Spring 4-20-2015

Producing Quality Barley for the Malting Industry

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PRODUCING QUALITY BARLEY FOR THE MALTING INDUSTRY

by

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A Doctoral Document

Presented to the Faculty of

The College of Agricultural Sciences and Natural Resources

In Partial Fulfillment of Requirements

For the Degree of Doctor of Plant Health

Major: Doctor of Plant Health

Under the Supervision of Professor Gary L. Hein

Lincoln, Nebraska

April 2015

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University of Nebraska, 2015

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The University of Nebraska – Lincoln Doctor of Plant Health program requires each student to fulfill a professional internship over the last summer of the program. For my internship, I worked as a Barley Scientist Intern for MillerCoors and Golden Malting in Golden, Colorado. During this internship I gained a fundamental understanding of malting barley production with a significant emphasis on grain quality. Barley produced for malting must fulfill strict industry quality standards before it is accepted by the head maltsters. These quality standards include: a high germination rate, low moisture content, protein content within an acceptable range and the grain must be free from disease and insect damage. Malting barley quality attributes are directly correlated with the conditions in which the barley was produced.

This paper provides a brief history of barley domestication and describes why barley became the grain of choice for malting and the subsequent making of beer. The three main steps of malting (steeping, germination, and kilning) are outlined to emphasize the role quality plays in the malting process. A description of farming practices that contribute to these quality attributes is presented, along with recommendations that may enhance crop production. This document has a special emphasis on the management of the Russian Wheat Aphid (*Diurpahis noxia* Mordvilko) as well as the fungal diseases Fusarium Head Blight (*Fusarium graminearum* Schwabe) and Ergot (*Claviceps purpurea*

(Fr.) Tul.). This pest and these pathogens can, under ideal environmental conditions, compromise crop yield and diminish grain quality.

ACKNOWLEDGEMENTS

I would like to express my appreciation for the people in my life that have continually encouraged and supported me throughout my Doctor of Plant Health (DPH) program. I am grateful for the patience, understanding, and constructive nature of my advisor, Dr. Gary L. Hein. My committee members, Dr. Robert Wright, Dr. Gerard Adams, Dr. Tom Hoegemeyer, and Dr. Roger Elmore, were instrumental in guiding me through this process. Dr. Loren Giesler, provided me a number of different research experiences that strengthened my ability as a researcher and reinforced my skills in plant pathology.

I want to especially mention Dr. Bill Baxendale and Betsy. Without these people I would have never discovered my potential and may have never sought higher education. I am extremely grateful to my parents, Mark and Susie Oser, for their continued encouragement, patience, and support during my education. I also thank my brother, Travis Oser, who has held down the fort this whole time.

I extend my appreciation to Doctor Plant Health faculty and staff as well as my fellow graduate students. I thank you all for everything. I wouldn't have been able to do it without you!

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CHAPTER 1

BARLEY: THE GRAIN OF CHOICE FOR MALT

Barley (*Hordeum vulgare* L.) belongs to the grass family (*Poaceae*) and is a very important global food and economic cereal grain crop. Between 2010 and 2013 barley ranked fifth worldwide in crop production, averaging over 134 million tons per year, following maize (*Zea mays* L.), rice (*Oryza sativa* L.), wheat (*Triticum spp*. L.), and soybean (*Glycine max* L. Merr.)(FAO 2014). Currently, 60-70% of the barley that is produced is used for animal feed, 30-40% is used in malting to make beer and whiskey, and only 2-4% is directly consumed by humans (Newman and Newman 2008).

Barley is a short season, early maturing crop that is tolerant to drought, alkali, and saline soils. There are cultivars of barley that are planted in the spring and there are winter cultivars that are planted in the fall. Winter cultivars need to undergo a vernalization (cold exposure) period before they can complete their life cycle and flower in the spring. Most cultivars (spring and winter) can germinate in under five days provided temperatures are conducive to plant growth and development (12 – 24 ˚C) (Stark 2003b). After emerging, spring barley matures within 60-90 days, making it a desirable crop in regard to water and nutrient use efficiency (Shewry and Ullrich 2014, Anderson et al. 2015). Barley is self pollinating. Both the pollen and the ovary mature at the same time to ensure fertilization. Spring cultivars generally do not open their florets while undergoing

fertilization (cleistogamy); whereas, winter cultivars are chasmogamous and open their florets during this time (OGTR 2008).

Barley used for animal feed is grown in regions where adverse climatic conditions limit cultivation of more popular feed grains (e.g. maize). Feed barley is generally not irrigated, and minimal inputs are invested into the crop because crop value is much lower than malting barley. Cultivars grown for malting are expected to produce grain that adheres to specific quality standards. Therefore, they are generally produced under more intensive management practices that include irrigation, fertility amendments, and the implementation of pest/pathogen mitigation strategies.

Domestication and Malt History

Barley is believed to be one of the first eight Neolithic founder crops (primary domesticates) domesticated over 10,000 years ago in the Fertile Crescent of Mesopotamia (Zohary et al. 2012). Modern cultivated barley (*H. vulgare* subsp. *vulgare*) is a direct descendant of the wild subspecies progenitor *H. vulgare* subsp. *spontaneum*. Primitive domesticates of *H. vulgare* subsp. *spontaneum* had three spikelets per node along the axis of the floral rachis, but only the central spikelet was fertile, resulting in the inflorescence having two rows of fertile grain (*Figure* 1.1) (Zohary et al. 2012). Through domestication humans selected for desired traits, and by 6000 B.C. six-row (three fertile spikelets) cultivars begin to show up in archeological excavation sites (*Figure* 1.1) (Simpson and Ogorzaly 2001). As domestication progressed, other desirable

traits were selected, such as, a non-brittle rachis in which the spikelets did not shatter on maturity and hull-less kernels that are easier for human consumption.

Dried barley grains are very hard and require processing prior to human consumption. It is believed that prehistoric people experimented with preparation techniques that made the grain easier to eat. They initially ate the grain raw after removing the husk or they ground the grain into a powder and mixed it with water to make pastes and porridges (Piperno et al. 2004). As time went on, it was discovered that toasting the grains on hot stones would make them easier to grind and provided a more appetizing flavor. As civilizations developed, barley became an integral part of daily life. Early people learned that soaking and sprouting the grain before drying made the grain easier to grind into flour. This flour was used in making barley breads. The dough made from this flour provided a hospitable environment for yeast to thrive, and it was often left to ferment (Simpson and Ogorzaly 2001). Through fermentation, yeast would digest available sugars, reducing the amount of tannins that cause irritation to the stomach and digestive tract. Fermentation also liberates nutrients and amino acids from the starchy dough (Katz and Maytag 1991). The practice of soaking and sprouting the barley prior to use became the precursor to the malting process and the production of beer.

The earliest documented use of barley to make beer is from Sumerian cuneiform tablets that date back to the third millennium BCE (3000–2001 BCE) (Damerow 2012). Around 1800 BCE, the "Hymn to Ninkasi" was carved into clay tables and preserved. Ninkasi was the Sumerian goddess of brewing, and the

hymn provided a detailed recipe for beer. The recipe describes making "bappir" (Sumerian bread) from barley, spreading it out on a mat, soaking the resultant "malt" in a jar, and ending up with beer (Katz and Maytag 1991). Although this process can be achieved using other small grains (e.g. wheat, corn, emmer, or rice) as malts, barley has historically been preferred because it has desirable characteristics that are lacking or deficient in other grains.

One such characteristic is that the grain retains its husk during malting. This is important at the end of the brewing process because the husks collect at the bottom of the mashing tank forming a filter bed through which the wort (sugar water extracted from the grain) passes before going on to the brew kettle (Simpson and Ogorzaly 2001). This process in which the wort is separated from the residual grain is called lautering. The husks also contribute to the flavor profile of the beer. Another desirable characteristic is the quantity of enzymes produced within a germinating barley grain. These enzymes sufficiently convert starches into sugars making them available for fermentation. Other grains produce the same enzymes but not to the extent of barley and often require the addition of supplemental enzymes during fermentation (Simpson and Ogorzaly 2001).

Seed Characteristics

The barley seed contains the necessary resources to initiate a barley plant. These resources include carbohydrates, proteins, and lipids. These raw materials

are packaged in a quiescent state until imbibition takes place and stimulates germination.

A barley seed is a dry indehiscent fruit called a caryopsis. The outermost protective layers of the caryopsis are the lemma and palea that make up the husk. The husk accounts for approximately 10-15% of the dry weight of the grain and is made up of cellulose, arabinoxylan (a hemicellulose), lignin, and silica (Höije et al. 2005). These sturdy cellular structures are resistant to enzymatic degradation during mashing and provide a structurally sound filter bed during lautering. Beneath the husk is the pericarp and testa that are fused and form the seed coat of the grain. Together, the husk and seed coat protect the grain from desiccation and decomposition via microorganisms.

Directly beneath the testa is the aleurone layer. This layer constitutes approximately 5% of the total dry weight of the kernel (Newman and Newman 2008). As the grain matures, the aleurone layer accumulates lipids and becomes desiccation tolerant. This enables the cells to remain alive but in a quiescent state after seed maturation and through dormancy (Shewry and Ullrich 2014). Upon imbibition, the embryo produces hormones that stimulate aleurone cells to release enzymes, including amylases, glucanases, and proteases. These enzymes catalyze the mobilization of stored energy reserves within the endosperm. Unlike maize and wheat, where the aleurone is only one cell layer thick, barley has three cell layers (Shewry and Ullrich 2014). A larger aleurone layer enables barley to produce enough hydrolytic enzymes to achieve sufficient modification of

endosperm cells, and this capability forms the basis for its use in brewing (Buttrose 1963, Walbot 1994).

The endosperm is made up of inactive starch granules and constitutes 70 - 80% of the dry kernel weight (Newman and Newman 2008, Shewry and Ullrich 2014). Two size fractions of starch granules are embedded within a protein matrix within the endosperm (*Figure* 1.2). The first fraction includes large starch granules (A-type) that measure approximately 10-48µm in diameter and make up 90% of the starch within the grain. The second fraction includes small starch granules (B-type; 1-10µm) that constitute the additional 10% (*Figure* 1.2) (Goering et al. 1973). At the onset of germination these proteins and starches become degraded by the enzymes that are released from the aleurone layer. These raw materials (e.g. sugars, amino, and fatty acids) are transported to the embryo where they are utilized to nourish the developing plant.

The scutellum is a layer of specialized tissue that separates the endosperm from the embryo. This region, often referred to as a modified cotyledon, is the initial site of protein synthesis. These specialized carrier proteins, embedded in the epithelial cells of the scutellum, transport liberated glucose, peptides, and amino acids from the endosperm to the developing embryo (Waterworth et al. 2000). As modification continues and products are transferred to the embryo, the scutellum increases in size to maintain contact with the dissolving endosperm (Tillich 2007).

The embryo is located at the proximal end of the barley grain. It is relatively small and only contributes 4% of the total dry weight of the kernel

(Mallett 2014). The first step toward germination is initiated when the seed is exposed to water. The embryo becomes hydrated, resumes metabolic function, and releases hormones that stimulate the reanimation of the scutellum and aleurone layer. All of the genetic information that determines the morphology and physiology of the future plant is packaged within the embryo. The plant genome directs and coordinates cell proliferation so that primordial tissues develop into organs. The synchronization of these organs produces a functioning seedling which emerges from the soil.

