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Host-preferential *Fusarium graminearum* gene expression during infection of wheat, barley, and maize

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ABSTRACT

Fusarium graminearum is a broad host pathogen threatening cereal crops in temperate regions around the world. To better understand how *F. graminearum* adapts to different hosts, we have performed a comparison of the transcriptome of a single strain of *F. graminearum* during early infection (up to 4 d post-inoculation) of barley, maize, and wheat using custom oligomer microarrays. Our results showed high similarity between *F. graminearum* transcriptomes in infected wheat and barley spike tissues. Quantitative RT-PCR was used to validate the gene expression profiles of 24 genes. Host-specific expression of genes was observed in each of the three hosts. This included expression of distinct sets of genes associated with transport and secondary metabolism in each of the three crops, as well as host-specific patterns for particular gene categories such as sugar transporters, integral membrane protein PTH11-like proteins, and chitinases. This study identified 69 *F. graminearum* genes as preferentially expressed in developing maize kernels relative to wheat and barley spikes. These host-specific differences showcase the genomic flexibility of *F. graminearum* to adapt to a range of hosts.

Introduction

The fungal pathogen *Fusarium graminearum* infects cereal crops, causing Gibberella ear rot (GER) and stalk rot of maize and Fusarium head blight (FHB) in small grain cereals such as wheat, barley, and oats. The infection of floral structures reduces grain quality and contaminates the grain with mycotoxins. Several mycotoxins, including trichothecenes and the phytoestrogenic compound zearalenone, are detected in *F. graminearum*-contaminated cereals and maximum mycotoxin levels have been imposed by many countries to protect the food and feed supply (van Egmond *et al.* 2007). Although

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Abbreviations; FHB, Fusarium head blight; GER, Gibberella ear rot; DON, deoxynivalenol; NRPS, nonribosomal peptide synthase; PKS, polyketide synthase; TS, terpenoid synthase

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fungicide treatments and improved agronomic practices can help to reduce *F. graminearum* in low to moderate infection years, *F. graminearum*-resistant cereals are required to prevent devastating losses and reduce mycotoxin contamination during epidemics.

Germination of *F. graminearum* usually takes place within 6–12 h of plant contact and hyphae initially grow asymptomatically to form hyphal networks on the surface of floral tissues (Bushnell *et al.* 2003). Subcuticular hyphae and bulbous infection hyphae were perceived by 48–72 h on inoculated, detached wheat (Rittenour & Harris 2010). Compound appressoria (including lobate appressoria and infection cushions) were observed as penetrating infection structures in inoculated wheat florets (Boenisch & Schäfer 2011). In wheat, the fungus spreads from floret to floret through vascular tissues in the rachilla and rachis while in barley infection is usually confined to the initial infected spikelet (Bushnell *et al.* 2003). Macroconidia or ascospores can infect maize ears by landing on exposed silks or entering directly through bird or insect-damaged kernels (Sutton 1982). Global transcriptome profiling of *F. graminearum* during infection of barley spikes and wheat spikes, stalks, crown, and coleoptiles has been monitored using Affymetrix gene chips (Güldener *et al.* 2006; Stephens *et al.* 2008; Guenther *et al.* 2009; Lysøe *et al.* 2011; Zhang *et al.* 2012). Studies profiling gene expression in barley and wheat spikes detected 10 007 and 7777 probe sets, respectively, during the initial 196 h after inoculation (Lysøe *et al.* 2011). Between 416 and 799 *F. graminearum* genes expressed specifically *in planta* were identified when comparing gene expression profiles from infected spikes to those from mycelium grown under different *in vitro* conditions (Güldener *et al.* 2006; Guenther *et al.* 2009; Lysøe *et al.* 2011). Of the *in planta* specific genes, subsets were tentatively designated as wheat and barley specific; however, uncertainties remained concerning the specificity of those gene lists because the wheat and barley gene expression profiles were assessed under different experimental conditions (Lysøe *et al.* 2011). Zhang *et al.* (2012) compared fungal transcriptomes between laser-captured hyphae growing within the wheat coleoptile and during an equivalent growth time point *in vitro* and identified 344 genes preferentially expressed *in planta*. They observed that the fungus metabolizes stored lipids via the fatty acid oxidation and glyoxylate pathways to compensate for nutrient limitation during early infection (Zhang *et al.* 2012).

This study expands *F. graminearum* gene expression profiling to an additional monocot host, maize, and re-examines the lists of genes specifically expressed in wheat and barley by comparing expression profiles from samples grown and treated under the same experimental conditions.

Materials and methods

Host plant inoculation

Fusarium graminearum strain DAOM180378 (obtained from the Canadian Collection of Fungal Cultures, Ottawa, ON) was used for the inoculation of the three *F. graminearum*-susceptible host monocots. DAOM180378 was isolated from naturally infected maize ears in Ottawa, ON in 1981 by G.A. Neish. Maize inbred B73 was grown on Central Experimental Farm fields, Ottawa, ON in 2004 and 2006. Eleven days after sib-crossing, B73 developing kernels were inoculated through the husks using a four-pin inoculator device, injecting 3–4 kernels per puncture with either a macroconidia suspension (5×10^5 spores mL^{-1}) or modified Bilay's media (control), as described previously (Reid *et al.* 1992; Mohammadi *et al.* 2011). Fields were irrigated daily to maintain humidity. The spring wheat cv. Roblin and 6-row barley cv. Encore were grown in a controlled-environment cabinet with 16 h light (20 °C) and 8 h dark (16 °C). At mid-anthesis, each biological replicate of six wheat spikes were point inoculated by pipetting 10 μL of a 1×10^5 spores mL^{-1} *F. graminearum* macroconidia suspension between lemma and palea of two primary florets in each spikelet. All fully developed spikelets on each spike were inoculated. In the case of barley, each fully developed floret of two central rows was inoculated at mid-anthesis while florets from laterals rows were removed from the rachis. In each inoculated barley floret, the tip of the lemma, including the awn, was cut off and 1000 *F. graminearum* macroconidia were pipetted inside the floret. Mock inoculation using water was carried out in parallel. Inoculated wheat and barley plants were misted overhead for 20 s every 30 min for 2 d to maintain high humidity during the initial infection period and then moved to a non-misted bench in the same growth room. Infected ears or spikelets were harvested 1, 2, and 4 d post-inoculation, flash-frozen in liquid nitrogen, and stored at −80 °C.

Microarray protocol and analysis

After grinding plant tissue with liquid nitrogen using a mortar and pestle, total RNA was extracted by using the guanidine isothiocyanate-caesium chloride method (Ohan & Heikkila 1995). Prior to labelling, RNA quality was verified using a 2100 BioAnalyzer (Agilent Technologies Canada Inc, Mississauga, ON, Canada). For each treatment and time point, hybridizations were done on three biological replicates, directly comparing fungal-inoculated and mock-inoculated samples, with two reverse-dye technical replicates for each biological sample. Total RNA (500 ng) was labelled using reagents and the procedure described in the 'Two-color Quick Amp Labeling' protocol version 5.7 (Agilent Technologies), except that only half the amounts of reagents were used in Step 2 of the labelling reaction. Samples were hybridized to a custom *Fusarium graminearum* 4 × 44 K oligomer microarray (Agilent Technologies) (NCBI GEO record # GPL11046 – *F. graminearum* 4 × 44 K) with up to three individual 60-mers for each of 13 918 predicted genes. Hybridizations were incubated overnight at 65 °C in a Robbins 400 oven outfitted with an Agilent hybridization oven rotator. The standard wash protocol was used. Slides were scanned on a Genepix 4200a (Molecular Devices, Inc., Sunnyvale, CA) at 5 μ resolution. Images were quantified using Genepix Pro 6.0 (Molecular Devices).

