AGRONOMIC PRACTICES THAT IMPACT GRAIN QUALITY FACTORS OF DURUM WHEAT

(*TRITICUM TURGIDUM* L. *VAR. DURUM*)

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Agronomic Practices That Impact Grain Quality Factors of Durum Wheat

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

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ABSTRACT

Durum wheat is a type of wheat primarily used for pasta production. North Dakota is the leading producer of durum wheat in the US with average yields of 2700 kg ha^{-1}. Durum wheat price discounts are common and occur due to disease, heavy metal contamination, and environmental issues that impact grain quality. Studies were conducted in order to determine how agronomic approaches might impact durum quality. Experiments were conducted in order to determine what impact planting date, cultivar, and seeding rate had on the agronomic performance and quality of end-use traits. In general, a delay in planting date resulted in a significant decrease in yield and test weight for all cultivars. Cultivars differed for many of the end-use traits evaluated such as protein content, falling number, and vitreous kernel. Seeding rate had little impact on the traits evaluated. No combination of planting date and cultivar was identified that consistently resulted in grain marketed as US Grade 1 hard amber durum (HAD), or 'choice durum'. Cultivar selection remains the best option for maintaining end-use traits. The effect of Zn fertilizer source and placement on grain Cd were evaluated. Treatments evaluated had no negative impact on grain yield or test weight. The foliar application of 1.1 kg Zn ha^{-1} Zn-EDTA in combination with 33 kg N ha^{-1} in the form of UAN applied at Feekes 10 growth stage (boot stage) resulted in the lowest grain Cd, and highest grain Zn, Fe and protein and represents an approach of biofortification for durum wheat.

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DEDICATION

To the loves of my life,

Aidan Marcus

&

Ella Suzanne

"The days are long, but the years are short."

~Gretchen Rubin

PREFACE

This dissertation was written in three separate chapters to be submitted for publication. Chapter 1 is a General Introduction and Literature Review. Chapters 2 and 3 are designed to be stand-alone journal articles and include an Introduction, Materials and Methods, Results and Discussion and Literature Cited. General conclusions are presented at the end of the dissertation. Due to the similarity of information, some repetition among chapters was unavoidable.

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CHAPTER 1. GENERAL LITERATURE REVIEW

Introduction

Durum wheat (*Triticum turgidum* L. *var. durum* Desf.) is a market class of wheat commonly grown in North Dakota. In 2014, 567,000 hectares of durum wheat were planted with an average yield of 2700 kg ha^{-1} (NASS, 2014). North Dakota produces approximately 60% of all U.S. durum wheat, but has previously produced more than 87%. The variability in production can be attributed to market price and quality concerns. Also, durum wheat production in ND has declined in recent years due to acreage competition with HRSW. Generally, HRSW is less vulnerable to the quality discounts associated with durum wheat. Durum wheat has many grain quality characteristics that are required for suitable pasta production. High protein content, density and gluten strength make ND durum wheat the choice for pasta production worldwide with approximately 35% of the durum wheat produced in ND exported internationally (J. Petersen, ND Wheat Commission, personal communication). Durum wheat production in ND has declined in recent years due to competition with hard red spring wheat (HRSW) (*Triticum aestivum* L.) and other crops. Generally, HRSW is less vulnerable to the quality discounts associated with durum wheat. Optimal planting date and selection of cultivars with favorable stress responses are agronomic management factors that could also maximize durum wheat yield and quality (Pfeiffer et al., 2000).

The most important disease of durum wheat that can impact quality in ND is Fusarium Head Blight (*Fusarium graminearum* Schwabe) (FHB) (McMullen et al., 2008). Most of the quality discounts associated with FHB are due to the presence of a pathogen-produced mycotoxin in the grain called deoxynivalenol (DON). DON can impact human and animal health by causing nausea, vomiting and diarrhea (Sobrova et al., 2010). Durum wheat producers in ND have seen an increase in management requirements needed to produce high quality grain. One of the few options growers have for FHB management is the use of a fungicide protectant. However, timing and coverage of the fungicide can be

very important to the success in managing this disease and DON levels associated with it. Currently, durum wheat cultivars have little genetic resistance to FHB.

Another potential quality issue with durum wheat production and marketing in ND is grain seed cadmium (Cd). Cadmium is a toxic heavy metal found in the environment that can impact human health. Some ND soils have been identified with relatively high diethylenetriaminepentaacetic acid (DTPA)-extractable Cd. Durum wheat tends to accumulate more Cd than other grain crops grown in ND.

There are two major risk categories associated with high Cd durum wheat. First is the potential health risk associated with consuming food high in Cd. The other risk is the opportunity to market durum wheat internationally. International standards limit the amount of Cd in food (CAC, 2009). Currently, the acceptable level of Cd in wheat grain is 0.2 mg kg^{-1} (CAC, 2009). It would be beneficial for ND producers to have management options available to help ensure that Cd levels in durum wheat grain do not exceed the established limits in order for it to be accepted worldwide.

Agronomic practices that minimize Cd accumulation in harvested grain might be an option. The use of low Cd accumulating durum wheat cultivars is an important management option for ND producers. Currently, there are few low Cd accumulating durum wheat cultivars available for ND producers and other management techniques are needed in order to meet international standards. Additionally, the interaction of cultivars with management practices could be a way to ensure that even lower Cd levels can be achieved. Agronomic management factors might be a way for producers to maintain optimal grain seed quality in regards to Cd accumulation and DON in harvested grain.

The research reported herein was conducted in order to determine ways to maximize durum quality, while using profitable agronomic practices. The specific objectives of this research was to 1) determine how planting date, seeding rate, and cultivar impact agronomic traits and pasta quality 2) determine how the type and placement of Zn fertilizer might impact grain seed Cd levels and 3) determine an appropriate timing for a foliar Zn fertilizer application that will reduce grain Cd levels and

also fit into current durum wheat management practices. The results of these studies will enable recommendations to be developed which will aid producers in applying agronomic practices in order to minimize negative grain quality factors.

Literature Review

Durum Wheat Quality

Durum wheat quality requirements differ depending on the end user (Troccoli et al., 2000). Producers, seed companies, grain buyers, grain millers, the pasta industry, and consumers evaluate durum wheat quality from different perspectives. However, durum wheat is primarily grown for producing high-quality pasta. Quality attributes of harvested grain such as density, high protein content, and gluten strength are required to produce high-quality pasta. Production factors can impact harvested durum wheat quality. Some of these factors include proper disease management and limiting heavy metal accumulation.

Cultural practices such as cultivar grown, field selection, crop rotation, fertility, seeding rate, and planting date can impact harvested grain quality. Planting date and seeding rate of durum wheat can vary based on producer and could impact the quality of harvested grain due to the environmental conditions during grain filling and harvest. Numerous diseases can affect durum wheat, but the disease with the most impact in ND is currently FHB or scab. Most of the quality discounts associated with FHB are due to the presence of a pathogen-produced mycotoxin in the grain called DON. Management of FHB is commonly done with the use of protectant fungicide applications. These applications are essential due to limited genetic resistance.

Heavy metal contamination can impact durum wheat quality and marketability. The European Union (EU) is the most stringent in their regulation of heavy metal contaminants such as Cd. For instance, the use of Cd in non-food items, such as filler in the production of metal jewelry, is prohibited. The EU also threatens to lower the level of acceptable contamination in some food imports. This threat

is of concern to ND durum wheat producers that export grain to the EU. Approximately, one-third of ND durum wheat is exported each year (J. Petersen, ND Wheat Commission, personal communication). Some of the countries in the EU that import ND durum wheat include Italy, Germany, Belgium and Spain. Currently, the Codex Allimentarius Commission of the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) has set the acceptable level of Cd in wheat grain of 0.2 mg kg^{-1} (CAC, 2009).

Durum wheat grades are determined by the Federal Grain Inspection Service (FGIS) and the USDA Grain Inspection, Packers, and Stockyards Administration (USDA, 2013). The grades of durum wheat are considered when marketed and determined based on many quality factors. The majority of the 2014 durum wheat crop was graded #2 Amber Durum (AD), while the 2015 crop was graded #1 Hard Amber Durum (HAD) (ND Wheat Commission, 2015). Essentially, HA durum wheat has a minimum test weight of 772 kg m^{-3}, less than 2% damaged kernels, and has a maximum limit of 0.4% foreign material, 3% broken kernels and defects (USDA, 2013). Disease damaged kernels can affect test weight and damaged and broken kernels. Hard amber durum wheat also has 75% or more of hard vitreous kernels. Grain kernels are considered hard vitreous due to higher protein content which is the primary factor affecting pasta texture (Dexter et al., 1988).

Quality characteristics important to pasta production from durum wheat include total extraction, semolina extraction, ash content, specks, protein content, and mixogram score (Troccoli et al., 2000). The total extraction is the portion of the durum wheat kernel that can be milled into flour or semolina. Semolina extraction is the portion of semolina taken from the kernel. Ash content is a measure of mineral content in the flour or semolina extracted. Specks are bran that escaped the cleaning and purifying process. These specks in finished pasta products can be perceived as a contaminant. Durum wheat high in disease or foreign material is most likely to cause specks in semolina. High protein content in durum wheat is highly correlated with high gluten strength. High gluten strength

improves cooking quality. The gluten quality of semolina can be determined by evaluating the mixogram curve. Mixogram ratings are based on a 1 to 8 scale, with a higher value indicating dough quality.

Agronomic Practices

Agronomic practices such as proper field selection, crop rotation, cultivar, planting date, seeding rate, fertility, weed management, and integrated pest management (IPM) are all important factors to consider when producing high-quality durum wheat. A critical factor for producing high-quality durum wheat is cultivar grown. New cultivars of durum are released based on the potential to have higher yields, better disease resistance profiles, and better end-use quality (Royo et al., 2009). Unfortunately, durum wheat quality in recent years in ND has been most impacted by FHB. Little genetic resistance is available in current durum wheat cultivars.

Numerous studies have been conducted involving the effect of N on durum wheat yield and quality. Black and Siddoway (1977), at Mandan, ND, found that yield response due to N and P fertilization was greatest for early and medium seeding dates than for the late seeding date for hard red spring and durum wheat. 'Olaf' spring wheat and 'Crosby' durum wheat were planted at a rate of 70 kg ha^{-1} and at a depth of 4.5 cm. Test weight of Crosby was not impacted by planting date. Additionally, Sing and Jain (2000) found that durum wheat yield in India was greatest at normal planting dates (November 15) and with increasing N and irrigation. Grain protein, ß-carotene, and sedimentation value were highest for the latest planting date (December 2). A genotype x N input interaction was observed for several characteristics such as shoot biomass at anthesis, grain yield, and straw yield of early planted durum wheat in California (Ehdaie and Waines, 2001). Early planting did not have a significant advantage over optimum planting for the traits evaluated, except more N was removed from the soil with early planting.

Seeding rate trials have been previously conducted in ND. However, many of these trials were conducted with cultivars no longer grown. Quick and Wilkens (1975) suggested early planting dates and

seeding rates between 84 and 100 kg ha^{-1} for optimum grain yield and quality when grown in ND. Riveland et al. (1979) at Williston, ND, determined that yield of hard red spring and durum wheat was maximized at 247 plants m^{-2} and only when yield potential was greater than 2350 kg ha^{-1}. Average HRSW yields across ND during the early 1970s were close to 2350 kg ha^{-1}. Most recently, Hanson and Lukach (1992) working near Langdon, ND, found that test weight and protein were not impacted by a change in seeding rate across locations in north eastern ND. A seeding rate of at least 141 kg ha^{-1} was suggested to optimize yield.

Cultivar, nitrogen rate, seeding date, and soil type have been previously evaluated to determine the effect of Cd accumulation in durum wheat grain. Additionally, management of toxic, heavy metals might be possible through agronomic practices that limit Cd uptake during grain fill and by the use of low Cd accumulating lines. Most of the cultivars of durum wheat grown in ND do not contain the allele for low Cd accumulation. Perilli et al. (2010) found Cd and protein were affected by N application. In most years, the Cd concentration increased with N application, but large differences were observed in years and seeding date which indicated an environmental effect. The authors suggested multiple management practices would enable producers to maximize grain protein and minimize grain Cd. Seeding rate could impact grain yield by increasing the number of spikes per area. Grain yield, post-heading N accumulation, and N remobilization were highest at a seeding rate of 400 seeds m^{-2} (Arduini et al., 2006). The cultivar x seeding rate interaction was not significant for any factors evaluated.

Edhaie et al. (2001) found that durum wheat genotypes responded differently to planting date and N rate when grown at three planting dates in California. No genotype performed consistently across planting dates. Planting date also could impact semolina and pasta quality due to environmental changes during grain filling. Increased protein content observed in later sown durum wheat was suggested as a reason for increased dough strength from harvested grain (Motzo et al., 2007). These authors also observed a decrease in gluten index when planting of durum wheat was delayed and

proposed that temperatures higher than 30˚C at grain filling might affect the gluten polymerization process. In a similar study, Fois et al. (2011) found that gluten index increased as temperatures rose to 30˚C and then decreased under high temperatures when grown in the Mediterranean. In addition, spaghetti firmness and protein were positively correlated, but independent of planting date. As a result of this study, these researchers suggested that later planting dates might be a way of increasing pasta cooking quality by increasing protein.

Cadmium management research conducted includes the use of cultivar, application of fertilizer, tillage, planting date and crop rotation. The use of genetic resistance and favorable agronomic practices can reduce Cd accumulation in durum wheat (Grant et al., 2007). Low-Cd accumulating durum wheat cultivars have been developed (Clarke et al., 2006). However, these might not be available to producers or have desirable agronomic characteristics.

The application of micronutrient fertilizers or soil amendments to limit grain Cd has been studied. Choudhary et al. (1995) found that durum wheat tends to accumulate Cd in roots, leaves, stems and grain, regardless of fertilizer treatment when grown under greenhouse conditions. Cadmium concentrations were highest in roots and lowest in grain for both the low and high Cd accumulating durum wheat genotypes. Soil-applied Zn fertilizer lowered Cd concentration in all plant parts tested. However, the application of foliar Zn had little effect on Cd levels. Additionally, they found that application of soil-applied N and P fertilizer with Zn fertilizer decreased plant Cd (Choudhary et al., 1995).

Zinc fertilizer is available in many different forms and could be applied by producers at different growth stages of durum wheat. Since Zn can compete with Cd for uptake by plants, this might be a way to manage Cd levels in the grain. Application of foliar Zn significantly reduced grain Cd levels in durum wheat in cultivars tested in Montana in dryland production in some years (Eckhoff, 2010). Higher rates

of Zn (18.7 L ha^{-1} of chelated Zn-ethylenediaminetetraacetic acid) (EDTA) resulted in lower grain Cd levels. The addition of humic acid with the foliar Zn treatment did not affect seed Cd levels.

Application of soil-applied $ZnSO_4$ and ground-up rubber had no significant effect on grain cadmium level, grain yield or protein content on two durum wheat cultivars grown on high Cd soils in Arizona (Wang et al., 2011). Oliver et al., (1994) found that the Cd concentration in wheat grain could be decreased by up to 50% by the addition of 2.5 to 5.0 kg Zn ha^{-1} on soils marginal for Zn. Others found that soil-applied Zn reduced Cd concentration in durum wheat, but foliar applied Zn did not (Choudhary et al., 1995).

The application of Zn fertilizer as a means of reducing Cd uptake has also been studied in other crops with mixed results. Bell et al. (1998) studied agronomic practices that minimized Cd uptake of peanut in Australia. Management practices such as liming and application of soil-applied Zn fertilizer had no significant effect on grain Cd. However, Bell et al. (1998), hypothesized that the application of Zn fertilizer might be useful in slightly responsive cultivars that could be near the maximum permitted concentration of Cd. These studies suggested that site selection based on soil type and fertilizer type were the most effective way to manage Cd accumulation in peanut. Jiao et al. (2004) found an antagonistic effect of Zn on Cd uptake and distribution within flax (*Linum usitatissimum* L.) and durum wheat. These researchers also suggested possible explanations for the effect of Zn on Cd uptake and distribution including 1) Zn and Cd are in the same group in the periodic table and might compete with each other for exchange sites on root surfaces and for transport within the plant 2) Zn maintains the root-cell plasma membrane integrity with Zn-deficiency resulting in more Cd movement into the plant and 3) Zn-deficiency could increase root exudation of amino acids, sugars and phenolics and increase Cd availability in the soil.

In addition to Zn, other micronutrients have been evaluated for their ability to limit Cd accumulation in harvested grain. The use of Mg as a potential Cd suppressant was evaluated in rice

(*Oryza sativa* L.) and winter wheat. Kikuchi et al. (2008) found that an application of MgO at 2250 kg ha^{-1} decreased plant available Cd. The decrease was attributed to the increase in soil pH; the soil Cd concentration was significantly negatively correlated to soil pH. In Cd-polluted rice fields the application of MgO would reduce Cd contamination in harvested grain. The application of MgO (2250 kg ha^{-1}) and Mg silicate (MgO-SH-A) (2250 kg $ha^{-1}$and 4500 kg ha^{-1}) was evaluated for suppression of Cd uptake of winter wheat in a wheat-rice rotation (Kikuchi et al., 2009). The MgO application significantly suppressed the accumulation of Cd in winter wheat.

As previously stated, management practices such as planting date and fertilizer type can influence grain Cd concentration in durum wheat. In Manitoba, Canada, Perilli et al. (2010) found that environment factors, specifically soil type, had a significant impact on grain Cd. Furthermore, the researchers found that the application of N fertilizer increased the Cd concentration in harvested grain. Early and middle planting dates generally had higher grain Cd than later planting dates. They hypothesized that the increased Cd was the result of the increased yields associated with the earlier planting dates.

Fusarium Head Blight

Quality concerns associated with durum wheat production in ND are often attributed to disease issues of the harvested grain. The most devastating disease of durum wheat in recent years that impacts quality is FHB, or scab, caused by the fungus *Fusarium graminearum*. As previously stated, quality discounts associated with FHB are due to the presence of a pathogen-produced mycotoxin in the grain called DON or the due to damaged kernels which can result in lower test weights of harvested grain. The mycotoxin DON is a member of the trichothecenes family of mycotoxins and can impact human and animal health by causing nausea, vomiting, diarrhea, abdominal pain, headache, dizziness, and fever in animals (Sobrova et al., 2010). DON is the most prevalent trichothecenes found in small grains worldwide (Ovando-Martinez et al., 2013). DON is also referred to as vomitoxin, because when grain

with high levels of DON is fed to pigs, they vomit. Human exposure is directly through foods such as cereal grains or indirectly through foods of animal origin (kidney, liver, milk, eggs).

Environmental conditions and a lack of genetic resistance to FHB in durum wheat cultivars have resulted in a decrease in the quality of harvested grain. In the late 1990s, FHB began to have a major impact on durum wheat production in ND. FHB epidemics during this time resulted in significant yield loss and quality reductions (McMullen, 2008.). Significant research activities at the time began in order to help control and/or manage FHB with major areas of research focused on genetic resistance and fungicide efficacy. The additional cost and management needed to produce high-quality durum wheat is one reason for the decrease in durum acres in ND.

Durum wheat producers in ND have seen an increase in management requirements needed to produce high quality grain. One of the few options growers have for FHB management is the use of a fungicide protectant. Currently, durum wheat has little genetic resistance to FHB and producers rely primarily on fungicide application to control FHB. Fusarium head blight management and DON accumulation are not always highly correlated and even with a fungicide application, durum wheat quality might be severely impacted (McMullen, 2008). However, timing and coverage of fungicide can be very important in managing this disease and the DON levels associated with it.

Quality concerns of FHB

Severe yield losses can occur with FHB, but the major impact of this disease is on grain quality. Fusarium infection can influence grain quality by producing shriveled and lightweight, pinkish seed. These grain kernels are often referred to as 'tombstone' kernels due to their chalky and lifeless appearance (Aakre et al., 2005). Most of the FHB-related quality discounts in durum wheat are associated with the presence of a fungal produced mycotoxin in the grain called DON.

DON has been detected in many cereal grains and their associated food products including flour, bread, breakfast cereals, noodles, infant foods, pancakes, malt, and beer (Yazar et al., 2008). One

characteristic of DON that can impact food quality and subsequent human consumption is its ability to withstand high temperatures during processing. DON is stable between the temperatures of 170 to 350˚C. However, DON levels are reduced in cooked pasta because of leaching (Manthey et al., 2004) but DON reduction is not observed during frying DON-contaminated food. The presence of DON in fermented beer has been studied. DON levels in Holland and Germany ranged from 26 to 41 mg L^{-1} to greater than 200 ng ml^{-1}, respectively (Schothorst and Jekel, 2003).

Toxins from grain can not only impact processed food, but the damage from FHB can affect the pasta quality of grain by impacting kernel weight and test weight resulting in less semolina yield. Semolina is the portion of durum wheat grain used for pasta production. Dexter et al. (1997) found that Fusarium damaged grain resulted in changes to semolina and subsequent pasta color. They also found Fusarium damaged grain had weaker gluten strength, but not enough to change the overall pasta quality.

The disease pressure and subsequent damage to durum wheat is environmentally dependent. Favorable weather conditions, a susceptible host plant, and the FHB fungus are needed for infection of durum wheat. In 2015, the environmental conditions were less favorable for FHB development compared to recent years. In 2014 and 2015, the average DON for ND and MT durum wheat was 2.1 and 0.8 mg kg^{-1}, respectively (ND Wheat Commission, 2015).

Genetic resistance

In other market classes of wheat, such as HRSW, genetic resistance has been the most effective method to manage FHB. Genetic resistance generally is effective in many environmental conditions and does not have the extra cost associated with a fungicide application. Resistant FHB cultivars of HRSW have been released (Mergoum et al., 2007). Unfortunately, durum wheat is more susceptible to FHB than many of the HRSW market classes (McMullen et al., 2008).

Incorporating resistance in durum wheat from HRSW and other wheat market classes has had limited success. Substitution lines derived from wild emmer (T. turgidum L. var. *dicoccoides*) were developed in order to incorporate possible resistance to FHB (Stack et al., 2002). Each line had a chromosome pair substituted from a corresponding chromosome pair in 'Langdon' durum wheat. Results suggested that the genes affecting FHB resistance were present on different chromosomes.

Efforts to identify another source of genetic resistance to FHB from durum wheat are ongoing (Royo et al., 2009). Kianian et al. (2012) evaluated Tunisian-derived durum wheat populations. They found no correlation between disease incidence and DON or disease severity and DON. However, they did identify transgressive segregates from the population and efforts to incorporate the resistance into durum wheat breeding material were identified as a main objective.

Mapping of quantitative trait loci (QTL) for FHB resistance in durum wheat is limited. Three back-cross mapping populations between a FHB-resistant wild emmer line and three durum wheat cultivars were analyzed for QTL detection with QTL validation (Buerstmayr et al., 2012). A QTL with the largest effect on FHB-resistance was mapped to chromosome 4B and suggested that some-FHB-resistance genes are common between wheat species. The moderately resistant FHB-lines identified in the study were used to pyramid FHB resistance into adapted durum wheat lines.

FHB management

With little genetic resistance available in durum wheat, producers must manage FHB with other methods, including the use of an IPM program. Many studies evaluating a single factor for FHB management have been conducted with limited success. Studies using multiple management strategies including cultivar, fungicide, and crop rotation have been evaluated in the Northern Great Plains. These studies determined the lowest field severity and DON with highest yield and test weight were achieved using multiple management strategies (McMullen et al., 2008).

Additional IPM strategies to minimize FHB have been evaluated. Sweets (2012) evaluated the importance of crop sequence, cultivar, and fungicide application for FHB management of soft red winter wheat (SRWW) in Missouri. DON and FHB levels were significantly reduced when SRWW was planted after soybean rather than corn. May et al. (2014) found that foliar fungicide treatments resulted in higher kernel weight, grain yield, and test weight compared to the no fungicide treatment. The results showed that fungicide treatment can result in a yield increase without an improvement in grain quality. They also found that of the factors tested, cultivar of durum wheat had the most significant impact on grain damage caused from Fusarium.

The use of fungicides for the control of FHB damage, along with less susceptible durum wheat cultivars, can help decrease the risk of FHB damage in durum wheat. However, chemical control of FHB can be expensive and proper application timing is necessary in order to maximize its effectiveness. Optimum application timing for FHB control in durum wheat is early flowering (Feekes 10.51 growth stage) (Large, 1954; Hofman et al., 2000). Uniform fungicide trials conducted on multiple wheat market classes across six states determined that a triazole fungicide applied at Feekes 10.51 growth stage had the lowest FHB index values (McMullen et al., 2008). Field experiments in Italy determined that a double treatment of a strobilurin fungicide at stem elongation and at heading resulted in an increase in yield (32%) and FHB control (11%) and a decrease in DON contamination (45%) compared to the untreated control (Blandino et al., 2009.)

