ABSTRACT

WHELAN, JOSHUA CRAIG. Nitrogen Management of Malting Barley in North Carolina. (Under the direction of Dr. Amy Johnson)

Barley, *Hordeum vulgare* L. has historically been grown throughout the southeast as a source of animal feed. However, increases in craft breweries in North Carolina (NC), 300+, has produced a demand for locally sourced, malt quality barley and an opportunity for farmers to achieve premium prices. Quality criteria demanded by the malting industry consists of a grain protein content within 9.5-12.5% and a grain plumpness of greater than 80%. Protein content and plumpness have been correlated to nitrogen (N) rates with higher N rates causing increased protein content and decreased plumpness. The objective of this study was to determine N application rates which optimize both yield and quality parameters across NC. Experimental plots of Violetta 2-row winter barley were planted at four locations across the state, representing NC's different physiographic regions during the 2017/2018 and 2018/2019 growing seasons. A randomized complete block design was used with experimental treatments consisting of six total nitrogen (N) rates, ranging from 0 to 167 kg N ha^{-1} (0-150 lbs N ac^{-1}), split applied at three different growth stages. Grain yield and malt quality indicators, including grain protein and kernel plumpness, were measured to determine the optimal N rate with respect to yield and malting quality goals. Plant tissue N was measured at specific growth stages in order to assess the potential of using tissue N as a predictor of final yield and protein content. Results demonstrate that increased N rates generally caused significantly higher barley yields but that yield plateaued at an N rate of 135 kg N ha^{-1} (120 lbs ac^{-1}) in all site-years save one. Greatest yields were found in sites having medium to fine textured soils, while sandier sites did poorly. Location and year-to-year variability greatly affected barley yield although malt quality parameters remained within acceptable limits in the majority of site-years except at the highest N

rate regardless of N rate or environmental factors, suggesting that Violetta is well adapted to NC. Tissue N content was highly variable from year to year and by location and did not consistently correlate with quality parameters. Additional studies are needed to determine whether N application timing can be further optimized and to evaluate the adaptability of other malting barley varieties for NC.

Nitrogen Management of Barley for Malt Quality in the Mountains, Piedmont, Sandhills, and Coastal Plain areas of North Carolina

by
Joshua Craig Whelan

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Soil Science

Raleigh, North Carolina
2020

APPROVED BY:

____
Dr. Amy Johnson
Committee Chair

____
Dr. Deanna Osmond

____
Dr. Luciano Gatiboni

____
Dr. Angela Post

____
Dr. Kristin Hicks
External Member

DEDICATION

This thesis is for my grandfather Gerald Whelan, the man whose passion for agriculture is what inspired mine, to Caroline James, my wonderful fiancé and future wife, and my parents Craig and Jill Whelan.

BIOGRAPHY

Joshua Craig Whelan was born on September 28, 1995. He was raised and lived in Prairie du Rocher, Illinois on a small farm in the Mississippi River Bottoms, until attending college at Southern Illinois University in Carbondale, Illinois. Here he received a Bachelor's of Science in Crop, Soil, and Environmental Management with a specialization in Soil Science, as well as being exposed to research agriculture for the first time. His interest in helping farmers like his own family led to a research assistantship at North Carolina State University, where he hopes his research will help growers thrive.

ACKNOWLEDGMENTS

Sincere thanks to my committee, Dr. Amy Johnson, Dr. Deanna Osmond, Dr. Kristin Hicks, Dr. Angela Post, and Dr. Luciano Gatiboni. Thanks especially to Dr. Johnson for her help in writing this thesis, and all the roadblocks that came with it. I also really appreciate the spreading of turkey plasma on a windy day. Thanks also to all the NCDA&CS regional agronomists who helped to make this statewide, labor intensive project work. Especially to Chris Jernigan without whose help nitrogen applications may have gone quite a bit differently. Thank you to the NCDA&CS plant analysis lab for all the wet plant tissue samples you had to run, and the labeling of stakes and bags. Thank you too Wes Childres for counting all those tillers. Thank you to Hartwick College and Aaron MacLeod for the assistance in running barley quality tests. Thanks to Dr. David Dickey for helping me with statistics even in retirement. Thank you to the Small Grain Extension group in all your help harvesting. A special thanks to my fiancé Carrie for nagging me into completion, I could not have done it without you. Also, a thanks to the North Carolina Tobacco Trust fund Commission, and to all my colleagues who helped me take tissue samples despite the weather.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: REVIEW OF LITERATURE

Barley Production Worldwide

Barley, *Horedeum vulgare* L., is one of 31 *Horedeum* species from the tribe Triticeae which also includes wheat, rye, and oats (Bothmer et al. 2003). Barley was domesticated from its wild relative *Horedeum spontaneum* and has been cultivated for over 10,000 years with the likely origin being the Fertile Crescent region (Badr 2000). Barley has been grown more widely than any other cereal grain and is capable of growing in regions that are unfavorable to other cereals.

Barley grows best in areas having temperate climates with long, cool and moderate dry growing seasons (Bothmer et al. 2003). Though barley will withstand heat in dry climates, and high humidity in cool climates, it struggles in climates that are both hot and humid largely due to the presence of disease (Klages 1942). Barley's adaptability has caused it to be the fourth most cultivated cereal crop worldwide, being grown over much of the Earth (Poehlman 1985, Newman and Newman 2006, Widmar 2018). The three main uses of barley are animal feed, human food and alcohol, with the predominant use differing by region.

Barley in Africa and the Middle East has been historically grown as a human food source (Newman and Newman 2008). Although it has been an important crop throughout history in all of North Africa, where poor soils and low rainfall often make barley the only harvestable cereal; barley production has gradually consolidated in Morocco with 40% of total African barley being produced here (Poehlman 1985).

Barley in Europe has historically prospered due to the cool climate and fertile soils (Poehlman 1985). This has resulted in the European Union being the number one producer of barley in the world (Newman and Newman 2008). Although the greatest portion of barley is used for animal feed, use for the production of alcohol is also important throughout (Newton et al. 2011).

Barley production in North America accounts for around 11% of total world production and is mainly grown as a spring crop in the cooler climate of Canada (Poehlman 1985). Canada's Prairie Province of Alberta, Manitoba, and Saskatchewan produce the bulk of the country's barley (Poehlman 1985). Though most of Canadian barley is grown for animal feed as 6-row varieties, around 20% is grown for the production of alcohol. Traditionally 2-row varieties were used for producing alcohol in Europe but Canada has primarily uses 6-row varieties for this purpose as better malting 6-row varieties have been developed (Poehlman 1985).

Similar to Canada, barley in the United States (US) is mostly grown as a spring crop where cold winters and mild springs are normal, but it can also be grown as a winter crop in the southeast with a milder winter. Barley is seen as a cheap source of animal feed with 65% of total barley produced being used as such, although it cannot compare to corn in feed efficiency (Poehlman 1985, Newman and Newman 2006). Barley, however, is superior to other cereals for the production of alcohol. Roughly 30% of barley grown in the US is used for alcohol production (Poehlman 1985, Newman and Newman 2006). Barley production has historically moved westward across the US, starting in the Northeast, moving to states like Illinois and Ohio, and finally reaching areas like Minnesota and the Dakotas (Poehlman 1985). This is partially due to the development of new varieties that are more suited for the cooler environment of the Upper Midwest and Great Plains. Today, the top barley producing states are Montana, North Dakota, and Idaho (Mosley 2019).

Barley Growth Cycles

The growth cycle of barley is similar to other cereal grains such as wheat, rye, and oats. According to the Zadoks system of plant developmental stages, primary and secondary growth stages are specified and determined by morphological differences in the plant (Zadoks et al. 1974). This system consists of ten primary stages (0-9) and 100 secondary stages, with ten secondary stages within each primary stage (Fig. 1.1). Primary growth stages recognize large changes in morphology, such as tillering, stem extension, heading, and grain ripening and consist of: (0) germination, (1) seedling growth, (2) tillering, (3) stem elongation, (4) booting, (5) inflorescence emergence, (6) anthesis, (7) milk development, (8) dough development, and (9) ripening (Zadoks et al. 1974). Secondary growth stages represent slight changes within a primary growth stage. For example, within the primary stage of tillering, GS 20-29 on the Zadoks scale, the difference between secondary stages 20 and 21 is an increase of a single tiller (Zadoks et al. 1974, Jackson and Williams 2006; Table 1.1).

Growth stages of interest include Growth Stage (GS) 13, GS 25, GS 30, GS 45, GS 50-75, and GS 75-100 (Table 1.1). Growth Stage 13 is considered to be the three leaf stage and is seen shortly after germination (Zadoks et al. 1974, Jackson and Williams, 2006). At Growth Stage 25 the main stem has five tillers and is seen after the air temperature reaches 50° F (Zadoks et al. 1974, Jackson & Williams, 2006). At Growth Stage 30 jointing and stem elongation begins, and this is when the majority of nitrogen (N) is required (Zadoks et al. 1974, Jackson and Williams, 2006, Weisz et al. 2013). At this growth stage, the grain head begins to form although it is still underground (Zadoks et al. 1974, Jackson and Williams 2006, Weisz et al. 2013). Growth Stage 45 is referred to as boot stage, which is when the seed head is completely encased by the flag leaf, directly before heading occurs (Zadoks et al. 1974). Vegetative growth is minimal past GS 45 and reproductive growth takes precedence (Jackson and Williams 2006, Weisz et al. 2013). Seed head emergence, flowering and grain fill occur between GS 50-75, while grain ripening takes place during Growth Stages 75-100 (Zadoks et al. 1974, Jackson and Williams 2006). Grain reaches harvest maturity at GS 92 and stems begin to collapse by GS 94 (Zadoks et al. 1974, Jackson and Williams, 2006)

Barley, as well as certain other small grains such as wheat, rye, and triticale, begins reproductive growth at the boot stage. The inflorescence, or flowering structure, occurs as a spike, which is made up of individual spikelets. A spike begins to emerge from the flag leaf at GS 50 and is completely emerged by GS 59 (Zadoks et al. 1974). The spike is composed of alternating nodes on either side of the rachis along the length of the spike. A spikelet forms at each of these nodes and is made up of one or more flowers referred to as florets (Fig. 1.2) Barley contains a single floret per spikelet with three spikelets per node (Jackson and Williams 2006, Wrigley 2017). The row orientations of barley are determined by the fertility of the florets in each spikelet. In 6-row barley all florets are fertile resulting in three seeds per spikelet and six spikelet rows going up the spike (Jackson and Williams 2006, Wrigley 2017). In 2-row barley the two lateral florets are sterile, giving just one seed per spikelet, giving the impression of only two rows of spikelets on either side of the spike (Fig. 1.3).

Barley as a Source for Malt

Barley is best known for its use as an ingredient in alcoholic beverages, with 15% of world barley acreage being grown for alcohol production (Magliano et al. 2014). Barley’s importance to alcohol is due to its wide geographical adaptation, ease of processing, and economical pricing (Magliano et al. 2014). In order to produce alcohol, the starches contained in the endosperm of the barley seed must be converted to simple sugars, a process known as malting. These sugars are then used by yeast during the fermentation process of the brewing procedure. The basic malting technique was developed thousands of years ago, but today this transformation occurs through a controlled germination method.

The malting process consists of three main stages: steeping, germination, and kilning (Briggs 1998). During the steeping process the barley grain is subjected to cycles of submergence in water and dry periods of “air rest” over a period of two days (Briggs 1998, Freeman 1999). This initiates seed imbibition, which begins the germination process while also allowing for release of carbon dioxide (Freeman 1999).

After steeping, germination is allowed to proceed at an air temperature of 14-18°C and high humidity to prevent moisture loss (Freeman 1999). The conversion of endosperm starch to simple sugars occurs when germination begins, as enzymes that breakdown starch are activated within the seed (Paterson et al. 2003, Izydorczyk and Edney 2017). This process is referred to as endosperm modification. Germination must be stopped before the grain begins to sprout, which is done by drying and heating the seed, a process known as kilning (Briggs 1998, Paterson et al. 2003). The grain is first dried at room temperature, reducing moisture and inactivating seed enzymes. After air drying, the grain is heated to 100°C, in a process known as curing (Freeman 1999). The degree of curing establishes the color and flavor for a particular finished beer product. Kilning takes 24 to 36 hours to complete and the grain will have a final moisture content of around 4% (Freeman 1999). After the last stage of kilning, the barley is considered “malted” and can be stored until sale to brewers.

Both 6-row and 2-row barley are used for the production of malt with the type used depending on geography (Briggs 1998, Freeman 1999). In Europe 2-row barley has historically been grown for malting because of its naturally lower protein content, lighter color and greater volume of fermentable sugars compared to 6-row barley (Briggs 1998, Freeman 1999). In contrast, 6-row barley is typically used to make malt in the US and Canada because those varieties are more suitable for the climate of North America (Freeman 1999). Overall, the choice of malt barley variety is based on brewer preference, but a recent interest in European style beer by the craft beer industry has created a market for malt quality 2-row barley in the US.

Quality Standards

Barley grown for malt must meet strict quality standards. These standards are set to ensure a quality final product and to improve the efficiency of converting the barley into malt (Briggs 1998, Edney et al. 2012, MacLeod 2016, Izydorczyk and Edney 2017). To assess the quality of malting barley, the following factors are examined: germination rate, protein content, grain moisture, grain plumpness, varietal uniformity and disease (MacLeod 2016, Izydorczyk and Edney 2017, Table 1.2). Less than ideal quality of the raw barley, such as high protein or low plumpness, can negatively impact the malt, which may cause hazy color, an off taste, and a decreased volume of usable end product (Briggs 1998, Freeman 1999, MacLeod 2016). Malt quality barley can demand high premiums by malt houses, up to $8-$12 per bushel of barley, whereas barley sold for animal feed in the Southeast sells for 90% of the current price of corn. Producing barley grain that meets the malting quality standards, therefore, offers a farmer a potentially lucrative value-added product above feed barley.

For malting to be successful, the raw barley seed must have a high germination rate (MacLeod 2016, Izydorczyk and Edney 2017). The standard germination test performed by the malting industry is called Germination Energy and is used to determine how much of the barley seed will germinate in the malt house (MacLeod 2016). This test is completed over a period of 72 hours with any germinated seed being removed each 24-hour period. An acceptable result is seed having at least 95% germination after 72 hours (MacLeod 2016, Izydorczyk and Edney 2017). Germination also needs to occur uniformly, because uneven germination lowers quality and quantity of the usable end product (Briggs 1998). Maintaining a uniform germination rate over an entire malt is often an indicator of varietal purity. Different varieties of barley have different levels of dormancy, and malts should always use a single variety of barley (Freeman 1999, Paterson et al. 2003). Another reason for the requirement of varietal purity is that many varieties, even varieties within a specific row orientation, can have naturally varying protein levels (Grant et al. 1991, McKenzie et al. 2005, Edney et al. 2012, O'Donovan et al. 2015). Therefore, it is impermissible within the industry to blend grains of different protein contents, as it leads to uneven germination and differing malt quality (Freeman 1999, AMBA, 2011, MacLeod 2016, Izydorczyk and Edney 2017).

Plump kernels are desirable because they have a higher starch content, which translates to a greater amount of malt extract, the fermentable sugars (Freeman 1999, Edney et al. 2012, MacLeod 2016, Izydorczyk and Edney 2017). High kernel plumpness increases the total volume of beer produced per weight of malt by increasing the total extract available (Edney et al. 2012, MacLeod 2016, Izydorczyk and Edney 2017). Ideally, greater than 80% of kernels should be stopped by a 6/64$^{\text{ths}}$ sieve for 2-row barley (MacLeod 2016, Izydorczyk and Edney 2017). Plumpness levels below this may have insufficient starch to produce a quality malt.

The protein content of malting barley is one of the most important determinants of the quality of the finished malt. While endosperm proteins are required for the production of starch degrading enzymes, a strict range for barley protein levels is necessary to prevent the deleterious effects of excessive protein. High seed protein can lead to poor endosperm modification, a reduced malt extract volume, and cloudy or discolored beer (Freeman 1999, Izydorczyk and

Edney 2017). Protein and starch occupy the endosperm during grain fill and, thus, protein levels have an inverse relationship with the amount of convertible starch. On the other hand, low protein can also be problematic as there may be insufficient enzymatic activity to convert endosperm starches to fermentable sugars (Freeman 1999, AMBA 2011, MacLeod 2016, Izydorczyk and Edney 2017). Agronomic practices, such as N fertilization, have been shown to affect protein content of grain kernels (Freeman 1999, O'Donovan et al. 2011, Sainju et al. 2013, Stevens et al. 2015) and thus are important considerations when growing barley for the malt market.

Nitrogen Effects on Yield and Quality

Barley grain's plumpness and protein content are greatly affected by agronomic practices such as seeding rate, N fertilization and variety (Wade and Frommet 2003, O'Donovan et al. 2011, Edney et al. 2012, Izydorczyk and Edney 2017). Management of N fertilizer is especially important to the quality of the barley as it can have the greatest effect of any agronomic practice (Gooding 2017, Shrestha and Lindsey 2019). Higher N fertilizer applications are not, surprisingly, associated with greater grain yield of malting barley (Grant et al. 1991, Weston et al. 1993, O'Donovan et al. 2011, Edney et al. 2012, Sainju et al. 2013, Magliano et al. 2014, Stevens et al. 2015). However, greater than ideal protein contents and lower plumpness levels have been shown to result from high N fertilization rates (Wade and Froment, 2003, O'Donovan et al. 2011, Edney et al. 2012, Magliano et al. 2014, Stevens et al. 2015). This makes malting barley a difficult crop to fertilize to obtain its full potential yield without sacrificing the quality required by the malting industry. If barley grain does not meet the quality standards of the maltsters, the only option is to sell it as livestock feed rather than at the premium price that malt quality barley can obtain.

While the N rate that satisfies both yield goal and quality requirements will vary depending on location and climate, O'Donovan et al. (2015) working in western Canada, found that a total of 90 kg N ha^{-1} (80 lb N ac^{-1}) applied at planting met the protein standards set by the Brewing and Malting Barley Research Institute in Canada for 2-row spring-planted varieties. However, N rates above 60 kg total N ha^{-1} (54 lb N ac^{-1}) led to a significant reduction in kernel plumpness, an indication of reduced malting quality. Also working in western Canada, Edney et al (2012), showed that the critical N rate at which grain protein reached unacceptable levels was above 90 kg N ha^{-1}, which is similar to the findings of O'Donovan et al. (2011, 2015). Working in western China, Wang et al. (2007) also found that increasing N rates significantly decreased the amount of malt extract by increasing protein content. While higher N rates may increase protein content and decrease seed quality, low protein varieties of malting barley exist and would theoretically allow high yields without sacrificing quality. Weston et al. (1993), compared two typical malting barley varieties for North Dakota, Robust and Bowman, with two varieties bred for low protein. Nitrogen rates greater than 150 kg N ha^{-1} (134 lb N ac^{-1}) led to unacceptably high protein content in the Robust and Bowman varieties, while the low protein varieties did not exceed protein limits at rates as high as 200 kg N ha^{-1} (178 lb N ac^{-1}) (Weston et al 1993). However, the low protein varieties had significantly lower yields than that of the normal varieties (Weston et al. 1993).

Other Nutrients and Seeding Rate

Practices such as seeding rate and additions of nutrients other than N, particularly phosphorus (P), potassium (K) and sulfur (S), can also impact barley yield and quality. Phosphorus is important for germination and early plant growth in small grains (Weisz et al. 2013) and also promotes winter hardiness. This is especially important for barley grown in the winter as it is more susceptible to cold damage than wheat (Poehlman 1985). Phosphorus additions have been shown to increase yield when applied to barley, although in some cases this effect can lower the number of plump kernels (McKenzie et al. 2005).

