The Effect of Genotype and Growing Environment on the Gluten Strength and End-Use Quality of CWRS Wheat

BY

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LIST OF ABBREVIATIONS

AC	Ash content
BB	Bran Content
CC	Cloud cover
CWD	Crop water demand
CWRS	Canada western red spring
DAA	Days after anthesis
DA30	Days above 30 ^{o}C
DON	Deoxynivalenol
DV	Diurnal temperature variation
Etc	Crop evapotranspiration
FP	Flour protein content
FY	Flour yield
FDK	Fusarium damaged kernels
GDD	Growing degree days
GP	Grain protein content
GMP	Glutenin macro polymer
GS	Glutenin Subunits
GSH	Glutathione reduced
GSI	Gluten Strength index
GSSG	Glutatione oxidized
HMWG	High molecular weight glutenins
HPLC	High performance liquid chromatography
LMW	Low molecular weight
MAX	Maximum daily temperature
MDT	Mixing development time
MEAN	Mean daily temperature variation
MIN	Minimum daily temperature
NDV	Normalized diurnal temperature variation
PAGE	Polyacrylamide gel electrophoresis
PAR	Photosynthetically active radiation
PBW	Peak band width
PDR	Peak dough resistance
PSSG	Protein bound glutathione
RP	Reversed phase
SC	Shorts content
SDS	Sodium dodecyl sulfate
SE	Size exclusion
SP	Soluble prolamins
SR	Solar Radiation
TKW	Thousand kernel weight
TOA	Top of the atmosphere radiation
TP	Total precipitation
TW	Test weight

UPP	Unextractable polymeric protein
WAP	Work at peak
WIP	Work input to peak

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ABSTRACT

The goal of this study was to develop a comprehensive understanding of genotype and environmental influences on gluten strength which is a critical asset of CWRS wheat. Replicated field trials in optimally fertilized sites were carried out in two years involving nine CWRS cultivars grown in nine locations. Wheat properties that were evaluated included: grade and degrading factors, milling quality, gluten strength using a 10-g mixograph, flour protein content (FP) and composition including soluble prolamins (SP) and HMW glutenin (HMWG). Weather data was acquired at each growing site. Cultivars varied widely in grade, and there was a progressive and practical decrease on average, in FP and gluten strength as grade decreased. Variation of FPC was remarkable (10–17%) and was primarily attributed to weather effects. Gluten strength, e.g. measured by mixograph work at peak dough development was unrelated to FPC, but was strongly related to HMWG/FPC (r = 0.87) and HMWG/SP (r = 0.86), and therefore to the molecular size distribution of gluten proteins. The contribution of genotype to total variance of gluten strength was about 80% greater than that for growing location. Novel analysis of weather data from seeding to maturity yielded multivariate regression models that explained > 90% of variation in FP and gluten strength. Models included many weather parameters at time points prior to anthesis; if validated, these models would have commercial importance both to farmers and for wheat marketing. The study highlighted the importance and utility of using a G x E approach to evaluate gluten strength and other attributes of CWRS wheat. It can be concluded that many site-years of data are required to accurately establish the gluten strength and quality of wheat varieties. In that regard, the current protocol for evaluation of bread wheat lines in wheat registration trials may be deficient.

1.0 INTRODUCTION

Canada Western Red Spring (CWRS) wheat grown in the Prairie region, spanning over 1,780,650 km^2, is subject to substantial variation in growing conditions causing wide variation in gluten strength within and among genotypes. This can result in a significant deviation in the expected performance of a wheat shipment, based on its grade and protein content. Deficient and variable quality of wheat shipments can be economically consequential, and can include reduced revenue for farmers and grain companies and lowered reputation of the CWRS wheat brand. The rationale for this thesis research stems from concerns prominently raised in 2012 by international and domestic customers, that CWRS wheat was exhibiting relatively low and variable gluten strength. Marketing of wheat in western Canada changed substantially beginning in 2012 with the exiting of the Canadian Wheat Board as the exclusive seller of milling quality wheat, and abandonment of the quota delivery system which helped minimize regional differences in the quality of wheat grown by farmers via blending of relatively small deliveries of wheat across the growing region. Now, wheat is marketed by several grain companies sourcing wheat from farmers who typically deliver their entire harvest into large elevators which results in rail or vessel shipments that accentuates regional differences in wheat quality.

This research project was initiated in order to develop a scientific and comprehensive understanding of the relative influence of genotype and environment on gluten strength which is the key property of wheat flour doughs that affects breadmaking quality in general. While genotype driven differences in gluten strength are generally very well understood, the nature of environmental effects are, surprisingly, largely unknown. Many studies in the past few decades have reported on the relative influence of genotype, environment and their interactions on wheat

breadmaking quality. The latter largely depends on the unique composition of wheat gluten proteins. Wheat gluten is comprised of two complex protein fractions, gliadins and glutenins. Together, they impart the essential viscoelastic properties to dough. Gliadins, which are the main storage protein of wheat endosperm, are monomeric proteins due to their inability to form inter-molecular disulphide bonds between different polypeptides. In contrast, glutenin is a polydisperse polymer of very large average molecular size whose structure and functionality depends mainly on inter-molecular disulphide bonds between polypeptide subunits. Polymeric glutenin is comprised of high molecular weight (HMW) and low molecular weight (LMW) subunits, whose proportions and amounts relative to gliadin protein can be influenced by environmental conditions during the growing season. It is widely accepted that the composition and concentration of glutenin subunits in flour, having substantial influence on the molecular size distribution of the polymer, are the determining factors for gluten strength.

Environmental conditions affecting the end-use quality of wheat, and gluten strength specifically, can be separated into abiotic (e.g. weather and soil fertility) and biotic factors (e.g. *Fusarium* Head Blight and other organisms such as wheat midge). Currently, a moderate amount of research exists on the effects of various abiotic conditions during crop growth on gluten strength. Abiotic conditions, especially soil N, tend to be most influential on total grain protein content whose variation can in turn alter the proportions of flour protein sub-fractions. Unfortunately, the majority of these studies have only examined extremes of environmental conditions, such as heat stress or nutrient deficiencies. There is a large gap of knowledge concerning the effects of intermediate ranges of e.g. temperature and precipitation, which persist for the majority of the growing season, and that reflect the most common weather conditions in the Canadian Prairies.

Similarly, there is relatively little research on the effects of biotic environmental factors, such as *Fusarium* damage or wheat midge infestation, on gluten strength. There is evidence from a few studies that indicate decreased breadmaking quality as a result of proteolytic activity of *Fusarium* (Dexter et al. 1996; Eggert et al. 2011; Nightingale et al. 1999) and some insects (Sivri et al. 1998; Sivri et al. 1999) that hydrolyzes gluten proteins. Degradation of HMW glutenin, hence reduction in average molecular size of polymeric glutenin, by proteases should be measurable and should adversely affect gluten strength. However, many of these studies were conducted by intentional introduction of the pest to the crop. The effects of naturally occurring levels of biotic factors on gluten strength are rarely studied.

1.1 Objectives

The specific objectives of the research were:

1. Characterize by ANOVA proportions of variance contributed by genotype, growing location and their interaction on gluten strength and related properties including wheat, milling, dough mixing and protein content and composition.
    a. Perform ANOVA with and without the cultivar Glenn to determine the relative influence of Glenn on results; Glenn has distinctly high gluten strength in the set of wheats that were studied.
2. Determine if there is a moderating effect of grade on gluten strength variation and level as assessed by dough mixing and protein composition. An appropriate analysis should separate samples into two groups: 1) top milling grades (No. 1 and 2 CWRS) and 2) lower grades, i.e. No. 3 CWRS, Feed and Sample Account grades.
3. Establish relationships between gluten strength assessed by dough mixing and protein content and composition. Results should distinguish effects of crop years (2015 and 2016).
4. Analyze nature of location effects on protein content and key gluten strength parameters (including gluten strength index, high molecular weight glutenin content, mixograph work input) using a new analysis protocol to normalize the varying days to maturity across growing sites into constant calendar day intervals. Weather parameters included both modelled and derived variables that are indices of temperature, precipitation and solar radiation.

2.0 LITERATURE REVIEW

2.1 Introduction

Wheat represents a large proportion of Canada's crop production, with 25 million tonnes of wheat produced annually from 2000-2014 (FOASTAT 2017). Wheat of bread-making quality typically has a narrow range of protein content and quality. Protein quality encompasses both qualitative and quantitative aspects of protein composition. Gluten is the essential protein in wheat that imparts viscoelastic properties to bread dough. It is composed of monomeric gliadins and polymeric glutenins. An understanding of the contribution of each fraction to gluten strength is essential for evaluating the bread-making quality of a wheat cultivar or sample of flour. The influence of genetics, environment and their interactions on gluten quality has been widely reported (Caffe-Treml et al. 2011; Dupont and Altenbach 2003; Graybosch et al. 1995; Koga et al. 2016b; Malik et al. 2013; Naeem et al. 2012; Preston et al. 2001; Triboi et al. 2000; Triboi et al. 2006; Zhu and Khan 2001), although the exact nature of these influences are unclear. While it is well known that gluten composition is governed by genetics (i.e. mainly which subunits are present), the relative amounts of the protein's constituents are determined by environmental influences.

The focus of this literature review is to discuss factors causing variation in wheat bread-making quality. This will include the effects of gluten and its fractions, and numerous abiotic and biotic environmental factors. Abiotic factors include variations in weather (temperature, precipitation, sunlight), soil conditions (mineral nutrition, salinity) in addition to atmospheric carbon dioxide concentrations. Biotic factors will focus on the degradation effects caused by *Fusarium* Head Blight and wheat midge. Understanding the influences of these factors on gluten strength is to deliver a consistent product of expected quality to the purchasers of Canadian

wheat, and to develop and improved understanding of the effects of the growing environment on gluten strength.

2.2 Wheat Gluten

Gluten is the essential wheat component responsible for the viscoelastic properties of dough. The gliadin fraction of gluten is responsible for the viscous behaviour of the dough, whereas the glutenin fraction imparts elastic properties. During dough mixing, the mechanical stress initially induces hydration of flour particles and interactions within and among flour constituents. However, dough development is mainly as ascribed to gluten proteins. As the components of flour continue to hydrate and interact, resistance to mixing continues to increase up to a maximum at which point an optimal protein network is formed. The stronger the flour, at a given protein content, the more it resists mixing, resulting in longer time to attain the highest resistance. Over-mixing, or mixing past this point, will start to break down the hydrated network. Strong doughs, once mixed to peak resistance, will have better stability to over-mixing compared to weaker doughs. Mixing a dough to peak resistance is important for the formation of the continuous protein network that will surround starch granules present in flour. This network is essential for entrapment of CO_2 gas produced by yeast during fermentation. The gas holding capacity of dough relates to the strength of gluten. A strong protein network can contain more gas, resulting in higher loaf volumes. Loaf volume is an important property of breadmaking quality wheats.

Gluten or dough strength reflects the quality of gluten in flour. However, gluten protein quality for e.g. breadmaking is difficult to define or precisely specify. It requires an appropriate amount and balance of glutenin and gliadin proteins. The basic quality of glutenin is determined

by which subunits are present, and the relative amounts of these subunits in the total glutenin polymer fraction. Gliadin proteins are thought to be a diluent of glutenin, and the relative amounts of gliadin and glutenin vary with protein content. To summarize, gluten strength is a reflection of protein content and composition. Understanding the characteristics of gluten that promote or reduce quality is important to help identify cultivars that are desirable for the production of bread and those that are not. Once gluten quality characteristics are understood, research can be directed towards examining how and why gluten strength varies due to environmental factors

2.2.1 Gluten Fractionation and Classification

Wheat flour is primarily a mix of starch and protein. Approximately 85% of the latter comprises the gluten protein fractions gliadin and glutenin. Osborne (1907) was one of the first to separate wheat protein into constituent fractions based on sequential solubility in water (albumins), dilute salt solutions (globulins), 70% aqueous ethanol (prolamins), and dilute acid or alkali solutions (termed glutelin). It is important to note that the term prolamins is commonly used today to describe the totality of gluten proteins which includes both monomeric gliadins and polymeric glutenin molecular weight (Shewry et al., 1986). The Osborne procedure was subsequently modified to begin fractionation with a dilute salt solution, rather than water, to limit extraction of gliadins which precipitate in the presence of salt (Chen and Bushuk, 1970). This fractionation gave five solubility fractions: water-soluble proteins (albumins), salt-soluble proteins (globulins), aqueous ethanol-soluble proteins (gliadins), dilute acetic acid-soluble proteins (soluble glutenins), and insoluble glutenin or residue protein. The acetic-acid soluble and insoluble fractions expressed as proportions of total flour protein were later shown to be well

correlated negatively and positively, respectively, with breadmaking quality including bread loaf volume and gluten strength measured by farinograph dough development time (Orth and Bushuk, 1972). Interestingly, despite the significant correlations, this modified Osborne procedure was later shown to produce glutenin fractions, both soluble and insoluble, that were significantly contaminated with gliadin protein (Dupuis et al. 1995; Fu et al. 1996).

Solubility fractionation procedures have proven useful to obtain more or less pure fractions of wheat proteins. However, isolation of pure fractions is difficult as gliadin and glutenin have overlapping solubility properties, and total glutenin resists solution in non-reducing solvents due to its very large molecular size. A major breakthrough in wheat protein research was the use of reducing agents to solubilize glutenin by disrupting disulphide bonds (Balls and Hale, 1936; Sullivan et al. 1940), although the subunit nature of glutenin was not known at the time.

Solubility fractionation procedures continued to evolve to improve the purity of resulting proteins. Rather than using the classic solvent 70% ethanol, Byers et al. (1983) found using solutions of 50% 1-propanol extracted more protein, and using 50% 1-propanol plus reducing agent (mercaptoethanol) gave virtually complete extraction of storage proteins (i.e. gliadin and glutenin). Marchylo et al. (1989) removed albumins and globulins with a salt solution, followed by extraction of prolamins from the residue using 50% 1-propanol and finally reduction of the insoluble glutenin into its subunits using dithiothreitol (DTT) in the aqueous alcohol solvent. Significantly, the same authors also selectively precipitated glutenin and some omega gliadins from the 50% 1-propanol soluble prolamin fraction by increasing the propanol concentration to 65%. This procedure showed some cross contamination between monomeric gliadin and glutenin as the 50% 1-propanol extract contained gliadin and some glutenin, while the final insoluble

glutenin fraction still contained some gliadin. Presumably, starting the fractionation with salt solution led to this cross contamination problem (Dupuis et al. 1995; Fu et al. 1996).

A different approach than using alcohol to initially extract flour proteins was introduced by Graveland et al. (1982) who used 1.5% solutions of the detergent sodium dodecyl sulphate (SDS). The researchers discovered that wheat proteins could be extracted from flour in a 1.5% SDS solution which, after ultracentrifugation, left a supernatant of SDS soluble protein over an insoluble gel comprising SDS insoluble glutenin; the latter being a higher molecular weight fraction of glutenin. The SDS gel protein, unextractable in SDS solution, is analogous to what was referred to as glutenin macropolymer (GMP) in other work (Weegels et al. 1996; Weegels et al. 1997). This basic approach using SDS as the initial solvent was exploited by a number of Australian researchers who used size exclusion chromatography to quantify both the SDS soluble fraction of wheat protein as well as the insoluble fraction (UPP) which was rendered soluble by sonication without using a reducing agent (Singh et al. 1990; Batey et al. 1991; Gupta et al. 1993). The SDS-extractable polymeric protein (EPP) and UPP fractions were further characterized by SDS-PAGE (Gupta et al. 1993). Although SE-HPLC and SDS-PAGE were techniques both used previously to determine relative amounts of monomeric and polymeric protein fractions, this work revealed that compositional differences in the EPP and UPP fractions may contribute to differences in gluten strength (Gupta et al. 1993). The SDS-sonication-SE-HPLC procedure remains a popular, albeit resource heavy and relatively complex, method that is mainly used to quantify HMW polymeric glutenin or its proportion in a sample of wheat or flour.

There are many variations to methods of fractionation of gluten proteins to study and quantify wheat protein composition e.g. using electrophoresis, chromatography, proteomics and genomics. The challenge remains that wheat proteins are highly complex and heterogeneous,

and that polymeric glutenin cannot be studied in its native state due to its very large and polydisperse molecular size. (MacRitchie, 2010). Regardless of the different approaches available to fractionate gluten proteins, there is a consensus about their correct nomenclature as prolamins that vary in molecular size and their nature as either monomers or polymers (Table 2.1).

Table 2.1. Summary of the types and characteristics of gluten proteins[a]

Components	Mr (% total)	Polymers or monomers	Partial amino acid composition (mol%)
HMW Prolamins			
HMW-GS	65-90,000 (6-10%)	Polymers	30-35% Gln 10-16% pro, 15-20% Gly, 0.5-1.5% Cys, 0.7-1.4%Lys
S-rich prolamins			
ϒ-gliadins	30-45,000 (70-80%)	Monomers	30-40% Gln, 15-20% Pro, 2-3% Cys, <1.0% Lys
α-gliadins		Monomers	
B & C-type LMW-GS[b]		Polymers	
S-poor prolamins			
ω-gliadins	30-75,000 (10-20%)	Monomers	40-50% Gln, 20-30% Pro, 8-9% Phe, 0-0.5% Lys, o-<0.5% Cys[c]
D-type LMW-GS[b]		Polymers	

Mr = molecular weight
[a]From (Shewry and Halford 2002)
[b]B-type LMW-GS are a group of S-rich proteins, C-type LMW-GS are polymeric α- and β- gliadins, D-type LMW-GS are polymeric ω-gliadins
[c] Cysteine is found in D-type LMW-GS but not ω-gliadins

2.2.2 Gliadins

Gliadins represent approximately 48-52% of flour protein (Sapirstein and Fu 1998). Using starch gel electrophoresis, Woychik et al. (1961) were the first to separate gliadins into its four monomeric fractions: α-, β-, γ- and ω-gliadins. In general, SDS-PAGE reveals a higher average molecular weight for ω-gliadins (~44 -72 kDa), whereas α-, β- and γ-gliadins are smaller with molecular weights ranging from 32-42 kDa (Shewry et al. 1986). Most of the γ- and all of the ω-gliadins are coded on the short arm of chromosome 1 (*Gli-1)* (Jackson et al. 1983). The alleles found on the *Gli-1* loci are tightly linked to LMW glutenin alleles found on *Glu-3* (Tatham and Shewry, 1995). The remaining γ- as well as α- and β-gliadins are coded on the short arm of chromosome 6, known as *Gli-2* (Masci et al. 1993).

Gliadins are unable to form inter-molecular di-sulfide bonds; consequently, they are monomeric proteins. However, gliadins are capable of forming intra-molecular bonds through sulfhydryl groups located on cysteine residues; with the exception of ω-gliadins that do not contain cysteine residues (Shewry and Tatham 1997; Wieser 2007). As a result, ω-gliadins are referred to as sulfur-poor prolamins, whereas the α-, β- and γ- gliadins containing high levels of cysteine are termed sulfur-rich prolamins. γ-gliadins are capable of forming four intermolecular disulfide bonds between its 8 cysteine residues, where α-gliadins only contain six cysteine residues, therefore form 3 bonds (Shewry and Tatham 1997).

The monomeric nature of gliadins and their relatively small molecular weights appears to contribute negatively to dough strength and bread handling properties. The negative effect of gliadins on gluten strength has been demonstrated in numerous studies (Barak et al. 2014; Fido et al. 1997; Khatkar et al. 2002; Uthayakumaran et al. 2001). Fido et al. (1997) suggested that the weakening effect of gliadins is largely due to interaction with glutenin in the dough. As gliadins are capable of forming non-covalent hydrogen bonds, they may disrupt the amount of polymer-polymer interactions, and conversely favour the formation of polymer-monomer hydrogen bonds. This gliadin and glutenin interface would result in a weaker glutenin polymeric network. This suggestion is supported by the results of Kuktaite et al. (2004a), in which ω-gliadins were found to be entangled non-covalently within glutenin during dough mixing. In addition to observing evidence of this monomer-polymer interaction, Fu et al. (1996) hypothesize that such an interaction may serve as an indicator of gluten strength. Strong flours will exhibit weaker interactions between gliadins and glutenins, making them easy to separate by solubilisation procedures. However, weak flours exhibit more extensive levels of monomer-polymer bonding, and separation of the two gluten fractions becomes more difficult.

A study by Khatkar et al. (2002) purified total and individual gliadins fractions to be reincorporated into a standard base flour. The purity of these fractions was assessed prior to incorporation into the flour. The effects of gliadins on the mixing properties of gluten were analyzed. A decrease in mixograph mixing time, mixing stability and work input was reported for addition of total (17.6, 25.3 and 22.0% decrease), α- (21.8, 14.8 and 20.2% decrease), β- (17.6, 21.3 and 15.6% decrease), γ-(9.4, 16.8 and 12.8% decrease) and ω- (5.9, 13.5 and 5.5% decrease) gliadins respectively, when incorporated at 1% w/w flour basis level. The lower level of interaction displayed by ω-gliadins was attributed to their lack of cysteine bonds, which resulted in this fraction having the smallest effect on gluten strength.

The influence of gliadins on dough properties was assessed by Barak et al. (2014) in a similar recombination study. Addition of gliadins at levels of 2% of flour resulted in significant ($p < 0.05$) changes in every parameter measured other than mixograph peak band width, which did not change regardless of the amount of gliadin incorporated. As would be expected, high levels of gliadin (10 g/100 g flour) drastically lowered the quality of the dough. The percent decrease for mixograph dough development time, dough stability and peak energy as well as texture analyzer parameters dough hardness and consistency upon the addition of 10% gliadins was 42.3%, 56.2%, 53.6%, 30.8% and 63.6% respectively. Additionally, the incorporation of 10% gliadins results in a 39.9% increase in mixograph dough softening degree. The weakening effect displayed by gliadins was attributed to an interaction between the native base flour gluten, as well as an alteration of the balance between gliadin and glutenin.

Despite the general agreement that gliadins contribute negatively to dough strength, there have been some studies where certain gliadins appear to enhance strength. Such a case was found for γ-gliadins in a study conducted by Gil-Humanes et al. (2012) using transgenic wheat varying

in γ-gliadin content. In this study, γ-gliadins were found to be positively correlated (r = 0.43, $p<0.1$) to mixograph dough development time, and negatively correlated to SDS sedimentation value (r = -0.42, $p<0.1$). The authors hypothesized that the influence of γ-gliadins may be due to its repetitive domain being rich in α-helices and β-turns, thus conferring elasticity.

RP-HPLC analysis of 113 winter and spring wheat cultivars discovered certain poor bread-making cultivars lacked a certain peak that eluted in good bread-making varieties (Wang et al. 2008). This peak was determined to be ω-gliadins, with molecular weight range of 44-55kDa. It was suggested that the mechanism behind the enhanced bread-making capabilities of cultivars containing this ω-gliadin was due to the heightened hydrogen bonding capacity of the protein, as it is rich in glutamine residues. The augmented hydrogen bonding may allow it to support aggregation of a high molecular weight gluten polymer during dough development, despite not being covalently bound.

Overall, it is generally conceded that gliadins contribute negatively to, i.e. weaken, dough strength. There is potentially a minor quality improving effect supplied by certain γ- or ω-gliadins, but this needs to be further evaluated in order to contradict the overwhelming evidence that gliadins negatively affect gluten strength.

2.2.3 Glutenins

Unlike gliadins, glutenins are polymers, whose basic structure is stabilized by inter-molecular disulfide bonds. The reduction of these disulfide bridges separates glutenin subunits from one another so that they may be extracted in aqueous alcohol solutions (Shewry et al. 1986). The reduced form of glutenin yields two fractions, high (HMW) and low molecular weight (LMW) subunits. LMW subunits are sulfur-rich prolamins; whereas HMW subunits,

despite containing cysteine residues, are not classified as such (Shewry and Tatham 1997). The LMW subunits are about 3 times more abundant than HMW (Gianibelli et al. 2001), whereas glutenins as a whole represent 22-48% flour protein (Fu and Sapirstein 1996). The subunits of glutenin may also follow a different nomenclature as introduced by Payne and Corfield (1979b), in which glutenin is split into A, B and C-type fractions. The A-type fraction represents the HMW subunits of glutenin, whereas B- and C-type fractions include the LMW subunits. Table 2.1 refers to a general summary of gluten proteins.

2.2.3.1 High Molecular Weight Glutenin Subunits

Most genotypes contain between 3-5 HMW glutenin subunits, that are genetically coded on the long arm of chromosomes 1A, 1B and 1D (Lawrence and Payne 1983), otherwise known as *Glu-1*. Each chromosome codes an x or y type subunit, the x type subunit being larger than the y type with a range of 80-130kDa. *Glu-1Ay* is almost always silent, where *Glu-1Ax* and *Glu-1Bx* are expressed only in certain cultivars. The HMW subunits were first characterized by Lawrence and Payne (1983). The subunits are named numerically, according to their mobility in SDS-PAGE. *Glu-1Ax* can code subunits 1 or 2*, *Glu-1D(x+y)* most often codes either (2+12) or (5+10) (Lawrence and Payne 1984) where *Glu-1B(x+y)* has many possible variations. Although varying between individual subunits, HMW-GS generally contain between 4-7 cysteine residues (Morel and Bonicel 1996).

The high molecular weight subunits of glutenin appear to be the most important factor in determining the strength of the dough. This is thought to be largely due to their high molecular size that allows them to form very big aggregates, sometimes referred to as the glutenin macropolymer (GMP). GMP is a specific term used to describe insoluble glutenin prepared from

SDS. A high correlation (r=0.80, p<0.001) has been found between large (>100 um) GMP particles and HMW-GS (Chen et al. 2013). There are many studies that correlate the amount of total HMW glutenin (in some cases termed un-extractable polymeric protein -UPP), and the GMP with gluten strength (Weegels et al. 1995(Gupta et al. 1992; Gupta et al. 1993; Kuktaite et al. 2004a; Sapirstein and Fu 1998). Very small difference in the quantity of glutenin can result in a difference of strength (Dupuis et al. 1996).

The composition of HMW-GS can cause an impact on gluten quality supplementary to total HMW glutenin content. Lawrence et al. (1988) found that subunits coded on the *Glu-D1* and *Glu-B1* had a much greater impact on gluten strength than those coded on *Glu-A1*. However, this effect may merely be because D and B loci each code for two subunits, whereas only one is coded on A. Yang et al. (2014) analyzed the relative contributions of *Glu-1* to gluten functionality by comparing gluten and GMP parameters in ion-beam mutants lacking either D, B or A loci. Deletion of *Glu-D1* resulted in the most significant decreases in SDS sedimentation volume, swelling index of gluten, HMW – and LMW-GS content, in addition the largest increase in the gliadin/glutenin ratio; followed by *Glu-B1* then lastly *Glu-A1* will the lowest magnitude of influence. From these results, it was concluded the relative influence of the *Glu-1* loci on gluten strength is as follows: D>B>A. In terms of individual subunits, the pair 5+10 on *Glu-D1* and 17+18 on *Glu-B2* yield much stronger gluten than the alternative subunits: 2+12 and 20 respectively (Lawrence et al. 1987). The rankings of individual subunits within their Glu-1 loci in terms of their contributions to dough strength can be found in

Table 1.2. Influence of individual glutenin subunits on dough strength[a]

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Table 1.2. Influence of individual glutenin subunits on dough strength[a]

Loci	Subunit Ranking
Glu-A1	1 > 2* > Null
Glu-B1 (x+y)	(7 + 8) ≥ (13 + 16) > (17 + 18) = (7 + 9)
Glu-D1 (x+y)	(5 + 10) > (2 + 12) > (4 +12)

[a] Adapted from (He et al. 2005; Rasheed et al. 2014; Wrigley et al. 2009)

A significant parameter in regards to the effect of HMW glutenin subunits is the relative proportion of HMW to LMW subunits. As this ratio increases, an improvement in extensograph Rmax and farinograph dough development time was noted (Gupta et al. 1992). This is because the quantities of HMW and LMW subunits affect the size distribution of the aggregated glutenin polymer (Gupta and Macritchie 1994). Essentially, the larger the polymer, the better the gluten strength. Depending on the extraction method, the ratio of HMW:LMW may also be represented by a ratio of glutenin to gliadin (as LMW-GS are usually extracted alongside gliadins). In cases where separation of LMW-GS and gliadin is achieved, the glutenin to gliadin ratio serves as its own indicator of gluten strength. Uthayakumaran et al. (1999) altered the glutenin:gliadin ratio by adding gluten to the flour from which it had been extracted. Although the ratio was altered, the total protein content was kept constant. The findings demonstrated that as the ratio between glutenin and gliadin increased, so did the gluten strength. Alternatively, Fido et al. (1997) noted that the negative effect on dough strength seen by the addition of gliadins to a base flour was the result of an unfavourable alteration of the glutenin:gliadin ratio.

2.2.3.2 Low Molecular Weight Glutenin Subunits

There are three types of LMW glutenin subunits: B, C and D. B- and C-types were first distinguished by Payne and Corfield (1979a) using electrophoresis. The B-type had the highest molecular weight, ranging from 42-51 kDA, whereas C-type LMW subunits appear to be related to γ- and α-gliadins. Two-dimensional electrophoresis by Jackson et al. (1983) later revealed a highly acidic D-type which included LMW subunits similar to ω- gliadins. On average, LMW

subunits have a molecular weight ranging from 20-45 kDa (D'Ovidio and Masci 2004). LMW subunits are coded on the short arm of chromosome 1, as well as chromosome 3 (Gupta and Macritchie 1994; Jackson et al. 1983). Supplemental nomenclature of LMW-GS may also be given based on allele location, i.e. *Glu-1a* or *Glu-3m*, “a” and “m” referring to mobility’s in SDS-PAGE.

It is difficult to study the effects of LMW-GS due to their similar solubility to gliadin. Even upon proper separation, the addition of isolated LMW-GS would impart dough-weakening effects comparable to the addition of gliadins. This is shown in a study by Weegels et al. (1995), in which 0.2% LMW proteins were added to a flour, leading to poor handling conditions of dough. Therefore, in order to understand the effects of LMW-GS, their interaction with HMW-GS in flour must be analyzed. Once LMW-GS are evaluated in their native state (i.e. complexed with HMW-GS), there are many studies that show a positive effect for particular subunits. For example: 42kDa subunits imparting enhanced bread-making quality (Maruyama-Funatsuki et al. 2004); allele *Glu-A3m* positively affect dough extensibility and resistance (Gupta et al. 1989); *Glu-B3h* augmenting mixing properties, dough strength and loaf volume (Wang et al. 2016).

2.3 Abiotic Environmental Factors

2.3.1 Temperature

2.3.1.1 Moderate Temperatures (< 30 ºC)

An analysis of Canadian climatic conditions over 1950-2003 by Vincent and Mekis (2006) shows that there has been a significant increase in warm temperature events over the twentieth century. In addition to increased daily temperatures, Canadian wheat is subject to large climatic variability simply due to the wide range of locations in which it is grown. Higher average temperatures during the growing season can yield compositional changes in wheat.

High temperatures during the grain filling period are positively correlated with protein content, and inversely correlated to yield (DuPont et al. 2006a; Ferreira et al. 2012; Gibson et al. 1998; Johansson et al. 2005). Simulation modeling of Australian wheat yields by Asseng et al. (2011) discerned that variations as small as 2 ºC from average temperatures can alter grain production by as much as 50%. The most substantial effects are seen when temperatures reach >34 ºC during leaf senescence. Accompanying yield loss, the grain filling period is reduced and grain desiccation occurs more rapidly (Altenbach et al. 2002; Gibson et al. 1998; Koga et al. 2015; Zhang et al. 2013). A review by Dupont and Altenbach (2003) attributed the increased rate of grain filling to augmented enzymatic and metabolic activity, up to temperatures of 30 ºC.

The increase in protein content that arises from high temperatures is the result of a reduced rate of starch accumulation (Johansson et al. 2005), whereas the accumulation of nitrogen is less sensitive to temperatures (Smith and Gooding 1996). Smith and Gooding (1996) developed a multiple linear regression model that accounted for 78.6% of the variation in crude protein for England wheat grown from 1974-1993. The model included temperature in ºC from

June 25 – July 15, nitrogen (kg N ha^{-1}) and mm of rainfall from March 5- May 27 as well as May 28 – July 8, with coefficients of 0.3386, 0.01210, 0.00735 and 0.00625 respectively.

Randall and Moss (1990) examined correlations between average, maximum and minimum daily temperatures (from anthesis to harvest) and wheat quality for field and glasshouse samples of 7 wheat varieties over a six year period at 4 separate locations. They found that increasing temperatures up to a threshold of 30 ^{o}C yielded stronger dough, however periods of three days or more past this threshold decreased strength. The temperature treatments were normalized by subjecting trials to the varying temperature combinations at either 15, 28 or 31 DAA. Interestingly, the magnitude of temperature-induced effects on dough strength was decreased the later the treatment was applied in the growing season.

Borghi et al. (1995) and Flagella et al. (2010) found the dough strengthening effect ceased once daily maximum temperatures passed 35 ^{o}C, rather than 30 ^{o}C as discussed previously. In the former study, temperature treatments were imposed by plastic tunnel covering over plots or delayed sowing. All treatments were optimally fertilized and irrigated. They found cultivars that were sowed late were exposed to highest growing season temperatures (< 30 ^{o} C) early in the grain filling period. At one of the locations, the late sowed cultivars demonstrated the highest strength, measured by an alveographic P/L ratio (0.75). The P/L ratio for normal and early sowing which were exposed to cooler grain filling temperatures was lower at 0.66 and 0.58 respectively. In the same study, the locations where frequent daily maximum temperatures exceeded 35 ^{o}C during grain filling, a weakening effect was observed, seen as a decrease from control P/L ratio (0.55 vs 0.66 of control).

Flagella et al. (2010) found a similar gluten strengthening effect when daily maximum temperatures ranged from 30-35 ^{o}C. The study analyzed two durum wheat cultivars in Foggia,

Italy over three growing seasons with lengths of 196, 199 and 168 days. The plots were fertilized prior to planting, and either irrigated or rainfed. Over the three seasons, gluten strength, measured as gluten index, was found to be significantly correlated ($r=0.71$, $p<0.01$) to the number of days where maximum temperatures ranged from 30-35 ^{o}C. The number of days with maximum temperatures between 30-35 ^{o}C was additionally found to be correlated to total glutenin (50% 1-propanol insoluble), HMW-GS and LMW-G concentrations measured by SE-HPLC, with positive correlations of 0.82, 0.94 and 0.93 ($p<0.01$) respectively.

Ferreira et al. (2012) reported that high temperatures (< 30 ^{o}C) applied either throughout the entire growing season, throughout grain filling or during the desiccation period favoured the formation of SDS-insoluble glutenin polymers. In the case of this study, temperatures did not exceed 27.5^{o}C. Moldestad et al. (2014) and Koga et al. (2015) similarly observed a higher concentration of unextractable polymeric protein (UPP) that was related to increases in temperature. In the latter, one of two spring wheat cultivars exhibited temperature resistance, demonstrating high %UPP regardless of temperature.

There is a general agreement between studies that up to a threshold temperature of approximately 30 ^{o}C there is an improvement in gluten quality. This is additionally reported by (Malik et al. 2013; Moldestad et al. 2011; Moldestad et al. 2014; Spiertz et al. 2006). Accordingly, a study by Koga et al. (2016b) using winter wheat field trials found the most favourable rheological properties arose with average temperatures ranging from 18-19 ^{o}C during July grain-filling period. These grain-filling temperature conditions occurred in three of the eight years for the experiment yielding extensograph Rmax values from 0.51 – 0.90 N. The remaining years in which cooler July grain filling temperatures (< 18 ^{o}C average) occurred had lower Rmax values ranging from 0.09 – 0.68 N.

Johansson et al. (2005) described an optimal temperature range of 15-20 ^{o}C to achieve high yield alongside desirable protein content, although there was no mention of the recommended temperature range required for acceptable protein quality. In fact, the results of Moldestad et al. (2011) suggest that the temperature range put forth by Johansson and colleagues is not desirable for optimal gluten strength. The four-year study analyzing 4 Norwegian spring wheat varieties observed weakened gluten resistance (extensograph Rmax) as diurnal temperatures dropped below 18 ^{o}C. Partial least squares regression analysis of all four years of the data found that mean temperature during the growing season explained 55% of the variation in Rmax. The highest Rmax values, ranging from 0.13 to 1.12 N, were observed when mean temperatures were higher (18-21 ^{o}C) in the first half of grain filling.

In disagreement with previous results, Stone et al. (1997) reported a decrease in dough strength as a result of increased yet moderate temperatures. Of note, the temperature treatments of 21/16 ^{o}C and 27/22 ^{o}C were applied at 15 days after anthesis (DAA), whereas up until this point, plants were grown under 18/13 ^{o}C day-night regimes. Therefore, the decrease in dough strength observed may be due to temperatures rising much higher than the growth temperatures in which the plants were acclimatized. The authors themselves attribute the decrease in dough strength to be the result of relatively high night temperatures (22 ^{o}C). Additionally, the single variety examined in this case was particularly heat-sensitive, possibly contributing to the discrepancies between results.

2.3.1.2 High Temperatures (> 30 ^{o}C)

Heat stress occurs in wheat that is subject to temperatures above 35 ^{o}C, and induces compositional changes in wheat. The quality implications of heat-stress on wheat are conflicting,

although there appears to be more results indicating a gluten weakening effect. As a result of high temperatures during grain filling, both losses (Blumenthal et al. 1991b; Borghi et al. 1995; Corbellini et al. 1998; DuPont et al. 2006a; Dupont et al. 2006b; Gibson et al. 1998; Graybosch et al. 1995; Guzman et al. 2016; Li et al. 2013b; Randall and Moss 1990; Skylas et al. 2002; Stone et al. 1997) and improvements (Skylas et al. 2002; Spiertz et al. 2006; Tahir et al. 2006) in dough quality have been reported, the former despite an increase in protein content. Blumenthal et al. (1991a) found a negative correlation between dough strength (Extensograph Rmax) and number of hours above 35 ^{o}C (r= -0.79) in a two year study containing both field and greenhouse experiments. In the field experiments, naturally occurring heat stress timing was varied by altering sowing date, so that early sowing heat stress occurred from 35-49 DAA whereas late sowing heat-stress occurred 18-26 DAA, depending on the variety, and lasted for roughly 4-6 days. In the greenhouse experiments a day/night temperature stress of 40/21 ^{o}C was imposed at 11 or 23 DAA for 48 hours.

In regards to loaf volume, (Dupont et al. 2006b; Li et al. 2013b) reported expansion, whereas (Blumenthal et al. 1991b) found damaging effects from heat stress. In a climate-controlled greenhouse experiment, Dupont et al. (2006b) examined the effect of 24/17, 37/17 and 37/28 ^{o}C day/night temperature regimes on one hard red spring wheat variety grown under varying levels of nitrogen (N), phosphorus (P), potassium (K) and sulfur (S) fertilization. Increased loaf volume was observed as a result of the 37/28 ^{o}C temperature regime with no added fertilizer, however was similar in value to the 24/17 ^{o}C regime with added NPK. They suggested the improving effect of heat stress on loaf volume arose from the increase in protein content, as both treatments yielded comparable flour protein contents (~14-16%).

A field study by Li et al. (2013b) imposed heat stress to 15 common wheat cultivars by sowing approximately one month after the optimum date, so that temperatures frequently rose above 30 ^{o}C, and even higher than 35 ^{o}C during grain filling. Both optimal fertilization and irrigation were applied so there were no other limiting factors for this treatment. Heat stress resulted in a significantly (p<0.05) higher loaf volume (841.3 mm) compared to the control (797.8 mm). Alternatively, wheat quality parameters mixograph peak time and alveograph P/L ratio were significantly decreased relative to the control; -0.3 min and -0.18 respectively.

Blumenthal et al. (1991b) collected quality data from eleven different field trials that included five wheat cultivars from four locations over a number of years. Meteorological data was collected for the sites to relate the number of hours above 35 ^{o}C from anthesis to harvest to wheat quality data. In general, all 19 out of the 20 cultivar-location combinations were negatively correlated to loaf volume (r = -0.22 to -0.76) and extensograph Rmax (r= -0.23 to-0.83), however only roughly half of these correlations were statistically significant. They speculated the loss of loaf volume and gluten strength was related to compositional changes in protein fractions.

As temperatures rise, gliadin synthesis occurs at an augmented rate resulting in large increases of monomeric proteins (Blumenthal et al. 1993; Ciaffi et al. 1996a; Johansson et al. 2005; Stone et al. 1997; Stone and Nicolas 1995). To monitor changes in grain protein synthesis during heat-stress, Blumenthal et al. (1991a) followed ^{14}C labeled amino acids 12 and 24 hours after wheat heads were subjected to either a 4 or 8 hour heat treatment at 40 ^{o}C. The results demonstrated that the accumulation of gliadins progressed at an increased pace in the four varieties studied. Blumenthal et al. (1990) previously revealed that gliadin genes contain heat-shock elements activated upon exposure to heat stress, possibly explaining why wheat grown in

high temperatures contain higher percentages of gliadin. It was additionally thought that heat-shock proteins might contribute to loss of dough strength. Blumenthal et al. (1998) later disproved this theory by incorporating heat shock protein 70 into dough at higher than naturally occurring levels. There were no dramatic decreases in mixograph parameters that would offer an explanation for heat-shock protein induced dough weakening. They concluded the effects of heat on wheat quality must therefore be attributed to compositional changes in gluten.

As high temperatures enhance the rate of gliadin synthesis, the gliadin/glutenin ratio is increased; as assessed in an unreduced (Blumenthal et al. 1993; Blumenthal et al. 1991a; Ciaffi et al. 1996a) and reduced (Ciaffi et al. 1996a) SDS-buffer by SE-HPLC, as well as based on solubility in 0.3 M NaI-7.5% 1-propanol (Tahir et al. 2006). In the case of Gibson et al. (1998), increases in the gliadin/glutenin ratio assessed in SDS buffer by SE-HPLC were only significant (p<0.10) when heat treatment was applied from 10 DAA until ripening. With a constant night temperature of 20 ^{o}C, day temperature treatments of 20, 25, 30 and 35 ^{o}C yielded gliadin/glutenin ratios of 0.95, 0.96, 1.00 and 1.04 respectively. When the same treatments were applied from 15 and 20 DAA until ripening the effects on this ratio were insignificant.

The effect of heat stress on the gliadin/glutenin ratio significantly varies between cultivars (Blumenthal et al. 1993; Stone and Nicolas 1995). Stone and Nicolas (1995) exposed 75 wheat cultivars to 3-day periods of 40 ^{o}C temperatures at 10 or 30 DAA .Heat exposure 10 DAA decreased the gliadin:glutenin ratio by 1.9%, where there was an 1.1% increase from exposure 30 DAA. However, changes in gliadin:glutenin were only statistically significant when looking at individual varieties. The differences between the response of individual varieties may explain some of the contradicting results. In addition, Corbellini et al. (1998) reported that wheat

is most sensitive when subject to heat stress between early-to-middle of the grain filling period and when this period lasted for more than 10 days.

Ciaffi et al. (1996a) observed that the amount of total glutenins remained constant as a result of high temperatures. However, the quantities of the SDS insoluble fractions were significantly lowered by heat and balanced by an increase in SDS-soluble glutenin fraction. Based on these results, they speculated that temperatures do not alter the synthesis of glutenin. Rather, high temperatures inhibit the formation of disulfide bonds and folding of the polymer within the kernel, preventing the formation of large insoluble aggregates. Corbellini et al. (1998) later observed a similar trend, in which total polymeric synthesis of 2 bread wheat cultivars was relatively unaffected (2% decrease), but the HPLC quantified proportions of insoluble glutenins dropped from 26.5% to 22.5%. It was proposed the decrease in total polymeric protein occurred because the increase in soluble glutenin was not large enough to fully account for the loss insoluble glutenin (soluble glutenin peaks 1 and 3 increased by 2.3% and 1.3% respectively). These results are congruent with the mechanism discussed by Johansson et al. (2013) in which heat alters the enzymatic activity of protein disulfide isomerase, thus affecting gluten quality. It was demonstrated by Every et al. (2003) that protein disulfide isomerase participates in the formation of large molecular weight glutenin polymers. Alternatively, DuPont et al. (2006a) described that the mechanism behind any temperature induced decreases in glutenin may arise because of temperature interfering with the transport or production of amino acids, similarly to how the synthesis of starch is reduced in the amyloplast.

As a result of the influence of heat on glutenin, the molecular size distribution generally decreases, reflected in an increase of LMW-GS at the expense of HMW-GS (Ciaffi et al. 1996a; Corbellini et al. 1998; DuPont et al. 2006a; Naeem et al. 2012; Stone and Nicolas 1995). It

follows that decreases in both HMW:LMW glutenin subunit ratio (Don et al. 2005; DuPont et al. 2006a), GMP concentration (Wardlaw et al. 2002; Zhang et al. 2013) and %UPP (Irmak et al. 2008) have been reported. However, alternative effects of heat shock on glutenin have been proposed. In the case of Dupont et al. (2006b) few changes in protein composition were noted, however one major LMW-GS decreased as a result of high temperatures. Additionally, DuPont et al. (2006a) reported an increase in sulfur-poor and decrease in sulfur-rich proteins. Spiertz et al. (2006) recounted heat stress induced increases in GMP content and average particle size, where Tahir et al. (2006) found increases in both soluble and insoluble gluten fractions.

In order to distinguish between the drastically differing changes in glutenin content reported as a result of heat stress, Table 2.2 outlines the experimental conditions for a number of studies. Observable in Table 2.2, all cases but one (Balla et al. 2011), increases in HMW-GS and large polymeric glutenin alongside decreases in LMW-GS and small polymeric glutenin occurred when fertilization was included in the experimental design. A possible explanation for this is that HMW glutenin content is unrelated to increases in temperature during the growing season; rather, it is related to the availability of nitrogen during its accumulation. As protein synthesis increases as a result of high temperatures, more nitrogen would be required for assimilation into proteins (Weidner and Ziemens 1975). Thus, under nitrogen regimes that would be sufficient for protein synthesis at lower temperatures, slight deficiencies could occur when the plant in exposed to these high temperature conditions. The lack of fertilization at high temperatures during growing season conditions would result in a decrease in the amount of large polymers formed, whereas fertilization application provides sufficient nitrogen for the synthesis of these large glutenin polymers. Additionally, the increases or decreases in large glutenin polymers appear to be compensated by either the respective loss or gain of smaller (LMW-GS)

polymers. A similar idea was proposed by Triboi et al. (2003), in which variations in protein fraction concentrations were not affected by post-anthesis temperatures, but rather the amount of nitrogen accumulated during grain filling

Table 2.2. Experimental conditions for temperature induced changes in polymeric glutenin of spring, winter and durum wheat.

Temperature Conditions[a]	Optimal Fertilization	Optimal Irrigation	Fractionation Procedure	Effect on polymeric gluten[b]	Reference
5 or 10 days of 40 C at early, middle or late grain filling alone or together	-	-	SE HPLC – SDS Buffer	Decrease in HMW-GS (%) Increase in LMW-GS (%) Decrease in HMW/LMW	(Corbellini et al. 1998)
37/28 C from anthesis to maturity; control 24/17 C	With and without NPK	Yes	RP HPLC – 6M TrisHCl Buffer SDS-PAGE	Increase* in HMW-GS (%) No effect in LMW-GS (%) Increase* in HMW/LMW *only when compared to 0 NPK 24/17 C, no significant effect vs 24/17 +NPK	(DuPont et al. 2006a)
35/20 C for 72hrs 25DAA, 40/25 C for 72 hrs 25 DAA or 40/25 C from 25DAA to maturity; control 20/16 C	-	Yes	SE HPLC – SDS Buffer	Decrease in UPP%	(Irmak et al. 2008)
72hrs of 30/18 or 35/20 C at 16 DAA, 35/20 or 20.25 at 16 DAA until maturity or 40/25 at 25 DAA until maturity, control 20/16	-	Yes	RP HPLC – 50% isopropanol	Decrease in GMP (%) Increase in LMW/GS Decrease in LMW/HMW	(Don et al. 2005)
All plants grown at 18/13 until 14 DAA then 40/16 C from 15-19DAA, followed by either 21/16, 27/22 or 30/25 from 20DAA until maturity, control 21/16 from 15DAA-maturity.	Yes	Yes	SE-HPLC – SDS Buffer	No significant changes in SDS-insoluble and SDS-soluble polymers	(Stone et al. 1997)
32/24 C applied end of spikelet initiation and/or anthesis, control 20/12 at end of spikelet initiation increasing to 24/16 at anthesis	Yes	Yes and drought stress	SDS-PAGE	Decrease in HMW-GS and GMP when heat stress alone at anthesis or combined at stem elongation and anthesis Increase in HMW-GS and GMP when drought stress at stem elongation and heat stress at anthesis	(Zhang et al. 2013)
38 C for 5 hrs/day at either 7-10 DAA or 12-20 DAA	Yes	Yes	SDS-Buffer	Increase in GMP	(Lu et al. 2013)
10hrs of 40 C daily from 29-31 DAA, control 18/13 C	-	Yes	SE-HPLC - SDS Buffer	Decrease in large glutenin polymer	(Blumenthal et al. 1995)
15 days of 35/20 at the 12th day after heading, control 24/20	-	Yes and drought stress	SE-HPLC - SDS Buffer	Increase in UPP% with heat alone Increase in UPP% for 3/5 genotypes (2/5 decreased) with combined heat and drought stress	(Balla et al. 2011)
37/28 C from anthesis to harvest, control 24/17	With and without N fertilization	Yes	2-DE Gel Analysis – SDS Buffer	Increase in HMW-GS Decrease in LMW-GS	(Hurkman et al. 2013)
32/15 C for 72h at soft dough stage, control 22/15 C	Yes	Yes	SE-HPLC – SDS Buffer	Decrease in soluble LPP Increase in insoluble LPP Decrease in soluble SPP Decrease in insoluble SPP	(Labuschagne et al. 2016)
38 C for 4 hours from 18-21 DAA, control average air temperature of 23.8 C	Yes	Yes	2-DE Analysis – 70 % ethanol	Decrease in two LMW-GS spots Increase in one LMW-GS No other significant changes	(Majoul-Haddad et al. 2013)
32/15 C for 3 days at soft dough stage, control 22/15 C	Yes	-	SE-HPLC - SDS Buffer	Decrease in soluble LPP	(Labuschagne et al. 2009)

[a] Temperatures are reported as day/night conditions

[b]LMW= low molecular weight, HMW = high molecular weight, GS = glutenin subunits, UPP = unsoluble polymeric protein, GMP = glutenin macropolymer, LPP = large polymeric protein, SPP = small polymeric protein

The discrepancies in temperature-induced variations of glutenin content observed can additionally be attributed to cultivar heat-tolerance. Skylas et al. (2002) subjected a heat-susceptible and heat-tolerant cultivar to heat stress of 40 ^{o}C days and 25 ^{o}C nights for 2 days at 15 DAA. Potted plants were grown in a 1:1 mixture of vermiculite and pearlite and frequently watered. As a result of heat stress, the susceptible variety exhibited a decrease in dough strength whereas the heat-tolerance variety became stronger. Proteome analysis revealed 7 heat shock proteins in the tolerant cultivar that were not present in susceptible one. It appears that lines containing HMW-GS characteristic of good bread-making quality (i.e. *Glu-D* 5+10) are more resistant to heat stress than lines with poor bread-making quality (*Glu-D* 2+12) (Gibson et al. 1998; Irmak et al. 2008; Naeem et al. 2012), although Don et al. (2005) reported only one out of the two 5+10 cultivars displayed higher heat resistance. In this study, near-isogenic lines of two cultivars differing in their HMW-GS composition were grown in well-watered pots within growth chambers. Heat treatment of 40/25 ^{o}C from 16 DAA anthesis to maturity resulted in 55.4, 43.6, 54.0, and 51.1% decreases in GMP content for varieties Lance 2+12, Lance 5+10, Warigal 2 +12 and Warigal 5+10 respectively. This was in comparison to control samples grown at 20/16 ^{o}C for the entire growing season. Although both 5+10 near-isogenic lines displayed the smallest loss in GMP, the loss of Warigal 5 +10 was nearer to the loss experienced by 2 +12 lines.

Interestingly, Spiertz et al. (2006) reported that cultivars containing 5+10 subunits were more responsive to a 3 day 38/20 ^{o}C heat shock treatment than the counter subunit composition of 2 +12, in that the more response cultivars favoured the formation of the GMP. This effect of heat shock on the cultivars was amplified when plants were acclimatized to cooler (18/13 C vs. 25/20 C) growing conditions prior to treatment. Nevertheless, cultivars with Glu-Dx5 HMW-GS are thought to be more resistant due an extra cysteine residue, which could potentially allow for

faster polymerization (Naeem et al. 2012; Rasheed et al. 2014). Increased concentrations of the reactant (S-H) found on cysteine promote formation of disulfide bonds. As the formation of the polymer is sped up, aggregation may occur before high temperatures can interfere (Irmak et al. 2008). Therefore, lines with HMW-GS 2+12 have later formation of the polymer, and are more at risk of being affected by high temperatures.

An additional source of variation emerges when ambient growth temperatures prior to heat stresses differ. Spiertz et al. (2006) analyzed three spring wheat cultivars with day/night temperatures of either 18/13 ^{o}C or 25/20 ^{o}C in a glasshouse experiment. During the grain filling period, plants were exposed to a 3-day heat shock treatment with day/night temperatures of 38/20 ^{o}C. They found that ambient growth temperatures of 18/13 ^{o}C preceding the shock greatly amplified the magnitude of heat shock effects. Similar results were reported by Don et al. (2005). This is due to the plant's ability to acclimatize to the temperature of environment in which it is grown (Berry and Bjorkman 1980). Yamasaki et al. (2002) demonstrated that the optimum temperature for wheat photosynthesis was directly related to the growth temperatures, (i.e. wheat grown at 25 ^{o}C had optimum photosynthesis at 25-30 ^{o}C). It follows that wheat grown under cool conditions would be more sensitive to high temperature variations, whereas plants grown under warmer conditions exhibit more acclimatization to heat.

2.3.2 Water Availability

Water is an essential tool in plant growth as it is responsible for the uptake of nutrients and takes part in several metabolic processes. Where there are many studies examining extreme variations in water conditions (i.e. drought, even less with excess water), there exists a lack of knowledge on how mid-range water quantities affect gluten properties. These mid-range

quantities would be more relevant in assessing the implications of precipitation patterns that occur naturally. Nevertheless, it is still important to understand effects of extreme water conditions on wheat quality.

Water stress (or drought) increases the protein concentration of wheat (Flagella et al. 2010; Gooding et al. 2003; Kharel et al. 2011; Li et al. 2013b; Ozturk and Aydin 2004; Saint Pierre et al. 2008; Zhao et al. 2009a), also reflected by an increase in grain nitrogen content (Bahrani et al. 2011; Jiang et al. 2009). The increased protein content is the result of a concentration effect. As water stress reduces yield and grain size (Houshmand et al. 2014), protein represents a higher percentage of the mature kernel (Guzman et al. 2016). In terms of bread-making, drought increases gluten strength (Flagella et al. 2010; Guzman et al. 2016; Kharel et al. 2011; Li et al. 2013a; b) and improves breadmaking quality (Ozturk and Aydin 2004; Zhao et al. 2009b). However, Li et al. (2013b) found that loaf volume decreased in response, but this decrease was not statistically significant.

In a study by Koga et al. (2016b), Norwegian wheat field trials throughout 2005-2013 exhibited highest Rmax values (0.51-0.91 N compared to 0.2-0.68 N) and therefore most desirable gluten quality during warm (average temp 18-19 C) and dry seasons compared to cool conditions with frequent precipitation. In this case, the decreased gluten strength observed during the wet years were associated with a high fungal protease activity. Similarly, Erekul and Kohn (2006) compared bread-making quality of winter wheat grown in 2003 and 2004. The warm and dry year (average temperature ~10.3-11.2 ^{o}C; average precipitation ~395.5-420.6mm) yielded crop with higher crude protein content, sedimentation value, gluten index and wet gluten compared to the colder wet year (average temperature ~8.9-9.3 ^{o}C; average precipitation

~500mm). However, soil type for the two locations in this study was a significant factor in determining wheat quality parameters. The quality ranking of the four cultivars remained the same regardless of the weather conditions; i.e. cultivars with the highest ranking breadmaking performance consistently held the highest gluten index, sedimentation value and wet gluten content between years and locations.

Cultivar variation in wheat quality response to drought has been reported by Li et al. (2013a). In this study, the well-watered control was irrigated pre-planting, in addition to auxiliary treatments when the top soil (60cm) profile was depleted of 50% of its water reserves. The drought stress treatment received irrigation before sowing, and a single auxiliary treatment 30-days post-planting. Seven durum wheat cultivars were analyzed over two growing seasons. Increases in flour SDS-sedimentation volume (9.94 to 10.43 mL), mixograph peak time (2.91 to 3.06 min) and gluten swelling index (4.53 to 4.69) were reported as a result of drought stress, although only the change in gluten swelling index was significant ($p<0.05$). When these parameters were presented for individual genotypes, the magnitude of change varied extensively between cultivars. The change in SDS sedimentation volume, mixograph peak time and gluten swelling index in comparison to the control ranged from -0.13 to +1.25 mL, -0.24 to +0.70 min and +0.02 to +0.27 respectively. Singh et al. (2008) additionally reported that the response of dimethyl formaldehyde soluble and insoluble proteins as a result of drought stress applied at 8 and 15 DAA was cultivar dependent.

Gu et al. (2015) was the first to analyze the response of wheat kernels to water deficit by integrated proteome analysis. They identified 285 newly expressed proteins in the endosperm and embryo combined. The embryonic proteins were related to the wheat's defense response,

whereas endosperm proteins related to protein synthesis; both exhibiting increases in energy metabolism related enzymes. HMW-GS *Glu-D*y12 increased in expression, which they related to an improvement in bread-making quality.

Giuliani et al. (2015) similarly used a proteomic approach in order to assess the changes in gluten protein composition as a result of drought applied at anthesis for two durum wheat cultivars. Proteins were fractionated based on solubility in SDS buffer solution, then quantified using 2-DE analysis on molecular weight-basis, and further digested to undergo liquid chromatography and mass spectrometry identification. The two cultivars differed greatly in their reaction to water stress. The changes in 2D gel electrophoresis percent volume as a result of water stress for variety Ciccio were -0.91, +1.16, -9.56 and +0.13 for HMW-GS, ω-gliadins and LMW-D subunits, the remaining LMW-GS and gliadins respectively. For variety Svevo, the 2-DE percent volume changes for the same protein fractions were +2.23, +0.56, -4.34 and +0.62 respectively. Based on these results, it would appear that variety Ciccio would experience a decrease in breadmaking quality, where the opposite would occur for variety Svevo.

Flagella et al. (2010) compared irrigated and rainfed environments for two durum wheat cultivars over three growing seasons. Under irrigated conditions, water was applied whenever the first 0.4m of top soil reached 50% of its available water. The irrigated and rainfed concentrations of SDS-soluble gliadins, 50% 1-propanol insoluble glutenins, HMW-GS, LMW-GS, UPP, and HMW/LMW- GS ratio can be found in Table 2.4. From these results, it appears that water deficit conditions lead to favourable alterations of gluten protein fractions, with significant increases in HMW-GS, total glutenins and HMW/LMW–GS ratio.

Table 2.3. Changes in protein fraction concentrations for two durum wheat cultivars grown under irrigated and rainfed environments over three growing seasons.

Protein fraction[a]	Irrigated	Rainfed	Significance
Gliadins (mAU mg^{-1} protein)	213,400	204,870	ns
Glutenins (mAU mg^{-1} protein)	71,222	76,519	$p<0.05$
HMW-GS (mAU mg^{-1} protein)	8,784	10,534	$p<0.01$
LMW-GS (mAU mg^{-1} protein)	62,438	65,985	ns
UPP (%)	43	46	ns
HMW/LMW	0.14	0.16	$p<0.01$

[a]HMW-GS= high molecular weight glutenin subunits, LMW-GS=low molecular weight glutenin subunits, UPP= unextractable polymeric protein
Data from (Flagella et al. 2010)

Contradictory to the preceding study, results presented by Jiang et al. (2009) and Jia et al. (2012) indicated that drought reduced the levels of HMW-GS. In the former study, drought (45-50% of soil water reserve) applied from anthesis to maturity resulted in lower GMP and HMW-GS (soluble in 62.5 mMol L^{-1} TrisHCl buffer solution) content of two winter wheat cultivars at maturity. Jia et al. (2012) applied three irrigation treatments to two winter wheat cultivars over two growing seasons. All treatments received 75 mm of irrigation at jointing, however the remaining treatments received two supplemental 75 mm applications before wintering and at anthesis, and the third treatment an additional 75 mm during grain filling. This resulted in a total of 75, 225 and 300 mm of supplemental water for the three treatments respectively. The 225 mm treatment resulted in significantly ($p<0.05$) higher wet gluten content, farinograph dough development and stability time, loaf volume, 60% ethanol insoluble HMW-GS content and HMW/LMW –GS ratio, compared to the other two treatments. Interestingly, both the 75 mm and 300 mm irrigation treatments had similar values for the aforementioned parameters. They reported that both drought and excess water alters breadmaking quality of winter wheat.

Jia et al. (2012) suggested that the effect of excess water availability is related to the aggregation of glutenin, due to its effect on both the starting point and duration of the desiccation period. There has been evidence that this period is closely related to the polymerization of glutenin (Carceller and Aussenac 1999; 2001). Alternatively, Ferrise et al. (2015) proposed that the insolubilization of polymeric glutenin in SDS may be directly related to the continuity of the grain dehydration state at maturity, rather than the rate of desiccation. Regardless, increased water availability after anthesis may alter the proportions of SDS-insoluble glutenins, as the grain dehydration process is modified. This hypothesis was reflected in the results of Jia et al. (2012) by a decrease in 60% ethanol insoluble glutenin particle size, polymerization index and HMW:LMW glutenin subunit ratio as a result of excess moisture conditions. Congruently, Robertson et al. (2009) reported nitrogen deficiency as a result of waterlogging. Similar results from waterlogging were observed by Jiang et al. (2009) in which HMW-GS and GMP to protein ratios and accumulation rates were decreased.

Bahrani et al. (2011) reported that post-anthesis drought stress reduced nitrogen accumulation. However, this was not reflected as a loss in grain protein content as the remobilization efficiency of nitrogen stores accumulated during vegetative stages of wheat growth increased (13%) with drought. N remobilization efficiency was calculated by the difference in N content from anthesis to maturity as a percentage of the total N content at anthesis. Consequently, this puts a lot of dependence on the N content stored prior to anthesis. Nitrogen fertilization appears to help alleviate some of these effects, also seen by (Kharel et al. 2011). The effects of water-nitrogen interactions on the size distribution of glutenin was analyzed by (Li et al. 2011), where there was a significant W x N interaction for proportion of

protein fractions and total protein content, the interaction had no significance on the glutenin subunits, which were determined largely by genotype.

Zhang et al. (2013) found that early drought applied at spikelet ignition, may be able to alleviate some of the negative effects on the HMW-GS quantities (separated by SDS-PAGE) seen as a result of heat stress. The combination of drought at spikelet initiation and heat stress at anthesis resulted in up-regulation of HMW-GS synthesis and increased GMP concentration, where both of these were reduced by heat stress applied singularly or doubled at spikelet initiation and anthesis. This phenomena was also observed by Flagella et al. (2010), in which temperature stress and water deficit that occurred simultaneously during the growing season resulted in an improvement of gluten strength and increased the molecular size distribution of glutenin.

The timing of water stress appears to alter how drought affects wheat breadmaking quality as well. Ozturk and Aydin (2004) found that drought applied late from milky ripe to maturity had more significant effects on quality than when applied earlier from second node to beginning of milky ripe (Feekes scale). Congruently, Gooding et al. (2003) reported water stress applied later resulted in increased SDS-sedimentation volume relative to stress applied prior to the end of grain filling. Alternatively, Flagella et al. (2010) reported that glutenin and protein content were highest in years with the most rain during grain filling. Likewise, Lopez-Bellido et al. (2001) found rainfall (up to maximum of 80mm) during grain accumulation increased protein content.

There are many contradictions in the literature in regards to the effects of water availability on the breadmaking quality of wheat. The problem lies within the variability of

experimental design between studies. Between locations, the water holding capacity of the varying soil types will not be the same. This may result in different levels of available moisture for the same quantity of incoming water, whether it be supplied by irrigation or rainfed environment. There also appears to be many interacting factors, including temperature, soil nitrogen supplies and timing of water related activities in addition to a genotype-dependent response to water availability. Nonetheless, there appears to be a strengthening effect as a result of decrease water availability, especially warm-dry environments in comparison to cool-wet ones, although this is not always the case. Despite the nature of the effects being unclear, it seems as though water stress applied later in the growing season will yield more significant results. Overall, much more research is needed to clarify the mechanisms and outcomes of water availability on the breadmaking quality of wheat.

2.3.3 Soil Conditions

2.3.3.1 Nitrogen

Biologically available nitrogen is generally present in soils as either ammonium or nitrate. The major form of nitrogen varies depending on the soil type; aerobic soils contain more nitrate whereas acidic soils, ammonium (Xu et al. 2012). Wheat roots uptake nitrogen in either form, by using specified transporters. After uptake, nitrate is ultimately converted to ammonium by the enzyme nitrate reductase, where ammonium is assimilated directly (Xu et al. 2012). Ultimately the ammonium from either source is converted to amino acids that are later incorporated into proteins. As a result, nitrogen is essential for the formation of gluten.

One of the indisputable effects of nitrogen fertilization on wheat is that it increases the total grain and flour protein content (Boehm et al. 2004; Bole and Dubetz 1986; DuPont et al. 2006a; Fuertes-Mendizabal et al. 2010; Luo et al. 2000; Pechanek et al. 1997; Tea et al. 2004; Triboi et al. 2000). In examining environmental effects on quantity and quality of gluten in two varieties, sites and growing seasons, Triboi et al. (2000) concluded that nitrogen supply was the most influential factor determining protein content and composition. More available nitrogen allows for increased assimilation into protein. In a multiple-linear regression analysis of England wheat grown from 1974-1993, Smith and Gooding (1996) suggested that a single treatment of 80 kg ha^{-1} applied N increases crude protein content by 1%, as determined by stepwise regression where nitrogen was the best one variable model. Lopez-Bellido et al. (2001) described nitrogen fertilizer as the best strategy available for farmers who want to improve breadmaking quality of wheat, which, in this study, was evaluated by alveograph.

The increased protein content results in increased monomeric gliadins, as the two are highly correlated (Jia et al. 1996; Labuschagne et al. 2006; Saint Pierre et al. 2008; Triboi et al. 2000). Likewise, Fuertes-Mendizabal et al. (2010) found the rate of 60%-ethanol soluble gliadin synthesis to be larger than that of insoluble glutenin, with gliadin content being proportional to the amount of available nitrogen ($r=0.93$, $p<0.05$). Similarly, Tea et al. (2004) discovered a significant increase in all gluten fractions as a result of supplemental nitrogen fertilization, however the quantities of soluble gliadins (0.3 M NaI with 7.5% 1-propanol) increased much more than insoluble glutenins compared the control; +10.9 mg g^{-1} in gliadins compared to +1.25 mg g^{-1} and +1.44 mg g^{-1} for LMW-GS and HMW-GS respectively. In this study, 60, 120 and 40 kg N ha^{-1} was applied at tilling, ear and flag leaf emergence respectively for all treatments

(including the control). For nitrogen treatment, an additional 20 kg N ha^{-1} at anthesis was applied.

It follows that alongside an augmented rate of gliadin synthesis there is a corresponding increase in the ratio of gliadin to glutenin (Doekes and Wennekes 1982; Fuertes-Mendizabal et al. 2010; Saint Pierre et al. 2008; Triboi et al. 2000) and decrease in the opposing glutenin:gliadin ratio (Jia et al. 1996), with grain N content accounting for 60.9% of the variability of this ratio (Fuertes-Mendizabal et al. 2010). Doekes and Wennekes (1982) quantified gliadins (70% ethanol soluble) and glutenins (insoluble) by electrophoresis. Nitrogen was applied as 30 kg of calcium ammonium nitrate ha^{-1} at tillering, followed by 0, 30 or 60 kg ha^{-1} at both stem extension and beginning of anthesis. The gliadin to glutenin ratio increased by 0.84 on average, for the 5 varieties analyzed. Likewise, Fuertes-Mendizabal et al. (2010) noted an increase in the gliadin (60% ethanol soluble) to insoluble glutenin ratio of +0.521 and +0.755 for N fertilizations levels of 100 and 180 kg ha^{-1} respectively, when compared to the control (0 kg ha^{-1}). In this study nitrogen treatments were applied as ammonium nitrate split into either 1-3 single or split applications at tillering, stem elongation or flagleaf emergence.

Both Saint Pierre et al. (2008) and Jia et al. (1996) quantified glutenins and gliadins by SE-HPLC as SDS-unextractable, and SDS-extractable proteins respectively. In the former study, all plots received 170 kg N ha^{-1} at stem elongation, and only half received an additional 170 kg N ha^{-1} at early milk stage near the end of stem elongation. Samples that received a single N treatment had an average gliadin:glutenin ratio of 1.29 that increased to 1.34 for samples that receiving a total of 340 kg N ha^{-1}; this change was statistically significant ($p < 0.05$). Jia et al.

(2012) reported a significant negative correlation (r= -0.53, $p<0.001$) for the inverse glutenin to gliadin ratio when nitrogen fertilization was applied.

All of the aforementioned studies noted an increase in the gliadin/glutenin ratio as a result of nitrogen fertilization. However, there are instances in which this ratio was unaffected. Triboi et al. (2000) did not report any significant change in the gliadin to glutenin ratio as nitrogen fertilization levels increased from 0-200 kg ha^{-1}. Alternatively, Pechanek et al. (1997) reported cultivar inconsistencies in the response of gliadin (60% ethanol soluble) to glutenin (60% ethanol insoluble) ratio quantified by RP-HPLC and SDS-PAGE. In this study, the three varieties analyzed displayed both an increase and decrease in this ratio. For example, the gliadin to glutenin ratio for variety Renan decreased from 2.90 to 2.50 and variety Capo increased from 2.45 to 2.80 for nitrogen applications of 0 kg N ha^{-1} and 180 kg N ha^{-1} respectively. The 180N treatment was split into three applications of 60 kg N ha^{-1} of ammonium nitrate shortly before tillering, shooting and heading. Therefore, the reason for the differences in the reaction of the gliadin/glutenin may be due to differing genotype responses to nitrogen.

Where it is generally acknowledged that nitrogen fertilizer application increases total gliadins, the effect of supplemental nitrogen on the individual gliadin fractions remains unclear. Both Triboi et al. (2000) and Pechanek et al. (1997) reported gliadin fractions increasing at different rates between samples as total gliadins increased. In the former study, individual RP-HPLC gliadin peak areas increased alongside total gliadins (r=0.8-0.9), however, when the peaks areas were taken as a percent of total gliadin area, individual peaks both increased and decreased. Thus nitrogen fertilization altered the proportions of gliadin subunits, as similarly observed by Branlard and Triboi (1983) alongside an increase in grain protein content. Pechanek et al. (1997)

similarly reported high positive correlations (r=0.83-0.93, $p<0.001$) between all gliadin subunits and total protein content, with the exception of ω-5 gliadin.

Nitrogen fertilization has conflicting effects on the quantities of glutenin. There have been reports where nitrogen has no effect (Doekes and Wennekes 1982; Luo et al. 2000; Scheromm et al. 1992), increases total glutenin (Eggert et al. 2010; Fuertes-Mendizabal et al. 2010; Park et al. 2014; Triboi et al. 2000), only LMW-GS (Peltonen and Virtanen 1994) or solely HMW-GS (Labuschagne et al. 2006). Where no effect of nitrogen fertilization was observed (Doekes and Wennekes 1982; Luo et al. 2000; Scheromm et al. 1992), the authors of these studies speculated that glutenin quantities of the varieties analyzed were cultivar dependent, rather than altered by environmental influences. Scheromm et al. (1992) and Jia et al. (1996) both reported HMW-GS composition as unaltered by nitrogen fertilization levels, however the quantities of their aggregates changed. From these results, it was suggested that nitrogen influences the aggregation and polymorphism of the HMW-GS, although this was thought to be dependent on both cultivar and growing environment. The HMW- to LMW-GS ratio has been reported to increase (Park et al. 2014), decrease (Pechanek et al. 1997) or remain the same (Triboi et al. 2000) as a result of nitrogen fertilization. Nitrogen does not appear to affect the content of albumins and globulins (Doekes and Wennekes 1982; Pechanek et al. 1997; Tea et al. 2004)

In addition to the effect of nitrogen fertilization on protein fraction concentrations, there appears to be a significant effect on loaf volume (Boehm et al. 2004; Doekes and Wennekes 1982; Massoudifar et al. 2014; Peltonen and Virtanen 1994) as well as an influence on dough rheological properties. In the same study previously described, Fuertes-Mendizabal et al. (2010)

reported a significant improvement in dough extensibility, tenacity and strength that they attributed to the small increase in HMW-GS, allowing for a greater degree of polymerization. However, nitrogen levels of 220 μg N grain^{-1} were required to sufficiently increase glutenin levels so that they improve bread-making quality.

Boehm et al. (2004) assessed the influence of N fertilizer levels on fresh and frozen dough qualities for four cultivars over two years. Compared to the control, the low level application of nitrogen (67.2 kg N ha^{-1}) was sufficient in significantly improving fresh and frozen dough quality, as assessed by farinograph water absorption, arrival and peak times, although extensograph curve area results were not significant.

Luo et al. (2000) found a significant increases in all tested quality parameters (hardness, SDS sedimentation, mixograph mid-line to peak) as a result of 200 kg ha^{-1} nitrogen applied at either booting or flowering stages for fourteen wheat cultivars. Improved breadmaking quality was also reported by Peltonen and Virtanen (1994), where 110 kg ha^{-1} of four different N fertilizers were applied at sowing to three breadmaking wheat cultivars. The improvement in breadmaking ability was seen in loaf volume and farinograph mixing time and stability. The results were attributed to the large increase (+3.78 to 4.44 g 100g flour^{-1}) in LMW-GS that resulted from N fertilization, which was much larger than increases seen in HMW-GS, α-, β- and ω-gliadins. Additionally, LMW-GS were most significantly correlated to the three quality parameters over all other gluten fractions. The small improvements in quality seen as a result of additional nitrogen are suggested to be due to increases in the ratio of HMW:LMW glutenin subunits; when this is not the case, as a result of increased flour protein content (Scheromm et al. 1992).

2.3.3.1.1 Factors Affecting the Influence of Soil Nitrogen on Wheat Quality

The effect of nitrogen on the end-use quality of wheat appears to be variable depending on growth conditions and genetics of the wheat. Cultivar differences in response to nitrogen have been reported by (Boehm et al. 2004; Labuschagne et al. 2006; Peltonen and Virtanen 1994; Scheromm et al. 1992; Triboi et al. 2000). Essentially, the extent to which nitrogen fertility may alter gluten quality depends on the variety of wheat. Duric et al. (2010) applied 0 to 180 kg ha^{-1} N in increments of 30, with one third of the total applied nitrogen incorporated before sowing and the remainder top dressed, to eight wheat varieties. Four of the varieties contained poor breadmaking subunit composition *Glu-D1* 2+12, and the remainder containing good breadmaking subunit pair 5 +10. The higher levels of nitrogen fertilization resulted in significant ($p < 0.05$) increases in the wet gluten content of all cultivars, with HMW-GS 2+12 cultivars and 5 +10 ranging from 14.3-21% and 18.7-22% at 0 kg N ha^{-1} respectively, increasing up to 23.3-30.2% and 28.7-32% with nitrogen application. A sensory evaluation using wet dough stretched over a ruler was used to measure extensibility and adhesiveness. The response of these parameters to nitrogen treatment was very cultivar specific. However, a significant correlation (r=0.82) was found between nitrogen and wet gluten strength only between 5 +10 wheat cultivars. It was suggested that these varieties have greater efficiencies in assimilating nitrogen. Duric et al. (2010) reported that poor bread-making quality cultivars containing *Glu-D1* pair 2+12 did not have much of a response to N rates, whereas the cultivar containing the good bread-making pair 5+10 actually decreased in quality and increased in extensibility as nitrogen was

added. Saint Pierre et al. (2008) reported that wheat of comparable bread-making quality responded similarly to nitrogen treatment.

One of the biggest environmental influences on nitrogen fertility effectiveness is temperature. Malik et al. (2013) were able to explain 36% of the variation in SDS unextractable polymeric protein by the amount of N applied at spike formation and anthesis. However, this was only seen when temperatures were low (17/14 ^{o}C), compared to moderate day/night temperatures of 25/21 ^{o}C. 20 mg N plant $^{-1}$ at anthesis was able to explain 12% (low) to 36% (moderate) of the variation of soluble proteins. DuPont et al. (2006a) found that the addition of NPK fertilizer from anthesis to maturity increased protein synthesis similarly to when wheat was subject to heat stress/high temperatures during the growing season. This augmented protein synthesis only occurred with moderate day/night temperatures of 24 /17 ^{o}C. At high temperatures of 37/28 ^{o}C there was no effect of NPK.

Both moderate temperatures and nitrogen application increase the protein content of wheat (refer to temperature and nitrogen sections). There appears to be an interaction between the two parameters in regards to their influence on wheat end-use quality. Lower temperatures observed by Kharel et al. (2011) between years (18.3 ^{o}C average vs. 21.3 ^{o}C from flowering to grain filling) decreased the mineralization of nitrogen in the soil, making it less accessible to the crop. This may explain why nitrogen fertilization at lower temperatures produces a more pronounced effect on wheat protein and quality aspects. Alternatively, wheat may only be able to synthesize protein up to a maximum value. Past this point, the higher protein content that arises with hot conditions may simply be due to decreased starch synthesis and lower kernel weight that increases the final protein percentage of the grain.

Supplementary to temperature, Massoudifar et al. (2014) found an interaction between water availability and N levels on the quality traits of bread in a two-year study conducted in Quchan, Iran. The highest protein and SDS sedimentation values occurred when nitrogen was applied at 210 kg N ha^{-1} and irrigated at 130 mm. Irrigation was applied as-needed throughout the entire growing season as assessed by an evaporation pan. In addition, they found that nitrogen fertilization could potentially improve quality when drought stress occurs. Kharel et al. (2011) also found there to be a beneficial relationship between nitrogen and water levels. When soil mineralization levels of N were 99 kg N ha^{-1}, the lowest protein content and farinograph dough quality parameters peak, arrival and departure time, stability and time to breakdown were seen in adequate water conditions; drought actually improved the quality in comparison.

Timing of nitrogen application appears to have a small effect on nitrogen wheat-quality implications. Labuschagne et al. (2006) reported no increase in protein fractions when 30 kg ha^{-1} nitrogen was applied at flag leaf compared to when 80 and/or 60-120 kg N ha^{-1} were applied at and six weeks after planting respectively. Contradictory to this study, DuPont et al. (2006a) observed that nitrogen application at anthesis promoted the accumulation rate of sulfur poor proteins, thought to be caused by an increased regulation of glutamine and proline as a result of nitrogen application. Luo et al. (2000) found that application timing wasn't necessarily significant, but applying nitrogen at flowering compared to booting produced more pronounced effects on quality parameters.

Dai et al. (2014) reported that as plant density increased from 135 to 405 plants m^{-2}, the root density of wheat as well as nitrogen uptake increases significantly; this occurs at every depth of soil. Increased plant density may be advantageous when nitrogen leaches deep into the

soil, however the root length density decreases the further the soil depth. Likewise Zhang et al. (2016b) reported an increase in protein content and fractions, glutenin polymerization degree, dough development time and stability and loaf volume when plant density increased from 120 to 189 to 240 plants m^{-2} at nitrogen levels of 240 kg N ha^{-1}. Consequently, when no nitrogen fertilization was applied, all of these parameters decreased, negatively affecting rheological and bread-making qualities.

2.3.3.1.2 N:S Ratio

Although sulfur does not have an influence on the accumulation of N, nitrogen levels impact sulfur accumulation (Randall et al. 1981; Tea et al. 2004). Using istopes S^{34} and N^{15}, Tea et al. (2004) found that where N equally accumulates into monomers, LMW-GS and HMW-GS, S preferentially accumulated in LMW-GS. However, the combination of N and S resulted in a better incorporation of storage protein. The combined treatment significantly increased %UPP, which was not observed in each individual treatment, suggesting an interaction between the fertilizers. Flaete et al. (2005) additionally observed increases in high molecular weight polymeric proteins when S fertilization (10-46 kg ha^{-1}) was applied and levels of N were high (300 kg ha^{-1}).

Grove et al. (2009) stated that despite an increase in total protein caused by the addition of N (200 vs 300 kg ha^{-1} split application), the rheological properties of the dough declined due to a deficiency of S. At the 300 kg N ha^{-1} level, when no sulfur was applied, the N:S ratio, SDS sedimentation volume and extensograph Rmax and extensibility values were 20.7, 60 mL, 14.25 g and 60.51 mm respectively. However, at the same nitrogen level, when sulfur was applied to achieve an N:S ratio of 16.6, the quality parameters increased to 72 mL, 14.84 g and 79.11 mm,

despite a comparable protein content (15.1 vs. 15.3%). Similarly, Luo et al. (2000) found that the best improvements in dough properties were seen when N and S were applied together. Tea et al. (2005) reported the foliar S application resulted in weakened dough strength and consistency, where both parameters were improved as a result of N application. Wooding et al. (2000) described an increase in work input driven by nitrogen fertilization that was undesirable for commercial scale baking. In this study, work input was analyzed by both mixograph and industrial scale mixers. For the industry scale mixing, the high work input achieved by high (225 kg ha^{-1}) nitrogen fertilization levels resulted in too much heat being produced, negatively affecting the quality of the dough. However, the dough could be softened to optimal levels for mixing by the addition of S fertilization, with N:S ~12.5 desirable to keep work input to preferred industry levels. However, this level is fairly low for Canadian soil. More typical, Flaete et al. (2005) proposed an N:S ratio of 15, similar to the suggested S:N level of <0.060 of Uthayakumaran et al. (2007), in order to prevent quality defects resulting from insufficient amounts of sulfur.

2.3.3.2 Sulfur

Sulfur is important for end-use quality of wheat as it is incorporated into sulfur containing amino acids: methionine, cysteine. This is seen by a 27% and 14% increase in cysteine and methionine when sulfur fertilization is applied, in comparison to solely nitrogen application (Habtegebrial and Singh 2009). Cysteine is particularly important as it is responsible for the formation of disulfide bridges that allow for the accumulation of strength-conferring glutenin macropolymers. Sulfur is also present in wheat as the thiol glutathione (GSH), which

can occur as free-reduced, oxidized or protein-bound forms and is present at levels of 144-256 nmol g^{-1} in freshly milled wheat flours (Chen and Schofield 1995a; Schofield and Chen 1995).

The main effect of sulfur fertility is reflected in the quantities of sulfur-rich and sulfur-poor proteins. In sulfur deficient soils there is an increase in the sulfur-poor ω-gliadins and HMW-GS and a decrease in sulfur rich LMW-GS and γ-gliadins with slight decreases in α-gliadins. Wieser et al. (2004) analyzed the effect of sulfur fertilization on protein subunit contents of wholemeal flour. The percent changes in concentration for gluten fractions can be found in Fig 2.1.

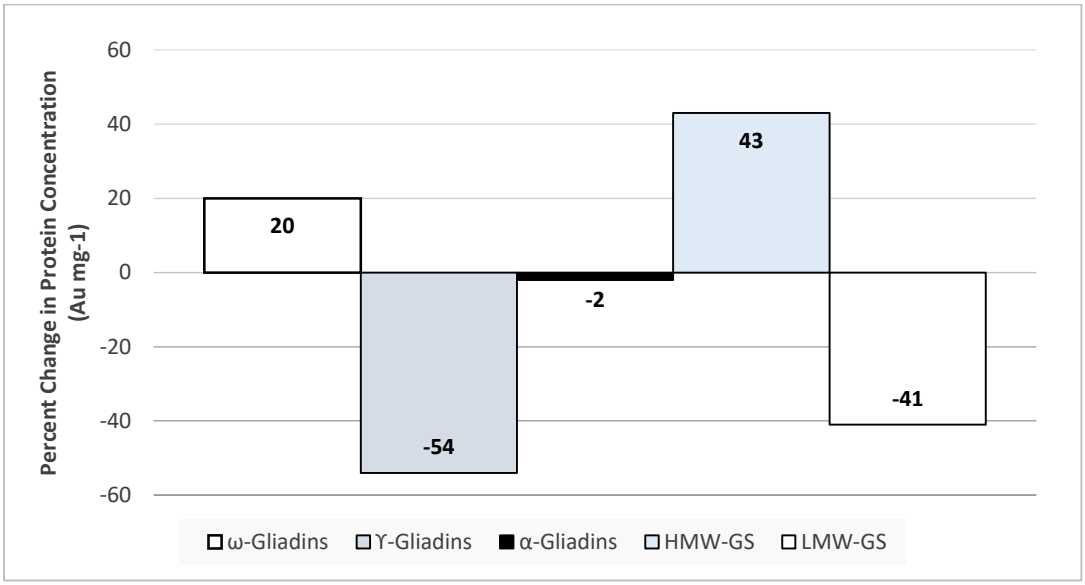

Figure 2.1 Percent change in protein subunit concentrations in sulfur deficient soils compared to adequate soil concentrations (23.9 mg kg^{-1} S vs. 0 mg kg^{-1} S), data from Wieser et al (2004).

There appears to be a more substantial increase in ω-gliadins in comparison to HMW-GS under sulfur deficient conditions (Wrigley et al 1984), presumably attributed to HMW-GS containing a small amount of cysteine residues, whereas ω-gliadins lack any (Table 2.1). The opposite changes in protein quantities occur when sulfur is readily available and applied to soils

(Grove et al. 2009; Tea et al. 2004; Zorb et al. 2009). The addition of sulfur does not appear to alter total protein content (Wieser et al. 2004; Zorb et al. 2009) as the loss of one fraction is compensated by the gain of the other. This is not the case without adequate S, where the "compensation gain" or assimilation of sulfur-rich proteins cannot occur if sulfur is limiting, as it would prevent the formation of cysteine residues (Zorb et al. 2009).

Sulfur deficiency can occur as the result of hot conditions during grain filling, too much nitrogen present, or insufficient soil supply (DuPont et al. 2006a). A deficiency in S results in the alteration of the HMW:LMW glutenin subunit composition, generally increasing this ratio (Macritchie and Gupta 1993; Naeem et al. 2012; Wieser et al. 2004) leading to stronger doughs (DuPont et al. 2006a; Macritchie and Gupta 1993) or in some instances, when too strong, presumably bucky (too elastic) properties (Naeem et al. 2012). However, sulfur is still required to attain optimal bread quality. Some reports have indicated a positive correlation of grain sulfur content to loaf volume (Zhao et al. 1999b; Zorb et al. 2009). A review by Zhao et al. (1999a) suggested that sulfur content may be a better predictor of loaf volume than grain nitrogen concentration. Flaete et al. (2005) and Wooding et al. (2000) reported increased grain S content proportional to the amount of applied N, while S application was kept constant, however applied S had no effect on grain N content. However, in this study, grain S content was significantly correlated ($r=0.97$, $p<0.001$) to SDS-sedimentation volume, whereas grain nitrogen was not. Too little sulfur could also be detrimental to dough quality, especially when nitrogen is readily available for protein assimilation. When the S:N ration drops below 0.006, a sharp increase in ω-gliadins is reported (Uthayakumaran et al. 2007), having potential implications on gluten strength. This may arise from insufficient cysteine residue available for formation of a large gluten polymer, or simply an unfavourable alteration of the gliadin to glutenin ratio. To avoid

any defects that may arise with sulfur deficiency, grain should contain at least 1.00 mg S g^{-1} (Uthayakumaran et al. 2007).

Conversely, the addition of S has been reported to weaken dough and promote elasticity (De Ruiter and Martin 2001; Flaete et al. 2005; Tea et al. 2005; Zhao et al. 1999b). Interestingly, Tea et al. (2004) reported similar levels of %UPP in control samples and those fertilized with sulfur, however the sulfur samples had weaker dough characteristics. The weakening effect of sulfur addition has been corresponded to the change in protein composition, as well as an increase of thiols within the dough that prevent protein disulfide-sulfhydryl exchange during mixing. It follows that there is an intermediate level of sulfur that will confer optimum gluten strength, that comes back to maintaining a proper S:N ratio

2.3.3.2.1 Flour Thiols

Levels of thiols within flour are thought to influence the breadmaking quality of wheat due to interactions with flour constituents that occur during mixing. Zorb et al. (2009) reported that increased sulfur fertilization raised levels of flour glutathione (GSH). Likewise, Reinbold et al. (2008) found the level of GSH in flour to be proportional to the supply of S via fertilization. GSH presumably weakens dough by interfering with the association of disulfide bridges that stabilize the protein network. Frater and Hird (1963) provided evidence for the interaction of GSH with gluten by measuring the total amount of thiols present in the dough. When mixed into the dough, GSH disappears followed by a corresponding weakening of dough, however the total thiol content of the system did not change. This occurred similarly when the oxidized form of glutathione was added (GSSG), suggesting that GSSG becomes reduced within the matrix to

GSH, allowing it to interrupt disulfide network and bonding to protein (PSSG). The reduction of GSSG to GSH is catalyzed by glutathione reductase, intrinsically present within wheat.

There is evidence that intrinsic glutathione levels within flour decreases over time. Chen and Schofield (1996) analyzed the content of GSH, GSSG and PSSG in wheat flour over time, stored at 20 C. For variety Mercia, the freshly milled and 10-day storage levels can be found in Table 2.5. Ten days of storage resulted in substantial decreases in all forms of glutathione, however these levels remained stable until 40 days of storage. The loss in not only GSH, but also GSSG and PSSG suggest that no GSH was lost to oxidation processes, and that the intrinsic level within the flour actually decreases over time. Similarly, Ewart (1988) noted significant decreases in total and accessible thiols over the course in logarithmic days after milling. The magnitude of the decrease can be seen in Figures 1-3 of the article (Ewart, 1998). Additional papers where decreases in thiol concentrations as a result of storage have been reported (Hahn and Grosch 1998; Sarwin et al. 1992; Schofield and Chen 1995; Tsen and Dempster 1963).

Table 2.5. GSH, GSSG and PSSG[a] content (nmol g^{-1}) "as-is" in cv. Mercia flour directly and 10 days[b] after milling.

	GSH	GSSG	PSSG
Freshly milled	149	61	87
10 days after milling	89	17	70
% Decrease	40%	72%	20%

[a]GSH=reduced glutathione, GSSG=oxidized glutathione, PSSG=protein bound glutathione
[b]Samples were stored at 20 C
Data from (Chen and Schofield 1996)

By following levels of glutathione using reversed phase HPLC, Rhazi et al. (2003) reported that the ratio of GSSG to GSH increased at the same point in the grain desiccation period where unextractable polymeric protein begin to form. This resulted in increased protein bound glutathione (PSSG). It follows that at maturity, 85% of PSSG was represented as polymeric protein bound glutathione. The formation of glutathione bound to polymeric protein

decreased the molecular weight distribution of glutenin (in comparison to unbound polymeric protein), rendering it soluble. These results suggest that PSSG regulates the polymerization degree of glutenin, also reported by (Li et al. 2004b). Similarly, Tea et al. (2005) found that decreased levels of PSSG raised the molecular weights of polymeric proteins and increased the proportion of the unextractable polymeric protein fraction. Chen and Schofield (1995b) observed higher levels of PSSG in the glutenin fraction (897 nmol/g of protein) compared to gliadins (76 nmol/g protein). Li et al. (2004b) also reported PSSG bound to both monomeric and polymeric proteins, and that levels of PSSG were higher and significantly different in poor bread-making varieties compared to good ones.

Tea et al. (2005) applied foliar S fertilization at levels of 5 or 10 kg h^{-1} alone or in combination with 30 kg N h^{-1}. S application increased the levels of GSSG, GSH, their ratio and PSSG. At their highest concentrations during the growing season (during the 10 kg S ha^{-1} treatment), the levels of GSH and GSSG were increased by 0.8 and 2.8 nmol grain $^{-1}$ as well as 4.6 and 5.2 nmol grain $^{-1}$ for varieties Trémie and Soissons respectively, relative to the control. The GSSG to GSH ratio increased 23-25% with the same level of sulfur application. Alternatively, nitrogen application decreased the level of these parameters, as shown in Figure 3 of the article. The significant correlations (p<0.05) between glutathione and dough mixograph parameters varied between cultivars, however, they were always negative for midline peak time, midline peak value, and midline time integral 12 minutes after mixing, ranging from-0.67 to -0.73, -0.67 to -0.77 and -0.69 to -0.79 between genotypes respectively. In contrast, a positive correlation was seen for mixograph weakening slope, with correlations of 0.69 and 0.74 for the two genotypes.

Conflicting results regarding glutathione's implications on dough strength were reported by Li et al. (2004a) who did not find any clear correlations that indicated free or bound thiols changed dough rheological or baking properties. However, they suggested that the endogenous levels of glutathione were too low to significantly affect gluten quality, which may explain the conflict to other studies.

Every et al. (2006) reported that GSH/GSSG was negatively correlated to loaf volume and crumb texture properties. Correlations of GSH and GSSG to loaf volume and texture varied from -0.66 to -0.92 and -0.58 to -0.88 respectively without ascorbic acid, however ranged from -0.51 to -0.93 and -0.27 to -0.89 when ascorbic acid was added. The addition of ascorbic acid appears to improve these effects, however this was not consistent for all four genotypes analyzed, as can be seen by the range of correlations. The conflicting results in regards to the improver action of ascorbic acid can be explained by genotype variation in response to the additive. Every et al. (2008) reported that good breadmaking cultivars with allelic composition *GluD1* 5+10 and high gluten strength (as determined by mixograph work input) responded much less to the addition of ascorbic acid compared to cultivars with low gluten strength and allelic composition *GluD1* 2+12. Mechanisms of ascorbic acid's improver effect on GSH have been reviewed by Grosch and Wieser (1999).

Despite unclear negative correlations between flour thiols and dough strength, the addition of ascorbic acid, or any oxidative improver, may alleviate any negative effects imparted by glutathione. Accordingly, it is common practice for bread-makers to use oxidants, due to their improver effect on loaf volume (Yamada and Preston 1992), which may alleviate any negative effects should they occur. Additionally, thiol concentrations within flour appear to decrease over

time, therefore the effects dough weakening effects from a commercial breadmaking perspective may be irrelevant, however more research is needed in order to confirm this.

2.3.3.3 Other Minerals

Harper and Paulsen (1969) related soil deficiencies in various combinations of macronutrients N, P, K, Ca, Mg, S and Fe to decreased activities of enzymes involved in the assimilation of nitrogen and amino acid synthesis in addition to reduced nitrate content. From these results, it appears there is potential for the concentration in wheat kernels or flour of certain minerals to have implications on gluten quality. As nitrogen and sulfur have already been discussed, the influence of other minerals will be examined, although very few studies have been reported.

The availability of minerals will differ due to cropping conditions, such as soil type. Wilkes et al. (2010) grew three related wheat genotypes in either grey vertosol or red kandosol soil in Narrabri, Australia, with complete or zero tillage. Before sowing, the soil concentrations of N P and K were measured, and fertilized so that equal mineral concentrations were found in both treatments. Sites were close in proximity to eliminate variability due to weather conditions. They found soil type was the most influential factor affecting protein content, with grey vertosol soil yielding higher percentages of total (+5.3), TRIS-buffer soluble (+0.3) and insoluble (+0.8-1.3) protein. Based on these results, it appears that soil type alters the bioavailability of nutrients.

Fertilization with phosphorus up to levels of 50 kg ha^{-1} raised the yield of wheat from 1.3 (Sweeney et al. 2000) to 5.0 (Zhang et al. 2017a) Mg ha^{-1}. Brennan and Bolland (2009) reported that when P is supplied, it raised the amount of N required to attain 90% maximum yield,

however N concentrations had no effect on P requirements. As would be expected from a yield increase, Zhang et al. (2017a) found fertilization of 50 kg P ha^{-1} or more resulted in significant reductions in grain protein content. Brennan and Bolland (2009) did not find any interaction between P and protein content, however the maximum level of P used in the study was 40 kg P ha^{-1}.

Ni et al. (2014) applied phosphorus levels of 0, 40, 100 and 160 kg P ha^{-1} to two winter wheat varieties in order to evaluate the effects of phosphorus on HMW-GS. The wheat was grown over the 2008/09 and 2009/10 growing seasons, and nitrogen levels of either 112.5 or 225 kg N ha^{-1} were applied. Phosphorus application resulted in favoured synthesis of larger glutenin subunits, forming macropolymer particles of >100 μm, although the effects were diminished at the increased nitrogen level (225 kg N ha^{-1}). Applied phosphorus resulted in percent increases in SDS-PAGE determined total HMW-GS ranging from 11.1-55.4% between different genotype and nitrogen regimes, relative to the control. They concluded that there was a significant interaction between N and P on the regulation of HMW-GS synthesis.

Potassium has been shown to increase wheat yield under limiting conditions (Chapman and Mason 1969; Sweeney et al. 2000). However, Sweeney et al. (2000) suggest that the response of yield to potassium fertilizer may be related to chloride, which is a component of KCl fertilizer. Separately, Lv et al. (2017) found that potassium foliage application significantly decreased the grain filling period of wheat cultivars Zhoumai 22 and Xinong 538 grown in waterproof mobile sheds. Due to this effect, it is plausible that the end-use quality may be affected by potassium. This has only been examined in one study to date. Lu et al. (2014) studied the effects of 120 kg K ha^{-1} either in full with basal application, or split equally as basal followed

by topdressing. They found that the split potassium treatment improved wheat quality by increasing the glutenin to gliadin ratio as well as molecular size distribution of the glutenin macropolymer in comparison to full basal application. There were no differences in total protein between K treatments. As a result of the compositional change in gluten, dough mixing properties and loaf volume were improved.

One other study was reported, relating the effects of NPK mineral fertilization supplemented with foliar Cu, Mn and Zn application indirectly to the breadmaking quality wheat (Stepien and Wojtkowiak 2016). Total gliadin concentrations were increased as a result of Cu supplementation, increasing from 30,770 to 32 393 mAu s^{-1} relative to the control. The most noticeable changes were seen in ω- and α/β- gliadins, in which control concentrations (2,101 and 16,601 mAu s^{-1}) were notably increased as a result of Cu supplementation (2,493 and 17,421 mAu s^{-1}). Conversely, Zn supplementation reduced the concentration of gliadin fractions (-2,249 mAu s^{-1}) and increased HMW-GS (+3,401 mAu s^{-1}) and LMW-GS(+1,561 mAu s^{-1}), favouring the synthesis of high molecular weight glutenin subunits. As a result, the gliadin to glutenin ratio was decreased from 0.96 to 0.77. Finally, Mn fertilization increased all gluten fractions, having the biggest impact on ω-gliadins (+418 mAu s^{-1}) and HMW-GS (+1,658 mAu s^{-1}). Gaj et al. (2013) utilized linear and stepwise regression to related wheat mineral nutrient content during stem elongation to protein and gluten content. The former regression analysis portrayed that phosphorus, nitrogen, magnesium and iron were positively related to protein and gluten content, whereas zinc was the only mineral found to negatively impact the proteins. The coefficient of determination for protein and gluten content equations were 0.46 and 0.48 respectively (p<0.000). With stepwise regression, magnesium content was found to be the most significant

one variable model, with positively relationship to both protein (R2=0.15, p<0.000) and gluten (R2=0.15, p<0.000) content.

2.3.3.4 Salinity

Soil salinity is an agricultural problem that arises in hot and dry regions, and areas under irrigation (Shrivastava and Kumar 2015). All stages of plant development are affected by salinity as it imposes osmotic and oxidative stresses, nutrient deficiency, ion toxicity and reduces plant water uptake ultimately killing cells (Shrivastava and Kumar 2015). The oxidative stress imposed by high soil salinity levels is due to the increase in production of reactive oxygen species (Esfandiari and Gohari 2017). To analyze the effects of soil salinity on gluten proteins, Zhang et al. (2016a) applied six salt treatments to potted plants beginning 10 DAA until maturity. The treatments ranged from 0 to 0.75% NaCL, in increments of 0.15%, as a w/w percentage of dry soil weight. They reported that increasing salt stress decreased grain yield, while increasing the HMW-GS concentration (determined by SDS-PAGE) and GMP larger than 10 μm; more substantially when concentrations were higher than 0.45%. Compared to the control, 0.75% salinity decreased the volume distribution percentage of GMP particles less than 10 μm by 26.53% where the total GMP content increased by 36.14% in the same salt treatment. Similarly, as a result of salt and drought stress, Houshmand et al. (2014) observed significant increases in grain protein content (+23.6 g kg^{-1}), SDS sedimentation volume (+17.3 mL) as well as wet (+11 g kg^{-1}) and dry (13 g kg^{-1}) gluten content, with salinity imposing greater effects than drought.

2.3.4 Atmospheric Carbon Dioxide

Atmospheric levels of carbon dioxide are on the rise as a result of climate change. 2015 CO_2 emissions across the Canadian prairies, in the megatonnes of carbon dioxide equivalent for greenhouse gas emissions, were 274.1, 75.0 and 20.8 for Alberta, Saskatchewan and Manitoba respectively (Environment and Climate Change Canada 2017). High atmospheric CO_2 levels cause wheat yields to rise as a result of increased photosynthesis and wheat productivity (Blumenthal et al. 1996; Högy and Fangmeier 2008; Kimball et al. 2001; Pleijel et al. 1999). Due to a dilution effect, protein and nitrogen levels decrease at the expense of yield (Blumenthal et al. 1996; Fernando et al. 2014; Kimball et al. 2001; Pleijel et al. 1999; Wieser et al. 2008).

Reductions in wheat processing quality may be predicted solely due to the loss in protein content. At atmospheric CO_2 levels of 550 μL L^{-1} (Fernando et al. 2014; Kimball et al. 2001; Panozzo et al. 2014; Wieser et al. 2008) and 700 μL L^{-1} (Blumenthal et al. 1996) there is deterioration in dough rheological properties and loss of loaf volume. Fernando et al. (2014) reported decreases in mixograph peak time (- 0.2 min), peak height (- 0.3 AU) and loaf volume (- 4 cm^3) at CO_2 levels of 550 μL L^{-1} for two cultivars compared to ambient CO_2 levels, where ANOVA listed CO_2 as a significant influential factor for peak height and bread volume (p<0.01) and for peak time (p<0.05). At the same CO_2 concentration, Panozzo et al. (2014) observed significant (p<0.01) interactions between CO_2 and farinograph dough development time, extensigraph extensibility, loaf volume and amylograph peak viscosity. For these four quality parameters, the decrease due to elevated (550 μL L^{-1}) CO_2 levels for the 3-years study ranged from 0.3-0.9 min, 0.4 -1 cm, 45-79 cm^3 and 63-121 BU respectively, for three wheat breadmaking cultivars.

In a field study analyzing two wheat cultivars, Blumenthal et al. (1996) applied ambient (350 μL L^{-1}) and elevated (700 μL L^{-1}) CO_2 levels during the growing seasons. Elevated CO_2 resulted in significant decreases in mixograph peak resistance (- 56), farinograph dough development time (- 4.2 min), extensibility (- 73 mL). However, no significant changes in SDS-extractable and –unextractable glutenin proteins were seen. Alternatively, (Wieser et al. 2008) reported decreases in all protein fractions, reflected in Table 2.6. The glutenin subunits were more largely affected by elevated levels of CO_2, with HMW-GS having a larger decrease than LMW-GS. In terms of the gliadins, the magnitude of the reduction was in ω-> α -> γ –gliadins. Hogy et al. (2013) observed a similar decrease in ω-, α - and γ -gliadins however no effect was reported on the concentrations albumins/globulins, total glutenins, or glutenin subunits.

Table 2.6. Relative proportion (HPLC Absorbance Units mg flour^{-1}) of wheat protein fractions as affected by elevated CO_2 levels with 168 kg ha^{-1} applied nitrogen.

	Gliadin[a]	ω-gli	α -gli	γ –gli	Glutenin[b]	HMW	LMW	SDS	GMP
Ambiant CO_2(350 μL L^{-1})	784	43	331	369	425	115	296	1422	204
Elevated CO_2(550 μL L^{-1})	628	28	261	305	360	85	260	1181	166

[a]Gliadins determined as fraction soluble in 60% ethanol
[b]Glutenins determined as insoluble in 60%ethonal, followed by extraction in 50% 1-propanol with reducing and alkylating agent.
Gli=gliadin, HMW= High molecular weight glutenin subunits, LMW = Low molecular weight glutenin subunits, SDS= SDS soluble protein, GMP= glutenin macropolymer (i.e. SDS insoluble protein)
Data from (Wieser et al. 2008)

Nitrogen fertilization has been hypothesized as a potential mechanism to remedy lower protein content arising as a result of high CO_2 levels. Both Wieser et al. (2008) and Kimball et al. (2001) studied the effects of increased CO_2 levels in the presence of N with conflicting results. The detrimental effects of CO_2 as noted by Wieser et al. (2008) were exacerbated as the N fertilization increased from 84 to 168 kg N ha^{-1} . At the high nitrogen application level, HMW-GS and ω- and α-gliadins were lowered by more than 20% (Table 2 in article).

Where Wieser et al. (2008) reported that negative effects of high CO_2 levels were enhanced by an increased level of nitrogen application, Kimball et al. (2001) found drastically poorer bread quality with nitrogen deficient conditions (15-70 kg ha^{-1}), resulting in decreases of 36% and 26% in protein content and loaf volume respectively compared to 5% and 2% when nitrogen was not a limiting factor (350 kg ha^{-1}). The contradicting results make it unclear to judge the interaction effect between nitrogen application level and CO_2; Hogy et al. (2013) speculated that increasing nitrogen fertilization will not help to counteract the decreased protein content caused by augmented CO_2 levels. However, as climate change is also accompanied by rising temperatures, Blumenthal et al. (1996) predicted that increased grain protein synthesis that

occurs with higher temperatures may alleviate some of the loss in protein. However, it may not remedy the situation entirely. Wieser et al. (2008) suggested that the breeding of extra high protein wheat may be necessary in order to maintain breeding quality standards in the future, due to the potentially reduced quality from rising atmospheric CO_2 levels.

2.3.5 Solar Radiation

Solar radiation is one of the major driving forces of plant development. The intensity of light affects photosynthate allocation and thus photosynthesis in cropping systems (Wang et al. 2003). Of the total incoming solar radiation, or incidence radiation, photosynthesis is adapted for wavelengths that fall within the visible light range (400-700 nm) (Wetzel 2001). In agrometeorological terms, this range is called photosynthetically active radiation (PAR). The actual incidence level of PAR available to the crop will vary based on time of year, geological coordinates, and many environmental factors such as cloud cover.

It is generally conceded that reduced levels of incidence radiation subsequently reduces wheat yield (grain number) by slowing the rate of photosynthesis, at a maximum effect when shading occurs approximately 30 days preceded anthesis. One of the initial reports of this was by Fischer (1985), and many later studies have demonstrated the same trend (Abbate et al. 1997; Demotes-Mainard and Jeuffroy 2004; Savin and Slafer 1991; Thorne and Wood 1987). These studies cover a wide range of radiation intensity, water availability (rainfed & irrigated), temperature, cultivar, locational and planting density variations, in addition to varying nitrogen fertilizer levels and modes of application. Estrada-Campuzano et al. (2008) later identified a more narrow time period in which grain yield was more significantly affected. This study reduced incident ration by 67% by installing black nylon nets above plots during five different

stages in growth development. The installation of the nets provided negligible (-0.3 C) temperature differences. PAR, measured using a linear ceptometer, was positively correlated to grain yield ($R^2 = 0.69$, $p<0.001$) and above ground dry matter maturity ($R^2 = 0.90$, $p<0.001$). The highest magnitude in yield reduction was seen when shading was imposed within 2 weeks prior and 1 week after anthesis (correlating to stem elongation – mid grain filling).

In contrast, Li et al. (2010a) installed layers of polyethylene screens from jointing to maturity that reduced 8, 15 and 23%, of incidence radiation to two winter wheat cultivars. The low intensity shading (8% inhibition) actually enhanced the growth, seen as significant increases in yield (19 and 109 kg ha^{-1}), TKW (0.2 and 0.4 g) and biomass (41 and 88 kg ha^{-1}) for both genotypes compared to the control. This was explained by an enlarged leaf area index, therefore larger canopy, which would enhance the capacity of the plant to capture light. This was observed over all shading treatments, however only in this low level of shading (8%) was the increased canopy able to compensate for yield losses. As the level of shading increased to 23%, there was a significant loss of yield (347 and 391 kg ha^{-1}), TKW (2.4 and 3.6 g) and biomass (509 and 1,117 kg ha^{-1}) for both genotypes respectively, compared to the control. Similar results were found by Mu et al. (2010), in which the yield of two wheat cultivars was reduced by 16.2% and 25.8% when 33% inhibition of incidence radiation was imposed polyethylene screens from jointing to maturity.

Due to the large implications of solar radiation on wheat yield, it follows that implementation of shading during the growing season would have some effects on wheat quality. Li et al. (2010b) found that 65% shading applied after anthesis altered the starch granule characteristics of wheat. However, the interaction between incidence radiation and gluten

performance and composition has rarely been studied. One of the first to test the effect of altered radiation levels on wheat quality was Zu et al. (2004), where increased levels (supplement of 10-15 kJ m^{-2}) of ultraviolet-B (280-315 nm) light (outside the range for optimum photosynthesis) was applied for 7 hours daily from three-leaf to ripening stages using filtered sunlamps. Unfortunately, results varied widely between the 10 cultivars analyzed and no clear trends were seen from this study.

Later, Yong-Geng et al. (2005) implemented 50% inhibition of PAR to two wheat cultivars by using white fabric, at three difference stages (1-10, 11-20, 21-30 DAA) post anthesis. Although the yield, test weight and nitrogen distribution were all significantly decreased, shading resulted in improved dough quality parameters: water absorption and dough stability time ($p<0.05$). This was explained by significant ($p<0.05$) increases in protein, wet gluten, GMP and glutenin content, in addition to an increase in the glutenin/gliadin ratio. These effects were most significant when shading was applied during late grain filling stage (21-30 DAA). Comparably, Sandana et al. (2009) found increased (66%, $p<0.05$) grain nitrogen concentration when black nets capturing 90% incidence radiation were installed over wheat plots from anthesis to maturity.

In an additional study, Li et al. (2012) inhibited 8, 15 and 23% of incidence radiation to 2 wheat cultivars using polyethylene screens from jointing to maturity. Shading implementation resulted in improved quality characteristics, with significant ($p<0.05$) increases in wet gluten content (0.97 and 3.21%), SDS-sedimentation volume (4.14 and 5.5 mL) and farinograph dough development time (0.68 and 0.8 min) for both cultivars respectively, compared to the control. There were significant decreases in farinograph softening degree (7 and 9 FU) and gluten index

(0.06 and 0.05). It was difficult to explain the improved dough quality parameters by changes in gluten composition, as the results were not consistent between cultivars or shading treatments. Interestingly, 8% light inhibition yielded the highest HMW-GS and GMP content (ug grain^{-1}) between all three treatments. This can be explained by the early formation of HMW-GS at 10DAA with a higher accumulation rate for the 8% shading treatment, where the 15 and 13% shading treatments did not begin accumulating HMW-GS until 15 DAA. HMW-GS often accumulates to higher concentrations in the grain when its formation begins earlier (Deng et al. 2006; Jiang et al. 2009; Yue et al. 2007).

