Pathogen profile

# Heading for disaster: *Fusarium graminearum* on cereal crops

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## SUMMARY

The rapid global re-emergence of *Fusarium* head blight disease of wheat and barley in the last decade along with contamination of grains with mycotoxins attributable to the disease have spurred basic research on the fungal causal agent. As a result, *Fusarium graminearum* quickly has become one of the most intensively studied fungal plant pathogens. This review briefly summarizes current knowledge on the pathogenicity, population genetics, evolution and genomics of *Fusarium graminearum*.

**Taxonomy:** Based on the sexual state *Gibberella zeae* (Schwein.) Petch: Superkingdom Eukaryota; Kingdom Fungi; Phylum Ascomycota; Subphylum Pezizomycotina; Class Sordariomycetidae; Subclass Hypocreomycetidae; Order Hypocreales; Family Nectriaceae; Genus *Gibberella*.

**Host range:** The pathogen is capable of causing head blight or 'scab' on wheat (*Triticum*), barley (*Hordeum*), rice (*Oryza*), oats (*Avena*) and *Gibberella* stalk and ear rot disease on maize (*Zea*). The fungus also may infect other plant species without causing disease symptoms. Other host genera cited for *Gibberella zeae* or *F. graminearum sensu lato* (see below) are *Agropyron*, *Agrostis*, *Bromus*, *Calamagrostis*, *Cenchrus*, *Cortaderia*, *Cucumis*, *Echinochloa*, *Glycine*, *Hierochloe*, *Lolium*, *Lycopersicon*, *Medicago*, *Phleum*, *Poa*, *Schizachyrium*, *Secale*, *Setaria*, *Sorghum*, *Spartina* and *Trifolium*.

**Disease symptoms and signs:** For wheat, brown, dark purple to black necrotic lesions form on the exterior surface of the florets and glume (Fig. 1). Although these lesion symptoms sometimes are referred to as scab, they are not formally related to the hyperplasia and hypertrophic epidermal growth associated with other scab diseases such as apple scab. Peduncles immediately below the inflorescence may become discoloured brown/purple. With time, tissue of the inflorescence often becomes blighted, appearing bleached and tan, while the grain within atrophies. Awns often become deformed, twisted and curved downward. In barley, infections are not always readily apparent in the field. Infected spikelets may show a browning or water-soaked appearance. Infected barley kernels show a tan to dark brown discolour-

ation that can be similar to that caused by other kernel blighting organisms. During prolonged wet periods, pink to salmon-orange spore masses of the fungus are often seen on infected spikelets, glumes and kernels in both wheat and barley. For maize ear rot, infection occurs by way of colonizing silk and thus symptoms first appear at the ear apex. White mycelium, turning pink to red with time, colonizes kernels and may progress basipetally, covering the entire ear.

**Useful websites:** www.broad.mit.edu/annotation/fungi/fusarium/mips.gsf.de/genre/proj/fusarium/
www.cdl.umn.edu/scab/gz-consort.html
www.scabusa.org/

## INTRODUCTION

### *Fusarium* head blight

The fungal pathogen *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schweinitz) Petch], is the most common causal agent of *Fusarium* head blight (FHB) in North America and many other parts of the world. This destructive disease, commonly but perhaps inappropriately known as scab, affects wheat, barley and other small grains both in temperate and in semitropical areas. The disease has the capacity to destroy a potentially high-yielding crop within a few weeks of harvest (McMullen *et al.*, 1997). FHB was first described in 1884 in England and was considered a major threat to wheat and barley during the early years of the twentieth century (Muriuki, 2001; Stack, 1999, 2003). Since then, FHB has increased worldwide and recent outbreaks have been reported in Asia, Canada, Europe and South America. FHB has been identified by CIMMYT as a major factor limiting wheat production in many parts of the world (Stack, 1999). In the United States, FHB has reached epidemic levels in several years during the last decade, causing yield losses and price discounts resulting from reduced seed quality (Windels, 2000). For example, direct and secondary economic losses due to FHB for all crops in the Northern Great Plains and Central United States were estimated to be $2.7 billion from 1998 to 2000 alone (Nganje *et al.*, 2002).

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**Fig. 1** Field-grown wheat inflorescence showing symptoms of *Fusarium* head blight. The third spikelet from the bottom shows a darkened necrotic lesion ('scab') whereas the second and fifth spikelets demonstrate tissue bleaching ('blight') symptoms. Photograph courtesy of Jacki Morrison, USDA ARS Cereal Disease Laboratory.