Cadmium

Heavy metal contamination from soil Cd has the potential to impact durum wheat quality. This is of specific interest to ND durum wheat production, quality, and marketing. Many soils found in ND naturally contain high levels of soil Cd and durum wheat is a known accumulator. As previously discussed, a large amount of durum wheat from ND is exported to international markets and could

impact trade. The effects of consuming high levels of Cd are a concern to human health as most durum wheat is consumed as a human food product.

Cadmium is a heavy metal, considered the most toxic trace element in the environment (Page et al., 1987). Its rapid uptake and accumulation in animals is of particular concern. Some of the disorders caused by Cd include kidney damage and skeletal disorders. It is of interest to note that these serious disorders occur due to Cd toxicity, but there are limited standards correlating human blood or urine Cd measurements with clinical toxicity (Bernhoft, 2013). The level of Cd in blood and urine are not sufficient indicators of Cd toxicity as most Cd is deposited in major organs such as the liver and kidneys. High toxicity can result in significant illness and even death in humans.

Humans can develop Cd accumulation via inhalation or ingestion. For example, Itai itai disease is the result of poisoning caused by food ingestion and inhalation of Cd. This disease was first documented due to the mass Cd poisoning of mining workers in Japan during the 1970's (Kobayashi, 1978). Some symptoms of itai-itai disease include softening of bones and kidney failure. Cigarette smoking is considered to be the most significant source of human cadmium exposure (Bernhoft, 2013).

Cadmium is naturally found in soil, water and air. Cadmium toxicity in the air can be measured by evaluating plant samples. These plant samples are then used as an index to determine airborne Cd pollution. Researchers evaluated Cd concentration in moss (*Hypnum cupressiforme* L.) to determine Cd pollution in the air from factories in Sweden (Shacklette, 1972). Locations with industries producing heavy-metal emissions tend to have higher Cd concentrations in moss. The U.S. Geological Survey found that the Cd concentration in Spanish moss (*Tillandsia usneoides* L.) could be used to determine the degree and type of air pollution at a particular location. Standard plant samples are maintained by the National Institute of Standards and Technology as reference material for Cd analysis.

Soil factors affecting Cd uptake

Cadmium level in the soil depend on many factors. Plant uptake of Cd is affected by soil properties, crop species and cultivar grown, fertilizers, agronomic practices and properties of the Cd metal source (Chaney, 2010). Soil properties that have the largest impact on Cd availability include pH, salinity, cation exchange capacity (CEC), organic matter and concentrations of other nutrients such as N, P and Zn. Most agricultural soils in the U.S. have a Cd concentration of 0.1 to 1.0 mg kg^{-1} based on 1N HCl extraction (Page et al., 1987). Young soils tend to have more Cd than highly weathered soils. Soil with parent material such as glacial till and alluvium are naturally high in Cd. Cadmium is released into water through weathering of rocks or released into the air from volcanoes or forest fires (Tran and Popova, 2013).

A major soil survey of 937 samples determined that the North Central region of the U.S., including ND, had a range of 0.20 to 0.94 mg kg^{-1} Cd with a mean of 0.37 mg kg^{-1} (Holmgren et al., 1993). Soils from the western and north central states tend to be higher in Cd than the rest of the U.S. Other studies have found that soils from the western and north central states tend to be higher in Cd than the rest of the U.S., with levels in ND varying from 0.01 to 0.31 mg kg^{-1} in DTPA-extractable Cd (Franzen et al., 2006). Additionally, they found that extractable Cd concentrations in the soil were lower in upland and sloping sites compared to lowland and depressional locations in ND. They suggested modified harvest of grain which segregates grain from low-lying areas and upland areas as a way to separate potentially high Cd grain and minimize marketing issues.

In addition to parent material from which the soil was formed, soil Cd levels can be increased due to Cd added from contaminated fertilizer products and soil amendments, Cd deposited from the atmosphere, and the Cd removed by crop production (Page et al., 1987). The addition of P fertilizer to crop land might increase the concentration of Cd in the soil if contaminated with Cd, depending on the source of P (Cook and Morrow, 1995). Fertilizers produced from sedimentary phosphate rock tend to

have more Cd contamination than other sources, but it also depends on processing techniques used (Roberts, 2014).

Other soil factors that can affect Cd uptake include pH and salinity. Soil properties such as pH can influence the amount of Cd taken up by crops. Generally, Cd concentration of plant tissue decreases as the pH of the soil increases when all other soil factors remain unchanged (Kirkman, 2006). An increase in soil pH increases Cd adsorption and reduces its extractability. Adams et al. (2004) found that total soil Cd and pH were the most significant factors affecting grain Cd concentration in wheat and barley (*Hordeum vulgare* L.). Soil salinity was identified as a soil factor that could enhance the availability of Cd in the soil (Chaney, 2010). Currently, ND has over 2.35 M hectares of soil considered saline (Brennan and Ulmer, 2012) which might impact Cd accumulation in crops known to accumulate Cd. In neutral soil and alkaline soils, Cl was identified as a factor associated with Cd uptake (Chaney, 2010). The presence of Cl caused more Cd to dissolve in nutrient solution and resulted in more Cd uptake.

Chloride is an essential nutrient for plants, particularly small grains, and research describing the impact of Cl fertilizer on crops has been conducted. In barley, Goos et al. (1987) found that fertilizing with KCl significantly reduced common root rot severity when grown in ND. Additionally, they found that grain yield was significantly increased with KCL fertilization at one site. Some studies found that Cl fertilizer rates had no effect on crops Cd. Evidence of enhanced Cd uptake of some wheat species were caused by elevated salinity or Cl. Other studies in wheat identified the mechanism for increased Cd uptake associated with Cl was induced Zn-deficiency (Khoshgoftarmanesh et al., 2006). The addition of Zn fertilizer caused a significant reduction in Cd uptake. In potato (*Solanum tuberosum* L.), the largest variation in tuber Cd content was correlated to water-extractable Cl (McLaughlin et al., 1994a). Chlorine was added to the irrigation water used to produce the potatoes. The elevated Cl levels mobilized the Cd in the soil and increased its plant availability. They also reported that the Cd content of the potato tubers was negatively correlated to the EDTA-extractable Zn in the soil.

Cadmium in durum wheat

Most plant species contain some amount of Cd; however, this level is usually low and Cd is not considered an essential micronutrient for normal growth and development. Cadmium toxicity in plants causes inhibition and abnormalities of normal plant growth and development (Tran and Popova, 2013). Cd toxicity in plants also affects photosynthesis, mineral nutrition, reactive oxygen species (ROS) formation, and changes in gene expression. In most cases, Cd contamination sometimes occurs along with a significantly higher Zn contamination (Chaney, 2010). This was evident in the itai-itai disease outbreak caused by contamination from Zn mining activities. Zinc and Cd also have similar properties in soils and in plants when they are absorbed and translocated to plant shoots and seeds.

Environmental conditions and genetics impact the level of Cd in durum wheat grain. Cieśliński et al. (1996) found that Cd accumulation and distribution in various tissues of durum wheat were strongly affected by both soil type and cultivar. Depending on soil type, these researchers also found that the durum wheat grain contained 21 to 36% of the total Cd taken up by the plant. Genetic differences in rhizosphere processing of Cd and Cd transport processes *in vivo* were suggested by the group as potential reasons for the wide range (0.0017 to 0.268 mg kg^{-1}) of Cd in accumulation in grain.

Studies on the ability of durum wheat genotypes to accumulate Cd indicated large variations and suggested a genetic effect (Hart et al., 2006; Harris and Taylor, 2013). They found low Cd near-isogenic lines (NILs) retained more Cd in roots and transported less Cd to the grain. The concentration of Cd in roots was 2.5 times greater for low Cd NILs than for high Cd NILs. The restriction of root-to-shoot transport of Cd translocation was significantly different (p<0.001) between the low and high Cd NILs. The timing of Cd accumulation in grain was strongly related to grain biomass accumulation in both low and high Cd NILs (r = 0.98 and 0.91, respectively).

Due to the genetic differences among durum wheat genotypes, plant breeding techniques aided in the development of low Cd accumulating cultivars. Developing low Cd durum wheat using low grain

Cd as a selection criterion began in Canada in the mid-1990s (Clarke et al., 2010). This was initiated because of international limits on Cd concentration in food. Characterization of near-isogenic lines of durum wheat found that no differences in root Cd uptake, but the low-Cd accumulating line had decreased movement from roots to shoots, which resulted in less Cd in harvested grain (Hart et al., 2006). A major gene was discovered that is responsible for the accumulation of Cd in durum wheat and has the designation of *Cdu1* (Clarke et al., 2010). The allele for low Cd accumulation is not pleiotropic and does not affect any major economic traits (Clarke et al., 2002). AC Strongfield durum wheat was released in Canada due to its superior agronomic performance, quality attributes, and reduced grain cadmium concentrations (Clarke et al., 2006). AC Strongfield contains the allele for low cadmium concentration.

Newly released cultivars, such as Joppa and Carpio (Elias et al., 2015), were developed for the ND growing region by North Dakota State University (NDSU). Both possess quality attributes that are superior to previously released durum wheat cultivars (E. Elias, personal communication). However, **n**either 'Carpio' nor 'Joppa' contain the gene for low cadmium, which could impact the marketing of these cultivars to the EU if grown in areas with high soil Cd. In 2015, approximately 80% of durum wheat produced in ND was with cultivars developed and released by NDSU (NASS, 2014). Low Cd content and the *cdu1* allele is currently an important selection criterion for cultivar development at NDSU (E. Elias, personal communication).

Cadmium in additional plant species

Information on Cd uptake, accumulation, and transport is important to understand in order to develop management practices that can be used to limit Cd in harvested grain. In general, dicotyledon plants accumulate more Cd than graminaceous plants mainly due to the chemical composition of root exudates (Chaney, 2010). The ferrous transporter system in dicot crops also transport Zn, Cd, Cu and Mn. Some of the known major Cd accumulating crops are grown in ND and their ability to accumulate Cd

in harvested grain may limit marketability and international trade. Even though cereal crops generally contain less grain Cd than dicot plants, cereal crops do vary in their accumulation of grain Cd. Sunflower (*Helianthus annus* L.) and flax are two dicots grown in ND that are known Cd accumulators. Other dicot crops that are potential Cd accumulators include peanut (*Arachis hypogaea* L.), potato, and soybean (*Glycine max* L. Merr.). Potato and soybean are also widely grown in ND. Flax, sunflower, and soybean are oilseed crops grown primarily for their oil; little whole grain is directly consumed by humans. Direct human consumption of durum wheat is more of a concern. It is important to limit the amount of Cd in staple food crops such as rice, potato, and wheat as accumulation over time could lead to Cd toxicity and these crops are consumed in large quantities. Durum wheat is the most problematic cereal crop in regards to Cd accumulation grown in ND. Rye (*Secale cereal* L.), barley (*Hordeum vulgare* L.), and oat (*Avena sativa* L.) tend to accumulate less Cd in harvested grain than durum wheat (Perilli et al., 2010).

Potato is consumed directly by humans and makes up a significant portion of people's diets worldwide. Its consumption might be of concern when tubers are consumed with high levels of Cd. Potato can accumulate Cd and can directly impact human absorption of Cd. McLaughlin et al. (1994b) evaluated different potato cultivars for Cd content in tubers under field conditions at three sites in Australia. They reported that the range of Cd concentrations found between sites was greater than the range of Cd concentrations between cultivars in each site. In most locations, a significant difference in tuber Cd concentration was identified. However, regression analyses determined that the Cd content in the tubers was sensitive to environmental conditions such as soil type and weather (McLaughlin et al., 1994b). Differential distribution of Cd within the potato plant rather than differential uptake was responsible for cultivar differences when grown under greenhouse conditions (Dunbar et al., 2003). These researchers found more Cd in roots than in leaves. The same differential distribution of Cd within a plant was also identified in durum wheat (Harris and Taylor, 2013).

Cadmium uptake, translocation, and subsequent levels in harvested grain have been studied in many field crops including sunflower. Results of these studies have indicated genetic differences among sunflower genotypes. The results from a screening of 200 sunflower lines at locations in ND and MN found large variations in Cd levels at different growth stages (Li et al., 1997). Leaf Cd at the sunflower growth stage R5 (Schneiter and Miller, 1981) was the best predictor of harvested grain Cd even when grown in different soils (r = 0.44 to 0.59). However, Li et al. (1997) found that the variation in leaf Cd accumulation was not the result of simple inheritance. Cadmium concentration at the seedling stage was not a good indicator of grain Cd (r = 0.19 to 0.50). This could be the result of different genes controlling uptake and accumulation. Conversely, the Cd and Zn levels in the young leaves of lettuce (*Lactuca sativa* L.) and spinach (*Spinacea oleracea* L.) were more closely related to that of soil concentrations than the concentration in older leaves (McKenna et al., 1993). Using leaf samples to determine Cd levels in edible food parts could be specific to the species being evaluated. Soil type and characteristics impact sunflower grain Cd. The effect of soil chloride, sulfate, and other soil factors on Cd concentration in sunflower was examined by Li et al. (1994). They found that sunflower kernel Cd levels were highly correlated with DTPA-extractable Cd (p<0.001) at all soil depths tested and with clay content in sub soils. Soil sulfate did not affect Cd uptake, however, soil chloride levels were correlated with grain Cd. The results indicated that soil chloride concentration was a factor in sunflower Cd uptake.

Another important crop grown in ND that can accumulate Cd is flax. Commercial cultivars and plant introductions of flax were screened in field plots near Fargo, ND for grain Cd by Li et al. (1997). They detected significant differences between cultivars in grain Cd (p<0.01). These researchers found that the average Cd concentration for the 14 commercial flax cultivars was 1.21 mg kg^{-1}. The range for the 60 plant introduction samples ranged from 0.14 to 1.37 mg kg^{-1}. The wide range of Cd values in this study indicated potential for selecting low Cd lines in a breeding program. The amount of Cd found in flax is generally higher than that of durum wheat. Jaio et al. (2004) found that flax contained higher

levels of Cd in both the shoot and grain compared to durum wheat due to different Cd transport pathways when evaluated in growth chambers. Potential reasons for the differences in transport pathways between flax and durum wheat could be attributed to differences in species type and the general differences between monocot and dicot root systems. The root exudates of dicots also could enhance Cd solubility as previously described.

Rice is a common staple food crop around the world. Similar to durum wheat, rice also is a monocot species that is a known Cd accumulator. Rice might have similar Cd uptake and translocation to durum wheat. Rice genotypes also vary in the accumulation of Cd in harvested grain. Arao and Ae (2003) evaluated rice genotypes for Cd concentration when grown in two soil types under upland or paddy conditions in Japan. The differences detected among genotypes were large and rankings were consistent across years and soil types. The researchers found that the grain Cd levels were highest and ranged from 1.42 to 4.95 mg kg^{-1} when rice was grown in Annaka soil type under upland conditions. The amount of HCl-extractable Cd in the Annaka soil type and the Fuchu soil type used in the research was 7.4 and 0.9 mg kg^{-1}, respectively. Grown under greenhouse conditions, 20 rice genotypes were evaluated for Cd, Fe, Zn, Mn, Cu, and Mg uptake and accumulation by Liu et al. (2003). They detected significant differences of the minerals evaluated in both roots and leaves. Based on correlation among minerals, their results indicated that Cd adsorption was cooperative between Fe, Mn, and Cu in rice plants. Additionally, Liu et al. (2003) found that the uptake and accumulation of Cd in rice also interacted with Fe, Zn and Cu.

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CHAPTER 2. PLANTING DATE, SEEDING RATE, AND CULTIVAR IMPACT AGRONOMIC TRAITS AND PASTA QUALITY OF DURUM WHEAT

Abstract

Durum wheat (*Triticum turgidum* L. *var. durum* Desf.) is a market class of wheat that is subject to price discounts in the marketplace if quality standards are not met. This study was conducted in order to determine how certain agronomic practices might impact durum wheat quality. The effects of planting date (PD), cultivar, and seeding rate on agronomic and pasta quality traits were investigated in field trials conducted near Hettinger and Minot, ND in 2014 and 2015. The interaction of PD and cultivar was significant for many of the traits evaluated. Yield had a significant PD x cultivar interaction or PD and cultivar effect in all environments. Test weight had significant PD x cultivar interaction at all environments. In general, a delay in PD resulted in a significant decrease in yield and test weight for all cultivars. However, Carpio yield was higher in high yielding environments while Joppa yield and test weight was more adversely affected by a delay in PD. Seeding rate had no consistent effect on any agronomic or quality trait. Protein content, kernel yellow pigment content, falling number (FN), and vitreous kernels were more dependent on cultivar, regardless of PD and environment, and were consistent with previous quality reports. Semolina extraction, gluten index (GI), and wet gluten (WG) values tended to increase and then decrease with a delay in PD. These data continue to support that cultivar selection is the critical component for obtaining high-yielding, high-quality durum wheat. However, PD and environment can impact certain agronomic and end-use traits, regardless of cultivar grown.

Introduction

Durum wheat is a market class of wheat commonly grown in North Dakota. In 2014, 567 000 ha were planted with an average yield of 2700 kg ha^{-1} (NASS, 2014). Currently, North Dakota produces approximately 60% of all U.S. durum wheat, but has previously produced more than 87%. The variability

in its production can be attributed to market price and quality concerns. Durum wheat production in ND has declined in recent years due to competition for acres with hard red spring wheat (HRSW) (*Triticum aestivum* L.). Generally, HRSW is less vulnerable to the quality discounts than is durum wheat.

Durum wheat quality requirements differ depending on the end user (Troccoli et al., 2000). Producers, seed companies, grain buyers, grain millers, the pasta industry, and consumers evaluate durum wheat quality from different perspectives. Durum wheat is primarily grown for producing high-quality pasta (Troccoli et al., 2000). Quality attributes of harvested grain such as density, high protein content, and gluten strength are required to produce high-quality pasta and result in ND durum wheat being marketed worldwide. Many production and environmental factors impact the quality of harvested durum wheat. Adjusting PD, applying foliar fungicide, and cultivar selection are a few production practices that a producer might employ to manage disease in durum wheat. Optimal planting date and selection of cultivars with favorable stress responses are agronomic management factors that can maximize durum wheat yield and quality (Pfeiffer et al., 2000).

Durum wheat grades are determined by the Federal Grain Inspection Service (FGIS) and the USDA Grain Inspection, Packers, and Stockyards Administration (USDA, 2013) and are important to final market price. The grades of durum wheat are considered when marketed and determined based on quality factors. Subclasses within grades of durum wheat are marketed based on the percentage of vitreous kernels. The majority of the 2014 durum wheat crop was US grade 2 Amber Durum (AD), while the 2015 crop was US grade 1 Hard Amber (HAD) durum wheat (ND Wheat Commission, 2015). Essentially, HAD has a minimum test weight of 772 kg m^{-3}, less than 2% damaged kernels, and has a maximum limit of 0.4% foreign material, 3% broken kernels and defects, and 75% or more hard vitreous kernels (USDA, 2013). Disease and mishandling of grain can cause damaged and broken kernels which can lower test weight. Grain kernels are considered hard vitreous because of high protein content and the absence of purpinoline proteins that interact with starch granules (Dexter et al., 1988).

Milling characteristics that are used to characterize the functionality of durum wheat include total extraction, semolina extraction, ash content, specks, protein content, and mixogram score (Troccoli et al., 2000). The total extraction is the portion of the durum wheat kernel that can be milled into flour or semolina. Semolina extraction is the portion of semolina taken from the kernel. Ash content is a measure of mineral content in the flour or semolina extracted. Specks are considered negative in the processing of durum wheat and are commonly the result of bran that escapes the cleaning and milling process. Durum wheat that is highly diseased or high in foreign material is most likely to cause specks in semolina. High gluten strength of processed durum wheat results in better quality of pasta (Troccoli et al., 2000). The gluten quality of semolina can be determined via sedimentation tests, gluten index, or SE-HPLC analyses. Mixogram ratings are used to evaluate dough properties and are based on a 1 to 8 scale, with a higher value indicating stronger mixing characteristics.

Decisions such as field selection, crop rotation, cultivar, PD, seeding rate, soil fertility, weed management, and integrated pest management (IPM) are all important factors to consider when producing high-quality durum wheat. The ability of a producer to maximize yield is an economic concern for producers. However, the quality and market grade of grain produced has a significant impact on market price. One factor for producing high-quality durum wheat is cultivar grown (Ransom et al., 2016). Planting date and seeding rate of durum wheat can vary widely based on individual producer and environmental conditions. These choices also might impact the quality of harvested grain due to the environmental conditions during grain filling and harvest and are somewhat dependent on PD (Edhaie et al. 2001).

Breeding programs in the U.S. and Canada have as primary objectives the identification cultivars with higher yield potential, better disease resistance profiles, and better end-use quality (Royo et al., 2009, Clarke et al., 1998, and Elias and Manthey, 2016). Unfortunately, durum wheat quality in recent years in ND has been regularly impacted by Fusarium head blight (*Fusarium graminearum* Schwabe)

(FHB or scab) (McMullen et.al, 2008). Most of the quality discounts associated with FHB are due to the presence of a pathogen-produced mycotoxin in the grain called deoxynivalinol (DON). Proper management of FHB is commonly achieved with the use of protectant fungicide applications. These applications are essential due to limited genetic resistance in current durum wheat cultivars (Clarke et al., 2010 and Buerstmayr et al., 2012).

Numerous agronomic studies have been conducted involving the effect of N on durum wheat yield and quality. Black and Siddoway (1977) found that yield response due to N and P fertilization was significantly higher for early and medium seeding dates than for the late seeding date for hard red spring and durum wheat. Additionally, the seeding date x NP-fertilization interaction was significant and indicated a decreasing response to fertilization with delayed planting. Seeding date did not significantly impact grain protein or test weight of durum wheat. Additionally, Sing and Jain (2000) found that durum wheat yield in India was greatest at normal seeding dates and with increasing N and irrigation. Flour characteristics such as grain protein, ß-carotene, and sedimentation value were highest for the latest planting date. A genotype x N input interaction was observed for several characteristics of early sown durum wheat (Ehdaie and Waines, 2001). This interaction was also present in the optimum and late planting date for grain yield.

Seeding rate trials to determine the optimum rate to maximum yield have been previously conducted in ND. However, many of these trials were conducted with cultivars no longer grown. Based on data, Quick and Wilkens (1975) suggested early planting dates and seeding rates between 84 and 100 kg ha^{-1} for optimum grain yield and quality. Riveland et al. (1979) determined that yield of hard red spring and durum wheat was maximized at 247 plants m^{-2} but only when yield potential was greater than the 1970s average yield of 2350 kg ha^{-1}. More recently, Hanson and Lukach (1992) found that test weight and protein were not impacted by a change in seeding rate across locations in north eastern ND. A seeding rate of at least 141 kg ha^{-1} was suggested to optimize yield. Seeding rate could impact grain

yield by increasing the number of spikes per area (Arduini et al., 2006). Grain yield, post-heading N accumulation, and N remobilization were highest at a seeding rate of 400 seeds m^{-2}. The cultivar x seeding rate interaction was not significant for any factors evaluated in their study.

Previous studies indicated that seeding rates can influence yield in HRSW. Riveland et al. (1979) found that HRSW yields in western ND were maximized at 247 plants m^{-2} when yield potential was greater than 2345 kg ha^{-1}. Cultivars responded similarly to seeding rate when comparing test weight and grain protein. Seeding rate had a significant effect on HRSW yield in Canada (Lafond, 1996). Increasing yield was identified as a function of more spikes produced due to more plants established. However, the tillering ability of a cultivar did not compensate for inadequate plant stands. Additionally, Mehring (2016) evaluated 12 HRSW cultivars for optimal seeding rate in eastern ND and MN. He found that when averaged across environments the highest yield was obtained at 3.5 mill seeds ha^{-1}, and subsequent regression analysis found that the predicted optimum rate to be 3.6 mill seeds ha^{-1}.

The effect of PD on quality of durum wheat at harvest has been reported. Edhaie et al. (2001) found that durum wheat genotypes responded differently to planting date and N rate when grown at three planting dates in California. No genotype consistently ranked the best across PD. Planting date also impacted semolina and pasta quality due to environmental changes during grain filling. Increased protein content observed in later sown durum wheat was suggested as a reason for increased dough strength (Motzo et al., 2007). These authors also observed a decrease in gluten index when planting of durum wheat was delayed and proposed that temperatures higher than 30˚C during grain filling might affect the gluten polymerization process. In a similar study, Fois et al. (2011) found that gluten index increased as temperatures rose to 30˚C and then decreased under higher temperatures. In addition, spaghetti firmness and protein content were positively correlated, but independent of planting date. As a result of this study, these researchers suggested that later PD might be a way of increasing pasta cooking quality by increasing overall protein.

