FUSARIUM HEAD BLIGHT IN WHEAT: STUDY OF FIELD RESISTANCE AND MYCOTOXIN ACCUMULATION IN MILLED FRACTIONS OF SOFT RED AND SOFT WHITE WINTER WHEAT

By

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ABSTRACT

FUSARIUM HEAD BLIGHT IN WHEAT: STUDY OF FIELD RESISTANCE AND MYCOTOXIN ACCUMULATION IN MILLED FRACTIONS OF SOFT RED AND SOFT WHITE WINTER WHEAT

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Fusarium head blight, caused by *Fusarium* spp. is a major threat to the wheat industry, affecting both yield and grain quality. Additionally, production of the mycotoxin deoxynivalenol (DON) in infected grain is a cause of severe losses. A higher proportion of toxin accumulates in the outer layers of kernel which is often removed by milling. To evaluate genotypic differences for toxin accumulation in different tissues of infected grain, we compared 39 locally adapted soft winter lines (21 white and 18 red) representing range of FHB resistance. DON was quantified in a whole grain sample and milled fractions of bran and flour. On average, white lines accumulated significantly higher quantities of DON in all fractions compared to red, while the ratio DON-bran/ DON-flour was lower ($p<0.05$). The 39 lines were also evaluated for different measures of FHB resistance in the field using two different artificial inoculation methods. Since the amount and duration of moisture available to infected wheat heads influences visible infection and toxin accumulation, a bagging method after spray inoculation (SB) was compared with misting treatment that followed grain spawn inoculum (GS). The methods were compared based on measures of Incidence, Severity, Index, Fusarium damaged kernels (FDK) and DON accumulation in whole grain. The methods were significantly correlated ($p<0.05$) in ranking genotypes for DON accumulation and FDK, while environmental and genotypic effects were observed in the other measures.

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INTRODUCTION

Wheat is one of the most important agricultural crops, with a global production of 690 million tons in 2011 (International Grains Council., 2012). In Michigan, both soft red and soft white winter wheat are grown; with a production value estimated at 341.7 million dollars in 2011 (National Agricultural Statistics Service, 2012). Michigan is one of the largest producers of white wheat in the Great Lakes region. Wheat bran is a vital source of dietary fiber, and increasing numbers of consumers are choosing whole wheat or bran-rich products for their health benefits. A fiber rich diet promotes digestion, reduces cholesterol, minimizes the risk of diabetes and helps in weight control (Kroon et al., 1997). White wheat bran lacks the bitter tannin present in red wheat and therefore can be used to produce more appealing whole wheat products (Boswell and Shroyer, 2000). More flour can be produced from white wheat because it can be milled closer to the bran without negatively affecting color or flavor. Bran from white wheat is a valuable commodity as it provides better flavor and color characteristics in the finished product, and is preferred by consumers (Zhang and Moore, 1999).

Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum* (Schwabe), is one of the major fungal diseases affecting small grains. A number of severe and moderate FHB epidemics over the past two decades have caused severe economic losses to the wheat and barley industry worldwide (McMullen et al., 1997; Snijders, 1990). In addition to yield loss due to shriveled kernels, FHB also results in poor grain quality and mycotoxin contamination (McMullen et al., 1997; Parry et al., 1995). Accumulation of mycotoxins such as deoxynivalenol (DON), nivalenol, and zealerone is a major concern with *Fusarium* contaminated wheat. Exposure to DON may cause diarrhea, vomiting, leukocytosis, gastrointestinal hemorrhage, and even shock like syndrome or death at higher doses. Long-term chronic exposure can affect growth, immune function and reproduction (Pestka and Smolinski 2005). The Food and Drug

Administration (FDA, 1993) has established guidelines for the use of mycotoxin-contaminated grain, with DON contamination higher than 1ppm considered unfit for human consumption. Wheat pericarp color (red or white) has been found to play a role in DON contamination, with reports of high toxin accumulation in white genotypes (Knott et al., 2008).

Recent studies have shown that a higher fraction of toxins accumulates in the outer layers or the bran (pericarp and aleurone layers) of infected kernels (Nishio et al., 2010; Edwards et al., 2011, Abbas et al., 1985). Processes such as cleaning of infected grain to remove chaff, and milling to remove bran reduce DON quantities.in the flour. Bran and shorts, the byproducts of milling that have high DON contamination, are often used as a part of animal feed. Toxin residues can remain in animal products that can be harmful to humans (D'Mello et al., 1999).

Wheat heads are most susceptible to FHB when they are at anthesis (Feekes stage 10.5-10.5.1) when the anthers are extruded. Climate conditions play an important role in determining the incidence and severity of the disease, with warm moist conditions in the spring increasing the risk of FHB epidemics. No-tillage cropping systems, crop rotations with non-host crops and fungicides have been used as ways of reducing FHB disease and subsequent DON accumulation. Although agronomic and chemical methods of FHB control are widely utilized, an integrated approach with the use of genetic resistance provides the best solution for control. Breeding programs use artificial inoculation systems to screen varieties for resistance to incidence (type I) and spread of infection (type II) (Schroeder and Christensen, 1963). Several methods have been described to measure the visual symptoms of FHB in the field to rank and select varieties for FHB resistance. Measurements such as % incidence, % severity and % Fusarium-damaged kernels (FDK – a post-harvest measure) assess multiple types of host resistances. Reports from state performance trials and uniform scab nurseries do not always agree on correlation of these

measures with mycotoxin accumulation in the grain, which is the biggest problem associated with FHB. Moreover, field screening methods and measurements may not be accurate in prediction of disease because they often represent a confounded effect from different resistance types. Some disadvantages of these methods include the variable effect of environment and the inability to measure or control the exact amount of inoculum per spike. It has been shown that the availability of moisture after inoculation affects disease severity and toxin accumulation in grains (Cowger et al., 2009; Culler et al., 2007; Gautam and Dill-Macky, 2008). Thus, choice of screening method and misting treatment post-inoculation can have a variable effect on the ranking of genotypes based on resistance to FHB or DON accumulation.

A higher fraction of DON accumulates in the bran compared to the flour (Abbas et al., 1985; Dexter et al., 1996; Nishio et al., 2010). Although it is standard practice to measure DON contamination in whole grain, toxin accumulation in bran or flour fractions has not been studied to identify presence of genotypic differences. Since white wheat varieties are more valuable for their bran, comparison of red and white genotypes is necessary to evaluate and identify white genotypes with low level DON accumulation in the bran.

For this study, 39 commercial varieties and advanced experimental lines, including 21 soft white and 18 soft red winter lines, representing a range of FHB resistance based on Michigan state performance trials (MSPT, 2007), were selected for the following objectives:

1. To estimate variability for toxin accumulation in bran and flour fractions of milled wheat in locally adapted commercial and MSU experimental lines, and evaluate differences in the proportion of toxin in these fractions based on toxin accumulation in whole grain within groups of red and white soft winter wheat.

2. To evaluate and compare two different methods of inoculation in the field: grain spawn (GS) inoculum followed by misting, and spray inoculation followed by bagging (SB). I hypothesized that since the SB method provides a more controlled environment post-inoculation, with a fixed amount of inoculum applied over each plot at the time of flowering, and each plot receiving the same humidity treatment through bagging, a more consistent measure of type 1 and type 2 resistance can be obtained.
3. To evaluate the role of grain color in the prediction of FHB resistance as assessed visually, toxin accumulation, and DON partitioning between bran and flour.
4. To identify lines with visible resistance to FHB, and lower toxin accumulation in whole grain and milled fractions among soft white and soft red winter wheat cultivars and elite experimental lines adapted to Michigan.

REVIEW OF LITERATURE

1. Wheat

1.1. Wheat: Evolution, History and Importance

Wheat (*Triticum aestivum*) originated approximately 10,000 years ago in the Fertile Crescent (present day southeastern Turkey –northern Syria) (Lev-Yadun et al., 2000). Wheat and barley constituted the principal grain stock upon which old world agriculture was founded. Wheat is one of the earliest plants to be domesticated (Harlan and Zohary, 1966); Nevo, 2002), and it contributed to the emergence of agriculture and was ultimately responsible for the increase in human population by enabling the production of food in large quantities (Nevo, 2002).

The common hexaploid wheat (*T. aestivum*) (AABBDD) and its several sub-species contain 21 pairs of chromosomes with seven pairs belonging to each of A, B, and D genomes. *Triticum aestivum* came into existence after the domestication of tetraploid wheat species, due to a hybridization event, followed by chromosome doubling between the tetraploid *T. turgidum* (AABB) and *T. tauschii* (*Ae. tauschii*) (DD) contributing the D genome (Kihara 1944) (Figure 1). *Triticum zhukovskyi* (AABBGG) evolved from *T. timopheevi* (AAGG), and they comprise another evolutionary lineage. The A genomes of all tetraploid species were contributed by *T. monococcum*) and *T. urartu*. The second genome of tetraploid wheat, B in *T. turgidum* and F in *T. timopheevi*, were contributed by *T. speltoides* (Drovak and Zhang 1990). Tetraploid wheat species in cultivation today are durum wheat and emmer, which share the AABB genome and are inter-fertile. The genetic structure and self-pollination behavior of polyploid wheat species facilitates

genetic diversity through mutation or hybridization. Being polyploid, common wheat can tolerate the presence of gene loci in multiple doses.

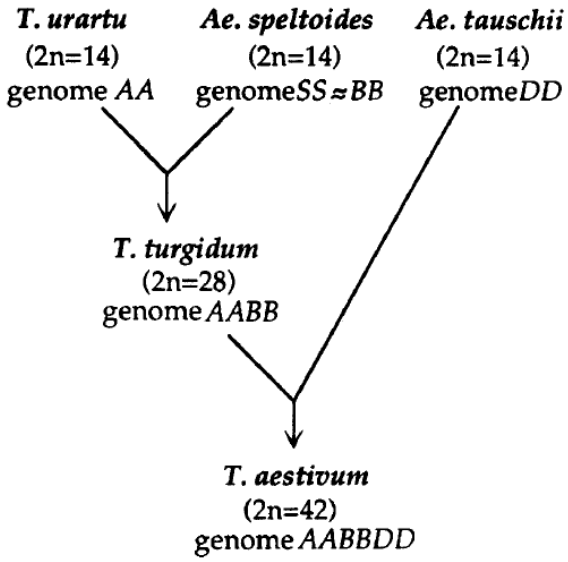

Figure 1. Schematic diagram for evolution of the hexaploid *T. aestivum.* Taken from Feldman et al. (1997)

Wheat is one of the world's largest and most important food crop, with a global production of 690 million tons in 2011 (International Grains Council, 2012). The United States is the fourth largest producer of wheat, with approximately 2 billion bushels (54.41 million tons) produced in 2011 (NASS 2011). In the US, wheat production has declined since the 1980s, as wheat acreage began to decrease. Projections from the USDA-ERS (2011) suggest that although higher yields are obtained, this trend will continue over the next few years as yield gains have not managed to offset the declining acreage (USDA-ERS, 2011). Thus, to meet the growing demands for wheat, continued improvements are necessary in yield and resistance to biotic and abiotic stresses. However, continual selective breeding has resulted in accelerated loss of genetic diversity in the recent decades. For wheat improvement programs to succeed, traditional breeding approaches

should be complemented by non-traditional methodologies that enhance the existing genetic variation. Gene banks have been established to store and utilize the available wild genetic resources. Dynamic *in situ* hybridization (within gene pools and geographic regions) is suggested as a way to maintain, screen, and efficiently utilize the diversity of wild relatives.

1.2.Introducing Genetic Variability in Wheat

Finding sources of germplasm with desired traits among cultivated crops has been a limiting factor in making suitable improvements. One of the consequences of founder effect in hexaploid wheat is the restricted genetic variability, i.e. only a fraction of genetic variability from the parental population is represented (Feldman, 2001). Useful genetic resources can be found among uncultivated (wild) plants, and these genes for resistance to biotic and abiotic stresses can be introduced into existing food crops through wide crosses (Mujeeb-Kazi, 1995). Researchers at International Maize and Wheat Improvement Center (CIMMYT) have pursued bread wheat improvement through interspecific and intergeneric hybridization.

Interspecific hybrids are produced by crossing bread wheat with diploids from *Triticum*/*Aegilops* spp. that have high genetic proximity to the A, B, and D genomes. Related diploid species include *T. tauschii* for the D genome; *T. monococcum* for the A genome; and *T. speltoides* and related accessions for the B genome. *Triticum tauschii* has been utilized for making interspecific crosses, as a wide range of resistances or tolerances to biotic or abiotic stresses have been identified: Karnal bunt (*Tilletia indica*), scab (*Fusarium graminearum*), spot blotch (*Helminthosporium sativum* syn. *Bipolaris sorokiniana*), leaf rust (*Puccinia recondita*), stripe rust (*P. striiformis*), salinity, and

drought (Valkoun et al. 1990; Gilchrist 1999; Mujeeb- Kazi 1999). Variability from *T. tauschii* (DD) has been effectively exploited by screening the accessions for desired traits and making direct transfers by crossing selected accessions with *T. aestivum* (AABBDD) or by crossing with *T. turgidum* (AABB) (Mujeeb-Kazi, 1995). Synthetic hexaploid populations have been created via crosses between *T. tauschii* and *T. turgidum*.

Intergeneric crosses have been explored as a source of introducing genetic variability for wheat improvement by crossing between annual bread wheat species (*Triticum* and *Aegilops* spp.) with different perennial species of *Triticae*. The perennials are important because their natural habitats provide the possibility that they could be potent sources of resistance for several biotic and abiotic stresses. Very low success rate is associated with intergeneric hybridizations (Mujeeb-Kazi, 1995) as the species involved in intergeneric crosses are genomically diverse and rather difficult to cross. Even when successfully combined, the resulting hybrids exhibit little or no intergenomic chromosome association (Mujeeb-Kazi, 1995). Other disadvantages include cross incompatibility, poor vigor in F1, and hybrid sterility. It is general practice to use the higher ploidy species as the female parent to avoid imbalances between the chromosome numbers of the embryo and endosperm and it is generally easier to emasculate the hexaploid *T. aestivum* than the tightly invested florets of most alien species.

1.3. Classes of Wheat and Economic Importance

Wheat is classified based on observable physical distinctions. Wheat is described as either red or white, based on the color of the outer layers of the kernel. A bran color of reddish brown is referred to as red, while yellow or tan colored bran is referred to as white. Hard and soft wheat are classified based on the texture or hardness of seed, usually

explained by allelic differences at a single locus *Ha* on chromosome 5D (Worland and Snape, 2001). The Spring/ Winter growing habit is determined by the sensitivity of the alleles present at the vernalization response loci (Vernalization sensitivity gene- *Vrn3*, Vernalization insensitivity genes- *Vrn-A1*, *Vrn-B1*, *Vrn D-1*, *Vrn-B4*). Spring type alleles are dominant and are insensitive to cold treatment, while the recessive winter alleles normally require at least six weeks of vernalizing temperatures before flowering (Worland and Snape 2001). Spring varieties are planted in spring and harvested in fall. Winter wheat is planted in the fall and harvested in the following summer.

Different classes of wheat are identified for marketing efficiency by exporting countries. The US system recognizes six broad classes of wheat: Hard Red Winter (HRW), Soft Red Winter (SRW), Hard Red Spring (HRS), Soft White (SW), Hard White (HW), and Durum. In Michigan, winter varieties of soft white (SWW) and soft red are of great economic importance, cultivated over 560,000 acres and with production value estimated at $164 million in 2009 (National Agriculture Statistics Service, 2009). Production of SWW in North America is limited to the Great Lakes area (Michigan, New York and Ontario) and the Pacific North-west (Washington, Oregon and Idaho). Given declining acreage of white wheat in New York and Ontario (Johnson, 2011), Michigan is now the major producer of white wheat in the eastern part of the country, catering to the needs of the local cereal industry. Comparison of genetic diversity in SRW and SWW wheat grown in the Eastern US shows the SWW gene pool has developed from a narrow genetic background which is due to the use of only a few elite parents in breeding SWW (Ward 1997).

