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ABSTRACT

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Deoxynivalenol (DON) is a potent mycotoxin and virulence factor produced by *Fusarium graminearum*. We examined the expression of the core DON biosynthetic gene *Tri5* during wheat head infection of susceptible and resistant cultivars and susceptible cultivars treated with strobilurin fungicides (e.g., azoxystrobin). DON was quantified to correlate expression with toxin accumulation. The highest *Tri5* expression relative to housekeeping genes occurred at the infection front. As infection progressed, earliest-infected kernels showed diminished relative *Tri5* expression but *Tri5* expression never ceased during the 21 days observed. Azoxystrobin treatment showed no significant effect on either relative *Tri5* expression or DON quantity. The resistant cultivar 'Alsen' showed minimal spread of the fungus, with no fungus detected by day 21. DON was not detected in significant quantities in Alsen in the later stages sampled. In Wheaten, DON levels were negligible at 8 days post-inoculation (dpi), with detectable DON at later-sampled time points. *Tri5* was detected even in fully senesced kernels 21 dpi. Our data demonstrate the presence of *Tri5* transcripts in a susceptible cultivar over a much longer time period than has been previously documented. This suggests the ability of the fungus to rapidly resume toxin biosynthesis in dried infected grain should conducive environmental conditions be present, and provides a possible mechanism for high DON levels in asymptomatic grain.

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch) is a devastating pathogen of small grains, causing head blight and crown rot of wheat and barley and ear rot and stalk rot of maize, as well as opportunistically infecting other cereal crops (rice, oat, rye, etc.) (42). In addition to loss of crop productivity and biomass, *F. graminearum* produces several mycotoxins that render harvested grain unfit for consumption; notably, the trichothecene mycotoxins deoxynivalenol (DON) and its acetylated derivatives (3ADON and 15ADON), nivalenol (NIV); and the estrogenic polyketide zearalenone (9,11).

The DON biosynthetic pathway is well characterized (9,37). In *F. graminearum,* 12 biosynthetic genes are found in a core cluster on chromosome 2, with three additional genes (*Tri1*, *Tri101*, and *Tri15*) located elsewhere (chromosomes 1, 4, and 3, respectively) (28). The sequenced strain of *F. graminearum*, PH-1, lacks functional *Tri13* and *Tri7*, leading to the production of DON and the concomitant inability to produce NIV (20,29). Tri5, encoded by a core cluster gene, catalyzes the formation of trichodiene from farnesyl pyrophosphate, the first step in trichothecene biosynthesis. Consequently, *Tri5* is an attractive target for mutagenesis (2,36) and for molecular detection of trichothecene-producing *Fusarium* spp. (22,34,38,44,47). Several studies have focused on *Tri5* expression to unravel specific aspects of the *F. graminearum*–host interaction. Stephens and colleagues (41) used Affymetrix GeneChips to examine *Fusarium* gene expression during crown rot of wheat, and reported significant upregulation of *Tri5* and *Tri14* in planta (compared with mycelial culture). Zhang and colleagues (48) compared *Tri5* expression between carbendazin-resistant and -sensitive *F. graminearum* in shake culture and found higher expression levels in the resistant strain. Gardiner et al. (14) showed that *Tri5* is strongly expressed in the rachis tissue of wheat, implicating DON in the early spread through the head. Early biosynthesis of wheat polyamines during infection suggests that these host compounds are important inducers of DON (13).

DON is produced primarily during plant infection, although DON production can be induced in vitro under special conditions (31). An infection time course in barley using the Fusarium GeneChip identifies DON biosynthetic genes among those uniquely expressed in planta (compared with genes expressed during growth in complete and carbon- and nitrogen-starved media) (20). DON and NIV are the only mycotoxins known to act as virulence factors and, in wheat, have been shown to be essential for virulence in head blight (2,10,36) but not for crown rot (33). Furthermore, DON has been shown to be essential for the spread of *F. graminearum* from floret to rachis in wheat by preventing host cell wall reinforcement (26). However, the same study showed limited spread of *F. graminearum* in barley, with or without DON production (26). DON production has not been demonstrated in rice (16), and DON is produced but not essential for infection in maize (1,36). In another study, DON-deficient mutants were unable to spread beyond the inoculated spikelet in wheat but did not differ significantly from the wild type in their ability to cause disease in rice and barley (30).

