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Predicting Farinograph Stability of Wheat Flour with Mixograph and Glutomatic Tests

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PREDICTING FARINOGRAPH STABILITY OF WHEAT FLOUR WITH MIXOGRAPH AND GLUTOMATIC TESTS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This thesis is dedicated to my dog, Tuff. He moved a thousand miles away with me, came with me on several 13-hour long road trips, he always had his head in my lap while I was writing, and is always excited to see me.

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CONTENTS

ABBREVIATIONS

AACC: American Association of Cereal Chemists

AIC: Akaike Information Criterion

DDT: Dough Development Time

DOS: Degree of Softening

GI: Gluten Index

GWAS: Genetic Mapping with complex structured populations

HRSW: Hard Red Spring Wheat

INTEG: Integral

MPT: Midline Peak Time

MPV: Midline Peak Value

MPW: Midline Peak Width

MRV: Midline Right Value

MTI: Mixing Tolerance Index

N: Sample Size

NIR: Near-Infrared Reflectance

MIR: Mid-Infrared Reflectance

RSME: Root Mean Square Error

R^2: R-squared; coefficient of determination

S: Stability

StdDev: Standard Deviation

WA: Water Absorption

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ABSTRACT

PREDICTING FARINOGRAPH STABILITY OF WHEAT FLOUR WITH MIXOGRAPH AND GLUTOMATIC TESTS

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Hard red spring wheat (*Triticum aestivum* L.; HRSW) flour is typically used to produce wheat-based foods where dough strength is a key quality component. Maintaining acceptable levels of dough strength is an important goal in the development of new HRSW cultivars. In a commercial setting, dough strength is often measured as Farinograph stability, though due to various resource constraints, stability is often predicted in breeding programs via other methods like the Mixograph. The objective of this research was to combine Glutomatic with Mixograph data to determine whether Farinograph stability predictions might be improved over the use of Mixograph data alone. Five hundred and forty flour samples of 33 to 48 HRSW genotypes grown at three locations over years 2015 – 2019 were subjected to Farinograph, Mixograph, and Glutomatic tests. Stepwise linear regression methods and pairwise correlation was used to select independent variables from the combined dataset to predict Farinograph stability. Including Glutomatic data with that of the Mixograph may assist breeders in selecting HRSW breeding lines and cultivars with sufficient levels of dough strength.

Midline peak time, midline peak integral, and gluten index were found to be the most significant predictors of Farinograph stability. Stability was affected by the

environment from year to year. Analysis on genotype averages was found to be the most useful and least effected by environmental interactions.

CHAPTER 1: LITERATURE REVIEW

1.1 Introduction

Wheat (*Triticum aestivum L.*) is one of the 'big three' cereal crops (along with rice and corn) with approximately 600 million tons harvested each year (Shewry, 2009). Wheat has a wide range of cultivation from Russia to Argentina, tropic regions and sub-tropic regions (Shewry, 2009). Significant wheat exporting areas of the world include the United States, Canada, Austrialia, The Black Sea Region, Europe, and Argentina (Sharma et al., 2015). Wheat has a wide variety of uses from human food to livestock feed, and in many cultures and religions wheat bread is of significance (Shewry, 2009). There are over 620 million tons of wheat grown worldwide every year (Dubcovsky & Dvorak, 2007). Approximately 95% of wheat grown is common wheat used for breadmaking and pastries, and the remaining amount is durum wheat used for pasta products. Wheat consumption represents about 1/5 of the world's caloric intake (Dubcovsky & Dvorak, 2007). The other 5% of wheat grown is durum wheat, which is often used to make pastas; einkorn and other hulled wheats such as emmer are of minor economic importance (Dubcovsky & Dvorak, 2007).

In the United States, wheat is the third crop for both value and acreage, behind corn and soybeans (Vocke & Ali, 2014). Unlike other crops, wheat has distinct varieties that are meant to be produced across different regions during different seasons. This causes a variation in the costs and competitiveness of wheat with other crop species in the United States (Vocke & Ali, 2014). Along with productivity of the wheat plant, the value of wheat lies in its flour quality that affects milling and breadmaking (Briggle & Reitz, 1963). Producing a wheat crop in which the sale price of the wheat outweighs the cost is

important to keep farmers growing wheat and not switching to a potentially more valuable crop.

1.2 Origin of Domesticated Wheat

Wheat in the genus *Triticum* originated in Asia and parts of Africa where wheat as we know it today evolved from wild grasses (Beldrok et al., 200 C.E.). *Triticum aestivum* is a 42-chromosome wheat that is believed to have descended from *Aegilops squarrosa* (*Triticum tauschii*) and *Triticum dicoccoides*; the use of colchicine aided in the discovery of *T. tauschii* as a parent (Beldrok et al., 200 C.E.).

Before using cereals to make bread, they were used to make porridges which is believed to be the first form of cereals being used as a human food source (Beldrok et al., 200 C.E.). Sumerians were the first to bake unleavened bread sometime around 6000 B.C. (Beldrok et al., 200 C.E.). The Egyptians were the ones who began using yeast they created from brewing beer in their bread around 3000 B.C. as well as developing a bread oven that could bake multiple loaves at one time (Beldrok et al., 200 C.E.).

Domestication of crop species makes them dependent on human interaction with their cultivation (Peng et al., 2011a). Wheat is a universal cereal crop and was among one of the first domesticated crop plants dating back 10,000 years ago (Peng et al., 2011a). Domestication of wheat led it to become more susceptible to environmental stress, pests, and diseases (Peng et al., 2011a). One major change in wheat development was the resistance to shattering created by humans. Wild wheat spikelets were free threshing, and the spikelet would fall to the ground when it was mature. The arrow like shape of the glume would help it to penetrate the soil (Peng et al., 2011a). Today, wheat is bred to not shatter which causes it to rely on humans to thresh and replant the seeds. Domestication

of *T. aestivum*, wheat commonly grown for bread, began from a cross between domesticated emmer wheat (*T. dicoccum*) and goat grass (*Aegilops tauschii*) (Peng et al., 2011).

Today, wheat cultivars usually refer to two species: hexaploid bread wheat, *Triticum aestivum* (2n = 6x = 42, AuAuBBDD), and tetraploid durum wheat, *T. durum* (2n = 4x = 28, AuAuBB) (Peng et al., 2011a). A wild diploid wheat (*T. urartu*, 2n=2x=14, genome AA) formed a hybrid with goat grass (*Aegilops speltoides*, 2n=2x=14, genome BB) which produced wild emmer wheat (Peng et al., 2011b). This resulted in the AABB genome of emmer wheat (*T. dicoccum*) (Peng et al., 2011b). *T. dicoccum* was then crossed with another species of goat grass, *Ae. tauschii* (2n=2x=14, genome DD), which produced *T. spelta* (2n=6x=42, genome AABBDD) (Peng et al., 2011b). Natural mutation evolved into the free-threshing durum and bread wheats we have today.

1.3 Wheat Classification

There are three commonly grown types of wheat in the United States: winter wheat, spring wheat, and durum (Bond & Liefert, 2019). In the US, winter wheat represents 70-80% of the production (Bond & Liefert, 2019). Followed by spring wheat with approximately 25% of production and durum wheat making up the remainder (Bond & Liefert, 2019). Wintertime temperatures help determine which kind of wheat will be planted in any given location (Vocke & Ali, 2014). Winter wheat is planted in the fall and establishes before going dormant during the winter, it is then harvested in the summer. Spring wheat is planted during the spring and is harvested in the late summer into fall (Vocke & Ali, 2014). Winter wheat has a higher yield potential because of its longer

growing season; most US spring wheat is grown in areas where the winter would be too cold and would kill dormant seeds (Vocke & Ali, 2014).

There are five major classes of wheat: hard red winter wheat (HRWW), hard red spring wheat (HRSW), soft red winter wheat (SRWW), white wheat and durum (Bond & Liefert, 2019). Hard red spring wheat accounts for approximately 25% of US production and is valuable because of its high protein levels which are useful for specialty breads and blending with lower protein wheat for loaf bread (Bond & Liefert, 2019; Vocke & Ali, 2014). Hard red winter wheat makes up approximately 40% of US production and is a high protein wheat commonly used for bread flour in the Great Plains (Bond & Liefert, 2019; Vocke & Ali, 2014). Soft red winter wheat accounts for 15-20% of wheat production and is used for cakes, cookies and crackers (Bond & Liefert, 2019; Vocke & Ali, 2014). White wheat, spring or winter, makes up 10-15% of production and is used for noodles, crackers, cereals, cookies, white crusted bread, and other wheat products that use low-protein flour (Bond & Liefert, 2019; Vocke & Ali, 2014). Durum wheat, making up 3-5% of production, is used in pasta production (Bond & Liefert, 2019; Vocke & Ali, 2014).

1.4 Wheat Grain Quality

Wheat grain quality depends on the suitability of the grain for its intended processes and products (Högy & Fangmeier, 2008). The quality of the grain for its purpose could include milling performance, dough rheology, baking quality, nutritional value and storage properties (Högy & Fangmeier, 2008). Grain quality relates to how successful wheat flour preforms in both consumer products and in industrial processes;

improving wheat quality increases desirability of consumer products (Mergoum et al., 2009).

The properties of wheat dough and its baking qualities can vary between different cultivars and can be influenced by abiotic stresses like a high temperature (Maphosa et al., 2015). High temperatures during grain filling have been known to decrease the dough strength (Maphosa et al., 2015; Randall & Moss, 1990). There are many variables that play a role in determining the quality of a wheat flour, such as physical properties, protein content and composition, and starch content (Bonfil & Posner, 2012). There are several proteins in the endosperm that have shown to be associated with flour and dough quality. These proteins include puroindolines, serpins, glutenins, and gliadins (Maphosa et al., 2015). Seed storage protein content is one of the best protein indicators of baking quality, but variation in the protein content alone does not explain all variation, protein quality is also a big factor (Bonfil & Posner, 2012).

Rheology is the study of how materials deform, flow, or fail when there is a force applied to them (Amjud, Shehzad, Hussain, Shabbir, Khan, & Shoaib. 2013). Dough rheology is an important tool to measure the stress in the dough, which is related to the gluten network (Amjud et al. 2013). The rheological properties of wheat are significant in determining the way doughs behave during handling and on the quality of the finished product (Mani et al., 1992). Mixing alters the rheological properties of dough; it rapidly hydrates flour particles, develops the gluten matrix, and aerates the dough (Mani et al., 1992). As the proteins in the dough become hydrated, they create fibrils that create a matrix and resistance to extension increases (Mani et al., 1992). A dough's full breadmaking potential is at the optimum point of dough development where there are no

intact flour particles, only a random mixture of protein fibrils with starch granules (Mani et al., 1992). Past the optimum mixing point, the dough begins to break down where it becomes wet and sticky (Mani et al., 1992).

Another important wheat characteristic for quality is kernel hardness. Soft kernels are easy to break, which can result in a large number of intact starch granules after milling a fine flour (Pasha et al., 2010). Harder wheats produce coarser flours that have more broken granules of starch, higher levels of starch damage, and consume more power on the flour mill (Pasha et al., 2010). Harder wheats are better for leavened breads because the broken starches absorb more water. Soft wheats are more suitable for cookies, cakes, and pastries because of their lower protein content and less starch damage (Pasha et al., 2010).

Interactions between carbohydrates and proteins influence the quality of flour and is related to the hardness of the endosperm (Pasha et al., 2010). The proteins that remain adsorbed to the outside of starch granules are the glutenins and gliadins, storage proteins (Pasha et al., 2010). Starch granule-associated proteins (SGAPs) are tightly bound to the surface, these proteins are biologically different from plant storage proteins (Goldner & Boyer, 1989; Pasha et al., 2010). Puroindoline, or friabilin, is prominent in soft wheat, hard wheat has a faint band, and it is lacking in durum (Pasha et al., 2010). Friabilin is a starch granule protein that is linked to both the texture and the quality of wheat.

1.5 Wheat Flour

The rheological properties of wheat flour are significant in influencing the quality of the final baked product. Mixing is one way the dough is severely altered; mixing hydrates the flour, incorporates air into the mixture, and develops the gluten (Mani et al.,

1992). The maximum potential of the dough's breadmaking ability is achieved at the optimum point of dough development (Mani et al., 1992). The properties of the dough system are related to the gluten; nonprotein components interact with the gluten and contribute significantly to the theological properties of the gluten (Mani et al., 1992).

Wheat flour is made up of several constituents: starch, non-starch polysaccharides, protein, lipids, and whole wheat flour contains other byproducts. These components enhance and define the properties of wheat flour as discussed in the following sections.

1.5.1 Starch

Starch is the most abundant wheat component making up 63-72% of the grain and is found in the endosperm (Van Der Borght et al., 2005). The starch granules come in two sizes: large- lenticular A-type granules and small- spherical B-type granules (Van Der Borght et al., 2005). An important property of starch is its ability to absorb water which results in a gelatinization and loss of the granular organization (Blazek & Copeland, 2008)

Starch is the most important reverse polysaccharose and is abundant in many plants (Goesaert et al., 2005). Starch is mostly composed of the glucose polymers: amylose and amylopectin (Goesaert et al., 2005). Amylose is a linear molecule, and amylopectin is a large branched molecule (Goesaert et al., 2005). Starches that have a higher level of amylose content are of nutritional interest because they are slow digesting, which is associated with beneficial physiological effects (Blazek & Copeland, 2008). Breeding for a higher amylose content has been successful in other crops like corn and

rice, but due to wheat's hexaploidy, it is difficult to combine mutations in genes for encoding amylose increase (Blazek & Copeland, 2008).

During the milling process, approximately 5-8% of wheat flour starch granules are damaged (Van Der Borght et al., 2005). This damage to the starch increases the water absorption during dough mixing, there is also an increase in enzymic degradation (Van Der Borght et al., 2005). Hard wheats tend to have a higher content of damaged starch because they do not mill into flour as easily as the soft wheats (Barrera et al., 2007). In doughs with little or no added sugar, the damaged starch should be enough so that there is a good production of yeast gas, but not so much starch damage that there are dough handling problems (Barrera et al., 2007). Starch damage is much more detrimental to the quality of the soft wheats used for cookies and cakes. Damaged starch can reduce the size of a cookie (Barrera et al., 2007).

1.5.2 Protein

About 12% of wheat grain is composed of proteins found in the endosperm (Van Der Borght et al., 2005). These proteins can be divided into two groups: gluten and non-gluten proteins. Non-gluten proteins make up 15-20% of the total protein in wheat grain and consist of water soluble albumins and iwater nsoluble globulins (Van Der Borght et al., 2005). Gluten proteins, which make up the remaining 80-85% of the protein, are made up of gliadins and glutenins (Van Der Borght et al., 2005).

The non-water soluble proteins form a viscoelastic network that allows the dough to retain yeast fermentation gases and help to produce an aerated baked good (Van Der Borght et al., 2005). Protein content in hard wheats can be a good indicator of how well

the wheat will perform in the baking of yeast breads (Stiegert & Blanc, 1997). However, the quality of the protein also contributes to a better end quality (Stiegert & Blanc, 1997).

Gluten is the protein responsible for the visco-elastic properties that allow wheat dough to be processed into baked and other goods (Cesevičiene & Butkute, 2011). Gluten is responsible for determining wheat baking quality by conferring water absorption capacity and cohesivity, viscosity and elasticity of wheat flour doughs (Cesevičiene & Butkute, 2011; Ionescu & Stoenescu, 2010). Gliadins and glutenins are needed for producing the balance of viscous and elastic properties in both gluten and in the dough (Song & Zheng, 2007).

