Review

On the trail of a cereal killer: recent advances in *Fusarium graminearum* pathogenomics and host resistance

KEMAL KAZAN*, DONALD M. GARDINER AND JOHN M. MANNERS

CSIRO Plant Industry, Queensland Bioscience Precinct, St Lucia, Brisbane, Qld 4067, Australia

SUMMARY

The ascomycete fungal pathogen *Fusarium graminearum* (sexual stage: *Gibberella zeae*) causes the devastating head blight or scab disease on wheat and barley, and cob or ear rot disease on maize. *Fusarium graminearum* infection causes significant crop and quality losses. In addition to roles as virulence factors during pathogenesis, trichothecene mycotoxins (e.g. deoxynivalenol) produced by this pathogen constitute a significant threat to human and animal health if consumed in respective food or feed products. In the last few years, significant progress has been made towards a better understanding of the processes involved in *F. graminearum* pathogenesis, toxin biosynthesis and host resistance mechanisms through the use of high-throughput genomic and phenomic technologies. In this article, we briefly review these new advances and also discuss how future research can contribute to the development of sustainable plant protection strategies against this important plant pathogen.

INTRODUCTION

The fungal pathogen *Fusarium graminearum sensu stricto* (O'Donnell *et al.*, 2004) is the causative agent of Fusarium head blight (FHB) disease on wheat and barley and ear rot of maize. Major FHB epidemics that have occurred in the last two decades have established *F. graminearum* as a major global pathogen of cereals. The *F. graminearum* infection cycle starts with the macroconidia of the fungus overwintering in the soil or on plant debris. The widespread adoption of no-till practices and stubble retention in recent years most probably accounts for the increased occurrence of FHB. The fungus produces fruiting bodies, called perithecia, and ascospores released from these perithecia infect cereal heads during flowering, especially under conducive climatic conditions (e.g. high humidity during flowering). Infection by the pathogen results in reduced grain yield as well as shrivelled and mycotoxin-contaminated grains that are not suitable for human or animal consumption. Interestingly, *F. graminearum* is not just

a specialized pathogen of the cereal head, but can also cause crown rot (Stephens *et al.*, 2008) and root rot (Henkes *et al.*, 2011; Lanoue *et al.*, 2010), diseases which may be important in some environments.

Given the economic importance of this pathogen, *F. graminearum* and related *Fusarium* species are currently under intense investigation. This review aims to provide an overview of recent developments, particularly those derived from new genomics-based technological approaches, and is intended to complement previously published reviews (Goswami and Kistler, 2004; Trail, 2009; Walter *et al.*, 2010; Xu and Nicholson, 2009) covering earlier studies of *F. graminearum* infection biology.

NEW INSIGHTS INTO THE *F. GRAMINEARUM* COLONIZATION PROCESS

Biotrophy versus necrotrophy

Over the years, one contentious issue has been whether *F. graminearum* exhibits a biotrophic lifestyle during the initial stages of infection of floral tissues (Brown *et al.*, 2010; Trail, 2009). A recent detailed microscopic study of the *F. graminearum* infection process in wheat heads (Brown *et al.*, 2010) found no indication of necrotrophy at the initial stages of infection, as the advancing *F. graminearum* hyphae remained in the intercellular spaces of wheat rachis cells before subsequent intracellular growth, which presumably leads to subsequent cell death and necrosis (Fig. 1). Therefore, *F. graminearum* may be classified as a hemibiotrophic pathogen, although Brown *et al.* (2010) concluded that, during floral infection, *F. graminearum* probably displays a unique lifestyle different from previously described fungal lifestyles. Similarly, crown infection by *F. graminearum* also seems to follow a hemibiotrophic lifestyle, characterized by a relatively long symptomless period, followed by massive tissue necrosis and a rapid increase in fungal biomass (Stephens *et al.*, 2008). To our knowledge, no microscopic investigation of root infection by *F. graminearum* has been reported (Henkes *et al.*, 2011; Lanoue *et al.*, 2010), but recent microscopic analysis of wheat root infection by the related pathogen *Fusarium culmorum* has shown the development of rapid necrosis that was visible within a few days of inoculation (Beccari *et al.*, 2011).

*Correspondence: Email: kemal.kazan@csiro.au

Fig. 1 A simplified model of *Fusarium graminearum*–wheat interaction. *Fusarium graminearum* produces effectors, cell wall-degrading enzymes (see Kikot *et al.*, 2009 for a review) and toxins to colonize wheat. The plant responds to infection by producing defence-related hormones, pathogenesis-related (PR) proteins, reactive oxygen and proteins involved in cellular detoxification. Although some of the plant's responses help to restrict infection, others can potentially be exploited by the pathogen to aid pathogenicity. See text for further details. Fg, *F. graminearum*.

New penetration mechanisms

Recent research utilizing detached wheat glumes in an infection assay has revealed new penetration mechanisms by *F. graminearum*. Rittenour and Harris (2010) observed two different hyphal structures, called 'subcuticular hyphae' and 'bulbous infection hyphae', on detached and infected wheat glumes. Of these two structures, the bulbous infection hyphae were thought to be specific to infection, partly because the development of these hyphae was dependent on the fungal *GPMK1* gene (Rittenour and Harris, 2010) encoding a mitogen-activated protein kinase (MAPK). *GPMK1* has been shown previously to be involved in *F. graminearum* pathogenicity (Jenczmionka *et al.*, 2003; Urban *et al.*, 2003). *Fusarium graminearum* can also form lobate appressoria and infection cushions during the infection of florets through caryopses and husks (Boenisch and Schäfer, 2011). However, the formation of these structures was not affected in a *tri5* mutant deficient in trichothecene production (Boenisch and Schäfer, 2011), consistent with the notion that these toxins are not necessary for the initial infection of wheat heads. Specialized infection structures were not observed during crown infection by *F. graminearum* (Stephens *et al.*, 2008), and Beccari *et al.* (2011) did not observe any bulbous hyphae during the infection of wheat roots by *F. culmorum*. This suggests that different penetration strategies may be used by these pathogens when infecting different tissues.

Tissue-specific invasion strategies

The use of *F. graminearum* strains expressing reporter genes, combined with confocal microscopy, has been instrumental in expanding our understanding of the *F. graminearum* infection processes. For instance, using *F. graminearum* transformed with a *TRI5* promoter-GFP gene construct, Ilgen *et al.* (2009) showed that *TRI5* expression, an indicator of the toxin deoxynivalenol (DON) biosynthesis, was activated in a tissue-specific manner in inoculated wheat heads. The reporter gene driven by the *TRI5* promoter was not expressed on anthers, which are the initial targets of the pathogen during floral infection. However, during the spread of the pathogen from inoculated to uninoculated spikelets (4–7 days post-inoculation), extensive reporter gene expression was detected in the rachis node, suggesting that the rachis tissue elicits DON biosynthesis in the pathogen (Ilgen *et al.*, 2009). This finding is consistent with earlier reports that, at least in wheat, the rachis constitutes a formidable barrier to the spread of *F. graminearum*, and that DON biosynthesis in the pathogen is required to overcome this major obstacle (Jansen *et al.*, 2005; Maier *et al.*, 2006).

Recent genetic data also support the importance of the rachis tissue in *F. graminearum* infection processes. The introduction of the long arm of chromosome 7E (7EL) from *Thinopyrum elongatum*, a wild relative of wheat, to hexaploid Chinese Spring (CS) provided a significant increase in resistance to *F. graminearum*.

(Miller *et al.*, 2011). Comparative analysis of the infection process using a green fluorescent protein (GFP)-transformed *F. graminearum* strain showed that the advance of infectious hyphae from inoculated spikelets to adjacent spikelets was blocked in the rachis node of CS-7EL, and this blockage was associated with the deposition of a brown substance, the nature of which is currently unknown (Miller *et al.*, 2011). The authors have also noted the possibility that the differences observed in rachis internode lengths between CS and CS-7EL might have contributed to the 7EL-mediated *F. graminearum* resistance in CS (Miller *et al.*, 2011).

The analysis of crown infection by *F. graminearum* identified at least three distinct stages of infection (spore germination, infection and initial colonization of leaf epidermis and extensive colonization of crown), and microarray analysis revealed that different *F. graminearum* genes were expressed in these distinct stages of crown rot infection, indicating the complexity of the processes involved in initial penetration and spread within the infected tissue (Stephens *et al.*, 2008). A comparative analysis of independent microarray data revealed overlapping fungal gene expression patterns between early stages of crown infection and head infection by *F. graminearum* (Stephens *et al.*, 2008). Some fungal pathogens, such as *Magnaporthe oryzae*, the causative agent of blast disease in rice, are known to exhibit a tissue-adapted invasion strategy (Marcel *et al.*, 2010). It would therefore be interesting to functionally test whether *F. graminearum* uses the same pathogenicity genes required for FHB to infect vegetative tissues, such as crowns and roots, or similar strategies to infect head, root and crown tissue.

Trichothecenes do not appear to be required to cause visible disease symptoms during crown infection in bread wheat, but do contribute to full fungal biomass production and stem colonization (Desmond *et al.*, 2008a; Mudge *et al.*, 2006). The involvement of trichothecene as a virulence factor in *F. culmorum*-mediated crown rot disease on durum wheat has also been demonstrated recently (Scherf *et al.*, 2011). The silencing of *TRI6*, a transcription factor known to regulate the expression of toxin biosynthesis genes in *F. culmorum*, led to reduced DON production and crown rot symptom development. In contrast, some of the transformants that showed unexpectedly high DON and *TRI6* transcript levels, despite being transformed with a *TRI6* silencing construct, showed increased symptom development, suggesting that *TRI6* and DON play a role in the infection of the crown/stem tissue during crown rot disease development (Scherf *et al.*, 2011).

Host-specific invasion strategies

Differences in the *F. graminearum* pathogenicity processes required for FHB on different host species are known to exist. For instance, *F. graminearum*-produced DON does not seem to act as a virulence factor on barley (Maier *et al.*, 2006). To understand this

host-specific adaptation, the *F. graminearum* genes expressed during the infection of wheat and barley have been compared (Lysøe *et al.*, 2011a) and, surprisingly, found to be substantially different. Recently, FHB disease caused by *F. graminearum* in the model cereal *Brachypodium distachyon* has been demonstrated (Peraldi *et al.*, 2011). Interestingly, although *F. graminearum* does not directly infect leaves of wheat and *Arabidopsis* without wounding and the addition of exogenous DON (Chen *et al.*, 2006), it is able to directly penetrate and infect leaf tissues of *Brachypodium* (Peraldi *et al.*, 2011), again suggesting that host-specific infection strategies may be employed by these pathogens.

FORWARD AND REVERSE GENETIC ANALYSES OF *F. GRAMINEARUM* GENES

The availability of a genome sequence (Cuomo *et al.*, 2007) and an efficient genetic transformation system for *F. graminearum* has facilitated forward genetic analyses of gene function, and transposon-, plasmid- and restriction enzyme-mediated integrations (REMI) have been employed to generate *F. graminearum* insertion mutant libraries and to identify pathogenicity mutants (Dufresne *et al.*, 2008; Seong *et al.*, 2005). Recently, Baldwin *et al.* (2010a) generated 5000 *F. graminearum* transformants by random plasmid insertion, and screened 1000 mutants from this collection for altered pathogenicity. This screen identified eight mutants, named 'disease-attenuated *F. graminearum*' or '*daf*', with reduced virulence towards wheat heads. One of the mutants further characterized was *daf10* (disease-attenuated *F. graminearum10*), which contained a large deletion of 350 kb from the end of chromosome 1. This deletion is predicted to harbour 146 genes, some of which may be involved in toxin production. Consistent with this, the *daf10* deletion mutant did not produce DON and, as expected, showed reduced virulence towards wheat in inoculation assays (Baldwin *et al.*, 2010a). Another mutant generated through random plasmid insertion was *top1*, affected in a gene encoding a topoisomerase 1 enzyme (Table 1). This enzyme is involved in uncoiling DNA during transcription and DNA replication, and seems to be required for fungal sporulation and virulence assessed in wheat ear infection assays (Baldwin *et al.*, 2010b).