The threat posed by this fungus is multifaceted. It causes yield and quality losses due to sterility of the florets and formation of discoloured, withered and light test-weight kernels. These characteristics cause difficulties for marketing, exporting and processing infected grain. Additionally, infected grains may contain significant levels of trichothecenes and the oestrogenic mycotoxin, zearelanone, which are hazardous to animals, thus making the grain unfit for food or feed (McMullen *et al.*, 1997). Trichothecene toxins such as deoxynivalenol (DON), commonly known as vomitoxin, are sesquiterpenoids that are potent inhibitors of eukaryotic protein biosynthesis. Acute adverse effects of the toxin in animals include food refusal, diarrhoea, emesis, alimentary haemorrhaging and contact dermatitis (Bennett and Klich, 2003). In humans, *F. graminearum* has been linked to alimentary toxic aleukia and Akakabi toxicosis, illnesses characterized by nausea, vomiting, anorexia and convulsions. Perhaps as expected for inhibitors of protein synthesis, chronic exposure to

trichothecenes has wide-ranging effects, including neurological disorders and immunosuppression (Bennett and Klich, 2003). Plant cultivars highly resistant to the disease or tolerant to the toxin currently are not available and the use of fungicides for controlling the disease is limited by cost, difficulty in efficient application to wheat heads and an incomplete understanding of factors that influence disease development (McMullen *et al.*, 1997; Pirgozliev *et al.*, 2003).

### Life cycle

Primary inoculum for this disease comes from infected plant debris on which the fungus overwinters as saprophytic mycelia. In spring, warm moist weather conditions are favourable for the development and maturation of conidia and perithecia that produce ascospores concurrently with the flowering of cereal crops (Markell and Francl, 2003). The sticky ascospores are forcibly discharged from mature perithecia (Trail *et al.*, 2002) formed on the surface of crop debris (e.g. corn stubble) and dispersed by wind, rain or insects to host plants (Parry *et al.*, 1995; Sutton, 1982).

A comprehensive review of the FHB infection process recently has been published (Bushnell *et al.*, 2003). Briefly summarized, deposition of spores on or inside spike tissue initiates the infection process; wheat heads are most susceptible to infection during anthesis (Sutton, 1982). The fungus initially does not penetrate directly through the epidermis. Rather, hyphae develop on the exterior surfaces of florets and glumes, allowing the fungus to grow toward stomata and other susceptible sites within the inflorescence (Bushnell *et al.*, 2003). Hyphae may also form peculiar lobed structures between cuticle and epidermal cell wall on the surface of inoculated glumes (Pritsch *et al.*, 2000). Such subcuticular growth on the glume, lemma and palea is thought to serve as a mechanism for fungal spread and, in the case of adaxial floret surfaces, probably leads to direct penetration of epidermal cells (Bushnell *et al.*, 2003). Other avenues for direct entry include stomata and underlying parenchyma, partially or fully exposed anthers, openings between the lemma and palea of the spikelet/floret during dehiscence (Bushnell *et al.*, 2003; Lewandowski and Bushnell, 2001) and through the base of the wheat glumes where the epidermis and parenchyma are thin-walled. Once inside the floret, the anthers, stigmas and lodicules are most easily colonized.

The principal mode of fungal spread in wheat from floret to floret inside a spikelet and from spikelet to spikelet is through the vascular bundles in the rachis and rachilla (Ribichich *et al.*, 2000). Several changes in the vascular bundles have been noted that may cause the xylem and phloem tissues in the infected rachis to become at least partially dysfunctional leading to premature death of the spikelet. Under wet conditions mycelia can spread over the exterior surfaces of the glume, lemma and palea in both wheat and barley (Bushnell *et al.*, 2003). The fungus appears to

have a brief biotrophic relationship with it host before switching to the necrotrophic phase. This necrotrophic stage is associated with an increase in vigour of colonization by the fungus and eventually plant death leads to thorough colonization of the host substrate. In some instances, *F. graminearum* also may colonize plant tissues asymptomatically, such as stalks of corn (Bushnell *et al.*, 2003), or can be isolated from nonsymptomatic grass hosts (Farr *et al.*, 1989; Inch and Gilbert, 2003).

## SPECIES DEFINITIONS AND GENETICS

### Definition of species

Until recently, the name '*Fusarium graminearum*' was used to describe what we now know to be a polyphyletic grouping of distinct fungal species. Prior to 1999, homothallic fungi causing *Fusarium* head blight of wheat and barley were known as *F. graminearum* 'group 2' to distinguish them from the heterothallic fungus causing crown and foot rot on grains, which was called *F. graminearum* 'group 1' (Francis and Burgess, 1977). It is now known that these fungi are separate biological and phylogenetic species, each producing their own characteristic sexual state. The former 'group 1' is now known as *Fusarium pseudograminearum* and has a sexual stage called *Gibberella coronicola* (Aoki and O'Donnell, 1999). Much of the literature on root rotting strains of '*F. graminearum*' probably involved studies on *F. pseudograminearum*. Excluding consideration of *F. pseudograminearum*, *F. graminearum* 'group 2' is now known to be a monophyletic species complex consisting of at least nine separate phylogenetic species, some of which are localized on particular continents or geographical regions (O'Donnell *et al.*, 2000, 2004).

