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Effects of Rain and Simulated Rain on Deoxynivalenol Levels in Grain and Chaff of Winter Wheat with Fusarium Head Blight

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Abstract

Wheat grain affected by Fusarium head blight (FHB) contains the mycotoxin deoxynivalenol (DON) that is harmful to humans and animals. Reducing the amount of DON in grain is the goal of management practices for FHB so it is important to understand the factors affecting DON in grain. Some studies on the effects of late-season moisture found increases in DON while others found decreases due to leaching. The objectives of this study were to determine effects of late-season rain and misting on DON concentration in wheat spike tissues and to quantify the amount of DON leached from spikes. Field experiments were conducted on susceptible and moderately resistant wheat cultivars affected by FHB utilizing spike holders to catch water leaching through groups of spikes, rain shelters to protect plots from rain and misting, and a rainfall simulator to apply simulated rain. A critical component of these experiments was to have groups of spikes with similar levels of DON at the beginning of experiments, and methods were developed to make groups as similar as possible and to statistically test for similarity such that dissimilar groups could be eliminated to improve the accuracy of results. Groups of spikes were either not treated or treated with various amounts of rain/simulated rain, and water, grain and chaff were analyzed for DON concentrations. DON was detected in all water samples, indicating that leaching of DON is common. Similar percentages of DON leached from most spike samples that received a particular rain treatment, indicating that the amount leached is proportional to the amount in the sample. Chaff and scabby grain had the highest concentrations of DON and the greatest reductions with rain treatments. Compared to grain from plots protected with rain shelters, grain from comparable plots that were exposed to rain and misting had lower concentrations of DON, indicating that late-season rain reduces DON in grain. A common practice of drying wet samples in a grain dryer was found to degrade a

portion of the DON. These results contribute to understanding the role of late-season moisture on DON concentrations in spike tissues and could be beneficial in identifying resistant cultivars to breeders.

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Introduction

Fusarium head blight (FHB), also known as head scab, is one of the most devastating diseases of wheat worldwide. Several *Fusarium* species cause FHB in wheat, but *Fusarium graminearum* Schwabe was primarily responsible for recent epidemics in the USA and elsewhere (Bai and Shaner 2004., McMullen et al., 1997). FHB is sporadic in the United States, but it can be severe when the weather is favorable. Since 1991, FHB outbreaks have been common and widespread, primarily in the eastern United States, affecting both yield and quality of wheat (McMullen et al., 1997). FHB can be recognized in the field as premature bleaching of infected spikelets and the production of orange spore-bearing sporodochia at the base of the glumes. Pinkish, fluffy fungal growth can also be seen during wet weather (Calpas et al., 2003).

FHB usually decreases yield, but associated mycotoxins are of more serious concern in the wheat market. Mycotoxins lower the value of the grain and cause difficulty in marketing, exporting and processing of grain (McMullen et al., 1997). *F. graminearum* produces trichothecene mycotoxins such as deoxynivalenol (DON) and nivalenol (NIV), which are toxic to human and animals. In cereals, DON is the most prevalent mycotoxin because the DON chemotypes of *F. graminearum* predominate worldwide, whereas NIV chemotypes are more geographically restricted and less frequent. Therefore, more attention is generally focused on DON than on NIV (Yoshida et al., 2010).

Wheat cultivars resistant to FHB are associated with lower levels of DON accumulation than susceptible cultivars (Mesterházy et al., 2003; Miller at al., 1985). The reduced level of DON in cultivars expressing resistance to FHB may be due to the host's resistance 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 degradation of DON (Miller et al., 1985).

DON is a virulence factor that is produced primarily during colonization of spike tissue and is essential for further colonization of wheat spikes after initial infection (Proctor at al., 1995, Bai et al., 2001). DON inhibits defense mechanisms of host plants and promotes the spread of *F. graminearum* in wheat spikes (Jansen et al., 2005). The production of DON decreases once *F. graminearum* fully colonizes the spike tissue or disease development stops due to plant maturity or unfavorable environmental conditions (Trail et al., 2011). Production of DON has been reported to be influenced by environmental factors, primarily moisture (Hope et al., 2005). Moisture in the form of rainfall or relative humidity, during and shortly after anthesis, has been linked to higher FHB incidence, severity, and DON accumulation (Rohácik and Hudec, 2005; Tuite et al., 1990).

In two consecutive years, Lemmens et al. (2004) evaluated ten wheat lines for FHB severity and DON concentration using four inoculation techniques and two misting regimes: no misting and misting for 26 days after inoculation (dai). The four inoculation techniques gave similar results. Averaged across wheat lines and inoculation techniques, misting for 26 dai resulted in significantly higher FHB severity but significantly lower DON. Susceptible lines with the highest FHB severities tended to have the greatest DON reductions in the misted treatment. The authors attributed this decrease in DON to a higher incidence of premature tip wilting resulting from rachis infection that cut off the flow of water and nutrients to the portion of the spike beyond the infection. This premature wilting causes symptom similar to those of infected florets, but the wilted florets are not infected and thus have no DON.

In two consecutive years, Culler et al. (2007) evaluated three spring wheat cultivars for FHB severity, percentage of visibly scabby kernels (VSK), and DON using low and high inoculum rates as well as standard (15-16 days) and extended (31-32 days) misting regimes. DON concentration in grain was measured at soft dough, hard dough, hard kernel, and harvest ripe stages. DON concentrations were affected by cultivar, inoculum rate, misting regime, and their interactions. When the effects of misting regime on DON concentration in grain were compared across the year × inoculum level × cultivar × growth stage interaction means (48 paired comparisons between standard and extended misting), 41 comparisons had numerically lower DON values with extended misting, but only nine of these differences were statistically significant ($P < 0.05$). The reduction in DON was most consistent and greatest in the high inoculum treatment during the year with the greatest rainfall between soft dough stage and harvest. Plots in this treatment and year had the highest DON concentrations at soft dough stage, and the DON concentrations for all three cultivars in both standard and extended misting treatments decreased from soft dough to harvest. Although the authors expected the extended misting to increase FHB severity and DON, they speculated that the observed decreases in DON with extended misting could have been due to disease development without DON accumulation or to leaching of DON from spikes in water. The fact that DON concentration decreased the most in the year with the most rainfall near the end of the season supports the conclusion that leaching may have been involved.

In two consecutive years, Cowger et al. (2009) evaluated eight winter wheat cultivars for FHB severity, percentage of *Fusarium*-damaged kernels (FDK), and DON concentration in grain under four misting treatments (0, 10, 20, and 30 days of misting after inoculation at flowering). The statistical analysis presented by the authors used year as a fixed effect, and year and all

possible interactions with year were highly significant ($P < 0.0002$). However, the analysis could have been simplified by considering year as a random effect. If year was a random effect, the effects of cultivar and mist treatment on DON concentrations in kernels at harvest were highly significant ($P < 0.0001$), but the cultivar x mist interaction was not significant ($P = 0.100$). There were trends for susceptible cultivars to have higher DON concentrations than moderately resistant cultivars under all mist treatments and for DON concentration of all cultivars to increase as the duration of the mist treatment increased. The authors concluded that their study was the first to show that increased duration of misting increases FHB severity, FDK, and DON. In one of two years, there was a significantly lower DON concentration across all cultivars with 30 days of misting compared to 20 days of misting. This decrease in DON with misting near the end of the season was similar to the results reported by Culler et al. (2007); however, the authors attributed this decrease in DON with 30 days of misting to lower FHB severities in these plots that may have been caused by confounding effects of *Soilborne wheat mosaic virus*.

