Fusarium graminearum Infection and Deoxynivalenol Concentrations During Development of Wheat Spikes

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ABSTRACT

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Fusarium head blight (FHB) affects whole spikes of small grain plants, yet little is known about how FHB develops following infection, or about the concentration or progression of the mycotoxin deoxynivalenol (DON) in non-grain spike tissues. *Fusarium* mycotoxin levels in whole small-grain spikes are of concern to producers of whole-crop silage, as well as users of straw containing chaff for animal bedding or winter livestock rations. A 2-year field experiment was performed in Kinston, NC to reveal the time course of FHB development. Eight winter wheat cultivars with varying levels of FHB resistance were used in the 2006 experiment, and four of them were used in 2007. Plots were spray-inoculated with *Fusarium graminearum* macroconidia at mid-anthesis. Four durations of post-anthesis mist were applied: 0, 10, 20, or 30 days. Spike samples were collected and bulked by plot at 15, 25, 35, 45, 55, and 65 days after anthesis (daa); samples were separated into grain, glume, and rachis fractions. Increasing durations of post-anthesis moisture elevated grain

DON and reduced the effect of cultivar on DON, presumably by affecting the expression of resistance, in all spike tissues. *Fusarium*-damaged kernels increased from early kernel-hard to harvest-ripe in both years. Percent infected kernels increased from medium-milk to harvest-ripe. During grain-fill, DON concentrations declined in grain but increased in rachises and glumes, peaking at early kernel-hard, before declining. Higher mean and maximum DON levels were observed in rachises and glumes than in grain. Estimated whole-spike DON peaked at early kernel-hard. In a high-FHB year, whole-plant harvest for forage should be conducted as early as possible. Straw that may be consumed by livestock could contain significant amounts of DON in chaff, and DON can be minimized if straw is sourced from low-symptom crops. Cultivar FHB resistance ratings and disease data should be useful in predicting whole-spike DON levels. Overall, associations between grain DON levels in harvest-ripe and prior samples were stronger the later the prior samples were collected, suggesting limits to the possibility of predicting harvest-ripe grain DON from earlier levels.

Additional keywords: scab.

Fusarium head blight (FHB, or scab) is a damaging disease of wheat (*Triticum aestivum*) and other small grains worldwide. In the United States, the most important causal agent is *Gibberella zeae* (anamorph *Fusarium graminearum*) (22). FHB lowers grain yield and contaminates grain with mycotoxins such as deoxynivalenol (DON). In the United States, the Food and Drug Administration (FDA) has set 1 $\mu\text{g g}^{-1}$ as the recommended ceiling for DON in finished wheat products such as flour, bran, and germ. For feed to be consumed by animals, FDA advisory limits are 5 $\mu\text{g g}^{-1}$ for dairy cattle and swine (for swine, scabby grain must comprise $\leq 20\%$ of the diet) and 10 $\mu\text{g g}^{-1}$ for beef cattle and chickens (for chickens, scabby grain must comprise $\leq 50\%$ of the diet).

DON levels in mature wheat grain have been well studied. DON and *Fusarium*-damaged kernels (FDK) at harvest-ripeness are generally correlated with visual symptoms (31) and are elevated by increasing numbers of wet days following anthesis (6). However, there is relatively less information about how DON concentrations change during the course of grain fill.

In the few published reports on DON concentrations and grain infection prior to harvest, various patterns have been observed. In the following examples, disparate results were reported. In field experiments with one winter wheat cultivar in a warm dry year in Ottawa, where spikes were inoculated at anthesis with a conidial suspension, Miller and Young (24) found that DON and ergosterol concentrations in wheat spikes peaked 6 weeks after inoculation, then declined more than threefold by 9 weeks after inoculation. The authors hypothesized that the decline was due to the breakdown of DON by plant enzymes. It was likely on that basis that Teich (38) indicated that DON levels, detectable at ≈ 3 days after inoculation (dai), peak ≈ 6 weeks after infection and then begin to decline, reaching a constant level before harvest maturity. In Ontario, Scott et al. (35) sampled five soft winter wheat fields planted to unspecified cultivars with relatively high natural FHB incidences at the three time points of 13 to 20, 6 to 13, and 2 to 9 days before harvest. There was a significant drop in DON from the first to the second sampling, which the authors thought might be due to metabolism by host enzymes. Mean DON levels in this study were quite low (≈ 0.5 to $3.5 \mu\text{g g}^{-1}$ at the first date and ≈ 0.09 to $0.17 \mu\text{g g}^{-1}$ at harvest). In Braunschweig, Germany, a susceptible winter wheat cultivar was inoculated in the field at mid-anthesis, and mean DON levels increased steadily from 0 mg kg^{-1} at 9 dai to $\approx 9 \text{ mg kg}^{-1}$ at 44 to 51 dai, and then declined to slightly $< 8 \text{ mg kg}^{-1}$ by 58 dai (20). The researchers speculated that the initial increase in DON concentration was due to precipitation during the first 3 weeks after inoculation. In field plots in Kentucky, Argyris et al. (1) established an intense FHB epidemic using inoculation and irrigation, and found that percent infected

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*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains one supplemental table.

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kernels (PIK) began to rise at ≈ 18 days after anthesis (daa) and reached levels of 90 to 100% by 40 daa. In that epidemic, DON levels remained fairly constant from ≈ 28 to 50 daa although, in the last 10 days, DON declined somewhat in the most susceptible cultivar. Culler et al. (7) sampled spikes of three spring wheat cultivars with varying levels of FHB resistance that were inoculated at anthesis in misted field plots in each of 2 years. They found no clear pattern to grain DON concentrations from early dough, hard dough, kernel hard, and harvest-ripe stages.

Some evidence suggests that very wet conditions may result in low DON levels relative to visible blight symptoms. Lemmens (17) inoculated winter wheat lines from mid-anthesis to 16 daa at two locations in each of 2 years, with one block mist irrigated for 42 daa and the other not irrigated. Visible symptoms and yield reduction were more severe after mist irrigation than in non-irrigated plots but DON was lower; it was unclear whether mist irrigation had produced a smaller initial DON accumulation or had deepened the decline of DON. Some wheat genotypes reacted to mist irrigation with an increase in grain DON content, whereas others responded with a decrease. In their 2-year field experiment, Culler et al. (7) compared 15 to 16 days to 31 to 32 days of post-flowering mist, and found that the harvest-ripe grain DON concentration under extended mist irrigation was significantly lower.

DON concentrations in wheat spikes are affected by the timing of infection and colonization. DON appears to play a key role in the spread of *F. graminearum* from the wheat floret into the rachis (11). As *F. graminearum* spreads from an infected spikelet up and down the wheat spike, DON biosynthesis is maximal near the infection front, before symptom development, and diminishes in kernels once they have been colonized (10).

The dynamic nature of DON concentrations in small-grain spikes is likely also partly plant-mediated. Wheat may convert DON to DON-3-glucoside, which is only detected by tandem mass spectrometry (5). As a percentage of DON concentration, DON-3-glucoside concentration was measured at 4 to 12% in naturally contaminated wheat samples (5) and 14% in randomly selected hard red spring wheat samples (33). FHB-resistant wheat lines produce a high ratio of DON-3-*O*-glucoside to DON (18). The quantitative trait locus *Qfhs.ndsu-3BS* is closely associated with FHB resistance (40) and has been hypothesized to encode a DON-glucosyl-transferase or its expression regulator (18). Differences in glucosyl-transferases may contribute to the phenotype of more rapidly or profoundly decreasing DON levels during grain-fill.