The research reported herein was conducted in order to determine ways to maximize durum quality, while using profitable agronomic practices. The specific objective was to determine how PD, cultivar, and seeding rate impact agronomic traits and subsequent pasta quality. The results of this study will enable recommendations to be developed which will aid producers in applying agronomic practices in order to minimize negative grain quality aspects.

Materials and Methods

Experiments were conducted near Hettinger, ND (46˚ 00' 40" N, 102˚ 38' 40" W) and Minot, ND (48˚ 10' 55" N, 101˚ 17' 46W) in 2014 and 2015. The soils were a Shambo- and an Aastad-loam in Hettinger and Minot, respectively (Table 2.1) (USDA-NRCS, 2016). Experiments at both locations were seeded directly into the stubble from the previous year using a no-till seeder. In Minot, the previous crops were flax (*Linum usitatissimum* L.) and field pea (*Pisum sativum* L.) in 2014 and 2015, respectively. In Hettinger, the previous crops were HRSW and lentil (*Lens culinaris* Medik) in 2014 and 2015, respectively. The size of each experimental unit was 10.7 m^{-2} (1.6 m wide x 6.7 m long) and 11.6 m^{-2} (1.52 x 7.6 m) in Hettinger and Minot, respectively. Row spacing was 17.8 cm at both locations.

Table 2.1. Soil series†, taxonomy, and slope at Hettinger and Minot, ND, in 2014 and 2015.

Location	Soil Series	Soil Taxonomy‡	Slope
			%
Hettinger	Shambo-loam	Fine-loamy, mixed, superactive, frigid Typic	0-2
Minot	Aastad-loam	Fine-loamy, mixed, superactive, frigid Calcic	0-3

† Soil data obtained from (USDA-NRCS, 2016).
‡ Soil taxonomy listed on individual lines based on hyphenated soil series name.

All plots were maintained using best management practices (Wiersma and Ransom, 2012) and fertilized based on yield potential and soil test results (Table 2.2) for each location (Franzen, 2014). All soil tests were conducted by the NDSU Soil Testing Laboratory, Fargo, ND using approved and standard practices (North Central Region Research Publication, 2015). Soil Cd was determined by Agvise Laboratories, Northwood, ND using EPA digestion method 3050 (EPA, 2012). Total Cd in soil was then determined using optical emission spectrometry. Herbicide and fungicide applications were applied as

needed. The herbicide and rate varied on weed species present at each location and were applied according to product label recommendations (Zollinger et al., 2016). A fungicide application of 100 g ha^{-1} active ingredient of prothioconazole + tebuconazole to control FHB was made at Feekes 10.51 growth stage (Large, 1954) at all locations and years. A plot combine harvester with an approximately 1.52 m wide head was utilized to harvest durum wheat in experimental untis when grain moisture was near 13% (Table 2.3).

The durum wheat cultivars, Divide, Carpio, and Joppa, evaluated in this experiment were developed by the North Dakota State University (NDSU) durum wheat breeding program and released by the North Dakota Agricultural Experiment Station (Elias and Manthey, 2007 and 2016) (Elias et al., 2015). Divide was selected for these experiments because it was grown on the largest area in ND for the previous seven consecutive years, accounting for 30% of the acreage in 2015 (ND Wheat Commission, 2015). Joppa and Carpio were selected based on their recent availability to ND producers and excellent end-use qualities.

The experimental design was a randomized complete block with a split-plot arrangement. Treatments were replicated four times. Whole plots consisted of PD (planting date) (see Table 2.3 for actual dates). Durum wheat cultivars (Carpio, Divide, and Joppa) and seeding rates (222, 297, and 371 viable seeds per m^{-2}) comprised subplots and were arranged in a two-way factorial arrangement.

Table 2.2. Soil factors measured prior to planting at Hettinger and Minot, ND in 2014 and 2015.

Location/Year	Depth	NO_3-N	P	K	pH	OM	Zn	Fe	Cl	Cd
	cm	kg ha^{-1}	mg kg^{-1}	mg kg^{-1}		%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Hettinger										
2014	0-15	34	33	345	6.0	2.8	0.88	75	3.5	1.3
	15-30	27	7	175	7.3	2.4	0.32	25	9.0	
2015	0-15	81	35	625	6.4	3.8	2.17	64	10.6	0.7
	15-30	34	8	200	7.0	2.6	0.37	24	13.9	
Minot										
2014	0-15	138	18	365	4.4	3.2	0.74	86	32.9	0.3
	15-30	28	2	167	6.0	2.2	0.20	35	59.1	
2015	0-15	88	13	310	5.9	3.7	1.23	62	26.3	0.5
	15-30	20	4	140	6.7	2.4	0.39	32	22.2	

Table 2.3. Planting and harvest date of durum wheat at Hettinger and Minot, ND in 2014 and 2015.

Year/Loc	Planting Date	Harvest Date	Year/Loc	Planting Date	Harvest Date
2014			*2015*		
Hettinger	May 2	August 28	Hettinger	April 14	August 17
	May 15	September 4		April 29	August 17
	May 27	September 8		May 13	August 20
	June 9	October 14		May 27	September 4
Minot	May 14	September 9	Minot	April 29	August 28
	May 27	September 16		May 11	August 28
	June 4	October 9		May 26	September 12
	June 17	October 24		June 9	September 12

Data Collection

Plant height was determined by averaging two measurements per plot (using a tape measure). The plant height was calculated based on the distance from the soil to the top of the plant's spike at physiological maturity. Grain yield from harvested field plots was recorded on a clean-grain basis corrected to 13% seed moisture content. Test weight was measured using methodology specified by the AACC International (AACC, 2000) (Approved Method 55-10.01). Protein content of whole durum wheat samples was determined using a Perten Instruments DA7200 NIR analyzer (Springfield, IL). Thousand kernel weight was determined by calculating the number of kernels in a 10 g sample and converting data to the weight of 1000 kernels. Percent large kernels were determined by sieving a 100 g sample and determining the weight of kernels that remained on the top of a 2.92 mm sieve (Shuey, 1960). Vitreous kernel was the percentage of 100 kernels cut with a farinator having a vitreous endosperm. The AACC International procedures for yellow pigment color (pigment) content (Method 14-50.01), polyphenol oxidase (PPO) (Method 22-85.01), milling semolina extraction/yield (semolina) and semolina protein content (semolina-p) (Method 26-50.01), gluten index (GI) and wet gluten (WG) (Method 38-12.02), falling number (FN) (Method 56-81.03), and ash content (Method 08-01.01) were consistent with AACC International standards (AACC, 2000).

Statistical Analysis

Analysis of variance was conducted for each of the variables measured. Environments were considered homogenous when the ratio (F_{max}) of the effective error variance for each trait was less than 10-fold (Tabachnick and Fidell, 2001). Combined analyses of variance across environments were calculated for all variables. Data for each environment were analyzed using PROC GLM procedure of SAS software (SAS Institute, 2010). Planting date, cultivar, and seeding rate were considered fixed effects. Replications and years were considered random effects. Mean comparisons using *F*-protected LSD were made to separate PD, cultivar and seeding rate where F-tests indicated significant differences existed ($p < 0.05$). LSDs for the combined analyses were calculated based on the methodology described by Carmer et al. (1989).

Results and Discussion

Average air temperatures and precipitation from April to September for each year and location are provided in Tables 2.4 and 2.5, respectively. The average daily temperature for the 2014 growing season was only 69 and 97% of the long-term average in Hettinger and Minot, respectively. Alternatively, the 2015 daily average temperature in Hettinger and Minot was slightly above the long-term average at 103 and 106% of normal, respectively. Precipitation totals during the 6-month growing

Table 2.4. Average air temperature (˚C) for Hettinger and Minot, ND in 2014 and 2015.

Location/Year	April	May	June	July	Aug.	Sep.	6-mo. ave	% Long-term Average
Hettinger								
2014	4	12	15	19	19	14	10.6	69
2015	6	10	18	21	21	18	15.7	103
Minot								
2014	3	12	17	19	19	15	14.2	97
2015	6	11	19	21	20	16	15.5	106

Source: NDAWN (2016)

Table 2.5. Precipitation (mm) for Hettinger and Minot, ND in 2014 and 2015.

Location/Year	April	May	June	July	Aug.	Sep.	6-mo. ave	% Long-term Average
Hettinger								
2014	31.8	40.6	130.0	21.6	131.3	32.8	64.7	119
2015	24.6	102.5	131.3	25.7	47.2	23.0	59.1	109
Minot								
2014	51.1	41.6	187.1	41.4	114.0	21.1	76.1	135
2015	11.9	79.2	155.0	46.3	27.8	39.8	60.0	106

Source: NNDAWN (2016)

season were above the long term average at both locations for both years. In 2014, the months of June and August had the most precipitation at both locations. In 2015, the months of May and June had the most precipitation at both locations.

Planting date had a significant impact on yield at all environments. When environments were combined, yield decreased as PD advanced from first to last PD. With each delay in PD, yield decreased by 329, 504, and 672 kg ha^{-1} across all cultivars and seeding rates, respectively (Table 2.6). Yield was the only agronomic factor evaluated that significantly differed based on PD across locations. The G X E interaction was significant at all locations for most traits evaluated. Therefore, each individual location will be discussed separately. Based on the differences among PD, emergence date, heading date, and harvest date, the growing environments were quite different and therefore, the combined analyses is of little value, however, some general trends were observed (Table 2.6). These data indicate that even in different environmental conditions, planting durum wheat early is the best option for maximizing yield regardless of cultivar or environment. The impact of G X E interactions on traits such as protein content and gluten quality (Troccoli et al., 2000) and yellow pigment, grain yield, brown index (Schulthess et al., 2013) in durum wheat have been documented

The effects of individual environments for PD X cultivar interactions for agronomic traits can be found in Tables 2.7 to 2.10. The PD x cultivar interaction was significant for yield in the Minot 2014 and Hettinger 2015 environments ($p<0.01$ and $p<0.001$, respectively). Other environments had significant PD

and cultivar effects, but no significant interaction. This indicates that PD and the durum wheat cultivar selected are each important factors when it comes to yield. The yield of each cultivar followed the trend of Joppa > Carpio > Divide (Table 2.6) and was consistent with regional and variety trial results conducted from 2008 to 2014 (Elias and Manthey, 2016). Joppa was the highest yielding cultivar at all locations for PD 1, except in the Hettinger 2014 environment where Carpio was higher yielding by 188 kg ha^{-1}. The Hettinger 2014 environment was the highest yielding environment of those included in the study and Carpio was the highest yielding at all PDs. It is possible, that when Joppa and Carpio are planted in a high yielding environment, Carpio could yield more than Joppa.

All cultivars experienced a yield reduction when PD was delayed in all environments; however, the yield reduction for Joppa at PD 4 was much greater than Carpio or Divide in all environments (Tables 2.7 to 2.10). This would indicate when producers are faced with a delay in planting due to weather conditions; selecting Carpio or Divide would be a better choice than Joppa, if maximizing yield is the primary objective. However, if the yields are expected to be high, perhaps due to exceptional, but not limiting soil water contents, Carpio might be the preferred cultivar as observed in the Hettinger 2014 environment.

Table 2.6. Impact of main effects on planting date, cultivar, and seeding rate on agronomic traits[a] associated with durum wheat in Minot and Hettinger, ND, in 2014 and 2015.

	Ht	TW	Yield	Pro	Kwt	FN	Large	Vit
Planting	cm	kg m^{-3}	kg ha^{-1}	g kg^{-1}	g 1000^{-1}	s	%	%
1	84	770	4340	137	44.1	460	70.4	67.8
2	90	768	4011	135	44.1	440	68.6	73.8
3	88	749	3507	136	40.1	417	59.1	65.4
4	83	726	2835	148	42.6	385	63.5	71.5
LSD[z]	NS	NS	73	NS	NS	NS	NS	NS
Cultivar								
Carpio	86	755	3722	137	43.8	437	72.8	66.9
Divide	87	750	3554	143	42.3	444	65.0	68.9
Joppa	86	754	3742	137	42.0	369	58.4	73.1
LSD	NS	2	NS	1	1.0	NS	4.6	5.0
Seeding								
222	86	753	3642	139	43.2	425	66.7	69.7
297	86	754	3722	139	42.6	426	65.3	70.2
371	86	753	3662	139	42.3	426	64.2	69.0
LSD	NS	NS	NS	NS	0.9	NS	2.2	NS
CV	4.9	1.5	8.6	4.3	4.4	7.3	7.1	9.4

[a] Ht = plant height, TW = test weight, Pro = protein, Kwt = weight of 1000 seeds, FN = falling number, Large = kernels remaining on a 2.92 mm sieve, Vit = vitreous kernel, NS= not significant at p=0.05 level

[z] LSD was calculated to compare all levels of planting date according to Fisher's Protected LSD ($p \leq 0.05$); CV = coefficient of variation

Table 2.7. Effect of planting date and cultivar interaction on agronomic traits[a] associated with durum wheat in Minot, ND in 2014.

Date	Cultiv	Ht	TW	Yield	Pro	Kwt	FN	Large	Vit
		cm	kg m^{-3}	kg ha^{-1}	g kg^{-1}	g 1000^{-}	s	%	%
May 2	Carpi	71	739	2694	155	45.3	282	81.2	96.9
	Divide	78	735	2647	158	44.9	340	79.4	98.3
	Joppa	73	752	2876	159	43.4	255	74.1	98.4
May 15	Carpi	80	766	2479	154	47.3	370	83.4	88.9
	Divide	84	758	2560	160	42.9	454	73.0	94.8
	Joppa	81	754	2049	156	45.1	390	74.4	95.6
May 27	Carpi	86	754	2150	152	46.3	447	82.4	90.4
	Divide	86	739	2103	159	43.3	474	73.1	93.3
	Joppa	87	752	2022	153	43.8	314	71.7	91.7
June 9	Carpi	87	753	1518	158	45.2	275	82.5	84.0
	Divide	85	755	1559	166	43.5	265	75.4	89.5
	Joppa	87	757	1781	157	44.3	172	71.9	92.0
LSD_1[z]		NS	12	470	4	2.0	46	4.3	2.7
LSD_2		NS	1	141	1	0.5	14	1.1	0.8

[a] Ht = plant height, TW = test weight, Pro = protein, Pig = pigment, PPO = polyphenol oxidase, Kwt = weight of 1000 seeds, FN = falling number, Large = kernels remaining on a 2.92 mm sieve, Vit = vitreous kernel, NS= not significant at p=0.05 level

[z] LSD_1 was calculated to compare all levels of planting date according to Fisher's Protected LSD ($p \leq 0.05$); [y] LSD_2 was calculated to compare all levels of cultivar for the same planting date according to Fisher's Protected LSD ($p \leq 0.05$).

Table 2.8. Effect of planting date and cultivar interaction on agronomic traits[a] associated with durum wheat in Hettinger, ND in 2014.

Date	Cultivar	Ht	TW	Yield	Pro	Kwt	FN	Large	Vit
		cm	kg m^{-3}	kg ha^{-1}	g kg^{-1}	g 1000^{-1}	s	%	%
May 14	Carpio	88	785	5899	119	46.6	480	76.5	84.4
	Divide	89	786	5463	121	42.4	453	59.1	89.6
	Joppa	88	782	5711	119	43.7	409	59.8	87.4
May 27	Carpio	89	772	5516	117	50.5	320	87.3	70.4
	Divide	93	775	5241	117	49.9	277	85.0	79.2
	Joppa	95	766	5489	114	48.4	190	81.7	81.1
June 4	Carpio	80	755	4697	121	40.5	266	69.7	86.0
	Divide	81	753	4381	122	36.1	313	53.1	88.7
	Joppa	82	761	4435	114	38.5	277	58.3	92.0
June 17	Carpio	78	694	3460	135	47.7	401	80.2	74.4
	Divide	78	665	2903	140	46.7	382	74.9	72.5
	Joppa	81	674	3192	133	46.5	280	72.5	74.4
LSD_1[z]		NS	6	121	3	0.6	10	2.1	4.1
LSD_2		NS	5	128	2	0.6	8	1.0	2.3

[a] Ht = plant height, TW = test weight, Pro = protein, Pig = pigment, PPO = polyphenol oxidase, Kwt = weight of 1000 seeds, FN = falling number, Large = kernels remaining on a 2.92 mm sieve, Vit = vitreous kernel, NS= not significant at p=0.05 level

[z] LSD_1 was calculated to compare all levels of planting date according to Fisher's Protected LSD ($p \leq 0.05$); [y] LSD_2 was calculated to compare all levels of cultivar for the same planting date according to Fisher's Protected LSD ($p \leq 0.05$).

Table 2.9. Effect of planting date and cultivar interaction on agronomic traits[a] associated with durum wheat in Minot, ND in 2015.

Date	Cultivar	Ht	TW	Yield	Pro	Kwt	FN	Large	Vit
		cm	kg m^{-3}	kg ha^{-1}	g kg^{-1}	g 1000^{-1}	s	%	%
April 14	Carpio	88	764	3796	146	45.6	571	78.2	27.8
	Divide	88	772	3474	153	44.5	578	75.1	17.9
	Joppa	85	763	4038	138	43.4	549	65.1	44.2
April 29	Carpio	97	772	3433	128	43.4	546	70.2	52.3
	Divide	94	775	3097	139	43.7	561	68.2	61.5
	Joppa	92	769	3353	131	42.5	517	58.1	59.6
May 13	Carpio	93	758	3333	130	42.1	451	68.6	20.2
	Divide	97	745	3144	144	39.2	472	53.9	8.8
	Joppa	95	749	3501	137	39.9	412	46.5	25.1
May 27	Carpio	82	746	3601	143	41.4	441	66.5	50.7
	Divide	89	731	3467	144	40.1	463	55.2	56.8
	Joppa	83	752	3716	139	39.7	452	50.6	55.5
LSD_1[z]		4	13	NS	11	2.0	NS	7.7	11.7
LSD_2		2	5	NS	4	0.7	NS	2.7	4.0

[a] Ht = plant height, TW = test weight, Pro = protein, Pig = pigment, PPO = polyphenol oxidase, Kwt = weight of 1000 seeds, FN = falling number, Large = kernels remaining on a 2.92 mm sieve, Vit = vitreous kernel, NS= not significant at p=0.05 level

[z] LSD_1 was calculated to compare all levels of planting date according to Fisher's Protected LSD ($p \leq 0.05$); [y] LSD_2 was calculated to compare all levels of cultivar for the same planting date according to Fisher's Protected LSD ($p \leq 0.05$).

Table 2.10. Effect of planting date and cultivar interaction on agronomic traits[a] associated with durum wheat in Hettinger, ND in 2015.

Date	Cultivar	Ht	TW	Yield	Pro	Kwt	FN	Large	Vit
		cm	kg m^{-3}	kg ha^{-1}	g kg^{-1}	g 1000^{-1}	s	%	%
April 29	Carpio	90	786	5046	126	43.5	551	72.1	58.6
	Divide	88	785	4999	130	43.8	539	70.2	54.2
	Joppa	87	785	5469	122	42.0	516	54.3	56.1
May 11	Carpio	91	773	4744	133	39.0	549	54.4	64.5
	Divide	93	776	5080	140	39.0	553	49.9	68.2
	Joppa	91	770	5100	133	37.8	560	37.5	68.7
May 26	Carpio	89	730	4011	132	36.8	539	55.3	53.0
	Divide	90	746	3910	139	38.2	518	46.9	65.8
	Joppa	89	743	4421	132	36.4	520	29.1	69.8
June 9	Carpio	80	701	3144	150	40.0	497	55.7	68.1
	Divide	75	716	2809	157	39.1	465	48.4	62.4
	Joppa	84	732	2882	148	36.9	520	28.4	77.4
LSD_1[z]		2	8	222	NS	NS	20	5.1	8.2
LSD_2		1	3	128	NS	NS	12	2.3	2.7

[a] Ht = plant height, TW = test weight, Pro = protein, Pig = pigment, PPO = polyphenol oxidase, Kwt = weight of 1000 seeds, FN = falling number, Large = kernels remaining on a 2.92 mm sieve, Vit = vitreous kernel, NS= not significant at p=0.05 level

[z] LSD_1 was calculated to compare all levels of planting date according to Fisher's Protected LSD ($p \leq 0.05$); [y] LSD_2 was calculated to compare all levels of cultivar for the same planting date according to Fisher's Protected LSD ($p \leq 0.05$).

Ehdaie et al. (2001) found that durum wheat genotypes evaluated in California responded differently to PD. Compared to the optimum planting date, the early planting date had no effect on yield. However, the grain yield of delayed PD was similar to the yield of the optimum date. Black and Siddoway (1977) evaluated HRS and durum wheat response to PD and NP-fertilization after fallow. They found that grain yield did not differ significantly between the first and second PD, but were significantly lower for the last seeding date. Early seeding of both wheat classes was important to maximize response to N-P fertilization. A decrease in response to fertilization was observed with delayed seeding. All PDs in the current study were fertilized when planted. The delay in yield response to PD in the current study also could have been affected by a decrease in the response to fertilization. Additionally, the durum wheat cultivars evaluated are considered daylength-sensitive. The delay in PD could result in less

vegetative growth and utilization of soil nutrients prior to reproductive stages which could result in a decrease of yield.

Seeding rate had little impact on the yield or other ergonomically important traits evaluated in the current study across all locations (Table 2.6). Previous studies conducted in ND showed a yield response to seeding rate (Hanson and Lukach, 1992; Riveland, 1979); however, the durum wheat cultivars evaluated in these studies are no longer grown. In addition, those trials were conducted in the Langdon, ND area (Hanson and Lukach, 1992) and far northwest ND near Williston, ND and were seeded at different seeding rates compared to those examined in the current experiments. These differences might be why these authors recorded yield with changes to seeding rate. In the current trial, the seeding rates evaluated were 222, 297, and 371 viable seeds per m^{-2}. Significant differences could have been more difficult to detect in the current trial compared to previous trials because the differences in seeding rates were not as extreme and did not include a seeding rate lower than standard practices. The seeding rates in the current series of experiments represent seeding rates commonly utilized by ND durum wheat producers (Ransom et al., 2016).

The cultivar x seeding rate interaction was significant for yield in both Minot environments. The yield average in Minot in 2014 and 2015 was 2217 and 3494 kg ha^{-1}, respectively. These values are significantly lower than the average yield values in Hettinger of 4770 and 4300 kg ha^{-1} in 2014 and 2015, respectively. These data suggest that there might be a significant cultivar x seeding rate interaction for yield in environments that have yield values that are below 3494 kg ha^{-1}. In both Minot environments, an increase in seeding rate tended to result in an increase in grain yield. Overall, the seeding rates evaluated had limited effect on the agronomic and quality traits for the cultivars evaluated at all locations. The seeding rate of 297 plants m^{-2} resulted in the highest average yield values for Carpio and Joppa. The yield advantage compared to the next highest seeding rate of 222 plants m^{-2} and was approximately 2% and not significant.

Arduini et al. (2006) evaluated the effect of different seeding rates (200, 250, and 400 seeds m^{-2}) on grain yield and N uptake of durum wheat cultivars Creso, Simento, and Svevo evaluated in Italy and found that the seeding rate x cultivar interactions were not significant for any of traits evaluated. Accordingly, Ghaffari et al. (2001) found that in temperate climates such as the United Kingdom, an optimal seeding rate was between 300 and 450 seed m^{-2} for winter wheat. In HRSW, Carr et al. (2003) found no significant interactions for seeding rate x cultivar or tillage x seeding rate x cultivar for yield near Dickinson, ND. These authors hypothesized that a significant interaction did not occur because soil moisture was similar in the tillage systems evaluated.

Mehring (2016) found that diverse cultivars of HRSW differed in their response to seeding rate, due to the diverse genetic background in the study cultivars. The durum wheat cultivars evaluated in the current study represent a narrow genetic background compared to the HRSW cultivars studied by Mehring. Environment played a significant role in Mehring's research to determine the optimum seeding rate of HRSW. His study found that high yielding environments benefit from a reduced seeding rate. In the current studies, no significant PD x seeding rate interaction for yield was observed for any environment suggesting when PD is delayed; a higher seeding rate might not be beneficial. Therefore, based on these data, the seeding rates of the durum wheat cultivars evaluated could be lowered to a seeding rate of 222 seeds m^{-1} to minimize the costs associated with the extra seed at the higher seeding rates while adversely affecting yield. A significant interaction between cultivar and seeding rate was found at both Minot environments. When comparing the results of the two sites over both years, the interaction observed was not consistent and appears to be environmentally or year dependent.

There was an interaction between PD and cultivar in all environments for test weight. Industry standard test weight of 772 kg m^{-3} was achieved for the first two PDs at all locations except for Minot in 2014. Discounts associated with test weight can begin when values drop below 746 kg m^{-3} at which point durum wheat is rated as US Grade 2. Planting dates 3 and 4 at all locations resulted in test weights

that would be subject to this discount. In regional and university trials conducted for 39 site years, the trend was for Joppa and Carpio to have higher test weights than Divide (Elias and Manthey, 2016). A similar trend was observed in test weight as was observed for the later PDs across locations. However, the test weight of Divide was similar or higher than Carpio and Joppa at the early PDs, but was subject to a larger decrease than these two cultivars with a delay in PD.