The objective of malting is to exploit the physiological processes that the germinating seed undergoes on its way to becoming a seedling. These processes liberate sugars, proteins, and amino acids, which brewers use to nourish the yeast in fermentation.
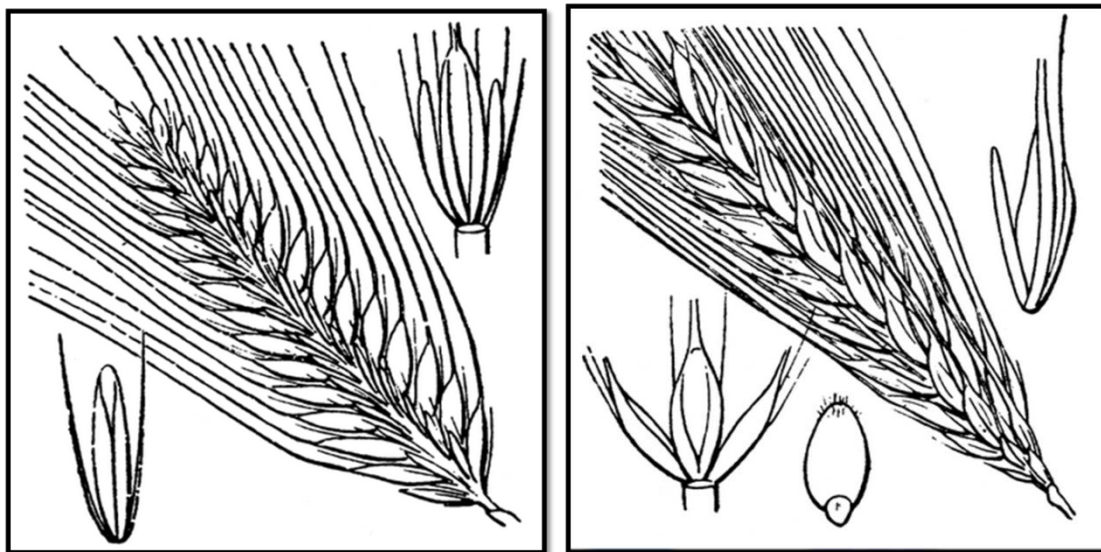
Figure 1.1 – Barley Rachis Morphology: 2-row barley head (left) and 6-row barley head (right). Note the single fertile floret on the 2-row variety and the three fertile florets on the 6-row. Adapted from the French Botanical Network. www.tela-botanica.org.
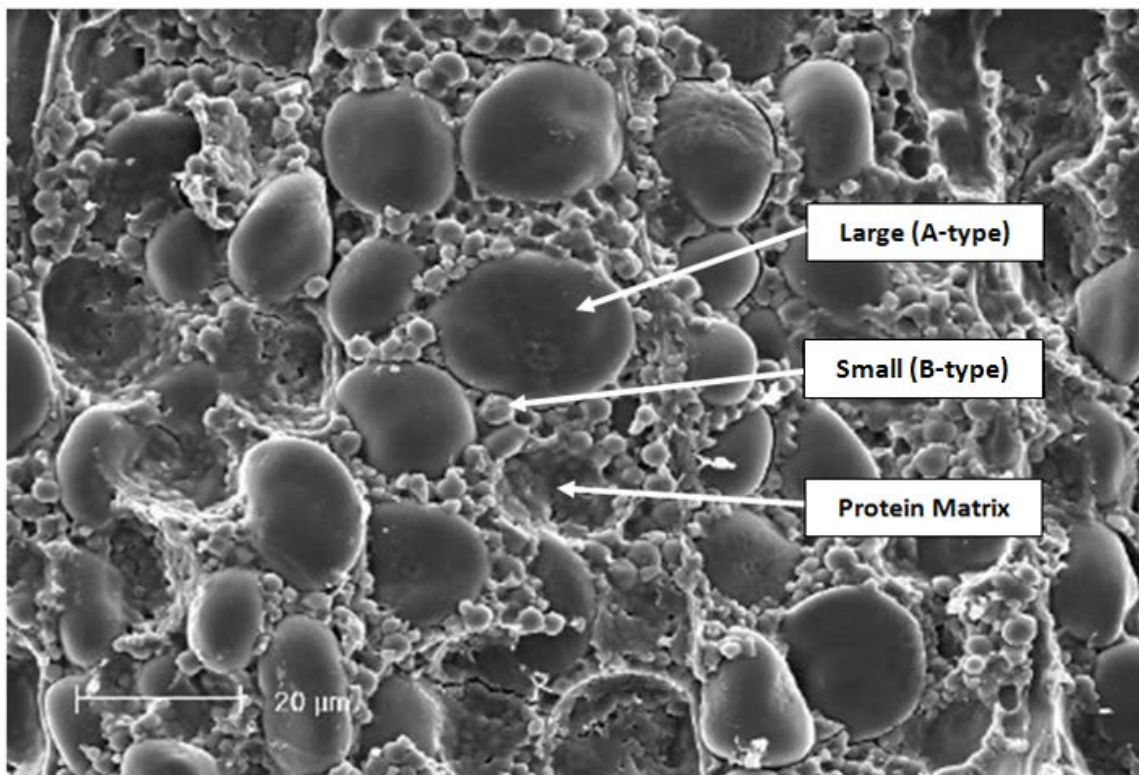
Figure 1.2 – Scanning electron micrograph of barley endosperm. Depicting both large (A-type) and small (B-type) starch granules embedded within the protein matrix. Micrograph provided courtesy of John L. Black. http://www.regional.org.au/au/abts/2001/m3/black.htm.

CHAPTER 2

MALTING

The function of the barley seed is to produce a growing barley plant. In malting, the processes of germination and growth are exploited to liberate and obtain a bounty of fermentable products that can be used for making beer. Malting begins with the acquisition of quality grain. As the grain arrives at the malt house, the moisture content should not exceed 12% (UK Malt 2011). This prevents the growth of mold and decreases the likelihood of mycotoxin build up within the stored grain. The grain must also retain its viability and have a germination rate of 98% before being approved for the malting process (UK Malt 2011). This ensures uniform germination so that grain modification is consistent throughout the batch. Nitrogen and protein content are also important attributes in assessing gain quality. Bamforth and Barclay (1993) indicate that acceptable nitrogen and protein levels in six-row malting barley are between 1.8–2.0% nitrogen and 11.3–12.5% protein. Low nitrogen content may not fulfill the needs of yeast during fermentation, while high protein content decreases the volume of starch within the endosperm cavity (Shewry and Ullrich 2014). The grain must also be free from disease, pre-harvest sprout damage, chemical damage, and insect damage. If these grain quality standards are satisfied, the grain is approved by the head maltster and allowed to begin the three stages of malting: steeping, germination, and kilning.

Steeping

Steeping is the first step in malting and involves submerging dried grain in water to increase the moisture content to stimulate germination. In modern industrialized malt houses, the grain is mixed with water into slurry at temperatures between 14 -16 °C (Schwarz and Li 2011). The slurry is then transferred to flat-bottom steeping vessels with perforated floors (*Figure* 2.1). Over the course of 24 - 40 hours, the steep will undergo a minimum of two or three water changes that are interspersed with ventilated air rests between tank refills. When the grain is submerged, air is often pushed up through the slurry to aerate the mixture. Depending on the style of the steeping vessel, air is drawn down (can be pushed up) through the grain bed during air rests to replenish oxygen that is needed by the respiring grain and remove built up carbon dioxide (Schwarz and Li 2011).

The length of time between immersion and air reset cycles varies among malt houses, grain variety, seasonal changes, and crop year, but it is generally between 4-12 hours (Schwarz and Li 2011). Barley varieties that have starch granules that are loosely packed into the protein matrix of the endosperm have more open spaces in which water can diffuse more freely (Bamforth and Barclay 1993). This hydrates the grain more expeditiously than varieties that have compacted endosperm. This increased rate of hydration (i.e. less compacted endosperm) decreases the length of the steeping period, and is considered a desirable characteristic in malting (Palmer and Harvey 1977).

During the first immersion, the dry outer grain layers rapidly take up water but minimal metabolic activity occurs (Kunze et al. 2004). The grain tends to take up water quickly while submerged, but internal distribution through the endosperm occurs during the air rests. Despite the grains initial uptake of water, the first immersion is considered to be a cleaning cycle. As the steep tank fills with water, dirt and debris float to the top, and the tank is overflowed to eliminate these impurities (Schwarz and Li 2011).

Over the course of the steep tank immersions and air rests, the grain reaches 35% moisture content, and the embryo resumes metabolic activity and begins to synthesize gibberellic acid (GA) (Fincher 2010, Schwarz and Li 2011). Water and GA diffuse through the seed from the proximal end of the grain toward the distal tip, gradually activating the aleurone layer. Once activated, the aleurone layer releases hydrolytic enzymes (glucanases, proteases, and amylases) into the endosperm cavity, and modification commences. Although 35% moisture content is enough water to stimulate germination, steeping continues until the grain reaches a final moisture content of 42-48% (Schwarz and Li 2011). This higher moisture level is required for uniform enzyme diffusion throughout the endosperm, promoting adequate modification of resources to support germination.

Germination

Maltsters visually inspect the grain and know when it has reached the appropriate moisture content by the emergence of the coleorhizae, referred to as the "chit" (Shewry and Ullrich 2014). Once the chit has emerged and the grain

has been moved to the germination beds (*Figure* 2.2), it is imperative that the grain remains moist and has adequate air circulation. Modern germination beds, such as a Saladin box (*Figure* 2.3) or circular germination chambers (*Figure* 2.4), are designed to maintain temperature and provide aeration and moisture to the developing grain. Both the Saladin box and the circular chamber are constructed of stainless steel and have perforated floors to allow humidified air to be drawn through the grain bed. The air is often heated to maintain temperatures at 16-20˚C (Bamforth and Barclay 1993). The Saladin box is rectangular in shape and has large vertical open augers attached to a carriage that moves across the top of the grain bed (*Figure* 2.5). The circular germination beds use the same auger mechanism with the carriage moving around the compartment on a center pivot. These augers turn the grain bed every 8-12 hours to keep the rootlets from matting together (Shewry and Ullrich 2014). This ensures that air and moisture reach all of the germinating seed throughout the profile of the grain bed.

The goal of germination in malting is to synthesize enzymes that initiate the modification of endosperm components to make them available for brewing. The quiescent barley grain is composed of stabilized raw materials, including carbohydrates, proteins, and lipids. Through germination, these raw materials are modified by enzymatic activity into smaller components, such as sugars, amino, and fatty acids (Shewry and Ullrich 2014). These smaller components are generally utilized by the developing embryo. In brewing, these components are essential to the proper nourishment of yeast during fermentation (White and Zainasheff 2010).

During modification, the cell walls of the endosperm become degraded, the protein matrix surrounding starch granules breaks down, and some of the starchy complexes are hydrolyzed. The rate of modification is dictated by the ease with which the endosperm becomes hydrated, the time it takes the aleurone layer to undergo protein synthesis, and the availability of substrates in relation to their respective enzymes.

The endosperm cell walls are the first physical structures that enzymes come into contact with after they are released from the aleurone layer. These cell walls are composed of up to 75% beta (β)-glucan, hemicellulose (arabinoxylan) (20%), and a small amount of cellulose (2%) (Fincher 2010). Beta-glucans are made of strings of β-glucose molecules that are linked by β-1$\rightarrow$3 and β-1$\rightarrow$4 bonds (Burton et al. 2010). If high levels of these long chains of sugars are left intact, they lead to an increase in viscosity of the wort and can "gum" up filtration systems (Loi et al. 1987, Burton et al. 2010, Mallett 2014). The amount of β-glucan remaining after malting indicates the level of modification that was achieved. Malt with lower levels of β-glucan generally achieved a higher level of modification. Measures of β-glucan dictate malt quality. Maltsters will use these values as indicators to determine adjustments that can be made during steeping and germination.