Potassium influences test weight and oil content, prevents lodging, and can increase drought and disease tolerance in small grains (Weisz et al. 2013). However, without the pressures of disease or drought, K applications may have no impact on yield or quality (McKenzie et al. 2003, 2004, 2005).

Sulfur in small grains is important to grain protein, kernel weight, kernel size, yield, and test weight (Weisz et al. 2013). Sulfur is also needed for utilization of N and, thus, N:S ratios play an important determining factor in applications of N. Ratios greater than 15 indicate that S is limiting (Weisz et al. 2013). Although S has shown no effect on the quality of barley in general (McKenzie et al. 2005), additions of S are known to cause increases in yield for small grains in NC (Oates and Kamprath 1985).

Seeding rates for hulled barley in NC are recommended at between 0.9 and 1.6 million seeds ac^{-1}, with the higher end being for more intensely managed fields (Weisz et al. 2013). Ohio State extension recommends a malting barley seeding rate of between 1.2 and 1.6 million seeds ac^{-1} (Culman et al. 2018). Researchers in Western Canada have found that a seeding rate of approximately 1.2 million seeds ac^{-1} maximizes yields while maintaining malting quality (O'Donovan et al. 2015). High seeding rates can lower levels of grain protein, thus improving endosperm modification and increasing malt extract production, but yields may be greater at lower seeding rates (Edney et al. 2012).

Nitrogen Application Timing

Small grains grown in spring in Western Canada and the Northern Great Plains typically receive all N at or shortly after planting. Winter small grains in the southeast receive N two to three times during the growing season. Generally a starter application is used and represents only a fraction of the total N applied. The largest N application generally occurs during "spring green up" as this is the time of greatest crop uptake. In addition a small winter application in January or February may be recommended in NC for wheat if tiller density is not ideal (Weisz et al 2013).

Starter N fertilizer applications are typically applied before GS 13 and the beginning of winter dormancy in order to promote early root growth and prevent winter kill (Alley et al. 2009, Culman et al. 2018). Winter wheat recommendations in NC call for the application of 17-34 kg ha^{-1} (15-30 lb ac^{-1}) as a starter due to the fact that early N stress can lead to low tillering, thereby lowering yields (Weisz et al. 2013). A caveat to this is when planting late. In this case, no starter N is recommended because low temperatures will prevent tillering, thereby making starter N application uneconomical (Weisz et al 2013).

In terms of barley specifically, research in Virginia has shown a benefit of starter N to root and stand development, and yield when winter planted (Alley et al. 2009). They found that 28 to 34 kg N ha^{-1} (25-30 lb N ac^{-1}) was an ideal rate of starter N, unless a significant amount of residual soil N exists, in which case starter N should be reduced (Alley et al. 2009). In Ohio 22 to 34 kg N ha^{-1} (20-30 lb N ac^{-1}) is recommended at planting for winter planted malting barley (in order to promote early root growth and improve winter hardiness (Culman et al 2018)

In the southeastern United States, winter application of N to small grains may be recommended at Zadoks GS 25, depending on tillering density (Weisz et al. 2013). As no data exists for a critical tiller density of barley at GS 25, Virginia has suggested that wheat protocols be followed (Alley et al. 2009). Virginia and NC recommend that N only be applied at GS 25 if tiller density is less than 100 tillers per square foot (Alley et al. 2009, Weisz et al. 2013). In NC, if tiller density is at or below 20 tillers per square foot, 56 to 78 kg N ha^{-1} (50-70 lb N ac^{-1}) should be applied to the crop (Weisz et al 2013). Researchers in Virginia suggest 56 kg N ha^{-1} (50 lb N ac^{-1}) if tiller density is at or below 50 tillers ft^2 (Alley et al. 2009). They also state that a small application of 34 N kg ha^{-1} (30 lb N ac^{-1}) can be made if tiller counts are close to 100 tillers ft^2 but wheat shows signs of N deficiency such as yellowing. Growth Stage 25 is the last chance to increase the number of tillers of the crop before stem extension begins (Weisz et al. 2013).

At GS 30, small grains' requirements for N are greatly increased as vegetative growth intensifies after winter dormancy via stem elongation (Zadoks et al. 1974). Therefore, this is a critical time for nitrogen applications (Weisz et al. 2013). For malting barley, Ohio recommends an 84 to 101 kg N ha^{-1} (75-90 lb N ac^{-1}) application in the spring around GS 30 with the option of splitting applications between GS 25 and growth stages up to GS 45, cautioning that no N applications past GS 45 should be used (Culman et al. 2018). The reason for this is because growth switches from high N-need vegetative growth, to remobilization of N within the plant during reproductive growth. Virginia's extension program recommends that no more than 112 kg N ha^{-1} (100 lb N ac^{-1}) should be applied to winter barley between GS 25 and GS 30 due to the potential for lodging (Alley et al. 2009). In the case of heavy rains, especially on sandy soils, N applications should be adjusted upward by between 11 and 22 kg N ha^{-1} (10-20 lb N ac^{-1}).

Plant Tissue analysis for Nitrogen

Because of the concern with high N levels in the production of malt barley, it may be beneficial to track N uptake in barley vegetative tissue throughout the growing season. Nitrogen tissue testing has been used to help increase N use efficiency of small grains (Lemaire et al.

2008) and been shown to be able to predict yield in small grains. There may be a potential for using an N tissue test in early spring to estimate the amount of N additions needed to reach full yield potential without exceeding quality standards.

Plant analysis (plant tissue testing or leaf analysis) determines the total elemental content of a specific plant part (Jones and Case 1990). Plant tissue analysis may indicate if a plant contains the necessary concentrations of essential nutrients for optimum plant growth (McGinnis 2014). Interpretation of the analysis is usually based upon critical values, which is the value at which elemental concentrations are considered sufficient to prevent a reduction in growth or yield (Jones and Case 1999). Measured plant tissue concentration can be compared to these critical values and recommendations for further nutrient addition can be made. In NC, the North Carolina Department of Agriculture and Consumer Services (NCDA&CS) provides recommendations for final spring N application rates on wheat based on biomass N critical values and total biomass collected at GS 30. These recommendations are based on the work of Weisz et al (2013) which established three yield response curves depending on the measured biomass density—low, medium and high. The applicable response curve is then used along with the measured N% in the leaf tissue to determine the recommended final N application rate (Fig.1). This approach has worked well in NC for high yielding wheat (Fig. 1.3). Scant research has been conducted to determine critical levels for barley for optimum yield and less for malt quality. Zhao (2014) and Alley et al. (2009) were able to create critical curves for plant tissue-N for winter barley in China and Virginia respectively, although these curves were designed to optimize yield and not quality.

CHAPTER 2: NITROGEN RATE AND QUALITY TRIALS

Introduction

Barley, *Hordeum vulgare* L., is the fourth most grown crop worldwide by acreage, and is one of the most widely adapted cereal grains (Newman and Newman 2006). The majority of barley grown in the United States (US) is utilized for animal feed, although roughly 25-30% is used for alcohol production (Newman and Newman 2006). Barley grown for malt must attain strict quality standards set by the malting industry which ensures proper flavor and color development of the final product (AMBA 2011). Two of the most important quality parameters include grain protein and kernel plumpness with plump barley and minimum levels of protein leading to the maximum amount of malt extract produced per volume of grain (Edney et al. 2012). Recommendations for 2-row malting barley sold in the US are protein contents of 9.5-12.5%, and kernel plumpness of less than 80% (AMBA 2011, MacLeod 2016). High levels of grain protein can lead to a lower starch content and poor endosperm modification (Freeman 1999). Plump kernels contain more starch which relates directly to the amount of available fermentable sugars released during the malting process (Izydorczyk and Edney 2017).

Protein content and plumpness can be greatly influenced by the variety of barley grown as well as agronomic practices (Weston et al., 1993, Edney et al. 2012, O'Donovan et al. 2015). Nitrogen (N) fertilization can be a major determinant of final grain quality. Although yields are generally increased with higher N fertilization, increasing N rates can also result in decreased malt quality, namely high grain protein and low plumpness (O'Donovan et al 2015). Producers aim to maximize yield but must also maintain quality standards in order to receive a price premium for their barley, making N fertilization challenging in this crop. The majority of research pertaining to the effect of N rate on the malt quality of barley have been performed in colder climates such as Canada and the northern Grain Plains where barley is grown as a summer crop and on cultivars bred for these colder climates (Poehlman 1985, Weston et al. 1985, Edney et al. 2012; O'Donovan et al.2015). In the US 80% of the barley grown is spring planted (Dill-Macky et al. 2015) and little data is available for winter malting barley grown in the Southeast.

North Carolina (NC), as in other states, has experienced rapid growth in the craft beer industry which has created a potential market for locally sourced malt and an opportunity for farmers to receive a premium price. A lack of research for winter malting barley grown in the southeast makes it difficult for growers to produce a quality crop, as well as creating a need for research of N rates in the Southeast. The objective of this study was to determine N rates which will maximize winter barley grain yield while maintaining malt quality standards under NC conditions. A secondary objective involved the investigation of whether plant tissue N can be used as a tool to predict final yield and/or protein content of barley grain. The results of this study will provide NC growers with needed recommendations for N rates that optimize both yield and quality of malting barley.

Materials and Methods

A N rate study on Violetta, a 2-row variety of winter malting barley (*Hordeum vulgare* L.) was conducted during the 2017/2018 and 2018/2019 growing seasons, hereafter referred to as 2018 and 2019 respectively, at agricultural research stations in four different physiographic regions of North Carolina: (1) Mountains Horticultural Crops Research Station = mountains; (2) Piedmont Research Station = piedmont; (3) Sandhills Research Station = sandhills; and (4) Upper Coastal Plain Research Station = coastal plain (Fig 2.1) for a total of eight site-years. These locations differ by typography and soils and were included to examine how this variety of malting barley performed under different conditions. At all research stations, other than the sandhills, the specific field on which study plots were located changed between the two study years but care was taken to choose fields with the same mapped soil series. The soil series and tillage were as follows: mountains: Codorus loam with conventional tillage; piedmont: Lloyd clay loam with no-till, sandhills: Ailey loamy sand with conventional tillage; and coastal plain: Norfolk loamy sand with conventional tillage (Table 2.1).

Prior to each growing season, soil samples were taken from each field from a depth of 0-15 centimeters (0-6 inches) and analyzed by the NC Department of Agriculture & Consumer Services (NCDA&CS) Soil Testing Lab. Amendments for soil nutrients (other than N) and pH were added in accordance with North Carolina (NC) recommendations for winter small grains at each location (Table 2.1). Soil N was not tested as it is not normally tested in NC, and it was assumed that all study sites started with no residual N. Sulfur (S) was added at a rate of 34 kg S ha^{-1} (30 lb ac^{-1}) to all plots to avoid S deficiencies, which are common in small grains in the southeast, and to prevent elevated N:S ratios that may inhibit plant assimilation of N and S. Barley was planted in bulk at a seeding rate of 4.2 million seeds ha^{-1} (1.7 mil seeds ac^{-1}) with a 19 cm (7.5 in) row spacing and at a depth of 2.5 to 4 cm (1-2 in). Planting in the mountains occurred on 10/167/17 and 10/31/18 following corn and soybeans respectively; in the piedmont on 10/16/17 and 10/26/17 following grain corn and corn silage respectively; in the sandhills on 10/13/17 and 10/17/18 following sweet potato and barley respectively; and in the coastal plain on 10/13/17 and 10/17/18 following tobacco and cotton respectively (Table 2.2). Plots measured 3m by 15m (10ft by 50 ft) and 1m (3ft) alleys were established between plots. Plots were laid out in a randomized complete block design including four replications and treatments consisting of six total N rates of 0, 34, 67, 101, 135, 168 kg N ha^{-1} (0, 30, 60, 90, 120, 150 lb N ac^{-1}) cumulatively applied at three applications. Nitrogen fertilizer was topdressed as urea ammonium nitrate (30-0-

0) with a CO_2 backpack sprayer having a spray width of 3m. The N rates (Table 2.3) were applied at three Zadoks' Growth Stages (GS) GS10 (three leaf) GS10, GS25 (tillering), and GS30 (jointing).

The GS10 applications were made between early to late November, depending on the year and location, GS25 applications were made mid to late February and GS30 applications were made late February to mid-March (Table 2.2).

At GS25 and GS30, fifty to 100 grams of aboveground plant tissue was randomly collected from a minimum of six locations in each plot in accordance with the NCDA&CS protocol for winter small grains tissue sampling (McGinnis et al. 2014) (Table 2.2). Samples were collected prior to, but on the same day as, N applications. At GS45 (boot stage), 100 to 150 flag leaves were randomly collected from each plot, also in accordance with NCDA&CS protocol for winter small grains tissue sampling. Leaf tissue samples were analyzed by oxygen combustion gas chromatography (NA1500s2; CE Elantech Instruments; Lakewood, NJ) (AOAC 1990b; Campbell 1992) by the NCDA&CS for tissue N. Tiller counts were taken at GS25 on only the first rep in both 2018 and 2019. Sampling methodology changed from location to location in 2018. At the mountains location in 2018, tillers were counted 3 times in 30 cm increments. At the piedmont location in 2018, tillers were counted 3 times in 1 m increments, but one counter was listed as overcounting. The sandhills in 2018 was missing all tiller counts. At the coastal plain location in 2018, tillers were counted 3 times in 1 m increments. In 2019, all locations were counted 3 times in 30 cm increments. See Table A.2 Due to only the first rep being sampled, and the variation in sampling methodology mentioned previously the tiller data was not analyzed or used in the remainder of the study.

All sites received Prothioconazole (Prosaro© Bayer Crop Science) as a disease (Fusarium head blight) preventative at a rate of 0.6 L ha^{-1} (8.5 oz ac^{-}) shortly after GS 45 flag leaf sampling in mid-April (Table 2.2). Herbicide treatments varied by location with the mountain site receiving 43 ml ha^{-1} (0.6 oz ac^{-1}) of Thifensulfron-methyl and Tribenuron-methyl (Harmony Extra SG© FMC) in mid-March of 2018. The piedmont site received a burndown application of Glyphosate (Roundup© Bayer Crop Science) at a rate of 2.3 L ha^{-1} (1 qt ac^{-1}) and a postemergence application of Thifensulfron-methyl and Tribenuron-methyl (Harmony Extra SG© FMC) at a rate of 43 ml ha^{-1} (0.6 oz ac^{-1}) in late February in both 2018 and 2019. In late February of the 2019 growing season, the sandhills site received an herbicide application of Thifensulfron-methyl and Tribenuron-methyl (Harmony Extra SG© FMC) at a rate of 43 ml ha^{-1} (0.6 oz ac^{-1}). The coastal plain received no herbicide applications in either year of the study.

Barley grain was harvested by NC State Small Grains Extension with a Wintersteiger small plot combine, equipped with a HarvestMaster© grain gauge on 6/9/18 and 5/29/19 in the mountains, 6/7/18 and 5/28/19 in the piedmont, 6/8/18 and 5/31/19 in the sandhills, and 6/2/18 and 6/5/19 in the coastal plain (Table 2.2). Only the middle 2-m of each plot were harvested. Grain samples were obtained from within the combine, and moisture and test weight were measured by the grain gauge. Yield was calculated using moisture content and sample weight data and was normalized to a standard moisture content of 14.5%. Grain samples were weighed and dried in a forced air oven to <13% moisture before being shipped to Hartwick College's Craft Food and Beverage Labs (Oneonta, New York) for quality testing.

Grain protein was measured using a near infrared reflectance spectrometer (FOSS Infratec™ Grain Analyzer) following the American Society of Brewing Chemists (ASBC) Barley-7D protocol (2009). This method is based upon a Kjeldahl-N digestion procedure. For this study, suitable protein levels that would lead to acceptable malt quality were defined as between 10 and 12% total grain protein. The recommended protein content of the American Malting Barley Association (AMBA) is between 9.5 and 12.5% grain protein (MacLeod 2016). To determine kernel plumpness, barley seed was sieved through an industry standard 6/64th sized sieve, with the kernels being caught considered to meet plumpness criteria. In the current study, a goal of 85% of kernels caught by the 6/64th sieve was used, but AMBA recommendations are that a minimum of 80% of kernels should be caught by the sieve (MacLeod 2014). Plumpness was measured on sieved samples by a Pfeuffer Sortimat using a 100g sample size and 3-minute shaking time, following the ASBC Barley-2C protocol (2009).

Other quality parameters including Rapid Visco Analysis (RVA) (ASBC Barley-12B), Deoxynivalenol (DON), and germination energy at 4 ml (GERM4) (ASBC Barley-3C) were also analyzed by Hartwick College. Rapid Visco Analysis is used to measure mild to moderate sprout damage and is given in units of Rapid Visco Units (RVU). The results of this analysis can be used to determine storability of barley (Macleod 2006). Deoxynivalenol (DON), also known as vomitoxin, is a mycotoxin that infects grains and is associated with certain species of Fusarium. Germination energy at 4ml is a measure of germinated kernels with 4ml of water. Acceptable levels for these quality indicators are: (1) RVU > 120; (2) DON < 1ppm; and (3) germination energy at 4ml > 95%.

Data Analysis

Grain yield, protein content, kernel plumpness, tissue N, RVU, and GERM4 were statistically analyzed using analysis of variance (ANOVA), and mean separations were performed using Tukey’s honestly significant difference (HSD) test at the $p = 0.05$ level. Protein, plumpness, yield, tissue N, RVA, and GERM4 were considered random effects. Nitrogen rate (treatment), year, and locations were considered independent variables, and the interactions of treatment×year, treatment×location, year×location, and treatment×year×location were also analyzed. All analyses were performed using PROC GLM in SAS 9.4 (Cary, NC).

If a significant treatment effect was found for yield as determined by analysis of variance, the data was fit to a linear and a linear plateau model using PROC GLM and the best fit was selected to determine the optimum N rate for maximum yield. The PROC GLM function was chosen over the more common PROC NLIN so that the N rate at which the yield breakpoint occurs is chosen from one of the discrete experimental N rates based on the best fit rather than estimating from continuous data.

Results

Grain Yield

Yield was analyzed against the fixed factors of treatment (N rate), year (two-year study) and location (four). Analysis of variance resulted in a three-way interaction of treatment×location×year (p=0.0014) for yield (Table 2.4). Because the experiment was explicitly established in four different locations (e.g. environments that included differing soil series, rainfall, and temperature), further analysis was conducted separately for each location.

Nitrogen rate had a significant main effect (p<0.0001) at the mountain, piedmont and sandhills locations, but there was no effect of N rate at the coastal plain location (p=0.1050) (Table 2.5). The significant effect of N rate at the three locations was expected as N rate has been shown to significantly impact the yield of malting barley (McKenzie et al. 2005, O'Donovan et al. 2011). The lack of a significant N rate effect at the coastal plain site was likely due to the high variability in yield data seen in 2018. The cause of this variability is unknown and cannot be attributed to weather or management.

Mean yield increased with increasing N rates at the mountain, piedmont, and sandhills locations up to a rate of 135 kg N ha^{-1} (120 lb N ac^{-1}) (Table 2.6). There was no significant difference in yield within a location between the 0 kg N ha^{-1} (0 lb N ac^{-1}) and 34 kg N ha^{-1} (30 lb N ac^{-1}) treatments (Table 2.6). This result suggests that 34 kg N ha^{-1} (30 lb N ac^{-1}) of starter N did not enhance yield, an outcome that was also seen in tissue N measurements that are discussed subsequently. Lack of response to starter N has been demonstrated by other researchers (Malik et al., 2012, Wiesz et al., 2013). Yield from the highest N rate (168 kg N ha^{-1};150 lb N ac^{-1}) was not statistically different than the 135 kg N ha^{-1} (120 lb N ac^{-1}) treatment at the mountain or sandhills locations, while there was no significant difference between the highest three N rates at the piedmont site (Table 2.6). Mean yields were highest at the mountain location, followed by the piedmont location; the sandhills location had the lowest yield, producing 50% or less of the average yield of the other two locations that had significant N rate effects (Table 2.6). These lower yields are attributed to the exceptionally sandy nature of soils in this region compared to the loam and clay loam of the mountain and the piedmont locations, respectively.