Glidians affect the viscous properties of dough and glutenins, expressed by G' (storage) and G" (loss moduli) are responsible for the elasticity and strength (Song & Zheng, 2007). Gliadins are monomeric proteins that can be solubilized in alcoholic solutions (Graybosch et al., 1996). An increase in gliadin content will produce a weak, sticky, inelastic gluten (Wrigley et al., 2006). A sulfur deficiency can cause changes in dough quality by upsetting the normal balance of gliadin and glutenin content (Wrigley et al., 2006).

Flours that have a higher glutenin content tend to be strong, tough, elastic, and have non-adhesive gluten proteins (Wrigley et al., 2006). Larger glutenin molecules require a longer mixing time to achieve full dough development due to their increased surface area (Wrigley et al., 2006). A balance between glidians and glutenins is required to form a desirable dough.

Gliadens come in several different subunit groups (α-; β-; γ-; ω-gliadins) which are all controlled by genes in the complex Gli-1 and Gli-2 loci (Mergoum et al., 2009).

Glutenen have high- (HMW-GS) and low- (LMW-GS) molecular weight subunits and are controlled in the complex Glu-1 and Glu-3 loci (Mergoum et al., 2009). HMW-GS contributes to the dough strength, and LMW-GS and ω-gliadins contribute to dough extensibility and ciscosity (Mergoum et al., 2009). Allelic variations mainly at Glu-1, Glu-3, and Gli-1 are where most variation in strength and extensibility in dough mixing is found (Mergoum et al., 2009).

An increase in the amount of glutenin-to-gliadin can lead to a longer mixing time (Uthayakumaran et al., 1999). Most likely this increase led to a reduced resistance breakdown or to an increased tolerance to overmixing (stability) (Uthayakumaran et al., 1999). An increase in the glutenin-to-gliadin ratio was also associated with an increase in the resistance to extension and a decrease in extensibility (Uthayakumaran et al., 1999). An increase in the glutenin-to-gliadin ratio also showed a significant increase in loaf height, which is an important quality in the production of pan bread (Uthayakumaran et al., 1999). The glutenin-to-gliadin ratio was found to be negatively correlated with dough development time (DDT), dough stability, gluten index, and protein content (Barak et al., 2013).

Protein content and the glutenin-to-gliadin ratio both have different roles in the determination of dough and bread quality (Uthayakumaran et al., 1999). Glutenins have a strong negative correlation with peak viscosity, breakdown viscosity, and pasting temperature while gliadins have a positive correlation with breakdown viscosity, setback, and final viscosity (Barak et al., 2013).

1.5.3 Non-starch polysaccharides

Wheat grain also contains non-starchy polysaccharides which are present in cell walls of the endosperm and in the bran (Van Der Borght et al., 2005). These polysaccharides are arabinoxylans, cellulose, and arabinogalactan-peptides; arabinoxylans are the most abundant (Van Der Borght et al., 2005).

Although the non-starch polysaccharides are a minor component of wheat, they significantly affect the physical properties of dough (Sasaki et al., 2000). The pentosans are composed of arabinoxylans and xylans. Water insoluble arabinoxylan is known to influence the dough characteristics as well as baking performance because of its water-binding capacity as well as its high viscosity (Sasaki et al., 2000). This positively affects the dough quality while adding water insoluble arabinoxylans will decrease loaf volume (Sasaki et al., 2000).

Cellulose is the most abundant organic compound found in nature; it comprises over 50% of all the carbon in vegetation (Choct, 1997). Cellulose is insoluble in water and has a high molecular weight. It is also believed that cellulose is identical in chemical composition regardless of the source (Choct, 1997). In cereals, cellulose can be recovered from the insoluble residue left after extraction of cell wall material (Choct, 1997).

1.5.4 Lipids

Two percent of the wheat grain is lipids and can be classified as either starch lipids or free and bound non-starch lipids depending on their extraction conditions (Van Der Borght et al., 2005). Non-starch lipids compose 75% of the lipids and are predominantly triacylglycerols and are sometimes digalactosyl diacylglycerols (Van Der Borght et al., 2005). The starch lipids, making up the remaining 25%, are majorly

lysophospholipids (Van Der Borght et al., 2005). The lipids interact with the dough and gluten; most of the non-starchy lipids bind to gluten during the dough mixing process (Van Der Borght et al., 2005).

Lipids also effect the relationship between the proteins and water; defatting gluten can improve the water uptake (Song & Zheng, 2007). In dough mixing, the lipid interaction is mainly with the gluten proteins which results in structural modification (Addo & Pomeranz, 1991). Lipids in the gluten are bound mainly to the glutenin proteins (approximately 20%) while only 1.5% of lipids bind to gliadin (Chung et al., 1978).

1.5.5 Byproducts

There are several byproducts created when wheat is milled into flour: bran, shorts and middlings. Bran is the outer seed coat of the wheat kernel (Figure 1) which has a high nutrient content but is typically used in animal feed and not human consumption (Balandrán-Quintana et al., 2015). Bran is sometimes incorporated into fiber-rich foods like cereals and baked goods because of its dietary fiber and B vitamins (Balandrán-Quintana et al., 2015). Although bran is a good source of protein, nutrients and fiber, adding it to bread affects bread quality(Kaprelyants et al., 2013).

1.6 Wheat flour quality measurement

1.6.1 Farinograph

The Brabender Farinograph measures the rheological behaviors of dough (Diósi et al., 2015). The farinograph uses arbitrary units called Brabender units to incorporate torque to dough mixing (Diósi et al., 2015). The Farinograph measures several different rheological behaviors in wheat flour dough: arrival time, peak time, mixing tolerance index, departure time, stability, and water absorption capacity. The Farinoraph is widely

used to predict the functionality of flour and provides information in determining the quality of a cereal grain (Saleh et al., 2016). Dough performance during quality testing is associated with changes in the chemical composition and structural changes in the gluten network formation of a flour (Saleh et al., 2016). The Farinograph is considered a major quality testing instrument in the baking industry; it measures the plasticity and mobility of a dough that is subjected to gentle mixing at a constant temperature and speed (Saleh et al., 2016). Typically on the Farinograph test, water is added to a sample of 50g or 300g of wheat flour that has a 14% moisture content until the consistency reaches 500 Brabender units (BU) (Okuda et al., 2016).

Farinograph arrival indicates of the rate of flour water uptake and protein content (Diósi et al., 2015; Saleh et al., 2016). The arrival time is the amount of time in minutes it takes for the center of the Farinograph curve to reach the 500-BU line (*AACC Approved Methods of Analysis, 11th Ed. Method 54-21.02. Rheological Behavior of Flour by Farinograph: Constant Flour Weight Procedure. Cereals & Grains Association, St. Paul, MN, U.S.A.*, 2011). Water absorption is important to the breadmaking process, typically the water content in a bread dough is 65% (Okuda et al., 2016). If the water content is low, the mixing time will increase. Bread volume is more effected by a water content that is too low than it is a water content that is too high (Okuda et al., 2016). Bread volume is reduced when the water content falls below approximately 45% (Okuda et al., 2016).

The dough development peak time is an indication of the flour development (mixing) time (Diósi et al., 2015; Saleh et al., 2016). The peak time begins when water is added to when the dough reaches its maximum consistency (Wheat Marketing Center Inc, 2004). The dough development peak time measurement from the Farinograph gives

an indication of the optimum mixing time under standardized conditions (Wheat Marketing Center Inc, 2004).

Mixing tolerance index (MTI) is the difference between the Brabender unit value at the top of the curve 5 minutes after the peak time (Wheat Marketing Center Inc, 2004). The MTI indicates the extent of which a dough will break down and soften during the mixing process (Wheat Marketing Center Inc, 2004).

The departure time is the time when the top of the curve leaves the 500-BU line (Wheat Marketing Center Inc, 2004). This measurement indicated when the dough is beginning to break down and soften, this is an indication of what the dough's consistency will be like during processing (Wheat Marketing Center Inc, 2004).

Farinograph stability is the difference in the time between arrival and departure (Wheat Marketing Center Inc, 2004). Stability is a good indication of the dough strength and indicated the amount of time a dough will remain at its maximum consistency (Wheat Marketing Center Inc, 2004).

The Farinograph procedure is as follows using large (300g flour) or small (50g) flour bowls (*AACC Approved Methods of Analysis, 11th Ed. Method 54-21.02. Rheological Behavior of Flour by Farinograph: Constant Flour Weight Procedure. Cereals & Grains Association, St. Paul, MN, U.S.A.*, 2011):

Procedure

1) Adjust the Farinograph thermostat to a temperature of 30 ± 0.2 ° , this needs to be maintained. Check the temperature of the circulating water, check that the water is circulating freely through the hose and bowl jackets, and confirm that the flow pattern matches the equipment manual.

2) Adjust the position of the base plate to be horizontal, and then fix the four foot-screws with their locknuts.
3) Check that the chart paper is exactly horizontal. Two small plates on spring-loaded hinges are the guides for the paper and can be adjusted.
4) To clean, at the end of each test, while the machine is running, add dry flour to the bowl to make a dough with a consistencey of 800-900BU within 1 minute of mixing with the test dough. Stop the machine, unscrew the bowl, discard the dough, and scrape the bowl with a plastic spatula. Clean the bowl with a damp cloth and wipe completely dry.

Constant flour weight procedure for large and small bowls

1) Sensitivity: There are four sensitivity settings, two choices of position linkage between balance levels, and two choices of weights (400 and 1000). Chose the correct sensitivity for the bowl size.
2) Zero position of the scalehead pointer: Adjust the scalehead pointer to the zero position when the instrument is running at 63±2 rmp with the mixer empty.
3) Adjustment of bandwidth: The damping device should be adjusted after the oil in the damping chamber has been at temperature for 1 hour or more and after the damping piston has been moved up and down several times. Raise the dynamometer until the scalehead pointer indicates 1,000BU. Measure the amount of time it takes for it to go from 1,000BU to 100 BU (should be 1±0.2s).

Large bowl procedure

1) Turn the thermostat and circulating pump on 1 hour before use

2) Determine the flour moisture content and keep the flour in moisture proof containers
3) Place 300 ±0.1g of flour (14% moisture basis) in the bowl
4) Fill the large burette with room temperature water making sure the tip is full, and that the automatic zero adjustment is functioning.
5) Set the pin-point to 9 minutes, turn the machine on to the 63rpm setting and then run for 1 minute until the zero-minute line is reach. Then begin to add water to the right front corner of the bowl from the burette to the expected absorption of the flour. When the dough begins to form, scrape the sides of the bowl with a plastic scraper working counterclockwise. Cover the bowl with the plexiglass cover to prevent any evaporation. If the mixing curve will be higher than 500BU. Add more water. This will be used to estimate the absorption for the next attempt.
6) The first titration rarely has a curve with the maximum resistance centered at 500BU. In the next titration adjust the absorption up or down until it is within 20 of 500BU.
7) In the final titration, add the water within 25s of opening the burette. Let the instrument run until an adequate curve is produced for evaluation. Then, lift the pen from the paper, and clean the bowl.
8) Report the absorption values to the nearest 0.1%, and calculate the absorption on a 14% moisture basis using the following equation:

$$Absorption\% = \frac{(x + y - 300)}{3}$$

X=mL of water needed to produce a curve with maximum consistency centered on the 500BY line

Y= g of flour used

Small bowl procedure

The same method is used except that 50±0.1g of flour is added. Titration is conducted with a small burette instead of a large one. The absorption rate is calculated with the following equation:

$$Absorption\% = 2(x + y - 50)$$

X= mL of water needed to produce a curve with maximum consistency centered on the 500BY line

Y= g of flour used

Farinogram interpretation is derived from the Farinograph curves, an example of which is shown in (Figure 2).

1.6.2 Mixograph

The Mixograph records the dough and gluten properties of a wheat flour by measuring the resistance of a dough to mixing (Wheat Marketing Center Inc, 2004). Mixograph output includes water absorption, peak time, peak width, peak value, and peak right value. The Mixograph curve indicated the strength of the gluten, optimum dough development time, and mixing tolerance (Wheat Marketing Center Inc, 2004). The peak time illustrates the dough development time which begins when the recorder is started and ends when the dough has reached its maximum consistency (Wheat Marketing Center Inc, 2004). The Mixograph mixing tolerance is the resistance of a dough to breaking down during continuous mixing, this measurement is expressed as a score

relative to a control (Wheat Marketing Center Inc, 2004). Weak gluten flours have shorter peak times and less of a mixing tolerance than in strong gluten flours (Wheat Marketing Center Inc, 2004).

The Mixograph method is as follows (*AACC Approved Methods of Analysis, 11th Ed. Method 54-40.02. Physical Dough Tests: Mixograph Method. Approved 1999. Cereals & Grains Association, St. Paul, MN, U.S.A.*, 199 C.E.):

1) The moisture content of the flour should be determined, then weigh the flour samples (10 or 35g on a 14% moisture basis) to 0.01g. The flour should be kept in moisture proof containers.
2) Room temperature needs to be maintained at 25±1° for 24 hours a day. The equipment, flour, and water should be at room temperature. The mixing bowl can be soaked with water between samples but should be dried before the next use.
3) After long idle periods, two or three mixograms of standard flour should precede the other recordings.
4) Transfer weighed flour to the dry mixograph bowl. This can be aided with a camel-hair brush.
5) With a tongue depressor or spatula, move the flour between two bowl pins to create a triangular shaped hole in the middle.
6) Before starting the mixogram, be sure the ink is running freely from the pen.
7) The mixogram should be started on a major arc and run for a fixed time (typically 8-10 minutes).

8) Place the bowl in position on the Mixograph, dispense the water from an automatic pipet, lower the mixing head, and start recording the mixogram.

The dough absorption is calculated using the following equation (14% moisture basis):

$$y = 1.5x + 43.6$$

X= percent of flour protein content

Y= Percent absorption of water

1.6.3 Glutomatic

The glutomatic measures the wet gluten quantity and quality of a wheat flour (Wheat Marketing Center Inc, 2004). Gluten gives wheat dough its elasticity and extensibility characteristics. We gluten reflects the protein content and is commonly a required specification for end-users (Wheat Marketing Center Inc, 2004). Wet gluten is determined by washing the flour in a salt solution which removes starch and other solubles, the remaining residue is the wet gluten (Wheat Marketing Center Inc, 2004). The wet gluten is centrifuged and forced through a sieve, the percentage of gluten left on the sieve is measured as the Gluten Index (GI) (Wheat Marketing Center Inc, 2004). The gluten index is an indicator of the gluten strength, a high GI indicated strong gluten (Wheat Marketing Center Inc, 2004).

The Glutomatic procedure is as follows (*AACC Approved Methods of Analysis, 11th Ed. Method 38-12.02. Gluten: Wet Gluten, Dry Gluten, Water-Binding Capacity, and Gluten Index Approved 2000. (AACC Approved Methods of Analysis, 11th Ed. Method 54-40.02. Physical Dough Tests: Mixograph Method. Cere*, 2000):

Gluten washing flour

1) Place the 88-µm polyester screen in the washing chamber. On top of the screen place the plastic chamber wall with the cylindrical insertion tool inside. Wash from top to bottom to remove any leftover debris.
2) Add wash liquid to the washing chamber to wet the polyester screen. Hit the screen three times on your hand covered with a cloth to remove excess water. Add 10±0.01g of well mixed flour onto the screen that contains a film of liquid to prevent the flour from falling through.
3) Add 4.8ml of wash solution from a dispenser while holding the chamber at about a 30° angle. Shake the chamber gently in circular motions to spread the liquid over the sample.
4) Assemble the washing chamber onto the Glutomatic and start it for a 20s dough mixing and 5min gluten washing cycle. The wash liquid flow rate should be 5-56ml/min.
5) At the end of the cycle, remove the gluten from the chamber without tearing to place it in a centrifuge.

