WHEAT DOCKAGE CONTENT: ANALYSIS OF DOCKAGE AND ITS RELATION TO FUSARIUM HEAD BLIGHT

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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

MASTER OF SCIENCE

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ABSTRACT

Hard red spring wheat crop grown in different locations in the US were surveyed for the mycotoxin deoxynivalenol (DON). DON is often found in wheat that is infected with the plant fungal disease Fusarium head blight. The contamination of wheat by DON is a major wheat industry concern since it affects human and livestock health. Furthermore, DON reduces wheat grain yield and quality. In this study, DON was measured using gas chromatography with electron capture detection (GC-ECD) in 1353 HRS wheat samples collected from 2013-2015. Results indicate that there was positive significant correlation ($P<0.001$) between DON content and damaged kernels (0.635) and total defects (0.445). However, for the three-year average, DON content had a weak positive significant correlation ($P<0.001$) with the percentage of wheat dockage (0.111). Overall, DON production had an effect on kernel damage and total defects, but DON production was not impacted by the percentage of wheat dockage.

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LIST OF ABBREVATIONS

ACS..American chemical society

DHV...Dark hard and vitreous

DNS..Dark northern spring

DON...Deoxynivalenol

FDA..Federal Drug Administration

FDK..Fusarium damaged kernels

FGIS...Federal Grain Inspection Service

FHB..Fusarium head blight

FN ....Falling number

GC-ECD...Gas chromatography – electron capture detector

HPLC ....High performance liquid chromatography

HRS..Hard red spring

lb/bu ....Pounds per bushel

NIR...Near infra-red

NS ....Northern spring

ppm ....Parts per million

RS...Red spring

SEM ....Scanning electron microscopy

TKW ....Thousand kernel weight

USDA...United States Department of Agriculture

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INTRODUCTION

Fusarium Head Blight (FHB), also known scab, is a disastrous fungal plant disease that has been effecting quality and safety of many small grains in North America and the rest of the world. There have been several epidemic outbreaks of FHB occurring in the US since 1996 and FHB has been classified as a “re-emerging disease” for the period between 1991 and1996 (McMullen et al. 2012). Rick Ward (quoted by Leonard and Bushnell, 2003) said, “Fusarium head blight is one of the most intractable diseases that agricultural scientists have ever encountered.” FHB affects small grains negatively by causing significant yield loss, reducing grain quality and producing mycotoxins (Leonard and Bushnell 2003). In many regions, there are a limited number of suitable wheat varieties that have any effectual resistance to FHB infection (Gilbert and Haber 2013).

Many studies have identified that deoxynivalenol (DON) is the most detected *Fusarium* mycotoxin with FHB. The contamination of small grains by DON cause safety, quality and economic problems. DON is also called vomitoxin because of its harmful effects on human and livestock health. For example, human consuming wheat products contaminated with DON may experience symptoms such as vomiting, headaches, fever and nausea. In addition, DON may have toxic effects on digestive system of swine and other monogastric animals (Schmale and Bergstrom 2003). Also, FHB can cause significant yield loss and reduce wheat quality. The size, shape and color of wheat are usually affected by DON development, which results in having what is called “tombstones” or “damaged kernels”. Damaged kernels can lower the quality of wheat end-products in many ways. In addition to quality problems of FHB, this disease can cause serious economic problems. For example, in the US since 1990, wheat and barley farmers have cost above three billion dollars as a result of FHB outbreaks (Schmale and Bergstrom 2003).

There are many factors influencing FHB development and DON accumulation in small grains cereals from an agricultural perspective. The understanding of these factors may benefit in developing strategies that can help in reducing FHB development and DON accumulation in grains before harvest. As a result, the harmful impact of DON on human and animal health might be reduced, and quality and economic issues could be avoided. Wegulo (2012) listed several factors that have shown correlation with DON accumulation. Wegulo (2012) reported that FHB intensity is the main cause that affects DON accumulation and this cause can be impacted by many environmental factors such as temperature, moisture, and relative humidity (RH). The climate change can significantly impact these factors through cases such as extreme drought or rainfall (Wegulo 2012).

It is important to highlight that several factors can affect FHB development before harvest, including farming conditions. Wheat dockage is a component in wheat fields that should be considered in assessing causes of FHB. The objective of this research was to find if wheat dockage is related to FHB. We analyzed DON content in HRS wheat for 2013-2015 Crop Surveys using GC-ECD to find if there is correlation between DON production and wheat dockage. Wheat quality parameters were measured according to Federal Grain Inspection Service (FGIS) to evaluate correlations between DON production and the quality parameters of HRS wheat.

OVERALL GOAL AND OBJECTIVES

Overall Goal

The overall goal of this research was to determine DON content using GC-ECD to find correlations between DON content and the percentage of wheat dockage. The correlations between wheat quality parameters grouped by classification and state have also been investigated.

Hypotheses

- The increase in the percentage of wheat dockage will increase DON levels.
- By analyzing DON levels for different survey crop years, DON levels will vary according to the year.
- By analyzing DON levels for growing state, DON levels will vary according to the growing state.
- The increase in DON levels will decrease wheat kernel quality parameters.

Specific Objectives

- To determine whether wheat dockage had an effect in DON content in wheat samples.
- To determine the relationship between DON level and wheat kernel quality parameters.

LITERATURE REVIEW

Fusarium Head Blight (FHB)

FHB is caused mainly by the fungus *Fusarium graminearum,* also known as *Gibberella zeae*. The fungus is able to lay dormant in the residues of small grains and corn. Growth and sporulation of the fungus on the crop residue will occur during the growing season if there are extended periods of wet weather (McMullen et al. 2012). Also, FHB infection will increase greatly in fields with higher amounts of crop residue, especially if the residue is left on the surface. Burying infected crop residues can reduce infection, however the *F. graminearum* pathogen can survive for several years (Leplat et al. 2013). Infection takes place during the flowering and early kernel development stages of the plant growth. Rain and elevated humidity are favorable conditions for FHB development (Delwiche and Hareland 2004). The main symptom of FHB appears in the plant head before maturity as bleaching of some of the florets. Moreover, bleaching of the entire spike or head may occur during severe infections (Burrows et al. 2012). Deoxynivalenol (DON) is considered one of the main mycotoxins associated with FHB. This mycotoxin affects human and livestock health and it is subject to regulatory limits by the U. S. Food and Drug Administration (FDA) (Burrows et al. 2012).

Deoxynivalenol (DON)

DON is one of the most common mycotoxins (Figure 1) found in grains and their subsequent products. The first description of DON was established in 1972 after Japanese men consumed moldy barley (Sobrova et al. 2010). Studies have shown that DON levels vary during different crop years and are associated with kernel quality in wheat. The growing region also was found to have significant effect on DON content in wheat (Simsek et al. 2013). However, wheat cultivar and the level of susceptibility to FHB infection may have a larger effect than the growing year or location on DON levels in wheat samples (Ovando-Martínez et al. 2013).

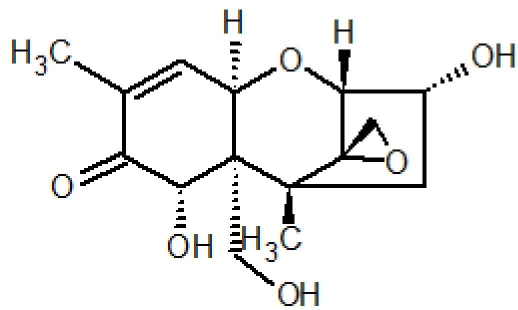

Figure 1. Structure of deoxynivalenol (DON)

This type B trichothecene (DON) is considered a food safety risk because many studies have shown that DON was heat-stable (Simsek et al. 2012; Sobrova et al. 2010). Sobrova (2010), wrote that when heating between 170°C to 350°C, there were no reduction of DON concentration after 30 min at 170°C. Moreover, DON is stable during frying DON-contaminated food in oil and there was no observed reduction of DON concentration. In contrast, DON is water-soluble and research showed reduction in DON levels as a result of cooking in water during boiling of pasta (Manthey et al. 2004; Sobrova et al. 2010). Some studies on DON in baked products have shown an increase in DON content during fermentation and after baking bread (Simsek et al. 2012). Simsek et al. (2012) also determined that treatment with enzymes (xylanase, protease, cellulase) increased DON levels in whole wheat meal samples. This may be due to the presence of bound forms of DON (Simsek et al. 2012).

Impact of FHB on Wheat Kernel Quality

FHB contamination has a negative impact on grain quality. Many studies have shown that FHB affects grain kernels negatively which may lead to low quality end-use products and health problems (Delwiche et al. 2005). Kernels affected by FHB can be easily distinguishing through visible symptoms (Figure 2).

Figure 2. Samples of healthy wheat kernels (a) and fusarium infected wheat kernels containing tombstone kernels (b)
*Blue circle indicates tombstone kernels

FHB affects the quality of wheat from farm to fork. The diseased seeds with F. *graminearum* will have poor growth which leads to slow emergence and they may have high chances of being affected by seedling blight disease. Infected seedlings look reddish-brown and they lack vigor. In addition, they will tiller poorly during wheat planting, yield loses occur in different percentages according to the susceptibility of the cultivars. In the susceptible cultivars the damage may reach 50 to 80% of developing spikelet. In addition, during harvesting the affected shrunken and lightweight kernels are not retained by combine fans and sieves (Jones and Mirocha 1999).

FHB impacts wheat physical characteristics with a visual symptom that can be clearly identified. Fusarium infected kernels are called *Fusarium*-damaged kernels (FDK), scabby or tombstone. These terms usually describe reduced kernel size with low densities and white, pink, chalky or pale gray color (Jones and Mirocha 1999). A study of scanning electron microscopy

(SEM) images of FHB infected wheat kernels showed that the FHB fungal growth caused significant damaged to the wheat kernels, starch granules and protein matrix in the kernel endosperm. The SEM images of the infected wheat kernels also show visible hyphae growth between starch granules and through the endosperm and bran layers (Jackowiak et al. 2005).

The infection by FHB affects wheat kernel quality by producing the mycotoxin DON, as well as producing enzymes that negatively affect the whole wheat and the end-use product quality (Simsek et al. 2012; Wang et al. 2005b). Research has shown that DON concentration has a positive relationship with the percentage of the infected plants with F. *graminearum*. Love and Seitz (1987), studied the correlation between DON and the concentration and the percentage of infected wheat at 12 locations in two years. The researchers found strong correlations between DON and the percentage of infected wheat for both years (r=0.97 and 0.76) (Love and Seitz 1987).

DON and Public Health

Even though FHB is a major food safety and quality issue, we are fortunate that DON has been the most associated trichothecenes identified with FHB. Other trichothecenes such as nivalenol, HT-2 toxin and T-2 toxin are 10 to 20 times more toxic than DON (Leonard and Bushnell, 2003). Mycotoxin and DON toxicity may be acute or chronic depending on level and length of exposure. DON and other mycotoxins are so prevalent in global agriculture that they are present at some level in most people’s diets (De Ruyck et al. 2015). In human, DON does not create a significant risk to public health. However, there are some reported cases such as short-term nausea and vomiting. Also, in other cases there were reported diarrhea, abdominal pain, headache, dizziness and fever (Sobrova et al. 2010). Fusarium mycotoxins are the most common and damaging toxins to animal health and productivity. DON and other Fusarium mycotoxins can result in acute effects such as chronic diseases and death. More commonly the ingestion of low levels of DON over longer periods of time will cause metabolic, immunological and physiologic diseases (Escrivá et al. 2015).

DON is one of the most well-known and highly regulated mycotoxins The FDA has advisory levels for consuming DON for human and livestock. DON in all finished wheat products (flour, bran and germ) should be limited to 1 ppm. For animals, of 5-10 ppm has been established. Determination of DON is a wheat quality testing method that can be used to determine the quality of wheat. (Wu et al. 2014).

The largest concern regarding DON contamination is in regards to the perceived risks of DON contamination in important staple food products. There have been animal trials showing that DON poisoning results in emesis in acute dosages, while lower doses case anorexia, immunotoxicity and retards growth (Pestka 2010). Other conditions such as alimentary toxic aleukia, acute gastroenteritis, growth impairment and Kashin-Beck disease have been associated with consumption of DON contaminated foods in human populations around the world (Wu et al. 2014). Although vomiting is a common symptom of DON poisoning, the level required to induce vomiting in humans is not known (Wu et al. 2014). For the most part DON levels in food are monitored closely and several surveys have been done to determine the occurance of mycotoxins in human food supplies (Cirillo et al. 2003; Omurtag and Beyoğlu 2007; Pralatnet et al. 2016; Tanaka et al. 1990; Tran and Smith 2013).

Economic Impact of FHB

Recently, FHB has become a global agricultural issue as the disease that has an impact on worldwide economics. FHB has been ranked by the USDA as the second most devastating plant disease in US agricultural history after the stem rust epidemics of the 1950s (Windels, 2000).

During the 1990s outbreak of FHB, the estimated loss of wheat and barley were over $3 billion. At that time, about 500 million bushels of wheat which valued about $ 2.5 billion were lost (Windels, 2000). FHB has not only affected the US agriculture, but also Asia, Canada, Europe and South America have been affected by recent outbreaks of FHB (Windels, 2000). For instance, during the 1990s outbreak, wheat producers in Canada encountered economic losses of about 220 million in US dollars, and from 1993 to 1998 the losses were estimated at about S300 million.

FHB pathogens pose two main threats to wheat quality and safety which affect the economic values of infected small grains. First, the grain quality and yield may show a significant reduction as a result of the “tombstone” kernels. Second, scabby grain that contain FHB mycotoxins are usually unsuitable for food and feed (Mc Mullen, et al 1998). As a result of FHB outbreaks in the US, $10 million were invested in public and private funding in new FHB research and education in order to better understand the disease aspects. Moreover, FHB has influenced changings in agricultural policies. (Windels, 2000).

Wheat Kernel Quality Testing Methods

Wheat is the one of the most important grains for millions of people’s diet. In wheat industry, many aspects around wheat quality can be determined through qualitative and quantitative tests. These tests are usually performed in order to have measurements that can help in predicting functionality and quality of whole grain, flour, dough, and end-use products (Dowell et al. 2006). In the US, there are standards that have been established by FGIS to determine wheat kernel quality using several factors. Some of these are grading factors that affect the numerical grades U.S. No. 1 through U.S. No. 5 and some are not.

Hard and Vitreous

One of the important grade-determining factors in assessing wheat kernel quality is vitreousness. The vitreousness of wheat kernels can be determined manually by examination of the appearance of the cut surface of kernels endosperm. The ability of reflecting the light is a factor that helps to classify vitreous kernels and nonvitreous kernels. The endosperm of vitreous kernels appears glasslike and translucent while nonvitreous kernels lack translucency and are light-colored (Baasandorj et al. 2016).

Shrunken and Broken Kernels

Shrunken and broken kernels is a grade-determining used to determine wheat quality. Shrunken and broken kernels may affect the quality of wheat by lowering the flour yield since they have many endosperm which was not filled completely during endosperm development (Gaines et al. 1998). Also, shrunken and broken kernels may be highly correlated with DON consecrations as a result of the infection by FHB. Shotwell et al. (1985) found significant ($P<0.05$) correlation between the level of shrunken and broken kernels and DON levels in wheat.

Damaged Kernels

USDA uses damaged kernels as a grade-determining factor that assesses many types of damaged kernels such as diseased kernels, heat damaged, mold-damaged and sprout-damaged. Damaged kernels affect the appearance of the flour negatively by decreasing yield increasing ash and decreasing sanitary quality (Regnier 2004). Damaged kernels have been associated with FHB infection and DON content in many previous researches. A strong significant ($P<0.05$) correlation has been found between the level of damaged kernels and DON content in wheat (Shotwell et al. 1985). Since tombstone kernels or FDK would be considered damaged kernels, there would be a logical connection between the amount of damage kernels and the level of DON in wheat.

Contrasting Class

Wheat types have different end-uses products. Different types of wheat have "Contrasting Classes" which means there is a contrast in use. For example, soft red winter wheat flour is suitable for cake mixes while flour from durum wheat is suitable spaghetti making. As a result, the contrasting class of soft red winter wheat is any durum wheat (Evans et al. 1997). Habernicht et al. (2002) tested the milling and baking quality of contaminated hard red spring wheat and hard white spring wheat contaminated with different types of wheat such as soft white spring wheat, durum wheat, hard wheat and hull-less barley. Habernicht et al. (2002) found that the low levels of the contamination of hard red spring wheat and white spring wheat with soft white wheat and hull-less barley impacted the end used quality of the flour yield negatively; on the other hand, higher levels of contamination impacted loaf volume.