Previous studies have reported an increase in DON production in wheat treated with strobilurin fungicides such as azoxystrobin (5,47). Another study (35) reported increased DON in wheat plots treated with azoxystrobin alone or in combination with triazoles as opposed to plots treated with triazoles alone. Additionally, strobilurins are known to delay plant senescence (3). If expression of DON biosynthetic genes is associated with green tissue and

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tissue remains green longer, then the appearance of higher levels of DON in strobilurin-treated wheat could be explained by the delayed senescence. Here, we tested whether higher levels of DON are found in strobilurin-treated wheat, and whether DON biosynthetic genes are mainly expressed in green tissue.

In this study, we used Fusarium Affymetrix GeneChip data to build a profile of DON biosynthetic gene expression during wheat infection. We then used quantitative reverse transcript polymerase chain reaction (qRT-PCR) to evaluate the expression of *Tri5*, the first committed step in DON production, during an infection time course, collecting data day by day and kernel by kernel in 'Wheaten', a susceptible cultivar of spring wheat. A relevant subset of time points was additionally examined in Wheaten treated with azoxystrobin, and in the resistant cultivar 'Alsen'. Alsen possesses the quantitative trait locus FHB1, which is associated with resistance to Fusarium head blight and, specifically, to the effects of DON (45). Furthermore, representative kernels were sampled to evaluate the relationship between *Tri5* expression levels and DON quantity. We found that transcription of DON biosynthetic genes in the fungus was initiated within 24 h of infection and did not cease throughout the time points examined. Furthermore, resistant Alsen appeared to be able to eliminate the fungus over time. Our findings suggest a possible mechanism for high DON levels in asymptomatic grain.

MATERIALS AND METHODS

Fungal strains and growth conditions. The strain of *F. graminearum* used for this study, Michigan field isolate PH-1 (FGSC 9075; NRRL 31084) (43), has had its genome sequenced (7). The genome sequence and annotation are publicly available (http://www.broad.mit.edu/annotation/genome/fusarium_graminearum/MultiHome.html; http://mips.gsf.de/genre/proj/FGDB). *F. graminearum* was maintained as mycelia and conidia (5×10^5 conidia ml^{-1}) in 35% glycerol at –80°C and on sterile soil at –20°C.

Wheat cultivars and inoculation of plants. Seed of spring wheat (*Triticum aestivum* L.) Wheaten and Alsen were planted in 9-cm clay pots (4 seeds per pot) and maintained in the greenhouse at ≈24°C with supplemental lighting. Wheat and kernel developmental stages were characterized using the Zadoks two-digit scale (46). Plants were inoculated 2 to 3 days after heading (prior to anthesis; Zadoks 59) by pipetting 10 µl of a 5×10^5 conidia ml^{-1} suspension into a spikelet at the midpoint of the rachis, as in Guenther and Trail (19). One plant per pot was mock inoculated with 35% glycerol. Plants were then placed in a mist chamber in the greenhouse at 24°C for 3 days before being returned to the greenhouse bench.

Harvest. Beginning 6 days postinoculation (dpi), kernels from susceptible Wheaten were collected, progressing outward from the inoculation point (IP). The rachis node immediately above the IP was designated +1, the node above that +2, and so on, while nodes descending from the IP were designated –1, –2, –3, and so on. All kernels from a given position (e.g., +1) from 12 plants were pooled, flash frozen in a dry ice/ethanol bath, lyophilized, and stored at –80°C until RNA isolation. Collection continued daily through 14 dpi. At harvest, presence or absence of visible infection of the kernels and of the awns were noted. Kernels from resistant Alsen were harvested at 8, 11, 14, 17, and 21 dpi; for the 17- and 21-dpi plants, the first and second centimeter of stem below the head were also separately harvested.

