	1908 bc	930 a	1098 b	768 bcd	932 d	1420 fg	
AC Crystal	CPS-R	1870 bcd	1673 bc	2396 bcde	1980 b	823 a	1295 b	619 d	912 d	1446 efg	
LSD(0.05)§		287	346	263	173	405	404	395	291	169	
Red¶		1850	1739	2394	1994	951	1381	805	1046	1520	
CWSWS		2188	1917	2696	2267	944	1398	692	1011	1639	
Triticale		2193	1955	2512	2220	1059	1394	1233	1229	1724	
P value		<0.001	<0.001	<0.001	<0.001	0.090	<0.001	<0.001	<0.001	<0.001	

† TRIT, spring triticale; CWSWS, Canada western soft white spring wheat; CWRS, Canada western red spring wheat; CWGP, Canada western general purpose candidate; CPS-W, Canada prairie spring white wheat; CPS-R, Canada prairie spring red wheat.

‡ Means followed by the same letter in a column are not significantly different ( $P < 0.05$ ; Bonferroni adjustment).

§ Adjusted LSD(0.05) can be used only to compare cultivar means.

¶ Red includes CPS-R, CWRS, and CWGP (Hoffman).

A multivariate representation of the means in a biplot was used to identify overall cultivar and class differences for all crop responses. Mean principal component scores for treatments for the first two principal components were plotted as points in the ordination space, and eigenvectors (correlation between the transformed and original data) for crop responses were plotted as points at the end of vectors projecting from the origin. The coincidence, or lack of coincidence, of points and vectors (the length of a vector indicates the strength of the relationship) on the ordination space suggested crop response variable associations with the cultivars. The Western Prairies biplot most closely

corresponded with the representation across all agroecozones (Fig. 2). Triticale and the CWRS and CPS wheat cultivars tended to deviate farthest from the origin, and these two groups of cultivars were most polarized among all 10 cultivars, mainly along the first, horizontal principal component. This distinction among cultivars corresponded with protein-related and test-weight vectors often aligning with CWRS and CPS cultivars, while yield-related vectors coincided with triticale and Hoffman. In the eastern Canada regions, Hoffman was most polarized from the other cultivars. The CWSWS cultivars generally positioned closer to the origin than the other cultivars; this indicated

**Table 9. Residual starch concentration means for data collected in six agroecological zones and provinces across Canada from 2007 to 2009.**

Cultivar	Class†	Western Prairies	Eastern Prairies	Parkland	Western Canada	Ontario	Quebec	Maritimes	Eastern Canada	Overall
		g kg <sup>-1</sup>								
AC Ultima	TRIT	61 b‡	114 ab	39 a	71 abc	71 b	46 a	164 a	94 ab	83 abc
Pronghorn	TRIT	74 ab	74 c	44 a	64 c	65 b	38 a	62 cd	55 b	59 c
Tyndal	TRIT	94 ab	88 bc	51 a	78 abc	82 ab	53 a	98 bcd	77 ab	78 abc
AC Andrew	CWSWS	108 ab	124 a	50 a	94 ab	122 a	58 a	79 bcd	87 ab	90 ab
AC Sadash	CWSWS	122 a	117 ab	53 a	97 a	121 a	56 a	121 ac	99 ab	98 a
Bhishaj	CWSWS	99 ab	101 ac	51 a	84 abc	81 ab	42 a	109 ac	78 ab	81 abc
AC Superb	CWRS	100 ab	111 ab	58 a	90 abc	103 ab	63 a	40 d	69 ab	79 abc
Hoffman	CWGP	96 ab	121 a	52 a	90 abc	92 ab	49 a	85 bcd	76 ab	83 abc
5700PR	CPS-R	79 ab	74 c	47 a	67 bc	85 ab	59 a	70 cd	71 ab	69 bc
AC Crystal	CPS-R	81 ab	73 c	49 a	68 bc	117 a	52 a	141 ab	103 a	86 abc
LSD(0.05)§		47	32	43	28	41	63	65	48	28
Red¶		89	95	52	78	99	56	84	80	79
CWSWS		109	114	52	92	108	52	103	88	90
Triticale		76	92	45	71	73	45	108	75	73
P value		0.001	0.001	0.967	<0.001	0.044	0.961	0.007	0.029	0.001

† TRIT, spring triticale; CWSWS, Canada western soft white spring wheat; CWRS, Canada western red spring wheat; CWGP, Canada western general purpose candidate; CPS-W, Canada prairie spring white wheat; CPS-R, Canada prairie spring red wheat.