To ensure embryo survival, endosperm cell walls need to be rapidly hydrolyzed during germination so essential components within the endosperm can be liberated. One of the suite of enzymes released by the aleurone layer is Beta (β)-glucanase. β-glucanase hydrolyzes the β-glucan that is woven into the

endosperm cell walls and loosens the barriers between the aleurone layer and the internal structure of the endosperm (Burton et al. 2010). With the cell walls degraded, the protein matrix and starch granules within the endosperm are available for additional enzyme activity.

The most prevalent storage proteins in the endosperm are a class of proteins called hordeins. Hordeins account for 30-50% of the total nitrogen content within the grain (Shewry and Tatham 1990). When these proteins are hydrolyzed by proteolytic enzymes that have been released from the aleurone layer, they provide peptides and amino acids for the growing embryo. For brewers, the amount of free amino nitrogen (FAN) is a measure of amino acids and small peptides that are available in the malt to support yeast during fermentation (White and Zainasheff 2010).

As mentioned above (Chapter 1), the starch contained in the endosperm cavity is divided into two size fractions, the A-type (large 10-48µm) and B-type (small 1-10µm) (Goering et al. 1973). Both sizes of these granules are composed of amylose and amylopectin. Amylopectin is made up of many short (6-20 glucose units) clusters of glucose molecules connected by α-1$\rightarrow$4 (the number 1 carbon [glucose 1] binds to oxygen which binds with the number 4 carbon [glucose 2] in α position) and α-1$\rightarrow$6 glycosidic bonds (Shewry and Ullrich 2014). These molecules are highly branched and can be as large as one million glucose units in size (Shewry and Ullrich 2014). Amylose is a much smaller compound being made up of approximately 2000 glucose units (Mallett 2014). Glucose linkages in amylose molecules are linearly connected by α-1$\rightarrow$4

glycosidic bonds, and therefore, are un-branched in nature. In both the A and B-type starch granules, amylopectin constitutes approximately 80% of the starch granule, and amylose makes up the additional 20% (Jane et al. 1992).

During starch modification, the aleurone layer synthesizes both alpha (α)-amylase and beta (β)-amylase. These enzymes attack the long chains of amylopectin and amylose. Alpha-amylase can hydrolyze any α-1→4 glycosidic bond along the glucose chain, provided the bond is not within one glucose molecule of an α-1→6 bond (Mallett 2014). Alpha-amylase is considered the starch molecule "chopper", because it chops the molecule in various places along the chain of glucose units (Mallett 2014). This results in chains of glucose that are variable in length. Beta-amylase can only hydrolyze α-1→4 bonds at the ends of the glucose chains that are within three glucose molecules of an α-1→6 bond (Mallett 2014). Beta amylase "nibbles" on the ends of the chains and generally results in the liberation of the disaccharide, maltose (Mallett 2014). For brewing, the malt starches need to be broken down to glucose, maltose, and in some cases maltotriose for yeast utilization in fermentation. Most of the starch hydrolysis occurs in the first stage of the brewing process, known as mashing. Larger sugars cannot be fermented and will carry over into the beer, resulting in a "sweeter" flavor profile.

As modification proceeds, the resultant malt extract (simple sugars, dextrins, amino acids, and proteins) becomes increasingly more available for embryo utilization. If allowed to continue, fermentable material can be lost and the malt is considered to be overmodified. To prevent this, maltsters visually

inspect this "green malt" to determine how modified the endosperm components have become. Ideal modification has occurred when the acrospire (the first shoot) has grown to approximately 75% of the grains length (Schwarz and Li 2011). In the malting process, germination generally takes between four and five days, at which point the green malt is moved to the kiln and modification is discontinued (Bamforth and Barclay 1993, Papazian 2003).

Kilning

Once it has been determined that the green malt has reached ideal modification it is transferred to a kilning chamber. Like germination beds, modern kilns are constructed of stainless steel, have perforated floors, and can be circular or rectangular in shape. During kilning, the green malt is evenly spread across the floor, and hot air is blown through the grain bed (*Figure* 2.6). This process kills the embryo and terminates germination while preserving the malt extracts and retaining its enzyme activity.

Over the course of approximately 24 hours, the green malt undergoes two major phases. The grain is dried (withering) and then cured. Withering takes place over the first 10 hours of kilning at temperatures ranging from 50-75˚C (Schwarz and Li 2011). The purpose of withering is to slowly remove the moisture from the grain so that modification is arrested and the enzymes stop working but are not denatured. As hot air passes through the grain bed, the air cools as it picks up moisture from the grain and the grain begins to dry. Eventually the moisture is dissipated and hot air permeates through the grain bed.

This is called a "breakthrough", and once this is uniform throughout the kiln, curing can commence (Mallett 2014).

Curing entails gradually increasing the temperature to between 80-110°C (depending on malt style) over the remainder of the kilning time (Bamforth and Barclay 1993). This enables the maltsters to develop the desired malt color and aroma for the particular malt they are creating. When kilning is completed, the dried rootlets are removed and the malt is relocated to a storage facility. Often malt is aged for up to 28 days to allow residual moisture to become uniformly distributed throughout the grain (Mallett 2014).

After the ageing process, the finished malt has reached a moisture content of approximately 3-5% (Bamforth and Barclay 1993). This moisture content keeps enzymatic activity dormant and the malting process (steeping, germination, and kilning) has decreased the likelihood of spoilage. The malt is then stored in climate controlled storage bins prior to being transferred to the brew house. These storage bins maintain a climate that is low in humidity (less than 10.5%) and temperatures of 10 - 20°C (Queensland 2015). Breweries do not generally store more than a few days worth of malt at the brew house because malt storage increases the brewer's expenses. Therefore, malt is stored onsite at the malt house until the brewery calls in an order.

Figure 2.1 – Filling Steep Tank with slurry (water and barley). Photograph taken by Haley Oser at Golden Malting in Golden, CO.

Figure 2.2 – Steeped grain being transferred to the germination bed. Photograph taken by Haley Oser at Golden Malting in Golden, CO.

Figure 2.3 – Saladin Box (germination bed). Photograph taken by Haley Oser at Golden Malting in Golden, CO.

Figure 2.4 – Circular construction germination bed. Photograph courtesy of Mont Stuart.

Figure 2.5 – Saladin box demonstrating large vertical open augers. Carriage moves across the top of the grain bed turning the grain. Photograph taken by Haley Oser at Golden Malting in Golden, CO.

Figure 2.6 – Kiln drying down green malt. Green malt is evenly spread across the floor, and hot air is blown up through the grain bed. Photograph taken by Haley Oser at Golden Malting in Golden, CO.

CHAPTER 3

BARLEY PRODUCTION

Barley produced for the malting industry is generally limited to selected cultivars approved by the American Malting Barley Association (AMBA). The AMBA's mission is "to encourage and support an adequate supply of high quality malting barley for the malting, brewing, distilling, and food industries and increase understanding of malting barley" (AMBA 2011a). Barley varieties approved by the AMBA are selected on the basis of their agronomic characteristics (e.g. yield, disease resistance, etc.) and their malt quality attributes (e.g. germination efficiency, plumpness, low protein, etc.) (AMBA 2011a). In malting barley production, malt quality factors are more important than yield; therefore, these cropping systems are managed more intensely than fields producing feed grade barley. In executing these practices, producers pay special attention to planting times and conditions, nutrient management, growth stages, irrigation and harvest.

Planting

Malting barley in the United States is primarily grown in the temperate regions of Colorado, Montana, Idaho, Wyoming, and North Dakota (AMBA 2011b). These states have an ideal climate for barley production. Barley is a cool season crop that will yield the best under conditions in which the vegetative and early reproductive growth coincide with moderate temperatures.

In the spring, barley should be planted as soon as soil temperatures ensure uniform germination and emergence. Barley will germinate when soil temperatures are

above 5˚C, but optimum temperatures for emergence and growth are 12 - 24˚C (Stark 2003b, McVay et al. 2008). Planting in the northwestern regions of the United States begins mid-February and can continue through mid-April. Early planting reduces the risk from drought stress, high temperatures later in the season, diseases, and insect infestations (McVay et al. 2008). Delayed planting increases the risk of high protein content in the grain and can result in decreased yields. Reduced grain quality can ultimately lead to rejection at the elevator.

Fukai et al. (1990) conducted a study in Australia on the effect of planting density on biomass accumulation and yield. They found that higher planting densities (120 plants/m^2) resulted in increased tiller production per unit area, higher rates of biomass accumulation, earlier heading, and higher yields when compared to lower planting densities (36 plants/m^2) . With more tillers per meter squared and higher biomass accumulation, canopy closure occurred sooner resulting in less water loss from the soil surface. This resulted in a reduction in overall evapotranspiration and more water for plant utilization through the season (Fukai et al. 1990). In Montana and Idaho, barley producers use planting population rates that range from 75-240 plants/m^2 (7-23 plants/ft^2) which is approximately 68–90 lbs (seed)/acre (Stark 2003b, McVay et al. 2008). These rates vary depending on cropping system, growing region, management tactics, soil type, plant variety, and irrigation practices. If growers plant a variety that does not tiller well, production may benefit from a higher planting rate; whereas, lower densities might be employed under dryland conditions, such as pivot corners.

Germination and emergence proceed more readily when the seed is planted in a fine textured but firm seedbed (Stark 2003b). Seeds should be planted at a depth of 1-1.5

inches with row spacing of 6-8 inches (Stark 2003b, McVay et al. 2008). Growers generally plant barley using grain drills. Modern grain drills provide precision planting, even seed spacing, uniform depth, and ensure adequate seed-soil contact.

Fertility Management

Although barley can grow in alkaline, saline, and nutrient deprived soils, malt quality barley requires optimum fertility management. To characterize nutrient availability in a field, soil samples should be taken one to two weeks prior to planting. Provided the field has a uniform soil type, samples should be taken from various locations and mixed together into a subsample that represents the major soil characteristics (i.e. texture, structure, and nutrient content) across the field. Samples taken from fields with variable soil types should be kept separate so site-specific nutrient content can be quantified. Soil tests can also provide information about the amount of soil organic matter (SOM) and pH. These soil attributes can indicate the availability of minerals and nutrients within the soil and can elucidate the effects of fertility amendments. Soil texture also plays a role in nutrient availability. Coarse-textured soils often leach easily and do not retain nutrients for long periods of time. Fine-textured soils have more surface area and higher cation exchange capacity, and thus, they can better retain nutrients (Singer and Munns 2006). It is important to keep soil characteristics in mind when establishing a fertility management plan.

Nitrogen

To obtain grain protein levels between 11.3%-12.5% (malt quality standard), the amount of available nitrogen (N) in the soil should be carefully monitored through regular soil testing. Soil N content is influenced by the current crop, previous fertilizer applications, previous year's crop, soil type, level of organic matter, irrigation, and other management practices. To determine the amount of N that needs to be applied to a field (or field section in a site-specific scenario), the grower should determine a reasonable estimated yield for the crop. This estimate is generally based on historical yield values or can be provided by the seed distributor. Next the grower should convert the concentrations of inorganic N (NO_3-N and NH_4-N) from the soil test to pounds of N per acre. From a N recommendation table, take the amount of available inorganic N present and subtract it from the recommended amount needed to reach the target yield potential (*Figure* 3.1) (Brown and Stark 2003, Jacobsen et al. 2005). These values usually take into account the amount of SOM and the rate it mineralizes, making nitrogen available to the plant. For example, in the Fertilizer Guidelines for Montana Crops, they assume a regional average organic matter level of 2% which is incorporated into their N recommendation values (Jacobsen et al. 2005). McVay et al. (2008) suggest that malting barley needs 1.2 lb N per target yield bushel to ensure vigorous plant growth and maintain protein content within quality standards. If the protein content of previous barley crops were in excess of the malt quality standards it has been suggested to reduce the recommend N application amount by 20 lbs/acre across all target yields (Jacobsen et al. 2005).

