Factors Affecting Fusarium Head Blight Development and Trichothecene Accumulation in *Fusarium*-infected Wheat Heads

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Pravin Gautam

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Ruth Dill-Macky, PhD

JUNE 2010

Acknowledgements

I would like to express my heartfelt gratitude to Dr. Ruth Dill-Macky for the opportunity and support towards this degree. Sincere appreciation goes to her for her excellent guidance and advice in this research and other academic area.

My thanks are due to Drs. Brian Steffenson, James Kurle and James A. Anderson for graciously accepting to serve on my doctoral committee and for providing valuable comments and suggestions as and when necessary.

I would like to extend thank to Dr. Yanhong Dong, Mycotoxin laboratory at University of Minnesota for help in mycotoxins analyses. I am thankful to the Small Grain Pathology Lab members; Beheshteh Zargaran and Tracy Scanlan, and former lab members Amar Elakkad and Karen Wennberg, for their valuable assistance in conducting experiments. Without them my research wouldn't have been completed successfully. My sincere thanks go to Susan Durham, Utah State University and Dr. Aaron Rendahl, University of Minnesota for providing assistance in statistical analysis. Last but not least, I would like to thank the US Wheat and Barley Scab Initiative for funding this research.

Dedication

This dissertation is dedicated to three persons who shaped me to become who I am. First, my late father Revati Raman Gautam, who always dreamed me to be educated to the highest level. Second, my mother Shobha Gautam, who devoted her life to educate her children. Finally, my wife Muna Kadariya, who supported in my each and every endeavor despite a painful separation during my doctoral period.

Abstract

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* Schwabe, is an economically important disease as it results in yield loss and quality losses of infected grain and the accumulation of mycotoxins produced by the invading fungus. Environmental factors, host genetics, and isolate aggressiveness impact FHB development and subsequently trichothecene production and accumulation. Though it is well established that moisture around anthesis promotes FHB development and trichothecene accumulation, the role of moisture, either in the form of rainfall or mist-irrigation during the period from anthesis to harvest has been largely overlooked. A three year field experiment was conducted in 2006, 2007 and 2008 to examine the influence of environmental factors, especially moisture, host resistance, and pathogen variation with respect to mycotoxin production capacity and pathogen aggressiveness, on infection, FHB development and mycotoxin production and accumulation *in planta*. In mature harvested grain FHB severity, visually scabby kernel (VSK) and mycotoxin concentration were significantly higher in Wheaton (FHB susceptible) than in the other two cultivars examined, Alsen and 2375. Although FHB severities were not significantly different in plots receiving different durations of mist-irrigation, VSK were significantly lower in the treatments receiving the least amount of mist-irrigation (14 DAI) than for treatments receiving mist-irrigation for longer periods, suggesting that extended periods of moisture promote disease development. DON concentration in harvested grain was, however, significantly lower in the treatment receiving the longest duration of mist-irrigation than those treatments receiving less water. In the whole head samples, which were collected 0, 7, 11, 14, 21, 28 and 41 days after inoculation, DON and other trichothecenes either declined with increased durations of mist-irrigation or remained low while water was being applied by the misting system. However, trichothecene accumulation was observed to increase after the cessation of mist-irrigation, with increases being most pronounced for the treatments with shorter mist-irrigation periods. The largest reduction in DON observed as a result of extended mist-irrigation periods was seen in the susceptible cultivar Wheaton.

The influence of host resistance and pathogen variation on infection, FHB infection, disease development and mycotoxin accumulation *in planta* was examined in the series of greenhouse experiments utilizing point and spray inoculations. The levels of FHB severity and mycotoxins were higher in spray inoculated experiment than point inoculation in all cultivars examined. Wheaton (FHB susceptible) had the highest FHB severity and levels of mycotoxins. Alsen (moderately resistant to FHB) had significantly lower FHB severities, DON, 15-ADON,

3-ADON and NIV than either 2375 or Wheaton. Though there were no significant differences in initial infection among cultivars examined, Alsen had reduced spread of FHB symptoms from initial infection presumably due to type II resistance. DON production did not peak in all treatments, but where evident, the peak was earlier in 2375 (11 dai) than Alsen and Wheaton (21 or 14 dai). Multiple peaks and declines in DON levels were also evident. The performance of isolates was highly variable, though generally isolates Butte86Ada-11 and B63A were the most aggressive isolates and 49-3 and B45A were the least.

The impact of free moisture, such as that from irrigation systems or rainfall, on mycotoxin accumulation was evaluated in greenhouse experiments. Despite the similar levels of FHB severity observed, the levels of mycotoxins were significantly less in the plants that received a single six hour wetting treatment compared to the respective control. The loss of DON and other mycotoxins was evident in all cultivars examined. Further, DON and 15-ADON were detected in run-off water.

The results of these studies suggest that the availability of free moisture such as from mist-irrigation or rainfall may increase FHB severity and VSK, although DON and other trichothecene concentrations may be concomitantly reduced. Leaching appears to contribute to reductions in DON following wetting events.

Table of Contents

List of Tables

List of Figures

Chapter 1

General Introduction

Fusarium head blight (FHB or scab), a major disease of wheat and other small grains, is incited by several species of *Fusarium* (Atanasoff, 1920; Parry et al., 1995; Stack and McMullen, 1985). *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch], *F. culmorum* W. G. Smith (Sacc.) and *F. avenaceum* (Fr.) Sacc. (teleomorph *G. avenacea* R. J. Cooke), however, are the most common causal agents (Parry et al., 1995). In North America, *F. graminearum* is the primary species causing FHB (McInnes and Fogelman, 1932; Wilcoxson et al., 1988). Temperature appears to be a critical criterion for the distribution of the major *Fusarium* species. While *F. graminearum* is more common in warmer and humid regions of the world, including North America, Australia and central Europe and southern Europe (Mesterházy, 2003; Parry et al., 1995), *F. culmorum* is more frequently isolated in cooler and humid areas including north-western Europe (Parry et al., 1995; Snijders et al., 1990b).

Though FHB has not been considered a widespread disease (Parry et al., 1995), in the last two decades it has re-emerged worldwide as a disease of importance (Windels, 2000). Because of its multifaceted effects on crops (Atanasoff, 1920) it has been estimated to have caused economic losses of $2.7 billion in the northern Great Plains and central US from 1998 to 2000 (Nganje et al., 2002). Although FHB has been common, severe and well documented in the past, recent epidemics have resulted in increased concern, greater public interest and expanded research efforts.

Warm temperatures and extended periods of moisture on plant surfaces around anthesis are essential for the infection and colonization of wheat tissues by *F. graminearum* and the first symptoms of water soaked lesions appear within 2-4 days after infection (Atanasoff, 1920, Xu, 2003). Kernels from infected spikelets are generally smaller and lighter having a shriveled and chalky appearance referred to as ‘tombstones’ or scabby kernels (Abramson et al., 1987; Dickson and Mains, 1929; Johnson and Dickson, 1921; Parry et al., 1995). Formation of tombstones reduces the yield significantly and *Fusarium* damaged grain tends to exhibit weaker dough properties and unsatisfactory baking quality, making the marketing and processing of the grain difficult (Bechtel et al., 1985; Dexter et al., 1996; Dexter et al., 1997; Wang et al., 2005). In addition to yield losses, the quality of infected grain is compromised due to the production of a range of mycotoxins, including deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), and nivalenol (NIV), by the FHB pathogens (Nasri et al., 2006; Pirgozliev et al., 2003). These mycotoxins are hazardous to humans and animals, thus making

highly infected grain unfit for food or feed. Wheat from US production fields infected by *Fusarium* have routinely tested positive for most of these mycotoxins (Abramson et al., 1987; McMullen et al., 1997). DON is considered the most important toxin produced by *F. graminearum* and is regulated in the grain and wheat based food products by US federal agencies.

FHB resistant wheat is reported to be associated with lower levels of DON contamination than FHB susceptible wheat (Mesterházy et al., 2003; Miller et al., 1985; Wilde and Miedaner, 2006). Whether the reduced DON in cultivars expressing FHB resistance is due to the resistance of the host to initial infection (type I resistance, Schroeder and Christensen, 1963), spread of the fungus in the spike (type II resistance, Schroeder and Christensen, 1963) or modes of resistance either preventing DON synthesis or promoting the degradation of DON (Miller et al., 1985; Miller and Arnison, 1986) has not been clearly established. To facilitate the evaluation and selection of resistant germplasm and to assess commercial crop quality, researchers frequently utilize correlations between visual FHB severity assessments in the field and DON concentrations to predict DON in harvested grain (Arsenuik et al., 1999; Groth et al., 1999; Jones and Mirocha, 1999). Although correlations are generally significant, correlation coefficients are frequently low and vary greatly among locations and years. The reasons for this variability are not fully understood, but may be associated with toxin production capacity of prevalent strains within the *Fusarium* population, the type and level of host plant resistance to FHB and/or the environmental conditions prevailing between the time of initial infection and harvest.

Environmental factors, primarily moisture, have been reported to have an important influence on toxin production (Hope et al., 2005). Moisture in the form of rainfall or relative humidity, during anthesis or shortly thereafter has been linked to higher FHB incidences, higher FHB severities and DON accumulation (Abramson et al., 1987; Atanasoff, 1920; Rohácik and Hudec, 2005; Tuite et al., 1990). However, the specific environmental factors triggering, regulating or influencing mycotoxin synthesis and accumulation in the infected host are not well understood, (Mesterházy, 1999).

The application of extended supplemental moisture between anthesis and the time of disease rating, generally around 21 days after inoculation (dai), is common in field nurseries screening wheat germplasm for resistance to FHB. Supplemental moisture is generally provided in the form of mist-irrigation. The possible impact of supplemental moisture and rainfall occurring after disease rating on the accumulation of *Fusarium*-mycotoxins has been largely ignored, however. It has been reported that continuous mist-irrigation for 3 days, after inoculation with *F. culmorum*, increased DON in harvested wheat compared to either of the overnight or one

full day misting (Lacey et al., 1999). Lemmens et al. (2004) used two levels of mist-irrigation treatments (no irrigation and irrigation for 26 days after flowering) and reported a decrease in the DON level in some wheat lines while observing an increase in others. Culler et al. (2007) also reported relatively lower levels of DON with greater environmental moisture, especially in the susceptible hard red spring wheat cultivar, Wheaton. They observed generally lower DON concentrations under misting treatments which ran for 31-32 days after inoculation compared to misting treatments for 15-16 days after inoculation. The differences in DON levels were, however, not significant at all sampling times. In contrast, Cowger et al. (2009) reported increases in FHB severities, *Fusarium* damaged kernels (FDK) and DON levels with the increased durations of mist-irrigation from 0 to 30 days post-anthesis.

Several studies have reported a positive correlation between isolate aggressiveness and DON production by *F. graminearum* and *F. culmorum* (Gang et al., 1998; Hestbjerg et al., 2002; Mesterházy, 2002). However, DON is not essential for pathogenesis (Dyer et al., 2005; Eudes et al., 2001; Proctor et al., 1995). The time from inoculation until the initiation of production of DON reported for wheat varies from 26 hrs (Chen et al., 1996) to 4 days (Savard et al., 2000), which supports findings that DON is not required for initial infection and colonization.

The first objective of this study was to examine the influence of environmental factors, especially moisture, host resistance types, and pathogen variation with respect to mycotoxin production capacity and pathogen aggressiveness, on infection, FHB development and mycotoxin production and accumulation *in planta*. The second objective of this study was to examine the impact of free environmental moisture, such as from rainfall or simulated by irrigation systems on disease development and mycotoxin production and accumulation *in planta*. Specifically, the second objective aimed to determine if a single wetting event could result in the leaching of mycotoxins produced *in planta* from head tissues.

Chapter 2

Literature Review

2.1 History

Fusarium head blight (FHB) of wheat and other small grains usually occurs in warm and humid cereal growing regions of the world (Schroeder and Christensen, 1963; Wilcoxson et al., 1992). The disease was first described by W. G. Smith in England in 1884 (McInnes and Fogelman, 1932). In North America, it was first reported by J. C. Arthur (Arthur, 1891). Fusarium head blight was reported in Ohio, Delaware, Indiana, Iowa, Pennsylvania, and Nebraska before 1900 (Stack, 2003). In Minnesota, the earliest FHB outbreaks reported were in 1905, 1907 and 1915 (MacInnes and Fogelman, 1923). Most of the Midwestern states were also affected by epidemics in 1917 among which Illinois, Indiana and Ohio were hardest hit. By 1919 the disease had been detected in 31 states, with its distribution covering most of the central and eastern states (Atanasoff, 1920). A major outbreak of FHB occurred in 1919 with the greatest loss of wheat reported in Illinois and Iowa (Dickson and Mains, 1929; Stack, 2003). In this epidemic Minnesota recorded losses in wheat production over 10% (Johnson and Dickson, 1921). In 1925 and 1928 outbreaks of FHB were again severe in Minnesota. Barley was heavily affected in a 1928 outbreak (Dickson and Mains, 1929). In the tri-state region of Minnesota, North Dakota and South Dakota, FHB was severe in the southern and eastern parts in 1932 and barley crops in Minnesota and Iowa were affected (Christensen and Stakman, 1935). Outbreaks continued across the country in 1940's and 1950's. Minnesota had serious outbreaks of FHB in 1939, 1941, 1944 and 1945 (Hanson et al., 1950). During the 1970's and 1980's, FHB epidemics were sporadic (Halfon-Meiri et al., 1979; Tuite et al., 1990), although the disease appeared in eastern and western Canada, and several states of the US including Minnesota (Sutton, 1982; Stack and McMullen, 1985; Wilcoxson et al., 1988). Epidemics which occurred during the 1990's, mostly across the eastern half of the US, are of prime importance in the history of the FHB. In 1993, Minnesota, North Dakota, South Dakota and the Canadian province of Manitoba were devastated by FHB epidemics. According to McMullen et al. (1997) the 1993 epidemic caused the greatest loss in any single year due to plant disease in the tri-state area and thus rivals the stem rust epidemics that ravaged the Upper Midwest in the early 1900's (Eversmeyer and Kramer, 2000). A severe epidemic occurred in 1996 on soft red and soft white winter wheat in Iowa, Arkansas, Louisiana, Ohio, Indiana, Illinois, Wisconsin, Michigan, New York and the Canadian province of Ontario (McMullen et al., 1997). Several soft red wheat producing states east of Mississippi River were ravaged by FHB in 2003 (USBWSI, 2004). In 2007, FHB disease was generally low

across the US. However, isolated disease outbreaks were serious in Nebraska and Kansas (USBWSI, 2007b). The situation repeated in 2008 with Nebraska and Kansas being hardest hit while much of the rest of the US reported little FHB (USBWSI, 2008).

2.2 Economic importance

Fusarium head blight occurs almost every year but is generally limited to relatively few wheat and barley crops. Thus FHB is not recorded as a widespread disease (Parry et al., 1995). In recent years, however, FHB has re-emerged worldwide as a disease of economic importance (Windels, 2000) with enormous economic impact because of its multifaceted effects on crops (Atanasoff, 1920). The impact of FHB starts right after germination as *Fusarium* infection of seed can result in reduced germination and post emergence seedling blight (Bechtel et al., 1985; Jones, 1999). However, FHB cannot be seed transmitted through *Fusarium*-infected seeds (Jones, 1999). In addition to yield losses caused by the FHB, the presence of mycotoxins in infected grain further exacerbates disease losses (McMullen et al, 1997). In the 1917 disease outbreak, wheat yield loss was 288.8 megagram (Mg) and was attributed to several species of *Fusarium* (Atanasoff, 1920). In the 1928 epidemics, there were yield losses of 20% and 15% in barley and wheat, respectively (Stack, 2003). Yield losses in wheat due to the FHB epidemics during 1990's in the US was over 18.4 Mg valued at ca. $2.5 billion. Similarly, barley producers lost $400 million in the same time (Windels, 2000). The epidemics of 1990's in the tri-state area were so serious that there was a net loss in revenue per harvested acre of wheat in the Red River Valley area of North Dakota and Minnesota every year from 1993 to 1998 with exception of Minnesota in 1996 (Windels, 2000). Estimated direct and secondary economic losses by FHB in wheat and barley in the northern Great Plains and central US was $2,679 million from 1998 to 2000 (Nganje et al., 2002). As a consequence of these losses to FHB, land planted to barley from 1991 to 1999, decreased by 77%, 53% and 84% in Minnesota, North Dakota and South Dakota, respectively. Similarly, the area planted to wheat decreased by 6%, 5% and 7% in Minnesota, North Dakota and South Dakota, respectively (NASS, 2009). Many farmers abandoned farming as an occupation and wheat crops became rotational crops and the barley crop was almost wiped out from Minnesota (McMullen, 2003). The decrease in wheat and barley planting from 1991 to 1999 can be attributed primarily due to yield losses caused by FHB and associated quality losses due to mycotoxin accumulation in the infected grain (Windels, 2000).

2.3 Causal organisms

Head blight disease is caused by several species of the fungal genus *Fusarium* (Atanasoff, 1920; Parry et al. 1995; Stack and McMullen, 1985). *Fusarium* species that are reported to cause head blight to various extent in cereals are *F. acuminatum* Ellis & Kellerm. (teleomorph *G. acuminata* Wollenw.), *F. avenaceum* (Fr.) Sacc. (teleomorph *G. avenacea* R.J. Cooke), *F. crookwellense* Burgess, Nelson & Toussoun, *F. culmorum* W.G. Smith (Sacc.), *F. dimerum* Penzig, *F. equiseti* (Cda.) Sacc., *F. equiseti* v. *bullatum* (Sherb.) Wollenw. (teleomorph *G. intricans* Wollenw.), *F. graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch], *F. lateritium* Nees (teleomorph *G. baccata* (Wallr.) Sacc.), *F. merismoides* Corda, *F. moniliforme* Sheldon (synonyms *F. verticillioides* (Sacc.) Nirenberg) (teleomorph *G. moniliformis* Wineland), *F. orthoceras* v. *longius* (Sherb.) Wollenw., *F. oxysporum* v. *aurantiacum* (Lk.) Wollenw., *F. poae* (Peck) Wollenw., *F. proliferatum* (Matsush.) Nirenberg ex Gerlach &Nirenberg, *F. redolens* Wollenw., *F. sambucinum* Fuckel (teleomorph *G. pulicaris*), *F. scirpi* Lamb. Et Fautr., *F. semitectum* Berk. & Ravelnel, *F. solani* (Mart. pr. p) App. et Wollenw., *F. sporotrichioides* Sherb, *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas, *F. tricinctum* (Corda) Sacc., *Microdochium nivale* (Fries) Samuels & Hallet [synonym *Fusarium nivale* (Fr.) Ces.] (teleomorph *Monographella nivalis*) (Abramson et al. 1987; Atanasoff, 1920; Duthie et al., 1986; Liddell, 2003; Parry et al., 1995; Schroeder and Christensen, 1963; Stack, 2003; Stack and McMullen, 1985; Tuite et al., 1990; Vargo et al., 1981; Wilcoxson et al., 1988; Wong et al. 1992).

Though FHB can be incited by several species of *Fusarium*, *F. graminearum*, *F. culmorum* and *F. avenaceum* are the most common causal agents (Parry et al., 1995). In North America, especially in Minnesota, *F. graminearum* is the primary species causing head blight (McInnes and Fogelman, 1932; Wilcoxson et al., 1988). Temperature is a critical factor for the distribution of the primary causal agents among the major *Fusarium* species. While *F. graminearum* is more common in warmer and humid regions of the world, including North America, Australia and central Europe and southern Europe (Mesterházy, 2003; Parry et al., 1995), *F. culmorum* dominates in cooler and humid areas including north-western Europe (Parry et al., 1995; Snijders et al., 1990b). Of several species isolated from infected wheat heads in North Dakota, *F. graminearum* was present in 68% (Stack and McMullen, 1985). Similarly, *F. graminearum* was isolated from 75% of the heads along with 15 other species isolated from heads collected in farm fields and experimental plots throughout Minnesota (Wilcoxson, 1988). When Tuite et al. (1990) analyzed FHB infected winter wheat samples from Indiana, 96% were infected

with *F. graminearum*. However, when the FHB severity is low, other species are reported to become dominant (Shaner, 2003).

2.4 Sign and symptoms

The symptoms of FHB are similar in all the affected cereals (Dickson and Mains, 1929; Parry et al., 1995). The first symptoms, water-soaked lesions of 2-3 mm in length, appear (Atanasoff, 1920) within 2-4 days after infection under favorable conditions, mostly at the base of the middle spikelets in the middle of the head (Stack, 2003). In water-soaked lesions in the glumes, the veins have a darker olive-green color than the area between veins (Atanasoff, 1920). Soon after the water soaking appears, symptoms spread to the rachis. Through the rachis the fungus can rapidly spread up, down and horizontally in the spike (Parry et al., 1995; Wiese, 1987). Frequently salmon to pink colored fungal growth and orange colored sporodochia can be seen at the base of the spikelets or along the edge of glumes (Arthur, 1891; Johnson and Dickson, 1921, Parry et al 1995). Under humid conditions water-soaked lesions may turn brown to purplish-brown with or without a bleached center (Bennett, 1931). Similarly, early infected spikelets give a 'scabbed' appearance due to formation of blue-black perithecia under prolonged moist and humid conditions (Mathre, 1982). If the environment is dry, the water-soaked symptoms turn the typical color of ripe head (Atanasoff, 1920). In most of the cases in susceptible cultivars of wheat, fungal growth in the rachis causes vascular occlusion cutting off the nutrient and water supply to spikelets above the point of infection (Atanasoff, 1921; Bai and Shaner, 1996). This results in healthy spikelets above the infection point drying out and turning to the color of mature heads, (Johnson and Dickson, 1921; Stack, 2003). Such dried spikelets shrink and appress to the rachis. Grains do not form or do not develop fully on such spikelets depending upon the stages of grain at which vascular tissues become dysfunctional. This phenomenon is more evident in susceptible cultivars of wheat (Bai and Shaner, 1996). In some cases one or a few vascular bundles remain uninfected continuing the nutrient supply to the spikelets above the infected spikelets allowing the formation of normal grain (Atanasoff, 1920).

In addition to the floret, characteristic signs and symptoms of FHB also develop in kernels. Symptom development in kernel depends on the time of infection. In severely infected and early infected spikelets, kernels do not develop. If kernels develop, they are smaller and light weight with a shriveled and chalky appearance and are referred to 'tombstones' or 'Fusarium damaged kernels' (FDK) or scabby kernels (Abramson, 1987; Dickson and Mains, 1929; Johnson and Dickson, 1921; Parry et al., 1995). If the infection occurs towards late stage of the kernel

development, kernels may achieve normal size and weight, but may look discolored with pinkish areas (Atanasoff, 1920).

2.5 Biology of *Fusarium graminearum*

F. graminearum belongs to the fungal phylum Ascomycota (Webster and Weber, 2007). The teleomorph or sexual stage of *F. graminearum* is *Gibberella zeae* (Schwein) Petch [syns. *G. roseum*] (Booth, 1971). *G. zeae* produces dark purple perithecia, which appear black. Perithecia are generally 140-250 µm in diameter and contain narrowly clavate and thin walled asci. The asci bear eight ascospores which are 3 or more septate, 20-29 µm in length and 3.5-4.5µm in width (Samuels et al., 2001; Webster and Weber, 2007). Each perithecium may bear up to 45,000 ascospores (Khonga and Sutton, 1988).

Burgess et al. (1975) described two groups of *F. graminearum*; group 1 and group 2. Group 1 isolates were described as homothallic and unable to produce perithecia on potato dextrose agar (PDA) and carnation leaf agar (CLA) media although fertile perithecia have been reported to occur in group 1 (Burgess et al., 1975). Group 2 isolates were described as homothallic and readily able to form fertile perithecia on PDA and CLA media (Burgess et al., 1975; Francis and Burgess, 1977). Group 1 isolates were associated with crown rot of wheat and group 2 isolates were primarily associated with stalk rot of maize and head blight of wheat (Burgess et al., 1975; Purss, 1971). It was suggested that *F. graminearum* group 1 evolved as soil borne pathogens and group 2 as air borne pathogens (Sutton, 1982). Later Aoki and O'Donnell (1999) reclassified group 1 isolates as *F. pseudograminearum* (teleomorph *G. coronicola* Aoki and O'Donnell) based on phylogeny analysis of DNA sequence of β-tubulin introns and exons from both groups 1 and 2. Further it was suggested that the morphology of macroconidia can be used reliably to distinguish these two species. While the conidium of *F. graminearum* is widest at upper region, conidium of *F. pseudograminearum* is widest at the midregion of their length (Aoki and O'Donnell, 1999; Francis and Burgess, 1977).

When *F. graminearum* is grown in PDA media, white mycelial colonies grow fast and develop a characteristic color of pink to dark purple (Samuels et al., 2001). *F. graminearum* produce classic foot-cells and banana shaped, 3-5 septate macroconidia on (Liddel, 2003; Seifert, 2001). Unlike some of other *Fusarium*, *F. graminearum* do not produce microconidia (Burgess et al., 1994; Liddel, 2003; Nelson et al., 1983). The size of macroconidia ranges from 41 to 60 µm in length and 4.5 to 5 µm in width (Samuels et al., 2001). Often *F. graminearum* produce chlamydospores, a thick walled structure formed from macroconidia and/or mycelium, when they

are in contact with soil (Sitton and Cook, 1981). Chlamydospores are globose to oval in shape, 8-12 μm in diameter and pale brown in color. They are generally produced in pairs or short chains (Gerlach et al., 1982). Though the function of chlamydospores in other microorganisms is for survival, its function in *F. graminearum* is largely unknown.

Conidia, ascospores, chlamydospores and hyphal fragments may serve as inoculum for infection (Dill-Macky, 2003). Ascospores are the principle inoculum in natural conditions. The infection effectiveness is not significantly different for ascospores and conidia (Stack, 1989; Scholz and Steffenson, 2001), thus the use of macroconidia is comparable to ascospores in experiments. Most experiments conducted with *F. graminearum* use conidia as inoculum because of their hydrophilic nature, ease of production, the ability to quantify inoculum and because they are economic to produce with respect to labor and financial resource (Dill-Macky, 2003). Hyphal fragments cannot be quantified thus its use as inoculum is limited.

Ascospores are forcibly ejected from asci and perithecia (Webster and Weber, 2007). Once they are ejected, they are dispersed by wind (Sutton, 1982). Ascospores have a diurnal discharge pattern which peaks from 1800 to 2400 hours (Paulitz, 1996). The discharge of ascospores is dependent on environmental factors, primarily moisture and temperature. It has been hypothesized that humidity promotes ascospore release through the mechanical rupture of the ascus due to swelling of mucilage around ascospores. However, Tschanz (1975) suggested that drying of perithecia and the substrate on which perithecia form, is required for the forcible ejection of ascospores and maximum ascospores discharge occurs at 16ºC. Though the moisture in the form of humidity or rainfall is required for perithecia development, it is not required for ascospore release. Paulitz (1996) showed that rainfall of >5mm during the day or relative humidity of >80% during the day, suppressed the ascospores ejection. However, ascospores will ooze into the gelatinous matrix around the ostiole, the opening of perithecia, when moisture is prevalent.

In contrast to ascospores, conidia are primarily dispersed by rain splash (Sutton, 1982), thus the distance of their dispersal is limited. Intense rainfall and large raindrops are required to splash macroconidia 70-90 cm above the ground surface to infect wheat head (Madden, 1992). Therefore, macroconidia are generally not splashed in one event from plant residues on the ground to the wheat head (Jenkinson and Parry, 1994). However, spores have been recorded to be splashed up to 100 cm (Paul et al., 2004). While macroconidia are also produced in infected heads later in the season, secondary inoculum has less importance in disease development (Fernando et al., 1997).

2.6 Mycotoxins production

The major concern from FHB, in addition to yield loss, is the production of mycotoxins by *Fusarium* in infected grain. Mycotoxins are secondary metabolites that are not essential for survival of the fungus (Desjardins et al, 1993). They are toxic against many different organisms, thus called mycotoxins (Webster and Weber, 2007). Different species of *Fusarium* produce trichothecene mycotoxins (type A or type B) and the estrogenic mycotoxin zeralenone (ZEN). Type A trichothecene include, T-2, H-2, and diacetoxyscirpenol (DAS); and type B trichothecenes include deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON) and fusarenon-X (FUS-X) (Nasri et al., 2006; Pirgozliev et al., 2003). North American strains of *F. graminearum* produce 15-ADON and ZEN, in addition to DON. Most Japanese and Australian strains produce NIV, FUS-X, and ZEN or DON, 3-ADON, and ZEN (Miller et al., 1991). In addition to above mentioned toxins, *F. graminearum* has been reported to produce other secondary metabolites including 4-acetamido-2-butenoic acid, butenolide, diacetodeoxynivalenol, 3,15-dihydroxy-12,13-epoxytrichothec-9-ene-8-one, monoacetoxyscirpenol, and neosolaniol (Marasas et al., 1984). Wheat and barley from US production fields infected by *Fusarium* have routinely tested positive for most of these mycotoxins (Abramson et al., 1987; McMullen et al., 1997). Deoxynivalenol is, however, considered the most important toxin produced by *F. graminearum*, though it is less toxic than other trichothecene. The DON concentration is regulated in grain and wheat based food products by US federal agencies. The US, Canada and several European countries have set 2 mg kg^{-1} as the acceptable limit of DON in wheat grain for human consumption (Bai et al., 2001).

Mycotoxins are hazardous to animals, thus making highly infected grain unfit for food or feed. There is long history of mycotoxosis due the consumption of moldy grain by humans and animals. One of the most notorious cases was the outbreak of alimentary toxic aleukia (ATA), in humans, in Russia during 1932. Symptoms included weakness, vertigo, nausea, vomiting, and diarrhea in milder cases to severe skin rashes and necrotic lesions of the gastrointestinal tract in severe cases, often resulting in death (Dounin, 1926; Joffe, 1986). Though the disease and cause was mysterious during the outbreak, after two decades it was identified that the trichothecene T-2 produced by *F. sporotrichioides* was associated with ATA (Desjardins, 2006). Similarly, outbreaks of human toxicosis during 1890, 1901, 1914, 1920 and 1923 in Japan were associated with grains contaminated with the red mold disease, akakabi-byo. Symptoms included nausea, vomiting, diarrhea, headache, dizziness and visual hallucinations. However, it was not fatal like ATA. Though there is no published proof, it was suspected that DON and NIV from *F.*

graminearum and other *Fusarium* species were associated with the toxicosis (Marasas et al., 1984).

Feed refusal by swine in the USA in 1928 was the first incident that was linked to *F. graminearum* and the toxin DON. In 1928 wheat, barley and oats grains were unsellable due to the FHB epidemic. *Fusarium*-infected grain was used as animal feed. It was reported that swine fed with infected grain became sick with vomiting and refused to eat. Thus DON became known as 'the refusal factor' or 'vomitoxin' (Desjardins, 2006). Later the effect of DON was proved experimentally by Christensen and Kernkamp (1936). They showed that as little as 13 g of heavily FHB infected barley could induce vomiting in a 132 pound pig. In addition to animals and human, *Fusarium* toxins are phytotoxic. The phytotoxicity of mycotoxins produced by several species of *Fusarium* has been demonstrated in pepper, corn, tomato, banana, wheat and several other crops (Bruins et al., 1993; Joffe, 1986; Shimada and Otani, 1990; Wang and Miller, 1988). However, the toxicity among trichothecene differ more than 1000 fold (Rotter et al., 1996). The phytotoxicity of DON results in the inhibition of seed germination and growth of wheat seedlings (Wakulinski, 1989) and the toxin has an inhibitory effect on mitosis (Packa, 1991). The mode of action of trichothecene, including DON, is to inhibit ribosomal protein synthesis in plants. The double bond at C9-C10 and presence of epoxy ring at C-12, 13 in DON is required for its inhibitory action. Inhibition of protein synthesis is achieved through the binding of DON to the 60S subunit of eukaryotic ribosomes and interference with the activity of peptidyltransferase (Ehrlich and Daigle, 1987).

The production and accumulation of DON in infected wheat heads has been shown to be varied after infection until harvest. When Evans et al. (2000) inoculated barley heads with *F. graminearum* macroconidia, DON was detected as early as 36 hrs after infection. The same study has indicated that DON can be produced in large amounts at 72 hours of infection, being especially apparent following the appearance of necrotic symptoms. Others have reported start of DON production from 26 hours after infection (Chen et al., 1996) up till 4 days (Savard et al., 2000). Miller and Young (1985) reported the start of the production and accumulation of DON in infected heads at about three days after infection, DON increased until six weeks and declined thereafter before it reached a constant level before grain maturity. A similar decline in DON level prior to harvest has been reported in barley (Prom et al., 1999) and from naturally infected wheat fields (Scott, 1984). Some studies have indicated the decline of DON started earlier than six weeks (Argyris et al., 2003; Culler et al, 2007).

Based on gold labeling of an antibody against DON, Kang and Buchenauer (1999) suggested that DON is synthesized in metabolically active areas of fungal hyphae and that the mitochondrion is the organelle of toxin production. DON can be translocated from the site of production to nearby tissues with no fungal invasion due to its water solubility (Desjardins, 2006). Snijders and Krechting (1992) found DON in kernels without fungal invasion. They suggested that the DON was translocated from chaff invaded by fungal hyphae. DON can be transported acropetally and basipetally from the site of infection facilitated by fungal growth and translocation through xylem and phloem (Kang and Buchenauer, 1999). However, accumulation of DON is usually higher in spikelets, rachis and rachilla below the point of infection than above (Savard et al., 2000). Among the different parts of the head, the rachis tends to have the highest concentration of DON (Savard et al., 2000). It might be the case that the rachis is heavily contaminated because, fungal hyphae and DON spread to adjacent spikelets through the rachis and rachilla.

As DON is phytotoxic, it is expected that it might have role in pathogen aggressiveness. The role of DON in pathogen aggressiveness has not been well defined. A close relationship has been demonstrated between toxin production and pathogenic changes in different tissues, symptom appearance and the colonization of the pathogen in the wheat spike (Kang and Buchenauer, 1999). While some studies have reported a positive correlation between aggressiveness and DON production by *F. graminearum* and *F. culmorum* (Gang et al., 1998; Hestbjerg et al., 2002; Mesterházy, 2002), others suggest no correlation or an inconsistent correlation (Adam & Hart, 1989). A rapid increase of the disease has reported to occur due to the inhibition of enzymatic activity by DON in susceptible hosts (Snijders, 1994). Several studies have indicated that though the DON is involved in *Fusarium* aggressiveness, it is not essential to pathogenesis (Desjardins et al., 1996; Dyer et al., 2005; Eudes et al., 2001). Variation in the time till the initiation of production of DON suggests that DON might not be required for initial infection and colonization. The role of mycotoxins in the virulence of *F. graminearum* on wheat was examined by Proctor et al. (1995) utilizing trichothecene deficient mutants induced by disruption of *TRI5* which encodes a trichodiene synthase, the first enzyme in the trichothecene biosynthesis pathway. They observed that the mutant was still pathogenic to wheat but less aggressive than the wild-type. A similar result was observed when the *TRI14*, a gene with putative function of positive regulator of DON synthesis and possible role in the export of DON outside of mycelia, was mutated (Dyer et al., 2005). DON producers are reported to be twice as aggressive as NIV producing *F. graminearum* strains (Cumagun et al., 2004). Similar results

were obtained when chemotypes and virulence of 246 strains of *F. graminearum* from Nepal was analyzed (Desjardins et al., 2004).

The pathogenic variability of *F. graminearum* has been well known since 1929 (Tu, 1929) and confirmed in subsequent studies (Akinsanmi et al., 2006; Gang et al., 1998; Goswami and Kistler, 2005; Xue et al., 2004). Variability for trichothecene production exists naturally in *F. graminearum* populations (Burgess et al., 1996). Tóth et al. (2005) observed wide variation in DON production (54 - 11471 mg kg^{-1}) within different isolates of *F. graminearum* when inoculated into sterilized rice medium. Almost all isolates they tested were pathogenic despite high variability in aggressiveness. Similar results were observed when 66 isolates of *F. graminearum* associated with FHB from North Carolina were analyzed for in vitro DON production and pathogenicity on three cultivars of soft red winter wheat (Walker et al., 2001).

2.7 Impact on grain quality

In addition to yield losses and mycotoxin contamination, *Fusarium* infection causes several chemical compositional changes in the wheat grain impacting desired dough and baking quality. Bechtel et al. (1985) indicated that *Fusarium* infection of wheat grain results into the enzymatic degradation of starch granules, storage proteins, and cell walls. *Fusarium* produces proteolytic enzymes, which are required for its successful colonization and utilization of nitrogen and carbon sources (Wang et al., 2005). Proteolytic enzymes hydrolyze endosperm proteins during dough mixing and fermentation and results in weaker dough, decreased loaf volume and unsatisfactory bread quality (Dexter et al., 1996; Dexter et al., 1997, Nightingale et al., 1999). *Fusarium* infected grains are low in gluten, which contribute to dough viscosity and extensibility (Dexter et al., 1997; Wang et al., 2005). Infected grains are high in free nitrogen and amino acids which results in intensive browning of the baked loaf surface. Further, *Fusarium* infection results into the withered and light test-weight kernels making marketing and processing of the grain difficult (Goswami and Kistler, 2004).

2.8 Epidemiology

The growth stage of the wheat plant is an important factor impacting infection, subsequent FHB symptom development and DON accumulation. Infection of wheat heads at the kernel watery ripe or early milk stage results in higher visually scabby kernel (VSK) and DON accumulation than infection occurring towards anthesis and late milk (Del Ponte et al., 2007). Wheat heads are however vulnerable to *Fusarium* infection until the dough stages (Anderson,

1948; Atanasoff, 1920) and cultivars differ in terms of their susceptibility at different growth stages (Schroeder and Christensen, 1963). Difference in the most susceptible stage, in terms of infection and DON accumulation, has also been shown in barley (Yoshida et al., 2007). Susceptibility of wheat at anthesis and thereafter has been often linked to the protrudence of anthers (Strange and Smith, 1971). Anthers are rich in choline and glycinebetaine which are reported to have stimulatory effect on fungal hyphal extension (Strange et al., 1974). Thus anthers may have role in stimulating fungal growth and providing a point of entrance into the spikelets. Other factors promoting susceptibility during anthesis are the temporary opening of florets allowing airborne inoculum and fungal hyphae to enter, and the expansion of crevices between palea and lemma due to enlargement of the caryopsis (Bushnell et al., 2003).

Environmental factors, especially moisture and temperature are important for FHB development and DON accumulation. Each *Fusarium* species has its own environmental requirements for growth and development. Thus, even though several species can be detected in an FHB infested area, only a few species will predominate (Osborne and Stein, 2007). Generally, *F. graminearum* mycelia grow rapidly between 25ºC and 27ºC and are inhibited below 3ºC and above 35ºC (McInnes and Fogelmann, 1923). The optimum temperature for FHB infection and symptom development is 25ºC and FHB incidence increases rapidly when the temperature range from 20 to 30ºC (Andersen, 1948; Rossi et al. 2001). Similar reports of higher FHB symptoms evident at higher temperatures have been published by other researchers (Brennan et al., 2005). Environmental stresses influence FHB development and DON production which are often unrelated to total fungal biomass (Hope et al., 2005). Environmental factors triggering, regulating or influencing mycotoxin synthesis and accumulation in the infected host are not well understood (Mesterházy et al., 1999). Martins and Martins (2002) reported that more DON was produced on maize following incubation for 35 days at 28ºC compared to 22ºC. In the same experiment the production of DON decreased in incubation treatments longer than 35 days and DON was not detected in treatments at 37ºC.

The impact of moisture on FHB development and DON production and accumulation has been studied more than temperature. FHB epidemics appear to coincide with rainfall at and after flowering. McMullen et al. (1997) reported that in the epidemic year 1993, the month of July received the highest rainfall in wheat growing regions of South Dakota, North Dakota and Minnesota. In these regions in July wheat flowers and begins grain fill. Similarly, high rainfall was related to a FHB epidemic in 1980 in Canada (Sutton, 1982). Similar linkage between FHB and rainfall during anthesis or shortly thereafter are indicated by other studies (Abramson et al.,

1987; Atanasoff, 1920; Rohácik and Hudec, 2005; Tuite et al., 1990). Hope et al. (2005) studied the relationship of water activity (a_w) of the kernel with the *Fusarium* infection and DON accumulation. They indicated that below an a_w of 0.90 (19-20% seed moisture), DON production by *F. graminearum* and *F. culmorum* was inhibited. However, Birzele et al. (2000) suggested that DON was produced in natural field grain at 17% grain moisture (a_w of 0.8-0.85). Due to its prime role in FHB development and DON accumulation, several studies have studied moisture impact with extended periods of moisture either in the form of mist irrigation or bagging heads. Hart et al. (1984) studied the accumulation of DON in both greenhouse and field wheat in response to different moisture durations created by bagging heads with plastic bags after inoculation. The accumulation of DON increased with increased durations of bagging until 96 hours after infection in the greenhouse experiment. However, in field samples, DON accumulation decreased after 72 hours of bagging. Lacey et al (1999) also reported increased DON with increased moisture durations when they inoculated wheat heads with *F. culmorum*. However, they utilized continuous mist irrigation over 3-6 days after inoculation instead of plastic bags. Lemmens et al. (2004) found a differential reaction of wheat grain to DON accumulation with environment moisture. They tested two levels of mist-irrigation treatments (no irrigation and irrigation for 26 days after flowering) and observed a decrease in the DON level in some wheat lines receiving irrigation while observing an increase in others. Similarly, when Culler et al. (2007) applied mist-irrigation treatments and sampled multiple times after inoculation, they found less DON accumulation in the infected heads, especially in a susceptible variety, in the treatment mist-irrigated for longer durations when compared to the treatments receiving misting for shorter durations. Though the results were not significant for all sampling times, lower DON contamination was generally observed despite the increases in both disease levels and VSK. Cowger et al. (2009) reported variable results for FHB severity and DON to mist-irrigation treatments. In one year of the study FHB severity and DON increased with the duration of mist irrigation, while in next year both FHB severity and DON decreased in the longest misted treatment. Thus the impact of extended moisture period on FHB development and DON accumulation is not well understood. The impact of moisture appears to be compounded by temperature. Hart et al., (1984) reported the concentration of DON in the field lower compared to greenhouse studies, which the authors attributed to a possible spike in temperature inside the plastic bags used in the field. Several studies have indicated that with decreasing temperature, increased durations of moisture are required for FHB development (Pugh et al., 1933; Rossi et al.,

2001). Thus the FHB incidence and severity, and final DON concentration in the infected heads is probably a result of complex interactions between the host, the pathogen and the environment.

Crop rotation and cropping patterns also are considered important factors influencing FHB development. It was reported that FHB incidence and DON concentrations were higher in winter wheat planted after corn than after soybeans in the province of Ontario in Canada (Teich and Hamilton, 1985). Corn and other cereals are also major hosts of *F. graminearum*. Thus wheat following corn or wheat in a rotation provides an opportunity for *Fusarium* to overwinter in residues and serve as inoculum for the next growing season (Wilcoxson et al., 1988). Several studies have shown evidence of increased FHB incidences and DON accumulation in wheat following wheat or wheat following corn (Holbert et al., 1919; Koehler et al., 1924; Windels and Kommedahl, 1984). The situation is further aggravated by the minimum or zero tillage practices, in which crop resides are left on the soil surface (Dill-Macky and Jones, 2000).

2.9 Genetics of FHB resistance

Resistance to FHB is conferred mainly by physiological and morphological components. Schroeder and Christensen (1963) reported two components of physiological resistance in wheat to FHB; type I resistance or the resistance against initial infection and type II or resistance against spread of infection within the spike. Type I resistance can be detected by spray inoculating heads and measuring the FHB incidence while type II resistance can be detected based on spread of infected spikelets upward and downward after single centrally located floret in a spike is inoculated. Wheat lines may possess either type I and/or type II (Schroeder and Christensen; 1963). Three other types of physiological resistances have been proposed; resistance to kernel infection (Mesterházy, 1995; Mesterházy et al., 1999), FHB tolerance (Mesterházy, 1995; Mesterházy et al., 1999) and resistance to toxin accumulation (Miller et al., 1985). However, a debate is still going on for codifying them in standardized list of resistance (Bushnell, 2002).

Variation in wheat genotypes for FHB resistance exists worldwide and the best resistances are generally found in three gene pools; spring wheats from Brazil, spring wheats from China and Japan, and winter wheats from Europe (Snijders, 1990). FHB resistant wheat cultivars include the Eastern European winter wheats ‘Arina’, ‘Praag-8’ and ‘Renan’. In spring wheat ‘Sumai-3’ and its derivatives from China, ‘Nobeoka Bozu’ and ‘Sin Chunaga’ and their derivatives from Japan, and ‘Frontana’ and ‘Encruzilhada’ from Brazil are reported to carry FHB resistance (Ruckenbauer et al., 2001; Snijders, 1990; Zhang et al., 2008). In addition, the winter wheat ‘Ernie’ and ‘Freedom’, and spring wheat ‘2375’ are considered the best US source of FHB

resistance (Rudd et al., 2001). Zhang et al. (2008) analyzed 1035 spring wheat worldwide accessions from the United States Department of Agriculture (USDA) National Small Grains Collection, noting those with moderate level of disease in the field, European resistant lines generally showed higher level of resistance in terms of VSK and DON. Sumai 3 has been shown to have type II FHB resistance (Hartel et al., 2004; Kolb et al., 2001). Because of its high combining ability and yield, Sumai 3 and its derivatives have been widely utilized as sources of resistance by breeding programs in the US and worldwide (Bai and Shaner 1994; Ruckenbauer et al., 2001). One concern with the use of a few resistance sources is that this has narrowed down the genetic base, increasing the possibility of wheat becoming more vulnerable to changes in the pathogen population (Ruckenbauer et al., 2001). Thus searches for FHB resistant genes has begun in wild relative and progenitor to identify sources with comparable resistance levels to 'Sumai 3' (Hartel et al., 2004; Oliver et al., 2005; Oliver et al., 2007).

The genetics of FHB resistance is complex due to its polygenic nature. Studies have indicated that FHB resistance is controlled by two to six genes, complicating its study and breeding for resistant cultivars (Singh et al., 1995; Snijders, 1990; Van Ginkel et al., 1996). Due to its quantitative nature of inheritance, and that FHB resistance is highly influenced by environment it is difficult to estimate the number of gene or to identify quantitative trait loci (QTL) (Groth et al., 1999; Kolb et al., 2001). The first major QTL reported to confer FHB resistance was on chromosome 3B (*Fhb1* syn. *Qfhs.ndsu-3BS*) detected in a population from the cross of resistant cultivar 'Sumai3' and the moderately susceptible cultivar 'Stoa' (Waldron et al., 1999). Other QTLs identified are on chromosome 2A (2AL), 4B (4BL) and 6B (6BS) (Waldron et al., 1999), 6A (6AS) and 3A (3AL) (Anderson et al., 2001). Among these QTLs, 3BS explained phenotypic variation from 15.4% (Waldron et al., 1999) to 60% (Buerstmayr et al., 2002).

Several QTLs have been reported to be linked with DON accumulation in FHB infected head. QTLs on chromosomes 2DS and 5AS were reported to be significantly associated with low DON independent of FHB severity (Somers et al., 2003). Similarly, Ma et al. (2006) reported a QTL on chromosome 5A, which explained 12.4% of DON variation independent of FHB severity in the recombinant inbred lines (RILs) from a cross of 'Wangshuibai' and 'Annong 8455'. The same study also reported other QTLs on chromosomes 2A (minor effect on both FHB severity and DON) and 3B (major effect on FHB severity and minor effect on DON). Other QTLs reported are on 1AL and 2AS detected in double haploid population from a cross between 'Arina' and 'NK93604' (Semagn et al., 2007). QTL 1AL explained 27.9% variation in DON and was also

associated with FHB severity. QTL 2AS explained 26.7% variation and was associated only with low DON. Recently Abate et al. (2008) reported three more QTLs associated with low DON and low Fusarium damaged kernel (FDK) in a RILs population from a cross between 'Ernie' and 'MO 94-317'.

In addition to physiological mechanisms of FHB resistance, agronomic characteristics including short plant height and the presence of awns have also been linked to increased FHB incidence and severity (Buerstmayr et al., 2000; Mesterházy, 1995; Ransom, 2008). Higher spikelet density in heads has also been linked to a higher probability of FHB because of increased humidity in the head (Rudd et al., 2001).

It is reported that, on average, FHB resistant wheat is associated with lower levels of DON than FHB susceptible wheat (Wilde and Miedaner, 2006). According to Arsenuik et al. (1999), the correlation is stronger ($r = 0.74$) for spring wheat than winter wheat ($r = 0.54$). In the same study, the deviation from linearity was higher for wheat than rye or triticale, when the relationship between FHB and DON was considered. In a greenhouse study by Bai et al. (2001) FHB ratings, in terms of the proportion of scabby kernels (PSS; $r = 0.65$) and area under disease progression curve (AUPDC; $r = 0.75$), were reported to be significantly and positively correlated with DON levels. Several other researchers have reported that resistant cultivars have lower DON levels than susceptible cultivars (Mesterházy et al., 2003; Miller et al., 1985; Wilde and Miedaner, 2006). Whether the reduced DON in cultivars expressing FHB resistance is due to the resistance of the host to initial infection, spread of the fungus in the spike (Schroeder and Christensen, 1963) or modes of resistance conferring either the prevention of DON synthesis or the degradation of DON (Miller et al., 1985; Miller and Arnison, 1986) has not been clearly established. To facilitate germplasm evaluation and selection, researchers utilize correlations between visual FHB severity assessments in the field and DON concentrations to predict DON in harvested grain (Arsenuik et al., 1999; Groth et al., 1999; Jones and Mirocha, 1999). Although correlations are frequently significant, correlation coefficients are often low and may vary greatly among locations and years. The reasons for this variability are not fully understood, but may be associated with the toxin production capacity of prevalent strains within the *Fusarium* population, the type and level of host plant resistance to FHB and/or environmental conditions prevailing between the time of initial infection and harvest.

2.10 FHB management

Because of the lack of FHB resistant wheat cultivars, chemical control has been widely utilized in the US over the past decade. The effectiveness of fungicides differs depending upon the environment and the cultivars upon which they are used (Hollingsworth et al., 2008). Several fungicides have been tested, among which tebuconazole, propiconazole, carbendazim, and benomyl have shown promising results (Mesterházy, 2003). However, fungicides results are not consistent. When Milus and Parsons (1994) tested several fungicides including tebuconazole and propiconazole, none of them reduced FHB severity and DON concentration or increased yield. However, Balaž et al. (2008) reported a significant reduction in FHB severity and an increase in yield. They treated wheat plants during flowering with several fungicides including epoxyconazole, thiophanate-methyl, carbendazim, flusilazole, trifloxystrobin, cyproconazole, tebuconazole, triadimenol, spiroxamine, procholraz, and propiconazole. Boyacioglu et al. (1992) reported reduction of DON by 80% with triadimefon, while propiconazole and thiobendazole were not effective in reducing DON. Several other researchers have indicated inconsistent fungicide results (Horsely et al., 2006; McMullen, 1994). Timing of application and application technology appear important for successful results when applying fungicides. Fungicides have also been tested for their effects on residue colonization by *F. graminearum* and have shown promising results to reduce residue colonization (Beare et al., 1993), although fungicides might also impact residue colonization by residue decomposing or other *Fusarium*-competitive microorganisms. Lack of accurate disease forecasting methods and the high cost of fungicide application limit the use of fungicides for FHB management (McMullen et al., 1997).

Tillage practices and residue management have been considered vital for the control of FHB epidemics as *Fusarium* is a residue borne pathogen. Dill-Macky and Jones (2000) showed that FHB incidence, severity and DON concentration was significantly higher in wheat following corn with no-till cultivation. Moldboard plowed plots had lower FHB incidence and severity than chisel plowed and no-tilled plots. Since moldboard plowing inverts the soil layer burying crop residues, the survival of *Fusarium* is reduced. Pereyra and Dill-Macky (2008) found that *G. zeae* can survive in several gramineae plant residues which subsequently act as inoculum sources. They showed that the survival of *G. zeae* decreased rapidly in wheat and barley residue compared to corn. Considering the fact that *Fusarium* survives in surface crop residues, especially gramineae crops, FHB may be managed by rotating with non-host crops and by proper burial of residues.

In the search for disease management strategies, biological control has emerged as one possibility. Several microorganism including bacteria (*Bacillus* spp., *Kluyvera cryocrescens*, *Lysobactor* spp., *Paenibacillus fluorescens*, *Pantoea agglomerans*, and *Pseudomonas fluorescens*), yeasts (*Cryptococcus* spp., *Rhodotorula* spp., and *Sporobolomyces roseus*) and filamentous fungi (*Trichoderma harzianum* and *T. virens*) have shown potential for the control of *F. graminearum* (Bacon and Hinton, 2007; Corio da luz et al., 2003; Jochum et al., 2006). However, problems encountered in biocontrol agents include their viability, delivery mechanisms, suitability of application with fungicides and inconsistent results (Yuen et al., 2007; Yuen, 2008).

It appears therefore that the development and use of resistant host is a most economical and environmentally safe strategy for disease management (Ruckenbauer et al., 2001). Efforts on FHB resistance breeding have been augmented worldwide, although progress in the development of FHB resistant cultivars has been slow. Several QTLs associated with FHB and DON resistances have been used to provide partial resistance to FHB. Efforts are ongoing for pyramiding multiple QTLs to a single cultivar (Shi et al., 2008). Similarly, several studies are focused on identifying transgenic wheat by incorporating plant defense antifungal proteins like thaumatine-like proteins (Chen et al., 1999; Mackintosh et al., 2007). Though the results of some of these studies have been promising in the greenhouse experiment, they have failed to prove significant in field environments (Anand et al., 2003). Wheat cultivars with partial resistance are available for commercial cultivation, but immune cultivars are lacking.

In conclusion it appears that FHB cannot be controlled by any single measure of fungicides, resistant cultivar or residue management strategies. An integrated approach utilizing several of the available mechanisms seems most likely to provide effective control of FHB (McMullen et al., 1997; Yuen and Schoneweis, 2007).

Chapter 3

Impact of Post-Inoculation Moisture on Fusarium Head Blight (FHB) Development and Trichothecene Accumulation in Spring Wheat

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* Schwabe, is an economically important disease as it results in yield and quality losses of infected grain and the accumulation of mycotoxins produced by the invading fungus. Environmental factors, host genetics and isolate aggressiveness impact FHB development and subsequently trichothecene production and accumulation. Though it is well established that moisture around the anthesis period promotes FHB development and trichothecene accumulation, the role of moisture, either in the form of rainfall or mist-irrigation during the period from anthesis to harvest has been largely overlooked. The objective of this study was to examine the influence of environmental factors, especially moisture, host resistance, and pathogen variation with respect to mycotoxin production capacity and aggressiveness, on infection, FHB disease development and mycotoxin production and accumulation *in planta*. The field experiments, conducted in 2006-2008, were a split-split-plot design with five replications. Main plots were the duration of mist-irrigation after inoculation [14, 21, 28 and 35 days after inoculation (DAI)]. Sub-plots were wheat cultivars. Three wheat cultivars used were Alsen (moderately resistant with the resistance derived from Sumai 3), 2375 (moderately susceptible) and Wheaton (susceptible). Sub-sub-plots were *F. graminearum* isolates (49-3, 81-2, B45A, B63A, and Butte86ADA-11) differing in terms of their aggressiveness and DON production capacity in addition to a mock-inoculated water control. Plots were inoculated twice, at anthesis and 3 days after the first inoculation with macroconidial inoculum (1×10^5 conidia ml^{-1}). FHB severity was assessed 21 dai by counting the total and visually symptomatic spikelets in 20 arbitrarily selected heads per plot. Visually scabby kernels (VSK), deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) and nivalenol (NIV) were determined on grain harvested at maturity. Additionally, in 2007 and 2008, whole heads (10 per plot) were sampled at 0, 7, 11, 14, 21, 28 and 41 dai. These heads were dried and the entire head ground and analyzed for DON, 15-ADON, 3-ADON and NIV. Severity, VSK and the DON concentration of mature grain, were significantly higher ($P < 0.05$), across all isolates, in the susceptible wheat cultivar Wheaton than in the other two cultivars examined. Although FHB severities were not significantly different in plots receiving different durations of mist-irrigation ,VSK were significantly lower ($P < 0.05$) in the treatments receiving the least amount of mist-irrigation (14 DAI) than for treatments receiving mist-irrigation for longer

periods, suggesting that extended periods of moisture promote disease development. DON was however significantly lower ($P < 0.05$) in the 35 DAI misting treatment than those treatments receiving less water. In the whole head samples, DON and other trichothecene either declined with increased durations of mist-irrigation or remained low while water was being applied by the misting system. However, trichothecene accumulation was observed to increase after the cessation of mist-irrigation, with increases being most pronounced for the treatments with shorter mist-irrigation periods. The largest reduction in DON observed as a result of extended mist-irrigation periods was seen in the susceptible cultivar Wheaton. Our results suggest that longer durations of moisture after inoculation, either from mist-irrigation or rainfall, may increase the FHB severity and VSK, although DON and other trichothecene concentrations may be concomitantly reduced. Leaching may explain the reduction of DON observed in extended misting duration treatments.

3.1 Introduction

Fusarium head blight (FHB or scab), a destructive disease of wheat and other small grains in warm and humid cereal growing regions of the world, is primarily incited by *F. graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] (Bai et al., 2001; Doohan et al., 2003; Schroeder and Christensen, 1963; Wilcoxson et al., 1992). The reemergence of FHB (Windels, 2000) was estimated to cause direct and secondary economic losses of $2.7 billion in wheat and barley in the northern Great Plains and central US from 1998 to 2000 (Nganje et al., 2002). Although FHB has been common, severe and well documented in the past, recent epidemics have resulted in increased concern, greater public interest and expanded research efforts.

Warm temperatures and extended periods of moisture on plant surfaces around anthesis favor the infection and colonization of wheat tissues by *F. graminearum*. In addition to yield losses, the quality of infected grain is compromised due to the production of a range of mycotoxins, including deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), and nivalenol (NIV), by infecting *Fusarium* (Nasri et al., 2006; Pirgozliev et al., 2003). These mycotoxins are hazardous to humans and animals, thus making highly infected grain unfit for food or feed. Wheat from US production fields infected by *Fusarium* have routinely tested positive for most of these mycotoxins (Abramson et al., 1987; McMullen et al., 1997). Deoxynivalenol is considered the most important toxin produced by *F. graminearum* and is regulated in grain and wheat based food products by US federal agencies.

The US, Canada and several European countries have set less than 2 mg kg^{-1} as the acceptable limit of DON in wheat grain for human consumption (Bai et al., 2001). In addition *Fusarium* damaged grain tends to be withered with light test-weight kernels exhibiting weak dough properties and unsatisfactory baking quality, making marketing and processing of the grain difficult (Bechtel et al., 1985; Dexter et al., 1996; Dexter et al., 1997; Wang et al., 2005).

FHB resistant wheat is reported to be associated with lower levels of DON than FHB susceptible wheat (Wilde and Miedaner, 2006). According to Arsenuik et al. (1999), the correlation between FHB visual symptoms and DON was stronger (r = 0.74) for spring wheat than winter wheat (r = 0.54). Other researchers have also observed that resistant cultivars generally have lower DON levels than susceptible cultivars (Mesterházy et al., 2003; Miller et al., 1985; Wilde and Miedaner, 2006). Whether the reduced DON in cultivars expressing FHB resistance is due to the resistance of the host to initial infection, spread of the fungus in the spike (Schroeder and Christensen, 1963) or modes of resistance conferring either the prevention of DON synthesis or the degradation of DON (Miller et al., 1985; Miller and Arnison, 1986) has not been clearly established. To facilitate the evaluation and selection of resistant germplasm and to assess commercial crop quality, researchers frequently utilize correlations between visual FHB severity assessments in the field and DON concentrations to predict DON in harvested grain (Arsenuik et al., 1999; Groth et al., 1999; Jones and Mirocha, 1999). Although correlations are generally significant, correlation coefficients are frequently low and vary greatly among locations and years. The reasons for this variability are not fully understood, but may be associated with toxin production capacity of prevalent strains within the *Fusarium* population, the type and level of host plant resistance to FHB and/or the environmental conditions prevailing between the time of initial infection and harvest.

Environmental factors, primarily moisture, have been reported to have an important influence on toxin production and DON production is often unrelated to total fungal biomass (Hope et al., 2005). Moisture in the form of rainfall or relative humidity, during anthesis or shortly thereafter, has been linked to higher FHB incidences, higher FHB severities and DON accumulation (Abramson et al., 1987; Atanasoff, 1920; Rohácik and Hudec, 2005; Tuite et al., 1990). Epidemics are generally associated with the occurrence of rainfall during wheat anthesis (McMullen et al., 1997; Sutton, 1982). However, the specific environmental factors triggering, regulating or influencing mycotoxin synthesis and accumulation in the infected host are not well understood, (Mesterházy et al., 1999). The final toxin concentration in the kernel is likely the

result of complex interactions between the host, the pathogen and the environments (Lemmens et al., 2004).

It has been reported that continuous mist irrigation over 3 days after inoculation with *F. culmorum* increased DON in harvested wheat (Lacey et al., 1999). Lemmens et al. (2004) reported differential DON accumulation in wheat grain when environmental moisture conditions differed. When Culler et al. (2007) applied mist-irrigation treatments and sampled multiple times after inoculation, he found less DON accumulated in the infected heads, especially in a susceptible variety, in the extended mist-irrigated treatment when compared to the standard mist-irrigation treatment. The DON results were, however, not significant at all sampling times despite observed increases in both disease levels and yield reduction in the extended mist-irrigation treatments. The results reported by Cowger et al., (2009) somewhat contradict those of Culler et al. (2007) and Lemmens et al. (2004). Cowger et al. (2009) observed increases in FHB severity, *Fusarium* damaged kernels and DON levels with increased durations of mist-irrigation from 0 to 30 days post-anthesis.

The role of DON in pathogen aggressiveness has not been well defined. While some studies have reported a positive correlation between aggressiveness and DON production by *F. graminearum* and *F. culmorum* (Gang et al., 1998; Hestbjerg et al., 2002; Mesterházy, 2002), others suggest no significant correlation or inconsistent correlation (Adam & Hart, 1989). However, there appears to be agreement that DON is not essential for pathogenesis (Dyer et al., 2005; Eudes et al., 2001; Proctor et al., 1995).

The pathogenic variability of *F. graminearum* has been well known since 1929 (Tu, 1929) and exists naturally in *F. graminearum* populations (Burgess et al., 1996). Variability in the capacity of *F. graminearum* with respect to aggressiveness and DON production has been reported both *in vitro* and *in vivo* (Tóth et al., 2005; Walker et al., 2001). An understanding of the impact of the diversity of *F. graminearum* on resistance expression and DON accumulation is useful for evaluating the risks of various FHB control strategies. Recent epidemics and emerging reports of changes in the types of mycotoxins produced by the *Fusarium* population (Gale et al., 2007; Jennings et al., 2004) have raised questions about the significance of changes in the genetic structure of the *F. graminearum* population at the regional and global scale. An assessment of the impact of the diversity and change in diversity of the pathogen population on FHB development is crucial if effective control practices are to be developed.

The objective of this study was to examine the influence of environmental factors, specifically moisture, host resistance, and pathogen variation with respect to mycotoxin

production capacity and aggressiveness, on infection, FHB development and mycotoxin production and accumulation *in planta*.

3.2 Materials and methods

3.2.1 Inoculum production

Single isolates of *F. graminearum* representative of the range in aggressiveness and DON production capacity of the isolates available in the small grains pathology collection at University of Minnesota were used. Five different isolates (Appendix 1) were used; three collected from wheat fields (49-3, 81-2, and Butte86Ada-11) and two collected from barley fields (B63A, and B45A) in Minnesota. The isolates were maintained in soil and transferred to mung bean agar media (MBA: 40 g mung beans boiled for 23 minutes in 1000 ml of Millipore filtered water [screen size 0.22 µm; Milli Q Biocell, Millipore Corporation, France], filtered through two layers of gauze pads, adjusted to 1 L with Millipore filtered water, 15 g of Difco agar [Bectin, Dickinson and Company, Sparks, MD 21152] and autoclaved). Isolates in MBA were allowed to grow for 7 days under fluorescent and UV lights (12 h: 12 h light: dark cycle) at room temperature (22-24ºC). On the seventh day, ten milliliters of Millipore filtered water per plate was added in cultured isolate plates and rubbed with sterile L-shaped glass rod to loosen macroconidia. The ensuing spore suspension was filtered through a two layers of cheesecloth to reduce the number of mycelial fragments in the suspension and transferred into a sterile beaker and final volume was made equal to ca. 40 ml by adding Millipore filtered water. The spore suspension was used to inoculate 20 MBA plates per isolate (1.5 ml per plate), which were then incubated at room temperature for seven days as described earlier. On the seventh day, macroconidia were harvested by washing MBA plates with ca. 20 ml of deionized (DI) water per plate using a CO_2 powered backpack sprayer fitted with flat-fan spray tip (TeeJet SS8003; Spraying Systems Co., Wheaton, IL) at an operating pressure of ca. 276 kPa. The spore suspension was filtered through one layer of cheesecloth to remove mycelia fragments. The spore concentration was determined using a hemacytometer and the inoculum concentration adjusted to 8×10^5 spores ml^{-1} inoculum was stored in 1L Nalgene® polyethylene bottles (Nalgene Nunc International Co., Rochester, NY) at -80ºC until used for inoculation.

3.2.2 Field experiment design

Experiments were split-split-plots with five replications and established at the Experiment Station of University of Minnesota at Saint Paul, MN during the summers of 2006,

2007 and 2008. Main plots were mist irrigation treatments with four levels [mist irrigation from inoculation until 14, 21, 28, and 35 days after inoculation (DAI)], sub plots were the wheat genotypes and the sub-sub plots were the different isolates of *F. graminearum*. Cultivars within mist irrigation main plots and isolates within cultivar sub-plots were assigned randomly.

Three wheat cultivars, varying in terms of resistance to FHB were used in the experiment; Alsen (Frohberg et al., 2006), previously identified as a source of FHB resistance and known to carry *Fhb1* from the Chinese wheat Sumai 3; 2375 a moderately susceptible cultivar with a non-Asian source of resistance; and Wheaton (Busch et al., 1984) the susceptible check. Two row plots of the three wheat cultivars were planted in mid-April each year of the study. Each plot was 2 m long with the inter-row distance of 30 cm. In order to reduce the cross contamination of isolates, one row buffer plots of moderately resistant cultivar, Alsen, was planted between each experimental plot. Mist irrigation main plots were separated by six meters of an oat buffer. When wheat plants were at 3-4 leaf stage, the field was sprayed with the herbicide Bronate® (3,5-dibromo-4-hydroxybenzonitrile, 0.086 L a.i./ha; Bayer Crop Science, Research Triangle Park, NC) to control broadleaf weeds. Similarly, the insecticide, Di-syston 8® (disulfoton, 0.082 L a.i./ha; Bayer Crop Science, Research Triangle Park, NC), was sprayed to control aphids and other insects. Hand weeding throughout the growing season supplemented weed control. During the late booting stage, a micro sprinkle mist irrigation system (DAN 8000 series with rotating spinner, NETAFIM® Irrigation Inc., Altamonte Springs, FL) was assembled. Risers were at a height of 1 m. The mist-irrigation system was programmed, using a programmable timer, to run for 9 min every hour starting at 1700 and ending at 0700. The system ran for total of 14 misting periods each day or 126 minutes, and delivered 10.7 mm of water per day.

3.2.3 Inoculation

On the day of inoculation, stored inoculum was thawed and tested for germination by plating 0.5 ml of inoculum in potato dextrose agar (PDA) plates and counting germinated macroconidia after 8 hours. For inoculation, 1 L of inoculum (8×10^5 spores ml^{-1}) was mixed with 7 L of water (making final concentration of 1×10^5 spores ml^{-1}). Approximately 20 ml of Tween-20 (polysorbate; Fisher Biotech, Fair Lawn, NJ) was added to the diluted inoculum as a wetting agent. All experimental plots were inoculated at anthesis (mid-June) and 3 days later. Inoculum was dispensed using a CO_2-powered backpack sprayer operating at the pressure of ca. 276 kPa, fitted with flat-fan spray tip (TeeJet SS8003; Spraying Systems Co., Wheaton, IL), at the rate of

30 ml per meter of row. Mist irrigation was started immediately following inoculation for 10 min to prevent the rapid drying of inoculum. Mist irrigation treatments were imposed following the first inoculation.