A genealogical concordance phylogenetic species recognition approach was used to identify species limits among strains morphologically identified as *F. graminearum*. DNA sequence comparisons were used to determine if concordant monophyletic genealogies of orthologous regions occurred at independent loci. Such concordance is the basis for recognition of phylogenetic species limits (Taylor *et al.*, 2000). Using 99 strains gathered from a variety of substrates from around the world, seven biogeographically structured species were discovered (O'Donnell *et al.*, 2000). More recent work containing a larger collection of strains and DNA sequence comparisons from a total of 11 genes at six independent genetic loci support the existence of nine distinct, cryptic species within the *F. graminearum* species complex (O'Donnell *et al.*, 2004; Ward *et al.*, 2002). These species now have been formally named, with *F. graminearum sensu stricto* retained for the species most commonly associated with *Fusarium* head blight worldwide. *Fusarium asiaticum* (formerly known as *F. graminearum* lineage 6) is the most common cause of *Fusarium* head blight in certain regions of China and other parts of Asia

(Gale *et al.*, 2002; O'Donnell *et al.*, 2000, 2004). Other species of the *F. graminearum* complex have been isolated from diverse hosts and studies are underway to determine if these too are capable of causing *Fusarium* head blight (Goswami and Kistler, 2002). For the remainder of this review, we use the name *F. graminearum* in the sense of O'Donnell *et al.* (2004) and recommend its strict use when referring to FHB pathogens.

### Genetics

The sexual state of *F. graminearum*, *Gibberella zeae*, is a homothallic ascomycete. Homothallism arises from the fact that alternative forms of the mating type (MAT) locus (idiomorphs), normally found in separate nuclei in heterothallic fungi, are juxtaposed at the same locus in *G. zeae* (Yun *et al.*, 2000). Deletion of either idiomorph from *F. graminearum* eliminates self-fertility although deletion strains retain the ability to outcross (Lee *et al.*, 2003). All mating type genes are subject to strong purifying selection in *F. graminearum*, *F. pseudograminearum* and related *Fusarium* species, including those for which a sexual stage is unknown (O'Donnell *et al.*, 2004).

*F. graminearum* contains four chromosomes that can be visualized by cytological methods (Taga *et al.*, 2003) but are too large to be resolved clearly by standard methods of pulsed field electrophoresis. Strains can outcross in culture (Bowden and Leslie, 1999) even though population genetic analysis indicates that the rate of outcrossing may be limited in nature (see below). Outcrosses have been made in culture between *F. graminearum* strains and between *F. graminearum* and other species within the *F. graminearum* species complex.

Gale and colleagues have constructed a genetic map using a cross between the strain of the fungus used for whole genome sequencing (see below), PH-1 (NRRL 31084), and a closely related strain from Minnesota, USA, 00-676 (NRRL 34097) (see www.broad.mit.edu/annotation/fungi/fusarium/markers.html). Currently over 99.8% of the DNA sequence has been anchored to the genetic map by way of 237 genetic markers, 164 of which are sequence-tagged sites. Only approximately 70 kb of the 36-Mb genome assembly is currently not linked to the map. All but three genetic markers of the 1154-cM map are colinear with the assembly; the three disagreements, among closely linked genes, are minor. Four major blocks of DNA sequences joined by genetic linkage groups thus have been described, in agreement with four chromosomes detected cytologically (L. R. Gale *et al.*, unpublished data).

A previous genetic map (Jurgenson *et al.*, 2002) now has been determined to have resulted from an interspecific cross between strains of *F. graminearum* and *F. asiaticum* (O'Donnell *et al.*, 2004). The cross exhibited profound recombination suppression and segregation distortion consistent with interspecific hybridization. For example, more than half the progeny show no

recombination for four of the nine linkage groups described. Two additional linkage groups show recombination patterns consistent with large-scale chromosome inversions (Jurgenson *et al.*, 2002). With the development of codominant and sequence-tagged markers for this cross (Cumagun *et al.*, 2004), it will be interesting to see how this genetic map may be used to determine chromosome differences that have arisen between the parental species.

### Trichothecene biosynthesis and evolution

Much of the biochemical characterization of the trichothecene toxin biosynthetic pathway has been conducted in the fungus *Fusarium sporotrichioides*. Comparative studies have shown very similar genes are operative in *F. graminearum* except where differences would be expected based on acetylation patterns of the trichothecenes (Kimura *et al.*, 2003; Lee *et al.*, 2002) or oxygenation at positions C-4 and C-8 (Lee *et al.*, 2002; McCormick *et al.*, 2004; Meek *et al.*, 2003).