Similarly, an earlier screen of 6500 antibiotic-resistant mutants generated by REMI identified several pathogenicity mutants (Seong *et al.*, 2005), including the *ftl1* mutant defective in the transducin- β -like protein FTL1 (Table 1). The *ftl1* mutant has been characterized in detail recently and displays defects in its ability to move from infected anthers to flowers. Although no alterations in DON biosynthesis were evident in the mutant, the *ftl1* mutant showed an increased sensitivity to plant defensins (Ding *et al.*, 2009), which might explain the reduced virulence phenotype of the mutant.

Recent gene knock-out mutants of *F. graminearum* from both forward and reverse genetic experiments (Table 1) can be placed

Table 1 Examples of recently characterized *Fusarium graminearum* genes and their roles in fungal pathogenesis.

Gene	Function	Mutant phenotype	Effect on pathogenicity	Reference
<i>Npc1</i>	Involved in sterol trafficking	Contain more ergosterol than wild-type	Reduced pathogenicity	Breakspear <i>et al.</i> (2011)
<i>Mid1</i>	Stretch-activated calcium-permeable ion channel	Reduced ascospore discharge, reduced growth and abnormal ascospores	No effect	Cavinder <i>et al.</i> (2011)
<i>FgStuA</i>	Transcriptional regulator	Impaired spore production and reduced production of secondary metabolites	Reduced pathogenicity	Lysøe <i>et al.</i> (2011b)
<i>HDF1</i>	Histone deacetylase	Reduced toxin production	Reduced pathogenicity	Li <i>et al.</i> (2011a)
<i>FgERG24B</i>	Sterol C-14 reductase	Increased sensitivity to amine-containing fungicides	No effect	Liu <i>et al.</i> (2011a)
<i>CYP51A</i> , <i>-B</i> and <i>-C</i>	14- α demethylases	<i>CYP51A</i> and <i>CYP51C</i> deletion mutants show increased sensitivity to fungicides containing sterol demethylation inhibitor	Not determined	Liu <i>et al.</i> (2011b)
<i>FgATG15</i>	Autophagy-like lipase	Aberrant fungal growth and aerial hyphae	Reduced pathogenicity	Nguyen <i>et al.</i> (2011)
<i>FgPac1</i>	Transcription factor	Reduced development under neutral and alkaline pH, and an earlier <i>Tri</i> gene induction and toxin accumulation at acidic pH	Unknown	Merhej <i>et al.</i> (2011a)
<i>FgVe1 (Velvet)</i>	Fungal development, secondary metabolism and virulence	Aberrant growth and conidiation, reduced aurofusarin biosynthesis, aberrant <i>Tri</i> gene expression and reduced toxin production	Reduced virulence	Merhej <i>et al.</i> (2011c)
<i>aurT</i> , <i>aurZ</i> and <i>aurS</i>	Biosynthesis of the red polyketide pigment aurofusarin	Aberrant pigment phenotypes	Not determined	Frandsen <i>et al.</i> (2011)
<i>ZIF1</i>	b-ZIP transcription factor unique to filamentous ascomycetes	Reduced toxin production	Reduced pathogenicity	Wang <i>et al.</i> (2011)
<i>FgRrg-1</i>	Response regulator implicated in the high osmolarity in glycerol (HOG) pathway	Increased sensitivity to osmotic stress mediated by NaCl, KCl, sorbitol or glucose, and to metal cations Li ⁺ , Ca ²⁺ and Mg ²⁺	Reduced pathogenicity	Jiang <i>et al.</i> (2011)
<i>FgTOP1</i>	Topoisomerase I required to relax supercoiled DNA	Defects in sporulation	Reduced pathogenicity	Baldwin <i>et al.</i> (2010b)
<i>FgTEP1b</i>	Tensin-like phosphatase 1 involved in phosphatidylinositol pathway	Increased sensitivity to lithium; reduced conidia production	Reduced pathogenicity	Zhang <i>et al.</i> (2010)
<i>FgPtc1</i>	Type 2C protein phosphatase	Mycelium growth in response to lithium	Reduced pathogenicity	Jiang <i>et al.</i> (2010)
<i>ROA</i>	Encodes a novel gene required for normal ascospore morphogenesis and discharge	Abnormal ascospores	Not determined	Min <i>et al.</i> (2010)
<i>FTL1</i>	Encodes a transducin- β -like gene acting as a component of the Set3 complex involved in late stages of ascospore formation	Significantly reduced in conidiation	Reduced pathogenicity	Ding <i>et al.</i> (2009)
<i>Ssk1</i> and <i>Hog1</i>	Histidine kinase two-component response regulator and mitogen-activated protein kinase, respectively	Aberrant reproductive development	Reduced pathogenicity	Oide <i>et al.</i> (2010)
<i>CCH1</i>	L-type calcium ion channel	Ascospore discharge and mycelial growth	No effect	Hallen and Trail (2008)
<i>TRI6</i> and <i>TRI10</i>	Transcriptional regulators of trichothecene biosynthesis	Reduced toxin production	Reduced pathogenicity	Seong <i>et al.</i> (2009)
<i>ZRA1</i>	ABC transporter involved in zearalenone biosynthesis	Reduced zearalenone production	Not determined	Lee <i>et al.</i> (2011)
<i>EBR1</i>	A novel Zn2Cys6 transcription factor	Reduced growth	Reduced pathogenesis	Zhao <i>et al.</i> (2011)

into broad categories. Some of the mutations affect fungal development without having any effect on virulence. For instance, knock-out mutants of the *Mid1* gene, encoding a calcium ion channel, caused reduced ascospore discharge, reduced growth and abnormal ascospores, but had no effect on virulence (Cavinder *et al.*, 2011), although in the field this mutation would probably reduce inoculum fecundity. In the great majority of knock-out mutants described, fungal development, toxin biosynthesis and virulence were all affected. Recent examples include mutants of regulatory genes *FgStuA*, encoding a transcription factor (Lysøe *et al.*, 2011b), and *FgATG15*, encoding an autophagy-like lipase involved in growth and the production of aerial hyphae (Table 1; Nguyen *et al.*, 2011).

Gene knock-out mutant analysis has also identified genes that affect different steps in *F. graminearum* pathogenicity processes. For instance, in the *hdf1* mutant deficient for the *HDF1* gene encoding a histone deacetylase, the initial penetration process was not altered, but the mutant failed to spread within wheat heads (Table 1; Li *et al.*, 2011b). The *hdf1* mutant also showed reduced DON levels, supporting the proposed role of DON in pathogen spread. A correlation between reduced DON levels and compromised pathogen spread within infected heads has also been observed in other *F. graminearum* mutants. For instance, *daf10* (Baldwin *et al.*, 2010a), *zif1* (Wang *et al.*, 2011) and *Fgatg15* (Nguyen *et al.*, 2011) all show reduced DON levels, as well as reduced virulence towards wheat heads (Table 1).

Although most gene knock-outs described so far have reduced toxin biosynthesis and attenuated virulence, mutants that show increased toxin biosynthesis and virulence have also been identified. For instance, knock-out mutants of the *TRI6*-regulated genes *FGSG_00007* and *FGSG_10397*, predicted to encode a putative cytochrome P450 monooxygenase and a protein with a partial terpene cyclase domain, respectively, showed elevated DON levels, as well as increased virulence (Gardiner *et al.*, 2009a), suggesting that these two loci act as negative regulators of toxin biosynthesis. The product of the *FGSG_10397* locus was later found to be a longiborneol synthase required for the production of culmorin, a tricyclic sesquiterpene known to be produced by *F. graminearum* and designated as *CLM1* (McCormick *et al.*, 2010). Disruption of *CLM1* in *F. graminearum* resulted in not only complete loss of culmorin production, but also in an increase in 15-acetyldeoxynivalenol (15ADON) levels (Gardiner *et al.*, 2009a; McCormick *et al.*, 2010). Increased DON levels and virulence have also been found in the recently described *pac1* mutant compromised in the sensing of extracellular pH (Table 1; Merhej *et al.*, 2011a).

FUSARIUM GRAMINEARUM TRANSCRIPTOME, PROTEOME AND METABOLOME ANALYSES

The advent of genome-scale microarrays (Becher *et al.*, 2011; Güldener *et al.*, 2006) has enabled the analysis of the *F. graminearum* transcriptome to better understand the fungal infection programmes on diverse tissues and hosts (Hallen-Adams *et al.*, 2011; Lysøe *et al.*, 2011a; Stephens *et al.*, 2008), as well as in different *F. graminearum* mutants (Baldwin *et al.*, 2010a; Gardiner *et al.*, 2009a; Hallen and Trail, 2008; Lee *et al.*, 2011; Seong *et al.*, 2009). Microarray analysis of global gene expression in *Tri6* and *Tri10* deletion mutants affected in toxin biosynthesis and fungal virulence revealed hundreds of genes potentially regulated by these two transcription factors (Seong *et al.*, 2009).

Proteome studies conducted in *F. graminearum* have identified a number of secreted proteins that may act as effectors during fungal growth in culture and infection of wheat heads (Paper *et al.*, 2007). More than 50% of *F. graminearum* proteins identified during infection contained putative secretion signals (Paper *et al.*, 2007), suggesting that these proteins may have effector functions, although this remains to be tested experimentally. Another proteomics study has observed changes in the accumulation of 135 of the 435 detected *F. graminearum* proteins when *F. graminearum* was grown on a medium that promotes trichothecene biosynthesis (Taylor *et al.*, 2008). Although most of these proteins were functionally unknown, their sheer numbers are an indication of the complex processes associated with mycotoxin biosynthesis in *F. graminearum*. The broad metabolic impact of DON production has also been further demonstrated via a comparative analysis of the DON-deficient *tri5* mutant with wild-type *F. graminearum*

using both transcriptome and metabolome approaches, where widespread alterations in a number of carbon and nitrogen metabolism pathways were observed (Chen *et al.*, 2011).

IN SILICO PREDICTION OF *F. GRAMINEARUM* PATHOGENICITY GENES

Large-scale analysis of protein–protein interaction networks (interactome) has the potential to reveal new insights into *F. graminearum* pathogenesis. Towards this aim, Zhao *et al.* (2009) used computational methods to predict protein–protein interactions in *F. graminearum* based on the assumption that *F. graminearum* proteins that are evolutionarily conserved will also show conserved interaction patterns similar to those known for well-studied species, such as *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Homo sapiens*, *Musculus musculus*, *Schizosaccharomyces pombe* and *Escherichia coli*. The predicted *F. graminearum* interactome database currently contains about 52% of the *F. graminearum* proteome, consisting of 3745 proteins and 27 102 predicted interactions. Of the three predicted protein–protein interactions, one of the interactions between a phosphoprotein phosphatase 3-R catalytic chain and a calcineurin B was verified experimentally using yeast two-hybrid experiments (Zhao *et al.*, 2009).

The availability of both microarray and predicted protein interaction data has led to a systems biology approach to computationally predict *F. graminearum* pathogenicity genes (Liu *et al.*, 2010). In this work, genes that are predicted to interact with previously described *F. graminearum* pathogenicity genes (Zhao *et al.*, 2009) and are differentially expressed during infection were identified. Genes involved in the G-protein-coupled receptor and MAPK signalling pathways that are known to affect the pathogenesis process (Oide *et al.*, 2010; Zhang *et al.*, 2011) were represented among the *in silico*-identified *F. graminearum* genes. Also identified were *F. graminearum* genes with homology to those known to affect pathogenesis in other fungi. This approach shows promise to narrow down the number of candidate genes that can be further functionally tested.

FUSARIUM GRAMINEARUM EFFECTORS AND VIRULENCE FACTORS

Pathogen effectors usually contain amino-terminal secretion signals and, after secretion by pathogens, either remain in the extracellular spaces of the host plant or enter host cells and have the ability to modify (i.e. suppress) host defences. Analysis of the *F. graminearum* genome has indicated that 1250 genes encode secreted proteins in this pathogen, and it is possible that a substantial number of these proteins are effectors (reviewed by Schmidt and Panstruga, 2011). In addition, recent microarray

analyses have revealed that 43% of the genes expressed in the pathogen during infection contain putative secretion signals, when compared with only 12.8% of the genes containing such sequences in the whole genome, suggesting that large numbers of *F. graminearum* effectors may be deployed during infection (Lysøe *et al.*, 2011a). However, to date, no report has described the virulence-associated roles for any effector from *F. graminearum*.