Wet gluten content and gluten index

1) Place the wet gluten from wash chamber into a separate gluten index cassette in a centrifuge.
2) Centrifuge for 30sec at 6000±5rpm for 1min.
3) Remove the gluten from the cassette. With a spatula, remove the gluten that has passed through the sieve. Weigh the gluten to the nearest 0.01g. Leave the gluten on the scale.

4) With tweezers, remove the gluten that is remaining on the top of the sieve and weigh for the total wet gluten.

Dry gluten content and water binding in wet gluten

1) Take the total amount of wet gluten and place it in the center of a lower heating surface or a dryer.
2) Close the dryer and dry at 150° for 4min.
3) With tweezers, remove the dry gluten and weigh to the nearest 0.01g.

The calculations for total wet gluten, gluten index, dry gluten, and water binding in wet gluten are as follows:

$$Wet\ gluten\ content\%\ (14\%\ moisture\ basis) = \frac{total\ wet\ gluten(g) * 860}{100 - \%sample\ moisture}$$

$$Gluten\ index = \frac{wet\ gluten\ remaining\ on\ sieve(g) * 100}{total\ wet\ gluten(g)}$$

$$Dry\ gluten\ content\%\ (14\%\ moisture\ basis) = \frac{total\ dry\ gluten(g) * 860}{100 - \%sample\ moisture}$$

$$Water\ binding\ capacity\ (water\ bound\ in\ wet\ gluten)\% = wet\ gluten\ content\% - dry\ gluten\ content\%$$

1.7 Environment

Environment is known to have a significant impact on the end-use quality of wheat cultivars, but the magnitude of the genotype by environment (GxE) interactions is unclear (Peterson et al., 1986). Temperature during the growing season, temperature during grain fill, distribution of precipitation, late season frost, and the duration of the grain fill have all been shown to be impactors of the end use quality of wheat flour (Peterson et al., 1986). Busch used regression analysis that characterized the bread making response and stability of hard red spring wheat grown in multiple environments,

there were significant differences found for quality traits (Busch et al., 1969; Peterson et al., 1986). There are many environmental factors that can play a role in wheat quality, but some of the main factors are temperature, soil nutrients, and soil moisture.

Warmer than normal temperatures can alter the plant's functions and productivity, short heat stress above 35°C during the post anthesis period can reduce grain weight and grain quality (Sial et al., 2005). With normal temperatures, genotypes that are typically relatively late in heading had better vegetative growth, which was reflected in the plant height and more internodes (Sial et al., 2005). Also associated with this vegetative growth was an increased number of spikelets per spike and in the number of seeds per spike (Sial et al., 2005). High temperatures late in the season decrease grain yield significantly, with an average loss of 8.9% according to Tahir et al, although the percentage of loss varied significantly across different genotypes (2006).

Planting date can also have an effect on the end-use quality of wheat. Late planting of varieties that require a higher number of days for maturation, grain filling (68 to 90 days for grain fill), and for heading had a lower grain weight due to forced heat stress maturation (4 to 10 days earlier than usual) (Sial et al., 2005). When the same genotypes were planted at optimal conditions, the grain filling period was higher (42 to 55.5 days) (Sial et al., 2005). Under late planting, vegetative growth was stunted resulting in shorter plants with less spikelets and seeds per spike (Sial et al., 2005). Sial et al also found that the percent of protein was significantly influenced by changes in planting date, the proteins are synthesized at higher rates under heat stress so later planting dates increased the protein content (Sial et al., 2005).

Soil moisture was the main factor affecting the protein content of Thatcher wheat in a study conducted by Sosulski et al in 1962. With a high moisture regimen (moisture was always above 17%) and up to 200lbs of nitrogen per acre failed to increase the protein content above 12.7% (Sosulski et al., 1962). When the wheat was allowed to go through drier periods, the protein content was over 20%. They concluded that lower levels of moisture with high available nitrogen increased the wheat protein content (Sosulski et al., 1962).

1.8 Breeding for quality improvement

In the beginning of wheat domestication, there was likely no human involvement in breeding wheat for milling or quality, just natural selection and some human intervention for choosing the best landraces for cultivation (Kiszonas & Morris, 2017). Often people and nature were selecting the landraces that survived freezes, disease, and drought (Kiszonas & Morris, 2017). Vilmorin in 1859 advocated for the selection of individual plants to purify lines, now known as pure line selection, but at the farmer's level, selecting landraces was still predominantly how wheat was improving at the time (Kiszonas & Morris, 2017). Improvement in knowledge in genetics and improved methods for flour milling and transportation in the early 1900's had an influence on the improvement of wheat (Kiszonas & Morris, 2017). In the United States and Canada, expansion of hard red spring wheat helped to inspire new methods for flour milling (Kiszonas & Morris, 2017).

Early measurement for wheat quality was almost synonymous with bread quality where studies were aimed at finding wheats with flours that made the best bread (Kiszonas & Morris, 2017). In 1884 Richardson found a relationship between the quality

of bread and the quantity of gluten present (Kiszonas & Morris, 2017; Richardson, 1884). Thatcher in 1907 began researching into wheat flour quality that kickstarted into the research of today (Kiszonas & Morris, 2017; Thatcher, 1907).

Along with a higher yield, today there is demand to produce wheat cultivars that offer end-use optimized flours, more nutrition, and higher quality wheat products (Battenfield et al., 2018). There are different end uses needed for different products in different settings. A home baker requires a different wheat quality than an industrial baker. Similarly, different products such as leavened bread or noodles also need different kinds of wheat flours with different quality parameters. Breeding strategy and priorities must be used with the cultivars intended use in mind as well as to fit the demand of the target market (Mergoum et al., 2009).

Determining the quality of wheat flour includes measurements on the wheat grain, flour, dough, and the final product which all need to be assessed by the wheat breeding program (Battenfield et al., 2018). This process can be limited or made more difficult by the amount needed for a sample or by the cost of each different assessment. There are some grain tests that can be done on a small scale, quickly, and cheaply, but dough rheology and end-use tests need larger quantities of grain to mill into flour which can restrict their use in advanced breeding stages (Battenfield et al., 2018).

Genetic mapping with complex structured populations (GWAS) has become more common with but is limited in breeding programs because of time and resource constraints (Battenfield et al., 2018). One benefit of GWAS is that it does not require structured mating, instead large diverse samples are needed to associate genomic markers to phenotypic variation (Battenfield et al., 2018). Statistical power of GWAS can be

strengthened by combining the results from several populations through meta-analysis (Battenfield et al., 2018). GWAS can be used as an approach for insight into genetic basis of some of the most important traits in wheat breeding programs which can enable more robust breeding approaches to compliment Quantitative trait loci mapping (QTL) (Battenfield et al., 2018).

Electrophoresis (sodium dodecyl sulfate polyacryl-amide gel electrophoresis, SDS-PAGE) is used to identify allelic variations at Glu-1, Glu-3, and Gli-1 to characterize parental lines (Mergoum et al., 2009). Information on glutenin subunit and gliadin composition assists breeders in choosing crosses that are aimed at achieving allelic combinations that are known to contribute to dough properties needed for producing bread products (Mergoum et al., 2009). Electrophoresis is a small scale, high throughput testing method that can be used on breeding lines for improving wheat quality.

Marker assisted selection (MAS) is a better option than electrophoresis for other traits such as grain color, hardness, and proteins and is used on plant tissue before the seed sets. This can help quickly eliminate breeding lines before harvesting occurs meaning less grain testing can be done to be more cost effective (Mergoum et al., 2009). MAS has proven itself to be useful for germplasm characterization and in the manipulation of DNA markers in genomic regions that are involved in trait expressions (Hoisington & Ribaut, 1998). MAS has also been a successful tool used in moving desired alleles from wild relatives into elite cultivars as well as in the process of selecting for parental lines (Hoisington & Ribaut, 1998). These molecular markers are used to identify and tag desired genes and to create linkage maps (Mohan et al., 1997). There are

several requirements for MAS to be successful in a breeding program: the markers should be closely linked to the desired trait, the screening technique should be easily replicable, and economical (Mohan et al., 1997). MAS is advantageous for several agronomic traits that can otherwise be difficult to tag such as pathogen resistance, insect and nematode resistance, abiotic stress resistance, quality parameters, and quantitative traits (Mohan et al., 1997).

CHAPTER 2: PREDICTING FARINOGRAPH STABILITY USING MIXOGRAPH AND GLUTOMATIC PARAMETERS

2.1 Introduction

Wheat (*Triticum aestivum L.*) is one of the 'big three' cereal crops (along with rice and corn) with approximately 600 million tons harvested each year (Shewry, 2009). Wheat has a wide range of cultivation from Russia to Argentina, tropic regions and sub-tropic regions (Shewry, 2009). Significant wheat exporting areas of the world include the United States, Canada, Australia, The Black Sea Region, Europe, and Argentina (Sharma et al., 2015). Wheat has a wide variety of uses from human food to livestock feed, and in many cultures and religions wheat bread is of significance (Shewry, 2009). There are over 620 million tons of wheat grown worldwide every year (Dubcovsky & Dvorak, 2007). Approximately 95% of wheat grown is common wheat used for breadmaking and pastries. Wheat consumption represents about 1/5 of the world's caloric intake (Dubcovsky & Dvorak, 2007). The other 5% of wheat grown is durum wheat, which is often used to make pastas. Einkorn and other hulled wheats, such as emmer, are of minor economic importance (Dubcovsky & Dvorak, 2007).

In the United States wheat is the third crop for both value and acreage behind corn and soybeans (Vocke & Ali, 2014). Unlike other crops, wheat has distinct varieties that are meant to be produced across different regions during different seasons, this causes a variation in the costs and competitiveness of wheat with other crop species in the United States (Vocke & Ali, 2014). Along with productivity of the wheat plant, the value of wheat lies in its flour quality that affects milling and breadmaking (Briggle & Reitz,

1963). Producing a wheat crop in which the sale price of the wheat outweighs the cost is important to keep farmers growing wheat and not switching to a potentially more valuable crop.

2.2 Materials and Methods

2.2.1 Field plot and sample preparation

All grain used for analysis was derived from Advanced Yield Trial (AYT) entry plots grown within the South Dakota State University Spring Wheat Breeding and Genetics program. Trials were grown in seven to 10 locations each year, though for the purpose of this study, samples were collected from three locations in each of the years 2015-2019 (Table 1). All AYTs were fashioned as a randomized complete block design composed of three replications at each location. Composite samples of each genotype selected for analysis were created by combining grain from each replication into a single container prior to milling. Thirty-three to 48 samples were milled each year, and a total of 540 were subjected to Farinograph, Mixograph and glutomatic tests to measure end-use quality potential.

2.2.2 Flour quality determination

Quality determination was performed as described by (Caffe-Treml et al., 2011). Grain samples were tempered at 15% moisture for at least 16 hours before being milled in a Quadramat Jr. Mill (C.W. Brabender Instruments, South Hackensack, NL). Flour was collected from a rotating US #60 sieve (250-µm aperture). Byproducts (bran and shorts) were discarded and not used in this study. Protein content with a 14% moisture basis was determined by the NIR Systems 6500 Monochromators (Foss, Laurel, MD). The amount of water added to each sample was determined by the estimates obtained by the NIR.

The Gluten Index Method, as described in AACC Approved Methods of Analysis 38-12.02 was determined by the Glutomatic. The Mixograph was also conducted on all samples as described in ACC Approved Methods of Analysis 54-40.02. The Farinograph using the ACC Approved Methods of Analysis 54-21.02 was conducted at the Northern Crops Institute Fargo, North Dakota.

2.2.3 Data Analysis

2.2.3.1 Stepwise regression

All statistical analyses were carried out using SAS-JMP version 14.0.0 (SAS Institute,2018). Parameters tested in this study include stability (S), midline peak time (MPT), midline peak value (MPV), midline peak width (MPW), midline peak integral (INTEG), gluten index (GI), and Gluten left on the sieve after centrifuging by gluten index (GOODXGI).

Stepwise linear regression methods in JMP were used to create two models to predict the Farinograph stability (S in seconds) measurement. Stepwise regression was performed on all 540 samples. All other analyses were performed on averaged datasets to reduce variability: one averaged by genotype and the other averaged by year genotype.

Stepwise regression is a method of variable selection for the purpose of specifying a linear regression model (Agostinelli, 2002). Stepwise regression allows a computer to select the best predictors from a set of potential variables to predict an outcome (Malek et al., 2007). There is an issue with using stepwise regression to create prediction models. Stepwise regression can produce an inflated R-squared, which can lead to an inaccurate test of statistical significance (Malek et al., 2007). Stepwise regression has the potential to fail at taking into account how many variables were considered in its analysis (Malek

et al., 2007). Hierarchical analysis can be used alongside stepwise regression to allow the researcher to have some control over the order of entry of predictor variables (Malek et al., 2007). Stepwise regression with hierarchical analysis was used to create Model 1 and Model 2 using JMP. Bidirectional stepwise regression with a 0.5 and 0.25 stopping p-value were used to create the original model; then, the model was cleared of any remaining non-significantly contributing values.

2.2.3.2 Pairwise correlation analysis

Pairwise correlation analysis was conducted on the raw data for all 540 samples, as well as on the data averaged by genotype year and by genotype. Pairwise correlation gives correlation coefficients for all parameters for all observations that have no missing values. This was used to aid in the creation of Model 3 containing the parameters that were significantly correlated in a positive linear fashion.

2.3 Results

2.3.1 Summary statistics of all parameter values by genotype and genotype year

The summary statistics for all parameters measured can be seen in table 2. Summary statistics for each parameter used by each genotype can be seen in tables 7-14.

2.3.2 Correlation between parameters, by genotype and by genotype year

Pairwise correlation tables were created in JMP for all 15 parameters, all 15 parameters by genotype, and all 15 parameters by genotype year in Tables 3, 4, and 5 respectively. There was a high correlation between stability and MPT (0.57), INTEG (0.55), and GI (0.57) when looking at the complete unaveraged dataset with all 540 samples (Table 3). When comparing the pairwise correlation coefficients for the data averaged by genotype, stability had a high correlation with MPT (0.74), INTEG (0.73),

and GI (0.70) (Table 4). Pairwise correlations for genotype by year show stability to be correlated with MPT (0.61), INTEG (0.57), and GI (0.62) (Table 5). Pairwise correlation was also done on all 15 parameters by genotype year subsetted by year. In 2015 stability had a high correlation with MPT (0.68), INTEG (0.69), GI (0.63), and GOODXGI (0.65). In 2016 stability was highly correlated with MPT (0.51), INTEG (0.51), and GI (0.66). In 2017 stability was correlated with MPT (0.54), INTEG (0.57), and GI (0.54). In 2018 stability was correlated with MPT (0.77), INTEG (0.82), GI (0.63), and GOODXGI (0.60). In 2019 stability was correlated with MPT (0.77), INTEG (0.89), GI (0.73), and GOODxGI (0.74). All correlation coefficients with stability considered, there were three that were always correlated: midline peak time, midline peak integral, and gluten index

2.3.3 Stability prediction

Averaging the measurements for genotype or genotype over years reduces the effects of the environment and variables are more likely to have a genetic effect on the response variable (Lu, 2017). Prediction models were created using stepwise regression to select variables for model 1 without GI and model 2 with GI, model 3 was created with the parameters that were significantly correlated to stability in every case.

$$Model\ 1 = MPV + MPW + MRV + MPT$$

$$Model\ 2 = MPV + MPW + MRV + MPT + GI$$

$$Model\ 3 = MPT + INTEG + GI$$

Model 1 containing midline peak value, midline peak width, midline right value, and midline peak time explained 55.7% of stability in 2015, 42% in 2016, 40.2% in 2017, 62.8% in 2018, and 78% in 2019 (Table 5; Figure 6). Across all years model 1 explained

47.9% of stability (Table 5; Figure 7). Across genotypes model 1 explained 57% of the stability measurement (Table 5; Figure 3).