Three strain-specific profiles of trichothecene metabolites, called 'chemotypes' (Anderson *et al.*, 1989), have been observed for individuals of the *F. graminearum* species complex and related species (Ward *et al.*, 2002). Strains produce predomi-

nantly either DON or its C-4 oxygenated derivative, nivalenol; DON producers also make acetyl ester derivatives of DON at the three-, and 15-position oxygens. Thus three trichothecene chemotypes are observed: NIV (nivalenol producers), 3ADON (DON producers that also make predominantly 3-acetyl,deoxynivalenol) and 15ADON (DON producers that also make predominantly 15-acetyl,deoxynivalenol). Approximately one half of the genes required for the biosynthesis of trichothecenes are located on a 25-kb gene cluster (Kimura *et al.*, 2001). The 'Tri cluster' (Fig. 2) contains a central core of genes involved in the biosynthesis of the basic trichothecene structure (e.g. *Tri4* and *Tri5*) and flanking genes probably involved in structural differences that are the basis for the chemotypes described above (e.g. *Tri3* and *Tri13*).

Strain-specific chemotype differences are not well correlated with the overall evolutionary history of head blight pathogens, but allelic polymorphisms within the trichothecene biosynthetic gene cluster are strongly associated with chemotype differences (O'Donnell *et al.*, 2000; Ward *et al.*, 2002). The basis for this discord between species and chemotype evolution is that polymorphism within the trichothecene cluster is transspecific and has apparently been maintained by balancing selection acting on chemotype differences originating in the ancestor of head blight pathogens (Ward *et al.*, 2002). However, the mechanism
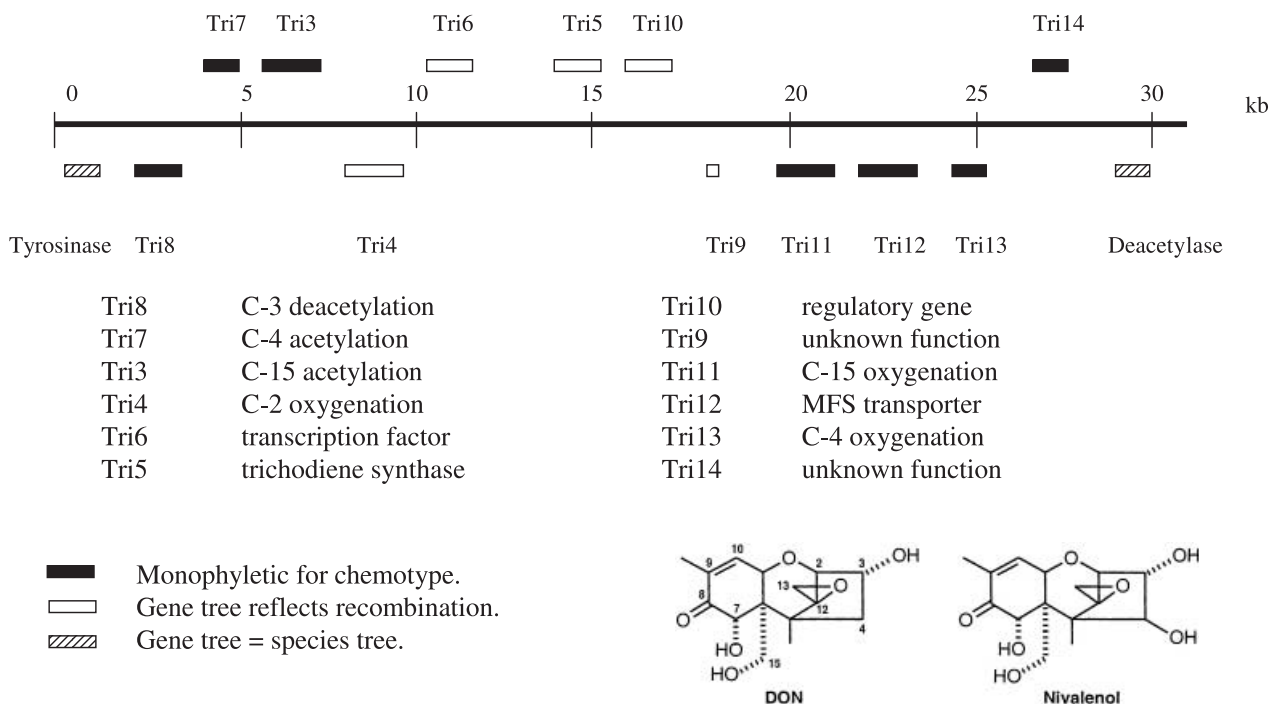


**Fig. 2** Diagram of the trichothecene biosynthetic (Tri) gene cluster of a nivalenol-producing strain of *F. graminearum*. (Top) Boxes indicate the relative positions of genes on the Watson (above ticked line) or Crick (below line) strand. In 15ADON strains, both *Tri7* and *Tri13* are pseudogenes. In 3ADON strains, *Tri 8* and *Tri13* are pseudogenes and *Tri7* is deleted. The putative tyrosinase and deacetylase at the 5′ and 3′ ends lie outside the Tri cluster. Below this is a key of the function of each gene and a legend indicating the evolutionary history of genes within and outside the cluster. At the bottom right are structures of the major trichothecenes produced by *F. graminearum*, DON and nivalenol.