Moisture

The determination of moisture content is an important step in assessing wheat and flour quality since other tests depend on moisture content. The common percentage of moisture is 14% and moisture content is a sign of the grain storability. Lower moisture content indicates more grain stability during storage. A high moisture content (higher than 14.5%) may attract insects, bacteria and mold (Shelton and Martin 2008).

Protein

Protein content is a key factor in wheat quality that has been associated with some wheat processing properties such as water absorption and gluten strength. The texture and appearance of finished-products may be associated to protein content. Different levels of protein content are desired to different wheat products. For instance, cakes and snacks which are considered tender or crisp wheat products may require low protein content, while pan bread and heart bread which are considered chewy texture products may require high protein content (Shelton and Martin 2008). Portion content might be affected by FHB infection which lead to lower end-use products. Nightingale et al. (1999), found that proteolytic enzymes that can be found in FDK are able to digest storage protein in durum and bread wheat which resulted in decreased loaf volume and weaker dough.

Falling Number

Falling number is an old wheat quality testing technique that has been developed by Hagberg, (1960) and Hagberg, (1961) and Perten (1964) to determine α-amylase activity in grain (Mares and Mrva 2008). Low falling number indicates grain with high α-amylase activity which may lead to several quality problems in processing, storage and end use quality; as a result, large economic losses may occur in grain marketing (Shelton and Martin 2008). Product quality can be affected by level of enzyme activity. Some products may need some level of enzyme activity such as yeast in bread dough which requires sugars to progress well. When there is a high amount of enzyme activity, that means that too much sugar and too little starch are present. Because starch offers the supporting structure of bread, too much activity leads to a sticky dough during processing and poor texture in the finished products. In measuring falling number, enzymes can be added to the flour to compensate when the falling number too high. However, enzymes cannot be removed from flour or wheat if the falling number is too low which leads to serious quality problems that makes the flour unusable (Edwards et al. 1989; Shelton and Martin 2008).

Foreign Material

Foreign material is a grade-determining factor that can be determined manually in wheat samples after the removal of dockage and shrunken and broken kernels. In the process of determining foreign material, all material other than wheat should be removed (Shelton and Martin, 2008). There are many types of foreign materials that can be found in wheat samples such as weed seeds and stones. Foreign material may have the same size and weight of wheat, which result in difficulties in removing them. As result, foreign material may stay in the wheat and affect price of wheat and the overall “grade (BASF, 2003).

Test Weight

Test weight is important quality grading factors of wheat since it is related to the degree of soundness of wheat. There are several factors may affect test weight such as such as whether condition, moisture content, kernel size and density. The presence of DON may affect wheat test weight (Dexter and Edwards 1998). A significant ($P<0.05$) negative correlation has been found between test weight and DON content in wheat (Shotwell et al. 1985).

Thousand Kernel Weight (TKW)

Thousand-kernel weight (TKW) is a method that is used by wheat breeders and flour millers to measure the mass of the wheat kernel. It is used as a match to test weight to better describe wheat kernel composition and potential flour extraction. In general, wheat with a higher TKW is likely to have a better possible flour extraction (Shelton and Martin, 2008).

Wheat Dockage

In grain, dockage is a matter that can be easily removed from grain kernels by using official cleaning equipment to classify wheat to the highest grade that it qualifies (Paliwal et al. 2003). The remain materials, non-grain, in the cleaned grain samples are considered foreign material. The amount of foreign materials is important since there are specific acceptable levels of foreign materials in grain marketing (Paliwal et al. 2003). Wheat dockage usually contain unwanted materials such as wheat heads, chaff, weed seeds and broken wheat kernels (Figure 3).

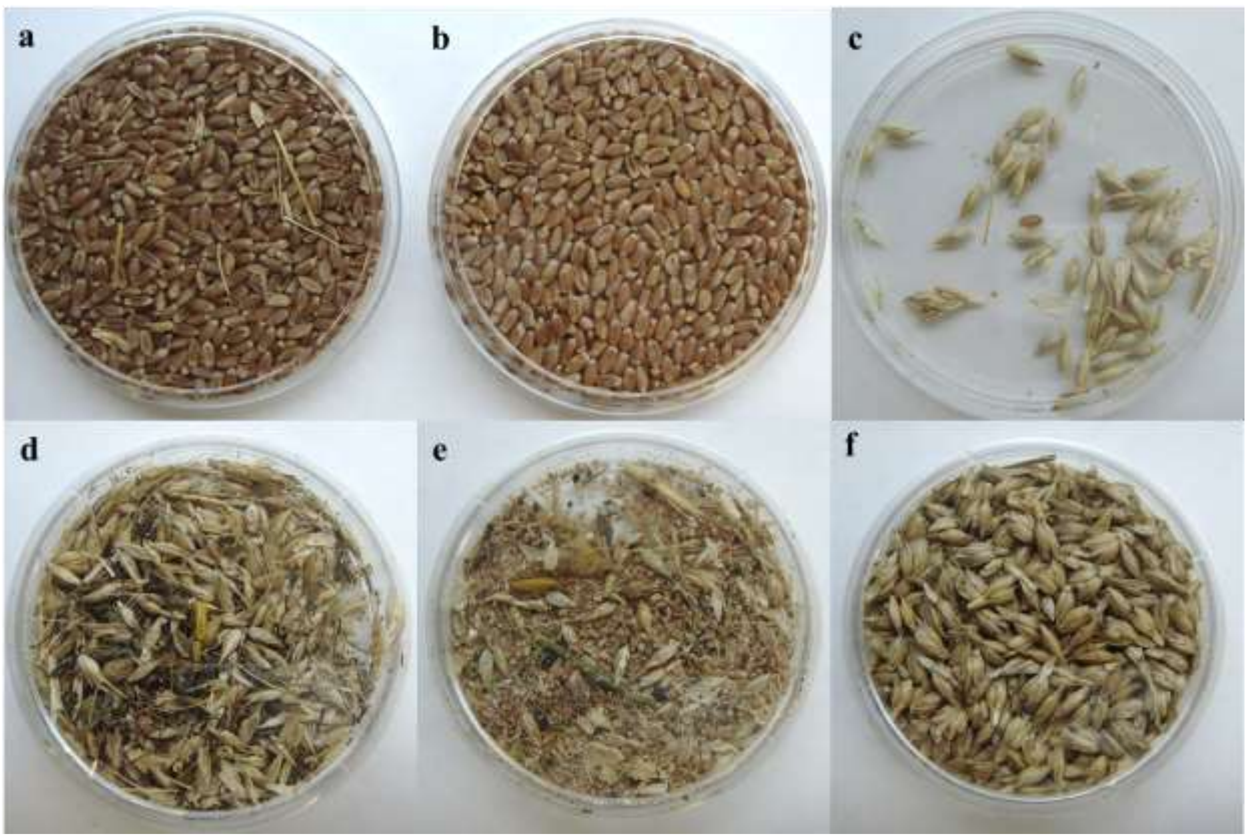

Figure 3. Images of different types of dockage commonly found in wheat samples
*a = uncleaned wheat sample, b = cleaned wheat sample, c = wheat chaff, d = dockage containing chaff, bug parts and weed seeds, e = dockage containing chaff, broken wheat kernels and weed seeds, f = unthreshed wheat

In wheat, cleaning dockage is a non-grading factor that does not impact the numerical grade; however, cleaning dockage is a main step at the beginning of the grading procedure to remove all the materials that is not wheat in order to move to the grading and non-grading factors classification (Simsek et al. 2013). There are several advantages of identifying dockage components. For instance, the identification of dockage will benefit in deciding appropriate automated cleaning when it is necessary (Paliwal et al. 2003). Also, the analyzing of the dockage components may help in improving weed control. In addition, for the contamination of grain by insect, the understanding of the influence of dockage in grain should lead to better insects control measures (Mcgregor 1964).

Measuring Dockage

Dockage can be measured by a screening device called Carter Day Dockage Tester (Figure 4) that is used to separate many sizes of cereal kernels from unwanted material such as chaff, weed seeds, and other foreign materials.

Figure 4. Carter-Day dockage tester

When performing the dockage determination, material that passes over the riddle should be checked for threshed or unthreshed kernels and sprouted kernels of wheat (Figure 5).

Threshed and sprouted kernels that pass over the riddle are not considered dockage. All such kernels should be returned to the dockage-free sample. Threshed kernels of wheat are kernels with either no glumes attached or not more than one glume attached (GIPSA 2006).

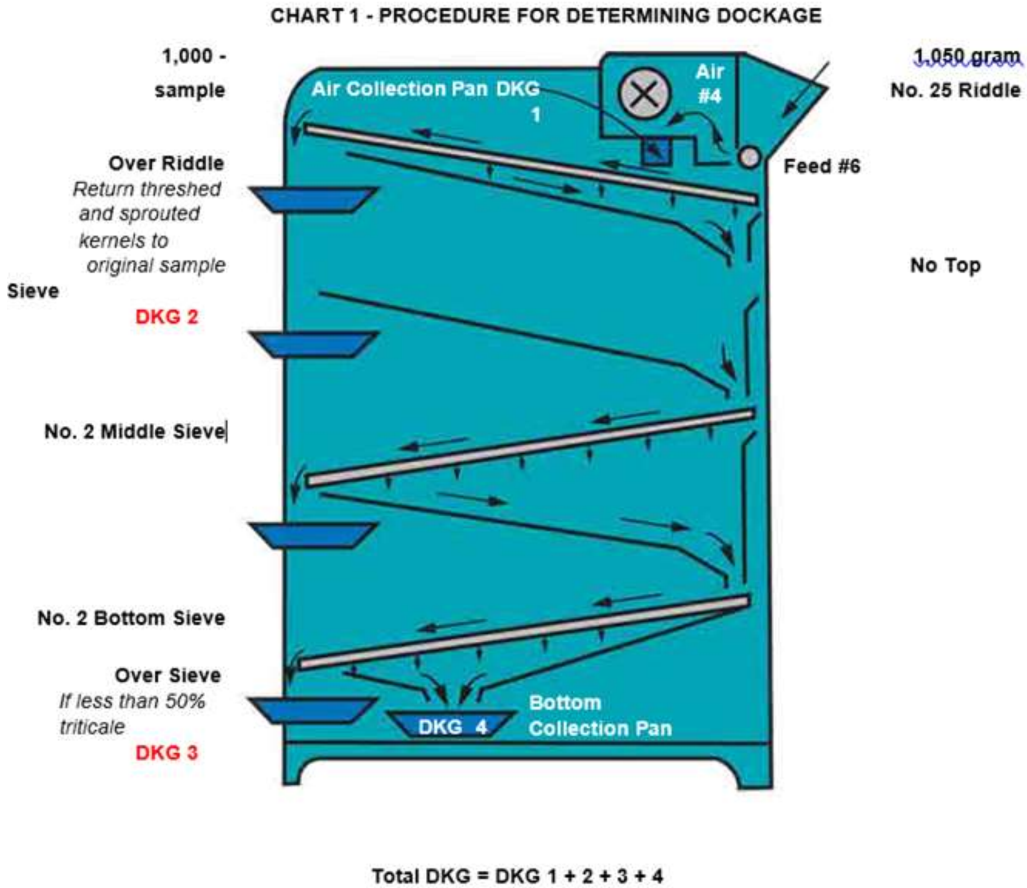

Figure 5. Schematic of dockage testing procedure using a dockage tester (GIPSA 2006)

MATERIALS AND METHODS

Materials

The Crop Survey samples included hard red spring (HRS) wheat between 2013-2015 that used as raw material. A total of 443, 460, 450 samples were selected as wheat grader samples from the 2013, 2014 and 2015 HRS wheat crop surveys, respectively and used in this study. The samples were collected from 16 regions in the four states that grow HRS wheat in the US which are Minnesota (MT), Montana, North Dakota (ND) and South Dakota (SD). Wheat dockage levels were determined in 436 HRS wheat samples obtained from the Department of Plant Sciences at North Dakota State University. Chemicals for determination of DON were purchased from Sigma-Aldrich (St. Louis, MO, USA). These chemicals were at of least the ACS grade. Water and acetonitrile for extraction of DON were HPLC grade and were from EMD Millipore (Billerica, MA, USA).

Methods

DON

DON was determined according to the method of Tacke and Casper (1995). The DON was extracted from 1 g of ground wheat using 8 ml of acetonitrile: water (84:16). The extraction was done by shaking the sample on an orbital shaker for one hour at 150 revolutions per minute RPM . After extraction the liquid extract (4 ml) was passed through a clean-up column (Extract clean C18-L, GRACE, IL, USA) and then 2 ml of the filtrate was evaporated under nitrogen at 55°C until dry. The samples were then derivitized wtih TMSI-TMCS (100ul, 100:1). After the addition of the internal standard solution (1 ml, 0.5mg/L Mirex in isooctane), water (1ml) was added. The tubes were shaken for 10 minutes and then allowed to separate. The top layer (isooctane) was removed and transferred to vials for analysis by gas chromatography with electron capture detection (GC-ECD). The samples were analyzed with an Agilent (Santa Clara, CA, USA) 6890 GC with cool on-column inlet, HP-5 column (30m, 0.25 mm and 0.25 um) and ECD detector. Helium was used as the carrier gas and argon-methane was used as the makeup gas (Simsek et al. 2013; Tacke and Casper 1995).

Kernel Vitreousness

The percentage of dark hard and vitreous (DHV) kernels was determined by manual inspection of 15 grams of wheat. The wheat sample was free from shrunken and broken kernels. Some minor defects such as bleached, cracked or checked were considered vitreous.

Shrunken and Broken Kernels

The percent of shrunken and broken kernels was determined by placing 250 grams of wheat on a 0.064 × 3/8 inch (1.626 mm × 9.545 mm) oblong-hole sieve. The sieve with wheat was shaken mechanically 30 times from side to side. The machine used to sieve the sample, a Strand Sizer, had a stroke counter and started and stopped in the same position. One complete stroke took approximately 1 second.

Damaged Kernels

Portions of the wheat sample were visually inspected-50 grams for heat damage and 15 grams for other damage types-to determine whether any kernels have been materially discolored or damaged by physical or biological factors.

Contrasting Classes

Contrasting classes is a grade-determining factor that is assessed on a 15-gram portion after the removal of dockage and shrunken and broken kernels.

Contrasting classes are:

1. durum wheat, soft white wheat, and unclassed wheat in classes of hard red spring wheat and hard red winter wheat.

2. hard red spring wheat, hard red winter wheat, hard white wheat, soft white wheat, soft red winter wheat, and unclassed wheat in the class durum wheat.
3. durum wheat and unclassed wheat in the class soft red winter wheat.
4. durum wheat, hard red spring wheat, hard red winter wheat, soft red winter wheat, and unclassed wheat in the classes hard white wheat and soft white wheat.

Moisture

Moisture was measured with the Grain Analysis Computer Model 2100 (GAC 2100), (Dickey-John Corporation, Auburn, IL, USA), which is the official moisture meter for the national inspection and weighing system. The GAC 2100 was calibrated to the USDA air-oven method (1 hour at 130° C).

Protein

The protein content was measured by near infrared (NIR) using a FOSS (Eden Prairie, MN, USA) Infratec 1241.

Falling Number

The falling number was determined using a Perten (Hägersten, Sweden) FN 1900 falling number apparatus with tube shaker (AACCI approved method 65-80.03). Ground wheat (7 g) was combined with distilled (25 ml) water in glass viscometer tube and shaken to form a slurry. The stirrer was added to the viscometer tube before placing the tube in the boiling water bath (100°C). The viscometer tube was used to mix the sample in the boiling water bath for 60 seconds before being dropped. The falling number was recorded in seconds as the time for the stirrer to fall a measured distance after the initiation of stirring (AACC-I 2009).

Foreign Material

Foreign material was determined on a 50-gram portion after the removal of dockage and shrunken and broken kernels. All material other than wheat was removed.

Total Defects

Total defects were considered to be the sum of damaged kernels, foreign material, and shrunken and broken kernels.

Test Weight

In measuring test weight per bushel, wheat was required to fill a level Winchester bushel measure 2,150.42 cubic-inch (35.24-liter) capacity. The determination of test weight per bushel was made for 1,350 grams of wheat by using approved tools which has a kettle capacity of one dry quart (1.101 liter). The sample was poured into the closed hopper placed over the kettle. The valve was rapidly opened to let the wheat to fill the kettle. The excess wheat was removed by a standard stroker with flat sides that was hold in both hands in a vertical position. The kettle is carefully placed on the scale stage. The weight was read by an electronic scale that changes the gram weight to either pounds per bushel or kilograms per hectoliter.