1.4.Differences between Red and White Wheat

Red and white colored wheat differ in the color of the seed coat. Pericarp color is controlled by three independent homoeologous genes *R-A1, R-B1,* and *R-D1*, located on chromosomes 3A, 3B, and 3D (Metzger and Silbaugh, 1970). The color genes are additive, and red color is dominant to white. Thus, presence of a single dominant allele is sufficient for red color. The alleles *R-A1b, R-B1b,* and *R-D1b* result in red color while white grain color is controlled by the recessive alleles *R-A1a, R-B1a,* and *R-D1a.* In many regions, white wheat suffers from the lack of desirable traits and poor agronomic performance due, in part, to limited breeding efforts in white wheat. The genetics of kernel color makes it difficult to breed white wheat, and introduce desirable traits from red lines (Sherman et al., 2008). For example, in a cross between a three gene red line and a white seeded line, there is only a 1/64 chance of getting a progeny which has recessive alleles at all three loci. White wheat suffers from relatively lower yield and higher susceptibility to diseases. White grain color is also genetically associated with susceptibility to Pre-harvest Sprouting (PHS) and subsequent starch degradation compared to Red (Bassoi and Flintham, 2005; Groos et al., 2002; Himi et al., 2002) and is also more susceptible to accumulation of mycotoxins produced during *Fusarium* infection. However, superior color characteristics (Miskelly, 1984) and possible advantages in milling and end use quality (Taylor et al., 2005) make white wheat attractive for a variety of products.

1.5. Wheat Kernel Composition and Milling Fractions

The wheat kernel is composed of three parts: bran, endosperm and germ. The bran consists of 7 layers that make up the hard protective coating on the kernel and is a

concentrated source of dietary fibers. The aleurone layer is the largest portion of bran and contains concentrated vitamins, minerals and other nutrients. The endosperm constitutes 83-84% of the weight of the grain and consists of starch granules embedded in a protein matrix within large thin walled cells (Yamazaki 1981). The germ contains the plant embryo and accounts for only 2-3% of the grain's dry weight. In the milling process the pericarp and aleurone layer are removed from the wheat kernel and these anatomical structures are separated into the milling fractions known as bran and shorts, respectively. Commercial milling produces multiple fractions of flour and bran and respective particle sizes vary depending on the mill producing it. Milling procedure and efficiency may vary for hard and soft wheat, as when using roller mills, hard wheat is milled easily leaving the bran intact and using successive rolls to scrape the endosperm away from the bran (Yamazaki 1981).

1.6. Wheat Bran and Whole Wheat Products

Consumers today are health conscious, and the consumption of high fiber foods increasing. Wheat bran is recognized as an important source of fiber, and use of refined flour has shifted to whole grain flour. A number of whole wheat and wheat bran products are available in the market, with several dietary and nutritional benefits. Higher fiber content in the diet promotes digestion, reduces cholesterol, minimizes risk of diabetes, and helps in weight control. Bran also supplies a high amount of antioxidants and micronutrients, and reduces the risk of colon cancer (Kroon et al. 1997).

Addition of bran to flour for bread making results in altered loaf volumes, changes in texture and crumb size (Galliard and Gallagher, 1988; Gan et al., 1992; Moder et al., 1984; Zhang and Moore, 1999). While the bran particle size contributes to different

dough rheological properties and bread making quality, other factors are important for consumer acceptance. Based on a consumer panel, general acceptability of products using bran from white wheat was significantly higher, as it results in bread with a uniform and lighter crust color (Zhang and Moore, 1999). Seed coat from red wheat contains a higher concentration of tannin-catechin and polyphenol oxidase (Park et al., 1997) that may have a negative effect on the flavor of the end product.

2. Fusarium Head Blight

2.1. History and Economic Importance

Fusarium head blight (FHB) or "Scab" is an important fungal disease affecting small grains (Yi et al., 2001). With a number of severe and moderate epidemics over the past two decades, FHB has caused severe economic losses to the wheat and barley industry worldwide (McMullen et al., 1997; Snijders, 1990). Occurrence of FHB results in loss of yield, poor grain quality resulting from shriveled and "tombstone" kernels, and mycotoxin accumulation (McMullen et al., 1997; Parry et al., 1995).

2.2. Causal Organisms

Causal organisms of natural infection have been identified as *Fusarium* spp., such as *F. graminearum, F. culmorum, F. avenaceum, F. moniliforme, F. equisetti, F. acuminatum* and *F. semitectum,* and *Microdochium nivale*. The most prominent of these are: *F. graminearum* (Schwabe), which is responsible for FHB in North America, and *F. culmorum* (W. G. Smith), which has been identified as the cause of FHB in Europe. *Fusarium* spp. have a wide host range including maize and small grain cereals like wheat, oats and barley.

2.3. Infection and Progress of Disease

Conidia (asexual spores) of *F. culmorum*, and conidia and ascospores (produced by sexual reproduction in perithecia) of *F. graminearum* growing on debris (grain, straw etc.) from previous infected crops provide the primary source of inoculum (Birzele et al., 2002; Snijders and Perkowski, 1990) that are dispersed (to the wheat head) through rain splashes (Fernandez et al., 1993; Parry et al., 1995). The role of cultural practices in FHB infections has been reviewed by Champeil et al. (2004). Higher frequency of FHB is associated with the use of maize, durum wheat or oats in the crop rotation cycle prior to wheat (Champeil et al., 2004; Dill-Macky and Jones, 2000). Limited soil tillage increases the frequency of head blight, whereas deep tillage (ploughing) decreases it (Dill-Macky and Jones, 2000; McMullen et al., 1997). Effects of irrigation and canopy density have been investigated since as inoculum is primarily dispersed by splashing, a low canopy density and presence of irrigation can favor spore dispersal (Mesterhazy, 1995). Wheat plants have a restricted period of susceptibility during which *F. graminearum* requires relatively high temperatures (20-30°C) and 48-60h surface wetness (Lacey et al., 1999; Snijders and Perkowski, 1990). Rainfall also assists in conidial dispersal and, coinciding with the availability of wheat heads at anthesis in late spring, provides suitable conditions for colonization. FHB infection is highest for inoculation during the short period of anthesis; little or no disease is observed for inoculations between head emergence and anthesis; and visible disease symptoms decrease steeply for inoculations after mid anthesis (Lacey et al., 1999).

Fusarium spores germinate on the abaxial surface of the glumes and in the floral cavity within 12 hours post inoculation (hpi), giving rise to unbranched hyphae that

frequently come in contact with stomata (Pritsch et al., 2000; Wanjiru et al., 2002). *Fusarium graminearum* has been reported to penetrate the adaxial surface and the stomatal opening of the floral brackets such as the glume, lemma and palea (Pritsch et al., 2000; Zhang et al., 2008). The ovary and the floral brackets are invaded by 36 hpi, while the host cell walls and middle lamella in the vicinity of the hyphae demonstrate reduced cellulose, xylan and pectin (Wanjiru et al., 2002). Inter- and intracellular hyphae are present throughout the ovary and floral brackets by 76 hpi (Pritsch et al., 2000; Wanjiru et al., 2002; Zhang et al., 2008). Hyphae spread down, and to a lesser extent up, the rachis (Jansen et al., 2005; Wanjiru et al., 2002), and then spread down the plant (Kang and Buchenauer, 2000; Zhang et al., 2008).

It has been suggested that initial FHB infection occurs on extruded anthers (Pugh et al., 1933; Strange and Smith, 1969; Strange and Smith, 1971), where the presence of growth stimulants promotes *F. graminearum* growth (Strange and Smith 1969; Strange and Smith 1971). Structure of florets, either open (induced by hot temperatures and drought conditions) or closed (induced by low temperatures and moist conditions) play an important role in infection process. However, the role of anther extrusion (AE) is not absolute and in spite of correlations, selecting for low AE could result in loss of some resistant sources (Skinnes et al., 2008).

Before maturity, wheat heads infected with FHB appear water-soaked, lose their chlorophyll and become straw-colored. When the heads are mature, it is difficult to distinguish infection symptoms from natural senescence. In case of severe infection, pinkish-red mycelium and conidia develop and the infection spreads through the entire head. Infected kernels become shriveled and discolored with a white, pink or light brown

pericarp (Bottalico and Perrone, 2002). Infections occurring in early stages of growth may result in seeds not being formed while late infections do not exhibit visual symptoms of colonization. A large amount of mycotoxin can be produced even in the lack of visible infection (Del Ponte 2007; Cowger 2010).

Several agronomic, chemical and biological methods of control have been suggested for FHB management in wheat. Growing resistant germplasm, use of non-host crops in rotation cycle, tillage practices are known to reduce the risk of Fusarium head blight. Triazole fungicides containing tebuconazole are currently the most effective (Homdork et al., 2000; Mesterhazy and Bartok, 1997), when applied during heading to cover all the spikes. Biological control agents offer another strategy of FHB management.

2.4. Production of Mycotoxins

Many *Fusarium* spp. have the potential to produce secondary metabolites, including mycotoxins in grain (Parry et al., 1995) reducing grain quality and rendering it unfit for consumption. Mycotoxins produced by *Fusarium* spp. are typically Type A or Type B trichothecenes and zearalenone (ZON). The major Type A trichothecenes associated with *Fusarium* include T-2 toxin (T-2) and HT-2 toxin (HT-2). Deoxynivalenol (DON), a Type B trichothecene, is the most prevalent toxin associated with FHB and is often regarded as an "indicator-toxin" for FHB. Several countries including Austria, Canada, Romania, Russia and the USA have established tolerance limits for DON in cereals and finished products (FAO/WHO 2001). Other Type B trichothecenes such as Nivalenol (NIV), 3-O-acetyl 4-deoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) have also been identified in FHB infected grains. Zearalenone is an uterotropic and estrogenic compound (Foroud and Eudes 2009). Based

on the major trichothecene produced by a given strain, *Fusarium* spp. are classified as DON or NIV chemotypes, with NIV chemotypes mainly associated with maize (Sydenham et al., 1991). DON chemotypes of *F. graminearum* are classified into two types: DON-chemotype IA, producing DON and 3-ADON (from warmer regions, including European strains) and DON-chemotype IB, producing DON and 15-ADON (from slightly cooler regions, including mostly American strains) (Miller et al., 1991). Different levels of aggressiveness and pathogenicity of *F. graminearum* isolates have been attributed to the trichothecene producing potential of the isolate (Mesterhazy, 2002). Isolates without the ability to produce trichothecenes were able to cause initial FHB infection, but spread of the disease across a wheat spike was not observed (Bai et al., 2002). Toxin contamination is also influenced by time of harvest. A study of mycotoxin concentrations in wheat after inoculation with *F. culmorum* showed that DON was detected in higher concentrations in earlier stages of development and it did not increase in mature grain, while ZON was produced at a later stage of growth and in lower amounts (Matthaus et al., 2004).

Trichothecene biosynthesis is well studied and the responsible TRI gene cluster is known (Kimura et al., 2007). Mutants with disrupted TRI5 gene (coding for trichodiene synthase) have dramatically reduced virulence and are no longer able to spread through the rachis. Ilgen et al. (2009) studied the induction of trichothecene biosynthesis using lines that were GFP tagged under the control of endogenous promoter of TRI5. Most fluorescence was observed at the rachis nodes, suggesting an important role of these structures in spread of infection. TRI5 mutants were not able to overcome the heavy cell wall thickenings of the rachis node (Ilgen et al., 2009).

2.5. Deoxynivalenol

The US Food and Drug Administration (FDA) regulates the use of mycotoxin contaminated wheat, and grain with DON concentration higher than 1 ppm is declared unfit for human consumption. For use as livestock feed, DON concentration in the finished feed should not be higher than 5 ppm (swine, dairy) and 10 ppm (poultry). Long term surveys of DON contamination of naturally infected wheat grain have shown presence of DON in quantities higher than the allowable limits. Mycotoxins have been associated with acute mycotoxicoses in livestock, and to a lesser extent in human (IARC1993). Acute administration of DON causes decreased feed consumption and emesis (Creppy, 2002). Symptoms of exposure to DON in animals include diarrhea, vomiting, leukocytosis, and gastrointestinal hemorrhage, with extremely high doses resulting in shock-like syndrome and death (Pestka and Smolinski, 2005). Toxins in diet not only cause serious health risks the animals, but toxin residues can remain in products that can be harmful to humans (D'Mello et al., 1999). On a molecular level, DON inhibits protein synthesis by binding to ribosomes and activating critical cellular kinases (Pestka et al., 2004). Long-term chronic exposure can affect growth, immune function (enhancement or suppression) and reproduction (Pestka, 2007; Pestka and Smolinski, 2005). Because it is heat tolerant, DON is not eliminated in finished food products through cooking, baking or brewing (Scott 1991). Dry milling of wheat (process of removing bran) results in DON reduction of up to 40% in flour, and 60% for highly contaminated grains (Pestka, 2007). Quantification of DON in different fractions of milled wheat shows that a higher fraction of the toxin accumulates in the bran (Rupp, 2002; Thammawong et al., 2010; TrigoStockli et al., 1996).

Surveys have identified DON as the most frequent contaminant associated with FHB in wheat all over the world, although variation has been observed in quantities of DON in infected grain (Bai et al., 2001; Creppy, 2002). Moisture levels, temperature and the timing of infection also determine the levels of DON produced. A direct prediction of toxin contamination cannot be made based on incidence of FHB, crop yield loss, or scabby grain severity (Bottalico and Perrone, 2002). In inoculation experiments, FHB infection and DON accumulation in grains varied with infection time. Contamination with DON peaked for inoculations at mid-anthesis, and small amounts of DON were produced in case of later inoculations, continuing up to the late milk- early dough stages, even in the absence of visual symptoms (Lacey et al., 1999). Periods of rain soon after anthesis and late infections favor low-symptom, high-DON scenario (Del Ponte 2007; Cowger 2010).

2.6. Types of Host Resistance

Several mechanisms of resistance, both active (physiological) and passive (morphological) are known to help against FHB infections. Passive mechanisms include plant height (dwarfness increases susceptibility), presence of awns (presence increases severity), flowering type (AE promotes susceptibility), and infection avoidance by early flowering in the boot stage (Mesterhazy, 1995). Five types of active mechanisms are summarized in Table 1.

Table 1. Different mechanisms of host resistance to FHB described in wheat

Resistance	Description
Type I	Resistance to initial infection (Schroeder and Christensen, 1963)
Type II	Resistance to disease spread (Schroeder and Christensen, 1963)
Type III	Resistance to kernel infection (Mesterhazy, 1995)
Type IV	Tolerance against FHB and trichothecenes (Mesterhazy, 1995)
Type V	Resistance to trichothecene accumulation (Miller et al., 1985)

Resistance to initial FHB infection (Type I) and resistance to the spread of disease from the point of infection (Type II) have been studied extensively and are used in breeding programs to evaluate genotypes in greenhouse and field. Type I resistance is measured as the percentage of spikelets exhibiting symptoms upon exposure to the pathogen. Plants are typically sprayed (at anthesis) with microconidial or ascosporic suspensions and high humidity is maintained by bagging infected heads or by mist irrigation for a few days after inoculation. An alternative method is using grain spawn inoculum, where infected wheat or corn is dispersed in the field to mimic natural conditions for infection (Rudd et al., 2001); Dill-Macky 2003). Resistance is measured as "incidence" (percentage of diseased spikes), "severity" (percentage of infected spikelets on diseased spikes) and "index" (product of incidence and severity). The disease index, the best estimate of resistance to FHB in the field, is a combination of Type I and Type II resistances (Rudd et al., 2001). Assessment of type I resistance is not accurate, and is often confounded with Type II resistance in the field. Environmental conditions in the field are variable and the amount of inoculum per spikelet is not consistent and cannot be measured, making it difficult to precisely assess Type I resistance (Mesterhazy et al., 2008; Rudd et al., 2001; Dill-Macky 2003). Availability of moisture post infection is shown to enhance FHB, FDK and DON accumulation, suggesting an influence of screening method on ranking and selection of genotypes (Cowger, 2009).