Because increasing N rate generally resulted in increased yields at three of the four locations, yields were fit to linear and linear-plateau models. The linear-plateau model has historically been used in NC because it is more conservative; that is, it provides for the greatest yield for the least amount of N (Anderson and Nelson, 1987; Crozier et al., 2014). The coastal plain location was not fit to either model as N rate was not significant. A linear plateau model best fit the data at the mountain (r^2=0.954, p=0.0008), piedmont (r^2=0.961, p=0.0006) and sandhills (r^2=0.974, p=0.0003) sites, and all three location showed a threshold at an N rate of 135 kg N ha^{-1} (120 lb N ac-1) (Figs. 2.2-2.4). The maximum yield at the mountain location was 5218 kg ha^{-1} (97 bu ac^{-1}), while the piedmont site had a maximum yield of 4674 kg ha^{-1} (87 bu ac^{-1}). Yield was much lower at the sandhills location compared to the other two sites, with a maximum yield of 1901 kg ha^{-1} (34 bu ac^{-1}). The nitrogen factors were 1.24, 1.38, and 3.53 lb N bu^{-1} for the mountains, piedmont, and sandhills sites, respectively. The linear plateau models fit well with results of the mean separation test, which showed no significant difference between the 135 kg N ha^{-1} (120 lb N ac^{-1}) and 167 kg N ha^{-1} (150 kg N ac^{-1}) treatments (Table 2.6). O'Donovan et al. (2011) used a quadratic equation to fit yields of barley in Canada because of yield depression at higher N rates. Their optimum N rate was 120 kg N ha^{-1} (107 lb N ac^{-1}) which yielded approximately 4800 kg ha^{-1} (89 bu ac^{-1}) of barley grain. Optimum barley yields at the NC locations were greater (mountain), approximately the same (piedmont), and lower (sandhills) than the Canadian yields.

A year main effect was seen at all locations (p<0.0001) (Table 2.5). Mean yield was significantly lower in 2019 compared to 2018 at all locations with the exception of the mountain site (Table 2.7). The mean yield at the piedmont location decreased from 4267 kg ha^{-1} (79.3 bu ac^{-1}) in 2018 to 3141 kg ha^{-1} (58.4 bu ac^{-1}) in 2019. This may have been caused by increased rainfall at the piedmont in 2019, with over 38 cm (14.9 in) more rainfall than in 2018. The mean yield at the sandhills location decreased from 1701 kg ha^{-1} (31.6 bu ac^{-1}) in 2018 to 548 kg ha^{-1} (10.2 bu ac^{-1}) in 2019. As in the piedmont, yield was likely affected by the greater rainfall that occurred during the 2019 growing season compared to the 2018 growing season, an increase of 59 cm (23 inches). Yield at the coastal plain location decreased from 3817 kg ha^{-1} (71.0 bu ac^{-1}) to 1397 kg ha^{-1} (26.0 bu ac^{-1}) in 2018 and 2019, respectively (Table 2.7). The opposite effect of year was seen at the mountain location, with 2019 yield (4725 kg ha^{-1}; 87.8 bu ac^{-1}) being nearly double that of 2018 (2405 kg ha^{-1}; 44.7 bu ac^{-1}) (Table 2.7).

Malt Quality Indicators

Grain quality measurements, such as protein content, plumpness, RVA, DON, and germination energy at 4ml (GERM4) are important factors determining the marketability of barley used for malting. Some of these quality parameters can be greatly affected by N application, especially protein content and plumpness. In general, an increase in N rate leads to lower malt quality (Wade and Froment, 2003, O'Donovan et al. 2011, Edney et al. 2012, Magliano et al. 2014, and Stevens et al. 2015). Therefore, the challenge for malt barley producers is to apply adequate N to maintain high yields while still meeting the quality standards set by the malting industry.

Analysis of grain protein content indicated a significant treatment×location×year interaction (Table 2.4); therefore, locations were analyzed separately. Nitrogen rate had a significant impact on grain protein content at the mountain location (p<0.0001) (Table 2.5), and although there was a significant treatment effect, only the highest N rate of 168 kg ha^{-1} (150 lb ac^{-1}) had a mean protein content that was significantly different than the other N rates (Table 2.6). Mean protein content at the mountain site fell below the lower protein quality limit of 1000 mg g^{-1} (10%) for all N rates with the exception of the highest N rate. The American Malting Barley Association uses a slightly broader standard for protein content, 950 mg g^{-1} to 1250 mg g^{-1} (9.5-12.5%) than was used in the present study. Mean protein content for most N rates still fell below this standard. While lower protein content is generally valued over higher protein by malt houses, protein content below 950 mg g^{-1} (9.5%) can result in lower enzymatic activity in the seed leading to less conversion of starches to sugars (MacLeod 2016). Year did not have a significant effect on protein in the mountain site (p=0.8451) (Table 2.5), and annual mean protein in both years was below the 1000 mg g^{-1} (10%) goal (Table 2.7).

The piedmont location exhibited a significant treatment×year interaction (p=0.0009) for protein content (Table 2.5), and protein content did not follow a discernible pattern of higher levels with increasing N rates in either study year (Fig. 2.5). All N rates in each year had protein contents that fell below the upper standard limit; at N fertilizer rates $\geq$101 kg N ha^{-1} protein contents were above 1000 mg g^{-1}. Nitrogen rates $\leq$ 67 kg N ha^{-1} were slightly below the minimum protein content in 2018 (Fig. 2.5). However, in these treatments the upper limit of the error bars fit in the minimum protein content, indicating this quality requirement could be reached in these treatments.

At the sandhills location a significant treatment×year interaction (p=0.0036) was also present for protein content (Fig. 2.6). Due to the lack of harvestable yield for the two lowest N rates at this site in 2019, no protein data was available at the 0 and 34 kg N ha^{-1} (0-30 lb N ac^{-1}) rates for this year. Protein content exceeded the upper limit of protein levels desired by commercial maltsters at the highest N rate in both study years, while desired protein levels were below 1000 mg g^{-1} for N rates $\leq$ 101 kg N ha^{-1} (Fig. 2.6).

As in the mountain location, N rate had a significant effect on protein in the coastal plain (p<0.0001) (Table 2.5). Mean protein increased with N rate as has been seen in previous studies such as O'Donovan et al. in Canada (2011). Protein content at all N rates, except for the 168 kg N ha^{-1} (150 lb N ac^{-1}) fell within the desired range of 1000 mg g^{-1} to 1200 mg g^{-1} (10-12%) (Table 2.6). While the mean of the 135 kg N ha^{-1} (120 lb N ac^{-1}) rate met the protein requirement, the variance reached above the 1200 mg g^{-1} (12%) requirement. This location also had a significant year main effect (p=0.0060) with the mean protein in 2018 (1040 mg g^{-1}) being significantly lower than in 2019 (1100 mg g^{-1}). This is contrary to the yield results where yield was significantly higher in 2018 than 2019.

Kernel plumpness exhibited a significant location×year interaction and locations were analyzed separately. When analyzing by location, no treatment (N rate), year, or treatment×year effects were seen for kernel plumpness in the mountains (Table 2.5). Plumpness levels at the mountain site were above the set standard of 8500 mg g^{-1} (85%) at all N rates and in both years (Tables 2.6, 2.7).

As was the case with protein content at the piedmont study site, plumpness had a significant treatment×year effect (p=0.0076) (Table 2.5). Kernel plumpness showed a decreasing pattern with increasing N rate, although mean plumpness values were above the 8500 mg g^{-1} (85%) minimum standard at all N rates in both years (Fig. 2.7).

Significant treatment (p<0.0001) and year (p<0.0001) effects for plumpness were observed at the sandhills location (Table 2.5). In general, plumpness decreased with increasing N rate falling below the 8500 mg g^{-1} (85%) limit at $\geq$ 135 kg ha^{-1} (120 lb ac^{-1}) treatments (Table 2.6). Year affected plumpness at the sandhills site, where mean plumpness in 2018 was above the quality limit at 9410 mg g^{-1} (94.1%) but below the 8500 mg g^{-1} goal in 2019 at 7190 mg g^{-1} (71.9%) (Table 2.7). In 2019 the plumpness is also below the broader limit of 8000 mg g^{-1} (80%) that the AMBA recommends (MacLeod 2016). The lower plumpness values in 2019 are most likely related to the extremely low grain yield that occurred at this site as yield and plumpness are intrinsically linked.

Plumpness was significantly affected by year (p<0.0001) but not N rate (p= 0.8377) at the coastal plain site. This was because plumpness in 2018 (9710 mg g^{-1} or 97.1%) was much greater than plumpness in 2019 (8610 mg g^{-1} or 86.1%) (Table 2.7). While no N rate effect occurred, mean plumpness at all N application rates was above 8500 mg g^{-1} (85%).

Overall, there was a treatment×location×year interaction for RVA (*p*=0.0050, Table A.6) and a significant location×year interaction for GERM4 (*p*=0.0111 Table A.6). When analysis was performed by location, only the piedmont site showed any significant independent variable effect, with year having an impact on RVA and GERM4 (p<0.0001) (Table A.7). RVA is acceptable at levels > 120 RVUs, DON < 1.0 ppm, and GERM4 $\geq$ 9500 mg g^{-1} (95%). While many of the samples were at acceptable levels there were some exceptions. For the entire study all but one sample was above the 120 RVU goal for RVA. All but 50 samples were above the 9500 mg g^{-1} (95%) GERM4 goal, but 39 of those samples were above 9000 mg g^{-1} (90%). The DON levels were well below the 1.0 ppm acceptance criteria with all but 28 samples being below the method detection limit of 0.1 ppm. The 28 samples that exceeded 1.0 ppm were from the piedmont site in 2019 where fungicide application was immediately followed by a rain event.

Prior studies suggest that malt quality, namely high protein and low kernel plumpness, suffers at high N rates (Wade and Froment, 2003, O'Donovan et al. 2011, Edney et al. 2012, Magliano et al. 2014, Stevens et al. 2015). In the current study increasing N rate resulted in significant increases in protein at all locations except the piedmont site. Significant decreases in plumpness due to higher N rates only occurred at the piedmont and sandhills locations (Table 2.6). Despite the significant increases in protein with increasing N, only the highest N rate of 168 kg N ha^{-1} (150 lb N ac^{-1}) at the sandhills and coastal plain locations had protein contents above the 1000 mg g^{-1} (10%) limit (Figure 2.6, Table 2.6 respectively). Kernel plumpness remained above the 8500 mg g^{-1} (85%) standard at all rates and three of the four locations (Table 2.6). The sandhills location was an exception where harvested barley had plumpness values below 8500 mg g^{-1} (85%) at $\geq$ 135 kg N ha^{-1} (120 lb N ac^{-1}) N rates (Table 2.6). When results for this study location were separated by year, however, only 2019 result showed mean plumpness levels (7190 mg g^{-1}) below the standard limit (Table 2.7).

The lack of consistent results showing increasing protein and decreasing kernel plumpness as N rate increases suggest that a factor other than N rate may be controlling grain protein and plumpness. The variety used in this study, Violetta, appears to have a relatively low protein content and relatively high and stable kernel plumpness that is less affected by N rate than would be expected when grown in NC. Though there have been few studies examining the performance of Violetta, other researchers have shown that variety has a significant impact on the quality of malting barley (Varvel et al. 1987, Edney et al. 2012). Varvel et al. (1987) and Edney et al. (2012) found that while N rate did have a significant effect on protein and plumpness, the effect could be greatly lessened if the variety used was bred for high malt quality.

Plant Tissue Nitrogen

Barley tissue N content at three growth stages was analyzed by analysis of variance similarly to yield and quality. The three growth stages at which tissue N was examined were: GS25 (tillering), GS30 (jointing), and GS45 (booting). There was a significant treatment×location×year interaction at the GS45 sampling (p<0.0001), while there were significant location×year interactions for both GS25 (p<0.0001) and GS30 (p<0.0001) sampling times (Table 2.4). As with the previous results, further analysis of tissue N concentrations at different growth stages was conducted by location.

Nitrogen rate had a significant effect on tissue N at GS30 and GS45 for each of the four locations, but only affected tissue N at GS25 at one of the four sites (Table 2.5). The sandhills location was the only site where a significant relationship between N rate and tissue N occurred at GS25 (p=0.0346) (Table 2.5). The lack of an N rate effect at GS25 for the other three locations suggests that the starter N fertilizer rate of 34 kg N ha^{-1} (30 lb N ac^{-1}) applied shortly after planting (GS10) had little effect on final barley yield tissue N content, a result also found by Malik et al. (2012). Weisz et al. (2013) states that when winter small grains are planted later than the recommended dates in NC, starter N may provide no benefit. In this study barley was planted at the mountain site two and four weeks later in 2018 and 2019, respectively, than the recommended planting window for the mountain region, September 25-30 (Wiesz et al. 2013). In 2019 the piedmont site was also planted a week later than recommended by Wiesz et al. (2013). These late planting dates may explain the lack of a treatment effect at GS25 for these two locations. It is unknown what may have caused the GS25 effect at the sandhills. There was a general trend of higher tissue N contents as N rate increased for GS30 and GS45 samplings. At GS30, the mountain and piedmont sites had greater tissue N concentration at N rates of $\geq$ 67 kg N ha^{-1} (60 lb N ac^{-1}) relative to the two lowest N rates, 0 kg N ha^{-1} and 34 kg N ha^{-1} (30 lb N ac^{-1}) (Table 2.8). The relationship between N rate and tissue N at GS 30 at the sandhills and coastal plain locations was not consistent. At GS 45, tissue N concentration at all sites showed a general pattern of higher levels with increasing N rate (Table 2.8).

Tissue N concentrations at GS25 by location ranged from a low of 26 mg g^{-1} and 15 mg g^{-1} in 2018 and 2019, respectively, to a high of 35 mg g^{-1} and 31 mg g^{-1} in 2018 and 2019 respectively (Table 2.9). At GS30, tissue N concentrations were variable among locations with the low ranging from 16 mg g^{-1} and 15 mg g^{-1} in 2018 and 2019, respectively, and the high ranging from 41 mg g^{-1} to 31 mg g^{-1} in 2018 and 2019, respectively (Table 2.9). At GS45, tissue N was less variable among locations with the low ranging from 27 mg g^{-1} and 26 mg g^{-1} in 2018 and 2019, respectively, and the high ranging from 33 mg g^{-1} to 30 mg g^{-1} in 2018 and 2019, respectively (Table 2.9). As was the case with yield, levels of tissue N were higher at the mountain and piedmont locations than at the lower yielding sandhills and coastal plain sites.

It is noteworthy to examine the change in tissue N content among locations over the growing season. While tissue N contents varied widely among location at the GS25 (20-45 mg g^{-1}) and GS30 (14-39 mg g^{-1}) samplings, the range was much narrower by the GS45 (21-37 mg g^{-1}) sampling, which occurred just before the plant entered reproductive growth. In addition, while the piedmont and mountain sites had higher tissue N content than the sandier sites (coastal plain and sandhills) at the two earlier growth stage samplings (GS25 and GS30), by GS 45 no single location stood out as having greater concentrations than any other location. This result is despite the large variation in grain yield by location.

All locations had a significant year effect at GS25 (Table 2.5), exhibiting greater tissue N concentrations in 2018 than in 2019 (Table 2.9); the range in mean tissue N concentration in 2018 was from a low of 26 mg g^{-1} to a high of 35 mg g^{-1}, while in 2019 it ranged from a low of 15 mg g^{-1} to a high of 33 mg g^{-1} at this sampling time. Because of the delayed planting at the mountain location, the first N application was applied 11 days later in 2019 (November 26) than in 2018 (November 15). However, the sampling at GS25 occurred on nearly the same dates in both 2018 (February 26) and 2019 (February 24). This resulted in barley grown in the 2018 season having 163 more growing degree days than barley grown in 2019, which may have influenced the greater tissue N in 2019 at the mountain location.

Unlike results at the GS25 sampling when all locations had a significant year effect, only the piedmont and sandhills locations showed a significant year effect (p<0.0001) at the GS 30 sampling. At the piedmont location tissue N was greater in 2018 than 2019 while the opposite was true for the sandhills (Table 2.9).

Tissue N at GS45 was significantly greater in 2018 as compared to 2019 at the mountains (p=0.0403), piedmont (p<0.0001), and coastal plain (p<0.0001) locations, but not the sandhills location (p=0.277) (Table 2.9). In the mountains the higher mean tissue N in 2018 versus 2019 was counter to the higher yield values for this site as there were significantly lower yields in 2018 than 2019.

Conclusions

Yield of malting barley was significantly affected by N rate and generally increased up to an N rate of 135 kg N ha^{-1}. The highest nitrogen rate (168 kg ha^{-1}) did not result in higher yields and was negatively affected by lodging at some locations. Linear plateau models consistently indicated that 135 kg N ha^{-1} was the best N rate for optimizing yield across locations and years. Location was a predominant factor as yields were lower at sites having coarser textured soils, namely the sandhills and coastal plain sites. Year also had a large effect as yield was greatly increased in the mountains from 2018 to 2018, but yields were greatly reduced in 2019 compared to 2018 at the other three locations.

Prior studies suggest that malt quality, namely protein and kernel plumpness, are negatively impacted by high N rates (Wade and Froment, 2003, O'Donovan et al. 2011, Edney et al. 2012, Magliano et al. 2014, Stevens et al. 2015). The current study showed increasing protein with increased N content at all locations except the piedmont. Mean protein was only outside the malt quality index of 1200 mg g^{-1} (12%) at the sandhills and coastal plain, and then only at the highest N rate of 168 kg N ha^{-1} (150 lb N ac^{-1}). Mean plumpness was above the 8500 mg g^{-1} (85%) goal at all locations at all rates except at the sandhills at the highest two rates in 2019. The lack of consistent results showing increasing protein and decreasing kernel plumpness as N rate increases suggest that a factor other than N rate may be controlling grain protein and plumpness.

The variety used in this study, Violetta, appears to have a relatively low protein content and relatively high and stable kernel plumpness that is not greatly affected by N rate. Though there have been few studies examining the performance of Violetta, other researchers have shown that variety has a significant impact on the quality of malting barley (Varvel et al. 1987, Weston et al. 1993, Edney et al. 2012). Varvel et al. (1987), Weston et al. (1993), and Edney et al. (2012) found that while N rate did have a significant effect on protein and plumpness, the effect could be greatly lessened if the variety used was bred for high malt quality. There was some indication from these results that quality may start to deteriorate at the highest N rate (165 kg N ha^{-1}). This coupled with the consistent results of linear plateau model points to an N recommendation of 135 kg N ha^{-1} for Violetta malt barley grown in NC. It is also suggested that more studies be performed to determine if other varieties of barley respond to N similarly in NC.

Biomass N at GS25 was not affected by N rate with the exception of the sandhills location. However, at GS30 and GS45 plant tissue N generally increased with N rate. This result suggests that tissue sampling at the two later growth stages may result in a more accurate representation of biomass N. Levels of tissue N at all measured growth stages were generally higher at the mountain and piedmont locations and lower at the two sandier-textured sites, namely the sandhills and coastal plain, and were greater in 2018 than 2019.

In addition to researching how other barley varieties perform under varying rates of N as mentioned above, more site years are needed to account for climatic variability and unpredictability. Future research should also examine the timing of N applications and whether the number of split applications can be reduced from three to two. Research is set to continue on this project for more site-years in hopes of providing more in-depth fertilizer recommendations to NC growers.