Thousand Kernel Weight

The wheat sample (500 g) was prepared by removing all dockage, shrunken and broken kernels and other foreign material. The sample was then divided on a mechanical divider. A 50 g sample was used for testing. A mechanical seed counter was used to count ten grams of wheat and the number of kernels in ten grams was converted to thousand kernel weight.

Dockage

The amount of dockage in the wheat samples was determined using a Carter-Day dockage tester (Minneapolis, MN, USA). The air control was set to 4 and the feed control was set to 6. The number two plastic riddle for HRS was placed in the riddle carriage and number two sieves were inserted into the middle and bottom sieve carriages. The sample was poured into the feed hopper after starting the dockage tester. The dockage was considered to be the aspirated material collected in the air collection pan, material over the riddle (except for threshed and sprouted kernels) and material in the bottom collection pan. Material passing over the bottom sieve, if containing less than 50% wheat kernels, was also considered dockage. If 50% or more wheat kernels passed over the bottom sieve, the material was returned to the cleaned wheat.

Data Analysis

The mean, median, mode, minimum and maximum values were calculated using Excel. The correlation coefficients were also calculated with Excel. The degrees of freedom (df) for 2013, 2014, 2015 and 2013-2015 were 441, 431, 428 and 1304, respectively. Tableau Public software v. 9.2 was used to prepare figures depicting the relationships between DON content and wheat quality parameters grouped by classification and state.

RESULTS AND DISCUSSION

Wheat Sample Collection

The HRS wheat crop survey of was conducted from regions that grow the most of HRS wheat in the United States (US) which includes North Dakota (ND), South Dakota (SD), Montana (MT) and Minnesota (MN) (Figure 6). In the US, the FGIS standardizes the assessment of the condition and quality of wheat through established grading and non-grading factors.

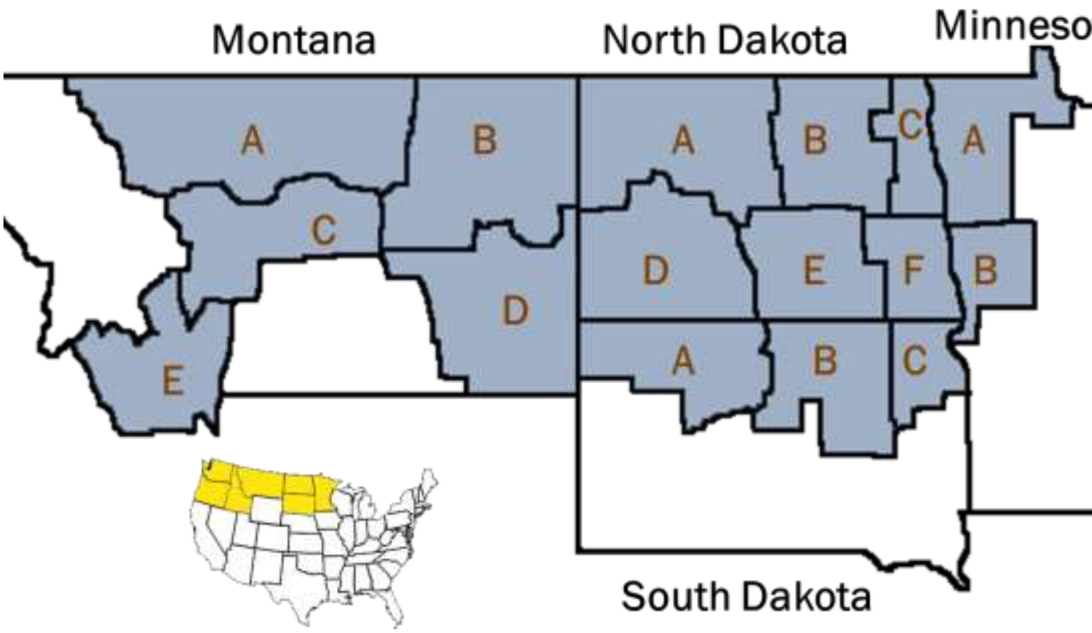

Figure 6. Four-state crop reporting areas for the hard red spring wheat crop survey sample collection

The area where the HRS wheat samples were collected represents a wide range of growing conditions. Weather patterns, soil conditions, types of common weeds, disease pressures and other factors vary widely across the four state growing region. Typically, the eastern (ND B, C, E and F; SD B and C; MN A and B) half of the growing region tends to have more moisture while the western (ND A and D; SD A; MT A, B, C, D and E) part of the growing region is dry and can be more prone to drought. The growing conditions may affect the amount and types of dockage present, as well as the level of *F. graminearum* infection and DON content.

Wheat Dockage Content

Cleaning wheat from dockage is an important step in order to move to the grading wheat procedure and processing wheat. Cleaning dockage procedure results in removing all non-wheat materials that may affect the quality and the safety of milling and processing wheat. Unwanted materials usually come from wheat fields. Wheat dockage usually contain chaff, shrunken and broken kernels and weed seeds; also, other gain such as corn and soybean might be found in the wheat dockage and they perhaps come from grain elevators. In general, wheat dockage components are lighter than grains and have higher moisture content which may increase the susceptibility of spoilage as these lighter particles accumulate on the storage structure (Ravikanth et al. 2015).

In addition, the presence of insects might be a grain safety issue that is related to the percentage of dockage. Mcgregor (1964) found that the percentage of wheat dockage is related positively to the presence of insects, particularly *Tribolium castaneum* (red flour beetle). The importance of identifying the dockage components in grain sample could help in developing right cleaning methods in assigning grain grade (Wang and Paliwal 2006). Figure 7 shows the percentage of HRS wheat crop survey samples containing various types of dockage.

It can be seen that 42.8% of the samples did not have any type of weed or other seeds; they just contained chaff or other types of dockage. About 29.7% of the wheat samples contained ergot. Green foxtail and yellow foxtail were the most prevalent types of weed seeds found in the wheat samples.

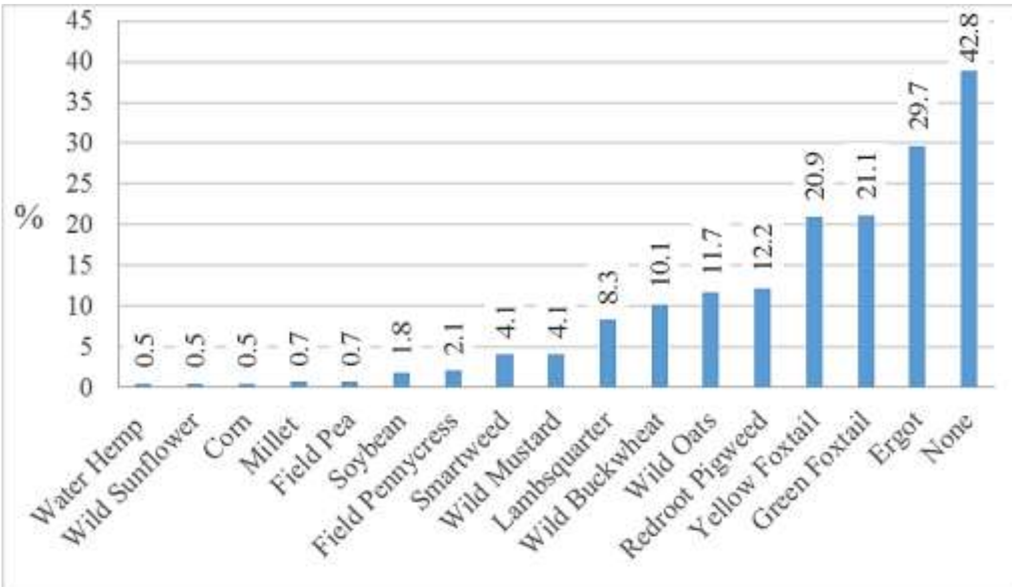

Figure 7. Percentage of hard red spring wheat crop survey samples containing various types of dockage

Ergot is the result of infection of the wheat by *Claviceps purpurea* and reduces yield and grain quality. Ergot is a food safety concern since the fungus produces alkaloid toxins. The alkaloid toxins can cause many negative effects on humans and livestock (McMullen and Stoltenow 2002; Tittlemier et al. 2015). The alkaloid toxins produced by the ergot fungus are amide or cyclic tripeptide derivatives of lysergic acid. If the sclerotium (ergot bodies) are present in wheat samples, they may cause severe ergotism in humans or mycotoxicosis in animals (Fajardo et al. 1995). Fajardo et al. (1995) found that cleaning grain with a Carter-Day dockage tester may not be effective for removing the sclerotium from wheat and the alkaloids associated with ergot were stable during processing of flour into bread, pasta and noodles. The high presence of ergot in the wheat samples may cause a significant problem with the safety of the wheat samples collected for the HRS crop survey.

The presence of weed seeds and seeds from other crops may impact the quality and safety of the wheat. *Fusarium* species not only infects wheat and other grasses, but broad-leaved weeds as well. Also, increased FHB infection has been shown to occur in crops with high weed density (Edwards 2004). Jenkinson and Parry (1994) were able to isolate 226 *Fusarium* isolates from fourteen of the fifteen weed species found in their study. Broad leaved weeds may serve as alternate host for several *Fusarium* species, which can then spread the infection to the wheat crops (Jenkinson and Parry 1994). Overall, the presence of weed seeds and other dockage is detrimental to the quality and safety of wheat.

Wheat Kernel Quality

The wheat kernel quality from the 2013, 2014 and 2015 HRS wheat crop surveys is presented in Table1. There were no differences observed in some of the grading factors such as percent shrunken and broken kernels, foreign materials and test weight. However, the values of percent damaged kernels and total defects in 2014 were higher than 2013 and 2015 crop surveys. The maximum values of damaged kernels were 4.9, 8.8 and 3.5% for 2013, 2014 and 2015, respectively. Damage to wheat kernels may be from heat, frost, sprout, insects, smut, mold or scab (Shelton and Martin 2008). The majority of the samples from the HRS wheat crop quality surveys for 2013-2015 did not contain any foreign material or damaged kernels. Shrunken & broken kernels and dockage were the most common defects in the wheat samples from the HRS wheat crop quality samples. The levels of dockage ranged from 0 to 8%, 0 to 6% and 0 to 6.7% for 2013, 2014 and 2015, respectively. Wheat kernels damaged from FHB are considered damaged but not counted as dockage by grain inspectors (Jones and Mirocha 1999).

As previously discussed (Figure 7) most of the dockage in the samples was chaff, while ergot, green foxtail, yellow foxtail, redroot pigweed, wild oats, wild buckwheat and lambsquarter made up the majority of the remaining dockage in the samples. The presence of Ergot in wheat samples presents a clear food safety risk. Because of the toxic alkaloids produced by the fungus (Tittlemier et al. 2015).

However, the presence of weed and other seeds may also result in higher FHB infection and DON contamination. The amount of dockage in wheat may have a relationship to the likelihood of FHB infection and DON contamination. Shotwell et al. (1985), found a significant correlation between the dockage content and DON content of winter wheat samples. In addition, there was a strong relationship between U.S. grade and the DON level in winter wheat samples. The samples which had better grades had much lower DON levels than samples with grades of U.S. number 3, 4 or 5 (Shotwell et al. 1985).

Test weight, 1000 kernel weight and percent dark hard and vitreous (DHV) are three other important wheat quality factors. The average test weight was slightly higher in 2013 (62.3 lb/bu) compared to 2014 (60.8 lb/bu) and 2015 (61.4 lb/bu). Although the test weights were slightly higher in 2013, the range test weights of the wheat samples over the three year collection period were very similar. There are many factors that may lower the test weight of wheat, one of which is the presence of tombstone kernels from FHB infection (Jones and Mirocha 1999). Since tombstone kernels are considered damaged kernels (Shelton and Martin 2008), but not dockage, they are not removed prior to determination of test weight.

Nevertheless, the 1000 kernel weight, which did not vary greatly over the period from 2013-2015, will not be affected by the presence of tombstone kernels in the wheat sample. This is because tombstone kernels, and all damaged kernels, are removed prior to determination of 1000 kernel weight. The percent DHV showed more variation than test weight during each year and over the three-year collection period. The mean percent DHV was 73.0, 60.0 and 77.1% for 2013, 2014 and 2015, respectively. The majority of the wheat samples collected from 2013-2015 had at least 98% DHV. Wheat kernels that are affected by FHB are not considered vitreous (Wang et al. 2002), so samples with high FHB infection will have lower DHV content. Overall, these physical characteristics of the wheat are important quality factors that may be affected by FHB infection.

There are also some wheat composition factors that were evaluated for assessment of wheat quality. These include wheat moisture, wheat protein, falling number and DON content (Table 1). Moisture content is an important factor for grain quality and grain storage (Abramson et al. 2005). The moisture content of the wheat samples did not vary greatly between years. The mean moisture content was 12.5% over the 2013 to 2015 collection period. The range of moisture content was 7.7 to 14.6% for the three years. Moisture content can have an impact on crop spoilage, fungal growth and mycotoxin levels during on-farm or elevator storage (Abramson et al. 2005).

Harvesting high moisture wheat may be required to protect the crop from damage due to wet weather conditions. However, if the wet grain is not dried after harvest there is increased risk of deterioration by microorganisms and fungus which pose a food safety risk (Karunakaran et al. 2001). Karunakaran et al. (2001) determined that there was visible mold growth at 24 days of storage on wheat with 17% moisture content when stored at 25°C. Another study also showed that DON levels increased up to 2,186 ug/kg in wheat with 20% moisture content was stored for a period of four weeks (Birzele et al. 2000). The improper storage of wheat with high moisture content may lead to significant food safety risks as well as monetary loss for wheat producers. The moisture content of the wheat samples in this work is well below 17%, so poses much lower risk for mold and bacterial growth.

Table 1. Wheat quality and deoxynivalenol content of hard red spring wheat samples collected from 2013 to 2015

		Shrunken & Broken	Foreign Material	Damaged Kernels	Total Defects	Dockage	Test Weight	1000 Kernel Weight	DHV	Moisture	Protein	FN	DON
		%	%	%	%	%	lb/bu	g	%	%	%	Sec	ppm
2013 (n = 443)	**Mean**	0.8	0.0	0.2	0.9	0.8	62.3	32.7	73.0	12.5	13.6	421	0.2
	Median	0.5	0.0	0.0	0.7	0.5	62.5	32.2	79.0	12.8	13.7	423	0.0
	Mode	0.3	0.0	0.0	0.4	0.7	62.9	31.3	98.0	13.9	13.7	423	0.0
	Minimum	0.0	0.0	0.0	0.0	0.0	54.1	20.2	2.0	9.0	9.9	242	0.0
	Maximum	4.9	2.4	4.9	7.6	8.0	66.1	51.3	99.0	13.9	17.7	572	11.0
2014 (n = 433)	**Mean**	0.7	0.0	0.4	1.1	0.7	60.8	32.7	60.0	12.6	13.6	370	0.6
	Median	0.4	0.0	0.1	0.7	0.4	61.2	32.7	67.0	13.0	13.6	376	0.0
	Mode	0.3	0.0	0.0	0.4	0.4	61.7	31.3	97.0	13.9	15.4	380	0.0
	Minimum	0.1	0.0	0.0	0.1	0.0	52.2	20.7	2.0	7.0	9.9	175	0.0
	Maximum	4.8	0.4	8.8	9.8	6.0	65.2	46.3	99.5	13.9	17.9	582	13.1
2015 (n = 430)	**Mean**	0.8	0.0	0.2	1.0	0.7	61.4	32.0	77.1	12.0	14.3	411	0.4
	Median	0.6	0.0	0.0	0.8	0.5	61.7	31.4	91.0	12.1	14.3	413	0.0
	Mode	0.3	0.0	0.0	0.3	0.1	61.7	29.2	99.0	13.9	14.3	395	0.0
	Minimum	0.0	0.0	0.0	0.0	0.0	54.5	23.3	2.0	7.7	10.5	239	0.0
	Maximum	5.6	4.4	3.5	5.6	6.7	65.6	44.2	99.0	14.6	18.5	539	18.5
3 Year Average (n = 1306)	**Mean**	0.8	0.0	0.2	1.0	0.7	61.5	32.4	69.9	12.5	13.8	401	0.4
	Median	0.5	0.0	0.0	0.7	0.5	61.8	32.2	81.0	12.7	13.9	405	0.0
	Mode	0.3	0.0	0.0	0.4	0.1	61.7	31.3	98.0	13.9	13.7	392	0.0
	Minimum	0.0	0.0	0.0	0.0	0.0	52.2	20.2	2.0	7.0	9.9	175	0.0
	Maximum	5.6	4.4	8.8	9.8	8.0	66.1	51.3	99.5	14.6	18.5	582	18.5

DHV = Dark hard and vitreous, FN = Falling number, DON = Deoxynivalenol

Wheat protein content is an important wheat quality factor, but does not have a strong connection to food safety issues. However, FHB infection can decrease protein content in wheat (Wang et al. 2005b). The mean protein contents of the wheat samples were 13.6, 13.6 and 14.3% for 2013, 2014 and 2015, respectively. The protein contents were lowest in the wheat samples collected in 2013 and highest in 2015. Overall, the wheat samples collected had a wide range of protein content. The two factors that most likely had the largest impact on protein content would be growing location and wheat variety.

