Effects of Genotype, Weather and FHB Fungicide/Pre-harvest Glyphosate on Wheat Quality and Gluten Strength for Breadmaking

by

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ABSTRACT

Canada Western Red Spring (CWRS) wheat is composed of genotypes that have relatively uniform instrinsic quality characteristics. Variation in growing season weather strongly affects CWRS quality, including its gluten strength, but relatively little is known about the effects of pesticide applications. The first study examined effects of genotype, growing location weather and two common pesticides on the grade and quality, including gluten strength of six CWRS wheat genotypes with a wide range of gluten strength characteristics in plot trials across Manitoba, Saskatchewan and Alberta in 2015, 2016 and 2017. Pesticide treatments were fungicide (Prothioconazole/Tebuconazole) applied at anthesis (F) for Fusarium head blight (FHB) mitigation, glyphosate applied pre-harvest (G), both F and G combined (FG) and an untreated control (C). Gluten strength was evaluated using dough mixing and gluten protein composition. Precipitation from seeding to anthesis was closely related to Fusarium damaged kernels (FDK). Site-year and genotype were the most significant factors affecting all quality parameters. In contrast, pesticide treatments had a small impact on the parameters associated with gluten strength. The second study examined the effects of delayed harvest on the grade and quality, including gluten strength, of four CWRS wheat genotypes with a wide range of gluten strength characteristics in plot trials at four locations in Manitoba in 2017. At each location, there were four different harvest dates including harvest at physiological maturity or two weeks prior to normal harvest (H1), normal harvest date at optimal moisture content of 13 to 15% (H2), four weeks after maturity (H3) and six weeks after maturity (H4). Substantial rainfall following H2 significantly and negatively affected grain quality and grades for H3 and H4. However, gluten strength increased slightly for H3 and H4. While harvest date was

statistically significant for many wheat quality parameters including gluten strength, contributions to total variance were very small and often lower than residual error. It was concluded that neither pesticide treatment when used as recommended, nor delayed harvest, were significant sources of variation of gluten strength for CWRS wheat.

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1. INTRODUCTION

1.1 Background Information

1.1.1 The Importance of Wheat

Wheat is one of the most important and influential crops grown around the world. Production of wheat has been a key component of agriculture for thousands of years, and its supply and utilization have continued to grow each year (Blandino and Reyneri, 2009). In 2017 alone, there were 760 million tonnes of wheat produced on a global scale (AMIS Market Database, 2018). As wheat is such a valuable, and widely used crop, it is critical that its production and quality remain a top priority for producers. This is particularly the case for Western Canadian wheat producers as wheat is a consistent, staple crop grown across the prairies.

One of the most common products created from wheat is bread. The gluten proteins in wheat are the unique characteristics which are key factors for breadmaking performance (Canadian Grain Commission, 2016). Gluten strength is essential for breadmaking quality as it establishes dough mixing requirements, allows doughs to rise during fermentation and is generally considered to be positively related to loaf volume. Gluten can be broken down into two key protein components, gliadin and glutenin. Gliadin contributes to dough or gluten viscosity, while glutenin contributes the elasticity property of gluten. Breadmaking requires the correct balance of both of these two proteins. It's the glutenin fraction that is mainly responsible for differences in quality among different genotypes or samples of wheat.

Gluten strength of bread wheat can vary substantially between growing seasons, and locations, and this affects the consistency of the wheat quality that bakers and millers require to produce similar products from shipment to shipment and from year to year. Wheat of a given class and protein content with variable gluten strength creates processing issues for breadmaking which reduces its overall value for producers.

Some of the factors that are currently known to cause gluten strength variability include wheat genotype, growing season environment, and crop fertilizer inputs such as nitrogen and sulphur. Growing season weather conditions cannot be controlled but management practices such as genotype, and crop fertilizer inputs can be controlled by the producer. There are also other commonly used management practices that may have an effect on gluten strength variability including pesticide applications such as fungicides applied at the anthesis stage to control Fusarium head blight, and pre-harvest applications of glyphosate. Both of these pesticide management practices are commonly used in Western Canada and have been widely adopted in other areas of the world. However, it has not been determined whether these practices have a significant effect on wheat quality, and in particular wheat gluten strength.

1.1.2 Wheat Protein

The storage proteins of wheat endosperm that form gluten can be classified as prolamins comprising monomeric gliadin (i.e. single chain polypeptides) and glutenin which is one of the largest proteins in nature (Wrigley, 1996). Glutenin is comprised of high molecular weight (HMW) and low molecular weight (LMW) subunits which aggregate via disulphide bonds

(Shewry et al., 1986). Depending on the composition of subunits and their interactions, the glutenin polymer that forms during grain development can also vary in molecular size which in turn has a strong impact on dough rheological properties such as gluten strength and baking performance (Southan and MacRitchie, 1999; Wieser and Zimmermann, 2000). Gliadin contributes to the viscosity of gluten and dough extensibility and is considered a diluent of glutenin which contributes to elasticity of gluten and dough. Accordingly, gluten or dough is often described as being viscoelastic. There needs to be a balance between the glutenin and gliadin, as they both contribute to important rheological properties. Glutenin, and in particular its HMW fraction, is typically insoluble in many solvents such as alcohols and dilute acetic acid, or detergents such as sodium dodecyl sulphate, and is frequently referred to in the literature as "insoluble glutenin". Gliadins on the other hand are typically soluble in these solvents, and are typically referred to as soluble protein or soluble prolamins. The ratio of HMW glutenin to gliadin, i.e. ratio of IG to SP, is a determinant of gluten strength and in this thesis research is called the Gluten Strength Index or GSI (Isaak et al., 2019). While the content of glutenin and gliadins in particular in a wheat or flour sample is influenced by environmental factors, the GSI is mainly a genotypic property.

1.1.3 Genotype Effects on Wheat Dough Strength

Molecular weight distribution (MWD) of wheat proteins, particularly glutenin has a major role in physical dough properties (Southan and MacRitchie, 1999). Glutenin is a polydisperse polymer, meaning that the glutenin fraction in a given wheat sample or genotype is composed of a range of polypeptides varying in molecular size or weight. Glutenin of larger average

molecular weight is directly related to dough strength (Southan and MacRitchie, 1999). Two factors that can directly affect the MWD of glutenin are genotype and environmental (Southan and MacRitchie, 1999). Nine major gene loci in hexaploid or common wheat are responsible for coding of wheat gluten proteins, i.e. three *Glu-1*, three *Gli-1/ Glu-3* and three *Gli-2 loci*, with each one having allelic variants (Southan and MacRitchie, 1999). These alleles can be altered through breeding and their proportions can vary due to environmental conditions (Southan and MacRitchie, 1999). Accordingly, this variation has consequences on gluten protein viscoelasticity and related functional properties (Southan and MacRitchie, 1999). It is important that breeding programs take into account the variation in these loci to attempt to manipulate the MWD glutenin (and in turn gluten strength), and also to consider the effects of environmental factors on glutenin (Southan and MacRitchie, 1999).

1.1.4 Impacts of Weather Variation on Wheat Quality

Every growing season, weather conditions can be very different at any given location in Western Canada, resulting in changes to the quality of wheat across locations and from the same location from year-to-year. This can become a challenging situation for both the milling and baking industries as they strive to produce consistent, high quality products every year (Peterson et al., 1998) from the same class and grade of wheat.

One of the main environmental parameters during crop development that has been found to have a significant effect on end-use quality is temperature (Zhu and Khan, 2001). Increased temperatures causing heat stress have been reported to decrease the glutenin to gliadin ratio due to the fact that gliadin continues to be synthesized during heat stress whereas glutenin

does not (Zhu and Khan, 2001). While heat stress conditions are uncommon in the Canadian Prairies, temperature provides an example of one environmental factor's influence on protein synthesis and quantitative composition. Environment as a whole (i.e. precipitation, temperature, soil, etc.) was found to have a greater influence than genetics for flour protein content (Zhu and Khan, 2001), but this outcome depends on the mix of varieties in any particular study. This was also found for other quality parameters related to a mixograph analysis (see Section 1.1.9), such as measurements of peak height and slope, and bandwidth at peak mixing time. The composition of protein into soluble glutenin and gliadin fractions can be influenced by the environment and subsequently affect dough mixing properties (Zhu and Khan, 2001).

Gooding et al. (2003) studied the controlled environmental effects of both rainfall and temperature in relation to grain development, grain specific weight (used to determine bulk density of grain), protein content, Hagberg Falling Number, SDS-Sedimentation volume and Sulphur content. Both drought stress and high temperatures (28/20^{o}C (day/night)) during grain filling increased grain protein content (Gooding et al., 2003). In field trials, there was much more variability in grain quality, and the effects were less clear compared to those in a controlled environment (Gooding et al., 2003). When soil moisture content was increased from 44% to 75% of field capacity in the controlled environment, there was a resulting linear increase in grain yield, mean grain weight and specific weight (Gooding et al., 2003). During the linear phase of grain growth, i.e. the time during which grain weight is increasing after anthesis, increased temperatures reduced the time for grain filling and reduced final grain weight

(Gooding et al., 2003). These are a few examples from one study showing how different environmental factors can change grain quality.

Protein composition, gluten strength and baking quality parameters are all critical factors for bread wheat, but grain quality, including grade and the type of degrading factors is also important because it determines the value of wheat delivered by producers to grain companies. Grain quality is especially susceptible to the weather conditions during the growing season. Campbell et al. (1981) studied environmental effects on grain quality during different growing periods of wheat. The growth stage during which the weather was found to have the greatest impact on the number of kernels was the boot stage, and for kernel weight, it was the anthesis stage (Campbell et al., 1981). The conditions that proved to be optimal for high yielding grain were cool temperatures, high soil fertility and low moisture stress (Campbell et al., 1981). In contrast, the conditions for poorest grain yield were hot temperatures, low fertility and high moisture stress (Campbell et al., 1981). When high moisture stress occurred in either the boot stage, or boot and tillering stages there was a decrease in yield potential (Campbell et al., 1981). Increased temperatures (over 27 ^{o}C) at boot stage also had a negative effect on yield (Campbell et al., 1981). In regards to protein content, the effects of temperature on yield were the reverse of the effects on yield (i.e. when yields were highest, the protein content was lowest). Under hot temperatures, high fertility and high moisture stress from boot stage to crop maturity, protein content was highest (Campbell et al., 1981). The lowest protein content occurred under low temperatures with medium fertility and moisture stress from late flowering to crop maturity (Campbell et al., 1981). This relationship between environmental effects on

protein can be attributed to protein synthesis increasing with higher temperatures (Campbell et al., 1981). This study also considered nitrogen fertility, and its effect on grain yield and protein. The use of nitrogen increased the number of seeds per plant at every temperature setting during the study; in addition, it increased grain protein content (Campbell et al., 1981). Soil fertility, especially nitrogen, has the ability to increase yield and improve the quality of wheat (see Section 1.1.6).

In some years, the harvest date of a crop may be adversely affected and delayed due to inclement weather (Czarnecki and Evans, 1986). Wheat is considered to reach physiological maturity at maximum dry matter content which marks the end of seed filling period (Rondanini et al., 2007) at which time the moisture content of the grain is typically within the range 33 - 41% (Calderini et al., 2000). However, there is still a period of time before the crop is deemed to be harvest ready or "harvest ripe" (Farrer et al., 2006). The ideal harvest grain moisture content for wheat is 13% to 15% (Farrer et al., 2006). During the period prior to the crop reaching the correct moisture content, many areas across Western Canada can experience inclement weather with large amounts of precipitation that hinder farmers' ability to harvest their wheat. When harvest is delayed there can be substantial losses in yield, grade and grain quality (Farrer et al., 2006). Some of the causes for these losses include lodging, shattering, hail damage, and increased grain wetting and drying in the field (Farrer et al., 2006). Delayed harvest can significantly decrease thousand kernel weight and test weight as well as increase levels of Fusarium damaged kernels and deoxynivalenol (see Section 1.1.7) (Farrer et al., 2006). Christensen and Legge (1984) studied the effects of windrowing and direct combining, at 5%

kernel moisture content intervals from 45% to 15% on the grain quality and yield of two cultivars. When the kernel moisture content was above 35%, there were negative results for yield, thousand kernel weight, Falling Number and grade (Christensen and Legge, 1984) for both windrowing and direct combining. The windrow conditions caused a larger increase in shattering losses than direct combining, due to increased handling and weathering of the individual swaths (Christensen and Legge, 1984). There was a distinct loss in grade for both harvesting methods at kernel moisture levels of 20% or higher primarily because of mildew, green kernels and sprouting (Christensen and Legge, 1984). The test weight of wheat was reduced substantially after it endured periods of wetting and drying in the field after the optimal harvest date passed (Farrer et al., 2006). The seed coat became rough and degraded after these adverse conditions, which reduced the test weight and flour extraction potential of the grain (Farrer et al., 2006). Although large precipitation events been related with test weight reduction, there are differences between cultivars (Czarnecki and Evans, 1986). The reductions in test weight can be categorized in two different ways, kernel density and packing efficiency (Czarnecki and Evans, 1986). The density is primarily affected by the environment, while the packing efficiency is affected mainly by genotype (Czarnecki and Evans, 1986).

Farrer et al. (2006) found that wheat protein content was not significantly affected by adverse weather conditions and delayed harvest. Similarly, Christensen and Legge (1984) found little effect on protein, no matter which harvest timing and method were applied. In addition, there were few milling and baking parameters for which delayed harvest dates had a negative effect. Farrer et al. (2006) found five milling and baking quality parameters that were negatively

affected by the adverse conditions. These were grain Falling Number, grain DON levels, clear flour yield and farinograph breakdown time (measurement of the dough's ability to retain its structure). Climatic differences in humidity and temperature in association with rainfall may have different results for different areas of North America (Farrer et al., 2006). Higher humidity during a delayed harvest time period can increase the significance of the harvest delay on Falling Numbers (Farrer et al., 2006). In the study by Christensen and Legge (1984), the alpha-amylase activity levels differed in each year of the study under different weather conditions. The Falling Numbers increased as the kernel moisture content levels decreased for the direct combine method, but with the windrow method, Falling Numbers were similar between different kernel moisture content levels (Christensen and Legge, 1984).

1.1.5 Genotype and Environment Interactions on Wheat Quality

Wheat flour properties and their baking quality parameters are controlled by the distribution and solubility of monomeric and polymeric proteins (Daniel and Triboi, 2002). Genetics play a large role in determining the distribution and ratio of gluten protein components, e.g. insoluble to soluble protein. Other factors such as temperature and precipitation also have the ability to affect this ratio (Daniel and Triboi, 2002). Inconsistencies in wheat quality between growing seasons can be attributed to weather and genotype as well as their interactions with one another (Peterson et al., 1998). If these interactions were better understood, there could be opportunity to improve grain sourcing and blending to meet the specifications required by millers and bakers (Peterson et al., 1998).

Peterson et al. (1998) analyzed the interactions between environment and genotype, and found that both cultivars (genotype) and growing locations (environment), and their interaction contributed to diversity in quality characteristics of the wheat (Peterson et al., 1998). When growing temperatures were greater than 32^{o}C, mixograph peak time, absorption, pup loaf volume and SDS sedimentation volume were all significantly affected. In an apparent contradiction to the observation that wheat protein synthesis increased with higher temperatures (Campbell et al., 1981), Peterson et al. (1998) found that increased temperature stress (high temperatures) decreased glutenin concentration and increased levels of LMW salt insoluble and HMW salt soluble proteins (Peterson et al., 1998). Those are just some of the many results from the study, and overall it was found that environmental factors had a larger effect on quality parameters than genotype (Peterson et al., 1998). The interaction between genotype and environment also had a significant effect on wheat quality, but explained a lower proportion of total variance than either genotype and environment (Peterson et al., 1998).

The interactions between genotype and environment can also signficantly affect baking quality parameters (Ames et al., 1999). In a study of the different effects of genotype, environment and genotype*environment interaction, the effect of genotype was much more significant than that of environment and gentoype*envirornment interaction effects for the majority of baking quality parameters (Ames et al., 1999). For genotypes with strong gluten characteristics, the gluten index and gluten viscoelasticty were both significantly affected by genotype, while the environment effect was minor. In terms of protein content, both the genotype and the genotype*environmental interaction were highly significant, and had a major impact. While

protein content was affected by the genotype*environment interactions, the highly significant impact on gluten index from genotype showed that this effect was the primary cause of the differences, while the environmental effect was relatively stable in this analysis (Ames et al., 1999).

1.1.6 Effects of Crop Fertilization on Wheat Quality

Boehm et al. (2004) examined the impact of nitrogen fertilizer on grain protein content (GPC) and frozen dough quality. The effects of three nitrogen fertilizer treatments (0, 67.2 and 134.4 kg ha^{-1}) on GPC, and wheat quality characteristics were analyzed to determine if there was a difference between frozen dough products and fresh baked bread products. There were significant differences in GPC for all of the cultivars in the study between 0 and 67.2 kg/ha N treatments, but no significant difference for the higher N treatment (Boehm et al., 2004). Farinograph analysis showed a significant increase in water absorption with the application of 67.2 kg/ha N, but no significant results for the other two nitrogen treatments (Boehm et al., 2004). Farinograph arrival and peak times were significantly shorter for the 0 kg/ha N treatment, but there were no significant differences between the other two treatments (Boehm et al., 2004). There were no consistent differences in extensigraph results across any of the four cultivars in the study for any of the nitrogen treatments (Boehm et al., 2004). These results suggest that dough rhetorical properties including gluten strength were unaffected by variation in protein content arising from the nitrogen applications. Mean loaf volumes increased with higher levels of nitrogen treatments and the increases were significant between the 0 and 134.4 kg/ha treatments, but not the 0 and 67.2 kg/ha treatments (Boehem et al.,

2004). For the frozen doughs, the 0 kg/ha nitrogen treatment resulted in the lowest mean loaf volume, whereas the loaf volumes for the increased nitrogen treatments were significantly larger, but the mean values for each of these different treatments remained similar (Boehm et al., 2004). The implication of this result could be that typical rates of fertilizer application will be adequate to produce a quality loaf volume (Boehm et al., 2004). The loaf volume of bread made from frozen dough was significantly lower than that from fresh dough (Boehm et al., 2004). Overall, it was found that adding nitrogen fertilizer resulted in higher GPC, farinograph water absorption and longer farinograph arrival and peak times for all of the cultivars in the study, and these effects were only seen between the 0 and 67.2 kg/ha treatments (Boehm et al., 2004). Thus, typical nitrogen applications of 67.2 kg/ha were adequate to produce a significant change in the quality of the wheat for breadmaking.

Bole and Dubetz (1986) examined the effects of both nitrogen fertilizer applications and irrigation on soft white spring wheat. The acceptable maximum protein content for soft spring wheat in the domestic Canadian market is below 10.5%, so it is important for growers to stay below that value (Bole and Dubetz, 1986). Nitrogen and irrigation impact both protein content and yield, with yield with an inverse relationship between yield and protein content. Irrigation up to the ripening stage increased yield, but decreased the protein content in the first two study years, while in the last two years there were no significant effects (Bole and Dubetz, 1986). Nitrogen fertilizer application increased protein content for all four study years and increased yield for three of the four study years (Bole and Dubetz, 1986). The year in which yield did not increase, the soil contained a large amount of NO_3-N in the top 60cm (145 kg/ha)

(Bole and Dubetz, 1986). When the amount of soil plus nitrogen fertilizer was large with low irrigation, the protein content was above 10.5% (Bole and Dubetz, 1986). However, with the addition of available soil nitrogen, coming from both fertilizer application and high levels of soil N, and irrigation maintaining available water in the root zone until maturity, the protein content remained within the accepted target range (Bole and Dubetz, 1986).

Luo et al. (2000) looked at the effect of both nitrogen and sulphur fertilization and their interaction with genotype on wheat glutenins and quality parameters. As previously mentioned, having both a high yielding and good bread quality wheat is important for the current wheat market, and it is also important to have stability within quality parameters (Luo et al., 2000). Increases to both yield and protein content can be achieved through improved fertilization management, and in particular nitrogen fertilization (Luo et al., 2000). Nitrogen fertilizer was found to enhance the uptake of sulphur, which then led to better optimum mixing time using a mixograph analysis (Luo et al., 2000). It was found that the genotype effect was significant for all measured parameters, whereas fertilizer treatment effects were only significant for half of the measured quality parameters (Luo et al., 2000). Throughout the trial, nitrogen fertilizer significantly increased both protein content as well as grain hardness, whereas sulphur fertilization alone had no significant effect on any of the quality parameters analyzed (Luo et al., 2000). Glutenin, and its subunits of high-molecular weight (HMW) and low-molecular weight (LWM) glutenin were strongly influenced by genetics and variation was attributed to genotype (Luo et al., 2000). Fertilizer treatments of nitrogen and sulphur were found to have no significant effect on the HMW and LWM glutenins, but their quantity was

slightly increased by late nitrogen fertilization (not significant) (Luo et al., 2000). While differences between genotypes were the main driving forces behind the variation in quality parameters, interactions between genotypes and fertilizer treatments did significantly affect whole meal and white flour protein percentage, hardness and mid-line peak values within samples (Luo et al., 2000).

1.1.7 Effects of Fusarium Head Blight and Fungicides on Wheat Quality

Wheat is produced for human as well as animal consumption and there must be measures taken to ensure the grain is safe to consume for both. Worldwide, Fusarium head blight (FHB) has been a disease of concern within susceptible small grain cereals since the end of the 19th century (Champeil et al., 2004). The cause of this disease is related to a variety of different pathogen strains. Some can be considered a health concern to both humans and livestock due to the presence of mycotoxins found in the infected grain (Dexter et al., 1996). In Western Canada, *F graminearum* is one of the primary strains related to FHB infection (Dexter et al., 1996). One of the mycotoxins produced by *F graminearum* is deoxynivalenol (DON), and the consumption of this mycotoxin can result in toxicosis in both humans and livestock (Wegulo, 2012). Food and health safety has been an increasing concern since the 1990s, and mycotoxins associated with FHB are considered an element of alimentary risk for cereal products (Champeil et al., 2004).

FHB infected grain can cause not only health problems, but also lead to reduced grain yield and quality. Some symptoms of FHB infection include premature bleaching of spikes, spikes fallen

on the ground prematurely, and shriveled or chalky kernels within spikes (Wegulo, 2012). These then lead to a decrease in grain quality, with FHB negatively affecting starch granules, storage proteins and cell walls of the grain (Dexter et al., 1996). When these grain components are affected, it can reduce the milling and baking quality of the grain, and result in a loss for producers, millers and bakers (Dexter et al., 1996). During the period of 1991-1997, there was an estimated $1.3 billion lost due to FHB infection in wheat and barley crops in the U.S. (Wegulo, 2012).

Dexter et al. (1996) analyzed the effects of Fusarium head blight on wheat kernels. In damaged kernels the effects of FHB were clearly seen in both protein content and wet gluten content. There were moderate decreases in both of these quality parameters (Dexter et al., 1996). High performance liquid chromatography (HPLC) analysis revealed the proportion of glutenins decreased in damaged kernel samples compared to clean samples. This agreed with the results from a previous study which showed that a decrease in glutenins within hard red spring wheat was caused by *F. graminearum* infection (Dexter et al., 1996). There was little effect on the gliadin proteins when comparing the clean and damaged samples. The significant effects on glutenins with no significant effects on gliadins implied that the reason for these differences was the rate at which the two proteins are synthesized (Dexter et al., 1996). Immaturity also plays a role in the impacts of FHB infection on wheat gluten strength. Glutenin is synthesized earlier and more rapidly than gliadin during the later stages of kernel development, and the infection of FHB takes place in the early milk to early dough stage, which stops the grain development at that point in time (Dexter et al., 1996). The effect of FHB on glutenins resulted

in poor baking performance, as glutenin is the key polymer in wheat that contributes to the unique ability of wheat to be used for breadmaking (Dexter et al., 1996).

Eggert et al. (2011) analyzed the effects of Fusarium infection on wheat storage protein gluten and the gliadin and glutenin fractions in the context of an *in vitro* study. Fusarium infection, and the proteases produced by the fungus, lead to substantial degradation of gluten proteins and the loss of dough functionality (Eggert et al., 2011). These proteases were mainly trypsin-like serine proteases which cut at the lysine or arginine amino acid within proteins, and are part of the exo-proteome of the fungus (Eggert et al., 2011). When gluten was incubated with the Fusarium proteases for a time of 4 h, there was a 17% decrease in gliadins and 80% decrease in glutenins, whereas for incubation of 24 h, there was a complete loss of gliadins and the loss of typical glutenin fractions (Eggert et al., 2011). In the scenario of a 4 h incubation period, the greater loss of glutenins shows the preferred digestion of glutenins in comparison to gliadins (Eggert et al., 2011). Within the glutenin protein fractions, there was a stronger impact of fungal proteases on the HMW glutenin subunits in comparison to low molecular weight (LMW) subunits of glutenin, with degradation of 97% and 42%, respectively (Eggert et al., 2001). This could be attributed to the larger quantity of lysine or arginine in HMW glutenin subunits (Eggert et al., 2011). The preferred digestion of HMW glutenin subunits by proteases has negative effects on the breadmaking quality of the wheat, as this fraction of glutenin is strongly related to the elastic properties of dough that are positively related with a high baking volume and high dough quality (Eggert et al., 2011). Over all of the incubation periods (two, four and eight hours) of the study, the Fusarium proteases also lead to a decrease in the total of gliadin

fractions (Eggert et al., 2011). In a comparison of purified glutenins and purified gliadins, the gliadins were degraded to a greater extent (Eggert et al., 2011). SDS-PAGE was used to confirm the higher solubility of protein fractions from glutenin digestion (Eggert et al., 2011). SDS-PAGE showed two fragments after the 8-hour incubation period, and these fragments were from the glutenin digestion by fungal proteases, and were most likely extracted with the gliadin fraction within the samples (Eggert et al., 2011). After confirming with Reverse Phase-HPLC, it was found that the destruction of gliadins was masked by the destruction of glutenin fragments, as both fractions are co-detected within the gliadin fraction (Eggert et al., 2011). It is important to note that both gliadins and glutenins, and in particular HMW glutenin subunits, were degraded by the Fusarium proteases. Eggert et al. (2011) recommended further research for more advanced methods of gliadin and glutenin characterization.

Wang et al. (2005) investigated the effects of Fusarium infection on gluten proteins, as well as the properties of fungal protease produced by *Fusarium culmorum* in a study where wheat spikes were artificially infected with Fusarium spores. Temperature and pH played a role in the activity of protease related to *F. culmorum* infection (Wang et al., 2005). Maximum activity of the protease was found at 50^{o}C and a pH range of 6.0-8.0, which caused impairment in storage proteins during processing procedures of the grain (Wang et al., 2005). Fusarium infection did not lead to a decrease in protein content, but it did result in other storage substances being degraded (Wang et al., 2005). In addition, when infection rates were highest, there was an increase in free amino acid concentration (Wang et al., 2005) from 33% to 139%, depending on the amino acid, compared to samples with a light infection rate (Wang et al., 2005). HMW

glutenin subunits decreased substantially, in comparison to LMW glutenin subunits and gliadins, within highly infected samples (Wang et al., 2005). The reasoning behind this large decrease in HMW glutenin subunits in relation to gliadin, was that glutenins are more rapidly synthesized during the later stages of kernel maturation, thus resulting in higher levels of gliadin, and lower levels of glutenin (Wang et al., 2005). *F. culmorum* produced produced protease which was insensitive to the influence of both temperature and pH (Wang et al., 2005). Fungal enzymes impaired wheat storage proteins, resulting in higher concentrations of free amino acids, and decreased amounts of glutenins (especially HMW glutenin subunits) within the more highly infected samples (Wang et al., 2005).

There are many management practices that can be employed to reduce widespread FHB infection including cultural, biological and pesticide controls, as well as selecting wheat with increased resistance to infection (Dweba et al., 2017). Fungicides have been shown to effectively manage FHB infection and DON contamination within cereal crops such as wheat (Blandino et al., 2006; Blandino and Reyneri, 2009). Two fungicide classes that are commonly used are triazoles and strobilurins, with triazoles being the most effective in terms of controlling FHB and DON levels within a field (Wegulo, 2012). When triazoles are applied both FHB and DON levels are reduced (Blandino and Reyneri, 2009). This reduction aids in obtaining higher quality grain, with higher yields (Blandino and Reyneri, 2009). However, strobilurin applications are not recommended for FHB control due to the fact that they can lead to increases in DON. (Wegulo, 2012).

When applying fungicides, it is critical for the application to be done at the correct rate and at anthesis to obtain maximum FHB control (Wegulo, 2012). Anthesis is a critical time for fungicide application because the anthers are the main site of primary infection (Wegulo, 2012). Blandino et al. (2006) found similar results, specifically that under both rainy and dry conditions, a fungicide application at time of anthesis resulted in yield increases of 23.8% and 16.9%, respectively (Blandino et al., 2006).

The effectiveness of fungicides to control FHB infection is impacted by other factors including cultivar resistance, climate, economic return, fungicide type and management (frequency and timing of application) (Dweba et al., 2017). Inherent resistance to FHB varies among cultivars with some having moderate resistance to FHB infection and others being more susceptible to infection. Susceptible cultivars will have higher levels of DON in their kernels, and are more prone to hyphal invasion (Wegulo, 2012). In terms of climate, diseases occur at higher levels in moist, hot conditions, rather than hot and dry conditions. FHB and DON levels are higher in years with more moisture, but are also percent reduction in FHB by fungicide is higher during these years. Blandino et al. (2006) found that the application of fungicide at anthesis led to a significant increase in grain yield, and clearer differences between treatments and timing during the wet, cool year of 2002, versus the hot, dry conditions of 2003. Application of fungicide at anthesis is critical, with an opportunity window of about seven days for application, as it ensures that the plant is protected when it is most vulnerable to hyphal invasion and disease development. An appropriate and properly-applied fungicide can lower the risk of FHB and mycotoxin which will then result in lowering the risk of wheat with lower gluten strength.

FHB is known to adversely affect wheat milling and baking quality in hard red spring wheat samples manipulated to contain especially high levels of FDK that would not be considered to be of milling quality (Dexter et al., 1996). When durum wheat was the focus of study (Dexter et al., 1996) similar results were obtained, i.e. only for severely damaged kernels (i.e. samples hand picked to contain 100% FDK) were substantial effects on quality found. In that study, a decrease in FDK from "as is" levels (avg. ~ 4%) to handpicked clean samples (FDK ~ 0.2%) resulted in a significant increase in durum wheat test weight, SDS sedimentation volume (an assay related to protein quality), and significant decrease in DON. No significant differences in gluten index (an assay of gluten strength) or mixograph development time was found. In a study of FHB quality effects on CWRW wheat (Hatcher et al., 2003) similar results were found. For high FDK levels (between 5 – 10%) there were weakening effects on gluten strength observed. There were no effects on flour ash or protein content.

1.1.8 Effects of Pre-Harvest Glyphosate on Wheat Quality

Pre-harvest glyphosate application is a common management practice across Western Canada, and elsewhere around the world. The two most common benefits are increased weed control and uniform dry-down in crops. Both perennial and annual weeds can cause issues in cereal crops, as they increase the moisture content of harvested wheat, as well as reduce harvest speed, and grain cleaning efficiency (Manthey et al., 2004). These issues reduce the value of wheat to producers as a result of increased dockage and foreign material in their grain deliveries which leads to a lower price (Manthey et al., 2004). The use of a pre-harvest glyphosate has the ability to kill both annual and perennial weeds, and facilitates more rapid

grain harvest, especially when there are moist soil conditions promoting a high level of weeds in a field (HGCA, 2008). Glyphosate aids in the dry-down of the crop as it interrupts the shikimic acid pathway through inhibition of 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase in the growing regions of the plant (Manthey et al., 2004). Although glyphosate is not a true desiccant, it can aid in the uniform dry-down of crops once they reach physiological maturity in their life cycle. An even crop dry-down can help producers to harvest their crop in unfavorable weather conditions.

It is important that the application instructions on the label are followed correctly, and that the pesticide is applied at the correct crop stage to ensure maximum yields, optimum combining time, and minimum residues in the grain (Glyphosate Task Force, 2018). Studies to evaluate pre-harvest glyphosate applications, and different timings of the application have shown, repeatedly, that an application before recommended timing will have a negative effect on the yield, and grain quality. Yenish and Young (2000) found that glyphosate applied at the milk stage of grain development resulted in a 20-77% decrease in yield depending on year, variety and glyphosate application rate. Not only did this early application affect the overall yield of the crop, but it also reduced kernel weight, and seedling germination (Yenish and Young, 2000). The treatments with a higher rate of glyphosate applied at milk stage had a 23% lower germination level than those which had a lower rate of glyphosate applied (Yenish and Young, 2000). This was not consistent for all years of the study, but the results showed there is reason for concern and serves as a reminder to use the recommended application rate. When glyphosate was applied at the hard dough (HD) development stage in the study, the stage in

which the application is recommended to occur, the pesticide did not affect the quality of wheat seeds or the germination levels in subsequent years (Yenish and Young, 2000).

Very few studies have reported the effects of glyphosate application on the physical or chemical properties of wheat. Yenish and Young (2000) observed no impact of pre-harvest glyphosate application relative to untreated control on test weight of two soft wheats grown in two years in Washington, regardless of timing of application (i.e. soft or hard dough stage) or rate (0.62 or 0.84 kg ha^{-1} active ingredient). Manthey et al. (2004) studied the application of glyphosate (0.84 kg ha^{-1} active ingredient) at both the soft dough (SD) and hard dough (HD) stages for one hard red spring (HRS) wheat grown at two locations in North Dakota. At the SD stage only, they observed significant lowering of test weight, kernel weight, large kernel content, and flour protein content and a small but significant increase in flour ash. Manthey et al. (2004) also showed that pre-harvest application of glyphosate positively affected gluten strength. They observed significant increases in SDS sedimentation volume and mixograph development time with application at the SD stage and significant increases for gluten index, farinograph stability and full formula dough mixing time with application at both the SD and HD stages. More recently Malalgoda et al. (2020) studied the effects of pre-harvest glyphosate (1.1 kg ha^{-1} active ingredient) applied at the SD and HD stages to two HRS wheats grown in three locations in North Dakota on a range of wheat properties. Similar to results reported by Manthey et al. (2004), Malalgoda et al. (2020) observed positive effects of glyphosate application at the SD stage on gluten strength-related parameters including gluten index, farinograph stability, lower farinograph mixing tolerance index and full formula dough mix time.

The effectiveness of glyphosate as a desiccant (it is not registered for this purpose) has been studied on wheat crops. Calvino et al. (2002) found that the use of glyphosate and paraquat herbicides resulted in larger grain moisture decreases than in scenarios with no herbicide application. This was analyzed by comparing herbicide application at different growth stages prior to physiological maturity. It was shown that herbicides were able to significantly accelerate grain drying, and this resulted in the advancement of harvest maturity (Calvino et al., 2002). However, with the expedited dry-down of a crop, there may also be some reduction in the grain mass. When glyphosate was applied at 45% moisture content there was a 9% reduction of grain mass but a reduction of only 2.5% with application at 40% moisture content compared to a control treatment (Calvino et al., 2002). This demonstrates the importance of correctly assessing physiological maturity for pre-harvest glyphosate application and not applying too early.

Darwent et al. (1993) examined the effects of pre-harvest glyphosate applications and their interactions between location and seed moisture at time of application. The rate of glyphosate application and the seed moisture at time of application had the largest effect on yield. Higher moisture content (41-60% moisture) at the time of glyphosate application led to a greater reduction in yield, in comparison to lower moisture contents (25-44%) (Darwent et al., 1993). This is due to the fact that when glyphosate was applied at the higher moisture level, the crop did not mature fully, and kernels were negatively affected, reducing yields (Darwent et al., 1993). The interaction between location and rate of glyphosate was also found to be significant, with some locations responding more significantly to the glyphosate application,

whereas others responded more to a windrowed control treatment (Darwent et al., 1993). The location, rate of glyphosate, seed moisture and location x seed moisture class all had significant effects on seed yields (Darwent et al., 1993). This study was able to highlight the interactions between herbicide management practices, and locations which is related to environmental conditions.

Clarke (1981) studied the effects of applications of three chemicals including glyphosate on the pre-harvest drying of wheat. The experiment which included windrowing as a treatment was carried out in Southwestern Saskatchewan over three years. This study was conducted to determine which management practice would work most efficiently to speed up crop dry-down. Windrowing dried the crop down to a moisture level suitable for threshing (i.e. 14-15%) in three days in the year (1978) with hot and dry weather conditions, whereas it took 9 days in the crop year (1980) when plots experienced much cooler and wetter growing conditions. Despite the different weather conditions, the chemical treated plots including glyphosate did not dry down faster than the untreated counterparts, whereas windrowing advanced the wheat to safe moisture levels for combining by about 2 days in each of the three years of the study. Clarke (1981) concluded that windrowing was a more effective management practice than using chemical treatments to reduce grain moisture content. It was also pointed out that chemical treatments could have a use for crop dry-down in situations where the crop was not evenly mature, but this could result in loss of yield and lower test weight when the treatment was applied to a wheat crop with a high proportion of immature heads (Clarke, 1981).

The literature shows that a pre-harvest glyphosate application has the ability to alter grain yield and quality if the recommended timing of the application is too early. It is extremely important for farmers to follow label directions to minimize the consequences for grain yield and test weight which is a grading factor, as well as seed germination for the next season.

1.1.9 Gluten Strength Analysis

There are a great many methods reported in the literature to assess or predict the gluten strength of wheat or samples of wheat flour. Many of these are physical methods that directly measure dough rheological properties, while others are chemical or biochemical in nature that typically quantify the concentration of important fractions of gluten proteins such as gliadin and glutenin which are responsible for the viscoelastic properties of dough. This thesis research implemented both strategies, one using the mixograph to measure gluten strength of doughs, while the other being a small-scale biochemical evaluation of gluten protein composition.

The mixograph is a pin-mixing style of dough mixer commonly used by wheat breeders in North America to evaluate gluten strength and dough mixing requirements of relatively small samples of flour. Compared to the farinograph, it provides a more discriminating assessment of dough properties under conditions of relatively high mixing energy which is very appropriate for evaluating relatively strong-mixing wheats such as those used in this thesis research. Modern mixographs like the one used in this research are computerized and generate mixing curves (e.g. Figure 1.1) which plot dough resistance or torque as a function of time. Some key parameters that are computed by the instrument's software are more or less related to gluten strength and include dough mixing time to peak development (DDT or MDT), peak band width

(PBW), work input to peak dough development (WIP) and peak dough resistance (PDR) and work at peak (WAP) which is the product of MDT * PBW (PDR and WAP are not shown in Fig. 1.1). Work input and development time are the two technological parameters most often used to describe gluten strength in the literature.

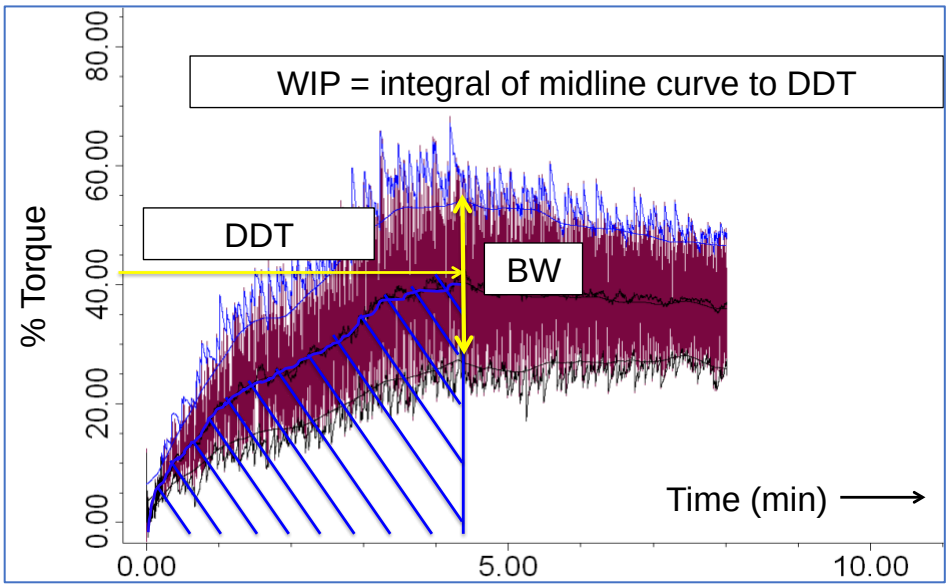


Figure 1.1 Sample mixograph analysis curve, with key characteristics used to determine gluten strength, including dough development time (DDT), work input to peak (WIP) and band width (BW).

A recent study by Isaak et al. (2019) provided the basis for evaluating gluten strength in this thesis research which required efficient and effective methods given the very large number of samples. Isaak et al. (2019) evaluated flours of 19 genotypes of hard red winter wheats varying in gluten strength using a 2-g mixograph and a UV spectrophotometric test of gluten protein (Sapirstein and Johnson, 2000) to measure alcohol-soluble endosperm protein (SP, mostly gliadins and some LMW glutenin polymer) and alcohol-insoluble protein corresponding to

HMW polymeric glutenin. This study showed that mixograph development time and work input had a different pattern of variation compared to bandwidth and peak resistance in relationships to protein content and composition. Whereas mixograph peak resistance and bandwidth were relatively highly correlated with both SP content and total flour protein ($r \sim 0.70$ or greater depending on mixing conditions), mixing time and work input were poorly correlated with SP content ($r < 0.30$), and correlations to flour protein were considerably lower ($r \sim 0.50$). On the other hand, HMW glutenin concentration in flour was highly correlated with all four mixograph parameters. The most striking differentiation of mixograph parameters that was found by Isaak et al. (2019) was in relation to a novel protein composition parameter based on the ratio of HMW glutenin to SP content, which the authors termed the gluten strength index or GSI. The authors explained that GSI is a unitless measure of protein quality related to the molecular size distribution of gluten proteins, given that the molecular size of HMW glutenin is substantially greater than that of gliadin protein. The authors found that mixograph work input and dough development time was highly correlated with GSI ($r = 0.86$ averaged across mixograph conditions). In contrast, the corresponding correlations to bandwidth and peak dough resistance were much lower ($r = 0.33$). Taken together, the results of the study by Isaak et al. (2019) highlighted the different influences of protein quality vs. protein content on mixograph parameters as well as differences between protein quality, protein content and mixograph parameters to measure or predict gluten strength.

1.2 Study Rationale and Objectives

This thesis research is part of a larger project initiated in 2015 to study the influence of genotype, weather and the growing environment, and crop management on gluten strength and the sustainability of Canada Western Red Spring (CWRS) wheat as a premium wheat class in the Prairie region. The rationale for the project as a whole originated from concerns of lower gluten strength and lack of consistency in gluten strength in shipments of CWRS wheat that were raised in the 2013 crop year by many domestic and international customers as reported at Prairie Grain Development Committee meetings in that year. The varietal makeup of CWRS wheat and specifically the presence of relatively weak varieties, was proposed by the Canadian Grain Commission as the main issue. However, another relevant factor is variable gluten strength due to differences in weather conditions across the Prairie region and the nature of the wheat supply chain which causes wheat to flow from farmers to customers in relatively tight packages of quality that reflects regional differences in growing conditions. The first part of the project research (Courcelles, 2019) focused on genotype (G) and environmental (E) influences on gluten strength variation of leading CWRS varieties which built substantially on earlier work reported by Jarvis (2006), Finlay et al. (2007) and Jarvis et al. (2008).

The research in this thesis focused on select crop management factors as a potentially important source of variation in gluten strength and wheat quality in general. In contrast to traditional G x E studies, there has been very little research reported on the impacts of widely used management practices such as pesticide application (e.g. fungicides and glyphosate) on wheat end-use quality, and gluten strength in particular. In addition, the impact of delayed

harvest on wheat gluten strength is not known. Since variability in CWRS wheat quality including gluten strength, is a key concern affecting its value to producers, millers and bakers, it is imperative to consider all potential factors. This thesis research examines the effects of two different pesticide management practices as well as delayed harvesting to determine their relative importance on wheat grade and select grading factors, grain quality, milling quality, gluten strength and protein composition of different CWRS wheat genotypes grown in different locations in Western Canada over three years. The specific objectives below are addressed in this thesis:

1. To determine the significance of the impact of two commonly-used pesticide applications on the quality, gluten strength and underlying protein composition of CWRS wheat including:
    - fungicide (Prothioconazole/Tebuconazole) applied at anthesis to control Fusarium Head Blight and,
    - pre-harvest application of glyphosate,
2. To determine the significance of genotype and growing season weather on the quality and gluten strength of CWRS wheat and compare it to the significance of pesticide application,
3. To assess the relationships between protein content and composition and the rheological properties of dough, and
4. To determine the impact of harvest delays on the quality and gluten strength of CWRS wheat.

1.3 References

Ames, N. P., Clarke, J. M., Marchylo, B. A., Dexter, J. E. and Woods, S. M. 1999. Effect of Environment and Genotype on Durum Wheat Gluten Strength and Pasta Viscoelasticity. *Cereal Chemistry* **76(4)**: 582-586.

AMIS (Agricultural Market Information System). 2018. Market Database. http://www.amis-outlook.org/home/en/

Blandino, M., Minelli, L. and Reyneri A. 2006. Strategies for the chemical control of *Fusarium* head blight: Effect on yield, alveographic parameters and deoxynivalenol contamination in winter wheat grain. *European Journal of Agronomy* **25**: 193-201.

Blandino, M. and Reyneri, A. 2009. Effect of fungicide and foliar fertilizer application to winter wheat at anthesis on flag leaf senescence, grain yield, flour bread-making quality and DON contamination. *European Journal of Agronomy* **30**: 275-282.

Boehm, D. J., Berzonsky, W. A. and Bhattacharya, M. 2004. Influence of nitrogen fertilizer treatments on spring wheat (Triticum aestivum L.) flour characteristics and effect on fresh and frozen dough quality. *Cereal Chemistry* **81**: 51-54.

Bole, J. B. and Dubetz, S. 1986. EFFECT OF IRRIGATION AND NITROGEN-FERTILIZER ON THE YIELD AND PROTEIN-CONTENT OF SOFT WHITE SPRING WHEAT. *Canadian Journal of Plant Science* **66**: 281-289.

Calderini, D., Abeledo, L.G., and Slafer, G.A. **2000.** Physiological maturity in wheat based on kernel water and dry matter. 2000. *Agronomy Journal* **92(5)**: 895-901. https://doi.org/10.2134/agronj2000.925895x

Calvino, P.A., Studdert, G.A., Abbate, P.E., Andrade, F.H. and Redolatti, M. 2002. Use of non-selective herbicides for wheat physiological and harvest maturity acceleration. *Field Crops Research* **77**: 191-199.

Campbell, C.A., Davidson, H.R. and Winkleman, G.E. 1981. Effect of Nitrogen, Temperature, Growth Stage and Duration of Moisture Stress on Yield Components and Protein Content of Manitou Spring Wheat. *Canadian Journal of Plant Science* **61**: 549-563.

Canadian Grain Commission. 2016. Gluten's role in bread baking performance. https://www.grainscanada.gc.ca/en/

Champeil, A., Doré, T. and Fourbet, J.F. 2004. Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by Fusarium in wheat grains. *Plant Science* **166**: 1389-1415.

Christensen, J. V. and Legge, W. G. 1984. Effect of Harvest Time and Drying Method on the Yield, Quality and grade of the Hard Red Spring Wheat in Northwest Alberta. *Canadian Journal of Plant Science* **64**: 617-623.

Clarke, J.M. 1981. Effect of Diquat, Paraquat and Glyphosate on Pre-harvest Drying of Wheat. *Canadian Journal of Plant Science* **61**: 909-913.

Courcelles, J.R. 2019. The Effect of Genotype and Growing Environment on the Gluten Strength and End-Use Quality of CWRS Wheat. M.Sc. thesis, University of Manitoba. http://hdl.handle.net/1993/33653 .

Czarnecki, E. and Evans, L. E. 1986. Effect of Weathering During Delayed Harvest on Test Weight, Seed Size, and Grain Hardness of Wheat. *Canadian Journal of Plant Science* **66**: 473-482.

Daniel, C. and Triboi, E. 2000. Effects of Temperature and Nitrogen Nutrition on the Grain Composition of Winter Wheat: Effects on Gliadin Content and Composition. *Journal of Cereal Science* **32**: 45-56.

Darwent, A.L., Kirkland, K.J., Townley-Smith, L., Harker, K.N., Cessna, A.J., Lukow, O.M. and Lefkovitch, L.P. 1993. Effect of pre-harvest applications of glyphosate on the drying yield and quality of wheat. *Canadian Journal of Plant Science* **74**: 221-230.

Dexter, J.E., Clear, R.M. and Preston, K.R. 1996. Fusarium Head Blight: Effect on the Milling and Baking of Some Canadian Wheats. *Cereal Chemistry* **73(6)**: 695-701.

Dweba, C.C., Figlan, S., Shimelis, H.A., Motaung, T.E., Sydenham, S., Mwadzingeni, L. and Tsilo, T.J. 2017. Fusarium head blight of wheat: Pathogenesis and control strategies. *Crop Protection* **91**: 114-122.

Eggert, K., Rawel, H. M. and Pawelzik, E. 2011. In vitro degradation of wheat gluten fractions by Fusarium graminearum proteases. *European Food Research and Technology* **233**: 697-705.

Farrer, D., Weisz, R., Heiniger, R. Murphy, J. P. and Pate, M. H. 2006. Delayed Harvest Effect on Soft Red Winter Wheat in the Southeastern USA. *Agronomy Journal* **98**: 588-595.

Finlay, G.J., Bullock, P.R., Sapirstein, H.D., Naeem, H.A., Hussain, A., Angadi, S.V. and DePauw, R.M. 2007. Genotypic and environmental variation in grain, flour, dough and bread making characteristics of western Canadian spring wheat. *Canadian Journal of Plant Science* **87**: 679–690.

Glyphosate Task Force. 2018. Clarification of Pre-harvest uses of glyphosate, The advantages, best practices and residue monitoring. [online] https://www.glyphosate.eu/system/files/sidebox-files/clarification_of_pre-harvest_uses_of_glyphsate_en_0.pdf. (verified 7 Sep 2019)

Gooding, M.J., Ellis, R.H., Shewry, P.R. and Schofield, J.D. 2003. Effects of Restricted Water Availability and Increased Temperature on the Grain Filling, Drying and Quality of Winter Wheat. *Journal of Cereal Science* **37**: 295-309.

Hatcher, D.W., Anderson, M.J., Clear, R.M., Gaba, D.G and Dexter, J.E. 2003. Fusarium head blight: Effect on white salted and yellow alkaline noodle properties. *Canadian Journal of Plant Science* **83**: 11-21.

HGCA (Home-Grown Cereals Authority). 2008. Information Sheet for Pre-harvest glyphosate application to wheat and barley. http://adlib.everysite.co.uk/resources/000/250/760/ IS02.pdf

Isaak, C., Sapirstein, H. and Graf, R. 2019. Effects of water absorption and salt on discrimination of wheat gluten strength assessed by dough mixing and protein composition. *Journal of Cereal Science* **89**: 102752.

Jarvis, C.K. 2006. Growing season weather impacts on breadmaking quality of Canada western red spring wheat grown in producer fields across western Canada. MSc thesis, University of Manitoba, Winnipeg (2006). Available: http://hdl.handle.net/1993/287 .

Jarvis, C.K. Sapirstein, H.D., Bullock, P.R., Naeem, H.A., Angadi, S.V., and Hussain, A. 2008. Models of growing season weather impacts on breadmaking quality of spring wheat from producer fields in western Canada. *Journal of the Science of Food and Agriculture* **88**: 2357–2370.

Luo, C., Branlard, G., Griffin, W. B. and McNeil, D. L. 2000. The effect of nitrogen and sulphur fertilisation and their interaction with genotype on wheat glutenins and quality parameters. *Journal of Cereal Science* **31**: 185-194.

Malalgoda, M. Ohm, J. B., Ransom, J. K. Howatt, K. and Simsek, S. 2020. Effects of pre-harvest glyphosate application on spring wheat quality characteristics. *Agriculture* **10(4)**: 111.

Manthey, F.A., Charkraborty, M., Peel, M.D. and Pederson, J.D. .2004. Effect of pre-harvest applied herbicides on breadmaking quality of hard red spring wheat. *Jornal of the Science of Food and Agriculture* **84**: 441-446.

Peterson, C.J., Graybosch, R.A., Shelton, D.R. and Baenziger, P.S. 1998. Baking quality of hard winter wheat: Response of cultivars to environment in the Great Plains. *Euphytica* **100**: 157-162.

Rondanini, D., Savin, R. and Hall, A. 2007. Estimation of physiological maturity in sunflower as a function of fruit water concentration. *European Journal of Agronomy* **26:** 295-309.

Sapirstein and Johnson 2000. A rapid spectrophotometric method for measuring insoluble glutenin content of flour and semolina for wheat quality screening. In: Shewry, P.R. and Tatham, A. (Eds.), Wheat Gluten. Royal Society of Chemistry, Cambridge, UK, pp. 307–312.

Shewry, P.R., Tatham, A.S., Forde, J., Kreis, M. and Miflin, B.J. 1986. The classification and nomenclature of wheat gluten proteins: A reassessment. *Journal of Cereal Science* **4(2)**: 97-106.

Southan, M. and MacRitchie, F. 1999. Molecular Weight Distribution of Wheat Proteins. *Cereal Chemistry* **76(6)**: 827-836.

Wang, J. H., Wieser, H., Pawelzik, E., Weinert, J., Keutgen, A. J. and Wolf, G. A. 2005. Impact of the fungal protease produced by Fusarium culmorum on the protein quality and breadmaking properties of winter wheat. *European Food Research and Technology* **220**: 552-559.

Wegulo, S.N. 2012. Factors Influencing Deoxynivalenol Accumulation in Small Grain Cereals. *Toxins* **4**: 1157-1180.

Wieser, H. and Zimmermann, G. 2000. Importance of amounts and proportions of high molecular weight subunits of glutenin for wheat quality. *European Food Research and Technology* **210(5)**: 324-330.

Wrigley, C. W. **1996.** Giant proteins with flour power. *Nature* **381(6585)**: 738-739.

Yenish, J.P. and Young, F.L. 2000. Effect of Pre-harvest Glyphosate Application on Seed and Seedling Quality of Spring Wheat (Triticum aestivum). *Weed Technology* **14**: 212-217.

Zhu, J. and Khan, K. 2001. Effects of Genotype and Environment on Glutenin Polymers and Breadmaking Quality. *Cereal Chemistry* **78**: 125-130.

2. EFFECTS OF GENOTYPE, WEATHER AND FHB FUNGICIDE/PRE-HARVEST GLYPHOSATE ON CWRS GRADE, GRAIN QUALITY AND FLOUR QUALITY

2.1 Abstract

Every year, Western Canadian wheat crops experience different growing season weather conditions which affect the quality of the grain produced. In addition, crop management, such as pesticide application, may affect wheat quality, but the impact is largely unknown. The quality characteristics of six commercial CWRS genotypes (Glenn, Carberry, Cardale, CDC Stanley, Stettler and Harvest) were evaluated in this study during the 2015, 2016 and 2017 growing seasons at four different locations across the prairies to capture a wide range of growing season weather conditions. Four pesticide treatments were applied to the field plots for each site-year including a control (untreated), Prothioconazole/Tebuconazole fungicide applied at anthesis for Fusarium head blight (FHB) control, pre-harvest glyphosate applied at physiological maturity, and applications of both fungicide and pre-harvest glyphosate. Grain quality analysis included test weight, thousand-kernel weight (TKW), protein content, grade, and degrading factors including Fusarium damaged kernels (FDK). After grain analysis was completed, the wheat was milled to produce flour samples which were analyzed for ash content, flour protein content and flour yield. Under the generally warmer and drier conditions in 2015 and 2017 compared to 2016, the wheat had higher grades, lower FDK, higher test weight and higher TKW. Wheat protein content values varied across all three years of the study. There were fewer differences in flour quality between years than other parameters of the study. The difference in temperature between locations and years was small and the effect of rainfall appeared to be the main environmental factor affecting variation. Wheat genotype was the most significant factor affecting the grain and flour quality parameters. Site-year,

reflecting the variation in growing season weather, was also a significant factor affecting quality. The pesticide treatment effect was significant for only some of the quality parameters analyzed and contributed less than 2% to total variance of all grain and flour parameters. The effect of the pesticide treatments on quality displayed no consistent pattern across all years and locations.

2.2 Introduction

Wheat, a food staple across the world, is produced both for human and animal consumption. Production for both purposes has been increasing (Blandino and Reyneri, 2009). In 2017 alone, there were 760 million tonnes of wheat produced on a global scale (AMIS Market Database, 2018). Wheat is such a valuable and widely used crop, it is critical that production and wheat quality remain a top priority for producers.

Every year the growing season weather conditions differ across Western Canada, resulting in changes to the quality of wheat from the same location from year-to-year. The wheat supply chain has evolved to increasingly deliver grain to market in relatively uniform packages of quality that reflects regional differences in growing conditions. This can create challenges for both millers and bakers whose modern, high throughput operations are based on expectations of high levels of uniformity of grain and flour quality (Peterson et al., 1998).

Campbell et al. (1981) found that weather conditions at anthesis had the greatest impact on kernel weight. They also found an inverse effect of weather conditions on yield versus grain

protein content. The cool temperatures, high fertility and low moisture stress conditions that were optimal for high yielding grain, produced the lowest grain protein content (Campbell et al., 1981). The impacts of weather on wheat quality are complicated by their interaction with variation in wheat genotype. Peterson et al. (1998) found that both cultivars and growing locations as well as their interaction contributed to diversity in quality characteristics for all of the quality parameters analyzed in their study.

Weather and genotype impact on wheat quality are difficult to quantify because they have both direct and indirect effects. An example is their combined impact on the level of Fusarium head blight (FHB) infection at a given location. There is a range of FHB resistance in wheat genotypes as well as a substantial variation in the level of FHB disease pressure as a result of weather variation. In Western Canada, Fusarium *graminearum* is one of the primary species that causes FHB infection in wheat (Dexter et al., 1996). It produces the mycotoxin deoxynivalenol (DON) which, if consumed, can result in toxicosis in both humans and livestock (Wegulo, 2012). Higher levels of FHB and DON in wheat negatively impacted bread-making quality (Dexter et al., 1996). Thus, both genotype and weather can impact wheat quality directly, as well as indirectly, through their influence on other factors such as FHB infection.

Crop management also has an impact on wheat quality. Additional nitrogen fertilizer increases grain protein content (Campbell et al., 1981, Bole and Dubetz, 1986, Luo et al., 2000) which is desireable for breadmaking wheats owing to the close relationship between flour protein content and bread loaf volume. Producers also use pesticides to minimize downgrading factors

and improve wheat quality, such as fungicide application at anthesis to reduce FHB infection as well as pre-harvest glyphosate for weed control. Triazole fungicide application at anthesis has been shown to reduce both FHB and DON levels in wheat and aid in obtaining higher quality grain with higher yields (Blandino and Reyneri, 2009). Pre-harvest glyphosate application provides increased weed control and uniform dry-down which reduces dockage and foreign material in grain deliveries (Manthey et al., 2004). For both of these management practices, correct timing of application is critical. Fungicide application must be done at anthesis for maximum efficacy because the anthers are the main site of primary infection (Wegulo, 2012). Studies of pre-harvest glyphosate used at different levels of moisture content near wheat maturity have shown repeatedly that applications outside the recommended timing will have a negative effect on yield and grain quality (Darwent et al., 1993, Yenish and Young, 2000, Calvino et al., 2002, Manthey et al., 2004). However, there is very little known about the impacts of these pesticide applications on wheat quality when they are applied at the recommended rates and timings.

In an effort to improve our understanding of the factors impacting wheat quality, this study examined the effects of genotype, weather and two widely-used pesticide practices on the quality of wheat and the flour produced. The objective was to determine whether these two pesticide practices had a statistically significant effect on several grain and flour quality parameters in comparison to the level of significance for the effects from both site-year and genotype.

2.3 Methods

2.3.1 Field Study

During the growing seasons of 2015, 2016 and 2017, six CWRS wheat genotypes were grown in four locations across Western Canada including Lethbridge, Alberta; Indian Head Agricultural Research Foundation (IHARF), Saskatchewan; Carberry, Manitoba and St. Adolphe (Kelburn Farm), Manitoba (Figure 2.1). The Carberry 2015 and St. Adolphe 2016 locations were not harvestable, due to mistakes during seeding and pesticide applications, leaving 10 site-years in total for this study. These site-years provided a broad, representative sample of growing season weather conditions typical for the Canadian prairies. The six genotypes, listed from strongest to weakest in terms of gluten strength were Glenn (GL), Carberry (CR), Cardale (CD), CDC Stanley (SN), Stettler (ST) and Harvest (HA). These varieties represent a wide range of genotypic variation in gluten strength for Canadian bread wheat. It should be noted that the designation of the Harvest genotype was re-classified from CWRS to Canada Northern Hard Red effective August 1, 2018, after this study had been completed (https://www.grainscanada.gc.ca/en/grain-quality/variety-lists/2017/2017-45.html).

Each genotype at each location received four treatments: Prothioconazole/Tebuconazole fungicide applied at anthesis (F), glyphosate applied pre-harvest (G), a treatment with both F and G (FG) and a control with no pesticide application (C). The pesticide treatments were applied according to label recommendations for rate and timing (Table 2.1). The products were Roundup Weathermax with Transorb2 (applied at 0.902 kg ha^{-1} glyphosate) and Prosaro EC (applied at 0.100 kg ha^{-1} for both Prothioconazole and Tebuconazole). The same lots of each

product were used at all study locations. The experimental design was a randomized complete block design with split plot for pesticide treatments. The main plot was the treatment with the genotypes as the sub-plots. Each field location was fertilized to optimum rates, which were dependent on management practices at each study site. Herbicides were applied as required at each location. Principal phenological dates for seeding, anthesis, maturity and harvest for each location are outlined in Table 2.2. The anthesis dates were observed in individual plots and a trial mean was determined for timing of fungicide application. A mean trial maturity date was based on observations from individual plots using “kernel hard” or Zadoks scale 91 to define maturity.

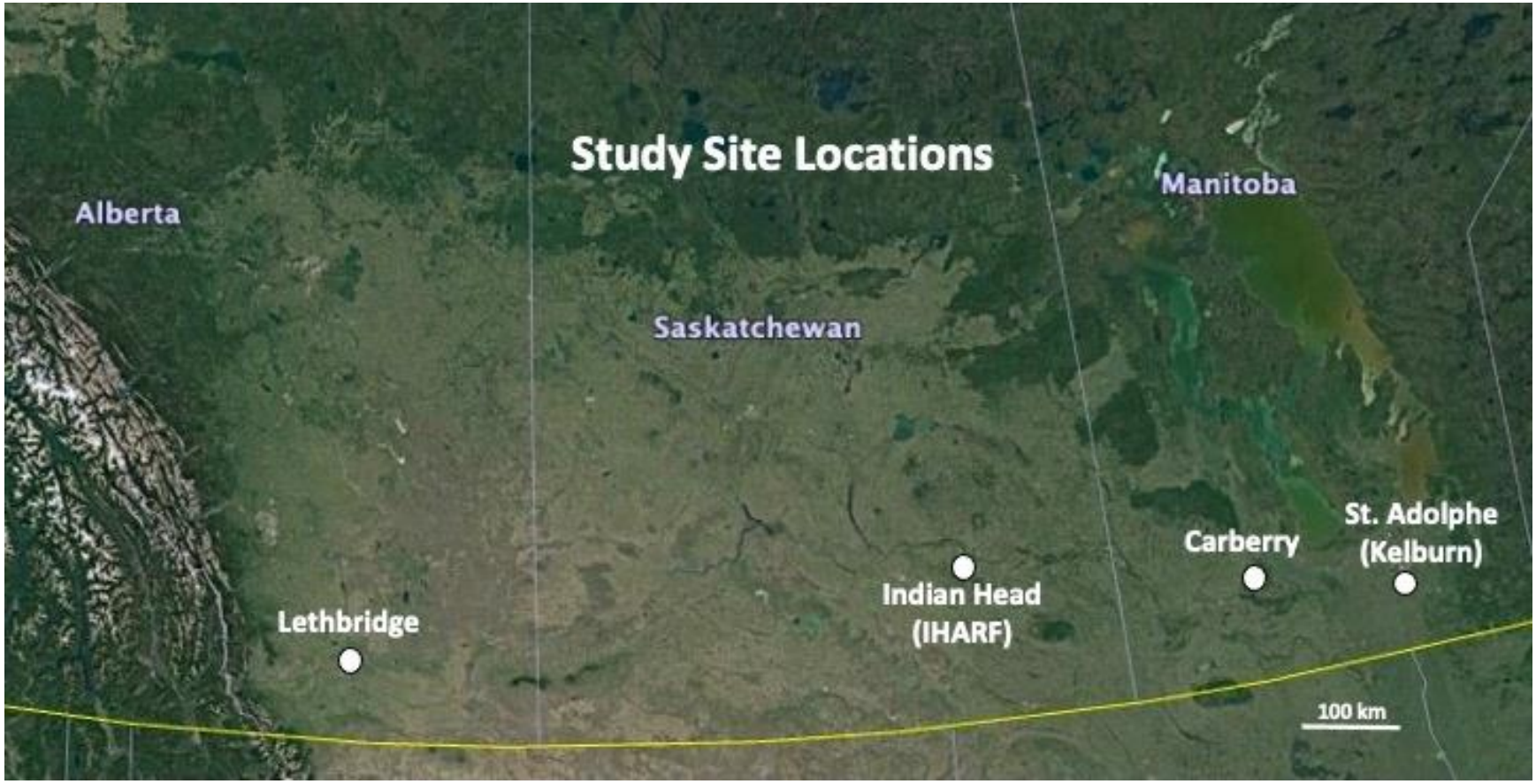


Figure 2.1 Map of the study sites in Western Canada for the 2015, 2016 and 2017 growing seasons.

Table 2.1 Label recommendations for FHB Fungicide (Prothioconazole/Tebuconazole) and Pre-Harvest Glyphosate.

Prothioconazole/Tebuconazole			
Crop	Purpose	Rate (mL/ha)	Remarks
Wheat (spring, winter and durum)	Suppression of: Fusarium head blight	800	"Apply fungicide as a preventative spray within the time period from when at least 75% of the wheat heads on the main stem are fully emerged to when 50% of the heads on the main stem are in flower. Application at this timing will also control the listed leaf diseases. Apply by ground or aerial application equipment. For ground application, apply specified dosage in a minimum of 100 L of water per hectare. For aerial application, apply specified dosage in a minimum of 50 L of water per hectare."

Pre-Harvest Glyphosate			
Crop	Purpose	Rate (mL/ha)	Remarks
Wheat	Late season weed control	1670	"Apply only when the crop has 30 percent or less grain moisture content. This stage typically occurs 7 to 14 days before harvest Apply pre-harvest at 1.67 litres per hectare in 50 to 100 litres per hectare of clean water, by ground application only. Do not apply by air."

Table 2.2 Phenology dates for wheat development in 2015, 2016 and 2017 at each field location.

Location	Year	Seeding Date	Anthesis Date[a]	Maturity Date[a]	Harvest Date
Indian Head	2015	May 3	Jul 7	Aug 13	Aug 25
	2016	May 4	Jul 6	Aug 6	Aug 17
	2017	May 4	Jul 7	Aug 16	Aug 28
Lethbridge	2015	May 6	Jul 2	Aug 4	Aug 20
	2016	May 5	Jul 7	Aug 11	Aug 31
	2017	May 5	Jul 5	Aug 5	Aug 14
St. Adolphe	2015	May 5	Jul 3	Aug 7	Aug 21
	2017	May 18	Jul 17	Aug 21	Sep 12
Carberry	2016	May 4	Jul 8	Aug 16	Sep 1
	2017	May 11	Jul 12	Aug 15	Aug 22

[a]Trial mean date

2.3.2 Meteorological Data Collection and Analysis

Weather data were collected from weather stations located within close proximity to each field site. The data included daily maximum, minimum, and average temperatures, as well as precipitation. The weather parameters were aggregated into phenological time periods at each site-year to summarize the mean temperature and accumulated precipitation for the periods from seeding to anthesis, from anthesis to maturity, maturity to harvest, and from seeding to harvest.

2.3.3 Analysis of Grain Quality

The grain from each plot was harvested and approximately 1.5 kg per plot was shipped to the University of Manitoba for analysis. The grain was first placed in frozen storage (-30 ^{o}C) for approximately four days to kill any live insects that could cause contamination. The grain was then taken from storage, equilibrated to room temperature, cleaned using a Carter Day Dockage Tester, and analyzed for grain protein content by NIR and graded by an experienced

inspector following the Canadian Grain Commissions Official Grain Grading Guide (Canadian Grain Commission 2015). The grading factors that were analyzed included test weight, grain moisture content, ergot-damaged kernels, midge-damaged kernels, Fusarium damaged kernels and sprouting. All of the samples were cleaned using a Carter Day Dockage Tester. A 400 g sample of the grain that was scheduled to be milled was analyzed using NIR (Foss Infratec™ 1241 Grain Analyzer, Hillerød, Denmark). Moisture content of the samples was determined at this time to guide tempering conditions prior to milling. Thousand kernel weights were determined using a seed counter and balance.

2.3.4 Analysis of Flour Quality

Two of the four field replicates from each genotype, location and treatment were randomly selected for milling. A 500 g subsample of each milling replicate was tempered overnight which was initially facilitated using a custom-built rotating conveyor (Hydrol Conveyor Co. Inc, Kansas, USA) as described below. The wheat moisture content for tempering was pre-determined to be to 14.5 ± 0.2% which in turn resulted in production of flour at a target moisture content of 14.0 ± 0.2%. The amount of water used for tempering was determined from the NIR readings performed about two days prior to milling. Water was added to each milling sample at the required amount and the lid of the jar was tightly sealed then shaken by hand until all of the kernels were water-coated. The jar was then placed on the conveyor and turned, on average, one rotation per second for about 10 min until the grain no longer adhered to the walls of the jar. The jars were removed from the conveyor, allowed to stand overnight for moisture equilibration, and milling started the next morning.

The jars of seed were put through a Seedburo moisture tester (Des Plaines, IL, USA) to measure the moisture content. A Brabender Quadrumat Senior experimental mill (Duisburg, Germany) comprising separate break and reduction machines, was used to mill approximately 500 g of tempered wheat per sample (Figure 2.2). The mill was customized and procedure used as described by Jeffers and Rubenthaler (1977) who developed a very effective protocol for high sample throughput. The mill was turned on and run for approximately 90 minutes to allow the machine to warm up and for the rolls to reach a consistent temperature determined using a Fischer Scientific Traceable IR Sensor Temperature Gun pointed at the center spindle of each roll. Grain samples were poured into the feeder section of the break side of the mill, with product put through the first of two sieving machines (Sampl-Sifter model, Great Western Manufacturing, Leavenworth, KS) (Figure 2.3) using no. 35 and 100 sieves (W.S. Tyler Co., Mentor, OH) to separate three fractions: flour, bran and middlings. The sieving times for separation of bran and middlings was 1 and 2 min, respectively. Break flour was weighed and set aside. The bran was weighed and then discarded, while the middlings, which were retained on top of the no. 100 sieve, were fed onto the reduction mill machine using a vibratory feeder (Syntron Co. Magnetic Feed F-TO-C) controlled (speed 4/10) by a Syntron PowerPulse WT Material Handling controller. The product from the reduction process which contained a mixture of flour and non-flour millfeed (shorts) was processed on a second sieving machine for 3 min to separate flour and shorts using a no. 9 sieve. Throughout the entire milling process, care was taken to ensure that all of the pieces of both the mill and the sieves were carefully cleaned to prevent contamination between samples using compressed air and brushes.

Figure 2.2 Brabender Quadrumat Senior experimental mill.

Figure 2.3 Sieves (500 and 150 μm) used to separate the bran and middlings during milling.

The break and reduction flour yield (%), and recovery of bran, shorts, ratio of break-to-reduction flour were all recorded. Typical yields of flour, bran and shorts were 72%, 21% and

4.8%, respectively. The straight grade flour, reported at ~14% moisture basis (mb) was stored in polyethylene zipped bags and allowed to mature (22-24°C) for at least one month before any further analysis.

