

Type I host resistance and Trichothecene Accumulation in *Fusarium*-infected Wheat Heads

Pravin Gautam and Ruth Dill-Macky
Department of Plant Science, South Dakota State University,
Brookings, SD 57007, USA

Abstract: Problem statement: The objective of this study was to examine the influence of the type I host resistance and pathogen variation with respect to mycotoxin and aggressiveness, on infection, FHB disease development and mycotoxin accumulation in planta. **Approach:** Greenhouse experiments were conducted utilizing a spray inoculation method. Five single isolates of *F. graminearum* were tested. Three wheat cultivars were used; Alsen (moderately resistant), 2375 (moderately susceptible) and Wheaton (susceptible). At anthesis spikes were spray inoculated to run off with inoculum at the concentration of 10,000 conidia ml⁻¹. Inoculated whole spikes were sampled at different growth stages up to soft dough. Kernels of sampled spikes were dissected from the spike and analyzed for mycotoxins. **Results:** The highest FHB severity and mycotoxin accumulation was observed in the susceptible cultivar Wheaton. Though deoxynivalenol (DON) did not peak and decline in all experiments, when a peak in the DON content was present it was earlier in 2375 (early milk) than in either Alsen (early dough) or Wheaton (late milk). 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 disease severity and mycotoxins accumulation. The levels of 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and nivalenol were less than 4.5, 0.8 and 0.3% to that of DON in a given sample. **Conclusion:** DON levels appear to provide a more precise measure of mycotoxin contamination of grain. 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. Screening of cultivars likely requires both greenhouse and the field testing.

Key words: Fusarium Head Blight (FHB), deoxynivalenol, nivalenol, acetyldeoxynivalenol, type I resistance, *Fusarium graminearum*, mycotoxin contamination

INTRODUCTION

Fusarium Head Blight (FHB) in North America is primarily caused by *F. graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] (Bai *et al.*, 2001; Parry *et al.*, 1995; Schroeder and Christensen 1963). 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 in floret and spread to the rachis adjacent to it (Parry *et al.*, 1995; Wiese, 1987). Vascular occlusion is common in susceptible cultivars 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). Grain either does not form, or does not develop fully, in infected spikes. 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 (Dexter *et al.*, 1996; Wang *et al.*, 2005).

A range of mycotoxins, including deoxynivalenol (DON), 15 acetyldeoxynivalenol (15 ADON), 3 acetyldeoxynivalenol (3 ADON) and nivalenol (NIV) has been reported to be produced by *F. graminearum* in infected tissues (Nasri *et al.*, 2006). Mycotoxins are hazardous to human and animal health and over the past decade wheat has routinely tested positive for most of these mycotoxins several wheat growing regions (McMullen *et al.*, 1997). Though the role of DON in

Corresponding Author: Ruth Dill-Macky, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA

the aggressiveness of individual isolates is not fully understood, it appears that DON is not essential for pathogenesis (Dyer *et al.*, 2005; Proctor *et al.*, 1995). Absence of DON in *F. graminearum* conidia indicates that it is 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 accumulation continues 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 *et al.*, 1984). Some studies have indicated that DON begins to decline even earlier than six weeks after inoculation (Culler *et al.*, 2007).

Schroeder and Christensen (1963) described two components of physiological resistance to FHB. 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). Spray inoculation method is used in breeding programs to evaluate large host populations in field nurseries and in the greenhouse evaluations for type I resistance (Rudd *et al.*, 2001).

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 wheat (Mesterházy *et al.*, 2003; Miller *et al.*, 1985).

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

MATERIALS AND METHODS

Two series of greenhouse experiments were established in a randomized complete block design at the Saint Paul campus of the University of Minnesota in spring 2007 and fall 2008. 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, 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 which served as the susceptible check. Five single spore isolates (49-3, 81 2, Butte86Ada 11, B63A and B45A) of *F. graminearum* were used in addition to a mock-inoculated control.

Seven plants per pot were planted in a soil-less growing media in magnum square plastic pots and maintained in the greenhouse at 20-23°C. 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, Marysville OH). Plants were watered every 2-4 days as necessary.