The falling number test was done to provide an indicator of sprout damage and enzyme activity in wheat samples. Samples with a falling number above 300 seconds are considered sound. The presence of excess enzymes will greatly affect dough and end product quality in wheat (Shelton and Martin 2008). The majority of the wheat samples collected between 2013 and 2015 were sound and had falling numbers above 300 seconds. The mean falling numbers for 2013, 2014 and 2015 were 421, 370 and 411 seconds, respectively. The falling numbers were lower in 2014, which was most likely due to pre-harvest sprouting. Infection with FHB can also result in low falling number. Studies have shown significant correlations between level of FHB infection and protease and amylase activities (Wang et al. 2005a; Wang et al. 2005b). Overall, the falling number is an important quality factor that indicates damage that, if too severe, may result in reduction in price or rejection of the wheat.

Correlation of DON with Wheat Quality Parameters

The correlation between DON content, dockage and wheat quality for HRS wheat samples collected in 2013 is given in Table 2. The low correlation coefficient of 0.026 indicates no significant correlation between percent dockage and DON content which shows that the dockage component did not have a relationship with DON production in 2013 crop survey. There was a moderate positive significant correlation (0.393, $P<0.001$) between DON content and damaged kernels. DON also had a weak positive significant correlation (0.203, P<0.001) with total defects. These correlations are to be expected since FHB damaged kernels are considered part of the damaged kernel determination and the amount of damaged kernels are part of the total defects determination. Shotwell et al. (1985) found similar relationships between the DON contents and levels of damaged kernels and total defects in winter wheat samples. There was also weak significant negative correlations between DON and test weight (-0.111, P<0.05) and percent DHV (-0.126, P<0.01). The presence of tombstone kernels will reduce test weight and growing conditions that favor FHB will also favor non-vitreous kernels.

The correlation between DON content, dockage and wheat quality for HRS wheat samples collected in 2014 is presented in Table 3. In addition, there was no correlation between DON and the most of wheat quality parameters. However, there was strong positive significant (P<0.001) correlation between DON content and damaged kernels (0.724) and a moderate positive significant (P<0.001) correlation between DON and total defects (0.560).The higher correlation might be attributed to the higher mean concentration of DON (0.6 ppm) in the 2014 crop survey; compared to DON concentration (0.2ppm) in 2013 crop survey.

Table 2. Correlation between deoxynivalenol content, dockage and wheat quality for hard red spring wheat samples collected in 2013

	DON	SHRUNKEN AND BROKEN	FOREIGN MATERIAL	DAMAGED KERNELS	TOTAL DEFECTS	DOCKAGE
DON	1.000	-0.035	0.082	0.393***	0.203***	0.026
SHRUNKEN AND BROKEN	-0.035	1.000	0.045	0.010	0.787***	0.239***
FOREIGN MATERIAL	0.082	0.045	1.000	0.287***	0.337***	0.135**
DAMAGED KERNELS	0.393***	0.010	0.287***	1.000	0.605***	-0.020
TOTAL DEFECTS	0.203***	0.787***	0.337***	0.605***	1.000	0.196***
DOCKAGE	0.026	0.239***	0.135**	-0.020	0.196***	1.000
TEST WEIGHT	-0.111*	-0.359***	-0.106*	-0.270***	-0.443***	-0.136**
1000 KERNEL WEIGHT	-0.003	-0.456***	-0.099*	0.000	-0.371***	-0.228***
DHV	-0.126**	0.116*	0.034	-0.242***	-0.032	0.089
MOISTURE	0.117*	-0.302***	0.013	0.127**	-0.165***	-0.009
PROTEIN	0.073	0.103*	0.105*	0.084	0.142**	0.043
FN	-0.034	0.090	-0.028	-0.182***	-0.032	0.086

DON = Deoxynivalenol, DHV = Dark hard and vitreous, FN = Falling number

*, ** and *** correspond to α = 0.05, 0.01 and 0.001, respectively; degrees of freedom = 441

Table 3. Correlation between deoxynivalenol content, dockage and wheat quality for hard red spring wheat samples collected in 2014

	DON	SHRUNKEN AND BROKEN	FOREIGN MATERIAL	DAMAGED KERNELS	TOTAL DEFECTS	DOCKAGE
DON	1.000	-0.010	-0.016	0.724***	0.560***	0.068
SHRUNKEN AND BROKEN	-0.010	1.000	0.078	0.022	0.629***	0.068
FOREIGN MATERIAL	-0.016	0.078	1.000	-0.033	0.053	0.051
DAMAGED KERNELS	0.724***	0.022	-0.033	1.000	0.790***	0.061
TOTAL DEFECTS	0.560***	0.629***	0.053	0.790***	1.000	0.091
DOCKAGE	0.068	0.068	0.051	0.061	0.091	1.000
TEST WEIGHT	-0.118*	-0.519***	-0.102*	-0.254***	-0.518***	-0.221***
1000 KERNEL WEIGHT	0.011	-0.536***	-0.023	-0.002	-0.330***	-0.187***
DHV	0.023	0.014	0.048	-0.168***	-0.121*	0.158**
MOISTURE	0.051	-0.150**	-0.179***	0.125**	0.000	0.048
PROTEIN	0.023	0.073	0.051	0.074	0.104*	0.182***
FN	0.051	-0.097*	0.046	-0.082	-0.121*	-0.194***

DON = Deoxynivalenol, DHV = Dark hard and vitreous, FN = Falling number

*, ** and *** correspond to α = 0.05, 0.01 and 0.001, respectively; degrees of freedom = 431

Table 4 shows the correlation between DON content, dockage and wheat quality for HRS wheat samples collected in 2015. There was a weak positive significant (P<0.001) correlation between the DON content and percent dockage in the HRS wheat samples from the 2015 crop survey. Similar to the 2014 crop survey the DON content in 2015 showed a stronger relationship to the damaged kernel and total defects content in the wheat samples. The correlation between DON and damaged kernels was 0.699 while the correlation between DON and total defects was 0.463, which were both very highly significant (P<0.001) at moderate levels. In addition, there was correlation between DON and the some of the wheat quality parameters, such as shrunken and broken kernels (0.102, P<0.05), test weight (-0.274, P<0.001) and 1000 kernel weight (-0.185, P<0.001).

Table 5 shows the correlation between DON content, dockage and wheat quality for HRS wheat samples collected in 2013-2015. In general, for the three year average, the DON content had a weak positive significant (P<0.001) correlation with percent dockage (0.111). For all samples collected from 2013-2015, there was positive significant (P<0.001) between DON content and damaged kernels (0.635) and total defects (0.445). The DON content also had a significant (P<0.001) negative correlation with test weight. There was also a very weak negative significant (P<0.05) correlation between 1000 kernel weight and the DON content for the HRS crop survey samples collected in 2013-2015.

The correlations for each year are somewhat similar to each other with just slight differences in the significance level of the correlations and the strength of the relationships between the DON levels, dockage and wheat quality parameters.

Table 4. Correlation between deoxynivalenol content, dockage and wheat quality for hard red spring wheat samples collected in 2015

	DON	SHRUNKEN AND BROKEN	FOREIGN MATERIAL	DAMAGED KERNELS	TOTAL DEFECTS	DOCKAGE
DON	1.000	0.102*	0.005	0.699***	0.463***	0.227***
SHRUNKEN AND BROKEN	0.102*	1.000	-0.020	0.017	0.807***	0.054
FOREIGN MATERIAL	0.005	-0.020	1.000	-0.021	0.174***	0.051
DAMAGED KERNELS	0.699***	0.017	-0.021	1.000	0.550***	0.186***
TOTAL DEFECTS	0.463***	0.807***	0.174***	0.550***	1.000	0.162***
DOCKAGE	0.227***	0.054	0.051	0.186***	0.162***	1.000
TEST WEIGHT	-0.274***	-0.285***	-0.068	-0.312***	-0.417***	-0.185***
1000 KERNEL WEIGHT	-0.185***	-0.417***	0.038	-0.153**	-0.408***	-0.085
DHV	0.018	0.082	-0.029	-0.116*	-0.010	-0.043
MOISTURE	-0.047	-0.289***	0.032	0.063	-0.177***	0.086
PROTEIN	0.041	0.009	-0.006	0.046	0.037	-0.139**
FN	-0.010	0.081	-0.060	-0.016	0.048	-0.016

DON = Deoxynivalenol, DHV = Dark hard and vitreous, FN = Falling number

*, ** and *** correspond to α = 0.05, 0.01 and 0.001, respectively; degrees of freedom = 428

Table 5. Correlation between deoxynivalenol, dockage and wheat quality for hard red spring wheat samples collected in 2013-2015

	DON	SHRUNKEN AND BROKEN	FOREIGN MATERIAL	DAMAGED KERNELS	TOTAL DEFECTS	DOCKAGE
DON	1.000	0.020	0.012	0.635***	0.445***	0.111***
SHRUNKEN AND BROKEN	0.020	1.000	0.009	0.001	0.715***	0.121***
FOREIGN MATERIAL	0.012	0.009	1.000	0.048	0.168***	0.071**
DAMAGED KERNELS	0.635***	0.001	0.048	1.000	0.682***	0.057*
TOTAL DEFECTS	0.445***	0.715***	0.168***	0.682***	1.000	0.138***
DOCKAGE	0.111***	0.121***	0.071*	0.057*	0.138***	1.000
TEST WEIGHT	-0.199***	-0.354***	-0.052	-0.289***	-0.456***	-0.155***
1000 KERNEL WEIGHT	-0.056*	-0.475***	-0.015	-0.025	-0.359***	-0.174***
DHV	-0.035	0.096***	0.004	-0.193***	-0.061*	0.084**
MOISTURE	0.038	-0.323***	0.012	0.111***	-0.149***	0.011
PROTEIN	0.035	0.088**	0.034	0.057*	0.108***	0.045
FN	-0.039	0.034	-0.010	-0.149***	-0.076**	-0.033

DON = Deoxynivalenol, DHV = Dark hard and vitreous, FN = Falling number

*, ** and *** correspond to α = 0.05, 0.01 and 0.001, respectively; degrees of freedom = 1304

Some of the differences in correlation from year to year may be related to the proportions of samples that did not have any DON and the number of samples which had DON present. In 2013, only 14.7% of the HRS wheat samples that were tested had DON present. While in 2014 and 2015 the percent of samples containing DON were 30.5% and 21.2%, respectively. Overall from 2013-2015, 22.0% of the HRS wheat samples that were tested had DON.

In the wheat industry, the association between DON concentration and the percent of FDK is well known phenomenon. The strong positive correlations that have been found in this study between DON concentration and Fusarium damaged kernels can be supported by many previous studies. Simsek et al. (2013) reported that the DON content in the HRS wheat crop survey of 2011 and 2012, from the same region of our samples in this study, was highly correlated with kernel damage for both years.

Also, Shotwell et al. (1985) stated that there was a high correlation between the DON content and the percent damaged kernels that was contaminated by mold. Also, Wong et al. (1995) reported a very high correlation of 0.95 between the DON content and Fusarium-damaged kernels in 11 Chinese and Canadian wheat cultivars that had been inoculated with F. graminearum. Boyacioglu (1992) stated a correlation of 0.71 between DON concentration and the number of FDK. In addition, Manthey et al. (2004) found that the contamination of durum wheat by DON content was positively correlated with damaged kernels. Tacke and Casper (1995) made a study that mixed Fusarium infected kernels and healthy kernels in different percentage and they found that the increment in DON concentration related positively to the increment of Fusarium-damaged percentage.

The other major finding of this study is the correlation between the DON concentration and the percent total defect which has been established by many other studies as well as the DON concentration and Fusurium damaged kernels. Shotwell et al. (1985) found high correlation between DON content and total defects. In addition, Manthey et al. (2004) al found that the contamination of durum wheat by DON was correlate positively to the total defects.

Relationship between DON, Wheat Quality and HRS Wheat Grade and Subclasses

The average DON (ppm) and test weight (lb/bu) for each HRS wheat grade broken down by subclass for 2013 to 2015 is given in Figure 8. The values of DON content for 2013 to 2015 averaged across US grade and subclass ranged between 0.0 to 4.0 ppm. Overall, for all of the HRS subclasses, the lowest DON content was observed in grade one and ranged from 0.0 to 0.5 ppm and the highest DON content was observed in grade four and ranged from 1.5 to 4.0 ppm. In all of the subclasses, as the DON content increased the average test weight (lb/bu) decreased. Even though Table 5 did not show a strong correlation between DON content and test weight, the DON content may impact the quality of HRS wheat negatively by decreasing the test weight.

Although there are many factors that affect the grade for HRS wheat samples, it seems that the DON content (level of FHB infections) had a large impact of the wheat quality and grade of the samples in this study. Gilbert and Tekauz (1995) determined that cleaning and removal of tombstone kernels may improve the grade for HRS wheat samples. Optical sorting systems are one method for removal of tombstone kernels. There is a strong relationship between the DON level and weight percent of FDK in wheat. Delwiche et al. (2005) were able to significantly reduce DON in soft wheat samples after removal of tombstone kernels by an optical sorter. This method may also improve the test weight of the wheat sample. However, the presence of FHB and DON not only effect the grade and wheat quality, but also poses a serious food safety risk.

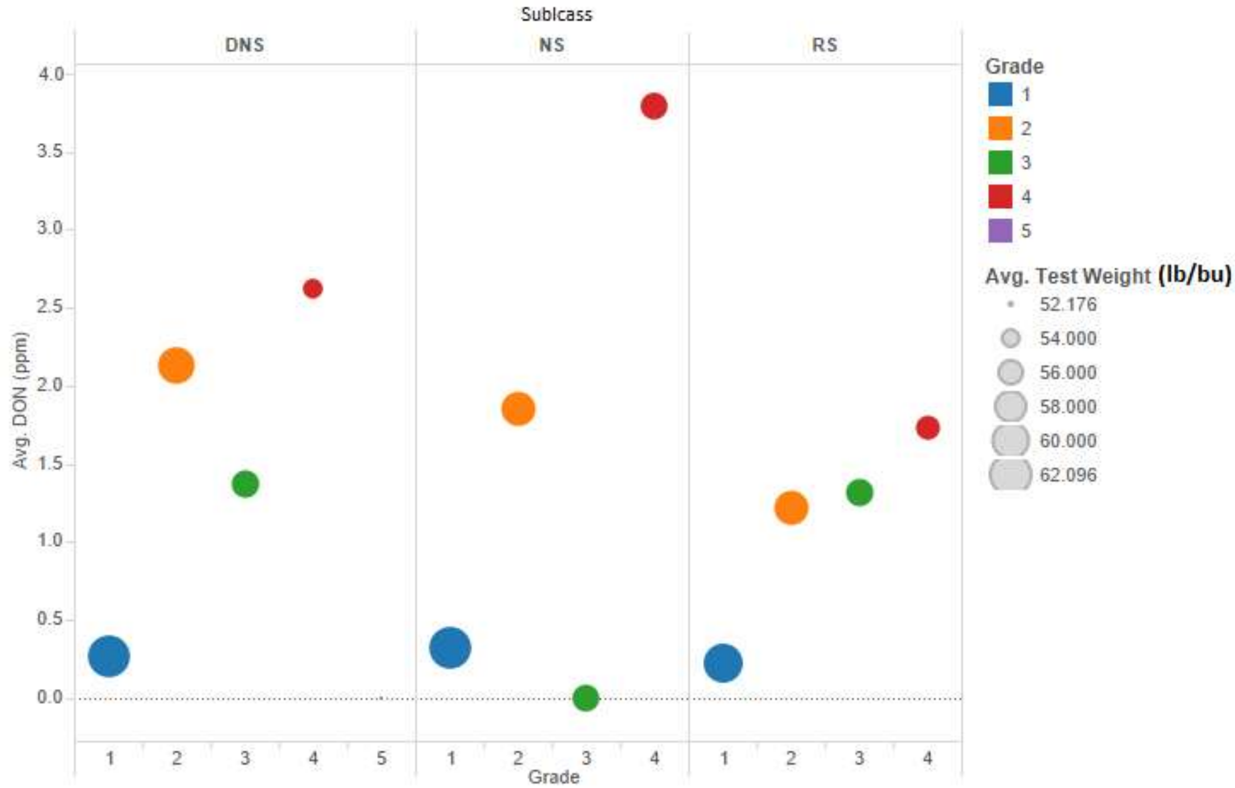

Figure 8. Average deoxynivalenol (DON, ppm) and average test weight (lb/bu) for 2013-2015 grouped by grade and subclass
*Color of circles indicates grade, size of circles indicates average test weight

Figure 9 shows the relationship between the average of DON ppm and average percent dockage for each grade broken down by subclass from 2013-2015. The figure shows that the highest percent dockage was in subclass DNS grade 5 at 0.0 ppm DON. Moreover, in NS subclass, the highest level of DON content was related to the smallest percent dockage. Also, in subclass DNS, the increase in DON content did not related to higher dockage percent. This supports the result shown in Tables 2, 3 and 4 which stated that the increase in dockage percent might not be related to the increase of DON content. In general, the amount of dockage present and the amount of FDK will affect the grade a sample of wheat (Tittlemier et al. 2013).