Flour moisture content analysis utilized a 3g flour sample which was placed in an air oven for 65 minutes at a temperature of 130^{o}C. Flour ash content was determined by putting samples in a muffle furnace set for 590^{o}C (AACC, 2000 Method 08-01). The furnace was turned on and warmed up for one hour, after which the samples were placed in the oven. The furnace turned off automatically nine hours later and the door left closed until the temperature had fallen to 140 ^{o}C or about seven hours later. Samples were then removed and placed in a desiccator until they were weighed.

2.3.5 Statistical Methodology

Statistical analyses were completed using SAS Software, version 9.4 for all grain and flour quality parameters. Global analysis of variance combining all site-years was conducted for all of the grain and flour quality parameters measured. This analysis provided evidence on the relative effects of factors on the quality parameter being assessed. The factors for the ANOVA were as follows: genotype, pesticide treatment, site-year, block (site-year), genotype*pesticide, site-year*genotype, site-year*pesticide, site-year*genotype*pesticide and residual) had the largest effect on the parameter being assessed. The ANOVA provided additional information in regards to mean square error, percent of variance associated with the effect and test significance (at varying levels).

All parameters were tested for normality before continuing with statistical analysis. It was determined that FDK values were not normally distributed, so the values were transformed using Transformed FDK = log(FDK+0.5). The FDK values were back-transformed for reporting.

The SAS MIXED procedure was run for individual site-years to produce a Type III Analysis of Variance table. The combination of the year and location into site-year considers every site in each year as unique. The MIXED procedure was used to assess the contributions of genotype, treatment, genotype*treatment, site-year, block(site-year), genotype*site-year, treatment*site-year and treatment*genotype*site-year. These analyses differentiated the significant differences in quality parameters between individual site-years.

2.4 Results

2.4.1 Growing Season Environmental Conditions

Growing conditions were very different among the years and locations of the study across the Canadian prairies. During the three years of the study, precipitation and mean temperatures varied between locations and stages of the growing season (Table 2.3). Generally, the majority of rainfall occurred during the periods of seeding to anthesis and anthesis to maturity. The 2016 season had the largest amount of precipitation in comparison to the two other years. In some cases, the differences between years were dramatic. For example, rainfall at the Lethbridge location in 2016 was almost double the amount in 2015 and 2017. The 2015 total precipitation was greater than in 2017 at the three common study sites for those years. For

example, at the Kelburn location, there was 344.4 mm of rain in 2015, and only 160.5 mm in 2017.

Table 2.3 Precipitation by growing season period and total growing season for all locations and study years. Study site locations are shown in Figure 2.1.

Location	Year	Seeding-Anthesis (mm)	Anthesis-Maturity (mm)	Maturity-Harvest (mm)	Seeding-Maturity (mm)	Seeding-Harvest (mm)
IHARF	2015	58.8	122.1	25.5	180.9	206.4
	2016	145.6	111.8	12.8	257.4	270.2
	2017	81.3	26.1	5.4	107.4	112.8
Lethbridge	2015	56.4	34.7	5.5	91.1	96.6
	2016	74.2	95.0	11.6	169.2	180.8
	2017	71.3	4.4	6.7	75.7	82.4
Kelburn	2015	183.4	149.8	11.2	344.4	344.4
	2017	103.4	47.4	9.7	160.5	160.5
Carberry	2016	182.3	78.9	16.1	277.3	277.3
	2017	86.8	37.2	4.3	128.3	128.3

Among the different years of the study, relatively small differences were apparent in the mean temperatures (Table 2.4). For the majority of sites, the mean temperatures remained relatively similar between the different years of the study with no large changes within each location and time period. The Lethbridge site was the only location to show consistent differences in mean temperature across all three years with 2016 values consistently the lowest, slightly higher values in 2015 and the highest values in 2017. The differences in accumulated precipitation were much more prominent than those for mean temperatures. However, these data do not reveal temperature (or precipitation) variations for shorter time periods, especially during the early stages of crop development, which are known to influence grain yield and protein content (Boonchoo et al., 1998, Zhang et al., 2012) and ultimately protein composition, which is well known to be affected by nitrogen supply dynamics during grain development (Daniel and Triboi

2000, 2001, Triboi et al., 2000). Courcelles (2019), in a companion study to this one, showed that wheat protein content was strongly related to growing degree days (base temp = 15 ^{o}C) at the seedling stage and maximum daily air temperature around the flag leaf stage, whereas the concentration of HMW glutenin protein was strongly related with both growing degree days (base temp = 21 ^{o}C) and total precipitation at a time period around the flag leaf stage.

Table 2.4 Mean temperature by growing season period for all locations and study years. Study locations are shown in Figure 2.1

Location	Year	Seeding-Anthesis (^{o}C)	Anthesis-Maturity (^{o}C)	Maturity-Harvest (^{o}C)	Seeding-Maturity (^{o}C)	Seeding-Harvest (^{o}C)
IHARF	2015	13.5	18.3	15.4	15.9	15.7
	2016	15.9	18.7	17.9	17.3	17.5
	2017	14.2	17.6	16.5	15.9	16.1
Lethbridge	2015	14.4	19.7	19.7	17.1	17.9
	2016	14.0	17.6	17.2	15.8	16.3
	2017	15.7	20.3	18.2	18.0	18.1
Kelburn	2015	15.2	20.3	20.0	17.8	18.5
	2017	16.3	19.5	16.7	17.9	17.5
Carberry	2016	15.5	18.9	17.0	17.2	17.1
	2017	15.2	18.3	17.7	16.8	17.1

2.4.2 Grain Analysis Results

The global ANOVA for the grain analyses for all site-years shows that site-year was a highly significant factor for all four grain parameters and it contributed the largest amount to total variance (Table 2.5). The effect of genotype was also significant for all four parameters; its contribution to variance exceeded the residual contribution for test weight and thousand kernel weight but not for %FDK or %GPC. The site-year*genotype interaction was also significant for all four grain parameters, but its contribution to variance exceeded the residual

contribution only for %FDK. Most notably, the effects of pesticide treatment and its interactions, while statistically significant in some cases, were very small and contributed less than 2% to total variance of these grain parameters and never exceeded the residual contribution for any of them. Thus, site-year, genotype and its interactions had more statistically and biologically important effects on the grain parameters than pesticide treatment and its interactions.

Table 2.5 Global analysis of variance for percent Fusarium damaged kernels (%FDK), test weight, thousand kernel weight (TKW) and grain protein concentration percent (%GPC) for all site-years.

		%FDK			Test Weight			TKW			%GPC		
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F	MS	PV	Pr>F	MS	PV	Pr>F
Genotype	5	0.6075	5.60	0.0013 **	386.3705	19.85	<.0001 ***	165.2351	9.05	<.0001 ***	15.2631	13.83	<.0001 ***
Pesticide treatment	3	0.3731	2.06	0.0025 **	14.1424	0.44	0.0420 *	25.2921	0.83	0.0052 **	0.3659	0.20	0.7743
Site-year	9	4.0152	66.59	<.0001 ***	723.1448	66.89	<.0001 ***	683.5000	67.39	<.0001 ***	23.7769	38.79	<.0001 ***
Block (Site-year)	30	0.02111	1.17	<.0001 ***	9.5579	2.95	<.0001 ***	8.1423	2.68	<.0001 ***	1.4468	7.87	<.0001 ***
Genotype*Pesticide	15	0.0173	0.48	0.0840	0.4718	0.07	0.7007	2.6184	0.43	0.0006 ***	0.0828	0.23	0.6784
Site-year*Genotype	45	0.1254	10.40	<.0001 ***	6.8280	3.16	<.0001 ***	16.7620	8.26	<.0001 ***	1.7323	14.13	<.0001 ***
Site-year*Pesticide	27	0.0605	3.01	<.0001 ***	4.5178	1.25	<.0001 ***	4.7647	1.41	<.0001 ***	0.9852	4.82	<.0001 ***
Site-year*Geno*Pest	135	0.0109	2.70	<.0001 ***	0.6070	0.84	0.6476	0.9153	1.35	0.9410	0.1038	2.54	0.9851
Residual	690	0.0063	8.00	-	0.6412	4.55	-	1.1376	8.69	-	0.1407	17.59	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at *P* < 0.05, 0.01, and 0.001, respectively

2.4.2.1 Percent Fusarium damaged Kernels. FHB levels in cereal grain is known to vary significantly between growing seasons as a result of differences in growing season weather with large outbreaks characteristically occurring in wet years and locations (McMullen et al., 2012). This pattern occurred during this study as well. There was a significant, positive relationship between total precipitation from seeding to anthesis and %FDK (Figure 2.4) as would be expected. This is an important reason for the significant main effect of site-year on %FDK.

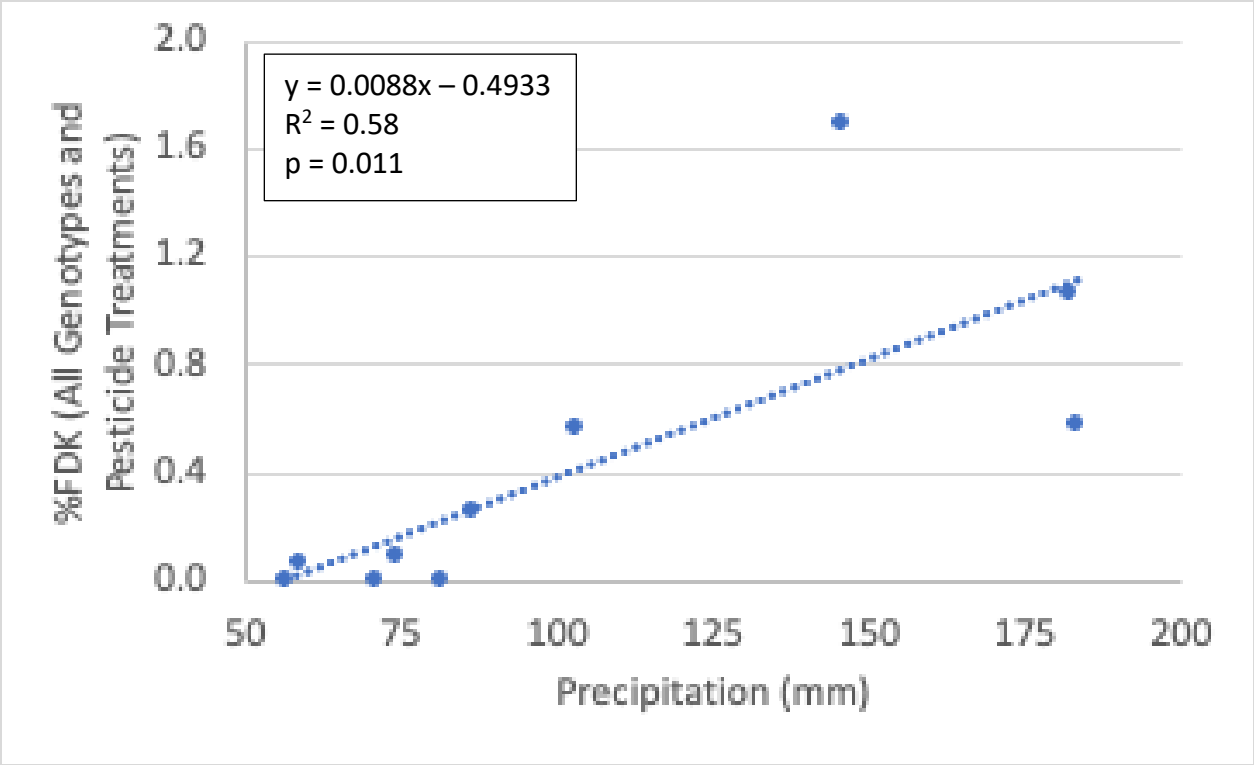


Figure 2.4 %Fusarium damaged kernels (%FDK) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

The significant genotype main effect and the significant interactions for %FDK in Table 2.5 are likely related to the differences in FHB resistance between the CWRS wheat genotypes in the study. According to Seed Manitoba (2019), Carberry and Cardale are both rated moderately resistant;

Glenn is rated intermediate; CDC Stanley is rated moderately susceptible and Harvest and Stettler are rated susceptible (Table 2.6). The FHB resistance ratings by genotype generally aligned with the %FDK levels observed by genotype (Figure I.2).

Table 2.6 Fusarium disease resistance ratings by genotype (Seed Manitoba 2019).

Genotype	Rating[a]
Carberry	MR
Cardale	MR
Glenn	I
Harvest	S
Stettler	S
Stanley	MS

[a]MR-moderately resistant, I-intermediate, MS-moderately susceptible, S-susceptible.

Differences in FHB resistance between genotypes could also impact their response to fungicide application and the subsequent %FDK levels in the grain. Prothioconazole/Tebuconazole fungicide application at anthesis is registered to suppress FHB. However, in some circumstances it may not decrease FDK if it just suppresses the disease enough that infected kernels are retained in the harvested grain rather than being lost during threshing. Despite its limitations, the %FDK levels in the grain samples were used to calculate a measure of fungicide efficacy (FE) for each genotype and location in all three years of the study as per equation 2.1.

$$FE = (FDK\ Control - FDK\ Fungicide) / FDK\ Control \quad [2.1]$$

The FDK Control and FDK Fungicide for each site-year was the mean of the four replicates. If FDK Control was equal to zero, then FE was set to zero. Both 2015 and 2017 had very low levels of FDK resulting in many locations and genotypes with efficacy ratings of zero due to the lack of the disease in the control plots (Table 2.7). Generally, FE was higher at site-years with higher %FDK levels and for genotypes that were moderately susceptible or susceptible to FDK. This would explain the

significant 2-way interaction between site-year and pesticide treatment on %FDK as well as the significant 3-way interaction between genotype, site-year and pesticide treatment on %FDK (Table 2.5). The negative values in Table 2.7 show instances where fungicide application increased FDK. These could be the circumstances when the fungicide suppressed the pathogen to the extent that diseased kernels are large enough to remain in the threshed grain and not lost through the back of a combine. As previously mentioned, %FDK can be an imperfect measure of fungicide efficacy.

Table 2.7 Location means[a] for fungicide efficacy by genotype and year.

Genotype	Carberry	Cardale	Glenn	Harvest	CDC Stanley	Stettler
			2015			
IHARF	0.00	0.25	0.25	0.50	-0.25	-2.73
Lethbridge	0.00	0.00	0.00	0.00	0.00	0.00
Kelburn	-0.67	0.59	0.06	0.82	0.75	0.89
			2016			
IHARF	0.38	0.68	0.35	0.15	0.41	0.11
Lethbridge	0.42	0.17	0.25	0.20	0.83	0.05
Carberry	-0.01	0.24	-0.86	0.34	0.13	0.07
			2017			
IHARF	0.00	0.00	0.00	0.00	0.00	0.00
Lethbridge	0.00	0.00	0.00	0.00	0.00	0.00
Kelburn	0.64	0.46	0.10	0.83	0.54	0.72
Carberry	0.31	-0.10	0.21	0.34	0.90	0.32

[a]Mean value of FE = (Mean FDK Control of 4 reps – Mean FDK Fungicide of 4 reps)/Mean FDK Control of 4 reps

The variation in %FDK by site-year and genotype for the control and fungicide treatments is illustrated in Figure 2.5. The effects of site-year are readily apparent as are the genotype differences as a result of varying FHB resistance, plus the variation in %FDK levels as a result of fungicide application both across and within site-years. The higher %FDK for Stettler and Harvest are consistent with their lower FHB resistance levels. The variation in fungicide efficacy is also

apparent with much lower %FDK in fungicide treatments versus controls in Kelburn 2015 and Kelburn 2017 compared to IHARF 2016 and Carberry 2016. Figure 2.5 illustrates why a 3-way interaction between genotype, site-year and fungicide treatment would be expected.

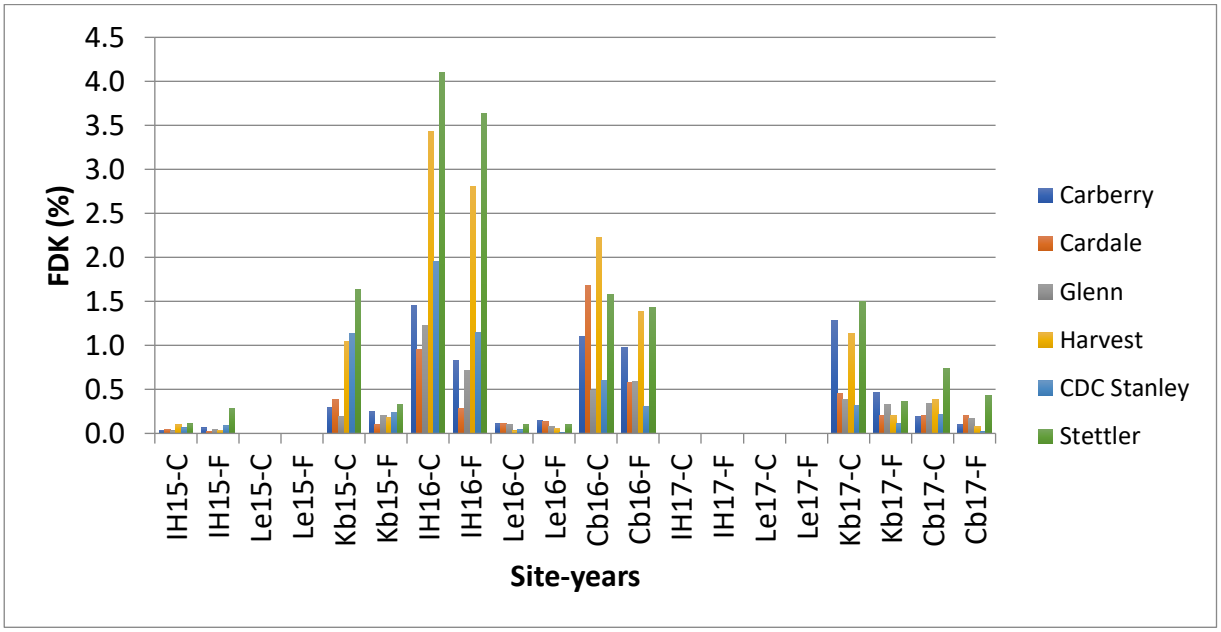


Figure 2.5. Percentage of Fusarium damaged kernels (%FDK) by location and genotype for the control ("C") and FHB fungicide ("F") treatments for IH (IHARF), Le (Lethbridge), Kb (Kelburn), Cb (Carberry), in 15 (2015), 16 (2016), 17 (2017). Values are means of four replicates.

Type III Analysis of Variance showed that the two Fusarium susceptible genotypes, Stettler and Harvest, had significantly higher mean %FDK than Cardale, Glenn, and CDC Stanley (Table 2.8, Figure I.2) when all site-years and pesticide treatments were combined. This would be expected, as explained previously, by the lower FHB resistance of Stettler and Harvest. Stettler and Harvest usually had the highest %FDK levels in the Control (C) treatment at site-years that had high FHB pressure (Figure 2.5). Stettler had significantly higher %FDK across all pesticide treatments and site-years than Carberry, Cardale, Glenn and CDC Stanley, while Harvest %FDK levels were statistically

higher than Cardale, Glenn and CDC Stanley (Table 2.8). The genotypes rated MR through MS did not show statistically significant differences in FDK levels across years and locations. A significant site-year*genotype interaction resulted in genotype as a significant factor affecting %FDK for only seven out of the 10 site-years (Table 2.8). The three site-years for which genotype was not significant were those with zero or near zero %FDK.

Pesticide treatment significantly affected %FDK for IHARF 2016, Lethbridge 2016, Kelburn 2015, Kelburn 2017 and Carberry 2017 (Table 2.8). These were the site-years with the highest %FDK levels (Figure 2.5), which explains the significant site-year*pesticide treatment interaction in Table 2.5. It should be noted that the genotype*treatment interaction for %FDK was significant for only Lethbridge 2016, Kelburn 2015 and Kelburn 2017 (Table 2.8). The F or the FG treatment significantly reduced %FDK compared to the C treatment across all genotypes and site-years, combined (Table 2.8). There was no significant difference between the F and FG treatments globally or for any individual site-year.

Table 2.8 Means comparisons and ANOVA for percent Fusarium damaged kernels by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	0.04 B[f]	0.48 D	0.00	0.02	0.11 AB	0.00	0.19 C	0.31 CD	0.60 B	0.14 B	0.16 D
Carberry	0.04 B	1.05 BC	0.00	0.00	0.13 A	0.00	0.29 BC	0.73 A	1.16 A	0.15 B	0.27 C
Glenn	0.03 B	0.74 CD	0.00	0.00	0.08 BC	0.00	0.21 C	0.40 BC	0.55 B	0.20 B	0.18 D
Harvest	0.05 B	2.93 A	0.00	0.00	0.05 CD	0.00	0.52 AB	0.52 B	1.38 A	0.20 B	0.34 B
CDC Stanley	0.05 B	1.31 B	0.00	0.00	0.04 D	0.00	0.62 A	0.21 D	0.58 B	0.11 B	0.22 CD
Stettler	0.15 A	3.41 A	0.01	0.00	0.08 BC	0.00	0.83 A	0.80 A	1.52 A	0.56 A	0.46 A
Pesticide treatment											Mean
C[g]	0.06	1.88 A	0.00	0.00	0.08 AB	0.00	0.62 A	0.73 A	1.06	0.31 A	0.34 A
G[h]	0.05	1.37 AB	0.00	0.02	0.10 A	0.00	0.68 A	0.76 A	1.00	0.26 AB	0.32 A
FG[i]	0.04	1.18 B	0.00	0.00	0.06 B	0.00	0.27 AB	0.26 B	0.83	0.16 BC	0.21 B
F[j]	0.08	1.21 B	0.00	0.00	0.08 AB	0.00	0.19 B	0.26 B	0.76	0.15 C	0.21 B
Site-year Mean	0.06	1.41	0.00	0.01	0.08	0.00	0.44	0.50	0.91	0.22	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	0.4526	0.5170	<.0001 ***	1.000	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	
Pesticide	0.4505	0.0068 **	0.4363	0.1952	0.0202 *	0.4363	0.0037 **	<.0001 ***	0.0874	0.0009 ***	
Geno*Pest	0.5670	0.6945	0.4673	0.6156	0.0022 **	1.000	0.0038 **	<.0001 ***	0.0510	0.3677	
Block	0.0205 *	0.0341 *	0.4363	0.4363	0.7586	0.4363	0.0695	0.4502	0.9029	0.8631	
Block*Pest	0.2119	0.2150	0.4501	0.6339	0.5089	1.000	0.0091	0.0929	0.6992	0.7688	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

2.4.2.2 Test Weight. Test weight showed a significant main effect for genotype, pesticide treatment and site-year (Table 2.5). Growing season precipitation for both seeding to maturity (Figure IV.4) as well as seeding to anthesis (Figure IV.5) were significantly, inversely correlated to test weight. There was no significant correlation between test weight and growing season temperature. Higher growing season precipitation was also positively correlated to higher %FDK (Figure 2.4), which would reduce kernel size and density. Thus, site-year was a significant factor and explained the largest percentage of variance for test weight, with 66.89% (Table 2.5). A possible cause as to why genotype had a significant effect on test weight could be due to the variation in kernel size between varieties. Although the pesticide treatment main effect was significant, it was not strong and explained only 0.44% of the variance in test weight (Table 2.5). Similar to site-year, the significance of pesticide treatment was likely an indirect result of its impact on %FDK (see Section 2.4.4.1), which would affect kernel size and density. This is also the most likely reason for the significant site-year*genotype and site-year*pesticide interactions. (Table 2.5). Variation in the effect of pesticide treatment on %FDK between genotypes and site-years (Table 2.7, Figure 2.5) would also impact kernel size and density.

The pesticide treatment effect caused slightly higher test weight for the F and FG treatments compared to the control for all site-years and genotypes combined (Table 2.9, Figure I.5). The site-year*pesticide interaction was significant, because pesticide treatment was a significant factor for test weight at only five of the 10 individual site-years (Table 2.9). In contrast, genotype was significant for test weight at all 10 site-years (Table 2.9) with the 2nd largest contribution to variance after site-year (Table 2.5). In regards to genotype trends, Glenn was the genotype with consistently

highest values for test weight, while the Harvest and CDC Stanley genotypes had the lowest values. Only the Kelburn 2015 test weights for the Carberry, Harvest, CDC Stanley and Stettler genotypes fell below the minimum threshold of 75 kg hL^{-1} required for a grade of No. 1 CWRS (Table 2.9).

Table 2.9 Means comparisons and ANOVA for test weight (kg hL^{-1}) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	80.58 D	76.31 C	80.69 E	82.66 C	79.36 E	78.71 E	72.56 D	81.33 D	75.90 C	79.38 D	78.75 D
Carberry	82.46 B[f]	77.44 B	83.31 B	83.55 B	81.44 B	82.24 B	75.82 B	82.96 B	78.62 B	82.25 B	81.01 B
Glenn	84.22 A	80.21 A	84.54 A	84.56 A	83.29 A	83.50 A	78.08 A	84.38 A	81.66 A	84.13 A	82.86 A
Harvest	81.71 C	75.67 D	81.59 D	82.60 C	79.95 D	80.55 C	74.69 BC	81.82 C	76.34 C	80.92 C	79.58 C
CDC Stanley	80.96 D	76.69 C	80.51 E	82.09 D	78.53 F	78.19 E	74.55 BC	81.47 CD	75.84 C	79.81 D	78.86 D
Stettler	82.08 BC	76.41 C	82.69 C	83.58 B	80.96 C	79.81 D	73.46 CD	82.62 B	76.49 C	80.81 C	79.89 C
Pesticide Treatment											Mean
C[g]	81.39 C	76.23 C	82.25	83.18 A	80.70	80.61	73.84 A	82.12 A	77.01	81.11	79.84 B
G[h]	82.11 B	77.12 B	82.10	83.21 A	80.68	80.70	74.15 A	82.30 A	77.73	80.83	80.09 AB
FG[i]	82.77 A	77.95 A	82.25	82.98 A	80.35	80.09	75.80 A	82.61 A	77.48	81.33	80.36 A
F[j]	81.73 BC	77.20 B	82.29	83.33 A	80.62	80.60	75.65 A	82.69 A	77.68	81.59	80.34 A
Site-year Mean	82.00	77.12	82.22	83.17	80.59	80.50	74.86	82.43	77.48	81.22	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	
Pesticide	0.0002 ***	<.0001 ***	0.0952	0.0091 **	0.2737	0.2537	0.0276 *	0.0171 *	0.2844	0.0843	
Geno*Pest	0.4771	0.1630	<.0001 ***	0.5812	0.0746	0.3775	0.4718	0.4772	0.8731	0.6105	
Block	0.2752	0.0654	0.0015 **	0.0008 ***	0.0487	0.0007 ***	0.0012 **	0.0170 *	0.2023	0.1091	
Block*Pest	0.0955	0.5186	0.8433	0.9745	0.0022 **	0.0102 *	0.0494 *	0.1746	0.2010	0.2165	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

2.4.2.3 Thousand Kernel Weight. Thousand kernel weight (TKW), similar to test weight, showed a significant main effect for genotype, pesticide treatment and site-year (Table 2.5). However, unlike test weight, TKW did not show any significant correlations to either growing season precipitation (see Figures in Appendix IV) or growing season temperature (see Figures in Appendix V). Thus, the origin of the site-year effect on TKW is not apparent in either of these key weather conditions. The variation in kernel size between varieties probably explains the significant genotype effect (Table 2.5) similar to test weight. Again, the significant pesticide treatment effect was not strong, explaining only 0.83% of the variance in TKW (Table 2.5). As with test weight, it is likely that the indirect effect of pesticide treatment on %FDK explains the significant pesticide treatment effect on TKW. Variation in the effect of pesticide treatment on %FDK between genotypes and site-years (Table 2.7, Figure 2.5) would also impact TKW and explain the significant site-year*genotype and site-year*pesticide interactions (Table 2.5). TKW was the only grain parameter that showed a significant genotype*pesticide interaction. Again, the variation in the effect of pesticide on %FDK (Table 2.7, Figure 2.5) is likely behind this effect.

TKW was higher for the F and FG treatments compared to the control across all site-years and genotypes, combined (Table 2.10, Figure I.3). Pesticide treatment was a significant factor for TKW at six of the 10 individual site-years (Table 2.10). Again, genotype was a significant factor for TKW at all 10 site-years (Table 2.10) with the 2nd largest contribution to variance after site-year (Table 2.5). The Carberry genotype had the highest TKW values, while CDC Stanley had the lowest. The genotype*pesticide treatment interaction was significant for TKW at two of the site-years, Lethbridge 2016 and Kelburn 2015.

Table 2.10 Means comparisons and ANOVA for thousand kernel weight (g) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	32.80 C	28.95 A	26.69 C	35.28 C	32.56 C	26.95 CD	28.02 C	32.75 ABC	29.37 C	28.67 B	30.20 C
Carberry	33.81 B[f]	27.98 B	29.77 A	37.35 A	34.60 A	29.30 A	30.79 A	33.77 A	31.31 AB	31.33 A	32.00 A
Glenn	32.37 D	28.03 B	29.95 A	34.92 C	32.64 C	28.49 B	29.72 AB	32.59 ABC	31.70 A	32.27 A	31.27 B
Harvest	34.31 A	28.00 B	28.12 B	35.28 C	33.52 B	27.75 BC	29.59 B	33.34 AB	31.70 A	31.03 A	31.26 B
CDC Stanley	31.92E	26.88 C	24.97 D	32.25 D	29.90 D	23.92 E	29.72 AB	31.46 C	31.96 A	28.01 B	29.10 D
Stettler	34.05 AB	28.25 AB	27.90 B	36.30 B	32.87 BC	26.79 D	28.13 C	31.56 BC	30.54 B	28.88 B	30.52 C
Pesticide treatment											Mean
C[g]	32.69 B	27.24 B	26.98 B	35.27	32.39 A	27.31	29.17	32.21 A	30.32 A	30.01	30.36 C
G[h]	33.01 AB	27.59 B	28.61 A	35.14	32.42 A	27.27	29.21	32.15 A	30.73 A	29.50	30.56 BC
FG[i]	33.60 A	28.64 A	28.91 A	35.40	32.90 A	26.70	29.30	32.98 A	31.81 A	30.48	31.07 A
F[j]	33.54 A	28.59 A	27.09 B	35.11	33.01 A	27.51	29.64	32.97 A	31.52 A	30.13	30.91 AB
Site-year Mean	33.21	28.02	27.90	35.23	32.68	27.20	29.33	32.58	31.10	30.03	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	0.0032 **	<.0001 ***	<.0001 ***	
Pesticide	0.0022 **	0.0004 ***	0.0016 **	0.7907	0.0403 *	0.4693	0.8966	0.0208 *	0.0021 **	0.1569	
Geno*Pest	0.1414	0.2634	0.2033	0.3006	0.0114 *	0.7848	0.0040 **	0.9995	0.7062	0.3082	
Block	0.1059	0.1670	0.8812	0.4346	0.0036 **	0.0031 **	0.0885	0.0151 *	0.0035 **	0.0411 *	
Block*Pest	0.0133 *	0.3580	0.0035 **	0.0233 *	0.3407	<.0001 ***	<.0001 ***	0.9801	0.3381	0.5150	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

2.4.2.4 Grain Protein Concentration. Site-year and genotype main effects were both significant for grain protein concentration percentage (GPC%). However, unlike the other grain parameters, pesticide treatment did not have a significant impact on GPC%. Growing season precipitation for both seeding to maturity (Figure IV.10) as well as seeding to anthesis (Figure IV.11) had a significant, positive correlation to GPC% but there was no significant correlation to growing season temperature. This is the same type of weather effect noted for %FDK and it suggests that higher rainfall led to higher %FDK which increased GPC%. It is possible that the lower yields associated with higher %FDK resulted in higher GPC%. The inverse relationship between yield and protein has been known for some time (e.g. Terman et al., 1969) and it could be the factor behind this effect. Genotypic variation in GPC% is the cause behind the genotype main effect. It is also part of the reason for the significant site-year*genotype interaction on GPC% (Table 2.5). The site-year*pesticide interaction is likely a result of the variation in FHB control between site-years (Table 2.7, Figure 2.5).

Across all site-years and genotypes combined, pesticide treatment was not significant for GPC% (Table 2.11, Figure I.7). The site-year*pesticide treatment interaction occurred because GPC% was significantly impacted by pesticide treatment at only five of 10 individual site-years (Table 2.11). However, effects of pesticide treatment on GPC was generally not substantial across the study overall with only a small contribution to variance from the main effect or its interactions (Table 2.5). Consistent with the other grain parameters, site-year and genotype were highly significant factors that affected GPC% for all 10 site-years (Table 2.11) with large contributions to variance. Trends between the genotypes and their mean GPC values varied between site-years. For the majority of

site-years Stettler had the highest GPC values, whereas Harvest and CDC Stanley had the lowest values. The site-year* genotype interaction contributed 14.13% of total variance (Table 2.5) and was slightly larger than the contribution from genotype. This further shows the significant effect of site-year on GPC%, as was also shown in the analysis of individual site-years. Genotype values of GPC% varied between the different site-years, and there were not as many obvious trends in genotype GPC% between site-years.

Table 2.11 Means comparisons and ANOVA for grain protein concentration (%) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	15.51 B	16.03 B	14.57 A	14.36 BC	14.79 D	14.70 C	15.81 B	15.26	15.84 B	15.05 BC	15.19 B
Carberry	14.86 D[f]	15.61 C	14.52 A	15.01 A	15.39 C	15.40 B	15.27 C	15.29	15.43 C	15.06 BC	15.18 BC
Glenn	14.68 E	15.34 D	14.51 A	14.87 A	15.67 B	15.41 B	15.15 C	15.31	15.26 C	14.94 CD	15.11 BC
Harvest	14.99 C	16.04 B	13.90 B	14.23 C	15.76 B	15.29 B	15.50 BC	15.03	15.44 C	14.73 D	15.09 BC
CDC Stanley	14.83 D	15.92 B	13.93 B	14.09 C	14.86 D	14.76 C	15.58 BC	15.15	16.19 A	15.18 B	15.05 C
Stettler	15.95 A	16.77 A	14.70 A	14.80 AB	16.39 A	16.21 A	16.53 A	15.64	16.13 A	15.58 A	15.87 A
Pesticide treatment											Mean
C[g]	15.31 A	16.13 A	14.40	14.15 A	15.32 A	15.59 A	15.73	15.42	15.77	15.17	15.30
G[h]	15.26 AB	15.99 AB	14.38	14.26 A	15.41 A	15.27 AB	15.55	15.36	15.58	15.22	15.23
FG[i]	14.95 B	15.92 AB	14.25	15.15 A	15.58 A	15.12 B	15.75	15.23	15.72	14.98	15.27
F[j]	15.02 AB	15.77 B	14.38	14.68 A	15.61 A	15.20 AB	15.53	15.11	15.79	15.00	15.21
Site-year Mean	15.14	15.95	14.35	14.56	15.48	15.29	15.64	15.28	15.71	15.09	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	0.0935	<.0001 ***	<.0001 ***	
Pesticide	0.0191 *	0.0380 *	0.1322	0.0187 *	0.0011 **	0.0224 *	0.5304	0.8337	0.4325	0.0644	
Geno*Pest	0.0020 **	0.1427	0.3297	0.6872	0.3915	0.5048	0.0997	0.9322	0.1655	0.5989	
Block	0.3334	0.2929	0.2205	0.0043 **	0.0002 ***	0.1213	0.7187	0.1590	0.0542	0.2183	
Block*Pest	<.0001 ***	0.0010 ***	0.6362	0.0004 ***	0.6112	0.0121 *	0.0272 *	0.0001 ***	0.0003 ***	0.0430 *	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

2.4.2.5 Grade. The CWRS grades for the grain samples varied by both location and year (Table 2.12) with %FDK being the most common factor affecting grade (Figure 2.6). FDK damage was higher at the site-years in 2016 than 2015 and 2017 (Figure 2.5). The mean grade by location ranged across genotypes from 1.0 to 4.88 in 2016 compared to 1.00 to 2.75 in 2015 and 1.00 to 2.63 in 2017. The %FDK for 2015 ranged across genotypes from 0.00 to 0.83 % compared to 0.11 to 3.41% in 2016 and 0.00 to 0.80% in 2017 (Table 2.8). Less precipitation during the 2015 growing season suppressed Fusarium pressure at many sites. The wetter conditions for 2016, in comparison to 2015, resulted in lower CWRS grades (Table 2.12) and higher levels of %FDK especially at the IHARF and Carberry locations (Table 2.8). In 2017, precipitation was lower and temperatures were higher on average than those in 2016 (Table 2.3). Wheat samples from Lethbridge 2017 all graded No. 1 CWRS and from Indian Head 2017 were mainly No. 1 CWRS with no Fusarium damaged kernels. In 2017, the Kelburn and Carberry locations experienced higher total precipitation (161 and 128 mm, respectively) than the other two locations in 2017 (Lethbridge 82 mm, IHARF 113 mm) and genotype samples from Kelburn and Carberry both experienced slight to moderate downgrading as a result of higher FDK (Table 2.12).

Table 2.12 Location means[a] by year and genotype for Canada Western Red Spring (CWRS) grade[b].

Genotype	IHARF	Lethbridge	Kelburn	Carberry
2015				
Cardale	1.00	1.00	2.25	-[c]
Carberry	1.00	1.00	2.00	-
Glenn	1.00	1.00	2.00	-
Harvest	1.00	1.00	2.25	-
CDC Stanley	1.13	1.00	2.25	-
Stettler	1.25	1.00	2.75	-
Average	1.06	1.00	2.25	-
2016				
Cardale	3.88	1.13	-	3.25
Carberry	3.88	1.00	-	3.00
Glenn	4.13	1.13	-	2.50
Harvest	4.50	1.00	-	3.00
CDC Stanley	4.88	1.00	-	2.13
Stettler	4.38	1.00	-	3.25
Average	4.27	1.04		2.85
2017				
Cardale	1.00	1.00	1.63	1.25
Carberry	1.00	1.00	2.25	1.00
Glenn	1.00	1.00	2.00	1.00
Harvest	1.00	1.00	1.75	1.38
CDC Stanley	1.00	1.00	1.25	1.25
Stettler	1.13	1.00	2.63	2.13
Average	1.02	1.00	1.92	1.33

[a]Mean of four replicates of four pesticide treatments, [b]Feed grade was given a value of 4, Harvest genotype was graded as CWRS (Harvest was moved to the Canada Northern Hard Red class on 1 Aug 2018), [c]Data not available due to spray application errors (Carberry 2015) and planting errors (Kelburn 2016).

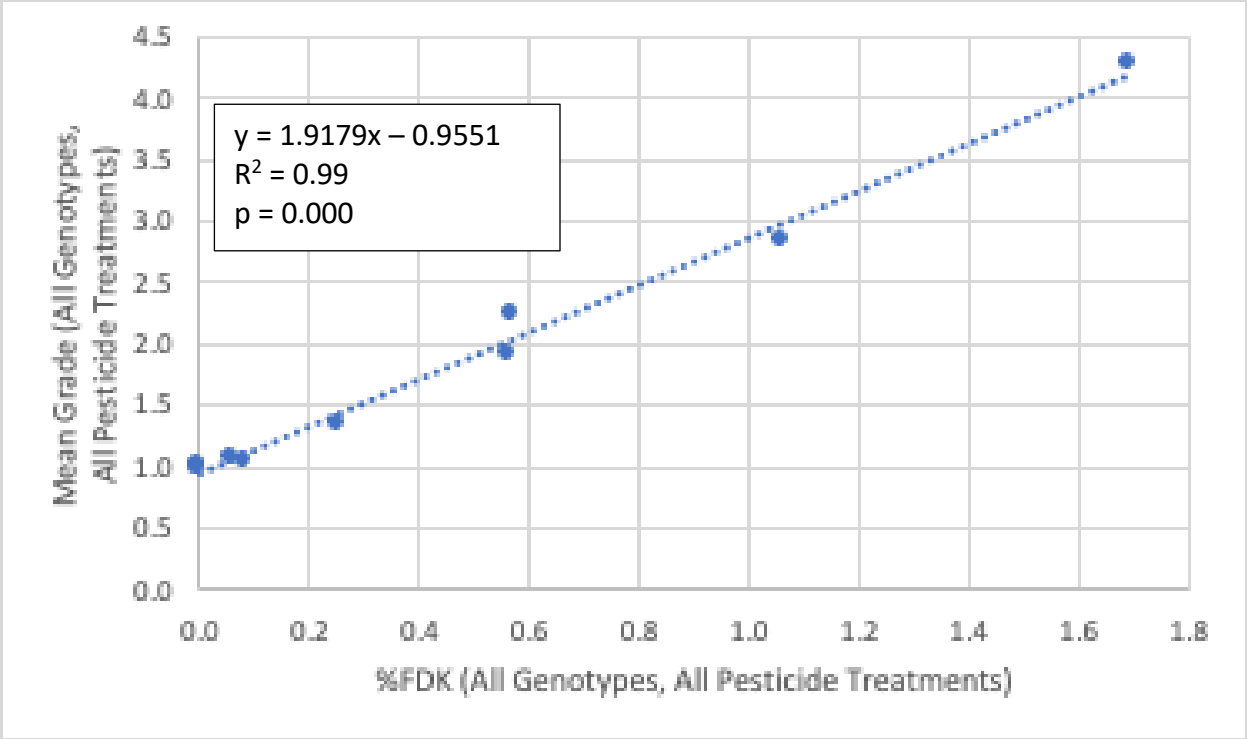


Figure 2.6 %Fusarium damaged kernels (%FDK) averaged across all genotypes and pesticide treatments versus mean grade averaged across all genotypes and pesticide treatments by site-year.

2.4.3 Flour Analysis Results

The ANOVA for the flour parameters across all site-years showed that the main effects on flour quality from site-year, genotype and their interactions were all significant (Table 2.13), similar to the results for the grain parameters. Site-year was a highly significant factor for all three flour parameters and it contributed the largest amount to the total variance. Flour ash showed a significant, positive correlation to growing season rainfall from seeding to anthesis, anthesis to maturity and seeding to maturity, but did not show any correlation to growing season temperature (see Figures in Appendix IV). Thus, growing season precipitation, again was an important driver of the site-year effect for flour ash. However, neither flour yield nor flour protein showed any significant correlations to either growing season precipitation or

temperature (see Figures in Appendix IV). In the case of flour protein, this was somewhat surprising because grain protein and flour protein are strongly related and there were significant correlations between grain protein and growing season precipitation. Thus, the origin of the site-year effects on flour yield and flour protein is not apparent in either of these key weather conditions.

It is important to note that there was a significant site-year*genotype interaction for all three flour parameters with a contribution to variance that exceeded the residual. This indicates that the flour quality responses among genotypes also differed among the site-years. Effects of genotype as well as site-year*genotype interaction were also significant except that the contributions to variance from these effects were less than that for site-year (Table 2.13). Differences in kernel size between genotypes were the most likely reason for these effects. Pesticide treatment was significant only for flour ash and its site-year*pesticide interaction was significant for flour ash and flour protein, but none of these accounted for a large proportion of variance (Table 2.13).

Table 2.13 Global analysis of variance of flour ash, flour protein and flour yield for all site-years.

		Flour Ash			Flour Protein			Flour Yield		
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F	MS	PV	Pr>F
Genotype	5	0.0312	13.98	<.0001 ***	11.9830	20.08	<.0001 ***	18.1309	8.79	<.0001 ***
Pesticide treatment	3	0.0024	0.64	0.0064 **	0.3641	0.37	0.5419	0.5576	0.16	0.1728
Site-year	9	0.0879	70.87	<.0001 ***	11.8066	35.60	<.0001 ***	83.4111	72.77	<.0001 ***
Block (Site-year)	30	0.0004	1.08	<.0001 ***	0.5799	5.83	<.0001 ***	0.4384	1.27	0.0083 **
Genotype*Pesticide	15	0.0003	0.40	0.0505	0.1788	0.90	0.0945	0.2616	0.38	0.4124
Site-year*Genotype	45	0.0017	7.01	<.0001 ***	1.0948	16.51	<.0001 ***	1.7481	7.63	<.0001 ***
Site-year*Pesticide	27	0.0005	1.14	<.0001 ***	0.4984	4.51	<.0001 ***	0.3118	0.82	0.2057
Site-year*Geno*Pest	135	0.0002	2.09	0.1571	0.1144	5.17	0.9764	0.2502	3.27	0.3995
Residual	210	0.0001	2.78	-	0.1569	11.04	-	0.2409	4.90	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

2.4.3.1 Flour Ash. The genotype effect was significant for flour ash at all 10 individual site-years (Table 2.14). There was a wide range in flour ash levels across site-years with Kelburn 2015 and IHARF 2016 values being notably high. This is likely related to the low test weight values at these locations, a result to be expected based on previous research (Marshall et al., 1986, Schuler et al., 1995). Pesticide treatment was significant for flour ash at the IHARF 2015, IHARF 2016, Carberry 2016 and Carberry 2017 site-years. There was a significant genotype*pesticide treatment interaction at only the IHARF 2016 site. The effect of pesticide treatment on flour ash would likely be related to its impact on %FDK and the subsequent effects on test weight during milling. The fungicide treatment, without glyphosate, slightly, but significantly reduced flour ash below the control and glyphosate treatments across all site-years combined (Figure I.9). However, this effect was observed at only one of 10 individual site-years (Carberry 2017), resulting in pesticide treatment*site-year interaction. This interaction was also due to glyphosate application reducing flour ash at only one site, IHARF 2016. However, across all site-years, combined, pesticide treatment and its interaction with site-year had very low contributions to variance. In contrast, site-year and genotype effects were highly significant across all site-years, combined, with the first and second largest contributions to variance, respectively (Table 2.13). The genotypes Cardale, Harvest and Stettler had the statistically highest flour ash (Table 2.14, Figure I.10).

Table 2.14 Means comparisons and ANOVA for flour ash (%) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	0.45 AB	0.49 B	0.40 A	0.42 A	0.43 A	0.43 A	0.57 A	0.44 BA	0.45 B	0.41 A	0.45 A
Carberry	0.42 C[f]	0.44 D	0.38 B	0.38 BC	0.41 AB	0.39 B	0.49 CD	0.43 B	0.42 C	0.39 BC	0.42 B
Glenn	0.41 C	0.45 C	0.38 B	0.39 B	0.39 BC	0.39 B	0.48 D	0.41 C	0.42 C	0.38 CD	0.41 BC
Harvest	0.45 A	0.54 A	0.37 B	0.41 B	0.42 A	0.42 A	0.53 B	0.46 A	0.48 A	0.39 AB	0.45 A
CDC Stanley	0.39 D	0.47 C	0.36 C	0.37 C	0.38 C	0.39 B	0.52 BC	0.39 C	0.40 D	0.36 D	0.40 C
Stettler	0.44 B	0.51 B	0.38 B	0.39 B	0.42 A	0.41 AB	0.54 AB	0.44 AB	0.46 AB	0.41 A	0.44 A
Pesticide treatment											Mean
C[g]	0.43 AB	0.49 A	0.37	0.40	0.41	0.40	0.52	0.44	0.45 A	0.40 A	0.43 A
G[h]	0.44 A	0.47 B	0.38	0.39	0.42	0.40	0.53	0.44	0.44 AB	0.40 A	0.43 A
FG[i]	0.43 AB	0.48 B	0 38	0.40	0.41	0.40	0.52	0.43	0.44 AB	0.38 B	0.43 AB
F[j]	0.41 B	0.49 AB	0.38	0.39	0.40	0.41	0.51	0.42	0.43 B	0.38 B	0.42 B
Site-year Mean	0.43	0.48	0.38	0.39	0.41	0.40	0.52	0.43	0.44	0.39	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	0.0002 ***	<.0001 ***	0.0001 ***	<.0001 ***	<.0001 ***	0.0003 ***	<.0001 ***	<.0001 ***	
Pesticide	0.0468 *	0.0353 *	0.3336	0.1026	0.1145	0.6078	0.0654	0.0923	0.0439 *	0.0186 *	
Geno*Pest	0.1978	0.0191 *	0.2431	0.7182	0.2927	0.2504	0.4075	0.9028	0.3401	0.8757	
Block	0.9725	0.2324	0.7121	0.2346	0.0445 *	0.0961	0.0073 **	0.7103	0.2108	0.2322	
Block*Pest	0.0314 *	0.5544	0.8287	0.8187	0.7007	0.0784	0.7986	0.7586	0.2552	0.6927	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

2.4.3.2 Flour Yield. Genotype was a significant factor at nine of the 10 site-years (Table 2.15), the exception being Kelburn 2015, resulting in a site-year*genotype interaction (Table 2.13). The low test weight at Kelburn 2015 (Table 2.9) is likely the reason for low flour yield at this location (Table 2.14), based on previous research (Marshall et al 1986, Schuler et al 1995) and also the most likely reason why genotype was not a significant factor for flour yield at this location. CDC Stanley had the highest flour yield while Glenn and Carberry had the lowest (Table 2.15, Figure I.14). Pesticide treatment and its interactions with site-year and variety were not significant factors affecting flour yield across all genotypes and site-years, combined (Table 2.13). Similarly, pesticide treatment did not affect flour yield at any individual site-years (Table 2.15).

Table 2.15 Means comparisons and ANOVA for flour yield (%) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	73.71 C	70.98 A	72.98 A	72.04 B	72.96 A	71.47 AB	69.40	72.59 BC	72.82 AB	73.28 B	72.22 B
Carberry	73.40 CD[f]	68.85 B	72.46 BC	72.04 B	71.08 D	71.52 AB	70.15	72.62 BC	72.30 BC	72.24 C	71.67 C
Glenn	73.01 D	69.06 B	72.03 C	70.90 C	71.10 D	71.86 AB	69.64	72.36 C	72.03 C	72.31 C	71.43 C
Harvest	74.37 AB	69.38 B	72.91 AB	72.37 AB	72.15 BC	71.89 A	70.08	72.66 BC	73.18 A	73.90 AB	72.28 B
CDC Stanley	74.59 A	71.29 A	73.23 A	73.21 A	72.71 AB	71.74 AB	70.56	73.07 AB	73.44 A	74.21 A	72.79 A
Stettler	73.81 BC	69.44 B	72.78 AB	72.62 AB	71.79 C	71.25 B	68.89	73.44 A	72.74 ABC	73.47 B	72.02 B
Pesticide treatment											Mean
C[g]	73.68	69.68	72.80	72.30	72.11	71.88	69.72	72.87	72.55	73.45	72.10
G[h]	73.97	69.67	72.72	72.40	72.08	71.59	69.85	72.89	72.99	73.12	72.12
FG[i]	73.79	70.02	72.74	72.29	71.87	71.47	69.79	72.78	72.81	73.17	72.06
F[j]	73.83	69.96	72.67	71.79	71.80	71.54	69.79	72.63	72.65	73.19	71.98
Site-year Mean	73.82	69.83	72.73	72.20	71.97	71.62	69.79	72.79	72.75	73.23	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	0.0009 ***	<.0001 ***	0.0364 *	0.1247	0.0049 **	0.0004 ***	<.0001 ***	
Pesticide	0.4973	0.0142	0.8103	0.1032	0.3518	0.3589	0.9952	0.8078	0.1169	0.3271	
Geno*Pest	0.4778	0.6078	0.1782	0.1857	0.6295	0.5437	0.7214	0.1677	0.2033	0.4264	
Block	0.2788	0.0038 **	0.7162	0.3302	0.7142	0.1442	0.5762	0.8186	0.0633	0.2240	
Block*Pest	0.0540	0.9903	0.0849	0.7018	0.3775	0.2583	0.2406	0.2465	0.3720	0.3960	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

2.4.3.3 Flour Protein. Consistent with the other flour parameters, genotype was a significant factor for flour protein at nine of 10 site-years (Table 2.16) with a large contribution to variance (Table 2.13). Similar to grain protein, Stettler flour protein was significantly higher than all of the others; however, there was no significant difference in the flour protein levels in the other five genotypes (Table 2.16, Figure I.12). Pesticide treatment did not have a significant impact on flour protein across all site-years and genotypes, combined (Table 2.13, Figure I.11). However, flour protein was significantly reduced by fungicide application at one of 10 individual site-years, Carberry 2017 (Table 2.16) resulting in a significant site-year*pesticide interaction. Overall, however, the effects of pesticide treatment and its interactions on flour protein were small and inconsistent, resulting in a very small contribution to variance (Table 2.13). The genotype mean values for flour protein varied across all site-years with no specific trends observed.

Table 2.16 Means comparisons and ANOVA for flour protein (%) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	14.75 B	15.52 B	13.87 AB	13.61 B	14.25 C	14.15 C	15.04 B	14.57	14.90 B	14.29 B	14.50 B
Carberry	13.94 D[f]	14.52 C	13.62 BC	14.23 AB	14.61 C	14.99 B	14.61 BC	14.67	14.39 C	14.12 B	14.37 B
Glenn	14.18 CD	14.55 C	13.70 B	14.45 A	15.33 B	15.15 B	14.44 C	14.48	14.42 C	14.17 B	14.50 B
Harvest	14.52 B	15.29 B	13.11 D	13.63 AB	15.57 B	15.10 B	14.81 BC	14.62	14.89 B	14.24 B	14.58 B
CDC Stanley	14.22 C	15.36 B	13.29 CD	13.78 AB	14.53 C	14.10 C	14.95 BC	14.47	15.59 A	14.53 B	14.49 B
Stettler	15.54 A	16.13 A	14.05 A	14.39 AB	16.39 A	16.07 A	15.73 A	15.10	15.68 A	15.23 A	15.45 A
Pesticide treatment											Site-year Mean
C[g]	14.68 AB	15.39	13.60	13.62	14.93 A	15.12	15.07	14.77	15.00	14.61 A	14.70
G[h]	14.75 A	15.25	13.72	13.82	15.10 A	14.99	14.86	14.92	14.89	14.61 A	14.69
FG[i]	14.33 B	15.22	13.51	14.50	15.24 A	14.77	15.15	14.41	15.04	14.24 B	14.65
F[j]	14.35 B	15.06	13.60	14.12	15.19 A	14.83	14.64	14.51	14.99	14.26 B	14.56
Site-year Mean	14.53	15.23	13.61	14.02	15.12	14.93	14.93	14.65	14.98	14.43	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	0.0102 *	<.0001 ***	<.0001 ***	0.0004 ***	0.7159	<.0001 ***	<.0001 ***	
Pesticide	0.0443 *	0.0660	0.4059	0.1701	0.0087 **	0.4309	0.1649	0.7016	0.6567	0.0287 *	
Geno*Pest	0.2820	0.2299	0.5409	0.2782	0.6448	0.9981	0.2205	0.7192	0.1626	0.8822	
Block	0.2959	0.0594	0.4907	0.0043 **	0.0011 **	0.5973	0.8382	0.9774	0.0044 **	0.0494 *	
Block*Pest	0.0192 *	0.1765	0.2198	0.0803	0.7702	0.3958	0.1436	0.1365	0.0991	0.6640	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

2.4.4 Impacts of Weather on Grain and Flour Quality

Site-year accounted for more of the variance in grain and flour parameters than any other factor in the study, amounting to 36-73% of the total variance across all measurements. Over the ten site-years of the study, one set of growing season weather conditions were monitored per site-year. This is insufficient to warrant a detailed analysis because of the limited number of data. However, the 10 values that were measured for a given weather parameter or time interval at each site-year provided a general indication of growing season weather impacts on the grain and flour properties across all genotypes and pesticide treatments. Regression analysis between the precipitation and temperature conditions for each of the 10 growing seasons (Tables 2.3 and 2.4) and the site-year mean grain parameters (Tables 2.8, 2.9, 2.10 and 2.11) and flour parameters (Tables 2.14, 2.15 and 2.16) is compiled in Appendices IV and V.

The analysis showed that growing season precipitation regressions with some of the grain and flour quality parameters were significant (see Figures in Appendix IV), but growing season mean temperature was not (see Figures in Appendix V). Thus, variation in precipitation between site-years appeared to be the main distinguishing feature of the growing season and the factor related most strongly to the quality parameters.

Grade deteriorated with increasing precipitation during the seeding to anthesis time period mainly as a result of the increased %FDK levels with higher precipitation in the same period (Figure 2.5). Percent FDK was one of the main degrading factors in the wheat samples (Figure 2.6) and levels were high enough (Table 2.8) for many samples to grade No.2 CWRS (i.e. 0.3 to

1.5%), some to grade No. 3 (i.e. 1.5 to 4%) and a few to grade CWRS Feed (i.e. 4% or more). It has been known for decades that warm, moist environmental conditions promote development of Fusarium head blight in wheat (Anderson, 1948), so these results are expected.

Test weight declined strongly with higher growing season precipitation, again in the seeding to anthesis period. Similar to grade, this effect is likely related to increased %FDK, which is known to decrease test weight (Salgado, 2014). Grain protein concentration and flour ash both increased with higher levels of growing season precipitation from seeding to maturity. The main reasons behind these relationships are not clear.

2.5 Discussion

Site-year was the most critical factor affecting grain and flour quality. Thus, the variation in weather conditions between locations and years was behind most of the variation in the parameters that were measured. Weather impacts grain and flour quality directly as well as indirectly through its effect on secondary factors such as pathogen pressure (e.g., Fusarium head blight). Site-year contributed the largest percent of variance for %FDK, test weight, TKW and grain protein concentration and for flour ash, flour yield and flour protein content (range from 35.60% to 72.77%).

Genotype was the second most critical factor affecting grain and flour quality. Genotype contributed between 5.60% to 19.85% of total variance for the grain quality parameters and between 8.79% and 20.08% to the variance of the flour parameters. Each genotype has its own

inherent genetic basis for wheat quality and each can exhibit a different response to changes in environmental conditions. For example, the genotypes in the study also have varying levels of FHB resistance and experienced different levels of infection within individual site-years, as well as different responses to fungicide application. Harvest and Stettler, which are rated as susceptible to FHB, generally had the highest %FDK levels in untreated control plots when average FDK levels for a site-year were above ~1% (Figure 2.4). Since %FDK affects grain and flour quality, the effect of genetic resistance to FHB varies with the FHB pressure which is, in turn, controlled by environmental conditions.

The impact of applications of FHB fungicide at anthesis and/or pre-harvest glyphosate on wheat quality were the key focus of the study since their impact on CWRS wheat quality was previously unknown. Unlike the impact of genotype and site-year, the FHB fungicide and pre-harvest glyphosate treatments did not play a major role in grain and flour quality across all locations. The pesticide treatment main effect and all of its interactions did not contribute more than the residual to the variance in any of the grain and flour parameters. In other words, the four pesticide treatments did not have a statistically or biologically important impact on grain or flour quality.

Although fungicide application at anthesis is a widely-used management practice to mitigate the impact of FHB, this study showed that fungicide efficacy for reducing %FDK was highly variable among site-years (Table 2.7). With a fungicide application for FHB control a reduction of %FDK was observed at only four of the 10 site-years. In six of the 10 site-years, there was no

significant reduction in %FDK by a pesticide treatment that included a fungicide in comparison to the control (Table 2.8). Most of the unresponsive sites were also the site-years with zero or very low FDK levels. Thus, in 2016, when there was an increase in FHB pressure, the majority of site-years showed some level of fungicide efficacy, but it also varied with genotype with a tendency for higher efficacy in varieties with lower FHB resistance (Table 2.7). Thus, the effectiveness of fungicide application at anthesis for FHB control was higher in years with higher FHB pressure and in varieties that are rated as moderately susceptible or susceptible to FHB. Part of the reason for the limited effects of fungicide treatment on grain and flour quality was a result of the low FHB levels in half of the site-years. However, even in the individual site-years with higher levels of %FDK, the impact of any pesticide treatment on %FDK was small by comparison to genotype.

There is not a single case where glyphosate application reduced grain or flour quality compared to the control or where glyphosate plus fungicide reduced grain or flour quality compared to the fungicide, alone. A comparison of glyphosate and fungicide treatments on the different grain and flour quality parameters, does not show any trends between the different pesticide treatments or between the different site-years. While there were some quality parameters with a significant pesticide treatment effect or pesticide interaction, their limited contributions to variance show that their impact was minimal.

2.6 Conclusions

The main purpose of this study was to determine the impact of two pesticides in comparison to genotype and growing season weather on wheat grade, and several grain and milling quality properties. The results showed that both FHB fungicide treatment and pre-harvest application of glyphosate and their interactions were minor factors in comparison to the impacts of weather variation between site-years and genotype which were both statistically significant and contributed the largest proportion of variance in wheat and flour quality.

The small impact of fungicide treatment on grain and flour quality can be partially explained by the practical limitations of fungicide efficacy to mitigate damage caused by FHB across this range of site-years, where FHB pressure was highly variable. Only four of the 10 site-years had a significant reduction in %FDK by either the F and/or the FG treatment, which was a limitation of the study. If FHB pressure had been consistently high across the site-years, the effects of fungicide treatment on grain and flour quality would likely be more statistically and biologically significant.

All label directions for the glyphosate applications were followed in this study, with applications near the time of physiological maturity. Results showed that glyphosate generally did not affect the measurements of grain and flour quality tested. This protocol was followed in the study although, for practical reasons, the glyphosate treatment was performed on entire blocks of wheat genotypes considering their average date of physiological maturity. This would be similar to the situation in producer fields where crop development is never completely uniform.

More importantly perhaps, are the reports in the literature that glyphosate application at physiological maturity has no dry down or desiccation effect on the grain (Clarke, 1981; Darwent et al., 1994). This aspect is also relevant to the next chapter on gluten strength as the timing of formation of high molecular weight polymeric glutenin (the fraction closely related to gluten strength) is skewed to the later stages of kernel development and is related to rapid moisture loss (Stone and Nicolas, 1996; Carceller and Aussennac, 1999; Shewry et al., 2009; Koga et al., 2017) which continues after the hard dough stage or physiological maturity is reached. Therefore, the following chapter will look more in-depth at the protein and gluten strength parameters of bread wheat quality.

2.7 References

AACC International, 2000. Approved Methods of the AACC, 10th ed. Method 08-01, ash-basic method. AACC International, St. Paul, MN.

AMIS (Agricultural Market Information System) 2018. Market Database. http://www.amis-outlook.org/home/en/

Andersen, A.L. 1948. The development of *Gibberella zeae* headblight of wheat. *Phytopathology* **38**: 599–611.

Blandino, M. and Reyneri, A. 2009. Effect of fungicide and foliar fertilizer application to winter wheat at anthesis on flag leaf senescence, grain yield, flour bread-making quality and DON contamination. *European Journal of Agronomy* **30**: 275-282.

Bole, J. B. and Dubetz, S. 1986. Effect of irrigation and nitrogen-fertilizer on the yield and protein-content of soft white spring wheat. *Canadian Journal of Plant Science* **66**: 281-289.

Boonchoo, S; Fukai, S; Hetherington, SE. 1998. Barley yield and grain protein concentration as affected by assimilate and nitrogen availability. *Australian Journal of Agricultural Research* **49(4)**: 695-706.

Calvino, P.A., Studdert, G.A., Abbate, P.E., Andrade, F.H. and Redolatti, M. 2002. Use of non-selective herbicides for wheat physiological and harvest maturity acceleration. *Field Crops Research* **77**: 191-199.

Campbell, C.A., Davidson, H.R. and Winkleman, G.E. 1981. Effect of Nitrogen, Temperature, Growth Stage and Duration of Moisture Stress on Yield Components and Protein Content of Manitou Spring Wheat. *Canadian Journal of Plant Science* **61**: 549-563.

Canadian Grain Commision. 2015. Official Grain Grading Guide. https://www.grainscanada.gc.ca/en/grain-quality/official-grain-grading-guide/official-grain-grading-guide-2018-en.pdf

Carceller, J. L. and Aussenac, T. 1999. Accumulation and changes in molecular size distribution of polymeric proteins in developing grains of hexaploid wheats: role of the desiccation phase. *Australian Journal of Plant Physiology* **26**: 301-310.

Clarke, J.M. 1981. Effect of Diquat, Paraquat and Glyphosate on pre-harvest drying of wheat. *Canadian Journal of Plant Science* **61(4):** 909-913.

Courcelles, J.R. 2019. The Effect of Genotype and Growing Environment on the Gluten Strength and End-Use Quality of CWRS Wheat. M.Sc. thesis, University of Manitoba. http://hdl.handle.net/1993/33653

Daniel, C. & Triboi, E. 2000. Effects of Temperature and Nitrogen Nutrition on the Grain Composition of Winter Wheat: Effects on Gliadin Content and Composition. *Journal of Cereal Science* **32:** 45-56.

Daniel, C. & Triboi, E. 2001. Changes in wheat protein aggregation during grain development: effects of temperatures and water stress. *European Journal of Agronomy* **16:** 1-12.

Darwent, A.L., Kirkland, K.J., Townley-Smith, L., Harker, K.N., Cessna, A.J., Lukow, O.M. and Lefkovitch, L.P. 1993. Effect of pre-harvest applications of glyphosate on the drying yield and quality of wheat. *Canadian Journal of Plant Science* **74**: 221-230.

Dexter, J.E., Clear, R.M. and Preston, K.R. 1996. Fusarium Head Blight: Effect on the Milling and Baking of Some Canadian Wheats. *Cereal Chemistry* **73(6)**: 695-701.

Jeffers, H.C. and Rubenthaler, G.L. 1977. Effect of roll temperature on flour yield with Brabender quadrumat experimental mills. *Cereal Chemistry* **54**: 1018-1025.

Koga, S., Bocker, U., Wieser, H., Koehler, P., Uhlen, A.K., Moldestad, A. 2017. Polymerisation of gluten proteins in developing wheat grain as affected by desiccation. *Journal of Cereal Science* **73**: 122-129.

Luo, C., Branlard, G., Griffin, W. B. and McNeil, D. L. 2000. The effect of nitrogen and sulphur fertilisation and their interaction with genotype on wheat glutenins and quality parameters. *Journal of Cereal Science* **31**: 185-194.

Manthey, F.A., Charkraborty, M., Peel, M.D. and Pederson, J.D. 2004. Effect of pre-harvest applied herbicides on breadmaking quality of hard red spring wheat. *Journal of the Science of Food and Agriculture* **84**: 441-446.

Marshall, D.; Mares, D.; Moss, H. and Ellison, F. 1986. Effects of grain shape and size on milling yields in wheat. II Experimental studies. *Australian Journal of Agricultural Research* **35**: 619-630.

McMullen, M., Bergstrom, G, De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G and Van Sanford, D. 2012. A unified effort to fight an enemy of wheat and barley: Fusarium head blight. *Plant Disease* **96**: 1712-1728.

Peterson, C.J., Graybosch, R.A., Shelton, D.R. and Baenziger, P.S. 1998. Baking quality of hard winter wheat: Response of cultivars to environment in the Great Plains. *Euphytica* **100**: 157-162.

Salgado, J., Madden, L. and Paul, P. 2014. Quantifying the Effects of Fusarium Head Blight on Grain Yield and Test Weight in Soft Red Winter Wheat. *Phytopathology* **105**. 10.1094/PHYTO-08-14-0215-R.

Seed Manitoba. 2019. https://www.seedmb.ca/digital-edition/

Schuler, S.; Bacon, R.; Finney, P; and Gbur, E. 1995. Relationship of test weight and kernel properties to milling and baking quality in soft red winter wheat. *Crop Science* **35**: 949-953.

Shewry, P.R., Underwood, C., Wan, Y.F., Lovegrove, A., Bhandari, D., Toole, G., Mills, E.N.C., Denyer, K., Mitchell, R.A.C. 2009. Storage product synthesis and accumulation in developing grains of wheat. *Journal of Cereal Science* **50**: 106-112.

Stone, P. J. and Nicolas, M. E. 1996. Effect of timing of heat stress during grain filling on two wheat varieties differing in heat tolerance .2. Fractional protein accumulation. *Australian Journal of Plant Physiology* **23**: 739-749.

Terman, G.L., R.E. Ramig, A.F. Dreier, and R.A. Olson. 1969. Yield– protein relationships in wheat grain, as affected by nitrogen and water. *Agronomy Journal* **61**: 755–759.

Triboi, E., Abad, A., Michelena, A., Lloveras, J., Ollier, J.L., Daniel, C. 2000. Environmental effects on the quality of two wheat genotypes: 1. quantitative and qualitative variation of storage proteins. *European Journal of Agronomy* **13:** 47–64.

Wegulo, S.N. 2012. Factors Influencing Deoxynivalenol Accumulation in Small Grain Cereals. *Toxins* **4**: 1157-1180.

Yenish, J.P. and Young, F.L. 2000. Effect of Pre-harvest Glyphosate Application on Seed and Seedling Quality of Spring Wheat (Triticum aestivum). *Weed Technology* **14**: 212-217.

Zhang, H., Turner, N.C. and Poolea, M.L. 2012. Increasing the harvest index of wheat in the high rainfall zones of southern Australia. *Field Crops Research* **129**: 111-123.

3. EFFECTS OF GENOTYPE, WEATHER AND FHB FUNGICIDE/PRE-HARVEST GLYPHOSATE ON CWRS PROTEIN COMPOSITION AND DOUGH RHEOLOGICAL PROPERTIES

3.1 Abstract

Gluten strength is one of the key properties of breadmaking wheats and is the foundation for the long-standing reputation of the CWRS wheat brand. Differences in weather conditions over a growing season as well as variation in gluten strength among varieties in the class may be problematic for millers and bakers who desire consistency in wheat or flour shipments. Additionally, crop management, such as pesticide application, may also affect gluten strength, but the impact is largely unknown. The main objective of this study was to evaluate the effects of two widely used pesticides on wheat quality for breadmaking with a focus on gluten strength. Six commercial CWRS wheat genotypes were grown in replicated field trials in 2015, 2016 and 2017 at four locations across the prairies. Weather conditions were typically variable among site-years. Four pesticide treatments were applied to the field plots for each site-year including a controle (untreated), Prothioconazole/Tebuconazole fungicide applied at anthesis for Fusarium head blight (FHB) control, pre-harvest glyphosate applied at physiological maturity and applications of both fungicide and pre-harvest glyphosate. Gluten strength was evaluated on doughs using a mixograph and in relation to gluten protein composition. Results showed no significant pesticide treatment main effect or genotype*pesticide treatment interaction. There was a significant site-year*pesticide treatment interaction as a result of differing response to the treatments between site-years but it explained very little of the variance. The impact of pesticide treatment on gluten strength or constituent protein fractions of gluten such as gliadin and glutenin was very small compared to genotype and site-year differences. Genotype effects reflected the known gluten strength characteristics of the genotypes. An analysis of site-year

variation of dough and protein composition parameters revealed that dough mixing parameters such as MDT, WIP and WAP were highly variable between site-yars. Variation in dough mixing properties for parameters most closely aligned with gluten strength has commercial implications as gluten strength is not a grading factor. The main weather parameter of importance was the association of reduced gluten strength-related mixograph and protein composition parameters at site-years with higher growing season precipitation. The relatively higher levels of FDK associated with higher precipitation may be a causal factor in this result.

3.2 Introduction

Wheat is one of the most important crops grown across the world. Its production has been a key component of agriculture for thousands of years, and its supply and utilization continue to grow each year (Blandino and Reyneri, 2009). One of the most common products created from wheat is bread. The gluten proteins in wheat are essential for breadmaking performance and confer the basis of gluten strength which is a critical property that determines dough mixing requirements, allows doughs to rise during fermentation and is positively related to loaf volume. Gluten strength of bread wheat can vary substantially between years and locations, and this affects the consistency of the wheat quality that bakers and millers require to produce similar products from year to year. It is important to stakeholders that CWRS wheat has consistent gluten strength from shipment to shipment to support the overall value of the brand and the longstanding reputation of western Canadian wheat producers as reliable suppliers of bread wheat of consistently high quality.