Inoculum was prepared by culturing *Fusarium* isolates in mung bean agar (MBA) for seven days under fluorescent and UV lights (12 h: 12 h light: dark cycle) at room temperature (22- 24°C) as described by Gautam (2010). 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 -80°C until needed.

The treatments included five isolates of *F. graminearum* 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 CO₂-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 at mid anthesis, watery ripe, early milk, late milk, early dough and soft dough stages (GS 65, 71, 73/74, 77/78, 83 and 85/86 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 -20°C. 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 -20°C until prepared for mycotoxin analysis.

Samples were analyzed for mycotoxin following Mirocha (1998) with some modifications. Samples were separated into two categories; those weighing <2 g (set 1) and those weighing ≥2-5 g (set 2). The procedure for sample preparation for the two sets were same but with different volumes of chemicals.

Set 1/2 samples were extracted in 4 ml /20 ml acetonitrile: water (84:1 v:v) extraction solvent by shaking for 1/ 2 hours, respectively. 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 25 100 µl⁻¹ of TMS reagent (TMSI:TMCS, 100:1), shaken for 10 minutes followed by addition of 100 µl⁻¹ ml Mirex isooctane solution (4mg L⁻¹) and 200 µl⁻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, 3-ADON, 15-ADON and NIV in samples were determined based on the calibrated standards.

Data for FHB severity, DON and 3-ADON were natural log transformed to achieve homoscedasticity. Data for 15-ADON and NIV 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 as the runs were significantly different. 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).

RESULTS

Disease severity. Visual symptoms of FHB had developed by the end of anthesis (GS 68/69) in all cultivars examined though the FHB severities were recorded from watery ripe stage (GS 71). By GS 71, high FHB severities were observed in all three cultivars (Fig. 1).

Neither 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 early dough (GS 83) and 49-3 at soft dough (GS 85/86), all isolates resulted in 100% FHB severity at early dough (GS 83) and soft dough (GS 85/86) stages. 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 early dough (GS 83). Similarly, all isolates except for Butte86Ada-11 resulted in 100% FHB severity in Wheaton by early dough (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 (up to early milk) FHB severities were similar in all three cultivars. From late milk (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 early dough stage (GS 83) in 2375 and Wheaton.

Deoxynivalenol: DON was detected in the initial sampling at kernel watery ripe stage (GS 71). None of the isolates consistently resulted in the highest or the lowest DON levels in all treatment combinations (Fig. 2). 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 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 peaked at either late milk (GS 77/78) or early dough (GS 83) and then declined in all cultivar and isolate treatments with exceptions.

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).