responsible for balancing selection within the trichothecene biosynthetic cluster has not been determined. It has been suggested that some form of temporal or spatial heterogeneity in selective pressure may exist, such that the optimal trichothecene chemotype varies by environment (e.g. host) or changes over time (Ward *et al.*, 2002). Different chemical forms of the trichothecene molecule (e.g. DON and 3ADON) show different toxicities toward plants and animal cells (Anderson *et al.*, 1989; Kimura *et al.*, 1998). Recent reports also suggest that strains of the *F. graminearum* species complex that produce NIV may be more aggressive toward corn but less aggressive to wheat than DON-producing strains (Carter *et al.*, 2002; Cumagun *et al.*, 2004). These results indicate that there may be important consequences for the fitness and aggressiveness of FHB pathogens of different chemotype on particular hosts.

Recombination between chemotype clusters has been extremely limited at either end, but not for genes in the middle of the trichothecene biosynthetic gene cluster (Ward *et al.*, 2002). In order to explain this unusual pattern, recombination among genes at the ends of the cluster must be either suppressed or strongly selected against. The central genes are probably those involved in processes common to the synthesis of all trichothecene molecules. For example, *Tri5* is the gene encoding trichodiene synthase, the enzyme for sesquiterpenoid cyclization and the first committed step in the trichothecene biosynthetic pathway (Hohn and Beremand, 1989). Likewise, *Tri4* (Hohn *et al.*, 1995) encodes a cytochrome P-450 monooxygenase involved in oxygenation of the two-position carbon required for the C-2, C-11 ether linkage forming the essential trichothecene oxygen heterocycle. Conversely, genes at either end of the biosynthetic cluster evolve in a manner strictly correlated with chemotype and it is these genes that probably encode proteins that determine chemotype differences. For example, *Tri3* encodes a 15-*O*-acetyl transferase (McCormick *et al.*, 1996) that seemingly would be essential for 15ADON production and the 15ADON chemotype. *Tri13*, by contrast, encodes the oxygenase responsible for C-4 hydroxylation and thus the NIV chemotype (Lee *et al.*, 2002). Recombination between the central, shared genes of the trichothecene gene cluster has been demonstrated (Ward *et al.*, 2002) and probably occurs because the genes serve the same function in each cluster. The reason why recombination is not observed between the outer regions of the NIV, 3ADON and 15ADON gene clusters is currently unclear. Comparison of the entire biosynthetic gene cluster for strains of different chemotype, however, explains the likely reasons for the differences in the trichothecene pathway end-products. Both DON chemotype gene clusters contain a nonfunctional *Tri13* pseudogene consistent with the inability of these strains to produce the C-4 oxygenated nivalenol (Kimura *et al.*, 2003; Lee *et al.*, 2002). Likewise, DON chemotype gene clusters lack a functional *Tri7* gene; it is a pseudogene in 15ADON clusters and is deleted in 3ADON clusters (Kimura *et al.*, 2003; Lee *et al.*, 2002). Presumably the gene for the enzyme involved in acetyl ester formation at the four-position oxygen is relieved of selective constraint in genotypes lacking the ability to synthesize trichothecenes oxygenated at position four. 3ADON clusters also contain a presumed transcribed *Tri8* pseudogene and loss of the encoded three-position deacetylase may contribute directly to the 3ADON chemotype (Kimura *et al.*, 2003). Finally, *Tri3*, involved in C-15 acetylation, also has been shown to be free of selective constraint in 3ADON gene clusters (Ward *et al.*, 2002).

Flanking the trichodiene synthase gene (*Tri5*) in all chemotypes are genes involved in the positive regulation of the Tri cluster, *Tri6* and *Tri10*. *Tri6* is a $Cys_2His_2$ zinc-finger protein involved in transcription activation of trichothecene biosynthetic genes, including genes outside of the Tri cluster (Hohn *et al.*, 1999; Matsumoto *et al.*, 1999; Proctor *et al.*, 1995; Tag *et al.*, 2001). *Tri6* binds the sequence TNAGGCCT found in the promoter of ten toxin biosynthetic genes (Hohn *et al.*, 1999; McCormick *et al.*, 2004) and binding is essential to transcriptional up-regulation (Hohn *et al.*, 1999). *Tri10* also is required for trichothecene biosynthesis and expression of genes involved in its biosynthesis, apparently acting upstream of *Tri6* (Tag *et al.*, 2001). Recently it has been shown that *Tri6* and *Tri10* each also positively regulate genes involved in isoprenoid biosynthesis (including hydroxymethylglutaryl CoA synthase, acetyl-CoA acetyl transferase, and mevalonate kinase) and other genes unlinked to the Tri cluster with unknown function (Peplow *et al.*, 2003). These authors concluded that many other *Tri10*- and *Tri6*-regulated genes, including additional genes involved in isoprenoid and trichothecene biosynthesis, are yet to be identified.