Chen et al. (2013) imposed black shading nets that inhibited 40% incidence radiation at three periods after anthesis (1-10, 11-20, 21-30 DAA) to two winter wheat cultivars. They reported an increase in GMP content for all treatments. However, shading imposed at early grain filling (1-10DAA:) favoured the formation of small particles. Variety Jinan17 having proportions of 0.996 and 0.005 for small (<100 um) and large (>100 um) particles respectively. Alternatively, shading imposed later in the season (11-20 and 21-30 DAA) favoured the formation of large particles. Where proportions for small and large particles in the same variety were 0.962 and 0.033 for shading applied 11-20 DAA and 0.968 and 0.032 for shading applied 21-30 DAA respectively. Similarly, the concentrations of total and individual HMW-GS increased when shading was applied, with more significant effects as the treatment was applied later in the grain filling period. In all cases, the maturation period (anthesis-maturity) was shortened by 1-3 days. Since it has been reported that shorter maturation periods leads to higher accumulation of polymeric protein (Malik et al. 2011), this may explain why shading applied after anthesis increased concentrations of HMW-GS and GMP.

It is difficult to compare between studies due to the extreme variations of incidence radiation that arises between geographical locations. A way to allow for comparison would be to record the energy level (joules per s or per m^2) of PAR. However, the few studies that measure the qualitative properties of gluten rarely recorded these values, and those that do have not attempted to correlate the level of PAR to gluten properties. Despite this, it appears that reduced solar radiation may have a positive implication on gluten strength, with more significant influences later in the grain filling period, albeit a negative influence on yield. Nevertheless, this area has rarely been studied, and much more research is needed.

2.4 Biotic Environmental Factors

2.4.1 Fusarium Damage

Fusarium Head Blight (FHB) is a fungal disease in wheat. The most prevalent species infecting North American wheat are *Fusarium geaminearum* and *F. culmorum*. FHB infection results in economic losses as it significantly reduces dry weight, germination and seed quality (Argyris et al. 2003). Infection is identifiable by shriveled bleached or orange/pink kernels. *Fusarium* damage is also a food safety concern as the fungus produces toxins that are able maintain harmful levels in wheat-products even after production. Deoxynivalenol (DON) is the dominant toxin, however others may be produced including: nivalenol, T-2 toxin and zearalenone (Wegulo 2012). The implications of DON on wheat quality will be assessed later.

In addition to being a food safety hazard, *Fusarium* damage has ramifications on the bread-making quality of wheat. The fungus produces proteinases that hydrolyze wheat gluten. Pekkarinen and Jones (2002) purified an enzyme from a gluten culture medium of *F. culmorum*.

The proteinase was inactivated at 50ºC and inhibited by soybean trypsin inhibitor. These results led the authors to refer to the enzyme as trypsin-like. In addition, the C-terminus sides of arginine and lysine were preferentially hydrolyzed on substrate B purothionin. The proteinase was most stable at pH 6-7, ambient temperatures (24-40 C) and enzyme stability increased in the presence of Ca^{2+} ions. Alternatively, Nightingale et al. (1999) found the proteinase derived from infected kernels and pure cultures of *F. graminearum* to be inhibited by p-chloromercuribenzoate and not soybean trypsin inhibitor. There results suggest an alkaline protease, rather than trypsin-like, although the two species may differ in the proteinase that is produced.

In an analysis of winter wheat samples from 2005-2013, Koga et al. (2016b) found that wet years, during which there was continuous and repeated rainfall during maturation and harvest yielded weak dough. The poor-quality was related to protease activity, suspected to be derived from *Fusarium*, as conditions for fungal development were favourable during those years.

Using light and electron microscopy, Bechtel et al. (1985) examined the effect of the fungus on wheat kernels. *Fusarium* infection was concentrated in the aleurone and pericarp tissues, however hyphae extended to the endosperm. In cases where the disease severity was high, the hyphae hit the germ. Starch granules, cell wall and storage proteins were all aggressively destroyed. In the same study, SDS-PAGE revealed a decrease of intensity of the HMW bands as the level of infection increased. The same microscopy technique was applied by Nightingale et al. (1999). Similar to the images of Betchel et al (1985), the endosperm protein was significantly degraded.

Electron microscopy provided visible confirmation that *Fusarium* infection degrades the protein of the endosperm. These changes in gluten may alter the viscoelastic properties of dough. One of the first to note a change in dough properties was Dexter et al. (1996). The sample kernels were hand sorted into damaged, "as is" or cleaned to reduce *Fusarium* presence. *Fusarium* damage in samples ranged from 1.5-10.7% between cultivars, some flours were mixed to include up to 20% damaged kernels. The mixing properties of the dough remained relatively normal, however rendered sticky and difficult to handle. Damaged kernels that were analyzed by reversed phase HPLC showed a reduction of glutenin, with no effect on gliadins.

In a later study, Dexter et al. (1997) assessed the pasta-making quality of durum wheat downgraded due to *Fusarium* damage. The severity of *Fusarium* damaged kernels ranged from 2.1-9.2%, with clean samples (control) having no more than 0.3%. Although the protein content was unaffected, the concentration of glutenins decreased. Although this decrease is not quantified, SE-HPLC chromatograms captioned in this study clearly demonstrate a loss in glutenin protein, especially in comparison to gliadins. This loss of glutenin resulted in lower gluten strength, although this difference was not large enough to change pasta textural properties. This is contrary to Hatcher et al. (2003) who noted a moderate reduction of dough strength during mixing followed by loss of noodle texture at *Fusarium* levels of 0.5-9.6% FDK.

The tendency of *Furasium* to hydrolyze glutenin was also noted by Boyacioglu and Hettiarachchy (1995). They achieved light and moderate levels of infection by spraying inoculum of *F. graminearum* 2 days after anthesis, although the exact severity was not specified. The compositional changes that arose from light infection were mostly seen in wheat carbohydrates. However, moderate infection resulted in an 80% decrease of glutenin, although a

6% increase in total wheat protein content was seen overall. Protein fractionation was completed based on solubility in 70% ethanol and quantified by Kjeldahl. A loss in quality was observed, assessed by a 6% and 15% decrease in SDS sedimentation volume for light and moderately infected whole flour samples respectively. The increase in protein content, despite glutenin degradation, was attributed to fungal metabolism of carbohydrates as a carbon source for the synthesis of proteins. The same hypothesis was suggested by Hamilton and Trenholm (1984) with starch specifically being the substrate.

Further losses in breadmaking quality as a result of disease have been reported. At levels of 20% *Fusarium* damage, there was a loss of dough functionality seen by decreases in dough consistency and resistance (measured by farinograph and extensograph) as well as a reduction in loaf volume (Nightingale et al. 1999). Although the numerical values of the decreases were not reported in this study, the loss of breadmaking quality can be seen in extensograph and farinograph figures. Wang et al. (2005) noted impaired dough quality in which infection grade was significantly correlated to dough mixing stability ($r=-0.81$, $p<0.01$), resistance ($r=0.82$, $p<0.01$), softening ($r=0.81$, $p<0.01$) and elasticity ($r=-0.72$, $p<0.05$) as well as mm height of loaf ($r=-0.99$, $p<0.05$). The change of these parameters in their respective order from light to severely damaged flour samples is as follows: dough stability -1.42 min decrease, dough resistance -1.62 min decrease, dough softening -70 valirograph units (VU) increase, dough elasticity -11 VU decrease and loaf height -1.2 mm decrease. As the level of *Fusarium* damaged increased, a decrease in the relative quantity of glutenin of 7.6% and 19.1% for moderate and severely damaged kernels respectively was reported, compared to lightly damaged kernels. Additionally, there was an observed increase in free amino acid concentration, ranging from 33.3% to 139.9% from light to severely infected samples depending on the amino acid of interest. The amino acids

were said to be degradation products of *Fusarium* protease activity, thus explaining the increase. A similar observation was recorded by (Nightingale et al. 1999). In contrast, Beyer and Aumann (2008) reported an average 0.13% decrease in total amino acid content for every 1% increase in *Fusarium* damage when compared to sound kernels, in particular glutamic acid. Conversely, individual levels of alanine, lysine and tyrosine increased with percentage *Fusarium* damaged kernels. This suggests that *Fusarium* preferentially metabolizes specific amino acids, while the others remain as degradation products, contributing to the free amino acid composition of the kernel.

Eggert et al. (2010) analyzed compositional changes in emmer and conventional wheat as a result of natural and artificial *Fusarium* infection. Artificially inoculated samples had a higher severity of the disease, as determined by a DON increase of 0.9-8.6 mg kg^{-1} between cultivars and locations. Wheat glutenin was highly susceptible when compared to emmer, and both 60%-ethanol insoluble HMW- and LMW-GS decreased as a result of the infection, as determined by RP-HPLC. They also found an increase in the proteins extractable at 60% ethanol alongside gliadins, which they contributed to the degradation products of glutenin hydrolysis. There was a slight (~ 0.2 mg kg^{-1}) decrease in the amount of ω-gliadins. However, this only occurred with certain cultivars, and it was speculated that the larger size of these gliadins resulted in their entanglement within the glutenin polymeric network. Therefore, they were hydrolyzed based on their association with glutenin. There was no influence on the albumin and globulin content.

In a later study, Eggert et al. (2011) examined the digestion of gluten proteins *in vitro* by *F. graminaerum* for 0, 2, 4, 8 and 24 h. The fractions were isolated based on solubility in 60% ethanol and quantified by RP-HPLC and SDS-PAGE. They found the larger molecular weight

fractions were hydrolyzed, preferentially; HMW-GS>LMW-GS>gliadins accordingly. Contrary to previous studies, gliadin fractions were digested. They suggested the reason that this had not been seen before is that glutenin degradation products are collected and go through HPLC analysis concurrently. The glutenin fragments may then elute and be falsely categorized as gliadin fractions, leading to the perception that total gliadins increase. As an increase in gliadins was observed in this study, this explanation seems the most likely.

Regardless if the digestion of gliadins is masked as suggested by Eggert et al. (2011) or they are unaffected by the proteinases, there clearly exists preferential degradation of the glutenin fractions. The effects of gluten hydrolyzation on dough rheological properties and breadmaking quality is less clear. Instances where the severity of *Fusarium* damaged kernels are similar yield contradictory results. One aspect that has not been examined is the duration between maturity until harvest. As seen by Eggert et al. (2011) a longer incubation period with fungal proteinase results in an increased severity of gluten degradation. Analogously, a crop allowed to sit in the field past maturity, could allow more time for the enzyme to act under optimal conditions. However, this would require favourable conditions (excess rainfall, high relative humidity, etc) for the moisture content to remain high enough to sustain enzymatic activity. In this instance, protease activity would be a more valuable parameter to correlate with deteriorations in quality rather than the percentage of damaged kernels. Interestingly, Pekkarinen and Jones (2002) reported increased stability of the proteinase in the presence of calcium ions. The potential relationship between the grain calcium content and the protease activity would be a new topic for research.

2.4.1.1 Deoxynivalenol and Inhibition of Protein Synthesis

Deoxynivalenol (DON) is a type B tricothene neurotoxin produced by *Fusarium* spp, commonly found in wheat. Due to its ability to withstand the processing stages in the production of flour, DON concentration in wheat is a serious food safety concern. Upon ingestion, DON alters brain neurochemicals, induces vomiting and reduces food intake (Rotter et al. 1996). Levels of DON in wheat are generally found to be correlated to the severity of *Fusarium* damage (Dexter et al. 1996). The tolerance of *Fusarium* damage in Canadian wheat is 0.25% for No.1 CWRS up to 4% for CW Feed (Canada Grains Commission 2016).

Much of the research surrounding DON lies in examining the toxilogical effects of DON towards animals and humans. Rotter et al. (1996) and Pinton and Oswald (2014) have good reviews that discuss the effects of DON dose and frequency in animals with inferences to humans. Unfortunately, these studies do little when drawing comparisons to plant systems, as the two organisms may differ in their metabolism and uptake of DON (Rocha et al. 2005). Of more interest is research analyzing the effects of DON on eukaryotic cells. In the cell, DON is known to indirectly inhibit protein synthesis by binding to the large (60S) subunit of the 80S ribosome. This binding interferes with the activity of peptidyl transferase, inhibiting elongation and transcription of proteins (Carter et al. 1976; Cole et al. 1981; Cundliffe et al. 1974; Foroud et al. 2016; Harris and Gleddie 2001; Pestka et al. 2004; Pinton and Oswald 2014; Rotter et al. 1996; Thompson and Wannemacher 1986; Walter and Doohan 2011; Wei et al. 1974). X-ray crystallography of yeast cells clearly demonstrate the A site of the peptidyl transferase center being bound by DON (de Loubresse et al. 2014). This suggests DON may have negative influences on wheat quality by inhibiting the production of gluten.

Wang and Miller (1988) analyzed the effect of DON concentrations on coleoptile tissue growth. This field study examined 14 wheat cultivars inoculated at anthesis. The results suggest that the two *Fusarium* resistant varieties were 10 to 1000 times more tolerant to DON concentration in comparison to susceptible ones. This was attributed to multiple plant defense mechanisms in which wheat tissues first prevent fungal hyphael invasion further into the crop and are actually capable of degrading the mycotoxin. Despite this, coleoptile tissue growth was inhibited by most varieties at tricothene concentrations of 10^{-6} M (13.3-36.7% inhibition, $p<0.05$), where all cultivars were inhibited at concentrations of 10^{-3} M (61.0-100% inhibition, $p<0.01$). Similarly, Bruins et al. (1993) reported a decrease in coleoptile tissue growth at DON concentrations of 10^{-5} M in 41 wheat varieties.

Contradicting results were noted by Hamilton and Trenholm (1984) in which wheat samples containing >0.6 mg kg^{-1} DON exhibited increased crude protein content, crude fiber and true available amino acids compared to samples where DON concentrations fell below 0.05 mg kg^{-1}. However, the results of this study may not be reliable, as there was no control and the six spring and winter wheat cultivars analyzed would have varying intrinsic protein contents. Therefore, the differences observed among the variables measured may have resulted from genotypical variation, with DON concentration being coincidental.

Nishiuchi et al. (2006) analyzed the effects of DON on wheat growth by injecting the trichothene into 4-5 week old *Arabidopsis thaliana* plants grown in a greenhouse with 16 light hours per day at 22 ^{o}C. Protein synthesis was determined by measuring the incorporation of ^{3}H-leucine into plants. The effects of DON on protein synthesis are displayed in Fig. 2.2. Although concentrations of 0.01 uM were sufficient to begin reduction in protein synthesis, it did not

induce the plant's defense mechanism until concentrations of 10 uM. These results suggest that DON inhibits protein translation at low levels without the induction of elicitors, as is not the case with other tricothenes such as T-2 toxin. Concentration dependent induction of defense genes in wheat was also observed by Desmond et al. (2008) in which 100 mg L^{-1} DON was required for defense genes to be detectable in wheat.

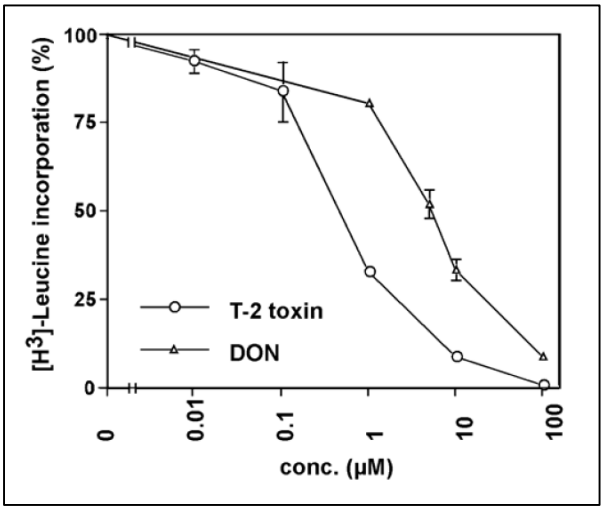

Figure 2.2. Effect of T-2 and deoxynivalenol (DON) concentration on *Arabidopsis thaliana* protein synthesis (from Nishiuchi et al, 2006)

Masuda et al. (2007) conducted a similar study in which the effects of DON on *Arabidopsis thaliana* were analyzed. Seeds were subjected to a three or 7 day vernalization period at 22 ^{o}C with 16 light hours day^{-1}. After which, plants were transferred to an agar growth medium were they were inoculated with DON. Even at high concentrations (10uM), DON did not inhibit germination. However, 10 uM DON severely inhibited root elongation and slightly inhibited shoot growth. This suggests that DON preferentially inhibits root growth, as shown in Fig 2.3. The inhibition of root growth may impact the plant's ability to assimilate nutrients.

Figure 2.3. Effect of 0, 15 and 30 uM (left to right) DON on root and shoot elongation in *Arabidopsis thaliana* (from Masuda et al 2007)

Although no studies have been conducted, based on the inhibitory effects of protein synthesis and plant tissue growth that arises with increasing DON levels, there may be a potential for the toxin to alter the gluten quality of wheat. However, a study must be conducted that relates the concentration of DON to defects in gluten quality, independent of *Fusarium* levels.

2.4.2 Insect Damage

Wheat bug is a pentatomid insect that feeds on the kernels of wheat. Some genera that impose problems in bread-making are *Eurygaster, Aelia* and *Nysius* (Hariri et al. 2000). Of these, *Eurygaster* spp. and *Aelia* spp. are most commonly studied. Both nymph and adult bugs feed on wheat by injecting salivary proteinases and amylases into the kernel that allows them to remove

nutritive components of the endosperm. Despite the loss of endosperm constituents, feeding does not stop the maturation process from occurring (Hariri et al. 2000). However, kernel aesthetics are altered and the extent varies depending on the growth stage at which wheat is attacked. If the kernel contents are still fairly liquid, such as in the water-ripe or milk-ripe stages, a dark spot will appear at the point of penetration and the kernel will depress around this point (Hariri et al. 2000). Once this kernel matures, damage is visible on the surface, distinguished by a pale area surrounding the darkened puncture point (Every 1992). Kernels attacked during the milk stage or prior are easier to separate from the sound kernels because of the visible dent. Kernels attacked at later stages do not deform and are difficult to separate from sound kernels, making quality losses more severe as they would commonly be included with sound samples (Koksel et al. 2009).

Every (1992) outlined the extent of damage required for a cultivar to be considered "bug-damaged". Proteinase levels, visible damage and SDS-sedimentation values were used to assess the samples. Each of the three parameters had good relationships with one another. High quality bread cultivars were less susceptible to defects from the damage, therefore the requirements for high and low quality cultivars differ. The "bug-damaged" limits of proteinase level, visible damage and SDS-sedimentation value were 280 U g^{-1}, 9% and 26 mL and 100 U g^{-1}, 4% and 21 mL for high and low quality cultivars respectively. The effects of bug damage on wheat as proposed by Koksel et al. (2009) include: low seed germination, decreased yield and poor gluten quality.

A later study by Every (1993) purified the proteinase derived from wheat samples damaged from *Nysius huttoni*. The highest level of purification they could achieve was 10^6 U g^{-1},

indicating that enzyme levels in wheat can reach ~1ppm in the highest. The enzyme was able to maintain stability over a pH range of 2.5-11, and at 50 ^{o}C for up to 20 minutes, but was inhibited by two separate serine proteinase inhibitors. These results indicate that wheat bug has a serine proteinase. When bread was baked with partially purified enzyme (20,000 U in 50 g sound flour), it produced loaves of poor quality similar to those baked with bug-damaged wheat. Actual deterioration of the protein matrix was seen in in scanning electron micrograph images produces by Torbica et al. (2014) showing a non-cohesive protein structure as a result of *Eurygaster* spp. and *Aelia* spp. damage.

Kretovich (1944) was one of the first to define the poor baking quality that is manifested as a result of wheat bug damaged grain. He described the dough as sticky and void of elasticity, difficult to shape resulting in flat loaves with poor texture. This description is reflected in the study previously cited (Every 1993) in which loaf volumes were reduced by 31% and 38% compared to the control, when baked with sound flour containing purified proteinase and bug-damaged flour respectively. Both had poor texture quality, with the most severe results seen in bread baked from bug-damaged wheat.

Torbica et al. (2014) examined the effects of six Serbia wheat varieties grown in two locations with significantly varying levels of *Eurygaster* and *Aelia* spp. infestation. Every variety grown in the bug-infested location demonstrated a higher farinograph softening degree as well as extensograph and alveograph deformation energies that were either low or not measurable.

The effects of bug damage on the production of wheat flat bread was examined by Hariri et al. (2000). The physicochemical properties of wheat were altered by as few as 5% damaged kernels. At levels of 20%, the dough was unsuitable for flat bread production. This was reflected

in 60% and 85% reductions in farinograph development times and stability values respectively, and an increase of 300% for mixing tolerance index. Gluten quality parameters of semolina samples were substantially deteriorated as *Eurygaster* spp. damage increased from zero, medium (20%) to high (40%) levels of damage (measured by wholemeal Pelshenke test and mixograph and farinograph analysis of flour doughs).

Sivri et al. (1998) related the loss of quality to the compositional changes that arise in *Eurygaster maura* damaged wheat. The enzyme affected both gliadin and glutenin, with HMW-GS being the most susceptible even disappearing completely after 120 minutes of incubation. As the length of the incubation period increased, some new bands appeared on acid-page electrophoregrams similar in size to small and large gliadins. These were thought to be the degradation products of gluten. Some cultivars varied in their susceptibility to bug damaged. These results are similar to those of Rosell et al. (2002) in which all proteins fractions decreased in wheat damaged by *Aelia* and *Eurygaster* spp.. In the latter study, glutenins (HMW and LMW) were heavily affected, and new peaks appeared in the ω-gliadin regions of electrophoregrams.

The degradation of glutenin (measured as decreased content of 50% 1-propanol insoluble protein) specifically was studied by Sivri et al. (1999). This was achieved by mixing damaged (*Eurygaster* spp.) and sound kernels at a 2:1 ratio and incubating in water for 0, 30, 60 and 120 minutes. More than an 80% decrease in total glutenin, HMW-GS and LMW-GS was seen when incubation times were greater than 30 minutes. Four of the six cultivars exhibited a significant decrease in the 50% 1-propanol insoluble HMW:LMW glutenin subunit ratio, indicating a intercultivar difference for bug protease resistance. Subsequently, Sivri et al. (2002) reported similar results in which Turkish bread wheat samples were incubated with proteinase extract

from *Eurygaster* spp. damaged wheat. There was once again a decrease in 50% 1-propanol insoluble glutenin, with HMW-GS having larger reductions than LMW-GS. However, the gluten deterioration was not as substantial; incubation time of 60 minutes resulted in 43-65% reductions rather than 80% previously seen. Once again, intercultivar differences were noted with higher quality, i.e. stronger, wheat being the most resistant.

Aja et al. (2004) incubated wet gluten from damaged wheat for intervals of : 0, 1, 2, 3, 7 and 24 hours. The amount of total gluten remained the same, however the gluten index steadily decreased the longer the incubation time, a characteristic of protein hydrolysis. The products of this hydrolysis were soluble in water, and the first 3 hours of incubation revealed products of molecular weights 15,000 – 30,000 DA. However, in order to analyze the hydrolysis products a reducing agent was used. When this step was omitted, the molecular weight range changed to 27,000 - 42,000 DA with some aggregates higher than 200,000 DA, indicating the presence of disulfide sulfide bonds, as determined by SE-HPLC and SDS-PAGE. These results indicated that the proteinase had endoproteolytic activity.

Subsequently, Salis et al. (2010) suggested that an undetermined reducing agent was present in the bug saliva, and that degraded gluten alongside salivary enzymes. In their experiment, they incubated 70% sound wheat with 30% damaged for 0, 1 and 3 hours at either 4 or 45 ^{o}C. Regardless of incubation temperature, there was a significant decrease in 50% 1-propanol insoluble glutenins. Contradictory to temperature-independent glutenin hydrolysis, gliadin degradation was temperature dependent and occurred more substantially at elevated temperatures (45 ^{o}C). When samples were incubated at 4 ^{o}C, even up to 3 hours, gliadin concentration was unchanged compared to sound wheat. Enzyme activity was correlated to

temperature; therefore, as temperature increased, degradation of gliadins also increased. The presence of salivary reducing agents is feasible as rapid glutenin hydrolysis but not gliadin hydrolysis was seen even at the lower temperature.

2.5 Conclusions and Final Thoughts

Gluten, the storage protein in wheat, is the essential component of flour that imparts viscoelastic properties to bread dough. In order to be applicable for baking use, flour must exhibit a certain level of gluten quality. Gluten quality is a combination of the content and composition of the constituent proteins, and is reflected as strength of the dough during mixing. Gliadin and glutenin are the two fractions of gluten. Gliadins are monomeric proteins, which contribute negatively to gluten strength due to their low molecular size. However, some research has suggested that certain γ- or ω- gliadins may enhance dough strength, although more research must be done to confirm these findings. After reduction, polymeric glutenin can be separated into two constituents: HMW and LMW subunits. HMW-GS appear to be the most influential determinant of gluten strength. They are certainly a much better studied group of proteins. Essentially, the larger the average molecular size the stronger the dough. LMW-GS are difficult to evaluate due to their molecular size being similar to gliadins, resulting in similar solubility properties. It follows that LMW-GS and gliadins can be extracted together, if not separated beforehand. Making matters more complex are the interactions between certain LMW- and HMW-GS occurring intrinsically within dough, which are thought to contribute to the polymerization or aggregation of a large glutenin polymer that confers strength. In addition to the contributions of specific gluten fractions, the relative proportions HMW- and LMW-GS as

well as glutenin and gliadin are important to gluten quality. In both cases, stronger doughs arise from higher amounts of the larger fractions, HMW-GS and glutenin polymer respectively.

While genetics govern which gliadin and glutenin subunits are present in a given genotype, environmental factors during crop development can alter the relative proportions of these subunits and/or fractions. Of the abiotic environmental factors, temperature, soil nitrogen status, soil water availability and atmospheric carbon dioxide are the most influential factors to determine wheat protein content. Changes in protein content ultimately alter the relative quantities of individual gluten fractions. These changes are reflected in rheological and bread-making properties, in which increased nitrogen fertility and drought conditions appear to improve whereas high growing temperatures and carbon dioxide levels lower gluten quality. Sulfur content is important to regulate the concentration of sulfur-poor ω-gliadins and HMW-GS and sulfur-rich LMW-GS, α- and γ-gliadins. An excess or deficiency of sulfur results in overly extensible or bucky dough properties, respectively. In order to prevent degradation in gluten quality as a result of sulfur levels, a N:S ratio of approximately 12.5-15 is required. Other soil minerals and salinity levels can alter gluten subunit quantities; however, more research is required to define the cause of these effects.

Gluten is subject to degradation by biotic environmental factors such as *Fusarium* and wheat bug. *Fusarium* is a fungal disease that is a food safety concern due to the production of deoxynivalenol, a neurotoxin. Both *Fusarium* and wheat bug produce proteinases that invade wheat kernels, and hydrolyze gluten protein. Glutenin, due to its very large molecular size, is preferentially hydrolyzed, although incubation studies have shown that gliadin can degraded as well if the proteinase activity lasts long enough. Enzyme hydrolysis of glutenin results in sticky

and weakened dough properties, where the effects of wheat bug are more well defined than those of *Fusarium.* It is suspect that *Fusarium* mycotoxin DON may additionally decrease gluten quality by inhibition of protein synthesis through ribosomal bonding, although this hypothesis has never been tested in relation to wheat breadmaking quality.

It is difficult to precisely characterize the effects of environmental factors during the growing season on wheat processing quality and dough strength, as there are many potential factors which naturally occur at similar times. The results of greenhouse and controlled experiments may not extrapolate to field conditions, and field conditions contain numerous variables with many interactions, making it difficult to conclusively delineate clear relationships. The impact of factors such as soil nutrients other than N and S, have been essentially ignored in the literature up until recently. Furthermore, the response of wheat to environmental factors can vary by genotype, rendering it more difficult to compile precise conclusions. Needless to say, there is no shortage of additional research that needs to be carried out to develop a better understanding of the great many factors, in addition to their interactions, on their influence on gluten strength.

3.0 MATERIALS AND METHODS

3.1 Field Setup

Certified seed of nine wheat varieties were obtained from seed SECAN, FP Genetics, Seed Depot, Viterra-CPS and Canterra (Table 3.1). The varieties were grown in nine locations across Western Canada over the 2015 and 2016 growing seasons (Fig 3.1). Experimental layout was a randomized complete block design with four replications using a seeding rate of $300m^{-2}$ with varying plot sizes per location (Table 3.1). All sites were optimally fertilized based on soil tests to eliminate soil fertility issues and differences across sites. Herbicides and fungicides were applied as needed based on weed and disease pressure. At each site, anthesis and maturity was observed or modelled according to Mkhabela et al. (2016) and/or Saiyed et al. (2009). Dates of principal phenology points in the two growing seasons are presented in Table 3.1. The nine genotypes along with their abbreviations are as follows: Brandon (BR), Carberry (CR), Cardale (CD), Glenn (GL), Harvest (HA), Stanley (SN), Stettler (ST), Unity (UN) and Utmost (UT). Cultivars were selected to provide a range in field performance, gluten strength, *Fusarium* resistance and midge tolerance. The glutenin allelic composition of all cultivars is presented in Table 4.1.

Table 3.1. Seed suppliers for each CWRS wheat varieties.

Seed Suppler	Variety
SECAN	Unity VB
	Stettler
	AAC Brandon
	Carberry
FP Genetics	Harvest
	CDC Utmost
Seed Depot	Cardale
Proven Seed	CDC Stanley
Canterra	Glenn

Table 3.2. Plot size of CWRS wheat trials in nine Canadian Prairie locations.

Location	Plot Size (m^2)
AAFC Brandon	3.6
AAFC Morden	3.6
JRI Kelburn	12
AAFC Swift Current	5
AAFC Indian Head	5
Hucl Saskatoon (Goodale)	5.4
AAFC Lethbridge	3.1
Spaner Edmonton	5.4
AAFC Beaverlodge	4.32

Figure 3.1. Map of the Canadian Prairie region showing field site locations for CWRS wheat varieties grown in 2015 and 2016.

Table 3.3. Principal phenological dates for 2015 and 2016 field trials.

Location	Abbreviation	Geographical Coordinates	Year	Seeding	Anthesis	Maturity	Harvest
Beaverlodge	BE	(55.20° N, 199.40° W)	*2015*	May 15	July 8	Sept. 1	Sept. 25
			2016	May 10	July 15	Sept. 11	Sept. 22
Brandon	BR	(49.90° N, 99.95° W)	*2015*	May 15	July 10	Aug. 18	Sept. 2
			2016	May 9	July 4	Aug. 12	Sept. 2
Edmonton	ED	(53.69° N, 113.62° W)	*2015*	May 21	July 10	Aug. 18	Sept. 12
			2016	May 9	July 9	Aug. 17	Sept. 7
Goodale	GO	(52.17° N, 106.72° W)	*2015*	May 11	July 4	Aug. 10	Aug. 28
			2016	May 6	July 2	Aug. 14	Sept. 8
Indian Head	IH	(50.55° N, 103.65° W)	*2015*	May 25	July 20[a]	Aug. 26[b]	Sept. 29
			2016	May 9	July 10	Aug. 16	Aug. 30
Kelburn	KE	(49.69° N, 97.13° W)	*2015*	May 5	July 3[a]	Aug. 7[b]	Aug. 21
			2016	May 5	July 5	Aug. 10	Aug. 31
Lethbridge	LE	(49.70° N, 112.77° W)	*2015*	May 6	July 2	Aug. 4	Aug. 20
			2016	May 5	July 7	Aug. 11	Aug. 31
Morden	MO	(49.19° N, 98.08° W)	*2015*	May 23	July 11[ab]	Aug. 7[ab]	Sept. 1
			2016	May 9	July 2	Aug. 3	Sept. 1
Swift Current	SC	(50.27° N, 107.73° W)	*2015*	May 12	July 6	Aug. 9	Sept. 10
			2016	May 4	July 6	Aug. 17	Sept. 21

Table 3.4. Glutenin allelic composition* of CWRS wheat varieties.

Genotype	Glu-A1	Glu-B1	Glu-D1	Glu-A3	Glu-B3	Glu-D3
Brandon	b	c	d	e	h	c
Carberry	b	c	d	d	h	a
Cardale	b	c	d	e	g	c
Glenn	b	c	d	d	h	c
Harvest	b	c	d	f	h	c
Stanley	b	c	d	e	b	c
Stettler	b	c	d	e	h	a
Unity	b	c	d	e	g	c
Utmost	a	al	d	e	h	a

*Glutenin alleles classified by catalogue with Shigen Database (Mishima, Japan).

3.2 Meteorological Data Collection and Agrometerological Analysis

Weather parameters used in this study (Table 3.5) that were directly measured were daily maximum, minimum and average temperature, as well as total precipitation. These data were acquired from nearby weather stations maintained by Alberta Agriculture and Forestry (ED), Environment Canada (BR, MO, IH, SC, LE and BE), Manitoba Agriculture (KE), and Weather Innovations (GO) (Chatham, ON). Solar radiation data for Kelburn and Morden was recorded and obtained from Manitoba Agriculture, however the nearest available weather station to the latter was located 13 km away in Winkler, MB. Agriculture and Agri-Food Canada provided observed solar radiation data for Brandon, Beaverlodge, Lethbridge and Swift Current, whereas Edmonton data was provided by Alberta Agriculture and Forestry. Solar radiation data was not available for Indian Head or Goodale. Derived weather variables (Table 3.5) are specified along with their equations.

Table 3.5. Weather variable abbreviations

Weather Variable	Abbreviation	Equation
Maximum Temperature (°C)	Max	-
Minimum Temperature (°C)	Min	-
Mean Temperature (°C)	Mean	-
Diurnal Variation (°C)	DV	$= Max - Min$
Weighted Diurnal Variation	WDV	$= Mean\ (Max - Min)$
Growing Degree Day (base temperature 0-24 °C)	GDD_b	$= \sum_{Stage\ 1}^{Stage\ 2} \frac{Max+Min}{2} - Base\ Temperature$
Total Precipitation (mm)	TP	-
Evapotranspitation (mm)	Eto	$= 0.0023 \times 0.4080 \times TOA \left(\left(\frac{Max + Min}{2}\right) + 17.8\right) \times (Max - Min)^{0.5}$
Crop Water Demand (mm)	Etc	$= Eto\ x\ K_C$*
Crop Water Deficit (mm)	CWD	$= TP - Etc$
-	TP*Etc	= TP x Etc
-	TP/Etc	= TP/Etc
Solar Radiation (MJ m^{-2} day^{-1})	SR	-
Sun-Earth Distance	Dr	$= 1 + 0.033\cos(\frac{2\pi J_D\ **}{365})$
Solar Declination Angle	δ	$= 0.409\sin(\frac{2\pi J_D}{365} - 1.39)$
Sunset Hour Angle	ω	$= \arccos(-\tan(latitude)\tan(\delta)$
Top of the Atmosphere Radiation Radiation (MJ m^{-2} day^{-1})	TOA	$= \left(\frac{24 \times 60}{\pi}\right) 0.082 Dr(\omega \sin(latitude)\sin(\delta) + \cos(latitude)\cos(\delta)\sin(\omega))$
Cloud Cover (MJ m^{-2} day^{-1})	CC	$= TOA - SR$

*Kc = 0.3 at seeding, linear increase to Kc = 1.15 at crop emergence to anthesis, linear decrease to Kc = 0 at soft dough stage to maturity

**J_D = Day number of the year (i.e January 1st = 1, December 31 = 365)

3.3 Grain Quality Analysis and Milling

Upon receipt, ~1.5 kg of samples were placed in frozen storage (-30 °C) to eliminate live insect contamination. Cleaning of samples, grain protein content (GP) determination and official grading were conducted at Intertek (Winnipeg, MB). A Carter Day Dockage Tester was used to clean samples according to the Canadian Grain Commission's (CGC) Grain Grading Guide (Canadian Grain Commission 2015). The same guide was utilized for the official grading of samples, determining test weight (TW) as well as the level of *Fusarium* damaged kernels (FDK), midge damage, sprout damage (total and severe) and ergot. Grain protein content and moisture content were additionally determined using 400 g samples in a Foss Infratec™ 1241 Grain Analyzer at setting CWAD CPSW at both Intertek (Winnipeg, MB) and the Grain Quality Lab

(Winnipeg, MB). Thousand kernel weight (TKW) was derived using an International Marketing and Design Corporation Old Mill 850-3 seed counter. Three hundred kernels were counted in triplicate then converted to weight per thousand. Random human checks were additionally conducted to ensure accuracy. Deoxynivalenol (DON) analysis was conducted on samples using Neogen ELISA kits, with a sensitivity level of 0.5 ppm (Veratox for DON 5/5) and 0.1 ppm (Veratox for DON High Sensitivity) in 2015 and 2016 respectively. The 2015 Edmonton, Lethbridge and Swift Current sites did not undergo DON analysis due to the low or absent level of FDK.

Wheat tempering was facilitated by the use of a 4 ft section of a Hytrol 199-CRR conveyor (Hytrol Co., Jonesboro, Arkansas), with rolls covered in PVC rubber for extra grip of tempering jars (Fig 3.2). Using this system eliminated what has been in the past, a largely manual procedure. Typically, up to 25 wheat samples were tempered for milling the next day. Wheat in up to 15 jars (see specification below) could be tempered simultaneously with 3 jars per row in 5 rows. Each jar contained about 500 g wheat. Tempering jars were composed of food grade PET plastic (Uline, product S-18076, dimensions 5.125-inch-wide (4-inch-wide opening), 7 inch tall). Initial experiments indicated that milling 500 g wheat resulted in a moisture loss (wheat to flour) of about 0.5%. Accordingly, wheat was tempered to 14.5% moisture which resulted in flour having a MC of 14.0 +/- 0.2%.

Figure 3.2. Hytrol Power Flex 40 conveyor roll with sample container.

The procedure for tempering was as follows: to 500 g wheat, the appropriate amount of water was added based on wheat moisture determined by NIRS using a Foss Infratec™ 1241 Grain Analyzer system. Each jar was securely closed with the screw on lid, and manually shaken briefly until all seed was wetted, and then placed on the roller conveyor which turned the jars at a rate of about 1 rotation per second; the conveyor had a nominal specification of product movement of 90 feet/min at 60 hertz. Tempering jars were rotated until no more wheat adhered to the walls of the PEWT jars. Subsequently, the jars containing wheat were moved to shelves for tempering overnight (17-20 hr; range is time frame for milling next morning).

Milling was conducted using a Brabender Quadrumat senior mill (Fig 3.3) containing separate break and reduction heads, modified from Jeffers and Rubenthaler (1977) described as follows. This milling modification entailed removal of all the sieving components configured with the commercially available mill supplied by the manufacturer and replaced with a manual sieving protocol which greatly enhanced sample throughput. In preparation for milling, the mill

was operated for 90 min to allow the rolls to reach a consistent temperature. The latter was measured using a Fischer Scientific Traceable IR Sensor Temperature Gun aimed at the center spindle of each roll. The typical temperatures of 1st to 4th rolls of the break and reduction units were 33 ºC to 36 ºC and 39 ºC and 38 ºC respectively.

Figure 3.3. Brabender Quadrumat Senior experimental mill.

The sieving procedure involved the use of two oscillatory sieving machines (Sampl-Sifter model, Great Western Manufacturing, Leavenworth, KS) (Fig 3.4). One of these machines was dedicated for separating bran and middlings from flour using no. 35 and no. 100 sieves, respectively; 12 x 3 inch, stainless steel (W.S. Tyler Co., Mentor, OH). The sieving time was one and two min for separation of bran and middlings respectively. Wheat was fed onto the first break rolls falling at a natural rate through the open door of the mill. Middlings on top of the no.

100 sieve were manually fed into a vibratory feeder (Fig 3.5) (Syntron Co. Magnetic Feed (F-TO-C) controlled (speed 4 /10) by a Syntron PowerPulse WT Material Handling controller, down a chute of dimensions 12”L x 1 ¾”W, x 1”H, which in turn fed the grist into the reduction section of the mill break head. Product from the reduction step was sieved to separate shorts from flour using a no. 90 sieve for 3 min. Break and reduction flours were combined. The weights of flour, bran and shorts were recorded. Typical yields were 72%, 21%, and 4.8%, respectively. The milling of a single sample was complete in ~15 min.

Figure 3.4 Great Western Manufacturing sieve unit.

Figure 1. Syntron Magnetic Vibratory Feeder into reduction side of mill.

3.4 Biochemical Testing

Flour after milling was stored in polyethylene bags for one month to mature at room temperature. The final flour moisture and ash content was determined (AACC Approved Method 44-15.02 and 08-01.01). Flour protein content (FP) was determined by combustion nitrogen analysis (Nx5.7) (AACC Approved Method 46-30.01) and reported at 14% moisture basis. Flour testing was performed at least in triplicate for each milling sample. Soluble prolamin (SP) enriched protein and high molecular weight glutenin (HMWG) content of flour were determined as described by Sapirstein and Johnson (2000) with some modifications. The SP fraction comprised mainly gliadins, but also low molecular weight (LMW) glutenin and monomeric albumins and globulins (Fu and Sapirstein, 1996). Flour (50 mg) was extracted at room

temperature for 15 min with 1 mL 50% (v/v) 1-propanol (HPLC grade) in a 2 mL microcentrifuge tube with intermittent vortexing (~ every 5 min). Samples were then centrifuged (3 min, 5,000 x g) and the resulting supernatant was decanted. This extraction was then repeated with pellet disrupted using a glass rod to increase extraction efficiency. The tube was centrifuged for 3 min and 15,000 x g. The pooled supernatants constituted the SP fraction. Insoluble prolamin, i.e. HMW glutenin was extracted from the SP fraction residue with 1 mL 0.1% DTT (w/v) in 50% 1-propanol for 30 min at 55 °C. The SP residue was initially disrupted with a glass rod and vortexed for 5 s. Samples were subsequently vortexed at 10 min intervals and just prior to centrifugation which was carried out for 3 min at 15,000 x g. The microcentrifuge tube was inverted once to ensure extract homogeneity. Quantification of SP and HMW glutenin was done by UV absorbance at 214 nm using a 10 mm path length semi-micro quartz cuvette and a matching cuvette for the reference blank (50% 1-propanol). An aliquot of each protein fraction was diluted 50-fold with 50% 1-propanol (30 µL supernatant:1,470 µL 50% 1-propanol) in a microcentrifuge tube and vortexed for 5 s prior to measurement of UV absorbance. A protein calibration curve was established by using a stock solution of PWG-gliadin sourced from van Eckert et al. (2006). The ratio of HMW glutenin to SP content is herein referred to as gluten strength index or GSI. Current research ongoing in our laboratory in the context of genotype by environment studies of gluten strength of CWRS wheat varieties has indicated that the GSI parameter is strongly associated with gluten strength as evaluated by mixograph work input to peak development.

3.5 Empirical Rheological Testing

Mixograph analysis was performed in triplicate, using a Dynamic Machines Co. (Winnipeg, MB) torque sensing 10 g mixograph (Fig 3.6). Torque calibration was conducted with a 200 g weight. Flour was placed into the water-jacketed bowls along with 6.0 mL of a 2.5% (w/v) salt solution to achieve constant absorption of 60% and salt concentration of 1.5% (flour basis). Mixograph bowls and salt solution were equilibrated to 30 ^{o}C prior to use. This temperature was maintained during mixing using a circulating water bath. Mixograph curves were analyzed with RAR-P2M software (RAR Software Systems, Winnipeg, MB). Fig 3.7 shows a sample mixograph curve indicating parameters used. The latter were mixing development time (MDT), peak dough resistance (PDR), peak band width (PBW) and work input to peak (WIP) all derived based on the top line of the mixograph curve. A final parameter, work at peak resistance (WAP) was used and calculated as MDT*PBW (%Torque*min), which is independent of PDR with higher repeatability and precision (Appendix C).

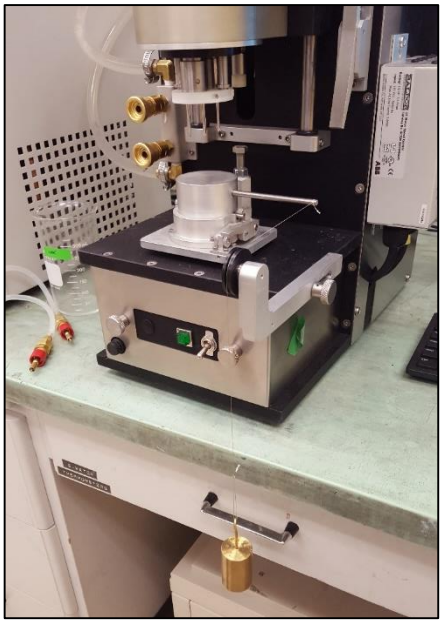

Figure 3.6. Dynamic Machines Co. torque sensing 10g mixograph with 200 g calibration weight.

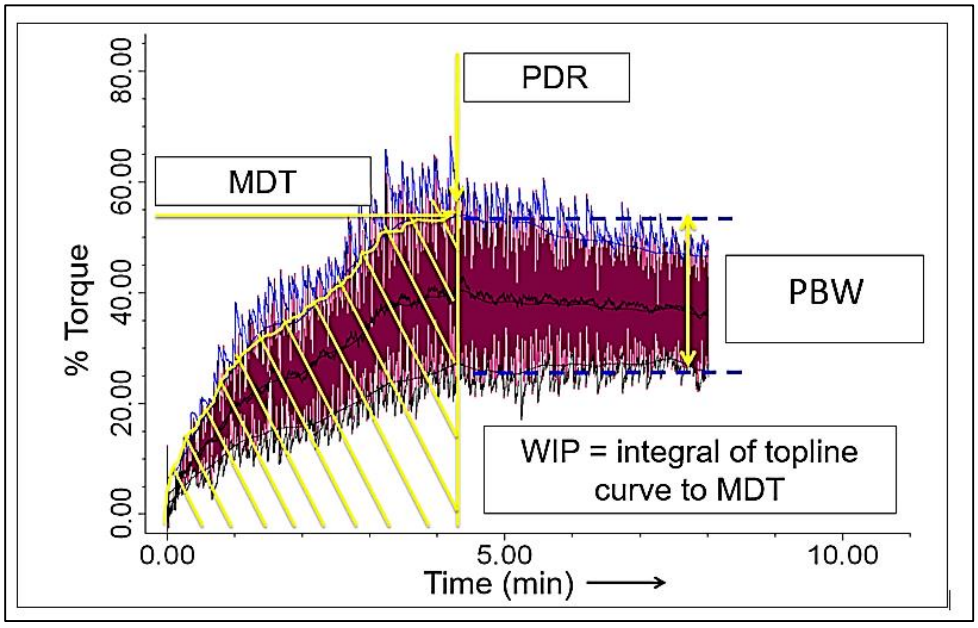

Figure 3.7. Sample mixograph curve delineating derivation of parameters mixing development time (MDT), peak dough resistance (PDR), peak band width (PBW) and work input to peak (WIP).

3.6 Experimental Design Statistical Analysis

Field trials were organized in a randomized complete block design with four replications per location. Two of these replications were randomly selected for milling, and samples within a location were milled in a random order. Statistical analysis was done using SAS software, version 9.4. All SAS codes can be found in Appendix A. Analysis of variance was conducted using two different procedures. The GLIMMIX procedure was used with replication as a random effect and location and genotypes as fixed when 2015 and 2016 crop years were analyzed individually. The growing seasons were also combined, in this case replication, location, year and interaction effects were assigned as random effects with genotype as the sole fixed effect. Significant differences ($p < 0.05$) between location and genotype means were determined using the Tukey-Kramer method. In Chapter 4.3, significant differences between grades were assessed at a lower level of significance ($p < 0.10$). Mean squares were derived from ANOVA analysis in a similar manner, except the MIXED procedure was used to yield Type III sum of squares. The variance contribution of genotype, location, year, replication and their interactions was taken as the sum of squares for the respective effect as a percentage of the total model sum of squares. The same ANOVA procedure was applied in Section 4.3, however grade was included as a fixed effect, with significant differences ($p < 0.10$) determined using the Tukey-Kramer method. In Sections 4.2 and 4.3, variety Glenn was removed from the sample set and ANOVA was determined normally. All parameters were initially tested for normality by the residual plots statement within the GLIMMIX procedure. Transformed variables included DON and FDK, by taking the square root of the concentration after a constant value had been added. Linear

regression and correlation analysis were conducted by using the Microsoft Excel's data analysis toolpak.

Weather analysis was conducted using stepwise regression using the STEPWISE procedure with the maxr statement to maximize R^2 values. Parameters were assessed for normality based on homogeneity of variances. It is important to note that there existed a higher number of explanatory variables than observations. In addition, weather data is highly collinear which violates one of the requirements for regression analysis, although the nature of the stepwise regression procedure does not place variables that are collinear within the same model. That being said, the models created using the stepwise regression procedure were not intended to predict any of the gluten strength parameters analyzed. Rather, the intention of the regression analysis was to get a preliminary, simple assessment of the nature of environmental effects on gluten strength. This procedure yielded linear regression models that included the best one, two, three and four variable models to explain variation in the selected gluten strength parameter. The variables included in the models were weather parameters (Table 3.5) compiled into calendar day intervals from planting to maturity. Either the average or cumulative sum of the weather variable was used for each group. The number of intervals examined and their abbreviations were two (1 and 2), four (Q1, Q2, Q3, Q4), six (H1, H2,…,H6), eight (O1, O2, … , O8), ten (D1, D2, … , D10) and twelve (T1, T2, … , T12). Intervals were calculated as follows (RAR Software Systems; Winnipeg, MB):

$$Days_{Start-to-End} = DayNumber_{Maturity} - DayNumber_{Planting}$$

$$Start_{Group1} = DayNumber_{Planting}$$

$$End_{Group1} = Integer\left(DayNumber_{Planting} + \left(1 \times \frac{Days_{Start-to-End}}{No.of\ Divsions}\right)\right) - 1 \ ;$$

$$Start_{Group2} = End_{Group1} + 1$$

$$End_{Group2} = Integer\left(Start_{Group2} + \left(2 \times \frac{Days_{Start-to-End}}{No.of\ Divsions}\right)\right) - 1 \ ;$$

$$Start_{Group3} = End_{Group2} + 1$$

$$End_{Group3} = Integer\left(Start_{Group3} + \left(2 \times \frac{Days_{Start-to-End}}{No.of\ Divsions}\right)\right) - 1 \ ;$$

$$\dots$$

$$Start_{N-1} = End_{N-2} + 1$$

$$End_{N-1} = Integer\left(Start_{N-1} + \left((N-1) \times \frac{Days_{Start-to-End}}{No.of\ Divsions}\right)\right) - 1 \ ;$$

$$Start_{N} = End_{N-1} + 1$$

$$End_{N} = DayNumber_{Maturity} \ ;$$

Where DayNumber, Start, and End are day number of the year from 1 to 365.

4.1 Variation in Canada Western Red Spring Wheat Quality

4.1.1 Grade and Degrading Factors

The grades of individual genotypes ranged between 1.7-2.3 and 2.2-3.5 in 2015 and 2016 respectively (Table 4.1.1). It is important to note that the statistical analysis of Grade is not valid due to the nominal nature of the data, however is presented for illustrative purposes. Stettler demonstrated the lowest mean grade in both years, whereas Glenn had the highest mean grade in both 2015 and 2016. The large standard deviations for each cultivar indicate a large difference in grade between locations for the same genotype, most notably in 2016 (Table 4.1.1). It follows that large differences in grade for location (averaged across genotypes) was observed in both 2015 and 2016 (Table 4.1.2), ranging from 1.0-2.8 and 1.2-4.8 respectively. It is noteworthy that the 2016 crop year produced lower grades on average (2.6) than those in 2015 (1.9), in addition to the majority of samples grading CWRS no.2 (132), compared to CWRS no.1 (85), CWRS no.3 (47), feed grade (42) and below feed (18). Despite this, Lethbridge, Edmonton and Goodale remained the highest grading locations between both years, particularly Lethbridge grading CWRS no.1 for all samples in 2015. Generally, the standard deviations for location were much smaller than those for genotypes (range and CVs for all parameters are documented in Appendix B.1). Considering all samples, grade ranged from 1.0 to 5.0 (Appendix B.1).

Table 4.1.1. Genotype means and standard deviations of DON and CWRS wheat degrading factors.

Genotype	Year	Grade[a]	FDK (%)	DON[b] (ppm)	Ergot (%)	Midge (%)	Sprout (%)
Brandon	*2015*	1.78 ± 0.81	0.19 ± 0.33	0.55 ± 0.56	-	1.14 ± 2.23	0.01 ± 0.03
	2016	2.83 ± 1.25	0.62 ± 0.68	1.00 ± 1.24	0.03 ± 0.05	1.43 ± 1.53	0.35 ± 0.34
Carberry	*2015*	1.89 ± 0.76	0.24 ± 0.36	0.42 ± 0.55	0.03 ± 0	0.99 ± 1.49	-
	2016	2.50 ± 1.15	0.67 ± 0.79	1.47 ± 1.86	0.04 ± 0.05	1.16 ± 1.36	0.43 ± 0.89
Cardale	*2015*	2.00 ± 0.77	0.20 ± 0.48	0.22 ± 0.34	-	1.24 ± 1.64	0.01 ± 0.02
	2016	2.50 ± 1.54	0.37 ± 0.30	0.72 ± 0.98	0.05 ± 0.14	1.68 ± 1.73	0.42 ± 0.67
Glenn	*2015*	1.72 ± 0.83	0.28 ± 0.35	0.43 ± 0.65	0.01 ± 0	1.11 ± 1.95	0.01 ± 0.03
	2016	2.17 ± 1.20	0.60 ± 0.94	1.41 ± 1.96	0.01 ± 0.02	0.87 ± 1.15	0.46 ± 0.76
Harvest	*2015*	1.94 ± 0.64	0.35 ± 0.46	0.93 ± 0.77	0.01 ± 0	0.34 ± 0.58	-
	2016	3.00 ± 1.37	1.26 ± 1.87	2.32 ± 2.66	0.05 ± 0.05	0.73 ± 0.81	0.15 ± 0.30
Stanley	*2015*	2.00 ± 0.59	0.35 ± 0.46	0.81 ± 0.76	-	0.61 ± 1.04	0.01 ± 0.05
	2016	2.72 ± 1.13	1.26 ± 1.87	1.71 ± 2.15	0.04 ± 0.06	0.82 ± 0.80	0.11 ± 0.15
Stettler	*2015*	2.33 ± 1.08	0.70 ± 0.85	1.95 ± 1.67	-	0.80 ± 0.99	0.06 ± 0.18
	2016	3.50 ± 1.29	2.10 ± 2.64	3.14 ± 3.22	0.01 ± 0.01	1.12 ± 0.93	0.75 ± 1.24
Unity	*2015*	1.72 ± 0.57	0.28 ± 0.42	0.63 ± 0.64	0.02 ± 0	0.16 ± 0.15	-
	2016	2.33 ± 1.53	0.73 ± 1.32	1.56 ± 1.96	0.04 ± 0.06	0.52 ± 1.08	0.23 ± 0.59
Utmost	*2015*	2.00 ± 0.84	0.46 ± 0.67	1.45 ± 1.41	-	0.31 ± 0.44	-
	2016	2.61 ± 1.29	1.19 ±1.59	2.08 ± 2.26	0.00 ± 0.01	0.69 ± 0.61	0.14 ± 0.37

FDK = Fusarium damaged kernels, DON = Deoxynivalenol

[a]The statistical analysis of grade is not valid due to the nominal nature of the data, however is presented for illustrative purposes

[b]DON analysis was not conducted in 2015 for Edmonton, Lethbridge and Swift Current and had different levels of sensitivity for 2015 and 2016 analysis; 0.5 and 0.1 ppm respectively.

Table 4.1.2. Location means and standard deviations of DON and CWRS wheat degrading parameters.

Location	Year	Grade[a]	FDK (%)	DON[b] (ppm)	Ergot (%)	Midge (%)	Sprout (%)
Beaverlodge	*2015*	2.17 ± 0.71	0.17 ± 0.52	0.00 ± 1.02	-	0.11 ± 1.35	0.01 ± 0.07
	2016	3.39 ± 1.04	0.15 ± 0.29	0.00 ± 0.00	0.00 ± 0.00	0.82 ± 0.58	1.57 ± 1.15
Brandon	*2015*	2.39 ± 0.61	0.77 ± 0.62	0.93 ± 1.07	0.01 ± 0.01	0.70 ± 0.64	0.02 ± 0.04
	2016	4.83 ± 0.38	4.06 ± 2.27	5.72 ± 1.77	0.13 ± 0.13	0.93 ± 0.58	0.08 ± 0.11
Edmonton	*2015*	1.78 ±0.43	0.01 ± 0.02	-	-	0.54 ± 0.32	0.00 ± 0.00
	2016	2.11 ± 1.02	0.08 ± 0.08	0.01 ± 0.03	0.02 ± 0.01	0.72 ± 0.41	0.27 ± 0.24
Goodale	*2015*	1.11 ± 0.32	0.03 ± 0.05	0.00 ± 0.00	-	0.09 ± 0.11	0.01 ± 0.02
	2016	1.72 ± 0.67	0.39 ± 0.27	0.73 ± 0.63	0.00 ± 0.00	0.53 ± 0.32	0.12 ± 0.11
Indian Head	*2015*	2.78 ± 0.43	0.77 ± 0.40	1.73 ± 1.15	-	3.49 ± 2.36	0.01 ± 0.05
	2016	3.50 ± 0.99	2.29 ± 1.75	4.27 ± 2.10	0.02 ± 0.02	3.34 ± 1.73	0.62 ± 0.93
Kelburn	*2015*	1.72 ± 0.67	0.36 ± 0.39	0.60 ± 0.55	-	0.19 ± 0.06	0.00 ± 0.00
	2016	2.00 ± 0.77	0.48 ± 0.28	1.82 ± 0.96	0.00 ± 0.00	0.51 ± 0.30	0.09 ± 0.07
Lethbridge	*2015*	1.00 ± 0.00	0.00 ± 0.01	-	-	0.20 ± 0.21	0.00 ± 0.00
	2016	1.17 ± 0.38	0.02 ± 0.04	0.08 ± 0.05	0.00 ± 0.00	0.17 ± 0.17	0.00 ± 0.00
Morden	*2015*	2.67 ± 0.69	0.95 ± 0.58	1.66 ± 0.85	-	0.87 ± 0.68	0.02 ± 0.04
	2016	2.72 ± 1.07	0.83 ± 0.75	1.99 ± 0.98	0.02 ± 0.02	0.42 ± 0.16	0.11 ± 0.13
Swift Current	*2015*	1.78 ± 0.43	0.00 ± 0.00	-	-	0.14 ± 0.13	0.08 ± 0.18
	2016	2.72 ± 0.96.	0.63 ± 0.34	0.77 ± 0.68	0.04 ± 0.02	1.56 ± 1.23	0.17 ± 0.20

FDK = Fusarium damaged kernels, DON = Deoxynivaloenol

[a]The statistical analysis of grade is not valid due to the nominal nature of the data, however is presented for illustrative purposes

[b]DON analysis was not conducted in 2015 for Edmonton, Lethbridge and Swift Current and had different levels of sensitivity for 2015 and 2016 analysis; 0.5 and 0.1 ppm respectively.

ANOVA results for separate years (Table 4.1.3) demonstrated that location effects contributed much more to the variance of grade than did genotype, as would be expected. Despite this, the genotype effect was highly significant in both years ($p < 0.001$). Genotypes that differed significantly in Grade, FDK and DON in 2016 are shown graphically in Appendix D.3 – D.6. It can be seen that while differences among a few genotypes were statistically significant, their practical significance is probably not compelling due to the relatively lower percent contribution to overall variance. The effect of genotype by location interaction (GxL) was also significant in both years ($p < 0.001$). That replication was significant for grade and FDK indicates that plot location in a field can influence these parameters, although the variance contributions were minimal. Considering all samples, FDK and DON ranged from 0.0% to 7.80% and 0.0 ppm to 0.56 ppm, respectively (Appendix B.1).

Interestingly, ANOVA of combined years (Table 4.1.4) yielded different results than when years were analyzed individually. While location still exhibited the highest percentage of variance contribution, its effect, as well as that for genotype, was not significant. Rather, it was location by year interaction (LxY) and genotype by location by year interaction (GxLxY) that was significant ($p < 0.0001$) as well as for replication ($p < 0.01$). Presumably, the significant effect of location observed in individual years was captured in the LxY interaction as the years were combined in the analysis. This suggests that each location-year combination acts as its own unrelated "site-year", reflecting the different weather/environmental conditions of locations that ultimately resulted in grade variability. In addition, the significant effect of replication on grade indicates that field location can affect grade, as was observed for the ANOVA when years were separated. Similarly, the contribution of replication to grade variance was minimal (0.99%, Table

4.1.4). Therefore, although statistically significant, the effect of replication did not have any practical significance.

Table 4.1.3. Percent variance contribution of mixed model effects on CWRS grade and select degrading factors for separate years.

	Year	Genotype	Location	GxL	Rep	Residual
Grade[a]	*2015*	4.70***	57.68****	24.31****	3.01*	10.29
	2016	8.10****	61.55****	19.75***	0.69 NS	9.91
FDK	*2015*	4.82****	71.14****	17.79****	1.13**	5.11
	2016	8.20****	72.87****	15.12****	`0.20 NS	3.61
DON	*2015*	6.93****	58.11****	4.98 NS	0.37 NS	29.62
	2016	7.21****	86.66****	4.66****	0.02 NS	1.44

[a]The statistical analysis of grade is not valid due to the nominal nature of the data, however is presented for illustrative purposes
*, **, *** and **** significant at p<0.5, p<0.01, p<0.001 and p<0.0001 respectively
FDK = Fusarium damaged kernels, DON = Deoxynivalenol, NS = no significance

Table 4.1.4. Percent variance contribution of mixed model effects on CWRS grade and select degrading factors for combined years.

	Genotype	Location	Year	G x L	G x Y	L x Y	GxLxY	Rep	Residual
Grade[a]	5.12 *	45.77 NS	9.55 NS	10.42 NS	1.35 NS	8.75****	8.81****	0.99**	9.24
FDK	5.45 NS	55.40 NS	9.34 NS	10.58***	0.86 NS	10.00****	4.25****	0.22 NS	3.91
DON	7.11***	61.86 NS	13.48 *	4.62 NS	0.41 NS	7.98****	2.15****	0.03 NS	2.36

[a]The statistical analysis of grade is not valid due to the nominal nature of the data, however is presented for illustrative purposes
*, **, *** and **** significant at p<0.05 p<0.01, p<0.001 and p<0.0001 respectively
FDK = Fusarium damaged kernels, DON = Deoxynivalenol, NS = no significance

Variation in grade is reflected by the varying presence of degrading factors. Table 4.1.2 presents results for FDK, DON (not a degrading factor), ergot, midge and sprout damage in both 2015 and 2016 locations averaged over genotypes. In general, there was a higher degree of damage reflected in all degrading factors in 2016 compared to 2015 (Appendix B.1), with the exception of FDK in Beaverlodge as well as the midge in Indian Head, Lethbridge and Morden. FDK was the predominant degrading factor as it was generally present in field samples for both years, with the exception of Edmonton and Lethbridge. In 2015, the relatively few *Fusairium* Head Bight (FHB) infected samples did not contain more than 0.05% FDK, whereas in 2016 Lethbridge and Edmonton samples never exceeded 0.1% and 0.3% FDK respectively. In

contrast, Brandon and Indian Head 2016 displayed exceptionally high levels of FDK at 4.1% and 2.3% respectively. These values are much higher than those for other locations which never exceed 1.0% FDK. Individual genotype samples in Brandon 2016 ranged from 0.8 -7.8% and from 0.8 - 6.3% in Indian Head 2016. Genotype values averaged across locations for degrading factors and DON (Table 4.1.1) show that the variation of *Fusarium* damage among genotypes was relatively low, ranging from 0.19 - 0.46% and 0.37- 1.39% FDK in 2015 and 2016, respectively. These values exclude Stettler, which displayed notably higher FDK levels of 0.79% (2015) and 2.10% (2016). These results are in line with Stettler's "poor" rating for FHB resistance and recommendation to be grown in western Saskatchewan and Alberta by its seed owner, Secan (Anonymous 2016).

The effect of location by (Table 4.1.3) was highly significant ($p < 0.0001$) for FDK and contributed ~70% of the variation. Although the variance contributions of genotype and GxL effects were much smaller, they were highly significant ($p < 0.0001$). In 2015, the replication effect for FDK was also significant ($p < 0.01$). However, similar to grade, the percent variance contribution of the replication effect was very small (1.1%). ANOVA for FDK showed significant interactions for GxL ($p < 0.001$), LxY ($p < 0.0001$) and GxLxY ($p < 0.0001$) (Table 4.1.4). While not significant, location still exhibited the largest contribution to variance in FDK at 55.4%.

Mycotoxin analysis for DON was conducted on the majority of samples, excluding Edmonton, Lethbridge and Swift Current in 2015 due to the low prevalence of FDK. Similar to FDK, DON concentration was generally higher in 2016 in comparison to 2015 (Table 4.1.1 and Tabl3 4.1.2). This is expected, as the production of DON results from *Fusarium* infection. It

follows that DON and FDK were strongly and positively correlated ($R^2 = 0.80$, $p<0.0001$) (Fig 4.1.1). As the 2016 crop year displayed higher levels of FHB infection, the protocol for DON analysis was changed using an ELISA kit with higher sensitivity (0.5 ppm in 2015, 0.1 ppm in 2016) to enhance detection of differences among genotypes and locations. In 2016, the locations displaying the highest DON concentrations were congruent with locations exhibiting the highest levels of FDK: Brandon (5.7 ppm) and Indian Head (4.3 ppm). For both growing seasons, Beaverlodge, Edmonton and Lethbridge were relatively free of DON; the highest genotype sample contained 0.2 ppm (Lethbridge 2016). DON ranged from 0.2 ppm to 2.0 ppm and 0.4 ppm to 2.6ppm across genotypes for 2015 and 2016, respectively (Table 4.1.1). Averaged across locations, Stettler and Cardale were genotypes with the highest and lowest DON concentrations, respectively in both 2015 and 2016.

ANOVA for DON for separated years displayed a similar trend to both FDK and grade, i.e. location effects were highly significant ($p < 0.001$) with location contributing the highest proportion to variance (Table 4.1.3). However, the location variance contribution was notably higher in 2016 than 2015, 86.7% and 58.1%, respectively. This could be explained by the omission of certain locations for DON analysis in 2015 (due to low or absent FDK levels) that may have decreased the contribution of the location effect. The residual effect in 2015 displayed an unusually high contribution to DON variance (29.6%), suggesting either an external effect not specified in the ANOVA was responsible for a large portion of the DON variation in that year or a large degree of experimental error. This may be a result of the lower degree of sensitivity used in 2015 (0.5 ppm vs 0.1 ppm in 2016), which could have introduced a higher source of error in comparison to 2016. Otherwise, both genotype and GxL interaction were significant effects ($p < 0.001$) for both years, with the exception of GxL 2015 which did not have as significant effect on

DON. Analysis of variance for combined years demonstrated significant effects for both LxY and GxLxY ($p < 0.0001$) (Table 4.1.4). Location effects, while not significant, remained the highest contributor to variance in DON at 61.9%. Genotype displayed a significant effect ($p<0.001$) on DON concentration. As a significant genotype effect was not observed in ANOVA for Grade or FDK, the significant effect of genotype on DON concentration may have resulted due to the high levels of DON observed in Stettler in comparison to the other genotypes.

Ergot was not highly prevalent in either year. In 2015, the only location that tested for ergot was Brandon, with four samples ranging from 0.006 to 0.028% (Table 4.1.2). However, Brandon in 2016 contained much higher levels of ergot, ranging from 0.00 to 0.56%. Because of this, many of the samples from Brandon 2016 did not even meet CWRS Feed grade standards. Samples from all other locations in 2016 did not exceed 0.08%. There were no observable trends in genotype ergot levels; all were relatively low ranging from 0.01 to 0.05% (Table 4.1.4)

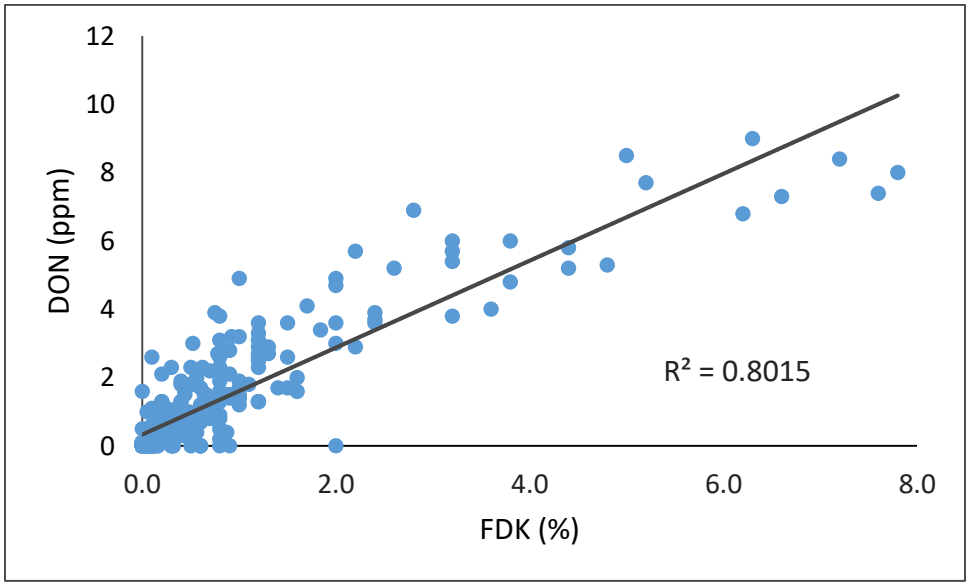

Figure 4.1.1 Relationship between FDK and DON including all samples from 2015 and 2016.

Midge damage was prevalent in both years, ranging from 0.09 – 3.49% in 2015 and 0.17 – 3.34% in 2016 across locations (Table 4.1.2). Indian Head displayed notably higher levels of midge damage; individual samples ranging from 0.3 – 8.0% and 0.3 – 6.0% in 2015 and 2016, respectively. In addition, Swift Current in 2016 displayed relatively high levels of midge damage ranging from 0.2 - 3.7%, however in 2015 low levels of midge damage was observed (0 - 0.5%). Midge damage ranged from 0.16% to 1.24% and 0.52% to 1.68% across genotype in 2015 and 2016, respectively. As expected, the two midge tolerant varieties exhibited the lowest mean levels of midge damage; 0.2% and 0.5% for Unity, and 0.3% and 0.7% for Utmost in 2015 and 2016, respectively (Table 4.1.1). Cardale and Brandon had the highest level of midge damage of 1.2% and 1.1% in 2015 and 1.7% and 1.4% in 2016, respectively.

Sprout damage ranged from 0.00 – 0.08% and 0.00 – 1.57% across locations in 2015 and 2016, respectively (Table 4.1.2).The range of sprout damage across genotypes was 0.01% to 0.06% in 2015 and 0.14% to 0.75% in 2016 (Table 4.1.3). There were no observable trends for sprout damage over locations or genotypes in both years.

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4.1.2 Grain Quality Factors

Test weight (TW) ranged from 78.6 to 82.7 kg/hL and 78.4 to 82.9 kg/hL across genotypes for 2015 and 2016, respectively (Table 4.1.5). Glenn displayed significantly ($p < 0.05$) higher TW than the other cultivars across years. This was consistent with Glenn having the highest grade, averaged across locations and can be shown in Table 4.1.7. TW is one of the grading factors for CWRS. For locations, TW ranged between 77.3 and 83.3 kg/hL in 2015 as well as 77.4 and 81.1 kg/hL in 2016 (Table 4.1.6). There were no observable trends in TW

across locations. Considering all samples TW ranged from 73.2 kg/hL to 85.8 kg/hL (Appendix B.1).

Table 4.1.5. Genotype means and standard deviation of test weight, thousand kernel weight and grain protein content.

2015	BR	CB	CD	GL	HA	SN	ST	UN	UT
TW (kg/hL)	80.25^{B}	80.74^{B}	78.59^{D}	82.74^{A}	79.27^{C}	78.51^{D}	79.25^{C}	80.53^{B}	78.81^{CD}
	± 2.57	± 2.38	± 3.12	± 1.87	± 2.49	± 2.61	± 3.08	± 2.29	± 2.67
TKW (g)	34.90^{A}	34.19^{AB}	32.92^{CD}	32.80^{D}	34.06^{ABC}	32.21^{D}	33.00^{CD}	33.31^{BCD}	33.22^{BCD}
	± 2.97	± 3.55	± 3.54	± 2.95	± 2.78	± 2.81	± 3.49	± 3.19	± 3.46
GP (%)	14.95^{B}	15.17^{AB}	15.09^{AB}	14.99^{AB}	14.63^{C}	14.43^{C}	15.31^{A}	14.53^{C}	14.56^{C}
	± 1.11	± 1.08	± 1.30	± 1.11	± 1.50	± 1.28	± 1.44	± 1.44	± 1.36
2016	BR	CB	CD	GL	HA	SN	ST	UN	UT
TW (kg/hL)	80.40^{BC}	80.02^{C}	78.43^{EF}	82.91^{A}	78.88^{D}	78.29^{F}	78.79^{DE}	80.61^{B}	78.42^{EF}
	± 0.98	± 0.99	± 1.31	± 1.10	± 1.59	± 1.53	± 1.53	± 1.29	± 1.51
TKW (g)	33.33^{A}	32.46^{ABC}	31.50^{DE}	32.33^{BCD}	33.02^{AB}	31.33^{E}	31.86^{CDE}	31.93^{CDE}	32.34^{BCD}
	± 2.97	± 3.58	± 3.00	± 3.05	± 2.90	± 3.37	± 2.91	± 2.76	± 2.91
GP (%)	14.49^{B}	14.54^{B}	14.68^{B}	14.42^{B}	14.81^{B}	14.77^{B}	15.34^{A}	13.97^{C}	14.56^{B}
	± 1.36	± 1.42	± 1.62	± 1.60	± 1.78	± 1.76	± 1.80	± 1.75	± 1.54

TW = Test Weight, TWK = Thousand Kernel Weight, GP = Grain Protein Content
Means in the same row with different letter groupings are statistically different according to Tukey-Kramer method ($p<0.05$)

Table 4.1.6. Location mean and standard deviation of test weight, thousand kernel weight and grain protein content.

2015	BE	BR	ED	GO	IH	KE	LE	MO	SC
TW (kg/hL)	80.37^{C}	79.57^{D}	82.56^{B}	82.58^{B}	77.77^{F}	76.67^{G}	83.33^{A}	77.28^{E}	78.55^{E}
	± 2.84	± 1.35	± 1.16	± 1.23	± 1.30	± 1.93	± 1.11	± 2.00	± 1.59
TKW (g)	34.13^{C}	32.73^{D}	38.39^{A}	36.50^{B}	32.53^{D}	29.53^{E}	35.76^{B}	29.47^{E}	31.57^{D}
	± 3.33	± 1.49	± 2.06	± 0.90	± 1.60	± 1.23	± 2.04	± 1.15	± 2.10
GP (%)	13.92^{E}	16.19^{A}	15.77^{B}	15.63^{B}	14.93^{C}	15.91^{AB}	14.56^{D}	12.22^{F}	14.54^{D}
	± 1.30	± 0.44	± 0.42	± 0.38	± 0.98	± 0.44	± 0.67	± 0.47	± 0.63
2016	BE	BR	ED	GO	IH	KE	LE	MO	SC
TW (kg/hL)	81.09^{A}	77.41^{E}	80.83^{A}	79.66^{BC}	79.78^{BC}	79.45^{CD}	80.04^{B}	79.37^{CD}	79.15^{D}
	± 1.14	± 1.59	± 1.53	± 1.42	± 1.82	± 1.25	± 1.47	± 2.00	± 2.43
TKW (g)	37.58^{A}	28.02^{F}	35.97^{B}	32.33^{C}	31.11^{DE}	31.08^{DE}	31.62^{CD}	31.92^{C}	30.47^{E}
	± 1.32	± 1.15	± 1.37	± 1.05	± 1.17	± 1.79	± 1.97	± 0.99	± 1.49
GP (%)	14.83^{D}	16.17^{A}	14.97^{CD}	15.72^{AB}	12.32^{E}	15.48^{B}	15.21^{BCD}	15.38^{BC}	11.49^{F}
	± 0.56	± 0.57	± 0.59	± 0.53	± 0.92	± 0.53	± 0.44	± 0.57	± 0.70

TW = Test Weight, TWK = Thousand Kernel Weight, GP = Grain Protein Content
Means in the same row with different letter groupings are statistically different according to Tukey-Kramer method ($p<0.05$)

Table 4.1.7 Correlation matrix between grain, grade and degrading quality parameters.

	Grade	TW	TKW	GP	Ergot	Midge	FDK	Sprout	DON
Grade	1.00								
TW	-0.44	1.00							
TKW	-0.33	0.68	1.00						
GP	-0.11	0.06	0.21	1.00					
Ergot	0.53	-0.37	-0.46	0.12	1.00				
Midge	0.37	-0.14	-0.13	-0.23	0.07	1.00			
FDK	0.70	-0.38	-0.48	-0.02	0.42	0.22	1.00		
Sprout	0.40	0.09	0.22	-0.09	-0.16	0.17	0.08	1.00	
DON	0.68	-0.39	-0.53	-0.04	0.47	0.26	0.91	0.00	1.00

FDK = Fusarium damaged kernels, DON = Deoxynivalenol, TW = Test Weight, TWK = Thousand Kernel Weight, GP = Grain Protein Content

ANOVA for TW showed significant effects ($p < 0.001$) of genotype, location and GxL in 2015 and 2016 (Table 4.1.8). Interestingly, replication significantly ($p < 0.001$) affected TW only in 2015, despite its small percent variance contribution of 0.5%. This is similar to the results of Bhatt and Derera (1975) in which replication within sites was a significant ($p < 0.01$) effect on TW. The most significant contributor to TW variance differed between years, i.e. location in 2015 (69.8%) and genotype in 2016 (56.2%). This may be due to 2015 locations having much larger ranges than that found in 2016, therefore rendering it a more significant effect for that year. ANOVA for combined years showed similar variance contributions of genotype and location at 31.0% and 35.0%, respectively (Table 4.1.9), although only genotype was significant ($p < 0.0001$). The ANOVA for combined years additionally revealed significant ($p < 0.0001$) LxY, GxLxY and replication effects on TW.

Table 4.1.8 Percent variance contribution of mixed model effects on grain quality factors for separate years.

	Year	Genotype	Location	GxL	Rep	Residual
TW	*2015*	20.25****	69.75****	8.01****	0.54***	1.45
	2016	56.24****	27.64****	14.13***	0.20 *	1.79
TKW	*2015*	5.81****	76.08****	12.51***	0.69 NS	4.91
	2016	3.78****	80.28****	12.36***	0.27 NS	3.31
GP	*2015*	5.26****	81.90****	10.33***	0.07 NS	2.45
	2016	3.92****	86.47****	6.16**	0.02 NS	3.43

*, **, *** and **** significant at p<0.05, p<0.01, p<0.001 and p<0.0001 , respectively
TW = Test Weight, TKW = Thousand Kernel Weight, GP = Grain Protein Content, NS = no significance

Table 4. Percent variance contribution of mixed model effects on grain quality factors for combined years.

	Genotype	Location	Year	G x L	G x Y	L x Y	GxLxY	Rep	Residual
TW	31.04***	35.01 NS	0.14 NS	6.03 NS	0.37 NS	20.89***	4.54***	0.40***	1.58
TKW	4.44*	55.82 NS	3.14 NS	8.69*	0.35 NS	18.98***	3.94***	0.43*	4.20
GP	3.43 NS	48.92 NS	0.63 NS	6.33***	1.40***	34.93***	1.49 NS	0.03 NS	2.84

*, **,*** and **** significant at p<0.05, p<0.01, p<0.001 and p<0.0001 , respectively
TW = Test Weight, TKW = Thousand Kernel Weight, GP = Grain Protein Content, NS = no significance

Thousand kernel weights (TKW) ranged from 32.8 g to 34.9 g and 31.3 g to 33.3 g across genotypes in 2015 and 2016, respectively (Table 4.1.5). Cultivar Brandon displayed the highest TKW in both 2015 and 2016, although not statistically higher ($p < 0.05$) than Carberry or Harvest in either year. The TKW ranges across locations were more than those across genotypes, ranging from 29.5 g to 38.4 g in 2015 and 28.0 g to 37.6 in 2016 (Table 4.1.6). With the exception of Beaverlodge, Kelburn and Lethbridge, TKW was generally larger in 2015 than 2016. Individual varieties varied widely in TKW, which ranged for individual samples, from 26.2 – 43.3g with a mean of 32.8 ± 3.2 g.

ANOVA indicated a significant ($p < 0.0001$) genotype, location and GxL interaction effect for TKW in both 2015 and 2016 (Table 4.1.8). Location contributed the most to TKW variance at 76.1% and 80.3% for 2015 and 2016, respectively. This was expected due to the large range of kernel size across locations. Erekul and Kohn (2006) reported significant changes in

TKW due to many environmental conditions including water deficit, above-average temperatures and plant densities. It follows that location remained the largest contributor of TKW variance (55.8%) in ANOVA for combined years, however it was no longer significant (Table 4.1.9). However, genotype remained significant ($p < 0.01$), as well as GxL, GxY and LxY interactions ($p < 0.0001$), when years were combined.

Grain protein content (GP) variation among genotypes was relatively small and ranged from 14.5% to 15.3% in 2015 and 14.0% to 15.3% in 2016 (Table 4.1.5). Cultivar Stettler had the highest grain protein content across years, and was significantly higher ($p <0.05$) than that for the other cultivars in 2016. Despite the small differences in mean grain protein content between genotypes, the range between samples across locations was very large (10.1 – 17.7%) and surprisingly so, as field sites were optimally fertilized. Sample variation in protein content ranged from 12.2% to 16.2% in 2015 and 11.5% to 16.3% in 2016. Interestingly, the Brandon location had the highest grain protein content in both years (Table 4.1.6). A reason for this outcome was unclear, especially considering that the ranking of locations for GP was completely different in 2015 to 2016 (Fig 4.1.2). This indicates that the physical location of the crop, including soil type, had no bearing on GP, whose variation appears to be the result of weather effects which varied considerably across locations and years (discussed later). The significant effect of growing location or environment on GP is reflected in the 2015 and 2016 ANOVA (Table 4.1.8, $p < 0.0001$). For individual years, location accounted for approximately 82% of the total variance in GP (Table 4.1.8). Additionally, ANOVA of combined years showed significant effects ($p < 0.0001$) of LxY, GxL and GxY interactions on GP (Table 4.1.9). In particular, LxY contributed substantially (35.9%) to the variance of GP, close to the amount contributed by location (48.9%). This is similar to the LxY interaction effect observed for ANOVA of grade, in

which each “site-year” was, in effect, an individual location, encompassing a different set of environmental conditions.

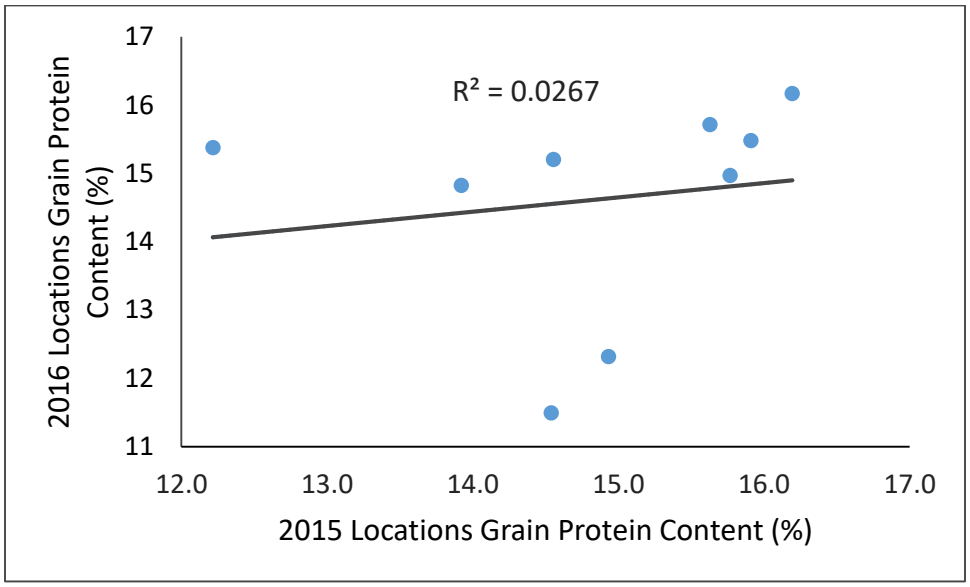

Figure 4.1.2. Relationship between mean grain protein contents for 2015 and 2016 growing sites.

4.1.3 Milling Quality

As is discussed below, the milling quality of samples overall was very consistent regardless of genotype, location and year, and although some parameters were statistically significant, the practical significance of differences was marginal. Flour yield (FY) did not exhibit large variation across genotypes, ranging from 71.5% to 73.6% in 2015 and 70.5% to 73.0% in 2016 (Table 4.1.10). This range is similar to that displayed across locations from 70.4% to 74.0% and 70.6% to 72.7% in 2015 and 2016, respectively (Table 4.1.11). There did not exist any relationship for FY ranking, averaged, across genotypes, between locations in 2015 and 2016, indicating that growing location did not influence FY. In contrast, the relationship between genotype mean values for FY ranked similarly between years, as reflected by $R^2 = 0.86$ ($p<0.001$; result not shown), despite the small 2% range in genotype means. This indicates a

strong influence of genotype on flour yield. Despite the small range exhibited by genotype and location, individual genotype samples varied more widely from 66.5% - 75.4% (Appendix B.1).

Table 4.1.10 Genotype means and standard deviation for milling quality parameters.

2015	BR	CB	CD	GL	HA	SN	ST	UN	UT
FY (%)	73.13^{AB}	72.03^{CD}	72.62^{BC}	71.46^{D}	73.06^{AB}	73.56^{A}	72.15^{CD}	72.95^{AB}	72.49^{BC}
	± 1.01	± 1.28	± 1.25	± 0.80	± 1.07	± 1.17	± 1.14	± 1.86	± 1.47
SC (%)	4.64^{CD}	4.99^{ABC}	4.83^{BCD}	5.40^{A}	5.01^{ABC}	3.82^{E}	4.74^{BDC}	5.19^{AB}	4.53^{D}
	± 0.55	± 0.62	± 0.65	± 0.47	± 0.66	± 0.52	± 0.57	± 0.64	± 0.43
BC (%)	21.32^{DEF}	22.09^{ABC}	21.58^{CDE}	22.18^{AB}	21.04^{EF}	21.66^{BCD}	22.27^{A}	21.01^{F}	22.07^{ABC}
	± 1.07	± 1.02	± 1.22	± 0.64	± 0.98	± 0.93	± 1.07	± 1.51	± 1.29
AC (%)	0.443^{BCDE}	0.425^{E}	0.485^{A}	0.437^{CDE}	0.467^{AB}	0.436^{DE}	0.463^{ABC}	0.458^{BCD}	0.431^{E}
	± 0.04	± 0.04	± 0.08	± 0.06	± 0.06	± 0.05	± 0.06	± 0.04	± 0.05
2016	BR	CB	CD	GL	HA	SN	ST	UN	UT
FY (%)	72.78^{AB}	71.25^{D}	72.41^{BC}	70.53^{E}	72.01^{C}	73.03^{A}	71.36^{D}	72.95^{AB}	72.18^{C}
	± 0.81	± 0.75	± 0.66	± 0.93	± 0.94	± 1.16	± 0.88	± 1.86	± 1.05
SC (%)	4.51^{EF}	4.96^{CD}	4.76^{DE}	5.59^{A}	5.46^{AB}	4.22^{F}	4.85^{DE}	5.21^{BC}	4.71^{DE}
	± 0.51	± 0.62	± 0.61	± 0.72	± 0.74	± 0.53	± 0.58	± 0.56	± 0.59
BC (%)	21.63^{C}	22.71^{A}	21.77^{BC}	22.75^{A}	21.49^{C}	21.69^{C}	22.79^{A}	20.95^{D}	22.06^{B}
	± 0.64	± 0.60	± 0.64	± 0.77	± 0.75	± 1.31	± 0.74	± 0.62	± 0.78
AC (%)	0.361^{DE}	0.368^{CD}	0.388^{B}	0.350^{F}	0.403^{A}	0.359^{E}	0.397^{A}	0.383^{B}	0.371^{C}
	± 0.02	± 0.02	± 0.02	± 0.02	± 0.30	± 0.03	± 0.03	± 0.20	± 0.03

FY = Flour Yield, SC = Shorts Content, BC = Bran Content, AC = Ash Content
Means in the same row with different letter groupings are statistically different according to Tukey-Kramer method ($p<0.05$)

Table 4.1.11 Location means and standard deviation for milling quality parameters.

2015	BE	BR	ED	GO	IH	KE	LE	MO	SC
FY (%)	73.4^{AB}	72.1^{D}	74.0^{A}	73.5^{AB}	72.2^{D}	70.4^{E}	72.5^{CD}	72.4^{CD}	73.0^{BC}
	± 1.37	± 1.07	± 1.05	± 0.87	± 1.09	± 1.48	± 0.71	± 0.69	± 0.57
SC (%)	4.50^{DE}	5.00^{BC}	4.69^{CD}	4.97^{BC}	5.04^{B}	5.07^{B}	5.57^{A}	4.13^{F}	4.20^{EF}
	± 0.71	± 0.71	± 0.52	± 0.73	± 0.68	± 0.57	± 0.48	± 0.40	± 0.45
BC (%)	21.9^{C}	21.9^{C}	20.6^{F}	20.7^{F}	21.9^{C}	23.6^{A}	21.1^{EF}	22.5^{B}	21.6^{CD}
	± 1.17	± 0.69	± 0.77	± 0.59	± 0.87	± 1.19	± 0.64	± 0.64	± 0.36
AC (%)	0.42^{D}	0.45^{C}	0.42^{D}	0.45^{C}	0.49^{B}	0.52^{A}	0.40^{DE}	0.51^{AB}	0.38^{E}
	± 0.06	± 0.03	± 0.02	± 0.03	± 0.03	± 0.07	± 0.02	± 0.02	± 0.01
2016	BE	BR	ED	GO	IH	KE	LE	MO	SC
FY (%)	72.3^{BCD}	71.6^{E}	72.6^{AB}	72.3^{ABCD}	70.6^{F}	72.7^{A}	72.4^{ABC}	72.1^{CD}	71.8^{DE}
	± 1.24	± 0.72	± 1.17	± 1.33	± 0.96	± 1.31	± 0.80	± 0.92	± 0.77
SC (%)	5.85^{A}	4.44^{E}	4.64^{DE}	4.87^{CD}	5.45^{B}	4.46^{E}	4.64^{DE}	4.73^{DE}	5.19^{BC}
	± 0.84	± 0.41	± 0.70	± 0.71	± 0.51	± 0.48	± 0.47	± 0.51	± 0.50
BC (%)	20.9^{C}	22.9^{A}	21.8^{B}	21.8^{B}	22.8^{A}	21.8^{B}	21.9^{B}	22.0^{B}	21.9^{B}
	± 0.73	± 0.62	± 0.86	± 1.05	± 0.74	± 0.99	± 0.63	± 0.84	± 0.54
AC (%)	0.37^{C}	0.39^{B}	0.36^{DE}	0.35^{E}	0.41^{A}	0.40^{AB}	0.36^{DE}	0.39^{B}	0.36^{DC}
	± 0.02	± 0.03	± 0.02	± 0.02	± 0.03	± 0.02	± 0.01	± 0.03	± 0.02

FY = Flour Yield, SC = Shorts Content, BC = Bran Content, AC = Ash Content
Means in the same row with different letter groupings are statistically different according to Tukey-Kramer method ($p<0.05$)

ANOVA for FY showed significant effects ($p < 0.0001$) of genotype, location and GxL interactions in 2015 and 2016, with the exception of GxL in 2015 ($p < 0.01$) (Table 4.1.12). Location contributed most to variance of FY in 2015 (51.3%), whereas genotype was the largest contributor in 2016 (44.3%). ANOVA for combined years had genotype as a significant effect ($p < 0.001$) for FY as well as the largest variance contributor (Table 4.1.13, 27.5%), similarly reported by Bergman et al. (1998). In addition, YxL and GxL interactions were significant for FY ($p < 0.0001$), whereas location was not significant.

Table 4.1.12. Percent variance contribution of mixed model effects on milling parameters for separate years.

	Year	Genotype	Location	GxL	Rep	Residual
FY	*2015*	20.49***	51.27***	17.33*	0.24 NS	10.67
	2016	44.28***	26.32***	22.45***	0.44 NS	6.51
SC	*2015*	37.83***	37.36***	15.38*	0.25 NS	9.18
	2016	33.03***	37.92***	18.73**	0.19 NS	10.13
BC	*2015*	15.92***	59.44***	15.71**	0.26 NS	8.66
	2016	42.65***	35.97***	21.19***	0.13 NS	0.06
AC	*2015*	10.15***	65.48***	14.80*	0.74 NS	8.84
	2016	6.99***	88.98***	3.22***	0.01 NS	0.81

*, ** and *** significant at p<0.01, p<0.001 and p<0.0001 , respectively
FY = Flour Yield, SC = Shorts Content, BC = Bran Content, AC = Ash Content, NS = No significance

Table 4.1.13 Percent variance contribution of mixed model effects on milling parameters for combined years.

	Genotype	Location	Year	G x L	G x Y	L x Y	GxLxY	Rep	Residual
FY	27.54***	19.54 NS	4.36 NS	9.20 NS	1.51 NS	19.54****	9.20****	0.23 NS	8.89
SC	33.52***	12.91 NS	0.68 NS	8.84 NS	1.86 NS	24.00****	8.34****	0.21 NS	9.65
BC	24.02****	28.31 NS	1.75 NS	10.27 NS	1.36 NS	19.75****	7.19****	0.14 NS	7.21
AC	8.22**	28.07 NS	40.45***	4.97 NS	1.14*	7.73****	4.13***	0.16 NS	5.13

*, **, *** and **** significant at p<0.05, p<0.01, p<0.001 and p<0.0001 , respectively
FY = Flour Yield, SC = Shorts Content, BC = Bran Content, AC = Ash Content, NS = No significance

Bran content (BC) varied from 21.0% to 22.3% and 21.0% to 22.8% across genotypes in 2015 and 2016, respectively (Table 4.1.10). This is similar to the variation exhibited across locations which ranged from 20.6% to 23.6% in 2015 and 20.9% to 22.9% in 2016 (Table 4.1.11). For all samples, BC ranged from 19.7-26.3% (Appendix B.1). Similar to flour yield, there were no trends in location rankings between years. However, a comparison of genotype

means averaged across the nine locations in 2015 and 2016 yielded a close relationship (R^2 = 0.83, $p<0.001$; result not shown) indicating a strong genotype influence on this milling quality parameter. ANOVA for BC showed location as the largest contributor of variance in 2015 (59.4%), whereas genotype contributed the most in 2016 (42.7%) (Table 4.1.12). In the ANOVA for combined years, location remained the largest variance contributor at 28.3%, although location was not significant (Table 4.1.13). The contribution of genotype to total variance was very similar (24.0%) and highly significant ($p < 0.0001$), similar to the values of LxY and GxLxY interactions and their respective variance contributions.

The content of shorts (SC), which represents the milling by-product on the reduction side of the mill varied from 3.8% to 5.0% and 4.2% to 5.6% across genotypes in 2015 and 2016 , respectively (Table 4.1.10). This is similar to the variation observed across locations, which ranged from 4.1% to 5.6% in 2015 and 4.4% to 5.9% in 2016 (Table 4.1.11). Despite the small range of SC for genotype and location means, the range of SC for individual genotype samples was much larger from 3.2-7.7% (Appendix B.1). ANOVA for SC showed significant effects ($p < 0.0001$) of both genotype and location in 2015 and 2016 (Table 4.1.12). In addition, GxL interaction was significant for FY across years ($p < 0.01$). Location and genotype both contributed similarly to the total variance of SC, i.e. 37.4% and 37.8% and 37.9% and 33.0% in 2015 and 2016, respectively. ANOVA of combined years showed genotype as a significant effect (Table 4.1.13, $p < 0.001$) as well as the largest contributor to SC variation (33.5%). When years were combined, location was no longer significant for FY, however, LxY and GxLxY interactions were significant ($p < 0.0001$).

Ash content (AC) varied from 0.38% to 0.52% and 0.35% to 0.41% across locations in 2015 and 2016, respectively (Table 4.1.11). In general, 2015 samples had higher ash content than those in 2016, with individual genotype samples ranging from 0.36% to 0.75% (average = 0.45%) in 2015 and 0.36% to 0.54% (average = 0.44%) in 2016 (Appendix B.1). AC variation across genotypes was similar to that of location, ranging from 0.43% to 0.49% and 0.35% to 0.40% in 2015 and 2016, respectively (Table 4.1.10). There was a significant relationship (R^2 = 0.60, p <0.05) for AC between 2015 and 2016 locations averaged across genotypes, indicating a location specific influence (most likely weather) on flour ash content. The effect of location on ash content has been previously reported in spring wheat (Lukow and McVetty 1991), which is not surprising given that soil profiles will likely effect mineral concentrations in plants. Similarly, there was a significant relationship (R^2 = 0.62, $p < 0.05$) for AC between 2015 and 2016 for genotypes averaged across locations, reflecting a genotype influence on this milling quality parameter. ANOVA for AC indicated that location accounted for 65.5% and 89.0% of the total variance in 2015 and 2016, respectively (Table 4.1.12). For both years, genotype, location and GxL effects were significant ($p < 0.0001$), with the exception of GxL in 2015 ($p < 0.01$). Interestingly, ANOVA for combined years showed year as significant ($p < 0.001$) and the largest contributor to total variance in AC at 40.5% (Table 4.1.13). This is different from other milling parameters, in which the growing year represented only a small proportion of variance and was not significant factor. Nonetheless, LxY and GxYxL interactions were still significant ($p <$ 0.0001) for ash content, in addition to genotype ($p < 0.01$). A similar result was reported by Caffe-Treml et al. (2011) where environment accounted for 63% of the variance in ash content.

4.1.4 Mixograph Parameters

Mixing development time (MDT) averaged over genotypes varied from 2.42 min to 3.67 min and 2.17 min to 3.48 min in 2015 and 2016, respectively (Table 4.1.14). Glenn had significantly ($p < 0.05$) higher values than the other genotypes in both years which reflects its higher gluten strength compared to the other genotypes in this study. Across locations, MDT on average ranged from 2.29 min to 4.12 min in 2015 and 2.14 min to 2.88 min in 2016. Accordingly, the range of MDT values was considerably higher in 2015 (Table 4.1.15), with individual samples ranging from 1.8-5.6 min (average = 2.9 min) and 1.5-4.6 min (average = 2.5 min) in 2015 and 2016, respectively (Appendix B.1). That 2015 was a better crop year in terms of grade may have contributed to samples expressing their full potential of gluten strength, especially at the higher end. There was no correlation ($R^2 = 0.06$, no significance) for MDT for the same growing locations over the two crop years, whereas there was a strong relationship for MDT between genotypes grown in 2015 and 2016 ($R^2 = 0.71$, $p<0.01$; results not shown).

Table 4.1.14. Genotype means and standard deviation for mixograph quality parameters.

2015	BR	CB	CD	GL	HA	SN	ST	UN	UT
MDT (min)	2.75^{C}	3.08^{B}	2.86^{C}	3.67^{A}	2.42^{D}	3.14^{B}	2.51^{D}	2.80^{C}	2.74^{C}
	± 0.64	± 0.6	± 0.75	± 0.58	± 0.48	± 0.84	± 0.57	± 0.73	± 0.45
PBW (%T)	26.97^{DE}	27.43^{CD}	28.51^{BC}	29.49^{AB}	27.16^{DE}	25.81^{F}	29.73^{A}	26.08^{ED}	30.32^{A}
	± 2.68	± 2.69	± 2.42	± 3.43	± 3.38	± 3.03	± 3.6	± 3.48	± 4.24
PDR (%T)	55.7^{BC}	53.72^{D}	55.27^{BCD}	56.61^{AB}	54.17^{CD}	49.98^{E}	57.64^{A}	51.42^{E}	58.07^{A}
	± 5.37	± 5.37	± 5.37	± 6.67	± 6.3	± 5.99	± 6.87	± 6.3	± 6.7
WIP (%T*min)	126.26^{BC}	135.65^{B}	130.11^{B}	173.64^{A}	106.7^{D}	131.46^{B}	118.95^{C}	119.46^{C}	126.23^{BC}
	± 21.89	± 20.54	± 25.88	± 16.84	± 14.94	± 21.07	± 17.64	± 20.6	± 14.4
WAP (%T*min)	72.99^{C}	83.35^{B}	80.24^{B}	107.11^{A}	64.49^{D}	79.1^{B}	73.22^{C}	71.33^{C}	82.1^{B}
	± 11.54	± 12.68	± 16.45	± 14.17	± 8.21	± 12.6	± 10.25	± 11.72	± 11.21
2016	BR	CB	CD	GL	HA	SN	ST	UN	UT
MDT (min)	2.57^{C}	2.85^{B}	2.56^{C}	3.48^{A}	2.03^{F}	2.30^{DE}	2.17^{EF}	2.51^{CD}	2.28^{E}
	± 0.32	± 0.38	± 0.29	± 0.56	± 0.28	± 0.36	± 0.21	± 0.25	± 0.3
PBW (%T)	28.23^{DE}	27.97^{EF}	28.80^{CDE}	29.77^{BC}	29.54^{BCD}	28.31^{DE}	30.35^{AB}	26.77^{F}	31.23^{A}
	± 2.71	± 2.63	± 3.36	± 2.94	± 3.76	± 3.25	± 3.42	± 3.19	± 4.15
PDR (%T)	56.36^{B}	53.02^{CD}	56.05^{B}	55.86^{B}	55.44^{B}	54.82^{BC}	58.66^{A}	51.38^{D}	60.01^{A}
	± 5.33	± 5.12	± 6.12	± 6.74	± 7.62	± 5.99	± 7.11	± 7.16	± 7.97
WIP (%T*min)	125.90^{B}	131.00^{B}	121.79^{BC}	176.13^{A}	94.11^{E}	108.85^{D}	107.00^{D}	112.22^{CD}	112.94^{CD}
	± 15.16	± 15.25	± 11.86	± 24.29	± 13.8	± 14.03	± 7.39	± 10.92	± 13.48
WAP (%T*min)	71.92^{C}	78.91^{B}	73.16^{C}	102.4^{A}	59.13^{F}	64.12^{EF}	65.30^{DE}	66.58^{DE}	70.50^{CD}
	± 7.46	± 8.17	± 7.13	± 13.02	± 7.17	± 5.71	± 5.57	± 6.04	± 9.65

MDT = Mixing development time, PBW = Peak band width, PDR = Peak Dough Resistance, WIP = Work input to peak, T = Torque

Means in the same row with different letter groupings are statistically different according to Tukey-Kramer method ($p<0.05$)

Table 4.1.15 Location means and standard deviation for mixograph quality parameters

2015	BE	BR	ED	GO	IH	KE	LE	MO	SC
MDT (min)	3.13^{BC}	2.42^{EF}	2.40^{EF}	2.50^{E}	2.29^{F}	3.26^{B}	2.81^{D}	4.12^{A}	3.04^{C}
	± 0.72	± 0.39	± 0.33	± 0.48	± 0.39	±0.61	± 0.40	± 0.61	± 0.45
PBW (%T)	29.4^{B}	28.7^{BC}	32.3^{A}	29.3^{B}	26.8^{E}	27.6^{CD}	28.0^{C}	21.2^{D}	28.3^{BC}
	± 3.54	± 2.31	± 1.75	± 2.16	± 1.88	± 2.37	± 2.44	± 1.43	± 2.67
PDR (%T)	55.7^{BCD}	56.4^{BC4}	63.2^{A}	57.1^{B}	54.8^{CD}	54.0^{D}	55.6^{BCD}	41.1^{E}	54.8^{CD}
	± 6.49	± 3.52	± 3.34	± 3.04	± 4.06	± 3.70	± 4.00	± 2.75	± 3.67
WIP (%T*min)	141.3^{AB}	114.1^{D}	122.5^{CD}	119.9^{D}	103.1^{E}	149.2^{A}	131.9^{BC}	148.3^{A}	138.2^{A}
	± 25.93	± 18.96	± 19.87	± 24.51	± 20.40	± 24.78	± 20.37	± 19.13	± 21.73
WAP	91.9^{A}	69.3^{D}	78.0^{BC}	72.7^{DC}	60.9^{E}	89.2^{A}	78.6^{B}	87.3^{A}	85.9^{A}
(%T*min)	± 16.52	± 11.81	± 13.49	± 12.20	± 10.03	±15.77	± 15.36	± 13.34	± 13.71
2016	BE	BR	ED	GO	IH	KE	LE	MO	SC
MDT (min)	2.24^{DE}	2.14^{E}	2.72^{AB}	2.73^{AB}	2.69^{AB}	2.61^{BC}	2.39^{CD}	2.35^{DE}	2.88^{A}
	± 0.30	± 0.36	± 0.56	± 0.58	± 0.46	± 0.59	± 0.37	± 0.35	± 0.63
PBW (%T)	34.2^{A}	30.6^{B}	28.7^{C}	30.2^{B}	26.0^{D}	28.6^{C}	30.6^{B}	28.2^{C}	23.8^{E}
	± 1.97	± 1.75	± 2.20	± 2.16	± 2.09	± 2.33	± 2.19	± 1.55	± 2.29
PDR (%T)	66.1^{A}	60.1^{B}	53.7^{E}	59.4^{BC}	47.3^{F}	55.7^{D}	58.0^{C}	55.9^{D}	45.5^{F}
	± 2.97	± 3.61	± 2.82	± 4.78	± 4.10	± 3.13	± 3.03	± 3.08	± 3.86
WIP (%T*min)	127.4^{ABC}	107.6^{E}	131.1^{AB}	137.6^{A}	118.0^{CDE}	126.6^{BC}	112.8^{DE}	109.6^{DE}	119.3^{CD}
	± 21.44	± 18.96	± 29.77	± 27.21	± 26.99	± 29.31	± 19.36	± 18.65	± 30.34
WAP	76.4^{B}	65.2^{E}	77.8^{AB}	81.8^{A}	69.9^{CDE}	73.8^{BC}	73.0^{BCD}	65.8^{E}	68.0^{DE}
(%T*min)	± 11.24	± 10.01	± 17.66	± 14.72	± 15.05	± 14.13	± 13.17	± 10.05	± 14.45

MDT = Mixing development time, PBW = Peak band width, PDR = Peak Dough Resistance, WIP = Work input to peak, T = Torque

Means in the same row with different letter groupings are statistically different according to Tukey-Kramer method ($p<0.05$)

ANOVA of mixograph parameters showed considerable variation in the respective contributions of genotype and growing location to total variance (Table 4.1.16 and Table 4.1.17) with WIP and WAP having the highest variance contributions for genotype (~53%, years combined), whereas PBW and PDR had the highest variance contribution for locations (~38%, years combined). In separate years, these parameters were consistent in this regard, in contrast with MDT which differed between years in the relative contributions of genotype and location to variance. In the analysis below, each of these parameters will be discussed in turn.

ANOVA of MDT showed significant effects ($p < 0.0001$) for genotype and location and in both 2015 and 2016, and significant GxL interaction ($p < 0.0001$) in 2015 only (Table 4.1.16). In 2015, location contributed the most to variance in MDT (59.8%), whereas the largest variance

contributor in 2016 (60.3%) was genotype. ANOVA for data of combined years yielded genotype as a significant effect ($p < 0.01$) as well as the largest variance contributor at 31.7%, whereas location was not significant (Table 4.1.17). Bergman et al. (1998) found genotype to contribute to ~60% of the total variance in mixing peak time. Interestingly, LxY interaction contributed substantially (24.8%) to the variation in MDT and was significant ($p<0.0001$, Table 5). In addition, GxL and GxLxY interactions were significant ($p < 0.0001$), although their respective contributions to variance did not exceed 3.9%. The importance of genotype by environment interaction in determining MDT has been shown by Baker and Kosmolak (1977).

Table 4.1.16. Percent variance contribution of mixed model effects on mixograph parameters for separate years.

	Year	Genotype	Location	GxL	Rep	Residual
MDT	*2015*	23.27****	59.83****	12.72****	0.24 NS	3.94
	2016	60.32****	20.53****	11.01*	0.35 NS	7.79
PBW	*2015*	18.76****	63.95****	12.42****	0.30 NS	4.57
	2016	13.69****	66.18****	13.62****	0.06 NS	6.45
PDR	*2015*	14.08****	74.14****	8.46****	0.16 NS	3.16
	2016	11.98****	75.95****	8.97****	0.15 NS	2.95
WIP	*2015*	40.24****	37.02****	15.55****	0.43 NS	6.75
	2016	70.85****	13.13****	8.87*	0.15 NS	7.00
WAP	*2015*	43.46****	37.88****	12.84****	0.47 NS	5.36
	2016	70.10****	13.52****	10.22***	0.37 NS	5.79

*, **, *** and **** significant at p<0.05, p<0.01, p<0.001 and p<0.0001, respectively
MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, WIP = work input to peak, NS = No significance

Table 5. Percent variance contribution of mixed model effects on mixograph parameters for combined years.

	Genotype	Location	Year	G x L	G x Y	L x Y	GxLxY	Rep	Residual
MDT	31.71***	17.65 NS	7.60 NS	7.09**	2.17***	24.83****	3.82**	0.08 NS	5.04
PBW	14.97***	38.33 NS	2.27 NS	8.64**	1.29*	24.47****	4.39***	0.09 NS	5.54
PDR	12.78**	37.42 NS	0.54 NS	7.44****	1.47****	35.09****	2.02*	0.09 NS	3.15
WIP	54.42****	11.41 NS	2.79 NS	7.33**	1.77**	11.63****	4.05*	0.09 NS	6.50
WAP	52.42****	12.68 NS	4.70 NS	6.79 NS	1.70**	11.65****	4.58****	0.23 NS	5.26

*, **, *** and **** significant at p<0.01, p<0.001 and p<0.0001, respectively
MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, WAP = work at peak resistance, WIP = work input to peak, NS = No significance

Peak band width (PBW) averaged over genotypes ranged from 25.81 %Torque to 30.32 %Torque and 26.77 %Torque to 31.23 %Torque in 2015 and 2016, respectively (Table 4.1.14);

Utmost had the highest value for PBW in both years. The variation across locations was much larger, from 21.2 %Torque to 32.3 %Torque in 2015 and 26.0 %Torque to 34.2 %Torque in 2016 (Table 4.1.15). The individual sample range for PBW was 18.4-38.8 %Torque (Appendix B.1). Peak dough resistance (PDR) averaged across genotypes ranged from 49.98 %Torque to 58.07 %Torque and 51.38 %Torque to 60.01 %Torque in 2015 and 2016, respectively (Table 4.1.14). PDR averaged across locations ranged from 41.1 %Torque to 63.2 %Torque in 2015 and 45.5 %Torque to 66.1 %Torque in 2016 (Table 4.1.15). Individual samples varied from 34.3-72.4 %Torque (Appendix B.1).

ANOVA results for PBW and PDR showed significant genotype, location and GxL interactions effects ($p < 0.01$) in 2015 and 2016 (Table 4.1.16). For both parameters, location had the largest variance contribution in 2015 and 2016 at approximately 64% and 74% for PBW and PDR, respectively. ANOVA data of combined years showed location as the highest contributor to variance in PBW and PDR at approximately 38% for both parameters, despite no longer having any significance as an effect (Table 4.1.17). LxY interaction was highly significant ($p < 0.0001$) for both parameters, and the second highest contributor to variance in PDR (35%).