TABLES

Table 1.1 - Zadoks' growth scale adapted from Jackson and Williams 2006.

Growth Stage	Zadoks Scale
planting	00
emergence	10
first leaf	11
second leaf	12
third leaf	13
tillering	21-29
jointing	31
flagleaf	37-39
boot	40-47
heading	51-59
flowering	61-69
grain formation	71
milk	72-79
soft dough	81
hard dough	85
harvest	92

Table 1.2 - Quality indicators set forth by the American Malting Barley Association (AMBA, 2006).

Protein	Plumpness	Germination Rates	Moisture
9.5-12.5%	85% Kernals stopped by a 6/64th seive	Greater than 95%	Less than 13.5%

Table 2.1 – Description of study locations, including: , soil series, tillage, soil test results: humic matter (HM), weight/volume (W/V), cation exchange capacity (CEC), base saturation (BS), exchangeable acidity (Ac), pH, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S).

Mountain, Codorus loam (Fine-loamy, mixed, active, mesic Fluvaqauentic Dystrudept), conventional tillage											
Growing Season	HM%	W/V g cm^{-3}	CEC cmolc kg^{-1}	BS%	Ac cmolc kg^{-1}	pH	P mg kg^{-1}	K mg kg^{-1}	Ca cmolc kg^{-1}	Mg cmolc kg^{-1}	S mg kg^{-1}
2017/18	0.66	1.06	8.3	84	1.3	6.4	108.7	151.2	4.8	1.8	18.1
2018/19	0.27	1.51	1.8	67	0.6	5.6	108.9	40.1	0.9	0.2	5.4
Piedmont, Lloyd clay loam (Fine, kaolinitic, thermic Rhodic Kanhapludult), no-till											
Growing Season	HM%	W/V g cm^{-3}	CEC cmolc kg^{-1}	BS%	Ac cmolc kg^{-1}	pH	P mg kg^{-1}	K mg kg^{-1}	Ca cmolc kg^{-1}	Mg cmolc kg^{-1}	S mg kg^{-1}
2017/18	0.27	0.93	8.9	77	2.0	5.6	127.7	90.4	4.6	2.1	23.7
2018/19	0.32	1.1	7.1	81	1.3	6.2	82.9	152.8	3.6	1.8	13.1
Sandhills, Ailey loamy sand (Loamy, kaolinitic, thermic Arenic Kanhapludult), conventional tillage											
Growing Season	HM%	W/V g cm^{-3}	CEC cmolc kg^{-1}	BS%	Ac cmolc kg^{-1}	pH	P mg kg^{-1}	K mg kg^{-1}	Ca cmolc kg^{-1}	Mg cmolc kg^{-1}	S mg kg^{-1}
2017/18	0.36	1.02	11.0	92	0.8	7	112.9	241.5	7.0	2.5	13.6
2018/19	0.51	1.38	2.8	75	0.7	5.9	153.0	31.2	1.5	0.5	7.0
Coastal Plain, Norfolk loamy sand (Fine-loamy, kaolinitic, thermic Typic Kandiudult), conventional tillage											
Growing Season	HM%	W/V g cm^{-3}	CEC cmolc kg^{-1}	BS%	Ac cmolc kg^{-1}	pH	P mg kg^{-1}	K mg kg^{-1}	Ca cmolc kg^{-1}	Mg cmolc kg^{-1}	S mg kg^{-1}
2017/18	0.51	1.3	4.2	74	1.1	5.8	322.2	90.2	2.2	0.6	17.0
2018/19	0.27	1.51	1.8	67	0.6	5.6	108.9	40.1	0.9	0.2	5.4

Table 2.2 – Management and rainfall at each location.

Planting date	Harvest date	Date GS10	Date GS25	Date GS30	Date GS45	Fungicide, date & amount applied	Growing season rainfall		Previous Crop
						Mountains			
10/17/17	6/9/18	11/15/17	2/26/18	3/5/18	4/19/18	Prosaro 4/20/18 0.6 L/ha (8.5 oz/ac)	133.5 cm	(52.6 in)	Corn
10/31/18	5/29/19	11/26/18	2/24/19	3/11/19	4/11/19	Presario 4/15/19 0.6 L/ha (8.5 oz/ac)	145.2 cm	(57.2 in)	Soybeans
						Piedmont			
10/16/17	6/7/18	11/14/17	2/16/18	02/28/18	4/13/18	Prosaro 4/14/18 0.6 L/ha (8.5 oz/ac)	79.7 cm	(31.4 in)	Corn
10/26/18	5/28/19	11/27/18	2/23/19	3/10/19	4/11/19	Prosaro 4/12/19 0.6 L/ha (8.5 oz/ac)	117.7 cm	(46.3 in)	Corn silage
						Sandhills			
10/13/17	6/8/18	11/7/17	2/16/18	3/12/18	4/23/18	Prosaro 4/24/18 0.6 L/ha (8.5 oz/ac)	67.8 cm	(26.7 in)	Sweet potato
10/17/18	5/31/19	11/10/18	2/21/19	3/14/19	4/10/19	Prosaro 4/11/19 0.6 L/ha (8.5 oz/ac)	127.0 cm	(50.0 in)	Barley
						Coastal Plain			
10/13/17	6/2/18	11/12/17	2/16/18	3/16/18	4/16/18	Prosaro 4/12/18 0.6 L/ha (8.5 oz/ac)	79.3 cm	(31.2 in)	Tobacco
10/17/18	6/5/19	11/9/18	2/20/19	3/14/19	4/9/19	Prosaro 4/11/19 0.6 L/ha (8.5 oz/ac)	78.6 cm	(30.9 in)	Cotton

Table 2.3 – Treatment nitrogen rates and timing of applications at three Zadoks' growth stages.

Total N applied kg ha^{-1}	Growth Stage 10 kg ha^{-1}	Growth Stage 25 kg ha^{-1}	Growth Stage 30 kg ha^{-1}
0	0	0	0
34	34	0	0
67	34	34	0
101	34	34	34
135	34	34	67
168	34	34	101

Table 2.4 – *P*-values from analysis of variance for yield, protein, kernel plumpness, and tissue nitrogen at three Zadok's growth stages (GS) with nitrogen (N) rate, location, and year as fixed effects. Significant (*p* < 0.05 effects are indicated in bold.

	Yield	Protein	Plumpness	GS 25	GS 30	GS 45
N Rate	**<0.0001**	**<0.0001**	**<0.0001**	0.4071	**<0.0001**	**<0.0001**
Location	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Year	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0086**	**<0.0001**
N Rate × Year	0.5862	**0.0114**	0.8527	0.1572	0.6698	**<0.0001**
Location × Year	**<0.0001**	**0.0005**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0222**
N Rate × Location × Year	**0.0014**	**0.0004**	0.2166	0.8396	0.9548	**<0.0001**

Table 2.5 – *P*-values from analysis of variance by location for yield, protein, kernel plumpness, and tissue nitrogen at three Zadok's growth stages (GS) with nitrogen rate and year as fixed effects. Significant ($p < 0.05$) effects are indicated in bold.

	Mountain		
	N Rate	Year	N Rate × Year
Yield	**<0.0001**	**<0.0001**	0.1869
Protein	**<0.0001**	0.8451	0.0871
Plumpness	0.1817	0.1332	0.0649
GS25	0.744	**0.0003**	0.3131
GS30	**<0.0001**	0.8054	0.0984
GS45	**<0.0001**	**0.0403**	0.2860
	Piedmont		
	N Rate	Year	N Rate × Year
Yield	**<0.0001**	**<0.0001**	0.1226
Protein	**0.0011**	**<0.0001**	**0.0009**
Plumpness	**<0.0001**	0.0795	**0.0076**
GS25	0.8308	**<0.0001**	0.5088
GS30	**<0.0001**	**<0.0001**	0.6021
GS45	**<0.0001**	**<0.0001**	0.7781
	Sandhills		
	N Rate	Year	N Rate × Year
Yield	**<0.0001**	**<0.0001**	0.6724
Protein	**<0.0001**	0.1028	**0.0036**
Plumpness	**<0.0001**	**<0.0001**	0.4457
GS25	**0.0346**	**<0.0001**	0.2616
GS30	**0.0033**	**<0.0001**	0.9077
GS45	**<0.0001**	0.277	0.3543
	Coastal Plain		
	N Rate	Year	N Rate × Year
Yield	0.105	**<0.0001**	0.6725
Protein	**<0.0001**	**0.006**	0.4411
Plumpness	0.8337	**<0.0001**	0.9125
GS25	0.8979	**<0.0001**	0.7894
GS30	**<0.0001**	0.5421	0.3453
GS45	**<0.0001**	**<0.0001**	0.5375

Table 2.6 - Nitrogen rate effects on mean yield, protein, and plumpness by location. Standard deviations are shown in parentheses.

Mountain			
N rate kg ha-	Yield kg ha^{-1}	Protein mg g^{-1}	Plumpness mg g^{-1}*
0	2590 (915) ab	910 (32) a	9520 (190)
34	3260 (571) ab	900 (45) a	9510 (120)
67	3580 (1110) bc	880 (26) a	9530 (120)
101	4160 (1170) c	900 (47) a	9530 (130)
135	5470 (780) d	955 (53) a	9430 (170)
168	5340 (766) d	1040 (88) b	9290 (440)
Piedmont			
N rate kg ha-	Yield kg ha^{-1}	Protein mg g^{-1}	Plumpness mg g^{-1}
0	2620 (1246.9) a	1070 (110) ab	9680 (73) a
34	2650 (1025.3) a	1050 (130) a	9580 (82) ab
67	3430 (1097.3) ab	1040 (86) a	9510 (140) ab
101	4010 (687.0) bc	1020 (56) a	9540 (130) ab
135	4710 (823.1) c	1060 (44) ab	9420 (170) b
168	4810 (844.6) c	1150 (68) b	9140 (200) c
Sandhills			
N rate kg ha-	Yield kg ha^{-1}	Protein mg g^{-1}	Plumpness mg g^{-1}
0	170 (249) a	960 (29) ab	9430 (230) a
34	314 (376) a	930 (30) a	9390 (210) a
67	1010 (698) b	970 (120) ab	8120 (1590) bc
101	1400 (843) c	1040 (80) bc	8810 (1010) ab
135	1830 (913) d	1110 (67) c	8480 (1170) bc
168	2030 (934) d	1290 (64) d	7780 (1420) c
Coastal Plain			
N rate kg ha-	Yield kg ha^{-1}	Protein mg g^{-1}	Plumpness mg g^{-1}*
0	1890 (1480)	1060 (58) ab	9130 (1010)
34	2660 (1890)	1000 (61) b	9120 (710)
67	2470 (1380)	1000 (49) b	9380 (490)
101	2920 (1920)	1050 (69) ab	9190 (620)
135	2520 (1280)	1110 (110) bc	9080 (790)
168	3190 (1330)	1210 (100) c	9050 (700)

*Means with the same letter in a column are not significantly different according to Tukey's HSD test at p<0.05. * denotes no significant N rate main effect.*

Table 2.7 - Effect of year on mean yield, protein, and plumpness by location. Standard deviations are shown in parentheses.

Mountain			
Year	Yield kg ha^{-}	Protein mg g^{-1}*	Plumpness mg g^{-1}*
2018	2410 (1200) a	930 (91)	9420 (290)
2019	4730 (1210) b	930 (52)	9510 (130)
Piedmont			
Year	Yield kg ha^{-}	Protein mg g^{-1}	Plumpness mg g^{-1}*
2018	4270 (1100) b	1020 (90) a	9450 (150)
2019	3140 (1220) a	1120 (63) b	9510 (270)
Sandhills			
Year	Yield kg ha^{-}	Protein mg g^{-1}*	Plumpness mg g^{-1}
2018	1700 (996) b	1030 (160)	9410 (270) a
2019	547 (540) a	760 (100)	7190 (910) b
Coastal Plain			
Year	Yield kg ha^{-}	Protein mg g^{-1}	Plumpness mg g^{-1}
2018	3820 (1190) b	1040 (71) a	9710 (100) a
2019	1400 (596) a	1100 (125) b	8610 (610) b

*Means with the same letter in a column are not significantly different according to Tukey's HSD test at $p<0.05$. * denotes no significant year main effect.*

Table 2.8 - Mean tissue N by N rate at Zadok's growth stage (GS) 25, 30 and 45 by location. Standard deviations are shown in parentheses.

N rate kg ha^{-1}	GS 25 mg g^{-1}*	GS 30 mg g^{-1}	GS45 mg g^{-1}
Mountain			
0	33.0 (5.0)	22.0 (1.0) a	28.0 (1.0) a
34	34.0 (6.0)	25.0 (3.0) a	29.0 (2.0) a
67	32.0 (3.0)	31.0 (3.0) b	29.0 (1.0) a
101	33.0 (5.0)	29.0 (4.0) b	29.0 (2.0) a
135	33.0 (4.0)	32.0 (2.0) b	32.0 (2.0) b
168	34.0 (4.0)	31.0 (2.0) b	36.0 (2.0) c
Piedmont			
N rate kg ha^{-1}	GS 25 mg g^{-1}*	GS 30 mg g^{-1}	GS45 mg g^{-1}
0	44.0 (6.0)	32.0 (6.0) a	28.0 (3.0) a
34	44.0 (9.0)	32.0 (5.0) a	28.0 (3.0) a
67	44.0 (7.0)	37.0 (5.0) b	30.0 (3.0) ab
101	45.0 (7.0)	37.0 (7.0) b	33.0 (3.0) bc
135	44.0 (8.0)	39.0 (7.0) b	34.0 (2.0) cd
168	45.0 (6.0)	39.0 (6.0) b	36.0 (3.0) d
Sandhills			
N rate kg ha^{-1}	GS 25 mg g^{-1}	GS 30 mg g^{-1}	GS45 mg g^{-1}
0	25.0 (6.0) b	18.0 (4.0) a	24.0 (3.0) a
34	22.0 (5.0) ab	17.0 (8.0) a	21.0 (3.0) a
67	21.0 (4.0) ab	25.0 (5.0) b	22.0 (2.0) a
101	22.0 (6.0) ab	23.0 (4.0) ab	26.0 (3.0) a
135	21.0 (6.0) ab	22.0 (2.0) ab	32.0 (3.0) b
168	20.0 (5.0) a	23.0 (5.0) ab	34.0 (7.0) b
Coastal Plain			
N rate kg ha^{-1}	GS 25 mg g^{-1}*	GS 30 mg g^{-1}	GS45 mg g^{-1}
0	21.0 (8.0)	15.0 (1.0) ab	27.0 (2.0) a
34	21.0 (7.0)	14.0 (1.0) b	27.0 (2.0) a
67	21.0 (7.0)	18.0 (2.0) c	27.0 (2.0) a
101	21.0 (7.0)	16.0 (1.0) bc	30.0 (3.0) ab
135	21.0 (7.0)	17.0 (2.0) c	32.0 (6.0) b
168	22.0 (8.0)	18.0 (1.0) c	37.0 (4.0) c

*Means with the same letter in a column are not significantly different according to Tukey's HSD at $p<0.05$. * denotes no significant N rate main effect.*

Table 2.9 – Effect of year on mean tissue N at Zadok's growth stage (GS) 25, 30, and 45 by location. Standard deviations are shown in parentheses.

Mountain			
Year	GS 25 mg g^{-1}	GS 30 mg g^{-1} *	GS 45 mg g^{-1}
2018	35.0 (4.0) b	28.0 (3.0)	31.0 (4.0) b
2019	31.0 (3.0) a	28.0 (4.0)	30.0 (3.0) a
Piedmont			
Year	GS 25 mg g^{-1}	GS 30 mg g^{-1}	GS 45 mg g^{-1}
2018	33.0 (2.0) b	41.0 (4.0) b	33.0 (3.0) b
2019	30.0 (3.0) a	31.0 (4.0) a	30.0 (4.0) a
Sandhills			
Year	GS 25 mg g^{-1}	GS 30 mg g^{-1}	GS 45 mg g^{-1}
2018	26.0 (3.0) b	18.0 (3.0) a	27.0 (7.0)
2019	18.0 (3.0) a	25.0 (6.0) b	26.0 (4.0)
Coastal Plain			
Year	GS 25 mg g^{-1}	GS 30 mg g^{-1}*	GS 45 mg g^{-1}
2018	28.0 (3.0) b	16.0 (2.0)	32.0 (5.0) b
2019	15.0 (2.0) a	15.0 (2.0)	28.0 (4.0) a

*Means with the same letter in a column are not significantly different according to Tukey's HSD test at $p<0.05$. * denotes no significant year main effect.*

FIGURES

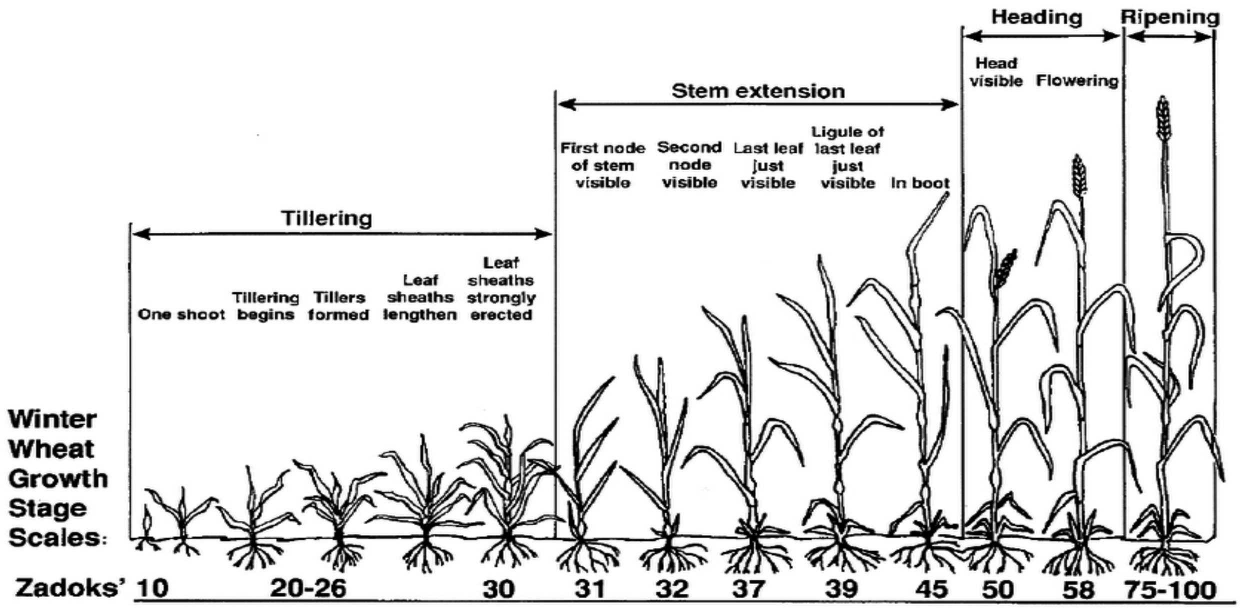
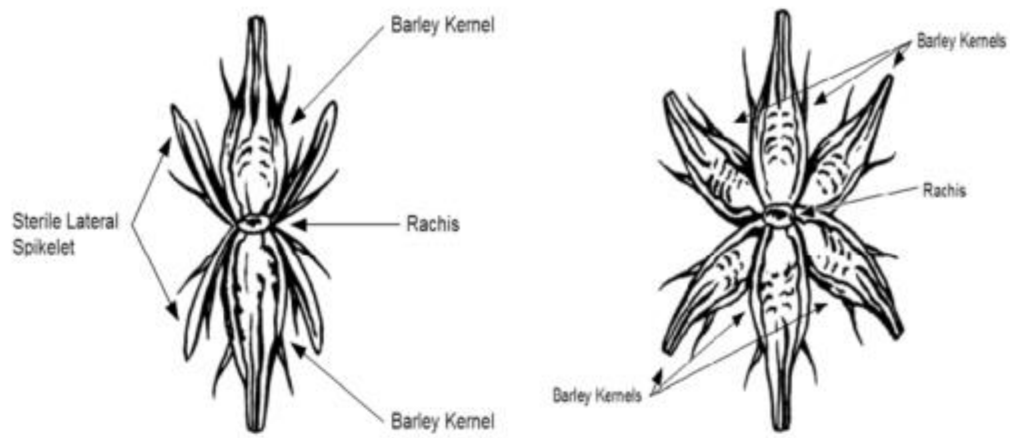


Figure 1.1 - Zadoks' system of growth stages for small grains, from Basden 2006.