Fusarium spp. infect not only the kernels but also the entire spike of the small-grain plant, and the implications for animal husbandry are, as yet, poorly understood. Wheat or barley straw is used as litter for turkey production (9), and the raising of pigs on straw is gaining in favor (26). Elevated mycotoxin levels in wheat straw used for swine can cause problems because swine consume part of their bedding (e.g., weaner pigs in straw-based systems obtain $\approx 12\%$ of their total feed intake from straw) (2). Cereal straw and chaff is used as a source of roughage (fiber) in dairy and beef cattle rations, and can provide $\leq 60\%$ of dry matter feed for wintering beef cows, with chaff comprising $\leq 21\%$ of the total material and having a higher feed value than stems (8,9,21).

It is known that DON levels are higher in chaff than in kernels of small grains. For example, Miller et al. (25) found that wheat chaff from inoculated field plots of both resistant and susceptible cultivars had ≈ 10 times the DON of wheat kernels. Rottinghaus et al. (32) found that DON concentrations in chaff from inoculated field plots were poorly correlated with cultivar resistance level, and were approximately four times higher than DON in straw and grain.

Wheat and other small grains are often a partial or primary source of ensiled forage in diets fed to dairy and beef cattle (14). For ensiling of whole-crop cereals, nutrition is maximized by har-

vesting plants at the milk to soft-dough stage, or ≈ 250 to 450 g kg^{-1} dry matter (3,4,12,36), although the practical goal of maximizing dry-matter yield may encourage slightly later harvest, at medium dough (37). Whole-crop wheat is sometimes harvested substantially later (700 to 800 g kg^{-1} dry matter) and ensiled with alkaline additives to increase digestibility (13). Because DON concentrations are apparently changing in developing grain, they may also be changing from anthesis onward throughout small-grain spikes, yet nothing is known about these temporal dynamics. Mycotoxins are known to survive the ensiling period and remain a risk to animal health (41).

Thus, DON concentrations appear to be dynamic during grain-fill but, to date, preharvest data are inconsistent and available only for grain. A better understanding of the factors that determine harvest-ripe DON levels may help with DON management. To understand FHB development and the progress of DON concentrations in different wheat spike fractions during the grain-fill period, we conducted a field experiment using multiple wheat cultivars with varying resistance levels, as well as different durations of post-anthesis moisture.

MATERIALS AND METHODS

A 2-year field experiment was conducted to assess the changes in FDK, infected kernels, and DON in three wheat spike fractions (rachis, glumes, and grain) over the course of grain development.

Field experiment location and design. The field experiment was performed in a misted nursery at the Cunningham Research and Extension Center in Kinston, NC. Experimental details were described by Cowger et al. (6); the same plots were used for that study and the presently described one. The same experiment (with different randomizations in the 2 years) was performed in each of two successive field seasons, 2005–06 and 2006–07, hereafter referred to as 2006 and 2007, respectively. In each year, the experiment was planted in a conventionally tilled field (full primary and secondary tillage with burying of crop residues) following corn or soybean.

In the experiment, there was a single section of the field each for misting durations of 0, 10, 20, and 30 daa. Due to resource limitations, the durations of misting were present each with a single repetition. Each section consisted of three replicate blocks. Cultivars were randomly assigned to plots within each block, so that each cultivar was subjected to each duration of post-anthesis misting in three replicate plots. Plots were planted with a five-row plot drill, and measured 3.1 m in length and 1.2 m in width after trimming ends for evenness of length.

Cultivars for the experiment (Table 1) were chosen as previously described (6) from among regionally adapted lines with similar maturities and a range of putative resistance types (type I, or resistance to primary infection; type II, or resistance to subsequent colonization; or both) (23). For the moderately resistant (MR) cultivars, resistance types were postulated based on incidence, severity, and DON data from the 2000 to 2003 Uniform Southern Soft Red Winter Wheat FHB Screening Nurseries (27–30). VA01W-99 was an elite line with pedigree FFR525/93-52-55 (Massey*3/Balkan//Saluda) that had shown a high level of FHB resistance in field experiments (J. Chen and C. Griffey). In the second year, only four of the cultivars used in the first year were included due to effects of a spring freeze on the earliest-maturing cultivars.

Inoculation and misting. Four isolates of *F. graminearum* with proven pathogenicity to wheat were selected to produce inoculum for the field experiment, and were increased individually in aerated 20-liter carboys of Mung bean tea. Mung beans were steeped in boiled, distilled H_2O at a rate of 40 g liter^{-1} for 15 min. The tea was filtered through cheesecloth, autoclaved, and inoculated with macroconidia to provide starter cultures. Carboys containing 12 liters of tea were spiked with 500 ml each of the starter

cultures, then aerated with sterile air for ≈ 1 week before centrifugation at 4,000 rpm for 3 min.

A suspension of 10^5 macroconidia ml^{-1} was used to inoculate field plots. The suspension was composed of equal proportions of spores of the four isolates, and plots were inoculated at mid-anthesis using a backpack sprayer. Mid-anthesis was considered to be the day on which 50% of the heads of a given cultivar had extruded anthers; thus, cultivars were inoculated on different dates within a season. The dates of inoculation were 13 to 19 April 2006 and 23 to 25 April 2007.

Roberts 435/436 mist nozzles (Mid-Atlantic Irrigation, Farmville, VA) with 1.4-mm-diameter orifices were mounted on risers at a height of 0.91 m above the ground and used to provide post-anthesis misting. Individual lateral irrigation lines were activated or deactivated to furnish the different durations of post-anthesis mist. Misting occurred for 2 min during each 20-min period for 3 h each in the morning and afternoon (8:00 to 11:00 a.m. and 2:00 to 5:00 p.m.), for a total of 36 min per day, using a programmable timer. Actual flow delivered was $0.275 \text{ mm min}^{-1}$, taking sprinkler overlap into account. Post-anthesis mist periods (0, 10, 20, or 30 daa) started during the inoculation period (17 April 2006 and 23 April 2007).

Buffer plots could not be planted between all treatments due to limited misted nursery space. However, buffer strips of the MR 'NC-Neuse' were planted to avoid interference of irrigation treatments, and borders of NC-Neuse were planted around the experiment.

Disease assessment and sample collection. The assessment of disease severity was conducted on each cultivar 20 days after it had been inoculated. Forty heads were chosen blindly in each plot, avoiding outside rows and terminal 0.3-m portions of plots. Severity was assessed as the percentage of diseased spikelets in the 40 heads (diseased spikelets/total spikelets $\times 100$).

To assay kernel symptoms and DON, 30 spikes were blindly harvested from each plot and bulked by plot on each of six dates ≈ 10 days apart, from milk stage to ≈ 20 days after harvest-ripeness. The collections were made 15, 25, 35, 45, 55, and 65 daa, with the first collection from all plots on a single day 15 days after the mid-point of the 6-day interval between the earliest and latest inoculation dates in 2006, the second collection 10 days later, and so on. In 2007, there was a 2-day interval between the earliest and latest inoculation dates; samples were collected at

≈ 10 -day intervals starting 15 days from the mid-point of that 2-day interval. The outer edges and 0.5 m at the ends of each plot were avoided to minimize edge effects. From each plot, spike samples were manually separated into grain, glume, and rachis fractions. In 2006, the grain fraction was weighed; in 2007, all fractions were weighed.