Gelin et al. (2007) found that preharvest sprouting (PHS) can reduce test weight and also cause specks in pasta resulting in poor pasta color and could be the cause of the decrease in test weight with a delay in PD as observed in the 2015 environments. The most significant decrease in test weight occurred between the first two PDs and last two PDs in the Hettinger 2015 environment. In Minot 2014, a significant PD x cultivar and cultivar x seeding rate interaction for test weight was observed that did not occur in any other environment. Carpio and Joppa had higher test weights of 761 and 759 kg m^{-3}, respectively when the seeding rate was 222 seeds m^{-2}. No differences in test weight were observed for Divide at any seeding rate. For the PD x seeding rate interaction, PD 1, 2, and 4 had higher test weights at the 297 seeds m^{-2} seeding rate, but this seeding rate was not significantly different than the other seeding rates for PD 3. Again, these interactions might be a result of this particular environment which is similar to the cultivar x seeding rate interaction observed for yield at this environment. Based on regional quality reports (ND Wheat Commission, 2015), test weight averages for both 2014 and 2015 were not subject to discounts. Individual environments could have seen discounts associated with test weight as observed in Minot 2014 and Hettinger 2015 environments.

The effects of PD, cultivar, and seeding rate on grain protein content were not similar among environments. Planting data impacted protein content in all environments except Minot 2014. Cultivar was significantly different for protein content in all environments. Seeding rate was significant for protein content in both 2014 environments. Protein content in durum wheat is related to genotype, environmental conditions, and G X E interactions and could be the cause of the differences observed

among environments. Motzo et al. (2007) observed that with delayed planting date, mean grain weight declined, but mean grain protein increased from 10.7 to 14.7%. Previous research by Black and Siddoway (1977) did not find a significant effect of PD on protein content or test weight, while Sing and Jain (2000) found that a delay in PD resulted in the highest grain protein, ß-carotene, and sedimentation values. High temperatures and low humidity during grain filling could also result in increased grain protein content.

All locations had a similar trend for cultivar rankings, with Divide having the highest protein content, regardless of planting date. Divide also was the lowest yielding cultivar at most locations and PDs. Breeding for high protein cultivars is complicated by the inverse relationship between protein and grain yield (Royo et al., 2009; Clarke et al., 2010). Ehdaie and Waines (2001) calculated a negative significant relationship between grain yield and protein in durum wheat based on correlation coefficients ranging from -0.59 to -0.66, depending on PD and N input. In the current study, a negative relationship between protein content and yield occurred across cultivars (r= -0.16, n = 144) (Table 2.11). Based on regional durum nursery testing and NDSU durum variety trials conducted from 2008 to 2014, Divide had a higher average grain protein concentration of 146 g kg^{-1} compared to Carpio and Joppa at 144 and 140 g kg^{-1}, respectively (Elias and Manthey, 2016). Average protein values for the ND growing region in 2014 and 2015 were 131 and 136 g kg^{-1} at 12% moisture, respectively (ND Wheat Commission, 2015). In the current research, Divide had an average grain protein of 143 g kg^{-1}, compared to Carpio and Joppa that both had an average of 137 g kg^{-1}.

Key quality traits can influence the functionality of durum wheat and its market value. These traits can significantly impact the quality of the end-product goods and determines the value of the grain being purchased by end users. Some of these quality traits are used to determine direct discounts to producers if their grain does not meet industry standards. Quality traits that can be used as a basis for discounting grain delivered to the elevator include FN and vitreous kernels. The determination of falling

number (AACC, 2000) is used as an indicator of α-amylase activity and kernel sprouting. In this study, both FN and vitreous kernels had significant PD x cultivar interactions in all environments except 2015 Minot. In the 2015 Minot environment, planting date and cultivar were factors that differed significantly for FN and vitreous kernels. There was no significant relationship between FN and percentage vitreous kernel ($r = 0.07$, $n = 144$) (Table 2.11).

Preharvest sprouting (PHS) can reduce end-use quality of durum wheat. One method used to indicate of PHS is FN where a value above 400 sec indicates that conditions throughout the desiccation process were adequate, while a lower FN value can indicate the presence of α-amylase. Based on the year, discounts associated with FN usually begin when numbers are below 330 sec. Particular PD and environments were above this critical limit except for 2014 planting dates 1 and 4, and 2 and 3, in Minot and Hettinger, respectively (Tables 2.7 to 2.10). Generally, the FN scores were much higher in 2015 than in 2014 for all PDs and cultivars. The environmental conditions in 2014 might have led to PHS for these PDs and locations. Gelin et al. (2007) found no significance for the cutting date (date the spike was removed from stem) x cultivar interaction for FN. However, the effect of cutting date could have been limited by the procedures used to store grain prior to evaluation. The cutting dates in the previous study were only 10 days apart. In the current study, harvest dates differed by up to five weeks (Table 2.3). Based on the regional durum wheat quality report (ND Wheat Commission, 2016), the average FN for 2014 and 2015 were 276 and 414 secs, respectively. According to Elias and Manthey (2016), Carpio and Divide had significantly higher FN than Joppa in long term trials. Similar results were found in this research indicating that regardless of PD and environment, the cultivar Joppa might be more prone to PHS than the other two cultivars.

Grain produced from all PDs and cultivars in the 2014 environments had sufficient levels of vitreous kernels to be graded hard amber durum (HAD), except for PD 4 in Hettinger which would be graded as amber durum (AD) (Tables 2.7 to 2.10). However, no planting dates or cultivars evaluated in

2015 in Minot would be rated as HAD or AD, except for Divide in PD 2 which would be graded as AD. All PDs and cultivars evaluated would only be graded as 'durum' which would result in significant quality discounts when sold in the marketplace. Percent vitreous kernels were lower in Hettinger in 2015 than 2014, however, they were not nearly as low as those in Minot in 2015. The grain produced in Hettinger 2015 would be graded as AD, except for PD 1 which would have resulted in a lower grade of 'durum' for all cultivars evaluated. More precipitation fell during the month of May in 2015 than in 2014. With the much early planting dates in 2015 compared to in 2014, the excess moisture might have caused the N fertilizer to move below the root zone and limit N uptake during tillering. Also, the loss of N through leaching during early growth might have resulted in less vitreous kernels during grain harvest.

Vitreousness is a quality characteristic of durum wheat that affects the yield of semolina during milling. Processors require vitreous kernels in order to maintain high levels of semolina. When the durum wheat grain heads are subject to high relative humidity one to three days prior to harvest, a reduction in vitreous kernels can result (Sandhu et al., 2009). Subclasses of durum wheat grading are based on a separate marketing factor of percentage vitreous kernels. Vitreous kernels can be influenced by G X E interactions and Beleggia et al. (2013) found that the interaction of G X E was significant on metabolite composition and durum wheat grain quality. Carpio had slightly higher kernel vitreousness compared to Divide and Joppa across 30 site years (Elias and Manthey, 2016), but in the current research Joppa had the highest average percent vitreous kernels of 73%. Carpio and Divide had 67 and 69% vitreous kernels, respectively.

Table 2.11. Pooled correlation values for agronomic and quality traits of trials conducted in Hettinger and Minot, ND in 2014 and 2015.

	TW	Yield	Pro	Pig	PPO	Kwt	Large	FN	Vit	Ash	Sem	Semp	GI	WG
Ht	0.21^{NS}	0.07^{NS}	-0.06^{NS}	0.54***	-0.05^{NS}	-0.11^{NS}	-0.28**	0.16^{NS}	-0.6***	-0.7***	0.7***	0.4***	0.48***	0.02^{NS}
TW		0.07^{NS}	-0.07^{NS}	0.2^{NS}	0.14^{NS}	0.19^{NS}	0.003^{NS}	-0.05^{NS}	-0.33**	-0.26*	0.32**	0.04^{NS}	0.36***	-0.12^{NS}
Yield			-0.16^{NS}	-0.6***	-0.07^{NS}	0.03^{NS}	0.09^{NS}	0.18^{NS}	0.58***	0.62***	-0.54***	-0.55***	-0.48***	-0.01^{NS}
Pro				-0.31**	-0.37***	-0.56***	-0.30**	-0.19^{NS}	0.03^{NS}	0.16^{NS}	-0.32**	0.65***	-0.27**	0.53***
Pig					0.56***	0.25*	0.24*	-0.08^{NS}	-0.8***	-0.6***	0.66***	0.41***	0.78***	-0.45***
PPO						0.71***	0.88***	-0.01^{NS}	-0.55***	-0.13^{NS}	0.16^{NS}	-0.03^{NS}	0.5***	-0.77***
Kwt							0.81***	0.06^{NS}	-0.27**	-0.14^{NS}	0.13^{NS}	-0.39***	0.34***	-0.64***
Large								0.09^{NS}	-0.3**	0.05^{NS}	-0.05^{NS}	-0.2^{NS}	0.21^{NS}	-0.66***
FN									0.07^{NS}	-0.19^{NS}	0.4***	-0.1^{NS}	0.13^{NS}	-0.09^{NS}
Vit										0.61***	-0.62***	-0.53***	-0.69***	0.39***
Ash											-0.72***	-0.29**	-0.65***	0.14^{NS}
Sem												0.26*	0.72***	-0.21^{NS}
Semp													0.23*	0.37***
GI														-0.62***

*, **, *** is significant at p<0.05, 0.01, and 0.001 level, respectively, NS=not significant at

[a] Ht = plant height, TW = test weight, Pro = protein, Pig = pigment, PPO = polyphenol oxidase, Kwt = weight of 1000 seeds, FN = falling number, Large = kernels remaining on a 2.92 mm sieve, Vit = vitreous kernel, Sem = semolina, Semp = semolina protein, GI = gluten index, WG = wet gluten

No PD at any environment evaluated resulted in a consistently high percentage of vitreous kernels (Tables 2.7 to 2.10). The mean percent vitreous kernels for 2014 and 2015 were 74 and 91%, respectively. Based on regional quality reports (ND Wheat Commission, 2016) the average percent vitreous kernels for the ND growing region was 67 and 95% in 2014 and 2015, respectively. Joppa consistently had the highest percent vitreous kernels, regardless of PD for all environments which would be an option for durum wheat producers if maintaining a high percentage of vitreous kernels is the main objective. Based on these data vitreous kernels tended to be higher in 2014 compared to 2015 environments and could be a result of environmental conditions at each location rather than the region as a whole. Additionally, specific rainfall events at grain fill and physiological maturity might have impacted the percent vitreous kernels. Most likely, the environmental conditions just prior to harvest had the most effect on vitreous kernels. Unfortunately, this also suggests that PD, cultivar grown, and seeding rate are relatively ineffective ways available to growers to maintain vitreous kernels alone. Percentage of vitreous kernels is often determined by N. For example, more N was needed in order to achieve a given percentage of vitreous kernels as the proportion of N applied at tillering was increased (Anderson, 1985). Additionally, vitreous kernels increased with nitrogen fertilization supply when there was no variation among the different cultivars (Samson et al., 2005). However, early harvest associated with an early PD may favor higher percentage of vitreous kernels. Harvest dates in 2014 were in September and October compared to harvest dates in August for 2015. The 2014 harvest dates generally received less precipitation after maturity and prior to harvest and could have had an impact on quality of vitreous kernels.

Additional milling characteristics important to pasta processors and end-users were largely not influenced by PD, seeding rate, or environment and were specific to the cultivar. For example, the PD X cultivar interaction was not significant for kernel ash content for any environment. Mean values for quality traits of cultivars grown at each environment and can be found in Table 2.12. Kernel yellow

pigment values were consistent for each cultivar, with Divide having the lowest pigment values and with Carpio having the highest values in most environments. Previous research has identified, a strong genotypic effect on semolina yellowness, while brown index or semolina brightness were mainly determined by the environment (Schultness, et al., 2013). Planting date did have some effect on kernel yellow pigment content at some locations, but no general trend was observed.

Polyphenol oxidase (PPO) activity is a major cause of the undesirable brown color of grain and flour of durum wheat and is considered an end-use quality defect. Significant differences among cultivars were identified in each environment (Table 2.12). Carpio had significantly higher PPO activity compared to Divide or Joppa in all environments. Total ash is an important quality trait for the milling industry. No significant differences in percent ash content were identified in this study, however, the percent ash in the current study was higher than reported in regional quality reports from the same years (ND Wheat Commission, 2015).

Semolina yield significantly increased from planting date 1 to 3, but tended to decrease in planting date 4 in 2014. In 2015, no trend existed for semolina yield based on PD (data not shown). Significant differences among cultivars were identified (Table 2.12). In 2014, the percent semolina extracted from Joppa and Carpio was higher than semolina extracted from Divide. The percentage of semolina extracted was much higher in 2015 than 2014, but the general trend was for greater extraction from Carpio and Joppa except for in the Hettinger 2015 environment. These results are consistent with long-term trials (Elias and Manthey, 2016). Semolina quality based on regional reports (ND Wheat Commission, 2015) found semolina extraction to be near 65% in both 2014 and 2015. In the current study, the semolina extraction was not only considerably lower in 2014 than 2015, but no semolina extracted was as high as reported from the regional reports. The regional report consists of a much larger sample size and may be the reason for the differences observed. Additionally, the type of mill used for semolina extraction was different for this research than that of the regional report. The current

Table 2.12. Effect of cultivar on quality traits[a] associated with durum wheat grown in Minot and Hettinger, ND in 2014 and 2015.

Environment Cultivar	Pig	PPO	Ash	Sem	Sem-p	GI	WG
			%	%	g kg^{-1}	%	%
Minot 2014							
Carpio	9.3	0.40	1.5	51.7	13.1	95.5	32.6
Divide	7.8	0.07	1.6	50.6	13.2	82.5	35.0
Joppa	8.5	0.08	1.6	51.3	13.1	86.0	34.9
LSD[z]	0.2	0.02	NS	0.5	0.1	1.7	0.5
CV	4.4	26.2	5.6	2.5	1.7	4.7	3.6
Hettinger							
Carpio	9.5	0.42	1.6	49.5	10.7	98.2	22.2
Divide	7.9	0.06	1.6	49.1	10.7	97.0	25.0
Joppa	8.5	0.08	1.6	50.4	10.4	95.2	26.4
LSD	0.1	0.01	NS	0.6	0.2	0.6	0.9
CV	3.0	18.6	4.8	3.1	3.6	1.4	9.1
Minot 2015							
Carpio	10.7	0.37	1.5	58.7	12.3	91.9	32.8
Divide	8.9	0.07	1.5	58.7	12.9	68.3	37.1
Joppa	10.4	0.08	1.6	59.2	14.6	80.6	34.3
LSD	0.2	0.03	NS	NS	NS	2.5	1.1
CV	4.0	42.8	4.3	2.5	71.7	7.7	7.7
Hettinger							
Carpio	10.9	0.32	1.7	57.8	12.6	94.3	30.6
Divide	8.8	0.06	1.7	58.3	13.0	67.9	36.1
Joppa	11.3	0.05	1.7	57.3	12.3	87.9	32.3
LSD	0.1	0.01	NS	NS	0.1	2.0	0.9
CV	2.8	22.2	3.7	6.9	2.3	5.9	6.7

[a] Pig = pigment, PPO = polyphenol oxidase, Sem = semolina extracted, Sem-p = semolina extracted protein, GI = gluten index, and WG = wet gluten, NS= not significant at p=0.05 level

[z] LSD_1 was calculated to compare all levels of planting date according to Fisher's Protected LSD ($p \leq 0.05$);

study used a Brabender Quadromat Jr. Mill (South Hackensack, NJ) for semolina extraction which tends to have lower semolina yields (F. Manthey, personal communication).

When evaluating gluten index (GI) and wet gluten (WG), Carpio consistently had the highest GI at all locations and PDs with values greater than 80% indicating very strong gluten (Table 2.12). Divide was most impacted by planting date and was not consistent across PDs and locations with some GI

values indicating only strong gluten. Planting dates 1 and 4 resulted in the largest decrease in GI especially for 2015 environments. As PD was delayed, Motzo et al. (2007) found that GI decreased and found temperatures greater than 30˚C at grain filling could result in a negative effect on gluten polymerization. Differences in PD would result in different temperatures during grain filling, however, no trends between PD 1 and 4 were observed. A similar trend in a reduction in GI was seen for Joppa in 2015; however, GI values for Joppa in 2014 were not as impacted by PD and were similar to the Carpio GI values except for PD 1 in Minot. The GI values observed in the current research based on cultivar was consistent with previous reports (Elias and Manthey, 2016) where Carpio and Joppa had significantly higher GI than Divide.

The wet gluten (WG) values were more consistently relative to grain protein. A strong relationship between WG and protein existed in the current trials ($r = 0.53$, $n = 144$) (Table 2.11). Divide had the highest protein and WG at all PDs in the 2015 environments. In 2014, Joppa had the highest WG even though Divide had higher grain protein. Wet gluten percent was similar across locations. Based on regional quality reports, the average WG in 2014 was 32.5% compared to 35.9% in 2015 (ND Wheat Commission, 2015). A similar trend in higher WG values in 2015 was observed in the current research. In an effort to identify the relationship between gluten protein and quality, Fois et al. (2011) evaluated the gliadin-to-gluten ratio of the durum wheat cultivars Trigu murru and Svevo. The Mediterranean environment significantly affected this ratio, but could not be explained by the variation in temperature from the different planting times.

Conclusions

Planting date and cultivar interactions had the greatest impact on the agronomic and quality traits evaluated at all environments. The performance of individual cultivars evaluated in this study are similar to previous yield and quality reports generated from the same growing region. In the highest yielding environments, Carpio yield was superior to that of Divide and Joppa. When planting was

delayed, however, the yield of Joppa tended to decrease more than either Carpio or Divide. Caprio or Divide should be grown when planting is delayed. These data also indicate that a through a range of environmental conditions, planting durum wheat early is the best option for maximizing yield regardless of cultivar or environment. Current selection and evaluation practices in durum wheat breeding programs should develop cultivars for specific environments and also for different PDs. The interaction of PD X cultivar had a significant effect on test weight, which was the only wheat grading quality parameter evaluated in this study. Overall, the general trend of test weight values for each cultivar were consistent with previous reports. Early PDs resulted in test weight values at or above the US grade 1 minimum standard for all three cultivars. Delayed planting resulted in lower test weight values lower resulting in grain rated as US grade 2. Environment significantly impacted percent vitreous kernels. All cultivars evaluated were subject to loss of vitreousness caused by precipitation and humidity around maturity and harvest. No specific planting date, cultivar, or experimental location consistently produced durum that would be graded as hard amber durum (HAD). Characteristics such as protein content, FN, kernel yellow pigment content, and GI were more dependent on cultivar than environment, although some effect of environment was observed. Much of the end-use data generated in this research is consistent with regional and multi-year evaluations currently conducted and was not impacted by changes in PD or seeding rate.

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CHAPTER 3. EFFECT OF FERTILIZER SOURCE AND TIMING OF APPLICATION ON CADMIUM UPTAKE OF DURUM WHEAT

Abstract

Durum wheat (*Triticum turgidum* L. *var. durum* Desf.) is a market class of wheat grown in ND that tends to accumulate Cd in harvested grain under certain environmental conditions. Due to potential international marketing concerns, ND durum wheat producers require strategies that might limit grain Cd accumulation in grain. These trials were conducted in order to determine the impact of type and placement of Zn fertilizer on grain seed Cd levels and to determine the best timing of foliar Zn-EDTA resulting in lowest grain Cd. Foliar Zn-EDTA applied at Feekes 10 growth stage had the lowest grain Cd of 0.97 mg kg^{-1} when evaluating different fertilizer sources and application timings. Application of 22.4 kg ha^{-1} KCl with the seed at planting resulted in the highest grain Cd of 0.151 mg kg^{-1} and might be a concern when environmental conditions are conducive for Cd uptake from soil. Applying 1.1 kg Zn ha^{-1} as foliar Zn-EDTA in combination with 33 kg N ha^{-1} at Feekes 10.54 growth stage resulted in significantly lower grain Cd, and significantly higher grain Zn, Fe, and protein content. No treatments in either trial negatively impacted grain yield, test weight, or protein content. The treatments that most reduced grain Cd resulted in the most benefits from a production, marketing, and nutritional standpoint and represents an agronomic approach to biofortification of durum wheat.

Introduction

Durum wheat (*Triticum turgidum* L. *var. durum* Desf.) is a premium market class of wheat commonly grown in North Dakota. In 2014, 567 000 ha were planted with an average yield of 2700 kg ha^{-1} (NASS, 2014). Currently, North Dakota produces approximately 60% of all U.S. durum wheat, but has previously produced more than 87%. Approximately 35% of the durum wheat produced in ND is exported internationally and heavy metal toxicity due to Cd accumulation in grain has the potential to impact trade (J. Petersen, ND Wheat Commission, personal communication). International

standards limit the amount of Cd in food. Currently, the Codex Allimentarius Commission of the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) has set the acceptable level of Cd in wheat grain of 0.2 mg kg^{-1} (CAC, 2009).

Durum wheat is the most problematic cereal crop in regards to Cd accumulation grown in ND. Rye (*Secale cereal* L.), barley (*Hordeum vulgare* L.), and oat (*Avena sativa* L.) tend to accumulate less Cd in harvested grain than durum wheat (Perilli et al., 2010). In general, dicotyledon plants accumulate more Cd than graminaceous plants mainly due to the ferrous transport system which also can aid in the uptake of Cd (Chaney, 2010). Some of the known major Cd accumulating crops are grown in ND and are a concern for international marketing standards. Sunflower (*Helianthus annus* L.) and flax (*Linum usitatissimum* L.) are two dicots grown in ND that are known Cd accumulators. Other dicot crops that are potential Cd accumulators include peanut (*Arachis hypogaea* L.), potato *(Solanum tuberosum* L.), and soybean (*Glycine max* L.). Direct human consumption of durum wheat is more of a concern than crops grown for their oil. It is important to limit the amount of Cd in staple food crops such as rice, potato, and wheat as accumulation over time could lead to Cd toxicity and these crops are consumed in large quantities. Itai itai disease was the result of poisoning caused by food ingestion and inhalation of Cd and was first documented due to the mass Cd poisoning of mining workers in Japan during the 1970's (Kobayashi, 1978).

The use of genetic resistance and favorable agronomic practices can reduce Cd accumulation in durum wheat (Grant et al., 2007). Low-Cd accumulating durum wheat cultivars have been developed (Clarke et al., 2006). However, these might not be available to producers or have desirable agronomic characteristics. Developing low Cd durum wheat using low grain Cd as a selection criterion began in Canada in the mid-1990s (Clarke et al., 2010). This was initiated because of international limits on Cd concentration in food. Hart et al. (2006) suggested that movement of Cd from roots to shoots was the cause of differential Cd partitioning in two durum wheat near-isogenic lines. A major gene was

discovered that is responsible for the accumulation of Cd in durum wheat and it is designated as *Cdu-1* (Clarke et al., 2010). The allele for low Cd accumulation is not pleiotropic and does not affect any major economic traits (Clarke et al., 2002). AC Strongfield durum wheat was released in Canada due to its superior agronomic performance, quality attributes, and reduced grain cadmium concentrations (Clarke et al., 2006). AC Strongfield contains the allele for low cadmium concentration. Newly released cultivars, such as Joppa and Carpio (Elias et al., 2015 and Elais and Manthey, 2016), were developed for the ND growing region by North Dakota State University (NDSU). Both possess quality attributes that are superior to previously released durum wheat cultivars (E. Elias, personal communication)**.** Neither Carpio nor Joppa contain the gene for low cadmium and could impact the marketing of these cultivars to the EU if grown in areas with high soil Cd. In 2015, approximately 80% of durum wheat produced in ND was with cultivars developed and released by NDSU (NASS, 2014). Low Cd content and the *cdu1* allele are currently important selection criterion for cultivar development at NDSU (E. Elias, personal communication).

Plant uptake of Cd is affected by soil properties, crop species and cultivar grown, fertilizers, agronomic practices and properties of the Cd source (Chaney, 2010; Hart et. al, 2006). Soil properties that have the largest impact on Cd availability include pH, salinity, Cl, cation exchange capacity (CEC), organic matter and concentrations of other nutrients such as N, P and Zn. Other soil factors that can affect Cd uptake include pH and salinity. Soil properties such as pH can influence the amount of Cd taken up by crops. Generally, Cd concentration of plant tissue decreases as the pH of the soil increases when all other soil factors remain unchanged (Kirkman, 2006). An increase in soil pH increases Cd adsorption and reduces extractability. Adams et al. (2004) found that total soil Cd and pH were the most significant factors affecting grain Cd concentration in wheat and barley. Soil salinity was identified as a soil factor that could enhance the availability of Cd in the soil (Chaney, 2010). Currently, ND has over 2.35 M hectares of soil considered saline (Brennan and Ulmer, 2012) which might impact Cd accumulation in

crops known to accumulate Cd. In neutral and alkaline soils, Cl concentration was identified as a factor associated with increased Cd uptake (Chaney, 2010). The presence of Cl caused more Cd to dissolve in nutrient solution and resulted in more Cd uptake.

