For Blighted Waves of Grain: *Fusarium graminearum* in the Postgenomics Era

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Fungi display astounding diversity in their pathogenicity. We categorize them as biotrophs or necrotrophs and as obligate or facultative pathogens, but these categories do not reflect their polymorphic nature. Our approach to controlling disease would be better served by improving our understanding of the complete life cycle of the fungal pathogen, from infection and colonization to overwintering, using all of the tools at our disposal. A handful of fungal plant pathogens are now being studied at this level, including *Fusarium graminearum* (sexual stage, *Gibberella zeae*), the causal agent of head blight of wheat (*Triticum aestivum*), oat (*Avena sativa*), and barley (*Hordeum vulgare*) and ear and stalk rot of maize (*Zea mays*). Although *F. graminearum* is arguably one of the best studied fungal plant pathogens, the genetic bases of its life cycle and pathogenicity are poorly defined. This article focuses on the contributions that genomics and postgenomic studies are making to our understanding of the entire life cycle of this important pathogen. The genome of this fungal pathogen cannot be viewed in isolation, just as the fungus does not exist in isolation. Genomics can be used to elucidate *F. graminearum*'s interaction with its hosts, leading to a clearer picture of its ecological niche.

Fusarium head blight is a global problem. *F. graminearum* is the most prominent causal agent of head blight in the United States, Canada, and Europe (McMullen et al., 1997). A study of a worldwide collection of *F. graminearum* isolates has resulted in the proposed division of the original species into 11 distinct species based on phylogenetic analyses of DNA sequences (O'Donnell et al., 2004; Starkey et al., 2007). The name *F. graminearum* was retained for the proposed species predominantly causing head blight in the United States and Europe. The proposed division of *F. graminearum* has not been accepted by all *Fusarium* researchers (Leslie and Bowden, 2008). Other *Fusarium* species, including *F. culmorum*, *F. poae*, *F. pseudograminearum*, and *F. avenaceum*, may also be associated with kernel blight and related diseases throughout the world. Interestingly, individual species may predominate in specific grain hosts, regions of the world, or under certain climatic conditions.

F. graminearum produces several mycotoxins, including deoxynivalenol (DON) and derivatives, zearalenone, fusarin C, and aurofusarin. Although head blight causes low grain weight, the primary economic and health consequences of the disease are due to mycotoxin contamination, primarily DON. DON is a potent protein biosynthesis inhibitor affecting the digestive system and major organ function in humans and animals when ingested in sufficient quantities (Council for Agricultural Science and Technology, 2003). DON levels are regulated in food supplies of many countries; for example, the European Community limits DON levels to 0.5 μg g^{-1} for cereals and the United States limits DON levels to 1 μg g^{-1} for finished products for human consumption (Council for Agricultural Science and Technology, 2003). In the upper midwestern United States, DON levels frequently exceed this limit. In barley, the presence of *F. graminearum* can also lead to uncontrolled foaming (gushing) of beer caused by an unknown fungal component. Recently, the presence of *F. graminearum* in maize has resulted in high levels of zearalenone and other mycotoxins in the "distillers' dried grains with solids," remnants from maize-based ethanol production fed to cattle and pigs (Wu and Munkvold, 2008). Zearalenone causes estrogenic effects in animals, including humans, and is regulated in some countries. Although zearalenone is of concern to the U.S. Food and Drug Administration, there are currently no regulatory standards limiting its levels in grain (Council for Agricultural Science and Technology, 2003).

CHARACTERISTICS OF THE GENOME SEQUENCE OF *F. GRAMINEARUM*

The genome sequence was publicly released by the Broad Institute in 2003 (www.broad.mit.edu/annotation/genome/fusarium_group/MultiHome.html). The genome sequence release has greatly stimulated research activity on *F. graminearum*. An average of 50 articles per year were published between 2004 and 2007, compared with an average of 20 from 1999 through 2002, prior to the genome's release (ISI Web of Science; search criterion: *Fusarium graminearum* in the title). The genome size (36.1 Mb) is typical for a filamentous fungus. It contains genes encoding 13,937

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predicted proteins (mips.gsf.de/genre/proj/FGDB/) distributed over four chromosomes. Of these genes, 2,001 are not similar to those of any other sequenced organism (orphans) and 5,812 have homology to proteins of unknown function. There are fewer repeat sequences and an unusually low number of paralogous genes in comparison with genome sequences of other filamentous ascomycetes (Cuomo et al., 2007). Some fungi retain the capability to render duplicated sequences nonfunctional by "repeat induced point mutation" (RIP; Galagan and Selker, 2004). RIP, which is active during meiosis, riddles both copies of duplicated genes with point mutations. The existence of a RIPing mechanism has been demonstrated in *F. graminearum* and may explain the lack of multicopy sequences in the genome (Cuomo et al., 2007).