3.2.4 Disease rating, sampling and VSK analysis

FHB severity was assessed visually 21 dai by counting the total and the number of infected spikelets in 20 arbitrarily selected heads in each plot (10 heads per plot row). Grain was harvested mechanically at maturity (late July: 2007; early August: 2006 and 2008), machine threshed (at a low blowing speed in order to retain most of the scabby kernels), and dried for 5 days at 95ºC. Dried grains were re-threshed with a belt thresher to separate heavily infected grains from the heads. In 2007 belt-threshed grains were also aspirated (Grain Aspirator 63; Grain Machinery Manufacturing Corp., Miami, FL) to clean the grain. Twenty five gram sub samples were taken from belt threshed and aspirated (2007 only) using a Boerner divider (Seedburo Equipment Company, Chicago, IL) and hand cleaned to remove rachis, glumes, weed seeds and other trash from the sample. The percentage of VSK was determined following the procedure of Jones and Mirocha (1999) in 2006 samples and with some modifications in 2007 and 2008 on the 25 g sub-samples. While the original VSK scale is based on 0 to 50% percent scale, majority of Wheaton samples had VSK readings of more than 50% in 2007 and 2008. In these years the VSK scale was extended to 100% in increments of 10%. Extension of the scale was based on the 0-50% scale but VSK was compared to healthy grain and vice versa. Following the assessment of VSK the same sub-samples were submitted to University of Minnesota's Mycotoxin Laboratory for DON analyses.

In 2007 and 2008, 10 random heads (5 heads per plot row) from each experimental plot were harvested on the day of the first inoculation and subsequently at 7, 11, 14, 21, 28, and 41 dai (2007) or 39 dai (2008) and immediately froze at -20ºC until further processing. The 39 and 41 dai samples are referred to as 41 dai samples henceforth for simplicity. Sampled heads were assessed for growth stage according to Zadoks et al. (1974). The visually symptomatic kernels and total kernel number were counted for samples up to 21 dai. Samples were air dried at 95ºC for 72 hours, ground and analyzed for mycotoxin. Wet and dry weight of head samples were taken before and after drying, respectively. Date, days from planting and growth stages (Zadoks et al., 1974) at each sampling are presented in Appendix 2.

3.2.5 Trichothecene analysis

The trichothecene analyses were done according to Mirocha et al. (1998) as modified by Fuentes et al. (2005). For harvested grain, samples were prepared by grinding for 2 min with a Stein laboratories mill (Model M-2, Stein laboratories Inc., Atchison, KS). Head samples were ground with a Wiley® mini mill (Thomas Scientific, Swedesboro, NJ). Four grams of the ground samples were extracted in 16 ml acetonitrile: water (84:1 v:v) extraction solvent by shaking for 1 hour. The extract was filtered through a column packed with C18: Aluminum oxide (1:3). One milliliter of filtrate was evaporated and completely dried under nitrogen. Dried samples were derivatized by the addition of 100 µl of trimethylsilyl (TMS) reagent (TMSI:TMCS, 100:1, Pierce Chemical Co., Rockford, IL), shaken for 10 min followed by addition of 1 ml Mirex isooctane solution (4mg L^{-1}) and 1 ml high performance liquid chromatography (HPLC) water, vortexed, and the upper transparent isooctane layer transferred to a vial for use in a gas chromatography-mass spectrometry (GC-MS). Derivatized solution was analyzed using GC-MS (Shimadzu Model QP 2010, Shimadzu Corporation, Kyoto, Japan). The concentrations of DON, 3ADON, 15ADON and NIV in samples were determined based on standards.

3.2.6 Data analysis

FHB severity for each spike was calculated by dividing the number of symptomatic spikelets by total number of spikelets and multiplying the result by 100. Data for DON, 15-ADON, 3-ADON and NIV obtained on whole head samples were adjusted to fresh weight values by multiplying with the ratio of wet to dry weight of heads. FHB severity and VSK data were squared transformed, DON, 15-ADON, 3-ADON and NIV data were natural log transformed to achieve homoscedasticity. Data were analyzed using PROC MIXED procedure in SAS v 9.0 (SAS Institute, Cary, NC). Each year data were analyzed separately as the year and its interaction effects for most of the variables were significant in combined analyses (Appendix 3). Means were separated using LSD and the output were letter grouped using SAS macrocode PDMIX800 (Saxton, 1998). Spearman rank correlation analyses were carried out using PROC CORR in SAS. Graphs were created using OriginPro 8.1 SR0 (OriginLab Corporation, Northampton, MA).

3.3 Results

3.3.1 Growing season and weather

The growing season was the longest (103 days) in 2006 and the shortest (98 days) in 2008 (Appendix 3). In 2007, the growing season was 99 days long. The growing season in 2007 began earlier than in either 2006 or 2008 with planting being completed eight days earlier than in 2006 and 11 days earlier than in 2008. The days required for each cultivar to reach anthesis also differed in the three years of the study. Anthesis period (start to completion of anthesis) for Alsen and 2375 coincided with each other in each year of the study. In Wheaton, anthesis occurred 1-2 days after the beginning of anthesis in Alsen and 2375. However, the anthesis period of the three cultivars used, which was generally 3-5 days long, overlapped. This overlapping of anthesis period allowed the inoculation of all three cultivars on the same day in each year of the study. Anthesis occurred 61 days after planting in 2006, after 57 days in 2007 and after 58 days in 2008. Wheat reached full maturity and was harvested 42 days after the first inoculation (at anthesis) in 2006 and 2007. In 2008, harvest was 40 days after the first inoculation.

Daily average temperatures varied more in 2006 than in either 2007 or 2008 (Figure 3.1). In 2008 the daily average temperature varied the least of the three years in the study. The growing season in 2006 was generally warmer than in either 2007 or 2008, though the three week period immediately after planting in 2006 and 2008 were cooler than the equivalent period in 2007. The period from three weeks before inoculation until harvest was warmest in 2006 with daily average temperatures ranging between 15ºC to 30ºC. The same periods in 2007 and 2008 were cooler with daily average temperatures ranging between 15ºC to 25ºC.

The period of 10 days after inoculation (growth stages equivalent up to early milk or medium milk stage) are considered the most important for successful infection and disease development by *Fusarium* in wheat (Shaner, 2003). In 2006, the daily average temperatures during this 10 day period were 19-25ºC and following this between 25 and 30ºC until harvest. Of the 42 days from inoculation to harvest in 2006, 23 days had daily maximum temperatures above 30ºC. In 2007 and 2008, the average daily temperatures during the 10 day period after inoculation were 22-26ºC and 18-27ºC, respectively. Daily average temperatures in the growing season from 10 dai till harvest in 2007 and 2008 generally remained at 20-25ºC, with 17 and 11 days, respectively, having daily maximum temperatures above 30ºC.

Total precipitation and the distribution of rainfall differed among the three years of the study. Total precipitation in the 2006 growing season (planting till harvest) was 425 mm, while the precipitation was 175 mm in 2007, and 202 mm in 2008 (Figure 3.1). The distribution pattern

for the precipitation was more uniform in 2006 though there were two major (> 22 mm) precipitation events, one nine days before inoculation and another six days before harvest. These two precipitation events contributed to 42% of the total precipitation in 2006. There was only one major precipitation event in 2007, nine days before harvest, which contributed 18% to the total precipitation for the season. In 2008, there was one major precipitation event two days after planting, which contributed 11% to the total precipitation during the growing season.

In 2006, there were 11 precipitation events providing 203 mm of precipitation during the period between inoculation and harvest. For the same period in 2007, there were 10 precipitation events totaling 77 mm and in 2008 nine precipitation events and a total of 63 mm of precipitation. Of these rainfall events, five in 2006 and two each in 2007 and 2008 exceeded the daily output of the mist-irrigation system (10.7 mm day^{-1}). During the 10 day period after inoculation, there were only two minor precipitation events (total of 4.3 mm) in 2006, the first occurring on the day of inoculation and the other 5 dai. For the same period in 2007, there were three minor precipitation events, at 1 dai, 3 dai and 6 dai totaling 21.8 mm. In 2008, there were two minor precipitation events (one on the day of inoculation and the second 1 dai) resulting in 3 mm of precipitation. Thus, in addition to the total of 107 mm water applied through mist-irrigation system over the 10 day period after inoculation 2006 received 4% more water, while 2007 and 2008 received 20% and 3% more water, respectively, than was provided by the mist-irrigation system.

3.3.2 Grain harvested at maturity

3.3.2.1 Disease Severity

The severity of FHB was generally lowest in 2006 and highest in 2007 (Figures 3.2A and 3.3, Appendix 4). Average disease severities, when combined across mist-irrigation, wheat cultivar and pathogen isolate treatments, were 31% (2006), 45% (2007) and 39% (2008).

There were no significant differences in the FHB severities among the mist-irrigation duration treatments in any year of the study. This result was expected as only the 14 DAI mist-irrigation treatments had been imposed when disease severity was assessed 21 dai.

The disease severity of the susceptible cultivar Wheaton differed significantly from the other cultivars in each year of the study. In all three years, Wheaton had significantly higher disease severities (2006: 56%; 2007: 70%; 2008: 57%) than Alsen (2006: 17%; 2007: 31%; 2008: 31%) and 2375 (2006: 19%; 2007: 33%; 2008: 30%). In general, cultivar 2375 had slightly higher disease severities than Alsen; however, these differences were not statistically significant in any year of the study.

The disease severities in the mock-inoculated plots were significantly less compared to the *Fusarium*-inoculated plots, indicating that there were generally no significant sources of exogenous inoculum. In 2006, however, average severities of up to 7.7% were recorded in the mock-inoculated plots. This fairly high level of disease in the control treatment might be due either to cross-contamination from drift of inoculum during inoculation or from sources of inoculum external to the trial. Given the fact that the disease severities in 2006 were generally low, and DON was not present in the majority of the control plots, contamination from drifting inoculum seems most likely. However, the level of disease in the mock-inoculated plots compared to *Fusarium* inoculated plots was very low, indicating that any cross-contamination would have had a negligible impact on the treatments. In general, isolates 49-3 and B45A resulted in higher disease severities compared to the three other isolates tested. Plots inoculated with isolate 49-3, however, had lower disease severities in 2008 compared to the other isolates included in the study. Isolates Butte86Ada-11 and 81-2 generally resulted in lower disease severities compared to the other isolates tested.

Based on Spearman's rank correlations, the rank order of isolates for FHB severity were significantly correlated among cultivars within same year of the study ($P < 0.05$). When the rankings of isolates in cultivars were compared between years, the rankings of the isolates in 2007 for cultivar 2375 were significantly correlated with the rankings of isolates for disease severity in all cultivars in 2006. Other rankings were not significantly correlated between years.

3.3.2.2 Visually scabby kernel (VSK)

In general, the VSK was lowest in 2006, which was also the year with the lowest FHB severity. The average VSK (combined across mist-irrigation treatments, cultivars and isolates) was 8% in 2006, 40% in 2007 and 38% in 2008 (Figures 3.2B, 3.4 and 3.5, Appendix 5).

Mist-irrigation duration treatments had a significant impact on VSK in 2007 and 2008, but not in 2006. In 2007 and 2008, longer periods of mist-irrigation resulted in increased VSK values. When combined over cultivars and isolates, the average VSK values were generally greater in the longest mist-irrigation duration (35 DAI) and least in the shortest duration of mist-irrigation (14 DAI). In both 2007 and 2008, VSK increased significantly in the cultivars Alsen and 2375 with increased durations of mist-irrigation (Appendix 5). In Wheaton in 2007, though there were numerical increases in VSK values with longer periods of mist-irrigation, this increase was not statistically significant from the 14 DAI to the 28 DAI mist-irrigation treatments. In 2008, a numerical increase in VSK in Wheaton from the 28 DAI to the 35 DAI mist-irrigation

duration was also observed but was not significant. No trend in VSK with increase in the duration of mist-irrigation treatment was observed in 2006. The percentage increases in VSK from the 14 DAI to the 21 DAI mist-irrigation duration treatments were 22% in 2007 and 58% in 2008. The percentage increases in VSK from the 21 DAI to the 28 DAI mist irrigation treatments were 30% and 33% in 2007 and 2008, respectively. The percentage increase in VSK was least between the 28 DAI and the 35 DAI irrigation duration treatments (2007: 13%, 2008: 21%).

VSK was significantly higher in the susceptible cultivar Wheaton in all mist-irrigation treatments in each year of study compared to the other cultivars. The VSK values (combined across mist-irrigation treatments and isolates) for Wheaton were above 60% in 2007 and 2008, but were less than 15% in 2006, the year with lowest FHB severity (Appendix 5). In general, 2375 had higher VSK values than Alsen in all years, except in 2006 where Alsen had slightly higher VSK values. The cultivar 2375 had higher VSK values than Alsen under the longer duration of moisture treatments (21, 28 and 35 DAI in 2007, and 28 and 35 DAI in 2008). On average, the percentage increase in VSK with one week longer mist-irrigation duration was the highest in cultivar 2375 (2007: 47%, 2008: 76%), intermediate in Alsen (2007: 36%, 2008: 67%) and the lowest in Wheaton (2007: 13%, 2008: 20%).

All isolates resulted in significantly higher VSK values than the water control irrespective of the mist-irrigation duration and wheat cultivar treatments (Figures 3.2, 3.4 and 3.5, Appendix 5). However, none of the isolates resulted in consistently higher or lower VSK values across all mist-irrigation durations and cultivars in any year of the study. In general, plots inoculated with isolates B45A and B63A had higher VSK values and the plots inoculated with isolate Butte86Ada-11 had the lowest VSK values in each year of the study. Increased VSK values were generally seen with all isolates in all cultivars under longer duration of mist-irrigation. Exceptions included isolates Butte86aAda-11 (in Alsen between the 14 DAI and 21 DAI irrigation treatment), B63A (in Wheaton between the 28 DAI and 35 DAI irrigation treatment) in 2007. In 2008, exceptions were isolates B45A (in Alsen between the 28 DAI and 35 DAI irrigation treatment), Butte86Ada-11 (in 2375 between the 28 DAI and 35 DAI irrigation treatments), 49-3 (in Alsen and 2375 between the 28 DAI and 35 DAI irrigation treatment) and 81-2 (in 2375 between the 28 DAI and 35 DAI irrigation treatment and Wheaton between the 21 DAI and 28 DAI irrigation treatment). However, these decreases in VSK were not statistically significant. When average percentage increase in VSK with increase in duration of mist-irrigation was considered, none of the isolates had a consistently higher or lower increase in VSK.

3.3.2.3 Deoxynivalenol (DON)

The level of DON was the lowest in 2006 and the highest in 2007 (Figures 3.2C, 3.4 and 3.5, Appendix 6). The low DON levels observed in 2006 reflects the low FHB severity and VSK also seen in that year. Similarly, the 2007 DON results appear to reflect the high FHB severity and VSK. The average DON levels (combined across mist-irrigation treatments, cultivars and isolates) in the three years of study were 26.7 μg g^{-1} (2006), 37.5 μg g^{-1} (2007) and 33.0 μg g^{-1} (2008).

The mist-irrigation treatments significantly impacted DON accumulation levels in all three years of the study except in 2006. The DON level increased with longer durations of mist-irrigation with the exception of the 35 DAI mist-irrigation treatments in 2007 and 2008. In the 35 DAI mist-irrigation treatments, the average DON levels (combined across cultivars and isolates) were significantly lower compared to the 28 DAI treatments in each of the years studied. The percentage increase in DON was highest between the 14 DAI and 21 DAI mist-irrigation treatments in 2008 (84%) and between the 21 DAI and the 28 DAI mist-irrigation treatments in 2007 (15%). Deoxynivalenol levels in the 35 DAI irrigation treatments were more than 30% lower compared to 28 DAI mist-irrigation treatments (2007: 40%, 2008: 33%). Though the effect of mist-irrigation was not significant in 2006, the level of DON in the 35 DAI treatments observed was significantly lower by 37% than the 28 DAI treatment when the means compared using an LSD test.

The susceptible cultivar Wheaton had the highest DON level in each year of the study. The average DON levels (combined across mist-irrigation and *Fusarium* isolate treatments) in Wheaton were 24.2 μg g^{-1} and 38.4 μg g^{-1} in 2007 and 2008, respectively. In 2006, which was the year with the lowest FHB severity and VSK, the DON concentration in Wheaton averaged only 1.9 μg g^{-1}. In general, the cultivar 2375 (2006: 0.6 μg g^{-1}, 2007: 10.9 μg g^{-1}, 2008: 16.2 μg g^{-1}) had lower DON levels than Alsen (2006: 0.6 μg g^{-1}, 2007: 10.5 μg g^{-1}, 2008: 18.5 μg g^{-1}); however, the DON levels in Alsen and 2375 did not differ statistically in any year of the study. Increases in the DON levels in the 28 DAI mist-irrigation treatments compared to the 14 DAI mist-irrigation treatments were the highest in cultivar 2375 (2007: 111%, 2008: 153%), intermediate in Alsen (2007: 31%, 2008: 144%) and the least in Wheaton (2007: 25%, 2008: 98%). In contrast, the decline in DON levels were seen between the 28 DAI and 35 DAI mist-irrigation treatments which were the least in 2375 (2007: 27%, 2008: 19%), intermediate in Alsen (2007: 42%, 2008: 29%) and the highest in Wheaton (2007: 46%, 2008: 41%).

Only trace amounts of DON accumulated in the mock-inoculated plots. The plots inoculated with isolate 49-3 had significantly higher DON levels irrespective of mist-irrigation and wheat cultivar treatments in each year of the study. Generally, isolate Butte86Ada-11 resulted in the lowest DON levels in all irrigation and wheat cultivars treatment combinations. With longer durations of mist-irrigation, up to the 28 DAI treatment, increased DON levels were evident with all isolates and in all cultivars, with some exceptions. The exceptions in 2007 included isolates B45A, B63A and 49-3 in Alsen which had lower levels of DON in the 21 DAI mist-irrigation treatments compared to the 14 DAI mist-irrigation treatments In Wheaton in 2007, all isolates, except B63A, resulted in lower DON levels in the 28 DAI mist-irrigation treatments compared to the 21 DAI mist-irrigation treatments. In Wheaton in 2008 isolates 81-2, B45A, B63A and 49-3 also resulted in lower DON levels in the 28 DAI mist-irrigation treatments than in 21 DAI mist-irrigation treatments; however, none of these declines in Alsen and Wheaton were statistically significant. All isolates had lower DON levels in the 35 DAI irrigation treatments compared to the 28 DAI treatments and the differences were statistically significant with the exception of 6 of the 30 isolate/cultivar treatments. These exceptions included three treatments inoculated with isolate 49-3 (Alsen in 2008 and 2375 in 2007 and 2008), two treatments inoculated with isolate B45A (Alsen and 2375 in 2008), and the 2375 treatment inoculated with isolate B63A in 2008. In general, plots inoculated with isolates B45A and Butte86Ada-11 in 2007 and 2008, respectively, recorded the largest drop in DON in the 35 DAI mist-irrigation treatments compared to 28 DAI treatments. The 49-3 inoculated treatments generally had the smallest drop in DON levels, when the mist-irrigation duration was increased from 28 DAI to 35 DAI, in each year of the study.

3.3.2.4 15-acetyldeoxynivalenol (15-ADON)

The level of 15-ADON in the grain harvested at maturity was consistently lower in 2006 than in either of the other two years in the study (Figures 3.2D, 3.6 and 3.7, Appendix 7). 15-ADON was detected only in 16 out of 360 samples in 2006. The highest and lowest levels of 15-ADON detected in 2006 were 0.58 $\mu g\ g^{-1}$ and 0.06 $\mu g\ g^{-1}$, respectively. In 2007 and 2008, 15-ADON was detected in all grain samples except those from the control (mock-inoculated) treatments. The levels of 15-ADON were less than 7.2% that of the DON detected in the same samples. The average 15-ADON levels (combined across mist-irrigation, wheat cultivar and *Fusarium* isolate treatments) were 15.2 $\mu g\ g^{-1}$ in 2007 and 24.4 $\mu g\ g^{-1}$ in 2008.

There was no significant impact of mist-irrigation on the 15-ADON levels in 2006. The mist-irrigation treatments did have a significant impact on the 15-ADON levels in 2007 and 2008. When combined across cultivar and isolate treatments, 15-ADON levels increased significantly between treatments with incrementally longer durations of mist-irrigation, except, between the 28 DAI and 35 DAI mist-irrigation treatments. The 15-ADON levels were higher by 6% (2007) and 43% (2008) in the 21 DAI mist-irrigation treatments compared to the 14 DAI treatments. The 15-ADON level in the 28 DAI mist-irrigation treatments were lower compared to the 21 DAI treatments and declined further from the 28 DAI treatments to the 35 DAI mist-irrigation treatments. The levels of 15-ADON were 56% (2007) and 43% (2008) lower in the 35 DAI mist-irrigation treatment compared to the 28 DAI treatment. In both the 2007 and 2008 35 DAI mist-irrigation treatments, 15-ADON levels were also significantly lower than in all other mist-irrigation duration treatments in the grain harvested at maturity.

The susceptible cultivar Wheaton had significantly higher levels of 15-ADON in the grain at harvest compared to the other cultivars. The mean 15-ADON levels (combined across mist-irrigation and isolate treatments) in Wheaton were 0.9 $\mu g\ g^{-1}$ (2007) and 1.4 $\mu g\ g^{-1}$ (2008). There were no significant differences in the level of 15-ADON among the cultivars Alsen (2007: 0.4 $\mu g\ g^{-1}$, 2008: 0.5 $\mu g\ g^{-1}$) and 2375 (2007: 0.4 $\mu g\ g^{-1}$, 2008: 0.6 $\mu g\ g^{-1}$). Cultivar 2375 had the largest increase in the levels of 15-ADON between the treatments receiving mist-irrigation durations of 14 DAI and 21 DAI (2007: 52%, 2008: 72%). The decline of 15-ADON in the 35 DAI mist-irrigation treatments compared to the 28 DAI mist-irrigation treatments were largest in the cultivar Wheaton (2007: 63%, 2008: 50%).

The *Fusarium* isolate did influence the levels of 15-ADON detected. Treatments inoculated with isolate 49-3, generally had the highest levels of 15-ADON and the treatments inoculated with isolate Butte86Ada-11 the lowest 15-ADON levels. Except for isolates B45A and B63A, in the 2007 Alsen treatments, all isolates resulted in an increased level of 15-ADON level in the 21 DAI mist-irrigation treatments compared to the 14 DAI mist-irrigation treatments. Lower levels of 15-ADON were seen in the 35 DAI mist-irrigation treatments compared to the 28 DAI treatments irrespective of the isolates tested. In general, plots inoculated with the isolate 49-3 had the smallest decline in 15-ADON levels in 35 DAI mist-irrigation treatments compared to the 28 DAI mist-irrigation duration treatments. No specific isolate had the largest decline in 15-ADON in the 35 DAI mist-irrigation treatments compared to the 28 DAI mist-irrigation treatments in all cultivars studied in each year of the study.

3.3.2.5 3-acetyldeoxynivalenol (3-ADON)

The levels of 3-ADON were only tested in the harvested grain in 2007 and 2008 (Figures 3.6 and 3.7, Appendix 8). The maximum levels of 3-ADON detected were 0.26 μg g^{-1} in 2007 and 0.79 μg g^{-1} in 2008. The 3-ADON level never reached over 3% of the DON detected in a given sample.

Though the levels of 3-ADON detected were very low, the mist-irrigation treatments did have a significant impact on the levels detected, although the changes were not consistent among the two years when 3-ADON was measured. In 2007, 3-ADON either declined or did not change with increasing durations of mist-irrigation, except in the cultivar 2375 where the 3-ADON level was higher in the 21 DAI mist-irrigation treatments compared to the 14 DAI treatments. By contrast in 2008, the levels of 3-ADON were higher in the treatments with longer durations of mist-irrigation until the 28 DAI treatment. Though in general the levels of 3-ADON in the 35 DAI mist-irrigated treatment were lower than the 28 DAI mist-irrigated treatment (Alsen: 23%, 2375: 19%, Wheaton: 30%) in 2008, the difference was significant only for the susceptible cultivar Wheaton.

The levels of 3-ADON in both years examined were significantly higher in the susceptible cultivar Wheaton compared to Alsen and 2375. The level of 3-ADON in Alsen and 2375 were not significantly different in 2007. In 2008, however, the level of 3-ADON differed significantly among all three cultivars with the levels being lowest in 2375 (0.11 μg g^{-1}).

In general isolates 49-3 and Butte86Ada-11 produced the highest and the lowest 3-ADON levels, respectively, in both 2007 and 2008. Other isolates were similar with each other in terms of the 3-ADON levels they produced, however, the levels of 3-ADON were not likely high enough to allow for the detection of statistically significant difference among the isolates.

3.3.2.6 Nivalenol (NIV)

The levels of NIV in harvested grain samples were tested in 2007 and 2008 (Figure 3.8, Appendix 9). The maximum level of NIV detected was 0.39 μg g^{-1} in Wheaton under the 28 DAI mist irrigation duration treatment in 2007. In the samples in which NIV was detected, NIV levels were less than or equal to 2.1% to that of DON levels detected in a sample.

The levels of NIV were progressively higher in treatments with longer durations of mist-irrigation until the 28 DAI treatment. In the 35 DAI mist-irrigation duration treatment, NIV levels for Alsen and Wheaton were significantly lower than in the 28 DAI mist-irrigation duration treatments. The levels of NIV in Alsen in the 35 DAI mist-irrigation treatments were lower by

67% (2007) and 46% (2008) than in 28 DAI mist-irrigation treatments. In Wheaton the NIV levels were lower by 55% (2007) and 43% (2008) in the 35 DAI treatment compared to the 28 DAI mist-irrigation treatments.

The susceptible cultivar Wheaton had significantly higher NIV levels compared to Alsen and 2375. The levels of NIV in both years in Wheaton were however less than 0.40 μg g^{-1}. In general, the levels of NIV did not differ significantly among Alsen and 2375. The levels of NIV detected in Alsen and 2375 were never above 0.15 μg g^{-1} in 2007 and 0.17 μg g^{-1} in 2008.

In general isolate 49-3 resulted in higher NIV levels in all cultivar and mist-irrigation treatments. Other isolates were generally similar in terms of NIV production producing very low levels of NIV which likely contributed to the non-significant differences observed.

3.3.2.7 Correlations

The correlation coefficients between FHB severity, VSK, DON, 15-ADON, 3-ADON and NIV were generally high and significant in each year of the study (Tables 3.1 and 3.2). Overall, the correlations between FHB severity and VSK were strong compared to the correlations between the FHB severity and the other variables examined. Irrespective of the mist-irrigation duration treatments, the correlation coefficients between FHB severity and VSK were not less than 0.60 ($P < 0.01$) in any year of the study. In general, FHB severity and VSK were more closely correlated in treatments with increased periods of irrigation until the 28 DAI treatments.

The correlation between FHB severity and 15-ADON was stronger compared to the other mycotoxins tested in 2007 and 2008. In 2006, the correlation coefficient was higher between FHB severity and DON than between FHB severity and 15-ADON. The correlation coefficients between FHB severity and DON generally increased with increased duration of mist-irrigations. However, in the 35 DAI mist-irrigation duration treatments, correlation coefficients were lower compared to all other mist-irrigation duration treatments in each year of the study. The numerical value of correlation coefficients between FHB severity and 15-ADON were similar irrespective of mist-irrigation duration treatments (2007: 14 DAI $r = 0.64$, 21 DAI $r = 0.80$, 28 DAI $r = 0.66$; 2008: 14 DAI $r = 0.72$, 21 DAI $r = 0.73$, 28 DAI $r = 0.72$, $P \leq 0.01$) with exceptions. These exceptions included the correlation between FHB severity and 15-ADON in the 35 DAI mist-irrigation duration treatments in 2007 ($r = 0.46$, $P \leq 0.01$) and 2008 ($r = 0.57$, $P \leq 0.01$), where the correlation coefficients were lower compared to the rest of the mist-irrigation duration treatments. Though there was no specific trend in the correlations between FHB severity and the other toxins

tested (3-ADON and NIV), the correlation coefficients were generally lower for the 35 DAI mist-irrigation treatments compared to other mist-irrigation duration treatments except for the 28 DAI mist-irrigation duration treatments in 2007, where the correlation coefficient for FHB severity and NIV was lower ($r = 0.32$, $P \leq 0.01$) than that of the 35 DAI mist-irrigation duration treatment ($r = 0.40$, $P \leq 0.01$).

The correlations of VSK with DON were slightly stronger than the correlations of FHB severity with DON. This was to be expected as the DON analysis was done on the same grain sample used to determine VSK and FHB severities were measured in the field 18-20 days before harvest. The correlation coefficients of VSK with DON declined with increased durations of mist-irrigation although all correlation coefficients were over 0.43 ($P \leq 0.01$). In the 35 DAI mist-irrigation treatments, the correlation coefficients of VSK with DON were lower compared to the other irrigation duration treatments. The correlations of VSK with 15-ADON was either weaker or remained stable with increased durations of mist-irrigation durations with the lowest correlation coefficient recorded in the 35 DAI mist-irrigation duration treatments in 2007 ($r = 0.35$, $P \leq 0.01$) and 2008 ($r = 0.52$, $P \leq 0.01$). The correlations of VSK with 3-ADON also weakened with increased durations of irrigation with the lowest correlation coefficients being observed in the 35 DAI mist-irrigation duration treatments in 2007 and 2008. However, the correlation coefficient of VSK with 3-ADON in the 35 DAI misting treatment in 2008 was non-significant. For NIV, the correlation with VSK became stronger with the increase in duration of mist-irrigation. However, the correlation coefficients were lowest but significant in the 35 DAI mist-irrigation duration treatments compared to the rest of the mist-irrigation duration treatments.

The correlations of DON with 15-ADON and 3-ADON became weaker with increases in the duration of irrigation in both 2007 and 2008, but never went below 0.80 (for DON and 15-ADON) and 0.54 (for DON and 3-ADON). The correlations of DON with NIV did not show a distinctive pattern in 2007. In 2008, the correlation coefficient between DON and NIV increased numerically with increases in the duration of mist-irrigation except in the 35 DAI mist-irrigation treatment where the correlation coefficient was lower than for the 28 DAI mist-irrigation duration treatment. The correlations of DON with 15-ADON were stronger than the correlation of DON with either 3-ADON or NIV. Similarly, the correlations of DON with 3-ADON were stronger than the correlations with either 15-ADON or NIV. The correlations of NIV were stronger with DON than with 15-ADON and in turn by those with 3-ADON.

3.3.3 Whole head samples

3.3.3.1 Disease severity

The whole head samples were taken from each plot 0, 7, 11, 14, 21, 28 and 41 dai. The 0 dai sampling corresponds to the day of the first inoculation while the sampling 41 dai corresponds to the day before the harvest of mature grain, though the final samplings in 2008 was 39 dai due to the early maturity of the plots. Samples on 0, 7 and 11 dai were collected from one of the irrigation duration treatments in each year of the study (21 DAI mist-irrigation duration treatment in 2007, 28 DAI mist-irrigation duration treatment in 2008), as the mist-irrigation duration treatments were equivalent during that period. For the FHB severity analyses, data from the corresponding mist-irrigation treatment (21 DAI in 2007 and 28 DAI in 2008) were also used in 14 and 21 dai head samplings.

FHB severities, which were recorded in samples taken from 7 dai to 21 dai, when combined across cultivar and isolate treatments, increased significantly in each successive sampling except from the 11 dai to the 14 dai samplings in 2007 (Appendix 10). Though there was an increase in FHB severity between the 11 dai and the 14 dai in 2007, it was not statistically significant. Disease severities were higher at the 7 dai sampling in 2007 compared to 2008 and increased rapidly from 7 to 11 dai. However in 2008, the FHB severities remained at a low level until 14 dai after which FHB severities increased rapidly (Figure 3.9). The greater rainfall that occurred within a 10 day period after inoculation in 2007 (21.8 mm) compared to 2008 (3 mm) might have contributed to the early increase in FHB severities observed in 2007.

Average FHB severities, when combined across isolates, increased in all three cultivars until the last sampling (21 dai) when disease severity was also assessed on whole plots in the field, except in the resistant cultivar Alsen in 2007. In Alsen, average FHB severity (combined across isolates) increased only until 11 dai. FHB severities were statistically similar in Wheaton (susceptible) and Alsen (resistant) at the first two samplings (7 and 11 dai), and both cultivars had significantly higher FHB severities at these sampling dates than did 2375. At later samplings (14 and 21 dai), the FHB severity was significantly higher in Wheaton than in either Alsen or 2375. Alsen generally had a higher FHB severity than 2375.

When the individual *Fusarium* isolates were examined, it was evident that all isolates resulted in significantly higher FHB severities 21 dai in Wheaton in 2007 compared to other sampling dates. In cultivar 2375 in 2007 all isolates, except Buttte86Ada-11, resulted in higher FHB severities at 14 dai, though they were not statistically different to those at 11 and 21 dai. In Alsen (2007), except isolate 49-3, the highest FHB severities were observed 11 dai, after which

FHB severities were lower numerically in subsequent samplings, although they were not statistically different to the FHB severities at 11 dai. In contrast to 2007, in 2008 all isolates resulted in increased FHB severities in all three cultivars in subsequent samplings until 21 dai. No specific isolate resulted in the highest FHB severity at 7 and 11 dai samplings in both year of the study. At the 14 and 21 dai samplings, isolate 49-3 in 2007 and isolate B45A in 2008 resulted in generally higher FHB severities in all three cultivars. The highest FHB severities recorded were 39.9% in Alsen 14 dai, 37.3% in 2375 21 dai and 72.6% in Wheaton 21 dai in the plots inoculated with isolate 49-3 in 2007. None of the isolates consistently resulted in the lower FHB severities across sampling days or cultivars in either year of the study.

3.3.3.2 Deoxynivalenol (DON)

Deoxynivalenol was detected from 7 dai, though some isolates did not produce detectable levels of DON in some cultivars until 11 dai in 2007 (Appendix 11). The level of DON increased significantly from the 7 dai to the 11 dai samplings in all cultivar and isolate treatments in 2007 and 2008. At the 7 dai sampling, cultivars were statistically similar for DON levels (2007: 0.03 $\mu g\ g^{-1}$ in Alsen, 0.02 $\mu g\ g^{-1}$ in 2375, 0.02 $\mu g\ g^{-1}$ in Wheaton; 2008: 0.33 $\mu g\ g^{-1}$ in Alsen, 0.24 $\mu g\ g^{-1}$ in 2375, 0.33 $\mu g\ g^{-1}$ in Wheaton). Of the two samplings (7 and 11 dai) before the imposition of mist-irrigation treatments, the DON level was highest in Wheaton in 2007 (36.5 $\mu g\ g^{-1}$) and in Alsen in 2008 (8.86 $\mu g\ g^{-1}$) at the 11 dai samplings. Isolate 49-3 resulted in the highest DON levels in all three cultivars in 2007. In the 7 and 11 dai samplings in 2008, no single isolate consistently resulted in the highest DON levels across all cultivar treatments.

When the DON levels were analyzed in whole heads samples after the first mist-irrigation treatments were imposed, Wheaton had significantly higher levels of DON at each sampling date in both 2007 and 2008 (Appendix 12) than the other two cultivars. In general, Alsen had higher levels of DON than 2375 in most of the treatments, although both cultivars had statistically similar DON levels in the sampling days from 14 dai to 41 dai in both years of the study.

Two representative isolates, 49-3 and Butte86Ada-11, are used to present the DON accumulation profiles over time (Figure 3.10). Isolate 49-3 was selected because, in general it resulted in significantly higher DON levels compared to other isolates in the study. Butte86Ada-11 was selected as it generally resulted in the lowest DON levels and in most cases it was similar to the other isolates, except 49-3.

The DON accumulation profiles across sampling days were different in 2007 and 2008. In 2007 the treatments which received mist-irrigation only up to 14 DAI were increased levels of DON recorded in each subsequent sampling after the cessation of mist-irrigation. This increase in the DON level after 14 dai was most evident in susceptible cultivar Wheaton. Isolate 49-3 resulted in higher DON compared to Butte86Ada-11 in all samplings in all cultivars. When all isolates were considered, levels of DON peaked at 41 dai (GS 93) in all three cultivars in the treatment receiving 14 DAI mist-irrigation. In the treatment which received mist-irrigation up to 21 DAI, DON peaked at 14 dai (GS 75) in Alsen and 2375 and declined thereafter, until the cessation of mist-irrigation on 21 DAI. DON levels were then observed to increase in samplings after the mist-irrigation had ceased. In Wheaton, though the irrigation was ended 21 DAI, DON levels continued to increase reaching their highest level 28 dai (GS 85) and declining thereafter. Similarly in the treatment receiving mist-irrigation up to 28 DAI, DON levels peaked at 14 dai (GS 75) for Alsen and Wheaton and then either remained at same level or declined in subsequent samplings until the 28 dai sampling. After mist-irrigation ceased in the 28 DAI treatment, DON level increased in subsequent samplings. However for cultivar 2375, DON levels peaked at 41 dai (GS 93). In the treatments receiving mist-irrigation duration up to 35 DAI, DON levels in all three cultivars generally peaked 14 dai (GS 75) although the peak was at 21 dai (GS 80) in case of the Wheaton/Butte86Ada-11 treatment and DON levels declined in subsequent samplings.

In contrast to 2007, the DON accumulation profile differed in 2008. Mist-irrigation did impact the DON levels and treatments receiving longer duration of mist-irrigation generally had lower DON levels in 2008. In the treatment that received mist-irrigation up to 14 DAI, DON levels increased rapidly after the cessation of mist-irrigation with exception of the sampling 28 dai (GS 85). A similar pattern of lower DON levels until the cessation of mist-irrigation was evident in the treatments which received mist-irrigations up to 21, 28 and 35 DAI. In all mist-irrigation duration treatments, DON levels increased in subsequent samplings after the end of mist-irrigation. Unlike 2007, DON levels peaked 41 dai (GS 93) in all three cultivars in all mist-irrigation treatments, except Wheaton in the 35 DAI misting treatment where the DON levels peaked 21 dai (GS 80) and decreased thereafter.

3.3.3.3 15-acetyldeoxynivalenol (15-ADON)

Except in the cultivar Alsen inoculated with isolate 81-2 (0.02 $\mu g\ g^{-1}$) in 2007, 15-ADON was not detected in the 7 dai samples (Appendix 13). In 2008, 15-ADON was detected 7 dai in the Alsen samples inoculated with isolates 81-2 (0.05 $\mu g\ g^{-1}$), B45A (0.02 $\mu g\ g^{-1}$), in the 2375

samples inoculated with isolate 81-2 (0.04 μg g^{-1}) and in the Wheaton samples inoculated with isolates 81-2 (0.02 μg g^{-1}) and 49-3 (0.01 μg g^{-1}). The levels of 15-ADON were significantly higher in the 11 dai samples compared to the 7 dai samples in all three cultivars. Wheaton recorded significantly higher amounts of 15-ADON (2.6 μg g^{-1}) compared to Alsen (0.91 μg g^{-1}) and 2375 (0.83 μg g^{-1}) at the 11 dai samplings in 2007. However, Wheaton (0.28 μg g^{-1}) was not significantly different to Alsen (0.32 μg g^{-1}) although they were both significantly different to 2375 (0.17 μg g^{-1}) for the 15-ADON concentration at the 11 dai sampling in 2008.

In samples harvested after the imposition of mist-irrigation duration treatments (14, 21, 28 and 41 dai), Alsen and 2375 had significantly lower levels of 15-ADON than Wheaton (Appendix 14). Cultivars Alsen and 2375 were statistically similar for 15-ADON at all sampling days, except 41 dai in 2007 and 2008. Cultivar 2375 had significantly higher 15-ADON levels than Alsen in the 41 dai samplings in 2007 and 2008.

Isolate 49-3 was, in general, the highest 15-ADON producer in all three cultivars at all sampling dates. Butte86Ada-11 generally resulted in the lowest levels of 15-ADON. Two isolates, 49-3 and Butte86Ada-11, are presented for comparing 15-ADON production profiles in different cultivars and mist-irrigation duration treatments over time (Figure 3.11). Similar to the DON accumulation profiles, 15-ADON profiles were also impacted by the mist-irrigation treatments. In all mist-irrigation treatments except in 35 DAI in 2007, 15-ADON increased over time with the level being highest in the 41 dai sampling. In the treatments receiving 35 DAI of mist-irrigation, 15-ADON levels declined between successive samplings from 14 dai to 41 dai, except from 14 dai to 21 dai in Wheaton and Alsen inoculated with Butte86Ada-11 and in 2375 inoculated with 49-3. Although the overall 15-ADON level increased over time, the levels were lower where the mist-irrigation was being applied. In all treatments, a significant increase in the 15-ADON level occurred after the end of misting. Overall, the levels of 15-ADON were lower in the treatment receiving the longer duration of mist-irrigations. Cultivar Wheaton had higher 15-ADON levels compared to Alsen and 2375. Alsen and 2375 did not differ significantly for the levels of 15-ADON at any sampling date. Isolate 49-3 generally resulted in highest 15-ADON levels irrespective of the cultivar examined.

3.3.3.4 3-acetyldeoxynivalenol (3-ADON)

3-ADON was detected only in the head samples collected on or after 11 dai (Appendix 15). Wheaton had significantly higher level of 3-ADON (0.82 μg g^{-1}) than Alsen (0.25 μg g^{-1}) and

2375 (0.25 μg g^{-1}) at 11 dai in 2007. However in 2008, the 3-ADON levels in Wheaton (0.17 μg g^{-1}) was significantly higher than 2375 (0.05 μg g^{-1}), but not Alsen (0.13 μg g^{-1}).

In samplings at 14, 21, 28 and 41 dai, the susceptible cultivar Wheaton had significantly higher levels of 3-ADON compared to Alsen and 2375, except in the 41 dai samplings from the 35 DAI mist-irrigation treatment in 2008 (Appendix 16). In the 41 dai samplings from the 35 DAI mist-irrigation treatment in 2008, all three cultivars had statistically similar levels of 3-ADON. At all sampling dates the levels of 3-ADON in Alsen and 2375 were statistically similar, though the levels were numerically higher for 2375 in 2007 and numerically higher for Alsen in 2008.

In general, isolates did not produce significantly different 3-ADON levels in Alsen and 2375. However, in the susceptible cultivar Wheaton, isolates differed significantly for the amount of 3-ADON produced. Similar to the results for DON and 15-ADON, isolate 49-3 generally produced the highest 3-ADON levels and Butte86ADA-11 the lowest. The 3-ADON accumulation profile of these two isolates is presented (Figure 3.12). The profiles of 3-ADON production over time differed between the two years of the study, although the impact of mist-irrigation treatments on the levels of 3-ADON in both years was evident. In all mist-irrigation treatments, except the 35 DAI mist-irrigation duration treatment in 2007, the levels of 3-ADON increased over time resulting in higher 3-ADON levels at 41 dai compared to 14 dai. In the 2007 exception, the levels of 3-ADON detected 41 dai was lower than that of the 14 dai samples. In 2007, the levels of 3-ADON peaked 21 dai and declined from that point until harvest, though it should be noted that the final 3-ADON level at the 41 dai sampling was higher than those from the 14 dai samples in the 14, 21 and 28 DAI mist-irrigation duration treatments. Generally, however treatments receiving longer durations of mist-irrigation had lower levels of 3-ADON compared to the treatments receiving shorter durations of mist-irrigation. In 2008, a continuous increase in levels of 3-ADON up to 41 dai samplings was observed and the levels of 3-ADON were generally lower in the treatments which received a shorter duration of mist-irrigation at any given sampling point.

Wheaton had significantly higher levels of 3-ADON compared to Alsen and 2375 in all sampling dates in each year of the study. Alsen and 2375 had similar 3-ADON levels at all samplings dates. Isolate 49-3 generally resulted in higher 3-ADON levels in Wheaton at a given sampling date compared to Alsen and 2375. Similarly, isolate Butte86Ada-11 generally resulted in higher 3-ADON levels in Wheaton compared to Alsen and 2375. The exceptions were at 41 dai in 2007, where in the treatment receiving mist-irrigation up to 35 DAI, the levels of 3-ADON were higher in Alsen and 2375 inoculated with isolate 49-3 and in 2008, where Alsen inoculated

with isolate 49-3 had higher levels of 3-ADON than Wheaton inoculated with Butte86Ada-11. Similarly, at the 14 dai sampling date in the 14 DAI mist-irrigation treatment, both Alsen and 2375 inoculated with isolate 49-3 had higher levels of 3-ADON compared to the Butte86Ada-11 inoculated Wheaton treatment.

3.3.3.5 Nivalenol (NIV)

Nivalenol was not detected in samples collected 7 dai in either 2007 or 2008 (Appendix 17). The 11 samplings where NIV was detected were in Wheaton inoculated with isolates 81-2 (0.12 μg g^{-1}) and 49-3 (0.13 μg g^{-1}) in 2007 and with isolate Butte86Ada-11 in both 2007 (0.06 μg g^{-1}) and 2008 (0.02 μg g^{-1}); and in Alsen inoculated with 49-3 (0.06 μg g^{-1}) in 2007.

In sampling dates (14, 21, 28 and 41 dai) after the imposition of mist-irrigation duration treatments, Wheaton had significantly higher levels of NIV compared to both Alsen and 2375 (Appendix 18). Alsen and 2375 were not statistically different for their levels of NIV. In general, isolates produced no NIV or very low level of NIV (≤0.16 μg g^{-1}) in Alsen and 2375, the exception being isolate 49-3 (≤0.40 μg g^{-1}). In Wheaton, most of the isolates resulted in detectable levels of NIV (≤1.16 μg g^{-1}) by 14 dai (Figure 3.13). Due to the undetectable or very low levels of NIV in Alsen and 2375, no specific trend was observed across sampling dates. However, mist-irrigation duration treatments did have an impact on NIV levels. There were higher levels of NIV in 41 dai sampling dates compared to 14 dai samplings except for plots receiving the 28 or 35 DAI durations of mist-irrigation in 2007. Generally, the levels of NIV were lower in the treatments receiving the longer durations of mist-irrigation, despite the increase observed in the NIV levels across the sampling dates.

3.3.3.6 Correlations

The correlations of FHB severity, DON, 15-ADON, 3-ADON and NIV in whole heads samples were analyzed in the samples harvested at 7, 11, 14 and 21 dai in both 2007 and 2008 (Table 3.3). The correlations of FHB severity and mycotoxins tested in the 7 dai samplings were either low (≤0.47), non-significant or not calculated due to absence of 3-ADON and NIV from the samples. This was expected as the 7 dai time point was early in the infection period and mycotoxins were not produced and accumulated in large amounts by then. In the rest of the samplings, the correlation of FHB severity and DON was high (≥0.72, $P < 0.0.1$) for all sampling dates and in both years. The correlation of FHB severity with 15-ADON and 3-ADON were comparable ($0.49 \geq r \leq 0.84$, $P < 0.0.1$) with those for DON. The correlation of FHB severity and

NIV were low (0.30≥ r ≤0.61, P < 0.01) in 2007 and mostly non-significant (0.01≥ r ≤0.17) in 2008 except for the 21 dai samplings, where the correlation coefficient was 0.39 (P < 0.01). Where correlations among mycotoxins were tested, correlations were generally higher among DON, 15-ADON and 3-ADON than with NIV.

3.3.4 Whole head samples versus mature harvested grain

The mature grain was harvested from the experiments conducted in 2006, 2007 and 2008. Whole heads were only sampled from the plots in 2007 and 2008. Not surprisingly, whole head samples had much higher levels of DON, in some cases these were up to five times those in grain harvested at maturity. The proportion of DON in whole head samples compared to that in harvested grain was higher in 2375 than in Alsen and Wheaton. The proportion of 3-ADON in whole head samples than harvested grain was higher compared to the proportion of other mycotoxins tested. The levels of 15-ADON and 3-ADON were 77 and 11 fold higher, respectively in whole head samples to that in grain harvested at maturity. Similar to the findings for DON, the proportion of 15-ADON and 3-ADON in whole head samples compared to that in grain harvested at maturity was higher in 2375. NIV was present in whole head samples were 19 fold higher to that in mature harvested grain. Although the highest proportion of NIV in whole heads compared to grain was found in cultivar 2375, generally, NIV was either in very low amount or absent in 2375 and Alsen. The proportion of all mycotoxins tested in whole head samples were higher in the treatments receiving shorter durations of misting (14 and 21 DAI) than in the treatments which received longer durations of mist-irrigation (28 and 35 DAI). The decline observed in the proportion of DON, 15-ADON and 3-ADON in whole heads compared to harvested grain with increasing durations of mist-irrigation was higher for 2375 than Alsen and Wheaton.

The correlations between the levels of mycotoxins tested in whole head samples and mature harvest grain were high and significant for all toxins except NIV (Table 3.4). The correlation coefficients for the correlation between the levels of a toxin in whole head samples and mature harvested grain ranged from 0.75 to 0.91 (P < 0.01) for DON and 15-ADON. The correlation coefficients for 3-ADON between whole head samples and harvested grain ranged from 0.36 to 0.76 (P < 0.01). For NIV, the correlations were generally low, although they were significant (0.44≥ r ≤0.61, P < 0.01), except for those treatments receiving the longer durations of mist-irrigation (28 and 35 DAI in 2007, 35 DAI in 2008), where the correlations were non-significant. The correlation between the levels of a mycotoxin in whole head samples and mature

harvested grain declined with an increase in the duration of mist-irrigation for 15-ADON and 3-ADON in 2007. No apparent trend of decline in the correlation was observed for other mycotoxins in 2007 or for all mycotoxins examined in 2008.

3.4 Discussion

That environmental condition in conjunction with the host and pathogen influence disease development is a foundational tenet in plant pathology. The availability of moisture either in the form of rainfall, dew or relative humidity during anthesis or shortly thereafter has been linked to FHB development and DON accumulation in wheat (Abramson et al., 1987; Atanasoff, 1920; Rohácik and Hudec, 2005). Past FHB epidemics in North America have been linked with the occurrence of rainfall during wheat anthesis (McMullen et al., 1997; Sutton, 1982). Extended supplemental moisture in the form of mist-irrigation is regularly applied to promote FHB development in field nurseries screening wheat germplasm for FHB resistance. However, supplemental moisture is generally provided only between anthesis and the time of disease rating in the field, generally from 18 to 21 dai, and the influence of rainfall occurring after this time is largely overlooked. This study was designed to evaluate the impact of moisture from anthesis to harvest on FHB development and the accumulation of mycotoxins. The study also provided information on the variation in FHB severity and DON accumulation resulting from individual pathogen isolates across a range of host resistance levels.

In this three year study, FHB severity was not significantly different between treatments receiving different durations of moisture provided. This was to be expected, as only the 14 DAI mist-irrigation duration treatments had been imposed when FHB severities were assessed in the field. Although there were no statistical differences in FHB severities observed in the 14 and 21 DAI mist-irrigation treatments, the FHB severities in the 21 DAI mist-irrigation treatments were numerically higher. This suggests that FHB severities are likely to be greater when moisture is available for longer periods following inoculation, although after disease assessment (21 dai, GS 80) the symptoms of FHB are masked by the natural senescence of the head tissues.

The levels of VSK, evaluated in grain harvested at maturity, were significantly higher in the treatments which received longer durations of mist-irrigation and lowest in the treatment receiving the shortest period of misting. Presumably the additional moisture in treatments with longer durations of misting provided an environment conducive for fungal growth and disease development which contributed to the observed increase in VSK. These results agree with the results obtained by Cowger et al. (2009) in *F. graminearum*-inoculated winter wheat. They

reported lower FHB severities and Fusarium damaged kernels (FDK, percentage of visually scabby kernel in 100 seeds collected randomly from each plot) in treatments receiving 0 and 10 days of misting after anthesis compared to those treatments receiving 20 and 30 days of misting. Increases in FHB severities in mist-irrigated plots compared to non-misted plots have also been reported by Lemmens et al. (2004), although in their study the *Fusarium* damage to grain was not examined. Culler et al. (2007) also reported higher FHB severities and VSK in *F. graminearum*-inoculated wheat receiving mist-irrigation for 31-32 days after inoculation compared to treatments receiving misting for only 15-16 days.

The results from the present study support the theory that the both symptom development and grain colonization increase with longer periods of moisture after inoculation. Since mycotoxin analyses were conducted on the same samples that were used for VSK analysis, it was our initial hypothesis that the DON levels would similarly increase in treatments with longer periods of misting. However, the DON levels in the harvested grain were consistent with the VSK results only for the three treatments providing shorter durations of mist-irrigation. Unlike VSK, DON levels in the treatments receiving the longest duration of misting (35 DAI) were significantly lower than those from the treatment with next longest duration of mist-irrigation (28 DAI). The same trend was observed for the other mycotoxins examined in this study with 15-ADON, 3-ADON and NIV levels being lower in treatments receiving longer durations of misting. The reduction of mycotoxins was most readily discernible in the susceptible cultivar Wheaton. Lower levels of DON following extended durations of mist-irrigation were also reported by Lemmens et al. (2004) when non-misted treatments were compared to plots which received misting for ca. 27 min per day for the period of 42 dai. They reported lower concentrations of DON in grain grown under misting, compared to grain from non-misted plots. It should also be noted that the disease level in their study was reported as being higher in the plots under misting. Similarly, Culler et al. (2007) also reported lower levels of DON with greater environmental moisture, especially in the susceptible cultivar, Wheaton. They observed generally lower DON concentrations under misting treatments for 31-32 days after inoculation compared to misting treatments which ran only for 15-16 days. The lower levels of DON observed in these experiments contrasts with the results reported by Cowger et al. (2009). In the first year of a two year study Cowger et al. (2009) reported higher DON levels in the treatment receiving misting for 30 days post-anthesis compared to the treatments receiving mist-irrigation for 0, 10 and 20 days. However in the second year they reported a lower level of DON in the treatment receiving irrigation for 30 days post-anthesis compared to the treatment receiving irrigation for 20 days

post-anthesis. They attributed the lower DON levels to lower levels of FHB in the treatment receiving 30 days of misting. The mean FHB severity in the susceptible cultivar in the treatment receiving 30 days post-anthesis was ca. 30% lower than treatments receiving either 10 or 20 days post-anthesis irrigation. The lower FHB severities appear likely to contribute to the low DON levels observed in the plots receiving irrigation for 30 days post-anthesis compared to the plots receiving irrigation up to 20 days post-anthesis, however, in some cultivars Cowger et al. (2009) reported decreases in DON levels despite an increase in FHB severities. They observed lower DON levels in plots receiving 30 days of post-anthesis irrigation compared to those plots receiving irrigation for only 20 days. By contrast, in the current study differences in FHB severities between the treatments receiving 14 DAI and the rest of the treatments, which received irrigation at least up to disease assessment (GS 80), were generally not statistically different. The lower amount of DON observed in the 35 DAI mist-irrigation treatment in the current study cannot be attributed to the FHB severity differences observed between the mist-irrigation duration treatments. Lacey et al. (1999) also reported increased DON levels with increased durations of misting. However, they studied misting only up to the three days after inoculation, thus their results cannot be compared directly with the results of this study.

The time course profiles of the accumulation of DON and its derivatives across the duration of grain development furthers our understanding of how the toxins observed in mature harvested grains are influenced by environmental moisture. Although the DON profiles, and those of the related toxins, were different in 2007 and 2008, the levels of toxins in whole heads were significantly impacted by mist-irrigation. In 2007, once the levels of DON peaked, the ongoing availability of moisture then dictated if the level stayed the same, declined or increased before the grain ripened. Generally, the DON levels either remained stable or declined when mist-irrigation was continued. Increases in DON levels were generally observed only after misting ceased in a given treatment. In 2008, DON levels increased between inoculation and the final sampling (41 dai, GS 93), however, the DON was low until the irrigation ceased and then increased rapidly. The other toxins examined (15-ADON, 3-ADON and NIV) showed the similar profiles to that of DON, even though the concentrations of these toxins were significantly lower.

Since differences observed in FHB severities between the different mist-irrigation duration treatments were low and non-significant, other factors must have contributed to the lower levels of DON and other toxins observed in the harvested grain. It is suggested that the decline in DON toward harvest may have been promoted by leaching of mycotoxins from the host tissues. DON and its derivatives are water soluble compounds (Bensassi et al., 2010; Böhm

et al., 2008; Cahill et al., 1999; Hazel and Patel, 2004; Kang and Buchenauer, 1999). Water solubility allows DON, 15-ADON and 3-ADON to be transported through phloem explaining the detection by Kang and Buchenauer (1999) of mycotoxins in wheat head tissues not invaded by *Fusarium* hyphae. A similar finding was also been reported by Snijders and Kretching (1992). It appears plausible that DON, and its derivatives, may be leached from plant tissues contributing to the lower levels of these compounds detected in tissues subjected to extended wet periods. Further, increases in DON following the cessation of irrigation likely reflect accumulation following production from continuing fungal activity in the wheat tissues. It is speculated that any DON leached before 28 DAI may have been masked by ongoing toxin production by the fungus; however, once the plant begins senescence, the rate of fungal growth and thus DON production is likely reduced, thus making losses of DON from plant tissues detectable.

Alternatively, the observed lower DON levels might be influenced by differences in the maturity of wheat kernel. DON levels have been reported to peak before harvest and then decline as grain matures. In the present study, a peak in DON accumulation was observed either 14 dai (GS 75) or 21 dai (GS 80), after which DON levels either increased again, decreased or stayed same depending upon the continuation of mist-irrigation treatments.

The peaking of DON in grain sometime before harvest followed by a decline till harvest has been reported by other researchers. Scott et al. (1984) reported a decline in DON levels before harvest in naturally infected commercially grown winter wheat in the province of Ontario, Canada. They observed a decline in the DON levels as the grain matured and the decrease was consistent across all fields evaluated. Miller and Young (1985) also reported that DON peaked six weeks after infection and declined thereafter until it reached a constant level before harvest. Others have also reported DON peaking in wheat and declining thereafter (Argyris et al., 2003; Teich, 1989). Similar declines in DON levels before harvest has been reported in barley by Prom et al. (1999), and in corn by Miller et al. (1983). In the current study, except in Wheaton, a decline of DON was not generally observed across the growth period.

In this study, except for Wheaton in the treatments receiving longest duration of irrigation, a DON peak followed by a decline was not observed. DON levels in Alsen and 2375 generally peaked at 41 dai (GS 93) or at harvest. However, in the treatment receiving longest durations of irrigation in one year of the study, DON did peaked at 14 dai. In Wheaton DON peaked at either 14 dai (GS 75) or 21 dai (GS 80) mostly in the treatments with longer durations of mist-irrigation. DON peaks at different growth stages with respect to the resistance levels of the wheat sometime before harvesting was also reported by Culler et al. (2007). In their study, the

growth stages at which DON peaked was not different among mist-irrigation duration treatments for either Alsen or 2375, but was at an earlier growth stage for Wheaton, in the treatment with longer duration of misting.

The resistance of wheat to *Fusarium* can affect the development of FHB and toxin accumulation. In the present study, susceptible cultivars tended to have consistently and significantly higher FHB severities, VSK, and mycotoxin accumulation compared to the cultivars examined with some level of resistance. The impact of genetic resistance on FHB development and toxin accumulation has been well studied. Atanasoff (1920) reported differences in susceptibility to FHB in spring wheat cultivars planted in the same field and treated the same agronomically. Other studies have shown both higher FHB severity and DON levels in susceptible cultivars than cultivars with some level of resistance (Mesterházy et al., 2003; Mesterházy et al., 2005; Miller et al., 1985; Schroeder and Christensen, 1963; Wilde and Miedaner, 2006). Snijders and Krechting (1992) studied the level of fungal biomass and DON accumulation in resistant and susceptible cultivars of winter wheat by collecting *F. culmorum* infected head samples 4 and 8 weeks after inoculation. They reported that the levels of mycotoxins were consistently higher in kernels of more susceptible cultivars, a finding similar to the current study. They also reported that the DON levels declined significantly between samplings in the susceptible cultivars. In contrast, the resistant lines in their study had similar or slightly higher levels of DON at 8 weeks compared to 4 weeks after inoculation. Interestingly, the level of fungal biomass measured as ergosterol, increased in both resistant and susceptible lines in between subsequent sampling dates. The same pattern of DON accumulation was observed in the present study. Irrespective of misting duration, DON levels were generally highest at the final sampling (41 dai, GS 93) in the cultivars with some resistance. In the susceptible cultivar Wheaton, DON levels peaked sometime before harvest and declined in one year of the study. However, in the second year DON peaked 21 dai (GS 80), declined and peaked again at 41 dai.

FHB severities and mycotoxin levels were similar for all three cultivars early in the sampling period. However, either after 11 dai (GS 72) or 14 dai (GS 75), both the FHB visual symptoms and toxin levels increased dramatically in the susceptible cultivar. In Alsen and 2375, the levels of DON were more stable and increases over time were modest. This suggests that the cultivars which have some levels of resistance are able to limit the spread of disease in the head (type II resistance as defined by Schroeder and Christensen, 1963), limit the infection of the kernel (Mesterházy, 1995), or resist toxin accumulation either by limiting the production or by metabolizing (Miller et al., 1985). Alsen is considered moderately resistant to FHB and DON

accumulation and documented to have FHB severities and DON levels approximately half that of the 2375 (Frohberg et al., 2006). Alsen is also believed to have type II resistance derived from 'Sumai 3', a Chinese hexaploid wheat cultivar (Hartel et al., 2004; Kolb et al., 2001). In the present study 2375, generally did not differ significantly from Alsen for either FHB severity, VSK or the level of any of the trichothecene tested, although slightly higher FHB severities, VSK and DON were seen in treatments with longer durations of misting. Under shorter durations of misting Alsen tended to have slightly higher FHB severities, VSK, DON and 15-ADON than 2375 and while the levels of 3-ADON and NIV were comparatively higher in 2375. These results agree with those reported by Culler et al. (2007), where they also found slightly higher FHB severities and DON in Alsen than 2375 in treatments receiving shorter durations of misting. Under longer duration of misting the reverse was true. Culler et al. (2007) suggested that high disease pressure may result in similar levels of disease and toxins detected in moderately resistant and moderately susceptible cultivars. The concentration of inoculum used in this experiment was same as the inoculum concentration used in high concentration treatments (1×10^5 macroconidia ml^{-1}) in the experiments conducted by Culler et al. (2007). They indicated that the resistance levels of more resistant cultivars were overcome by the high inoculum pressure. Argyris et al. (2003) also reported being able to overwhelm resistance in a high disease pressure environment. This suggests that the current FHB resistance levels are not sufficient to avoid economic losses from FHB under high disease pressure (Liu and Anderson, 2003).

In the current study, in addition to observing a rapid increase in the trichothecene levels in a susceptible cultivar following inoculation, the decline in trichothecene levels was also most dramatic in the susceptible cultivars under treatments with extended durations of mist-irrigation. That this decline was most readily observed in the susceptible cultivar suggests that higher concentrations of trichothecene may be more amenable to leaching.

In the present study, whole head samples were collected at different time intervals in addition to the grain samples harvested at maturity. The last samplings of whole heads (41 dai) were the day before the harvest of the plots. Thus, the trichothecene levels in whole heads 41 dai can be compared with the levels in grain harvested at maturity. Concentrations of trichothecene were higher in the whole head samples as it included other parts of wheat head in addition to the grain. The results demonstrate that the proportion of DON, 15-ADON and 3-ADON in harvested grain compared to whole heads was lowest in cultivar 2375 of the three cultivars examined. This may suggest that the 2375 has a mechanism to reduce the spread of *Fusarium* to the grain from the other head tissues including the lemma, palea and rachis thus resulting in reduced

trichothecene contamination of grain. Alternatively, there might be a mechanism to prevent the synthesis of or promote the degradation of trichothecene. The ratio of trichothecene in the whole heads to those in harvested grain declined with increasing durations of mist-irrigation. This supports the hypothesis that trichothecene may be leached out by water as it would be expected that leaching would be more effective from glumes and the other external tissues of the head which are thinner. As the grain forms inside the lemma and palea, it is expected that water would first leach trichothecene from these tissues.

The production of mycotoxins, especially DON, by *Fusarium* isolates is considered important to the aggressiveness of an isolate although the production of mycotoxins is not essential for pathogenicity (Desjardins et al., 1996; Dyer et al., 2005; Eudes et al., 2000; Proctor et al., 1995). A delay in the time when DON can first be detected following inoculation (Chen et al., 1996; Evans et al., 2000; Savard et al., 2000; Teich et al., 1989) also supports the hypothesis that DON is not required for initial infection and colonization. The results of the current study agree with these studies, as DON was first detected in whole heads sampled at either 11 or 14 dai.

Isolate variability for pathogenicity and toxin production has been well established in *F. graminearum* (Akinsanmi et al., 2006; Bai and Shaner, 1996; Carter et al., 2002; Gang et al., 1998; Goswami & Kistler, 2005; Tóth et al, 2005; Tu, 1929; Xue et al, 2004). In this study, the five isolates examined differed in their capacity to produce disease symptoms and mycotoxins in field inoculated plants. The isolates differed for each of the disease parameters measured, although isolates did not rank consistently for FHB severity, VSK and DON. Based on FHB severity, VSK, DON, 15-ADON, and 3-ADON assessment in this study, the isolates used in these experiments can be broadly divided into two groups. The first group includes isolate 49-3 and second group includes the remaining four isolates. Generally, isolates inciting higher FHB severities did not generate higher VSK levels. Neither of the isolates which produced the highest FHB severities or VSKs produced the highest level of DON. Isolate 49-3 was associated with the highest levels of DON and other toxins in all treatments despite generating comparatively lower FHB severities and VSK levels than the other isolates examined. Therefore, it is speculated that there must be other factors besides DON, which contribute to isolate aggressiveness. Variation in FHB severity, VSK and mycotoxins accumulation among isolates helps explain the inconsistent and frequently low correlations among these variables observed in nurseries for germplasm evaluation and selection (Malla, 2005).

The results of this study also support the hypothesis that isolates are non-host specific. The isolates obtained from barley host (B45A and B63A) resulted in comparatively higher FHB severities and VSK levels in the three wheat cultivars examined. The observed variation of isolates was more evident in the cultivars with some resistance than in the susceptible cultivar, a finding that agrees with Bai and Shaner (1996). The variation in an isolate for traits observed in the present study was not stable in the three years of the study, especially for FHB severity and VSK. For DON, only isolate 49-3 had a consistent response across treatment combinations and years. While the other four isolates examined generally did not differ statistically from one another for DON production, their rankings changed in different treatment combinations. The variable nature of the five isolates used in the study for the variable examined under different conditions further support the fact that the pathogen is not race specific. Interestingly, isolate 49-3 was the poorest for spore production. The spore concentration obtained when harvesting inoculum of 49-3 was never above 6.6×10^5 spores ml^{-1}. However the other isolates routinely produced inoculum well above 8×10^5 spores ml^{-1}. The spore germination of the five isolates examined were similar ranging between 87-95%. Therefore, isolates spore production capacity cannot be used to predict the aggressiveness of an isolate.

Isolate Butte86Ada-11 is the isolate best characterized in the Small Grain Pathology Laboratory at the University of Minnesota. This isolate was reported to be an aggressive isolate (Evans et al., 2000). However, in the current study, Butte86Ada-11 was the least aggressive isolate in terms of FHB severity, VSK, DON, 15-ADON, 3-ADON and NIV production capacity. This isolate was collected in 1995 from a wheat field in northwestern Minnesota. It is recognized that *F. graminearum* isolates can lose their ability to sporulate if maintained in culture by mycelial transfer and similarly they may lose their ability to produce mycelia if maintained by single-spore transfer (Dill-Macky, 2003). Further, mutation of *F. graminearum* isolates in culture is common resulting in changes in aggressiveness and mycotoxin production capacity (Tu, 1930). It is therefore possible that the aggressiveness of Butte86Ada-11 has lessened in the 14 years since its collection. For this reason, it is recommended to evaluate isolates in collections frequently for aggressiveness and toxin production capacity *in planta*. Further, it is suggested to regularly obtain isolates of *F. graminearum* from naturally infected wheat fields and to incorporate these new isolates into inoculum mixtures used for screening purposes. This ensures that the populations of *F. graminearum* prevalent in the field are represented in inocula used for resistance screening.