The uniqueness of wheat for breadmaking derives from a balance of two protein-related rheological properties, viscosity and elasticity (Bushuk, 1985). Viscosity is associated with the monomeric proteins of wheat endosperm, mainly gliadins, which are single-chain polypeptides. The elasticity of dough derives from the glutenin component, which comprises polydisperse polymers of disulfide-bonded polypeptides. Glutenin is one of nature's largest proteins (Wrigley, 1996). Numerous protein solubility schemes have been reported to fractionate gliadin and glutenin which are fundamentally different proteins in terms of structure and function (Sapirstein and Fu, 1998). Strong evidence exists in the literature that the amount of unextractable polymeric protein following direct extraction of flour with 50% 1-propanol is closely related to dough or gluten strength (Fu and Sapirstein, 1996; Sapirstein and Johnson, 2000; Isaak et al., 2019). The insolubility of glutenin using non-reducing solvents such as diluted propanol derives from glutenin's very large molecular size. The propanol-soluble fraction comprises all the gliadin proteins. A small but significant amount of glutenin is also extracted along with the gliadins (Fu and Sapirstein, 1996) using 50% 1-propanol. Soluble glutenin is presumed to comprise polymers of smaller size than those of insoluble glutenin and have reduced functionality in keeping with the hypothesis that only glutenin proteins above a certain molecular size contribute to dough strength (Southan and MacRitchie, 1999). Accordingly, separating propanol-soluble gliadins plus soluble glutenins from insoluble glutenin, as was done in the present study, should result in an effective sorting of wheat samples according to gluten strength, as has been reported (Isaak et al., 2019).

Gluten strength can vary due to genotype and environmental factors and their interactions. At the genetic level, there are nine major complex loci that contain the many structural genes that code for the gliadin proteins and HMW and LMW subunits of glutenin. The totality of these genes and expressed polypeptides are responsible for the functional properties of wheat. When these genes are altered, whether through breeding or their expression is affected by environmental conditions during crop development, there are resulting impacts on protein composition (Southan and MacRitchie, 1999). For example, increased temperatures causing heat stress result in a decrease in the ratio of glutenin to gliadin and, in turn, gluten strength (Peterson et al., 1998, Zhu and Khan, 2001). However, it has also been found that the effect of genotype was much more significant than that of environment and genotype*environment interaction for the majority of baking quality parameters that were tested (Ames et al., 1999). The relative importance of genotype vs. environment outcomes in any study largely depends on the genetic diversity of the wheat varieties used and the degree to which growing conditions vary. Accordingly, the relative effects of genotype and environment can vary widely in different studies.

Weather and genotype exert both direct and indirect effects on wheat quality. An important indirect effect is their combined impact on the level of Fusarium head blight (FHB) infection at a given location. FHB has been a disease of global concern in susceptible small grain cereals since the end of the 19th century around the world (Champeil et al., 2004). In Western Canada, Fusarium *graminearum* is one of the primary fungal species that causes FHB infection in wheat (Dexter et al., 1996). It produces a mycotoxin called deoxynivalenol (DON), which is considered

an element of alimentary risk for cereal products (Champeil et al., 2004). Some symptoms of FHB infection include premature bleaching of spikes, and shriveled or chalky kernels within spikes (Wegulo, 2012). Because FHB can degrade starch granules, storage proteins and cell walls (Dexter et al., 1996) milling and baking quality of the grain can be reduced, resulting in financial losses for producers, millers and bakers (Dexter et al., 1996). In one of the earliest studies on the effects of FHB on wheat protein composition and quality (Dexter et al., 1996) glutenin concentration decreased by about 28% on average for four varieties of HRS wheat in handpicked samples of fusarium damaged kernels (FDK) compared to cleaned (CL) samples free of visible damage. The contrast in gluten strength between the two types of samples (FDK vs. CL) was reflected in reduced farinograph development time and stability (7 to 1.25 min), and reduced mixograph developmemt time (18 to 11 min). Bread prepared from a 20:80 blend of flour from DK wheat with flour from CL wheat was reduced in volume by about 25%. These outcomes have been clearly attributed to proteolytic enzymes of Fusarium that prefentially hydrolyze polymeric glutenin when the affected wheat flour or semolina is processed during bread- or pasta-making (Dexter et al., 1996, 1997, Nightingale et al., 1999, Wang et al., 2005, Eggert et al., 2011).

Producers strive to maximize both the yield and quality of CWRS wheat during its production because both will contribute to higher revenues from the sale of the grain. Pesticides are commonly used to improve wheat quality, such as fungicide application at anthesis to reduce FHB infection as well as pre-harvest glyphosate for weed control. Triazole fungicide application at anthesis has been shown to reduce both FHB and DON levels in wheat and aid in obtaining

higher quality grain with higher yields (Blandino and Reyneri, 2009). Pre-harvest glyphosate application provides increased weed control and uniform dry-down which reduces dockage and foreign material in grain deliveries (Manthey et al., 2004). Fungicide application must be done at anthesis to obtain the maximum efficiency because the anthers are the main site of primary infection (Wegulo, 2012). There have been very few studies on the effects of pre-harvest application of glyphosate on wheat quality. Two independent studies involving a few varieties of U.S. HRS wheat in North Dakota growing sites reported an increase in gluten strength as reflected by several related parameters. Manthey et al. (2004) saw significant increases in gluten index, farinograph stability and full formula mixing time when glyphosate was applied at both the soft dough (SD) and hard dough (HD) stages. Similar results were reported by Malalgoda et al. (2020a) who found significantly positive effects of glyphosate application at the SD stage for gluten index, farinograph stability, full formula mix time, and lower values for farinograph mixing tolerance which is a parameter inversely related to gluten strength. In another report on the effects of pre-harvest glyphosate application on protein components of wheat endosperm, there were significant and large decreases, relative to untreated control, in the molecular size of SDS soluble and insoluble fractions isolated from flour of wheat treated at both the SD and HD stages (Malalgoda et al., 2020b). This result was at odds with those in Malalgoda et al. (2020a) as reduction in molecular size of the HMW fraction of SDS insoluble protein, which should correspond to HMW glutenin, would be expected to result in lowering of gluten strength by the methods used. The authors did not explain the apparent discrepancy between these results.

Given the prevalence of FHB in western Canada, there is widespread use of fungicides for its suppression. The literature is clear that FHB can adversely affect wheat quality and gluten strength. It is plausible that when farmers use fungicides to mitigate FHB to improve yield and grade, that the reported negative effects on gluten strength caused by FHB would be reduced as well. While there is some literature on the effects of pre-harvest application of glyphosate on wheat quality and gluten strength, the scope of published research is very limited. Accordingly, the objective of this study was to develop a better understanding of the degree to which these pesticides, when used for their intended purposes, affect wheat quality, gluten strength and protein composition, and whether genotype and/or growing conditions are interacting factors.

3.3 Methods

3.3.1 Field Study

During the growing seasons of 2015, 2016 and 2017 there were six CWRS wheat genotypes grown in four locations across Western Canada (Figure 2.1). The six genotypes, listed from strongest to weakest in terms of gluten strength were Glenn (GL), Carberry (CR), Cardale (CD), CDC Stanley (SN), Stettler (ST) and Harvest (HA). These varieties represent a wide range of intrinsic genotypic variation in gluten strength for CWRS wheat. It should be noted that the Harvest genotype was delisted from the CWRS class by the Canadian Grain Commission and designated to the Canada Northern Hard Red class effective August 1, 2018, after this study had been completed (https://www.grainscanada.gc.ca/en/grain-quality/variety-lists/2017/2017-45.html). The reclassification of Harvest was due to its relatively weak gluten strength

compared to other CWRS varieties. The Carberry 2015 and Kelburn 2016 locations were not harvestable, leaving ten site-years in total for this study. These locations provided a representative sample of growing season weather conditions typical for the Canadian prairies. Each genotype at each location received four pesticide treatments: Prothioconazole/Tebuconazole fungicide applied at anthesis (F), glyphosate applied pre-harvest (G), a treatment with both F and G (FG) and a control with no pesticide application (C). The field design at each design at each study site was a RCBD with split plots for pesticide treatments, and four replicates for each genotype and treatment. The main plot was the pesticide treatment with the genotypes as the sub-plots. Further details of the field study are described in Section 2.3.1.

3.3.2 Meteorological Analysis

Weather data were collected from weather stations located within close proximity to each field site. The data included daily maximum, minimum, and average temperatures, as well as precipitation. The weather parameters were aggregated into phenological time periods at each site-year to summarize the temperature and precipitation for the periods from seeding to anthesis, from anthesis to maturity, maturity to harvest, and from seeding to harvest.

3.3.3 Gluten Strength Analysis

Two of the four field replicates were randomly selected from each genotype and location for milling as described in Section 2.3.4. Mixograph analysis was peformed in triplicate on each flour sample using a Dynamic Machines Co. (Winnipeg, MB) torque sensing 10 g computerized

mixograph (Figure 3.1) with a water-jacketed mixing bowl. Torque readings were facilitated by a strain gauge attached to the base of the mixing bowl. The mixograph's strain gauge was calibrated for torque measurement using a 200 g weight to establish a precise reading of 50% torque. The linearity of response was confirmed by adding another 200 g weight which yielded a 100% torque reading. Flour samples and the mixing bowl were equilibrated to 30°C prior to mixing which was likewise carried out at 30°C. Flour was mixed with 6.0 mL of a 2.5% salt water solution (30°C) to achieve constant absorption of 60% and salt concentration of 1.5% (flour basis). Dough mixing was carried out at 30°C. The suitability of these mixing conditions for discrimination of gluten strength was established by Isaak et al. (2019).

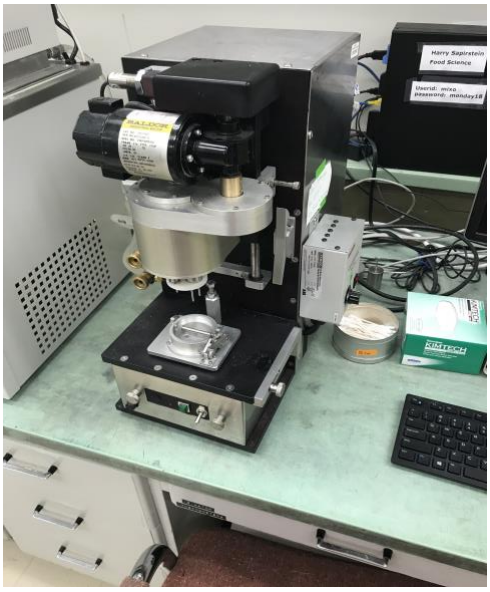


Figure 3.1 Dynamic Machines Co. torque sensing 10 g mixograph.

Figure 3.2 Water bath set up for mixograph analysis (water bath outlined in red box).

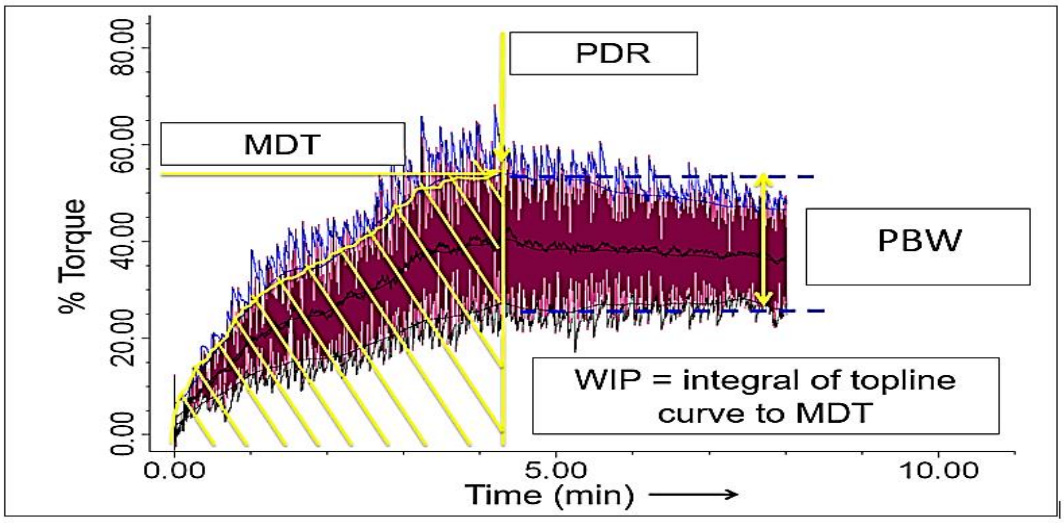


Figure 3.3 Sample mixograph curve, indicating parameters analyzed (PDR=peak dough resistance, PBW=peak band width, MDT=mixograph development time, and WIP=work input to peak).

Mixing speed was 92 rpm which was controlled using a laser tachometer aimed at the rotating spindle of the mixing head to which a small strip of reflective tape was attatched. The software used to analyze the mixograph curves was RAR-P2M UTe® software (RAR Software Systems, Winnipeg). The software generated mixing curves and computed a range of rheological

parameters (Figure 3.3). The parameters used in this study were mixograph development time (MDT), peak dough resistance (PDR, %Torque), peak band width (PBW, %Torque), work input to peak (WIP, %Torque*min) and work at peak (WAP, which was calculated as MDT*PBW in units of %torque*min).

3.3.4 Protein Composition Analysis

The protein composition was analyzed using the method of Sapirstein and Johnson (2000) as adapted by Isaak et el. (2019). This procedure used a sequential fraction scheme with 50% 1-propanol (v/v) without and with the dislulphide bond reducing agent 0.1% dithiothreitol (DTT), to extract soluble protein and "insoluble" glutenin (IG), respectively. The soluble protein (SP) fraction contained total gliadins and small proportion of glutenin which was presumed to be of relatively low molecular weight. The IG fraction that was solubilized with the addition of DTT contained HMW polymeric glutenin. The composition of these fractions has been previously characterized (Fu and Sapirstein 1996, Fu et al., 1996, Sapirstein and Fu, 1998). 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, 5000×g) and the resulting supernatant was decanted. This extraction was then repeated with the pellet disrupted using a glass rod to increase extraction efficiency. The tube was centrifuged for 3 min and 15,000×g. The pooled supernatants constituted the SP fraction. The insoluble residue containing IG or HMW glutenin was extracted with 1 mL of 0.1% (w/v) DDT (Millipore-Sigma, USA, Cat 233155) in 50% 1-propanol for 30 min at 55 ^{o}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 immediately before centrifugation at 15,000 × g

for 3 min at 22 ^{o}C. The microcentrifuge tube was inverted once to ensure extract homogeneity. Quantification of SP and IG was done by UV absorbance at 214 nm using a 10 mm path length semi-micro quartz cuvette (Hellma Analytics, Markham, ON) and a matching cuvette for the reference blank composed of 50% 1-propanol. UV absorbance for both the SP and IG fractions was converted to protein concentration (% of flour basis) using a calibration curve as described by Isaak et al. (2019). Results for five protein composition parameters were generated: SP (% of flour), IG (% of flour), SP/flour protein content (FP), IG/FP and IG/SP which was termed the gluten strength index (GSI).

3.3.5 Statistical Analysis

Statistical analysis ANOVA was performed using SAS Software, version 9.4. Global analysis of variance combining all site-years was performed using the SAS MIXED procedure for all of the dough mixing and protein parameters analyzed. The MIXED procedure was also used for individual site-year analysis to produce a Type III ANOVA. Details are described in Section 2.3.5 and sample SAS code is presented in Appendix VIII.

3.4 Results

Growing season weather conditions during the study were previously discussed in Section 2.4.1. with descriptions in Tables 2.3 and 2.4. The presentation of the results for this chapter will refer to this section in Chapter 2.

3.4.1 Rheological Properties

Effects of site-year and genotype were significant for all rheological properties (as well being the largest sources of variance in all cases (Tables 3.1 and 3.2). There was a distinction among the mixograph properties in the total amount of variance contributed by genotype and the growing environment (i.e., site-year) depending on the underlying influence of protein content or protein quality. PDR and PBW are highly related to protein content (Isaak et al., 2019) and would therefore be relatively sensitive to growing conditions. Genotype contributed approximately 12.02% and 20.16% to total variance for PDR and PBW, respectively (Table 3.1) compared with site-year contributions of 68.72% and 38.56%, respectively. In contrast, WIP and WAP which are more closely linked with genotype and more accurately measure gluten strength, the genotype contribution to total variance was considerably greater at approximately 45.88% and 44.90%, respectively, and more than the variance from site-year (Table 3.2). Compared to PDR and PBW, mixograph MDT was more influenced by genotype than by site-year (contributions to total variance of 36.78% and 46.22%, respectively Table 3.2), but the values indicate that MDT was more influenced by growing conditions compared to WIP and WAP. Both PDR (Figure V.25) and PBW (Figure V.28) had a significant negative correlation to mean temperature from seeding to maturity, as well as PBW and mean temperature from anthesis to maturity (Figure V.30). However, the physical basis for these relationships is not known. It is very important to note that the main effect of pesticide treatment was not significant for any of the five rheological properties measured.

The site-year*genotype interaction was statistically significant for all five mixograph properties, but the contribution to total variance was very similar to that for the residual factor, so the effect was practically inconsequential. The significance likely is related to the site-year*genotype interaction on grain protein concentration (Table 2.5) and flour protein (Table 2.13) as a result of the impact of growing season rainfall. The site-year*pesticide treatment interaction was also significant for all five mixograph properties, but contributed less to total variance than the residual. This was likely related to the variation in FHB control between site-years (Table 2.7, Figure 2.5) and the impact on both grain protein concentration and flour protein. The relative rankings of the pesticide treatments changed between different site-years and caused crossover interactions because the pesticide response was not consistent across site-years.

Table 3.1 Global analysis of variance for peak dough resistance (PDR) and peak band width (PBW) for all site-years.

			PDR			PBW	
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F
Genotype	5	432.8405	12.02	<.0001 ***	127.9360	20.16	<.0001 ***
Pesticide Treatment	3	9.8080	0.16	0.3623	5.5294	0.52	0.2701
Site-year	9	1375.1998	68.72	<.0001 ***	135.9216	38.56	<.0001 ***
Block (Site-year)	30	11.1020	1.85	0.0011 **	2.9627	2.80	0.1221
Genotype*Pesticide	15	5.2528	0.44	0.2177	1.0231	0.48	0.9199
Site-year*Genotype	45	25.4573	6.36	<.0001 ***	7.9248	11.24	<.0001 ***
Site-year*Pesticide	27	8.8494	1.33	0.0019 **	4.0126	3.41	0.0032 **
Site-year*Geno*Pest	135	4.0605	3.04	0.9434	1.9230	8.18	0.8099
Residual	210	5.2229	6.09	-	2.2112	14.64	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at *P* < 0.05, 0.01, and 0.001, respectively

Table 3.2 Global analysis of variance for mixing development time (MDT), work input to peak (WIP) and work at peak (WAP) for all site-years.

		MDT			WIP			WAP		
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F	MS	PV	Pr>F
Genotype	5	17.1866	36.78	<.0001 ***	46613.0000	45.88	<.0001 ***	16466.0000	44.90	<.0001 ***
Pesticide Treatment	3	0.1443	0.19	0.3111	214.7617	0.13	0.4324	97.1134	0.16	0.2157
Site-year	9	11.9987	46.22	<.0001 ***	22425.0000	39.73	<.0001 ***	8326.5243	40.87	<.0001 ***
Block (Site-year)	30	0.1031	1.32	0.0220 *	131.9467	0.78	0.5280	42.7531	0.70	0.6301
Genotype*Pesticide	15	0.0857	0.55	0.0527	153.3166	0.45	0.0863	57.6825	0.47	0.0948
Site-year*Genotype	45	0.2678	5.16	<.0001 ***	404.5982	3.58	<.0001 ***	155.1081	3.81	<.0001 ***
Site-year*Pesticide	27	0.1156	1.34	0.0008 ***	227.4343	1.21	0.0007 ***	61.2717	0.90	0.0325 *
Site-year*Geno*Pest	135	0.0495	2.86	0.9246	96.1657	2.56	0.9867	47.8529	2.72	0.9477
Residual	210	0.0622	5.59	-	137.1657	5.67	-	47.8529	5.48	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.1.1. Peak Dough Resistance. Individual site-year analysis showed that genotype had a significant effect on peak dough resistance for nine of ten site-years with the exception of Kelburn 2017 (Table 3.3). The Stettler, Glenn, and Cardale genotypes had the highest PDR values and Harvest, Carberry and Stanley had the lowest (Table 3.3, Figure II.18). Neither pesticide treatment nor its interaction with genotype were statistically significant at any of the site-years (Table 3.3).

Table 3.3 Mean comparisons and ANOVA for peak dough resistance (%Torque) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	55.40 B[f]	61.44 AB	50.70 AB	53.25 BC	60.22 C	47.83 B	51.93 B	43.16	57.50 AB	48.59 BC	53.04 C
Carberry	51.82 C	56.05 D	46.52 B	55.09 AB	58.85 CD	47.40 BC	48.99 B	42.34	53.18 C	46.95 C	50.70 D
Glenn	54.38 B	59.76 BC	53.83 A	58.14 A	65.78 A	53.74 A	49.05 B	46.20	57.35 AB	51.20 AB	54.94 B
Harvest	53.99 B	57.62 CD	44.72 B	52.51 BC	63.03 B	47.62 B	50.76 B	43.82	56.89 B	45.93 C	51.68 D
CDC Stanley	50.34 C	59.43 BC	49.53 AB	50.60 C	57.23 D	43.03 C	49.77 B	42.39	57.46 AB	46.14 C	50.60 D
Stettler	58.65 A	62.54 A	50.81 AB	56.40 AB	66.73 A	53.42 A	56.56 A	46.08	60.00 A	52.95 A	56.44 A
Pesticide Treatment											Mean
C[g]	54.50 AB	59.99	48.88	53.06	61.27	48.76	51.44	44.36	56.99	49.36	52.89
G[h]	55.16 A	58.57	49.08	53.93	62.72	48.49	51.35	45.05	56.59	50.00	53.07
FG[i]	53.45 B	59.79	50.44	56.06	61.97	49.50	51.59	43.22	57.64	47.30	53.12
F[j]	53.29 B	59.54	49.00	54.28	61.94	48.62	50.32	43.35	57.03	47.84	52.51
Site-year Mean	54.10	59.47	49.35	54.33	61.98	48.84	51.18	44.00	57.06	48.63	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	0.0135 *	0.0005 ***	<.0001 ***	0.0007 ***	0.0009 ***	0.1018	0.0084 **	0.0003 ***	
Pesticide Treatment	0.0408 *	0.5161	0.7125	0.2544	0.3845	0.2972	0.4731	0.3418	0.9855	0.0698	
Geno*Pest	0.9085	0.2876	0.8407	0.5503	0.2523	0.9452	0.5409	0.8962	0.8503	0.4135	
Block	0.7043	0.5176	0.4557	0.0067	0.3977	0.2743	0.9136	0.9664	0.7218	0.0769	
Block*Pest	0.5112	0.0434	0.6579	0.4328	0.2112	0.9884	0.6299	0.4147	0.2390	0.5161	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.1.2. Peak Band Width. Genotype had a significant effect on peak band width for seven of ten site-years with the exception of Kelburn 2015, Kelburn 2017 and Carberry 2016 (Table 3.4). Again, the Stettler and Glenn genotypes had the highest PBW values and Harvest, Carberry and Stanley had the lowest (Table 3.4, Figure II.20). Neither pesticide treatment nor its interaction with genotype were statistically significant at any of the site-years (Table 3.4).

Table 3.4 Mean comparisons and ANOVA for peak band width (%Torque) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	28.41 AB[f]	30.89 A	29.56 A	26.20 BC	29.65 BC	25.33 B	26.56	23.66	28.91	27.17 B	27.63 B
Carberry	26.53 BC	27.66 BC	28.60 AB	27.75 ABC	29.70 BC	25.70 B	25.89	23.69	27.46	26.51 B	26.95 BC
Glenn	28.12 AB	29.85 AB	30.45 A	29.78 A	33.61 A	29.15 A	26.04	26.62	29.38	27.77 B	29.03 A
Harvest	26.43 BC	27.36 C	27.60 AB	26.00 BC	31.38 B	26.32 B	26.54	24.85	28.11	26.11 B	27.06 BC
CDC Stanley	25.13 C	28.87 ABC	26.25 B	25.36 C	28.79 C	23.02 C	26.58	24.03	29.05	26.54 B	26.38 C
Stettler	29.40 A	30.60 A	28.83 AB	28.65 AB	34.15 A	29.24 A	29.03	26.51	30.80	29.53 A	29.69 A
Pesticide Treatment											Mean
C[g]	27.60	29.00	28.74	27.00	30.78	26.22	27.05	24.93	29.22	27.66	27.77
G[h]	28.30	29.04	28.06	26.58	31.96	27.01	26.86	25.42	28.91	28.05	28.05
FG[i]	26.72	29.89	28.10	28.86	31.23	26.25	26.89	24.60	29.11	26.36	27.82
F[j]	26.73	28.90	29.30	26.71	30.89	26.36	26.30	24.62	28.57	27.02	27.53
Site-year Mean	27.34	29.21	28.55	27.29	31.21	26.46	26.78	24.89	28.95	27.27	
Type III Analysis of Variance											
Genotype	0.0002 ***[k]	0.0114 **	0.0106 *	0.0016 **	<.0001 ***	0.0002 ***	0.2099	0.0605	0.0606	0.0010 ***	
Pesticide	0.4354	0.1748	0.5040	0.0537	0.2471	0.1293	0.4265	0.4623	0.6068	0.0422	
Geno*Pest	0.7348	0.6362	0.1185	0.8791	0.5975	0.9276	0.7873	0.9185	0.5413	0.4929	
Block	0.2991	0.4822	0.5581	0.0163 *	0.2940	0.7059	0.5139	0.4630	0.7777	0.4565	
Block*Pest	0.3067	0.9160	0.0350 *	0.7672	0.4248	0.9975	0.5421	0.6290	0.7111	0.5951	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.1.3. Mixing Development Time. Genotype had a significant effect on mixing development time at all ten site-years (Table 3.5). Glenn, the strongest genotype in the set (Courcelles, 2019) had the highest values for mixing development time (Table 3.5, Figure II.12). Neither pesticide treatment nor its interaction with genotype were statistically significant at any of the site-years (Table 3.5).

Table 3.5 Mean comparisons and ANOVA for mixing development time (min) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	2.40 D[f]	1.98 C	3.55 CD	2.77 B	2.46 B	2.94 BC	3.34 BC	2.39 BC	2.42 C	3.12 B	2.73 C
Carberry	3.07 B	2.27 B	4.01 B	2.70 B	2.46 B	3.20 AB	3.50 B	2.57 BC	2.76 B	3.24 B	2.99 B
Glenn	3.83 A	2.65 A	4.63 A	3.91 A	3.12 A	3.40 A	4.42 A	3.58 A	3.19 A	4.35 A	3.70 A
Harvest	2.20 D	1.48 E	3.44 CD	2.69 B	1.88 D	2.34 D	2.83 CD	2.23 C	2.02 D	2.90 B	2.40 D
CDC Stanley	2.63 C	1.72 D	3.73 BC	3.00 B	2.16 C	3.29 A	2.86 CD	2.68 B	2.20 CD	2.93 B	2.72 C
Stettler	2.22 D	1.69 D	3.27 D	2.64 B	2.04 CD	2.68 C	2.48 D	2.34 BC	2.07 D	2.83 B	2.42 D
Pesticide Treatment											Mean
C[g]	2.73	2.00	3.71	2.95	2.39	3.09	3.37	2.62	2.47	3.13	2.84
G[h]	2.65	1.99	3.80	3.13	2.34	2.91	3.10	2.50	2.47	3.32	2.82
FG[i]	2.80	1.91	3.78	2.71	2.32	2.98	3.03	2.66	2.40	3.30	2.79
F[j]	2.72	1.96	3.80	3.01	2.37	2.93	3.45	2.74	2.42	3.17	2.86
Site-year Mean	2.73	1.97	3.77	2.95	2.36	2.98	3.24	2.63	2.44	3.23	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	
Pesticide	0.1220	0.8030	0.9076	0.1066	0.5999	0.6155	0.4279	0.1986	0.7936	0.3341	
Geno*Pest	0.4002	0.8952	0.2714	0.8292	0.5701	0.0709	0.1439	0.5827	0.8842	0.1715	
Block	0.0519	0.1067	0.2530	0.1193	0.4726	0.5946	0.8328	0.4569	0.9072	0.4603	
Block*Pest	0.9405	0.6355	0.0127 *	0.2648	0.6624	0.0386	0.0076 **	0.6759	0.6846	0.3595	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.1.4. Work Input to Peak. Genotype had a significant effect on work input to peak at all ten site-years (Table 3.6). Again, Glenn, the strongest genotype, had the highest values for WIP (Table 3.6, Figure II.14). Neither pesticide treatment nor its interaction with genotype were statistically significant at any of the site-years (Table 3.6).

Table 3.6 Mean comparisons and ANOVA for work input to peak (%Torque*min) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	107.75 CD[f]	97.74 BC	163.66 BC	125.94 B	118.35 B	117.25 B	150.22 B	84.51 C	113.81 BC	128.10 B	120.10 C
Carberry	133.25 B	106.82 B	173.68 B	126.62 B	117.94 B	126.59 B	149.57 B	91.63 BC	122.83 B	132.33 B	128.37 B
Glenn	176.90 A	134.96 A	229.39 A	193.85 A	165.76 A	152.01 A	195.58 A	143.70 A	156.55 A	191.35 A	173.36 A
Harvest	97.72 D	65.56 E	143.71 D	120.15 B	93.43 D	89.00 C	124.02 C	79.50 C	95.02 D	114.15 B	102.27 E
CDC Stanley	111.26 C	81.97 D	165.38 BC	132.48 B	101.07 CD	121.47 B	126.86 BC	98.02 B	106.26 CD	118.11 B	116.41 CD
Stettler	106.58 CD	84.68 CD	150.72 CD	125.44 B	107.41 BC	117.09 B	118.80 C	89.42 BC	101.26 CD	125.94 B	112.96 D
Pesticide Treatment											Mean
C[g]	123.15	97.72	170.13	134.95	117.36	125.42	151.86	99.05	116.45	132.21	126.51
G[h]	121.03	96.18	169.57	145.51	117.56	115.47	137.69	94.16	117.20	142.36	125.64
FG[i]	125.13	91.72	173.48	128.45	116.73	121.47	135.04	97.98	115.21	133.52	123.77
F[j]	118.99	95.53	171.17	140.75	117.64	119.91	152.12	100.00	114.96	131.90	126.40
Site-year Mean	122.08	95.29	171.09	137.42	117.32	120.58	144.18	97.69	115.96	135.00	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	
Pesticide	0.1560	0.7147	0.8981	0.0983	0.9978	0.3219	0.4023	0.2954	0.7415	0.3663	
Geno*Pest	0.2634	0.7540	0.2823	0.6569	0.3877	0.5627	0.1933	0.5162	0.8832	0.4365	
Block	0.0719	0.1104	0.3312	0.3877	0.5361	0.5698	0.8203	0.1457	0.8489	0.3455	
Block*Pest	0.9393	0.5336	0.0092 **	0.3355	0.2476	0.4123	0.0192 *	0.8895	0.9552	0.2498	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.1.5. Work at Peak. Genotype had a significant effect on work at peak at all ten site-years (Table 3.7). Glenn had the highest overall values for WAP (Table 3.7, Figure II.16) and also had WAP values that were significantly greater than for all other genotypes at all site-years. Neither pesticide treatment nor its interaction with genotype were statistically significant at any of the site-years (Table 3.7).

Table 3.7 Mean comparisons and ANOVA for work at peak (%Torque*min) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	68.00 C[f]	61.00 B	103.73 BC	71.83 B	72.79 B	74.37 B	88.49 BC	56.32 BC	69.91 B	84.96 B	74.94 C
Carberry	81.61 B	62.83 B	113.89 B	75.07 B	72.64 B	82.14 B	90.61 B	60.43 BC	75.59 B	86.45 B	80.34 B
Glenn	107.61 A	78.92 A	138.27 A	115.90 A	104.57 A	98.69 A	115.22 A	94.91 A	93.50 A	119.93 A	106.38 A
Harvest	58.13 D	40.48 D	93.69 C	69.47 B	59.59 C	61.22 C	75.92 BCD	55.12 C	56.69 D	75.82 B	64.57 E
CDC Stanley	66.16 C	49.74 C	97.77 C	76.28 B	61.49 C	75.79 B	75.87 CD	63.69 B	63.76 C	77.69 B	70.79 D
Stettler	65.11 CD	51.75 C	93.54 C	74.12 B	70.47 B	77.89 B	71.52 D	61.97 BC	63.73 C	83.64 B	71.44 D
Pesticide Treatment											Mean
C[g]	75.78	58.02	105.54	79.79	73.35	80.41	91.17	65.25	72.10	86.53	78.48
G[h]	74.86	57.76	107.21	83.08	74.69	78.42	82.50	63.71	71.30	93.41	78.57
FG[i]	74.67	57.14	104.32	78.15	72.73	77.65	81.34	65.69	69.77	86.54	76.84
F[j]	72.45	56.89	110.19	80.77	73.59	76.93	90.08	66.98	68.95	85.85	78.42
Site-year Mean	74.44	57.45	106.82	80.45	73.59	78.35	86.27	65.41	70.53	88.08	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	
Pesticide	0.4870	0.9191	0.5581	0.5262	0.7849	0.6328	0.5615	0.2696	0.2242	0.2665	
Geno*Pest	0.8862	0.3592	0.3528	0.8181	0.1038	0.1880	0.1607	0.5546	0.4104	0.2788	
Block	0.4984	0.1706	0.5147	0.3548	0.1489	0.7128	0.7535	0.1574	0.4552	0.4387	
Block*Pest	0.5487	0.3811	0.1568	0.4681	0.2164	0.1391	0.0097 **	0.8274	0.8133	0.1959	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.2 Protein Composition

Site-year and genotype had significant effects and were the largest sources of variance for insoluble glutenin (IG) and soluble prolamin (SP) (Table 3.8) as well as gluten strength index (GSI), IG/flour protein and SP/flour protein (Table 3.9). Unlike the mixograph parameters, genotype was the largest source of variance for all protein composition parameters except SP/flour protein. There was a significant, positive correlation between SP/flour protein and precipitation from anthesis to maturity (Figure IV.54), which was the only significant correlation amongst any of the protein composition parameters and the weather parameters. Genotype also had a significant effect on both grain protein concentration (Table 2.11) and flour protein (Table 2.16) but site-year had a larger contribution to variance in both cases. Thus, genotype has a stronger impact on protein composition than protein content. Similar to the rheological properties, pesticide treatment had no significant effects on any of the protein composition measures. The genotype*pesticide interaction was significant for the IG/FP parameter, however it had a minimal contribution to variance, much smaller than the residual value.

The site-year*genotype interaction was significant for all five protein composition parameters but contributed more to variance than the residual only for SP. Similar to the rheological properties, the significance likely is related to the site-year*genotype interaction on grain protein concentration (Table 2.5) and flour protein (Table 2.13) as a result of the impact of growing season rainfall. The site-year*pesticide treatment interaction was significant for all but the SP/flour protein parameter, but contributed less to total variance than the residual for the other four parameters. This interaction was likely related to the variation in FHB control

between site-years (Table 2.7, Figure 2.5). As previously discussed, the relative rankings of the pesticide treatment effect changed between site-years, leading to crossover interactions as a result of the variable response to the different pesticide applications between years and locations.

Table 3.8 Global analysis of variance for insoluble glutenin (IG) and soluble prolamin (SP) for all site-years.

			IG			SP	
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F
Genotype	5	6.5260	63.86	<.0001 ***	27.5407	41.02	<.0001 ***
Pesticide Treatment	3	0.0127	0.07	0.7786	0.2811	0.25	0.5909
Site-year	9	0.6532	11.51	<.0001 ***	9.5757	25.67	<.0001 ***
Block (Site-year)	30	0.0320	1.88	0.0553	0.4071	3.64	<.0001 ***
Genotype*Pesticide	15	0.0206	0.60	0.1785	0.1534	0.69	0.4336
Site-year*Genotype	45	0.0847	7.46	<.0001 ***	0.8190	10.98	<.0001 ***
Site-year*Pesticide	27	0.0349	1.85	0.0009 ***	0.4346	3.50	<.0001 ***
Site-year*Geno*Pest	135	0.0151	3.99	0.9856	0.1500	6.03	0.1965
Residual	210	0.0214	8.78	-	0.1316	8.23	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

Table 3.9 Global analysis of variance for gluten strength index (GSI), insoluble glutenin/flour protein (IG/FP) and soluble prolamin/flour protein (SP/FP) for all site-years

		GSI			IG/FP			SP/FP		
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F	MS	PV	Pr>F
Genotype	5	0.1278	70.34	<.0001 ***	0.0372	65.26	<.0001 ***	0.0219	3.71	<.0001 ***
Pesticide Treatment	3	0.0006	0.19	0.1514	0.0002	0.20	0.1263	0.0001	0.01	0.8717
Site-year	9	0.0161	15.99	<.0001 ***	0.0065	20.62	<.0001 ***	0.2995	91.31	<.0001 ***
Block (Site-year)	30	0.0003	0.84	0.2528	0.0001	0.89	0.2290	0.0003	0.32	0.1699
Genotype*Pesticide	15	0.0002	0.34	0.2647	0.0001	0.47	0.0196 *	0.0001	0.05	0.9944
Site-year*Genotype	45	0.0008	3.92	<.0001 ***	0.0003	4.40	<.0001 ***	0.0007	1.12	<.0001 ***
Site-year*Pesticide	27	0.0003	0.88	0.0189 *	0.0001	0.86	0.0044 **	0.0004	0.33	0.2505
Site-year*Geno*Pest	135	0.0002	2.50	0.9432	0.0000	2.10	0.9981	0.0003	1.77	0.0978
Residual	210	0.0002	5.00	-	0.0001	5.20	-	0.0002	1.77	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.2.1. Insoluble Glutenin. Genotype had a significant effect on insoluble glutenin at all ten site-years (Table 3.10). Glenn had the highest values for IG (Table 3.10, Figure II.2) among all other genotypes at all site-years, with IG values that were significantly greater than other genotypes except for IHARF 2016, Lethbridge 2015, Lethbridge 2017 and Kelburn 2015 where the genotype Carberry was equivalent (regarding the Tukey Kramer analysis) (Table 3.10). Pesticide treatment was significant at only IHARF 2017 and the genotype*pesticide treatment interaction was significant at only IHARF 2016 (Table 3.10). Regarding IG values for IHARF 2017, the IG value for the FG treatment was slightly smaller than those for the F and G treatments.

Table 3.10 Means comparisons and ANOVA for insoluble glutenin (%) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	3.63 C[f]	3.58 B	3.58 C	3.35 C	3.60 D	3.47 B	3.78 BC	3.45 C	3.66 BC	3.62 CD	3.58 C
Carberry	3.96 B	3.70 A	3.93 B	3.87 AB	4.03 B	4.08 A	4.08 AB	3.80 B	3.80 B	3.85 B	3.91 B
Glenn	4.15 A	3.81 A	4.25 A	4.17 A	4.55 A	4.06 A	4.24 A	4.10 A	4.12 A	4.20 A	4.17 A
Harvest	3.44 D	3.01 D	3.47 CD	3.45 C	3.68 CD	3.45 B	3.73 C	3.39 C	3.42 D	3.46 D	3.45 D
CDC Stanley	3.38 D	3.12 D	3.37 D	3.43 C	3.36 E	3.33 B	3.60 C	3.40 C	3.56 C	3.56 CD	3.41 D
Stettler	3.62 C	3.36 C	3.57 C	3.60 BC	3.85 BC	3.86 A	3.81 BC	3.54 BC	3.57 C	3.66 C	3.64 C
Pesticide Treatment											Mean
C[g]	3.69	3.44	3.70 AB	3.52	3.80	3.77	3.91	3.61	3.69	3.78	3.69
G[h]	3.76	3.44	3.76 A	3.62	3.83	3.70	3.81	3.62	3.64	3.78	3.70
FG[i]	3.67	3.44	3.62 B	3.72	3.85	3.64	3.86	3.65	3.71	3.61	3.68
F[j]	3.66	3.39	3.70 AB	3.72	3.89	3.72	3.88	3.58	3.71	3.73	3.70
Site-year Mean	3.69	3.43	3.70	3.65	3.84	3.71	3.87	3.62	3.69	3.73	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	0.0003 ***	<.0001 ***	<.0001 ***	<.0001 ***	
Pesticide	0.3370	0.7974	0.0151 *	0.1362	0.4838	0.2278	0.8976	0.9645	0.5552	0.0641	
Geno*Pest	0.6285	0.0102 *	0.8591	0.7315	0.8665	0.9852	0.3183	0.8523	0.2970	0.9119	
Block	0.2615	0.2710	0.2593	0.0530	0.1106	0.6945	0.9424	0.9996	0.4546	0.3388	
Block*Pest	0.3448	0.0091 **	0.9434	0.6501	0.4821	0.4611	0.1099	0.0101 *	0.1468	0.7535	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.2.2. Soluble Prolamin. Genotype had a significant effect on soluble prolamin at all ten site-years (Table 3.11). Stettler had the highest values for SP (Table 3.11, Figure II.6) while Glenn and Carberry had the lowest values. The effect of pesticide treatment was significant for only one site-year, IHARF 2015; however, the differences between the pesticide treatments were not substantial enough to be detected by means comparisons (Table 3.11).

Table 3.11 Means comparisons and ANOVA for soluble prolamin (%) by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	10.68 B[f]	11.25 B	9.93 B	9.65 B	10.60 C	10.29 C	10.62 BC	10.51 AB	10.86 BC	10.30 B	10.48 C
Carberry	9.57 D	10.32 C	9.51 B	10.35 AB	10.95 C	10.65 C	10.12 CD	10.31 B	10.41 CD	9.89 C	10.20 D
Glenn	9.61 D	10.23 C	9.62 B	10.02 B	11.05 C	10.63 C	9.73 D	10.08 B	10.31 D	9.85 C	10.11 D
Harvest	10.40 BC	11.36 B	9.74 B	9.82 B	11.66 B	11.58 B	10.46 BC	10.77 AB	11.18 B	10.53 B	10.75 B
CDC Stanley	10.20 C	11.14 B	9.82 B	10.05 B	11.09 C	10.40 C	10.69 B	10.70 AB	11.83 A	10.43 B	10.64 BC
Stettler	11.76 A	12.28 A	10.76 A	10.91 A	12.65 A	12.43 A	11.90 A	11.37 A	12.16 A	11.70 A	11.79 A
Pesticide Treatment											Mean
C[g]	10.53 A	11.19	9.82	9.89	11.13	11.21	10.61	10.75	10.98	10.73	10.69
G[h]	10.52 A	11.08	10.04	9.94	11.29	11.09	10.49	10.79	11.17	10.50	10.70
FG[i]	10.22 A	11.19	9.76	10.58	11.52	10.83	10.89	10.51	11.09	10.30	10.68
F[j]	10.21 A	10.93	9.97	10.12	11.40	10.86	10.35	10.44	11.26	10.27	10.59
Site-year Mean	10.37	11.10	9.90	10.13	11.33	11.00	10.59	10.62	11.13	10.45	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	0.0138 *	<.0001 ***	<.0001 ***	<.0001 ***	0.0177 *	<.0001 ***	<.0001 ***	
Pesticide	0.0392 *	0.0518	0.2895	0.0940	0.1797	0.3144	0.0995	0.6270	0.1209	0.1548	
Geno*Pest	0.6062	0.0933	0.0249 *	0.4851	0.2184	0.9185	0.2444	0.7002	0.3568	0.0159 *	
Block	0.0264	0.0135	0.3090	0.0065	0.4221	0.5798	0.6081	0.9242	0.0606	0.2239	
Block*Pest	0.9177	0.4921	0.0288	0.3700	0.1457	0.6329	0.2730	0.3966	0.5180	0.0043 **	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.2.3. Gluten Strength Index. Genotype had a significant effect on gluten strength index at all ten site-years (Table 3.12). Glenn had the highest values for GSI (Table 3.12, Figure II.10) at all site-years, with values that were greater than all other genotypes, except at IHARF 2016, Lethbridge 2017 and Kelburn 2015 where the genotype Carberry was equivalent (regarding the Tukey Kramer analysis) (Table 3.12). Neither pesticide treatment nor its interaction with genotype were statistically significant at any of the site-years (Table 3.12).

Table 3.12 Means comparisons and ANOVA for gluten strength index by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	0.34 C[f]	0.32 B	0.36 C	0.35 BC	0.34 C	0.34 B	0.36 B	0.33 C	0.34 C	0.35 C	0.34 C
Carberry	0.41 B	0.36 A	0.42 B	0.37 B	0.37 B	0.38 A	0.40 A	0.37 B	0.37 B	0.39 B	0.38 B
Glenn	0.43 A	0.37 A	0.44 A	0.42 A	0.41 A	0.38 A	0.44 A	0.41 A	0.40 A	0.43 A	0.41 A
Harvest	0.33 C	0.26 D	0.36 C	0.35 BC	0.32 CD	0.30 C	0.36 B	0.32 C	0.31 D	0.33 DE	0.32 D
CDC Stanley	0.33 C	0.28 C	0.34 CD	0.34 C	0.30 D	0.32 BC	0.34 BC	0.32 C	0.30 D	0.34 CD	0.32 D
Stettler	0.31 D	0.27 CD	0.33 D	0.33 C	0.30 D	0.31 C	0.32 C	0.31 C	0.29 D	0.32 E	0.31 E
Pesticide Treatment											Mean
C[g]	0.35	0.31	0.38	0.36	0.34	0.34	0.37	0.34	0.34	0.35	0.35
G[h]	0.36	0.31	0.38	0.36	0.34	0.34	0.37	0.34	0.33	0.36	0.35
FG[i]	0.36	0.31	0.37	0.35	0.34	0.34	0.36	0.35	0.34	0.35	0.35
F[j]	0.36	0.31	0.37	0.37	0.34	0.34	0.38	0.34	0.33	0.37	0.35
Site-year Mean	0.36	0.31	0.37	0.36	0.34	0.34	0.37	0.34	0.33	0.36	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	
Pesticide	0.0727	0.9503	0.6399	0.3788	0.4502	0.6237	0.5669	0.9072	0.1218	0.1323	
Geno*Pest	0.4398	0.1433	0.1888	0.6797	0.8455	0.9616	0.4212	0.8036	0.9502	0.8418	
Block	0.1457	0.5603	0.2818	0.7547	0.0239	0.8493	0.9110	0.6662	0.4252	0.0243	
Block*Pest	0.2262	0.1259	0.0589	0.5689	0.7960	0.8418	0.1298	0.9690	0.7686	0.4697	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.2.4. Insoluble Glutenin/Flour Protein. Genotype had a significant effect on insoluble glutenin/flour protein at all ten site-years (Table 3.13). Glenn had the highest values for IG/FP (Table 3.13, Figure II.4) amongst all other genotypes at all site-years, and the IG/FP values for Glenn were greater than for all other genotypes except at Lethbridge 2017 and Kelburn 2015 where the genotype Carberry was equivalent (regarding the Tukey Kramer analysis) (Table 3.13). Pesticide treatment was not significant at any site-year and its interaction with genotype was statistically significant at only IHARF 2016 (Table 3.13).

Table 3.13 Means comparisons and ANOVA for insoluble glutenin/flour protein by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	0.247 C[f]	0.230 C	0.258 C	0.248 C	0.253 C	0.246 B	0.251 B	0.238 C	0.245 C	0.253 C	0.247 C
Carberry	0.283 B	0.254 B	0.289 B	0.271 B	0.276 B	0.273 A	0.279 A	0.259 B	0.265 B	0.273 B	0.272 B
Glenn	0.293 A	0.262 A	0.310 A	0.291 A	0.297 A	0.268 A	0.295 A	0.282 A	0.285 A	0.296 A	0.288 A
Harvest	0.237 D	0.197 E	0.264 C	0.254 BC	0.237 D	0.228 C	0.251 B	0.232 C	0.229 D	0.243 CD	0.237 D
CDC Stanley	0.237 D	0.203 DE	0.253 C	0.248 C	0.231 D	0.237 BC	0.241 B	0.235 C	0.228 D	0.245 CD	0.236 D
Stettler	0.233 D	0.208 D	0.255 C	0.246 C	0.235 D	0.240 BC	0.240 B	0.234 C	0.228 D	0.240 D	0.236 D
Pesticide Treatment											Mean
C[g]	0.252	0.224	0.272	0.260	0.255	0.250	0.260	0.244	0.247	0.259	0.252
G[h]	0.256	0.226	0.274	0.261	0.254	0.247	0.257	0.243	0.245	0.259	0.252
FG[i]	0.257	0.227	0.268	0.255	0.253	0.247	0.255	0.253	0.247	0.254	0.252
F[j]	0.255	0.226	0.272	0.263	0.257	0.251	0.266	0.247	0.248	0.262	0.255
Site-year Mean	0.255	0.226	0.272	0.260	0.255	0.249	0.259	0.247	0.247	0.259	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	
Pesticide	0.3044	0.8899	0.4363	0.8536	0.8652	0.6779	0.5524	-[l]	0.7881	0.2758	
Geno*Pest	0.2241	0.0026 **	0.6622	0.7638	0.6607	0.9411	0.5932	0.8655	0.2883	0.9906	
Block	0.7121	0.6970	0.2010	0.3357	0.1057	0.8459	0.8718	0.2812	0.7614	0.6652	
Block*Pest	0.3734	0.0028 **	0.4611	0.4204	0.2917	0.5676	0.2082	0.9855	0.1378	0.6391	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [l]Value unavailable

3.4.2.5. S**oluble Prolamin/Flour Protein.** Genotype had a significant effect on insoluble glutenin/flour protein at nine of the ten site-years with the exception of Kelburn 2017 (Table 3.14). As would be expected, Stettler had the highest values for SP/FP (Table 3.14, Figure II.8) amongst all other genotypes at all site-years, but Harvest and/or CDC Stanley were equivalent at some site-years (regarding the Tukey Kramer analysis) (Table 3.14). Pesticide treatment was significant at only Carberry 2016 (Table 3.14). While the Tukey Kramer analysis shows no differences between the pesticide treatments, there was a slightly significant pesticide effect for Carberry 2016, likely because the F test can sometimes indicate significance when a means separation analysis does not detect any.

Table 3.14 Means comparisons and ANOVA for soluble prolamin/flour protein by genotype[a] and by pesticide treatment[b] for individual site-years.

	IHARF[c] 2015	IHARF 2016	IHARF 2017	Leth[d] 2015	Leth 2016	Leth 2017	Kelburn 2015	Kelburn 2017	Carb[e] 2016	Carb 2017	
Genotype											Mean
Cardale	0.725 B[f]	0.723 BC	0.565 C	0.713 BC	0.742 AB	0.567 B	0.706 BC	0.563	0.726 BC	0.565 B	0.660 C
Carberry	0.685 C	0.710 C	0.563 C	0.727 AB	0.749 AB	0.563 B	0.693 CD	0.555	0.726 BC	0.560 B	0.653 C
Glenn	0.678 C	0.703 C	0.574 BC	0.698 C	0.722 B	0.556 B	0.673 D	0.557	0.711 C	0.559 B	0.643 D
Harvest	0.716 B	0.743 AB	0.585 AB	0.722 BC	0.750 AB	0.593 A	0.706 BC	0.571	0.749 A	0.571 AB	0.670 B
CDC Stanley	0.716 B	0.725 BC	0.578 ABC	0.726 AB	0.762 A	0.575 AB	0.715 B	0.570	0.756 A	0.562 B	0.669 B
Stettler	0.758 A	0.762 A	0.594 A	0.751 A	0.771 A	0.593 A	0.756 A	0.577	0.777 A	0.590 A	0.692 A
Pesticide Treatment											Mean
C[g]	0.717	0.727	0.571	0.725	0.746	0.577	0.702	0.565	0.730 A	0.575	0.664
G[h]	0.713	0.726	0.581	0.721	0.746	0.575	0.705	0.563	0.748 A	0.565	0.664
FG[i]	0.711	0.731	0.571	0.728	0.755	0.571	0.718	0.572	0.734 A	0.567	0.666
F[j]	0.711	0.726	0.582	0.717	0.750	0.575	0.707	0.561	0.752 A	0.565	0.664
Site-year Mean	0.713	0.727	0.576	0.723	0.749	0.575	0.708	0.565	0.741	0.568	
Type III Analysis of Variance											
Genotype	<.0001 ***[k]	0.0003 ***	0.0062 **	0.0021 **	0.0033 **	0.0002 ***	<.0001 ***	0.0762	0.0056 **	0.0050 **	
Pesticide	0.2787	0.1084	0.2485	0.4757	0.7803	0.9270	0.3628	0.3202	0.0373 *	0.5371	
Geno*Pest	0.2254	0.3142	0.1756	0.5296	0.5551	0.4876	0.0866	0.7078	0.7479	0.3737	
Block	0.0538	0.0692	0.3484	0.5434	0.9444	0.0851	0.6844	0.1649	0.3047	0.3342	
Block*Pest	0.4929	0.9054	0.0939	0.2193	0.2969	0.2076	0.0668	0.8204	0.7692	0.1541	

[a]Mean of four replicates of four pesticide treatments, [b]mean of four replicates of six genotypes, [c]Indian Head Agricultural Research Foundation, [d]Lethbridge, [e]Carberry, [f]Values followed by the same upper case letter in columns by genotype and by pesticide treatment are not significantly different at p=0.05, [g]Control treatment, [h]Glyphosate treatment, [i]Fungicide plus Glyphosate treatment, [j]Fungicide treatment, [k]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

3.4.3 Mixograph-Protein Composition Comparison

There have been very few studies on relationships between breadmaking properties and protein composition of Canadian wheat varieties. The present research, like its companion study (Courcelles, 2019), provided an important opportunity to obtain new insights and advance knowledge on these relationships, especially considering the context of the research and the wheat samples used. In this study, two of the most popular pest management practices used in western Canadian wheat production were assessed for their impact on gluten strength determined by dough mixing and protein composition of gluten proteins.

In Figures 3.4 and 3.5, the peak dough resistance and peak band width parameters are shown in relation to the gluten strength index. Clearly, these mixograph parameters are not related to GSI (and, in turn are not related to gluten strength) but more likely due to the relatively large positive influence of flour protein content on mixograph PDR and PBW (Isaak et al., 2019). The range of GSI values are shifted lower in 2016 compared to the other two years. This appears to reflect the impact of generally higher precipitation in 2016 (Table 2.3) or could be an indirect effect of precipitation via higher Fusarium damaged kernels in 2016 compared to FDKs in both 2015 and 2017 (Figure 2.8).

The mixograph parameters of MDT, WIP and WAP showed a strong, positive relationship to GSI (Figures 3.6, 3.7 and 3.8) similar to the results of Courcelles (2019) and Isaak et al. (2019).

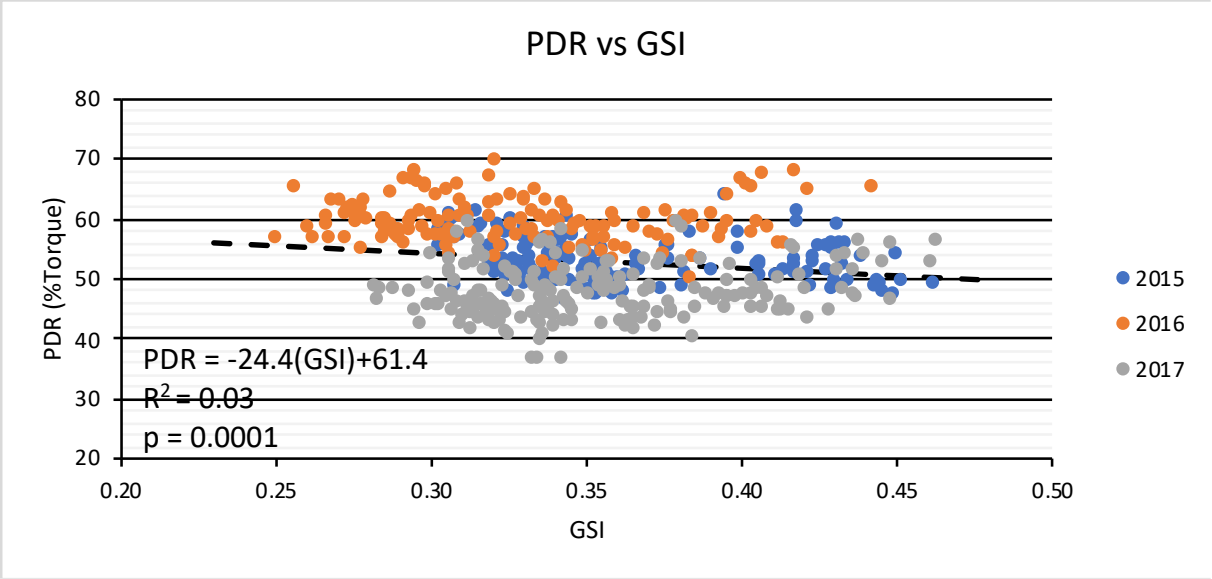


Figure 3.4 Linear regression between PDR (Peak Dough Resistance) and GSI (Gluten Strength Index).

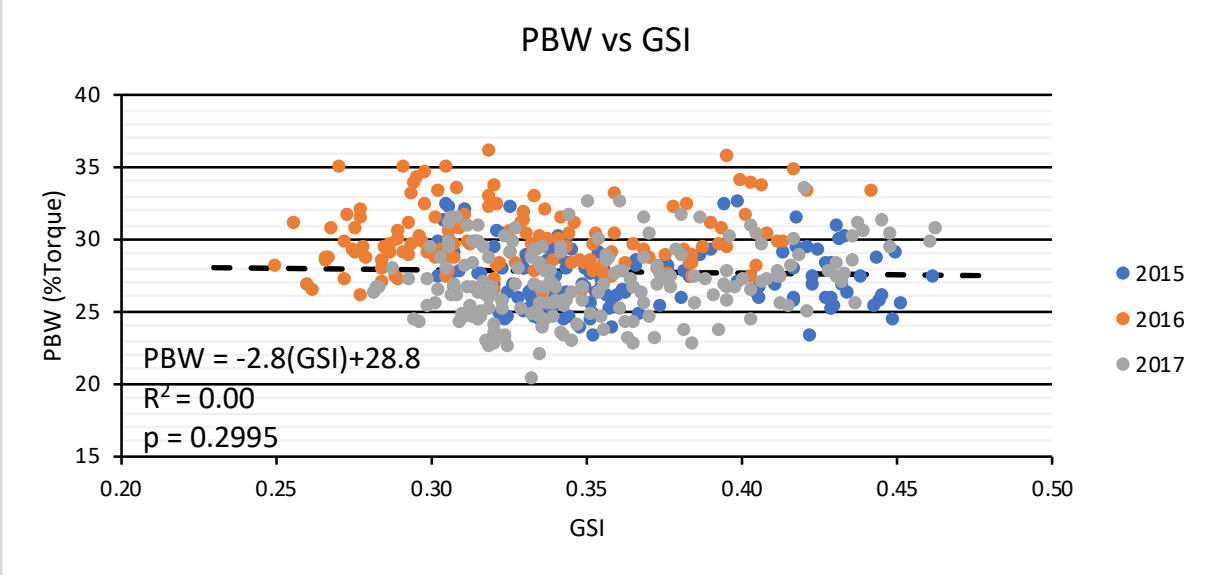


Figure 3.5 Linear regression between PBW (Peak Band Width) and GSI (Gluten Strength Index).

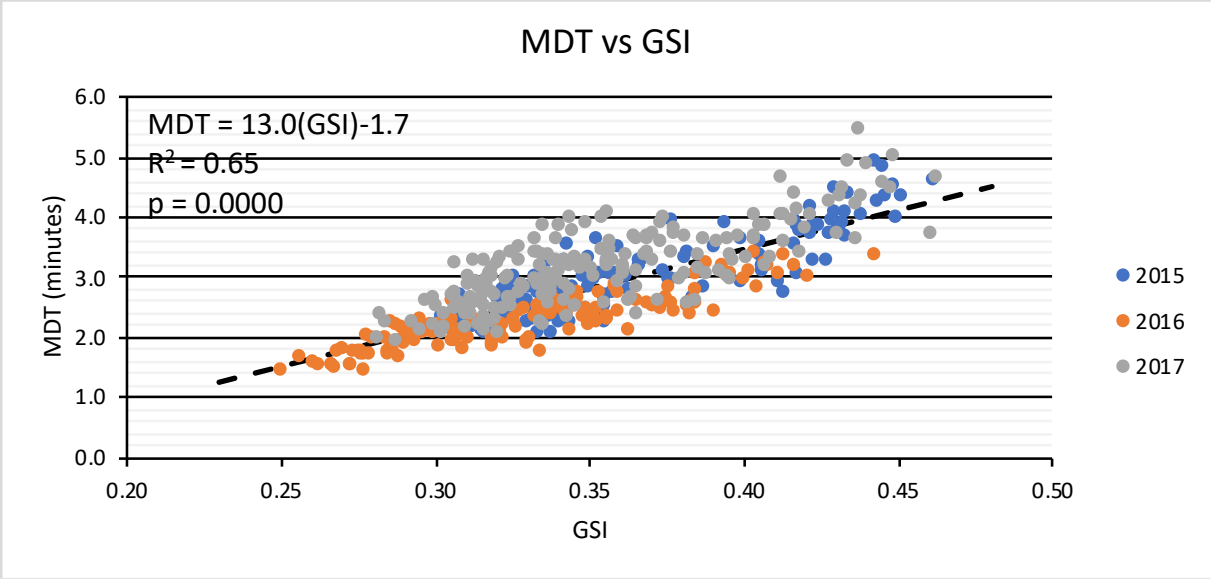


Figure 3.6 Linear regression between MDT (Mixing Development Time) and GSI (Gluten Strength Index).

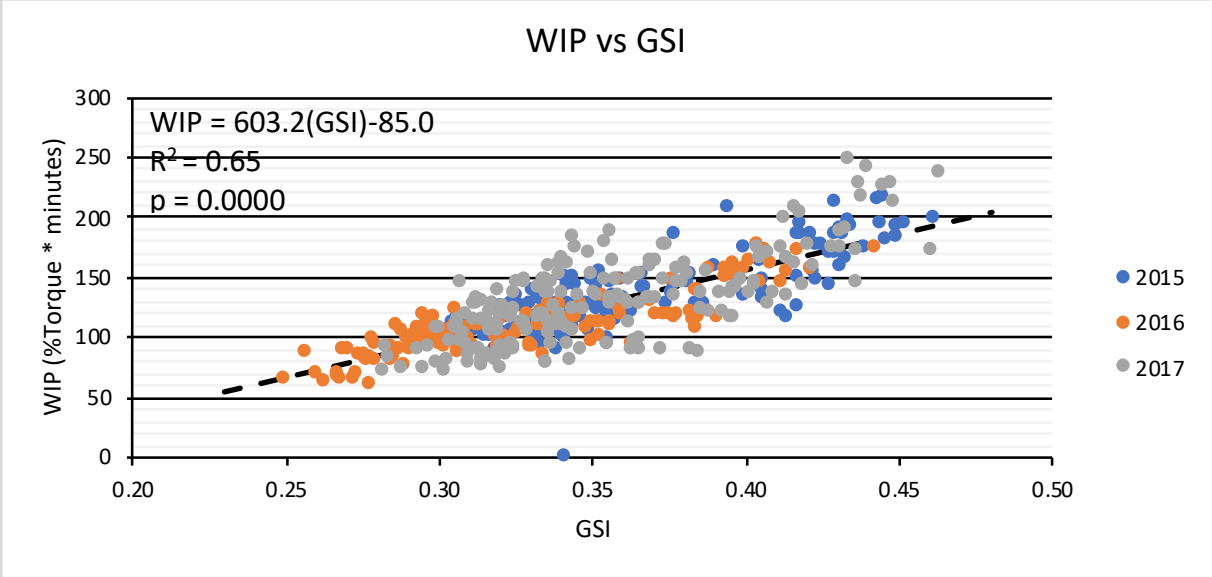


Figure 3.7 Linear regression between WIP (Work Input to Peak) and GSI (Gluten Strength Index).

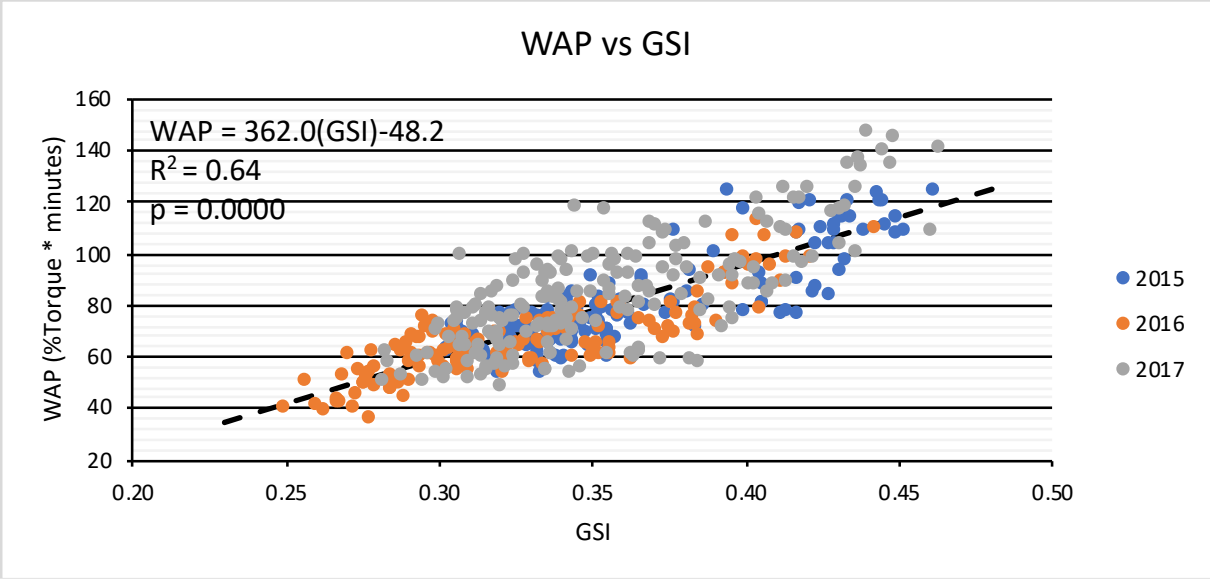


Figure 3.8 Linear regression between WAP (Work At Peak) and GSI (Gluten Strength Index).

Figures 3.9 through 3.13 show the relationship of Insoluble Glutenin/Flour Protein (IG/FP) to the five mixograph parameters, PDR, PBW, MDT, WIP and WAP. The results are very similar to the comparisons of the same mixograph parameters with GSI above. The peak dough resistance and peak band width had very poor correlation to IG/FP, but there was a moderately strong correlation of mixing development time, work input to peak and work at peak.

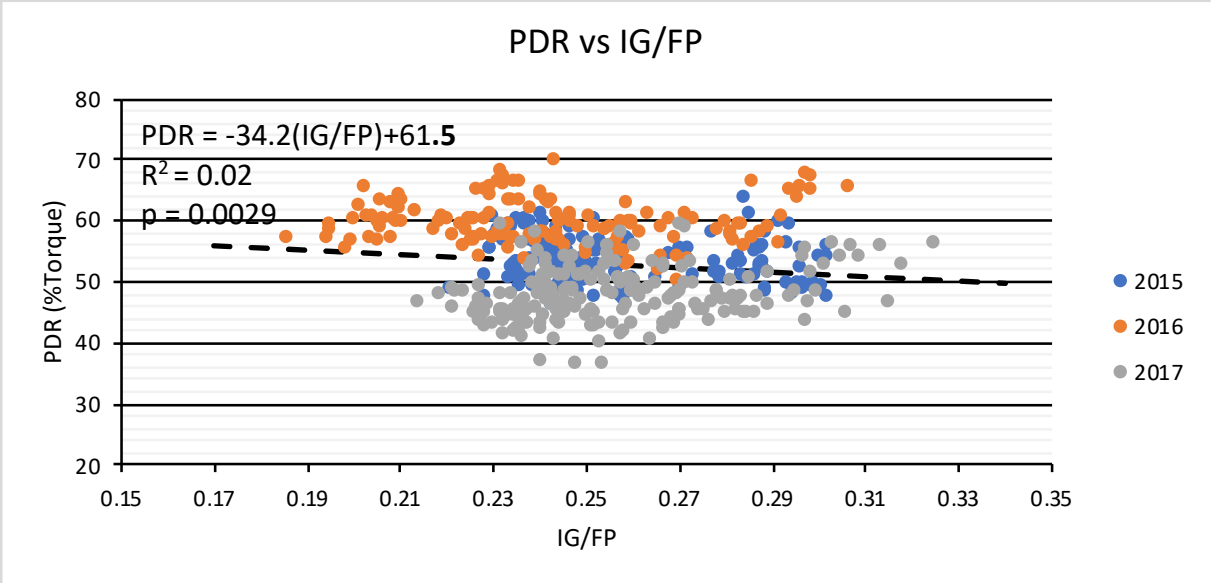


Figure 3.9 Linear regression between PDR (Peak Dough resistance) and IG/FP (Insoluble Glutenin/Flour Protein).

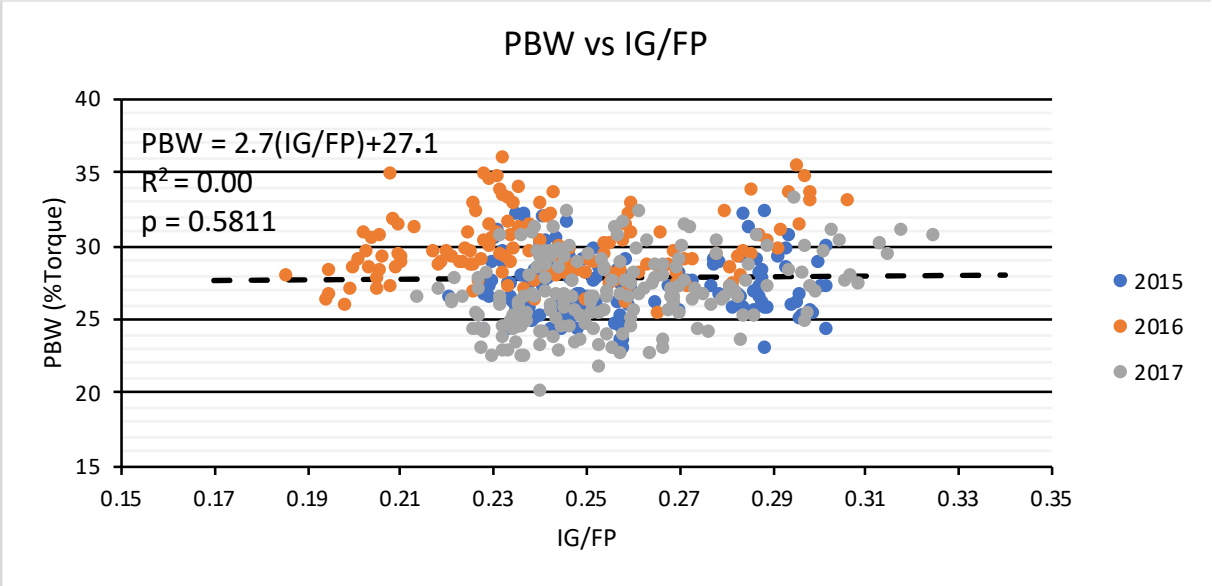


Figure 3.10 Linear regression between PBW (Peak Band Width) and IG/FP (Insoluble Glutenin/Flour Protein).

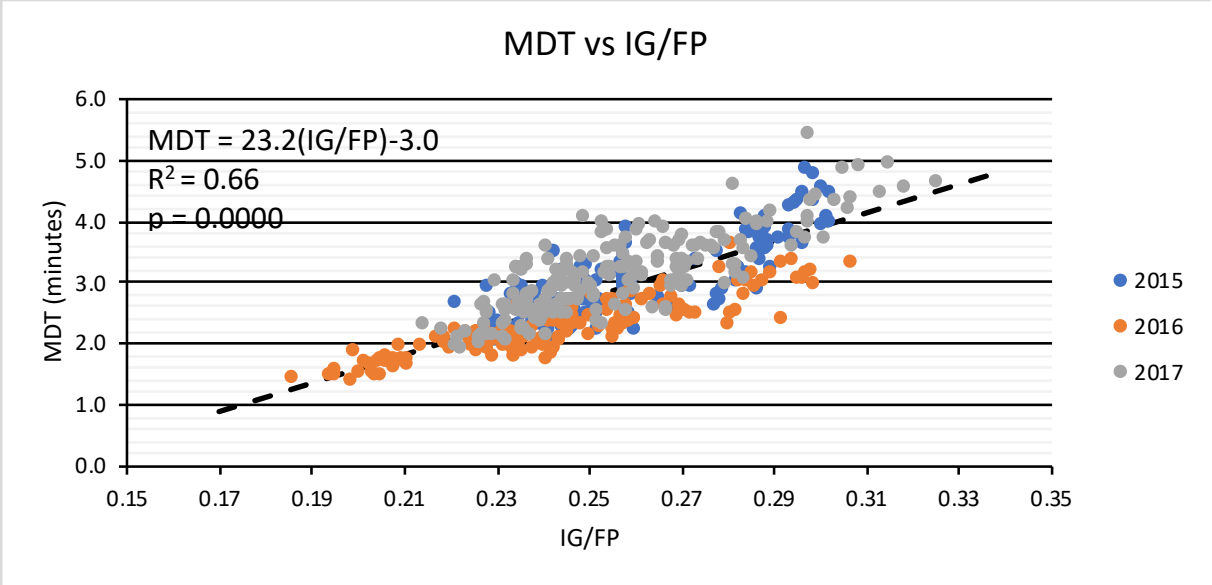


Figure 3.11 Linear regression between MDT (Mixing Development Time) and IG/FP (Insoluble Glutenin/Flour Protein).