Two-row barley head **Six-row barley head**

Figure 1.2 - Comparison of 2-row and 6-row barley spikelet anatomy, from Hertrich 2013.

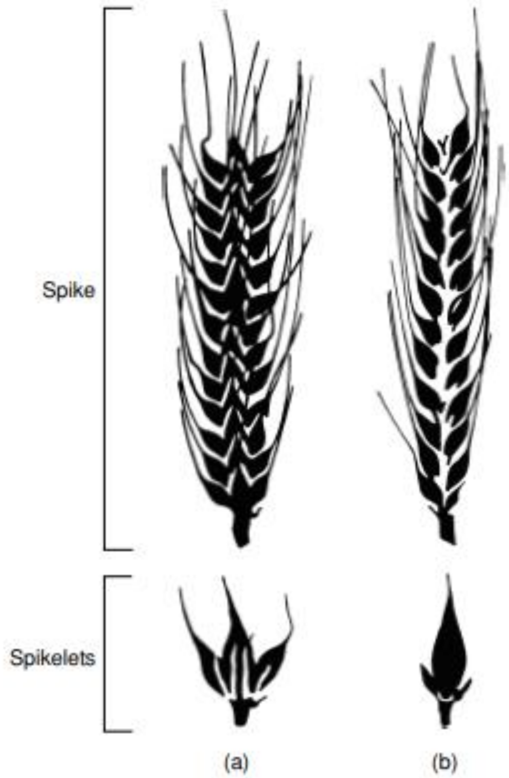


Figure 1.3 - Barley spikes and spikelets. (a) Six-rowed barley, (b) Two-rowed barley. Reproduced from MacGregor 2003.

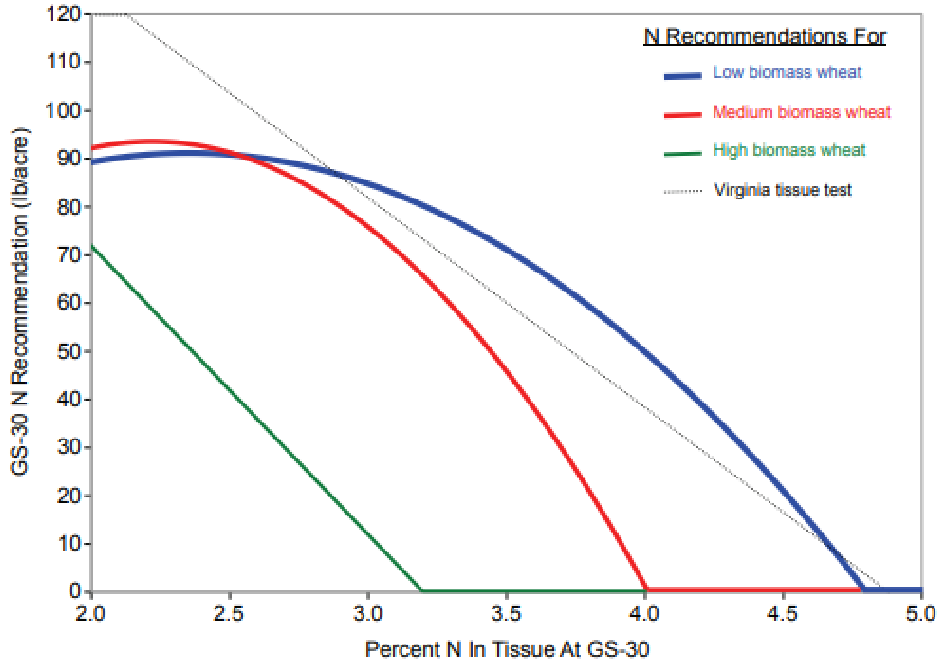


Figure 1.4 - Tissue nitrogen curves for making fertilizer recommendations to wheat at GS 30 in North Carolina.

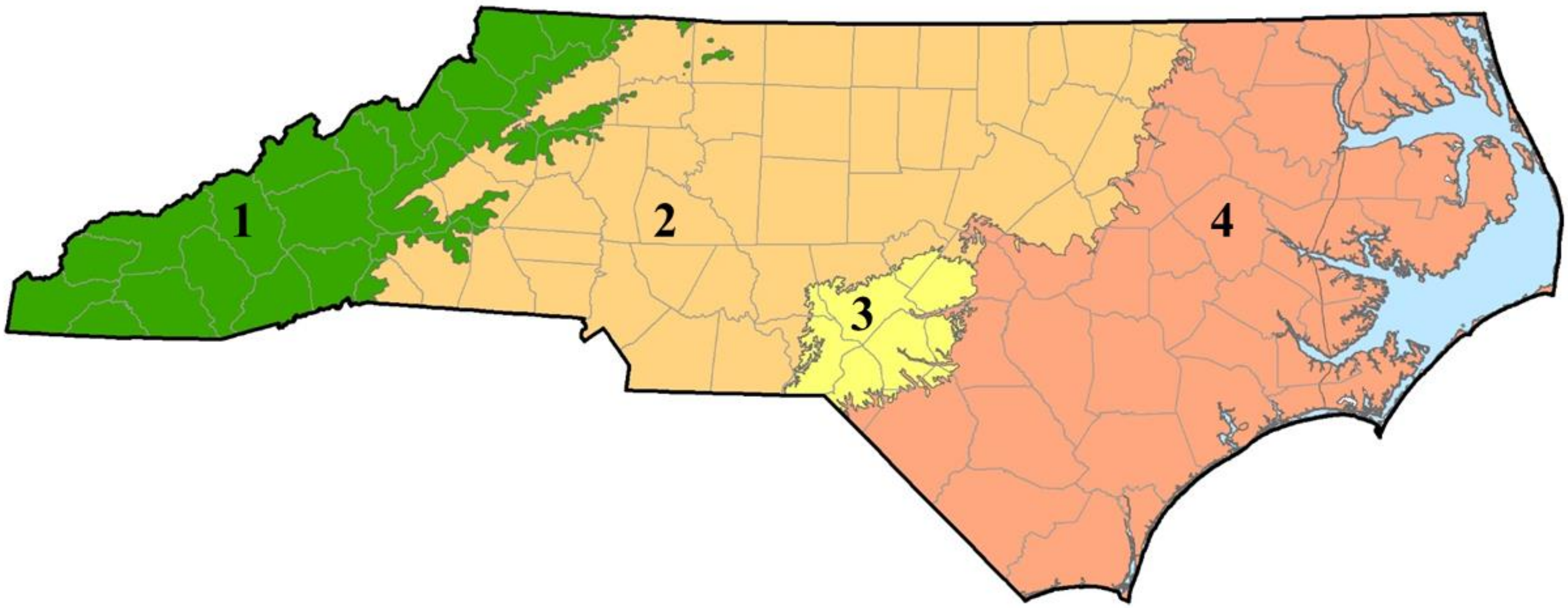


Figure 2.1. Map of North Carolina with experiment locations: (1) Mountain Horticultural Crops Research Station. (2) Piedmont Research Station. (3) Sandhills Research Station. (4) Upper Coastal Plain Research Station.

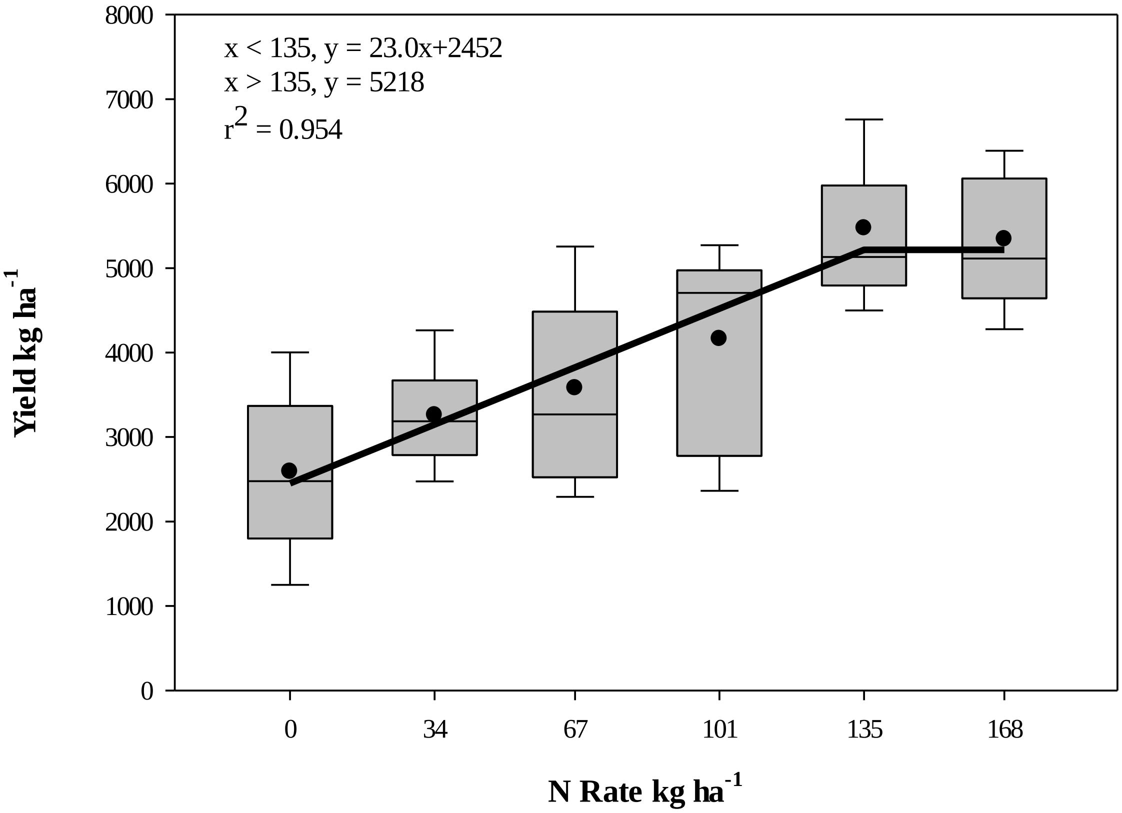


Figure 2.2 – Barley yield linear-plateau model for the mountain location.

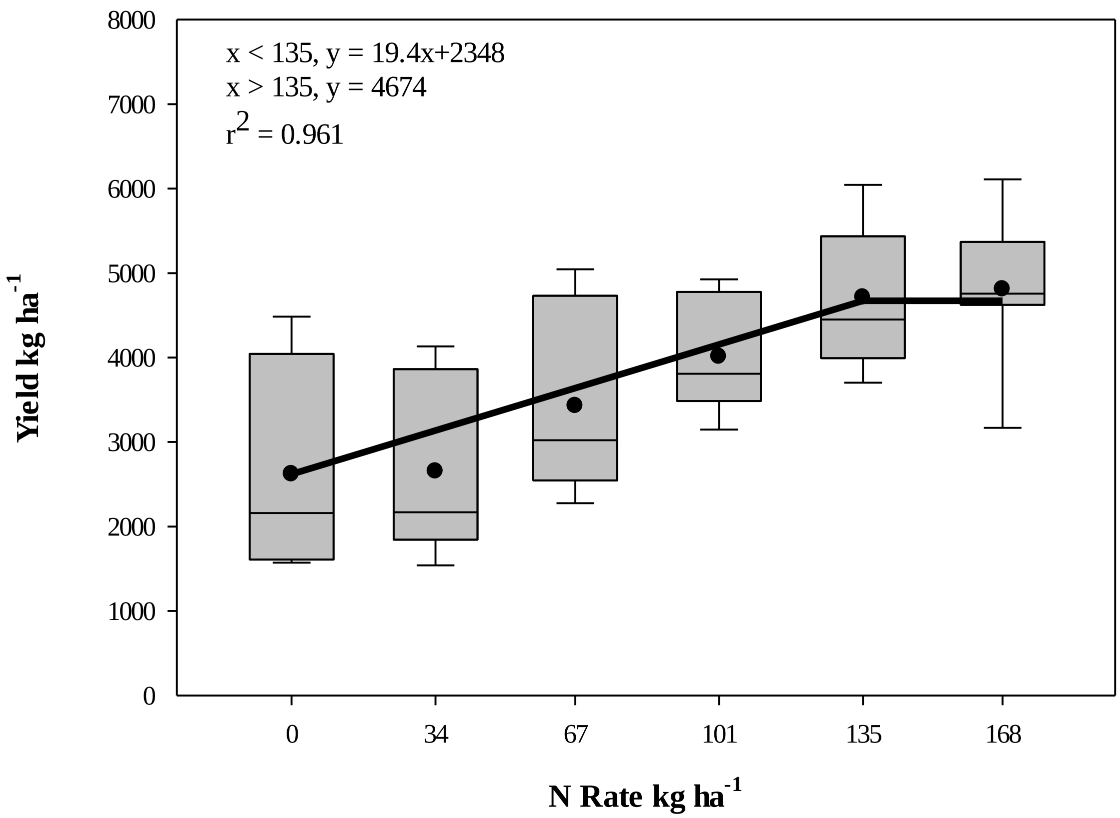


Figure 2.3 - Barley yield linear-plateau model for the piedmont location.

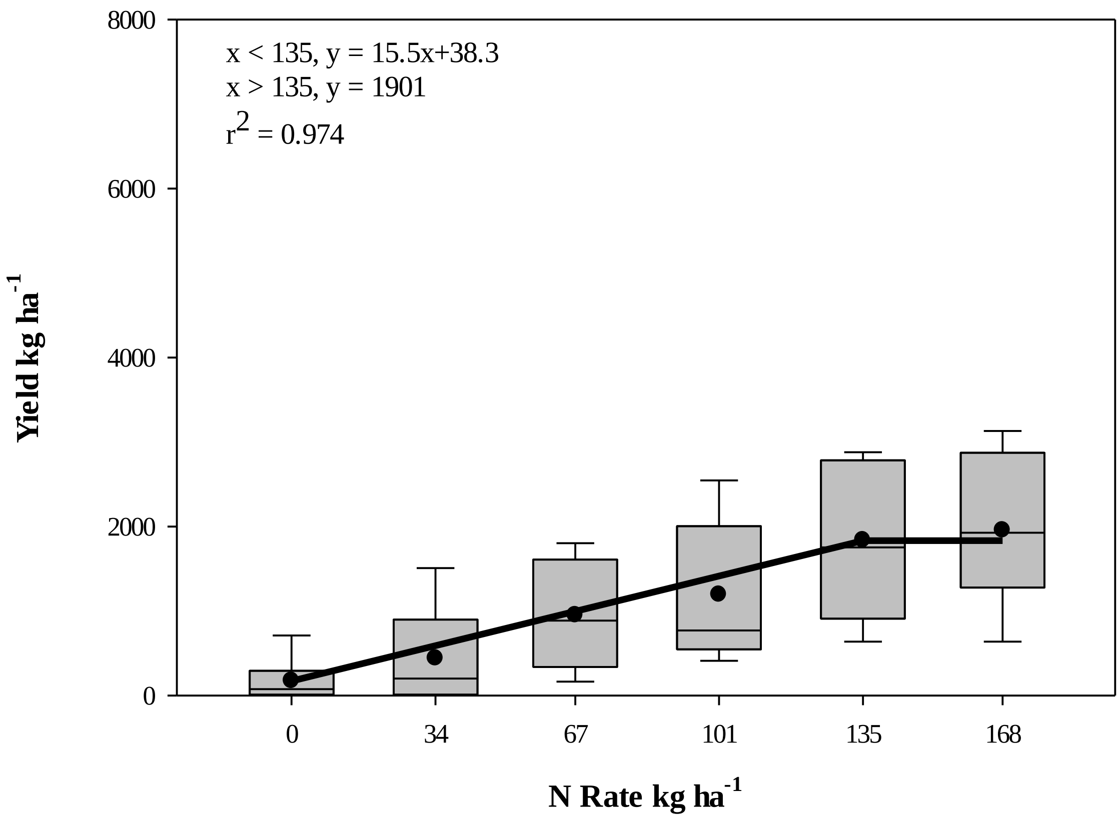


Figure 2.4 - Barley yield linear-plateau model for the sandhills location.

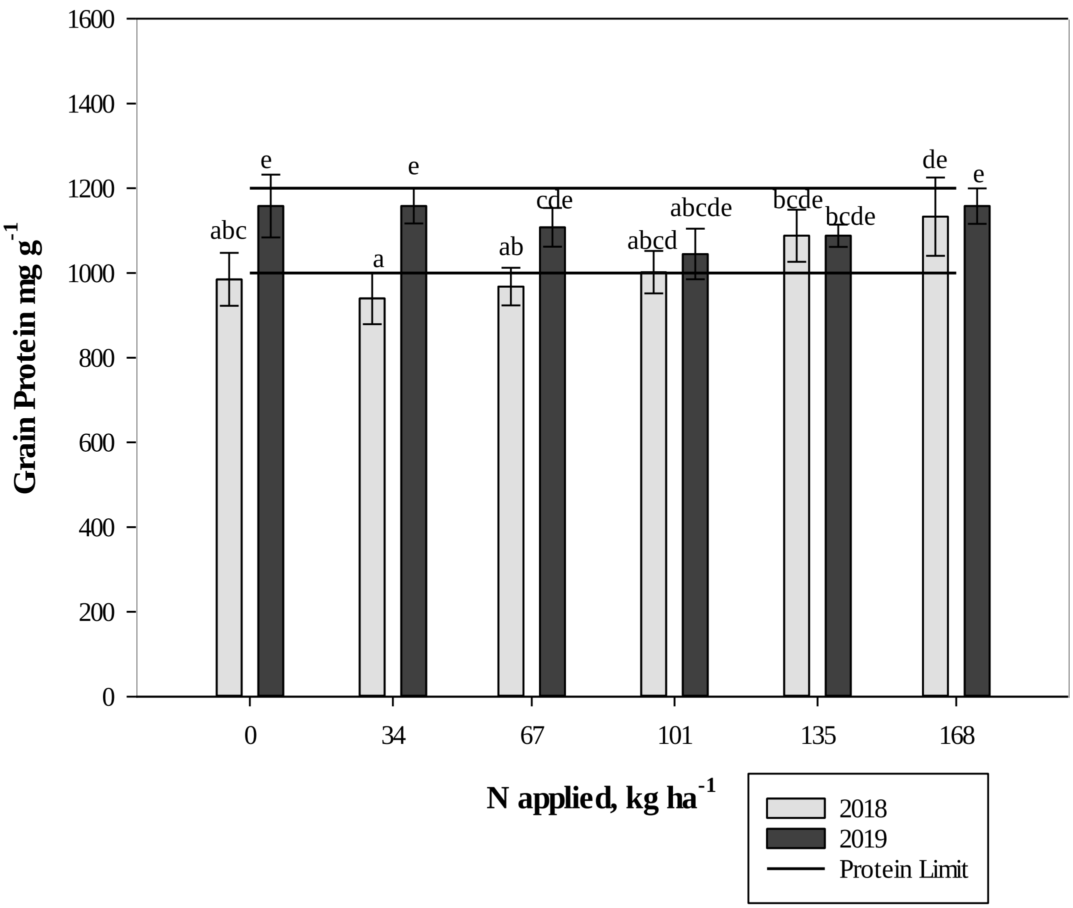


Figure 2.5 - Barley grain protein by N rate×year interaction at the piedmont location with standard deviations. Significant difference between means at the *p*<0.05 level is indicated by different letters.