In the present study, all isolates produced 15-ADON, 3-ADON and NIV in addition to DON. However, the concentrations of 15-ADON, 3-ADON and NIV were not above 7.1%, 2.8% and 2.1%, respectively, of the DON levels. Thus, the isolates used may be considered primarily DON producers. High amounts of DON relative to 15-ADON and 3-ADON have been reported elsewhere (USWBSI, 2007; Tanaka et al., 1988). Ichinoe et al. (1983) divided *F. graminearum* population to two groups; one that produces NIV and one that produces DON and its derivatives. The DON producing chemotype was further divided into 3-ADON and 15-ADON chemotypes based on the acetylated DON produced by *Fusarium* (Miller et al., 1991). Recently the classification of *F. graminearum* populations has been extended to the molecular basis (Lee et al., 2002), with groups classified based on the presence or absence of certain genes in trichothecene gene cluster (Desjardins, 2006; Gale et al., 2007; Ichinoe et al, 1983; Jennings et al., 2004). Isolates belonging to DON chemotype were assumed not to produce NIV and isolates belonging to NIV chemotype were assumed not to produce DON (Ichinoe et al., 1983; Marasas et al., 1984; Yang et al., 2008). Often acetylated versions of DON are reported to be present only when the sample has a sufficiently high level of DON (Abbas et al., 1988). It is recognized that these different genotypes can inhabit the same host tissue producing all respective mycotoxins (Gale et al., 2007; Jennings et al., 2004). The NIV chemotypes have recently been identified as being common as the DON chemotype in the North American region (Gale et al., 2007). The *F. graminearum* which produce DON are reported as being twice as aggressive as NIV producers (Cumagun et al., 2004). If this is true, DON producing isolates may infect and colonize wheat heads faster, and in turn may produce higher amount of DON than less aggressive NIV producing isolates. It should be noted that NIV is more biologically active, being up to 10 times more toxic to animals compared to DON (Mirocha et al., 1985). The presence of NIV and DON in individual samples infected with *Fusarium* was reported by Tanaka et al., (1988). However, they did not reported whether NIV and DON were produced by single isolates or different isolates infecting same head. In the current study, individual isolates produced DON, 15-ADON, 3-ADON, and NIV in plants. Based on the observed levels of DON, 15-ADON, 3-ADON, and NIV, all five isolates used in this experiment can be classified as belonging to the DON producing 15-ADON chemotype. This supports the finding by Mirocha et al., (1989) that isolates from the USA, specifically Minnesota, in addition to DON, produce primarily 15-ADON rather than 3-ADON. As reported by Mirocha et al. (1989) isolate R5245 produced both 3-ADON and 15-ADON in addition to DON. Ward et al., (2002) later classified R5245, the same isolate used by Mirocha et al. (1989), as a 3-ADON chemotype. Isolates belonging to the NIV chemotype, as reported by

Ward et al (2002), can produce DON, 3-ADON and 15-ADON. Similarly the reverse is true with DON. Therefore, chemotype designation of a *F. graminearum* isolate as defined by Ward does not define the trichothecene produced by an isolate either in rice culture or *in planta*. Genotyping of cultures can offer clues as to the relative amounts of trichothecene likely to be produced by the isolate but not the actual amounts produced. Despite its comparatively low toxicity, DON is the most important among trichothecene produced by *Fusarium*, as it is produced at high levels irrespective of 3-ADON or 15-ADON levels. Further the production of DON appears more stable than compared to either 3-ADON or 15-ADON, thus DON levels provide a more precise measure of mycotoxin contamination. Therefore, measures of DON, which are widely used in wheat breeding programs to select for resistance to *Fusarium*, appear well justified.

The high cost of mycotoxin testing and the presence of correlation between FHB severity and DON levels allow researchers to utilize correlations between visual FHB severity assessments in the field and DON concentrations to predict the DON levels in harvested grain (Arsenuik et al., 1999; Groth et al., 1999; Jones and Mirocha, 1999). The correlation between FHB severity and DON was high in the current study. However, the correlation declined in the treatments with increased duration of misting. Thus if supplemental moisture, in the form of mist-irrigation, is utilized to facilitate disease development in breeding nurseries, it is expected that the moisture will impact the level of DON in grain depending upon the resistance levels of host plant. As DON levels decline most rapidly in susceptible cultivars, breeders should be cautious when utilizing these correlations particularly in locations where significant rainfall events occur following disease assessment. The correlation of VSK with DON may provide a more reliable estimation of DON as the correlation between these variables was higher than the correlation between FHB severity and DON. However, the correlation of VSK with DON also declined in treatments with increased duration of moisture in the later stages of grain fill. This suggests the need for caution in interpreting results from misted nurseries and high rainfall sites. Since the correlations of DON with 15-ADON and 3-ADON are high and the amount of acetylated DON derivatives is very low, selection for low DON alone will result in the selection for genotypes with low contamination with acetylated DON derivatives. Thus the extra expense to select and test for low 15-ADON and 3-ADON appears not to be justified.

Models have been developed in North America to forecast both FHB development and DON accumulation in infected grains. The first model, DONcast, was developed in Canada, primarily for forecasting DON (Hooker et al., 2002a; Hooker et al., 2002b) and has been commercialized for wheat. DONcast was developed utilizing environmental parameters measured

from seven days pre- to 10 days post-anthesis. Specifically, the model considers temperature and rainfall parameters before heading and rainfall data after heading (Hooker et al., 2002a). The model has been validated and can explain up to 73% of the variation in DON levels in harvested grain. A model to predict the risk of FHB epidemics has been developed in the US with cooperation of several universities in the Midwest region (De Wolf et al., 2003). The model relies primarily on temperature, relative humidity and rainfall data collected in the seven days prior to anthesis and the 10 days period beginning at anthesis. The model has been widely deployed and validated and demonstrates 75-80% accuracy in predicting the development of FHB epidemics (Prandini et al., 2009). This model does not, however, predict DON. Both models utilize the moisture information between seven days pre-anthesis to 10 days post-anthesis. The result of this study suggest that the accumulation of DON to be impacted by the rainfall at any time after anthesis, therefore, the inclusion of moisture parameters beyond 10 days post-anthesis will likely increase the accuracy of the current models used to predict trichothecene toxins in wheat.

Table 3.1. Spearman's rank correlations of Fusarium head blight (FHB) severity, visually scabby kernels (VSK), deoxynivalenol (DON), and 15-acetyldeoxynivalenol (15-ADON) of mature grain harvested in 2006.

	VSK	DON	15-ADON
	14 DAI		
Severity	0.76**	0.69**	0.20
VSK		0.85**	0.22
DON			0.29*
	21 DAI		
Severity	0.65**	0.75**	-
VSK		0.78**	-
DON			-
	28 DAI		
Severity	0.84**	0.81**	0.34**
VSK		0.79**	0.37**
DON			0.45**
	35 DAI		
Severity	0.73**	0.63**	-
VSK		0.64**	-
DON			-

** Significant at $P < 0.01$

* Significant at $P < 0.05$

Table 3.2. Spearman's rank correlations of Fusarium head blight (FHB) severity, visually scabby kernels (VSK), deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) and nivalenol (NIV) of mature grain harvested in 2007-2008.

			2007		
	VSK	DON	15-ADON	3-ADON	NIV
	14 DAI				
Severity	0.71**	0.65**	0.64**	0.56**	0.50**
VSK		0.81**	0.79**	0.65**	0.47**
DON			0.97**	0.75**	0.66**
15-ADON				0.71**	0.63**
3-ADON					0.58**
	21 DAI				
Severity	0.75**	0.77**	0.80**	0.53**	0.61**
VSK		0.78**	0.75**	0.59**	0.55**
DON			0.92**	0.76**	0.71**
15-ADON				0.68**	0.68**
3-ADON					0.63**
	28 DAI				
Severity	0.76**	0.57**	0.66**	0.49**	0.32**
VSK		0.74**	0.72**	0.60**	0.55**
DON			0.87**	0.71**	0.68**
15-ADON				0.67**	0.53**
3-ADON					0.52**
	35 DAI				
Severity	0.82**	0.55**	0.46**	0.38**	0.40**
VSK		0.53**	0.35**	0.29*	0.29*
DON			0.80**	0.54**	0.70**
15-ADON				0.42**	0.64**
3-ADON					0.39**
			2008		
	14 DAI				
Severity	0.70**	0.68**	0.72**	0.65**	0.61**
VSK		0.74**	0.79**	0.60**	0.63**
DON			0.92**	0.76**	0.76**
15-ADON				0.75**	0.73**
3-ADON					0.67**
	21 DAI				
Severity	0.71**	0.66**	0.73**	0.53**	0.58**
VSK		0.82**	0.80**	0.51**	0.69**
DON			0.88**	0.64**	0.85**
15-ADON				0.62**	0.75**
3-ADON					0.65**
	28 DAI				
Severity	0.76**	0.69**	0.72**	0.52**	0.65**
VSK		0.72**	0.80**	0.27*	0.70**
DON			0.87**	0.65**	0.93**
15-ADON				0.40**	0.79**
3-ADON					0.61**
	35 DAI				
Severity	0.64**	0.35**	0.57**	0.21	0.39**
VSK		0.43**	0.52**	0.06	0.43**
DON			0.85**	0.54**	0.87**
15-ADON				0.35**	0.77**
3-ADON					0.45**

** Significant at $P < 0.01$

* Significant at $P < 0.05$

Table 3.3. Spearman's rank correlation of Fusarium head blight (FHB) severity, deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) and nivalenol (NIV) in whole head samples harvested 7, 11, 14 and 21 dai in 2007-2008.

	DON	15-ADON	3-ADON	NIV	DON	15-ADON	3-ADON	NIV
			2007				2008	
	7 dai							
Severity	-0.14^{NS}	-0.03^{NS}	-	-	0.36**	0.46**	-	-
DON		0.25*	-	-		0.38**	-	-
15-ADON			-	-			-	-
3-ADON				-			-	-
	11 dai							
Severity	0.83**	0.76**	0.74**	0.30**	0.80**	0.68**	0.68**	0.17^{NS}
DON		0.95**	0.91**	0.38**		0.83**	0.78**	0.03^{NS}
15-ADON			0.87**	0.37**			0.50**	-0.02^{NS}
3-ADON				0.37**				0.21*
	14 dai							
Severity	0.74**	0.78**	0.81**	0.61**	0.86**	0.77**	0.78**	-0.01^{NS}
DON		0.92**	0.88**	0.74**		0.88**	0.92**	-0.03^{NS}
15-ADON			0.86**	0.73**			0.72**	-0.03^{NS}
3-ADON				0.72**				-0.03^{NS}
	21 dai							
Severity	0.85**	0.49**	0.84**	0.43**	0.72**	0.68**	0.70**	0.39**
DON		0.59**	0.98**	0.53**		0.93**	0.89**	0.50**
15-ADON			0.60**	0.61**			0.80**	0.47**
3-ADON				0.54**				0.48**

** Significant at $P < 0.01$
* Significant at $P < 0.05$
^{NS} Non-significant

Table 3.4 Spearman's rank correlation of deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) and nivalenol (NIV) in whole head samples harvested 41 dai and harvested mature grain subjected to four mist-irrigation duration treatments (14, 21, 28 and 35 DAI) in 2007-2008.

	Whole head samples							
	DON	15-ADON	3-ADON	NIV	DON	15-ADON	3-ADON	NIV
Mature harvested grain			2007				2008	
	14 DAI							
DON	0.90**	0.89**	0.70**	0.61**	0.76**	0.79**	0.58**	0.52**
15-ADON	0.89**	0.89**	0.70**	0.63**	0.73**	0.76**	0.54**	0.50**
3-ADON	0.90**	0.89**	0.71**	0.58**	0.72**	0.71**	0.57**	0.52**
NIV	0.79**	0.78**	0.65**	0.59**	0.58**	0.56**	0.53**	0.44**
	21 DAI							
DON	0.91**	0.91**	0.76**	0.65**	0.87**	0.84**	0.68**	0.74**
15-ADON	0.87**	0.85**	0.72**	0.66**	0.86**	0.89**	0.64**	0.73**
3-ADON	0.87**	0.90**	0.72**	0.59**	0.84**	0.82**	0.76**	0.71**
NIV	0.68**	0.69**	0.54**	0.58**	0.70**	0.61**	0.51**	0.61**
	28 DAI							
DON	0.90**	0.84**	0.68**	0.76**	0.87**	0.84**	0.64**	0.82**
15-ADON	0.85**	0.82**	0.64**	0.73**	0.72**	0.81**	0.43**	0.65**
3-ADON	0.78**	0.74**	0.59**	0.71**	0.79**	0.75**	0.76**	0.76**
NIV	0.07^{NS}	0.06^{NS}	0.09^{NS}	-0.05^{NS}	0.53**	0.54**	0.37**	0.54**
	35 DAI							
DON	0.90**	0.85**	0.48**	0.66**	0.76**	0.80**	0.48**	0.67**
15-ADON	0.78**	0.75**	0.50**	0.63**	0.75**	0.80**	0.40**	0.64**
3-ADON	0.50**	0.41**	0.36**	0.31**	0.61**	0.63**	0.54**	0.58**
NIV	0.11^{NS}	0.15^{NS}	-0.06^{NS}	0.15^{NS}	0.19^{NS}	0.21^{NS}	0.10^{NS}	0.10^{NS}

** Significant at $P < 0.01$
* Significant at $P < 0.05$
^{NS} Non-significant

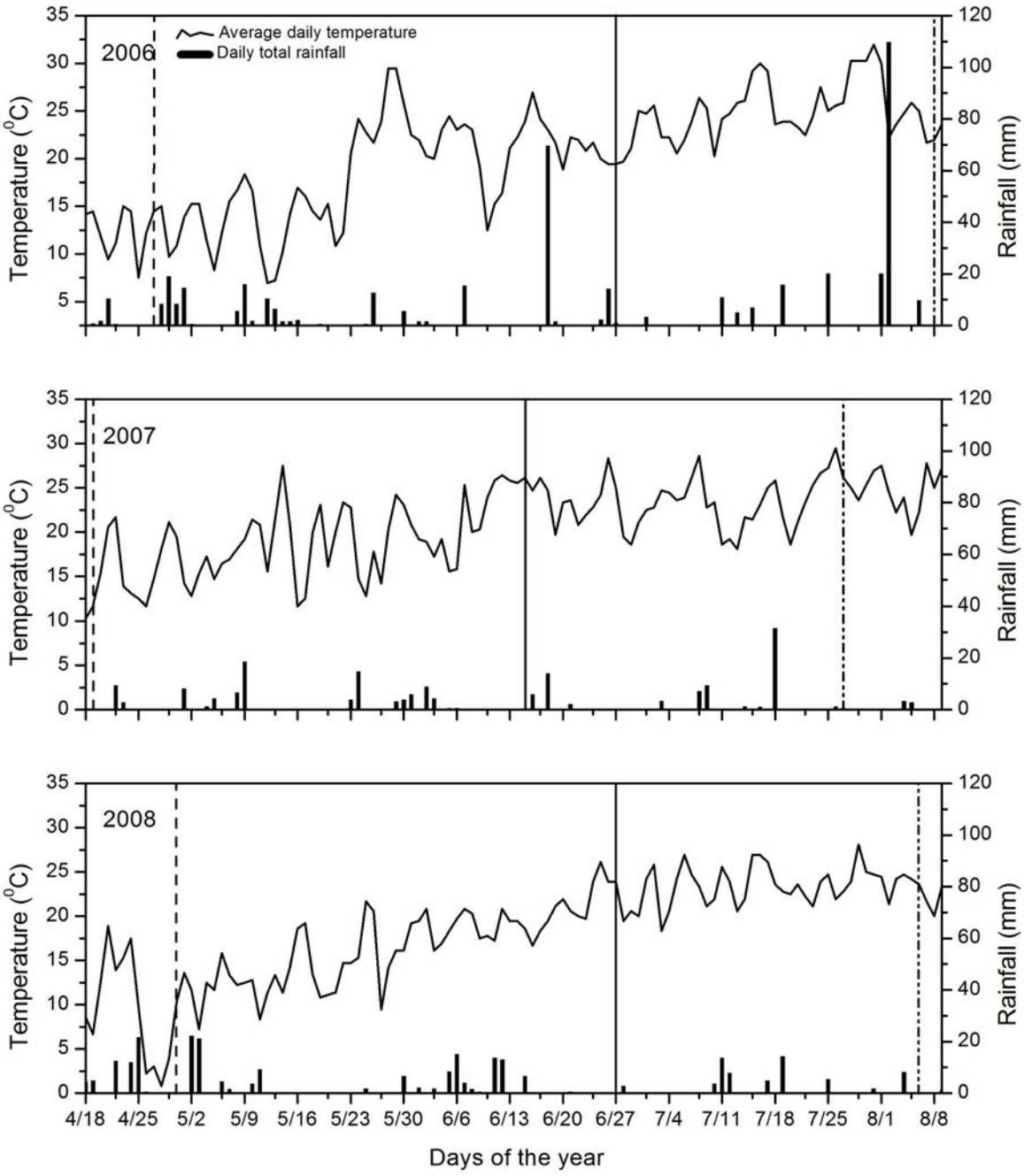

Figure 3.1. Average daily temperature and daily rainfall in 2006-2008. Vertical lines represents the day of planting (– – –), inoculation (——), and harvest of mature grain (– · · –).

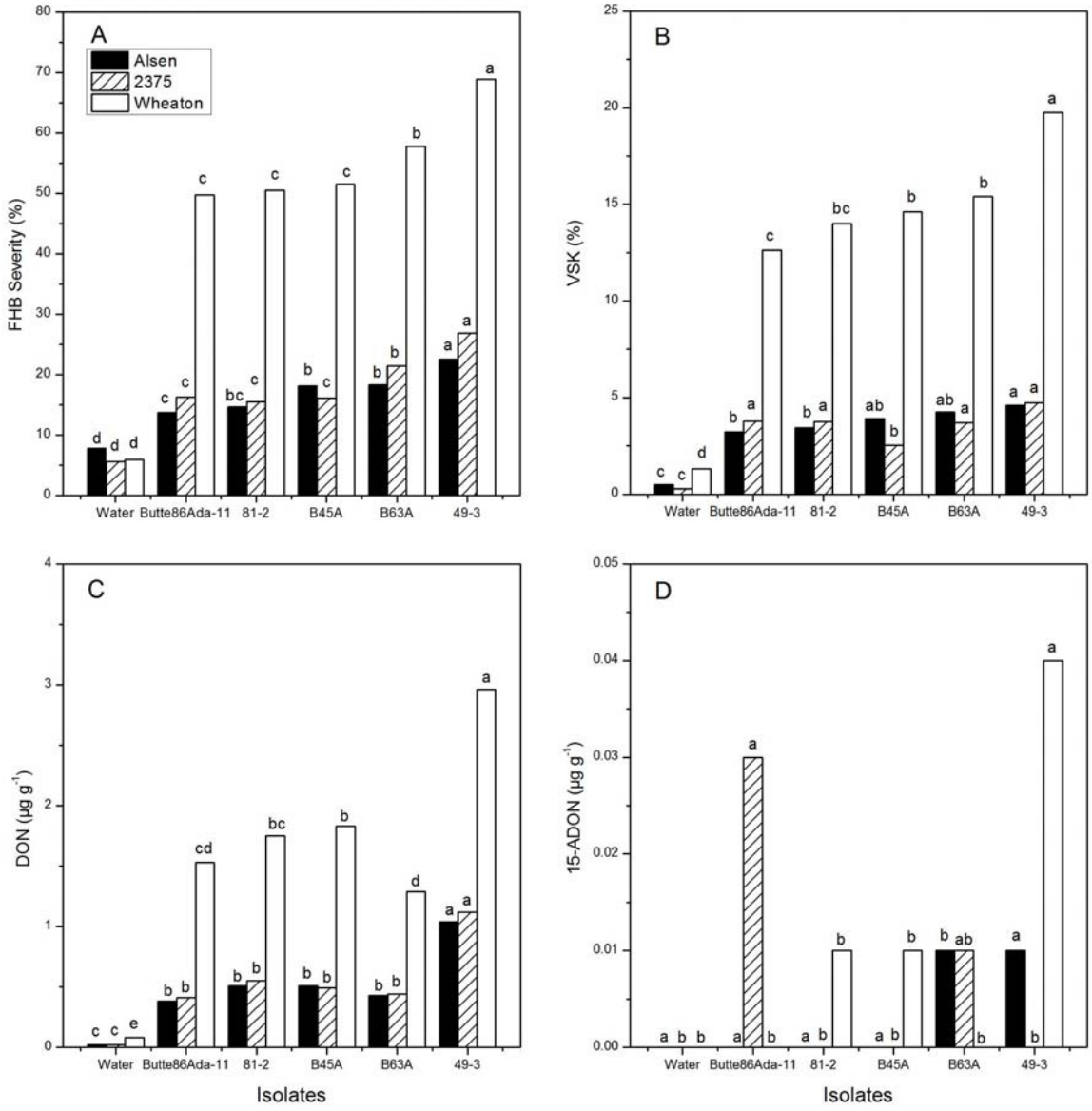

Figure 3.2. Fusarium head blight (FHB) severity (%) (A), percentage visually scabby kernels (VSK) (B), deoxynivalenol (DON, µg g^{-1}) (C), and 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) (D) in grain harvested at maturity for three wheat cultivars (Alsen, 2375 and Wheaton) inoculated with five isolates of *F. graminearum* and a non-inoculated water control in 2006. Isolates with same lowercase letter within each cultivar are not significantly different ($P < 0.05$).

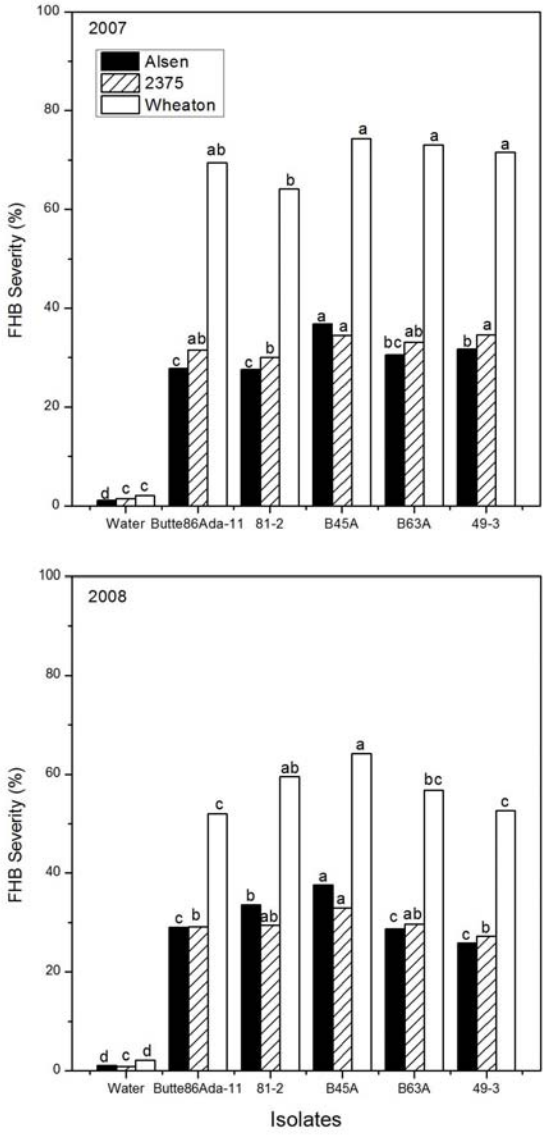

Figure 3.3. Fusarium head blight (FHB) severities (%) in grain harvested at maturity for three wheat cultivars (Alsen, 2375 and Wheaton) inoculated with five isolates of *F. graminearum* and a non-inoculated water control in 2007 and 2008. Isolates with same lowercase letter within each cultivar are not significantly different ($P < 0.05$).

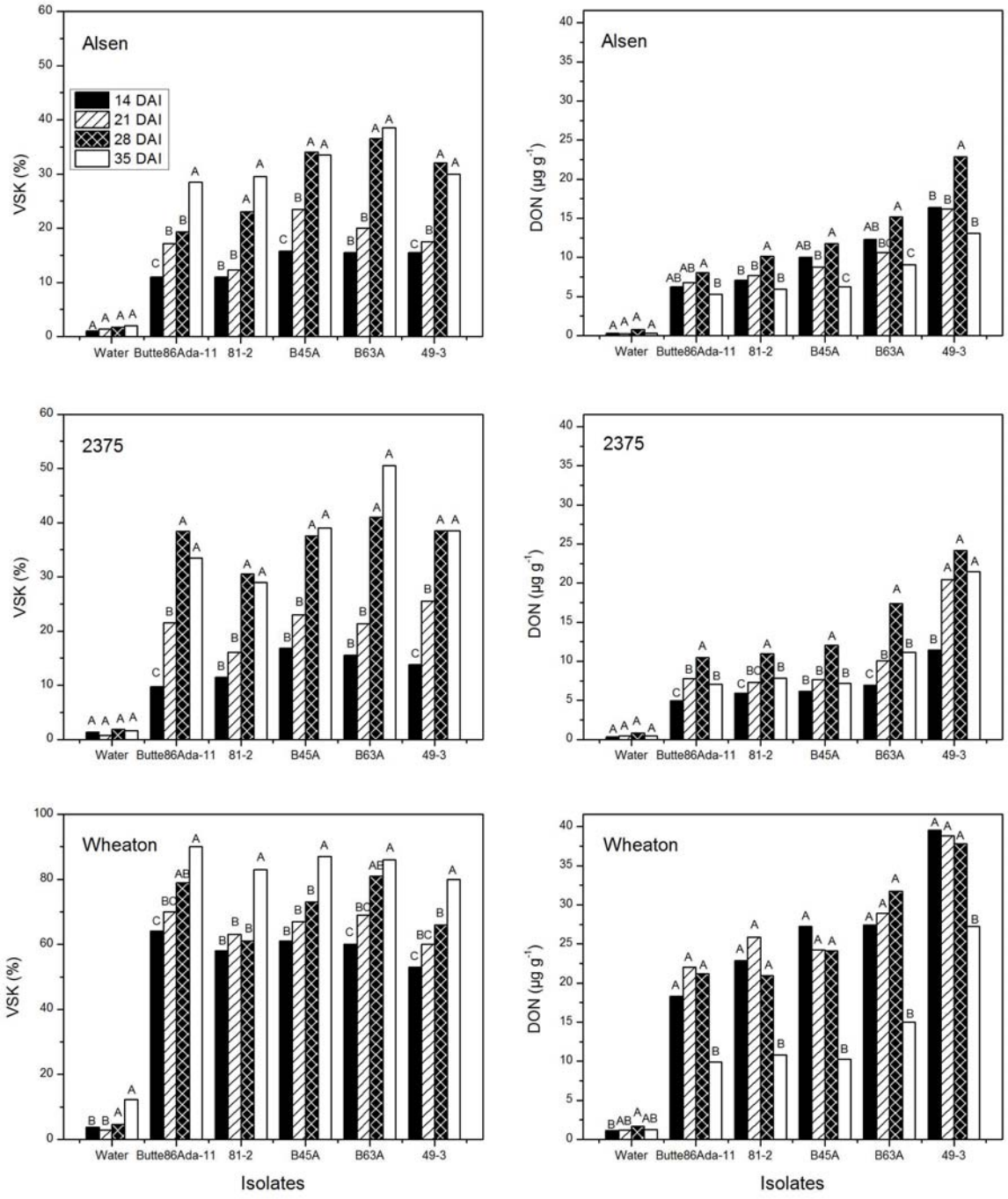

Figure 3.4. Percentage visually scabby kernels (VSK) and deoxynivalenol (DON, µg g^{-1}) in grain harvested at maturity for three wheat cultivars (Alsen, 2375 and Wheaton) subjected to four mist irrigation duration treatments (14, 21, 28, 35 DAI) in 2007. VSK and DON levels for mist-irrigation duration treatments with same uppercase letter within each isolate and cultivar are not significantly different ($P < 0.05$).

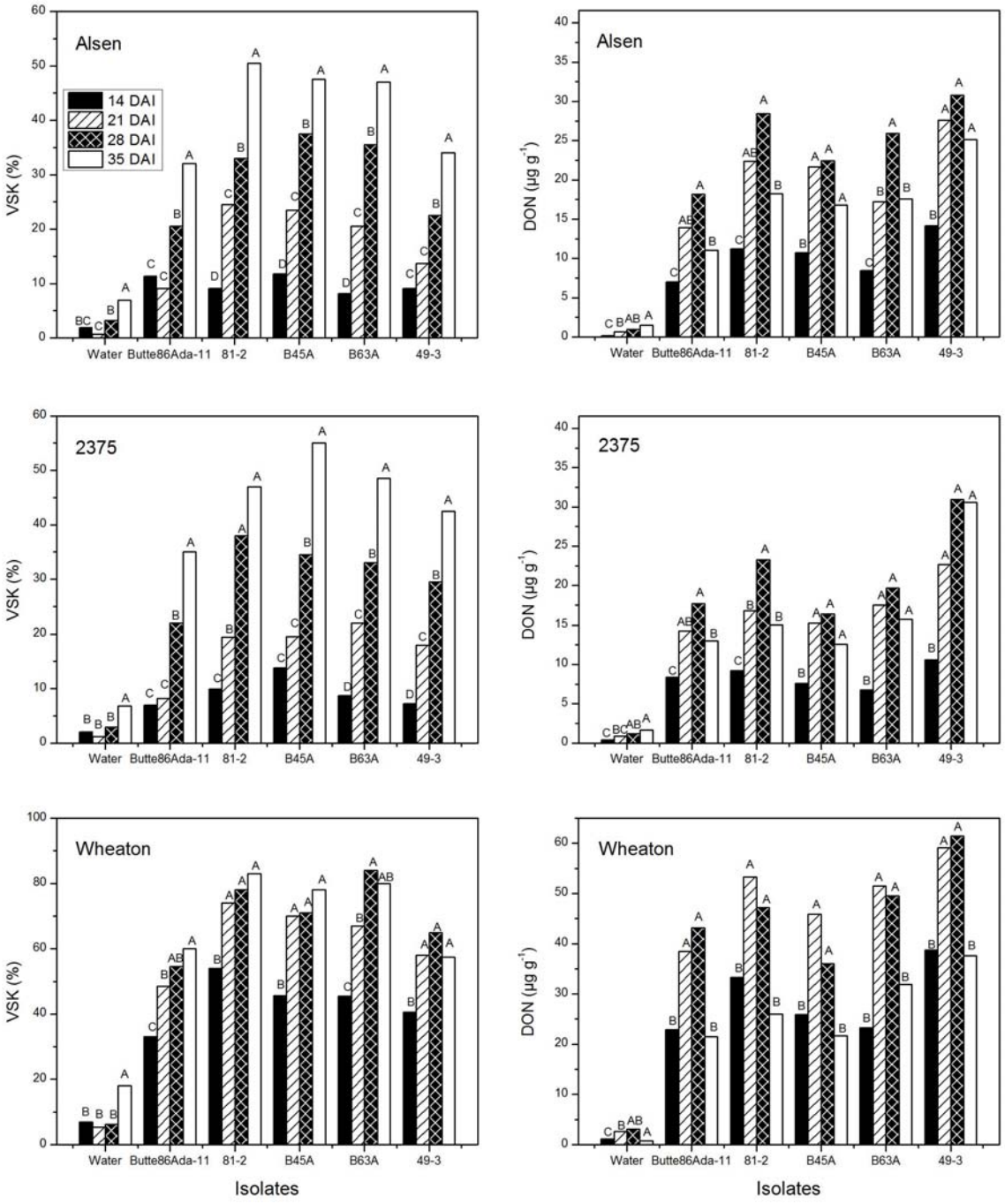

Figure 3.5. Percentage visually scabby kernels (VSK) and deoxynivalenol (DON, µg g^{-1}) in grain harvested at maturity for three wheat cultivars (Alsen, 2375 and Wheaton) subjected to four mist irrigation duration treatments (14, 21, 28, 35 DAI) in 2008. VSK and DON levels for mist-irrigation duration treatments with same uppercase letter within each isolate and cultivar are not significantly different ($P < 0.05$).

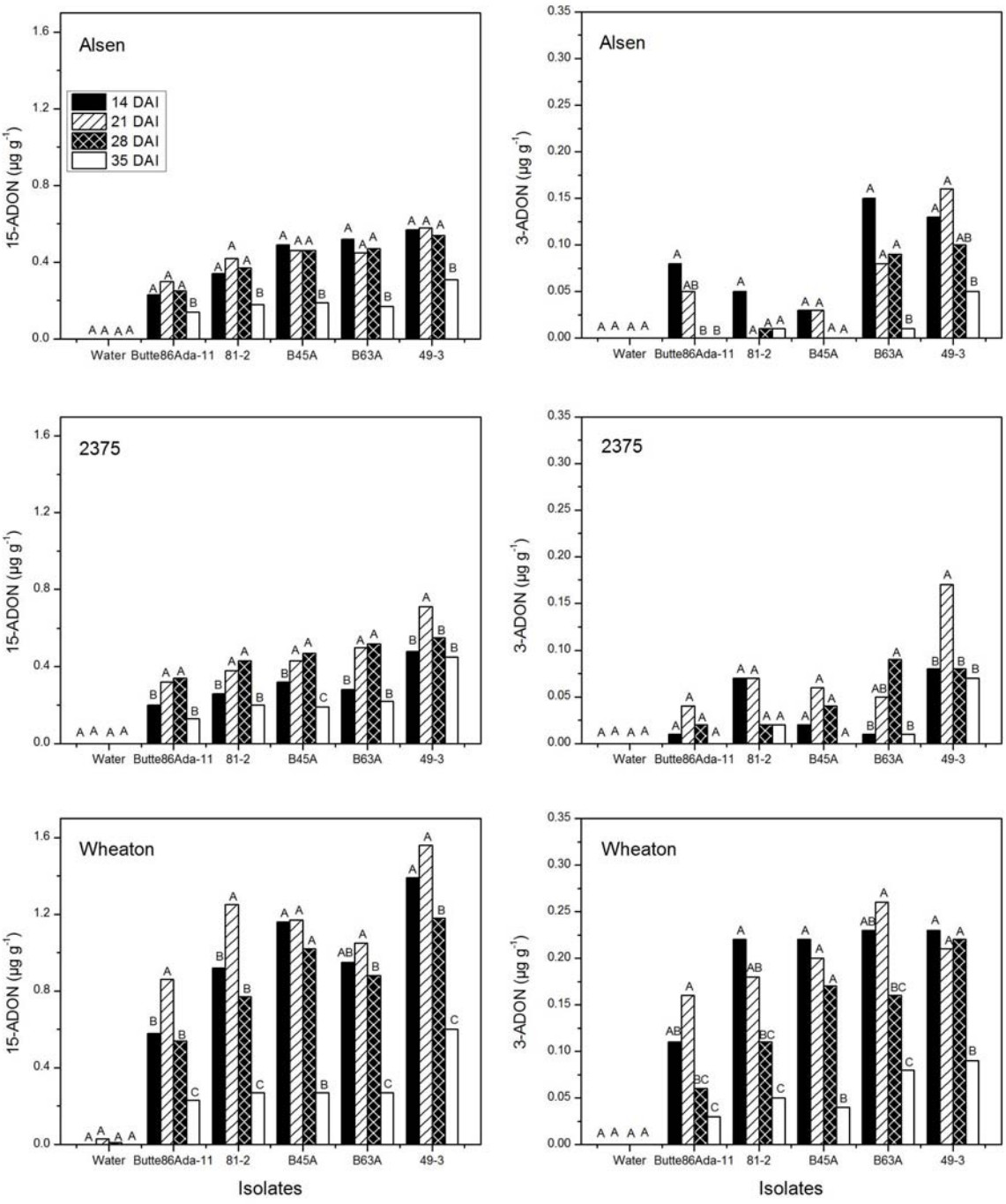

Figure 3.6. 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) and 3-acetyldeoxynivalenol (3-ADON, µg g^{-1}) in grain harvested at maturity of three wheat cultivars (Alsen, 2375 and Wheaton) subjected to four mist irrigation duration treatments (14, 21, 28, 35 DAI) in 2007. 15-ADON and 3-ADON levels for mist-irrigation duration treatments with same uppercase letter within each isolate and cultivar are not significantly different ($P < 0.05$).

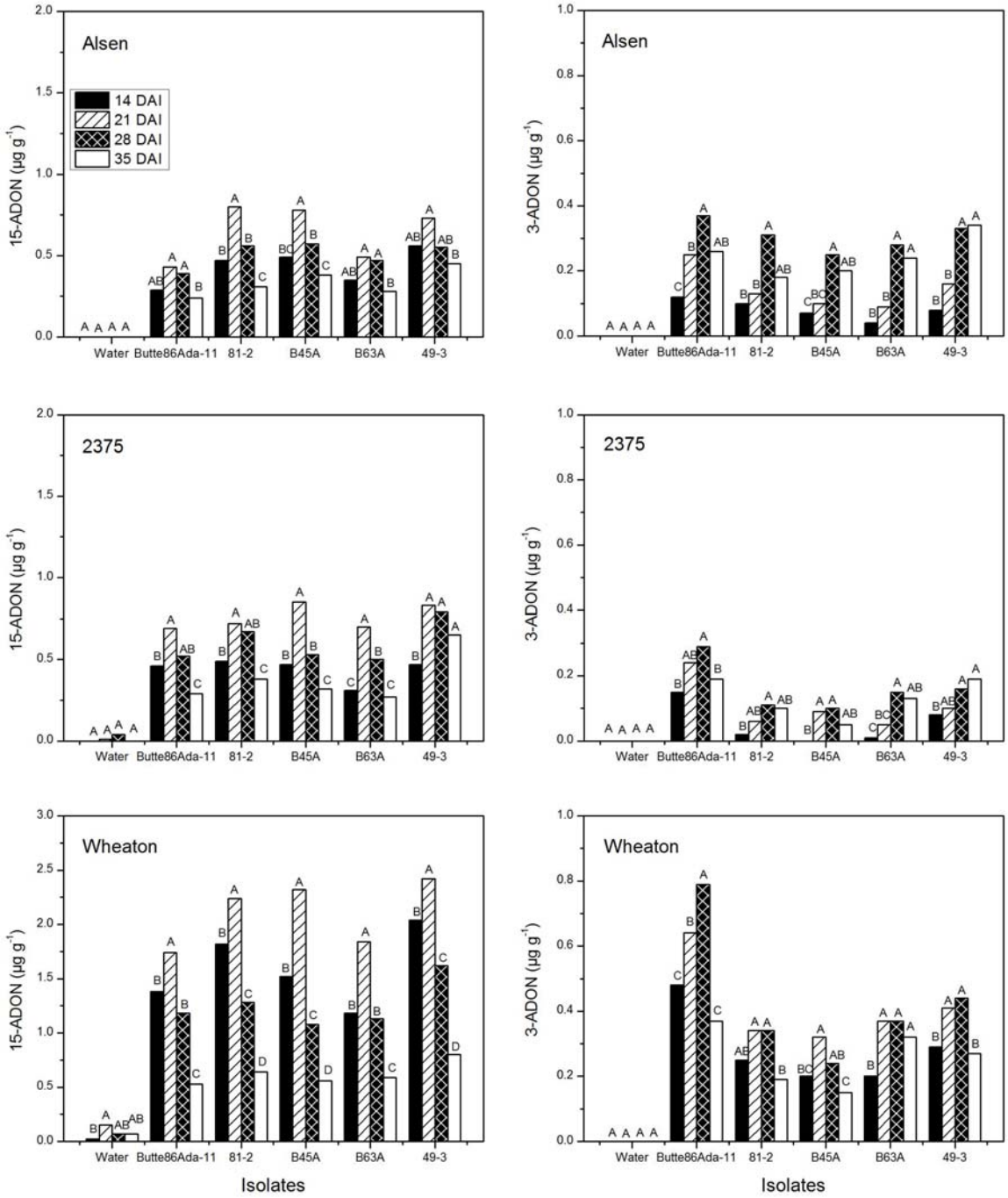

Figure 3.7. 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) and 3-acetyldeoxynivalenol (3-ADON, µg g^{-1}) in grain harvested at maturity from three wheat cultivars (Alsen, 2375 and Wheaton) subjected to four mist irrigation duration treatments (14, 21, 28, 35 DAI) in 2008. 15-ADON and 3-ADON levels for mist-irrigation duration treatments with same uppercase letter within each isolate and cultivar are not significantly different ($P < 0.05$).

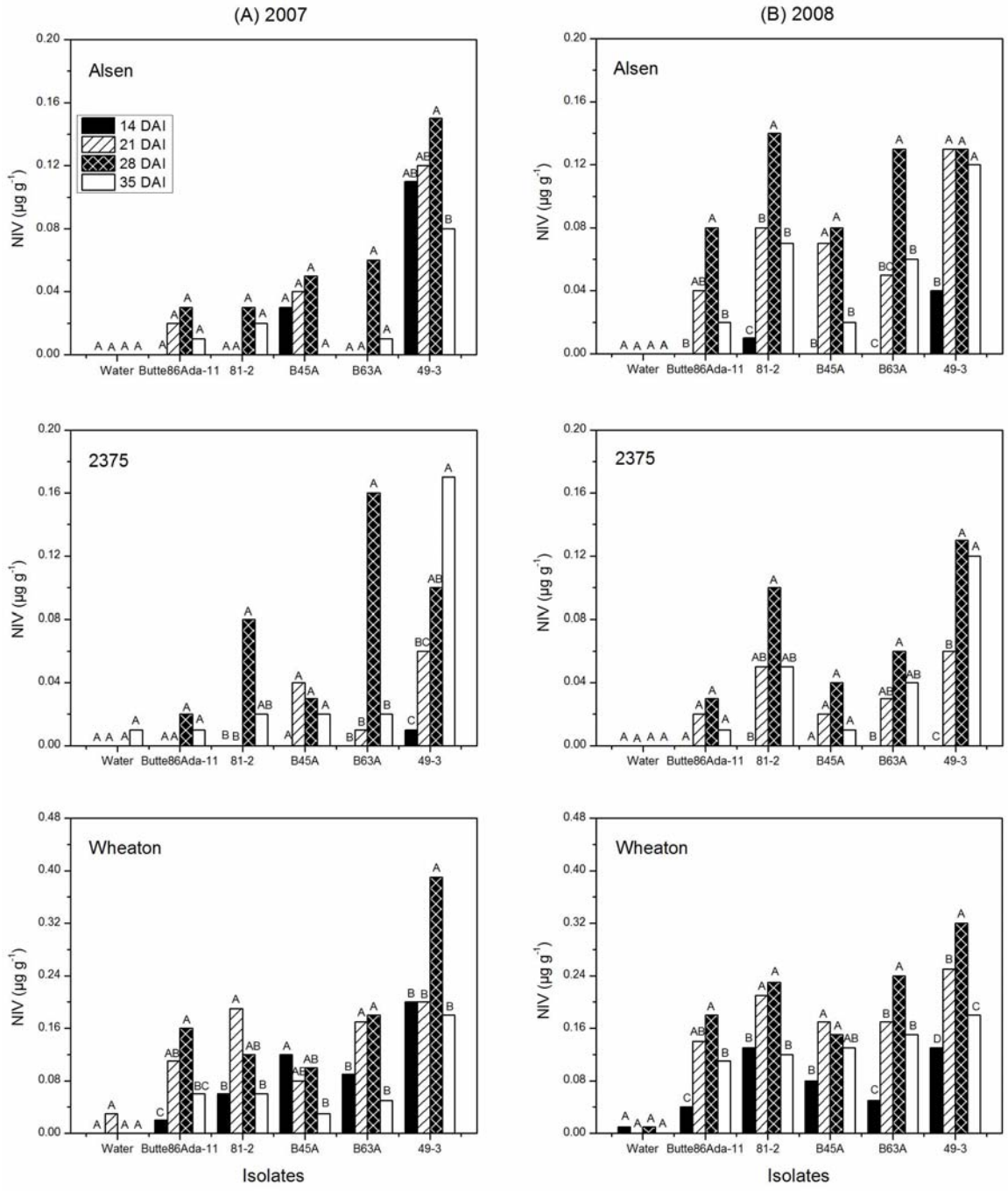

Figure 3.8. Nivalenol (NIV) concentrations (µg g^{-1}) in grain harvested at maturity for three wheat cultivars (Alsen, 2375 and Wheaton) subjected to four mist irrigation duration treatments (14, 21, 28, 35 DAI) in 2007 (Panel A) and 2008 (Panel B). Nivalenol levels for mist-irrigation duration treatments with same uppercase letter within each isolate and cultivar are not significantly different ($P < 0.05$).

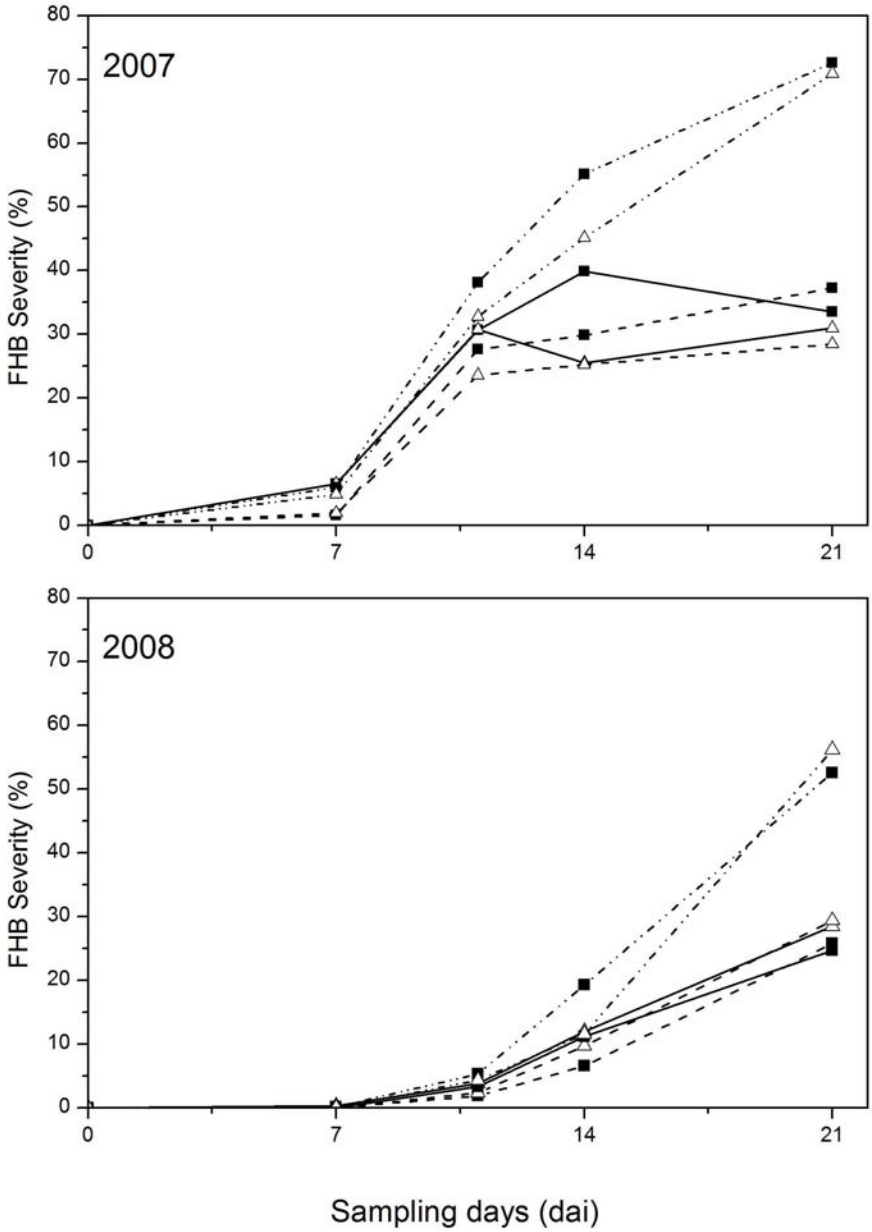

Figure 3.9. FHB severity (%) in whole head samples of Alsen (——), 2375 (− − −) and Wheaton (− · · −) inoculated with *F. graminearum* isolates 49-3 (■) and Butte86Ada-11 (Δ) subjected to mist-irrigation duration treatments 21 DAI in 2007 and 28 DAI in 2008 sampled 0, 7, 11, 14, and 21 dai in 2007 and 2008.

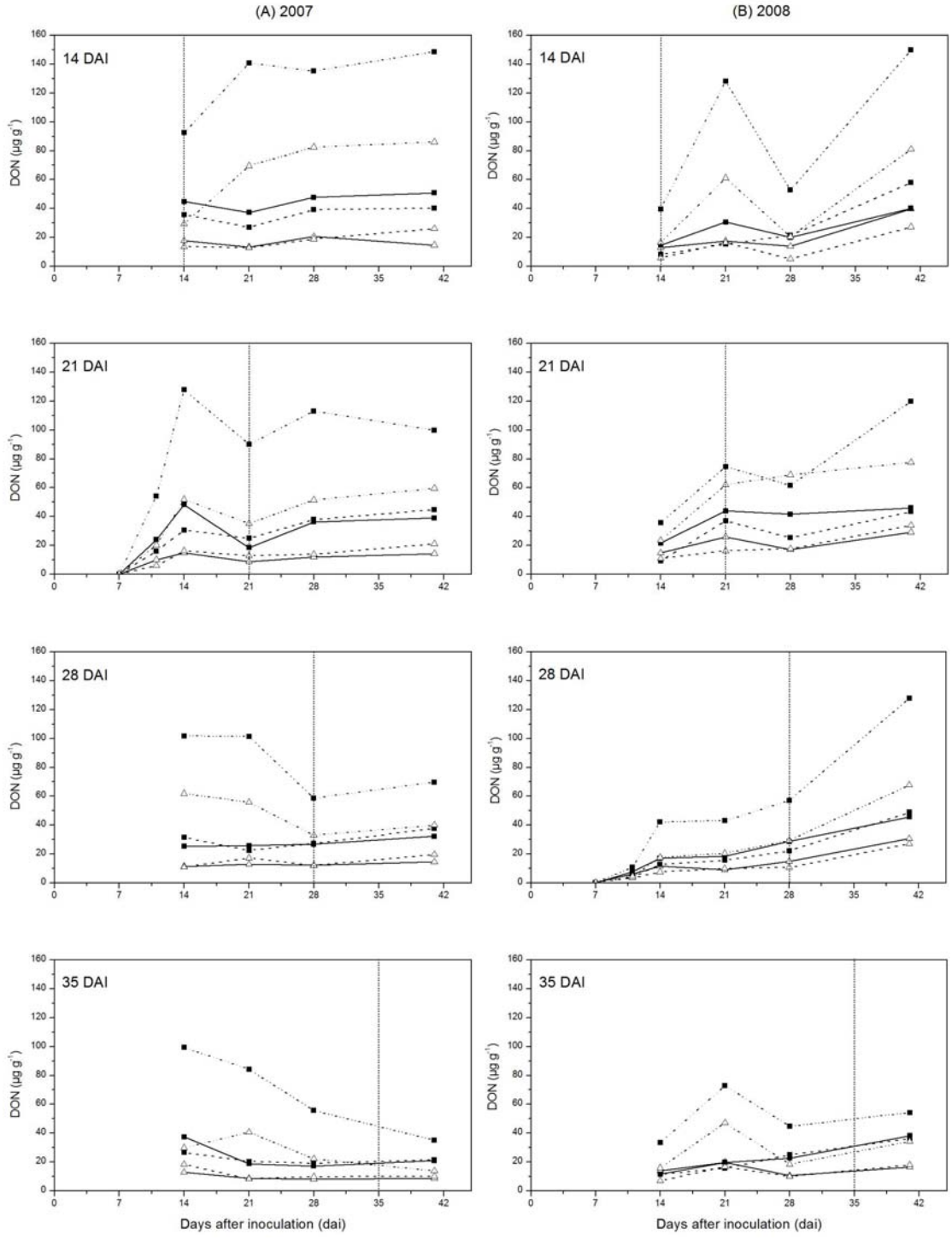

Figure 3.10. Deoxynivalenol (DON, µg g^{-1}) in whole head samples of Alsen (——), 2375 (– – –) and Wheaton (– · · –) inoculated with *F. graminearum* isolates 49-3 (■) and Butte86Ada-11 (Δ) subjected to four mist-irrigation duration (14, 21, 28 and 35 DAI) treatments sampled 7, 11, 14, 21, 28 and 41 dai in 2007 (Panel A) and in 2008 (Panel B). Area to the left of the vertical dashed line indicates the duration of mist-irrigation.

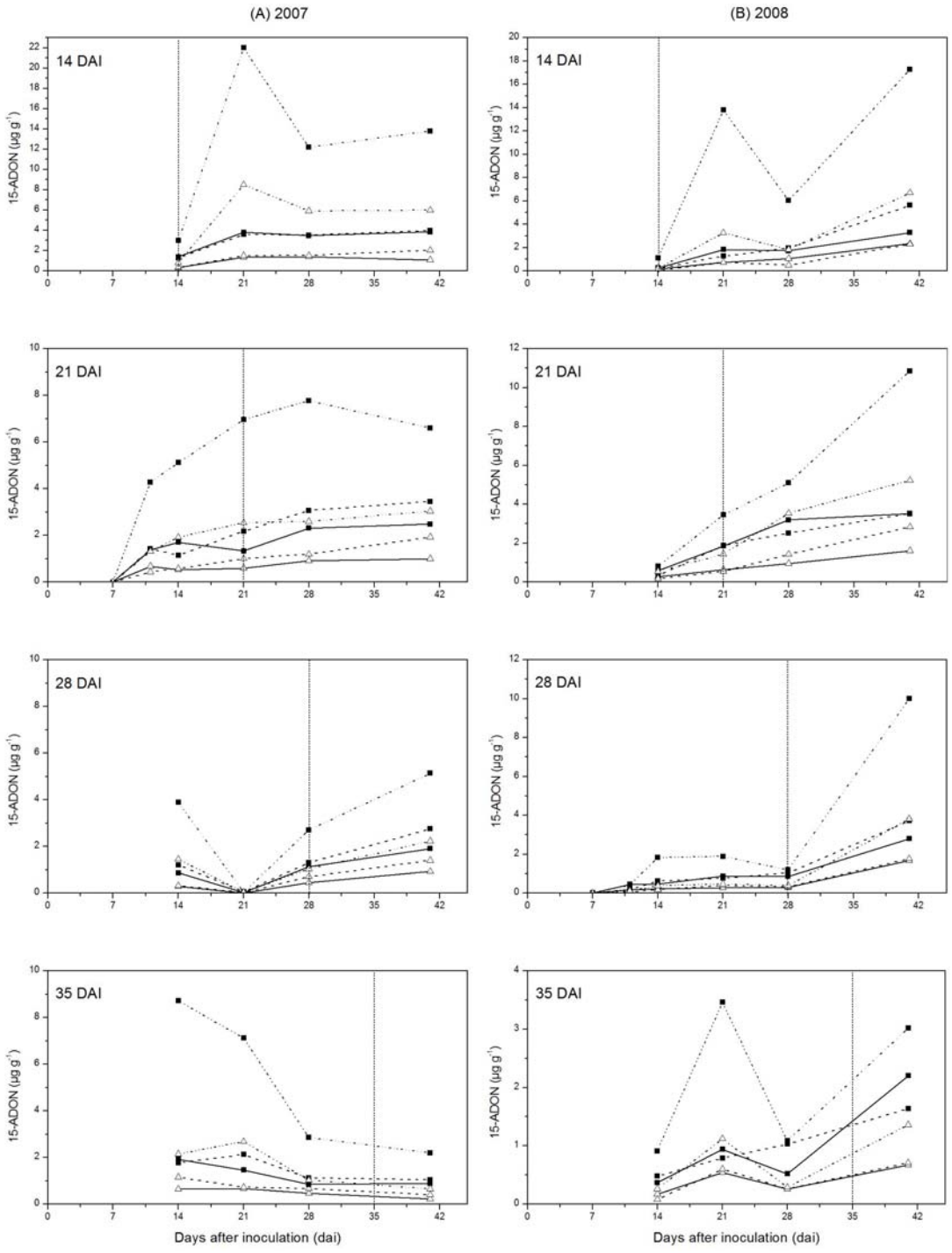

Figure 3.11. 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) in whole head samples of Alsen (——), 2375 (– – –) and Wheaton (– · · –) inoculated with *F. graminearum* isolates 49-3 (■) and Butte86Ada-11 (Δ) subjected to four mist-irrigation duration (14, 21, 28 and 35 DAI) treatments sampled 7, 11, 14, 21, 28 and 41 dai in 2007 (Panel A) and in 2008 (Panel B). Area to the left of the vertical dashed line indicates the duration of mist-irrigation.

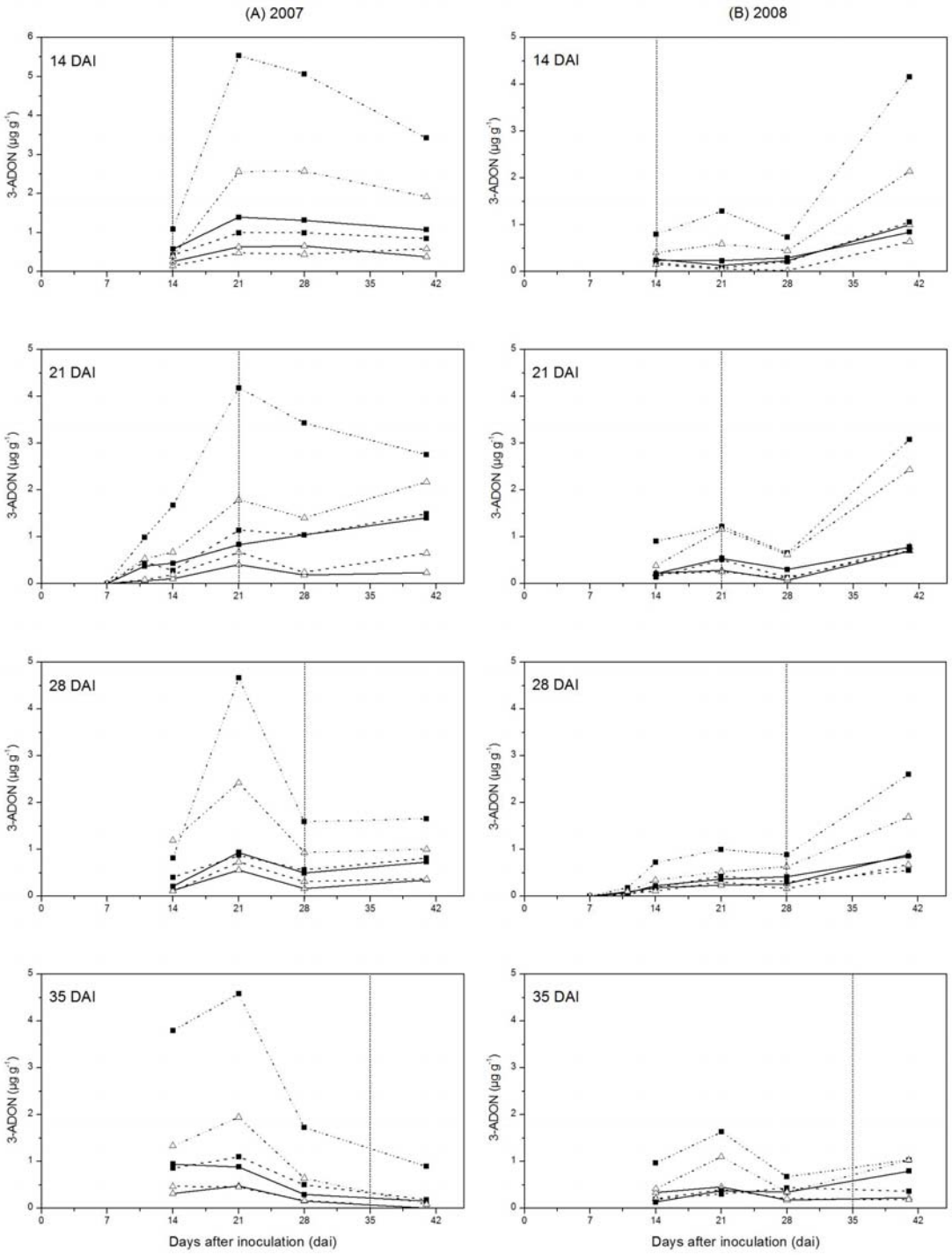

Figure 3.12. 3-acetyldeoxynivalenol (3-ADON, µg g^{-1}) in whole head samples of Alsen (——), 2375 (– – –) and Wheaton (– · · –) inoculated with *F. graminearum* isolates 49-3 (■) and Butte86Ada-11 (Δ) subjected to four mist-irrigation duration (14, 21, 28 and 35 DAI) treatments sampled 7, 11, 14, 21, 28 and 41 dai in 2007 (Panel A) and in 2008 (Panel B). Area to the left of the vertical dashed line indicates the duration of mist-irrigation.

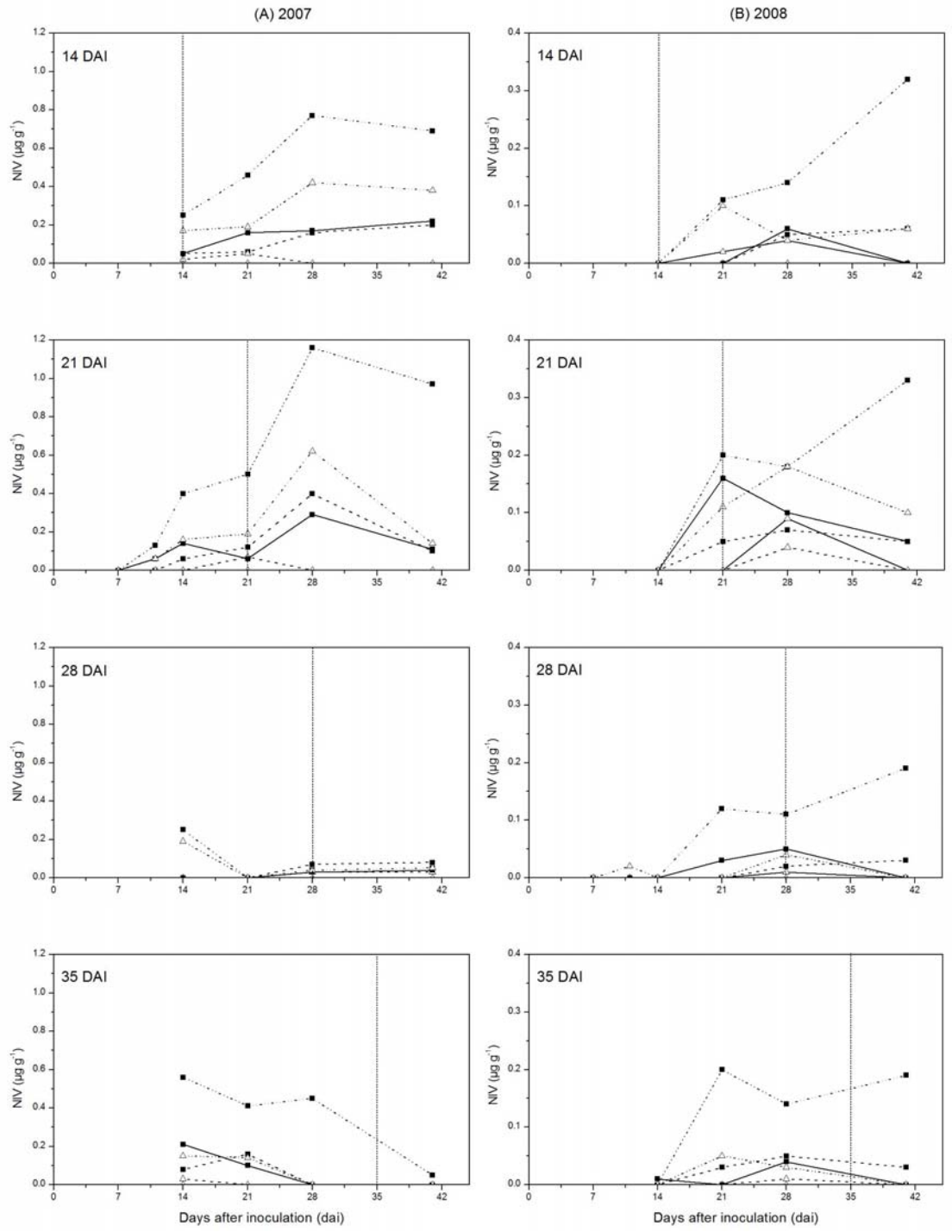

Figure 3.13. Nivalenol (NIV, µg g^{-1}) in whole head samples of Alsen (——), 2375 (– – –) and Wheaton (– · · –) inoculated with *F. graminearum* isolates 49-3 (■) and Butte86Ada-11 (Δ) subjected to four mist-irrigation duration (14, 21, 28 and 35 DAI) treatments sampled 7, 11, 14, 21, 28 and 41 dai in 2007 (Panel A) and in 2008 (Panel B). Area to the left of the vertical dashed line indicates the duration of mist-irrigation.

Chapter 4

Fusarium Head Blight Development and Trichothecene Accumulation in *Fusarium*-Infected Wheat Heads

F. graminearum Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] is the predominant causal agent of Fusarium head blight, an economically important disease of wheat, in North America. Fusarium head blight is favored by warm and humid environments at and shortly after anthesis. Fusarium head blight results in yield losses and quality losses in infected grain due to the accumulation of mycotoxins produced by the invading fungus. The objective of this study was to characterize the influence of different *F. graminearum* isolates and host resistance on FHB development and mycotoxin accumulation. Two series of greenhouse experiments were established, each a randomized complete block design with five replications, one series utilized a point inoculation method and the second a spray inoculation method. Five single isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum* which varied in aggressiveness and DON production capacity were tested in addition to a mock-inoculated control. Three wheat cultivars were used; Alsen (FHB-resistant, Sumai 3 derived), 2375 (FHB-moderately resistant) and Wheaton (FHB-susceptible). In the point-inoculation experiments, ca. 1000 conidia were placed into a central spikelet of spikes at anthesis. In the spray inoculation experiments, spikes were spray inoculated to run off (inoculum @ 10,000 conidia ml^{-1}) at anthesis. Inoculated plants were incubated in dew chamber for 72 hours to facilitate disease development. Point-inoculated central spikelets were sampled 0, 3, 7, 11, 14 and 21 dai. In spray-inoculated experiment, whole spikes were sampled 0, 7, 11, 14, 21 and 30 dai. FHB severities were assessed at each sampling in both experiments. Central spikelets sampled from the point-inoculated experiments were analyzed for mycotoxins. In spray-inoculated experiments, kernels of sampled spikes were dissected from the spike and analyzed for mycotoxins. The level of FHB severity and mycotoxins were higher in all cultivars examined in the spray-inoculated experiment than in point-inoculated. The susceptible cultivar Wheaton had both the highest FHB severity and mycotoxin accumulation in the both point and spray-inoculated experiments. Alsen had significantly lower FHB severities, DON, 15-ADON, 3-ADON and NIV than either 2375 or Wheaton. In the point inoculation experiment, the FHB severity in Alsen never exceeded 25%, which was not unexpected as it possesses resistance to spread of FHB within a spike. The spread of symptoms both below and above the inoculated central spikelet was significantly higher in 2375 and Wheaton than Alsen. Though the spread of symptoms below the point of inoculation

was similar for Wheaton and 2375, the spread above the point of inoculation was higher in Wheaton than 2375. Though DON did not peak and decline in all experiments, when a peak in the DON content was present it was earlier in 2375 (11 dai) than in either Alsen or Wheaton (21 or 14 dai). All isolates examined produced DON, 15-ADON, 3-ADON and NIV. The levels of 15-ADON, 3-ADON and NIV were comparatively lower than DON and isolates used in these experiments can be designated as belonging to the 15-ADON chemotype. Though the isolates did not rank similarly in all experiments and in all cultivars, generally isolates Butte86Ada-11 and B63A were more aggressive and isolates 49-3 and B45A were less aggressive in terms of their capacity to cause FHB disease and accumulate mycotoxins.

4.1 Introduction

Fusarium head blight (FHB) is an economically important disease of cereals, including wheat. Though several species of *Fusarium* can incite FHB, *F. graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] is the predominant causal agent in North America (Bai et al., 2001; Parry et al.; 1995; Schroeder and Christensen, 1963; Stack and McMullen, 1985; Wilcoxson et al., 1992). Fusarium head blight re-emerged worldwide in the 1990's (Windels, 2000) causing losses of ca. $2.7 billion to wheat and barley producers in the northern Great Plains and central United States (Nganje et al., 2002). A decline in the area planted to barley and wheat since the early 1990's (NASS, 2009) has been primarily attributed to FHB (McMullen, 2003).

The infection and colonization of wheat by *F. graminearum* is favored by warm temperature and extended periods of moisture around anthesis. The first symptoms of FHB, water soaked lesions 2-3 mm in length, appear (Atanasoff, 1920) within 4 days of infection under favorable conditions and are most readily evident at the base of the middle floret in spikelets located in the middle of the head (Stack, 2003). Soon after the water soaked symptoms appear, the lesions spread to the rachis adjacent to the floret. The fungus then spreads vertically in the spike (Parry et al., 1995; Wiese, 1987). Generally in susceptible cultivars vascular occlusion occurs following infection of the rachis (Atanasoff, 1920, Bai and Shaner, 1996) and results in the death of tissues above the infection point, which turn color to that of mature heads (Johnson and Dickson, 1921; Stack, 2003). Thus, grain either does not form, or does not develop fully, in infected spikes; though the extent of grain fill is dependant on the stage of the grain when the vascular tissues became dysfunctional. *Fusarium*-infected grain tends to be shriveled with light test-weight and kernels which exhibit weak dough properties and unsatisfactory baking quality.