In two consecutive years, Gautam and Dill-Macky (2012a) evaluated FHB severity, VSK, and DON concentration in grain using three spring wheat cultivars, five DON-producing isolates of *F. graminearum*, and four mist treatments (14, 21, 28, and 35 days of misting after inoculation at flowering). DON concentrations in harvested grain were significantly affected by cultivar, isolate, mist treatment, and all possible interactions, except in one year when the three-way interaction was barely non-significant ($P = 0.0558$). When the cultivar × isolate × mist treatment interaction means were compared across years, there was a trend for DON concentration to increase as mist duration increased from 14 to 28 day and then decrease from 28 days to 35 days. Of the 30 year × cultivar × isolate comparisons, 25 had a statistically significant decrease in DON concentrations between 28 and 35 days of misting. An increased duration of misting

increased VSK, but DON increased only from 14 to 28 days of misting and then decreased at 35 days of misting. The authors speculated that this decrease in DON between 28 and 35 days of misting was due to leaching of DON in water and that the pathogen had stopped producing DON as plants approached maturity. They also speculated that the DON could have been translocated to other parts of the spike (chaff or rachis), but these tissues were not evaluated for DON.

A greenhouse study was conducted by Gautam and Dill-Macky (2012b) to determine if a single misting event could leach DON from FHB-infected spikes of spring wheat cultivars. The experiment was done using three spring wheat cultivars, two DON-producing isolates of *F. graminearum*, and one 6-h mist treatment at four growth stages (7, 14, 21 and 28 days after flowering). At each growth stage, they assessed the FHB severity on four marked primary spikes from each of ten pots of each cultivar-isolate combination. Five pots of each cultivar-isolate combination were randomly assigned to the mist treatment in a dew chamber with a water bath at the bottom that was fitted with two sprinkler nozzles at the top. The other five pots were kept as non-misted controls. Each cultivar with two isolates and five replications (pots) was misted separately. A water sample was collected from the water bath at 3- and 6-h after the start of the mist treatment. The DON concentration was analyzed from a bulk of four whole spikes per pot and from the 3- and 6-h water samples. The experiment was done twice. FHB severities were higher in run 1 than in run 2, and cultivar '2375' had higher severities at all growth stages compared to the other two cultivars. DON concentrations in spikes were significantly affected by cultivar and isolate. When cultivar × isolate interaction means were compared across runs, the DON concentrations were significantly lower in spikes from plants that received 6-h of misting than in spikes from control plants except at 7 dai when DON concentrations in both misted and control plants were statistically similar. The amount of DON in run-off water was higher from

cultivar ‘2375’ than the other two cultivars. Based on their findings, authors concluded that free water such as misting can leach DON from spike tissues.

Cowger and Arellano (2013) evaluated the effects of four post-anthesis mist durations (0, 10, 20 and 30 days after anthesis (daa)) on FDK, kernel infection, and DON in grain, glumes and rachises of eight cultivars in 2006 and four cultivars in 2007 at six growth stages. The total amount of water applied as misting was approximately 10 mm spanning a 6-h period each day. Mist duration × growth stage and growth stage × cultivar interactions were significant ($P < 0.02$) in both years for DON concentration in grain. The DON concentration in grain was highest at 15 or 25 daa and decreased towards later stages. Unlike DON concentration in grain, the concentration of DON in rachis and glumes increased during later growth stages. Across cultivars and mist durations, rachises had highest DON concentrations followed by glumes and grain. The authors concluded that the reduction of DON is likely due to conversion of DON to glucosylated forms of DON which are not detected in assays for DON.

Schaafsma et al. (2002), proposed a model “DONcast” for predicting DON concentrations in mature grain. This model was based on weather variables such as daily rainfall, daily minimum and maximum air temperatures, and hourly relative humidity. DON was responsive to weather in three critical periods around heading. During the first critical period (4 to 7 days before heading) DON generally increased with the number of days with > 5 mm of rainfall and decreased with number of days with a low temperature < 10ºC. The DON concentration increased in the second critical period (3 to 6 days after flowering) with number of days receiving > 3 mm of rain, but decreased with the number of days exceeding 32ºC. At the third critical stage (7 to 10 days after anthesis), temperature had no effect on DON concentration and DON concentration increased with the number of days having > 3 mm of rainfall. Although the

DONcast model was able to predict up to 72% of the variation for DON concentration in wheat samples when a threshold of 1.0 mg kg^{-1} was taken into consideration and 83% of variability at 2.0 mg kg^{-1} (Schaafsma et al., 2007), it takes account of rainfall only from 7 days before to 10 days after anthesis. Based on the findings of Culler et al. (2007), Cowger et al. (2009) and Gautam and Dill-Mackey (2012a, 2012b), DON concentrations also are influenced by rainfall near maturity. Therefore, to increase the accuracy of models used to predict trichothecene toxin in wheat, inclusion of a moisture parameter beyond 10 days post-anthesis should be taken into consideration (Gautam and Dill-Macky, 2012a, 2012b).

There have been numerous attempts to use FDK level to predict DON concentrations in grain. In each of 6 years, Beyer et al. (2007) mixed healthy kernels with FDK to make samples containing 0%, 20%, 40%, 60%, 80% and 100% FDK and determined the DON concentration of each sample. Within each year, there was a high positive correlation between FDK and DON, but across years the slopes of the regression lines varied by a factor of 11.6. Likewise, when FDK data were used to predict DON concentration in soft red winter wheat cultivars and breeding lines across seven environments, DON was positively correlated with FDK in each environment (R^2 = 0.40 to 0.76) but the regression coefficients ranged from 0.16 to 0.76 (a 4.8-fold difference) across the environments (Milus, unpublished data). These differences in regression coefficient values among years may be at least partially attributed to late-season rainfall.

Lines from northern and southern soft winter wheat, hard red winter wheat and hard red spring wheat breeding programs are evaluated annually in inoculated and misted FHB nurseries across multiple locations to identify lines that are resistant and have low DON levels (U.S. Wheat and Barley Scab Initiative). Determining the effect of late-season rain events on DON concentration in grain of cultivars with a range of resistance to FHB will help to improve the

efficiency of selection for low DON among breeding lines, improve the DON prediction model and help to explain the different relationships between FDK and DON across years and environments.

The objectives of this study were to determine the effects of late-season rain and misting on DON concentration in wheat spike tissues and to quantify the amount of DON leached from spikes.

Materials and Methods

2012 Experiment. A field experiment was planted on 24 October 2011 as a randomized complete block with eight cultivars and four replications at the University Farm in Fayetteville, AR. Three susceptible cultivars (Coker 9835, 26R20 and 26R22) and five moderately resistant cultivars (Beretta, Ricochet, Oakes, Jamestown and ARGE 03-1145-9) were included. Individual plots were 1.5 m × 6.1 m, planted with 100 g of seed at the rate of 10.9 g m^{-2}, and bordered by a buffer plot of Jamestown on each side.

One DON-producing isolate (AR Fg-4) of *F. graminearum* was used as inoculum. Macroconidia were produced in mung bean broth as described by Desjardins et al. (1996). Briefly, 200 ml of mung bean broth in 500-ml flasks was inoculated with a single PDA plug of the isolate and incubated on a shaker (140 rpm) at room temperature. After 4 days, the spore concentrations were determined in all flasks using a hemacytometer, and the flasks with a concentration $\geq 10^5$ spores ml^{-1} were bulked in 1-liter plastic containers and frozen at -20ºC. For each inoculation of field plots, inoculum was thawed in a refrigerator at 5ºC, and the concentration of macroconidia was adjusted to 1×10^5 spores ml^{-1} using a hemacytometer.