Array results files (.gpr) were imported into Acuity 4.0 (Molecular Devices) and normalized by a linear, ratio-based method using the spike-in control (+)E1A_r60_1 (spotted twenty times on each array), which has a cy5:cy3 ratio of 1:1, as reference. Two datasets were formed with all arrays and the data types were changed to F532–B532 and

F635−B635 to remove false signals from dust or other contaminants. Probes that did not have intensities higher than 250 in at least one of nine replicates for each dataset were removed. Cross-hybridizing plant sequences were eliminated by comparing mock- and *Fusarium*-inoculated hybridization intensities and removing 1055 probes with (F635 median−B635) >100 in 50 % of arrays. For each plant host, expression data is in Ratio of Medians (treatment vs mock) and probe data was retained which exhibited a) intensity >100 in at least two replicates; b) Ratio of Medians >2 and not <0.75 in at least three replicates. Oligomers were assigned to genes and Funcat categories in the FGDB database version 3.2 of the Munich Information Center for Protein Sequences (MIPS) (ftp://ftpmips.gsf.de/FGDB/), as well as alternate gene calls from Ma *et al.* (2010). Array data has been deposited at NCBI (GEO accession #GSE37886).

Quantitative PCR

All PCR primers are listed in Supplementary Table 1. The relative fungal biomass in infected plant samples was estimated using two assays. The amount of *Fusarium graminearum* genomic DNA relative to plant DNA was measured by qPCR. Total genomic DNA was extracted using the Nucleon Phytopure DNA extraction kit (Amersham Bioscience, Quebec, Canada). For *F. graminearum* DNA quantification, three housekeeping genes, *GAPDH* (*FGSG_16627*), *β-tubulin* (*FGSG_09530*), and elongation factor-1 (*FGSG_08811*) were amplified in parallel with one wheat reference gene, heterogeneous nuclear ribonucleoprotein Q (*hn-RNPQ*, Ta.10105) which was used for normalization of the data separately for each host. Fungal biomass was also estimated using RT-qPCR by measuring the relative transcript levels of the same three *F. graminearum* housekeeping genes, with normalization to two plant housekeeping genes, heterogeneous nuclear ribonucleoprotein Q (*hn-RNPQ*, Ta.10105) and *GAPDH* (Ta.16204), for which primers recognize conserved sequences in wheat, barley, and maize.

For quantitative RT-qPCR analyses, RNA samples were treated with an RNase-free DNase (Qiagen, Mississauga, Canada) and purified using the RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. Next, RNA samples were normalized relative to each other following precise measurement with the Quant-iT RiboGreen RNA assay kit (Invitrogen, Carlsbad, CA, USA) in triplicate on a single microtiter plate. cDNA synthesis was performed using the RETROscript kit (Ambion Inc, TX, USA), following the manufacturer's instructions.

RT-qPCR assays were performed using the Brilliant SYBR-Green QPCR kit (Agilent Technologies). The relative level of expression of *F. graminearum* genes during the development of fungal infection in wheat, barley, and maize plants was measured relative to the expression of three *F. graminearum* housekeeping genes listed above, which were used as a reference for normalization of the data. Data normalization and rescaling was performed as described previously (Wang *et al.* 2010).

Mycotoxin quantitation

An aliquot of ground tissue from each sample was freeze-dried and weighed prior to quantification of the mycotoxin deoxynivalenol (DON). DON analysis was performed on mock and *Fusarium graminearum*-infected tissues using a DON-specific antibody and ELISA analysis (Savard *et al.* 2000). The DON concentration is the average of two technical replicates; when the two measurements differed by more than 10 %, the sample analysis was repeated.

Phylogenetic analysis

Multiple sequence alignment of protein sequences were performed with the Clustal W program, available in Geneious Pro 5.6.4 (http://www.geneious.com), using BLOSOM Protein Weight Matrix, where gap penalties were set at the default values. The construction of a phylogenetic tree, using a maximum likelihood approach, was performed with PhyML 2.1.0 (Guindon & Gascuel 2003).

Results

Fungal biomass and mycotoxin accumulation in three hosts

To compare the transcriptome of *Fusarium graminearum* during the early infection of three different plant hosts, we inoculated wheat and barley spikes, and maize ears with the same 15-ADON-producing strain of *F. graminearum* and collected tissues at 1, 2, and 4 d after inoculation. We chose varieties that are each considered quite susceptible to *F. graminearum* within their crop. Wheat and barley florets were grown and point inoculated under the same environmental conditions in growth cabinets. Due to inconsistent GER development indoors and to optimize the fungal-to-plant biomass ratio, direct kernel inoculation was used to infect maize ears in the field.

Fungal biomass was estimated in the infected samples from the three cereal hosts by quantifying three *F. graminearum* housekeeping genes relative to plant genomic DNA using quantitative PCR (qPCR). At 4 d post-inoculation, *F. graminearum* biomass was significantly higher in wheat than in both barley and maize, with barley exhibiting the lowest levels of *F. graminearum* infection (Fig 1A). Similar results were also obtained when transcript levels of the three *F. graminearum* genes were compared to the relevant plant housekeeping gene by RT-qPCR (Supplementary Fig 1).

The amount of the mycotoxin DON produced in the three susceptible hosts was measured in the 4 d samples (Fig 1B). A substantial amount of DON had accumulated in all three hosts by day 4 after inoculation, following the trend observed with fungal biomass. However, relative to the wheat samples, the levels of DON measured in the barley and maize samples were higher than expected when compared to the relative amounts of fungal biomass for the three hosts.

Comparison of Fusarium graminearum *transcriptome in three hosts*

Fungal transcriptome profiling was carried out using a custom-designed Agilent oligomer array containing up to three individual 60-mers for each *F. graminearum* predicted gene. Three biological replicates of inoculated tissues were compared in dual-dye hybridizations to mock-inoculated samples using gene expression ratios, to eliminate from analysis oligomers

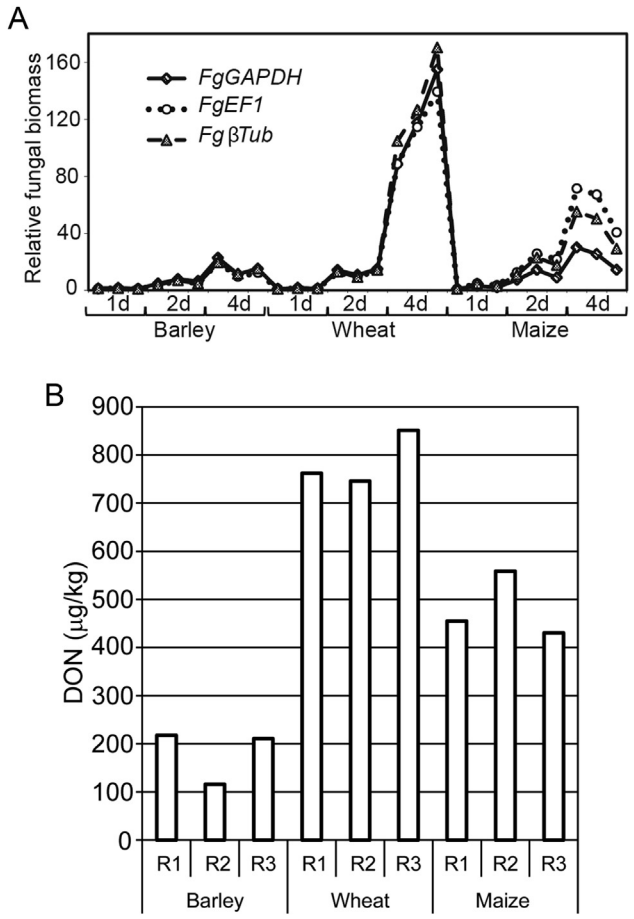

Fig 1 – Comparison of the level of infection and DON deposition by *F. graminearum* in barley, wheat, and maize samples. (A) The relative fungal biomass was estimated using qPCR on genomic DNA to test one plant-specific and three fungal-specific housekeeping genes at 1, 2, and 4 dpi. (B) Quantification of the mycotoxin DON in the three plant hosts at 4 dpi. R1-3, biological replicates 1–3.