Fungicide treatment. Spring wheat Wheaten was grown and inoculated as above. The wheat was treated with the azoxystrobin fungicide Quadris (Syngenta, Greensboro, NC) at boot (Zadoks 45) and again immediately following removal from the mist chamber (Zadoks 65). A second set of plants was inoculated simultaneously as an untreated control. Kernels from treated and untreated plants were harvested at 8, 11, 14, 17, and 21 dpi; for the 17- and 21-dpi plants, the first and second centimeter of stem below the head were also separately harvested. Although we followed the manufacturer's guidelines for application of the fungicide to wheat, it should be noted that Quadris is not approved for treatment of Fusarium head blight.

RNA extraction. RNA extraction utilized the lithium chloride-based method described by Goswami et al. (17). Following ethanol precipitation, 100 µg of RNA was brought to 88 µl in RNase-free water and treated with DNase I (Roche, Basel, Switzerland) for 15 min at 37°C. The sample was then purified with an RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

qRT-PCR. RNA (3 µg) was used in a 20-µl first-strand cDNA synthesis reaction using an AffinityScript QPCR cDNA Synthesis Kit (Agilent, Santa Clara, CA) following the manufacturer's instructions. For each template, a control reaction was also run without reverse transcriptase. The following primers were designed to amplify from the trichodiene synthase gene (*Tri5*; FGSG_03537), a necessary component of the DON pathway, the housekeeping genes for glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*; FGSG_16627) and translation elongation factor 1 α (*EF1A*; FGSG_08811) from *F. graminearum*, and the housekeeping gene for actin from wheat (AY663392.1): *Tri5* F, TCTATGGCCCAAGGACCTGTTTGA and *Tri5* R, TGACCC AAACCATCCAGTTCTCCA; *GAPDH* F, CTACATGCTCAAG TACGACTCTTCC and *GAPDH* R, GCCGGTCTCGGACCA CTTG; *EF1A* F, AAAGACCCTCCTTGAGGCCATTGA and *EF1A* R, ACTTCAGTGGTGACGTTGGAAGGA; and wheat *ACT1* F, ACGTGGATATCAGGAAGG and wheat *ACT1* R, ACATCTGTTGGAAAAGTGC. Optimal primer concentrations were determined empirically, with *Tri5* and *GAPDH* reactions using 1 pmol each primer, while *EF1A* and *ACT* reactions used 10 pmol. Reactions (25 µl total) were prepared using Brilliant II SYBR Green QPCR Master Mix (Agilent), following the manufacturer's instructions. These reactions were run on an ABI Prism 7000 Sequence Detection System, running ABI 7000 System SDS Software (Applied Biosystems, Carlsbad, CA) for 40 cycles followed by a dissociation cycle, according to standard protocols. Three biological replicates were performed for every kernel position and time point.

Affymetrix GeneChip analysis. GeneChip data from previous studies on barley and wheat (18,20) and in culture (21) were normalized using RMA in R and Bioconductor (15,24,25), as described by Hallen et al. (21). Wheat inoculations in the previous study (18) were done similarly to those described here. Barley inoculations were accomplished by spraying heads with a spore suspension (20). The genes involved in the trichothecene biosynthetic pathway, along with three housekeeping genes (*GAPDH*, *EF1A*, and β-tubulin [FGSG_09530]) were used to generate a heat map using heatmap.2, part of the gplots package in R (http://cran.r-project.org/web/packages/gplots/index.html).

DON quantification. DON measurements were taken for a subset of kernels intended to represent different stages of infection. One kernel per rachis node was set aside for DON quantification by gas chromatography (GC), while the other kernel was used for qRT-PCR. Each kernel was weighed, extracted, and quantified separately. Kernels were ground in liquid nitrogen and DON was extracted overnight at room temperature in an orbital shaker at 180 rpm with 10 ml of high-performance liquid chromatography-grade acetonitrile/water (84:16) per kernel. Samples were centrifuged at 2,400 rpm at 21°C for 10 min, and the supernatant was collected. From each sample, 4 ml was eluted through a column packed with 0.5 g of a mixture consisting of 28 g of Darco G-60 charcoal, 20.1 g of aluminum oxide, and 1 g of celite 545, and followed by an additional 1 ml of acteonitrile/water (84:16). Cleaned extracts were evaporated to dryness overnight under warm nitrogen. Evaporated samples were prepared for analysis by dissolving in 100 µl of 4-dimethylaminopyridine (2 mg ml^{-1}, in toluene; Mirex at 1 µg ml^{-1} [Chem Service,