However, dockage and DON are not necessarily directly related since there are many types of dockage that can be present in a wheat sample that may or may not affect the DON level.

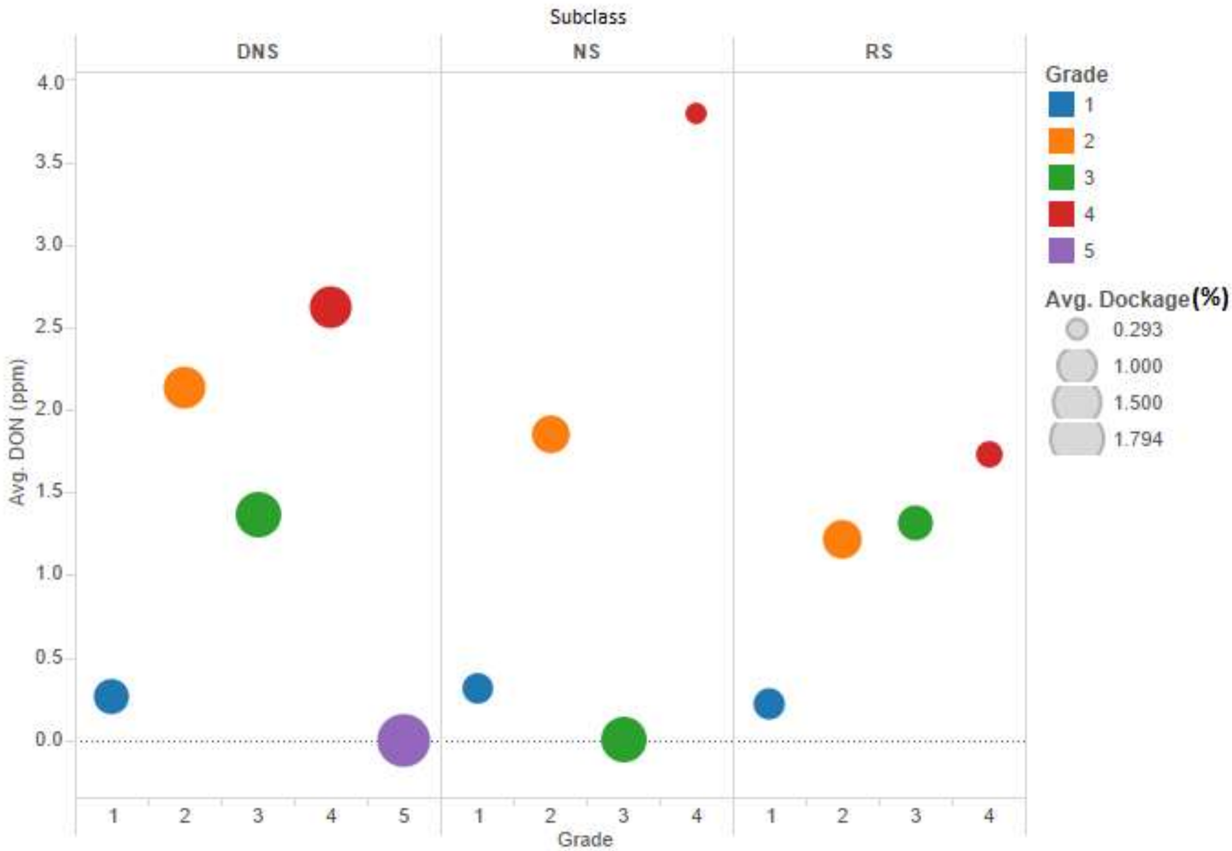

Figure 9. Average deoxynivalenol (DON, ppm) and average dockage (%) for 2013-2015 grouped by grade and subclass
*Color of circles indicates grade, size of circles indicates average dockage

The percent damaged kernels is an important grading factor for wheat, and depending on the type of damage may be highly related to DON content. The results of this study show there is a strong relationship between the percent damaged kernels and DON levels (Figure 10). The DON content and percent damaged kernels were both higher in wheat with higher grades. However, the samples with a grade of 5 and subclass DNS had very low DON and very low damaged kernels. In the case of the samples with US grade 5 subclass DNS, the grade was a result of high dockage and not damaged kernels or FHB infection. The highest levels of damaged kernels and DON were seen in the samples that had US grades of 4 and NS subclass. All of the samples with a US grade of 1, regardless of subclass, had average DON levels less than 0.5ppm. The samples with grades of 2 or higher that had more than 0.1% damaged kernels had DON contents higher than 1.0 ppm. All samples with a US grade of 4 had average DON contents greater than 1.5ppm.

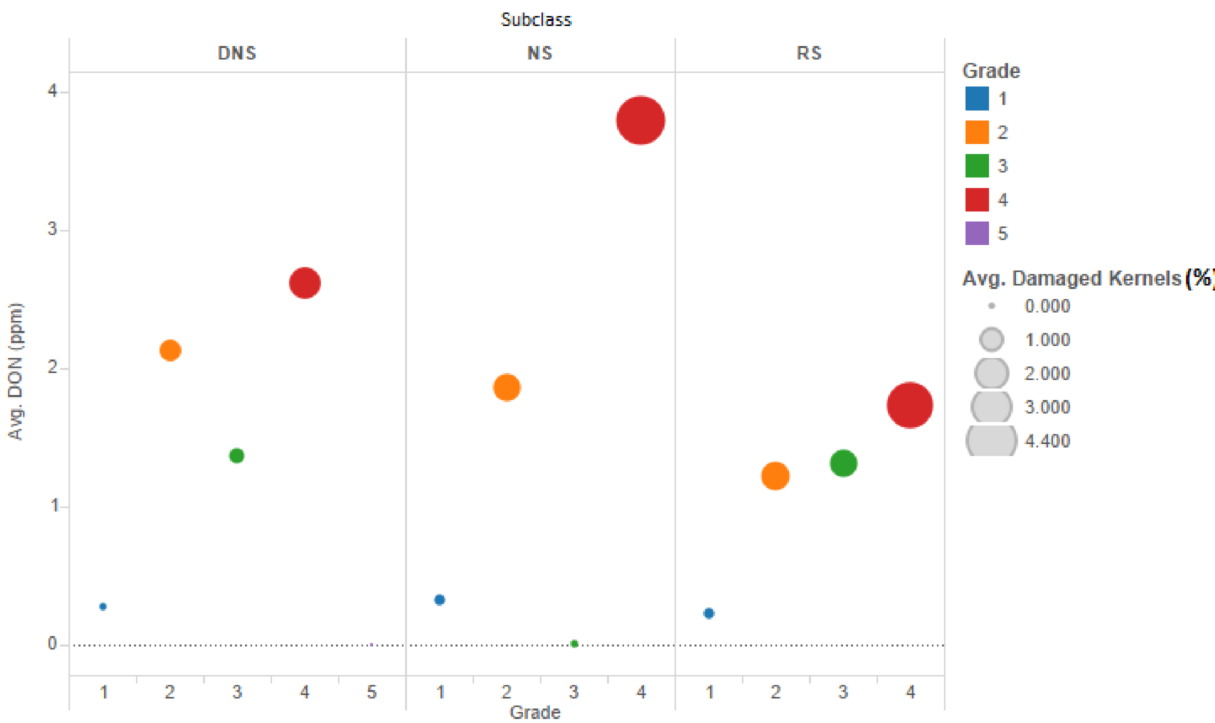

Figure 10. Average deoxynivalenol (DON, ppm) and average % damaged kernels for 2013-2015 grouped by grade and subclass
*Color of circles indicates grade, size of circles indicates average damaged kernels

High levels of FHB infection will increase the amount of damaged kernels and the DON level in wheat samples. The FHB and damaged kernels may affect the ability of producers to store their grains. If the producers had the ability to clean and remove FDK from their wheat, it would allow for better storage conditions and reduced food safety risks. When grain with high FHB damage is stored, the DON levels will increase during storage (Wilcke et al. 1999).

Figure 11 shows the average (DON, ppm) and average shrunken and broken kernels (%) for each grade broken down by subclass for 2013-2015. In general, the figure does not show any association between DON content and the average percent of shrunken and broken kernels. Being a grading factor, the amount of shrunken and broken kernels will affect the grade given to a sample of wheat.

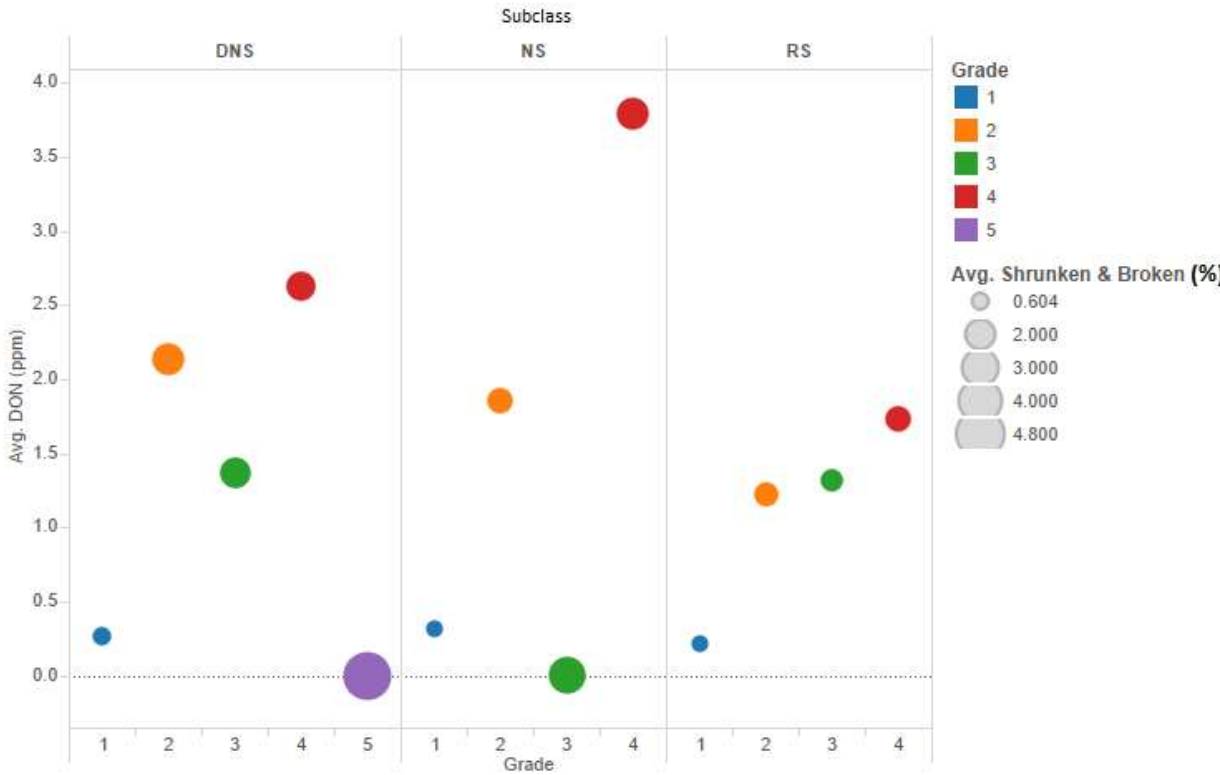

Figure 11. Average deoxynivalenol (DON, ppm) and average shrunken and broken (%) for 2013-2015 grouped by grade and subclass
*Color of circles indicates grade, size of circles indicates average of shrunken and broken kernels

The average percent of shrunken and broken kernels in samples with a grade of 1 was less than 1%. The average shrunken and broken contents of the samples with grades of 2-5 varied, because there are other factors such as damaged kernels, foreign material and other factors that affect the grade of a wheat sample. The samples with a grade of 5 and subclass DNS, which had no DON, had the highest amount of shrunken and broken kernels (4.8%). The shrunken and broken kernels was the biggest factor effecting the grade of the samples in that grade and subclass (5, DNS). Shotwell et al. (1985) found a weak significant ($P<0.05$) correlation ($r = 0.18$) between DON content and shrunken and broken kernels in winter wheat samples. However, the results of this study show no relationship between those two factors in HRS wheat.

The average of DON ppm and foreign material (%) for each grade broken down by subclass for 2013-2015 is given in Figure 12. In general, the figure does not show any association between DON content and foreign material percent; however, in some grades the increase in DON content shows an increase in the foreign material percent such as grade 3 subclass DNS and grade 2 subclass NS.

The HRS crop survey samples collected from 2013-2015 did not contain a large proportion of foreign material. The highest foreign material content was 0.2% in the samples with US grade 2 subclass NS. The average foreign material in samples with grade 3 subclass DNS was 0.09%, and the rest of the samples contained less than 0.01% foreign material, on average. Although foreign material did not have a connection with DON content in this study, it may pose a food safety risk, as well as result in losses for wheat producers and millers. Samples with high foreign materials result in reduced grade and thus reduced price for the wheat producer. Foreign material including things like glass or stones, can damage milling equipment if it is not properly removed (Zayas et al. 1989). Also, the presence of insects or animal filth will be a food safety risk. Overall, the samples in this study did not contain a substantial amount of foreign material and the amount of foreign material was not correlated with DON content.