Thus FHB makes marketing and processing of the grain difficult (Bechtel et al., 1985; Dexter et al., 1996; Dexter et al., 1997; Wang et al., 2005).

In addition to impacting grain weight, *F. graminearum* produces a range of mycotoxins, including deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), and nivalenol (NIV) in infected tissues (Nasri et al., 2006; Pirgozliev et al., 2003). These mycotoxins are hazardous to human and animal health and in the United States over the past decade wheat has routinely tested positive for most of these mycotoxins (Abramson et al., 1987; McMullen et al., 1997).

The role of DON in the aggressiveness of individual isolates is not fully understood, but it appears that DON is not essential for pathogenesis (Alexander et al., 1997; Dyer et al., 2005; Eudes et al., 2001; Proctor et al., 1995). DON is not present in *F. graminearum* conidia and thus not essential to the initial infection of the wheat plant (Evans et al., 2000). Production of DON in infected cereals has been reported to start between 26 hours (Chen et al., 1996) and four days (Savard et al., 2000) after infection. Miller and Young (1985) reported that DON could be detected in infected heads about three days after infection with DON accumulation continuing for about six weeks after infection and then declining before reaching a constant level before harvest. A similar peak in DON, followed by a decline toward grain maturity, has been reported in barley (Prom et al., 1999) and in naturally infected wheat fields (Scott, 1984). Some studies have indicated that DON begins to decline even earlier than six weeks after inoculation (Argyris et al., 2003, Culler et al., 2007).

Resistance to FHB is conferred mainly by physiological and morphological components. Schroeder and Christensen (1963) described two components of physiological resistance which are now widely accepted in the literature. The first of these is known as Type I resistance which is defined as resistance to initial infection by the pathogen, and the second, type II is defined as resistance against the spread of infection within the spike. Type I resistance is detected by spray-inoculating heads and measuring the FHB incidence at early stages of infection. Type II resistance is generally detected by assessing the spread of infection up and down following the point-inoculation of a centrally located floret in a spike. Wheat lines may possess either type I or II individually or in combination (Schroeder and Christensen; 1963). While spray inoculation methods are used in breeding programs to evaluate large host populations in field nurseries and in the greenhouse evaluations for type I resistance, point-inoculation is used extensively in greenhouse evaluations to assess specifically for type II resistance (Miedaner, 1997; Rudd et al, 2001). The resistance identified by point and spray inoculation experiments appear to be

generally poorly correlated (Miedaner et al., 2003). Other physiological resistances have been proposed including; resistance to kernel infection (Mesterházy, 1995; Mesterházy et al., 1999), FHB tolerance (Mesterházy, 1995; Mesterházy et al., 1999) and resistance to toxin accumulation (Miller et al., 1985). Irrespective of the resistance mechanism, FHB resistant wheats are generally reported to be associated with lower levels of DON than susceptible wheats (Mesterházy et al., 2003; Miller et al., 1985; Wilde and Miedaner, 2006). To facilitate the evaluation and selection of resistant germplasm demonstrating economic levels of resistance, researchers frequently utilize visual assessments of FHB severity in the field and the DON content of harvested grain (Arsenuik et al., 1999; Groth et al., 1999; Jones and Mirocha, 1999;).

Substantial pathogenic variability exists in the natural populations of *F. graminearum* (Burgess et al., 1996). Variability in the capability of *F. graminearum* with respect to aggressiveness and DON production has been reported both *in vitro* and *in vivo* studies of the pathogen (Walker et al., 2001; Tóth et al., 2005). Variability in the toxin producing capability has been reported in different populations of *F. graminearum* (Gale et al., 2007; Jennings et al., 2004). An assessment of the impact of the diversity and change in diversity of the pathogen population on FHB development is crucial if effective long-term control practices are to be developed.

The objective of this study was to examine the influence of the type of host resistance, and pathogen variation with respect to mycotoxin and aggressiveness, on infection, FHB disease development and mycotoxin accumulation *in planta*.

4.2 Materials and methods

Two series of greenhouse experiments were established in a randomized complete block design but were carried out utilizing different inoculation methods. In fall 2006, a greenhouse experiment (run 1), in which a point inoculation method was utilized, was repeated in spring 2007 (run 2). Similarly, a greenhouse experiment, in which plants were a spray inoculated, was conducted in spring 2007 (run 1) and repeated in fall 2008 (run 2). Each greenhouse experiment consisted of five replications with a replication consisting of two pots, each with five plants, three of which were inoculated. Three wheat cultivars varying in terms of their resistance to FHB were used in the experiment. The moderately resistant (MR) cultivar Alsen (Frohberg et al., 2006), previously identified as a source of FHB resistance derived from the Chinese wheat Sumai 3; 2375, a moderately susceptible (MS) cultivar with a non-Asian source of resistance; and Wheaton (Busch et al., 1984) which served as the susceptible check. Five single spored isolates (49-3,

81-2, Butte86Ada-11, B63A and B45A) of *F. graminearum* were used as in the field study (Chapter 3) in addition to a mock inoculated control.

4.2.1 Greenhouse planting

Seeds, seven per pot, were planted in a soil-less growing media (Metromix 200; Sun Gro Horticulture Canada Ltd, Seba Beach AB) in magnum square plastic pots (15×15×16.5 cm, Belden Plastics Co., St. Paul, MN). Plants were maintained in the greenhouse at 20-23ºC. In addition to natural daylight, 16 h of supplemental light (Lumalax high pressure sodium lamp, LU400/Eco S51; mean lumens: 45000; Osram Sylvania Ltd., Denvers, MA), starting at 0500 and ending at 2100, was provided.

When the plants were at 3-4 leaf stage (GS 14, Zadoks et al., 1974), pots were thinned to five plants per pot and fertilized with Osmocote (14-14-14 N-P-K; 5.8 g per pot; The Scotts Company, Marysvile OH). After thinning and fertilization, pots were treated with Bayleton (triadimefon 0.008 g a.i./pot; Bayer Crop Science Co., Germany) to prevent the development of powdery mildew and Marathon (imidacloprid 0.02 g a.i./pot; Olympic Horticultural Products Co., mainland, PA) to prevent aphids and other insect pests. Plants were watered every 2-4 days as necessary.

4.2.2 Inoculum preparation

The five *F. graminearum* isolates were cultured on mung bean agar (MBA) plates for seven days at room temperature as described in Chapter 3. On the seventh day, ten milliliters of Millipore filtered water (screen size 0.22µm; Milli Q Biocell, Millipore Corporation, France) per plate was added in cultured isolate plates and rubbed with a sterile L-shaped glass rod to loosen macroconidia. The ensuing spore suspension was filtered through a two layers of cheesecloth to reduce the number of mycelial fragments in the suspension and transferred into a sterile beaker. The spore suspension was used to inoculate five MBA plates per isolate (1.5 ml per plate), which were then incubated at room temperature for seven days. On the seventh day, conidia were harvested into ca. 10 ml of Millipore filtered water per plate as described earlier. The resulting spore suspensions were adjusted to a concentration of 1×10^5 spores ml^{-1} and stored in 20 ml Wheaton® polyethylene liquid scintillation vials (Wheaton Industries Inc., Millville, NJ) at -80ºC until needed.

4.2.3 Inoculation, disease assessment and sampling

4.2.3.1 Point inoculation

The treatments included five isolates of *F. graminearum* and a mock inoculated water control. Six primary spikes (three spikes per pot) were inoculated per replication. At anthesis (GS 65) a central spikelet per spike was inoculated with 10 µl of inoculum (~1,000 macroconidia; 1×10^5 macroconidia ml^{-1}). Inoculum was delivered into the central floret of the spikelet using a repeating dispenser (Hamilton 83700, PB600-1 dispenser; Hamilton Company, Reno, NV). The control treatment was injected with 10 µl of Millipore filtered water. Inoculated plants were incubated in a dew chamber (100% relative humidity; 16 h fluorescent light) for 72 hours. Plants were returned to the greenhouse following the dew period. Three inoculation dates were needed to accommodate the variation in anthesis among the cultivars.

Inoculated spikelets, one arbitrarily selected spike per replicate, were sampled at 0, 3, 7, 11, 14 and 21 dai (GS 65, 69, 71, 73, 75, and 83 respectively). Sampled spikelets were placed in 1.5 ml gas chromatography (GC) vials and stored at -20ºC until prepared for DON analysis. The total number of spikelets per spike and the number of visually symptomatic spikelets per spike were assessed before sampling and used to calculate FHB severity. FHB severity for each spike was calculated by dividing the number of symptomatic spikelets by the total number of spikelets and multiplying the result by 100. In addition, the numbers of symptomatic spikelets below and above the inoculated spikelet were assessed.

4.2.3.2 Spray inoculation

The treatments for the spray inoculated experiments included five isolates of *F. graminearum* used in the point inoculation experiments and a mock-inoculated water control. Six primary spikes per replication were inoculated for each isolate and the control. Prior to use inoculum was thawed and diluted to 10,000 macroconidia ml^{-1}. Selected primary heads were inoculated with a CO_2-powered airbrush (Model VL, Paasche® Airbrush Company, Chicago, IL) until run off. Inoculated plants were incubated in a dew chamber (100% relative humidity; 16 h fluorescent light) for 72 hours. Following the dew period, plants were returned to the greenhouse. Three inoculation dates were needed to accommodate the variation in anthesis among the cultivars.

Inoculated spikes were sampled 0, 7, 11, 14, 21 and 30 dai (GS 65, 71, 73, 75, 83, and 85 respectively). The total number of spikelets per spike and the number of symptomatic spikelets per spike were assessed visually and used to calculate FHB severity. Whole heads were sampled

and stored in plastic bags at -20ºC. Kernels were separated manually from all the sampled spikes. Kernels from the spikes from all five replications were bulked and ground in a mortar and pestle with liquid nitrogen. Ground samples were kept at -20ºC until prepared for mycotoxin analysis.

4.2.4 Trichothecene analysis

Mycotoxin analyses of point inoculated samples were done following Mirocha (1998) with some modifications. Samples were extracted in 2 ml acetonitrile: water (84:16, v:v) shaken for 24 hours and filtered through a column prepared with C18: aluminum oxide (1:3). The filtrate was dried under nitrogen. Extracted dried samples were derivatized by the addition of 25 µl of trimethylsilyl (TMS) reagent (TMSI:TMCS, 100:1, Pierce Chemical Co., Rockford, IL), shaken for 10 min followed by the addition of 150 µl Mirex isooctane solution (4mg L^{-1}) and 150 µl high performance liquid chromatography (HPLC) water and vortexed. The upper transparent isooctane layer was then transferred to a new GC vial. The derivatized solution was analyzed for DON, 3ADON, 15ADON and NIV based on the calibrated standards using gas chromatography-mass spectrometry (GC-MS, Shimadzu Model QP 2010, Shimadzu Corporation, Kyoto, Japan).

Samples from spray inoculation treatments were analyzed for mycotoxin following Mirocha (1988) with some modifications. Samples were separated into two categories; those weighing < 2 g (set 1) and those weighing ≥ 2 g to 5 g (set 2). The procedure for sample preparation for the two sets were same but with different volumes of chemicals.

Set 1 samples were extracted in 4 ml acetonitrile: water (84:1 v:v) extraction solvent by shaking for 1 hour. The extract was filtered through a column packed with C18: aluminum oxide (1:3). One milliliter of the filtrate was evaporated under nitrogen. Dried samples were derivatized by the addition of 25 µl of TMS reagent (TMSI:TMCS, 100:1), shaken for 10 minutes followed by addition of 100 µl Mirex isooctane solution (4mg L^{-1}) and 200 µl HPLC water and vortexed. The upper transparent isooctane layer was then transferred to a GC vial. The derivatized solution was analyzed using GC-MS.

Set 2 samples were extracted in 20 ml acetonitrile: water (84:1 v:v) extraction solvent by shaking for 2 hours. The extract was filtered through a column packed with C18: aluminum oxide (1:3). One milliliter of the filtrate was evaporated under nitrogen. Dried samples were derivatized by the addition of 100 µl of TMS reagent (TMSI:TMCS, 100:1), shaken for 10 minutes followed by addition of 1 ml Mirex isooctane solution (4mg L^{-1}) and 1 ml HPLC water and vortexed. The upper transparent isooctane layer was then transferred to a GC vial. The derivatized solution was

analyzed using GC-MS. Concentrations of DON, 3ADON, 15ADON and NIV in samples were determined based on the calibrated standards.

4.2.5. Data analysis

Data for FHB severity, DON, 15-ADON, 3-ADON and NIV from the point-inoculated experiments, and FHB severity, DON and 3-ADON from the spray-inoculated experiments were natural log transformed to achieve homoscedasticity. Data for 15-ADON and NIV from the spray-inoculated experiments were analyzed untransformed. Data were analyzed using the PROC MIXED procedure for randomized complete block design in SAS v 9.0 (SAS Institute, Cary, NC). Data from different runs were analyzed separately except for DON in the point-inoculated experiments, where the runs were not significantly different and thus data were combined. Means were separated using LSD and the outputs were letter grouped using SAS macrocode PDMIX800 (Saxton, 1998). Spearman rank correlation analyses were carried out using PROC CORR in SAS. Graphs were created using OriginPro 8.1 SR0 (OriginLab Corporation, Northampton, MA).

4.3 Results

4.3.1. Disease severity

4.3.1.1. Point inoculation

Symptoms of FHB severity developed in all treatments of Alsen, 2375 and Wheaton by 3 dai (GS 68/69) except in 2375 and B63A inoculated Wheaton in run 2 of the experiment where symptoms were visible by 7 dai (GS 71, Figure 4.1). The mock-inoculated plants did not develop any symptoms of FHB, indicating that no contamination occurred during incubation in the dew chamber (Appendix 19).

In general isolate Butte86Ada-11 incited the highest and 49-3 the lowest FHB severities in all three cultivars examined. Generally the FHB severities resulting from the isolates at 11 dai (GS73/74), 14 dai (GS 77/78) or 21 dai (GS 83) were significantly greater than the FHB severities observed either at 3 dai (GS 68/69) or 7 dai (GS 71).

The relative aggressiveness of isolates was evaluated by determining the ability of a given isolate to increase FHB severity from 3 dai (GS 68/69) to 21 dai (GS 83). Isolate Butte86Ada-11 was generally the most aggressive isolate with increases in the FHB severities of 13-18% in Alsen, 70-73% in 2375 and 95% in Wheaton. No isolate consistently ranked as the least aggressive. All isolates were less aggressive in Alsen with the increases in FHB severity by not more than 17%. Except for isolates Butte86Ada-11, B45A and 49-3 in run 1, all isolates were

more aggressive in the susceptible cultivar Wheaton, than in 2375 and Alsen, with an increase in the FHB severity from 3 dai (GS 68/69) to 21 dai (GS 83) by more than 50%. Isolates Butte86Ada-11, B45A and 49-3 in the run 1 were more aggressive in 2375 than Wheaton with the increases of the FHB severity more than 64% from 3 dai (GS 68/69) to 21 dai (GS 83).

Differences in the spread of isolates were also observed in terms of spread of FHB symptoms above and below the point of inoculation (Figure 4.2, Appendix 19). Isolate Butte86Ada-11, which generally resulted in the most disease overall, generally resulted in the greatest spread of symptoms above (Alsen: 0.80, 2375: 3.80, Wheaton: 6.20) and below (Alsen: 1.50, 2375: 6.40, Wheaton: 6.60) the inoculated spikelet. Isolate 49-3 generally resulted in the least spread of symptoms above (Alsen: 0.00, Wheaton: 3.50) and below (Alsen: 0.00, 2375: 4.30, Wheaton: 5.40) the point of inoculation, although isolate 81-2 resulted in the least spread above the point of inoculation (0.90 spikelet) in 2375. All isolates examined spread more rapidly below than above the point of inoculation with the phenomenon being most conspicuous in 2375.

When combined across isolates, FHB severities in Alsen increased significantly until either 7 dai (GS 71) or 11 dai (GS 73/74), after which the FHB severities either remained stable or increased minimally. By 11 dai (GS 71) and afterwards FHB severities in 2375 (15-74%) and Wheaton (12-97%) were significantly higher than in Alsen (7-24%). The FHB severities of Wheaton and 2375 generally were not statistically different. At earlier growth stages (GS 68-71), FHB severities were generally higher in Alsen than 2375 and Wheaton.

The differences in the spread of visual symptoms were also observed in cultivars (Figure 4.2, Appendix 19). When combined across isolates, the mean number of spikelets showing visible symptoms of FHB in Alsen was not more than one spikelet above the inoculated central spikelet. In 2375 and Wheaton FHB symptoms spread up to two and five spikelets, respectively, above the inoculated central spikelet. The spread of FHB symptoms below the inoculated spikelet was the lowest in Alsen (1 spikelet) compared to that of either Wheaton (6 spikelets) or 2375 (5 spikelets). While the spread of FHB symptoms was more down than up from the inoculation point in 2375, FHB symptoms spread equally above and the below the point of inoculation in Wheaton.

4.3.1.2. Spray inoculation

Visual symptoms of FHB had developed by 3 dai (GS 68/69) in all cultivars examined though the FHB severities were recorded from 7 dai (GS 71). By 7 dai (GS 71), high FHB

severities were observed in all three cultivars (Figure 4.3, Appendix 20). The FHB severities observed were higher in run 2 than run 1.

No one isolate consistently resulted in higher FHB severities across different growth stages in the cultivars examined. In run 1, isolate 49-3 generally resulted in the lowest FHB severity at all growth stages in 2375 and Wheaton, and isolate B45A resulted in the lowest FHB severity in Alsen. In Wheaton in run 1, except isolates 49-3 and B45A at 21 dai (GS 83) and 49-3 at 30 dai (GS 85/86), all isolates resulted in 100% FHB severity at 21 dai (GS 83) and 30 dai (GS 85/86). In run 2, isolate Butte86Ada-11 resulted in the least FHB severity in all cultivars and at all sampling dates. No isolate generated 100% FHB severity in Alsen. All isolates except for Butte86Ada-11 and B63A resulted in 100% FHB severity in 2375 by 21 dai (GS 83). Similarly, all isolates except for Butte86Ada-11 resulted in 100% FHB severity in Wheaton by 21 dai (GS 83).

The FHB severities observed, when combined across isolates, were higher in Wheaton (run 1: 30-100%, run 2: 18-100%) and 2375 (run 1: 35-100%, run 2: 62-100%) than Alsen (run 1: 25-82%, run 2: 50-99%). At earlier growth stages (GS 71-73/74) FHB severities were similar in all three cultivars. From 14 dai (GS 77/78), the FHB severities observed in 2375 and Wheaton were statistically similar and both cultivars generally had significantly higher FHB severities than Alsen. FHB severity often reached 100% by 21 dai (GS 83) in 2375 and Wheaton.

4.3.2. Deoxynivalenol (DON)

4.3.2.1. Point inoculation

A combined analysis was carried out for DON data from runs 1 and 2. Deoxynivalenol was detected from the initial sampling 3 dai (GS 68/69) in all cultivars examined (Figure 4.4, Appendix 19).

Isolate 49-3 generally resulted in lower levels of DON in all cultivars and at all growth stages sampled. No one isolate consistently resulted in the highest DON levels. Generally, isolates B63A and Butte86Ada-11 were the higher DON producing isolates. DON levels declined from 14 (GS 83) to 21 dai (GS 77/78) for isolates Butte86Ada-11, B63A and 81-2.

DON levels detected ranged from of 9 to 317 µg g^{-1} in Alsen, 14 to 372 µg g^{-1} in 2375 and 3 to 397µg g^{-1} in Wheaton over the sampling period up to 21 dai (GS 83). Alsen had significantly lower DON levels compared to either of 2375 or Wheaton at all growth stages. The levels of DON were not statistically different in 2375 and Wheaton. The DON levels, when combined across isolates, increased up to 14 dai (GS 77/78) in Alsen and decreased thereafter,

though the decline was not statistically significant. In 2375, DON levels significantly increased until 11 dai (GS 73/74) and then declined thereafter, though again the decline was not statistically significant. The levels of DON increased in Wheaton until 21 dai (GS83), though the increases observed were not statistically significant after 11 dai (GS 73/74).

4.3.2.2. Spray inoculation

DON was detected in the initial sampling at 7 dai (GS 71). The levels of DON were generally higher in run 2 than 1. None of the isolates consistently resulted in the highest or the lowest DON levels in all treatment combinations (Figure 4.5, Appendix 20). Since the replicated samples were bulked before DON analyses, statistical difference between isolates in each cultivar could not be analyzed. When data were combined across cultivars, isolates 49-3 and B45A generally resulted in significantly lower DON levels compared to the other isolates in run 1. Similarly, isolates Butte86Ada-11 and B45A generally resulted in significantly lower levels of DON in run 2. Isolates Butte86Ada-11 and 81-2 in run 1, and isolate 49-3 in run 2 generally produced in the highest DON levels in all cultivars at all growth stages. DON generally peaked at either 14 dai (GS 77/78) or 21 dai (GS 83) and then declined in all cultivar and isolate treatments with exceptions. Exceptions included isolate B63A in 2375 and Wheaton in run 1, isolate 49-3 in Alsen and 2375 in run 2 and 81-2 in 2375 in run 2, where the levels of DON levels continued to increase until the last sampling.

The DON levels were generally higher in Wheaton (run 1: 12-543 µg g^{-1}, run 2: 5-695 µg g^{-1}) and 2375 (run 1: 15-425 µg g^{-1}, run 2: 95-782 µg g^{-1}) than Alsen (run 1: 3-94 µg g^{-1}, run 2: 11-329 µg g^{-1}). Generally DON levels were higher in later samplings (11 to 30 dai) than 7 dai (GS 71). The levels of DON detected in all three cultivars examined reached the highest level either at early dough stage (GS 83) or before that and then declined thereafter. However, these declines observed in DON levels were not statistically significant except Alsen in run 1.

4.3.3. 15-acetyldeoxynivalenol (15-ADON)

4.3.3.1. Point inoculation

15-acetyldeoxynivalenol was most readily detected in run 1. In run 2, 15-ADON was detected only in a few samples which were not more than 0.03 µg g^{-1} in any sample, therefore, data from run 2 were not analyzed. As seen for DON, 15-ADON was detected by 3 dai (GS 68/69) in all cultivars examined (Figure 4.6, Appendix 19). While the levels of 15-ADON

detected reached 63% of the levels of DON detected in a given sample, more than 99% of samples had 15-ADON levels less than 50% of the DON level of that sample.

Isolate 49-3 produced the least 15-ADON in all cultivars and generally at all growth stages. Although no one isolate consistently resulted in the highest 15-ADON levels, Butte86Ada-11, 81-2 and B63A generally resulted in higher levels of 15-ADON than the other isolates tested. The level of 15-ADON generally peaked around either 11 dai (GS 73/74) or 14 dai (GS 77/78) for all isolates.

The levels of 15-ADON generally were significantly higher in 2375 (1-80 μg g^{-1}) than in either of Wheaton (1-47 μg g^{-1}) or Alsen (1-42 μg g^{-1}). When combined across isolates, the levels of 15-ADON generally peaked at either 11 dai (GS 73/74, Alsen and 2375) or 14 dai (GS 77/78, Wheaton). However, the changes in 15-ADON levels after 11 dai (GS 73/74) were not statistically significant.

4.3.3.2. Spray inoculation

15-acetyldeoxynivalenol was detected by 7 dai (GS 71) in all cultivars examined (Figure 4.7, Appendix 20). The levels of 15-ADON were not more than 4.5% of the DON levels detected in a given sample.

Though none of the isolates consistently resulted in the highest level of 15-ADON in all cultivars or at all growth stages, 81-2 was generally a higher 15-ADON producing isolate, especially in Wheaton in run 1. Isolate 49-3 produced higher 15-ADON levels than the other isolates in run 2. No specific isolate was the lowest 15-ADON producer in run 1, but in run 2 isolate Butte86Ada-11 consistently resulted in the least amount of 15-ADON. None of the isolates produced 15-ADON in Alsen in 21 and 30 dai (GS 83 and 85/86).

When combined across isolates, Wheaton (run 1: 0-14 μg g^{-1}, run 2: 0-14 μg g^{-1}) and 2375 (run 1: 0-7 μg g^{-1}, run 2: 1-34 μg g^{-1}) had higher levels of 15-ADON compared to Alsen (run 1: 0-1 μg g^{-1}, run 2: 0-3 μg g^{-1}). Though Wheaton had higher levels of 15-ADON in run 1, 2375 had higher levels of 15-ADON in run 2. The levels of 15-ADON in Alsen in run 1 declined after 11 dai (GS 73/74), but continued to increase until 30 dai (GS 85/86) in run 2. In 2375 the levels of 15-ADON declined either after 21 dai (GS 83, run 1) or increased until 30 dai (GS 85/86, run 2). In Wheaton, 15-ADON levels increased until 21 dai (GS 83) and declined thereafter.

4.3.4. 3-acetyldeoxynivalenol (3-ADON)

4.3.4.1. Point inoculation

3-acetyleoxynivalenol was detected only in run 1 (Figure 4.8, Appendix 19) and was not more than 3.5% of the level of DON detected in a given sample. 3-ADON in all cultivars examined was detected by 7 dai (GS 71).

All isolates examined resulted in 3-ADON accumulation in all three cultivars except B45A in 2375. None of the isolates consistently produced the highest or the lowest 3-ADON levels in all treatments. By 7 dai (GS 71) all isolates except 49-3 produced 3-ADON in Wheaton. In Alsen and 2375 the isolates tested generally did not produce 3-ADON until 11 dai (GS 73/74).

Wheaton generally had higher 3-ADON levels (0-4 $\mu g\ g^{-1}$) than either 2375 (0-5 $\mu g\ g^{-1}$) or Alsen (0-3 $\mu g\ g^{-1}$). The level of 3-ADON peaked either 7 dai (GS 71, 2375) or 11 dai (GS 73/74, Alsen and Wheaton).

4.3.4.2. Spray inoculation

3-acetyldeoxynivalenol was detected by 7 dai (GS 71) except in Alsen in run 1where 3-ADON was not detected in entire growth period (Figure 4.9, Appendix 20). Whenever 3-ADON was present it was, however, not more than 0.8% of the DON level in a given sample.

No specific isolate resulted in the highest or the lowest 3-ADON levels in all treatments. Isolate B45A run 1 in 2375, and isolates B45A and 49-3 in Wheaton in run 1 did not result in detectable levels of 3-DON. All isolates resulted in the detectable levels of 3-ADON in run 2.

The ranges of 3-ADON detected were 0-0.5 $\mu g\ g^{-1}$ in Alsen, 0-1 $\mu g\ g^{-1}$ (run 1) and 0-4 $\mu g\ g^{-1}$ (run 2) in 2375, and 0-3 $\mu g\ g^{-1}$ (run 1) and 0-4 $\mu g\ g^{-1}$ (run 2) in Wheaton. Except in 2375 in run 1 and Alsen in run 2, whenever 3-ADON was present, its level increased significantly at later growth stages (14-30 dai, GS 77/78-85/86) compared to the levels at 7 (GS 71) and 11 dai (GS 73/74).

4.3.5. Nivalenol (NIV)

4.3.5.1. Spray inoculation

Nivalenol was not tested in point inoculated samples. In spray inoculated samples, NIV was detected mostly in run 2 (Figure 4.10, Appendix 20). In run 1, NIV was detected only at 7 dai (GS 71) in 2375 inoculated with isolate 81-2 (0.24 $\mu g\ g^{-1}$). In run 2, NIV was detected in all cultivars and was detected by 7 dai (GS 71) in all cultivars except in Wheaton, where NIV was not detected until 11 dai (GS 73/74).

Isolate Butte86Ada-11 produced the least NIV at all growth stages in Alsen. Isolate B45A tended to produce the least amount of NIV in 2375. Isolates 49-3 and Butte86Ada-11 were the lowest NIV producers in Wheaton. No specific isolate resulted in the highest levels of NIV in all experiments.

The levels of NIV detected were up to 0.98 μg g^{-1} in Wheaton. In Alsen and 2375, the NIV levels were up to 0.16 μg g^{-1} and 0.44 μg g^{-1}, respectively. The levels of NIV detected were not more than 0.3% of the level of DON detected in a given sample.

4.3.6. Correlations

4.3.6.1. Point inoculation

Though FHB severity was assessed in whole heads and the mycotoxins were analyzed only in the inoculated centrally located spikelets, the correlations between FHB severity and DON were high ($r \geq 0.67$, $P < 0.01$) in sampling dates 11 dai (GS 73/74) and onward (Table 4.1). In the first run of the experiment, the correlation of FHB severity with DON and 15-ADON generally increased with the maturity of grain. The correlations between FHB severity and 15-DON were stronger ($r = 0.52$-0.77) than the correlations between the FHB severity and DON ($r = 0.41$-0.75). However, in run 2, though the correlations between FHB severity and DON increased with the maturity of grain, it was lower 21 dai (GS 83, $r = 0.69$) compared to 11 dai (GS 73/74, $r = 0.78$) and 14 dai (GS 77/78, $r = 0.80$). The correlations of FHB severity with 15-ADON in run 2 were weak ($0.19 \leq r \geq 0.44$), being non-significant at 7, 11, and 14 dai.

The correlations between DON and 15-ADON were not consistent between runs. The correlation coefficients were very high ($r \leq 0.89$) in first run of the study, however in the second run, the correlation coefficients were generally less than 0.1 and non-significant. The correlation coefficients for DON with 3-ADON were low but significant ($r < 0.35$, $P < 0.05$).

4.3.6.2. Spray Inoculation

The correlations between FHB severity and DON (Table 4.2) obtained in the spray inoculated experiments were high and significant ($r \geq 0.65$, $P<0.01$). The correlations between FHB severity and 15-ADON were generally also high and significant ($r \geq 0.73$, $P < 0.01$). The correlations between FHB severity and 3-ADON was initially non significant, however after 11 dai (GS 73/74) became significant ($r \geq 0.48$, $P < 0.05$). The correlations of FHB severity with DON, 15-ADON and 3-ADON were stronger with the maturity of grain and the highest correlation coefficients were either at 21 dai (GS 83) or 30 dai (GS 85/86). The correlation

between FHB severity and NIV were also significant in the second run with the coefficients ranging between 0.45 and 0.89. The correlations between DON and its acetylated derivatives and NIV were also generally significant.

4.4 Discussion

These greenhouse experiments were conducted to examine the influence of pathogen variation and host resistance on FHB infection, disease development and mycotoxin accumulation *in planta*. The two series of experiments were conducted utilizing point and spray inoculation methods, respectively.

Variability in the relative aggressiveness of pathogen isolates, defined by the increase in FHB severity from the first appearance of visible symptoms to the last sampling date, were evident in both the point and spray inoculated experiments. Generally, an isolate was either more or less aggressive, in both the point- and spray-inoculated experiments. Generally isolates Butte86Ada-11 and B63A were the most aggressive and isolates 49-3 and B45A were the least aggressive, relative to the other isolates tested. Variability in the relative aggressiveness of *F. graminearum* isolates has been reported in earlier studies examining FHB development in wheat (Akinsanmi et al., 2006; Bai and Shaner, 1996; Xue et al., 2004).

Variations in the capacity of *F. graminearum* isolates to produce DON in sterilized rice culture have been reported by Carter et al. (2002) and Tóth et al. (2005). Variability in the capacity of *F. culmorum* isolates to produce DON and NIV in rye has also been reported (Gang et al., 1998). In this study, the variation of an isolates ability to produce mycotoxin was evidenced by a differential accumulation of toxins both in the point-inoculated and spray-inoculated wheat heads. The ability of an isolate to accumulate DON and its derivatives generally corresponded with the aggressiveness of a given isolate. Though the ranking of isolates in different cultivars varied, the same isolates generally resulted in either higher or lower levels of mycotoxins across all cultivars examined. This result reiterates the importance of selecting appropriate isolates for screening of wheat for FHB resistance. The use of mixtures of isolates, representative of the local population, in resistance screening appears advisable in order to avoid the misinterpretation of a cultivar's resistance with the use of single isolate.

It should be noted that Butte86Ada-11 was the least aggressive isolate in the field experiments (Chapter 3) for all disease parameters examined. Isolate 49-3, which was the least aggressive in this greenhouse study, as indicated by the FHB severity and mycotoxin accumulation data, was the most aggressive for mycotoxin accumulation in the field conditions.

The discrepancy in the performance of isolates between the greenhouse and field experiments might be due to differences in the prevailing environmental conditions. In the greenhouse study, environmental parameters including temperature are optimized for host crop development, and fungal and disease development. In contrast, in the field fungi have to survive and incite disease amid the prevailing environmental conditions. This indicates that isolates vary in terms of their aggressiveness independent of host resistance, and this variation may depend on the prevailing environmental conditions. Environmental conditions appear to be key to infection, disease development and mycotoxin accumulation in FHB. Isolates that appeared to be less aggressive in the field but which were highly aggressive in the greenhouse may be poor competitors in nature, which may explain the low correlation obtained between greenhouse and the field experiments here and in studies by Bai et al. (2001) and Malla (2005).

Though NIV was not examined in the point-inoculated experiments, all isolates examined produced 15-ADON and 3-ADON in addition to DON. NIV was also examined in the spray inoculated experiments. The concentrations of 15-ADON, 3-ADON and NIV, in both point and spray experiments was very low compared to the level of DON. Comparatively higher amounts of DON compared to 15-ADON and 3-ADON have also been reported by others (Burlakoti et al., 2008; Tanaka et al., 1988) in North America. Based on the observed levels of DON, 15-ADON, 3-ADON, and NIV, all five isolates examined in this study would be grouped in the 15-ADON chemotype of *F. graminearum*. This result agrees with Mirocha et al., (1989) who suggested that in addition to DON, *F. graminearum* isolates from North America produce 15-ADON rather than 3-ADON.

The ability of an isolate to produce DON has been assumed to be positively linked with the aggressiveness of *Fusarium* isolates but not their pathogenicity (Desjardins et al., 1996; Dyer et al., 2005; Eudes et al., 2001; Proctor et al., 1995). The *F. graminearum* isolates which produce DON are reported as being generally twice as aggressive as NIV producers (Cumagun et al., 2004). However, NIV is biologically more important with regard to the safety of food and feed as it is up to 10 times more toxic to animals compared to DON (Mirocha et al., 1985). Despite its comparatively low toxicity, DON is generally produced in higher concentration in wheat grain than NIV. In the current study, the level of DON appears to be both greater and more stable than the levels of either 3-ADON or 15-ADON detected. Therefore, DON levels appear to provide a more precise measure of mycotoxin contamination of grain.

Subgroups of the 15-ADON producing population of *Fusarium graminearum* in the upper Midwest have recently been identified by Gale et al. (2007). The identified sub-population,

[Upper Midwestern (UMW) 15-ADON population] is reported to be less diverse, in terms of the mean number of alleles per locus, gene diversity across all loci and pairwise differences between multilocus RFLP genotypes, than the currently predominating 15-ADON mid-western (MW) sub-population. Members of this UMW 15-ADON sub-population were described as being more aggressive, in terms of their ability to produce deoxynivalenol in the greenhouse, than the MW 15-ADON sub-population (Gale et al., 2006). Quirin (2010) genotyped all isolates used in this study, except 49-3, classifying isolates Butte86Ada-11, B45A and 81-2 as belonging to the Midwestern (MW) 15-ADON population. Isolate B63A was identified as belonging to the UMW 15-ADON population. In our study the isolate B63A generally resulted in the higher DON levels and higher FHB severity than other isolates examined in both the point and the spray inoculation experiments. The result from our study agrees with Gale et al. (2006) in that members of UMW 15-ADON population appear more aggressive than the MW 15-ADON population. However, further study with the inclusion of multiple isolates from UMW 15-ADON population is required to confirm these preliminary findings.

The three cultivars examined in this study differ significantly in terms of their resistance to FHB development and mycotoxin accumulation. The moderately resistant cultivar Alsen had significantly less disease development than either 2375 or Wheaton in both the point- and spray-inoculated experiments. In the point-inoculated experiment, the visible symptoms of FHB spread to non-inoculated spikelets as quickly as 7 dai. In Alsen, the FHB symptoms were generally limited to the inoculated spikelets. Though Wheaton and 2375 generally had similar FHB severities across growth stages, the difference in pattern of spread of symptoms was striking. In Wheaton FHB symptoms spread equally above and the below the point of inoculation, while in 2375 the spread of symptoms was greater below the point of inoculation. The observed discrepancy in the spread of FHB might be due to the fact that *Fusarium* infection and growth cause vascular occlusion, especially in susceptible cultivars, thus spikelets above the inoculation point wilt and die more readily. Thus although the fungus did spread more rapidly downward in 2375 and Wheaton, vascular occlusion in Wheaton may have led to equal symptom spread both upward and downward from the point of inoculation.

Cultivar performance was not consistent between the point- and spray-inoculated experiments. In the spray inoculation experiments, Alsen had FHB severities ranging from 25 to 99% across growth stages examined. However, following point-inoculation the severity of FHB never reached above 25% in Alsen with visible symptoms generally being limited to the inoculated spikelet. At early growth stages (GS 68/69-71; 3-7 dai) the FHB severity in Alsen was

often higher than Wheaton and 2375, irrespective of the inoculation method. Similarly, the levels of DON and other mycotoxins were frequently higher in Alsen at earlier stages of disease development. Thus it appears that the resistance in Alsen limits the spread of fungus rather than limiting initial infection and that this resistance is most clearly expressed starting at 7 dai.

QTLs for FHB resistance, which explain up to 60% of the variation for type II resistance have been identified in the Chinese wheat Sumai-3 and its derivatives (Hart et al., 2004). Since Alsen is derived from Sumai-3, and possesses the resistance allele at the 3BS QTL, it can be assumed that it also possesses type II resistance.

Wheaton and 2375 performed similarly with respect to both FHB severity and mycotoxin accumulation. At the early stages of disease development, 2375 appeared to have accumulated more mycotoxins than Wheaton. This suggests that 2375 may possess some mechanism to either prevent the production or accumulation of DON or that it has the ability to degrade DON and other mycotoxins. Berthiller et al. (2007) and Lemmens et al. (2005) reported that some FHB resistant cultivars may possess mechanisms to metabolize DON and convert it to deoxynivalenol-3-β-glucopyranoside (D3G). As D3G was not tested in this study we are unable to distinguish between reduced toxin production by the pathogen and toxin degradation by the host.

The levels of DON in infected wheat heads have been reported to decline sometime before harvest (Argyris et al., 2003; Culler et al., 2007; Teich, 1989). Miller and Young (1985) reported that the level of DON peaked six weeks after inoculation and declined after this point until harvest. In the current study, a peak and subsequent decline in DON was observed in all three cultivars, although this pattern was not consistently evident in all experiments. Further, in many cases, the DON accumulation profiles were variable with multiple peaks and declines being observed throughout the progression of the disease. When a peak in the level of DON was observed in the spray-inoculated experiments, the peak generally occurred at early dough (GS 83) in Alsen and Wheaton and around early milk (GS 73/74) stages in 2375. In the point inoculated experiments DON was observed to peak around the late milk stage (GS 77/78) in Alsen and Wheaton and at early milk (GS 73/74) in 2375. The earlier decline in DON levels in 2375 might be due to the presence of a mechanism to detoxify DON.

In contrast to this greenhouse study, in field the experiments (Chapter 3), Alsen and 2375 performed similarly for all disease parameters examined, including FHB severity, VSK level, and mycotoxin accumulation. Further, the levels of DON detected in greenhouse experiments were higher by at least 100 $\mu g\ g^{-1}$ than those detected in the field experiments. The discrepancies in the

mycotoxin levels, observed in the greenhouse and field experiments, may be explained by differences in the environmental conditions between the field and greenhouse. Since the greenhouse is optimized for early and faster disease development, DON production would also start earlier, as evident in the presence of DON at the 3 dai sampling in the current study. Detection of DON at 24 hours after inoculation has been reported by Chen et al. (1996) which was 48 hours earlier than the first samplings in the current study.

Differences in sample size in the field and the greenhouse experiments may also have led to the observed differences in mycotoxin levels between the field and the greenhouse. In the point inoculation greenhouse experiments, only the inoculated spikelets were sampled for mycotoxin analyses. In the spray inoculation experiments the inoculated heads were sampled and all kernels in the spike were included in the mycotoxin analyses. The field experiments included the two meters plot harvested mechanically. Therefore due to the larger sample size, data from the field were less variable compared to the greenhouse study. Further, in the field experiments, the grain harvested for mycotoxin analysis included both infected and uninfected heads which were threshed mechanically. Although every attempt was made to reduce the loss of highly infected kernels, very light grain might have been blown out of the thresher and grain from uninfected heads may have led to a dilution of DON levels in the harvested grain.

The results of this study indicate that *F. graminearum* isolates rank similarly in different resistance levels of wheat. However, isolates may vary significantly in terms of their aggressiveness and mycotoxin production in the greenhouse and the field. Therefore characterization of isolates based solely on greenhouse experiments cannot provide accurate information on an isolate. Cultivars with type II resistance are suitable for limiting both FHB development and DON accumulation. Deployment of QTL and/or gene responsible for type II resistance in commercial cultivars could provide significant reductions in FHB development and DON accumulation. It should be noted that the performance of moderately susceptible cultivars was highly variable when compared in the greenhouse and the field experiments. Therefore, screening of cultivars likely requires both greenhouse and the field testing.

Table 4.1. Spearman's rank correlations for Fusarium head blight (FHB) severity, deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) detected in centrally located spikelets of three wheat cultivars point inoculated with five *Fusarium graminearum* isolates sampled 3, 7, 11, 14, and 21 dai in greenhouse experiments run 1 and 2.

	DON	15-ADON	3-ADON	DON	15-ADON	3-ADON
	Run 1			Run 2		
	3 dai (GS 68/69)					
Severity	0.41**	0.52**	-	0.18^{NS}	0.44**	-
DON		0.91**	-		0.49**	-
15-ADON			-			-
	7 dai (GS 71)					
Severity	0.60**	0.60**	0.02^{NS}	0.45**	0.21^{NS}	-
DON		0.95**	0.35**		0.07^{NS}	-
15-ADON			0.32**			-
	11 dai (GS 73/74)					
Severity	0.67**	0.71**	0.26*	0.78**	0.20^{NS}	-
DON		0.90**	0.27*		0.09^{NS}	-
15-ADON			0.18^{NS}			-
	14 dai (GS 77/78)					
Severity	0.70**	0.75**	0.27**	0.80**	0.19^{NS}	-
DON		0.89**	0.31**		0.01^{NS}	-
15-ADON			0.29**			-
	21 dai (GS 83)					
Severity	0.75**	0.77**	0.30**	0.69**	0.23*	-
DON		0.92**	0.29**		0.04^{NS}	-
15-ADON			0.24*			-

** Significant at $P \leq 0.01$

* Significant at $P \leq 0.05$

^{NS} Non-significant

Table 4.2. Spearman's rank correlations for Fusarium head blight (FHB) severity, deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) and nivalenol (NIV) detected 7, 11, 14, 21 and 30 dai in the spray inoculated wheat heads in greenhouse experiments run 1 and 2.

	DON	15-ADON	3-ADON	NIV	DON	15-ADON	3-ADON	NIV
			Run 1				Run 2	
	7 dai (GS 71)							
Severity	0.91**	0.78**	0.40^{NS}	0.40^{NS}	0.92**	0.87**	-0.22^{NS}	0.85**
DON		0.79**	0.40^{NS}	0.40^{NS}		0.97**	-0.07^{NS}	0.86**
15-ADON			0.07^{NS}	0.07^{NS}			-0.02^{NS}	0.81**
3-ADON				1.00**				-0.19^{NS}
	11 dai (GS 73/74)							
Severity	0.91**	0.79**	0.56*	-	0.65**	0.73**	0.48*	0.54*
DON		0.91**	0.77**	-		0.91**	0.26^{NS}	0.87**
15-ADON			0.79**	-			0.27^{NS}	0.78**
3-ADON				-				0.45^{NS}
	14 dai (GS 77/78)							
Severity	0.94**	0.34^{NS}	0.50*	-	0.89**	0.89**	0.58*	0.58*
DON		0.52*	0.55*	-		0.95**	0.67**	0.79**
15-ADON			0.56*	-			0.76**	0.67**
3-ADON				-				0.68**
	21 dai (GS 83)							
Severity	0.94**	0.83**	0.81**	-	0.95**	0.95**	0.85**	0.89**
DON		0.85**	0.82**	-		0.99**	0.85**	0.84**
15-ADON			0.95**	-			0.86**	0.83**
3-ADON				-				0.85**
	30 dai (GS 85/86)							
Severity	0.97**	0.92**	0.85**	-	0.79**	0.90**	0.89**	0.88**
DON		0.94**	0.88**	-		0.94**	0.85**	0.90**
15-ADON			0.94**	-			0.95**	0.94**
3-ADON				-				0.91**

** Significant at $P \leq 0.01$
* Significant at $P \leq 0.05$
^{NS} Non-significant

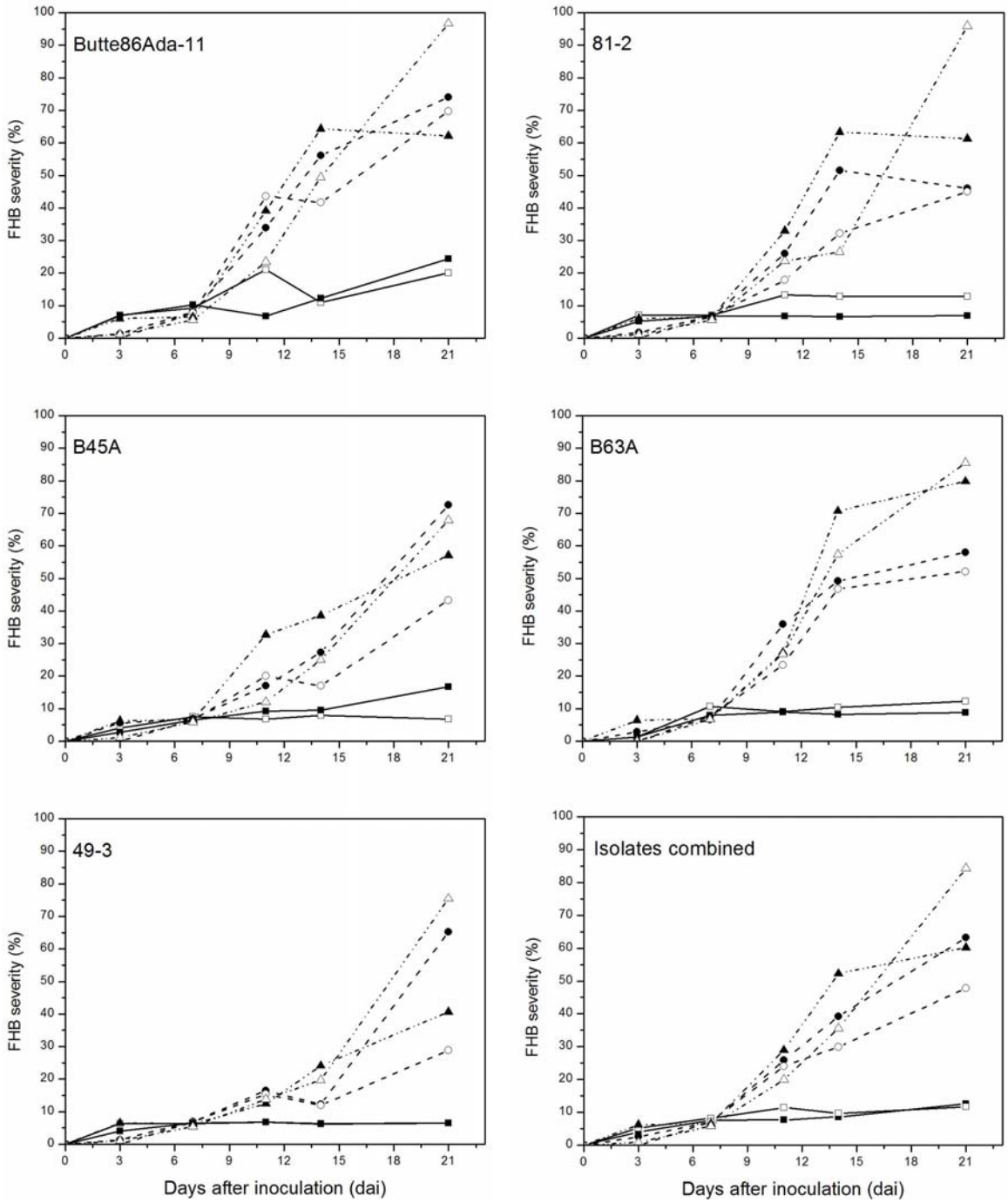

Figure 4.1. Fusarium head blight (FHB) severity (%) observed 0, 3, 7, 11, 14 and 21 dai in spikes of Alsen (——), 2375 (– – –) and Wheaton (– · · –) after point inoculation of a centrally located spikelet with five isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum*. The first and the second runs of the greenhouse experiments are indicated by the closed and the open symbols, respectively.

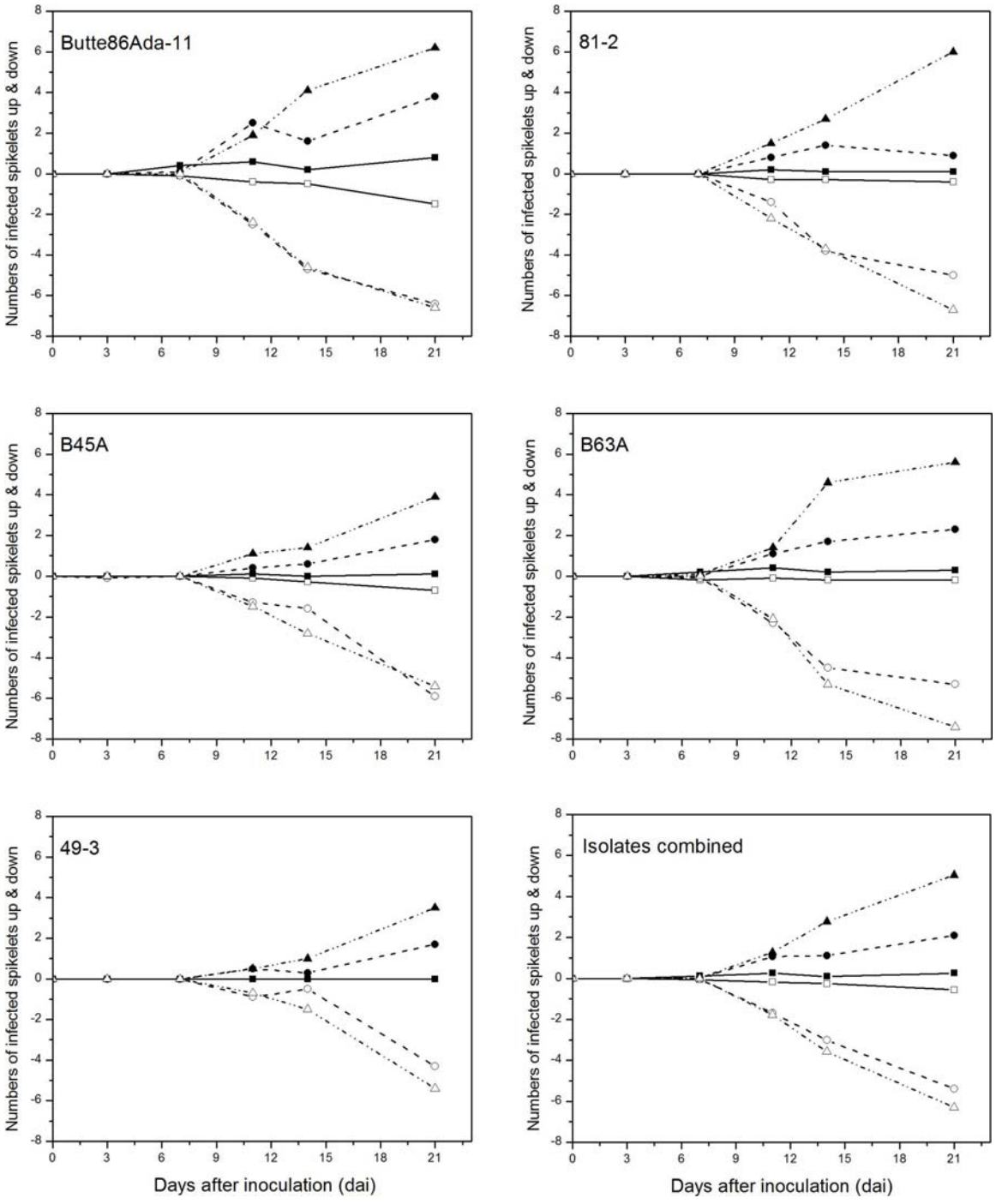

Figure 4.2. Number of symptomatic spikelets observed 3, 7, 11, 14, 21 and 30 dai in spikes of Alsen (——), 2375 (– – –) and Wheaton (– · · –) spray inoculated with five isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum*. The numbers of spikelets above and below the inoculated spikelet is indicated by the closed and the open symbols, respectively.

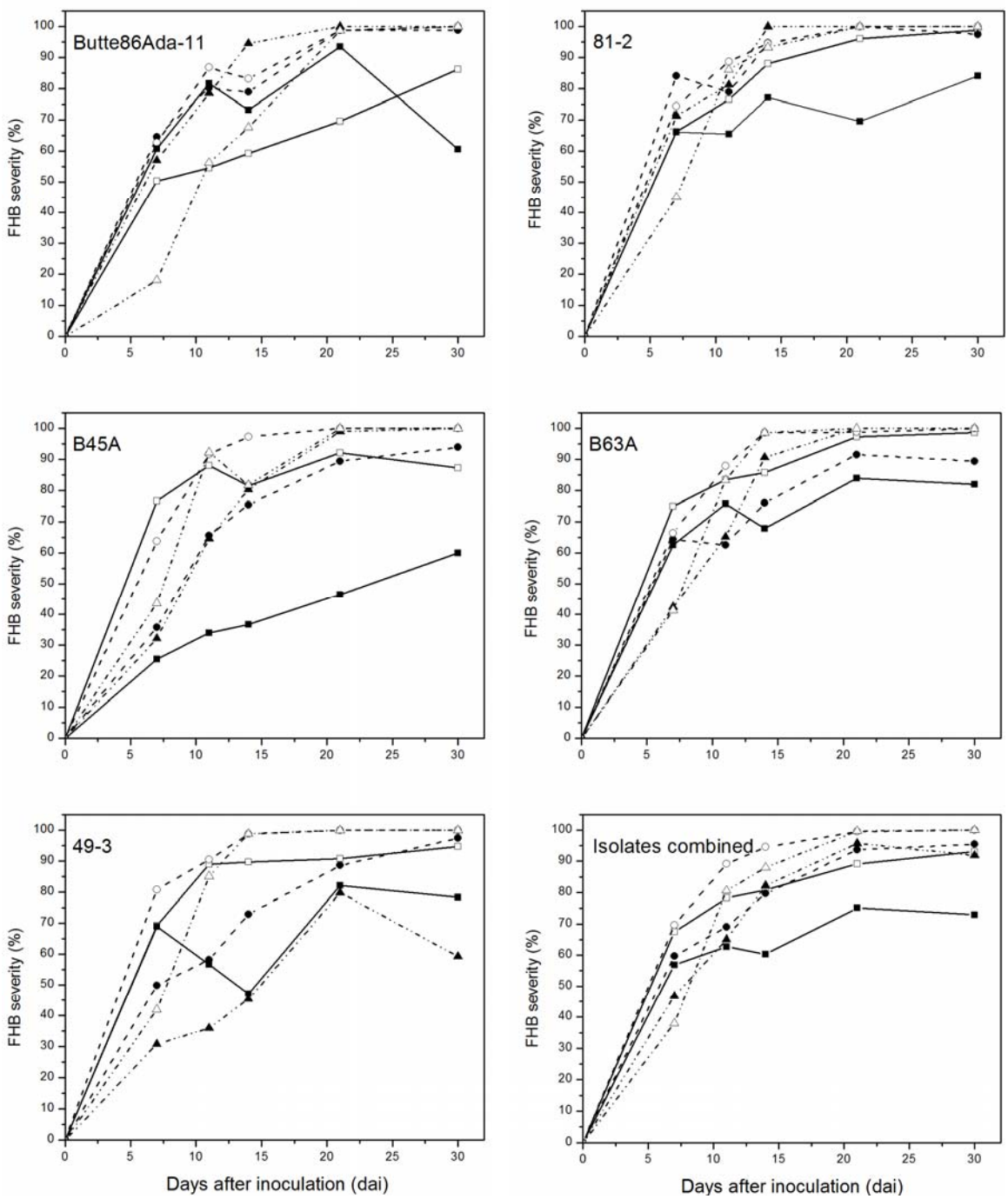

Figure 4.3. Fusarium head blight (FHB) severity (%) observed 0, 7, 11, 14, 21 and 30 dai in spikes of Alsen (——), 2375 (− − −) and Wheaton (− · · −) spray inoculated with five isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum*. The first and the second runs of the greenhouse experiments are indicated by the closed and the open symbols, respectively.

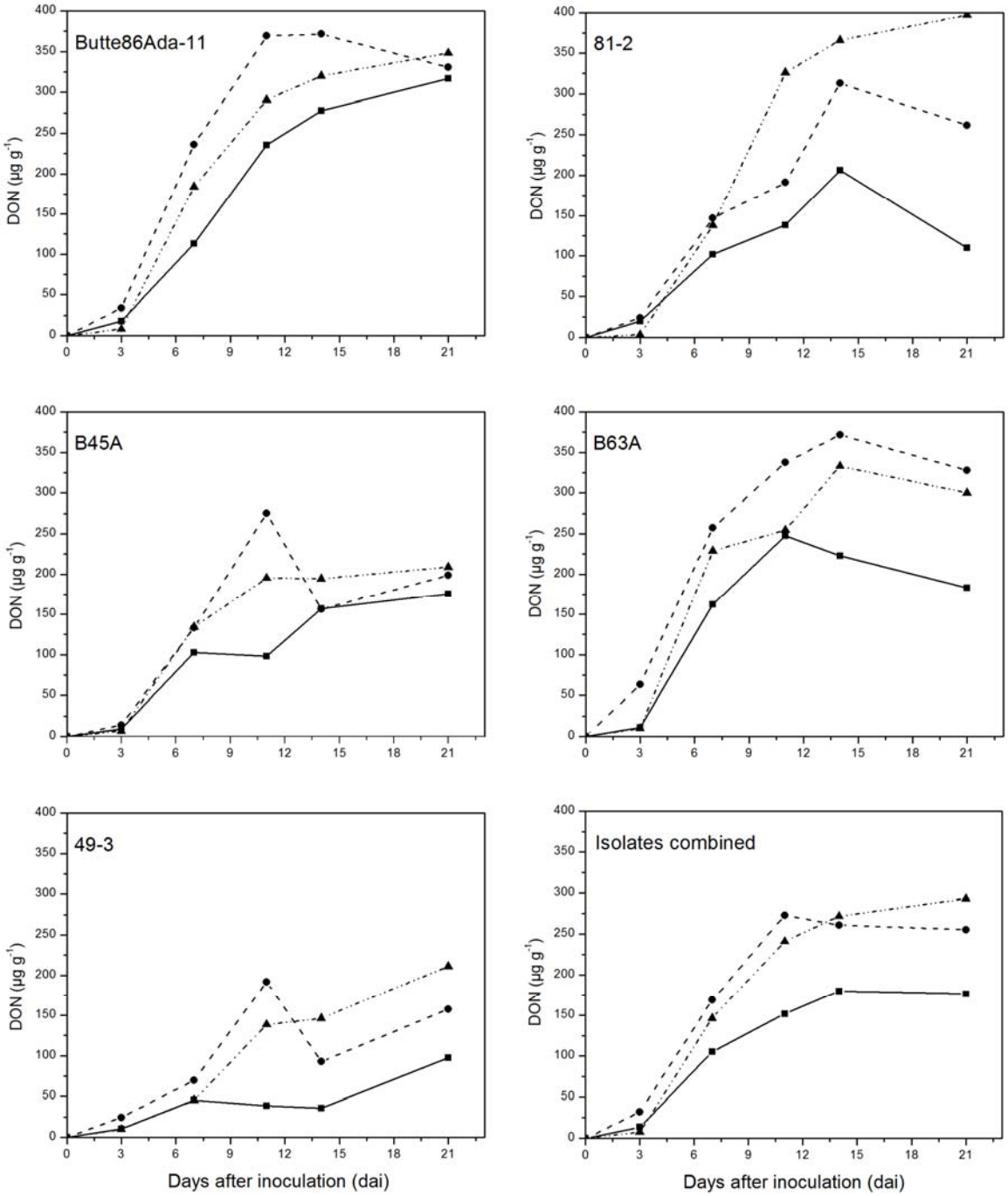

Figure 4.4. Deoxynivalenol (DON, µg g^{-1}) detected 0, 3, 7, 11, 14 and 21 dai in point inoculated centrally located spikelets of Alsen (——), 2375 (– – –) and Wheaton (– · · –) inoculated with five isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum*. Data from runs 1 and 2 are combined.

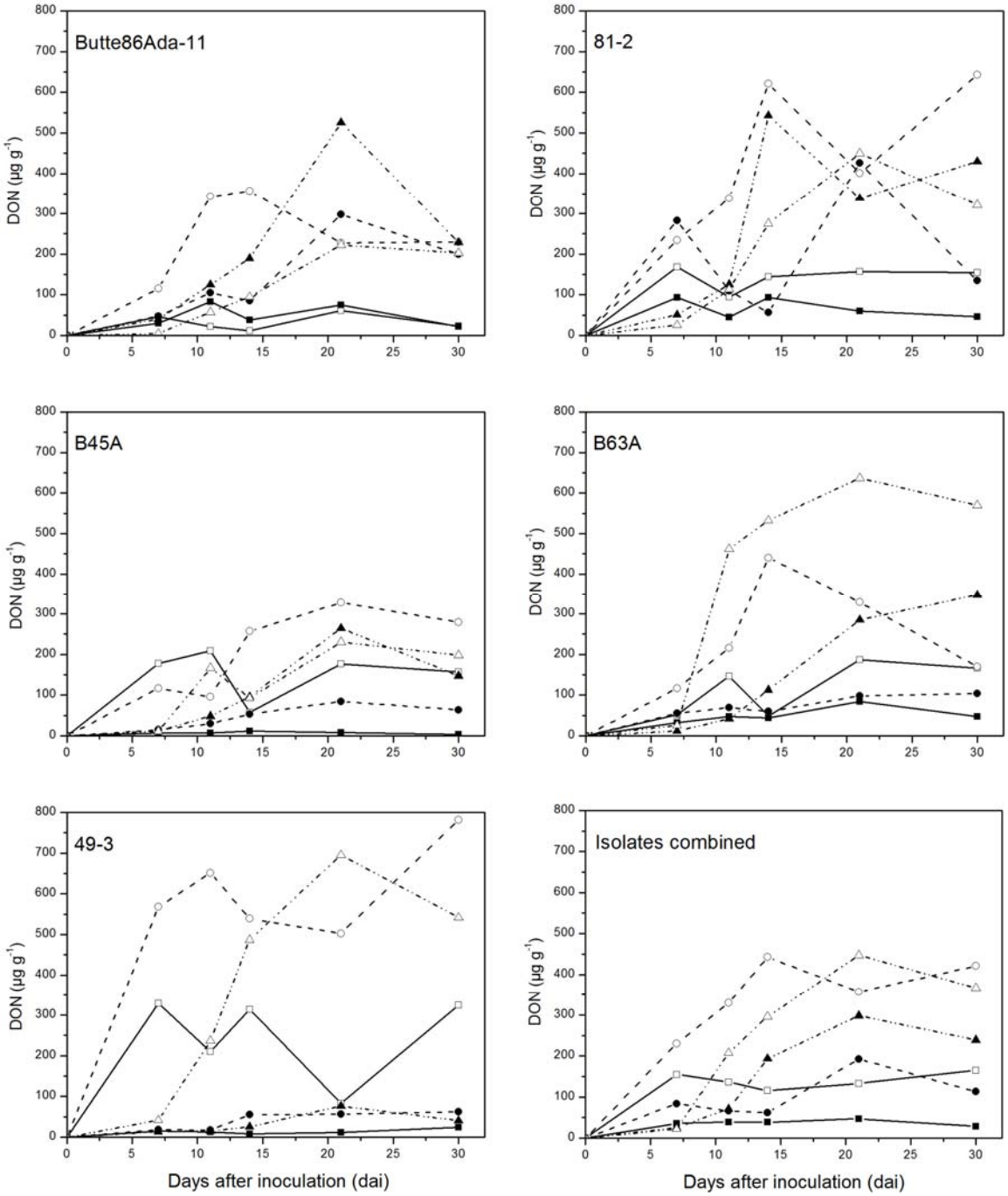

Figure 4.5. Deoxynivalenol (DON, µg g^{-1}) detected 0, 7, 11, 14, 21 and 30 dai in kernels from spikes of Alsen (——), 2375 (– – –) and Wheaton (– · · –) spray inoculated with five isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum*. The first and the second runs of the greenhouse experiments are indicated by the closed and the open symbols, respectively.

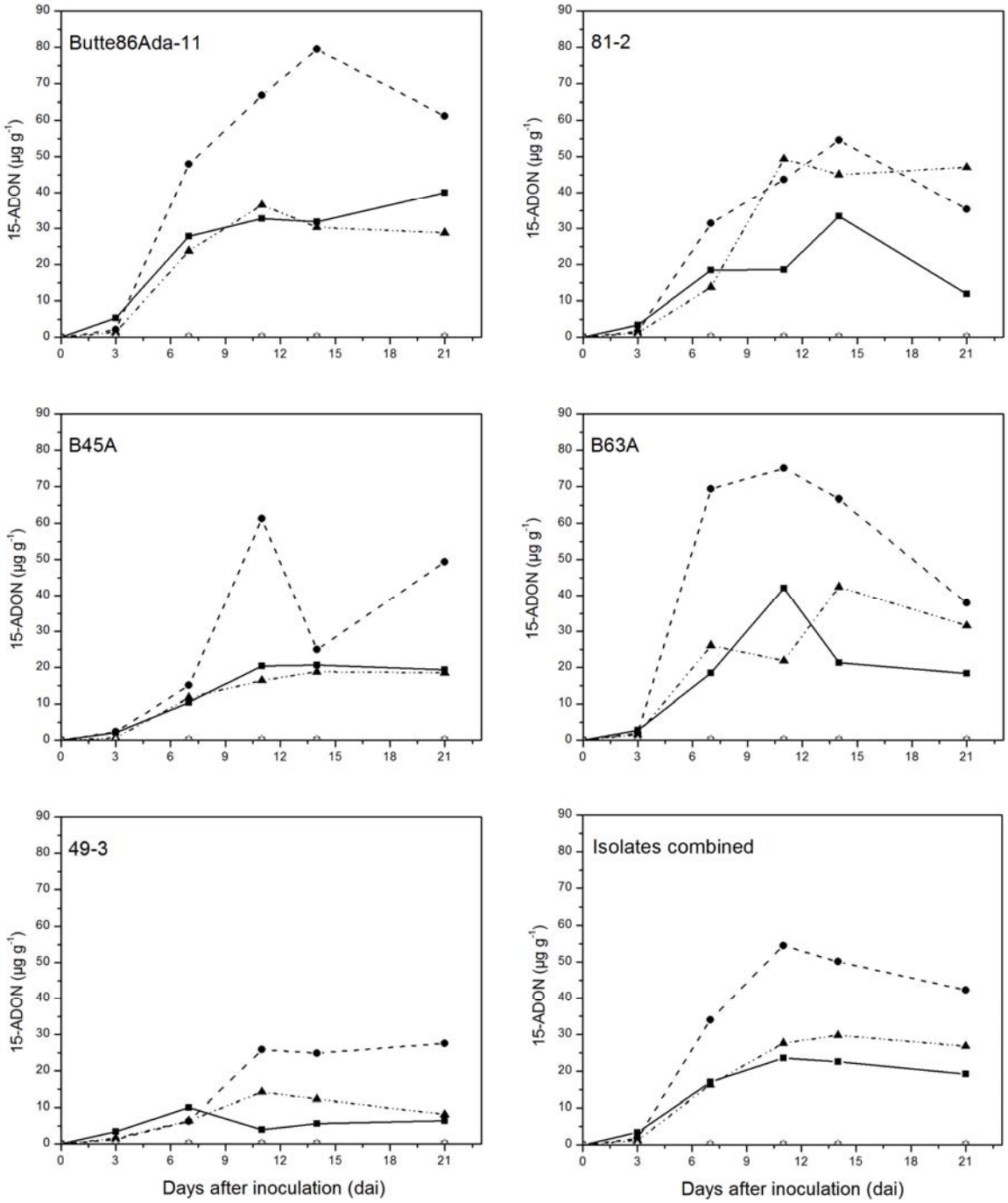

Figure 4.6. 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) detected 0, 3, 7, 11, 14 and 21 dai in point inoculated centrally located spikelets of Alsen (——), 2375 (– – –) and Wheaton (– · · –) inoculated with five isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum*. The first and the second runs of the greenhouse experiments are indicated by the closed and the open symbols, respectively.