‡ Means followed by the same letter in a column are not significantly different ( $P < 0.05$ ; Bonferroni adjustment).

§ Adjusted LSD(0.05) can be used only to compare cultivar means.

¶ Red includes CPS-R, CWRS, and CWGP (Hoffman).

that these cultivars were generally moderate with regard to the traits tested in this study. Moreover, starch-related and ethanol-concentration vectors tended to position closest to the CWSWS cultivars, especially in the western Canada agroecological zones. In eastern Canada, starch- and ethanol-related variables had greater coincidence with the triticales (Pronghorn and AC Ultima) and Hoffman. Pentosan concentration was associated with Tyndal in the western agroecological zones and with all triticale cultivars in the Parkland agroecological zone and Quebec. The Maritimes and Ontario tended to deviate from these trends because the CPS cultivar AC Crystal and the CWSWS cultivar Bhishaj were associated with the pentosan concentration vector.

### Performance Stability and Variance

The random effect of site captured the variability among location  $\times$  year combinations not explained by the fixed effect of region. The site  $\times$  cultivar variance estimate was always highly significant ( $P < 0.01$ ) (Table 11). The percentage of the total site variance accounted for by this interaction often varied around 10 to 15% but was as high as 24 to 31% for protein, pentosan, and residual starch and starch concentrations.

Mean vs. CV biplots were used to further explore and understand the response variability relative to the mean responses (Fig. 3 and 4). Overall, Hoffman wheat and AC Ultima and Pronghorn triticale produced consistent, maximum yields in most areas, although Pronghorn was positioned closely only to the quadrant associated with high and consistent yields. Tyndal and the CWSWS cultivars were less consistent but comparatively high yielding. The CWRS cultivar AC Superb and the CPS cultivars produced lower grain yields, with CPS red wheat yields being variable and AC Superb yields being consistent. Hoffman clearly showed that it is highly adapted (Group I: maximum crop response with low CV across sites) in all regions except the Maritimes and Eastern Prairies. Similar to Hoffman, at least one

**Table 10. Means for grain and ethanol characteristics averaged across six agroecological zones and provinces across Canada from 2006 to 2009.**

Cultivar	Class†	Starch conc.	Ethanol conc.	
		g kg <sup>-1</sup>	L Mg <sup>-1</sup>	
AC Ultima	TRIT	606 ab‡	139 a	372 a
Pronghorn	TRIT	609 ab	140 a	375 a
Tyndal	TRIT	583 c	133 c	356 c
AC Andrew	CWSWS	608 ab	139 a	373 a
AC Sadash	CWSWS	616 a	139 a	372 a
Bhishaj	CWSWS	607 ab	140 a	374 a
AC Superb	CWRS	587 c	134 bc	358 bc
Hoffman	CWGP	604 ab	139 a	373 a
5700PR	CPS-R	597 bc	139 a	373 a
AC Crystal	CPS-R	603 ab	138 ab	368 ab
LSD(0.05)§		15	2	11
Red¶		598	137	368
CWSWS		610	139	373
Triticale		599	137	368
P value		<0.001	<0.001	<0.001