Phosphorus and Potassium

Phosphorus (P) and Potassium (K) are needed to achieve optimum tillering and plant growth. The amount of P and K in the soil can be determined through soil sampling and testing. If soil test levels are below the "critical level" of 16 ppm P, and 250 ppm K, amendments should be made (McVay et al. 2008). After identifying the concentration of P and K (ppm) in the soil, growers can determine how much needs to be applied by referencing regional nutrient recommendation tables similar to the one provided by Montana State University in Fertilizer Guidelines for Montana Crops (*Figures* 3.2 and 3.3). It is recommended that starter applications of 10-20 lbs. per acre of P and K be applied at planting because cool, wet soils generally limit their availability (Jacobsen et al. 2005). Amendments should be placed up to 2 inches below the seed so that the emerging roots will readily come into contact with the nutrients (McVay et al. 2008).

Sulfur and Micronutrients

Sulfur (S) requirements for barley are generally $1/15^{th}$ (7%) that of N (Brown and Stark 2003). If the soil test indicates that S content is less than 10 ppm, it is recommended to apply 15-20 pounds per acre (Mahler and Guy 2007). Irrigation water often contains a sufficient amount of S and should be tested before making an application. Soil pH should be taken into account when deciding which form of S is applied. Elemental S can lower the pH of a soil and should be used cautiously on neutral and acidic soils (McVay et al. 2008).

Micronutrient deficiencies are generally rare in barley production but should not be excluded as a culprit if symptomology appears. Dell (1981) found that barley grown

in copper deficient soils often had underdeveloped lignifications in the endothecia (inner wall of a pollen grain), and this resulted in sterile pollen grains. If a deficiency is detected, liquid amendments can be made foliarly or granule amendments can be applied in furrow at planting (McVay et al. 2008).

Development and Growth Staging

Barley production has become increasingly more intensive over the years. Many management decisions regarding the application of fertilizers, pesticides, and growth regulators are dependent on the developmental stage of the crop. To accurately schedule management inputs and employ tactical control measures (i.e. insect or pathogen) it is essential for the producer to know the specific growth stages of the crop. A number of cereal grain staging systems have been employed for describing the developmental stages of barley. Currently, the Zadoks system is the most universally recognized system that is used for describing growth stages of cereal grains (*Figure 3*.4) (Anderson et al. 2015). The Zadoks system is a two-digit code where the first number designates the principal stage of development. The principal stages include; 0) germination, 1) Seedling growth, 2) Tillering, 3) Stem extension, 4) Booting, 5) Heading, 6) Anthesis, 7) Milk development, 8) Dough development, and 9) Ripening. The second number of the code (also 0-9) divides each principal group into incremental development stages. For example, a plant that is in growth stage Z23 is tillering (2nd primary stage) and has the main shoot plus 3 tillers (3rd secondary stage). Fertility amendments and herbicide applications are generally completed during tillering, and disease management is critical from stem extension through heading (OMAFRA 2013). It is also important to

understand the critical times during plant growth that the plant should not undergo water stress.

Irrigation

Irrigation management is one of the most important factors affecting malting barley yield and quality. Morgan and Riggs (1980) found that drought induced yield reduction was greatest when the drought stress was imposed at heading. Drought stress at this developmental stage significantly reduced the amount and size of grains along the developing rachis (Morgan and Riggs 1981). They also determined that the quality of malt extracts was compromised when the plants experienced drought stress during grain development (Morgan and Riggs 1981). It has been demonstrated that cereal grains that undergo drought stress during development tend to accumulate higher quantities of storage proteins during seed fill (Parchin and Shaban 2014). Growers are encouraged to monitor soil moisture throughout the season to ensure their crop has sufficient water. Monitoring can be conducted using soil moisture sensors, such as neutron probes, time domain reflectometry (TDR), tensiometers, or gypsum blocks (McVay et al. 2008). These instruments enable the grower to easily determine the soil moisture in many locations across the field. With this information, irrigation strategies can be developed to ensure the crop has adequate water during critical growth and developmental stages.

Evapotranspiration (ET) is the loss of water from the soil via plant transpiration and evaporation from the soil surface. This value can be translated into the amount of water required by a crop at any given time. Spring barley, grown in southern Idaho, has a seasonal ET of 15-19 inches (Stark 2003a). In mid-June to mid-July barley is generally

flowering and water demands can be as much as 0.30 inches per day (Stark 2003a, McVay et al. 2008). It is important to monitor the amount of available water in the soil profile and irrigate when levels fall below the amount needed by the crop during specific growth stages.

The amount of water a soil can hold is called the water holding capacity (WHC) and is represented in inches of water per foot of soil (in/ft). The soil profile is made up of layers that differ in their soil characteristics (i.e. texture, structure, SOM, etc.). Each of these layers has a different WHC and the sum of the WHCs of all layers is the total available soil moisture (ASM). Soil properties vary by depth; therefore, when determining the ASM, measurements should be taken from a three foot profile (McVay et al. 2008). If the amount of water applied to the soil exceeds the ASM, water will move beyond the root zone and vital minerals and nutrients will be lost to leaching (McVay et al. 2008). Stark (2003a) recommends not allowing the ASM to drop below 50% at any point during the growing season, especially during tillering and flowering. By maintaining the ASM at 50%, water use efficiency can be achieved and crop quality and yield can be ensured.

Harvest and Storage

Maltsters will pay premiums for barley that meets all of the quality standards and has been harvested in good condition and properly stored. Malting barley reaches physiological maturity at 35% moisture and must be dried down to less than 12% for proper storage (McVay et al. 2008). After maturity some yield losses can occur due to pre-harvest sprout damage or shattering. It is important to know when the crop reaches

moisture levels suitable for harvesting to mitigate these pre-harvest losses. Some

varieties are more susceptible to pre-harvest sprout damage than others; therefore, variety

selection can help minimize this loss. Cutting and windrowing the barley at 20-30%

moisture content and allowing it to dry in the field can also decrease losses (AMBA

2011b). Growers can also directly harvest the grain and put it into drying storage bins but

this has its own set of risks that should be assessed. Grain that is too moist (greater than

18%) when harvested has the potential to be damaged in the thresher, and grain that is too

dry can potentially shatter (AMBA 2011b). At harvest the ideal moisture content of the

grain is dependent on harvesting strategy (e.g. direct combine, windrow, etc.) and storage

facilities.

Arinze et al. (1994) conducted a study on grain that was directly harvested and

dried in storage bins. Their research addressed the effects of harvesting at different

moisture contents and drying the grain using different air temperatures. They found that

grain viability decreased when malting barley was dried in bins under temperatures

exceeding 30-40˚C (Arinze et al. 1994). They recommend not harvesting the grain until

it reaches at least 22% (no more) and ventilating it with air that does not exceed 25˚C to

maintain grain quality (Arinze et al. 1994).

Maintaining quality in malting barley is the primary criteria in which growers

base their production decisions. Decisions regarding planting times, fertility

management, irrigation schedules, and harvesting all contribute to the quality of the

grain. In addition to making good production decisions, growers often face challenges in

managing insect pests and pathogens. These pests and pathogens can have deleterious

effect on the both grain quality and yield. The next chapter (Chapter 5) discusses some

of these pest/pathogen challenges and presents some management strategies that may help mitigate reductions in quality and yield.

Feed and malt barley N guidelines based on soil analysis.

BARLEY - FEED		BARLEY - MALT	
Yield Potential (bu/a) *	Available N (lbs/a) **	Yield Potential (bu/a) *	Available N (lbs/a) **
40	64	60	72
60	96	70	84
80	128	80	96
100	160	90	108
120	192	100	120
140	224	110	132
		120	144

* Attainable yield with *all* growth factors optimized.
** Fertilizer N = Available N - soil analysis NO_3-N.

Figure 3.1 – Nitrogen Recommendation Table. Amended from: Fertilizer Guidelines for Montana Crops – Jacobsen et al. 2005.

Phosphorus fertilizer guidelines based on soil analysis.					
Crop	Olsen P Soil Test Level (ppm)				
	0	4	8	12	16*
	P Fertilizer Rate (lbs P$_2$O$_5$/a)				
Alfalfa	140	110	75	40	0
Alfalfa-Grass	55	50	40	25	10
Barley-Feed/Malt	50	40	30	20	10
Bean	30	25	20	15	5
Buckwheat	45	35	30	20	10
Canola	45	40	35	30	25
Corn-Grain	100	80	60	40	20
Corn-Silage	80	65	50	35	25
Flax	35	30	20	15	10
Grass	45	35	30	20	5
Lentil, Chickpea and Pea	35	30	25	20	15
Millet	40	35	25	20	5
Oat	45	35	30	25	20
Potato	170	145	115	75	20
Safflower	50	40	30	20	10
Soybean	60	50	40	25	5
Sugarbeet	85	70	55	40	10
Sunflower	35	30	25	20	15
Wheat-Spring	50	45	35	30	20
Wheat-Winter	55	50	45	40	35

Figure 3.2 – Phosphorus Recommendation Table. Amended from: Fertilizer Guidelines for Montana Crops – Jacobsen et al. 2005.

Potassium fertilizer guidelines based on soil analysis.						
Crop	**K Soil Test Level (ppm)**					
	0	50	100	150	200	250
	K Fertilizer Rate (lbs K$_2$O/a)					
Alfalfa	240	205	170	140	95	30
Alfalfa-Grass	80	70	60	50	40	25
Barley-Feed	75	65	55	45	30	20
Barley-Malt	90	80	65	50	35	25
Bean	45	40	35	25	15	5
Buckwheat	60	50	40	30	20	5
Canola	45	40	35	30	25	20
Corn-Grain	135	120	100	80	50	20
Corn-Silage	145	125	110	85	60	35
Flax	45	40	35	30	25	20
Grass	80	70	60	45	30	15
Lentil, Chickpea and Pea	45	40	35	30	25	20
Millet	65	55	45	35	20	5
Oat	100	85	70	55	40	25
Potato	300	250	215	165	100	25
Safflower	65	55	45	35	25	15
Soybean	90	75	60	45	30	15
Sugarbeet	120	100	80	60	40	20
Sunflower	55	50	45	40	35	30
Wheat	135	115	90	70	40	10

Figure 3.3 – Potassium Recommendation Table. Amended from: Fertilizer Guidelines for Montana Crops – Jacobsen et al 2005.