Other genes involved in trichothecene biosynthesis in *F. graminearum* have been identified outside the Tri biosynthetic gene cluster, including *Tri1*, a cytochrome P-450 that may be involved in oxygenation of trichothecenes at either position C-7, C-8 or at both positions (McCormick *et al.*, 2004), and *Tri101* (Kimura *et al.*, 1998), a trichothecene 3-*O*-acetyltransferase.

## Population genetics

Genetic diversity studies on members of the *F. graminearum* species complex have been undertaken on strains gathered from around the world, including China (Gale *et al.*, 2002), Nepal (Carter *et al.*, 2000), parts of Europe (Miedaner *et al.*, 2001; Waalwijk *et al.*, 2003), Canada (Miedaner *et al.*, 2001) and parts of the USA (Walker *et al.*, 2001; Zeller *et al.*, 2003, 2004). Although these studies, recently reviewed by Gale (2003), have provided some insight into the population biology of *F. graminearum*, many have suffered from an incomplete recognition of phylogenetic species within the species complex, making inference on diversity and gene flow problematic. High genotypic diversity has been found within populations of

*F. graminearum* in the USA and this has been interpreted as evidence for frequent and ongoing sexual outcrossing in this homothallic fungus (Walker *et al.*, 2001; Zeller *et al.*, 2004). Additionally, little or no population subdivision has been observed in the sampling of fields separated by hundreds of kilometres (Zeller *et al.*, 2003, 2004).

In contrast to these findings, Gale *et al.* (2003) found evidence for persistent population subdivision within *F. graminearum* in the USA that is correlated with the profile of trichothecene toxins produced by these strains (chemotype). Although most North American strains of *F. graminearum* have been determined to produce predominantly 15ADON as their major acetylated form of DON, a small sampling of isolates of the fungus from North Dakota and Minnesota obtained in 1999 and 2000 produced 3ADON as their major acetyl derivative. When these 3ADON strains were considered together as a subpopulation they displayed considerable evidence of isolation from the larger, 15ADON population. Owing to the limited geographical distribution of the data and differentiation of the data from the larger 15ADON population, Gale *et al.* (2003) suggested that the 3ADON strains are a relatively recent introduction to North America and that they have not yet been assimilated into the resident population. Because this population differentiation could persist over the course of at least a year, the rate of outcrossing among strains of *F. graminearum* in the USA is not considered to be high (Gale *et al.*, 2003).

## GENOMIC STUDIES

### ESTs and the whole genome shotgun assembly

Genomics research on *F. graminearum* was initiated in the late 1990s with the public release of databases of Expressed Sequence Tags (ESTs). EST libraries were created from the fungus grown in culture or from plants infected with the fungus. Trail *et al.* (2003b) reported on the analysis of three cDNA libraries consisting of a total of 7996 ESTs estimated to code for 2110 genes. Among the three libraries, two were generated from carbon- (C-) and nitrogen- (N-) starved mycelia and one was generated from cultures of maturing perithecia (P). As expected, the C and N libraries exhibited similar, but not identical, patterns of gene expression whereas the perithecial library contained a dissimilar suite of expressed genes. Comparisons were made with ESTs from the related fungi *Neurospora crassa* and *Magnaporthe grisea* and the genomic sequence of *N. crassa*. Based on BLASTX comparisons, 53.8% of *F. graminearum* ESTs from the three libraries had homologues in the *N. crassa* genome at $P < $ e-5 (47.0% at $P < $ e-10). Kruger *et al.* (2002) described an EST database containing 4838 sequences obtained from a cDNA library created using heads of Sumai 3, a partially resistant wheat cultivar, infected with *F. graminearum*. Their identification of fungal genes was primarily based on BLASTX annotations to proteins from fungal species in GenBank and sequence comparisons withn the publicly available *F. graminearum* ESTs. Although this library contained many sequences apparently unique to *Fusarium*/wheat interaction, only a small fraction of ESTs, roughly 2% of the non-redundant sequence set, were attributable to the fungus.

Other approaches also are being used to study the gene expression pattern during disease development on wheat. These include production of subtracted cDNA libraries from wheat heads inoculated with a highly aggressive strain and a less aggressive strain (Goswami *et al.*, 2002) or a noninfected (mock inoculated) treatment (Goswami *et al.*, 2003). Differences in overall gene expression were observed comparing the highly aggressive and less aggressive strain during pathogenesis. However, only a very low percentage of *Fusarium* ESTs were recovered from these libraries, as determined by matches to the *F. graminearum* genome sequence. By contrast, using subtracted libraries made from *Fusarium*-infected vs. mock inoculated plants, nearly 25% of the ESTs were of fungal origin (Goswami *et al.*, 2003). Several of these potentially may be involved in pathogenicity based on predicted function of the genes corresponding to ESTs.