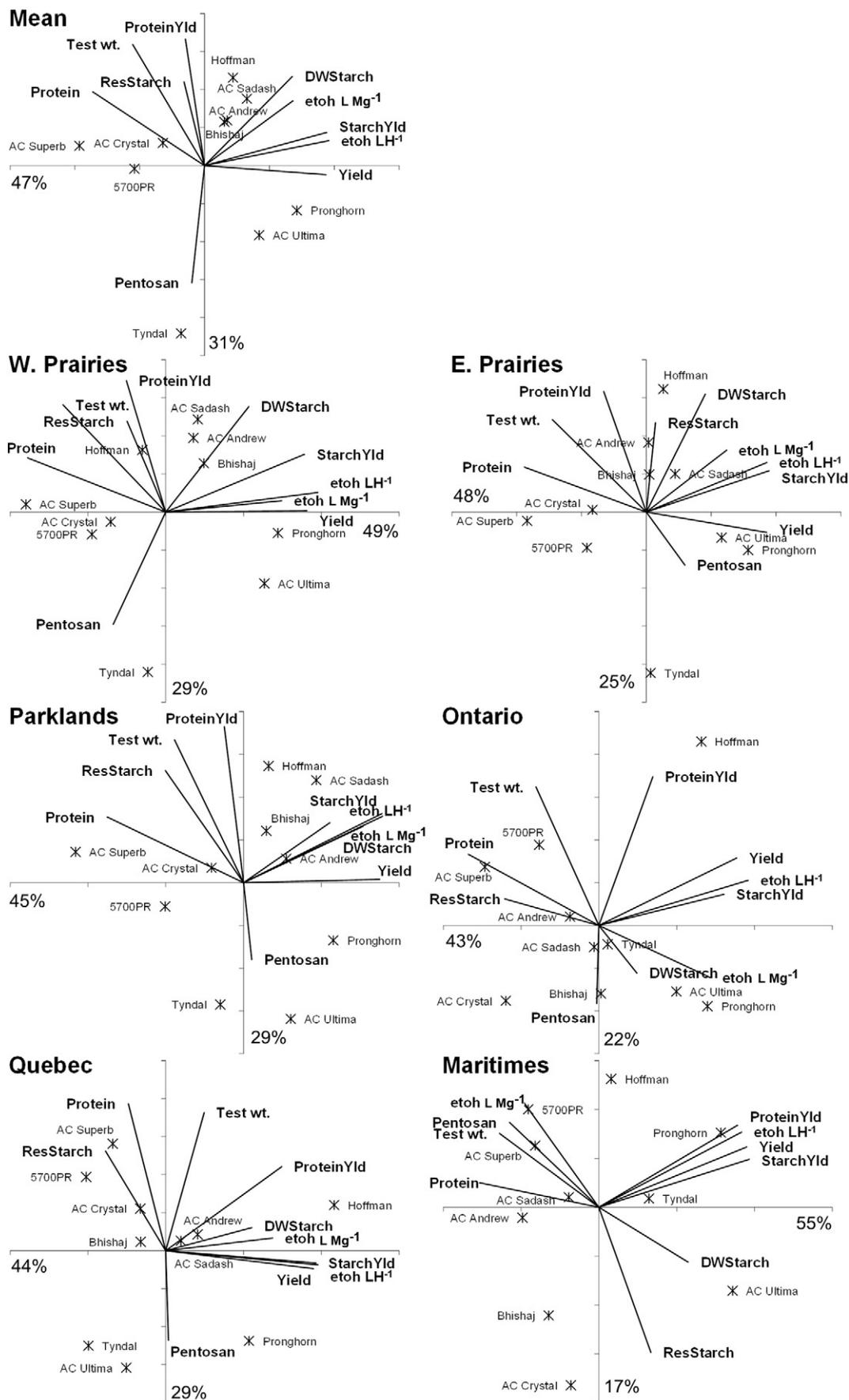
† TRIT, spring triticale; CWSWS, Canada western soft white spring wheat; CWRS, Canada western red spring wheat; CWGP, Canada western general purpose candidate; CPS-W, Canada prairie spring white wheat; CPS-R, Canada prairie spring red wheat.

‡ Means followed by the same letter in a column are not significantly different ( $P < 0.05$ ; Bonferroni adjustment).