Some secondary metabolites, such as the phytotoxin coronatine from the phytopathogenic bacterium *Pseudomonas syringae*, act as effectors in modulating host defences (Schulze-Lefert and Panstruga, 2011). Similarly, DON acts as a virulence factor promoting *F. graminearum* aggressiveness on wheat. Although DON is known to act as a eukaryote protein synthesis inhibitor *in vitro* (Pestka, 2010), how DON acts on plant cells to affect virulence is currently unknown. Exogenous application of DON to wheat and *Arabidopsis* triggers both programmed cell death and strong defence gene expression (Desmond *et al.*, 2008a; Nishiuchi *et al.*, 2006). In animal cells, DON also triggers programmed cell death and an immune reaction via a ribotoxic shock process (Pestka, 2010). The DON-mediated activation of immune responses in both animals and plants requires MAPK signalling pathways (Nishiuchi *et al.*, 2006; Pestka, 2010) that are known to act downstream from pattern recognition receptors (Tena *et al.*, 2011). Therefore, DON may be considered as an *F. graminearum* 'effector' with a cross-kingdom action.

WHAT INDUCES TOXIN BIOSYNTHESIS IN *F. GRAMINEARUM*?

Given the importance of DON as a virulence factor, the regulation of its biosynthesis has received considerable attention (Merhej *et al.*, 2011a). Importantly, DON biosynthesis in *F. graminearum* appears to be much higher during infection than during growth in culture, suggesting that signals generated *in planta* may play important roles in the triggering of DON biosynthesis (Mudge *et al.*, 2006; Voigt *et al.*, 2005). There is evidently a complex spectrum of possible regulatory signals for toxin production in infected plants, but an over-arching observation from this body of work is that *F. graminearum* appears to stimulate host stress and defence responses and to recognize these as cues for the activation of mycotoxin biosynthesis. Table 2 contains examples of compounds that regulate *TRI* gene expression and DON biosynthesis in *F. graminearum in vitro*. Some of the DON-inducing compounds identified are naturally present in the plant or induced during infection (see below). However, the evidence that DON-inducing compounds are present at the appropriate concentration in a given tissue during infection is mostly unavailable.

Amine inducers

To identify nutritional inducers of DON biosynthesis in *F. graminearum*, Gardiner *et al.* (2009b) used an indicator *F. graminearum*

Inducer molecule	Mode of action	Reference
Polyamines	Activates <i>Tri</i> gene expression and toxin biosynthesis	Gardiner <i>et al.</i> (2009b)
Sugars (sucrose, 1-kestose and nystose)	Activates <i>Tri4</i> and <i>Tri5</i> expression and DON and 3ADON biosynthesis	Jiao <i>et al.</i> (2008)
Precocenes and piperitone from the essential oils of <i>Matricaria recutita</i> and <i>Eucalyptus dives</i>	Inhibits <i>Tri4</i> , <i>Tri5</i> , <i>Tri6</i> and <i>Tri10</i> expression and DON biosynthesis	Yaguchi <i>et al.</i> (2009)
Magnesium ions	Inhibits <i>Tri5</i> , <i>Tri6</i> and <i>Tri12</i> expression and toxin biosynthesis	Pinson-Gadais <i>et al.</i> (2008)
Reactive oxygen (e.g. H ₂ O ₂)	Activates <i>Tri</i> gene expression and toxin biosynthesis	Ponts <i>et al.</i> (2006; 2007); Audenaert <i>et al.</i> (2010)
pH	Low pH activates <i>Tri</i> gene expression and toxin biosynthesis	Gardiner <i>et al.</i> (2009c); Merhej <i>et al.</i> (2010)
Ferulic acid	Inhibits <i>Tri5</i> expression and DON biosynthesis	Boutigny <i>et al.</i> (2009)
Cinnamic-derived acids and phenolic acids	Ferulic acid and coumaric acid activates DON biosynthesis	Ponts <i>et al.</i> (2011)
Cobalt chloride	Induces <i>TRI4</i> and <i>TRI6</i> expression and DON biosynthesis	Tsuyuki <i>et al.</i> (2011)
Warm temperature (e.g. 25 °C)	Induces trichothecene production	Kokkonen <i>et al.</i> (2010)
Salt (NaCl) in culture	Inhibits <i>Tri5</i> expression and DON biosynthesis	Ochiai <i>et al.</i> (2007)

3ADON, 3-acetyldeoxynivalenol.

Table 2 Examples of molecules acting as inducers or repressors of trichothecene mycotoxin (e.g. deoxynivalenol, DON) biosynthesis in *Fusarium graminearum*.

Fig. 2 Examples of strategies aimed at the high-throughput identification of novel compounds that inhibit fungal growth (A) (Schreiber *et al.*, 2011) or induce mycotoxin biosynthesis (B) (Gardiner *et al.*, 2009b). See text for additional details. GFP, green fluorescent protein; *Fg*, *Fusarium graminearum*.

strain expressing the *GFP* reporter gene under the control of the *TRI5* gene promoter. In this work, commercially available ‘phenotype array’ plates containing different nitrogen sources were initially used to grow the *TRI5*-GFP *F. graminearum* strain (Fig. 2). The effects of selected GFP-inducing nitrogen sources on DON biosynthesis were further verified in larger scale experiments. Interestingly, the nitrogen sources that most effectively induced DON biosynthesis were ornithine, agmatine and putrescine, all being part of polyamine biosynthesis, a well-established stress response pathway in plants. In the follow-up work, it was further shown that polyamine gene expression and polyamine levels are stimulated by *F. graminearum* infection (Gardiner *et al.*, 2010b). This has led to the hypothesis that *F. graminearum* exploits a stress-induced polyamine pathway in the host plant to activate toxin production and enhance virulence.

pH

The acidity of the culture medium is another factor that promotes DON biosynthesis in the pathogen (Gardiner *et al.*, 2009c; Merhej *et al.*, 2010). It has been proposed that the fungal pH sensing

system may be involved in pH-mediated alteration of DON biosynthesis (Gardiner *et al.*, 2009c). Indeed, the PacC transcription factor, a key component of the fungal regulatory system involved in sensing extracellular pH, negatively regulates *TRI* gene expression and toxin production. The *FgDPac1* deletion mutant showed an earlier induction of *TRI* gene expression and toxin accumulation under low pH (Table 1; Merhej *et al.*, 2011b).

Reactive oxygen

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), have been identified as one of the signals that induce DON biosynthesis in the pathogen. Interestingly, the addition of H_2O_2 to *F. graminearum* cultures leads to relatively late induction of *TRI* gene expression (2–4 days after treatment) and toxin accumulation (Ponts *et al.*, 2006). It seems likely that the induction of *TRI* gene expression by H_2O_2 requires several downstream metabolic or signalling steps for DON induction. The ROS-degrading enzyme catalase suppresses *TRI* gene expression and toxin accumulation (Ponts *et al.*, 2007), and several H_2O_2 -generating fungicides have been reported to induce *TRI* gene expression and DON

biosynthesis (Audenaert *et al.*, 2010). H₂O₂ is also generated in the host by exogenously applied DON (Desmond *et al.*, 2008a), and it is possible that a feedback loop exists in which increased cellular ROS in the host further stimulates DON biosynthesis.

Phenolic acids

Ponts *et al.* (2011) have investigated the effect of five phenolic acids (ferulic, *p*-coumaric, caffeic, syringic and *p*-hydroxybenzoic acid) on DON biosynthesis. Of the phenolic acids tested, ferulic acid and coumaric acid enhanced toxin production, whereas *p*-hydroxybenzoic acid treatment showed a reduction (Table 2). Interestingly, Hamzehzarghani *et al.* (2005, 2008) have identified higher levels of cinnamic acid and ferulic acid in resistant wheats compared with susceptible genotypes. These compounds induce toxin biosynthesis, but also show antifungal activity on *F. graminearum* (Bollina *et al.*, 2010; Ponts *et al.*, 2011). These defensive metabolites may act as cues for the pathogen to boost toxin production in an attempt to stimulate host cell death, which allows infection to continue.

HOST GENES EXPRESSED DURING PATHOGENESIS

Transcriptome studies

So far, there have been several microarray studies in both wheat and barley, investigating which host genes are induced during infection (reviewed by Bischof *et al.*, 2011). Overall, the classes of host genes induced by *F. graminearum* overlap substantially with those found in many other plant-microbe interactions. In these microarray studies, gene expression patterns, either in the same genotype with and without infection or in genetically diverse genotypes that display differences in FHB resistance, have been analysed (e.g. Foroud *et al.*, 2011). The use of isogenic lines that differ in quantitative trait loci (QTLs) conditioning FHB would be a better alternative than the use of genetically diverse lines to pinpoint possible resistance functions.

One of the QTLs associated with type-II FHB resistance (i.e. resistance to spread within the spike) is the *Fhb1* locus located on the short arm of the 3B chromosome in wheat. The mechanism by which *Fhb1*-mediated resistance occurs is currently unknown and, to identify potential mechanisms of *Fhb1*-mediated resistance, *F. graminearum*-responsive gene expression in isogenic wheat lines that differ by this QTL have been compared using microarray analyses (Golkari *et al.*, 2009; Jia *et al.*, 2009). Although large numbers of wheat genes responded to *F. graminearum* infection in both susceptible and resistant near-isogenic lines (NILs), only a small subset of 14 genes showed differential expression between NILs that differed by the presence and absence of the *Fhb1* locus (Jia *et al.*, 2009). Future analyses of these candidate genes may

reveal new insights into the potential mechanism(s) of resistance conferred by the *Fhb1* locus. Remarkably, in another recent microarray study that compared isogenic pairs of three QTLs known to confer quantitative resistance to FHB in barley, different sets of differentially expressed genes were found, suggesting that the mechanisms of resistance conferred by different QTLs might be distinct (Jia *et al.*, 2011). Ding *et al.* (2011) analysed a disease-susceptible wheat mutant with its partially resistant wild-type using proteome and transcriptome analyses, as well as measurements of the defence regulators salicylic acid (SA) and jasmonic acid (JA). They postulated that early induction of the SA defence pathway was critical for resistance in wheat.

The mycotoxin DON is one of the best-known *F. graminearum* virulence factors and causes programmed cell death and has defence-inducing activities (Desmond *et al.*, 2008a; Masuda *et al.*, 2007; Nishiuchi *et al.*, 2006). Therefore, recent work has also focused on the transcriptome responses triggered by DON in the host (Walter *et al.*, 2008). Using a custom-made wheat cDNA array, it was found that, among other things, DON modulates JA biosynthesis and signalling as the wheat homologues of the *Arabidopsis* MYC2 and 12-oxophytodienoic acid (OPDA) were up-regulated (Walter and Doohan, 2011). Perhaps, because of the toxicity of DON to plant cells, DON exposure also triggers strong expression of genes potentially involved in DON detoxification. For instance, treatment of barley heads with DON induced the expression of genes encoding ABC transporters, uridine diphosphate (UDP)-glucosyltransferases, cytochrome P450s and glutathione-S-transferases, plant enzymes that are potentially involved in DON detoxification (Gardiner *et al.*, 2010a; Li *et al.*, 2010).