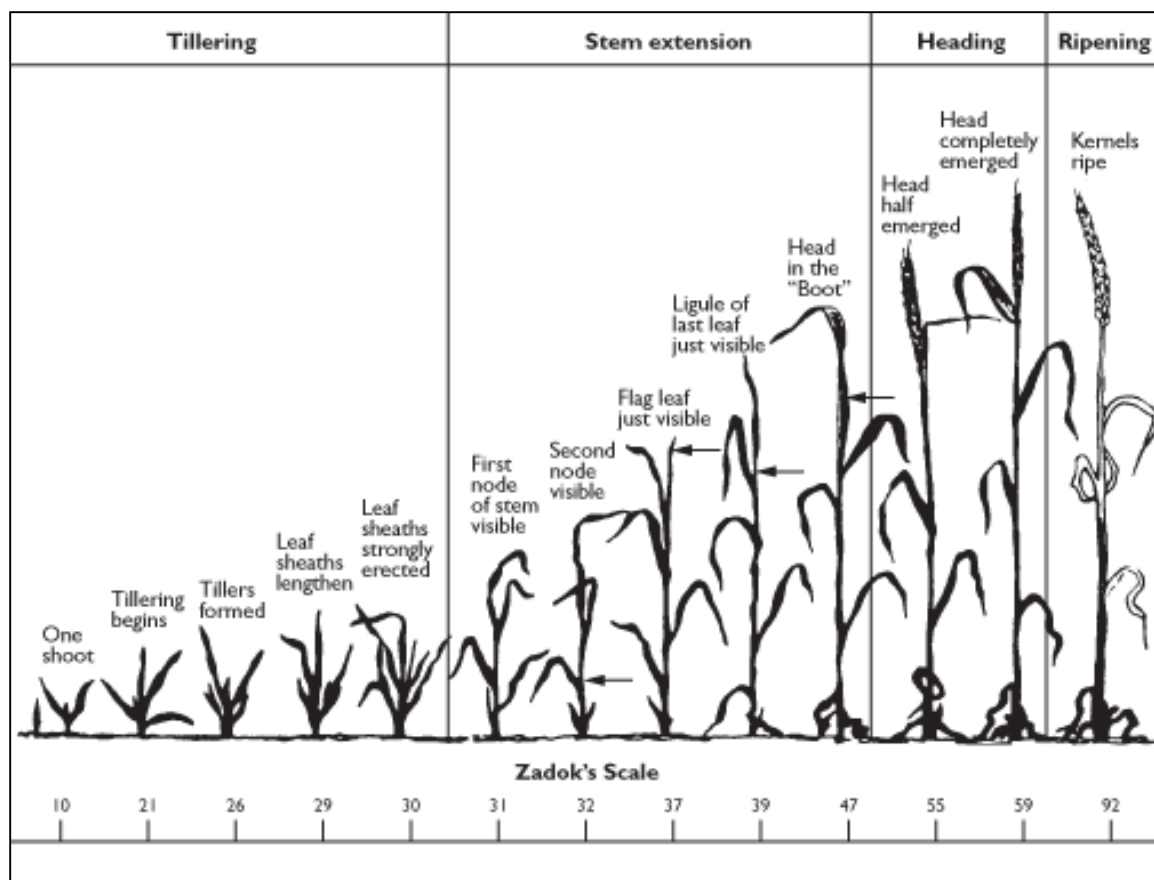| Tillering | | | | | Stem extension | | | | | Heading | | Ripening |

Figure 3.4 – Zadok's scale for cereal growth and development stages. Figure courtesy of ©Queen's Printer for Ontario, 2013.

CHAPTER 4

PEST AND PATHOGEN MANAGEMENT CHALLENGES THAT IMPACT

MALTING BARLEY PRODUCTION

A Common Pest

In barley production, insects are generally not a major factor, although

environmental conditions favoring an increase in populations can lead to significant

damage (McVay et al. 2008, OGTR 2008).   Some insect pests that have been reported to

cause periodic economic losses in barley include: Haanchen mealybug (*Trionymus

haancheni* McKenzie), Cereal leaf beetle (*Oulema melanopus* L.), Russian wheat aphid

(*Diuraphis noxia* Mordvilko), Pale Western Cutworm (*Agrotis orthogonia* Morrison),

and the Army cutworm (*Euxoa auxiliaries* Grote) (McVay et al. 2008). This section will

provide an overview of the Russian wheat aphid, a pest that has a life history and

behavioral characteristics that make detection difficult and management challenging.

Severe infestations of Russian wheat aphid can compromise plant health, diminish barley

yield, and reduce grain quality.

Russian Wheat Aphid

The Russian wheat aphid (RWA) (*Diurpahis noxia* Mordvilko) (Hemiptera:

Aphididae) can be a serious pest of wheat and barley. In North America, it was first

identified in Texas in 1986 and quickly spread through the western Great Plains and

intermountain regions of the United States and into Canada (Mornhinweg et al. 2006).

Cumulative economic losses since its introduction are estimated to be in the billions of

dollars (Mirik et al. 2009). The injury incurred to host plants from the RWA is a complex domino effect of developmental, morphological, physiological, and biochemical responses.

The aphid feeds within the leaf whorl where leaves are young and a high concentration of photosynthate is present (Macedo et al. 2003). The insect taps into the phloem with its proboscis and injects a toxin into the plant that prevents the leaf from unfurling (Smith et al. 1991). Continued feeding results in leaf purpling, the development of yellowish-white streaks, and prostrate growth of the tillers (*Figure* 4.1) (Macedo et al. 2003). Plants eventually become stunted, and at heading, the awns become trapped in the rolled leaves and cause the rachis to become curved or "fish-hooked" upon emergence (Hein et al. 2005). When heads become fish-hooked, grain fill is compromised causing a decrease in yield potential and grain quality.

The RWA is a small insect, measuring 1.6-2.1 mm in length (Hodgson and Karren 2008). They are light green in color, have an elongated spindle-shaped body, and short antennae (Hein et al. 2005, Hodgson and Karren 2008). Distinctive characteristics include the lack of visible cornicles (siphunculi) and the presence of both a cauda and a supracaudal process which gives the appearance of a "double tail" (*Figure* 4.2) (Aalbersberg et al. 1987b). Females reproduce parthenogenetically (asexual reproduction), are viviparous (live birth), and give birth to both winged (alate) and wingless (apterous) young. The alate morphology occurs when the host plant is under stress and is no longer suitable to sustain the colony. One female can produce 72-81 nymphs provided temperatures remain between 5-20 ˚C (Aalbersberg et al. 1987a, Tolmay 2006). After 10 days, nymphs can reach maturity and begin reproducing. This

rapid rate of reproduction increases the difficulty of management if infestations go unidentified.

Barley and wheat are the main economic hosts of the RWA in which the insect can complete its entire life cycle. Alternate hosts can also support aphid populations during seasonal changes when barley and wheat are not being grown. Alternate hosts include several grass species such as Crested wheatgrass (*Agropyron cristatum* (L.) Gaertn), Intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & Dewey), and Canada wild rye (*Elymus cnandensis* L.) (Hodgson and Karren 2008). The RWA will survive on alternate host plants until suitable barley and wheat host plants are available. In winter wheat and barley production, the aphids will move into the newly emerging crop in the fall. Heavy infestations compromise the plants, making them more susceptible to winter kill (Hein et al. 2005). Plants that survive and sustain aphid colonies through the winter become reservoirs for population increase in the spring. When the winter cultivated host crops are harvested in the spring, aphids move into spring wheat or barley cultivars, and/or onto alternate hosts, where they will feed for the rest of the summer.

Often producers will see symptomology on the plants, but will not detect the presence of the aphid infestation because the colony is within the immature rolled leaves. This enclosure not only protects the aphids from detection but also prevents biological control agents from gaining access to the colony (Carver 1989). The protective rolled leaf also protects the colony from contact insecticide treatments, making management of this cereal grain pest challenging.

Crop Resistance

Because of the limited cost effectiveness of insecticides and biological control agents against RWA, plants exhibiting resistant characteristics are being sought. A substantial amount of research is underway to develop resistant cultivars of both wheat and barley. Wheat has received the majority of the attention as a number of RWA resistant genes have been identified (e.g. Dn1 to Dn9 and Dnx) (Liu et al. 2005). Resistant barley cultivars have been identified but the mechanisms that confer this resistance are not well understood. Gutsche et al. (2009) investigated the physiological response of resistant and susceptible barley cultivars to RWA feeding. Their research indicated that RWA feeding has an impact on the plants ability to assimilate carbon, hence affecting the physiology of the Calvin Cycle (dark reactions) within the plant. They found that the resistant cultivar was able to compensate for this deficiency for a longer period of time as compared to the susceptible cultivar.

Although there is a tremendous amount of work going into developing resistant varieties, different biotypes of RWA have been identified (Haley et al. 2004). When deploying resistance in a RWA management plan, it is important to understand that available resistant varieties may not be resistant to all RWA biotypes. The identification of resistant characteristics and utilization of resistant germplasm coupled with appropriate chemical use and cultural practices can greatly reduce RWA populations and enhance long-term control measures.

Chemical Control

The use of insecticides to effectively control the RWA has presented a significant challenge to producers. Due to the feeding habit of these insects, chemical control measures have been limited to systemic insecticides (e.g. disulfoton and dimethoate), insecticides with vapor action (e.g. chlopyriphos), and some insecticidal seed treatments (e.g. imidacloprid) (Tolmay 2006).

Pest management strategists recommend refraining from chemical use until RWA populations have reached economic thresholds. Fields that have 5-10% infested tillers prior to boot, 10-20% after boot, and greater than 25% after flowering would benefit from an insecticide application (McVay et al. 2008). Often infestations are isolated to discrete areas within a field. In this case, thresholds should still be utilized, but spot treatments of the infested area are advised (Hein et al. 1989, Peairs et al. 2012). This enables the producer to limit aphid populations while ensuring natural enemy populations are maintained.

RWAs often move in from infested host plants along the borders of field and can often be detected in these areas prior to an epidemic. The immigration of flying RWA in both the spring and fall can be unpredictable, therefore scouting fields regularly will aid in identifying potential threats. Producers that detect RWA in their fields should monitor infestation density and use appropriate economic thresholds to make treatment decisions. Advantages of early detection include minimizing crop damage and maintaining effectiveness of chosen control methods. Under continued infestation pressures that warrant insecticide use, it is important to rotate modes of action in order to prevent the evolution of chemical resistance.

Cultural Practices

Cultural practices can be very useful and effective in reducing population size and spread of the RWA. Tactics that include the use of resistant cultivars can limit the rate that RWA populations can increase. This, coupled with crop rotations that alternate between host and non-host species, will greatly reduce insect pressure. Controlling volunteer wheat and barley will eliminate refuges between rotations (McVay et al. 2008). Managing the presence of alternate hosts on the edges of fields can also reduce RWA populations.

Adjusting planting dates can reduce the severity of the impact of RWA infestations. Planting spring grains as early as possible enables the plant to reach a more mature growth stage before immigration of RWA. This enables the plant to better withstand an infestation and deters the aphids from establishing colonies (Hein et al. 1989, Hodgson and Karren 2008). It is also recommended to avoid early planting of winter cultivars to extend RWA oversummering period. This will allow the crop to become established and achieve maximum vigor going into the winter with decreased insect pressure (Peairs et al. 2012). Selecting cultivars that are well-suited for the growing area and maintaining plant health by reducing water and nutrient stress will make the plant less susceptible to RWA damage. Combining cultural techniques can significantly reduce RWA densities and preserve crop health and yield potential.

Biological Control

A number of different RWA natural enemies have been detected. These natural enemies include predators such as: ladybird beetles (*Coccinella magnifica* Redtenbacher),

green lacewings (*Chrysoperla* spp. L.), parasitic wasps (*Diaeretiella rapae* M'Intosh, *Alloxysta* spp., *Asaphes* spp., and *Aphelinus* spp.), and syrphid fly larvae, and parasitic fungal pathogens (*Entomophthora chromaphidis* Burger & Swain, *Pandora neoaphidis* Remaudiere & Hennebert, and *Conidiobolus obscures* Hall & Dunn) (Carver 1989, Wraight et al. 1993). Although there are many biological agents that feed on or parasitize RWA they are generally not effective in controlling populations. This is in part due to their low numbers and inability to gain access to colony because of the protection of the rolled leaf. Current research is underway to find better ways of implementing these natural enemies into an integrated management program.

Although insects are rarely responsible for major economic losses in barley production, they can cause serious damage under favorable conditions. When favorable conditions result in increased pest populations it is advantageous to have an integrated pest management (IPM) plan already in place. An effective IPM plan is an ecologically based strategy that reduces populations below levels that cause economic damage. This reduction in population density is achieved by incorporating multiple management tactics such as the use of resistant varieties, cultural practices, biological control, and selective pesticides. Utilizing an ecologically based IPM plan reduces pest pressure while maintaining environmental quality.

Fungal Pathogens

Plant diseases can severely impact malting barley quality and yield. Disease control is largely based on preventative measures because chemical controls are often ineffective or not economical after an infection has begun. There are a number of

pathogens that cause disease in barley. Many of them infect the root system and prevent the uptake of water and nutrients. Others infect seedlings, preventing them from emerging or becoming established. Some diseases cause foliar damage that compromises the plants ability to efficiently conduct photosynthesis. This paper will cover two fungal pathogens that infect the floral rachis of barley. Both of these pathogens compromise grain quality and produce mycotoxins that are harmful if consumed by humans. In malting barley production there is very little tolerance for these diseases and if they are detected the grain is likely to be rejected at the elevator.