At flowering stage, five cultivars with similar flowering times were selected with each cultivar replicated only three times because of poor stands in some plots. The selected plots were

inoculated three times on 6, 7, and 9 April 2012, when plants of the five cultivars were at mid-anthesis (Zadoks growth stage 65 (Zadoks et al., 1974)) to a few days after mid-anthesis. A CO_2-powered backpack sprayer (Bellspray Inc., Opelousas, LA) equipped with three pairs of flat fan nozzles angled 30 degrees downward from horizontal and oriented to spray forward and backward to maximize spike coverage was used to apply the inoculum. Each plot was sprayed with 250 ml of inoculum in 1% Tween 20. To promote infection, inoculations were performed in the early evening, and mist irrigation was applied for 2 min immediately after inoculation and for 2 min per hour between 00:00 and 08:00 h. After each inoculation, the residual inoculum was dilution plated on PDA to determine the concentration of viable spores.

At 2 weeks after flowering, each plot was divided into two subplots: - one covered from rain with a movable rain shelter made with a piece of acrylic greenhouse panel, while the other subplot was not covered. The covered subplot had an area of 1.2 m × 1.5 m and was covered during rains and misting events. Because of abnormally dry conditions, plots were misted several times to simulate rain events. The mist system with S31N12 nozzles (Isaacs & Associates, Inc., Walla Walla, WA) was on 6.1-m centers within rows and 4.6-m centers between rows. Four rain gauges were installed at plant height to determine the amount of water from rain and misting events. The plants in both subplots were supported with stakes and strings as needed to keep them from lodging.

At harvest time, fifty spikes were harvested randomly from each covered and not covered subplot immediately before and immediately after a misting event with 75 mm of water. The total amount of water applied in the form of rain or mist in uncovered subplots from the establishment of rain shelter to harvesting is summarized in Table 1. Each sample of 50 spikes was placed into a labeled cloth bag and was dried immediately in a grain dryer at 65ºC for 3

days. All 50 spikes were threshed by hand in bulk and separated into grain, chaff and rachis portions. The total number of kernels, number of scabby kernels, weight of scabby kernels and total weight of kernels (to nearest 0.01 g) were recorded. Chaff, rachis, and healthy and scabby grain from each sample were bulked separately, but only healthy and scabby grains were ground separately and sent for DON analysis at the University of Minnesota Mycotoxin Laboratory.

2013 Experiments. For experiment 1, spikes were collected from field plots at Newport, AR, on 28 May at hard dough stage (Zadoks growth stage 87). Three-hundred-sixty spikes with a similar size and severity were hand harvested from each of two susceptible cultivars (Coker 9835 and Cropland Genetics 554W) and two moderately resistant cultivars (Bess and Jamestown). Spikes were placed in labeled zip-lock bags and transported on ice in ice chests to reduce further fungal growth. Upon arrival in Fayetteville, AR, samples were placed in a cold room at 6ºC. On 29 May, spikes from each cultivar were weighed individually, grouped by weight, and allocated to 12 experimental units containing 20 spikes, in which all experimental units had similar weights. Standardizing these factors should result in similar levels of FDK and total DON among the experimental units that were randomly assigned to treatments. The 12 experimental units were assigned randomly to three treatments: no treatment, treatment 1 (natural rain + simulated rain) and treatment 2 (treatment 1 followed by additional simulated rain). The 20 spikes comprising an experimental unit were placed in a spike holder made from an inverted 1-liter plastic bottle (Smart Water, Whitestone, NY) with the bottom removed. A wire mesh of same diameter as the bottle was placed inside to hold the spikes vertically above water that collected in the spike holder. Spike holders were held in a plywood rack during treatments.

After the natural rain treatment, a simulated rain treatment was done to complement the desired level of water application as a first rain treatment. For simulated rain treatments, a

rainfall simulator was set up in the field as described by Dufault and Isard (2010). Briefly, a Fulljet 1/2HH-SS50WSQ nozzle (50 WSQ) (Spraying Systems Co. Wheaton, IL) was supported at a height of 3.6 m above the ground. The rainfall rate was adjusted to approximately 100 mm h^{-1} at 8 kPa. Water collected in spike holders was measured and a 10-ml aliquot was saved in a labeled 20-ml vial for DON analysis immediately after treatment 1 and treatment 2. Spikes from each spike holder were placed in labeled envelopes. Immediately after the experiment, all spike samples were dried in a grain dryer at 65ºC for 3 days. Water samples were frozen at -80ºC within 3 h of collection and were freeze dried in a lyophilizer (Labconco Corporation, Kansas City, MO) at -45ºC and 10^{-3} MBAR vacuum for 48 h.

All twenty spikes from each spike holder were threshed by hand in bulk and separated into grain and chaff (chaff + rachis). The total number of kernels, number of scabby kernels, weight of scabby kernels and total weight of grains (to nearest 0.01 g) were recorded. Chaff and grain from each sample were bulked and ground separately. All grain, chaff and water samples were sent for DON analysis to the University of Minnesota Mycotoxin Laboratory. Quantification of DON was done through gas chromatography-mass spectrometry (GC-MS) method.

For experiment 2, the cultivars were the same as those in experiment 1. Samples were harvested on 14 June at harvest-ripe stage (Zadoks growth stage 92), and the procedures were similar to experiment 1 except that treatment 1 was only natural rain, and grain was separated into scabby and healthy portions and analyzed separately for DON. For experiment 3, spikes of two susceptible cultivars (26R20 and 26R22) and two moderately resistant cultivars (Ricochet and Beretta) were collected from field plots at University Farm, Fayetteville, AR, on 3 June at soft dough stage (Zadoks growth stage 85). Procedures were as described above for experiment 1

except that treatment 1 was only natural rain. The amount of rain and simulated rain for each of the three experiments is summarized in Table 2.

For experiment 4, eight cultivars were planted on 23 Oct 2012 as described for the 2012 experiment, except that 120 g of seed was used to plant each plot at the rate of 13 g m^{-2}. At jointing stage (Zadoks growth stage 32), *F. graminearum*-infested corn kernels (isolate AR Fg-4) were scattered in all plots at the rate of 37 g m^{-2}. Two susceptible cultivars (26R20 and 26R22) and two moderately resistant cultivars (Beretta and Ricochet) were flowering at the same time and were selected for the experiment. Plots of the selected cultivars were spray-inoculated on 8 and 9 May, 2013 when plants were at mid-anthesis and again on 13 May as described previously. Each plot was divided into two subplots 2 wk after flowering; one not covered and other covered by a rain shelter during rain and misting as described for the 2012 experiment. A summary of the rain and misting events received by the uncovered subplots but not the covered subplots is given in Table 3.

Within each cultivar, 60 spikes of similar size and disease severity were tagged 37 days after flowering in both covered and uncovered subplots when spikes were still green for disease assessment. On 25 June (51 days after flowering), 60 tagged spikes were harvested by hand from covered and not covered subplots and divided into two similar experimental units with 20 spikes by weighing as described previously. The experimental units were randomly assigned to treatments (none or 50 mm simulated rain). The simulated rain procedures were as described for experiment 1 except that a smaller Fulljet 3/8HH-SS24WSQ (24WSQ) (Spraying System Co., Wheaton, IL) nozzle was used and the rainfall rate was adjusted to approximately 60 mm h^{-1} at 8 kPa. The non-treated spikes were not dried in the grain dryer as they were already dry.