Model 2 containing midline peak value, midline peak width, midline right value, midline peak time, and gluten index explained 55.7% of stability in 2015, 46.8% in 2016, 40.6% in 2017, 57.4% in 2018, and 75.2% in 2019 (Table 5; Figure 8). Across all years model 2 explained 51.2% of stability (Table 5; Figure 9). Model 2 across genotype averages explained 60.9% of the stability measurement (Table 5; Figure 4).

Model 3 containing midline peak time, midline peak integral, and gluten index explained 44.5% of stability in 2015, 42.3% in 2016, 32.4% in 2017, 50.6% in 2018, and 55% in 2019 (Tables 5; Figure 10). Across all genotype years model 3 explained 46.4% of stability (Table 5; Figure 11). Across all genotypes model 3 explained 61.3% of stability (Table 5; Figure 5).

2.4 Discussion

2.4.1 Stability

Farinograph stability is often used to determine the strength of a dough, a stability prediction can aid a hard-red spring wheat breeder in making selections by determining if the dough strength for a variety is acceptable or not. The Farinograph stability of a dough is an indication of the amount of time the dough remains at its maximum consistency while mixing and is a good indication of dough strength (Koppel & Ingver, 2010), Dough with a good strength will have a stability time of 4-12 minutes; satisfactory dough will be stable for approximately 6 minutes (Koppel & Ingver, 2010). In an industrial setting, doughs with a mixing time that is too long or too short can cause

issues. Flours that have short mixing times can cause problems in processes that require long formation times (Koppel & Ingver, 2010).

Stability was significantly correlated with MPT and INTEG from Mixograph tests and with GI from Glutomatic tests (Model 3). Together these three parameters across genotypes explained 61.3% (R-square of 0.613) of variation in predicting the stability measurement. There are environmental influences on stability. There was significant changes in the R-square values for these predictors across the years 2015-2019 (0.45, 0.43, 0.32, 0.51, and 0.55 respectively). Across all genotype years, the R-square for this model was 0.46.

Model 1 showed to be the weakest of the three considered models, MPV, MPW, MRV, and MPT. This model also showed stability to be affected by year with R-square values ranging from 0.56, 0.42, 0.40, 0.63, and 0.78 (2015-2019) respectively. Across all genotype years, this model explained 47.9% of stability. Across genotypes, 57% of the stability measurement was explained.

The variability in stability across years noted in this study confirms data discussed by Koppel & Ingver in 2010. The stability measurement varies more by year than it does on genotype (Koppel & Ingver, 2010).

Akaike information criterion (AIC) values estimates the in-sample prediction error of a model. Overall, models conducted on data averaged by genotype had lower AIC values than models used on data averaged by genotype year (Table 15). Model 3 by genotype average had the lowest AIC value of all three models by genotype average (1074.4), but Model 2 had the lowest AIC value of all three models by genotype year average (2390.07). Model 3 across genotype averages also had the lowest root mean

square error (RSME) overall models by genotype average, of 165.03 (Table 6). Model 2 by genotype average was close behind with an RSME of 165.87.

2.4.2 Mixograph parameters

INTEG was found to predict 26-78% of farinograph stability across genotype years with an average of 33% prediction and a correlation coefficient ranging from 0.55-0.72 across genotype years. The integral is the area under the curve from the beginning until the peak time is reached.

MPT explained 26-59% of stability across genotype years with an average of 37%. MPT across genotypes explained 55% of stability with a correlation coefficient ranging from 0.57-0.74. The MPT measurement begins when the mixer starts and continues until the dough is at its maximum consistency.

MPV across genotype years explained 0.04-3% of stability with an average of 0.00002%. Across genotypes, MPV explained 0.9% of stability. MPV has a correlation coefficient of -0.1- 0.10 with stability.

MPW is a measurement illustrating the strength and elasticity of a dough. Wide peaks are stronger and more elastic than narrow ones. 0.7-20% of stability across years with an average of 1% was explained by the peak width. Across genotypes, MPW explained 0.00002% of stability. MPW has a correlation coefficient of -0.005 – 0.18 with stability.

MRV explained 0.1-12% of stability with an average of 2%. Across genotypes MRV explained 0.2% of stability. MRV has a correlation coefficient of 0.04-0.16 with stability. The MRV is expressed as a percentage and is the height of the curve 2 minutes after the peak time is reached.

Szafrańska created a prediction equation for stability using Mixograph parameters differing from those mentioned above. A regression model containing water absorption (WA), torque measures at 8, 10, 12, and 14 minutes, and time (Szafrańska. 2015). This model produced adjusted R-square values ranging from 0.644 to 0.724 depending on the type of flour used (Szafrańska. 2015).

Environmental changes were found to have an impact on different Mixograph parameters in this study which is in line with the conclusions made in a 2000 study from Guttieri et al. A drop in moisture altered the Mixograph MPT but did not affect MPV (Guttieri et al., 2001). The MPT was longest in flours that came from grain experiencing a severe moisture deficit. Flours from a grain grown under well-watered deficit had a much lower peak time than the latter (3.7 and 2.9 min respectively) (Guttieri et al., 2001). There are also significant changes in the MPV in different environmental conditions, different cultivars, and different cultivars with different environmental conditions (Guttieri et al., 2001).

2.4.3 Glutomatic

With only the GI measurement across years, 29-53% of stability can be predicted with an average of 38.8%. Across genotypes, GI can predict 48.6% of stability. GI has a correlation coefficient of 0.57- 0.69 with stability.

GOODXGI has a correlation coefficient of 0.42-0.46 with stability. Across all years, GOODXGI explained 17- 54% of stability with an average of 0.21%. Across all genotypes, GOODXGI explained 17% of stability.

GI is an expression of the weight percentage of wet gluten remaining, and is heavily influenced by environmental factors and variety (Bonfil & Posner. 2012). While

GI is an indicator of the quality of the wheat, using it as a sole indicator can cause issues. There is not a correlation between GI and other quality parameters such as protein or loaf volume, both of which are important indications of end use quality (Bonfil & Posner. 2012). Weak glutens typically will have a GI of less than 30%, normal GI ranges from 30-80% and strong gluten will have a GI of over 80% (Šekularac, Torbica, Živančev, Tomić, & Knežević. 2018).

2.4.4 Other prediction methods

Near-infrared (NIR) and mid-infrared (MIR) have been used to predict Farinograph parameters such as WA, DDT, degree of softening (DOS), and stability (Chen, Ye, & Zhao. 2017). Using NIR and MIR Chen et al. reported R-square values for WA, DDT, stability, and DOS of 0.96, 0.94, 0.95, and 0.94 respectively. The time to predict Farinograph parameters in this study was very rapid, taking approximately 10 minutes (Chen, Ye, & Zhao. 2017).

WA is the amount of water that is required during mixing to achieve the desired dough consistency and have optimal gluten development, like stability WA is sometimes predicted in breeding programs (Fu, Wang, & Dupuis. 2017). The GlutoPeak instrument from Brabender was found to have a positive linear relationship with Farinograph WA with an R-square value of 0.97 (Fu, Wang, & Dupuis. 2017).

2.4.5 Variance inflation factor

The variance inflation factor (VIF) is a quantification of how much the variance is inflated (PennState. 2018). When VIF is 1 there is no correlation between the predictor and there is no inflation, VIFs over 4 should be further investigates, and VIFs over 10 show multicollinearity that needs to be corrected (PennState. 2018). The VIF scores for

all three models on the dataset of 540 hard red spring wheat samples can be seen in table 16. Every model contains VIF scores that are over 10 and need to be corrected to provide an accurate prediction equation for stability.

2.5 Conclusion

Hard red spring wheat end-use quality is dependent on genotype, environment, and their interactions. Using Farinograph stability on early breeding lines can prove useful to hard red spring wheat breeders to find lines that have a sufficient dough strength. Both Mixograph and Glutomatic parameters prove to be useful in the prediction of Farinograph stability to determine dough strength.

Mixograph parameters can prove useful to be present in a prediction equation for farinograph stability. Model 3 appears to be the best predictor for stability; it contains midline peak time and midline peak integral. Midline peak time explained 26-59% of stability across years with an average of 37%. Midline peak time across genotypes explained 55% of stability. Across years midline peak integral explained 26-78% of stability with an average of 33%. The third parameter in the third model is gluten index from the glutomatic, which appears to be the most significant predictor variable present. With only the GI measurement across years 29-53% of stability can be predicted with an average of 38.8% Across genotypes GI can predict 48.6% of stability. When the three parameters are used for prediction it can explain 61% (R-square of 0.61) of variation for the stability measurement. The AIC value for model 3 across genotype averages is 1074.4, the lowest of all three models by genotype average. VIF scores in each model are high and would need to be corrected to come up with a model that could accurately

predict Farinograph stability, but the predictive models can still be useful to a HRSW breeder for screening for acceptable levels of dough strength.

Using Mixograph and Glutomatic parameters can prove useful in helping hard red spring wheat breeders make selections for lines that have a sufficient dough strength early on in the breeding program. This can save both time and money in a breeding program from culling lines that do not meet the requirements of the breeder.

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Year	Location		
2015	Brookings, SD	Selby, SD	Watertown, SD
2016	Brookings, SD	Selby, SD	Groton, SD
2017	Letcher, SD	Watertown, SD	Groton, SD
2018	Brookings, SD	Miller, SD	Groton, SD
2019	Groton, SD	Selby, SD	Watertown, SD

Locations of all AYT samples grown from 2015-2019 in the South Dakota State University Spring Wheat Breeding and Genetics program

Table 2: Simple statistics on observed parameters for all 540 samples

	N	DF	Mean	Std Dev	Sum	Minimum	Maximum
S (sec)	540	539	1015.37	388.41	548301	180	2820
ML PK TIME	534	533	5.73	2.03	3060	2.01	16.34
ML PK VAL	534	533	50.27	4.45	26843.5	39.87	70.83
ML PK W	534	533	22.39	4.93	11954.58	9.90	37.51
ML R VAL	534	533	47.71	3.86	25476.29	38.08	64.83
INTEG	534	533	200.40	60.49	107012	66.17	465.50
GI	534	533	91.10	8.03	49127.73	46.68	99.69
GOOD X GI	534	533	327.35	47.94	174804.1	101.81	470.72

S(sec): Stability in seconds; ML PK T: Midline peak time; ML PK V: Midline peak value; ML PK W: Midline peak width; ML R V: Midline right value; INTEG: Integral; GI: Gluten Index; GOODXGI. N: Sample size; DF: Degrees of freedom; StdDev: Standard deviation.

Table 3: Pairwise correlations for all 540 AYT samples

	S (sec)	MPT	MPV	MPW	MRV	INTEG	GI	GOOD X GI
S (sec)	1	0.57	-0.06	0.18	0.10	0.55	0.57	0.43
MPT	0.57	1	-0.43	-0.23	-0.24	0.96	0.58	0.19
MPV	-0.06	-0.43	1	0.58	0.95	-0.26	0.04	0.39
MPW	0.18	-0.23	0.58	1	0.521	-0.214	0.04	0.44
MRV	0.10	-0.23	0.95	0.52	1	-0.04	0.25	0.48
INTEG	0.55	0.96	-0.26	-0.21	-0.048	1	0.65	0.27
GI	0.57	0.58	0.04	0.04	0.25	0.65	1	0.75
GOOD X GI	0.43	0.19	0.39	0.44	0.48	0.29	0.75	1

S(sec): Stability in seconds; MPT: Midline peak time; MPV: Midline peak value; MPW: Midline peak width; MRV: Midline right value; INTEG: Integral; GI: Gluten Index; GOODXGI

Table 4: Pairwise correlations for data averaged by genotype.

	S (sec)	MPT	MPV	MPW	MRV	INTEG	GI	GOOD X GI
S (sec)	1	0.74	-0.1	-0.01	0.05	0.73	0.7	0.42
MPT	0.74	1	-0.31	-0.32	-0.15	0.95	0.71	0.26
MPV	-0.01	-0.31	1	0.69	0.96	-0.12	0.14	0.6
MPW	-0.01	-0.32	0.69	1	0.62	-0.23	-0.02	0.56
MRV	0.05	-0.15	0.96	0.62	1	0.09	0.332	0.67
INTEG	0.73	0.95	-0.12	-0.23	0.09	1	0.81	0.4
GI	0.7	0.70	0.14	-0.02	0.33	0.81	1	0.7
GOOD X GI	0.42	0.26	0.6	0.56	0.67	0.4	0.69	1

S(sec): Stability in seconds; MPT: Midline peak time; MPV: Midline peak value; MPW: Midline peak width; MRV: Midline right value; INTEG: Integral; GI: Gluten Index; GOODXGI

Table 5: Pairwise correlations for data averaged by genotype year.

	S (sec)	MPT	MPV	MPW	MRV	INTEG	GI	GOOD X GI
S (sec)	1	0.61	0.004	0.11	0.16	0.57	0.62	0.46
MPT	0.61	1	-0.28	-0.33	-0.05	0.95	0.7	0.25
MPV	0.004	-0.28	1	0.63	0.94	-0.09	0.13	0.49
MPW	0.11	-0.33	0.63	1	0.5	-0.28	-0.03	0.49
MRV	0.16	-0.05	0.94	0.5	1	0.19	0.37	0.56
INTEG	0.57	0.95	-0.09	-0.28	0.19	1	0.76	0.33
GI	0.62	0.7	0.13	-0.03	0.37	0.76	1	0.72
GOOD X GI	0.46	0.25	0.49	0.49	0.56	0.37	0.72	1

S(sec): Stability in seconds; MPT: Midline peak time; MPV: Midline peak value; MPW: Midline peak width; MRV: Midline right value; INTEG: Integral; GI: Gluten Index; GOODXGI

Table 6: Model summary statistics

Model	Multiple Regression		
	R2	RMSE	Mean of Response
Model 1			
Genotype Averages	0.57	173.10	1038.38
Genotype year (2015)	0.56	191.27	957.93
Genotype year (2016)	0.42	183.61	980.30
Genotype year (2017)	0.40	188.20	1210.21
Genotype year (2018)	0.63	156.81	1031.12
Genotype year (2019)	0.78	104.84	801.94
Genotype Year Average	0.48	203.57	1014.76
Model 2			
Genotype Averages	0.61	165.87	1038.38
Genotype year (2015)	0.56	191.08	957.93
Genotype year (2016)	0.47	175.83	980.30
Genotype year (2017)	0.41	187.61	1210.21
Genotype year (2018)	0.57	167.779	1031.12
Genotype year (2019)	0.75	111.36	801.94
Genotype Year Average	0.51	196.99	1014.76
Model 3			
Genotype Averages	0.61	165.03	1038.38
Genotype year (2015)	0.45	213.97	957.93
Genotype year (2016)	0.42	183.08	980.30
Genotype year (2017)	0.32	200.16	1210.21
Genotype year (2018)	0.51	180.76	1031.12
Genotype year (2019)	0.55	150.09	801.94
Genotype Year Average	0.46	206.53	1014.76

R-squared, root mean square error (RMSE), and mean of response statistics for all three models across genotype averages, genotype years, and genotype year averages.