To determine the percentage of FDK, 100 kernels were selected arbitrarily from the sample for each plot and visually inspected for gray-white or pink discoloration and mycelial growth. Grain was assayed for DON concentration by enzyme-linked immunosorbent assay using EZ-Tox DON Kits (Diagnostix, Mississauga, ON, Canada), with detection limits of 0.5 to $5 \mu\text{g g}^{-1}$; for results exceeding $5 \mu\text{g g}^{-1}$, samples were diluted and reanalyzed appropriately.

The PIK in the 2006 and 2007 samples was determined by surface sterilizing 25 arbitrarily chosen kernels per sample for 2 min in 5% bleach, and plating the kernels on Komada's selective medium (15). Kernels were incubated 7 days in a growth chamber at 22°C on a 12-h light-and-dark cycle. Identification of infected kernels was based on fluffy peach-white mycelial growth and characteristic discoloration of the medium beneath the kernels. Spore morphology was determined microscopically where necessary to confirm infection by *F. graminearum*.

Statistical analysis. In each year, the experiment followed a nested or hierarchical design, with duration of post-anthesis moisture (mist duration) as a main-unit (single-repetition) factor; subunits (blocks) nested within each mist-duration main-unit, three repetitions for each; and cultivar as a sub-subunit factor, with all cultivars represented within each block. Sample date was a repeated-measures fixed-effect factor, with a number of samples taken from each plot. Mist duration, cultivar, and sample were crossed fixed factors. Data were analyzed following a linear mixed model with mist duration, cultivar, sample dates, and their interactions as fixed effects, and block nested within mist duration and interactions involving block as random effects. Sample dates were considered repeated measures and their variability was modeled as a heterogeneous compound symmetry, with the subject identified by the combination mist duration–block–cultivar, where six repeated measures were taken at 10-day intervals. This covariance structure estimated a separate residual variance for each sample date and common compound symmetry coefficient.

TABLE 1. Disease severity in soft red winter wheat cultivars inoculated with *Fusarium graminearum* macroconidia at mid-anthesis and subjected to varying durations of post-anthesis mist in a field nursery in Kinston, NC

Cultivar	Number ^x	Date ^y	Severity (%) at each mist duration (days after anthesis) ^z				
			0	10	20	30	Mean
2006							
Ernie	9600360	14 April	1.0	1.9	6.4	8.5	4.5 a
VA01W99	Experimental	13 April	2.4	8.2	14.3	10.3	8.8 ab
NC-Neuse	200400303	19 April	1.3	8.8	15.1	11.1	9.1 ab
V-Tribute	632689	15 April	2.9	9.0	14.0	18.0	11.0 b
C9474	9400188	19 April	1.3	16.1	17.7	16.1	12.8 b
USG 3650	W711	19 April	3.2	14.5	26.3	14.1	14.5 bc
C9184	200200135	17 April	11.0	27.1	34.9	41.8	28.7 c
USG 3592	200400110	15 April	5.7	23.2	59.8	50.4	34.8 c
Mean	...	...	3.6 a	13.6 ab	23.6 b	21.3 b	...
2007							
NC-Neuse	...	25 April	1.2 a	4.9 a	9.6 a	5.0 a	5.2
C9474	...	23 April	0.2 a	7.3 a	8.8 a	7.5 ab	6.0
USG 3650	...	23 April	0.9 a	15.5 b	40.0 b	6.6 ab	15.8
C9184	...	25 April	4.3 a	92.4 c	90.2 c	16.7 b	50.9
Mean	...	...	1.6	30.0	37.1	9.0	...

^x Plant introduction or Plant Variety Protection tagging number.

^y Inoculation date. Each cultivar was inoculated at 50% anthesis.

^z Severity was calculated as (diseased spikelets/total spikelets) $\times 100$, 40 spikes per plot. In 2006, the mist duration–cultivar interaction was not significant ($P = 0.214$). In 2007, the interaction was significant ($P < 0.0001$); simple-effects tests indicated that cultivar differences were significant at every mist duration but mist duration differences were only significant for USG 3650 and C9184. Within a year–column or year–row combination, means followed by the same letter are not significantly different ($P \leq 0.05$).

Due to the small number of years (two) and environmental differences between the years, each year was analyzed separately. A combined analysis, with year treated as a fixed-effect factor, showed a nonsignificant effect for mist duration when tested against the year–mist duration interaction, with P values for DON of 0.10 (grain), 0.10 (glumes), and 0.9 (rachis). Significant ($\alpha = 0.05$) interaction effects were year–sample date, year–mist duration–sample date, and mist duration–cultivar (year) for all three measures of DON (grain, glumes and rachis). Within each year, no test for the mist-duration main effect was considered.

To determine the effects of the independent experimental factors on grain weight, PIK, FDK, and DON, a general linear mixed model was fitted to the data using the MIXED procedure in SAS (version 9.1.3; SAS Institute, Cary, NC). Inspection of residual plots indicated the need for square-root transformation of FDK and DON data to attain normality and homogeneity of variances. The restricted maximum likelihood method was used to calculate variance components in the mixed model. Fixed effects were estimated by solving the mixed-model equations. The Kenward-Roger correction was used to reduce bias in the estimated covariance matrix of fixed and random effects and apply a Satterthwaite-type adjustment in the calculation of denominator degrees of freedom for the approximated F statistic used in testing the fixed effects. Least-squares means separation was performed using Tukey's test and the pdmix800.sas macro (34).

The statistical model was given by $y_{ijkl} = \mu + \alpha_i + b_{ij} + \gamma_k + (\alpha\gamma)_{ik} + g_{ijk} + \omega_l + (\alpha\omega)_{il} + (\gamma\omega)_{kl} + (\alpha\gamma\omega)_{ikl} + e_{ijkl}$, where α_i is the fixed effect for mist duration, $i = 1, 2, 3, 4$; b_{ij} is the random effect of the j th block within the i th level of mist duration, $j = 1, 2, 3$, $b_{ij} \sim N(0, \sigma_b^2)$; γ_k is the fixed effect of cultivar, $k = 1, 2, 3, \dots, K$ ($K = 8$ for 2006 and $K = 4$ for 2007); $(\alpha\gamma)_{ik}$ is the fixed interaction effect between mist duration and cultivar for the i th mist duration and k th cultivar; g_{ijk} is the random effect associated with the cultivar–block (mist duration) interaction, for the k th cultivar, j th block, and i th mist duration, $g_{ijk} \sim N(0, \sigma_g^2)$; ω_l is the fixed effect for sample date, $l = 1, 2, \dots, 6$; $(\alpha\omega)_{il}$ is the fixed interaction effect between mist duration and sample date, for the i th mist duration and l th sample date; $(\gamma\omega)_{kl}$ is the fixed interaction effect between cultivar and sample date, for the k th cultivar and l th sample date; $(\alpha\gamma\omega)_{ikl}$ is the fixed interaction effect between mist duration, cultivar, and sample date, for the i th mist duration, k th cultivar, and l th sample date; and e_{ijkl} is the random effect associated with the l th sample date for the observational unit identified by the k th cultivar, j th block, and i th mist duration, $e_{ijkl} \sim N(0, \sigma_e^2)$.

When necessary, an alternative model was used that took into account the heterogeneity of the random effects, and a separate residual variance was estimated for each sample date, as follows: $y_{ijkl} = \mu + \alpha_i + b_{ij} + \gamma_k + (\alpha\gamma)_{ik} + \omega_l + (\alpha\omega)_{il} + (\gamma\omega)_{kl} + (\alpha\gamma\omega)_{ikl} + e_{ijkl}$.