Statistical Analysis. For the 2012 experiment, the experimental design was a split-split plot in which the whole plot was a randomized complete block consisting of four cultivars and four replications, rain shelter (covered or not covered) was the split plot factor, and simulated rain treatment (yes or no) was the split-split plot factor. To determine if the experimental units within sub-subplots sampled before or after simulated rain treatment were similar, the percentage FDK was analyzed. DON concentrations in healthy grain and scabby grain were analyzed to determine the effects of cultivar, rain shelter and simulated rain treatment. Data were analyzed using PROC MIXED of SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

For experiments 1, 2, and 3 in 2013, data for FDK and total DON in spikes were analyzed to determine if experimental units within a cultivar were similar. To determine the effects of rain/simulated rain and cultivar on DON, DON concentrations in grain and chaff and the percentage of DON leached from spikes were analyzed as completely randomized design with four replications. For experiment 4 in 2013, FDK and total DON in spikes were analyzed to test for similarity between the two experimental units from sub-subplots that were randomly assigned to the control (no rain) and 50-mm simulated rain treatments. DON concentrations in healthy grain, scabby grain, total grain, and chaff were analyzed as described for the 2012 experiment to determine the effects of rain shelter, simulated rain, and cultivar. To determine the relationship between the amount of rain/simulated rain and the percentage of DON leached from spikes, regression analysis was performed with Microsoft Excel 2007 version 12.0 using the mean percentage of DON leached from spikes of each cultivar in each rain/simulated rain treatment across the four experiments in 2013.

Results

2012 Experiment. Although the inoculum concentration ranged from 0.9 to 1.8×10^5 cfu ml^{-1}, the precipitation, relative humidity and temperature were not favorable for Fusarium head blight and little head blight developed during the season. The statistical test for similarity of experimental units between simulated rain treatments for FDK showed no significant difference and was considered to be similar (Table 4).

For DON concentration in scabby grain, statistical analysis showed significant ($P < 0.05$) effects of simulated rain treatment, rain shelter and cultivar × rain shelter interaction (Table 5). DON concentration was higher in 26R22 and Ricochet among the cultivars in covered subplots whereas Beretta and Oakes had higher DON concentrations in subplots that were not covered (data not shown). DON concentration in scabby grain was significantly lowered after 75 mm of simulated rain treatment and in subplots that were not covered during late-season rain and misting events (Table 6). For DON concentration in healthy grain, rain shelter × simulated rain treatment interaction was significant ($P < 0.05$). DON concentration in healthy grain was significantly lower after 75 mm of simulated rain treatment in covered subplots whereas it did not change in uncovered subplots after 75 mm of simulated rain treatment (data not shown). For DON concentration in total grain, both the main effects and its interactions were not significant (Table 5).

Similarity of Experimental Units in 2013 Experiments 1, 2 and 3. FDK percentage was significantly different ($P < 0.05$) across the 12 experimental units randomly assigned to the rain/simulated rain treatments for Bess in experiment 1 and for Coker 9835 and Cropland Genetics 554W in experiment 2 (Table 7). Likewise, the experimental units for Bess and Jamestown in experiment 1 and Coker 9835 and Cropland Genetics 554W in experiment 2 were

significantly different for total DON in spikes (Table 7). Therefore, these cultivars were eliminated from analyses in these experiments to ensure that treatment comparisons were among experimental units with similar total DON levels, but these cultivars were not eliminated from the comparison of percentage DON leached from spikes.

Effects of Rain/Simulated Rain Treatments on DON Concentration in Grain and Chaff. For DON concentration in grain, cultivar and treatment effects were significant ($P < 0.05$) only in experiment 3 (Table 8). The DON concentration decreased significantly after the 70-mm simulated rain treatment and was higher for 26R22 and Ricochet than for 26R20 and Beretta (Table 9). For the DON concentration in chaff, the main effects of cultivar and treatment were significant ($P < 0.0001$) in all three experiments (Table 8). There was a significant ($P < 0.02$) cultivar × treatment interaction in experiment 2. Although this interaction was statistically significant, DON concentrations for all cultivars decreased with successive rain/simulated rain treatment, and there was no change in the ranking of cultivars across the treatments. The DON concentration in chaff decreased significantly after both rain/simulated rain treatments in experiments 1 and 2 and after the 70-mm treatment in experiment 3 (Table 9). In experiment 2 in which grain was sorted into healthy and scabby portions, cultivar and treatment effects were not significant for healthy grain whereas both main effects were significant ($P < 0.002$) for scabby grain. DON concentration in scabby grain decreased significantly after 6 mm of rain, and Bess had a higher DON concentration than Jamestown (Table 10).

Percentage of DON Leached from Spikes after Rain/Simulated Rain Treatments. For the percentage of total DON leached from spikes, the effect of rain/simulated rain treatment was significant in all three experiments ($P < 0.0001$), whereas the cultivar effect was significant in experiments 3 and 4 ($P < 0 .001$) (data not shown). The percentage of DON leached from spikes

was proportional to the amount of water as rain/simulated rain and was similar across 25 of 28 cultivar comparisons (Table 11). The regression between the percentage of DON leached from spikes to amount of water applied as rain/simulated rain indicated that, on average 0.84% of the DON is leached from spikes for every centimeter of rain/simulated rain (Fig 1).

2013 Experiment 4. The inoculum ranged from 1.5 to 2.5 × 10^5 cfu ml^{-1}, and the weather was more favorable for FHB development than in 2012. There were enough diseased spike samples to conduct the experiment.

Similarity of Experimental Units in 2013 Experiments 4. Statistical analysis of FDK and total DON in experimental units of 20 spikes showed that the experimental units assigned to the simulated rain treatments were not significantly different for FDK but were significantly different ($P < 0.0001$) for total DON (Table 12). For three of the four cultivars, non-treated samples had significantly higher total DON than samples exposed to simulated rain in both covered and uncovered subplots (Table 13). This difference is most likely due to degradation of DON while drying spike samples in the grain dryer. Therefore data were re-analyzed by treatment to determine the effects of rain shelter. The design was a split plot in which the whole plot was a randomized complete block of four cultivars and four replications and the split-plot was rain shelter

DON Concentration in Healthy Grain, Scabby Grain, Overall Grain and Chaff in 2013 Experiment 4. Statistical analysis showed that rain shelter had a significant effect ($P < 0.0001$) on the concentration of DON in scabby grain, healthy grain, total grain, and chaff for both non-treated and simulated rain-treated samples (Table 14). Uncovered subplots received 286 mm more rain and misting than the covered subplots and had significantly lower DON concentrations

in scabby grain, healthy grain, total grain, and chaff than covered subplots in both non-treated and simulated rain treated samples (Table 15).

For the control treatment (no simulated rain), there was a significant ($P < 0.05$) cultivar × rain shelter interaction for DON concentration in total grain and chaff (Table 14). DON concentration in total grain was highest for 26R22 and lowest for Ricochet in both covered and not covered subplots. However, the ranking of 26R20 and Beretta changed between covered and not covered subplots even though these two cultivars were not significantly different for DON concentration (data not shown). For DON concentration in chaff, Ricochet had a significantly lower DON concentration than other three cultivars in covered subplot, whereas cultivars were not significantly different for DON concentration in subplots that were not covered (data not shown).

For the 50-mm simulated rain treatment, there was significant cultivar × rain shelter interaction for DON concentration in total grain (Table 14). Again, the ranking of 26R20 and Beretta changed, but not 26R22 and Ricochet, between covered and not covered subplots as described for the control treatment (data not shown). For DON concentration in chaff, DON concentrations in 26R20, 26R22 and Beretta were similar, whereas Ricochet had a significantly lower DON concentration.

Discussion

DON was leached from blighted wheat spikes of all experimental units that were subjected to a rain/simulated rain event in this study, indicating that leaching of DON from wheat spikes with FHB is likely a common phenomenon under field conditions. The percentage of DON leached from these spikes during a rain/simulated rain event was similar across 25 of 28 cultivar comparisons, indicating that the amount of DON leached is proportional to the amount in the spikes and that DON leaches at a similar rate from most cultivars. Across all experiments, the DON concentration in chaff averaged 2.7 times more than the DON concentration in grain, indicating that most of the DON was associated with the chaff rather than the grain. Higher association of DON with chaff was also shown by Cowger and Arellano (2013) where rachis and glumes had higher DON concentration than grain. Chaff had the greatest and most consistent reductions of DON following rain/simulated rain events and between covered and not covered subplots in experiment 4, indicating that most of the leached DON came from the chaff rather than the grain.