Fusarium Head Blight

Fusarium head blight (FHB) (*Fusarium graminearum* Schwabe (anamorph)) (*Gibberella zeae* (Schwein) Petch (teleomorph) (Ascomycota), also known as scab, is a plant disease caused by the pathogen *Fusarium graminearum*. This pathogen is a destructive disease of barley that is most severe in growing regions that exhibit high temperatures and relative humidity during heading. These regions include the states of the upper Midwest (Minnesota, North and South Dakota, Illinois, Indiana, and Ohio), Montana, and into Canada (Manitoba) (Agrios 2005, Burrows 2012). This pathogen decreases yield, reduces grain quality, and produces a mycotoxin that makes grain unusable. Prior to 1993, brewers in the United States had obtained 75% of their malting barley from North Dakota, South Dakota, and Minnesota. In 1993 these states experienced a FHB epidemic that has since diminished their ability to grow malting barley in the region. Between 1993 and 1998 barley producers lost a total of $406

million in net revenue due to this disease. These states have collectively lost 73% of the nationwide malting barley market (Windels 2000).

The primary symptom exhibited as a result of FHB infection is bleaching of the floral rachis accompanied with a pinkish mold (sporodochia) growing at the base of the spikelets (*Figure* 4.3). The pathogen infects the spikelets making the developing kernels shrivel and turn white (scabby). These scabby kernels are rarely viable, resulting in poor malt performance, and therefore, the grain is rejected at the elevator. *F. graminearum* produces deoxynivalenol (DON), a harmful mycotoxin if consumed by human or livestock. This mycotoxin, often referred to as "vomitoxin", causes nausea (vomiting), gastroenteritis, diarrhea, immunosuppression, and fever (Sobrova et al. 2010). The U.S. Food and Drug Administration (FDA) has imposed strict regulations on the amount of DON that can be present in grain. Products that are directly consumed by humans cannot have more than 1ppm DON; whereas, grain that is going for animal feed cannot have more than 5-10 ppm (Burrows 2012). Malting barley has even tighter industry standards, requiring DON levels to be under 0.5 ppm (Windels 2000). Beer brewed from grain with elevated DON levels is susceptible to spontaneous "gushing" (Mallett 2014). It is undesirable at the bar, and generally frowned upon in the industry, to have a vomitoxin in your beer that makes it gush upon opening.

Fusarium head blight spores are introduced into a field via wind or through infected seed. Once in the field, this pathogen overwinters on infested crop residue from the previous year. Infectious spores reside on the residue within either asexual sporodochia or sexual (*G. zeae*) perithecia (Tekauz et al. 2000). Under favorable conditions, macroconidia or ascospores are forcibly ejected from the sporodochia or

perithecia, respectively. Infection can occur any time from floral anthesis through the soft dough stage of kernel development. Spores enter the plant through natural openings (e.g. stomata) and need to be in contact with soft tissues such as the pistil or stamen to elicit an infection (Leonard and Bushnell 2003). Once the spores make contact with receptive tissues, they germinate, and hyphae invade the developing seed. Successful infection entails hyphal penetration of the seed coat, endosperm colonization, and eventual seed death (Leonard and Bushnell 2003).

The success of this pathogen is dependent on extended periods of high relative humidity (>90%) and warm temperatures (between 15-30˚C) before, during, and after anthesis. If inoculum is present, chances of infection increase if there has been three or more rain/irrigation events from anthesis until 3-5 days post-anthesis (Burrows 2012). To maintain profitability, it is important for a grower to scout fields regularly to identify infection early on. If an infection is identified, management strategies to reduce inoculum or prevent future outbreaks can be employed. These strategies include an integrated approach that utilizes the adoption of less susceptible cultivars, fungicides, cultural practices, and forecasting.

Crop Resistance

Unlike wheat, identifying sources of resistance to FHB has been challenging in barley. One cultivar, 'Quest', has been released from the University of Minnesota (UMN) (McMullen et al. 2012). Breeders at UMN identified resistant traits that trace back to China and Switzerland and were able to introduce these characteristics into Quest (McMullen et al. 2012). Although Quest has been developed there is generally a lack of

resistant varieties for growers to choose from. Without having varieties that are resistant

to FHB, growers have discontinued barley production in regions where environmental

conditions perpetuate disease.

Cultural Practices

Most cultural practices are focused on avoiding or limiting exposure of vulnerable

tissues to *F. graminearum* infection. This pathogen survives saprophytically on the

residue of plants, such as corn and other small cereal grains (Leonard and Bushnell

2003). One method of avoiding infection is to manage infected plant debris. This can be

achieved by mechanically chopping residue to expedite the decomposition rate, or

employing tillage practices that bury infested residue (McMullen et al. 2012). Crop

rotations that incorporate the use of non-host species can significantly reduce inoculum

load. Combining crop rotations with residue management practices has been shown to

decrease FHB infestations up to 30% (McMullen et al. 2012).

The spores of *F. graminearum* are disseminated from the source of primary

inoculum via wind and water. Burrows (2012) recommends ensuring the crop is

adequately watered prior to anthesis and suspending irrigation practices immediately

before and throughout the time period during anthesis. Other growers have spread the

risk from FHB by staggering planting dates or using cultivars with different maturation

times. This ensures variable flowering dates, reducing the chance that weather conditions

favoring *F. graminearum* infection would affect their entire crop (McMullen et al. 2012).

Although cultural practices can reduce the amount of local inoculum, *F.

graminearum* spores can be carried by wind currents for long distances. If the

atmospheric inoculum load is high, the reliance on long distance predictive models and the preventative use of fungicides become increasingly more important when managing FHB.

Fungicide Intervention

The U.S. Wheat and Barley Scab Initiative established research plots across multiple states where Fusarium head blight was a problem. Through the Uniform Fungicide Trials, researchers found that locally systemic demethylation inhibitor fungicides (i.e. triazole, prothioconazole, and metconazole) were the most effective in suppressing outbreaks of *F. graminearum* infection (52% control compared to untreated check; (Mueller 2006, McMullen et al. 2012). The timing of application and coverage of vulnerable tissues was critical in achieving control. Barley undergoes anthesis while the head is still in the leaf sheath (boot). If a field has a history of FHB or long distance predictive models indicate that FHB risk is high, it is recommended that fungicides be applied before the head emerges from the boot (McMullen et al. 2012). The locally systemic fungicide will penetrate the floral tissues and provide added protection in the event of high inoculum loads.

Ergot

Ergot (*Claviceps purpurea* (Fr.) Tul.) (Ascomycota) is a plant disease caused by the fungal pathogen *Claviceps purpurea*. This pathogen infects the floral spikelets of cereal grains and grasses. Upon infection, the florets become replaced by the development of fungal masses called sclerotia (*Figure* 4.4). These sclerotia produce

toxic alkaloids that cause ergotism if consumed by humans or animals. Symptoms of ergotism include seizures, spasms, psychosis, and vasoconstriction resulting in insufficient blood flow to the extremities (Peraica et al. 1999). Often the lack of blood supply to these tissues results in the loss of limbs. Under severe disease pressure, ergot infections can cause 5-10% crop yield loss and severely impact the quality of the grain (McMullen and Stoltenow 2002, Wegulo and Carlson n.d.). Malting barley is considered "ergoty" when more than 0.1% by weight of the grain are sclerotia (McMullen and Stoltenow 2002). Grain containing excessive amounts of sclerotia is subject to rejection, rendering the entire crop worthless.

 C. purpurea ascospores are dispersed by wind and must come into contact with open florets in order to initiate infection (McVay et al. 2008). Chasmogamous (open florets) varieties are more susceptible to ergot infection than cleistogamous (closed florets) varieties. Under environmental stress, such as drought and excessive heat, barley pollen can become sterile. Cleistogamous florets that do not become fertilized as a result of sterile pollen often open to increase the chances for pollination, and this makes them more susceptible to ergot infection (Barkley 2008). Barley grown in copper deficient soils has shown to have a decrease in pollen fertility which increases the propensity of the florets to open (Dell 1981). Increased ergot infection has been strongly correlated with soils that are copper deficient (Barkley 2008).

 C. purpurea infection begins with the ascospores being dispersed by wind, rain splash, or insects and deposited on spikelets. The spores land on the stigma, hydrate, and germinate. The germ tube releases the enzyme catalase, which catalyzes the decomposition of hydrogen peroxide, suppressing the host defenses (Agrios 2005).

Infection ensues as the germ tube grows down the pistil tissue and into the ovary. Within seven days the pathogen has established a mycelial mass (sphacellum) and formed sporodochia that begin to ooze honeydew (sticky sweet liquid) impregnated with conidial spores (Agrios 2005). The honeydew drips out of the spikelets and onto other open florets perpetuating secondary infection. Insects are attracted to the honeydew to feed. As they feed conidia adhere to their abdomen and are then transferred to uninfected spikelets (Lemon 1992). As the sphacellum matures, it becomes a hard, purplish-black, mycelial mass called sclerotia. Sclerotia mature within the same time frame as the developing grain. At harvest, the sclerotia remain on the rachis and end up mixed in the harvested grain. If the grain is intended for seed stock, the infested seed will act as inoculum the following year. Sclerotia that fall to the ground go dormant and overwinter until ideal conditions stimulate germination.

Spring moisture triggers the sclerotia in the soil to germinate. Multiple mushroom-shaped fruiting bodies grow from the fungal mass. The stromatic ("mushroom" cap) heads of these fruiting bodies are covered with perithecia that are packed with ascospore filled asci (Agrios 2005). As the fruiting bodies mature, and take up water, turgor pressure builds within the asci. Eventually ascospores are discharged from the asci and are disseminated by wind, water, or insects and the disease cycle starts over (Kirby 1998).

The success of this fungal pathogen is dependent on host susceptibility, environmental conditions, and timing of infection. The manipulation of any of these elements by the use of fungicide intervention, resistance varieties, cultural practices, and biological control can disrupt the disease cycle and eliminate future ergot infestations.

Fungicide Intervention

Fungicides are not generally an economically feasible management option in ergot infested fields. Puranik and Mathre (1971) conducted a study that looked at foliar fungicide applications as well as soil drench systemic fungicide applications. They identified that the foliar applied fungicides have to be in direct contact with the surface of the ovary prior to infection to be effective. Barley varieties that open their florets generally hold them open for 10-15 days, at which time protective fungicides should be applied (Puranik and Mathre 1971). This becomes difficult, and increasingly expensive, if multiple applications are needed. Additionally, spray applications do not effectively penetrate the florets yielding potentially wasted chemicals without achieving the desired protection. Puranik and Mathre (1971) did observed a 35-56% decrease in infection when 1-2 lbs/acre was diluted with 100 gallons of water and sprayed three different times over a period of 18 days (Puranik and Mathre 1971). Although disease pressure was decreased, it is important to consider whether the cost of fungicide, coupled with application expenses, economically warrants fungicide use. It is recommended that alternative management strategies including the utilization of resistant varieties and cultural practices be employed when controlling ergot.

Crop Resistance

Most commercially grown barley cultivars have been bred for improved agronomic performance and quality attributes, and this has resulted in the reduction of genetic diversity across elite lines (OGTR 2008). Pageau et al. (1994) conducted a study comparing the level of resistance to ergot across thirteen different commercially grown

barley cultivars. The results indicated that genetic variation in ergot resistance may be attributed to morphological and physiological characteristics (Pageau et al. 1994). Susceptibility may decline after pollination due to biochemical changes that alter stigmatic acceptance after the ovary has been fertilized (Pageau et al. 1994). Other resistance mechanisms include physical attributes such as chasmogamy or fertilization that occurs while the head is still in the leaf sheath. Chasmogamy provides resistance protecting the ovary with the floral structures and avoiding infection. Currently there are no barley varieties that are rated as being ergot resistant (McMullen and Stoltenow 2002).