The corresponding assumption was that, for each observational unit, $e_{ijkl} \sim N(0, R)$, where R , the covariance matrix between the set of six sample date measurements, was represented by

$$R = \begin{bmatrix} \sigma_1^2 & & & & & \\ \sigma_1\sigma_2\rho & \sigma_2^2 & & & & \\ \sigma_3\sigma_1\rho & \sigma_3\sigma_2\rho & \sigma_3^2 & & & \\ \sigma_4\sigma_1\rho & \sigma_4\sigma_2\rho & \sigma_4\sigma_3\rho & \sigma_4^2 & & \\ \sigma_5\sigma_1\rho & \sigma_5\sigma_2\rho & \sigma_5\sigma_3\rho & \sigma_5\sigma_4\rho & \sigma_5^2 & \\ \sigma_6\sigma_1\rho & \sigma_6\sigma_2\rho & \sigma_6\sigma_3\rho & \sigma_6\sigma_4\rho & \sigma_6\sigma_5\rho & \sigma_6^2 \end{bmatrix}$$

with ρ representing the common correlation between any two measurements and σ_l^2 the variance associated with measurements taken at the l th sample date. The Kenward-Roger correction was used to reduce bias in the estimated covariance matrix of fixed and random effects, and a Satterthwaite-type adjustment was applied in the calculation of denominator degrees of freedom for

the approximated F statistic used in testing the fixed effects (19,39). Covariance parameter estimates for FDK, PIK, and DON are given in Supplemental Table 1.

An analysis was conducted to determine potential association of DON levels of each spike fraction (grain, glumes, and rachis) with DON levels in the other spike fractions and with index, FDK, and PIK, after accounting for the effect of experimental factors. This was done by calculating partial correlation coefficients using the multivariate analysis of variance procedure in SAS.

A covariance analysis was also performed to evaluate the relationships of DON levels at sample 5 (harvest-ripeness) with DON levels at each of the prior sampling points. The purpose was to explore whether determination of grain DON prior to harvest could be useful in forecasting DON levels at harvest. The analysis was performed on square-root-transformed DON in the 2006 data set only, because it was larger than the 2007 dataset and allowed for better estimation of the fixed and random effects. For this analysis, mist duration was considered a fixed-effect factor. The model fitted included a separate intercept and slope for each mist duration level. Cultivar was treated as a random-effect factor, because the tested cultivars possessed diverse resistance phenotypes and the question of interest was the relationship of harvest-ripe DON to DON at earlier stages, using a range of cultivar responses. The standard error for calculated slopes (regression coefficients) included the variability associated with cultivar effects on square-root-transformed DON. For each level of mist duration, the statistical model was $y_{ijk} = \mu + \alpha_i + b_{ij} + g_k + (\alpha g)_{ik} + \beta_{il} + x_{ijk}^1 + e_{ijk}$, where α_i was the fixed effect for mist duration, $i = 1, 2, 3, 4$; b_{ij} was the random effect of the j th block within the i th level of mist duration, $j = 1, 2, 3$, $b_{ij} \sim N(0, \sigma_b^2)$; g_k was the random effect of cultivar, $k = 1, 2, 3, \dots, 8$, $g_k \sim N(0, \sigma_g^2)$; $(\alpha g)_{ik}$ was the random effect of the interaction of mist duration and cultivar, $(\alpha g)_{ik} \sim N(0, \sigma_{\alpha g}^2)$; β_{il} was the regression coefficient for the covariable $X^1 = \sqrt{\text{DON}}$ at l th sample date, in the i th level of mist duration, $l = 1, 2, 3, 4, 5, 6$; and e_{ijk} represented the uncontrolled random effects associated with the observation identified by the i th mist duration, j th repetition, and k th cultivar, $e_{ijk} \sim N(0, \sigma_e^2)$.

The model estimated a separate intercept and slope for each mist duration level. The hypotheses tested were (i) H_0 : no mist duration effect (i.e., a common intercept was enough), H_a : $\alpha_i = 0$, $i = 1, \dots, 4$; and (ii) H_0 : slopes for all mist durations equaled 0, H_a : $b_{il} = 0$, $i = 1, \dots, 4$.

Total DON amounts in wheat spikes were estimated for the different sample dates as follows. Weights for the three spike fractions (glumes, grain, and rachises) were available for 2007 but not for 2006. The weights of the spike fractions were averaged across cultivars and misting treatments for each sample date, and the relative proportions of fraction weights on each date in 2007 were used for estimates in both years, on the assumption that the physiological process of spike development is relatively invariant. Total DON per spike was then estimated by weighting the concentration of DON in each spike fraction by the proportion of total spike weight contributed by that fraction, and summing the results.

RESULTS

The spring 2006 season was more conducive to FHB than the spring 2007 season, primarily due to more frequent rains in the 10- to 20-mm-per-day range in the post-anthesis period (Fig. 1). The period of anthesis and grain development was considerably drier in 2007 than in 2006: altogether, between mid-anthesis and the 55-daa sample date (harvest-ripeness), there was 166 mm of rain in 2006 and 94 mm of rain in 2007.

In the analysis of FDK, PIK, and DON, the three measures of grain disease, year interacted significantly with sample date in each case. Therefore, data are presented for the 2 years separately (Table 2).

Severity. Assessments of visual symptoms were conducted at approximately the point at which the 20-daa mist treatment finished (Table 1). Thus, the values for the 30-daa mist treatment were not expected to differ significantly from those for the 20-daa treatment.

Mist duration and cultivar did not interact significantly in 2006 ($P = 0.214$). Mist duration and cultivar each had a significant effect on severity ($P \leq 0.0004$). As expected, the two cultivars chosen for their susceptibility ('C9184' and 'USG 3592') had higher levels of severity, although they were not significantly different from 'USG 3650'.

In 2007, the interaction was significant ($P < 0.0001$); a simple-effects test indicated that cultivar differences were significant for each duration of mist applied but mist duration differences were only significant for USG 3650 and C9184. The low severities for USG 3650 and C9184 under the 30-daa mist treatment may have been due to patchy occurrence of *Wheat soilborne mosaic virus* (WSBMV).

Weight. In both years, mean 30-spike grain weights increased to a maximum in the third sample, collected at early kernel hard stage (Fig. 2A and B). After that, grain weights declined slightly, as was expected as the crop dried down. In both years, grain weights were significantly related to cultivar and mist duration ($P \leq 0.0003$).

Proportions of the weights of the various spike fractions, averaged across cultivars and misting treatments for 2007, are shown in Figure 3. The weight of the grain fraction relative to those of

glumes and spikes increased until the 35-daa sample, when it leveled out for the remainder of the season.

Kernel damage. Percent FDK was assessed for the 35- through 65-daa samples in both years (Fig. 2C and D), the kernels in the previous samples being too small to accurately detect differences by visual inspection. Sample date interacted significantly with mist duration in both years ($P \leq 0.007$). Misting had a strong elevating effect on FDK in 2006 ($P \leq 0.0001$ at all sample dates, simple-effects test) but less so in 2007 ($P \geq 0.071$ for all samples except 55 daa, $P = 0.014$, simple-effects test) (Fig. 2C and D). Post-anthesis mist tended to elevate FDK in the 45- and 55-daa samples, and lack of post-anthesis mist was associated with the lowest level of FDK at all sampling points.

Sample date interacted significantly with cultivar in 2006 but not in 2007 (Table 2). Cultivars differed significantly for FDK at all sample dates in both years ($P \leq 0.033$, simple-effects test).