Type II resistance is evaluated on individual plants, by injecting a quantifiable amount of inoculum into individual spikelets at anthesis, with high humidity maintained

for a few days after inoculation. Resistance is measured by the number of infected spikelets, typically below the initial point of infection (Rudd et al., 2001).

Types III, IV and V of resistance cannot be quantified directly. Resistance to kernel infection can be assessed and reported as Fusarium damaged kernel (FDK) evaluation. DON quantification in grains gives a measure of tolerance against trichothecenes. Since toxin accumulation is a major concern in FHB infected grain, several studies have been conducted to correlate visible symptoms with DON contamination (Beyer 2007). Type II resistance and FDK contamination are not correlated with DON contamination (Cowger 2010, Beyer 2007) and a large effect of environment and genotype are responsible for inconsistent results over years (Foroud and Eudes 2009). Type V resistance, resistance to trichothecene accumulation can be subdivided into two classes: a) class 1- genotypes are able to chemically modify or degrade trichothecenes, and b) class 2- genotypes that have the ability to inhibit trichothecene synthesis (Boutigny et al., 2008)

MATERIALS AND METHODS

1. Plant Materials

Thirty nine commercial varieties and MSU advanced lines were selected for the study (Table 1). The lines included 18 soft red and 21 soft white winter lines well adapted to Michigan. A wide range of FHB resistance levels (including DON contamination levels) was represented within each group according to previous data from the Michigan State Performance Trial (MSPT, 2007).

2. Planting of Field Trial

Field study was carried out over three years- 2009, 2010 and 2011, with two locations planted each year (Table 1). The 39 lines were planted in each location in an alpha lattice design with three replications. All locations had Capac loam type soil, and 0-3 percent slope, except the MSU Clarksville research center in 2009 which had a Lapeer sandy loam soil type and 2-6 percent slope. A Hege 95 planter was used to plant 5 ft long plots at MSU agronomy research farm in 2009 (4 rows at 15in row spacing, 0.5in seed spacing) while 6 ft or 12 ft long plots were planted in all other locations using an Almaco heavy duty grain drill (ALMACO, Nevada, Iowa) (6 rows at 7.5 in row spacing, 2 million seeds per acre, ~4.2 in seed spacing). All plots were trimmed to a length of 6 ft before heading began in spring. Fertilizer: 196lbs of 46-0-0 (urea) (corresponding to 90 lbs N) and Herbicide: 0.5 oz Harmony Extra (dupont), plus non-ionic surfactant were applied once in the spring at all locations. Flowering date was recorded for each plot as the number of days past January 1 when 50% of the wheat spikes in the plot had reached anthesis.

Each location was artificially inoculated with *Fusarium graminearum* using the grain spawn (GS) inoculum or spray and bag (SB) method, as shown in Table 2 and described below.

2.1.Inoculation

Plots were inoculated with *Fusarium graminearum* isolate PH1 (NRRL31084) during flowering using one of two methods: grain spawn inoculum (*F. graminearum* colonized cereal grain spread in the field) followed by artificial misting, or spray inoculation with conidial spores followed by 48 hours of bagging (Table 2). See below for details of the two different inoculum types.

2.2. Spray Inoculation- Liquid Inoculum

Inoculum was generated by culturing *Fusarium graminearum* isolate PH1 (NRRL31084) in Carboxymethyl Cellulose (CMC) liquid medium (Cappelli and Peterson, 1965; Cappelli and Peterson, 1971). Ten microliters of stock culture was added to autoclaved media (100 ml in 250 ml flasks). The culture was shaken at 200-250 rpm and 25°C for four days. Mycelia were removed by filtration with a sterile cheese cloth. Spore count was taken using a haemocytometer and the culture was diluted to a final concentration of 50,000 spores/ml. One drop of Tween 20 was added per 100 ml culture before inoculations.

Plots were sprayed uniformly with 15 ml of 50000spores/ml liquid inoculum on the day of flowering using a sprayer from R&D sprayers (BELLSPRAY INC., Opelousas, LA) fitted with TeeJet 8002VS nozzles (TeeJet Technologies, Springfield, IL). The nozzles were adjusted at a 20° angle and one nozzle at the extreme right end was plugged to cover the plot width of 4ft. To ensure an even spread of inoculum per plot,

pressure was maintained at 40 psi and the spray-boom was held at a height of 2ft over the wheat heads (Figure 2). Transparent plastic bags of 43 x 48in size and 0.98 mil (25 micron) thickness (Grainger Inc., Lake Forest, IL) (4 per plot) were used to cover the plots and secured at the base of plants using twist ties. Before use, folded bags were punched at the top to allow for air flow and ventilation to the plants. All bags were removed after 48 hours.

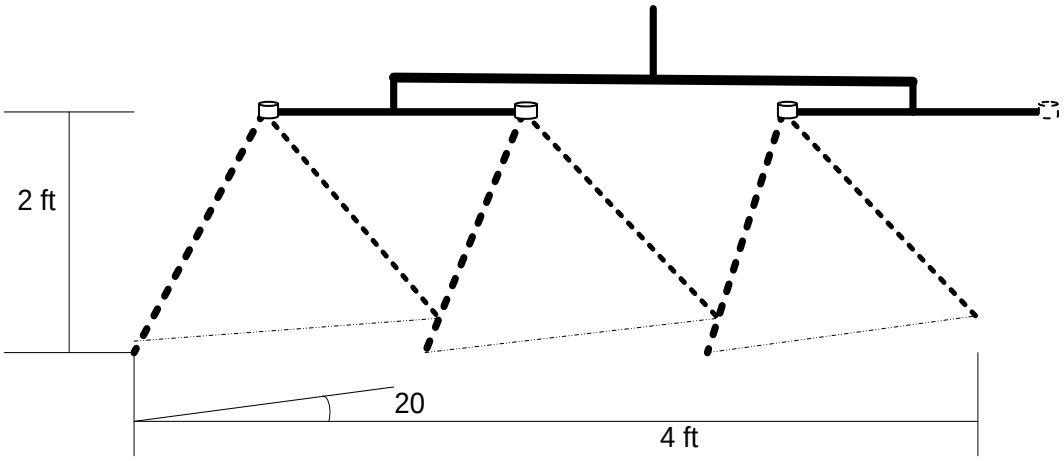

Figure 2. Modifications in spray equipment: Adjustments in the hand held spray boom. Figure not to scale.

2.3.Grain Spawn Inoculum

Grain spawn inoculum was prepared in two batches about 2 weeks apart, using wheat and barley mixture in 2009 and 2010; and using only wheat in 2011. Per batch, seed (30lbs per acre) was divided into 21 milk jugs (800-900 ml seeds per jug); covered with distilled water and soaked overnight. The seed was then drained of excess water and autoclaved for two 45 minutes cycles (seed was cooled overnight between runs). The sterile grains were inoculated with liquid *F. graminearum* culture (as prepared in 2.2) at a rate of 10-15 million spores/ jug. The jugs were shaken well and incubated at room temperature for 3 weeks, with shaking provided every alternate day. The infected grain

was then transferred to sterile aluminum trays and allowed to dry in a biohazard hood for a week (the grain was stirred every day to allow even drying and break any clumps). Once dry, the inoculum was stored in buckets at room temperature until ready to spread in the field.

The inoculum was spread evenly over the plots (two handfuls sprinkled over each plot) twice: at approx. 2 weeks prior to anthesis, and at the start of anthesis. A misting system was set up using overhead sprinklers, running for 2 minutes every hour from 7 am to 10 pm and one run at 2 am. Misting was provided from the time of first inoculum application to help in the development of spores and continued through anthesis to help in spore dispersal.

3. Visual Evaluation of Disease

In the field, visual symptoms were noted at 14 and 21 days post inoculation (dpi) of each plot. In case of the GS method, the day of flowering was treated as the day of inoculation. In 2011, disease ratings were taken only at 21 dpi as visual symptoms were not obvious at earlier days.

Incidence- Number of wheat heads that are infected with FHB out of an arbitrary collection of 10 heads. Reading taken 3 times per plot on a single day, averaged and reported as percentage.

Severity- Number of spikelets infected per infected wheat head. Reading taken 3 times per plot on a single day, averaged and reported as percentage.

FHB Index-FHB index was calculated as Index = [% Incidence x % Severity]/100

4. Post-Harvest Sample Handling and Analysis

All plots were harvested after the plants had completely senesced, using an Almaco SPC40 harvester (ALMACO, Nevada, Iowa). The air passing through the sieves was lowered to prevent loss of scabby seeds. The grain was then cleaned using an air column cleaner (CB-2A, AGRICULEX Inc., Guelph, Ontario, Canada) and the ventilation scale was set at five. All chaff was picked out by hand before milling and toxin analysis. Cleaned grain was stored at 4°C before milling.

4.1.Fusarium Damaged Kernel (FDK) Scoring

A scoop of approximately 1000 kernels was sub-sampled from the cleaned samples, for FDK rating. Each sample was spread evenly in a 5.5in x 5.5in x .8in white plastic weigh-boat, and compared to standard checks placed in similar containers. Separate sets of checks (0-100%) were created for red and white lines, by mixing suitable number of scabby and healthy heads. For example, 30% check has 300 scabby and 700 healthy seeds.

4.2.Milling and Toxin Analysis

A subsample of 10g of cleaned grain was taken for quantification of deoxynivalenol (DON) in the whole grain. The whole grain sample was ground to an approximate 20 mesh size in a Stein Mill (Model M-2, Fred Stein Laboratories Inc., Atchison, KS) at University of Minnesota. Another subsample of 100g was milled to separate bran and flour fractions. A Quadrumat Jr. Mill (Brabender® GmbH & Co. KG, Germany) with a screen with mesh size 240 microns was used for milling. Moisture content was measured before milling, and was found to vary between 11-12%. % Flour yield was calculated as 100 x [flour weight (g)]/ [flour weight (g) + bran weight (g)] (Nishio, 2010). This calculation accounts for the loss of screenings after 10 min of

milling. An average flour yield of 67.84% was observed. A whole grain, bran and flour sample of each plot were sent to the DON testing laboratory at University of Minnesota where DON was quantified using Gas chromatography Mass Spectrometry (GC-MS) (Fuentes et al. 2005;(Mirocha et al., 1998).

Apart from the measured DON quantities in bran (DON-bran), flour (DON-flour), and whole grain (DON-std), DON in entire kernel (DON-kernel) and DON partitioning ratio in flour were calculated for each variety as described by Nishio et al. (2010)

DON-kernel = [DON-flour x % flour yield /100] + [DON-bran x (100 - %flour yield)/100]

DON partitioning ratio = [(DON-flour/ DON-kernel) x % Flour yield] (Nishio 2010)

5. Statistical analysis

From a total of 720 possible data points (120 plots x 6 environments), visual data from 33 plots were missing due to flooding damage, error in or missed inoculation, or excessive heat damage due to bagging. Four of 720 samples were missing for DON quantification in whole grain (total DON response variable). Samples from the year 2009 were stored for a year at 4°C before being milled, and 55 samples from environment 1 (replication 3 and some random) 30 samples from environment 2 (random) had to be discarded due to molding. Therefore, for the calculation of simple means for DON-bran, DON-flour, DON-kernel and DON partitioning ratio, a separate data set consisting of environments 2-6 (Table 1) was used.

Each year x location combination was treated as an environment (6 levels). To maintain homogeneity of data during analysis, flowering dates were classified into five levels (1-5) nested within each environment. The alpha lattice blocking, replication,

environment and flowering date were used as random factors in the model, to evaluate the fixed effects of variety and method on the response variables. Simple means for effect of variety (genotype) and method were calculated using proc mixed in SAS 9.2. Data was found to be near normal; and variance was not homogenous across all years. Data was transformed according to the λ value predicted for the variable by box-cox transformation, when required. A total of 9 entries were removed as outliers based on the Cook's distance calculated for each variable by the "influence" command in proc mixed. Data from 2009 spray and bag method was found to heavily influence the mean estimates of varieties, and hence was treated as a third method "SB1". For comparisons between Red and White groups, student's t test was used.

RESULTS

1. Comparison of Inoculation Methods

Weather conditions varied substantially over the period of study. Considerable effect of environment, due to atypical temperatures in 2010 and 2011, was observed on flowering dates and FHB infection. The average daytime temperature during the period of inoculation was 70.9°F in 2009, 76,7°F in 2010, and 82.05°F in 2011. A maximum recorded temperature during this period was 75.02°F in 2009, 87.8 °F in 2010 and 87.59°F in 2011. In 2011, the temperature and relative humidity in bagged and un-bagged plots over the period of inoculation was measured using data loggers. Temperature inside bags crossed 100°F, when the outside temperature was above 85°F. Four such hot days, when the temperature crossed 100°F in bagged plots, were observed in 2011. Data loggers were not used for temperature measurement in 2010, but from data obtained from the Michigan automated weather network for stations nearest to the field locations, it is estimated that there were three hot days during the period of inoculation. Heat stress was observed in plots inoculated with SB method in both years. Heads with visible signs of heat damage were not included in the calculation of visual symptoms, and removed from the plot before harvest.

The conditions in 2009 were cooler and more suited for the SB treatment making it was easier to see differences in visual symptoms between plots in the field. For the measured response variables, mean values for all response variables observed in 2009 were much higher, and had a higher variance than in years 2010 and 2011. In order to maintain the integrity of data and reduce errors, SB inoculation in 2009 at Clarksville (environment 1) was treated as a third method – SB1. Overall analysis of variance

(ANOVA) was significant for the effect of variety and the interaction term variety x inoculation method at α=0.01, but was not significant for inoculation method alone. Grain color was not treated as a fixed effect in the analysis. However, varieties and their simple means for the disease response measures were sorted by grain color, and the two groups were compared using student's t test.

1.1. Comparison of Response Variables

Correlation coefficients between the different measures of diseases response within each method are summarized (Table 3). For the GS method of inoculation, all visual response measures except incidence were significantly correlated ($p \leq 0.05$) with DON accumulation for red genotypes. Studying the rankings of red genotypes, the measures index and severity were found to correlate significantly with DON, FDK and each other ($p \leq 0.05$). Incidence was found to correlate significantly with index and FDK, but not DON or severity ($p \leq 0.05$). In white genotypes, only FDK showed a significant correlation with DON contamination ($p \leq 0.05$). Incidence and severity measures from the field correlated with index, as expected from index calculation.

Methods SB and SB1 were expected to perform in a similar way, as they represent the same method with possible heat stress in SB. Both SB and SB1 methods ranked white genotypes consistently over all response measures. FDK, index, incidence and severity showed a significant positive correlation ($p \leq 0.05$) with DON contamination in white lines. All visual measures were also found to correlate with one another ($p \leq 0.05$). Comparing red genotypes, DON contamination correlated with FDK, index, incidence, and severity in SB1 and FDK, index and severity in SB. In SB1, index, incidence, and severity correlated among themselves ($p \leq 0.05$) but not with FDK.