Proteome studies

So far, several proteome studies have been conducted to identify differentially expressed proteins in infected grains of wheat (Dornez *et al.*, 2010; Wang *et al.*, 2005; Zhou *et al.*, 2005, 2006), barley (Geddes *et al.*, 2008; Yang *et al.*, 2011; Zantinge *et al.*, 2010), maize (Mohammadi *et al.*, 2011) and wild relatives of wheat (Eggert *et al.*, 2011; Yang *et al.*, 2010a, b) and barley (Eggert and Pawelzik, 2011). However, very little overlap, other than the accumulation of some well-described pathogenesis-related (PR) proteins (e.g. chitinases and peroxidases, etc.), has been observed for the *F. graminearum*-induced proteins detected in these studies. It was proposed that differences observed in different studies could be a result of the use of different inoculation methods and growth conditions (Yang *et al.*, 2010a). In addition, proteome studies appear to suffer from the lack of sensitivity in detecting changes in low-abundance proteins. This may explain why many potential *F. graminearum* effectors were not identified in fungal proteomic studies, although microarray experiments often revealed that mRNAs encoding secreted proteins were induced during pathogenesis in *F. graminearum*.

Metabolome studies

A number of metabolome studies have also been conducted in *F. graminearum*-infected hosts. In a recent study, Kumaraswamy *et al.* (2011a) identified a total of 626 metabolites whose levels were altered by *F. graminearum* in two barley genotypes that showed different levels of quantitative resistance to FHB. The majority of these metabolites could be grouped into fatty acid, phenylpropanoid and flavonoid pathways. Among the metabolites belonging to the fatty acid pathway, methyl jasmonate (MeJA), as well as the JA precursors linolenic and linolenic acids, were particularly notable, and increased accumulation of MeJA following *F. graminearum* challenge in the resistant barley genotype relative to the susceptible genotype has led to the proposal that the JA pathway is the predominant defence signalling pathway operating in barley against *F. graminearum* (Kumaraswamy *et al.*, 2011a).

NEW INSIGHTS INTO HOST RESISTANCE FROM MODEL PATHOSYSTEMS

Earlier research has established that *F. graminearum* can infect *Arabidopsis* (Urban *et al.*, 2002), and this model pathosystem has been exploited to dissect the potential roles of signalling pathways in host resistance to *F. graminearum*. In *Arabidopsis*, the SA pathway appears to be required for FHB resistance. The *Arabidopsis* SA signalling mutants, *npr1* and *eds11*, as well as the SA-deficient mutant *sid1* and transgenic *nahG* plants display increased susceptibility when *F. graminearum* or *F. culmorum* are inoculated on leaves or floral organs, respectively (Cuzick *et al.*, 2008; Makandar *et al.*, 2010). In contrast, the JA pathway appears to mediate disease susceptibility based on the increased disease resistance observed in the receptor mutant *coi1* (Makandar *et al.*, 2010; Schreiber *et al.*, 2011). This is consistent with recent observations on defence pathway induction in resistant and susceptible wheats (Ding *et al.*, 2011).

Similar to JA signalling, the ethylene (ET) signalling pathway is also required for susceptibility to *F. graminearum* in both *Arabidopsis* and wheat. The *Arabidopsis* ET signalling mutants *ein2*, *ein3* and *etr1* show smaller necrotic lesions than do wild-type plants when their leaves are inoculated with *F. graminearum*. In contrast, ET-overproducing mutants, *eto1* and *eto2*, as well as the *ctr1* mutant with constitutively activated ET signalling display enhanced disease susceptibility. Furthermore, transgenic wheat heads silenced for the expression of the wheat *EIN2* (*ETHYLENE INSENSITIVE2*) gene were more resistant to FHB. This suggests that ET signalling is exploited by *F. graminearum* to cause disease in both monocotyledonous and dicotyledonous plants (Chen *et al.*, 2009). Although it is not known how JA and ET pathways provide susceptibility to *F. graminearum*, one reason may be the potential involvement of these pathways in pathogen-induced senescence responses (Thatcher *et al.*, 2009).

PLANT PROTECTION AND TOXIN REDUCTION STRATEGIES

Antifungal transgenic approaches

Recent studies have shown that transgenic wheat independently expressing a pectin methyl-esterase inhibitor (Volpi *et al.*, 2011), an antifungal plant defensin (AFP) (Li *et al.*, 2011b), a class II chitinase from barley (Shin *et al.*, 2008) and polygalacturonase-inhibiting protein (Ferrari *et al.*, 2011) provides quantitative FHB resistance under glasshouse conditions. Of these proteins, chitinase, AFP2 and polygalacturonase directly inhibit pathogen growth, whereas pectin methyl-esterase inhibitor-mediated resistance is associated with an inability of *F. graminearum* to grow on methyl-esterified pectin. The pyramiding of multiple transgenes with different modes of action may be a way to achieve stronger and more durable resistance.

More recently, a new class of sesquiterpenoid phytoalexins, called zealexin, with inhibitory activities against *F. graminearum* has been identified in maize, responding to attack by *F. graminearum* as well as synergistically in jasmonate-treated plants (Hufaker *et al.*, 2011). Purified zealexin inhibited *F. graminearum* growth in physiologically active concentrations, indicating that transgenic enhancement of the levels of this phytoalexin can be beneficial to reduce cob rot in maize.

Mycotoxin reduction strategies

As discussed above, various polyamines and phenolic acids have been shown to be strong inducers of DON biosynthesis in *F. graminearum*, and the inhibition of the biosynthesis of these host metabolites in the host plant may lead to reduced toxin levels. However, these host metabolites are involved in other functions, such as resistance to other stresses, and therefore their inhibition would need to be spatially and temporally restricted (e.g. in the developing grain tissue and only during infection) to reduce possible collateral effects.

Another way of reducing DON levels is to express genes that are responsible for DON detoxification (Karlovsy, 2011). Previous research has identified an *Arabidopsis* UDP-glucosyltransferase (UGT) involved in DON detoxification (Poppenberger *et al.*, 2003). More recently, several DON-inducible UGTs have been identified in wheat (Desmond *et al.*, 2008b) and barley (Gardiner *et al.*, 2010a), and a barley gene product was able to detoxify DON. In a separate study, Schweiger *et al.* (2010) expressed DON-inducible barley UGT cDNAs in yeast and tested the resistance of recombinant yeast strains to DON. In these assays, one of the barley UGTs, called *HvUGT13248*, conferred DON resistance in yeast. However, the effects of both barley genes on disease and DON deposition in transgenic cereals have yet to be demonstrated. The formation of DON–glutathione

conjugates may also help to reduce the effect of DON on plant tissue (Gardiner *et al.*, 2010a).

The identification of the cellular targets of DON would also potentially increase resistance if such targets could be modified to reduce toxin sensitivity. A recent example of this strategy is the simultaneous reduction of DON accumulation and the enhancement of *F. graminearum* resistance under field conditions in wheat transgenically expressing a truncated form of the yeast ribosomal protein L3 (Di *et al.*, 2010). The authors have speculated that the expression of yeast protein possibly interferes with the targeting of the wheat L3 protein by DON, and hence reduces the cellular sensitivity to DON. Indeed, DON is known to bind to eukaryotic ribosomes and to interfere with the protein synthesis apparatus (Pestka, 2010). To identify other potential targets of trichothecene action in an unbiased manner, McLaughlin *et al.* (2009) employed a large-scale screen to identify individual yeast mutants showing increased mycotoxin resistance. This screen identified several mutants deficient for genes involved in mitochondrial translation and mitochondrial membrane function, suggesting that these functions confer toxin sensitivity (McLaughlin *et al.*, 2009). It remains to be determined whether similar processes provide sensitivity to trichothecene mycotoxins in plant cells.

Novel defence compounds

The external application of novel compounds with effects on pathogen growth is also a promising approach to reduce disease incidence. A recent example of this strategy is the complete prevention of disease development in both wheat and maize by the external application of CNI-1493, a compound that inhibits fungal deoxyhypusine synthase (DHS) activity without affecting grain development (Woriedh *et al.*, 2011). The *F. graminearum* DHS gene, which is strongly induced during the early stages of infection, is involved in hypusine biosynthesis required for the activity of protein eukaryotic initiation factor-5A (eIF-5A), and eIF-5A is thought to transport fungal mRNAs required for pathogenesis out of the nucleus to facilitate their translation by ribosomes (Woriedh *et al.*, 2011). Despite its promise in disease prevention, the application of CNI-1493 under field conditions seems to be unlikely because of possible nondiscriminatory toxic effects on other organisms.

Using a high-throughput screening system in which *Arabidopsis* leaves were incubated with suspected defence-activating small molecules (Fig. 2), Schreiber *et al.* (2011) identified sulphamethoxazole and the indole alkaloid gramine as providing protection against *F. graminearum* in the assay. These two compounds were also effective in providing protection in wheat when sprayed onto heads prior to inoculation. Although possible protection mechanisms afforded by these compounds are not known, this finding certainly suggests the relevance of the *Arabidopsis* pathosystem to *F. graminearum*–wheat interaction. Recent research in our

laboratory has shown that gramine can be highly toxic to *F. graminearum* if the tests are conducted on agarose medium, but not on agar (D. M. Gardiner, unpublished work), as conducted by Schreiber *et al.* (2011). This is probably because sulphated polysaccharides in agar may bind amines such as gramine.

Biological control

Inhibition of FHB through the use of biocontrol organisms may offer environmentally friendly disease control compared with chemical treatments. So far, some success in disease reduction has been reported with bacterial strains *Bacillus* spp. and *Pseudomonas* spp. under controlled conditions (He *et al.*, 2009). Recent research has provided new insights into a *Pseudomonas*-mediated disease protection system for *F. graminearum*. Using a split root inoculation system and carbon tracer elements, Henkes *et al.* (2011) demonstrated that the infection of roots by *F. graminearum* results in the distribution of more and less carbon to uninfected and infected roots, respectively, perhaps in an attempt to reduce fungal growth before antifungal defences can kick in. In contrast with plants inoculated directly with *F. graminearum* through the roots, barley plants primed with *Pseudomonas fluorescens* before inoculation did not show the same patterns of distorted distribution of carbon allocation and were also less affected developmentally in response to infection (Henkes *et al.*, 2011). This study provides a possible explanation of the nature of processes operating in the host plant in response to root infection by *F. graminearum*, but it is not known whether nutrient supply pathways are affected in other plant parts.

Another study compared the *F. culmorum*-triggered global gene expression patterns in barley heads that were either primed or nonprimed with the concurrent application of FHB biocontrol strain *P. fluorescens* and *F. culmorum*. As suspected, the application of biocontrol bacteria altered the expression patterns of large numbers of genes, including genes encoding lipid transfer proteins and protease inhibitors. This study also implicated the JA pathway as a modulator of *P. fluorescens*-mediated priming against *F. culmorum* infection in barley (Petti *et al.*, 2010). Together, these studies suggest that biological control agents for *F. graminearum* may act through the stimulation of intrinsic resistance in the host rather than direct inhibitory effects on the pathogen.

Resistance breeding

Although this review has focused mainly on the molecular aspects of the host–pathogen interaction, significant progress has also been made in recent years in the genetic dissection of *F. graminearum* resistance in the host. For example, large numbers of QTLs controlling resistance to *F. graminearum* have been identified in both wheat and barley (reviewed by Buerstmayr *et al.*, 2009;

Handa *et al.*, 2008), and isogenic lines that differ by the presence and/or absence of some QTLs have been produced. The mechanisms of resistance conditioned by these QTLs are currently unclear and, as progress is made in wheat genome sequencing, the genomic regions harbouring these QTLs can certainly be better defined and their effects on disease resistance studied.

Breeding selections made on the basis of resistance-associated host metabolites (both induced during *F. graminearum* infection and having *in vitro* inhibitory activity against *F. graminearum*) could be a way to speed up the selection process if reliable associations between these metabolites as biomarkers and host resistance can be established (Bollina *et al.*, 2011). For example, in a relatively small-scale metabolomic study, phenylalanine, *p*-coumaric acid, jasmonate, linolenic acid and DON levels have been associated with resistance in a small subset of barley genotypes (Kumaraswamy *et al.*, 2011b).

FUTURE PERSPECTIVES

New *F. graminearum* genomes?