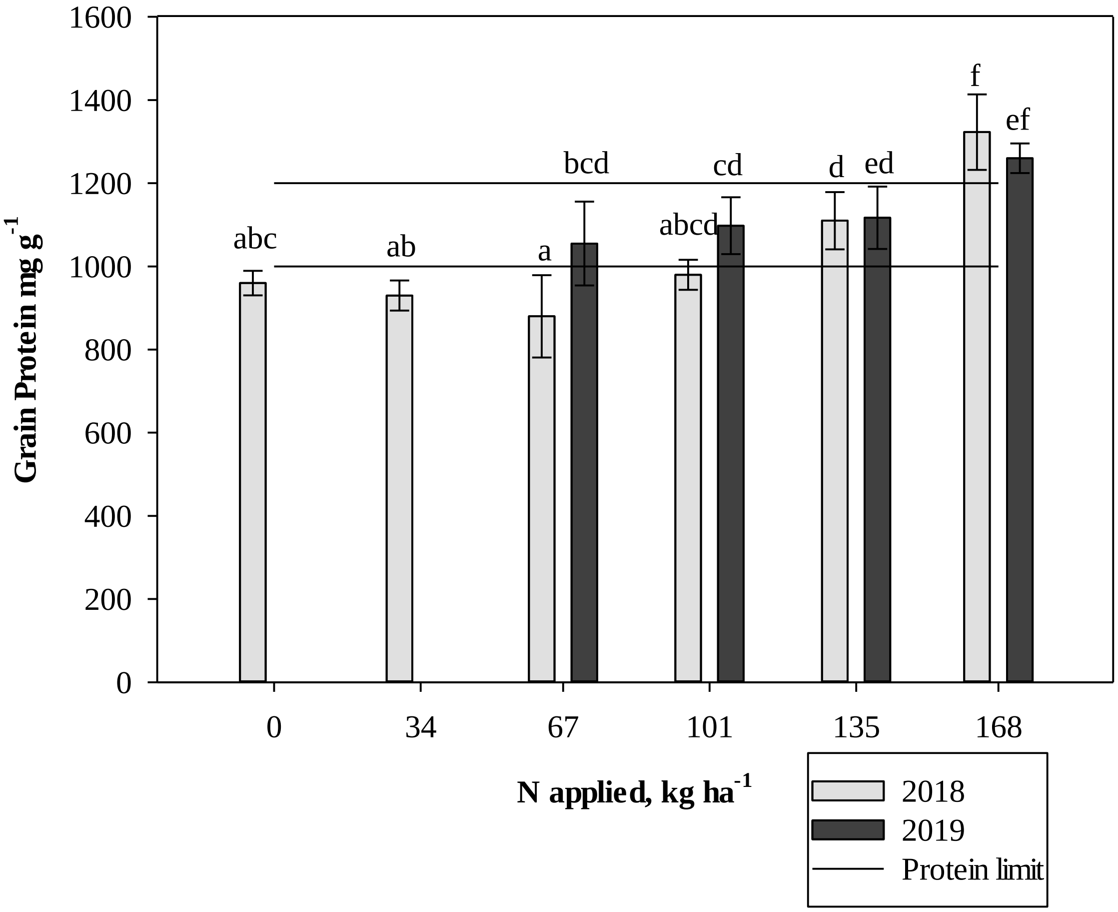


Figure 2.6 - Barley grain protein by N rate×year interaction at the sandhills location with standard deviations. Significant difference between means at the *p*<0.05 level is indicated by different letters.

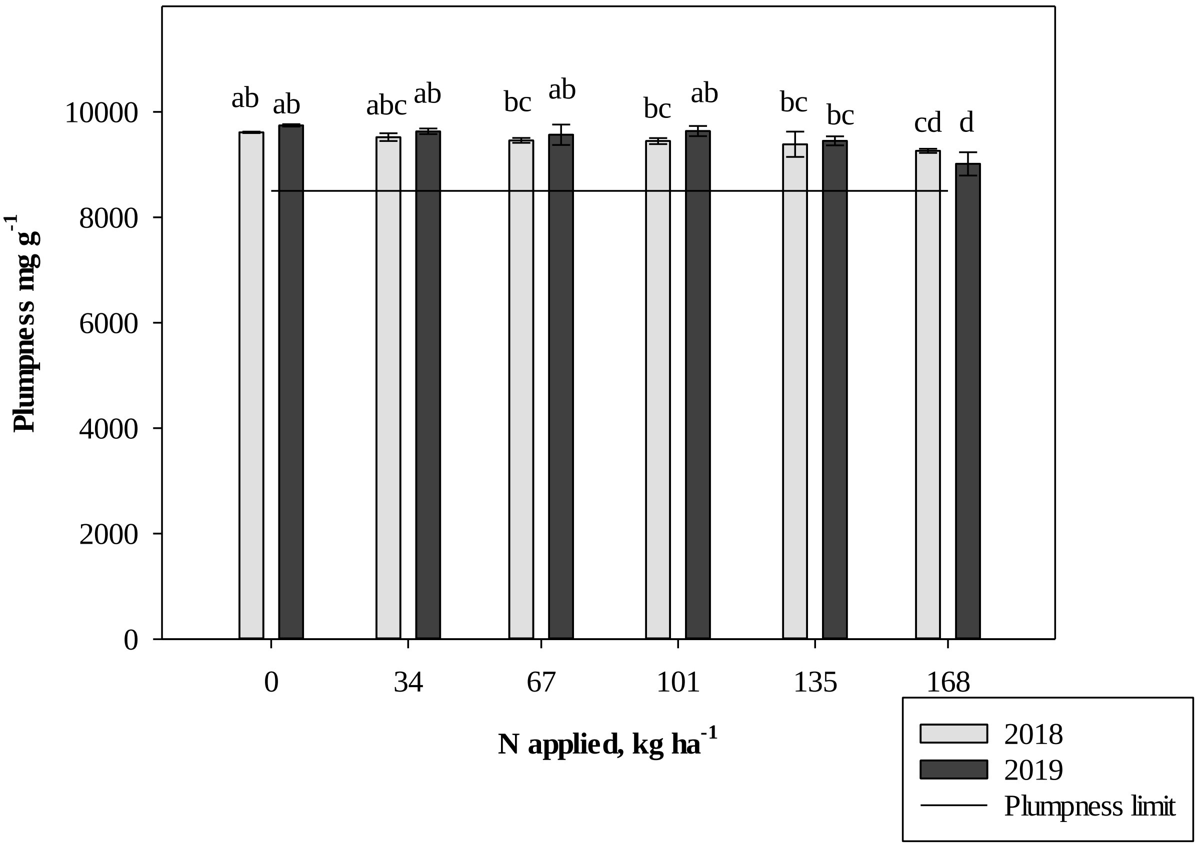


Figure 2.7 - Barley kernel plumpness by treatment×year interaction at the piedmont location with standard deviations. Significant difference between means at the p<0.05 level is indicated by different letters.

REFERENCES

Alley, M.M. T.H. Pridgen, D.E. Brown, J.L. Hammons, and R.L. Mulford. 2009. Nitrogen fertilization of winter barley: principles and recommendations. Publication 424-801 Virginia Polytechnic Institute and State University.

American Malting Barley Association, Inc. (AMBA) 2011. Malting Barley Quality Requirements. http://ambainc.org/media/AMBA_PDFs/Pubs/Production/QualityBrochure.pdf

Anderson, R.L., and L.A. Nelson. 1987. Linear-plateau and plateau-linear-plateau models useful in evaluating nutrient responses. North Carolina State Univ. Tech. Bull. 283. North Carolina State Univ., Raleigh.

[AOAC] Association of Official Analytical Chemists. 1990. AOAC official method 972.43: microchemical determination of carbon, hydrogen, and nitrogen. In: Official methods of analysis. 15th ed. Arlington (VA): AOAC International. p 341.

ASBC Methods of Analysis, online. Barley Method 2C. Kernel Plumpness. (2009). American Society of Brewing Chemists, St. Paul, MN, U.S.A. doi: 10.1094/ASBC Barley2C

ASBC Methods of Analysis, online. Barley Method 7D. Barley Protein. (2009). American Society of Brewing Chemists, St. Paul, MN, U.S.A. doi: 10.1094/ASBC Barley7D.

Badr, A. K. M, R. Sch, H. El Rabey, S. Effgen, H. H. Ibrahim, C. Pozzi, W. Rohde, F. Salamini, 2000. On the origin and domestication history of barley (*Hordeum vulgare*). Molecular Biology and Evolution 7(4):499–510. doi:10.1093/oxfordjournals.molbev.a026330.

Basden, T.J. A.O. Abaye, and R.W. Taylor. 2006. Crop production. Chapter 5 in: Haering, K.C. and G.K. Evanylo (eds.), The Mid-Atlantic nutrient management handbook. Mid-Atlantic Regional Water Program publication. MAWP 06-02.

von Bothmer, R., K. Sato, H. Knupffer, T. van Hintum. 2003. Chapter 1: Barley diversity - an introduction. In R. von Bothmer, T. van Hintum, H. Knupffer, & K. Sato (Eds.), Diversity in Barley : (Hordeum vulgare) 1st ed. Elsevier Science. pp.3-8 https://doi.org/10.1016/S0168-7972(03)80004-X.

von Bothmer, R., K. Sato, T. Komatsuda, S. Yasuda, G. Fischbeck. 2003. Chapter 2: The domestication of cultivated barley. In R. von Bothmer, T. van Hintum, H. Knupffer, & K. Sato (Eds.), Diversity in barley : (Hordeum vulgare) 1st ed. Elsevier Science. pp.9-27 https://doi.org/10.1016/S0168-7972(03)80004-X.

Briggs, D.E. 1998. Malts and Malting. Blackie Academic & Professional, London.

Bulman, P. and D.L. Smith. 1994. Post-heading nitrogen uptake, retranslocation, and partitioning in spring barley. Crop Sci. 34:977-984. https://dx.doi.org/10.2135/cropsci1994.0011183X003400040028x.

Campbell CR. 1992. Determination of total nitrogen in plant tissue by combustion. In: Plank CO, editor. Plant analysis reference procedures for the southern region of the United States. Athens (GA): Georgia Cooperative Extension Service. p 20–2. Southern Cooperative Series Bulletin 368. Available at www.ncagr.gov/agronomi/pdffiles/sera368.pdf.

Crozier, C.R., R.J. Gehl, R.W. Heiniger, and D.H. Hardy. 2014. Nitrogen management for high population corn production in wide and narrow rows. Agron. J. 106:66–72. doi:10.2134/agronj2013.0280.

Culman, S., L. Lindsey, M. Loux, P. Paul, and E. Stockinger. 2018. Management of Ohio Winter Malting Barley. https://stepupsoy.osu.edu/sites/hcs-soy/files/malting%20barley%20spring%202018%20final.pdf.

Dill-Macky, R. G. Gusmini, G. Muehlbauer, C. Sheaffer, K. Smith, M. Smith, B. Steffenson, M.S. Wells, J. Wiersma. 2015.Winter barley. Forever Green. University of Minnesota.

Edney, M.J. J.T. O'Donovan, T.K. Turkington, G.W. Clayton, R. Mckenzie, P. Juskiw, G.P. Lafond, S. Brandt, C.A. Grant, K.N. Harker, E. Johnson, and W. May. 2012. Effects of seeding rate, nitrogen rate and cultivar on barley malt quality. Journal of the Science of Food and Agriculture 92(13): 2672–2678.

Freeman, P.L. 1999. Chapter III: Barley and malting. In J.T, McCabe (Eds.), The Practical Brewer: A Manual for the Brewing Industry, 3rd ed. Master Brewers Association of the Americas. 53–74.

Gooding, M. 2017. Chapter 18: The effects of growth environment and agronomy on grain Quality. In C. Wrigley, D. Miskelly, & I. Batey (Eds.), Cereal Grains: Assessing and Managing Quality: Second Edition 2nd ed. 493–512. Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100719-8.00004-8.

Grant, C.A., L.E. Gauer, L.D. Bailey, and D.T. Gehl. 1991. Protein production and nitrogen utilization by barley cultivars in response to nitrogen fertilizer under varying moisture conditions. Canadian Journal of Plant Science 71(4): 997–1009.

Hertrich, J. 2013. Topics in brewing: malting barley. Technical Quarterly. 29–41., doi:10.1094/tq-50-1-0331-01.

Izydorczyk, M.S. and M. Edney. 2017. Chapter 9: Barley: grain-quality characteristics and management of quality requirements. In C. Wrigley, D. Miskelly, & I. Batey (Eds.), Cereal Grains: Assessing and Managing Quality: 2: 195–234. Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100719-8.00004-8.

Jackson, L. and J. Williams. 2006. Small Grain Production Manual Part 2: Growth and Development of Small Grains. University of California Division of Agriculture and Natural Resources, 8165, 1–7. http://anrcatalog.ucdavis.edu.

Jones, J.B. and V.W. Case. 1990 Chapter 15: Sampling, handling, and analyzing plant tissue samples. In R.L. Westerman (Ed.), Soil Testing and Plant Analysis, 3: 389-427. Soil Science Society of America.

Klages, K.H.W. 1942. Ecological Crop Geography. The Macmillan Co., New York.

Lemaire, G., M.H. Jeuffroy, and F. Gastal. 2008. Diagnosis tool for plant and crop N status in vegetative stage. European Journal of Agronomy 28(4): 614–624.

MacGregor, A.W. Barley. Encyclopedia of Food Sciences and Nutrition. 379–382. doi:10.1016/b0-12-227055-x/00081-x.

MacLeod A. Understanding malting barley quality. 2016. Michigan State University Extension.

Magliano, P.N., P.H. Prystupa, and F. Gutiérrez-Boem. 2014. Protein content of grains of different size fractions in malting barley. Journal of the Institute of Brewing. Available at https://onlinelibrary.wiley.com/doi/epdf/10.1002/jib.161.

Malik, A., L. Holm, and E. Johansson. 2012. Soil and starter fertilizer and its effect on yield and protein composition of malting barley. Journal of Soil Science and Plant Nutrition. 12. 835-849. 10.406/S0718-95162012005000036.

McGinnis, M., C.S. Stokes, and B. Cleveland. 2014. NCDA&CS Plant Tissue Analysis Guide. Plant/Waste/Solution/Media Analysis Section Agronomic Division. N.C. Dept. of Agriculture & Consumer Services.

McKenzie, R.H. E. Bremer, L. Kryzanowski, A.B. Middleton, E.D. Solberg, D. Heaney, G. Coy, and J. Harapiak. 2003. Yield benefit of phosphorus fertilizer for wheat, barley and canola in Alberta. Canadian Journal of Soil Science 83(4): 431–441.

McKenzie, R.H. A.B. Middleton, L. Hall, J. Demulder, and E. Bremer. 2004. Fertilizer response of barley grain in south and central Alberta. Canadian Journal of Soil Science 84(4): 513–523.

McKenzie, R.H. A.B. Middleton, and E. Bremer. 2005. Fertilization, seeding date, and seeding rate for malting barley yield and quality in southern Alberta. Canadian Journal of Plant Science 85(3): 603–614.

Mosley, J. 2019. Acreage: Barley Area Planted and Harvested – States and United States: 2018 and 2019. United States Department of Agriculture (USDA) Agriculture Counts, edited by Lance Honig. June 28, 2019. 9. ISSN: 1949-1522.

Newman, C. W. and R. K. Newman. 2006. A brief history of barley foods. Cereal Foods World. 51. 4-7. doi:10.1094/cfw-51-0004.

Newman, R.K. and C.W. Newman. 2008. Barley for Food and Health. John Wiley and Sons Inc, New Jersey.

Newton, A. C., A.J. Flavell, T.S. George, P. Leat, B. Mullholland, L, Ramsay, C. Revoredo-Giha, J. Russell, B.J. Steffenson, J.S. Swanston, W.T.B. Thomas, R. Waugh, P.J. White, and I.J. Bingham. Crops that feed the world 4. Barley: a resilient crop? Strengths and weaknesses in the context of food security. Food Security, 3(2), 141–178. doi: 10.1007/s12571-011-0126-3.

North Carolina Dept. of Agriculture & Consumer Services, Agronomic Division NCDA&CS. Plant Tissue Sampling for Wheat http://www.ncagr.gov/agronomi/documents/Wheattissuesamplinghandout.pdf.

Oates, K.M. and E.J. Kamprath. 1985. Sulfur fertilization of winter wheat grown on deep sandy soils. Soil Science Society of America Journal 49(4): 925.

O' Donovan, J.T. T.K. Turkington, M.J. Edney, G.W. Clayton, R.H. McKenzie, P.E. Juskiew. 2011. Seeding rate, nitrogen rate, and cultivar effects on malting barley production. Agronomy Journal 103:709–716. doi:10.2134/agronj2010.0490.

O'Donovan, J.T. C.A. Grant, R.E. Blackshaw, K.N. Harker, E.J. Johnson, and Y. Gan. 2014. Rotational effects of legumes and nonlegumes on hybrid canola and malting barley. Agronomy Journal 106:1921–1932. doi:10.2134/agronj14.0236.

O'Donovan, J.T. Y. Anbessa, C.A. Grant, A.L. Macleod, M.J. Edney, M.S. Izydorczyk, T.K. Turkington, P.E. Juskiw, G.P. Lafond, W.E. May, K.N. Harker, E.N. Johnson, B.L. Beres, T.A. Mcallister, E.G. Smith, and W. Chapman. 2015. Relative responses of new malting barley cultivars to increasing nitrogen rates in western Canada. Canadian Journal of Plant Science 95(5): 831–839.

Paterson, A., J.S. Swanston, and J.R. Piggott. Chapter 1: The production of fermentable extracts from cereals and fruits. In Lea, A.G.H., J.R. Piggott. 2003. Fermented Beverage Production. 2nd ed. Kluwer Academic/Plenum Publishers, New York, NY.

Poehlman, J.M. 1985 Chapter 1: Adaptation and distribution. In D.C. Rasmussson, D.R. Buxton, E.S. Horner, J.J. Mortvedt, D.A. Fuccillo (Eds.), Barley. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Publishers, Madison Wisconsin. 1–18.

Sainju, U.M. A.W. Lenssen, and J.L. Barsotti. 2013. Dryland malt barley yield and quality affected by tillage, cropping sequence, and nitrogen fertilization. Agronomy Journal 105:329-340. doi:10.2134/agronj2012.0343.

Scharf, P.C. M.M. Alley, and Y.Z. Lei. 1993. Spring nitrogen on winter wheat: I. Farmer-field validation of tissue test based rate recommendations. Agronomy Journal 85(6): 1181 Available at https://dl.sciencesocieties.org/publications/aj/abstracts/85/6/AJ0850061181.

Shrestha, Raj K. and Laura E. Lindsey. 2019. Agronomic management of malting barley and research needs to meet demand by the craft brew industry. Agronomy Journal, vol. 111, no. 4, 23 May 2019, pp. 1570–1580. doi:10.2134/agronj2018.12.0787.

Stevens, W.B. U.M. Sainju, T. Caesar-Tonthat, and W.M. Iversen. 2015. Malt barley yield and quality affected by irrigation, tillage, crop rotation, and nitrogen fertilization.

United States Standards for Barley, Title 82 Federal Register Part 20541. August 1, 2017.