Table 7: Summary statistics for the stability measurement by genotype

	Stability		
	Mean	StdDev	Range
ADVANCE	1091.33	388.26	1272.00
BOOST	1221.87	556.39	2172.00
BRICK	1196.20	394.07	1480.00
BRIGGS	555.80	226.03	839.00
FALLER	951.47	381.32	1499.00
FOCUS	1019.93	308.26	1218.00
FOREFRONT	935.27	252.35	840.00
LCS-TRIGGER	759.33	273.20	864.00
OXEN	1044.20	311.57	983.00
PREVAIL	902.93	272.69	974.00
SD4393	948.67	272.63	660.00
SD4403	962.83	253.56	690.00
SD4416	1006.17	215.10	630.00
SD4465	1145.11	398.00	1040.00
SD4472	1056.00	437.30	1174.00
SD4492	1308.67	395.91	1020.00
SD4493	588.17	166.00	394.00
SD4514	1125.00	232.56	570.00
SD4529	716.00	326.91	1028.00
SD4539	1088.58	188.63	614.00
SD4543	1012.67	180.42	450.00
SD4546	1264.78	308.45	848.00
SD4557	1117.67	321.77	756.00
SD4575	700.50	226.10	599.00
SD4579	943.89	275.25	780.00
SD4582	769.00	410.31	1002.00
SD4587	1043.17	396.24	990.00
SD4595	1199.56	450.54	1357.00
SD4624	977.83	329.78	848.00
SD4625	977.00	397.19	1510.00
SD4650	1450.00	315.12	630.00
SD4676	1150.00	617.98	1230.00
SD4681	1450.00	586.60	1140.00
SD4689	1320.00	226.50	450.00
SD4692	1270.00	595.73	1170.00
SD4693	1370.00	424.62	750.00
SD4702	1280.00	454.31	840.00
SD4703	1560.00	670.15	1290.00
SD4706	1157.83	412.59	1230.00
SD4707	977.67	200.49	462.00
SD4708	1553.50	475.27	1230.00
SD4711	1039.67	292.59	810.00
SD4719	1075.33	248.25	690.00
SD4720	789.00	314.13	930.00
SD4721	1088.33	181.30	570.00
SD4729	1060.00	255.15	510.00
SD4732	1100.00	424.62	750.00
SD4735	1500.00	599.25	1170.00
SD4738	1640.00	636.63	1170.00
SD4740	1466.33	433.90	1290.00
SD4742	1690.00	396.11	780.00
SD4744	920.00	425.68	840.00
SD4745	1067.50	223.81	571.00
SD4746	1279.00	387.81	930.00
SD4747	1170.00	467.65	810.00
SD4748	1462.67	355.79	742.00
SD4752	1006.83	123.90	330.00
SD4771	731.33	210.16	554.00
SD4772	712.67	53.15	94.00
SD4773	915.33	277.74	755.00
SD4775	849.00	243.42	682.00
SD4792	908.00	228.95	456.00
SD4816	1254.33	156.03	284.00
SD4840	879.67	96.72	191.00
SD4842	899.00	102.37	204.00
SD4843	772.33	56.96	113.00
SD4844	697.67	70.22	135.00
SD4848	1304.33	133.36	266.00
SD4849	1092.67	99.61	192.00
SD4852	1014.67	195.22	369.00
SD4854	712.67	97.73	195.00
SD4855	887.00	12.77	25.00
SD4870	671.33	61.03	119.00
SD4871	871.67	48.17	96.00
SD4873	688.00	63.79	127.00
SD4874	946.33	67.04	119.00
SD4876	534.00	102.24	204.00
SELECT	1167.47	331.65	1046.00
STEELE-ND	888.42	365.79	1080.00
SURPASS	1078.80	331.92	1200.00
SY-VALDA	650.33	29.50	59.00
TRAVERSE	496.73	262.81	1140.00

The mean, standard deviation, and range of stability for all varieties.

Table 8: Summary statistics for the MPT measurement by genotype

	Midline Peak Time		
	Mean	StDev	Range
ADVANCE	6.44	0.89	2.29
BOOST	5.43	1.19	3.13
BRICK	7.15	1.01	2.56
BRIGGS	3.76	0.78	2.17
FALLER	5.56	1.36	3.48
FOCUS	5.72	0.85	1.92
FOREFRONT	5.50	0.90	2.39
LCS-TRIGGER	4.55	0.06	0.12
OXEN	5.82	0.88	2.22
PREVAIL	4.93	0.91	2.08
SD4393	7.01	1.37	1.94
SD4403	5.20	0.53	0.75
SD4416	6.59	0.72	1.02
SD4465	5.68	0.36	0.71
SD4472	6.14	0.35	0.49
SD4492	7.25	0.26	0.37
SD4493	4.93	0.48	0.68
SD4514	7.53	0.36	0.50
SD4529	4.57	0.28	0.54
SD4539	6.25	1.20	2.61
SD4543	6.14	0.93	1.31
SD4546	7.20	0.52	1.01
SD4557	7.96	1.01	1.42
SD4575	5.66	0.07	0.10
SD4579	5.11	0.16	0.32
SD4582	5.27	0.71	1.01
SD4587	5.34	0.29	0.41
SD4595	5.98	0.38	0.75
SD4624	6.51		0.00
SD4625	6.31	1.65	3.37
SD4650	6.08		0.00
SD4676	6.10		0.00
SD4681	5.76		0.00
SD4689	8.74		0.00
SD4692	7.92		0.00
SD4693	6.98		0.00
SD4702	5.88		0.00
SD4703	6.74		0.00
SD4706	6.01	1.03	1.46
SD4707	5.28	1.25	1.76
SD4708	7.50	1.28	1.81
SD4711	5.44	0.90	1.27
SD4719	5.93	0.80	1.13
SD4720	4.12	0.66	0.94
SD4721	4.17	0.84	1.18
SD4729	6.99		0.00
SD4732	5.68		0.00
SD4735	10.48		0.00
SD4738	9.42		0.00
SD4740	6.65	0.43	0.61
SD4742	6.44		0.00
SD4744	6.12		0.00
SD4745	6.26	1.79	2.53
SD4746	7.30	1.52	2.15
SD4747	6.54		0.00
SD4748	7.26	0.93	1.32
SD4752	6.78	2.08	2.95
SD4771	4.51	0.30	0.42
SD4772	4.49		0.00
SD4773	4.24	0.15	0.21
SD4775	3.73	0.48	0.67
SD4792	3.42		0.00
SD4816	5.39		0.00
SD4840	5.17		0.00
SD4842	5.00		0.00
SD4843	4.70		0.00
SD4844	4.09		0.00
SD4848	5.66		0.00
SD4849	6.39		0.00
SD4852	4.88		0.00
SD4854	3.57		0.00
SD4855	5.21		0.00
SD4870	4.43		0.00
SD4871	5.17		0.00
SD4873	3.73		0.00
SD4874	4.64		0.00
SD4876	3.96		0.00
SELECT	5.47	0.74	1.82
STEELE-ND	4.92	1.14	2.77
SURPASS	8.02	1.79	4.69
SY-VALDA	4.41		0.00
TRAVERSE	2.96	0.52	1.10

The mean, standard deviation, and range of MPT for all varieties.

Table 9: Summary statistics for the MPV measurement by genotype

	Midline Peak Value		
	Mean	StDev	Range
ADVANCE	47.51	2.44	6.40
BOOST	51.58	2.97	7.33
BRICK	49.13	2.14	5.65
BRIGGS	48.78	1.94	5.07
FALLER	47.77	1.57	3.91
FOCUS	49.97	0.65	1.57
FOREFRONT	49.36	1.12	2.71
LCS-TRIGGER	47.97	2.36	4.52
OXEN	51.41	1.25	3.27
PREVAIL	48.69	1.37	3.49
SD4393	50.33	0.61	0.87
SD4403	50.01	0.07	0.10
SD4416	47.90	0.18	0.25
SD4465	48.51	1.27	2.53
SD4472	49.02	0.62	0.88
SD4492	48.73	0.57	0.81
SD4493	48.72	2.58	3.65
SD4514	51.00	0.73	1.03
SD4529	54.60	3.14	5.52
SD4539	54.32	1.09	2.34
SD4543	53.77	1.17	1.66
SD4546	50.48	0.81	1.54
SD4557	45.56	0.93	1.31
SD4575	49.68	0.66	0.93
SD4579	46.71	2.80	5.59
SD4582	49.96	0.38	0.54
SD4587	51.14	0.45	0.63
SD4595	53.54	1.34	2.56
SD4624	48.78		0.00
SD4625	46.54	2.03	4.87
SD4650	53.40		0.00
SD4676	49.61		0.00
SD4681	52.52		0.00
SD4689	46.71		0.00
SD4692	48.75		0.00
SD4693	48.45		0.00
SD4702	53.17		0.00
SD4703	46.00		0.00
SD4706	50.96	0.78	1.11
SD4707	50.94	1.44	2.04
SD4708	47.76	1.21	1.71
SD4711	54.81	1.20	1.70
SD4719	49.97	1.87	2.65
SD4720	56.59	1.54	2.18
SD4721	57.18	2.50	3.54
SD4729	49.79		0.00
SD4732	47.82		0.00
SD4735	49.57		0.00
SD4738	48.41		0.00
SD4740	46.83	0.21	0.30
SD4742	50.68		0.00
SD4744	51.62		0.00
SD4745	60.83	7.66	10.83
SD4746	53.76	6.15	8.69
SD4747	47.69		0.00
SD4748	50.03	3.09	4.37
SD4752	51.00	4.56	6.45
SD4771	50.05	2.65	3.75
SD4772	50.41		0.00
SD4773	46.17	3.26	4.61
SD4775	52.32	0.48	0.69
SD4792	62.77		0.00
SD4816	46.34		0.00
SD4840	48.12		0.00
SD4842	52.30		0.00
SD4843	53.07		0.00
SD4844	55.78		0.00
SD4848	59.40		0.00
SD4849	51.05		0.00
SD4852	58.89		0.00
SD4854	54.19		0.00
SD4855	47.29		0.00
SD4870	52.99		0.00
SD4871	43.78		0.00
SD4873	56.11		0.00
SD4874	49.88		0.00
SD4876	48.36		0.00
SELECT	48.89	2.18	4.94
STEELE-ND	52.73	3.49	7.34
SURPASS	47.77	0.18	0.46
SY-VALDA	47.38		0.00
TRAVERSE	48.52	1.62	4.19

The mean, standard deviation, and range of MPV for all varieties

Table 10: Summary statistics for the MPW measurement by genotype

	Midline Peak Width		
	Mean	StDev	Range
ADVANCE	19.41	2.81	7.29
BOOST	23.52	4.27	10.68
BRICK	21.57	3.76	8.28
BRIGGS	19.58	4.67	10.71
FALLER	19.73	4.32	9.34
FOCUS	22.36	4.72	12.46
FOREFRONT	20.19	4.34	9.39
LCS-TRIGGER	23.70	0.48	0.86
OXEN	22.45	2.64	6.79
PREVAIL	20.98	3.49	8.07
SD4393	18.92	0.55	0.78
SD4403	20.52	2.27	3.20
SD4416	16.12	0.61	0.86
SD4465	19.05	2.26	4.04
SD4472	18.23	1.10	1.56
SD4492	19.85	0.02	0.03
SD4493	14.80	2.23	3.15
SD4514	20.10	0.17	0.25
SD4529	21.82	1.01	1.93
SD4539	23.80	1.84	4.36
SD4543	22.93	0.32	0.45
SD4546	22.05	3.82	7.53
SD4557	14.92	0.43	0.61
SD4575	17.58	1.30	1.84
SD4579	17.54	0.58	1.05
SD4582	17.96	0.29	0.41
SD4587	21.08	2.73	3.86
SD4595	24.29	1.41	2.79
SD4624	17.96		0.00
SD4625	20.59	3.35	8.20
SD4650	24.92		0.00
SD4676	22.47		0.00
SD4681	26.14		0.00
SD4689	23.82		0.00
SD4692	20.88		0.00
SD4693	22.49		0.00
SD4702	24.73		0.00
SD4703	20.56		0.00
SD4706	25.74	1.69	2.39
SD4707	25.05	0.92	1.31
SD4708	23.32	0.10	0.14
SD4711	26.59	0.73	1.03
SD4719	25.64	2.91	4.12
SD4720	26.23	3.43	4.85
SD4721	27.45	1.14	1.61
SD4729	22.46		0.00
SD4732	21.83		0.00
SD4735	23.11		0.00
SD4738	23.22		0.00
SD4740	22.84	0.97	1.38
SD4742	24.91		0.00
SD4744	22.94		0.00
SD4745	29.00	6.39	9.04
SD4746	26.62	5.16	7.30
SD4747	19.10		0.00
SD4748	24.85	3.91	5.53
SD4752	24.95	5.09	7.20
SD4771	25.83	2.76	3.91
SD4772	27.83		0.00
SD4773	22.52	3.06	4.33
SD4775	27.67	0.10	0.15
SD4792	32.96		0.00
SD4816	22.28		0.00
SD4840	24.57		0.00
SD4842	27.66		0.00
SD4843	25.82		0.00
SD4844	28.92		0.00
SD4848	32.27		0.00
SD4849	28.49		0.00
SD4852	33.40		0.00
SD4854	28.72		0.00
SD4855	25.10		0.00
SD4870	27.71		0.00
SD4871	24.32		0.00
SD4873	28.02		0.00
SD4874	27.51		0.00
SD4876	26.86		0.00
SELECT	21.72	4.04	10.21
STEELE-ND	23.40	4.98	10.40
SURPASS	20.67	3.43	7.68
SY-VALDA	22.80		0.00
TRAVERSE	19.22	4.19	9.90

The mean, standard deviation, and range of MPW for all varieties

Table 11: Summary statistics for the MRV measurement by genotype

	Midline Right Value		
	Mean	StDev	Range
ADVANCE	45.55	2.42	5.38
BOOST	49.35	1.83	4.27
BRICK	47.65	1.83	4.51
BRIGGS	45.45	1.38	3.49
FALLER	45.51	0.59	1.44
FOCUS	47.42	0.88	2.31
FOREFRONT	46.53	0.68	1.76
LCS-TRIGGER	45.20	2.57	4.77
OXEN	49.15	1.22	2.95
PREVAIL	46.04	1.60	4.39
SD4393	48.52	0.40	0.56
SD4403	48.23	0.14	0.20
SD4416	46.11	0.17	0.24
SD4465	46.41	1.74	3.28
SD4472	46.87	0.66	0.94
SD4492	47.64	0.31	0.44
SD4493	46.73	2.23	3.16
SD4514	49.45	0.57	0.81
SD4529	51.41	3.17	6.11
SD4539	51.62	1.25	2.60
SD4543	52.11	0.76	1.08
SD4546	49.08	1.21	2.39
SD4557	44.66	0.77	1.08
SD4575	47.89	0.68	0.97
SD4579	44.87	3.19	6.37
SD4582	48.10	0.10	0.15
SD4587	49.05	0.19	0.27
SD4595	51.63	1.58	2.77
SD4624	47.07		0.00
SD4625	43.84	2.23	4.91
SD4650	51.00		0.00
SD4676	46.65		0.00
SD4681	49.56		0.00
SD4689	45.09		0.00
SD4692	46.21		0.00
SD4693	46.34		0.00
SD4702	50.17		0.00
SD4703	44.24		0.00
SD4706	48.25	0.41	0.57
SD4707	47.86	0.21	0.30
SD4708	45.99	0.82	1.15
SD4711	50.92	0.36	0.51
SD4719	47.97	1.16	1.64
SD4720	51.65	0.63	0.90
SD4721	53.18	1.70	2.40
SD4729	47.93		0.00
SD4732	44.98		0.00
SD4735	47.33		0.00
SD4738	46.91		0.00
SD4740	44.98	0.19	0.27
SD4742	49.23		0.00
SD4744	48.28		0.00
SD4745	55.43	6.17	8.72
SD4746	51.09	5.95	8.42
SD4747	44.81		0.00
SD4748	48.12	3.12	4.41
SD4752	48.05	3.11	4.41
SD4771	45.84	1.85	2.62
SD4772	47.13		0.00
SD4773	42.53	2.42	3.43
SD4775	47.91	0.64	0.91
SD4792	55.84		0.00
SD4816	43.97		0.00
SD4840	46.27		0.00
SD4842	49.12		0.00
SD4843	48.55		0.00
SD4844	50.85		0.00
SD4848	56.43		0.00
SD4849	49.17		0.00
SD4852	55.52		0.00
SD4854	51.55		0.00
SD4855	45.23		0.00
SD4870	49.87		0.00
SD4871	42.71		0.00
SD4873	52.57		0.00
SD4874	47.61		0.00
SD4876	44.55		0.00
SELECT	46.99	2.11	5.10
STEELE-ND	50.17	2.20	4.83
SURPASS	46.12	0.62	1.45
SY-VALDA	44.37		0.00
TRAVERSE	43.54	1.86	4.57

The mean, standard deviation, and range of MRV for all varieties.