When healthy grain was analyzed separately from scabby grain, the concentration of DON in scabby grain decreased significantly after most rain/simulated rain treatments and between covered and not covered subplots in both years, whereas DON concentration in healthy grain decreased significantly only between covered and not covered subplots in 2013. Significant reduction of DON in total grain only occurred after the 70-mm rain/simulated rain event in experiment 3 and in experiment 4 between covered and not covered subplots that differed by 286 mm of cumulative rain and misting. The consistent loss of DON from chaff and scabby grain indicates that the effect of rain/simulated rain is greater on the spike tissues with more DON concentration. The effect of leaching on DON concentration in total grain depends on the

percentage of FDK. Little DON will be leached at low percentage of FDK, whereas much DON will be leached at high percentage of FDK.

In addition to loss of DON from spikes due to leaching, results from this study indicate that some DON was also degraded, especially when wet spikes were dried at elevated temperature. When the percentage of DON that leached from blighted spikes of all cultivars in all relevant experiments was plotted against the amount of rain/simulated rain that leached trough the spikes (Fig. 1), the Y-intercept value for the regression line was 5.6, indicating that 5.6% of the DON was leached with no water. A more likely explanation is that some of the DON in the treated spikes degraded while the spikes were being dried in a grain dryer at 65ºC. The percentage of DON leached from spikes was calculated as [µg DON in water /µg DON in spikes + µg DON in water] × 100. If the measured amount of DON in spikes was less than the actual amount in the spikes immediately after the rain/simulated rain event (i.e. some of the DON degraded before it could be measured), then the percentage of DON leached from the spikes would be calculated to be higher than it should have been, and this appears to be the case for the data presented in Fig. 1. This degradation of DON during the drying of wet spikes at elevated temperature is supported by the cultivar comparisons in experiment 4, in which experimental units exposed to 50 mm of simulated rain and then dried at 65ºC for 3 days had significantly lower values for total DON compared to otherwise similar experimental units that were not exposed to simulated rain and not dried in the grain dryer (Table 13). Degradation of DON in dry and moist barley kernels after 3 days at 80ºC was shown by Abramson et al. (2005). The DON concentration in moistened barley declined 32% and 15% after 3 days in experiment 1 and 2 respectively and in dry barley the DON concentration declined 27% and 8% after 3 days in experimental 1 and 2, respectively.

Because DON concentration in harvested grain is an important criterion for evaluating the FHB resistance of wheat cultivars and breeding lines, the procedure to conduct these evaluations should be designed to obtain the most accurate results. Cultivars and breeding lines are commonly evaluated for FHB resistance in inoculated and misted field nurseries, but the timing, amount of misting, and duration of wet spikes varies considerably among field nurseries in different breeding programs and years. Based on the findings of this study, stopping mist treatments after kernel hard stage, after which DON production in grain decrease considerably, would be preferable to continuing mist treatments beyond this time because later misting increases the probability of leaching and degradation of DON. The finding of this study showed that similar percentages of DON were leached from most cultivars such that cultivars with the most DON in grain will lose the most DON due to leaching. With this scenario, two cultivars with large differences in DON at the time DON production ceases will have progressively similar differences with successive leaching events. Because DON appears to be more susceptible to degradation under wet than dry conditions, late-season misting also increases the probability for DON degradation in grain. Furthermore, the rates of degradation among cultivars in this study appeared to differ such that the ranking of cultivars for DON concentration in grain could be affected. For experiments requiring the most accurate estimates of DON levels in water or wet wheat spikes, the water and spikes should be frozen at -80ºC as quickly as possible and then lyophilized for DON analysis.

To minimize possible distortions of DON concentration in grain among entries in FHB screening nurseries, it would be preferable to harvest naturally dried grain as soon as possible after entries first reach harvest dryness. Delaying harvest increases the probability of leaching and degradation of DON in the field. Harvesting at high moisture and drying at elevated

temperature increases the probability for DON degradation. Because all entries in screening nurseries are not likely to cease DON production and reach harvest dryness at the same time, and not all nurseries are likely to be harvested under naturally dried conditions at the optimal time, leaching and degradation of DON likely will continue to affect screening results. However, knowing which factors are most likely to affect DON concentration in grain will allow these factors to be minimized or at least made as similar as possible across all entries in a test or within replications of a test to improve the accuracy of the results. Evaluations performed as well as possible across multiple environments should be used to obtain the most reliable estimates of DON concentration in grain.

Based on the findings of this study, grain from commercial wheat fields affected by FHB likely loses some DON between the time when DON production ceases and harvest. Heavy rain events during this time are likely to leach significant amount of DON from grain, especially from the scabby grain that has most of the DON. The effect of leaching on DON concentration in harvested grain likely would depend on the proportion of scabby grain that is retained versus the proportion that is blown out of the combine at harvest. Higher proportions of retained scabby grain are likely to be associated with greatest effects of leaching but also higher DON concentrations. If all of the scabby grain could be blown out of the combine at harvest, the effect of leaching likely would be minimal, and DON concentration in the grain likely would be insignificant. Schenzel et al. (2012) detected DON in drainage water samples collected from FHB-infected wheat fields, which confirms that it is likely DON leaches in commercial fields.

The DONcast model proposed by Schaafsma et al. (2002) takes the rainfall consideration from 7 days before flowering to 10 days after flowering to predict the DON concentration in grain. However, the results of this study, consistent with the results of Gautam and Dill-Macky

(2012b) and Schenzel et al. (2012), show that DON is leached out during rain events. Thus to predict the DON concentration more accurately in wheat grain, the rainfall before harvesting should also be taken into account.

Literature Cited

1. Abramson, D., House, J. D., and Nyachoti, C. 2005. Reduction of deoxynivalenol in barley by treatment with aqueous sodium carbonate and heat. Mycopathologia160:297-301.

2. Bai, G. H., and Shaner, G. 2004. Management and resistance in wheat and barley to Fusarium head blight. Annu. Rev. Phytopathol. 42:135-161.

3. Bai, G. H., Desjardins, A. E., and Plattner, R. D. 2001. Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but do not cause disease spread in wheat spikes. Mycopathologia 153:91-98.

4. Beyer, M., Klix, M. B., and Verreet, J. A. 2007. Estimating mycotoxin contents of *Fusarium*-damaged winter wheat kernels. International Journal of Food Microbiology 142:370-374.

5. Calpas, J., Howard, R., Turkington, K., Clear, R., and Evans, I. 2003. Fusarium head blight of barley and wheat. Agri-fact. Revised. Agdex 110/632- 1.

6. Cowger, C., and Arellano, C. 2013. *Fusarium graminearum* infection and deoxynivalenol concentrations during development of wheat spikes. Phytopathology 103:460-471.

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

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

9. Desjardins, A. E., Proctor, R. H., Bai, G., McCormick, S. P., Shaner, G., Buechley, G., and Hohn, T. M. 1996. Reduced virulence of trichothecene-non producing mutants of *Gibberella zeae* in field tests. Mol. Plant-Microbe Interact. 9:775-781.

10. Dufault, N. S., and Isard, S. A. 2010. A portable rainfall simulator for evaluating the wet deposition of plant pathogens. Applied Engineering in Agriculture 26:71-78.

11. Gautam, P., and Dill-Macky, R. 2012a. Impact of moisture, host genetics and *Fusarium graminearum* isolates on Fusarium head blight development and trichothecene accumulation in spring wheat. Mycotoxin Res. 28:45-58.

12. Gautam, P., and Dill-Macky, R. 2012b. Free water can leach mycotoxins from *Fusarium*-infected wheat heads. Journal of Phytopathology, 160:484-490.