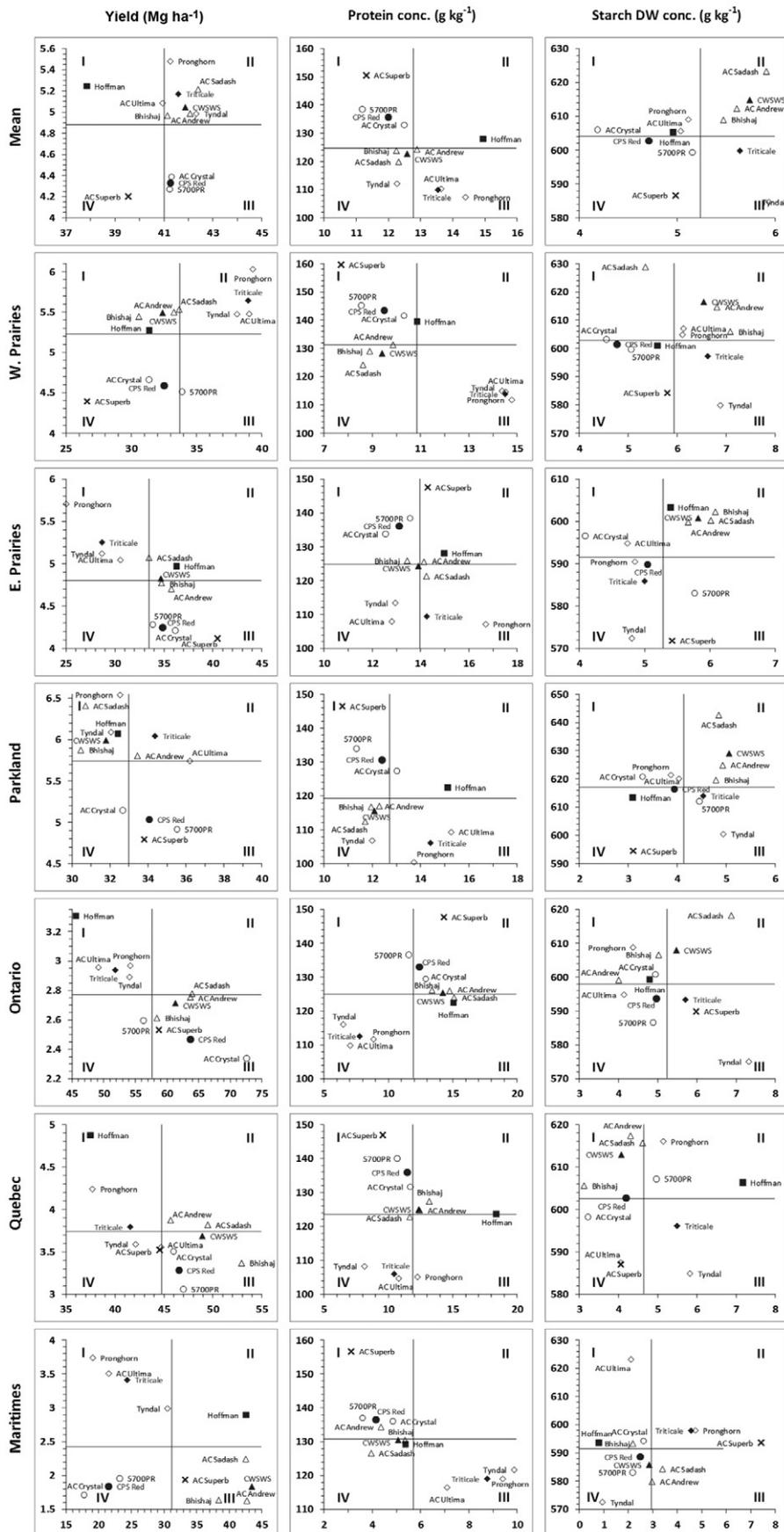
§ Adjusted LSD(0.05) can be used only to compare cultivar means.