Work input to peak (WIP) average across genotypes ranged from 106.7 %Torque*min to 173.6 %Torque*min and 94.1 %Torque*min to 176.1 %Torque*min in 2015 and 2016, respectively (Table 4.1.14). In both years, Glenn and Harvest yielded statistically significant ($p < 0.05$) highest and lowest values for WIP, respectively. Carberry consistently had the second highest value for WIP across years, despite not being statistically different from Brandon, Cardale and Utmost (2015 only). It follows that 2015 and 2016 genotype values for WIP were highly correlated ($R^2 = 0.90$, $p < 0.0001$) with one another (Fig 4.1.3). There existed a much

smaller range in WIP averaged across locations, from 103.1 %Torque*min to 149.2 %Torque*min in 2015 and 107.6 %Torque*min to 137.6 %Torque*min in 2016 (Table 4.1.15). There were no observable trends in WIP values across locations between years (Fig 4.1.4). Considering all samples WIP ranged from 70.2-211.9 %Torque*min (Appendix B.1).

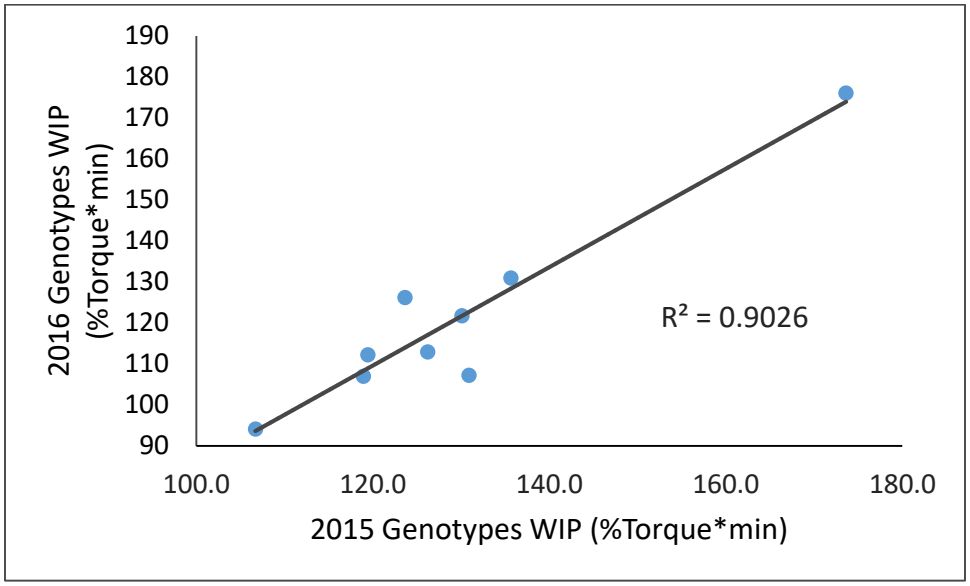

Figure 4.1.3 Relationship between WIP for 2015 and 2016 genotypes. Each symbol represents a mean of 18 field replications.

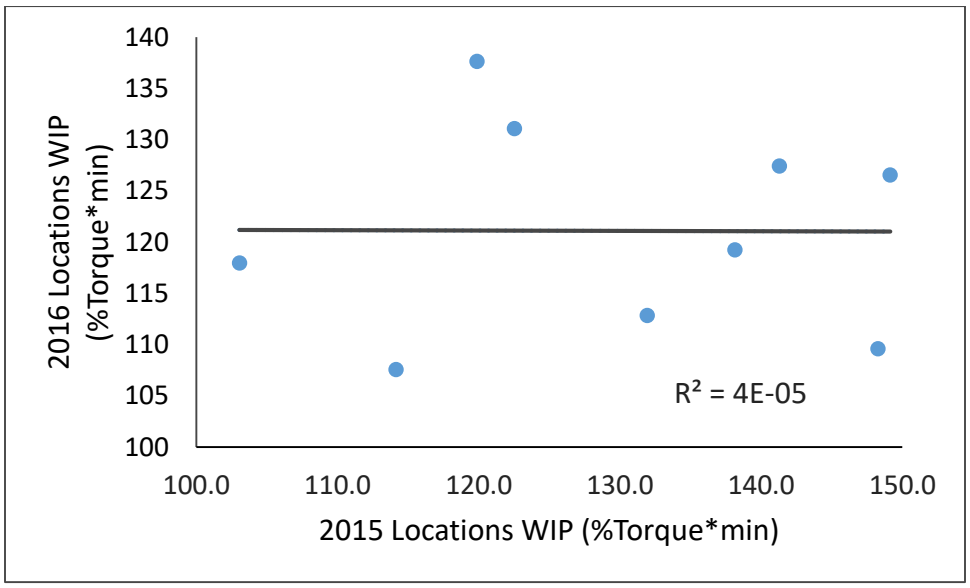

Figure 4.1.4. Relationship between WIP for 2015 and 2016 growing sites. Each symbol represents a mean of 18 field replications.

WAP is an alternate parameter closely related to WIP by correlation (previously explained in Materials and Methods) that measures the product of MDT and PBW and is therefore independent of and unrelated PDR (as well as PBW, refer to Table 4.4.2). Considering all samples WAP ranged from 48.6-136.4 %Torque*min (Appendix B.1). WAP averaged across genotypes ranged from 64.5 %Torque*min to 107.1 %Torque*min and 59.1 %Torque*min to 102.4 %Torque*min in 2015 and 2016, respectively (Table 4.1.14). In both years, Glenn's WAP value was significantly ($p < 0.05$) higher than those of the other genotypes. In contrast, the variety Harvest had the lowest WAP values averaged across locations and years, but was significantly ($p < 0.05$) lower than the other genotypes in 2015 only. In addition, Carberry had the second highest value for WAP in 2015 and 2016, and was significantly ($p < 0.05$) different than the other genotypes in 2016. Compared to genotype WAP, location WAP had a much smaller range in values, from 60.9%Torque*min to 91.9 %Torque*min in 2015 and 65.2

%Torque*min to 81.8 %Torque*min in 2016 (Table 4.1.15). The smaller range for location WAP was due to lower maximum WAP values compared to genotype WAP. Genotype values for WAP averaged across locations in 2015 and 2016 were strongly correlated ($R^2 = 0.86$, $p < 0.001$; Fig 4.1.5). In contrast, there was no relationship in WIP values for locations averaged across genotypes between years (Fig 4.1.6). This further highlights the very strong influence of growing location environment on gluten strength.

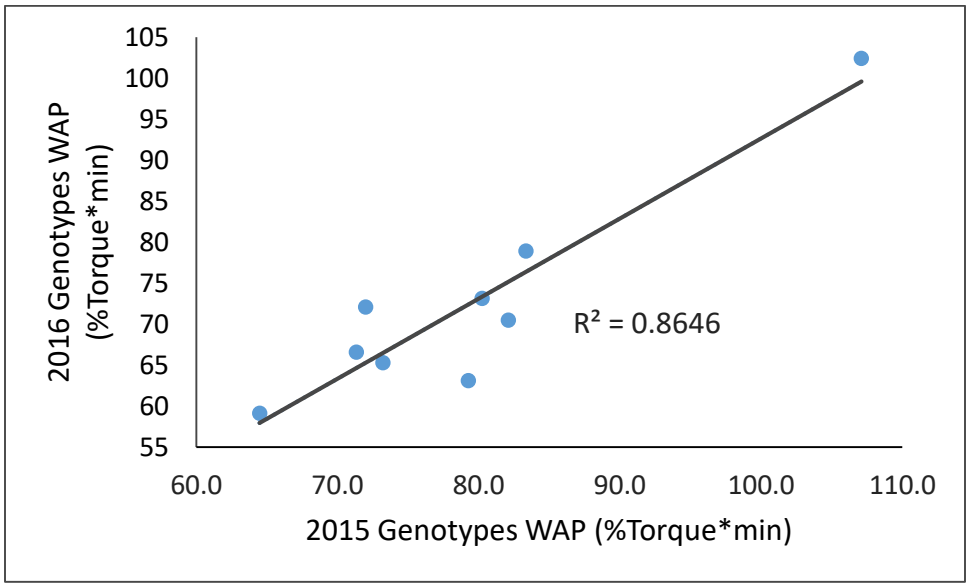

Figure 4.1.5. Relationship between WAP for 2015 and 2016 genotypes. Each symbol represents a mean of 18 field replications.

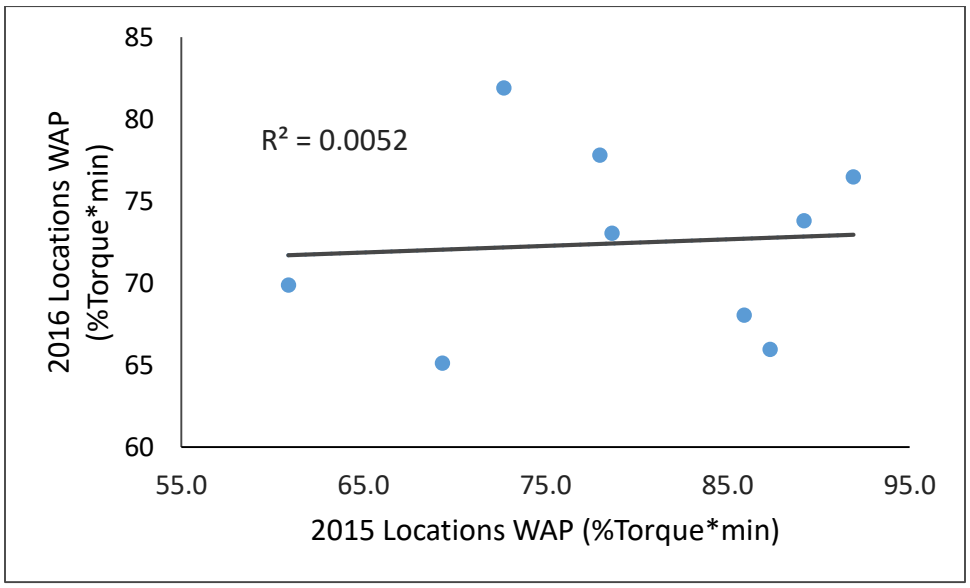

Figure 2. Relationship between WAP for 2015 and 2016 WAP growing sites. Each symbol represents a mean of 18 field replications.

ANOVA of WIP and WAP yielded similar results for the two parameters (Table 4.1.16). Genotype contributed the largest portion of variance for both years, accounting for approximately 40% of the variation in both WIP and WAP in 2015, and approximately 70% of the variation in 2016. In 2015, the contribution to variance of location was very similar to that of genotype, at ~37% for both parameters. In both crop years, genotype and location effects of WIP and WAP were significant ($p < 0.0001$). GxL interaction for WAP was significant ($p < 0.001$ and $p < 0.0001$) in 2016 and 2015, respectively. ANOVA of combined years showed genotype as the largest contributor to WIP and WAP variance, accounting for approximately 53% for the two parameters; genotype was a significant effect ($p<0.0001$, Table 4.1.17). For both WIP and WAP, LxY and GxY interactions were significant ($p < 0.0001$ and $p < 0.01$, respectively), whereas GxL interaction was significant ($p < 0.01$) for WIP and GxLxY interaction was significant ($p < 0.0001$) for WAP.

4.1.5 Protein Fractions

Results for flour protein content (FP) were very similar to those for grain protein content, as would be expected; the correlation between GP and FP for individual samples when data was combined across years was r = 0.99. Considering all samples, FP ranged from 9.7-17.1% (Appendix B.1). FP varied more among locations than genotypes, indicative of a large environmental influence that is reflected in the ANOVA results (discussed later). FP ranged from 11.41% to 15.68% and 10.94% to 15.63% across locations for 2015 and 2016, respectively (Table 4.1.18). Brandon had the highest FP location average for both years. Across genotypes, FP ranged from 13.90% to 14.97% in 2015 and 13.55% to 15.01% in 2016 (Table 4.1.19). Stettler displayed significantly ($p < 0.05$) higher FP values than the other genotypes in both years. Location accounted for the largest proportion of FP variance at 81%, 86% and 47% in 2015, 2016 and combined year analysis, respectively (Table 4.1.20 and Table 4.1.21). Location was a significant ($p < 0.0001$) effect on FP in 2015 and 2016, but not when the years were combined. ANOVA of combined years showed the LxY interaction was significant ($p < 0.0001$) on FP, accounting for 37% of its variation. The significant ($p < 0.01$) influence of environment has been reported by Budak et al. (2003), in which the mean square value for environment was 87.6, where the value for all other effects never exceeded 4.59, as well as by Caffe-Treml et al. (2011) where environment account for 60% of the total variance in protein content.

Table 4.1.18. Locations means and standard deviation for protein composition quality parameters.

2015	BE	BR	ED	GO	IH	KE	LE	MO	SC
FP (%)	13.28^{F}	15.68^{A}	15.47^{AB}	14.97^{C}	14.41^{D}	15.17^{BC}	13.98^{E}	11.41^{G}	14.05^{E}
	± 1.39	± 0.40	± 0.54	± 0.53	± 1.03	± 0.50	± 0.64	± 0.46	± 0.69
SP (%)	9.66^{F}	11.25^{AB}	11.51^{A}	10.99^{BC}	10.67^{D}	10.91^{CD}	10.18^{E}	8.32^{G}	10.16^{E}
	± 1.09	± 0.55	± 0.69	± 0.76	± 0.65	± 0.61	± 0.55	± 0.36	± 0.57
SP/FP (%)	72.71^{CD}	71.74^{D}	74.37^{A}	73.35^{ABC}	74.10^{AB}	71.90^{D}	72.85^{BCD}	72.95^{BCD}	72.38^{CD}
	± 2.66	± 2.72	± 2.91	± 2.90	± 2.48	± 2.49	± 1.91	± 2.03	± 2.89
HMWG-(%)	3.57^{D}	3.77^{B}	3.89^{A}	3.76^{B}	3.37^{E}	3.97^{A}	3.62^{CD}	2.95^{F}	3.71^{BC}
	± 0.42	± 0.29	± 0.35	± 0.20	± 0.33	± 0.29	± 0.36	± 0.24	± 0.33
HMWG/FP (%)	26.87^{A}	24.04^{D}	25.16^{C}	25.19^{C}	23.36^{E}	26.22^{B}	25.86^{B}	25.88^{B}	26.39^{AB}
	± 2.15	± 1.86	± 1.88	± 2.04	± 1.62	± 2.30	± 1.78	± 1.69	± 2.08
GSI	0.331^{CD}	0.307^{E}	0.363^{A}	0.356^{AB}	0.319^{DE}	0.349^{B}	0.347^{B}	0.328^{CD}	0.333^{C}
	± 0.039	± 0.037	± 0.034	± 0.041	± 0.029	± 0.040	± 0.031	± 0.032	± 0.040
2016	BE	BR	ED	GO	IH	KE	LE	MO	SC
FP (%)	14.51^{D}	15.63^{A}	14.48^{D}	15.29^{AB}	11.43^{E}	14.93^{BC}	14.85^{CD}	14.81^{CD}	10.94^{F}
	± 0.58	± 0.64	± 0.63	± 0.71	± 0.87	± 0.65	± 0.52	± 0.64	± 0.66
SP (%)	10.50^{BC}	11.13^{A}	10.19^{C}	10.77^{AB}	8.46^{D}	10.71^{B}	10.85^{AB}	10.58^{BC}	7.99^{E}
	± 0.51	±0.66	± 0.68	± 0.86	± 0.66	± 0.86	± 0.68	± 0.70	± 0.68
SP/FP (%)	72.40^{AB}	71.17^{B}	70.44^{B}	70.46^{B}	74.05^{A}	71.49^{AB}	73.07^{AB}	71.49^{AB}	73.03^{AB}
	± 2.53	± 2.58	± 2.69	± 3.78	± 3.62	± 3.66	± 3.38	± 2.93	± 4.78
HMWG (%)	3.47^{C} 0.31 ± 0.27	3.40^{C} ± 0.26	3.67^{B} ± 0.20	3.80^{A} ± 0.28	2.69^{D} ± 0.31	3.71^{AB} ± 0.23	3.74^{AB} ± 0.35	3.45^{C} ± 0.27	2.64^{D} ± 0.23
HMWG/FP (%)	23.93^{DC}	21.75^{E}	25.42^{A}	24.91^{AB}	23.53^{CD}	24.87^{AB}	25.23^{A}	23.33^{D}	24.20^{BC}
	± 1.61	± 1.77	± 1.80	±2.18	± 2.09	± 2.05	± 2.33	± 1.88	± 2.24
GSI	0.371^{A}	0.336^{C}	0.339^{C}	0.345^{C}	0.316^{D}	0.366^{A}	0.356^{B}	0.356^{B}	0.366^{A}
	± 0.027	± 0.033	± 0.035	± 0.047	± 0.039	± 0.044	± 0.045	± 0.036	± 0.040

FP = Flour protein content, SP = Soluble prolamin content in flour, SP/FP = Soluble prolamin content in FP, HMWG = High molecular weight glutenin in flour, HMWG/FP = High molecular weight glutenin content in FP, GSI = gluten strength index
Means in the same row with different letter groupings are statistically different according to Tukey-Kramer method ($p<0.05$)

Table 4.1.19. Genotype means and standard deviations for protein composition quality parameters.

2015	BR	CB	CD	GL	HA	SN	ST	UN	UT
FP (%)	14.23^{BCD}	14.35^{BC}	14.49^{B}	14.42^{B}	14.05^{CD}	13.90^{D}	14.97^{A}	14.01^{D}	14.00^{D}
	± 1.23	± 1.18	± 1.32	± 1.23	± 1.60	± 1.34	± 1.54	± 1.53	± 1.44
SP (%)	10.64^{B}	10.08^{E}	10.43^{BCD}	9.98^{E}	10.28^{CDE}	10.13^{DE}	11.35^{A}	10.31^{CDE}	10.46^{BC}
	± 0.95	± 0.84	± 0.91	± 0.87	± 1.15	± 1.01	± 1.29	± 1.18	± 1.13
SP/FP (%)	74.78^{AB}	70.25^{E}	71.99^{D}	69.22^{E}	73.21^{CD}	72.83^{CD}	75.76^{A}	73.56^{BC}	74.75^{AB}
	± 1.28	± 1.68	± 1.50	± 1.57	± 1.58	± 1.93	± 2.04	± 2.23	± 1.72
HMWG-(%)	3.55^{C}	3.96^{B}	3.64^{C}	4.19^{A}	3.41^{D}	3.36^{D}	3.57^{C}	3.36^{D}	3.58^{C}
	± 0.31	± 0.31	± 0.28	± 0.36	± 0.35	± 0.27	± 0.38	± 0.28	± 0.41
HMWG/FP (%)	24.95^{DE}	27.67^{B}	25.17^{CD}	29.10^{A}	24.36^{EF}	24.20^{F}	23.86^{F}	24.06^{F}	25.59^{C}
	± 1.27	± 1.69	± 1.39	± 1.30	± 1.63	± 1.00	± 1.36	± 1.23	± 1.13
GSI	0.33^{D}	0.35^{C}	0.39^{B}	0.42^{A}	0.33^{D}	0.33^{D}	0.32^{E}	0.33^{D}	0.34^{C}
	± 0.02	± 0.02	± 0.03	± 0.02	± 0.02	± 0.02	± 0.02	± 0.02	± 0.02
2016	BR	CB	CD	GL	HA	SN	ST	UN	UT
FP (%)	13.85^{CDE}	14.08^{BCD}	13.70^{DE}	13.85^{CDE}	14.27^{B}	14.45^{B}	15.01^{A}	13.55^{E}	14.11^{BC}
	± 1.40	± 1.65	± 1.44	± 1.65	± 2.13	± 1.66	± 1.91	± 1.82	± 1.68
SP (%)	10.19^{BCD}	9.49^{EF}	9.90^{CD}	9.38^{F}	10.26^{BC}	10.54^{B}	11.20^{A}	9.81^{DE}	10.39^{B}
	± 0.98	± 1.01	± 1.07	± 1.14	± 1.48	± 1.12	± 1.37	± 1.14	± 1.16
SP/FP (%)	73.61^{AB}	67.61^{C}	72.31^{AB}	67.75^{C}	72.00^{B}	73.05^{AB}	74.74^{A}	72.59^{AB}	73.76^{AB}
	± 2.22	± 3.97	± 3.84	± 1.90	± 2.20	± 1.89	± 1.90	± 2.51	± 2.33
HMWG (%)	3.34^{CD}	3.61^{B}	3.40^{C}	3.89^{A}	3.24^{D}	3.23^{D}	3.37^{C}	3.09^{E}	3.41^{C}
	± 0.37	± 0.45	± 0.39	± 0.50	± 0.55	± 0.39	± 0.48	± 0.43	± 0.46
HMWG/FP (%)	24.09^{C}	25.68^{B}	24.8^{C}	28.06^{A}	22.71^{D}	22.40^{D}	22.44^{D}	22.82^{D}	24.18^{C}
	± 1.15	± 1.83	± 1.37	± 1.52	± 1.49	± 1.12	± 1.32	± 1.18	± 1.60
GSI	0.328^{D}	0.343^{C}	0.380^{B}	0.415^{A}	0.316^{DE}	0.307^{EF}	0.300^{F}	0.315^{E}	0.328^{D}
	± 0.02	± 0.20	± 0.03	± 0.03	± 0.02	± 0.02	± 0.02	± 0.02	± 0.03

FP = Flour protein content, SP = Soluble prolamin content in flour, SP/FP = Soluble prolamin content in FP, HMWG = High molecular weight glutenin in flour, HMWG/FP = High molecular weight glutenin content in FP, GSI = gluten strength index
Means in the same row with different letter groupings are statistically different according to Tukey-Kramer method ($p<0.05$)

Table 4.1.20. Percent variance contribution of mixed model effects on protein fractions and GSI for separate years.

	Year	Genotype	Location	G x L	Rep	Residual
FP	*2015*	5.24***	80.98***	10.94***	0.12 NS	2.72
	2016	5.86***	85.85***	6.14***	0.06 NS	2.10
SP	*2015*	12.15***	71.52***	12.23***	0.08 NS	4.03
	2016	17.66***	70.07***	7.65**	0.22 NS	4.40
SP/FP	*2015*	58.39***	10.02***	19.85**	0.63 NS	11.12
	2016	47.02***	10.69**	15.55 NS	2.01 NS	24.73
HMWG	*2015*	39.90***	48.73***	8.14***	0.16 NS	3.07
	2016	20.15***	71.20***	6.09***	0.01 NS	2.55
HMWG/FP	*2015*	62.49***	24.55***	9.35***	0.13 NS	3.48
	2016	62.37***	23.47***	7.58 NS	0.09 NS	6.50
GSI	*2015*	71.50***	18.29***	7.84***	0.10 NS	2.28
	2016	73.48***	16.22***	6.07*	0.23 NS	4.00

*, ** and *** significant at $p<0.01$, $p<0.001$ and $p<0.0001$, respectively

FP = Flour protein content, SP = Soluble prolamin content in flour, SP/FP = Soluble prolamin content in FP, HMWG = High molecular weight glutenin in flour, HMWG/FP = High molecular weight glutenin content in FP, GSI = gluten strength index
NS = No significance

Table 4.1.21. Percent variance contribution of mixed model effects on protein fractions and GSI for combined years.

	Genotype	Location	Year	G x L	G x Y	L x Y	GxLxY	Rep	Residual
FP	3.87 NS	46.55 NS	0.29 NS	6.00****	1.64****	37.37****	1.89***	0.05 NS	2.34
SP	13.78***	37.05 NS	1.37 NS	7.33****	1.92****	34.15****	0.03 NS	0.04 NS	4.33
SP/FP	48.58****	5.44 NS	2.39 NS	9.70 NS	1.63 NS	5.04****	7.02 NS	1.06 NS	19.15
HMWG	26.31****	37.29 NS	5.80 NS	4.34**	0.56 NS	20.87****	2.18***	0.05 NS	2.61
HMWG/FP	55.84****	16.00 NS	8.29*	4.43 NS	1.04*	6.44****	3.23**	0.10 NS	4.62
GSI	69.55****	10.93 NS	2.66 NS	3.38 NS	0.56 NS	6.38****	3.25****	0.11 NS	3.19

*, **, *** and **** significant at p<0.05, p<0.01, p<0.001 and p<0.0001, respectively
FP = Flour protein content, SP = Soluble prolamin content in flour, SP/FP = Soluble prolamin content in FP, HMWG = High molecular weight glutenin in flour, HMWG/FP = High molecular weight glutenin content in FP, GSI = gluten strength index, NS = No significance

The influence of location and genotype on soluble prolamin (SP) content varied based on how the data was reported. When SP was expressed as a percentage of flour (and not a percentage of flour protein), much like GP or FP, location had a larger influence than genotype to total variance (Table 4.1.20 and Table 4.1.21). This was reflected in the larger range of values across locations in comparison with genotypes (Table 4.1.18 and Table 4.1.19). Across locations, SP ranged from 8.32% to 11.51% and 7.99% to 11.13% in 2015 and 2016, respectively. Whereas across genotypes, SP ranged from 9.98% to 11.35% in 2015 and 9.38% to 11.20% in 2016. Accordingly, lower minimum values were observed for locations means compared with genotype means. In both years, Stettler had the highest SP content, while Glenn had and the significantly ($p < 0.05$) the lowest SP values compared to the other genotypes. Genotype values in 2015 and 2016 were moderately correlated ($R^2 = 0.56$, $p < 0.05$; result not shown). Across individual samples, SP ranged from 6.9-13.3% (Appendix B.1).

By reporting SP per unit protein (i.e. SP/FP) observations were different, as might be expected by eliminating the influence of protein content. Protein content was one of the most variable parameters affected by growing location, thus eliminating its influence magnified the effect of genotype. In this case, the proportion of total variance contributed by genotype in SP/FP

in 2015, 2016 and combined yeas was 58.5%, 47.0% and 48.6%, respectively (Table 4.1.20 and Table 4.1.21). Considering all samples, SP/FP ranged from 65.8% to 80.49% (Appendix B.1), thus represents the protein fraction in highest concentration in flour (or wheat). For location averaged across genotypes, SP/FP ranged from 71.74% to 74.37% in 2015 and 70.44% to 74.05% in 2016 (Table 4.1.18). For genotypes averaged across locations, SP/FP ranged from 69.22% to 75.76% and 67.61% to 74.74% in 2015 and 2016, respectively (Table 4.1.19). For both years, Stettler and Glenn had the highest and lowest values for SP/FP, respectively. Genotype values for SP/FP were highly correlated across years ($R^2 = 0.89$, $p < 0.001$; results not shown).

The influence of location and genotype on the variation of high molecular weight glutenin (HMWG) followed a similar pattern to SP, in which location had a larger influence on HMWG expressed as a concentration of flour whereas genotype was more influential for HMWG expressed per unit protein (HMWG/FP). It deserves mentioning that HMWG/FP was the most potent protein quality related measure of gluten strength as it yielded very strong relationships to mixograph WIP and WAP across growing seasons. Over all samples and crop years, HMWG ranged from 2.2% to 4.5% (Appendix B.1). For genotypes averages across locations, HMWG ranged from 3.36% to 4.19% and 3.09% to 3.89% in 2015 and 2016, respectively (Table 4.1.19). Similarly, over all samples and crop years HMWG/FP ranged from 20.1% to 30.9% (Appendix B.1). For genotypes averaged across locations, HMWG/FP ranged from 23.86% to 29.10% in 2015 and 22.44% and 28.06% in 2016 Accordingly, HMWG comprised about 25% of total FP on average. For HMWG and HMWG/FP, Glenn had the highest concentrations ($p < 0.05$) in both years (Table 4.1.19). Stettler had the lowest HMWG and HMWG/FP values, however Stettler was not significantly different from the other

genotypes. Genotype HMWG and HMWG/FP were highly correlated across years ($R^2 = 0.95$, $p < 0.0001$ and $R^2 = 0.93$, $p < 0.0001$, respectively; Fig 4.1.7 and Fig 4.1.8). However, location means for HMWG and HMWG/FP across years were not correlated (Fig 4.1.9 and Fig 4.1.10). Location HMWG ranged from 2.95% to 3.97% in 2015 and 2.64% to 3.80% in 2016, respectively (Table 4.1.18). Location HMWG/FP means ranged from 23.36% to 26.87% and 21.75% to 25.42% in 2015 and 2016, respectively (Table 4.1.18).

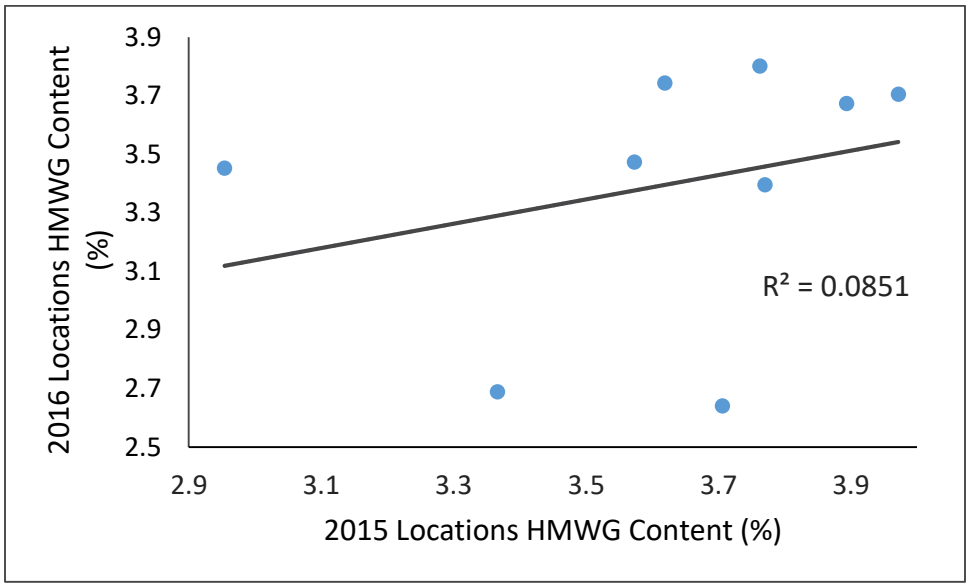

Figure 4.1.7. Relationship between HMWG concentrations for 2015 and 2016 growing sites. Each symbol represents a mean of 18 field replications.

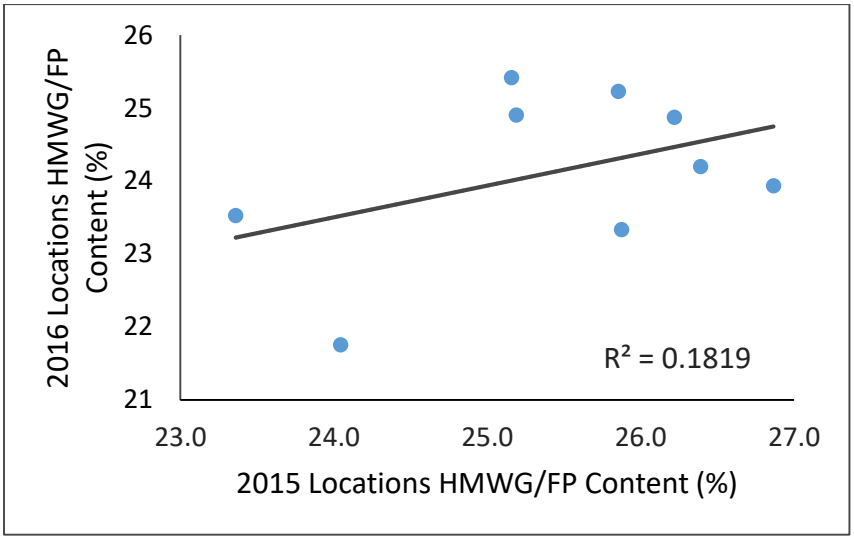

Figure 4.1.8. Relationships between HMWG/FP concentrations for 2015 and 2016 HMWG/FP growing sites. Each symbol represents a mean of 18 field replications.

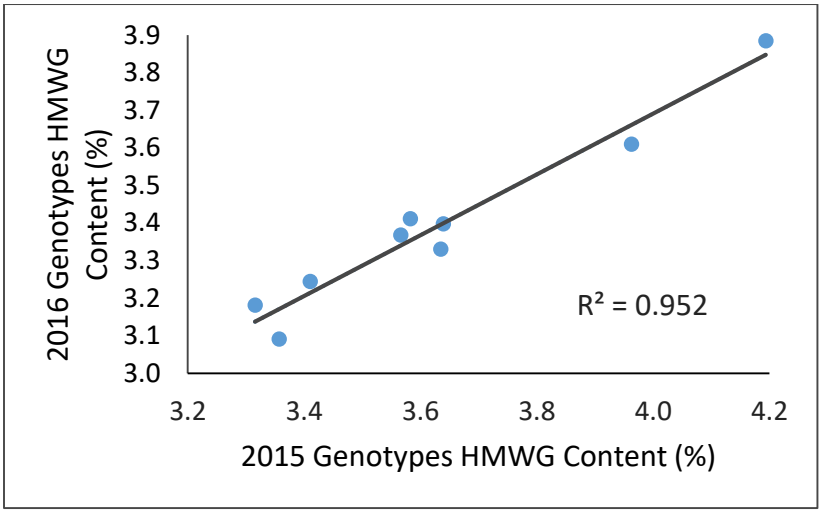

Figure 4.1.9. Relationship between HMWG concentrations for 2015 and 2016 genotypes. Each symbol represents a mean of 18 field replications.

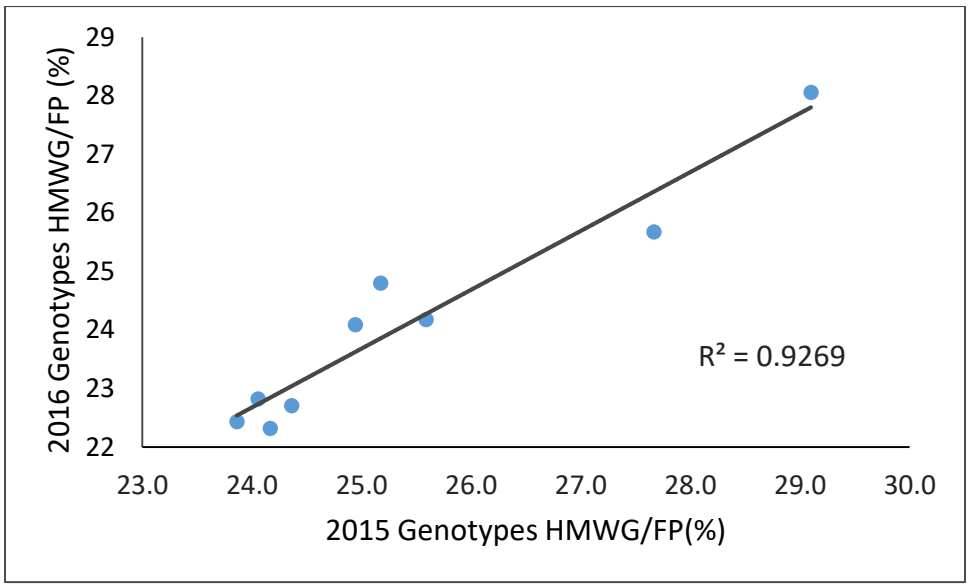

Figure 4.1.10. Relationship between HMWG/FP concentrations for 2015 and 2016 HMWG/FP genotypes. Each symbol represents a mean of 18 field replications.

ANOVA results for HMWG and HMWG/FP yielded similar results in which all effects were significant ($p < 0.0001$) in 2015 and 2016, with the exception of GxL interaction for HMWG/FP in 2016 (Table 4.1.20). Similar results for the complementary storage protein fraction SP, location contributed the largest proportion of total variance for HMWG of 49% and 71% for 2015 and 2016, respectively. In contrast, for HMWG/FP genotype contributed the highest proportion of total variance of 62% for both years. Similar results were obtained when years were combined, i.e. location had the largest variance contribution for HMWG (37.29%), despite not being a statistically significant, while genotype HMWG/FP contributed the most to total variance of 55.84% and was as a significant effect ($p < 0.0001$, Table 4.1.21).

Gluten strength index (GSI) results were very similar to those for HMWG/FP. Over all samples and crop years, GSI ranged from 0.27 to 0.46 (Appendix B.1). For genotypes averaged across locations, GSI varied from 0.32 to 0.42 and 0.30 to 0.42 across genotypes in 2015 and

2016, respectively (Table 4.1.19). In both years, Glenn had the highest ($p < 0.05$) GSI values, whereas Stettler had the lowest values. Genotype GSI was highly correlated across years ($R^2 = 0.98$, $p < 0.001$; Fig 4.1.11). The location GSI mean range was much smaller, from 0.31 to 0.36 in 2015 and 0.32 to 0.37 in 2016 (Table 4.1.18). No observable trend existed between location GSI values across years (Fig 4.1.12). ANOVA results showed genotype as a significant effect ($p < 0.0001$) for GSI, contributing to 72%, 74% and 70% of the total variance in 2015, 2016 and combined years, respectively (Table 4.1.20 and Table 4.1.21).

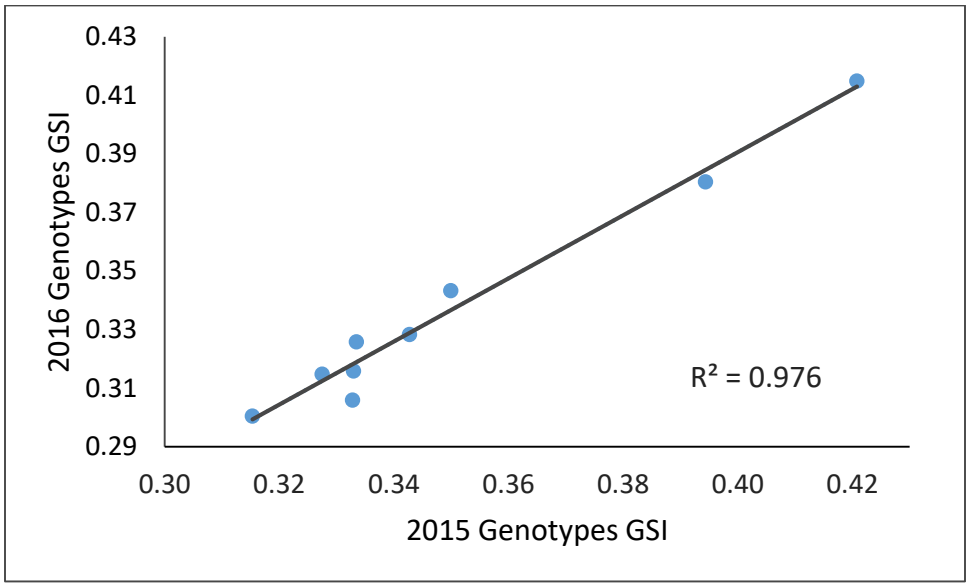

Figure 4.1.11. Relationship between GSI for 2015 and 2016 GSI genotypes. Each symbol represents a mean of 18 field replications.

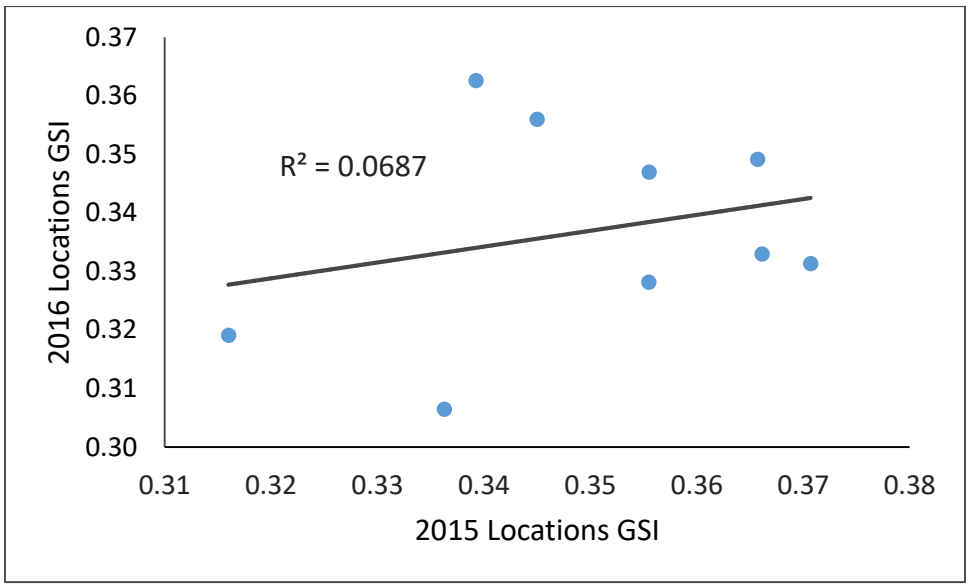

Figure 4.1.12. Relationship between GSI for 2015 and 2016 GSI growing sites. Each symbol represents a mean of 18 field replications.

4.2 Significance of Removing Cultivar Glenn on Analysis of Variance Results for Gluten Strength

As can be seen from previous results, variety Glenn was consistently and significantly higher in gluten strength compared to the other eight genotypes in this study. Accordingly, it was of interest to determine whether Glenn may have altered the influence of genotype on ANOVA results. For this reason, Glenn was removed from the data set and the ANOVA was redone.

ANOVA results for 2015 locations for protein content, protein composition and mixograph parameters (Table 4.2.1) show that genotype was highly significant for all parameters ($p < 0.0001$) except for FP which had a lower level of significance ($p < 0.01$). This result is similar to the ANOVA results that included Glenn (Table 4.1.16 and Table 4.1.20) Despite Glenn being removed, genotype accounted for the highest contribution to total variance of SP/FP, HMWG/FP and GSI. Similar to previous results, genotype did not contribute the highest portion of variance for WIP and WAP, as location contributed 51.8% and 45.5% of total variance, respectively. The significance of the GxL interaction remained regardless of the removal of Glenn (Table 4.2.1).

Table 4.2.1. Percent variance contribution of 2015 effects on protein composition and mixograph parameters with variety Glenn removed.

	Genotype	Location	Rep	GxL	Residual
FP	5.57*	80.46**	0.14 NS	11.01**	2.82
SP	11.81**	72.26**	0.11 NS	11.78**	4.05
SP/FP	46.93**	13.17*	0.65 NS	24.82*	14.43
HMWG	24.26**	61.06**	0.18 NS	10.33**	4.18
HMWG/FP	42.67**	36.64**	0.08 NS	15.33**	5.28
GSI	53.29**	29.57**	0.10 NS	13.16**	3.88
MDT	11.43**	68.13**	0.25 NS	15.67**	4.52
PBW	18.50**	64.41**	0.14 NS	12.29**	4.65
PDR	16.34**	70.52**	0.24 NS	9.64**	3.25
WIP	15.73**	51.84**	0.44 NS	23.07**	8.92
WAP	29.70**	45.46**	0.44 NS	18.14**	6.26

*, ** are significant at p<0.01 and P,0.0001 respectively. NS = no significance

FP = Flour protein content, GSI = Gluten strength index, HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

Similar to 2015 results, ANOVA of 2016 grown samples (Table 4.2.2) confirmed the significant ($p<0.0001$) effect of genotype on all parameters when Glenn was removed from the data set. Genotype remained the highest contributor to total variance for SP/FP, IG/FP, GSI, MDT, WIP and WAP. However, the level of statistical significance for 2016 GxL changed for numerous parameters. Without Glenn, GxL interaction significantly affected FP, HMWG and PDR at $p < 0.0001$, PBW at $p < 0.001$, SP at $p < 0.01$ as well as GSI at $p < 0.05$. Other parameters did not show a significant GxL interaction. The results of GxL interaction of FP, SP/FP, HMWG, HMWG/FP and PDW did not change from when Glenn was included in the analysis.

Table 4.2.2 Percent variance contribution of 2016 effects on protein composition and mixograph parameters with variety Glenn removed.

	Genotype	Location	Rep	GxL	Residual
FP	6.17****	85.46****	0.08 NS	6.24****	2.05
SP	15.09****	72.11****	0.28 NS	7.80**	4.72
SP/FP	36.99****	13.81****	1.96 NS	16.95 NS	30.29
HMWG	9.82****	80.52****	0.01 NS	6.83****	2.82
HMWG/FP	41.53****	35.19****	0.17 NS	11.95 NS	11.16
GSI	57.80****	26.51****	0.40 NS	8.52*	6.77
MDT	41.56****	29.15****	0.92 NS	15.56 NS	12.81
PBW	14.31****	66.37****	0.13 NS	12.82***	6.36
PDR	13.34****	75.11****	0.12 NS	8.49****	2.94
WIP	44.33****	24.10****	0.42 NS	14.92 NS	16.24
WAP	41.74****	26.51****	0.83 NS	16.96 NS	13.96

*, **, *** and **** are significant at p<0.05, p<0.01, p<0.001 and P,0.0001 respectively. NS = no significance
FP = Flour protein content, GSI = Gluten strength index, HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

ANOVA for combined years (absent Glenn) showed that genotype was significant for all parameters except FP (Table 4.2.3). The effect of genotype was highly significant ($p <0.0001$) for HMWG and GSI. Genotype was also highly significant for SP/FP, PBW and HMWG/FP (p

<0.001) as well as SP, WIP, WAP and PDR ($p<0.01$) and MDT ($p<0.05$). Genotype continued to contribute the highest proportion of total variance for GSI, SP/FP, HMWG/FP, WIP, WAP and MDT. It is important to note that although genotype remained the highest contributor to variance for these parameters, the percent variance decreased upon the removal of Glenn. Fig 4.2.1 shows the difference in the percent variance contribution of genotype with Glenn removed in ANOVAs for 2015, 2016 and combined years. It is clear from this result that the magnitude of the genotype effect was decreased upon the removal of Glenn by approximately 0 to 21% and 0 to 32% for protein related and mixograph parameters, respectively. The decrease in genotype variance was most prominent for those related to gluten strength such as WIP, WAP, HMWG/FP, GSI and MDT. Interestingly, the proportion of genotype variance to total variance upon removal of Glenn from the data set was unaffected for FP, SP, PBW and PDR. Compared to genotype variance, the opposite effect was observed for location variance as the proportion of total variance (Fig 4.2.2), wherein location contribution to variance was increased by approximately 5-15% for HMWG/FP, GSI, MDT, WIP and WAP.

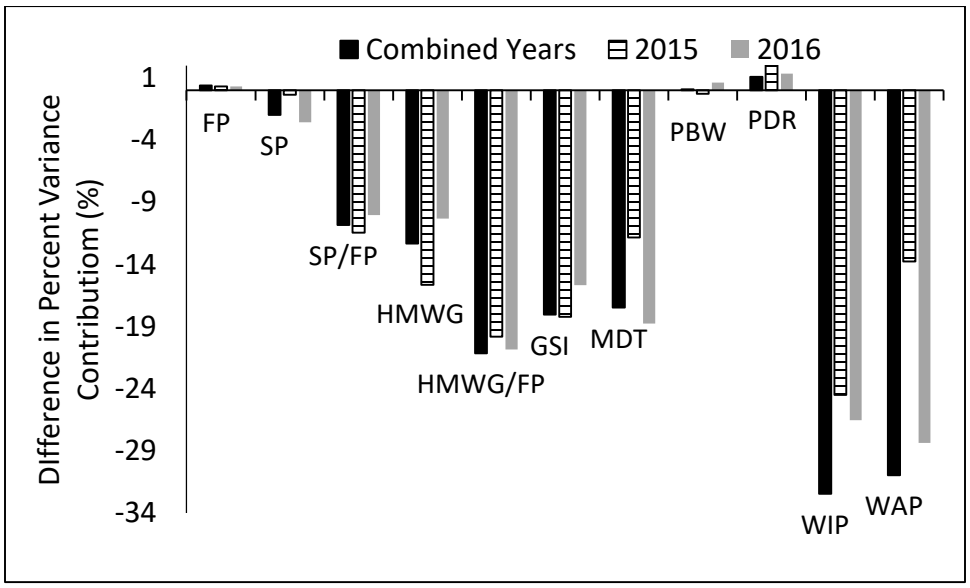

Figure 4.2.1. Difference in the percent variance contribution of ANOVA genotype effect with the removal of CWRS variety Glenn for combined years' data (blue) as well as 2015 (orange) and 2016 (grey) alone.

Table 4.2.3. Percent variance contribution of combined years' effects on protein composition and mixograph parameters with variety Glenn removed.

	Genotype	Rep	Year	Location	LxY	LxG	GxY	LxGxY	Residual
FP	4.27 NS	0.04 NS	0.14 NS	47.34 NS	36.28****	6.03****	1.57****	1.95***	2.39
SP	11.82**	0.06 NS	0.96 NS	38.53 NS	33.05****	7.22****	1.84****	2.04 NS	4.49
SP/FP	37.74***	1.05 NS	2.42 NS	6.23 NS	7.50****	11.67*	2.03 NS	7.46 NS	23.89
HMWG	13.99****	0.03 NS	6.35 NS	44.31 NS	23.89****	5.21**	0.61 NS	2.45**	3.16
HMWG/FP	34.70***	0.10 NS	12.81**	23.20 NS	8.63****	6.97 NS	1.66*	4.78**	7.15
GSIp	51.50****	0.18 NS	5.10 NS	17.22 NS	10.02****	5.59 NS	0.85 NS	4.41***	5.14
MDT	14.23*	0.07 NS	10.95 NS	22.31 NS	29.96****	9.00**	2.74***	4.63**	6.11
PBW	15.07***	0.02 NS	2.56 NS	38.70 NS	24.45****	8.28**	1.28*	4.10**	5.52
PDR	13.87**	0.09 NS	0.82 NS	37.52 NS	34.15****	6.91****	1.36***	2.12**	3.16
WIP	21.97**	0.11 NS	6.71 NS	19.39 NS	19.98****	11.94*	2.40*	6.46*	11.04
WAP	21.45**	0.16 NS	9.26 NS	21.80 NS	18.69****	10.43*	2.94**	6.66**	8.62

*, **, ***, **** significant at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively. NS = No significance

FP = Flour protein content, GSI = Gluten strength index, HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

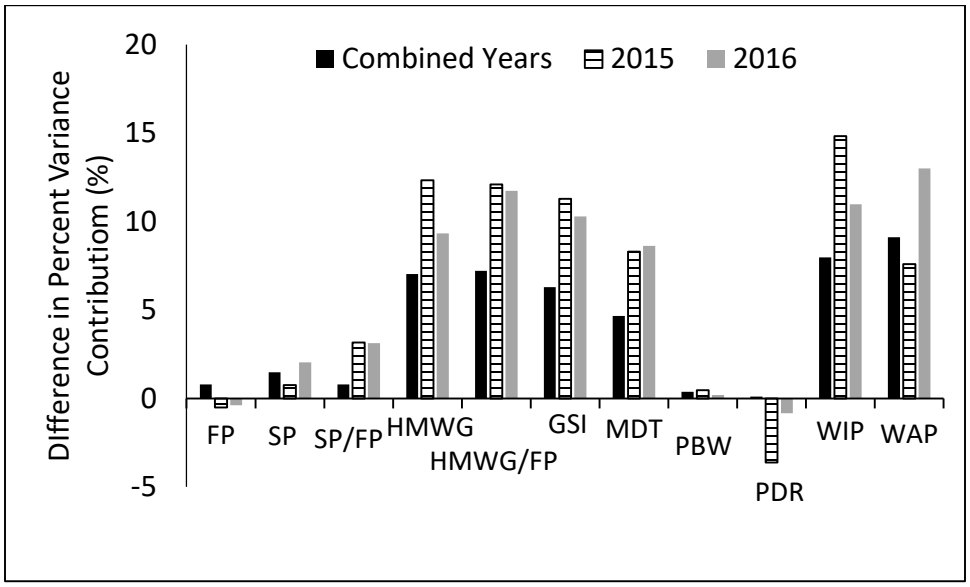

Figure 4.2.2. Difference in the percent variance contribution of ANOVA location effect with the removal of CWRS variety Glenn for combined years' data (blue) as well as 2015 (orange) and 2016 (grey) alone.

Despite the removal of Glenn, many of the genotype interaction effects remained significant. GxL was significant for FP, SP and PDR ($p < 0.0001$), HMWG, MDT and PBW ($p < 0.01$) and SP/FP, WIP and WAP ($p < 0.05$). The remaining parameters did not show significant GxL interactions. Interestingly, the magnitude of GxL effect appeared to increase upon the removal of Glenn from the analysis. This can be seen in Fig 4.2.3, in which the percent variance contribution of GxL without Glenn was greater than when Glenn was included in the ANOVA for 2015 and 2016 as well as for combined years. However, this increase was small; the maximum being 7.5% for WIP in 2015. There was no change in the variance contribution of GxL for FP, SP, PBW and PDR with Glenn removed from the analysis. The difference in percent variance contribution of GxY and GxLxY effects upon the removal of Glenn can be seen in Figure and Figure , respectively. The magnitude of the influence of these interactions did not

appear to change; the largest difference was a 2.4% increase in the variance contribution of GxLxY for WIP. Regardless, both interactions were significant for the majority of parameters, with the exception of GxL on SP/FP, IG, GSI as well as GxLxY on SP and SP/FP. Similarly, the proportion of total variance for YxL increased by approximately 2-8% for most parameters (Fig 4.2.6).

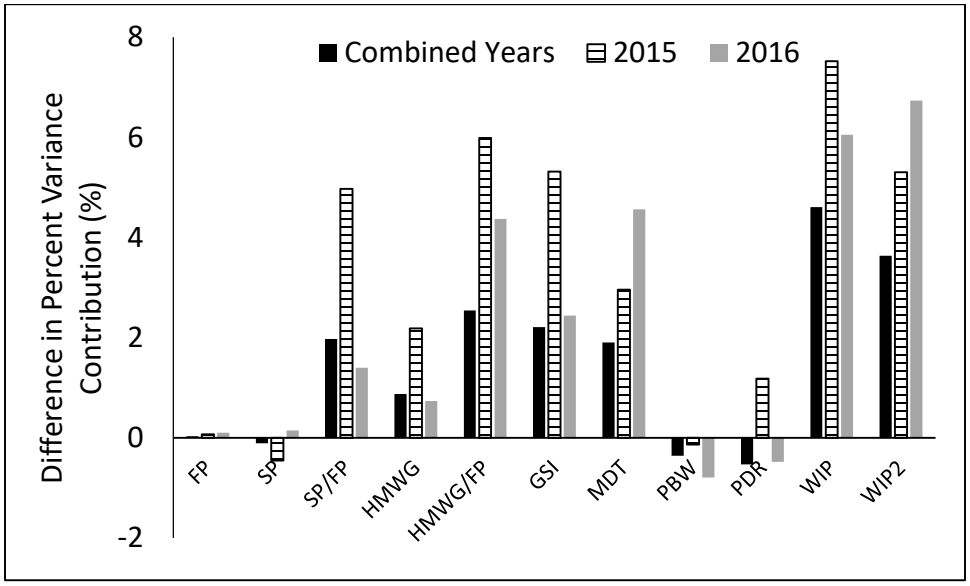

Figure 4.2.3. Difference in the percent variance contribution of ANOVA GxL effect with the removal of CWRS variety Glenn for combined years' data (black) as well as 2015 (lined) and 2016 (grey) alone.

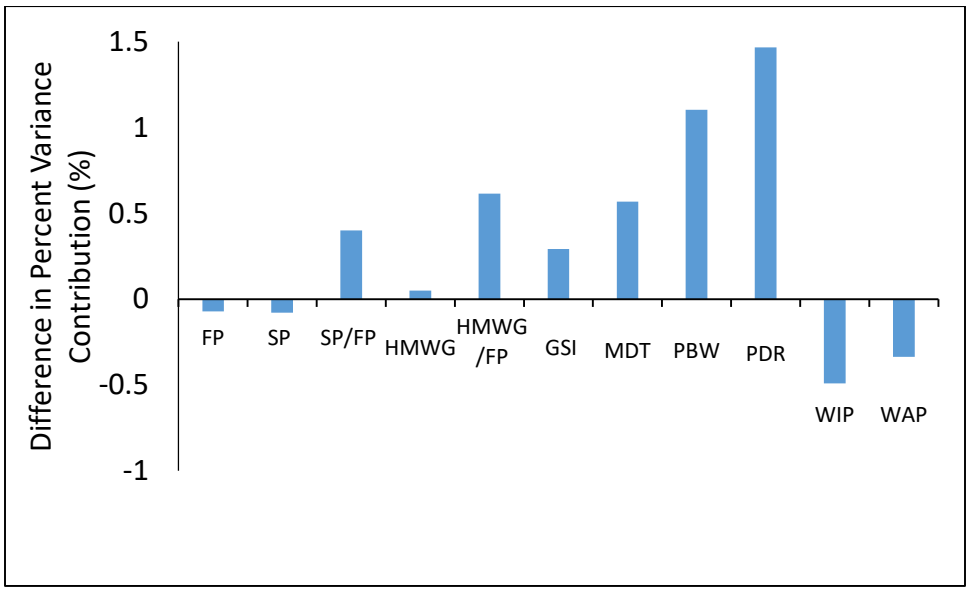

Figure 4.2.4. Difference in the percent variance contribution of ANOVA GxY effect with the removal of CWRS variety Glenn for combined years' data.

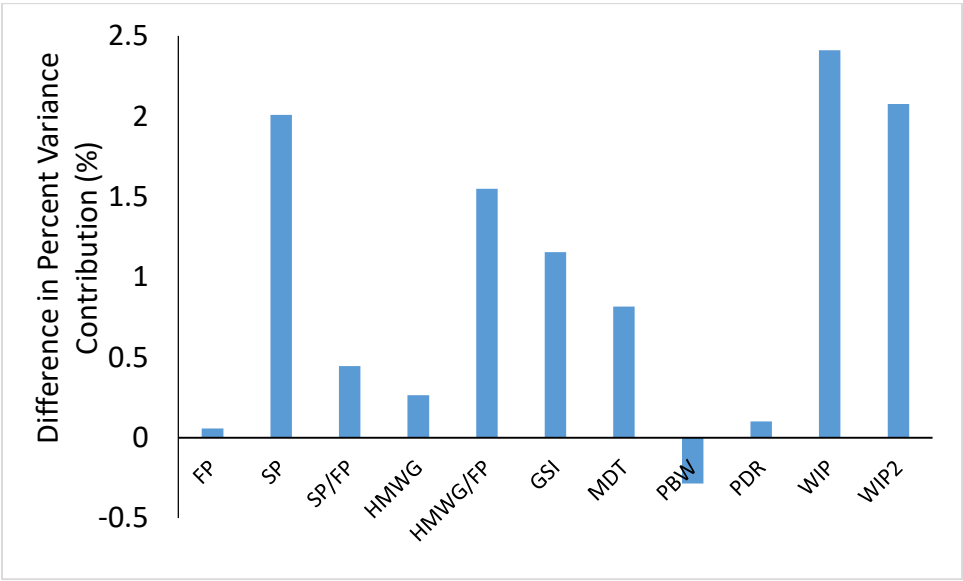

Figure 4.4.5 Difference in the percent variance contribution of ANOVA GxYxL effect with the removal of CWRS variety Glenn for combined years' data.

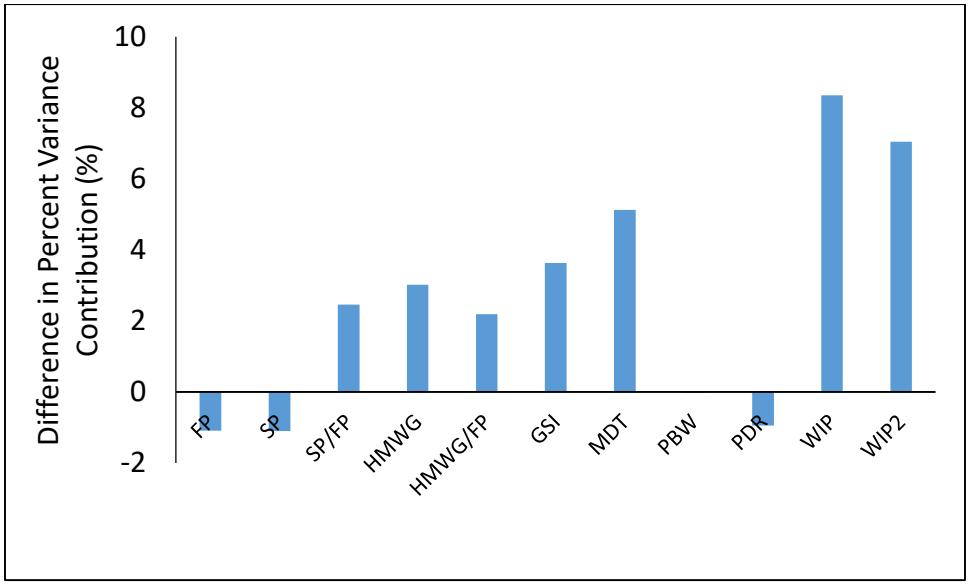

Figure 4.2.6. Difference in the percent variance contribution of ANOVA YxL effect with the removal of CWRS variety Glenn for combined years' data.

4.3 Effect of Wheat Grade on Gluten Strength

4.3.1 Effect of Sample Grade on Parameters Related to Gluten Strength

The effect of grade on mean, range and CV values for the various wheat quality parameters that were assessed based on combined year results are presented in Table 4.3.1, Table 4.3.2 and Table 4.3.3, respectively. Tukey-Kramer range test was used to evaluate the significance of mean differences among grades; none was found at a significance level of $p<0.10$. Despite the lack of significance, there were noticeable trends consistent with grade lowering for the following parameters: HMWG, HMWG/FP, GSI, WIP and WAP. It should be underscored that all these parameters reflect gluten strength. Accordingly, as sample grade was lowered on average, gluten strength declined. For these parameters the relative decreases from No. 1 to Feed grade was 15.6%, 17.9%, 16.7%, 22.6% and 22.9% for HMWG, HMWG/FP, GSI, WIP and WAP, respectively. ANOVA showed grade to be a significant factor ($p<0.05$) on GSI only, contributing 0.33% of total variance compared to more prominent factors such as genotype and location. Grade as an effect was not significant for any other parameter, and never contributed to more than 0.60% of the variance in any of the protein composition and mixograph parameters analyzed. Similar to observations on mean parameter values, there was a noticeable trend in the ranges of some quality parameters with grade reduction, particularly for HMWG, HMWG/FP, GSI, WIP and WAP (Table 4.3.2). In each case, with two exceptions, as the sample grade decreased, the maximum and minimum values of the data range decreased. The exceptions were HMWG/FP and WIP where for grade No. 2 samples, the minimum value was higher than that for CWRS No.1. In addition, the maximum values of WAP did not follow this decreasing trend. The coefficients of variation for each parameter in this analysis (Table 4.3.3) did not show any trend related to sample grade. It is important to note that some of the variation between

grades could also have occurred due to the varying number of samples within each class. CWRS No.2 contained a notably higher number of samples (132), followed by CWRS No.1 (85), CWRS No.3 (47), feed grade (42) and below feed (18).

Table 4.3.1 CWRS grade means for protein composition and mixograph parameters.

	No.1	No.2	No.3	Feed	Below Feed
FP (%)	14.59	14.12	13.89	13.58	14.99
SP (%)	10.51	10.21	10.15	9.85	10.85
SP/FP (%)	72.02	72.35	73.14	72.71	72.49
HMWG (%)	3.77	3.53	3.37	3.21	3.18
HMWG/FP (%)	25.88	25.04	24.43	23.68	21.25
GSI	0.36	0.35	0.33	0.33	0.30
MDT (min)	2.79	2.80	2.73	2.49	2.11
PBW (%Torque)	29.26	28.09	27.12	28.96	29.94
PDR (%Torque)	56.63	54.56	53.01	55.78	58.12
WIP (%Torque*min)	132.66	127.49	120.89	119.46	102.64
WAP (%Torque*min)	81.07	77.24	72.23	70.97	62.49
	-	-	-	-	-
Number of Samples	85	132	47	42	18

FP = Flour protein content, GSI = Gluten Strength Index, HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

Table 4.3.2. CWRS grade ranges for protein composition and mixograph parameters.

	CWRS no.1	CWRS no.2	CWRS no.3	Feed	Below Feed
FP (%)	12.5 – 16.4	9.8 -16.7	10.8 – 16.6	9.9 – 16.25	10.2 – 17.5
SP (%)	9.0 -13.0	6.9 – 13.3	7.8 – 12.8	7.3 – 11.8	7.8 – 13.1
SP/FP (%)	63.9 – 79.2	62.2 – 79.6	67.1 – 78.0	62.0 – 78.6	65.6 – 76.5
HMWG (%)	3.21 – 4.53	2.21 – 4.51	2.44 – 4.28	2.26 – 4.15	2.34 -3.64
HMWG/FP (%)	21.4 – 30.4	21.0 – 30.8	20.9 – 30.2	20.8 – 27.6	19.8 – 23.4
GSI	0.29 – 0.46	0.28 – 0.45	0.27 – 0.43	0.27 -0.41	0.27 – 0.35
MDT (min)	1.77 – 4.22	1.87 – 5.45	1.78 - 4.94	1.87 – 3.92	1.53 – 2.83
PBW (%Torque)	24.3 – 34.7	18.7 – 36.2	18.4 – 33.2	20.5-38.8	22.9 – 34.2
PDR (%Torque)	47.4 – 67.6	35.6 – 71.1	36.6 – 64.4	41.7 – 72.4	40.8 – 68.2
WIP (%Torque*min)	81.8 – 206.4	79.7 – 211.9	79.2 – 193.2	73.5 – 185.1	70.2 – 118.1
WAP (%Torque*min)	54.6 – 136.4	48.7 – 122.3	49.5 – 114.3	50.7 – 107.4	48.6 - 73.4
	-	-	-	-	-
Number of Samples	85	132	47	42	18

FP = Flour protein content, GSI = Gluten Strength Index, HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

Table 4.3.3. CWRS grade coefficients of variation for protein composition and mixograph parameters.

	CWRS no.1	CWRS no.2	CWRS no.3	Feed	Below Feed
FP (%)	4.8	11.4	13.4	13.6	11.2
SP (%)	7.5	11.8	14.2	13.6	10.5
SP/FP (%)	4.6	4.3	3.9	4.7	3.4
HMWG (%)	9.4	12.9	12.5	16.0	10.8
HMWG/FP (%)	8.9	7.9	8.4	7.0	5.5
GSI	12.5	10.3	10.6	9.4	7.7
MDT (min)	22.2	23.2	28.9	19.6	15.6
PBW (%Torque)	8.6	12.9	12.6	16.4	8.9
PDR (%Torque)	6.9	12.8	12.9	16.5	11.1
WIP (%Torque*min)	22.4	24.3	22.3	19.2	12.7
WAP (%Torque*min)	22.3	14.3	21.8	18.5	10.8
	-	-	-	-	-
Number of Samples	85	132	47	42	18

FP = Flour protein content, GSI = Gluten Strength Index, HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

The effect of sample grade on relationships between WAP and HMWG/FP and WAP and GSI is shown in Fig 4.3.1. Somewhat similar to above-noted observations, as the grade decreased, there were noticeably fewer samples with relatively high values for the charted parameters. However, grade did not appear to have a large effect on the R^2 values of the relationships, although for samples grading lower than feed, the lowest R^2 values of 0.56 and 0.48 were obtained for the influence of HMWG/FP and GSI, respectively, on WAP. The lower range of data values and possible deviation from normality can also affect correlation and R^2 values. However, all the relationships shown in Fig 4.3.1 were statistically significant at p values indicated in the Figure caption. This was not surprising especially given the high R^2 values for relationships involving grade No.1, No. 2 and No. 3 samples.

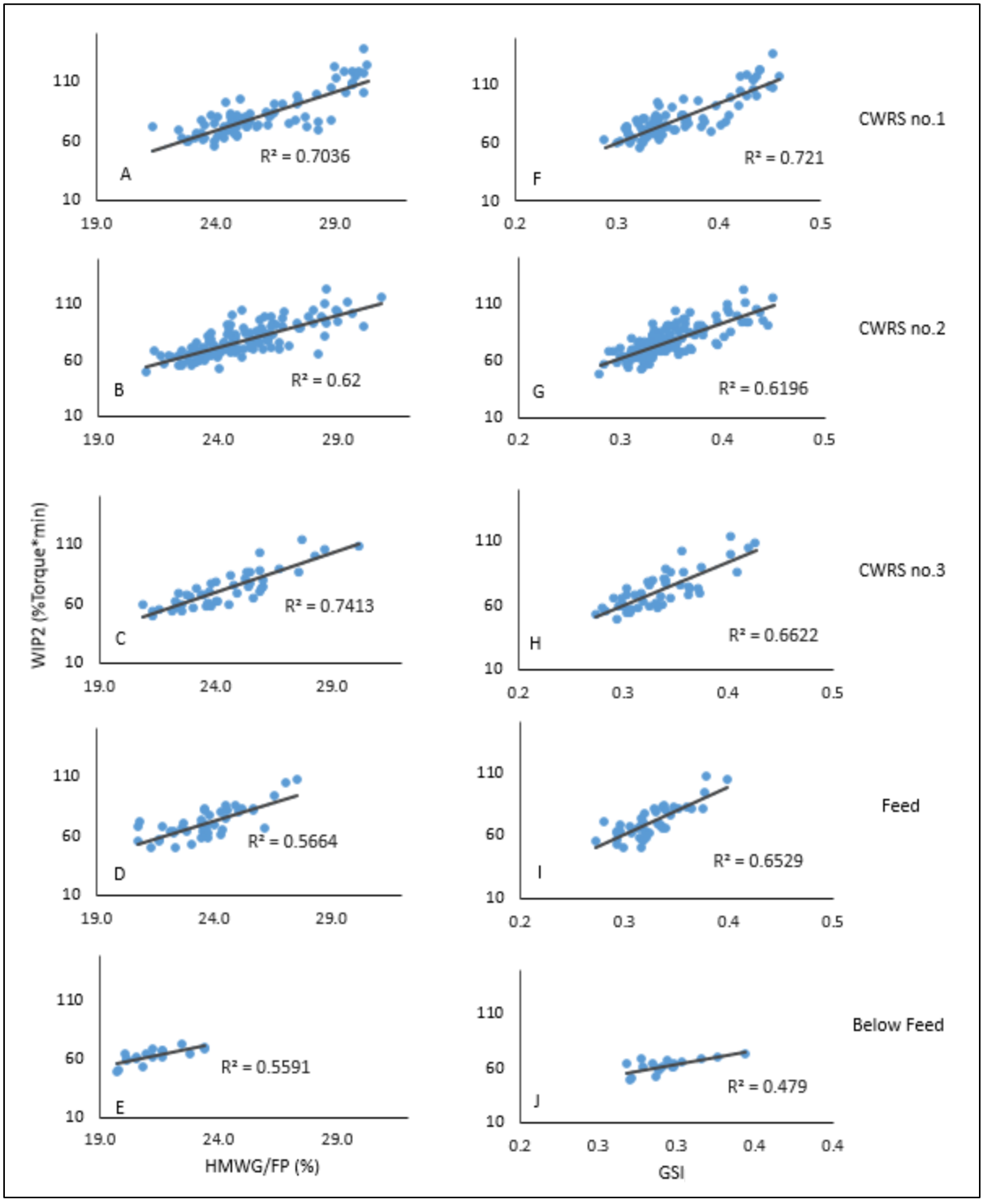

Figure 4.3.1. Relationships between WAP and HMWG/FP (left: A-E) and WAP and GSI (right: F-J) for samples grading CWRS No.1 (A,F), No.2 (B,G), No.3 (C,H), feed (D,I) and below feed (E,J). Each symbol represents mean value of three analytical replications. All relationships were significant (p <0.0001) with the exception of E (p <0.001) and J (p <0.01).

4.3.2 Analysis of Variance of Canada Western Red Spring No.1 and No.2 Protein Composition and Mixograph Parameters

Analysis of variance was conducted on samples grading CWRS No.1 and No.2 to assess the effects of genotype, growing location and interactions on milling grade samples only, as well as to compare those results to those obtained when all samples were included. In total, 217 samples were included in the analysis, 126 and 91 from 2015 and 2016, respectively. Table 4.3.4 provides the number of samples included in this analysis by location and genotype. In general, Edmonton, Goodale, Kelburn Lethbridge and Swift Current locations provided a similar number of samples ranging from 29 to 36. The largest number of milling grade samples came from the Lethbridge site which was generally free from effects of degrading factors in 2015 and 2016. The number of No. 1 and No. 2 grade samples from Beaverlodge, Morden, Brandon and Indian Head were lower, i.e. 19, 17, 12 and 7 respectively which reflects increasing influence of degrading factors, most notably FDK, in those locations. For genotypes, there was no noticeable grade differentiation, with one exception (Stettler), as the number of milling grade samples was relatively high, i.e. from 23 to 30 samples/genotype. The variety Stettler was much more susceptible to degrading factors compared to the other genotypes in the study, as only 12 samples of 36 were graded No. 1 or No. 2.

Table 4.3.4. Count of locations and genotype included in analysis of variance of CWRS no.1 and no.2 wheat samples.

Location	Count	Genotype	Count
Beaverlodge	19	Brandon	23
Brandon	12	Carberry	25
Edmonton	32	Cardale	26
Goodale	34	Glenn	26
Kelburn	31	Harvest	23
Lethbridge	36	Stanley	27
Indian Head	7	Stettler	12
Morden	17	Unity	30
Swift Current	29	Utmost	25

Table 4.3.5 presents the percent variance contributions of mixed model effects for protein composition and mixograph parameters for No. 1 and No. 2 CWRS samples. Genotype was the largest contributor to total variance for SP/FP (45.7%), HMWG/FP (70.8%), GSI (79.1%), MDT (37.8%), WIP (58.2%) and WAP (61.1%). In contrast, location contributed the most to total variance in FP and SP of 46.9% and 34.8%, respectively. These results were very similar to those involving the complete sample set. Where the results differed from the complete sample set was that for milling grade samples, genotype and location effects were similar in their contribution to total variance of HMWG, PBW and PDR at ~20-30% each (Table 4.3.5). This contrasts with results for the complete sample set, where location accounted for the largest proportion of total variance (~40%) for the parameters and genotype accounted for only 26% of the total variance in HMWG and 15% for PBW and PDR. Compared to the complete sample set, the contribution of genotype to variance of HMWG, HMWG/FP, GSI, MDT, PDR, PBW and WAP increased once only milling grade samples were included in the analysis. This increase ranged from 5.5% to 15.7%, the highest affected parameter being PDR, following by HMWG/FP in which the percent variance contribution of genotype rose by 14.6%. The aforementioned increase in the percent variance contribution of genotype for the select parameters was compensated by a decrease for the effect of location to the total variance. The contribution of genotype to total variance for the

remaining parameters did not vary by more than three percent compared to the complete sample set.

Table 4.3.5. Percent variance contribution of mixed model effects for mixograph and protein composition of No. 1 and No. 2 CWRS wheat samples.

	Genotype	Location	Rep	Year	GxL	GxY	LxY	GxLxY	Residual
FP	3.71 NS	46.91 NS	0.13 NS	0.32 NS	7.10**	2.75***	34.47****	2.14***	2.47
SP	14.90*	34.84 NS	0.01 NS	1.13 NS	9.07**	3.43****	28.62****	2.38 NS	5.62
SP/FP	45.73***	4.35 NS	0.85 NS	1.28 NS	12.73 NS	2.14 NS	6.29**	8.21**	18.43
HMWG	35.20****	30.15 NS	0.09 NS	2.77 NS	4.77 NS	1.02 NS	20.96****	2.14 NS	2.89
HMWG/FP	70.46****	5.82 NS	0.08 NS	3.38*	5.76 NS	1.72 NS	4.39***	3.66**	4.74
GSI	79.06****	5.26 NS	0.13 NS	0.47 NS	5.10 NS	1.30 NS	4.63****	0.63***	3.42
MDT	37.76**	13.54 NS	0.23 NS	5.20 NS	8.76 NS	3.82**	21.08****	3.75*	5.86
PBW	28.50**	25.42 NS	0.31 NS	1.81 NS	8.19 NS	3.43**	21.52****	4.69**	6.14
PDR	20.44**	30.11 NS	0.11 NS	0.10 NS	9.37****	2.34****	31.72****	1.60 NS	4.20
WIP	58.21****	6.71 NS	0.12 NS	1.78 NS	8.43 NS	2.89**	11.58****	3.61 NS	6.66
WAP	61.05****	5.90 NS	0.74*	2.94 NS	8.32 NS	1.71 NS	10.45****	3.38*	5.51

FP = Flour protein content, GSI = Gluten Strength Index , HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

*, **, ***, **** signifciant at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively, NS = not significant

ANOVA was also conducted on No.1 and No.2 CWRS samples of 2015 and 2016 individually (results not shown). There were only two notable differences in the results compared to when the data was combined over growing seasons. Similar to when years were combined, there was an increase in the genotype contribution to total variance of HMWG/FP. However, this increase was larger for the individual years. In addition, the percent contribution of genotype to total variance of MDT decrease in 2015 and 2016 compared to the combined year analysis. Similar to the results of the combined years, the direction and magnitude of these differences in genotype variance contribution was accompanied by an opposing change in the contribution of location to total variance.

ANOVA of milling grade sample results vs. complete sample set was additionally conducted without Glenn in order to evaluate outcomes without this distinctly strong gluten genotype (Table 4.3.6). By way of reminder, for the complete sample set, when Glenn was

removed, ANOVA results showed that the magnitude of the genotype effect was decreased most notably for parameters related to gluten strength. Similarly, when only No. 1 and No. 2 grade samples were subjected to the same ANOVA, the contribution of genotype to total variance decreased for all parameters except FP, PDR and PBW (these 3 parameters have high co-variance, as reflected by correlations of r = 0.69, r = 0.78 and r = 0.94 for FP to PBW, FP to PDR and PBW to PDR, respectively). This decrease ranged from 2.4% for SP to 34.7% for WIP. Once Glenn was removed from the complete sample set, WIP also had the largest decrease in genotype contribution to total variance. For milling grade samples, this decrease in genotype influence to total variance was accompanied by small increases in all other effects and interactions, none of them very large. In the case of the Glenn removal from the complete sample set, the decrease in genotype influence to total variance was accompanied by an increase of similar magnitude in location influences. Despite these changes, genotype remained a significant ($p<0.05$) effect on all parameters other than FP and SP as well as the highest contributor to variance in SP/FP, HMWG/FP, GSI, PBW, WIP and WAP.

Table 4.3.6. Percent variance contribution of mixed model effects for mixograph and protein composition of No.1 and No.2 CWRS wheat samples with genotype Glenn removed.

	Genotype	Location	Rep	Year	GxL	GxY	LxY	GxLxY	Residual
FP	4.42 NS	47.17 NS	0.12 NS	0.09 NS	7.06**	2.44***	33.97****	2.13**	2.59
SP	11.43 NS	37.16 NS	0.01 NS	0.58 NS	9.08**	3.02***	30.30****	2.34 NS	6.08
SP/FP	31.57**	5.57 NS	0.87 NS	1.04 NS	14.75 NS	2.26 NS	10.11**	9.98 NS	23.87
HMWG	21.21***	37.90 NS	0.10 NS	2.94 NS	5.62 NS	1.32 NS	24.75****	2.24 NS	3.92
HMWG/FP	48.69**	9.44 NS	0.13 NS	6.47* NS	9.84 NS	2.93 NS	7.87***	6.51**	8.13
GSIp	58.30***	10.20 NS	0.27 NS	1.46 NS	7.84 NS	1.69 NS	9.71****	5.10***	5.43
MDT	14.11 NS	18.82 NS	0.28 NS	9.00 NS	11.78 NS	4.16 NS	29.11****	5.29**	7.45
PBW	28.97**	26.53 NS	0.00 NS	2.63 NS	6.27 NS	2.95 NS	22.01****	4.82**	5.81
PDR	21.68***	31.18 NS	0.10 NS	0.37 NS	7.92***	1.86***	31.31****	1.66 NS	3.92
WIP	19.75*	12.99 NS	0.41 NS	5.49 NS	15.93 NS	3.38 NS	22.58****	6.88 NS	12.61
WAP	22.30*	14.83 NS	0.58 NS	6.98 NS	14.85 NS	3.23 NS	19.78****	6.33 NS	11.13

FP = Flour protein content, GSI = Gluten Strength Index , HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

*, **, ***, **** signifciant at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively, NS = not significant

4.3.3 Analysis of Variance of Lower Grade Samples < No. 2 Canada Western Red Spring for Protein Content and Composition and Mixograph Parameters

Analysis of variance was additionally conducted on the 107 remaining samples grading No. 3 CWRS and below. Of these 107 samples, only 36 (34%) were from the 2015 year which was a more favourable growing season in terms of weather and degrading factors in the Prairie region. The number of samples by genotype and location included in this sample set is presented in Table 4.3.7. Indian Head, Brandon, Morden and Beaverlodge generally had the highest number of samples of lower grades, ranging from 17 to 29. For the remaining locations, the number of samples included in this analysis were relatively low ranging from 2 (Goodale) to 7 (Swift Current). It is interesting to note that that Lethbridge did not have any samples that graded below No. 2 CWRS, so this location was not included in the analysis. The majority of genotypes were included at similar numbers of samples, ranging from 9 to 13, except for Unity (6 samples) and Stettler (24 samples) with grades lower than No. 2 CWRS.

Table 4.3.7. Count of locations and genotype included in analysis of variance of CWRS no.3, feed and below wheat samples.

Location	Count	Genotype	Count
Beaverlodge	17	Brandon	13
Brandon	24	Carberry	11
Edmonton	4	Cardale	10
Goodale	2	Glenn	10
Kelburn	5	Harvest	13
Lethbridge	0	Stanley	9
Indian Head	29	Stettler	24
Morden	19	Unity	6
Swift Current	7	Utmost	11

Table 4.3.8 presents the variance contribution of ANOVA for all samples grading below No. 2 CWRS. For protein related parameters, results are very similar to those obtained for top milling grade samples (compare with Table 4.3.5). Excluding FP and SP (which is strongly related to FP, r = 0.95), genotype contributed the highest proportion of variance and was

significant for SP/FP, HMWG, HMWG/FP and GSI although the level of significance was lower. For mixograph parameters, there was similarity in ANOVA results between analyses for mixograph parameters for upper and lower milling grade samples (compare Table 4.3.5 and Table 4.3.8), although only for WIP was genotype a significant effect for lower grade samples, despite having a large contribution to total variance for WAP (40.8%). Similar to the analysis for top milling grade samples, location was not significant although it contributed the highest proportion of total variance for FP, SP, HMWG, as well as mixograph parameters (MDT, PBW and PDR). Interestingly, LxY interaction notably decreased in significance for lower grade samples, where this interaction was previously highly significant ($p<0.0001$) for all parameters, this was only found for FP, SP and PDR in the analysis of low grade samples. Presumably this outcome can be explained by the strong response of FP to environmental effects (i.e. weather) compared to the other parameters, where SP and PDR are strongly related to FP.

Table 4.3.8. Percent variance contribution of mixed model effects for mixograph and protein composition of CWRS no.3 wheat, feed grade and below.

	Genotype	Location	Rep	Year	GxL	GxY	LxY	GxLxY	Residual
FP	4.65 NS	55.00 NS	0.20 NS	0.24 NS	5.38*	1.40 NS	29.89****	0.31 NS	2.92
SP	13.01*	46.81 NS	0.32 NS	0.30 NS	5.84***	1.71**	28.60****	0.12 NS	3.28
SP/FP	43.56*	9.62 NS	3.92 NS	0.00 NS	13.51 NS	4.99 NS	2.63 NS	1.42 NS	20.36
HMWG	20.98**	54.12 NS	0.03 NS	2.72 NS	4.80 NS	0.68 NS	12.18***	0.47 NS	4.03
HMWG/FP	45.82***	24.00 NS	0.45 NS	8.31 NS	5.80 NS	1.53 NS	6.06**	0.67 NS	7.36
GSI	63.23***	17.81 NS	0.15 NS	5.01 NS	5.04 NS	2.06 NS	3.02*	0.67 NS	3.01
MDT	25.80 NS	26.35 NS	0.22 NS	7.85 NS	7.82 NS	3.27 NS	22.38**	1.82*	4.49
PBW	5.02 NS	56.32 NS	0.39 NS	6.89 NS	12.07 NS	1.05 NS	11.90**	1.23 NS	5.12
PDR	7.52 NS	53.33 NS	0.16 NS	4.16 NS	7.24**	1.15*	22.97****	0.20 NS	3.27
WIP	47.59*	19.93 NS	0.65 NS	1.24 NS	10.75 NS	2.50 NS	8.55**	1.53 NS	7.26
WAP	40.78	23.79 NS	0.57 NS	2.14 NS	10.46 NS	5.21 NS	9.01*	2.21*	5.83

FP = Flour protein content, GSI = Gluten Strength Index , HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

*, **, ***, **** signifciant at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively, NS = not significant

The difference between ANOVA for low grade samples and the complete set for genotype, location and LxY effects is illustrated in Fig 4.3.2. The contribution of genotype decreased minimally by approximately 5 to 10% for almost all the protein composition and

mixograph parameters with the exception of FP and SP, which were unaffected. In general, there was a small decrease in the variance contribution of the LxY interaction, however larger decreases were observed for FP (-7.5%), SP (-5.6%). HMWG (-8.7%) and PDR (-23.2%). In contrast, for lower grade samples, the variance contribution of location to total variance increased for all parameters, the increase ranging from 4.2% (SP/FP) to 18.9% (PDR). This result appears to originate from the fact that lower grade samples arise due to adverse growing conditions at locations, which should increase the influence and contribution of location to parameter results.

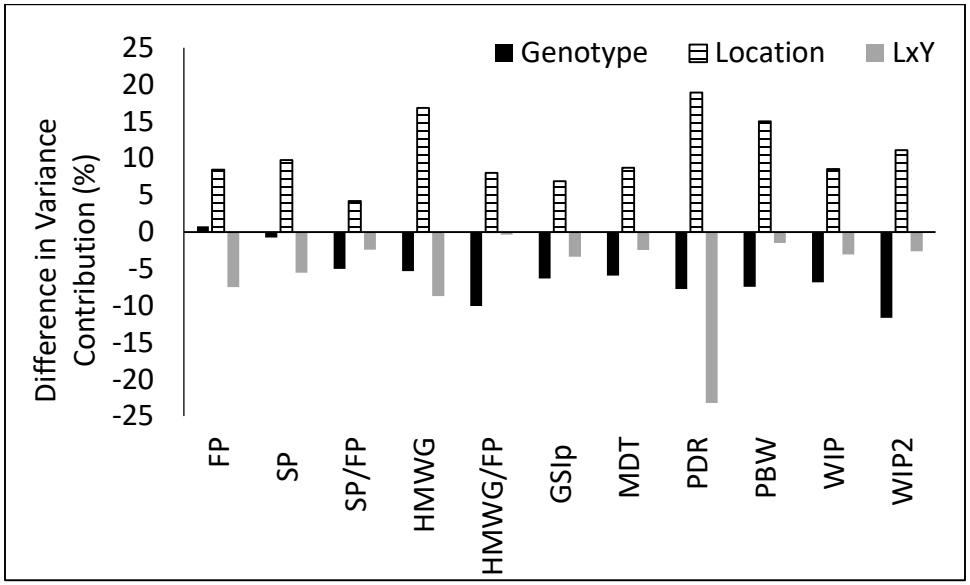

Figure 4.3.2. Differences from ANOVA in contribution to total variance of genotype (black), location (lined) and location x year interaction (grey) for indicated quality parameters for low grading samples (< No. 2 CWRS) compared to all samples.

Unlike the analysis for No.1 and No. 2 CWRS samples, the ANOVA results for lower grade samples grown in 2015 and 2016 when analyzed individually differed from one another as well as from the ANOVA based on combined years. The percent variance contribution of mixed

model effects for 2015 and 2016 growing seasons are presented in Table 4.3.9. In all cases, the differences between years occurred mainly for genotype and location effects.

Table 4.3.9. Percent variance contribution of mixed model effects for 2015 and 2016 protein composition and mixograph parameters for low grade (< No.2 CWRS) samples.

	Year	Genotype	Location	GxL	Rep	Residual
FP	*2015*	11.96 NS	81.27****	4.90*	0.21 NS	1.67
	2016	5.79**	87.10****	4.48 NS	0.20 NS	2.44
SP	*2015*	12.34 NS	79.94****	5.62*	0.16 NS	1.94
	2016	15.41****	76.64****	4.76 NS	0.34 NS	2.85
SP/FP	*2015*	69.90***	13.51*	7.01 NS	3.73 NS	5.85
	2016	46.78****	9.39 NS	16.34 NS	4.34 NS	23.15
HMWG	*2015*	44.64**	46.01***	5.05 NS	0.70 NS	3.60
	2016	15.72****	76.78****	4.39 NS	0.11 NS	3.00
HMWG/FP	*2015*	49.89***	41.28****	3.59 NS	1.24 NS	4.01
	2016	54.70****	27.95****	7.89 NS	0.53 NS	8.93
GSI	*2015*	66.08****	28.16***	3.88*	0.26 NS	1.63
	2016	73.46****	15.79****	6.84 NS	0.13 NS	3.79
MDT	*2015*	18.82 NS	70.26****	6.84 NS	0.35 NS	3.73
	2016	49.09****	30.74****	14.07**	0.62 NS	5.48
PBW	*2015*	10.03 NS	76.16****	6.40 NS	3.11 NS	4.30
	2016	6.45 NS	75.92****	13.39***	0.59 NS	3.65
PDR	*2015*	10.21 NS	80.74****	5.27 NS	0.24 NS	3.55
	2016	7.90**	83.49****	6.15**	0.13 NS	2.34
WIP	*2015*	35.83*	47.78**	8.94 NS	0.45 NS	7.00
	2016	66.29****	13.56**	12.52 NS	1.12 NS	6.51
WAP	*2015*	32.96*	52.96***	8.85 NS	0.14 NS	5.09
	2016	63.12****	14.42**	14.80*	1.54 NS	6.13

FP = Flour protein content, GSI = Gluten Strength Index , HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

*, **, ***, **** signifciant at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively, NS = not significant

The difference between 2015 and 2016 sample sets of lower grade in terms of percent variance contribution to total variance for genotype and location is presented in Fig 4.3.3. In general, location and genotype had relatively similar effects in the two growing seasons on the variation of FP, SP, PBW and PDR; all these parameters have relatively high covariance as reflected by correlations (Table 4.4.2). However, for the 2015 sample set, location had a larger contribution to total variance for all other parameters with the exception of HMWG where in 2016, location contributed 30.8% more to total variance. Otherwise, the contribution of location to total variance was lower and ranged from 4.1% (SP/FP) to 39.5% (MDT).

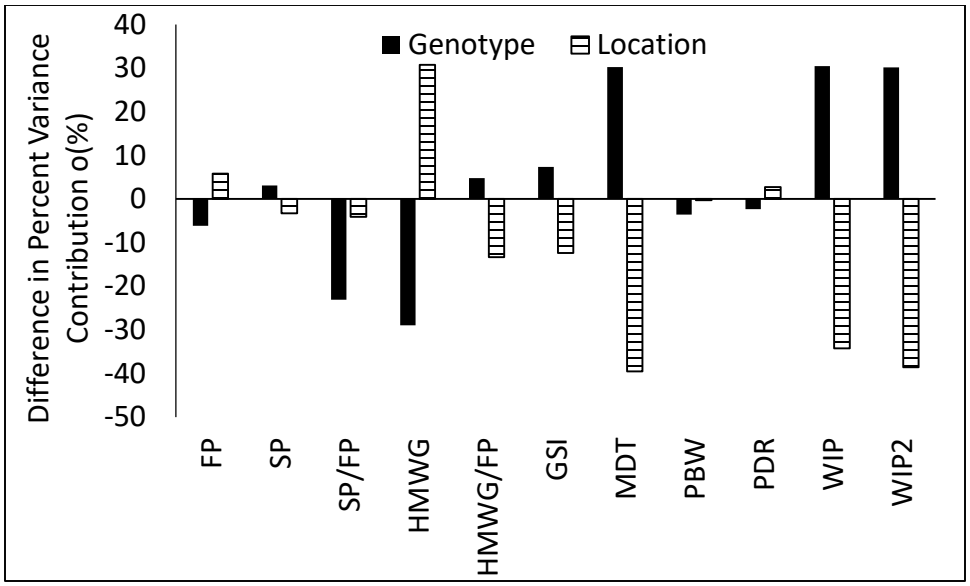

Figure 4.3.3. Differences from ANOVA in contribution to total variance of genotype (black), location (lined) for indicated quality parameters for 2016 low grading samples (< No. 2 CWRS) compared to 2015.

ANOVA results for samples of lower grade also differed from corresponding results for samples of higher grade. The difference between these data sets in terms of the contribution of genotype, location and LxY effects is shown in Fig 4.3.4. The contribution of genotype decreased, with a concomitant increase in the contribution of location for low grade samples for the majority of quality parameters with the exception of FP, SP and SP/FP. This result is similar to that shown in Fig 4.3.2, and can be similarly explained by the adverse weather conditions more likely experienced by lower grading samples. The contribution of genotype decreased in 2015 compared to 2016 from 10.6% (WIP) to 24.6% (HMWG/FP). Similarly, the contribution of LxY interaction decreased, although the magnitude of the decrease was lower. The largest decreases in variance contribution of LxY were observed for HMWG (-8.7%), PBW (-9.6%) and PDR (-8.7%). Conversely, the contribution of location effect to total variance of low grade

samples increased, and ranged from 5.3% (SP/FP) to 30.9% (PBW). The remaining effects and interactions on other parameters did not vary by more than 5%.

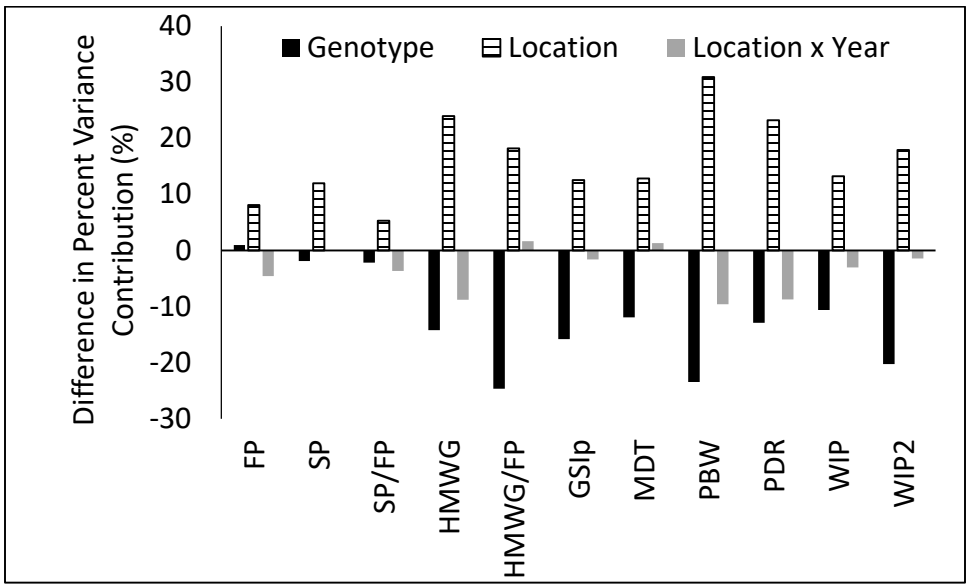

Figure 4.3.4. Differences from ANOVA in contribution to total variance of genotype (black), location (lined) and location x year interaction (grey) for indicated quality parameters for low grading samples (< No. 2 CWRS) compared to all samples.

The last aspect of the ANOVA involving high and low grade samples was to evaluate the effect of removing Glenn from the lower grade sample set, i.e. for samples < No. 2 CWRS. In contrast to the results for the top milling grade samples (refer to Table 4.3.6) where the deletion of Glenn resulted in decreased genotype influence on total variance for almost all parameters, the results did not vary substantially when Glenn samples were deleted. However, a decrease in the contribution of genotype to total variance still occurred for HMWG, HMWG/FP, GSI, MDT, WIP and WAP, and ranged from 11.2% (HMWG) to 26.7% (WAP) (Table 4.3.10). Similar to previous results, the decrease in contribution of genotype to total variance upon the removal of Glenn was compensated by an increase (3.0 -11.0%) in the variance contributed by location, as well as small increases (<5%) contributed by the remaining effects.

Table 4.3.10. Percent variance contribution of mixed model effects for protein composition and mixograph parameters for low grade samples (< No. 2 CWRS) without genotype Glenn.

	Genotype	Location	Rep	Year	GxL	GxY	LxY	GxLxY	Residual
FP	4.65 NS	55.18 NS	0.29 NS	0.36 NS	5.27*	1.37 NS	30.03****	0.32 NS	2.52
SP	11.91*	47.50 NS	0.38 NS	0.50 NS	5.83***	1.61**	29.09****	0.11 NS	3.06
SP/FP	37.26*	10.83 NS	4.13 NS	0.06 NS	15.22 NS	4.84 NS	3.05 NS	1.69 NS	22.91
HMWG	9.78*	62.56 NS	0.10 NS	2.87 NS	5.65 NS	0.77 NS	13.98***	0.61 NS	3.67
HMWG/FP	25.02**	33.42 NS	0.26 NS	13.08 NS	7.28 NS	1.36 NS	8.65**	0.96 NS	9.97
GSIp	44.83**	26.91 NS	0.15 NS	9.50*	6.74 NS	1.67 NS	4.74*	1.09 NS	4.37
MDT	14.31 NS	29.33 NS	0.38 NS	10.23 NS	8.70 NS	3.76 NS	27.15*	2.20*	3.95
PBW	4.97 NS	59.39 NS	0.38 NS	5.92 NS	10.71 NS	0.18 NS	12.20**	1.30 NS	4.95
PDR	7.44 NS	52.71 NS	0.21 NS	4.64 NS	7.08**	1.13*	23.42****	0.21 NS	3.17
WIP	24.26 NS	30.88 NS	1.48 NS	2.64 NS	13.35 NS	3.26 NS	13.86**	2.39 NS	7.86
WAP	14.12 NS	33.63 NS	1.41*	4.59 NS	11.20 NS	13.15 NS	13.12*	3.31**	5.47

FP = Flour protein content, GSI = Gluten Strength Index , HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WIP = Work input to peak

*, **, ***, **** signifciant at $p<0.05$, $p<0.01$, $p<0.001$ and $p<0.0001$ respectively, NS = not significant

4.4 Relationships Among Wheat Quality Parameters

4.4.1 Relationships Between Grain and Milling Quality Parameters with Protein Composition and Mixograph Quality Parameters

Correlations between grain and milling quality with protein composition parameters are presented in Table 4.4.1. Most of the correlations are weak, however for a few, there were some interesting trends. Only those factors with correlations higher than r = 0.25 are considered in this discussion. That said, milling parameters (flour yield, and yield of bran and shorts) were unrelated to protein composition outcomes. However, ash content which measures flour refinement mainly, was weakly and negatively related to HMWG/FP (r = -0.33) and GSI (r= -0.30) and bran content was weakly and positively related to MDT (r = 0.31).

Several degrading factors were associated with changes in protein composition including grade, test weight, FDK and DON (DON is not a degrading factor, but is correlated with FDK; Fig 4.1.1) and ergot to a lesser extent. Test weight (TW) was positively correlated with HMWG, HMWG/FP and GSI. The latter all have commonality with glutenin content, so it appears that the bulk density of wheat is related to the content of glutenin in wheat or flour; i.e. the higher the test weight, the higher the glutenin content or HMW glutenin per unit protein. This outcome may be an indirect one and could have arisen from a relationship between grade (and e.g. FDK as the predominant degrading factor) and test weight which is a separate degrading factor for CWRS wheat. Minimum test weights for CWRS No.1, No.2, No.3 and Feed wheat are 75, 72, 69 and 65 kg/hL, respectively. The other degrading factors were negatively correlated with HMWG, HMWG/FP and GSI, i.e. all protein composition parameters related to glutenin. Midge damage was negatively related (r = -0.31) with HMWG only. Interestingly and in contrast, SP content

was apparently not affected by grade or grading factor as correlations were very low (Table 4.4.1).

Table 4.4.1. Simple correlations between grain quality and milling parameters with protein composition and mixograph parameters (n = 161)[a]

	FP	SP	SP/FP	HMWG	HMWG/FP	GSI	MDT	PBW	PDR	WIP	WAP
Grade [b]	-0.14	-0.08	0.21	-0.45	-0.48	-0.43	-0.26	-0.01	-0.02	-0.30	-0.33
FDK	-0.06	0.00	0.19	-0.36	-0.45	-0.40	-0.21	-0.05	-0.07	-0.28	-0.30
DON (n = 134)	-0.08	0.00	0.25	-0.40	-0.49	-0.44	-0.24	-0.08	-0.08	-0.32	-0.35
Midge (n = 159)	-0.25	-0.22	0.09	-0.31	-0.14	-0.14	-0.07	-0.16	-0.16	-0.11	-0.18
Sprout (n = 159)	-0.09	-0.07	0.08	-0.12	-0.07	-0.08	-0.11	0.25	0.21	0.05	0.02
Ergot (n = 75)	0.07	0.04	-0.11	-0.16	-0.33	-0.22	-0.16	-0.11	-0.08	-0.20	-0.22
GP	0.99	0.92	-0.16	0.74	-0.10	-0.03	-0.45	0.68	0.76	-0.13	-0.05
TW	0.08	0.00	-0.24	0.36	0.44	0.42	0.08	0.28	0.24	0.30	0.32
TKW	0.26	0.27	0.04	0.30	0.12	0.07	-0.26	0.45	0.46	-0.04	0.01
FY	0.12	0.20	0.24	-0.04	-0.19	-0.24	-0.22	0.06	0.14	-0.27	-0.23
BC	-0.09	-0.14	-0.15	0.03	0.15	0.17	0.31	-0.16	-0.22	0.27	0.24
SC	-0.03	-0.09	-0.17	0.07	0.12	0.15	-0.09	0.18	0.13	0.06	0.04
AC	-0.03	0.02	0.13	-0.25	-0.33	-0.30	-0.03	-0.27	-0.24	-0.21	-0.25

[a]Sample size of 161 unless otherwise noted. [b] Although data is nominal, grade was included in the correlation matrix for illustrative purposes. DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, TKW = Thousand Kernel Weight, WAP = Work at Peak,WIP = Work Input to Peak. When n = 161, coefficients with | r | > 0.161, 0.206, 0.0.258, 0.300 are significant at $p < 0.05$, 0.01, 0.001 and 0.0001, respectively. When n = 159, coefficients with | r | > 0.160, 0.208 and 0.308 are significant at $p < 0.05$, 0.01 and 0.0001, respectively. When n = 134, coefficients with | r | > 0.237, 0.318 and 0.348 are significant at $p < 0.001$ and 0.0001, respectively. When n = 148, coefficients with | r | > 0.329 are significant at $p < 0.01$

The strongest relationships for HMWG/FP were with Grade, FDK and DON with correlations of r = -0.48, -0.45 and -0.49, respectively. The correlations of these three factors with GSI were slightly lower (r = -0.43, -0.40 and -0.44, respectively). Fig 4.4.1 and 4.4.2 chart the relationships between HMWG/FP and FDK and DON, respectively. For FDK, the R^2 value was higher in the 2016 crop year ($R^2 = 0.28$, $p < 0.0001$) when FDK was more prevalent across the Prairie region and growing sites of this study. In 2015, there were no samples with greater than 2% FDK (Fig 4.4.1) but in 2016 FDK levels extended up to about 8%. These results may indicate that HMWG/FP is only affected by higher levels of *Fusarium* damage. For DON, the R^2 for HMWG/FP in 2015 and 2016 were 0.18 and 0.26, respectively ($p < 0.0001$). For the remaining degrading factors, midge damage was negatively related to HMWG content (r = -0.3, Table 4.4.1) and ergot damage was negatively related to HMWG/FP (r = -0.33). Sprout damage was not a significant factor affecting protein composition in this study based on the very low correlations that were found (Table 4.4.1).

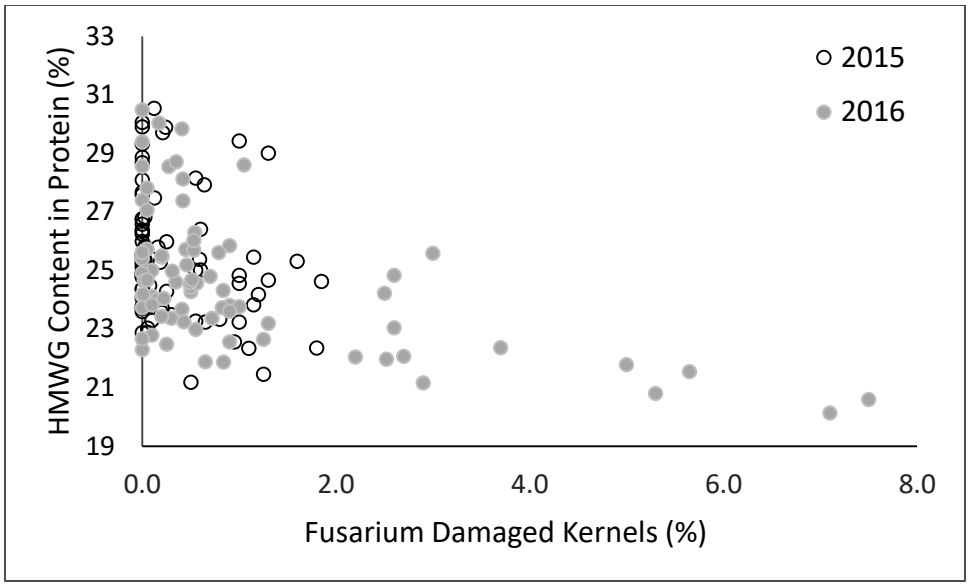

Figure 4.4.1. Relationship between fusarium damaged kernels (FDK) and HMWG content (HMWG/FP) for 2015 (empty) and 2016 (grey) CWRS wheat genotypes. Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.0603 and 0.2805, respectively.

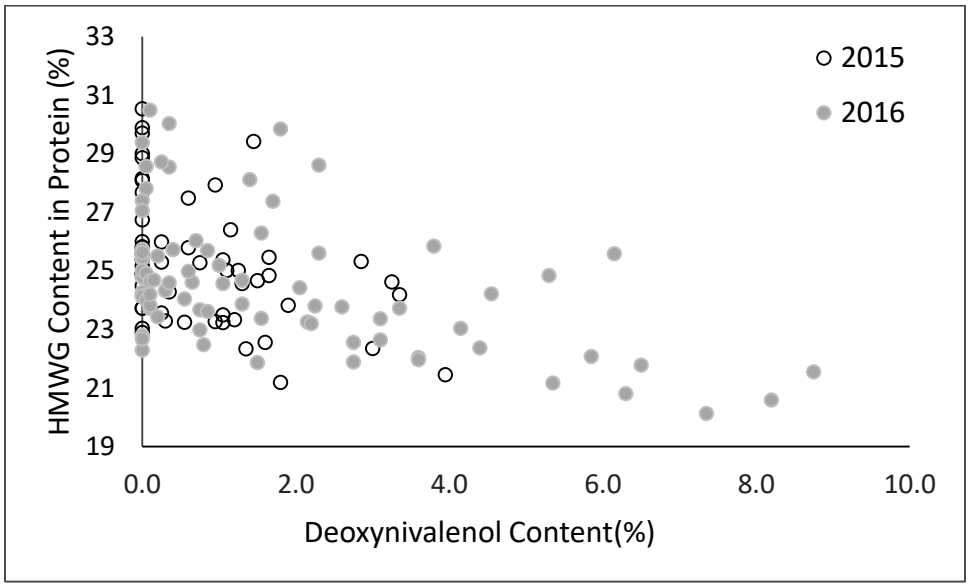

Figure 4.4.2. Relationship between dexoynivalenol and HMWG content (HMWG/FP) for 2015 (empty) and 2016 (grey) CWRS wheat genotypes. Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.1758 and 0.2525 respectively.

With a few exceptions, there were generally weak to non-existent relationships between dough mixing properties and grade and degrading factors and milling quality parameters, with the exception of grain protein content which was negatively related to MDT (r = -0.45), and was more strongly and positively related to PDR and PBW (r = 0.76 and r = 0.68, respectively, Table 4.1.1). Test weight (TW) was positively correlated with PBW, WIP and WAP (r = 0.28, 0.30 and 0.32, respectively). The higher correlations between TW and WIP and WAP are in line with the correlations observed between TW and e.g. HMWG/FP and GSI (r = 0.44 and r = 0.42, respectively) as previously noted. As observed, this outcome may be an indirect one and could have arisen from a relationship between grade (and e.g. FDK as the predominant degrading factor) and test weight which is a separate degrading factor for CWRS wheat.

Flour yield was negatively and weakly related to WIP (r = -0.27), whereas bran content was positively but weakly correlated with MDT, WIP and WAP (r = 0.31, 0.27, -.24, respectively, Table 4.1.1).

4.4.2 Correlations Among and Between Protein Composition and Mixograph Quality Parameters

Both soluble prolamins (SP) and high molecular weight glutenin (HMWG) accumulate as endosperm storage protein during grain filling, to comprise the majority of protein in wheat and flour. It follows that both soluble prolamins and HMWG should be highly correlated with flour protein content (FP). Fig 4.4.3 shows those relationships for genotype samples in the present study in the complete data set including 2015 and 2016 grown samples. The range of protein content (9.9% to 16.5%) due almost entirely to location-related effects was remarkable (Fig 4.4.4). Interestingly, the correlation between SP and FP (r = 0.95) were much higher than that for HMWG (0.74; Table 4.4.2). This suggests that gliadin protein which predominates in the SP

fraction (Fu and Sapirstein, 1996) is a more significant and/or efficient sink for storage protein compared to glutenin, the majority of which is HMW and therefore insoluble by the method used to quantify it in this study. That SP is a more efficient storage protein fraction than HMWG is shown in Fig 4.4.3, it can be seen that the slope of SP is clearly different and greater than that for HMWG. A similar result has been reported (Blumenthal et al. 1993; Ciaffi et al. 1996a; Huebner et al. 1990; Labuschagne et al. 2009; Saint Pierre et al. 2008; Stone et al. 1997; Tea et al. 2004; Triboi et al. 2000). Interestingly, the relationship between FP and HMWG appears to cease at around 13.5%, after which there is no increase in HMWG as sample FP content increases. However, HMWG and FP had a positive correlation ($R^2 = 0.67$, results not shown) for samples below 13.5%. This suggests that at higher levels of FP, glutenin no longer functions as storage protein. This appears to be a result not previously reported in the literature. SP and HMWG were not highly correlated (r = 0.57)

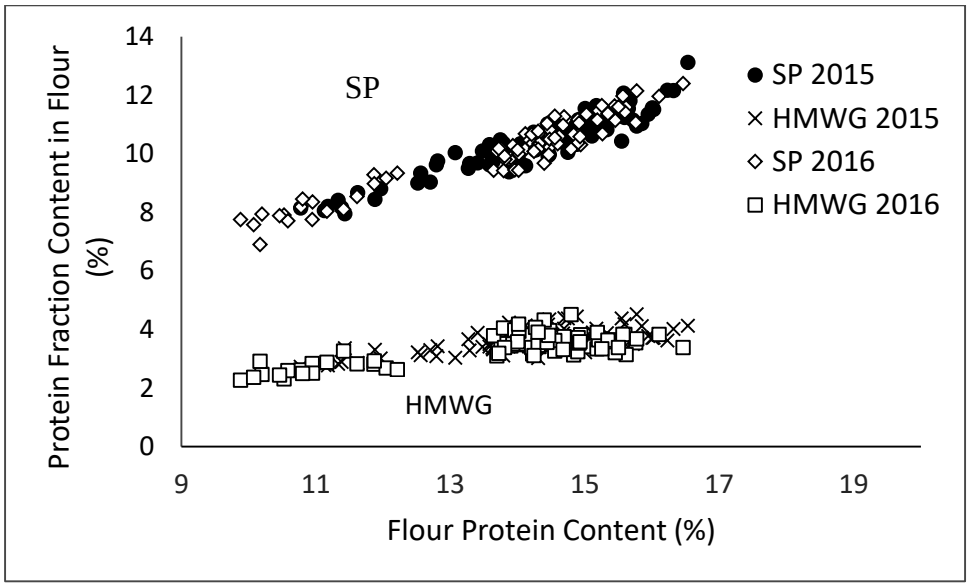

Figure 4.4.3. Relationships between soluble prolamin and HMW glutenin with flour protein content. Each symbol represents a genotype mean of two field replications. R^2 values for 2015 SP and HMWG were 0.8831 (black circle) and 0.4719 (cross) as well as 0.9092 (empty diamond) and 0.5817 (empty square) for 2016, respectively.