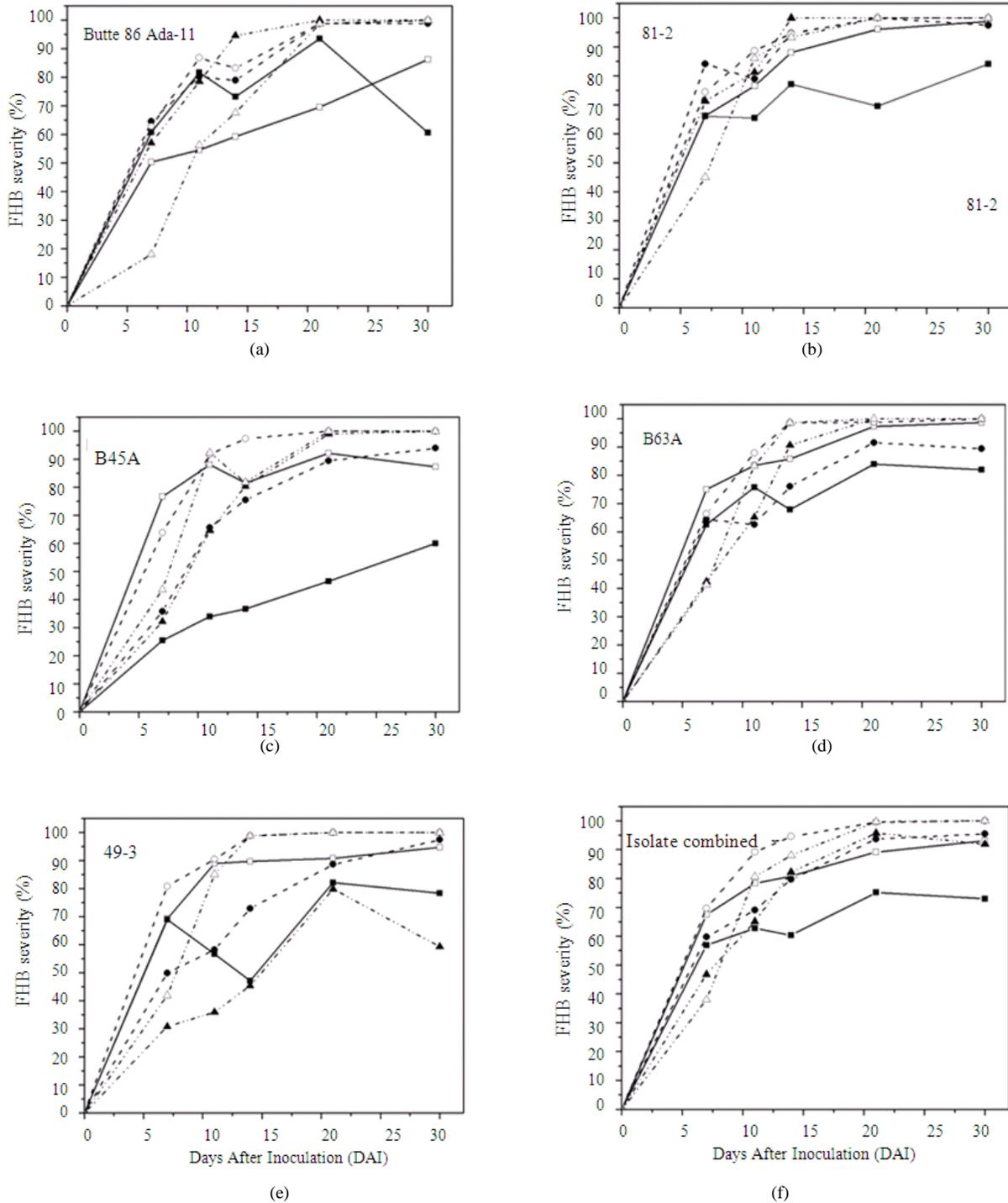


Fig. 1: Fusarium head blight (FHB) severity (%) observed 0 dai (mid anthesis), 7 dai (kernel watery ripe), 11 dai (early milk), 14 dai (late milk) and 21 dai (early dough) and 30 dai (soft dough) in spikes of Alsen (—), 2375 (---) and Wheaton (- · -) spray inoculated with five isolates (Butte86Ada-11, 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