Westchester, PA] was used as an internal standard), and 50 µl of heptafluorobutyric acid (Campbell Scientific, Logan, UT) was added as a derivitizing agent. Samples were mixed well and incubated at 65°C for 1 h. The reaction was neutralized with 1 ml of 3% sodium bicarbonate and further diluted with 150 µl of toluene. The toluene-based portion of the sample was decanted into an autosampler vial for analysis. Each sample (1 µl) was injected onto a Shimadzu GC-17A model gas chromatograph (Shimadzu Scientific, Columbia, MD). The separation and detection system consisted of an Rtx-5 Crossbond column (15 m, 0.25-mm i.d., 0.25-µm df) (Restek Corporation, Bellefonte, PA) with a 5-m guard and an ECD ^{63}Ni detector. Samples were run alternately with DON-spiked wheat standards as calibration controls. A split injection was used, with a ratio of 1:25. The carrier gas was ultrapure helium at a flow rate of 1.3 ml min^{-1}, while zero-grade nitrogen was used for the make-up gas. The injector was set at 300°C and detector temperature was 310°C. Oven temperature was initially 80°C and increased by 65°C min^{-1} to 171°C, 1.2°C min^{-1} to 182°C, 45°C min^{-1} to 228°C, and 0.1°C min^{-1} to 229°C (182°C was used to detect DON and 229°C was used to detect the internal standard, Mirex). DON was quantified by comparison with a standard curve of DON analytical standard (Biopure) obtained from Romer Labs (Union, MO). DON quantities (ppm) were averaged across eight kernels for any given treatment.

RESULTS

GeneChip analyses. A heat map showing expression of DON biosynthetic genes and some representative housekeeping genes during an in vitro developmental time course on carrot agar, and during barley infection and wheat stem colonization, is given in Figure 1. DON biosynthetic genes were expressed predominantly in planta, with the earliest expression detected at 48 h post-inoculation in barley. In wheat stems, DON biosynthetic gene expression was detected in the infection front and just behind the infection front in the "water-soaked" zone but not in senescent wheat. Housekeeping genes *EF1A* and *GAPDH* were highly expressed throughout.

Course of visible infection. Infection was visible at the inoculated kernel upon removal from the mist chamber (3 dpi); the developing kernel (watery ripe; Zadoks 71) was shriveled and discolored. Rarely, white-to-pink mycelial growth was visible on the kernel or glume. No macroscopically visible damage was detected on any other kernel at 4 dpi, including the kernel adjacent to the IP. For susceptible Wheaten, macroscopically visible symptoms (beyond the IP) first evinced at 8 dpi, both above and below the IP. Symptoms progressed outward from the IP, with some infection detectable at all kernel positions by 14 dpi (Fig. 2). We did not differentiate between different types of fungal-induced damage to the wheat kernel, which included water soaking, discoloration, shriveling, drying (distinct from the loss of moisture that occurs naturally during maturation), and the presence of superficial mycelia. Bleaching of the glumes and a bending and distortion of the awn tracked or shortly preceded the manifestation of symptoms on the kernels.

As infection progressed in Wheaten (14 dpi and afterward), kernels above the IP became increasingly difficult to harvest. The combination of progressing fungal infection and probable diminished water transport, due to fungal hyphae infiltrating and plugging the vascular tissue, resulted in severely stunted kernels (average mass <10 mg/kernel dry weight compared with >20 mg for equivalent kernels in healthy and mock-inoculated plants). Beginning at 16 dpi, the stem immediately below the wheat head had begun to exhibit water-soaking symptoms in some plants.

Wheaten plants were treated with the azoxystrobin at boot and immediately following removal from the mist chamber (shortly after anthesis and 3 dpi). Development of visible symptoms did not differ between fungicide-treated and untreated plants.