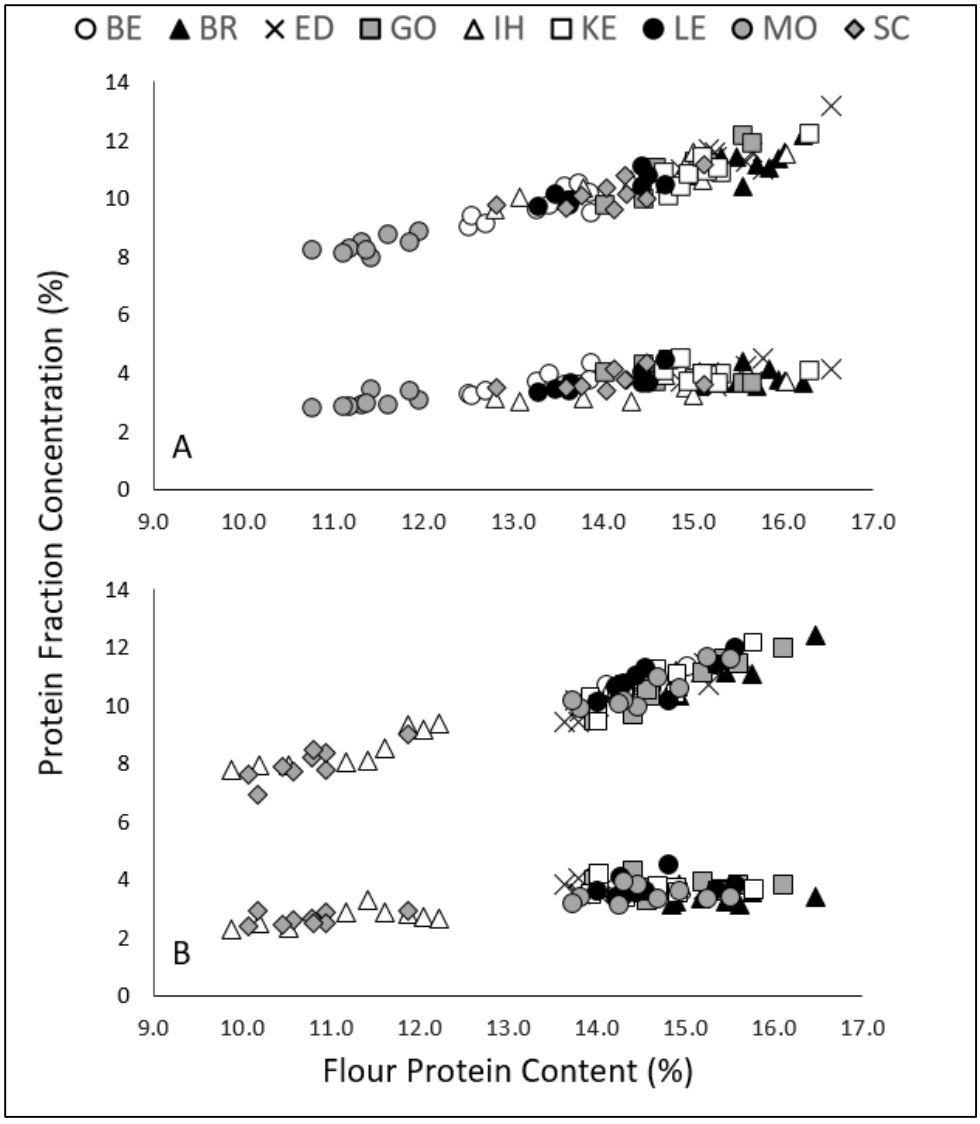

Figure 4.4.4. Relationships between soluble prolamin (topline as per Fig 4.4.3) and HMW glutenin (bottomline as per Fid 4.4.3) with flour protein content and locations highlighted. Each symbol represents a genotype mean of two field replications for 2015 (A) and 2016 (B).

Table 4.4.2. Simple correlations between and among protein composition and mixograph quality parameters (n = 161)

	FP	SP	SP/FP	HMWG	HMWG/FP	GSI	MDT	PBW	PDR	WIP	WAP
FP	1.00										
SP	0.95	1.00									
SP/FP	-0.12	0.20	1.00								
HMWG	0.74	0.57	-0.49	1.00							
HMWG/FP	-0.12	-0.31	-0.60	0.58	1.00						
GSI	-0.06	-0.30	-0.78	0.60	0.97	1.00					
MDT	-0.46	-0.60	-0.48	0.10	0.72	0.71	1.00				
PBW	0.69	0.69	0.00	0.59	0.03	0.02	-0.48	1.00			
PDR	0.79	0.79	0.03	0.61	-0.06	-0.06	-0.53	0.94	1.00		
WIP	-0.15	-0.33	-0.58	0.44	0.83	0.83	0.87	-0.06	-0.12	1.00	
WAP	-0.06	-0.23	-0.57	0.54	0.87	0.86	0.81	0.11	0.00	0.96	1.00

FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, WAP = Work at Peak, WIP = Work Input to Peak. Coefficients with $| r | > 0.204$ and 0.304 are significant at $p < 0.01$and 0.0001, respectively.

Fig 4.4.5 shows as result similar to Fig 4.4.3 to highlight differences between the strongest and weakest genotypes, Glenn and Harvest respectively. It is important to note that both genotypes were had a wide range in protein content and fractions. Of greater important related to gluten strength were the quantitatively small differences (<2%) in the concentrations of SP and HMWG fractions, where Glenn had consistently lower and higher protein compared to Harvest in the SP and HWMG fractions, respectively. Clearly, small differences in content of polymeric glutenin with similar subunit composition (Table 2.4) can result in substantial

differences in dough strength and mixing requirements as was observed in this study and by Dupuis et al (1996).

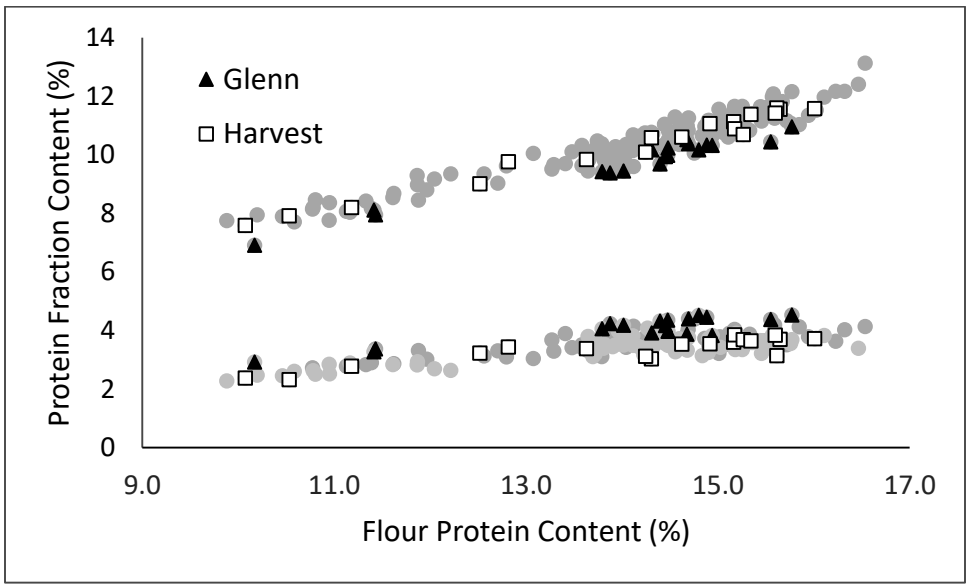

Figure 4.4.5. Relationships between soluble prolamins (top line) and HMWG (bottom line) content to flour protein content with varieties Glenn (triangles) and Harvest (squares) highlighted. Each symbol represents a genotype mean of two field replications.

Interestingly, SP per unit protein, i.e. SP/FP and HMWG/FP were negatively and positively correlated, respectively with a number of protein compositional parameters, most notably GSI and, in turn, three mixograph measures of gluten strength (MDT, WIP and WAP, refer to Table 4.4.2). Accordingly, SP/FP and HMWG/FP are genotype properties as the effect of growing location which very strongly influences FP has been largely eliminated. Fig 4.5.6 and Fig 4.5.7 illustrate the inverse and direct relationships between SP/FP and HMWG/FP and GSI; both significant ($p<0.0001$). The distinct protein composition of cultivar Glenn relative to other genotypes is highlighted as having distinctly low and high content of SP and HMWG expressed on a per unit protein basis. It can also be seen that the relationship between GSI and SP/FP (R^2 = 0.61) has more scatter than that for HMWG/FP (R^2 = 0.98). A likely reason for this difference is

that the HMWG fraction represents pure glutenin protein (Fu and Sapirstein, 1996), whereas the SP fraction contains all monomeric protein including propanol-soluble albumin and globulin protein. These are mainly metabolic proteins and would not be expected to behave as storage protein, but likely would be negatively associated with gluten strength, as only glutenin protein of a certain molecular size threshold would contribute positively to strength (Southan and MacRitchie 1999).

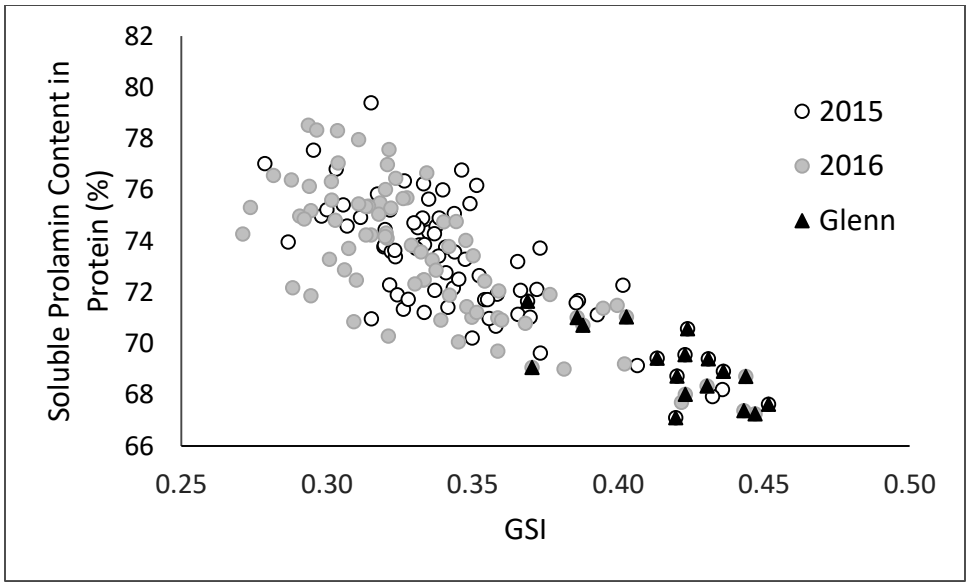

Figure 4.4.6. Relationship between soluble prolamin content in protein and GSI for 2015 (empty circles) and 2016 (grey circles) genotype means with variety Glenn highlighted (triangles). Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.6135 and 0.6142 respectively.

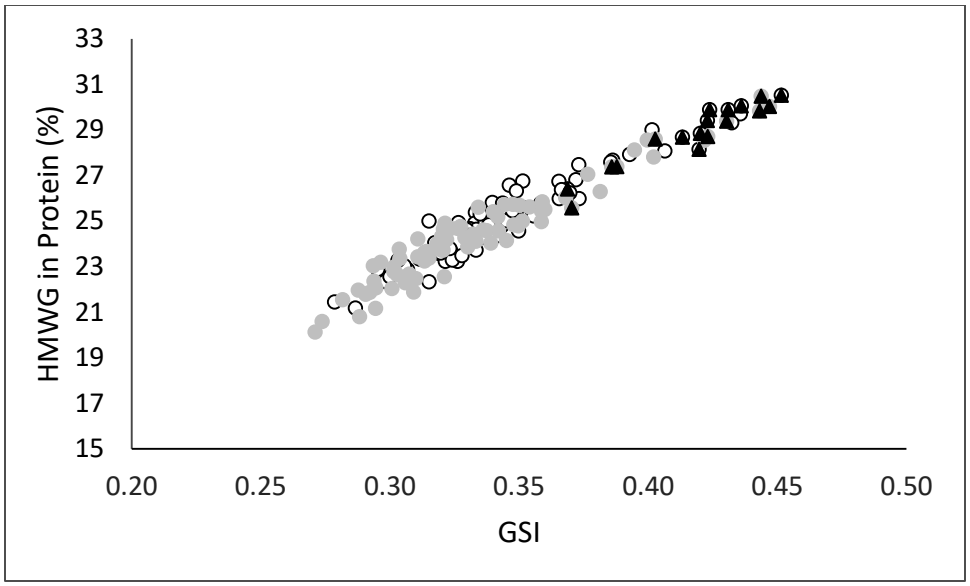

Figure 4.3.7. Relationship between HMWG content in protein and GSI for 2015 (empty circles) and 2016 (grey circles) wheat genotype means with variety Glenn highlighted (triangles). Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.9345 and 0.9361 respectively.

Work input and development time are the two technological parameters most often used to describe gluten strength in the literature when mixograph results are assessed. In this study, a new parameter, WAP (work at peak), was additionally included which as previously mentioned is a peak height independent measure of dough strength at peak time. These three mixograph properties, especially WIP and WAP, were closely related (r = 0.96) and were clearly different from PBW and PDR which were uncorrelated with WIP (r = -0.06 and r = -0.12) and WAP (r = 0.11 and r = 0.00) and negatively correlated with MDT (r = -0.48 and r = -0.53, respectively). Fig 4.4.8 illustrates the close relationship between WIP and WAP by growing year, and also the distinctive gluten strength of the variety Glenn. The relevance of these mixograph parameters (WIP, WAP, and MDT) to gluten strength was most apparent when correlations were determined with protein content (FP) and composition (Table 4.4.2). The soluble prolamin fraction, which is comprised mainly of gliadins, had relatively high correlation with mixograph peak dough

resistance (r = 0.79, Fig 4.4.9) and bandwidth (r = 0.69,Fig 4.4.10) but was negatively and more weakly correlated with WAP (r = -0.23), WIP (r = -0.33) and MDT (r = -0.59, Fig 4.4.11). This pattern of variation was similar to that for FP in which SP is the predominant fraction (Fig 4.4.12), except that there was no correlation between FP and WAP and WIP (r = -0.06 and -0.15, respectively). A similar result was reported by Bergman et al. (1998) in which flour protein content was positively correlated with mixograph peak height (r = 0.55, $p < 0.001$) however was not significantly correlated to mixograph peak time. The contrast in correlations was reversed for the GSI and HMWG/FP which were as highly correlated with WIP (r = 0.83 and r = 0.83, respectively) and WAP (r= 0.86 and r = 0.87, respectively) and MDT (r = 0.71 and r = 0.72, respectively) to a lesser extent, and uncorrelated with PBW (r = 0.02 and r = 0.03, respectively) and peak dough resistance (r = -0.06 and r = -0.06, respectively, Table 4.4.2). The strong relationships between WIP and GSI (Fig 4.4.13), WAP and GSI (Fig 4.4.14), WIP and HMWG/FP (Fig 4.4.15) and WAP and HMWG/FP (Fig 4.4.16) are graphically shown to further underscore the relevance of these protein fractions to gluten strength as well as the effectiveness of the straightforward and efficient fractionation procedure used in the study to screen wheat genotypes or flour samples for gluten strength. This distinction between mixograph PDR and PBW, and WIP, WAP and MDT further illustrates the different influences of protein quality (e.g. GSI and HMWG/FP parameters) and protein content on mixograph parameters. The near zero correlations between FP and GSI and HMWG/FP (Table 4.4.2) further supports this distinction. In contrast, there was relatively high covariance between FP and SP (r = 0.95) and HMWG (r = 0.74), an unsurprising result especially for SP which is comprised mainly of gliadin protein which is the principal storage protein of wheat endosperm as noted previously in this study (Fig 4.4.3) and other reports (Blumenthal et al. 1993; Ciaffi et al. 1996a; Huebner et al. 1990;

Labuschagne et al. 2009; Saint Pierre et al. 2008; Stone et al. 1997; Tea et al. 2004; Triboi et al. 2000). In the aforementioned figures, varieties Glenn and Carberry are highlighted as they represent the maximum and minimum thresholds for CWRS wheat variety registration trials

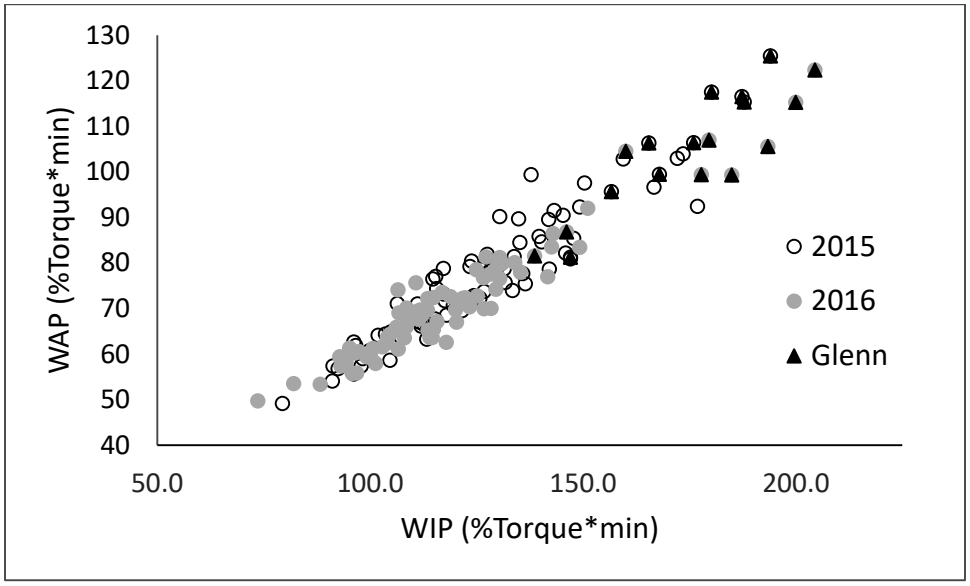

Figure 4.4.8. Relationship between WIP and WAP for 2015 (empty circles) and 2016 (grey circles) genotype means with variety Glenn highlighted (triangles). Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.9083 and 0.9316, respectively.

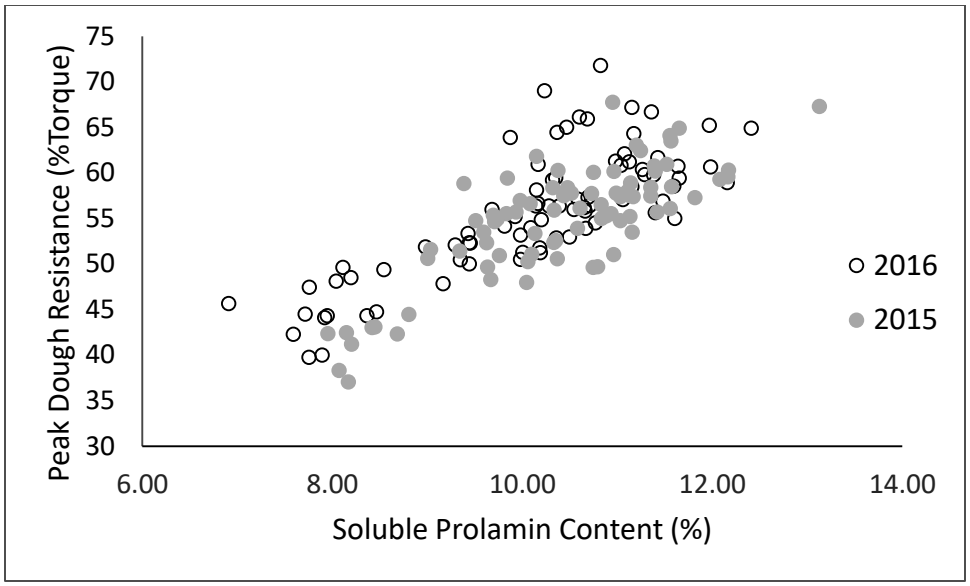

Figure 4.4.9. Relationship between soluble prolamin content and peak dough resistance for 2015 (grey circles) and 2016 (empty circles) genotype means). Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.6506 and 0.6379, respectively.

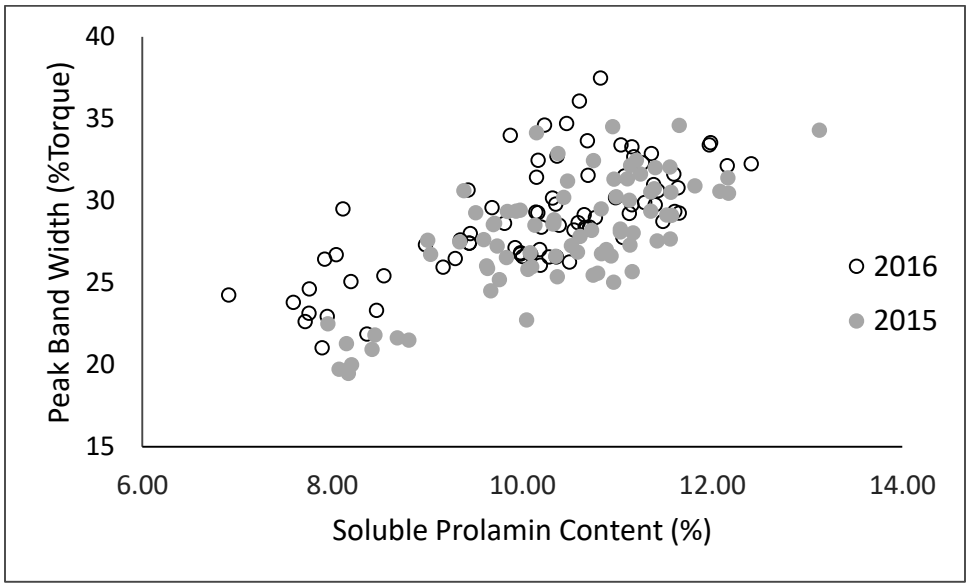

Figure 4.4.10. Relationship between soluble prolamin content and peak band width for 2015 (grey circles) and 2016 (empty circles) genotype means). Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.5311 and 0.5048, respectively.

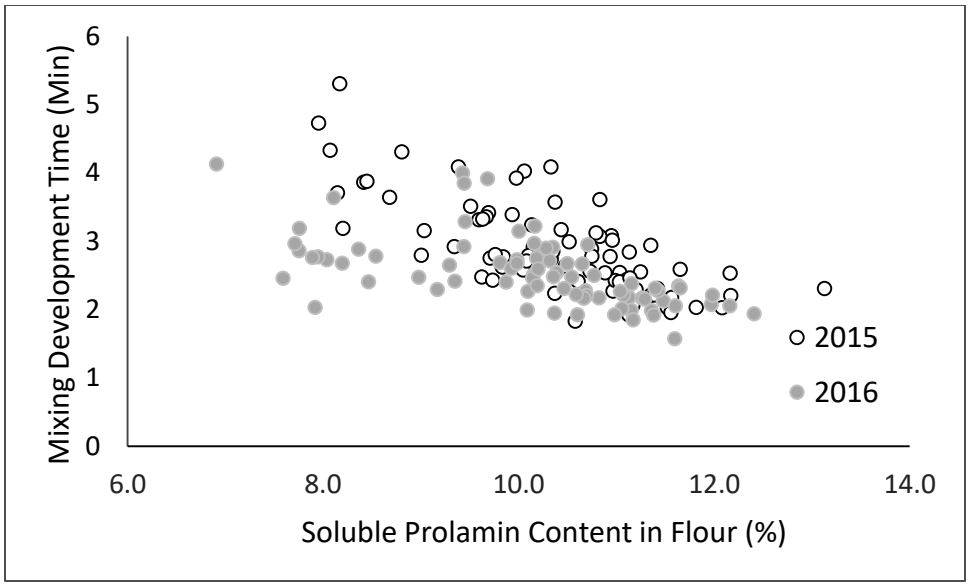

Figure 4.4.11. Relationship between mixing development time (MDT) and soluble prolamin content (SP) for 2015 (empty) and 2016 (grey) genotype means. Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.5604 and 0.3468, respectively.

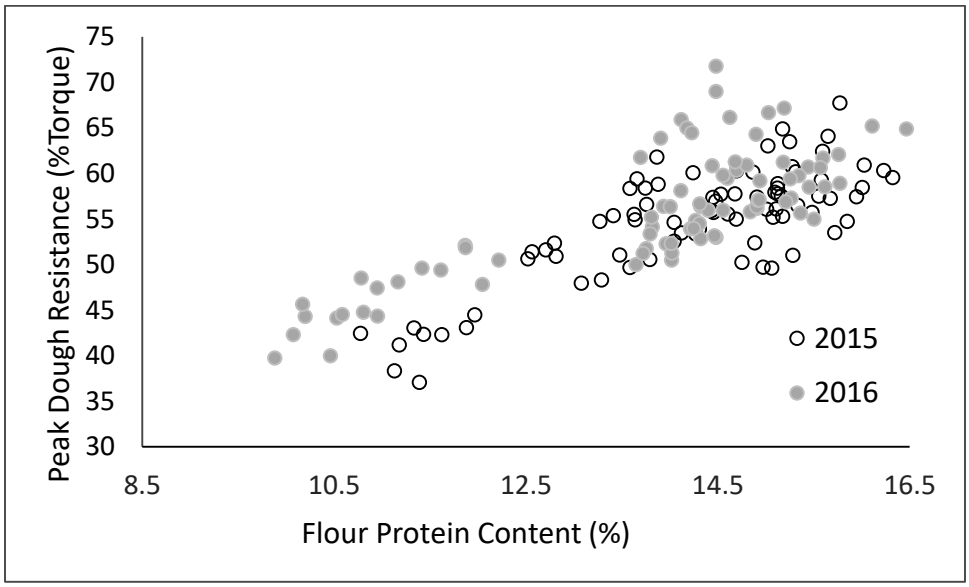

Figure 4.4.12. Relationship between peak dough resistance (PDR) and flour protein content (FP) for 2015 (empty) and 2016 (grey) genotype means. Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.6454 and 0.6439, respectively.

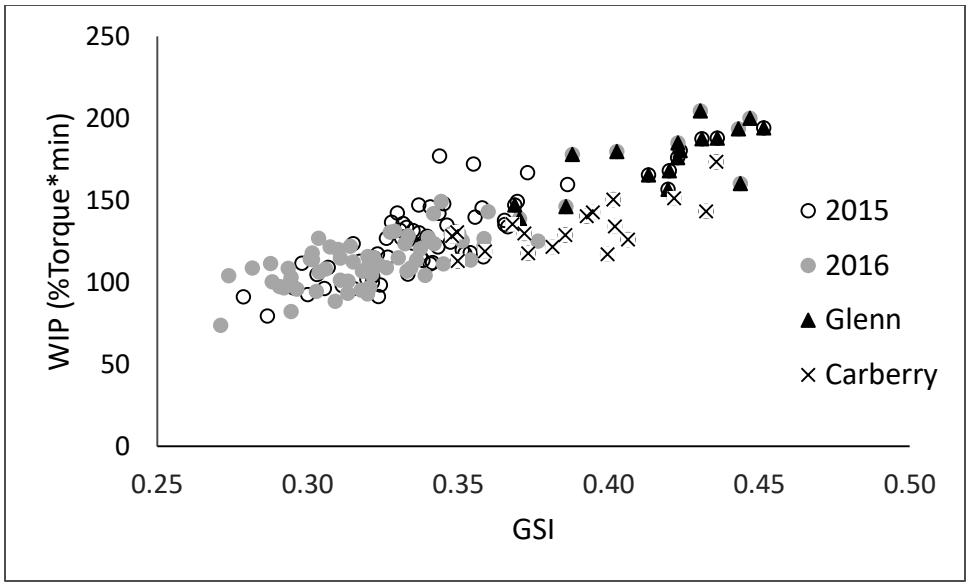

Figure 4.4.13. Relationship between WIP and GSI for 2015 (empty circles) and 2016 (grey circles) genotype means with varieties Glenn (triangles) and Carberry (crosses) highlighted. Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.6599 and 0.7103, respectively.

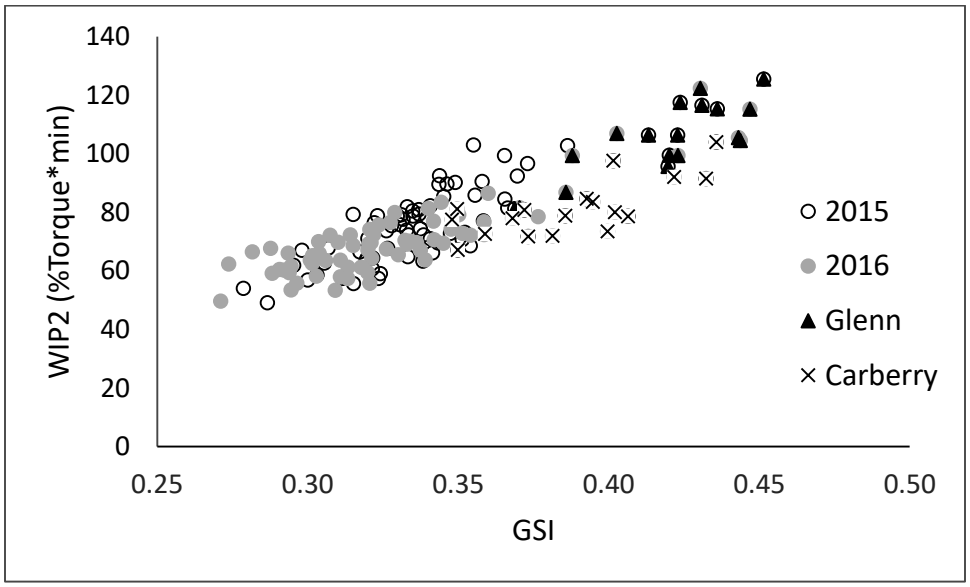

Figure 4.4.14. Relationship between WAP and GSI for 2015 (empty circles) and 2016 (grey circles) genotype means with varieties Glenn (triangles) and Carberry (crosses) highlighted. Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.7123 and 0.7528, respectively.

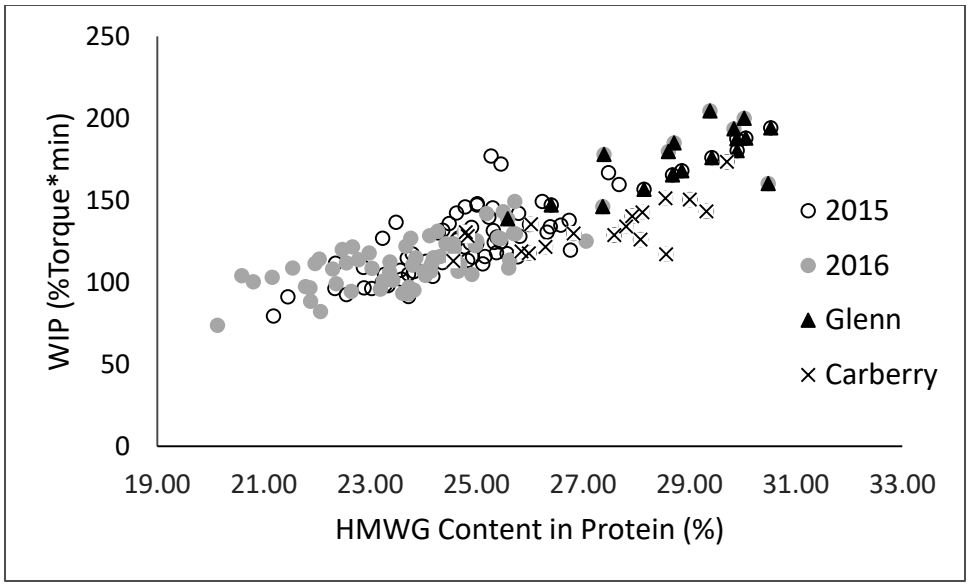

Figure 4.4.15. Relationship between WIP and HMWG/FP for 2015 (empty circles) and 2016 (grey circles) genotype means with varieties Glenn (triangles) and Carberry (crosses) highlighted. Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.6854 and 0.6797, respectively.

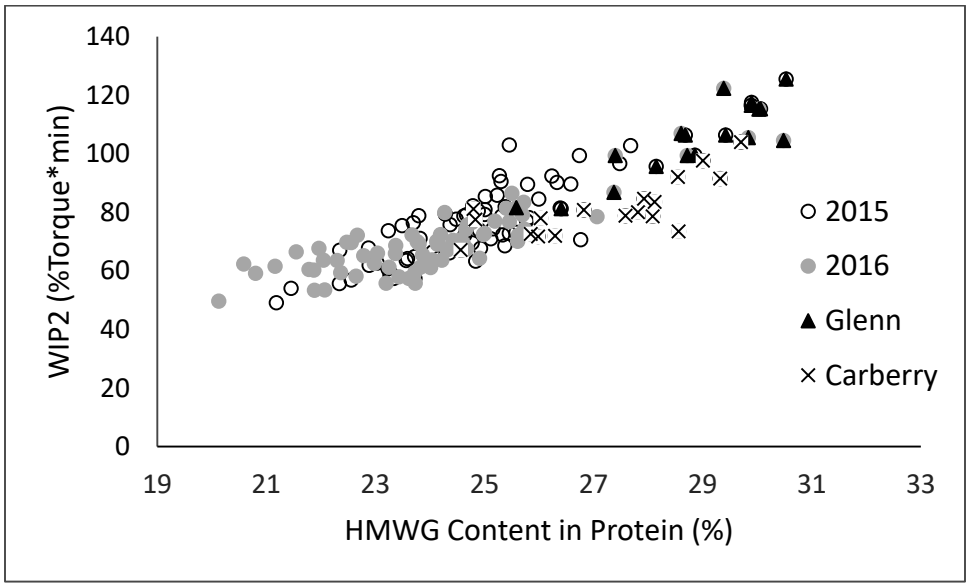

Figure 4.4.16. Relationship between WAP and HMWG/FP for 2015 (empty circles) and 2016 (grey circles) genotype means with varieties Glenn (triangles) and Carberry (crosses) highlighted. Each symbol represents a mean of two field replications. R^2 values from 2015 and 2016 were 0.7593 and 0.7381, respectively.

4.4.3 Stepwise Regression Analysis to Enhance Prediction of Mixograph Parameters Based on Protein Content and Composition

As noted above, simple correlation analysis yielded relatively high and compelling correlations between mixograph parameters of gluten strength (MDT, WIP, WAP, refer to Table 4.4.2) and two related parameters of protein composition related mainly to the molecular size of glutenin (HMWG/FP and GSI) whose variation is due mainly to genetic factors (Table 4.1.21). It was of interest to determine the relationships between these mixograph parameters and protein composition based on multivariate linear regression in order to evaluate the extent to which these mixograph parameters could be potentially predicted using readily acquired protein data. To this end, stepwise regression analysis was applied, and results are shown In Table 4.4.3. The list of independent protein related variables that were analyzed was as follows:

FP, SP, SP/FP, HMWG, HMWG/FP, GSI

Table 4.4.3. Stepwise regression analysis of mixograph MDT, WIP and WAP with protein related independent variables

MDT				**WIP**			
Number of Variables	Variables	R^2	Probability[a]	Number of Variables	Variables	R^2	Probability
1	HMWG/FP	0.48	p<0.0001	1	HMWG/FP	0.62	p<0.0001
2	SP HMWG/FP	0.65	p<0.0001	2	HMWG/FP GSI	0.65	p<0.0001
3	SP HMWG/FP GSI	0.66	p<0.0001	3	SP/FP HMWG/FP GSI	0.67	p<0.0001
4	SP SP/FP HMWG HMWG/FP	0.67	p<0.0001	4	SP/GP HMWG HMWG/FP GSI	0.68	p<0.0001
WAP							
Number of Variables	Variables	R^2	Probability				
1	HMWG/FP	0.67	p<0.0001				
2	HMWG/FP GSI	0.71	p<0.0001				
3	SP/FP HMWG/FP GSI	0.72	p<0.0001				
4	SP SP/FP HMWG/FP GSI	0.72	p<0.0001				

[a]Probability represents the level of significance for the regression model. FP = Flour Protein Content, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, SP = Soluble Prolamin, WAP = Work at Peak, WIP = Work Input to Peak

The results from the multivariate regression analysis (Table 4.4.3) confirm that HMWG/FP is the most efficient parameter to predict variation of WIP and WAP (R^2 = 0.62 and R^2 = 0.67, respectively). In both cases, the single variable regression models' R^2 values were

only slightly increased (0.03 and 0.04, respectively) by the addition of a second variable (GSI) into the model. Any remaining variables that were included in the model did not significantly increase the R^2 of the models. HMWG/FP in conjunction with SP was able to account for 65% of the variation in MDT in a two variable regression model, after which the addition of more variables did not significantly increase the R^2 value ($R^2 = 0.67$, four variable model). The predicted vs. observed values from the one to four variable regression models of MDT, WIP and WIP are presented in Figs 4.4.17, 4.4.18 and 4.4.19, respectively. Visibly, the charts do not look much different as the number of variables included in the model increase, except perhaps for slightly less scatter among data points.

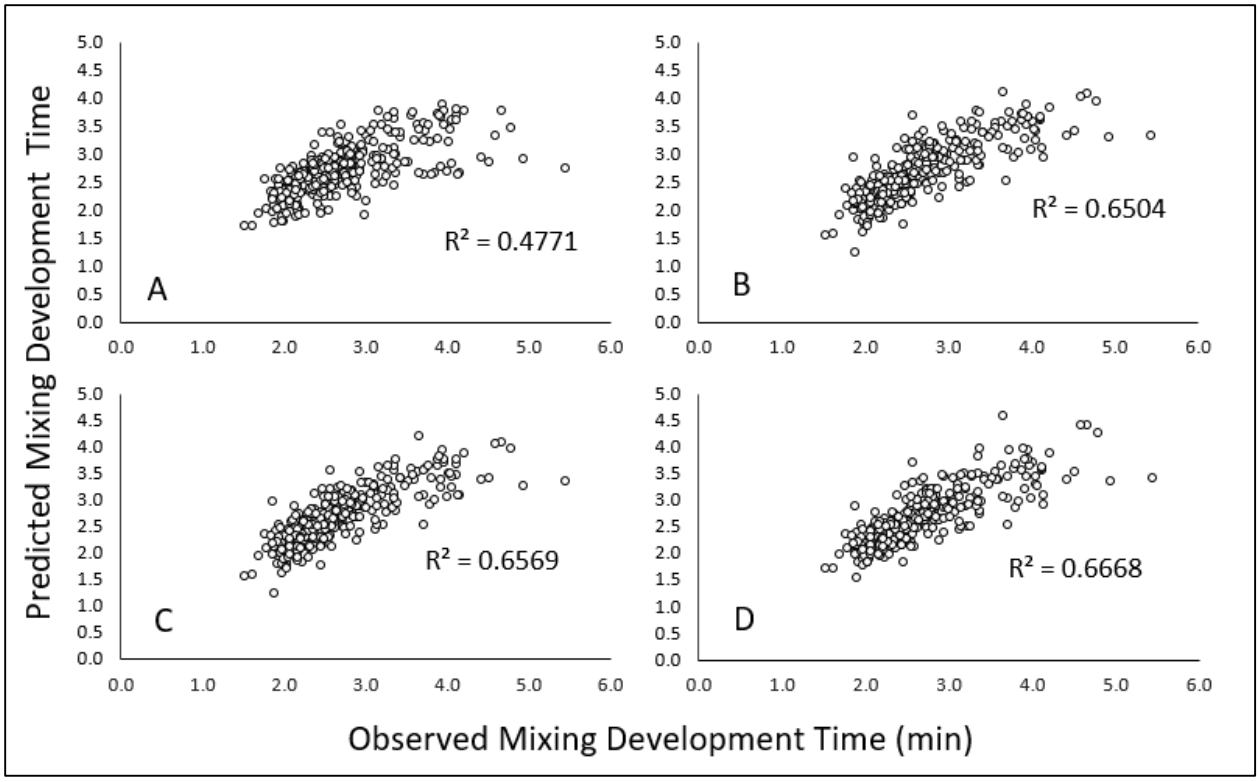

Figure 4.4.17. Relationship between predicted and observed mixing development times for multivariate regression analysis including one (A), two (B), three (C) and four (D) variables in the model.

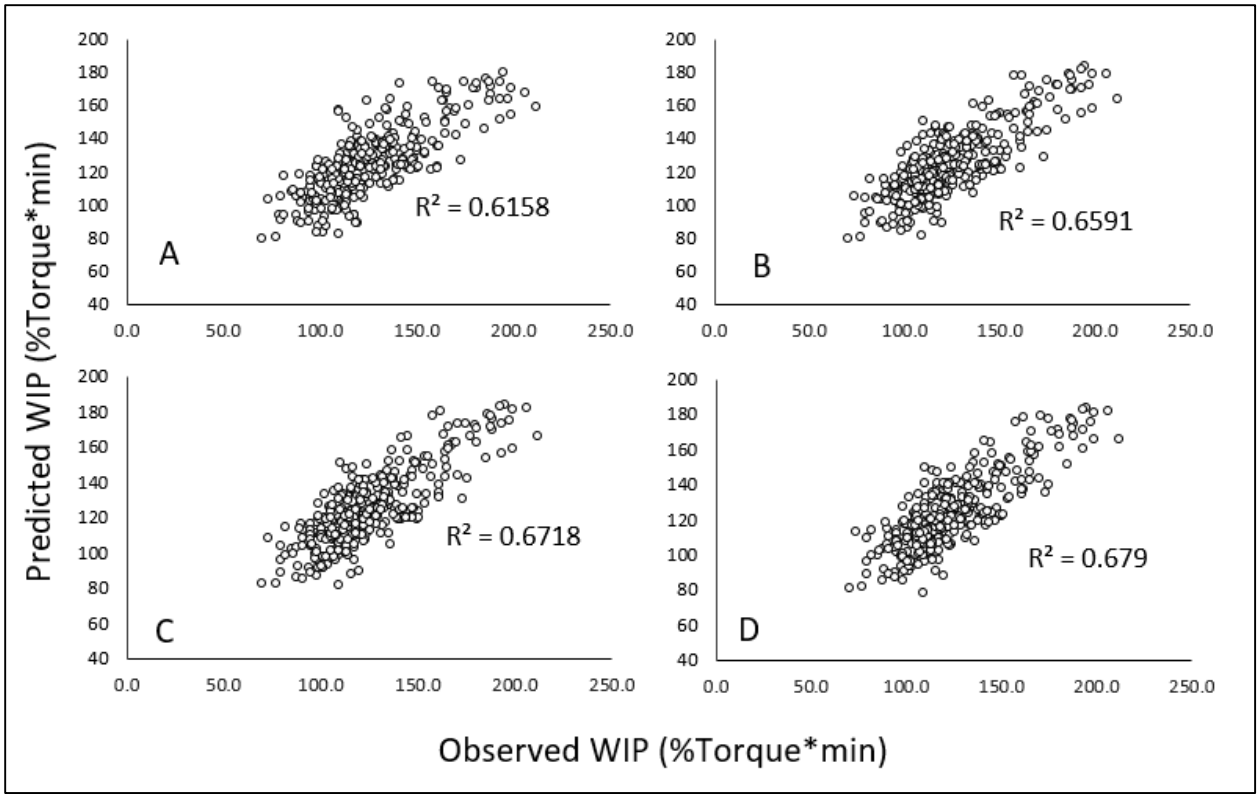

Figure 4.4.18 Relationship between predicted and observed work input to peak (WIP) for multivariate regression analysis including one (A), two (B), three (C) and four (D) variables in the model.

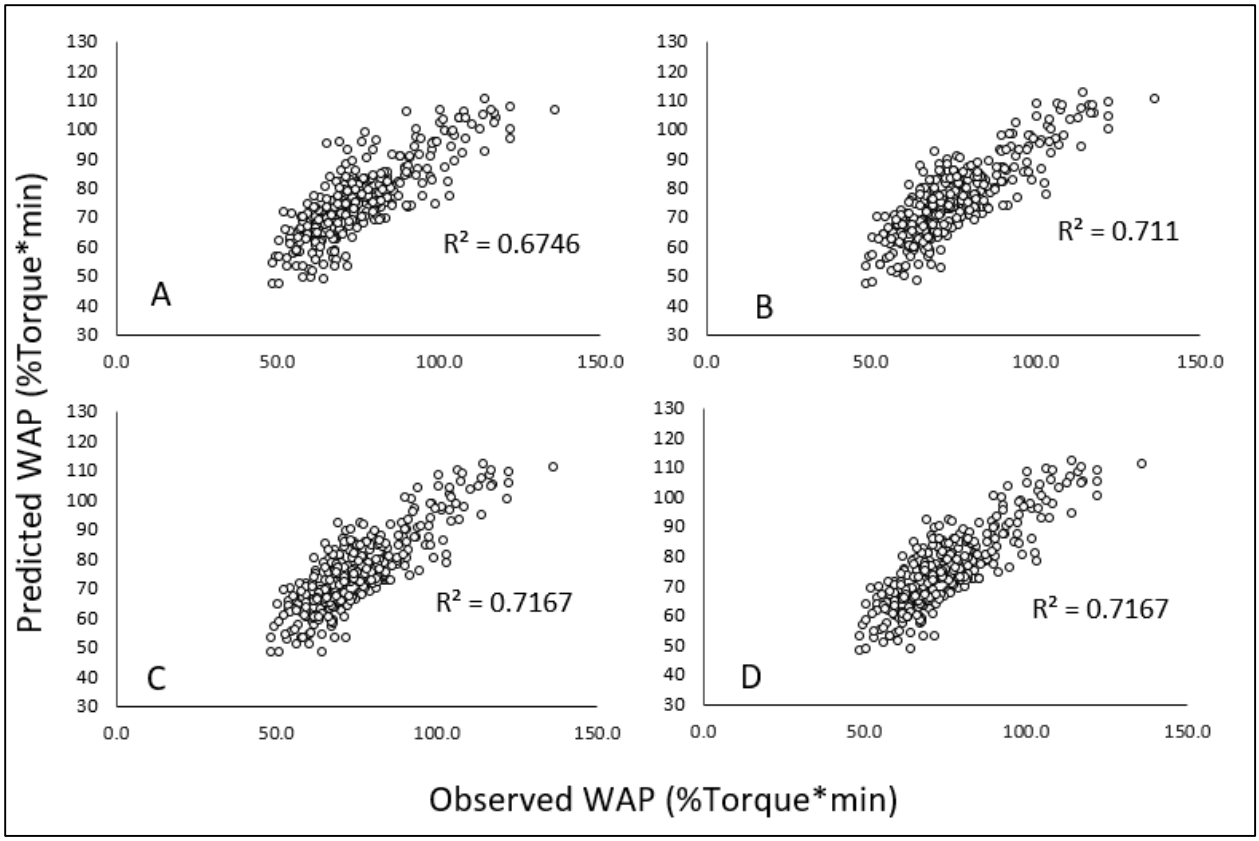

Figure 4.4.19. Relationship between predicted and observed work at peak (WAP) for multivariate regression analysis including one (A), two (B), three (C) and four (D) variables in the model.

4.5 Weather Interval Analysis and Relation to Gluten Strength

CWRS wheat grown in the Canadian Prairie region is subject to large variation in environmental conditions. These weather conditions during the growing season have a strong influence on the breadmaking quality of wheat, gluten strength in particular. Although the extent of this influence has been largely studied, it is difficult to relate qualitative changes in gluten strength to particular weather incidents due to the variable length of exposure of wheat crops to weather during crop development. Weather data can be compiled into time periods based on detailed phenology, but this must either be observed (which is challenging) or modeled (which is less accurate). The goal of this study was to develop an improved understanding of the significant variation of gluten strength of CWRS wheat grown in different locations in the Prairie region using an alternate approach to analyzing weather data. This approach compiles weather data from planting to maturity into a fixed number of intervals, thus normalizing the length of the growing season between sites, allowing the identification of critical time intervals that maximize correlations to protein content and gluten strength

4.5.1 Variation in Planting to Maturity Weather Conditions Between Site Years.

Each site-year combination generally experienced different weather conditions which undoubtedly influenced wheat end-use quality. Differences in content of total protein, SP and HMWG in flour to a lesser extent, were the most obvious quality-related parameters that were affected by weather at individual growing locations. In general, 2015 was a warmer, drier year in the Prairie region compared to 2016 which experienced cooler temperatures and more precipitation on average. Table 4.5.1 reports the average and cumulative values of select weather

variables from planting to maturity for the 2015 and 2016 locations used in this study. Indian Head was not included in the weather analysis; as solar radiation data was not available. The variability of weather conditions across locations can be seen in the extreme range of days to maturity, from 76 (Morden 2015) to 124 (Beaverlodge 2016). The cooler temperatures in 2016 are reflected in longer days to maturity and lower mean temperatures for all locations except 2016 Kelburn, where the mean temperature was 0.9 ^{o}C higher than in 2015. In general, Beaverlodge was the coolest location with mean temperatures of approximately 14 ^{o}C both years. Beaverlodge was followed by Edmonton, Lethbridge and Swift Current, with mean and minimum temperatures ranging between 15.3-16.7 C and 8.2-9.9 C respectively. Conversely, Morden was the warmest location in both growing years, with 2015 and 2016 mean temperatures of 19.2 and 17.9 ^{o}C, respectively. Moreover, Morden had the highest daily minimum temperatures, which averaged 13.3 and 12.2 ^{o}C in 2015 and 2016, respectively, whereas all other locations had daily minimum temperatures averaging below 11.5 C. The growing degree days (GDD; base 0) of all site-year combinations ranged between 1441 (Edmonton 2015) and 1743 (Kelburn 2016), where solar radiation levels ranged from 1642 MJ m^{-2} day^{-1} (Goodale 2015) and 2497 m^{-2} day^{-1} (Lethbridge 2016).

All locations received higher levels of precipitation in 2016, again, with the exception of Kelburn where 333 mm and 301 mm of rain occurred in 2015 and 2016, respectively. Across sites, there was a wide range in precipitation levels, from 91-333 mm in 2015 and 171-403 mm in 2016. Crop water demand was relatively similar for all locations in both years, with the exception of Beaverlodge and Morden. The former location had the highest levels of evapotranspiration of 420 mm in 2015 and 404 mm in 2016, whereas the latter had the lowest evapotranspiration of 328 and 338 mm in 2015 and 2016, respectively. All other locations had

water demand levels in a small range, from 369-404 mm. The wide ranging precipitation between site-years, was reflected in similarly wide-ranging crop water deficit values. Locations with less than 100 mm water deficit were Beaverlodge 2016 (-1 mm), Kelburn 2015 (-42 mm) and 2016 (-74 mm), Morden 2015 (-30 mm) and Swift Current 2016 (-25 mm). Conversely, the highest water deficits were experienced in Lethbridge 2015 (-308 mm), Edmonton 2015 (-275 mm) and Brandon 2015 (-256 mm).

Table 4.5.1. Average[a] and cumulative[b] values of weather variables from planting to maturity for 2015 and 2016 Canadian Prairie locations.

Location	Year	Days to Maturity	Max Temperature[a] (C)	Mean Temperature[a] (C)	Min Temperature[a] (C)	GDD_0[b]	Total Precipitation[b] (mm)	Crop Water Demand[b] (mm)	Crop Water Deficit[b] (mm)	Solar Radiation[b] ($MJ\ m^{-2}day^{-1}$)
Beaverlodge	*2015*	110	21.5	14.8	8.0	1627	191	420	-229	2284
	2016	124	19.3	13.3	7.3	1649	403	404	-1	2363
Brandon	*2015*	95	24.6	17.4	10.2	1652	131	390	-256	1949
	2016	96	23.2	16.8	10.4	1612	237	378	-142	1873
Edmonton	*2015*	89	24.0	16.7	8.4	1441	123	398	-275	1877
	2016	100	22.0	15.3	8.2	1510	267	394	-127	2101
Goodale	*2015*	92	23.3	17.2	10.3	1547	191	369	-178	1642
	2016	101	23.3	17.2	10.9	1725	223	378	-155	1725
Kelburn	*2015*	94	23.8	17.1	10.4	1608	333	375	-42	1790
	2016	97	24.4	18.0	11.5	1743	301	375	-74	1775
Lethbridge	*2015*	91	23.9	16.4	8.8	1490	91	399	-308	2376
	2016	99	22.2	15.3	8.5	1519	171	404	-233	2497
Morden	*2015*	76	25.2	19.2	13.3	1460	145	328	-184	1694
	2016	86	23.6	17.9	12.2	1540	308	338	-30	1815
Swift Current	*2015*	90	23.8	16.5	9.3	1487	125	391	-266	2029
	2016	106	21.9	15.9	9.9	1685	363	388	-25	2350

4.5.2 Correlation Between Weather Parameters (Planting to Maturity) and Gluten Strength

Table 4.5.2 presents correlations matrix between weather conditions from planting to maturity and select gluten strength parameters. There were few compelling correlations. Most noteworthy were consistent negative correlations, albeit not statistically significant, between min daily temperature and protein content-related parameters, e.g. FP, SP, HMWG and PDR of r = -0.17, -0.20, -0.29 and -0.55 ($p < 0.5$), respectively. This indicates that cooler night-time temperatures were associated with higher protein contents, averaged over genotypes and site-years. Similar results have not been previously reported, however nitrogen fertilization efficiency has been shown to increase as a result of cooler temperatures (DuPont et al. 2006a; Weidner and Ziemens 1975). Also, there was a weak negative correlation between min daily temperature and HMWG/FP (r = -0.20) and WAP (r = -0.16), indicating an inverse association between low night time temperature during the growing season and gluten strength. This relationship was further supported by negative relationships between GDD_0 and SP/FP (r = -0.45), HMWG/FP (r = -0.32), GSI (r = -0.17), MDT (r = -0.23) and WAP (r = -0.19), although none of these were statistically significant. Similar relationships were observed between GDD_5 and the aforementioned parameters, with the correlations weakening as the base temperature used to calculate GDD increased (Table). Evidently, growing locations with lower average temperatures favour decreased gluten strength, most likely the result of gliadin being the more efficient store of nitrogen in wheat (refer to Fig 4.4.3).

Regarding precipitation-related parameters, results indicated that growing sites with lower levels of precipitation were associated with higher gluten strength (Table 4.4.2), an observation that was further supported by correlations with crop water demand (positive r-

values, related to reduced precipitation) and water deficit (negative r-values, related to increased precipitation). In fact, for key parameters of gluten strength (HMWG/FP, GSI, and WAP), correlations with total precipitation and crop water deficit were moderately higher than those for temperature-related weather parameters, suggesting that precipitation-related differences among growing sites were relatively more important determinants of gluten strength compared with temperature-related influences. For example, compare correlations between HMWG and total precipitation (r = -0.38) or crop water deficit (r = -0.41) and GDD_0 (r = -0.32). or between WAP and total precipitation (r = -0.23) or crop water deficit (r = -0.26) and GDD_0 (r = -0.19).

Solar radiation (SR) had the strongest influence on grain and flour protein content, and was negatively associated to both with r= -0.33 and r = -0.28, respectively. Subfractions of flour protein, i.e. SP and HMWG were similarly affected with correlations of r = -0.26 and r = -0.21, respectively. This result may be related to the presumed positive effect of solar radiation on photosynthesis and crop yield (Estrada-Campuzano et al. 2008; Wang et al. 2003) and inverse relationship that is generally found between wheat yield and protein (DuPont et al. 2006a; Ferreira et al. 2012; Gibson et al. 1998; Johansson et al. 2005). The fact that solar radiation had negative correlations to minimum, mean and maximum temperatures was of concern (Table 4.5.2) and indicative that there may have been some discrepancies between the pyranometers used to record daily solar radiation at each growing location.

Table 4.5.2. Correlations between average and cumulative daily weather variables from planting to maturity between site-year means of gluten strength parameters (n = 16).

	Days to Maturity	Max Temperature	Mean Temperature	Min Temperature	GDD_0	Total Precipitation	Crop Water Demand	Crop Water Deficit	Solar Radiation
Days to Maturity	1.00								
Max Temperature	-0.90	1.00							
Mean Temperature	-0.86	0.92	1.00						
Min Temperature	-0.67	0.70	0.91	1.00					
GDD_0	0.58	-0.24	-0.10	0.11	1.00				
Total Precipitation	0.57	-0.56	-0.30	0.01	0.57	1.00			
Crop Water Demand	0.68	-0.57	-0.81	-0.92	0.13	-0.09	1.00		
Crop Water Deficit	0.38	-0.40	-0.10	0.23	0.52	0.97	-0.32	1.00	
Solar Radiation	0.58	-0.65	-0.79	-0.72	-0.04	0.06	0.71	-0.11	1.00
GP	-0.01	0.15	0.04	-0.11	0.09	-0.10	0.13	-0.12	-0.33
FP	0.03	0.10	-0.01	-0.17	0.09	-0.09	0.17	-0.13	-0.28
SP	0.01	0.10	-0.03	-0.21	0.01	-0.15	0.21	-0.19	-0.26
SP/FP	-0.14	0.04	-0.06	-0.22	-0.45	-0.34	0.17	-0.37	0.22
HMWG	-0.02	0.15	-0.05	-0.29	-0.07	-0.28	0.31	-0.34	-0.21
HMWG/FP	-0.13	0.11	-0.05	-0.20	-0.32	-0.38	0.24	-0.41	0.12
GSI	-0.09	0.11	-0.02	-0.14	-0.17	-0.27	0.19	-0.30	0.04
MDT	-0.40	0.35	0.37	0.39	-0.23	-0.19	-0.35	-0.10	-0.18
PBW	-0.08	0.07	0.06	0.01	-0.07	0.13	-0.14	0.15	-0.16
PDR	0.46	-0.39	-0.48	-0.55	0.13	0.06	0.50	-0.06	0.11
WIP	-0.07	0.11	0.07	0.05	-0.08	-0.08	-0.03	-0.07	-0.14
WAP	-0.02	0.02	-0.08	-0.16	-0.19	-0.23	0.18	-0.26	-0.02

FP = Flour protein content, GDD = Growing degree day, GP = Grain protein, GSI = Gluten strength index, HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WAP = Work at peak, WIP = Work input to peak. Correlations with $|r| >$ 0.516, 0.651, 0.787 and 0.858 are significant at $p < 0.05$, 0.01, .001 and 0.0001, respectively.

Table 4.5.3. Correlations between growing degree day accumulation from planting to maturity with various base temperatures and gluten strength parameters (n = 16).

	GDD_5	GDD_8	GDD_{12}	GDD_{15}	GDD_{18}	GDD_{21}	GDD_{24}
GDD_5	1.00						
GDD_8	0.93	1.00					
GDD_{12}	0.75	0.93	1.00				
GDD_{15}	0.62	0.85	0.98	1.00			
GDD_{18}	0.47	0.72	0.90	0.97	1.00		
GDD_{21}	0.34	0.58	0.79	0.88	0.96	1.00	
GDD_{24}	0.45	0.61	0.72	0.77	0.82	0.88	1.00
GP	0.11	0.12	0.12	0.12	0.09	0.07	0.15
FP	0.08	0.08	0.07	0.06	0.02	0.00	0.08
SP	0.01	0.01	0.02	0.02	0.01	0.00	0.08
SP/FP	-0.45	-0.40	-0.29	-0.20	-0.05	-0.01	-0.04
HMWG	-0.08	-0.06	-0.01	0.03	0.05	0.08	0.09
HMWG/FP	-0.30	-0.24	-0.13	-0.04	0.07	0.15	0.02
GSI	-0.15	-0.10	-0.03	0.03	0.09	0.16	0.04
MDT	0.00	0.14	0.26	0.33	0.41	0.44	0.31
PBW	-0.02	0.05	0.13	0.12	0.08	0.04	0.02
PDR	-0.16	-0.32	-0.42	-0.44	-0.44	-0.42	-0.36
WIP	-0.04	-0.01	0.05	0.11	0.19	0.26	0.12
WAP	-0.22	-0.21	-0.15	-0.08	0.03	0.10	-0.02

FP = Flour protein content, GDD = Growing degree day, GP = Grain protein, GSI = Gluten strength index, HMWG = High molecular weight glutenin, MDT = Mixing development time, PBW = Peak band width, PDR = Peak dough resistance, SP = Soluble prolamins, WAP = Work at peak, WIP = Work input to peak. Correlations with $|r| > 0.571$, 0.721, 0.746 and 0.829 are significant at $p < 0.05$, 0.01, .001 and 0.0001, respectively.

4.5.3 Stepwise Regression of Weather Data in Normalized Time Intervals in Relation to Select Wheat Quality Parameters

This results section documents outcomes of relationships between select wheat quality parameters (wheat and flour protein content, HMWG/FP, WAP) and weather data acquired in fixed time intervals between seeding to maturity by stepwise regression analysis. As explained in Materials and Methods, the interval analysis approach represents a novel treatment of weather data which is designed to efficiently and objectively handle the often highly variable crop growing periods across the Prairie region, which can vary by as much as 40 days or more between sites. The interval analysis strategy is attractive as it does not rely on detailed phenological staging of the crop to establish a temporal map of crop development which is resource intensive and is in general, unrealistic in a large G x E study upon which this thesis

research was based. It is important to reiterate that the regression analysis used in this thesis was only to provide a preliminary understanding of some of the influence of weather on gluten strength. The models created using this stepwise regression analysis were not intended to predict any of the parameters analyzed, as some the underlying assumptions for regression analysis were not satisfied; the number of observations was less than the number of explanatory variables and weather data is highly collinear.

For this analysis, 6 different interval lengths were evaluated, viz. 2, 4, 6, 8, 10, 12, between seeding and date of maturity for each site-year. The date of maturity for each site (see Table 3.3) represented the average for the set of genotypes. The nomenclature used for interval periods depended on the number of intervals in the analysis. For example, the code or abbreviation of the last interval when subdividing 2 (halves), 4 (fourths), 6 (sixths), 8 (eighths), 10 (tenths) and 12 (twelves) intervals is B2, Q4, H6, O8, D10 and T12.

Given the presumed importance of anthesis as a major phenological stage, it was of interest to compare the different sites with regards to its staging. Fig 4.5.1 shows those anthesis points for the growing locations for both years based on 6 and 12 intervals, respectively. It can be seen that despite the wide range in growing season days to maturity, the dates of anthesis fell inside or close to the same interval point, i.e. interval 4 and 8 for the 6 and 12 interval analyses, respectively.

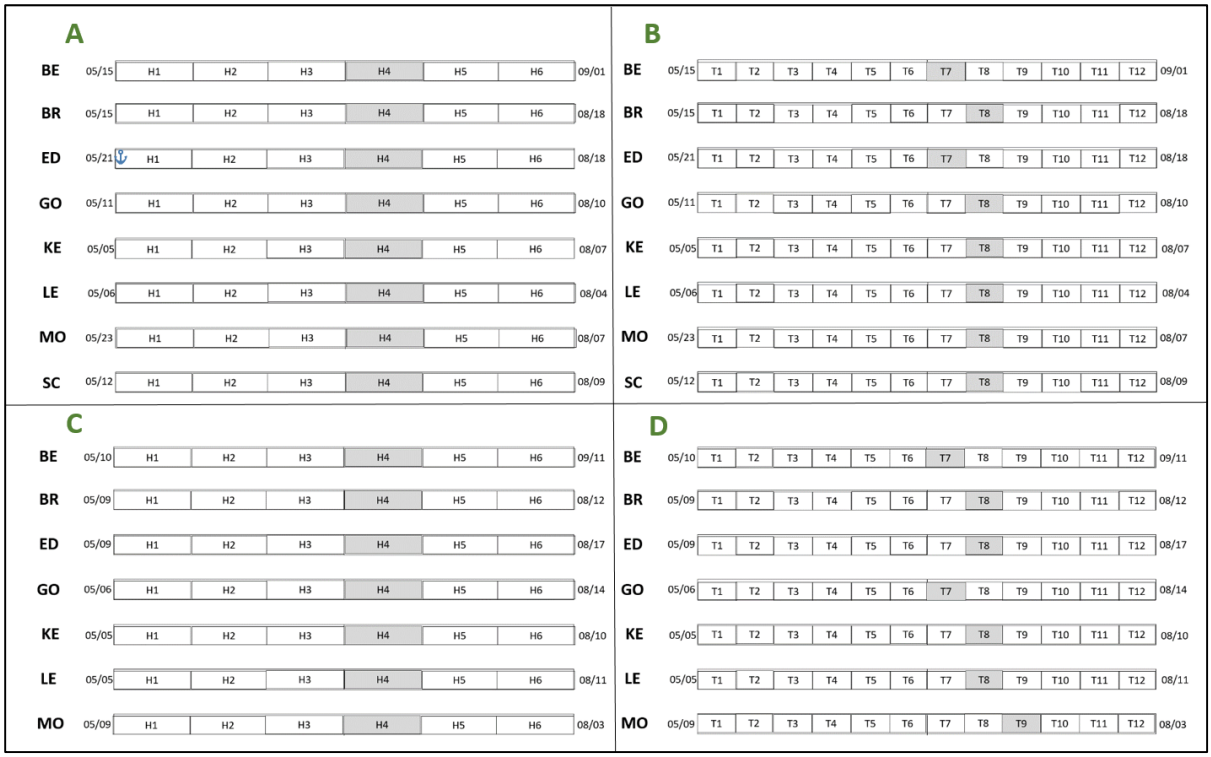

Figure 4.5.1. Growing seasons for locations in 2015 (top: A + B) and 2016 (bottom: C + D) split into six (left: A + C) and twelve (right: B + D) calendar day intervals. Planting and maturity dates are written to the left and right, respectively, with the highlighted interval indicating anthesis.

4.5.3.1 Stepwise Regression Analysis for Grain and Flour Protein Content

Stepwise regression results for grain protein content based on interval analysis of weather parameters is reported in Table 4.5.4. The regression models include the best 1, 2, 3, and 4 weather variables from planting to maturity compartmentalized into the specified calendar day intervals, from 2 to 12. It can be seen that as the number of intervals or divisions of the growing season increases, the weather variables change. This is likely a reflection that different weather-related conditions had different impacts on grain protein content depending on the lengths of the interval time periods. Additionally, as the number of intervals from planting to maturity increases, the various models become more robust in terms of higher R^2 and statistical significance, although the 10 and 12 interval models for grain protein had similar R^2 values and probabilities. What is particularly noteworthy in this analysis is the degree to which R^2 values continue increase as more variables enter the model. For example, in the 12 interval analysis (Table 4.5.4) R^2 increase from 0.47 (1 variable) to 0.72 (2 variables) to 0.89 (3 variables). These increments suggest that the variables entered at each step (e.g. from 2 to 3) complement well those already in the model and significantly if not substantially explain more of the variation in protein content attributable to weather. On this point, it can be seen that the best 4 variable models for 10 and 12 interval analysis of grain protein content explained 92% and 94% of the variation of grain protein content, respectively, which is an impressive result. That said, it remains to be determined whether the same weather models can adequately predict grain protein content of samples grown in different locations and/or years.

Many temperature-related variables were included in the best models selected by stepwise regression. With the exception of the 6 interval analysis, a temperature related parameter was the best single variable to explain variation in grain protein content, with R^2

values of 0.23, 0.16, 0.38, 0.48 and 0.47 for the 2, 4, 8, 10, and 12 interval analyses, respectively. The most frequently selected weather parameter was a moderate GDD temperature accumulation during the 1st interval of the growing season. Such a variable appeared in all models of 8, 10 and 12 interval analyses, i.e. GDD_{18} in the 8 interval analysis, and GDD_{15} in the 10 and 12 interval analyses. All were negatively associated with grain protein content, which may have occurred as a consequence of moderate GDD temperatures being positively associated with grain yield which is generally accepted to be inversely related with protein content. Additionally, GDD_{21} midway through the grain season (interval D6, 10 interval analysis), was negatively associated with GP in the 3 and 4 variable models. Likewise, GDD_{24} in interval B1 (2 interval analysis) was negatively associated with GP. The negative association between GP and warmer temperatures appeared to cap at a certain level, as variable DA30 (i.e. days above 30 ^{o}C) in intervals B1 (2 interval analysis), and D4 (10 interval analysis), as well as maximum temperature in interval D5 were all positively associated with GP. The diurnal variation in temperature (DV and WDV) was positively associated with GP during the intervals which corresponded to the halfway point in the growing season (i.e. B2, Q2 and O4); DV or WDV were not selected in models that involved subdividing the growing period by more than 8 intervals

Stepwise regression analysis of GP included few precipitation-related parameters in the models. However, crop water deficit (CWD) early in the growing season (intervals B1 and H2) showed a negative relationship to GP. Subsequently, CWD at interval H2 (6 interval analysis) was the best one variable model to explain variation in GP with an R^2 of 0.20. A negative association between GP and TP*Etc occurred during interval O7 in the four variable regression model. Four variable models of the 4 and 12 interval analysis reported positive associations between Etc (at Q4) and TP/Etc (at T12) to GP, respectively.

Solar radiation (SR) as well as top of the atmosphere radiation (TOA), were negatively associated with protein content. Again, this is presumably the result of the inverse relationship between yield and protein content, i.e. an increase in grain yield is to be expected as a result of the increased rate of photosynthesis that occurs when incident SR is higher (Estrada-Campuzano et al. 2008; Wang et al. 2003). SR and TOA were reported in the regression models for GP when the number of days in the intervals was smaller, i.e. 6 – 12 intervals throughout the growing season. The negative association between SR and TOA to GP occurred midway throughout the growing season (SR: O6 and T5, TOA: H5) was presented in the three and four variable models of the respective 6, 12 and 8 interval analysis. However, TOA early in the growing season (H1; 6 interval analysis) was positively related to GP. As SR and TOA were generally found to negatively impact GP, it follows that cloud cover (CC) was positively associated with GP, as shown in the 4, 3 and/or 2 variable models of the 2, 4 and 6 interval analyses at B1, Q3 and H1 interval points, respectively.

Results of stepwise regression analysis of flour protein content (Table 4.5.5) was extremely similar to that of GP both in terms of variables selected and R^2 outcomes, as expected due to the high correlation between the two parameters ($r = 0.99$). Regression models were able to explain up to 93% of the variation in flour protein content which was identical to the result for GP. Regardless of the number of intervals during the growing season, SR was negatively associated with FP at intervals B2, Q4, H3, H5, O6, S2 and T5. In contrast, for the GP regression models, SR was selected in the 6-12 interval analyses. Additionally, CWD had a positive influence on FP in the 4 variable model at D10 (10 interval analysis), whereas for GP regression models, CWD was always negative. It is unclear why this occurred.

Table 4.5.4. Stepwise regression analysis of grain protein content with weather variables compartmentalized into normalized calendar day intervals ranging from 2 – 12 intervals from planting to maturity. Variables in black and blue text denote positive and negative associations with grain protein content, respectively.

2 Intervals				**4 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	DA30 (B1)	0.23	p = 0.0590	1	WDV (Q2)	0.16	p = 0.1244
2	DA30 (B1), CWD (B1)	0.34	p = 0.0652	2	WDV (Q2), CC (Q3)	0.40	p < 0.05
3	CWD (B1), CC (B1) WDV (B2)	0.54	p < 0.05	3	WDV (Q2), CC (Q3), ETc (Q4)	0.56	p < 0.05
4	GDD24 (B1), CWD (B1), CC (B1) WDV (B2)	0.61	p < 0.05	4	WDV (Q2), WDV (Q3), CC (G3), Etc (Q4)	0.73	p < 0.01
6 Intervals				**8 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	CWD (H2)	0.20	p = 0.0866	1	GDD18 (O1)	0.38	p < 0.05
2	CC (H1), CWD (H2)	0.58	p < 0.01	2	GDD18 (O1), SR (O6)	0.70	p < 0.001
3	CC (H1), CWD (H2), TOA (H5)	0.68	p < 0.01	3	GDD18 (O1), DV (O4), SR (O6)	0.81	p < 0.001
4	CC (H1), TOA (H1), CWD (H2), TOA (G5)	0.86	p < 0.001	4	GDD18 (O1), GDD21 (O4), SR (O6), TP*Etc (O7)	0.89	p < 0.0001
10 Intervals				**12 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	GDD15 (D1)	0.48	p < 0.01	1	GDD15 (T1)	0.47	p < 0.01
2	GDD15 (D1), Max (D5)	0.79	p < 0.0001	2	GDD15 (T1), SR (T5)	0.72	p < 0.001
3	GDD15 (D1), Max (D5), GDD21 (D6)	0.88	p < 0.0001	3	GDD15 (T1), SR (T5), Mean (T7)	0.89	p < 0.0001
4	GDD15 (D1), DA30 (D4), Max (D5), GDD21 (D6)	0.92	p < 0.0001	4	GDD15 (T1), SR (T5), Min (T2), GDD18 (T7)	0.94	p < 0.0001

CC = Cloud cover, CWD = Crop water deficit, DA30 = Days above 30 C, DV = Diurnal variation, Etc = Crop water demand, GDD = Growing degree days, Max = Maximum temperature, SR = Solar radiation, TOA = Top of atmosphere radiation, TP = Total precipitation, WDV = Weighted diurnal variation. The abbreviation code for the last interval when subdividing the growing season into 2, 4, 6, 8, 10 and 12 intervals is B2, Q4, H6, O8, D10 and T12, respectively.

Table 4.5.5. Stepwise regression analysis of flour protein content with weather variables compartmentalized into normalized calendar day intervals ranging from 2 – 12 intervals from planting to maturity. Variables in black and blue text denote positive and negative associations with flour protein content, respectively.

2 Intervals				**4 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	DA30 (B1)	0.23	p = 0.0588	1	DV (Q2)	0.19	p = 0.0921
2	DA30 (B1), CWD (B1)	0.35	p = 0.0592	2	DV (Q2), SR (Q4)	0.45	p < 0.05
3	GDD18 (B1), DA30 (1), SR (B2)	0.49	p < 0.05	3	DV (Q2), TP*Etc (Q3), SR (Q4)	0.59	p < 0.05
4	GDD18 (B1), DA30 (B1), SR (B2), CWD (B2)	0.73	p < 0.01	4	DA30 (Q2), DV (Q2), TP*Etc (Q3), SR (Q4)		p < 0.01
6 Intervals				**8 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	DV (H3)	0.20	p = 0.0841	1	GDD18 (O1)	0.40	p = 0.0081
2	GDD18 (H1), SR (H5)	0.50	p < 0.05	2	GDD18 (O1), SR (O6)	0.69	p < 0.001
3	GDD18 (H1), DA30 (H1), SR (H3)	0.79	p < 0.001	3	GDD18 (O1), DV (O4), SR (O6)	0.82	p < 0.0001
4	GDD18 (H1), DA30 (H1), SR (H3), TP (H6)	0.83	p < 0.001	4	GDD18 (O1), SR (O6), DV (O7), GDD21 (O8)	0.87	p < 0.0001
10 Intervals				**12 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	GDD15 (D1)	0.50	p < 0.01	1	GDD15 (T1)	0.49	p < 0.01
2	GDD15 (D1), Max (D5)	0.81	p < 0.0001	2	GDD15 (T1), SR (T5)	0.73	p < 0.001
3	GDD15 (D1), Max (D5), GDD18 (D10)	0.88	p < 0.0001	3	GDD15 (T1), SR (T5), Mean (T7)	0.87	p < 0.0001
4	GDD15 (D1), SR (D2), Max (D5), CWD (D10)	0.93	p < 0.0001	4	GDD15 (T1), SR (T5), TP (T6), GDD15 (T7)	0.93	p < 0.0001

CWD = Crop water deficit, DA30 = Days above 30 C, DV = Diurnal variation, Etc = Crop water demand, GDD = Growing degree days, Max = Maximum temperature, SR = Solar radiation, TP = Total precipitation. The abbreviation code for the last interval when subdividing the growing season into 2, 4, 6, 8, 10 and 12 intervals is B2, Q4, H6, O8, D10 and T12, respectively.

4.5.3.2 Stepwise Regression Analysis of Soluble Prolamin Content

The results of the stepwise regression analysis of SP are presented in Table 4.5.6 and are very similar to those for GP and FP. This can be explained on the basis that SP being composed mostly of gliadin protein is the main storage protein fraction of wheat endosperm. Regression models were able to account for up to 93% of the variation in SP (Table 4.5.6) as shown in the 4 variable models using 10 and 12 intervals. Diurnal variation in temperature (DV and WDV) was positively associated with SP in the 2, 8 and 10 interval analyses (at B1 and B2, O4 and O7 as well as D5 and D7, respectively). Additionally, DV was the best 1 variable model to explain variation in SP in both 2 and 4 interval analyses, yielding R^2 values of 0.24 and 0.21, respectively. However, these models were not statistically significant. Positive relationships to SP were additionally reported for variable DA30 at H1 (6 interval analysis) in the 3 and 4 variable models, as well as minimum temperature at D1 in the 3 and 4 variable regression models of the 10 interval analysis.

Moderate temperature accumulation parameters, GDD_{15} and $GDD_{18.}$ early in the growing season negatively impacted SP, which was similar to results of grain and flour protein content. As SP is highly correlated with FP (Table 4.4.2), this is presumably due to the same inverse relationship between yield and protein content as noted previously. GDD_{18} at intervals H1 (6 interval analysis) and O1 (8 interval analysis) was the best 1 variable model to explain variation in SP with R^2 values of 0.24 (ns) and 0.45 ($p < 0.01$), respectively. When the growing season was divided into a greater number of intervals GDD_{15} accounted for 51% ($p < 0.10$) and 50% ($p < 0.01$) of SP variation in the 1 variable models at D1 (10 interval analysis) and T1 (12 interval analysis), respectively.

SR was found to be negatively associated with SP; again, presumably due to the high collinearity between FP and SP. The negative relationship between SR and SP appeared throughout the entire growing season, i.e. at B1, Q4, H3 and H5, O6, D2 and T5 in the 2, 4, 6, 8, 10 and 12 interval analyses, respectively. Conversely, CC was positively associated with SP (at B1) in the 3 variable regression model of the 2 interval analysis. Few precipitation-related parameters were selected in the regression models of SP; negative and positive associations between SP and CWD (at T9) and TP*Etc (at Q3) were found, respectively.

Stepwise regression models of SP/FP are presented in Table 4.5.7. Stepwise regression analysis of soluble prolamin content in protein (SP/FP) with weather variables compartmentalized into normalized calendar day intervals ranging from 2 – 12 intervals from planting to maturity. Variables in black and blue text denote positive and negative associations with SP/FP, respectively, and were able to account for up to 92% of the variation in this parameter. This unit-less and mainly genotype influenced protein quality parameter, which tracks mainly gliadin protein content per unit protein, was negatively correlated with gluten strength (Table 4.4.2) The influence of temperature-related parameters on SP/FP is unclear, but would be expected to be different from those related to SP; the very low correlation between SP and SP/FP ($r = 0.20$) supports this view. In contrast to the results for SP, GDD_{18} at the first interval of the growing season (H1 and D1) was positively associated with SP/FP throughout many of the models, i.e. from 1 to 4 variables e.g. in (Table 4.5.7). Additionally, other high-temperature related parameters at the start of the growing season, i.e. mean daily temperature at B1, DA30 at Q2, and GDD_{24} at T8 were positively related to SP/FP. In contrast, high temperature-related conditions at the end of the growing season were negatively related to SP/FP. This is shown in the 10 and 12 interval analyses where GDD_{24} (at D8 and T12, respectively) was negatively

related to SP/FP in the 3 and 4 variable regression models. In fact, all remaining temperature-related parameters not previously discussed that were selected by stepwise regression were negatively associated with SP/FP. This includes cold to moderate temperature-related GDD parameters ($GDD_{5\text{-}21}$) and Min early to midway throughout growing season (B1 and B2, Q2 and Q3, H2 and H3, O2 and O5, D3 and D4). Thus it appears that high temperature accumulation (> 18 ^{o}C) at the very first interval in the growing season positively influenced the concentration of SP/FP (and would be expected to negatively influence gluten strength), whereas similar high temperature conditions at the end of the growing season were negatively associated with SP/FP (would be expected to positively influence gluten strength. In addition, all other GDD parameters through the growing season were negatively related to SP/FP.

Variables relating total precipitation to crop evapotranspiration appeared to have a positive effect on SP/FP, particularly at the end of the growing season (TP/Etc at B2, Etc at T1 as well as TP*Etc at H5 and O6). However, no other precipitation-related parameters were included in the regression models of SP/FP. Interestingly, SR was positively related to SP/FP (at B2 and T4), whereas all protein content related parameters (GP, FP and SP) were negatively associated with solar radiation. As pointed out previously, SP/FP would not be expected to follow the same trends as for GP, FP and SP. It follows that CC negatively influence SP/FP in the 4 and/or 3 variable models of the 4 and 8 interval analyses.

Table 4.5.6. Stepwise regression analysis of soluble prolamin content in flour (SP) with weather variables compartmentalized into normalized calendar day intervals ranging from 2 – 12 intervals from planting to maturity. Variables in black and blue text denote positive and negative associations with SP, respectively.

2 Intervals				**4 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	DV (B1)	0.24	p = 0.0554	1	DV (Q2)	0.21	p = 0.0730
2	DV (B1), TP (B2)	0.39	p < 0.05	2	DV (Q2), SR (Q4)	0.44	p < 0.05
3	CWD (B1), CC (B1), DV (B2)	0.51	p < 0.05	3	DV (Q2), TP*Etc (Q3), SR (Q4)	0.59	p < 0.05
4	Min (B1), SR (B1), CWD (B1), TOA (B2)	0.71	p < 0.001	4	DA30 (Q2), DV (Q2), TP*Etc (Q3), SR (Q4)	0.73	p < 0.01
6 Intervals				**8 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R_2	Probability
1	GDD18 (H1)	0.24	p = 0.0568	1	GDD18 (O1)	0.45	p < 0.01
2	GDD18 (H1), SR (H5)	0.52	p < 0.01	2	GDD18 (O1), SR (O6)	0.70	p < 0.001
3	GDD18 (H1), DA30 (H1), SR (H3)	0.77	p < 0.001	3	GDD18 (O1), DV (O4), SR (O6)	0.83	p < 0.0001
4	GDD18 (H1), DA30 (H1), SR (H3), DA30 (H5)	0.82	p < 0.001	4	GDD18 (O1), CWD (O4), SR (O6), DV (O7)	0.88	p < 0.0001
10 Intervals				**12 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	GDD15 (D1)	0.51	p < 0.01	1	GDD15 (T1)	0.50	p < 0.01
2	GDD15 (D1), WDV (D5)	0.78	p < 0.0001	2	GDD15 (T1), SR (T5)	0.72	p < 0.001
3	Min (D1), GDD15 (D1), WDV (D5)	0.86	p < 0.0001	3	GDD15 (T1), SR (T5), Min (T7)	0.89	p < 0.0001
4	Min (D1), GDD15 (D1), SR (D2), GDD5 (D7)	0.92	p < 0.0001	4	GDD15 (T1), SR (T5), Min (T7), CWD (T9)	0.93	p < 0.0001

CC = Cloud cover, CWD = Crop water deficit, DA30 = Days above 30 C, DV = Diurnal variation, , Etc = Crop water demand, GDD = Growing degree days, Min = Minimum temperature, SR = Solar radiation, TOA = Top of atmosphere radiation, TP = Total precipitation, WDV = Weighted diurnal variation. The abbreviation code for the last interval when subdividing the growing season into 2, 4, 6, 8, 10 and 12 intervals is B2, Q4, H6, O8, D10 and T12, respectively.

Table 4.5.7. Stepwise regression analysis of soluble prolamin content in protein (SP/FP) with weather variables compartmentalized into normalized calendar day intervals ranging from 2 – 12 intervals from planting to maturity. Variables in black and blue text denote positive and negative associations with SP/FP, respectively.

2 Intervals				**4 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	GDD5 (B2)	0.33	$p < 0.05$	1	GDD12 (Q2)	0.45	$p < 0.01$
2	GDD5 (B2), SR (B2)	0.46	$p < 0.05$	2	GDD12 (Q2), DV (Q3)	0.60	$p < 0.01$
3	Mean (B1), GDD15 (B1), SR (B2)	0.70	$p < 0.01$	3	GDD12 (Q2),DA30 (Q2), DV (Q3)	0.71	$p < 0.01$
4	Mean (B1), GDD12 (B1), SR (B2), TP/Etc (B2)	0.84	$p < 0.001$	4	CC (Q1), GDD12 (Q2),DA30 (Q2), DV (Q3)	0.79	$p < 0.001$
6 Intervals				**8 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	GDD8 (H2)	0.56	$p < 0.001$	1	GDD18 (O2)	0.40	$p < 0.01$
2	GDD18 (H1), GDD8 (H2)	0.71	$p < 0.001$	2	GDD18 (O2), TP/Etc (O6)	0.59	$p < 0.01$
3	GDD18 (H1), GDD8 (H2), GDD21 (H3)	0.80	$p < 0.001$	3	CC (O3), Min (O5), TP/Etc (O6)	0.83	$p < 0.0001$
4	GDD18 (H1), GDD8 (H2), GDD21 (H3), TP*Etc (H5)	0.86	$p < 0.0001$	4	CC (O3), Min (O5), TP/Etc (O5), TP/Etc (O6)	0.90	$p < 0.0001$
10 Intervals				**12 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	GDD8 (D3)	0.50	$p < 0.01$	1	SR (T4)	0.44	$p < 0.01$
2	GDD18 (D1), GDD8 (D3)	0.70	$p < 0.001$	2	SR (T4), GDD5 (T8)	0.62	$p < 0.01$
3	GDD18 (D1), GDD8 (D3), GDD24 (D8)	0.87	$p < 0.0001$	3	SR (T4), GDD24 (T8), GDD24 (T12)	0.76	$p < 0.01$
4	GDD18 (D1), GDD8 (D3), GDD15 (D4), GDD24 (D8)	0.91	$p < 0.0001$	4	Etc (T1), SR (T4), GDD24 (T8), GDD24 (T12)	0.92	$p < 0.0001$

CC = Cloud cover, DA30 = Days above 30 C, DV = Diurnal variation, Etc = Crop water demand, GDD = Growing degree days, Mean = Mean temperature, Min = Minimum temperature, SR = Solar radiation, TP = Total precipitation. The abbreviation code for the last interval when subdividing the growing season into 2, 4, 6, 8, 10 and 12 intervals is B2, Q4, H6, O8, D10 and T12, respectively.

4.5.3.3 Stepwise Regression Analysis of High Molecular Weight Glutenin

Stepwise regression models for HMWG (Table 4.5.8) were able to account for up to 97% of the variation in the parameter. Unlike results for dependent variables GP, FP, SP and SP/FP, GDD was not the most prominent parameter selected. Still, GDD_{18} at Q1 (4 interval analysis) and GDD_5 at T7 (2 and 12 interval analyses) all negatively influence the concentration of HMWG. Whereas very high temperatures (days above 30 ^oC) in the first half of the growing season, which is relatively uncommon in the Prairie region, positively affected HMWG in 2, 4 and 6 interval analyses at B1, Q1 + Q2 and H2, respectively. That high temperatures during crop development before anthesis can affect the formation of glutenin in wheat grain underscores the complexity of wheat physiology and weather influences. On this point, DA30 (days above 30 ^oC) at B1 (2 interval analysis) was selected as the best 1 variable model to account for variation in HMWG with an R^2 of 0.47 ($p < 0.01$). Additionally, diurnal temperature variation was positively related to concentrations of HMWG. DV alone was able to account for 28% ($p < 0.05$), 34% ($p < 0.05$) and 42% ($p < 0.05$) of the variation in HMWG in regression models involving 4 (at Q1), 6 (at H3) and 10 (at D5) interval analyses, respectively.

A higher number of precipitation-related parameters were included in the regression models of HMWG, all of which were negatively related to HMWG. This was the case with the exception of TP (at H6), TP/Etc (at O5) and TP*Etc (at D10) in the 4, 3 and/or 2 variable regression models for 6, 8 and 10 interval analyses. Regardless, the negative influence of total precipitation (TP) alone at T3 in the one variable model of the 12 interval analysis accounted for 55% of the variation in HMWG ($p < 0.01$) and was negatively associated with HMWG in the 4 variable model of the 6 interval analysis at H2. Additionally, the best 1 variable model for HMWG in the 8 interval analysis was TP/Etc (at O4), with an R2 value of 0.43 ($p < 0.01$).

HMWG, being a storage protein fraction, albeit weakly, was positively associated with FP content (Table 4.4.2). Accordingly, as with SP content, HMWG displayed similar results to GP and FP, being negatively associated with SR throughout the entire growing season (at Q4, H5,O1, D5, D8 and D10), and positively associated with CC in the 4, 3 and/or 2 variable regression models for the 8 and 12 interval analyses. The 2 variable model of the 12 interval analysis including CC (T1, positively related) and TP (at T3, negatively related) accounted for 78% of the variation in HMWG ($p < 0.0001$). Again, this outcome seems surprising if not untenable as the interval periods selected by the regression analysis involving cloud cover and total precipitation occurred during the vegetative phase of crop development.

Stepwise regression models of HMWG/FP are presented in Table 4.5.9. Compared to protein composition parameters discussed previously in this chapter, HMWG/FP is distinct due to its close association with gluten strength. Like SP/FP, HMWG/FP is a unit-less protein quality parameter that was highly influenced by genotype. The maximum R^2 value in this regression analysis was 0.86 ($p < 0.0001$) in the 4 variable model of the 12 interval analysis. High temperature-related variables (DA30, GDD_{24}) in the last interval of the growing season were prominent in this model and were negatively related to HMWG/FP in the 4, 6, and 10 interval analyses, as well. High temperatures at the end of the growing season were also found to be negatively associated with SP/FP. Additionally, GDD_{21} at H2 (6 interval analysis) and DA at O5 (8 interval analysis) were negatively related to HMWG/FP. Remaining high temperature-related parameters both at the beginning (DA30 at B1, GDD_{18} at Q1 and O1) and middle (GDD_{21} at H5 and D5, Mean at O5, DA30 at D7) of the growing season were positively associated with HMWG/FP. Similar to the results for HMWG content, DV and WDV were positive associated with HMWG/FP, the former being the best 1 variable models in the 4 and 8 interval analyses

with R^2 values of 0.25 ($p < 0.05$) and 0.33 ($p < 0.05$), respectively. The influence of DV and WDV on HMWG/FP occurred approximately midway through the growing season, based on intervals selected, i.e. Q3, H2, H3, O6 and T9. In contrast to the results for the other protein-related parameters, SR was not prominently included in the HMWG/FP regression models.

Similar to the results for HMWG, precipitation-related parameters, included in many of the regression models, were negatively associated with HMWG/FP. An exception was for TP/Etc (at B2) in the 4 variable model of the 2 interval analysis. Otherwise, TP*Etc at B1 was the best 1 variable model for HMWG/FP, accounting for 18% (ns) of variation. Similarly, TP at about the midpoint in the growing season (H3, D5 and T6) was the best 1 variable model, with R^2 values of 0.30 ($p < 0.05$), 0.43 ($p < 0.01$) and 0.44 ($p < 0.01$) for the 6, 10 and 12 interval analyses, respectively. In the 4 variable model of the 12 interval analysis, the combination of precipitation (TP at T6), late-season high temperature conditions (GDD_{24} at T12 and DA30 at T12) and WDV (at T9) accounted for 86% of the variation in HMWG/FP ($p < 0.0001$).

Table 4.5.8. Stepwise regression analysis of high molecular weight glutenin content in flour (HMWG) with weather variables compartmentalized into normalized calendar day intervals ranging from 2 – 12 intervals from planting to maturity. Variables in black and blue text denote positive and negative associations with HMWG, respectively.

2 Intervals				**4 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	DA30 (B1)	0.47	$p < 0.01$	1	DV (Q1)	0.28	$p < 0.05$
2	DA30 (B1), CWD (B1)	0.60	$p < 0.01$	2	DA30 (Q1), DA30 (Q2)	0.48	$p < 0.05$
3	GDD21 (B1), DA30 (B1), CWD (B1)	0.75	$p < 0.001$	3	DA30 (Q1), DA30 (Q2), SR (Q4)	0.62	$p < 0.01$
4	GDD5 (B1), DA30 (B1), CWD (B1), CC (B2)	0.86	$p < 0.001$	4	GDD18 (Q1), DA30 (Q1), DA30 (Q2), SR (Q4)	0.78	$p < 0.01$
6 Intervals				**8 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	DV (H3)	0.34	$p < 0.05$	1	TP/Etc (O4)	0.43	$p < 0.01$
2	DV (H3), TP (H6)	0.61	$p < 0.01$	2	TP/Etc (O4), TP/Etc (O5)	0.62	$p < 0.01$
3	DA30 (H2), DV (H3), SR (H5)	0.75	$p < 0.001$	3	SR (O1), CC (O2), TP/Etc (O4)	0.85	$p < 0.0001$
4	TP (H2), DV (H3), TOA (H4), SR (H5)	0.89	$p < 0.0001$	4	SR (O1), CC (O2), TP/Etc (O4), DV (O7)	0.93	$p < 0.0001$
10 Intervals				**12 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	DV (D5)	0.42	$p < 0.01$	1	TP (T3)	0.55	$p < 0.01$
2	DV (D5), SR (D8)	0.70	$p < 0.001$	2	CC (T1), TP (T3)	0.78	$p < 0.0001$
3	DV (D5), SR (D10), TP*Etc (D10)	0.92	$p < 0.0001$	3	CC (T1), TP (T3), GDD5 (G7)	0.85	$p < 0.0001$
4	SR (D5), DV (D5), SR (D10), TP*Etc (D10)	0.97	$p < 0.0001$	4	CC (T1), TP/Etc (T3), GDD5 (T7), WDV (T7)	0.94	$p < 0.0001$

CC = Cloud cover, CWD = Crop water deficit, DA30 = Days above 30 C, DV = Diurnal variation, Etc = Crop water demand, GDD = Growing degree days, SR = Solar radiation, TP = Total precipitation, WDV = Weighted diurnal variation. The abbreviation code for the last interval when subdividing the growing season into 2, 4, 6, 8, 10 and 12 intervals is B2, Q4, H6, O8, D10 and T12, respectively.

Table 4.5.9. Stepwise regression analysis of high molecular weight glutenin content in protein (HMWG/FP) with weather variables compartmentalized into normalized calendar day intervals ranging from 2 – 12 intervals from planting to maturity. Variables in black and blue text denote positive and negative associations with HMWG/FP, respectively.

2 Intervals				**4 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	TP*Etc (B1)	0.18	p = 0.1023	1	DV (Q3)	0.25	p < 0.05
2	DA30 (B1), TP*Etc (B1)	0.24	p = 0.1687	2	GDD18 (Q1), DV (Q3)	0.44	p < 0.05
3	SR (B1), DA30 (B1), TOA (B1)	0.36	p = 0.1346	3	GDD18 (Q1), WDV (Q3), DA30 (Q4)	0.75	p < 0.001
4	SR (B1), DA30 (B1), TOA (B1), TP/Etc (B2)	0.60	p < 0.05	4	GDD12 (Q1), DA30 (Q1), WDV (Q3), DA30 (Q4)	0.82	p < 0.001
6 Intervals				**8 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	TP (H3)	0.30	p < 0.05	1	DV (O6)	0.33	p < 0.05
2	DV (H1), TP (H3)	0.44	p < 0.05	2	GDD18 (O1), DV (O6)	0.56	p < 0.01
3	DV (H3), GDD21 (H5), DA30 (H6)	0.64	p < 0.01	3	GDD18 (O1), DV (O6), TP (O8)	0.65	p < 0.01
4	GDD21 (H2), DV (H2), GDD21 (H5), DA30 (H6)	0.75	p < 0.01	4	GDD18 (O1), Mean (O5), DA30 (O5), DV (O6)	0.79	p < 0.01
10 Intervals				**12 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	TP (D5)	0.43	p < 0.01	1	TP (T6)	0.44	p < 0.01
2	TP (D5), GDD24 (D10)	0.63	p < 0.01	2	TP (T6), GDD24 (T12)	0.68	p < 0.001
3	GDD21 (D5), TP (D5), GDD24 (D10)	0.79	p < 0.001	3	TP (T6), WDV (T9), GDD24 (T12)	0.80	p < 0.001
4	GDD21 (D5), TP (D5), DA30 (D7), GDD21 (D10)	0.85	p < 0.001	4	TP (T6), WDV (T9), GDD24 (T12), DA30 (T12)	0.86	p < 0.0001

DA30 = Days above 30 C, DV = Diurnal variation, Etc = Crop water demand, GDD = Growing degree days, Mean = Mean temperature, TOA = Top of atmosphere radiation, TP = Total precipitation, WDV = Weighted diurnal variation. The abbreviation code for the last interval when subdividing the growing season into 2, 4, 6, 8, 10 and 12 intervals is B2, Q4, H6, O8, D10 and T12, respectively.

4.5.3.4 Stepwise Regression Analysis of Gluten Strength Index

GSI or HMWG/SP is a protein parameter that is closely related to HMWG/FP, as the only difference is in the denominator; SP makes up the majority of FP and represented 73% of FP on average. Accordingly, there were similarities in the stepwise regression results between those for HMWG/FP and GSI. Stepwise regression analysis of GSI account for up to 92% of the variation ($p < 0.0001$) in the 4 variable model of the 12 interval analysis (Table 4.5.10). Similar to the results of HMWG/FP, high temperature-related weather variables occurring at the last interval of the growing season were negatively related to GSI. This is reflected in variables selected in regression models of the 4, 6, 8 and 10 interval analyses, i.e. DA30 at Q4, H6, O7 and O8 as well as GDD_{21} at D10 (Table 4.5.10). All other temperature related parameters included in the regression models were positively related to GSI. The positive association of these high temperature variables to GSI occurred at the beginning (DA30 at B1 and Q1; GDD_{18} at B1 and O2; Max at Q3), midway (GDD_{21} at H5, D5) and later (GDD_{21} at D9; DA70 at D7) periods of the growing season. Additionally, low temperature accumulation at T7 (GDD_{5-8}) was positively associated with GSI in all the regression models of the 12 interval analysis, with GDD_8 accounting for 43% of the variation ($p < 0.01$) as the best on variable model. SR did not have a clear pattern of association with GSI; SR at Q1 and TOA at T12 were positively related, whereas TOA at T11 was negatively related to GSI.

Precipitation-related parameters were all negatively related to GSI. TP at the midway point of the growing season (H3 and D5) was the best 1 variable model with R^2 values of 0.26 ($p < 0.05$) and 0.38 ($p < 0.05$) in the 6 and 10 interval analyses, respectively. TP was also negatively related to GSI at T11 in 2, 3 and 4 variable models of the 12 interval analysis.

Table 4.5.10 Stepwise regression analysis of gluten strength index (GSI) with weather variables compartmentalized into normalized calendar day intervals ranging from 2 – 12 intervals from planting to maturity. Variables in black and blue text denote positive and negative associations with GSI, respectively.

2 Intervals				**4 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	DA30 (B1)	0.22	p = 0.0685	1	WDV (Q3)MMM (Q3)	0.25	p = 0.0506
2	DA30 (B1), TP*Etc (B1)	0.27	p = 0.1340	2	Max (Q3), DA30 (Q4)	0.42	p < 0.05
3	GDD18 (B1), Min (B1), GDD5 (B2)	0.56	p < 0.05	3	DA30 (Q1), TP (Q3), DA30 (Q4)	0.69	p < 0.01
4	GDD18 (B1), DV (B1), GDD5 (B2), WDV (B1)	0.61	p < 0.05	4	SR (Q1), DA30 (Q1), TP (Q3), DA30 (Q4)	0.80	p < 0.001
6 Intervals				**8 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	TP (H3)	0.26	p < 0.05	1	DV (O6)	0.31	p < 0.05
2	TP (H3), GDD21 (H5)	0.41	p < 0.05	2	GDD18 (O2), DV (O6)	0.60	p < 0.01
3	DV (H3), GDD21 (H5), DA30 (H6)	0.65	p < 0.01	3	GDD18 (O2), DV (O6), DA30 (O8)	0.69	p < 0.01
4	CWD (H3), GDD21 (H5), DV (H5), DA30 (H6)	0.79	p < 0.01	4	GDD18 (O2), DV (O6), DA30 (O8), DA30 (O7)	0.79	p < 0.01
10 Intervals				**12 Intervals**			
Number of Variables in Model	Variables	R^2	Probability	Number of Variables in Model	Variables	R^2	Probability
1	TP (D5)	0.38	p < 0.05	1	GDD8 (T7)	0.43	p < 0.01
2	TP (D5), GDD21 (D9)	0.58	p < 0.01	2	GDD8 (T7), TP (T11)	0.58	p < 0.01
3	GDD21 (D5), TP (D5), GDD21 (D10)	0.76	p < 0.001	3	GDD8 (T7), TOA (T11), TP (T11)	0.70	p < 0.01
4	GDD21 (D5), TP (D5), DA30 (D7), GDD21 (D10)	0.84	p < 0.001	4	GDD5 (T7), TOA (T11), TP (T11), TOA (T12)	0.92	p < 0.0001

CWD = Crop water deficit, DA30 = Days above 30 C, DV = Diurnal variation, Etc = Crop water demand, GDD = Growing degree days, Max = Maximum temperature, SR = Solar radiation, TOA = Top of atmosphere radiation, TP = Total precipitation. The abbreviation code for the last interval when subdividing the growing season into 2, 4, 6, 8, 10 and 12 intervals is B2, Q4, H6, O8, D10 and T12, respectively.

4.5.3.5 Stepwise Regression of Work at Peak

As previously noted, WAP or mixograph work after peak was the best measure of gluten strength by dough mixing, having slightly better repeatability precision compared to (Appendix C) and having marginally higher correlations with GSI and HMWG/FP. Stepwise regression analysis results for WAP are presented in Table 4.5.11. A maximum R^2 of 0.92 ($p < 0.0001$) was achieved in the 4 variable regression model from the 12 interval analysis. This was similar to the maximum R^2 values obtained for GSI and HMWG/FP parameters which are closely related to WAP. Many lower temperature-related parameters at the beginning of the growing season were positively associated with WAP, as reflected in regression models of the 6, 8 and 10 interval analyses as GDD_8 (at H1 and D1), GDD_5 (H1 and D1), minimum temperature (O1) and GDD_{12} (D1). Similarly, GDD_8 and GDD_5 were positively associated with WAP at D6 and O7, respectively. Variable DA30 at B1, Q1 and D8 were all positively related to WAP, whereas during the last interval in the 12 interval analysis (T12) and O4 there was a negative association. Weather parameters related to daily temperature variability were included in many of the regression models for WAP. DV at H1 (6 interval analysis) was negatively related to WAP. However, DV (at O6 and T3) and WDV (at Q3, H5 and T9) were positively associated with WAP. DV at O6 and T3 alone accounted for 31% ($p < 0.05$) and 43% ($p < 0.01$) of the variation in WAP, respectively. SR was not included in any of the regression models for WAP. All precipitation-related parameters were negatively associated to WAP at approximately the midway point of the growing season (Etc at B1; TP at Q3, H3 and T6; TP*Etc at H3 and D7; TP/Etc at H3) in the 2, 4, 6, 10 and 12 interval analyses.

Table 4.5.11. Stepwise regression analysis of work at peak (WAP) with weather variables compartmentalized into normalized calendar day intervals ranging from 2 – 12 intervals from planting to maturity. Variables in black and blue text denote positive and negative associations with WAP, respectively.

2 Intervals				**4 Intervals**			
Number of Variables in Model	Variables	R2	Probability	Number of Variables in Model	Variables	R2	Probability
1	Etc (B2)	0.14	p = 0.1566	1	DA30 (Q1)	0.18	p = 0.1018
2	DA30 (B1), GDD5 (B2)	0.22	p = 0.2015	2	DA30 (Q1), TP (Q3)	0.47	p < 0.05
3	GDD12 (B2), GDD21 (B2), DA30 (B1)	0.42	p < 0.05	3	DA30 (Q1), TP (Q3), GDD12 (Q4)	0.79	p < 0.001
4	GDD24 (B2), GDD18 (B2), GDD21 (B2), DA30 (B1)	0.55	p < 0.05	4	DA30 (Q1), DA30 (Q2), Min (Q3), TP (Q3)	0.88	p < 0.0001
6 Intervals				**8 Intervals**			
Number of Variables in Model	Variables	R2	Probability	Number of Variables in Model	Variables	R2	Probability
1	TP*Etc (H3)	0.22	p = 0.0649	1	DV (O6)	0.31	p < 0.05
2	DV (H1), TP (H3)	0.40	p < 0.05	2	Min (O1), DV (O6)	0.61	p < 0.01
3	GDD8 (H1), DV (H1), TP (H3)	0.56	p < 0.05	3	Min (O1), DV (O6), GDD5 (O7)	0.80	p < 0.001
4	GDD5 (H1), DV (H1), TP/Etc (H3), WDV (H5)	0.74	p < 0.01	4	Min (O1), DA30 (O4), DV (O6), TP/Etc (O8)	0.92	p < 0.0001
10 Intervals				**12 Intervals**			
Number of Variables in Model	Variables	R2	Probability	Number of Variables in Model	Variables	R2	Probability
1	CWD (D4)	0.29	p < 0.05	1	DV (T3)	0.43	p < 0.01
2	GDD12 (D1), GDD8 (D6)	0.57	p < 0.01	2	DV (T3), DA30 (T12)	0.69	p < 0.001
3	GDD5 (D1), GDD8 (D6), DA30 (D8)	0.75	p < 0.001	3	WDV (T3), TP (T6), DA30 (T12)	0.85	p < 0.0001
4	GDD8 (D1), GDD5 (D6), TP*Etc (D7), DA30 (D8)	0.87	p < 0.0001	4	Max (T3), TP (T6), WDV (T9), DA30 (T12)	0.93	p < 0.0001

CWD = Crop water deficit, DA30 = Days above 30 C, DV = Diurnal variation, Etc = Crop water demand, GDD = Growing degree days, Max = Maximum temperature, Min = Minimum temperature, TP = Total precipitation, WDV = Weighted diurnal variation. The abbreviation code for the last interval when subdividing the growing season into 2, 4, 6, 8, 10 and 12 intervals is B2, Q4, H6, O8, D10 and T12, respectively.

5.0 GENERAL DISCUSSION

The goal of this thesis research was to develop a comprehensive understanding of the relative influence of genotype and environment on gluten strength which is the key property of wheat flour doughs that affects breadmaking quality in general. Gluten strength specifically determines dough mixing requirements, is closely related to the capacity of doughs to rise during fermentation, and is positively related to loaf volume. This study evaluated a spectrum of factors related to or potentially related to gluten strength, i.e. properties related to grain, flour, dough mixing and protein composition. A total of 24 properties or variables were assessed including 9 for grain, 4 for flour, 5 for dough and 6 for protein content and composition. The basic design of the study involved nine CWRS wheat genotypes grown in replicated field trials over two years, 2015 and 2016. There were four main objectives of the study: 1) characterize (by ANOVA) the proportions of variance contributed by genotype, growing location and interactions on gluten strength; 2) determine if there is a moderating effect of grade on gluten strength and its variation; 3) establish relationships between gluten strength assessed by dough mixing and protein content and composition; and 4) analyze the nature of location, i.e. weather effects on protein content and key gluten strength-related parameters using a novel protocol to normalize days to maturity which varied widely across location-years into constant calendar day intervals instead of using phenology based intervals which were not specifically established in this study.

Environmental conditions varied widely among locations and between years in this study. It is important to note that the “location effect” is actually an environment effect related mostly to weather and weather-related environmental factors that resulted in wheat being downgraded,

e.g. on account of Fusarium or other damage. As well, location x year interaction is commonly used to classify environmental effect in many studies (Budak et al. 2003; Caffe-Treml et al. 2011; Finlay et al. 2007; Mariani et al. 1995). It follows that the three-way interaction, i.e. genotype x location x year can also be classified as a genotype x environment interaction.

No acute weather was experienced over the two years of field studies, i.e. there was no hail damage, temperatures were on the whole relatively moderate during the growing seasons, and no frost damage was incurred. While precipitation varied considerably across growing locations, there was no significant precipitation during the pre-harvest period that could have resulted in significant sprout damage that could have affected gluten strength. Despite the relatively normal range of weather the growing sites experienced in 2015 and 2016, what was particularly striking in the results was the effect of the growing environment on protein content that ranged in samples from 9.7% to 17.1% over both years. Averaging genotypes within a location, the range of protein contents across locations was more moderate, but still substantial, i.e. 10.9% to 15.7%. These results are even more compelling when considering that all field locations were optimized in terms of fertility based on soil tests that were carried out typically in the fall. Accordingly, variation in protein content was due mainly or entirely to weather influences on crop development during the growing season at each location which also could include weather x soil interactions which could have affected nitrogen mobilization from soil to vegetative matter and eventually grain but these aspects were not studied. Previous reports have noted significant interactions between plant nitrogen assimilation with varying temperature (DuPont et al. 2006a; Kharel et al. 2011; Weidner and Ziemens 1975) and water (Kharel et al. 2011; Massoudifar et al. 2014) levels. Perhaps even more compelling was the clear observation that protein content was completely unrelated to gluten strength. This is discussed later below.

ANOVA indicated that location was highly significant for all parameters when evaluated within a single growing season. When years were combined, the significant effect of location or the growing environmental was captured in location by year interactions. This was not surprising, as much of the environmental effect was due to the variation in weather conditions that differed between years. Furthermore, for all measured parameters, there was little to no relationship between individual locations in 2015 and 2016. This was a surprising result, as it suggests that soil type which distinguishes many growing locations from each other is not a factor that affects wheat quality for breadmaking.

Although the location effect was no longer significant in ANOVA of combined years, it did contribute the majority of variance for most of the grain, milling, mixograph and protein composition parameters, and in particular, flour and grain protein content. Many studies have found variation of protein content to be affected by environment rather than genetic influences (Budak et al. 2003; Caffe-Treml et al. 2011; Dencic et al. 2011; Eagles et al. 2002; Fenn et al. 1994; Gomez-Becerra et al. 2010; Mariani et al. 1995; Panozzo and Eagles 2000; Peterson et al. 1998; Peterson et al. 1986; Rharrabti et al. 2001; Surma et al. 2012; Zhu and Khan 2001). In turn, grain or flour protein content is largely driven by environmental factors during the growing season such as temperature (DuPont et al. 2006a; Ferreira et al. 2012; Johansson et al. 2005; Koga et al. 2015; Zhang et al. 2013), precipitation (Flagella et al. 2010; Kharel et al. 2011; Li et al. 2013b; Saint Pierre et al. 2008; Smith and Gooding 1996) and soil nitrogen levels (DuPont et al. 2006a; Luo et al. 2000; Tea et al. 2004; Triboi et al. 2000). Interestingly, location was a predominant contributor to variance of most parameters unrelated to gluten strength including protein content and content of constituent fractions SP and HMWG. Conversely, genotype was the main contributor of variance for parameters related to gluten strength, i.e. mixograph MDT,

WIP, and WAP, as well as protein composition parameters SP/FP (inversely related to gluten strength), and HMWG/FP and GSI which were directly related. These results are similar to other studies reported in the literature that gluten strength is largely controlled by genotype but will vary from location-to-location (Bhatta et al. 2017; Eagles et al. 2002; Fenn et al. 1994; Panozzo and Eagles 2000; Peterson et al. 1998; Preston et al. 2001).

There have been instances in which genotype did not have the largest effect on variation in gluten strength related parameters. Surma et al. (2012) found environment to contribute more than genotype to overall variance for wet gluten content, Zeleney sedimentation value and Alveograph W parameter. This study involved four locations across Poland and two NPK fertilization levels. The result for wet gluten content is understandable as gluten content is closely related to protein content (Hussain et al. 1998). Zeleny sedimentation value and especially Alveograph W measure gluten strength. However, in this study the 24 genotypes analyzed were all advanced breeding lines of winter wheat cultivar Tonacja that varied in their grain hardness. On the other hand, Baker and Kosmolak (1977) who assessed composite samples of 20-30 hard red spring wheat lines grown in four locations across Western Canada over two years, reported that genotype-by-environment interaction was the most important determinant of mixograph development time and loaf volume. Thus, genotype alone was not the most influential factor. In the current study, the genotype by environment interaction (GxYxL) did not account for more than 9.2% of the variance in any parameter, although that interaction was significant ($p < 0.05$) for all parameters except GP, SP and SP/FP. It is important to note that when there is significant interaction between genotype and environment, the effects of each factor alone cannot be considered without the other.