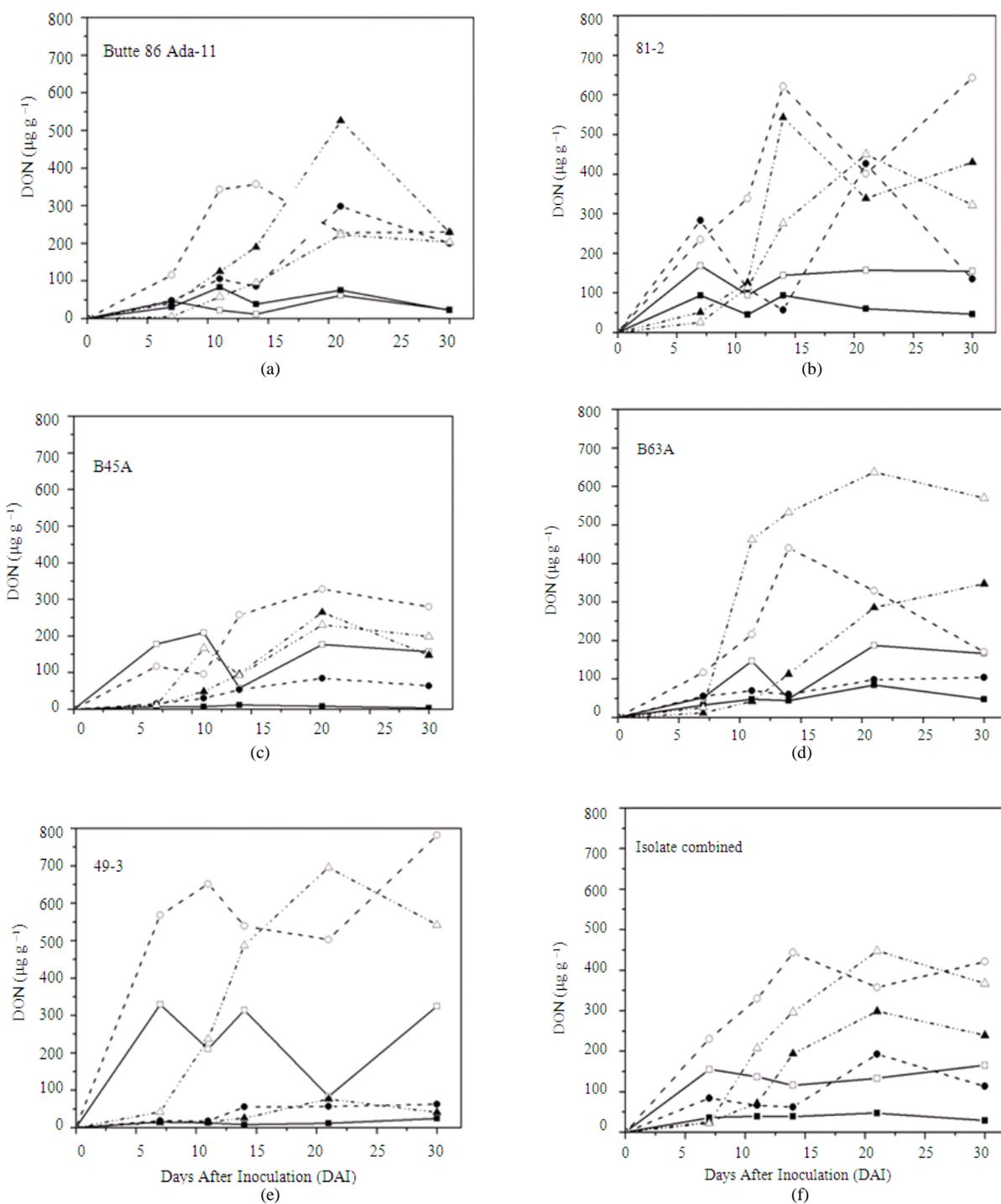


Fig. 2: Deoxynivalenol (DON, $\mu\text{g g}^{-1}$) detected 0 dai (mid anthesis), 7 dai (kernel watery ripe), 11 dai (early milk), 14 dai (late milk) and 21 dai (early dough) and 30 dai (soft dough) in kernels from spikes of Alsen (—), 2375 (---) and Wheaton (- · -) spray inoculated with five isolates (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

Table 1: 15-acetyldeoxynivalenol (15-ADON, $\mu\text{g g}^{-1}$) levels detected at GS 71, 73/74, 77/78, 83 and 85/86 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) | | | | | | | | | | |
| GS 71 | | GS 73/74 | | GS 77/78 | | GS 83 | | GS 85/86 | | |
| Alsen | | | | | | | | | | |
| Isolates | $\mu\text{g g}^{-1}$ | Rank | $\mu\text{g g}^{-1}$ | Rank | $\mu\text{g g}^{-1}$ | Rank | $\mu\text{g g}^{-1}$ | Rank | $\mu\text{g g}^{-1}$ | 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 ² 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 $P < 0.05$

Generally DON levels were higher in samplings after early milk stage than at kernel watery ripe (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.

15-Acetyldeoxynivalenol: 15-acetyldeoxynivalenol was detected by kernel watery ripe stage (GS 71) in all cultivars examined (Tables 1-2). 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 at early dough stage and afterwards.

When combined across isolates, Wheaton (run 1: 0-14 $\mu\text{g g}^{-1}$, run 2: 0-14 $\mu\text{g g}^{-1}$) and 2375 (run 1: 0-7 $\mu\text{g g}^{-1}$, run 2: 1-34 $\mu\text{g g}^{-1}$) had higher levels of 15-ADON compared to Alsen (run 1: 0-1 $\mu\text{g g}^{-1}$, run 2: 0-3 $\mu\text{g g}^{-1}$). 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 early dough (GS 73,74), but continued to increase until soft dough stage (GS 85/86) in run 2. In 2375 the levels of 15-ADON declined either after early dough (GS 83, run 1) or increased until soft dough stage (GS 85/86, run 2). In Wheaton, 15-ADON levels increased until early dough (GS 83) and declined thereafter.