Spring wheat Alsen, carrying FHB1-mediated head blight resistance, exhibited minimal development of symptoms of infection. The inoculated kernel was visibly infected upon removal from the mist chamber, as was the case for Wheaten. By 14 dpi, <20% of the kernels at the –1 and –2 position exhibited visible damage, and no other kernels were symptomatic. The greatest spread of infection observed was at 17 dpi, when kernels at +1, –1, –2, and –3 showed some damage. At no point (up to 21 dpi, the last day of data collection) were >20% of the kernels at any given position on an Alsen plant visibly infected.

qRT-PCR on *Tri5* gene expression. In Wheaten, fungal nucleic acids were detected as early as 6 dpi, preceding the development of visible symptoms by 2 days. At 6 dpi, transcripts for all three fungal genes (*GAPDH, EF1A*, and *Tri5*) were detected in the kernels immediately above and below the IP (kernel positions +1 and –1, respectively). Transcripts of the housekeeping genes *GAPDH* and *EF1A* were detected at kernels +2, –2, and –3, whereas *Tri5*, the trichodiene synthase gene, was not detected in these kernels. By 13 dpi, *Tri5* as well as transcripts of the representative housekeeping genes were detected in all kernels.

The highest *Tri5* expression relative to *GAPDH* (and *EF1A*) expression (cycle threshold [Ct] ratio) was observed in asymptomatic tissue at the infection front (Fig. 2); Tri5 transcript levels in these kernels were as high as or slightly higher than the levels of the housekeeping genes. As infection progressed, kernels closest to the IP showed diminished *Tri5* expression relative to housekeeping gene expression but *Tri5* expression was not seen to cease during the 21 days observed. Kernels from mock-inoculated plants were harvested at 10 and 21 dpi for a negative control, and no fungal gene transcripts were detected from these, whereas wheat actin transcripts were detected (data not shown).

Relative *Tri5* expression in azoxystrobin-treated Wheaten did not differ significantly from that in untreated Wheaten (data not shown). Resistant Alsen showed significant deviation in *Tri5* (and fungal housekeeping) gene expression in comparison with Wheaten (Fig. 2). *Tri5* was never detected in kernels below –3 or above +1, and *GAPDH* (and *EF1A*) expression did not extend below –4. Interestingly, for the final day of the time course, 21 dpi, no fungus was detected below –1, although *Tri5* and *GAPDH* expression had been detected at –2 on 11, 14, and 17 dpi, and at –3 on 17 dpi (three independent replicates, each consisting of at least 12 pooled kernels).

DON levels in kernels. DON quantities ranged from none detected (most kernels collected beyond the infection front) to 183 ppm (a single kernel of Wheaten 21 dpi, –8). Alsen kernels exhibited negligible quantities of DON, with the highest level being 32 ppm in a single kernel (8 dpi, –4; average across 8 dpi, –4 Alsen kernels = 10.2 ppm). DON was not detected in significant quantities in Alsen at any kernel position in the later stages sampled (11, 14, 17, and 21 dpi). In Wheaten, DON levels were negligible at 8 dpi, with detectable DON at 11, 14, 17, and 21 dpi. DON levels for kernel position –4 declined slightly over time (from 44.6 ppm at 11 dpi to 28.6 ppm at 21 dpi). DON levels averaged across kernels from representative positions (1, 4, and 8 nodes below the IP) are shown in Table 1. Wheaten plants treated with azoxystrobin did not differ significantly in DON levels from untreated plants. DON levels <20 ppm were considered not significant, because they were difficult to distinguish from background levels.

DISCUSSION

Analysis of expression data from multiple conditions in wheat and barley and in culture revealed a distinct pattern of expression for the DON biosynthetic genes. We investigated this further in both susceptible and resistant cultivars of wheat to determine the relationship of *Tri5* gene expression to progress of infection. Transcription was most active at the infection front before symp-

tom development, consistent with the role of DON in establishing infection and colonization. Interestingly, *Tri5* and fungal *GAPDH* and *EF1A* transcripts remained detectable, albeit reduced, in infected tissue of the susceptible cultivar after the plant tissue had completely senesced (wheat actin transcripts no longer detectable). In the resistant cultivar, initial fungal invasion was halted and the fungus appeared to retreat by the end of the sampling. The apparent correlation between early stages of plant infection and highest levels of DON biosynthetic gene transcripts suggested the potential value of the strobilurin fungicide in dissecting the interactions between fungus and plant in Fusarium head blight. However, no significant differences were observed between sus-