A review by Williams et al. (2008) examined 100 studies across the globe that assessed the influence of genotype, environment and their interactions on the quality of bread wheats. A concern raised in this review is that conclusions from GxE studies are, in general, limited due to deficiencies of the experimental design (often as a result of cost limitations). The authors found that generally, studies examining the GxE influences on wheat quality did not involve field trials spanned over a two or more-year period, did not involve a range of locations to properly capture the environmental conditions within their geographical range, did not include a sufficient a number of wheat varieties as well as did not measure a large number of quality attributes. This study sufficiently addressed two of these concerns surrounding GxE studies identified by Williams et al. (2008). By including growing sites across all Prairie provinces (spanning a range of ~400,000 km^2), the samples were exposed to the naturally occurring variation in environmental conditions experienced in the Canadian Prairies over the two-year period. Additionally, as previously mentioned, 24 wheat quality variables were measured, although only genotype of CWRS genotypes were used.

A possible issue with the magnitude of the genotype effect in this study is the distinctly higher gluten strength of the cultivar Glenn which was reflected in results for many gluten strength related parameters, i.e. HMWG, HMWG/FP, GSI, MDT, WIP and WAP (refer to Tables 4.1.14 and 4.1.19). Consequently, the contribution of genotype to overall variance may have been over estimated by an uneven sample distribution. To evaluate this possibility, ANOVA was redone with Glenn removed from the sample set. This reduced the relative magnitude genotype contribution to total variance for gluten strength parameters by about 15-34%. Specifically, the contribution of genotype to total variance decreased from 56% to 35% and 52% to 21% for HMWG/FP and WAP, respectively. The reduction in variance contribution due to genotype was

accounted for by increases in variance contributed by location (~5-15%), environment (~2-8%), genotype by location interaction (~2-8%) and genotype by environment interaction (~0.5-2.5%). Despite the lower contribution of genotype to total variance with Glenn removed from the analysis, genotype remained a significant contributor to total variance.

Table 5.1 provides a compilation of gluten strength rankings from ANOVA with and without years combined for several gluten strength related parameters, i.e. mixograph MDT, WIP and WAP and GSI and HMWG/FP. The result for the mixograph parameters indicate consistently the relative weakness of Harvest regardless of the growing season. Using the WAP parameter as arguably the most accurate measure of gluten strength using the mixograph test, and results from combined growing seasons 2015 and 2016 as the most accurate measure of genotype variation (due to greater number of site years and sampling of environmental effects) only Harvest was significantly lower in gluten strength compared with the variety Carberry, which in turn was not significantly different from the six remaining genotypes in the study. The comparison with Carberry is important and relevant as the gluten strength of Carberry is considered the minimum threshold for support of CWRS bread wheat lines in wheat registration trials. In those trials, the extensigraph Rmax parameter is used to assess gluten strength. Extensigraph Rmax and mixograph WIP and WAP are closely related parameters of gluten strength based on ongoing studies in our laboratory (H. Sapirstein, personal communication). Table 5.1 further shows that the ranking of gluten strength based on only one year's worth of trial data, despite the large number of growing locations used in the study, is different for genotypes of intermediate levels of strength, and for those genotypes the ranking varies between years. For example, using parameter WAP, in 2015, genotypes Stettler, Brandon and Unity were significantly different from Carberry and Harvest, while Utmost, Cardale and Stanley were not

different than Carberry in gluten strength. In 2016, a crop year which resulted in lower grades on average due to suboptimal weather conditions compared with 2015, Cardale, Brandon, Utmost, Unity, Stettler and Stanley were significantly different from Carberry and Harvest. Accordingly, the ranking of gluten strength of a given set of genotypes can vary from year to year, and decisions about whether one genotype is weaker or stronger than another requires careful consideration of all available data based ideally on results obtained in multi-year trials and appropriate number of growing locations. On this point, the Canadian Grain Commission recently delisted the varieties Unity and Harvest, and other cultivars, from the CWRS wheat class (effective August 1, 2018) in accordance with a CGC directive which began in 2016 to modernize Canada's wheat classes in order to maintain Canada's reputation as a consistent source of high quality milling wheat.

Table 5.1. Genotype mean rankings for gluten strength parameters in 2015, 2016 and combined years.

2015	MDT (min)		WIP (%Torque*min)		WAP (%Torque*min)		GSI		HMWG/FP	
	GL	3.67^{A}	GL	173.6^{A}	GL	107.1^{A}	GL	0.421^{A}	GL	29.1^{A}
	SN	3.14^{B}	CB	135.7^{B}	CB	83.4^{B}	CD	0.394^{B}	CB	27.7^{B}
	CB	3.08^{B}	SN	131.5^{B}	UT	82.1^{B}	CB	0.350^{C}	UT	25.6^{C}
	CD	2.86^{C}	CD	130.1^{B}	CD	80.2^{B}	UT	0.343^{C}	CD	25.2^{CD}
	UN	2.80^{C}	BR	126.3^{BC}	SN	79.1^{B}	BR	0.334^{D}	BR	25.0^{DE}
	BR	2.75^{C}	UT	126.2^{BC}	ST	73.2^{C}	SN	0.333^{D}	HA	24.4^{EF}
	UT	2.74^{C}	UN	119.5^{C}	BR	73.0^{C}	HA	0.333^{D}	SN	24.2^{F}
	ST	2.51^{D}	ST	119.0^{C}	UN	71.3^{C}	UN	0.327^{D}	UN	24.1^{F}
	HA	2.42^{D}	HA	106.7^{D}	HA	64.5^{D}	ST	0.315^{E}	ST	23.9^{F}
2016	MDT (min)		WIP (%Torque*min)		WAP (%Torque*min)		GSI		HMWG/FP	
	GL	3.48^{A}	GL	176.1^{A}	GL	102.4^{A}	GL	0.415^{A}	GL	28.1^{A}
	CB	2.85^{B}	CB	131.0^{B}	CB	78.9^{B}	CD	0.380^{B}	CB	25.7^{B}
	BR	2.57^{C}	BR	125.9^{B}	CD	73.2^{C}	CB	0.343^{C}	CD	24.8^{C}
	CD	2.56^{C}	CD	121.8^{BC}	BR	71.9^{C}	BR	0.328^{D}	UT	24.2^{C}
	UN	2.51^{CD}	UT	112.9^{CD}	UT	70.5^{CD}	UT	0.328^{D}	BR	24.1^{C}
	SN	2.30^{DE}	UN	112.2^{CD}	UN	66.6^{DE}	HA	0.316^{DE}	UN	22.8^{D}
	UT	2.28^{E}	SN	108.9^{D}	ST	65.3^{DE}	UN	0.315^{E}	HA	22.7^{D}
	ST	2.17^{EF}	ST	107.0^{D}	SN	64.1^{EF}	SN	0.307^{EF}	ST	22.4^{D}
	HA	2.03^{F}	HA	94.1^{E}	HA	59.1^{F}	ST	0.300^{F}	SN	22.4^{D}
Combined Years	MDT (min)		WIP (%Torque*min)		WAP (%Torque*min)		GSI		HMWG/FP	
	GL	3.57^{A}	GL	174.9^{A}	GL	104.8^{A}	GL	0.418^{A}	GL	28.6^{A}
	CB	2.96^{B}	CB	133.3^{B}	CB	81.1^{B}	CD	0.387^{B}	CB	26.7^{B}
	SN	2.71^{BC}	BR	126.1^{B}	CD	76.7^{B}	CB	0.347^{C}	CD	25.0^{C}
	CD	2.71^{BC}	CD	125.9^{B}	UT	76.3^{B}	UT	0.336^{CD}	UT	24.9^{CD}
	BR	2.66^{BC}	SN	120.2^{BC}	BR	72.5^{BC}	BR	0.331^{CD}	BR	24.5^{CDE}
	UN	2.66^{BC}	UT	199.6^{BC}	SN	71.6^{BC}	HA	0.324^{DE}	HA	23.5^{CDE}
	UT	2.51^{BC}	UN	115.8^{BC}	ST	69.3^{BC}	UN	0.321^{DE}	UN	23.4^{DE}
	ST	2.34^{C}	ST	113.0^{BC}	UN	69.0^{BC}	SN	0.320^{DE}	SN	23.3^{E}
	HA	2.22^{C}	HA	100.4^{C}	HA	61.8^{C}	ST	0.308^{E}	ST	23.1^{E}

Means with different letter groupings are significantly ($p < 0.05$) different according to Tukey-Kramer method.
BR = Brandon, CB = Carberry, CD = Cardale, FP = Flour protein, GL = Glenn, GSI = Gluten strength index, HA = Harvest, HMWG = High molecular weight glutenin, MDT = Mixing development time, SN = Stanley, ST = Stettler, UN = Unity, UT = Utmost, WAP = Work at peak, WIP = Work input to peak

An important objective of the study was to determine the potential influence of grade on gluten strength. Previous research published by the CGC has documented the effect of individual grading factors on wheat quality (Dexter et al. 1996; Dexter and Edwards 1998; Dexter et al. 1997; Dexter et al. 1985; Dexter et al. 1987; Fu et al. 2014; Nightingale et al. 1999; Preston et al. 1991). Results have shown that moderate to severe levels of degrading factors (FDK, mildew, midge, frost damage, sprout) have implications on baking performance of wheat. It is unclear from these reports whether wheat of lower grade, e.g. CWRS No. 3 vs No. 1 or 2, has lower gluten strength. This observation may not be relevant in the present study, as the main degrading factor was FDK (refer to Chapter 4.1). In this regard, there are suggestions in the literature that wheat containing significant levels of FDK would be expected to have lower gluten strength due to reduction in glutenin content (Nightingale et al. 1999; Wang et al., 2005; Eggert et al. 2011). The extent of effects of FDK in levels encountered commercially indicate detrimental effects on milling quality (Dexter et al. 1996), although the effect on dough strength and breadmaking quality was moderate at best (Dexter et al. 1996; Hatcher et al. 2003) and depended on genotype (Dexter et al. 1996). In this study, only a mild negative relationship was found between FDK (or DON) and protein composition or mixograph measures of gluten strength, with correlations of r = -0.45 and -0.30 for HMWG/FP and WAP, respectively. This was more evident in 2016 samples than those grown in 2015, as the disease pressure was much higher in 2016.

The effect of Grade on all wheat quality related parameters was statistically negligible. ANOVA including Grade as a fixed effect was found to significantly affect only GSI ($p < 0.05$), although the contribution of grade to total variance was low for all parameters (< 1%). However, as the grade decreased from CWRS no.1 to below feed grade (Table 4.3.1), there was a decrease in the mean values of HMWG (-15.6%), HMWG/FP (-17.9%), GSI (-16.7%), MDT (-24.4%),

WIP (-22.6%) and WIP2 (-22.9%), although these mean values were not statistically different from one another. While these results were not statistically significant, they may be practically relevant as they indicate that the grading system is successfully functioning to distinguish wheat on the basis of end-use quality.

In addition to an analysis of all milled samples regardless of grade, the sample set was divided into two groups, one of milling grade sample including only CWRS no.1 and no.2 wheat and the other samples grading CWRS no.3 and below. ANOVA results of milling grade samples were very similar to results that included the entire sample set. However, compared to milling grade samples, gluten strength parameters (MDT, WIP, WAP, HMWG/FP and GSI) in the lower grade (CWRS no.3 and below) set had lower contribution to total variance for both genotype (by ~ 5 – 10%) and LxY interactions (~ 1 – 10%), whereas the contribution of location to the total variance of these parameters was increased (~ 5 -20%). This outcome likely reflects the influence of weather on degrading factors in wheat.

The third objective of the research was to establish relationships between gluten strength assessed by dough mixing and protein content and composition. To that end, the large number of genotype samples and site years provided an excellent basis to understand the extent to which gliadin and glutenin fractions of gluten protein varied due to genotype and the growing environment. In that regard, perhaps one of the most interesting and compelling results of the thesis research was the clear pattern of variation of gliadin protein (measured as soluble prolamins) and glutenin (measured as HMW glutenin, with LMW glutenin residing in the SP fraction). Results showed that both SP and HMWG fractions increased with protein content, but not at the same rate. Variation of flour protein content was closely related to variation of SP, displaying a strong linear relationship ($r = 0.95$). In contrast, HMWG, was more weakly

correlated to FP (r = 0.74) and above about 13.5% FP content, there was little to no increase in HMWG, i.e. HMWG or glutenin was not functioning as a storage protein fraction. It appears that this result has not been previously reported. On the other hand, the result confirmed that gliadin protein was the principal storage protein of wheat endosperm. The higher response of gliadin vs glutenin protein to variation in total FP has been reported in many studies (Blumenthal et al. 1993; Ciaffi et al. 1996a; Huebner et al. 1990; Labuschagne et al. 2009; Saint Pierre et al. 2008; Stone et al. 1997; Tea et al. 2004; Triboi et al. 2000). About half of these studies involved fertility experiments where flour protein content was manipulated based on increasing soil nitrogen content. In the present study, the nature of the wide range of protein content of genotype samples derived from variation in weather across the growing locations, and importantly field sites were optimally fertilized to eliminate this source of environmental variation. Accordingly, the observed variation of gliadin and glutenin fractions in this study, including the unusually constant level of protein in HMWG above about 13.5% total protein could have a different nature compared to studies where fertility was an experimental variable.

There have been reports of negative correlations between flour protein content and dough strength; correlations to mixograph peak time ranging from r= -0.22 (Zhu and Khan 2001), - 0.88 (Hou et al. 1996) and -0.98 (Stone et al. 1997). Wooding et al. (2000) found flour protein content to be negatively correlated (r = -0.67) with farinograph breakdown, with no relationship to MDT (r = 0.04). Marchetti et al. (2012) reported flours with the same protein content displayed different levels of dough strength (assessed by farinograph and alveograph), which they related to differences in gliadin/glutenin ratios and genotype protein profiles. Likewise, this study showed no relationship between FP content and mixograph measures of gluten strength and key protein composition parameters such as GSI and HMWG/FP. However, a negative relationship

was found between SP/FP (i.e. gliadin content per unit protein) and mixograph MDT (r = - 0.48), WIP (r= - 0.58), and WAP (r = -0.57). This result is consistent with studies wherein dough strength assessed by mixograph decreased upon incorporation of purified gliadin fractions to a base flour (Barak et al. 2014; Fido et al. 1997; Khatkar et al. 2002; Macritchie 1987; Uthayakumaran et al. 2001). It has been observed that gliadins exhibit a plasticizing effect on dough due to their weakening effect on glutenin polymers (Delcour et al. 2012) through the disruption of intermolecular bonds. Also, there have been reports citing a reduction in dough strength as a result of an increase in the ratio of gliadin to glutenin (Dhaka and Khatkar 2015; Uthayakumaran et al. 1999; Zhu and Khan 2001). In this study, as noted above there was a clear increase in the ratio of SP to HMWG as a function of increasing total flour protein content (refer to Fig 4.4.3). This result was consistent with the strong positive relationship of GSI (i.e. the inverse of SP-to-HMWG) to mixograph parameters MDT (r = 0.71), WIP (r = 0.83) and WAP (r = 0.86), and also between HMWG/FP (closely related to GSI; replaces SP with FP) and the same mixograph measures of gluten strength.

These close relationships between e.g. HMWG/FP and e.g. mixograph WAP derive from small differences in HMWG content over about a <2% protein range, i.e. the large difference in mixograph WAP values between e.g. genotypes Harvest (WAP = 62 %Torque*min) and Glenn (WAP = 105 %Torque*min) stem from what appears to be a small quantitative difference in content of HMWG protein (Harvest = 3.3% and Glenn = 4.0%, flour basis). In contrast, on a protein basis, the difference between Harvest (HMWG/FP = 23.5%) and Glenn (HMWG/FP = 28.6%) was larger. Dupuis et al. (1996) and Goesaert et al. (2005) likewise concluded that small differences in HMWG can result in large differences in gluten strength.

Reporting SP and HMWG content as a percentage of protein instead of a percentage of flour resulted in considerable increase in correlations with gluten strength parameters. SP/FP was inversely related to GSI (r = -0.78), MDT (r = -0.48), WIP (r = -0.58) and WAP (r = -0.57), whereas HMWG/FP was closely related to GSI (r = 0.97), MDT (r = 0.72), WIP (r = 0.83) and WAP (r = 0.87). These results are in line with many studies reporting HMWG to be positively related to dough/gluten strength (Barak et al. 2014; Ciaffi et al. 1996b; Deng et al. 2006; Edwards et al. 2004; Gupta et al. 1993; Kuktaite et al. 2004a; Kuktaite et al. 2004b; Macritchie 1987; Singh and MacRitchie 2001; Suchy et al. 2003; Veraverbeke and Delcour 2002).

The final objective of this thesis research was the most challenging, i.e. unravel the nature of the substantial effects of the growing environment, i.e. weather, on protein content and key indicators of gluten strength including GSI, HMWG/FP, mixograph WAP. For this study, a novel protocol was developed and implemented as reported in Chapter 4.5. Basically, the growing season interval analysis protocol normalizes the number of days to maturity across sites whose variation can be substantial, if not extreme, as was experienced, e.g. from 76 (Morden 2015) to 124 days (Beaverlodge 2016). This approach is very different from conventional studies, in which weather data is compartmentalized based on crop development stages. However, phenological data must be either observed, which is resource intensive, or modeled, which is less accurate. In previous research involving six spring wheat genotypes grown in nine locations across two years, Finlay (2006) attempted to assess the crop developmental stage that was best correlated to wheat quality. The stages that were used included all combinations of crop development stages from planting, jointing, inflorescence, anthesis, 50% anthesis, milk and soft dough. No individual crop stage yielded a relationship higher than R^2 0.58 for wheat quality parameters FP, SP, HMWG, GSI, MDT and WIP, that were assessed in that study. Although the

correlations between the quality parameters and crop stage were relatively strong, each quality parameter appeared to be most affected by different crop development stages in a random manner. It was concluded that no single development stage clearly impacted any given wheat quality parameter including gluten strength. Rather, it was a combination of weather variables throughout the entire crop development period that generated satisfactory regression outcomes. Results obtained in the present study were similar, as reflected in the tabulations of multivariate regression analysis results in Tables 4.5.4-11. Importantly, compared to results shown in Table 4.5.2 comprising simple correlations between protein and gluten strength parameters and weather variables reflecting their average or cumulative effects over the entire growing, when the latter is divided into intervals of increasing number, R^2 values typically increased for the best single variable model. For example, the highest correlation between mixograph WAP and any weather variable quantified over the entire growing season was for CWD ($r = -0.26$ or $r^2 = 0.07$, Table 2). In contrast, Table 4.5.11 shows single variable models for WAP with r^2 values trending upwards from $r^2 = 0.14$ (2 intervals) to $r^2 = 0.43$ (12 intervals).

Some interesting trends in the correlation and regression analysis results between specific weather variables and gluten strength parameters are discussed below. For almost all protein content-related dependent variables (GP, FP, SP, and HMWG) solar radiation (SR) and crop water demand (CWD) were inversely related, e.g. simple correlations between HMWG and SR and CWD were $r = -0.21$ and $r = 0.31$, respectively (Table 4.5.2). SR was selected as a significant weather parameter related to GP, FP, SP and HMWG for all of the interval analyses and throughout all stages of the growing season (B2; Q4; H3 and H5; O1 and O6; D2, D5, D6 and D10; T5), whereas CWD was only included in the 2 and 6 interval analyses at B1, B2 and H2 for the same dependent variables. In contrast, cloud cover (CC) was positively related to

protein (GP, FP, SP and HMWG) selected as a significant weather parameter in regression models mostly early in the growing season, i.e. B1, Q3, H1, O2 and T1 of the 2, 4, 6, 8 and 12 interval analyses, respectively. Negative correlations between crop water use and grain protein content have been previously reported (Johansson and Svensson 1998; Pan et al. 2006; Smith and Gooding 1996). The weak inverse relationship between cumulative daily solar radiation over the growing season and protein content (r = -0.28) could be due to the increased rate of photosynthesis that would occur with higher levels of light, which would in turn promote the synthesis of starch in the developing wheat grain resulting in with higher yields (Estrada-Campuzano et al. 2008; Wang et al. 2003) and also leading to more plump kernels with lower protein content. Li et al. (2010a) reported that experimental shading of winter wheat increased the transportation of dry matter from vegetative tissue to the kernel during grain filling. Another study by the same research group indicated that shading the crop after anthesis resulted in a significant decrease in wheat yield (Li et al. 2010b), which would likely lead to an increase in protein content as a result of the inverse relationship typically found between yield and protein content (Fowler 2003; Terman et al. 1969).

In this study, regression models for GP, FP and SP had negative relationships to warm temperatures ($GGD_{15\text{-}24}$) in the first interval of the growing season of 1-4 variable regression models (at B1, O1, D1 and T1). For example, for dependent variable FP (Table 4.5.5), best variables GDD_{18} (O1), GDD_{15} (D1) and GDD_{15} (T1) explained 40%, 50% and 49%, respectively, of variation. Very similar results were obtained for SP (Table 4.5.6) which is the predominant component of FP. These results are interesting, but hard to explain, i.e. why warm temperatures so early in the crop development period (soon after seedling emergence) are so strongly and negatively related with FP content. The result can be explained if warm temperatures following

emergence are directly related to grain yield, hence would be negatively related to FP. It is generally well known that increases in protein content occur with warmer temperature (Johansson et al. 2005; Koga et al. 2016a; Uhlen et al. 2015). However, the majority of studies assessing effects of temperature conditions on protein content focus on post-anthesis temperature conditions. Jarvis et al. (2008) suggested that both temperature and precipitation factors prior to anthesis may affect grain yield and protein quality of wheat due to effects on the nitrogen source/sink relationships. A greenhouse experiment growing 17 winter wheat cultivars in one year found that pre-anthesis water deficit resulted in a decrease in the nitrogen source/sink ratio (Triboi et al. 2006). Similarly, it has been reported that wheat yield potential may be altered by environmental factors as early as tillering stage (Acevedo et al. 2002).

For parameters more closely and positively related with gluten strength, results showed that WAP (Table 4.5.11), HMWG/FP (Table 4.5.9) and GSI (Table 4.5.10) were negatively associated with hot temperatures at the last interval of the growing season (i.e closest to maturity). For example, number of days above 30 ^{o}C (DA30) and $GDD_{21\text{-}24}$ at Q4, H6, O8 D10, T12 were included in the 2, 3 and 4 variable regression models of the 4-12 interval analyses. It is interesting that when these same higher temperature parameters ($GDD_{21\text{-}24}$, DA30) were included in regression models earlier in the growing season, they were positively associated with HMWG/FP, GSI. For example, the 10 interval analysis 3 variable model that accounted for 76% of the variation in GSI comprised of GDD_{21} (D5), TP (D5) and GDD_{21} (D10); where GDD_{21} at D5 and D10 was positively and negatively related to GSI, respectively. These results suggest that high temperatures may only be negatively related to gluten strength when the hot conditions are experienced closest to the plant maturity, i.e. maximum dry weight, otherwise, a positive relationship exists.

A possible explanation for the negative response of gluten strength to high temperatures in the last interval of the grain development period may be related to the accumulation pattern of gluten in wheat. Where both gliadin and glutenin synthesis begins around 10 DAA, glutenin synthesis is much slower, only increasing in rate once gliadin synthesis ceases about midway through the grain filling process (Huebner et al. 1990; Panozzo et al. 2001; Stone and Nicolas 1996). It has been reported that LMW polymeric glutenin begins to be synthesized first, followed and overlapped by HMW glutenin at roughly 28 DAA (Gupta et al. 1996; Zhu and Khan 1999). Therefore, the high temperatures during late stages of kernel development may interfere with the synthesis of HMWG glutenin polymers. High temperatures during the grain maturation period have been reported to inhibit the formation of large polymeric proteins (Ciaffi et al. 1996a; Johansson et al. 2013) which could be explained by the effect of heat on the enzyme disulfide isomerase (Every et al. 2003) that is responsible for the formation of disulfide bonds and large protein aggregation

Regression models for gluten strength parameters (HMWG, HMWG/FP, GSI, WIP2) also found an inverse relationship to precipitation. Total precipitation (TP) was included in the regression models for these parameters at both the beginning (H2, T3) and close to midway point (Q3, H3, D5, T6) of the growing season. TP at the midway point of the growing season was found to be the best 1 variable model for the 12 interval analysis for HMWG (at T3, $R^2 = 0.55$). Similarly, TP at the midway point of the growing season was found to be the best 1 variable model for the 6, 10 and 12 interval analyses for HMWG/FP (at H3, D5 and T6 with $R^2 = 0.30$, 0.43 and 0.44, respectively) and also the 6 and 10 interval analyses for dependent variable GSI (at H3 and D5 with $R^2 = 0.26$ and 0.38, respectively). The strong influence of TP especially for protein parameters involving glutenin is compelling, as few studies have evaluated the effect of

precipitation on gluten strength. Rather, much of the literature has focused on the effects of drought stress which is a very different environmental condition with specific effects to attenuate starch synthesis (Yi et al. 2014; Zhang et al. 2017b) thereby increasing protein concentration including the concentration of glutenin. Not surprisingly, drought conditions have been found to increase gluten strength (Flagella et al. 2010; Guzman et al. 2016; Kharel et al. 2011; Li et al. 2013a; b). Additionally, water deficit at heading has been reported to increase nitrogen mobilization to the kernel, that is accompanied by a decrease in starch production (Xu et al. 2006; Yang and Zhang 2006). Conversely, it might be expected that increasing levels of precipitation during crop development, and even prior to anthesis, could result in a decrease of gluten strength, possibly related to increased grain yield and reduced (glutenin) protein content. Xu et al. (2006) reported reduced levels of nitrogen translocation to the kernel as a result of excessive irrigation. Results from our research group stemming from an unrelated G x E study indicated that wheat grown under irrigation had significantly weaker dough mixing properties and lower concentration of HMWG compared to wheat grown without irrigation (Sapirstein, unpublished).

It is difficult to compare the stepwise regression results of this study to multivariate regression reports in the literature as the method used to analyze growing season weather data was novel and unique. Jarvis et al. (2008) evaluated results of a G x E study involving two CWRS genotypes grown in two years by numerous farmers over the entire Prairie region, developing similar models using multivariate regression to maximize R^2 values with weather data split into four groups (similar to the 4 interval analysis of this study) based on crop development stages. They reported three variable regression models with R^2 values of 0.49, 0.44, 0.46 and 0.51 for FP, SP HMWG and WIP, respectively. In the present study, three variable

regression models in the context of 4 growing season intervals yielded somewhat comparable results, but with considerably higher R^2 values of 0.59, 0.59, 0.62 and 0.79 for FP, SP HMWG and WAP, respectively. Finlay (2006) carried out a replicated field study similar to the present study which involved six bread wheat genotypes, four Prairie growing sites over two years. That study reported 1 and 3 variable regression models to predict wheat quality parameters by compiling weather data in 18 crop development stage combinations based on planting, jointing, inflorescence, anthesis, 50% anthesis, milk and soft dough crop development stages. The 3 variable models generated by Finlay (2006) for GP, FP, SP, HMWG and HMWG/SP produced R^2 values of 0.87, 0.86, 0.73, 0.89 and 0.29, respectively. These results are analogous to the three variable regression models in this study with the 10 interval analysis, which yielded R^2 of 0.88, 0.88, 0.86, 0.92 and 0.76 for variables GP, FP, SP, HMWG and GSI, respectively. The 3 variable model for GP using 10 intervals was similar to a 3 variable model reported by Mkhabela et al. (2018) who adapted results from the field study of Finlay et al . (2007) using agricultural drought indicators to predict GP with an $R^2 = 0.83$.

The use of multivariate linear regression to establish relationships between weather conditions and wheat quality is challenging, as weather parameters are often highly inter-correlated. Mkhabela et al. (2010) developed regression models to predict wheat yield and quality by using drought indices. This same data was later re-analyzed using partial least squares regression (PLS) which is able to account for multi-collinearity between weather parameters, in addition to the high number of predictor variables compared to the number of observations (Mkhabela et al. 2018). They reported that linear regression of drought indicators to predict wheat quality yielded similar results to those obtained by partial least squares analysis. This provides validation for the use of stepwise regression in the current study as a method to evaluate

the effects of weather during the growing season on gluten strength. The PLS approach can provide greater confidence in prediction models when parameters are collinear, however results of PLS regression are typically more difficult to interpret, as all independent weather parameters form part of the model. A PLS analysis of the growing season interval results obtained in this study could provide an additional enhancement in understanding the complex relationship between wheat gluten strength and growing season weather.

6.0 CONTRIBUTION TO KNOWLEDGE AND CONCLUSIONS

This thesis research project has considerably extended knowledge and understanding of the effects of genotype and the growing environment on the gluten strength of contemporary CWRS wheat varieties. In total, 648 wheat samples encompassing nine genotypes, grown in nine sites over two years, were harvested, cleaned, and graded. Half of these samples were milled to flour, and measures of gluten strength were derived from about 1000 mixograph tests and over 2000 determinations of gluten protein composition, including replications. The number and spatial distribution of field locations that were used, totalling 18 site years, enabled the capture of a broad and realistic range of weather influences that wheat crops generally exposed to in the Prairie region. Considering all these aspects taken together, the results of this project represent the best available knowledge to date of the relative effects of genotype and environment that contribute to variation in gluten strength of CWRS wheats grown in western Canada, with results applicable to other breadmaking wheat classes.

The project also accommodated the refinement and validation of methodology pertinent to measurement of gluten strength. This methodology included a streamlined and small-scale strain-gauge based mixograph procedure for measuring gluten strength without the need to

optimize water absorption, and an efficient and effective protocol to distinguish and quantify gluten protein fractions most closely related to dough strength.

That wheat or flour protein content was determined to be unimportant in relation to gluten strength of CWRS wheat was clearly shown, and possibly for the first time. This result underscores a significant deficiency in the valuation of CWRS wheat, i.e. farmers are not compensated for what is arguably the most important asset of the CWRS wheat brand which is a moderately high level gluten strength providing a good balance of dough properties. While a "driveway" test of gluten strength is currently not available, research towards this end should be vigorously pursued.

Results of this study also highlighted the extent to which the growing environment, i.e. weather, regardless of the physical location of the crop, influenced protein content which ranged from about 11 to 17% for any given genotype. Whether the weather effect was directly related to nitrogen mobilization from soil to plant vegetative tissue and eventually to grain, or indirectly due to the effect of weather on grain yield which is inversely related to grain protein content, is not known. This question merits further study.

A G x E analysis of gluten strength was the key focus of this research. In contrast to the heavy influence of weather on protein content, the relative effects of genotype and environment on gluten strength was much more variable depending on the growing season and the type of parameter being assessed, i.e. protein composition or mixograph WAP, as well as the complement of genotypes evaluated by ANOVA. By way of reminder, the sample set used for the study included one genotype, Glenn, with distinctly strong gluten strength as reflected in mixograph work parameters and gluten protein composition (high levels of HMW glutenin per unit protein). Regardless of the growing season or whether the variety Glenn was included or not

in ANOVA, protein composition parameters related to dough strength were always influenced more by genotype than by growing location. For example, the ratios of G/E variance for HMWG/FP in 2015 and 2016 were 2.5 and 2.7, respectively, and 1.2 in both years with Glenn removed. This was not the case for gluten strength assessed by mixograph work parameters. In this case, the ratio of G/E variance for WAP in 2015 and 2016 were 1.1 and 5.2, respectively and 0.65 and 1.6 with Glenn removed. The large difference in those ratios between crop years is noteworthy. Removing Glenn from the sample set resulted in a lowering of G/E variance, which is to be expected. The large effect of growing season on G/E variance of mixograph WAP (or WIP) is more difficult to explain, especially given the absence of a corresponding effect on either GSI or HMWG/FP, i.e. protein composition determinants of gluten strength by dough mixing. Careful examination of weather data between 2015 and 2016 indicates that total precipitation was the most variable weather parameter in 2015 (CV= 45%) and was considerably more variable than that in 2016 (CV = 27%). Furthermore, TP was more closely correlated to WAP (r = -0.33) compared to any other single weather parameter. Also, multivariate analysis results indicated that weather parameters most closely related to gluten strength (HMWG, HMWG/FP, GSI, WAP) were inversely related to precipitation beginning to midway through the growing season. It seems plausible therefore that the higher variability of precipitation in 2015 could be an important factor explaining the lower G/E, i.e. higher E/G variance in 2015 compared to 2016. What can be concluded from this result is that when environmental influence on gluten strength increases relative to genotype, which can occur in different growing seasons, other factors besides the ratio of glutenin to gliadin (i.e. GSI) and HMWG/FP can increase in importance. In this regard, quantification of the amount of LMW glutenin, which was not determined in this study, could be an important factor to evaluate in future research.

The novel weather interval analysis protocol that was implemented in this study deserves close attention in future research. This new approach to analyze weather data across Prairie locations typically varying widely in days to maturity requires only two pieces of temporal data, seeding date and date of crop maturity; and the latter can be determined directly or by modelling. Stepwise regression analysis based on this approach yielded statistical models for potential prediction of protein content and gluten strength at least equal to those reported in the literature based on subdividing the growing season using crop phenology dates whose determination is highly resource intensive and impractical for routine applications.

The final aspect of this thesis research that warrants attention or comment concerns accuracy in determining gluten strength of genotypes when the growing environment is so variable. The basis for this determination in the thesis research was a classical genotype by environment approach using replicated field trials across a large expanse of the Prairie region over two years. Once appropriate data was collected on gluten strength, ANOVA was applied that generated an overall ranking of genotypes. Results showed that with the exception of the two genotypes at opposite ends of strength in this study, i.e. Harvest and Glenn, ranking of genotypes was highly complicated by the variable nature of weather across field sites within and between growing seasons. As was discussed in Results, in 2015 (using mixograph parameter WAP) varieties Stettler, Brandon and Unity were significantly different from Carberry and Harvest, whereas Utmost, Cardale and Stanley were not different. In 2016, Cardale, Brandon, Utmost, Unity, Stettler and Stanley were significantly different from Carberry and Harvest. Accordingly, the varieties Stanley, Brandon and Unity in both years of this study were significantly lower in gluten strength than Carberry which is the current check variety in wheat registration trials establishing the floor of gluten strength for support of a bread wheat line. On this point, the

Canadian Grain Commission recently delisted the variety Unity as well as Harvest from the CWRS wheat class (effective August 1, 2018) to protect the CWRS wheat brand. The varieties Stanley and Brandon remained. It is difficult to reconcile results of this thesis project with decisions made by the CGC as the processes and data used are so very different. However, it can be argued that the formal G x E methodology used in the present study to evaluate gluten strength is superior to that used by the CGC which apparently relied on cooperative registration trial data for those decisions. Those data derive from composites of genotypes grown in several trial locations in each of three years for bread wheat lines that advance for registration support. Results of individual locations are not considered, and results as a whole are not amenable for ANOVA or any conventional statistical analysis. All this should be a concern to wheat breeders.

It is the observation, documentation and analysis of environmental effects on wheat quality and gluten strength which help to contribute a robust understanding of those important properties upon which much of Canada's wheat marketing depends. G x E study of wheat quality traits is an infrequent undertaking in western Canada. This thesis research has generated considerable new knowledge in this area, but there is a pressing need for more science going forward, as quality and marketing issues related to weather challenge the sustainability of CWRS wheat as a premium wheat class in the Prairie Region.

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7.0 APPENDIX

Appendix A.1. Sample code for SAS 9.4 software

A.1.1. Sample GLIMMIX procedure for individual year analysis of flour protein content

```
proc import datafile="C:\Users\courcelj\Documents\SP1 Datafiles\SP1 for Statistics.xlsx"
     out=sp1 dbms=xlsx replace;
     Sheet="2016";
run;

ods pdf file="C:\Users\courcelj\Documents\My SAS Files\glimmix_FP16.pdf" notoc nogtitle;

Proc GLIMMIX data=sp1 plots=residualpanel;
   Class Location Genotype Rep;
   model FP = Location|Genotype/ddfm=Satterthwaite dist=normal;
   random Rep;
   LSMeans Location|Genotype / pdiff adjust=tukey lines ilink;
  slice Location*Genotype / sliceby=Genotype diff adjust=tukey lines ilink;
   slice Location*Genotype / sliceby=Location diff adjust=tukey lines ilink;

   run;

ods pdf close;
```

A.1.2. Sample GLIMMIX procedure for combined year analysis of WAP

```
dm'log;clear;odsresult;clear;';


proc import datafile="C:\Users\courcelj\Documents\SP1 Datafiles\2016 SP1 for Statistics.xlsx"
     out=sp1 dbms=xlsx replace;
     Sheet="Both";
run;

ods pdf file="C:\Users\courcelj\Documents\My SAS Files\GenotypeMeansss.pdf" notoc nogtitle;

Proc GLIMMIX data=sp1;
   Class Genotype Rep Location Year;
   model WIP2 = Genotype/ddfm=Satterthwaite;
   random Location Location*Genotype Rep Year Year*Location Year*Genotype Genotype*location*Year;
   LSMeans Genotype / pdiff adjust=tukey lines ilink;

   run;
```

A.1.3 Sample MIXED procedure for combined year analysis of flour protein content

```
proc import datafile="C:\Users\courcelj\Documents\SP1 Datafiles\SP1 for Statistics.xlsx"
     out=sp1 dbms=xlsx replace;
     Sheet="Both Years";
run;

ods pdf file="C:\Users\courcelj\Documents\My SAS Files\mixed_FP1516.pdf" notoc nogtitle;

Proc Mixed data=sp1 method=type3;
   Class Location Genotype Rep Year;
   model FP = Genotype/ddfm=Satterthwaite dist=normal;
   random Rep Year Location Genotype*Location Year*Genotype Year*Location Location*Genotype*Year;

   run;

ods pdf close;
```

A.1.4 Sample MIXED procedure for individual year analysis of work at peak

```
Proc MIXED data=sp1 method=type3;
   Class Genotype Rep Location;
   model WIP2 = Genotype|Location/ddfm=Satterthwaite;
   random Rep;

   run;
```

A.1.5 Sample STEPWISE produced for 2 weather interval analysis of flour protein content

```
proc import datafile="C:\Users\courcelj\Documents\My SAS Files\Weather For Statistics.xlsx"
     out=weather dbms=xlsx replace;
     Sheet="2 Intervals";
run;

ods pdf file="C:\Users\courcelj\Documents\My SAS Files\Stepwise_FP_2Intervals.pdf" notoc nogtitle;

 proc stepwise data=weather;
Model FP = MIN_G1 MAX_G1 MEAN_G1 GDD5_G1 GDD8_G1 GDD12_G1 GDD15_G1  GDD18_G1 GDD21_G1 GDD24_G1 ED_G1
SR_G1 DA30_G1 MAXmMIN_G1 MAXxMIN_G1 MEANxMAXmMIN_G1 MEANxMAXxMIN_G1 ETC_G1  TP_G1 TPxETC_G1 TPdETC_G1
CWD_G1 CC_G1 TOA_G1 MIN_G2 MAX_G2 MEAN_G2 GDD5_G2 GDD8_G2 GDD12_G2 GDD15_G2 GDD18_G2 GDD21_G2 GDD24_G2
ED_G2 SR_G2 DA30_G2 MAXmMIN_G2 MAXxMIN_G2 MEANxMAXmMIN_G2 MEANxMAXxMIN_G2 ETC_G2 TP_G2 TPxETC_G2
TPdETC_G2 CWD_G2 CC_G2 TOA_G2
/ maxr sle=0.1 sls=0.1 stop=4;
   run;

   ods pdf close;
```

Appendix B.1. Averages and ranges by year for all quality parameters.

	2015			2016		
	Mean	Min	Max	Mean	Min	Max
Grain Quality						
Grade	1.93	1.00	4.00	2.6	1.0	5.0
FDK (%)	0.34	0.00	2.40	0.99	0.00	7.80
Midge Damage (%)	0.74	0.00	8.00	1.00	0.00	6.00
Sprout Damage (%)	0.01	0.00	0.76	0.34	0.00	3.50
Ergot Damage (%)	0.01	0.01	0.03	0.03	0.00	0.56
DON Level (ppm)	0.82	0.00	4.1	1.71	0.00	9.00
Grain Protein (%)	14.85	11.40	17.00	14.62	10.10	17.70
TKW (g)	33.40	27.23	43.28	32.24	26.17	41.11
Test Weight (kg/hL)	79.86	73.20	85.80	79.64	75.10	84.2
Milling Quality						
Flour Yield (%)	72.61	66.54	75.36	72.04	68.72	74.93
Shorts Content (%)	4.80	3.20	6.92	4.20	3.58	7.74
Bran Content (%)	21.69	19.69	26.28	21.98	19.50	24.20
Ash Content (%)	0.45	0.36	0.75	0.44	0.36	0.54
Protein Quality						
Flour Protein (%)	14.27	10.55	16.67	13.84	9.69	17.14
SP (%)	10.41	7.77	13.27	10.13	6.90	13.14
SP/FP (%)	72.93	65.78	79.63	73.25	65.90	80.49
HMWG (%)	3.62	2.65	4.54	3.40	2.22	4.53
HMWG/FP (%)	25.44	21.01	30.85	24.58	20.11	30.78
GSI	0.35	0.27	0.45	0.34	0.27	0.46
Mixograph Quality						
MDT (min)	2.88	1.77	5.64	2.53	1.53	4.59
PBW (%Torque)	27.98	18.35	35.79	28.99	20.60	38.79
PDR (%Torque)	54.84	34.32	69.10	55.76	38.77	72.44
WIP (%Torque*min)	129.80	79.16	195.17	121.27	70.20	211.88
WAP (%Torque*min)	79.19	48.73	136.39	72.50	48.57	122.39

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, TKW = Thousand Kernel Weight, WIP = Work Input to Peak, WAP = Work at peak

Appendix B.2 Averages and genotype differences for all quality parameters for combined years.

	BR	CB	CD	GL	HA	SN	ST	UN	UT
Grain Quality									
Grade[1]	2.31^{AB}	2.19^{AB}	2.25^{AB}	1.94^{B}	2.47^{AB}	2.36^{AB}	2.92^{A}	2.03^{B}	2.31^{AB}
FDK (%)	0.41^{AB}	0.45^{AB}	0.29^{B}	0.44^{AB}	0.87^{AB}	0.8^{AB}	1.4^{A}	0.51^{AB}	0.82^{AB}
DON (ppm)	0.82^{DC}	1.05^{BCD}	0.52^{D}	1.02^{BCD}	1.76^{ABC}	1.35^{BCD}	2.66^{A}	1.19^{BCD}	1.83^{AB}
Midge[2] (%)	1.29	1.08	1.47	0.98	0.54	0.72	0.96	0.35	0.5
Sprout[2] (%)	0.18	0.23	0.22	0.25	0.08	0.06	0.4	0.12	0.07
Ergot[2] (%)	0.03	0.04	0.05	0.01	0.04	0.04	0.01	0.04	0
GP (%)	14.72^{A}	14.86^{A}	14.88^{A}	14.71^{A}	14.72^{A}	14.6^{A}	15.33^{A}	14.25^{A}	14.56^{A}
TKW (g)	34.12^{A}	33.33^{ABC}	32.21^{BC}	32.56^{ABC}	33.54^{AB}	31.77^{C}	32.43^{BC}	32.62^{ABC}	32.78^{ABC}
TW (kg/hL)	80.32^{B}	80.38^{B}	78.51^{C}	82.83^{A}	79.08^{C}	78.4^{C}	79.02^{C}	80.57^{B}	78.62^{C}
Milling Quality									
FY (%)	72.96^{A}	71.64^{C}	72.51^{AB}	71^{C}	72.54^{AB}	73.29^{A}	71.76^{BC}	72.89^{A}	72.33^{AB}
BC (%)	21.47^{CD}	22.4^{AB}	21.68^{BCD}	22.47^{AB}	21.27^{D}	21.67^{BCD}	22.53^{A}	20.98^{D}	22.07^{ABC}
SC (%)	4.57^{DE}	4.97^{ABCD}	4.8^{BCD}	5.5^{A}	5.24^{AB}	4.02^{E}	4.8^{BCD}	5.2^{ABC}	4.62^{CD}
AC (%)	0.402^{AB}	0.397^{B}	0.436^{A}	0.393^{B}	0.435^{A}	0.397^{B}	0.430^{AB}	0.421^{AB}	0.402^{AB}
Protein Quality									
FP (%)	14.04^{A}	14.22^{A}	14.1^{A}	14.14^{A}	14.16^{A}	14.18^{A}	14.99^{A}	13.78^{A}	14.05^{A}
SP (%)	10.41^{AB}	9.79^{B}	10.16^{B}	9.68^{B}	10.27^{AB}	10.33^{AB}	11.28^{A}	10.06^{B}	10.43^{AB}
SP/FP (%)	74.2^{AB}	68.9^{C}	72.15^{B}	68.48^{C}	72.6^{B}	72.94^{AB}	75.25^{A}	73.08^{AB}	74.25^{AB}
HMWG (%)	3.44^{CD}	3.79^{B}	3.52^{C}	4.04^{A}	3.33^{CD}	3.3^{CD}	3.47^{C}	3.22^{D}	3.5^{C}
HMWG/FP (%)	24.52^{CDE}	26.67^{B}	24.99^{C}	28.58^{A}	23.53^{CDE}	23.3^{E}	23.15^{E}	23.44^{DE}	24.88^{CD}
GSI	0.331^{CD}	0.347^{C}	0.387^{B}	0.418^{A}	0.324^{DE}	0.320^{DE}	0.309^{E}	0.321^{DE}	0.336^{CD}
Mixing Quality									
MDT (min)	2.66^{BC}	2.96^{B}	2.71^{BC}	3.57^{A}	2.22^{C}	2.72^{BC}	2.34^{C}	2.66^{BC}	2.51^{BC}
PBW (%T)	27.6^{BC}	27.7^{BC}	28.65^{ABC}	29.63^{AB}	28.35^{ABC}	27.06^{C}	30.04^{AB}	26.42^{C}	30.78^{A}
PDR (%T)	56.03^{ABC}	53.37^{BC}	55.66^{ABC}	56.24^{ABC}	54.81^{ABC}	52.4^{C}	58.15^{AB}	51.4^{C}	59.04^{A}
WIP (%T*min)	126.08^{B}	133.33^{B}	125.95^{B}	174.88^{A}	100.4^{C}	120.15^{BC}	112.97^{BC}	115.84^{BC}	119.58^{BC}
WAP (%T*min)	72.45^{BC}	81.13^{B}	76.7^{B}	104.76^{A}	61.81^{C}	71.61^{BC}	69.26^{BC}	68.96^{BC}	76.3^{B}

Means within the same row with different letter groupings are statistically different from one another ($p < 0.05$) based on the Tukey-Kramer method.

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, TKW = Thousand Kernel Weight, WIP = Work Input to Peak, WAP = Work at peak

[1]Statistical analysis on grade is not valid due to its nominal nature, however is presented for its illustrative value

[2]Mean differences were not conducted on midge, sprout and ergot as the data was not normally distributed

Appendix C.1 Coefficient of variation and ranges of quality parameters for genotypes averaged across locations.

Appendix C.1.1 Coefficient of variation and ranges of quality parameters for 2015 genotypes averaged across locations

	Brandon			Carberry			Cardale		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	45.48	1.00	3.00	40.15	1.00	3.00	38.35	1.00	4.00
FDK (%)	170.54	0.00	1.00	153.46	0.00	1.20	236.21	0.00	2.00
DON (ppm)	102.41	0.00	1.40	132.58	0.00	1.30	158.46	0.00	1.00
Midge (%)	196.56	0.00	8.00	150.65	0.10	5.10	132.21	0.00	6.60
Sprout (%)	282.29	0.00	0.10	.	0.00	0.00	412.31	0.00	0.10
Ergot (%)	.	.	.	.	0.03	0.03	.	.	.
GP (%)	7.64	12.10	16.4	7.11	12.90	16.80	8.62	12.40	16.90
TW (kg/hL)	3.20	75.70	83.8	2.95	76.90	84.20	3.97	73.20	82.40
TKW (g)	11.28	28.60	43.28	10.39	28.67	40.73	10.75	27.68	39.50
Milling Quality									
FY (%)	1.38	70.98	75.02	1.78	69.62	73.84	1.73	69.75	74.54
BC (%)	5.01	19.74	23.74	4.63	20.93	24.21	5.66	19.62	24.16
SC (%)	11.84	3.91	5.63	12.41	4.14	6.35	13.54	3.68	6.37
AC (%)	9.39	0.38	0.50	9.12	0.36	0.50	15.71	0.41	0.75
Protein Quality									
FP (%)	8.63	11.30	15.82	8.23	11.81	16.04	9.12	11.60	16.30
SP (%)	8.95	8.36	11.92	8.35	8.45	11.52	8.72	8.46	11.55
SP/FP (%)	1.71	72.39	76.90	2.40	67.67	73.28	2.08	68.98	74.23
IG (%)	8.84	2.79	4.05	7.88	3.24	4.45	7.78	3.01	3.95
IG/FP (%)	5.10	23.05	27.53	6.10	23.69	30.10	5.54	22.49	27.77
GSI	5.67	0.31	0.38	5.64	0.32	0.39	7.19	0.34	0.45
Milling Quality									
MDT (min)	23.14	2.01	4.06	19.64	2.20	4.11	26.13	1.90	4.42
PBW (%T)	9.93	20.63	31.35	9.80	21.63	32.09	8.48	22.22	31.5
PDR (%T)	9.64	42.72	64.89	9.55	43.03	62.58	9.72	40.28	61.46
WIP (%T*min)	17.34	95.10	175.39	15.14	99.22	180.91	19.89	91.23	164.77
WAP (%T*min)	15.81	56.96	97.60	15.21	60.06	106.28	20.5	55.57	107.39

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix C.1.2 Coefficient of variation and ranges of quality parameters for 2015 genotypes averaged across locations

	Glenn			Harvest			Stanley		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	47.99	1.00	3.00	32.87	1.00	3.00	29.70	1.00	3.00
FDK (%)	125.65	0.00	1.00	121.31	0.00	1.20	132.58	0.00	1.50
DON (ppm)	149.34	0.00	1.50	83.63	0.00	1.90	93.54	0.00	1.70
Midge (%)	176.00	0.00	6.00	170.21	0.00	2.40	170.75	0.00	4.40
Sprout (%)	273.25	0.00	0.10	.	0.00	0.00	412.31	0.00	0.20
Ergot (%)	.	0.01	0.01	.	0.01	0.01	.	.	.
GP (%)	7.43	12.20	16.20	10.24	11.60	16.40	8.86	11.80	16.00
TW (kg/hL)	2.26	79.70	85.80	3.15	76.10	84.40	3.32	73.60	82.20
TKW (g)	8.99	29.00	37.78	8.17	29.57	40.80	8.72	27.66	38.82
Milling Quality									
FY (%)	1.12	70.23	72.58	1.47	71.31	75.11	1.59	71.34	75.36
BC (%)	2.88	21.11	23.26	4.66	19.50	22.72	4.29	20.30	23.35
SC (%)	8.69	4.63	6.16	13.24	4.31	6.92	13.57	3.20	5.05
AC (%)	14.20	0.37	0.65	13.03	0.37	0.57	12.03	0.36	0.51
Protein Quality									
FP (%)	8.54	11.37	15.86	11.41	10.75	16.03	9.67	10.98	15.55
SP (%)	8.72	7.77	11.12	11.14	7.97	11.80	9.97	7.77	11.43
SP/FP (%)	2.26	65.78	71.71	2.16	70.98	76.27	2.65	69.31	76.25
IG (%)	8.57	3.26	4.53	10.15	2.76	3.88	8.10	2.75	3.74
IG/FP (%)	4.46	26.01	30.85	6.71	21.01	26.99	4.14	22.55	25.91
GSI	5.37	0.36	0.45	6.81	0.28	0.36	5.39	0.30	0.36
Milling Quality									
MDT (min)	15.80	2.63	4.79	19.92	1.77	3.53	26.90	2.12	5.45
PBW (%T)	11.63	21.91	35.57	12.43	18.45	32.62	11.75	18.98	32.41
PDR (%T)	11.79	42.26	69.10	11.63	38.06	64.55	11.97	35.60	59.34
WIP (%T*min)	9.70	129.38	195.17	14.01	79.16	127.33	16.03	87.64	173.41
WAP (%T*min)	13.23	72.86	136.39	12.74	48.73	79.51	15.93	54.20	103.46

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix C.1.3 Coefficient of variation and ranges of quality parameters for 2015 genotypes averaged across locations

	Stetter			Unity			Utmost		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	46.49	1.00	4.00	33.36	1.00	3.00	42.01	1.00	4.00
FDK (%)	121.11	0.00	2.40	148.50	0.00	1.60	145.79	0.00	2.00
DON (ppm)	85.46	0.00	4.10	103.04	0.00	2.00	97.02	0.00	3.60
Midge (%)	124.04	0.10	3.50	88.35	0.00	0.60	143.96	0.00	1.80
Sprout (%)	304.10	0.00	0.76	.	0.00	0.00	.	0.00	0.00
Ergot (%)	.	.	.	.	0.02	0.02	.	.	.
GP (%)	9.44	12.00	17.00	9.88	11.70	16.30	9.31	11.40	16.00
TW (kg/hL)	3.89	75.50	84.00	2.84	76.70	84.00	3.39	75.50	83.20
TKW (g)	10.57	27.23	38.90	9.56	28.50	39.13	10.43	27.99	37.90
Milling Quality									
FY (%)	1.58	69.38	74.00	2.54	66.54	75.36	2.02	67.39	74.16
BC (%)	4.82	21.05	24.68	7.20	19.29	26.14	5.86	20.78	26.28
SC (%)	12.06	3.74	5.70	12.26	4.18	6.15	9.44	3.94	5.46
AC (%)	12.94	0.39	0.55	9.35	0.38	0.53	10.49	0.37	0.51
Protein Quality									
FP (%)	10.27	11.42	16.67	10.94	10.88	15.86	10.32	10.55	15.32
SP (%)	11.35	8.59	13.27	11.40	7.79	11.82	10.77	8.05	12.03
SP/FP (%)	2.69	73.26	79.63	3.04	69.48	78.06	2.30	71.49	78.68
IG (%)	10.65	2.82	4.15	8.26	2.78	3.68	11.49	2.65	4.18
IG/FP (%)	5.71	21.35	25.94	5.13	22.28	26.17	4.41	23.05	27.32
GSI	6.38	0.27	0.34	6.63	0.30	0.37	4.83	0.31	0.37
Milling Quality									
MDT (min)	22.75	1.92	4.00	25.90	1.88	4.52	16.29	2.20	3.92
PBW (%T)	12.10	20.93	34.36	13.36	18.73	31.79	13.98	20.55	35.79
PDR (%T)	11.92	40.02	67.61	12.25	36.87	62.30	11.55	41.71	65.80
WIP (%T*min)	14.83	87.77	150.98	17.25	85.56	150.63	11.41	98.31	148.51
WAP (%T*min)	14.00	53.25	87.85	16.42	54.12	91.80	13.66	59.51	102.11

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix C.1.4 Coefficient of variation and ranges of quality parameters for 2016 genotypes averaged across locations

	Brandon			Carberry			Cardale		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	44.07	1.00	5.00	46.02	1.00	5.00	61.74	1.00	5.00
FDK (%)	108.73	0.00	2.40	117.82	0.00	3.20	81.73	0.00	1.00
DON (ppm)	124.19	0.00	3.60	127.11	0.00	5.70	136.04	0.00	3.20
Midge (%)	106.86	0.00	5.00	116.72	0.10	4.80	103.25	0.10	6.00
Sprout (%)	98.09	0.00	1.40	206.63	0.00	3.50	161.05	0.00	2.60
Ergot (%)	159.20	0.00	0.17	124.07	0.00	0.18	296.60	0.00	0.56
GP (%)	9.38	11.60	16.10	9.76	11.20	16.30	11.04	11.40	16.90
TW (kg/hL)	1.22	78.70	82.10	1.24	77.80	81.80	1.66	75.70	80.70
TKW (g)	8.91	29.67	41.11	11.04	27.17	39.44	9.54	27.22	38.22
Milling Quality									
FY (%)	1.11	71.27	74.33	1.05	69.77	72.38	0.92	71.36	73.40
BC (%)	2.97	20.30	22.63	2.66	21.72	23.82	2.92	20.76	22.89
SC (%)	11.40	3.81	5.41	12.44	4.08	6.50	12.74	4.16	6.68
AC (%)	4.21	0.34	0.39	4.74	0.34	0.40	4.37	0.35	0.42
Protein Quality									
FP (%)	10.12	10.93	15.53	11.74	10.76	16.25	10.54	10.27	15.54
SP (%)	9.60	8.10	11.41	10.63	7.02	10.92	10.79	7.50	11.17
SP/FP (%)	3.01	70.38	77.39	5.88	62.23	78.97	5.32	62.04	78.94
IG (%)	10.95	2.56	3.74	12.57	2.65	4.12	11.53	2.51	3.78
IG/FP (%)	4.78	21.63	26.12	7.12	22.52	28.90	5.54	20.80	26.48
GSI	5.58	0.30	0.36	4.87	0.32	0.37	7.03	0.34	0.42
Milling Quality									
MDT (min)	12.50	2.06	3.08	13.31	2.25	3.80	11.31	2.18	2.99
PBW (%T)	9.59	23.41	33.93	9.40	23.80	35.75	11.66	22.59	35.40
PDR (%T)	9.46	47.62	67.19	9.65	43.64	63.89	10.93	43.59	65.35
WIP (%T*min)	12.04	103.33	154.51	11.64	109.78	160.83	9.74	101.88	147.70
WAP (%T*min)	10.37	61.26	85.39	10.35	65.40	93.17	9.75	61.99	90.08

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix C.1.5 Coefficient of variation and ranges of quality parameters for 2016 genotypes averaged across locations

	Glenn			Harvest			Stanley		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	55.41	1.00	4.00	45.73	1.00	5.00	41.42	2.00	5.00
FDK (%)	156.73	0.00	3.20	161.93	0.00	7.60	148.98	0.00	6.20
DON (ppm)	139.53	0.00	6.90	114.53	0.00	7.40	126.16	0.00	6.80
Midge (%)	131.64	0.10	4.00	111.41	0.10	2.80	97.80	0.20	3.20
Sprout (%)	165.50	0.00	2.50	198.36	0.00	1.00	130.56	0.00	0.60
Ergot (%)	115.52	0.00	0.05	116.17	0.00	0.17	174.87	0.00	0.22
GP (%)	11.11	10.60	15.90	11.99	10.70	16.30	11.91	10.10	16.30
TW (kg/hL)	1.33	80.40	84.20	2.01	76.50	81.60	1.96	76.30	81.40
TKW (g)	9.44	28.44	38.67	8.77	28.50	38.50	10.77	26.17	37.28
Milling Quality									
FY (%)	1.32	68.71	71.90	1.31	70.12	73.44	1.59	71.28	74.93
BC (%)	3.37	21.47	24.20	3.47	20.39	22.62	5.20	19.50	23.22
SC (%)	12.90	4.78	7.38	13.62	4.77	7.74	12.46	3.58	5.22
AC (%)	5.04	0.31	0.37	7.89	0.36	0.45	7.35	0.31	0.41
Protein Quality									
FP (%)	11.93	10.00	15.30	14.94	9.91	16.07	11.52	10.63	15.87
SP (%)	12.10	6.90	10.49	14.43	7.35	11.81	10.66	7.84	11.75
SP/FP (%)	2.81	64.15	70.59	3.05	68.15	76.26	2.59	69.55	76.35
IG (%)	12.79	2.80	4.53	16.95	2.26	3.88	11.92	2.49	3.66
IG/FP (%)	5.42	24.59	30.22	6.56	19.75	24.72	5.00	20.18	24.30
GSI	7.22	0.37	0.46	7.78	0.27	0.36	5.50	0.28	0.35
Milling Quality									
MDT (min)	16.17	2.65	4.59	13.72	1.53	2.78	15.66	1.88	3.36
PBW (%T)	9.86	22.72	35.50	12.74	20.99	36.39	11.49	20.60	33.61
PDR (%T)	12.06	43.45	69.94	13.75	41.85	67.20	11.23	41.78	65.74
WIP (%T*min)	13.79	133.61	211.88	14.67	70.20	115.70	12.89	94.55	139.74
WAP (%T*min)	12.72	81.46	122.39	12.13	48.57	71.63	8.91	56.00	74.92

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix C.1.6 Coefficient of variation and ranges of quality parameters for 2016 genotypes averaged across locations

	Stetter			Unity			Utmost		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	36.99	1.00	5.00	65.74	1.00	5.00	49.39	1.00	5.00
FDK (%)	126.01	0.01	7.80	181.51	0.00	4.40	133.75	0.00	5.20
DON (ppm)	102.58	0.00	9.00	125.53	0.00	6.00	108.38	0.00	7.70
Midge (%)	82.94	0.00	4.20	208.27	0.00	4.80	88.28	0.00	2.70
Sprout (%)	165.79	0.00	3.50	257.23	0.00	2.50	254.89	0.00	1.60
Ergot (%)	151.71	0.00	0.05	159.11	0.00	0.20	149.72	0.00	0.02
GP (%)	11.71	11.50	17.70	12.54	10.40	15.70	10.57	11.00	16.00
TW (kg/hL)	1.57	76.10	80.30	1.60	77.90	82.40	1.93	75.10	81.10
TKW (g)	9.15	26.17	37.00	8.66	27.83	37.28	9.01	27.00	37.89
Milling Quality									
FY (%)	1.24	69.45	72.70	1.13	71.02	73.87	1.45	69.72	73.59
BC (%)	3.25	21.56	23.99	2.96	19.99	22.26	3.54	21.00	23.82
SC (%)	12.05	4.03	5.75	10.81	4.45	6.06	12.59	3.82	5.83
AC (%)	7.99	0.36	0.46	6.07	0.35	0.42	7.57	0.33	0.42
Protein Quality									
FP (%)	12.74	10.88	17.53	13.42	9.85	15.09	11.88	10.08	15.62
SP (%)	12.22	8.16	13.14	11.67	7.64	11.13	11.17	7.79	11.57
SP/FP (%)	2.54	72.08	78.57	3.46	69.08	77.93	3.15	70.33	79.11
IG (%)	14.27	2.44	3.94	13.97	2.22	3.52	13.63	2.40	3.94
IG/FP (%)	5.90	20.08	24.65	5.16	20.57	24.73	6.63	20.87	27.14
GSI	6.07	0.27	0.33	6.11	0.29	0.36	7.99	0.29	0.38
Milling Quality									
MDT (min)	9.53	1.89	2.65	10.07	2.16	3.02	13.14	1.70	2.78
PBW (%T)	11.27	22.17	33.92	11.90	21.02	32.70	13.29	22.26	38.79
PDR (%T)	12.13	44.06	68.16	13.94	38.77	62.29	13.29	42.29	72.44
WIP (%T*min)	6.91	86.04	119.92	9.73	89.36	129.36	11.93	88.57	133.00
WAP (%T*min)	8.54	52.58	74.57	9.08	52.34	76.78	13.68	52.94	83.50

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix C.2 Coefficient of variation and ranges of quality parameters for locations averaged across genotypes.

Appendix C.2.1 Coefficient of variation and ranges of quality parameters for 2015 locations averaged across genotypes

	Beaverlodge			Brandon			Edmonton		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	32.64	1.00	4.00	25.44	2.00	4.00	24.06	1.00	2.00
FDK (%)	288.36	0.00	2.00	80.51	0.10	2.40	291.04	0.00	0.05
DON (ppm)	.	0.00	0.00	115.19	0.00	3.70	.	.	.
Midge (%)	92.59	0.00	0.30	91.67	0.10	2.40	58.50	0.00	1.20
Sprout (%)	424.26	0.00	0.10	230.09	0.00	0.10	.	0.00	0.00
Ergot (%)	.	.	.	75.67	0.01	0.03	.	.	.
GP (%)	3.89	13.00	14.50	2.69	15.30	16.90	2.68	15.00	16.60
TW (kg/hL)	2.90	73.20	83.90	1.70	77.90	82.60	1.40	80.50	84.80
TKW (g)	5.91	28.22	36.32	4.54	30.45	36.07	5.36	34.72	43.28
Milling Quality									
FY (%)	1.34	71.34	75.11	1.48	69.62	73.72	1.41	71.84	75.36
BC (%)	4.14	19.50	23.26	3.13	20.72	23.10	3.76	19.29	21.86
SC (%)	10.48	3.23	5.09	14.23	3.95	6.35	11.08	3.52	5.43
AC (%)	8.74	0.36	0.48	6.50	0.40	0.50	3.77	0.40	0.45
Protein Quality									
FP (%)	4.25	12.27	13.98	2.53	14.93	16.41	3.48	14.64	16.67
SP (%)	5.72	8.82	10.48	4.90	10.25	12.59	5.96	10.78	13.27
SP/FP (%)	3.69	66.69	76.26	3.79	65.78	76.69	3.93	68.70	79.63
IG (%)	9.63	3.02	4.26	7.75	3.46	4.39	8.96	3.44	4.53
IG/FP (%)	6.82	24.63	30.85	7.74	21.78	28.30	7.46	22.85	28.91
GSI	10.04	0.33	0.45	10.93	0.30	0.43	9.92	0.30	0.42
Milling Quality									
MDT (min)	15.00	2.44	4.22	15.99	1.99	3.32	13.55	1.97	3.11
PBW (%T)	8.26	26.52	35.79	8.07	24.93	33.25	5.40	29.70	35.57
PDR (%T)	7.09	49.73	62.48	6.24	47.56	61.18	5.28	56.55	69.10
WIP (%T*min)	16.99	114.42	195.17	16.61	94.85	168.36	16.21	98.59	167.63
WAP (%T*min)	17.70	74.58	136.39	17.04	54.12	98.83	17.30	61.87	108.63

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix C.2.2 Coefficient of variation and ranges of quality parameters for 2015 locations averaged across genotypes

	Goodale			Indian Head			Kelburn		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	29.10	1.00	2.00	15.40	2.00	3.00	38.85	1.00	3.00
FDK (%)	150.57	0.00	0.15	52.33	0.10	1.70	109.28	0.00	1.40
DON (ppm)	.	0.00	0.00	66.49	0.00	4.10	91.29	0.00	1.70
Midge (%)	127.33	0.00	0.30	67.61	0.30	8.00	32.87	0.10	0.40
Sprout (%)	424.26	0.00	0.10	424.26	0.00	0.20	.	0.00	0.00
Ergot (%)	.	.	.	.	.	.	.	.	.
GP (%)	2.43	15.00	16.30	6.55	13.40	16.70	2.79	15.40	17.00
TW (kg/hL)	1.49	80.70	85.00	1.68	76.10	80.00	2.51	73.70	81.00
TKW (g)	2.46	34.68	38.05	4.91	30.33	35.07	4.18	27.23	31.98
Milling Quality									
FY (%)	1.19	71.74	74.88	1.51	70.23	74.23	2.10	66.54	72.00
BC (%)	2.84	19.74	21.52	3.99	20.39	23.19	5.05	22.21	26.28
SC (%)	14.64	3.86	6.92	13.56	3.53	5.81	11.32	4.06	6.09
AC (%)	6.14	0.42	0.50	5.55	0.46	0.55	14.39	0.46	0.75
Protein Quality									
FP (%)	3.51	14.04	15.85	7.12	12.67	16.30	3.27	14.51	16.41
SP (%)	6.92	9.70	12.37	6.06	9.47	11.59	5.58	9.96	12.21
SP/FP (%)	3.95	68.42	78.56	3.35	70.17	78.06	3.46	67.73	76.39
IG (%)	5.41	3.54	4.29	9.88	2.98	3.90	7.33	3.58	4.47
IG/FP (%)	8.12	22.62	29.48	6.93	21.01	26.80	8.76	23.39	30.39
GSI	12.01	0.29	0.43	9.14	0.27	0.37	11.02	0.32	0.44
Milling Quality									
MDT (min)	19.30	1.77	3.42	17.10	1.79	3.35	18.74	2.48	4.12
PBW (%T)	7.36	25.45	33.16	7.03	22.66	29.26	8.62	24.45	32.55
PDR (%T)	5.33	51.53	63.83	7.41	45.93	61.46	6.84	47.40	61.00
WIP (%T*min)	20.45	81.75	169.87	19.79	79.16	164.77	16.61	109.67	188.40
WAP (%T*min)	16.78	54.61	100.69	16.48	48.73	89.70	17.68	66.92	122.52

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix C.2.3 Coefficient of variation and ranges of quality parameters for 2015 locations averaged across genotypes

	Lethbridge			Morden			Swift Current		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	0.00	1.00	1.00	25.72	2.00	4.00	24.06	1.00	2.00
FDK (%)	424.26	0.00	0.05	61.23	0.05	2.20	.	0.00	0.00
DON (ppm)	.	.	.	51.18	0.50	3.60	.	.	.
Midge (%)	104.32	0.00	0.70	78.43	0.20	2.40	104.49	0.00	0.50
Sprout (%)	.	0.00	0.00	230.09	0.00	0.10	300.00	0.00	0.76
Ergot (%)	.	.	.	.	.	.	.	.	.
GP (%)	4.62	13.50	15.50	3.84	11.40	13.10	4.35	13.20	16.10
TW (kg/hL)	1.33	82.00	85.80	2.59	73.60	81.60	2.02	76.30	81.80
TKW (g)	5.69	31.77	39.57	3.78	27.66	31.39	6.64	29.13	38.82
Milling Quality									
FY (%)	0.97	70.85	73.66	0.96	71.43	73.73	0.78	71.81	73.89
BC (%)	3.02	19.62	22.04	2.82	21.28	23.56	1.66	20.93	22.27
SC (%)	8.68	4.39	6.37	9.66	3.25	4.77	10.62	3.20	4.94
AC (%)	6.12	0.37	0.46	4.43	0.47	0.55	3.55	0.36	0.42
Protein Quality									
FP (%)	4.55	12.91	14.98	3.99	10.55	12.33	4.90	12.75	15.86
SP (%)	5.41	9.49	11.27	4.37	7.77	9.15	5.57	9.45	11.74
SP/FP (%)	2.62	69.68	76.90	2.78	68.37	76.33	4.00	67.67	77.07
IG (%)	9.95	3.22	4.48	8.18	2.65	3.47	9.02	3.35	4.36
IG/FP (%)	6.89	24.32	30.05	6.54	24.04	30.17	7.89	23.67	30.21
GSI	8.59	0.32	0.43	8.88	0.33	0.43	10.95	0.32	0.45
Milling Quality									
MDT (min)	14.14	2.35	3.58	14.79	2.84	5.45	14.72	2.30	3.97
PBW (%T)	8.74	24.25	33.02	6.76	18.45	23.89	9.44	24.64	33.41
PDR (%T)	7.19	47.74	60.86	6.68	35.60	44.52	6.71	47.76	61.26
WIP (%T*min)	15.44	110.84	180.80	12.90	105.85	176.93	15.73	111.86	188.27
WAP (%T*min)	19.53	63.52	117.85	15.27	61.44	107.88	15.96	68.45	116.53

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix C.2.4 Coefficient of variation and ranges of quality parameters for 2016 locations averaged across genotypes

	Beaverlodge			Brandon			Edmonton		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	30.60	1.00	4.00	7.93	4.00	5.00	48.44	1.00	4.00
FDK (%)	194.37	0.00	0.90	56.10	0.80	7.80	103.94	0.00	0.30
DON (ppm)	.	0.00	0.00	30.94	2.30	8.40	291.04	0.00	0.10
Midge (%)	70.86	0.20	2.40	62.04	0.20	2.20	57.68	0.30	1.50
Sprout (%)	73.10	0.10	3.50	131.78	0.00	0.40	88.69	0.10	0.90
Ergot (%)	158.03	0.00	0.02	98.45	0.00	0.56	73.34	0.00	0.05
GP (%)	3.80	13.80	15.60	3.50	15.20	17.70	3.92	14.30	16.20
TW (kg/hL)	1.40	79.30	83.20	2.06	75.10	80.90	1.90	78.70	84.20
TKW (g)	3.50	35.72	41.11	4.12	26.17	29.67	3.81	33.28	38.56
Milling Quality									
FY (%)	1.71	70.20	74.93	1.00	70.34	72.76	1.61	70.80	74.38
BC (%)	3.49	19.50	21.78	2.72	21.81	23.99	3.97	20.56	23.19
SC (%)	14.35	4.56	7.74	9.25	3.61	5.03	15.01	3.72	6.06
AC (%)	4.09	0.34	0.39	8.06	0.34	0.45	6.18	0.31	0.39
Protein Quality									
FP (%)	4.00	13.72	15.40	4.09	14.82	17.53	4.38	13.67	15.68
SP (%)	4.88	9.56	11.40	5.97	10.15	13.14	6.64	9.13	11.71
SP/FP (%)	3.50	65.67	76.08	3.62	65.55	74.92	3.82	65.90	74.67
IG (%)	7.78	2.99	4.15	7.62	3.08	3.94	5.49	3.38	4.09
IG/FP (%)	6.72	21.33	27.12	8.12	19.75	25.74	7.08	22.83	29.02
GSI	8.29	0.29	0.40	10.75	0.27	0.38	9.75	0.32	0.44
Milling Quality									
MDT (min)	13.54	1.88	2.95	16.98	1.53	2.78	20.65	1.85	4.04
PBW (%T)	5.75	30.85	38.79	5.73	28.27	34.20	7.65	25.56	33.37
PDR (%T)	4.49	61.23	72.44	6.01	53.94	68.16	5.25	48.58	58.32
WIP (%T*min)	16.82	102.57	185.13	17.62	70.20	143.63	22.71	90.03	211.88
WAP (%T*min)	14.70	63.11	104.88	15.38	48.57	81.66	22.70	58.29	122.39

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix C.2.5 Coefficient of variation and ranges of quality parameters for 2016 locations averaged across genotypes

	Goodale			Indian Head			Kelburn		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	38.85	1.00	3.00	28.15	2.00	5.00	38.35	1.00	4.00
FDK (%)	67.90	0.14	1.30	76.31	0.50	6.30	58.97	0.00	0.92
DON (ppm)	85.61	0.20	2.70	49.22	1.80	9.00	52.66	0.40	3.90
Midge (%)	59.01	0.10	1.20	51.70	0.30	6.00	57.99	0.20	1.20
Sprout (%)	86.75	0.00	0.40	151.33	0.00	3.50	76.09	0.00	0.20
Ergot (%)	128.62	0.00	0.01	102.28	0.00	0.08	.	.	.
GP (%)	3.35	15.00	16.80	7.47	10.40	13.70	3.43	14.80	16.60
TW (kg/hL)	1.78	77.90	82.60	2.28	77.50	84.00	1.57	77.90	82.30
TKW (g)	3.24	30.50	34.83	3.78	29.28	32.61	5.77	28.44	33.89
Milling Quality									
FY (%)	1.84	68.71	73.87	1.36	69.27	72.17	1.79	69.42	74.04
BC (%)	4.83	20.51	24.20	3.24	21.75	23.95	4.56	20.57	24.07
SC (%)	14.67	3.96	5.94	9.35	4.59	6.29	10.71	3.81	5.42
AC (%)	5.59	0.31	0.38	6.97	0.37	0.46	5.23	0.36	0.43
Protein Quality									
FP (%)	4.62	14.27	16.63	7.61	9.85	12.72	4.36	14.14	16.42
SP (%)	7.99	9.42	11.99	7.84	7.54	9.61	8.03	9.38	12.81
SP/FP (%)	5.37	62.23	74.77	4.89	67.06	78.94	5.11	63.43	78.02
IG (%)	7.37	3.22	4.34	11.41	2.22	3.33	6.29	3.40	4.25
IG/FP (%)	8.75	21.37	29.45	8.87	20.79	28.26	8.24	22.73	29.77
GSI	13.22	0.31	0.46	12.25	0.27	0.40	12.57	0.29	0.45
Milling Quality									
MDT (min)	21.11	2.06	4.12	17.14	1.87	3.89	22.63	1.96	3.91
PBW (%T)	7.15	27.30	34.56	8.02	22.26	29.58	8.13	25.44	33.20
PDR (%T)	8.04	51.74	68.27	8.67	38.77	54.52	5.62	50.09	60.90
WIP (%T*min)	19.77	109.28	206.38	22.88	73.47	193.18	23.16	93.91	199.27
WAP (%T*min)	17.97	67.71	117.36	21.53	50.67	114.26	19.14	54.17	106.75

DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

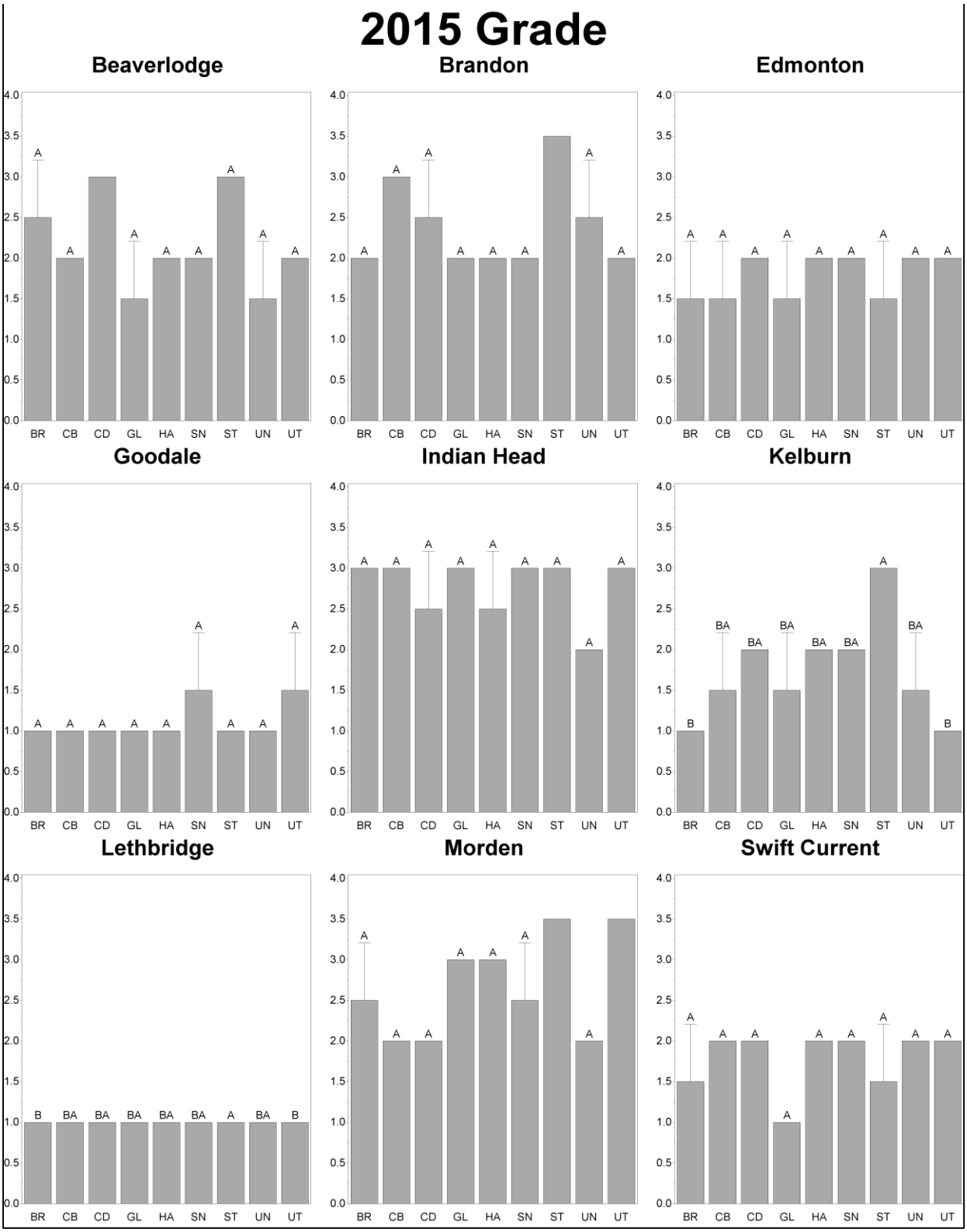
Appendix C.2.6 Coefficient of variation and ranges of quality parameters for 2016 locations averaged across genotypes

	Lethbridge			Morden			Swift Current		
	CV (%)	Min	Max	CV (%)	Min	Max	CV (%)	Min	Max
Grain Quality									
Grade	32.87	1.00	2.00	39.46	1.00	4.00	35.20	2.00	4.00
FDK (%)	188.65	0.00	0.10	90.98	0.10	3.20	54.01	0.20	1.50
DON (ppm)	61.74	0.00	0.20	49.23	0.50	3.80	88.24	0.10	2.60
Midge (%)	97.17	0.00	0.60	37.53	0.10	0.80	78.44	0.20	3.70
Sprout (%)	.	0.00	0.00	114.49	0.00	0.50	117.95	0.00	0.67
Ergot (%)	207.40	0.00	0.00	94.83	0.00	0.07	52.14	0.00	0.07
GP (%)	2.91	14.60	15.90	3.70	14.10	16.30	6.11	10.10	12.90
TW (kg/hL)	1.83	77.70	83.00	2.53	76.60	83.10	3.08	75.70	84.20
TKW (g)	6.22	26.78	34.72	3.09	30.39	33.72	4.89	27.22	32.44
Milling Quality									
FY (%)	1.10	71.12	73.69	1.27	70.45	73.59	1.07	70.29	73.09
BC (%)	2.88	20.62	22.76	3.82	20.51	23.22	2.47	20.84	22.97
SC (%)	10.02	3.58	5.24	10.81	4.06	5.70	9.70	4.46	6.45
AC (%)	3.39	0.34	0.38	6.74	0.36	0.44	4.65	0.34	0.39
Protein Quality									
FP (%)	3.48	14.24	15.91	4.32	13.59	15.87	6.03	9.91	12.21
SP (%)	6.24	9.86	12.11	6.64	9.66	11.77	8.55	6.90	9.10
SP/FP (%)	4.63	66.19	79.11	4.11	64.56	76.00	6.55	62.04	78.97
IG (%)	9.30	3.32	4.53	7.80	3.02	3.96	8.63	2.30	3.05
IG/FP (%)	9.24	22.81	30.22	8.04	20.85	27.46	9.25	20.80	28.48
GSI	12.95	0.31	0.45	11.08	0.28	0.40	12.10	0.28	0.44
Milling Quality									
MDT (min)	15.58	1.89	3.28	15.11	1.87	3.11	21.77	2.13	4.59
PBW (%T)	7.16	27.48	34.70	5.49	25.21	31.33	9.64	20.60	27.56
PDR (%T)	5.22	52.61	61.67	5.51	50.34	61.92	8.48	39.65	52.97
WIP (%T*min)	17.16	90.53	162.06	17.02	81.80	155.56	25.44	79.72	199.11
WAP (%T*min)	18.04	59.33	108.41	15.24	50.82	91.16	21.24	52.34	104.21

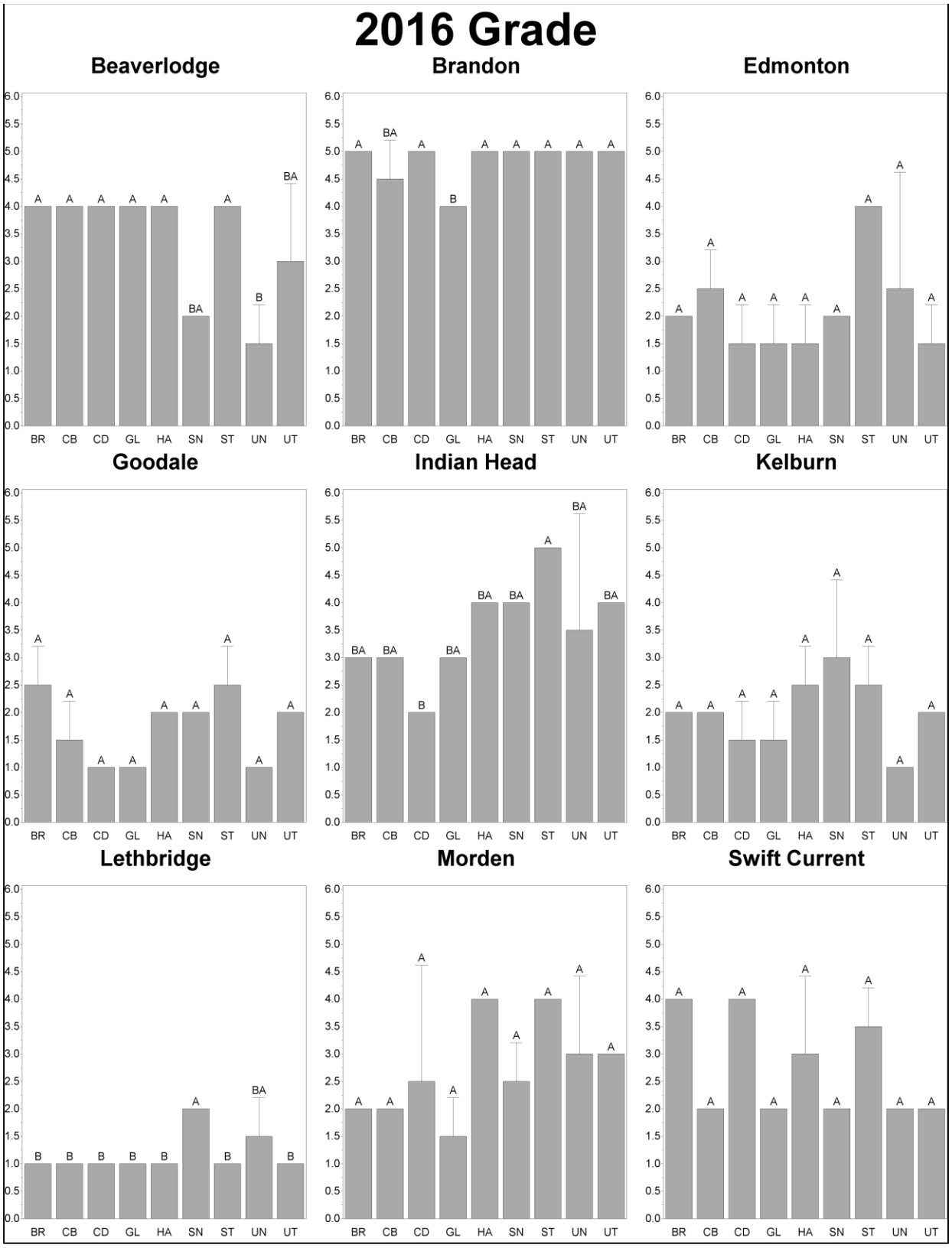
DON = Deoxynivalenol, FDK = Fusarium Damaged Kernels, FP = Flour Protein, GSI = Gluten Strength Index, HMWG = High Molecular Weight Glutenin, MDT = Mixing Development Time, PBW = Peak Band Width, PDR = Peak Dough Resistance, SP = Soluble Prolamins, T = Torque, TKW = Thousand Kernel Weight, WIP = Work Input to Peak

Appendix D. Wheat quality parameters genotype means within locations.

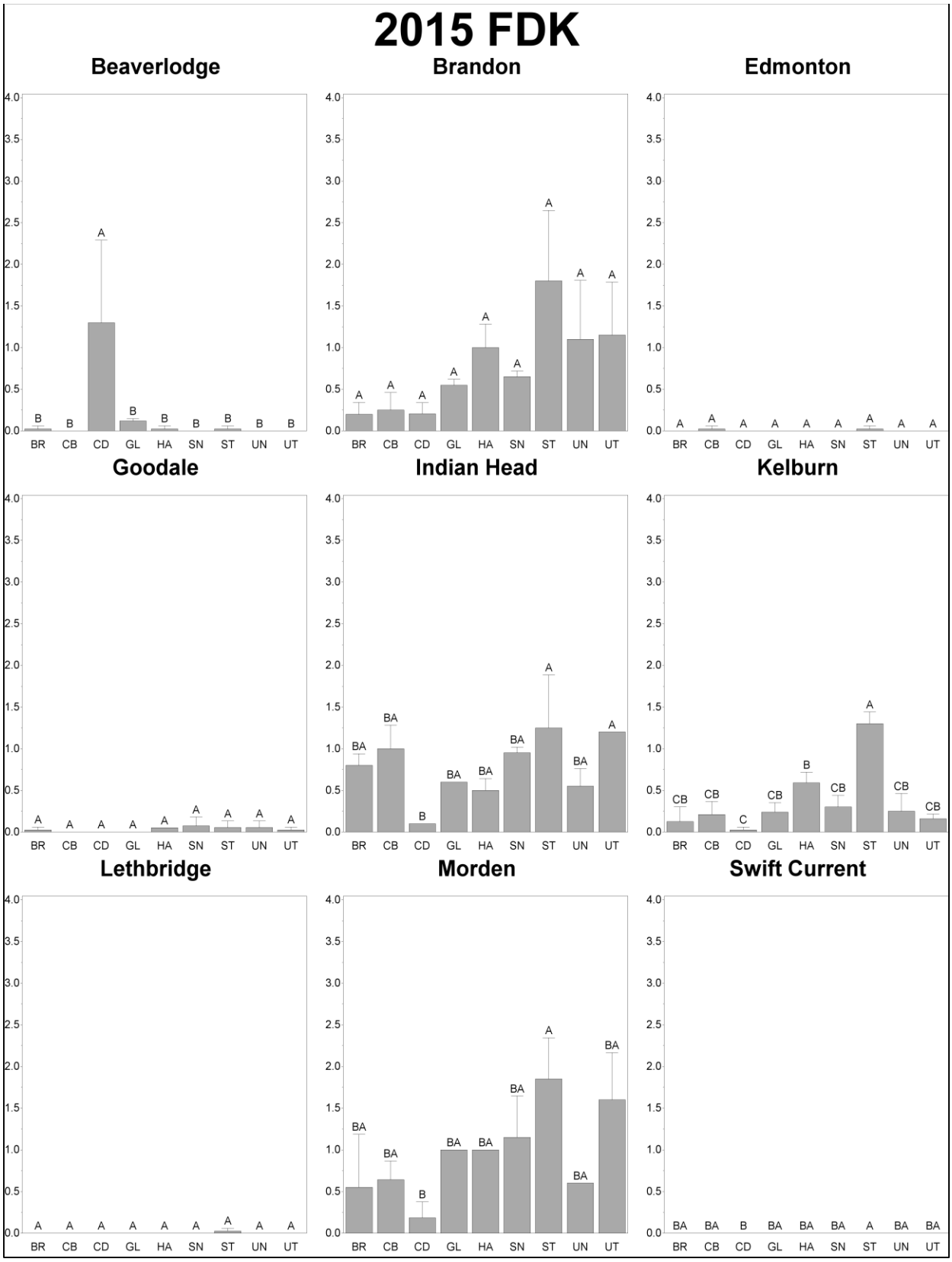
Appendix D.1 Average grade for 2015 genotypes within locations.



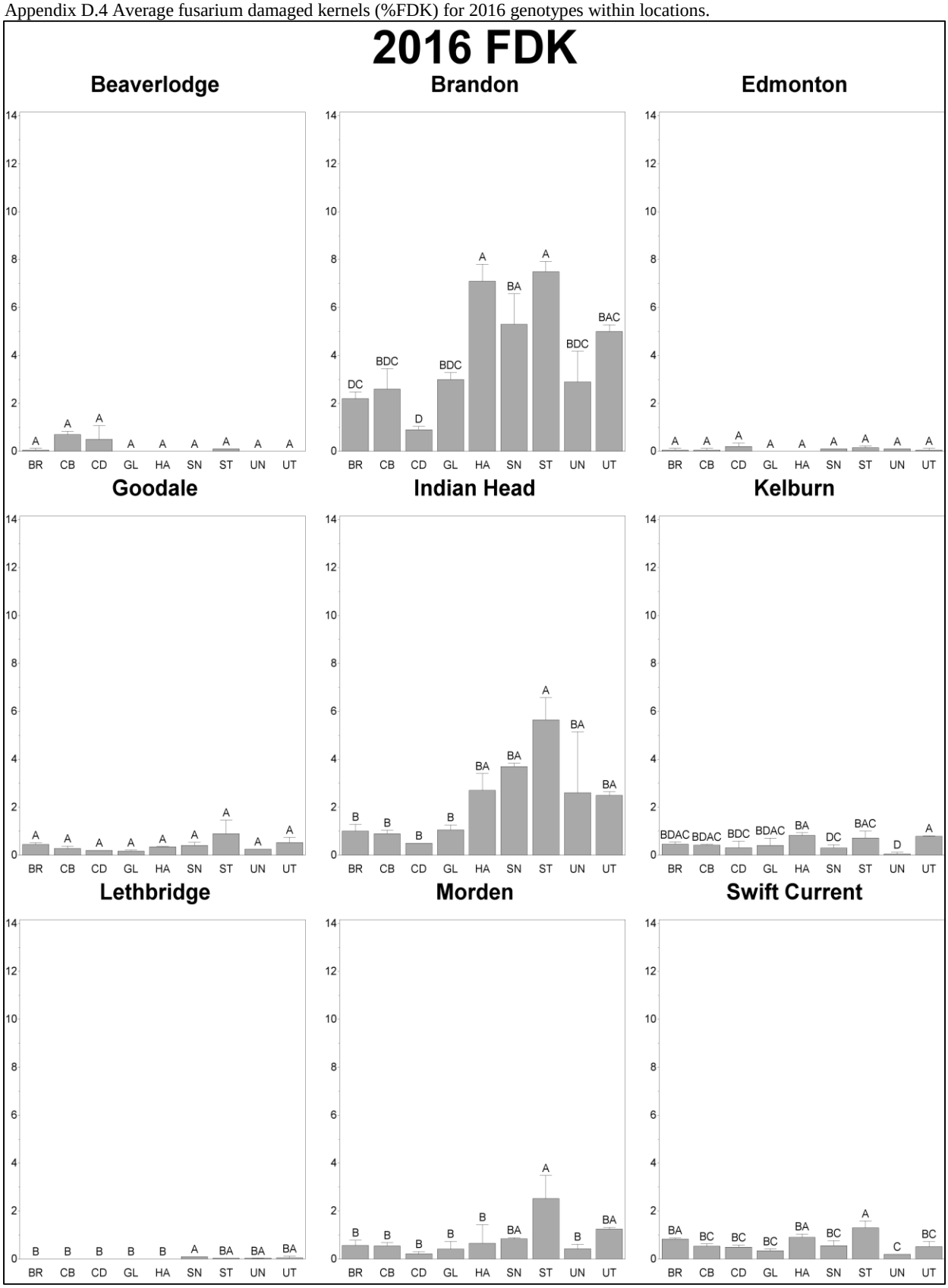
Appendix D.2 Average grade for 2016 genotypes within locations.



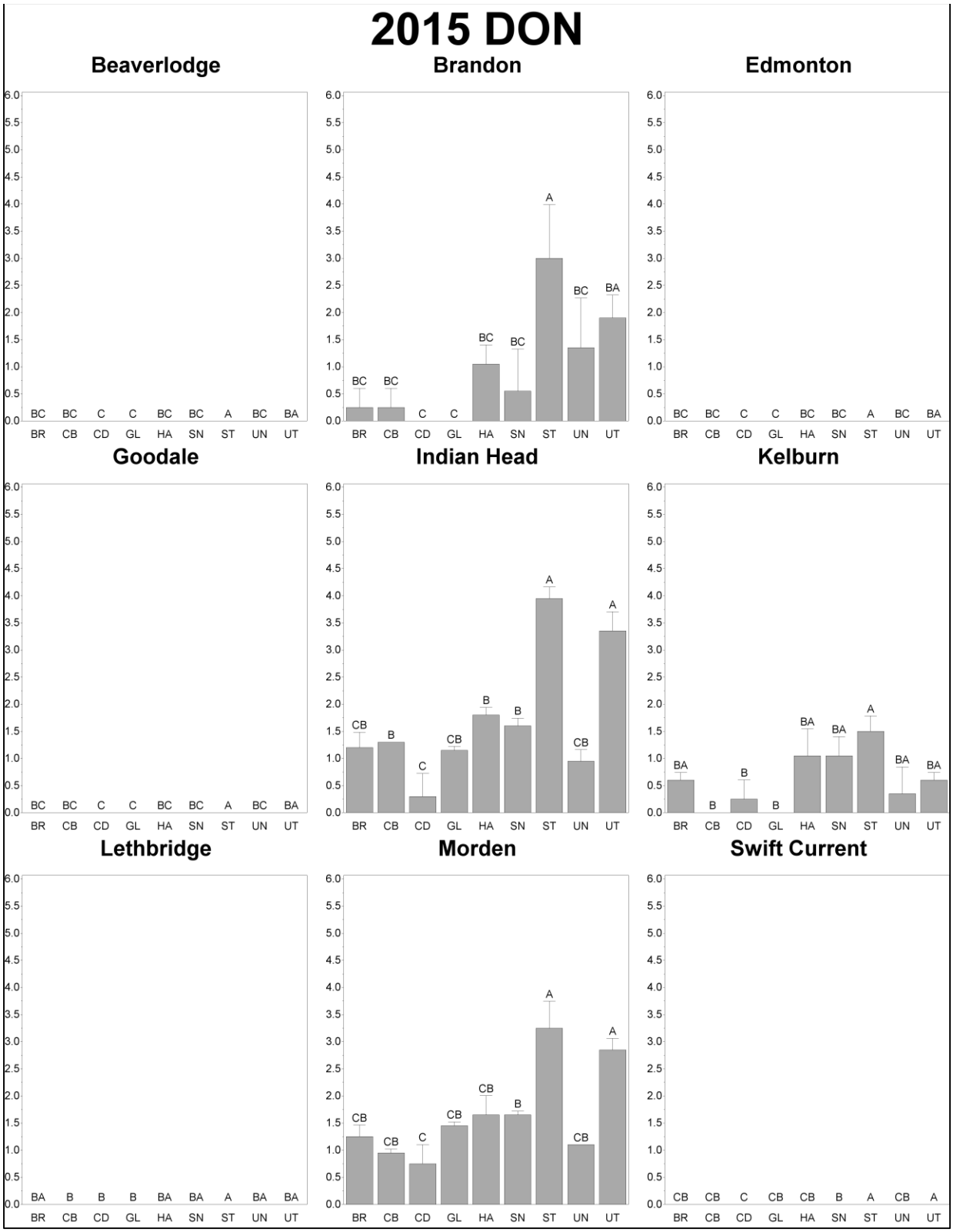
Appendix D.3 Average fusarium damaged kernels (%FDK) for 2015 genotypes within locations.



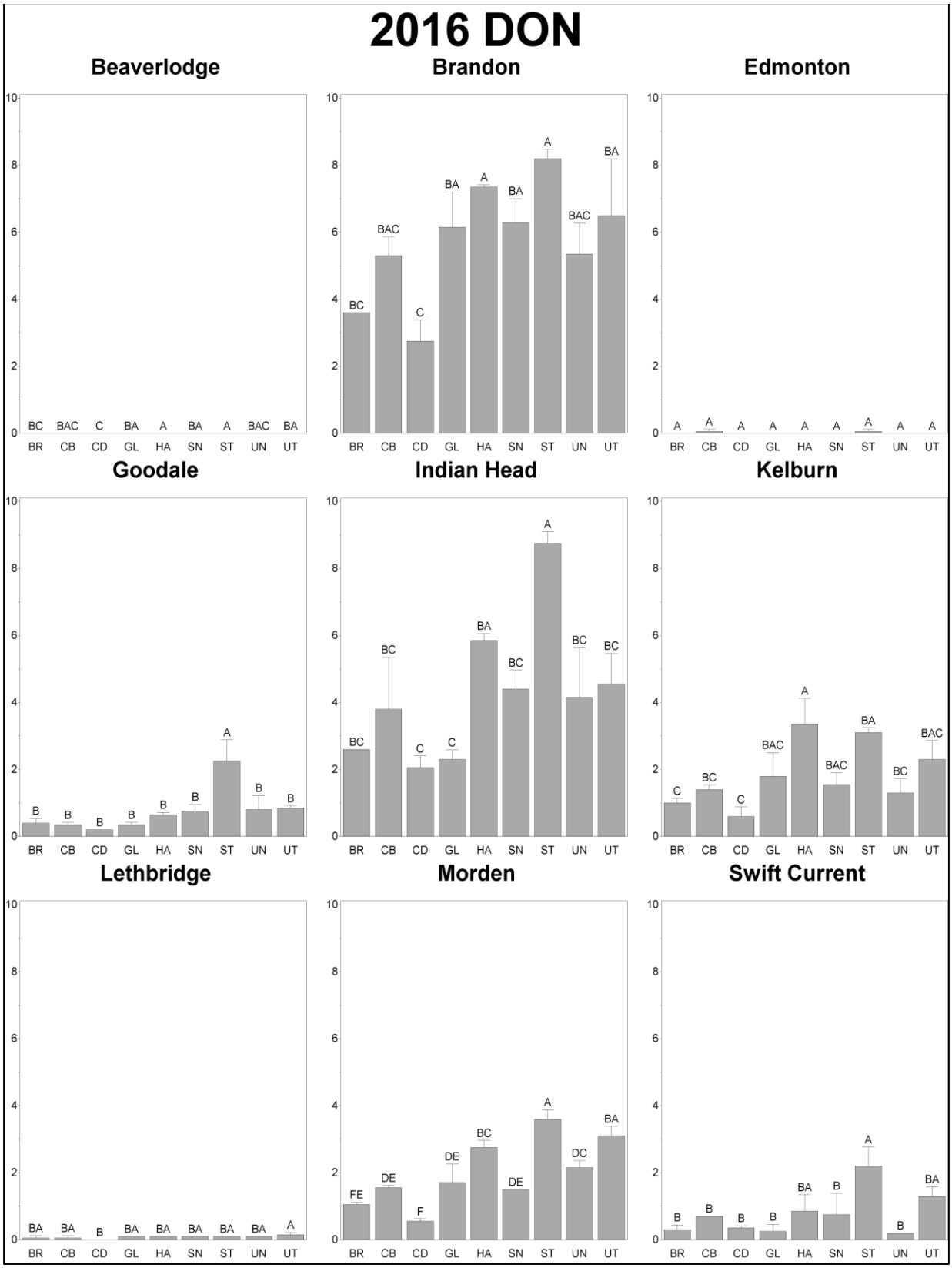
Appendix D.4 Average fusarium damaged kernels (%FDK) for 2016 genotypes within locations.



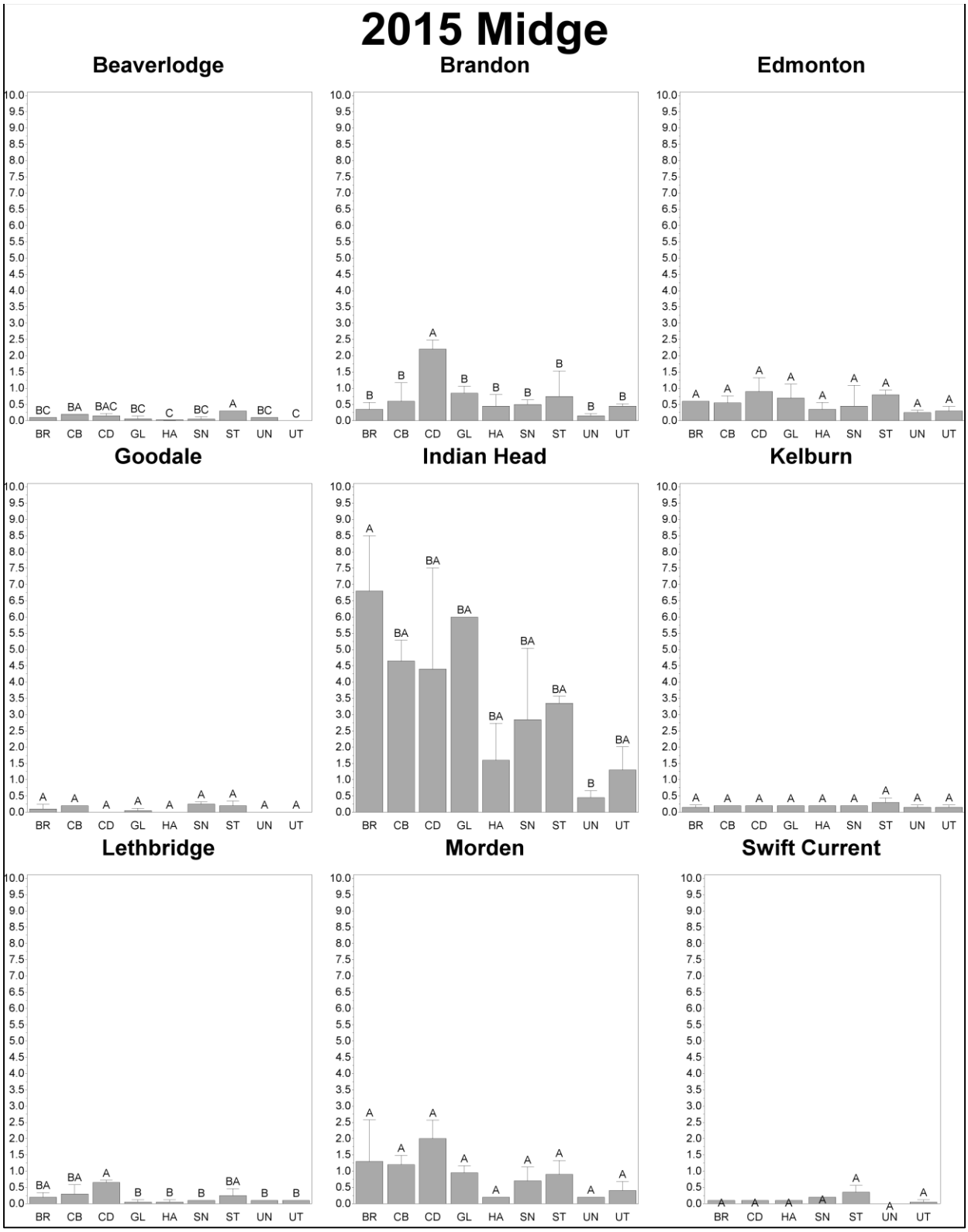
Appendix D.5 Average deoxynivalenol Content (DON, ppm) for 2015 genotypes within locations.



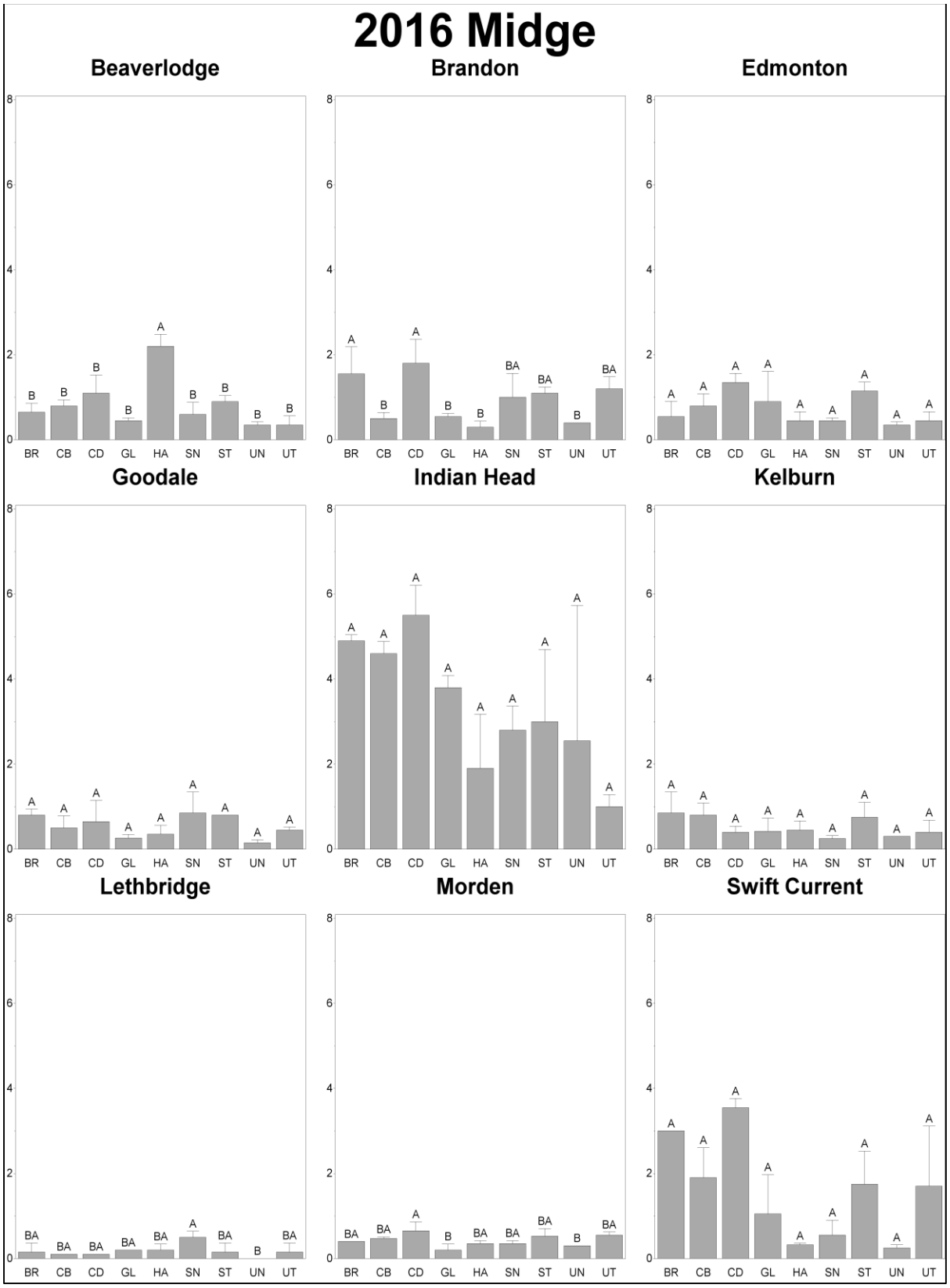
Appendix D.6 Average deoxynivalenol Content (DON, ppm) for 2016 genotypes within locations.