Most agricultural soils in the U.S. have a Cd concentration ranging from 0.1 to 1.0 mg kg^{-1} (Page et al., 1987). Young soils tend to have more Cd than highly weathered soils and soils with parent material such as glacial till and alluvium are natural sources of Cd. Soils in ND have been identified with relatively high diethylenetriaminepentaacetic acid (DTPA)-extractable Cd for these reasons. A major soil survey of 937 samples determined that the North Central region of the U.S., including ND, had a range of 0.20 to 0.94 mg kg^{-1} Cd with a mean of 0.37 mg kg^{-1} (Holmgren et al., 1993). Other studies have found that soils from the western and north central states tend to be higher in Cd than the rest of the U.S., with levels in ND varying from 0.01 to 0.31 mg kg-1 in DTPA-extractable Cd (Franzen et al., 2006). Additionally, Franzen et al. found that extractable Cd concentrations in the soil were lower in upland and sloping sites compared to lowland and depressional locations in ND. They suggested modified harvest of grain which segregates grain from low-lying areas and upland areas as a way to separate potentially high Cd grain and minimize marketing issues.

Many factors can influence the Cd levels in harvested crops in addition to the soil properties in which they are grown. Many fertilizers products and soil amendments can be contaminated with heavy metals and can be a source of potential Cd. The addition of P fertilizer to crop land might increase the concentration of Cd in the soil if the fertilizer is contaminated with Cd, depending on the source of P (Cook and Morrow, 1995). Fertilizers produced from sedimentary phosphate rock tend to have more Cd contamination depending on processing techniques (Roberts, 2014). Cadmium also can be deposited from the atmosphere and taken up by crops (Page et al., 1987). Cadmium is released into water through weathering of rocks or released into the air from volcanoes or forest fires (Tran and Popova, 2013).

Information on Cd uptake, accumulation, and transport is important to understand in order to develop management practices that can be used to limit Cd in harvested grain. Cadmium uptake, translocation, and subsequent accumulation in harvested grain have been studied in many field crops including sunflower. Results of these studies have indicated genetic differences among sunflower genotypes. The results from a screening of 200 sunflower lines at locations in ND and MN found large variations in Cd levels at different growth stages (Li et al., 1997). Leaf Cd at the sunflower growth stage R5 (Schneiter and Miller, 1981) was the best predictor of harvested grain Cd even when grown in different soils (r = 0.44 to 0.59). However, Li et al. (1997) found that the variation in leaf Cd accumulation was not the result of simple inheritance. The seedling stage was not a good indicator of grain Cd (r = 0.19 to 0.50) and might be the result of different genes controlling uptake and accumulation. Using leaf samples to determine Cd levels in edible food parts could be specific to species being evaluated. Soil type and characteristics impact sunflower grain Cd. The effect of soil chloride, sulfate, and other soil factors on Cd concentration in sunflower was examined by Li et al. (1994). They found that sunflower kernel Cd levels were highly correlated with DTPA-extractable Cd (p<0.001) at all soil depths tested and with clay content in sub soils. Soil sulfate did not affect Cd uptake, however, soil chloride levels were positively correlated with grain Cd. The results indicated that soil chloride concentration was a factor in sunflower Cd uptake.

Environmental conditions and genetics impact the level of Cd in durum wheat grain. Cieśliński et al. (1996) found that Cd accumulation and distribution in various tissues of durum wheat are strongly affected by both soil type and cultivar. Depending on soil type, these researchers also found that the durum wheat grain contained 21 to 36% of the total Cd taken up by the plant. Genetic differences in rhizosphere processing of Cd and Cd transport processes *in vivo* were suggested by the group as potential reasons for the wide range (0.0017 to 0.268 mg kg^{-1}) of Cd in accumulation in harvested grain.

Cultivar, nitrogen rate, planting date, tillage, fertilizer, and soil type were evaluated in previous research in order to determine the effect of Cd accumulation in durum wheat grain. According to Cieśliński et al. (1996), both cultivar and soil type interacts and contributes to Cd accumulation in durum wheat. The authors suggested an integrated management strategy of agronomic practices and proper cultivar selection to limit Cd in harvested grain. Management of toxic, heavy metals might be possible through agronomic practices that limit Cd uptake during grain fill and by the use of low Cd accumulating cultivars. Most of the cultivars of durum wheat grown in ND do not contain the allele for low Cd accumulation. As previously stated, management practices such as planting date and fertilizer type can influence grain Cd concentration in durum wheat. In Manitoba, Canada, Perilli et al. (2010) found that environment factors, specifically soil type, had a significant impact on grain Cd. Furthermore, the researchers found that the application of N fertilizer increased the Cd concentration in harvested grain. Early and middle planting dates generally had higher grain Cd than later planting dates. They hypothesized that the increased Cd was the result of the increased yields associated with the earlier planting dates. The authors suggested multiple management practices would enable producers to maximize grain protein and minimize grain Cd.

The application of micronutrient fertilizers or soil amendments to limit grain Cd has been studied. Choudhary et al. (1995) found that durum wheat tends to accumulate Cd in roots, leaves, stems and grain, regardless of fertilizer treatment when grown under greenhouse conditions. Cadmium concentrations were highest in roots and lowest in grain for both the low and high Cd accumulating durum wheat genotypes. Soil-applied Zn fertilizer lowered Cd concentration in all plant parts tested. However, the application of foliar Zn had little effect on Cd levels. Additionally, they found that application of N and P fertilizer with Zn fertilizer decreased plant Cd (Choudhary et al., 1995). Application of $ZnSO_4$ and ground-up rubber had no significant effect on grain cadmium level, grain yield or protein content on two durum wheat cultivars grown on high Cd soils in Arizona (Wang et al., 2011).

Oliver et al., (1994) found that the Cd concentration in wheat grain could be decreased by up to 50% by the addition of 2.5 to 5.0 kg Zn ha^{-1} on soils marginal for Zn. Others found that soil-applied Zn reduced Cd concentration in durum wheat, but foliar applied Zn did not (Choudhary et al., 1995).

Zinc fertilizer is available in many different forms and could be applied by producers at different growth stages of durum wheat. Since Zn can compete with Cd for uptake by plants, this might be a way to manage Cd. Application of foliar Zn significantly reduced grain Cd levels in durum wheat in cultivars tested in Montana in dryland production in some years (Eckhoff, 2010). Higher rates of Zn (1.1 kg Zn ha^{-1} of chelated Zn-ethylenediaminetetraacetic acid (EDTA) resulted in lower grain Cd levels. The application of Zn fertilizer has also been studied in other crops with mixed results. Bell et al. (1998) studied agronomic practices that minimized Cd uptake of peanut in Australia. Management practices such as liming and application of Zn fertilizer had no significant effect on grain Cd. However, Bell et al. (1998) hypothesized that the application of Zn fertilizer might be useful in slightly responsive cultivars that could be near the maximum permitted concentration. These studies suggested that site selection based on soil type and fertilizer type were the most effective way to manage Cd accumulation in peanut. Jiao et al. (2004) found an antagonistic effect of Zn on Cd uptake and distribution within flax and durum wheat. These researchers also suggested possible explanations for the effect of Zn on Cd uptake and distribution including: 1) Zn and Cd are in the same group in the periodic table and might compete with each other for exchange sites on root surfaces and for transport within the plant; 2) Zn maintains the root-cell plasma membrane integrity with Zn-deficiency resulting in more Cd movement into the plant; and 3) Zn-deficiency could increase root exudation of amino acids, sugars, and phenolics and increase Cd availability in the soil.

The research reported herein was conducted in order to determine ways to maximize durum quality, while minimizing the accumulation of grain Cd. The specific objective was to determine how the type and placement of Zn fertilizer might impact grain seed Cd levels and to determine an appropriate

timing for foliar Zn-EDTA fertilizer application that will reduce grain Cd levels and also fit into current durum wheat management practices. The results of this study will enable recommendations to be developed for recently released durum wheat cultivars in order to minimize the negative grain quality aspect of grain Cd and so that growers might continue to meet international marketing standards.

Materials and Methods

Experiments were conducted in 2014 and 2015. The first experiment was the source trial which evaluated the placement and type of Zn fertilizer. The second experiment was the timing trial which evaluated different application timing of foliar Zn. The source experiments were conducted near Crosby (48˚ 50' 43" N, 103˚ 18' 34" W), Hettinger, (46˚ 00' 40" N, 102˚ 38' 40" W) and Minot, ND (48˚ 10' 55" N, 101˚ 17' 46" W). The timing trials were conducted in Hettinger and Minot, ND. The soil types at each location were a Williams-loam, Shambo-loam, and Aastad-loam in Crosby, Hettinger, and Minot, respectively (Table 3.1) (USDA-NRCS, 2016). Experiments at all locations were seeded directly into the stubble from the previous year using a no-till seeder. The previous crops in Crosby were HRSW (*Triticum aestivum* L.) and flax in 2014 and 2015, respectively. In Hettinger the previous crops were HRSW green fallow (planted HRSW terminated prior to maturity) and lentil (*Lens culinaris* Medik) in 2014 and 2015, respectively. In Minot, the previous crops were flax and field pea (*Pisum sativum* L.) in 2014 and 2015, respectively. Experimental units were 6.1 m^{-2} (1.2 m wide x 4.9 m long), 10.7 m^{-2} (1.6 m wide x 6.7 m long), and 11.6 m^{-2} (1.52 m wide x 7.6 m long) in Crosby, Hettinger, and Minot, respectively. Row spacing was 17.8 cm at all locations.

Table 3.1. Soil series[†], taxonomy, and slope at Crosby, Hettinger, and Minot, ND in 2014 and 2015.

Location	Soil Series	Soil Taxonomy[‡]	Slope
			%
Crosby	Williams-loam	Fine-loamy, mixed, superactive, frigid Typic	0-3
Hettinger	Shambo-loam	Fine-loamy, mixed, superactive, frigid Typic	0-2
Minot	Aastad-loam	Fine-loamy, mixed, superactive, frigid Calcic	0-3

[†] Soil data obtained from (USDA-NRCS, 2016).

[‡] Soil taxonomy listed on individual lines based on hyphenated soil series name.

All plots were maintained using best management practices (Wiersma and Ransom, 2012) and fertilized with N-P-K based on yield potential and soil test values (Table 3.2) for each location (Franzen, 2014). All soil tests were conducted by the NDSU Soil Testing Laboratory, Fargo, ND using approved and standard practices (North Central Region Research Publication, 2015). Soil Cd was determined by Agvise Laboratories (Northwood, ND) using EPA digestion method 3050 (EPA, 2012). Total Cd in soil was then determined using optical emission spectrometry. Herbicide and fungicide applications were applied uniformly across all treatments. The herbicide and rate varied on weed species present at each location and were applied according to product label recommendations (Zollinger et al., 2016). A fungicide application of 100 g ha^{-1} active ingredient of both prothioconazole + tebuconazole to control FHB was made at Feekes 10.51 growth stage (Large, 1954) at all locations in the source trial. Type, rate, and placement of the fertilizer treatments used in the source trial can be found in Table 3.3. All treatments for the timing trial (Table 3.4) were applied using a CO_2 pressurized back-pack sprayer and hand-held spray boom. All sources of Zn applied in the study were from low Cd sources and were tested for Cd contamination. A plot combine harvester with a 1.52 m wide head was utilized to harvest durum wheat in experimental units when grain moisture was near 13%.

The durum wheat cultivars Carpio, Joppa, and AC Strongfield were evaluated in these experiments. Carpio and Joppa were all developed by the North Dakota State University (NDSU) durum wheat breeding program and released by the North Dakota Agricultural Experiment Station (Elias et al., 2015 and Elias and Manthey, 2016). AC Strongfield was developed at the Semiarid Prairie Agricultural Research Centre in Swift Current, SK, and registered by the Canadian Food Inspection Agency in 2004 (Clarke et al., 2006). AC Strongfield was released due to its agronomic performance and end-use quality factors. AC Strongfield was selected for the current research because it contains the *Cdu-1* gene for low Cd accumulation. Joppa and Carpio were selected for these experiments based on recent availability to

ND producers and excellent end-use qualities. Neither Carpio nor Joppa have the gene for low Cd accumulation.

Table 3.2. Soil factors measured prior to planting at Crosby, Hettinger, and Minot, ND in 2014 and 2015.

Locatio	Depth	NO_3-N	P	K	pH	OM	Zn	S	Fe	Cl	Cd
	cm	kg ha^{-1}	mg kg^{-}	mg kg^{-1}		%	mg kg^{-1}	kg ha^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Crosby											
2014	0-15	11	20	400	6.3	4.0	1.04	7	47	29	0.4
	15-30	8	4	336	7.2	3.0	0.30	3	26	42	
2015	0-15	14	16	645	7.1	4.0	1.20	24	27	6	0.8
	15-30	3	5	281	7.6	3.6	0.66	103	29	1	
Hetting											
2014	0-15	105	23	400	6.1	3.3	0.89	3	71	6	1.3
	15-30	30	5	225	7.5	2.5	0.30	17	17	23	
2015	0-15	81	35	625	6.4	3.8	2.17	11	64	11	0.7
	15-30	34	8	200	7.0	2.6	0.37	20	24	14	
Minot											
2014	0-15	49	14	385	5.0	3.9	1.14	44	83	42	0.4
	15-30	35	3	192	7.4	2.9	0.31	156	25	77	
2015	0-15	270	23	447	6.3	3.8	1.46	255	61	16	0.5
	15-30	130	8	220	7.3	2.5	0.54	355	20	9	

Table 3.3. Type, rate, and placement of fertilizer treatments for the source trial conducted in Crosby, Hettinger, and Minot, ND in 2014 and 2015.

Treatment	Fertilizer	Rate	Placement
1	KCl	9 kg Cl ha^{-1}	With seed at planting
2	KCl	9 kg Cl ha^{-1}	With seed at planting
	Zn-EDTA	1.1 kg Zn ha^{-1}	Feekes 10 growth stage
3	Zn-EDTA	1.1 kg Zn ha^{-1}	Feekes 10 growth stage
4	$ZnSO_4$	12 kg Zn ha^{-1}	Broadcast at seeding
5	$ZnSO_4$	12 kg Zn ha^{-1}	Broadcast at seeding
	KCl	9 kg Cl ha^{-1}	With seed at planting
6	Untreated	n/a	n/a

Table 3.4. Type, rate, and growth stage of foliar-applied treatments for the timing trial conducted in Crosby and Minot, ND in 2014 and 2015.

Treatment	Application	Rate	Feekes Growth Stage
1	Zn-EDTA	1.1 kg Zn ha^{-1}	4 (Tillering)
2	Zn-EDTA	1.1 kg Zn ha^{-1}	4
	Pyraclostrobin	161 g ha^{-1}	
3	Pyraclostrobin	161 g ha^{-1}	4
4	Zn-EDTA	1.1 kg Zn ha^{-1}	10 (Boot)
5	Zn-EDTA	1.1 kg Zn ha^{-1}	10
	Pyraclostrobin	100 g ha^{-1}	
	Tebuconazole	100 g ha^{-1}	
6	Pyraclostrobin	100 g ha^{-1}	10
	Tebuconazole	100 g ha^{-1}	
7	Zn-EDTA	1.1 kg Zn ha^{-1}	11.1 (Grain ripening)
8	Zn-EDTA	1.1 kg Zn ha^{-1}	10.54 (Late flowering)
9	Zn-EDTA	1.1 kg Zn ha^{-1}	10.54
	UAN[†]	33 kg N ha^{-1}	
10	UAN	33 kg N ha^{-1}	10.54
11	Untreated	n/a	n/a

[†]Urea-ammonium nitrate

Data Collection

Plant height was determined by averaging two measurements per plot using a tape measure. The plant height was calculated based on the distance from the soil to the top of the plant spike at physiological maturity. Grain yield from harvested field plots was recorded on a clean-grain basis corrected to 13% seed moisture content. Test weight was measured using methodology specified by the AACC International (AACC, 2000) (Approved Method 55-10.01). Protein content of whole durum wheat samples was determined using a Perten Instruments DA7200 NIR analyzer (Springfield, IL). Determination of DON levels in harvested grain were as previously described (Ovando-Martinez et al., 2015).

Cd, Fe, and Zn Determinations

Determination of grain Cd, Fe, and Zn analyses were performed as described by Thavaraja et al. (2015). In summary, approximately 0.5 g of each ground sample was weighed into a DigiTUBEs digestion tube (SCP Science, Quebec, Canada) and 6 mL of concentrated nitric acid (70%) was added to each tube.

Tubes were then placed into a programmable automated digestion system (Questron Technologies Corp, Mississauga, Canada). The temperature of the digestion block system was adjusted to 90°C for 60 minutes, followed by the addition of 3 mL of hydrogen peroxides and allowed to digest for an additional 15 minutes. Samples were further digested with 3 mL of 6 M hydrochloric acid for an additional 5 minutes to ensure complete digestion. Blank and standard reference samples were prepared and analyzed for each digestion run.

After digestion, samples were cooled to room temperature and filtered using DigiFILTER (SCP Science). Total volume of the filtrate was adjusted to 10 mL using nano-pure water. Cd, Fe, and Zn were then measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, iCAP 6500 Duo, Thermo Fisher scientific, USA). Standard Reference Material 1515 from the National Institute of Standards and Technology, Gaithersburg, MD and an acid blank sample were digested and analyzed with samples to provide an indication of accuracy. Cd, Fe, and Zn standards (CPI International, USA) were used to develop the standard curves (0 to 3 g L^{-1}). In addition to sample analyses, the Zn-EDTA applied in the field trial was analyzed for Cd and Zn concentration.

Experimental Design and Statistical Analysis

The experimental design for the timing trial was a randomized complete block with a factorial arrangement of cultivars x timing. Treatments were replicated four times. The experimental design for the source trial was a randomized complete block design with a split-plot arrangement. Whole plots were fertilizer source and the subplots were cultivars. Analysis of variance was conducted. Environments were considered homogenous when the ratio (F_{max}) of the effective error variance for each trait was less than 10-fold (Tabachnick and Fidell, 2001). Data for each environment were analyzed using PROC GLM procedure of SAS software (SAS Institute, 2010). F-tests were considered significant at $p<0.05$. Mean comparisons using *F*-protected least significant differences (LSD) were made where F-tests indicated significant differences existed ($p < 0.05$). Combined analyses of variance across

environments were calculated for all variables. LSDs for the combined analyses were calculated based on the methodology described by Carmer et al. (1989). Tests for collinearity among were performed using Pearson correlation coefficients from the PROC CORR procedure. PROC STEPWISE was performed in order to determine stepwise multivariate analysis.

Results and Discussion

Growing Conditions

Average air temperature and precipitation from April to September for each year and location are provided in Tables 3.5 and 3.6, respectively. The average daily temperature for the 2014 growing season was only 69 and 97% of the long term average in Hettinger and Minot, respectively. However, in Crosby the average daily temperature for the growing season was normal. Alternatively, the 2015 daily average temperature at all locations was slightly above normal. Precipitation totals during the 6-month growing season were above the long term average at all locations for both years. In 2014, the months of June and August had the most precipitation at all locations. In 2015, the months of May and June had the most precipitation at all locations.

Table 3.5. Average air temperature (˚C) for Crosby, Hettinger, and Minot, ND in 2014 and 2015.

Location/Year	Apr.	May	Jun.	July	Aug.	Sep.	6-mo.	% Long-
Crosby								
2014	3	12	16	19	19	14	14	100
2015	4	11	18	20	19	15	15	104
Hettinger								
2014	4	12	15	19	19	14	11	69
2015	6	10	18	21	21	18	16	103
Minot								
2014	3	12	17	19	19	15	14	97
2015	6	11	19	21	20	16	16	106

Source: NDAWN (2016)

Table 3.6. Precipitation (mm) for Crosby, Hettinger, and Minot, ND in 2014 and 2015.

Location/Year	Apr.	May	Jun.	July	Aug.	Sep.	6-mo.	% Long-
Crosby								
2014	35.8	60.5	130.0	31.2	61.2	35.3	59.0	121
2015	4.1	39.9	105.9	30.2	33.3	113.0	54.4	111
Hettinger								
2014	31.8	40.6	130.0	21.6	131.3	32.8	64.7	119
2015	24.6	102.5	131.3	25.7	47.2	23.0	59.1	109
Minot								
2014	51.1	41.6	187.1	41.4	114.0	21.1	76.1	135
2015	11.9	79.2	155.0	46.3	27.8	39.8	60.0	106

Source: NDAWN (2016)

Source Trial

The main objective of the source trial was to determine what impact the type and source of fertilizer would have on grain Cd. A secondary objective was to see if these treatments had an effect on other agronomic traits. DON and plant height data were not measured at all locations, so in combined analyses for these traits will not be discussed. All traits evaluated were impacted by environment. For the combined analyses, the locations of Crosby and Minot conducted in 2014 were not included. When evaluating individual locations, these numbers were not consistent with the other locations. Based on soil test Cd levels, these locations had the DTPA-extractable soil Cd levels of 0.4 mg kg^{-1} and therefore the treatment response was not consistent and minimal compared with the other locations.

The data reported are from locations where soil Cd levels are greater than 0.5 mg kg^{-1}. According to Page et al. (1987), the mean soil Cd level in the north central region of the US is about 0.37 mg kg^{-1} and therefore the results from these trials is important for most soils in ND. The highest levels of grain Cd observed in these studies were from the trials conducted in Minot and Hettinger in 2015. However, the soil values at Minot and Hettinger were 0.7 and 0.5 mg kg^{-1}, respectively, and lower than the other locations. The Hettinger 2014 environment had the most DTPA-extractable soil Cd with 1.3 mg kg^{-1}. A positive relationship between DTPA-extractable soil Cd and grain Cd levels has been determined

in most studies (Chaney, 2010). However, the relationship between soil Cd and grain Cd levels could not be directly established in these studies indicating that other environmental factors might be attributing to the differences observed.

Stepwise multiple linear regression was used to determine is determine if any of the soils factors evaluated could be used to predict grain Cd using a multivariable model. All soil factors evaluated at the four environments were considered. The best relationships (Eq. 1), included soil pH and soil Cl and

$$\text{Grain Cd} = 0.30441 + 0.06098 \text{ soil pH} + 0.00108 \text{ soil Cl} \quad \text{(Eq. 1)}$$

$$(R^2 = 0.9625, n = 4, P<0.05)$$

explained 96% of the variability of grain Cd. Grain Cd and soil Cl were measured in mg kg^{-1}. Soil pH contributed the most to the model. Although the relationship demonstrates an association between grain Cd and soil pH and Cl levels, the correlation does not provide causation. An unmeasured factor might be causing a simultaneous relationship. Additionally, few environments were evaluated (n =4).

Previous research has suggested that soil pH can greatly influence availability of Cd, but what type of relationship is variable. Generally, Cd concentration of plant tissue decreases as the pH of the soil increases when all other soil factors remain unchanged (Kirkman, 2006). An increase in soil pH increases Cd adsorption and reduces its extractability due to carbonates in soil which can precipitate Cd. Adams et al. (2004) found that total soil Cd and pH were the most significant factors affecting grain Cd concentration in wheat and barley.

Norvell et al. (2000) found a grain Cd of durum wheat could be predicted by DTPA-extractable soil Cd and log transformed salinity, but not pH ($R^2 = 0.664$, n = 124). Wu et al. (2002) determined that DTPA-extractable soil Cd and the natural logarithm of water-extractable Cl cold predict grain Cd ($R^2 = 0.858$). Smolders and McLaughlin (1996) found that soil Cl increased Cd uptake in Swiss chard (*Beta vulgaris* ssp. *cicla* (L.) Koch) due to the $CdCl^{-2}$ species formed in soil solution was more plant available than Cd alone and that the Cl in the soil enhanced Cd diffusion in plant roots. Wu et al. (2002) analyzed

durum wheat samples and found that locations with elevated grain Cd and soil Cl were distributed similarly. Locations with well-drained soil and less Cl resulted in grain with lower Cd. Conversely, locations that were poorly drained with elevated Cl produced higher grain Cd. In neutral and alkaline soils, Cl was identified as a factor associated with Cd uptake in plants (Chaney, 2010). They found the presence of Cl in the nutrient solution caused more Cd to dissolve and resulted in more Cd uptake by the plant. Enhanced Cd levels in potato tubers grown in Australia also were related to soil electrical conductivity (EC) and extractable Cl (R^2= 0.62, p<0.001) (McLaughlin et al., 1994).