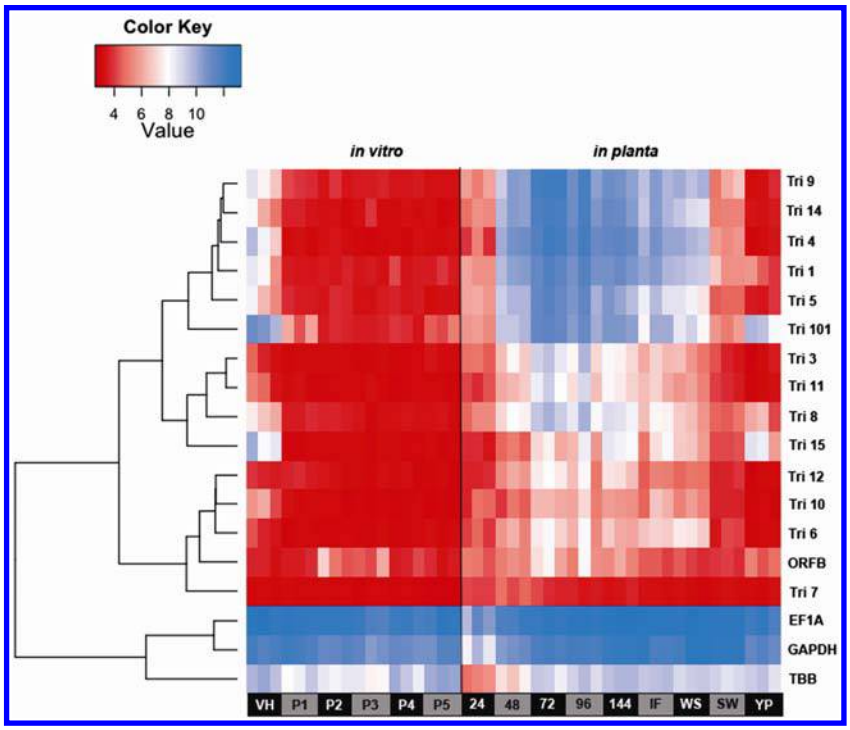

Fig. 1. Heat map of GeneChip data showing expression of trichothecene biosynthetic genes. Gene expression is given as $\log_2$-normalized intensity values. Housekeeping genes are included as controls: EF1A = transcription elongation factor 1 α, GAPDH = glyceraldehyde phosphate dehydrogenase, TBB = β-tubulin. In vitro growth conditions: VH = vegetative hyphae, P1 to P5 = sequentially developing perithecia, with P1 representing the earliest perithecial initials and P5 mature perithecia with ascospores. Numerical values in planta are hours postinoculation in barley heads. Other in planta values are taken from wheat stems: IF = infection front (asymptomatic); WS = water-soaking symptoms, just behind the infection front; SW = senescent, bleached wheat; and YP = young perithecia following incubation of stems in moist vermiculite.