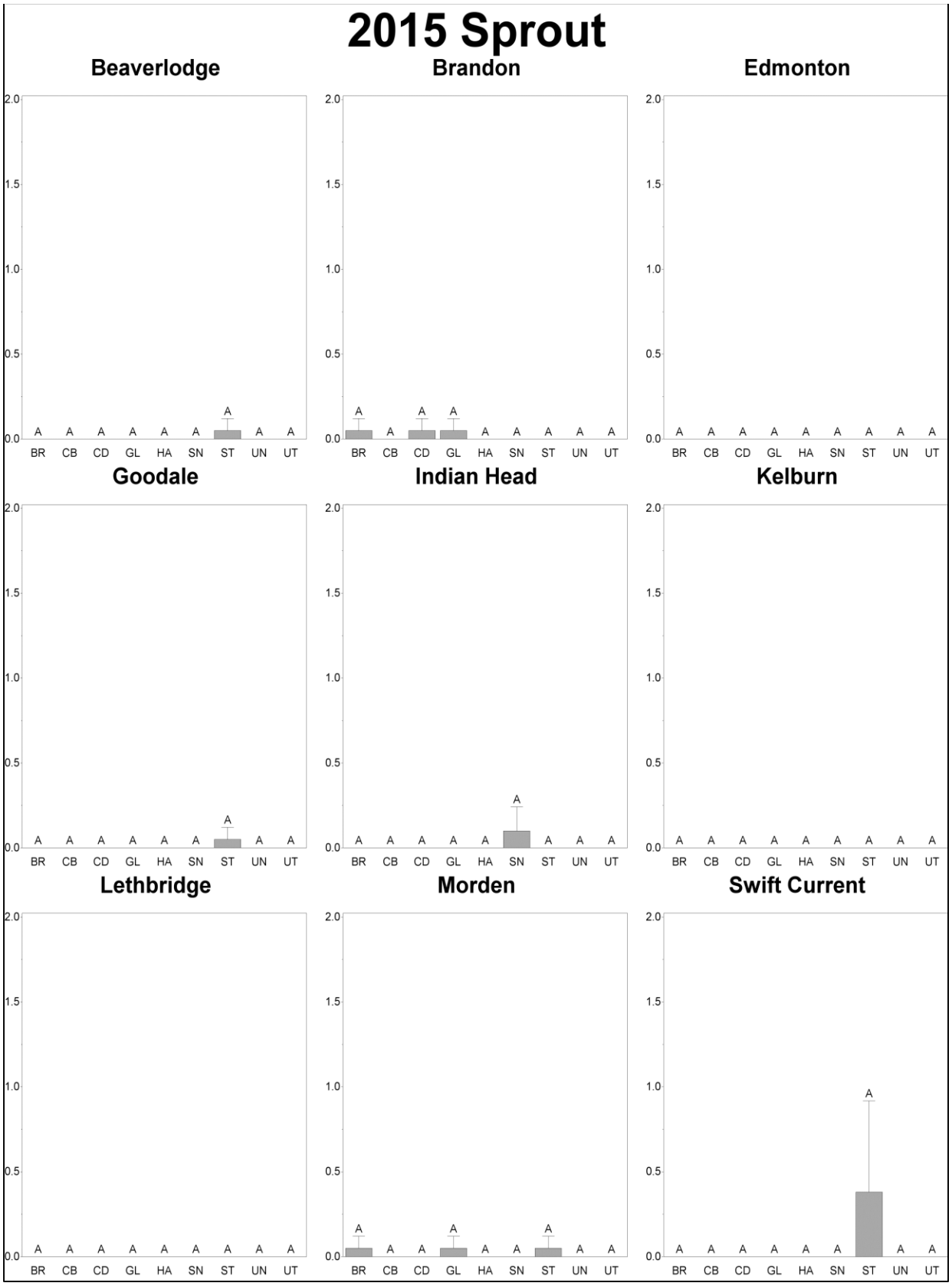
Appendix D.7 Average midge damage (%) for 2015 genotypes within locations.



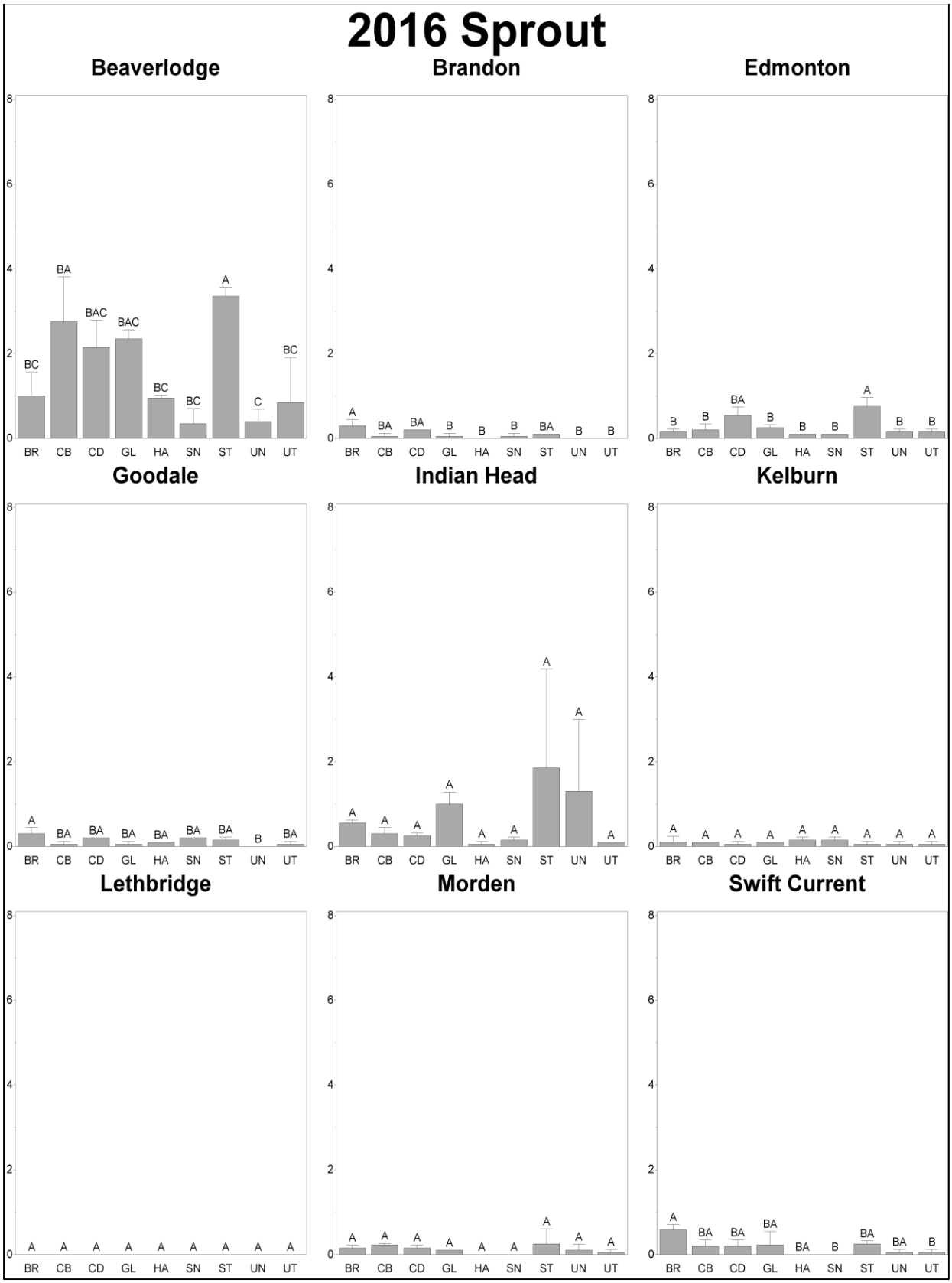
Appendix D.8 Average midge damage (%) for 2016 genotypes within locations.



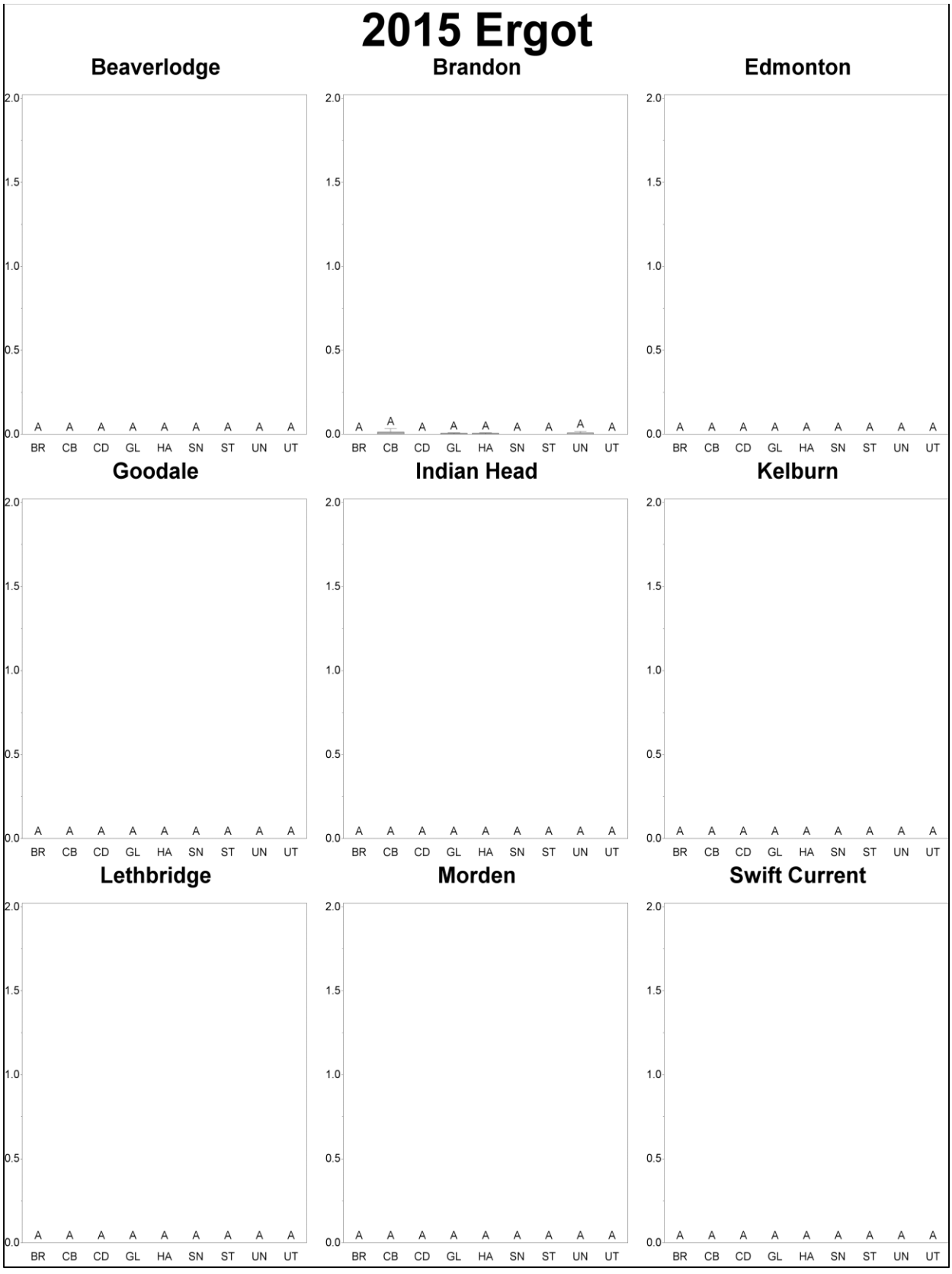
Appendix D.9 Average sprout damage (%) for 2015 genotypes within locations.



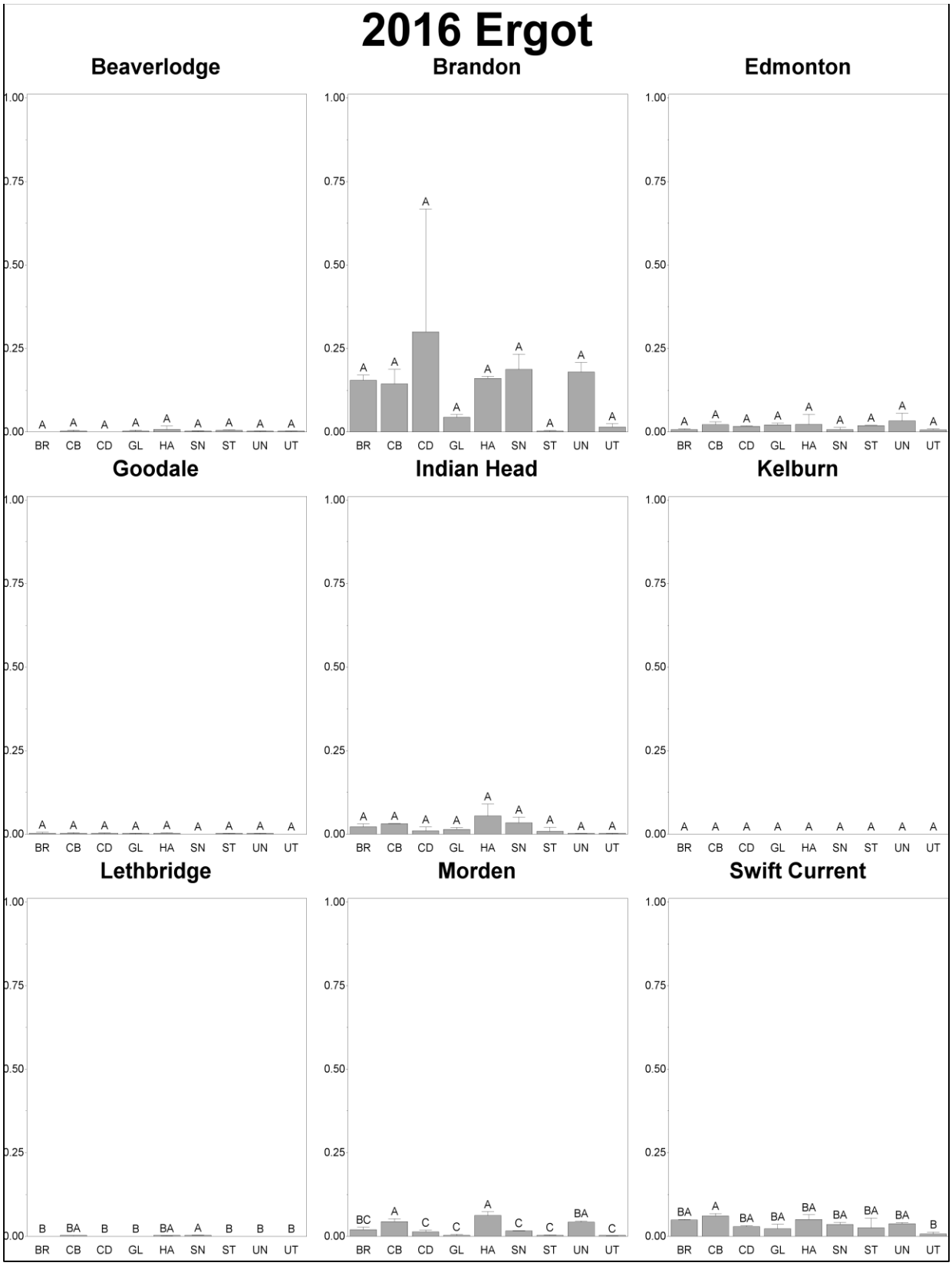
Appendix D.10 Average sprout damage (%)for 2016 genotypes within locations.



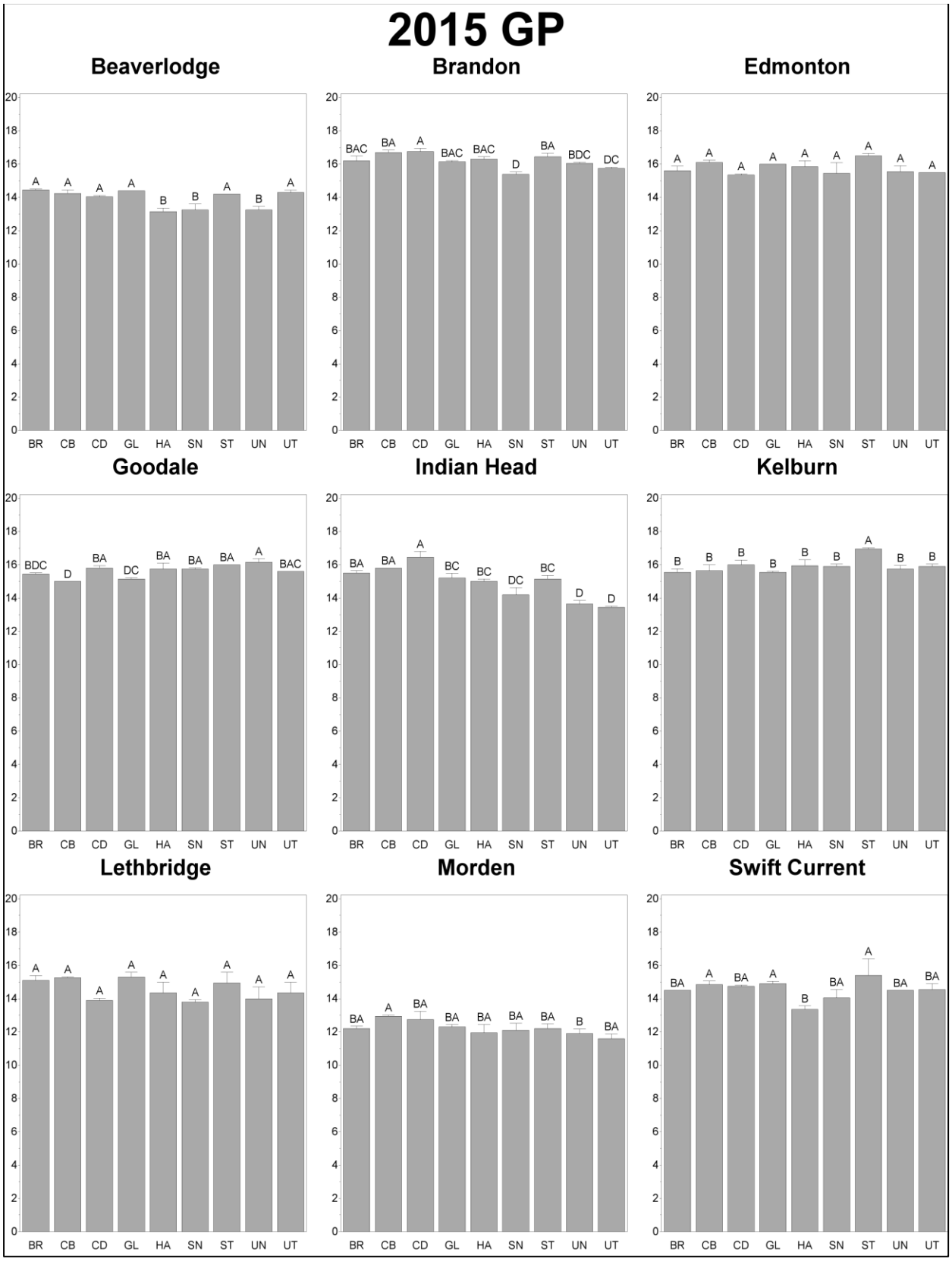
Appendix D.11 Average ergot damage (%) for 2015 genotypes within locations.



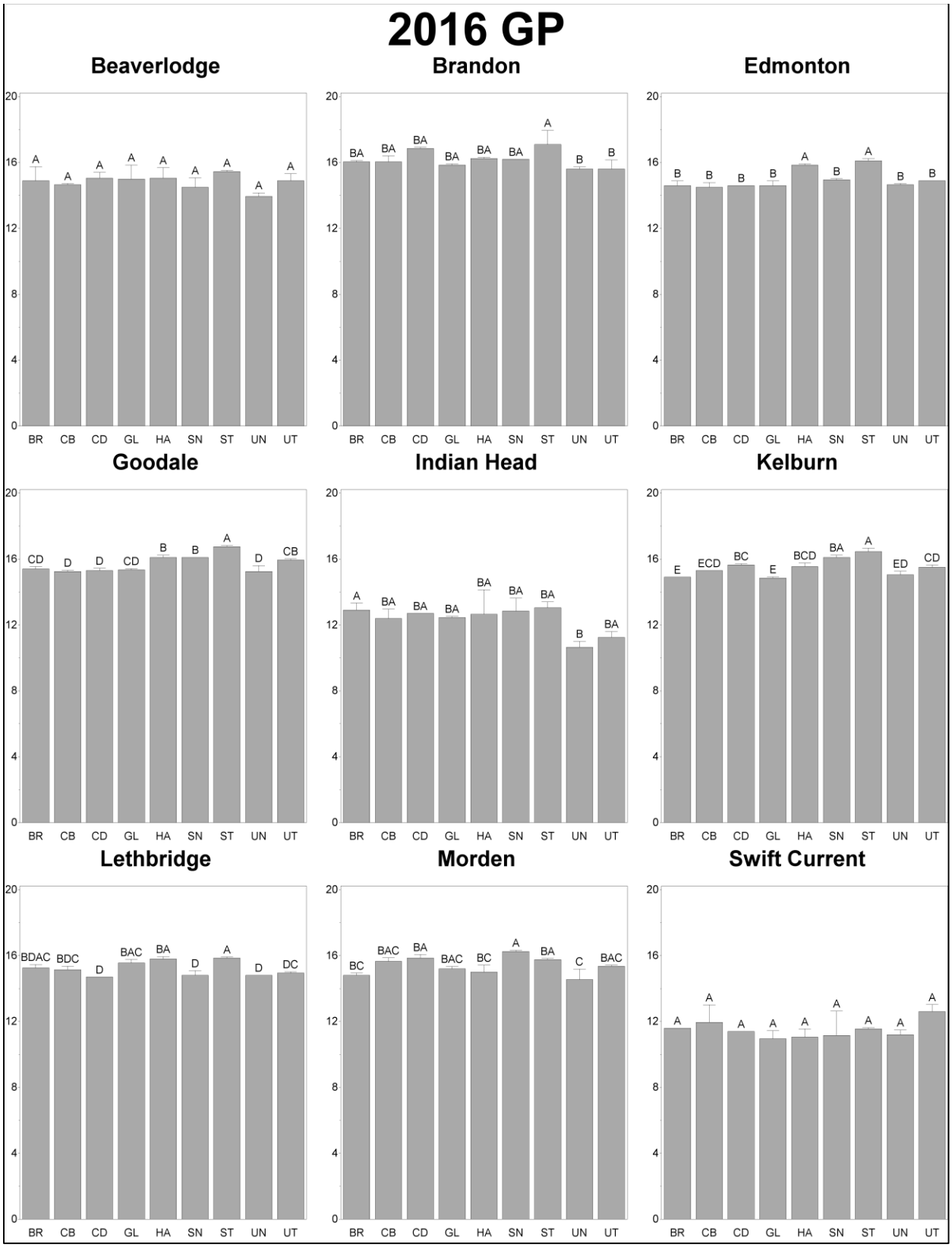
Appendix D.12 Average ergot damage (%) for 2016 genotypes within locations.



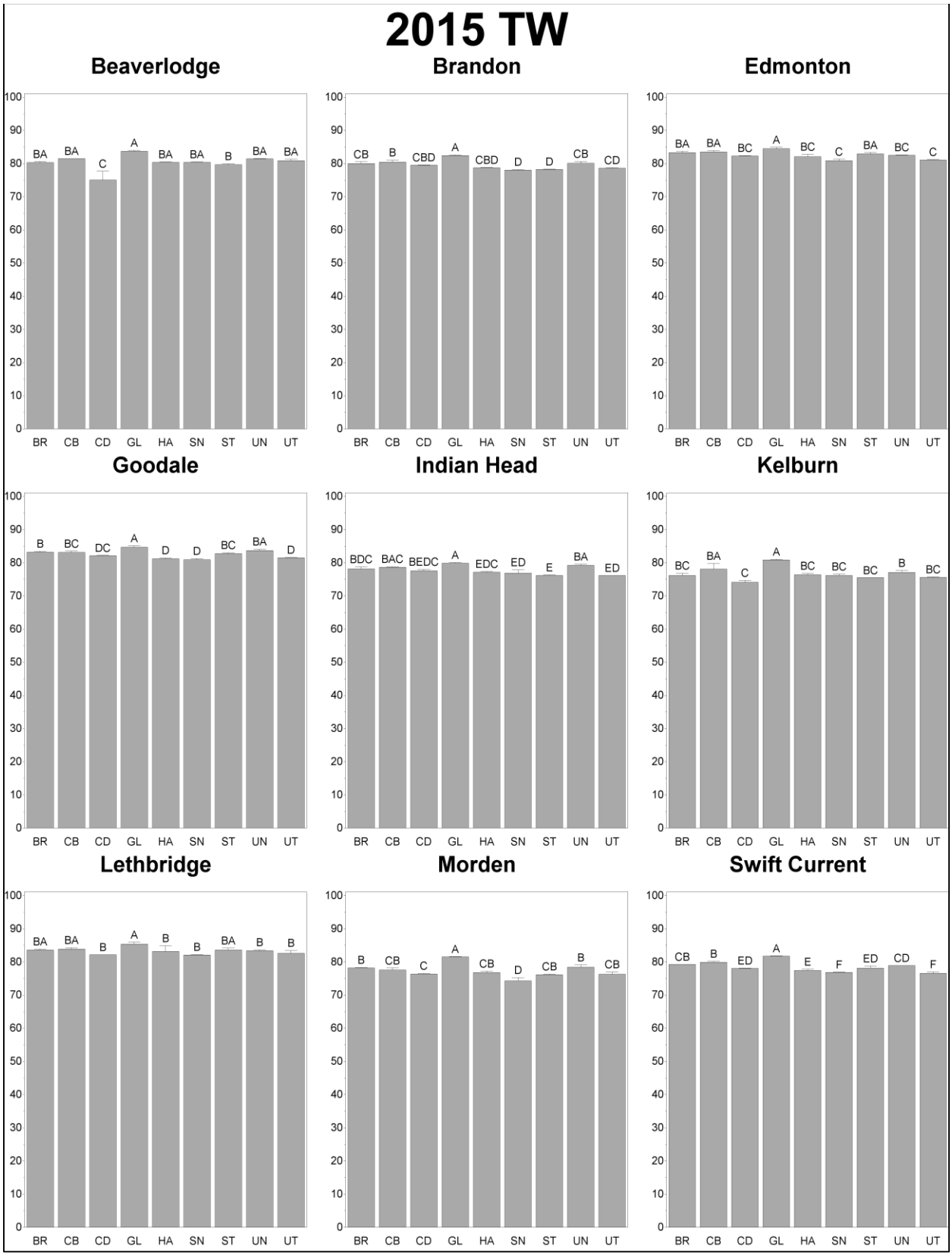
Appendix D.13 Average grain protein content (GP, %) for 2015 genotypes within locations.



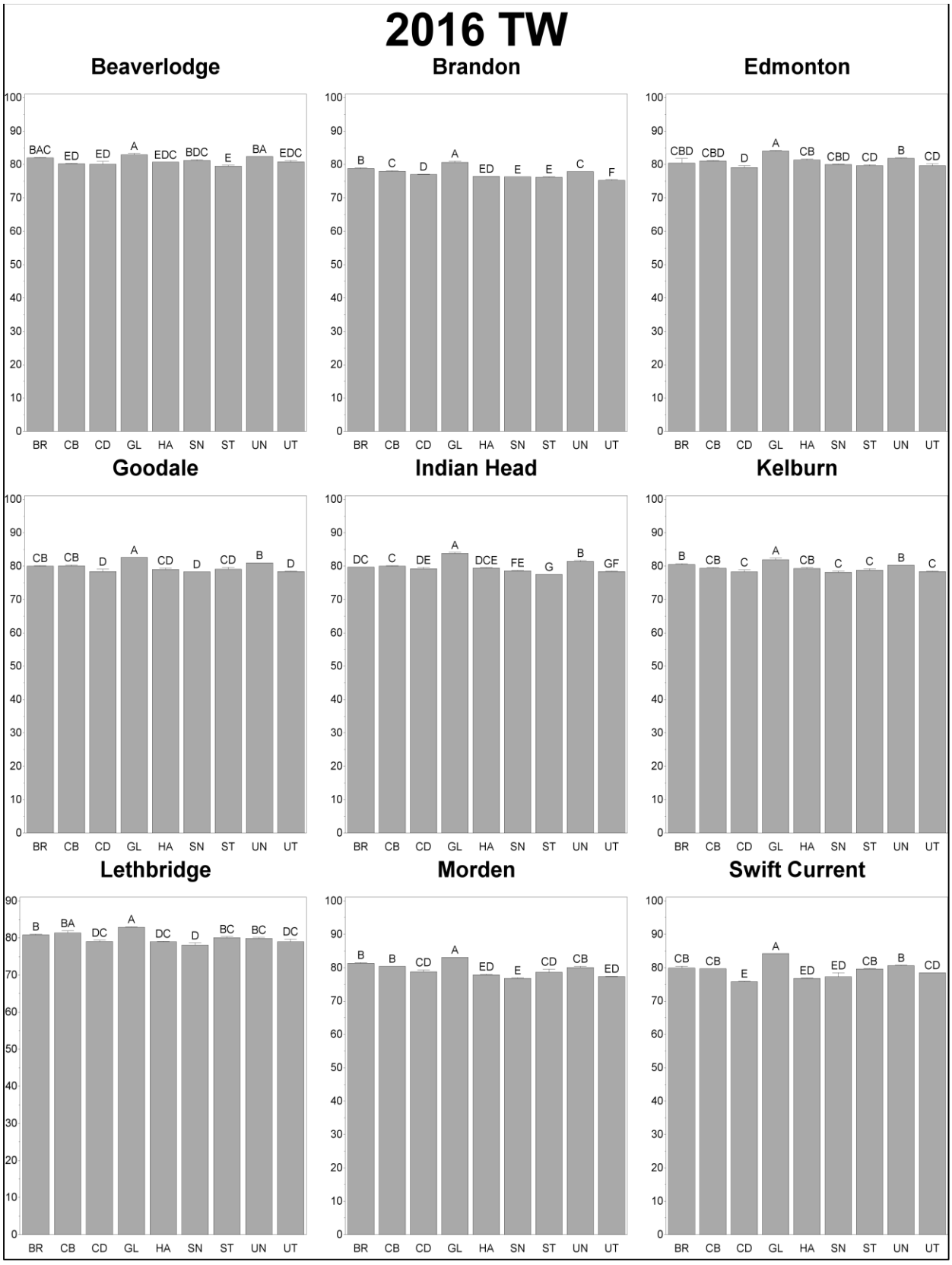
Appendix D.14 Average grain protein content (GP, %) for 2016 genotypes within locations.



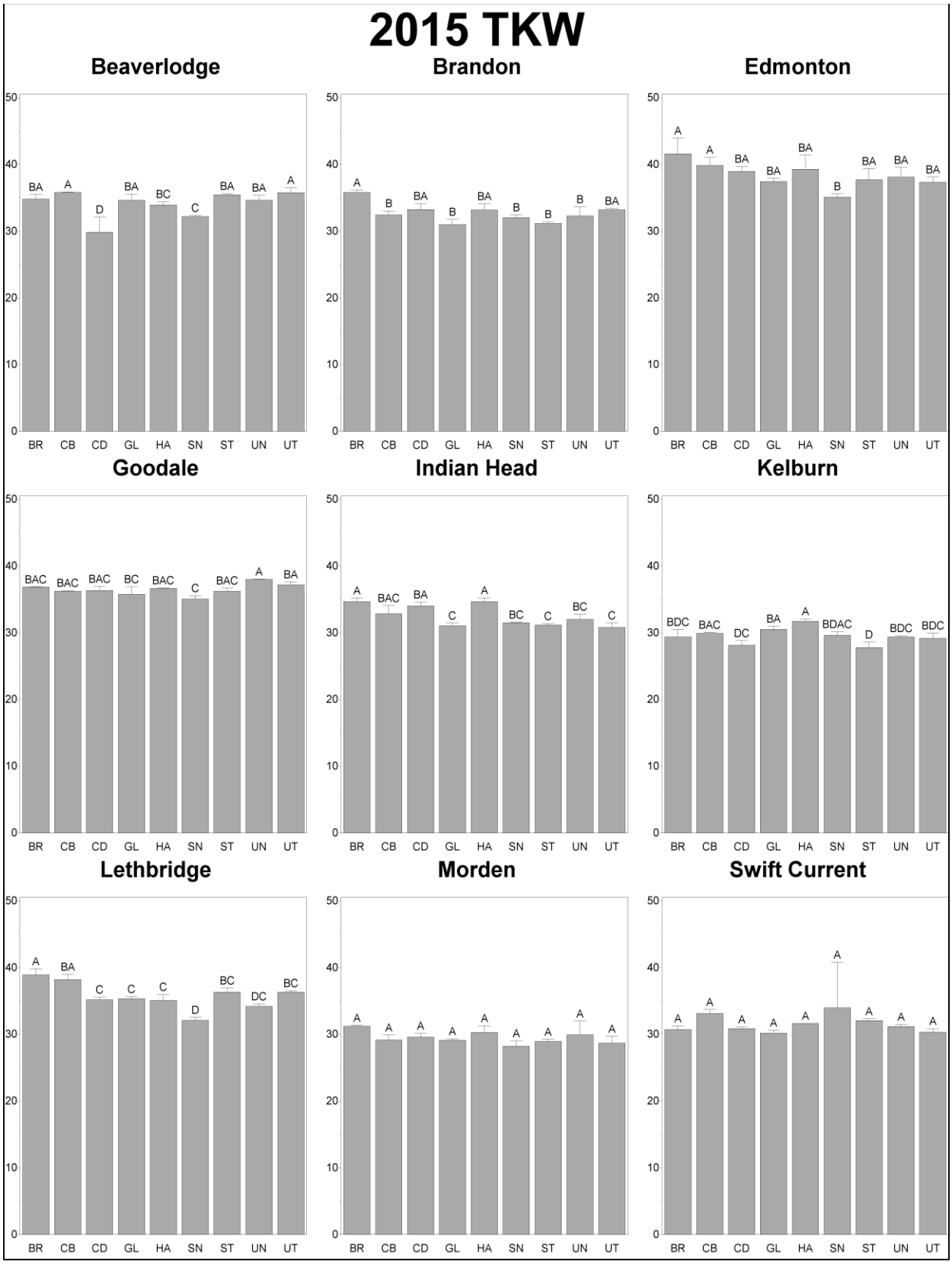
Appendix D.15 Average test weight (TW, kg/hL) for 2015 genotypes within locations.



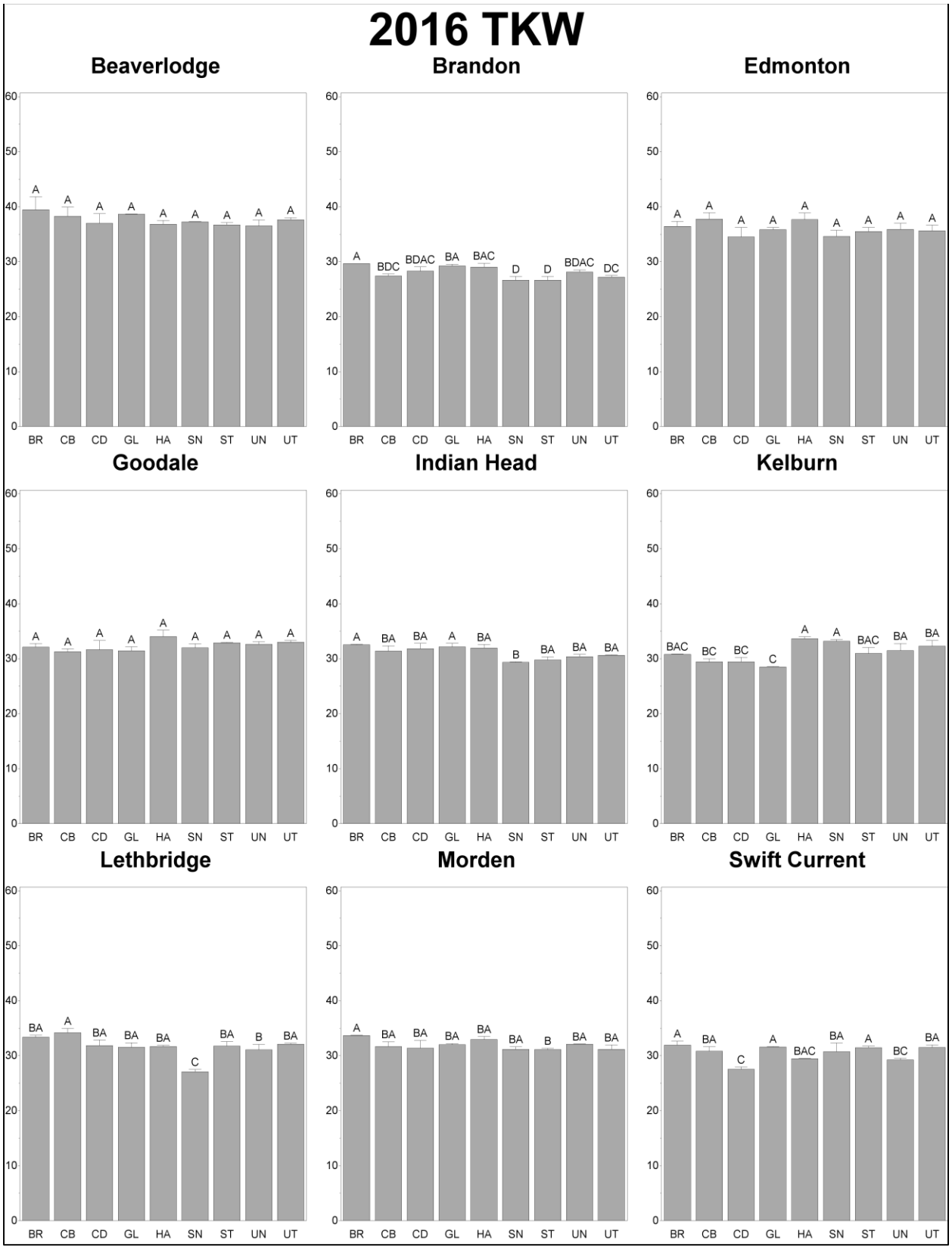
Appendix D.16 Average test weight (TW, kg/hL) for 2016 genotypes within locations.



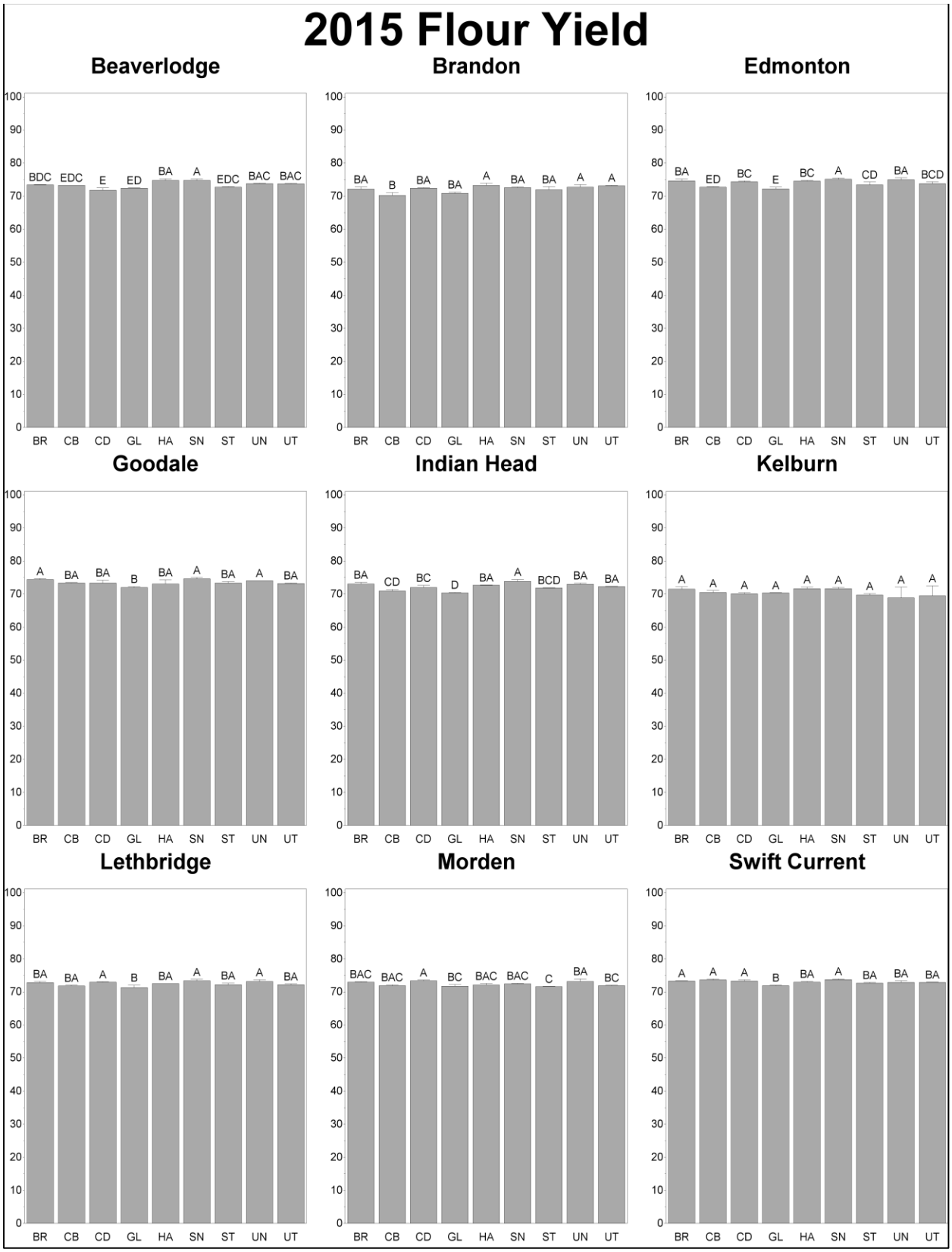
Appendix D.17 Average thousand kernel weight (TKW, g) for 2015 genotypes within locations.



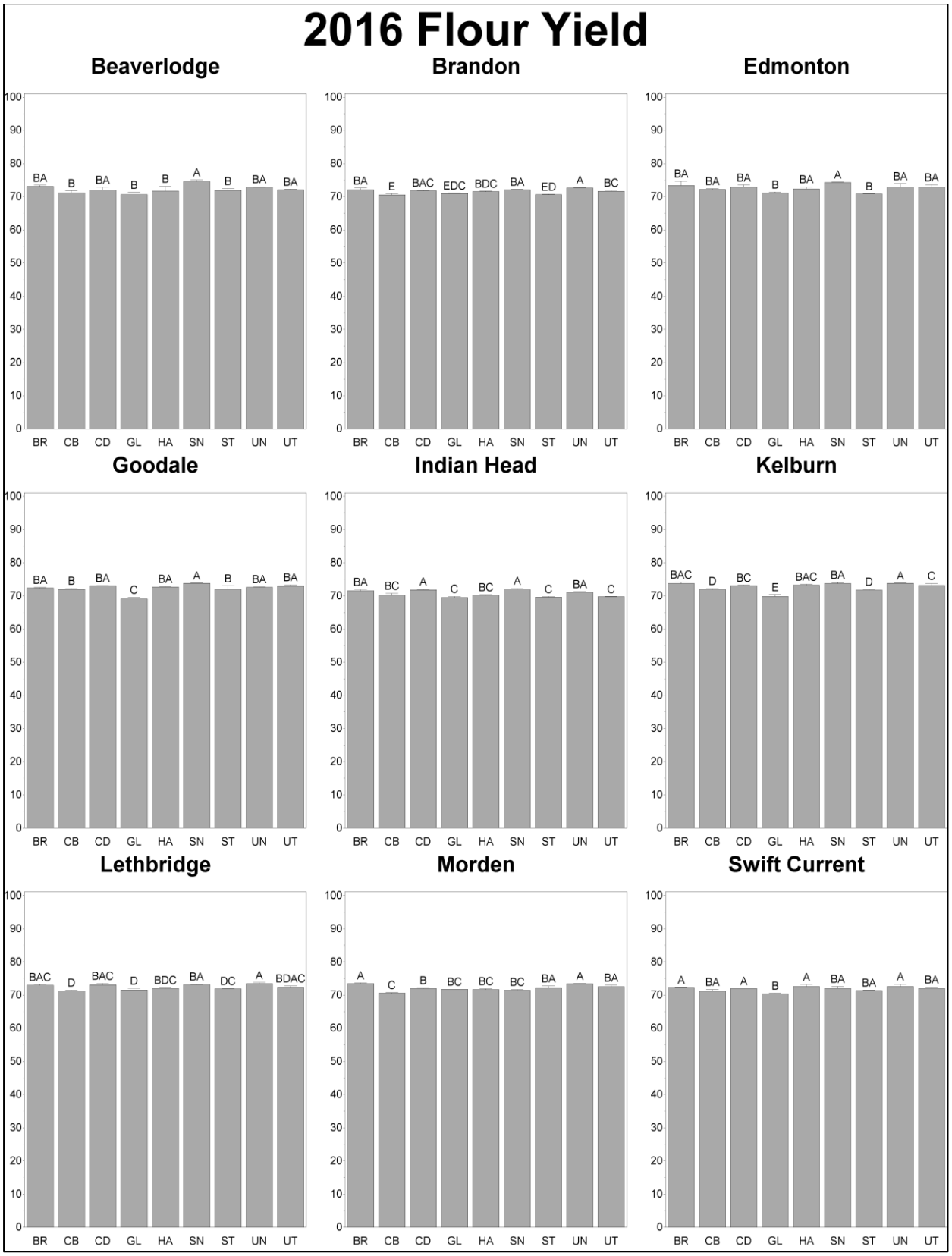
Appendix D.18 Average thousand kernel weight (TKW, g) for 2016 genotypes within locations.



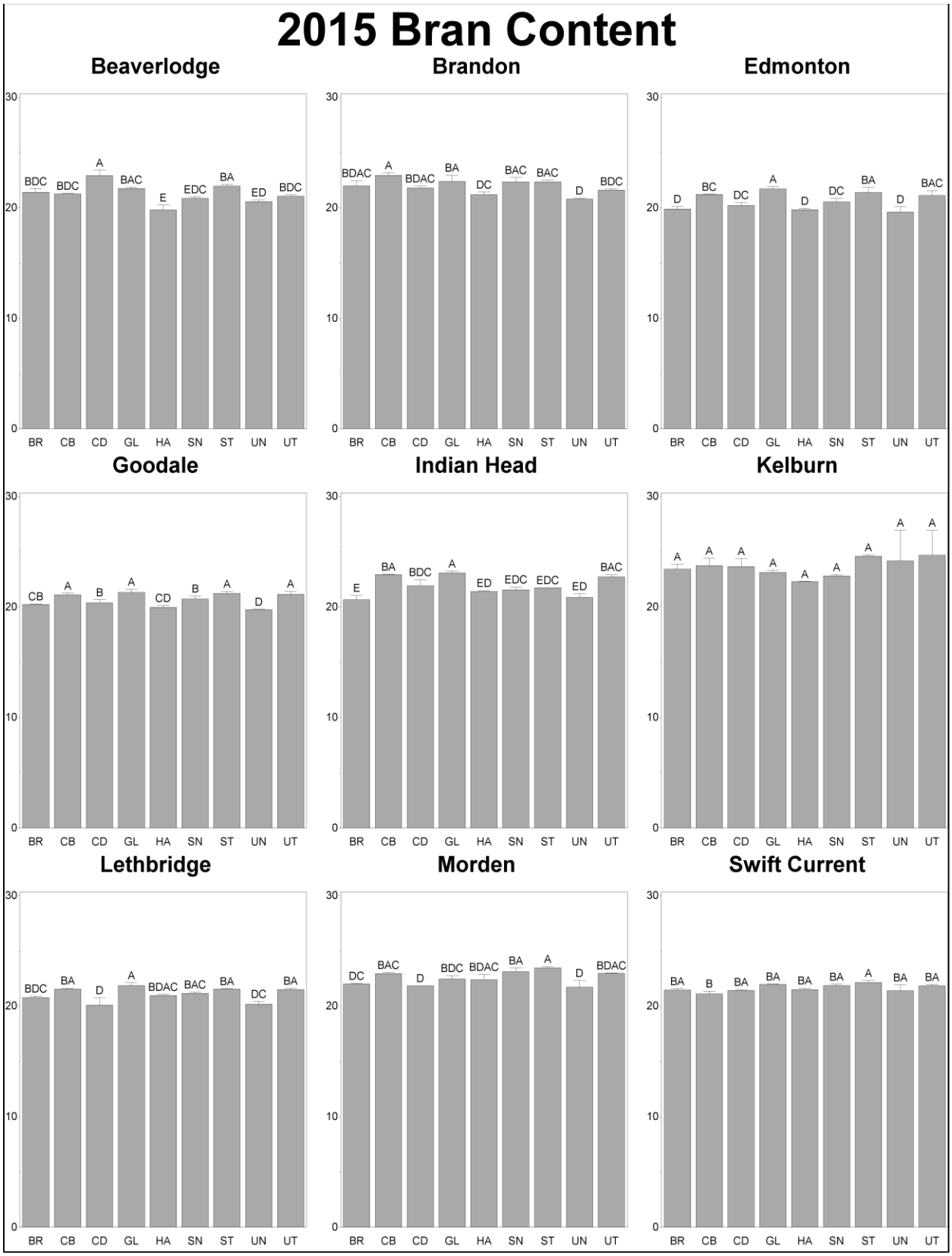
Appendix D.19 Average flour yield (%) for 2015 genotypes within locations.



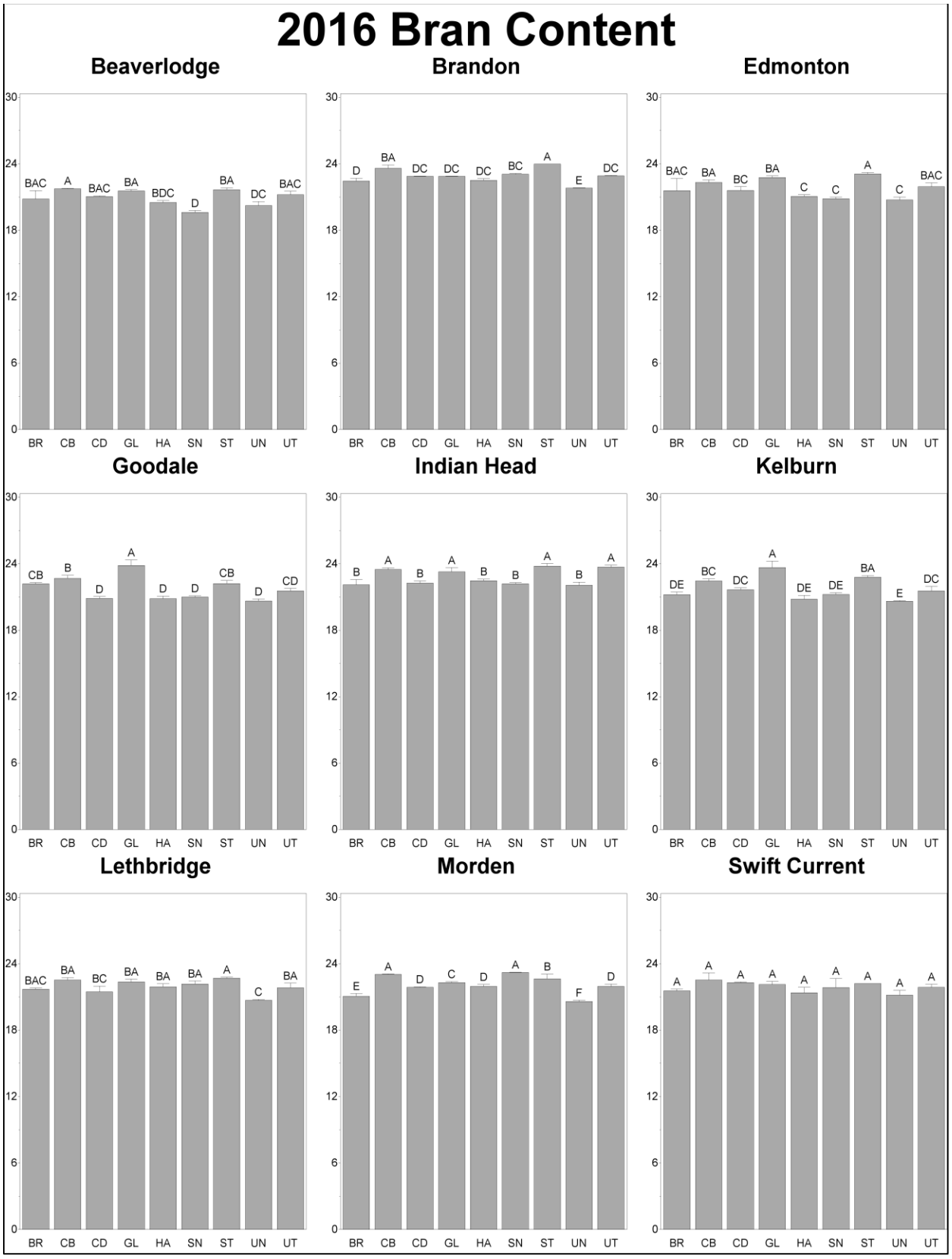
Appendix D.20 Average flour yield (%) for 2016 genotypes within locations.



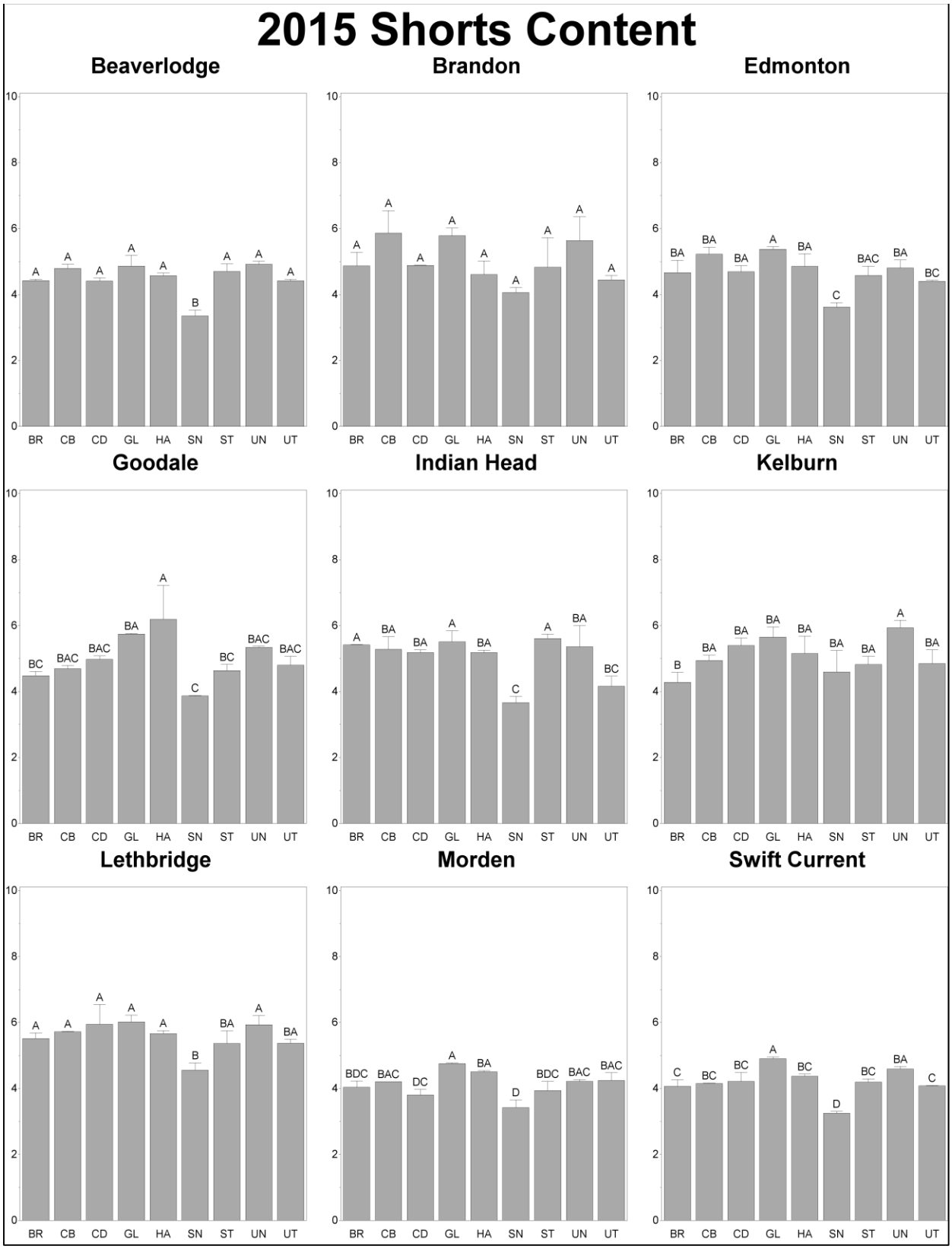
Appendix D.21 Average bran content (%) for 2015 genotypes within locations.



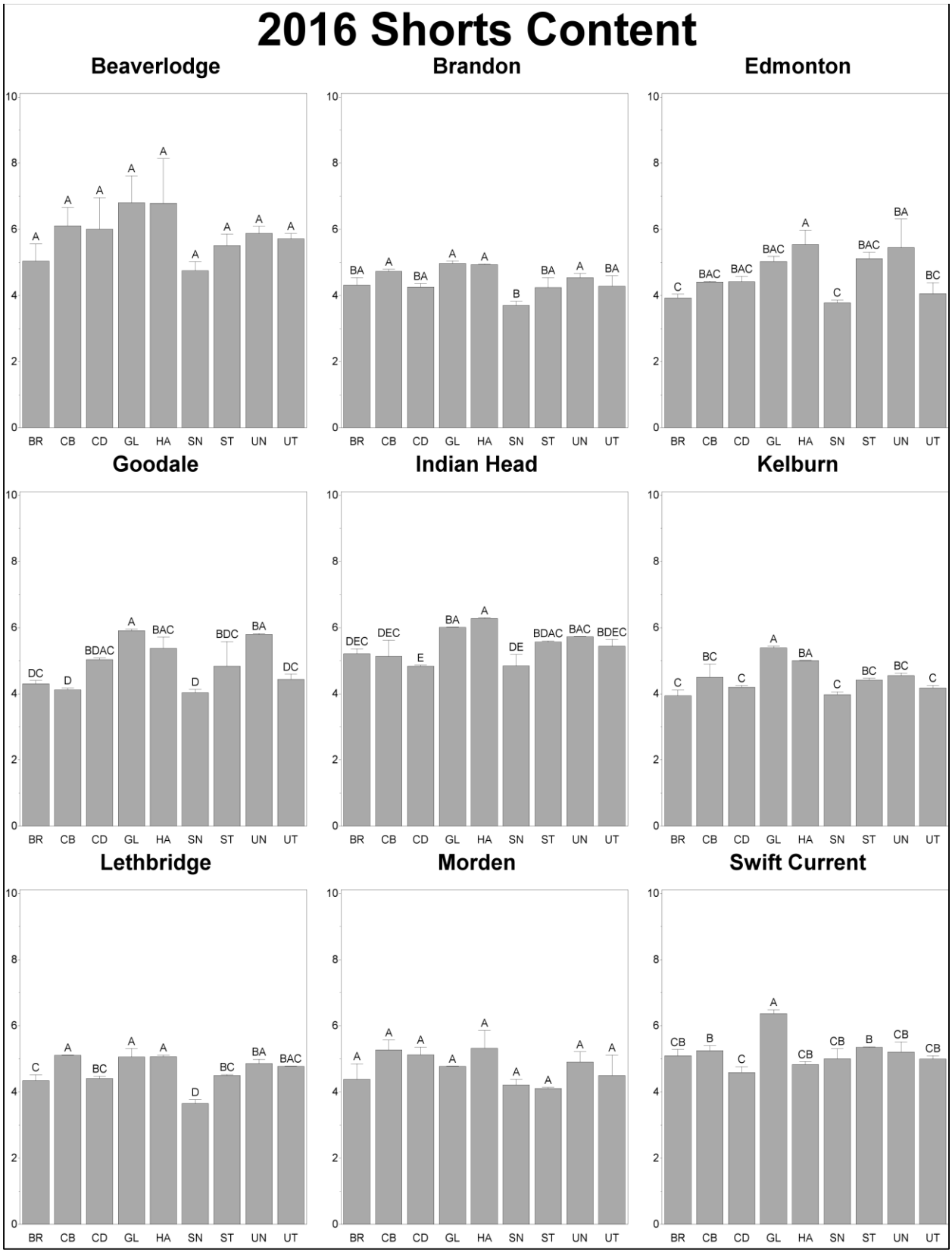
Appendix D.22 Average bran content (%) for 2016 genotypes within locations.



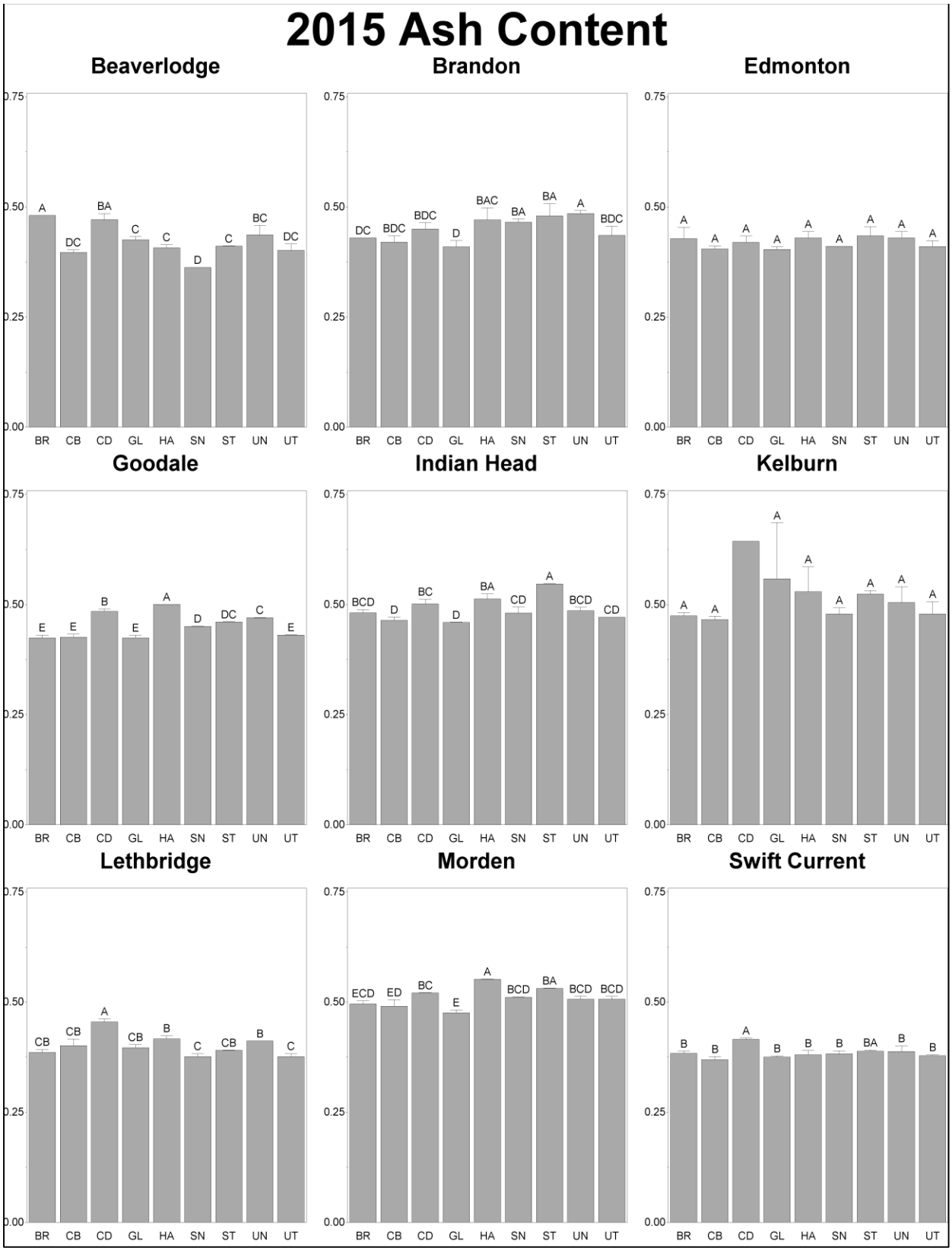
Appendix D.23 Average shorts content (%) for 2015 genotypes within locations.



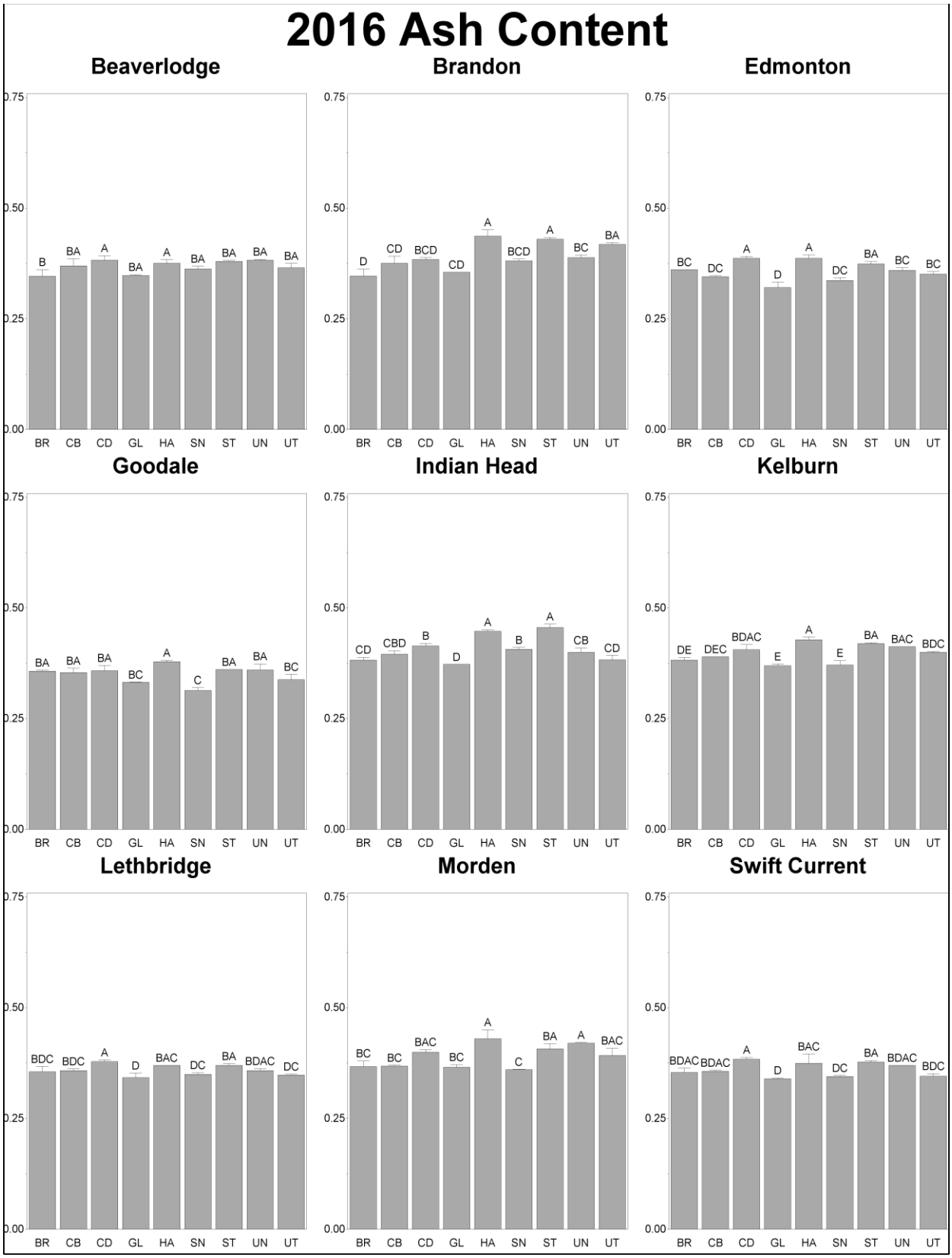
Appendix D.24 Average shorts content (%) for 2016 genotypes within locations.



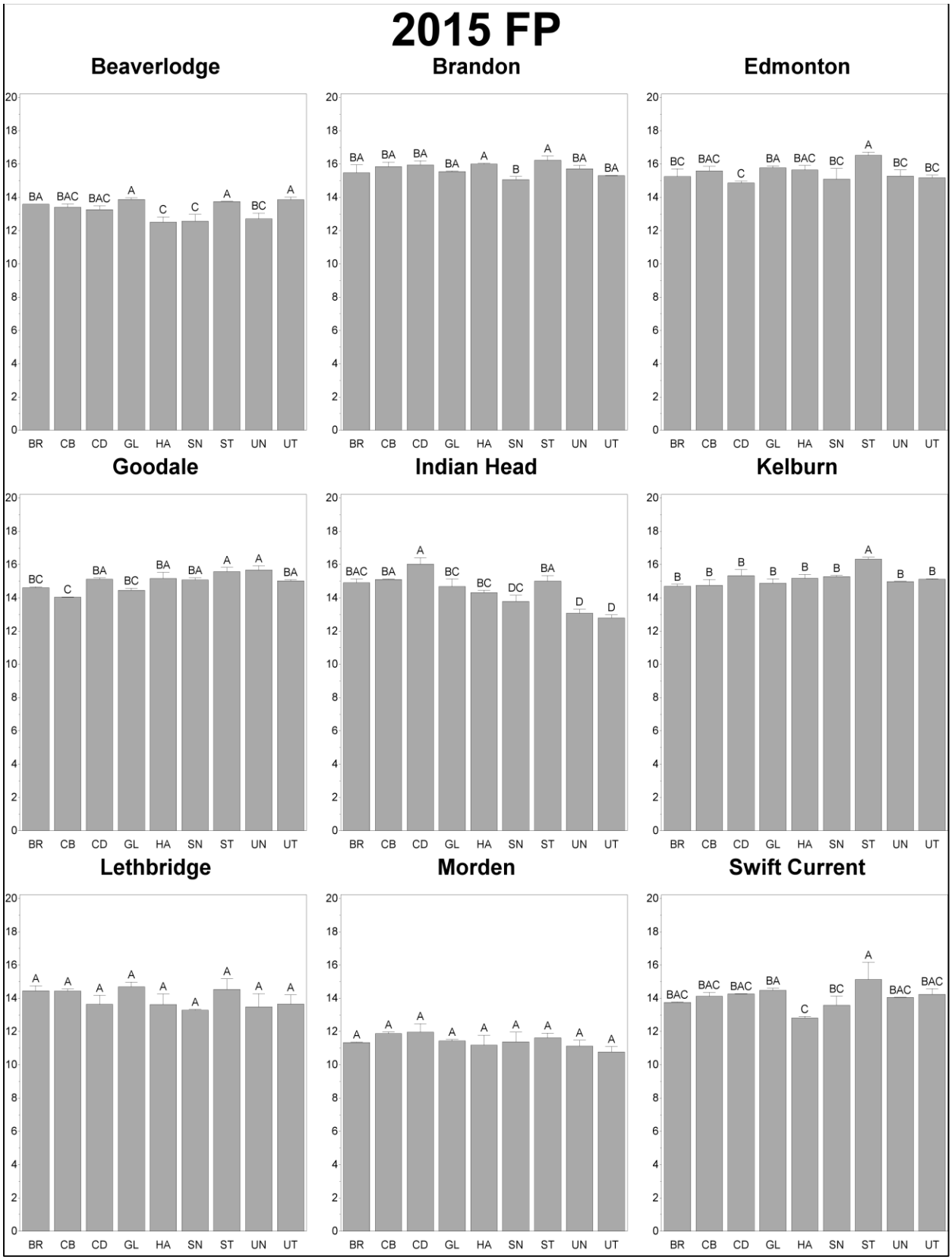
Appendix D.25 Average ash content (%) for 2015 genotypes within locations.



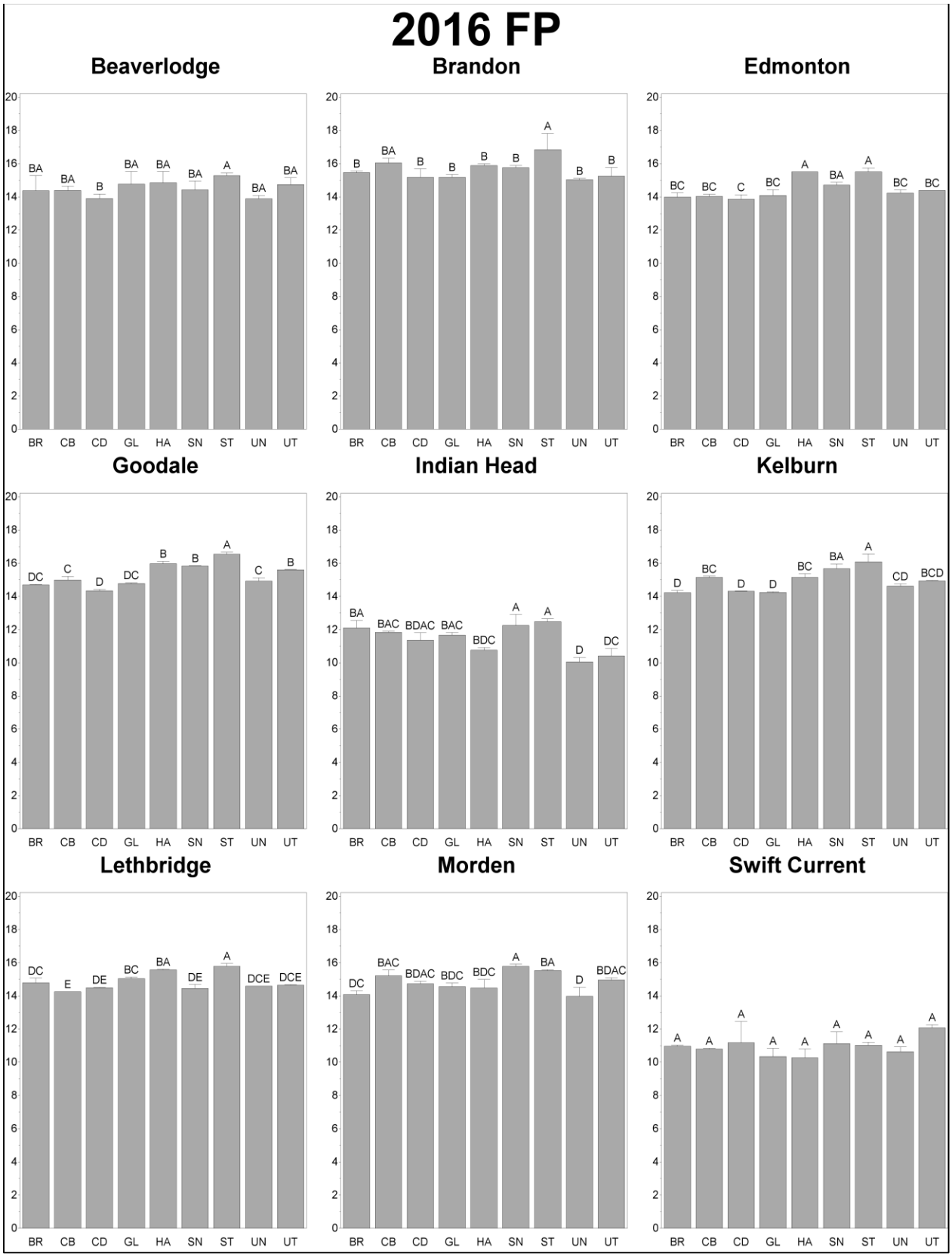
Appendix D.26 Average ash content (%) for 2016 genotypes within locations.



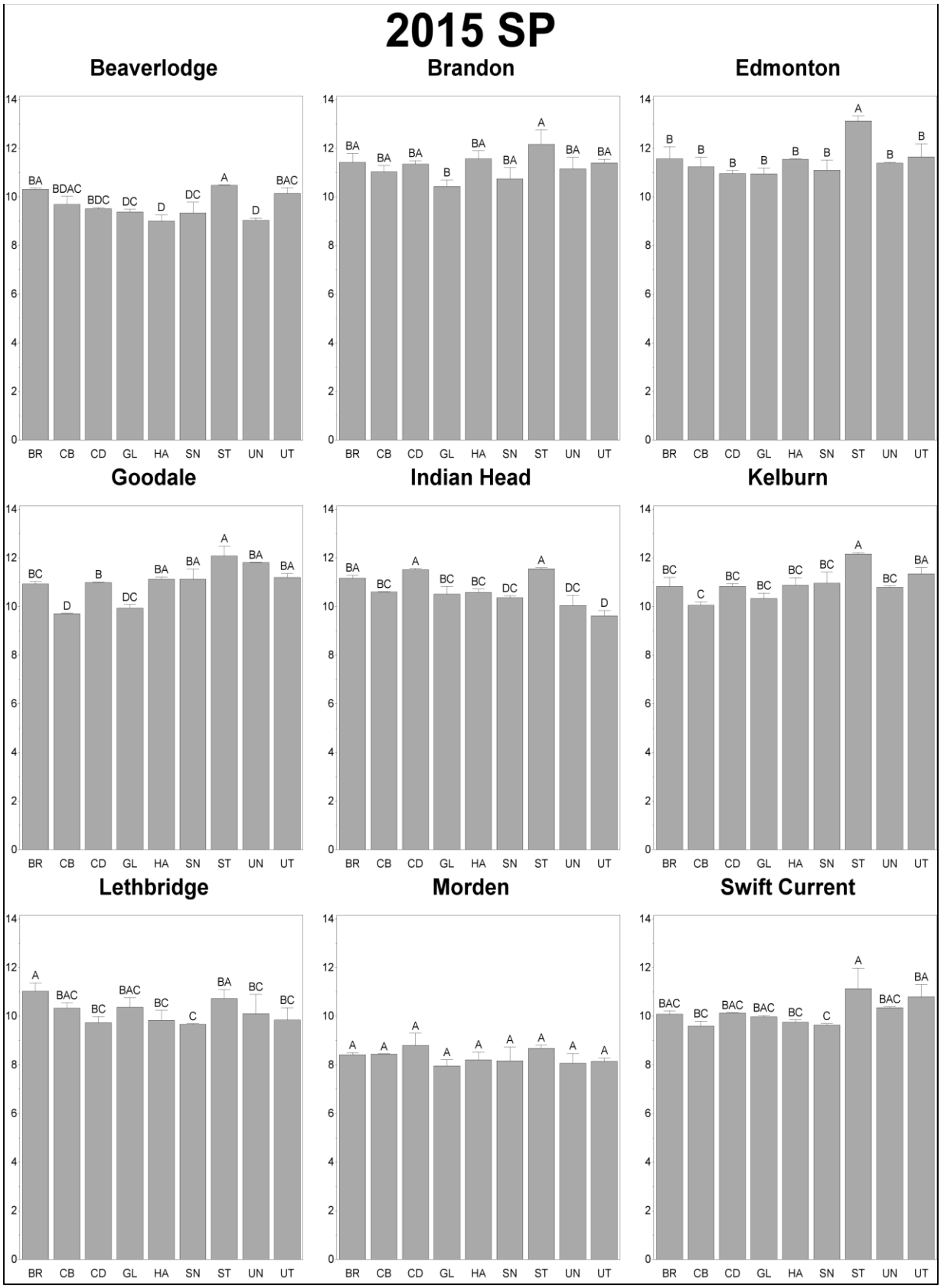
Appendix D.27 Average flour protein content (FP, %) for 2015 genotypes within locations.



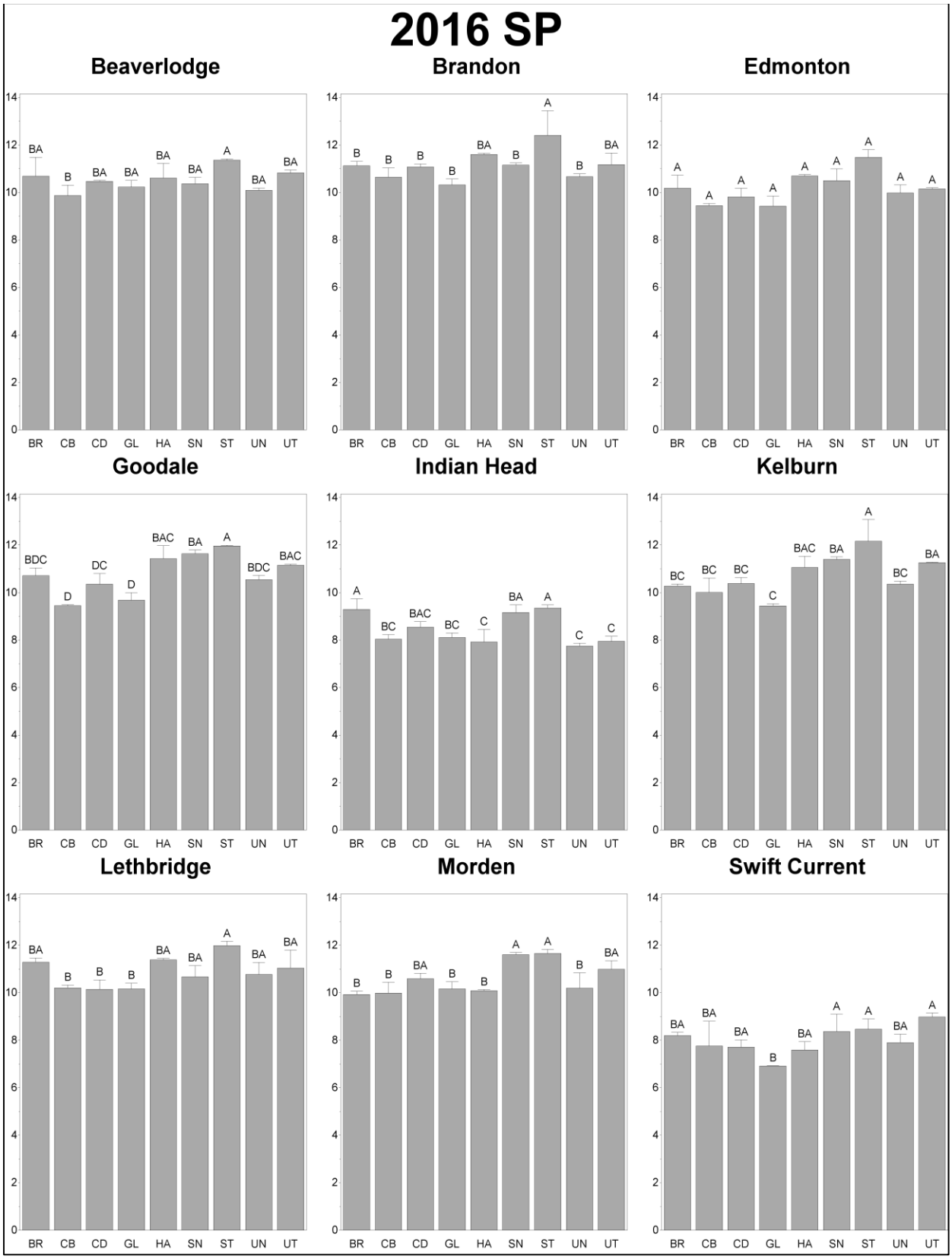
Appendix D.28 Average flour protein content (FP, %) for 2016 genotypes within locations.



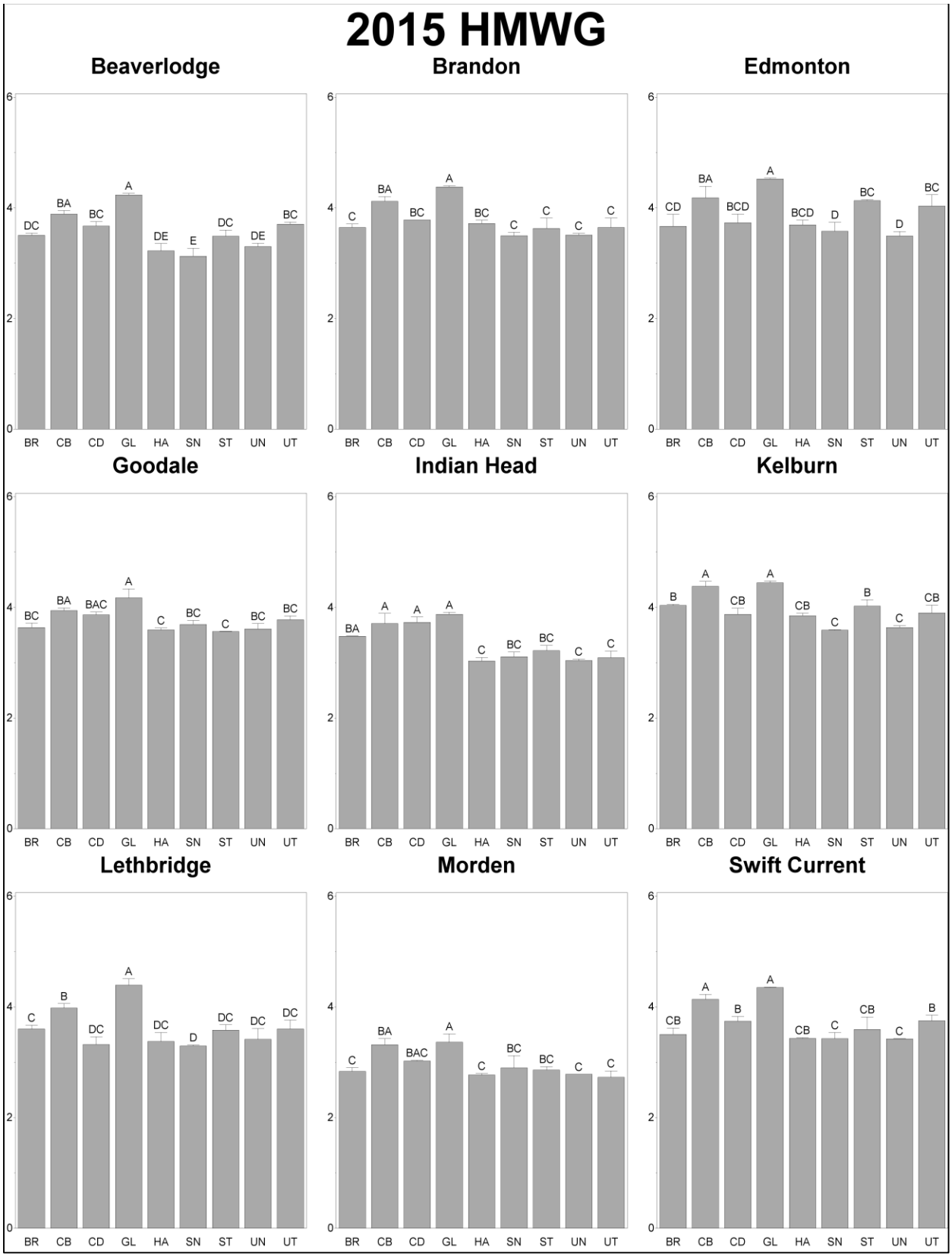
Appendix D.29 Average soluble prolamin content (SP, %) for 2015 genotypes within locations.



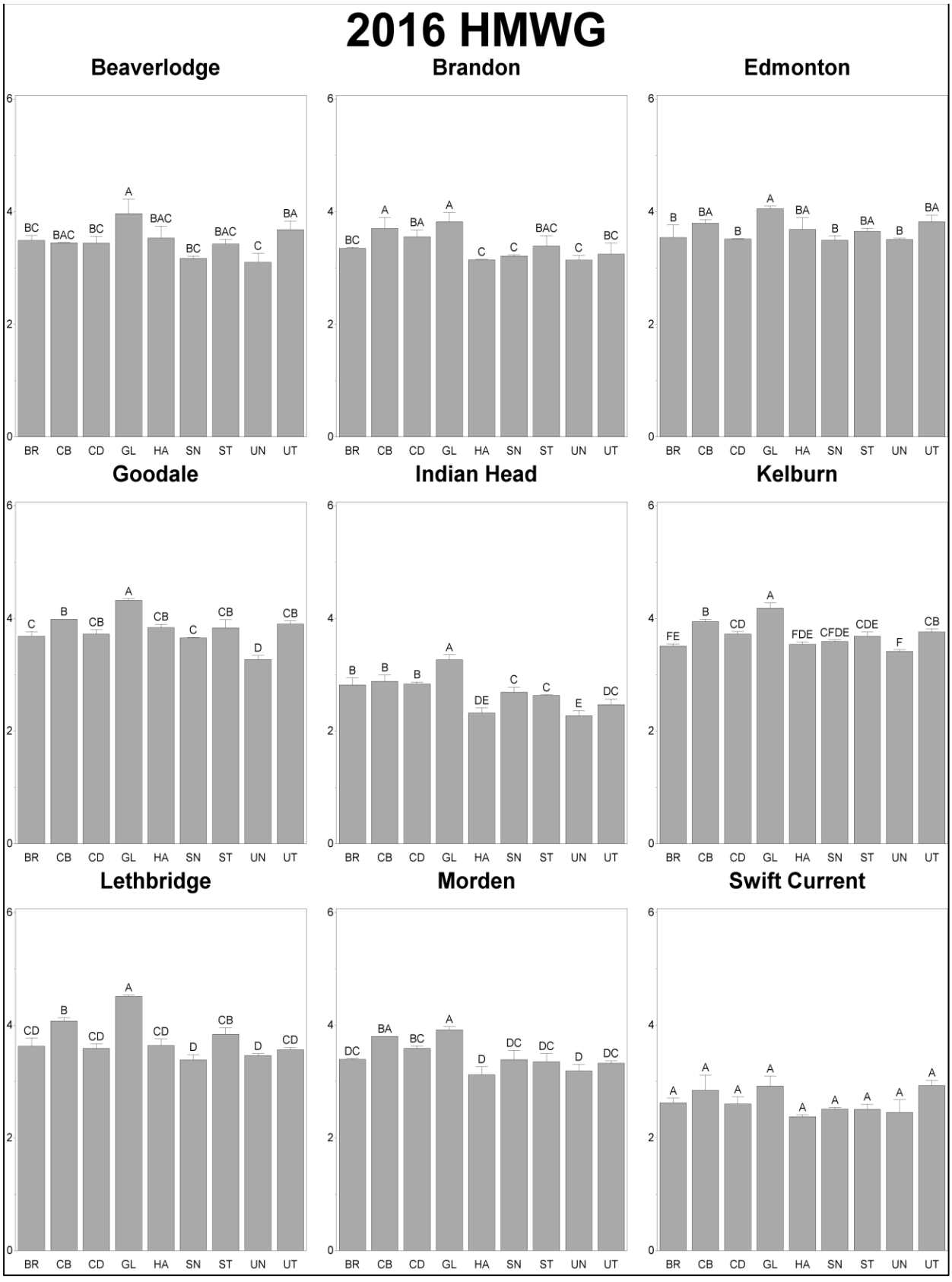
Appendix D.30 Average soluble prolamin content (SP, %) for 2016 genotypes within locations.



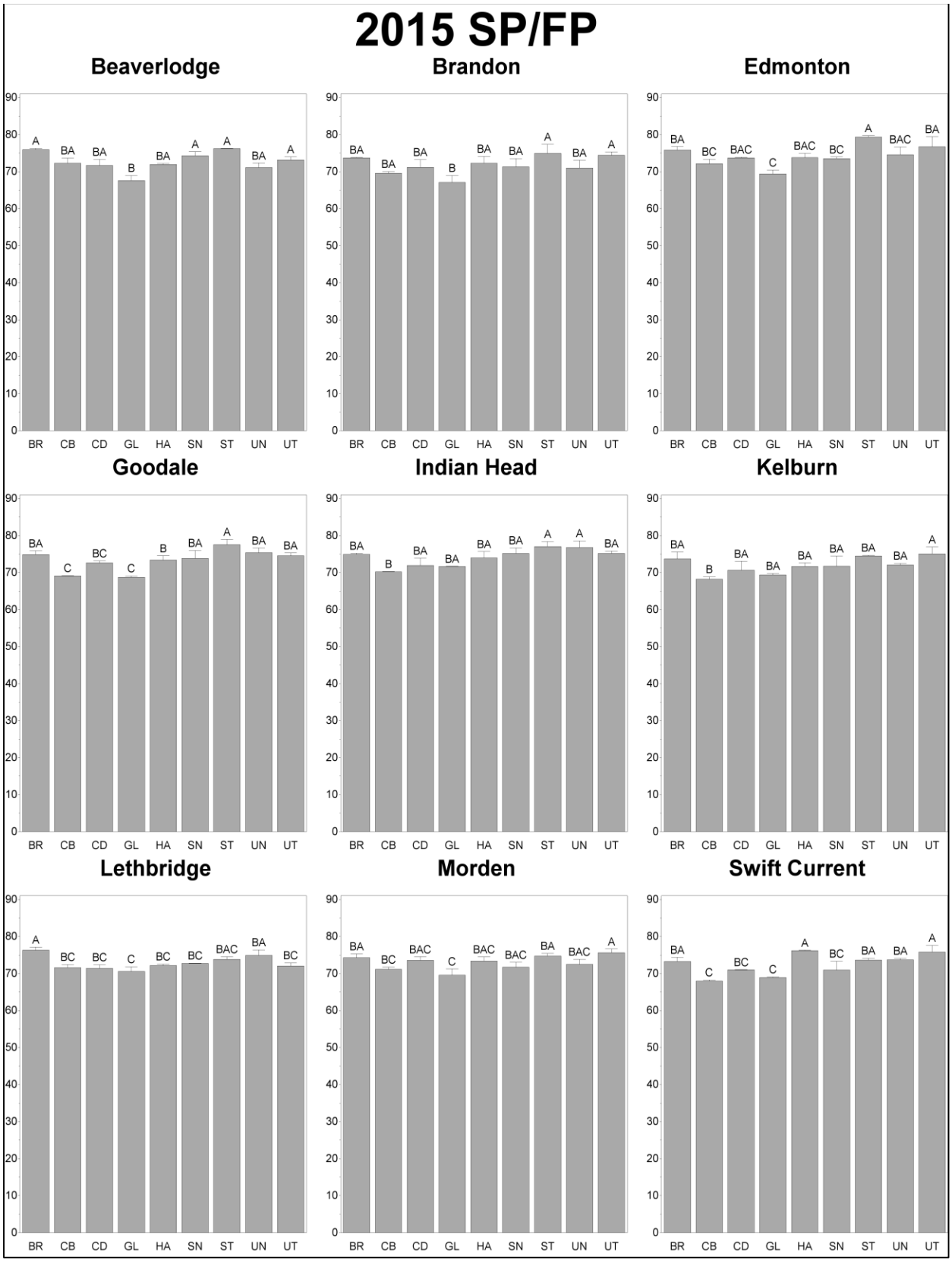
Appendix D.31 Average HMW glutenin content (HMWG, %) for 2015 genotypes within locations.



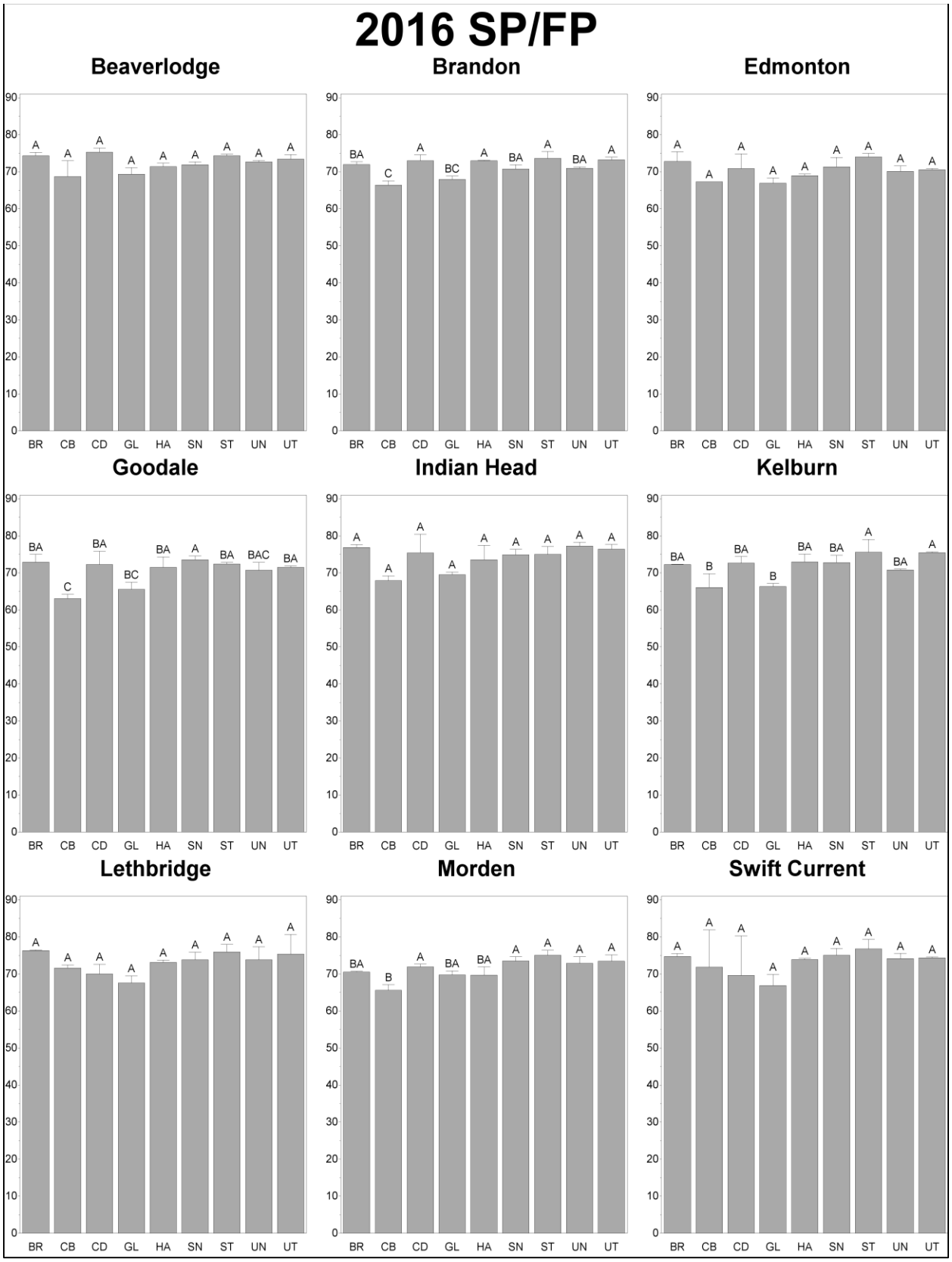
Appendix D.32 Average HMW glutenin content (HMWG, %) for 2016 genotypes within locations.



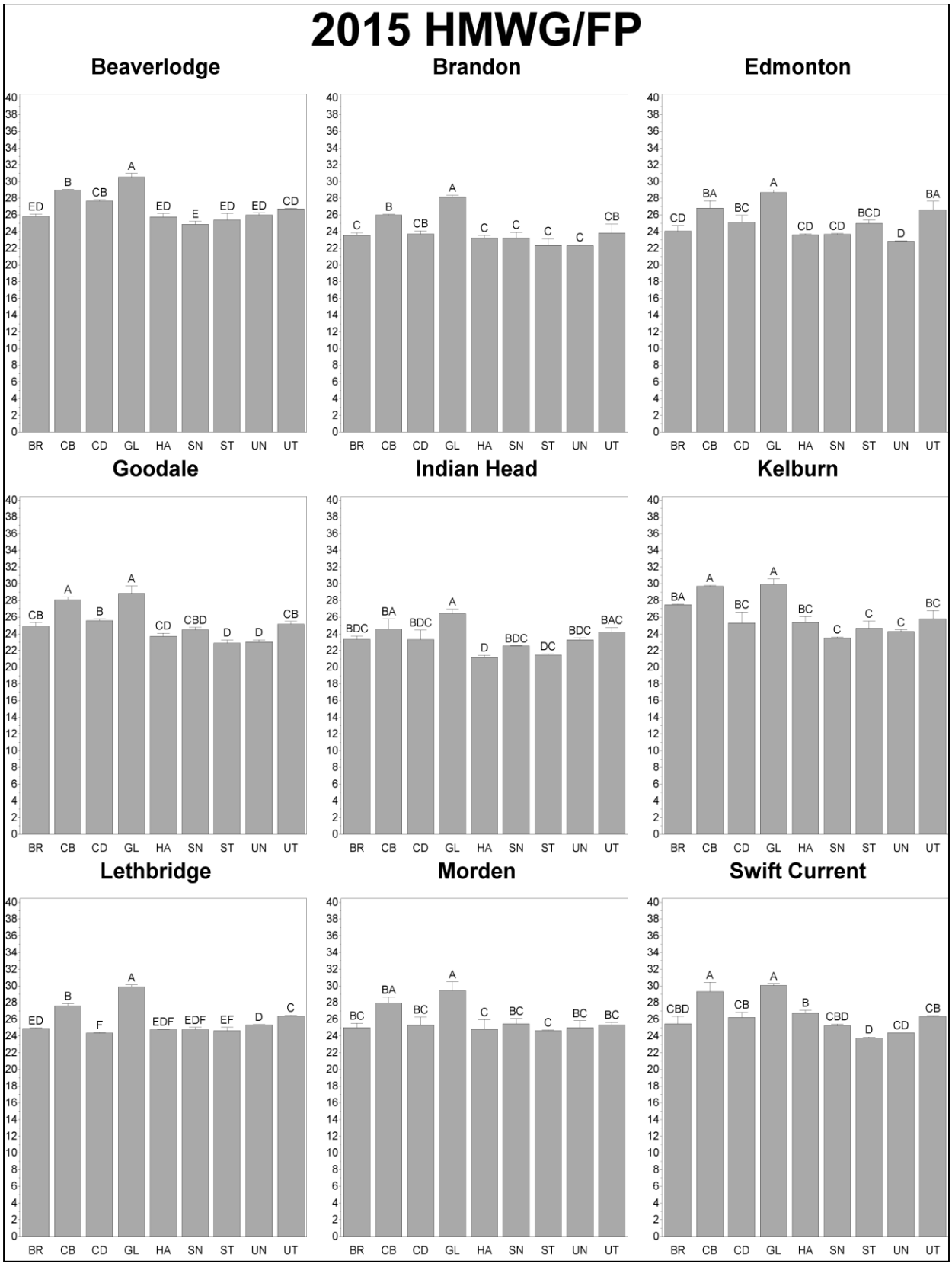
Appendix D.33 Average SP/FP (%) for 2015 genotypes within locations.



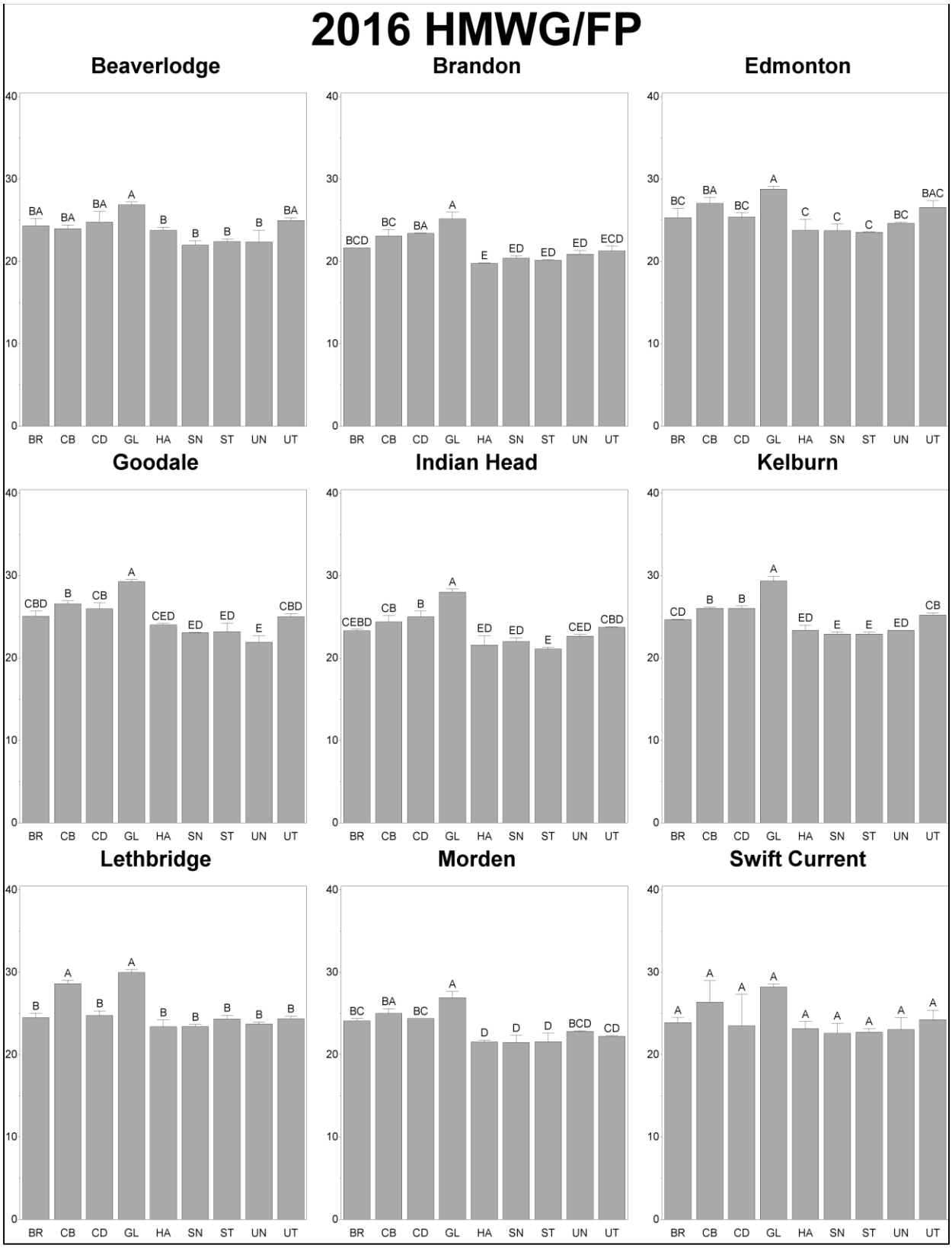
Appendix D.34 Average SP/FP (%) for 2016 genotypes within locations.



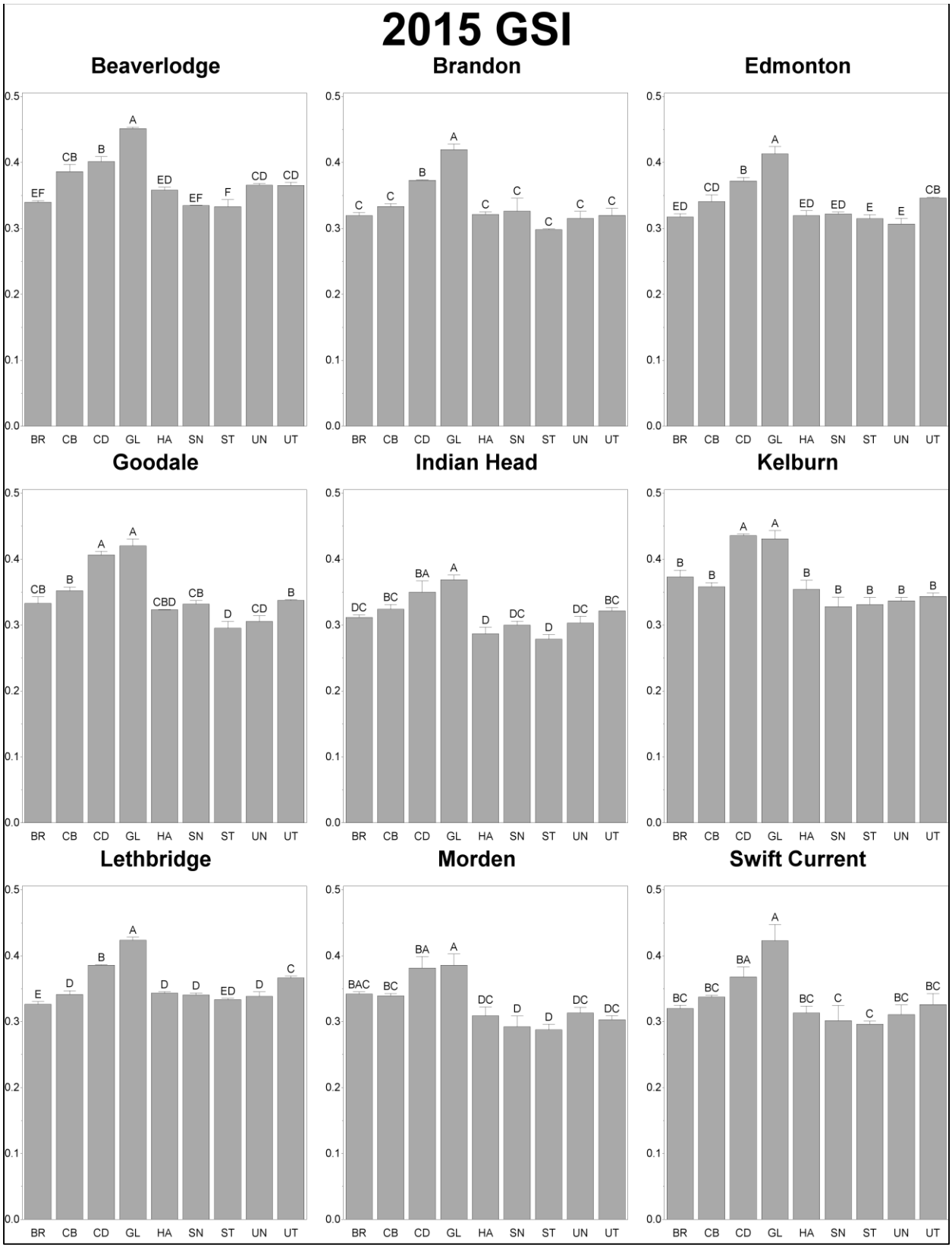
Appendix D.35 Average HMWG/FP (%) for 2015 genotypes within locations.



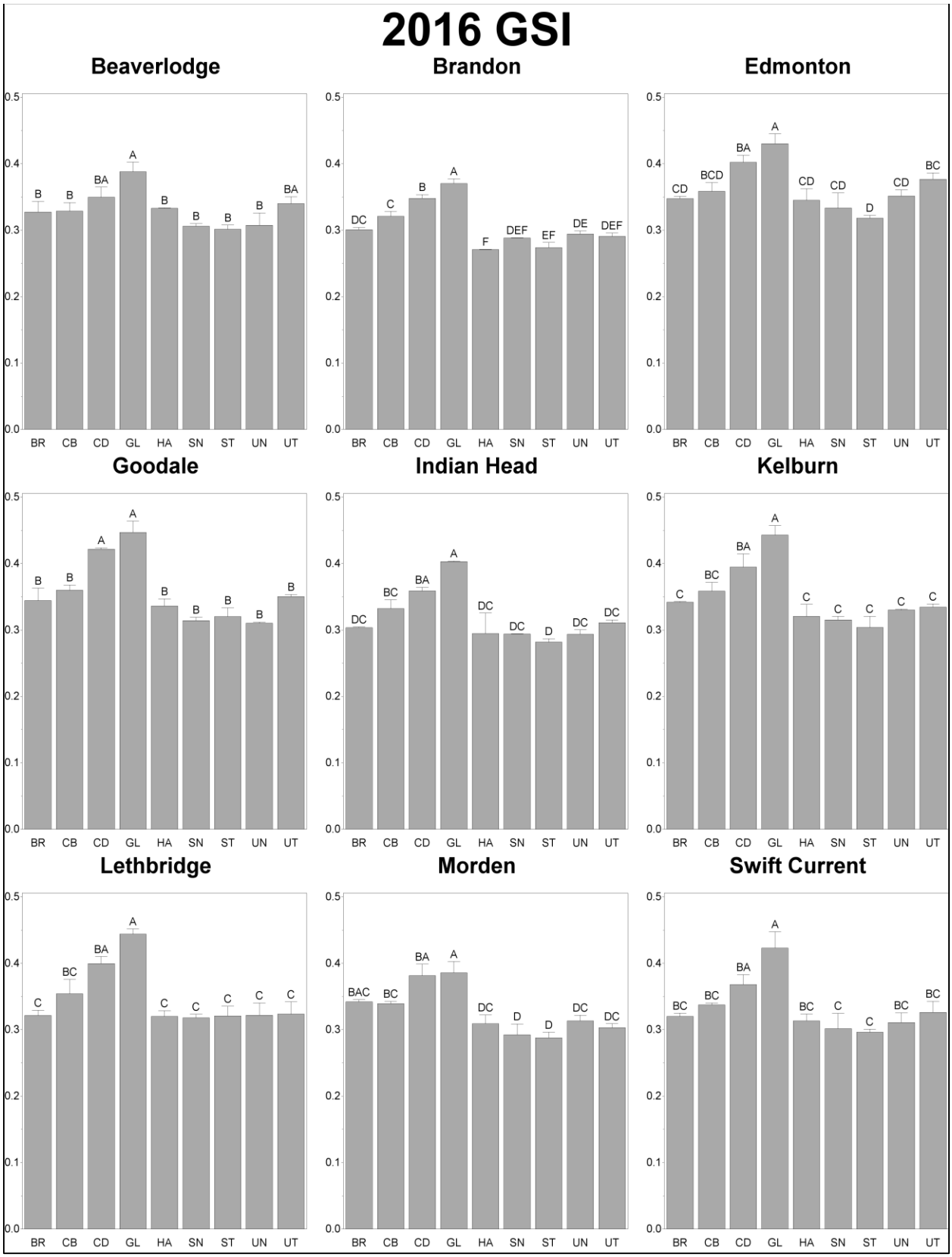
Appendix D.36 Average HMWG/FP (%) for 2016 genotypes within locations.