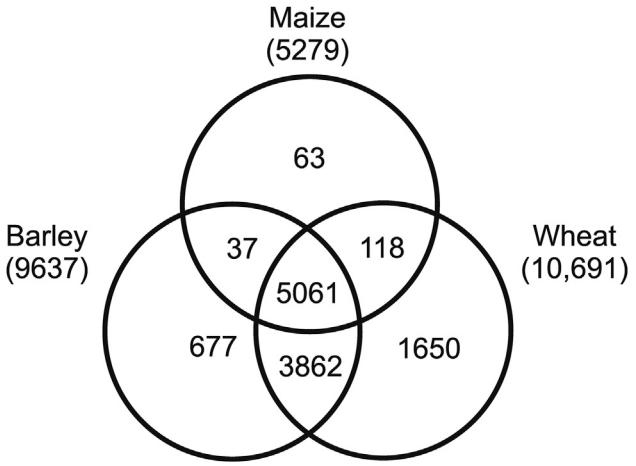

Fig 2 – Venn diagram showing the number of *F. graminearum* genes detected in infected wheat, barley, and maize. Numbers in brackets represent the total number of genes detected in each host. Genes in each category are listed in Supplementary Table 2d.

cross-hybridizing to plant transcripts. In our experimental design, gene expression ratios represented the level of expression relative to background as well as the fraction of fungal biomass contained in the infected samples. After filtering, a total of 27 575 oligomers representing over 10 752 fungal transcripts were perceived in the three cereal hosts. We detected 10 691, 9637, and 5279 *F. graminearum* transcripts in wheat spikes, barley spikes, and maize kernels, respectively (Fig 2, Supplementary Table 2d).

The *F. graminearum* transcriptomes in wheat and barley were very similar, with 8923 common genes detected (Fig 2). As the infection levels in wheat were higher than those in barley, it is likely that some of the genes detected only in wheat could also be expressed in barley, albeit at a level below the sensitivity of our procedure. Supporting this possibility, we have observed that the average expression ratio value for genes detected at 4 d was 45 in wheat while it was 15 in barley. However, the detection of significantly fewer *F. graminearum* genes in maize than in barley was unexpected as fungal biomass and average expression ratio (18 at 4 d in maize) were both higher in maize than in barley tissues.

We examined the data for genes showing host-preferential expression in maize. We eliminated some genes initially identified as maize-specific through manual data inspection and focused on 30 genes specifically expressed in maize. We also identified 39 other genes exhibiting expression signals considerably higher in maize than in wheat and/or barley, for a total of 69 maize preferentially expressed genes (Table 1). Applying the Funcat system (Ruepp *et al.* 2004) to annotate proteins into functional categories, 45 % (31) of the 69 genes encoded proteins of unknown function. One-fifth of the *F. graminearum* genes (14/69) that are preferentially expressed in maize under our conditions are predicted to be involved in transport, compared to 3 % of genes detected primarily in wheat or barley. The remaining genes were primarily in the categories of metabolism (primarily carbon and secondary metabolism), and in cell rescue, defence, and virulence.

Of the 1650 genes preferentially expressed in wheat, only 346 had a predicted function (Supplementary Table 2b). Gene annotation indicated involvement in biological processes associated with the cell cycle and differentiation, and with stress response; this included nucleic acid and protein synthesis/processing/degradation, cell wall biogenesis/degradation, sugar and lipid metabolism, signalling, and gene regulation, transport and secondary metabolite biosynthesis/degradation. Enrichment for genes associated with secondary metabolism and transport was observed. Of the 677 genes preferentially expressed in barley (Supplementary Table 2c), those with a predicted function also showed enrichment for cell wall biogenesis, secondary metabolism, and transport. However, genes associated with protein degradation, nucleic acid processing, signalling, and gene regulation were underrepresented in the genes preferentially expressed in barley when compared to those in wheat.

Most of the genes preferentially expressed in wheat or barley were detected with increasingly higher values as the infection progressed; however, a subset of genes expressed in wheat

Table 1 – *F. graminearum* genes preferentially expressed in developing maize ears. Genes in chromosomal (chr) order. Mean expression ratio relative to mock-inoculated treatment. Replicated gene expression profiles (ratio of medians) in three hosts are shown in Supplementary Table 2a–c.