Cultural Practices

Cultural practices have shown to be the most economic and effective strategies for managing ergot. These tactics include the utilization of clean seed stock, cultivars with resistance characteristics, management of alternate hosts, crop rotation, and tillage.

Ensuring that the seed stock is free of ergot sclerotia eliminates the threat of introducing ergot to a field that does not have a previous history of infestation (USASK n.d.). Using barley varieties that exhibit uniform heading, and do not open their florets can decrease chances of infection and reduce the establishment of secondary infections over the course of a growing season (Barkley 2008, USASK n.d.).

Careful management of secondary host weed species can reduce inoculum reservoirs. Annual bluegrass, meadow foxtail, tall fescue, and wild rye are common weed species that are susceptible to ergot infection (McMullen and Stoltenow 2002).

Ergot sclerotia can only undergo dormancy once and do not survive after one year. Crop rotational schemes that incorporate non-host species in the year following

heavy ergot infestation are recommended. It is important to control volunteer host species during this time (McMullen and Stoltenow 2002). Additionally, sclerotia do not survive if buried deeper than one inch. McMullen and Stoltenow (2002) suggest deep plowing fields that have had severe ergot infestations.

Biological Control

The shining flower beetle (*Acylomus pugetanus* Casey) has been identified as a potential ergot biological control agent. This beetle's life cycle is directly dependent on ergot sclerotium. *A. pugetanus* seeks out developing ergot sclerotia, feeds on the honeydew, and lays its eggs on the surface of the mycelial mass (Steiner Jr and Singh 1987). The larvae develop inside the mycelial mass, consume the fungal tissue, and render the mature sclerotium unviable. Sclerotia that are no longer viable do not sporulate the following season and the inoculum load is significantly reduced.

There are a number of plant pathogens that can compromise malting barley yield and quality. Management strategies that prevent the occurrence of disease or decrease inoculum load are preferred over treatment. Often crop rotations that incorporate non-host species can break the disease cycle and prevent future infection. Changing watering regimes or methods (e.g. drip vs. overhead pivot), can reduce the moisture within the canopy making the environment less hospitable for pathogens. Managing infected plant debris can have a substantial impact on the amount of inoculum that is present within a field. When disease does occur, prompt identification is critical so that alternative methods of management, such as selective fungicides, can be employed. In regard to

disease management, the efficiency at which the disease is identified and managed will

mitigate major epidemics.

Figure 4.1 – Russian wheat aphid infestation: Demonstrating leaf symptomology – leaf curling, purpling of tissue, and the development of yellowish-white streaks. Photograph taken by Frank Peairs, Colorado State University, Bugwood.org.

Figure 4.2 – Russian wheat aphid: Demonstrating the lack of visible cornicles and the presence of the "double tail" (cauda and supracaudal process). Photograph taken by Frank Peairs, Colorado State University, Bugwood.org.

Figure 4.3 – Fusarium Head Blight (*Fusarium graminearum* Schwabe) symptomology on barley. Photograph courtesy of North Dakota State University Plant Pathology Department. http://www.ag.ndsu.nodak.edu/aginfo/barleypath/fhbdisease.html

Figure 4.4 – Ergot (*Claviceps purpurea* (Fr.) Tul.) sclerotia on barley. Photograph courtesy of the Pilzegalerie http://www.pilzepilze.de/galerie/v/Lateinisch/C/claviceps/purpurea/3.html

CHAPTER 5

DOCTORAL INTERNSHIP

The University of Nebraska – Lincoln Doctor of Plant Health program requires each student to fulfill a professional internship over the last summer of the program. For my internship, I worked as a Barley Scientist Intern for MillerCoors and Golden Malting in Golden, Colorado.

MillerCoors is a joint venture between SABMiller and Molson Coors that began operating as a combined entity in 2008. This union made MillerCoors the second-largest beer company in the USA, offering brands such as: Miller Genuine Draft, Miller Lite, Miller High Life, Coors, Coors Light, Killian's, Blue Moon, Molson Canadian, Leinenkugel's, and many more (MillerCoors 2015). These beers are brewed in nine breweries scattered throughout the United States. Breweries are supplied with all of the raw materials needed to make the various beers through a large network of barley and hop growers that make up the MillerCoors supply chain (MillerCoors 2015).

MillerCoors focuses on procuring the highest quality raw materials for making their beer. This pursuit of quality ingredients was the basis for the establishment of the MillerCoors Grower Direct Program. Through this program the company contracts with 864 independent barley growers in Colorado, Idaho, Montana, and Wyoming (MillerCoors 2015). These growers produce as much as 75% of the barley that is used in the MillerCoors breweries across the United States. Farmers grow barley varieties that have been developed through the MillerCoors Barley Variety Development Program, and their barley is expected to meet the highest quality standards (MillerCoors 2015). Quality

standards for barley include plumpness, color, protein, and moisture, and it also must be damage and disease free (Rockhold n.d.). After harvest each year, grain that has met these quality standards, is stored in one of the seven MillerCoors owned grain elevators or one of seven secondary partner receiving stations. After reaching these facilities, barley is stored between 1-8 months until it is requested for processing.

Upon request, the barley is transferred via truck or railcar to the Golden Malting facility, located in Golden, Colorado. This malt house is the fourth largest in the nation and produces approximately 250,000 metric tons of malt per year (Mallett 2014).

At multiple points throughout the process of making malt, barley/malt samples are submitted to the barley quality lab which is located within the malt house in Golden, CO. Here, barley scientists and chemists quantify the germination potential of the seed and determine the chemical composition of the malt (e.g. diastatic power, alpha amylase, free amino nitrogen, and enzyme activity). These elements of malt are of primary interest so that processing consistency can be maintained and ultimate performance in the brew house can be predicted.

During the four months I worked for Golden Malting, I had the opportunity to participate in four research projects that involved lipoxygenase (LOX) activity: 1) through germination while making green malt (germinated seed); 2) in malted barley over the 28 day aging period; 3) across malted barley varieties; 4) in malted barley from the breeding program.

LOX is an enzyme that catalyzes the oxygenation of polyunsaturated fatty acids in germinating barley seed. This enzyme is of much interest in the brewing industry because it oxidizes the available linoleic acid, which is 54 percent of the unsaturated fatty

acid present in the geminating grain, and forms conjugated hydroperoxides (Youssef et al. 2012). Some of these hydroperoxide products further degrade, yielding the compound trans-2-nonenal (T2N). T2N is attributed to cause the cardboard, or papery, off flavors described in 'stale' beer (Barker et al. 1983).

The first LOX assay was conducted to determine LOX activity over the course of germination as it becomes green malt. Here, we measured LOX activity every 24 hours up to 96 hours during germination. We observed LOX activity increase up to 86% across tested varieties (*Figure* 5.1). Due to proprietary restrictions the specifics of these trials will not be discussed.

The second trial involved observing LOX activity in malted barley over a 28-day aging period. Samples were taken and assayed every seven days after the malt had been removed from the kiln. The data suggested that over the 28-day aging period, LOX activity decreased up to 40% across all surveyed varieties (*Figure* 5.2). Again, further investigation will be needed to determine a definitive outcome of the data.

After conducting the first two portions of this project, we wanted to see the trend in LOX activity over the course of the entire malting process. Here, I conducted LOX analysis on the quiescent barley grains of each of the selected varieties. I plotted the LOX activity of the quiescent grain along with the increase in LOX activity during germination and the decrease in LOX activity over the aging process. Combining these graphs enabled us to visually observe the trend in LOX activity over the entire malting process. There was an increase in LOX activity during germination, a sharp drop (~90%) in LOX activity as a result of kilning, and a continued gradual decline over the 28 day aging process (*Figure* 5.3).

The third project was a survey of LOX activity in aged malt from different barley varieties grown across growing regions. Preliminary data revealed that some varieties have higher LOX activity than others, but statistically significant differences were not observed, and it could not be determined if differences were correlated with growing region. Without further investigation and increased sample size, the information was valuable, but inconclusive.

The last project entailed surveying barley lines that had been derived through the MillerCoors breeding program. Fifty different samples were micro-malted (malted in small batches) and analyzed for LOX activity. The samples fell within one of three defined LOX activity categories (low, medium, and high) (*Figure* 5.4). Due to the diverse response of theses samples, green malt samples were tested to see if high LOX activity could be determined prior to the completion of the malting process. LOX activity in green malt was found to be equally high across all barley lines. These results indicated that lines with high LOX activity after micro-malting had enzymes that were more thermostable, and persisted to a greater extent through kilning. This information was relayed to the breeder to provide more information for barley parental line selection. The breeder has since initiated a more detailed genetic study on one of the lines that were determined to have low LOX activity after malting.

In addition to conducting the enzyme assays, I also obtained an in depth understanding of barley production with MillerCoors. Annually, MillerCoors hosts "Barley Days" in appreciation of their barley growers. Barley Days are held at MillerCoors owned grain elevators and are rotated on a year to year basis across growing regions. These events are community oriented, fun, and informative. MillerCoors

obviously provides beer for everyone, but there is also food, and informational booths to educate farmers on the importance of grain quality in the malting process. I had the opportunity to engage with growers and talk to them about their production practices. We discussed various management strategies and talked about sustainable production practices (e.g. crop rotations, tillage practices, etc.).

I also had the opportunity to visit the barley breeding research farm in Burley, Idaho where I met with the breeder and discussed variable aspects of the MillerCoors breeding program (*Figure* 5.5). From Idaho we traveled back to Colorado where I went to the agronomic research farm in Monte Vista. On this farm, they conduct various trials that contribute to the MillerCoors Sustainable Agriculture Program. Here, they identify the best management practices by employing sustainable options, and disseminate this information to their contract growers. Overall my summer internship with MillerCoors/Golden Malting was an excellent experience where I was able to obtain a complete understanding about malted barley, literally from the ground up!

Synthesis

The objective of the Doctor of Plant Health program is to produce plant practitioners with broad expertise and experience across the various disciplines (plant science, entomology, plant pathology, soil science, and agronomy) that impact plant health and production. The goal of the final internship experience is to enable the student to utilize their academically derived expertise in an applied setting. The doctoral document provides the student with an opportunity to take the information that was

gained over the course of the final internship, synthesize it, and elaborate on aspects of the experience that the student wants to more fully understand.

Working at MillerCoors/Golden Malting provided me with the opportunity to learn about malting barley production with an emphasis on the importance of grain quality. I was able to visit the various growing regions where malting barley is produced. Here, I had the opportunity to engage with barley growers and establish a fundamental understanding of barley production and learn about the challenges growers face on an annual basis. The time I spent working in the barley quality lab enlightened me to the importance of grain quality to the malting industry. Overall, this internship provided me with further insight into the influence production practices have on grain quality.

Through this doctoral document, I have synthesized the information I gained from my summer internship, and elaborated on some of the more finite aspects of production. Additionally, I addressed some of the seasonal challenges growers experience in regard to pest and pathogen management. The malting industry is dependent on barley that meets all of the industry quality standards. These quality attributes are directly correlated with production practices and pest/pathogen management.