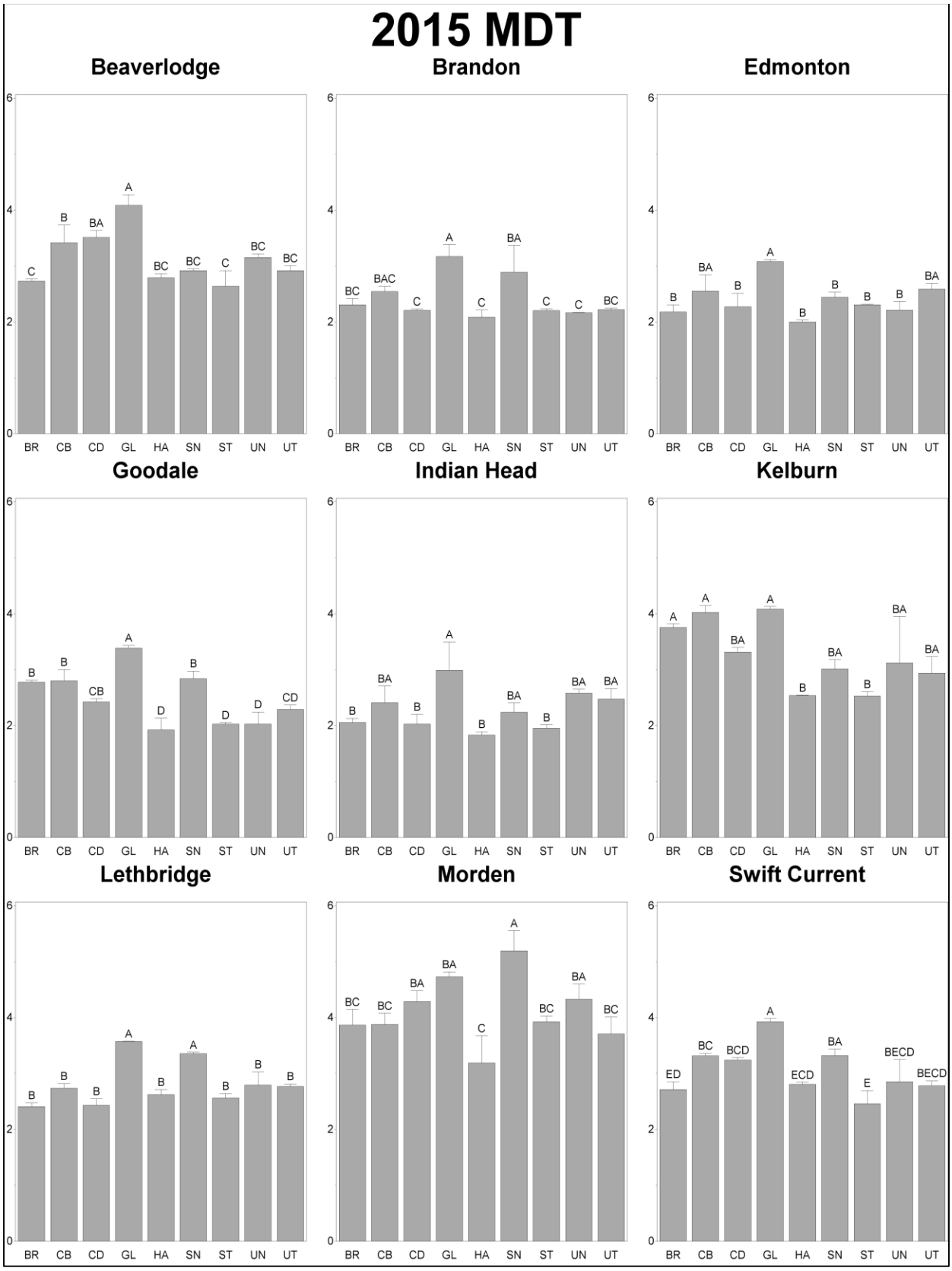
Appendix D.37 Average GSI for 2015 genotypes within locations.



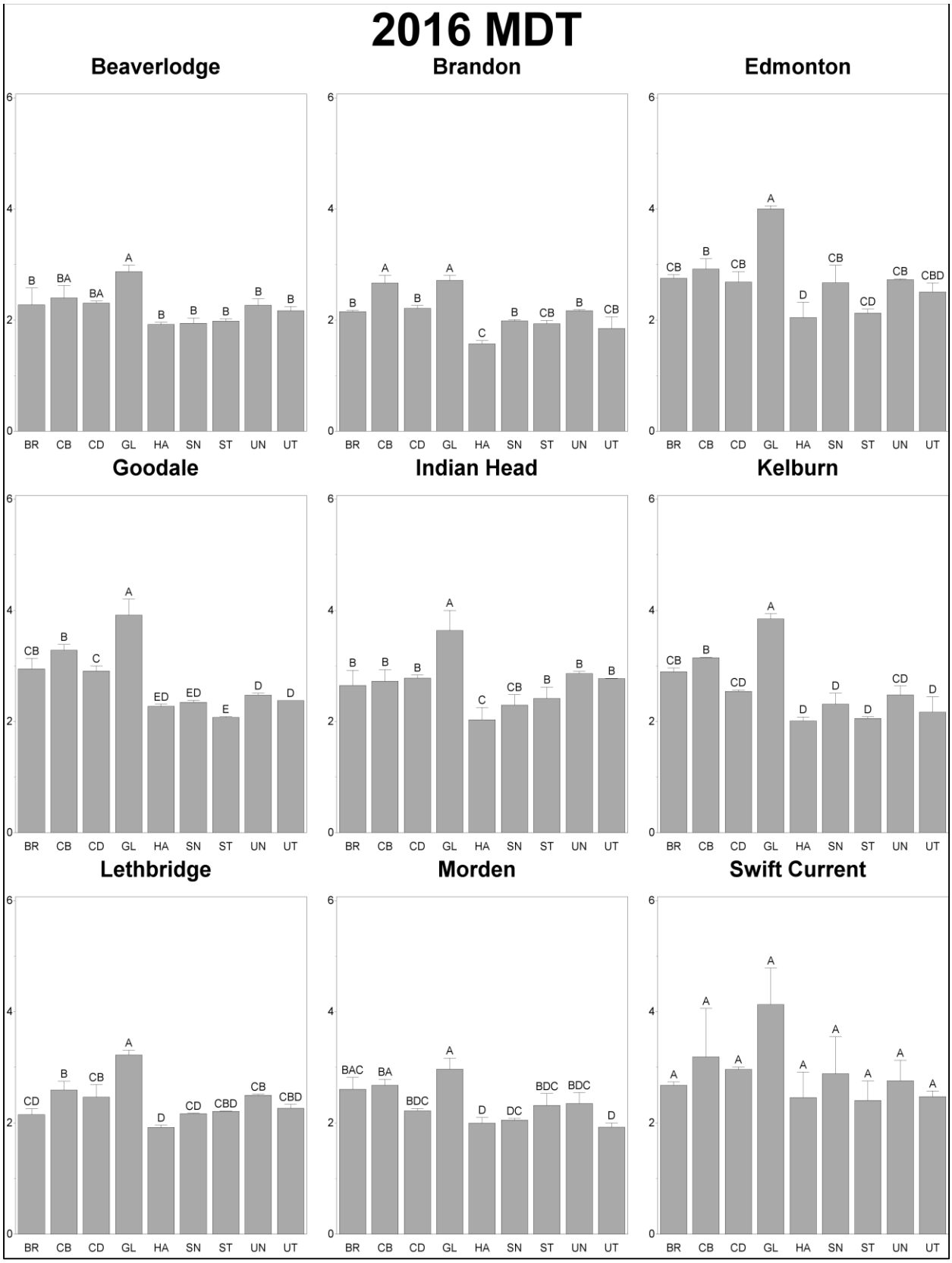
Appendix D.38 Average GSI for 2016 genotypes within locations.



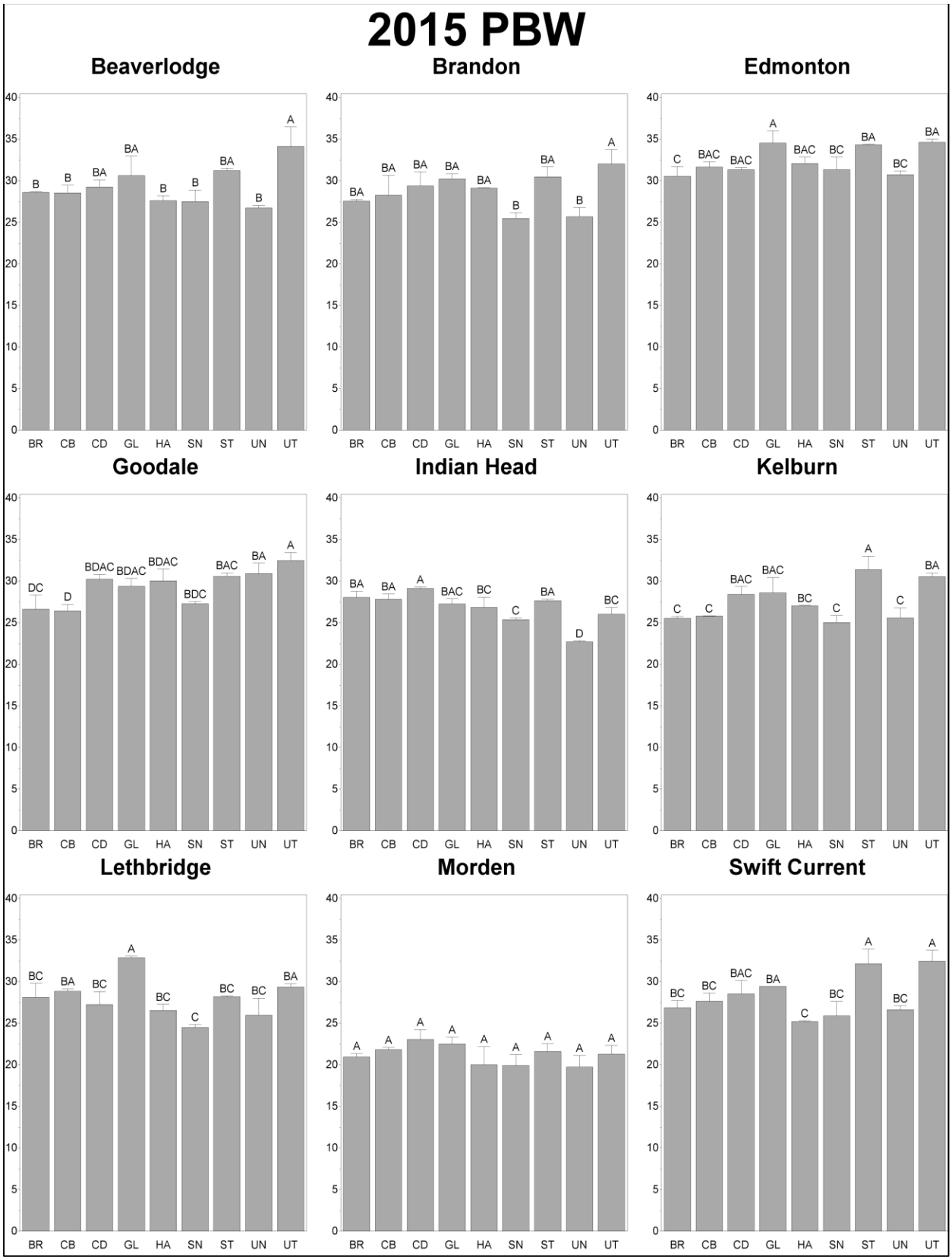
Appendix D.39 Average mixing development time (MDT, min) for 2015 genotypes within locations.



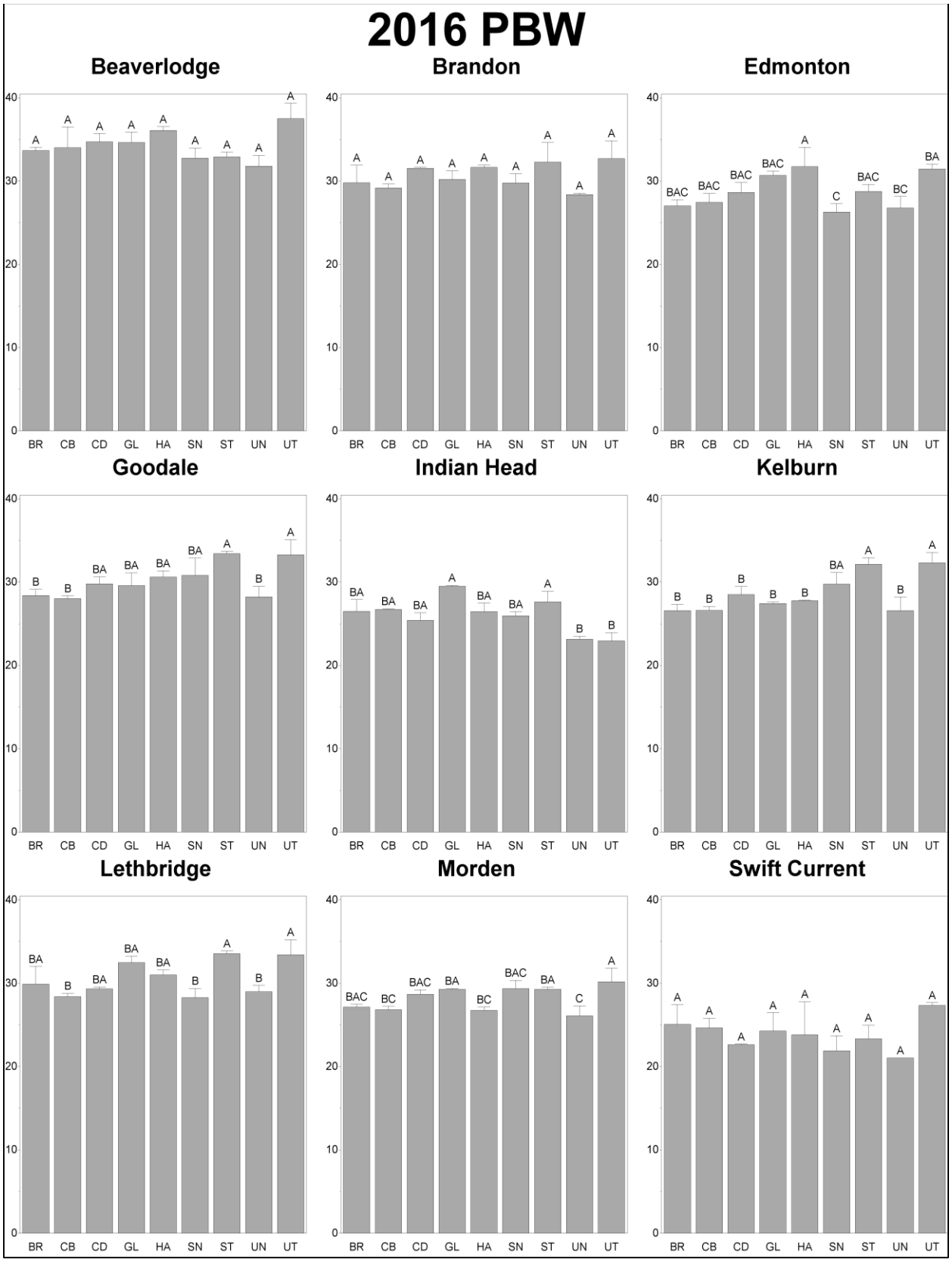
Appendix D.40 Average mixing development time (MDT, min) for 2016 genotypes within locations.



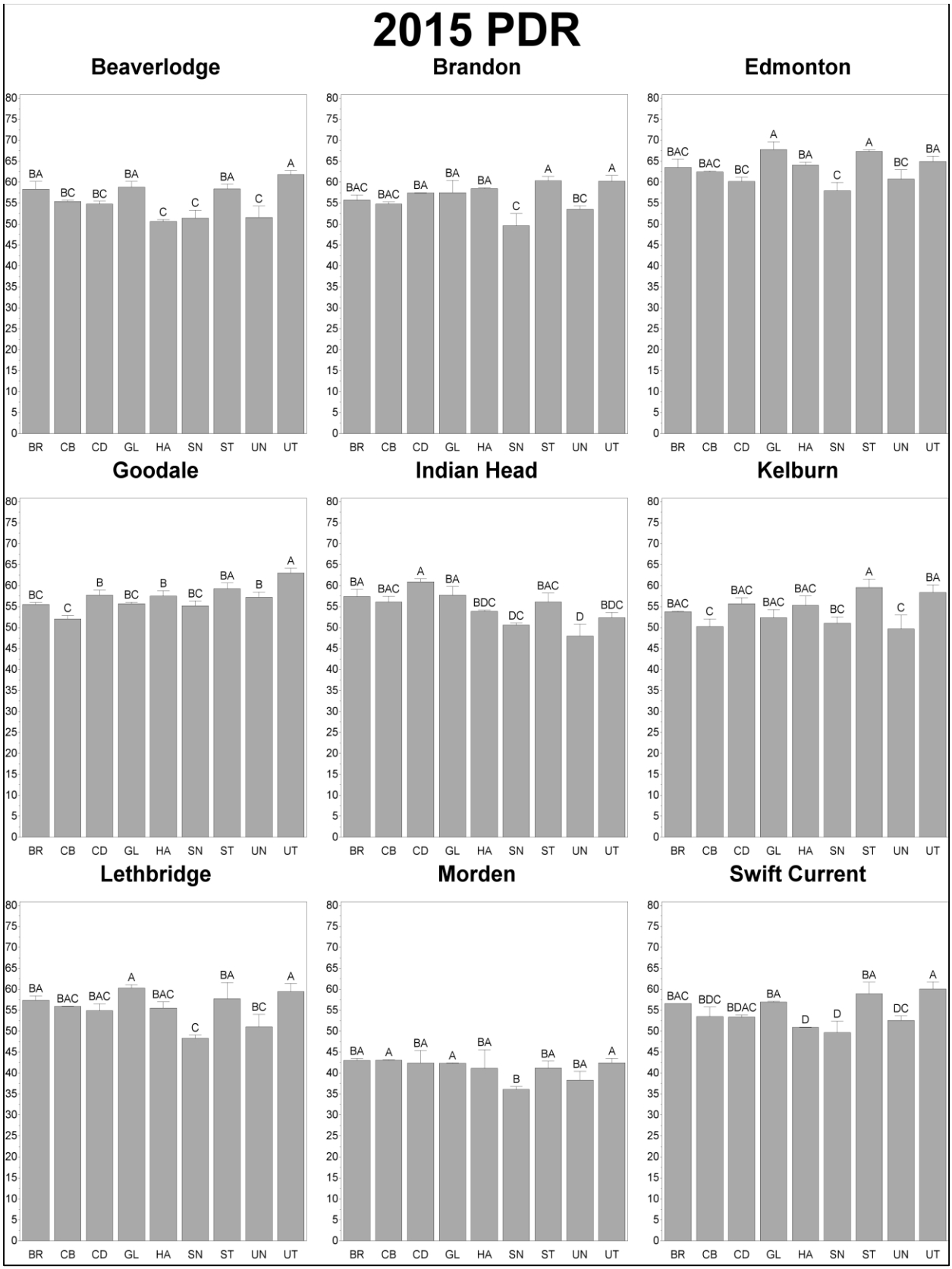
Appendix D.41 Average peak band width (PBW, %Torque) for 2015 genotypes within locations.



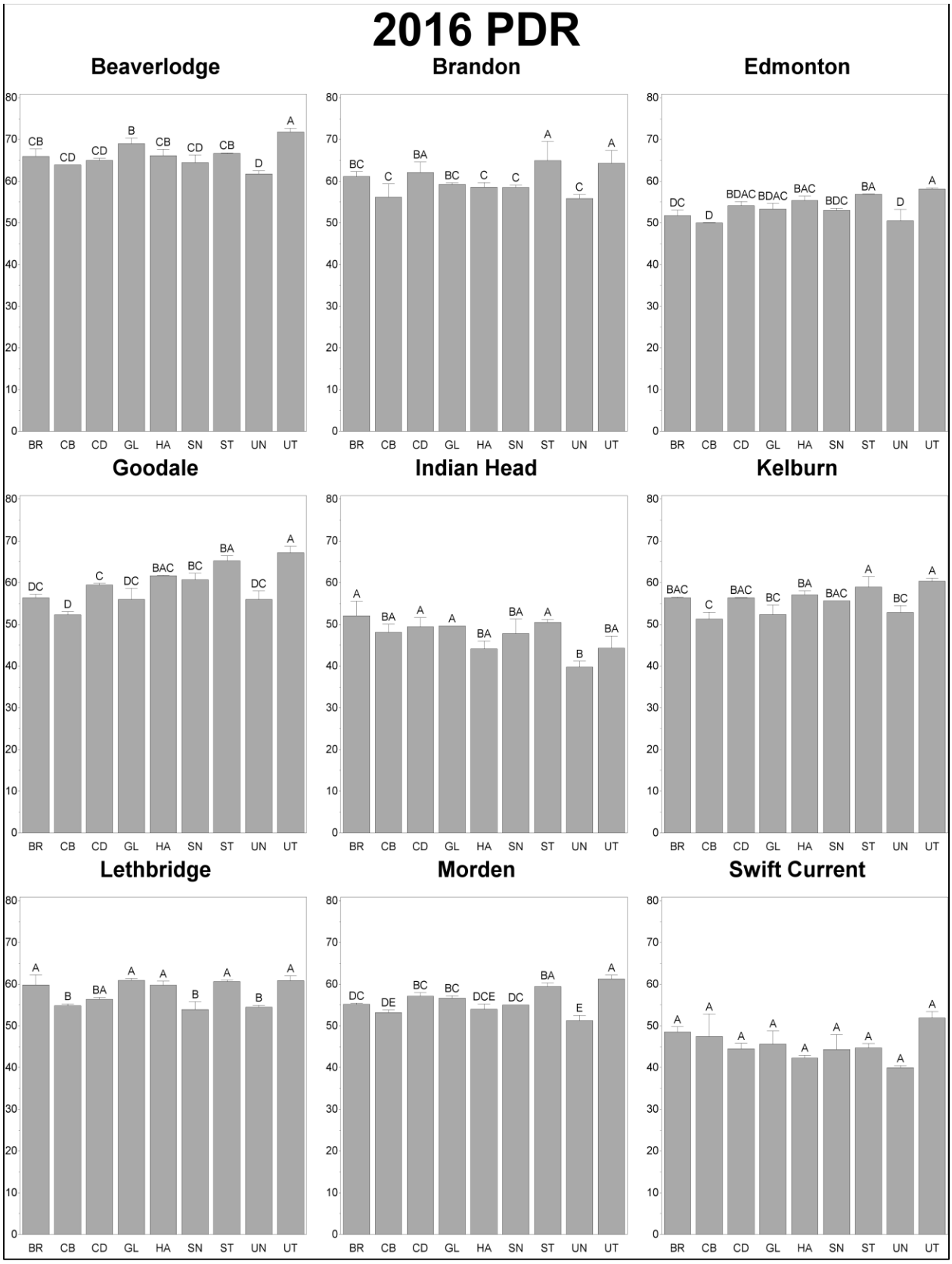
Appendix D.42 Average peak band width (PBW, %Torque) for 2016 genotypes within locations.



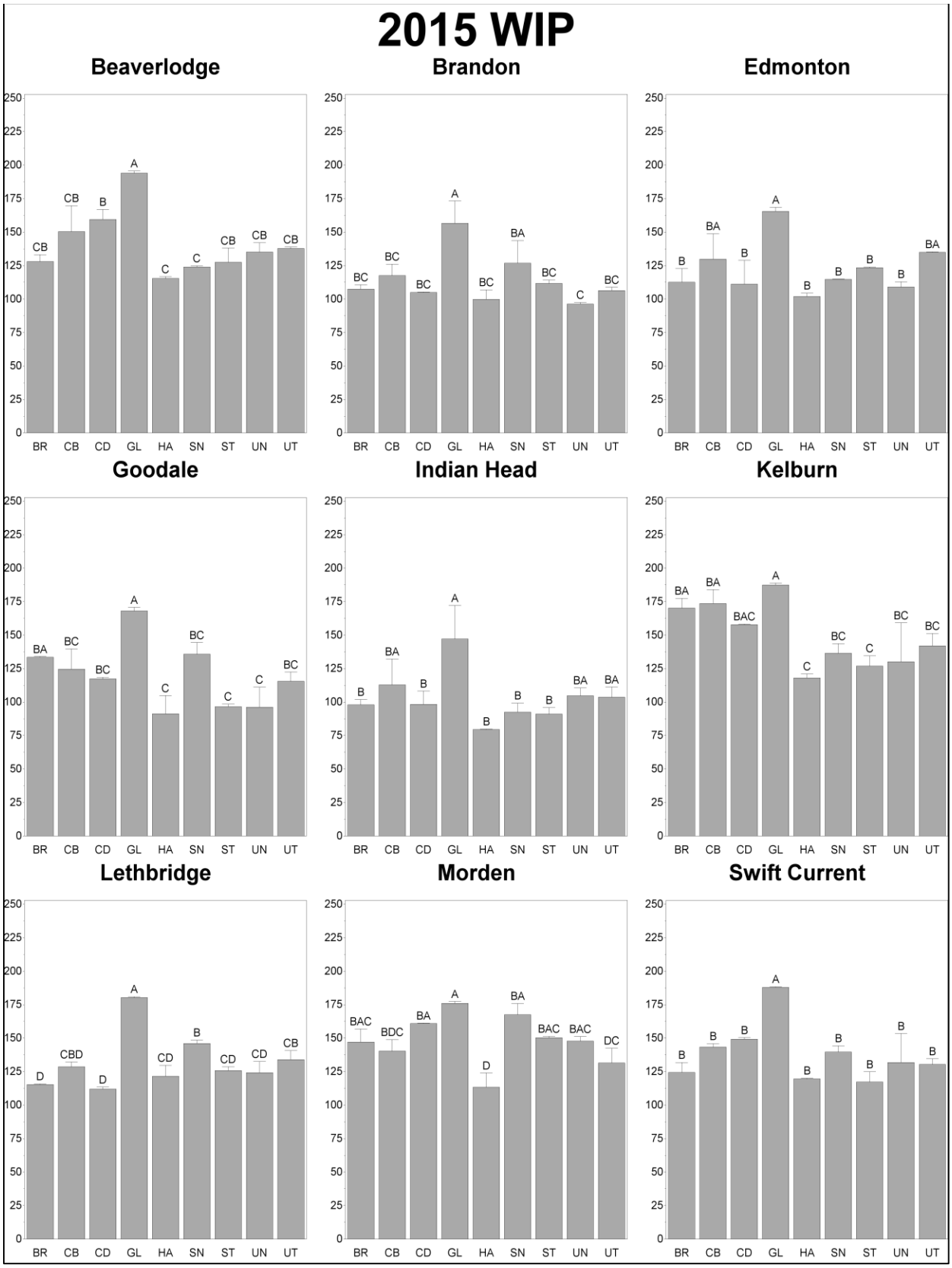
Appendix D.43 Average peak dough resistance (PDR, %Torque) for 2015 genotypes within locations.



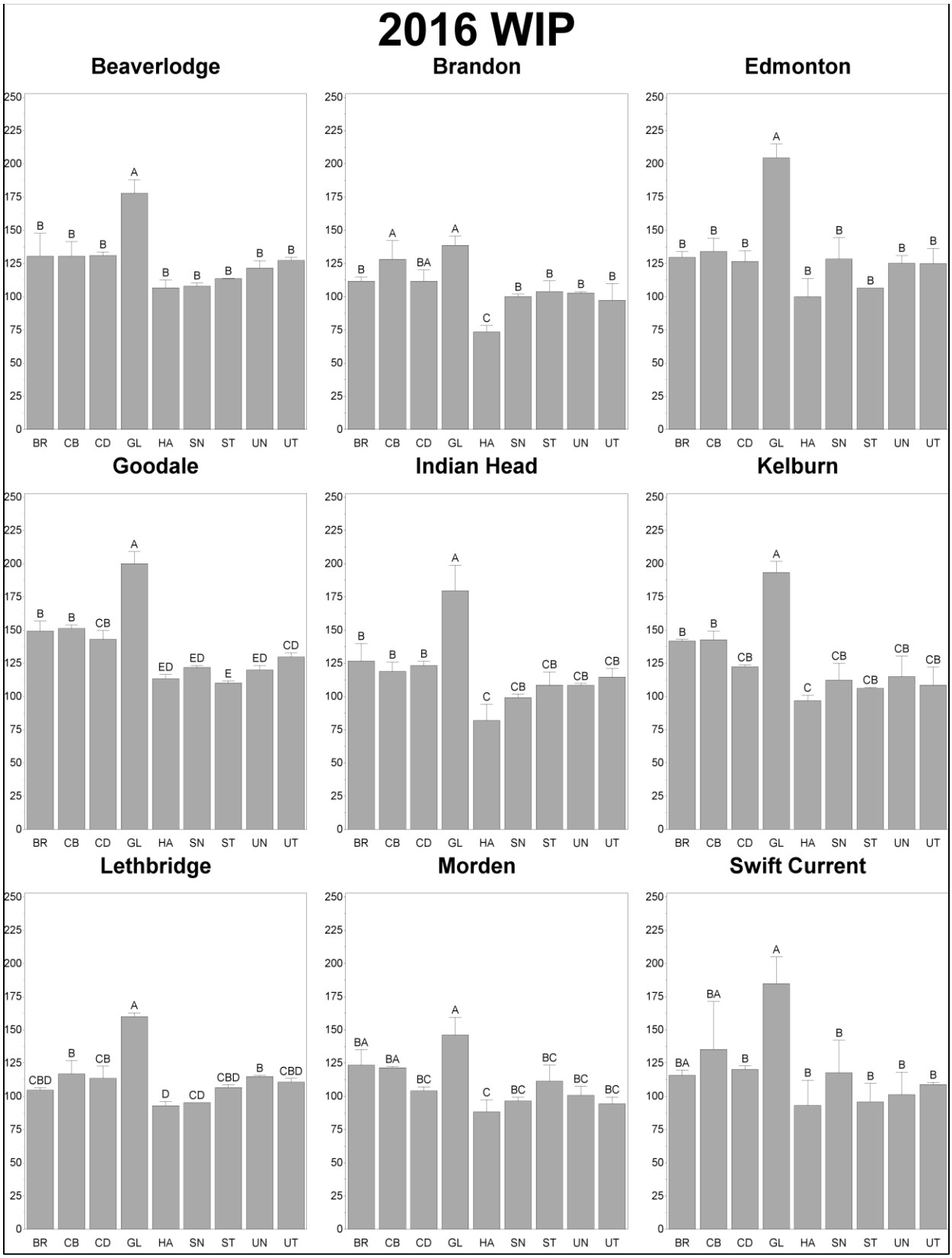
Appendix D.44 Average peak dough resistance (PDR, %Torque) for 2016 genotypes within locations.



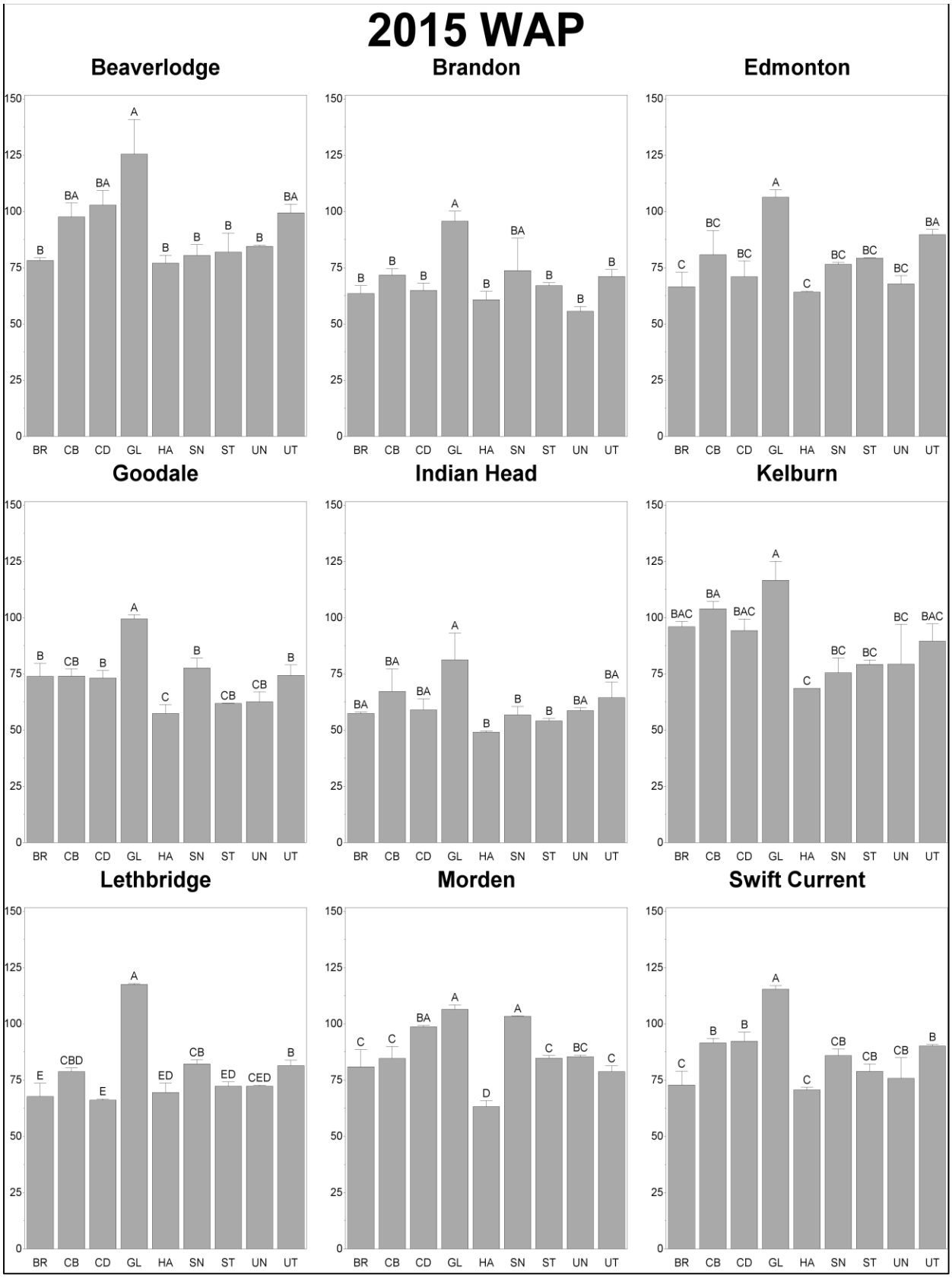
Appendix D.45 Average work input to peak (WIP, %Torque*min) for 2015 genotypes within locations.



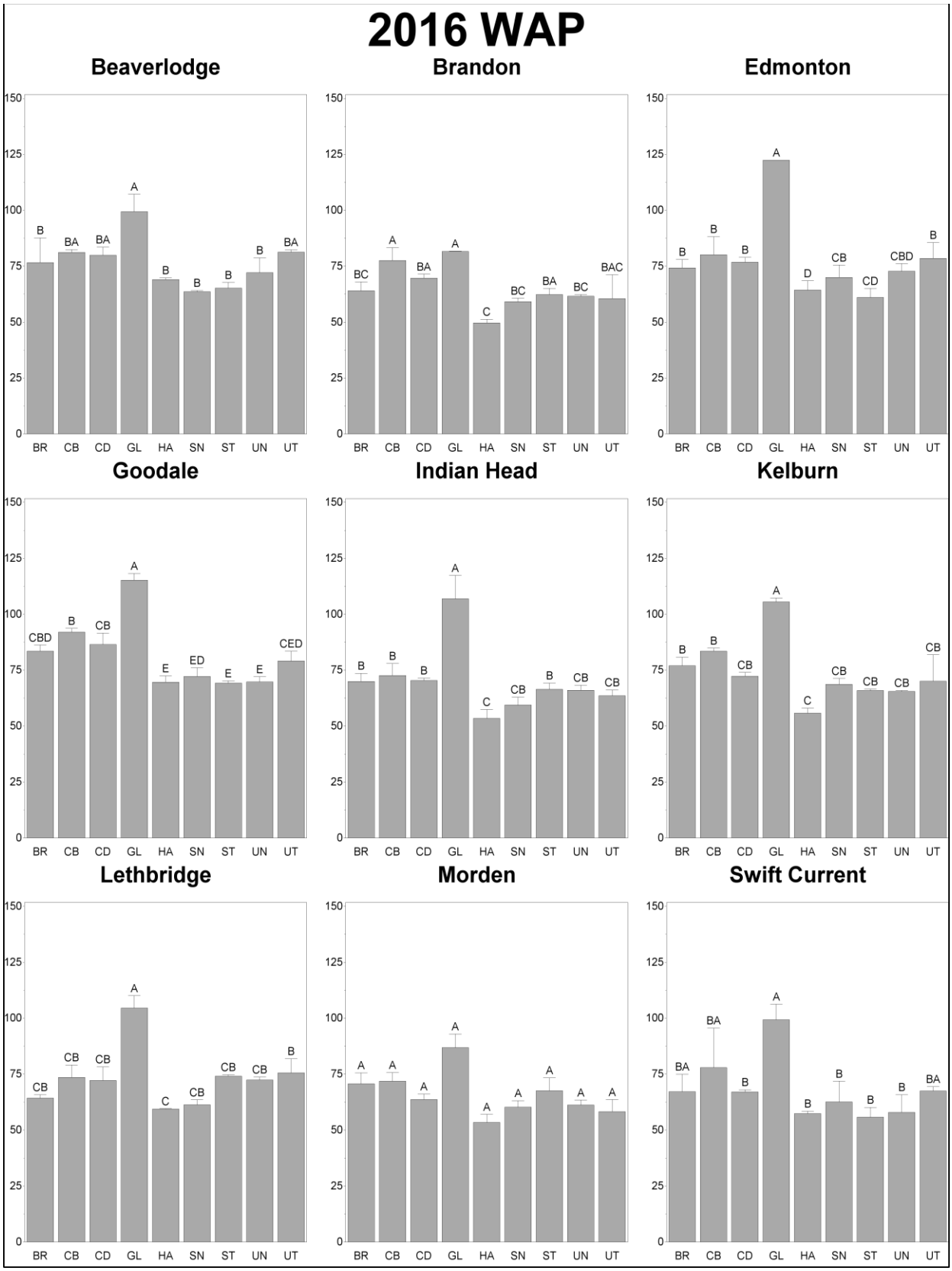
Appendix D.46 Average work input to peak (WIP, %Torque*min) for 2016 genotypes within locations.



Appendix D.47 Average work at peak (WAP, %Torque*min) for 2015 genotypes within locations.

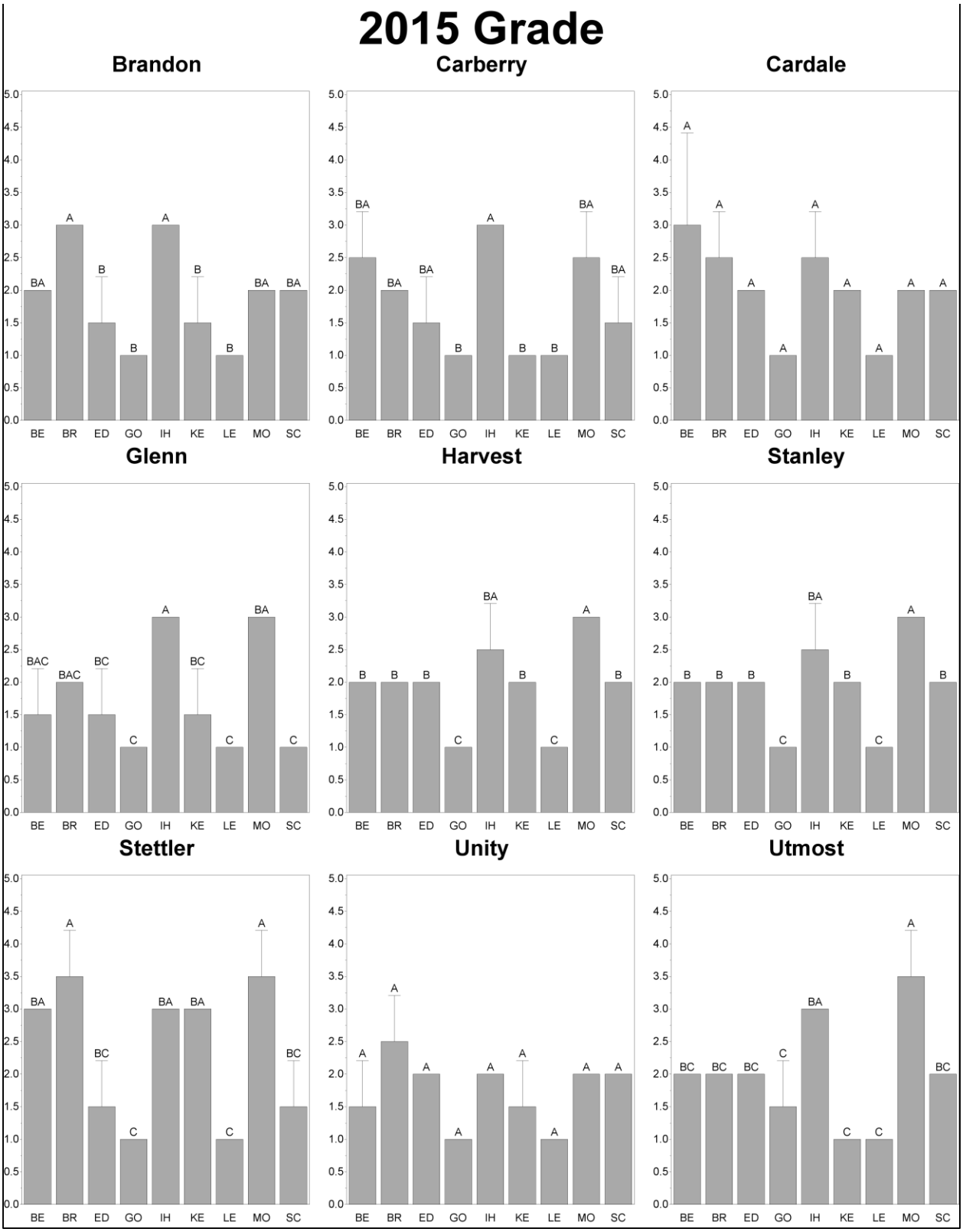


Appendix D.48 Average work at peak (WAP, %Torque*min) for 2016 genotypes within locations.

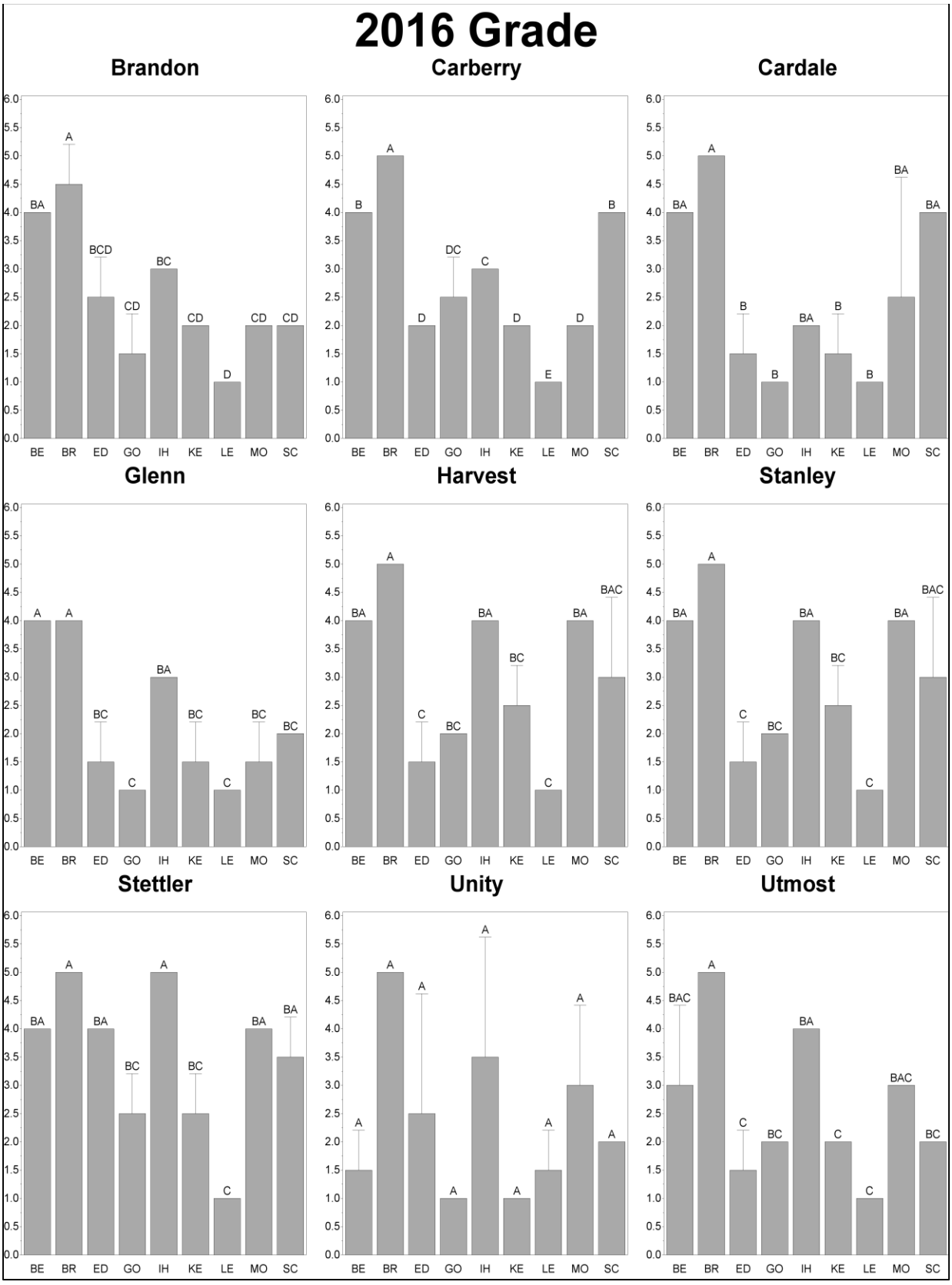


Appendix E. Wheat quality parameters location means within genotypes.

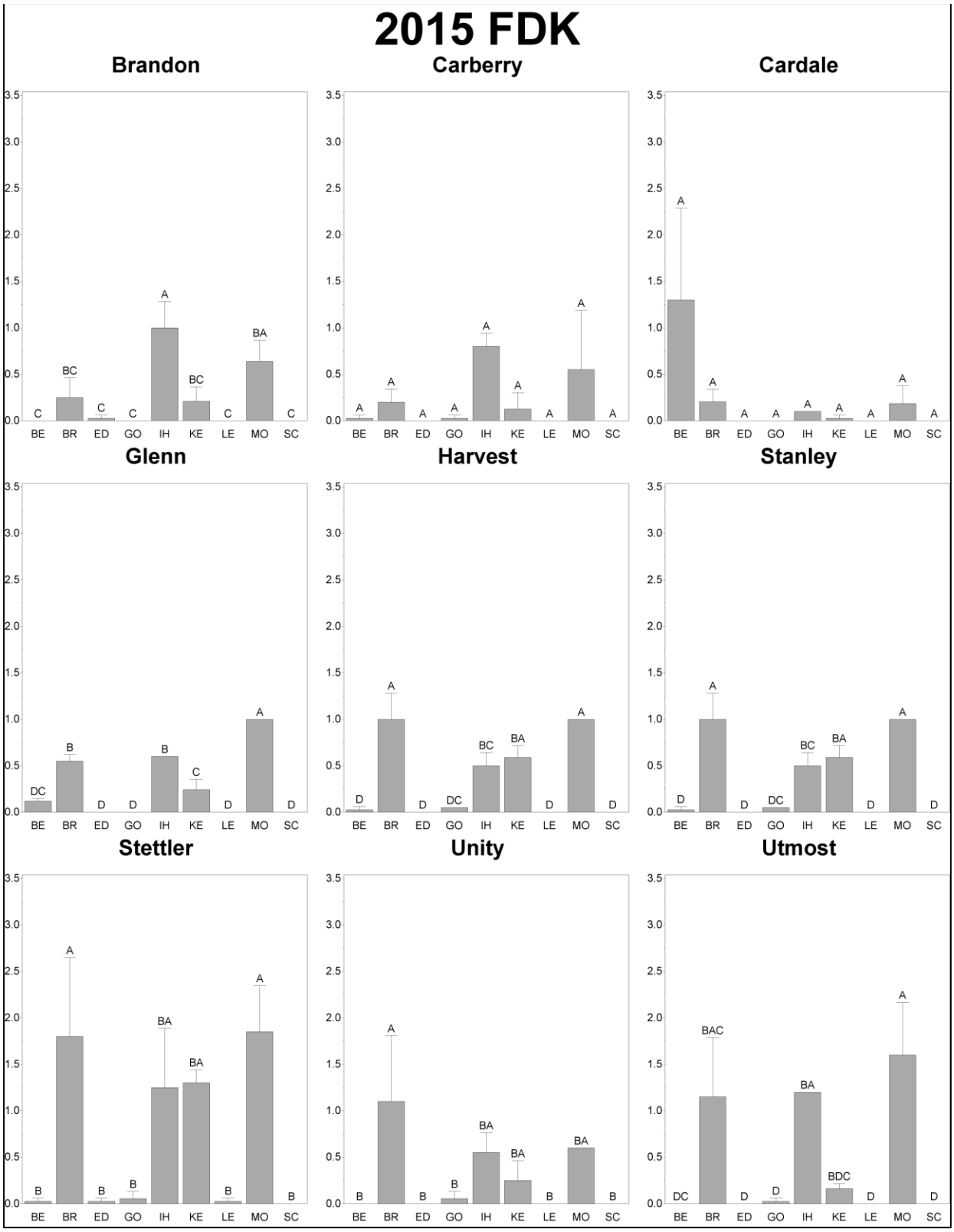
Appendix E.1 Average grade for 2015 locations within genotypes.



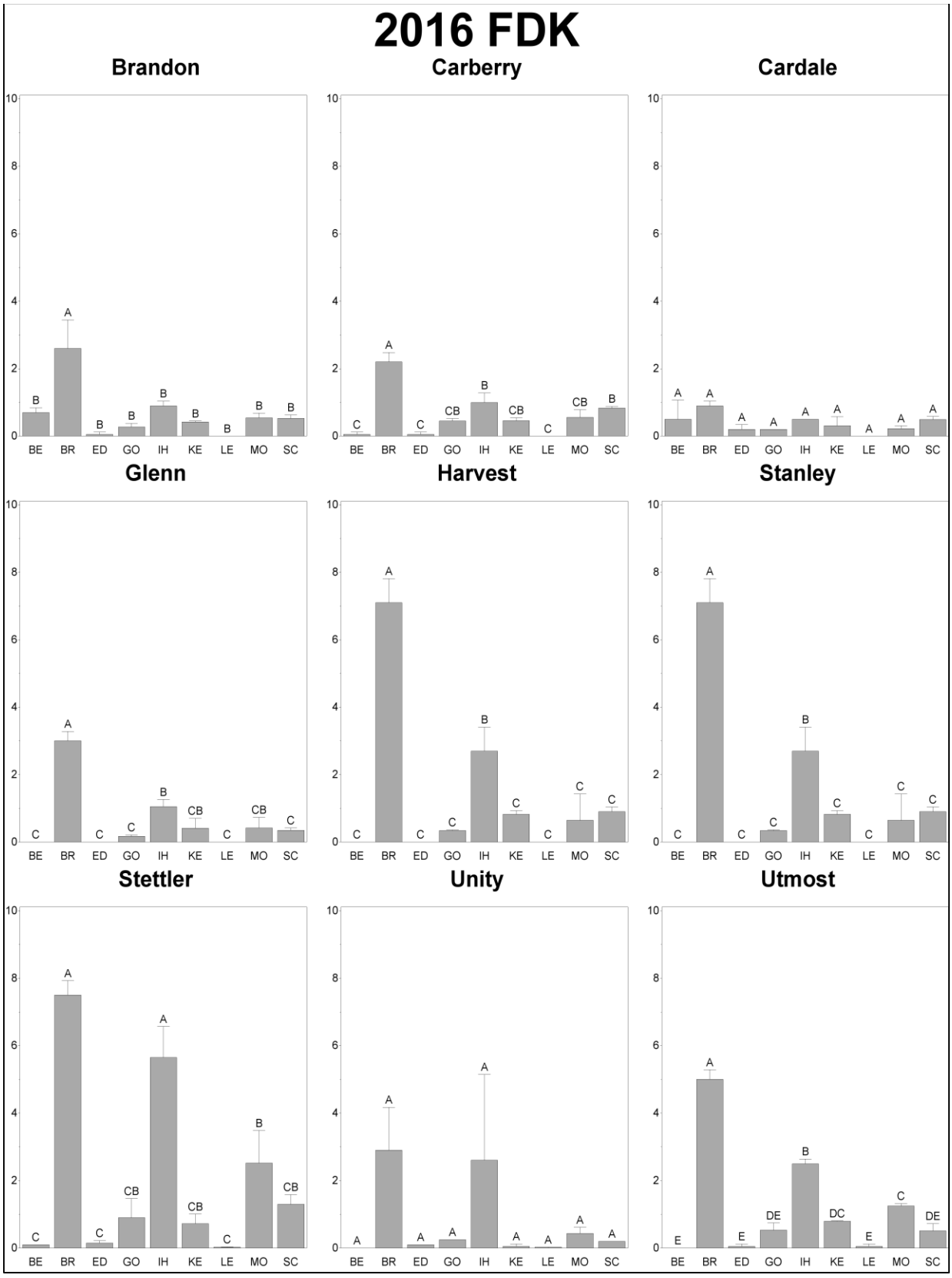
Appendix E.2 Average grade for 2016 locations within genotypes.



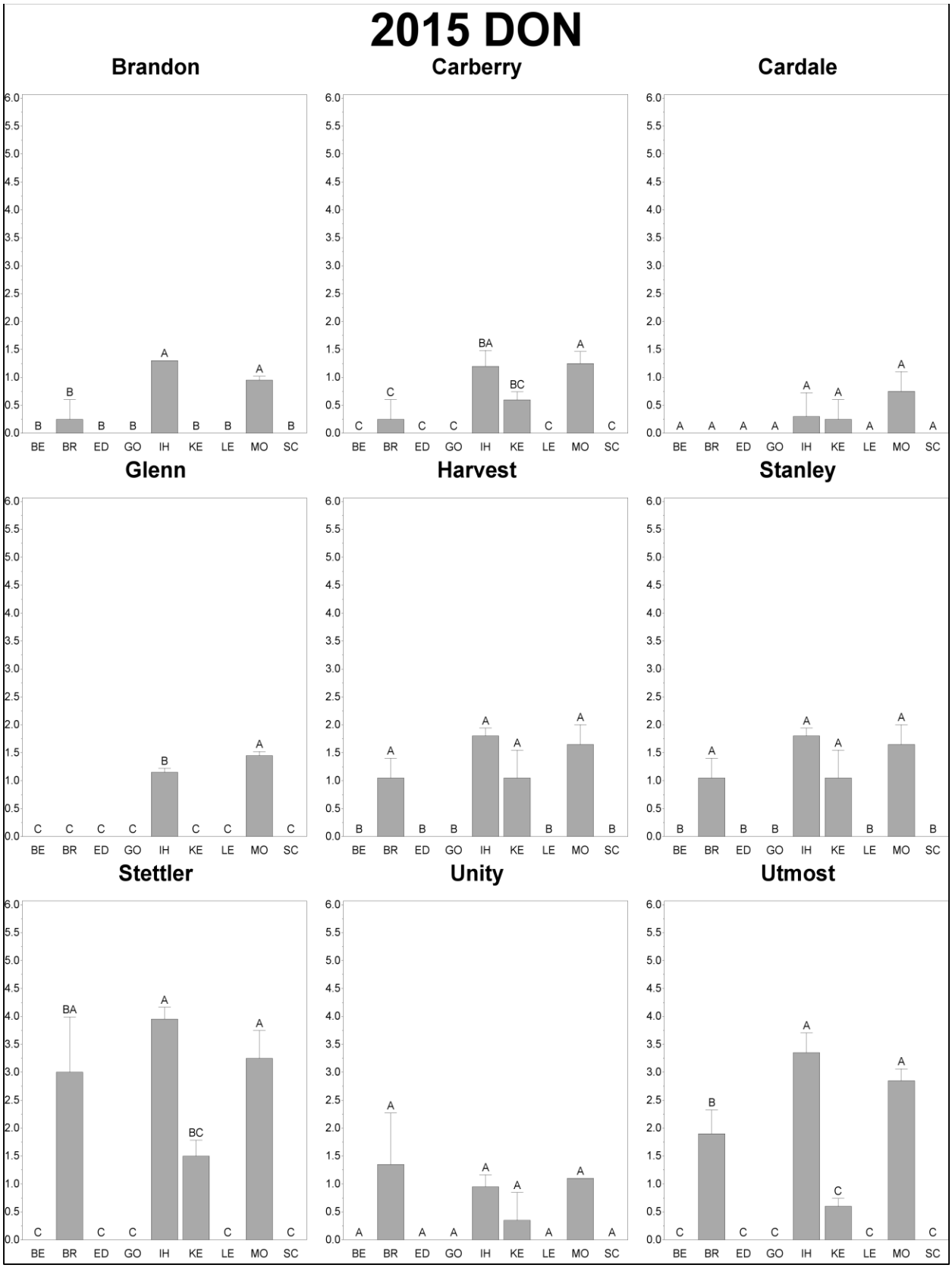
Appendix E.3 Average fusarium damaged kernels (%FDK) for 2015 locations within genotypes.



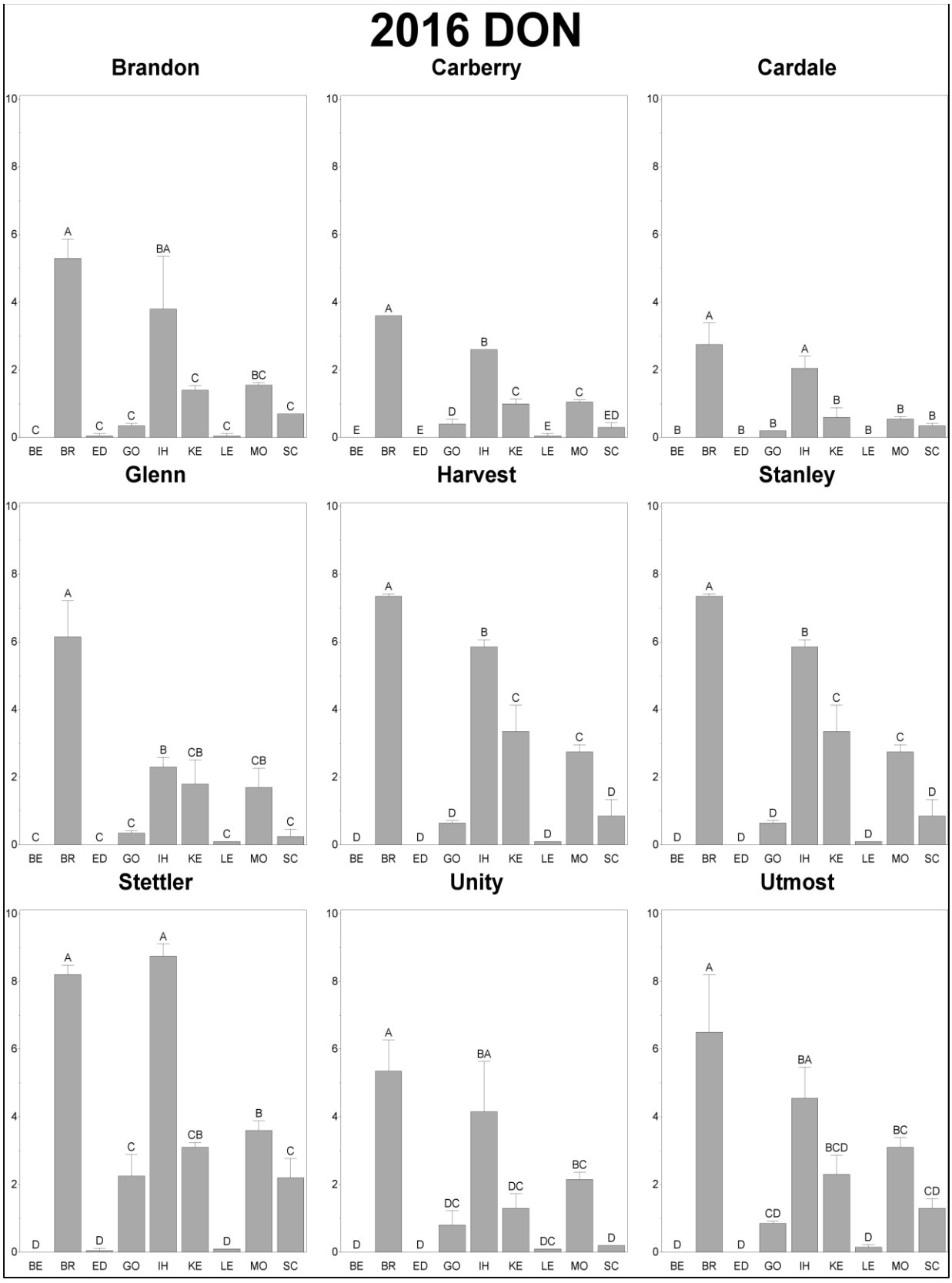
Appendix E.4 Average fusarium damaged kernels (%FDK) for 2016 locations within genotypes.



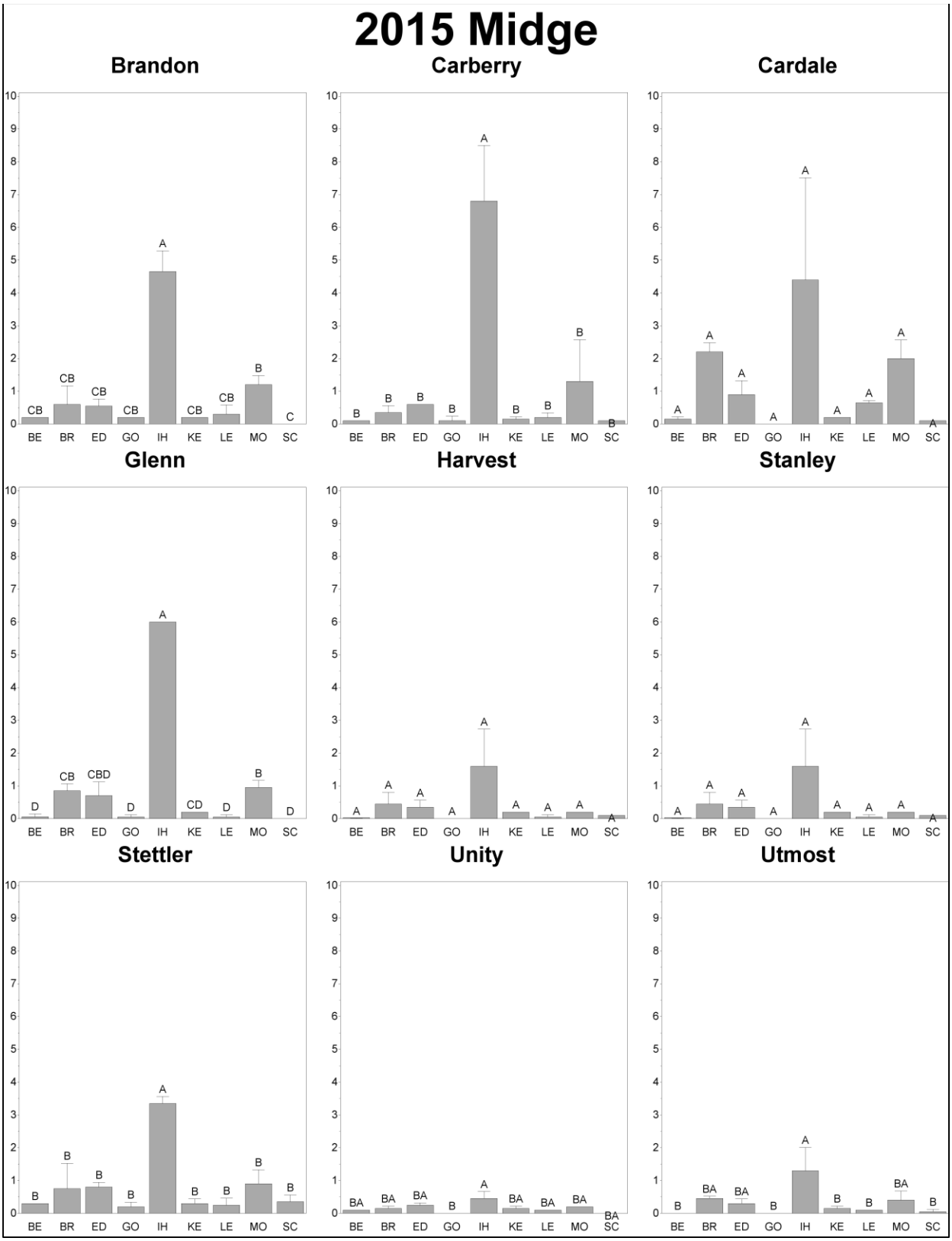
Appendix E.5 Average deoxynivalenol Content (DON, ppm) for 2015 locations within genotypes.



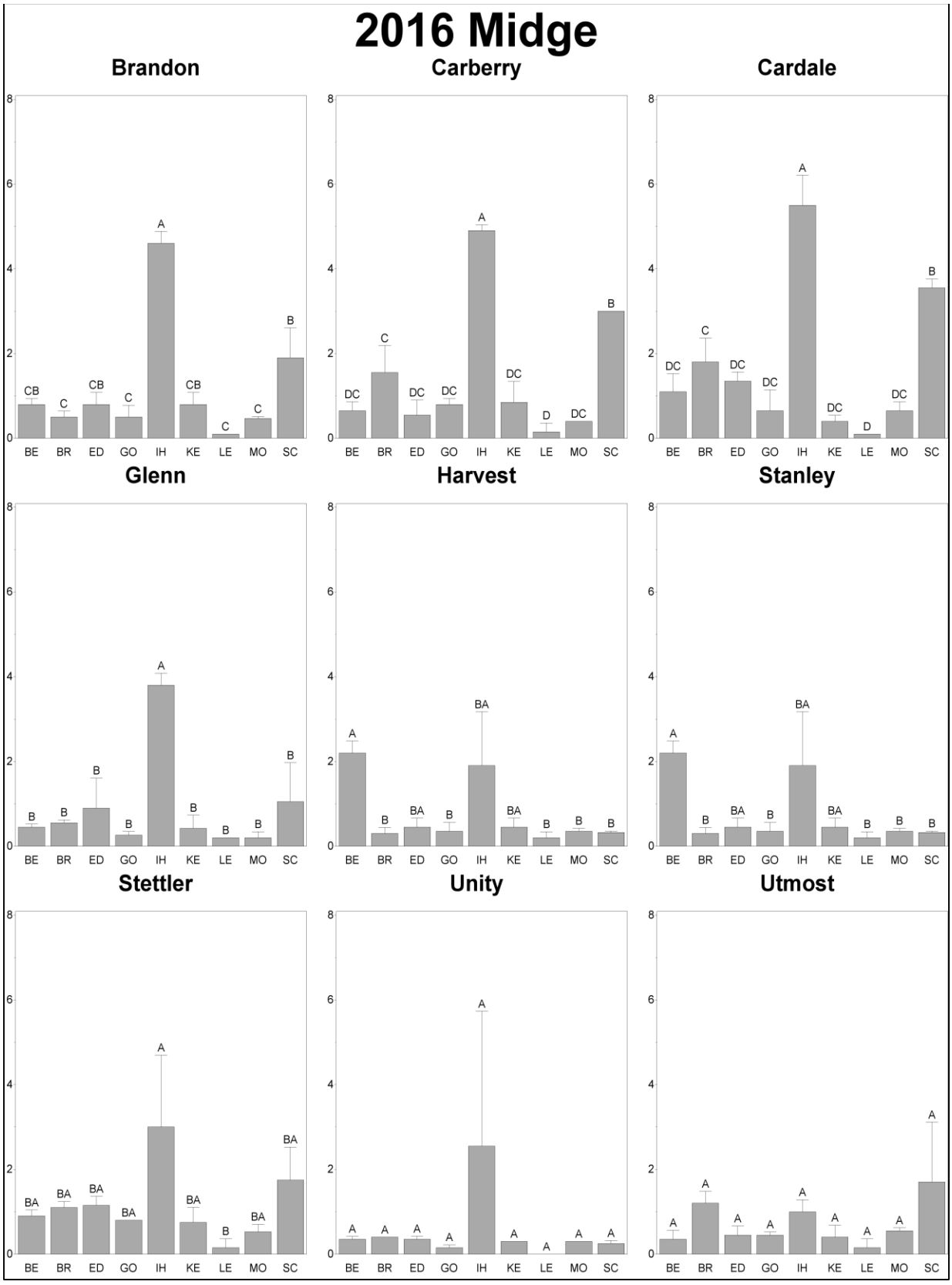
Appendix E.6 Average deoxynivalenol Content (DON, ppm) for 2016 locations within genotypes.



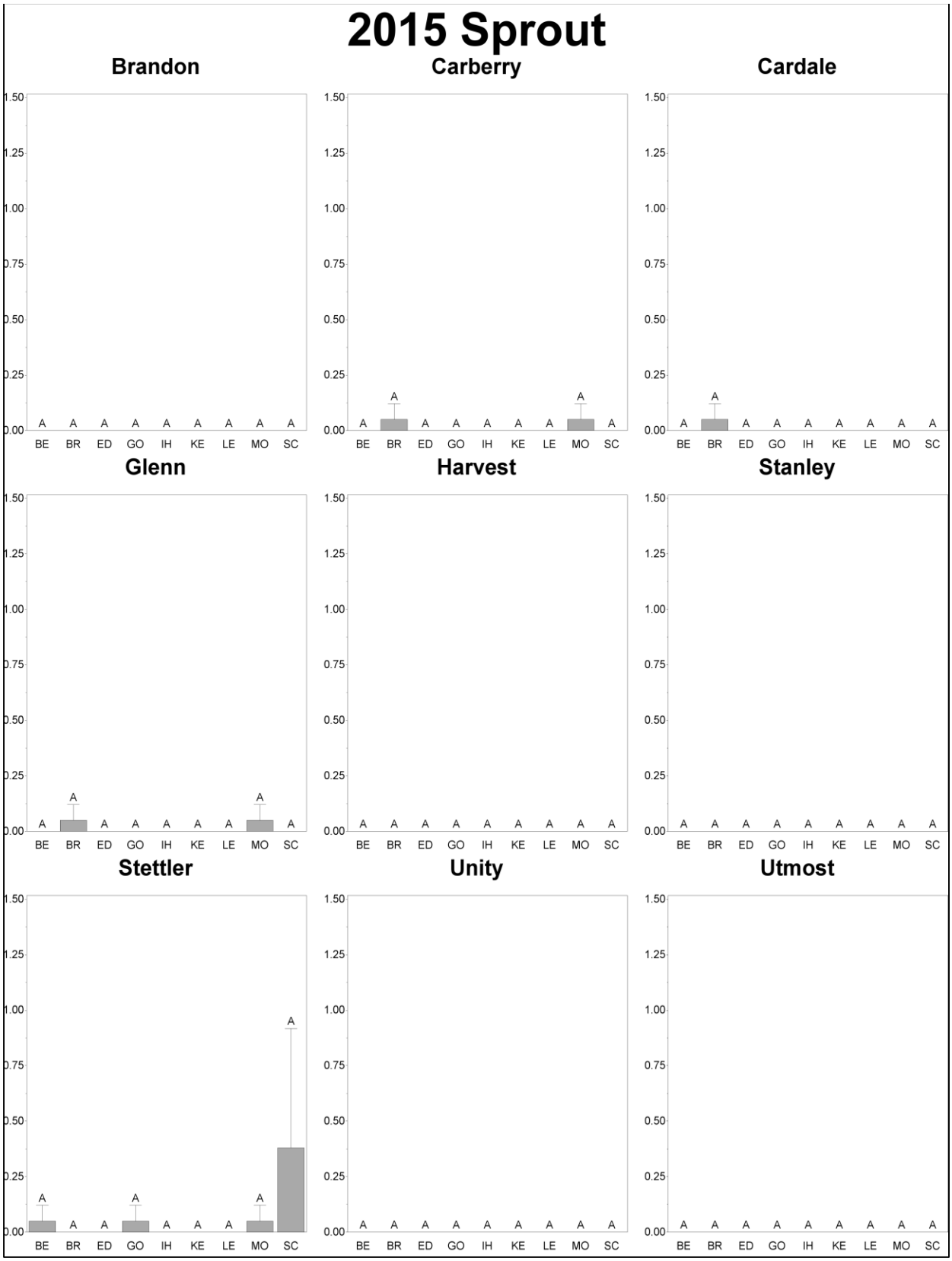
Appendix E.7 Average midge damage (%) for 2015 locations within genotypes.



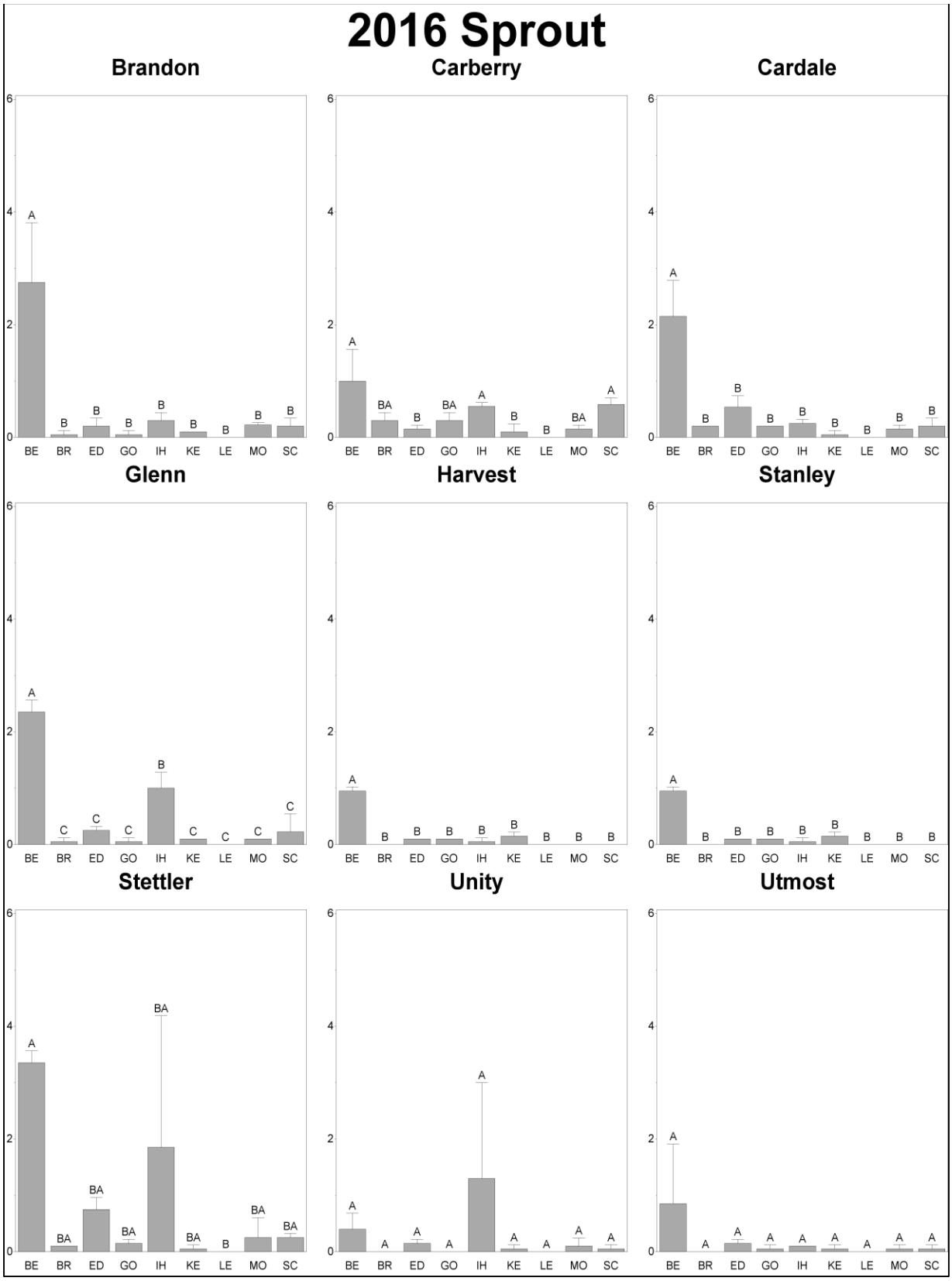
Appendix E.8 Average midge damage (%) for 2016 locations within genotypes.



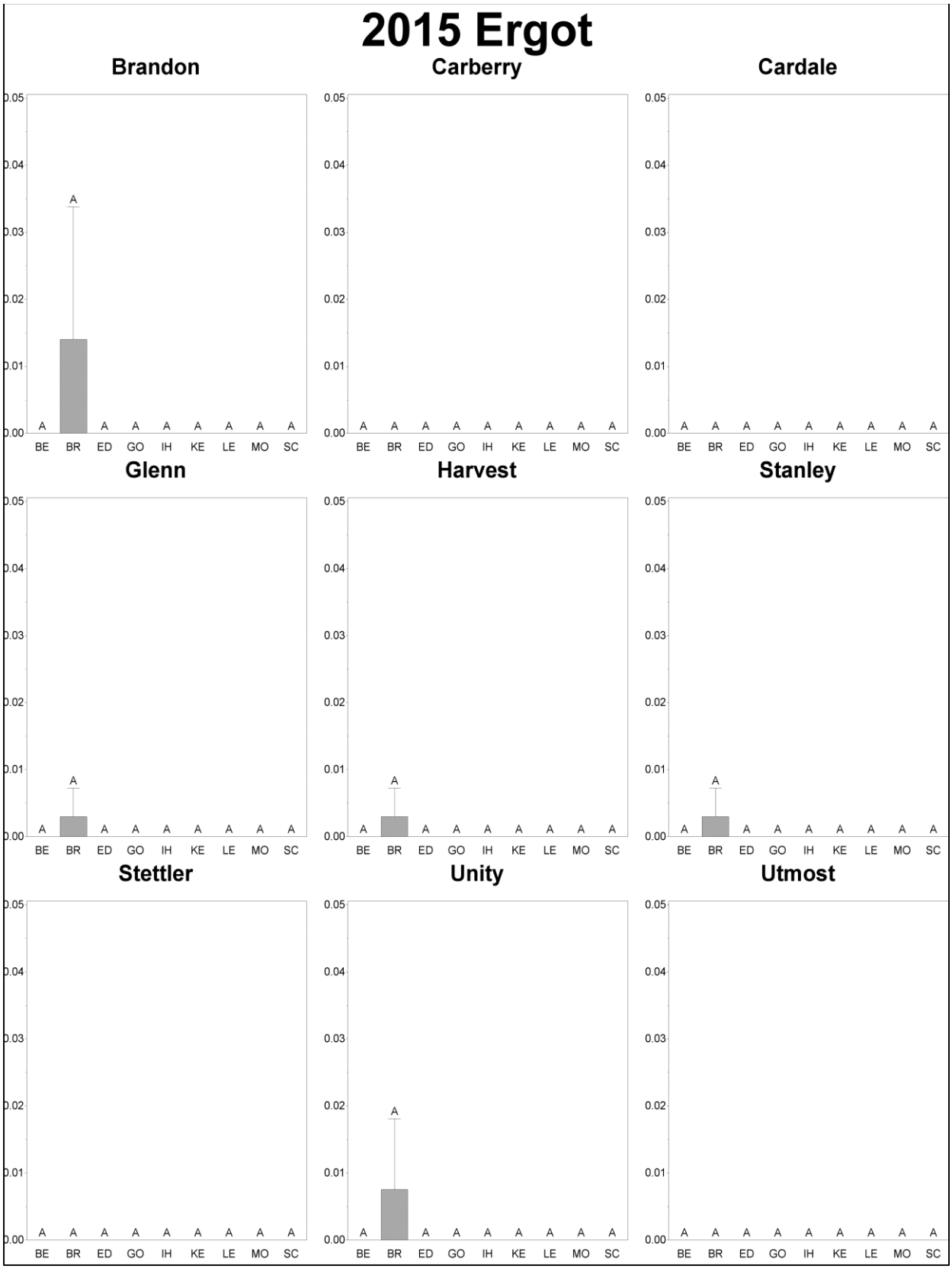
Appendix E.9 Average sprout damage (%) for 2015 locations within genotypes.



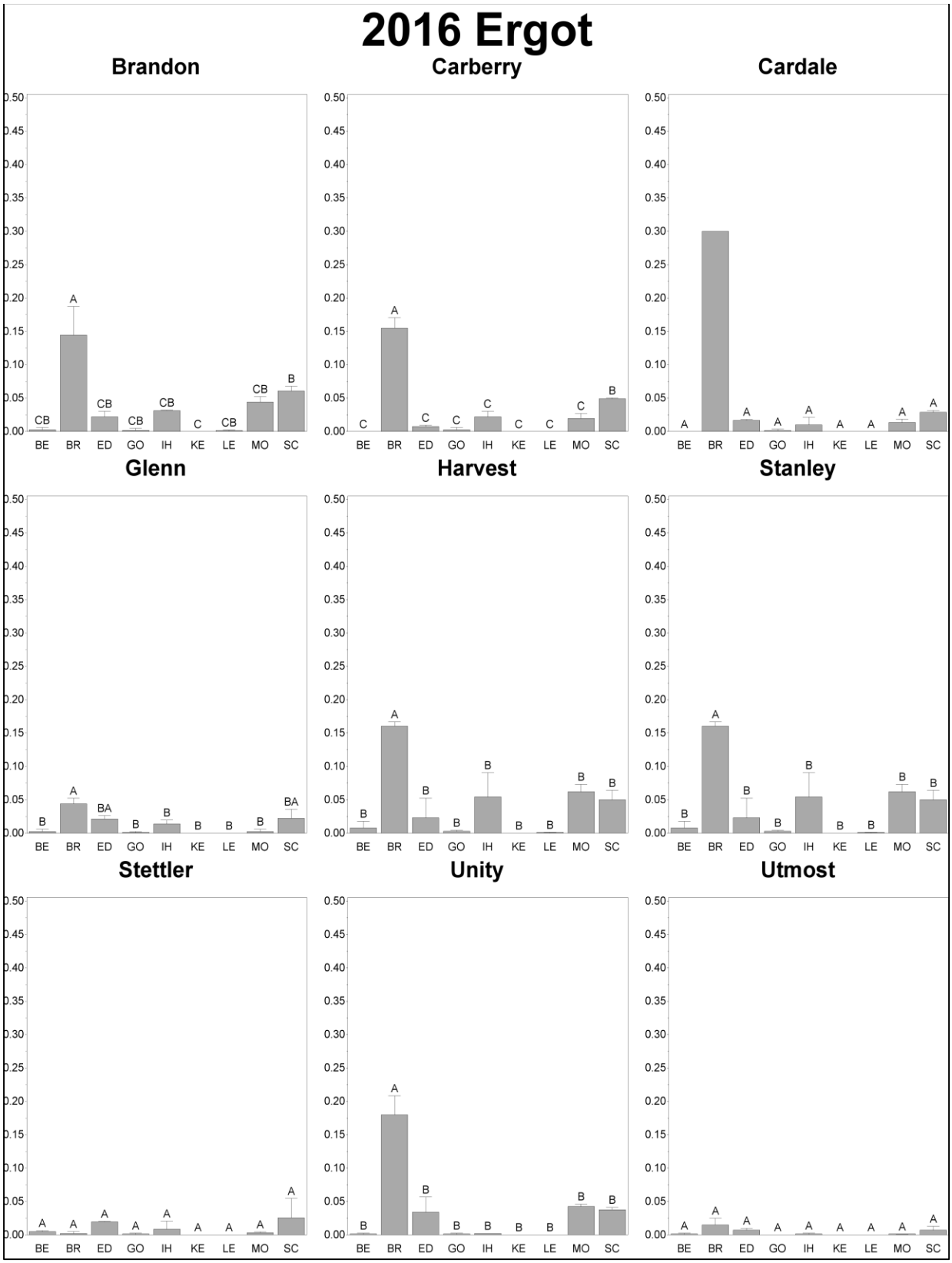
Appendix E.10 Average sprout damage (%)for 2016 locations within genotypes.



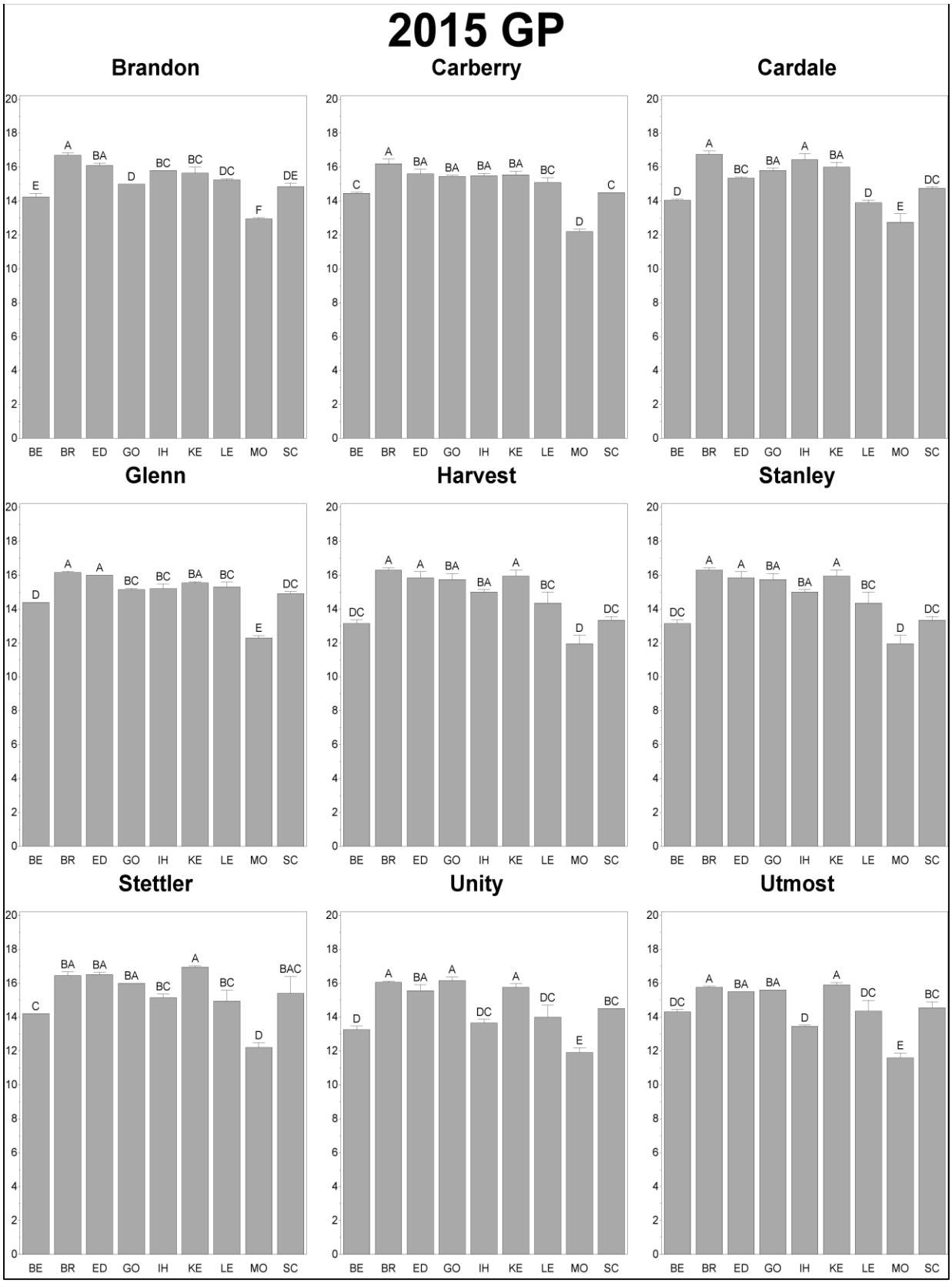
Appendix E.11 Average ergot damage (%) for 2015 locations within genotypes.



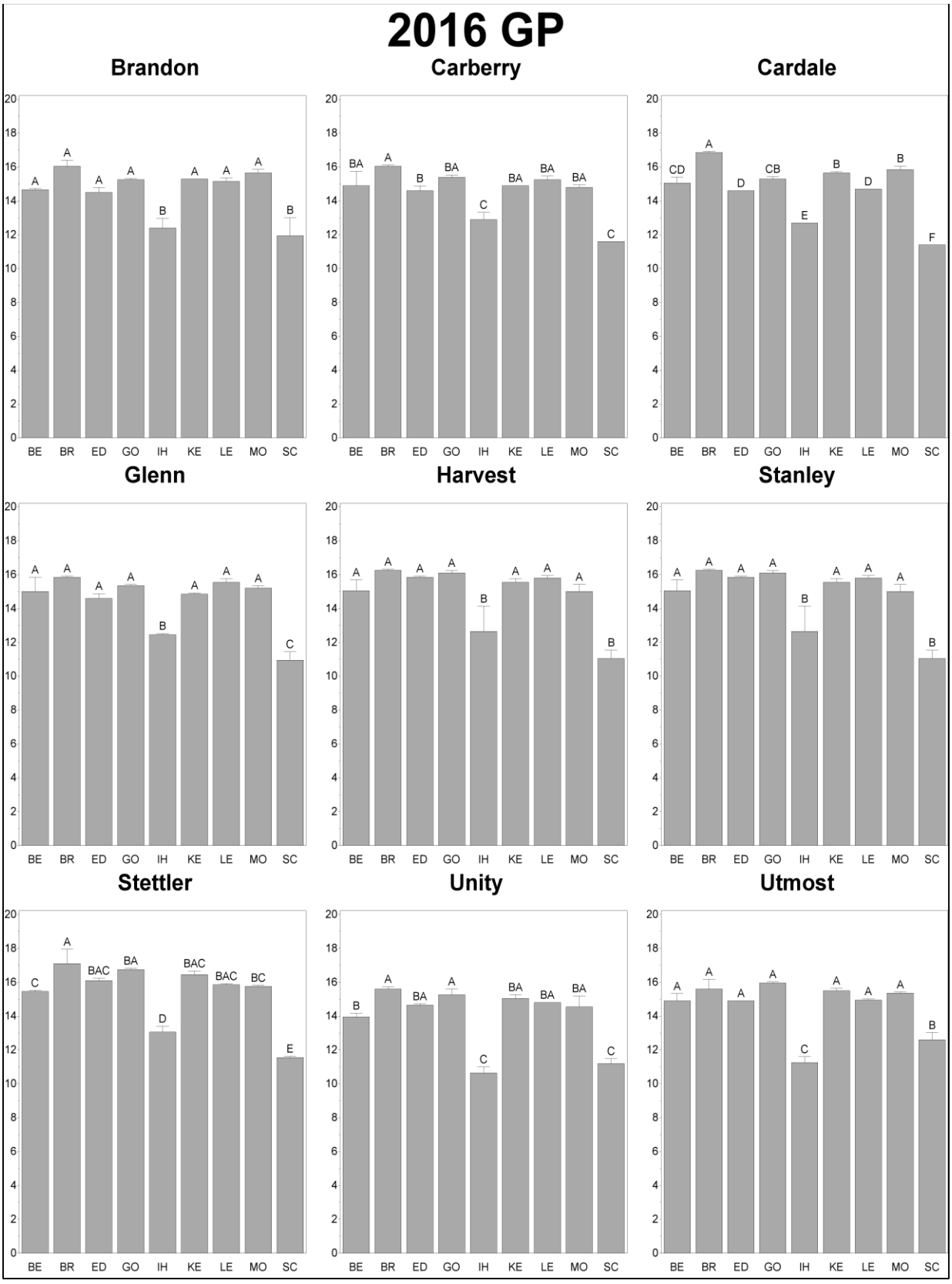
Appendix E.12 Average ergot damage (%) for 2016 locations within genotypes.



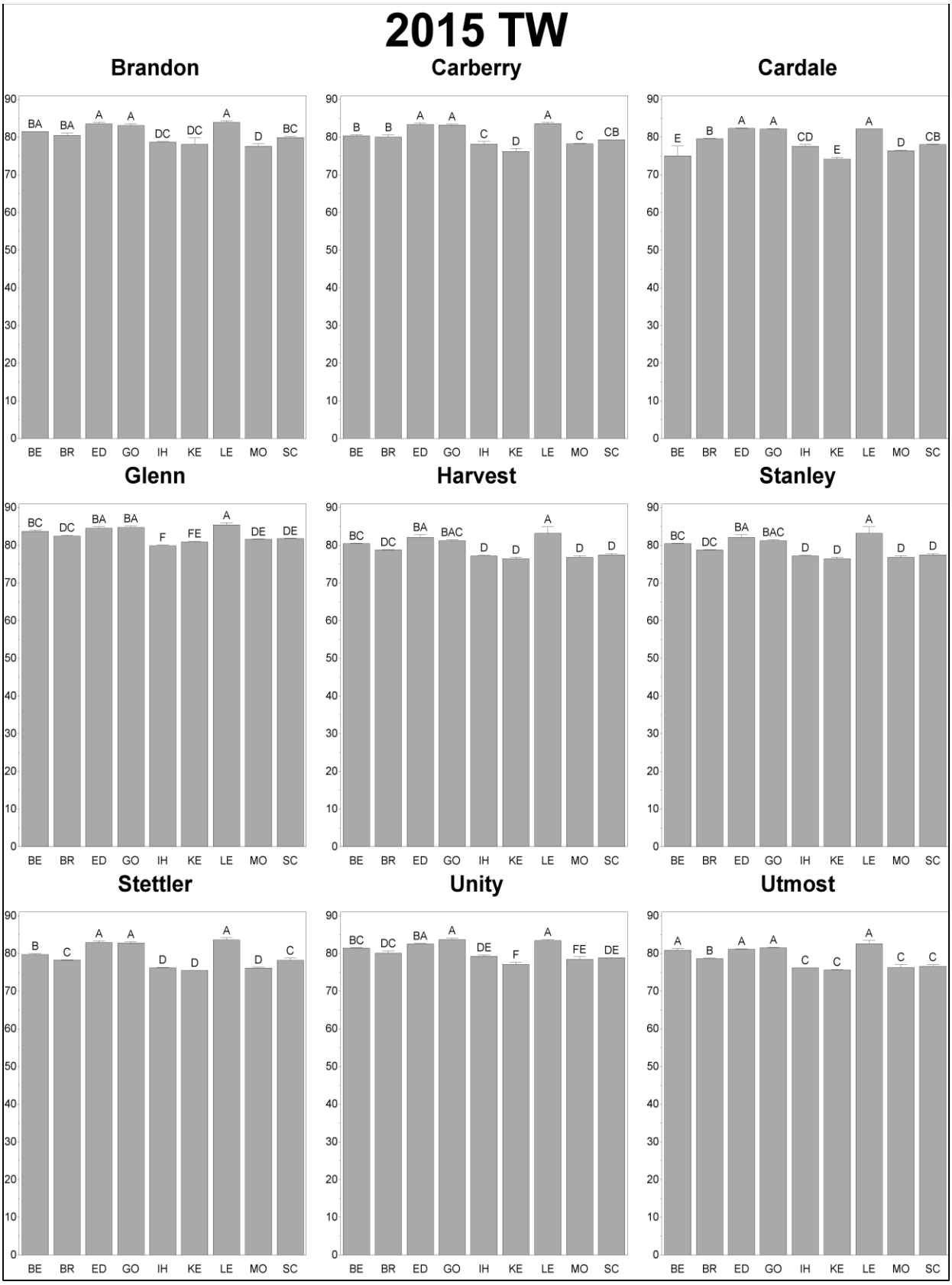
Appendix E.13 Average grain protein content (GP, %) for 2015 locations within genotypes.



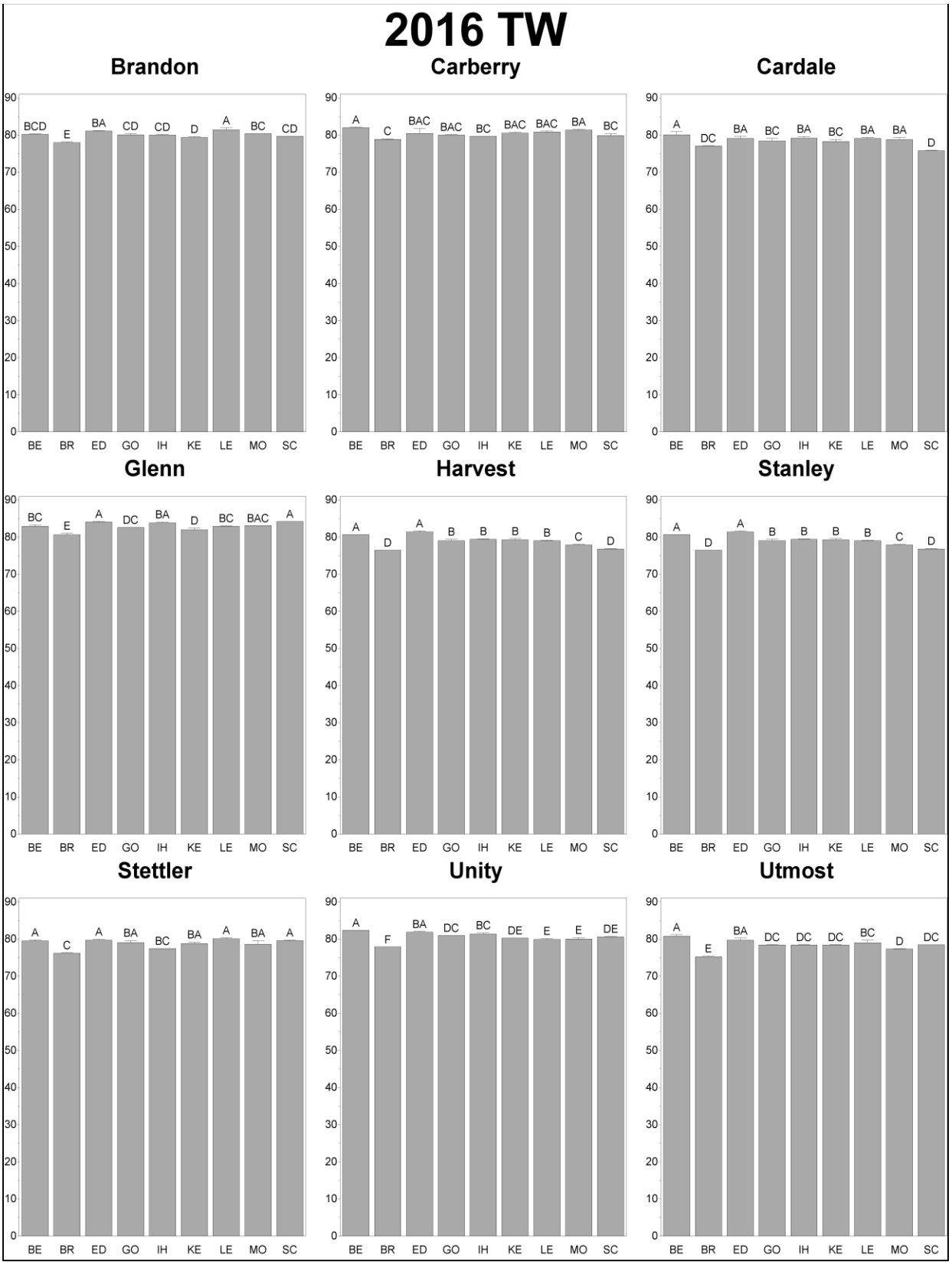
Appendix E.14 Average grain protein content (GP, %) for 2016 locations within genotypes.



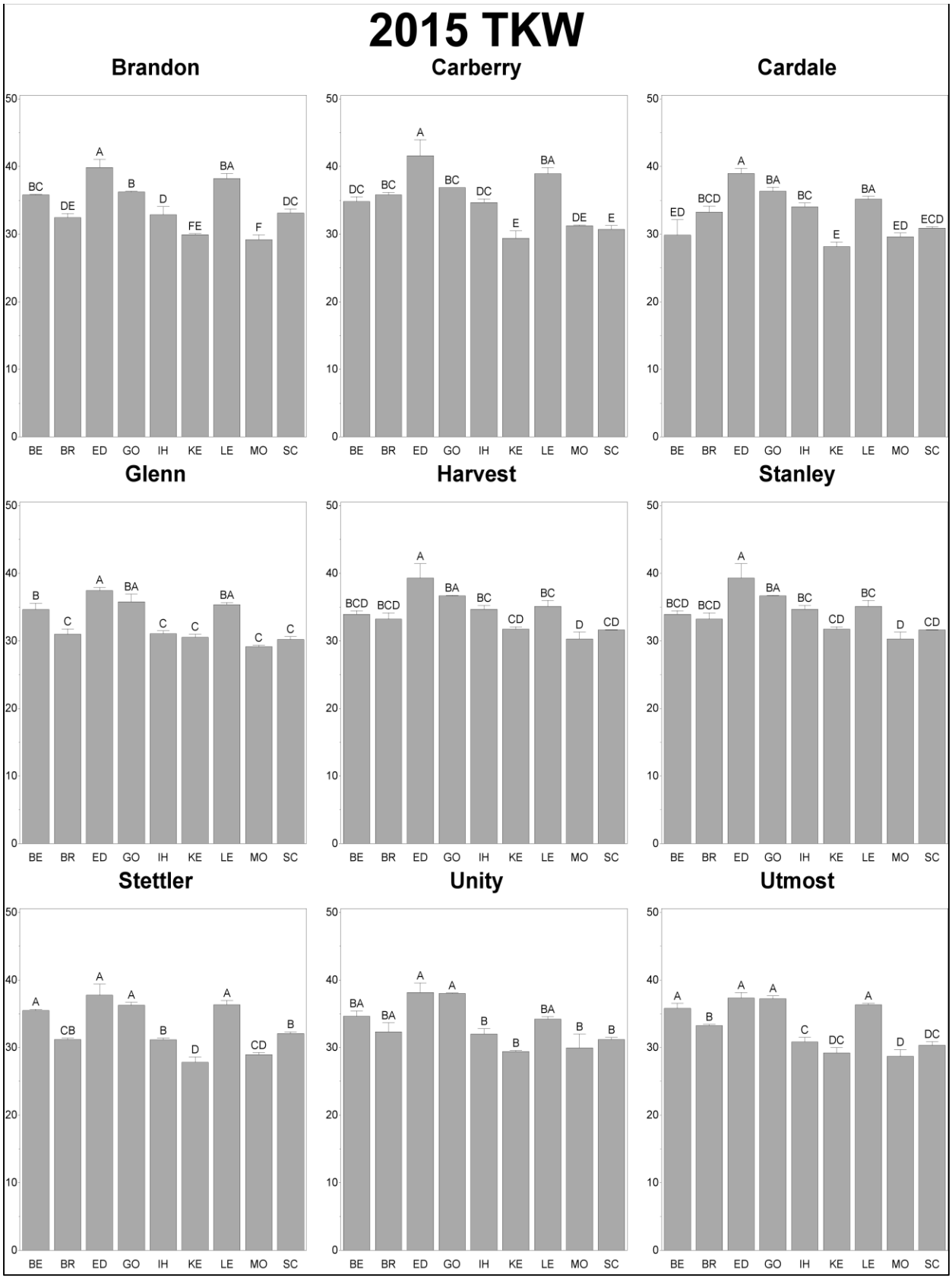
Appendix E.15 Average test weight (TW, kg/hL) for 2015 locations within genotypes.



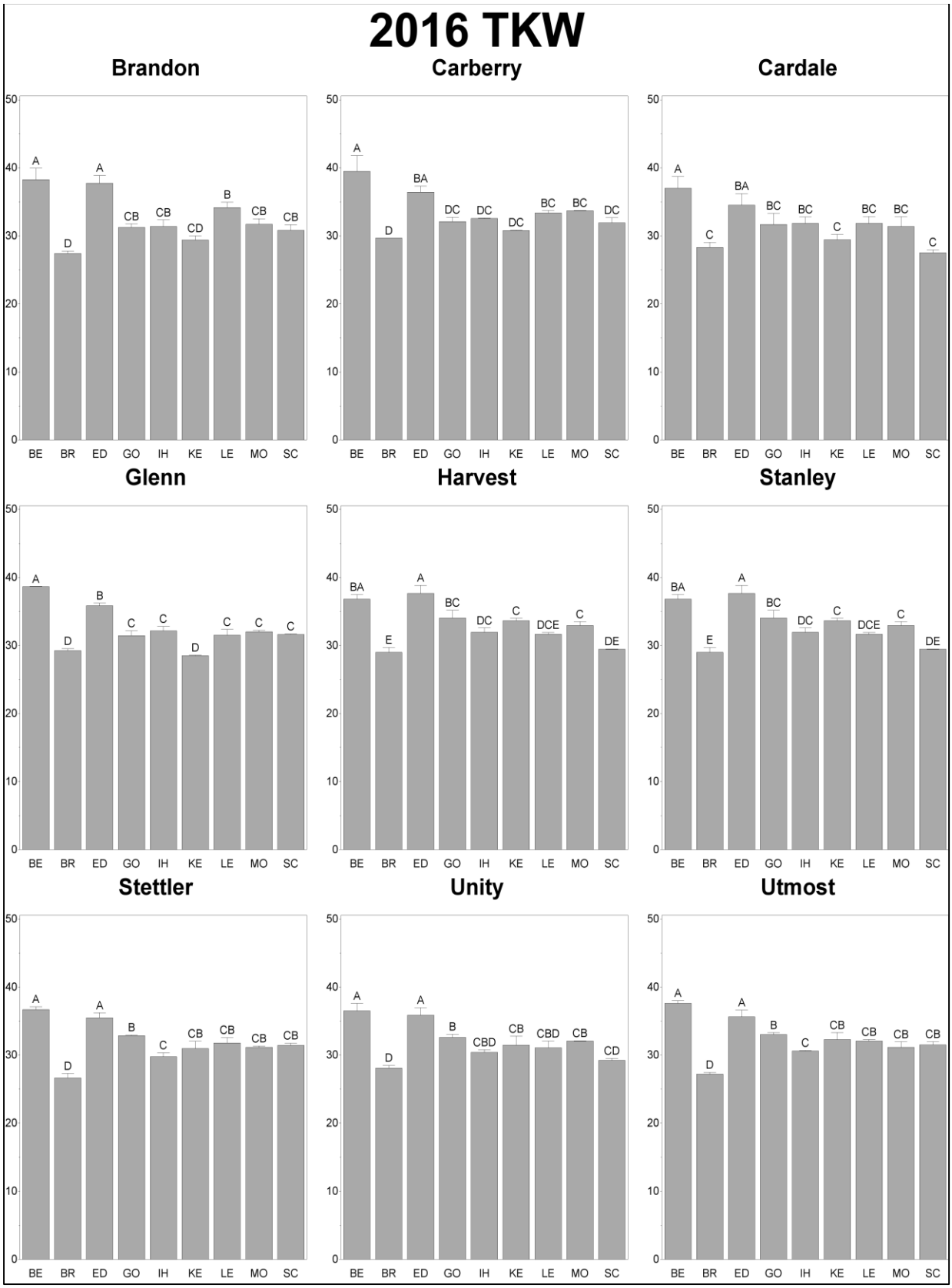
Appendix E.16 Average test weight (TW, kg/hL) for 2016 locations within genotypes.



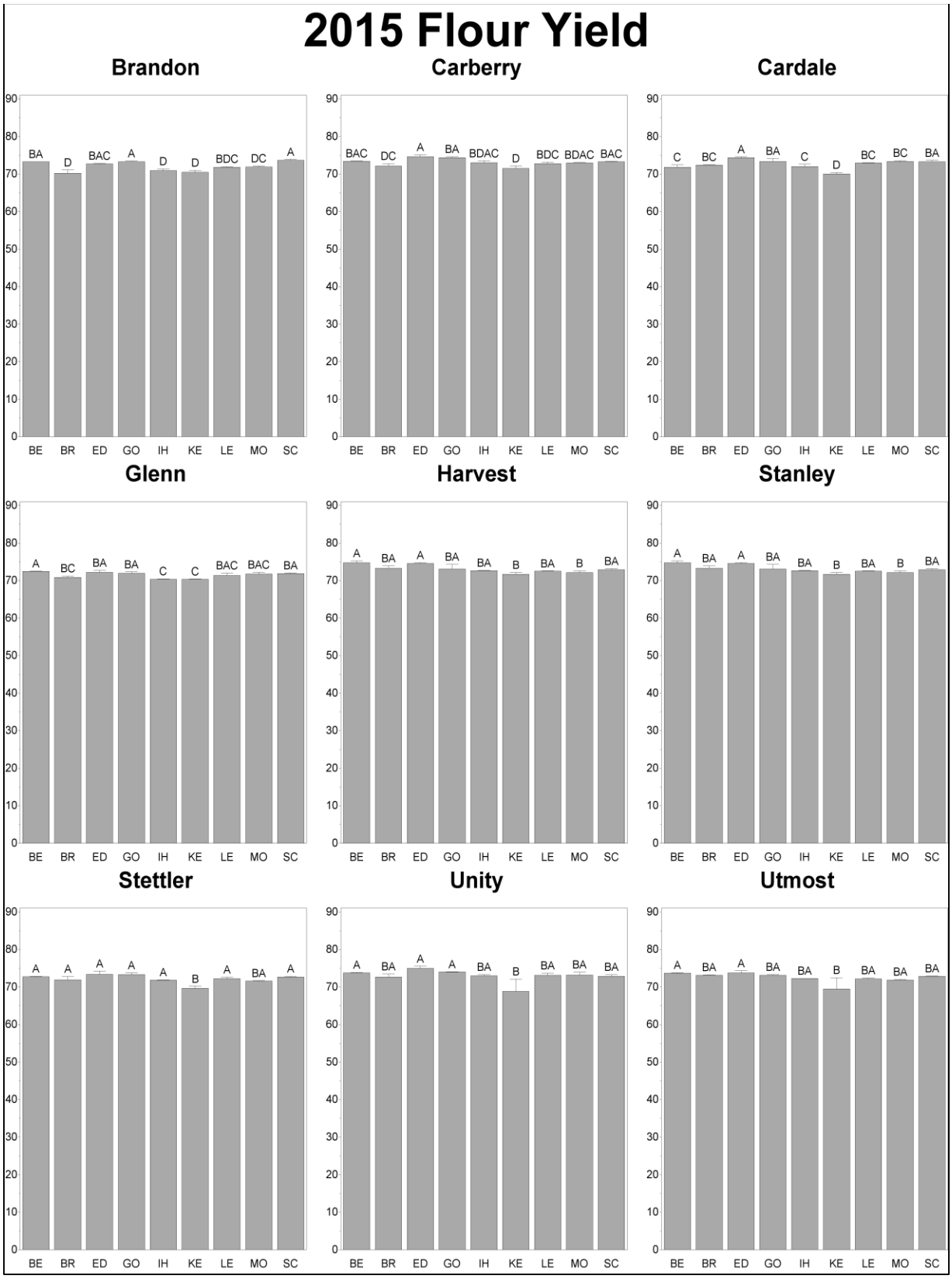
Appendix E.17 Average thousand kernel weight (TKW, g) for 2015 locations within genotypes.