Varvel, G. E., and Severson, R. K. (1987). Evaluation of cultivar and nitrogen management options for malting barley 1. Agronomy Journal. 79(3), 459–463. https://doi.org/10.2134/agronj1987.00021962007900030010x.

Wade, A. and M.A. Froment. 2003. Barley quality and grain size homogeneity for malting. Vol. 1. Agronomic effects on varieties. Project Rep. no. 320. Home Grown Cereals Authority, Stoneleigh Park, Kenilworth, Warwickshire, UK.

Wang, J., J.X. Chen, F. Dai, F.B. Wu, J.M. Yang, G.P. Zhang. Protein fractions in barley grains as affected by some agronomic factors and their relationships to malt quality. 2007. Cereal Research Communications, 35(1), 129–140., doi:10.1556/crc.35.2007.1.15.

Weisz, R. G. Ambrose, S. Bambra, C. Cowger, C. Crozier, W. Everman, R. Heiniger, D.J. Herbert, D. Jordan, P. Murphy, and D. Reisig. 2013. Small Grains Production Guide. Revised March 2013. North Carolina Cooperative Extension, College of Agricultural & Life Sciences, North Carolina State University, Raleigh, NC.

Weston, D.T. R.D. Horsley, P.B. Schwarz, and R.J. Goos. 1993. Nitrogen and planting effects on low-protein spring barley. Agronomy Journal 85:1170–1174. doi:10.2134/agronj1993.00021962008500060015x.

Wrigley, C. 2017. Chapter 4: Cereal-grain morphology and composition. In C. Wrigley, D. Miskelly, & I. Batey (Eds.), Cereal Grains: Assessing and Managing Quality: Second Edition 55–87. Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100719-8.00004-8.

Zadoks, J. C., T.T. Chang, C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Research, 14(6), 415–421. doi:10.1111/j.1365-3180.1974.tb01084.x.

Zhao, B. 2014. Determining of a critical dilution curve for plant nitrogen concentration in winter barley. Field Crops Research 160: 64–7.

APPENDICES

APPENDIX A

Table A.1 – Treatment nitrogen rates and timing of application at three Zadoks' growth stages. English units.

Total N applied lb a^{-1}	Growth Stage 10 lb a^{-1}	Growth Stage 25 lb a^{-1}	Growth Stage 30 lb a^{-1}
0	0	0	0
30	30	0	0
60	30	30	0
90	30	30	60
120	30	30	60
150	30	30	90

Table A.2 -Tiller counts methodology

Location	2018 tiller count methods	2019 tiller count methods
Mountains	30 cm, 1 rep, 3 counts	30 cm, 1 rep, 3 counts
Piedmont	1m, 1 rep, 3 counts, one person listed as overcounting	30 cm, 1 rep, 3 counts
Sandhills	Missing counts	30 cm, 1 rep, 3 counts
Coastal Plain	1m, 1 rep, 3 counts	30 cm, 1 rep, 3 counts

Table A.3 - Nitrogen rate effects on mean yield, protein, and plumpness by location. Standard deviations are in parentheses. English units

Mountain			
N rate (lb ac^{-1})	Yield bu ac^{-1}	Protein %	Plumpness %*
0	48.1 (17.0) a	9.1 (0.32) a	95.2 (1.9)
30	60.5(10.6) ab	9.0 (0.45) a	95.1 (1.2)
60	66.5 (20.6) bc	8.8 (0.26) a	95.3 (1.2)
90	77.3 (21.7) c	9.0 (0.47) a	95.3 (1.3)
120	101.7 (14.5) d	9.6 (0.53) a	94.3 (1.7)
150	99.3 (14.2) d	10.4 (0.88) b	92.9 (4.4)
Piedmont			
N rate (lb ac^{-1})	Yield bu ac^{-1}	Protein %	Plumpness %
0	48.7 (23.2) a	10.7 (1.10) ab	96.8 (0.73) a
30	49.3 (19.1) a	10.5 (1.30) a	95.8 (0.82) ab
60	63.7 (20.4) ab	10.4 (0.86) a	95.1 (1.4) ab
90	74.5 (12.8) bc	10.2 (0.56) a	95.4 (1.3) ab
120	87.6 (15.3) c	10.6 (0.44) ab	94.2 (1.7) b
150	89.4 (15.7) c	11.5 (0.68) b	91.4 (2.0) c
Sandhills			
N rate (lb ac^{-1})	Yield bu ac^{-1}	Protein %	Plumpness %
0	3.2 (4.6) a	9.6 (0.29) ab	94.3 (2.3) a
30	5.9 (7.0) a	9.3 (0.30) a	93.9 (2.1) a
60	18.8 (13.0) b	9.7 (1.2) ab	81.2 (15.9) bc
90	25.9 (15.7) c	10.4 (0.80) bc	88.1 (10.1) ab
120	34.1 (17.0) d	11.1 (0.67) c	84.8 (11.7) bc
150	37.7 (17.4) d	12.9 (0.64) d	77.8 (14.2) c
Coastal Plain			
N rate (lb ac^{-1})	Yield bu ac^{-1}	Protein %	Plumpness %*
0	35.1 (27.5)	10.6 (0.58) ab	91.3 (10.1)
30	49.4 (35.1)	10.0 (0.61) b	91.2 (7.1)
60	45.9 (25.7)	10.0 (0.49) b	93.8 (4.9)
90	54.3 (35.7)	10.5 (0.69) ab	91.9 (6.2)
120	46.8 (23.8)	11.1 (1.1) bc	90.8 (7.9)
150	59.3 (24.7)	12.1 (1.0) c	90.5 (7.0)

*Means with the same letter in a column are not significantly different according to Tukey's HSD test at p<0.05. * denotes variable that*

Table A.4 - Effects of year on mean yield, protein, and plumpness by location. Standard deviations are shown in parentheses. English units

Mountain			
Year	Yield bu ac^{-1}	Protein %	Plumpness %
2018	44.7 (22.4) a	9.3 (0.91)	94.2 (2.9)
2019	87.8 (22.6) b	9.3 (0.52)	95.1 (1.3)
Piedmont			
Year	Yield bu ac^{-1}	Protein %	Plumpness %
2018	79.3 (20.4) b	10.2 (0.90) a	94.5 (1.5)
2019	58.4 (22.7) a	11.2 (0.63) b	95.1 (2.7)
Sandhills			
Year	Yield bu ac^{-1}	Protein %	Plumpness %
2018	31.6 (18.5) b	10.3 (1.6)	94.1 (2.7) a
2019	10.2 (10.0) a	0.76 (1.0)	71.9 (9.1) b
Coastal Plain			
Year	Yield bu ac^{-1}	Protein %	Plumpness %
2018	71.0 (22.1) b	10.4 (0.71) a	97.1 (1.0) a
2019	26.0 (11.0) a	11.0 (1.3) b	86.1 (6.1) b

Means with the same letter in a column are not significantly different according to Tukey's HSD test at p<0.05.

Table A.5 - Mean tissue N by N rate at Zadok's growth stage (GS) 25, 30 and 45 by location. Standard deviations are in parentheses. English Units

Mountain			
N rate (lb ac^{-1})	GS 25 %	GS 30 %	GS 45 %
0	3.3 (0.5)	2.2 (0.1) a	2.8 (0.1) a
30	3.4 (0.6)	2.5 (0.3) a	2.9 (0.2) a
60	3.2 (0.3)	3.1 (0.3) b	2.9 (0.1) a
90	3.3 (0.5)	2.9 (0.4) b	2.9 (0.2) a
120	3.3 (0.4)	3.2 (0.2) b	3.2 (0.2) b
150	3.4 (0.4)	3.1 (0.2) b	3.6 (0.2) c
Piedmont			
N rate (lb ac^{-1})	GS 25 %	GS 30 %	GS 45 %
0	4.4 (0.6)	3.2 (0.6) a	2.8 (0.3) a
30	4.4 (0.9)	3.2 (0.5) a	2.8 (0.3) a
60	4.4 (0.7)	3.7 (0.5) b	3.0 (0.3) ab
90	4.5 (0.7)	3.7 (0.7) b	3.3 (0.3) bc
120	4.4 (0.8)	3.9 (0.7) b	3.4 (0.2) cd
150	4.5 (0.6)	3.9 (0.6) b	3.6 (0.3) d
Sandhills			
N rate (lb ac^{-1})	GS 25 %	GS 30 %	GS 45 %
0	2.5 (0.6) b	1.8 (0.4) a	2.4 (0.3) a
30	2.2 (0.5) ab	1.7 (0.8) a	2.1 (0.3) a
60	2.1 (0.4) ab	2.5 (0.5) b	2.2 (0.2) a
90	2.2 (0.6) ab	2.3 (0.4) ab	2.6 (0.3) a
120	2.1 (0.6) ab	2.2 (0.2) ab	3.2 (0.3) b
150	2.0 (0.5) a	2.3 (0.5) ab	3.4 (0.7) b
Coastal Plain			
N rate (lb ac^{-1})	GS 25 %	GS 30 %	GS 45 %
0	2.1 (0.8)	1.5 (0.1) ab	2.7 (0.2) a
30	2.1 (0.7)	1.4 (0.1) b	2.7 (0.2) a
60	2.1 (0.7)	1.8 (0.2) c	2.7 (0.2) a
90	2.1 (0.7)	1.6 (0.1) bc	3.0 (0.3) ab
120	2.1 (0.7)	1.7 (0.2) c	3.2 (0.6) b
150	2.2 (0.8)	1.8 (0.1) c	3.7 (0.4) c

Means with the same letter in a column are not significantly different according to Tukey's HSD at $p<0.05$.

Table A.6 – Effect of year on mean tissue N at Zadok's growth stage (GS)25, 30, and45 by location. Standard deviations are shown in parentheses. English Units

Mountain			
Year	GS 25 % N	GS 30 % N	GS 45 % N
2018	3.5 (0.4) b	2.8 (0.3)	3.1 (0.4) b
2019	3.1 (0.3) a	2.8 (0.4)	3.0 (0.3) a
Piedmont			
Year	GS 25 % N	GS 30 % N	GS 45 % N
2018	3.3 (0.2) b	4.1 (0.4) b	3.3 (0.3) b
2019	3.0 (0.3) a	3.1 (0.4) a	3.0 (0.4) a
Sandhills			
Year	GS 25 % N	GS 30 % N	GS 45 % N
2018	2.6 (0.3) b	1.8 (0.3) a	2.7 (0.7)
2019	1.8 (0.3) a	2.5 (0.6) b	2.6 (0.4)
Coastal Plain			
Year	GS 25 % N	GS 30 % N	GS 45 % N
2018	2.8 (0.3) b	1.6 (0.2)	3.2 (0.5) b
2019	1.5 (0.2) a	1.5 (0.2)	2.8 (0.4) a

Means with the same letter in a column are not significantly different according to Tukey's HSD at $p<0.05$

Table A.7 – *P*-values from analysis of variance for RVA, and germination energy at 4ml (GERM 4) with nitrogen rate, location, and year as fixed effects. Significant ($p < 0.01$) effects are indicated in bold.

	RVA	GERM4
N Rate	0.2392	0.0697
Location	**<0.0001**	**<0.0001**
Year	**<0.0001**	**<0.0001**
N Rate × Year	**0.0068**	0.7008
Location × Year	**0.0002**	**0.0111**
N Rate × Location × Year	**0.0050**	0.323

Table A.8 - P-values of analysis of variance for RVA, DON and germination energy at 4ml (GERM4) with nitrogen rate, and year as fixed effects at the piedmont location. Bold indicates significance at the *p*<0.01 level.

Piedmont			
	N Rate	Year	N Rate × Year
RVA	0.1557	**<0.0001**	0.4131
DON	0.2552	**<0.0001**	0.1158
GERM4	0.1408	**<0.0001**	0.6414

Table A.9 Effect of year on RVA, DON, and GERM4 at the piedmont location. Metric Units

Piedmont Research Station			
Year	RVA	DON ppm	GERM4 mg g^{-1}
2018	184.0 (13.3) b	<0.1 (0.0) b	9800 (110) b
2019	156.7 (9.7) a	1.5 (0.6) a	9380 (260) a

Means with the same letter in a column are not significantly different according to Tukey's HSD test at p<0.05.

Table A.10 Effect of year on RVA, DON, and GERM4 at the piedmont location. English Units

Piedmont Research Station			
Year	RVA	DON ppm	GERM4 %
2018	184.0 (13.3) b	<0.1 (0.0) b	98.0 (1.1) b
2019	156.7 (9.7) a	1.5 (0.6) a	93.8 (2.6) a

Means with the same letter in a column are not significantly different according to Tukey's HSD test at p<0.05.

Table A.11 - Correlation of barley tissue nitrogen content measured at three growth stages to protein content, yield, and plumpness in 2018 and 2019 and at all locations.

2018			
	Protein	Yield	Plumpness
GS25	-0.03943	0.46669	-0.23241
	NS	**	*
GS30	0.00552	0.45167	-0.32534
	NS	**	*
GS45	0.64415	0.64685	-0.28122
	**	**	*
2019			
	Protein	Yield	Plumpness
GS25	-0.15108	0.64903	0.61059
	NS	**	**
GS30	-0.03715	0.54635	0.17748
	NS	**	NS
GS45	-0.03999	0.66842	0.35727
	NS	**	*

***, *, and NS indicate p <0.0001, p < 0.05, and not significant (p > 0.05), respectively*

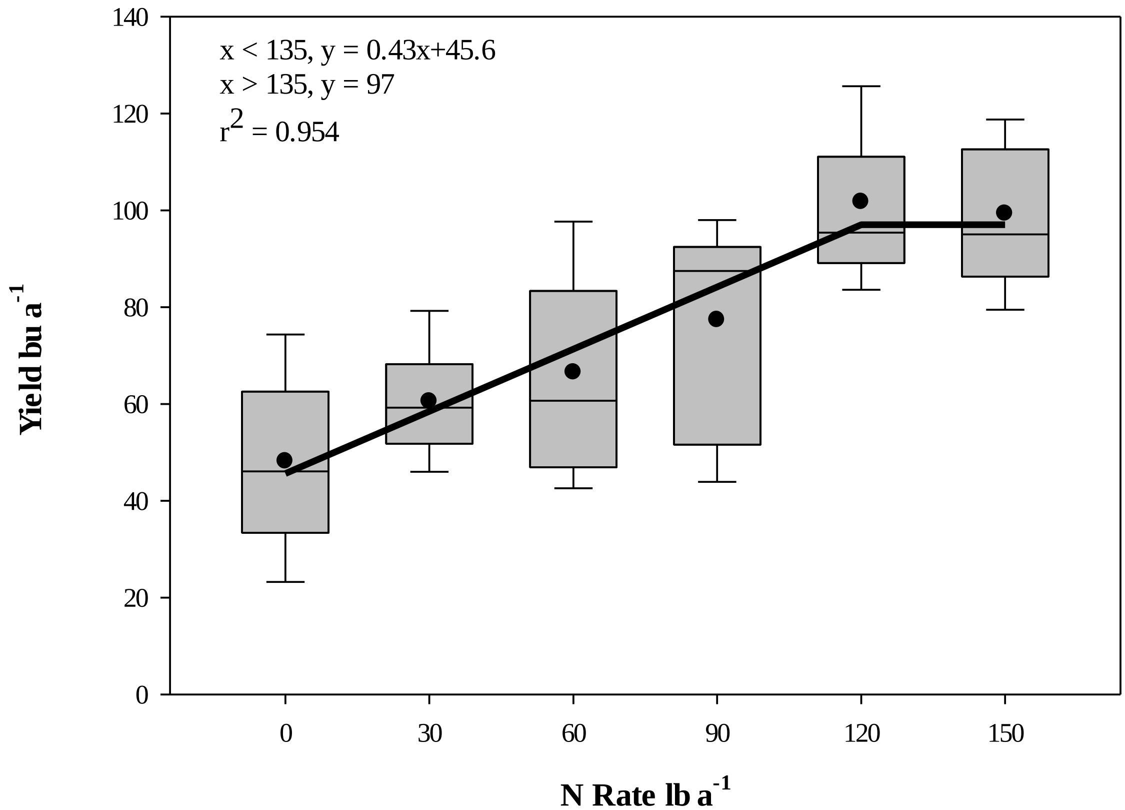


Figure A.1 - Barley grain yield linear-plateau model for the mountain location. English units.

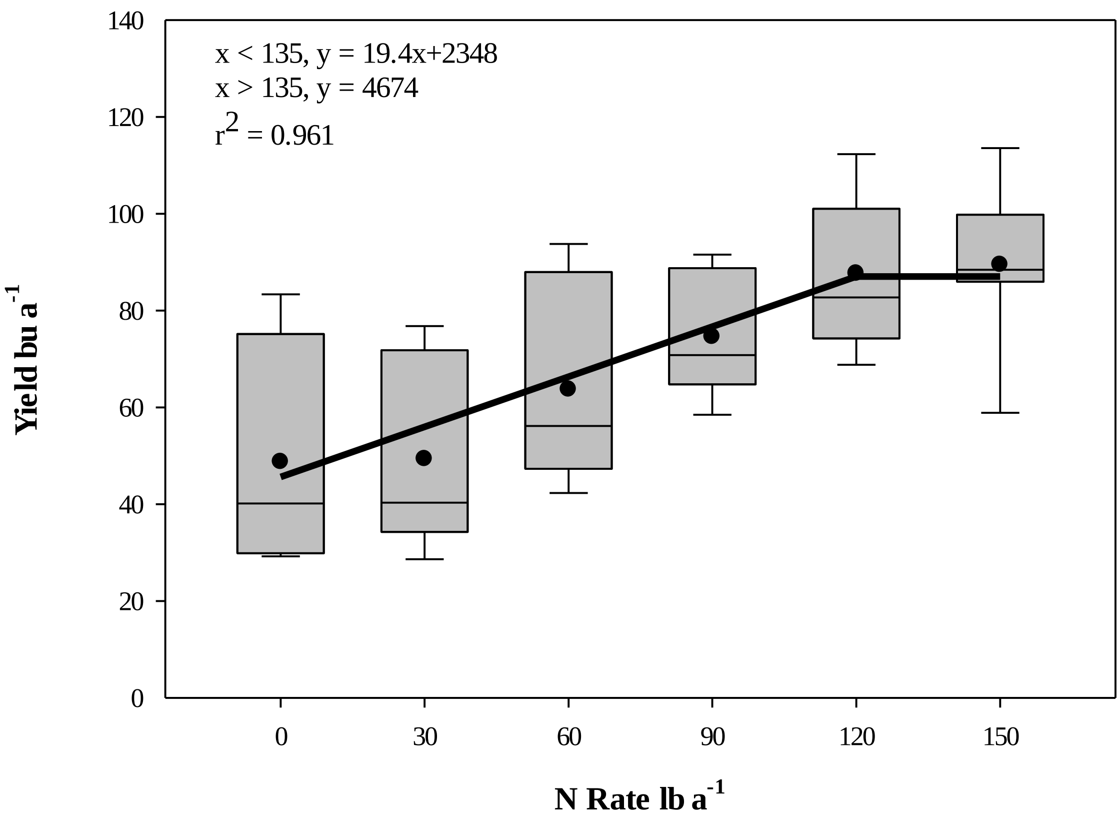


Figure A.2 - Barley grain yield linear-plateau model for the piedmont location. English units.