¶ Red includes CPS-R, CWRS, and CWGP (Hoffman).

triticale, usually Pronghorn or AC Ultima, displayed high adaptation to all regions except the Western Prairies. In this agroecological zone, high and variable grain yields were observed for the triticales. The CWSWS cultivars were not adapted (lower and/or more variable responses) to areas outside the Western Prairies and Parklands. The CPS and CWRS cultivars were frequently



**Fig. 2.** Biplot of agronomic, grain quality, and ethanol responses (vectors) relative to cultivars (points) generated with multidimensional preference analysis (generalized form of principal components analysis) for data collected at locations across Canada from 2006 to 2009. The percentage of variance explained by the first two principal components (x axis = first component and y axis = second component) is indicated on the respective axes.



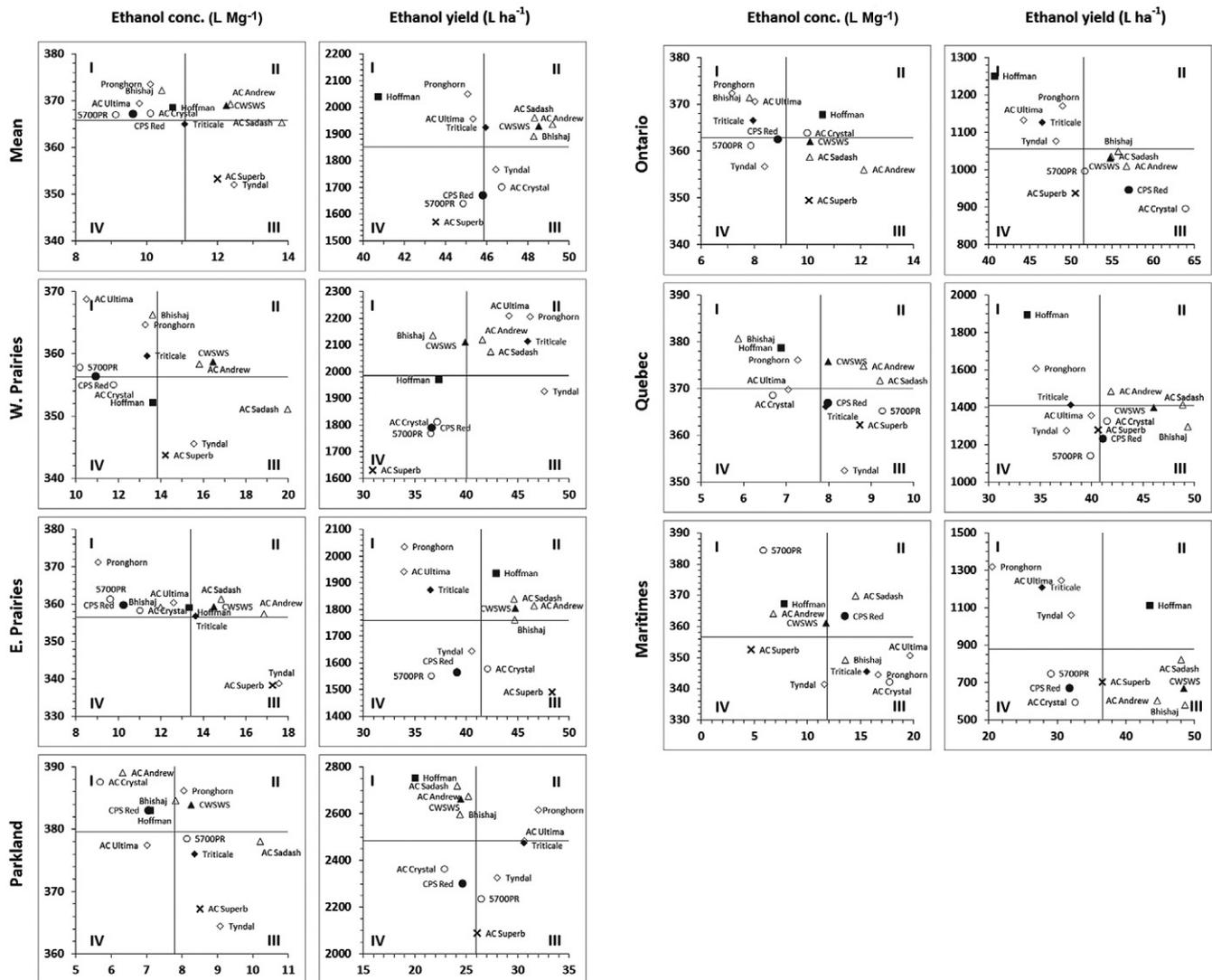
**Fig. 3.** Biplot (mean on y axis vs. CV on x axis) summarized across and by zone or province for yield ( $\text{Mg ha}^{-1}$ ), protein concentration ( $\text{g kg}^{-1}$ ), and starch dry weight concentration ( $\text{g kg}^{-1}$ ) data in six agroecological zones and provinces collected at locations across Canada from 2006 to 2009. In addition to cultivar estimates, estimates are also provided for the Canada western soft white spring (CWSWS) and Canada prairie spring red (CPS Red) wheat classes and indicated with different symbols. Grouping categories: Group I—high mean, low variability; Group II—high mean, high variability; Group III—low mean, high variability; Group IV—low mean, low variability.