In both years, sample was a significant source of variation, and percent FDK increased and peaked after the 35-daa sample (Table 2; Fig. 2C and D). Sample date was a larger source of variation in FDK in 2006 than in 2007, particularly due to the 30-daa mist treatment in 2006. FDK declined in the 65-daa sample, although more so in 2006 than in 2007.

PIK. The PIK was assayed for all six samples in 2006 and for all but the 15-daa sample in 2007 (Fig. 2E and F). Averaging across all independent variables, mean PIK was higher in 2006, the wetter year, than in 2007 (Fig. 2E and F), and it was higher at 55 daa (harvest-ripeness) in 2006 than in 2007 (63 versus 28%, $P < 0.05$).

Fig. 1. Mean temperature and rainfall during wheat flowering and grain development in 2006 and 2007 at a misted *Fusarium* head blight field nursery in Kinston, NC. Temperatures are means of the daily minima and maxima supplied by State Climate Office of North Carolina, NC CRONOS database. Inoculations with *Fusarium graminearum* at 10^5 macroconidia ml^{-1} were performed 14 to 19 April 2006 and 23 to 25 April 2007. Vertical lines numbered 1 to 6 indicate dates that wheat spike samples were collected (short dashes = 2006, long dashes = 2007).

Sample date interacted significantly with mist duration in both years (Table 2, $P \leq 0.034$). Misting increased PIK at all sample dates in 2006 ($P \leq 0.004$), and at 35, 55, and 65 daa in 2007 (simple-effects test, $P \leq 0.037$; $P \geq 0.243$ for 25 and 45 daa). That is, as with FDK, mist duration affected PIK in both years but the effect was greater in 2006 than in 2007 (Table 2; Fig. 2C and D).

Sample date interacted significantly with cultivar in both years ($P \leq 0.006$, Table 2). Cultivars differed significantly for PIK at all sample dates in 2006 ($P \leq 0.026$) and at all dates in 2007 except 35 daa ($P \leq 0.069$). Averaged across mist durations, the two most susceptible cultivars, USG 3592 and C9184, had the highest or second-highest PIK at all of the significantly differing sample dates prior to 65 daa in both years.

TABLE 2. Analysis of variance for grain sampled from soft red winter wheat cultivars at 10-day intervals from medium-milk to kernel-loosening after inoculation at mid-anthesis with *Fusarium graminearum* macroconidia in Kinston, NC, in 2006 and 2007^y

Year, variation ^z	<i>Fusarium</i> -damaged kernels			Percent infected kernels			Deoxynivalenol		
	df	F value	P value	df	F value	P value	df	F value	P value
2006									
Mist	3, n/a	...	...	3, n/a	...	...	3, n/a	...	...
Cult	7, 222	13.51	<0.0001	7, 53.3	13.48	<0.0001	7, 55.7	10.51	<0.0001
Mist × Cult	21, 222	1.77	0.0232	21, 52.2	1.75	0.0525	21, 55.3	1.64	0.0721
Date	3, 223	66.33	<0.0001	5, 139	222.57	<0.0001	5, 142	83.32	<0.0001
Mist × Date	9, 223	3.38	0.0007	15, 193	11.38	<0.0001	15, 195	16.12	<0.0001
Cult × Date	21, 222	3.29	<0.0001	35, 217	1.79	0.0064	35, 220	1.70	0.0121
Mist × Cult × Date	63, 222	1.48	0.0209	105, 202	0.75	0.9471	105, 205	1.11	0.2691
2007									
Mist	3, n/a	...	...	(3, n/a)	...	...	3, n/a	...	...
Cult	3, 118	80.24	<0.0001	3, 26.4	22.73	<0.0001	3, 26.4	44.18	<0.0001
Mist × Cult	9, 118	4.56	<0.0001	9, 26.3	1.50	0.2008	9, 26.3	1.47	0.2044
Date	3, 118	8.57	<0.0001	4, 67.3	99.60	<0.0001	4, 67.3	6.59	<0.0001
Mist × Date	9, 118	2.70	0.0067	12, 86.3	2.00	0.0338	12, 86.3	2.86	0.0009
Cult × Date	9, 118	1.47	0.1679	12, 86.3	6.08	<0.0001	12, 86.3	2.16	0.0126
Mist × Cult × Date	27, 118	2.46	0.0005	36, 90	1.06	0.3972	36, 90	1.10	0.3389

^y Eight cultivars in 2006 and four of those eight in 2007; data were square-root transformed. Within a year, there was no test for the mist-duration main effect, which was the main unit with a single repetition. df = degrees of freedom: numerator, denominator; n/a = not applicable. Covariance parameter estimates appear in Supplemental Table 1.

^z Year and fixed effect source of variation. Mist = mist duration, Cult = cultivar, and Date = sample date. Within a year, there was no test for the mist-duration main effect, which was the main unit with a single repetition.

Fig. 2. Least-squares means of grain weights and kernel assays across eight (2006) or four (2007) soft red winter wheat cultivars, sampled during grain-fill in a misted *Fusarium* head blight field nursery in Kinston, NC. Starting at mid-anthesis, four mist durations (0, 10, 20, or 30 days after anthesis [daa]) were applied. Samples of 30 spikes per plot were collected from three replicate plots per cultivar-mist-duration treatment at 15 daa (medium milk, Zadoks growth stage 75 [Z75]), 25 daa (hard dough, Z87), 35 daa (early kernel hard, Z90), 45 daa (kernel hard, Z91), 55 daa (harvest-ripe, Z92), or 65 daa (kernel loosening, Z93). In each pair of figures, data are for 2006 and 2007, respectively: **A and B**, grain weights; **C and D**, *Fusarium*-damaged kernels (FDK); and **E and F**, percent infected kernels. Within a figure, means above the same letter are not significantly different ($P \leq 0.05$, Tukey's test).

The largest source of variation in PIK was sample date (Table 2). PIK generally increased on successive sampling dates in 2006 through 55 daa (harvest-ripeness); averaging across cultivars and mist durations, means increased significantly on dates 2, 3, and 5 (Fig. 2E). In 2007, PIK was higher in the later samples than in the earlier ones (Fig. 2D). Values for the 45-daa sample in 2007 were

high (Fig. 2F), possibly related to the 42-mm rain event on 3 June (Fig. 1), 4 days before collection of the 45-daa sample.

DON. Averaging across cultivars, mean DON reached higher maximum concentrations in rachis and glume tissues than in grain for all mist durations in both years (Figs. 4 and 5). Whereas DON concentrations generally declined in grain from 15 or 25 daa

Fig. 3. Spike fractions by weight. Data averaged across four soft red winter wheat cultivars inoculated with *Fusarium graminearum* macroconidia at mid-anthesis, subjected to four post-anthesis mist durations, and sampled during grain-fill in a misted Fusarium head blight field nursery in Kinston, NC, in 2007.

Fig. 4. Least-squares means of deoxynivalenol (DON) in different spike fractions by misting treatment averaged across eight (2006) or four (2007) soft red winter wheat cultivars, sampled during grain-fill in a misted Fusarium head blight field nursery in Kinston, NC. Starting at mid-anthesis, four mist durations (0, 10, 20, or 30 days after anthesis [daa]) were applied. Samples of 30 spikes per plot were collected from three replicate plots per cultivar–mist-duration treatment at 15 daa (medium milk, Zadoks growth stage 75 [Z75]), 25 daa (hard dough, Z87), 35 daa (early kernel hard, Z90), 45 daa (kernel hard, Z91), 55 daa (harvest-ripe, Z92), or 65 daa (kernel loosening, Z93). In each pair of figures, data are for DON concentrations in 2006 and 2007, respectively: **A and B**, grain; **C and D**, rachis; and **E and F**, glumes. Within a figure, means above the same letter are not significantly different ($P \leq 0.05$, Tukey's test).

onward, DON levels generally reached their peak in the other spike tissues later (on average, at 45 daa).