1.2.Comparison of Methods

Comparison of inoculation methods for each response variable is summarized in Table 4. Significance of all correlation coefficients was tested at $\alpha=0.05$. Methods GS, SB and SB1 ranked varieties in a similar way for DON contamination. Correlation coefficients for all three methods for DON are significant in both red and white genotypes at $\alpha=0.05$. FDK scores of white genotypes in the three methods show significant correlation, while for red genotypes, SB method correlated with GS and SB1. Methods GS and SB1 were not significantly correlated for FDK scores in red genotypes. The three methods did not show much significant correlation with one another for field visual measures of index, incidence and severity among red genotypes. When comparing white genotypes, however, all methods are significantly correlated for measure of severity. Methods SB and SB1 were not correlated with GS for a measurement of incidence in red or white genotypes.

1.3.Comparison of Genotypes

Individual rankings of the 18 red and 21 white lines were not consistent across all response variables and methods. On classifying the red and white lines into three categories based on 33^{rd} and 66^{th} percentiles; it was found the same few lines were ranked in the “high” and “low” categories for most response variables in all the methods (Tables 5,6). Genotypes ranked in the middle group showed a lot of variation and were classified as better or worse depending on method of inoculation and measure of disease response. Simple means for each genotype were calculated for all response variables (Tables 7, 8).

A possible effect of flowering date was identified on mycotoxin accumulation in grain. Spray and Bagging method should provide a more controlled environment than misting for inoculation studies in the field, reducing the effect of environment.

1.4. Effect of Grain color

There is a significant effect ($\alpha < 0.01$) effect of color on the DON accumulation in whole grain as well as bran and flour samples. White lines consistently accumulated higher quantities of DON in the whole grain even though visual assessment ranked both red and white lines similarly. Effect of grain color on FDK, index, incidence and severity were not significant overall, or by method.

2. DON Accumulation in Milled Fractions

The calculated DON-kernel correlated positively ($p<0.01$) with DON-std (DON contamination in whole grain), which is the DON accumulation test routinely carried out by breeding programs for selection (Fig. 5). DON-std and DON accumulation in bran and flour fractions were correlated at $\alpha=0.01$. Regression analysis between DON-bran, DON-flour and DON-kernel showed positive linear relationships between DON-bran and DON-kernel ($R^2=0.97$ in white and $R^2= 0.95$ in red genotypes), and DON-flour and DON-kernel ($R^2=0.94$ in white and $R^2=0.96$ in red genotypes) (Fig. 6). A higher DON-kernel value corresponds to higher toxin concentrations in both bran and flour, irrespective of grain color.

Red and white genotypes were categorized into groups based on each of DON-bran, DON-flour, DON-kernel, Partitioning ratio and DON-std (Tables 9, 10). The categories were consistent in ranking the same lines as low or high based on 95% LSD values compared to lowest and highest values in each variable.

DON accumulation in bran fraction was consistently higher than in the flour in both red and white genotypes. DON quantities in bran were approximately twice of that in flour (on average, 2.24 in red and 1.95 in white genotypes). Genotypes tested showed a range DON ratio between bran and flour fractions: 1.88 to 2.58 in red, and 1.51 to 2.36 in white genotypes. ANOVA tests show a significant effect of genotype on DON ratio. There are varietal differences in both groups for DON ratio but the trait is not correlated with DON-std, DON-kernel, DON-bran and DON-flour (Tables 9, 10).

The partitioning ratio of DON in flour, calculated by weighing in the flour recovery % was also not significantly different between red and white genotypes, and could not be predicted by DON-std or DON-kernel (Fig. 7). The range of partitioning ratio of DON in flour varied between 44.54 to 54.24 in red, and 50.65 to 60.61 in white genotypes. White genotypes accumulated significantly higher ($p<0.01$) amounts of toxin in whole grain, bran and flour fractions, and calculated total (Fig. 8).

DISCUSSION

1. Choice of Inoculation Method

Resistance to FHB is multifaceted, best described as a combination of five different types of resistance (Mesterhazy, 1995). Host resistance has been extensively studied in wheat, using different inoculation methods and disease response measures in laboratory, greenhouse and field. Various traits of the disease such as % incidence (type I resistance), % severity (type II resistance), FHB index, FDK (type IV resistance) and mycotoxin contamination in grain (type III, V resistance), and their interactions have been studied extensively. A number of studies, including the uniform *Fusarium* nursery trials conducted by the US Wheat and Barley Scab Initiative (Sneller et al., 2011) measure these disease traits regularly and have reported a large effect of year and environment (Lemmens et al., 2004). Environment is recognized as the major factor that determines severity of natural and artificial infections (Parry et al., 1995).

Of all environmental factors, moisture/ humidity/ rainfall during and after infection is critical in determining the level of FHB. In this study, two methods of artificial inoculation in the field were explored. In GS, humidity is controlled by a misting system similar to Snijders and Perkowski (1990). Bagging of inoculated heads has been recommended by several researchers as an alternative method for studying FHB resistance in the field by providing a more stable microclimate for each genotype studied (Mesterhazy et al., 1999; Wang and Miller, 1988). A study of spray inoculation methods, comparing humidity treatment by irrigation (misting) and bagging found that providing optimal moisture conditions by bagging for a longer period of time resulted in more disease as compared to the treatment without bags (Lemmens et al., 2004).

The SB method was studied in a total of four environments, three of which were affected by heat stress. SB method in environment 1 (SB1), which is expected to be a more typical representation of the attributes of this inoculation treatment, resulted in significantly higher levels of visual disease as well as toxin compared to the GS method in the same year ($p<0.05$), following the same trend as Lemmens et al. (2004). Effects of inoculation method on any of the disease traits measured were not significant. However, a significant effect ($p<0.05$) of genotype and genotype-method interaction was detected for all response variables.

The methods GS, SB, and SB1 were compared for two groups of genotypes based on grain color (Table 4). In both red and white genotypes, the three methods were significantly correlated with one another for a measure of DON accumulation. The methods were also correlated when comparing FDK scores in both red and white genotypes (with the exception of GS and SB1 in red genotypes).

Within each method, DON accumulation was always found to strongly correlate ($p<0.05$) with FDK scores, contradictory to some reports (Mesterhazy et al., 1999; Perkowski et al., 2008). Severity was correlated with DON accumulation for both red and white genotypes in SB and SB1 methods. Incidence was significantly correlated ($p<0.05$) with DON in SB and SB1 method for white, and in only SB1 method for red genotypes. Similar studies, comparing various disease traits and measurements, have reported varying results, attributed mainly to the climate in particular years. Severity and DON accumulation were found to be significantly correlated by Wang and Miller (1988), Gilbert et al. (1993), which was not confirmed by Mesterhazy (1999). All disease traits were correlated ($p<0.05$) in SB and SB1 methods for white genotypes, while in GS

method, significant correlations ($p<0.05$) were observed between most disease traits in the case of red genotypes.

Disease symptoms such as incidence and severity, as well as DON accumulation, are positively influenced by the period of moisture availability during and following infection (Lacey et al., 1999; Cowger et al., 2009; Culler et al., 2007; Gautam and Dill-Macky, 2008). With the GS method, differences in flowering dates could have altered the misting duration for genotypes. The SB method allows a greater control on the timing of infection and humidity treatment in each plot (Prange et al., 2005). This can explain the consistency of FHB incidence in SB and SB1 methods over GS. It is possible that the SB method provides a better estimation of type I and type II resistance, as measured by incidence and in the field, especially in the case of white genotypes (incidence and severity significantly correlated with all other measures). The highest visible amount of disease was observed with the SB1 method, with presence of shriveled as well as scabby kernels in harvested samples. This could have played a role in biased FDK scores for red genotype.

Average flowering dates were obtained for each genotype, over all six environments and genotypes were classified into five groups of very early, early, moderate, late, very late flowering times. White genotypes had an even distribution of genotypes over the flowering groups, while red genotypes flowered relatively early, with the majority of genotypes flowering in early and moderate groups. This could explain some of the differences between methods when comparing red and white genotypes. SB and SB1 methods were more consistent in the ranking of white lines for both visual disease traits and DON accumulation. The flowering time of red lines, unfortunately,

coincided with the hot days observed in years 2010 and 2011. These differences in flowering dates could explain the difference in GS and SB methods for the visual measures of disease between red and white genotypes. The effect of only flowering date on the two methods is vague, as it is also confounded with genetic resistance and susceptibility to FHB. Lack of correlation between visible disease and toxin accumulation could be explained by infection in premature heads in the early flowering genotypes (Lemmens et al., 2004; Bai and Shaner, 1994). In premature wheat heads, FHB infection can cause wilting above the point of infection, giving the appearance of higher visual infection, but cause a reduction in DON accumulation (Bai and Shaner, 1994) Interestingly, superior white lines (multiple low rankings across different responses and methods), such as E6003 and E6055, had the earliest flowering dates in the group suggesting escape as a mechanism of resistance. Red genotypes Truman, and OH-02-126, ranking consistently in the low disease response group, were the last ones to flower in the red group.

SB method, with heat stress, resulted in very low estimates of all visible traits of FHB. However, significant amount of DON still accumulated in the grain showing that high level DON accumulation could result in grain with lower visible symptoms of disease. This observation is in agreement with results from Mesterhazy (1999), who suggested different modes of resistance (type III or V) could be playing a role in toxin accumulation.

Another aim of this study was to compare the red and white genotypes to each other. Effect of grain color was not significant (α=0.05) on the visual disease traits such as incidence, severity, index and FDK. Even though white and red genotypes were ranked

equivalent based on visual symptoms, white genotypes were found to accumulate significantly higher quantities of DON than red consistently over the three inoculation methods. Lastly, the genotypes were ranked into three categories of low, moderate and high-levels for each disease trait measured based on 25^{th} and 75^{th} percentile (Tables 7, 8). The best and poorest performing lines were found to be stable in their rankings across all disease traits in both grain color groups, while the varieties that ranked as moderate were found to be highly variable (Lewis, 2012). This behavior could be explained by presence of only partial resistance types as opposed to presence or absence of multiple types of resistance in the varieties ranked at extremes.

Bagging protocol in the field has been demonstrated by Prange et al.(2005) by bagging plots immediately after liquid inoculation for a period of 12h. (Prange et al., 2005). Mesterhazy (1999) used a similar bagging protocol after inoculating selected heads within a plot at early morning hours (Mesterhazy et al., 1999). But in our experience, SB method could be more useful and escape potential heat stress when inoculation and bagging are carried out later in the day when the temperature starts falling, and removed before noon the next day. This might ensure sufficient humidity treatment and avoid very high temperatures that could damage wheat heads as well as fungal spores. In summary, the SB method gave a fair estimate of DON accumulation and FDK, comparable to those obtained from the more traditional GS inoculum and misting method. There seems to be a lesser effect of environment as explained by the flowering date (in case of white genotypes), as was expected. SB method could be advantageous for testing in locations where misting systems are not available, and may be more consistent

in measuring type I and type II resistances, provided cool temperatures occur during the flowering period.

2. DON Accumulation in Milled Fractions

Most commercial applications of wheat involve milling and processing steps, as flour is the primary product of interest. Effects of cleaning, milling and other processing steps such as baking and extrusion have been extensively studied (Kushiro, 2008). Cleaning of contaminated wheat reduces DON levels down by removing chaff, husk, screenings, dust and other debris (Abbas et al., 1985; Lancova et al., 2008; Young et al., 1984). Plant tissue other than grain (e.g., husk, chaff and stems) can accumulate considerable quantities of fungal biomass and high amounts of toxin, which are removed during the process of cleaning (Young and Miller, 1985). Cleaning steps remove the majority of infected seeds, which are of lower density, thus reducing DON levels. In this experiment, samples were cleaned thoroughly to remove all unwanted plant tissue. Air settings were controlled during harvest and cleaning to avoid the loss of low-weight infected seeds and to obtain an accurate measure of toxin levels across genotypes. The process of milling, by the removal of bran and shorts, reduces DON levels considerably. Effect of milling on DON content in wheat has been widely studied, with multiple studies reporting a reduction of DON contamination after milling, suggesting that a higher concentration of DON accumulates in the bran (Abbas et al., 1985; Cheli et al., 2010; Dexter et al., 1996; Edwards et al., 2011; Lancova et al., 2008). Kushiro (2008) published a review of studies on the effects of cleaning, milling and cooking methods on mycotoxin content in wheat.

Commercial milling produces multiple streams of flour including break flour, reduction flour, bran and shorts. Study of industrially milled soft winter wheat in Canada concluded that milling led to a fractionation of DON, with increased levels in the outer kernel, with DON quantities decreasing in inner flour portions (Young et al., 1984). Comparing DON quantities in various milling fractions, Thammawong et al (2011) found that DON accumulation was highest in bran and shorts fractions at low, moderate and high-level contaminated grains. A high amount of DON remained in the flour, in high-level contaminated grains, even after milling (Thammawong et al., 2010). Reported DON quantities can vary substantially due to environment in a particular year, wheat characteristics, level of contamination, and mill type (Abbas et al., 1985; Edwards et al., 2011). However, all studies consistently reported higher DON concentrations in the bran (or shorts) fraction, consistent with our results (Abbas et al., 1985; Edwards et al., 2011; Lancova et al., 2008; Trucksess et al., 1996).

The relationship between location of fungal biomass and DON levels remains unclear. High levels of toxin in the bran are attributed to the presence of *F. graminearum* in the outer layers of the kernel (pericarp and aleurone tissues) (Bechtel et al., 1985). Young et al. (1984) reported a strong correlation between ergosterol (indicator of fungal biomass) and DON levels in infected grain, and suggested that greater fungal infection occurred at or near the surface of the kernel. In heavily infected kernels, hyphae were found throughout the starchy endosperm, which may explain the higher DON-flour obtained in such lines (Bechtel et al., 1985). Further investigation is needed to clearly understand the relationship between fungal biomass localization on the grain and the mechanism of DON accumulation and distribution.

Nishio et al. (2010) studied the DON level in 16 different lines, and found positive linear correlations ($p<0.01$) between DON level in bran and in kernels ($R^2=0.94$) and DON level in flour to DON level in kernel ($R^2=0.93$). Our findings are similar, with correlation of DON-bran to DON-kernel, and DON-flour to DON-kernel. For both red and white genotypes, DON-bran and DON-flour increased with an increase in DON-kernel. However, DON-bran levels increased at a higher rate with respect to DON-kernel ($y = 1.4833x + 0.087$, $R^2= 0.98$ in red, $y = 1.2747x+0.2877$, $R^2= 0.94$ in white) compared to DON-flour ($y = 0.7384x -0.053$, $R^2= 0.98$ in red, $y = 0.8551x-0.1215$, $R^2= 0.97$ in white).

Correlation of DON partitioning ratio with the total DON content in kernel has been reported (Nishio et al., 2010). Our results did not show a significant correlation between DON partitioning ratio in flour to DON-kernel (Fig. 7). DON partitioning ratio between bran and flour was not significantly affected by genotype ($\alpha=0.05$) and was found to be fairly constant within groups of red and white.

A significant effect of grain color was observed in DON-bran, DON-flour and DON-kernel ($p<0.01$), with higher quantities of DON accumulating in the white genotypes (Fig. 8). This finding agrees with results from Lewis et al. (2008) and Knott et al. (2008) studying toxin accumulation and FHB infection between the two grain colors groups (Knott et al., 2008; Lewis et al., 2008). Although quantities of DON in all fractions were higher, average DON-ratio (DON-bran/DON-flour) of white genotypes was significantly lower than red at $\alpha=0.05$. It was anticipated that the DON quantity in the bran of red wheat would be much lower, proportionally, since phenolic compounds present in the outer layers of red wheat have an anti-fungal property (Boutigny et al.,

2008; McKeehen et al., 1999). However, DON can be translocated away from the actual growth of the fungus. Since we did not quantity the fungus in these studies, we don't know if the accumulation of the DON in the bran of the red wheat is also associated with a similar proportion of the fungus.