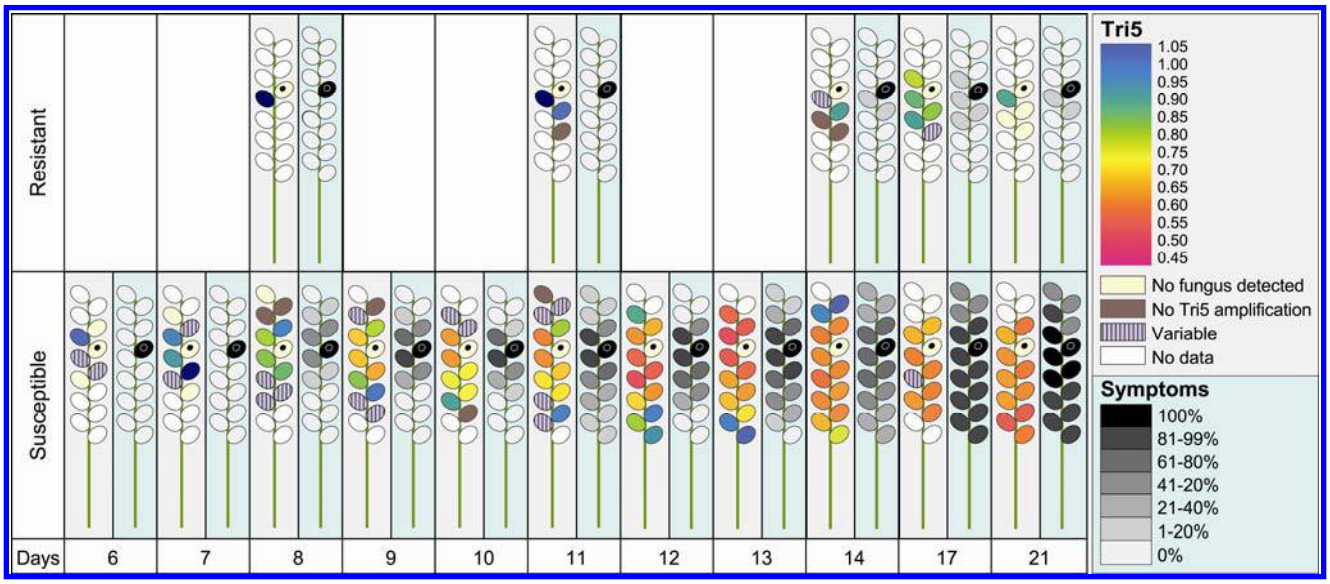

Fig. 2. Deoxynivalenol (DON) gene expression and percent kernel infection relative to inoculation point. Top row is 'Alsen' (carrying FHB1-mediated resistance) plants and bottom row is 'Wheaten' (susceptible). Numbers refer to days postinoculation. Transcript abundance of the DON biosynthetic gene *Tri5* relative to the housekeeping gene glyceraldehyde phosphate dehydrogenase (*GAPDH*), as determined by cycle threshold (Ct)*Tri5*/Ct*GAPDH*, is shown in color (left); blue indicates higher proportional *Tri5* transcript abundance, while red and pink indicate lower proportional abundance. *GAPDH* is constitutively expressed and is detected whenever the fungus is present. "Variable" kernel positions indicate that *Tri5* was detected in one or more replicates, while at least one replicate lacked *Tri5* or fungal gene expression. Percent kernels showing visible infection at harvest is shown in grayscale (right). Symptoms of infection included water-soaking at the point of attachment to the rachis, softening and rotting of the kernel, drying and shriveling of the kernel, and fungal hyphal growth. Inoculation point is indicated by the black spot on the middle right kernel.

ceptible spring wheat treated with azoxystrobin and untreated plants, in either visible symptom development, DON quantities, or *Tri5* (or fungal housekeeping) gene transcript abundance.

The striking increase in expression of several *Tri* genes (including *Tri5*) 72 h after inoculation of barley (Fig. 1) corresponds to the first appearance of lesions (4), when the fungus is just becoming abundant in the plant. The peak of expression of *Tri5* in the present study in wheat appears consistently at the infection front. In wheat, *Tri5* expression of invading hyphae was shown to be moderated by tissues in the head, with kernel tissue having strong inductive effects (23). Our results suggest that the inductive effect lessens as the kernel is increasingly colonized (Fig. 2). Regulation of the *Tri* pathway is complex, with positive and negative regulatory factors at play (12,40), as well as nutritional sources (27) and pH (32).

qRT-PCR studies reflect the relative transcript level of the genes of interest—in this case, *Tri5*—and not merely presence of genes that may or may not be expressed under study conditions. Schmidt-Heydt and colleagues (39) used the expression levels of six genes in the trichothecene pathway (*Tri4, Tri5, Tri6, Tri10, Tri12*, and *Tri13*) to derive a polynomial equation linking DON levels to gene expression in *F. graminearum* and *F. culmorum*, with an R^2 of 0.9542. Our study primarily examined expression of *Tri5* relative to fungal housekeeping genes, which yields results inconsistent with quantitative DON prediction. For example, in susceptible Wheaten, the highest DON level observed (158 ppm) was associated with a comparatively low Ct ratio (0.6), whereas higher Ct ratios—and, thus, higher relative *Tri5* expression—correlated with much lower DON levels (22 to 45 ppm). This is explicable by the fact that the highest relative *Tri5* expression was consistently observed at or near the infection front, where overall gene expression of both *Tri5* and housekeeping genes *GAPDH* and *EF1A* was comparatively low, due to the reduced presence of the fungus.