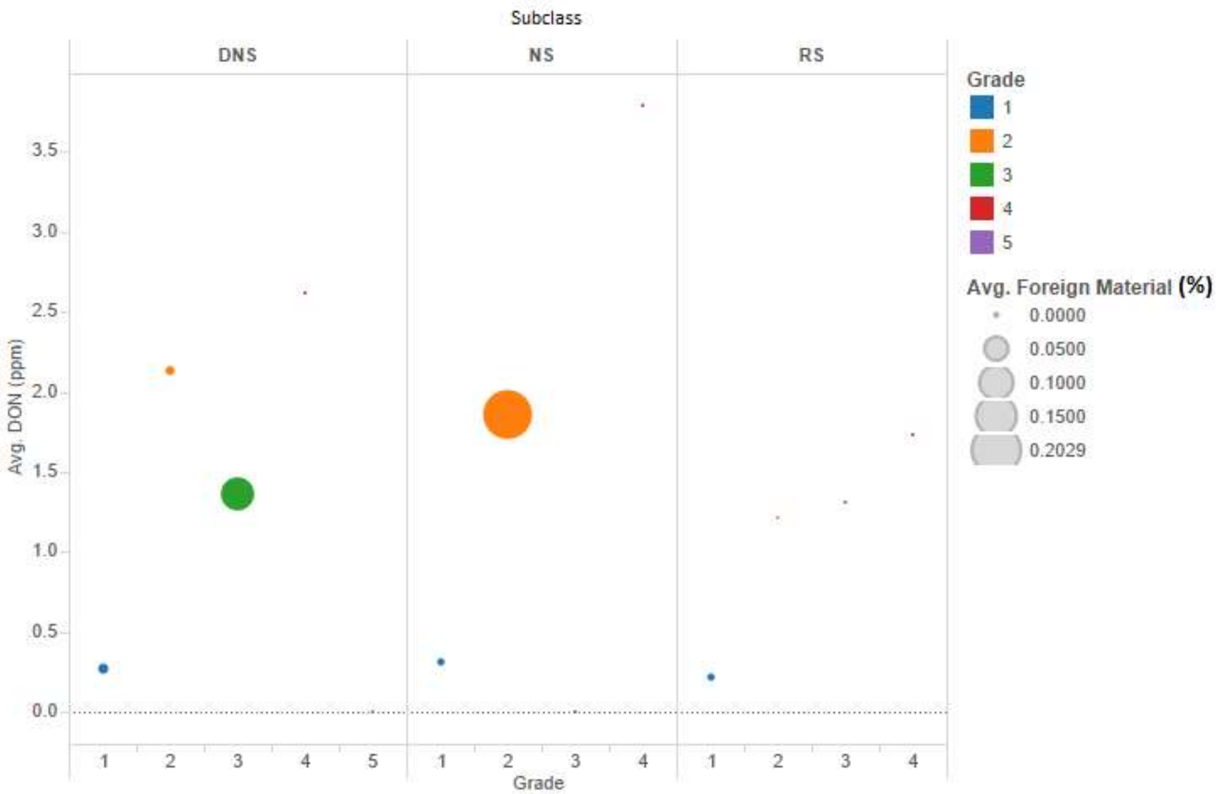

Figure 12. Average deoxynivalenol (DON, ppm) and foreign material (%) for 2013-2015 grouped by grade and subclass
*Color of circles indicates grade, size of circles indicates average foreign material

The total defects is a grading factor which is the sum of shrunken and broken kernels, foreign material and damaged kernels. Figure 13 shows the correlation between the DON content and the total defects. In the most of the HRS subclasses, the increase in DON content increased the percent of the total defects except few cases such as grade 5 in DNS subclass and grade 3 in NS subclass. This figure shows the moderate correlation trend between the average DON content and the total defects. The relationship between the DON content and total defects is significant since the total defects encompasses the amount of damaged kernels. However, the strength of the relationship between DON and total defects is lower than for damaged kernels since the total defects also includes shrunken and broken kernels and foreign material, which did not have correlation with DON content. The average total defects ranged from 0.79 to 6.55 among the various grade and subclass categories.

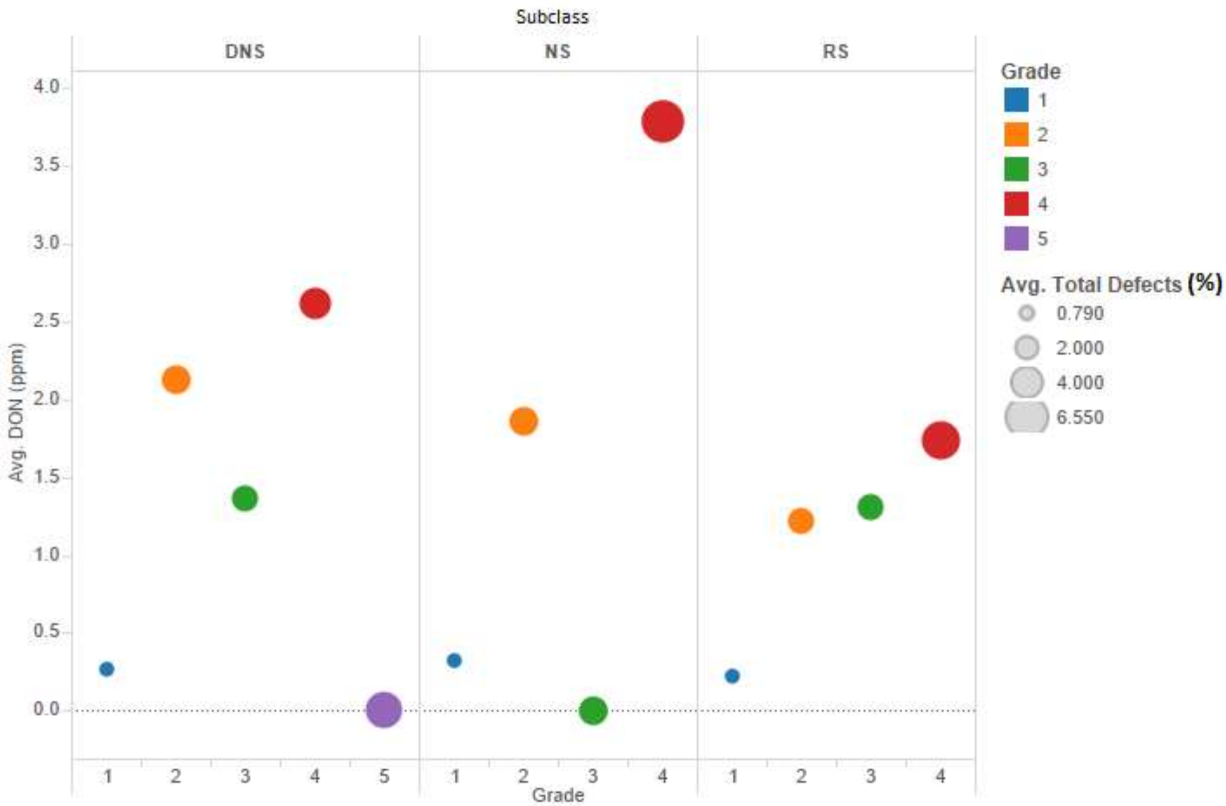

Figure 13. Average deoxynivalenol (DON, ppm) and average total defects (%) for 2013-2015 grouped by grade and subclass
*Color of circles indicates grade, size of circles indicates average total defects (%)

For the most part, samples with higher total defects had higher DON content. One exception being the group of samples with US grade 5 and subclass DNS. This is because the total defects in the samples in that group (5, DNS) were comprised mainly of shrunken and broken kernels. The amount of total defects and the DON content varied greatly across grade and subclass. The grade of wheat seemed to have a stronger relationship to DON content than the subclass. Which is reasonable, owing to the fact that FHB infection and the presence of tombstone kernels will affect the wheat grading factors, but not necessarily the vitreousness of the wheat. Wheat vitreousness is the only determining factor in the subclass for HRS wheat, and the DON content in the samples in this study did not have significant correlation with the percent of DHV kernels. Simsek et al. (2013) did not find any relationship between the DON content and wheat subclass (percent DHV kernels).

Correlation of DON with Wheat Quality Parameters and Growing Locations

The growing location of HRS wheat can also have an impact on the wheat quality, FHB infection and DON content. It was previously determined that the DON content of HRS wheat samples varied significantly between growing states and regions (Simsek et al. 2013). Average DON content for 2013 to 2015 grouped by states and growing regions ranged from 0.0 to 1.1 ppm (Figure 14). These averages are lower than when the samples were grouped based on US grade and subclass. This is because, when grouped by US grades, the lower grade samples tend to have a high proportion of FHB infected samples. While, when the samples are grouped by growing location there is a dilution effect due to a higher proportion of samples with no DON in each growing location.

The relationship between the average of DON (ppm) and average test weight (lb/bu) for each state and region from 2013 to 2015 is given in Figure 14. The variables showed differences between state and region for the relation between DON content and test weight (lb/ bu) indicating that HRS wheat samples from different states or regions might be impacted differentially by FHB infection according to the state's or region's growing conditions. In general, the highest average test weight was in ND (ND B, 62.35 lb/bu) and the lowest average in the test weights were in MT (MT C, 59.87 lb/bu). In MN region A and B and in ND region A, B, C, D, E and F, the test weight decreased as the DON levels increased. However, as the DON content was increasing in SD, the average of test weight did not change and the average test weights and DON levels were all low for all regions in Montana.

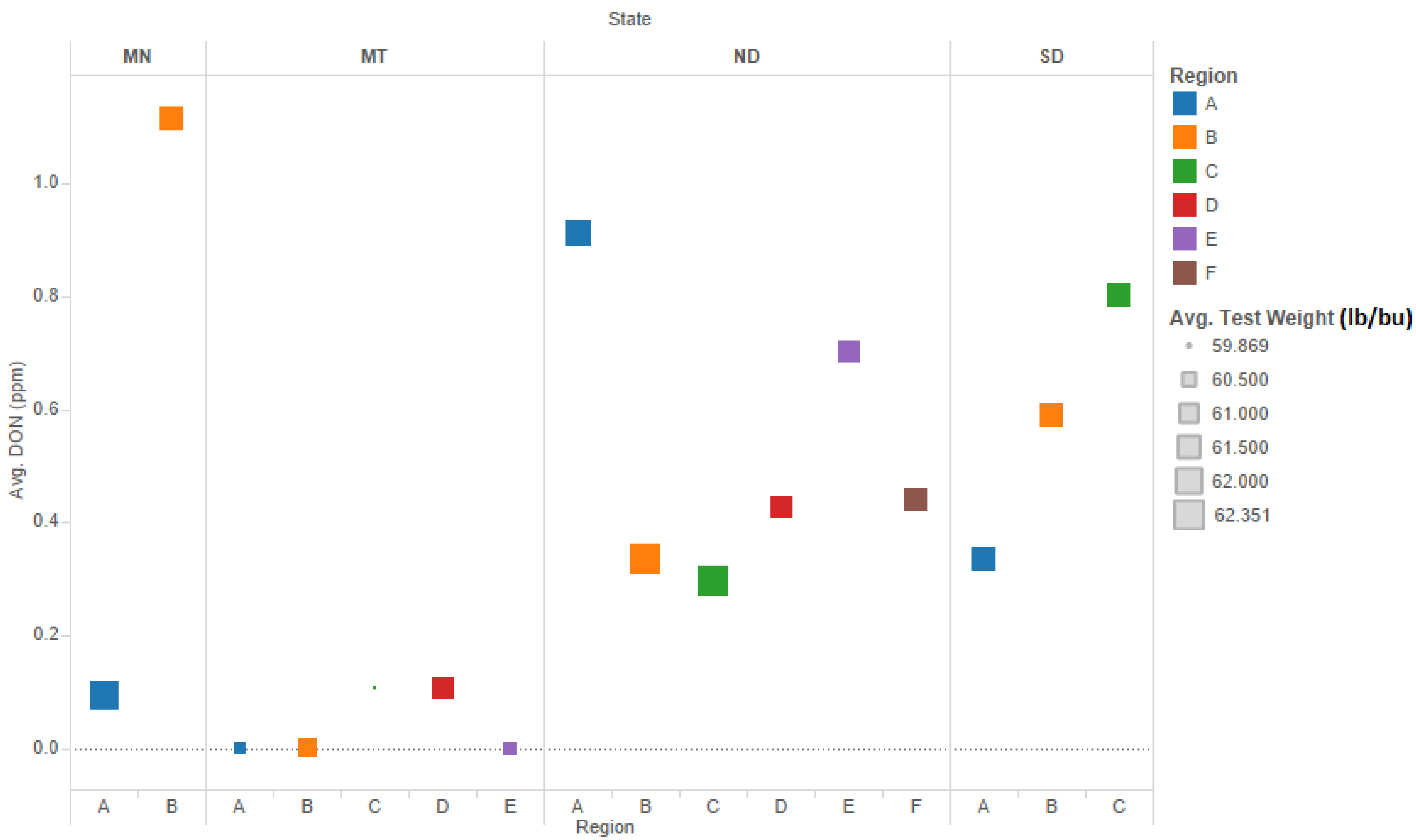

Figure 14. Average deoxynivalenol (DON, ppm) and average test weight (lb/bu) for 2013-2015 grouped by state and region
*Color of squares indicates region, size of squares indicates average test weight

If the FHB infection has results in the presence of tombstone kernels the test weight will be reduced. Tombstone kernels have lower density and reduced size compared to healthy HRS wheat kernels. However, other factors such as wheat variety and growing conditions can also affect test weight of HRS wheat samples (Jones and Mirocha 1999). Because of the large impact of other factors on test weight, the test weight may not be an effective indicator of the level of FHB or DON in HRS wheat samples.

The relationship between average DON (ppm) and average dockage (%) grouped by growing state and region is shown in Figure15. The average dockage content of samples grouped by state and growing region ranged from 0.51% to 1.24% for 2013-2015. There is a positive association between the amount of DON and the percent dockage for samples grown in Minnesota and North Dakota. For the samples from Minnesota and North Dakota, as the dockage increased, the DON level also increased. However, there is a negative relationship between the dockage percent and DON content in the samples grown in South Dakota. Also, there does not seem to be any relationship between dockage content and DON for samples grown in Montana.

The correlation between DON and dockage for samples collected from 2013-2015 (0.111) was weak but very highly significant ($P<0.01$). This shows that there is a relationship between DON content and dockage. However, there are many other contributing factors that affect the dockage and DON levels. Based on the results of the HRS wheat quality surveys from 2013-2015, the states and regions with samples having higher FHB infection and more variation in DON levels are more likely to show a relationship between DON and dockage. While, areas with low DON and less variation in DON content will not show a relationship between DON and dockage content.

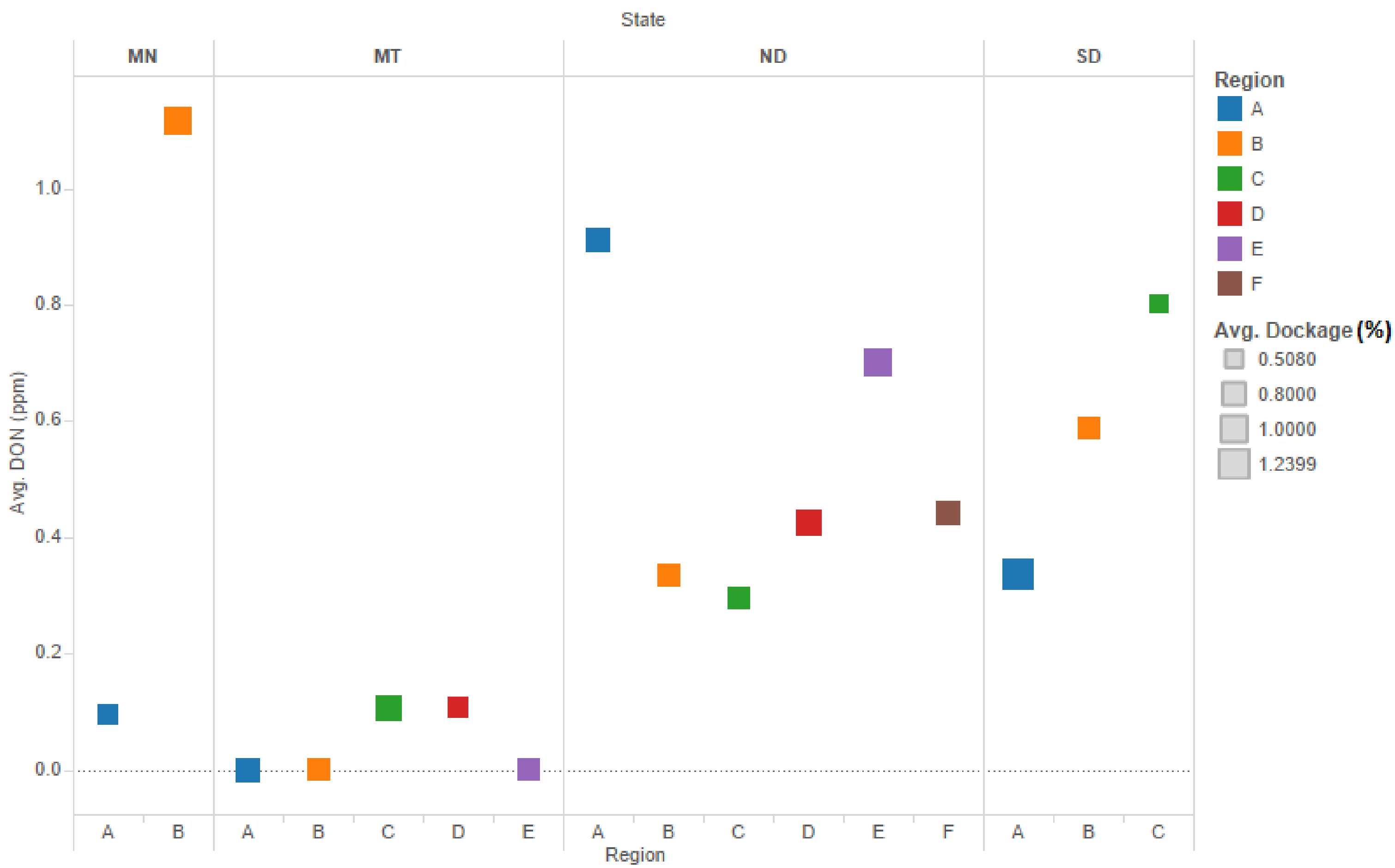

Figure 15. Average deoxynivalenol (DON, ppm) and average dockage (%) for 2013-2015 grouped by state and region
* Color of squares indicates region, size of squares indicates average dockage

The average DON contents and percent damaged kernels grouped by state and region are shown in figure 16. The average percent of damaged kernels for 2013-2015 grouped by state and region ranged from 0.0 to 0.66%. Since tombstone kernels from FHB infection are considered damaged kernels, the amount of damaged kernels may have a strong relationship with the DON content. In this study there was a very highly significant ($P<0.001$) correlation (0.635) between DON content and damaged kernels. Within each state there are different growing conditions and different levels of FHB infection that show varying trends with the amount of damaged kernels. The two growing regions in Minnesota had vastly different levels of DON and damaged kernels. However, samples grown in all regions of Montana showed very low levels of both DON and damaged kernels. The samples grown in North Dakota and South Dakota show a strong relationship between DON level and damaged kernels. It is likely that the most of the samples between 2013-2015 are FDK.