13. Hope, R., Aldred, D., and Magan, N. 2005. Comparison of environmental profiles for growth and deoxynivalenol production by *Fusarium culmorum* and *F. graminearum* on wheat grain. Lett. Appl Microbiol. 40:295-300.

14. Horevaj, P., Gale, L.R., and Milus, E. A. 2011. Resistance in winter wheat lines to initial infection and spread within spike by deoxynivalenol and nivalenol chemotypes of *Fusarium graminearum*. Plant Dis. 95:31-37.

15. Jansen, A., Wettstein, D., Schäfer, W., Kogel, K.–H., Felk, A., and Maier, F. J. 2005. Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase gene disrupted *Fusarium graminearum.* PNAS 102:16892-16897.

16. Lemmens, M., Buerstmayr, H., Krska, R., Schuhmacher, R., Grausgruber, H., and Ruckenbaur, P. 2004. The effect of inoculation treatment and long-term application of moisture on Fusarium head blight symptoms and deoxynivalenol contamination in wheat grain. Eur. J. Plant Pathol. 110:299-308.

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

18. McMullen, M., and Stack, R. W. 2011. A visual scale to estimate severity of Fusarium head blight in wheat. NDSU Extension Service (revised). PP 1095. http://www.ag.ndsu.edu/pubs/plantsci/smgrains/pp1095.pdf.

19. Mesterházy, Á., Bartók, T., and Lamper, C. 2003. Influence of wheat cultivar, species of *Fusarium*, and isolate aggressiveness on the efficacy of fungicides for control of Fusarium head blight. Plant Dis. 87:1107-1115.

20. Miller, J. D., and Young, J. C. 1985. Deoxynivalenol in an experimental *Fusarium graminearum* infection of wheat. Can. J. Plant Pathol. 7:132-134.

21. Proctor, R. H., Hohn, T. M., and McCormick, S. P. 1995. Reduced virulence of Gibberella zeae caused by disruption of a trichothecene toxin biosynthesis gene. Mol. Plant-Microbe Interact. 8:593-601.

22. Rohácik, T., and Hudec, K. 2005. Influence of agro-environmental factors on *Fusarium* infestation and population structure in wheat kernels. Ann. Agric. Environment. Med. 12:39-45.

23. Schaafsma, A. W., and Hooker, D. C. 2007. Climatic models to predict occurrence of *Fusarium* toxins in wheat and maize. International Journal of Food Microbiology 119:116-125.

24. Schaafsma, A. W., Hooker, D. C., and Tamburic-Ilincic, L. 2002. Using weather variables pre- and post-heading to predict deoxynivalenol content in wheat. Plant Dis. 86:611-619.

25. Schroeder, H. W., and Christensen, J. J. 1963. Factors affecting resistance of wheat to scab caused by *Gibberella zeae*. Phytopathology 53:831-838.

26. Schenzel, J., Forrer, H., Vogelgsang, S., Hungerbühler, K., and Bucheli, T. D. 2012. Mycotoxins in the environment: I. Production and emission from an agricultural test field. Environ. Sci. Technol. 46:13067-13075.

27. Trail, F., Wenner, N., Kuldau, G. A., and Hallen-Adams, H. E. 2011. Deoxynivalenol biosynthesis-related gene expression during wheat kernel colonization by *Fusarium graminearum*. Phytopathology 101:1091-1096.

28. Tuite, J., Shaner, G., and Everson, R. J. 1990. Wheat scab in soft red winter wheat in Indiana in 1986 and its relation to some quality measurements. Plant Dis. 74:959-963.

29. Yoshida, M., and Nakajima, T. 2010. Deoxynivalenol and nivalenol accumulation in wheat infected with *Fusarium graminearum* during grain development. Phytopathology 100:763-773.

30. Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415–421.

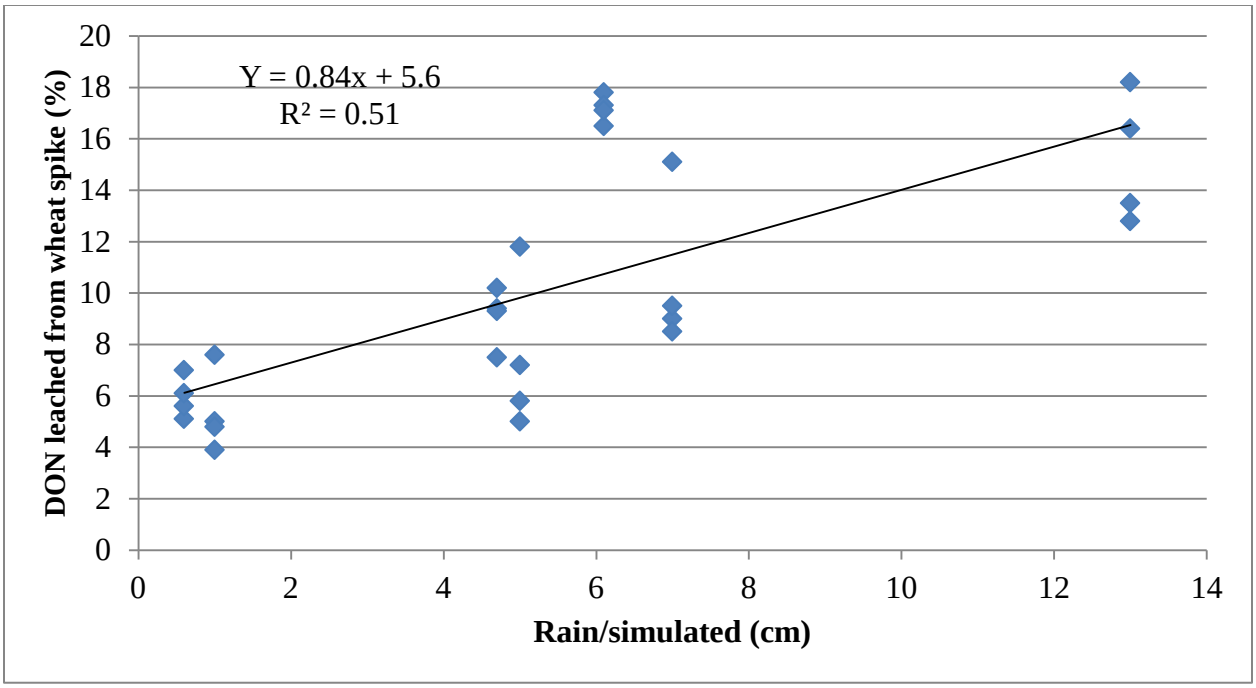

Fig 1**.** Relationship between the amount of rain/simulated rain (based on the amount of water collected in spike holders) and the percentage of total DON leached from experimental units of 20 wheat spikes across four experiments in 2013. Each point represents the mean of a cultivar in one rain/simulated rain treatment in an experiment.

Table 1. Date, time after flowering and amount of water from rain and misting events received by uncovered plots but not by the covered plots during the 2012 experiment.

Date	Days after flowering	Rain or mist[a]	Amount[b] (mm)
7 May	34	Rain	2
13 May	40	Mist	31
17 May	44	Mist	31
20 May	47	Mist	25
26 May	53	Mist	42
29 May	56	Rain	7
29 May	56	Mist	21
31 May	58	Rain	16
31 May	58	Mist	31
3 June	61	Mist	47
4 June	62	Rain	42
Total			295

[a]Rate of misting was 17 mm h^{-1}.
[b]Averaged across four rain gauges.

Table 2. Growth stage at which rain/simulated rain treatments were applied, treatment designation (Trt), description of the treatments, amount of rain/simulated rain applied and duration of wet spikes in four experiments during 2013.