Individual genotypes showed a range of DON ratio (DON-bran/ DON-flour), with overlapping values between the two grain color groups, ranging from 1.88 to 2.58 in red, and 1.51 to 2.36 in white genotypes. Previously, a ratio of 2.3-2.4 between DON-bran and DON-flour has been reported, from a study of two spring wheat cultivars (Tanaka, 1999).

Nishio et al. (2010) reported DON partitioning ratio in flour to be primarily related to the level of DON-kernel. In our study, no significant correlation was found between DON partitioning ratio and DON-kernel levels. The calculation of partitioning ratio takes the % flour recovery into account, suggesting that the amount of total DON retained in the flour, or removed in the form of bran, was not correlated with percent flour recovery (range of 54.03% to 71.44%), consistent with the findings of Nishio et al. (2010). Flour yield from a Quadrumat Jr mill was shown to be similar to that from an experimental or large scale mill (Meredith, 1967). Hence percent flour yield and DON partitioning ratio reported in this study are expected to be similar to those that would have been obtained from commercial milling. It has been previously shown that flour yield is not influenced by an increase in FHB damaged kernels in Canadian hard winter wheat (Dexter 1996), suggesting that the possibility of altered percent flour recovery due to presence of high-level infected (scabby) kernels, which in turn could have an effect on DON partitioning ratio, is unlikely.

Breeding programs select for DON contamination traits based on quantities estimated based on whole grain samples (DON-std). We found that DON-std can be used as a selection tool for lower quantities of DON-kernel, DON-flour as well as DON-bran. Comparing four different levels of FHB contaminated wheat, Thammawong (2011) concluded that at higher contamination levels, higher quantities of DON remained in the flour and were not removed by milling (Thammawong et al., 2010). Results from this study indicate the presence of toxins in the flour even at low-level DON contamination, in both red and white genotypes. This is consistent with the findings of (Lancova et al., 2008), who showed that although substantial part of DON accumulates in the bran, almost 40% of its original content is still left in the flour. The first break flour, obtained from the first set of break rolls in a commercial mill, was found to be the most contaminated (Lancova et al., 2008).

Even though wheat flour is the primary product of interest, bran from white wheat is an attractive commodity due to its superior flavor and taste characteristics. In Michigan, one of the major producers of white wheat in the great lakes region, wheat bran is valuable for the local cereal industry. Bran is used as a source of dietary fiber, as a component of high fiber breakfast cereals or mixed in with flour for bread recipes (Edwards, 2011). This report rejects the concept that DON fractionation in bran is higher in white genotypes. Although white genotypes accumulate significantly higher quantities of DON, DON ratios are comparable for red and white genotypes. Estimates of average DON ratio in white genotypes was found to be lower than in red genotypes. The white lines with low-level DON-std, MSU lines E6003 and E6055 and Pioneer 25W43, also had significantly lower levels of DON-bran. MSU lines E6003 and E6055 contained

DON levels in all fractions comparable to those in the best red genotypes Roane and Truman. These white lines are recommended for use as parents for breeding programs intending to improve FHB resistance in soft white lines.

Genetics of FHB resistance in white genotypes should be further investigated using populations derived from the use of the resistant lines identified in the study. The role of grain color related genes in attributing resistance to DON accumulation should be studied using a red x white population. Correlation of toxin accumulation in genotype with presence of fungal biomass in different kernel tissues can provide further insight into how DON is produced and translocated within the kernel in infected red and white wheat.

Table 2. List of 39 lines planted in the study and their pedigree information

Color	Variety	Pedigree
White	Ambassador	Pioneer 2737W/MSU Line D1148
	Aubrey	NA
	Augusta	Genesee/Redcoat/A2747//Yorkstar
	Caledonia	Ross Selection /3/(NY5207aB-2B-34) Burt // Genesee / CI 12658 /4/ Genesee
	Coral	MSU Line D3913/MSU Line D0331
	Crystal	Pioneer 2737W/MSU Line D1148
	Envoy	MSU Line DC076/Pioneer 2552
	Jewel	Pioneer 2552/Pioneer 2737W
	MSU D6234 (D6234)	X1291/C5107
	MSU D8006 (D8006)	Pioneer 2555/Lowell
	MSU Line E3024 (E3024)	(Caledonia, Geneva / Geneva)//NY85020-395
	MSU Line E5011B (E5011B)	Caledonia / NY88024-117
	MSU Line E5017 (E5017)	D6206 / Pioneer Brand 2552
	MSU Line E5024 (E5024)	MSU D6234 / Pioneer Brand 25W33
	MSU Line E5028 (E5028)	D6234 / Pioneer Brand 25W33
	MSU Line E5038 (E5038)	VA96W-403WS / D6277
	MSU Line E6003 (E6003)	VA96W-403-WS / W14
	MSU Line E6055 (E6055)	VA96W-403-WS / W14
	Pioneer 25W43 (25W43)	SC8021V2 (PI554142)/25W33//WBL0305A1
Red	Arena	NASW84-345/Coker9835//OH419/OH389
	Bravo	Madison/Twain
	Cooper	Pio.2510/Jackson
	FT Wonder	KANRED/SEVIER//RIDIT/3/YOGO[1318]
	Hopewell	Logan / Hart // 32270A / Rousalka /3/ TN1685 / IA22 // 6767 / 216-6-3
	Hyland Emmit (Emmit)	Pio.2510/Marilee
	MCIA Oasis	IL85-3132-1/Irena//OH449/VA86-54-290
	OH02-12686	Foster/Hopewell//OH581/OH569
	OH02-7217	NA
	OH04-213-39	Hopewell/IL94-6858
	OH04-264-58	OH645/Hopewell
	Pioneer 25R47 (25R47)	WBE2190B1(Frankenmuth/2555 sib//2551 sib)/WBA416H2(Houser/MO9545//W4034D/Augusta)//2552
	Pioneer 25R62 (25R62)	WBN0686C/WBJ0249B1
	Red Ruby	Pioneer 2552/Pioneer 2737W
	Roane	VA71-54-147(CI17449)/C68-15//IN65309C1-18-2-3-2
	Tribute	VA98W-593=VA92-51-39(IN71761A4-31-5-48//71-54-147/MCN1813)/AL870365 (CK747*2/Amigo)
	Truman	MO 11769/Madison

Note: NA= Not Available

Table 3. List of sites for artificial inoculation using Grain Spawn or spray and Bag methods over three years of field study

Environment	Year	Location	Inoculation Method	Flowering Dates
1	2009	MSU Agronomy Research Farm East Lansing, MI	GS	11-Jun to 25-Jun
2		MSU Clarksville Research Center Clarksville, MI	SB	1-Jun to 6-Jun
3	2010	MSU Agronomy Research Farm East Lansing, MI	SB	24-May to 1-Jun
4		"Ingham" test location Webberville, MI	SB	27-May to 3-Jun
5	2011	MSU Agronomy Research Farm East Lansing, MI	GS	1-Jun to 8-Jun
6		MSU Wheat Research Farm Mason, MI	SB	3-Jun to 8-Jun

Table 4. Correlation of different response measures within GS, SB and SB1 methods

Method=GS	DON-std	FDK	Index	Incidence	Severity
DON-std		0.70*	0.19	0.12	0.42
FDK	0.63*		0.10	0.09	0.33
Index	0.55*	0.49*		0.56*	0.79*
Incidence	0.39	0.57*	0.58*		0.34
Severity	0.53*	0.53*	0.77*	0.26	
Method=SB	**DON-std**	**FDK**	**Index**	**Incidence**	**Severity**
DON-std		0.80*	0.74*	0.60*	0.81*
FDK	0.83*		0.67*	0.67*	0.72*
Index	0.49*	0.61*		0.85*	0.93*
Incidence	0.46	0.60*	0.90*		0.82*
Severity	0.48*	0.63*	0.80*	0.61*	
Method=SB1	**DON-std**	**FDK**	**Index**	**Incidence**	**Severity**
DON-std		0.80*	0.76*	0.73*	0.67*
FDK	0.59*		0.70*	0.48*	0.67*
Index	0.62*	0.36		0.69*	0.95*
Incidence	0.52*	0.09	0.72*		0.54*
Severity	0.59*	0.38	0.99*	0.65*	

Note: Correlation coefficients for red genotypes are listed below the diagonal and those for white are above. * Significant with $\alpha<0.05$

Table 5. Correlation between GS, SB and SB1 methods for the response variables

	GS	SB	SB1
a.DON-std			
GS		0.52*	0.64*
SB	0.67*		0.69*
SB1	0.58*	0.69*	
b.FDK			
GS		0.44*	0.61*
SB	0.63*		0.53*
SB1	0.39	0.47*	
c.Index			
GS		0.44*	0.15
SB	-0.04		0.42
SB1	0.39	0.46	
d.Incidence			
GS		0.21	0.13
SB	-0.07		0.60*
SB1	0.44	0.61*	
e.Severity			
GS		0.70*	0.54*
SB	0.46		0.54*
SB1	0.37	0.29	

Note: Correlation coefficients for red genotypes are listed below the diagonal and those for white are above.
*Significant with $\alpha<0.05$

Table 6. Estimates of the response variables DON, FDK, Index, Incidence and Severity across the inoculation methods GS, SB and SB1 for 18 red genotypes

	GS					SB					SB1				
Genotype	DON	FDK	Index	Inc.	Sev.	DON	FDK	Index	Inc.	Sev.	DON	FDK	Index	Inc.	Sev.
25R47	2.52	16.73	6.80	34.82	13.73	1.36	6.53	5.86	29.84	14.60	8.56	23.09	23.69	93.93	26.37
25R62	1.48	20.49	8.77	43.77	12.98	1.41	4.18	2.41	21.18	8.59	8.94	5.08	19.94	96.83	20.39
Arena	2.92	18.56	13.67	38.48	24.74	3.36	7.99	5.19	33.42	10.40	12.74	33.26	68.32	101.14	71.52
Bravo	1.18	22.51	12.64	40.11	26.97	1.51	5.59	6.94	38.99	14.54	12.08	26.86	31.30	90.74	34.04
Cooper	2.78	25.57	11.78	45.34	19.66	1.65	8.15	4.67	29.10	13.68	11.01	30.51	27.56	88.95	30.99
Emmit	0.98	18.05	15.19	47.51	20.33	1.10	4.30	2.26	18.11	8.08	11.88	36.58	33.28	90.22	35.92
FT Wonder	1.33	10.27	11.28	44.98	19.20	0.90	3.61	1.36	13.53	7.99	9.93	43.53	12.68	68.58	18.81
Hopewell	2.35	21.48	16.74	43.17	22.85	1.75	6.59	2.87	21.58	11.10	15.62	24.03	34.72	94.22	38.41
MCIA Oasis	1.35	25.70	12.88	43.34	25.24	1.89	5.11	3.25	22.12	11.20	11.16	12.09	30.32	83.97	36.43
OH02-126	1.22	10.29	14.33	39.44	22.85	0.50	2.51	2.73	18.44	9.37	5.40	5.21	15.15	73.74	19.05
OH02-721	1.09	15.02	7.73	39.64	12.83	1.45	4.24	4.54	25.21	12.41	13.73	11.27	36.94	99.82	37.65
OH04-213	0.95	14.61	11.08	37.04	17.58	0.95	4.08	3.58	23.53	11.55	12.29	16.75	35.09	89.68	39.30
OH04-264	2.14	16.73	14.90	48.27	18.39	1.21	4.87	3.11	24.65	12.09	9.26	12.98	27.09	93.88	28.49
Red Amber	3.24	20.51	17.50	44.29	35.23	2.66	7.49	6.31	27.95	20.68	18.96	35.20	37.59	96.61	37.27
Red Ruby	3.54	26.59	19.85	52.73	24.68	1.73	5.03	3.61	20.89	14.36	16.28	40.91	41.75	97.86	43.77
Roane	0.74	5.94	8.87	46.06	11.00	0.65	3.85	4.81	32.14	12.72	6.97	9.58	27.81	97.06	31.01
Tribute	0.74	5.32	11.13	32.04	15.01	1.40	5.02	3.61	28.82	12.28	10.21	14.17	31.46	91.66	32.54
Truman	1.04	4.74	8.64	26.40	16.76	0.39	1.06	2.29	15.72	6.76	6.51	11.72	8.30	56.21	13.06
Mean	1.83	16.60	12.91	42.08	20.84	1.51	5.00	3.98	25.27	12.13	11.20	20.39	30.84	88.16	34.73
Std Error	0.22	1.65	0.94	1.66	1.64	0.17	0.44	0.33	1.42	0.74	0.83	2.65	2.94	3.35	3.00

Note: Inc. and Sev. stand for percent Incidence and Severity respectively. DON quantities are in ppm. FDK values are in percent.

Table 7. Estimates of the response variables ON, FDK, Index, Incidence and Severity across the inoculation methods GS, SB and SB1 for 21 white genotypes

	GS					SB					SB1				
Genotype	DON	FDK	Index	Inc	Sev	DON	FDK	Index	Inc	Sev	DON	FDK	Index	Inc	Sev
25W43	1.43	8.31	9.84	38.62	16.55	1.14	4.79	5.60	29.76	14.17	14.22	25.16	28.32	93.43	31.60
Ambassador	3.91	22.03	26.28	48.30	46.31	4.30	6.92	9.40	33.96	23.87	31.01	50.51	52.61	89.08	58.92
Aubrey	2.03	9.01	12.25	38.62	23.72	2.17	3.83	3.43	28.94	10.24	14.74	21.97	40.75	87.63	46.58
Augusta	3.95	12.09	20.94	45.61	48.10	5.17	7.17	10.78	41.45	24.57	16.34	28.69	43.75	68.98	63.86
Caledonia	3.94	23.62	25.88	54.22	43.24	3.31	7.02	3.99	23.02	13.18	17.23	24.21	50.91	81.38	63.63
Coral	2.07	18.65	19.13	44.74	28.77	2.24	4.65	3.66	27.30	13.32	14.36	25.41	39.32	84.44	45.02
Crystal	4.66	21.10	16.24	47.49	28.01	2.46	4.09	3.85	21.98	11.36	16.63	26.41	31.31	76.63	37.33
D6234	2.43	12.28	21.65	54.87	32.34	2.43	6.28	5.34	30.52	15.60	11.28	7.61	17.89	72.99	24.02
D8006	3.98	8.93	19.56	44.90	29.54	2.61	3.19	4.65	25.40	15.06	21.73	23.79	55.94	98.70	56.80
E3024	2.67	24.21	14.58	46.24	38.02	2.70	4.41	4.00	21.18	17.38	13.38	34.25	33.03	73.03	38.86
E5011B	1.87	18.14	10.58	49.63	15.23	2.43	5.32	5.48	34.95	16.48	16.82	23.47	50.77	95.00	52.99
E5017	2.51	13.88	13.58	54.40	20.57	3.26	5.89	4.56	25.38	13.88	16.46	20.94	19.51	91.26	20.36
E5024	3.59	23.27	21.76	53.34	30.43	2.06	5.25	3.50	24.39	13.08	17.30	11.96	19.74	82.92	21.45
E5028	3.43	18.26	20.31	38.62	30.49	2.36	4.40	3.29	25.02	12.23	16.44	24.25	29.85	89.17	31.93
E5038	8.52	36.19	9.98	38.62	30.74	3.46	6.45	2.90	20.58	10.82	24.84	51.55	63.63	91.02	70.29
E6003	0.75	12.74	9.13	38.62	14.46	0.41	0.82	0.63	6.39	2.80	3.76	8.53	0.22	13.13	3.55
E6055	0.88	11.83	13.38	38.62	18.75	0.82	1.87	2.42	18.19	7.09	4.68	8.77	-0.69	25.09	1.77
Envoy	4.15	21.85	15.28	44.17	29.26	1.72	4.94	4.64	26.49	13.15	14.93	26.95	37.57	97.54	38.21
Frankenmuth	2.73	10.06	19.86	54.97	34.30	2.28	3.34	3.65	23.13	13.99	9.93	17.78	24.83	50.26	48.87
Jewel	5.20	15.63	15.92	50.08	22.14	1.85	4.39	3.45	27.65	11.31	14.97	16.96	26.11	91.51	29.27
Lowell	1.80	12.03	13.57	36.84	25.44	1.78	4.13	4.86	29.03	12.48	16.25	21.94	64.57	88.64	71.58
Mean	3.10	16.87	16.24	46.07	28.16	2.36	4.74	4.37	25.48	13.34	15.59	25.09	34.18	79.06	39.37
Std Error	0.39	1.51	1.13	1.16	2.11	0.25	0.35	0.50	1.63	1.07	1.28	2.64	4.08	4.78	4.47

Note: Inc. and Sev. stand for percent Incidence and Severity respectively. DON quantities are in ppm. FDK values are in percent.