Another interesting trend among the samples is between the growing environments and the DON and damaged kernels. Overall, growing locations in the east generally have more favorable conditions for FHB infection and have higher DON levels (Simsek et al. 2013). Samples from Montana (western) and South Dakota (western and eastern) are consistent with this tendency. This is especially evident in the regions in South Dakota as one goes from west (region A) to east (region C), where the DON content and damaged kernels both increase from west to east. However, the regions in Minnesota which are both eastern locations have the highest variability in DON and damaged kernels. Also, there is variability among the DON and damaged kernels in the growing regions of North Dakota that do not conform to normal growing conditions for those areas.

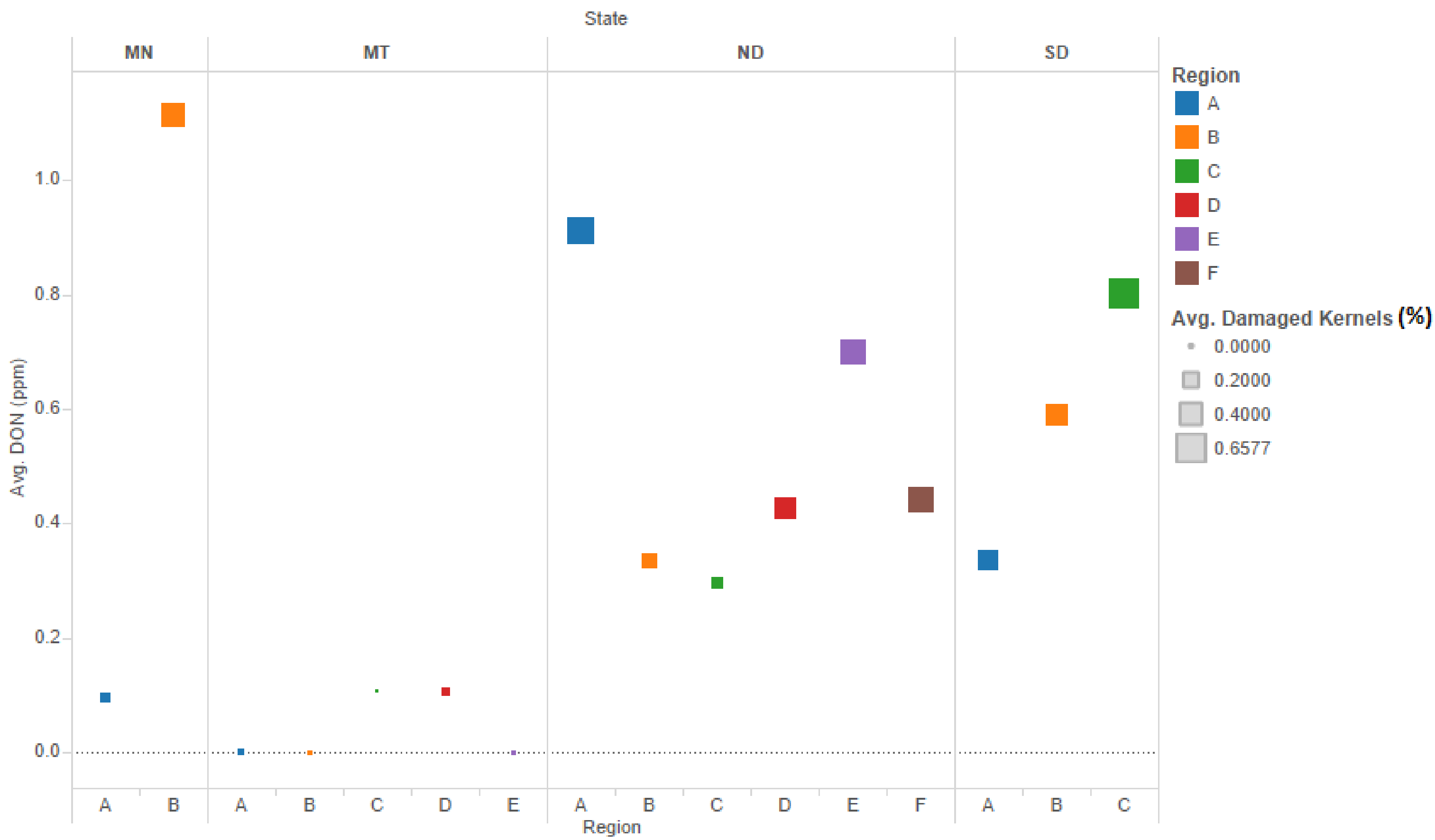

Figure 16. Average deoxynivalenol (DON, ppm) and average % damaged kernels for 2013-2015 grouped by state and region
* Color of squares indicates region, size of squares indicates average damaged kernels

The average DON and average shrunken and broken (%) grouped by state and region for 2013-2015 are shown in figure 17. The average shrunken and broken kernels for 2013-2015 grouped by state and region ranged from 0.46 to 1.64%. In the Minnesota growing regions, there was a direct relationship between the DON content and shrunken and broken kernels. However, it must be taken into consideration that there are only two regions with vastly different DON contents in Minnesota. All regions in Montana had low DON content and relatively high percent of shrunken and broken kernels. So, there is no connection between the two parameters for samples grown in Montana. When comparing DON and shrunken and broken kernels from samples grown in North Dakota, there is a weak relationship. Among the regions in North Dakota showing lower DON content, the percent of shrunken and broken kernels is also lower. The regions in South Dakota show the opposite relationship between DON and shrunken and broken kernels to those of Minnesota and North Dakota. In the three growing regions in South Dakota, the level of DON decreased as the percent of shrunken and broken kernels increased.

The average DON ppm and foreign material percent for 2013-2015 grouped by state and region is shown in figure 18. Overall the foreign material content of the samples collected from 2013-2015 was very low. The average foreign material content when grouped by state and region ranged from 0.0 to 0.03%. When grouped by state and region the results are similar to when the values are grouped by US grade and subclass. Minnesota A had the highest amount of foreign material but only had less than 0.1 ppm DON. Montana and South Dakota had similar results, with the regions with the highest foreign materials contents having the lowest DON contents. However, the region in North Dakota with the highest DON (0.91 ppm) content (ND-A) also had the highest percent of foreign material (0.02%) in that state.

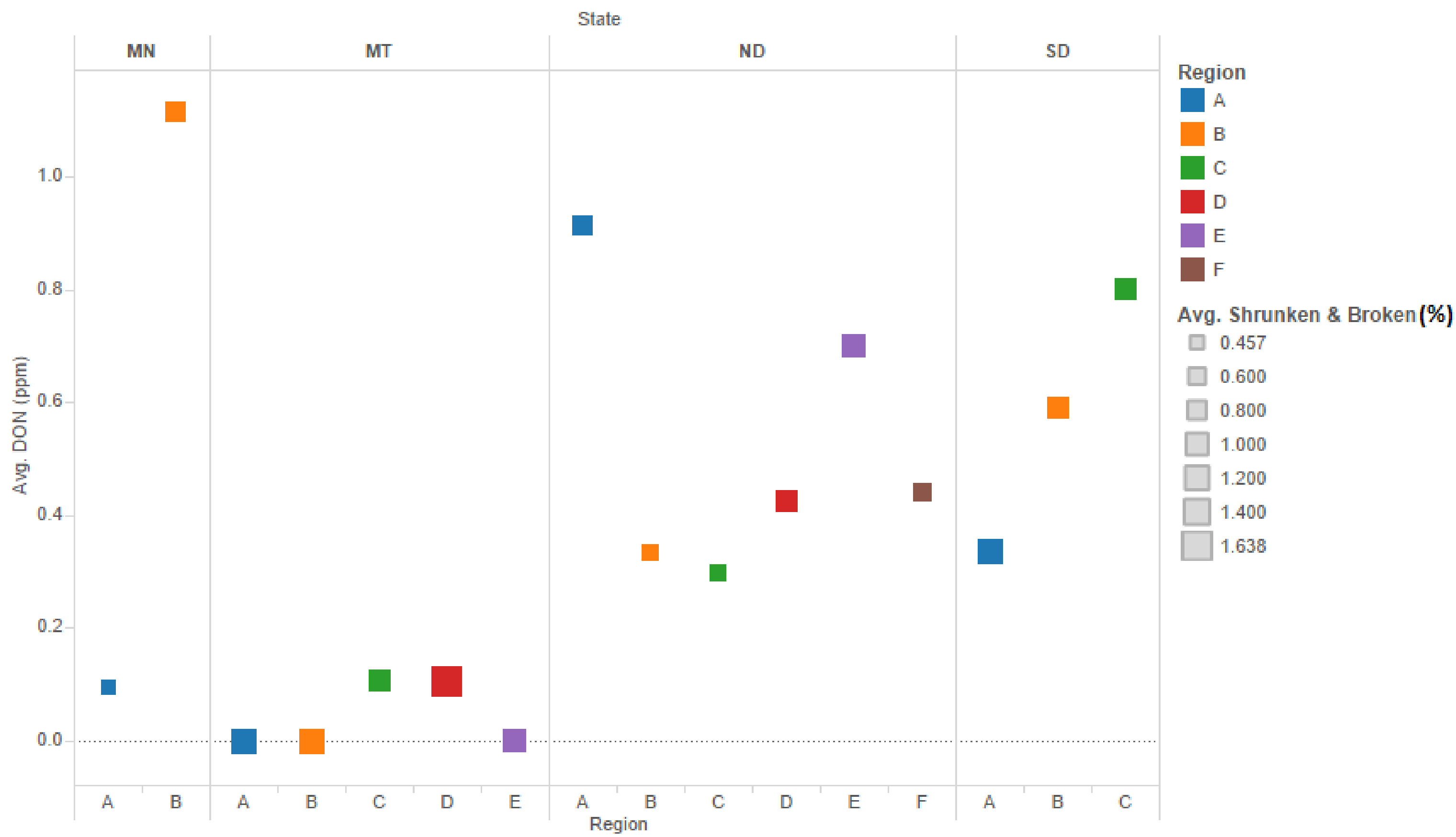

Figure 17. Average deoxynivalenol (DON, ppm) and average shrunken and broken (%) for 2013-2015 grouped by state and region
* Color of squares indicates region, size of squares indicates average of shrunken and broken kernels

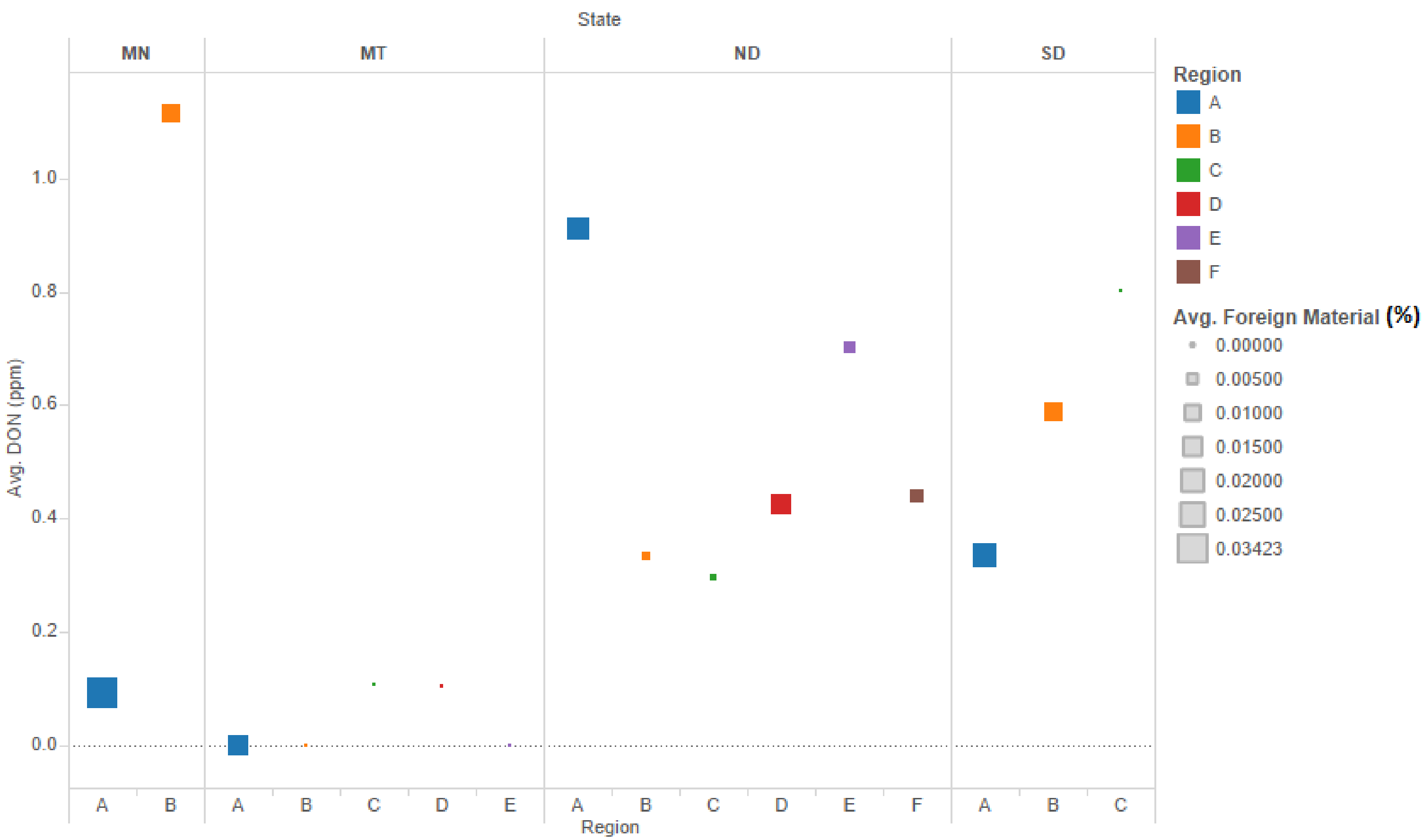

Figure 18. Average deoxynivalenol (DON, ppm) and foreign material (%) for 2013-2015 grouped by state and region
* Color of squares indicates region, size of squares indicates average foreign material

Overall, there are other factors that affect the presence of foreign material, as well as shrunken and broken kernels that are not necessarily related to FHB infection or DON contamination. The average DON ppm and average total defects percent for 2013-2015 grouped by state and region is shown in figure 19. The percent of total defects for 2013-2015 grouped by state and region ranged from 0.55 to 1.69%. There is some relationship between the DON content and the amount of total defects in the HRS wheat samples. This is because fusarium damaged kernels are considered damaged kernels which is part of the determination of total defects. However, since the total defects also includes parameters, such as shrunken and broken kernels and foreign material that do not have a significant relationship with DON the strength of this relationship is less than that of DON with percent damaged kernels.

The variation in DON among the regions in Minnesota and North Dakota show the strongest relationship between the DON content and total defects. The samples from Minnesota and North Dakota with higher total defects generally had higher DON content. The samples from Montana have very low DON content overall, so do not show a clear relationship with total defects. However, the samples from MT-D that had the highest total defects in Montana had slightly higher DON content than most of the other regions in Montana. For the samples grown in South Dakota there is some other factor contributing to total defects that is not related to FHB infection and so there is no relationship between total defects and DON content in the samples from South Dakota. In the two regions from South Dakota with the highest and lowest DON contents the amounts of total defects in those regions are very similar. South Dakota A had a DON content of 0.34 ppm and 1.52% total defects, while South Dakota C had 0.80 ppm DON and 1.50% total defects.