Exp	Growth stages	Trt	Description of treatment	Water (mm)	Duration of wet spikes (h)
1	Hard dough	1	Natural rain (two days) +simulated rain treatment using 50WSQ nozzle for 20 min.	47	40
		2	Simulated rain using 50WSQ nozzle for 50 min	47+83	40
2	Harvest ripe	1	Natural rain (overnight)	6	16
		2	Simulated rain using 50WSQ nozzle for 35 min.	6+55	16
3	Soft dough	1	Natural rain (overnight)	10	18
		2	Simulated rain using 50WSQ nozzle for 40 min	10+60	18
4	Harvest ripe	1	Simulated rain using 24WSQ nozzle for 100 min.	50	4

Table 3. Date, time after flowering and amount of water from rain and misting events received by uncovered plots but not by the covered plots during experiment 4 in 2013.

Date	Days after flowering	Rain or Mist[a]	Amount[b] (mm)
21 May	16	Rain	36
29 May	24	Rain	55
1 June	26	Rain	12
5 June	30	Rain	11
11 June	37	Mist	50[c]
12 June	38	Mist	50[c]
13 June	39	Mist	50[c]
17 June	43	Rain	10
18 June	44	Rain	12
Total			286

[a]Rate of misting was 17 mm h^{-1}.
[b]Averaged across four rain gauges.
[c]Amount was measured after 3 consecutive days with a similar misting schedule and was divided equally among the 3 days.

Table 4. Test for similarity of percentage of *Fusarium*-damaged kernels between randomly harvested 50-spike samples in two simulated rain treatments in the 2012 experiment.

		Fusarium Damaged Kernels (%)	
Source	df	F-value	P > F
Cultivar	4	3.8	0.0128
Rain shelter	1	8.0	0.0084
Treatment	1	1.0	0.3188
Cultivar × Rain shelter	4	23.3	<0.0001
Cultivar × Treatment	4	0.3	0.8501
Rain shelter × Treatment	1	1.1	0.3147
Cultivar × Treatment × Rain shelter	4	0.4	0.6539

Table 5. Statistical test for the main effects of cultivar, rain shelter and simulated rain treatment and their interaction for deoxynivalenol (DON) concentration in scabby grain, healthy grain and total grain of wheat cultivars at 65 days after flowering in the 2012 experiment.

		Deoxynivalenol concentration					
		Scabby grain		Healthy grain		Total grain	
Source	df	F-value	P > F	F-value	P > F	F-value	Pr > F
Cultivar (cul)	4	1.7	0.2531	3.7	0.0548	2.0	0.1858
Rain shelter (R.S.)	1	8.4	0.0157	4.5	0.0609	0.3	0.6197
Treatment (Trt)	1	4.7	0.0425	5.8	0.0262	2.0	0.1753
Cult × R.S.	4	4.8	0.0203	1.3	0.3424	0.4	0.7751
Cul × Trt	4	0.6	0.6499	1.6	0.2131	0.1	0.9949
R. S. × Trt	1	1.4	0.2576	5.5	0.0299	0.6	0.4560
Cul × Trt × R. S.	4	1.2	0.3355	0.7	0.6120	0.3	0.9076

Table 6. Deoxynivalenol concentration in healthy grain, scabby grain and total grain of randomly harvested 50-spike samples at 65 days after flowering from covered and not covered subplots and before and after 75 mm of simulated rain treatment in the 2012 experiment.

		Deoxynivalenol concentration (µg g^{-1})[a]		
Rain shelter	Simulated rain	Scabby grain	Healthy grain	Total grain
Covered		76.4 a	0.7 a	1.7 a
Not covered		60.8 b	0.4 a	1.8 a
	None	77.1 a	0.6 a	1.9 a
	75 mm	60.2 b	0.4 a	1.5 a

[a]Value for rain shelter or simulated rain within a column followed by same letter are not significantly different according to LSD test at P = 0.05.

Table 7. Test for similarity of percentage *Fusarium*-damaged kernels and total deoxynivalenol among the 12 experimental units (20-spike samples) of each cultivar that were allocated randomly to four replications of three rain/simulated rain treatments (Trt) in each of three experiments.

		Fusarium-damaged kernels (%)				Total deoxynivalenol in 20-spike sample (µg)			
Experiment	Cultivar	P > F	Trt 0[a]	Trt 1	Trt 2	P > F	Trt 0	Trt 1	Trt 2
1	CG554W	0.6354	37.2 a[b]	37.9 a	38.7 a	0.4361	2591.3 a	2511.8 a	2311.2 a
	Coker 9835	0.2961	45.4 a	46.2 a	47.3 a	0.2151	1843.7 a	1815.5 a	1522.9 a
	Bess	0.0270	9.6 b	12.1 a	9.5 b	0.0384	722.1 b	1060.8 a	894.3 b
	Jamestown	0.1510	9.4 a	11.6 a	10.8 a	0.0143	616.5 a	665.7 a	476.7 b
2	CG554W	0.0027	49.9 b	52.2 a	49.5 b	0.0008	765.8 a	715.2 a	550.5 b
	Coker 9835	0.0320	69.4 ab	67.1 b	70.4 a	0.0231	650.0 a	464.1 b	530.2 ab
	Bess	0.1927	14.6 a	15.7 a	16.5 a	0.1544	542.8 a	460.9 a	446.6 a
	Jamestown	0.4754	17.5 a	18.6 a	18.7 a	0.3305	281.4 a	263.9 a	233.0 a
3	26R20	0.5438	31.7 a	32.4 a	31.1 a	0.6742	805.4 a	733.0 a	736.0 a
	26R22	0.2354	32.9 a	34.2 a	33.6 a	0.3453	1195.8 a	1226.0 a	1044.3 a
	Beretta	0.2383	31.3 a	32.7 a	30.8 a	0.1174	1061.3 a	1049.1 a	844.3 a
	Ricochet	0.3978	31.5 a	32.8 a	33.1a	0.0559	1104.8 a	1130.9 a	828.0 a

[a] Treatment 0 = no rain/simulated rain; treatment 1 = 47 mm of natural + simulated rain, 6 mm of natural rain and 10 mm of natural rain during experiments 1, 2 and 3, respectively; treatment 2 = 130 mm of simulated rain, 61 mm of simulated rain and 70 mm of simulated rain during experiments 1, 2 and 3, respectively.

[b]Values within a cultivar and variable for each experiment followed by the same letter are not significantly different according to a LSD test at P = 0.05.

Table 8. Statistical tests for the main effects and interaction of cultivar and rain/simulated rain treatment on deoxynivalenol (DON) concentration in grain and chaff in experiments 1, 2 and 3 in 2013.

			DON concentration (µg g^{-1})			
			Grain		Chaff	
Experiment	Source	df	F-value	P > F	F-value	P > F
1	Cultivar	1	0.0	0.8893	27.1	<0.0001
	Treatment	2	1.8	0.2168	26.5	<0.0001
	Cultivar × Treatment	2	0.6	0.5452	2.3	0.1268
2	Cultivar	1	0.2	0.6962	246.7	<0.0001
	Treatment	2	1.6	0.2217	27.6	<0.0001
	Cultivar × Treatment	2	0.7	0.5143	5.3	0.0152
3	Cultivar	3	9.1	0.0001	39.2	<0.0001
	Treatment	2	5.7	0.0073	49.8	<0.0001
	Cultivar × Treatment	6	0.8	0.5656	0.9	0.4937

Table 9. Deoxynivalenol concentration in grain and chaff for cultivars and three rain/simulated rain treatments in three experiments in 2013.