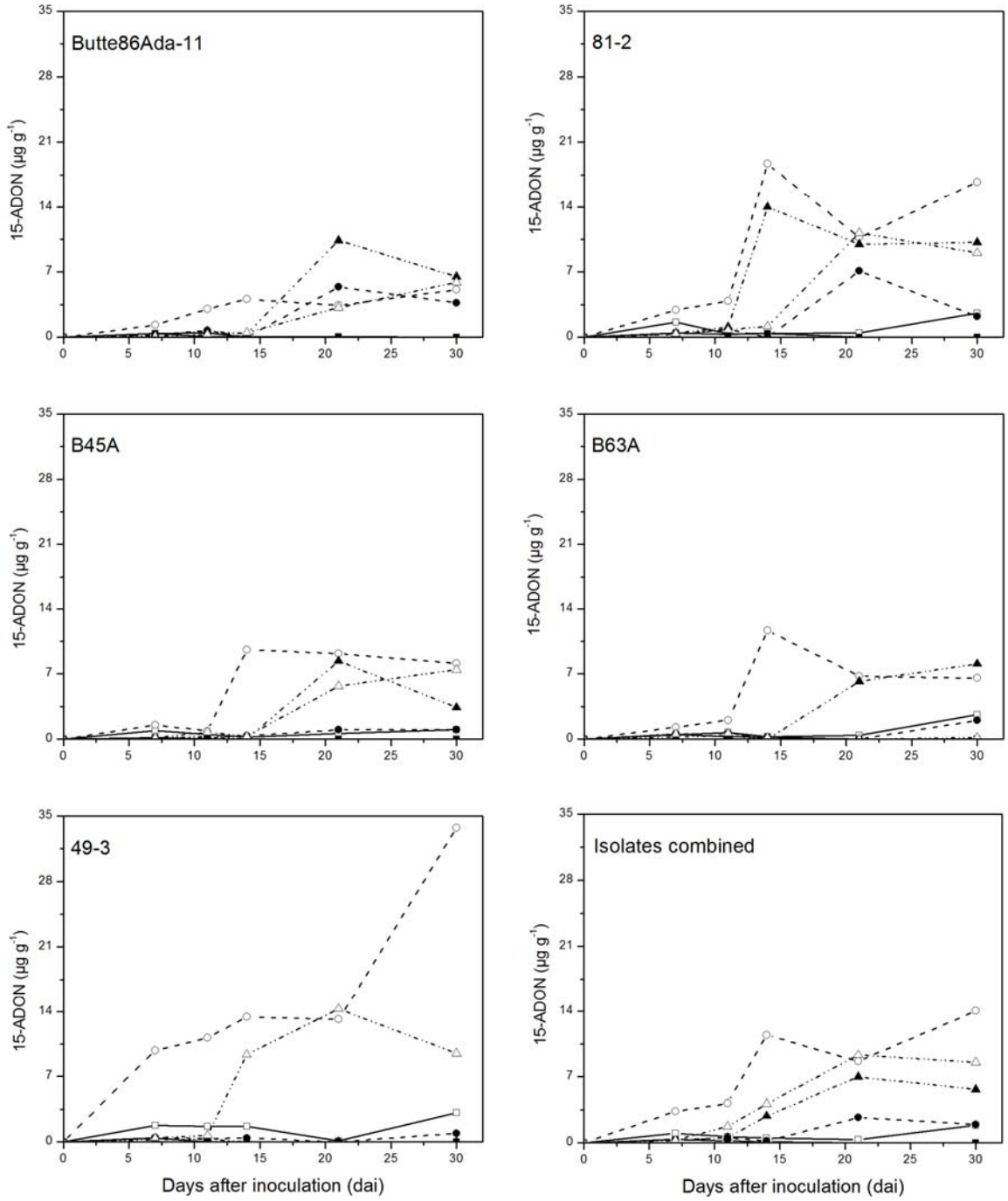

Figure 4.7. 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) detected 0, 7, 11, 14, 21 and 30 dai in kernels from spikes of Alsen (——), 2375 (– – –) and Wheaton (– · · –) spray inoculated with five isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum*. The first and the second runs of the greenhouse experiments are indicated by the closed and the open symbols, respectively.

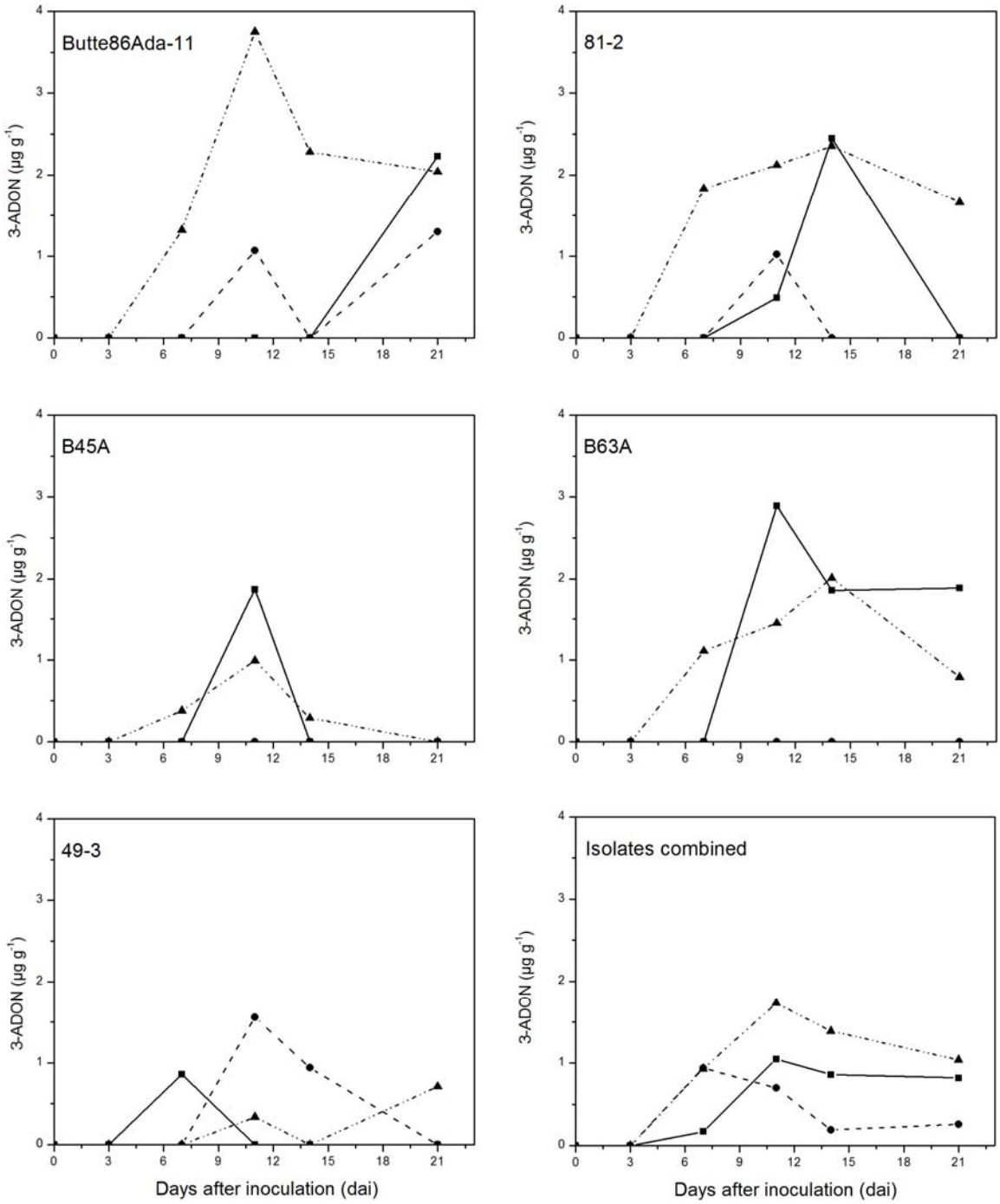

Figure 4.8. 3-acetyldeoxynivalenol (3-ADON, µg g^{-1}) detected 0, 3, 7, 11, 14 and 21 dai in point inoculated centrally located spikelets of Alsen (——), 2375 (– – –) and Wheaton (– · · –) inoculated with five isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum*. The first and the second runs of the greenhouse experiments are indicated by the closed and the open symbols, respectively.

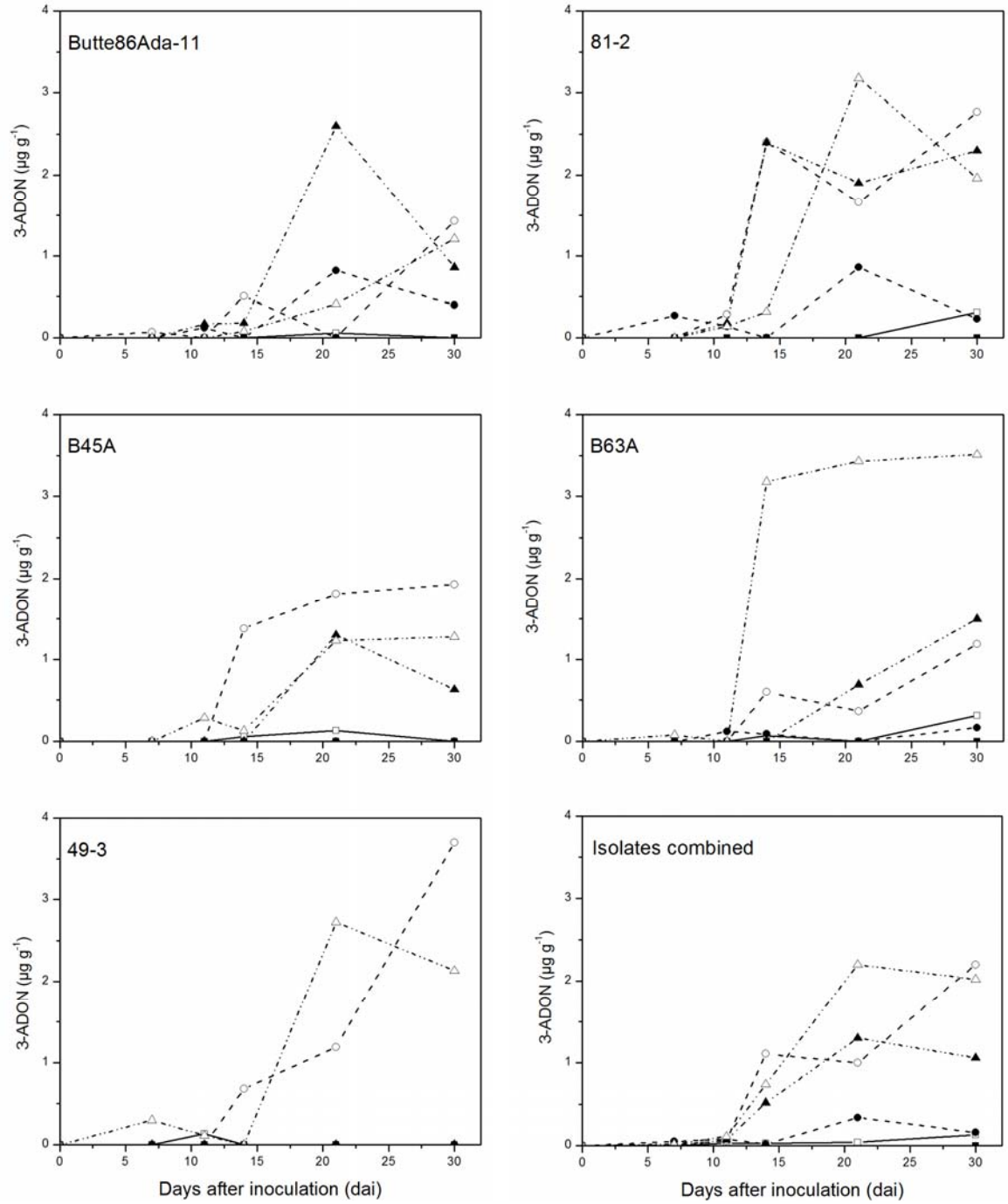

Figure 4.9. 3-acetyldeoxynivalenol (3-ADON, µg g^{-1}) detected 0, 7, 11, 14, 21 and 30 dai in kernels from spikes of Alsen (——), 2375 (– – –) and Wheaton (– · · –) spray inoculated with five isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum*. The first and the second runs of the greenhouse experiments are indicated by the closed and the open symbols, respectively.

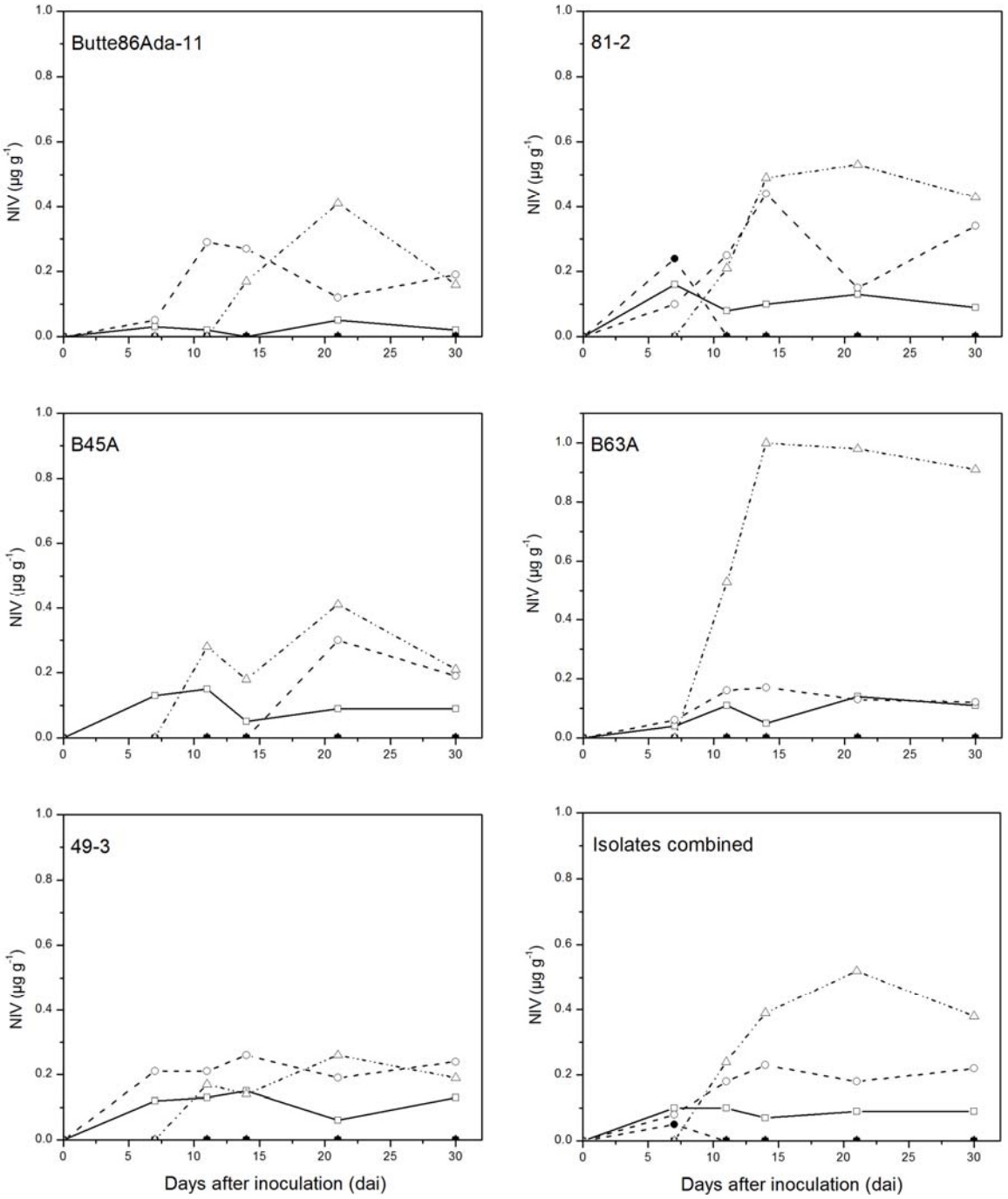

Figure 4.10. Nivalenol (NIV, µg g^{-1}) detected 0, 7, 11, 14, 21 and 30 dai in kernels from spikes of Alsen (——), 2375 (– – –) and Wheaton (– · · –) spray inoculated with five isolates (Butte86Ada-11, 81-2, B45A, B63A and 49-3) of *F. graminearum*. The first and the second runs of the greenhouse experiments are indicated by the closed and the open symbols, respectively.

Chapter 5
Evaluation of Impact of Single Wetting Event on Trichothecene Accumulation

Fusarium head blight (FHB) is an economically important disease of wheat and barley. In North America FHB is primarily caused by *Fusarium graminearum* Schwabe. The disease results in yield and quality losses in infected grain. Mycotoxins, toxic to humans and animals, are also produced in the infected grain by the invading fungus. Environmental factors, host genetics and isolate aggressiveness play an important role in FHB development and subsequent mycotoxins production and accumulation. The availability of free moisture during anthesis promotes FHB development and mycotoxin accumulation. However, the role of moisture, either in the form of rainfall or mist-irrigation at later stages of disease development such as from early dough stage to harvest has not been fully documented. The objective of this study was to examine the impact of free environmental moisture, such as from rainfall on disease development and mycotoxin production and accumulation *in planta*. Specifically, the study aims to determine if a single wetting event provided by an irrigation system could result in the leaching of mycotoxins from head tissues. Two runs of a greenhouse experiment established as a randomized complete block design with five replications were undertaken in spring 2009. Two single *F. graminearum* isolates (Butte86Ada-11 and 81-2) were used to inoculate spikes of three wheat cultivars: Alsen (FHB-resistant, Sumai 3 derived), 2375 (FHB-moderately resistant) and Wheaton (FHB-susceptible). At anthesis, plants were spray inoculated with macroconidial inoculum (5×10^4 conidia ml^{-1}) at the rate of 1.7 ml per pot. On each wetting event/sampling day, i.e. 7, 14, 21 or 28 dai, four primary spikes per pot were marked in 10 arbitrarily selected pots from each cultivar/isolate treatment combination and FHB severity was assessed. Five pots of each of the two cultivar/isolate treatments were transferred to the wetting chamber. A single wetting event was imposed by running irrigation continuously for six hours delivering the equivalent of a 132.8 mm rainfall event. The remaining five pots of each of each cultivar/isolate treatment were left on the greenhouse bench. At the end of wetting event, the marked spikes were sampled both from the plants which received the wetting treatment and those that did not and these spikes were analyzed for mycotoxins. Fifty milliliter samples of run-off water were taken 3 hours after the start of irrigation and immediately after the wetting treatment concluded, these samples were freeze dried and analyzed for mycotoxins. The results showed that FHB severities were similar in plants that received the wetting treatment and their comparable control. However, the levels of DON and other mycotoxins detected were significantly lower in the plants receiving a single wetting event

compared to the control. The differences between the levels of mycotoxins in the plants which went under a wetting treatment and control were higher at later growth stages. Alsen had significantly lower FHB severities and levels of DON, 15-ADON and 3-ADON than the other cultivars tested. DON and 15-ADON were detected in run-off water from the inoculated heads of all cultivars examined. Based on the results of this study, it can be concluded that DON and its derivatives produced *in planta* can be leached out from the host tissues by free water on contact with plant surfaces.

5.1 Introduction

Fusarium head blight (FHB) of wheat and barley in the United States is primarily caused by *F. graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] (Bai et al., 2001; Schroeder and Christensen, 1963; Wilcoxson et al., 1992). Although FHB has been common, severe and well documented in the past, its reemergence in the 1990's (Windels, 2000) resulted in economic losses of over $2.7 billion in the northern Great Plains and central United States between 1998 and 2000 (Nganje et al., 2002).

Warm temperatures and extended periods of moisture at and/or shortly after anthesis favor the infection and colonization of wheat tissues by *F. graminearum*. In severely infected and/or early infected spikelets, kernels may fail to develop. Grains that do develop in infected spikelets are generally smaller and lighter frequently being referred to as 'tombstones' or 'scabby kernels' because of their shriveled and chalky appearance (Abramson et al., 1987; Dickson and Mains, 1929; Johnson and Dickson, 1921; Parry et al., 1995). *Fusarium* damaged grain tends to exhibit weaker dough properties and unsatisfactory baking quality, making the marketing and processing of the grain from *Fusarium*-damaged crops difficult (Bechtel et al., 1985; Dexter et al., 1996; Dexter et al., 1997; Wang et al., 2005). *F. graminearum* produces a range of mycotoxins, including deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), and nivalenol (NIV) in infected grain (Nasri et al., 2006; Pirgozliev et al., 2003). Wheat from US production fields infected by *Fusarium* have routinely tested positive for most of these mycotoxins (Abramson et al., 1987; McMullen et al., 1997). These mycotoxins are phytotoxic (Ehrlich and Daigle, 1987; Packa, 1991) and also hazardous to humans and animals (Christensen and Kernkamp, 1936; Marassas et al., 1984), thus making infected grain unfit for food or feed.

The role of DON in determining the relative aggressiveness of isolates has not been well defined, but it is clear that DON is not essential for pathogenesis (Alexander et al., 1997; Dyer et al., 2005; Eudes et al., 2001; Proctor et al., 1995). The reported time from inoculation until the detection of DON *in planta* varies from 26 hrs (Chen et al., 1996) to 4 days (Savard et al., 2000), which supports studies indicating that DON is not required for initial infection and colonization. Miller and Young (1985) reported that the production and accumulation of DON in infected heads started about three days after infection, peaked at six weeks after infection and declined thereafter. Similar peaks in DON and declines before harvest have also been reported in barley (Prom et al., 1999) and in the naturally infected wheat fields (Scott et al, 1984). Some studies have indicated that DON may peak earlier than six weeks (Argyris et al., 2003; Culler et al., 2007).

Environmental factors, primarily moisture, have an important influence on mycotoxin production in *Fusarium*-infected cereals. Moisture in the form of rainfall or relative humidity, during or shortly after anthesis has been linked to higher FHB incidences, FHB severities and DON accumulation (Abramson et al., 1987; Atanasoff, 1920; Rohácik and Hudec, 2005; Tuite et al., 1990). The specific environmental factors triggering, mycotoxin synthesis and influencing accumulation in the infected host are, however, not well understood (Mesterházy, 1999), although the toxin concentration in harvested grain is likely the result of complex interactions between the host, the pathogen and the environment (Lemmens et al., 2004).

The application of supplemental moisture, in the form of mist-irrigation, is common in field nurseries screening wheat germplasm for resistance to FHB. Supplemental moisture is generally provided between anthesis, when most nurseries are inoculated, and the time of disease rating, generally at the early dough (GS 80-83) stage about three weeks after anthesis. The impact of precipitation after disease rating on the accumulation of *Fusarium*-mycotoxins, however, has been less studied. Lemmens et al. (2004), studied two different levels of mist-irrigation treatments (no irrigation and irrigation for 26 days after anthesis) and reported lower levels of DON in mist-irrigated wheat lines compared to that of plants that did not receive mist-irrigation. Culler et al. (2007) also reported lower levels of DON with increased environmental moisture, which was especially evident in the susceptible cultivar, Wheaton. They observed generally lower DON concentrations in grain developing under misting treatments which ran for 31-32 days after inoculation compared to 15-16 days misting treatments. The differences in DON were, however, not significant at all sampling times. In contrast Cowger et al. (2009) observed increases in FHB severities, *Fusarium* damaged kernels (FDK, percent of visually scabby kernel in 100 seeds

collected randomly) and DON levels when mist-irrigation was applied for 20 or 30 days post-anthesis compared to misting for 10 days post-anthesis or no misting.

In our earlier study (Chapter 3), the impact of supplemental moisture, in the form of mist-irrigation applied for 14, 21, 28 and 35 days after inoculation (DAI), on FHB disease development and mycotoxin accumulation was examined. Our results indicated that increased durations of misting increase the kernel infection as observed by the increased levels of visually scabby kernel (VSK). However, the levels of DON and other mycotoxins tested were frequently significantly lower in treatment which received misting for extended periods compared to the treatments receiving misting for lesser durations. Since the differences in FHB severities between the mist-irrigation duration treatments were small and non-significant, lower levels of DON observed in the treatment receiving mist-irrigation for 35 DAI cannot be attributed to differences in the FHB severities. As DON and its derivatives are water soluble compounds (Bensassi et al., 2010; Böhm et al., 2008; Cahill et al., 1999; Hazel and Patel, 2004; Kang and Buchenauer, 1999) it has been speculated that a decline in DON toward harvest may be promoted by the leaching of mycotoxins from the host tissues. This study was designed to test the hypothesis that free moisture can leach DON and other mycotoxins from the host tissues. The objective of this study was to examine the impact of free environmental moisture, simulated by irrigation systems on disease development and mycotoxin production and accumulation *in planta*. Specifically, the study aims to determine if a single wetting event could result in the leaching of mycotoxins from head tissues.

5.2 Materials and methods

The study was undertaken in spring 2009 and consisted of a greenhouse experiment established as a randomized complete block design and repeated over time. Each experiment had five replications with a replication consisting of a single pot of five plants. Wheat cultivar, pathogen isolates and wetting treatments were examined as factorial treatments. Three wheat cultivars; Alsen, 2375 and Wheaton, were included in the study as were two isolates of *F. graminearum*. The wetting treatments consisted of a single 6 hour wetting event.

The wheat was grown in the greenhouse as described in Chapter 4. The planting date for each cultivar was staggered so that anthesis of the three cultivars would not coincide. As only one misting chamber was available, staggered planting allowed the collection of run-off water from each cultivar separately.

The two isolates included in the study, Butte86Ada-11 and 81-2, were cultured in Petri plates on mung bean agar (MBA) and the macroconidia harvested from seven day old cultures as described previously (Chapter 3). The harvested inoculum was adjusted to a concentration of 8×10^5 macroconidia ml^{-1} and was stored in 1L Nalgene® polyethylene bottles (Nalgene Nunc International Co., Rochester, NY) at -20ºC until used.

Prior to use the inoculum was thawed, diluted to 5×10^4 spores ml^{-1} and polysorbate (Tween-20, Fisher Biotech, Fair Lawn, NJ; 20 ml per 8 L of inoculum) was added as a wetting agent. A 2 ml sample of inoculum was diluted to 1×10^5 spores ml^{-1} and tested for germination by spreading 0.5 ml of the diluted inoculum over the surface of potato dextrose agar (PDA) media in a Petri plate. The percentage of germinated macroconidia visible after 8 hour incubation at room temperature was recorded. The minimum germination observed in the study was 90%.

Plants were inoculated at anthesis (GS 65). Pots to be inoculated were set on the ground in 2 m long rows. The heads of these plants were inoculated with inoculum (5×10^4 spores ml^{-1}) at the rate of 1.7 ml per pot using a CO_2-powered backpack sprayer as described in Chapter 3. Inoculated plants were incubated in a dew chamber for 72 hours as described in Chapter 4. After the dew period, plants were returned to the greenhouse.

Wetting treatments consisted of a single 6 hour wetting event imposed 7, 14, 21 or 28 days after inoculation. Plants subjected to a wetting treatment were placed inside a wetting chamber [the dew chamber - internal dimension of 2.1 m (length) ×1.1 m (width) ×1.8 m (height) –including 0.3 m deep water bath at the bottom - fitted with a sprinkle irrigation system]. Two sprinkler nozzles (8Q-FLT 8’ Quarter Circle Flat Plastic MPR Nozzle; Nelson Irrigation Corporation, Walla Walla, WA) were placed in opposite corners of the dew chamber (2.1 m apart) and 1.2 m above the mesh surface on which the pots were placed. The wetting system delivered the equivalent of 132.8 mm irrigation over the 6 hour wetting period. The surface of the potting media in each pot was covered with plastic sheeting to prevent water entering into potting media, and to deflect the water flow to the water bath area below the mesh surface supporting the pots allowing for the collection of run-off water.

Ten pots from each cultivar/isolate treatment combination were arbitrarily selected on the date of each wetting event (7, 14, 12 or 28 dai). In each pot four primary spikes were marked on peduncle with permanent marker and growth staged according to Zadoks et al. (1974). FHB severity was assessed for all marked heads, by counting the total number of spikelets and numbers of symptomatic spikelets per head. FHB severity for each spike was calculated by dividing the number of symptomatic spikelets by total number of spikelets and multiplying the

result by 100. After the assessment of FHB severity, five pots of each of the two cultivar/isolate treatments were transferred to the wetting chamber. The single wetting event was imposed by running the irrigation system continuously for six hours. The remaining five pots of each of the two cultivar/isolate treatments were left on the greenhouse bench.

At the end of a wetting event, the marked spikes were sampled both from the plants which received the wetting treatment and those that did not. Four primary spikes sampled from a single pot were bulked and frozen immediately at -20ºC and kept until processed for mycotoxin analysis. Prior to mycotoxin analyses the head samples were dried under forced air at 95ºC for 72 hours. Wet and dry weight of the bulk head samples were taken before and after drying, respectively. The bulk head samples were then ground and analyzed for mycotoxins following the protocol of Mirocha (1998) as modified by Fuentes et al. (2005) as described in Chapter 4. The DON, 15-ADON and 3-ADON concentrations obtained for the spike samples were adjusted by multiplying by the ratio of wet to dry weight for each bulked head sample.

The run-off water collected below the plants was sampled two times during each wetting event. Fifty milliliter samples of run-off water were taken 3 hours after the start of irrigation and immediately after the wetting treatment concluded. Three replicate samples were collected at each sampling time. Sampled water was filtered through eight layers of cheesecloth to remove any plant parts and potting media, and stored in 50 ml polypropylene centrifuge tubes (Corning® Incorporated, Lowell, MA) at -20ºC until prepared for mycotoxin analysis. Frozen run-off water samples were prepared for mycotoxin analysis by being evaporated in a freeze-dryer (Virtis-Sentry; Model 24XDX48, The Virtis Company, Gardiner, NY) then the solids were re-suspended in 5 ml acetonitrile/water (84:16 v:v) and analyzed for mycotoxins as indicated above.

DON data was transformed using square root transformation, while the 15-ADON and 3-ADON data were log transformed to achieve homoscedasticity. FHB severity data was analyzed untransformed. Data were analyzed using PROC MIXED procedure for randomized complete block design in SAS v 9.0 (SAS Institute, Cary, NC). Since the two runs of the experiment were significantly different for all variables, separate analyses were carried out for each run. Means were separated using LSD and the output were letter grouped using SAS macrocode PDMIX800 (Saxton, 1998). Spearman rank correlation analyses were carried out using PROC CORR in SAS. Graphs were created using OriginPro 8.1 SR0 (OriginLab Corporation, Nortampton, MA).

5.3 Results

5.3.1. Disease severity

FHB severity was assessed before the imposition of a wetting event. Thus, the FHB severities observed in the plants that received a wetting treatment and those that did not, serve as check of the inoculation. Symptoms of FHB had developed in all inoculated heads by 3 dai (GS 68/69), although FHB severities were recorded only from 7 dai (GS 71) onward. Generally, FHB severities were higher in run 1 than run 2. By 7 dai (GS 71) FHB severities ranged from 47-88% in Alsen, 56-87%% in 2375 and 28-52%% in Wheaton (Appendix 21). FHB severities observed in plants that received a wetting event were statistically similar to plants that did not in all treatment combinations except for the 7 dai wetting of 2375 in run 1. In 2375 at 7 dai in the run 1, FHB severities were significantly higher in the plants that underwent the wetting treatment than plants in the control treatment.

Alsen had higher FHB severities at earlier growth stages [GS 71 (7 dai) and 77/78 (14 dai)] compared to that of either 2375 or Wheaton. As the wheat matured, 2375 and Wheaton, however, had higher FHB severities than Alsen. Wheaton had lower FHB severities than 2375 at all growth stages, however, these differences were generally not statistically significant. By 21 dai (GS 83) FHB severities were at or close to 100% in 2375 and Wheaton.

Isolates Butte86Ada-11 and 81-2 resulted in equivalent FHB severities in each cultivar. Though neither of the isolates generated 100% FHB severities in Alsen, FHB severities of 100% were recorded in 2375 and Wheaton by 21 dai (GS 83) in both runs.

5.3.2. Deoxynivalenol (DON)

DON was detected in inoculated heads in both runs at 7 dai (GS 71) and at levels of more than 20 $\mu g\ g^{-1}$ (Figure 5.1, Appendix 21). The levels of DON were generally higher in run 1 than 2. When combined over isolates and cultivars, DON levels in the plants receiving a wetting treatment were significantly lower than the control treatments except at 7 dai. In the wetting treatment imposed 7 dai (GS 71), the levels of DON were statistically similar to the control treatment.

The impact of a single wetting event on the DON was evident in each of the cultivars examined. DON levels were numerically lower in Alsen following each of the six hours wetting treatments when compared to the relevant control. In 2375 and Wheaton, DON levels were significantly lower in plants receiving a wetting treatment than the control treatments, except for the 7 dai (GS 71) treatment in both runs (Figure 5.1, Appendix 21), where the DON levels were

statistically similar. Alsen generally had significantly lower DON levels than either 2375 or Wheaton in both the control and wetting treatments at all growth stages examined. Wheaton generally had significantly higher levels of DON than 2375 in both the control and wetting treatments.

Inoculation with isolates Butte86Ada-11 and 81-2 generally resulted in statistically similar levels of DON. Isolate 81-2 did, however, tend to generate higher DON levels in 2375 and Wheaton, while isolate Butte86Ada-11 resulted in higher DON levels in Alsen in run 1. In run 2, inoculation with isolate Butte86Ada-11 generally resulted in higher DON levels in each of the three cultivars.

5.3.3. 15-acetyldeoxynivalenol (15-ADON)

The levels of 15-acetyldeoxynivalenol detected were at the range of 0.5-36 μg g^{-1}, which were not more than 19% of the DON level in a given sample. The levels of 15-ADON, when combined over cultivars and isolates, were significantly lower in plants receiving six hours of wetting (7 dai: 1.80 μg g^{-1}, 14 dai: 3.35 μg g^{-1}, 21 dai: 4.91 μg g^{-1}, 28 dai: 4.78 μg g^{-1}) than in the corresponding control (7 dai: 3.48 μg g^{-1}, 14 dai: 6.77 μg g^{-1}, 21 dai: 9.12 μg g^{-1}, 28 dai: 8.70 μg g^{-1}).

Except in Alsen at 28 dai (GS 85) in run 1, and at 7 and 21 dai (GS 71 and 85) in run 2, significantly lower levels of 15-ADON were detected in all cultivars receiving the wetting treatment compare to the corresponding control (Figure 5.2, Appendix 21). In the Alsen exceptions, though the 15-ADON was lower in plants receiving a single wetting event than the control, the difference was not statistically significant. Generally, Alsen had lower levels of 15-ADON than the other cultivars tested. The moderately susceptible cultivar 2375 had the highest levels of 15-ADON, except at 14 and 21 dai (GS 77/78 and 83) in the control treatment of the run 1, where Wheaton and Alsen, respectively, had the highest levels of 15-ADON. Isolates Butte86Ada-11 and 81-2 generally resulted in comparable levels of 15-ADON.

5.3.4. 3-acetyldeoxynivalenol (3-ADON)

The range of 3-ADON detected was 0.23-4.63 μg g^{-1} (Figure 5.3, Appendix 21) which meant that 3-ADON was not more than 2.2% of the DON detected in a given sample. When combined over cultivars and isolates, plants receiving a single wetting treatment had lower levels of 3-ADON than the control, though the differences were not statistically significant. Lower levels of 3-ADON were also apparent in each of the cultivars examined under wetting treatment.

Generally 2375 had the highest levels of 3-ADON in run 1 and Wheaton had the highest levels of DON in run 2. Isolates Butte86Ada-11 and 81-2 generally resulted in statistically similar levels of 3-ADON. However, isolate Butte86Ada-11 did tend to produce higher 3-ADON levels in Alsen and Wheaton, and isolate 81-2 generally produced higher 3-ADON levels in 2375.

5.3.5. Mycotoxins in run-off water

The water applied to the plants during each of the wetting events was collected and examined for the presence of mycotoxins. DON and 15-ADON were detected in the run-off water (Figure 5.4, Appendix 21) collected at each of the wetting events. These mycotoxins were detected in run-off water sampled both three hours after the start of a wetting event and immediately after the conclusion of the six hour wetting event. Generally higher amounts of DON were detected in the runoff water from cultivar 2375 and the least amount of DON was detected in water washed out from Alsen, reflecting the level of DON present in the plant tissues. The levels of DON detected in water was lower in water collected from the first wetting event (7 dai) compared to samples taken from wetting events conducted at later growth stages.

Trace amounts of 15-ADON were detected in the run-off water from 2375 ($\leq$0.03 $\mu g\ g^{-1}$) and Wheaton ($\leq$0.02 $\mu g\ g^{-1}$) though none was detected in the run-off water from Alsen.

5.3.6. Correlations

The correlation coefficients between FHB severity and DON (Table 5.1 and 5.2) were more than 0.4 and significant ($P < 0.05$) except for the control treatment at 14 dai (GS 77/78), and 14 and 21 dai (GS 77/78 and 83) wetting treatment in run 1, where the correlations were low ($r < 0.3$) and non-significant ($P < 0.05$). No specific pattern was evident in the correlation between FHB severity and DON with the growth stages of wheat. The correlation coefficient of FHB severity and DON usually declined from 7 to 14 dai (GS 71 to 77/78) but increased thereafter. However, in the six hours of wetting treatment in run 2, the correlation between FHB severity and DON declined from the first to the last wetting treatment.

The correlations between DON and 15-ADON were high ($r = 0.56$-0.89) and highly significant ($P < 0.01$). The correlation between DON and 3-ADON were also high ($r > 0.46$) and significant ($P < 0.05$), except in the 21 dai (GS 83) samplings for the wetting treatment in run 1, where it was low ($r = 0.07$) and non-significant.

5.4 Discussion

Favorable environmental conditions are key to disease development in the presence of a susceptible host and a virulent pathogen. Moisture is a vital environmental parameter facilitating infection and disease development by fungal pathogens (Agrios, 1997). The development of FHB and accumulation of DON in wheat has been linked with rainfall and/or high relative humidity at and shortly after anthesis (Abramson et al., 1987; Atanasoff, 1920; McMullen et al, 1997; Rohácik and Hudec, 2005; Sutton, 1982). The application of supplemental moisture, in the form of mist-irrigation, for approximately 21 days after inoculation at anthesis is accepted as a way of facilitating disease development and is now a standard practice in field nurseries screening wheat germplasm resistant for FHB (Rudd et al., 2001). However, the impact of free moisture after disease rating on the accumulation of mycotoxins has not been explored. The objective of this study was to examine the impact of free moisture, such as that from irrigation systems or rainfall, on FHB disease development and mycotoxin accumulation *in planta*.

In this greenhouse study, FHB severity was assessed before the plants were subjected to each of the six hours of wetting treatments applied. As disease progression is relatively slow we would not expect that FHB severity would change over the period that the plants were in the wetting chamber or expect that the wetting of plants would have an impact on the visible symptoms of FHB. It might however be assumed that a greater availability of free moisture may promote later fungal colonization and presumably also mycotoxin accumulation. In this study there were no differences in the FHB severities observed in the plants that received the wetting treatment and the control plants. In contrast, mycotoxin accumulation was significantly impacted by the wetting treatment, at each wetting events (7, 14, 21, or 28 dai). The levels of DON, 15-ADON and 3-ADON generally were significantly lower in the heads of plants that received a wetting treatment than in the respective control. The reductions in the levels of mycotoxins in plants that received a six hour wetting treatment were evident at each of the four wetting events. The difference in the levels of mycotoxins between plants that received a wetting treatment and the control increased both with the maturation of wheat and the overall level of mycotoxins present in the heads. This suggests that the impact of free moisture is dependent both on the concentration of toxins in plant tissues and perhaps physiological state of the plant tissues. It is considered that one route of loss of toxins from the tissues of plants receiving a single wet period may be leaching since DON and its derivatives are water soluble compounds (Bensassi et al., 2010; Böhm et al., 2008; Cahill et al., 1999; Hazel and Patel, 2004; Kang and Buchenauer, 1999). The water solubility of these mycotoxins allows DON, 15-ADON and 3-ADON to be transported

through phloem explaining the detection of mycotoxins by Kang and Buchenauer (1999) in wheat head tissues not invaded by *Fusarium* hyphae. A similar finding has also been reported by Snijders and Kretching (1992). In addition to the lower levels of DON and its derivatives in plants subjected to a wetting treatment, DON and 15-ADON were also detected in the run-off water collected during and after the wetting event. It should be noted that the leached mycotoxins were detected in 50 ml samples taken from the 576 L of water collected at the bottom of the misting chamber.

The role of genetic resistance in reducing FHB development and mycotoxin accumulation in wheat has been well studied. Atanasoff (1920) was among the first to report a difference in susceptibility to FHB in spring wheat cultivars, which he treated the same agronomically. Other studies have shown higher FHB severity (Schroeder and Christensen, 1963) and subsequently high DON levels in susceptible cultivars when compared to cultivars with some level of resistance (Mesterházy et al., 2003; Mesterházy et al., 2005; Miller et al., 1985; Wilde and Miedaner, 2006). Snijders and Krechting (1992) studied the level of fungal biomass and related this to DON accumulation in resistant and susceptible cultivars of winter wheat sampled 4 and 8 weeks after inoculation with *F. culmorum*. Their findings were similar to those of the present study, in that the levels of mycotoxins were consistently higher in kernels of the susceptible cultivars.

It has been reported that the level of DON in *Fusarium*-infected wheat peaks sometime before harvest maturity and then either declines or stays stable until harvest (Argyris et al., 2003; Culler et al., 2007; Teich, 1989). Miller and Young (1985) reported observing a peak in DON six weeks after inoculation, followed by a decline to harvest. In our study, a peak in DON followed by a decline was observed in all three cultivars, though not in all treatments. In Alsen and 2375 the highest DON level was consistently observed at 21 dai while in Wheaton the highest DON levels were frequently recorded one week earlier at 14 dai. More pronounced losses of DON from a susceptible cultivar may be due to higher initial levels of DON. Culler et al. (2007) reported a similar result with DON levels peaking earlier in susceptible cultivars in comparison to resistant cultivars.

The levels of FHB severities were high in all cultivars tested. The FHB severity in the moderately resistant cultivar Alsen also reached 99%. High FHB severity in Alsen, which was similar to the moderately susceptible and susceptible cultivars, was possibly due to high disease pressure, as the concentration of inoculum used was high. Argyris et al. (2003) and Culler et al.

(2007) reported that the resistance in moderately resistant cultivars can be overwhelmed by high inoculum pressure under highly favorable environmental conditions.

Models have been developed in North America to forecast both FHB development and DON accumulation in infected grains. The first model, DONcast, developed in Canada, primarily for forecasting DON (Hooker et al., 2002a; Hooker et al., 2002b) has been commercialized for use in wheat. The DONcast was developed utilizing environmental parameters measured from seven days pre- to 10 days post-anthesis. Specifically the model considers temperature and rainfall parameters before heading and rainfall data after heading (Hooker et al., 2002). The model has been validated and can explain up to 73% of the variation in DON levels in harvested grain. A model to predict the risk of FHB epidemics has been developed in the US with the cooperation of several universities in the Midwest region (De Wolf et al., 2003). This model relies primarily on temperature, relative humidity and rainfall data collected in the seven days prior to anthesis and the 10 day period beginning at anthesis. The model has been widely deployed and validation studies have demonstrated the model to have 75-80% accuracy in predicting the development of FHB epidemics (Prandini et al., 2009), although this model does not predict DON. Both models utilize the moisture information between seven days pre-anthesis to 10 days post-anthesis. The result of this study suggest that the accumulation of DON may be impacted by rainfall at any time after anthesis, therefore, the inclusion of moisture parameters beyond 10 days post-anthesis will likely increase the accuracy of models to predict trichothecene toxins in wheat.

Table 5.1. Spearman's rank correlations for FHB severity, deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON) and 3-acetyldeoxynivalenol (3-ADON) detected 7, 14, 21 or 28 dai in control and six hour wetting treatments in greenhouse experiment run 1.

	Control			Six hours of wetting		
	Alsen					
	DON	15-ADON	3-ADON	DON	15-ADON	3-ADON
	7 dai					
Severity	0.36^{NS}	0.55^{NS}	0.45^{NS}	-0.16^{NS}	0.13^{NS}	-0.20^{NS}
DON		0.65*	0.70*		0.70*	0.79**
15-ADON			0.89**			0.49^{NS}
	14 dai					
Severity	0.54^{NS}	-0.04^{NS}	0.13^{NS}	0.03^{NS}	-0.01^{NS}	0.08^{NS}
DON		0.55^{NS}	0.68*		0.59^{NS}	0.81**
15-ADON			0.55^{NS}			0.66*
	21 dai					
Severity	0.63*	0.63*	0.39^{NS}	0.38^{NS}	0.42^{NS}	0.58^{NS}
DON		0.66*	0.36^{NS}		0.84**	0.39^{NS}
15-ADON			0.67*			0.49^{NS}
	28 dai					
Severity	0.52^{NS}	0.19^{NS}	0.64*	0.13^{NS}	0.65*	0.16^{NS}
DON		0.83**	0.82**		0.65**	0.48^{NS}
15-ADON			0.75*			0.41^{NS}
	2375					
	7 dai					
Severity	0.71*	0.44^{NS}	0.47^{NS}	0.01^{NS}	0.03^{NS}	-0.10^{NS}
DON		0.76*	0.81**		0.68*	0.95**
15-ADON			0.75*			0.52^{NS}
	14 dai					
Severity	0.70*	0.65*	0.74*	0.22^{NS}	-0.03^{NS}	0.46^{NS}
DON		0.89**	0.89**		0.87**	0.90**
15-ADON			0.76*			0.72*
	21 dai					
Severity	0.45^{NS}	0.19^{NS}	0.42^{NS}	0.04^{NS}	0.18^{NS}	-0.14^{NS}
DON		0.92**	0.92**		0.76*	0.49^{NS}
15-ADON			0.91**			0.42^{NS}
	28 dai					
Severity	0.29^{NS}	-0.17^{NS}	-0.06^{NS}	-	-	-
DON		0.66*	0.79**		0.81**	0.48^{NS}
15-ADON			0.80**			0.53^{NS}
	Wheaton					
	7 dai					
Severity	0.91**	0.90**	0.33^{NS}	0.49^{NS}	-0.10^{NS}	0.26^{NS}
DON		0.90**	0.33^{NS}		0.18^{NS}	0.62^{NS}
15-ADON			0.54^{NS}			-0.07^{NS}
	14 dai					
Severity	0.56^{NS}	0.64*	0.36^{NS}	0.80**	0.77*	0.32^{NS}
DON		0.42^{NS}	0.71*		0.87**	0.65*
15-ADON			0.12^{NS}			0.42^{NS}
	21 dai					
Severity	0.12^{NS}	0.38^{NS}	0.25^{NS}	0.11^{NS}	-0.21^{NS}	-0.52^{NS}
DON		0.75*	0.75*		0.67*	0.39^{NS}
15-ADON			0.76*			0.50^{NS}
	28 dai					
Severity	-	-	-	-	-	-
DON		0.37^{NS}	0.61**		0.78**	0.85**
15-ADON			0.85**			0.96**

** Significant at P < 0.01, * Significant at P < 0.05

Table 5.2. Spearman's rank correlations for FHB severity, deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON) and 3-acetyldeoxynivalenol (3-ADON) detected 7, 14, 21 or 28 dai in control and six hour wetting treatments in greenhouse experiment run 2.

	Control			Six hours of wetting		
	Alsen					
	DON	15-ADON	3-ADON	DON	15-ADON	3-ADON
	7 dai					
Severity	0.56^{NS}	0.77**	0.15^{NS}	0.98**	0.98**	0.96**
DON		0.75*	0.71*		0.98**	0.94**
15-ADON			0.41^{NS}			0.92**
	14 dai					
Severity	0.90**	0.56^{NS}	0.84**	0.90**	0.89**	0.88**
DON		0.77**	0.85**		0.82**	0.98**
15-ADON			0.72*			0.75*
	21 dai					
Severity	0.68*	0.66*	0.73*	0.87**	0.76*	0.87**
DON		0.73*	0.95**		0.76*	1.00**
15-ADON			0.79**			0.76*
	28 dai					
Severity	0.66*	0.54^{NS}	0.44^{NS}	0.30^{NS}	-0.03^{NS}	0.18^{NS}
DON		0.81**	0.94**		0.66*	0.93**
15-ADON			0.78**			0.72*
	2375					
	7 dai					
Severity	0.50^{NS}	0.66*	0.73*	0.50^{NS}	0.58^{NS}	0.49^{NS}
DON		0.90**	0.87**		0.72*	0.71*
15-ADON			0.94*			0.56^{NS}
	14 dai					
Severity	-0.08^{NS}	0.27^{NS}	-0.13^{NS}	0.66*	0.70*	0.26^{NS}
DON		0.12^{NS}	0.59^{NS}		0.48^{NS}	0.52^{NS}
15-ADON			0.56^{NS}			0.14^{NS}
	21 dai					
Severity	0.41^{NS}	0.38^{NS}	0.40^{NS}	-0.34^{NS}	0.44^{NS}	0.69*
DON		0.58^{NS}	0.67*		0.49^{NS}	0.70*
15-ADON			0.90**			0.36^{NS}
	28 dai					
Severity	-	-	-	-	-	-
DON		0.73*	0.80**		0.20^{NS}	0.20^{NS}
15-ADON			0.93**			0.35^{NS}
	Wheaton					
	7 dai					
Severity	0.70*	0.50^{NS}	0.61^{NS}	0.78**	79**^{NS}	0.85**
DON		0.92**	0.82**		0.89**	0.94**
15-ADON			0.75*			0.82**
	14 dai					
Severity	0.82**	0.73*	0.76*	0.54^{NS}	0.55^{NS}	0.55^{NS}
DON		0.72*	0.90**		0.97**	0.93**
15-ADON			0.76*			0.96**
	21 dai					
Severity	-0.30^{NS}	-0.01^{NS}	-0.15^{NS}	0.31^{NS}	0.39^{NS}	0.29^{NS}
DON		0.81**	0.96**		0.88**	0.96**
15-ADON			0.92**			0.90**
	28 dai					
Severity	-	-	-	-0.29^{NS}	-0.41^{NS}	-0.29^{NS}
DON		0.65*	0.55^{NS}		0.62^{NS}	0.59^{NS}
15-ADON			0.81**			0.83**

** Significant at $P < 0.01$, * Significant at $P < 0.05$

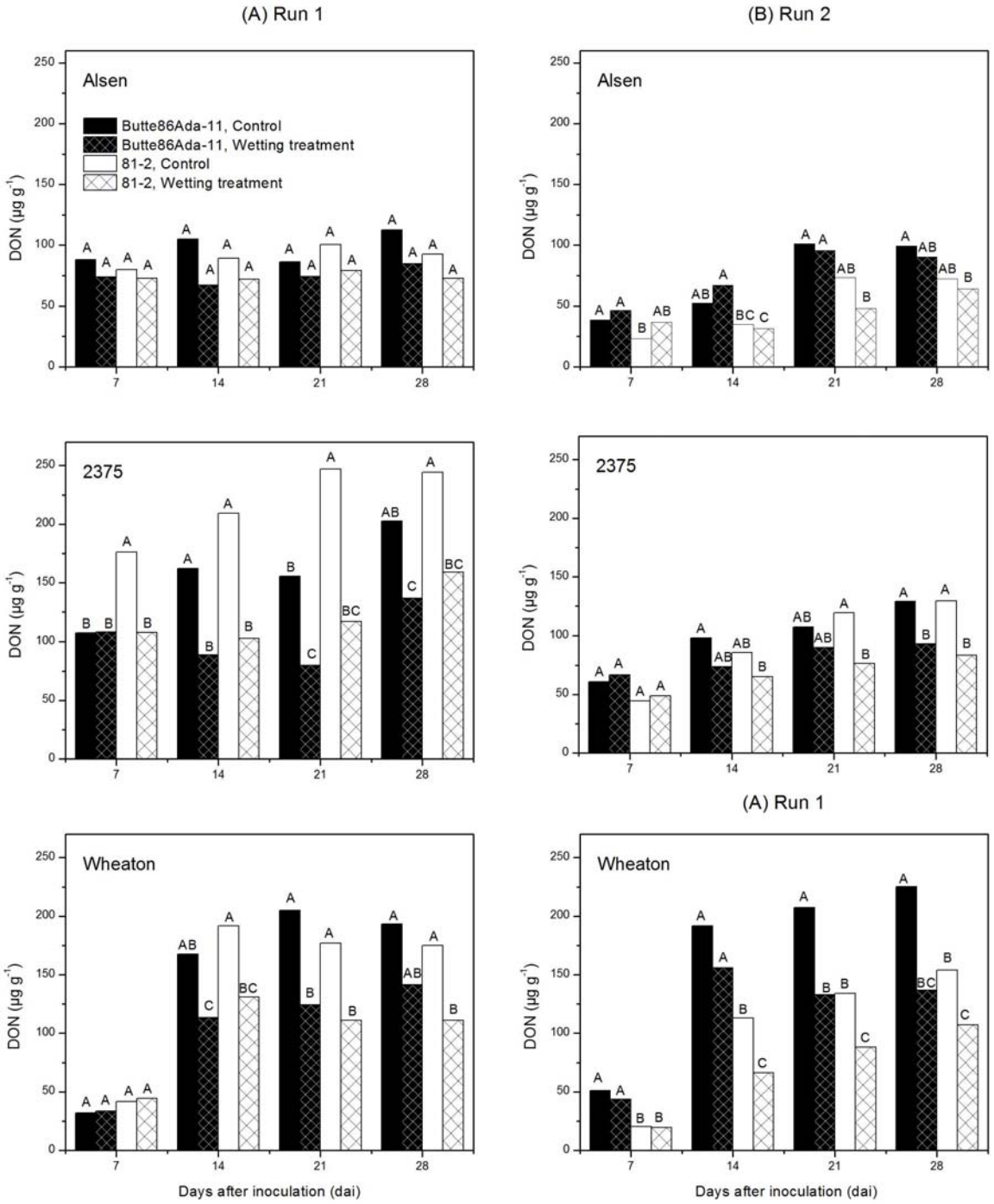

Figure 5.1. Deoxynivalenol (DON, µg g^{-1}) detected 7, 14, 21 and 28 dai in Alsen, 2375 and Wheaton inoculated with isolates Butte86Ada-11 and 81-2 of *F. graminearum* for control plants and plants subjected to a six hour wetting treatment. Runs 1 (panel A) and 2 (panel B) of the greenhouse experiment are shown. Same uppercase letters within each cultivar, run and wetting treatment indicates no statistical differences (P>0.05).

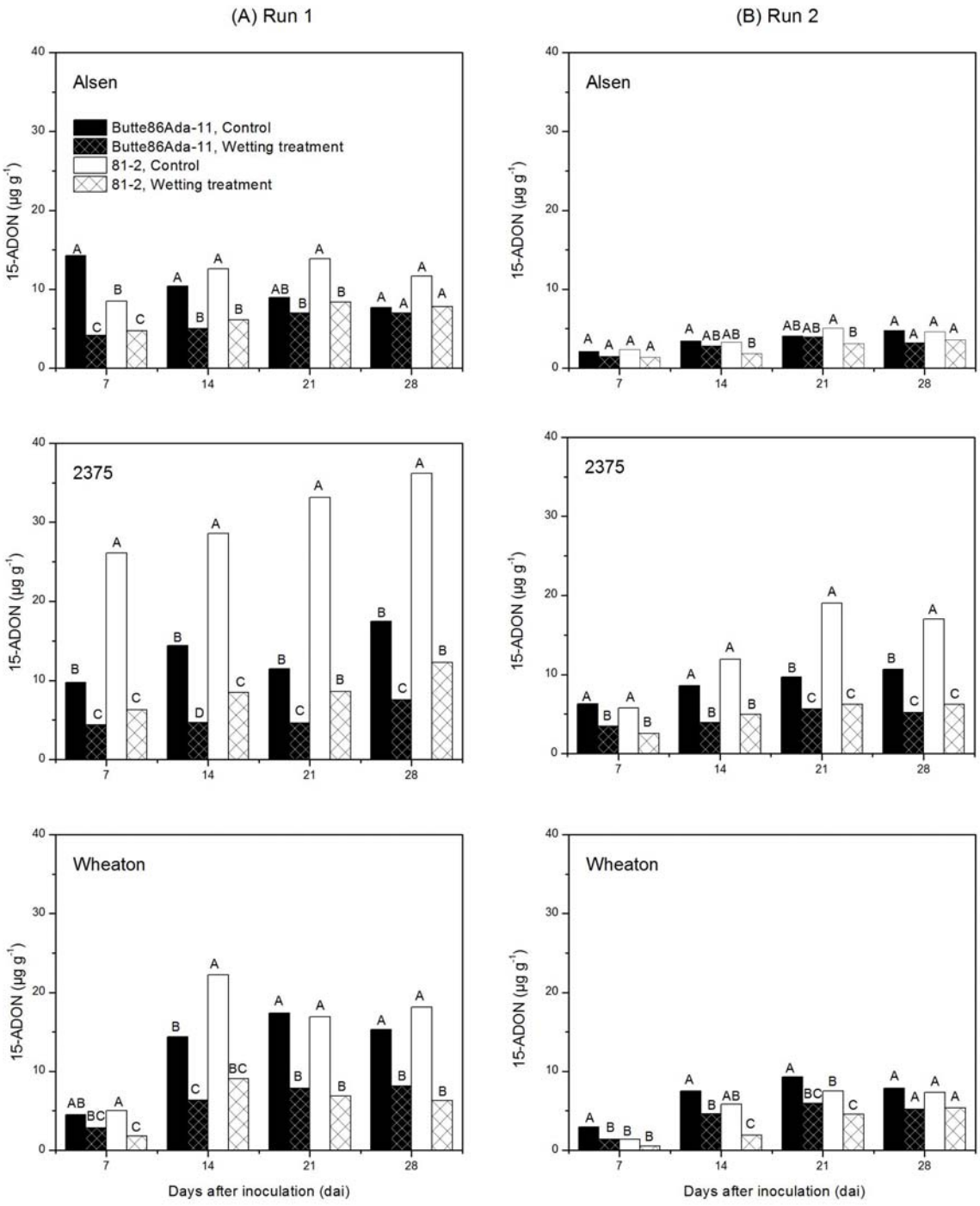

Figure 5.2 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) detected 7, 14, 21 and 28 dai in Alsen, 2375 and Wheaton inoculated with isolates Butte86Ada-11 and 81-2 of *F. graminearum* for control plants and plants subjected to a six hour wetting treatment. Runs 1 (panel A) and 2 (panel B) of the greenhouse experiment are shown. Same uppercase letters within each cultivar, run and wetting treatment indicates no statistical differences (P>0.05).

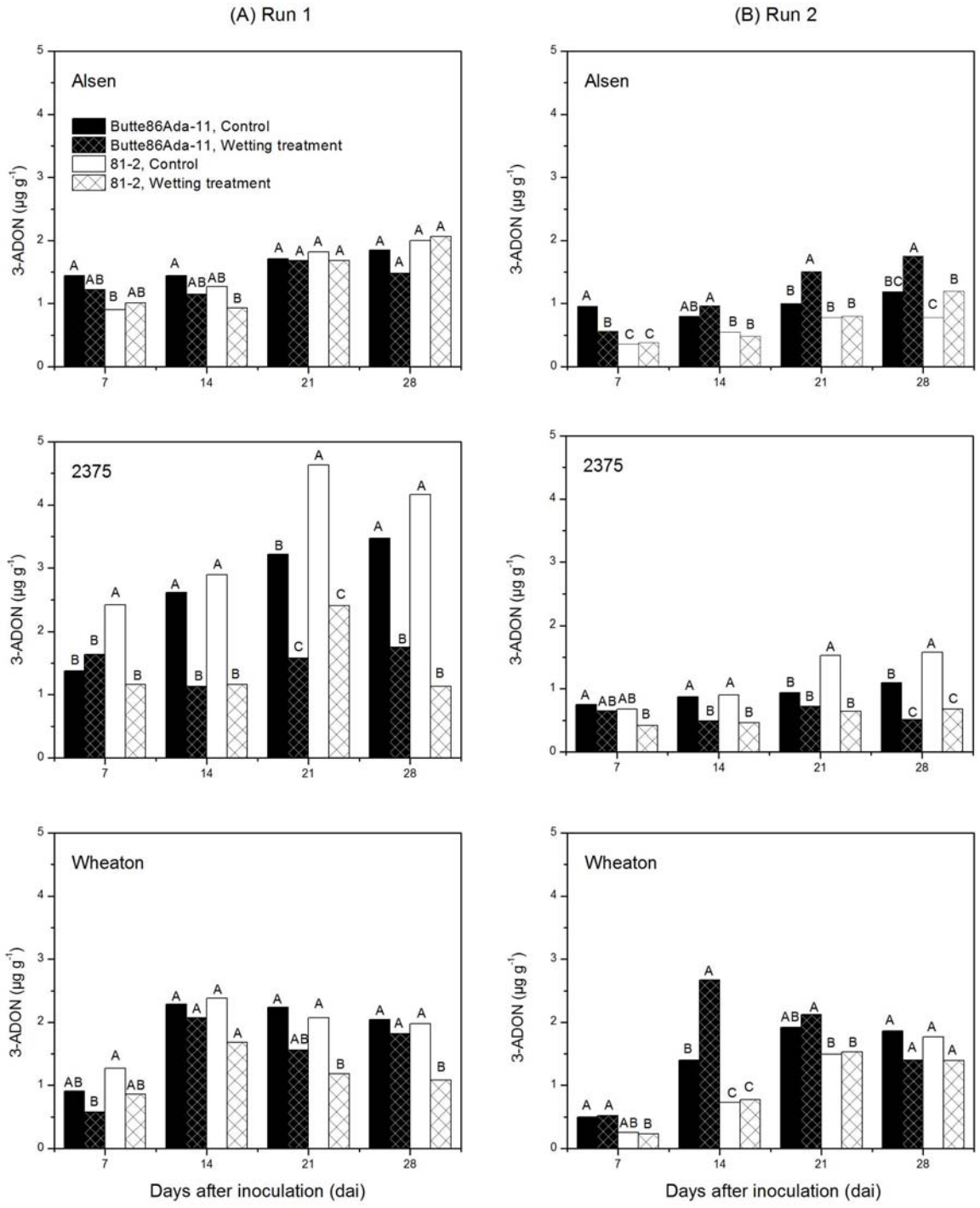

Figure 5.3 3-acetyldeoxynivalenol (3-ADON, µg g^{-1}) detected 7, 14, 21 and 28 dai in Alsen, 2375 and Wheaton inoculated with isolates Butte86Ada-11 and 81-2 of *F. graminearum* for control plants and plants subjected to a six hour wetting treatment. Runs 1 (panel A) and 2 (panel B) of the greenhouse experiment are shown. Same uppercase letters within each cultivar, run and wetting treatment indicates no statistical differences (P>0.05).

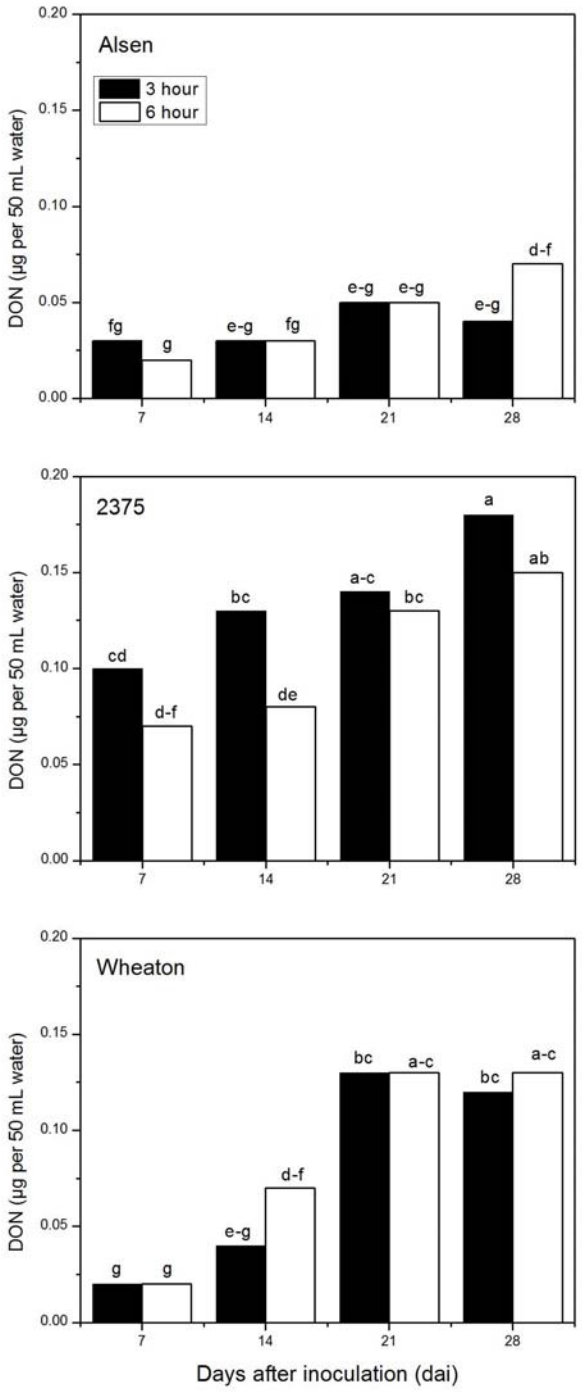

Figure 5.4 Deoxynivalenol (DON, µg per 50 ml of water) detected in run-off water from Alsen, 2375 and Wheaton inoculated with isolates of *F. graminearum* at anthesis and subjected to a six hour wetting event at 7, 14, 21 or 28 dai. Water samples were collected 3 hours after the start of irrigation (■) and immediately after the wetting treatment concluded (□). Same lowercase letters within each cultivar indicates no statistical differences (P>0.05).

Chapter 6

General Discussion

A series of field and greenhouse experiments were conducted to study the influence of moisture in the form of mist-irrigation, host resistance, and pathogen variation with respect to aggressiveness and mycotoxin production capacity, on Fusarium head blight (FHB) disease development and mycotoxin production and accumulation. In a three year field study, although FHB severity was not significantly different between treatments receiving different durations of irrigation, FHB severities in the 21 DAI mist-irrigation treatments were numerically higher than that of treatments receiving misting for 14 DAI. The levels of VSK, evaluated in grain harvested at maturity, were significantly higher in the treatments which received longer durations of mist-irrigation. The data obtained for FHB severity and VSK from the present study support the theory that both symptom development and grain colonization increase with longer periods of moisture after inoculation.

In the treatment receiving the longest duration of misting (35 DAI), DON levels in harvested grains were significantly lower than to 28 DAI durations of mist-irrigation. The same trend was observed for the other mycotoxins examined with levels of 15-ADON, 3-ADON and NIV being lower in treatments receiving longer durations of misting. The reduction of mycotoxins was more discernible in the susceptible cultivar Wheaton than in either Alsen or 2375. The lower levels of DON and other toxins were also apparent in the whole head sampled throughout head development. Lower levels of DON following extended durations of mist-irrigation have also been reported by Lemmens et al. (2004) and Culler et al. (2007). These results, however, contradict Cowger et al. (2009), who reported higher DON levels in the treatment receiving misting for 30 days post-anthesis compared to the treatments receiving mist-irrigation for 0, 10 and 20 days.

It is suggested that the decline in DON as observed in the current study toward harvest may have been promoted by leaching of mycotoxins from the host tissues given that DON and its derivatives are water soluble (Bensassi et al., 2010; Böhm et al., 2008; Cahill et al., 1999; Hazel and Patel, 2004; Kang and Buchenauer, 1999). Further, detection of DON in the wheat head tissues not invaded by *Fusarium* hyphae (Kang and Buchenauer, 1999; Snijders and Kretching, 1992) supports the hypothesis that DON, and its derivatives are mobile within plant tissues. The hypothesis is further supported by the results of the greenhouse experiments presented in this dissertation (Chapter 5) which assessed the impact of free moisture on FHB disease development and mycotoxin accumulation *in planta*. In these greenhouse experiments, the levels of DON and

its derivatives were significantly lower in the plants that were exposed to a six hour wetting treatment compared to plants that did not receive the wetting treatment. The phenomenon was evident in all cultivars examined. The results obtained from the field study (Chapter 3) in conjunction with the greenhouse studies (Chapter 5) support the hypothesis that free moisture can leach mycotoxins out from the plant tissues resulting in lower levels of these toxins in the plant at harvest.