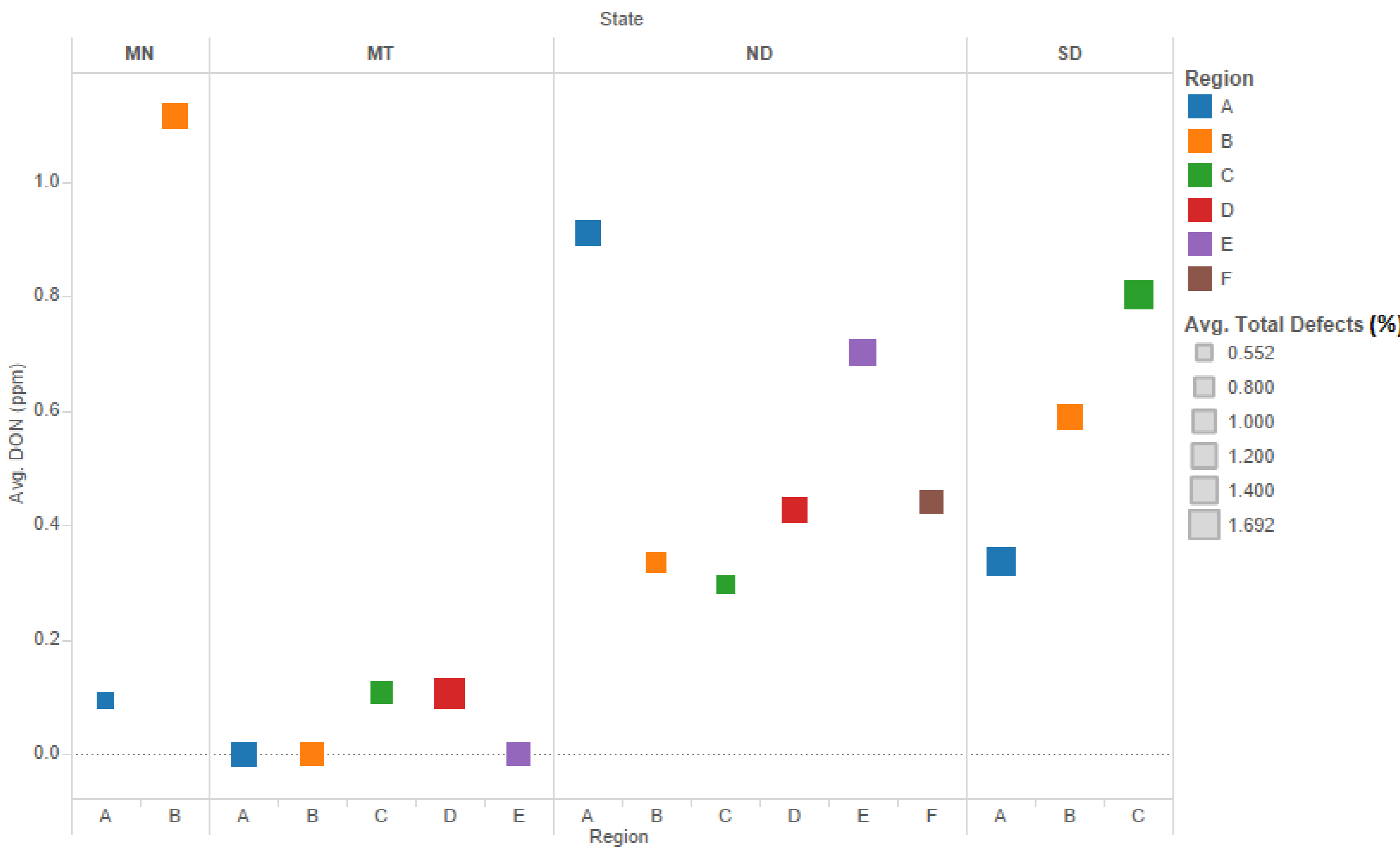

Figure 19. Average deoxynivalenol (DON, ppm) and average total defects (%) for 2013-2015 grouped by state and region
* Color of squares indicates region, size of squares indicates average total defects (%)

In the case of these two regions, South Dakota A had more shrunken and broken kernels and more foreign material than South Dakota A. Based on these results, the level of total defects may indicate the presence of FHB infection and DON contamination. However, too many other factors that affect this value, which are not related to FHB infection and DON. The amount of damaged kernels may be the best indicator of FHB infection and DON levels, especially if weather and growing conditions are known for the area where a HRS wheat sample may be grown.

CONCLUSION

FHB and its associated mycotoxin, especially DON, continue to cause health, quality and economic problems in the most of small-grain regions around the world. Some previous researchers found that FHB development was affected by the environmental conditions and locations. The understanding of FHB development factors could lead to better management strategies to minimize the disease development and the mycotoxin accumulation. In this study, we wanted to determine FHB development from an agricultural prospective by testing the whether wheat dockage percentage was related DON accumulation in HRS wheat samples. We found that DON content was different according to the survey crop year, which affects wheat quality parameters differently and we did not found a relationship between wheat dockage percentage and DON content. This study confirmed that DON content in wheat samples was not affected by the increase or decrease of the percent wheat dockage. Also, this study confirmed that DON content in wheat samples did not affect the majority of wheat quality parameters. However, we have found, as many previous researchers, DON content correlated positively with damaged kernels and total defects.

FUTURE RESEARCH

The next steps in this research would involve more on the measurements of different types of cleaning wheat dockage. Conditions would need to be improved in order to increase the effectiveness of cleaning wheat with diseased wheat kernels such as ergot kernels. Another step would be to study the relation of wheat dockage to other types of mycotoxins.

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APPENDIX

Table A1. ANOVA using state-region combinations as the independent variable and DON as dependent variable

Means with the same letter are not significantly different.						
Duncan Grouping				**Mean**	**N**	**StateReg**
		A		1.1151	57	MN_B
		A				
B		A		0.9117	171	ND_A
B		A				
B		A	C	0.8015	26	SD_C
B		A	C			
B	D	A	C	0.7010	63	ND_E
B	D	A	C			
B	D	A	C	0.5885	94	SD_B
B	D	A	C			
B	D	A	C	0.4411	44	ND_F
B	D	A	C			
B	D	A	C	0.4262	156	ND_D
B	D		C			
B	D		C	0.3362	29	SD_A
B	D		C			
B	D		C	0.3345	159	ND_B
B	D		C			
B	D		C	0.2963	100	ND_C
	D		C			
	D		C	0.1070	10	MT_C
	D		C			
	D		C	0.1062	13	MT_D
	D		C			
	D		C	0.0945	149	MN_A
	D					
	D			0.0000	104	MT_A
	D					
	D			0.0000	11	MT_E

Table A1. ANOVA using state-region combinations as the independent variable and DON as dependent variable (continued)

Means with the same letter are not significantly different.						
Duncan Grouping				**Mean**	**N**	**StateReg**
	D					
	D			0.0000	120	MT_B

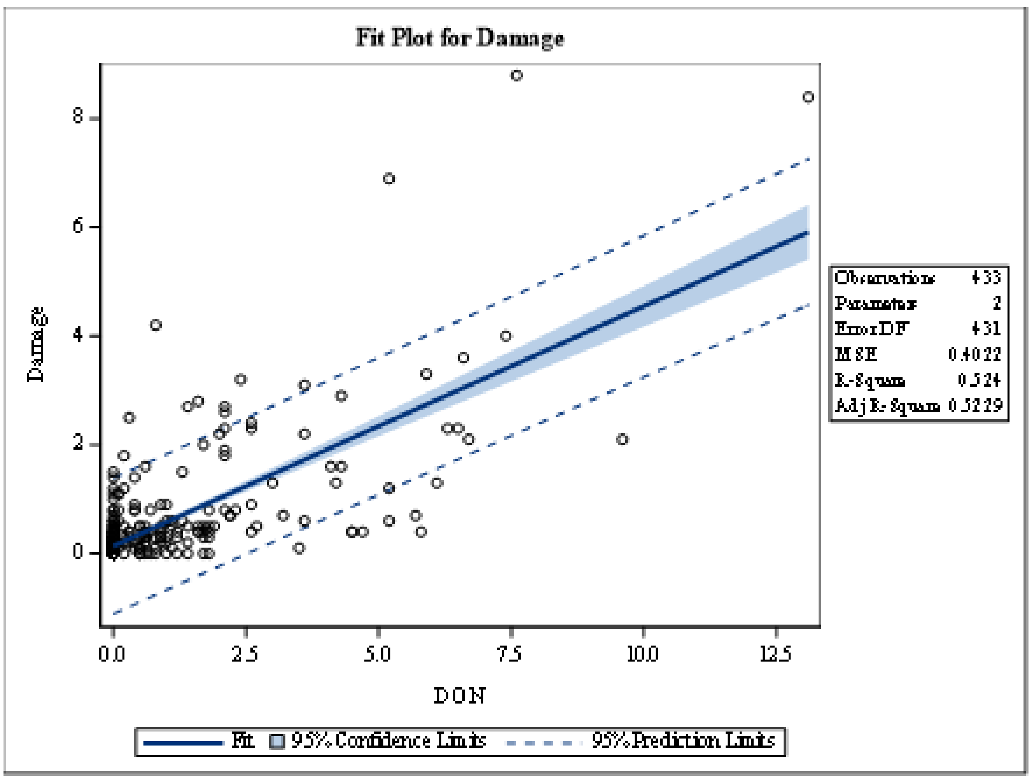

Figure A1. Simple regressions using DON as the independent variable and damage as the dependent variable for 2014

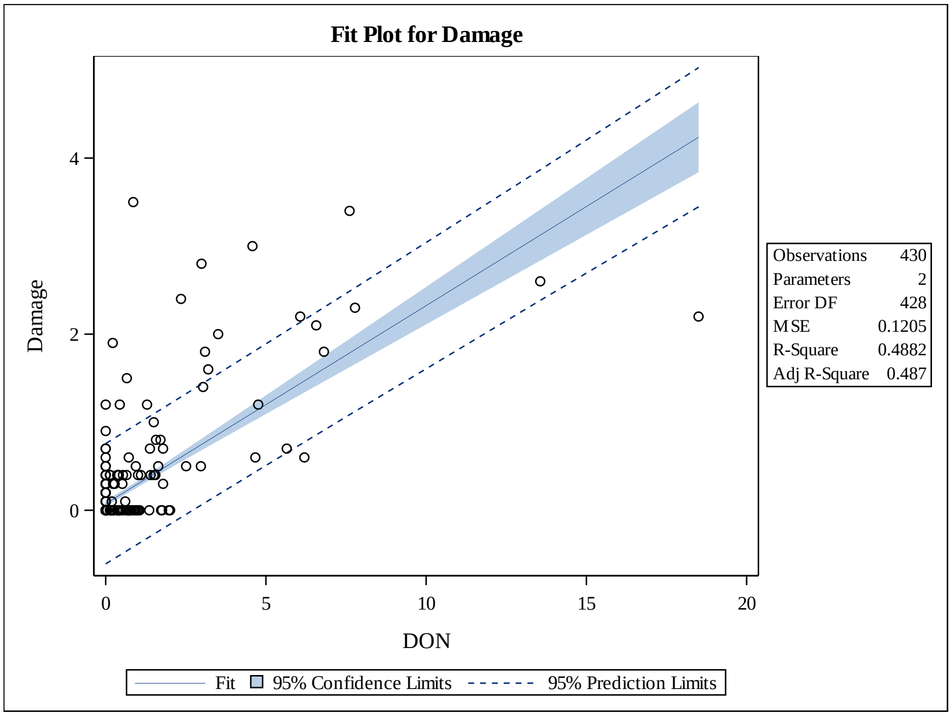

Figure A2. Simple regressions using DON as the independent variable and damage as the dependent variable for 2015

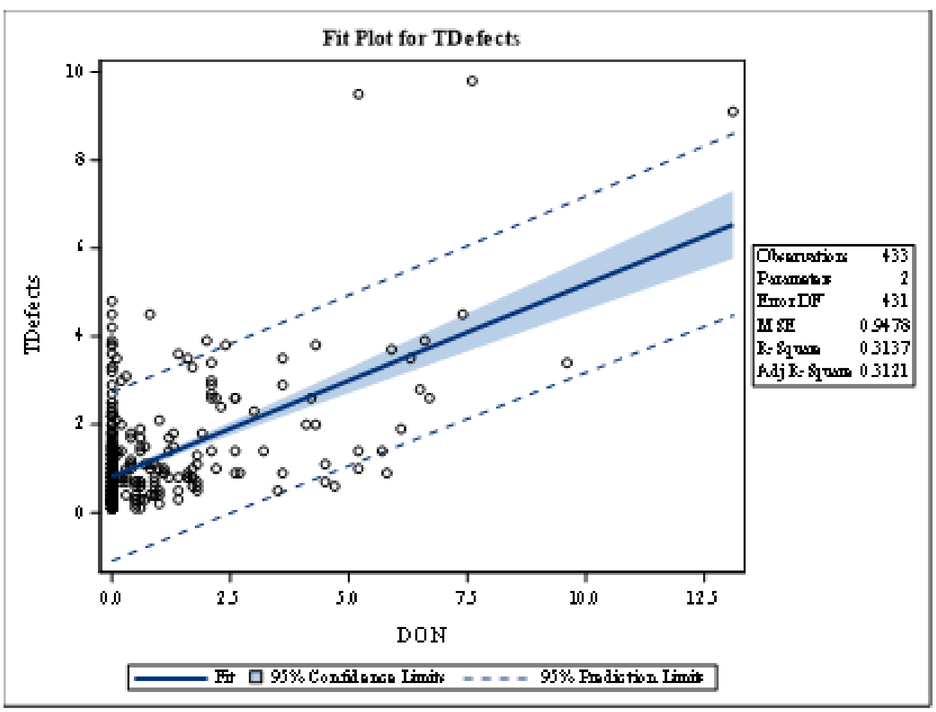

Figure A3. Simple regressions using DON as the independent variable and total defect as the dependent variable for 2014

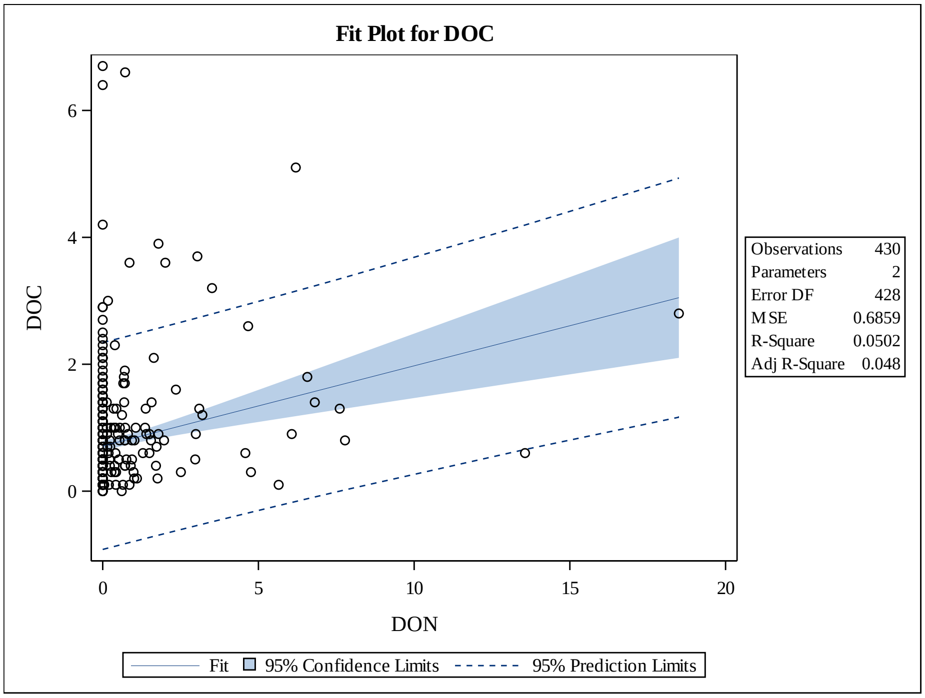

Figure A4. Simple regressions using DON as the independent variable and dockage as the dependent variable for 2014