Gene	Maize				Barley				Wheat				Short annotation
	#o	1 d	2 d	4 d	#o	1 d	2 d	4 d	#o	1 d	2 d	4 d	
Chr 1													
FGSG_00036	3	4.4	4.3	26.2	1	1.3	3.0	1.1	2	5.2	5.6	2.6	Fatty acid synthase
FGSG_15680	3	15.2	12.4	34.1	nd				2	7.4	8.0	1.1	Cytochrome P450
FGSG_11658	1	1.1	6.3	16.2	1				nd				Transcription factor
FGSG_15673	2	10.0	6.9	28.0	nd				2	3.1	4.1	1.1	NRPS8
FGSG_00043	3	12.4	10.9	50.6	1	1.2	1.6	1.4	3	5.1	5.0	0.7	Oxidoreductase
FGSG_00044	3	11.2	14.7	25.1	nd				3	8.3	4. 6	2.6	CHP
FGSG_00046	3	14.8	10.3	40.3	nd				3	5.8	4.9	1.5	Multidrug transporter
FGSG_00048	1	10.7	24.4	50.7	1	1.5	1.9	2.7	1	17.9	5.6	1.1	Flavonol synthase
FGSG_00049	2	21.5	48.0	70.0	2	2.5	1.5	1.3	2	10.0	4.5	2.1	Amino acid aminotransferase
FGSG_00099	1	10.3	37.5	1.0	nd				nd				Carbonic anhydrase
FGSG_00260	2	1.7	2.5	36.7	nd				1	1.8	2.7	6.5	Secreted protein
FGSG_11757	1	3.1	3.7	16.9	1				nd				CHP
FGSG_01196	1	1.4	1.6	5.9	nd				nd				Ca^{2+} transporting ATPase
FGSG_01335	1	1.5	1.2	3.6	nd				nd				CHP
FGSG_01729	1	1.1	3.4	6.5	nd				nd				CHP
FGSG_15892	1	1.6	7.4	12.5	nd				nd				Hypothetical protein
FGSG_12050	1	0.9	16.8	7.1	1				nd				Transferase
FGSG_02038	2	2.0	79.8	105.0	2	16.7	1.9	2.2	2	1.5	1.8	24.2	CHP
FGSG_02309	3	1.4	1.5	17.3	3	1.5	1.2	2.8	3	0.8	1.6	5.5	Secreted protein
FGSG_15598	1	0.5	1.0	30.3	nd				nd				Hypothetical protein
FGSG_17358	3	2.6	9.5	13.2	nd				nd				Polyamine transporter, Mfs1
FGSG_10433	3	14.8	21.5	102.2	3	20.6	2.5	2.7	3	1.1	4.5	6.3	Oxidoreductase
FGSG_10442	2	1.7	1.4	15.1	nd				nd				Secreted protein
FGSG_10576	3	2.2	8.0	14.6	nd				nd				CHP
FGSG_10617	3	42.0	67.8	139.3	1	1.2	4.4	10.8	1	2.2	4.6	7.3	Myxochelin iron transport
FGSG_10694	3	2.1	15.4	24.2	1	11.5	2.5	3.0	nd				CHP
FGSG_10695	3	16.2	45.8	59.2	2	10.6	2.7	1.8	nd				Cytochrome P450
Chr 2													
FGSG_04675	3	3.5	3.1	15.2	1	1.6	1.9	1.2	nd				Methyltransferase
FGSG_04545	2	1.3	5.3	2.5	nd				nd				CHP
FGSG_04544	1	1.1	3.6	5.8	nd				nd				Maltose permease
FGSG_15249	1	1.0	1. 5	2.6	nd				nd				Hypothetical protein
FGSG_04433	1	1.0	3.0	2.6	nd				nd				Glutathione S-transferase
FGSG_04207	1	1.0	1.1	4.9	nd				nd				Glutamine rich protein
FGSG_04204	3	3.7	7.5	42.2	3	2.6	2.9	6.1	3	2.8	6.4	12.2	Carboxylic acid transporter
FGSG_03984	3	2	17.6	26.2	3	11.0	4.8	5.3	3	1.4	2.6	5.3	Lactate 2-monooxygenase
FGSG_03752	2	4.7	8.6	28.4	nd				2	1.6	4.2	1.4	Methyltransferase
FGSG_03693	1	14.0	19.1	5.8	nd				1	3.2	3.0	0.7	Copper transporter
FGSG_03692	1	3.8	24.6	13.7	nd				1	2.1	4.8	3.9	Related to Fre1p and Fre2p
FGSG_03422	3	2.2	104.7	110.7	nd				3	1.6	4.2	7.0	Phenolic acid decarboxylase
FGSG_03295	2	7.3	44.2	90.9	2	1.4	3.0	8.0	2	3.1	5.9	9.2	Secreted protein
FGSG_03213	3	2.3	3.3	47.6	2	12.2	3.2	4.1	nd				Gibberellin 20-oxidase-like
FGSG_03208	1	1.2	6.7	10.0	1	1.1	3.5	2.0	1	2.1	1.7	1.5	Multidrug transporter
FGSG_16362	1	0.9	15.9	25.5	1	4.8	1.2	3.0	1	1.6	1.2	10.8	Multidrug transporter
FGSG_02908	1	0.8	2.9	86.5	nd				nd				Monooxygenase
FGSG_02903	3	20.3	42.0	70.9	3	13.4	2.1	3.4	3	15.9	16.8	0.74	Secreted protein
FGSG_02901	3	3.4	34.8	32.9	3	3.8	4.6	12.8	3	8.3	6.9	3.4	Sulfatase
FGSG_02869	1	1.8	8.7	17.2	nd				nd				Multidrug transporter
FGSG_17047	3	3.5	15.4	19.2	1				nd				Flavanoid reductase
FGSG_08343	3	62.7	38.7	138.9	3	8.4	2.7	11.9	3	16.7	15.4	3.1	H^+ transporting P-type ATPase
FGSG_13426	3	1.2	7.4	9.9	1				nd				Purine-cytosine permease
FGSG_17087	1	1.3	2.0	2.5	nd				nd				Cytochrome P450
Chr 3													
FGSG_04717	3	1.6	2.7	21.3	1	3.7	0.9	6.6	nd				Cytochrome P450
FGSG_04753	2	2.6	3.5	9.1	nd				nd				CHP
FGSG_04757	3	3.4	4.7	24.9	2	2.8	1.6	4.0	nd				Enoyl-CoA isomerase

(*continued on next page*)

Table 1 – (continued)

Gene	Maize				Barley				Wheat				Short annotation
	#o	1 d	2 d	4 d	#o	1 d	2 d	4 d	#o	1 d	2 d	4 d	
FGSG_05471	3	2.7	5.3	8.7	2	6.1	1.2	2.1	nd				Aspartic proteinase
FGSG_06046	2	1.2	6.2	12.1	1	0.6	5.7	3.3	nd				Oxidoreductase
FGSG_06286	1	2.0	0.6	6.0	nd				nd				DNA polymerase
FGSG_11011	2	2.0	18.3	9.6	nd				2	2.9	3.9	1.2	Endo-polygalacturonase
FGSG_10971	1	1.0	0.7	8.4	nd				nd				CHP
FGSG_11470	2	5.2	18.0	18.6	nd				1	1.2	2.5	2.0	CHP
FGSG_11310	3	6.0	11.3	17.5	3	2.3	2.4	6.9	3	6.0	5.1	1.7	Secreted protein
Chr 4													
FGSG_06533	1	1.6	1.0	4.6	nd				nd				CHP
FGSG_06612	3	15.3	27.4	54.0	3	3.7	3.8	11.1	3	2.3	5.4	22.8	Oxalate decarboxylase
FGSG_12977	1	2.0	5.0	14.2	1				nd				Helicase-like
FGSG_07532	1	3.2	2.5	1.0	nd				nd				CHP
FGSG_07809	3	3.4	7.2	17.2	nd				nd				CHP
FGSG_09090	1	0.9	5.4	2.9	nd				nd				Secreted protein
FGSG_09127	1	1.6	13.9	1.1	nd				nd				CHP
FGSG_09804	2	0.9	1.6	12.1	nd				nd				Multidrug transporter

#o = number of oligomers detected; nd = not detected; CHP = conserved hypothetical protein.

tissues presented a contrasting expression profile, with expression ratios above two in either or both of the 1 d and 2 d samples while their expression was not detected at 4 d (i.e. the ratio value mean for the three biological replicates was equal to or smaller than 1) (Supplementary Table 2e). Of the 225 wheat genes expressed preferentially earlier in infection, 42 had a predicted function and showed a functional distribution resembling that of the larger group of genes preferentially expressed in wheat. Similarly, among the genes detected only in barley, we observed a subgroup of 126 genes expressed preferentially at 1 d or 2 d (Supplementary Table 2f). Such a subgroup was not obvious among the genes detected only in maize.

RT-qPCR was used to validate the expression profiles of 24 genes, most of which were initially identified as preferentially expressed during maize infection or during wheat and barley infection. The transcript levels were normalized and rescaled relative to the expression levels of three fungal housekeeping genes, to compare expression between plant hosts. The resulting gene expression profiles generally supported the interpretation of the microarray analysis (Fig 3, Supplementary Fig 2). Many of the genes were clearly preferentially expressed during infection of maize (e.g. *FGSG_03208*, *FGSG_03422*, *FGSG_07809*, *FGSG_10617*, *FGSG_10694*, *FGSG_10695*) while others, such as a gene cluster including the nonribosomal peptide synthase gene NRPS8 (*FGSG_00036*, *FGSG_15673*, and *FGSG_00043*), were expressed in maize and, to a lesser extent, wheat tissues. As predicted, almost no expression was detected in maize for five genes (*FGSG_00060*, *FGSG_00096*, *FGSG_03264*, *FGSG_03981*, and *FGSG_07993*) originally identified as specific to wheat and barley.

Differential expression of gene family members during infection of three hosts

Many of the genes detected preferentially in specific hosts are part of larger gene families, suggesting that *Fusarium graminearum* uses host-specific gene expression of family members to modulate its primary response (Tables 2 and 3, Supplementary Table 3). For example, Table 2 summarizes the number of genes predicted to encode sugar transporters and permeases for which expression was detected in one or more of the three hosts, and the expression data for those genes is compiled in Supplementary Table 3a. With the exception of a possible maltose permease, no sugar transporter gene was detected only in maize. Fifteen sugar transporter genes expressed in maize were also expressed in barley and wheat, generally at high levels (Supplementary Table 3a), and coded for predicted broad-substrate spectrum sugar transporters rather than for more specialized ones. Our observations suggest that *F. graminearum* expresses many broad-substrate sugar transporters in all three hosts while it expresses a subset of broad- and specific-substrate sugar transporters only in wheat and/or barley.