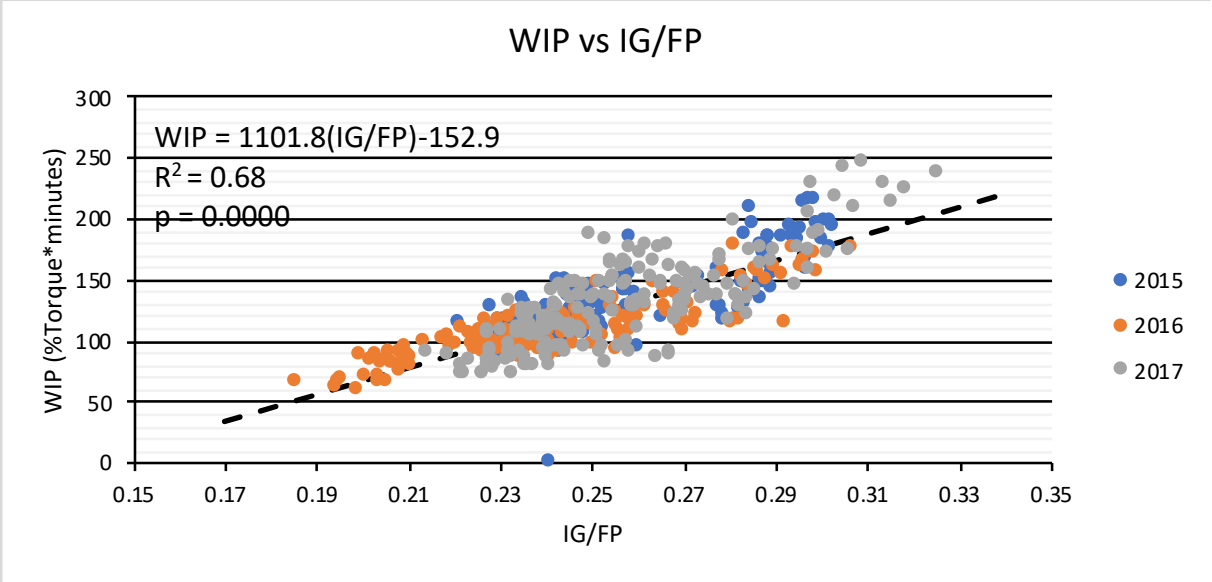


Figure 3.12 Linear regression between WIP (Work Input to Peak) and IG/FP (Insoluble Glutenin/Flour Protein).

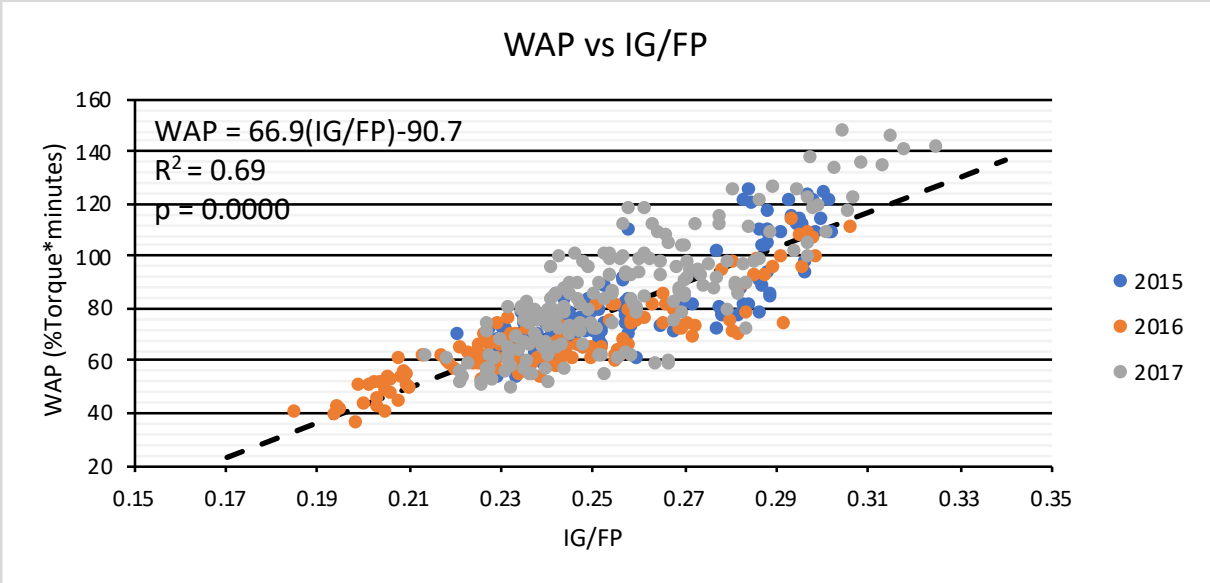


Figure 3.13 Linear regression between WAP (Work At Peak) and IG/FP (Insoluble Glutenin/Flour Protein).

3.5 Discussion

Higher precipitation levels were associated with increased PDR and PBW values by location (see Figures in Appendix IV). Most of the wettest site-years were in 2016, and this is when the highest PDR and PBW values tended to occur. The mechanism that underlies this relationship is not clear. There was no consistent ranking of average values by genotype for PDR and PBW (Tables 3.3 and 3.4, respectively).

The MDT, WIP and WAP showed much different results (Tables 3.5, 3.6 and 3.7, respectively). Increased precipitation was associated with lower values for these parameters (see Figures in Appendix IV). While the values differed year to year, there were also differences observed between the different study locations, in particular for the WIP and WAP values. In 2017, the

location with the highest average gluten strength was IHARF, with WIP and WAP means of 171.09 %Tq*min and 106.82 %Tq*min, respectively. In comparison, the IHARF location in 2016 had the lowest average gluten strength, with WIP and WAP means of 95.29 %Tq*min and 57.45 %Tq*min, respectively. These results highlight the substantial variation that can occur in dough mixing properties between locations in a single year. Genotype differences in gluten strength were generally very consistent across all study years and locations (Figure II.12, II14 and II.16, respectively) with order in ranking based on MDT, WIP and WAP parameters being Glenn > Carberry > Cardale ~= CDC Stanley and Stettler > Harvest. Glenn had the highest values for all three parameters, while the Harvest and Stettler genotypes had the lowest values. This ranking is very similar to that reported by Courcelles (2019) which matches expectations based on known gluten strength properties of these genotypes.

Genotype, site-year and site-year*genotype interaction were all significant for all of the mixograph parameters. Site-year contributed the largest amount to variance followed by genotype and then the site-year*genotype interaction. Although pesticide treatment and its interactions were occasionally significant, they did not contribute substantially to variance for any of the mixograph parameters. Results indicated that site-year (i.e., growing season weather) and genotype were the main determinants of gluten strength measured using the mixograph, whereas pesticide treatments were a minor factor. Site-year has been previously identified as a significant factor affecting grain protein concentration (Campbell et al., 1981), which is consistent with the results of this study. In addition, Gooding et al. (2003) showed mixing parameters were significantly affected by environmental conditions.

The most notable result for the mixograph analysis was that there was no significant effect of either pesticide treatment or genotype*pesticide treatment interaction at any individual site-year for any of the five parameters (Tables 3.1 and 3.2). There was a significant site-year*pesticide treatment interaction because the relative rankings for the mixograph parameters by pesticide treatment was different between site-years (Tables 3.3 to 3.7). This caused crossover interactions that explained a small amount of variance. Therefore, gluten strength was largely unaffected by the application of FHB fungicide at anthesis or pre-harvest application of glyphosate at the hard dough stage. However, higher FDK in affected site-years was associated with negative effects on grade and mixograph parameters of gluten strength, as well as reduced concentration of IG in flour and lower values for GSI. These associations were not specifically examined in this study, but are suggested in the results. For example, the IHARF 2016 site in particular had the highest FDK values in any site-year (Table 2.8). The majority of samples at this site graded Feed (Table 2.12). This site was also associated with the lowest values for mixograph MDT (Table 3.5), WIP (Table 3.6), WAP (Table 3.7), IG content (Table 3.10), GSI (Table 3.12), and IG/FP (Table 3.13), indicating the lowest gluten strength. These results are in line with expected effects of FHB to cause some proteolytic degradation of glutenin (Dexter et al., 1996, 1997, Nightingale et al., 1999, Wang et al., 2005, Eggert et al., 2011).

The protein composition results supported those from the mixograph analysis. The effects of genotype, site-year and the site-year*genotype interaction were all significant, for all of the protein composition parameters. Triboi et al. (2000) also found that genotype played a large

role in determining the distribution and ratio of protein fractions and that other environmental factors such as precipitation and temperature also affected the protein ratio, similar to the results of this study. The site-year*pesticide interaction was significant for some of the protein parameters but not all, and it did not contribute a large amount to variance. Again, pesticide treatment and its interactions did not contribute substantially to variance.

The strong positive relationships between protein composition parameters and MDT, WIP and WAP mixograph parameters demonstrated a consistent response of the two analytical methods used in this study for quantification of gluten strength. This is consistent with results from other studies (Wieser and Zimmerman, 2000, Isaak et al., 2019). The relationships between the protein composition parameters and select mixograph parameters also highlighted key differences in gluten strength between site-years in a consistent manner.

3.6 Conclusions

One of the most important results of this study was the relative scarcity of significant effects from FHB fungicide or glyphosate treatments on any of the dough mixing or protein composition parameters that were measured for any of the six CWRS wheat genotypes that were studied. The crossover interaction between site-year and pesticide treatment did not explain a substantial amount of the variance in these parameters. It can be concluded that fungicide application for FHB control and pre-harvest glyphosate application are not significant sources of gluten strength variability for CWRS wheat produced in Western Canada. In contrast, variation contributed by genotype as well as growing location weather between site-

years were very important determinants of CWRS gluten strength. This result is important as it supports one of the issues raised by wheat customers that led to the inititation of this GxE research project, i.e. problematic variation in gluten strength in commercial shipments of CWRS wheat. The grading system cannot control gluten strength as it is not a grading factor. That fact, combined with results of this study that reveal that gluten strength is one of the most variable properties of wheat, seems to indicate a problem that has yet to be resolved.

3.7 References

AACC International, 2000. Approved Methods of the AACC, 10th ed. Method 54-40.02, mixograph method for flour. AACC International, St. Paul, MN.

Ames, N.P., Clarke, J.M., Marchylo, B.A., Dexter, J.E., Woods, S.M. 1999. Effect of environment and genotype on durum wheat gluten strength and pasta viscoelasticity. *Cereal Chemistry* **76:** 582-586.

Blandino, M. and Reyneri, A. 2009. Effect of fungicide and foliar fertilizer application to winter wheat at anthesis on flag leaf senescence, grain yield, flour bread-making quality and DON contamination. *European Journal of Agronomy* **30**: 275-282.

Bushuk, W. 1985. Flour proteins: structure and functionality in dough and bread. *Cereal Foods World* **30**: 447-451.

Campbell, C.A., Davidson, H.R. and Winkleman, G.E. 1981. Effect of Nitrogen, Temperature, Growth Stage and Duration of Moisture Stress on Yield Components and Protein Content of Manitou Spring Wheat. *Canadian Journal of Plant Science* **61:** 549-563.

Canadian Grain Commission Official Grain Grading Guide. (2019). https://www.grainscanada.gc.ca/en/grain-quality/official-grain-grading-guide/official-grain-grading-guide-2018-en.pdf

Champeil, A., Doré, T. and Fourbet, J.F. 2004. Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by Fusarium in wheat grains. *Plant Science* **166**: 1389-1415.

Courcelles, J.R. 2019. The Effect of Genotype and Growing Environment on the Gluten Strength and End-Use Quality of CWRS Wheat. M.Sc. thesis, University of Manitoba. http://hdl.handle.net/1993/33653

Dexter, J.E., Clear, R.M. and Preston, K.R. 1996. Fusarium Head Blight: Effect on the Milling and Baking of Some Canadian Wheats. *Cereal Chemistry* **73(6)**: 695-701.

Dexter, J.E., Marchylo, B.A., Clear, R.M. and Clarke, J.M. 1997. Effect of Fusarium Head Blight on Semolina Milling and Pasta-Making Quality of Durum Wheat. *Cereal Chemistry* **74**: 519–525.

Eggert, K., Rawel, H. M. and Pawelzik, E. 2011. In vitro degradation of wheat gluten fractions by Fusarium graminearum proteases. *European Food Research and Technology* **233**: 697-705.

Fu, B. X. and Sapirstein, H. D. 1996. Procedure for isolating monomeric proteins and polymeric glutenin of wheat flour. *Cereal Chemistry* **73**: 143-152.

Fu, B. X., Sapirstein, H.D. and Bushuk, W. 1996. Salt-induced disaggregation/solubilisation of gliadin and glutenin proteins in water. *Journal of Cereal Science* **24**: 241-246.

Gooding, M. J., Ellis, R. H., Shewry, P. R. and Schofield, J. D. 2003. Effects of restricted water availability and increased temperature on the grain filling, drying and quality of winter wheat. *Journal of Cereal Science* **37:** 295-309.

Isaak, C. 2019. Comparison of physical and biochemical methods to evaluate the gluten strength of Canadian hard red winter wheats. M.Sc. thesis, University of Manitoba. http://hdl.handle.net/1993/33666

Malalgoda, M. Ohm, J. B., Ransom, J. K. Howatt, K. and Simsek, S. **2020a.** Effects of pre-harvest glyphosate application on spring wheat quality characteristics. *Agriculture* **10(4)**: 111.

Malalgoda, M. Ohm, J. B., Ransom, J. K., Howatt, K., Green, A., and Simsek, S. **2020b.** Pre-harvest glyphosate application during wheat cultivation: Effects on wheat starch physicochemical properties. *Journal of Agricultural and Food Chemistry* **68(2)**: 503-511.

Manthey, F.A., Charkraborty, M., Peel, M.D. and Pederson, J.D. .2004. Effect of pre-harvest applied herbicides on breadmaking quality of hard red spring wheat. *Journal of the Science of Food and Agriculture* **84**: 441-446.

Nightingale, M.J., Marchylo, B.A., Clear, R.M., Dexter, J.E. and Preston, K.R. 1999. Fusarium Head Blight: effect of fungal proteases on wheat storage proteins. *Cereal Chemistry* **76**: 150-158.

Peterson, C.J., Graybosch, R.A., Shelton, D.R. and Baenziger, P.S. 1998. Baking quality of hard winter wheat: Response of cultivars to environment in the Great Plains. *Euphytica* **100**: 157-162.

Sapirstein, H. D. and Fu, B. X. 1998. Intercultivar variation in the quantity of monomeric proteins, soluble and insoluble glutenin, and residue protein in wheat flour and relationships to breadmaking quality. *Cereal Chemistry* **75**: 500-507.

Sapirstein and Johnson 2000. A rapid spectrophotometric method for measuring insoluble glutenin content of flour and semolina for wheat quality screening. 307-312 in; Wheat Gluten. P.R. Shewry and A.S. Tatham, eds.

Southan, M. and MacRitchie, F. 1999. Molecular Weight Distribution of Wheat Proteins. *Cereal Chemistry* **76(6)**: 827-836.

Triboi, E., Abad, A., Michelena, A., Lloveras, J., Ollier, J.L., Daniel, C. 2000. Environmental effects on the quality of two wheat genotypes: 1. quantitative and qualitative variation of storage proteins. *European Journal of Agronomy* **13:** 47-64.

Wang, J. H., Wieser, H., Pawelzik, E., Weinert, J., Keutgen, A. J. and Wolf, G. A. 2005. Impact of the fungal protease produced by Fusarium culmorum on the protein quality and breadmaking properties of winter wheat. *European Food Research and Technology* **220**: 552-559.

Wegulo, S.N. 2012. Factors Influencing Deoxynivalenol Accumulation in Small Grain Cereals. *Toxins* **4**: 1157-1180.

Wieser, H. and Zimmermann, G. 2000. Importance of amounts and proportions of high molecular weight subunits of glutenin for wheat quality. *European Food Research and Technology* **210(5)**: 324-330.

Wrigley, C. W. **1996.** Giant proteins with flour power. *Nature* **381(6585)**: 738-739.

Zhu, J. and Khan, K. 2001. Effects of Genotype and Environment on Glutenin Polymers and Breadmaking Quality. *Cereal Chemistry* **78:** 125-130.

4. EFFECTS OF DELAYED HARVEST DATES ON CWRS GLUTEN STRENGTH

4.1 Abstract

Delayed harvest dates are a common occurrence in Western Canada as a result of inclement weather, especially fall precipitation. This can have negative impacts on the yield, grade and quality of wheat with deterioration of grading factors. While the effects of delayed harvest on grain quality are known, the effects on gluten strength are largely unknown. In this study, four commercial CWRS genotypes (Glenn, Carberry, Brandon and Harvest), with a range of gluten strength characteristics were grown at four different locations across Manitoba (Brandon, Carberry, Grosse Isle and Kelburn) in 2017. At each location there were four different harvest dates implemented: harvest date one (H1) was at physiological maturity, harvest date two (H2) was during the normal harvest period (grain moisture content between 13-15%), harvest date three (H3) was four weeks after physiological maturity and harvest date four (H4) was six weeks after physiological maturity. The grain from the different harvest dates was analyzed to determine the effect on grain and flour quality, as well as protein composition and rheological properties. The results showed that the delayed harvest had a significant negative impact on grain quality parameters, especially test weight, and increased the concentration of flour ash. However, the protein and rheological properties were minimally affected by harvest delays. Both the protein composition and rheological properties indicated a trend of slightly increased gluten strength in the samples from the delayed harvest.

4.2 Introduction

Harvest timing is a critical factor affecting wheat quality in Western Canada. In some years the harvest date may be delayed due to inclement weather (Czarnecki and Evans, 1986). A wheat crop will reach its physiological maturity when the dry matter content reaches a maximum. This coincides with moisture content of the grain reaching levels between 33% to 41% (Calderini et al., 2000). However, there is still a period of time before the crop is ready for harvest. The ideal grain moisture content for wheat being harvested is 13-15% moisture (Farrer et al., 2006). During the period period between maturity and harvest, wheat can experience inclement weather with large amounts of precipitation that can hinder farmers' ability to harvest wheat at the ideal moisture content. When harvest is delayed there can be substantial losses in yield, grade and grain quality (Farrer et al., 2006). Some of the causes for these losses in western Canada include lodging, shattering, sprout damage and frost damage.

Farrer et al. (2006) reported that delayed harvest date significantly reduced grain yield, test weight, flour falling number, percent of clear flour yield, and significantly increased grain deoxynivalenol (DON). Christensen and Legge (1984) also found little effect on grain protein content with harvest timings ranging from 45% to 15% grain moisture content. However, they observed that the falling numbers increased as grain moisture content at harvest decreased for wheat that was direct combined. Also, there was a loss in grade for wheat harvested at grain moisture levels of 20% or higher, primarily as a result of mildew, green kernels and sprouting (Christensen and Legge, 1984). It is challenging to harvest wheat with grain moisture content higher than about 20% which increases the likelihood of damaging the grain or even the

combine. Also, grain drying is essential for wheat harvested at high moisture content, in order to reduce spoilage and for farmers to avoid discounts for wheat that would be graded as tough (14.6-17.0% moisture content) or damp (>17.0% moisture content) (Canadian Grain Commission, 2019).

The number of studies on the effects of delayed harvest on wheat quality are few and there is very little information on the interacting effects of cultivar or site-year. For example, Czarnecki and Evans (1986) observed that although large precipitation events were related to reduced test weight, not all cultivars responded similarly. Farrer et al. (2006) observed in two of five field trials, that farinograph breakdown time, a measure of gluten strength, was significantly reduced for the delayed second harvest. In addition, these two trials produced wheat that contained the highest concentrations of deoxynivalenol (DON, 2.9 and 2.6 ppm) at the time of the second harvest which was 16 and 8 days, respectively, after the first. The DON levels reported indicate that FHB was likely quite severe in those trials. One of the effects of FHB is degradation of polymeric glutenin and/or gluten strength as has been previously reported (Dexter et al., 1996, 1997; Nightingale et al., 1999; Wang et al., 2005; Eggert et al., 2011) and suggested by results in Chapter 3. Fonad et al. (2008) studied the effects of harvest timing on the quality components of winter wheat grown in a single location in Hungary for two years for 12 wheat varieties that ranged in maturity and breadmaking quality. Weather in the time periods leading up to harvest and between optimum and delayed harvest dates were very different in the two years. In one year, the authors reported a downpour of 27 mm of precipitation between the two harvests. In the other year, the grain filling period saw

unspecified “extremely hot temperatures” and insignificant rain (6 mm) between the optimum and delayed harvest dates. The study monitored protein content, kernel hardness, wheat falling number, and two unspecified parameters of gluten quality. No consistent trend was found related to delayed harvest for most of the parameters as the effects of year and cultivar varied considerably. In the year with the hot grain filling period and dry harvest conditions, there was an apparent improvement in gluten strength. The authors concluded that delayed harvest along with harvest-time rains can seriously stress wheat quality, but the extent of effects depended strongly on cultivar and climatic conditions.

The objective of this study was to improve understanding of the effects of delayed harvest on wheat quality and gluten strength in the context of several leading varieties of CWRS wheat grown in Manitoba locations. Outcomes were evaluated for four harvest dates which extended past physiological maturity by up to six weeks, and included assessment of grain quality, flour quality, rheological properties, and protein composition parameters.

4.3 Materials and Methods

4.3.1 Field Study

In 2017, four study site locations across southern Manitoba (Grosse Isle, Kelburn Farm, Carberry and Brandon) were selected for their distance from one another, to maximize the potential variability in the weather between locations. At each location, four CWRS spring wheat genotypes (Harvest, Glenn, Carberry and Brandon) were grown to provide a range in gluten strength quality from Glenn (strongest) to Harvest (weakest). It should be noted that the

designation of the Harvest genotype was changed from CWRS to Canada Northern Hard Red effective August 1, 2018, after this study had been completed (https://www.grainscanada.gc.ca/en/grain-quality/variety-lists/2017/2017-45.html). Seed for planting the plots was received at the University, then weighed and packaged for each study site. The amount of seed used per site varied depending on seeding equipment and seeding practices, so the plot sizes varied by site (Table 4.1). The planned seeding rate for all sites was 300 seeds m^{-2} after considering the germination percentage and thousand kernel weight. At each site, the four genotypes were planted in a randomized complete block design with separate plots of each genotype to be harvested at four different times with four replicates per harvest per genotype. Table 4.1 has further information on the plot sizes, including length and width dimensions, as well as rows per plot and row spacing information. Due to an infestation of wild oats, the eastern half of the Carberry study location was not salvageable for quality analysis. The salvageable plots were repurposed as blocks and were split into smaller plots to provide replicates for each harvest of each genotype. The original Brandon and Glenn genotype plots that were salvageable were divided into three smaller separate plots. The harvest of the smaller plots was randomized to provide three different harvest times (maturity, four weeks after maturity, six weeks after maturity) with four replications per harvest time. The salvageable plots for the Carberry and Harvest genotypes were divided in half. The smaller plots were randomized for harvest at all four harvest times with four replications. Even with these changes, there was still enough grain from each harvest at Carberry to conduct quality analysis for the smaller plots.

Plots were seeded between May 2nd and the 19th, 2017 (Table 4.1). At the Brandon site there was fall rye seeded between the plots. Kelburn, Carberry and Grosse Isle had nothing between the plots, but there were guard rows of other spring wheat varieties on either side of the trial.

Table 4.1 Study location set up, including plot size, seeds per plots, and seeding and maturity dates.

Study Site Locations	Plot Size (m^2)	Plot Length (m)	Plot Width (m)	Rows Per Plot/ Row Spacing (inches)	Seeds per Plot	Seeding Date	Maturity Date (Harvest Date1)
Kelburn	12	7	1.28	7/7.1	3600	May 18th	August 24/17
AAFC Brandon[a]	3.6	4	1	5/7	1080	May 19th	August 30/17
Grosse Isle	9	6	1.5	7/7.5	2700	May 2nd	August 22/17
CMCDC Carberry[b]	8.5	5	1.5	4/12	2550	May 11th	August 28/17

[a]Agriculture and Agri-Food Canada, [b]Canada-Manitoba Crop Diversification Centre

4.3.2. Grain Harvest

The study utilized four harvest dates. The first harvest (H1) occurred approximately at physiological maturity, which was classified within this experiment as wheat with grain moisture content of 15% to 30%. The second harvest (H2) was two weeks after maturity with grain moisture expected to range from 13% to 15% (i.e., "dry" or ideal moisture for commercial harvesting), the third date (H3) was four weeks after maturity and the fourth (H4) was six weeks after maturity. Grain moisture content was measured prior to the first harvest date, by head sampling at the different locations, and then using an oven to dry the samples. Three head samples from each replicate for each genotype at all four sites were clipped and put in sealed plastic bags. At the University of Manitoba, the kernels were removed and weighed, then put into tins in a drying oven for 48 hours at a temperature of 60 °C. The kernels were re-weighed and the moisture content was determined by the difference in weight between the wet and dry grain as shown in equation 4.1. Head samples were taken at different times after senescence to determine the timing for H1 (Table 4.2). The harvest dates for locations are shown in table 4.3.

OD moisture % = (mass of water/mass of dry biomass) x 100 [4.1]

Table 4.2 Mean moisture content measurements for each genotype at all four locations on different sampling dates.

		Moisture Content (%)	
Brandon			
	August 25/17	August 30/17	
Glenn	57.27	18.38	
Brandon	67.85	35.13	
Harvest	55.00	22.03	
Carberry	58.00	27.79	
Carberry			
	August 25/17	August 28/17	
Glenn	14.21	11.03	
Brandon	14.40	10.75	
Harvest	12.83	11.13	
Carberry	12.90	11.09	
Grosse Isle			
	August 14/17	August 18/17	August 22/17
Glenn	40.12	21.65	20.55
Brandon	41.86	20.14	19.19
Harvest	26.14	15.00	15.33
Carberry	40.56	23.91	19.21
Kelburn			
	August 15/17	August 21/17	August 24/17
Glenn	50.75	15.82	25.52
Brandon	58.00	23.64	25.18
Harvest	47.00	18.00	27.62
Carberry	48.19	22.00	27.41

Table 4.3 Harvest dates for all four study locations.

	Harvest Date 1	Harvest Date 2	Harvest Date 3	Harvest Date 4
Brandon	August 30th	September 13th	September 29th	October 12th
Carberry	August 28th	September 11th	September 25th	October 10th
Grosse Isle	August 22nd	September 5th	September 19th	October 6th
Kelburn	August 24th	September 7th	September 21st	N/A[a]

[a]The last harvest was not completed at the Kelburn site due to geese having eaten the majority of the plots.

There were two different harvest methods used to collect grain from the plots. The first method consisted of using a sickle mower to cut down wheat in the plots, which was then bagged and stored in a drying room. Once the bags had been in the drying room for a period of at least two weeks, the samples were threshed using a stationary combine. The grain was collected from the combine and then returned to the drying room. The second harvest method involved transporting a plot combine to the study site and harvesting the grain directly from the plots on a specific harvest date. The grain collected from the combine at the study site was put into plastic mesh bags and then placed in the drying room.

The sickle mower harvest method was used for all of the plots at Carberry, and Brandon due to their small plot size, and distance from the University. It was much more efficient to load the small sickle mower than to bring the combine to these sites. The sickle mower was also used for harvest dates one (maturity), two (two weeks after maturity) and three (four weeks after maturity) at Grosse Isle. It was also used for harvest dates one and two at Kelburn Farm. This method was used at Grosse Isle and Kelburn for these harvest dates because it was not possible to manoeuvre the plot combine through the individual plots at the sites due to the limited amount of space between plots. The second harvest method was used for the fourth harvest date at Grosse Isle (six weeks after maturity), as well as the third harvest date at Kelburn Farms because there was space between plots after the earlier harvested plots had been removed. In addition, these two sites were close to the University making it more practical to transport the combine over the shorter distance.

The threshed grain remained in the drying room until it was processed. The grain from each plot, and each location was weighed and the total yield from each plot was calculated. After the yield had been recorded, the grain samples were reduced to 1600 g by removing excess grain from the original sample using a metal scoop. All samples were sent to the Intertek laboratory in Winnipeg for grading and cleaning.

4.3.3. Meteorological Analysis

Weather data were collected from weather stations located within close proximity to each study site, as well as weather stations at field sites. The data included daily maximum, minimum and average temperatures, as well as precipitation. At each study site, the weather data was aggregated into four time periods; seeding to H1, H1 to H2, H2 to H3 and H3 to H4.

4.3.4. Analysis of Wheat Quality

Full details of the quality analysis are described in Sections 2.3.3, 2.3.4, 3.3.3 and 3.3.4.

4.3.5. Statistical Methodology

Statistical analysis was carried out using SAS Software, version 9.4 (Appendix IX). Global Analysis of Variance was conducted to determine the proportion of variance contributed by each of the main effects and their interactions (genotype, harvest treatment, location, block (location), genotype*harvest, location*genotype, location*harvest, location*genotype*harvest and residual). The SAS MIXED procedure was used in order to produce a Type III Analysis of Variance table for individual locations. The Tukey Kramer method was utilized for means separation and to determine the statistical differences between genotypes and harvest dates.

All parameters were tested for normality before continuing with statistical analysis. It was determined that FDK values were not normally distributed, so the values were transformed using Transformed FDK = log(FDK+0.5). The FDK values were back-transformed for reporting.

4.4 Results

4.4.1. Weather Conditions

Precipitation data for 2017 at each location is presented in Table 4.4. From seeding until maturity, precipitation ranged from 130.7 mm (Carberry) to 190.2 mm (Grosse Isle). Between Harvest Dates 1 and 2 there was not much precipitation and the locations remained relatively dry during this time period. The time period between Harvest Dates 2 and 3 was when the weather conditions began to change and rainfall was much greater than during the previous time period. Between H2 and H3, the Brandon and Carberry locations had rainfall amounts of 74.4 and 80 mm, respectively. The Grosse Isle and Kelburn received less rainfall, with amounts of 19.2 and 19.7 mm, respectively. The period between H3 and H4 produced low rainfall for Brandon and Carberry (1.2 and 0.4 mm), while the rainfall amount at Grosse Isle was 56.5 mm. The Kelburn site was destroyed by geese during this time period, so there is no corresponding data available for the last harvest period at this site-year.

Table 4.4 Precipitation levels between different growth stages, and harvest dates, for four Manitoba locations in 2017.

Location	Seeding-Maturity (Harvest 1) (mm)	Harvest 1-Harvest 2 (mm)	Harvest 2-Harvest 3 (mm)	Harvest 3-Harvest 4 (mm)
Brandon	155.2	4.2	74.4	1.2
Carberry	130.7	3.4	80	0.4
Grosse Isle	190.2	10.3	19.2	56.4
Kelburn	160.5	6.3	19.7	N/A

4.4.2. Wheat Quality

The variation in CWRS grade and grading factors between the different locations of the study are illustrated in Figures 4.1 through 4.9 showing the mean values across all genotypes. Figure 4.1 shows the overall proportion of CWRS No.1 through CW Feed for all genotypes at all four locations by harvest date. For both Harvest Dates 1 and 2 the grades were predominantly CWRS No.1 and CWRS No.2, with very few samples of CWRS No.3 and CW Feed. For Harvest Dates 3 and 4, CWRS No.2 was the most common grade. Higher proportions of CWRS No.3 and CW Feed grade were harvested in H3 and H4 dates compared to the H1 and H2 dates and there were no samples that graded CWRS No.1. Thus, the grades deteriorated with progressively later Harvest Dates.

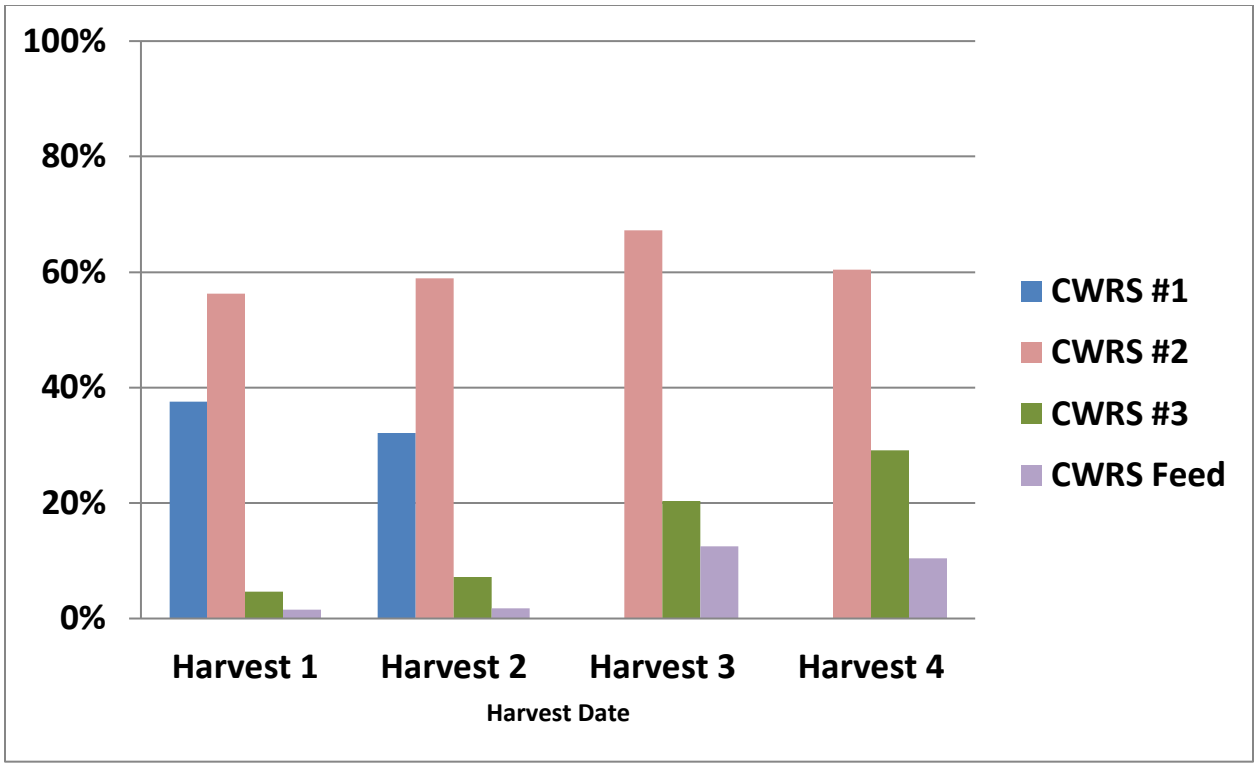


Figure 4.1 Percentage of samples with specific grades for combined genotypes by harvest dates for all locations.

The samples from the Brandon site were graded predominantly CWRS No.1 and No.2 (Figure 4.2). For the first two harvest dates the majority of samples were graded CWRS No.1. For the third and fourth harvests the grades were solely CWRS No.2.

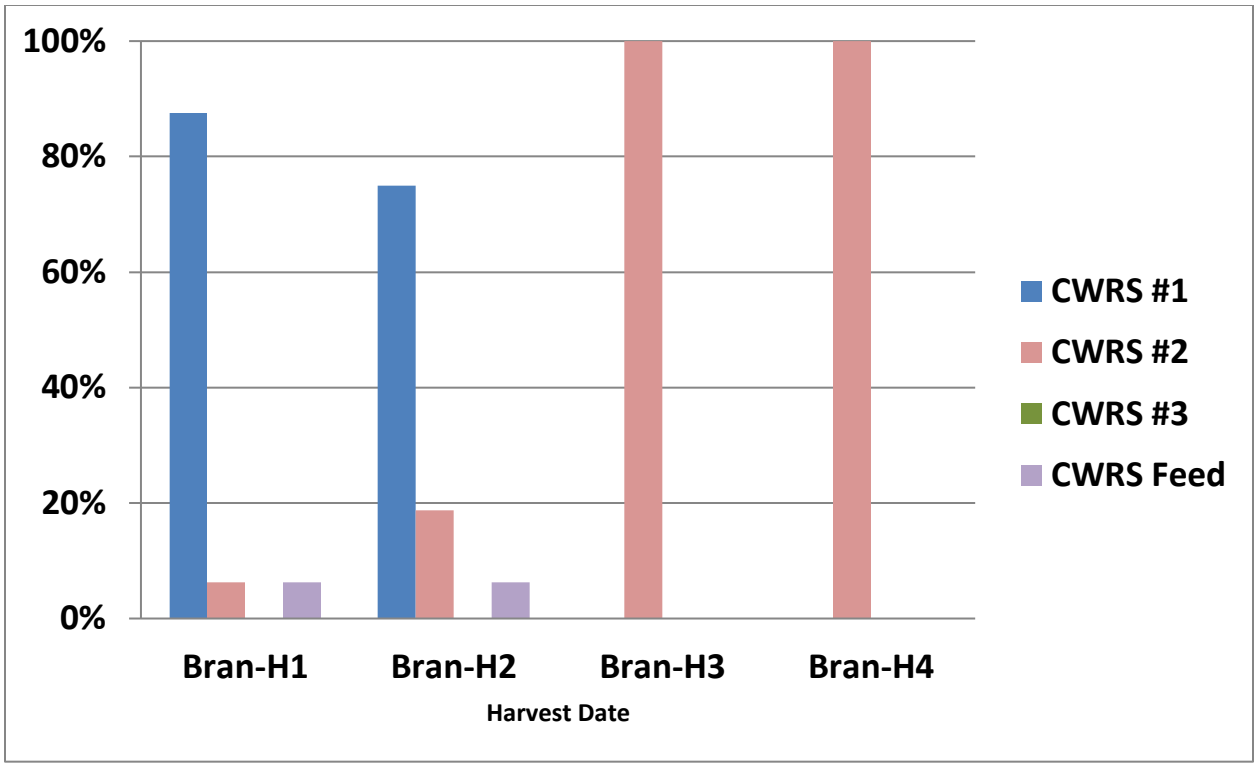


Figure 4.2 Percentage of samples with specific grades for combined genotypes by harvest date at Brandon.

The grading factor that was most predominant at the Brandon location, for each harvest date was midge damage (Figure 4.3). The percentage of midge damage observed in samples increased with each harvest date, with the highest value of 1.6% occurring for H4. The differences in midge damage by harvest date are not large and are most likely a result of random sample variation. Other grading factors observed were Fusarium damaged kernels (FDK) and sprout damage. These factors occurred at much lower levels of less than 0.2%. The low values for all of these grading factors are the reason that the grades for this location were mainly CWRS No.1 and No.2 for all harvest dates.

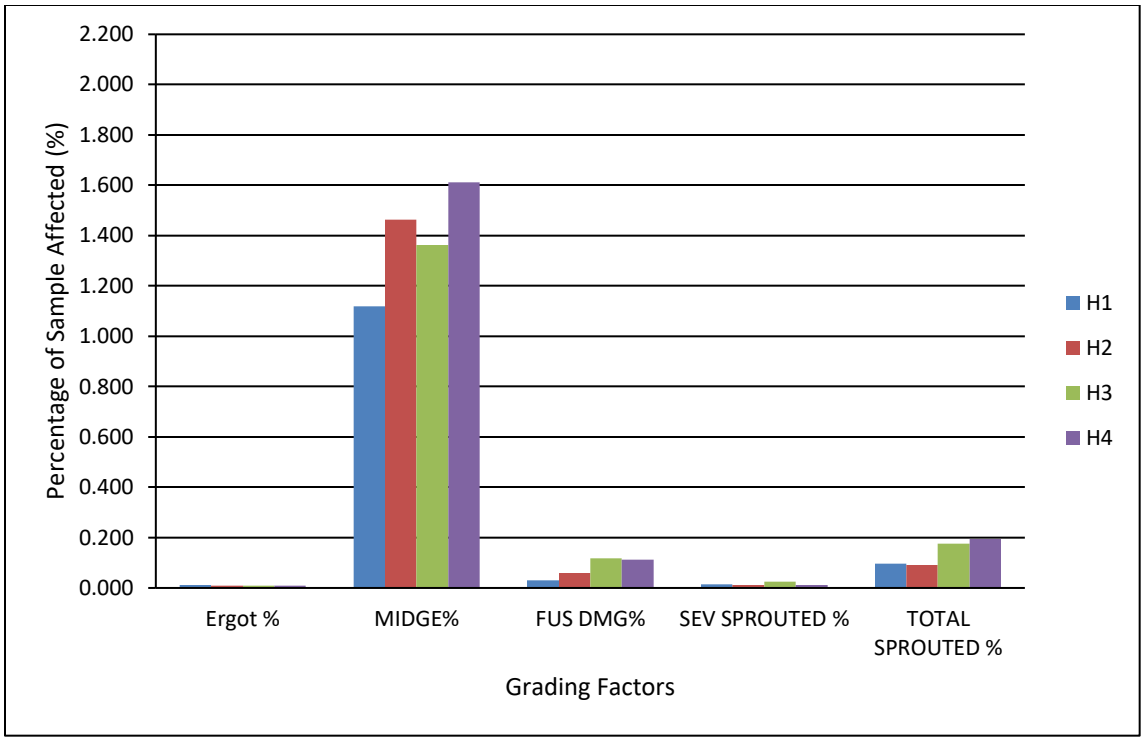


Figure 4.3 Distribution of grading factors and their effect on samples, for combined genotypes by harvest dates at Brandon. Grading factors include ergot, midge, Fusarium damage (FUS DMG), severely sprouted (SEV SPROUTED) and total sprouted.

The grades at the Carberry location (Figure 4.4) for the first two harvest dates were superior compared to the last two. The samples from H1 were mainly CWRS No.1 while CWRS No.2 was most prevalent for H2. For H3 and H4, there were no samples that graded CWRS No.1, and more than half of the samples graded CWRS No.3 and CWRS feed.

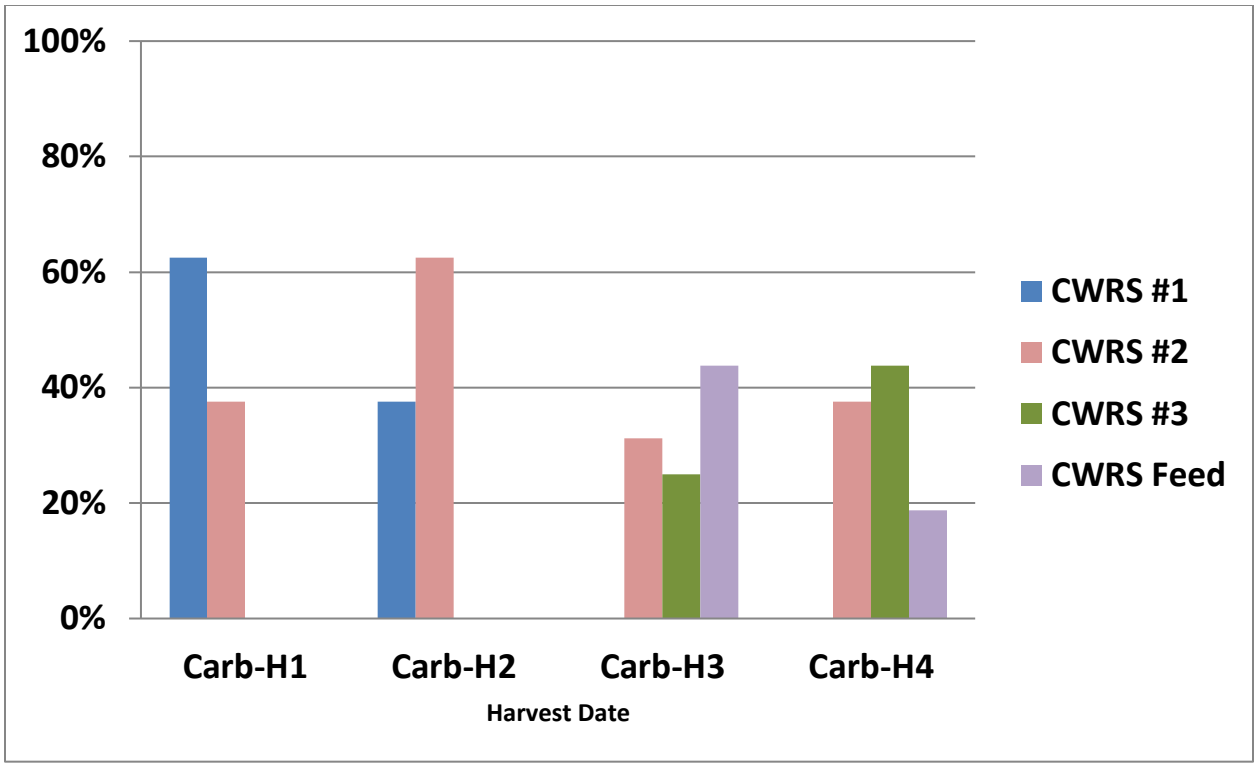


Figure 4.4 Percentage of samples with specific grades for combined genotypes by harvest date at Carberry.

The main grading factors affecting the Carberry location included midge damage, Fusarium damaged kernels, sprouted and severe sprouted (Figure 4.5). Midge damage was at a reasonably consistent level for all four harvests. The FDK grading factor was low and also remained similar between harvest dates. The most predominant change in grading factors was the total sprout damage. The total sprout damage was low or non-existent for the first two harvest dates, but much higher for H3 and H4. There was no severe sprout damage detected in the H1 and H2 samples, but some low levels were present in H3 and H4.

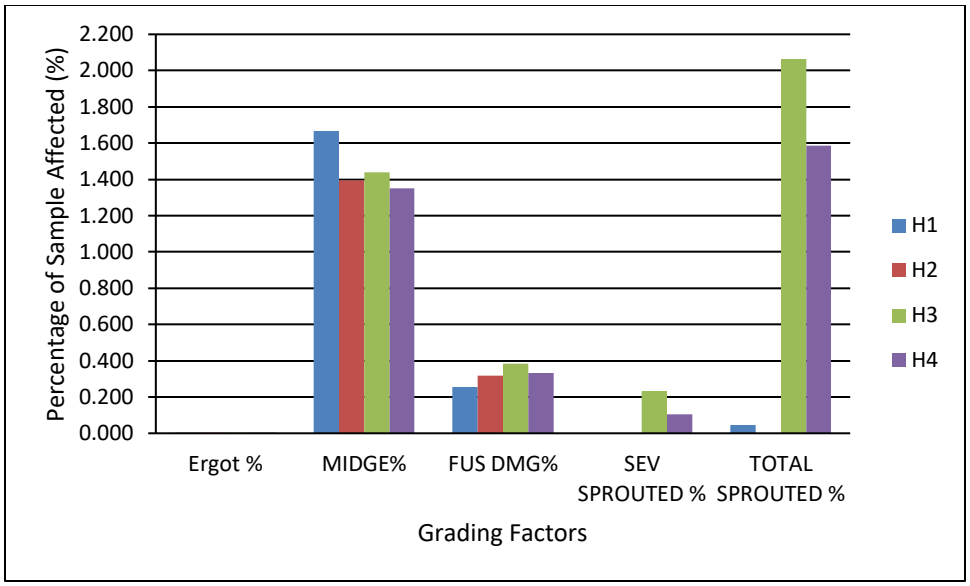


Figure 4.5 Distribution of grading factors and their effect on samples, for combined genotypes by harvest dates at Carberry. Grading factors include ergot, midge, Fusarium damage (FUS DMG), severely sprouted (SEV SPROUTED) and total sprouted.

A grade of CWRS No.2 was predominant in the samples of H1, H2 and H3 at the Grosse Isle location (Figure 4.6). The H4 samples graded mainly CWRS No.2 and CWRS No.3 and with a few samples graded as Feed.

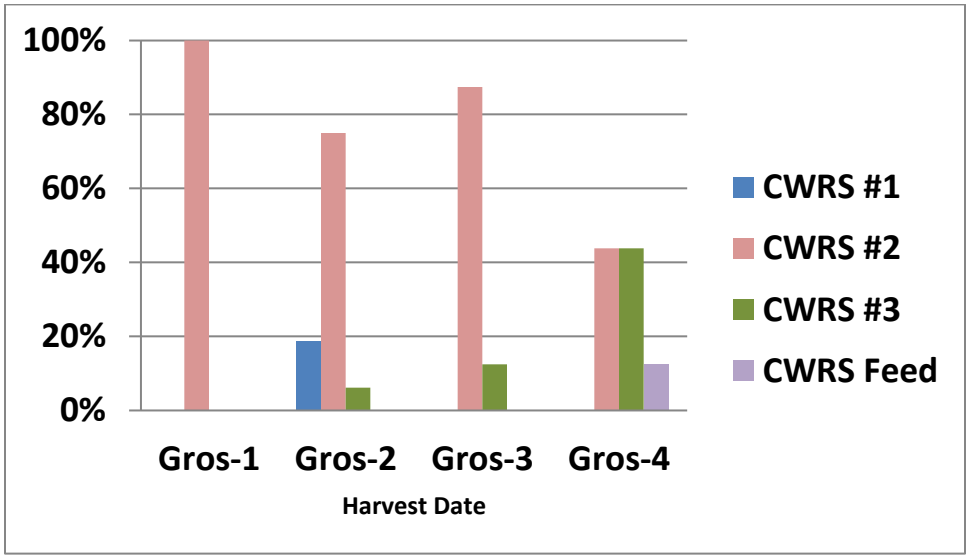


Figure 4.6 Percentage of samples with specific grades for combined genotypes by harvest date at Grosse Isle.

Similar to the previous two locations, midge damage was the main grading factor at Grosse Isle for all four harvest dates (Figure 4.7). Fusarium damaged kernels were, again at lower levels compared to the midge damage. Sprout and severe sprout damage was present at low levels for all four harvests. The slightly higher levels of FDK and sprout for H4 in comparison to the other harvest dates were sufficient to cause a deterioration in grade.

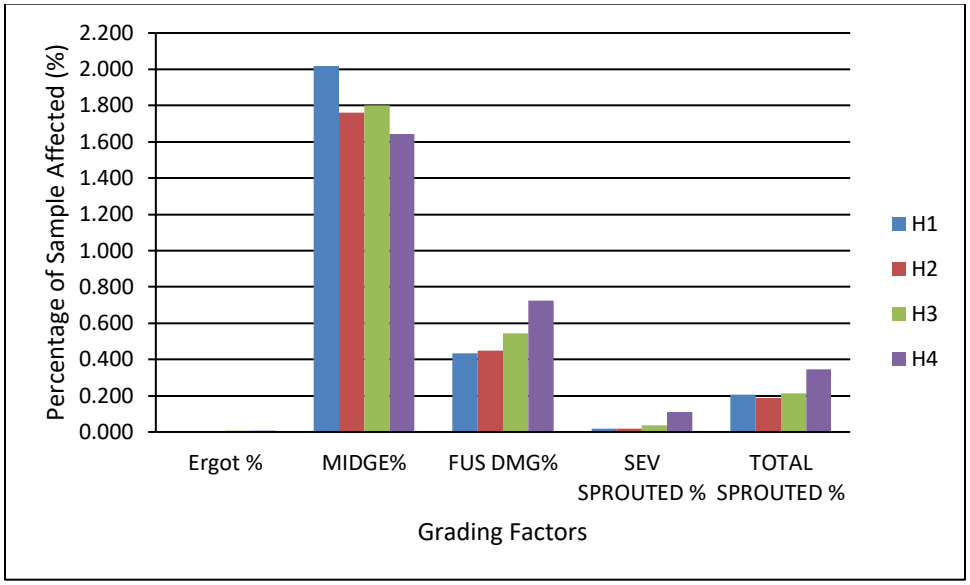


Figure 4.7 Distribution of grading factors and their effect on samples, for combined genotypes by harvest dates at Grosse Isle. Grading factors include ergot, midge, Fusarium damage (FUS DMG), severely sprouted (SEV SPROUTED) and total sprouted.

The grades for the Kelburn location (Figure 4.8) were almost identical for H1 and H2 with approximately 80% graded as CWRS No.2 and about 20% graded as CWRS No.3. For H3, just over 40% of the samples graded as CWRS No.2 with a similar amount graded CWRS No.3, and a small percentage of Feed grade samples. There were no samples for H4.

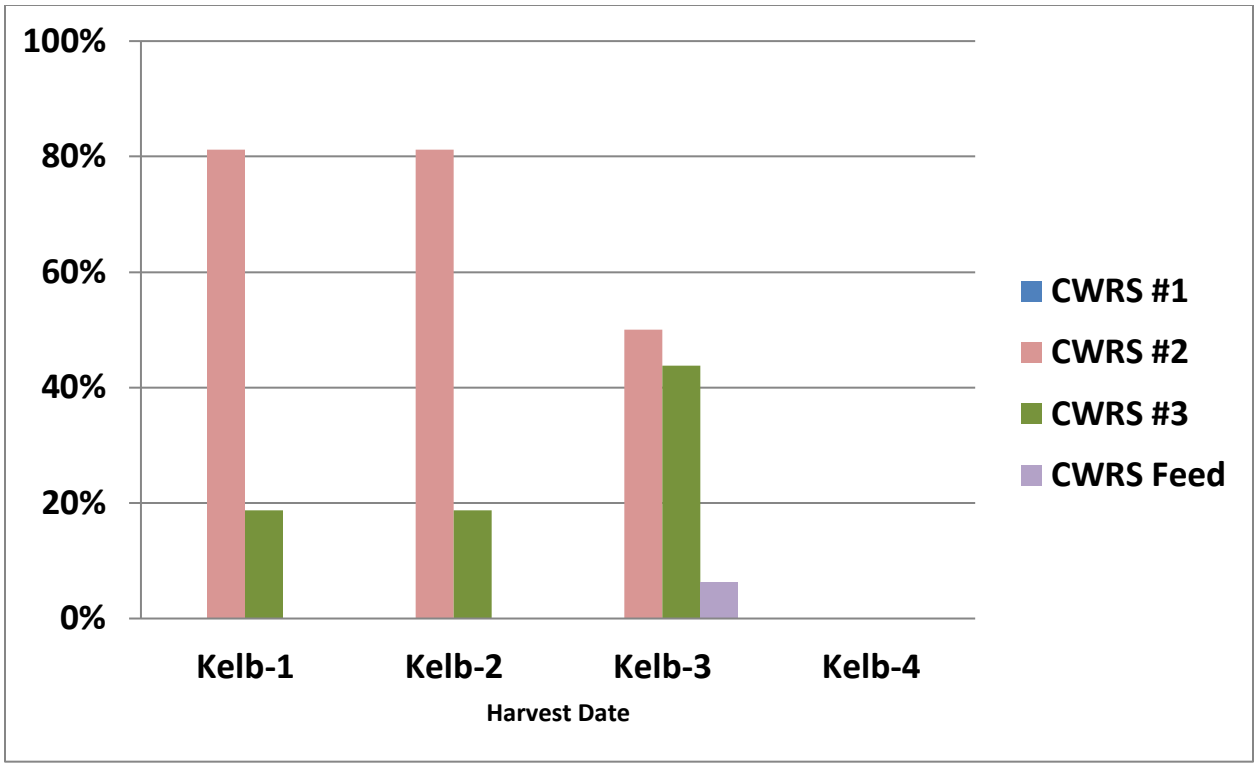


Figure 4.8 Percentage of samples with specific grades for combined genotypes by harvest date at Kelburn.

Midge damage was, again, the most common grading factor at Kelburn (Figure 4.9). Kelburn FDK values were the highest compared to the other locations and the slightly higher levels in H3 were sufficient to downgrade some samples to feed. There was also some low-level sprout damage for all three harvests, but no severely sprout-damaged grain.

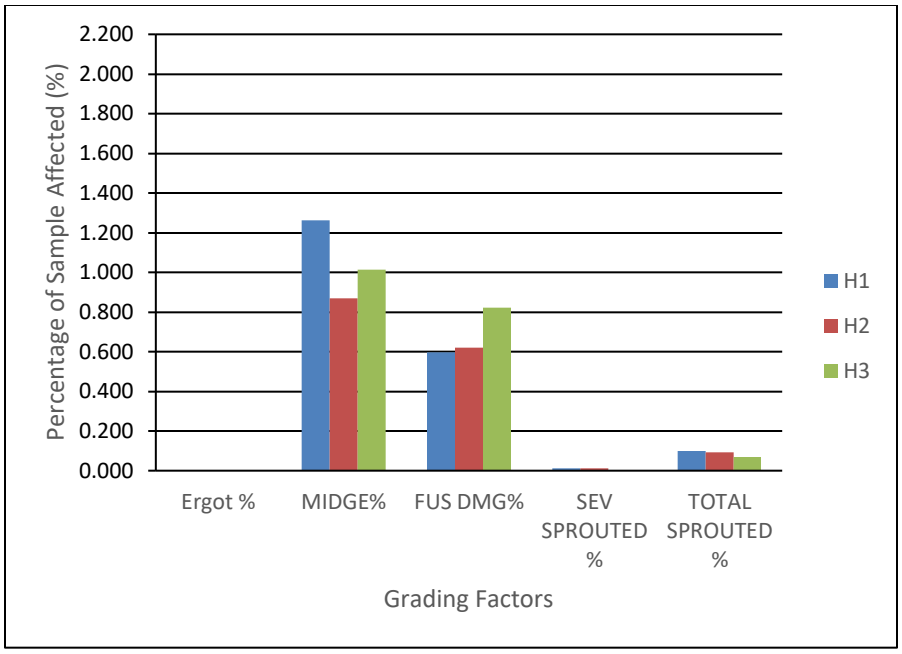


Figure 4.9 Distribution of grading factors and their effect on samples, for combined genotypes by harvest dates at Kelburn. Grading factors include ergot, midge, Fusarium damage (FUS DMG), severely sprouted (SEV SPROUTED) and total sprouted.

For FDK, TKW and %GPC, the location effect and location*genotype interaction contributed the largest amount to the total variance (Table 4.5). For the FDK and TKW parameters, the location effect was the largest source of variance, with 42.35 % and 25.64%, respectively. For %GPC, the location*genotype interaction was the largest contributor to variance at 30.11%, followed by the location effect at 16.90% and the block (location) effect at 15.39%. For these three parameters, the location*genotype interaction was highly significant (P<0.001). The test weight parameter was different. The effect of harvest date contributed the largest amount of variance, 55.52%, followed by the genotype effect, contributing 31.25%. Both of these factors were also highly significant (P<0.001). For the test weight parameter all of the main effects and their interactions were significant. The lack of significant genotype effects on %FDK, TKW and %GPC contrasts with the results of the pesticide treatment study (Table 2.5).

Table 4.5 Global analysis of variance for percent Fusarium damaged kernels (%FDK), thousand kernel weight (TKW), test weight and grain protein concentration percent (%GPC) for all sites.

		%FDK			TKW			Test Weight			%GPC		
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F	MS	PV	Pr>F	MS	PV	Pr>F
Genotype	3	0.3321	7.55	0.2595	5.1978	8.05	0.3740	55.7865	31.25	<.0001 ***	1.9783	12.72	0.3464
Harvest Date	3	0.1713	3.90	0.0084 **	1.6029	2.48	0.4711	99.0936	55.52	<.0001 ***	0.1240	0.80	0.6545
Location	3	1.8614	42.35	0.0097 **	16.5497	25.64	0.1028	13.7069	7.68	0.0042 **	2.6293	16.90	0.3694
Block (Location)	12	0.0400	3.64	0.7815	1.2669	7.85	0.0423 *	0.1473	0.33	0.0086 **	0.5985	15.39	<.0001 ***
Genotype*Harvest	9	0.0244	1.66	0.5014	1.8263	8.49	0.0034 **	0.5766	0.97	0.0014 **	0.1376	2.65	0.6632
Location*Genotype	9	0.2071	14.14	<.0001 ***	4.4010	20.45	<.0001 ***	1.1161	1.88	<.0001 ***	1.5614	30.11	<.0001 ***
Location*Harvest	8	0.0213	1.29	0.5754	1.7278	7.14	0.0058 **	0.9336	1.39	<.0001 ***	0.2207	3.78	0.3468
Location*Geno*Harvest	22	0.0254	4.23	0.9854	0.4497	5.11	0.7934	0.1221	0.50	0.0121 *	0.1845	8.70	0.0215 *
Residual	46	0.0609	21.23	-	0.6225	14.79	-	0.0555	0.48	-	0.0908	8.95	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at *P* < 0.05, 0.01, and 0.001, respectively

Type III analysis of variance for individual sites for %FDK (Table 4.6) showed that at the Brandon location, the genotype, genotype*treatment and block*treatment effects were all significant ($P<0.01$). The genotype effect was also significant at the Grosse Isle location. These were the only two locations to show significant effects for %FDK. Unlike the pesticide treatment study (Table 2.8), the differences in FDK levels by genotype in the harvest treatment study did not reflect their differences in FHB resistance (Table 4.6). The differences in %FDK by genotype between locations is also apparent in Table 4.6 and is a factor behind the significant genotype*location interaction (Table 4.5). Although the harvest treatment effect on %FDK was significant in the global analysis of variance (Table 4.5), it was not significant at any of the individual locations (Table 4.6). Mean %FDK by location was considerably different (Table 4.6) with a range from 0.09% at the Brandon location to 0.64% at the Kelburn location. This explains the significant location effect on %FDK in the global analysis of variance (Table 4.5).

Table 4.6 Means comparisons and ANOVA for percent Fusarium damaged kernels (%FDK) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	0.13 A[c]	0.18	0.50 A	0.50	0.32 AB
Carberry	0.10 A	0.34	0.67 A	0.58	0.40 A
Glenn	0.09 A	0.41	0.16 B	0.55	0.26 B
Harvest	0.03 B	0.42	0.66 A	0.96	0.44 A
Harvest Treatment					Mean
Harvest 1[d]	0.04	0.28	0.42	0.55	0.30
Harvest 2[e]	0.09	-[h]	0.36	0.60	0.28
Harvest 3[f]	0.09	0.38	0.53	0.76	0.42
Harvest 4[g]	0.12	0.34	0.60	-[i]	0.42
Site-year Mean	0.09	0.33	0.48	0.64	
Type III Analysis of Variance					
Genotype	0.0039 **[j]	0.1067	0.0230 *	0.5348	
Harvest treatment	0.6576	-[k]	0.3132	0.6172	
Genotype*Harvest	0.0046 **	0.3177	0.8198	0.9112	
Block	0.4400	0.0640	0.5540	0.9840	
Block*Harvest	0.0022 **	0.9842	0.4880	0.6202	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [k]Value unavailable

Type III analysis of variance for the test weight parameter (Table 4.7) showed both genotype and harvest treatment were highly significant at all four of the study locations, which is consistent with the results in Table 4.5. The effect contributing most to test weight variance was harvest date, followed by genotype. The genotype*harvest treatment interaction was also significant for Brandon, Grosse Isle and Kelburn (Table 4.7), consistent with the significant genotype*harvest interaction in Table 4.5. Thus, the test weight response to delayed harvest differed across the different genotypes. In Table 4.7, mean test weight values generally declined with each additional harvest date, with the highest for H1, followed by H2 which was significantly higher than both H3 and H4. In general, test weight declined as a result of delayed harvest, which is evident at all locations but the absolute mean values were still above the threshold of 75 kg hl^{-1} required for a CWRS No.1 grade. Overall, the Glenn genotype had the highest test weight values, followed by the Brandon and Carberry genotypes which were significantly higher than Harvest (Table 4.7). This mirrors the results of the pesticide treatment study in which the test weight was highest for Glenn followed by Carberry and then Harvest (Table 2.9). Across all locations, the main effects and interactions were significant for test weight (Table 4.5). Test weight is clearly sensitive to many factors including genotype, delayed harvest and growing season weather differences by location. However, the test weight response varied by genotype with harvest date, by genotype with location, by location with harvest date as well as between locations, with genotype and harvest date. Nevertheless, even though all of these 2- and 3-way interactions are statistically significant, in total, they account for less than 5% of the variance in test weight (Table 4.5).

Table 4.7 Means comparisons and ANOVA for test weight (kg hL^{-1}) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	82.13 B[c]	78.76 B	80.72 B	81.36 B	80.74 B
Carberry	81.84 B	79.47 B	80.82 B	81.34 B	80.85 B
Glenn	84.24 A	82.54 A	83.62 A	83.19 A	83.35 A
Harvest	80.33 C	78.94 B	79.66 C	80.44 C	79.77 C
Harvest Treatment					Mean
Harvest 1[d]	84.03 A	82.26 A	83.48 A	82.94 A	83.19 A
Harvest 2[e]	83.80 A	-[h]	82.40 B	82.47 B	82.62 B
Harvest 3[f]	80.40 B	78.48 C	80.08 C	79.34 C	79.59 C
Harvest 4[g]	80.30 B	79.03 B	78.86 D	-[i]	79.32 C
Site-year Mean	82.13	79.92	81.20	81.58	
Type III Analysis of Variance					
Genotype	<.0001 ***[j]	0.0005 ***	<.0001 ***	<.0001 ***	
Harvest Treatment	<.0001 ***	0.0013 **	<.0001 ***	0.0009 ***	
Genotype*Harvest	0.0301 *	0.7265	0.0149 *	0.0410 *	
Block	0.4240	0.1808	0.0184 *	0.6786	
Block*Harvest	0.4790	0.8267	0.0777	0.4346	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

The Type III analysis of variance for TKW (Table 4.8) showed that the effect of genotype was significant at both the Brandon and Kelburn locations. This was despite the relatively low contribution of genotype to variance of TKW (Table 4.5). The genotype*harvest interaction was also significant in the global ANOVA in Table 4.5. Although this interaction contributed less to total variance than the residual, it is an indication that the TKW for the four genotypes in this study responded differently to a delayed harvest. The main contributors to variance were location and the location*genotype interaction (Table 4.5). The mean values certainly differed between locations (Table 4.8), with Kelburn having the largest mean values across the all genotypes and Grosse Isle having the lowest mean values. Location also had the largest contribution to variance in the pesticide treatment study (Table 2.5), indicating that differences in growing season weather between locations is the most significant factor affecting TKW. However, the highest and lowest TKW values by genotype varied by location (Table 4.8), which explains the significant location*genotype interaction (Table 4.5). The third significant 2-way interaction identified in the global ANOVA, location*harvest, contributed less than half of the variance that was due to residual error and none of the differences between means for the harvest dates across locations were large enough to be statistically significant.

Table 4.8 Means comparisons and ANOVA for thousand kernel weight (g) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	33.48 A[c]	30.75	30.98	31.72 B	31.85 AB
Carberry	33.05 A	32.16	31.24	32.88 A	32.46 A
Glenn	31.65 B	31.80	30.22	32.31 AB	31.47 B
Harvest	30.30 C	31.33	30.55	32.90 A	31.38 B
Harvest Treatment					Mean
Harvest 1[d]	32.36	31.18	30.17	32.28	31.55
Harvest 2[e]	31.96	-[h]	31.10	32.17	31.85
Harvest 3[f]	31.85	31.13	30.54	32.91	31.61
Harvest 4[g]	32.31	32.22	31.18	-[i]	32.16
Site-year Mean	32.12	31.51	30.75	32.45	
Type III Analysis of Variance					
Genotype	0.0025 **[J]	0.1595	0.2692	0.0072 **	
Harvest Treatment	0.8465	0.2200	-[k]	0.1391	
Genotype*Harvest	0.2367	0.1753	0.6772	0.0106 *	
Block	0.1698	0.7585	0.0991	0.2263	
Block*Harvest	0.1501	0.1584	0.9734	0.0646	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [k]Value unavailable

Type III analysis of variance for the grain protein parameter (Table 4.9) showed no effect of harvest date as a main effect or as a factor in any of the 2-way interactions. Location, as a main factor, did not influence %GPC. These results differ from those of the pesticide treatment study, which showed a significant effect of location (Table 2.5). However, genotype affected %GPC at two of the four study locations (Brandon and Grosse Isle), resulting in a significant genotype*location interaction that is also the largest contributor to variance (Table 4.5). The significant 3-way interaction of location*genotype*harvest also indicated that the grain protein concentration by genotype varied not only with location but also by harvest date; however, the contribution to variance from this 3-way interaction is very small, less than that for the residual (Table 4.5).

Table 4.9 Means comparisons and ANOVA for grain protein concentration (%) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	14.79 AB[c]	14.89	13.82 B	14.36	14.46 B
Carberry	14.89 A	15.24	14.96 A	14.87	15.00 A
Glenn	14.35 B	15.00	14.75 A	14.70	14.71 AB
Harvest	13.16 C	14.86	14.75 A	15.09	14.37 B
Harvest Treatment					Mean
Harvest 1[d]	14.21	15.11	14.30	14.88	14.63
Harvest 2[e]	14.12	-[h]	15.03	14.74	14.67
Harvest 3[f]	14.43	14.91	14.39	14.65	14.54
Harvest 4[g]	14.44	14.98	14.56	-[i]	14.70
Site-year Mean	14.30	15.00	14.57	14.76	
Type III Analysis of Variance					
Genotype	0.0021 **[j]	0.4644	0.0015 **	0.0716	
Harvest Treatment	0.0914	0.9845	0.1293	0.5622	
Genotype*Harvest	0.6785	0.1228	0.3520	0.0452 *	
Block	0.6717	0.3751	0.0003	0.3995	
Block*Harvest	0.7348	0.1387	0.2537	0.3223	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

4.4.3 Flour Quality

Location was the largest source of variance for flour ash and flour yield, with 47.32% and 41.09%, respectively (Table 4.10). For both parameters, genotype contributed the next largest amount to variance followed by the location*genotype interaction, and both effects were significant. The factors affecting flour protein parameter differed, as the location*genotype interaction contributed the largest amount of variance, at 32.40%. This was followed by the location effect, 19.65% and the block (location) effect, 17.02%. The location*genotype interaction, the block (location) effect and the 3-way interaction of location*genotype*treatment were all significant. Harvest date had a significant effect on flour ash, but not on flour yield or flour protein.

For all of the flour quality parameters, the differences between locations (i.e., growing season weather) or interactions of genotype with location were the main source of variation. Genotype was also a large source of variation for the ash and flour yield parameters. The interaction between genotype and location was the largest contributor to variance for the flour protein parameter showing that genotypic variation in response to growing season weather had a substantial impact on flour protein. The lack of genotype effect on flour protein is consistent with the lack of effect noted for %GPC (Table 4.5), but differs from the pesticide treatment study which showed a significant and substantial impact of genotype on flour protein (Table 2.13). This likely because the genotypes Stettler and Stanley, which had significantly different %GPC in the pesticide study, were not included in the delayed harvest study.

Table 4.10 Global analysis of variance of flour ash, flour yield and flour protein for all sites.