Table 8. Overall ranking of 18 red genotypes by different response measures

Rank	DON	FDK	Index	Incidence	Severity
1	Truman	Emmit	25R62	Truman	25R62
2	OH02-126	Truman	Truman	OH02-126	Emmit
3	Roane	OH02-126	FT Wonder	FT Wonder	25R47
4	OH04-213	OH04-213	Tribute	Tribute	OH02-721
5	FT Wonder	Bravo	Roane	OH04-213	MCIA Oasis
6	OH02-721	Tribute	25R47	25R62	OH02-126
7	Tribute	Red Amber	OH02-721	Hopewell	Tribute
8	MCIAOasis	Hopewell	OH04-213	Emmit	OH04-213
9	Emmit	25R62	MCIA Oasis	MCIA Oasis	FT Wonder
10	25R62	Roane	OH02-126	25R47	Truman
11	25R47	Red Ruby	Emmit	OH02-721	Hopewell
12	Cooper	FT Wonder	Cooper	Red Ruby	Roane
13	Hopewell	25R47	Hopewell	Arena	OH04-264
14	Bravo	OH04-264	Arena	OH04-264	Bravo
15	OH04-264	OH02-721	OH04-264	Red Amber	Red Ruby
16	Arena	Arena	Bravo	Cooper	Cooper
17	Red Ruby	MCIA Oasis	Red Ruby	Roane	Red Amber
18	Red Amber	Cooper	Red Amber	Bravo	Arena

Table 9. Overall ranking of 21 white genotypes by different response measures

Rank	DON	FDK	Index	Incidence	Severity
1	E6003	E6003	E6003	E6003	E6003
2	E6055	E6055	E5038	E6055	E6055
3	25W43	Aubrey	25W43	E5038	25W43
4	Lowell	25W43	Aubrey	Lowell	Jewel
5	E5011B	Crystal	Lowell	D8006	E5011B
6	Aubrey	E5024	E6055	E3024	Aubrey
7	Coral	Lowell	Jewel	25W43	E5017
8	D6234	E3024	E5011B	Jewel	Lowell
9	Frankenmuth	E5011B	E5017	Caledonia	Crystal
10	E5024	D8006	E3024	Crystal	Coral
11	E3024	D6234	Envoy	Coral	E5038
12	Envoy	Coral	Crystal	E5024	Envoy
13	E5028	Jewel	Coral	Ambassador	E5028
14	E5017	E5017	D8006	Aubrey	D8006
15	D8006	Augusta	E5028	Envoy	E5024
16	Jewel	Frankenmuth	Frankenmuth	E5028	Frankenmuth
17	Crystal	Caledonia	E5024	E5017	D6234
18	Caledonia	Envoy	D6234	Frankenmuth	E3024
19	Ambassador	Ambassador	Caledonia	Augusta	Caledonia
20	Augusta	E5028	Augusta	D6234	Ambassador
21	E5038	E5038	Ambassador	E5011B	Augusta

Table 10. DON partitioning ratio in flour and DON accumulation levels in whole grain sample (DON-std), bran (DON-bran), flour (DON-flour) and total (DON-kernel) of red genotypes

Variety Name	DON-bran	DON-flour	DON-kernel	Partitioning Ratio	DON-std
Roane	1.50	0.74	0.97	50.72	0.78
Truman	1.45	0.68	0.95	48.27	0.88
OH02-126	1.98	0.81	1.23	44.54	0.96
Tribute	2.04	0.90	1.22	50.05	1.13
FT Wonder	2.23	1.08	1.45	48.30	1.24
OH04-213	2.03	0.99	1.33	50.84	1.25
Emmit	2.45	1.14	1.56	48.57	1.27
OH02-721	2.42	1.20	1.61	51.82	1.56
Bravo	2.68	1.50	1.84	53.44	1.57
25R62	2.44	1.16	1.58	51.96	1.64
MCIA Oasis	3.18	1.59	2.07	50.90	1.72
OH04-264	3.39	1.77	2.30	52.31	1.95
25R47	2.99	1.64	2.01	52.96	1.98
Hopewell	3.79	1.96	2.55	50.09	2.25
Cooper	3.53	1.57	2.21	49.41	2.31
Red Ruby	4.46	2.67	3.24	54.24	2.77
Arena	5.18	2.36	3.26	47.90	3.05
Red Amber	5.17	2.40	3.26	49.74	3.37
Mean	2.94	1.45	1.92	50.34	1.76
Std. Error	0.26	0.14	0.18	0.55	0.18
LSD (95%)	0.96	0.56	0.64	4.2	0.62

Note: Varieties are arranged in ascending order of DON-std. DON quantities are in ppm, Partitioning ratio is a percentage value.

Table 11. DON partitioning ratio in flour and DON accumulation levels in whole grain sample (DON-std), bran (DON-bran), flour (DON-flour) and total (DON-kernel) of white genotypes

Variety Name	DON-bran	DON-flour	DON-kernel	Partitioning Ratio	DON-std
E6003	1.27	0.69	0.85	56.41	0.67
E6055	1.86	1.32	1.47	60.36	0.99
25W43	2.56	1.90	2.26	54.39	1.30
Lowell	3.28	1.83	2.29	54.27	1.70
E5011B	4.19	2.24	2.89	53.48	1.91
Aubrey	3.11	1.80	2.24	55.47	2.05
Coral	3.51	1.65	2.22	50.65	2.19
D6234	3.36	1.74	2.24	54.62	2.34
Frankenmuth	3.66	2.09	2.67	52.73	2.49
E5024	3.36	1.91	2.37	56.84	2.65
E3024	4.81	2.36	3.13	52.04	2.76
Envoy	4.45	2.62	3.21	54.95	2.84
E5028	3.84	2.34	2.83	52.67	2.84
E5017	4.54	3.41	3.91	60.41	2.92
D8006	5.22	3.00	3.82	55.28	3.23
Jewel	4.12	2.67	3.15	56.94	3.39
Crystal	4.44	2.90	3.40	56.21	3.40
Caledonia	5.33	2.46	3.37	50.93	3.57
Ambassador	6.25	4.32	4.97	58.23	4.01
Augusta	6.98	3.89	5.07	50.89	4.58
E5038	6.65	4.48	4.99	60.61	5.86
Mean	4.13	2.46	3.02	55.16	2.75
Std. Error	0.32	0.21	0.24	0.66	0.26
LSD (95%)	1.42	0.90	0.96	3.8	1.08

Note: Varieties are arranged in ascending order of DON-std. DON quantities are in ppm, Partitioning ratio is a percentage value

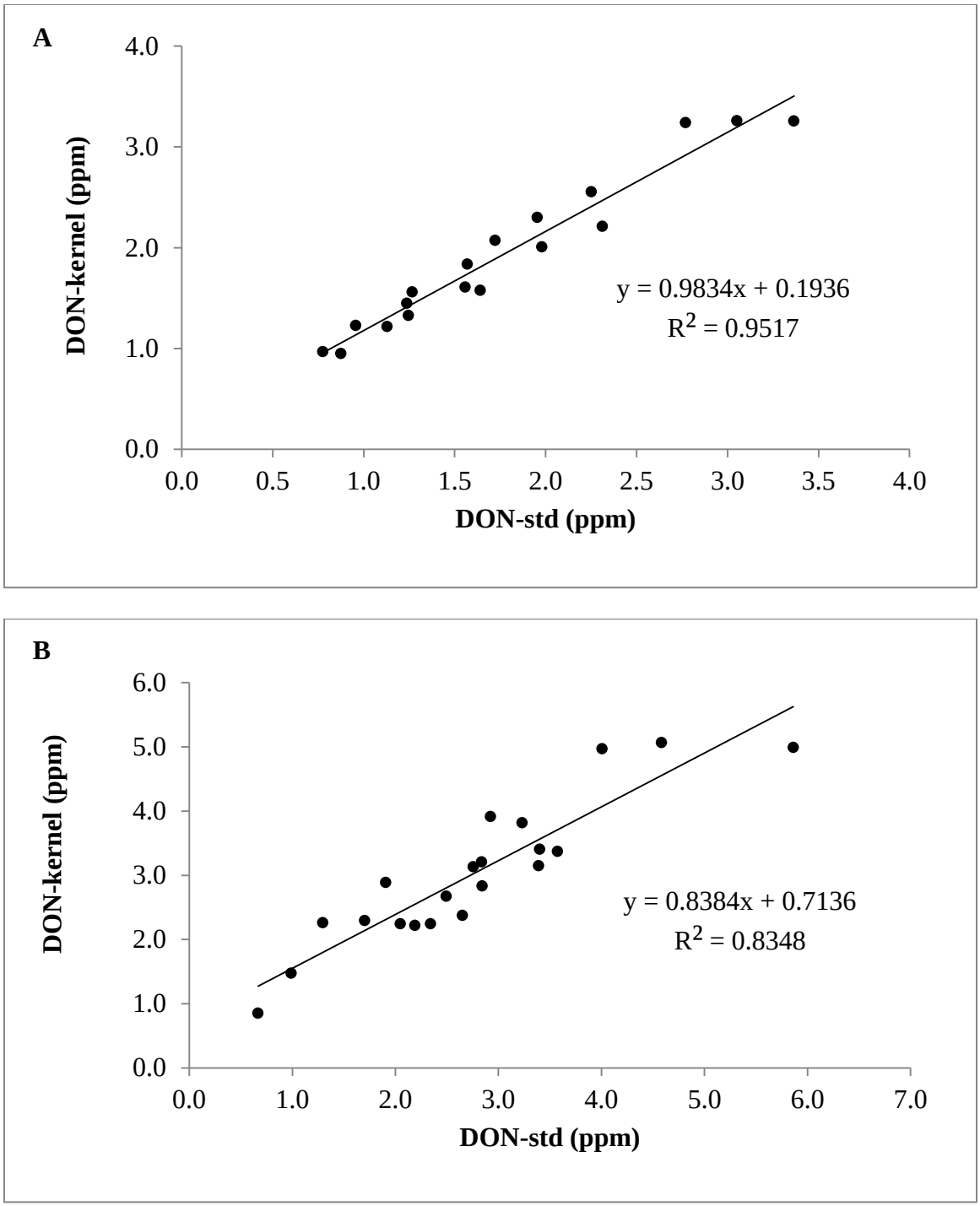

Fig. 3. Linear regression relationship between DON-kernel and DON-std in A. red and B. white genotypes

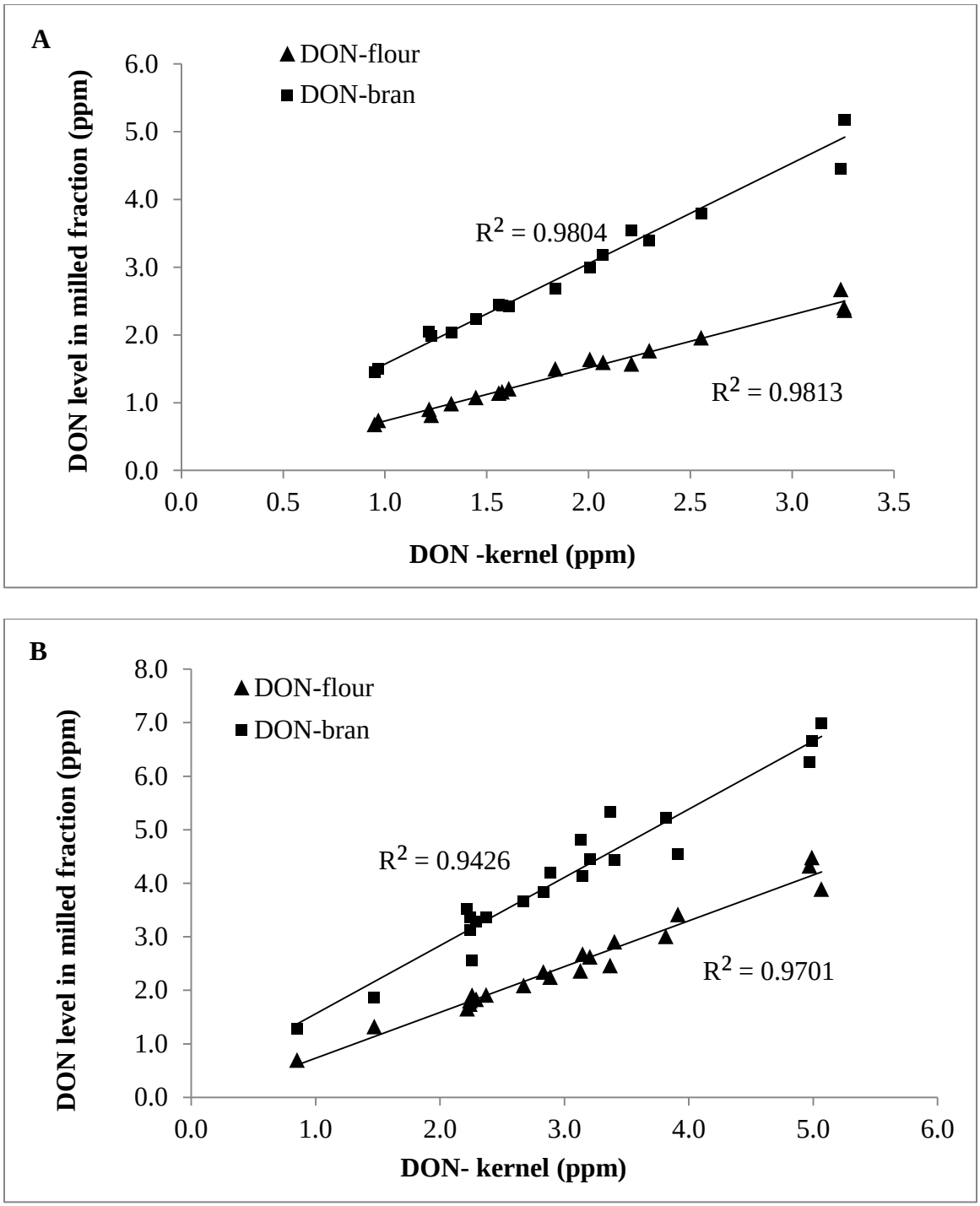

Fig. 4. Linear regression relationship between DON levels in milled fractions (DON-bran and DON-flour) with total DON levels (DON-kernel) in A. red and B. white genotypes