The availability of a sequenced *F. graminearum* genome (Cuomo *et al.*, 2007) has undoubtedly enhanced significantly our ability to uncover new insights into this pathogen. The sequencing of additional genomes from *F. graminearum* strains that differ by virulence and toxin production, etc., as well as from related species such as *F. culmorum* and *F. pseudograminearum*, would provide useful information about the evolution of pathogenicity and host specificity in these pathogens. Indeed, recent research has shown that the differential activity of TRI8, resulting from DNA sequence variations in the coding region of this gene, is a critical determinant of whether an *F. graminearum* isolate produces 3-acetyldeoxynivalenol (3ADON) or 15ADON. It appears that TRI8 has a C-15 esterase or C-3 esterase activity in 3ADON- and 15ADON-producing *F. graminearum* isolates, respectively (Alexander *et al.*, 2011). Recent technical developments (e.g. next-generation sequencing technologies) would make such sequencing efforts a highly feasible exercise.

Although additional *F. graminearum* genomes are currently not available, existing genome sequences from related *Fusarium* species have the potential to provide new insights into the evolution of *F. graminearum*. Indeed, comparative analysis of the *F. graminearum* genome with the recently completed genomes of *F. verticillioides*, *F. oxysporum* f.sp. *tomato* and *F. solani* (*Nectria haematococca*) has revealed substantial sequence similarity and conservation of the order of nearly 9000 genes in these three pathogens (Ma *et al.*, 2010). Interestingly, genes that are unique to each species are found in telomeric chromosomal regions that also show high sequence diversity and recombination rates and contain transposons. This has led to the suggestion that these regions of high variability have evolved through

telomeric fusion of ancestral chromosomes (reviewed by Rep and Kistler, 2010).

Functional analyses of host and pathogen genes

So far, only a few *F. graminearum* mutants have been analysed in detail. The availability of an *F. graminearum* gene-indexed mutation resource base, such as those for yeast and *Arabidopsis*, where knockout strains are systematically identified, deposited and distributed to the scientific community, would significantly speed up the analysis of gene function in *F. graminearum*.

The scale and throughput of stable transformation of wheat are limiting factors for the functional analysis of candidate host genes for resistance. An alternative to forward genetic analyses of gene function in wheat would be to employ reverse genetic studies. For instance, high-throughput methods for the identification of deletions in candidate wheat genes of interest have recently been developed (Fitzgerald *et al.*, 2010). To overcome the difficulty of host analysis, at least initially, model systems, such as yeast, *Arabidopsis* and *Brachypodium*, are currently being exploited. *Brachypodium*, being a close relative of wheat, offers new advantages over the *Arabidopsis* system. In addition to being a susceptible host to *F. graminearum* (Peraldi *et al.*, 2011), *Brachypodium* is transformable, has a sequenced genome and contains diverse germplasm and emerging functional genomic resources, such as T-DNA insertion lines, that can facilitate host gene function analyses.

New challenges

How farming practises and environmental shifts, such as climate change, may affect the evolution of virulence and toxin biosynthesis in *F. graminearum* is another area of future research. Recent research has shown that larger numbers of *F. graminearum* isolates with the 3ADON chemotype and with higher aggressiveness and greater levels of grain DON content can be found in current *F. graminearum* isolates than those collected between 1980 and 2000 in North Dakota (Puri and Zhong, 2010). Although the exact reason for this adaptation is unknown, it has been postulated that the use of resistant host genotypes and fungicides may have led to the development of more aggressive isolates (Puri and Zhong, 2010). Another study examining *F. graminearum* isolates throughout the USA has found that nivalenol (NIV)-producing *F. graminearum* isolates have already established in small-grain-growing regions of southern Louisiana (Gale *et al.*, 2011), despite earlier reports showing that such chemotypes were rare (Desjardins *et al.*, 2008). Although the potential toxicity of NIV versus DON toxin-producing *F. graminearum* isolates is still controversial (Gale *et al.*, 2011; Puri and Zhong, 2010), it will be important to maintain regular surveillance to monitor possible changes in toxin chemotypes.

FHB: STILL A LOOMING DISASTER?

In their nearly decade-old pathogen profile, Goswami and Kistler (2004) rightfully warned that *F. graminearum* and related pathogens could produce disastrous outcomes unless concerted efforts were devoted to a better understanding of the complex nature of these particular plant–pathogen interactions. As reviewed in this article, significant progress has been made towards this goal, although we still have a long way to go towards the development of a good understanding of *F. graminearum*–cereal interactions and sustainable plant protection strategies.

ACKNOWLEDGEMENTS

We apologize to colleagues whose relevant work could not be cited because of space restrictions, as well as our focus to review more recent aspects of this plant–microbe interaction. We thank anonymous reviewers for constructive comments on the manuscript.

NOTE ADDED IN PROOF

Recently, an *Fg* deletion mutant library comprising mutant strains for 657 putative *Fg* transcription factors has been constructed and the analysis of these mutants under various conditions revealed diverse mutant phenotypes, including alterations in mycelial growth, sexual development, conidia production, virulence, toxin production and stress responses (Son *et al.*, 2011). This library provides a useful resource for functional analysis of *Fg* transcription factor genes.

REFERENCES

- Alexander, N.J., McCormick, S.P., Waalwijk, C., van der Lee, T. and Proctor, R.H. (2011) The genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in *Fusarium*. *Fungal Genet. Biol.* **48**, 485–495.
- Audenaert, K., Callewaert, E., Höfte, M., De Saeger, S. and Haesaert, G. (2010) Hydrogen peroxide induced by the fungicide prothioconazole triggers deoxynivalenol (DON) production by *Fusarium graminearum*. *BMC Microbiol.* **10**, 112.
- Baldwin, T.K., Gaffoor, I., Antoniw, J., Andries, C., Guenther, J., Urban, M., Hallen-Adams, H.E., Pitkin, J., Hammond-Kosack, K.E. and Trail, F. (2010a) A partial chromosomal deletion caused by random plasmid integration resulted in a reduced virulence phenotype in *Fusarium graminearum*. *Mol. Plant–Microbe Interact.* **23**, 1083–1096.
- Baldwin, T.K., Urban, M., Brown, N. and Hammond-Kosack, K.E. (2010b) A role for topoisomerase I in *Fusarium graminearum* and *F. culmorum* pathogenesis and sporulation. *Mol. Plant–Microbe Interact.* **23**, 566–577.
- Beccari, G., Covarelli, L. and Nicholson, P. (2011) Infection processes and soft wheat response to root rot and crown rot caused by *Fusarium culmorum*. *Plant Pathol.* **60**, 671–684.
- Becher, R., Weihmann, F., Deising, H.B. and Wirsal, S.G. (2011) Development of a novel multiplex DNA microarray for *Fusarium graminearum* and analysis of azole fungicide responses. *BMC Genomics*, **12**, 52.
- Bischof, M., Eichmann, R. and Hückelhoven, R. (2011) Pathogenesis-associated transcriptional patterns in Triticaceae. *J. Plant Physiol.* **168**, 9–19.
- Boenisch, M.J. and Schäfer, W. (2011) *Fusarium graminearum* forms mycotoxin producing infection structures on wheat. *BMC Plant Biol.* **11**, 110.
- Bollina, V., Kumaraswamy, G.K., Kushalappa, A.C., Choo, T.M., Dion, Y., Rioux, S., Faubert, D. and Hamzehzarghani, H. (2010) Mass spectrometry-based metabolomics application to identify quantitative resistance-related metabolites in barley against *Fusarium* head blight. *Mol. Plant Pathol.* **11**, 769–782.
- Bollina, V., Kushalappa, A.C., Choo, T.M., Dion, Y. and Rioux, S. (2011) Identification of metabolites related to mechanisms of resistance in barley against *Fusarium graminearum*, based on mass spectrometry. *Plant Mol. Biol.* **77**, 355–370.
- Boutigny, A.L., Barreau, C., Atanasova-Penichon, V., Verdabonnon, M., Pinson-Gadais, L. and Richard-Forget, F. (2009) Ferulic acid, an efficient inhibitor of type B trichothecene biosynthesis and *Tri* gene expression in *Fusarium* liquid cultures. *Mycol. Res.* **113**, 746–753.
- Breakspear, A., Pasquali, M., Broz, K., Dong, Y. and Kistler, H.C. (2011) Npc1 is involved in sterol trafficking in the filamentous fungus *Fusarium graminearum*. *Fungal Genet. Biol.* **48**, 725–730.
- Brown, N.A., Urban, M., van de Meene, A.M. and Hammond-Kosack, K.E. (2010) The infection biology of *Fusarium graminearum*: defining the pathways of spikelet to spikelet colonisation in wheat ears. *Fungal Biol.* **114**, 555–571.
- Buerstmayr, H., Ban, T. and Anderson, J. (2009) QTL mapping and marker-assisted selection for *Fusarium* head blight resistance in wheat: a review. *Plant Breed.* **128**, 1–26.
- Cavinder, B., Hamam, A., Lew, R.R. and Trail, F. (2011) Mid1, a mechanosensitive calcium ion channel, affects growth, development, and ascospore discharge in the filamentous fungus *Gibberella zeae*. *Eukaryot Cell*, **10**, 832–841.
- Chen, F., Zhang, J., Song, X., Yang, J., Li, H., Tang, H. and Liao, Y.C. (2011) Combined metabolomic and quantitative real-time PCR analyses reveal systems metabolic changes of *Fusarium graminearum* induced by *Tri5* gene deletion. *J. Proteome Res.* **10**, 2273–2285.
- Chen, X., Steed, A., Harden, C. and Nicholson, P. (2006) Characterization of *Arabidopsis thaliana*–*Fusarium graminearum* interactions and identification of variation in resistance among ecotypes. *Mol. Plant Pathol.* **7**, 391–403.
- Chen, X., Steed, A., Travella, S., Keller, B. and Nicholson, P. (2009) *Fusarium graminearum* exploits ethylene signalling to colonize dicotyledonous and monocotyledonous plants. *New Phytol.* **182**, 975–983.
- Cuomo, C.A., Guldener, U., Xu, J.R., Trail, F., Turgeon, B.G., Di Pietro, A., Walton, J.D., Ma, L.J., Baker, S.E., Rep, M., Adam, G., Antoniw, J., Baldwin, T., Calvo, S., Chang, Y.L., Decaprio, D., Gale, L.R., Gnerre, S., Goswami, R.S., Hammond-Kosack, K., Harris, L.J., Hilburn, K., Kennell, J.C., Kroken, S., Magnuson, J.K., Mannhaupt, G., Mauceli, E., Mewes, H.W., Mitterbauer, R., Muehlbauer, G., Münsterkötter, M., Nelson, D., O'donnell, K., Ouellet, T., Qi, W., Quesneville, H., Roncero, M.I., Seong, K.Y., Tetko, I.V., Urban, M., Waalwijk, C., Ward, T.J., Yao, J., Birren, B.W. and Kistler, H.C. (2007) The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. *Science*, **317**, 1400–1402.
- Cuzick, A., Lee, S., Gezan, S. and Hammond-Kosack, K.E. (2008) NPR1 and EDS11 contribute to host resistance against *Fusarium culmorum* in *Arabidopsis* buds and flowers. *Mol. Plant Pathol.* **9**, 697–704.
- Desjardins, A.E., Busman, M., Manandhar, G., Jarosz, A.M., Manandhar, H.K. and Proctor, R.H. (2008) Distribution of the mycotoxins nivalenol and deoxynivalenol in naturally and experimentally infected maize. *J. Agric. Food Chem.* **56**, 5428–5436.
- Desmond, O.J., Manners, J.M., Schenk, P.M., Maclean, D.J. and Kazan, K. (2008b) Gene expression analysis of the wheat response to infection by *Fusarium pseudograminearum*. *Physiol. Mol. Plant Pathol.* **73**, 40–47.
- Desmond, O.J., Manners, J.M., Stephens, A.E., Maclean, D.J., Schenk, P.M., Gardiner, D.M., Munn, A.L. and Kazan, K. (2008a) The *Fusarium* mycotoxin deoxynivalenol elicits hydrogen peroxide production, programmed cell death and defence responses in wheat. *Mol. Plant Pathol.* **9**, 435–445.
- Di, R., Blechl, A., Dill-Macky, R., Tortora, A. and Tumer, N.E. (2010) Expression of a truncated form of yeast ribosomal protein L3 in transgenic wheat improves resistance to *Fusarium* head blight. *Plant Sci.* **178**, 374–380.
- Ding, L., Xu, H., Yi, H., Yang, L., Kong, Z., Zhang, L., Xue, S., Jia, H. and Ma, Z. (2011) Resistance to hemi-biotrophic *F. graminearum* infection is associated with coordinated and ordered expression of diverse defense signaling pathways. *PLoS ONE*, **6**, e19008.
- Ding, S., Mehrabi, R., Kote, C., Kang, Z., Wei, Y., Seong, K., Kistler, H.C. and Xu, J.R. (2009) Transducin beta-like gene FTL1 is essential for pathogenesis in *Fusarium graminearum*. *Eukaryot Cell*, **8**, 867–876.
- Dornez, E., Croes, E., Gebruers, K., Carpentier, S., Swennen, R., Laukens, K., Witters, E., Urban, M., Delcour, J.A. and Courtin, C.M. (2010) 2-D DIGE reveals changes in wheat xylanase inhibitor protein families due to *Fusarium graminearum* DeltaTri5 infection and grain development. *Proteomics*, **10**, 2303–2319.
- Dufresne, M., van der Lee, T., Ben M'Barek, S., Xu, X.D., Zhange, X., Liu, T.G., Waalwijk, C., Zhang, W.W., Kema, G.H.J. and Daboussi, M.J. (2008) Transposon