The Drug:H^+ Antiporter-1 (DHA1) proteins, a group of plasma membrane carriers within the Major Facilitator Superfamily (TC2.A.1.2 [http://www.tcdb.org/]), also showed differential expression of family members between hosts. The yeast DHA1 family includes four plasma membrane proteins, TPO1-4, which have been shown to be involved in cellular export of polyamines (Sá-Correia *et al.* 2009). Of the 22 DHA1-like members in the *F. graminearum* genome related to the yeast TPO family of proteins (Fig 4), five (*FGSG_02869*, *FGSG_03208*, *FGSG_04317*, *FGSG_16362*, and *FGSG_17358*) were preferentially expressed during infection of developing maize kernels (Tables 1 and 3, Fig 3, Supplementary Table 3b). *FGSG_04317* was initially identified as expressed in all three hosts, but RT-qPCR analysis indicated that gene expression is much more induced in maize (Supplementary Fig. 2).

Examination of other gene families also illustrated how the host environment influences the expression of other *F. graminearum* gene family members (Table 3, Supplementary Table 3b). Beta transducin-like (WD-40 repeat) proteins are often part of regulatory complexes involved in transcription regulation or signal transduction. One of the gene members of this family, *FGSG_00332*, detected in barley and wheat in our experiment, has been demonstrated to be essential for

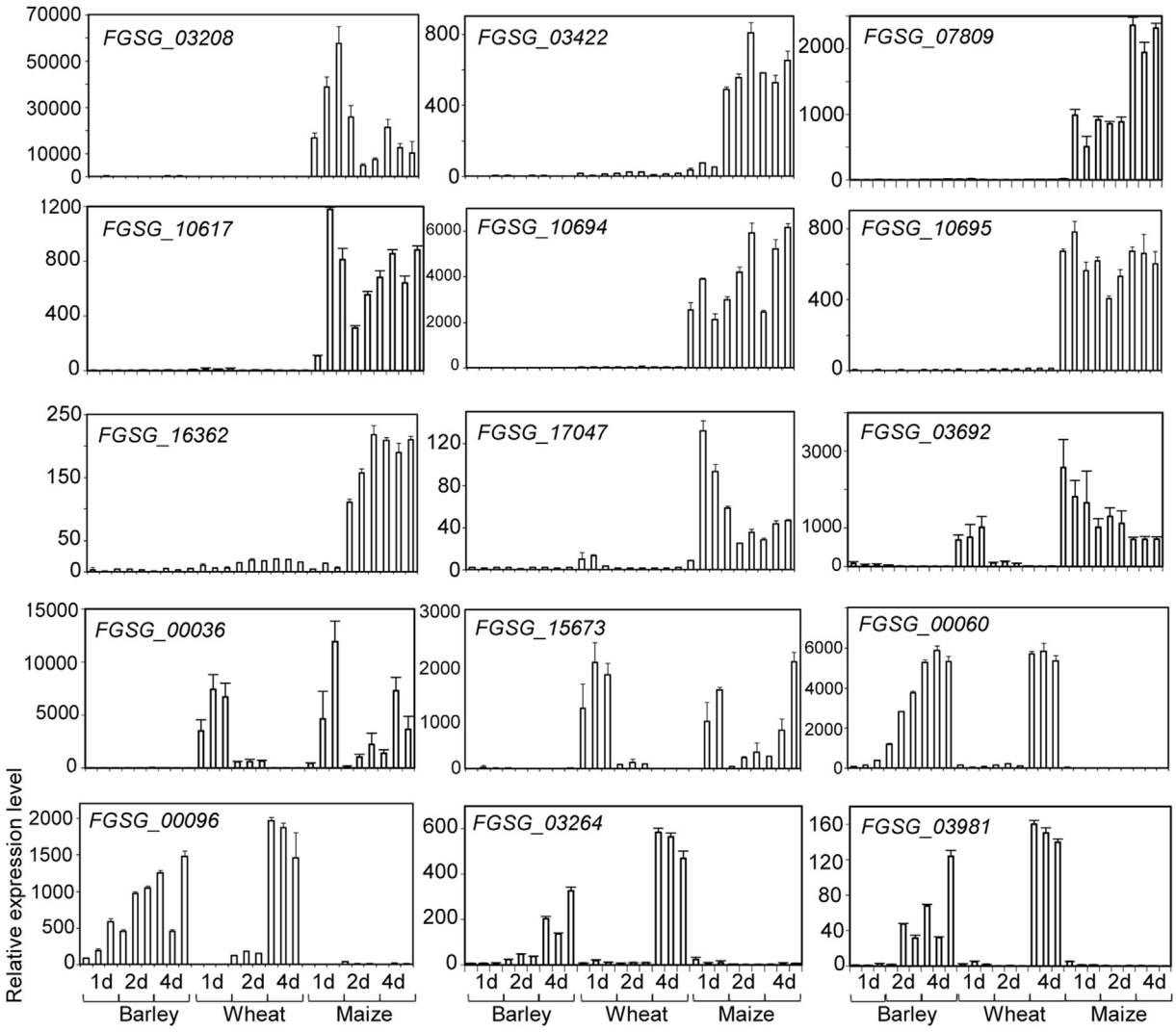

Fig 3 – Validation of expression profiles of selected *F. graminearum* genes *in planta* through RT-qPCR.

pathogenicity in wheat (Ding *et al.* 2009). There are 30 genes detected *in planta* encoding G-protein coupled receptors belonging to the integral membrane protein PTH11 class and many were exhibiting host-preferential expression; PTH11 has been shown to be important for appressorium formation and pathogenicity in *Magnaporthe grisea* (DeZwaan *et al.* 1999). The cell wall-modulating enzymes chitinases and endochitinases, are used by fungi to reshape their own chitin (Sámi

Table 2 – Number of predicted sugar transporter genes detected in each host or host combination categories. The focus is on transport of sugars most likely found in the hosts tissues infected by *F. graminearum* in this experiment.

Sugar transported[a]	Transport type[b]	Maize only	Barley only	Wheat only	Barley + Wheat	Maize, Barley + Wheat
Sugars	A	0	1	0	2	3
Hexoses	A	0	0	3	9	7
Monosaccharides	A	0	2	0	2	0
Sucrose	A	0	0	0	0	2
Maltose	P	1	0	2	8	1
Glucose	A	0	0	0	2	2
Galactose	A	0	0	0	3	0
Fucose	P	0	2	1	1	0
Sorbitol	A	0	0	1	1	0

a According to the annotation in FGDB database version 3.2 of the MIPS.
b Transport type: Active (A) by transporters, Passive (P) by permeases.

Table 3 – Number of genes detected in each host or host combination *in planta* for six distinct gene families, in relation to the total number of predicted *F. graminearum* genes for that family.