Currently, ND has over 2.35 M hectares of soil considered saline (Brennan and Ulmer, 2012) which might impact Cd accumulation in crops known to accumulate Cd such as durum wheat. This could be of particular interest for ND producers growing Carpio in saline areas, where more Cd might accumulate in harvested grain. Preceding crop also might affect the Cd found in grain. Crop rotation could be a way to minimize grain Cd levels under saline conditions. Khoshgoftarmanesh and Chaney (2007) found that grain Cd levels in HRSW were higher when grown after cotton (*Gossypium* L.) than when grown after sunflower. Grant et al. (1999) found that Cd was highest in wheat grown after sunflower when the sunflowers stalks were incorporated after harvest. The removal or incorporation of crop residue can impact the grain Cd levels of the preceding crop especially when the preceding crop is a known Cd accumulator such as sunflower or flax. Ideally, durum wheat grown in ND should not precede crops such as sunflower or flax when grown in saline conditions which consist mainly of Cl, especially under conventional tillage.

In the combined analyses, no significant treatment by cultivar interaction occurred for any trait evaluated. The mean values and LSD values for treatment can be found in Table 3.7. No significant differences among treatments was observed in any of the trials for test weight or yield. The application of the treatments evaluated did not have a negative impact on yield or test weight and should not be a concern to durum wheat producers. However, no test weight value was above the industry standard of

752 kg m^{-3}, but may be a result of the specific year and location. Eckhoff (2010) also found that $ZnSO_4$ and foliar Zn-EDTA treatments did not impact yield, test weight or protein. In the current study, treatment was significant for protein content (p<0.05). The lowest grain protein content was found with the soil-applied $ZnSO_4$ + KCl treatment at planting, but it was not significantly different from the foliar-applied Zn-EDTA, soil-applied $ZnSO_4$, or untreated. The soil-applied KCl + foliar Zn-EDTA treatment resulted in the highest grain protein of 144 g kg^{-1}.

Table 3.7. Combined average treatment means for the source trial conducted at Hettinger, ND in 2014 and Crosby, Hettinger and Minot, ND in 2015.

Treatment	TW	Yield	Protein	Cd	Fe	Zn	Cd	Fe	Zn
	kg m^{-3}	kg ha^{-1}	g kg^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg ha^{-1}	mg ha^{-1}	mg ha^{-1}
KCl	748	4105	140	0.151	33.1	27.0	621	134412	106961
KCl + Zn EDTA	743	4233	144	0.137	33.5	29.7	599	140991	125664
Zn EDTA	744	3810	132	0.097	31.5	29.1	394	120563	108187
$ZnSO_4$	743	3823	133	0.111	31.2	27.8	459	119583	103246
$ZnSO_4$ + KCl	752	3810	129	0.123	32.2	29.3	467	121174	106638
Untreated	749	3810	134	0.121	30.5	25.4	507	115077	86688
LSD (0.05)	NS	NS	8	NS	NS	NS	73	NS	NS
CV	2.1	7.0	5.3	22.1	11.6	8.8	25.3	13.9	13.7

TW= test weight

The foliar application of Zn-EDTA had the lowest grain Cd compared to all other treatments, although no significant differences among treatments was observed (Table 3.7). However, significant differences in total harvested grain Cd were observed. The total harvested grain Cd was 394 mg ha^{-1} which was significantly lower than all other treatments except for the soil-applied $ZnSO_4$. The application of foliar Zn-EDTA lowered the grain Cd approximately 25% compared to the untreated control. The soil-applied $ZnSO_4$ treatment had the second lowest grain Cd of the treatments evaluated. Eckhoff (2010) found that $ZnSO_4$ applied with seed at planting did not significantly decrease grain Cd compared to the untreated control. The $ZnSO_4$ in the current study was broadcast on the soil at planting. The differences in application might account for the differences observed in grain Cd. The untreated control had lower grain Cd and total harvested grain Cd than either the soil-applied KCL and soil-applied KCl + foliar Zn-

EDTA treatments. The highest grain Cd levels observed were below the current CAC acceptable level of Cd in wheat grain of 0.2 mg kg^{-1} (CAC, 2009). However, some specific treatment and locations did exceed this level. In Hettinger 2015, Carpio had an average grain Cd of 0.22 mg kg^{-1} which exceeds current acceptable levels in the EU.

Large differences in grain Cd were observed among environments in this study, indicating a strong environmental effect. No relationship between yield and grain Cd was observed and is similar to trends observed for durum wheat by others (Perilli et al. 2010). Arao and Ae (2003) evaluated rice genotypes for Cd concentration and detected large differences among genotypes, but the differences were consistent across years and soil types. They found that the grain Cd levels in rice genotypes were highest, ranging from 1.42 to 4.95 mg kg^{-1}, when rice was grown in soil with the highest levels of HCl-extractable Cd.

It is difficult to speculate how the treatments and cultivars evaluated in the current study might perform when evaluated in soils with higher levels of Cd that might have resulted in harvested grain Cd levels closer to maximum levels. Previous research suggests a decrease of about 25% in grain Cd levels even when grain Cd levels are higher and above maximum limits (Eckhoff, 2010). In her studies, grain Cd levels for the untreated controls averaged 0.245 mg kg^{-1} which is much higher than the CAC acceptable limit of 0.2 mg kg^{-1}. In the current study, when foliar Zn-EDTA was applied a decrease of about 20% in grain Cd was observed in the harvested grain compared to the untreated (Table 3.7). Total grain Cd harvested was about 22% lower when comparing the same treatments.

No significant differences were observed for grain Fe or Zn among treatments. Any treatment that included KCl applied at planting with the seed tended to have higher grain Fe than the other treatments (Table 3.7). Treatments which included an application of foliar Zn-EDTA tended to have higher grain Zn levels compared to the other treatments. The lowest grain Zn was found in the untreated control and indicates that all treatments increased Zn in the harvested grain even if the

treatment did not include a Zn component. Eckhoff (2010) found that the $ZnSO_4$ applied with seed at planting did not significantly increase Zn in harvested grain. However, when foliar Zn-EDTA was applied at Feekes 10 growth stage (boot) in addition to the application of $ZnSO_4$ at planting, a significant increase in Zn was found in harvested grain. In her study, an application of foliar Zn-EDTA alone was not applied. Based on the current data, the application of foliar Zn-EDTA would be the best treatment evaluated for lowering grain Cd and would have minimal negative effects on the desirable agronomic qualities.

Cultivar differed significantly for protein, Cd, Fe, and Zn (p<0.05) in the combined analyses (Table 3.8). No significant differences in test weight or yield were observed among cultivars. The yield of Joppa was higher than the yield of Carpio or AC Strongfield and is consistent with previous reports (Elias and Manthey, 2016).

Table 3.8. Combined average cultivar means for the source trial conducted at Hettinger, ND in 2014 and Crosby, Hettinger, and Minot, ND in 2015.

Treatment	TW	Yield	Protein	Cd	Fe	Zn	Cd	Fe	Zn
	kg m^{-3}	kg ha^{-1}	g kg^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg ha^{-1}	mg ha^{-1}	mg ha^{-1}
Carpio	753	3857	132	0.150	31.1	28.8	603	120425	105729
Joppa	746	4132	128	0.126	29.6	26.7	542	121941	107458
AC Strongfield	741	3810	145	0.094	35.3	28.7	378	133534	105504
LSD (0.05)	NS	NS	10	0.038	3.4	1.5	51	NS	NS
CV	2.1	7.0	5.3	22.1	11.6	8.8	25.3	13.9	13.7

TW= test weight

AC Strongfield had significantly lower Cd and significantly higher protein and Fe compared to Carpio and Joppa (Table 3.8). Carpio and Joppa were not significantly different from each other for these traits. Cadmium was significantly lower in AC Strongfield, followed by Joppa, which was significantly lower than Carpio. AC Strongfield contains the *cdu-1* gene for low Cd accumulation, so it is not surprising that the grain Cd levels were lower than the cultivars that do not contain the *cdu-1* gene. Eckhoff (2010) evaluated the durum wheat cultivars AC Strongfield and Alzada in her studies. Alzada does not contain the *cdu-1* gene and accumulated significantly more grain Cd than AC Strongfield in her

trials. In the current trial, Joppa was not significantly higher in grain Cd than AC Strongfield. Carpio was significantly higher than the other cultivars evaluated and could require additional management due to the potential for this cultivar to accumulate grain Cd above acceptable limits. The lowest grain Zn was found in Joppa which was significantly lower than Carpio or AC Strongfield, but these two cultivars were not significantly different from each other.

Jaio et al. (2004) observed an inverse relationship between grain Zn and Cd in durum wheat and suggested an inverse relationship between Zn and Cd for uptake and translocation sites. These current data did not reflect an inverse relationship between grain Zn and Cd. In this trial, the foliar Zn-EDTA treatment had the lowest grain Cd treatment but did not have the highest grain Zn. However, the inverse relationship between Zn and Cd in AC Strongfield was observed. Seed Zn of Carpio was not significantly different from that of AC Strongfield and its seed Cd was the highest of all cultivars. This indicates that these cultivars can differ in their ability to uptake Cd and Zn and that the *cdu-1* gene might be responsible for the inverse relationship observed in AC Strongfield.

A decrease of up to 50% of grain Cd was observed in HRSW after a soil application of 2.5 to 5.0 kg Zn ha^{-1} in soils that were marginally or severely Zn-deficient in Australia. Oliver at al. (1994) suggested an application of Zn fertilizer to minimize grain Cd when soil test values were below 2 mg kg^{-1} of Zn. In the present work, only the Hettinger 2014 environment would be considered marginally Zn-deficient based on soil test levels and only for crops sensitive to Zn-deficiency. The response of all traits evaluated to Zn fertilizer in the Hettinger 2014 environment was similar to the other environments, indicating that soil Zn was not a limiting production factor. Generally, soils in ND are considered Zn-deficient at levels below 0.5 mg kg^{-1} DTPA-extractable Zn for sensitive crops such as corn, potato, flax and edible beans. Durum wheat is not classified as a Zn sensitive crop in ND (D. Franzen, personal communication).

Li et al. (1994) examined the effect of soil chloride and sulfate on the Cd concentration of sunflower in ND. In their work, the soil pH range was narrow (7.3 to 8.1) and regression analysis showed no significant correlation with seed Cd and soil pH. However, previous work indicated that soil pH was important to controlling Cd with an increasing pH resulting in a decrease in grain Cd (Chaney, 2010). Based on soil sulfate levels, Li et al. (1994) found no statistically significant effect of soil sulfate on grain Cd, but did find that chloride levels were strongly correlated with Cd accumulation in sunflower. Adams et al. (2004) found that total soil Cd and pH were the most significant factors affecting grain Cd concentration in wheat and barley. A close evaluation of soil test results for each environment in the current research did not indicated relationships among grain Cd and soil pH, chloride, or sulfate

All treatments that included the application of KCl placed with seed at planting did result in much higher grain Cd levels than the untreated control in this study (Table 3.7). When KCl was applied with the seed, the resulting increase of Cd in the harvested grain was not reduced by the addition of foliar Zn-EDTA in the combination with KCl. The grain Cd level of the soil-applied KCl + $ZnSO_4$ treatment was 0.123 mg kg^{-1} and was similar the untreated control. The reason for the increase in grain Cd due to the application of KCl is most likely a result of the Cl component. Soil test results for all location showed adequate soil K for all environments and production was not limited be K deficiency. Potassium deficiencies are rare in ND and therefore rarely impact durum wheat production.

Timing Trial

The main objective of the timing trial was to determine at which growth stage an application of Zn-EDTA could reduce grain Cd to the greatest extent. In our study, the application of Zn-EDTA was applied to coincide with other application timings common in durum wheat production in ND in order to eliminate the added cost of application. Furthermore, the additional management timings were applied with and without Zn-EDTA in order to determine if any synergism or antagonism was present. Results for the timing trial are based on the combined analyses. Not all locations had DON and plant height data, so

the results for the combined analyses for these traits will not be discussed. The treatment by cultivar interactions for the combined analyses were not significant for any trait evaluated. Environment was significant for all traits evaluated (p<0.001). Treatments differed significantly for Zn (p<0.001), protein content and Cd (p<0.01), and Fe (p<0.05) (Table 3.9). Cultivar differed significantly for test weight, yield, protein content, Cd, and Fe (p<0.001), and Zn (p<0.05).

Treatment had no significant effect on test weight or yield, indicating that the application of any of these treatments on the cultivars evaluated would impact them similarly. Treatments containing foliar fungicide with and without foliar Zn-EDTA applied at Feekes 4 (tillering) or 10 (boot) growth stages tended to have higher yield than the similar treatments without fungicide. Test weight values for all treatments were lower than the industry standard of 772 kg m^{-3}. Protein was significantly higher for the treatments containing foliar-applied 33 kg ha^{-1} of N in the form of urea-ammonium nitrate (UAN). These values are consistent with previous research indicating that application of UAN at this growth stage can increase protein content approximately five to ten g kg^{-1} (Ransom et al., 2016). The application of foliar-applied Zn-EDTA in combination with UAN did not have an antagonistic effect on protein content.

Table 3.9. Combined harvested grain means for treatments across all cultivars and locations for the timing trial conducted in Crosby and Minot, ND in 2014 and 2015.

Treatment	Feekes	TW[†]	Yield	Protein	Cd	Fe	Zn	Cd	Fe	Zn
		kg m^{-3}	kg ha^{-1}	g kg^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg ha^{-1}	mg ha^{-1}	mg ha^{-1}
Zn-EDTA	4	737	2708	134	0.092	31.9	33.0	261	86058	88381
Zn-EDTA + py	4	736	2795	135	0.090	32.8	32.7	264	92481	90392
Py	4	722	2768	136	0.104	31.9	33.6	304	88689	90680
Zn-EDTA	10	728	2620	135	0.083	33.2	37.9	218	86627	97942
Zn-EDTA + py + teb	10	734	2815	135	0.082	34.4	36.9	233	97904	102543
Py+ teb	10	731	2735	135	0.103	31.7	34.2	291	86893	90466
Zn-EDTA	11.1	728	2735	136	0.110	34.0	38.5	320	93076	105323
Zn-EDTA	10.54	728	2815	136	0.091	33.8	37.0	270	95495	104679
Zn-EDTA + UAN	10.54	731	2762	140	0.085	34.3	39.0	242	92492	107553
UAN	10.54	732	2721	143	0.106	32.3	34.8	304	87621	93374
Untreated control	n/a	728	2681	134	0.101	31.5	32.3	281	83558	84202
LSD (0.05)		NS	NS	4	0.016	2.1	3.0	51	6424	9184
CV		2.6	15.5	5.9	45.9	14.7	18.4	57.3	21.9	23.7

[†] TW = test weight, Py = Pyraclostrobin, Teb = tebuconazole , UAN=Urea-ammonium nitrate

All treatments containing foliar-applied Zn-EDTA had significantly lower grain Cd than corresponding treatments without foliar-applied Zn-EDTA (Table 3.9). In addition, the inclusion of foliar Zn-EDTA in combination with fungicide or fertilizer did not have a synergistic or antagonistic effect on grain Cd levels; No significant differences were detected between these types of treatments compared to the treatment alone. The application of foliar Zn-EDTA made at Feekes 11.1 growth stage (grain ripening) resulted in the highest grain Cd of 0.110 mg g^{-1} and this value was more, but not significant compared to the untreated value of 0.101 mg g^{-1}. The foliar Zn-EDTA application with and without fungicide applied at Feekes 10 growth stage resulted in the lowest grain Cd of any treatments with values of 0.083 and 0.082 mg g^{-1}, respectively. Additionally, the treatment of foliar Zn-EDTA + UAN at Feekes 10.54 growth stage (late flowering) resulted in a similar grain Cd level of 0.085 mg g^{-1} and was not significantly different from Zn-EDTA treatments made at growth stage 4. The result of this study in regards to percent decrease in Cd due to application of foliar Zn-EDTA are similar to those found by Eckhoff (2010). The addition of foliar Zn-EDTA at Feekes 10 growth stage resulted in a significant decrease of approximately 25% in grain Cd. All treatments containing foliar-applied Zn-EDTA did increase grain Zn levels more than the untreated check.

Studies on the ability of durum wheat genotypes to accumulate Cd indicated large variations and suggested a genetic effect (Hart et al., 2006; Harris and Taylor, 2013). Harris and Taylor (2013) found that grain Cd content increased was highly correlated to dry weight accumulation and Cd content of harvested grain accumulated between 14 and 28 days post anthesis. The application of foliar Zn-EDTA during the Feekes 10 and 10.54 growth stages resulted in the lowest harvested grain Cd in the current study (Table 3.9). Application of foliar Zn-EDTA coinciding with the period of rapid grain biomass accumulation resulted in a preferential mobilization of Zn rather than Cd to grain.

The application of Zn in the timing experiment tended to increase the grain Zn levels which was not consistently observed in the source trial. However, in the source trial two different sources of Zn

fertilizer were applied, foliar Zn-EDTA and soil applied $ZnSO_4$ which might be the reason for the difference in response to Zn in harvested grain. However, the foliar Zn-EDTA treatment in source trial resulted in a similar decrease in seed Cd compared to the timing trial at the same growth stage. The most significant increase was at the Feekes 10.54 growth stage. Both treatments containing Zn at this stage had significantly higher grain Zn compared to the UAN treatment alone and an inverse relationship between grain Zn and Cd was observed with these treatments. A similar trend was observed at the Feekes 10 growth stage treatments, but not at the Feekes 4 growth stage treatments. This would suggest that at Feekes 10 and 10.54 growth stages there could be competition between Zn and Cd for uptake and translocation sites within durum wheat. The competition between uptake and translocation may be limited by Feekes 11.1 (grain ripening) growth stage. At this growth stage the grain Cd level of 0.110 mg kg^{-1} was statistically the same as the untreated control of 0.101 mg kg^{-1}. Eckhoff (2010) found a similar inverse relationship between Zn and Cd when foliar Zn was applied at Feekes 10 growth stage, but did not make any additional applications at other growth stages. Conversely, Perilli et al. (2010) did not find a direct correlation between grain Zn and Cd when evaluating the effect of N rate, seeding date, and soil type on Cd concentration of harvest durum wheat and identified the different levels of soil Zn as a possible cause. Other research does suggest an inverse relationship between grain Zn and grain Cd (Chaney, 2010). Treatments containing Zn resulted in higher grain Fe, but all treatments had more Fe than the untreated control. The highest grain Fe also tended to have the lowest grain Cd and highest grain Zn, regardless of treatment type or application timing.

A positive correlation value between yield and Cd indicates that the increased grain production did not necessarily lead to a dilution of Cd (Table 3.10). The foliar Zn-EDTA treatment applied at Feekes 10 growth stage was the lowest yielding treatment and had the lowest grain Cd content and lowest total harvest grain Cd content (Table 3.9). The highest yielding treatment was the foliar Zn-EDTA + pyraclostrobin + tebuconazole also applied at the Feekes 10 growth stage. However, this treatment had

the second lowest grain Cd content. These data support the lack of a dilution effect in regards to grain Cd and yield. Similar results were observed by Grant and Baley (1997) when evaluating N, P, and Zn management on grain yield and Cd in two durum wheat cultivars. Correlation values for the timing trial between Fe and Zn and Fe and Cd were 0.57 and -0.29 (p<0.001), respectively (n=528). The correlation value for Zn and Cd was -0.13 (p<0.01). The correlation values between protein and Zn or Fe were 0.57 and 0.40 (p<0.001), respectively. High correlation values between Zn and Fe with protein have previously been reported by Kutman et al. (2010). A dilution effect for grain yield and Cd, Fe, and Zn was not observed (Table 3.9 and 3.10).

Grain proteins may aid in the accumulation of Zn by increasing the storage capacity of grain for Zn. In durum wheat, recombinant chromosome substitution lines carrying the *Gpc-B1* allele accumulated more protein, Zn, Fe and Mn in harvested grain (Distelfeld et al., 2007). The *Gpc-B1* locus is associated with accelerated senescence and might contribute to the remobilization of nutrients in the plant to grain. Based on these values, the opportunity to increase Zn, Fe, and protein at the same time is possible. Additionally, the inverse relationship of Zn and Fe with Cd is of particular interest in regards to human health. Cadmium absorption by humans from various food sources tends to be reduced when the nutritional status of an individual is low in Zn, Fe, and Ca (Reeves and Chaney, 2008). Similar to durum wheat, rice a monocot species, is a known Cd accumulator. Liu et al. (2003) detected significant differences of the minerals evaluated in both roots and leaves of rice. Based on

Table 3.10. Pooled correlation values for harvested grain traits evaluated for the timing trials Conducted in Crosby and Minot, ND in 2014 and 2015.

	Yield	Protein	Cd	Fe	Zn
Test weight	0.45***	-0.58***	0.23***	-0.02^{NS}	-0.14**
Yield		-0.46***	0.21***	-0.11*	0.00^{NS}
Protein			0.29***	0.40***	0.29***
Cd				-0.29***	-0.13**
Fe					0.57***

*, **, *** is significant at p<0.05, 0.01, and 0.001 level, respectively, NS=not significant
n=528

correlation among minerals, their results indicated that Cd adsorption was cooperative between Fe, Mn, and Cu in rice plants and that the uptake and accumulation of Cd in rice also interacted with Fe, Zn and Cu. Increasing the micronutrient availability for crops which are consumed worldwide such as durum wheat would have multiple advantages.

Significant differences among the cultivars for test weight and yield (p<0.001) were observed and are similar to those observed in the source trial (Table 3.11). Carpio was significantly higher yielding than Joppa across locations which was significantly higher yielding than AC Strongfield. Based on previous long term trial data, Joppa tends to be higher yielding than Carpio, but not always significantly higher (Elias and Manthey, 2016). Also, based on the long term trial data, AC Strongfield tends to have significantly lower yield than Carpio or Joppa as observed in the current trial. The significant differences in protein content (p<0.001) values were similar to those observed in the source trial and are consistent with long term trial data (Elias and Manthey, 2016).

Table 3.11. Combined harvested grain means for cultivars across all treatments and locations for the timing trial conducted in Crosby and Minot, ND in 2014 and 2015.

Treatment	Test	Yield	Protein	Cd	Fe	Zn	Cd	Fe	Zn
	kg	kg ha^{-1}	g kg^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg ha^{-1}	mg ha^{-1}	mg ha^{-1}
Carpio	743	2903	130	0.111	32.9	35.1	336	95974	102183
Joppa	731	2782	131	0.096	31.3	34.5	275	86817	95502
AC Strongfield	719	2547	147	0.078	34.5	36.6	204	87453	90188
LSD (0.05)	4	66	2	0.008	1.1	1.5	27	3355	4769
CV	2.6	15.5	5.9	45.9	14.7	18.4	57.3	21.9	23.7

Significant differences in grain Cd (p<0.001), Fe (p<0.001), and Zn (p<0.05) were observed among cultivars (Table 3.11). The rank of cultivars for Cd, Zn, and Fe were similar to those in the source trial. AC Strongfield had the lowest grain Cd, but was not significantly different than Joppa. Carpio had significantly higher grain Cd than Joppa. Also, AC Strongfield had the highest grain Fe and Zn and both values were significantly higher than those for Carpio or Joppa. Joppa had significantly less grain Fe than Carpio.

Micronutrient decencies of Fe and Zn are a problem worldwide, especially in developing nations. Biofortification of durum wheat would be advantageous based on the large amount consumed by humans (Khoshgoftarmanesh et al., 2010). Durum wheat grain tends to have more protein, Zn, and Fe than bread wheat and a positive, close relationship between these traits has been identified (Cakmak et al., 2010) suggesting that genes controlling these traits are co-segregating. This relationship may aid plant breeders in the selection of these traits simultaneously. Foliar application of Zn and N may not contribute or prevent yield loss due to deficiencies of these elements, but it can increase the grain concentrations of N and Zn (Kutman et al 2010). They found that the foliar-applied Zn and N appear to act synergistically in improving grain Zn when both exist at sufficient levels. High N nutrition might also delay senescence and result in a longer period of time during grain-filling in order to accumulate Zn, Fe, Mn, and Cu and suggests a similar N-dependent mechanism for uptake and translocation of these elements. The current trials were conducted in soils already sufficient in Zn and were fertilized to meet crop N requirements and therefore, should not be considered nutrient deficient.