For all spike fractions, increasing durations of mist tended to reduce the effect of cultivar on DON in both years (Table 3). In other words, increasing numbers of post-anthesis wet days changed cultivar DON resistance rankings in all types of spike tissues. This trend was greater in 2006, the year in which mist duration tended to affect measures of disease, than in 2007, when it did not.

Grain. When averaged across samples, cultivars, and mist treatments, mean grain DON was nearly twice as high in 2006 as in 2007 (7.2 and 3.8 $\mu\text{g/g}$, respectively) and, at harvest ripeness, it

was 3.2 versus 2.3 $\mu\text{g g}^{-1}$. Averaging across cultivars and mist levels, grain DON levels were higher earlier in grain-fill and declined toward harvest in both years (Fig. 4A and B). Sample date was by far the largest source of variation in grain DON in 2006 and was significant in 2007 although, in the latter year, cultivar accounted for a larger share of the variation (Table 2). When the data from both years were combined, sample date interacted significantly with year, cultivar, and mist duration ($P \leq 0.0009$).

Mist duration interacted significantly with sample in both years ($P \leq 0.0009$). Mist duration had a significant effect ($P \leq 0.025$) on

Fig. 5. Least-squares means of deoxynivalenol (DON) in different spike fractions by cultivar averaged across four misting treatments for soft red winter wheat cultivars, sampled during grain-fill in a misted *Fusarium* head blight field nursery in Kinston, NC. Starting at mid-anthesis, four mist durations (0, 10, 20, or 30 days after anthesis [daa]) were applied. Samples of 30 spikes per plot were collected from three replicate plots per cultivar–mist-duration treatment at 15 daa (medium milk, Zadoks growth stage 75 [Z75]), 25 daa (hard dough, Z87), 35 daa (early kernel hard, Z90), 45 daa (kernel hard, Z91), 55 daa (harvest-ripe, Z92), or 65 daa (kernel loosening, Z93). In each pair of figures, data are for DON concentrations in 2006 and 2007, respectively: **A and B**, grain; **C and D**, rachis; and **E and F**, glumes. Moderately resistant cultivars: ‘C9474’, ‘Ernie’, ‘NC-Neuse’, ‘V-Tribute’, ‘USG 3592’, and ‘USG 3650’. Susceptible cultivars: ‘C9184’ and ‘USG 3592’. Within a figure, means above the same letter are not significantly different ($P \leq 0.05$, Tukey’s test).

TABLE 3. Effects of mist duration on cultivar differences in deoxynivalenol (DON) in three fractions of wheat spikes sampled at 10-day intervals from medium-milk to kernel-loosening after inoculation at mid-anthesis with *Fusarium graminearum* macroconidia in a 2-year field experiment in Kinston, NC

Year, mist (daa) ^y	Significance of cultivar effect on DON ^z								
	Grain			Glumes			Rachis		
	df	F value	P value	df	F value	P value	df	F value	P value
2006									
0	7, 52.7	5.23	0.0001	7, 16	21.35	<0.0001	7, 55.4	6.31	<0.0001
10	7, 57.3	7.48	<0.0001	7, 14.6	2.12	0.107	7, 56.6	2.31	0.039
20	7, 54.8	1.43	0.213	7, 13.7	1.98	0.133	7, 58.4	2.07	0.061
30	7, 56.6	1.35	0.245	7, 14.2	0.49	0.827	6, 60.7	1.76	0.122
2007									
0	3, 30.1	17.18	<0.0001	3, 5.6	19.04	0.002	3, 36.1	16.39	<0.0001
10	3, 28.9	13.52	<0.0001	3, 8.07	14.81	0.001	3, 36.2	8.96	0.0001
20	3, 29.8	12.29	<0.0001	3, 7.42	2.96	0.103	3, 35.5	6.44	0.0001
30	3, 28.9	5.47	0.004	3, 6.74	3.69	0.073	3, 35.5	6.82	0.0001

^y Mist duration in days after anthesis (daa). Data are from eight cultivars in 2006 and four of those eight in 2007.

^z Simple-effects test were conducted on data after square-root transformation to improve homogeneity of variance. Under Df (degrees of freedom), the numbers are numerator, denominator.

grain DON in all samples except the 35-daa sample in 2006 and the 55-daa sample in 2007 ($P = 0.095$ and 0.076 , respectively; simple-effects test). Misting affected grain DON levels more so in 2006 than in 2007 (Fig. 4A and B). In the non-misted plots, mean grain DON was approximately the same in the 2 years across most sampling points, and post-anthesis mist tended to elevate DON levels.

Cultivar interacted significantly with sample in both years ($P \leq 0.013$), and cultivars differed significantly for DON at all sample dates in both years (simple-effects test, $P \leq 0.006$). The susceptible C9184 had significantly higher DON ($P \leq 0.05$, data not shown) than the MR cultivars in the 15-daa sample in 2006 and in the 35-, 45-, and 55-daa samples in 2007; the susceptible USG 3592 had higher DON ($P \leq 0.05$, data not shown) than all MR cultivars except USG 3650 and 'VA01W99' at all sample dates in 2006 (Fig. 5A and B).

Rachises. Mean DON concentrations were $20 \mu\text{g g}^{-1}$ in 2006 and $17 \mu\text{g g}^{-1}$ in 2007. DON reached similar maximal levels (30 to $50 \mu\text{g g}^{-1}$) in rachis tissues in both the wetter and the drier year (2006 and 2007, respectively) (Fig. 4C and D). Sample date interacted with both mist duration and cultivar in both years ($P \leq 0.0002$). In 2006, the mist effect was significant in every sample (simple-effects test, $P \leq 0.002$), and peak rachis DON concentration lagged behind the end of misting by 15 to 25 days (Fig. 4C). In 2007, however, mist was only significant in the 35- and 55-daa samples (simple-effects test, $P \leq 0.002$; other samples, $P \geq 0.071$).

Cultivar was significant in all samples in 2007 ($P \leq 0.008$) and in all but the 35- and 45-daa samples in 2006 ($P \leq 0.005$). Looking across the samples, cultivar FHB resistance rankings by severity (Table 1) were not as well reflected in rachis DON levels as they were in grain DON (Fig. 5). For example, rachis DON levels in the MR USG 3650 attained high peaks in both years. However, 'C9474', one of the most resistant cultivars, did have among the lowest rachis DON levels in both years, and the susceptible USG 3592 had significantly more DON than the MR cultivars at the first two sampling points in 2006 ($P \leq 0.05$, data not shown). The susceptible C9184 did not attain the test-wide peak rachis DON concentration in 2006 but, in both years, it had high rachis DON concentrations at 55 daa.

In both years, mean rachis DON concentrations attained their peak at 45 daa (not different from 35 daa in 2006 and from 55 daa in 2007, $P = 0.05$). Rachis DON levels were lowest (5 to $15 \mu\text{g g}^{-1}$) at the earliest and latest sampling dates in both years. DON concentrations in rachises declined significantly from 45 daa to 65 daa.

Glumes. Sample date interacted significantly with mist duration ($P < 0.0001$) and cultivar ($P \leq 0.03$) in 2006 but only with cultivar in 2007 ($P = 0.01$). Misting increased DON at all dates in 2006 ($P \leq 0.0007$), except 25 and 65 daa (simple-effects test, $P = 0.079$ and 0.126 , respectively). In 2007, misting affected DON only at 25 daa ($P = 0.016$). The magnitude of the difference in 2006 was greatest in the 45-daa sample, collected on 31 May, soon after a rain event of 20 mm on 27 May (Fig. 1).