By having a complete understanding of how production practices contribute to the quality of malting barley, future efforts can be made toward resource management and sustainable production. Water is often a limited resource in regions where malting barley is grown. Management practices that maintain the available soil moisture, such as reduced tillage, precision irrigation, and eco-fallow may be options to investigate in the future. Implementing crop rotational schemes can often enhance the soil profile and contribute to nutrient retention. Cover cropping has the potential to reduce soil erosion

and prevent weed species from becoming established. To ensure a continual supply of

high quality barley for years to come, an integrated approach to sustainable production
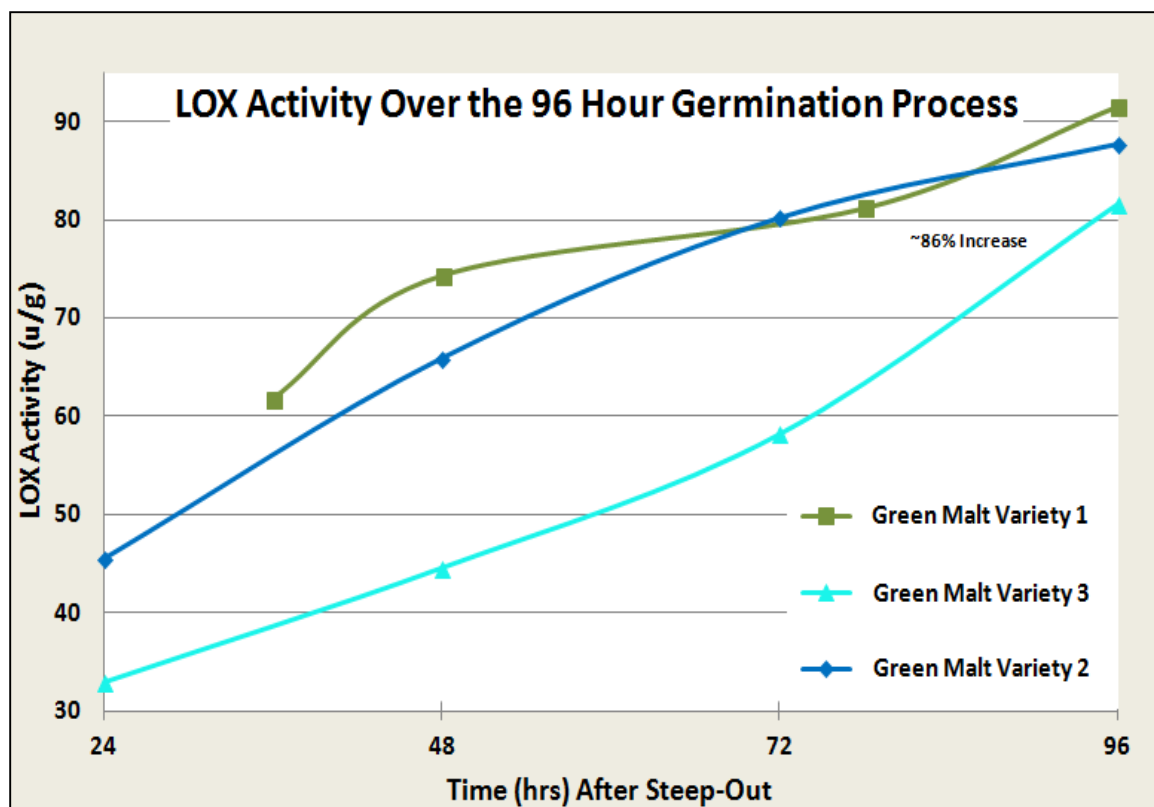
should be pursued.

Figure 5.1 - LOX activity over 96 hour germination process. Depicting ~ 86% increase across tested varieties.
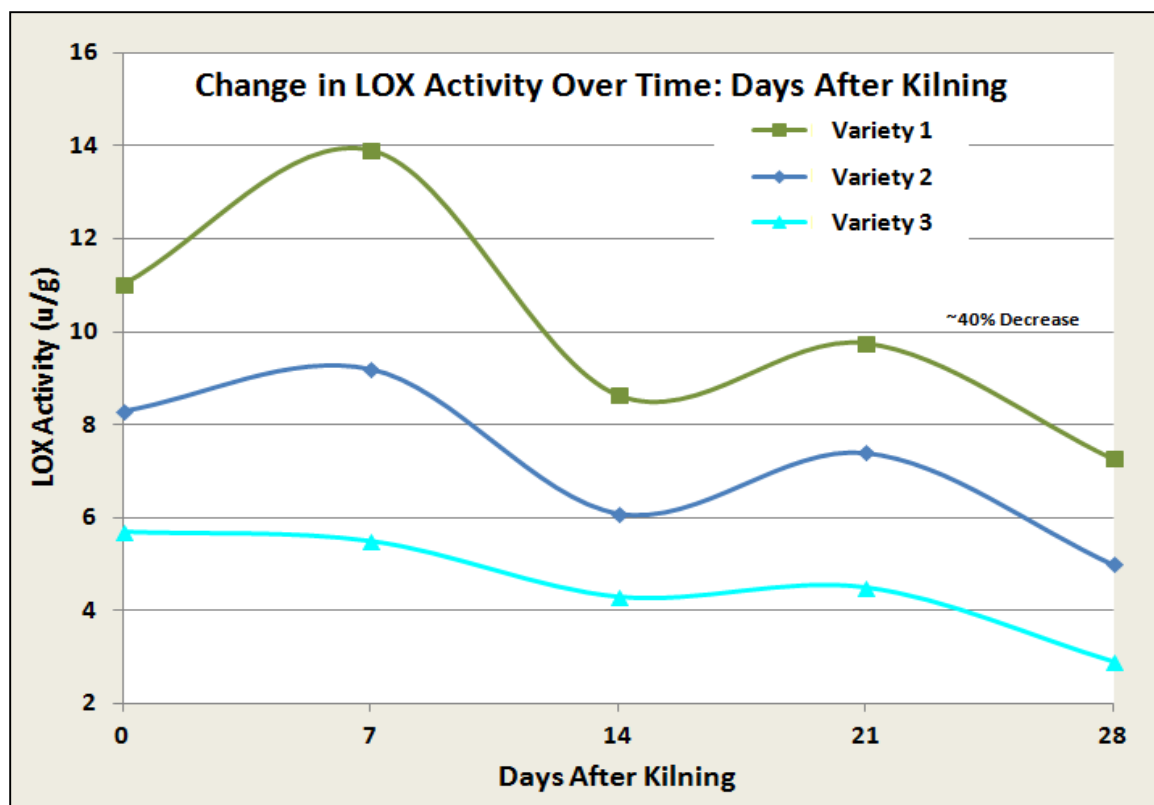
Figure 5.2 - LOX activity over 28 day aging process. Depicting ~ 40% decrease across all surveyed varieties.
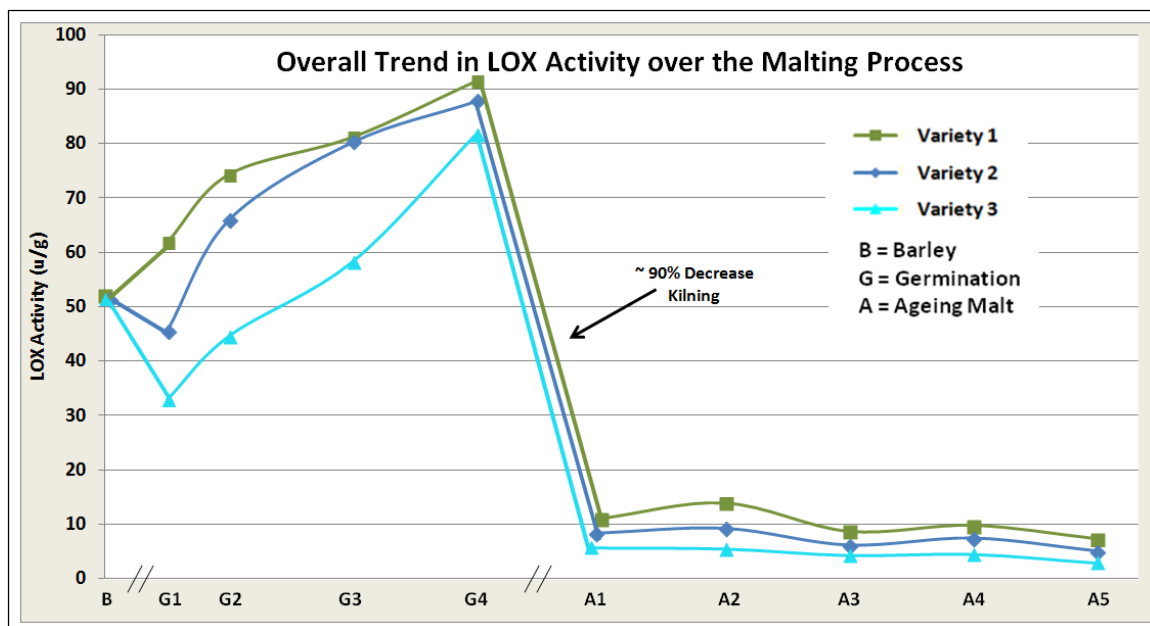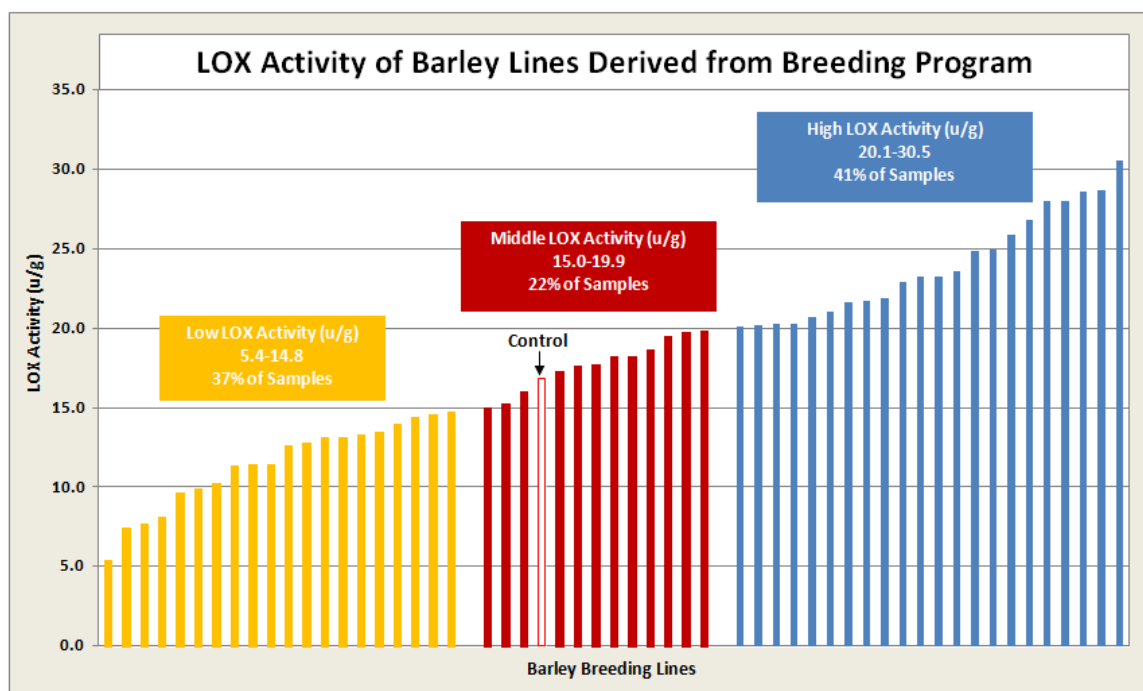
Figure 5.3 – Overall trend in LOX activity in tested varieties over the entire malting process. LOX is present in quiescent barley grain, increases during germination, drops ~90% over kilning, and continues to decrease during aging.

Figure 5.4 – LOX activity of different barley lines derived from the MillerCoors barley breeding program.   Samples fell within one of three defined LOX activity categories (low, medium, and high).

Figure 5.5 – MillerCoors breeding program Burley, Idaho. Photograph taken by Haley Oser.

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