Gene family	Maize only	Barley only	Wheat only	Maize + Wheat	Barley + Wheat	Maize, Barley, Wheat	Total # predicted genes
Drug:H^+ Antiporter-1	2	2	2	0	5	7	22
β transducing–like protein	–	2	6	1	4	2	17
Integral membrane, PTH11	–	5	9	–	13	3	30
Chitinases	–	1	4	–	3	–	10
Endochitinases	–	–	2	–	–	3	6
Epoxide hydrolases	–	–	3	–	1	3	9

et al. 2001). We observed eight chitinase genes expressed only in wheat and/or barley while three of five detected endochitinase genes were expressed in all three hosts. Epoxide hydrolases convert epoxides arising from aromatic compound degradation into dihydrodiols and could be used by *F. graminearum* to detoxify host aromatic compounds; seven epoxide hydrolase genes exhibited various expression patterns in wheat, maize, and barley. Overall, our results suggest that *F. graminearum* exploits the flexibility of its genome to modulate its gene expression profile to adapt to substrate differences or plant defences between diverse hosts.

Three clustered genes encoding KP4 killer toxin-like proteins (*FGSG_00060*, *FGSG_00061*, and *FGSG_00062*) were only detected in wheat and barley and RT-qPCR confirmed there was little or no expression of *FGSG_00060* in maize (Fig 3).

Expression of secondary metabolism genes

Bioinformatic analyses have predicted that the genome of *Fusarium graminearum* contains 19 nonribosomal peptide synthases (NRPS), 15 polyketide synthases (PKS), and 17 terpenoid synthases (TS), but only a fraction of these synthases are associated with known products (Gaffoor *et al.* 2005; Tobiasen *et al.* 2007; Ma *et al.* 2010; Hansen *et al.* 2012; Hansen *et al.* 2015). Expression of nine NRPSs and four PKSs was detected in all three hosts, although expression levels varied between hosts (Table 4, Supplementary Table 4a). For five NRPSs, and three PKSs, expression was detected in wheat and barley, but not maize, including PKS10 which is required for fusarin C biosynthesis. Finally, there was no or very little expression for four NRPSs (NRPS7, NRPS13, NRPS15, NRPS17), and five PKSs (PKS3, PKS4, PKS6, PKS9, PKS13). Turning to terpene synthases, trichodiene synthase (*TRI5*) (Desjardins & Proctor 2007) and longiborneol synthase (*CLM1*) (McCormick *et al.* 2010), required for trichothecene and culmorin biosynthesis, respectively, were expressed at high levels in all three hosts. Expression of *FGSG_06444*, the TS gene most closely related to *CLM1*, was also detected in all three monocots. Three other TS genes were detected at lower levels only in wheat and barley (*FGSG_01738*, *FGSG_17725*) or only in wheat and maize (*FGSG_16873*) (Table 4). Several of the TSs were predicted to be involved in essential cellular functions, e.g. prenyl transferases producing polyprenyl precursors; expression of these genes (*FGSG_04591*, *FGSG_06784*, *FGSG_09381*, and *FGSG_10933*) was detected in all three hosts (Table 4). Our expression results suggest that there are numerous undiscovered *F. graminearum* secondary metabolites produced by these synthases during the early stage of infection.

Recent bioinformatic and gene coregulation analyses (Sieber *et al.* 2014) have predicted 67 *F. graminearum* gene clusters involved in secondary metabolite biosynthesis. We examined expression profiles of these established or putative biosynthetic gene clusters (Supplementary Table 4b) to ascertain host influence on expression patterns. Expression of gene clusters demonstrated to be involved in the biosynthesis of trichothecenes (C03, C23), butenolide (C49), and culmorin (C59) was detected during the infection of all three monocot hosts. *FGSG_17598*, required for culmorin biosynthesis (L.H., unpubl. results), was the only gene of cluster 1 strongly expressed in all three monocots. Expression of all genes in the aurofusarin biosynthetic cluster (C13, *FGSG_02320 – FGSG_02330*) (Malz *et al.* 2005) was detectable at 2 dpi in wheat only, although that expression was transient; expression of a subset of these genes was detected in maize and barley. This is consistent with observations that visible accumulation of the red aurofusarin pigment occurs much later in infection.

Among the gene clusters not yet associated with a known product, expression of most of the NRPS, PKS, TS, and neighbouring genes was detected mainly in wheat and barley or solely in wheat. Sieber and associates had proposed that three novel gene clusters, C16, C62, and C64, were likely to be involved in the plant infection process (Sieber *et al.* 2014). In our study, clusters 16 (*FGSG_04596 – FGSG_04588*) and 64 (NRPS5/NRPS9, *FGSG_10995 – FGSG_17487*) were expressed in all three hosts while cluster 62 (*FGSG_10608 – FGSG_10617*) was expressed in wheat and barley only, with the notable exception of the NRPS-like gene *FGSG_10617*, which was strongly and preferentially expressed in maize (Fig 3). This observation supports the suspicion that *FGSG_10617* may not truly belong to cluster 62 (Sieber *et al.* 2014). Other gene clusters exhibiting notable preferential expression in wheat and barley included C06 (NPRS16, NRPS19), C22, and C48. In addition, cluster 2 surrounding and including NRPS8 was preferentially expressed in maize, with only transient expression at the onset of infection in wheat, as supported by RT-qPCR analysis (Supplementary Table 4b, Fig 3). We also noticed another small gene cluster (C68, *FGSG_09110 – FGSG_09113*) preferentially expressed in barley which includes an esterase and a pair of monooxygenases.

Discussion

We have performed a comparison of the transcriptome of a single strain of *Fusarium graminearum* while infecting three distinct plant hosts. Our results showed strong similarity

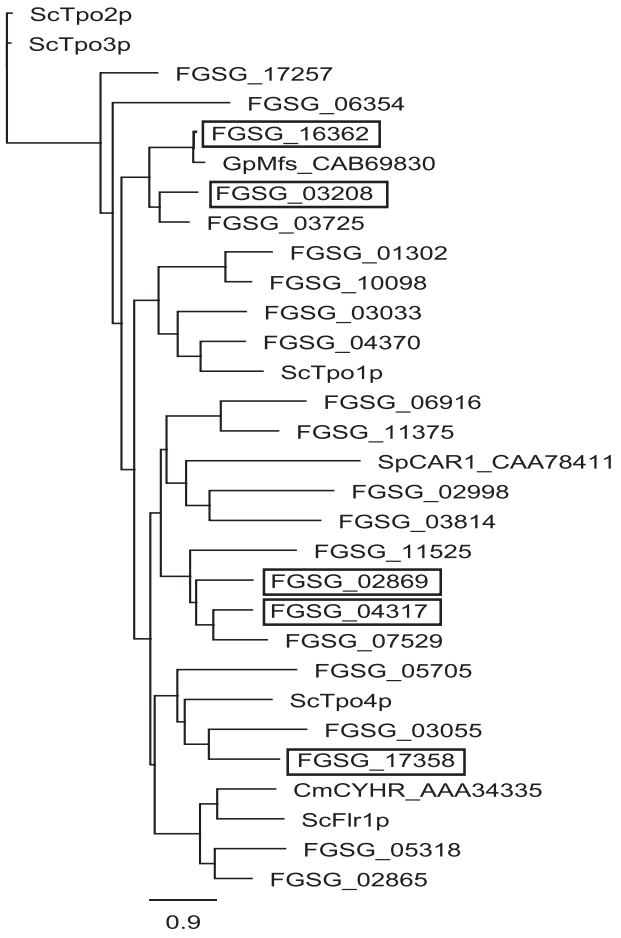

Fig 4 – Phylogenetic tree of known and predicted DHA1 transporter members of the Major Facilitator Superfamily in *F. graminearum* and other fungi. *F. graminearum* proteins extracted from gene loci version 3.2 (http://pedant.helmholtz-muenchen.de/pedant3htmlview/pedant3view?Method=start_method&Db=p3_p13839_Fus_grami_v32). Genbank accession numbers are provided in brackets for non-*F. graminearum* proteins: CmCYHR, *Candida maltosa* cycloheximide resistance protein (AAA34335); GpMfs1, *Fusarium sambucinum* major facilitator superfamily 1 (CAB69830); ScTpo, *Saccharomyces cerevisiae* polyamine transporter proteins (ScTpo1p, DAA09292; ScTpo2p, DAA08230; ScTpo3p, DAA11568; ScTpo4p, DAA11039); ScFlr1, *Saccharomyces cerevisiae* fluconazole transporter protein (DAA07129); SpCAR1, *Schizosaccharomyces pombe* amiloride resistance protein (CAA78411). The bar represents the number of substitutions per site. *F. graminearum* genes preferentially expressed in maize tissues are boxed.