Cultivars differed significantly for glume DON at all dates in both years (simple-effects test, $P \leq 0.021$), except at 35 and 45 daa in 2006 ($P \geq 0.164$), the same two samples in which cultivars did not differ significantly for rachis DON that year. Glume DON levels of the susceptible USG 3592 were significantly higher than those of MR cultivars in the 15-daa sample ($P \leq 0.05$, data not shown), and levels of both susceptible cultivars (USG 3592 and C9184) remained higher than average throughout both years (Fig. 5E and F). In 2007, DON in C9184 glumes was higher than that in C9474 in all samples; the other cultivars were intermediate ($P \leq 0.05$).

As with rachises, peak DON concentrations in glumes were found at the intermediate rather than the earliest and latest sampling dates (Fig. 4E and F). The samples with the highest mean DON were at 45 daa in 2006 and 35 daa in 2007. Mean glume

DON levels across samples, mist durations, and cultivars were significantly higher in 2006, the wetter year.

Whole-spike DON. In both years, estimated DON per spike was at its peak at 35 daa (Fig. 6). However, that peak was extended from 25 to 45 daa in 2006. The mean proportions of total DON contributed by grain and glumes were roughly the same at almost all sample points. Due to their relatively low weight and volume, rachises contributed a small share of total spike DON despite their high DON concentrations, especially early and late in grain-fill.

Associations. Across sample dates, the most consistently significant associations were between DON in grain and DON in glumes, and between grain DON and FDK (Table 4). Severity, which was assessed shortly before the 25-daa samples were collected, was significantly associated with DON in grain and glumes of both 25- and 55-daa samples in 2006 (Table 4) ($P \leq 0.08$). Severity was not associated with DON concentrations in rachises of either sample, however. In 2007, severity was not associated with DON in any spike fraction at either sample point.

The results of regression analysis to explore the relationship of grain DON at other sample dates with grain DON at 55 daa are shown in Table 5. There was a tendency for slopes to be more positive, and to differ significantly from zero, at 35 and 45 daa, compared with the earlier 15- and 25-daa stages.

DISCUSSION

This time-course study provides a new perspective on the progression of FHB symptoms during wheat spike development. Mean DON levels in wheat grain showed an overall declining trend during the period from medium milk (15 daa) to harvest-ripeness (55 daa). The decline is consistent with reports that DON biosynthesis occurs immediately following kernel infection, with DON acting as a virulence factor that allows fungal hyphae to move into the wheat rachis and spread to other florets (11). Initial kernel infections usually occur at and soon after anthesis, and the fungus then spreads through the head, infecting additional kernels over the following days. DON biosynthesis has been found (in a controlled environment) to be maximal at the infection front and before symptom development, and to drop after infection, shutting down by early dough (10), or ≈ 18 daa. From the current study, it appears the conditions for DON biosynthesis may prevail longer in glume and rachis tissues, at least in the field.

Once synthesized, DON is thought to be removed by host detoxification (18) or by the leaching action of water (7). From the present results, it can be seen that, as *Fusarium* spreads from spikelet to spikelet via the rachis, maximum DON concentrations in the rachis are not attained until close to harvest. Maximum DON concentrations are also not reached in the glumes until close to harvest, although this dynamic appears to be more dependent on post-anthesis moisture. Thus, it appears unlikely that leaching due to rain or irrigation water is the reason for DON decline in grain, given the different time course of DON in the glumes. The late-season decline of DON concentrations in non-grain spike tissues may have causes similar to those in grain. In particular, conversion to glucosylated forms of DON could explain this decline, especially if the biosynthesis of DON in rachises and glumes starts later or lasts longer than that in grain.

At the same time that DON levels were dropping, FDK and PIK increased in the present experiment until after harvest ripeness in 2006 and until harvest ripeness in 2007. Misting increased early DON levels, FDK, and PIK and decreased sample weights to a greater extent in 2006 than in 2007. FDK and PIK at harvest-ripe were elevated in plots where moisture was applied after anthesis compared with unmisted plots. PIK and grain DON were not associated. Considering these data, it appears that *Fusarium* continues to spread within spikes through harvest ripeness without a concomitant increase in FDK or grain DON. Evidently, as spike tissues mature and dry down, the fungus continues its

movement inside the head but is no longer able to colonize kernels as thoroughly as in earlier stages of grain fill. Moist conditions during grain-fill can alleviate the effects of increasing water shortage in the head, facilitating the ongoing process of kernel colonization needed for FDK. Without misting, FDK stayed relatively flat from early kernel-hard (35 daa) on, whereas PIK increased over time with or without misting. This suggests that less externally applied moisture is needed to allow fungal movement through the rachis for infection of additional kernels (PIK) than for colonization (FDK) and DON synthesis.

We found that, within wheat spikes, DON concentrations reached highest levels in the rachis; this is consistent with reports by researchers in New Zealand working with maize ears. Lauren and DiMenna (16) examined leaf and ear sections of maize infected with several *Fusarium* spp., including *F. graminearum*. They found that the most contaminated part of the ear was the rachis, with concentrations of DON plus nivalenol reaching 40 to 95 $\mu\text{g g}^{-1}$, whereas the highest DON concentration in kernels was 3.8 $\mu\text{g g}^{-1}$.

The results of this experiment confirm our earlier findings that increasing numbers of wet days in the post-anthesis period increase not only visible FHB symptoms but also kernel damage, the percentage of *Fusarium*-infected kernels, and DON levels in wheat spikes (6). Naturally, this relationship is substantiated for the volumes and durations of misting imposed; in this case, a maximum of 30 days at $\approx 10 \text{ mm day}^{-1}$. Longer durations or larger daily quantities of moisture could have a different effect.

What are the consequences of these findings for silage and forage producers? If FHB is observed or suspected, it is desirable to harvest whole plants on the early side (e.g., at milk rather than dough stage). The more conducive the year is to FHB, the closer to flowering one should aim to harvest.

The question arises whether variety ratings for FHB are useful in predicting whole-spike DON concentrations. Not surprisingly,

differences in visible symptoms, which, along with grain DON concentrations, are the main variable used to compile cultivar FHB resistance rankings, were more associated with DON in grain than with DON in other spike tissues. Severity assessments at the normal time, ≈ 20 days after mid-anthesis, were found to be associated with hard-dough levels of DON in grain and glumes in the higher-scab year but not in the lower-scab year. DON in grain was consistently associated with DON in glumes, and those are the two spike fractions contributing the majority of overall spike DON. On the whole, cultivar FHB resistance ratings should be useful in assessing which cultivars will have the highest levels of whole-plant DON when decisions must be made about harvest for silage. In other words, visible symptoms should have some relationship to whole-spike DON levels, especially in a bad scab year, when choices must be made whether to harvest whole crops for silage, or which fields or cultivars to ensile.

With respect to straw, it is clear that chaff can contain significant amounts of DON, as has been previously found. By weight, approximately half of total spike DON resides in glumes and rachises and, after the grain is harvested, that half remains in the chaff. Attention needs to be paid to the DON content of straw that may be consumed by animals. Averaged across mist-duration treatments, susceptible cultivars had higher DON concentrations in non-grain spike tissues than did MR cultivars at harvest. This has significance for use of wheat straw for bedding that might be eaten by livestock. It suggests that cultivar FHB ratings should be useful in choosing straw with minimal DON content. Straw harvested after prolonged post-anthesis rainy periods may have elevated concentrations of DON in the chaff. Although glume and rachis tissues would be a small proportion by mass of total straw, their high DON levels could be an issue in animal bedding or winter rations.