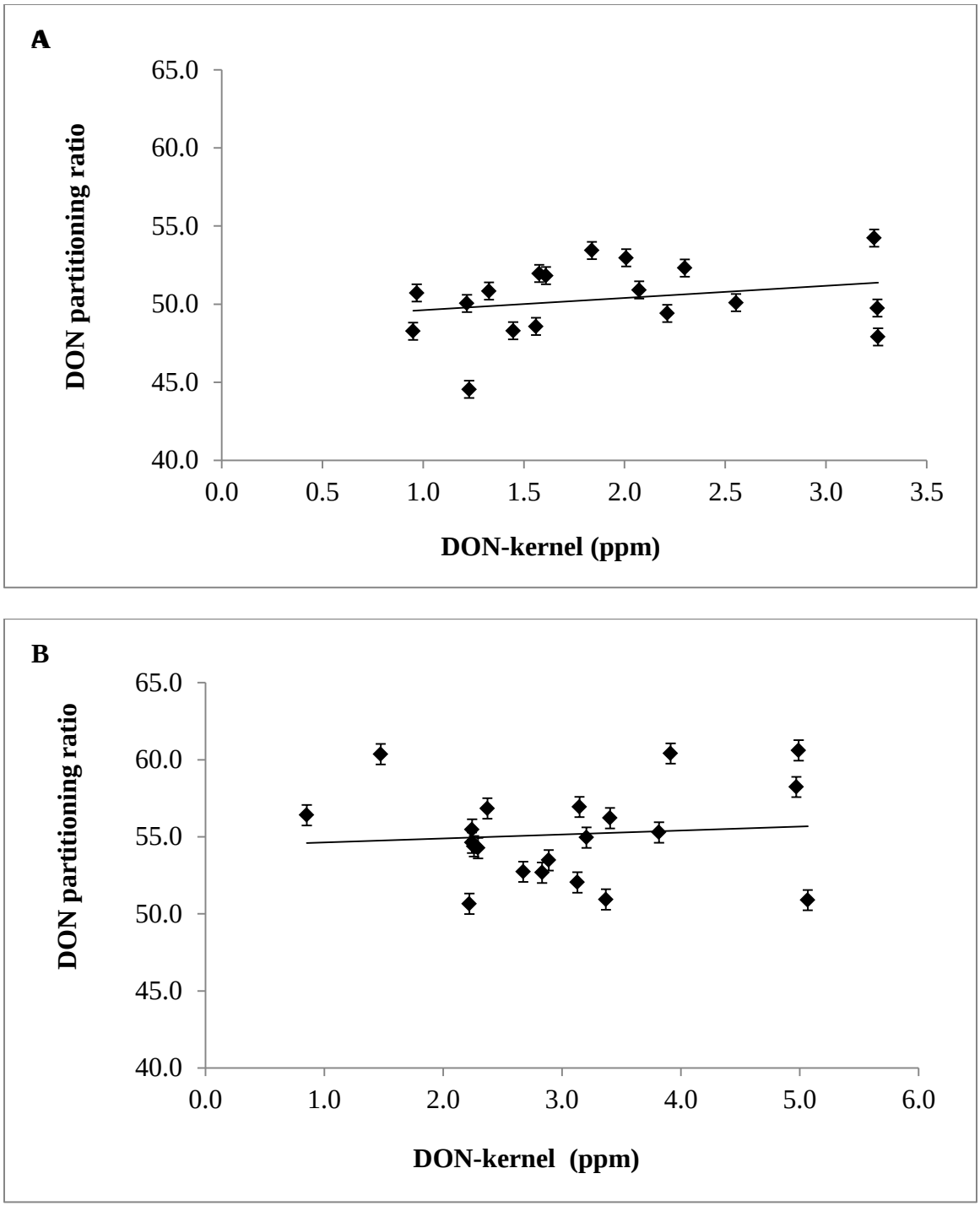

Fig. 5. Relationship between DON partitioning ratio and DON-kernel in A. red and B. white genotypes

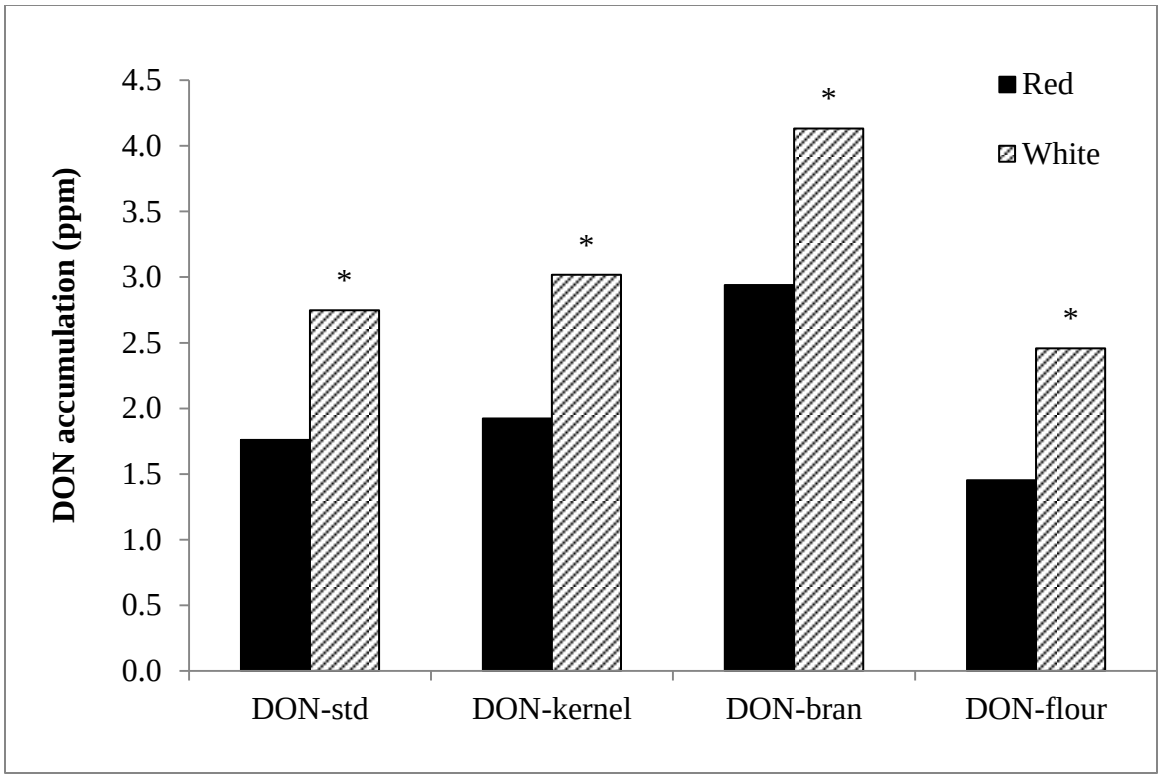

Fig. 6. Comparison of DON-std, DON-kernel, DON-bran and DON-flour between red and white genotypes.* shows statistically significant difference between the two groups (α=0.05).

REFERENCES

REFERENCES

Abbas, H.K., C.J. Mirocha, R.J. Pawlosky, and D.J. Pusch. 1985. Effect of cleaning, milling, and baking on deoxynivalenol in wheat. Applied and Environmental Microbiology 50:482-486.

Bai, G.H., and G. Shaner. 1994. Scab of wheat - prospects for control. Plant Disease 78:760-766.

Bai, G.H., Desjardins, A.E. and Plattner, R.D., 2002. Deoxynivalenol-nonproducing Fusarium graminearum causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia, 153(2): 91-98.

Bai, G.H., Plattner, R., Desjardins, A. and Kolb, F., 2001. Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breeding, 120(1): 1-6.

Bassoi, M.C. and Flintham, J., 2005. Relationship between grain colour and preharvest sprouting-resistance in wheat. Pesquisa Agropecuaria Brasileira, 40(10): 981-988.

Bechtel, D.B., L.A. Kaleikau, R.L. Gaines, and L.M. Seitz. 1985. The effects of Fusarium-graminearum infection on wheat kernels. Cereal Chemistry 62:191-197.

Beyer, M., Klix, M.B. and Verreet, J.A., 2007. Estimating mycotoxin contents of Fusarium-damaged winter wheat kernels. International Journal of Food Microbiology, 119(3): 153-158.

Birzele, B., Meier, A., Hindorf, H., Kramer, J. and Dehne, H.W., 2002. Epidemiology of Fusarium infection and deoxynivalenol content in winter wheat in the Rhineland, Germany. European Journal of Plant Pathology, 108(7): 667-673.

Boswell, M., and J. Shroyer. 2000. Hard white wheat: A comparison to hard red wheat. Kansas Wheat Commission, Manhattan.

Bottalico, A. and Perrone, G., 2002. Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. European Journal of Plant Pathology, 108(7): 611-624.

Boutigny, A.L., Richard-Forget, F. and Barreau, C., 2008. Natural mechanisms for cereal resistance to the accumulation of Fusarium trichothecenes. European Journal of Plant Pathology, 121(4): 411-423.

Cappelli.R, and J.L. Peterson. 1965. Macroconidium formation in submerged cultures by a non-sporulating strain of Gibberella zeae. Mycologia 57:962.

Cappelli.R, and J.L. Peterson. 1971. PH, nutrients and macrospore germination in Gibberella-zeae. Mycologia 63:641.

Champeil, A., Fourbet, J.F., Dore, T. and Rossignol, L., 2004. Influence of cropping system on Fusarium head blight and mycotoxin levels in winter wheat. Crop Protection, 23(6): 531-537.

Cheli, F., A. Campagnoli, V. Ventura, C. Brera, C. Berdini, E. Palmaccio, and V. Dell'Orto, 2010. Effects of industrial processing on the distributions of deoxynivalenol, cadmium and lead in durum wheat milling fractions. Lwt-Food Science and Technology 43:1050-1057.

Cook RJ, 1981. In: Nelson PE, Toussoun TA, Cook RJ (eds) *Fusarium*: diseases, biology and taxonomy. The Pennsylvania State University Press, University Park and London

Cowger, C. and Arrellano, C., 2010. Plump kernels with high deoxynivalenol linked to late Gibberella zeae infection and marginal disease conditions in winter wheat. Phytopathology, 100(7): 719-728.

Cowger, C., J. Patton-Ozkurt, G. Brown-Guedira, and L. Perugini. 2009. Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North Carolina winter wheat. Phytopathology 99:320-327.

Creppy, E.E., 2002. Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology Letters, 127(1-3): 19-28.

Culler, M.D., J.E. Miller-Garvin, and R. Dill-Macky. 2007. Effect of extended irrigation and host resistance on deoxynivalenol accumulation in Fusarium-infected wheat. Plant Disease 91:1464-1472.

Del Ponte, E.M., Fernandes, J.M.C. and Bergstrom, G.C., 2007. Influence of growth stage on Fusarium head blight and deoxynivalenol production in wheat. Journal of Phytopathology, 155(10): 577-581.

Dexter, J.E., R.M. Clear, and K.R. Preston. 1996. Fusarium head blight: Effect on the milling and baking of some Canadian wheats. Cereal Chemistry 73:695-701.

Dill-Macky, R. 2003. Inoculation methods and evaluation of Fusarium head blight resistance in wheat. Pages 184-210 In: Fusarium: Diseases, biology and taxonomy. P.E. Nelson, T.A. Toussoun & R.J. Cook, eds. APS Press.

Dill-Macky, R. and Jones, R.K., 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Disease 84:71-76.

D'Mello, J.P.F., Placinta, C.M. and Macdonald, A.M.C., 1999. Fusarium mycotoxins: a review of global implications for animal health, welfare and productivity. Animal Feed Science and Technology, 80(3-4): 183-205.

Dvorak, J. and H.B. Zhang (1990) Variation in repeated nucleotide sequences sheds light on the phylogeny of the wheat B and G genomes. Proc. Natl. Acad. Sci. USA 87: 9640-9644.

Dvorak, J., and Zhang, H.B. 1992. Reconstruction of the phylogeny of the genus *Triticum* from variation in repeated nucleotide sequences. Theor. Appl. Genet. 84, 419–429

Edwards, S.G., E.T. Dickin, S. MacDonald, D. Buttler, C.M. Hazel, S. Patel, and K.A. Scudamore. 2011. Distribution of Fusarium mycotoxins in UK wheat mill fractions. Food additives and contaminants part a-chemistry analysis control exposure & risk assessment 28:1694-1704.

Eggert, K., J. Hollmann, B. Hiller, H.P. Kruse, H.M. Rawel, and E. Pawelzik. Effects of Fusarium Infection on the Phenolics in Emmer and Naked Barley. Journal of Agricultural and Food Chemistry

FDA, 1993. http://www.ngfa.org/files/misc/Guidance_for_Toxins.pdf

Feldman M, Liu B, Segal G, Abbo S, Levy AA, Vega JM. 1997. Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. Genetics 147(3): 1381-1387

Feldman, M. 2001. The origin of cultivated wheat. In: The world wheat book. Edited by A.P. Bonjean and W.J. Angus. Laroisier Publishing, Paris. pp. 1–56.

Feldman, M., Lupton, F.G.H., and Miller, T.E. 1995. Wheats. In: Evolution of Crops, 2nd ed., J. Smartt and N.W. Simmonds, eds (London: Longman Scientific), pp. 184–192.

Fernandez, M.R., Fernandes, J.M. and Sutton, J.C., 1993. Effects of fallow and of summer and winter crops on survival of wheat pathogens in crop residues. Plant Disease, 77(7): 698-703.

Foroud, N.A. and Eudes, F., 2009. Trichothecenes in Cereal Grains. International Journal of Molecular Sciences, 10(1): 147-173.

Fuentes, R.G; Mickelson, H.R.; Busch, R.H.; Dill-Macky, R.; Evans, C.K.; Thompson, W.G.; Wiersma, J.V.; Xie, W.; Dong, Y.; Anderson, J.A. Resource Allocation and Cultivar Stability in Breeding for Fusarium Head Blight Resistance in Spring Wheat. *Crop Sci.* 2005, *45*, 1965-1972.

Galliard, T. and Gallagher, D.M., 1988. The effects of wheat bran particle-size and storage period on bran flavor and baking quality of bran flour blends. Journal of Cereal Science, 8(2): 147-154.

Gan, Z., Galliard, T., Ellis, P.R., Angold, R.E. and Vaughan, J.G., 1992. Effect of the outer bran layers on the loaf volume of wheat bread. Journal of Cereal Science, 15(2): 151-163.

Gautam, P., and R. Dill-Macky. 2008. Post inoculation moisture impacts deoxynivalenol accumulation in Fusarium graminearum-infected wheat. Cereal Research Communications 36:493-493.

Gilbert, J., A. Tekauz & E. Mueller, 1993. Occurrence of Fusarium head blight in Manitoba in 1992. Can Plant Dis Surv 73: 71–72

Groos, C. et al., 2002. Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a white x red grain bread-wheat cross. Theoretical and Applied Genetics, 104(1): 39-47.

Harlan, J.R. and Zohary, D., 1966. Distribution of Wild Wheats and Barley. Science, 153(3740): 1074-1080.

Himi, E., Mares, D.J., Yanagisawa, A. and Noda, K., 2002. Effect of grain colour gene (R) on grain dormancy and sensitivity of the embryo to abscisic acid (ABA) in wheat. Journal of Experimental Botany, 53(374): 1569-1574.