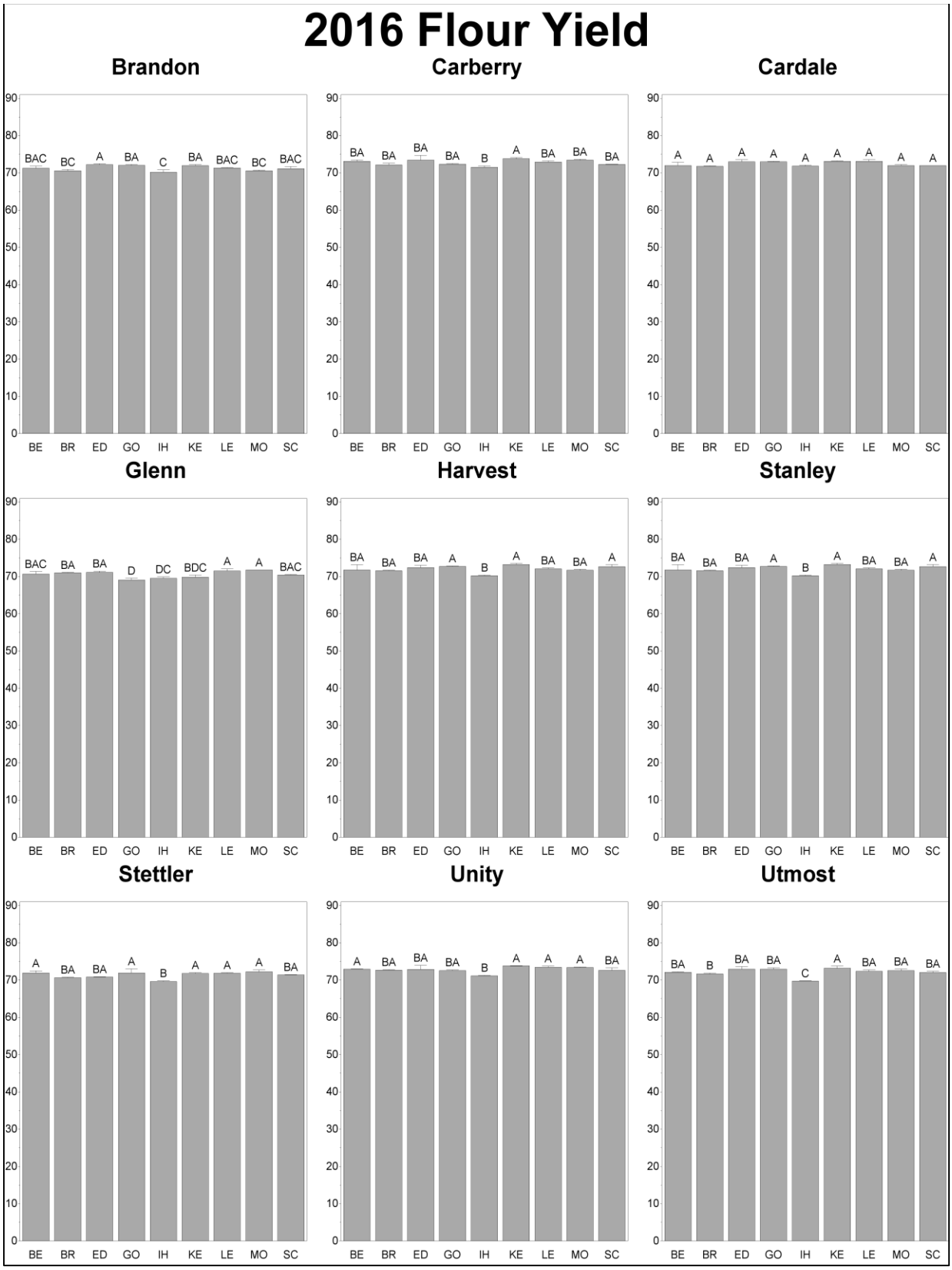
Appendix E.18 Average thousand kernel weight (TKW, g) for 2016 locations within genotypes.



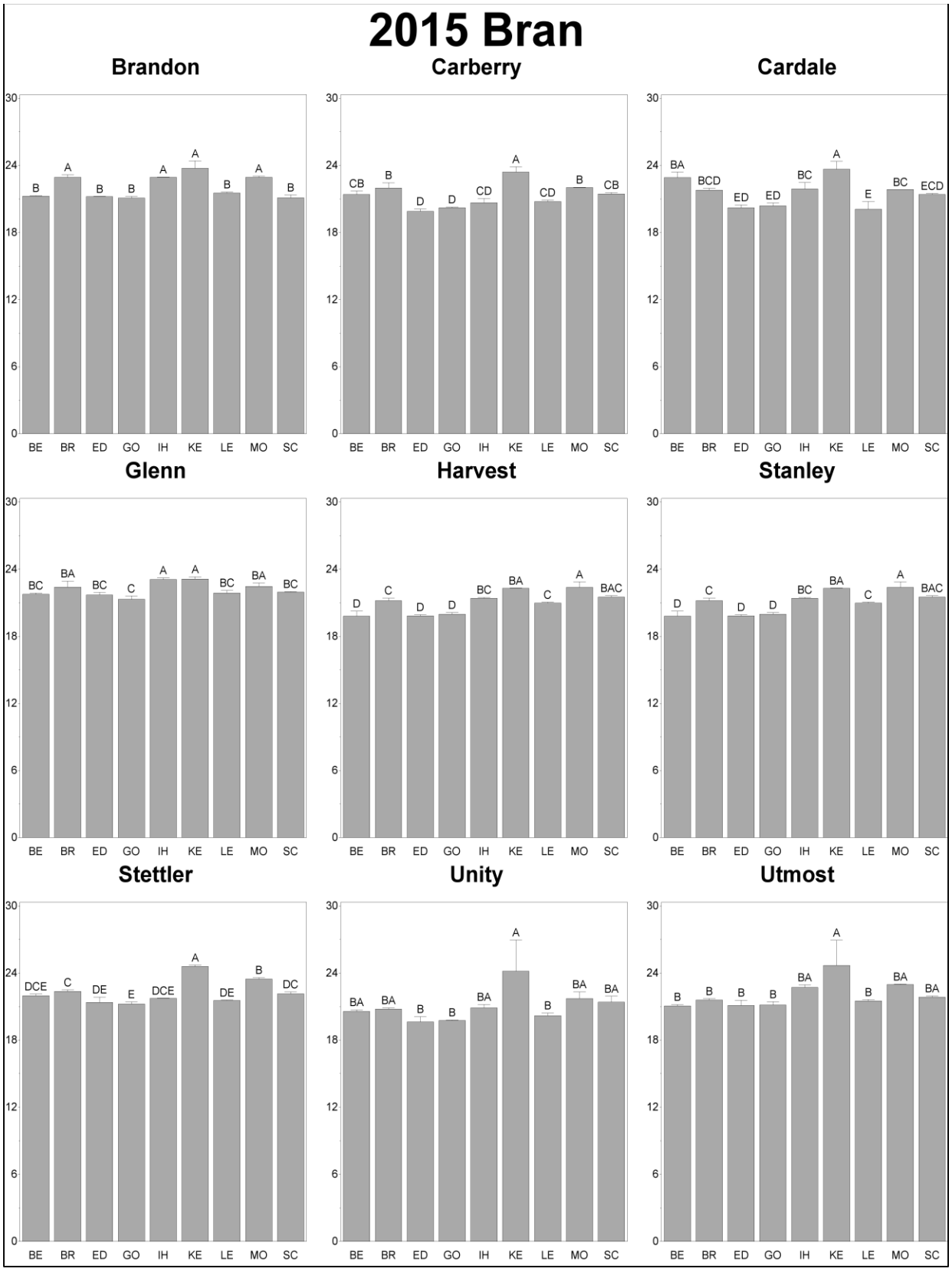
Appendix E.19 Average flour yield (%) for 2015 locations within genotypes.



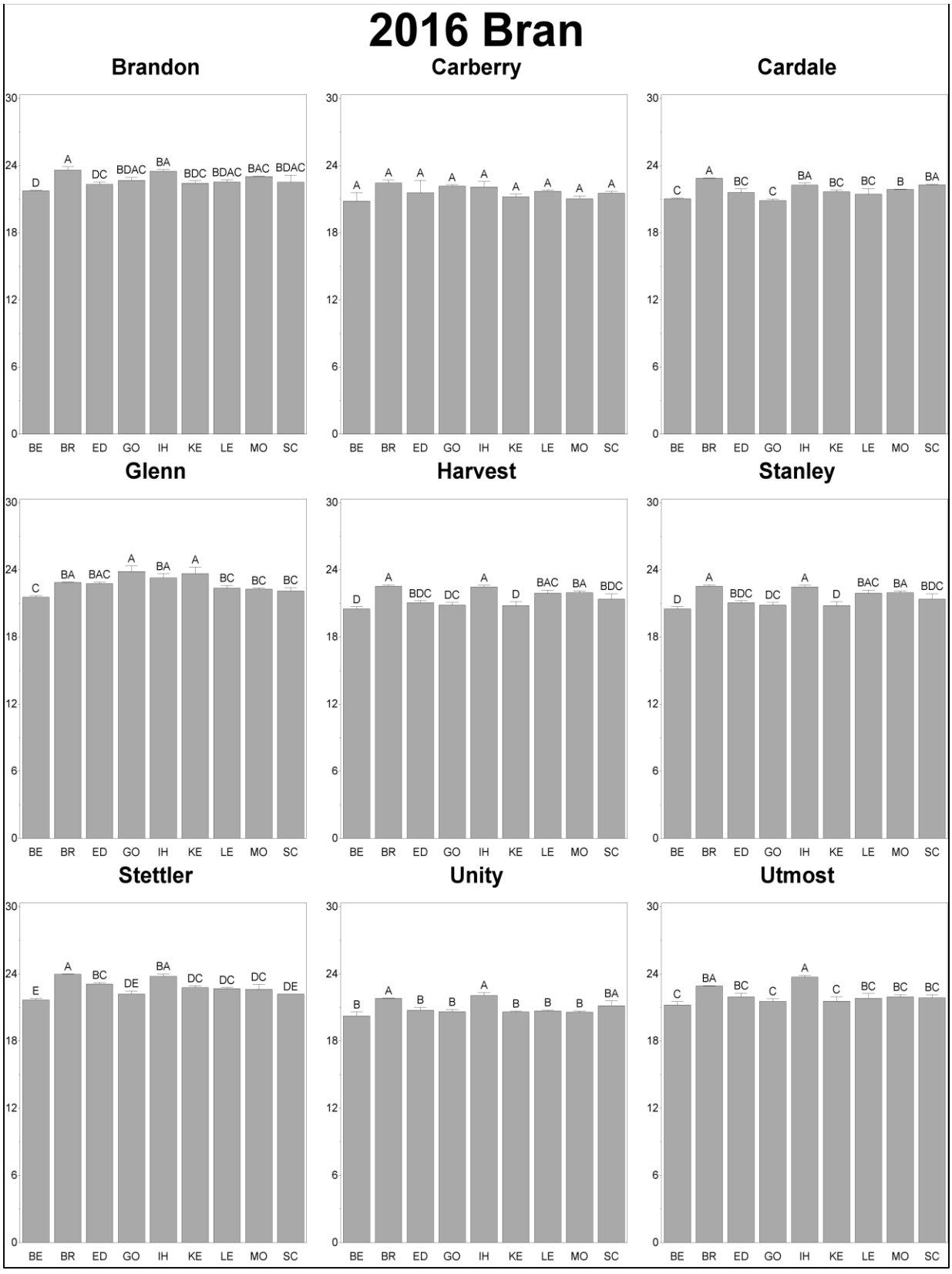
Appendix E.20 Average flour yield (%) for 2016 locations within genotypes.



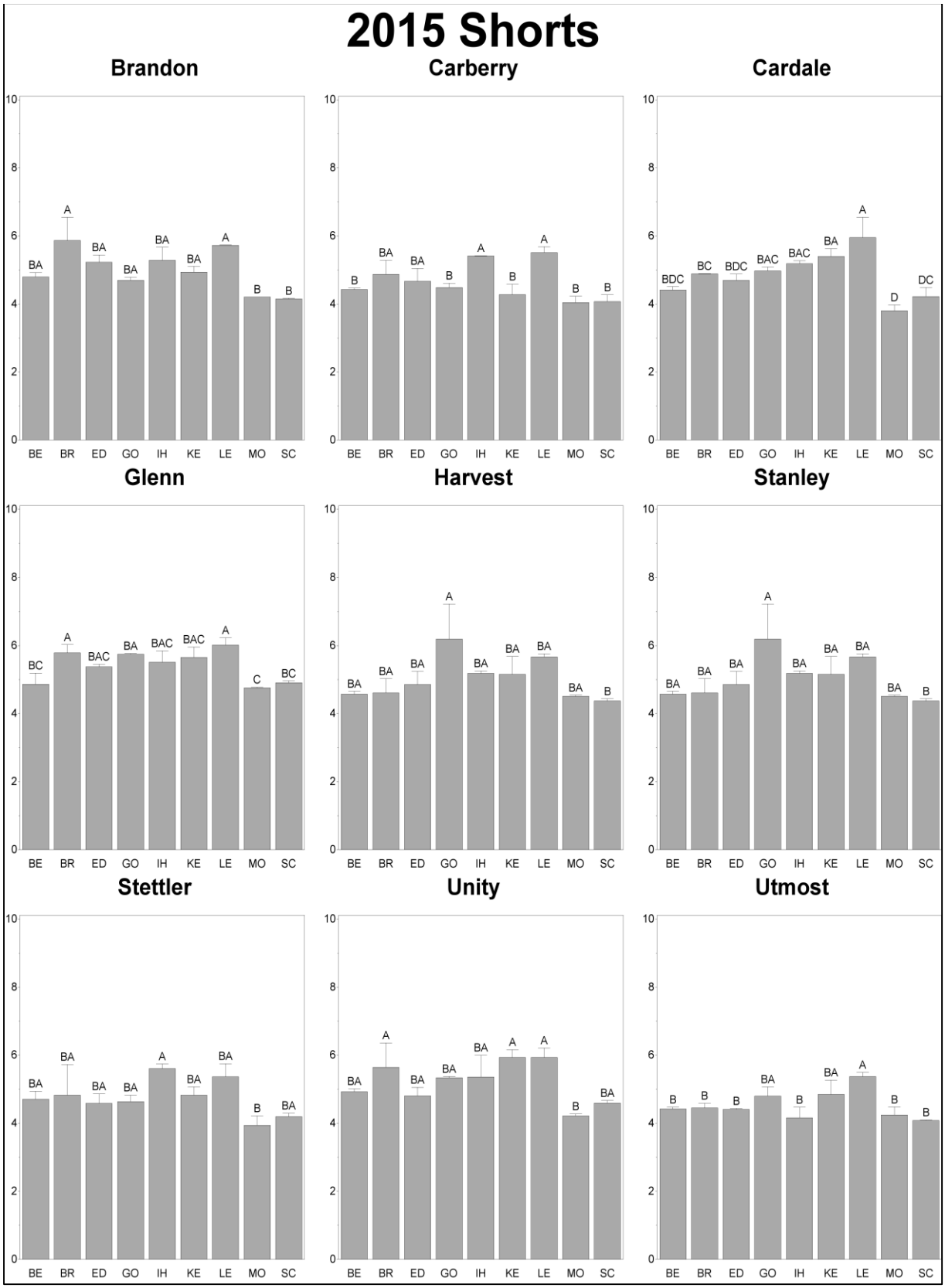
Appendix E.21 Average bran content (%) for 2015 locations within genotypes.



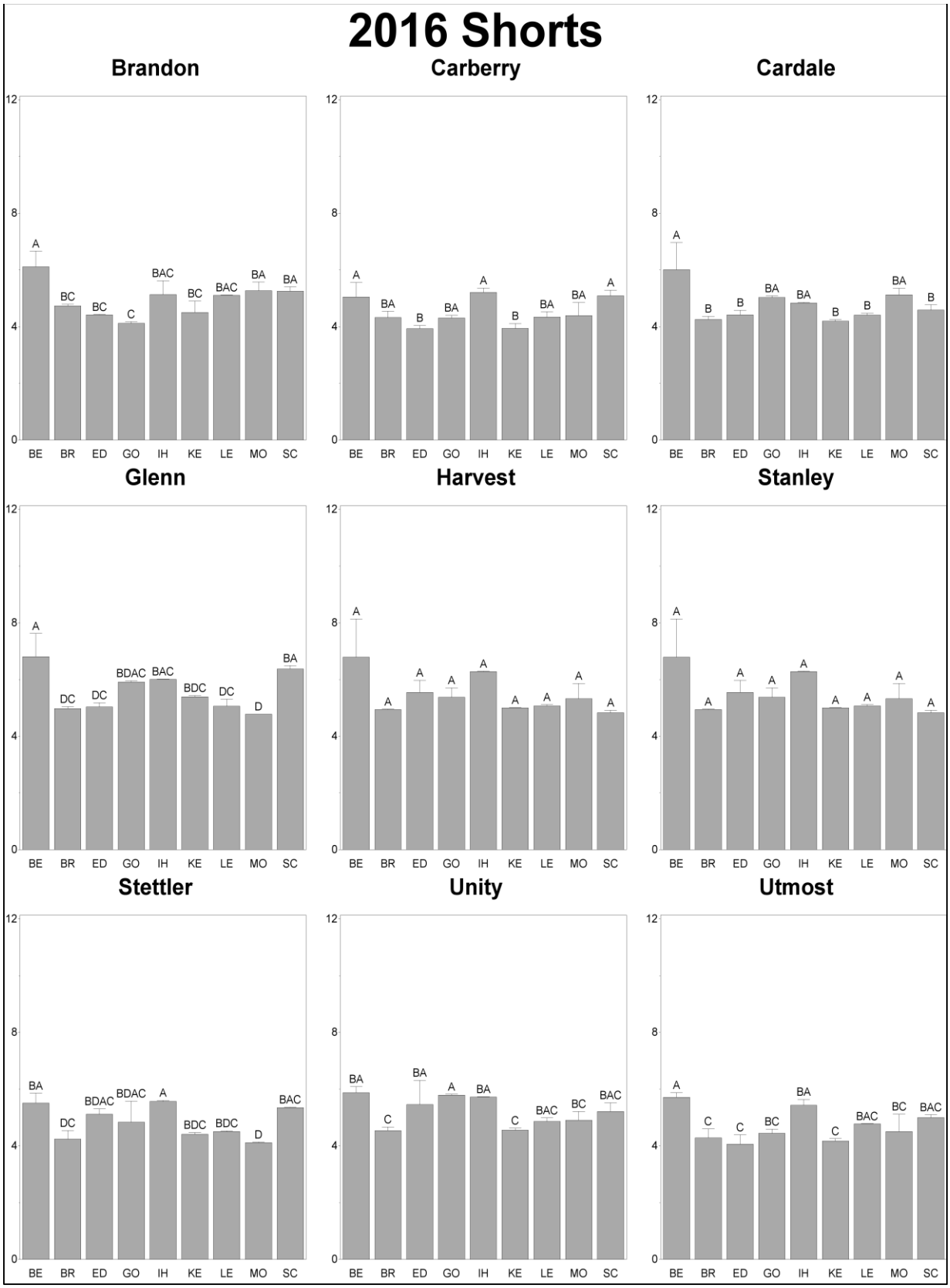
Appendix E.22 Average bran content (%) for 2016 locations within genotypes.



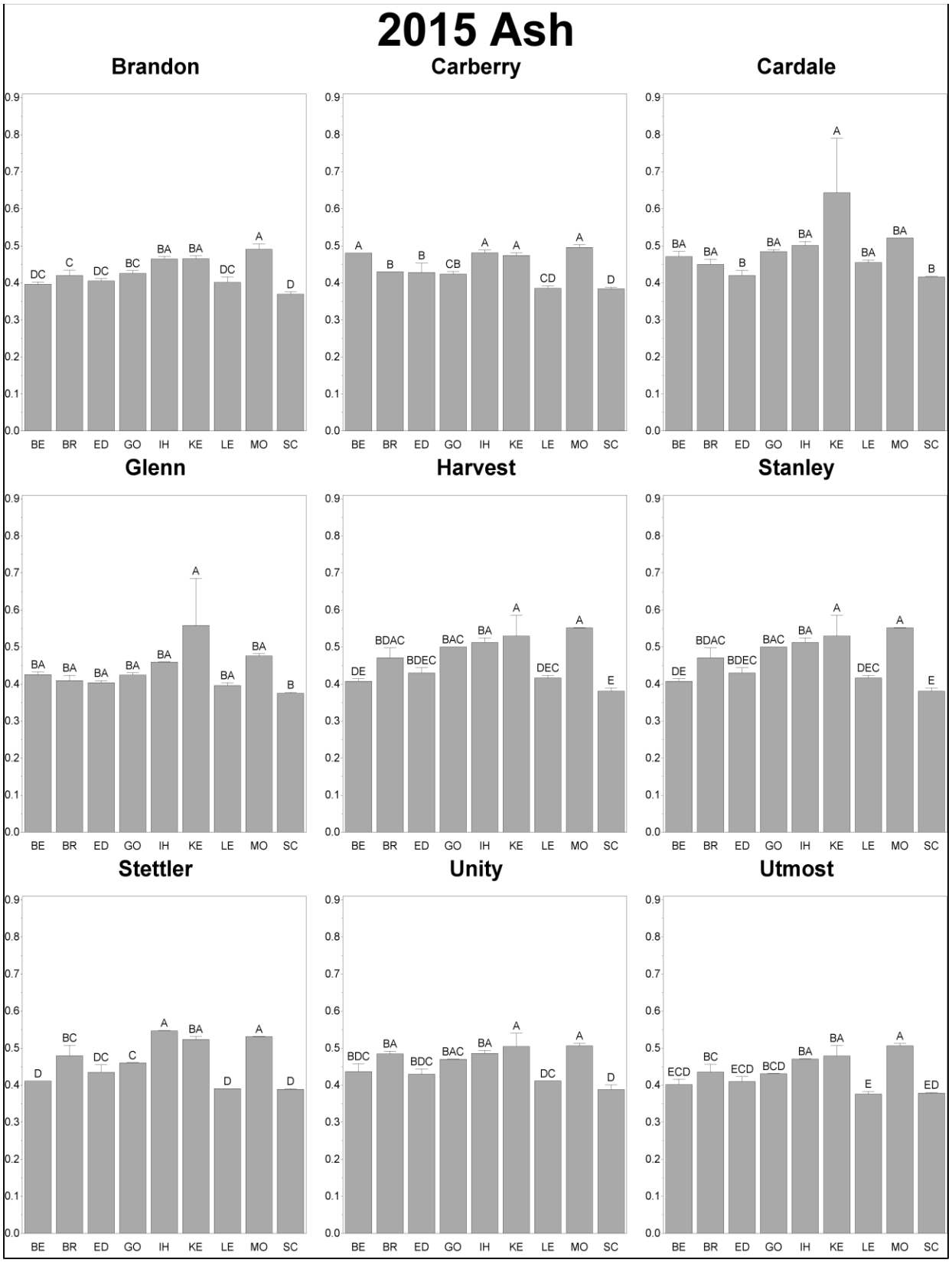
Appendix E.23 Average shorts content (%) for 2015 locations within genotypes.



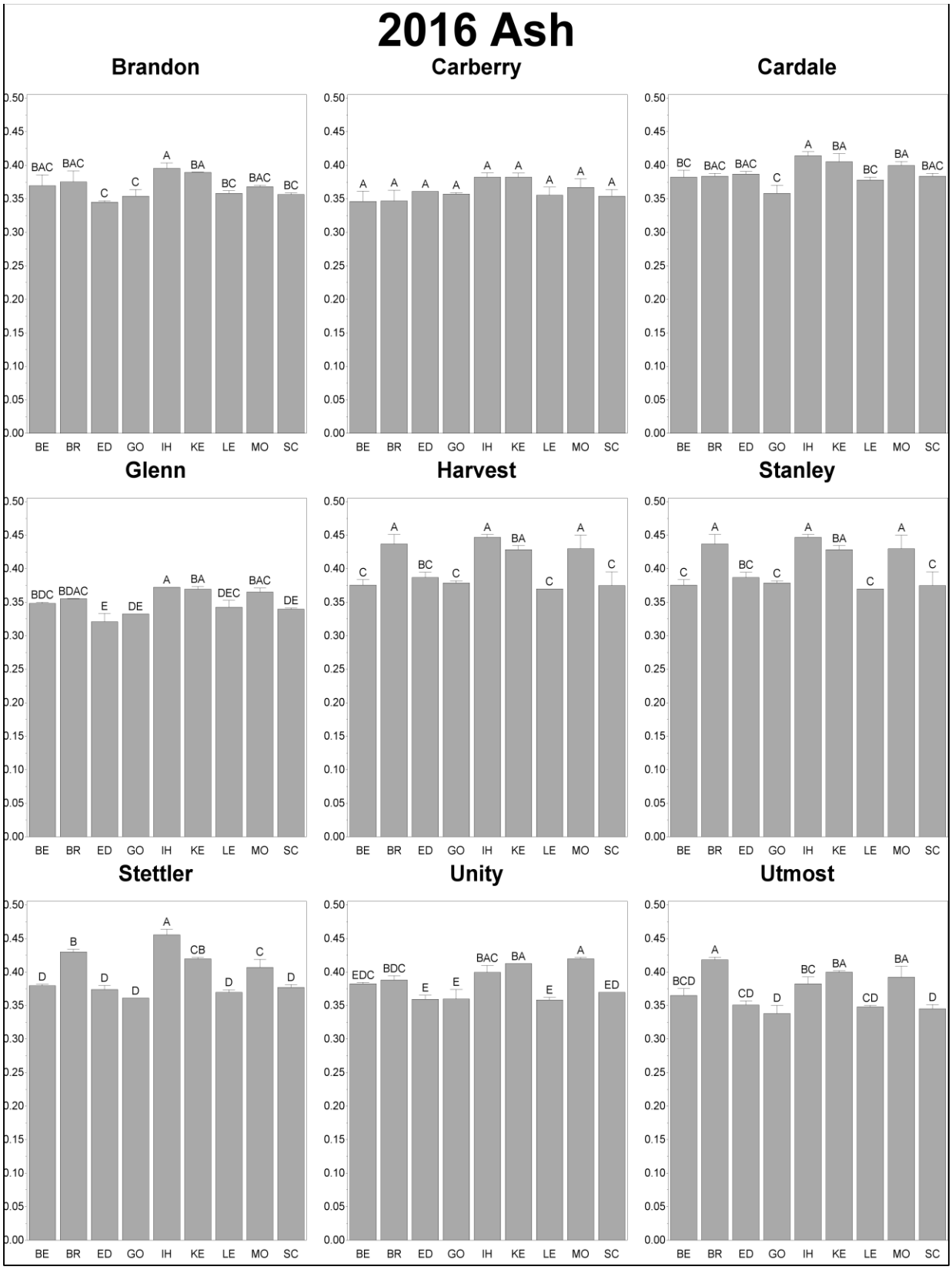
Appendix E.24 Average shorts content (%) for 2016 locations within genotypes.



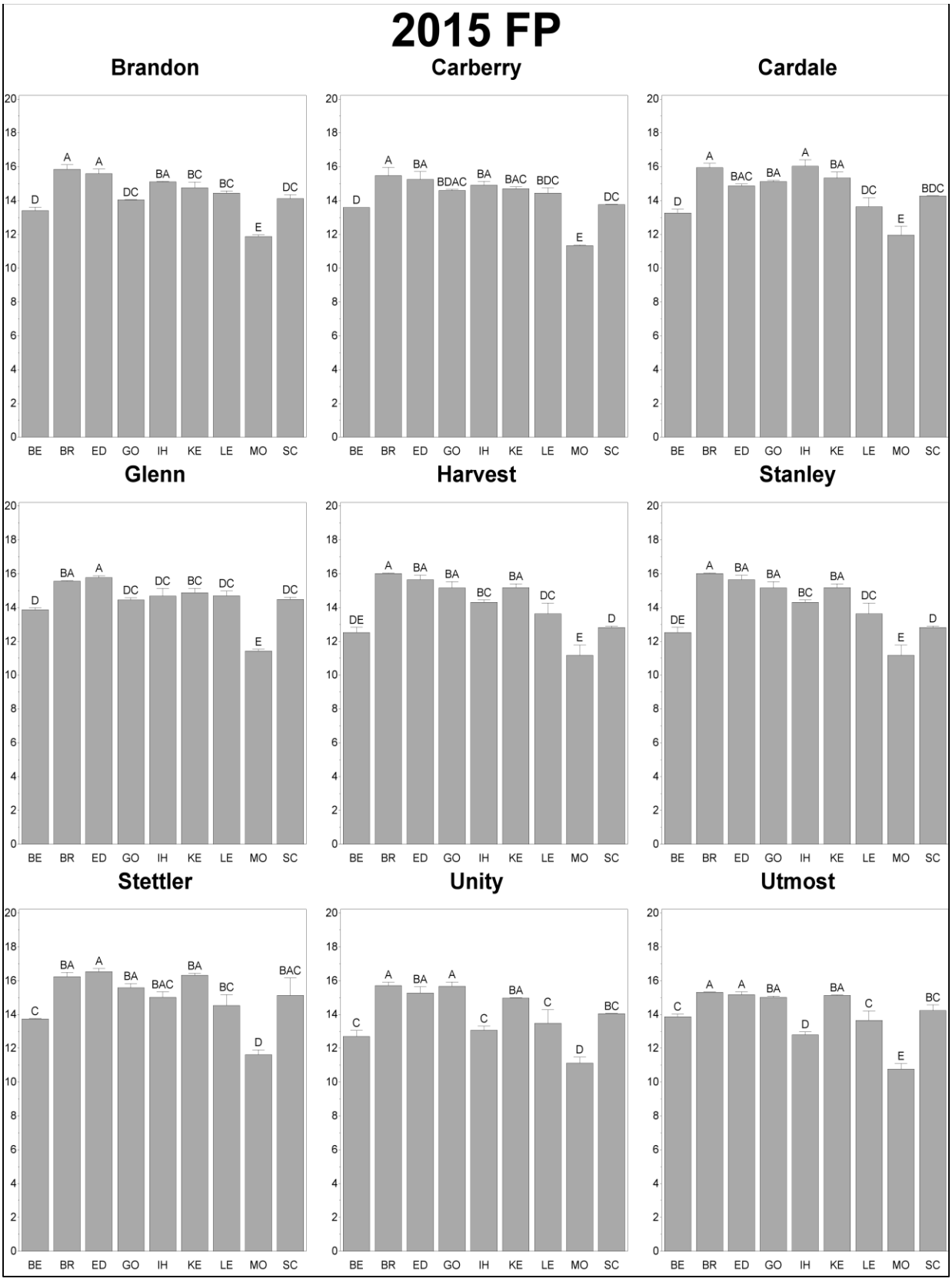
Appendix E.25 Average ash content (%) for 2015 locations within genotypes.



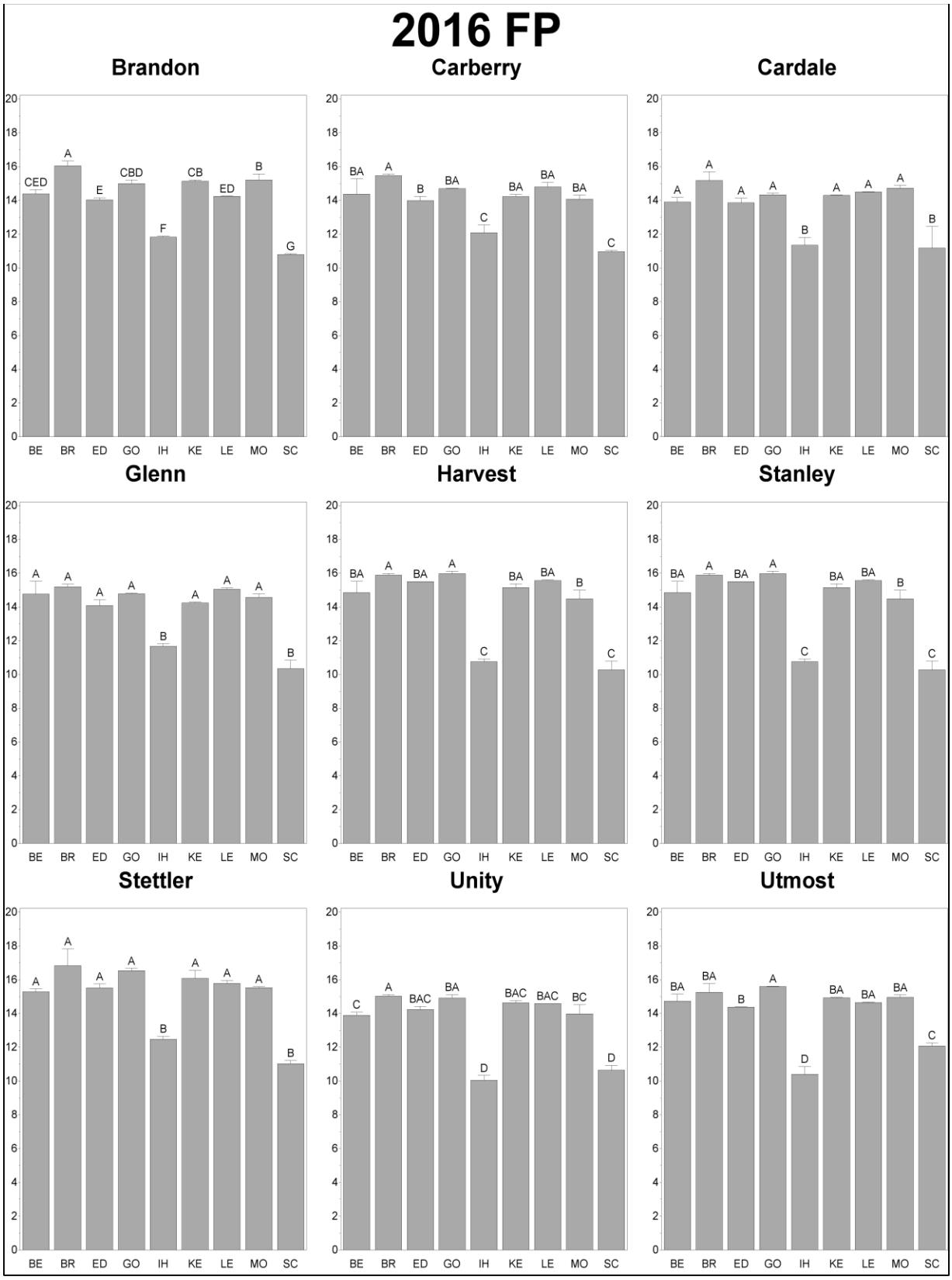
Appendix E.26 Average ash content (%) for 2016 locations within genotypes.



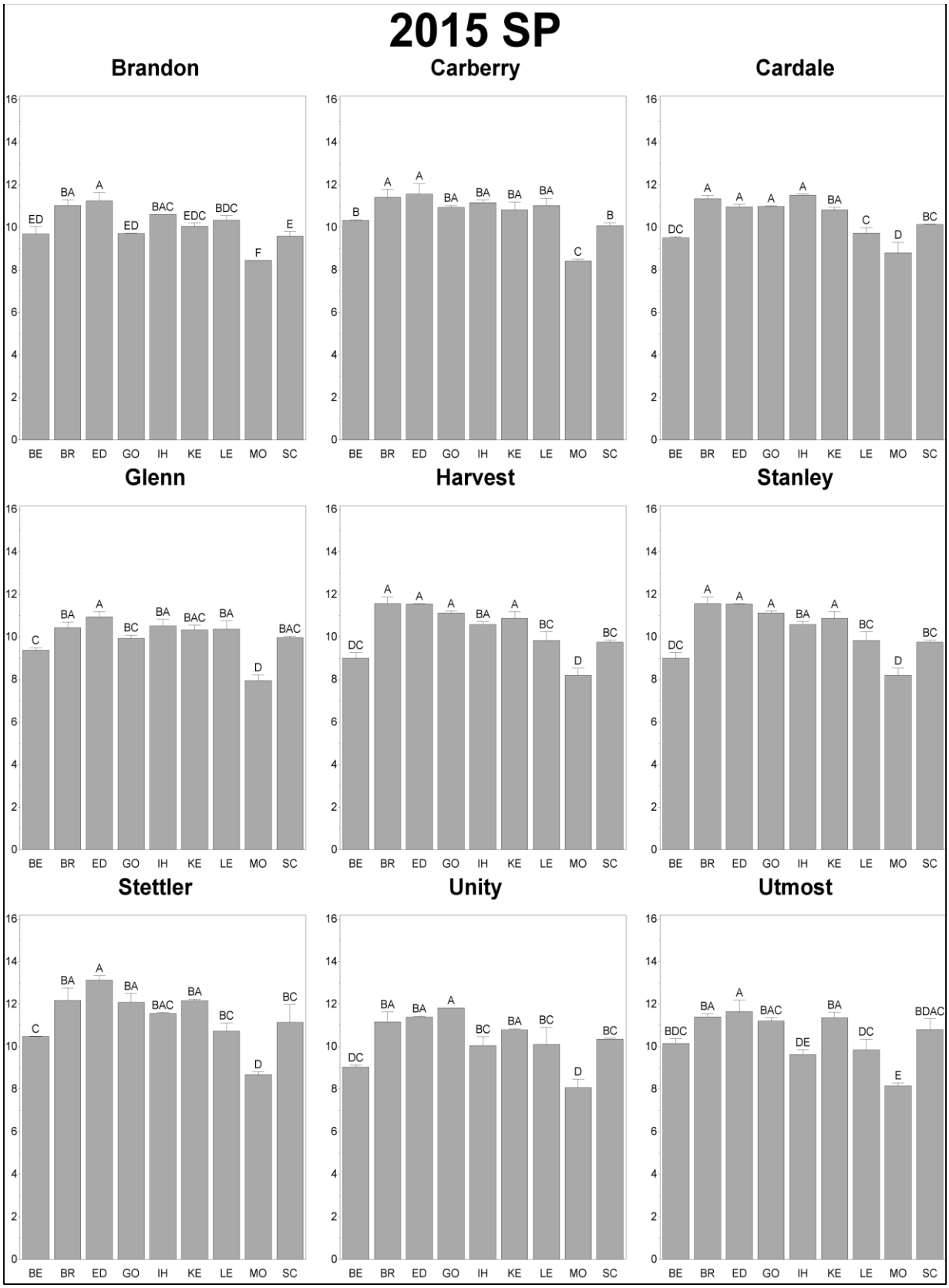
Appendix E.27 Average flour protein content (FP, %) for 2015 locations within genotypes.



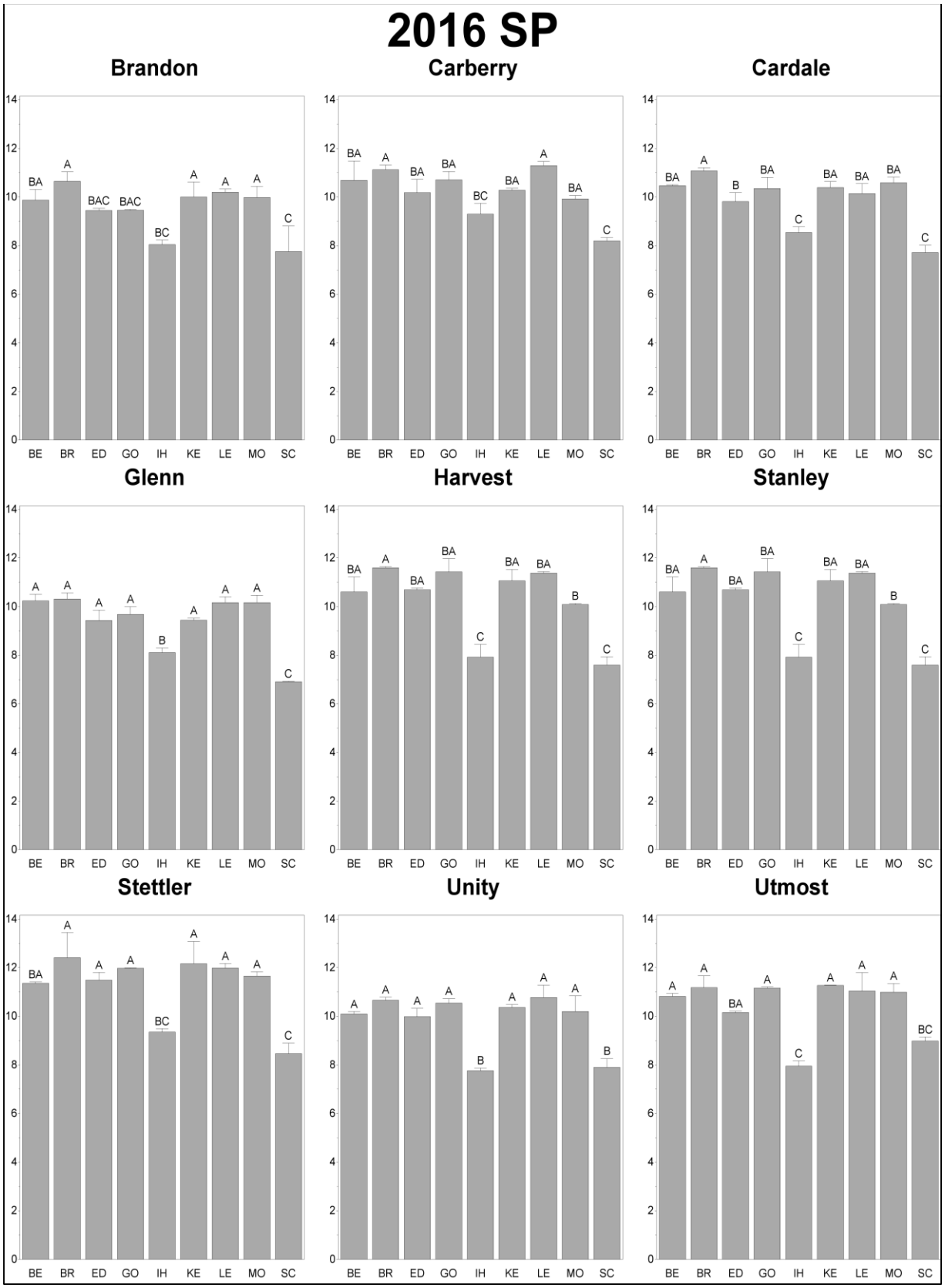
Appendix E.28 Average flour protein content (FP, %) for 2016 locations within genotypes.



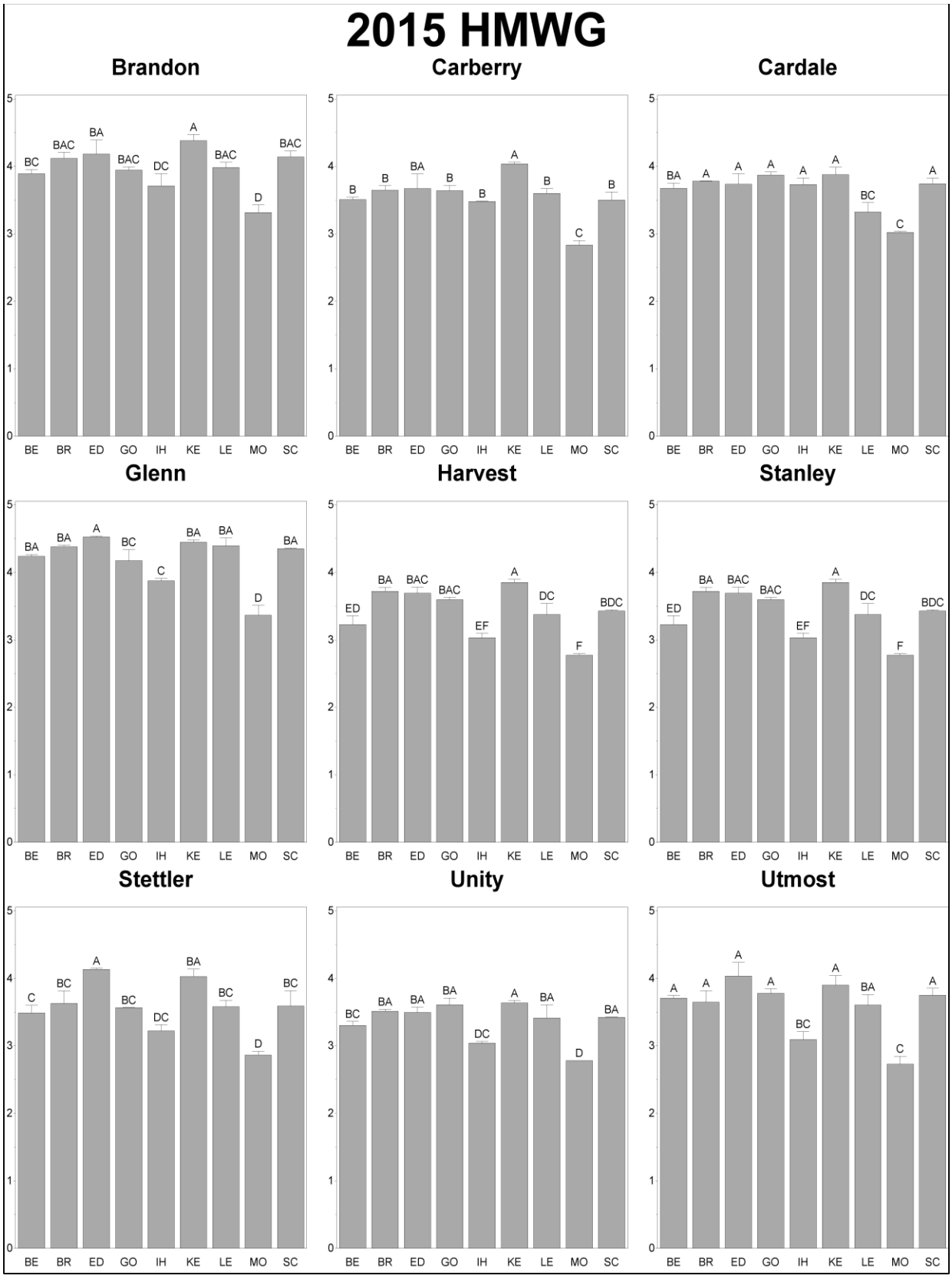
Appendix E.29 Average soluble prolamin content (SP, %) for 2015 locations within genotypes.



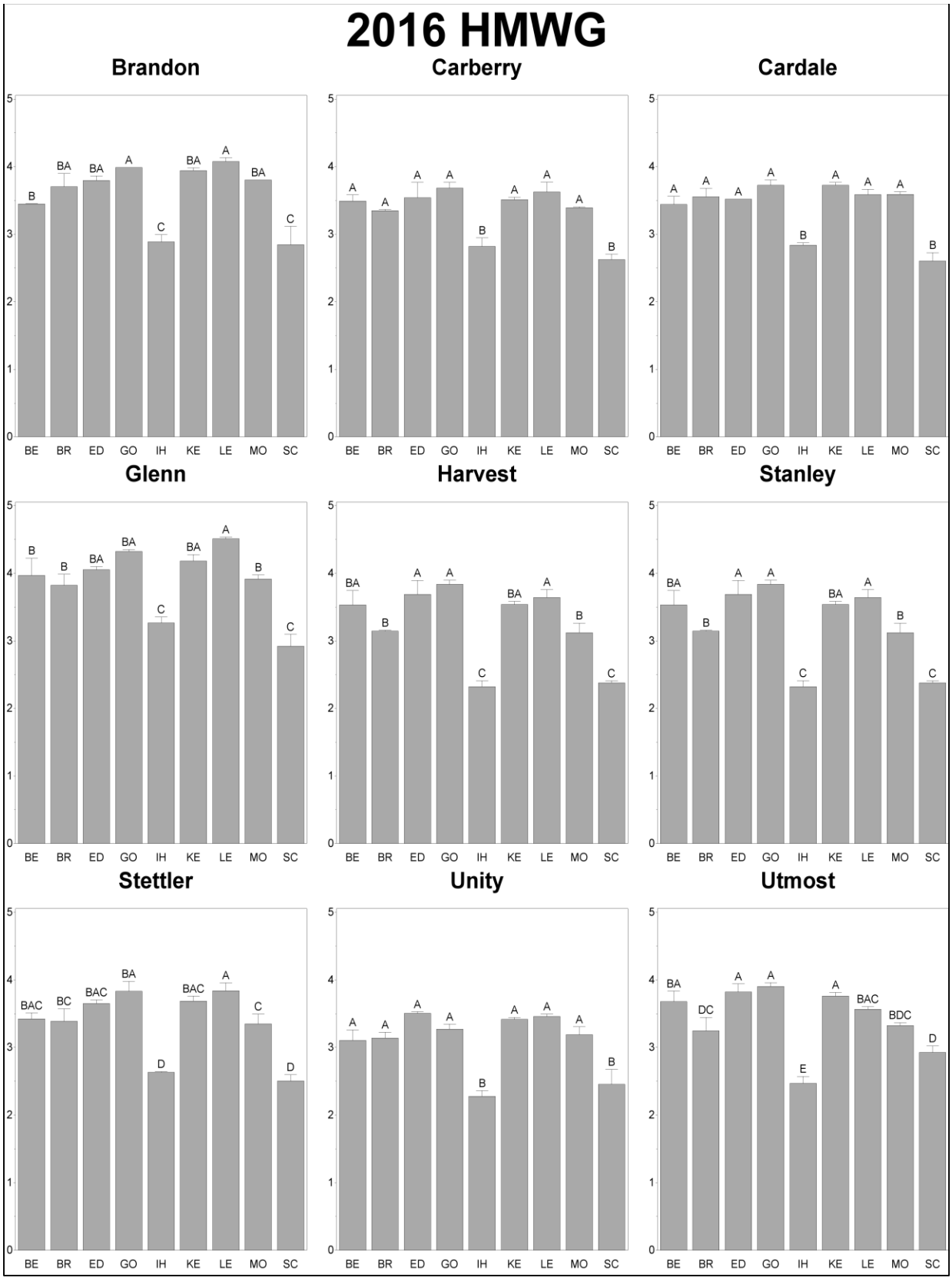
Appendix E.30 Average soluble prolamin content (SP, %) for 2016 locations within genotypes.



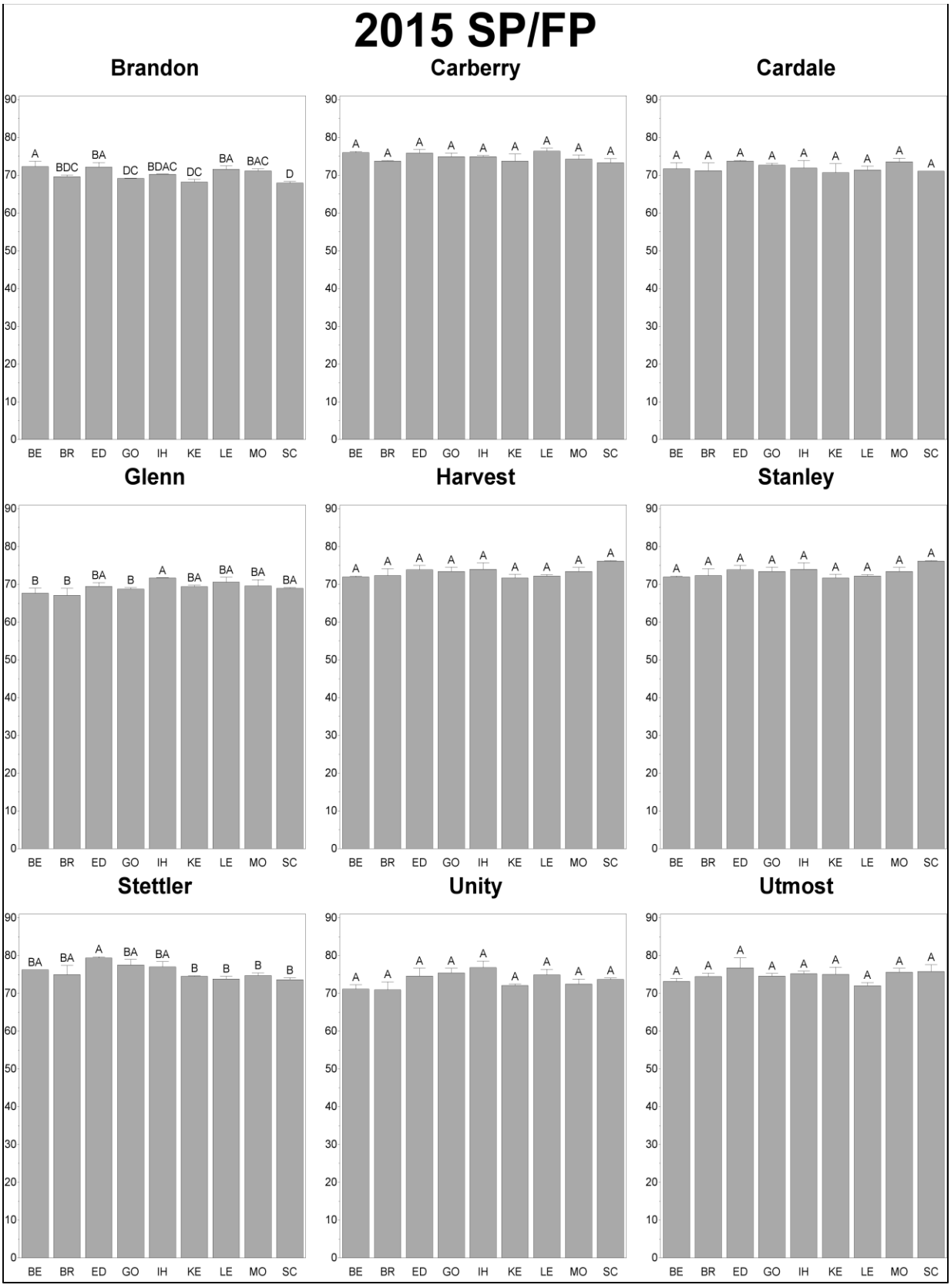
Appendix E.31 Average HMW glutenin content (HMWG, %) for 2015 locations within genotypes.



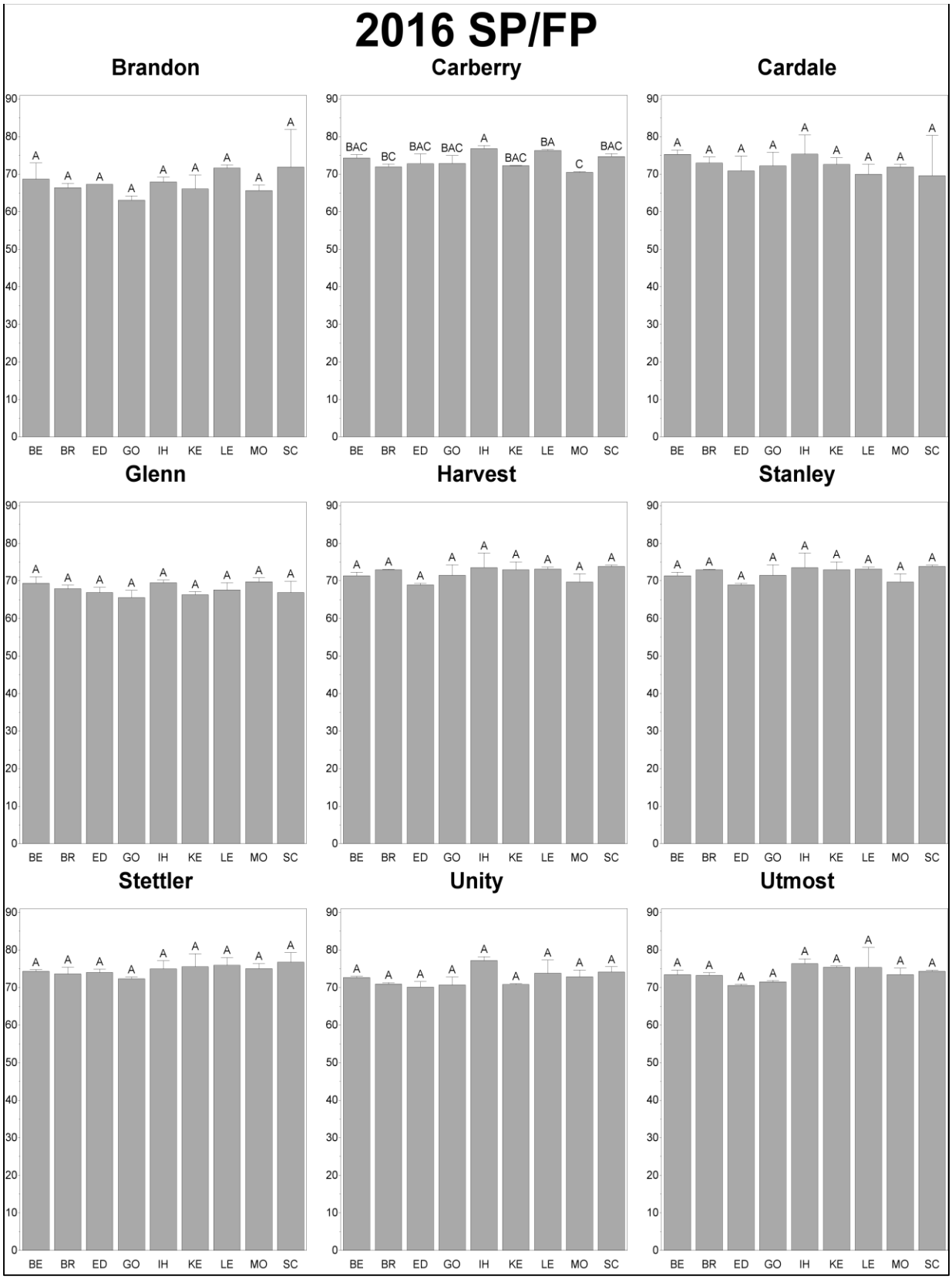
Appendix E.32 Average HMW glutenin content (HMWG, %) for 2016 locations within genotypes.



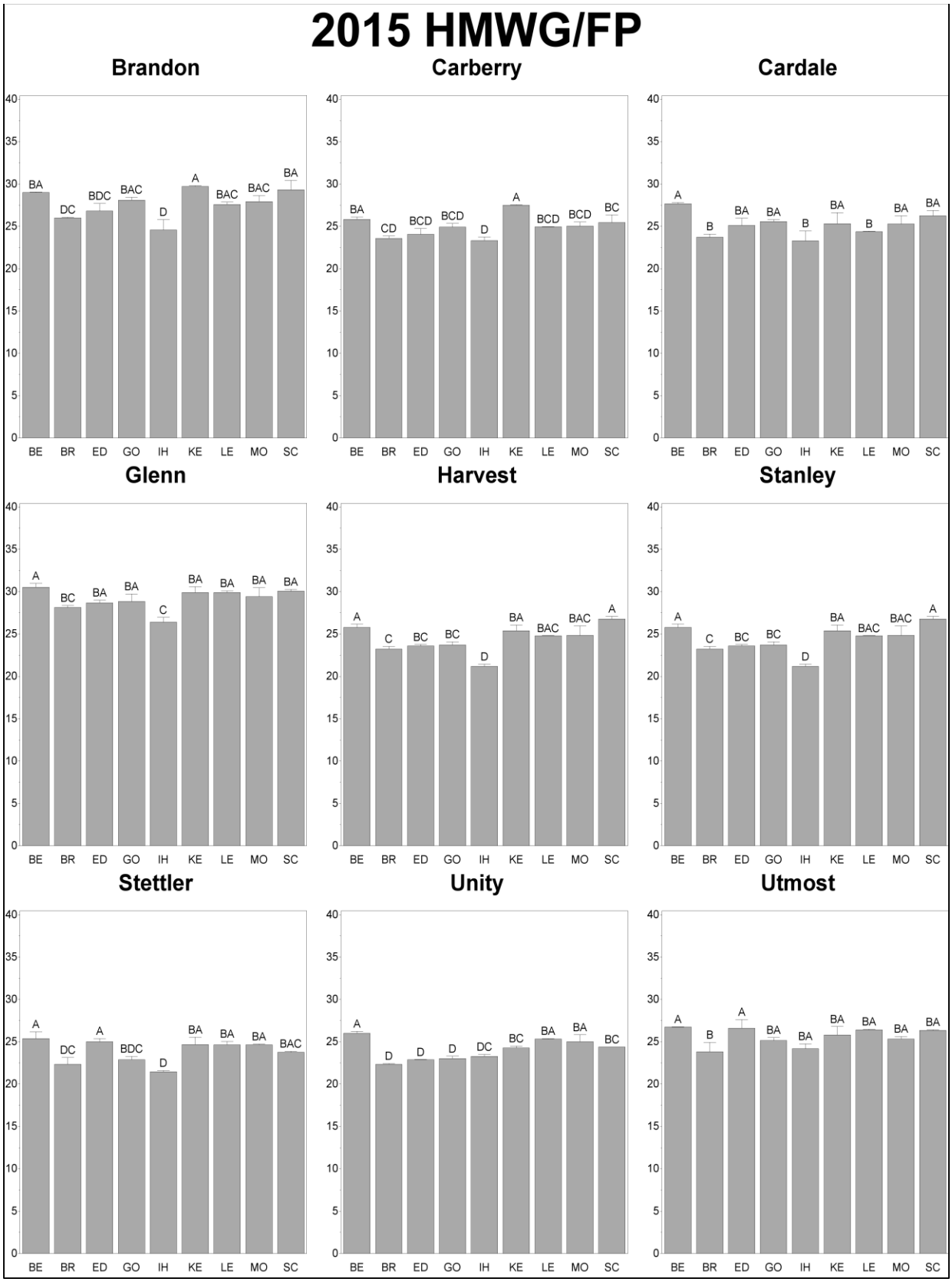
Appendix E.33 Average SP/FP (%) for 2015 locations within genotypes.



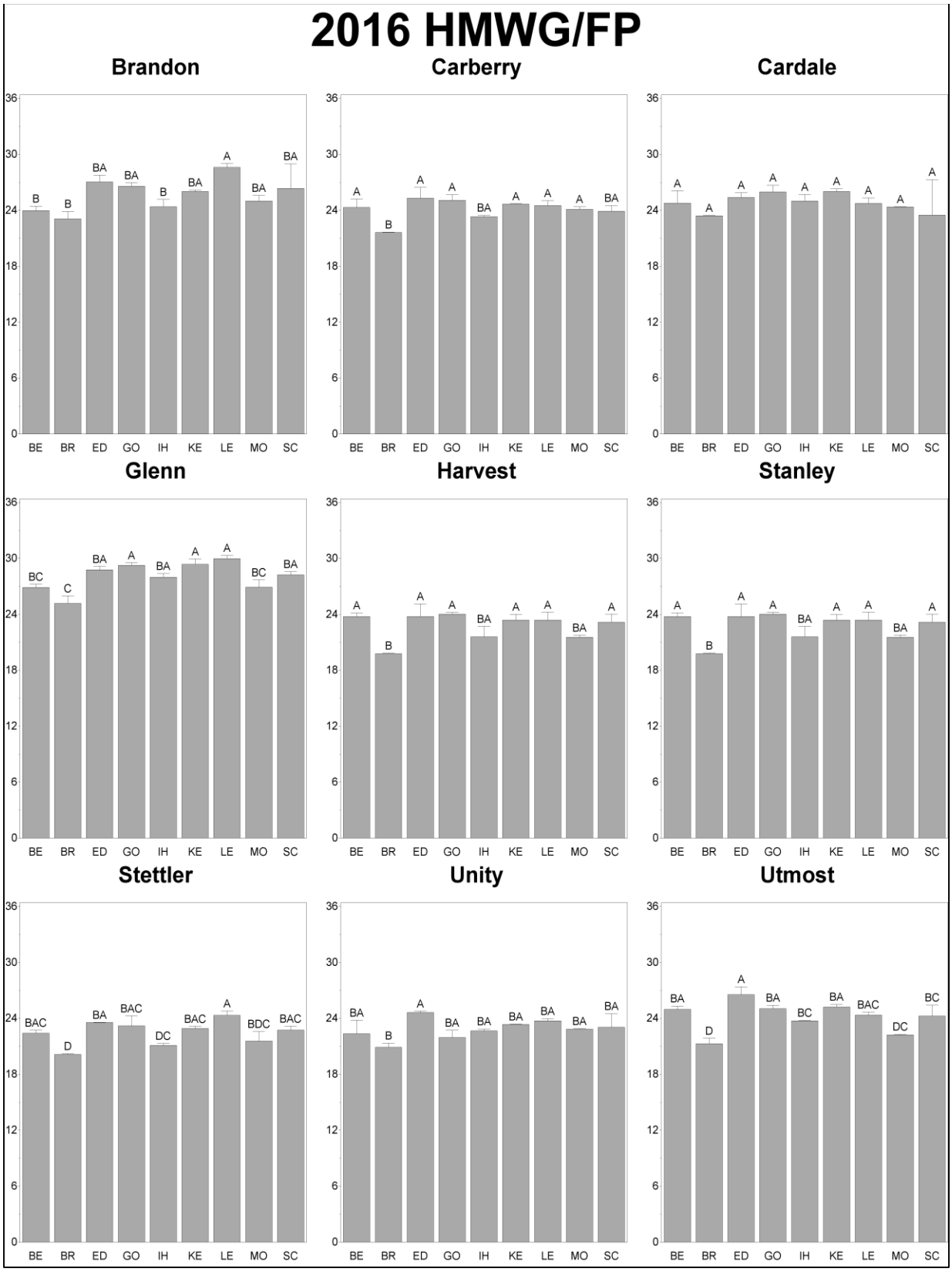
Appendix E.34 Average SP/FP (%) for 2016 locations within genotypes.



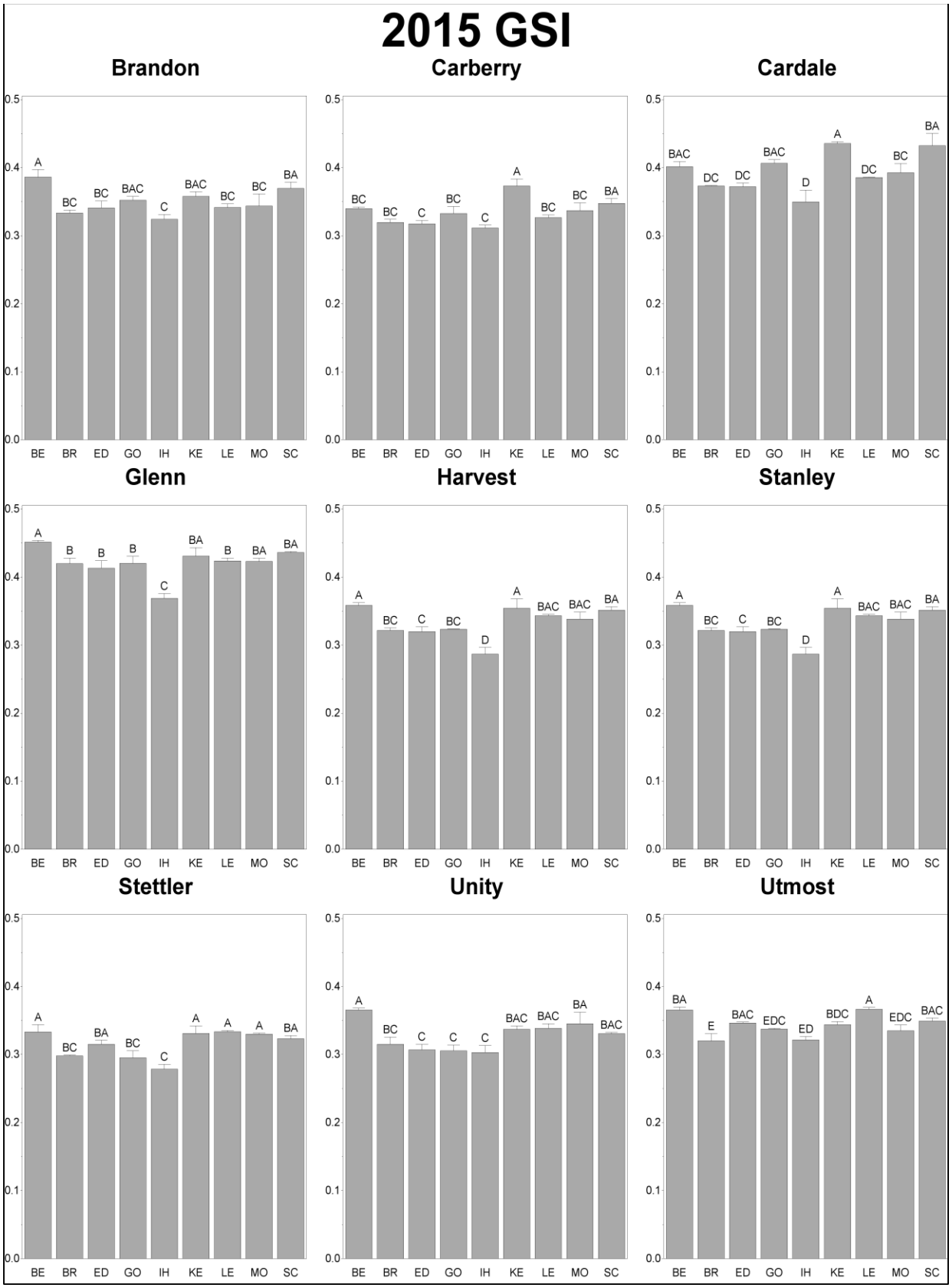
Appendix E.35 Average HMWG/FP (%) for 2015 locations within genotypes.



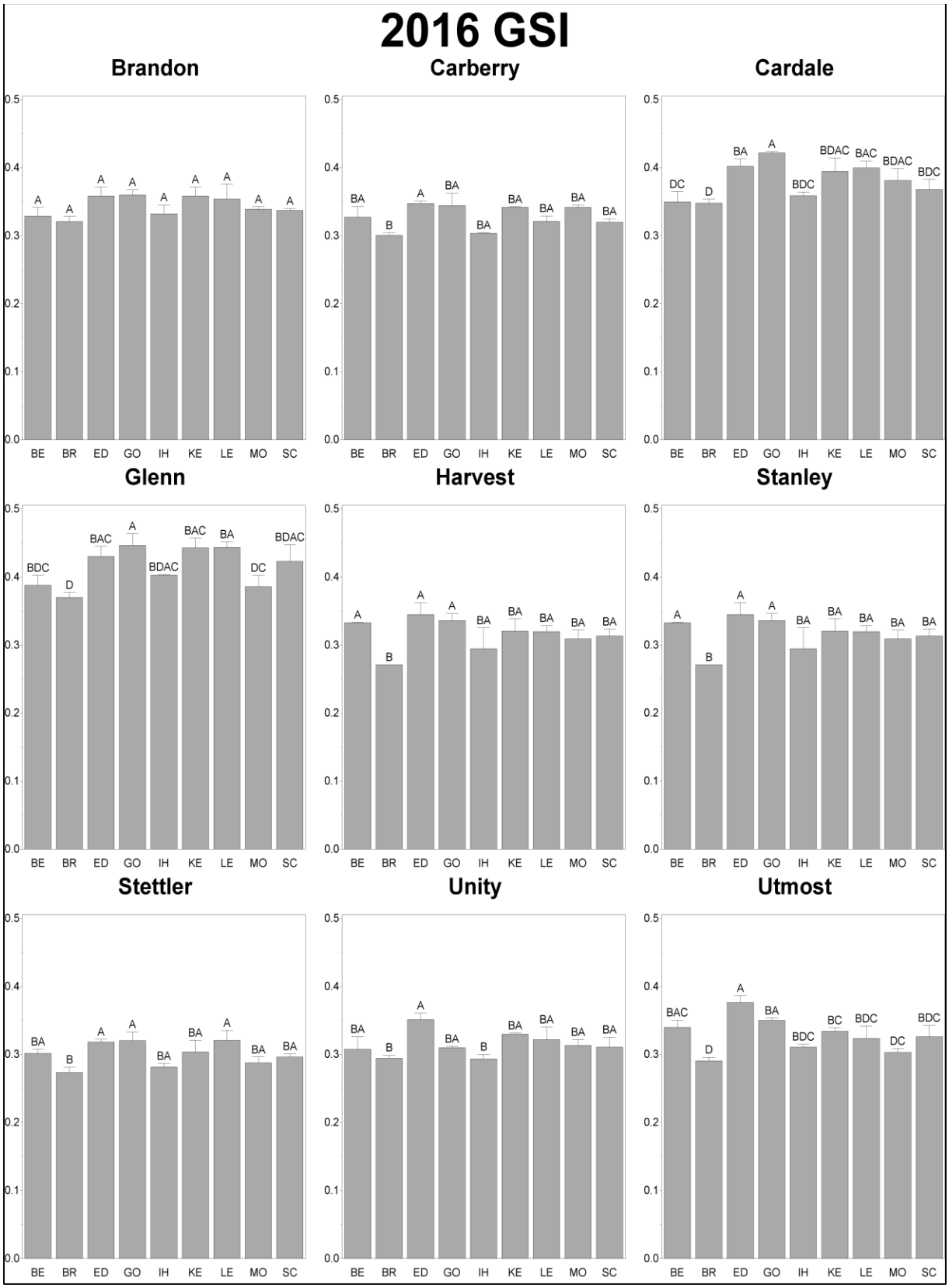
Appendix E.36 Average HMWG/FP (%) for 2016 locations within genotypes.



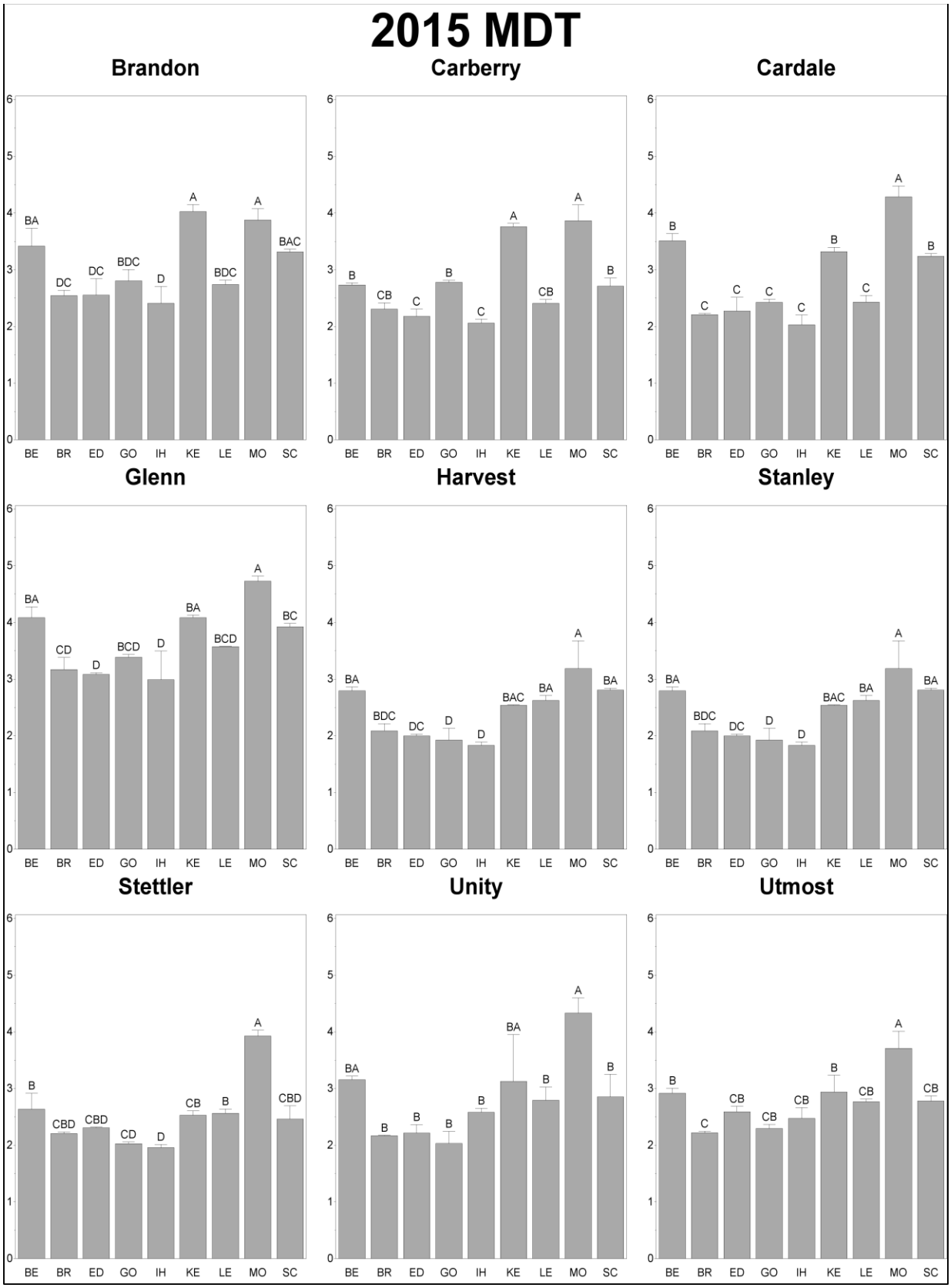
Appendix E.37 Average GSI for 2015 locations within genotypes.



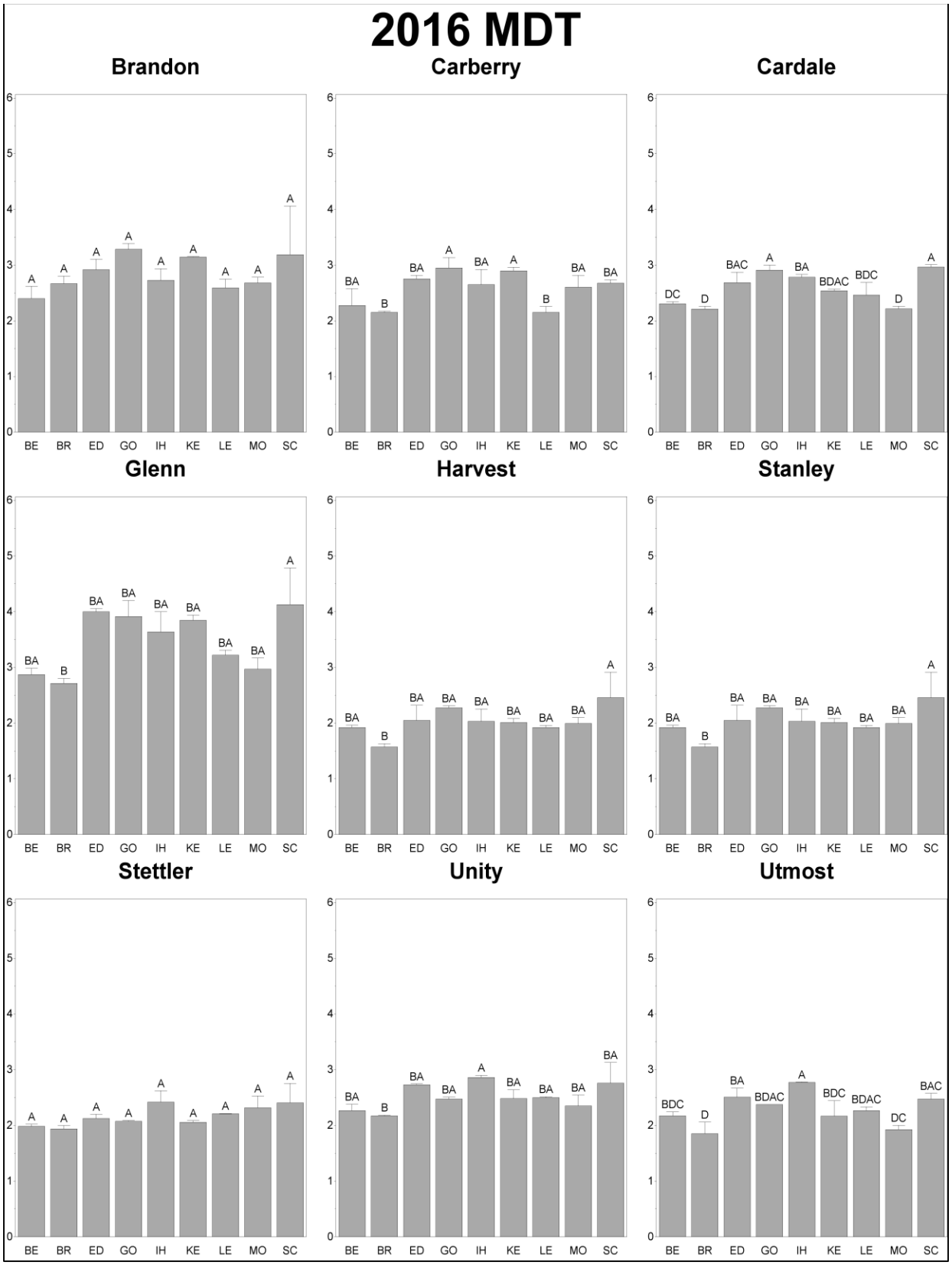
Appendix E.38 Average GSI for 2016 locations within genotypes.



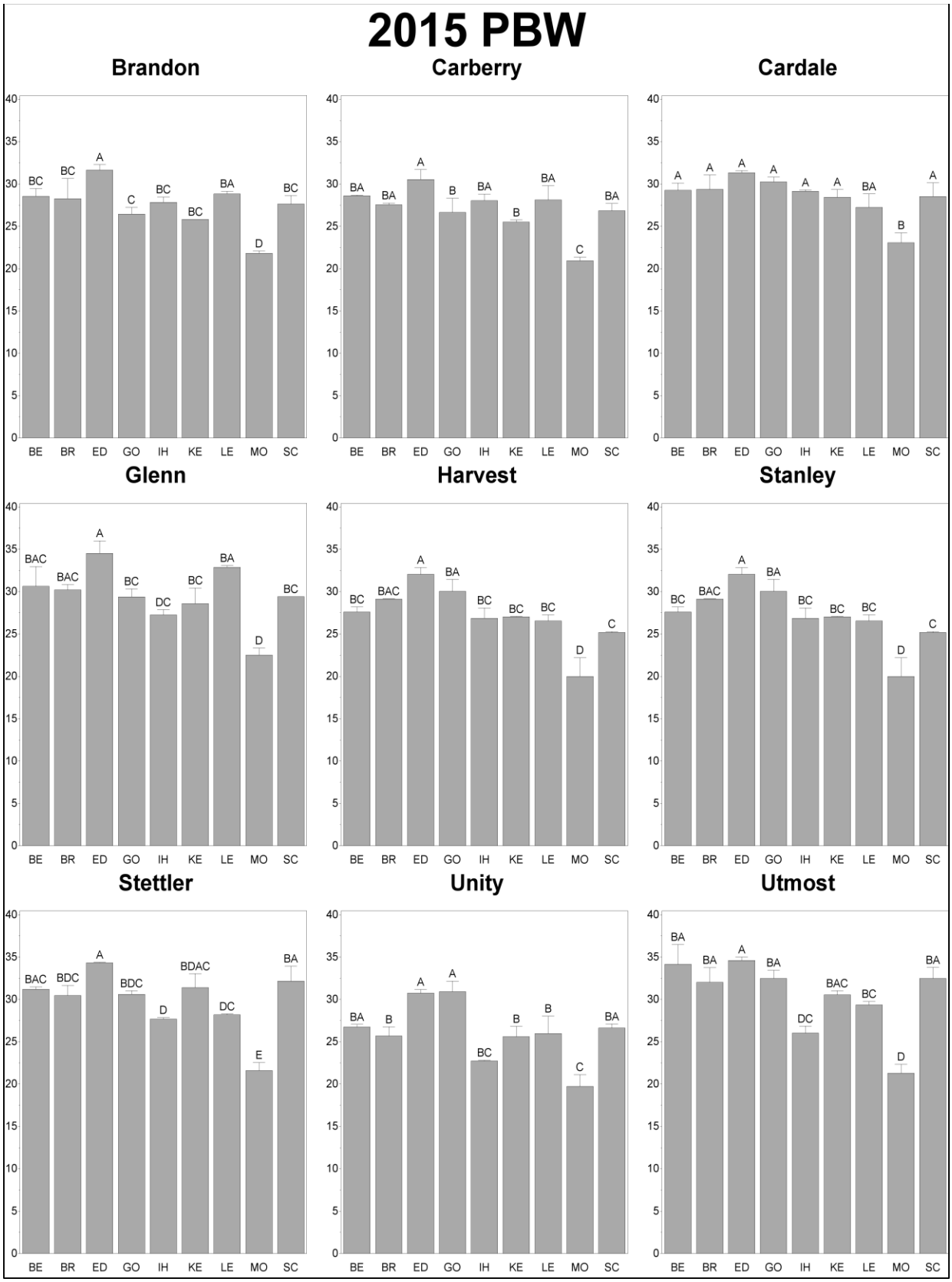
Appendix E.39 Average mixing development time (MDT, min) for 2015 locations within genotypes.



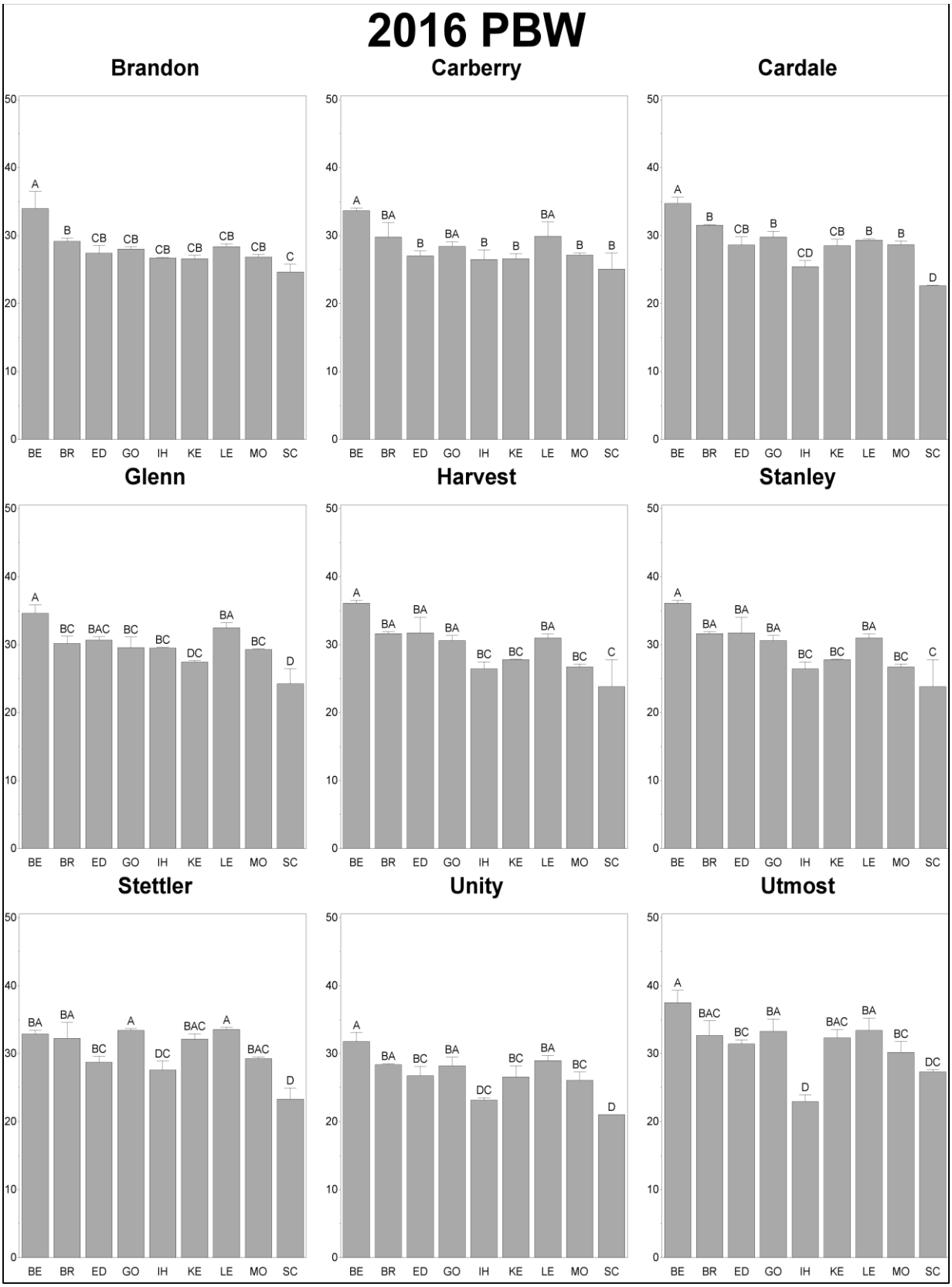
Appendix E.40 Average mixing development time (MDT, min) for 2016 locations within genotypes.



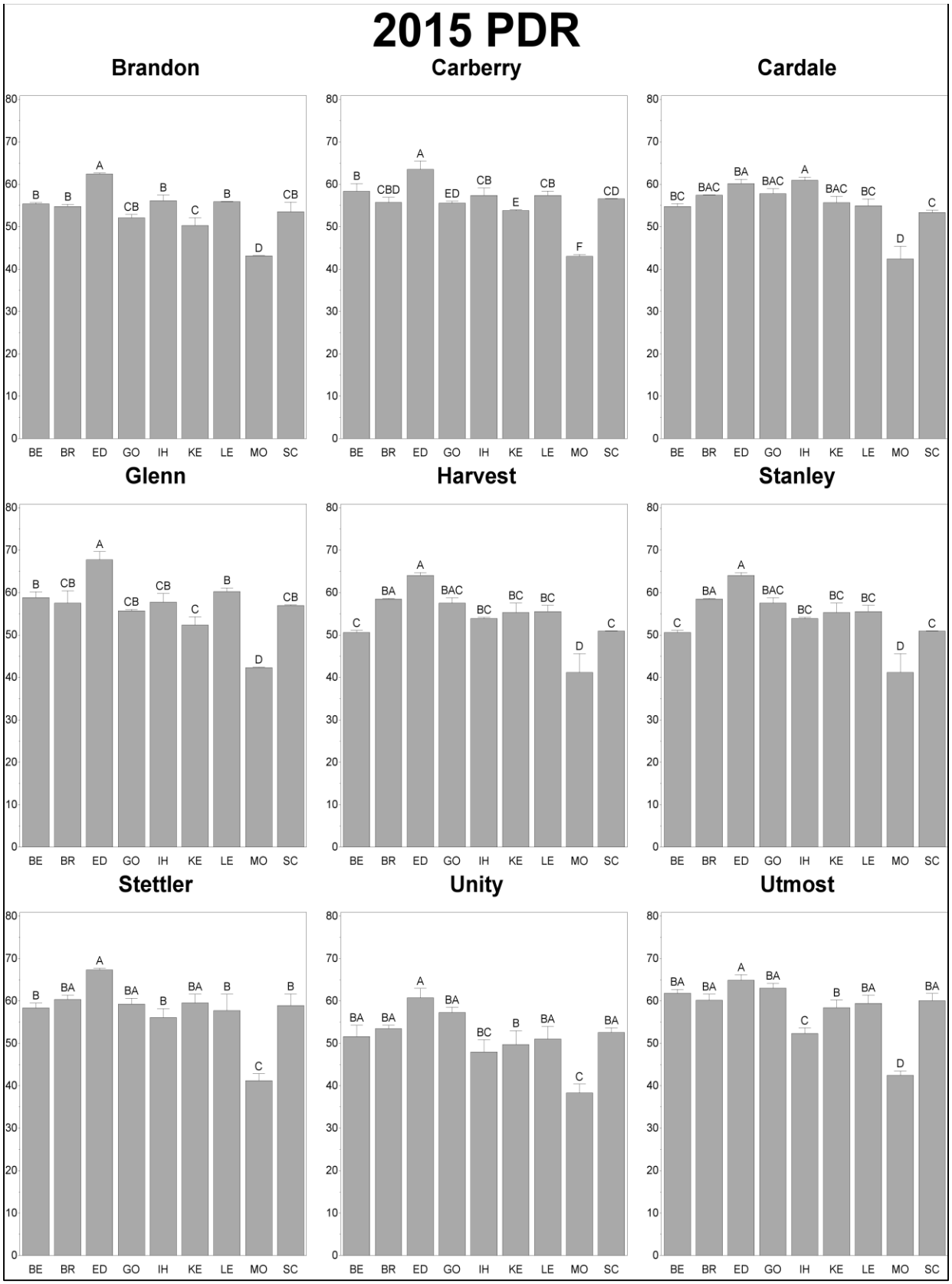
Appendix E.41 Average peak band width (PBW, %Torque) for 2015 locations within genotypes.



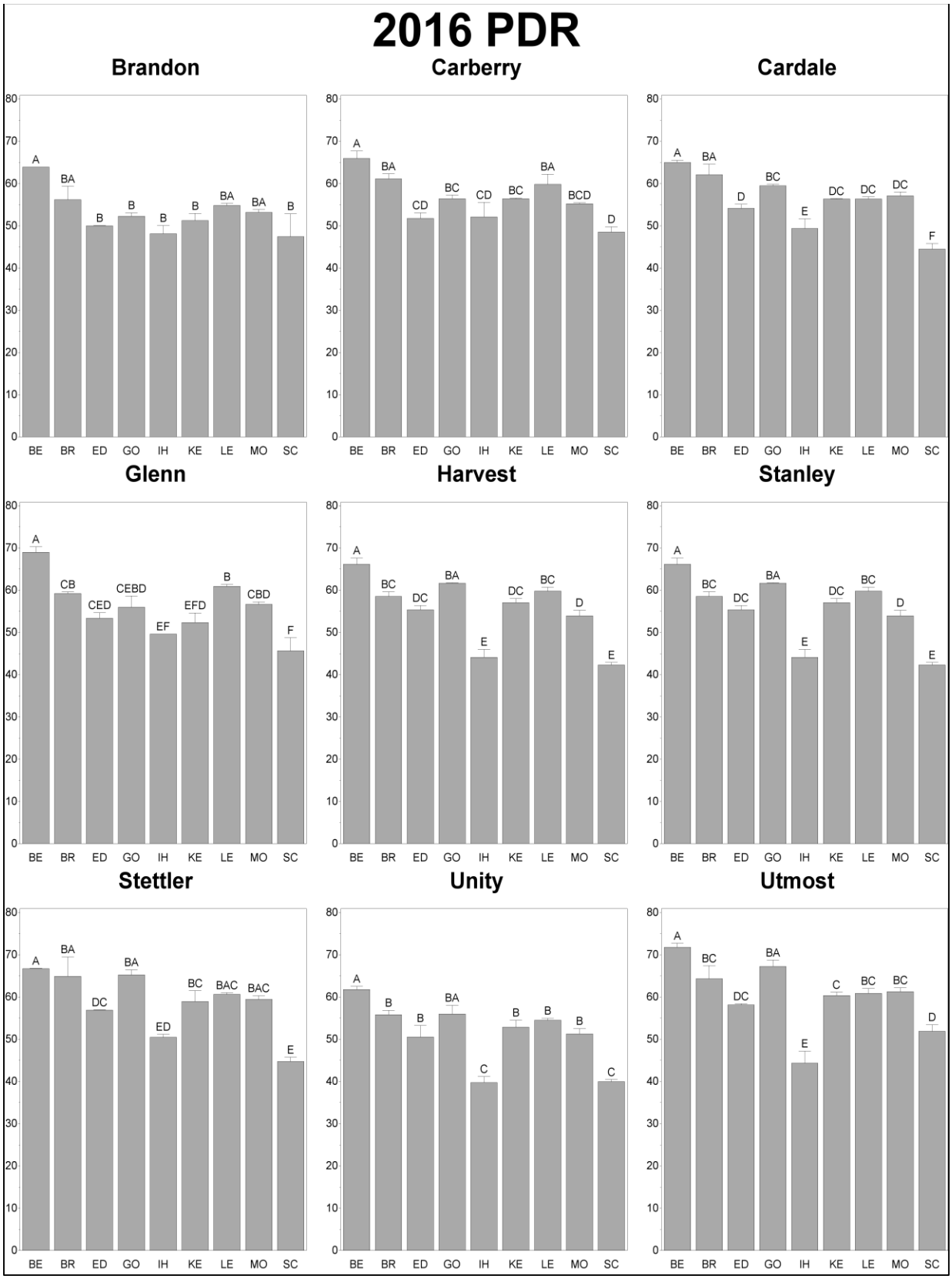
Appendix E.42 Average peak band width (PBW, %Torque) for 2016 locations within genotypes.



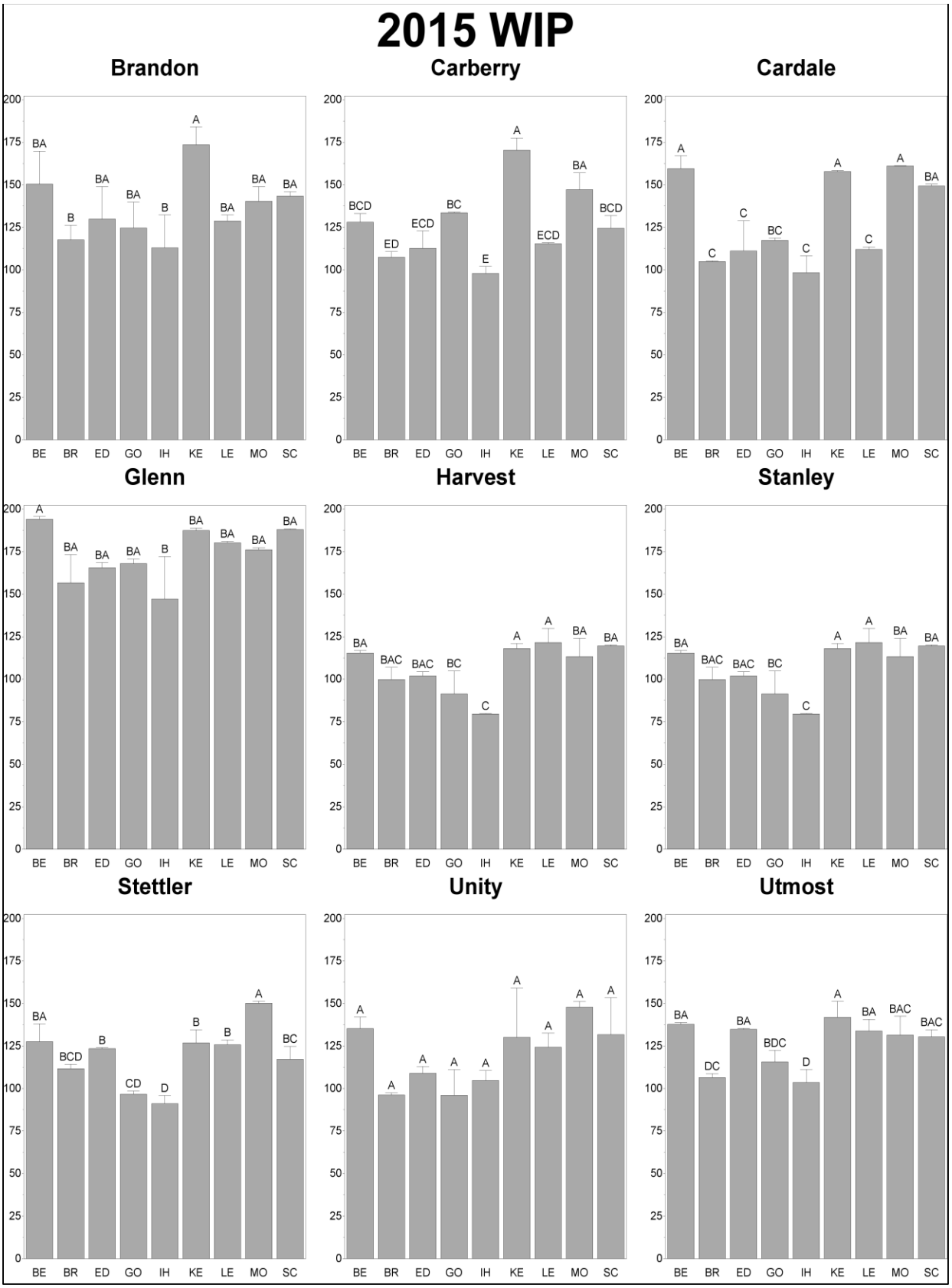
Appendix E.43 Average peak dough resistance (PDR, %Torque) for 2015 locations within genotypes.



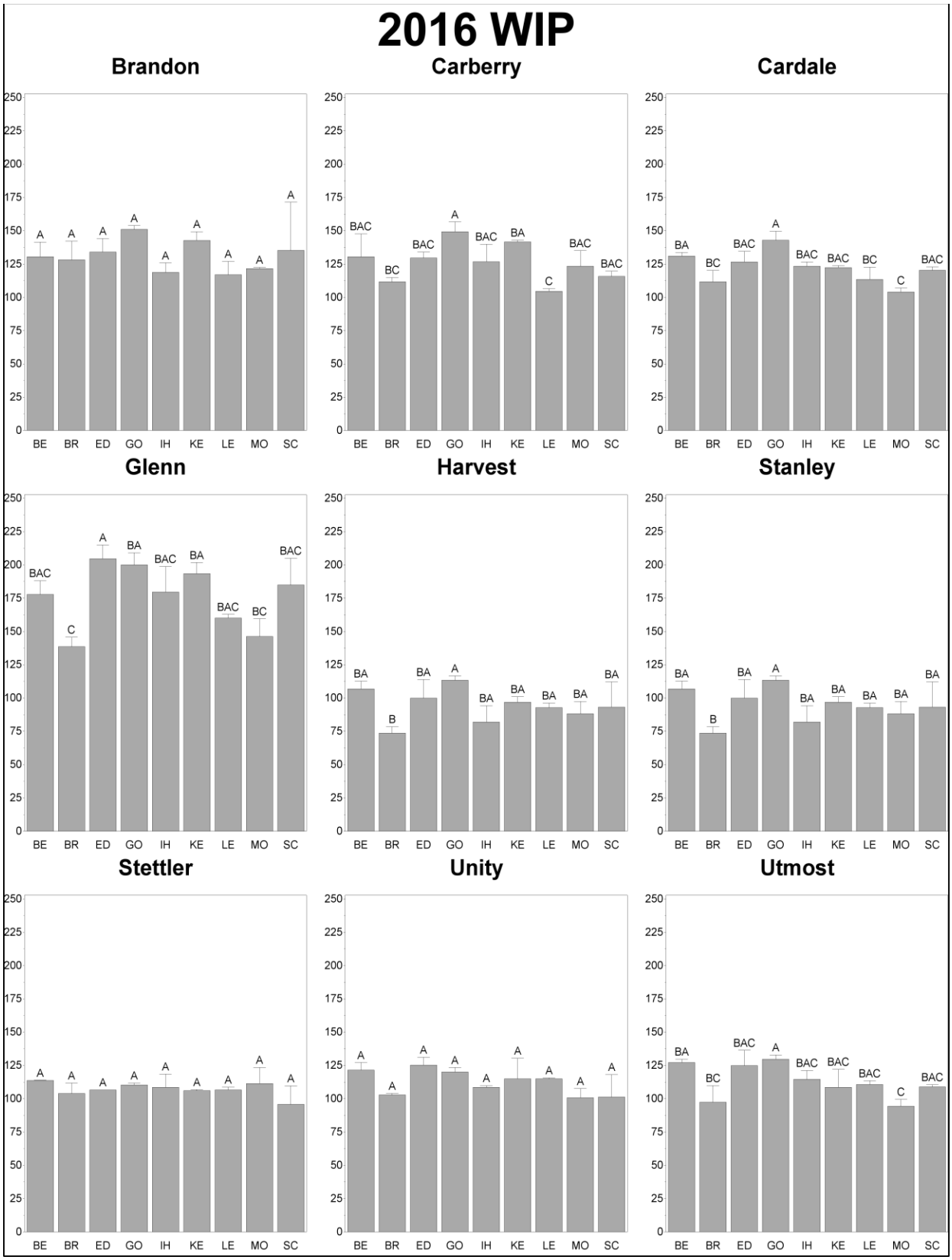
Appendix E.44 Average peak dough resistance (PDR, %Torque) for 2016 locations within genotypes.



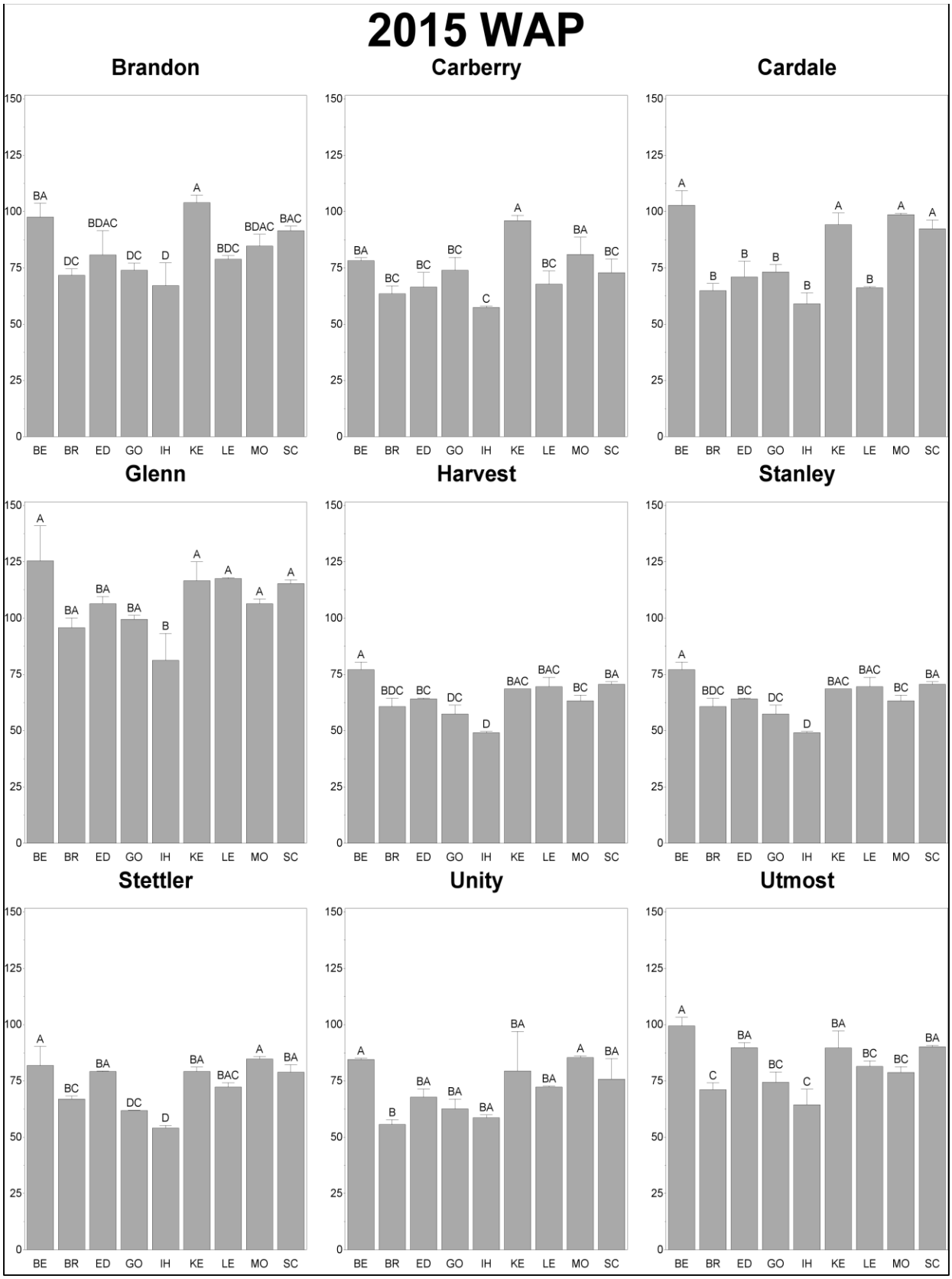
Appendix E.45 Average work input to peak (WIP, %Torque*min) for 2015 locations within genotypes.



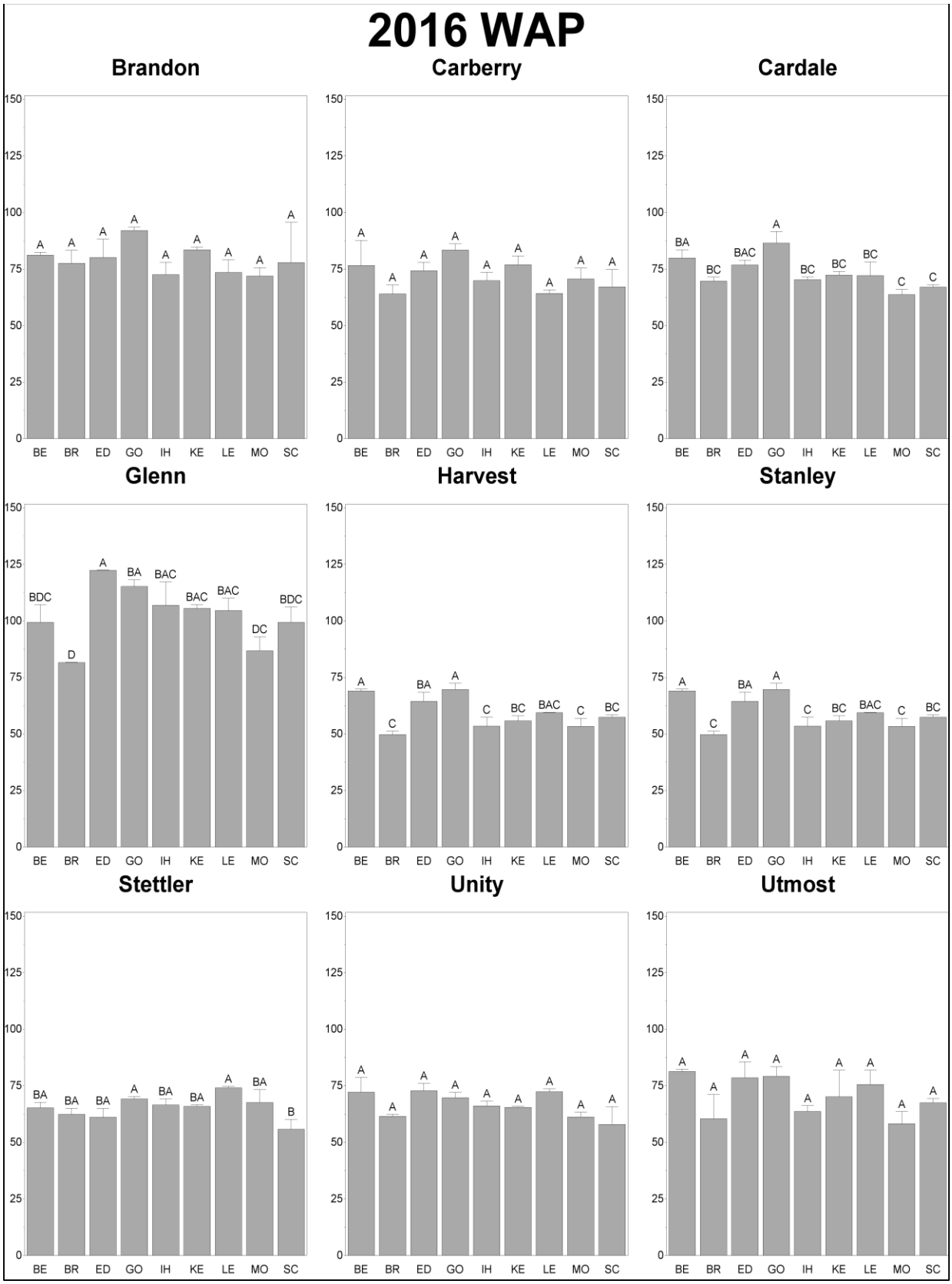
Appendix E.46 Average work input to peak (WIP, %Torque*min) for 2016 locations within genotypes.



Appendix E.47 Average work at peak (WAP, %Torque*min) for 2015 locations within genotypes.



Appendix E.48 Average work at peak (WAP, %Torque*min) for 2016 locations within genotypes.



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