		Ash			Flour Yield			Flour Protein		
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F	MS	PV	Pr>F
Genotype	3	0.0069	24.71	0.0039 **	9.5181	36.35	0.0017 **	1.0234	6.24	0.6467
Harvest Date	3	0.0024	8.57	0.0004 ***	0.1010	0.39	0.7744	0.3019	1.84	0.997
Location	3	0.0132	47.32	0.0003 ***	10.7606	41.09	0.001 ***	3.2214	19.65	0.3181
Block (Location)	12	0.0001	2.02	0.0898	0.1862	2.84	0.0166 *	0.6974	17.02	<.0001 ***
Genotype*Harvest	9	0.0002	1.96	0.0525	0.0586	0.67	0.7199	0.1148	2.10	0.8902
Location*Genotype	9	00007	7.79	<.0001 ***	0.7806	8.94	<.0001 ***	1.7705	32.40	<.0001 ***
Location*Harvest	8	0.0001	1.09	0.2333	0.2700	2.75	0.0164 *	0.1033	1.68	0.9052
Location*Geno*Harvest	22	0.0001	2.07	0.5136	0.0864	2.42	0.3681	0.2541	11.37	0.0006 ***
Residual	46	0.0001	4.46	-	0.0776	4.54	-	0.0824	7.70	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

Type III analysis of variance for ash (Table 4.11) showed that the genotype effect was significant for three of the four locations. The harvest treatment effect was also significant overall, with a small decline in flour ash with delays in harvest. These results were consistent with those observed in the global analysis of variance (Table 4.10). The genotype effect was the second largest contributor to variance, and it was significant at a level of 0.01. The harvest treatment effect was the third largest contributor to variance, at 8.57%. The Harvest genotype had the largest flour ash values overall, while the Brandon and Glenn genotypes had significantly lower values than Harvest overall and at 3 of 4 individual sites (Table 4.11). This pattern is consistent with genotype effect on flour ash in the pesticide study (Table 2.14). The location effect was the largest contributor to variance (Table 4.10). Location was also the largest source of variance for flour ash in the pesticide treatment study (Table 2.13). Thus, variation in growing season has consistently been the largest factor affecting this parameter. The significant decline in flour ash with delayed harvest (Table 4.11) is surprising considering that there was also a significant decline in test weight with delayed harvest (Table 4.7).

Table 4.11 Means comparisons and ANOVA for flour ash (%) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	0.34 B[c]	0.36	0.38 B	0.38 B	0.37 B
Carberry	0.35 AB	0.36	0.39 B	0.40 B	0.37 B
Glenn	0.35 AB	0.36	0.37 B	0.39 B	0.37 B
Harvest	0.36 A	0.38	0.43 A	0.43 A	0.40 A
Harvest Treatment					Mean
Harvest 1[d]	0.36 A	0.38 A	0.41	0.41	0.39 A
Harvest 2[e]	0.36 AB	-[h]	0.39	0.40	0.38 B
Harvest 3[f]	0.34 C	0.35 B	0.38	0.39	0.37 C
Harvest 4[g]	0.34 BC	0.36 AB	0.38	-[i]	0.37 BC
Site-year Mean	0.35	0.37	0.39	0.40	
Type III Analysis of Variance					
Genotype	0.0071 **[j]	0.0789	0.0007 ***	0.0045 **	
Harvest Treatment	0.0266 *	0.0086 **	0.1362	0.7001	
Genotype*Harvest	0.0308 *	0.1204	0.6205	0.9139	
Block	0.8933	0.0870	0.0248 *	0.3441	
Block*Harvest	0.0356 *	0.3328	0.8216	0.8791	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

Type III analysis of variance for flour yield (Table 4.12) showed a highly significant genotype effect across all locations. This is consistent with the significant genotype effect and its large contribution to variance in the global analysis of variance (Table 4.10) as well as that in the pesticide treatment study (Table 2.13). The Brandon genotype had the highest flour yield values at three of the four sites, and it was followed closely by the Harvest genotype (Table 4.12). The Carberry and Glenn genotypes had very similar mean flour yield values across all sites, and for the overall mean values. The Harvest genotype had higher flour yield values than Glenn and Carberry in the pesticide treatment study as well (Table 2.15). The harvest treatment effect was not significant for flour yield in the global analysis of variance (Table 4.10). At the Carberry location (Table 4.12) there was a significant harvest treatment effect, however the differences between means were not large enough to be detected by means comparison. The main differences in flour yield values occurred between the four different locations, as shown in the global analysis of variance (Table 4.10), where location had the largest contribution to variance. This is consistent with the pesticide treatment study in which the location effect also had the largest contribution to variance of flour yield (Table 2.13). The location*genotype and location*harvest interactions were both significant, but the latter contributed less to variance than the residual, indicating that it is not an important factor. The former is a result of the slightly different genotype effect on flour yield between locations. Thus, differences in growing season weather between locations is the most important factor affecting flour yield and delayed harvest has minimal impact.

Table 4.12 Means comparisons and ANOVA for flour yield (%) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	73.88 A[c]	74.03 A	73.13 A	73.56 A	73.66 A
Carberry	73.10 B	72.99 B	71.21 C	72.00 B	72.26 C
Glenn	72.33 C	72.93 B	71.61 C	72.20 B	72.26 C
Harvest	73.33 AB	74.19 A	72.18 B	72.38 B	73.02 B
Harvest Treatment					Mean
Harvest 1[d]	73.24	73.31A	72.04	72.59	72.78
Harvest 2[e]	72.86	-[h]	72.23	72.46	72.77
Harvest 3[f]	73.26	73.61 A	71.96	72.56	72.81
Harvest 4[g]	73.29	73.70 A	71.89	-[i]	72.85
Site-year Mean	73.16	73.54	72.03	72.54	
Type III Analysis of Variance					
Genotype	0.0012 **[j]	0.0003 ***	<.0001 ***	0.0091 **	
Harvest Treatment	0.2107	0.0196 *	0.3518	0.5429	
Genotype*Harvest	0.1587	0.1980	0.1441	0.8406	
Block	0.2472	0.0648	0.3102	0.3642	
Block*Harvest	0.0939	0.3349	0.1775	0.7194	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

Type III analysis of variance for flour protein (Table 4.13) showed that three of the four locations (Carberry being the exception) showed a significant genotype effect. The result was a significant location*genotype interaction (Table 4.10). The location*genotype interaction was the largest contributor to variance for the global analysis of variance. The reason is that the Harvest genotype had significantly higher flour protein than the Brandon genotype at two locations, but the Brandon genotype flour protein was higher than the Harvest genotype flour protein at one location (Table 4.13). None of the sites showed a significant harvest treatment effect (Table 4.13), and the harvest treatment effect did not significantly contribute to variance, overall (Table 4.10). There was a significant genotype*harvest interaction at the Kelburn location (Table 4.13) similar to wheat protein (Table 4.9), resulting in a significant 3-way interaction on flour protein (Table 4.10). However, delayed harvest was not a critical factor affecting flour protein, which was affected much more by differences in genotype response by location (Table 4.10).

Table 4.13 Means comparisons and ANOVA for flour protein (%) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	Mean
Genotype					
Brandon	13.92 A[c]	13.98	12.87 B	13.28 B	13.52 B
Carberry	13.83 A	14.34	13.90 A	13.77 AB	13.93 A
Glenn	13.50 A	14.34	13.89 A	13.95 AB	13.94 A
Harvest	12.41 B	14.20	13.96 A	14.43 A	13.65 AB
Harvest Treatment					Mean
Harvest 1[d]	13.49	14.40	13.57	14.06	13.88
Harvest 2[e]	13.33	-[h]	14.09	13.87	13.82
Harvest 3[f]	13.41	14.15	13.38	13.65	13.61
Harvest 4g	13.43	14.09	13.57	-[i]	13.72
Site-year Mean	13.41	14.21	13.65	13.86	
Type III Analysis of Variance					
Genotype	0.0079 **[j]	0.4271	0.0003 ***	0.0193 *	
Harvest Treatment	0.4568	0.6339	0.2170	0.3388	
Genotype*Harvest	0.6572	0.2285	0.0721	0.0474 *	
Block	0.2634	0.7054	<.0001 ***	0.3614	
Block*Harvest	0.9223	0.4310	0.1088	0.3737	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

4.4.4. Rheological Properties

The global analysis of variance for MDT, WIP and WAP (Table 4.14), showed that although the harvest treatment effect was statistically significant for all three rheological properties, the contribution of harvest date to variance was small and much less than that from the residual. The genotype effect on all three parameters was also significant, and it accounted for the largest contribution to variance (57.73% for MDT, 65.09% for WIP and 68.33% for WAP). The large difference in the instrinsic gluten strength between the strongest (Glenn) and weakest (Harvest) genotypes contributed to this result. The location effect was significant for the WIP and WAP parameters and contributed the second largest proportion of variance (ranging from 18.42% to 18.80% for WIP and WAP). These results are very similar to those from the pesticide treatment study (Table 3.2), except for the location effect on MDT in the pesticide treatment study, which was significant and the largest source of variance.

The genotype*location interaction was significant for all three parameters and contributed from 5.41% to 13.24% of the variance (Table 4.14), which, again, is similar to the results from the pesticide treatment study (Table 3.2). As mentioned in Chapter 3, WIP and WAP are the most accurate rheological measures of gluten strength and the significance of genotype reflects the large difference in strength characteristics between genotypes within the study.

Table 4.14 Global analysis of variance of MDT, WIP and WAP for all sites.

		MDT			WIP			WAP		
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F	MS	PV	Pr>F
Genotype	3	9.0725	57.73	0.0013 **	24924.00	65.09	<.0001 ***	11170.00	68.33	<.0001 ***
Harvest Date	3	0.5541	3.53	0.0022 **	448.00	1.17	0.011 *	139.60	0.85	0.0455 *
Location	3	2.0856	13.27	0.1073	7052.85	18.42	0.0115 *	3072.65	18.80	0.0035 **
Block (Location)	12	0.1190	3.03	0.0758	59.35	0.62	0.8931	35.09	0.86	0.598
Genotype*Harvest	9	0.0290	0.55	0.5262	95.55	0.75	0.2816	24.44	0.45	0.3407
Location*Genotype	9	0.6937	13.24	<.0001 ***	963.61	7.55	<.0001 ***	294.52	5.41	<.0001 ***
Location*Harvest	8	0.0443	0.75	0.2445	60.95	0.42	0.5736	32.88	0.54	0.1731
Location*Geno*Harvest	22	0.0313	1.46	0.9694	72.17	1.38	0.8805	20.21	0.91	0.9633
Residual	46	0.0660	6.44	-	114.89	4.60	-	41.15	3.86	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

Table 4.15 outlines the Type III analysis of variance for each location for MDT. Although the global ANOVA indicated a significant effect of harvest date on MDT (Table 4.14), the effect was relatively small, overall, and not large enough to create significant differences in harvest dates at any individual site. Overall, MDT was slightly larger for late harvest dates, compared to early harvest dates; however, delayed harvest did not have a large effect on MDT (Table 4.15). There was a highly significant genotype effect overall and for each of the four locations. MDT for the Glenn genotype was longer than for any of the other genotypes analyzed in the study, which reflects its intrinsicly strong gluten strength. Compared to Glenn, the Carberry and Brandon genotypes had significantly lower MDT with the Harvest genotype having the lowest values, except at the Brandon location, where the Brandon genotype had the lowest MDT value. These results are consistent with the significant genotype effect in the global analysis of variance for MDT, which identified a significant location*genotype interaction (Table 4.14). All other effects within the Type III analysis of variance were not significant at any of the locations (Table 4.15).

Table 4.15 Means comparisons and ANOVA for mixing development time (min) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	2.75 C[c]	2.96 BC	3.45 B	2.89 B	2.98 B
Carberry	3.55 B	3.09 B	2.81 C	2.84 B	3.10 B
Glenn	4.64 A	3.96 A	3.93 A	3.58 A	4.05 A
Harvest	3.31 BC	2.50 C	2.43 C	2.12 C	2.64 C
Harvest Treatment					Mean
Harvest 1[d]	3.41	3.02	2.98	2.79	3.05 B
Harvest 2[e]	3.37	-[h]	2.95	2.76	3.06 AB
Harvest 3[f]	3.73	3.17	3.37	3.03	3.33 A
Harvest 4[g]	3.73	3.19	3.33	-[i]	3.33 A
Site-year Mean	3.56	3.13	3.16	2.86	
Type III Analysis of Variance					
Genotype	0.0075 **[j]	0.0095 **	0.0007 ***	<.0001 ***	
Harvest Treatment	0.0553	0.6123	0.0884	0.3667	
Genotype*Harvest	0.9463	0.9086	0.7779	0.1417	
Block	0.4627	0.9805	0.0132	0.7398	
Block*Harvest	0.8026	0.5127	0.6396	0.1998	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

The Type III analysis of variance for individual sites for WIP (Table 4.16) was generally similar to that of MDT. Within the global and Type III analyses of variance, there were no significant means differences for harvest treatments or significant interactions with harvest treatment (Table 4.14 and Table 4.16). Thus, even though the global ANOVA identified an overall effect of harvest date on WIP, the impact of delayed harvest on WIP was not substantial. The genotype effect was highly significant, overall, and at all four study locations. Generally, Glenn had the largest WIP values, the Brandon and Carberry genotypes had similar values and the Harvest genotype had the smallest WIP values. However, the effect of genotype on WIP was somewhat different between locations (Table 4.16), which created a significant location*genotype interaction (Table 4.14). These differences explain the large contribution to variance for the genotype effect (Table 4.14) and mirror the genotype effect on WIP from the pesticide treatment study (Table 3.6). Unlike for MDT, the location effect on WIP was significant (Table 4.14). The Brandon location had the largest mean WIP values, while the Kelburn location had the lowest and the Carberry and Grosse Isle location values are similar.

Table 4.16 Means comparisons and ANOVA for work input to peak (%Torque*min) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	135.11 C[c]	140.51 B	151.14 B	123.77 B	136.58 B
Carberry	166.23 B	136.23 BC	124.20 C	120.71 B	137.29 B
Glenn	213.47 A	192.46 A	191.33 A	163.60 A	190.94 A
Harvest	146.90 BC	116.90 C	110.33 C	90.33 C	117.21 C
Harvest Treatment					Mean
Harvest 1[d]	160.91	144.17	138.15	123.80	141.84
Harvest 2[e]	160.11	-[h]	138.67	121.15	141.35
Harvest 3[f]	170.75	147.10	151.54	128.85	149.59
Harvest 4[g]	169.94	148.31	148.64	-[i]	149.24
Sitey-year Mean	165.43	146.53	144.25	124.60	
Type III Analysis of Variance					
Genotype	0.0030 **[j]	0.0030 **	0.0009 ***	<.0001 ***	
Harvest Treatment	0.1203	0.6966	0.3272	0.4851	
Genotype*Harvest	0.8987	0.8246	0.8635	0.1204	
Block	0.8987	0.9852	0.4422	0.4614	
Block*Harvest	0.6908	0.5097	0.8207	0.2738	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

The analysis for the work at peak parameter resulted in the same trends as observed for WIP. Within the global and Type III analyses of variance, there were no significant means differences for harvest treatments or significant interactions with harvest treatment (Table 4.14 and Table 4.17). Thus, even though the global ANOVA identified an overall effect of harvest date on WAP, the impact of delayed harvest on WAP was not substantial. Type III analysis of variance for individual sites for WAP (Table 4.17) showed that the only significant effect for all individual sites was for genotype and the location*genotype interaction. Again, Glenn generally had the largest WAP values across all individual sites as well as the overall mean and Harvest generally had the lowest values except for the Brandon location. However, the ranking of WAP for the genotypes varied somewhat across the various sites, due to a location*genotype interaction (Table 4.14 and 4.17). In the global analysis of variance (Table 4.14) for WAP, the genotype effect was highly significant, and was the largest contributor to variance, consistent with the results in Table 4.17. Similar to MDT and WIP, the location effect on WAP contributed the second largest amount to variance. Within the Type III analysis of variance there were no significant effects or interactions other than genotype at individual locations. Genotype was the dominant effect on WAP in the pesticide treatment study as well (Table 3.2); although in that case, there was a stronger location effect, likely because the data in that study were collected from a much larger number of site-years.

The important observation from the study of delayed harvest was no significant harvest treatment effect on WIP and WAP. There was a small but significant effect on MDT. Thus, the rheological properties of the wheat changed very little as a result of harvest delays.

Table 4.17 Means comparisons and ANOVA for work at peak (%Torque*min) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	79.03 C[c]	83.82 B	83.36 B	70.72 B	78.58 C
Carberry	102.52 B	89.12 B	74.74 BC	71.99 B	84.88 B
Glenn	133.96 A	126.67 A	119.18 A	100.13 A	120.48 A
Harvest	87.97 BC	75.92 B	67.30 C	56.48 C	72.51 D
Harvest Treatment					Mean
Harvest 1[d]	98.00	93.67	81.83	73.78	86.82
Harvest 2[e]	96.98	-[h]	84.67	74.21	87.20
Harvest 3[f]	104.60	94.47	88.49	76.49	91.11
Harvest 4[g]	103.90	93.51	89.59	-[i]	91.31
Site-year Mean	100.87	93.88	86.15	74.83	
Type III Analysis of Variance					
Genotype	0.0050 **[j]	0.0033 **	0.0001 ***	<.0001 ***	
Harvest Treatment	0.6209	0.9168	0.2479	0.4248	
Genotype*Harvest	0.9814	0.8746	0.6471	0.2954	
Block	0.5106	0.9672	0.7411	0.4130	
Block*Harvest	0.9867	0.3493	0.5303	0.4035	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

The WAP values in Figure 4.10 illustrate differences in gluten strength between the four different study locations and harvest treatments, averaged across all genotypes. An important result was the similarity of the WAP values of each harvest date for each location. There was no significant location*harvest interaction (Table 4.14). The WAP values were statistically the

same between harvests at all locations, although there was a pattern of slightly increasing values with later harvest dates.

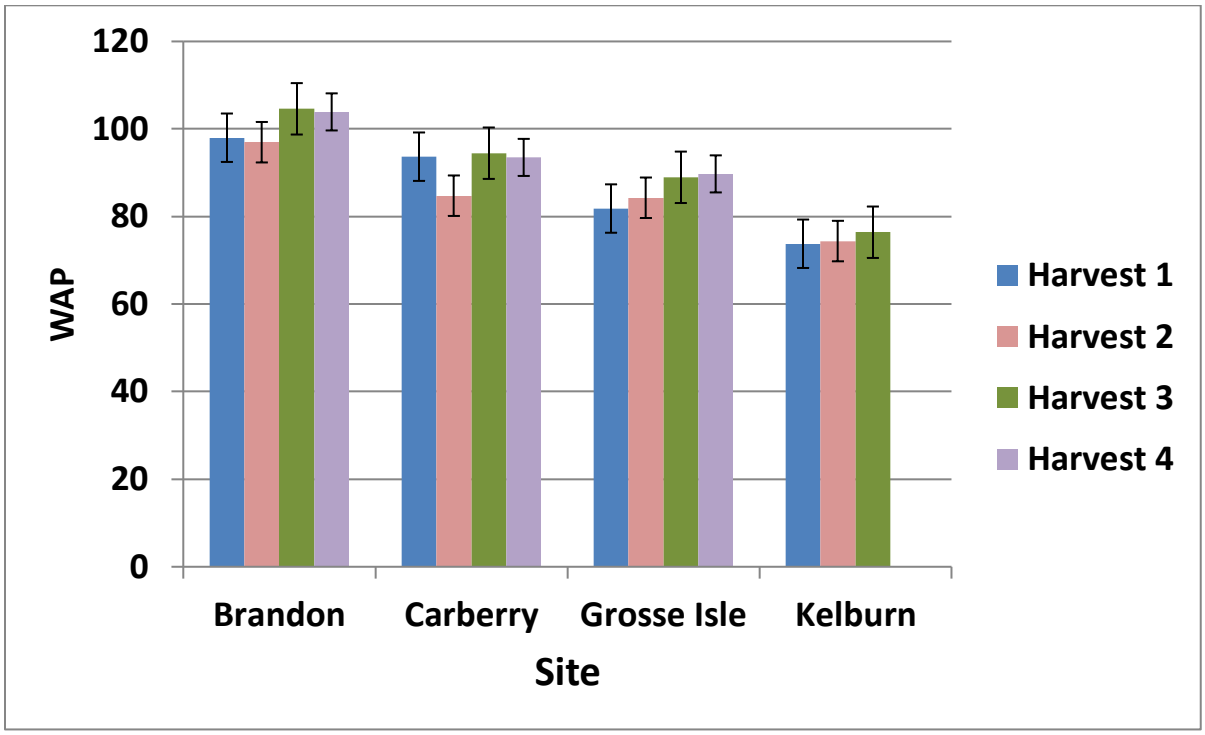


Figure 4.10 Work at peak (WAP) values for the four harvest dates, across all locations, with error bars showing the spread of values.

4.4.5. Protein Composition

Genotype had a significant effect and was the largest source of variance for insoluble glutenin (IG), IG/flour protein (IG/FP), soluble prolamin/flour protein (SP/FP) and the gluten strength index (GSI) (Tables 4.18 and 4.19). The location effect was the next largest contributor to variance for the IG, IG/FP and GSI parameters and was significant for all of these parameters. These results mirror those for the same parameters in the pesticide study in which genotype had a strong impact on protein composition (Tables 3.8 and 3.9). For SP, in the harvest delay study, the location*genotype effect was significant and the largest contributor to variance, at

31.97% (Table 4.19). The genotype, location and block (location) effects on SP had similar contribution to variance (ranging from 13.13% to 14.52%) but only the block (location) was significant. Although the delayed harvest treatment had a significant effect on IG/FP, SP/FP and GSI, it is important to note that it contributed less than 2% of total variance and was always smaller than the residual, for all parameters. Thus, delayed harvest had minimal impact on these protein composition parameters.

Table 4.18 Global analysis of variance of IG and IG/FP for all sites.

		IG			IG/FP		
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F
Genotype	3	2.6902	62.51	<.0001 ***	0.01062	59.24	<.0001 ***
Harvest Date	3	0.0228	0.53	0.1572	0.00031	1.74	0.0004 ***
Location	3	0.8613	20.01	0.0015 **	0.00410	22.90	0.0038 **
Block (Location)	12	0.0352	3.28	0.0193 *	0.00010	2.13	0.1258
Genotype*Harvest	9	0.0123	0.86	0.7852	0.00004	0.65	0.2841
Location*Genotype	9	0.0476	3.32	0.0507	0.00041	6.80	<.0001 ***
Location*Harvest	8	0.0100	0.62	0.8528	0.00002	0.22	0.8309
Location*Geno*Harvest	22	0.0206	3.52	0.1807	0.00003	1.20	0.9644
Residual	46	0.0151	5.36	-	0.00006	5.12	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

Table 4.19 Global analysis of variance of SP, SP/FP and GSI for all sites.

		SP			SP/FP			GSI		
	DF[a]	MS[b]	PV[c]	Pr>F[d]	MS	PV	Pr>F	MS	PV	Pr>F
Genotype	3	1.6152	13.13	0.3571	0.0194	65.48	<.0001 ***	0.04574	75.51	<.0001 ***
Harvest Date	3	0.1162	0.94	0.2641	0.0006	1.87	0.0179 *	0.00022	0.37	0.0267 *
Location	3	1.6362	13.30	0.4463	0.0008	2.71	0.5415	0.00710	11.72	0.013 *
Block (Location)	12	0.4464	14.52	<.0001 ***	0.0002	3.00	0.6785	0.00024	1.59	0.0458
Genotype*Harvest	9	0.0870	2.12	0.8643	0.0002	2.41	0.4281	0.00013	0.62	0.6237
Location*Genotype	9	1.3107	31.97	<.0001 ***	0.0003	3.22	0.2414	0.00102	5.05	0.0002 ***
Location*Harvest	8	0.0729	1.58	0.9022	0.0001	0.81	0.9078	0.00004	0.18	0.9711
Location*Geno*Harvest	22	0.1773	`0.57	0.0377 *	0.0002	5.55	0.7357	0.00016	1.92	0.2079
Residual	46	0.0952	11.86	-	0.0003	14.95	-	0.00012	3.03	-

[a]Degrees of freedom, [b]Mean square error, [c]Percent of variance associated with the effect, [d]F test significance where *, **, *** represent significant effects at *P* < 0.05, 0.01, and 0.001, respectively

Type III analysis of variance for individual sites, for the IG protein composition parameter (Table 4.20) confirmed the results of the global ANOVA. Neither the harvest date treatment nor the interactions with harvest date were significant for the IG parameter, overall, or at any of the locations. However, the genotype effect was significant overall and for all locations. This is consistent with the global analysis of variance that showed the genotype effect was highly significant, and the largest contributor to variance (Table 4.18), which is not surprising considering the variation in the gluten strength of the genotypes in this study. The mean IG values by genotype across combined sites showed a similar pattern to the rheological properties. The Glenn genotype had the largest IG value, while the Harvest genotype had the lowest. The genotype rank for IG values in Glenn, Carberry and Harvest in the pesticide treatment study showed a similar pattern (Table 3.10). In Table 4.18, the second largest contributor to variance was the location effect.

Table 4.20 Means comparisons and ANOVA for insoluble glutenin (%) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	3.51[c] B	3.56 B	3.27 C	3.22 B	3.38 C
Carberry	3.98 A	4.01 A	3.63 B	3.40 B	3.75 B
Glenn	4.02 A	4.22 A	4.04 A	3.77 A	4.03 A
Harvest	3.28 B	3.57 B	3.27 C	3.13 B	3.30 C
Harvest Treatment					Mean
Harvest 1[d]	3.66	3.84	3.49	3.42	3.60
Harvest 2[e]	3.62	-[h]	3.61	3.35	3.59
Harvest 3[f]	3.75	3.87	3.51	3.37	3.61
Harvest 4[g]	3.75	3.81	3.62	-[i]	3.66
Site-year Mean	3.70	3.84	3.56	3.38	
Type III Analysis of Variance					
Genotype	0.0015 **[j]	0.0010 ***	0.0001 ***	0.0117 *	
Harvest Treatment	0.4153	0.3252	0.2372	0.6060	
Genotype*Harvest	0.4443	0.1348	0.2407	0.7066	
Block	0.6547	0.0921	0.0025 **	0.5025	
Block*Harvest	0.2302	0.6390	0.7661	0.8177	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

The Type III analysis of variance for the IG/FP protein composition parameter (Table 4.21) follows a pattern that is similar to that for IG (Table 4.20). Neither the harvest treatment nor the genotype*harvest interaction effect were statistically significant at any of the locations. The harvest treatment effect (harvest date) was highly significant in the global analysis of variance (Table 4.18) but was not a large contributor to variance. This could explain why the means separation does not show statistical differences for IG/FP between harvest dates at any of the locations of the study (Table 4.21). The genotype effect on IG/FP was highly significant for each of the four locations and was also the largest contributor to variance (Table 4.18). Similar to WAP (Table 4.17), the Glenn genotype had the largest mean IG/FP values across all sites, followed by the Carberry genotype, the Brandon genotype and then the Harvest genotype with the lowest values (Table 4.21). This order for Glenn, Carberry and Harvest IG/FP values averaged across all sites is the same as that in the pesticide treatment study (Table 3.13). However, there were a few small differences in rankings of these genotypes across individual sites. These results are consistent with the significance of the effect of genotype and the location*genotype interaction on IG/FP in the global analysis of variance (Table 4.18). The location effect was the second largest contributor to variance of IG/FP.

Table 4.21 Means comparisons and ANOVA for insoluble glutenin/flour protein by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	0.251 B[c]	0.254 C	0.255 B	0.242 B	0.250 C
Carberry	0.287 A	0.279 B	0.261 B	0.247 B	0.269 B
Glenn	0.297 A	0.295 A	0.290 A	0.271 A	0.289 A
Harvest	0.264 B	0.252 C	0.234 C	0.217 C	0.243 D
Harvest Treatment					Mean
Harvest 1[d]	0.271	0.266	0.257	0.244	0.260
Harvest 2[e]	0.272	-[h]	0.256	0.242	0.260
Harvest 3[f]	0.278	0.274	0.262	0.247	0.265
Harvest 4[g]	0.279	0.270	0.265	-[i]	0.266
Site-year Mean	0.275	0.270	0.260	0.244	
Type III Analysis of Variance					
Genotype	0.0069 **[j]	<.0001 ***	<.0001 ***	0.0015 **	
Harvest Treatment	0.2705	0.3846	0.2084	0.7933	
Genotype*Harvest	0.5666	0.0897	0.3867	0.7785	
Block	0.3861	0.4482	0.0024	0.4139	
Block*Harvest	0.4043	0.0249 *	0.8334	0.6286	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

For the Type III analysis of soluble prolamin (SP), none of the main effects were significant for SP in the global analysis of variance (Table 4.19). This is the same result as for flour protein content (Table 4.10), which is expected because SP makes up the majority of flour protein. On an individual basis, the location and the genotype effects were not significant but did contribute approximately 13%, each, to total variance. Their interaction was more important than their individual effects. Neither the harvest date treatment nor the genotype*harvest interaction were significant for SP at any of the locations (Tables 4.19 and 4.22). The genotype effect was significant at all locations except Kelburn (Table 4.22), resulting in a location*genotype interaction (Table 4.18), which was the largest contributor to variance. There was also a significant location*genotype*harvest 3-way interaction with a very small contribution to variance.

Table 4.22 Means comparisons and ANOVA for soluble prolamin (%) by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	10.50 A[c]	10.48 A	9.53 B	9.79 AB	10.07 A
Carberry	9.68 B	9.96 AB	9.74 B	9.39 B	9.71 BC
Glenn	9.11 BC	9.78 B	9.67 B	9.34 B	9.51 C
Harvest	8.96 C	10.45 A	10.29 A	10.37 A	9.94 AB
Harvest Treatment					Mean
Harvest 1[d]	9.64	10.24	9.68	9.79	9.86
Harvest 2[e]	9.45	-[h]	10.09	9.77	9.80
Harvest 3[f]	9.56	10.14	9.57	9.61	9.70
Harvest 4[g]	9.60	10.11	9.90	-[i]	9.88
Site-year Mean	9.56	10.17	9.81	9.72	
Type III Analysis of Variance					
Genotype	0.0067 **[j]	0.0270 *	0.0142 *	0.0772	
Harvest Treatment	0.7156	0.9843	0.2586	0.5551	
Genotype*Harvest	0.4474	0.2526	0.5390	0.2494	
Block	0.6284	0.4109	0.0002 ***	0.9033	
Block*Harvest	0.4512	0.4600	0.4376	0.7840	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

Table 4.23 outlines the Type III analysis of variance for individual locations for SP/FP. Even though the harvest treatment effect was significant in the global ANOVA, there were no significant harvest treatment effects of harvest date on SP/FP at any of the locations (Table 4.23). However, the genotype*harvest treatment interaction was significant at the Carberry location. This relates to the global analysis of variance (Table 4.19) that shows a significant harvest treatment effect with a small contribution to variance and that the location*genotype*harvest interaction was the next largest contributor to variance (5.55%), even though it was not significant and less than the residual contribution. Again, the harvest date treatment displayed a very small effect on the SP/FP parameter in comparison to genotype. There was also a significant genotype effect overall and for each of the four locations. The Brandon genotype had the largest mean SP/FP value across all locations, followed by Harvest, then the Carberry genotype and, finally, Glenn, which had the lowest value of the four genotypes. This is the same ranking for SP/FP as that in the pesticide treatment student for Harvest, Carberry and Glenn (Table 3.14). The differences in SP/FP between the genotypes explains why the genotype effect was the largest contributor to variance in the harvest date study, at over 65% (Table 4.19).

Table 4.23 Means comparisons and ANOVA for soluble prolamin/flour protein by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	0.754 A[c]	0.752 A	0.743 A	0.734 A	0.745 A
Carberry	0.700 BC	0.691 C	0.703 B	0.681 BC	0.696 C
Glenn	0.676 C	0.683 C	0.696 B	0.673 C	0.683 D
Harvest	0.722 AB	0.737 B	0.735 A	0.717 AB	0.727 B
Harvest Treatment					Mean
Harvest 1[d]	0.716	0.712	0.713	0.698	0.710
Harvest 2[e]	0.709	-[h]	0.716	0.701	0.709
Harvest 3[f]	0.713	0.718	0.716	0.705	0.712
Harvest 4[g]	0.715	0.718	0.731	-[i]	0.720
Site-year Mean	0.713	0.716	0.719	0.701	
Type III Analysis of Variance					
Genotype	0.0052 **[j]	0.0001 ***	0.0025 **	0.0487 *	
Harvest Treatment	0.8149	0.8474	0.4012	-[k]	
Genotype*Harvest	0.2096	0.0205 *	0.2732	0.9550	
Block	0.3227	0.7730	0.7826	0.0418 *	
Block*Harvest	0.2431	0.0129 *	0.2449	0.9948	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [k]Value unavailable

The Type III analysis of variance for GSI (Table 4.24) shows that, similar to the other protein composition parameters, the effect of harvest treatment was much less important for GSI than the effect of genotype. Even though the effect of harvest date was statistically significant in the global ANOVA, its contribution to variance was less than 1% (Table 4.19), resulting in no differences between harvest treatment means overall or at any site (Table 4.24). In contrast, the genotype effect was highly significant for all four study sites and the genotype effect was a substantial contributor to variance of GSI, at over 75%, (Table 4.19). This was a consistent trend throughout the rheological property parameters and protein composition parameters analyzed. The mean values GSI for the four genotypes within the study show Glenn had the largest GSI values, followed by the Carberry genotype and the Harvest and Brandon genotypes with the lowest values. However, slight differences between genotype effects on GSI between locations (Table 4.24) explain the significant location*genotype interaction (Table 4.19). The Glenn, Carberry and Harvest genotypes showed the same order of significant differences in GSI in the pesticide treatment study (Table 3.12). Location also had significant effects on GSI, however, the contribution of location to variance was much smaller than for genotype.

Table 4.24 Means comparisons and ANOVA for gluten strength index by genotype[a] and by harvest treatment[b] for individual sites.

	Sites				
	Brandon	Carberry	Grosse Isle	Kelburn	
Genotype					Mean
Brandon	0.33 D[c]	0.34 C	0.34 C	0.33 C	0.34 C
Carberry	0.41 B	0.40 B	0.37 B	0.36 B	0.39 B
Glenn	0.44 A	0.43 A	0.42 A	0.40 A	0.42 A
Harvest	0.37 C	0.34 C	0.32 D	0.30 D	0.33 C
Harvest Treatment					Mean
Harvest 1[d]	0.38	0.38	0.36	0.35	0.37
Harvest 2[e]	0.39	-[h]	0.36	0.35	0.37
Harvest 3[f]	0.39	0.38	0.37	0.35	0.37
Harvest 4[g]	0.39	0.38	0.36	-[i]	0.37
Site-year Mean	0.39	0.38	0.36	0.35	
Type III Analysis of Variance					
Genotype	0.0001 ***[j]	<.0001 ***	<.0001 ***	0.0002 ***	
Harvest Treatment	0.5778	0.5062	0.7339	0.9331	
Genotype*Harvest	0.0465	0.0452	0.3261	0.5269	
Block	0.5131	0.4613	0.0434 *	0.6572	
Block*Harvest	0.0756	0.1655	0.5351	0.5118	

[a]Mean of four replicates of four harvest treatments, [b]Mean of four replicates of four genotypes, [c]Values followed by the same upper case letter in columns by genotype and by harvest treatment are not significantly different at p=0.05,[d]Harvest at physiological maturity, [e]Harvest when grain is dry, [f]Harvest 4 weeks after maturity, [g]Harvest 6 weeks after maturity, [h]Harvest date 2 was elimated due to missing data, [i]No data for Harvest date 4 due to predation by geese, [j]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively

Figure 4.11 illustrates the GSI values for each harvest date, at each of the study locations. While there were differences between the locations, the differences between each of the harvest dates were very small and not detectable with means separations. There was a tendency for a slight increase in GSI from the first two harvest dates, compared to the last two. These results are similar to those observed with the WIP and WAP mixograph parameters. The differences in protein composition between harvest dates were minimal and much less than the differences between genotype and location. This also supports the mixograph results that showed that gluten strength did not decline with later harvest dates, even though grades deteriorated. As harvest was delayed, the GSI values appeared to increase slightly after the second harvest date.

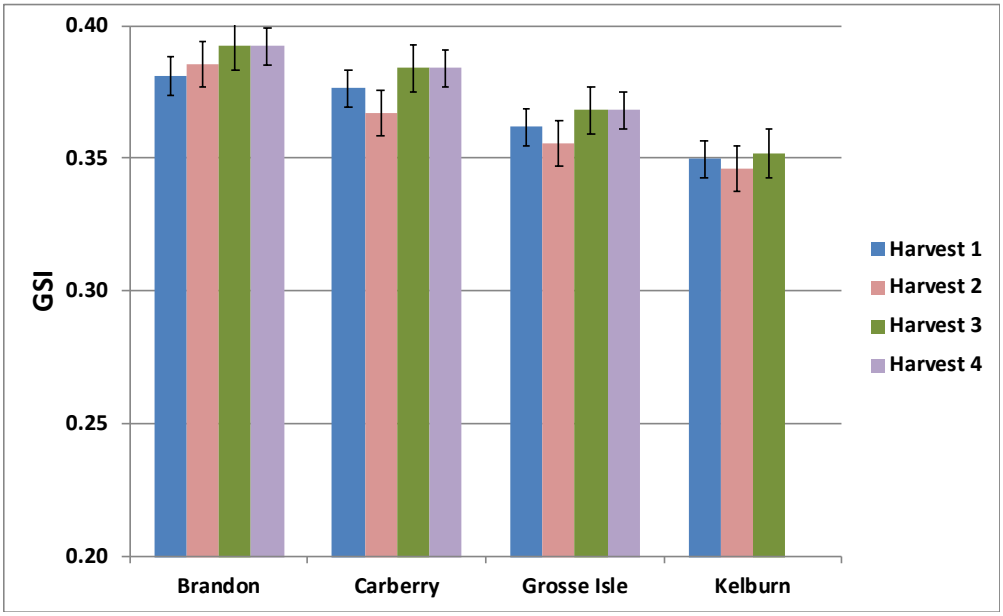


Figure 4.11 Gluten strength index (GSI) values for the four harvest dates, across all locations, with error bars showing the spread of values.

Figures 4.12 and 4.13 show the relationships between WAP and GSI, as well as WAP and IG/FP, by harvest date. These charts show relationships and ranges of values that are similar to those in the pesticide treatment study (Figures 3.8 and 3.13). Harvest dates 3 and 4 tended to be less prominent at lower values of WAP, GSI and IG/FP. The grain samples from H3 and H4, which had more time both to mature and to weather in the field, also had higher levels for these gluten strength indicators, in comparison to the samples from the earlier harvest dates.

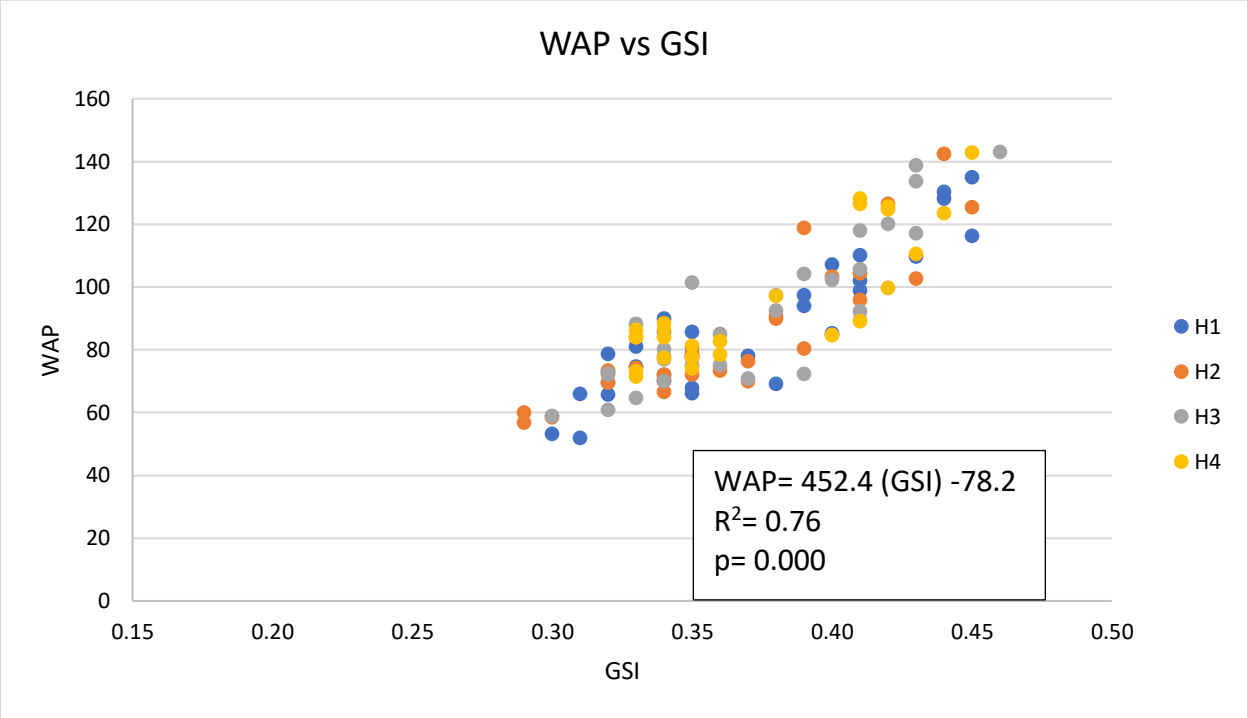


Figure 4.12 Relationship between work at peak (WAP) and gluten strength index (GSI) shown at the four harvest dates.

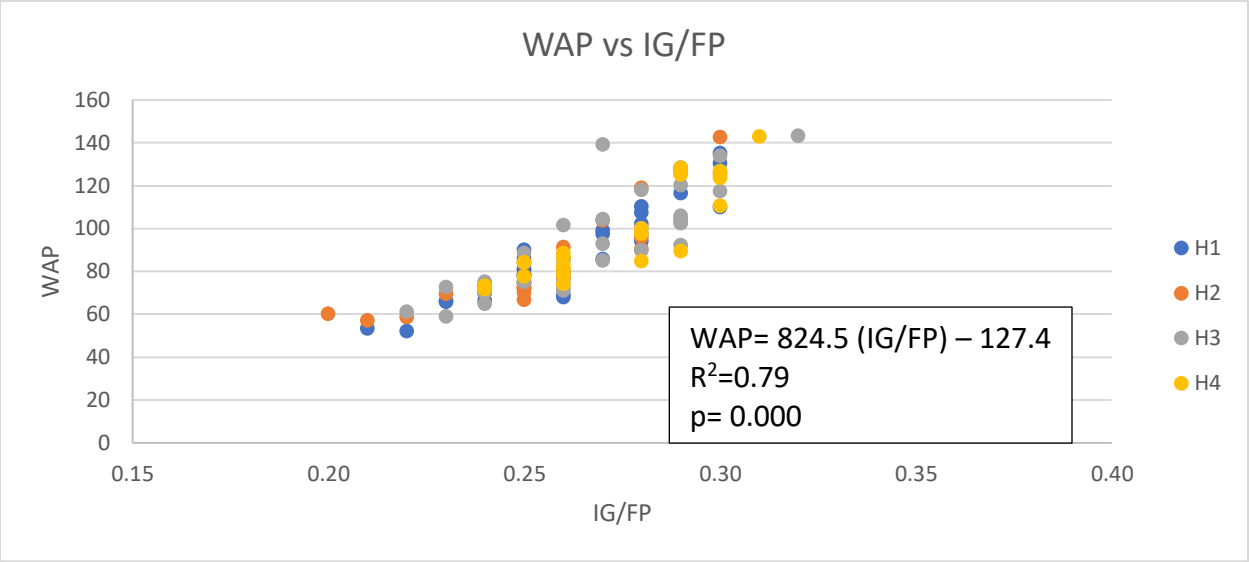


Figure 4.13 Relationship between work at peak (WAP) and insoluble glutenin/flour protein (IG/FP) shown at the four harvest dates.

Figures 4.14 and 4.15 show the same relationships between the WAP mixograph parameter, and the protein parameters of GSI and IG/FP, respectively, but by genotype. The genotypes known to have stronger gluten strength characteristics, Glenn and Carberry, had higher WAP values as well as higher GSI and IG/FP values compared to the Brandon and Harvest genotypes. The gluten strength pattern by genotype was consistent with the known gluten strength characteristics of each.

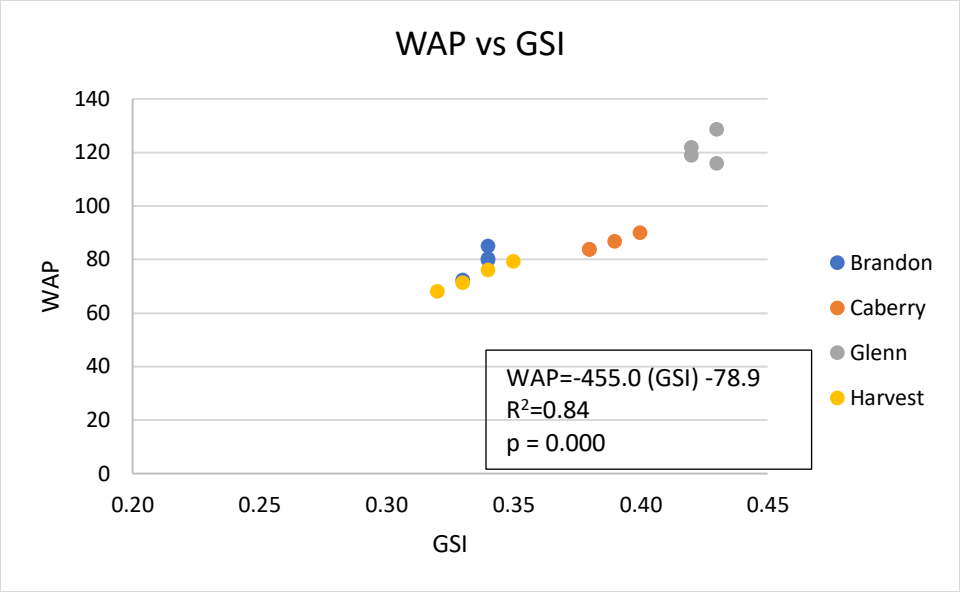


Figure 4.14 Relationship between work at peak (WAP) and gluten strength index (GSI), separated by genotype.

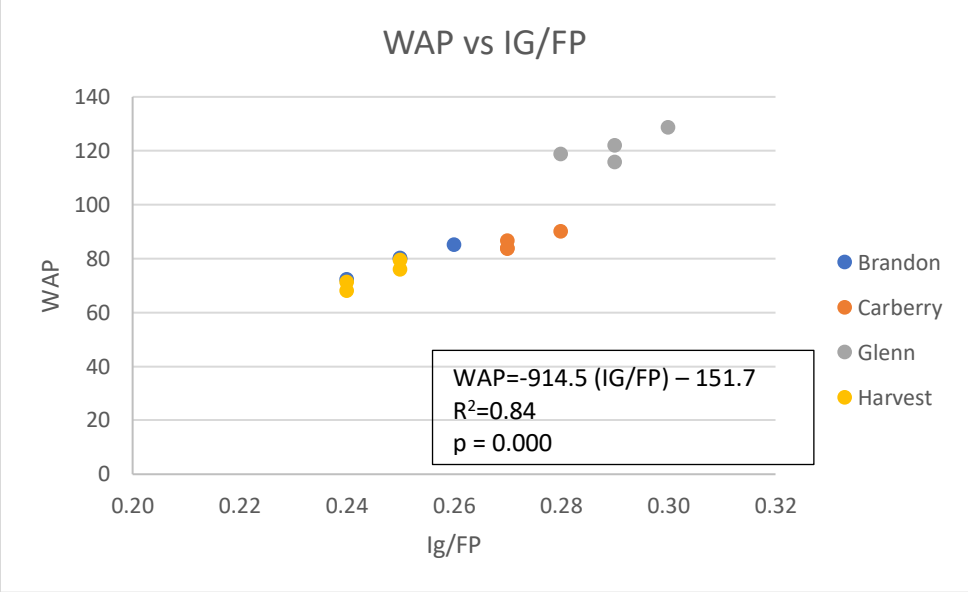


Figure 4.15 Relationship between work at peak (WAP) and insoluble glutenin/flour protein (IG/FP), separated by genotype.

4.5. Discussion

Harvesting a wheat crop when it is mature and as soon as it dries to a moisture content in the range of 13% to 15% is critical to ensure that the quality of the wheat is maintained. When harvest is delayed in the post-growing season, environmental conditions, especially significant precipitation, can adversely affect wheat quality. During the 2017 growing season, there was significant precipitation after the first two harvest dates of this study were completed. The precipitation after H2, the "normal" harvest period, resulted in poorer grades for the wheat samples from the delay in harvest. Between the third and fourth harvest dates rainfall was minimal at the Brandon and Carberry locations, but the Grosse Isle location received 56.4 mm of precipitation. The large rainfall events caused a noticeable deterioration of the grades for the wheat samples harvested afterwards. At the Grosse Isle location, the most serious downgrading occurred after Harvest 3, probably because this was also the location that experienced the greatest amount of precipitation between H3 and H4.

CWRS grades also varied between locations of the study due to the different growing season conditions at each location, and each location had its own unique grade distribution between harvest dates. All locations experienced midge damage, FDK and sprout damage, but in varying amounts that triggered slight to severe downgrading that was different at each location. The location effect as well as the location*genotype interaction were significant factors and the largest contributors to variance in the FDK, TKW and %GPC grain parameters (Table 4.5). Test weight was the only grain quality parameter to display a significant impact as well as a large contribution to variance from the delayed harvest treatment.

Genotype consistently affected all of the grain quality parameters at individual locations (Tables 4.6 to 4.9) and was the most consistent factor affecting grain quality parameters across harvests at these locations, but test weight was also significantly reduced by delayed harvest. This is consistent with results from Christensen and Legge (1984) who also found that test weight of grain was affected by the time of harvest. Farrer et al. (2006) also showed that a delayed harvest caused reductions in test weight that were closely related to the number of precipitation events observed during their study.

The location effect and the location*genotype interaction were also significant and the largest contributors to variance for flour quality (Table 4.10). However, the delayed harvest treatment had a significant effect only on flour ash. Since ash levels are related to test weight, this result is to be expected. Similar to the grain quality parameters, genotype also had the most consistent significant effect on flour quality by location. Through the analysis of means (Tables 4.11-4.13) the variation between locations was apparent. For the flour yield and protein parameters the Carberry site was found to have the largest mean values, while for flour ash, Grosse Isle and Kelburn had higher mean values, with the Brandon and Carberry sites having lower values.

The mixograph parameters, MDT, WIP and WAP all showed the genotype effect was by far the largest contributor to variance, followed by the location effect, and then location*genotype interaction (Table 4.14). Although the delayed harvest treatment was significant for all three parameters, the contribution to variance was small in all cases. In the Type III analysis of

variance, harvest date was not significant for any of the sites (for all parameters). Genotype was the only significant factor for all three parameters at the four individual locations. This was apparent in the means analysis of the mixograph parameters, there was a distinct pattern between the genotypes, with Glenn having the highest values, while Harvest generally had the lowest. For the individual locations, there was no significant harvest treatment effect on WIP and WAP and a very small effect on MDT, meaning that the delayed harvest was a minor factor for gluten strength. The WAP values for each of the harvest dates across all four of the locations (Figure 4.13) show differences by location but a limited amount of variation between harvest dates, within each location. Some locations showed a trend of increasing WAP values with later harvest dates, but the differences were relatively small.

The global analysis of variance for the protein composition parameters was similar to that for the mixograph parameters. Genotype and location caused the largest source variation for most of the parameters (Tables 4.18 and 4.19). The SP parameter was the outlier for the protein composition parameters, as it had the location*genotype interaction being the largest contribution to variance. Although the effect of delayed harvest treatment was significant in some cases, the contribution to variance was consistently small. Genotype was also the most consistent significant factor affecting the five protein composition parameters at the majority of locations. For the IG, IG/FP and GSI parameters the Glenn genotype was consistently the genotype with the highest mean values, while the Harvest genotype generally had the lowest. For the SP and SP/FP parameters this was reversed, as the Brandon and Harvest genotypes had the largest mean values, while the Glenn and Carberry genotypes had the lowest values. Even

though the global ANOVA identified harvest date as a statistically significant factor affecting IG/FP, SP/FP, and GSI, the contribution to variance was very small. Therefore, harvest treatment effects did not result in means differences for any of the protein composition parameters at any of the individual locations or collectively, across all locations.

There are many similarities between the results from this delayed harvest date study and the pesticide treatment study described in Chapters 2 and 3. The main effects contributing large amounts to the variance of the majority of the parameters analyzed in that study were related to the site-year or location and the genotype and not due to pesticide or harvest management practices. Thus, there was consistency in the results of the pesticide treatment study and the delayed harvest study in that both showed differing genetics and the variation in growing season weather conditions by location had the largest and most frequent significant contribution to variability in grain, flour, mixograph and protein composition parameters.

4.6. Conclusions

Delayed harvest dates had a significant impact on the grades of the wheat samples collected from each of the four locations. For locations and harvests that experienced significant rainfall beforehand, there were fewer CWRS No.1 samples and an increased number of lower grade samples. However, most of the quality parameters showed little response to the delayed harvest dates. The exceptions were test weight and flour ash, which were significantly reduced as a result of harvest delays. Although the delayed harvest treatment had a significant impact on both mixograph and protein composition, the contribution to variance was small. Thus,

delayed harvest had a limited impact on wheat gluten strength and, if anything, resulted in slightly stronger wheat samples.

The wheat grading system in Western Canada utilizes grading factors that can indicate adverse effects of biotic and abiotic factors on wheat quality. However, in this study, the reduced grades as a result of post-growing season precipitation did not result in reduced gluten strength. Both mixograph and protein composition analysis showed that gluten strength was, if anything, slightly stronger in the wheat samples from the delayed harvests. Thus, the downgrading of the samples due to delayed harvest did not accurately reflect their gluten strength characteristics.

4.7 References

Calderini, D.F., Abeledo, L.G., Slafer, G.A., 2000. Physiological maturity in wheat based on kernel water and dry matter. *Agronomy Journal* **92**: 895–901.

Canadian Grain Commission Official Grain Grading Guide. 2019.
https://www.grainscanada.gc.ca/en/grain-quality/official-grain-grading-guide/official-grain-grading-guide-2018-en.pdf

Christensen, J. V. and Legge, W. G. 1984. Effect of Harvest Time and Drying Method on the Yield, Quality and grade of the Hard Red Spring Wheat in Northwest Alberta. *Canadian Journal of Plant Science* **64**: 617-623.

Czarnecki, E. and Evans, L. E. 1986. Effect of Weathering During Delayed Harvest on Test Weight, Seed Size, and Grain Hardness of Wheat. *Canadian Journal of Plant Science* **66**: 473-482.

Dexter, J.E., Clear, R.M. and Preston, K.R. 1996. Fusarium Head Blight: Effect on the Milling and Baking of Some Canadian Wheats. *Cereal Chemistry* **73(6)**: 695-701.

Dexter, J.E., Marchylo, B.A., Clear, R.M. and Clarke, J.M. 1997. Effect of Fusarium Head Blight on Semolina Milling and Pasta-Making Quality of Durum Wheat. *Cereal Chemistry* **74**: 519–525.

Eggert, K., Rawel, H. M. and Pawelzik, E. 2011. In vitro degradation of wheat gluten fractions by Fusarium graminearum proteases. *European Food Research and Technology* **233**: 697-705.

Farrer, D., Weisz, R., Heiniger, R. Murphy, J. P. and Pate, M. H. 2006. Delayed Harvest Effect on Soft Red Winter Wheat in the Southeastern USA. *Agronomy Journal* **98**: 588-595.

Fonad, P., Acs, E., Cseuz, C. and Matuz, J. **2008.** Effects of harvest time on the quality components of winter wheat. *Cereal Research Communications* **36** Supplement: 127-130.

Nightingale, M.J., Marchylo, B.A., Clear, R.M., Dexter, J.E. and Preston, K.R. 1999. Fusarium Head Blight: effect of fungal proteases on wheat storage proteins. *Cereal Chemistry* **76**:150-158.

Wang, J. H., Wieser, H., Pawelzik, E., Weinert, J., Keutgen, A. J. and Wolf, G. A. 2005. Impact of the fungal protease produced by Fusarium culmorum on the protein quality and breadmaking properties of winter wheat. *European Food Research and Technology* **220**: 552-559.

5. GENERAL DISCUSSION AND OVERALL SYNTHESIS

5.1 Result Summary

This thesis research examined the effects of two different pesticide management practices as well as delayed harvesting on CWRS wheat quality. The overall objective of the research was to evaluate the relative contributions of genoptype and these management practices, and their interactions with the growing environment on grain quality, milling quality, gluten strength and protein composition. Particular emphasis was the development of an improved understanding of gluten strength because of its critical importance to CWRS breadmaking wheat.

In Chapter 2 and Chapter 3, the effects of pesticide applications on wheat, flour, dough and protein composition were studied in conjunction with growing environment conditions and genotype variation for three years (2015, 2016 and 2017) at four locations across Western Canada. The effects of four pesticide treatments (control, Prothioconazole/Tebuconazole fungicide, pre-harvest glyphosate and a combination of fungicide and pre-harvest glyphosate) were examined in responses of six CWRS genotypes having a broad range of gluten strength. The growing season weather conditions varied between years and locations which had a significant effect on the grain quality, flour quality, rheological properties and protein composition parameters. The analysis of site-year highlighted the significance of the differences in grain quality parameters between both locations and years. The weather condition with the highest variation across site-years was precipitation. The 2016 growing season generally had the highest amount of precipitation, followed by 2015 and then 2017. There was a considerable deterioration of the grade for wheat produced in 2016, in comparison

to 2015 and 2017 as a result of degrading from Fusarium damaged kernels (FDK) and midge damage. The genotypes used in this study had varying levels of susceptibility to Fusarium head blight (FHB), ranging from moderately resistant to susceptible. The fungicide efficacy ratings indicated that the effect of triazole fungicide application on %FDK was statistically significant for only five of the ten site-years. In five of the site-years, FDK levels were very low or zero in the untreated check and fungicide was not needed. However, over all site-years, the treatments that received fungicide at anthesis had statistically lower FDK than the treatments that did not.

Grain test weight, thousand kernel weight (TKW) and wheat protein also had lower values in the wetter site-years in comparison to the drier site-years. These results highlight the fact that while each year can have different weather conditions, each location will also differ within a given year as well, making a site-year effect an important consideration. Site-year contributed to the largest percent of variation, ranging from 35.60% to 72.77% for the wheat and flour quality parameters that were studied in Chapter 2. For each individual site-year, the genotype effect was highly significant for the FDK, test weight, TKW and wheat protein parameters for at least nine of the site-years. Next to site-year, genotype was the second most important factor contributing to variation in grain and flour quality parameters. Genotypes also varied in response to different weather conditions, due to their unique genetics. For example, the genotypes in this study had a range of resistance to Fusarium damage, which would be expected to create particularly large differences in wheat quality under growing conditions with high Fusarium disease pressure.

The effects of FHB fungicide and pre-harvest glyphosate application on CWRS quality were unknown prior to this study. The main effect of the pesticide treatments on FDK, test weight, TKW, grain protein, and flour ash was statistically significant overall, across all of the site years. However, the contribution of the pesticide treatments to the total variance was very small. There were also several grain and flour quality measurements that were affected by site-year*pesticide interactions. For the individual site-year analysis, the differences in pesticide treatment means were significant at three site-years for flour protein, four site-years for flour ash, five site-years for FDK, test weight, and grain protein, and six site-years for TKW. Most of these differences were due to fungicide application at site-years where there was substantial disease pressure from Fusarium. Throughout the study there was not a single instance where a glyphosate application caused a reduction in grain or flour quality when compared to the control treatment. Overall, the pesticide treatments did not influence grain and flour quality in a practical way because the pesticide treatment main effect and all interactions contributed a small amount to the total variance, which was usually less than the contribution from the residual.

In Chapter 3, the analysis of rheological properties of dough using a mixograph showed that the pesticide treatments did not cause a statistically significant main effect or contribute large proportions to the variance for the five mixograph parameters examined. The lack of significant pesticide treatment effects was evident from analysis of variance across both the individual site-years, as well as the combined site-years. This result indicated that applications of an FHB fungicide and/or pre-harvest glyphosate did not have a practical effect on dough

properites including those measuring gluten strength. In contrast, growing season conditions had large effects on dough rheological properties and protein composition. For the mixograph PDR and PBW parameters, higher precipitation values led to higher values. For the MDT, WIP and WAP parameters, the opposite was observed. The highest location means for the MDT, WIP and WAP parameters were observed when growing season precipitation was low. Genotypes were readily distinguishable in these dough mixing properties. The Glenn genotype had the highest values for the MDT, WIP and WAP parameters, followed by Carberry, Cardale, Stanley or Stettler and then Harvest. This ranking also corresponds to the known gluten strength characteristics of these genotypes. For analysis of variance for individual site-years, the genotype effect was highly significant for all of the mixograph parameters. This was also the case for the majority of site-years for PDR and PBW and all 10 site-years for MDT, WIP and WAP. For all mixograph parameters, genotype, site-year and their interaction, were all significant. Site-year was the effect that contributed the largest amount to variance, followed by genotype and then the site-year* genotype interaction. These results are consistent with those observed for grain and flour quality in Chapter 2. The variation in growing season weather between site-years as well as genotype were the main factors affecting mixograph measurements of gluten strength.

The effects of pesticide treatment, genotype and environment on protein composition were also reported in Chapter 3. Parameters included insoluble glutenin (IG), soluble prolamins (SP), the ratios of IG and SP with flour protein (FP), which are IG/FP and SP/FP, and the gluten strength index (GSI), which is the ratio of IG to SP. As observed for other response variables,

the pesticide treatments and their interactions contributed very little to total variance. The main effect of pesticides was not statistically significant for any of protein composition parameter. However, there were very small, but statistically significant effects of the site year*pesticide interaction for four of five protein composition measurements. However, the contribution to total variance from these interactions was very small and means separations could not detect significant differences between pesticide treatments, except for very small differences in IG at IHARF 2017, SP at IHARF 2015, and SP/FP at Carberry 2016. For each study year, the location means were relatively similar, but there were larger differences between the three years. Analysis of variance for individual site-years for the GSI, IG/FP, IG and SP parameters all showed that genotype was highly significant for eight or more of the 10 site-years. Genotype, site-year and their interaction, were significant for all protein parameters. For these parameters, with the exception of SP/FP, genotype contributed the largest amount to total variance, followed by site-year. These results are a consequence of the large differences among the different genotypes as well as large differences in growing season weather among the 10 site-years. Thus, the growing season weather had a significant effect on both the protein composition parameters as well as the mixograph parameters.

Chapter 3 also highlighted relationships between protein composition and mixograph parameters. There were significant, positive relationships between mixograph parameters that measure gluten strength (MDT, WIP and WAP) and two protein composition parameters (GSI and IG/FP). The gluten strength of the genotypes was reflected accurately by these mixograph parameters and the protein composition parameters. The mixograph vs protein composition

relationships also reflected the impacts of the different growing conditions on gluten strength across the three study years. The lower gluten strength values in 2016 compared to the 2015 and 2017 samples appeared to be related to increased precipitation in 2016 (Figures IV.40-IV.54) and the higher levels of FHB infection and FDK (Figure 2.5).

Chapter 4 reported the effects of delayed harvest dates on grain grade and quality, flour properties, rheological properties and protein composition parameters. Wheat grade varied among both the locations and the different harvest dates. Wheat at all locations experienced midge damage, sprout damage and FDK, but in varying amounts. As expected, wheat grades decreased as the harvest dates were progressively delayed. There was a substantial decline in sample grades at some sites and smaller levels of grade deterioration at others.

Delayed harvest significantly decreased test weight at all four locations. Location and location*genotype interaction were the largest contributors to variance for the FDK, TKW and %GPC. The impact of delayed harvest was significant for test weight, and was a large contributor to variance. Analysis of variance at individual locations showed that genotype was frequently significant for FDK, TKW, test weight and wheat protein.

For the flour quality parameters, harvest date was a large contributor to total variance for flour ash only. The genotype effect was significant at all locations for flour ash and flour yield, and for three locations for flour protein content. Global analysis of variance showed a highly significant genotype for flour ash and yield, but not for protein content. Similar to the grain

parameters, flour quality was also significantly impacted by the location and location*genotype interaction effects. These two effects were the largest contributors to variance.

Analysis of variance for mixograph parameters revealed a significant genotype effect for MDT, WIP and WAP, in both the global analysis of variance and for individual locations. The location effect and the location*genotype interaction were also highly significant in the global analysis of variance as well as large contributors to total variance. Thus, the genotype response of gluten strength varied with different growing season weather conditions, similar to the observations in the pesticide treatment study.

Results for protein composition parameters were similar to those for gluten strength. The effect of the delayed harvest, although statistically significant, accounted for a small amount of total variance. The genotype effect was highly significant for all five parameters at all individual locations. Global analysis of variance revealed that genotype was not significant for only SP. For the protein composition parameters, the location effect was also a significant contributor to variance.

5.2 Implications for CWRS Grade, Quality and Gluten Strength

Pesticide application is a common farming practice across Western Canada and is also utilized around the world. These pesticide applications help to improve yields, manage pests, and increase field productivity and plant health. The use of both a fungicide at anthesis and pre-harvest glyphosate are common for these reasons. They are used to reduce the risk of a cereal

crop being degraded by Fusarium damaged kernels, and penalized with excess dockage at the time of wheat delivery. This study determined that the grain quality parameters of FDK, TKW, test weight and wheat protein were those most affected by the pesticide treatments, but at very small and inconsistent levels of statistical and biological significance across individual site-years. Due mainly to variations in Fusarium pressure across site-years, the effect of pesticide treatment was inconsistent across different site-years with 5-6 of 10 site-years significantly affected by the fungicide treatment for these parameters. Flour ash and flour protein were also significantly affected by these pesticide treatments, but at only 3-4 site-years. There was a distinct lack of significant differences in parameters means for pesticide treatments on all of the mixograph parameters assessed in this study, showing that these pesticide treatments did not affect the gluten strength of the wheat. Pesticide treatments also did not have an effect on any of the protein composition parameters in the study. These are important results, as they rule out these pesticide treatments as potential source of gluten strength variation in CWRS wheat in Western Canada. It is critical to note that proper timing of these pesticide treatments is important and the pesticide treatments in this study were all applied as recommended. There may be adverse effects if the pesticides are applied at the incorrect growth stage of the wheat.

The main conclusion is that site-year, genotype and site-year*genotype interaction contribute the largest proportions of variance to the grain, flour, rheological and protein composition parameters. This result was consistent throughout the entire study. For grain, flour and rheological parameters, site-year was the largest contributor to variance and for the protein composition parameters (excluding SP/FP), genotype was the largest contributor to variance.

The site-year*genotype interaction effect was the third largest contributor to variance for all parameters analyzed. The consistency of these results shows that variation in growing season weather from year to year can be expected to have a significant impact on wheat quality including gluten strength. The effect of the pesticide applications was minimal, and there was no apparent effect on the gluten strength of the genotypes analyzed.

The effects of progressively-delayed harvest dates, as expected, caused CWRS grades to decline. Delayed harvest as shown by global analysis of variance was statistically significant for grain test weight, flour ash and flour yield, mixograph MDT, WIP and WAP, IG, IG/FP, SP/FP and GSI. It appears therefore that delayed harvest can affect many important attributes of wheat and flour quality, and dough and protein composition with a slight positive effect gluten strength. However, the contributions of all these parameters to total variance was very small and often smaller than the residual error. The decline in grade with harvest delay was clear and consistent across all sites (Figure 4.1) and for each individual site (Figures 4.4, 4.6, 4.8, 4.10). This outcome is important for producers. They lose revenue as a result of declines in wheat grade but variation in gluten strength does not impact their returns at the farm gate.

5.3 Implications for the Wheat Industry in Western Canada

The most important result of this study was that application of the pesticides studies, when applied according to label recommendations, did not significantly affect the gluten strength of the different CWRS wheat genotypes studied. However, the variation in growing season weather, had substantial effects on not only gluten strength but wheat quality in general. Site-

year dominated variance contributions for many parameters including %FDK, test weight, TKW, protein content, flour yield, flour ash, mixograph PDR, PBW, and MDT. Therefore, it should be expected that gluten strength of CWRS wheat shipments will vary between years and locations. More intense scrutiny of annual gluten strength variation by genotype and location could provide an opportunity to identify genotypes that are intrinsically less sensitive to the effects of growing season weather variation. The protein composition methods utilized in this study could lend themselves to this type of assessment or even provide a rapid driveway test of gluten strength at delivery could facilitate adoption of gluten strength as a grading factor. In addition, genotype is a key factor affecting CWRS gluten strength. As previously mentioned, the Harvest genotype was re-designated from CWRS to the CNHR wheat class in 2018 after this study was completed. The relative weakness of Harvest as shown in the results of this study indicate that the reclassification was justified. The changes and re-evaluations of genotypes already done by the Canadian Grain Commission to remove specific varieties from the CWRS class are important steps to maintaining the gluten strength quality desired in this wheat class and will continue to be required going forward.

Even though application of FHB pesticide and pre-harvest glyphosate did not affect CWRS gluten strength, it is in the best interests of the western Canadian agriculture industry to limit pesticide use to the greatest extent possible as a result of growing consumer and importer demand for pesticide-free products. For example, the FHB fungicide treatment in this study showed that there was a significant improvement in wheat quality as a result. However, by individual site-year, 5 of 10 site-years had any significant benefit in terms of reduced FDK. This

was because there was little or no Fusarium disease pressure at half of the site-years. If there is no disease present, then an improvement in grade from a fungicide application to control the disease cannot be expected. There is clearly an opportunity to use this treatment selectively, with resulting benefits to producers, who can reduce production costs, to consumers, who want more pesticide-free products and to the environment, which would experience reduced impacts of pesticide transfer from agricultural fields. It is also important to note that there are risk forecasts available for farmers to help them determine what potential FHB development might look like for the season. Considering the wide variation in growing season weather conditions between years and the potential impact on Fusarium head blight disease pressure, these risk forecast models should be utilized to assist farm-level decisions for FHB fungicide application in an effort to target their use more effectively across the Canadian prairies.

6. APPENDICES

Appendix I. Tukey-Kramer Groupings for Pesticide Treatment and Genotype Least Square Means from combined year analysis for Chapter 2.

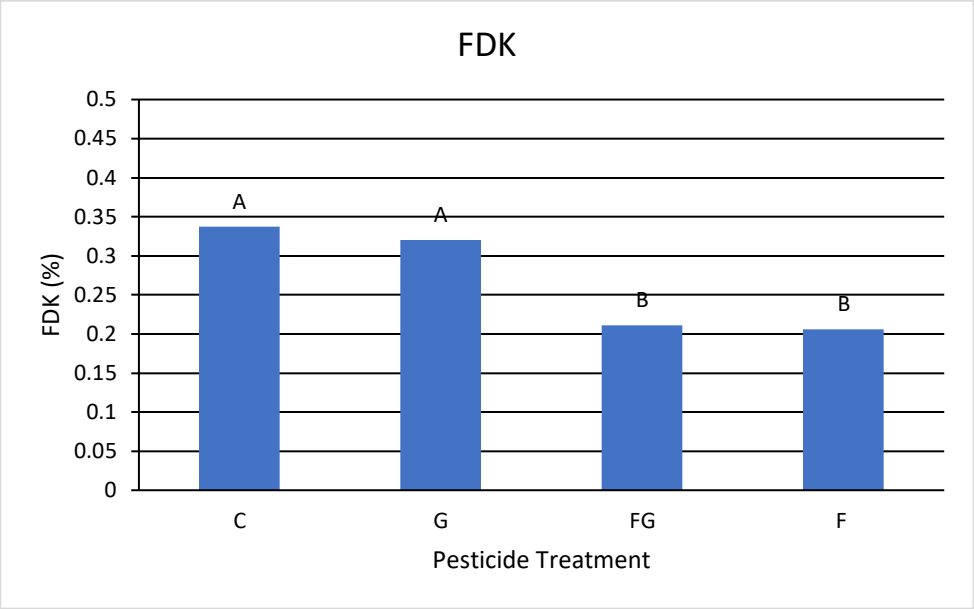


Figure I.1 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for FDK by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

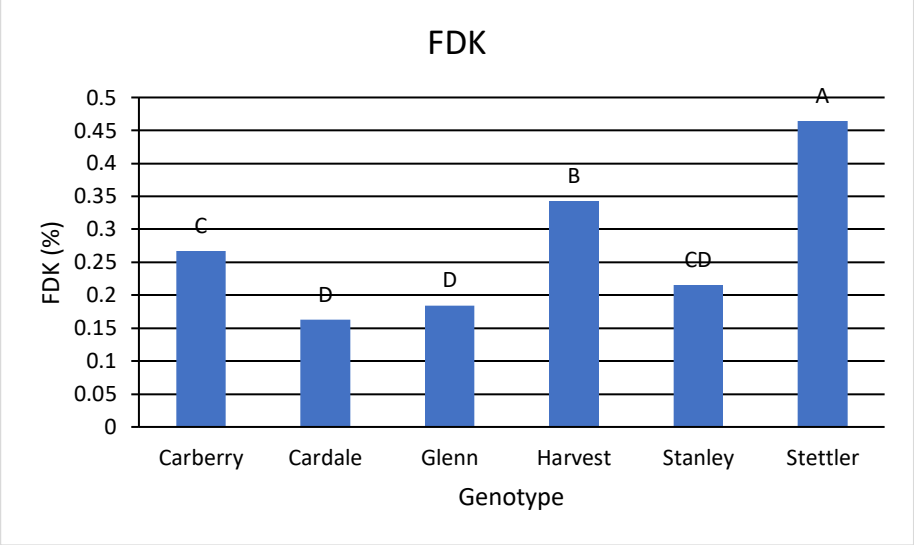


Figure I.2 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for FDK by genotype.