Several qPCR studies have used the presence and quantification of *Tri5* in genomic DNA as a marker for the presence of potentially trichothecene-producing *Fusarium* spp. (22,38,47). By providing a quantitative measure of *Fusarium* spp. present in the host plant, this qPCR data can be correlated with disease severity or, conversely, with grain yield and quality (22). Zhang and colleagues (47) reported a significant linear relationship between $\log_{10}$ *Tri5* DNA and DON quantity; however, arguably, their use of genomic DNA as a template suggests that the same relationship may have been observed had they used any single-copy *Fusarium* gene as their marker.

The resistant spring wheat Alsen exhibited minimal symptom development over 21 days; at the worst, kernels from immediately above to three below the IP were affected, and <20% of kernels at any given position showed damage. The 21-dpi plants exhibited a reduction in *Tri5* transcripts and fungal housekeeping gene transcripts compared with 17 and 14 dpi plants. The FHB1 resistance carried by Alsen is correlated with a resistance to DON accumulation, although the mechanism is not known. Our data show that relative expression of *Tri5* can be quite high in Alsen compared with Wheaten (Fig 2; Table 1) even relatively late in the infection, at day 17. This implies that the resistance mechanism may affect mycelial spread rather than transcription of DON biosynthetic genes. Investigations are in progress to further understand this observation and determine its significance for resistance.

The infection results for Wheaten clearly show that the infection spreads out from the IP both upward and downward. Previous work has shown that the infection front is mainly colonization of the xylem (19,26). By the time the infection reached the bottom of the head (12 dpi), the upper florets were severely shriveled, due to plugging of the vascular system, and the fungus stopped colonizing. The infection front continued past the lowest floret and began to move down the stem.

A major concern for wheat contamination is the presence of asymptomatic grain that has significant toxin levels. These grains are hard to eliminate, because they cannot be physically recognized and there is little understanding of how these infections develop. Other workers have shown that more mature kernels can become infected and show few symptoms but harbor significant DON levels (6,8). The present studies suggest that the highest DON may be associated with early infection. The data presented here show that genes for DON synthesis can be relatively highly expressed in very little mycelium, before symptoms develop. Furthermore, transcripts for DON biosynthetic genes remain even after host tissue senesces. This suggests that infections which, due to environmental or host conditions, remain small may have the potential to produce large amounts of DON in a small amount of mycelium, should environmental conditions permit. Further investigations will be geared toward understanding this interaction.

TABLE 1. Relative expression of Tri5 and deoxynivalenol (DON) accumulation (ppm) in representative kernels of susceptible and resistant wheat cultivars

Wheat, dpi, position[a]	*Tri5* expression (standard error)[b]	Kernels infected (%)	DON (ppm) (standard error)
W8, –4	ND	0	2.03 (1.19)
W11, –4	0.75 (0.07)	34	44.60 (10.83)
W17, –4	0.62 (0.05)	96	36.50 (12.70)
W21, –4	0.62 (0.05)	96	28.60 (4.95)
W11, –8	NM	11	3.43 (1.60)
W14, –8	0.78 (0.04)	25	21.90 (2.84)
W21, –8	0.60 (0.002)	91	158.00 (10.16)
A14, –1	0.68; IF	7	0.88 (0.58)
A17, –1	0.86 (0)	19	1.77 (0.63)
A21, –1	0.86 (0.01)	9	1.04 (0.74)
A8, –4	NM	0	10.20 (4.14)
A14, –4	ND	0	0.88 (0.54)
A17, –4	ND	0	2.05 (1.48)
A21, –4	ND	0	2.50 (1.25)

[a] W = susceptible 'Wheaten', and A = resistant 'Alsen' at days postinoculation (dpi) for each kernel position.

[b] ND = *Tri5* was not detected, NM = no measurement taken, and IF = infection front; one or more reps showed no detectable fungus, while one or more reps detected housekeeping genes and, in some cases, *Tri5*. Data from the rest of the head suggests no *Tri5* for W11, –8, and no fungus for the Alsen NMs. DON values <20 could not be reliably distinguished from background.

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