			DON concentration (µg g^{-1})[b]	
Experiment	Cultivar	Treatment[a]	Grain	Chaff
1	CG554W		45.5 a	129.2 a
	Coker 9835		44.7 a	101.1 b
	Mean		45.1	115.2
		None	39.1 a	139.7 a
		47 mm	52.6 a	114.4 b
		130 mm	43.6 a	91.5 c
2	Jamestown		8.4 a	15.7 b
	Bess		8.6 a	47.9 a
	Mean		8.5	31.8
		None	9.2 a	40.6 a
		6 mm	8.2 a	32.8 b
		61 mm	8.0 a	22.1 c
3	26R22		47.8 a	57.3 c
	26R20		31.0 b	61.8 b
	Ricochet		42.3 a	60.6 bc
	Beretta		31.3 b	75.8 a
	Mean		38.1	63.9
		None	42.1 a	69.9 a
		10 mm	40.6 a	66.9 a
		70 mm	31.5 b	54.8 b

[a]None = no rain/simulated rain treatment.

[b]Values for cultivars or treatments within a column and experiment followed by same letter are not significantly different according to a LSD test at P = 0.05.

Table 10. Deoxynivalenol concentration in healthy and scabby grain for cultivars and rain/simulated rain treatments in experiment 2.

		Deoxynivalenol concentration (µg g^{-1})[b]	
Cultivar	Treatment[a]	Healthy grain	Scabby grain
Bess		2.4 a	63.0 a
Jamestown		1.9 a	48.8 b
	None	2.1 a	65.6 a
	6 mm	2.1 a	54.4 b
	61 mm	2.3 a	47.7 b

[a]None = no rain/simulated rain treatment.

[b]Values for cultivars or treatments within a column followed by same letter are not significantly different according to a LSD test at P = 0.05.

Table 11. Percentage of total DON leached from 20-spike experimental units of cultivars during rain/simulated rain treatments in experiments 1, 2, 3 and 4 in 2013.

Experiment	Cultivar	Rain/simulated rain (mm)	Percentage of total DON leached in water
1	Bess	47	7.5 a[a]
	C-9835	47	9.4 a
	CG554W	47	9.3 a
	Jamestown	47	10.2 a
	Bess	130	12.8 a
	C-9835	130	16.4 a
	CG554W	130	18.2 a
	Jamestown	130	13.5 a
2	Bess	6	5.1 a
	C-9835	6	5.6 a
	CG554W	6	6.1 a
	Jamestown	6	7.0 a
	Bess	61	16.5 a
	C-9835	61	17.8 a
	CG554W	61	17.3 a
	Jamestown	61	17.1 a
3	26R20	10	7.6 a
	26R22	10	5.0 b
	Beretta	10	3.9 b
	Ricochet	10	4.8 b
	26R20	70	15.1 a
	26R22	70	9.2 b
	Beretta	70	9.3 b
	Ricochet	70	9.5 b
4[b]	Ricochet	50	11.8 a
	26R20	50	7.2 b
	26R22	50	5.8 b
	Beretta	50	5.0 b

[a]Values within a rain treatment followed by the same letter are not significantly different according to a LSD test at P = 0.05.

[b]Based on subplots that were not covered during rain and misting events similar to experimental units in experiments 1, 2 and 3.

Table 12. Test of similarity for percentage of *Fusarium*-damaged kernels (FDK) and total deoxynivalenol (DON) in 20-spike experimental units of two simulated rain treatments and two rain shelters in experiment 4.

		FDK		Total DON in spike	
Source	df	F-value	P > F	F-value	P > F
Cultivar (Cul)	3	17.9	0.0004	21.4	0.0002
Rain shelter (R.S.)	1	25.1	0.0003	301.8	<0.0001
Treatment (Trt)	1	1.0	0.3225	118.9	<0.0001
Cult × R.S.	3	1.6	0.2339	7.2	0.0051
Cul × Trt	3	0.4	0.7447	9.5	0.0003
R. S. × Trt	1	0.3	0.5923	4.8	0.0379
Cul× Trt × R. S.	3	0.4	0.7603	5.2	0.0066

Table 13. Percentage of *Fusarium*-damaged kernels (FDK), and total deoxynivalenol (DON) in 20-spike experimental units for two simulated rain treatments of four cultivars in covered and not covered subplots of experiment 4 in 2013, and difference in total DON between simulated rain treatments and percentage of DON estimated to have been lost from the simulated rain-treated experimental units during drying in a grain dryer.

Rain shelter	Cultivar	Simulated rain[a]	FDK (%)	Total DON (µg)[b]	Difference (None – 5 mm)	DON lost (%)
Covered	26R20	None	41.6a	1515.2 a	338	22.3
		50 mm	41.5 a	1176.9 b		
	26R22	None	44.8 a	1877.9 a	286	15.2
		50 mm	44.5 a	1591.5 b		
	Beretta	None	31.4 a	1755.8 a	586	33.4
		50 mm	31.5 a	1169.9 b		
	Ricochet	None	16.7 a	669.4 a	24	3.6
		50 mm	16.2 a	645.7 a		
Not covered	26R20	None	43.1 a	563.0 a	197	35.0
		50 mm	43.7 a	365.6 b		
	26R22	None	46.8 a	968.5 a	347	35.8
		50 mm	45.1 a	621.9 b		
	Beretta	None	36.0 a	789.4 a	204	25.8
		50 mm	34.9 a	585.9 b		
	Ricochet	None	18.7 a	277.6 a	82	29.5
		50 mm	18.3 a	195.3 a		

[a] None = no simulated rain and not dried in grain dryer, 50 mm = experimental units were treated with 50 mm of simulated rain and dried in grain dryer for 3 days at 65ºC.

[b]Values for simulated rain treatment within a column, cultivar and rain shelter followed by the same letter are not significantly different according to a LSD test at P = 0.05

Table 14. Statistical test for the main effects of cultivar and rain shelter and their interaction on deoxynivalenol (DON) concentration in scabby grain, healthy grain, total grain, and chaff of winter wheat cultivars with no simulated rain or a 50-mm simulated rain treatment at 51 days after flowering in experiment 4 in 2013.

A	No simulated rain		Deoxynivalenol concentration							
			Healthy grain		Scabby grain		Total grain		Chaff	
	Source	df	F-value	P > F	F-value	P > F	F-value	P > F	F-value	P > F
	Cultivar (Cul)	3	2.8	0.1003	2.9	0.0972	26.1	<0.0001	6.0	0.0158
	Rain shelter (R.S.)	1	33.4	<0.0001	59.9	<0.0001	158.7	<0.0001	208.3	<0.0001
	Cul × R.S.	3	2.1	0.1530	0.3	0.8030	8.0	0.0034	5.6	0.0125
B	50 mm simulated rain									
	Cultivar (Cul)	3	3.0	0.0886	2.8	0.1048	19.3	0.0003	5.4	0.0208
	Rain shelter (R.S.)	1	39.5	<0.0001	230.0	<0.0001	187.2	<0.0001	185.3	<0.0001
	Cul× R.S.	3	1.9	0.1824	1.3	0.3247	13.5	0.0004	1.9	0.1870

Table 15. Deoxynivalenol concentration in healthy grain, scabby grain, total grain and chaff from covered and not covered subplots of each simulated rain treatments in experiment 4 in 2013.

Simulated rain treatments	Rain shelter[a]	Deoxynivalenol concentration (µg g^{-1})[b]			
		Healthy grain	Scabby grain	Total grain	Chaff
None	Covered	5.9 a	168.5 a	54.9 a	142.7 a
	Not covered	2.2 b	100.2 b	35.1 b	40.4 b
50 mm	Covered	5.1 a	129.7 a	43.3 a	103.9 a
	Not covered	1.6 b	68.3 b	23.5 b	26.0 b

[a]Covered subplots were protected from rain and misting since 14 days after flowering where as not covered subplot were subjected to rain and misting totaling 286 mm of water during the same time.

[b]Values for covered and not covered subplots within a column and simulated rain treatment followed by the same letter are not significantly different according to a LSD test at P = 0.05.