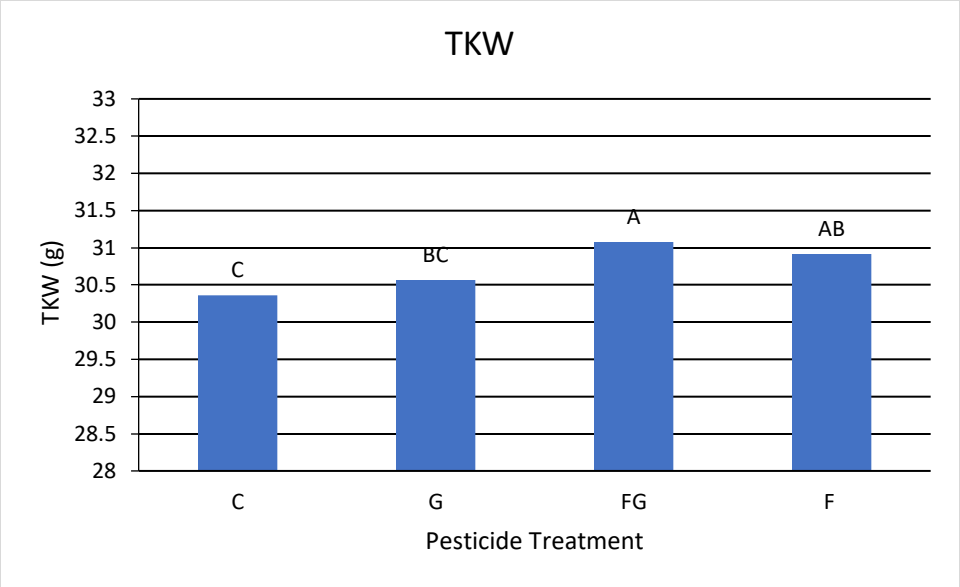


Figure I.3 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for TKW by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

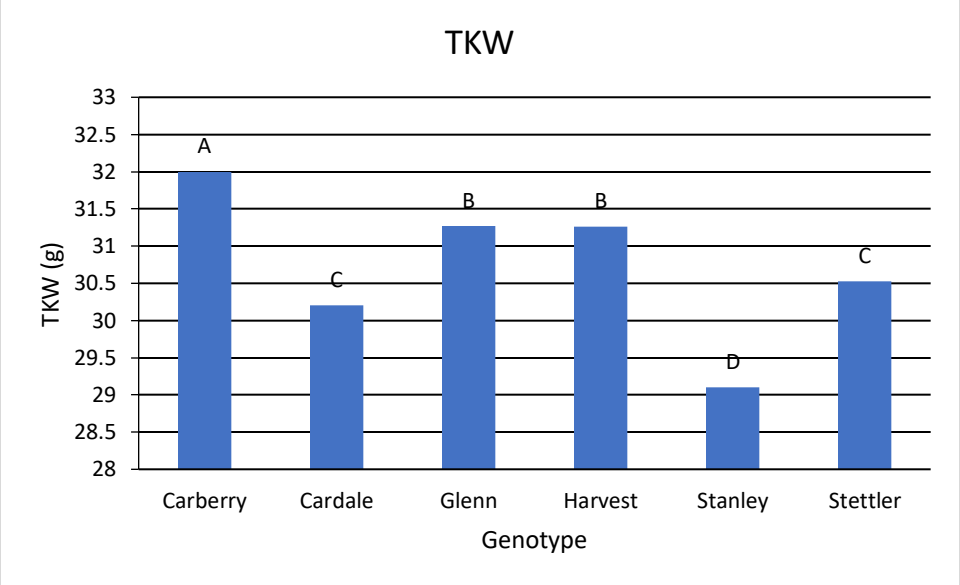


Figure I.4 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for TKW by genotype.

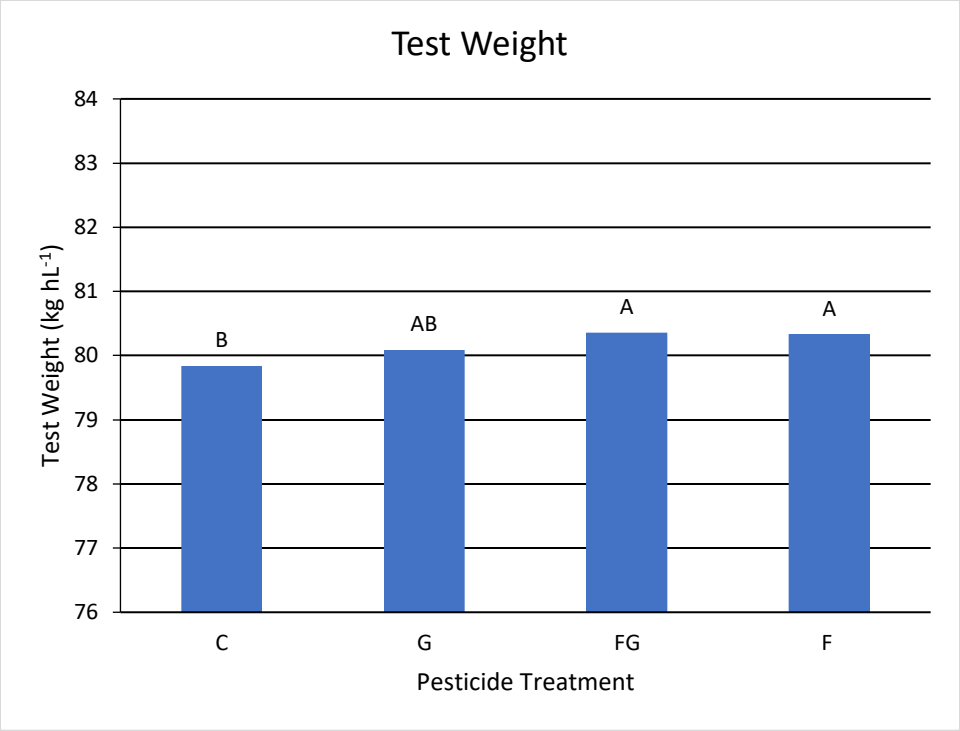


Figure I.5 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for test weight by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

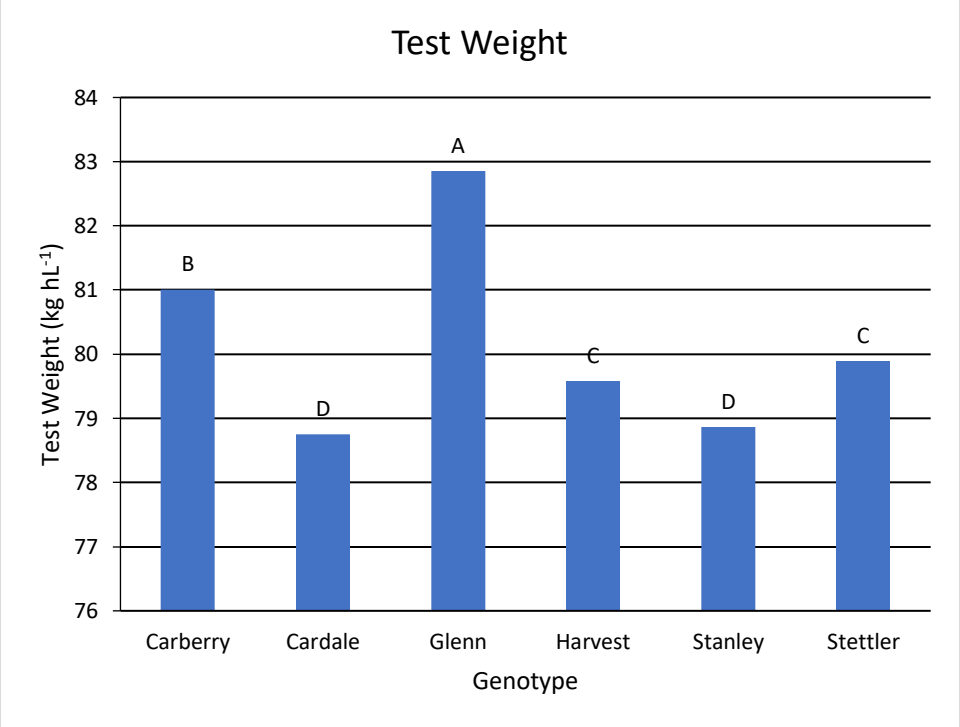


Figure I.6 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for test weight by genotype.

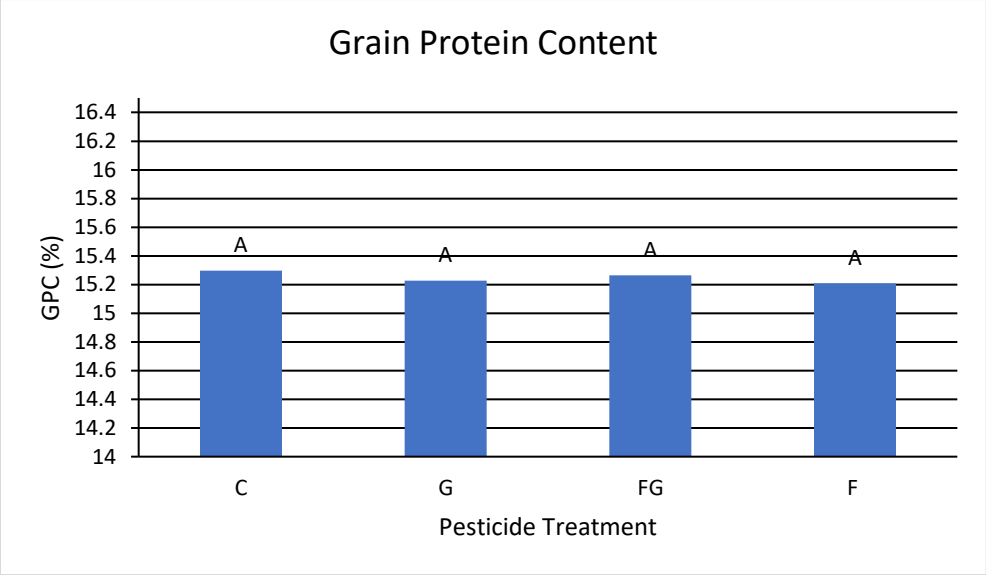


Figure I.7 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for grain protein content by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

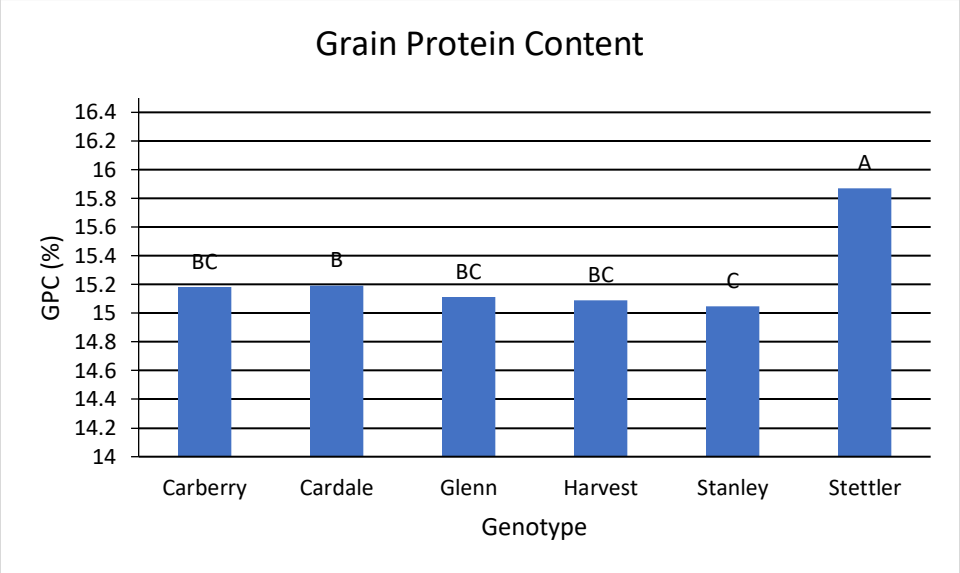


Figure I.8 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for grain protein content by genotype.

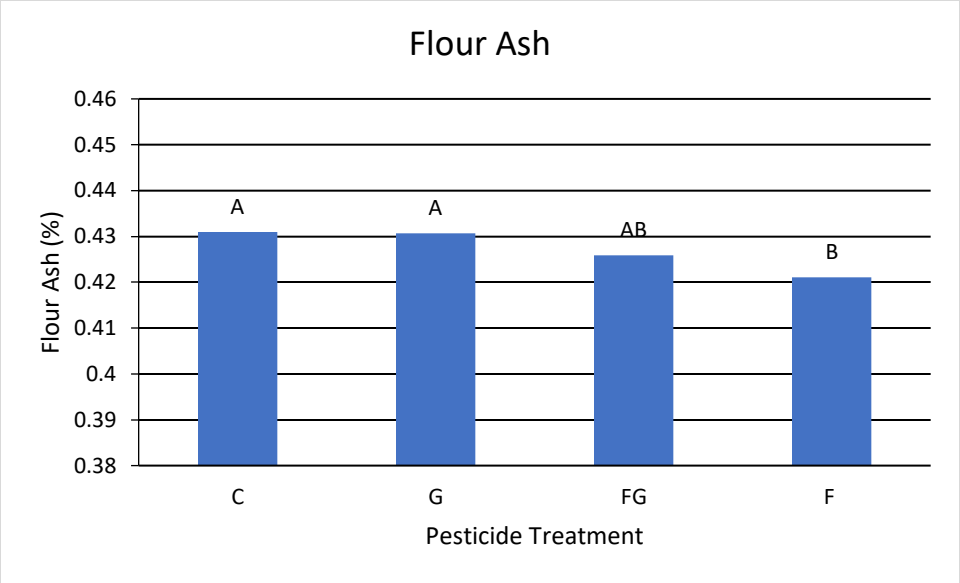


Figure I.9 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for flour ash by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

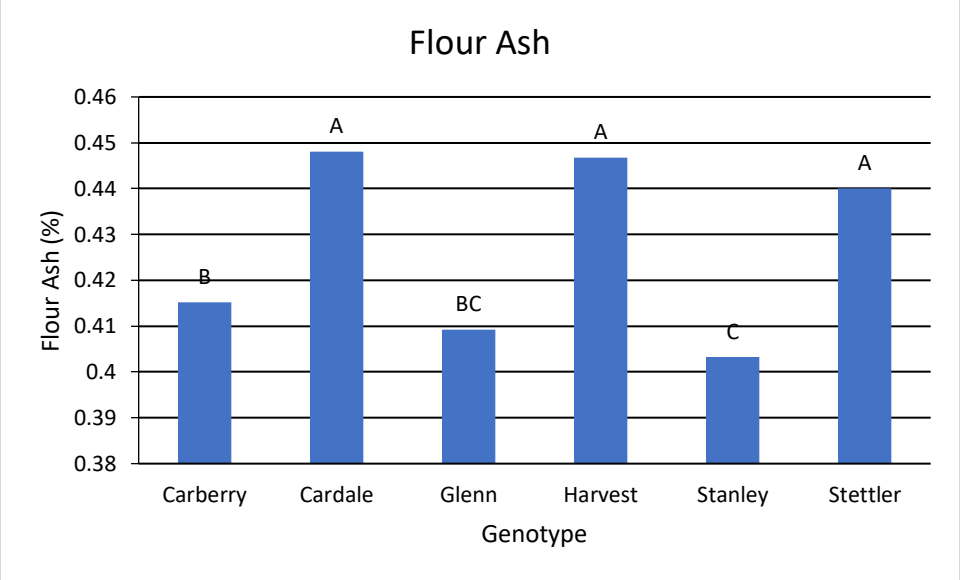
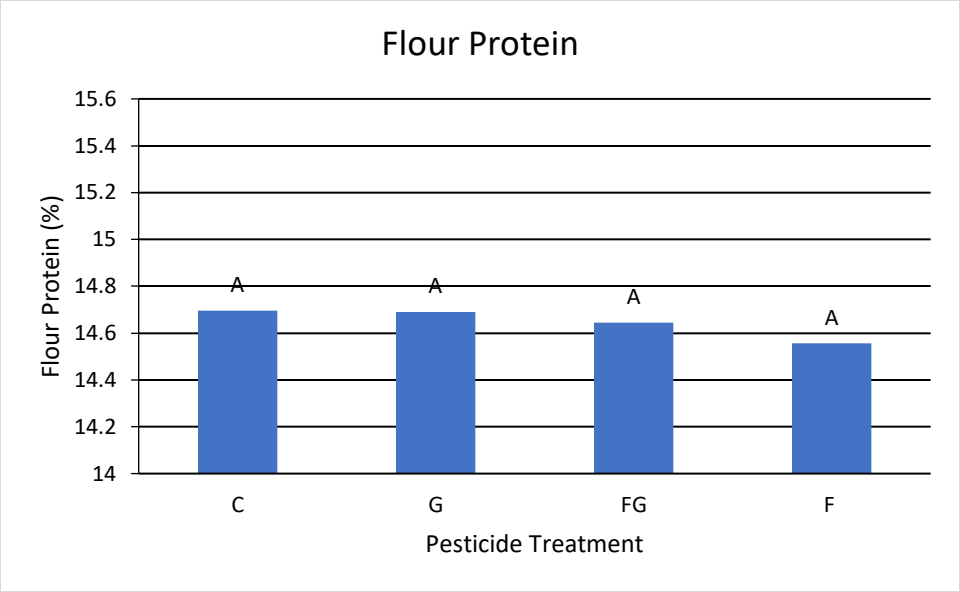


Figure I.10 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for flour ash by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).



I.11 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for flour protein separated by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

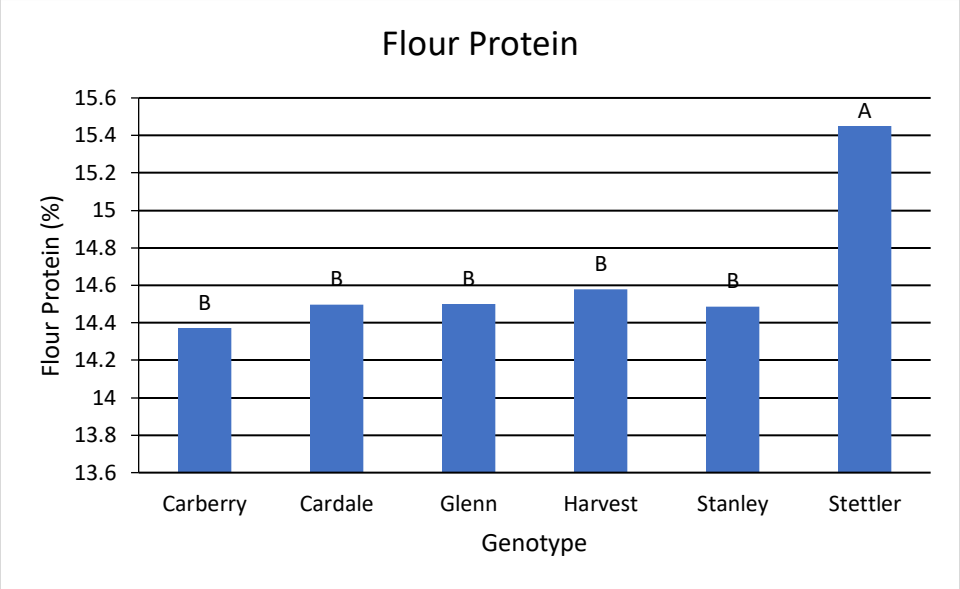


Figure I.12 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for flour protein separated by genotype.

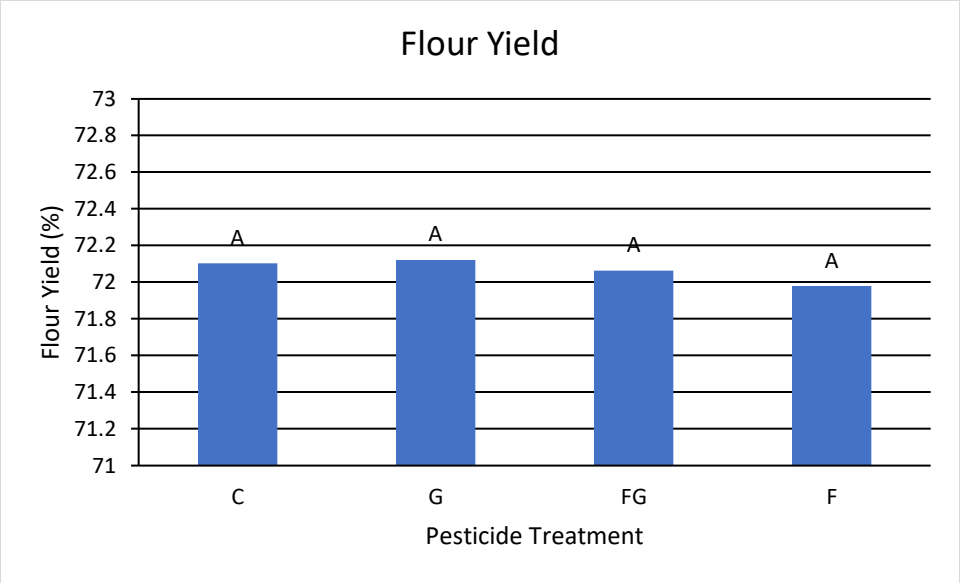


Figure I.13 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for flour yield by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

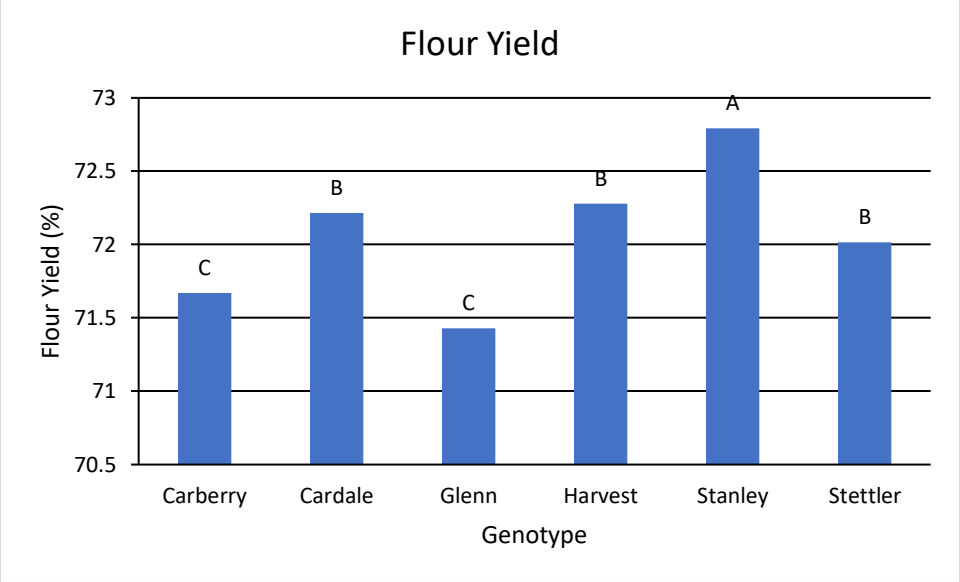


Figure I.14 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for flour yield by genotype.

Appendix II. Tukey-Kramer Groupings for Pesticide Treatment and Genotype Least Square Means from combined year analysis for Chapter 3.

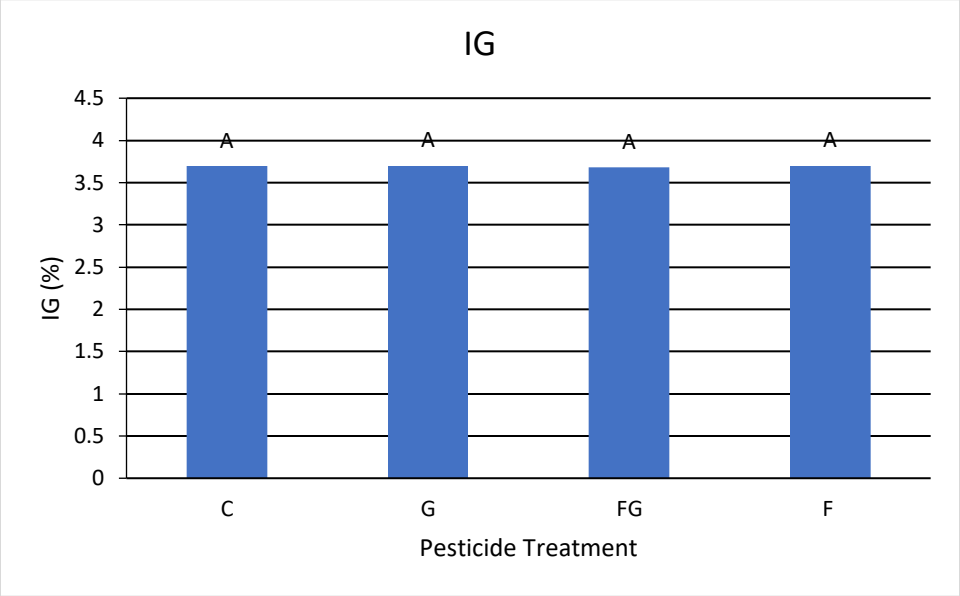


Figure II.1 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for IG by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

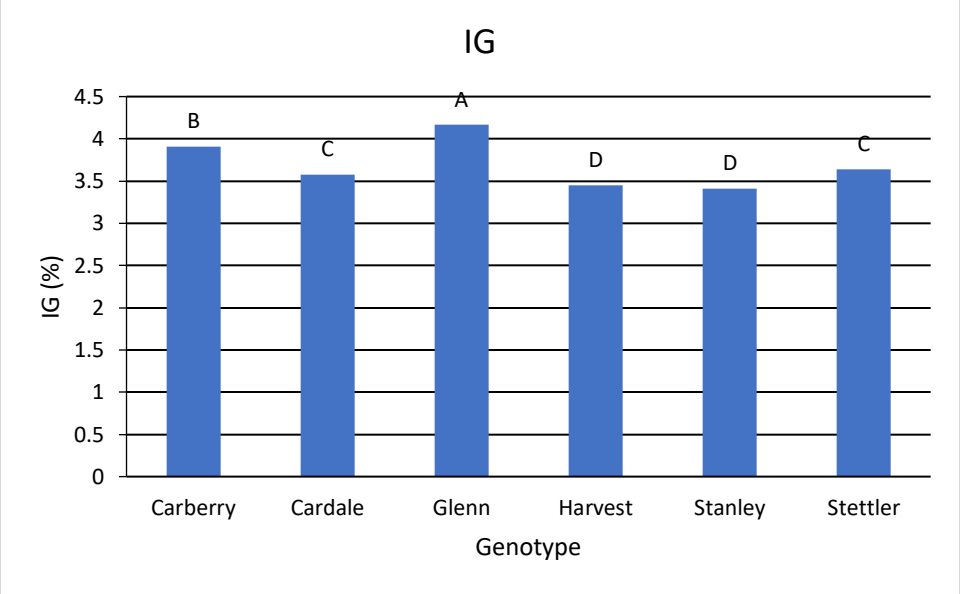


Figure II.2 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for IG by genotype.

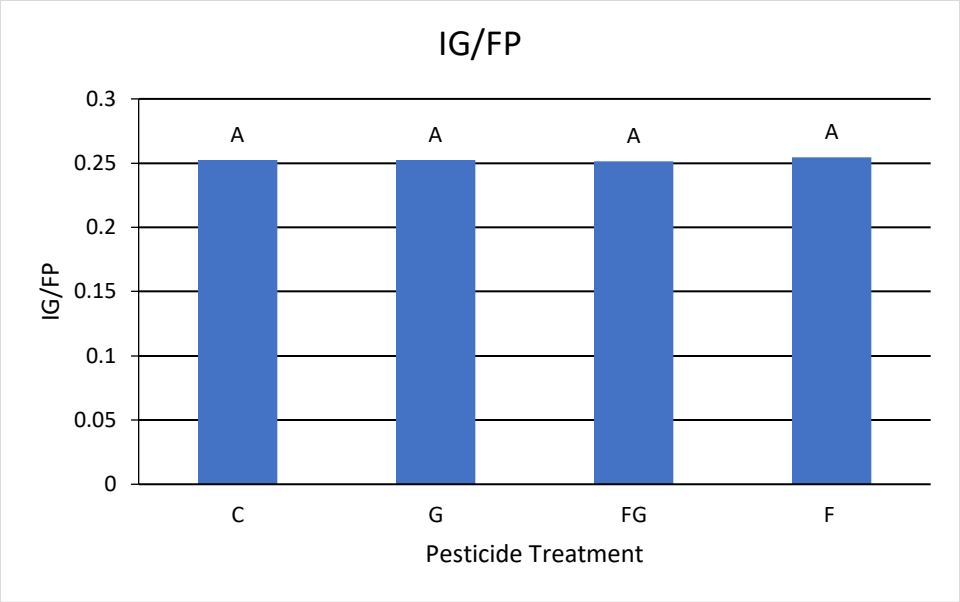


Figure II.3 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for IG/FP by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

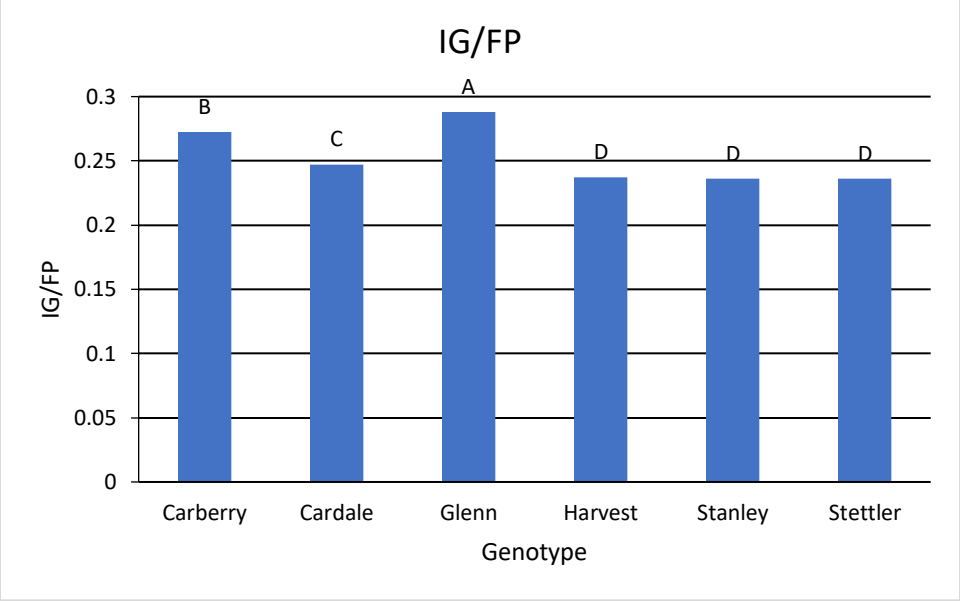


Figure II.4 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for IG/FP by genotype.

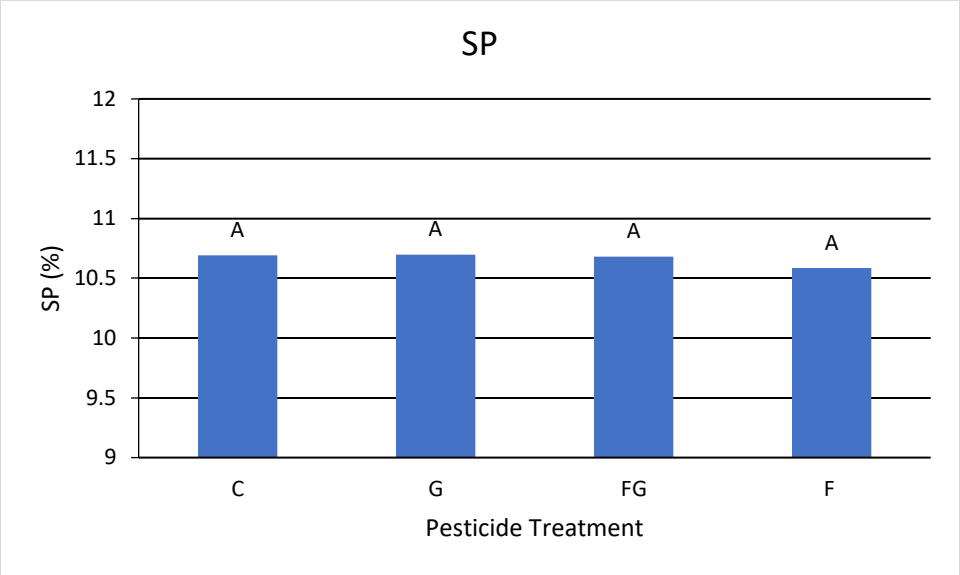


Figure II.5 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for SP by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

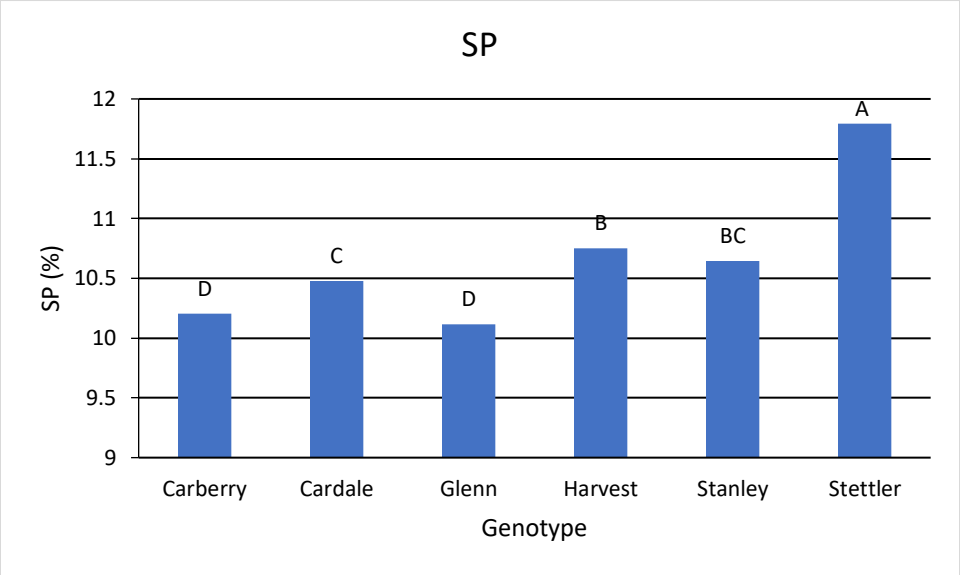


Figure II.6 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for SP by genotype.

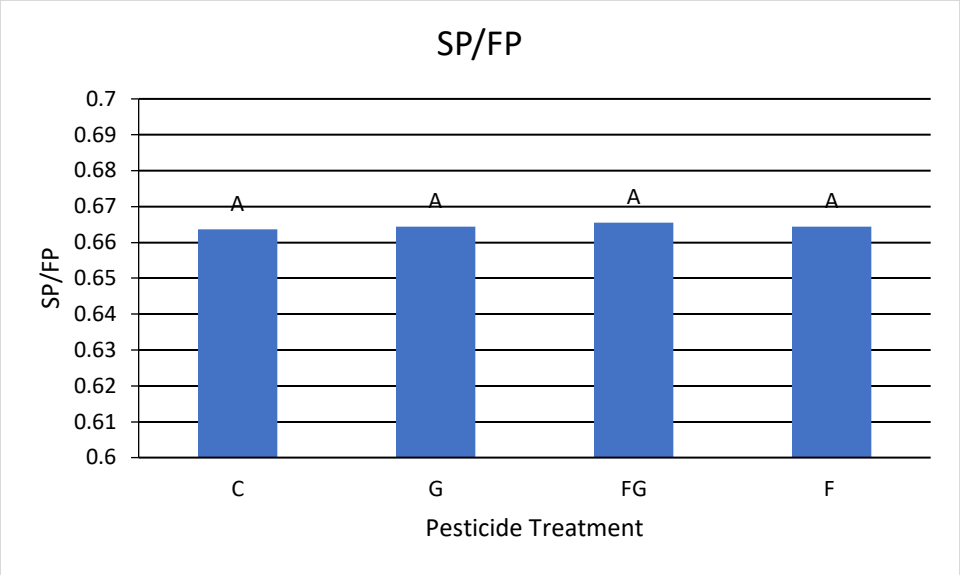


Figure II.7 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for SP/FP by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

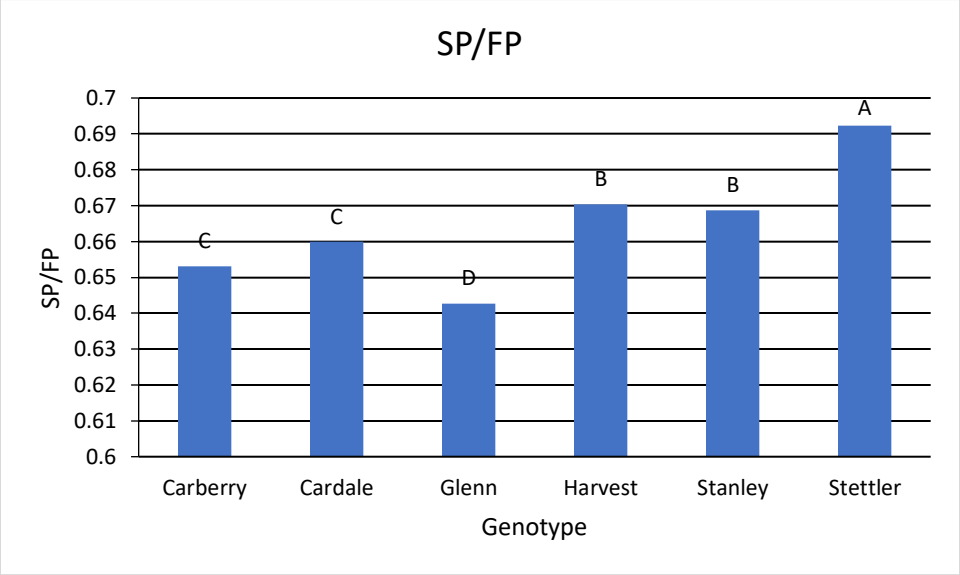


Figure II.8 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for SP/FP by genotype.

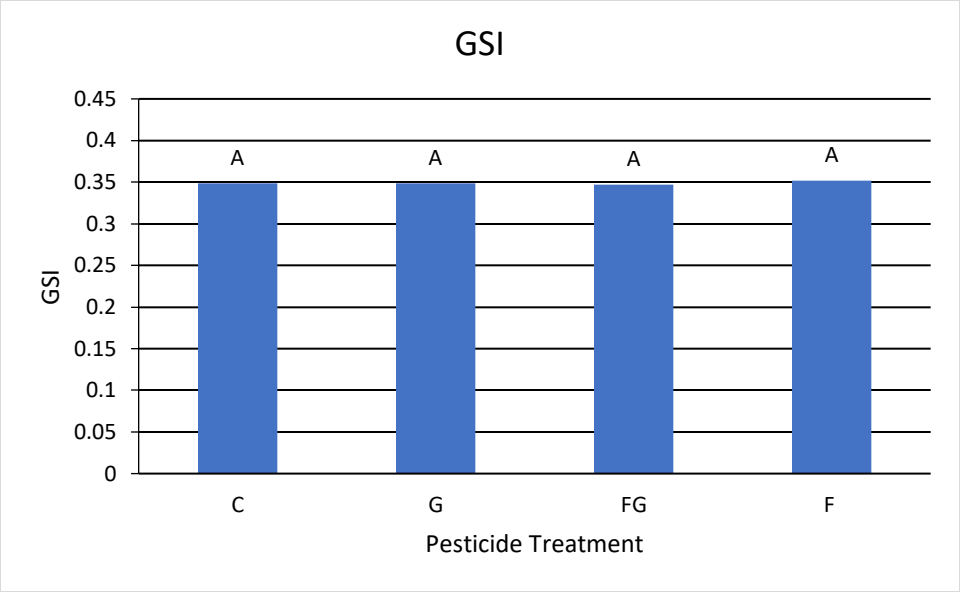


Figure II.9 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for GSI by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

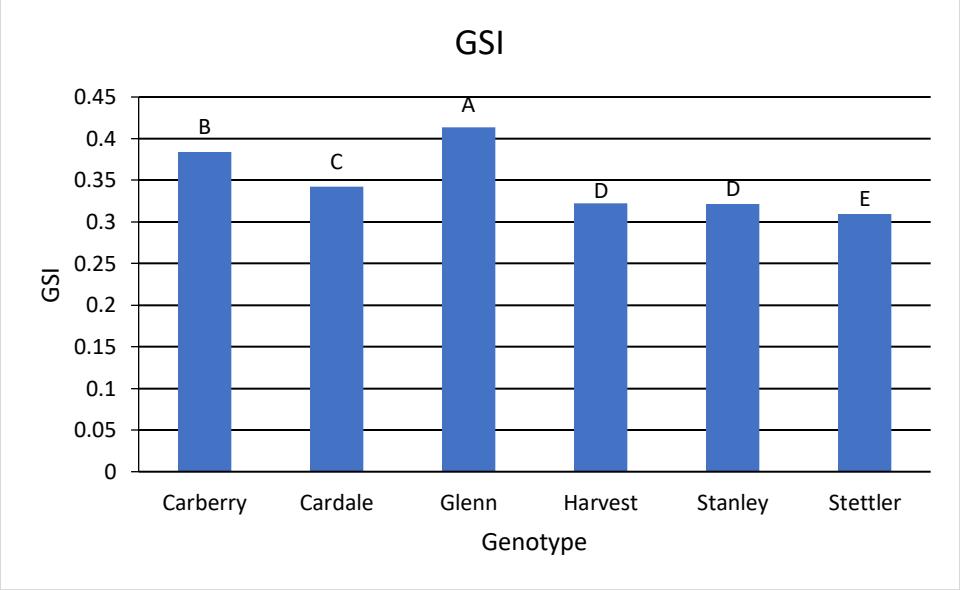


Figure II.10 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for GSI by genotype.

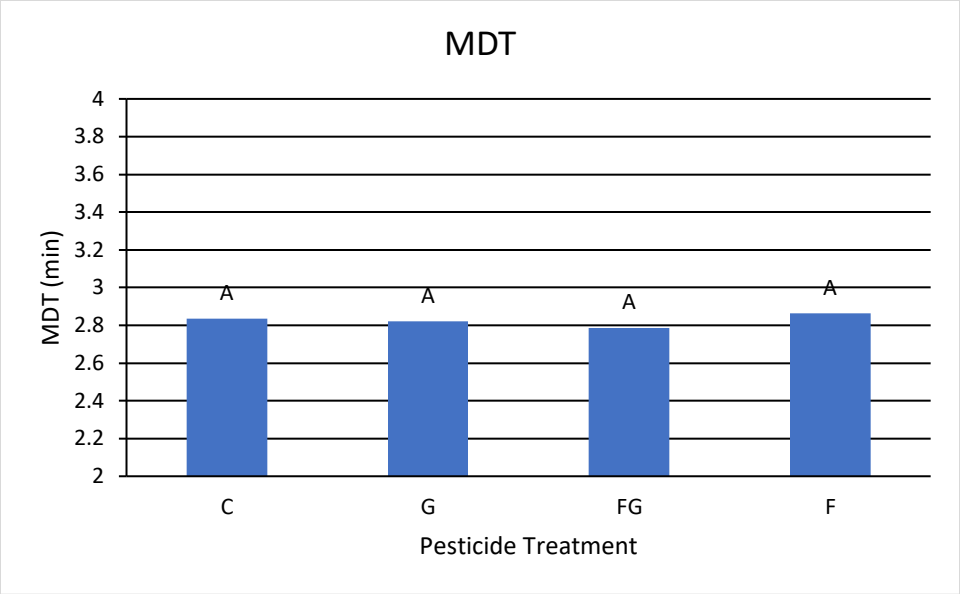


Figure II.11 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for MDT by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

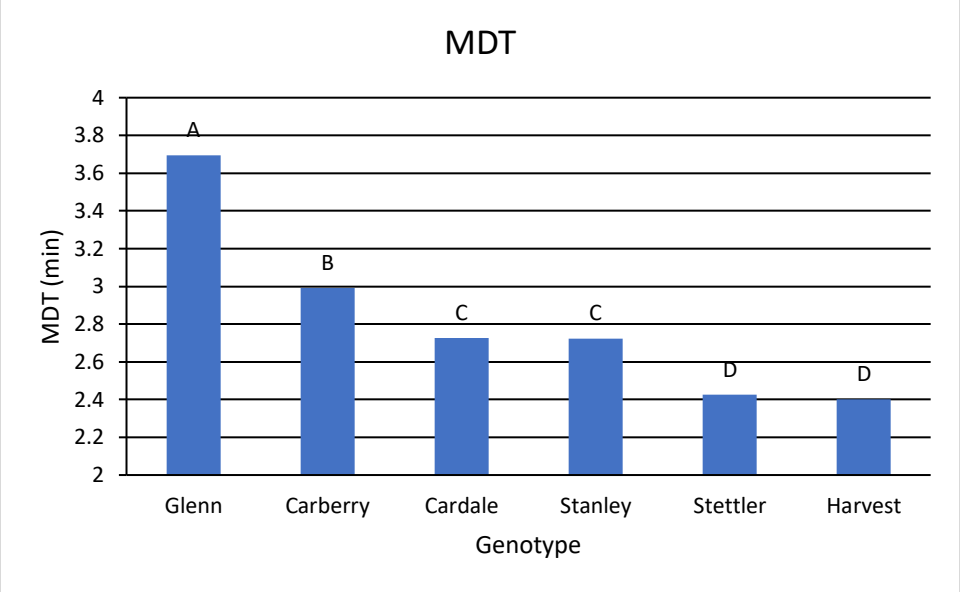


Figure II.12 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for MDT by genotype.

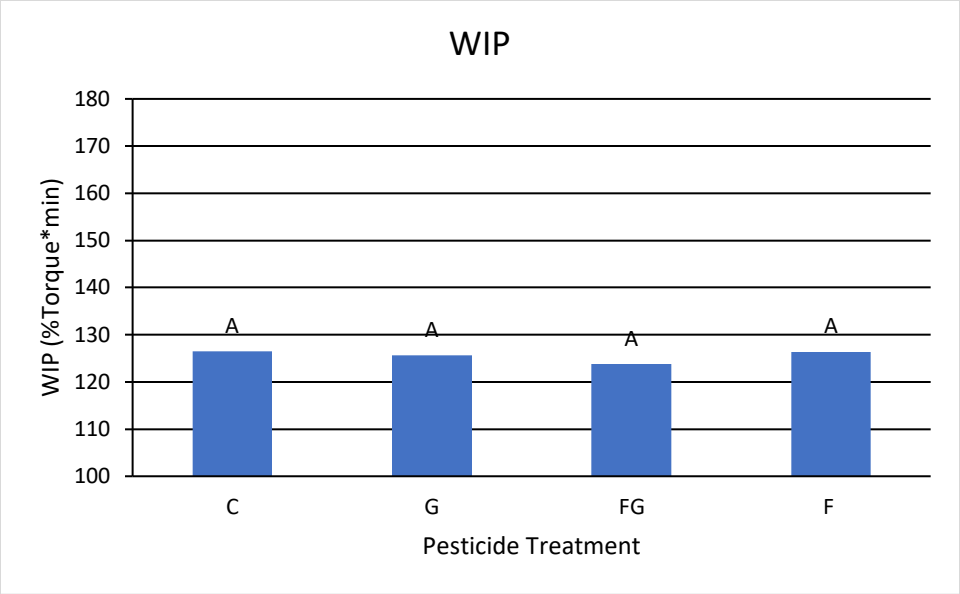


Figure II.13 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for WIP by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

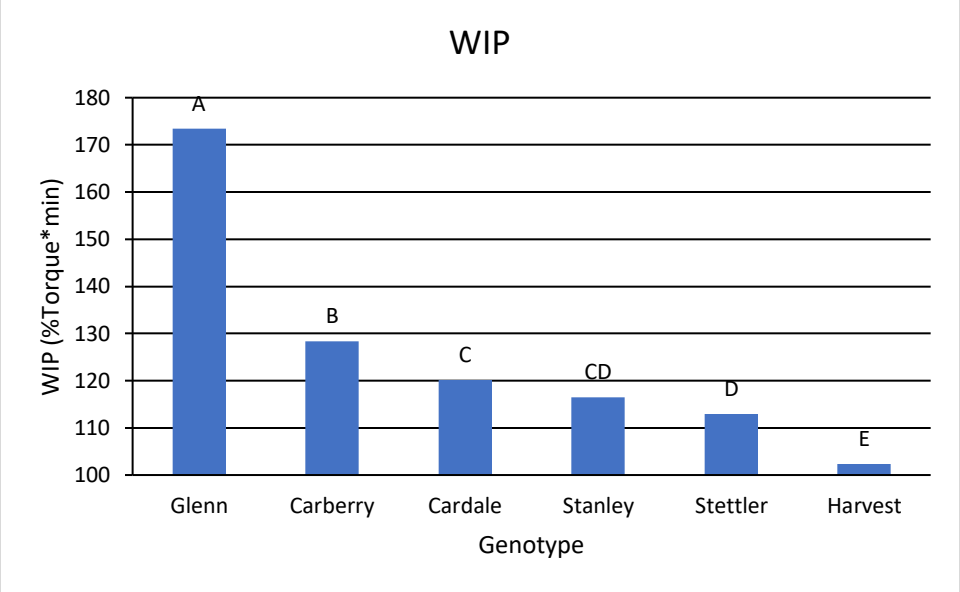


Figure II.14 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for WIP by genotype.

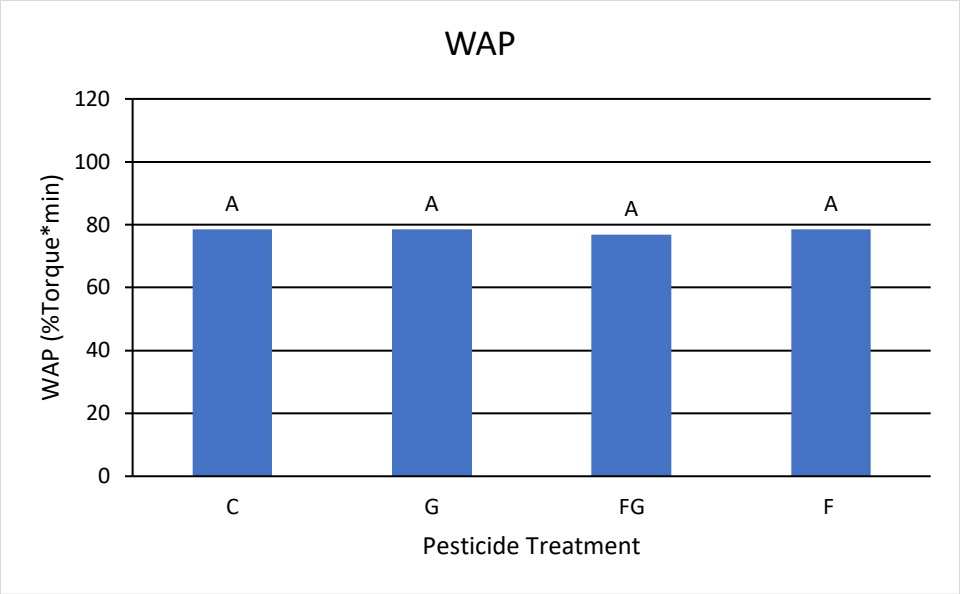


Figure II.15 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for WAP by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

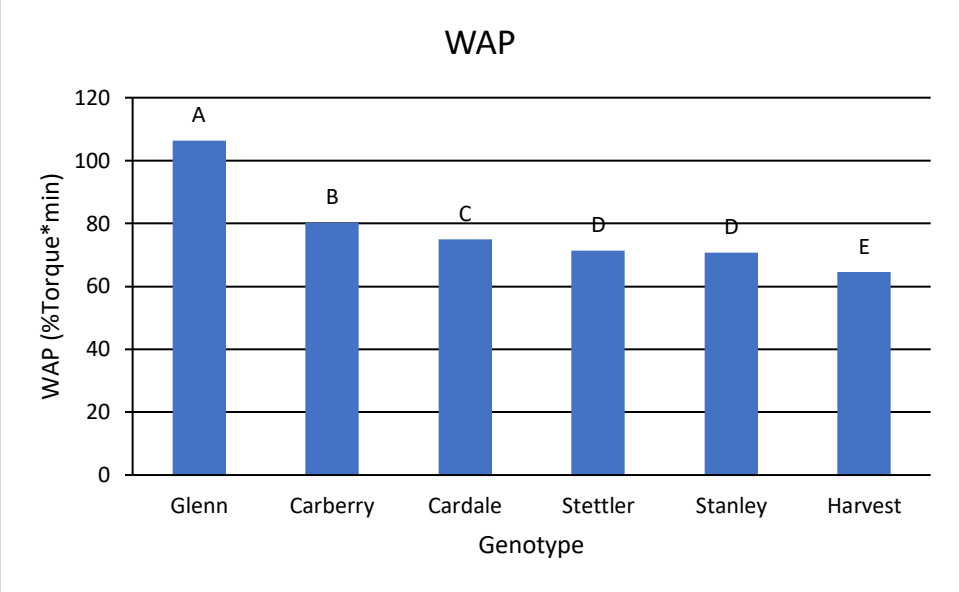


Figure II.16 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for WAP by genotype.

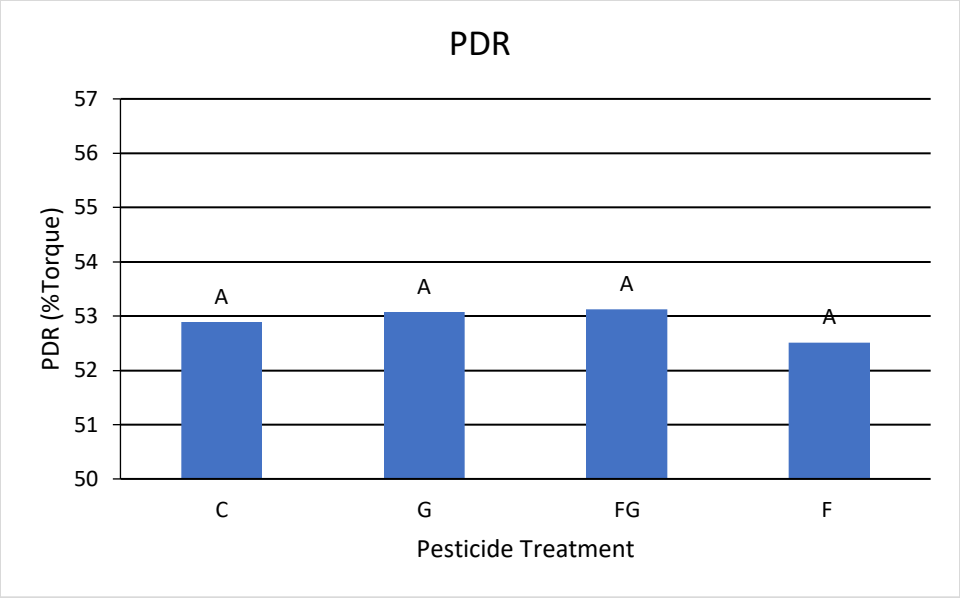


Figure II.17 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for PDR by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

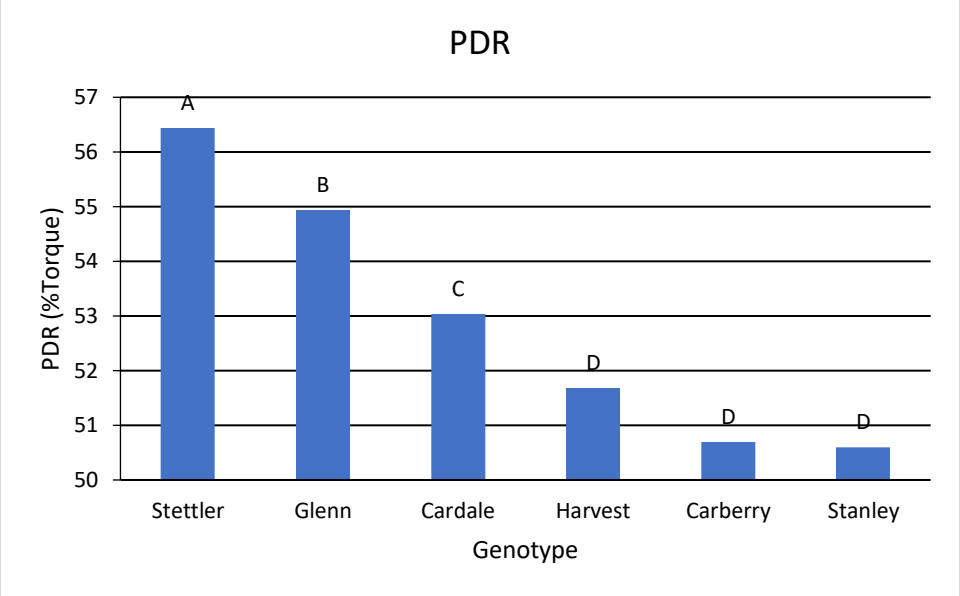


Figure II.18 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for PDR by genotype.

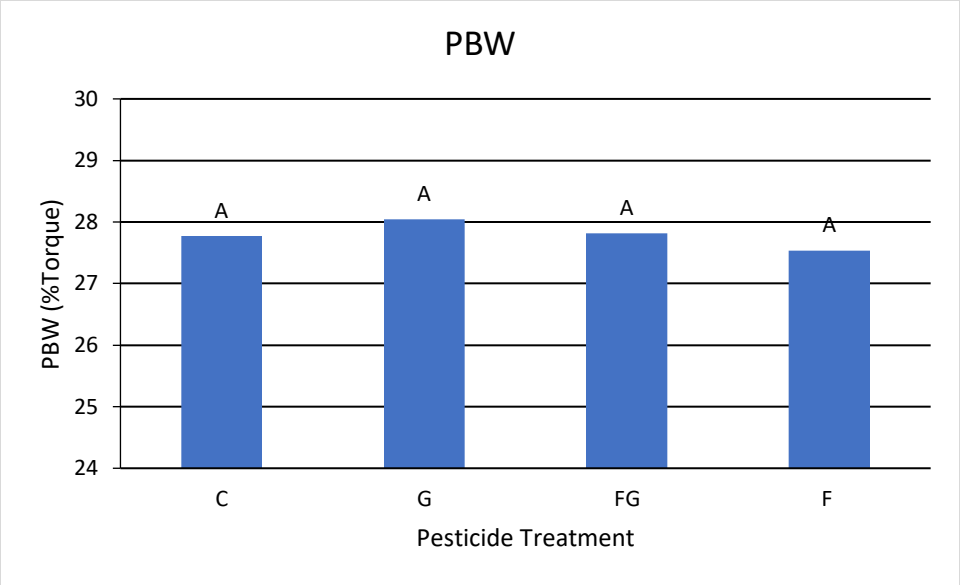


Figure II.19 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for PBW by treatment; Control (C), Glyphosate (G), Fungicide (F) and Fungicide plus Glyphosate (FG).

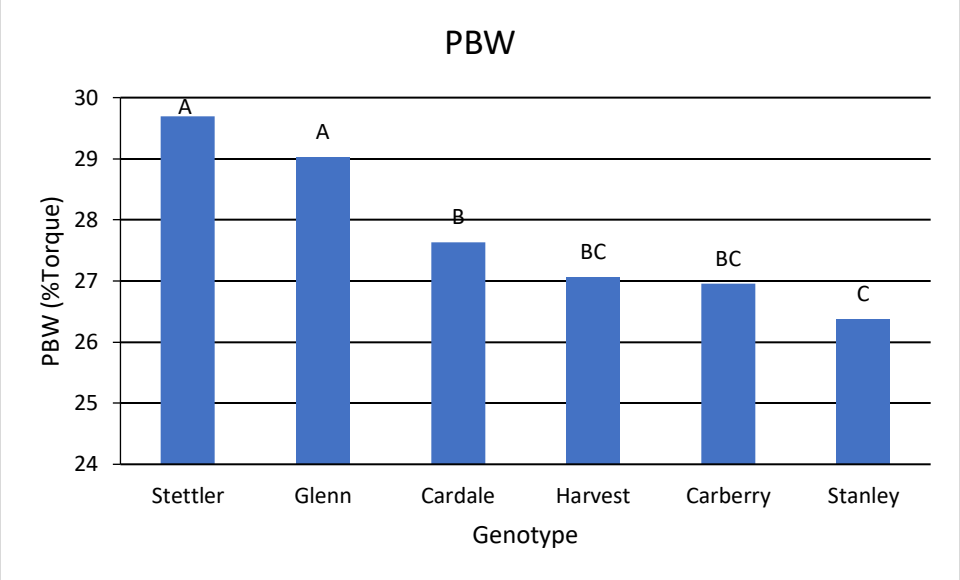


Figure II.20 Tukey-Kramer Grouping for Pesticide Treatment Least Squares Means from combined year analysis for PBW by genotype.

Appendix III. Least Square Means Tables for all analyzed parameters, from combined year analysis, for Chapter 4.

Table III.1 Least Squares Means for FDK (%), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		0.323891	0.07517	4.46	-1.12	0.3197
Genotype	**Carberry**		0.397780	0.07511	4.45	-0.62	0.5635
Genotype	**Glenn**		0.274283	0.07517	4.46	-1.48	0.2062
Genotype	**Harvest**		0.441868	0.07510	4.45	-0.35	0.7448
Treatment		**H1**	0.297517	0.06640	2.85	-1.48	0.2400
Treatment		**H2**	0.295921	0.06661	2.87	-1.49	0.2373
Treatment		**H3**	0.424443	0.06638	2.85	-0.51	0.6444
Treatment		**H4**	0.419200	0.06666	2.9	-0.55	0.6226

Table III.2 Least Squares Means for TKW (kg hL^{-1}), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		31.7597	0.5445	7.74	58.32	<.0001
Genotype	**Carberry**		32.4347	0.5422	7.62	59.82	<.0001
Genotype	**Glenn**		31.4923	0.5446	7.74	57.83	<.0001
Genotype	**Harvest**		31.4701	0.5423	7.63	58.03	<.0001
Treatment		**H1**	31.5370	0.4533	4.44	69.58	<.0001
Treatment		**H2**	31.8721	0.4600	4.66	69.29	<.0001
Treatment		**H3**	31.6255	0.4537	4.45	69.70	<.0001
Treatment		**H4**	32.1222	0.4837	5.33	66.40	<.0001

Table III.3 Least Squares Means for Test Weight (g), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		80.7316	0.4103	4.77	196.77	<.0001
Genotype	**Carberry**		80.8257	0.4095	4.74	197.36	<.0001
Genotype	**Glenn**		83.3509	0.4103	4.77	203.15	<.0001
Genotype	**Harvest**		79.7793	0.4095	4.74	194.80	<.0001
Treatment		**H1**	83.1876	0.3979	4.26	209.06	<.0001
Treatment		**H2**	82.5818	0.4001	4.34	206.40	<.0001
Treatment		**H3**	79.5785	0.3980	4.26	199.96	<.0001
Treatment		**H4**	79.3395	0.4168	4.96	190.37	<.0001

Table III.4 Least Squares Means for Grain Protein (%), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		14.4301	0.2715	10.3	53.16	<.0001
Genotype	**Carberry**		14.9981	0.2700	10.1	55.54	<.0001
Genotype	**Glenn**		14.6894	0.2716	10.3	54.09	<.0001
Genotype	**Harvest**		14.4309	0.2701	10.1	53.44	<.0001
Treatment		**H1**	14.6179	0.1761	4.04	83.01	<.0001
Treatment		**H2**	14.6779	0.1812	4.44	81.00	<.0001
Treatment		**H3**	14.5489	0.1764	4.06	82.49	<.0001
Treatment		**H4**	14.7038	0.1859	4.85	79.10	<.0001

Table III.5 Least Squares Means for Flour Ash (%), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		0.3651	0.01230	3.54	29.69	<.0001
Genotype	**Carberry**		0.3741	0.01229	3.53	30.45	<.0001
Genotype	**Glenn**		0.3669	0.01230	3.54	29.83	<.0001
Genotype	**Harvest**		0.3997	0.01229	3.53	32.53	<.0001
Treatment		**H1**	0.3887	0.01148	2.71	33.86	0.0001
Treatment		**H2**	0.3789	0.01152	2.74	32.89	0.0001
Treatment		**H3**	0.3674	0.01148	2.72	31.99	0.0001
Treatment		**H4**	0.3708	0.01156	2.79	32.08	0.0001

Table III.6 Least Squares Means for Flour Protein (%), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		13.4852	0.2928	11	46.06	<.0001
Genotype	**Carberry**		13.9199	0.2915	10.8	47.76	<.0001
Genotype	**Glenn**		13.9194	0.2929	11	47.52	<.0001
Genotype	**Harvest**		13.7203	0.2915	10.8	47.07	<.0001
Treatment		**H1**	13.8663	0.1830	3.79	75.75	<.0001
Treatment		**H2**	13.8287	0.1864	4.05	74.18	<.0001
Treatment		**H3**	13.6306	0.1833	3.82	74.36	<.0001
Treatment		**H4**	13.7193	0.1871	4.12	73.33	<.0001

Table III.7 Least Squares Means for Flour Yield (%), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		73.6297	0.3592	4.28	204.96	<.0001
Genotype	**Carberry**		72.2654	0.3586	4.25	201.50	<.0001
Genotype	**Glenn**		72.2723	0.3593	4.28	201.16	<.0001
Genotype	**Harvest**		72.9818	0.3586	4.25	203.49	<.0001
Treatment		**H1**	72.7860	0.3346	3.27	217.56	<.0001
Treatment		**H2**	72.7109	0.3363	3.32	216.23	<.0001
Treatment		**H3**	72.8002	0.3346	3.27	217.55	<.0001
Treatment		**H4**	72.8522	0.3410	3.51	213.64	<.0001

Table III.8 Least Squares Means for IG (%), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		3.3777	0.09928	5.08	34.02	<.0001
Genotype	**Carberry**		3.7516	0.09894	5.01	37.92	<.0001
Genotype	**Glenn**		4.0230	0.09933	5.09	40.50	<.0001
Genotype	**Harvest**		3.3076	0.09895	5.01	33.43	<.0001
Treatment		**H1**	3.5990	0.09275	3.91	38.80	<.0001
Treatment		**H2**	3.5892	0.09336	4.01	38.44	<.0001
Treatment		**H3**	3.6171	0.09281	3.92	38.97	<.0001
Treatment		**H4**	3.6546	0.09350	4.04	39.09	<.0001

Table III.9 Least Squares Means for IG/FP, using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		0.2502	0.007239	5.5	34.57	<.0001
Genotype	**Carberry**		0.2689	0.007229	5.48	37.19	<.0001
Genotype	**Glenn**		0.2887	0.007240	5.5	39.88	<.0001
Genotype	**Harvest**		0.2420	0.007231	5.48	33.46	<.0001
Treatment		**H1**	0.2595	0.006346	3.37	40.89	<.0001
Treatment		**H2**	0.2590	0.006361	3.39	40.71	<.0001
Treatment		**H3**	0.2653	0.006349	3.37	41.79	<.0001
Treatment		**H4**	0.2660	0.006361	3.4	41.81	<.0001

Table III.10 Least Squares Means for SP (%), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		10.0562	0.2368	11.2	42.46	<.0001
Genotype	**Carberry**		9.6936	0.2356	11	41.14	<.0001
Genotype	**Glenn**		9.4945	0.2370	11.2	40.06	<.0001
Genotype	**Harvest**		9.9906	0.2357	11	42.39	<.0001
Treatment		**H1**	9.8412	0.1318	4.17	74.68	<.0001
Treatment		**H2**	9.8103	0.1352	4.56	72.59	<.0001
Treatment		**H3**	9.7125	0.1321	4.21	73.50	<.0001
Treatment		**H4**	9.8711	0.1357	4.65	72.73	<.0001

Table III.11 Least Squares Means for SP/FP, using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		0.7446	0.003997	21.1	186.28	<.0001
Genotype	**Carberry**		0.6962	0.003964	20.3	175.63	<.0001
Genotype	**Glenn**		0.6833	0.003985	20.8	171.47	<.0001
Genotype	**Harvest**		0.7260	0.003963	20.4	183.18	<.0001
Treatment		**H1**	0.7092	0.002960	10.9	239.60	<.0001
Treatment		**H2**	0.7103	0.003020	11.6	235.16	<.0001
Treatment		**H3**	0.7117	0.002943	10.7	241.81	<.0001
Treatment		**H4**	0.7190	0.003077	12.4	233.70	<.0001

Table III.12 Least Squares Means Table for GSI, using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		0.3360	0.01002	6.02	33.54	<.0001
Genotype	**Carberry**		0.3867	0.009994	5.97	38.69	<.0001
Genotype	**Glenn**		0.4236	0.01002	6.03	42.28	<.0001
Genotype	**Harvest**		0.3327	0.009995	5.97	33.28	<.0001
Treatment		**H1**	0.3670	0.008356	3.14	43.92	<.0001
Treatment		**H2**	0.3670	0.008388	3.18	43.76	<.0001
Treatment		**H3**	0.3738	0.008362	3.14	44.71	<.0001
Treatment		**H4**	0.3711	0.008386	3.18	44.25	<.0001

Table III.13 Least Squares Means for MDT (min), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		3.0103	0.2037	8.33	14.78	<.0001
Genotype	**Carberry**		3.1046	0.2033	8.27	15.27	<.0001
Genotype	**Glenn**		4.0443	0.2037	8.33	19.85	<.0001
Genotype	**Harvest**		2.6138	0.2033	8.28	12.86	<.0001
Treatment		**H1**	3.0537	0.1461	2.98	20.91	0.0002
Treatment		**H2**	3.0591	0.1473	3.06	20.77	0.0002
Treatment		**H3**	3.3296	0.1462	2.99	22.78	0.0002
Treatment		**H4**	3.3307	0.1483	3.16	22.45	0.0001

Table III.14 Least Squares Means for WAP (%Torque*min), using PROC MIXED, and combined year analysis.

Effect	Genotype	Harvest Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Genotype	**Brandon**		78.9530	6.2320	4.53	12.67	0.0001
Genotype	**Carberry**		84.8600	6.2280	4.52	13.63	<.0001
Genotype	**Glenn**		119.98	6.2320	4.53	19.25	<.0001
Genotype	**Harvest**		72.5271	6.2278	4.52	11.65	0.0002
Treatment		**H1**	86.8627	5.5411	2.93	15.68	0.0006
Treatment		**H2**	87.2858	5.5572	2.96	15.71	0.0006
Treatment		**H3**	91.0609	5.5405	2.93	16.44	0.0006
Treatment		**H4**	91.1093	5.5819	3.01	16.32	0.0005

Appendix IV. Growing Season Precipitation- Relationships Between Tested Parameters and Precipitation Timing.