Isolate variability for pathogenicity and toxin production has been well established in *F. graminearum* (Akinsanmi et al., 2006; Bai and Shaner, 1996; Carter et al., 2002; Tóth et al, 2005; Xue et al, 2004). In this study, the five isolates examined differed in their capability to produce disease symptoms and mycotoxins in field inoculated plants. Isolates did not rank consistently for FHB severity, VSK and DON. Generally, isolates inciting higher FHB severities did not generate higher levels of VSK. None of the isolates resulting in the highest FHB severity or VSK produced the highest levels of DON. Isolate 49-3 was associated with the highest levels of DON and other toxins in all misting and cultivar treatments despite generating comparatively lower FHB severities and VSK levels to the other isolates examined. Therefore, we speculate that there must be other factors besides DON, which contribute to isolate aggressiveness.

Variability in an isolates' performance in terms of FHB severity and mycotoxins accumulation was also evident in the greenhouse studies. In the point- and spray-inoculated greenhouse experiments, isolates Butte86Ada-11 and B63A were generally more aggressive and isolates 49-3 and B45A were less aggressive in terms of their capacity to cause FHB. Variability of isolates for DON and DON derivatives in the greenhouse generally corresponded with the aggressiveness of the given isolate. Though the ranking of the isolates examined varied in different cultivars, they generally resulted in either high or low levels of mycotoxins across all cultivars, although the isolates were inconsistent in their response to a given cultivar. This result reiterates the suggestion to consider isolate variability in screening of wheat for FHB resistance. The use of mixtures of prevalent isolates for resistance screening is advisable in order to avoid the misinterpretation of resistance levels of cultivars with the use of single isolate.

Though the isolate Bute86Ada-11 was the least aggressive in the field experiments (Chapter 3) for all disease parameters examined, it was highly aggressive in the greenhouse experiments. Isolate 49-3, which was the least aggressive in the greenhouse studies with respect to FHB severity and mycotoxin accumulation capacity but was the most aggressive for mycotoxin accumulation in the field. The discrepancy in the performance of isolates between the greenhouse and field experiments might be due to differences in the prevailing environmental conditions. In

the greenhouse study, environmental parameters including temperature are optimized for host crop development, and fungal and disease development. In contrast, in the field fungi have to survive and incite disease amid changing environmental conditions. This indicates that isolates vary in terms of their aggressiveness independent of host resistance, and this variation may depend on the prevailing environmental conditions. Environmental conditions appear to be key to infection, disease development and mycotoxin accumulation in FHB. Isolates that appeared to be less aggressive in the field but which show high aggressiveness in the greenhouse may be poor competitors in nature, this may explain the low correlation obtained between greenhouse and the field experiments here and in other studies such as reported by Bai et al. (2001) and Malla (2005).

All isolates examined in this study produced 15-ADON and 3-ADON in addition to DON. NIV was also detected in the spray inoculated experiments. The concentrations of 15-ADON, 3-ADON and NIV were very low compared to that of the DON levels. Based on the observed levels of DON, 15-ADON, 3-ADON, and NIV, all five isolates used in this experiment can be classified into the DON producing 15-ADON chemotype. This result agrees with Mirocha et al., (1989) who suggested that in addition to DON, the *F. graminearum* isolates from North America primarily produce 15-ADON rather than 3-ADON. Recently subgroups of the 15-ADON producing population of *F. graminearum* [Upper Midwestern (UMW) 15-ADON] in the Upper Midwest have been identified by Gale et al. (2007). The UMW 15-ADON population, is reported to be less diverse, in terms of the mean number of alleles per locus, gene diversity across all loci and pairwise differences between multilocus RFLP genotypes, than the currently predominating 15-ADON mid-western (MW) sub-population. Members of this UMW 15-ADON sub-population were described as being more aggressive, in terms of their ability to produce DON in the greenhouse, than the MW 15-ADON sub-population (Gale et al., 2006). Quirin (2010) genotyped all isolates used in this study, except 49-3, classifying isolates Butte86Ada-11, B45A and 81-2 as belonging to the Midwestern (MW) 15-ADON population. Isolate B63A was identified as belonging to the UMW 15-ADON population. In our study, isolate B63A generally resulted in the higher DON levels and the higher FHB severity than other isolates examined in both the field and the greenhouse experiments. The result from our study agree with Gale et al. (2006) in that one isolate of UMW 15-ADON population we tested was generally more aggressive than the isolates belonging to the MW 15-ADON population included in the study. However, further studies with the inclusion of multiple isolates from UMW 15-ADON population would require to support this conclusion.

The high cost of mycotoxin testing and the presence of significant correlation between FHB severity and DON levels, allows researchers to utilize correlations between visual FHB severity assessments in the field and DON concentrations to predict the DON levels in harvested grain (Arsenuik et al., 1999; Groth et al., 1999; Jones and Mirocha, 1999). The correlation between FHB severity and DON was high in the current study. However, the correlation declined in treatments subjected to increased duration of misting prior to harvest. Thus if supplemental moisture, in the form of mist-irrigation, is utilized to facilitate disease development in breeding nurseries, it is expected that this moisture will impact the level of DON in grain and that the impact will depend upon the host resistance level. As DON levels decline most rapidly in susceptible cultivars, breeders should be cautious when utilizing these correlations particularly in locations where significant rainfall occurs following disease assessment. Since the correlations of DON with 15-ADON and 3-ADON are high and the amount of acetylated DON derivatives were very low, selection for low DON alone will also select for low contamination with acetylated DON derivatives. Thus the extra expense to select and test for 15-ADON and 3-ADON appears not to be justified.

The results from the current study may be used to refine disease development and/or DON accumulation models used in forecasting and disease risk assessment systems. In North America, two forecasting models have been developed. The first model, DONcast, was developed in Canada, primarily for forecasting DON (Hooker et al., 2002a; Hooker et al., 2002b). A model to predict the risk of FHB epidemics has been developed in the US (De Wolf et al., 2003). These models utilize information on moisture between seven days pre-anthesis and up to 10 days post-anthesis. The result of this study suggest that the accumulation of DON may be impacted by rainfall any time after anthesis, therefore, the inclusion of moisture parameters beyond 10 days post-anthesis will likely increase the accuracy of these models to predict DON in harvested grains.

Though the current study answered how the different durations of environmental moisture from anthesis to harvest impacted FHB and DON accumulation, several questions are still unanswered and are prospects for future research. The continuous mist-irrigation system that was applied in the current study may not represent real world situations. Therefore the application of intermittent moisture at different growth stages of wheat in the period between anthesis and harvest may provide better understanding of how moisture impacts FHB and mycotoxin accumulation. In addition to moisture, temperature is also an important factor in disease development and mycotoxin accumulation. It would be useful to assess how different temperature

regimes also impact FHB development and mycotoxin accumulation and further how temperature may modify the effect of moisture demonstrated in this study. Examination of wheat cultivars which vary in terms of their resistance levels may also provide precise information on how wheat genetics impacts mycotoxin accumulation. In the current study, though isolates used were variable in terms of their aggressiveness and mycotoxin accumulation capacity, the variability was frequently not statistically significant. Similarly the examination of isolates more variable in terms of their aggressiveness and mycotoxin production and/or inclusion of other species of *Fusarium* might help to better understand the dynamics of FHB disease and mycotoxin accumulation.

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Appendix 1. *Fusarium graminearum* isolates used in the study.

	Collection information					Spores concentration during inoculum production (spores ml^{-1})	
Isolates	SGP ID No*.	Host Crop	Year	County, State	Previous crop	2007	2008
Butte86Ada-11	10195001	Wheat	1995	Norman, MN	NA[w]	1017500	1470000
81-2	10102099	Wheat	2002	Mahnomen, MN	Soybean	1077500	1232500
B45A	10103013	Barley	2003	Polk, MN	NA	1496000	882500
B63A	10103023	Barley	2003	Norman, MN	NA	1568700	1383300
49-3	10102036	Wheat	2002	Becker, MN	Soybean	658700	565000

* SGP ID - Small Grain Pathology Lab's Identification Number

[w] NA - not available

Appendix 2. Analysis of variance (ANOVA) of Fusarium head blight (FHB) severity, visually scabby kernels (VSK), deoxynivalenol (DON), 15-acetyledoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) and nivalenol (NIV) of harvested grain samples of three wheat cultivars inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2006-2008.

		F value					
Sources of variation	df[w]	Severity	VSK	DON	15-ADON	3-ADON	NIV
Year (Yr.)	2/1	131.21**	3338.84**	12400.50**	4617.97**	333.03**	23.31**
Moisture (M)	3	2.22	300.85**	40.48**	88.67**	10.03**	40.00**
Cultivar (Cv.)	2	438.59**	1745.54**	939.33**	734.51**	128.53**	253.25**
Isolate (Iso.)	5	498.65**	661.60**	1369.58**	750.73**	76.73**	111.91**
Yr. x M	6/3	7.18**	93.97**	62.11**	98.20**	36.78**	5.90**
Yr. x Cv.	4/2	16.14**	72.49**	28.60**	318.01**	22.91**	5.76**
Yr. x Iso.	10/5	304.49**	288.58**	718.22**	194.67**	33.23**	7.06**
M x Cv.	6	0.56	7.23**	22.45**	41.30**	6.02**	6.72**
M x Iso.	15	0.91	6.75**	4.82**	14.40**	1.05	5.57**
Cv. X Iso.	10	14.47**	23.32**	8.57**	41.00**	4.97**	10.77**
Yr. x M x Cv.	12/6	0.80	7.08**	5.65**	18.43**	3.26**	1.81
Yr. x M x Iso.	30/15	0.96	2.82**	3.17**	5.37**	2.07*	1.16
Yr. x Cv. x Iso.	20/10	12.94**	13.32**	4.44**	10.26**	3.79**	1.26
M x Cv. x Iso.	30	0.79	2.22**	1.71*	3.90**	1.20	2.14**
Yr. x M x Cv. x Iso.	60/30	0.47	0.92	1.05	1.39*	1.00	1.28

* Significant at $P < 0.05$

** Significant at $P < 0.01$

[w] First number applies to FHB severity, VSK, DON and 15-ADON; second number applies to 3-ADON and NIV.

Appendix 3. Planting, inoculation, samplings and harvest dates for field trials in 2006, 2007 and 2008.

	2006		
	Date	Days from planting	Zadoks growth stage (GS)*
Planting	27th April	0	-
Bronate® & Di-syston8® spray	-	-	13-15
1st Inoculation & 0 dai sampling	27th June	61	64/65
2nd Inoculation	30th June	64	
7 dai sampling	-	-	70
11 dai sampling	-	-	72
14 DAI mist-irrigation ended & 14 dai sampling	11th July – no head sampling	75	75
21 DAI mist-irrigation ended & 21 dai sampling	18th July - no head sampling	82	80
28 DAI mist-irrigation ended & 28 dai sampling	25th July – no head sampling	89	85
35 DAI mist-irrigation ended	1st August	96	-
41 dai sampling	-	-	93
Harvest	8th August	103	93
	2007		
Planting	19th April	0	-
Bronate® & Di-syston8® spray	-	-	13-15
1st Inoculation & 0 dai sampling	15th June	57	64/65
2nd Inoculation	18th June	60	
7 dai sampling	22nd June	64	70
11 dai sampling	26th June	68	72
14 DAI mist-irrigation ended & 14 dai sampling	29 June	71	75
21 DAI mist-irrigation ended & 21 dai sampling	6th July	78	80
28 DAI mist-irrigation ended & 28 dai sampling	13th July	85	85
35 DAI mist-irrigation ended	20th July	92	-
41 dai sampling	26th July	98	93
Harvest	27th July	99	93
	2008		
Planting	30th April	0	-
Bronate® & Di-syston8® spray	-	-	13-15
1st Inoculation & 0 dai sampling	27th June	58	64/65
2nd Inoculation	30th June	61	
7 dai sampling	4th July	65	70
11 dai sampling	8th July	69	72
14 DAI mist-irrigation ended & 14 dai sampling	11th July	72	75
21 DAI mist-irrigation ended & 21 dai sampling	18 July	79	80
28 DAI mist-irrigation ended & 28 dai sampling	25th July	86	85
35 DAI mist-irrigation ended	1st August	93	-
41 dai sampling	5th August	97	93
Harvest	6th August	98	93

* Zadoks et al. (1974).

Appendix 4. Fusarium head blight (FHB) severity (%) of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control in 2006, 2007 and 2008.

	FHB Severity						
	2006						
	Alsen		2375		Wheaton		
Isolates	%	Rank	%	Rank	%	Rank	Avg[w].
Control	7.75 d[x] A[y]	1	5.54 d A	1	5.92 d A	1	6.40 d
Butte86Ada-11	13.67 c B	2	16.19 c B	4	49.69 c A	2	26.52 c
81-2	14.62 bc B	3	15.49 c B	2	50.47 c A	3	26.86 c
B45A	18.08 b B	4	16.07 c B	3	51.47 c A	4	28.54 c
B63A	18.24 b B	5	21.41 b B	5	57.78 b A	5	32.47b
49-3	22.53 a B	6	26.86 a B	6	68.86 a A	6	39.42 a
Avg.	17.43 N[y]		19.20 N		55.65 M		Int.[z]
	2007						
Control	1.10 d A	1	1.43 c A	1	2.10 c A	1	1.54 d
Butte86Ada-11	27.84 c B	3	31.51 ab B	3	69.49 ab A	3	42.95 bc
81-2	27.55 c B	2	30.02 b B	2	64.10 b A	2	40.56 c
B45A	36.86 a B	6	34.44 a B	5	74.26 a A	6	48.52 a
B63A	30.54 bc B	4	33.09 ab B	4	73.04 a A	5	45.56 b
49-3	31.74 b B	5	34.58 a B	6	71.52 a A	4	45.95 ab
Avg.	30.91 N		32.73 N		70.48 M		Int.
	2008						
Control	1.07 d A	1	0.86 c A	1	2.09 d A	1	1.34 e
Butte86Ada-11	29.06 c B	4	29.14 b B	3	52.03 c A	2	36.74 cd
81-2	33.61 b B	5	29.48 ab B	4	59.51 ab A	5	40.87 b
B45A	37.61 a B	6	32.97 a B	6	64.19 a A	6	44.92 a
B63A	28.79 c B	3	29.71 ab B	5	56.83 bc A	4	38.44 c
49-3	25.87 c B	2	27.18 b B	2	52.71 c A	3	35.25 d
Avg.	30.99 N		29.69 N		57.05 M		Int.[z]

[w] Average (cultivar means within each year excludes water treatment).
[x] Means followed by the same lowercase letter within column in each year are not significantly different at $P \leq 0.05$.
[y] Means followed by the same uppercase letter within row in each year are not significantly different at $P < 0.05$.
[z] Cultivar×isolate interaction significant in each year when Int. is present.

Appendix 5. Percentage visually scabby kernels (VSK) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control in 2006.

	Visually scabby kernel (VSK)						
	2006						
	Alsen		2375		Wheaton		
Isolates	%	Rank	%	Rank	%	Rank	Avg.[w]
Control	0.50 c[x] B[y]	1	0.28 c B	1	1.32 d A	1	0.70 d
Butte86*	3.22 b B	2	3.78 a B	5	12.62 c A	2	6.54 c
81-2	3.42 b B	3	3.75 a B	4	14.00 bc A	3	7.06 bc
B45A	3.92 ab B	4	2.52 b B	2	14.60 b A	4	7.01 c
B63A	4.25 ab B	5	3.70 a B	3	15.40 b A	5	7.78 b
49-3	4.60 a B	6	4.72 a B	6	19.75 a A	6	9.69 a
Avg.	3.88 N[y]		3.69 N		15.27 M		Int.[z]

[w] Average (cultivar means within each year excludes water treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each year are not significantly different at $P \leq 0.05$.

[y] Means followed by the same uppercase letter within row in each year are not significantly different at $P < 0.05$.

[z] Cultivar×isolate interaction significant in each year when Int. is present.

Appendix 5 continued. Percentage visually scabby kernels (VSK) of harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2007.

	Visually scabby kernel (VSK)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	%	Rank	%	Rank	%	Rank	%	Rank	Avg.[w]
Control	1.00 b[x] A[y]	1	1.40 d A	1	1.70 c A	1	2.00 c A	1	1.53 d
Butte86*	11.00 a C	2	17.10 bc B	3	19.30 b B	2	28.50 b A	2	18.98 c
81-2	11.00 a B	2	12.30 c B	2	23.00 b A	3	29.50 b A	3	18.95 c
B45A	15.80 a C	5	23.50 a B	6	34.00 a A	5	33.50 ab A	5	26.70 ab
B63A	15.50 a B	3	20.00 ab B	5	36.50 a A	6	38.50 a A	6	27.63 a
49-3	15.50 a C	4	17.50 a-c B	4	32.00 a A	4	30.00 b A	4	23.75 b
Avg.	13.76 n[z] P		18.08 n O		28.96 o N		32.00 o M		23.20 o[†]
	2375								
Control	1.30 d A	1	0.70 c A	1	1.90c A	1	1.60 d A	1	1.38 c
Butte86	9.70 c C	2	21.50 ab B	4	38.36 ab A	4	33.50 bc A	3	24.68 b
81-2	11.50 bc B	3	16.10 b B	2	30.50 b A	2	29.00 c A	2	21.78 b
B45A	16.80 a B	6	23.00 a B	5	37.50 ab A	3	39.00 b A	5	29.08 a
B63A	15.50 ab B	5	21.30 ab B	3	41.00 a A	6	50.50 a A	6	32.08 a
49-3	13.80 a-c C	4	25.50 a B	6	38.50 a A	5	38.50 bc A	4	29.08 a
Avg.	13.46 n P		21.48 n O		36.30 n N		38.10 n M		27.34 n
	Wheaton								
Control	3.80 c B	1	2.90 b B	1	4.60 d B	1	12.20 b A	1	5.88 c
Butte86	64.00 a C	6	70.00 a BC	6	79.00 a AB	5	90.00 a A	6	75.75 a
81-2	58.00 ab B	3	63.00 a B	3	61.00 c B	2	83.00 a A	3	66.25 b
B45A	61.00 ab B	5	67.00 a B	4	73.00 ab B	4	87.00 a A	5	72.00 a
B63A	60.00 ab C	4	69.00 a BC	5	81.00 a AB	6	86.00 a A	4	74.00 a
49-3	53.00 b C	2	60.00 a BC	2	66.00 bc B	3	80.00 a A	2	64.75 b
Avg.	59.20 m O		65.80 m NO		72.00 m N		85.20 m M		70.55 m
Mean[‡]	28.81 S		35.12 R		45.75 Q		51.77 P		

[w] Average (mist-irrigation treatment means within each cultivar excludes water treatment).

* Isolate Butte86Ada-11

[x] Means followed by the same letter within column in each mist-irrigation duration and cultivar are not significantly different at $P \leq 0.05$.

[y] Means followed by the same letter within row in each cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same uppercase letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Cultivar means (combined across isolates and mist-irrigation durations) followed by same letter are not significantly different at $P < 0.05$.

[‡] Mist-irrigation duration mean value combined across cultivars and isolates.

Appendix 5 continued. Percentage visually scabby kernels (VSK) of harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2008.

	Visually scabby kernel (VSK)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	%	Rank	%	Rank	%	Rank	%	Rank	Avg.[w]
Control	1.90 b[x]BC[y]	1	0.70 c C	1	3.20 c B	1	6.90 c A	1	3.18 c
Butte86*	11.30 a C	5	9.10 b C	2	20.50 b B	2	32.00 b A	2	18.23 b
81-2	9.10 a D	3	24.50 a C	6	33.00 a B	4	50.50 a A	6	29.28 a
B45A	11.80 a D	6	23.50 a C	5	37.50 a B	6	47.50 a A	5	30.08 a
B63A	8.10 a D	2	20.50 a C	4	35.50 a B	5	47.00 a A	4	27.78 a
49-3	9.10 a C	4	13.70 b C	3	22.50 b B	3	34.00 b A	3	19.83 b
Avg.	9.88 n P		18.26 n O		29.80 n N		42.20 n M		25.04 n[†]
	2375								
Control	2.00 c B	1	1.20 c B	1	2.90 c B	1	6.80 d A	1	3.23 d
Butte86	7.00 b C	2	8.20 b C	2	22.00 b B	2	35.00 c A	2	18.05 c
81-2	9.90 ab C	5	19.40 a B	4	38.00 a A	6	47.00 ab A	4	28.58 a
B45A	13.80 a C	6	19.50 a C	5	34.50 a B	5	55.00 a A	6	30.70 a
B63A	8.70 ab D	4	22.00 a C	6	33.00 a B	4	48.50 ab A	5	28.05 ab
49-3	7.20 b D	3	18.00 a C	3	29.50 a B	3	42.50 bc A	3	24.30 b
Avg.	9.32 n P		17.42 n O		31.40 n N		45.60 n M		25.94 n
	Wheaton								
Control	6.90 d B	1	5.40 c B	1	6.20 d B	1	18.00 c A	1	6.18 d
Butte86	33.00 c C	2	48.50 b B	2	54.50 c AB	2	60.00 b A	3	49.00 c
81-2	54.00 a B	6	74.00 a A	6	78.00 a A	5	83.00 a A	6	72.25 a
B45A	45.60 ab B	5	70.00 a A	5	71.00 ab A	4	78.00 a A	4	66.15 a
B63A	45.50 ab C	4	67.00 ab B	4	84.00 a A	6	80.00 a AB	5	69.13 a
49-3	40.50 bc B	3	58.00 b A	3	65.00 bc A	3	57.50 b A	2	55.25 b
Avg.	43.72 m O		63.50 m N		70.50 m M		71.70 m M		62.36 m
Mean[‡]	20.97 S		33.06 R		43.90 Q		53.17 P		

[w] Average (mist-irrigation treatment means within each cultivar excludes water treatment).

***** Isolate Butte86Ada-11

[x] Means followed by the same letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same letter within row in each cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same uppercase letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Cultivar means (combined across isolates and mist-irrigation durations) followed by same letter are not significantly different at $P < 0.05$.

[‡] Mist-irrigation duration mean value combined across cultivars and isolates.

Appendix 6. Deoxynivalenol (DON) concentration (µg g^{-1}) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control in 2006.

	Deoxynivalenol (DON)						
	2006						
	Alsen		2375		Wheaton		
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.02 c A	1	0.02 c A	1	0.08 e A	1	0.04 d
Butte86*	0.38 b B	2	0.41 b B	2	1.53 cd A	3	0.77 c
81-2	0.51 b B	4	0.55 b B	5	1.75 bc A	4	0.94 b
B45A	0.51 b B	4	0.49 b B	4	1.83 b A	5	0.94 b
B63A	0.43 b B	3	0.44 b B	3	1.29 d A	2	0.72 c
49-3	1.04 a B	5	1.12 a B	6	2.96 a A	6	1.71 a
Avg.	0.57 N[y]		0.60 N		1.88 M		Int.[z]

[w] Average (cultivar means within each year excludes water treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each year are not significantly different at $P \leq 0.05$.

[y] Means followed by the same uppercase letter within row in each year are not significantly different at $P < 0.05$.

[z] Cultivar×isolate interaction significant in each year when Int. is present.

Appendix 6 continued. Deoxynivalenol (DON) concentration (µg g^{-1}) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2007.

	Deoxynivalenol (DON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.30 d[x] A[y]	1	0.27 e A	1	0.81 e A	1	0.30 d A	1	0.42 f
Butte86*	6.26 c AB	2	6.80 d AB	2	8.06 d A	2	5.28 c B	2	6.60 e
81-2	7.08 c B	3	7.68 cd B	3	10.14 cd A	3	5.94 c B	3	7.71 d
B45A	10.04 b AB	4	8.78 bc B	4	11.74 c A	4	6.26 c C	4	9.21 c
B63A	12.28 b AB	5	10.62 b BC	5	15.20 b A	5	9.04 b C	5	11.79 b
49-3	16.38 a B	6	16.20 a B	6	22.88 a A	6	13.06 a B	6	17.13 a
Avg.	10.41 n N		10.02 n N		13.60 n M		7.92 o O		10.49 n[†]
	2375								
Control	0.35 d A	1	0.43 d A	1	0.81 d A	1	0.46 d A	1	0.51 d
Butte86	4.98 c C	2	7.78 c B	4	10.46 c A	2	7.06 c B	2	7.57 c
81-2	5.92 bc C	3	7.32 c BC	2	10.96 c A	3	7.86 c B	4	8.02 c
B45A	6.18 bc B	4	7.66 c B	3	12.04 c A	4	7.18 c B	3	8.27 c
B63A	6.96 b C	5	10.04 b B	5	17.38 b A	5	11.12 b B	5	11.38 b
49-3	11.46 a B	6	20.44 a A	6	24.18 a A	6	21.46 a A	6	19.39 a
Avg.	7.10 o O		10.65 n N		15.00 n M		10.94 n N		10.92 n
	Wheaton								
Control	1.12 d B	1	1.21 d AB	1	1.68 c A	1	1.25 d AB	1	1.31 e
Butte86	18.32 c A	2	22.00 c A	2	21.18 b A	3	9.92 c B	2	17.86 d
81-2	22.84 bc A	3	25.86 bc A	4	20.94 b A	2	10.80 c B	4	20.11 cd
B45A	27.22 b A	4	24.22 bc A	3	24.14 b A	4	10.28 c B	3	21.47 c
B63A	27.40 b A	5	28.90 b A	5	31.74 a A	5	15.02 b B	5	25.77 b
49-3	39.56 a A	6	38.82 a A	6	37.78 a A	6	27.22 a B	6	35.85 a
Avg.	27.07 m M		27.96 m M		27.16 m M		14.65 m N		24.21 m
Mean[‡]	14.86 Q		16.21 Q		18.59 P		11.17 R		

[w] Average (mist-irrigation treatment means within each cultivar excludes water treatment).

***** Isolate Butte86Ada-11

[x] Means followed by the same letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same letter within row in each cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same uppercase letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Cultivar means (combined across isolates and mist-irrigation durations) followed by same letter are not significantly different at $P < 0.05$.

[‡] Mist-irrigation duration mean value combined across cultivars and isolates.

Appendix 6 continued. Deoxynivalenol (DON) concentration (μg g^{-1}) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2008.

	Deoxynivalenol (DON)								
	Mist-irrigation duration treatmetns								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	Avg.[w]
Control	0.18 d[x] C[y]	1	0.67 d B	1	0.98 d AB	1	1.49 d A	1	0.83 e
Butte86*	7.04 c C	2	13.90 c AB	2	18.16 c A	2	11.04 c B	2	12.54 d
81-2	11.24 a C	5	22.38 a AB	5	28.44 ab A	5	18.26 b B	5	20.08 b
B45A	10.76 ab B	4	21.64 ab A	4	22.44 bc A	3	16.78 b A	3	17.91 bc
B63A	8.44 bc C	3	17.22 bc B	3	25.94 ab A	4	17.60 b B	4	17.30 c
49-3	14.14 a B	6	27.62 a A	6	30.80a A	6	25.14 a A	6	24.43 a
Avg.	10.32 n O		20.55 n NO		25.16 n M		17.76 n N		18.45 n[†]
	2375								
Control	0.40 d C	1	0.90 c BC	1	1.17 d AB	1	1.66 c A	1	1.03 d
Butte86	8.40 a-c C	4	14.24 b AB	2	17.66 c A	3	13.02 b B	3	13.33 c
81-2	9.22 ab C	5	16.78 b B	4	23.26 b A	5	15.04 b B	4	16.08 b
B45A	7.58 bc B	3	15.30 b A	3	16.34 c A	2	12.56 b A	2	12.95 c
B63A	6.74 c B	2	17.48 b A	5	19.68 bc A	4	15.78 b A	5	14.92 bc
49-3	10.58 a B	6	22.64 a A	6	30.92 a A	6	30.60 a A	6	23.69 a
Avg.	8.50 n N		17.29 n M		21.57 n M		17.40 n M		16.19 o
	Wheaton								
Control	1.14 d C	1	2.58 d B	1	3.06 d AB	1	3.94 d A	1	2.68 d
Butte86	22.88 c B	2	38.48 c A	2	43.18 bc A	3	21.44 c B	2	31.50 c
81-2	33.30 ab B	5	53.30 ab A	5	47.14 b A	4	26.02 bc B	4	39.94 b
B45A	25.90 bc B	4	45.82 a-c A	3	36.02 c A	2	21.64 c B	3	32.35 c
B63A	23.24 c B	3	51.50 ab A	4	49.54 ab A	5	31.92 ab B	5	39.05 b
49-3	38.76 a B	6	59.12 a A	6	61.48 a A	6	37.62 a B	6	49.25 a
Avg.	28.82 m N		49.64 m M		47.47 m M		27.73 m N		38.41 m
Mean[‡]	15.88 Q		29.16 PQ		31.40 P		20.96 R		

[w] Average (mist-irrigation treatment means within each cultivar excludes water treatment).

***** Isolate Butte86Ada-11

[x] Means followed by the same letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same letter within row in each cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same uppercase letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Cultivar means (combined across isolates and mist-irrigation durations) followed by same letter are not significantly different at $P < 0.05$.

[‡] Mist-irrigation duration mean value combined across cultivars and isolates.

Appendix 7. 15-acetyldeoxynivalenol (15-ADON) concentration (µg g^{-1}) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control in 2006.

	15 Acetyldeoxynivalenol (15-ADON)						
	2006						
	Alsen		2375		Wheaton		
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 a A	1	0.00 b A	1	0.00 b A	1	0.00 b
Butte86*	0.00 a B	1	0.03 a A	3	0.00 b B	1	0.01 ab
81-2	0.00 a A	1	0.00 b A	1	0.01 b A	2	0.00 b
B45A	0.00 a A	1	0.00 b A	1	0.01 b A	2	0.00 b
B63A	0.01 a A	2	0.01 ab A	2	0.00 b A	1	0.00 b
49-3	0.01 a B	2	0.00 b B	1	0.04 a A	3	0.02 a
Avg.	0.00 M		0.00 M		0.01 M		Int.[z]

[w] Average (cultivar means within each year excludes water treatment).

***** Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each year are not significantly different at $P \leq 0.05$.

[y] Means followed by the same uppercase letter within row in each year are not significantly different at $P < 0.05$.

[z] Cultivar×isolate interaction significant in each year when Int. is present.

Appendix 7 continued. 15-acetyldeoxynivalenol (15-ADON) concentration (μg g^{-1}) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2007.

	15-Acetyldeoxynivalenol (15-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	Avg.[w]
Control	0.00 d[x] A[y]	1	0.00 d A	1	0.00 d A	1	0.00 c A	1	0.00 e
Butte86*	0.23 c A	2	0.30 c A	2	0.25 c A	2	0.14 b B	2	0.23 d
81-2	0.34 b A	3	0.42 b A	3	0.37 b A	3	0.18 b B	4	0.33 c
B45A	0.49 a A	4	0.46 b A	5	0.46 ab A	4	0.19 b B	5	0.40 b
B63A	0.52 a A	5	0.45 b A	4	0.47 ab A	5	0.17 b B	3	0.40 b
49-3	0.57 a A	6	0.58 a A	6	0.52 a A	6	0.31 a B	6	0.50 a
Avg.	0.43 n M		0.44 n M		0.42 n M		0.20 n N		0.37 n[†]
	2375								
Control	0.00 d A	1	0.00 e A	1	0.00 d A	1	0.00 c A	1	0.00 e
Butte86	0.20 c B	2	0.32 d A	2	0.34 c A	2	0.13 b B	2	0.25 d
81-2	0.26 bc B	3	0.38 cd A	3	0.43 bc A	3	0.20 b B	4	0.32 c
B45A	0.32 b B	5	0.43 bc A	4	0.47 ab A	4	0.19 b C	3	0.35 bc
B63A	0.28 bc B	4	0.50 b A	5	0.52 ab A	5	0.22 b B	5	0.38 b
49-3	0.48 a B	6	0.71 a A	6	0.55 a B	6	0.45 a B	6	0.55 a
Avg.	0.31 o N		0.47 n M		0.46 n M		0.24 n N		0.37 n
	Wheaton								
Control	0.00 e A	1	1.56 e A	1	0.01 e A	1	0.00 c A	1	0.01 e
Butte86	0.58 d B	2	0.86 d A	2	0.54 d B	2	0.23 b C	2	0.55 d
81-2	0.92 c B	3	1.25 b A	5	0.77 c B	3	0.27 b C	3	0.80 c
B45A	1.16 b A	5	1.17 bc A	4	1.02 b A	5	0.27 b B	3	0.90 b
B63A	0.95 c AB	4	1.05 c A	3	0.88 bc B	4	0.27 b C	3	0.79 c
49-3	1.39 a A	6	1.56 a A	6	1.18 a B	6	0.60 a C	4	1.18 a
Avg.	1.00 m		1.18 m		0.88 m		0.33 n		0.85 m
Mean[‡]	0.66 Q		0.70 P		0.59 Q		0.26 R		

[w] Average (mist-irrigation treatment means within each cultivar excludes water treatment).

* Isolate Butte86Ada-11

[x] Means followed by the same letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same letter within row in each cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same uppercase letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Cultivar means (combined across isolates and mist-irrigation durations) followed by same letter are not significantly different at $P < 0.05$.

[‡] Mist-irrigation duration mean value combined across cultivars and isolates.

Appendix 7 continued. 15-acetyldeoxynivalenol (15-ADON) concentration (µg g^{-1}) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2008.

	15-Acetyldeoxynivalenol (15-ADON)								
	Mist-irrigation duration treatmetns								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.
Control	0.00 d[x] A[y]	1	0.00 c A	1	0.00 c A	1	0.00 d A	1	0.00 c
Butte86*	0.29 c AB	2	0.43 b A	2	0.39 b A	2	0.24 c B	2	0.34 b
81-2	0.47 ab B	4	0.80 a A	6	0.56 a B	5	0.31 bc C	4	0.53 a
B45A	0.49 ab BC	5	0.78 a A	5	0.57 a B	6	0.38 ab C	5	0.55 a
B63A	0.35 bc AB	3	0.49 b A	3	0.47 ab A	3	0.28 bc B	3	0.39 b
49-3	0.56 a AB	6	0.73 a A	4	0.55 a AB	4	0.45 a B	6	0.57 a
Avg.	0.43 n NO		0.64 n M		0.51 n N		0.33 n O		0.48 o
	2375								
Control	0.00 c A	1	0.01 b A	1	0.04 d A	1	0.00 c A	1	0.01 d
Butte86	0.46 a B	3	0.69 a A	2	0.52 bc AB	3	0.29 b C	3	0.49 bc
81-2	0.49 a B	5	0.72 a A	4	0.67 ab AB	5	0.38 b C	5	0.57 b
B45A	0.47 a B	4	0.85 a A	6	0.53 bc B	4	0.32 b C	4	0.54 b
B63A	0.31 b C	2	0.70 a A	3	0.50 c B	2	0.27 b C	2	0.44 c
49-3	0.47 a B	4	0.83 a A	5	0.79 a A	6	0.65 a A	6	0.69 a
Avg.	0.44 n O		0.76 n M		0.60 n N		0.38 n O		0.55 n
	Wheaton								
Control	0.01 e B	1	0.15 c A	1	0.07 c AB	1	0.07 c AB	1	0.08 e
Butte86	1.38 cd B	3	1.74 b A	2	1.18 b B	4	0.53 b C	2	1.21 d
81-2	1.82 ab B	5	2.24 a A	4	1.28 b C	5	0.64 ab D	5	1.49 b
B45A	1.52 bc B	4	2.32 a A	5	1.08 b C	2	0.56 b D	3	1.37 c
B63A	1.18 d B	2	1.84 b A	3	1.13 b B	3	0.59 b C	4	1.19 d
49-3	2.04 a B	6	2.42 a A	6	1.62 a C	6	0.80 a D	6	1.72 a
Avg.	1.59 m N		2.11 m M		1.26 m O		0.63 m P		1.40 m
Mean[‡]	0.82 Q		1.17 P		0.79 Q		0.45 R		

[w] Average (mist-irrigation treatment means within each cultivar excludes water treatment).

***** Isolate Butte86Ada-11

[x] Means followed by the same letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same letter within row in each cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same uppercase letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Cultivar means (combined across isolates and mist-irrigation durations) followed by same letter are not significantly different at $P < 0.05$.

[‡] Mist-irrigation duration mean value combined across cultivars and isolates.

Appendix 8. 3-acetyldeoxynivalenol (3-ADON) concentration (µg g^{-1}) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2007.

	3-Acetyldeoxynivalenol (3-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.
Control	0.00 d A	1	0.00 c A	1	0.00 b A	1	0.00 a A	1	0.00 c
Butte86*	0.08 bc A	4	0.05 bc AB	3	0.00 b B	1	0.00 a B	1	0.03 b
81-2	0.05 cd A	3	0.00 c A	1	0.01 b A	2	0.01 a A	2	0.02 bc
B45A	0.03 cd A	2	0.03 c A	2	0.00 b A	1	0.00 a A	1	0.02 bc
B63A	0.15 a A	6	0.08 b A	4	0.09 a A	3	0.01 a B	2	0.08 a
49-3	0.13 ab A	5	0.16 a A	5	0.10 a AB	4	0.05 a B	3	0.11 a
Avg.	0.09 n M		0.07 n MN		0.04 n NO		0.01 n O		0.05 n
	2375								
Control	0.00 b A	1	0.00 c A	1	0.00 c A	1	0.00 b A	1	0.00 c
Butte86	0.01 ab A	2	0.04 bc A	2	0.02 a-c A	2	0.00 b A	1	0.02 bc
81-2	0.07 ab A	4	0.07 b A	5	0.02 bc A	2	0.02 ab A	3	0.04 b
B45A	0.02 ab A	3	0.06 bc A	4	0.04 a-c A	3	0.00 b A	1	0.03 bc
B63A	0.01 b B	2	0.05 bc AB	3	0.09 a A	5	0.01 ab B	2	0.04 b
49-3	0.08 a B	5	0.17 a A	6	0.08 ab B	4	0.07 a B	4	0.10 a
Avg.	0.04 o MN		0.08 n M		0.05 n MN		0.02 n N		0.05 n
	Wheaton								
Control	0.00 c A	1	0.00 c A	1	0.00 d A	1	0.00 b A	1	0.00 d
Butte86	0.11 b AB	2	0.16 b A	2	0.06 cd BC	2	0.03 ab C	2	0.09 c
81-2	0.22 a A	3	0.18 b AB	3	0.11 bc BC	3	0.05 ab C	4	0.14 b
B45A	0.22 a A	3	0.20 ab A	4	0.17 ab A	5	0.04 ab B	3	0.16 ab
B63A	0.23 a AB	4	0.26 a A	6	0.16 ab BC	4	0.08 a C	5	0.18 a
49-3	0.23 a A	4	0.21 ab A	5	0.22 a A	6	0.09 a B	6	0.19 a
Avg.	0.20 m M		0.20 m M		0.14 m N		0.06 m O		0.15 m
Mean‡	0.11 P		0.11 P		0.08 Q		0.03 R		

[w] Average (mist-irrigation treatment means within each cultivar excludes water treatment).

* Isolate Butte86Ada-11

[x] Means followed by the same letter within column in each mist-irrigation duration and cultivar are not significantly different at $P \leq 0.05$.

[y] Means followed by the same letter within row in each cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same uppercase letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Cultivar means (combined across isolates and mist-irrigation durations) followed by same letter are not significantly different at $P < 0.05$.

[‡] Mist-irrigation duration mean value combined across cultivars and isolates.

Appendix 8 continued. 3-acetyldeoxynivalenol (3-ADON) concentration (μg g^{-1}) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated wtih five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2008.

	3-Acetyldeoxynivalenol (3-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	Avg.
Control	0.00 b A	1	0.00 c A	1	0.00 b A	1	0.00 c A	1	0.00 c
Butte86*	0.12 a C	6	0.25 a B	6	0.37 a A	6	0.26 ab AB	5	0.25 a
81-2	0.10 ab B	5	0.13 b B	4	0.31 a A	4	0.18 b AB	2	0.18 b
B45A	0.07 ab C	3	0.10 bc BC	3	0.25 a A	2	0.20 b AB	3	0.15 b
B63A	0.04 ab B	2	0.09 bc B	2	0.28 a A	3	0.24 ab A	4	0.16 b
49-3	0.08 ab B	4	0.16 ab B	5	0.33 a A	5	0.34 a A	6	0.23 a
Avg.	0.08 n N		0.15 n N		0.31 n M		0.24 m M		0.20 n
	2375								
Control	0.00 b A	1	0.00 b A	1	0.00 c A	1	0.00 c A	1	0.00 d
Butte86	0.15 a B	5	0.24 a AB	6	0.29 a A	6	0.19 a B	5	0.22 a
81-2	0.02 b B	3	0.06 b AB	3	0.11 bc A	3	0.10 a-c AB	3	0.07 c
B45A	0.00 b B	1	0.09 b A	4	0.10 bc A	2	0.05 bc AB	2	0.06 c
B63A	0.01 b C	2	0.05 b BC	2	0.15 b A	4	0.13 ab AB	4	0.08 c
49-3	0.08 ab B	4	0.10 b AB	5	0.16 b A	5	0.19 a A	5	0.13 b
Avg.	0.05 n N		0.11 n MN		0.16 o M		0.13 n M		0.11 o
	Wheaton								
Control	0.00 c A	1	0.00 c A	1	0.00 d A	1	0.00 c A	1	0.00 e
Butte86	0.48 a C	5	0.64 a B	6	0.79 a A	6	0.37 a C	6	0.57 a
81-2	0.25 b AB	3	0.34 b A	3	0.34 bc A	3	0.19 b B	3	0.28 c
B45A	0.20 b BC	2	0.32 b A	2	0.24 c AB	2	0.15 b C	2	0.23 d
B63A	0.20 b B	2	0.37 b A	4	0.37 bc A	4	0.32 a A	5	0.32 bc
49-3	0.29 b B	4	0.41 b A	5	0.44 b A	5	0.27 ab B	4	0.35 b
Avg.	0.28 m N		0.42 m M		0.44 m M		0.26 m N		0.35 m
Mean‡	0.14 R		0.22 Q		0.30 P		0.21 Q		

[w] Average (mist-irrigation treatment means within each cultivar excludes water treatment).

* Isolate Butte86Ada-11

[x] Means followed by the same letter within column in each mist-irrigation duration and cultivar are not significantly different at $P \leq 0.05$.

[y] Means followed by the same letter within row in each cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same uppercase letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

† Cultivar means (combined across isolates and mist-irrigation durations) followed by same letter are not significantly different at $P < 0.05$.

‡ Mist-irrigation duration mean value combined across cultivars and isolates.

Appendix 9. Nivalenol (NIV) concentration (µg g^{-1}) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2007.

	Nivalenol (NIV)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.
Control	0.00 b A	1	0.00 b A	1	0.00 b A	1	0.00 b A	1	0.00 b
Butte86*	0.00 b A	1	0.02 b A	2	0.03 b A	2	0.01 b A	2	0.01 b
81-2	0.00 b A	1	0.00 b A	1	0.03 b A	2	0.02 b A	3	0.01 b
B45A	0.03 b A	2	0.04 b A	3	0.05 b A	3	0.00 b A	1	0.03 b
B63A	0.00 b A	1	0.00 b A	1	0.06 b A	4	0.01 b A	2	0.02 b
49-3	0.11 a AB	3	0.12 a AB	4	0.15 a A	5	0.08 a B	4	0.12 a
Avg.	0.03 n MN		0.04 n MN		0.06 n M		0.02 n N		0.04 n
	2375								
Control	0.00 a A	1	0.00 a A	1	0.00 d A	1	0.01 b A	1	0.00 c
Butte86	0.00 a A	1	0.00 a A	1	0.02 cd A	2	0.01 b A	1	0.01 c
81-2	0.00 a B	1	0.00 a B	1	0.08 bc A	4	0.02 b AB	2	0.03 bc
B45A	0.00 a A	1	0.04 a A	3	0.03 cd A	3	0.02 b A	2	0.02 bc
B63A	0.00 a B	1	0.01 a B	2	0.16 a A	6	0.02 b B	2	0.05 b
49-3	0.01 a C	2	0.06 a BC	4	0.10 ab AB	5	0.17 a A	3	0.09 a
Avg.	0.00 n O		0.02 n NO		0.08 n M		0.05 no MN		0.04 n
	Wheaton								
Control	0.00 d A	1	0.03 d A	1	0.00 d A	1	0.00 b A	1	0.00 d
Butte86	0.02 cd C	2	0.11 bc AB	3	0.16 bc A	4	0.06 b BC	4	0.09 c
81-2	0.06 bc B	3	0.19 a A	5	0.12 bc AB	3	0.06 b B	4	0.11 bc
B45A	0.12 b A	5	0.08 cd AB	2	0.10 c AB	2	0.03 b B	2	0.08 c
B63A	0.09 b B	4	0.17 ab A	4	0.18 b A	5	0.05 b B	3	0.12 b
49-3	0.20 a B	6	0.20 a B	6	0.39 a A	6	0.18 a B	5	0.24 a
Avg.	0.10 m N		0.15 m M		0.19 m M		0.08 m N		0.13 m
Mean‡	0.04 R		0.07 Q		0.11 P		0.05 QR		

[w] Average (mist-irrigation treatment means within each cultivar excludes water treatment).

***** Isolate Butte86Ada-11

[x] Means followed by the same letter within column in each mist-irrigation duration and cultivar are not significantly different at $P \leq 0.05$.

[y] Means followed by the same letter within row in each cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same uppercase letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Cultivar means (combined across isolates and mist-irrigation durations) followed by same letter are not significantly different at $P < 0.05$.

[‡] Mist-irrigation duration mean value combined across cultivars and isolates.

Appendix 9 continued. Nivalenol (NIV) concentration (µg g^{-1}) in harvested grain samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated wtih five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments in 2008.

	Nivalenol (NIV)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.
Control	0.00 a A	1	0.00 c A	1	0.00 c A	1	0.00 d A	1	0.00 e
Butte86*	0.00 a B	1	0.04 b AB	2	0.08 b A	2	0.02 cd B	2	0.04 d
81-2	0.01 a C	2	0.08 b B	5	0.14 a A	4	0.07 b B	4	0.08 b
B45A	0.00 a B	1	0.07 b A	4	0.08 b A	2	0.02 cd B	2	0.04 cd
B63A	0.00 a C	1	0.05 b BC	3	0.13 a A	3	0.06 bc B	3	0.06 bc
49-3	0.04 a B	3	0.13 a A	6	0.13 a A	3	0.12 a A	5	0.11 a
Avg.	0.01 n O		0.08 n N		0.11 n M		0.06 n N		0.06 n
	2375								
Control	0.00 a A	1	0.00 b A	1	0.00 d A	1	0.00 c A	1	0.00 d
Butte86	0.00 a A	1	0.02 ab A	2	0.03 cd A	2	0.01 bc A	2	0.02 cd
81-2	0.00 a B	1	0.05 a AB	4	0.10 ab A	5	0.05 b AB	4	0.05 b
B45A	0.00 a A	1	0.02 ab A	2	0.04 cd A	3	0.01 bc A	2	0.02 cd
B63A	0.00 a B	1	0.03 ab AB	3	0.06 bc A	4	0.04 bc AB	3	0.03 bc
49-3	0.00 a C	1	0.06 a B	5	0.13 a A	6	0.12 a A	5	0.08 a
Avg.	0.00 n O		0.03 m N		0.07 m M		0.05 n MN		0.04 o
	Wheaton								
Control	0.01 c A	1	0.00 d A	1	0.01 d A	1	0.00 c A	1	0.00 e
Butte86	0.04 bc C	2	0.14 c AB	2	0.18 c A	3	0.11 b B	2	0.12 d
81-2	0.13a B	5	0.21 ab A	4	0.23 bc A	4	0.12 b B	3	0.17 b
B45A	0.08 b B	4	0.17 bc A	3	0.15 c A	2	0.13 b AB	4	0.13 cd
B63A	0.05 bc C	3	0.17 bc B	3	0.24 b A	5	0.15 ab B	5	0.15 bc
49-3	0.13 a D	5	0.25 a B	5	0.32 a A	6	0.18 a C	6	0.22 a
Avg.	0.08 m P		0.19 m N		0.22 m M		0.14 m O		0.16 m
Mean‡	0.03		0.10		0.14		0.08		

[w] Average (mist-irrigation treatment means within each cultivar excludes water treatment).

* Isolate Butte86Ada-11

[x] Means followed by the same letter within column in each mist-irrigation duration and cultivar are not significantly different at $P \leq 0.05$.

[y] Means followed by the same letter within row in each cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same uppercase letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Cultivar means (combined across isolates and mist-irrigation durations) followed by same letter are not significantly different at $P < 0.05$.

[‡] Mist-irrigation duration mean value combined across cultivars and isolates.

Appendix 10. Fusarium head blight (FHB) severity (%) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to a 21 DAI mist-irrigation duration and sampled from 7 to 21 dai in 2007.

	FHB Severity							
	Sampling							
	7 dai		11 dai		14 dai		21 dai	
	Alsen							
Isolates	%	Rank	%	Rank	%	Rank	%	Rank
Control	2.21 b[x] B[y]	1	12.97 c A	1	9.72 c AB	1	0.63 b B	1
Butte86Ada-11	6.48 a B	5	30.76 b A	3	25.49 b A	2	30.92 a A	4
81-2	5.52 a B	3	31.45 b A	4	29.58 b A	4	27.89 a A	3
B45A	4.97 ab B	2	41.66 a A	6	34.93 ab A	5	34.51 a A	6
B63A	6.07 a B	4	35.31 ab A	5	29.30 b A	3	27.32 a A	2
49-3	6.48 a B	5	30.62 b A	2	39.86 a A	6	33.52 a A	5
Avg.[w]	5.90 m[z] O		33.96 m M		31.83 n MN		30.83 n N	
	2375							
Control	0.51 b A	1	9.62 b A	1	4.50 b A	1	0.87 b A	1
Butte86Ada-11	1.90 a B	4	23.54 a A	3	25.22 a A	4	28.45 a A	3
81-2	3.42 a B	6	27.72 a A	6	25.59 a A	5	28.94 a A	2
B45A	1.65 a B	3	25.82 a A	4	22.11 a A	2	30.12 a A	5
B63A	3.04 a B	5	23.04 a A	2	22.98 a A	3	32.17 a A	4
49-3	1.52 a B	2	27.59 a A	5	29.81 a A	6	37.27 a A	6
Avg.	2.30 n O		25.54 n MN		25.14 o N		31.39 n M	
	Wheaton							
Control	0.25 b B	1	10.51 c A	1	5.00 d B	1	1.66 c B	1
Butte86Ada-11	4.81 a D	2	32.78 b C	3	45.13 b B	5	70.90 a A	5
81-2	4.81 a C	2	43.54 a B	5	40.90 bc B	4	59.42 b A	2
B45A	7.22 a C	4	45.06 a B	6	36.54 bc B	3	63.07 ab A	4
B63A	4.81 a C	2	31.65 b B	2	34.10 c B	2	69.55 ab A	3
49-3	5.95 a D	3	38.10 ab C	4	55.13 a B	6	72.63 a A	6
Avg.	5.52 m O		38.23 m N		42.36 m N		67.20 m M	
Mean[†]	4.58 O[‡]		32.58 N		33.11 N		43.14 M	

[w] Average (sampling day means combined across isolates excluding water treatment).

[x] Means followed by the same lowercase letter within column in each sampling day and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate in each year or in each sampling days in each year are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same lowercase letter within column in each sampling day are not significantly different at $P < 0.05$.

[†] Sampling day mean values combined across cultivars and isolates.

[‡] Sampling day means (combined across isolates and cultivars) followed by same uppercase letter within row are not significantly different at $P < 0.05$.

Appendix 10 continued. Fusarium head blight (FHB) severity (%) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and water control subjected to a 28 DAI mist-irrigation duration and sampled from 7 to 21 dai in 2008.

	FHB Severity							
	Sampling							
	7 dai		11 dai		14 dai		21 dai	
	Alsen							
Isolates	%	Rank	%	Rank	%	Rank	%	Rank
Control	0.12 a[x] A[y]	2	0.25 c A	1	0.54 c A	1	0.81 c A	1
Butte86*	0.26 a C	3	3.79 b C	3	11.95 ab B	5	28.46 b A	4
81-2	0.12 a C	2	6.14 ab BC	5	11.54 ab B	4	35.97 a A	5
B45A	0.12 a D	2	9.47 a C	6	16.38 a B	6	38.12 a A	6
B63A	0.00 a C	1	4.63 b B	4	9.80 b AB	2	27.25 b A	3
49-3	0.00 a C	1	3.28 bc C	2	11.14 ab B	3	24.63 b A	2
Avg.[w]	0.10 m[z] P		5.46 m O		12.16 n N		30.89 n M	
	2375							
Control	0.00 b A	1	0.13 c A	1	1.03 b A	1	0.77 c A	1
Butte86	0.00 b C	1	2.34 bc C	3	9.66 a B	4	29.36 ab A	5
81-2	0.52 a C	2	4.33 ab BC	5	7.82 a B	3	26.99 b A	4
B45A	0.00 b C	1	5.91 a BC	6	11.41 a B	5	34.68 a A	6
B63A	0.00 b C	1	2.41 bc BC	4	7.82 a B	3	26.28 b A	3
49-3	0.00 b C	1	1.84 bc BC	2	6.54 ab B	2	25.77 b A	2
Avg.	0.10 m P		3.37 n O		8.65 o N		28.62 n M	
	Wheaton							
Control	0.00 b A	1	2.37 c A	1	5.15 c A	1	0.80 c A	1
Butte86	0.00 b C	1	4.32 bc C	2	11.53 b B	2	56.20 ab A	4
81-2	0.37 a D	3	5.98 b C	4	17.91 a B	5	60.12 a A	5
B45A	0.00 b C	1	9.52 a B	6	13.62 ab B	3	61.84 a A	6
B63A	0.00 b D	1	7.02 ab C	5	17.79 a B	4	53.07 b A	3
49-3	0.13 ab C	2	5.28 bc C	3	19.26 a B	6	52.58 b A	2
Avg.	0.10 m P		6.42 m O		16.02 m N		56.76 m M	
Mean[†]	0.10 P[‡]		5.08 O		12.28 N		38.76 M	

[w] Average (sampling day means combined across isolates excluding water treatment).

* Isolate Butte86Ada-11

[x] Means followed by the same lowercase letter within column in each sampling day and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate in each year or in each sampling days in each year are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same lowercase letter within column in each sampling day are not significantly different at $P < 0.05$.

[†] Sampling day mean values combined across cultivars and isolates.

[‡] Sampling day means (combined across isolates and cultivars) followed by same uppercase letter within row are not significantly different at $P < 0.05$.

Appendix 11. Deoxynivalenol (DON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to a 14 DAI mist-irrigation duration and sampled from 7 to 11 dai in 2007 and 2008.

	Deoxynivalenol (DON)							
	2007				2008			
	Sampling							
	7 dai		11 dai		7 dai		11 dai	
	Alsen							
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.02 a[x] A[y]	2	0.22 c A	1	0.07 b A	1	0.05 d A	1
Butte86*	0.00 a B	1	10.15 b A	3	0.17 b B	3	6.02 c A	3
81-2	0.02 a A	2	9.19 b A	2	0.72 a B	5	11.71 ab A	5
B45A	0.02 a B	2	15.81 ab A	5	0.30 ab B	4	13.11 a A	6
B63A	0.00 a B	1	14.98 ab A	4	0.30 ab B	4	5.83 c A	2
49-3	0.03 a B	3	24.03 a A	6	0.16 b B	2	7.61 bc A	4
Avg.[w]	0.03 m[z] N		14.83 n M		0.33 m N		8.86 m M	
	2375							
Control	0.00 a A	1	0.32 c A	1	0.08 a A	1	0.19 c A	1
Butte86	0.02 a A	2	6.14 b A	2	0.21 a A	4	3.46 b A	3
81-2	0.00 a B	1	10.21 ab A	4	0.54 a B	6	6.33 a A	6
B45A	0.00 a B	1	10.66 ab A	5	0.26 a B	5	5.78 a A	5
B63A	0.00 a A	1	9.22 b A	3	0.09 a A	3	3.27 b A	2
49-3	0.05 a B	3	16.05 a A	6	0.08 a B	2	3.90 b A	4
Avg.	0.02 m N		10.45 n M		0.24 m N		4.55 n M	
	Wheaton							
Control	0.02 a A	2	0.21 d A	1	0.07 b A	1	0.03 c A	1
Butte86	0.03 a B	3	20.10 c A	2	0.07 b B	1	4.42 b A	2
81-2	0.00 a B	1	40.50 b A	4	0.70 a B	5	7.66 ab A	3
B45A	0.00 a B	1	40.98 b A	5	0.47 ab B	4	9.54 a A	5
B63A	0.03 a B	3	26.44 c A	3	0.08 b B	2	7.92 ab A	4
49-3	0.03 a B	3	54.27 a A	6	0.32 ab B	3	10.77 a A	6
Avg.	0.02 m N		36.46 m M		0.33 m N		8.06 m M	
Mean[†]	0.02 N[‡]		20.58 M		0.30 N		7.16 M	

[w] Average (sampling day means combined across isolates excluding water treatment).

* Isolate Butte86Ada-11

[x] Means followed by the same lowercase letter within column in each sampling day and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate in each year or in each sampling days in each year are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same lowercase letter within column in each sampling day are not significantly different at $P < 0.05$.

[†] Sampling day mean values combined across cultivars and isolates.

[‡] Sampling day means (combined across isolates and cultivars) followed by same uppercase letter within row are not significantly different at $P < 0.05$.

Appendix 12. Deoxynivalenol (DON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 14 dai in 2007.

	Deoxynivalenol (DON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.03 c[x] A[y]	1	0.04 c A	1	0.37 c A	1	0.28 c A	1	0.18 d
Butte86*	17.66 b A	2	14.96 bc A	2	11.42 bc A	2	12.89 bc A	2	14.23 c
81-2	18.06 b A	3	16.66 bc A	4	15.52 a-c A	3	13.81 bc A	3	16.01 bc
B45A	20.97 b A	5	19.32 b A	5	31.27 a A	6	25.33 ab A	5	24.22 b
B63A	20.73 b A	4	15.14 bc A	3	24.49 ab A	4	15.95 bc A	4	19.08 bc
49-3	44.83 a A	6	48.25 a A	6	25.29 ab B	5	37.34 a AB	6	38.93 a
Avg.[w]	24.45 n[z] M		22.87 n M		21.60 n M		21.06 n M		22.19 n
	2375								
Control	0.02 c A	1	0.03 b A	1	0.16 c A	1	0.30 b A	1	0.13 c
Butte86	13.67 bc A	3	16.28 ab A	4	10.91 bc A	2	18.28 a A	4	14.79 b
81-2	13.13 bc A	2	16.45 ab A	5	14.36 abc A	3	10.03 ab A	2	13.49 b
B45A	22.98 ab A	5	12.48 bc A	3	18.80 ab A	4	20.50 a A	5	18.69 b
B63A	14.10 bc A	4	11.79 b A	2	19.29 ab A	5	11.16 ab A	3	14.09 b
49-3	35.86 a A	6	30.55 a A	6	31.38 a A	6	26.76 a A	6	31.14 a
Avg.	19.95 n M		17.51 n M		18.95 n M		17.35 n M		18.44 n
	Wheaton								
Control	0.22 e A	1	0.16 d A	1	0.59 d A	1	0.51 d A	1	0.37 e
Butte86	29.33 d B	2	51.75 b A	5	61.83 bc A	3	29.81 c B	2	43.18 d
81-2	50.58 c A	3	45.84 bc A	4	45.06 c A	2	53.13 b A	5	48.65 cd
B45A	85.09 ab A	5	33.39 c B	2	84.74 a A	5	46.67 bc B	3	62.47 b
B63A	69.43 b A	4	42.92 bc C	3	63.41 b AB	4	50.47 b BC	4	56.55 bc
49-3	92.53 a B	6	127.75 a A	6	101.81 a B	6	99.39 a B	6	105.37 a
Avg.	65.39 m MN		60.33 m NO		71.37 m M		55.89 m O		63.25 m
Mean[†]	36.60 MN[‡]		35.57 MN		37.31 M		31.43 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 12 continued. Deoxynivalenol (DON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 21 dai in 2007.

	Deoxynivalenol (DON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.30 c[x] A[y]	1	0.29 b A	1	0.40 c A	1	0.19 b A	1	0.30 c
Butte86*	13.32 b A	2	8.62 ab A	3	12.86 bc A	3	8.57 ab A	3	10.84 b
81-2	13.75 b A	3	7.60 ab A	2	12.28 bc A	2	8.54 ab A	2	10.54 b
B45A	18.14 b A	4	12.10 ab A	5	17.63 ab A	4	12.04 ab A	5	14.98 b
B63A	23.74 b A	5	10.92 ab A	4	18.78 ab A	5	11.39 ab A	4	16.21 b
49-3	37.21 a A	6	18.46 a B	6	25.76 a AB	6	18.80 a B	6	25.06 a
Avg.[w]	21.23 n[z] M		11.54 n N		17.46 n MN		11.87 n N		15.53 n
	2375								
Control	0.00 c A	1	0.33 c A	1	0.32 b A	1	0.09 b A	1	0.19 c
Butte86	12.78 b A	3	12.79 a-c A	3	17.23 a A	3	8.30 ab A	2	12.78 b
81-2	12.00 bc A	2	9.53 bc A	2	13.38 a A	2	9.82 ab A	3	11.18 b
B45A	17.91 ab A	5	13.79 ab A	5	20.19 a A	5	9.91 ab A	4	15.45 b
B63A	16.45 b A	4	12.80 a-c A	4	17.52 a A	4	10.94 ab A	5	14.43 b
49-3	27.03 a A	6	25.01 a A	6	22.49 a A	6	20.34 a A	6	23.72 a
Avg.	17.23 n M		14.78 n M		18.16 n M		11.86 n M		15.51 n
	Wheaton								
Control	2.34 f A	1	1.11 d A	1	1.32 e A	1	1.07 d A	1	1.46 d
Butte86	69.56 d A	3	35.16 c C	2	55.80 cd B	3	40.71 c C	2	50.31 c
81-2	55.91 e A	2	39.14 bc B	3	44.94 d B	2	43.97 c B	4	45.99 c
B45A	106.22 b A	5	47.64 b C	4	64.08 bc B	4	43.54 c C	3	65.37 b
B63A	86.05 c A	4	48.64 b C	5	73.83 b AB	5	60.75 b BC	5	67.32 b
49-3	140.71 a A	6	90.05 a BC	6	101.36 a B	6	84.22 a C	6	104.09 a
Avg.	91.69 m M		52.13 m O		68.00 m N		54.64 m O		66.61 m
Mean[†]	43.39 M[‡]		26.15 O		34.54 N		26.12 O		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isoalte Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 12 continued. Deoxynivalenol (DON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 28 dai in 2007.

	Deoxynivalenol (DON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.27 c[x] A[y]	1	0.38 c A	1	0.59 c A	1	0.23 b A	1	0.37 c
Butte86*	20.65 b A	3	12.11 bc A	4	12.16 bc A	3	8.23 ab A	2	13.29 b
81-2	19.11 b A	2	11.88 bc A	3	11.27 bc A	2	8.87 ab A	3	12.78 b
B45A	22.32 b A	4	11.08 bc A	2	12.72 bc A	4	8.93 ab A	4	13.76 b
B63A	24.37 b A	5	19.88 b A	5	17.93 ab A	5	11.92 ab A	5	18.53 b
49-3	47.68 a A	6	36.26 a AB	6	26.80 a BC	6	17.17 a C	6	31.98 a
Avg.[w]	26.83 n[z] M		18.24 n MN		16.18 n N		11.02 n N		18.07 n
	2375								
Control	0.00 d A	1	0.51 c A	1	0.68 c A	1	0.57 b A	1	0.44 c
Butte86	18.86 c A	2	13.89 b A	4	12.05 bc A	2	9.85 ab A	2	13.66 b
81-2	19.59 c A	3	13.10 b A	3	14.58 bc A	4	10.06 ab A	3	14.33 b
B45A	32.49 ab A	5	12.51 bc B	2	12.77 bc B	3	11.12 ab B	4	17.22 b
B63A	24.32 bc A	4	18.99 b A	5	18.82 ab A	5	11.61 ab A	5	18.44 b
49-3	39.21 a A	6	37.92 a A	6	27.25 a AB	6	19.22 a B	6	30.9 a
Avg.	26.89 n M		19.28 n MN		17.09 n N		12.37 n N		18.91 n
	Wheaton								
Control	1.57 d A	1	2.23 d A	1	3.20 c A	1	1.14 d A	1	2.04 e
Butte86	82.44 c A	3	51.50 c B	2	32.96 b C	3	22.18 c C	2	47.27 d
81-2	70.43 c A	2	65.00 b A	5	33.64 b B	4	28.59 bc B	4	49.42 d
B45A	118.71 b A	4	63.59 bc B	4	30.09 b C	2	25.20 c C	3	59.40 c
B63A	125.31 ab A	5	61.23 bc B	3	41.25 b C	5	38.20 b C	5	66.50 b
49-3	135.23 a A	6	112.99 a B	6	58.80 a C	6	55.63 a C	6	90.66 a
Avg.	106.42 m M		70.86 m N		39.35 m O		33.96 m O		62.65 m
Mean[†]	53.31 M[‡]		36.13 N		24.21 O		19.11 O		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Bute86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 12 continued. Deoxynivalenol (DON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 41 dai in 2007.

	Deoxynivalenol (DON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.63 e[x] A[y]	1	1.05 c A	1	0.39 c A	1	0.30 b A	1	0.59 d
Butte86*	14.65 d A	2	14.29 b A	2	14.53 b A	2	8.71 b A	4	13.05 c
81-2	21.04 cd A	3	20.25 b A	5	15.91 b AB	3	7.21 b B	2	16.10 bc
B45A	36.70 b A	4	17.91 b B	3	16.99 b B	4	7.97 b B	3	19.89 b
B63A	30.49 bc A	5	19.41 b AB	4	23.45 ab A	5	10.86 ab B	5	23.30 b
49-3	50.82 a A	6	39.09 a A	6	32.24 a B	6	20.88 a B	6	35.76 a
Avg.[w]	32.54 n[z] M		22.19 n N		20.62 n N		11.13 n O		21.62 n
	2375								
Control	0.60 d A	1	1.64 c A	1	2.23 c A	1	0.48 c A	1	1.24 d
Butte86	26.07 bc A	3	21.11 b AB	2	19.70 b AB	3	10.04 bc B	4	19.23 bc
81-2	21.10 c A	2	21.49 b A	3	21.00 b A	4	9.55 bc A	3	18.29 c
B45A	33.70 ab A	5	22.14 b AB	4	18.80 b BC	2	8.80 bc C	2	20.86 bc
B63A	32.33 ab A	4	23.48 b AB	5	29.54 ab A	5	11.86 ab B	5	24.30 b
49-3	40.12 a A	6	44.71 a A	6	37.78 a A	6	21.49 a B	6	36.02 a
Avg.	30.66 n M		26.59 n M		25.36 n M		12.35 n N		23.74 n
	Wheaton								
Control	2.75 d A	1	3.61 c A	1	5.99 c A	1	1.47 c A	1	3.46 e
Butte86	86.07 c A	2	59.56 b B	3	40.02 b C	4	13.75 b D	2	49.85 d
81-2	112.31 b A	3	67.92 b B	4	39.63 b C	3	19.47 b D	4	59.83 bc
B45A	114.28 b A	4	57.69 b B	2	38.25 b C	2	14.21 b D	3	56.11 c
B63A	121.39 b A	5	65.13 b B	5	44.61 b C	5	20.56 b D	5	62.92 b
49-3	148.67 a A	6	99.83 a B	6	69.66 a C	6	35.19 a D	6	88.34 a
Avg.	116.54 m M		70.03 m N		46.43 m O		20.64 m P		63.41 m
Mean[†]	59.92 M[‡]		39.60 N		30.81 O		14.70 P		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 12 continued. Deoxynivalenol (DON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 14 dai in 2008.