The release of the complete *F. graminearum* genome sequence in May 2003 marked the beginning of a new era in the study of this pathogen. The whole genome shotgun (WGS) sequencing of a highly pathogenic *F. graminearum* strain designated NRRL 31084 (PH-1) was carried out at the Broad Institute, Center for Genome Research (www.broad.mit.edu/annotation/fungi/fusarium/). The strain for sequencing was chosen by consensus agreement of the *Gibberella zeae* International Genome Initiative (GIGI) (www.cdl.umn.edu/scab/gz-consort.html). The WGS sequencing of the genome involved using > 600 000 paired end reads from plasmid, fosmid and BAC libraries that were then assembled using the program Arachne (Batzoglou *et al.*, 2002) resulting in ~10× coverage of the genome. Although the assembly size was approximately 36 Mb, the total genome size probably is closer to 38 Mb as some elements, such as the genes for the major ribosomal RNA repeats and telomeres, are not included within the assembly.

The draft assembly is remarkably complete owing to the paucity of low-complexity DNA and near lack of repetitive sequences in the genome. After manual editing, the entire assembly currently is contained on only 28 scaffolds ranging in size from 3 kb to over 8.8 Mb with an average contig length of over 71 kb. This level of completion greatly minimizes the number of genes that may be missed due to gaps in the assembly.

Gene prediction currently is an inexact science and different prediction methods differ in the number of predicted genes (gene models) determined for the assembly. Regardless, all estimates indicate that the *F. graminearum* genome contains > 11 600 genes, making it the most gene-rich fungus yet described. The Broad

Institute has used their gene prediction pipeline to determine the location and structure of 11 640 genes in the *Fusarium* genome assembly. This pipeline uses a combination of the programs FGENESH and FGENESH+ (Salamov and Solovyev, 2000) modified by Softberry (www.softberry.com) with *Fusarium*-specific gene parameters and the GENEWISE gene prediction program (Birney *et al.*, 2004). Some users of these gene models, however, have noted differences between predicted genes and manually annotated sequences, including fusion of 5′ and 3′ exons as well as open reading frame (ORF) fusions. The Munich Information Center for Protein Sequences (MIPS) has presented a second predicted gene set with FGENESH utilizing more general 'mixed fungi' parameters. Although there is a measure of overlap for the gene models by these two methods, 7457 of the gene models are nonidentical. Additionally, both methods fail to predict hundreds of genes validated by EST data. Improvements to the predicted gene sets are being made by manual annotation by members of GIGI representing over 25 laboratories world-wide and further EST sequencing at the Broad Institute. MIPS currently is hosting web-access to the manual annotation as well as both the Broad Institute and the MIPS gene models.

## Functional genomics

The *Tri5* gene of the trichothecene biosynthetic gene cluster was the first pathogenicity gene of *F. graminearum* to be identified and verified by gene disruption. *Tri5* encodes a trichodiene synthase that catalyses the first committed reaction in the trichothecene biosynthetic pathway. *F. graminearum* mutants with a disrupted *Tri5* gene were shown to be unable to produce deoxynivalenol and to be drastically reduced in virulence (Bai *et al.*, 2001; Desjardins *et al.*, 1996; Proctor *et al.*, 1995).

Recently, genes for two mitogen-activated protein (MAP) kinases, *Mgv1* (Hou *et al.*, 2002) and *Map1* (Urban *et al.*, 2003)—also called *gpmk1* (Jenczmionka *et al.*, 2003)—have been identified and their role in pathogenicity demonstrated. MAP kinase-mediated signal transduction pathways play important roles in the pathogenicity of other fungi (Liu, 2002; Ortoneda *et al.*, 2004; Talbot, 2003). MAP kinases belong to a family of serine/threonine protein kinases which activate transcription factors that function in the regulation of mating, conidiation and conidia germination, appressorium and penetration peg formation, invasive hyphal growth and response to hyperosmotic stress and cell turgor (Lengeler *et al.*, 2000; Xu, 2000). *Mgv1* is homologous to the MAP kinase genes *Mps1* of *M. grisea* and *Slt2* from *Saccharomyces cerevisiae* (Xu *et al.*, 1998). *F. graminearum* mutants of this gene generated through gene replacement strategies exhibited female sterility, reduced vegetative growth on solid media but normal growth on liquid media, weak cell walls, self-incompatibility, substantially reduced virulence and reduced ability to produce mycotoxins. Thus, *Mgv1* is believed to be involved in multiple developmental processes related to sexual reproduction, plant infection and cell wall integrity (Hou *et al.*, 2002).