The application of fertilizer should be used to increase micro and macronutrients in grains when economical. Both soil and foliar applications of Zn fertilizers have improved grain Zn in durum wheat (Cakmak, 2010). In this study, the application of 1.1 kg Zn ha^{-1} in the form of foliar Zn-EDTA + 33 kg N ha^{-1} at Feekes 10.54 growth stage resulted in higher test weight and yield and significantly higher Zn, Fe, and grain protein compared to the untreated. In addition, this treatment resulted lowest grain Cd. The N application could have resulted in not only an increase in grain protein, but also Zn and Fe. Additionally, since Cd and Zn compete with one another in the plant, this also might be the reason for a decrease in seed Cd. Grain Fe concentrations can also be improved with increasing N supply and may share a similar N-dependent mechanism for uptake and translocation (Kutman, 2010). Some sources of Zn fertilizer commonly have Cd and other heavy metal contaminants so the source of these fertilizers should be monitored closely.

Conclusions

There are two major risk categories associated with high Cd durum wheat. First is the potential health risk associated with consuming food high in Cd. The second risk is the impact it can have on the opportunity for producers to market durum wheat internationally. Agronomic practices that minimize Cd accumulation in harvested grain might be an option to reduce these risks. The use of low Cd accumulating durum wheat cultivars is an important management option for ND producers, but the newest cultivars selected and released for ND do not contain the *cdu-1* gene for low Cd accumulation. The interaction of current cultivars with specific management practices could be a way to ensure that even lower Cd levels can be achieved. Agronomic management via the application of foliar Zn-EDTA might be a way for producers to maintain optimal grain seed quality in regards to Cd accumulation in harvested grain.

The application of soil-applied $ZnSO_4$ at planting did little to impact grain Cd or Zn levels in the source trial. Grain Cd was lowest with an application of 1.1 kg Zn ha^{-1} of foliar Zn-EDTA at Feekes 10 growth stage. The application of 9 kg Cl ha^{-1} in the form of KCl fertilizer at planting did increase grain Cd levels above untreated levels and could be a concern for ND producers growing durum wheat. A multivariate analysis found that grain Cd could be predicted by soil pH and soil Cl. Growing durum wheat in saline areas containing high levels of Cl have the potential to increase Cd levels above acceptable limits.

In the timing trial, the application of foliar Zn-EDTA at all growth stages except 11.1 resulted in lower grain Cd compared to the untreated control. Additionally, no antagonism was observed in grain Cd when foliar Zn-EDTA was used in combination with other foliar-applied fertilizer or fungicide applications. No treatment applied on Carpio or Joppa had lower grain Cd than AC Strongfield and suggests that growing durum wheat cultivars containing the *Cdu-1* gene is the best management option for producing grain with low Cd. Carpio tended to have the highest levels of grain Cd compared to Joppa

or AC Strongfield and might need additional management when grown in environments conducive to Cd accumulation such as saline areas. The application of 1.1 kg Zn ha^{-1} of foliar Zn-EDTA in combination with 33 kg N ha^{-1} of UAN at Feekes 10.54 growth stage resulted in the lowest grain Cd, and significantly higher grain Zn, Fe, and protein content compared to the untreated control. This approach represents a way for ND durum wheat producers to bio-fortify grain with desirable micronutrients.

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CHAPTER 4. GENERAL CONCLUSONS

Planting date and cultivar interactions had greatest impact on the agronomic and quality traits evaluated at all environments. The relative ranking of individual cultivars evaluated in this study is similar to previous yield and quality reports generated from the same growing region. In the highest yielding environments, Carpio yield was superior to Divide and Joppa. When planting was delayed, the yield of Joppa tended to decrease more than either Carpio or Divide. These data indicate that even in different environmental conditions, planting durum wheat early is the best option for maximizing yield regardless of cultivar or environment. No specific planting date, cultivar, or environment consistently produced durum that would be graded as HAD or 'choice milling durum'. Characteristics such as protein content, FN, kernel yellow pigment content, and GI were more dependent on cultivar than environment, although some effect of environment was observed.

The application of 12 kg Zn ha^{-1} of $ZnSO_4$ did not impact grain Cd or Zn levels in the fertilizer source trial. Additionally, the application of 9 kg Cl ha^{-1} in the form of KCl resulted in increased levels of grain Cd compared to the untreated control. The application of 1.1 kg Zn ha^{-1} of foliar Zn-EDTA resulted in lowest grain Cd compared to the untreated control and was similar to results observed in the timing trial. Multivariate analysis determined that soil pH and soil Cl were important factors to predict grain Cd.

In the timing trial, all applications of foliar Zn-EDTA can be combined with the application of other fertilizers and fungicides effectively. All application timings of Zn resulted in lower gain Cd than the untreated control except for at Feekes 11.1 growth stage. The application of 1.1 kg Zn ha^{-1} of foliar Zn-EDTA with 33 kg N ha^{-1} of UAN at Feekes 10.54 growth stage resulted in the lowest grain Cd, and significantly higher grain Zn, Fe, and protein content compared to the untreated control.

APPENDIX

Table A1. Mean squares and significance levels for the ANOVA for agronomic traits and pasta quality parameters, Minot, ND, 2014.

Source	df	Plant height	Test weight	Yield	Protein	Pigment	PPO	Kwt	Kpro	FN
Rep	3	499.3***	4.2***	388.3***	6.8***	0.6**	0.003	3.1	3.5***	22240.4***
A [Planting Date]	3	1235.9**	13.2*	1785.7**	2.1	31.9***	0.02***	3.9	0.8	264156.5***
Error (a)	9	138.4***	2.8***	173.7***	0.7***	0.8***	0.001	14.0***	0.5***	7466.7***
B [Cultivar]	2	44.9	3.4**	3.7	5.1***	28.2***	1.7***	75.6***	1.8***	121885.1***
A*B	6	51.8	3.2***	101.4**	0.6***	1.2***	0.02***	11.8***	0.4***	16356.6***
Error (b)	96	33.8	0.6	27.5	0.1	0.1	0.002	1.7	0.07	1148.3
C [Seeding Rate]	2	29.7	10.0***	150.2**	0.6*	0.2	0.0008	1.1	0.4**	114.3
A * C	6	36.5	3.4***	14.9	0.3	0.5**	0.0005	1.1	0.1	796.0
B * C	4	38.0	5.3***	181.8***	0.1	0.3	0.0004	0.7	0.03	1799.5
A * B * C	12	47.2	1.4*	70.3**	0.1	0.1	0.0009	1.6	0.08	586.3

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A1. Mean squares and significance levels for the ANOVA for agronomic traits and pasta quality parameters, Minot, ND, 2014 (continued).

Source	df	Large	Medium	Bottom	Vitreous	Ash	Semolina	Semolina-p	GI	WG	DoughL30
Rep	3	20.2*	17.1	0.2***	8.6	0.09***	171.6***	1.9***	226.5***	18.6***	36.2***
A [Planting Date]	3	38.9	39.6	0.01	551.0***	0.2	235.5**	1.3*	2711.6***	2.2	24.0*
Error (a)	9	63.9***	59.2***	0.1***	26.2***	0.2***	18.4***	0.2***	39.9*	4.1**	4.0
B [Cultivar]	2	1143.9***	1100.2***	0.5***	281.1***	0.004	13.9***	0.3**	2158.2***	88.3***	19.0***
A*B	6	52.0***	50.0***	0.05	38.1***	0.02*	7.7***	0.4***	243.7***	5.0**	4.5
Error (b)	96	7.3	6.9	0.02	3.5	0.007	1.7	0.05	17.1	1.5	2.2
C [Seeding Rate]	2	8.7	7.2	0.1***	7.4	0.03*	3.5	0.3**	23.9	3.8	1.4
A * C	6	5.9	6.0	0.003	5.8	0.001	3.9*	0.04	11.3	1.6	1.3
B * C	4	3.0	3.1	0.002	20.6***	0.004	1.8	0.01	27.3	1.1	2.3
A * B * C	12	4.2	4.3	0.02	9.8**	0.005	1.3	0.03	21.4	1.4	2.6

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A2. Mean squares and significance levels for the ANOVA for pasta quality parameters, Minot, ND, 2014.

Source	df	DoughLa	DoughLb	DoughHL30	DoughHa	DoughHb	DoughCIEL	DoughCIEa	DoughCIEb	DoughHL
Rep	3	1.3***	5.9**	47.3***	1.2***	2.8**	21.7*	3.5***	16.2*	26.2*
A [Planting Date]	3	2.6**	158.6***	31.6*	2.2**	49.1***	54.6	2.4	113.2***	65.6
Error (a)	9	0.2*	4.3**	5.3	0.2*	1.3**	25.9***	1.1**	5.1	30.8***
B [Cultivar]	2	0.9***	44.9***	25.0***	0.8***	16.0***	1.6	0.4	24.1*	2.0
A*B	6	0.5***	3.9*	5.9	0.4***	1.3*	11.8	0.6	7.5	13.9
Error (b)	96	0.1	1.3	2.9	0.1	0.6	5.8	0.3	5.7	6.8
C [Seeding Rate]	2	0.1	1.5	2.0	0.1	0.4	7.7	0.2	5.0	9.3
A * C	6	0.1	1.1	1.7	0.1	0.6	5.3	0.2	3.1	6.2
B * C	4	0.1	1.0	3.0	0.05	0.4	2.1	0.2	2.7	2.4
A * B * C	12	0.1	2.9*	3.4	0.1	1.2*	8.0	0.4	7.7	9.5

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A2. Mean squares and significance levels for the ANOVA for pasta quality parameters, Minot, ND, 2014 (continued).

Source	df	DoughHLHa	DoughHLHb	DoughCIEdiff	DoughCIEdiffa	DoughCIEdiffb	DoughHuntL	DoughHunta	DoughHuntb
Rep	3	2.7***	8.9*	55.4***	1.0**	7.1	72.2***	0.8***	3.7
A [Planting Date]	3	1.8	51.1***	122.8**	2.0*	5.4	153.9**	1.7*	0.8
Error (a)	9	0.8**	3.1	15.0***	0.4*	4.4	17.5***	0.3*	1.5
B [Cultivar]	2	0.3	6.2	27.9**	0.7*	25.3**	36.4***	0.6*	6.4*
A*B	6	0.5	2.9	6.6	0.1	5.2	7.9	0.1	2.0
Error (b)	96	0.2	2.7	4.2	0.2	4.0	5.0	0.13	1.6
C [Seeding Rate]	2	0.1	2.8	2.5	0.1	11.6	2.7	0.1	4.9
A * C	6	0.2	1.0	2.4	0.1	2.5	2.9	0.1	0.7
B * C	4	0.1	1.2	2.7	0.04	3.7	3.5	0.02	1.6
A * B * C	12	0.3	3.9	6.2	0.2	2.9	7.5	0.1	1.5

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A3. Mean squares and significance levels for the ANOVA for agronomic traits and pasta quality parameters, Hettinger, ND, 2014.

Source	df	Plant height	Test weight	Yield	Protein	Pigment	PPO	Kwt	Kpro	FN
Rep	3	72.6**	1.5	53.4	0.9*	0.2	0.0009	0.5	0.7*	1749.1**
A [Planting Date]	3	1427.7***	509.5***	10302.7***	28.5***	15.5***	0.06***	812.9***	19.9***	248493.2***
Error (a)	9	47.1**	0.8	11.1	0.4	0.3***	0.002	1.4	0.3	355.3
B [Cultivar]	2	82.1**	4.0*	423.7***	2.6***	29.5***	2.0***	86.1***	1.4**	84858.5***
A*B	6	20.6	4.6***	18.0	0.5	0.6***	0.06***	14.8***	0.5*	13287.6***
Error (b)	96	14.1	1.0	22.5	0.3	0.07	0.001	2.0	0.2	359.9
C [Seeding Rate]	2	8.0	0.5	60.8	0.6	0.5**	0.0009	4.8	0.4	1847.7**
A * C	6	6.8	0.2	5.6	0.7*	0.2*	0.002	3.8	0.2	1049.8*
B * C	4	3.3	1.1	30.2	0.9*	0.1	0.0009	2.5	0.5*	233.7
A * B * C	12	10.5	1.1	37.7	0.3	0.04	0.001	1.3	0.1	666.4

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A3. Mean squares and significance levels for the ANOVA for agronomic traits and pasta quality parameters, Hettinger, ND, 2014 (continued).

Source	df	Large	Medium	Bottom	Vitreous	Ash	Semolina	Semolina-p	GI	WG	DoughL30
Rep	3	16.4	27.8**	1.8***	24.2	0.07***	7.0*	0.6**	0.3	12.7	0.9
A [Planting Date]	3	4238.4***	3989.5***	6.5***	1988.5***	0.36***	680.5***	7.6***	21.1*	23.6**	17.3*
Error (a)	9	16.2*	13.7*	0.4	60.5	0.02**	2.6	0.2	4.5*	2.7	4.2
B [Cultivar]	2	1715.4***	1607.0***	2.6***	318.0***	0.0008	21.2***	0.9**	113.0***	220.3***	16.4**
A*B	6	197.3***	185.1***	0.3	96.6**	0.007	17.7***	0.3*	11.8***	21.8***	3.6
Error (b)	96	6.2	6.4	0.3	31.1	0.006	2.3	0.1	1.9	4.9	2.7
C [Seeding Rate]	2	72.4***	79.0***	0.3	46.0	0.003	0.2	0.4	1.9	12.2	1.5
A * C	6	16.0*	13.7	0.2	13.3	0.002	0.7	0.2	3.9	3.1	1.6
B * C	4	7.8	7.3	0.3	118.2**	0.008	1.7	0.6**	5.5*	5.4	4.9
A * B * C	12	13.3*	11.7	0.3	36.3	0.01*	0.7	0.1	4.3*	4.3	3.5

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A4. Mean squares and significance levels for the ANOVA for pasta quality parameters, Hettinger, ND, 2014.

Source	df	DoughLa	DoughLb	DoughHL30	DoughHa	DoughHb	DoughCIEL	DoughCIEa	DoughCIEb	DoughHL
Rep	3	0.1	1.6	1.2	0.1	0.5	2.4	0.4	3.2	3.0
A [Planting Date]	3	19.2***	173.4***	23.6*	16.5***	69.3***	71.8**	38.9***	84.7*	88.7**
Error (a)	9	0.3	4.0*	5.6	0.3	1.3*	6.4	0.9**	13.7**	7.7
B [Cultivar]	2	0.1	9.8**	22.3**	0.1	4.3**	32.4**	1.8**	6.5	39.9**
A*B	6	0.3*	9.4***	4.9	0.3*	3.1***	4.6	0.3	3.0	5.5
Error (b)	96	0.1	1.7	3.7	0.1	0.6	4.9	0.3	5.0	5.9
C [Seeding Rate]	2	0.1	11.5**	2.2	0.05	4.0**	5.2	0.3	28.7**	6.1
A * C	6	0.1	2.0	2.2	0.1	1.0	9.1	0.3	7.3	11.1
B * C	4	0.1	1.6	6.7	0.1	0.4	4.1	0.3	4.4	5.0
A * B * C	12	0.1	2.5	4.8	0.1	0.9	5.2	0.3	5.5	6.4

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A4. Mean squares and significance levels for the ANOVA for pasta quality parameters, Hettinger, ND, 2014 (continued).

Source	df	DoughHLHa	DoughHLHb	DoughCIEdiff	DoughCIEdiffa	DoughCIEdiffb	DoughHunterL	DoughHuntera	DoughHunterb
Rep	3	0.4	1.5	0.9	0.2	2.8	1.1	0.1*	1.1
A [Planting Date]	3	30.5***	39.7*	52.7**	3.9***	38.2*	65.2**	2.5**	10.4
Error (a)	9	0.7**	6.0**	6.1	0.3***	8.2	7.7	0.2***	3.1
B [Cultivar]	2	1.4**	0.6	92.0***	1.6***	4.9	117.7***	1.3***	1.7
A*B	6	0.2	0.7	3.8	0.03	3.5	4.8	0.03	1.1
Error (b)	96	0.2	2.2	3.8	0.1	4.5	4.7	0.05	1.6
C [Seeding Rate]	2	0.2	11.5**	6.8	0.1	8.6	8.4	0.1	3.6
A * C	6	0.3	3.9	4.1	0.1	5.2	4.8	0.1	2.5
B * C	4	0.2	2.2	3.2	0.1	4.9	4.0	0.1	1.7
A * B * C	12	0.3	2.5	2.3	0.1	5.2	2.9	0.1	1.8

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A5. Mean squares and significance levels for the ANOVA for agronomic traits and pasta quality parameters, Minot, ND, 2015.

Source	df	Plant height	Test weight	Yield	Protein	Pigment	PPO	Kwt	Kpro	FN
Rep	3	120.6***	1.6	191.9***	25.0***	0.5*	0.0009	28.8***	21.5***	16868.6***
A [Planting Date]	3	871.3***	40.1**	406.9**	11.8	1.2*	0.01	149.8**	11.0	134021.0**
Error (a)	9	45.0**	3.4**	43.3**	4.4***	0.3	0.003	14.4***	3.8***	13615.6***
B [Cultivar]	2	132.2***	1.3	347.5***	11.4***	42.1***	1.4***	38.4***	7.9***	16016.7***
A*B	6	63.4**	4.5***	22.7	2.1*	1.3***	0.02**	5.8	1.3*	1895.0
Error (b)	96	16.6	1.1	15.1	0.8	0.2	0.005	3.3	0.5	1519.3
C [Seeding Rate]	2	33.7	0.9	0.9	0.7	0.2	0.001	13.4*	0.9	2655.3
A * C	6	15.9	0.3	23.2	0.5	0.1	0.003	1.2	0.5	2136.3
B * C	4	49.4*	1.5	42.4*	0.6	0.1	0.001	1.5	0.1	1067.2
A * B * C	12	19.2	2.1*	24.9	0.5	0.2	0.004	5.3	0.5	2400.0

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A5. Mean squares and significance levels for the ANOVA for agronomic traits and pasta quality parameters, Minot, ND, 2015 (continued).

Source	df	Large	Medium	Bottom	Vitreous	Ash	Semolina	Semolina-p	GI	WG	DoughL30
Rep	3	183.5**	174.4**	0.4	6977.9***	0.05***	8.8**	41.4	204.5**	154.2***	5.0***
A [Planting Date]	3	2100.1**	1943.2**	4.7	12891.4***	0.03	62.8**	118.0	465.2*	73.4	0.1
Error (a)	9	208.9***	183.6***	1.5	480.5***	0.01**	5.0*	87.0	80.7*	37.9***	0.8*
B [Cultivar]	2	2913.5***	2448.1***	21.5***	1347.1***	0.004	3.3	67.4	6608.0***	223.4***	1.9**
A*B	6	125.2*	108.4**	2.6	660.4***	0.004	9.7***	97.4	140.8**	15.4	1.3**
Error (b)	96	42.9	34.5	1.4	96.0	0.005	2.2	90.5	38.1	7.2	0.4
C [Seeding Rate]	2	153.2*	151.0*	0.2	117.5	0.01	0.4	83.9	32.9	1.3	0.1
A * C	6	41.9	36.6	1.4	16.6	0.005	3.2	88.2	45.0	8.3	0.6
B * C	4	7.9	8.2	0.2	85.2	0.002	4.0	83.2	32.9	11.8	0.3
A * B * C	12	71.8	63.1	0.8	146.4	0.006	0.9	91.3	39.8	8.6	0.6

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A6. Mean squares and significance levels for the ANOVA for pasta quality parameters, Minot, ND, 2015.

Source	df	DoughLa	DoughLb	DoughHL30	DoughHa	DoughHb	DoughCIEL	DoughCIEa	DoughCIEb	DoughHL
Rep	3	0.6***	2.6**	7.2***	0.6***	0.6	15.8***	1.2***	1.6	20.6***
A [Planting Date]	3	0.1	7.7*	0.1	0.1	3.4*	14.4*	0.7	12.8**	19.0*
Error (a)	9	0.1*	1.8***	1.2*	0.1*	0.7**	2.3**	0.3*	1.0	3.0**
B [Cultivar]	2	1.5***	80.8***	2.7**	1.4***	36.5***	3.0*	4.8***	116.1***	3.9*
A*B	6	0.2***	1.2*	1.9**	0.2***	0.5*	0.7	0.3*	1.6	0.9
Error (b)	96	0.03	0.5	0.5	0.03	0.2	0.8	0.1	0.7	1.1
C [Seeding Rate]	2	0.0001	0.2	0.2	0.0002	0.1	0.9	0.1	0.7	1.2
A * C	6	0.1	1.0	0.9	0.1	0.4	1.1	0.1	0.7	1.5
B * C	4	0.02	0.5	0.5	0.02	0.2	1.4	0.2	0.3	1.8
A * B * C	12	0.03	0.4	0.9	0.03	0.1	0.5	0.2	0.8	0.6

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A6. Mean squares and significance levels for the ANOVA for pasta quality parameters, Minot, ND, 2015 (continued).

Source	df	DoughHLHa	DoughHLHb	DoughCIEdiff	DoughCIEdiffa	DoughCIEdiffb	DoughHunterL	DoughHuntera	DoughHunterb
Rep	3	1.1***	0.05	7.4***	0.2	5.3**	9.5***	0.1	2.5***
A [Planting Date]	3	0.6	4.9***	49.9**	1.9*	23.4***	64.4**	1.7*	15.1***
Error (a)	9	0.3**	0.3	6.2***	0.4**	1.0	8.1***	0.4***	0.7
B [Cultivar]	2	4.0***	37.0***	18.4***	4.2***	113.9***	23.9***	3.6***	33.3***
A*B	6	0.3*	0.6*	2.0	0.4*	4.5***	2.6	0.3*	1.4**
Error (b)	96	0.1	0.2	1.2	0.1	1.0	1.6	0.1	0.4
C [Seeding Rate]	2	0.1	0.4	0.7	0.1	0.9	0.9	0.05	0.5
A * C	6	0.1	0.3	1.1	0.1	1.5	1.4	0.1	0.6
B * C	4	0.1	0.1	1.3	0.2	0.6	1.7	0.1	0.1
A * B * C	12	0.1	0.2	1.1	0.2	1.1	1.4	0.1	0.4

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A7. Mean squares and significance levels for the ANOVA for agronomic traits and pasta quality parameters, Hettinger, ND, 2015.

Source	df	Plant height	Test weight	Yield	Protein	Pigment	PPO	Kwt	Kpro	FN
Rep	3	52.4***	2.1***	327.6***	0.9*	0.5***	0.002	48.9***	1.0***	19540.8***
A [Planting Date]	3	978.4***	158.4***	8281.7***	41.9***	11.9***	0.003*	237.3***	27.1***	21727.2***
Error (a)	9	16.6**	1.1***	37.9	0.3	0.1	0.0008	5.7	0.3**	1418.3
B [Cultivar]	2	47.9***	0.4	221.1***	8.1***	80.7***	1.1***	43.8**	8.8***	2817.1*
A*B	6	79.5***	5.4***	113.1***	0.2	0.4***	0.007***	4.6	0.3*	3982.1***
Error (b)	96	6.1	0.3	22.9	0.3	0.1	0.001	7.3	0.09	809.7
C [Seeding Rate]	2	1.0	0.2	42.0	0.2	0.2	0.002	30.9*	0.1	374.3
A * C	6	10.7	0.3	27.0	0.4	0.1	0.0007	5.9	0.2	829.7
B * C	4	0.7	0.3	22.2	0.05	0.05	0.001	4.1	0.02	752.6
A * B * C	12	6.9	0.6*	21.0	0.3	0.06	0.0003	8.0	0.2	310.8

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A7. Mean squares and significance levels for the ANOVA for agronomic traits and pasta quality parameters, Hettinger, ND, 2015 (continued).

Source	df	Large	Medium	Bottom	Vitreous	Ash	Semolina	Semolina-p	GI	WG	DoughL30
Rep	3	186.0***	165.3***	5.6*	2368.2***	0.03***	36.2	0.6***	488.4***	19.5*	0.8
A [Planting Date]	3	3834.2***	3210.1***	27.4***	1169.5*	0.2***	1.8	25.1***	2233.6**	362.1***	8.2**
Error (a)	9	92.9**	78.4**	1.1	237.2***	0.009*	19.6	0.2*	169.6***	7.9	0.9**
B [Cultivar]	2	6171.5***	5300.0***	33.3***	634.9***	0.03***	11.3	5.5***	8833.4***	373.2***	2.3***
A*B	6	95.5**	72.7**	4.5**	362.4***	0.006	8.8	0.3**	333.4***	9.4	0.4
Error (b)	94	31.5	24.2	1.5	44.1	0.004	15.8	0.08	24.6	4.9	0.3
C [Seeding Rate]	2	194.5**	150.6**	2.8	97.4	0.003	19.0	0.04	0.1	12.0	0.6
A * C	6	42.8	29.0	1.8	22.2	0.003	12.5	0.1	18.7	8.6	0.3
B * C	4	44.9	37.0	1.0	51.4	0.0006	14.2	0.01	7.6	14.6*	0.2
A * B * C	12	33.1	25.3	1.5	55.1	0.004	15.6	0.1	17.7	7.4	0.2

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A8. Mean squares and significance levels for the ANOVA for pasta quality parameters, Hettinger, ND, 2015.