**Table II. Variance estimates associated with the random effect of site (location × year combinations) for wheat and triticale cultivar traits collected across Canada from 2006 to 2009.**

Effect	Variance estimate									
	Yield	Test wt.	Protein conc.	Pentosan conc.	Starch yield	Residual starch conc.	Starch conc.	Ethanol conc. (g kg <sup>-1</sup> )	Ethanol conc. (L Mg <sup>-1</sup> )	Ethanol yield
Site	2.60**	13.1**	161**	106**	0.811**	3594**	433**	167**	38519**	1202**
Site × cultivar	0.25**	2.3**	66**	41**	0.126**	1105**	199**	23**	47575**	189**
Site × cultivar, %†	9	15	29	28	13	24	31	14	11	14

\*\* Significant at  $P < 0.01$ .

† Percentage of the total variance associated with the effect of site.



**Fig. 4. Biplot (mean on y axis vs. CV on x axis) summarized across and by agroecological zone for ethanol concentration (L Mg<sup>-1</sup>) and yield (L ha<sup>-1</sup>) in six agroecological zones and provinces collected at locations across Canada from 2006 to 2009. In addition to cultivar estimates, estimates are also provided for the Canada western soft white spring (CWSWS) and Canada prairie spring red (CPS Red) wheat classes and indicated with different symbols. Grouping categories: Group I—high mean, low variability; Group II—high mean, high variability; Group III—low mean, high variability; Group IV—low mean, low variability.**

highly adapted in all regions in terms of protein concentration (Fig. 3). It was apparent that AC Superb and Tyndal often were not adapted in terms of starch and ethanol concentration (Fig. 3 and 4); however, AC Ultima, AC Sadash, and AC Crystal exhibited clear adaptation in most regions for starch concentration (Fig. 3). The other CWSWS cultivars, Pronghorn triticale, and Hoffman also produced high starch concentration but with greater variability (Fig. 3). Ethanol yield trends, not surprisingly, followed closely with those for grain yield (Fig. 4).

## DISCUSSION

The ethanol production potential of triticale in this study can generally be summarized as: triticale (excluding Tyndal) = Hoffman red spring wheat = CWSWS > CPS > CWRS. Hoffman was not recommended for registration in western Canada in 2010 based on susceptibility to stem rust. For this reason, it was deemed that Hoffman was not a suitable ethanol feedstock alternative in western Canada. Although ethanol concentration differed only for the lesser performing Tyndal and AC Superb (Table 2), the mean vs. CV biplots (Fig. 4) suggest that the Pronghorn and AC Ultima triticales, Bhashaj CWSWS wheat, and the CPS class would provide consistently greater levels of ethanol concentration across most regions. The other CWSWS cultivars, AC Sadash and AC Andrew, were more variable in most regions, which indicates that utilization of this class outside the Parkland and Western Prairies could pose greater risk (i.e., variable supply) for ethanol plants, especially when compared with Pronghorn and AC Ultima.