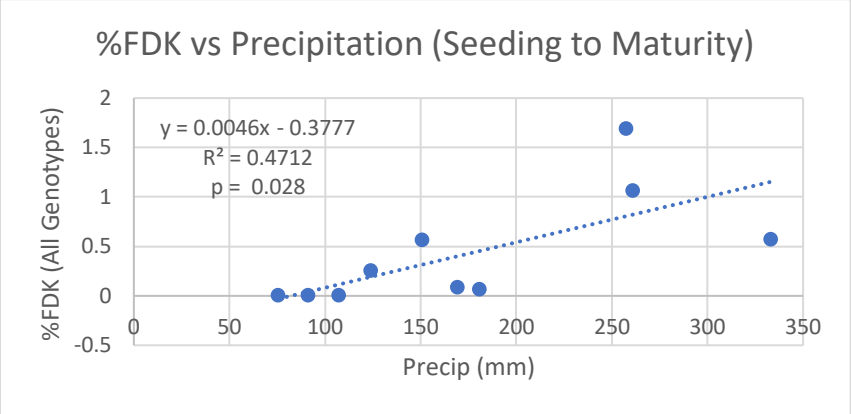


Figure IV.1 Fusarium damaged kernels (%FDK) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

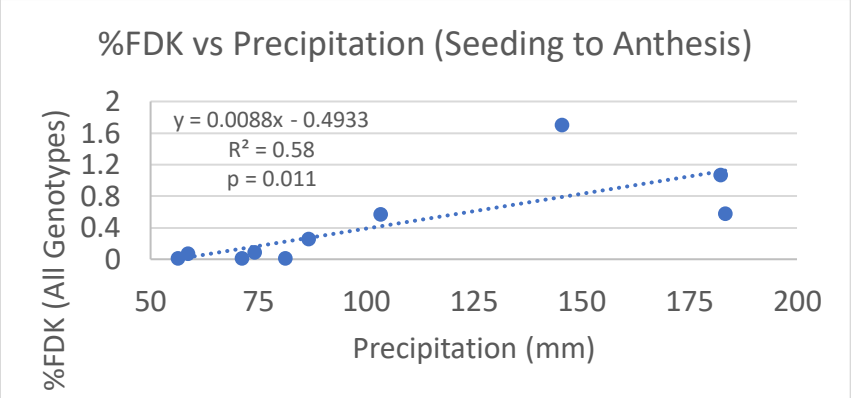


Figure IV.2 Fusarium damaged kernels (%FDK) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

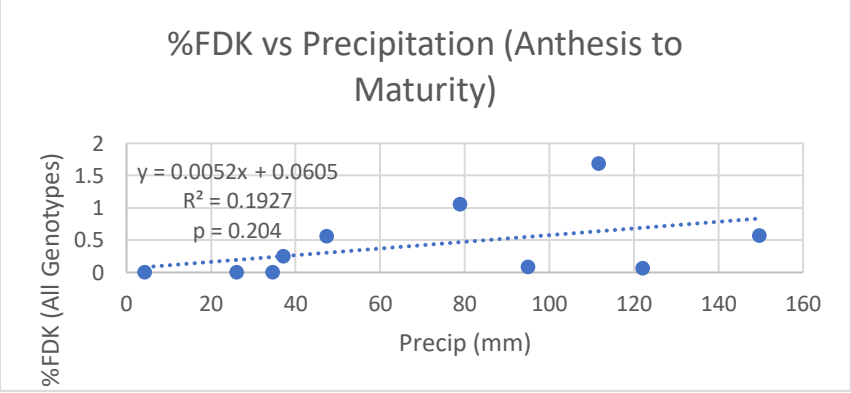


Figure IV.3 Fusarium damaged kernels (%FDK) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

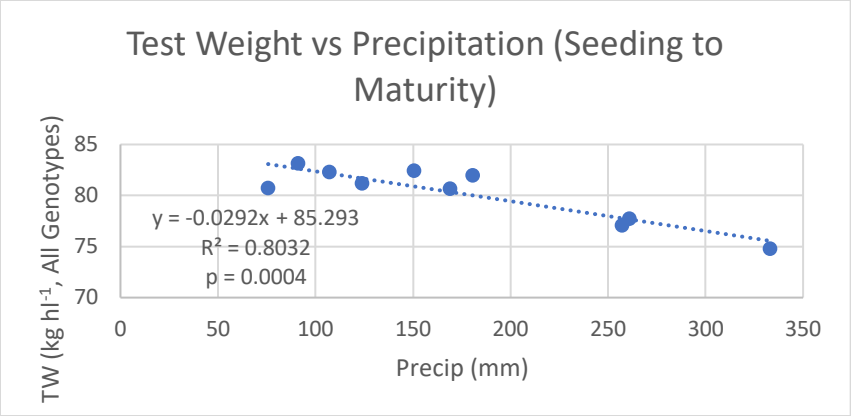


Figure IV.4 Test weight averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

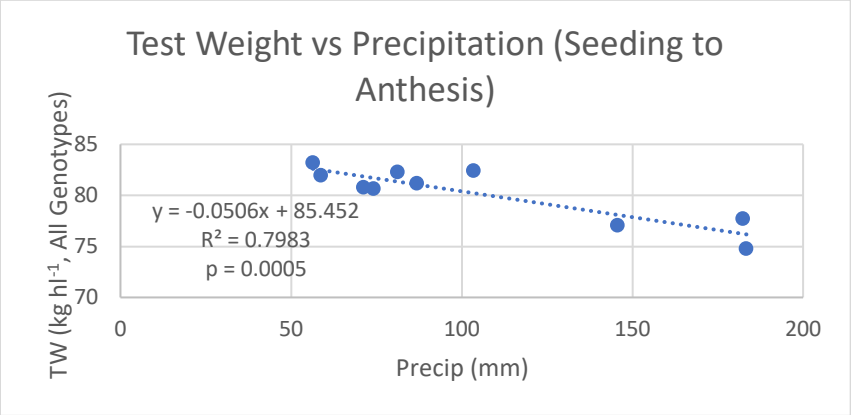


Figure IV.5 Test weight averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

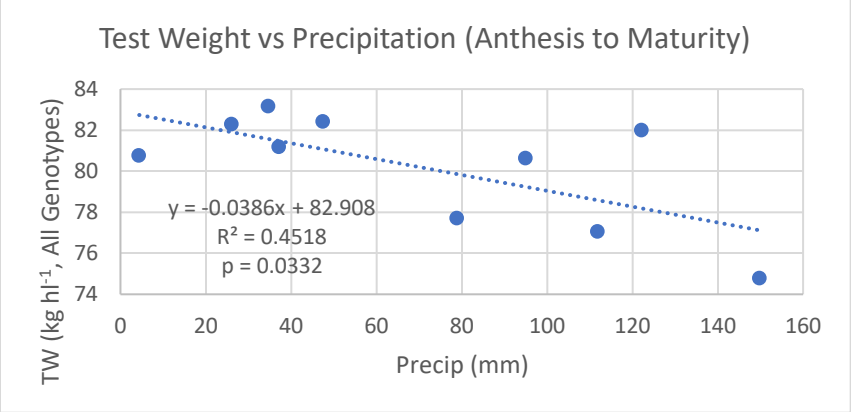


Figure IV.6 Test weight averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

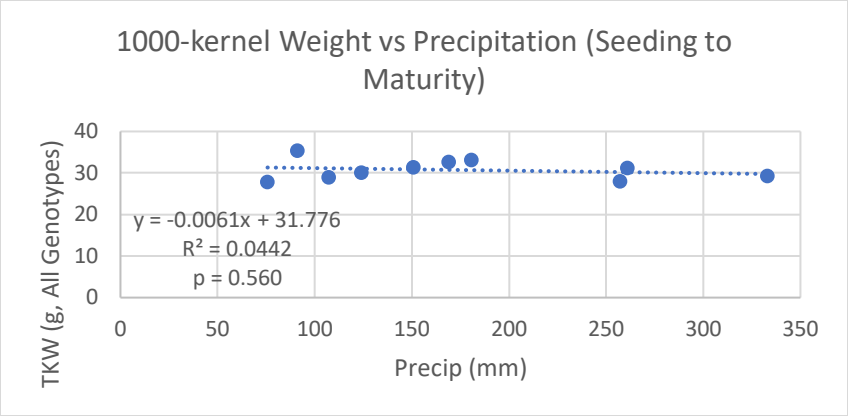


Figure IV.7 1000-kernel weight (TKW) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

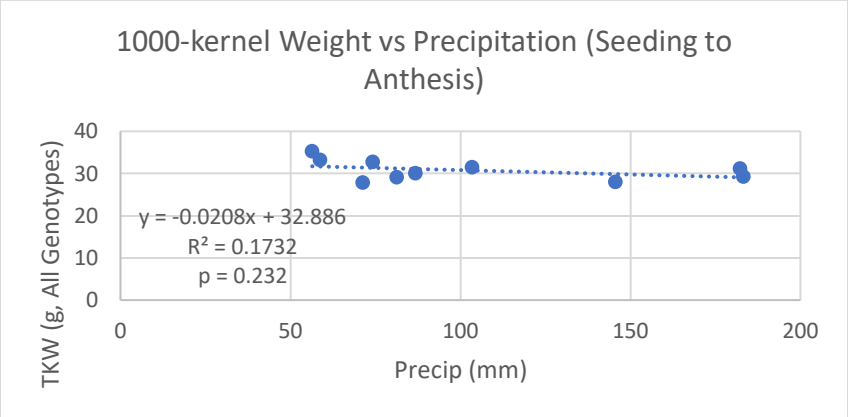


Figure IV.8 1000-kernel weight (TKW) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

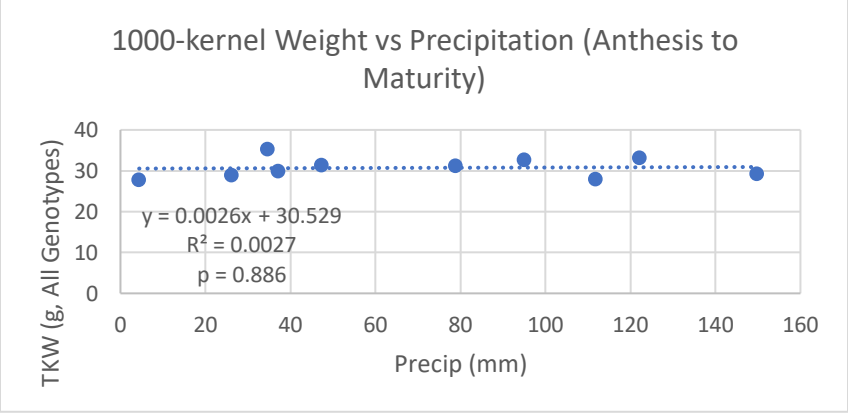


Figure IV.9 1000-kernel weight (TKW) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

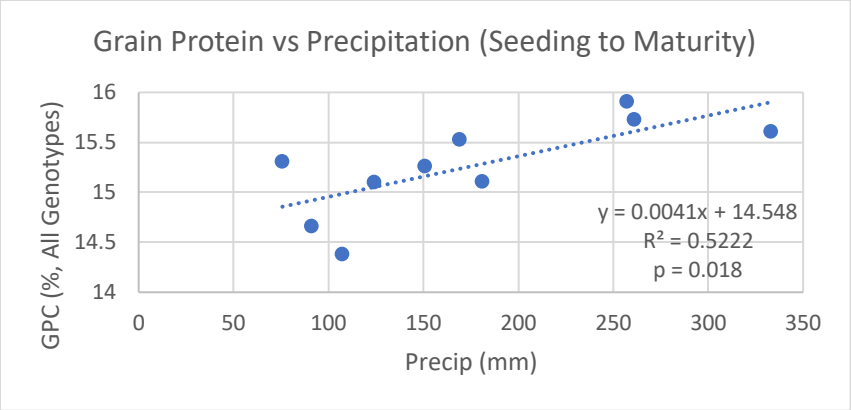


Figure IV.10 Grain protein content (%GPC) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

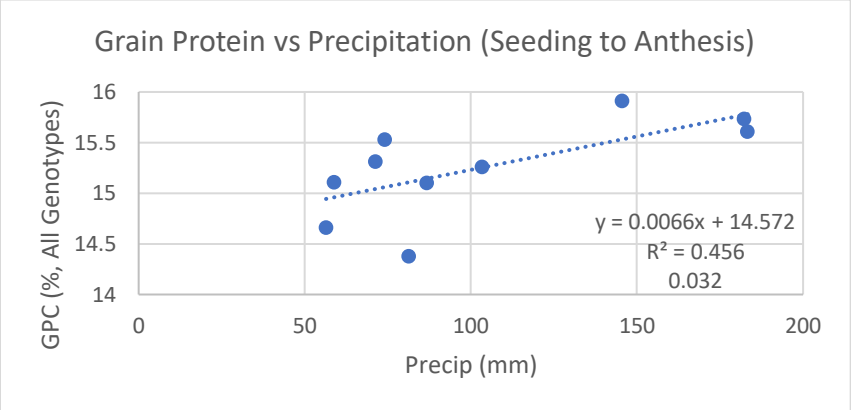


Figure IV.11 Grain protein content (%GPC) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

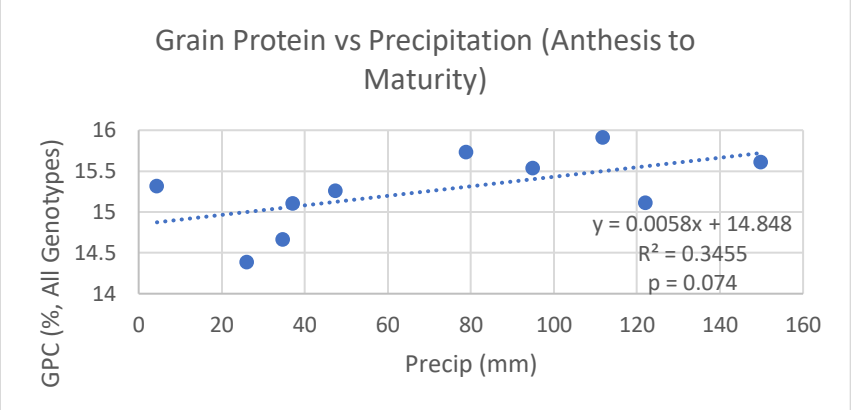


Figure IV.12 Grain protein content (%GPC) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

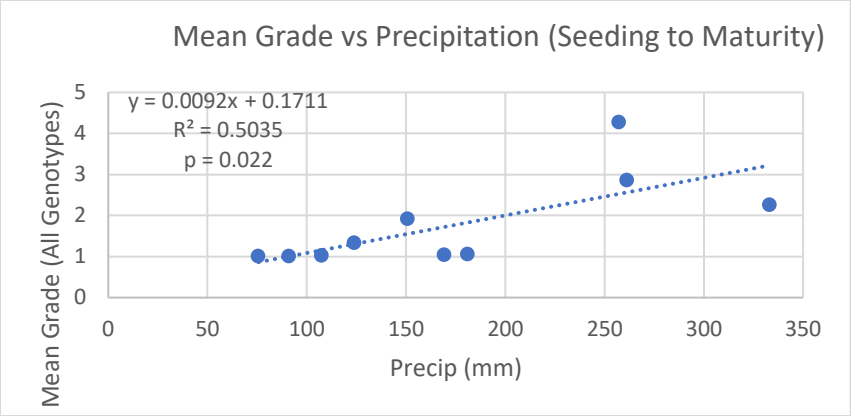


Figure IV.13 Mean grade averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

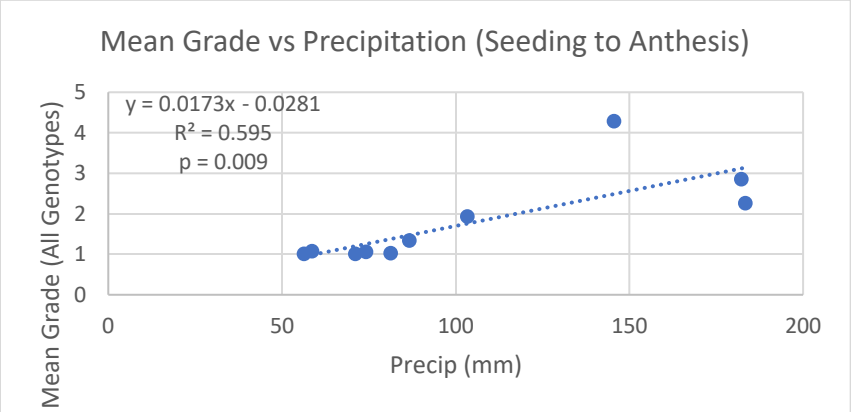


Figure IV.14 Mean grade averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

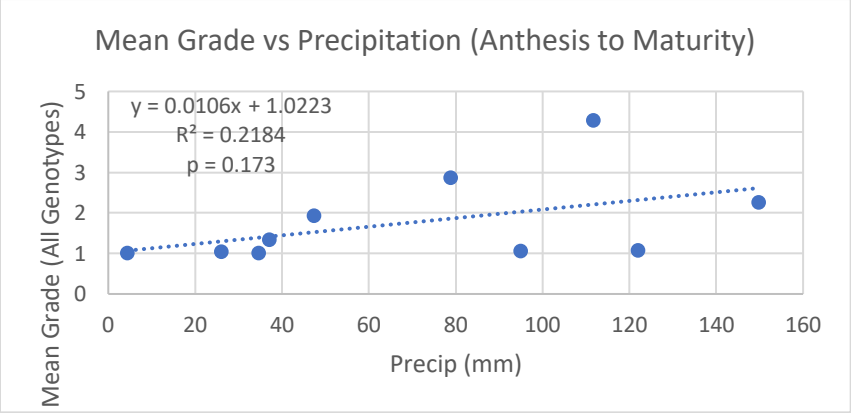


Figure IV.15 Mean grade averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

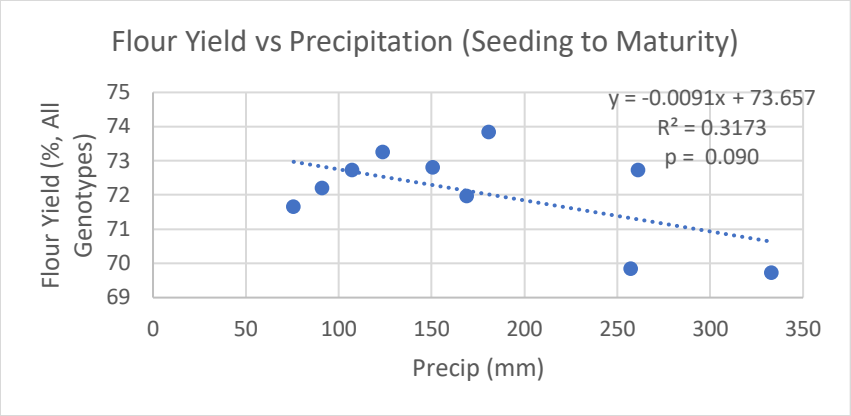


Figure IV.16 Flour yield averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

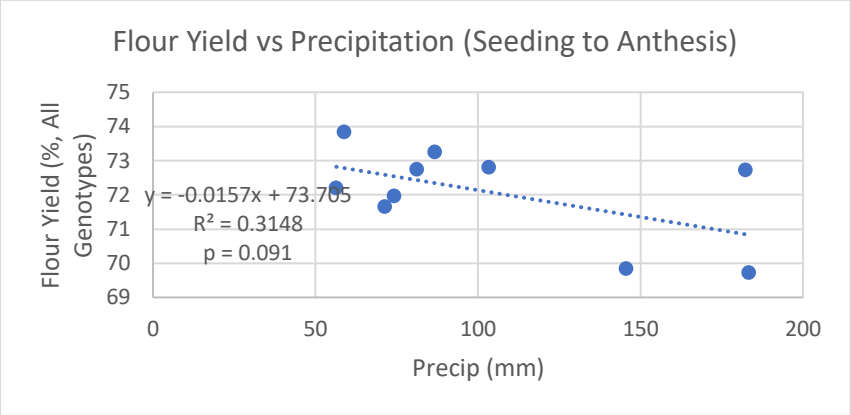


Figure IV.17 Flour yield averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

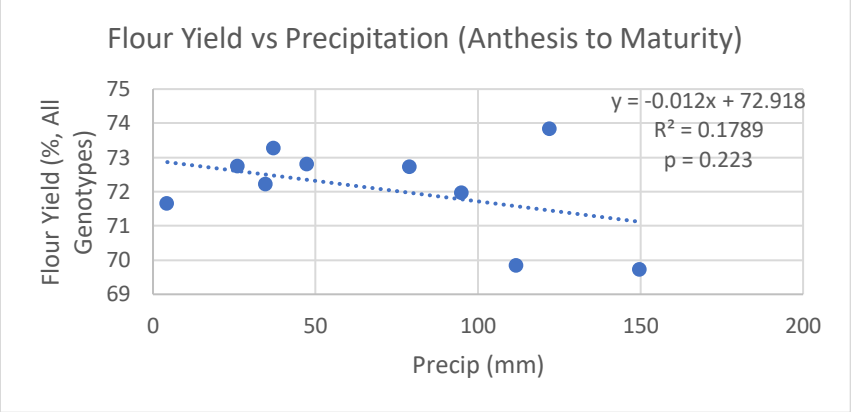


Figure IV.18 Flour yield averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

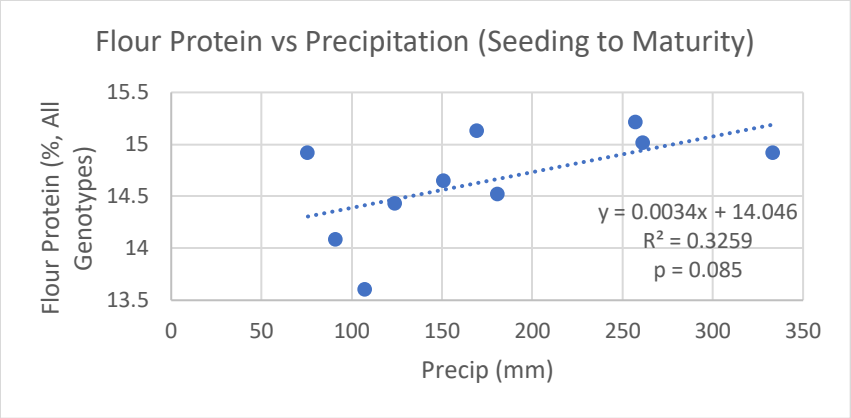


Figure IV.19 Flour protein averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

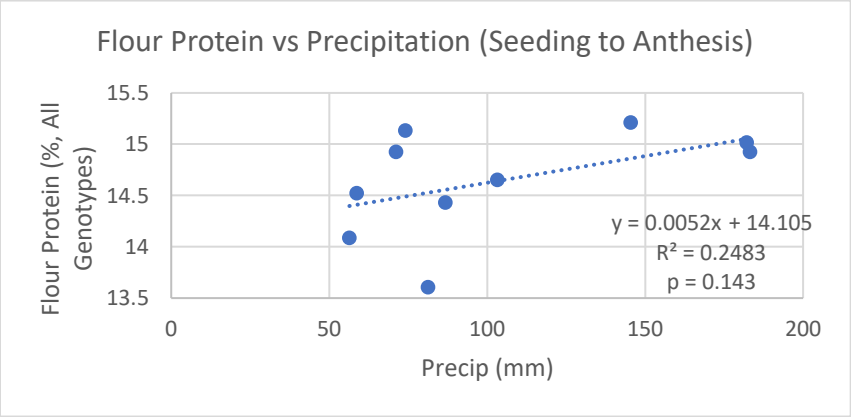


Figure IV.20 Flour protein averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

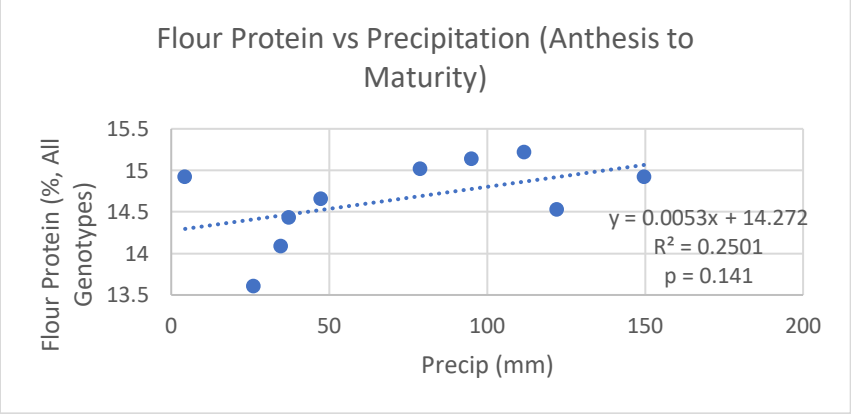


Figure IV.21 Flour protein averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

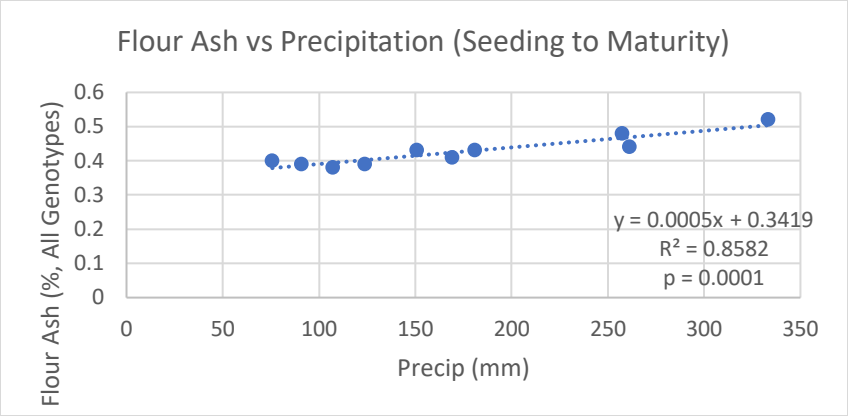


Figure IV.22 Flour ash averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

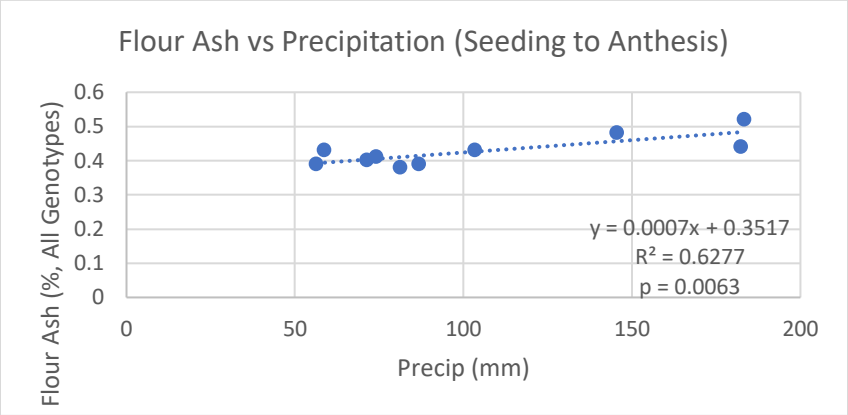


Figure IV.23 Flour ash averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

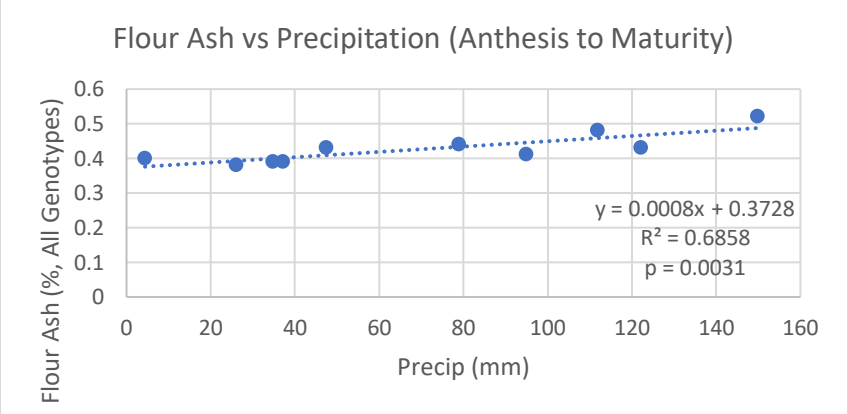


Figure IV.24 Flour ash averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

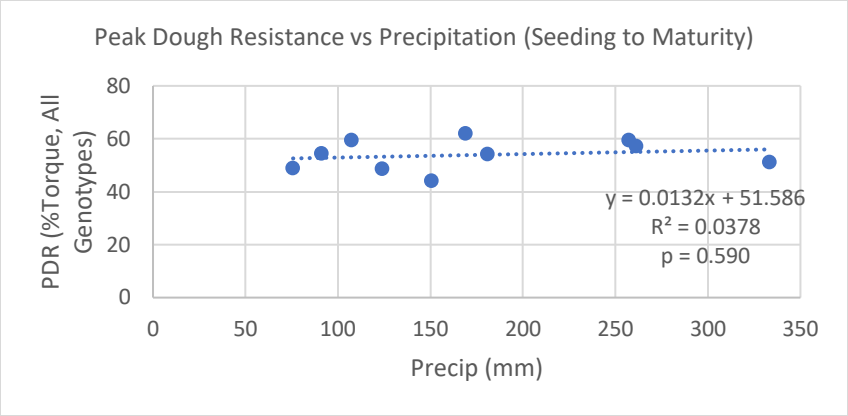


Figure IV.25 Peak dough resistance (PDR) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

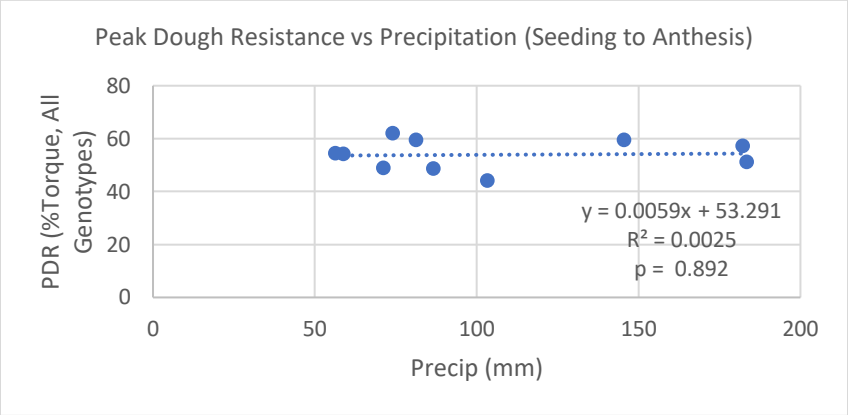


Figure IV. 26 Peak dough resistance (PDR) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

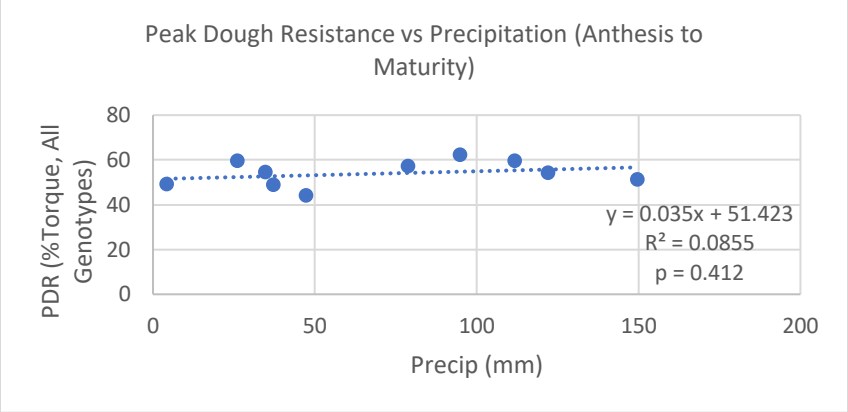


Figure IV.27 Peak dough resistance (PDR) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

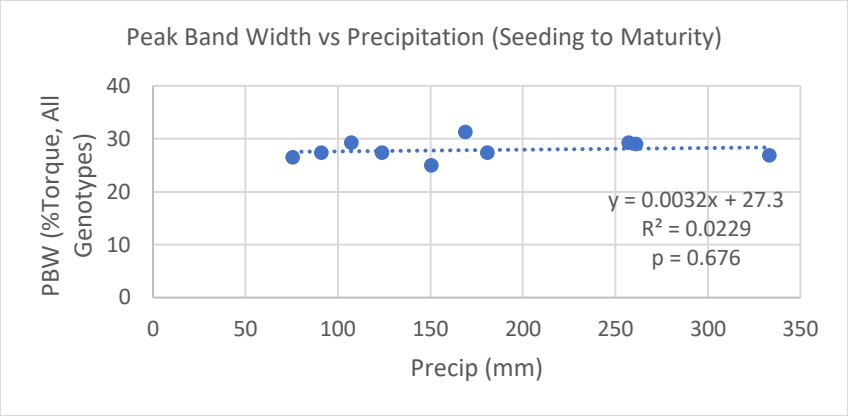


Figure IV.28 Peak band width (PBW) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

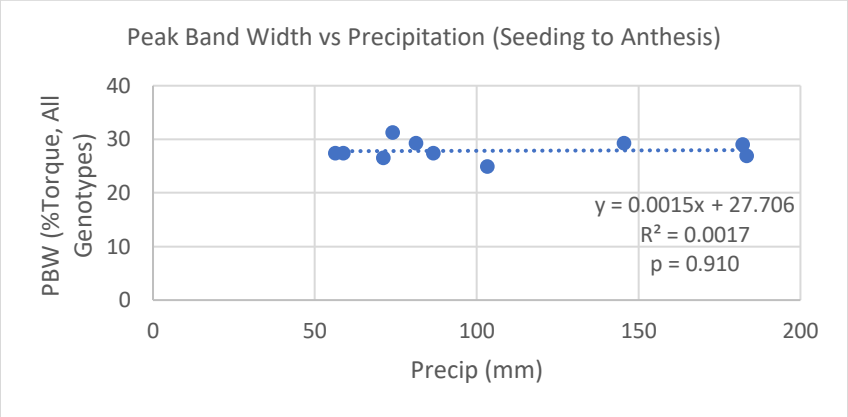


Figure IV.29 Peak band width (PBW) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

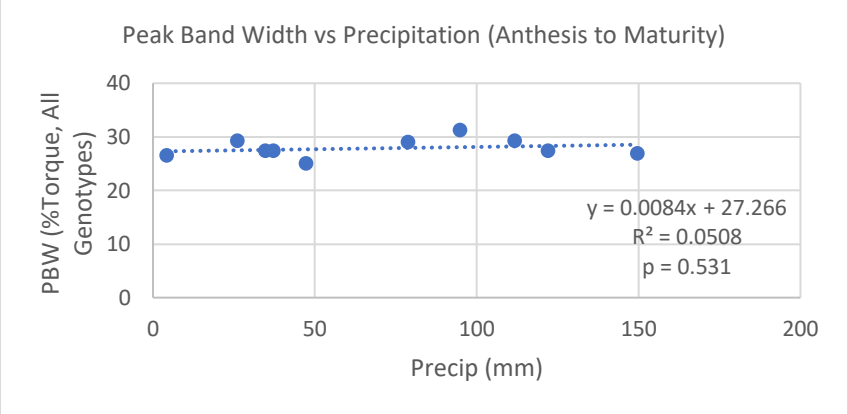


Figure IV.30 Peak band width (PBW) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

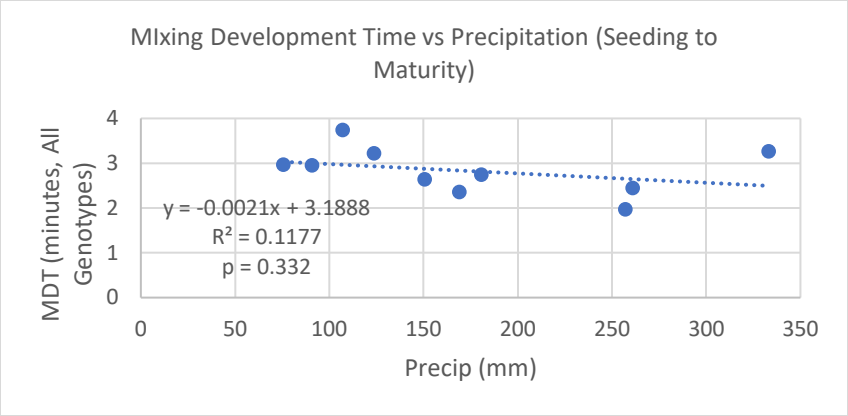


Figure IV.31 Mixing development time (MDT) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

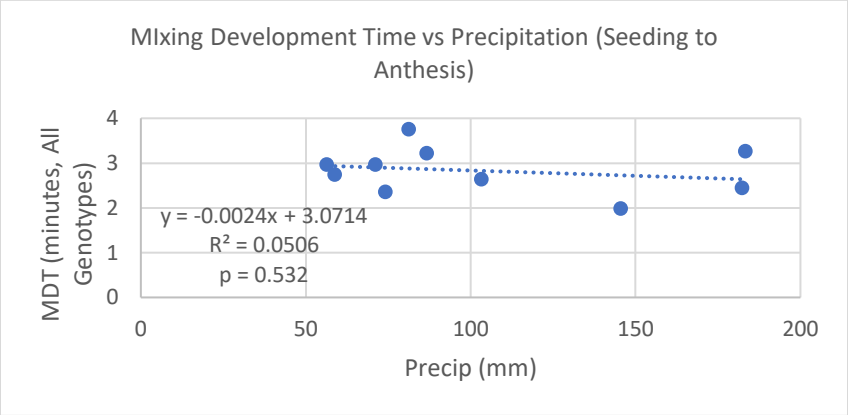


Figure IV.32 Mixing development time (MDT) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

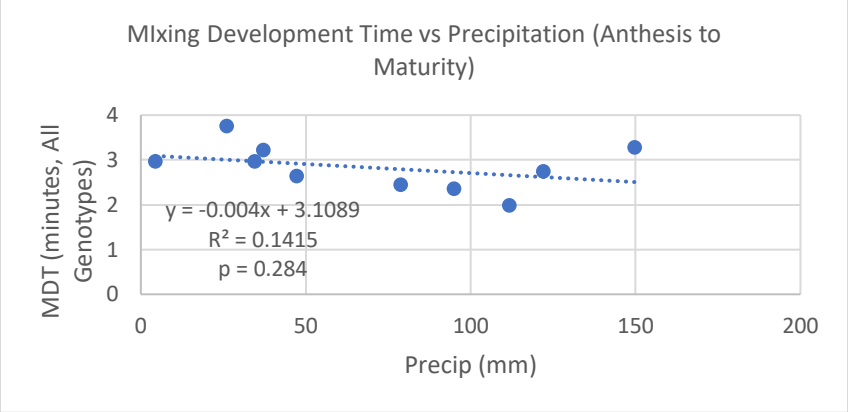


Figure IV.33 Mixing development time (MDT) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

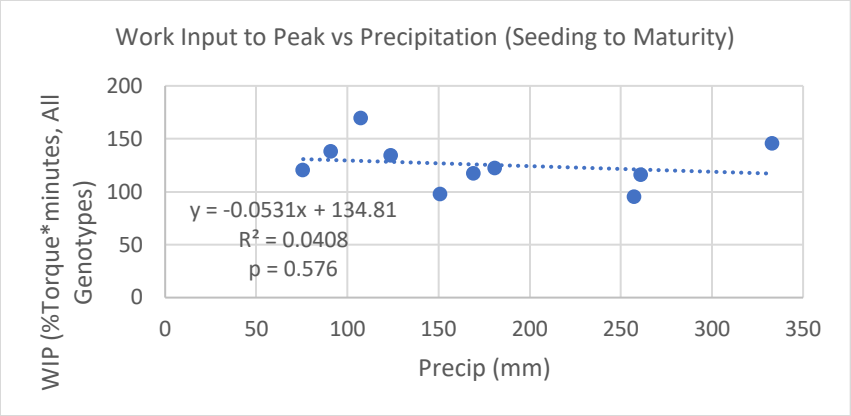


Figure IV.34 Work input to peak (WIP) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

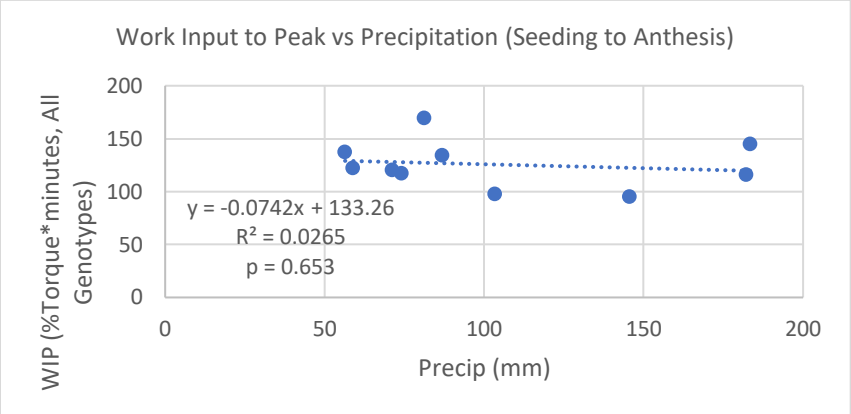


Figure IV.35 Work input to peak (WIP) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

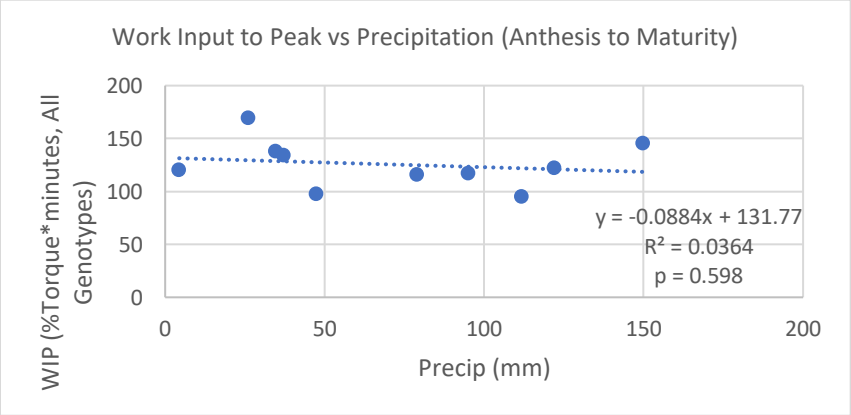


Figure IV.36 Work input to peak (WIP) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

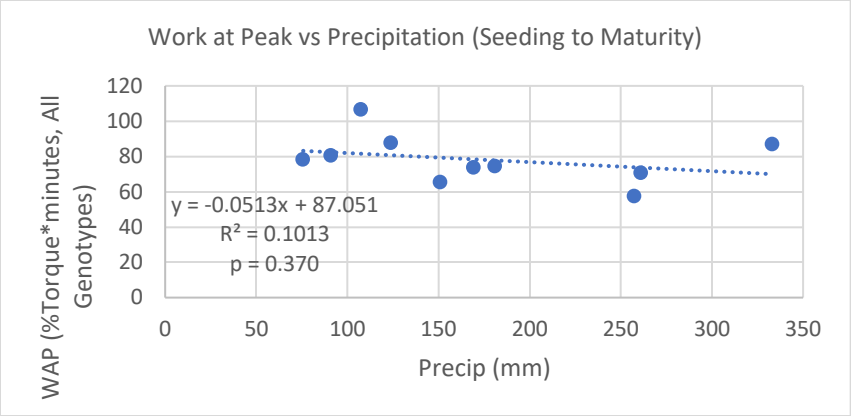


Figure IV.37 Work at peak (WAP) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

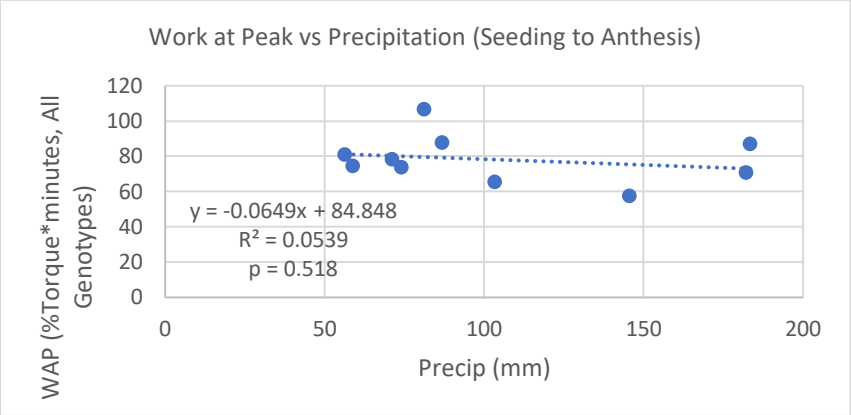


Figure IV.38 Work at peak (WAP) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

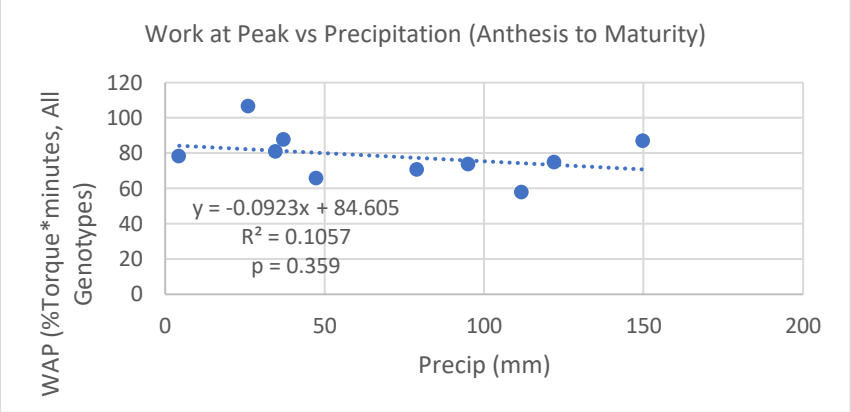


Figure IV.39 Work at peak (WAP) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

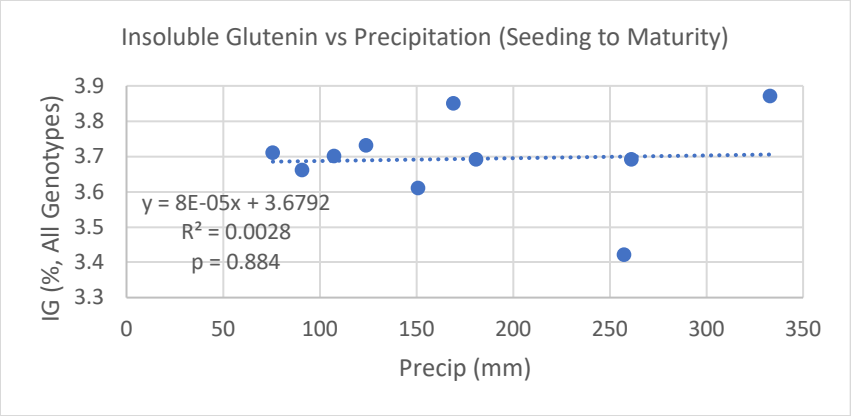


Table IV.40 Insoluble glutenin (IG) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

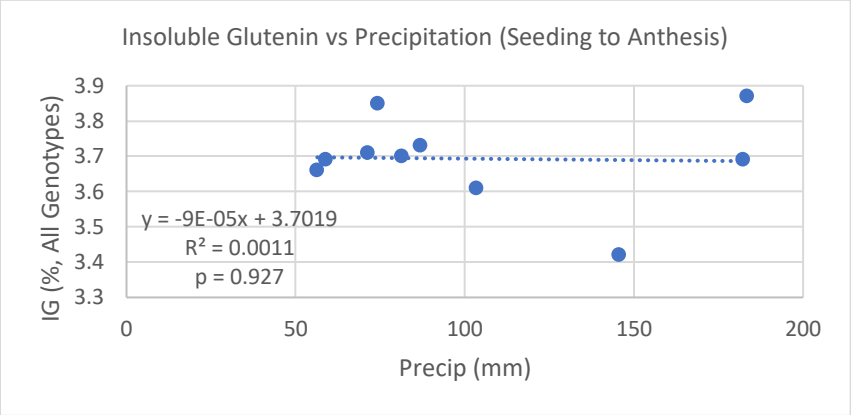


Figure IV.41 Insoluble glutenin (IG) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

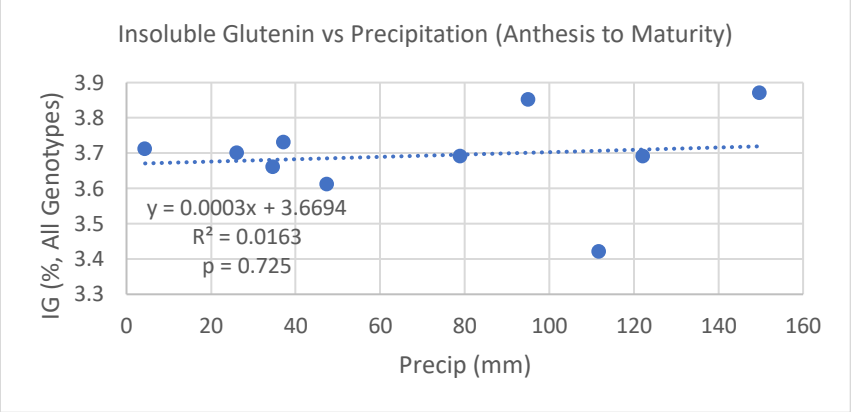


Figure IV.42 Insoluble glutenin (IG) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

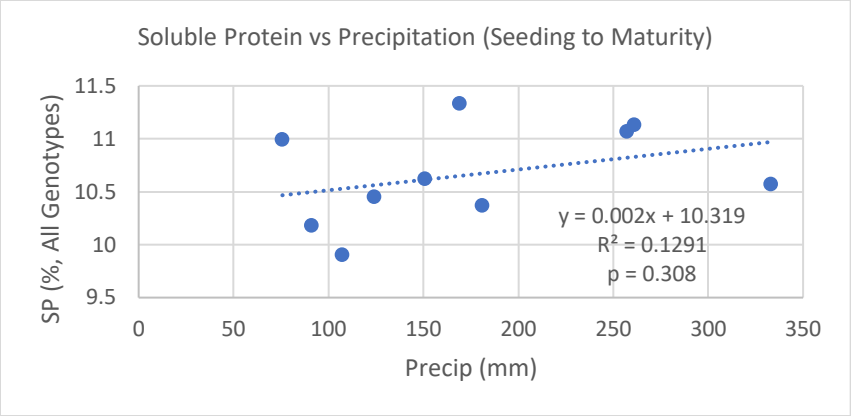


Figure IV.43 Soluble prolamin (SP) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

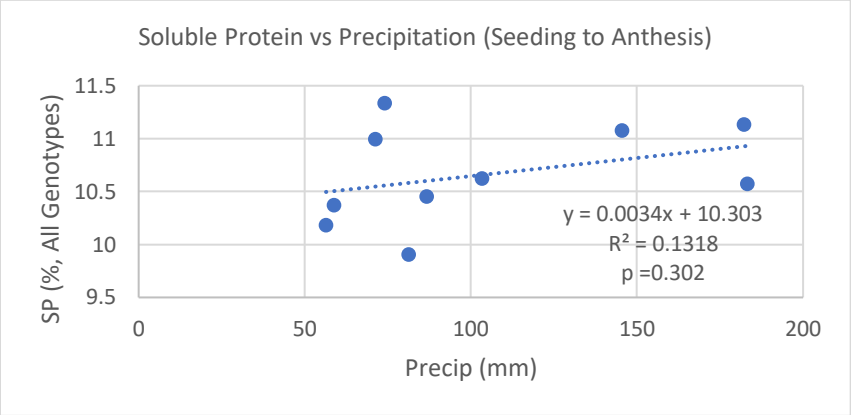


Figure IV.44 Soluble prolamin (SP) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

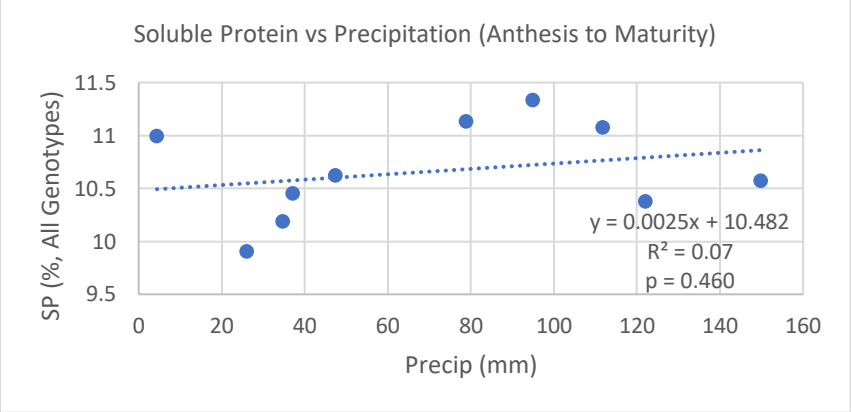


Figure IV.45 Soluble prolamin (SP) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

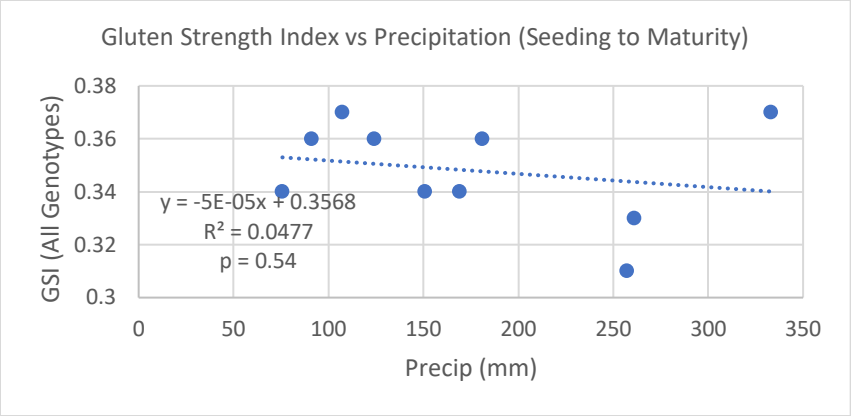


Figure IV.46 Gluten strength index (GSI) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

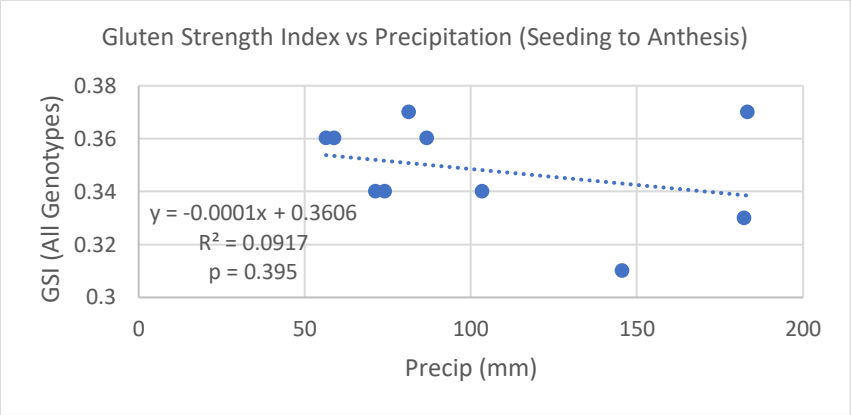


Figure IV.47 Gluten strength index (GSI) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

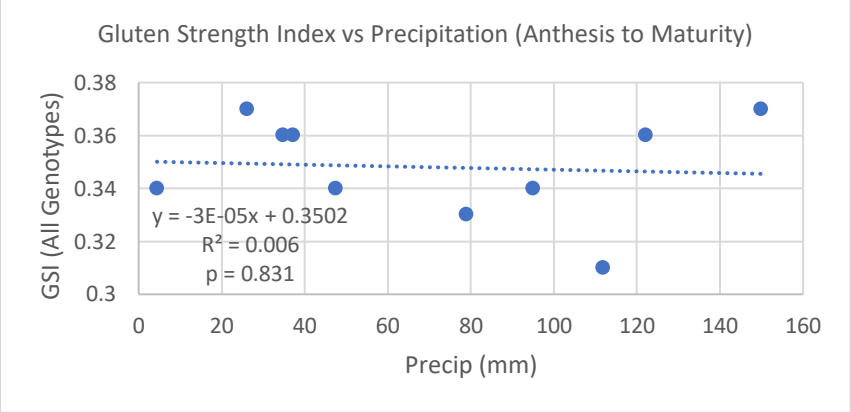


Figure IV.48 Gluten strength index (GSI) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

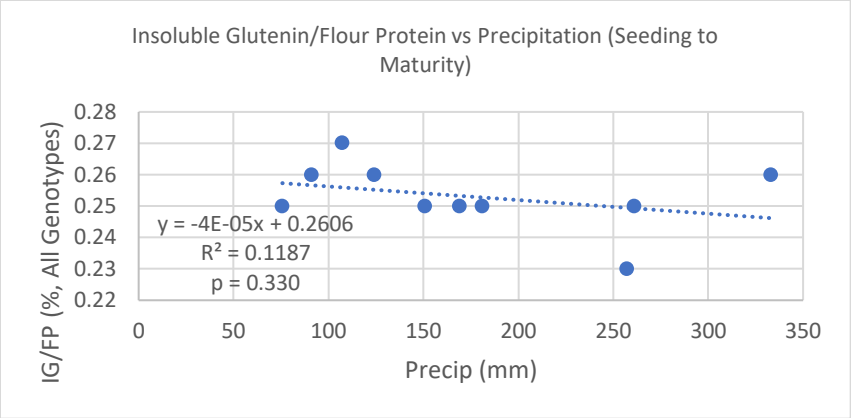


Figure IV.49 Insoluble glutenin/flour protein (IG/FP) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

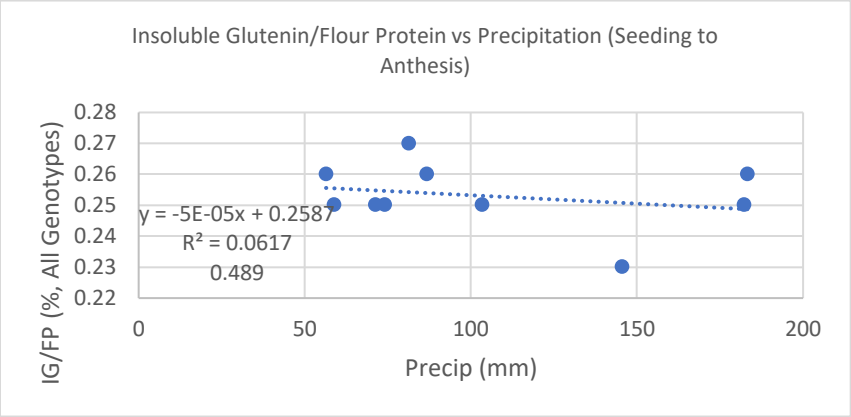


Figure IV.50 Insoluble glutenin/flour protein (IG/FP) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

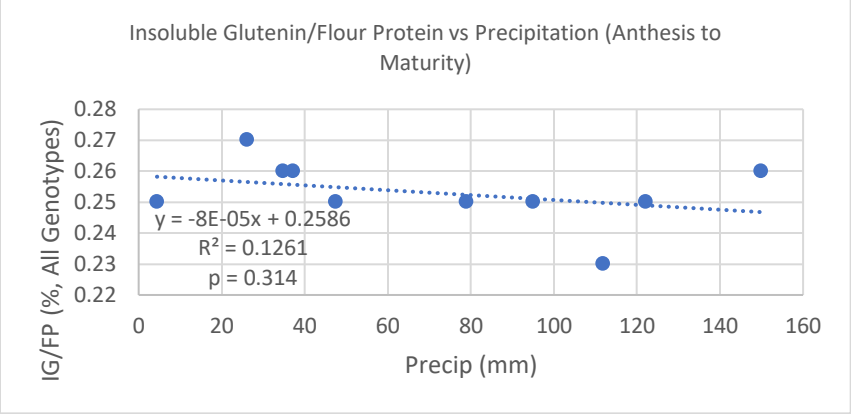


Figure IV.51 Insoluble glutenin/flour protein (IG/FP) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

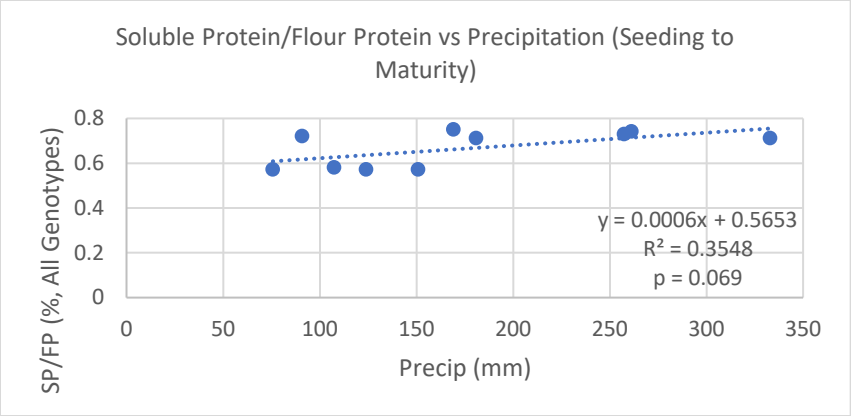


Figure IV.52 Soluble prolamin/flour protein (SP/FP) averaged across all genotypes and pesticide treatments versus precipitation from seeding to maturity for all site-years.

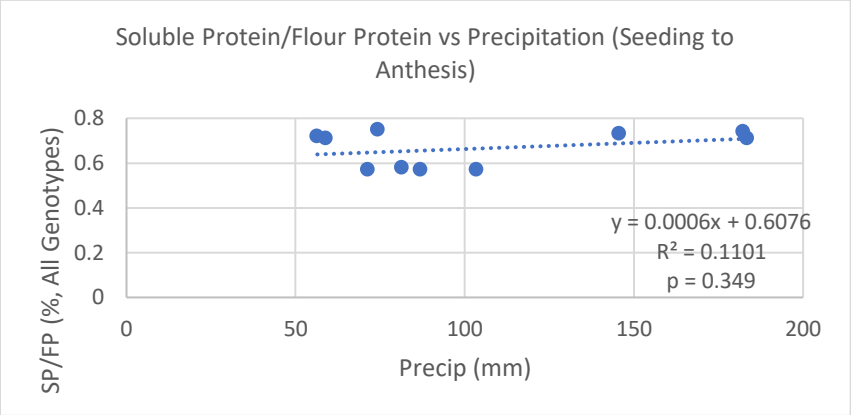


Figure IV.53 Soluble prolamin/flour protein (SP/FP) averaged across all genotypes and pesticide treatments versus precipitation from seeding to anthesis for all site-years.

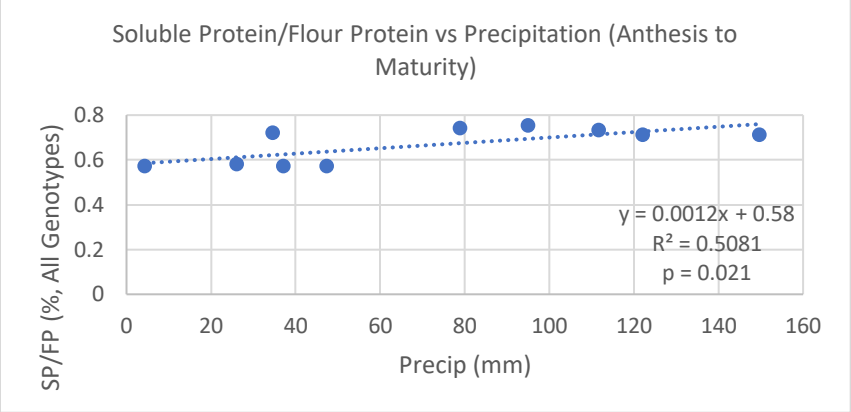


Figure IV.54 Soluble prolamin/flour protein (SP/FP) averaged across all genotypes and pesticide treatments versus precipitation from anthesis to maturity for all site-years.

Appendix V. Growing Season Temperature- Relationships Between Tested Parameters and Mean Temperatures during the Growing Season.