Source	df	DoughLa	DoughLb	DoughHL30	DoughHa	DoughHb	DoughCIEL	DoughCIEa	DoughCIEb	DoughHL
Rep	3	0.07	1.0*	1.1*	0.07	0.6*	2.9***	0.7***	3.7***	3.8***
A [Planting Date]	3	0.8**	22.3***	11.7**	0.7**	10.2***	40.5***	7.3***	56.7***	52.4***
Error (a)	9	0.07*	0.7	1.2**	0.1*	0.3	0.9**	0.1	0.5	1.2**
B [Cultivar]	2	3.3***	152.3***	3.3***	3.0***	67.9***	13.8***	10.5***	241.9***	17.9***
A*B	6	0.1**	3.3***	0.6	0.1*	1.5***	0.4	0.4***	4.1***	0.5
Error (b)	94	0.04	0.3	0.4	0.03	0.2	0.3	0.06	0.5	0.4
C [Seeding Rate]	2	0.04	1.2*	0.8	0.04	0.7*	0.3	0.06	2.0*	0.3
A * C	6	0.02	0.5	0.5	0.02	0.2	0.2	0.03	0.6	0.2
B * C	4	0.01	0.4	0.3	0.01	0.1	0.4	0.05	0.1	0.5
A * B * C	12	0.03	0.3	0.2	0.03	0.1	0.5	0.04	0.2	0.6

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A8. Mean squares and significance levels for the ANOVA for pasta quality parameters, Hettinger, ND, 2015 (continued).

Source	df	DoughHLHa	DoughHLHb	DoughCIEdiff	DoughCIEdiffa	DoughCIEdiffb	DoughHunterL	DoughHuntera	DoughHunterb
Rep	3	0.6***	0.9***	2.3	0.9***	9.0***	3.0	0.7***	2.1**
A [Planting Date]	3	6.3***	16.5***	67.6***	14.5***	41.6***	86.2***	12.1***	15.6***
Error (a)	9	0.09	0.2	1.2	0.2	0.7	1.5	0.1	0.3
B [Cultivar]	2	8.7***	79.6***	17.4***	11.7***	252.5***	22.3***	9.5***	79.3***
A*B	6	0.3***	1.2***	1.8	0.4**	4.3***	2.2	0.3**	1.5**
Error (b)	94	0.05	0.2	1.4	0.1	0.85	1.8	0.08	0.4
C [Seeding Rate]	2	0.04	0.4	0.4	0.03	0.9	0.5	0.02	0.2
A * C	6	0.02	0.2	1.4	0.06	1.6	1.8	0.05	0.7
B * C	4	0.04	0.1	0.8	0.1	0.8	1.0	0.09	0.4
A * B * C	12	0.03	0.1	0.9	0.05	0.7	1.2	0.04	0.4

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A9. Mean squares and significance levels for the ANOVA for agronomic traits and pasta quality parameters, for Hettinger and Minot, ND, 2014 and 2015.

Source	df	Plant height	Test weight	Yield	Protein	Pigment	PPO	Kwt	Kpro	FN
Experiment	3	1603.1***	21.8***	38477.3***	288.7***	119.6***	0.05***	915.2***	202.3***	1501941.8***
Rep (Experiment)	12	186.2***	2.5***	240.3***	8.4***	0.5***	0.001	20.3***	6.7***	15099.7***
A [Planting Date]	3	1671.8	373.8	13857.5*	46.8	11.6	0.01	498.2	9.7	153200.6
A * Experiment	9	947.2***	115.8***	2306.5***	12.5***	16.3***	0.03***	233.6***	16.4***	171773.5***
Error (a)	36	61.8***	2.0***	66.5***	1.4***	0.4***	0.002	8.9***	1.2***	5714.0***
B [Cultivar]	2	40.2	6.3	488.3	23.9**	152.9***	6.2***	176.2*	15.8**	129755.4
B * Experiment	6	88.9***	0.9*	169.2***	1.1**	9.4**	0.02***	21.9***	1.4***	31620.7***
Error (b)	384	17.6	0.8	22.0	0.4	0.1	0.002	3.6	0.2	958.6
C [Seeding Rate]	2	2.0	1.3	68.9	0.3	0.2	0.001	34.7*	0.1	146.0
C * Experiment	6	23.5	3.4***	61.7*	0.6	0.3*	0.001	5.3	0.6*	1615.9
A * B	6	43.2	6.8	72.3	0.5	1.1	0.01	4.5	0.9	2570.5
A * B * Experiment	18	57.4***	3.7***	61.0***	1.0	0.8***	0.03***	10.8***	0.5**	10925.8***
A * C	6	5.7	1.4	2.8	0.3	0.1	0.002	2.0	0.2	1607.3
A * C * Experiment	18	21.4	0.9	22.7	0.5	0.2*	0.001	3.3	0.3	1058.7
B * C	4	18.2	0.7	11.2	0.3	0.2	0.002	2.2	0.1	2535.8**
B * C * Experiment	12	24.4	2.4***	88.5***	0.4	0.1	0.001	2.2	0.2	435.2
A * B * C	12	14.0	1.0	27.6	0.3	0.1	0.001	3.8	0.2	710.1
A * B * C * Experiment	36	23.3	1.4**	42.1**	0.3	0.1	0.002	4.1	0.2	1085.8

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A9. Mean squares and significance levels for the ANOVA for agronomic traits and pasta quality parameters, for Hettinger and Minot, ND, 2014 and 2015 (continued).

Source	df	Large	Medium	Bottom	Vitreous	Ash	Semolina	Semolina-p	GI	WG	DoughL30
Experiment	3	19184.9***	15209.1***	235.1***	75556.1***	0.6***	3054.5***	220.7***	7385.3***	3271.8***	2146.8***
Rep (Experiment)	12	101.6***	96.1***	2.0**	2344.7***	0.06***	55.9***	11.1	229.9***	51.3***	10.7***
A [Planting Date]	3	3709.2	3357.3	8.7	1951.4	0.04	117.7	57.9	2533.5	132.0	5.4
A * Experiment	9	2164.4***	1938.3***	10.0***	4904.8***	0.3***	287.4***	31.4	972.1***	111.6***	14.7***
Error (a)	36	95.5***	83.7***	0.8	201.1***	0.06***	11.4***	21.9	73.7***	13.1***	2.5**
B [Cultivar]	2	9775.7**	8615.8**	36.9*	1885.2*	0.01	6.6	9.5	12239.7*	692.9*	10.1
B * Experiment	6	730.1***	618.1***	7.1***	231.8***	0.009	14.3*	21.6	1878.2***	72.0***	9.8***
Error (b)	381	21.9	17.9	0.8	43.5	0.005	5.5	22.7	20.4	4.6	1.4
C [Seeding Rate]	2	291.1*	272.1*	0.4	66.9	0.0004	1.9	21.2	16.8	6.6	0.7
C * Experiment	6	46.6*	39.1*	1.0	67.1	0.01*	7.1	21.1	14.0	7.6	1.0
A * B	6	154.5	125.9	3.0	184.9	0.01	15.3	22.2	282.5	23.8	1.0
A * B * Experiment	18	105.9	97.3***	1.5*	323.6***	0.008	9.5*	25.3	149.4***	9.3**	3.0**
A * C	6	13.2	10.2	0.3	24.1	0.001	2.2	21.6	31.6	5.3	0.4
A * C * Experiment	18	31.1	24.9	1.0	11.3	0.003	6.0	22.2	15.8	5.5	1.2
B * C	4	27.3	22.9	0.5	127.8	0.004	10.4	22.3	12.7	2.7	2.2
B * C * Experiment	12	12.2	11.0	0.3	49.0	0.003	3.8	20.5	20.2	10.1*	1.9
A * B * C	12	45.3	36.7	1.0	54.3	0.006	4.7	23.8	35.4*	6.3	1.8
A * B * C * Experiment	36	25.9	22.7	0.6	63.9	0.007	4.6	22.6	15.9	5.2	1.7

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A10. Mean squares and significance levels for the ANOVA for pasta quality parameters, for Hettinger and Minot, ND, 2014 and 2015.

Source	df	DoughLa	DoughLb	DoughHL30	DoughHa	DoughHb	DoughCIEL	DoughCIEa	DoughCIEb	DoughHL
Experiment	3	91.4***	125.0***	2944.9***	85.5***	40.4***	1378.1***	329.5***	3549.6***	1729.7***
Rep (Experiment)	12	0.5***	2.8***	14.2***	0.5***	1.1**	10.7***	1.5***	6.2*	13.4***
A [Planting Date]	3	4.7	10.8	7.4	4.1	3.7	14.0	7.63	34.2	17.5
A * Experiment	9	6.0***	117.1***	19.9***	5.2***	42.8***	55.8***	13.9***	77.8***	69.5***
Error (a)	36	0.2***	2.7***	3.3**	0.2***	1.0***	8.9***	0.6***	5.1**	10.7***
B [Cultivar]	2	4.4*	241.1**	13.2	3.9*	100.4**	16.2	10.1	239.0	20.3
B * Experiment	6	0.5***	16.0***	13.3***	0.4***	8.3***	11.5***	2.5***	50.6***	14.4***
Error (b)	381	0.08	1.0	1.9	0.07	0.4	3.0	0.2	3.0	3.6
C [Seeding Rate]	2	0.1	6.4	0.9	0.1	2.4	0.7	0.3	11.5	0.9
C * Experiment	6	0.03	2.6*	1.4	0.02	0.9*	4.5	0.1	8.2*	5.4
A * B	6	0.3	5.4	1.4	0.2	2.0	3.4	0.5	6.0	4.0
A * B * Experiment	18	0.3***	4.0***	4.0	0.3***	1.5***	4.7	0.4*	3.3	5.6
A * C	6	0.05	0.5	0.5	0.04	0.2	2.7	0.2	4.9	3.3
A * C * Experiment	18	0.09	1.4	1.6	0.08	0.7*	4.3	0.1	2.2	5.2
B * C	4	0.01	0.5	2.9	0.01	0.1	0.7	0.1	1.7	0.9
B * C * Experiment	12	0.06	1.0	2.5	0.06	0.3	2.4	0.2	2.0	3.0
A * B * C	12	0.08	1.7	2.4	0.06	0.6	4.5	0.2	2.7	5.4
A * B * C * Experiment	36	0.07	1.4*	2.3	0.06	0.6*	3.2	0.3	3.8	3.8

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A10. Mean squares and significance levels for the ANOVA for pasta quality parameters, for Hettinger and Minot, ND, 2014 and 2015 (continued).

Source	df	DoughHLHa	DoughHLHb	DoughCIEdiff	DoughCIEdiffa	DoughCIEdiffb	DoughHunterL	DoughHuntera	DoughHunterb
Experiment	3	269.5***	1546.6***	203794.9***	44.6***	55157.9***	164036.2***	36.4***	24442.2***
Rep (Experiment)	12	1.2***	2.8*	16.5***	0.6***	6.1**	21.4***	0.5***	2.3**
A [Planting Date]	3	6.2	10.2	112.5	8.2	29.9	140.9	6.9	20.3
A * Experiment	9	11.0***	34.0***	60.3***	4.8***	26.4***	76.4***	3.7***	7.3***
Error (a)	36	0.5***	2.4**	7.1***	0.3***	3.6	8.7***	0.3***	1.4
B [Cultivar]	2	8.3	73.1	16.0	8.5	254.1*	20.3	7.0	83.3*
B * Experiment	6	2.1***	17.0***	46.3***	3.2***	48.4***	59.6***	2.6	12.7***
Error (b)	381	0.2	1.3	2.7	0.1	2.6	3.3	0.09	1.0
C [Seeding Rate]	2	0.2	3.7	0.7	0.01	0.2	0.9	0.006	0.1
C * Experiment	6	0.1	3.8*	3.2	0.09	7.3*	3.9	0.07	3.0**
A * B	6	0.4	1.8	3.3	0.09	1.5	4.1	0.08	0.7
A * B * Experiment	18	0.3*	1.2	3.6	0.3**	5.3**	4.5	0.2	1.7*
A * C	6	0.2	2.1	2.4	0.1	1.6	2.9	0.08	0.8
A * C * Experiment	18	0.1	1.1	2.2	0.08	3.0	2.6	0.06	1.2
B * C	4	0.1	0.7	2.3	0.02	1.9	3.1	0.02	0.6
B * C * Experiment	12	0.1	1.0	1.9	0.1	2.7	2.4	0.09	1.1
A * B * C	12	0.1	1.5	3.6	0.1	2.1	4.4	0.1	0.9
A * B * C * Experiment	36	0.2	1.7	2.3	0.1	2.6	2.9	0.1	1.0

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A11. Mean squares and significance levels for the ANOVA for agronomic traits of the timing trial conducted near Crosby, ND in 2014.

ource	df	Height	TW	Yield	Protein	DON	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Rep	3	249.4***	0.04	338.6***	3.7***	6.4	74751***	7.0×10^{9}***	$4.7\ 10^{9}$**	0.01***	886***	715***
A [Treatment]	10	17.7	0.62	30.1	3.3***	2.0	12860	4.3×10^{8}	1.2×10^{9}	0.002	28	128
B [Cultivar]	2	235.5***	77.6***	1017.9***	85.5***	90.4***	69310**	8.4×10^{9}***	8.2×10^{9}***	0.002	8	70
A*B	20	10.5	1.0	30.7	0.5	2.2	8997	7.0×10^{8}*	1.2×10^{9}	0.0009	53	92
Error	96	17.6	0.6	21.1	0.5	2.4	12347	4.1×10^{8}	8.7×10^{8}	0.001	38	88

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A12. Mean squares and significance levels for the ANOVA for agronomic traits of the timing trial conducted near Minot, ND in 2014.

Source	df	Height	TW	Yield	Protein	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Rep	3	1123.7***	1.9	3846	1.0***	9.5×10^{5}***	3.6×10^{10}***	3.1×10^{10}***	0.04***	558.7***	223.1***
A [Treatment]	10	12.2	1.2	49	0.1	7.0×10^{4}	1.3×10^{9}*	3.4×10^{9}***	0.006	63.3*	306.1***
B [Cultivar]	2	97.2**	49.8***	598***	14.4***	2.1×10^{5}	4.2×10^{9}***	6.7×10^{9}***	0.009	133.5**	79.7**
A*B	20	7.5	1.8	15	0.1	5.4×10^{4}	4.5×10^{8}	5.0×10^{8}	0.005	25.4	31.4
Error	96	15.5	1.2	32	0.1	7.7×10^{4}	5.5×10^{8}	3.4×10^{8}	0.005	25.6	18.9

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A13. Mean squares and significance levels for the ANOVA for agronomic traits of the timing trial conducted near Crosby, ND in 2015.

Source	df	TW	Yield	Protein	DON	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Rep	3	14.9***	512.4***	19.6***	21.3***	74419***	3.0×10^{9}***	2.9×10^{9}***	0.01***	60**	59
A [Treatment]	10	1.3	25.6	3.4**	2.0	2258	3.2×10^{8}	5.2×10^{8}	0.004	78***	54
B [Cultivar]	2	51.1***	558.1***	55.5***	38.0***	44008***	6.1×10^{8}**	1.2×10^{9}	0.005***	207***	796***
A*B	20	1.9	20.1	2.5*	0.9	1040	1.4×10^{8}	5.2×10^{8}	0.00002	13	60
Error	96	1.4	31.9	1.2	1.3	1779	1.9×10^{8}	4.6×10^{8}	0.00002	15	44

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A14. Mean squares and significance levels for the ANOVA for agronomic traits of the timing trial conducted near Minot, ND in 2015.

Source	df	Height	TW	Yield	Protein	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Rep	3	5.2	17.0*	242*	1.9	100838***	5.4×10^{8}	1.4×10^{9}**	0.01**	129***	66*
A	10	27.6	6.0	46	1.3	28050**	3.5×10^{8}	7.2×10^{8}*	0.002	32*	42*
B [Cultivar]	2	185.1***	33.9**	30	24.5***	835499***	4.4×10^{9}***	8.9×10^{8}	0.7***	407***	47
A*B	20	15.3	2.7	35	1.0	8790	2.4×10^{8}	1.6×10^{8}	0.0007	15	17
Error	96	14.6	5.4	75	0.7	9856	4.0×10^{8}	3.3×10^{8}	0.0009	14	19

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A15. Mean squares and significance levels for the ANOVA for agronomic traits of the timing trials conducted near Crosby and Minot, ND in 2014 and 2015.

Source	df	TW	Yield	Protein	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Experiment	3	199.2***	15075***	118.4***	1759653***	6.9×10^{8}***	7.9×10^{10}***	0.08***	2416***	5943***
Rep (Experiment)	12	8.4***	1235***	6.6***	299240***	1.2×10^{10}***	9.9×10^{9}***	0.02***	409***	266***
A [Treatment]	10	3.2	35	3.5**	47386*	9.1×10^{10}*	2.9×10^{9}*	0.005**	57*	290***
A * Experiment	30	2.0	40	1.5***	22777	5.3×10^{8}	1.0×10^{9}**	0.002	48**	78**
B [Cultivar]	2	127.6***	1214***	150.6***	728441***	4.2×10^{9}***	5.9×10^{9}	0.05***	446***	206*
B * Experiment	6	28.8***	344***	9.9***	142670***	4.7×10^{9}***	3.9×10^{9}***	0.01***	104***	263***
A * B	20	1.9	36	0.8	21312	3.3×10^{8}	6.7×10^{8}	0.002	25	43
A * B * Experiment	60	1.8	21	1.1**	18818	4.0×10^{8}	5.7×10^{8}	0.002	27	53
Error	384	2.1	40	0.6	23549	3.8×10^{8}	5.1×10^{8}	0.04	23	43

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A16. Mean squares and significance levels for the ANOVA for the source trial conducted near Crosby, ND in 2014.

Source	df	Height	TW	Yield	Protein	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Rep	3	176**	3.1*	152.7**	0.7	30830***	7.9×10^{8}*	6.3×10^{8}	0.003***	109*	30
A [Cultivar]	5	296***	1.7	249**	2.2***	66797***	2.3×10^{9}*	2.1×10^{9}**	0.004***	58	73
Error (a)	15	21	0.8	35	0.3	7107	5.8×10^{8}*	4.3×10^{8}	0.00009	37	27
B [Treatment]	2	267**	51.6***	962***	65.2**	183050***	3.5×10^{8}	1.8×10^{9}*	0.007*	542***	318***
A * B	10	24	0.6	15	0.8*	5316	3.2×10^{8}	1.9×10^{8}	0.0005	32	19
Error (b)	36	32	0.7	29	0.4	4107	2.3×10^{8}	4.2×10^{8}	0.0004	27	33

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A17. Mean squares and significance levels for the ANOVA for the source trial conducted near Hettinger, ND in 2014.

Source	df	Height	TW	Yield	Protein	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Rep	3	52**	1.5***	172***	1.0***	20435***	2.1×10^{9}**	7.8×10^{8}	0.0007*	30	0.04
A [Cultivar]	5	22	0.9**	150***	1.2	21954**	1.5×10^{9}	$7.9 \times$	0.0006*	20	167***
Error (a)	15	17	0.1	16	0.5**	4682*	6.0×10^{8}	2.4×10^{8}	0.00001	21	7
B [Treatment]	2	532***	3.4***	59**	17.8***	589560***	$7.8 \times$	1.1×10^{8}	0.02***	372***	27**
A * B	10	17	0.2	2	0.1	1639	3.0×10^{8}	6.0×10^{8}	0.00005	10	11
Error (b)	36	11	0.2	10	0.2	2344	3.7×10^{8}	4.3×10^{8}	0.00004	11	4

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A18. Mean squares and significance levels for the ANOVA for the source trial conducted near Minot, ND in 2014.

Source	df	Height	TW	Yield	Protein	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Rep	3	143***	0.3	124*	0.2	10991	1.4×10^{8}	8.8×10^{8}**	0.0002	47	19
A [Cultivar]	5	277*	1.7	896***	0.2	36065***	6.8×10^{9}***	4.8×10^{9}***	0.0006	52**	241***
Error (a)	15	85***	2.4	58	0.5	2475	7.1×10^{8}	2.8×10^{8}	0.00003	9	7
B [Treatment]	2	202***	3.6	337***	0.03	36812*	2.6×10^{9}*	1.5×10^{9}***	0.001	66	4
A * B	10	25	1.1	12	0.4	6938	3.9×10^{8}	9.5×10^{7}	0.0009	48	11
Error (b)	36	20	2.0	37	0.6	6526	6.2×10^{8}	1.6×10^{8}	0.0009	31	9

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A19. Mean squares and significance levels for the ANOVA for the source trial conducted near Crosby, ND in 2015.

Source	df	TW	Yield	Protein	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Rep	3	2.4	103	4.5***	3713	1.0 x	1.8 x 107	0.002***	54*	146**
A [Cultivar]	5	3.2	367***	14.4***	83666***	3.3 x	1.2 x 109**	0.01***	54	33
Error (a)	15	1.6	23	1.2***	5584**	3.2 x 108*	2.4 x	0.001***	35*	27***
B	2	35.7***	248***	50.4***	84558***	1.0 x 108	2.8 x 108*	0.01***	483**	122**
A * B	10	1.1	35	0.7*	4884**	2.5 x 108	1.1 x 108	0.0008	16	6
Error (b)	36	1.2	13*	0.3	1694	1.5 x 108	6.1 x 107	0.0002	14	6

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A20. Mean squares and significance levels for the ANOVA for the source trial conducted near Hettinger, ND in 2015.

Source	df	Height	TW	Yield	Protein	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Rep	3	3.4	0.4	1	0.2	17665	4.3×10^8	4.4×10^8	0.0008	26**	29**
A [Cultivar]	5	13.3*	2.1**	21	5.8***	121039***	1.3×10^8	4.6×10^{8}*	0.006**	4	44**
Error (a)	15	3.8	0.4	15	0.2	15824	2.2×10^8	1.4×10^8	0.001	13*	9
B [Treatment]	2	29.1***	4.9***	900***	34.8***	1266955***	1.5×10^{9}***	6.6×10^{8}*	0.08***	234***	39**
A * B	10	3.8	0.5	90***	0.4	22366*	5.1×10^{8}***	3.6×10^{8}*	0.0006	3	3
Error (b)	36	2.3	0.3	15	0.2	9355	1.7×10^8	1.3×10^8	0.0006	6	6

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A21. Mean squares and significance levels for the ANOVA for the source trial conducted near Minot, ND in 2015.

Source	df	Test Wt.	Yield	Protein	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Rep	3	21**	212***	6.2**	29015	1.1×10^{9}	1.9×10^{9}**	0.003	51	75***
A [Cultivar]	5	7	534	4.6*	443087*	5.7×10^{9}	7.6×10^{9}**	0.02*	57*	131***
Error (a)	15	9*	386***	1.3	152307**	2.4×10^{9}***	1.3×10^{9}***	0.004*	15	12
B [Treatment]	2	2	60	0.4	33935	3.6×10^{8}	2.3×10^{8}	0.002	1	2
A * B	10	3	22	1.7	73740	5.2×10^{8}	1.0×10^{8}	0.004	26	4
Error (b)	36	4	29	1.4	52542	5.2×10^{8}	2.4×10^{8}	0.002	24	7

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.

Table A22. Mean squares and significance levels for the ANOVA for the source trials conducted near Hettinger, ND 2014 and Crosby, Hettinger, and Minot, ND in 2015.

Source	df	Test Wt.	Yield	Protein	Total Cd	Total Fe	Total Zn	Cd	Fe	Zn
Experiment	3	309***	34922***	9***	4295713***	2.0×10^{11}***	8.5×10^{10}***	0.07***	1388***	881***
Rep (Experiment)	12	6***	122***	3***	17707	1.1×10^{9}***	6.3×10^{8}**	0.001*	40**	62***
A [Treatment]	5	5	379	15*	356925*	4.6×10^{9}	7.1×10^{9}	0.02	61	122
A * Experiment	15	3*	233***	4***	104410***	1.9×10^{9}***	3.3×10^{9}***	0.006***	24	87***
Error (a)	60	3**	110***	1*	44599***	8.9×10^{8}***	4.9×10^{8}***	0.002***	21*	14***
B [Cultivar]	2	20	620	75*	1253753*	4.8×10^{9}	1.0×10^{8}	0.07*	816*	134*
B * Experiment	6	8***	204***	8***	232712***	1.6×10^{9}***	4.0×10^{8}	0.01***	92***	19**
Error (b)	140	2	17	1	16269	3.0×10^{8}	2.1×10^{8}	0.0007	14	6
A * B	10	1	41	1	38501	5.3×10^{8}	3.2×10^{8}	0.002	15	7
A * B * Experiment	30	1	36**	1	21393	3.4×10^{8}	2.9×10^{7}	0.001	13	6

*** Significant at the 0.001 probability level.

** Significant at the 0.01 probability level.

* Significant at the 0.05 probability level.