- tagging identifies novel pathogenicity genes in *Fusarium graminearum*. *Fungal Genet. Biol.* **45**, 1552–1561.
- Eggert, K. and Pawelzik, E. (2011) Proteome analysis of *Fusarium* head blight in grains of naked barley (*Hordeum vulgare* subsp. *nudum*). *Proteomics*, **11**, 972–985.
- Eggert, K., Zörb, C., Mühling, K.H. and Pawelzik, E. (2011) Proteome analysis of *Fusarium* infection in emmer grains (*Triticum dicoccum*). *Plant Pathol.* **60**, 918–928.
- Ferrari, S., Sella, L., Janni, M., De Lorenzo, G., Favaron, F. and D'Ovidio, R. (2011) Transgenic expression of polygalacturonase-inhibiting proteins in *Arabidopsis* and wheat increases resistance to the flower pathogen *Fusarium graminearum*. *Plant Biol.* DOI: 10.1111/j.1438-8677.2011.00449.x.
- Fitzgerald, T.L., Kazan, K., Li, Z., Morell, M.K. and Manners, J.M. (2010) A high-throughput method for the detection of homologous gene deletions in hexaploid wheat. *BMC Plant Biol.* **10**, 264.
- Foroud, N.A., Ouellet, T., Laroche, A., Oosterveen, B., Jordan, M.C., Ellis, B.E. and Eudes, F. (2011) Differential transcriptome analyses of three wheat genotypes reveal different host response pathways associated with *Fusarium* head blight and trichothecene resistance to the flower pathogen *Fusarium graminearum*. *Plant Pathol.* DOI: 10.1111/j.1365-3059.2011.02512.x.
- Frandsen, R.J., Schütt, C., Lund, B.W., Staerk, D., Nielsen, J., Olsson, S. and Giese, H. (2011) Two novel classes of enzymes are required for the biosynthesis of aurofusarin in *Fusarium graminearum*. *J. Biol. Chem.* **286**, 10 419–10 428.
- Gale, L.R., Harrison, S.A., Ward, T.J., O'Donnell, K., Milus, E.A., Gale, S.W. and Kistler, H.C. (2011) Nivalenol-type populations of *Fusarium graminearum* and *F. asiaticum* are prevalent on wheat in southern Louisiana. *Phytopathology*, **101**, 124–134.
- Gardiner, D.M., Kazan, K. and Manners, J.M. (2009a) Novel genes of *Fusarium graminearum* that negatively regulate deoxynivalenol production and virulence. *Mol. Plant–Microbe Interact.* **22**, 1588–1600.
- Gardiner, D.M., Kazan, K. and Manners, J.M. (2009b) Nutrient profiling reveals potent inducers of trichothecene biosynthesis in *Fusarium graminearum*. *Fungal Genet. Biol.* **46**, 604–613.
- Gardiner, D.M., Osborne, S., Kazan, K. and Manners, J.M. (2009c) Low pH regulates the production of deoxynivalenol by *Fusarium graminearum*. *Microbiology*, **155**, 3149–3156.
- Gardiner, D.M., Kazan, K., Praud, S., Torney, F.J., Rusu, A. and Manners, J.M. (2010b) Early activation of wheat polyamine biosynthesis during *Fusarium* head blight implicates putrescine as an inducer of trichothecene mycotoxin production. *BMC Plant Biol.* **10**, 289.
- Gardiner, S.A., Boddu, J., Berthiller, F., Hametner, C., Stupar, R.M., Adam, G. and Muehlbauer, G.J. (2010a) Transcriptome analysis of the barley–deoxynivalenol interaction: evidence for a role of glutathione in deoxynivalenol detoxification. *Mol. Plant–Microbe Interact.* **23**, 962–976.
- Geddes, J., Eudes, F., Laroche, A. and Selinger, L.B. (2008) Differential expression of proteins in response to the interaction between the pathogen *Fusarium graminearum* and its host, *Hordeum vulgare*. *Proteomics*, **8**, 545–554.
- Golkari, S., Gilbert, J., Ban, T. and Procnier, J.D. (2009) QTL-specific microarray gene expression analysis of wheat resistance to *Fusarium* head blight in Sumai-3 and two susceptible NILs. *Genome*, **52**, 409–418.
- Goswami, R.S. and Kistler, H.C. (2004) Heading for disaster: *Fusarium graminearum* on cereal crops. *Mol. Plant Pathol.* **5**, 515–525.
- Göldener, U., Seong, K.Y., Boddu, J., Cho, S., Trail, F., Xu, J.R., Adam, G., Mewes, H.W., Muehlbauer, G.J. and Kistler, H.C. (2006) Development of a *Fusarium graminearum* Affymetrix GeneChip for profiling fungal gene expression in vitro and in planta. *Fungal Genet. Biol.* **43**, 316–325.
- Hallen, H.E. and Trail, F. (2008) The L-type calcium ion channel cch1 affects ascospore discharge and mycelial growth in the filamentous fungus *Gibberella zeae* (anamorph *Fusarium graminearum*). *Eukaryot Cell*, **7**, 415–424.
- Hallen-Adams, H.E., Wenner, N., Kuldau, G.A. and Trail, F. (2011) Deoxynivalenol biosynthesis-related gene expression during wheat kernel colonization by *Fusarium graminearum*. *Phytopathology*, **101**, 1091–1096.
- Hamzehzarghani, H., Kushalappa, A.C., Dion, Y., Rioux, S., Comeau, A., Yaylayan, V., Marshall, W.D. and Mather, D.E. (2005) Metabolic profiling and factor analysis to discriminate quantitative resistance in wheat cultivars against *Fusarium* head blight. *Physiol. Mol. Plant Pathol.* **66**, 119–133.
- Hamzehzarghani, H., Paraniharan, V., Abu-Nada, Y., Kushalappa, A., Dion, Y., Rioux, S., Comeau, A., Yaylayan, V. and Marshall, W.D. (2008) Metabolite profiling coupled with statistical analyses for potential high-throughput screening of quantitative resistance to *Fusarium* head blight in wheat. *Can. J. Plant Pathol.* **30**, 24–36.
- Handa, H., Namiki, N., Xu, D.H. and Ban, T. (2008) Dissecting of the FHB resistance QTL on the short arm of wheat chromosome 2D using a comparative genomic approach: from QTL to candidate gene. *Mol. Breed.* **22**, 71–84.
- He, J., Boland, G.J. and Zhou, T. (2009) Concurrent selection for microbial suppression of *Fusarium graminearum*, *Fusarium* head blight and deoxynivalenol in wheat. *J. Appl. Microbiol.* **106**, 1805–1817.
- Henkes, G.J., Jousset, A., Bonkowski, M., Thorpe, M.R., Scheu, S., Lanoue, A., Schurr, U. and Röse, U.S. (2011) *Pseudomonas fluorescens* CH0 maintains carbon delivery to *Fusarium graminearum*-infected roots and prevents reduction in biomass of barley shoots through systemic interactions. *J. Exp. Bot.* **62**, 4337–4344.
- Huffaker, A., Kaplan, F., Vaughan, M.M., Dafoe, N.J., Ni, X., Rocca, J.R., Alborn, H.T., Teal, P.E. and Schmelz, E.A. (2011) Novel acidic sesquiterpenoids constitute a dominant class of pathogen-induced phytoalexins in maize. *Plant Physiol.* **156**, 2082–2097.
- Ilgen, P., Haderer, B., Maier, F.J. and Schäfer, W. (2009) Developing kernel and rachis node induce the trichothecene pathway of *Fusarium graminearum* during wheat head infection. *Mol. Plant–Microbe Interact.* **22**, 899–908.
- Jansen, C., von Wettstein, D., Schäfer, W., Kogel, K.H., Felk, A. and Maier, F.J. (2005) Infection patterns in barley and wheat spikes inoculated with wild-type and trichodiene synthase disrupted *Fusarium graminearum*. *Proc. Natl. Acad. Sci. USA*, **102**, 16 892–16 897.
- Jenczmionka, N.J., Maier, F.J., Losch, A.P. and Schäfer, W. (2003) Mating, conidiation, and pathogenicity of *Fusarium graminearum*, the main causal agent of the head-blight disease of wheat, are regulated by the MAP kinase gpmk1. *Curr. Genet.* **43**, 87–95.
- Jia, H., Cho, S. and Muehlbauer, G.J. (2009) Transcriptome analysis of a wheat near-isogenic line pair carrying *Fusarium* head blight-resistant and -susceptible alleles. *Mol. Plant–Microbe Interact.* **22**, 1366–1378.
- Jia, H., Millett, B.P., Cho, S., Bilgic, H., Xu, W.W., Smith, K.P. and Muehlbauer, G.J. (2011) Quantitative trait loci conferring resistance to *Fusarium* head blight in barley respond differentially to *Fusarium graminearum* infection. *Funct. Integr. Genomics*, **11**, 95–102.
- Jiang, J., Yun, Y., Fu, J., Shim, W.B. and Ma, Z. (2011) Involvement of a putative response regulator FgRrg-1 in osmotic stress response, fungicide resistance and virulence in *Fusarium graminearum*. *Mol. Plant Pathol.* **12**, 425–436.
- Jiang, L., Yang, J., Fan, F., Zhang, D. and Wang, X. (2010) The type 2C protein phosphatase FgPtc1p of the plant fungal pathogen *Fusarium graminearum* is involved in lithium toxicity and virulence. *Mol. Plant Pathol.* **11**, 277–282.
- Jiao, F., Kawakami, A. and Nakajima, T. (2008) Effects of different carbon sources on trichothecene production and Tri gene expression by *Fusarium graminearum* in liquid culture. *FEMS Microbiol. Lett.* **285**, 212–219.
- Karlovsky, P. (2011) Biological detoxification of the mycotoxin deoxynivalenol and its use in genetically engineered crops and feed additives. *Appl. Microbiol. Biotechnol.* **91**, 491–504.
- Kikot, G.E., Hous, R.A. and Alconada, T.M. (2009) Contribution of cell wall degrading enzymes to pathogenesis of *Fusarium graminearum*: a review. *J. Basic Microbiol.* **49**, 231–241.
- Kokkonen, M., Ojala, L., Parikka, P. and Jestoi, M. (2010) Mycotoxin production of selected *Fusarium* species at different culture conditions. *Int. J. Food Microbiol.* **143**, 17–25.
- Kumaraswamy, G.K., Bollina, V., Kushalappa, A.C., Choo, T.M., Dion, Y., Rioux, S., Mamer, O. and Faubert, D. (2011a) Metabolomics technology to phenotype resistance in barley against *Gibberella zeae*. *Eur. J. Plant Pathol.* **130**, 29–43.
- Kumaraswamy, G.K., Kushalappa, A.C., Choo, T.M., Dion, Y. and Rioux, S. (2011b) Mass spectrometry based metabolomics to identify potential biomarkers for resistance in barley against *Fusarium* head blight (*Fusarium graminearum*). *J. Chem. Ecol.* **37**, 846–856.
- Lanoue, A., Burlat, V., Henkes, G.J., Koch, I., Schurr, U. and Röse, U.S. (2010) De novo biosynthesis of defense root exudates in response to *Fusarium* attack in barley. *New Phytol.* **185**, 577–588.
- Lee, S., Son, H., Lee, J., Lee, Y.R. and Lee, Y.W. (2011) A putative ABC transporter gene, ZRA1, is required for zearelanone production in *Gibberella zeae*. *Curr. Genet.* **57**, 345–351.
- Li, X., Zhang, J.B., Song, B., Li, H.P., Xu, H.Q., Qu, B., Dang, F.J. and Liao, Y.C. (2010) Resistance to *Fusarium* head blight and seedling blight in wheat is associated with activation of a cytochrome P450 gene. *Phytopathology*, **100**, 183–191.
- Li, Y., Wang, C., Liu, W., Wang, G., Kang, Z., Kistler, H.C. and Xu, J.R. (2011a) The HDF1 histone deacetylase gene is important for conidiation, sexual reproduction, and pathogenesis in *Fusarium graminearum*. *Mol. Plant–Microbe Interact.* **24**, 487–496.
- Li, Z., Zhou, M., Zhang, Z., Ren, L., Du, L., Zhang, B., Xu, H. and Xin, Z. (2011b) Expression of a radish defensin in transgenic wheat confers increased resistance to *Fusarium graminearum* and *Rhizoctonia cerealis*. *Funct. Integr. Genomics*, **11**, 63–70.