	Deoxynivalenol (DON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.03 b[x] A[y]	1	0.60 c A	1	0.05 b A	1	0.35 b A	1	0.26 c
Butte86*	13.06 a A	4	14.74 b A	2	11.58 a A	3	13.97 a A	4	13.34 b
81-2	12.44 a B	3	21.86 ab A	4	12.52 a B	4	14.60 a AB	5	15.36 ab
B45A	11.64 a B	2	23.30 a A	6	17.57 a AB	6	19.31 a AB	6	17.96 a
B63A	19.01 a A	6	18.86 ab A	5	10.47 a B	2	12.98 a AB	3	15.33 ab
49-3	14.56 a AB	5	21.52 ab A	3	17.00 a AB	5	11.62 a B	2	16.18 ab
Avg.[w]	14.14 n[z] N		20.06 n M		13.83 n N		14.50 n N		15.63 n
	2375								
Control	0.02 b A	1	0.15 b A	1	0.04 b A	1	0.32 b A	1	0.13 b
Butte86	5.89 ab A	2	10.86 a A	4	7.58 ab A	4	7.08 ab A	3	7.85 a
81-2	6.62 ab A	3	9.26 a A	2	8.48 a A	5	8.07 ab A	5	8.11 a
B45A	9.46 a A	6	11.62 a A	5	5.74 ab A	2	7.31 ab A	4	8.53 a
B63A	7.61 ab AB	4	15.39 a A	6	6.63 ab BC	3	5.15 ab C	2	8.70 a
49-3	8.11 ab A	5	9.32 a A	3	12.70 a A	6	11.38 a A	6	10.38 a
Avg.	7.54 o M		11.29 o M		8.23 o M		7.80 o M		8.71 o
	Wheaton								
Control	0.06 d A	1	0.11 c A	1	0.38 d A	1	0.04 d A	1	0.15 d
Butte86	16.43 c A	2	23.51 b A	2	17.49 c A	2	15.95 c A	2	18.35 c
81-2	24.70 b A	4	32.65 a A	5	29.12 b A	5	17.57 c B	3	26.01 b
B45A	26.11 b A	5	31.10 ab A	3	22.98 bc A	3	25.78 ab A	5	26.49 b
B63A	16.52 c B	3	32.54 a A	4	24.62 bc AB	4	20.47 bc B	4	23.54 b
49-3	39.75 a AB	6	35.78 a AB	6	42.07 a A	6	33.61 a B	6	37.80 a
Avg.	24.70 m N		31.11 m M		27.26 m MN		22.68 m N		26.44 m
Mean[†]	15.46 N[‡]		20.82 M		16.44 N		14.99 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 12 continued. Deoxynivalenol (DON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 21 dai in 2008.

	Deoxynivalenol (DON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.06 b[x] A[y]	1	0.00 c A	1	0.11 b A	1	2.02 b A	1	0.55 c
Butte86*	17.48 ab A	3	25.95 b A	2	9.05 b A	2	19.74 a A	4	18.06 b
81-2	24.88 a A	5	35.73 ab A	3	31.66 a A	6	18.72 a A	3	27.75 ab
B45A	19.49 ab B	4	53.39 ab A	6	17.96 ab B	4	23.26 a B	6	28.53 a
B63A	15.95 ab B	2	39.42 ab A	4	10.07 b B	3	16.44 a B	2	20.47 ab
49-3	30.57 a AB	6	43.89 a A	5	18.39 a B	5	19.75 a B	5	28.15 a
Avg.[w]	21.67 n[z] N		39.68 n M		17.43 n N		19.58 n N		24.59 n
	2375								
Control	0.09 b A	1	0.70 c A	1	0.00 a A	1	0.05 a A	1	0.21 b
Butte86	16.48 ab A	3	16.30 bc A	2	9.89 a A	3	16.82 a A	6	14.87 a
81-2	21.75 a A	5	20.55 ab A	3	17.90 a A	6	16.50 a A	5	19.18 a
B45A	22.13 a A	6	27.41 ab A	5	8.30 a A	2	7.82 a A	2	16.42 a
B63A	17.86 ab A	4	21.40 ab A	4	13.68 a A	4	11.72 a A	3	16.17 a
49-3	15.48 ab B	2	37.09 a A	6	15.64 a B	5	15.75 a B	5	20.99 a
Avg.	18.74 n M		24.55 o M		13.08 n M		13.72 n M		17.53 o
	Wheaton								
Control	3.04 d A	1	0.40 b A	1	0.48 c A	1	0.63 e A	1	1.14 e
Butte86	61.12 c A	2	61.91 a A	2	20.44 b B	2	47.16 bc A	4	47.66 cd
81-2	81.00 b A	5	79.63 a A	6	32.61 ab B	4	28.25cd B	3	55.37 bc
B45A	68.98 bc a	4	78.00 a A	5	41.18 a B	5	59.06 ab AB	5	61.81 b
B63A	68.48 bc A	3	62.98 a A	3	21.28 b B	3	24.70 d B	2	44.36 d
49-3	128.29 a A	6	74.51 a B	4	43.04 a C	6	73.03 a B	6	79.72 a
Avg.	81.57 m M		71.41 m M		31.71 m O		46.44 m N		57.78 m
Mean[†]	40.66 M[‡]		45.21 M		20.74 N		26.58 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 12 continued. Deoxynivalenol (DON) concentration (μg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 28 dai in 2008.

	Deoxynivalenol (DON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	Avg.[w]
Control	5.03 a[x] A[y]	1	1.96 c A	1	0.40 b A	1	0.50 a A	1	1.97 c
Butte86*	13.74 a A	5	17.14 b A	2	15.01 ab A	2	10.57 a A	2	14.11 b
81-2	13.29 a A	4	27.50 ab A	5	23.71 a A	4	20.83 a A	4	21.33 ab
B45A	10.32 a A	3	25.38 ab A	3	25.51 a A	5	22.66 a A	5	20.97 ab
B63A	8.54 a A	2	26.26 ab A	4	17.14 ab A	3	14.79 a A	3	16.68 b
49-3	19.91 a A	6	41.58 a A	6	28.68 a A	6	22.77 a A	6	28.24 a
Avg.[w]	13.16 n[z] N		27.57 n M		22.01 n MN		18.32 n MN		20.27 n
	2375								
Control	0.04 a A	1	1.00 b A	1	0.39 a A	1	0.11 b A	1	0.39 c
Butte86	5.05 a A	2	17.62 ab A	2	10.77 a A	2	10.00 ab A	2	10.86 bc
81-2	6.37 a B	3	33.71 a A	6	18.96 a AB	5	12.24 ab AB	4	17.82 ab
B45A	9.76 a A	4	28.18 a A	5	14.74 a A	4	16.37 ab A	5	17.26 ab
B63A	12.96 a A	5	19.44 ab A	3	14.18 a A	3	10.60 ab A	3	14.29 ab
49-3	21.46 a A	6	25.29 a A	4	22.29 a A	6	25.04 a A	6	23.52 a
Avg.	11.12 n N		24.85 n M		16.19 n MN		14.85 n MN		16.75 n
	Wheaton								
Control	1.91 c A	1	7.30 A	1	1.87 c A	1	3.68 c A	1	3.69 c
Butte86	20.48 bc B	2	68.92 b A	3	29.40 b B	4	18.44 bc B	2	34.31 b
81-2	43.55 a B	4	83.50 ab A	4	36.08 ab B	5	36.49 ab B	5	49.91 a
B45A	53.49 a B	6	83.85 ab A	5	27.21 b C	3	29.28 ab C	3	48.46 a
B63A	32.75 ab B	3	95.32 a A	6	25.46 b B	2	35.77 ab B	4	47.33 a
49-3	52.71 a A	5	61.69 b A	2	57.21 a A	6	44.63 a A	6	54.06 a
Avg.	40.60 m N		78.66 m M		35.07 mN		32.92 m N		46.81 m
Mean[†]	21.63 N[‡]		43.69 M		24.42 N		22.03 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 12 continued. Deoxynivalenol (DON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 41 (39) dai in 2008.

	Deoxynivalenol (DON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	1.30 b[x] A[y]	1	1.11 c A	1	2.10 b A	1	2.57 c A	1	1.77 c
Butte86*	39.74 a A	3	29.25 b AB	2	30.61 a AB	2	16.63 bc B	2	29.06 b
81-2	43.13 a AB	5	56.18 a A	6	47.41 a AB	6	25.78 ab B	5	43.12 a
B45A	34.03 a AB	2	51.04 a A	5	38.79 a AB	3	23.02 a-c B	4	36.72 ab
B63A	45.30 a A	6	39.60 ab AB	3	42.82 a AB	4	21.48 a-c B	3	37.30 ab
49-3	40.10 a A	4	46.05 ab A	4	45.72 a A	5	38.15 a A	6	42.51 a
Avg.[w]	40.46 n[z] M		44.42 n M		41.07 n M		25.01 n N		37.74 n
	2375								
Control	0.34 c A	1	1.56 b A	1	3.29 c A	1	2.17 b A	1	1.84 c
Butte86	27.29 b A	2	33.89 a A	2	27.00 b A	3	17.98 ab A	2	26.54 b
81-2	53.03 ab A	5	34.92 a AB	3	43.27 ab AB	5	26.48 a B	5	39.43 a
B45A	47.89 ab A	4	42.81 a AB	4	26.65 b AB	2	20.89 ab B	3	34.56 ab
B63A	39.42 ab A	3	43.13 a A	5	33.12 ab A	4	23.65 a A	4	34.83 ab
49-3	57.95 a A	6	43.43 a A	6	48.85 a A	6	36.26 a A	6	46.42 a
Avg.	45.12 n M		39.64 n M		35.78 n MN		25.05 n N		36.40 n
	Wheaton								
Control	3.92 e A	1	7.77 c A	1	5.09 d A	1	5.93 b A	1	5.68 e
Butte86	80.97 d A	2	77.53 b A	2	67.72 bc A	4	34.18 a B	2	65.10 d
81-2	118.77 bc A	4	129.39 a A	6	80.06 b B	5	41.31 a C	4	92.38 b
B45A	124.13 b A	5	114.84 a A	4	67.60 bc B	3	34.83 a C	3	85.35 bc
B63A	102.01 c A	3	111.53 a A	3	58.47 c B	2	46.23 a B	5	79.56 c
49-3	149.67 a A	6	119.69 a B	5	127.85 a AB	6	54.30 a C	6	112.88 a
Avg.	115.11 m M		110.60 m M		80.34 m N		42.17 m O		87.05 m
Mean[†]	66.90 M[‡]		64.89 M		52.40 N		30.74 O		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 13. 15-acetyldeoxynivalenol (15-ADON) concentration (μg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to 14 DAI mist-irrigation duration and sampled from 7 to 11 dai in 2007 and 2008.

	15-acetyldeoxynivalenol (15-ADON)							
	2007				2008			
	Sampling							
	7 dai		11 dai		7 dai		11 dai	
	Alsen							
Isolates	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank
Control	0.00 a[x] A[y]	1	0.00 b A	1	0.00 b A	1	0.00 c A	1
Butte86*	0.00 a A	1	0.67 ab A	3	0.00 b B	1	0.18 bc A	3
81-2	0.02 a A	2	0.60 ab A	2	0.05 a B	3	0.39 ab A	4
B45A	0.00 a B	1	1.14 a A	5	0.02 a B	2	0.47 a A	6
B63A	0.00 a B	1	0.77 ab A	4	0.00 b A	1	0.11 c A	2
49-3	0.00 a B	1	1.37 a A	6	0.00 b B	1	0.44 a A	5
Avg.[w]	0.01 m[z] N		0.91 n M		0.02 m N		0.32 m M	
	2375							
Control	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 c A	1
Butte86	0.00 a A	1	0.43 ab A	2	0.00 a A	1	0.06 bc A	2
81-2	0.00 a B	1	0.78 ab A	4	0.04 a B	2	0.21 ab A	5
B45A	0.00 a B	1	0.79 ab A	5	0.00 a B	1	0.29 a A	6
B63A	0.00 a A	1	0.73 ab A	3	0.00 a A	1	0.08 abc A	3
49-3	0.00 a B	1	1.42 a A	6	0.00 a B	1	0.19 abc A	4
Avg.	0.00 m N		0.83 n M		0.01 m N		0.17 n M	
	Wheaton							
Control	0.00 a A	1	0.00 d A	1	0.00 a A	1	0.00 c A	1
Butte86	0.00 a B	1	1.31 c A	2	0.00 a A	1	0.12 bc A	2
81-2	0.00 a B	1	2.99 b A	5	0.02 a B	3	0.19 bc A	4
B45A	0.00 a B	1	2.66 b a	4	0.00 a B	1	0.25 ab A	5
B63A	0.00 a B	1	1.73 c A	3	0.00 a B	1	0.16 bc A	3
49-3	0.00 a B	1	4.28 a A	6	0.01 a B	2	0.42 a A	6
Avg.	0.00 m N		2.59 m M		0.01 m N		0.28 m M	
Mean[†]	0.00 N[‡]		1.44 M		0.01 N		0.26 M	

[w] Average (sampling day means combined across isolates excluding water treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each sampling day and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate in each year or in each sampling days in each year are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same lowercase letter within column in each sampling day are not significantly different at $P < 0.05$.

[†] Sampling day mean values combined across cultivars and isolates.

[‡] Sampling day means (combined across isolates and cultivars) followed by same uppercase letter within row are not significantly different at $P < 0.05$.

Appendix 14.15-aceyldeoxynivalenol (15-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 14 dai in 2007.

	15-acetyldeoxynivalenol (15-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 b[x] A[y]	1	0.00 b A	1	0.01 a A	1	0.00 c A	1	0.00 c
Butte86*	0.34 b A	2	0.52 b A	3	0.29 a A	2	0.64 bc A	2	0.45 bc
81-2	0.57 ab A	3	0.55 b A	4	0.54 a A	3	0.82 bc A	4	0.62 b
B45A	0.57 ab A	3	0.58 b A	5	0.94 a A	6	1.35 b A	5	0.86 b
B63A	0.63 ab A	4	0.46 b A	2	0.69 a A	4	0.79 bc A	3	0.64 b
49-3	1.38 a AB	5	1.70 a AB	6	0.86 a B	5	1.92 a A	6	1.47 a
Avg.[w]	0.70 n[z] M		0.76 n M		0.66 n M		1.10 n M		0.81 n
	2375								
Control	0.00 b A	1	0.02 b A	1	0.00 b A	1	0.00 c A	1	0.01 c
Butte86	0.37 b A	2	0.57 ab A	4	0.32 ab A	2	1.16 ab A	5	0.61 b
81-2	0.48 ab A	3	0.71 ab A	5	0.52 ab A	3	0.68 bc A	2	0.60 b
B45A	0.75 ab A	5	0.43 ab A	3	0.58 ab A	5	0.84 bc A	4	0.65 b
B63A	0.55 ab A	4	0.41 ab A	2	0.55 ab A	4	0.77 bc A	3	0.57 b
49-3	1.28 a A	6	1.15 a A	6	1.21 a A	6	1.77 a A	6	1.35 a
Avg.	0.69 n M		0.65 n M		0.64 n M		1.04 n M		0.76 n
	Wheaton								
Control	0.00 e A	1	0.22 c A	1	0.00 d A	1	0.01 e A	1	0.06 e
Butte86	0.81 de B	2	1.91 b A	5	1.45 c A	2	2.15 d A	2	1.58 d
81-2	1.58 cd B	3	1.80 b B	4	1.45 c B	2	4.31 b A	5	2.29 bc
B45A	2.78 ab A	5	1.47 b B	3	2.69 b A	4	3.50 bc A	4	2.61 b
B63A	2.04 bc B	4	1.38 b B	2	1.83 bc B	3	3.22 c A	3	2.12 c
49-3	2.98 a C	6	5.12 a B	6	3.88 a C	5	8.71 a A	6	5.17 a
Avg.	2.04 m N		2.34 m N		2.26 m N		4.38 m M		2.75 m
Mean[†]	1.14 N[‡]		1.25 N		1.19 N		2.18 M		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 14 continued. 15-acetyldeoxynivalenol (15-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 21 dai in 2007.

	15-acetyldeoxynivalenol (15-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		41 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.03 c[x] A[y]	1	0.03 b A	1	0.02 a A	2	0.00 b A	1	0.02 c
Butte86*	1.34 b A	2	0.59 ab AB	2	0.00 a B	1	0.67 ab AB	2	0.65 bc
81-2	1.55 b A	3	0.64 ab AB	3	0.03 a B	3	0.74 ab AB	3	0.74 b
B45A	2.04 b A	4	1.10 ab AB	5	0.03 a B	3	1.17 ab AB	5	1.09 ab
B63A	2.39 b A	5	0.74 ab B	4	0.10 a B	4	0.80 ab B	4	1.01 b
49-3	3.79 a A	6	1.33 a B	6	0.03 a C	3	1.46 a B	6	1.65 a
Avg.[w]	2.22 n[z] M		0.88 n N		0.04 m O		0.97 n N		1.03 n
	2375								
Control	0.00 c A	1	0.00 c A	1	0.00 a A	1	0.00 b A	1	0.00 c
Butte86	1.49 b A	3	1.01 abc A	4	0.02 a B	2	0.72 b A	2	0.81 b
81-2	1.42 b A	2	0.83 bc AB	2	0.00 a B	1	0.98 ab AB	4	0.81 b
B45A	2.31 ab A	5	1.47 ab A	5	0.00 a B	1	1.17 ab AB	5	1.24 b
B63A	2.23 b A	4	0.94 abc AB	3	0.00 a B	1	0.80 b B	3	0.99 b
49-3	3.60 a A	6	2.18 a B	6	0.06 a C	3	2.13 a B	6	1.99 a
Avg.	2.21 n M		1.29 n N		0.02 m O		1.16 n N		1.17 n
	Wheaton								
Control	0.11 e A	1	0.08 d A	1	0.02 a A	2	0.13 d A	1	0.09 e
Butte86	8.48 d A	3	2.54 c B	2	0.04 a C	3	2.68 c B	2	3.44 d
81-2	7.47 d A	2	3.30 c B	4	0.00 a C	1	4.09 b B	5	3.71 cd
B45A	13.01 b A	5	4.60 b B	5	0.00 a C	1	3.99 b B	4	5.40 b
B63A	10.02 c A	4	2.97 c B	3	0.04 a C	3	3.88 bc B	3	4.23 c
49-3	21.99 a A	6	6.96 a B	6	0.04 a C	3	7.12 a B	6	9.03 a
Avg.	12.19 m M		4.07 m N		0.02 m O		4.35 m N		5.16 m
Mean[†]	5.542 M[‡]		2.08 N		0.03 O		2.16 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†] Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 14 continued. 15-acetyldeoxynivalenol (15-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 28 dai in 2007.

	15-acetyldeoxynivalenol (15-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 c[x] A[y]	1	0.00 c A	1	0.00 b A	1	0.00 a A	1	0.00 c
Butte86*	1.39 b A	2	0.91 bc A	2	0.45 ab A	2	0.46 a A	3	0.80 b
81-2	1.50 b A	3	1.06 bc A	3	0.57 ab A	3	0.53 a A	4	0.92 b
B45A	1.71 b A	5	1.09 b A	4	0.79 ab A	5	0.58 a A	5	1.04 b
B63A	1.68 b A	4	1.27 ab A	5	0.58 ab A	4	0.21 a A	2	0.94 b
49-3	3.48 a A	6	2.31 a AB	6	1.14 a BC	6	0.84 a C	6	1.94 a
Avg.[w]	1.95 n[z] M		1.33 n MN		0.71 n N		0.52 n N		1.13 n
	2375								
Control	0.00 d A	1	0.00 c A	1	0.00 b A	1	0.06 a A	1	0.02 c
Butte86	1.52 c A	2	1.19 b A	2	0.70 ab A	2	0.66 a A	2	1.02 b
81-2	1.81 bc A	3	1.33 b A	3	0.83 ab A	3	0.69 a A	3	1.17 b
B45A	2.82 ab A	5	1.34 b B	4	0.93 ab B	5	0.75 a B	4	1.46 b
B63A	2.03 bc A	4	1.77 b AB	5	0.85 ab AB	4	0.66 a B	2	1.33 b
49-3	3.51 a A	6	3.07 a A	6	1.30 a B	6	1.12 a B	5	2.25 a
Avg.	2.34 n M		1.74 n MN		0.92 mn N		0.78 mn N		1.44 n
	Wheaton								
Control	0.23 e A	1	0.12 e A	1	0.26 c A	1	0.22 c A	1	0.21 e
Butte86	5.91 d A	2	2.59 d B	2	1.04 bc C	2	1.03 bc C	2	2.64 d
81-2	6.33 d A	3	4.44 b B	5	1.47 b C	4	1.56 b C	5	3.45 c
B45A	9.94 b A	5	4.34 bc B	4	1.60 b C	5	1.34 b C	4	4.31 b
B63A	8.22 c A	4	3.31 cd B	3	1.33 bc C	3	1.31 b C	3	3.54 c
49-3	12.18 a A	6	7.77 a B	6	2.70 a C	6	2.85 a C	6	6.38 a
Avg.	8.52 m M		4.49 m N		1.63 m O		1.62 m O		4.06 m
Mean[†]	4.27 M[‡]		2.52 N		1.09 O		0.97 O		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 14 continued. 15-acetyldeoxynivalenol (15-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 41 dai in 2007.

	15-acetyldeoxynivalenol (15-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.03 e[x] A[y]	1	0.00 c A	1	0.00 c A	1	0.00 a A	1	0.01 d
Butte86*	1.09 d A	2	1.00 b A	2	0.92 b A	2	0.22 a A	2	0.81 c
81-2	1.70 cd A	3	1.64 ab A	5	1.08 ab AB	3	0.24 a B	3	1.17 bc
B45A	2.94 ab A	5	1.42 b B	4	1.15 ab BC	4	0.27 a C	4	1.45 b
B63A	2.20 bc A	4	1.20 b BC	3	1.21 ab B	5	0.29 a C	5	1.23 bc
49-3	3.85 a A	6	2.48 a B	6	1.90 a B	6	0.88 a C	6	2.28 a
Avg.[w]	2.36 n[z] M		1.54 o N		1.25 o N		0.38 n O		1.38 o
	2375								
Control	0.00 d A	1	0.11 c A	1	0.28 c A	1	0.00 b A	1	0.10 c
Butte86	2.04 c A	3	1.92 b A	3	1.40 b A	2	0.40 ab B	3	1.44 b
81-2	1.99 c A	2	1.93 b A	4	1.77 b A	4	0.60 ab B	5	1.57 b
B45A	3.28 ab A	5	2.39 b AB	5	1.50 b B	3	0.28 ab C	2	1.86 b
B63A	2.66 bc A	4	1.86 b A	2	1.79 b A	5	0.51 ab B	4	1.71 b
49-3	3.98 a A	6	3.45 a AB	6	2.75 a B	6	1.05 a C	6	2.81 a
Avg.	2.79 n M		2.31 n MN		1.84 n N		0.57 n O		1.88 n
	Wheaton								
Control	0.14 e A	1	0.21 d A	1	0.74 c A	1	0.00 c A	1	0.27 e
Butte86	5.99 d A	2	3.04 c B	2	2.23 b B	2	0.65 bc C	2	2.98 d
81-2	9.52 bc A	4	4.69 b B	5	2.90 b C	4	1.15 b D	5	4.57 b
B45A	10.30 b A	5	4.39 b B	4	3.06 b C	5	0.74 bc D	3	4.62 b
B63A	8.67 c A	3	3.14 c B	3	2.38 b B	3	0.83 bc C	4	3.76 c
49-3	13.78 a A	6	6.60 a B	6	5.14 a C	6	2.20 a D	6	6.93 a
Avg.	9.65 m M		4.37 m N		3.14 m O		1.11 m P		4.57 m
Mean[†]	4.93 M[‡]		2.74 N		2.08 O		0.69 P		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 14 continued. 15-acetyldeoxynivalenol (15-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 14 dai in 2008.

	15-acetyldeoxynivalenol (15-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 a[x] A[y]	1	0.02 c A	1	0.00 c A	1	0.00 A	1	0.01 d
Butte86*	0.15 a A	2	0.26 bc A	2	0.21 bc A	2	0.17 A	2	0.20 c
81-2	0.28 a A	6	0.56 a A	4	0.35 ab A	4	0.38 A	5	0.39 ab
B45A	0.26 a B	5	0.59 a A	6	0.56 a AB	6	0.49 AB	6	0.48 a
B63A	0.24 a A	3	0.49 ab A	3	0.22 bc A	3	0.31 A	3	0.32 bc
49-3	0.25 a B	4	0.58 a A	5	0.45 ab AB	5	0.37 AB	4	0.41 ab
Avg.[w]	0.24 n[z] N		0.50 n M		0.36 n MN		0.34 n MN		0.35 n
	2375								
Control	0.00 a A	1	0.00 c A	1	0.00 c A	1	0.02 A	1	0.01 b
Butte86	0.04 a A	2	0.17 bc A	2	0.18 bc A	2	0.08 A	2	0.12 b
81-2	0.15 a A	3	0.41 ab A	5	0.42 ab A	5	0.20 A	4	0.30 a
B45A	0.24 a A	5	0.38 ab A	4	0.25 bc A	3	0.25 A	5	0.28 a
B63A	0.21 a AB	4	0.49 a A	6	0.27 bc AB	4	0.12 B	3	0.27 a
49-3	0.24 a B	5	0.32 b AB	3	0.61 a A	6	0.48 AB	6	0.41 a
Avg.	0.18 n M		0.35 n M		0.35 n M		0.23 n M		0.28 n
	Wheaton								
Control	0.00 e A	1	0.00 c A	1	0.01 d A	1	0.00 A	1	0.00 e
Butte86	0.21 de B	2	0.54 b A	2	0.37 c AB	2	0.26 Ab	2	0.35 d
81-2	0.52 bc C	4	0.90 a A	6	0.95 b AB	5	0.62 BC	4	0.75 b
B45A	0.71 b A	5	0.77 ab A	4	0.67 b A	3	0.66 A	5	0.70 b
B63A	0.26 cd B	3	0.63 ab A	3	0.76 b A	4	0.40 AB	3	0.51 c
49-3	1.11 a B	6	0.81 ab B	5	1.82 a A	6	0.91 B	6	1.16 a
Avg.	0.56 m N		0.73 m MN		0.91 m M		0.57 m N		0.69 m
Mean[†]	0.32 N[‡]		0.53 M		0.54 M		0.38 MN		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isoalte Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 14 continued. 15-acetyldeoxynivalenol (15-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 21 dai in 2008.

	15-acetyldeoxynivalenol (15-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 b[x] A[z]	1	0.00 c A	1	0.00 a A	1	0.04 a A	1	0.01 c
Butte86*	0.71 a A	2	0.64 ab A	2	0.26 a A	2	0.54 a A	2	0.54 bc
81-2	1.71 a A	5	1.67 ab A	4	1.39 a A	6	1.10 a A	5	1.47 a
B45A	1.29 a A	4	2.68 a A	6	0.99 a A	5	1.43 a A	6	1.60 a
B63A	1.03 a A	3	1.49 ab A	3	0.45 a A	3	0.59 a A	3	0.89 ab
49-3	1.83 a A	6	1.85 ab A	5	0.87 a A	4	0.94 a A	4	1.37 a
Avg.[w]	1.31 n[z] M		1.67 n M		0.79 m M		0.92 mn M		1.17 n
	2375								
Control	0.00 a A	1	0.05 b A	1	0.00 a A	1	0.00 a A	1	0.01 b
Butte86	0.74 a A	2	0.54 ab A	2	0.38 a A	2	0.60 a A	5	0.57 ab
81-2	1.45 a A	5	1.20 ab A	3	1.13 a A	6	1.00 a A	6	1.20 a
B45A	1.63 a A	6	1.75 a A	5	0.61 a A	3	0.49 a A	2	1.12 a
B63A	1.41 a A	4	1.28 ab A	4	0.86 a A	5	0.51 a A	3	1.02 a
49-3	1.27 a A	3	1.88 a A	6	0.76 a A	4	0.79 a A	4	1.18 a
Avg.	1.30 n M		1.33 n M		0.75 m M		0.68 n M		1.01 n
	Wheaton								
Control	0.21 e A	1	0.02 c A	1	0.00 b A	1	0.03 c A	1	0.07 e
Butte86	3.26 d A	2	1.44 c B	2	0.47 a B	2	1.12 bc B	3	1.57 d
81-2	7.83 b A	5	3.13 ab B	4	1.44 a B	4	1.37 bc B	4	3.44 b
B45A	5.98 c A	3	4.02 a B	6	1.81 a C	5	1.49 ab BC	5	3.33 b
B63A	6.42 bc A	4	1.97 bc B	3	0.71 a B	3	0.77 c B	2	2.47 c
49-3	13.79 a A	6	3.45 a B	5	1.88 a B	6	3.46 a B	6	5.65 a
Avg.	7.46 m M		2.80 m N		1.26 m O		1.64 m NO		3.29 m
Mean[†]	3.36 M[‡]		1.93 N		0.93 O		1.08 NO		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 14 continued. 15-acetyldeoxynivalenol (15-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 28 dai in 2008.

	15-acetyldeoxynivalenol (15-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.44 a[x] A[y]	1	0.16 c A	1	0.01 a A	1	0.04 a A	1	0.16 b
Butte86*	1.07 a A	5	0.95 bc A	2	0.30 a A	2	0.26 a A	2	0.65 ab
81-2	1.01 a A	4	2.37ab A	4	0.74 a A	4	0.71 a A	5	1.21 a
B45A	0.98 a A	3	2.47 ab A	5	0.95 a A	6	1.00 a A	6	1.35 a
B63A	0.63 a A	2	1.47 a-c A	3	0.38 a A	3	0.43 a A	3	0.73 ab
49-3	1.72 a AB	6	3.20 a A	6	0.86 a B	5	0.52 a B	4	1.58 a
Avg.[w]	1.08 n[z] MN		2.09 n M		0.65 m N		0.58 m N		1.10 n
	2375								
Control	0.00 a A	1	0.22 c A	1	0.07 a A	1	0.03 a A	1	0.08 b
Butte86	0.50 a A	2	1.43 bc A	2	0.32 a A	2	0.26 a A	2	0.63 ab
81-2	0.81 a B	3	3.82 a A	6	0.74 a B	4	0.64 a B	4	1.50 a
B45A	1.31 a AB	5	3.31 ab A	5	0.91 a B	5	0.73 a B	5	1.57 a
B63A	1.62 a A	4	2.16 a-c A	3	0.71 a A	3	0.45 a A	3	1.24 a
49-3	1.95 a A	6	2.52 ab A	4	1.04 a A	6	1.03 a A	6	1.64 a
Avg.	1.24 n N		2.65 n M		0.74 m N		0.62 m N		1.31 n
	Wheaton								
Control	0.04 d A	1	1.01 d A	1	0.07 a A	1	0.12 a A	1	0.31 d
Butte86	1.82 c AB	2	3.53 c A	2	0.37 a B	2	0.29 a B	2	1.50 c
81-2	4.56 ab A	4	5.77 ab A	5	0.78 a B	5	1.19 a B	6	3.08 ab
B45A	5.39 a A	5	7.58 a B	6	0.76 a C	4	0.85 a C	4	3.65 a
B63A	2.98 bc B	3	5.49 bc A	4	0.51 a C	3	0.55 a C	3	2.38 bc
49-3	6.03 a A	6	5.10 bc A	3	1.19 a B	6	1.09 a B	5	3.35 ab
Avg.	4.16 m N		5.49 m M		0.72 m O		0.79 m O		2.79 m
Mean[†]	2.16 N[‡]		3.41 M		0.70 O		0.67 O		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isoalte Bute86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 14 continued. 15-acetyldeoxynivalenol (15-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 41 (39) dai in 2008.

	15-acetyldeoxynivalenol (15-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.17 b[x] A[y]	1	0.17 c A	1	0.12 b A	1	0.13 a A	1	0.15 c
Butte86*	2.34 a A	2	1.61 b A	2	1.67 ab A	2	0.67 a A	2	1.57 b
81-2	3.46 a AB	5	4.31 a A	6	3.39 a AB	6	1.46 a B	5	3.16 a
B45A	2.98 a AB	3	4.05 a A	5	3.01 a AB	5	1.36 a B	4	2.85 a
B63A	3.55 a A	6	2.49 ab AB	3	2.02 ab AB	3	0.80 a B	3	2.22 ab
49-3	3.28 a A	4	3.51 ab A	4	2.80 a A	4	2.20 a A	6	2.95 a
Avg.[w]	3.12 o[z] M		3.19 n M		2.58 n M		1.30 mn N		2.55 o
	2375								
Control	0.15 c A	1	0.40 b A	1	0.76 b A	1	0.31 a A	1	0.41 c
Butte86	2.30 b A	2	2.83 a A	2	1.76 b A	2	0.71 a A	2 A	1.90 b
81-2	5.29 a A	5	3.49 a AB	4	4.02 a A	6	1.39 a B	5	3.55 a
B45A	4.97 a A	4	4.84 a A	6	3.12 ab AB	4	1.07 a B	4	3.50 a
B63A	3.79 ab A	3	3.31 a A	3	2.91 ab AB	3	0.90 a B	3	2.73 ab
49-3	5.62 a A	6	3.52 a AB	5	3.72 ab AB	5	1.64 a B	6	3.63 a
Avg.	4.39 n M		3.60 n MN		3.11 n N		1.14 n O		3.06 n
	Wheaton								
Control	2.47 e A	1	1.09 c A	1	0.71 d A	1	0.50 b A	1	1.19 d
Butte86	6.69 d A	2	5.23 b AB	2	3.81 c B	3	1.36 ab C	2	4.27 c
81-2	12.19 b A	5	11.13 a A	5	6.17 b B	5	2.47 ab C	5	7.99 b
B45A	12.88 b A	4	11.25 a A	6	6.09 b	4	2.44 ab C	4	8.17 b
B63A	9.50 c A	3	7.18 b B	3	3.01 c C	2	1.59 ab C	3	5.32 c
49-3	17.28 a A	6	10.85 a B	4	9.99 a B	6	3.02 a C	6	10.29 a
Avg.	11.71 m M		9.13 m N		5.81 m O		2.18 m P		7.21 m
Mean[†]	6.41 M[‡]		5.31 N		3.83 O		1.54 P		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 15. 3-Acetyldeoxynivalenol (3-ADON) concentration (μg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to 14 DAI mist-irrigation duration and sampled from 7 to 11 dai in 2007 and 2008.

	3-acetyldeoxynivalenol (3-ADON)							
	2007				2008			
	Sampling							
	7 dai		11 dai		7 dai		11 dai	
	Alsen							
Isolates	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank
Control	0.00 a[x] A[y]	1	0.00 c A	1	0.00 a A	1	0.00 c A	1
Butte86*	0.00 a A	1	0.06 bc A	2	0.00 a A	1	0.09 a-c A	3
81-2	0.00 a A	1	0.09 a-c A	3	0.00 a B	1	0.15 ab A	5
B45A	0.00 a B	1	0.34 ab A	4	0.00 a B	1	0.22 a A	6
B63A	0.00 a B	1	0.36 a A	5	0.00 a B	1	0.13 a-c A	4
49-3	0.00 a B	1	0.37 a A	6	0.00 a A	1	0.07 bc A	2
Avg.[w]	0.00 m[z] N		0.25 n M		0.00 m N		0.13 mn M	
	2375							
Control	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 a A	1
Butte86	0.00 a A	1	0.08 b A	2	0.00 a A	1	0.05 a A	4
81-2	0.00 a B	1	0.23 ab A	4	0.00 a A	1	0.09 a A	5
B45A	0.00 a B	1	0.25 ab A	5	0.00 a A	1	0.05 a A	4
B63A	0.00 a A	1	0.17 ab A	3	0.00 a A	1	0.03 a A	2
49-3	0.00 a B	1	0.46 a A	6	0.00 a A	1	0.04 a A	3
Avg.	0.00 m N		0.24 n M		0.00 m N		0.05 n M	
	Wheaton							
Control	0.00 a A	1	0.00 c A	1	0.00 a A	1	0.00 b A	1
Butte86	0.00 a B	1	0.53 b A	2	0.00 a B	1	0.10 ab A	2
81-2	0.00 a B	1	0.91 a A	4	0.00 a B	1	0.17 a A	3
B45A	0.00 a B	1	0.94 a A	5	0.00 a B	1	0.21 a A	6
B63A	0.00 a B	1	0.72 ab A	3	0.00 a B	1	0.20 a A	5
49-3	0.00 a B	1	0.98 a A	6	0.00 a B	1	0.18 a A	4
Avg.	0.00 m N		0.82 m M		0.00 m N		0.17 m M	
Mean[†]	0.00N[‡]		0.43 M		0.00 N		0.12 M	

[w] Average (sampling day means combined across isolates excluding water treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each sampling day and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate in each year or in each sampling days in each year are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same lowercase letter within column in each sampling day are not significantly different at $P < 0.05$.

[†] Sampling day mean values combined across cultivars and isolates.

[‡] Sampling day means (combined across isolates and cultivars) followed by same uppercase letter within row are not significantly different at $P < 0.05$.

Appendix 16. 3-acetyldeoxynivalenol (3-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (ALsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 14 dai in 2007.

	3-acetyldeoxynivalenol (3-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 b[x] A[y]	1	0.00 a A	1	0.00 a A	1	0.00 c A	1	0.00
Butte86*	0.25 ab A	3	0.10 a A	2	0.12 a A	2	0.31 bc A	2	0.20
81-2	0.16 ab A	2	0.17 a A	4	0.19 a A	3	0.41 bc A	4	0.23
B45A	0.25 ab A	3	0.24 a A	5	0.35 a A	6	0.74 ab A	5	0.40
B63A	0.25 ab A	3	0.16 a A	3	0.27 a A	5	0.35 bc A	3	0.26
49-3	0.57 a AB	4	0.43 a AB	6	0.21 a B	4	0.94 a A	6	0.54
Avg.[w]	0.30 n[z] M		0.22 n M		0.23 n M		0.55 n M		0.32 n
	2375								
Control	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 b A	1	0.00
Butte86	0.15 a A	2	0.19 a A	4	0.11 a A	2	0.46 ab A	5	0.23
81-2	0.15 a A	2	0.23 a A	5	0.11 a A	2	0.32 ab A	2	0.20
B45A	0.26 a A	3	0.14 a A	2	0.23 a A	3	0.38 ab A	3	0.25
B63A	0.15 a A	2	0.17 a A	3	0.34 a A	4	0.43 ab A	4	0.27
49-3	0.42 a AB	4	0.28 a B	6	0.40 a AB	5	0.85 a A	6	0.49
Avg.	0.23 n M		0.20 n M		0.24 n M		0.49 n M		0.29 n
	Wheaton								
Control	0.00 d A	1	0.05 c A	1	0.00 c A	1	0.00 e A	1	0.01
Butte86	0.39 cd C	2	0.67 b BC	4	1.19 ab AB	5	1.33 d A	2	0.90
81-2	0.85 bc B	3	0.56 b B	3	0.70 b B	2	2.80 b A	5	1.23
B45A	1.49 a B	6	0.29 bc C	2	1.45 a B	6	2.64 bc A	4	1.47
B63A	1.23 ab B	5	0.67 b C	4	1.09 ab BC	4	2.25 c A	3	1.31
49-3	1.09 ab C	4	1.67 a B	5	0.81 b C	3	3.79 a A	6	1.84
Avg.	1.01 m N		0.77 m N		1.05 m N		2.56 m M		1.35 m
Mean[†]	0.51 N[‡]		0.40 N		0.50 N		1.20 M		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isoalte Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 16 continued. 3-acetyldeoxynivalenol (3-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 21 dai in 2007.

	3-acetyldeoxynivalenol (3-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 c[x] A[y]	1	0.00 b A	1	0.00 b A	1	0.00 b A	1	0.00
Butte86*	0.63 b A	3	0.40 ab A	3	0.56 ab A	3	0.47 ab A	2	0.52
81-2	0.61 b A	2	0.32 ab A	2	0.47 ab A	2	0.51 ab A	3	0.48
B45A	0.77 b A	4	0.59 ab A	5	0.75 a A	5	0.72 a A	5	0.71
B63A	0.98 ab A	5	0.42 ab A	4	0.65 a A	4	0.53 ab A	4	0.65
49-3	1.39 a A	6	0.83 a A	6	0.93 a A	6	0.88 a A	6	1.01
Avg.[w]	0.88 n[z] M		0.51 n M		0.67 n M		0.62 n M		0.70 n
	2375								
Control	0.00 b A	1	0.00 c A	1	0.00 b A	1	0.00 c A	1	0.00
Butte86	0.47 ab A	3	0.67 ab A	4	0.73 a A	3	0.45 bc A	2	0.58
81-2	0.44 ab A	2	0.52 bc A	2	0.61 a A	2	0.58 a-c A	4	0.54
B45A	0.75 a A	5	0.71 ab A	5	0.99 a A	4	0.60 ab A	5	0.76
B63A	0.61 a A	4	0.61 ab A	3	0.73 a A	3	0.57 a-c A	3	0.63
49-3	0.99 a A	6	1.14 a A	6	0.87 a A	5	1.10 a A	6	1.03
Avg.	0.65 n M		0.73 n M		0.79 n M		0.66 n M		0.71 n
	Wheaton								
Control	0.21 e A	1	0.00 d A	1	0.00 e A	1	0.00 d A	1	0.05
Butte86	2.56 d A	3	1.79 c B	2	2.42 cd AB	3	1.94 c AB	2	2.18
81-2	2.28d AB	2	1.87 c B	3	1.90 d B	2	2.57 b A	4	2.16
B45A	3.86 b A	5	2.46 b BC	5	3.10 b B	5	2.32 bc C	3	2.94
B63A	3.21 c A	4	2.02 bc C	4	2.99 bc AB	4	2.57 b BC	4	2.70
49-3	5.53 a A	6	4.18 a B	6	4.66 a B	6	4.58 a B	5	4.74
Avg.	3.49 m M		2.46 m O		3.01 m N		2.80 m NO		2.94 m
Mean[†]	1.67 M[‡]		1.24 N		1.50 MN		1.36 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 16 continued. 3-acetyldeoxynivalenol (3-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four DAI mist-irrigation duration treatments and sampled 28 dai in 2007.

	3-acetyldeoxynivalenol (3-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 c[x] A[y]	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00
Butte86*	0.65 b A	3	0.18 b A	2	0.16 a A	3	0.15 a A	3	0.29
81-2	0.63 b A	2	0.00 b A	1	0.15 a A	2	0.11 a A	2	0.22
B45A	0.71 b A	4	0.20 b A	3	0.31 a A	5	0.17 a A	4	0.35
B63A	0.79 ab A	5	0.47 b A	4	0.19 a A	4	0.21 a A	5	0.42
49-3	1.31 a A	6	1.04 a AB	5	0.49 a B	6	0.29 a B	6	0.78
Avg.[w]	0.82 n[z] M		0.38 n M		0.26 n M		0.19 n M		0.41 n
	2375								
Control	0.00 c A	1	0.00 b A	1	0.00 b A	1	0.00 a A	1	0.00
Butte86	0.44 bc A	2	0.24 b A	4	0.31 ab A	4	0.16 a A	2	0.29
81-2	0.58 ab A	3	0.20 b A	3	0.28 ab A	3	0.23 a A	4	0.32
B45A	0.92 ab A	5	0.16 b B	2	0.17 ab AB	2	0.32 a AB	5	0.39
B63A	0.83 ab A	4	0.39 b A	5	0.31 ab A	4	0.18 a A	3	0.43
49-3	0.99 a A	6	1.04 a A	6	0.56 a A	5	0.50 a A	6	0.77
Avg.	0.75 n M		0.41 n M		0.33 n M		0.28 n M		0.44 n
	Wheaton								
Control	0.07 d A	1	0.00 d A	1	0.00 c A	1	0.00 c A	1	0.02
Butte86	2.57 c A	3	1.40 c B	2	0.93 b B	3	0.64 b B	2	1.39
81-2	2.36 c A	2	1.99 bc A	4	0.92 b B	2	0.96 b B	4	1.56
B45A	3.70 b A	4	2.19 b B	5	0.92 b C	2	0.68 b C	3	1.87
B63A	4.22 b A	5	1.58 c B	3	1.08 ab B	4	1.08 b B	5	1.99
49-3	5.06 a A	6	3.43 a B	6	1.59 a C	5	1.72 a C	6	2.95
Avg.	3.58 m M		2.12 m N		1.09 m O		1.02 m O		1.95 m
Mean[†]	1.72 M[‡]		0.97 N		0.56 N		0.49 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 16 continued. 3-acetyldeoxynivalenol (3-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 41 dai in 2007.

	3-acetyldeoxynivalenol (3-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 d[x] A[y]	1	0.00 c A	1	0.00 b A	1	0.00 a A	1	0.00
Butte86*	0.38 cd A	2	0.23 c A	2	0.34 ab A	2	0.00 a A	1	0.24
81-2	0.49 bc A	3	0.71 b A	4	0.47 a AB	3	0.00 a B	1	0.42
B45A	0.81 ab A	5	0.70 b A	3	0.47 a AB	3	0.00 a B	1	0.50
B63A	0.79 a-c A	4	0.73 b A	5	0.60 a A	4	0.00 a B	1	0.53
49-3	1.07 a AB	6	1.40 a A	6	0.73 a B	5	0.15 a C	2	0.84
Avg.[w]	0.71 n[z] M		0.75 n M		0.52 n M		0.03 n N		0.50 n
	2375								
Control	0.00 b A	2	0.00 c A	1	0.00 b A	1	0.00 a A	1	0.00
Butte86	0.58 a A	3	0.65 b A	2	0.36 b AB	2	0.00 a B	1	0.40
81-2	0.46 a AB	2	0.72 b A	3	0.44 a AB	3	0.00 a B	1	0.41
B45A	0.74 a A	4	0.74 b A	4	0.48 a AB	4	0.00 a B	1	0.49
B63A	0.78 a A	5	0.80 b A	5	0.73 a A	5	0.00 a B	1	0.58
49-3	0.84 a B	6	1.49 a A	6	0.81 a B	6	0.18 a C	2	0.83
Avg.	0.68 n M		0.88 n M		0.56 n M		0.04 n N		0.54 n
	Wheaton								
Control	0.05 d A	1	0.06 c A	1	0.20 c A	1	0.00 c A	1	0.08
Butte86	1.91 c A	2	2.17 b A	2	1.00 b B	2	0.08 c C	2	1.29
81-2	2.40 b A	3	2.25 b A	4	1.18 b B	3	0.36 bc C	4	1.55
B45A	2.58 b A	4	2.19 b A	3	1.00 b B	2	0.17 c C	3	1.49
B63A	3.20 a A	5	2.53 ab B	5	1.23 ab C	4	0.62 ab D	5	1.90
49-3	3.42 a A	6	2.75 a B	6	1.65 a C	5	0.89 a D	6	2.18
Avg.	2.70 m M		2.38 m M		1.21 m N		0.42 m O		1.68 m
Mean[†]	1.36 M[‡]		1.34 M		0.77 N		0.16 O		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 16 continued. 3-acetyldeoxynivalenol (3-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 14 dai in 2008.

	3-acetyldeoxynivalenol (3-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 c[x] A[y]	1	0.01 b A	1	0.00 b A	1	0.00 b A	1	0.01 c
Butte86*	0.26 ab A	4	0.22 a A	3	0.18 ab A	2	0.33 a A	6	0.25 ab
81-2	0.26 ab A	4	0.31 a A	4	0.22 ab A	4	0.30 a A	4	0.27 ab
B45A	0.19 bc A	2	0.36 a A	6	0.27 a A	5	0.31 a A	5	0.28 ab
B63A	0.49 a A	5	0.32 a AB	5	0.19 ab B	3	0.29 a AB	3	0.32 a
49-3	0.24 b A	3	0.21 a A	2	0.22 ab A	4	0.13 a A	2	0.20 b
Avg.[w]	0.29 n[z] M		0.28 n M		0.22 n M		0.27 n M		0.27 n
	2375								
Control	0.00 a A	1	0.01 b A	1	0.00 a A	1	0.00 a A	1	0.01 b
Butte86	0.15 a A	3	0.20 ab A	4	0.11 a A	4	0.20 a A	6	0.17 a
81-2	0.11 a A	2	0.12 ab A	2	0.12 a A	5	0.18 a A	4	0.13 a
B45A	0.19 a A	6	0.20 ab A	4	0.08 a A	2	0.14 a A	3	0.15 a
B63A	0.17 a A	4	0.30 a A	5	0.10 a A	3	0.11 a A	2	0.17 a
49-3	0.18 a A	5	0.14 ab A	3	0.18 a A	6	0.19 a A	5	0.17 a
Avg.	0.16 o M		0.19 n M		0.12 n M		0.16 n M		0.16 o
	Wheaton								
Control	0.00 c A	1	0.00 d A	1	0.00 c A	1	0.00 d A	1	0.00 e
Butte86	0.40 b A	2	0.38 c A	2	0.34 b A	2	0.41 c A	3	0.38 d
81-2	0.57 ab AB	4	0.64 b A	3	0.53 ab AB	4	0.35 c B	2	0.52 c
B45A	0.59 ab AB	5	0.65 b A	4	0.36 b B	3	0.70 b A	5	0.58 bc
B63A	0.46 b B	3	0.88 a A	5	0.68 a AB	5	0.69 b AB	4	0.68 b
49-3	0.79 a A	6	0.90 a A	6	0.72 a A	6	0.96 a A	6	0.84 a
Avg.	0.56 m MN		0.69 m M		0.53 m N		0.62 m MN		0.60 m
Mean[†]	0.34 M[‡]		0.39 M		0.29 M		0.35 M		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 16 continued. 3-acetyldeoxynivalenol (3-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 21 dai in 2008.

	3-acetyldeoxynivalenol (3-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 a[x] A[y]	1	0.00 c A	1	0.00 c A	1	0.02 b A	1	0.01 b
Butte86*	0.13 a A	2	0.28 b A	2	0.23 bc A	2	0.45 a A	6	0.27 a
81-2	0.26 a B	6	0.45 ab B	3	0.73 a A	6	0.39 ab B	4	0.46 a
B45A	0.21 a B	4	0.75 a A	5	0.43 ab B	5	0.48 a B	6	0.47 a
B63A	0.16 a A	3	0.54 ab A	4	0.26 bc A	3	0.38 ab A	3	0.34 a
49-3	0.23 a A	5	0.54 ab A	4	0.36 a-c A	4	0.37 ab A	2	0.38 a
Avg.[w]	0.20 n[z] N		0.51 n M		0.40 n MN		0.41 n MN		0.38 n
	2375								
Control	0.00 a A	1	0.00 b A	1	0.00 b A	1	0.00 a A	1	0.00 b
Butte86	0.05 a A	2	0.25 ab A	2	0.30 ab A	3	0.40 a A	6	0.25 a
81-2	0.15 a A	5	0.32 ab A	3	0.47 a A	6	0.33 a A	5	0.32 a
B45A	0.17 a A	6	0.48 a A	5	0.25 ab A	2	0.16 a A	2	0.27 a
B63A	0.13 a A	4	0.39 ab A	4	0.40 ab A	4	0.32 a A	4	0.31 a
49-3	0.08 a B	3	0.51 a A	6	0.43 ab AB	5	0.30 a AB	3	0.33 a
Avg.	0.12 n N		0.39 n M		0.37 n MN		0.30 n MN		0.29 n
	Wheaton								
Control	0.02 c A	1	0.00 d A	1	0.00 d A	1	0.00 d A	1	0.01 c
Butte86	0.59 b B	2	1.16 c A	2	0.52 c B	2	1.10 b A	4	0.84 b
81-2	0.93 ab B	5	1.49 ab A	5	0.73 a-c B	4	0.58 c B	2	0.93 b
B45A	0.79 b B	4	1.67 a A	6	1.11 a B	6	1.49 ab AB	5	1.27 a
B63A	0.72 b B	3	1.19 c A	3	0.59 bc B	3	0.67 c B	3	0.79 b
49-3	1.29 a AB	6	1.22 a AB	4	0.99 ab B	5	1.63 a A	6	1.28 a
Avg.	0.86 m NO		1.35 m M		0.79 m O		1.09 m N		1.02 m
Mean[†]	0.39 O[‡]		0.75 M		0.52 NO		0.60 MN		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 16 continued. 3-acetyldeoxynivalenol (3-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments sampled 28 dai in 2008.

	3-acetyldeoxynivalenol (3-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.07 a[x] A[y]	1	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.02 b
Butte86*	0.23 a A	5	0.07 a A	2	0.26 ab A	2	0.17 a A	2	0.18 ab
81-2	0.18 a A	4	0.26 a A	4	0.32 ab A	4	0.31 a A	4	0.27 a
B45A	0.16 a A	3	0.27 a A	5	0.37 ab A	5	0.36 a A	6	0.29 a
B63A	0.14 a A	2	0.22 a A	3	0.29 ab A	3	0.23 a A	3	0.22 a
49-3	0.29 a A	6	0.30 a A	6	0.41 a A	6	0.34 a A	5	0.33 a
Avg.[w]	0.20 n[z] M		0.22 n M		0.33 n M		0.28 n M		0.26 n
	2375								
Control	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 b A	1	0.00 b
Butte86	0.02 a A	2	0.11 a A	2	0.16 a A	2	0.20 ab A	2	0.12 ab
81-2	0.04 a A	3	0.28 a A	5	0.28 a A	4	0.20 ab A	2	0.20 a
B45A	0.10 a A	4	0.29 a A	6	0.28 a A	4	0.30 ab A	3	0.24 a
B63A	0.16 a A	5	0.21 a A	4	0.23 a A	3	0.17 ab A	5	0.19 a
49-3	0.21 a A	6	0.13 a A	3	0.30 a A	5	0.43 a A	4	0.27 a
Avg.	0.11 n M		0.20 n M		0.25 n M		0.26 n M		0.21 n
	Wheaton								
Control	0.00 c A	1	0.09 c A	1	0.02 c A	1	0.04 b A	1	0.04 c
Butte86	0.44 b A	2	0.62 b A	2	0.63 ab A	5	0.34 ab A	2	0.51 b
81-2	0.63 bAB	4	1.02 ab A	4	0.59 ab B	4	0.62 a AB	4	0.72 a
B45A	1.05 a A	6	1.17 a A	5	0.48 ab B	3	0.55 a B	3	0.82 a
B63A	0.62 b B	3	1.22 a A	6	0.45 b B	2	0.69 a B	6	0.75 a
49-3	0.73 ab A	5	0.65 b A	3	0.88 a A	6	0.67 a A	5	0.73 a
Avg.	0.69 m N		0.94 m M		0.61 m N		0.57 m N		0.70 m
Mean[†]	0.33 M[‡]		0.45 M		0.40 M		0.37 M		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

***** Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 16 continued. 3-acetyldeoxynivalenol (3-ADON) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 41 (39) dai in 2008.

	3-acetyldeoxynivalenol (3-ADON)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 c[x] A[y]	1	0.00 b A	1	0.00 b A	1	0.03 b A	1	0.01 b
Butte86*	1.00 ab A	4	0.70 a AB	2	0.89 a AB	6	0.22 ab B	3	0.70 a
81-2	1.46 a A	6	0.98 a AB	6	0.81 a AB	3	0.30 ab B	4	0.89 a
B45A	0.59 b A	2	0.88 a A	5	0.82 a A	4	0.53 ab A	5	0.71 a
B63A	1.23 ab A	5	0.82 a AB	4	0.80 a AB	2	0.19 ab B	2	0.76 a
49-3	0.84 ab A	3	0.78 a A	3	0.85 a A	5	0.79 a A	6	0.82 a
Avg.[w]	1.02 n[y] M		0.83 n MN		0.83 n MN		0.41 m N		0.77 n
	2375								
Control	0.00 b A	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 b
Butte86	0.64 ab A	2	0.78 a A	5	0.67 a A	5	0.18 a A	4	0.57 a
81-2	1.07 a A	6	0.58 ab AB	2	0.64 a AB	4	0.17 a B	3	0.62 a
B45A	0.99 a A	4	0.72 a AB	4	0.38 a AB	2	0.05 a B	2	0.54 a
B63A	0.70 ab A	3	0.80 a A	6	0.55 a A	3	0.24 a A	5	0.57 a
49-3	1.06 a A	5	0.71 a A	3	0.55 a A	3	0.36 a A	6	0.67 a
Avg.	0.89 n M		0.72 n MN		0.56 n MN		0.20 m N		0.59 n
	Wheaton								
Control	0.73 d A	1	0.12 b A	1	0.00 c A	1	0.00 b A	1	0.21 c
Butte86	2.14 c A	2	2.43 a A	2	1.69 b AB	5	1.03 a B	5	1.82 b
81-2	3.00 b A	4	2.71 a A	4	1.21 b B	3	0.58 ab B	3	1.88 b
B45A	3.01 b A	5	2.51 a A	3	1.12 b B	2	0.55 ab B	2	1.80 b
B63A	2.60 bc A	3	2.77 a A	5	1.30 b B	4	0.77 a B	4	1.86 b
49-3	4.16 a A	6	3.08 a B	6	2.60 a B	6	1.03 a C	5	2.72 a
Avg.	2.98 m M		2.70 m M		1.58 m N		0.79 m O		2.01 m
Mean[†]	1.63 M[‡]		1.42 MN		0.99 N		0.47 O		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 17. Nivalenol (NIV) concentration (μg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to 14 DAI mist-irrigation duration and sampled from 7 to 11 dai in 2007 and 2008.

	Nivalenol (NIV)							
	2007				2008			
	Sampling							
	7 dai		11 dai		7 dai		11 dai	
	Alsen							
Isolates	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank	μg g^{-1}	Rank
Control	0.00 a[x] A[y]	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
Butte86*	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
81-2	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
B45A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
B63A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
49-3	0.00 a A	1	0.06 a A	2	0.00 a A	1	0.00 a A	1
Avg.[w]	0.00 m[z] M		0.01 n M		0.00 m M		0.00 m M	
	2375							
Control	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
Butte86	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
81-2	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
B45A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
B63A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
49-3	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
Avg.	0.00 m M		0.00 n M		0.00 m M		0.00 m M	
	Wheaton							
Control	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 b A	1
Butte86	0.00 a A	1	0.06 ab A	2	0.00 a A	1	0.02 b A	2
81-2	0.00 a B	1	0.12 a A	3	0.00 a A	1	0.00 b A	1
B45A	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 b A	1
B63A	0.00 a A	1	0.00 b A	1	0.00 a B	1	0.07 a A	3
49-3	0.00 a B	1	0.13 a A	4	0.00 a A	1	0.00 b A	1
Avg.	0.00 m N		0.06 m M		0.00 m N		0.02 m M	
Mean[†]	0.00 N[‡]		0.02 M		0.00 M		0.00 M	

[w] Average (sampling day means combined across isolates excluding water treatment).

***** isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each sampling day and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate in each year or in each sampling days in each year are not significantly different at $P < 0.05$.

[z] Means (combined across isolates) followed by the same lowercase letter within column in each sampling day are not significantly different at $P < 0.05$.

[†] Sampling day mean values combined across cultivars and isolates.

[‡] Sampling day means (combined across isolates and cultivars) followed by same uppercase letter within row are not significantly different at $P < 0.05$.

Appendix 18. Nivalenol (NIV) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 14 dai in 2007.

	Nivalenol (NIV)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 a[x] A[y]	1	0.00 b A	1	0.00 a A	1	0.00 b A	1	0.00
Butte86*	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 b A	1	0.00
81-2	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 b A	1	0.00
B45A	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.07 b A	3	0.02
B63A	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.04 b A	2	0.01
49-3	0.05 a B	2	0.14 a A	2	0.00 a B	1	0.21 a A	4	0.10
Avg.[w]	0.01 n[z] N		0.03 n MN		0.00 n N		0.06 n M		0.03 n
	2375								
Control	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00
Butte86	0.02 a A	2	0.00 a A	1	0.00 a A	1	0.03 a A	4	0.01
81-2	0.00 a A	1	0.02 a A	2	0.00 a A	1	0.01 a A	2	0.01
B45A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.02 a A	3	0.01
B63A	0.00 a A	1	0.02 a A	2	0.00 a A	1	0.00 a A	1	0.01
49-3	0.05 a A	3	0.06 a A	3	0.00 a A	1	0.08 a A	5	0.05
Avg.	0.01 n M		0.02 n M		0.00 n M		0.03 n M		0.02 n
	Wheaton								
Control	0.00 c A	1	0.02 c A	1	0.00 c A	1	0.00 d A	1	0.01
Butte86	0.17 ab A	3	0.16 b A	4	0.19 ab A	5	0.15 c A	2	0.17
81-2	0.16 b B	2	0.12 b B	4	0.11 b B	2	0.33 b A	4	0.18
B45A	0.23 ab A	5	0.10 bc C	2	0.13 b BC	3	0.19 c AB	3	0.16
B63A	0.22 ab A	4	0.12 b B	3	0.16 b AB	4	0.19 c AB	4	0.17
49-3	0.25 a B	6	0.40 a B	5	0.25 a B	6	0.56 a A	5	0.37
Avg.	0.21 m N		0.18 m N		0.17 m N		0.28 m M		0.21 m
Mean[†]	0.08 N[‡]		0.08 N		0.06 N		0.13 M		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 18 continued. Nivalenol (NIV) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 21 dai in 2007.

	Nivalenol (NIV)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 b[x] A[y]	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00
Butte86*	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00
81-2	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00
B45A	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00
B63A	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00
49-3	0.16 a A	2	0.06 a AB	2	0.00 a B	1	0.10 a AB	2	0.08
Avg.[w]	0.03 n[z] M		0.01 n M		0.00 m M		0.02 n M		0.02 n
	2375								
Control	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 b A	1	0.00
Butte86	0.05 a A	2	0.07 b A	3	0.00 a A	1	0.00 b A	1	0.03
81-2	0.00 a A	1	0.05 ab A	2	0.00 a A	1	0.05 b A	3	0.03
B45A	0.00 a A	1	0.00 ab A	1	0.00 a A	1	0.00 b A	1	0.00
B63A	0.00 a A	1	0.05 ab A	2	0.00 a A	1	0.04 b A	2	0.02
49-3	0.06 a AB	3	0.12 a A	4	0.00 a B	1	0.16 a A	4	0.09
Avg.	0.02 n M		0.06 n M		0.00 m M		0.05 n M		0.03 n
	Wheaton								
Control	0.00 d A	1	0.00 d A	1	0.00 a A	1	0.00 d A	1	0.00
Butte86	0.19 c A	2	0.19 c A	2	0.00 a B	1	0.14 bc A	3	0.13
81-2	0.23 c A	3	0.21 c A	3	0.00 a C	1	0.06 c B	2	0.13
B45A	0.36 ab A	5	0.37 b A	5	0.00 a B	1	0.00 d B	1	0.18
B63A	0.26 bc A	4	0.27 bc A	4	0.00 a B	1	0.24 b A	4	0.19
49-3	0.46 a A	6	0.50 a A	6	0.00 a B	1	0.41 a A	5	0.34
Avg.	0.30 m M		0.31 m M		0.00 m O		0.17 m N		0.19 m
Mean[†]	0.12 MN[‡]		0.13 M		0.00 O		0.08 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 18 continued. Nivalenol (NIV) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 28 dai in 2007.

	Nivalenol (NIV)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 b[x] A[y]	1	0.00 b A	1	0.02 a A	2	0.00 a A	1	0.01
Butte86*	0.00 b A	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00
81-2	0.00 b A	1	0.00 b A	1	0.03 a A	3	0.00 a A	1	0.01
B45A	0.00 b A	1	0.00 b A	1	0.03 a A	3	0.00 a A	1	0.01
B63A	0.00 b A	1	0.16 a A	2	0.10 a A	4	0.00 a A	1	0.07
49-3	0.17 a AB	2	0.29 a A	3	0.03 a B	4	0.00 a B	1	0.12
Avg.[w]	0.03 n[z] M		0.09 n M		0.04 m M		0.00 n M		0.04 n
	2375								
Control	0.00 b A	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00
Butte86	0.00 b A	1	0.00 b A	1	0.03 a A	2	0.00 a A	1	0.01
81-2	0.00 b A	1	0.10 b A	3	0.00 a A	1	0.00 a A	1	0.03
B45A	0.16 a A	2	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.04
B63A	0.00 b A	1	0.06 b A	2	0.00 a A	1	0.00 a A	1	0.02
49-3	0.16 a B	2	0.40 a A	4	0.07 a B	3	0.00 a B	1	0.16
Avg.	0.06 n M		0.11 n M		0.02 m M		0.00 n M		0.05 n
	Wheaton								
Control	0.00 d A	1	0.00 d A	1	0.02 a A	2	0.00 b A	1	0.01
Butte86	0.42 bc B	3	0.62 b A	5	0.04 a C	3	0.00 b C	1	0.27
81-2	0.28 c A	2	0.41 c A	4	0.00 a B	1	0.06 b B	2	0.19
B45A	0.54 b A	5	0.27 c B	2	0.00 a C	1	0.06 b C	2	0.23
B63A	0.51 b A	4	0.36 c A	3	0.04 a B	3	0.13 b B	3	0.26
49-3	0.77 a B	6	1.16 a A	6	0.04 a D	3	0.45 a C	4	0.61
Avg.	0.50 m M		0.56 m M		0.02 m N		0.14 m N		0.31 m
Mean[†]	0.20 M[‡]		0.26 M		0.03 N		0.05 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 18 continued. Nivalenol (NIV) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 41 dai in 2007.

	Nivalenol (NIV)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 b[x] A[y]	1	0.00 a A	1	0.03 ab A	2	0.00 a A	1	0.01
Butte86*	0.00 b A	1	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00
81-2	0.00 b A	1	0.00 a A	1	0.04 ab A	3	0.00 a A	1	0.01
B45A	0.06 b A	2	0.00 a A	1	0.03 ab A	2	0.00 a A	1	0.02
B63A	0.00 b A	1	0.00 a A	1	0.13 a A	4	0.00 a A	1	0.03
49-3	0.22 a A	3	0.11 a AB	2	0.04 ab B	3	0.00 a B	1	0.09
Avg.[w]	0.06 n[z] M		0.02 n M		0.05 m M		0.00 m M		0.03 n
	2375								
Control	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00
Butte86	0.00 b A	1	0.00 a A	1	0.03 a A	2	0.00 a A	1	0.01
81-2	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00
B45A	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00
B63A	0.06 b A	2	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.02
49-3	0.20 a A	3	0.10 a AB	2	0.08 a AB	3	0.00 a B	1	0.10
Avg.	0.05 n M		0.02 n M		0.02 m M		0.00 m M		0.02 n
	Wheaton								
Control	0.06 c A	1	0.00 d A	1	0.03 a A	2	0.00 a A	1	0.02
Butte86	0.38 b A	2	0.14 c B	2	0.05 a B	3	0.00 a B	1	0.14
81-2	0.57 a A	3	0.42 b B	4	0.00 a C	1	0.00 a C	1	0.25
B45A	0.62 a A	5	0.23 c B	3	0.00 a C	1	0.00 a C	1	0.21
B63A	0.60 a A	4	0.49 b A	5	0.05 a B	3	0.00 a B	1	0.29
49-3	0.69 a B	6	0.97 a A	6	0.05 a C	3	0.05 a C	2	0.44
Avg.	0.57 m M		0.45 m N		0.03 m O		0.01 m O		0.30 m
Mean[†]	0.23 M[‡]		0.16 M		0.03 N		0.00 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 18 continued. Nivalenol (NIV) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 14 dai in 2008.

	Nivalenol (NIV)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 a[x] A[y]	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
Butte86*	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
81-2	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.02 a A	3	0.01 a
B45A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
B63A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.03 a A	3	0.01 a
49-3	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.01a A	2	0.00 a
Avg.[w]	0.00 m[n] N		0.00 m N		0.00 m N		0.01 m M		0.00 m
	2375								
Control	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
Butte86	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 A	1	0.00 a
81-2	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.02 a A	2	0.00 a
B45A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
B63A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
49-3	0.00 a A	1	0.00 a A	1	0.01 a A	2	0.00 a A	1	0.00 a
Avg.	0.00 m M		0.00 m M		0.00 m M		0.00 n M		0.00 m
	Wheaton								
Control	0.06 a A	2	0.00 a B	1	0.07 a A	2	0.00 a B	1	0.03 a
Butte86	0.00 b A	1	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 a
81-2	0.00 b A	1	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 a
B45A	0.00 b A	1	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 a
B63A	0.00 b A	1	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 a
49-3	0.00 b A	1	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 a
Avg.	0.00 m M		0.00 m M		0.00 m M		0.00 n M		0.00 m
Mean[†]	0.00 M[‡]		0.00 M		0.00 M		0.01 M		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 18 continued. Nivalenol (NIV) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 21 dai in 2008.