It would be useful to be able to predict DON levels in wheat at harvest time on the basis of DON levels measured earlier in the

Fig. 6. Total deoxynivalenol (DON) per spike, based on spike fractions by weight and DON concentrations by spike fraction. Data averaged across **A**, eight soft red winter wheat cultivars in 2006 and **B**, four cultivars in 2007, subjected to four post-anthesis mist durations and sampled during grain-fill in a misted field nursery in Kinston, NC.

grain-fill period. This would require that there be a demonstrable relationship between the two measures. Our results suggest that that relationship is affected by the number of moist days. The predictability of harvest-ripe DON by cultivar or in relationship to earlier DON concentrations tends to be diminished by increasing numbers of moist days in the post-anthesis period. Averaging across mist durations, it appears that DON is most predictive of harvest-ripe DON the closer in time that one samples to harvest-ripeness (i.e., sampling early in grain-fill may not provide an accurate estimate of grain DON concentrations at harvest). A model that includes visual symptoms and weather variables between anthesis and harvest, as well as early DON levels, might enhance the ability to predict DON at harvest.

Why did misting duration have less effect in 2007, the drier and later spring? It is possible that, in the drier year, amounts of mist were insufficient to affect disease development. Due to evaporation, applied mist may have been more subject to evaporation than in a wetter year and, thus, less available to affect disease development.

The mid-point of cultivar anthesis was used as the 0-daa mark although, in 2006, mid-anthesis varied by up to 6 days among

cultivars. Single dates for turning off misting zones were unavoidable, given resource limitations. Although this method may have introduced some variation, if that variation were important, the downward-trending data in Figure 5A (for example) would exhibit more of a “traveling wave” pattern, with multiple, horizontally staggered lines based on inoculation date. No such pattern is apparent.

Severity levels were lower in plots destined to be misted for 30 days than in those misted for 20 days in 2007 (Table 1), and grain DON in the 30-day-misted plots was low at the first sampling date (Fig. 4B). The 30- and 20-day plots had received the same duration of mist at index assessment (i.e., the last 10 of the 30 days of mist had not yet been imposed). Nevertheless, factors in the 30-day block were initially unfavorable to disease development, in particular patches of WSBMV that retarded anthesis.

To summarize, the progression of DON differs among wheat spike tissues, with concentrations declining in grain during grain-fill but peaking at approximately early kernel-hard in glumes and rachises. *Fusarium* continues spreading throughout the head during grain-fill but the ongoing production of FDK depends upon post-

TABLE 4. Selected associations of scab damage measures and deoxynivalenol (DON) in three fractions of wheat spikes sampled at 10-day intervals from medium-milk to kernel-loosening after inoculation at mid-anthesis with *Fusarium graminearum* macroconidia in a 2-year field experiment in Kinston, NC^y

Associations evaluated ^z	2006		2007		2006–07 combined	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Across sample dates						
DON _{grain} –DON _{glumes}	0.16	0.052	0.18	0.082	0.13	0.002
DON _{grain} –DON _{rachis}	0.03	0.761	0.26	0.011	0.13	0.002
DON _{glumes} –DON _{rachis}	–0.08	0.353	0.13	0.202	–0.02	0.650
DON _{grain} –PIK	–0.05	0.518	0.26	0.010	0.05	0.231
DON _{grain} –FDK	0.21	0.010	0.31	0.002	0.20	<0.0001
Sample 2 (hard dough, Z87)						
DON _{grain} –DON _{glumes}	0.42	0.003	0.43	0.031	...	...
DON _{grain} –DON _{rachis}	0.18	0.213	0.59	0.002	...	...
DON _{glumes} –DON _{rachis}	0.10	0.486	0.51	0.010	...	...
DON _{grain} –Severity	0.33	0.017	–0.07	0.747	...	...
DON _{glumes} –Severity	0.25	0.081	0.11	0.601	...	...
DON _{rachis} –Severity	0.13	0.371	0.33	0.107	...	...
DON _{grain} –PIK	–0.16	0.293	0.11	0.601	...	...
Sample 5 (harvest-ripe, Z92)						
DON _{grain} –DON _{glumes}	0.52	<0.0001	0.29	0.156	...	...
DON _{grain} –DON _{rachis}	0.31	0.026	0.30	0.152	...	...
DON _{glumes} –DON _{rachis}	0.23	0.097	0.25	0.227	...	...
DON _{grain} –Severity	0.20	0.135	0.04	0.851	...	...
DON _{glumes} –Severity	0.51	<0.0001	0.11	0.604	...	...
DON _{rachis} –Severity	0.01	0.926	–0.15	0.469	...	...
DON _{grain} –PIK	0.31	0.023	0.09	0.678	...	...
DON _{grain} –FDK	0.34	0.012	0.42	0.037	...	...

^y Data are from eight cultivars in 2006 and four of those eight in 2007, and were square-root transformed to improve homogeneity of variance. Correlations were assessed across cultivars and mist durations; *r* = partial correlation coefficient.

^z PIK = percent infected kernels; FDK = *Fusarium*-damaged kernels; severity was assessed at 20 days after anthesis (daa) or medium-dough stage. Sample dates were 15 daa (medium milk, Zadoks growth stage 75 [Z75]), 25 daa (hard dough, Z87), 35 daa (early kernel-hard, Z90), 45 daa (kernel hard, Z91), 55 daa (harvest-ripe, Z92), or 65 daa (kernel loosening, Z93). No data were available on PIK in 15-daa samples in 2007 and FDK in 15- and 25-daa samples in either year.

TABLE 5. Association of grain deoxynivalenol (DON) concentrations sampled at four points during grain-fill, with grain DON concentrations at harvest-ripe (sample 5, 55 days after anthesis [daa]) in eight soft red winter wheat cultivars grown in 2006 in Kinston, NC^y

Mist (daa) ^z	Medium-milk (15 daa)		Hard dough (25 daa)		Early kernel-hard (35 daa)		Kernel-hard (45 daa)	
	Slope (s.e.)	<i>P</i> value	Slope (s.e.)	<i>P</i> value	Slope (s.e.)	<i>P</i> value	Slope (s.e.)	<i>P</i> value
0	–0.017 (0.125)	0.895	0.432 (0.216)	0.051	0.431 (0.190)	0.026	0.409 (0.220)	0.069
10	0.397 (0.119)	0.001	0.138 (0.123)	0.264	0.364 (0.108)	0.001	0.420 (0.057)	0.010
20	...	...	–0.174 (0.172)	0.316	0.489 (0.337)	0.155	0.159 (0.176)	0.372
30	...	...	...	...	0.096 (0.118)	0.419	0.392 (0.130)	0.004

^y DON concentrations were square-root transformed to improve homogeneity of variance. *P* values are for the null hypothesis that the slope equals 0; s.e. = standard error; ... indicates results not reported in cases where mist treatments had not yet been fully imposed.

^z Mist duration.

anthesis moisture. Glumes and rachises account for approximately half the DON in wheat spikes, and DON from these sources must be taken seriously in small-grain straw that may be consumed by livestock. In bad FHB years, harvest of whole plants for silage should be conducted as soon as possible after flowering. Cultivar resistance ratings and observations of index at late-milk should be useful in making judgments about harvest of whole-plant silage and sourcing of straw.

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