- Liu, X., Tang, W.H., Zhao, X.M. and Chen, L. (2010) A network approach to predict pathogenic genes for *Fusarium graminearum*. *PLoS ONE*, **5**, 10.
- Liu, X., Fu, J., Yun, Y., Yin, Y. and Ma, Z. (2011a) A sterol C-14 reductase encoded by *FgERG24B* is responsible for the intrinsic resistance of *Fusarium graminearum* to amine fungicides. *Microbiology*, **157**, 1665–1675.
- Liu, X., Yu, F., Schnabel, G., Wu, J., Wang, Z. and Ma, Z. (2011b) Paralogous *cyp51* genes in *Fusarium graminearum* mediate differential sensitivity to sterol demethylation inhibitors. *Fungal Genet. Biol.* **48**, 113–123.
- Lysøe, E., Seong, K.Y. and Kistler, C. (2011a) The transcriptome of *Fusarium graminearum* during the infection of wheat. *Mol. Plant–Microbe Interact.* **24**, 995–1000.
- Lysøe, E., Pasquali, M., Breakspear, A. and Kistler, H.C. (2011b) The transcription factor *FgStuAp* influences spore development, pathogenicity, and secondary metabolism in *Fusarium graminearum*. *Mol. Plant–Microbe Interact.* **24**, 54–67.
- Ma, L.J., van der Does, H.C., Borkovich, K.A., Coleman, J.J., Daboussi, M.J., Di Pietro, A., Dufresne, M., Freitag, M., Grabherr, M., Henrissat, B., Houterman, P.M., Kang, S., Shim, W.B., Woloshuk, C., Xie, X., Xu, J.R., Antoniw, J., Baker, S.E., Bluhm, B.H., Breakspear, A., Brown, D.W., Butchko, R.A.E., Chapman, S., Coulson, R., Coutinho, P.M., Danchin, E.G.J., Diener, A., Gale, L.R., Gardiner, D.M., Goff, S., Hammond-Kosack, K.E., Hilburn, K., Hua-Van, A., Jonkers, W., Kazan, K., Kodira, C.D., Koehrsen, M., Kumar, L., Lee, K.H., Li, L., Manners, J.M., Miranda-Saavedra, D., Mukherjee, M., Park, G., Park, J., Park, S.Y., Proctor, R.H., Reguev, A., Ruiz-Roldan, M.C., Sain, D., Sakthikumar, S., Sykes, S., Schwartz, D.C., Turgeon, B.G., Wapinski, I., Yoder, O., Young, S., Zeng, Q., Zhou, S., Galagan, J., Cuomo, C.A., Kistler, H.C. and Rep, M. (2010) Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature*, **464**, 367–373.
- Maier, F.J., Miedaner, T., Haderl, B., Felk, A., Salomon, S., Lemmens, M., Kassner, H. and Schäfer, W. (2006) Involvement of trichothecenes in fusarioses of wheat, barley and maize evaluated by gene disruption of the trichodiene synthase (*Tri5*) gene in three field isolates of different chemotype and virulence. *Mol. Plant Pathol.* **7**, 449–461.
- Makandar, R., Nalam, V., Chaturvedi, R., Jeannotte, R., Sparks, A.A. and Shah, J. (2010) Involvement of salicylate and jasmonate signaling pathways in *Arabidopsis* interaction with *Fusarium graminearum*. *Mol. Plant–Microbe Interact.* **23**, 861–870.
- Marcel, S., Sawers, R., Oakeley, E., Angliker, H. and Paszkowski, U. (2010) Tissue-adapted invasion strategies of the rice blast fungus *Magnaporthe oryzae*. *Plant Cell*, **22**, 3177–3187.
- Masuda, D., Ishida, M., Yamaguchi, K., Yamaguchi, I., Kimura, M. and Nishiuchi, T. (2007) Phytotoxic effects of trichothecenes on the growth and morphology of *Arabidopsis thaliana*. *J. Exp. Bot.* **58**, 1617–1626.
- McCormick, S.P., Alexander, N.J. and Harris, L.J. (2010) CLM1 of *Fusarium graminearum* encodes a longiborneol synthase required for culmorin production. *Appl. Environ. Microbiol.* **76**, 136–141.
- McLaughlin, J.E., Bin-Umer, M.A., Tortora, A., Mendez, N., McCormick, S. and Turner, N.E. (2009) A genome-wide screen in *Saccharomyces cerevisiae* reveals a critical role for the mitochondria in the toxicity of a trichothecene mycotoxin. *Proc. Natl. Acad. Sci. USA*, **106**, 21 883–21 888.
- Merhej, J., Boutigny, A.L., Pinson-Gadais, L., Richard-Forget, F. and Barreau, C. (2010) Acidic pH as a determinant of *TRI* gene expression and trichothecene B biosynthesis in *Fusarium graminearum*. *Food. Addit. Contam. Part A: Chem. Anal. Control Expo. Risk Assess.* **27**, 710–717.
- Merhej, J., Richard-Forget, F. and Barreau, C. (2011a) Regulation of trichothecene biosynthesis in *Fusarium*: recent advances and new insights. *Appl. Microbiol. Biotechnol.* **91**, 519–528.
- Merhej, J., Richard-Forget, F. and Barreau, C. (2011b) The pH regulatory factor Pac1 regulates *Tri* gene expression and trichothecene production in *Fusarium graminearum*. *Fungal Genet. Biol.* **48**, 275–284.
- Merhej, J., Urban, M., Dufresne, M., Hammond-Kosack, K.E., Richard-Forget, F. and Barreau, C. (2011c) The velvet gene, *FgVel1*, affects fungal development and positively regulates trichothecene biosynthesis and pathogenicity in *Fusarium graminearum*. *Mol. Plant Pathol.* DOI: 10.1111/j.1364-3703.2011.00755.x.
- Miller, S.S., Watson, E.M., Lazebnik, J., Gulden, S., Balcerzak, M., Fedak, G. and Ouellet, T. (2011) Characterization of an alien source of resistance to *Fusarium* head blight transferred to Chinese Spring wheat. *Botany*, **89**, 301–311.
- Min, K., Lee, J., Kim, J.C., Kim, S.G., Kim, Y.H., Vogel, S., Trail, F. and Lee, Y.W. (2010) A novel gene, *ROA*, is required for normal morphogenesis and discharge of ascospores in *Gibberella zeae*. *Eukaryot Cell*, **9**, 1495–1503.
- Mohammadi, M., Anoop, V., Gleddie, S. and Harris, L.J. (2011) Proteomic profiling of two maize inbreds during early gibberella ear rot infection. *Proteomics*, **11**, 3675–3684.
- Mudge, A.M., Dill-Macky, R., Dong, Y., Gardiner, D.M., White, R.G. and Manners, J.M. (2006) A role for the mycotoxin deoxynivalenol in stem colonisation during crown rot disease of wheat caused by *Fusarium graminearum* and *Fusarium pseudograminearum*. *Physiol. Mol. Plant Pathol.* **69**, 73–85.
- Nguyen, L.N., Bormann, J., Le, G.T., Stärkel, C., Olsson, S., Nosanchuk, J.D., Giese, H. and Schäfer, W. (2011) Autophagy-related lipase *FgATG15* of *Fusarium graminearum* is important for lipid turnover and plant infection. *Fungal Genet. Biol.* **48**, 217–224.
- Nishiuchi, T., Masuda, D., Nakashita, H., Ichimura, K., Shinozaki, K., Yoshida, S., Kimura, M., Yamaguchi, I. and Yamaguchi, K. (2006) *Fusarium* phytotoxin trichothecenes have an elicitor-like activity in *Arabidopsis thaliana*, but the activity differed significantly among their molecular species. *Mol. Plant–Microbe Interact.* **19**, 512–520.
- Ochiai, N., Tokai, T., Takahashi-Ando, N., Fujimura, M. and Kimura, M. (2007) Genetically engineered *Fusarium* as a tool to evaluate the effects of environmental factors on initiation of trichothecene biosynthesis. *FEMS Microbiol. Lett.* **275**, 53–61.
- O'Donnell, K., Ward, T.J., Geiser, D.M., Kistler, H.C. and Aoki, T. (2004) Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. *Fungal Genet. Biol.* **41**, 600–623.
- Oide, S., Liu, J., Yun, S.H., Wu, D., Michev, A., Choi, M.Y., Horwitz, B.A. and Turgeon, B.G. (2010) Histidine kinase two-component response regulator proteins regulate reproductive development, virulence, and stress responses of the fungal cereal pathogens *Cochliobolus heterostrophus* and *Gibberella zeae*. *Eukaryot Cell*, **9**, 1867–1880.
- Paper, J.M., Scott-Craig, J.S., Adhikari, N.D., Cuomo, C.A. and Walton, J.D. (2007) Comparative proteomics of extracellular proteins *in vitro* and *in planta* from the pathogenic fungus *Fusarium graminearum*. *Proteomics*, **7**, 3171–3183.
- Peraldi, A., Beccari, G., Steed, A. and Nicholson, P. (2011) *Brachypodium distachyon*: a new pathosystem to study *Fusarium* head blight and other *Fusarium* diseases of wheat. *BMC Plant Biol.* **11**, 100.
- Pestka, J.J. (2010) Deoxynivalenol-induced proinflammatory gene expression: mechanisms and pathological sequelae. *Toxins*, **2**, 1300–1317.
- Petti, C., Khan, M. and Doohan, F. (2010) Lipid transfer proteins and protease inhibitors as key factors in the priming of barley responses to *Fusarium* head blight disease by a biocontrol strain of *Pseudomonas fluorescens*. *Funct. Integr. Genomics*, **10**, 619–627.
- Pinson-Gadais, L., Richard-Forget, F., Frasse, P., Barreau, C., Cahagnier, B., Richard-Molard, D. and Bakan, B. (2008) Magnesium represses trichothecene biosynthesis and modulates *Tri5*, *Tri6*, and *Tri12* genes expression in *Fusarium graminearum*. *Mycopathologia*, **165**, 51–59.
- Ponts, N., Pinson-Gadais, L., Verdal-Bonin, M.N., Barreau, C. and Richard-Forget, F. (2006) Accumulation of deoxynivalenol and its 15-acetylated form is significantly modulated by oxidative stress in liquid cultures of *Fusarium graminearum*. *FEMS Microbiol. Lett.* **258**, 102–107.
- Ponts, N., Pinson-Gadais, L., Barreau, C., Richard-Forget, F. and Ouellet, T. (2007) Exogenous H₂O₂ and catalase treatments interfere with *Tri* genes expression in liquid cultures of *Fusarium graminearum*. *FEBS Lett.* **581**, 443–447.
- Ponts, N., Pinson-Gadais, L., Boutigny, A.L., Barreau, C. and Richard-Forget, F. (2011) Cinnamic-derived acids significantly affect *Fusarium graminearum* growth and *in vitro* synthesis of type B trichothecenes. *Phytopathology*, **101**, 929–934.
- Poppenberger, B., Berthiller, F., Lucyshyn, D., Sieberer, T., Schuhmacher, R., Krška, R., Kuchler, K., Glössl, J., Luschnig, C. and Adam, G. (2003) Detoxification of the *Fusarium* mycotoxin deoxynivalenol by a UDP-glucosyltransferase from *Arabidopsis thaliana*. *J. Biol. Chem.* **278**, 47 905–47 914.
- Puri, K.D. and Zhong, S. (2010) The 3ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15ADON population in spring wheat. *Phytopathology*, **100**, 1007–1014.
- Rep, M. and Kistler, H.C. (2010) The genomic organization of plant pathogenicity in *Fusarium* species. *Curr. Opin. Plant Biol.* **13**, 420–426.
- Rittenour, W.R. and Harris, S.D. (2010) An *in vitro* method for the analysis of infection-related morphogenesis in *Fusarium graminearum*. *Mol. Plant Pathol.* **11**, 361–369.
- Scherm, B., Orrù, M., Balmas, V., Spanu, F., Azara, E., Delogu, G., Hammond, T.M., Keller, N.P. and Migheli, Q. (2011) Altered trichothecene biosynthesis in *TRI6*-silenced transformants of *Fusarium culmorum* influences the severity of crown and foot rot on durum wheat seedlings. *Mol. Plant Pathol.* **12**, 759–771.
- Schmidt, S.M. and Panstruga, R. (2011) Pathogenomics of fungal plant parasites: what have we learnt about pathogenesis? *Curr. Opin. Plant Biol.* **14**, 392–399.