With the exception of the Western Prairies, where greater variability was observed, Pronghorn consistently produced high grain yield, which means it was probably best adapted to produce stable, maximum ethanol yields across most of Canada. Hoffman and AC Ultima also displayed reasonable ethanol yield traits; however, Hoffman has stem rust susceptibility in western Canada and AC Ultima displayed a slightly narrower range of adaptation across the three agroecological zones. The CWRS and CPS classes are not as well suited to ethanol production relative to select triticales, the CWSWS class, and Hoffman. It was not surprising that the CWRS class is not suitable as an ethanol feedstock because this has been established in other studies (McLeod et al., 2010). Our findings for CPS, however, differ from the conclusions of McLeod et al. (2010) that both CPS red and white classes were superior to triticale at study sites in 1993 to 1996.

There are a few possibilities suggesting that the ethanol yield potential of triticale has improved in recent years. Also, innovations to fermentation technologies have evolved, along with new-generation enzymes that seem to have improved starch digestibility to the point that most cereal grain feedstocks, including those in this study, do not differ in terms of ethanol concentration (Gibreel et al., 2011). The residual starch values presented in this study would seem to support this argument because the values were generally similar among most cultivars. When elevated residual starch values were observed, they occurred in the CWSWS class, the preferred feedstock for ethanol plants. Inferior residual starch responses for CWSWS further support the use of select triticale as a suitable ethanol feedstock in Canada.

It is thought that advancements in cultivar development have overcome concerns about growing degree day requirements, elevated concentrations of pentosans, and other major

deficiencies responsible for a lack of utilization of triticale for ethanol plants. Our findings indicate that select triticales will be suitable for ethanol production, which corresponds with other studies conducted in the Parkland and the southern region of Alberta, Canada (Beres et al., 2010; Collier et al., 2013; Goyal et al., 2011). For example, Collier et al. (2013) reported that triticale could be successfully grown in regions that accumulate  $\geq 1700$  growing degree days and that modern cultivars appeared to have improved stress tolerance and grain quality. This provides evidence that these issues are no longer relevant if appropriate cultivars are utilized. Pronghorn and AC Ultima appear well suited for an ethanol end use because they were widely adapted and displayed pentosan levels similar to the preferred cultivars. Tyndal, however, would not be well suited due to elevated pentosan contents and longer growing degree day requirements—attributes that hindered the adoption of previous triticale cultivars (McLeod et al., 2010).

The generally small variance estimates relative to total variance for the random effect of site suggests that rank changes between cultivars, in exclusion of agroecological zone or region interactions, probably were not important. In the first study of this series (Beres et al., 2013), similar observations were reported for agronomic and disease traits with the exception of lodging, which had a site  $\times$  cultivar variance estimate of 31%. Entry difference variability may increase when the area expands beyond the agroecological zones; however, the area required to observe a response can be large. For example, a study assessing genotype  $\times$  region interactions for two-row barley cultivars also reported no notable adaptation to subregions; responses within western Canada were similar (Atlin et al., 2000). Regional adaptation occurred only when the area was expanded to all of western Canada vs. eastern Canada.

In conclusion, the high ethanol yield potential for triticale, along with relatively stable ethanol yields, reflects its potential as an ethanol feedstock in most regions of Canada. This broad range of adaptation and stability would also suggest that the potential extends into the northern and central Great Plains of the United States. There is greater perceived risk in growing triticale, however, due to diseases such as ergot, a lack of information regarding the fermentation efficacy of triticale, and the fact that crop insurance programs will not ensure triticale at a rate similar to CWSWS wheat (Keith Rueve, personal communication, 2011). There was greater potential for risk (in terms of additional variability) growing the preferred CWSWS cultivars AC Andrew and AC Sadash in all regions except the Parkland in western Canada. Compared with AC Andrew or AC Sadash, Pronghorn and AC Ultima appeared to have similar or greater ethanol production overall, in most regions, and with greater consistency. Therefore, the results of this study indicate that triticale offers advantages over CPS and CWSWS as an ethanol feedstock. Changing ethanol feedstock acreages from CPS and CWSWS wheats could occur when Canadian growers have a revenue insurance option to cover the price of risk of growing triticale.

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