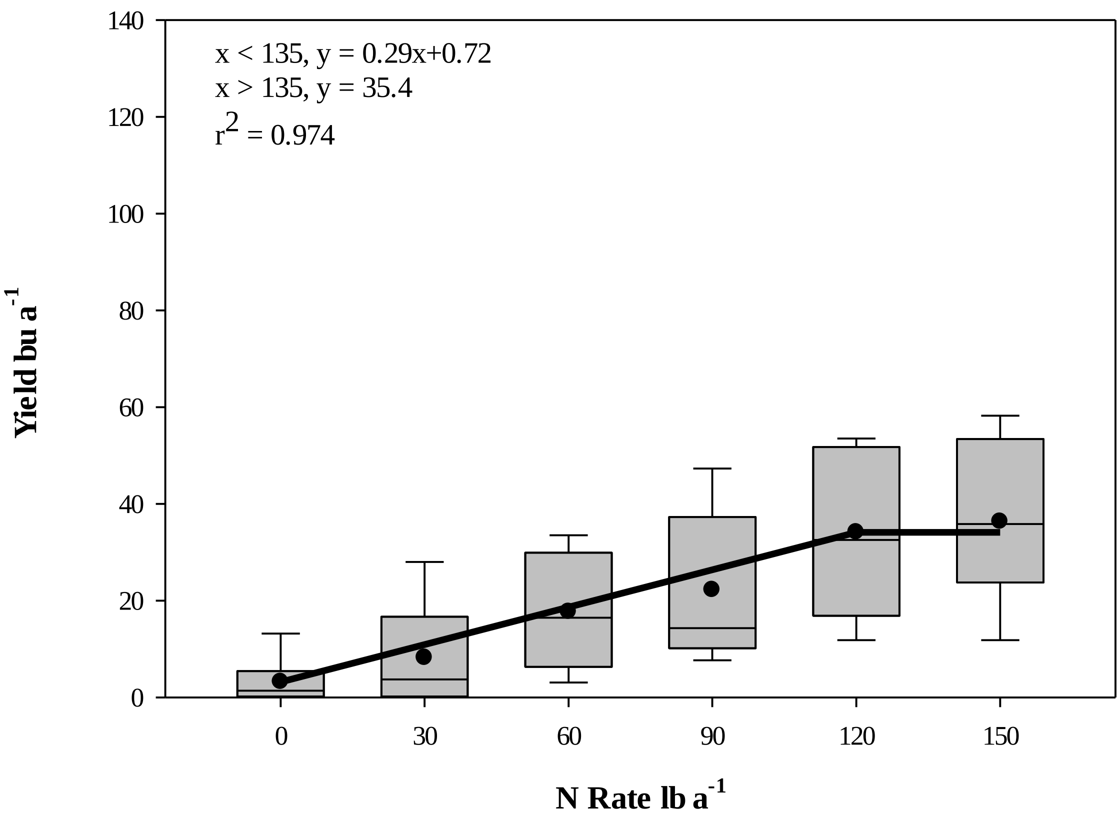


Figure A.3 - Barley grain yield linear-plateau model for the sandhills location. English units.

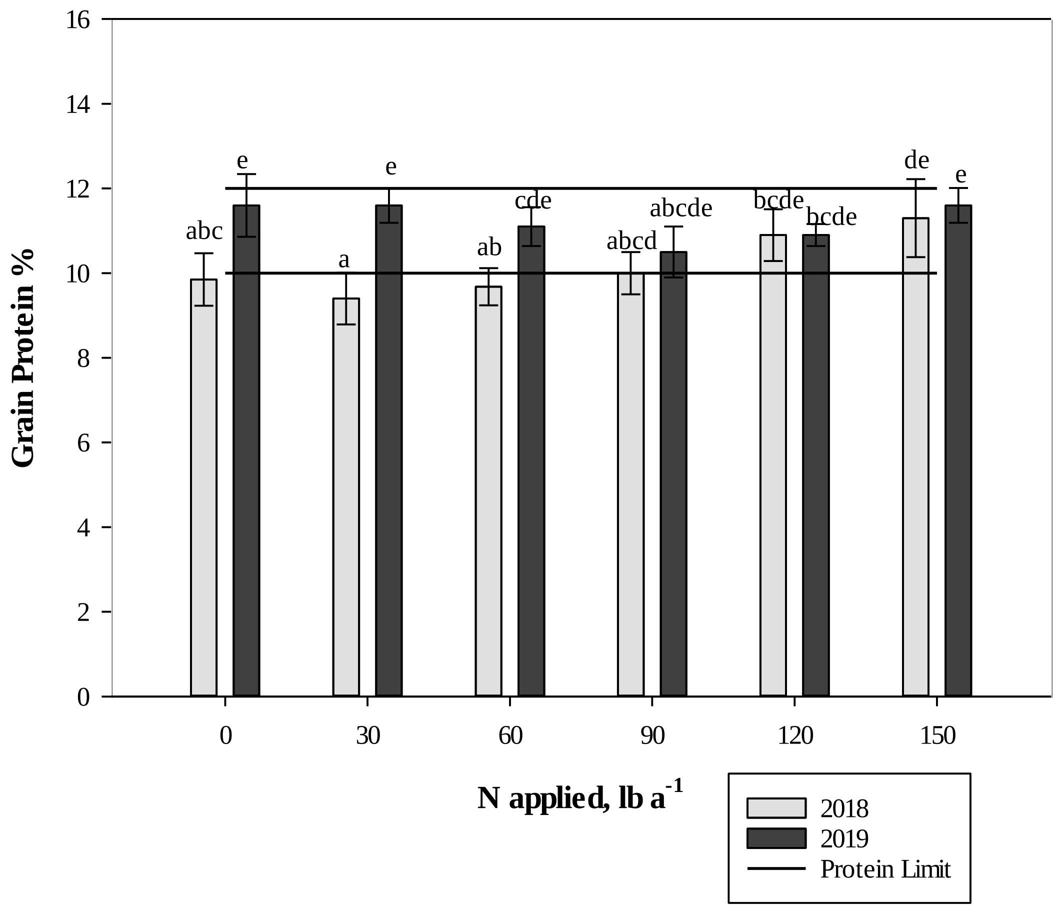


Figure A.4 - Barley grain protein by treatment×year interaction at the piedmont location with standard deviations. Significant difference between means at the *p*<0.05 level is indicated by different letters. English units.

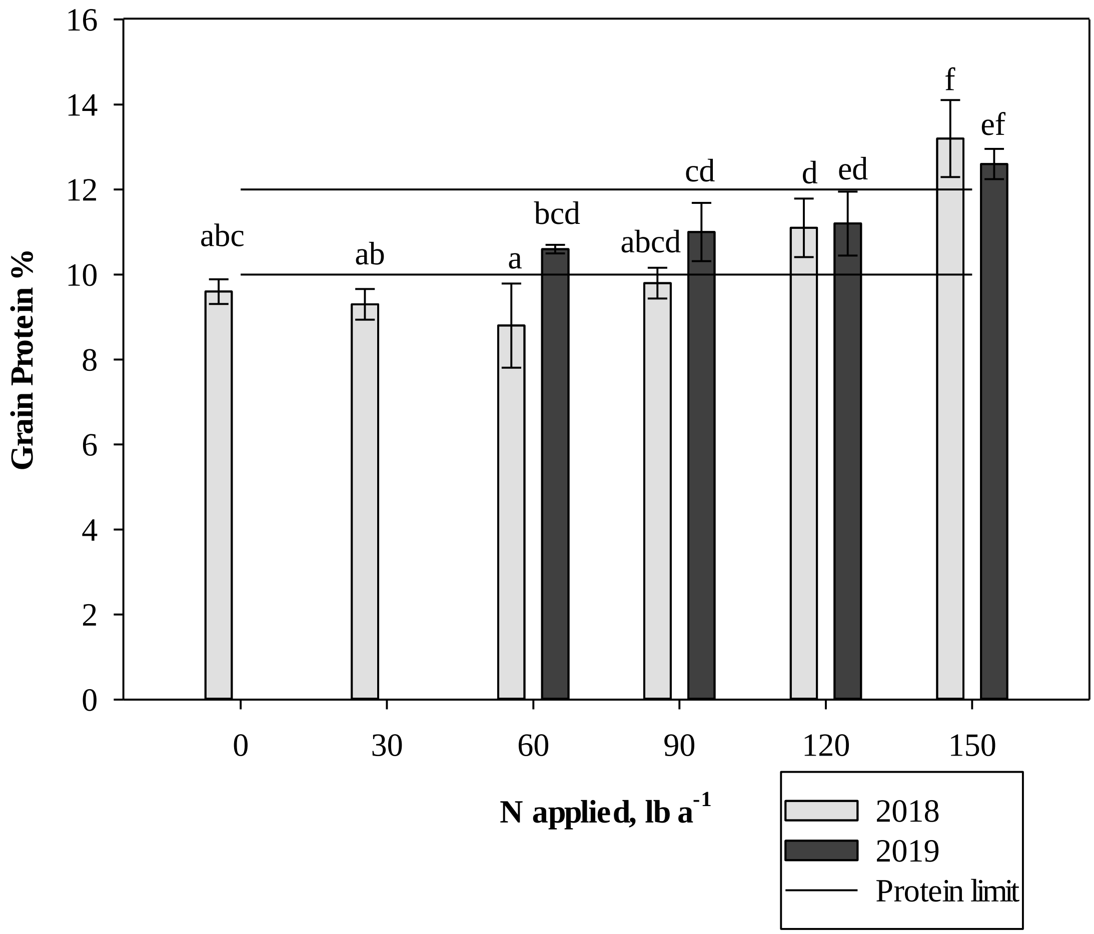


Figure A.5 - Barley grain protein by treatment×year interaction at the sandhills location with standard deviations. Significant difference between means at the $p<0.05$ level is indicated by different letters. English units.

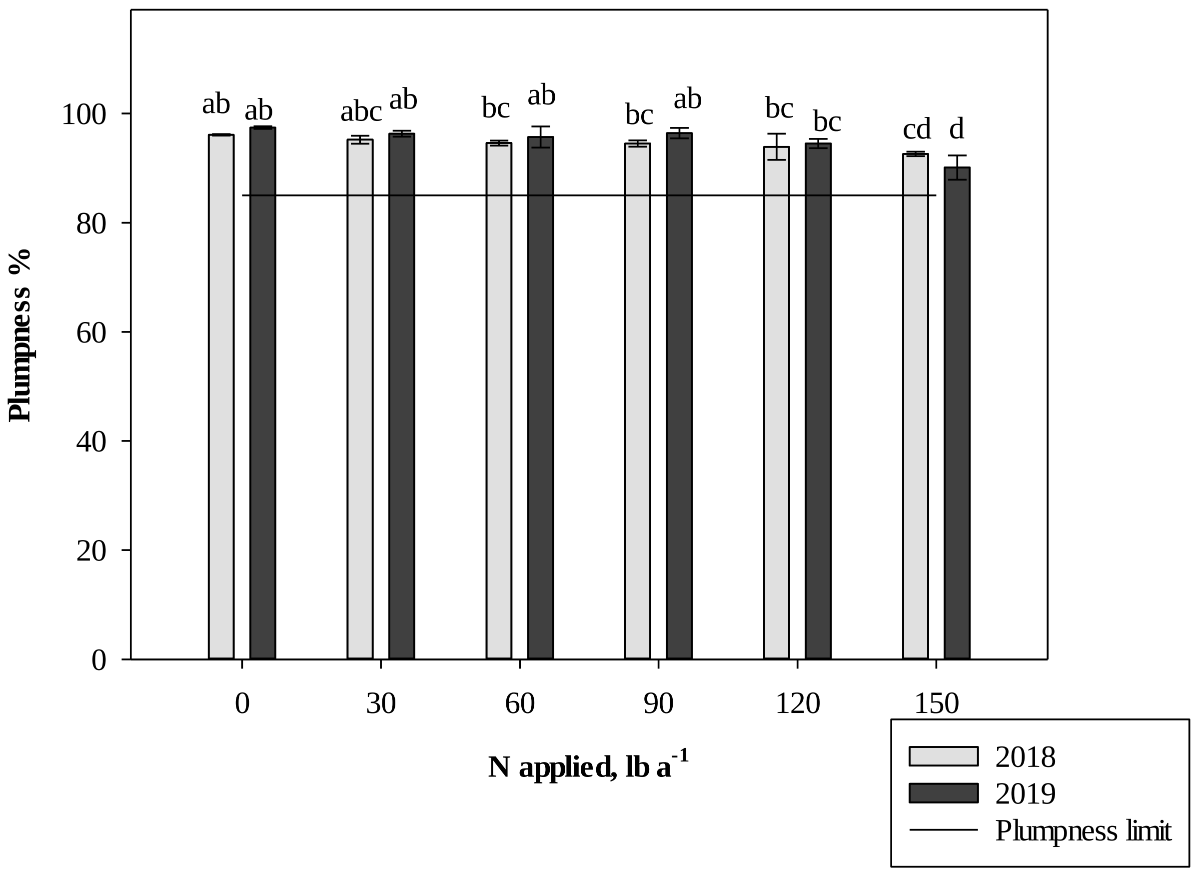
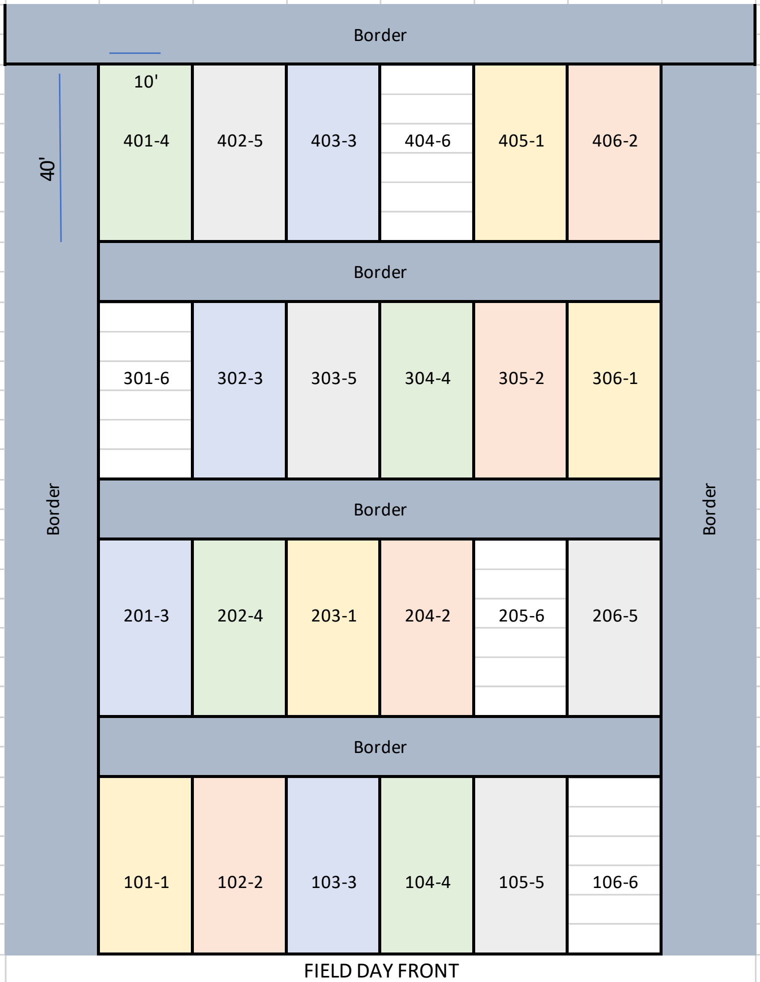


Figure A.6 - Barley kernel plumpness by treatment×year interaction at the piedmont location with standard deviations. Significant difference between means at the p<0.05 level is indicated by different letters. English units.



10 ft Borders
2 ft row between each rep.

Treatment (total lb/N)	Pre-plant	GS25	GS30
T1 (0)	0	0	0
T2 (30)	30	0	0
T3 (60)	30	30	0
T4 (90)	30	30	30
T5 (120)	30	30	60
T6 (150)	30	30	90

Trt1	0 lb	101-401
Trt2	30 lb	102-402
Trt3	60 lb	103-403
Trt4	90 lb	104-404
Trt5	120 lb	105-405
Trt6	150 lb	106-406

Figure A.7. Plot map with treatment layout all locations 2018.

Trt1	0 lb
Trt2	30 lb
Trt3	60 lb
Trt4	90 lb
Trt5	120 lb
Trt6	120 lb
Trt7	150 lb
Trt8	150 lb

Treatment (total lb/N)	Pre-plant	GS25	GS30
T1 (0)	0	0	0
T2 (30)	30	0	0
T3 (60)	30	30	0
T4 (90)	30	30	30
T5 (120)	30	30	60
T6 (120)	30	60	30
T7 (150)	30	30	90
T8 (150)	30	90	30

Dimensions 240 x 114 ft =
27,360 ft2
0.63 Ac

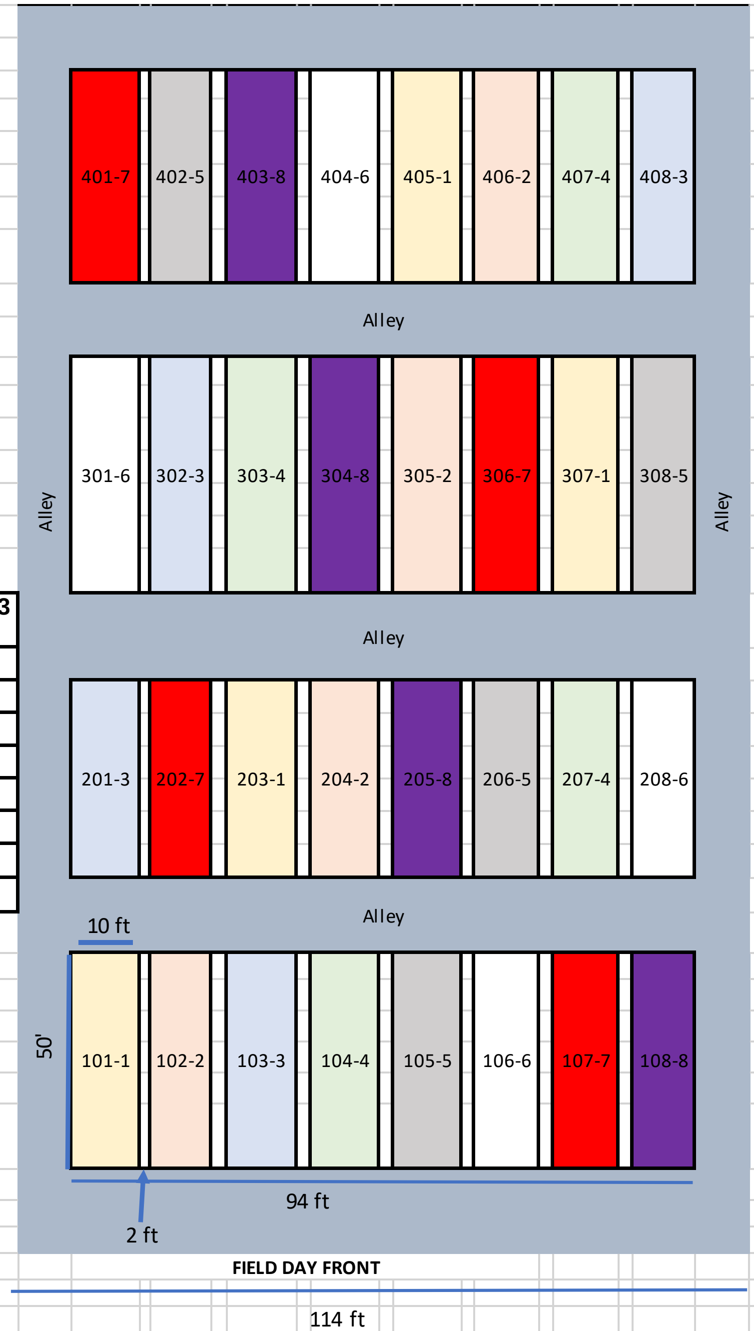


Figure A.8. Plot map with treatment layout all locations 2019. Treatments 7 and 8 left out of analysis due to being incomparable to previous year's results.