- Schreiber, K.J., Nasmith, C.G., Allard, G., Singh, J., Subramaniam, R. and Desveaux, D. (2011) Found in translation: high-throughput chemical screening in *Arabidopsis thaliana* identifies small molecules that reduce *Fusarium* head blight disease in wheat. *Mol. Plant–Microbe Interact.* **24**, 640–648.
- Schulze-Lefert, P. and Panstruga, R. (2011) A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. *Trends Plant Sci.* **16**, 117–125.
- Schweiger, W., Boddu, J., Shin, S., Poppenberger, B., Berthiller, F., Lemmens, M., Muehlbauer, G.J. and Adam, G. (2010) Validation of a candidate deoxynivalenol-inactivating UDP-glucosyltransferase from barley by heterologous expression in yeast. *Mol. Plant–Microbe Interact.* **23**, 977–986.
- Seong, K., Hou, Z.M., Tracy, M., Kistler, H.C. and Xu, J.R. (2005) Random insertional mutagenesis identifies genes associated with virulence in the wheat scab fungus *Fusarium graminearum*. *Phytopathology*, **95**, 744–750.
- Seong, K.Y., Pasquali, M., Zhou, X., Song, J., Hilburn, K., McCormick, S., Dong, Y., Xu, J.R. and Kistler, H.C. (2009) Global gene regulation by *Fusarium* transcription factors Tri6 and Tri10 reveals adaptations for toxin biosynthesis. *Mol. Microbiol.* **72**, 354–367.
- Shin, S., Mackintosh, C.A., Lewis, J., Heinen, S.J., Radmer, L., Dill-Macky, R., Baldridge, G.D., Zeyen, R.J. and Muehlbauer, G.J. (2008) Transgenic wheat expressing a barley class II chitinase gene has enhanced resistance against *Fusarium graminearum*. *J. Exp. Bot.* **59**, 2371–2378.
- Son, H., Seo, Y.-S., Min, K., Park, A.R., Lee, J., Jin, J.-M., Lin, Y., Cao, P., Hong, S.-Y., Kim, E.-K., Lee, S.-H., Cho, A., Lee, S., Kim, M.-G., Kim, Y., Kim, J.-E., Kim, J.-C., Choi, G.J., Yun, S.-H., Lim, J.Y., Kim, M., Lee, Y.-H., Choi, Y.-D. and Lee, Y.-W. (2011) A phenotype-based functional analysis of transcription factors in the cereal head blight fungus, *Fusarium graminearum*. *PLoS Pathog.* **7**, e1002310.
- Stephens, A.E., Gardiner, D.M., White, R.G., Munn, A.L. and Manners, J.M. (2008) Phases of infection and gene expression of *Fusarium graminearum* during crown rot disease of wheat. *Mol. Plant–Microbe Interact.* **21**, 1571–1581.
- Taylor, R.D., Saparno, A., Blackwell, B., Anoop, V., Gleddie, S., Tinker, N.A. and Harris, L.J. (2008) Proteomic analyses of *Fusarium graminearum* grown under mycotoxin-inducing conditions. *Proteomics*, **8**, 2256–2265.
- Tena, G., Boudsocq, M. and Sheen, J. (2011) Protein kinase signaling networks in plant innate immunity. *Curr. Opin. Plant Biol.* **14**, 1–11.
- Thatcher, L.F., Manners, J.M. and Kazan, K. (2009) *Fusarium oxysporum* hijacks COI1-mediated jasmonate signaling to promote disease development in *Arabidopsis*. *Plant J.* **58**, 927–939.
- Trail, F. (2009) For blighted waves of grain: *Fusarium graminearum* in the postgenomics era. *Plant Physiol.* **149**, 103–110.
- Tsuyuki, R., Yoshinari, T., Sakamoto, N., Nagasawa, H. and Sakuda, S. (2011) Enhancement of trichothecene production in *Fusarium graminearum* by cobalt chloride. *J. Agric. Food Chem.* **59**, 1760–1766.
- Urban, M., Daniels, S., Mott, E. and Hammond-Kosack, K.E. (2002) *Arabidopsis* is susceptible to the cereal ear blight fungal pathogens *Fusarium graminearum* and *Fusarium culmorum*. *Plant J.* **32**, 961–973.
- Urban, M., Mott, E., Farley, T. and Hammond-Kosack, K. (2003) The *Fusarium graminearum* MAP1 gene is essential for pathogenicity and development of perithecia. *Mol. Plant Pathol.* **4**, 347–359.
- Voigt, C.A., Schäfer, W. and Salomon, S. (2005) A secreted lipase of *Fusarium graminearum* is a virulence factor required for infection of cereals. *Plant J.* **42**, 364–375.
- Volpi, C., Janni, M., Lionetti, V., Bellincampi, D., Favaron, F. and D'Ovidio, R. (2011) The ectopic expression of a pectin methyl esterase inhibitor increases pectin methyl esterification and limits fungal diseases in wheat. *Mol. Plant–Microbe Interact.* **24**, 1012–1019.
- Walter, S. and Doohan, F. (2011) Transcript profiling of the phytotoxic response of wheat to the *Fusarium* mycotoxin deoxynivalenol. *Mycotoxin Res.* **27**, 221–230.
- Walter, S., Brennan, J.M., Arunachalam, C., Ansari, K.I., Hu, X., Khan, M.R., Trognitz, F., Trognitz, B., Leonard, G., Egan, D. and Doohan, F.M. (2008) Components of the gene network associated with genotype-dependent response of wheat to the *Fusarium* mycotoxin deoxynivalenol. *Funct. Integr. Genomics*, **8**, 421–427.
- Walter, S., Nicholson, P. and Doohan, F.M. (2010) Action and reaction of host and pathogen during *Fusarium* head blight disease. *New Phytol.* **185**, 54–66.
- Wang, Y., Liu, W., Hou, Z., Wang, C., Zhou, X., Jonkers, W., Ding, S., Kistler, H.C. and Xu, J.R. (2011) A novel transcriptional factor important for pathogenesis and ascospore germination in *Fusarium graminearum*. *Mol. Plant–Microbe Interact.* **24**, 118–128.
- Wang, Y., Yang, L., Xu, H., Li, Q., Ma, Z. and Chu, C. (2005) Differential proteomic analysis of proteins in wheat spikes induced by *Fusarium graminearum*. *Proteomics*, **5**, 4496–4503.
- Woriedh, M., Hauber, I., Martinez-Rocha, A.L., Voigt, C., Maier, F.J., Schröder, M., Meier, C., Hauber, J. and Schäfer, W. (2011) Preventing *Fusarium* head blight of wheat and cob rot of maize by inhibition of fungal deoxyhypusine synthase. *Mol. Plant–Microbe Interact.* **24**, 619–627.
- Xu, X. and Nicholson, P. (2009) Community ecology of fungal pathogens causing wheat head blight. *Annu. Rev. Phytopathol.* **47**, 83–103.
- Yaguchi, A., Yoshinari, T., Tsuyuki, R., Takahashi, H., Nakajima, T., Sugita-Konishi, Y., Nagasawa, H. and Sakuda, S. (2009) Isolation and identification of precenes and piperitone from essential oils as specific inhibitors of trichothecene production by *Fusarium graminearum*. *J. Agric. Food Chem.* **57**, 846–851.
- Yang, F., Jensen, J.D., Svensson, B., Jørgensen, H.J., Collinge, D.B. and Finnie, C. (2010a) Analysis of early events in the interaction between *Fusarium graminearum* and the susceptible barley (*Hordeum vulgare*) cultivar Scarlett. *Proteomics*, **10**, 3748–3755.
- Yang, F., Jensen, J.D., Spliid, N.H., Svensson, B., Jacobsen, S., Jørgensen, L.N., Jørgensen, H.J., Collinge, D.B. and Finnie, C. (2010b) Investigation of the effect of nitrogen on severity of *Fusarium* head blight in barley. *J. Proteomics*, **73**, 743–752.
- Yang, F., Svensson, B. and Finnie, C. (2011) Response of germinating barley seeds to *Fusarium graminearum*: the first molecular insight into *Fusarium* seedling blight. *Plant Physiol. Biochem.* **49**, 1362–1368.
- Zantinge, J., Kumar, K., Xi, K., Johns, M., Murray, A., Jones, T., Helm, J.H. and Juskiw, P. (2010) Comparison of barley seed proteomic profiles associated with *Fusarium* head blight reaction. *Can. J. Plant Pathol.* **32**, 496–512.
- Zhang, D., Fan, F., Yang, J., Wang, X., Qiu, D. and Jiang, L. (2010) FgTep1p is linked to the phosphatidylinositol-3 kinase signalling pathway and plays a role in the virulence of *Fusarium graminearum* on wheat. *Mol. Plant Pathol.* **11**, 495–502.
- Zhang, Y., Choi, Y.E., Zou, X. and Xu, J.R. (2011) The FvMK1 mitogen-activated protein kinase gene regulates conidiation, pathogenesis, and fumonisin production in *Fusarium verticillioides*. *Fungal Genet. Biol.* **48**, 71–79.
- Zhao, C., Waalwijk, C., De Wit, P.J., van der Lee, T. and Tang, D. (2011) EBR1, a novel Zn2Cys6 transcription factor, affects virulence and apical dominance of hyphal tip in *Fusarium graminearum*. *Mol. Plant–Microbe Interact.* DOI: 10.1094/MPMI-06-11-0158.
- Zhao, X.M., Zhang, X.W., Tang, W.H. and Chen, L. (2009) FPPI: *Fusarium graminearum* protein–protein interaction database. *J. Proteome Res.* **8**, 4714–4721.
- Zhou, W., Kolb, F.L. and Riechers, D.E. (2005) Identification of proteins induced or upregulated by *Fusarium* head blight infection in the spikes of hexaploid wheat (*Triticum aestivum*). *Genome*, **48**, 770–780.
- Zhou, W., Eudes, F. and Laroche, A. (2006) Identification of differentially regulated proteins in response to a compatible interaction between the pathogen *Fusarium graminearum* and its host, *Triticum aestivum*. *Proteomics*, **6**, 4599–4609.