	Nivalenol (NIV)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 a[x] A[y]	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a
Butte86*	0.02 a A	2	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.01 a
81-2	0.06 a A	4	0.02 b A	2	0.06 a A	3	0.02 a A	2	0.04 a
B45A	0.05 a A	3	0.09 ab A	3	0.00 a A	1	0.00 a A	1	0.04 a
B63A	0.00 a A	1	0.02 b A	2	0.00 a A	1	0.05 a A	3	0.02 a
49-3	0.00 a B	1	0.16 a A	4	0.03 a B	2	0.00 a B	1	0.05 a
Avg.[w]	0.03 n[z] M		0.06 n M		0.02 n M		0.01 n M		0.03 n
	2375								
Control	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
Butte86	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
81-2	0.00 a A	1	0.03 a A	2	0.00 a A	1	0.00 a A	1	0.01 a
B45A	0.00 a A	1	0.04 a A	3	0.00 a A	1	0.00 a A	1	0.01 a
B63A	0.00 a A	1	0.00 a A	1	0.02 a A	2	0.00 a A	1	0.01 a
49-3	0.00 a A	1	0.05 a A	4	0.00 a A	1	0.03 a A	2	0.02 a
Avg.	0.00 n M		0.02 n M		0.00 n M		0.01 n M		0.01 n
	Wheaton								
Control	0.03 b B	1	0.10 bc AB	2	0.05 ab AB	2	0.15 ab A	5	0.08 bc
Butte86	0.10 ab AB	2	0.11 a-c A	3	0.00 b B	1	0.05 bc AB	2	0.07 c
81-2	0.18 a A	5	0.18 ab A	4	0.09 ab AB	4	0.07 bc B	3	0.13 ab
B45A	0.15 a A	4	0.18 ab A	4	0.11 a A	5	0.10 a-c A	4	0.14 a
B63A	0.03 b A	1	0.06 c A	1	0.07 ab A	3	0.02 c A	1	0.05 c
49-3	0.11 a A	3	0.20 a A	5	0.12 a A	6	0.20 a A	6	0.16 a
Avg.	0.11 m MN		0.15 m M		0.08 m N		0.09 n N		0.11 m
Mean[†]	0.05 N[‡]		0.08 M		0.03 N		0.04 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 18 continued. Nivalenol (NIV) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments and sampled 28 dai in 2008.

	Nivalenol (NIV)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.02 a[x] A[y]	2	0.03 a A	1	0.00 a A	1	0.00 a A	1	0.01 b
Butte86*	0.04 a AB	4	0.09 a A	5	0.01 a AB	2	0.00 a B	1	0.04 ab
81-2	0.05 a A	5	0.06 a A	2	0.01 a A	2	0.01 a A	2	0.03 ab
B45A	0.03 a A	3	0.07 a A	3	0.03 a A	3	0.01 a A	2	0.04 ab
B63A	0.01 a A	1	0.08 a A	4	0.01 a A	2	0.00 a A	1	0.03 ab
49-3	0.06 a A	6	0.10 a A	6	0.05 a A	4	0.04 a A	3	0.06 a
Avg.[w]	0.04 n[z] N		0.08 n M		0.02 n N		0.01 n N		0.04 n
	2375								
Control	0.00 a A	1	0.00 b A	1	0.03 a A	3	0.00 a A	1	0.01 b
Butte86	0.00 a A	1	0.04 ab A	3	0.00 a A	1	0.01 a A	2	0.01 b
81-2	0.01 a B	2	0.11 a A	5	0.00 a B	1	0.01 a B	2	0.03 ab
B45A	0.03 a A	3	0.07 ab A	4	0.00 a A	1	0.01 a A	2	0.03 ab
B63A	0.03 a A	3	0.01 b A	2	0.00 a A	1	0.01 a A	2	0.01 b
49-3	0.05 a A	4	0.07 ab A	4	0.02 a A	2	0.05 a A	3	0.05 a
Avg.	0.02 n N		0.06 n M		0.00 n N		0.02 n N		0.03 n
	Wheaton								
Control	0.00 d B	1	0.11 b B	1	0.02 b A	1	0.00 c B	1	0.03 c
Butte86	0.04 cd B	2	0.18 ab A	3	0.04 ab B	3	0.03 bc B	2	0.07 b
81-2	0.14 ab A	4	0.15 ab A	2	0.09 ab A	5	0.09 ab A	4	0.12 a
B45A	0.18 a A	5	0.19 ab A	4	0.05 ab B	4	0.09 ab B	4	0.13 a
B63A	0.09 bc B	3	0.23 a A	5	0.03 ab B	2	0.08 a-c B	3	0.11 ab
49-3	0.14 ab A	4	0.18 ab A	3	0.11 a A	6	0.14 a A	5	0.14 a
Avg.	0.12 m N		0.19 m M		0.06 m O		0.09 m NO		0.11 m
Mean[†]	0.06 N[‡]		0.11 M		0.03 O		0.04 NO		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 18 continued. Nivalenol (NIV) concentration (µg g^{-1}) in whole head samples of three wheat cultivars (Alsen, 2375, Wheaton) inoculated with five *F. graminearum* isolates and a water control subjected to four mist-irrigation duration treatments sampled 41 (39) dai in 2008.

	Nivalenol (NIV)								
	Mist-irrigation duration treatments								
	14 DAI		21 DAI		28 DAI		35 DAI		
	Alsen								
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	Avg.[w]
Control	0.00 a[x] A[y]	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 b
Butte86*	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 b
81-2	0.10 a AB	2	0.13 a A	4	0.06 a AB	2	0.00 a B	1	0.07 a
B45A	0.00 a A	1	0.04 ab A	2	0.00 a A	1	0.00 a A	1	0.01 ab
B63A	0.00 a A	1	0.04 ab A	2	0.00 a A	1	0.00 a A	1	0.01 ab
49-3	0.00 a A	1	0.05 ab A	3	0.00 a A	1	0.00 a A	1	0.01 ab
Avg.[w]	0.02 n[z] M		0.05 n M		0.01 n M		0.00 m M		0.02 n
	2375								
Control	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
Butte86	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
81-2	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
B45A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
B63A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a
49-3	0.06 a A	2	0.05 a A	2	0.00 a A	1	0.03 a A	2	0.04 a
Avg.	0.01 n M		0.01 n M		0.00 n M		0.01 m M		0.01 n
	Wheaton								
Control	0.05 d A	1	0.08 c A	1	0.00 d A	1	0.00 b A	1	0.03 d
Butte86	0.06 d A	2	0.10 c A	2	0.06 cd A	2	0.00 b A	1	0.06 d
81-2	0.19 bc C	4	0.56 a A	6	0.32 b B	5	0.00 b D	1	0.27 b
B45A	0.29 ab A	5	0.30 b A	4	0.12 cd B	3	0.00 b B	1	0.18 c
B63A	0.13 cd A	3	0.24 b A	3	0.15 c A	4	0.00 b B	1	0.13 c
49-3	0.32 a BC	6	0.33 b B	5	0.58 a A	6	0.19 a C	2	0.36 a
Avg.	0.20 m N		0.31 m M		0.25 m MN		0.04 m O		0.20 m
Mean[†]	0.08 M[‡]		0.12 M		0.09 M		0.01 N		

[w] Average (Means across isolates in each cultivar and mist-irrigation duration treatments do not include water control treatment).

* Isolate Butte86Ada-11.

[x] Means followed by the same lowercase letter within column in each mist-irrigation duration and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within row in each isolate or cultivar are not significantly different at $P < 0.05$.

[z] Means (combined across isolates excluding water control) followed by the same lower letter in each mist-irrigation duration are not significantly different at $P < 0.05$.

[†]Mist -irrigation duration mean values combined across cultivars and isolates excluding water control.

[‡] Mist-irrigation duration treatment means (combined across isolates and cultivar treatmetns) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 19. Fusarium head blight (FHB) severity (%) observed in three wheat cultivars (Alsen, 2375, Wheaton) point inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 1.

	Fusarium head blight (FHB) severity									
	Sampling days after inoculation (dai)									
	3 dai		7 dai		11 dai		14 dai		21 dai	
	Alsen									
Isolates	%	Rank	%	Rank	%	Rank	%	Rank	%	Rank
Control	0.00 d[x] A[y]	1	0.00 b A	1	0.00 b A	1	0.00 b A	1	0.00 c A	1
Butte86*	6.91 a B	6	10.20 a B	6	6.81 a B	4	12.29 a AB	6	24.36 a A	6
81-2	5.19 ab A	5	6.80 a A	4	6.71 a A	2	6.68 a A	3	6.90 b A	3
B45A	2.68 bc B	3	6.43 a A	2	9.21 a A	6	9.47 a A	5	16.77 ab A	5
B63A	1.25 cd B	2	7.95 a A	5	8.94 a A	5	8.24 a A	4	8.76 b A	4
49-3	4.10 b B	4	6.50 a A	3	6.80 a A	3	6.26 a A	2	6.43 b A	2
Avg.[w]	4.02 n[z] N		7.58 m M		7.69 n M		8.59 o M		12.64 n M	
	2375									
Control	0.00 c A	1	0.00 b A	1	0.00 c A	1	0.00 c A	1	0.00 c A	1
Butte86	1.33 bc D	2	8.01 a C	5	33.92 a B	5	56.10 a AB	6	74.09 a A	6
81-2	1.67 bc D	3	6.76 a C	3	26.00 ab B	4	51.52 a A	5	46.10 b A	2
B45A	5.62 a E	5	6.95 a D	4	17.05 b C	3	27.23 a B	3	72.65 a A	5
B63A	2.86 ab D	4	6.60 a C	2	35.90 a B	6	49.14 a AB	4	58.04 ab A	3
49-3	1.33 bc C	2	6.95 a B	4	16.49 b B	2	12.20 b B	2	65.23 ab A	4
Avg.	2.56 o Q		7.05 m P		25.87 m O		39.24 n N		63.22 m M	
	Wheaton									
Control	0.00 b A	1	0.00 b A	1	0.00 c A	1	0.00 d A	1	0.00 c A	1
Butte86	6.03 a B	2	6.52 a B	5	39.16 a A	6	64.29 a A	5	62.15 ab A	5
81-2	6.10 a C	3	6.43 a C	3	33.00 a B	5	63.33 ab A	4	61.27 ab A	4
B45A	6.33 a C	4	6.51 a C	4	32.75 a B	4	38.67 bc AB	3	57.09 ab A	3
B63A	6.43 a C	5	6.87 a C	6	27.32 a B	3	70.74 a A	6	79.83 a A	6
49-3	6.50 a C	6	6.29 a C	2	12.43 b C	2	24.10 c B	2	40.66 b A	2
Avg.	6.28 m O		6.52 m O		28.93 m N		52.22 m M		60.20 m M	
Mean[†]	4.29 W[‡]		7.05 V		20.83 U		33.35 T		45.35 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 19 continued. Fusarium head blight (FHB) severity (%) observed in three wheat cultivars (Alsen, 2375, Wheaton) point inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 2.

	Fusarium head blight (FHB) severity									
	Sampling days after inoculation (dai)									
	3 dai		7 dai		11 dai		14 dai		21 dai	
	Alsen									
Isolates	%	Rank	%	Rank	%	Rank	%	Rank	%	Rank
Control	0.00 c[x] A[y]	1	0.00 b A	1	0.00 d A	1	0.00 b A	1	0.00 d A	1
Butte86*	7.14 a B	6	9.19 a B	5	21.06 a A	6	11.02 a B	5	20.12 a A	6
81-2	7.01 a A	5	7.09 a A	3	13.26 ab A	5	12.86 a A	6	12.86 ab A	5
B45A	4.01 b A	3	7.46 a A	4	6.79 bc A	3	7.94 a A	3	6.77 c A	3
B63A	1.25 c B	2	10.65 a AB	6	9.10 c B	4	10.43 a AB	4	12.20 bc A	4
49-3	6.42 a A	4	6.42 a A	2	6.77 bc A	2	6.34 a A	2	6.52 c A	2
Avg.[w]	5.17 m[z] O		8.16 m N		11.40 n MN		9.72 o MN		11.70 o M	
	2375									
Control	0.00 a A	1	0.00 b A	1	0.00 c A	1	0.00 c A	1	0.00 d A	1
Butte86	0.00 a D	1	7.85 a C	6	43.63 a B	6	41.67 a B	5	69.72 a A	6
81-2	0.00 a D	1	6.71 a C	3	17.86 b B	3	32.20 a A	4	44.98 ab A	4
B45A	0.00 a D	1	6.50 a C	2	20.00 b B	4	16.96 b B	3	43.33 bc A	3
B63A	0.00 a D	1	7.59 a C	5	23.42 b B	5	46.79 a A	6	52.13 ab A	5
49-3	0.00 a D	1	6.81 a C	4	15.41 b B	2	11.94 b BC	2	28.87 c A	2
Avg.	0.00 o P		7.09 m O		24.06 m N		29.91 n N		47.81 n M	
	Wheaton									
Control	0.00 a A	1	0.00 b A	1	0.00 c A	1	0.00 c A	1	0.00 b A	1
Butte86	1.18 a E	3	5.62 a D	4	23.51 a C	4	49.47 a B	5	96.73 a A	6
81-2	1.11 a D	2	5.58 a C	3	23.68 a B	5	26.55 b B	4	95.95 a A	5
B45A	1.11 a E	2	5.89 a D	5	12.12 b C	2	24.97 b B	3	67.94 a A	2
B63A	0.00 a E	1	6.69 a D	6	26.77 a C	6	57.37 a A	6	85.56 a A	4
49-3	1.11 a D	2	5.39 a C	2	13.79 b B	3	19.74 b B	2	75.54 a A	3
Avg.	0.90 n Q		5.83 m P		19.97 m O		35.62 m N		84.34 m M	
Mean[†]	2.02 W[‡]		7.03 V		18.48 U		25.08 T		47.95 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 19 continued. Deoxynivalenol (DON, µg g^{-1}) levels detected in three wheat cultivars (Alsen, 2375, Wheaton) point inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment. Data from run 1 and 2 are combined.

	Deoxynivalenol (DON)									
	Sampling days after inoculation (dai)									
	3 dai		7 dai		11 dai		14 dai		21 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00 c[x] A[y]	1	0.00 d A	1	0.00 d A	1	0.00 d A	1	0.00 d A	1
Butte86*	17.68 a C	6	113.22 ab B	5	235.55 a A	5	277.36 a A	6	316.64 a A	6
81-2	19.89 a C	5	101.69 b B	3	138.40 ab A	4	206.45 ab A	4	109.87 c B	3
B45A	9.39 b C	2	102.67 b AB	4	98.08 b B	3	156.73 b A	3	175.39 b A	4
B63A	10.77 ab B	4	162.29 a A	6	247.80 a A	6	223.08 ab A	5	182.87 ab A	5
49-3	10.29 ab C	3	45.33 c AB	2	38.85 c B	2	35.77 c B	2	97.57 c A	2
Avg.[w]	13.61 n[z] O		105.04 n N		151.74 n M		179.88 n M		176.47 n M	
	2375									
Control	0.00 c A	1	0.00 d A	1	0.00 c A	1	0.00 d A	1	0.00 c A	1
Butte86	34.29 ab B	5	236.29 ab A	5	369.37 a A	6	371.55 a A	5	330.52 a A	6
81-2	23.88 ab C	3	146.81 ab B	4	191.07 b B	2	313.41 a A	4	261.73 ab A	4
B45A	14.04 b C	2	133.36 b B	3	275.39 ab A	4	156.32 b AB	3	198.46 b AB	3
B63A	63.38 a B	6	257.32 a A	6	337.48 a A	5	371.90 a A	6	327.58 ab A	5
49-3	24.41 b C	4	69.92 c B	2	191.90 b A	3	95.85 c B	2	157.86 b A	2
Avg.	32.00 m O		168.74 m N		273.04 m M		261.21 m M		255.23 m M	
	Wheaton									
Control	0.00 c A	1	0.00 c A	1	0.00 c A	1	0.00 d A	1	0.00 c A	1
Butte86	8.34 a C	4	184.03 a B	5	290.75 a A	5	320.25 ab A	4	348.30 a A	5
81-2	3.24 b C	2	138.19 a B	4	326.32 a A	6	366.12 a A	6	396.88 a A	6
B45A	6.90 ab C	3	134.40 a B	3	195.12 b A	3	194.33 bc A	3	208.82 b AB	2
B63A	9.91 a C	5	229.07 a B	6	254.72 ab B	4	333.15 a A	5	300.13 a AB	4
49-3	9.94 Ca	6	45.46 b B	2	138.63 b A	2	146.35 c A	2	211.13 b A	3
Avg.	7.67 o O		146.23 m N		241.11 m M		272.04 m M		293.05 m M	
Mean[†]	17.76 U[‡]		140.00 T		221.96 S		237.71 S		241.58 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 19 continued. 15-cetyldeoxynivalenol (15-ADON, µg g^{-1}) levels detected in three wheat cultivars (Alsen, 2375, Wheaton) point inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 1.

	15-acetyldeoxynivalenol (15-ADON)									
	Sampling days after inoculation (dai)									
	3 dai		7 dai		11 dai		14 dai		21 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00 b A	1	0.00 c A	1	0.00 d A	1	0.00 c A	1	0.00 d A	1
Butte86*	5.31 a B	6	27.81 a A	6	32.75 ab A	5	31.81 a A	5	40.04 a A	6
81-2	3.38 a B	4	18.49 a A	5	18.62 b A	3	33.35 a A	6	11.84 bc A	3
B45A	2.14 a B	2	10.44 b A	3	20.41 b A	4	20.65 a A	3	19.32 ab A	5
B63A	2.79 a C	3	18.38 a B	4	42.09 a A	6	21.36 a AB	4	18.34 ab B	4
49-3	3.43 a A	5	10.05 b A	2	3.85 c A	2	5.61 b A	2	6.36 c A	2
Avg.[w]	3.41 m[z] N		17.03 n M		23.54 n M		22.56 n M		19.18 o M	
	2375									
Control	0.00 b A	1	0.00 d A	1	0.00 d A	1	0.00 c A	1	0.00 c A	1
Butte86	2.02 a C	5	47.91 a B	5	66.77 ab A	5	79.48 a A	6	61.13 a AB	6
81-2	1.56 a C	3	31.47 ab B	4	43.67 bc AB	3	54.53 a A	4	35.35 b AB	3
B45A	2.33 a D	6	15.06 bc C	3	61.29 ab A	4	24.90 b B	3	49.38 ab A	5
B63A	1.94 a C	4	69.29 a A	6	75.01 a A	6	66.60 a A	5	37.88 b B	4
49-3	1.18 ab B	2	6.22 c B	2	25.78 c A	2	24.81 b A	2	27.54 b A	2
Avg.	1.80 n O		33.99 m N		54.51 m M		50.06 m M		42.26 m M	
	Wheaton									
Control	0.00 b A	1	0.00 c A	1	0.00 d A	1	0.00 d A	1	0.00 d A	1
Butte86	8.86 a B	6	23.72 a A	5	36.55 ab A	5	30.28 ab A	4	28.79 ab A	4
81-2	1.23 a C	3	13.74 ab B	4	49.37 a A	6	45.04 a A	6	47.07 a A	6
B45A	0.70 ab	2	11.68 ab A	3	16.40 bc A	3	18.83 bc A	3	18.56 b A	3
B63A	1.57 a C	5	26.04 a B	6	21.83 b B	4	42.43 a A	5	31.62 ab AB	5
49-3	1.50 a B	4	6.42 b A	2	14.17 c A	2	12.24 c A	2	8.10 c A	2
Avg.	1.28 n O		16.32 n N		27.67 n M		29.76 n M		26.83 n M	
Mean[†]	2.17 U[‡]		22.45 T		35.24 S		34.13 S		29.42 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 19 continued. 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) levels detected in three wheat cultivars (Alsen, 2375, Wheaton) point inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 2.

	15-acetyldeoxynivalenol (15-ADON)									
	Sampling days after inoculation (dai)									
	3 dai		7 dai		11 dai		14 dai		21 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00 c[x] A[y]	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 b A	1
Butte86*	0.01 bc A	2	0.01 ab A	2	0.00 a A	1	0.00 a A	1	0.00 b A	1
81-2	0.01 bc AB	2	0.00 b B	1	0.01 a AB	2	0.01 a AB	2	0.02 a A	3
B45A	0.02 ab A	3	0.00 b B	1	0.01 a AB	2	0.01 a AB	2	0.00 b B	1
B63A	0.01 bc A	2	0.00 b A	1	0.01 a A	2	0.00 a A	1	0.01 ab A	2
49-3	0.03 a A	4	0.02 a AB	3	0.01 a B	2	0.01 a B	2	0.01 ab B	2
Avg.[w]	0.01 m[z] M		0.01 m M		0.01 m M		0.01 m M		0.01 m M	
	2375									
Control	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
Butte86	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
81-2	0.01 a A	2	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
B45A	0.01 a A	2	0.01 a A	2	0.00 a A	1	0.00 a A	1	0.01 a A	2
B63A	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
49-3	0.01 a A	2	0.01 a A	2	0.01 a A	2	0.01 a A	2	0.00 a A	1
Avg.	0.01 m M		0.00 m M		0.00 m M		0.00 m M		0.00 m M	
	Wheaton									
Control	0.00 b A	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
Butte86	0.02 a A	3	0.01 ab AB	2	0.00 a B	1	0.00 a B	1	0.00 a B	1
81-2	0.00 b A	1	0.01 ab A	2	0.00 a A	1	0.00 a A	1	0.00 a A	1
B45A	0.01 ab A	2	0.01 ab A	2	0.00 a A	1	0.01 a A	2	0.00 a A	1
B63A	0.01 ab A	2	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
49-3	0.02 a A	3	0.02 a A	3	0.01 a AB	2	0.01 a AB	2	0.00 a B	1
Avg.	0.01 m M		0.01 m M		0.00 m M		0.01 m M		0.00 m M	
Mean[†]	0.01 S[‡]		0.01 S		0.01 S		0.01 S		0.01 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 19 continued. 3-acetyldeoxynivalenol (3-ADON, µg g^{-1}) levels detected in three wheat cultivars (Alsen, 2375, Wheaton) point inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 1.

	3-acetyldeoxynivalenol (3-ADON)									
	Sampling days after inoculation (dai)									
	3 dai		7 dai		11 dai		14 dai		21 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00 a[x] A[y]	1	0.00 a A	1	0.00 c A	1	0.00 b A	1	0.00 b A	1
Butte86*	0.00 a B	1	0.00 a B	1	0.00 c B	1	0.00 b B	1	2.23 a A	3
81-2	0.00 a B	1	0.00 a B	1	0.49 bc A	2	2.45 a A	3	0.00 b B	1
B45A	0.00 a B	1	0.00 a B	1	1.87 ab A	3	0.00 b B	1	0.00 b B	1
B63A	0.00 a B	1	0.00 a B	1	2.89 a A	4	1.86 a A	2	1.89 a A	2
49-3	0.00 a A	1	0.86 a A	2	0.00 c A	1	0.00 b A	1	0.00 b A	1
Avg.[w]	0.00 m[z] N		0.17 n N		1.05 n M		0.86 n MN		0.82 mn MN	
	2375									
Control	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
Butte86	0.00 a A	1	0.00 b A	1	1.07 a A	3	0.00 a A	1	1.30 a A	2
81-2	0.00 a A	1	0.00 b A	1	1.02 a A	2	0.00 a A	1	0.00 a A	1
B45A	0.00 a A	1	0.00 b A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1
B63A	0.00 a B	1	4.51 a A	2	0.00 a B	1	0.00 a B	1	0.00 a B	1
49-3	0.00 a A	1	0.00 b A	1	1.56 a A	4	0.94 a A	2	0.00 a A	1
Avg.	0.00 m M		0.94 m M		0.70 n M		0.19 n M		0.26 n M	
	Wheaton									
Control	0.00 a A	1	0.00 c A	1	0.00 c A	1	0.00 c A	1	0.00 c A	1
Butte86	0.00 a C	1	1.32 ab B	4	3.75 a A	6	2.28 a AB	4	2.04 a AB	5
81-2	0.00 a C	1	1.83 a AB	5	2.12 b A	5	2.35 a A	5	1.66 ab AB	4
B45A	0.00 a A	1	0.38 bc A	2	0.99 bc A	3	0.29 bc A	2	0.00 c A	1
B63A	0.00 a B	1	1.11 ab AB	3	1.45 bc AB	4	2.01 ab A	3	0.79 a-c AB	3
49-3	0.00 a A	1	0.00 c A	1	0.34 bc A	2	0.00 c A	1	0.71 bc A	2
Avg.	0.00 m N		0.93 m M		1.73 m M		1.39 m M		1.04 m M	
Mean[†]	0.00 U[‡]		0.68 T		1.16 S		0.81 ST		0.71 ST	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 19 continued. Numbers of infected spikelets above the inoculated spikelet of three wheat cultivars (Alsen, 2375, Wheaton) point inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment. Data are combined for runs 1 and 2.

	Infected spikelets above the point of inoculation									
	Sampling days after inoculation (dai)									
	3 dai		7 dai		11 dai		14 dai		21 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00 a A	1	0.00 b A	1	0.00 b A	1	0.00 a A	1	0.00 b A	1
Butte86*	0.00 a C	1	0.40 a AB	3	0.60 a AB	5	0.20 a BC	3	0.80 a A	4
81-2	0.00 a A	1	0.00 b A	1	0.20 ab A	3	0.10 a A	2	0.10 b A	2
B45A	0.00 a A	1	0.00 b A	1	0.10 b A	2	0.00 a A	1	0.10 b A	2
B63A	0.00 a A	1	0.20 ab A	2	0.40 ab A	4	0.20 a A	3	0.30 b A	3
49-3	0.00 a A	1	0.00 b A	1	0.00 b A	1	0.00 a A	1	0.00 b A	1
Avg.[w]	0.00 m[z] N		0.12 m MN		0.26 o M		0.10 o MN		0.26 o M	
	2375									
Control	0.00 a A	1	0.00 a A	1	0.00 d A	1	0.00 c A	1	0.00 e A	1
Butte86	0.00 a D	1	0.10 a D	2	2.50 a B	6	1.60 a C	5	3.80 a A	6
81-2	0.00 a B	1	0.00 a B	1	0.80 bc A	4	1.40 a A	4	0.90 d A	2
B45A	0.00 a C	1	0.00 a C	1	0.40 c B	2	0.60 b B	3	1.80 bc A	4
B63A	0.00 a C	1	0.00 a C	1	1.10 b B	5	1.70 a B	6	2.30 b A	5
49-3	0.00 a C	1	0.00 a C	1	0.50 c B	3	0.30 bc BC	2	1.70 cd A	3
Avg.	0.00 m O		0.02 m O		1.06 n N		1.12 n N		2.10 n M	
	Wheaton									
Control	0.00 a A	1	0.00 a A	1	0.00 d A	1	0.00 d A	1	0.00 c A	1
Butte86	0.00 a D	1	0.00 a D	1	1.90 a C	6	4.10 a B	5	6.20 a A	6
81-2	0.00 a D	1	0.00 a D	1	1.50 ab C	5	2.70 b B	4	6.00 a A	5
B45A	0.00 a C	1	0.00 a C	1	1.10 b B	3	1.40 c B	3	3.90 b A	3
B63A	0.00 a C	1	0.10 Ca	2	1.40 ab B	4	4.70 a A	6	5.60 a A	4
49-3	0.00 a D	1	0.00 a D	1	0.50 c C	2	1.00 c B	2	3.50 b A	2
Avg.	0.00 m P		0.02 m P		1.28 m O		2.78 m N		5.04 m M	
Mean[†]	0.00 V[‡]		0.00 V		0.05 U		0.87 T		2.47 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 19 continued. Numbers of infected spikelets below the inoculated spikelet of three wheat cultivars (Alsen, 2375, Wheaton) point inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment. Data are combined for runs 1 and 2.

	Infected spikelets below the point of inoculation									
	Sampling days after inoculation (dai)									
	3 dai		7 dai		11 dai		14 dai		21 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00 a[x] A[y]	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 b A	1
Butte86*	0.00 a B	1	0.10 a B	2	0.40 a B	4	0.50 a BC	4	1.50 a A	5
81-2	0.00 a A	1	0.00 a A	1	0.30 a A	3	0.30 a A	3	0.40 b A	3
B45A	0.00 a A	1	0.00 a A	1	0.10 a A	2	0.30 a A	3	0.70 b A	4
B63A	0.00 a A	1	0.20 a A	3	0.10 a A	2	0.20 a A	2	0.20 b A	2
49-3	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 a A	1	0.00 b A	1
Avg.[w]	0.00 m[z] N		0.06 m MN		0.18 o MN		0.26 o M		0.56 o M	
	2375									
Control	0.00 a A	1	0.00 a A	1	0.00 d A	1	0.00 c A	1	0.00 e A	1
Butte86	0.00 a D	1	0.10 a D	2	2.50 a C	6	4.70 a B	6	6.40 a A	6
81-2	0.00 a D	1	0.00 a D	1	1.40 bc C	4	3.80 a B	4	5.00 c A	3
B45A	0.10 a C	2	0.00 a C	1	1.30 c B	3	1.60 b B	3	5.90 b A	5
B63A	0.00 a C	1	0.10 a C	2	2.30 b B	5	4.50 a A	5	5.30 bc A	4
49-3	0.00 a C	1	0.00 a C	1	0.90 c BC	2	0.71 bc C	2	4.30 c AC	2
Avg.	0.02 m O		0.04 m O		1.68 n N		3.02 n N		5.38 n M	
	Wheaton									
Control	0.00 a A	1	0.00 a A	1	0.00 d A	1	0.00 d A	1	0.00 d A	1
Butte86	0.00 a D	1	0.00 a D	1	2.40 a C	6	4.60 a B	5	6.60 a A	3
81-2	0.00 a D	1	0.00 a D	1	2.20 ab C	5	3.70 b B	4	6.70 a A	4
B45A	0.00 a C	1	0.00 a C	1	1.50 b B	3	2.80 c B	3	5.40 b A	2
B63A	0.00 a C	1	0.00 a C	1	2.10 ab B	4	5.30 a A	6	7.40 a A	5
49-3	0.00 a D	1	0.00 a D	1	0.70 c C	2	1.50 c B	2	5.40 c A	2
Avg.	0.00 m P		0.00 m P		1.78 m O		3.58 m N		6.30 m M	
Mean[†]	0.01 V[‡]		0.03 V		1.21 U		2.29 T		4.08 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 20. Fusarium head blight (FHB) severity (%) observed in three wheat cultivars (Alsen, 2375, Wheaton) spray inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 1.

	Fusarium head blight (FHB) severity									
	Sampling days after inoculation (dai)									
	7 dai		11 dai		14 dai		21 dai		30 dai	
	Alsen									
Isolates	%	Rank	%	Rank	%	Rank	%	Rank	%	Rank
Control	0.00 c[x] A[y]	1	0.00 c A	1	0.00 c A	1	0.00 c A	1	0.00 c A	1
Butte86*	60.81 a B	3	81.61 a A	6	73.22 a AB	5	93.43 a A	6	60.56 b B	3
81-2	66.08 a A	5	65.41 a A	4	77.05 a A	6	69.58 a A	3	84.03 a A	6
B45A	25.51 b B	2	33.97 b AB	2	36.67 b AB	2	46.54 b AB	2	59.98 b A	2
B63A	62.59 a A	4	75.69 a A	5	67.92 a A	4	83.91 a A	5	81.99 a A	5
49-3	68.96 a A	6	56.64 a A	3	47.06 b B	3	82.08 a A	4	78.33 a A	4
Avg.[w]	56.79 m[z] N		62.66 m MN		60.38 n N		75.11 n M		72.98 n M	
	2375									
Control	0.00 d A	1	0.00 b A	1	0.00 b A	1	0.00 b A	1	0.00 b A	1
Butte86	64.61 ab B	5	80.08 a A	6	78.89 a AB	5	98.82 a A	5	98.75 a A	6
81-2	84.11 a A	6	78.92 a A	5	94.67 a A	6	100.00 a A	6	97.50 a A	5
B45A	35.79 c C	2	65.58 a B	4	75.49 a A-C	3	89.42 a A	3	93.89 a A	3
B63A	64.40 ab A	4	62.55 a A	3	76.11 a A	4	91.54 a A	4	89.42 a A	2
49-3	49.85 bc C	3	58.21 a BC	2	72.95 a A-C	2	88.57 a A	2	97.39 a A	4
Avg.	59.75 m P		69.07 m OP		79.62 m NO		93.67 m MN		95.39 m M	
	Wheaton									
Control	0.00 d A	1	0.00 c A	1	0.00 c A	1	0.00 b A	1	0.00 c A	1
Butte86	57.01 ab B	5	78.51 a AB	5	94.61 a A	5	100.00 a A	4	100.00 a A	3
81-2	71.33 a A	6	81.23 a A	6	100.00 a A	6	100.00 a A	4	100.00 a A	3
B45A	32.16 bc C	3	64.63 a B	3	80.44 a AB	3	98.95 a A	3	100.00 a A	3
B63A	42.42 b C	4	65.16 a B	4	90.65 a AB	4	100.00 a A	5	100.00 a A	3
49-3	30.82 c C	2	35.97 b BC	2	45.34 b B	2	79.78 a A	2	59.31 b B	2
Avg.	46.75 n O		65.10 m N		82.21 m M		95.75 m M		91.86 m M	
Mean[†]	54.43 U[‡]		65.61 T		74.07 T		88.18 S		86.74 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 20 continued. Fusarium head blight (FHB) severity (%) observed in three wheat cultivars (Alsen, 2375, Wheaton) spray inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 2.

	Fusarium head blight (FHB) severity									
	Sampling days after inoculation (dai)									
	7 dai		11 dai		14 dai		21 dai		30 dai	
	Alsen									
Isolates	%	Rank	%	Rank	%	Rank	%	Rank	%	Rank
Control	0.00 c[x] A[y]	1	0.00 c A	1	0.00 c A	1	0.00 c A	1	0.00 b A	1
Butte86*	50.25 b C	2	54.61 b BC	2	59.23 b BC	2	69.55 b AB	2	86.22 a A	2
81-2	66.19 a A	3	76.58 a A	3	88.09 a A	5	96.08 a A	5	98.75 a A	6
B45A	76.64 a A	6	88.01 a A	5	81.50 ab A	3	92.08 ab A	4	87.32 a A	3
B63A	75.06 a A	5	83.42 a A	4	85.75 a A	4	97.32 a A	6	98.57 a A	5
49-3	69.19 a A	4	89.00 a A	6	89.73 a A	6	90.81 ab A	3	94.75 a A	4
Avg.[w]	67.47 m[z] O		78.33 n N		80.86 n MN		89.17 m MN		93.12 m M	
	2375									
Control	0.00 b A	1	0.00 b A	1	0.00 b A	1	0.00 b A	1	2.00 b A	1
Butte86	62.76 a B	2	86.65 a AB	2	83.24 a AB	2	98.75 a A	2	100.00 a A	2
81-2	74.45 a A	5	88.69 a A	4	94.57 a A	3	100.00 a A	3	100.00 a A	2
B45A	63.77 a B	3	91.67 a A	6	97.33 a A	4	100.00 a A	3	100.00 a A	2
B63A	66.38 a B	4	87.92 a AB	3	98.57 a A	5	98.75 a A	2	100.00 a A	2
49-3	80.81 a A	6	90.46 a A	5	98.75 a A	6	100.00 a A	3	100.00 a A	2
Avg.	69.64 m N		89.08 m M		94.49 m M		99.50 m M		100.00 m M	
	Wheaton									
Control	0.00 c A	1	0.00 c A	1	0.00 c A	1	0.00 b A	1	0.00 b A	1
Butte86	18.04 b C	2	56.23 b B	2	67.60 b B	2	98.67 a A	2	100.00 a A	2
81-2	45.07 a B	6	86.07 a A	5	93.33 a A	4	100.00 a A	3	100.00 a A	2
B45A	43.57 a B	5	92.25 a A	6	81.79 ab A	3	100.00 a A	3	100.00 a A	2
B63A	41.33 a B	3	83.36 a A	3	98.57 a A	5	100.00 a A	3	100.00 a A	2
49-3	41.94 a B	4	85.14 a A	4	98.82 a A	6	100.00 a A	3	100.00 a A	2
Avg.	37.99 n P		80.61 mn O		88.02 mn NO		99.73 m MN		100.00 m M	
Mean[†]	58.36 U[‡]		82.67 T		87.79 T		96.13 S		97.71 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P \leq 0.05$.

Appendix 20 continued. Deoxynivalenol (DON, µg g^{-1}) levels detected in three wheat cultivars (Alsen, 2375, Wheaton) spray inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 1.

	Deoxynivalenol (DON)									
	Sampling days after inoculation (dai)									
	7 dai		11 dai		14 dai		21 dai		30 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00	1	0.00	1	0.26	1	0.00	1	0.00	1
Butte86*	30.50	4	82.90	6	37.80	4	74.70	5	21.40	3
81-2	93.70	6	45.20	4	93.20	6	60.00	4	46.50	5
B45A	7.30	2	7.30	2	11.70	3	7.60	2	3.00	2
B63A	32.80	5	47.40	5	43.50	5	83.70	6	81.99	6
49-3	15.50	3	13.00	3	8.10	2	11.40	3	23.80	4
Avg.[w]	35.96 n[z] M[y]		39.16 n M		38.86 o M		47.48 o M		28.48 o N	
	2375									
Control	0.00	1	0.00	1	6.60	1	0.00	1	0.00	1
Butte86	47.90	4	104.80	6	85.60	6	297.90	5	199.70	6
81-2	283.10	6	109.20	5	57.10	4	425.40	6	135.00	5
B45A	15.00	2	29.50	3	53.50	2	84.70	3	63.90	3
B63A	55.30	5	69.30	4	59.80	5	98.30	4	103.70	4
49-3	18.20	3	17.70	2	55.30	3	56.30	2	62.60	2
Avg.	83.90 mO		66.10 mO		62.26 n NO		192.52 n MN		112.98 n M	
	Wheaton									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	40.20	5	125.20	5	189.70	5	525.70	6	228.90	5
81-2	51.20	6	125.30	6	542.60	6	338.60	5	100.00	3
B45A	12.30	3	48.30	4	96.30	3	264.90	3	147.30	4
B63A	11.90	2	41.90	3	112.90	4	285.70	4	347.30	6
49-3	13.60	4	15.50	2	25.60	2	76.30	2	40.50	2
Avg.	25.84 n P		71.24 m O		193.42 m N		298.24 m MN		238.72 m M	
Mean[†]	48.57 V[‡]		58.83 U		98.18 TU		179.41 S		126.73 T	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 20 continued. Deoxynivalenol (DON, µg g^{-1}) levels detected in three wheat cultivars (Alsen, 2375, Wheaton) spray inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 2.

	Deoxynivalenol (DON)									
	Sampling days after inoculation (dai)									
	7 dai		11 dai		14 dai		21 dai		30 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86*	45.99	2	21.94	2	11.40	2	61.30	2	23.64	2
81-2	168.77	4	94.25	3	143.76	5	157.41	4	154.92	3
B45A	178.32	5	209.32	5	57.69	4	176.37	5	157.09	4
B63A	51.81	3	146.59	4	48.98	3	186.95	6	165.77	5
49-3	328.99	6	209.62	6	313.81	6	83.55	3	324.47	6
Avg.[w]	154.77 m[z] M[y]		136.34 n MN		115.13 o N		133.11 n M		165.18 n M	
	2375									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	115.03	2	343.42	5	356.26	3	227.96	2	230.30	3
81-2	234.37	5	338.70	4	621.35	6	401.06	5	642.70	5
B45A	116.81	4	95.47	2	257.03	2	327.69	3	279.63	4
B63A	116.70	3	216.40	3	440.25	4	328.97	4	169.45	2
49-3	567.46	6	651.42	6	538.97	5	502.24	6	781.60	6
Avg.	230.07 m N		329.08 m MN		442.77 m M		357.59 m M		420.73 m M	
	Wheaton									
Control	0.00	1	0.04	1	0.00	1	0.00	1	0.00	1
Butte86	5.09	2	57.68	2	95.07	3	222.26	2	202.69	3
81-2	25.51	4	113.21	3	275.63	4	449.84	4	322.03	4
B45A	11.03	3	167.03	4	92.44	2	230.49	3	198.18	2
B63A	25.68	5	462.14	6	532.28	6	636.79	5	570.04	6
49-3	41.99	6	237.06	5	486.62	5	695.43	6	541.94	5
Avg.	21.86 n P		207.42 mn O		296.41 n NO		446.96 m M		366.98 m MN	
Mean[†]	135.57 V[‡]		224.28 U		284.77 TU		312.55 S		317.63 ST	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 20 continued. 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) levels detected in three wheat cultivars (ALsen, 2375, Wheaton) spray inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 1.

	15-acetyldeoxynivalenol (15-ADON)									
	Sampling days after inoculation (dai)									
	7 dai		11 dai		14 dai		21 dai		30 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86*	0.42	4	0.45	4	0.00	1	0.00	1	0.00	1
81-2	0.43	5	0.28	3	0.39	3	0.00	1	0.00	1
B45A	0.15	2	0.00	1	0.00	1	0.00	1	0.00	1
B63A	0.48	6	0.23	2	0.22	2	0.00	1	0.00	1
49-3	0.39	3	0.00	1	0.00	1	0.00	1	0.00	1
Avg.[w]	0.37 m[z] M[y]		0.19 m M		0.12 n M		0.00 o M		0.00 o M	
	2375									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.26	2	0.72	5	0.00	1	5.40	3	3.70	6
81-2	0.32	3	0.84	6	0.00	1	7.10	4	2.20	5
B45A	0.00	1	0.14	2	0.33	3	1.00	2	1.00	3
B63A	0.54	5	0.62	4	0.16	2	0.00	1	2.00	4
49-3	0.43	4	0.34	3	0.42	4	0.00	1	0.91	2
Avg.	0.31 m N		0.53 m MN		0.18 n N		2.70 n MN		1.96 n M	
	Wheaton									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.21	4	0.64	4	0.36	2	10.40	5	6.50	3
81-2	0.45	6	1.10	5	14.00	3	10.00	4	10.20	5
B45A	0.14	2	0.30	2	0.00	1	8.40	3	3.40	2
B63A	0.19	3	0.39	3	0.00	1	6.20	2	8.10	4
49-3	0.25	5	0.30	2	0.00	1	0.00	1	0.00	1
Avg.	0.25 m O		0.55 m NO		2.87 m N		7.00 m M		5.64 m M	
Mean[†]	0.31 T[‡]		0.42 T		1.06 T		3.23 S		2.53 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 20 continued. 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) levels detected in three wheat cultivars (Alsen, 2375, Wheaton) spray inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 2.

	15-acetyldeoxynivalenol (15-ADON)									
	Sampling days after inoculation (dai)									
	7 dai		11 dai		14 dai		21 dai		30 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86*	0.38	2	0.07	2	0.03	2	0.05	2	0.01	2
81-2	1.61	5	0.36	3	0.38	5	0.45	5	2.56	4
B45A	0.90	4	0.49	4	0.22	3	0.63	6	0.99	3
B63A	0.47	3	0.70	5	0.26	4	0.42	4	2.61	5
49-3	1.76	6	1.65	6	1.66	6	0.12	3	3.14	6
Avg.[w]	1.02 m M		0.65 m M		0.51 n M		0.34 n M		1.86 o M	
	2375									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	1.29	3	3.01	4	4.08	2	3.41	2	5.10	2
81-2	2.92	5	3.88	5	18.68	6	10.73	5	16.74	5
B45A	1.52	4	0.86	2	9.60	3	9.18	4	8.12	4
B63A	1.27	2	2.04	3	11.67	4	6.75	3	6.55	3
49-3	9.78	6	11.14	6	13.41	5	13.18	6	33.75	6
Avg.	3.35 m O		4.19 m O		11.49 m MN		8.65 m N		14.05 m M	
	Wheaton									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.09	2	0.28	2	0.48	3	3.17	2	5.90	2
81-2	0.37	5	0.76	5	1.18	4	11.20	4	9.05	4
B45A	0.15	3	0.75	4	0.29	2	5.70	3	7.47	3
B63A	0.33	4	6.19	6	9.39	6	12.26	5	10.85	6
49-3	0.41	6	0.69	3	9.35	5	14.31	6	9.47	5
Avg.	0.27 m O		1.73 m NO		4.14 n N		9.33 m M		8.55 n M	
Mean[‡]	1.55 O		2.19 O		5.38 N		6.11 MN		8.15 M	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 20 continued. 3-acetyldeoxynivalenol (3-ADON, µg g^{-1}) levels detected in three wheat cultivars (Alsen, 2375, Wheaton) spray inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 1.

	3-acetyldeoxynivalenol (3-ADON)									
	Sampling days after inoculation (dai)									
	7 dai		11 dai		14 dai		21 dai		30 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86*	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
81-2	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
B45A	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
B63A	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
49-3	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Avg.[w]	0.00 m[z] M[y]		0.00 m M		0.00 n M		0.00 o M		0.00 n M	
	2375									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.00	1	0.12	2	0.00	1	0.82	2	0.40	4
81-2	0.27	2	0.14	3	0.00	1	0.86	3	0.23	3
B45A	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
B63A	0.00	1	0.12	2	0.09	2	0.00	1	0.17	2
49-3	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Avg.	0.05 m M		0.08 m M		0.02 n M		0.34 n M		0.16 n M	
	Wheaton									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.00	1	0.17	2	0.18	2	2.60	5	0.86	3
81-2	0.00	1	0.18	3	2.40	3	1.90	4	2.30	5
B45A	0.00	1	0.00	1	0.00	1	1.30	3	0.63	2
B63A	0.00	1	0.00	1	0.00	1	0.69	2	1.50	4
49-3	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Avg.	0.00 mO		0.07 m NO		0.52 m N		1.30 m M		1.06 m M	
Mean[†]	0.02 T[‡]		0.05 T		0.18 T		0.54 S		0.41 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 20 continued. 3-acetyldeoxynivalenol (3-ADON, µg g^{-1}) levels detected in three wheat cultivars (Alsen, 2375, Wheaton) spray inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 2.

	3-acetyldeoxynivalenol (3-ADON)									
	Sampling days after inoculation (dai)									
	7 dai		11 dai		14 dai		21 dai		30 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86*	0.00	1	0.00	1	0.00	1	0.06	2	0.00	1
81-2	0.00	1	0.00	1	0.00	1	0.00	1	0.31	2
B45A	0.00	1	0.00	1	0.06	2	0.13	3	0.00	1
B63A	0.00	1	0.00	1	0.07	3	0.00	1	0.32	3
49-3	0.00	1	0.13	2	0.00	1	0.00	1	0.00	1
Avg.[w]	0.00 m[z] M[y]		0.03 m M		0.03 o M		0.04 o M		0.13 n M	
	2375									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.07	2	0.00	1	0.51	2	0.00	1	1.43	3
81-2	0.00	1	0.29	2	2.40	6	1.66	4	2.77	5
B45A	0.00	1	0.00	1	1.38	5	1.81	5	1.93	4
B63A	0.00	1	0.00	1	0.60	3	0.37	2	1.19	2
49-3	0.00	1	0.00	1	0.68	4	1.19	3	3.70	6
Avg.	0.01 m O		0.06 m O		1.11 m N		1.00 n N		2.20 m M	
	Wheaton									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.00	1	0.00	1	0.08	2	0.41	2	1.21	2
81-2	0.00	1	0.14	3	0.32	4	3.18	5	1.96	4
B45A	0.00	1	0.29	4	0.13	3	1.23	3	1.28	3
B63A	0.08	3	0.00	1	3.18	5	3.43	6	3.51	6
49-3	0.03	2	0.11	2	0.00	1	2.73	4	2.13	5
Avg.	0.02 m O		0.11 m O		0.74 n N		2.20 m M		2.02 m M	
Mean[†]	0.01 V[‡]		0.07 V		0.63 U		1.08 T		1.45 S	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 20 continued. Nivalenol (NIV, µg g^{-1}) levels detected three wheat cultivars (Alsen, 2375, Wheaton) spray inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 1.

	Nivalenol (NIV)									
	Sampling days after inoculation (dai)									
	7 dai		11 dai		14 dai		21 dai		30 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86*	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
81-2	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
B45A	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
B63A	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
49-3	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Avg.[w]	0.00		0.00		0.00		0.00		0.00	
	2375									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
81-2	0.24	1	0.00	1	0.00	1	0.00	1	0.00	1
B45A	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
B63A	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
49-3	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Avg.	0.05		0.00		0.00		0.00		0.00	
	Wheaton									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
81-2	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
B45A	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
B63A	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
49-3	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Avg.	0.00		0.00		0.00		0.00		0.00	
Mean[‡]	0.02		0.00		0.00		0.00		0.00	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 20 continued. Nivalenol (NIV, µg g^{-1}) levels detected in three wheat cultivars (Alsen, 2375, Wheaton) spray inoculated with five *F. graminearum* isolates and a water control in the greenhouse experiment run 2.

	Nivalenol (NIV)									
	Sampling days after inoculation (dai)									
	7 dai		11 dai		14 dai		21 dai		30 dai	
	Alsen									
Isolates	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank	µg g^{-1}	Rank
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86*	0.03	2	0.02	2	0.00	1	0.05	2	0.02	2
81-2	0.16	6	0.08	3	0.10	3	0.13	5	0.09	3
B45A	0.13	5	0.15	6	0.05	2	0.09	4	0.09	3
B63A	0.04	3	0.11	4	0.05	2	0.14	6	0.11	4
49-3	0.12	4	0.13	5	0.15	4	0.06	3	0.13	5
Avg.[w]	0.10 m M		0.10 n M		0.07 o M		0.09 n M		0.09 n M	
	2375									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.05	2	0.29	5	0.27	4	0.12	2	0.19	3
81-2	0.10	4	0.25	4	0.44	5	0.15	4	0.34	5
B45A	0.00	1	0.00	1	0.00	1	0.30	6	0.19	3
B63A	0.06	3	0.16	2	0.17	2	0.13	3	0.12	2
49-3	0.21	5	0.21	3	0.26	3	0.19	5	0.24	4
Avg.	0.08 m N		0.18 mn MN		0.23 n M		0.18 n MN		0.22 n MN	
	Wheaton									
Control	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1
Butte86	0.00	1	0.00	1	0.17	3	0.41	3	0.16	2
81-2	0.00	1	0.21	3	0.49	5	0.53	4	0.43	5
B45A	0.00	1	0.28	4	0.18	4	0.41	3	0.21	4
B63A	0.00	1	0.53	5	1.00	6	0.98	5	0.91	6
49-3	0.00	1	0.17	2	0.14	2	0.26	2	0.19	3
Avg.	0.00 m O		0.24 m N		0.39 m M		0.52 m M		0.38 m MN	
Mean[‡]	0.06 O		0.17 N		0.23 MN		0.26 M		0.23 MN	

[x] Means followed by the same lowercase letter within a column in each sampling date and cultivar are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within a row in each cultivar are not significantly different at $P < 0.05$.

***** Isolate Butte86Ada-11.

[w] Average (sampling days after inoculation means within each cultivar excludes water treatment).

[z] Means (combined across isolates) followed by the same uppercase letter within column in each sampling date are not significantly different at $P < 0.05$.

[†] Sampling days after inoculation mean value combined across cultivars and isolates.

[‡] Sampling date means (combined across isolates and cultivars) followed by same uppercase letter within a row are not significantly different at $P < 0.05$.

Appendix 21. Fusarium head blight (FHB) severity (%) observed 7, 14, 21 and 28 dai in Alsen, 2375 and Wheaton inoculated with two *F. graminearum* isolates (Butte86Ada-11 and 81-2) for control plants and plants subjected to a six hour wetting treatment in runs 1 and 2.

	Fusarium head blight (FHB) severity (%)							
	Sampling days after inoculation (dai)							
	RUN 1							
	Control				Six hour wetting			
Isolates	7 dai	14 dai	21 dai	28 dai	7 dai	14 dai	21 dai	28 dai
	Alsen							
Butte86*	80.47 k-n[x]	94.99 a-g	95.77 a-f	98.46 ab	87.66 b-k	91.62 a-j	96.66 a-e	98.54 a
81-2	84.21 g-l	86.01 e-k	91.83 a-j	96.47 a-e	82.95 i-m	83.39 h-m	90.26 a-k	97.00 a-d
Avg.[w]	82.34 G-I[y]	90.50 C-F	93.80 A-D	97.47 A-C	85.30 F-H	87.50 D-G	93.46 A-E	97.77 A-C
	2375							
Butte86*	72.67 mn	95.50 a-f	96.79 a-e	100.00 a	85.31 f-k	94.05 a-h	98.64 a	100.00 a
81-2	81.42 j-n	96.50 a-e	94.29 a-g	99.62 a	86.52 d-k	90.47 a-k	95.48 a-f	100.00 a
Avg.	77.05 I	96.00 A-C	95.54 A-C	99.81 AB	85.91 E-H	92.26 B-F	97.06 A-C	100.00 A
	Wheaton							
Butte86*	30.09 q	74.30 l-n	92.58 a-i	100.00 a	32.91 pq	71.24 n	93.76 a-i	100.00 a
81-2	41.18 p	90.37 a-k	98.23 ab	100.00 a	52.05 o	87.21 c-k	97.83 a-c	100.00 a
Avg.	35.63 J	82.34 G-I	95.40 A-C	100.00 A	42.48 J	79.23 HI	95.80 A-C	100.00 A
Mean[†]	65.01 V[z]	89.61 T	94.91 S	99.09 S	71.23 U	86.33 T	95.44 S	99.26 S
	RUN 2							
	Alsen							
Butte86*	52.19 jk	77.48 e-h	78.56 d-h	87.55 a-e	60.46 ij	80.03 d-g	81.00 d-f	87.09 a-e
81-2	47.89 jk	58.11 ij	68.97 f-i	83.67 de	50.63 jk	57.87 ij	67.83 g-i	81.99 d-f
Avg.	50.04 I	67.80 FG	73.77 EF	85.61 CD	55.55 HI	68.95 FG	74.42 EF	84.54 CD
	2375							
Butte86*	57.84 ij	90.56 a-d	97.53 a-c	100.00 a	65.85hi	88.34 a-e	97.04 a-c	100.00 a
81-2	56.34 ij	86.61 b-e	98.24 ab	100.00 a	58.12 ij	91.50 a-d	98.66 ab	100.00 a
Avg.	57.09 HI	88.59 CD	97.88 AB	100.00 Aa	61.98 GH	89.92 BC	97.85 AB	100.00 A
	Wheaton							
Butte86*	40.88 kl	85.14 c-e	97.61 a-c	100.00 a	39.76 kl	82.59 de	97.16 a-c	99.67 a
81-2	28.75 l	75.59 e-h	100.00 a	100.00 a	28.14 l	76.27 e-h	100.00 a	100.00 a
Avg.	34.81 J	80.36 DE	98.81 AB	100.00 A	33.95 J	79.43 DE	98.58 AB	99.83 A
Mean	47.31 U	78.92 T	90.15 S	95.20 S	50.49 U	79.43 T	90.28 S	94.79 S

[w] Average (sampling date means within each cultivar).

***** Isolate Butte86Ada-11

[x] Means followed by the same lowercase letter within sane run are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within same run are not significantly different at $P < 0.05$.

[†] Means - combined across isolates and cultivars.

[z] Means within same run followed by same uppercase letter are not significantly different at $P < 0.05$.

Appendix 21 continued. Deoxynivalenol (DON, µg g^{-1}) levels detected 7, 14, 21 and 28 dai in Alsen, 2375 and Wheaton inoculated with two *F. graminearum* isolates (Butte86Ada-11 and 81-2) for control plants and plants subjected to a six hour wetting treatment in runs 1 and 2.

	Deoxynivalenol (DON, µg g^{-1})							
	Sampling days after inoculation (dai)							
	RUN 1							
	Control				Six hour wetting			
	7 dai	14 dai	21 dai	28 dai	7 dai	14 dai	21 dai	28 dai
	Alsen							
Butte86*	88.21 j-n[x]	105.19 h-n	86.32 i-n	112.53 g-m	73.74 m-o	67.26 n-p	74.25 l-o	84.80 k-n
81-2	79.76 k-n	89.24 i-n	100.51 h-n	92.73 i-n	72.75 m-o	72.17 m-o	79.09 k-n	72.70 m-o
Avg.[w]	83.99 H-K[y]	97.22 F-J	93.41 G-K	102.63 F-I	73.25 JK	69.72 K	76.67 I-K	78.75 I-K
	2375							
Butte86*	107.55 h-m	162.24 b-f	155.49 b-g	202.56 ab	108.27 g-m	88.82 j-n	79.94 k-n	136.72 d-h
81-2	176.17 b-e	209.27 ab	247.06 a	244.17 a	108.00 g-m	102.74 h-n	117.28 f-k	159.04 b-f
Avg.[w]	141.86 DE	185.76 A-C	201.28 AB	223.36 A	108.14 E-H	95.78 G-K	98.61 F-J	147.88 CD
	Wheaton							
Butte86*	32.07 q	167.59 b-f	205.00 ab	193.32 a-c	33.60 q	113.35 g-m	124.28 e-j	141.24 c-h
81-2	41.81 pq	191.60 a-c	176.69 b-d	174.86 b-d	44.28 o-q	130.86 d-i	110.95 g-l	111.22 g-l
Avg.[w]	36.94 L	179.60 BC	190.85 AB	184.09 A-C	38.94 L	122.10 D-G	117.61 D-G	126.23 D-F
Mean[†]	87.59 VW[z]	154.19 S	161.85 S	170.03 S	73.44 W	95.87 UV	97.63 U	117.62 V
	RUN 2							
	Alsen							
Butte86*	38.61 q-s	52.69 m-r	101.48 e-i	99.61 e-i	46.58 p-s	67.17 j-p	95.65 f-j	90.27 f-k
81-2	23.13 t	34.99 r-t	73.46 i-o	72.35 i-n	36.62 r-t	31.45 st	48.07 o-s	64.26 k-p
Avg.[w]	30.87 P	43.84 L-O	87.47 G-I	85.98 G-I	41.60 M-P	49.31 L-N	71.86 I-K	77.26 H-J
	2375							
Butte86*	60.94 l-q	98.35 e-i	107.68 d-h	128.88 c-e	66.64 j-p	73.61 i-n	90.17 f-k	92.99 f-j
81-2	44.51 p-s	85.63 f-l	119.13 c-f	129.44 c-e	48.66 n-s	65.23 j-p	76.23 h-m	83.20 g-l
Avg.[w]	52.73 K-M	91.99 E-H	113.40 DE	129.16 CD	57.65 J-L	69.42 I-K	83.20 HI	88.09 F-I
	Wheaton							
Butte86*	51.05 n-s	191.73 ab	207.44 a	225.08 a	43.61 p-s	155.86 bc	132.80 c-e	136.59 cd
81-2	20.08 t	112.90 d-g	134.18 c-e	154.02 bc	19.66 t	66.27 j-p	88.23 f-l	107.17 d-h
Avg.[w]	35.56 N-P	152.31 BC	170.81 AB	189.55 A	31.64 OP	111.06 D-G	110.52 D-F	121.88 D
Mean	39.72 V	96.05 T	123.90 S	134.90 S	43.63 V	76.60 U	88.53 T	95.75 T

[w] Average (sampling date means within each cultivar).

***** Isolate Butte86Ada-11

[x] Means followed by the same lowercase letter within sane run are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within same run are not significantly different at $P < 0.05$.

[†] Means - combined across isolates and cultivars.

[z] Means within same run followed by same uppercase letter are not significantly different at $P < 0.05$.

Appendix 21 continued. 15-acetyldeoxynivalenol (15-ADON, µg g^{-1}) levels detected 7, 14, 21 and 28 dai in Alsen, 2375 and Wheaton inoculated with two *F. graminearum* isolates (Butte86Ada-11 and 81-2) for control plants and plants subjected to a six hour wetting treatments in runs 1 and 2.

	15-acetyldeoxynivalenol (15-ADON, µg g^{-1})							
	Sampling days after inoculation (dai)							
	RUN1							
	Control				Six hour wetting			
	7 dai	14 dai	21 dai	28 dai	7 dai	14 dai	21 dai	28 dai
	Alsen							
Butte86*	14.23 d-h[x]	10.38 f-k	8.93 g-m	7.70 j-n	4.15 qr	5.01 n-q	6.96 k-p	6.99 k-p
81-2	8.50 i-m	12.60 e-j	13.85 d-h	11.65 f-k	4.71 o-r	6.11 m-q	8.35 i-m	7.82 j-n
Avg.[w]	11.37 CD[y]	11.49 C	11.39 C	9.68 C-E	4.43 H	5.56 F-H	7.65 D-F	7.41 D-F
	2375							
Butte86*	9.78 f-l	14.41 d-f	11.50 e-j	17.46 c-e	4.38 p-r	4.68 p-r	4.63 o-r	7.59 j-o
81-2	26.09 a-c	28.59 ab	33.14 ab	36.21 a	6.28 m-q	8.49 i-m	8.59 h-m	12.29 d-i
Avg.[w]	17.94 B	21.50 AB	22.32 AB	26.83 A	5.33 GH	6.59 F-H	6.61 FG	9.94 C-E
	Wheaton							
Butte86*	4.49 qr	14.36 d-g	17.38 c-e	15.30 d-f	2.83 rs	6.34 m-q	7.83 j-n	8.14 i-m
81-2	5.02 o-q	22.24 bc	16.91 c-e	18.11 cd	1.79 s	9.04 g-m	6.86 k-p	6.29 l-q
Avg.[w]	4.75 H	18.30 B	17.15 B	16.71 B	2.31 I	7.69 D-F	7.34 D-F	7.22 EF
Mean[†]	11.35 T[z]	17.10 S	16.95 S	17.74 S	4.02 W	6.61 V	7.20 UV	8.19 TU
	RUN 2							
	Alsen							
Butte86*	2.12 s-v	3.47 m-s	4.10 j-q	4.77 i-p	1.51 uv	2.84 q-u	4.00 k-q	3.22o-s
81-2	2.37 r-v	3.28 o-t	5.08 g-o	4.59 i-p	1.38 v	1.87 t-v	3.11 p-t	3.55 l-r
Avg.[w]	2.25 H-J	3.38 FG	4.59 D-F	4.68 C-E	1.44 JK	2.36 HI	3.55 E-G	3.38 FG
	2375							
Butte86*	6.28 e-k	8.62 c-f	9.71 cd	10.66 cd	3.49 m-s	3.90 k-q	5.64 g-m	5.19 g-n
81-2	5.79 g-l	11.94 bc	19.01 a	16.97 ab	2.56 q-u	4.99 h-o	6.25 e-j	6.22 e-j
Avg.[w]	6.04 B-D	10.28 A	14.36 A	13.82 A	3.03 GH	4.44 D-F	5.94 B-D	5.70 B-D
	Wheaton							
Butte86*	2.92 p-t	7.48 d-h	9.29 c-e	7.87 d-g	1.37 vw	4.60 i-p	5.92 f-l	5.19 g-n
81-2	1.37 vw	5.81 g-m	7.52 d-h	7.34 d-i	0.52 w	1.88 t-v	4.57 i-p	5.34 g-n
Avg.[w]	2.15 IJ	6.64 BC	8.40 B	7.60 B	0.94 K	3.24 GH	5.24 CD	5.27 CD
Mean	3.48 V	6.77 T	9.12 S	8.70 S	1.80 W	3.35 V	4.91 U	4.78 U

[w] Average (sampling date means within each cultivar).

* Isolate Butte86Ada-11

[x] Means followed by the same lowercase letter within sane run are not significantly different at $P < 0.05$.

[y] Means followed by the same uppercase letter within same run are not significantly different at $P < 0.05$.

[†] Means - combined across isolates and cultivars.

[z] Means within same run followed by same uppercase letter are not significantly different at $P < 0.05$.

Appendix 21 continued. 3-acetyldeoxynivalenol (3-ADON, μg g^{-1}) levels detected 7, 14, 21 and 28 dai in Alsen, 2375 and Wheaton inoculated with two *F. graminearum* isolates (Butte86Ada-11 and 81-2) for control plants and plants subjected to a six hour wetting treatment in runs 1 and 2.

	3-acetyldeoxynivalenol (3-ADON, μg g^{-1})							
	Sampling days after inoculation (dai)							
	RUN 1							
	Control				Six hour wetting			
	7 dai	14 dai	21 dai	28 dai	7 dai	14 dai	21 dai	28 dai
	Alsen							
Butte86*	1.44 j-s[x]	1.44 j-s	1.71 g-p	1.85 f-m	1.22 o-v	1.15 p-v	1.68 g-q	1.48 j-s
81-2	0.90 u-w	1.27 m-v	1.82 g-o	2.00 f-l	1.01 s-w	0.93 t-w	1.68 g-q	2.06 e-k
Avg.[w]	1.17 I[y]	1.35 G-I	1.77 D-G	1.93 CD	1.12 I	1.04 IJ	1.68 D-H	1.77 D-G
	2375							
Butte86*	1.38 k-t	2.61 c-f	3.22 b-d	3.47 a-c	1.64 i-r	1.13 q-v	1.58 h-r	1.75 g-p
81-2	2.42 d-h	2.89 c-e	4.63 a	4.16 ab	1.16 q-v	1.16 q-v	2.31 e-i	2.40 d-g
Avg.[w]	1.90 C-F	2.75 B	3.92 A	3.82 A	1.40 HI	1.15 I	1.95 CD	2.08 CD
	Wheaton							
Butte86*	0.91 t-w	2.29 e-i	2.24 e-i	2.04 e-k	0.58 w	2.07 e-k	1.56 i-r	1.82 g-o
81-2	1.27 m-v	2.38 d-g	2.07 e-j	1.98 f-l	0.86 u-w	1.68 g-p	1.18 n-v	1.08 r-v
Avg.[w]	1.09 I	2.34 BC	2.16 B-D	2.01 CD	0.72 J	1.87 C-E	1.37 F-I	1.45 E-I
Mean[†]	1.38 V[z]	2.15 T	2.62 S	2.58 S	1.08 W	1.35 V	1.66 U	1.77 U
	RUN 2							
	Alsen							
Butte86*	0.96 h-l	0.80 j-n	1.00 g-l	1.19 e-j	0.56 m-r	0.97 h-l	1.51 c-f	1.75 b-d
81-2	0.36 r-t	0.55 m-r	0.78 j-n	0.78 j-n	0.38 q-t	0.48 n-t	0.80 j-n	1.20 e-i
Avg.[w]	0.66 I-K	0.68 H-J	0.89 GH	0.98 FG	0.47 KL	0.72 HI	1.16 D-G	1.47 A-C
	2375							
Butte86*	0.75 k-o	0.87 i-m	0.94 i-l	1.10 f-k	0.65 l-q	0.49 n-t	0.72 l-p	0.51 n-s
81-2	0.68 l-p	0.90 i-l	1.53 c-f	1.58 b-e	0.42 p-t	0.46 o-t	0.64 l-q	0.68 l-p
Avg.[w]	0.71 HI	0.88 GH	1.23 C-F	1.34 C-E	0.53 i-L	0.47 J-L	0.68 H-J	0.59 I-K
	Wheaton							
Butte86*	0.50 n-s	1.40 d-g	1.92 bc	1.86 b-d	0.52 n-s	2.67 a	2.12 ab	1.40 d-g
81-2	0.25 st	0.73 k-o	1.49 c-f	1.77 b-d	0.23 t	0.77 k-o	1.53 c-f	1.39 d-g
Avg.[w]	0.38 L	1.07 E-G	1.70 AB	1.82 A	0.37 L	1.72 AB	1.82 A	1.39 B-D
Mean	0.58 V	0.88 U	1.28 ST	1.38 S	0.46 V	0.97 U	1.22 T	1.15 T

[w] Average (sampling date means within each cultivar).

* Isolate Butte86Ada-11

[x] Means followed by the same lowercase letter within sane run are not significantly different at $P < 0.05$.

[y] Sampling date means followed by the same uppercase letter within same run are not significantly different at $P < 0.05$.

[†] Means - combined across isolates and cultivars.

[z] Means within same run followed by same uppercase letter are not significantly different at $P < 0.05$.

Appendix 21 continued. Deoxynivalenol (DON, µg per 50 ml of water) detected in run-off water from Alsen, 2375 and Wheaton inoculated with isolates Butte86Ada-11 and 81-2 of *F. graminearum* at anthesis and subjected to a six hour wetting event at 7, 14, 21 or 28 dai. Water samples were collected 3 hours after the start of irrigation and immediately after the wetting treatment concluded.

	Deoxynivalenol (DON, µg per 50 ml of water)			
	Sampling days after inoculation (dai)			
Hours	7 dai	14 dai	21 dai	28 dai
	Alsen			
3	0.03 fg[x]	0.03 e-g	0.05 e-g	0.04 e-g
6	0.02 g	0.03 fg	0.05 e-g	0.07 d-f
Avg.[w]	0.02 FG[y]	0.03 E-G	0.05 EF	0.06 DE
	2375			
3	0.10 cd	0.13 bc	0.14 a-c	0.18 a
6	0.07 d-f	0.08 de	0.13 bc	0.15 ab
Avg.	0.09 CD	0.10 BC	0.13 B	0.16 A
	Wheaton			
3	0.02 g	0.04 e-g	0.13 bc	0.12 bc
6	0.02 g	0.07 d-f	0.13 a-c	0.14 a-c
Avg.	0.02 G	0.06 DE	0.13 AB	0.13 AB
Mean[†]	0.04 U[z]	0.06 T	0.10 S	0.12 S

[w] Average (sampling date means within each cultivar).
[x] Means followed by the same lowercase letter are not significantly different at $P < 0.05$.
[y] Means followed by the same uppercase letter are not significantly different at $P < 0.05$.
[†] Means - combined across cultivars and hours.
[z] Means within same row followed by same uppercase letter are not significantly different at $P < 0.05$.