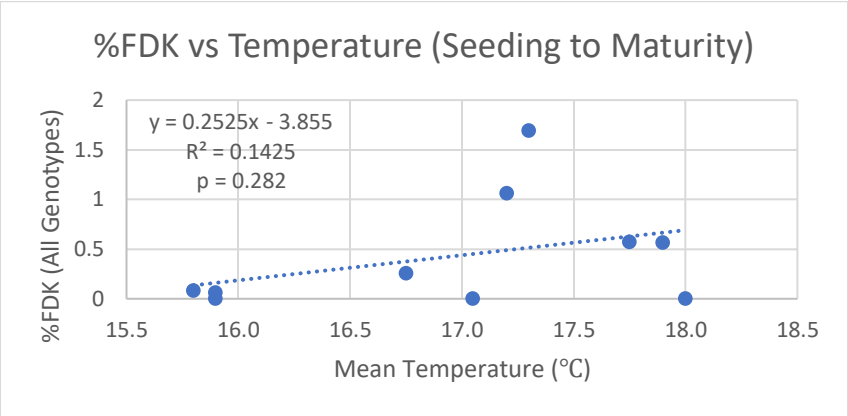


Figure V.1 Fusarium damaged kernels (%FDK) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

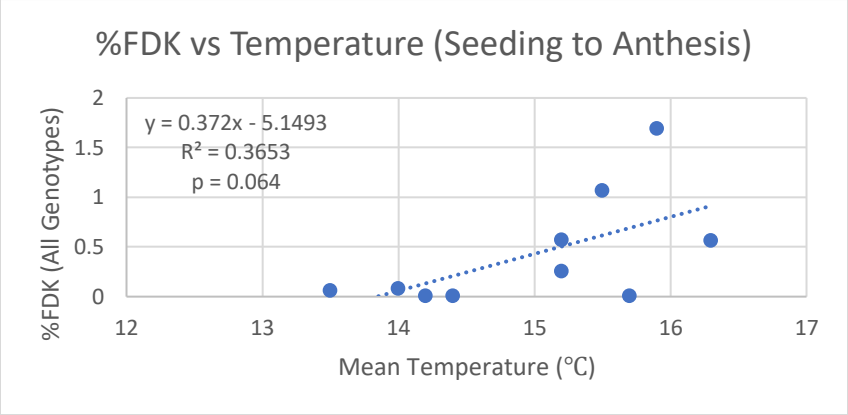


Figure V.2 Fusarium damaged kernels (%FDK) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

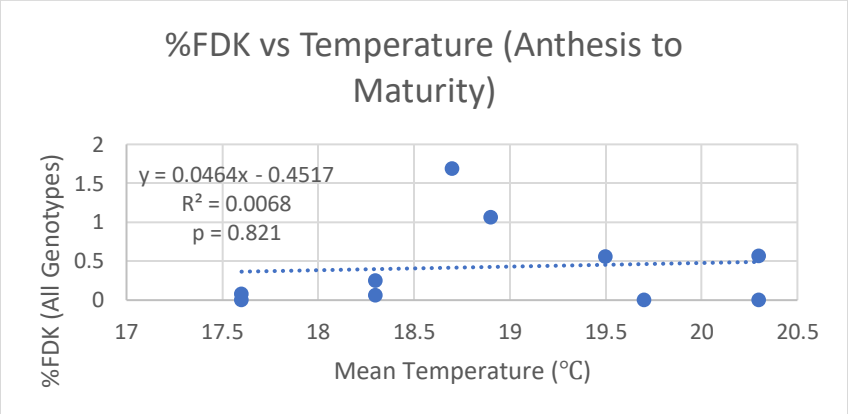


Figure V.3 Fusarium damaged kernels (%FDK) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

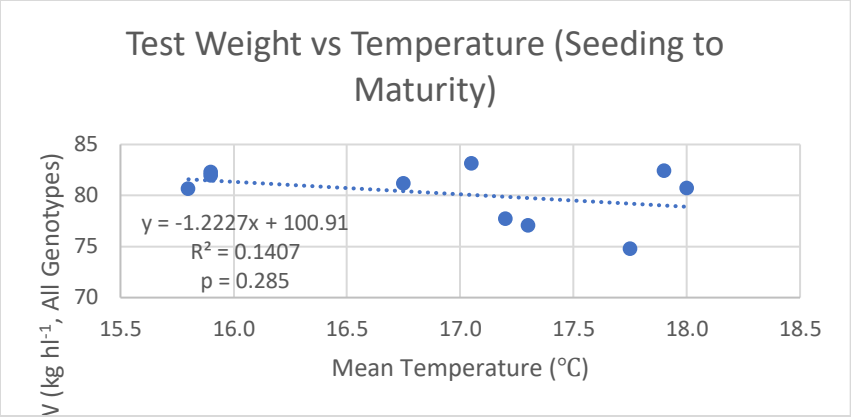


Figure V.4 Test weight averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

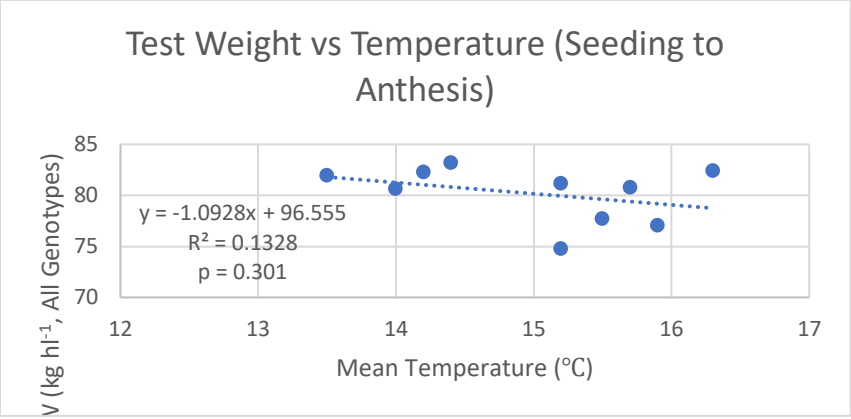


Figure V.5 Test weight averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

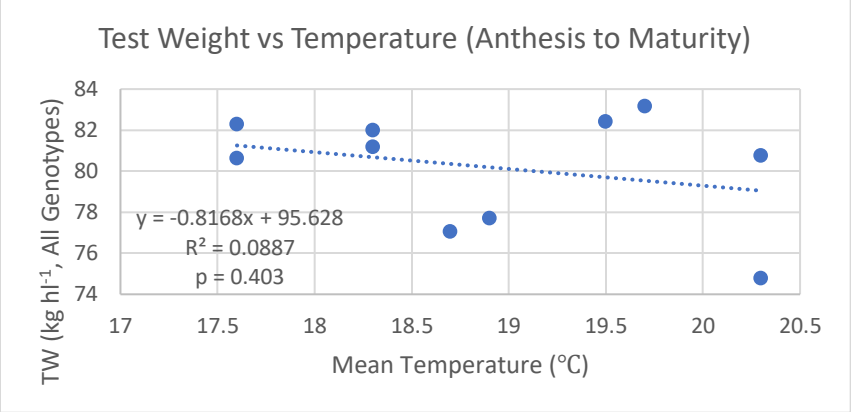


Figure V.6 Test weight averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

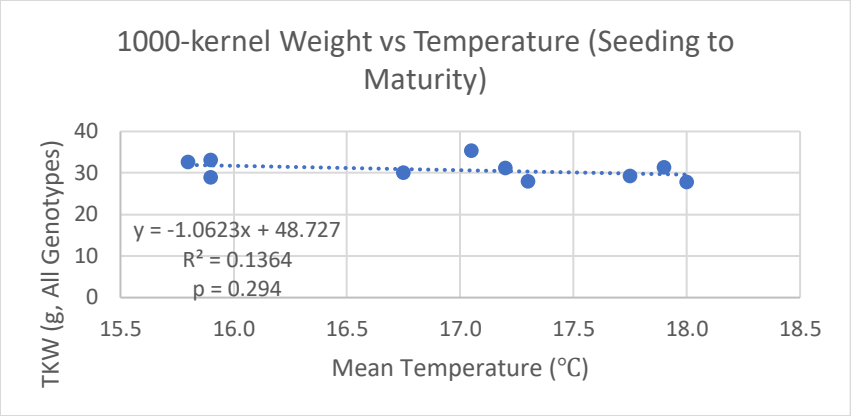


Figure V.7 1000-kernel weight (TKW) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

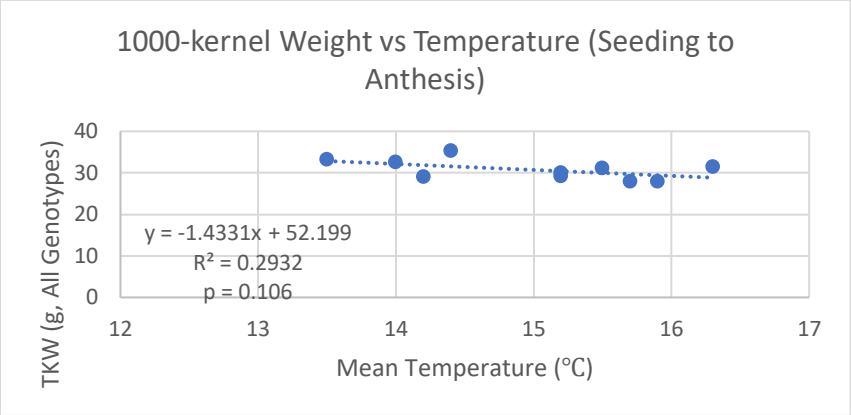


Figure V.8 1000-kernel weight (TKW) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

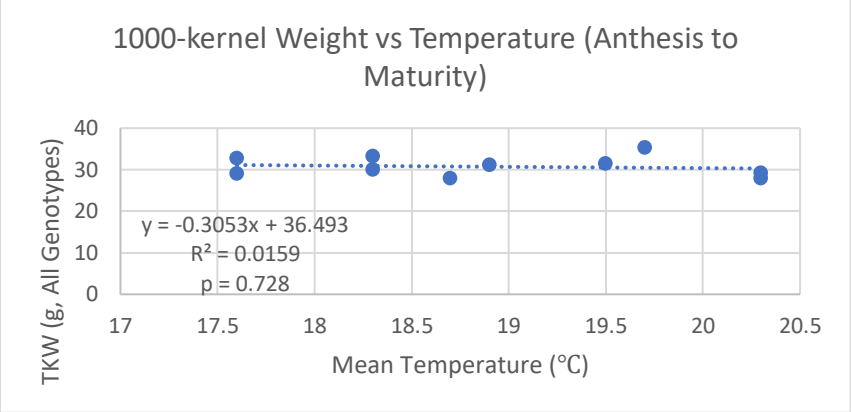


Figure V.9 1000-kernel weight averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

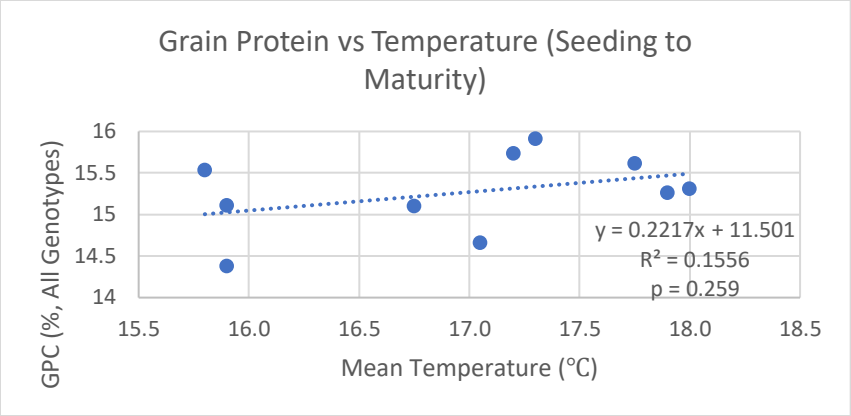


Figure V.10 Grain protein content (%GPC) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

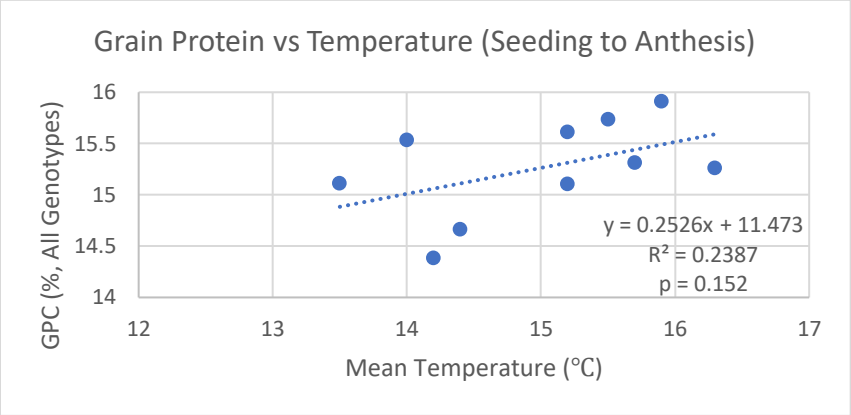


Figure V.11 Grain protein content (%GPC) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

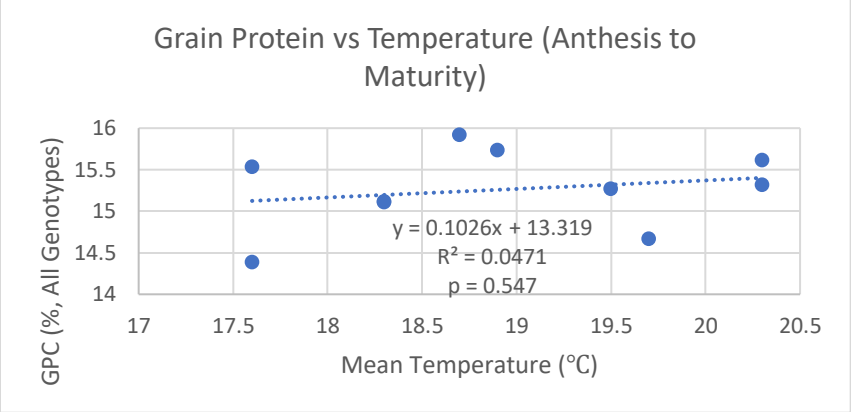


Figure V.12 Grain protein content (%GPC) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

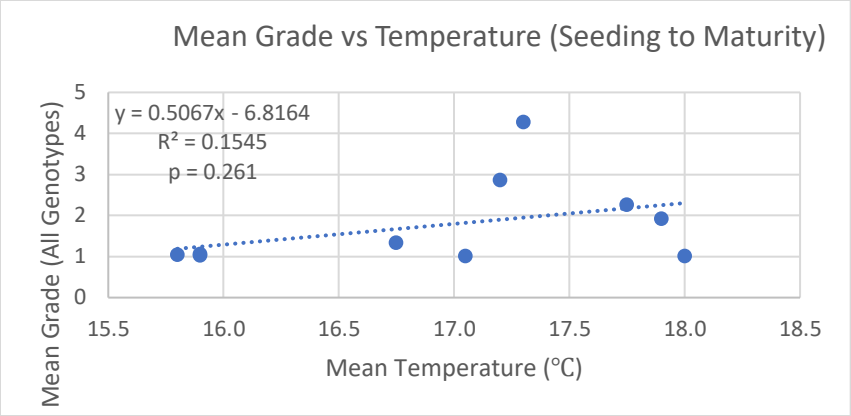


Figure V.13 Mean grade averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

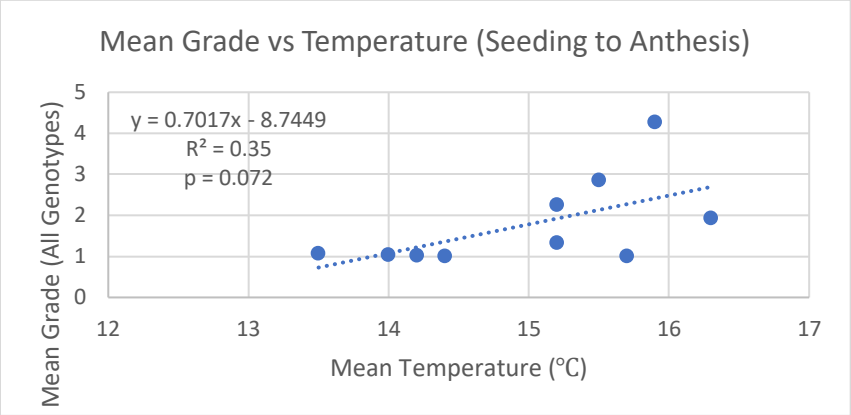


Figure V.14 Mean grade averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

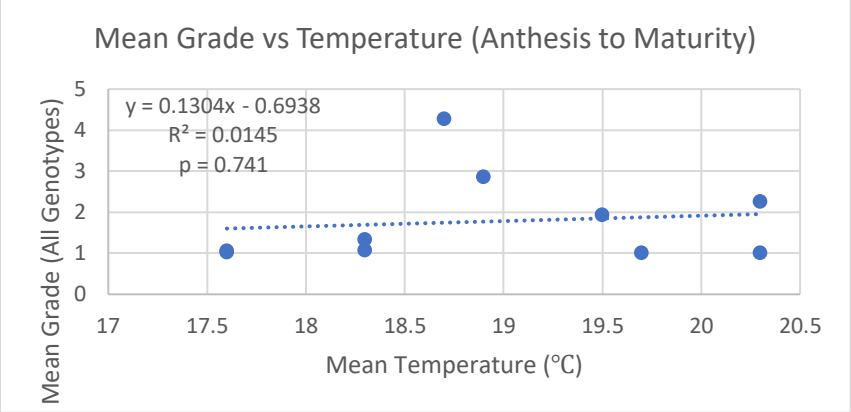


Figure V.15 Mean grade averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

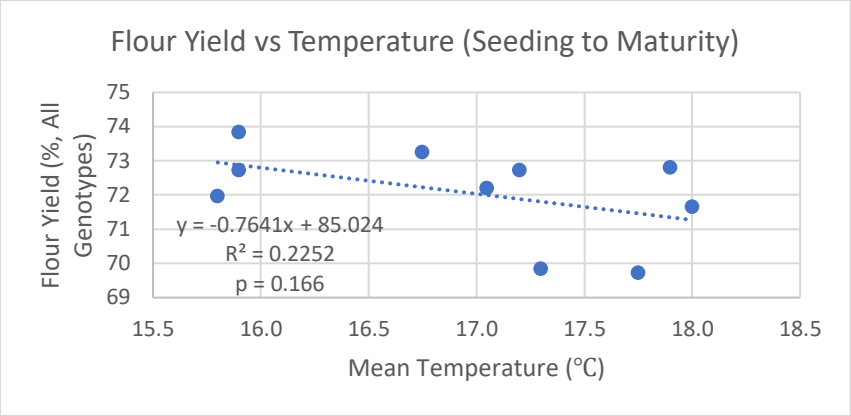


Figure V.16 Flour yield averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

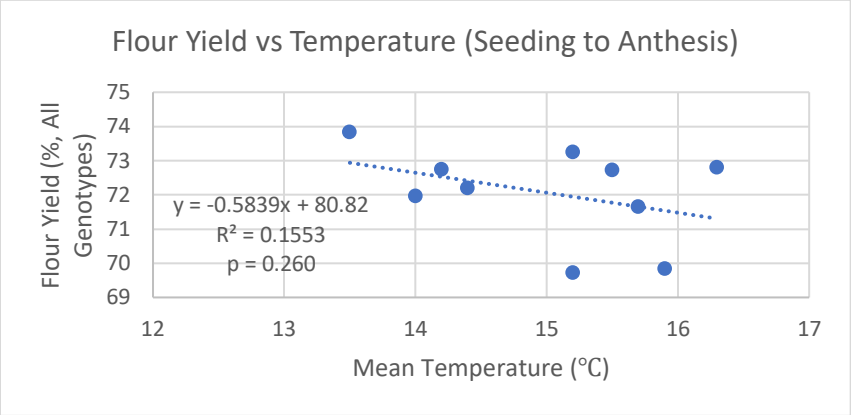


Figure V.17 Flour yield averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

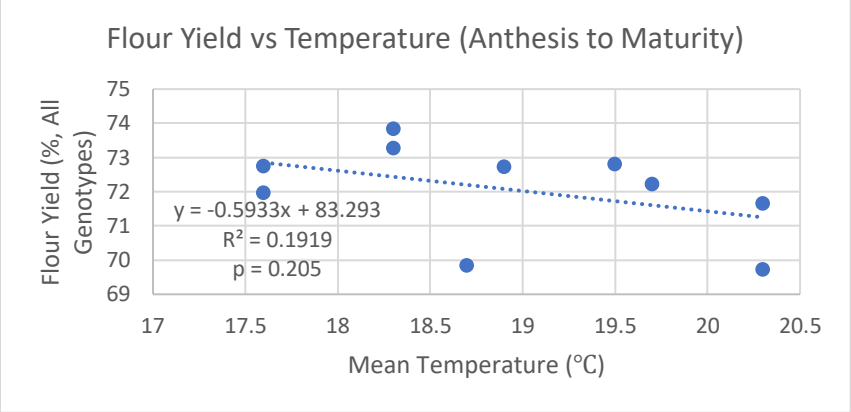


Figure V.18 Flour yield averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

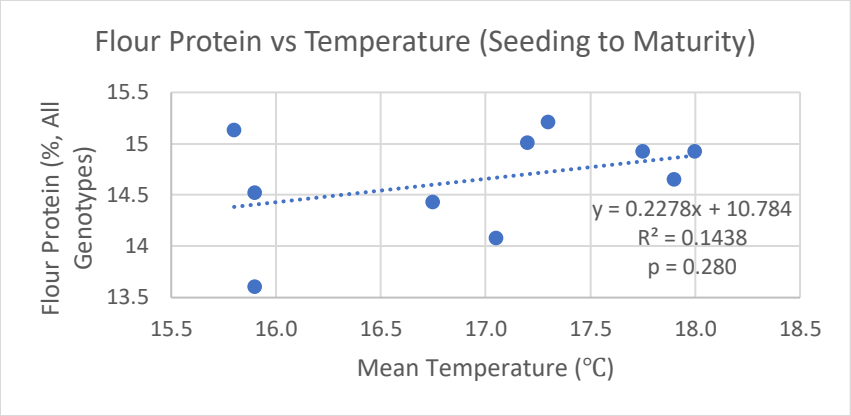


Figure V.19 Flour protein averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

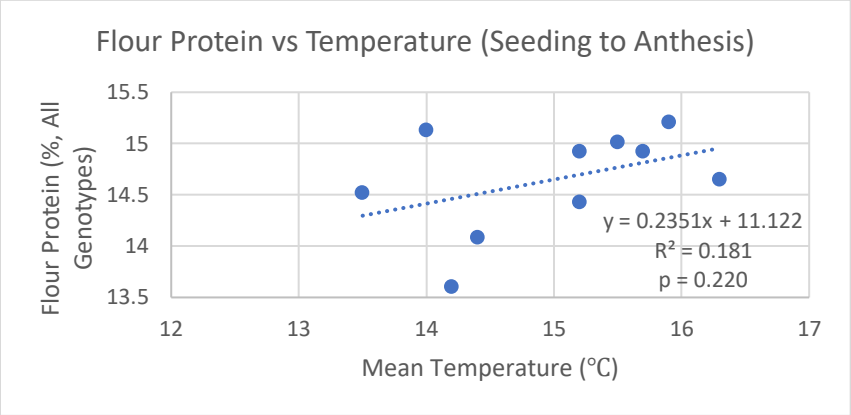


Figure V.20 Flour protein averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

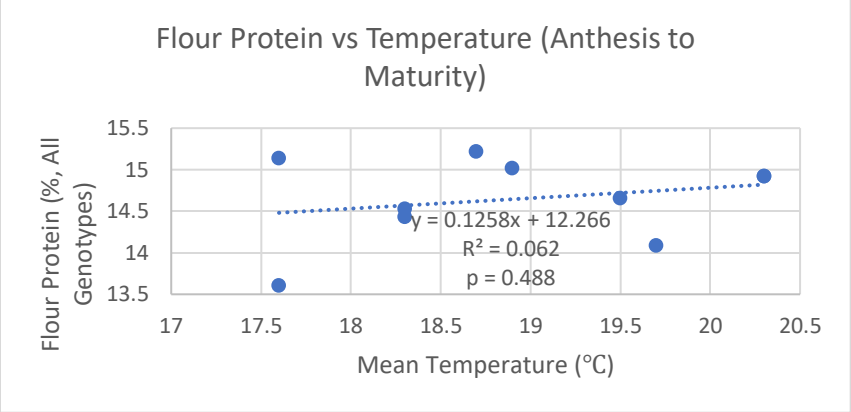


Figure V.21 Flour protein averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

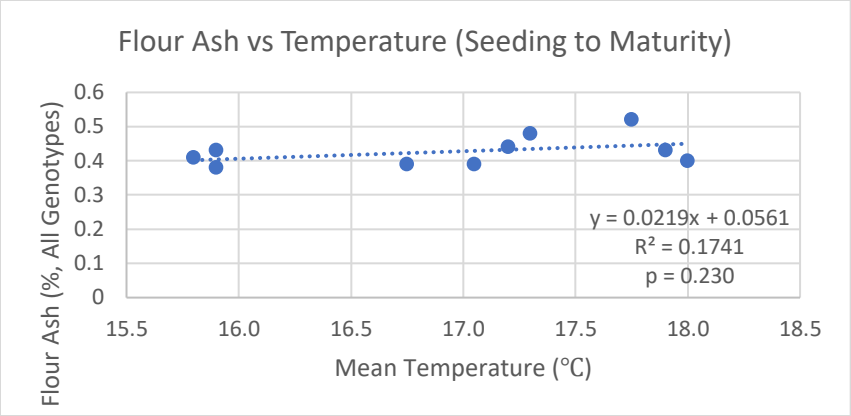


Figure V.22 Flour ash averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

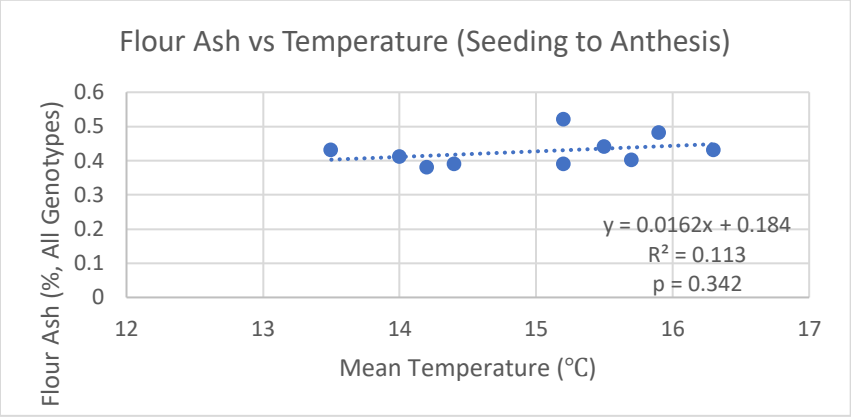


Figure V.23 Flour ash averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

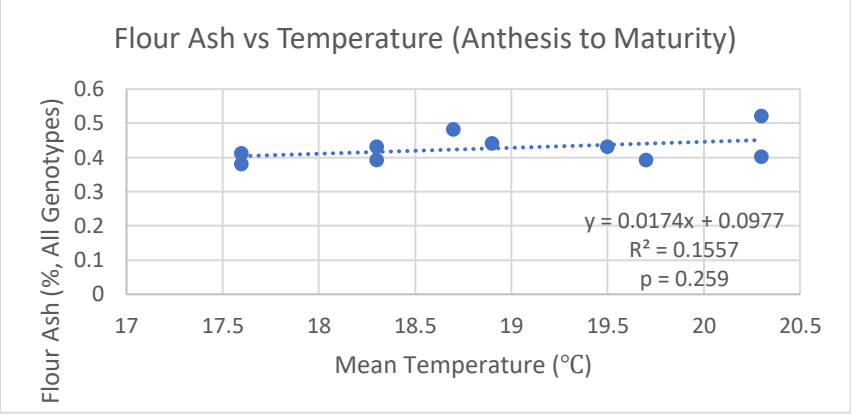


Figure V.24 Flour ash averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

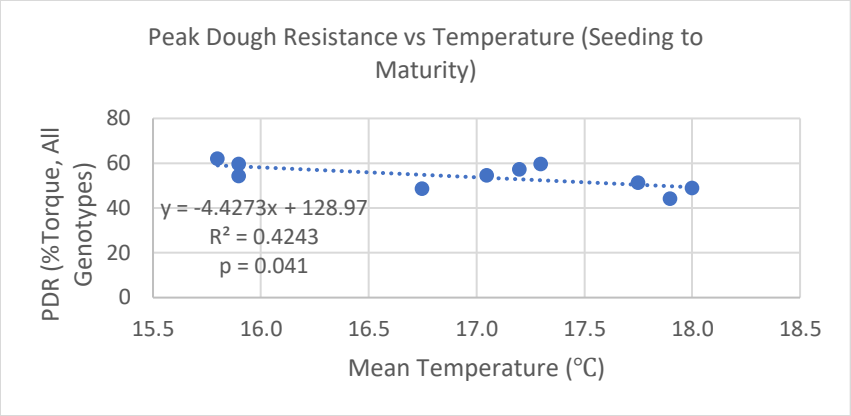


Figure V.25 Peak dough resistance (PDR) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

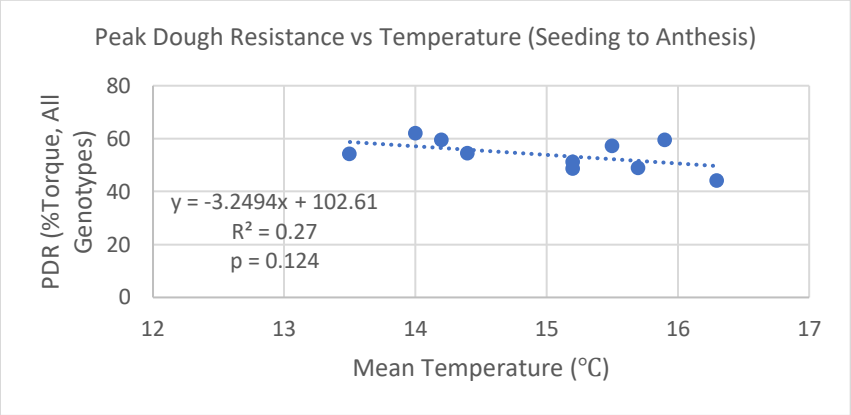


Figure V.26 Peak dough resistance (PDR) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

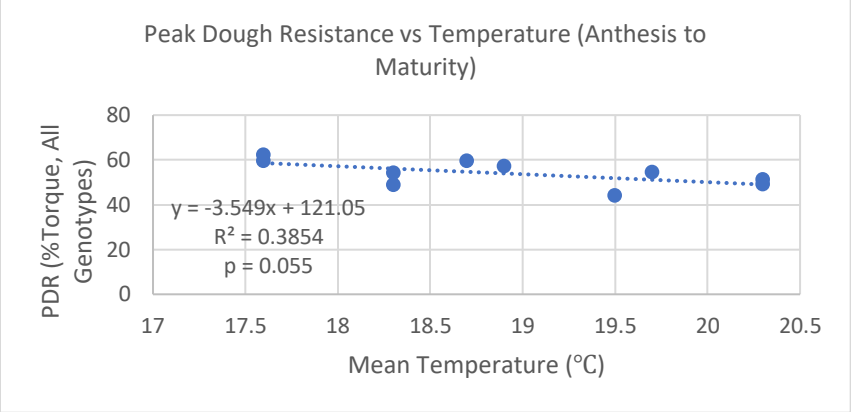


Figure V.27 Peak dough resistance (PDR) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

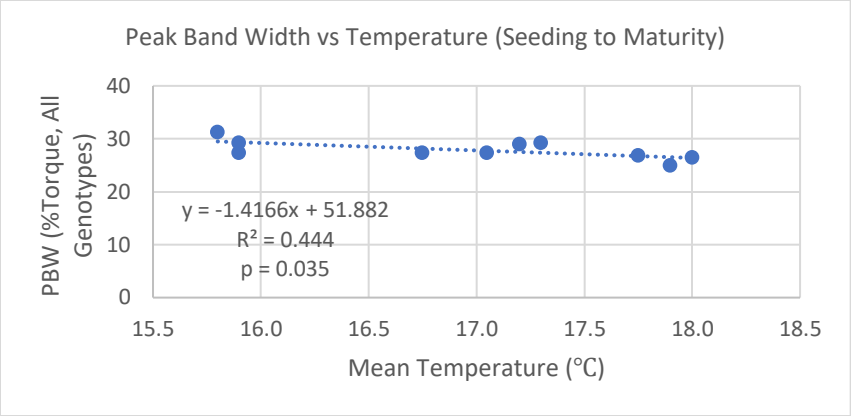


Figure V.28 Peak band width (PBW) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

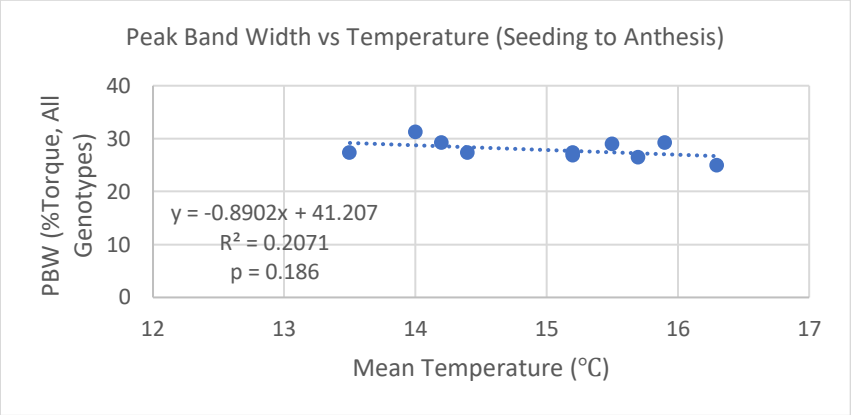


Figure V.29 Peak band width (PBW) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

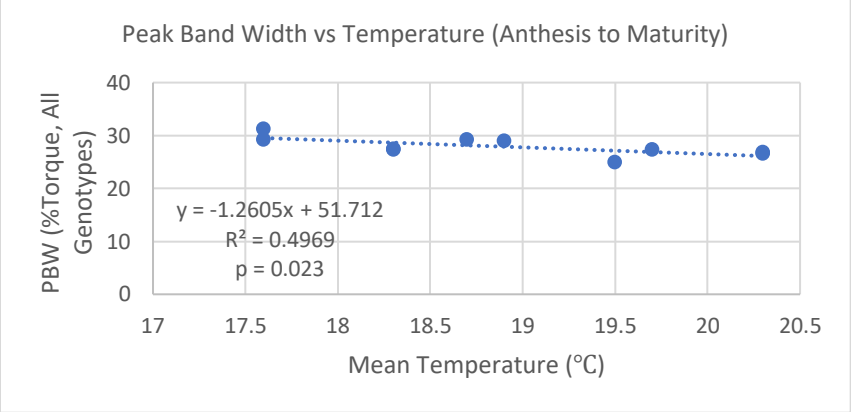


Figure V.30 Peak band width (PBW) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

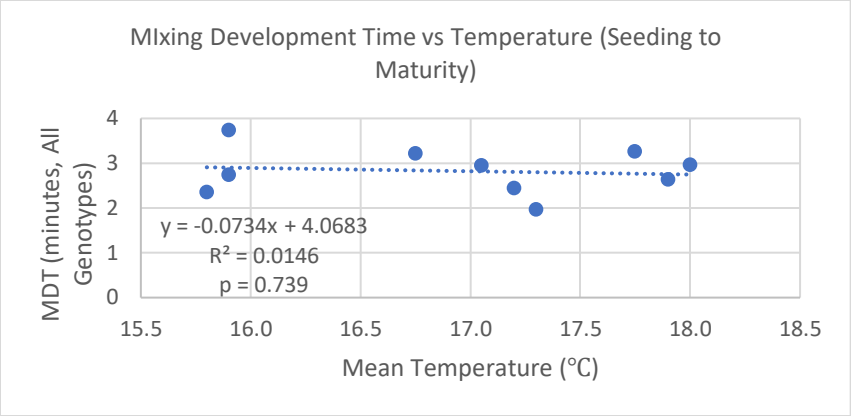


Figure V.31 Mixing development time (MDT) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

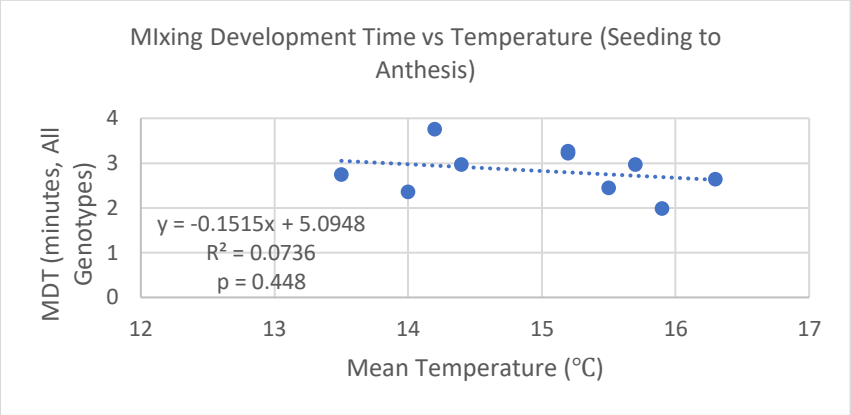


Figure V.32 Mixing development time (MDT) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

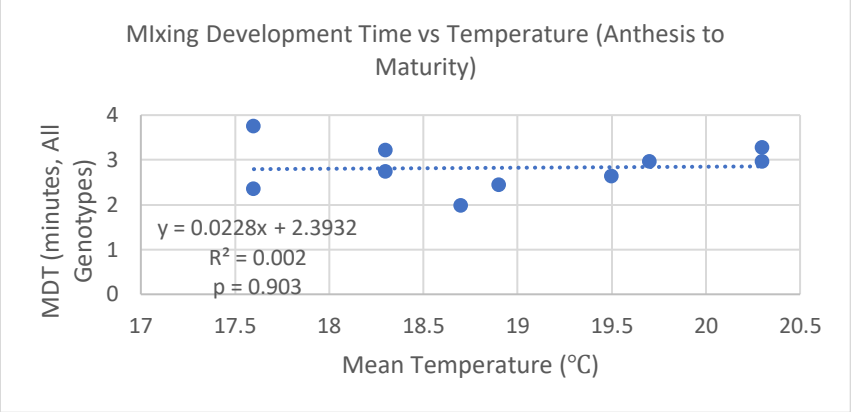


Figure V.33 Mixing development time (MDT) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

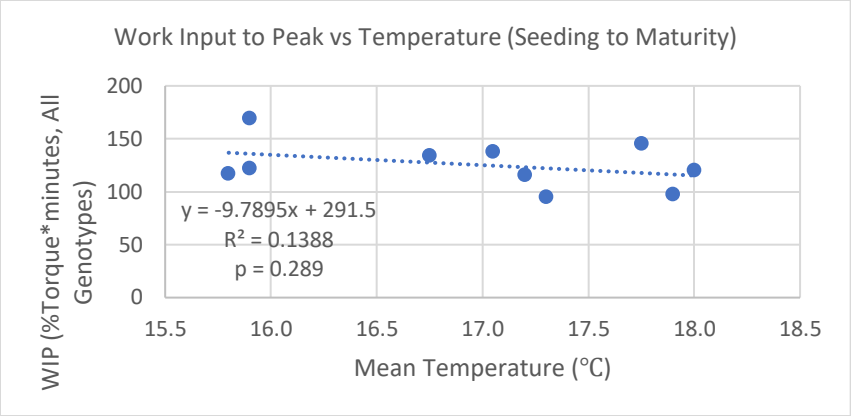


Figure V.34 Work input to peak (WIP) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

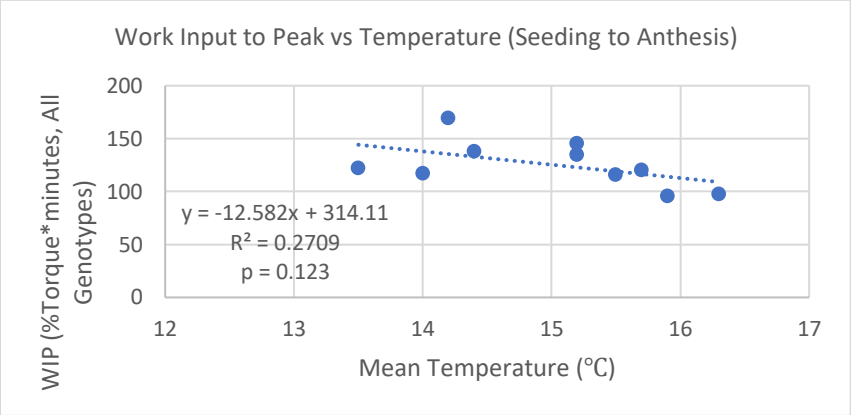


Figure V.35 Work input to peak (WIP) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

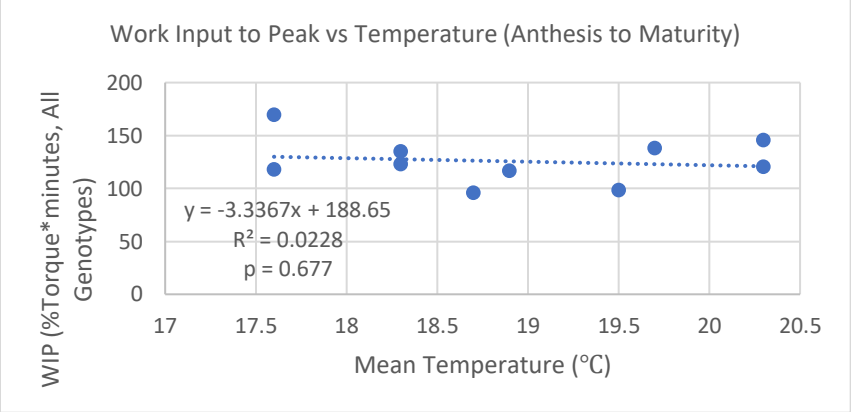


Figure V.36 Work input to peak (WIP) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

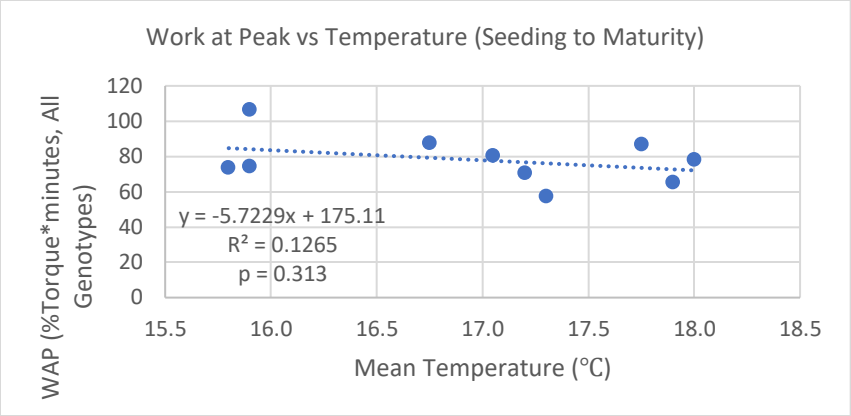


Figure V.37 Work at peak (WAP) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

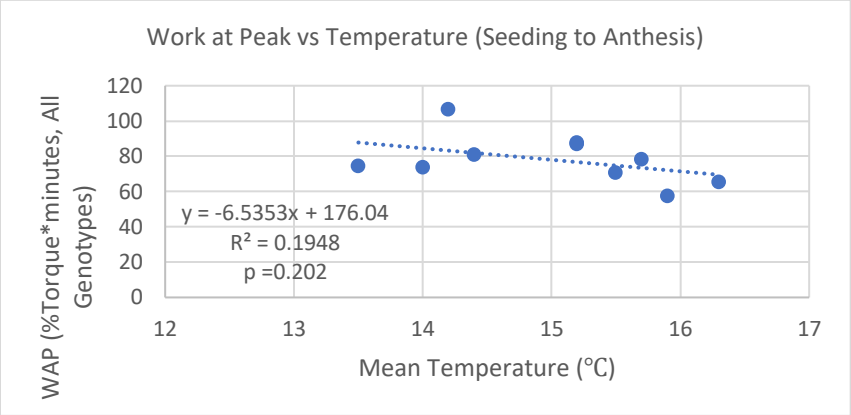


Figure V.38 Work at peak (WAP) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

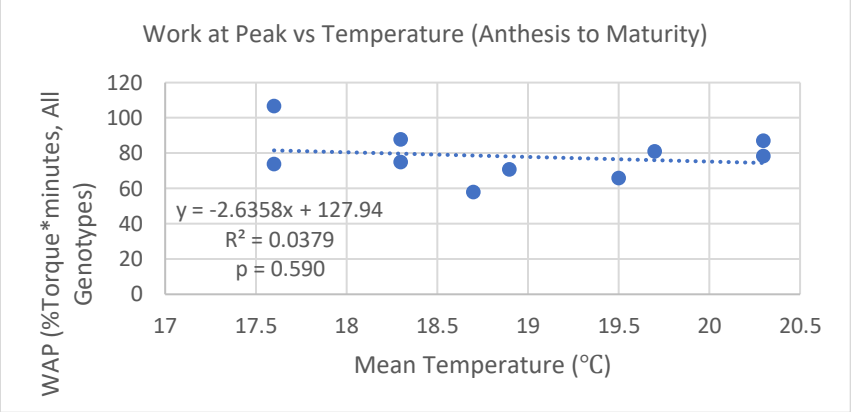


Figure V.39 Work at peak (WAP) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

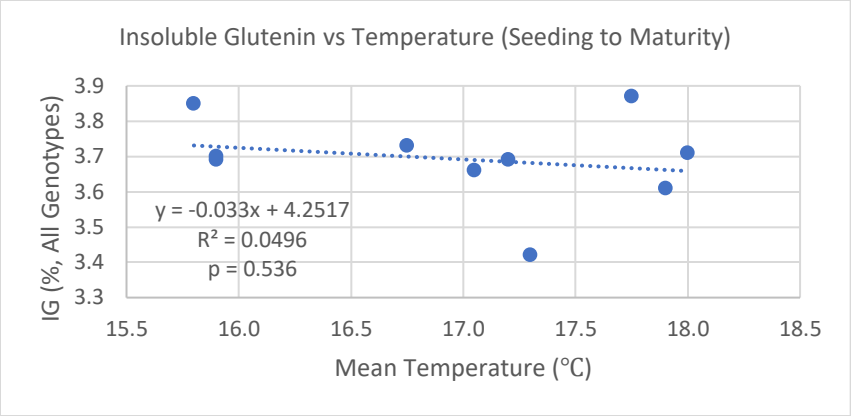


Figure V.40 Insoluble glutenin (IG) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

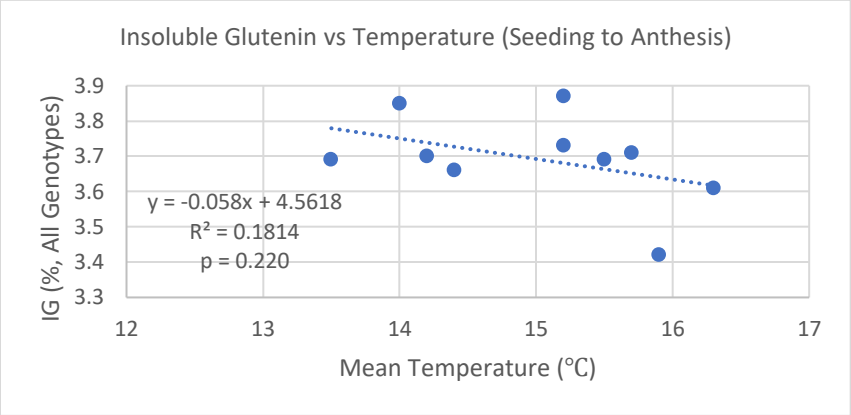


Figure V.41 Insoluble glutenin averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

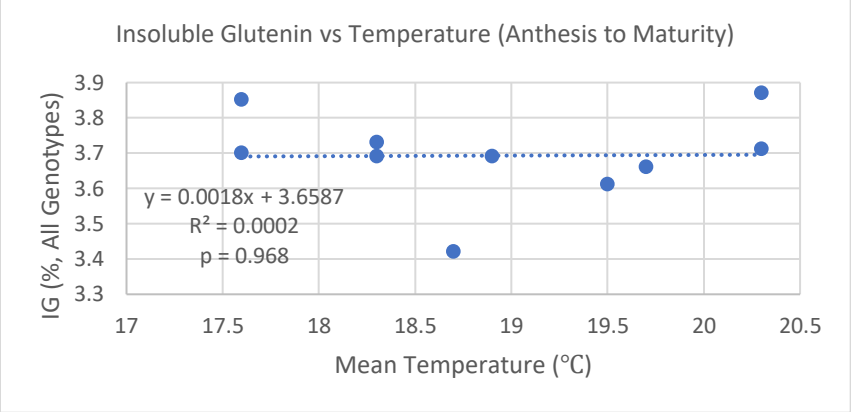


Figure V.42 Insoluble glutenin averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

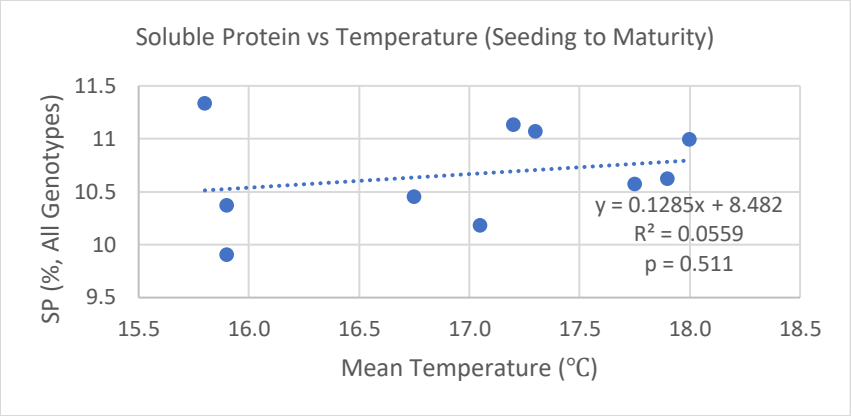


Figure V.43 Soluble prolamin (SP) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

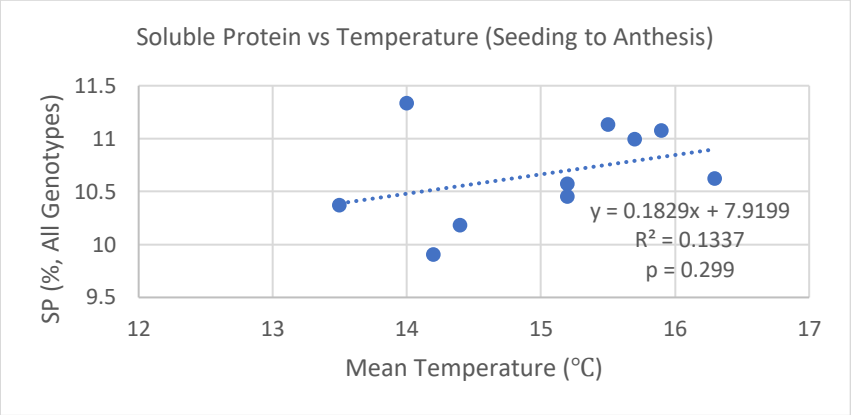


Figure V.44 Soluble prolamin (SP) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

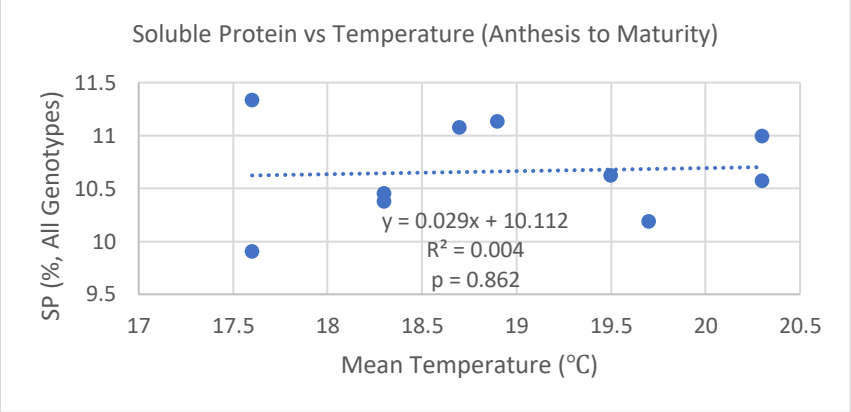


Figure V.45 Soluble prolamin (SP) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

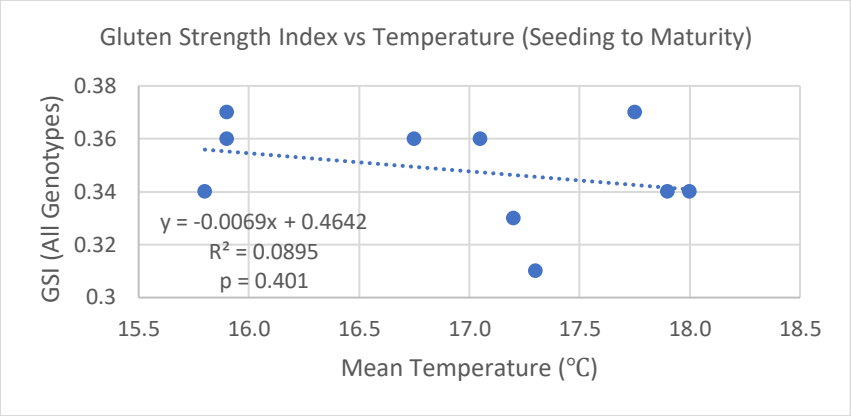


Figure V.46 Gluten strength index (GSI) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

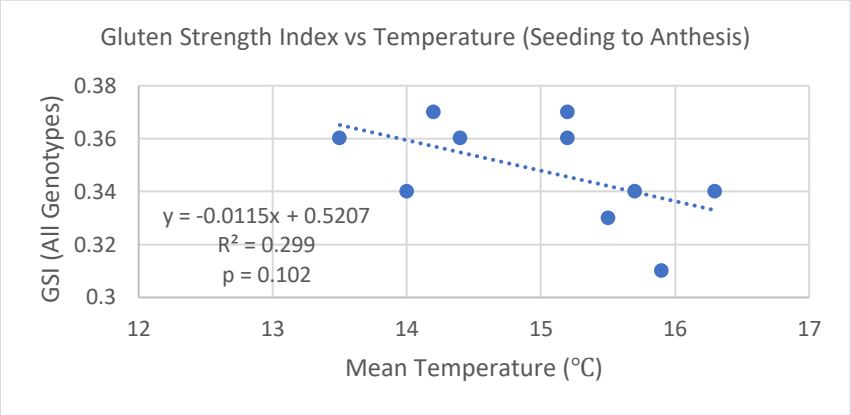


Figure V.47 Gluten strength index (GSI) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

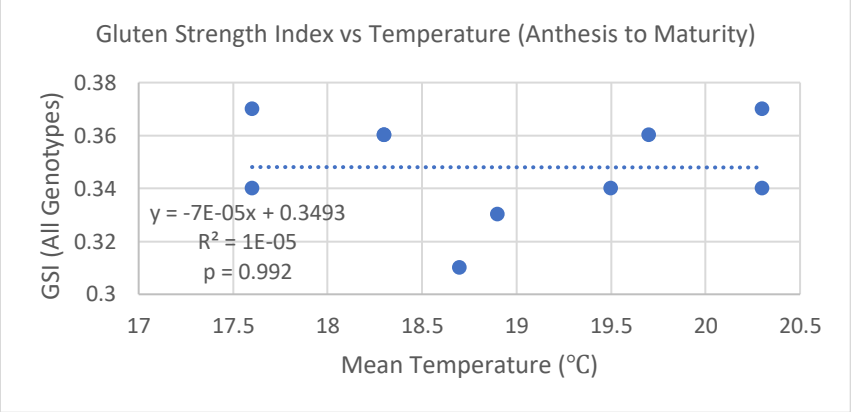


Figure V.48 Gluten strength index (GSI) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

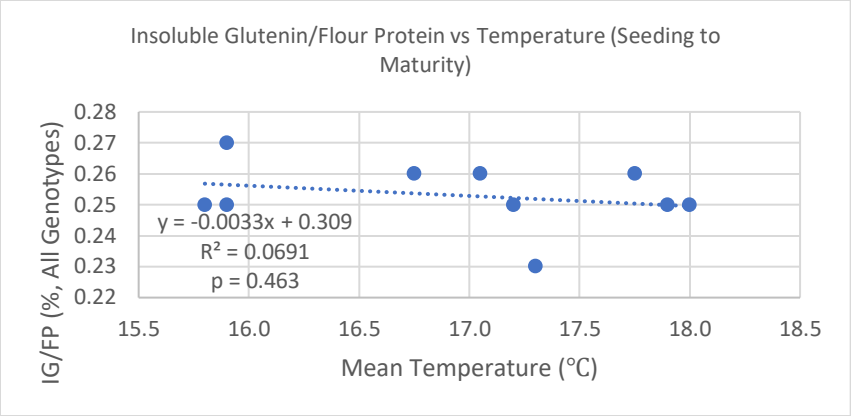


Figure V.49 Insoluble glutenin/flour protein (IG/FP) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

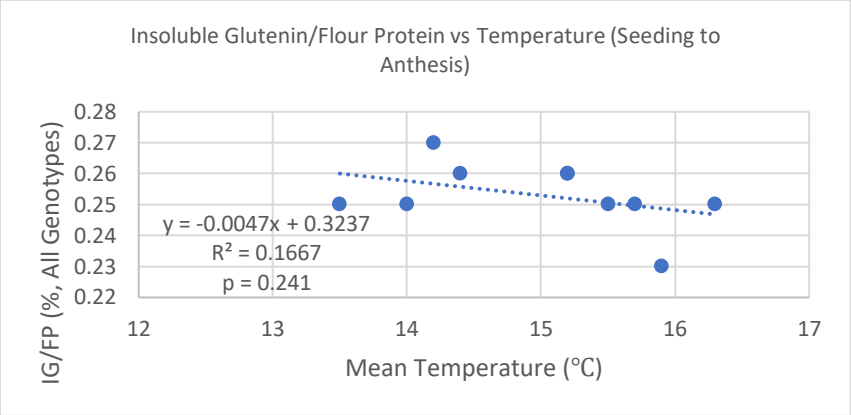


Figure V.50 Insoluble glutenin/flour protein (IG/FP) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

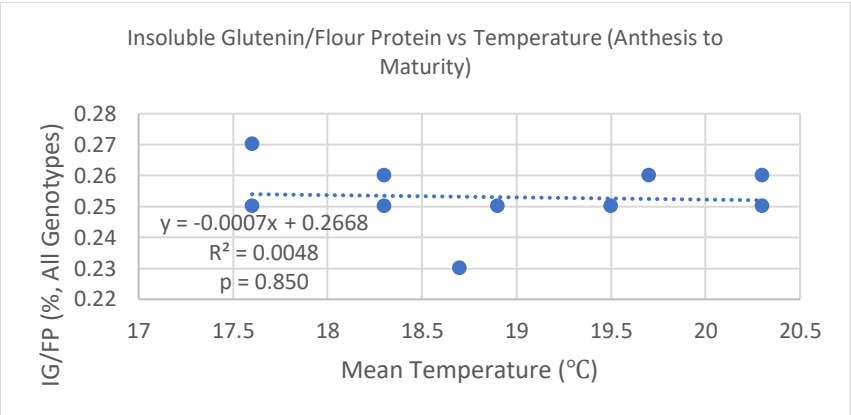


Figure V.51 Insoluble glutenin/flour protein (IG/FP) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

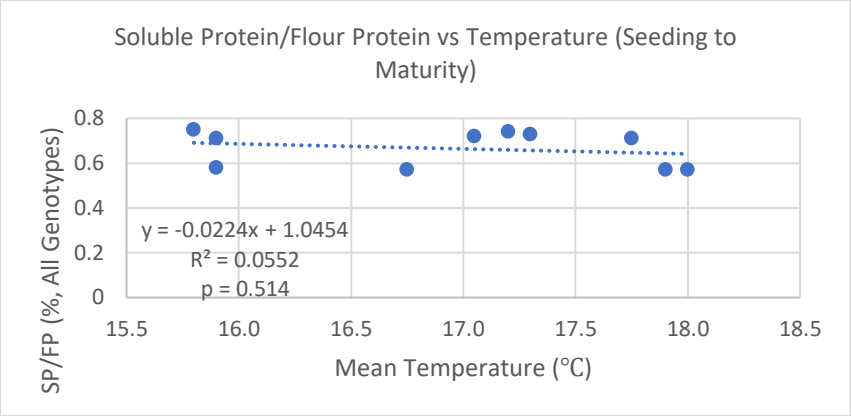


Figure V.52 Soluble prolamin/flour protein (SP/FP) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to maturity for all site-years.

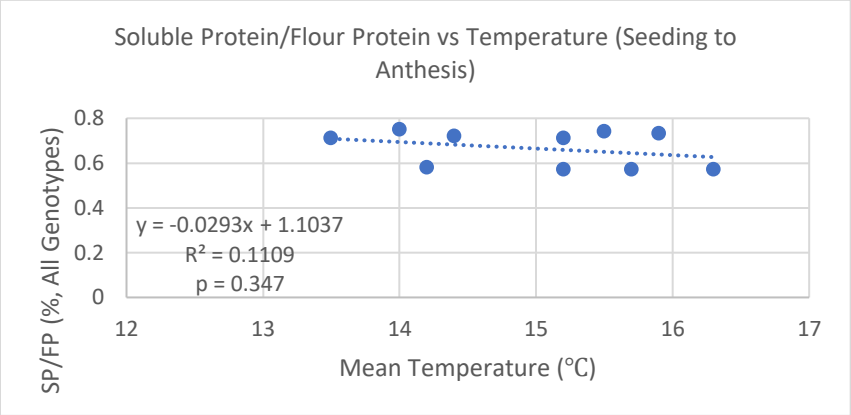


Figure V.53 Soluble prolamin/flour protein (SP/FP) averaged across all genotypes and pesticide treatments versus mean temperature from seeding to anthesis for all site-years.

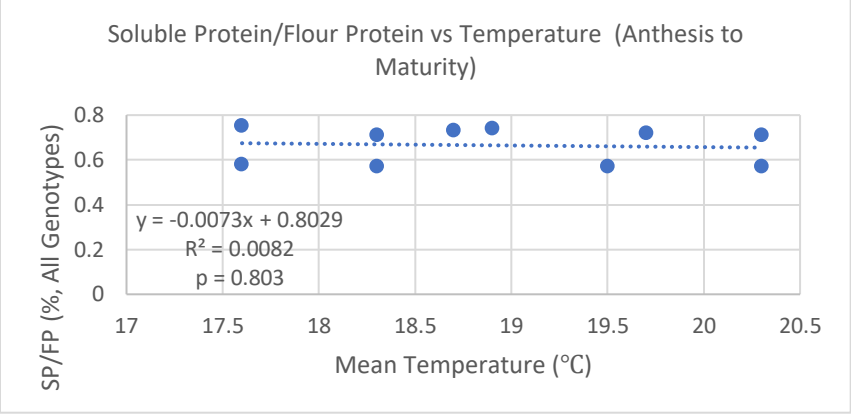


Figure V.54 Soluble prolamin/flour protein (SP/FP) averaged across all genotypes and pesticide treatments versus mean temperature from anthesis to maturity for all site-years.

Appendix VI. Additional SAS analysis for Chapter 2.

Table VI.1 Significance levels of Type III Fixed Effects (genotype, pesticide treatment and G x T) from combined year analysis for percentage of Fusarium damaged kernels (%FDK), test weight, thousand kernel weight (TKW) and grain protein concentration percentage (%GPC).

	Genotype	Treatment	G x T
%FDK	<.0001 ***[A]	<.0001 ***	0.0175 *
Test Weight	<.0001 ***	<.0001 ***	0.9428 Ns[B]
TKW	<.0001 ***	<.0001 ***	0.1625 ns
%GPC	<.0001 ***	0.5459 ns	0.9752 ns

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Table VI.2 Type III Fixed Effects (genotype, pesticide treatment and location) from individual year analysis for percentage of Fusarium damaged kernels (%FDK), test weight, thousand kernel weight (TKW) and grain protein concentration percentage (%GPC).

	Genotype			Treatment			Location		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
%FDK	<.0001 ***[A]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***
Test Weight	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	0.0187 *	<.0001 ***	<.0001 ***	<.0001 ***
TKW	<.0001 ***	<.0001 ***	<.0001 ***	0.0322 *	<.0001 ***	0.0154 *	<.0001 ***	<.0001 ***	<.0001 ***
%GPC	<.0001 ***	<.0001 ***	<.0001 ***	0.0058 **	0.1742 Ns[B]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Table VI.3 Type III Fixed Effects (genotype, pesticide treatment and location interactions) from individual year analysis for percentage of Fusarium damaged kernels (%FDK), test weight, thousand kernel weight (TKW) and grain protein concentration percentage (%GPC).

	Genotype x Treatment			Location x Genotype			Location x Treatment		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
%FDK	0.1524 ns[B]	0.1737 ns	0.0011 **[A]	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	0.0142 *	<.0001 ***
Test Weight	0.8820 ns	0.9605 ns	0.8575 ns	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	0.0049 **
TKW	0.0407 *	0.1822 ns	0.7430 ns	<.0001 ***	<.0001 ***	<.0001 ***	0.1776 ns	0.1497 ns	<.0001 ***
%GPC	0.7708 ns	0.9308 ns	0.6287 ns	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***	0.3704 ns

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Table VI.4 Type III Fixed Effects (genotype, pesticide treatment and G x T) from combined year analysis for flour ash, flour protein and flour yield.

	Genotype	Treatment	G x T
Ash	<.0001	0.0002	0.4786
	***[A]	***	ns
Flour Protein	<.0001	0.2823	0.7601
	***	Ns[B]	ns
Flour Yield	<.0001	0.4212	0.8879
	***	ns	ns

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Table VI.5 Type III Fixed Effects (genotype, pesticide treatment and location) from individual year analysis for flour ash, flour protein and flour yield.

	Genotype			Treatment			Location		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
Ash	<.0001	<.0001	<.0001	0.0002	0.0005	0.0003	<.0001	<.0001	<.0001
	***	***[A]	***	***	***	***	***	***	***
Flour Protein	<.0001	<.0001	<.0001	0.0456	0.2335	0.0005	<.0001	<.0001	<.0001
	***	***	***	*	ns	***	***	***	***
Flour Yield	<.0001	<.0001	<.0001	0.2974	0.5326	0.0290	<.0001	<.0001	<.0001
	***	***	***	Ns[B]	ns	*	***	***	***

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Table VI.6 Type III Fixed Effects (genotype, pesticide treatment and location interactions) from individual year analysis for flour ash, flour protein and flour yield.

	Genotype*Treatment			Location*Genotype			Location*Treatment		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
Ash	0.2822	0.0130	0.4028	<.0001	<.0001	<.0001	0.2351	0.0024	0.0006
	ns	*[A]	ns	***	***	***	ns	**	***
Flour Protein	0.4854	0.8764	0.3199	0.0001	<.0001	<.0001	<.0001	<.0001	0.8190
	ns	ns	ns	***	***	***	***	***	ns
Flour Yield	0.3339	0.1894	0.3333	0.0005	<.0001	<.0001	0.6844	0.0142	0.8156
	ns	ns	Ns[B]	***	***	***	ns	*	ns

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Appendix VII. Additional SAS analysis for Chapter 3.

Table VII.1 Type III Fixed Effects (genotype, pesticide treatment and G x T) from combined year analysis for peak dough resistance (PDR), peak band width (PBW), mixing development time (MDT), work input to peak (WIP) and work at peak (WAP).

	Genotype	Treatment	G x T
PDR	<.0001	0.3007	0.6992
	***[A]	ns[B]	Ns
PBW	<.0001	0.1418	0.9921
	***	ns	Ns
MDT	<.0001	0.3148	0.2971
	***	ns	Ns
WIP	<.0001	0.4302	0.3350
	***	ns	Ns
WAP	<.0001	0.3559	0.2442
	***	ns	Ns

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Table VII.2 Type III Fixed Effects (genotype, pesticide treatment and location) from individual year analysis for peak dough resistance (PDR), peak band width (PBW), mixing development time (MDT), work input to peak (WIP) and work at peak (WAP).

	Genotype			Treatment			Location		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
PDR	<.0001	<.0001	<.0001	0.0973	0.8225	0.4079	<.0001	<.0001	<.0001
	***[A]	***	***	ns[B]	ns	ns	***	***	***
PBW	<.0001	<.0001	<.0001	0.1256	0.2102	0.0712	0.1539	<.0001	<.0001
	***	***	***	ns	ns	ns	ns	***	***
MDT	<.0001	<.0001	<.0001	0.0177	0.0717	0.7862	<.0001	<.0001	<.0001
	***	***	***	*	ns	ns	***	***	***
WIP	<.0001	<.0001	<.0001	0.0565	0.3457	0.9250	<.0001	<.0001	<.0001
	***	***	***	ns	ns	ns	***	***	***
WAP	<.0001	<.0001	<.0001	0.2231	0.1188	0.5029	<.0001	<.0001	<.0001
	***	***	***	ns	ns	ns	***	***	***

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Table VII.3 Type III Fixed Effects (genotype, pesticide treatment and location interactions) from individual year analysis for peak dough resistance (PDR), peak band width (PBW), mixing development time (MDT), work input to peak (WIP) and work at peak (WAP).

	Genotype*Treatment			Location*Genotype			Location*Treatment		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
PDR	0.8940	0.9338	0.2102	<.0001	<.0001	<.0001	0.0068	0.1753	0.2291
	ns[B]	ns	ns	***[A]	***	***	**	ns	ns
PBW	0.9285	0.7902	0.8715	0.0003	<.0001	<.0001	0.00581	0.3109	0.4941
	ns	ns	ns	***	***	***	**	ns	ns
MDT	0.2337	0.5557	0.1705	<.0001	0.0101	<.0001	0.0034	0.9403	0.3790
	ns	ns	ns	***	*	***	**	ns	ns
WIP	0.0506	0.6581	0.1222	0.0012	0.0046	<.0001	0.0016	0.9564	0.2292
	ns	ns	ns	**	**	***	**	ns	ns
WAP	0.1549	0.3259	0.3347	0.0013	<.0001	<.0001	0.0480	0.8636	0.3306
	ns	ns	ns	**	***	***	*	ns	ns

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Table VII.4 Type III Fixed Effects (genotype, pesticide treatment and G x T) from combined year analysis for gluten strength index (GSI), insoluble glutenin/flour protein (IG/FP), insoluble glutenin (IG), soluble prolamin/flour protein (SP/FP) and soluble prolamin (SP).

	Genotype	Treatment	G x T
GSI	<.0001	0.1081	0.7122
	***[A]	ns[B]	ns
IG/FP	<.0001	0.0946	0.3352
	***	ns	ns
IG	<.0001	0.8246	0.7362
	***	ns	ns
SP/FP	<.0001	0.8740	0.9992
	***	ns	ns
SP	<.0001	0.3818	0.8279
	***	ns	ns

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Table VII.5 Type III Fixed Effects (genotype, pesticide treatment and location) from individual year analysis for gluten strength index (GSI), insoluble glutenin/flour protein (IG/FP), insoluble glutenin (IG), soluble prolamin/flour protein (SP/FP) and soluble prolamin (SP).

	Genotype			Treatment			Location		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
GSI	<.0001	<.0001	<.0001	0.0431	0.7451	0.3601	0.0084	<.0001	<.0001
	***[A]	***	***	*	ns[B]	ns	**	***	***
IG/FP	<.0001	<.0001	<.0001	0.0591	0.6984	0.4421	0.0152	<.0001	<.0001
	***	***	***	ns	ns	ns	*	***	***
IG	<.0001	<.0001	<.0001	0.5823	0.6114	0.0052	<.0001	<.0001	0.0002
	***	***	***	ns	ns	**	***	***	***
SP/FP	<.0001	<.0001	<.0001	0.3476	0.2988	0.9382	<.0001	<.0001	0.0003
	***	***	***	ns	ns	ns	***	***	***
SP	<.0001	<.0001	<.0001	0.0103	0.2773	0.0010	<.0001	0.0011	<.0001
	***	***	***	*	ns	***	***	**	***

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Table VII.6 Type III Fixed Effects (genotype, pesticide treatment and location interactions) from individual year analysis for gluten strength index (GSI), insoluble glutenin/flour protein (IG/FP), insoluble glutenin (IG), soluble prolamin/flour protein (SP/FP) and soluble prolamin (SP).

	Genotype*Treatment			Location*Genotype			Location*Treatment		
	2015	2016	2017	2015	2016	2017	2015	2016	2017
GSI	0.2357	0.6235	0.7539	<.0001	0.0062	<.0001	0.1647	0.4622	0.1142
	ns[B]	ns	ns	***[A]	**	***	ns	ns	ns
IG/FP	0.1704	0.4791	0.6622	0.0031	0.0045	<.0001	0.1846	0.8936	0.0051
	ns	ns	ns	**	**	***	ns	ns	**
IG	0.3608	0.3511	0.9244	0.2483	<.0001	<.0001	0.0371	0.1781	0.1206
	ns	ns	ns	ns	***	***	*	ns	ns
SP/FP	0.5385	0.4525	0.2472	0.0004	0.1389	0.3362	0.5263	0.1236	0.1206
	ns	ns	ns	***	ns	ns	ns	ns	ns
SP	0.5540	0.6588	0.1927	<.0001	<.0001	<.0001	0.0006	0.0139	0.5132
	ns	ns	ns	***	***	***	***	*	ns

[A]F test significance where *, **, *** represent significant effects at $P < 0.05$, 0.01, and 0.001, respectively, [B]ns = not significant

Appendix VIII. SAS code used for the analysis of grain quality and flour quality parameters in Chapter 2, and the mixograph and protein composition parameters in Chapter 3.

```
Data sp2;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\SAS Layout SP2
(Updated).xlsx"
out=sp2 dbms=xlsx replace;
sheet="Sheet1";
Proc glimmix data=sp2 plots=residualpanel;
Class siteyear genotype treatment block;
Model FlourYield =genotype treatment genotype*treatment;
        Random siteyear block(siteyear) treatment*block(siteyear);
      LSMEANS treatment | genotype/pdiff adj=tukey ilink lines;
Run;
```

Figure VIII.1 Example of GLIMMIX procedure code for combined year analysis of Flour Yield.

```
Data sp2;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\SAS Layout SP2
(Updated).xlsx"
out=sp2 dbms=xlsx replace;
sheet="Sheet1";
Proc glimmix data=sp2 plots=residualpanel;
Class location genotype treatment block;
Model FlourYield =genotype treatment location genotype*treatment
genotype*location treatment*location;
        Random block;
        LSMEANS treatment | genotype/pdiff adj=tukey ilink lines;
Run;
```

Figure VIII.2 Example of GLIMMIX procedure code for individual year analysis of Flour Yield.

```
Data sp2;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\SAS Single Year
Analysis.xlsx"
out=sp2 dbms=xlsx replace;
sheet="Sheet1";
Proc Mixed data=sp2 METHOD=TYPE3;
        Class Year Location Block Genotype Treatment;
        Model FlourYield=Genotype Treatment Genotype*Treatment/
DDFM=KENWARDROGER;
        Random Year Location Year*Location Block(Year*Location) Genotype*Year
Genotype*Location Genotype*Year*Location Treatment*Year Treatment*Location
Treatment*Year*Location;
        LSMEANS Genotype Treatment;
Run;
```

Figure VIII.3 Example of MIXED procedure code for combined year analysis, using year and location as separate parameters for Flour Yield.

```
Data sp2;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\SAS Single Year
Analysis.xlsx"
out=sp2 dbms=xlsx replace;
sheet="Sheet1";
Proc Mixed data=sp2 METHOD=TYPE3;
        Class Siteyear Block Genotype Treatment;
        Model FlourYield=Genotype Treatment Genotype*Treatment/
DDFM=KENWARDROGER;
        Random Siteyear Block(Siteyear) Genotype*Siteyear Treatment*Siteyear
Treatment*Genotype*Siteyear;
        LSMEANS Genotype Treatment;
Run;
```

Figure VIII.4 Example of MIXED procedure code for combined year analysis, using site-year for Flour Yield. This SAS code was used for the contribution of variance analysis.

```
Data sp2;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\SAS Single Year
Analysis (April).xlsx"
out=sp2 dbms=xlsx replace;
sheet="Kelburn2015";
Proc Mixed data=sp2 METHOD=TYPE3;
        Class Block Genotype Treatment;
        Model FlourYield =Genotype Treatment Genotype*Treatment/
DDFM=KENWARDROGER;
        Random Block Block*Treatment;
        LSMEANS Genotype Treatment;
Run;
```

Figure VIII.5 Example of MIXED procedure code for individual year analysis of Flour Yield.

```
Data sp2;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\SAS Single Year
Analysis (4 reps-April).xlsx"
out=sp2 dbms=xlsx replace;
sheet="IHARF2015";
Proc glimmix data=sp2 plots=residualpanel;
Class block genotype treatment;
Model TKW=genotype treatment genotype*treatment;
        Random block (treatment);
        LSMEANS treatment | genotype/pdiff adj=tukey ilink lines;
Run;
```

Figure VIII.6 Example of GLIMMIX procedure code for individual year Tukey Kramer analysis of TKW.

```
Data sp2;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\SAS Single Year
Analysis (4 reps-April).xlsx"
out=sp2 dbms=xlsx replace;
sheet="Sheet1";
Proc glimmix data=sp2 plots=residualpanel;
Class siteyear genotype treatment block;
Model TKW =genotype treatment genotype*treatment;
        Random siteyear block(siteyear) treatment*block(siteyear);
      LSMEANS treatment | genotype/pdiff adj=tukey ilink lines;
Run;
```

Figure VIII.7 Example of GLIMMIX procedure code for combined year Tukey Kramer analysis of TKW.

Appendix IX. SAS code used for the analysis of grain quality, flour quality, mixograph and protein composition parameters in Chapter 4.

```
Data sp6;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\ Subproject 6-
Complete Data Set (Final).xlsx"
out=sp6 dbms=xlsx replace;
sheet="2017";
Proc Mixed data=sp6 METHOD=TYPE3;
        Class Location Block Genotype Treatment;
        Model FlourYield=Genotype Treatment Genotype*Treatment/
DDFM=KENWARDROGER;
        Random Location Block(Location) Genotype*Location Treatment*Location
Treatment*Genotype*Location;
        LSMEANS Genotype Treatment;
Run;
```

Figure IX.1 Example of MIXED procedure code for combined site analysis of Flour Yield. This SAS code was used for the contribution of variance analysis.

```
Data sp6;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\Subproject 6-
Complete Data Set (Final).xlsx"
out=sp6 dbms=xlsx replace;
sheet="Brandon";
Proc Mixed data=sp2 METHOD=TYPE3;
        Class Block Genotype Treatment;
        Model FlourYield =Genotype Treatment Genotype*Treatment/
DDFM=KENWARDROGER;
        Random Block Block*Treatment;
        LSMEANS Genotype Treatment;
Run;
```

Figure IX.2 Example of MIXED procedure code for individual site analysis of Flour Yield.

```
Data sp6;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\Subproject 6-
Complete Data Set (Final).xlsx"
out=sp6 dbms=xlsx replace;
sheet="Sheet3";
Proc glimmix data=sp6 plots=residualpanel;
Class block genotype treatment;
Model TKW=genotype treatment genotype*treatment;
        Random block (treatment);
        LSMEANS treatment | genotype/pdiff adj=tukey ilink lines;
Run;
```

Figure IX.3 Example of GLIMMIX procedure code for individual year Tukey Kramer analysis of TKW.

```
Data sp6;
proc import datafile="C:\Users\perten\Desktop\Kate_Dorrian\Subproject 6-
Complete Data Set (Final).xlsx"
out=sp6 dbms=xlsx replace;
sheet="Sheet4";
Proc glimmix data=sp6 plots=residualpanel;
Class location genotype treatment block;
Model TKW =genotype treatment genotype*treatment;
        Random location block(location) treatment*block(location);
      LSMEANS treatment | genotype/pdiff adj=tukey ilink lines;
Run;
```

Figure IX.4 Example of GLIMMIX procedure code for combined year Tukey Kramer analysis of TKW.