Table 4 – Expression profiles for all detected NRPSs, PKSs, and TSs in three cereal hosts. Gene expression ratios: + = moderate; ++ = high; nd = not detected.

Gene loci	Gene (End product)	*In planta* expression		
		Maize	Barley	Wheat
FGSG_11026	*NRPS1* (malinochrome)	nd	+	++
FGSG_05372	*NRPS2* (ferricrocin)	+	+	++
FGSG_10523	*NRPS3*	nd	+	++
FGSG_02315	*NRPS4*	++	++	++
FGSG_17487	*NRPS5*	++	+	++
FGSG_03747	*NRPS6* (fusarinine)	++	+	++
FGSG_15673	*NRPS8*	++	nd	+
FGSG_10990	*NRPS9*	++	+	++
FGSG_06507	*NRPS10*	+	+	++
FGSG_03245	*NRPS11*	+	+	++
FGSG_17574	*NRPS12*	nd	++	++
FGSG_11395	*NRPS14*	+	+	++
FGSG_15872	*NRPS16*	nd	+	++
FGSG_17386	*NRPS18*	+	+	+
FGSG_15676	*NRPS19*	nd	+	+
FGSG_17387	*PKS1*	+	nd	+
FGSG_04694	*PKS2*	+	nd	+
FGSG_17168	*PKS3*; *PGL1*	nd	+	nd
FGSG_17677	*PKS5*	nd	+	++
FGSG_08208	*PKS6* (fusaristatin A)	nd	nd	+
FGSG_08795	*PKS7*	+	+	++
FGSG_03340	*PKS8*	++	+	+
FGSG_07798	*PKS10* (fusarin C)	nd	+	+
FGSG_01790	*PKS11*	nd	+	++
FGSG_02324	*PKS12*; *AUR1* (aurofusarin)	+	nd	+
FGSG_03964	*PKS14*	+	+	+
FGSG_04588	*PKS15*	+	+	+
FGSG_06444	*TS*	+	+	+
FGSG_08181	*TS*	nd	+	+
FGSG_10933	*TS*	+	+	+
FGSG_16873	*TS*	+	nd	+
FGSG_10397	*CLM1* (culmorin)	++	++	++
FGSG_15742	*TS*	nd	nd	+
FGSG_01738	*TS*	nd	+	+
FGSG_03066	*TS*	nd	+	+
FGSG_03494	*TS*	nd	nd	+
FGSG_03537	*TRI5* (trichothecenes)	++	++	++
FGSG_04591	*TS*	+	+	+
FGSG_06784	*TS*	+	++	++
FGSG_09381	*TS*	+	+	++
FGSG_17725	*TS*	nd	+	+

between the transcriptomes observed in infected wheat and barley spike tissues. Surprisingly, a significant difference in the number of genes with detectable expression was observed in *F. graminearum*-infected maize samples. Host-specific expression of genes was observed in each of the three hosts. This included expression of distinct sets of genes associated with transport and secondary metabolism in each of the three crops, as well as host-specific enrichment for particular gene categories (i.e. protein degradation, nucleic acid processing, signalling, and gene regulation in wheat; cell wall biogenesis in wheat and barley; carbon metabolism, cell rescue, defence and virulence in maize). These host-specific differences strongly suggest that *F. graminearum* has the genome flexibility to adapt to a range of host environments.

Although the intent was to provide similar environmental conditions, the different crop architecture and environmental requirements, especially between maize and the small grain cereals, necessitated some crop-specific conditions. We still employed the inoculation technique optimized for each crop for reproducible and optimal disease development. Inoculation of developing maize kernels was achieved through an injection method accompanied with wounding through the husk directly into the kernels. Therefore, some of the

observed maize host specificity may be due to the fungus responding to this wounded environment not experienced in the wheat and barley spikes. In addition, the field-grown maize plants experienced much higher fluctuations in temperature than the wheat and barley grown in growth cabinets. In both 2004 and 2006, temperatures on the Central Experimental Farm ranged from below 12 °C overnight (known to cause cold stress in maize) to ~26 °C during the day, both prior to and over the 4 d inoculation and collection time period (data not shown). However, it has been our experience that it is difficult to obtain consistent GER infection when maize is grown indoors relative to in the field. Additionally, wounding via weather-related events or insect damage is a known disease route for GER (Sutton 1982). Further experimentation will be necessary to establish the exact induction parameters and host specificity of these *F. graminearum* genes.

A comparison of our results with other transcriptomic and proteomic profiling of *F. graminearum* grown in the presence of plant material is summarized in Supplementary Table 5. Of the genes identified to be expressed specifically in wheat and not in barley by transcriptome analysis (Guenther *et al.* 2009; Lysøe *et al.* 2011), about 50 % of them were detected in two or three hosts in this study while about 15 % remained wheat-specific and 5 % were detected only in barley under our experimental conditions. A similar trend was observed for the genes identified as specific to barley by Lysøe *et al.* (2011). Among the genes identified in previous transcriptome studies as *in planta* specific, a majority of them (i.e. 63–88 %) were detected in at least two hosts in our experiment (Güldener *et al.* 2006; Stephens *et al.* 2008; Guenther *et al.* 2009; Lysøe *et al.* 2011; Zhang *et al.* 2012). Of the 69 genes identified as preferentially expressed in maize, 48 were either not detected or detected at very low levels in infected barley and/or wheat spikes according to Affymetrix transcriptome profiling (Lysøe *et al.* 2011). Expression of 23 of the 69 genes preferentially expressed in maize was not detected on solid media (Güldener *et al.* 2006). Comparing our results with proteomic profiling of *F. graminearum* grown in the presence of plant material indicated that the majority (i.e. 75–80 %) of the proteins detected in those studies corresponded to genes detected in all three hosts in our study (Supplementary Table 5) (Paper *et al.* 2007; Yang *et al.* 2012). Differences observed between the studies could be explained by differences between fungal strains, host varieties, environmental conditions, and/or transcriptome technology platforms. The comparison between multiple studies helped to discern a core list of genes expressed only during *in planta* growth (and not *in vitro*) in all transcriptome studies examined, including a group of genes that we observed to be expressed in all three hosts and another one observed only in wheat and barley tissues (Supplementary Table 6). The majority of these genes encode secreted proteins demonstrated or predicted to be involved in cuticle and cell wall degradation (Balcerzak *et al.* 2012; Brown *et al.* 2012) as well as genes involved in secondary metabolism, including three members each of the secondary gene clusters C64 and C66 (Sieber *et al.* 2014). Zhang *et al.* (2012) have shown that the predicted peptidoglycan deacetylase FGSG_10992, as well as a probable cellulose 1,4-β-cellobiosidase (FGSG_00571) and a putative endo-1,3(4)-β-glucanase (FGSG_00184) distinct from those identified here, contribute to the infection process on wheat coleoptiles. Intense activity in cell wall degradation by fungal pathogens has been proposed to serve both as support for penetration and supply of carbohydrates (Horbach *et al.* 2011).