Table 2: 15-acetyldeoxynivalenol (15-ADON, $\mu\text{g g}^{-1}$) levels detected at GS 71, 73/74, 77/78, 83 and 85/86 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) | | | | | | | | | | |
| | GS 71 | | GS 73/74 | | GS 77/78 | | GS 83 | | GS 85/86 | |
| | Alsen | | | | | | | | | |
| Isolates | $\mu\text{g g}^{-1}$ | 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 | |

* 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 $P < 0.05$

3-Acetyldeoxynivalenol: 3-acetyldeoxynivalenol was detected by kernel watery ripe (GS 71) except in Alsen in run 1 where 3 ADON was not detected in entire growth period (data not shown). 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-ADON.

The ranges of 3-ADON detected were 0-0.5 $\mu\text{g g}^{-1}$ in Alsen, 0-1 $\mu\text{g g}^{-1}$ (run 1) and 0-4 $\mu\text{g g}^{-1}$ (run 2) in 2375 and 0-3 $\mu\text{g g}^{-1}$ (run 1) and 0-4 $\mu\text{g g}^{-1}$ (run 2) in Wheaton. Whenever 3-ADON was present, its level increased significantly at late milk and afterwards compared to the levels at kernel watery ripe and early milk stages.

Nivalenol: Nivalenol was detected only in run 2 (data not shown). NIV was detected in all cultivars and was

detected by kernel watery ripe stage (GS 71) in all cultivars except in Wheaton, where NIV was not detected until early milk stage (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 $\mu\text{g g}^{-1}$ in Wheaton. In Alsen and 2375, the NIV levels were up to 0.16 and 0.44 $\mu\text{g g}^{-1}$, respectively. The levels of NIV detected were not more than 0.3% of the level of DON detected in a given sample.

DISCUSSION

In this study, the variation of an isolates ability to produce mycotoxin was evidenced by a differential accumulation of toxins in the spray-inoculated wheat

heads. Variations in the capacity of *F. graminearum* isolates to produce DON in sterilized rice culture have been reported by 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). 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.

In the current study all isolates examined produced 15-ADON, 3-ADON and NIV in addition to the DON. The concentrations of 15-ADON, 3-ADON and NIV were very low compared to the level of DON. This result agrees with Burlakoti *et al.* (2008) where they reported comparatively higher amounts of DON compared to 15-ADON and 3-ADON. 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* as suggested by Mirocha *et al.* (1989). 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 *F. 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. 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. 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. 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 (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. 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 our another study in the field (under preparation, unpublished) we found that 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 the current greenhouse experiments were higher by at least $100 \mu\text{g g}^{-1}$ than those in the field. The discrepancies in the mycotoxin levels, observed in the present greenhouse and the field study (unpublished), 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 in the samples at the end of anthesis 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 may also have led to the observed differences in mycotoxin levels between in this greenhouse study and the field condition. In the current study inoculated heads were sampled and all kernels in the spike were included in the mycotoxin analyses compared to normal field study procedure where sample size was large. Therefore due to the larger sample size, field data were less variable compared to our greenhouse study. Further, in field experiments, grain harvested for mycotoxin analysis included both infected and uninfected heads which were threshed mechanically, thus 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. Therefore characterization of isolates based solely on greenhouse experiments cannot provide accurate information on an isolate. It should be noted that the performance of moderately susceptible cultivars was highly variable when compared in the greenhouse and the field experiments (unpublished data). Therefore, screening of cultivars likely requires both greenhouse and the field testing.

CONSLUSION

DON levels appear to provide a more precise measure of mycotoxin contamination of grain. 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.

Screening of cultivars likely requires both greenhouse and the field testing.

ACKNOWLEDGEMENT

This material is based upon work supported by the US Department of Agriculture, under agreement No. 59-0790-4-096. This is a cooperative project with the US Wheat and Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of authors and do not necessarily reflect the view of the US Wheat and Barley Scab Initiative.

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