Table 12: Summary statistics for the INTEG measurement by genotype

	Integral		
	Mean	StDev	Range
ADVANCE	213.57	34.71	92.44
BOOST	200.89	41.36	112.16
BRICK	249.71	38.67	94.52
BRIGGS	135.25	29.94	79.69
FALLER	191.35	46.93	119.87
FOCUS	196.90	35.70	83.09
FOREFRONT	193.44	32.17	82.24
LCS-TRIGGER	155.16	8.79	15.69
OXEN	207.12	35.77	91.16
PREVAIL	167.07	33.22	79.71
SD4393	249.81	49.23	69.62
SD4403	188.25	18.54	26.21
SD4416	228.26	21.08	29.82
SD4465	194.59	21.69	43.36
SD4472	199.59	9.95	14.08
SD4492	261.10	2.10	2.96
SD4493	182.76	26.28	37.17
SD4514	271.78	16.23	22.96
SD4529	178.38	16.36	31.80
SD4539	232.56	43.67	103.32
SD4543	240.76	29.16	41.24
SD4546	258.29	29.13	58.06
SD4557	269.60	28.74	40.65
SD4575	206.87	4.45	6.30
SD4579	170.96	15.61	29.20
SD4582	196.57	22.57	31.92
SD4587	196.82	5.75	8.13
SD4595	225.50	22.91	45.78
SD4624	229.90		0.00
SD4625	192.19	50.89	101.80
SD4650	228.05		0.00
SD4676	192.78		0.00
SD4681	197.55		0.00
SD4689	265.50		0.00
SD4692	229.38		0.00
SD4693	229.11		0.00
SD4702	211.96		0.00
SD4703	226.24		0.00
SD4706	211.06	32.73	46.28
SD4707	183.98	34.83	49.26
SD4708	246.25	40.72	57.58
SD4711	194.43	27.40	38.75
SD4719	210.48	24.17	34.18
SD4720	159.63	20.60	29.14
SD4721	160.61	22.13	31.30
SD4729	238.54		0.00
SD4732	182.83		0.00
SD4735	310.22		0.00
SD4738	294.33		0.00
SD4740	227.62	15.60	22.06
SD4742	225.69		0.00
SD4744	211.33		0.00
SD4745	230.43	30.79	43.54
SD4746	251.15	24.80	35.07
SD4747	199.03		0.00
SD4748	235.36	13.23	18.71
SD4752	219.76	44.11	62.38
SD4771	148.54	4.58	6.48
SD4772	160.99		0.00
SD4773	133.15	1.59	2.26
SD4775	133.05	9.94	14.06
SD4792	140.48		0.00
SD4816	179.52		0.00
SD4840	182.95		0.00
SD4842	178.27		0.00
SD4843	163.99		0.00
SD4844	154.12		0.00
SD4848	233.04		0.00
SD4849	230.91		0.00
SD4852	197.52		0.00
SD4854	150.40		0.00
SD4855	175.34		0.00
SD4870	165.47		0.00
SD4871	176.42		0.00
SD4873	151.64		0.00
SD4874	179.42		0.00
SD4876	139.12		0.00
SELECT	197.36	33.70	80.53
STEELE-ND	190.95	43.29	104.32
SURPASS	265.66	67.40	173.81
SY-VALDA	151.83		0.00
TRAVERSE	101.68	18.73	43.65

The mean, standard deviation, and range of INTEG for all varieties

Table 13: Summary statistics for the GI measurement by genotype

	Gluten Index		
	Mean	StDev	Range
ADVANCE	97.01	0.89	2.30
BOOST	93.26	2.65	5.27
BRICK	96.51	1.63	4.22
BRIGGS	81.23	3.99	10.23
FALLER	94.25	3.21	8.18
FOCUS	90.25	2.98	6.46
FOREFRONT	93.97	2.13	5.85
LCS-TRIGGER	88.36	5.25	10.43
OXEN	96.39	1.41	3.31
PREVAIL	88.61	5.79	15.90
SD4393	97.58	1.12	1.58
SD4403	90.05	2.27	3.21
SD4416	96.12	0.51	0.73
SD4465	94.47	2.07	4.04
SD4472	94.43	3.45	4.87
SD4492	97.51	0.77	1.09
SD4493	90.72	4.63	6.55
SD4514	96.81	1.53	2.16
SD4529	91.99	2.03	3.93
SD4539	97.39	1.64	3.70
SD4543	96.82	1.11	1.58
SD4546	97.70	0.89	1.73
SD4557	95.87	1.41	1.99
SD4575	89.13	2.10	2.97
SD4579	86.37	6.47	12.20
SD4582	89.01	3.01	4.26
SD4587	91.28	2.98	4.21
SD4595	96.10	2.12	3.85
SD4624	91.28		0.00
SD4625	86.62	5.97	10.81
SD4650	98.34		0.00
SD4676	91.07		0.00
SD4681	96.22		0.00
SD4689	94.91		0.00
SD4692	96.43		0.00
SD4693	93.67		0.00
SD4702	97.01		0.00
SD4703	97.61		0.00
SD4706	96.93	0.93	1.31
SD4707	88.76	4.38	6.19
SD4708	96.30	0.08	0.12
SD4711	91.76	3.90	5.52
SD4719	96.51	1.67	2.36
SD4720	87.86	1.00	1.42
SD4721	91.68	3.84	5.43
SD4729	98.17		0.00
SD4732	89.03		0.00
SD4735	98.02		0.00
SD4738	96.53		0.00
SD4740	93.15	3.23	4.57
SD4742	97.71		0.00
SD4744	94.60		0.00
SD4745	98.06	0.20	0.28
SD4746	98.11	0.41	0.58
SD4747	92.48		0.00
SD4748	97.60	0.48	0.68
SD4752	94.92	1.52	2.15
SD4771	87.36	4.66	6.59
SD4772	82.41		0.00
SD4773	82.56	0.67	0.95
SD4775	87.55	10.08	14.25
SD4792	90.04		0.00
SD4816	88.04		0.00
SD4840	95.29		0.00
SD4842	86.22		0.00
SD4843	94.10		0.00
SD4844	85.83		0.00
SD4848	95.72		0.00
SD4849	97.98		0.00
SD4852	94.97		0.00
SD4854	85.09		0.00
SD4855	86.30		0.00
SD4870	91.38		0.00
SD4871	88.10		0.00
SD4873	91.06		0.00
SD4874	89.13		0.00
SD4876	80.78		0.00
SELECT	93.81	3.50	8.15
STEELE-ND	90.03	2.38	5.41
SURPASS	97.06	1.59	3.63
SY-VALDA	84.31		0.00
TRAVERSE	68.86	10.52	21.91

The mean, standard deviation, and range of GI for all varieties

Table 14: Summary statistics for the GOODXGI measurement by genotype

	Good X GI		
	Mean	StDev	Range
ADVANCE	332.45	36.47	83.21
BOOST	346.96	17.62	46.22
BRICK	352.65	26.33	56.81
BRIGGS	274.62	13.45	30.66
FALLER	331.27	22.34	57.51
FOCUS	326.05	22.21	60.16
FOREFRONT	336.56	16.48	37.65
LCS-TRIGGER	281.56	37.85	74.97
OXEN	347.56	26.92	67.01
PREVAIL	299.29	32.53	84.79
SD4393	335.37	1.93	2.73
SD4403	314.69	19.43	27.48
SD4416	331.85	21.31	30.14
SD4465	314.65	6.21	11.84
SD4472	323.49	24.67	34.88
SD4492	334.79	2.55	3.61
SD4493	288.77	35.19	49.77
SD4514	344.91	3.84	5.42
SD4529	323.56	11.17	20.93
SD4539	350.30	14.16	34.05
SD4543	346.13	0.24	0.34
SD4546	335.34	5.20	10.40
SD4557	286.40	19.14	27.07
SD4575	289.78	0.99	1.40
SD4579	294.38	43.70	83.52
SD4582	300.51	17.84	25.23
SD4587	322.78	28.50	40.30
SD4595	345.04	10.35	19.25
SD4624	314.04		0.00
SD4625	306.40	21.45	48.15
SD4650	346.81		0.00
SD4676	309.55		0.00
SD4681	352.85		0.00
SD4689	321.02		0.00
SD4692	337.24		0.00
SD4693	316.40		0.00
SD4702	339.89		0.00
SD4703	303.11		0.00
SD4706	352.61	36.96	52.27
SD4707	317.36	26.02	36.80
SD4708	360.86	42.64	60.31
SD4711	339.17	75.30	106.50
SD4719	349.71	30.31	42.86
SD4720	351.02	43.69	61.78
SD4721	360.56	24.62	34.82
SD4729	317.75		0.00
SD4732	316.95		0.00
SD4735	339.71		0.00
SD4738	346.21		0.00
SD4740	340.81	27.46	38.83
SD4742	329.35		0.00
SD4744	337.04		0.00
SD4745	409.12	58.68	82.99
SD4746	380.78	51.84	73.31
SD4747	317.10		0.00
SD4748	370.71	46.03	65.10
SD4752	369.15	34.06	48.16
SD4771	339.37	39.98	56.54
SD4772	293.71		0.00
SD4773	296.90	18.49	26.15
SD4775	327.89	72.19	102.09
SD4792	378.91		0.00
SD4816	325.85		0.00
SD4840	355.55		0.00
SD4842	314.98		0.00
SD4843	351.56		0.00
SD4844	318.29		0.00
SD4848	398.52		0.00
SD4849	378.94		0.00
SD4852	379.19		0.00
SD4854	331.92		0.00
SD4855	310.24		0.00
SD4870	358.60		0.00
SD4871	310.60		0.00
SD4873	374.87		0.00
SD4874	338.09		0.00
SD4876	277.70		0.00
SELECT	334.63	28.48	69.50
STEELE-ND	326.11	14.07	32.53
SURPASS	348.39	30.29	73.55
SY-VALDA	290.53		0.00
TRAVERSE	199.39	39.38	93.74

The mean, standard deviation, and range of GOODXGI for all varieties

Table 15: Model AIC values by genotype year average and genotype average

	AIC: Genotype Year Average	AIC: Genotype Averages
Model 1	2401.77	1083.07
Model 2	2390.07	1082.14
Model 3	2406.9	1074.4

AIC: Akaike information criterion

Table 16: VIF values for models 1, 2, and 3.

	Parameter	VIF
Model 1	MPT	1.80
	MPV	16.10
	MPW	1.52
	MRV	13.76
Model 2	MPT	2.29
	MPV	19.17
	MPW	1.52
	MRV	17.33
	GI	2.23
Model 3	INTEG	14.70
	MPT	12.84
	GI	1.79

VIF: Variance inflation factor, MPT: Midline peak time, MPV: Midline peak value, MPW: Midline peak width, MRV: Midline right value, GI: Gluten index, INTEG: midline integral.

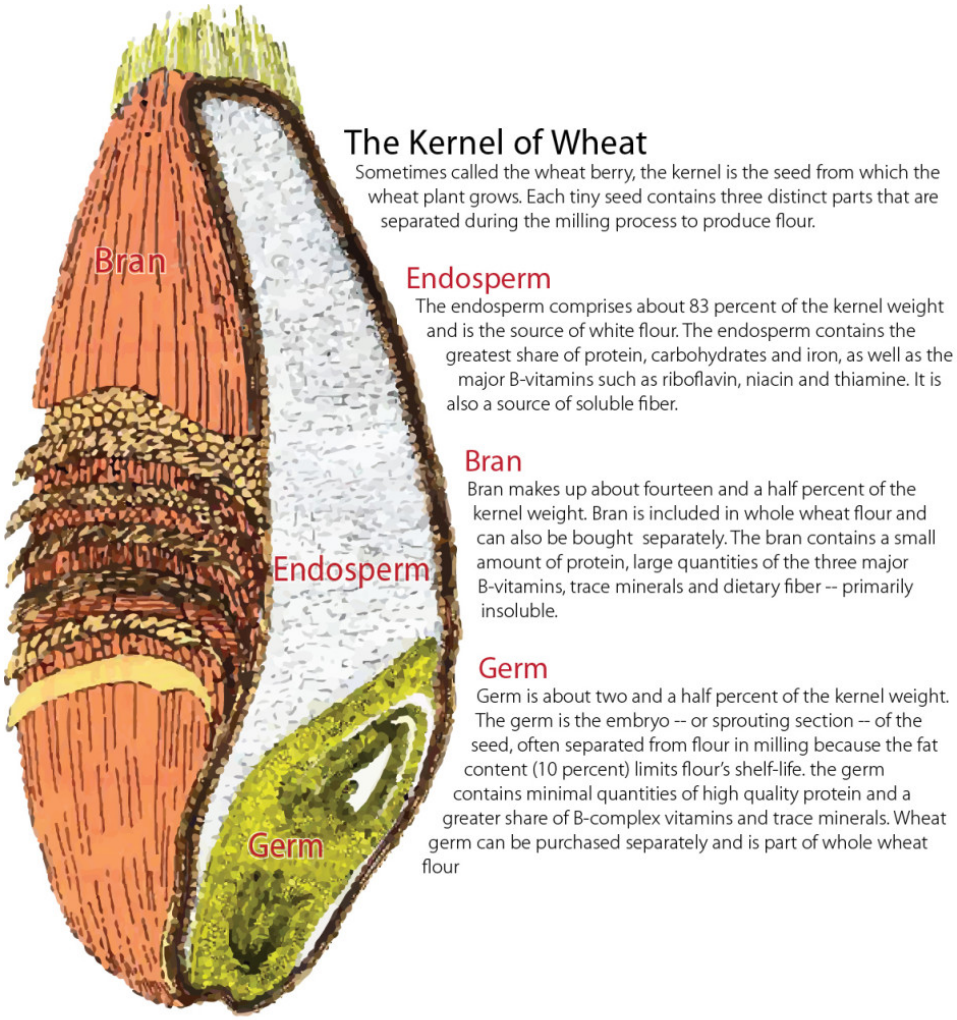

Figure 1: The anatomy of a wheat kernel: including bran, endosperm, and germ (Flour.Com, n.d.).