During early germination, fungi are more sensitive to environmental stresses. Plant pathogenic fungi can employ membrane transporter proteins of the major facilitator (MFS) and ATP-binding cassette (ABC) superfamilies to protect themselves against endogenous and exogenous toxins, including self-produced mycotoxins and plant defence compounds such as phytoalexins and phytoanticipins (Stergiopoulos *et al.* 2002). The list of *F. graminearum* genes preferentially expressed in the maize host includes a disproportionate number of genes involved in cellular transport, including five MFS DHA-1 transporters. MFS transporter proteins exploit electrochemical gradients to transport small molecules across membranes. Although MFS transporters are not as well conserved as ABC transporters, there is a close relationship between sequence homology and function as demonstrated by phylogenetic analysis (Stergiopoulos *et al.* 2002). Preferentially expressed during maize kernel infection, *FGSG_16362* and *FGSG_03208* encode proteins which share homology (88 % and 48 % amino acid sequence identity, respectively) with *Fusarium sambucinum* GpMfs (CAB69830), which is strongly induced in the presence of the potato phytoalexin rishitin (Del Sorbo *et al.* 2000).

FGSG_17358 is closely related to the yeast TPO4 protein, a transporter of polyamines including spermine, putrescine, and spermidine (Tomitori *et al.* 2001). Free and conjugated polyamines accumulate during early kernel development in maize prior to the inoculation time point of 11 d post-pollination (Liang & Lur 2002). Chilling stress causes polyamine levels to increase in maize embryos (Zheng *et al.* 2009). Thus, polyamines may be at higher concentrations in developing maize kernels inoculated in the field than in inoculated wheat and barley at mid-anthesis grown under more controlled conditions and may have induced higher than expected DON concentrations in the maize tissues. Since polyamines have been shown to ameliorate biotic and abiotic stresses in eukaryotic cells, the presence of polyamines may be beneficial to both the plant host and fungal pathogen.

Some *F. graminearum* genes preferentially expressed in maize kernels may be induced and recruited to detoxify metabolites such as phytoalexins more likely encountered in maize kernels than wheat and barley spikes. Zealexins are acidic sesquiterpenoid phytoalexins recently discovered in maize; zealexins exhibit antifungal activity and are known to be induced by *F. graminearum* (Huffaker *et al.* 2011). Six related ent-kaurane diterpenoid phytoalexins, named kauralexins, accumulate in maize tissue after attack by insects and fungi (Schmelz *et al.* 2011). The maize *An2* gene is strongly up-regulated during the infection of developing maize kernels by *F. graminearum* and encodes an *ent*-copalyl diphosphate synthase which has been proposed to be involved in kauralexin biosynthesis (Harris *et al.* 2005; Schmelz *et al.* 2011). *Fusarium graminearum* genes preferentially expressed in maize that could potentially contribute to secondary metabolite detoxification include: *FGSG_03422*, homologous to phenolic acid decarboxylases; *FGSG_03213* and *FGSG_11440*, related to gibberellin 20-oxidase; *FGSG_17047*, related to NADP-dependent

flavonoid reductases which modify ketone-containing plant secondary metabolites known as flavonoids; *FGSG_03984*, related to lactate 2-monooxygenase; and *FGSG_04717*, a cytochrome P450 monooxygenase.

Essential minerals such as copper and iron are generally found in lower concentrations in seeds, especially endosperm, relative to other plant tissues (White & Broadley 2009). Therefore, preferential expression of fungal genes potentially involved in copper and/or iron transport in maize (e.g. *FGSG_03692*, ferric/cupric reductase-like protein; *FGSG_03693*, related to the Ctr high affinity copper transporter family) may reflect differences in mineral acquisition by *Fusarium* between maize kernels and wheat and barley spikes. NRPS1, NRPS2, and NRPS6 are required for the biosynthesis of the siderophores malonichrome, ferricrocin, and fusarinine C/triacetylfusarinine C, respectively (Oide *et al.* 2006; Tobiasen *et al.* 2007; Haas *et al.* 2008; Hansen *et al.* 2012; Oide *et al.* 2015). Fusarinines and malinochrome can mediate extracellular uptake of iron (Fe^{3+}) while ferricrocin is associated with intracellular storage of iron in the fungal cells. Expression of *NRPS2* and *NRPS6*-associated gene clusters (C33, C21) was easily detected in all three hosts by day 1 or 2 after inoculation (Supplementary Table 4). In contrast, expression of the *NRPS1* gene cluster (C63) was detected only in wheat and barley. Next to *NRPS6*, the predicted acetyltransferase (*FGSG_03745*) and enoyl-CoA hydratase (*FGSG_16211*) genes are also expressed in all three hosts. These are candidate genes to produce and transfer the acetyl moiety required to convert fusarinine C to triacetylfusarinine C (Haas *et al.* 2008). It is interesting to note that, among the coexpressed genes neighbouring *NRPS1*, *NRPS2*, and *NRPS6*, there are genes related to *MirA* and/or a ferric reductase (Supplementary Table 4). The major facilitator MirA is required for uptake of siderophores in low iron concentration while ferric reductases such as Fre2p are part of an alternate uptake pathway for siderophores used in high iron conditions (Haas *et al.* 2008).

Our investigation of the gene expression patterns of NRPSs, PKSs, TSs and associated gene clusters in the three hosts revealed many potential biosynthetic gene clusters expressed in all three hosts, or preferentially expressed in one or two hosts. This analysis provides additional information to previous analyses (Ma *et al.* 2010; Sieber *et al.* 2014) which identified potential secondary metabolite gene clusters through bioinformatics and transcriptome comparisons between *in vitro* and *in planta* fungal culture. In our experiment, while expression of four NRPSs and associated gene clusters was detected in all three hosts, no PKS-associated gene cluster was clearly expressed during the initial 4 d of maize kernel infection (Table 4). Only PKS7 and PKS15 were significantly expressed in maize and no or only partial expression of the genes in their potential clusters was detected in that host. In contrast, most of the genes in the cluster C02 containing NRPS8 (*FGSG_11653* – *FGSG_00049*) (Gardiner *et al.* 2009) were strongly expressed during infection in maize while only transient expression was observed in wheat. Gardiner *et al.* (2009) have demonstrated that expression of the NRPS8 gene cluster and the trichothecene gene cluster are strongly induced *in vitro* by agmatine, an intermediate in polyamine biosynthesis associated with grain filling in cereals (Liang & Lur 2002; Liu *et al.* 2013) and a metabolite associated with defence in many plant species. Induction of expression of the *NRPS8* and trichothecene gene clusters may represent an example of how the pathogen exploits host metabolites to induce favourable infection conditions. Another predicted secondary metabolite synthase, *FGSG_10617*, encoding a monomodular NRPS-like protein with single adenylation and thiolation domains, was preferentially expressed in maize. Identification of the products generated by the *NRPS8* gene cluster and by *FGSG_10617* will be essential to elucidate their role during maize infection. For some of the NRPSs, PKSs, and TSs, compilation of nearby coexpressed genes suggests candidate genes for future studies to help elucidate their biosynthetic pathways.

In summary, our comparative transcriptomic analysis showed that the majority of genes expressed by *F. graminearum* when infecting maize, wheat and barley can be divided into three major groups: genes expressed in all three hosts, genes expressed preferentially in wheat and barley, and gene expressed preferentially in only one of the hosts. An examination of the expression profiles for secondary metabolite synthases of the NRPS, PKS and TS types and their neighbouring genes, as well as the expression profiles among members of selected gene families indicated that *F. graminearum* has considerable flexibility in its genome to modulate gene expression and to adapt to differences in substrate or plant defence metabolites between maize, wheat, and barley.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funbio.2015.10.010.

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