Cloning of *MAP1,* another MAP kinase, from *F. graminearum* followed by targeted deletion (Urban *et al.*, 2003) or disruption (Jenczmionka *et al.*, 2003) of the gene was conducted independently in two laboratories. Each study showed that the *MAP1* kinase plays an essential role in mating and pathogenicity. *MAP1* mutants were unable to produce aerial hyphae on minimal media, had reduced conidiation, were sexually sterile, being unable to produce perithecia, and were essentially nonpathogenic on wheat. *MAP1* belongs to the Fus3/Kss1 clade of extracellular signal-regulated MAP kinases. Orthologues have been shown to be involved in pheromone response as well as appressorium formation and invasive growth of *M. grisea* on rice (Xu and Hamer, 1996) and pathogenicity defects in a number of other fungi (Xu, 2000).

Another *F. graminearum* pathogenicity gene, *CPS1*, was first identified in the foliar maize pathogen *Cochliobolus heterostrophus* (Lu *et al.*, 2003). Mutation in the gene in *C. heterostrophus* led to a reduced virulence to corn, with mutants producing foliar lesions only 40% the size of those in wild-type plants. Based on genomic Southern blotting and searches of DNA databases, the gene also was found in a wide range of ascomycetous fungi, including *F. graminearum*. Based on its similarity to *CPS1* from *C. heterostrophus*, the *F. graminearum* gene was cloned and disrupted. Mutation of *CPS1* in *F. graminearum* also results in a great reduction in the ability to cause FHB on wheat (Lu *et al.*, 2003). *CPS1* possesses two AMP-binding motifs and is predicted to encode an enzyme capable of producing acyl adenyl conjugants of unknown substrates.

## FUTURE PROSPECTS

The rapid availability of the whole genome sequence of *F. graminearum* has begun a new chapter in research on *Fusarium* head blight. The *Fusarium* community has shown a great deal of cooperation in efforts for manual annotation and functional analysis of the genome. These international efforts, coordinated through GIGI, have invigorated the community based on mutual interests and shared goals. A custom Affymetrix GeneChip microarray designed from gene models derived from the draft assembly currently is under construction and should be available by the end of 2004. As the same microarray platforms are available for genomes of the most important hosts of *F. graminearum*, barley and wheat, extensive gene profiling of the complete disease interaction soon will be possible. Targeted gene disruption and deletion is straight-forward for *F. graminearum* and steps also have been taken by GIGI to coordinate the systematic genome-wide mutation of all predicted genes.

Comparative genomic studies for *Fusarium* species are in their infancy but show promise for providing an understanding of the

evolution of gene clusters (Ward *et al.*, 2002) and gene family expansions and extinctions in *F. graminearum* and other plant pathogens (Kroken *et al.*, 2003). When sequence assemblies for closely related *Fusarium* species become available, the reading frame conservation test (Kellis *et al.*, 2003) also will be the most robust statistic for gene prediction.

Much work remains to be done for a better understanding of the population dynamics of *F. graminearum*. In addition to simple diversity studies, other topics of population genetics need to be explored in depth. For example, tests for the frequency of sexual out-crossing in nature will determine the tempo by which the high levels of genotypic diversity observed in most population studies are generated (Gale, 2003). As distinct subpopulations of *F. graminearum* (Gale *et al.*, 2003) and new species of *Fusarium* capable of causing FHB (O'Donnell *et al.*, 2004) still are being discovered, their distribution and intercontinental movement will undergo intense scrutiny in the near future. The recent development of microsatellite markers for population studies of *F. graminearum* and *F. asiaticum* (Suga *et al.*, 2004) derived from the *Fusarium* sequence assembly will facilitate these population studies.

Functional analysis of the *F. graminearum* genome will immediately promote the discovery of potential antifungal or antitoxin targets. Several novel genes affecting pathogenicity (Seong *et al.*, 2003) and toxin production *in planta* (Trail *et al.*, 2003a) have been identified by reverse genetics. The genes for the biosynthetic pathway of the oestrogenic mycotoxin zearalenone also are being targeted for identification in reverse genetic screens. Forward genetic analysis of the 16 genes for polyketide synthases in *F. graminearum* (Kroken *et al.*, 2003) will provide insight into the potential of the fungus to produce novel secondary metabolites.

## SUMMARY OUTLOOK

**1** A high-quality draft sequence assembly and genetic map are available for *Fusarium graminearum*. Manual and automated annotation efforts for gene discovery are currently underway and the results of these efforts will be accessible on the web.

**2** Functional genomic analysis will be advanced by the imminent availability of an *F. graminearum* Affymetrix GeneChip microarray as well as the availability of similar arrays for both barley and wheat.

**3** Genome-wide mutagenesis studies are being initiated and co-ordinated by the highly interactive *Fusarium* research community.

**4** Discovery of new subpopulations of *F. graminearum* and new *Fusarium* species capable of causing FHB will create challenges for monitoring the migration and persistence of species and introgression of populations over time.

**5** Because of its dynamic research community and the development of research infrastructure and databases, *F. graminearum*

has become an outstanding organism for studying pathogenic plant–fungus interactions.

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