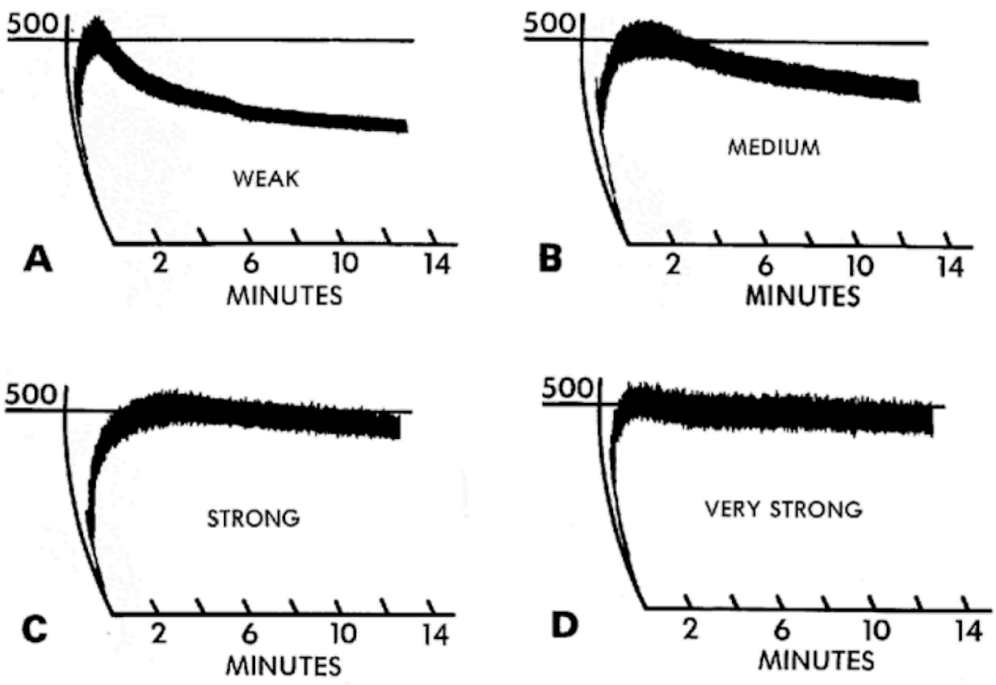

Figure 2: (*AACC Approved Methods of Analysis, 11th Ed. Method 54-21.02. Rheological Behavior of Flour by Farinograph: Constant Flour Weight Procedure. Cereals & Grains Association, St. Paul, MN, U.S.A.*, 2011). A: weak flour with an absorption of 54%, DDT of 1.25min, MTI of 180.; B: Medium strength flour with an absorption of 57%, DDT 2.75, MTI of 80; C: Strong flour with an absorption of 64.5%, DDT 5min, MTI of 30; D: Very strong flour, absorption 62.7%, DDT 1.75min, MTI 20.

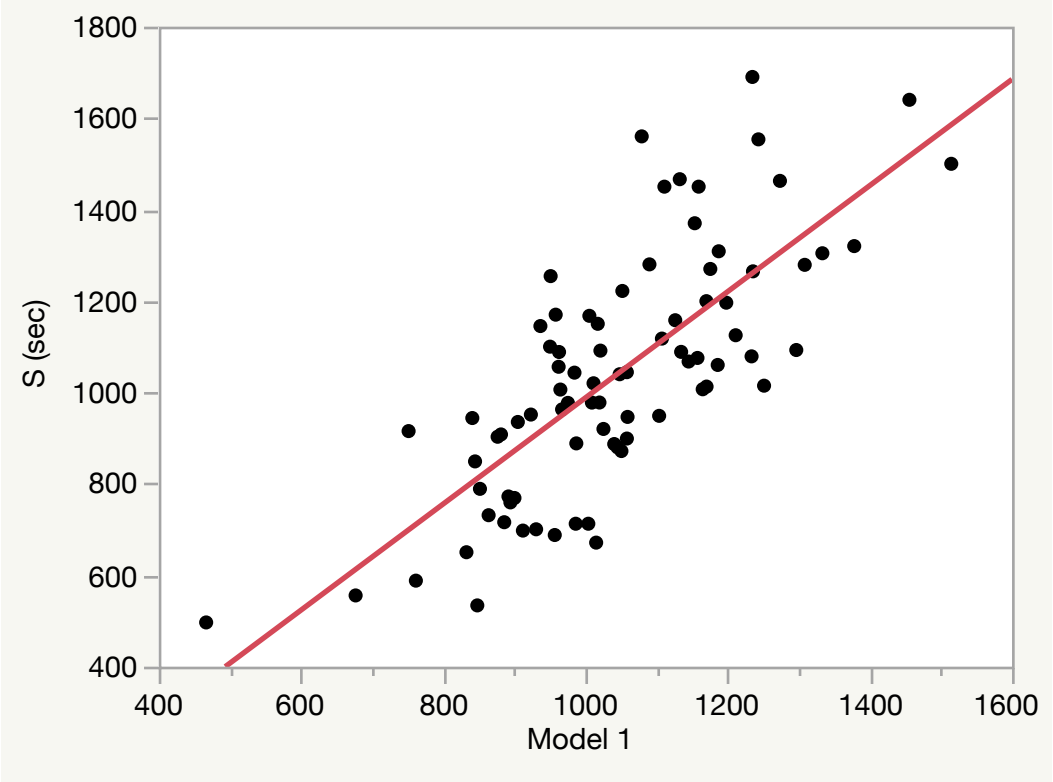

Figure 3: Model 1 Regression for averages over all location-years available for each genotype (points represent 3, 6, 9, 12, or 15 location-years, n = 82, R^2= 0.57).

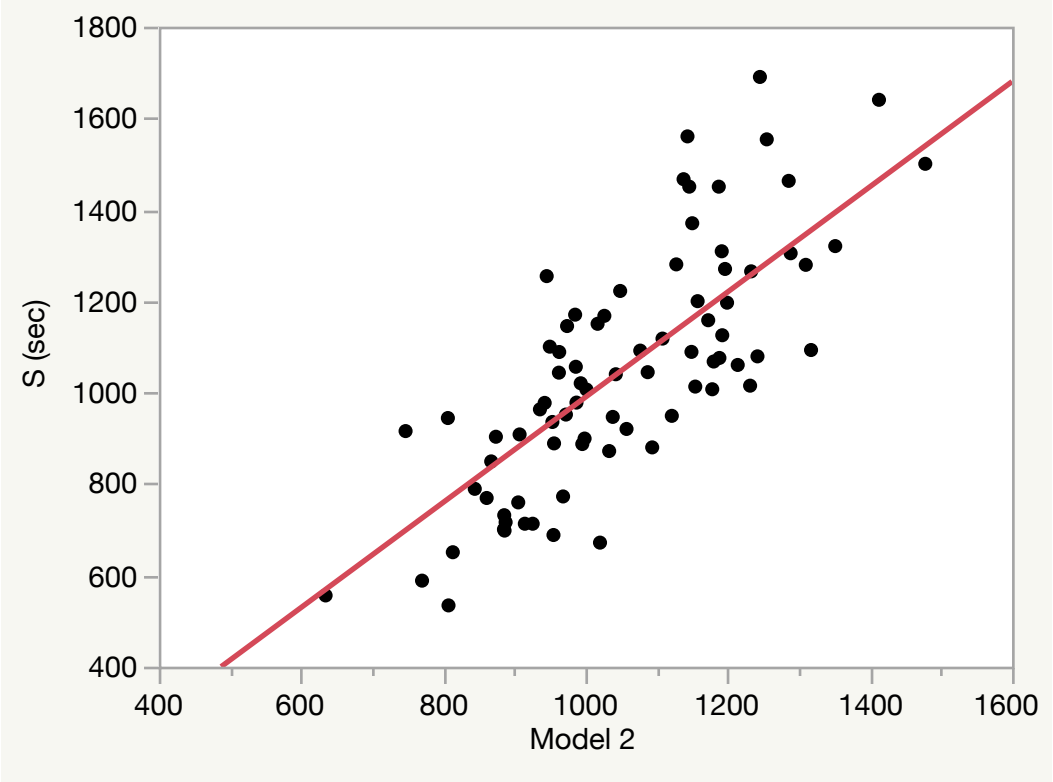

Figure 4: Model 2 Regression for averages over all location-years available for each genotype (points represent 3, 6, 9, 12, or 15 location-years, n = 82, R^2= 0.61).

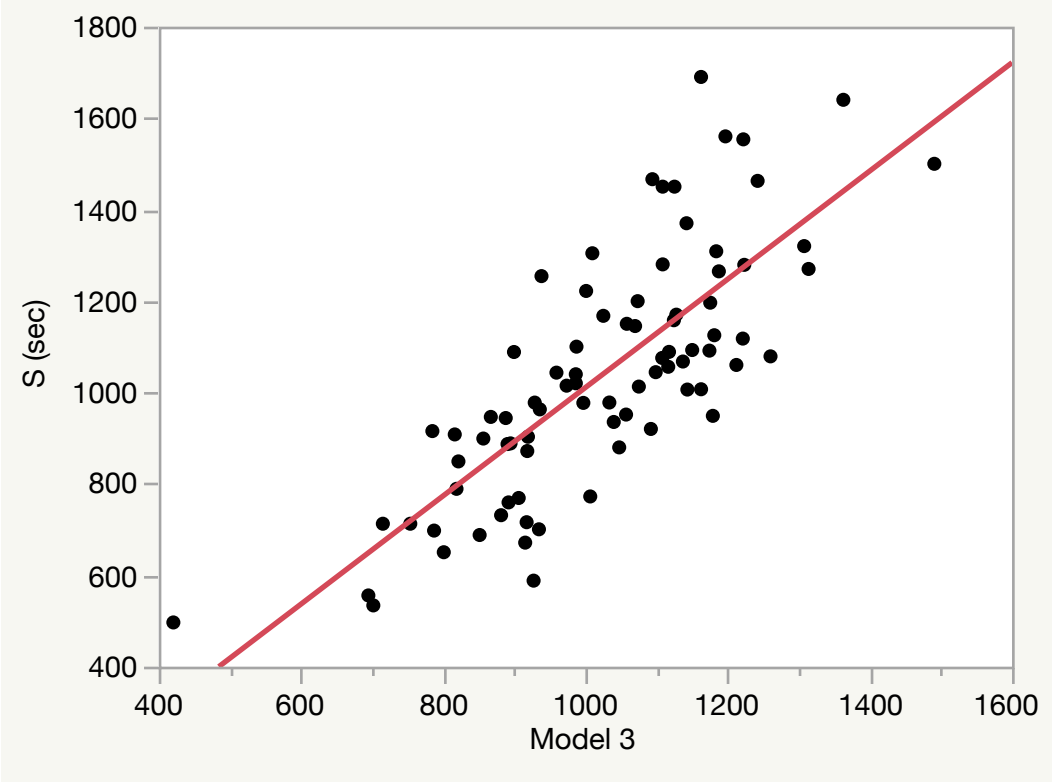

Figure 5: Model 3 Regression for averages over all location-years available for each genotype (points represent 3, 6, 9, 12, or 15 location-years, n = 82, R^2= 0.61).

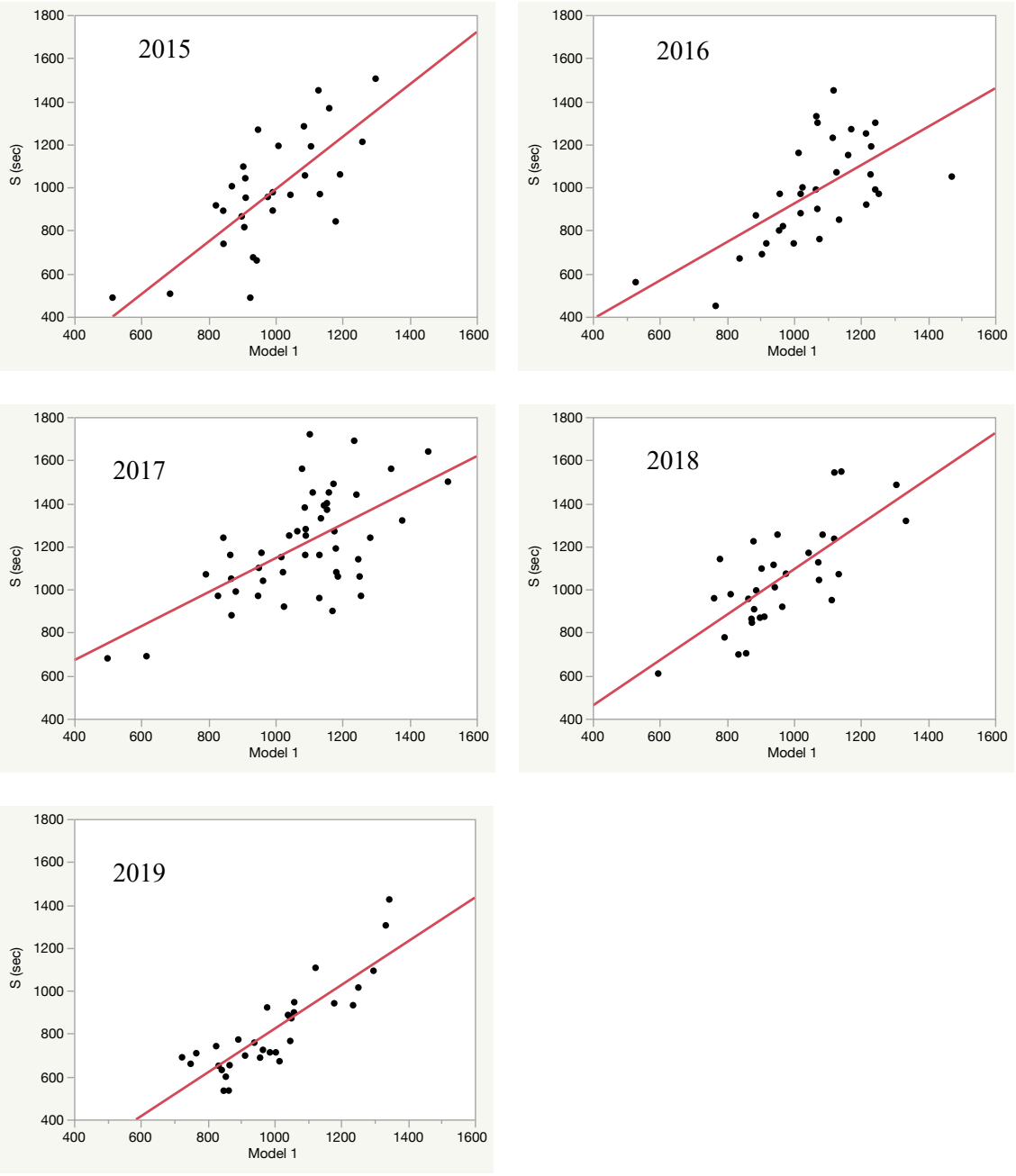

Figure 6: Model 1 Regression for yearly genotype averages from 2015-2019 (2015 n= 31, R^2= 0.55; 2016 n=33, R^2= 0.42; 2017 n=48, R^2= 0.40; 2018 n=33, R^2= 0.63; 2019 n=33, R^2= 0.78).