Homdork, S., Fehrmann, H. and Beck, R., 2000. Effects of field application of tebuconazole on yield, yield components and the mycotoxin content of Fusarium-infected wheat grain. Journal of Phytopathology-Phytopathologische Zeitschrift, 148(1): 1-6.

Ilgen, P., B. Hadeler, F.J. Maier, and W. Schafer. 2009. Developing Kernel and Rachis Node Induce the Trichothecene Pathway of Fusarium graminearum During Wheat Head Infection. Molecular Plant-Microbe Interactions 22:899-908.

International Grain Council, 2012. http://www.igc.int/en/grainsupdate/sd.aspx?crop=Wheat

Jansen, C. et al., 2005. Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted Fusarium graminearum. Proceedings of the National Academy of Sciences of the United States of America, 102(46): 16892-16897.

Jenkinson P, Parry DW, 1994. Splash dispersal of conidia of Fusarium culmorum and Fusarium avenaceum. Mycological Research 98, 506–10

Johnson, P. 2011. Personal communication.

Kang, Z. and Buchenauer, H., 2000. Ultrastructural and immunocytochemical investigation of pathogen development and host responses in resistant and susceptible wheat spikes infected by Fusarium culmorum. Physiological and Molecular Plant Pathology, 57(6): 255-268.

Kihara H, 1944. Discovery of the DD-analyser, one of the ancestors of Triticum vulgare (abstr) (in Japanese). Agric. Hortic.;19:889-890.

Kimura, M., T. Tokai, N. Takahashi-Ando, S. Ohsato, and M. Fujimura. 2007. Molecular and genetic studies of Fusarium trichothecene biosynthesis: Pathways, genes, and evolution. Bioscience Biotechnology and Biochemistry 71:2105-2123.

Knott, C.A., D.A. Van Sanford, and E.J. Souza. 2008. Comparison of selection methods for the development of white-seeded lines from red x white soft winter wheat crosses. Crop Science 48:1807-1816.

Kroon P.A, C.B Faulds, P Ryden, J.A Robertson, G Williamson, 1997. Release of covalently bound ferulic acid from fibre in human colon. J Agric Food Chem., 45 (1997), pp. 661–667

Kushiro, M. 2008. Effects of milling and cooking processes on the deoxynivalenol content in wheat. International Journal of Molecular Sciences 9:2127-2145.

Lacey, J., Bateman, G.L. and Mirocha, C.J., 1999. Effects of infection time and moisture on development of ear blight and deoxynivalenol production by Fusarium spp. in wheat. Annals of Applied Biology, 134(3): 277-283.

Lancova, K., J. Hajslova, M. Kostelanska, J. Kohoutkova, J. Nedelnik, H. Moravcova, and M. Vanova. 2008. Fate of trichothecene mycotoxins during the processing: Milling and baking. Food Additives and Contaminants 25:650-659.

Lemmens, M., H. Buerstmayr, R. Krska, R. Schuhmacher, H. Grausgruber, and P. Ruckenbauer. 2004. The effect of inoculation treatment and long-term application of moisture on Fusarium head blight symptoms and deoxynivalenol contamination in wheat grains. European Journal of Plant Pathology 110:299-308.

Lev-Yadun S,Gopher A, Abbo S., 2000. The cradle of agriculture. Science;288:1602-1603.

Lewis J, Siler L, Jiang GL, Ward RW, 2008. History of FHB Resistance Evaluation in Michigan State Performance Trial, Red vs. White. In: Canty SM, Clark A, Walton E, Ellis D,Mundell J, Van Sanford D (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2008 Dec 2-4 Indianapolis, IN. Lexington, KY: Univeresity of Kentucky, p. 174. Abstract and poster presentation.

Lewis, J., 2012. Personal communication.

Matthaus, K. et al., 2004. Progression of mycotoxin and nutrient concentrations in wheat after inoculation with Fusarium culmorum. Archives of Animal Nutrition, 58(1): 19-35.

McKeehen, J.D., Busch, R.H. and Fulcher, R.G., 1999. Evaluation of wheat (Triticum aestivum L.) phenolic acids during grain development and their contribution to Fusarium resistance. Journal of Agricultural and Food Chemistry, 47(4): 1476-1482.

McMullen, M., Jones, R. and Gallenberg, D., 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Disease, 81(12): 1340-1348.

Meredith, P. 1967. Evaluation of the Brabender Quadrumat Junior experimental flour mill for routine wheat testing. J. Sci. Food Agric.18:396-399.

Mesterhazy, A. and Bartok, T., 1997. Effect of chemical control on FHB and toxin contamination of wheat. Cereal Research Communications, 25(3): 781-783.

Mesterhazy, A., 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breeding, 114(5): 377-386.

Mesterhazy, A., 2002. Role of deoxynivalenol in aggressiveness of Fusarium graminearum and F-culmorum and in resistance to Fusarium head blight. European Journal of Plant Pathology, 108(7): 675-684.

Mesterhazy, A., T. Bartok, C.G. Mirocha, and R. Komoroczy. 1999. Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breeding 118:97-110.

Mesterhazy, A., Toth, B., Bartok, T. and Varga, M., 2008. Breeding strategies against FHB in winter wheat and their relation to Type I resistance. Cereal Research Communications, 36: 37-43.

Metzger, R.J. and Silbaugh, B.A., 1970. Location of genes for seed coat color in hexaploid wheat, Triticum-aestivum l. Crop Science, 10(5): 495-&.

Michigan automated weather network: http://www.agweather.geo.msu.edu/mawn/station.asp?id=msu

Miller, J.D., Greenhalgh, R., Wang, Y.Z. and Lu, M., 1991. Trichothecene chemotypes of 3 Fusarium species. Mycologia, 83(2): 121-130.

Miller, J.D., Young, J.C. and Sampson, D.R., 1985. Deoxynivalenol and fusarium head blight resistance in spring cereals. Phytopathologische Zeitschrift-Journal of Phytopathology, 113(4): 359-367.

Mirocha, C.J., E. Kolaczkowski, W.P. Xie, H. Yu, and H. Jelen. 1998. Analysis of deoxynivalenol and its derivatives (batch and single kernel) using gas chromatography mass spectrometry. Journal of Agricultural and Food Chemistry 46:1414-1418.

Miskelly, D.M., 1984. Flour components affecting paste and noodle color. Journal of the Science of Food and Agriculture, 35(4): 463-471.

Moder, G.J., Finney, K.F., Bruinsma, B.L., Ponte, J.G. and Bolte, L.C., 1984. Bread-making potential of straight-grade and whole-wheat flours of triumph and eagle-plainsman v-hard red winter wheats. Cereal Chemistry, 61(4): 269-273.

MSPT, 2007. http://www.css.msu.edu/varietytrials/wheat/Variety_Results.html

Mujeeb-Kazi, A., Hettel., G.P., 1995. Utilizing wild grass biodiversity in wheat improvement: 15 years of wide cross research at CIMMYT, CIMMYT Research Report

Mujeeb-Kazi, A., Silva, P, Fraga, H., Acevedo, E., Pagas, R. 1999. Bread wheat, durum wheat, and synthetic hexaploid wheat in saline and non-saline soils.

National Agricultural Statistics Service, 2012. http://www.nass.usda.gov/

Nevo, E. 1998. Genetic diversity in wild cereals: Regional and local studies and their bearing on conservation ex situ and in situ. Genetic Resources and Crop Evolution 45, no. 4: 355-370.

Nevo, E. 2002. Evolution of wild emmer and wheat improvement: population genetics, genetic resources, and genome organization of wheat's projenitor, *Triticum dicoccoides*. Berlin: Springer.

Nishio, Z., K. Takata, M. Ito, M. Tanio, T. Tabiki, H. Yamauchi, and T. Ban. 2010. Deoxynivalenol Distribution in Flour and Bran of Spring Wheat Lines with Different Levels of Fusarium Head Blight Resistance. Plant Disease 94:335-338.

Park, W.J. et al., 1997. Variation in polyphenol oxidase activity and quality characteristics among hard white wheat and hard red winter wheat samples. Cereal Chemistry, 74(1): 7-11.

Parry, D.W., Jenkinson, P. and McLeod, L., 1995. Fusarium ear blight (scab) in small-grain cereals - A review. Plant Pathology, 44(2): 207-238.

Perkowski, J., T. Basinski, M. Wiwart, M. Kostecki, M. Busko, and A. Matysiak. 2008. The effect of environmental conditions on ergosterol and trichothecene content of naturally contaminated oat grain. Annals of Agricultural and Environmental Medicine 15:271-276.

Pestka, J.J. and Smolinski, A.T., 2005. Deoxynivalenol: Toxicology and potential effects on humans. Journal of Toxicology and Environmental Health-Part B-Critical Reviews, 8(1): 39-69.

Pestka, J.J., 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. Animal Feed Science and Technology, 137(3-4): 283-298.

Pestka, J.J., Zhou, H.R., Moon, Y. and Chung, Y.J., 2004. Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. Toxicology Letters, 153(1): 61-73.

Prange, A., B. Birzele, J. Kramer, A. Meier, H. Modrow, and P. Kohler. 2005. Fusarium-inoculated wheat: deoxynivalenol contents and baking quality in relation to infection time. Food Control 16:739-745.

Pritsch, C., Muehlbauer, G.J., Bushnell, W.R., Somers, D.A. and Vance, C.P., 2000. Fungal development and induction of defense response genes during early infection of wheat spikes by Fusarium graminearum. Molecular Plant-Microbe Interactions, 13(2): 159-169.

Pugh, G.W., Johann, H. and Dickson, J.G., 1933. Factors affecting infection of wheat heads by Gibberella saubinetii. Journal of Agricultural Research, 46: 0771-0797.

Rudd, J.C., Horsley, R.D., McKendry, A.L. and Elias, E.M., 2001. Host plant resistance genes for Fusarium head blight: Sources, mechanisms, and utility in conventional breeding systems. Crop Science, 41(3): 620-627.

Rupp, H.S., 2002. Determination of deoxynivalenol in whole wheat flour and wheat bran. Journal of Aoac International, 85(6): 1355-1359.

Schroeder, H.W., and J.J. Christensen. 1963. Factors affecting resistance of wheat to scab caused by Gibberella zeae. Phytopathology 53:831-&.

Scott1991-http://www.amazon.com/Cereal-Grain-Mycotoxins-Quality-developments/dp/0444885544

Sherman, J.D., Souza, E., See, D. and Talbert, L.E., 2008. Microsatellite markers for kernel color genes in wheat. Crop Science, 48(4): 1419-1424.

Skinnes, H., Tarkegne, Y., Dieseth, J.A. and Bjornstad, A., 2008. Associations between anther extrusion and Fusarium Head Blight in European wheat. Cereal Research Communications, 36: 223-231.

Sneller C., Paul P., Herald L and Sugerman B, 2011. Report on the 2010-2011 Northern Uniform Winter Wheat Scab Nurseries

Snijders, C.H.A. 1990. Fusarium head blight and mycotoxin contamination of wheat, a review. Netherlands Journal of Plant Pathology 96:187-198.

Snijders, C.H.A. and Perkowski, J., 1990. Effects of head blight caused by Fusarium-culmorum on toxin content and weight of wheat kernels. Phytopathology, 80(6): 566-570.

Stack RW, 2003. History of Fusarium head blight with emphasis on North America. In: Leonard KJ, Bushnell WR, eds. Fusarium Head Blight of Wheat and Barley. St Paul, MN, USA: APS Press, 1–34.

Strange, R.N. and Smith, H., 1969. A growth stimulant for Fusarium-graminearum in wheat anthers which promotes head infection. Journal of General Microbiology, 59: R2-&.

Strange, R.N. and Smith, H., 1971. Fungal growth stimulant in anthers which predisposes wheat to attack by Fusarium-graminearum. Physiological Plant Pathology, 1(2): 141-&.

Sutton JC, 1982. Epidemiology of wheat head blight and maize ear rot caused by Fusarium graminearum. Canadian Journal of Plant Pathology 4, 195–209.

Sydenham, E.W., Marasas, W.F.O., Thiel, P.G., Shephard, G.S. and Nieuwenhuis, J.J., 1991. Production of mycotoxins by selected Fusarium-graminearum and F-crookwellense isolates. Food Additives and Contaminants, 8(1): 31-41.

Taylor, M.R., Brester, G.W. and Boland, M.A., 2005. Hard white wheat and Gold Medal Flour: General Mills' contracting program. Review of Agricultural Economics, 27(1): 117-129.

Thammawong, M., H. Okadome, T. Shiina, H. Nakagawa, H. Nagashima, T. Nakajima, and M. Kushiro. 2011. Distinct distribution of deoxynivalenol, nivalenol, and ergosterol in

Fusarium-infected Japanese soft red winter wheat milling fractions. Mycopathologia, 172(4): 323-330.

Thammawong, M., M. Okabe, T. Kawasaki, H. Nakagawa, H. Nagashima, H. Okadome, T. Nakajima, and M. Kushiro. 2010. Distribution of deoxynivalenol and nivalenol in milling fractions from Fusarium-infected Japanese wheat cultivars. Journal of Food Protection 73:1817-1823.

TrigoStockli, D.M., Deyoe, C.W., Satumbaga, R.F. and Pedersen, J.R., 1996. Distribution of deoxynivalenol and zearalenone in milled fractions of wheat. Cereal Chemistry, 73(3): 388-391.

Trucksess, M.W., D.E. Ready, M.K. Pender, C.A. Ligmond, G.E. Wood, and S.W. Page. 1996. Determination and survey of deoxynivalenol in white flour, whole wheat flour, and bran. Journal of Aoac International 79:883-887.

USDA-ERS, 2011. http://www.ers.usda.gov/briefing/wheat/2012baseline.htm

Wang, Y.Z., and J.D. Miller. 1988. Effects of Fusarium-graminearum metabolites on wheat tissue in relation to Fusarium head blight resistance. Journal of Phytopathology-Phytopathologische Zeitschrift 122:118-125.

Wanjiru, W.M., Kang, Z.S. and Buchenauer, H., 2002. Importance of cell wall degrading enzymes produced by Fusarium graminearum during infection of wheat heads. European Journal of Plant Pathology, 108(8): 803-810.

Worland T., and Snape J. W., 20101. Genetic basis of worldwide wheat varietal improvement. In: The World Wheat Book: A history of wheat breeding. Bonjean A.P., Angus W.J. eds

Yamazaki, W. T., and C. T. Greenwood. 1981. Soft wheat: production, breeding, milling, and uses. St. Paul, Minn: American Association of Cereal Chemists.

Yi, C., Kaul, H.P., Kubler, E., Schwadorf, K. and Aufhammer, W., 2001. Head blight (Fusarium graminearum) and deoxynivalenol concentration in winter wheat as affected by pre-crop, soil tillage and nitrogen fertilization. Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection, 108(3): 217-230.

Young, J.C., and J.D. Miller. 1985. Appearance of fungus, ergosterol and Fusarium mycotoxins in the husk, axial stem and stalk after ear inoculation of field corn. Canadian Journal of Plant Science 65:47-53.

Young, J.C., R.G. Fulcher, J.H. Hayhoe, P.M. Scott, and J.E. Dexter. 1984. Effect of milling and baking on deoxynivalenol (vomitoxin) content of eastern canadian wheats. Journal of Agricultural and Food Chemistry 32:659-664.

Zhang, D. and Moore, W.R., 1999. Wheat bran particle size effects on bread baking performance and quality. Journal of the Science of Food and Agriculture, 79(6): 805-809.

Zhang, X., T. Van De Lee, M. Dufresne, T.G. Liu, W.Z. Lu, D.Z. Yu, and H.X. Ma. 2008. Infection of green fluorescence protein-tagged Fusarium graminearum on wheat and barley spikes. Cereal Research Communications 36:465-469.