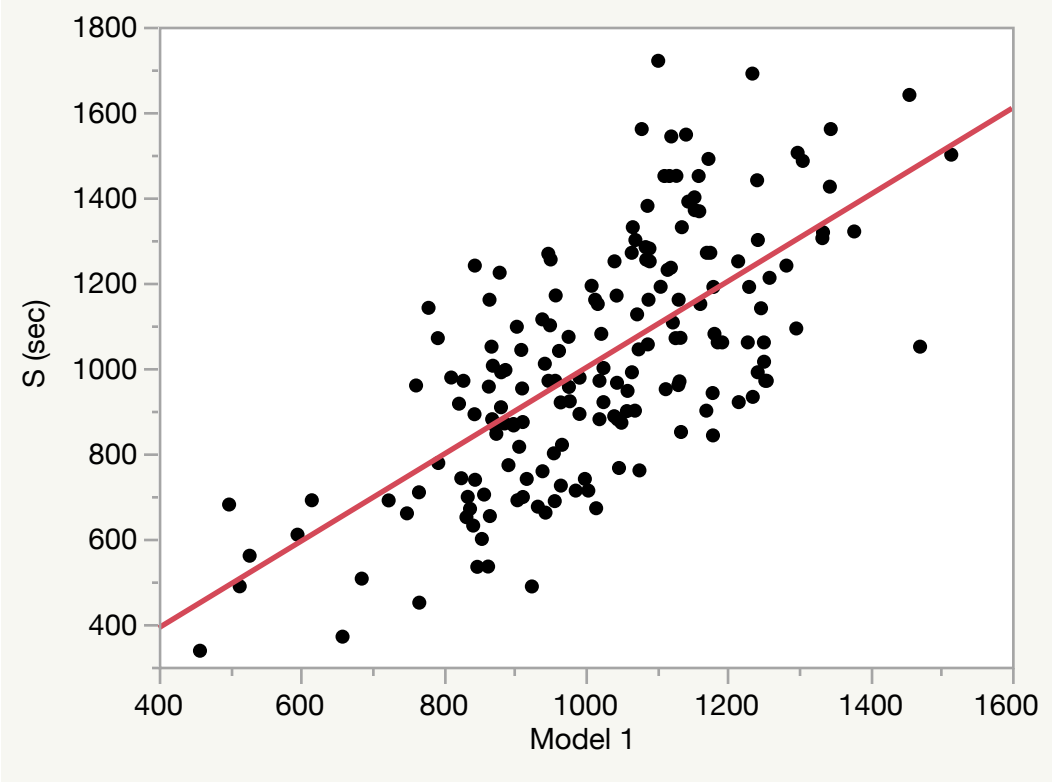

Figure 7: Model 1 Regression for yearly genotype averages over years (points represent 3 location-years, n = 178, R^2= 0.48).

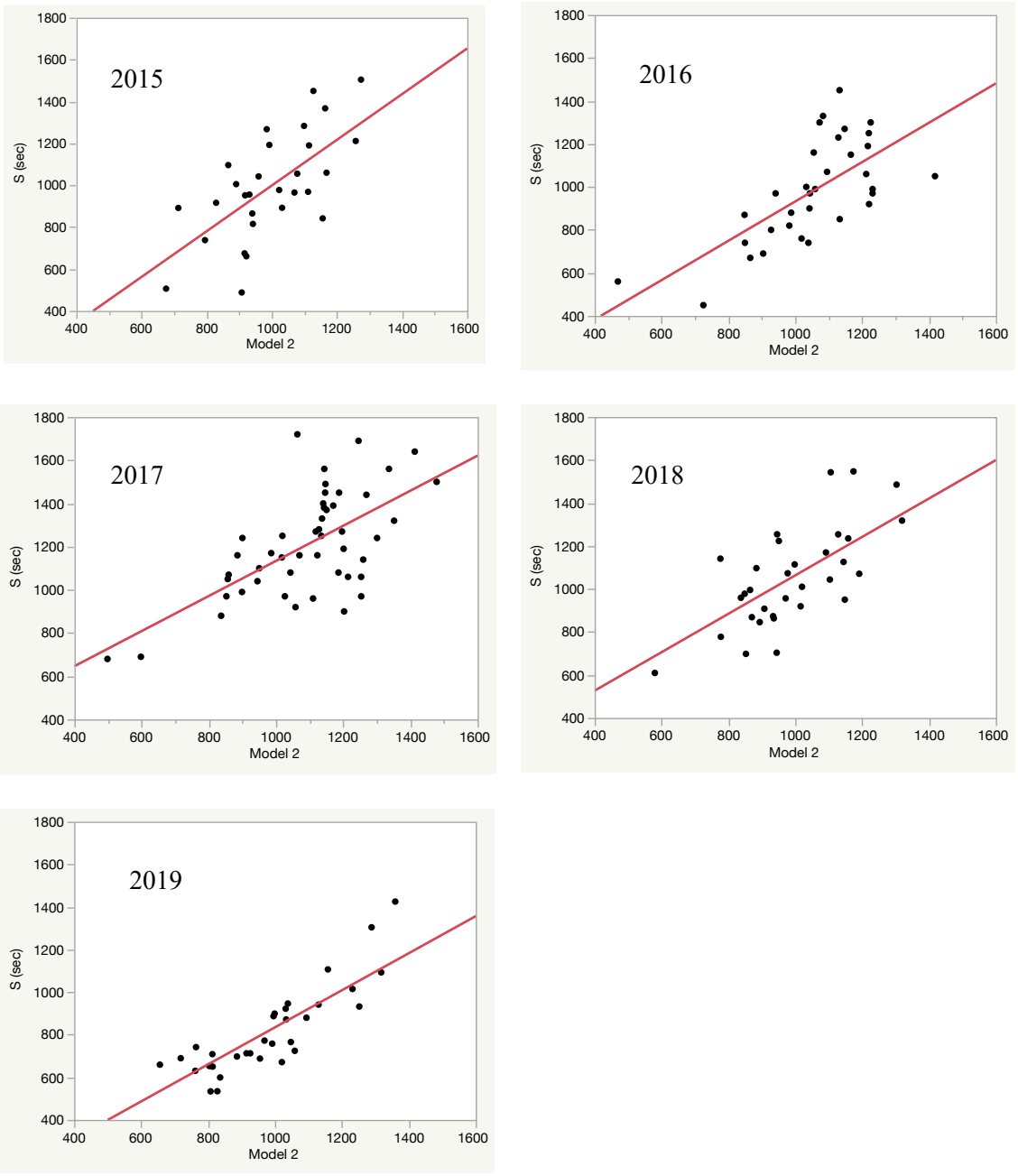

Figure 8: M Model 2 Regression for yearly genotype averages from 2015-2019 (2015 n= 31, R^2= 0.56; 2016 n=33, R^2= 0.47; 2017 n=48, R^2= 0.41; 2018 n=33, R^2= 0.57; 2019 n=33, R^2= 0.75).

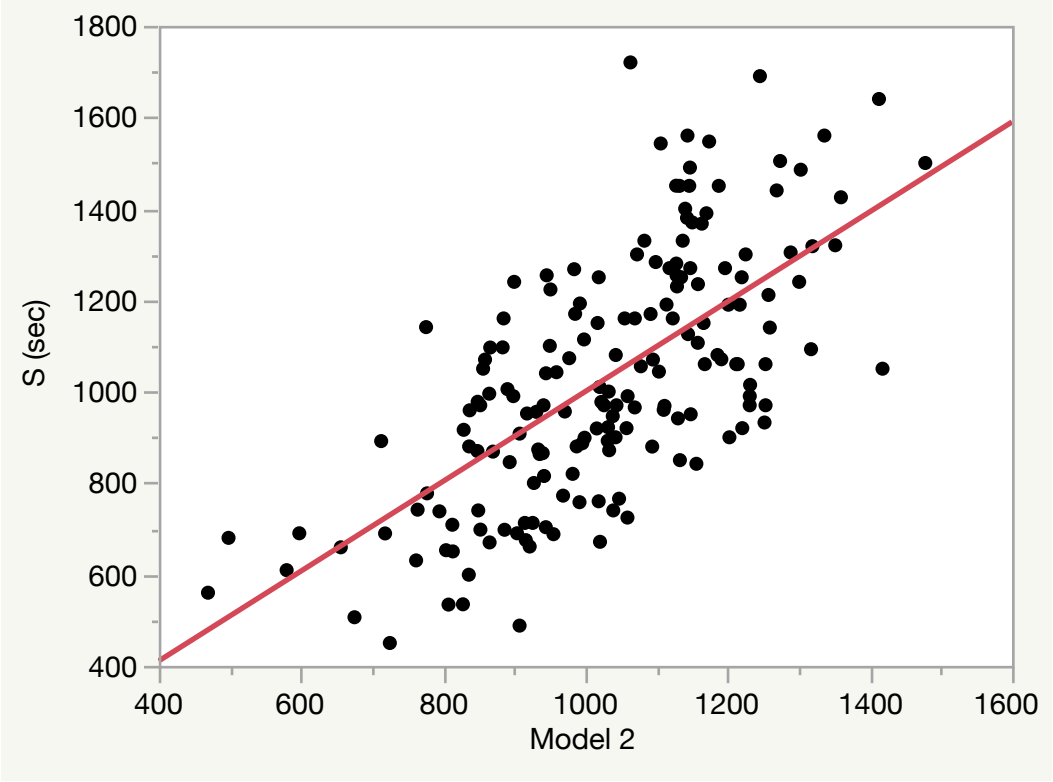

Figure 9: Model 2 Regression for yearly genotype averages over years (points represent 3 location-years, n = 178, R^2= 0.51).

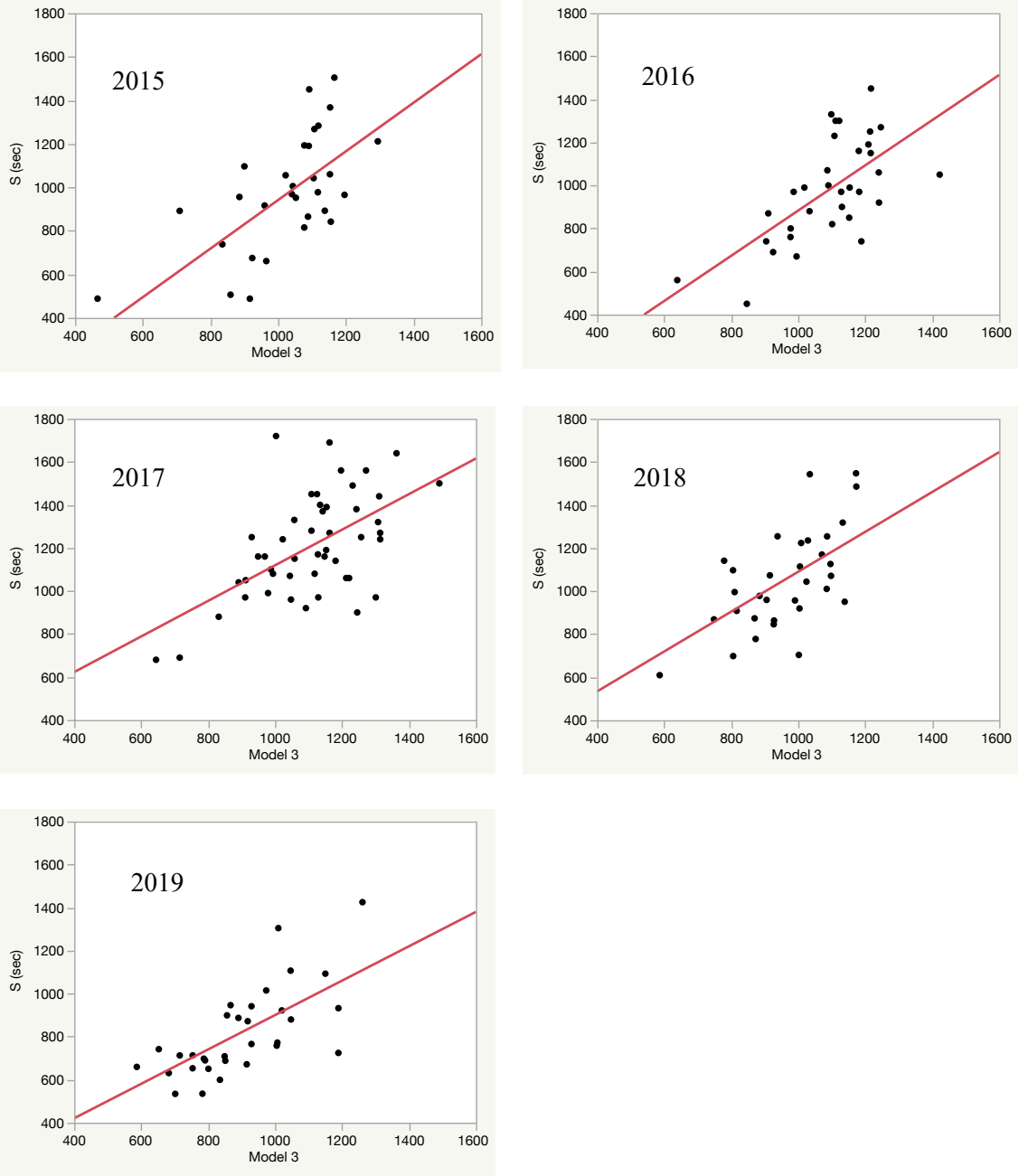

Figure 10: Model 3 Regression for yearly genotype averages from 2015-2019 (2015 n= 31, R^2= 0.46; 2016 n=33, R^2= 0.42; 2017 n=48, R^2= 0.32; 2018 n=33, R^2= 0.51; 2019 n=33, R^2= 0.55).

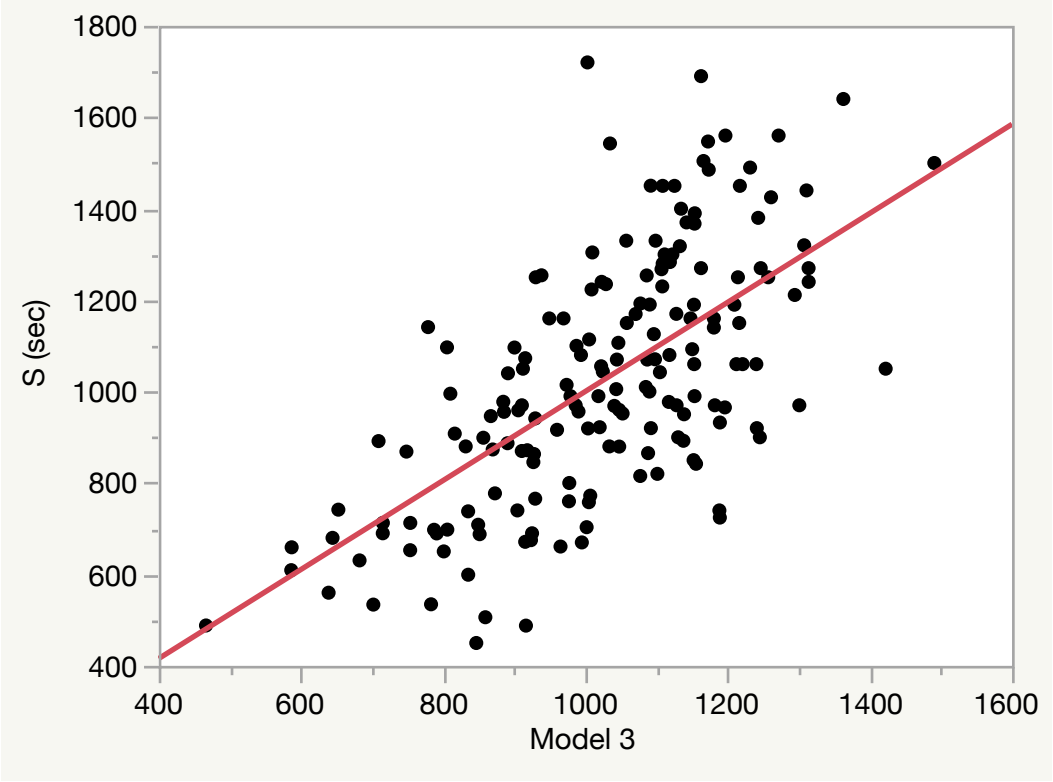

Figure 11: Model 3 Regression for yearly genotype averages over years (points represent 3 location-years, n = 178, R^2= 0.46).