The availability of the *F. graminearum* genome sequence provides the opportunity to understand how this pathogen has evolved to occupy its niche. The first step in this process was to identify regions of the genome with high sequence variability among strains. Single nucleotide polymorphism (SNP) analysis of the *F. graminearum* sequence was conducted using the two major U.S. experimental strains (PH-1, nearly complete sequence; GZ3639, 0.4× coverage) and resulted in the identification of regions of high SNP density that correspond with telomeric regions and central chromosomal regions (centromeres have not been identified; Cuomo et al., 2007). Genes associated with basal metabolism and those highly conserved are more amply represented in the low SNP regions. As has been noted in other organisms, subtelomeric regions appear to harbor a larger fraction of the genes necessary for niche specificity (Farman, 2007). In *F. graminearum*, high SNP regions are enriched for genes of pathogenicity, genes unique to *F. graminearum*, secreted proteins, and gene clusters involved in secondary metabolism (Cuomo et al., 2007). However, the telomeric regions are not well represented in the current genome sequence. More advanced sequencing methods, such as adaptations of the pyrosequencing procedure (i.e. 454 sequencing), which eliminate cloning as a step in sequencing, would be useful to completing these regions.

One of the most interesting outcomes of genome sequencing is the identification of genes that have limited phylogenetic distribution: the orphan genes. The 2,001 orphan genes identified in the genome sequence of *F. graminearum* are concentrated in the high SNP regions (Cuomo et al., 2007). A recent study of 122 bacterial genomes indicated that about 1.9% of the genes from any single genome are orphan genes (Wilson et al., 2005). A similar study has not been conducted for fungi, but it would be useful, considering the more complex life cycles of fungi compared with bacteria. However, the orphan genes of *F. graminearum* remain functionally elusive. Will genes for pathogenicity be well represented in these sequences? How many orphan genes will be related to other life cycle stages?

THE LIFE CYCLE OF *F. GRAMINEARUM*

A generalized disease cycle of *F. graminearum* in wheat is illustrated in Figure 1. In the wheat field, head blight disease is initiated by airborne spores landing on flowering spikelets, germinating, and entering the plant through natural openings such as the base of the lemma and palea or through degenerating anther tissues (for review, see Bushnell et al., 2003). At the infection front, the fungus grows intercellularly and asymptomatically (Bushnell et al., 2003; Guenther and Trail, 2005; Jansen et al., 2005), spreading through the xylem and pith. Although this stage has been referred to as biotrophic (Bushnell et al., 2003), the lack of intracellular growth is not in keeping with the traditional definition of biotrophy (Jansen et al., 2005). Behind the infection front the fungus spreads radially and necrosis begins as the fungus grows intracellularly and rapidly colonizes the tissue. Symptoms at this stage include water soaking, particularly of the chlorenchyma. Following water soaking, colonized tissue becomes bleached. Premature bleaching of head tissue is a characteristic of infected heads in the field, and bleached tissue may form a band of several florets in the center of the head, a typical symptom of head blight of wheat.

Following infection of wheat florets, the fungus expresses genes for DON biosynthesis almost immediately (Jansen et al., 2005). DON is a virulence factor in wheat, causing tissue necrosis (Proctor et al., 1995; Desjardins et al., 1996) and allows the fungus to spread into the rachis from florets in wheat (Jansen et al., 2005). Interestingly, DON is the only mycotoxin shown to be a virulence factor. Colonization of developing seeds is accompanied by DON accumulation, resulting in shriveled, undersized grain referred to as tombstones. There is some evidence that DON also functions as a virulence factor in maize (Harris et al., 1999), but in barley, spread of the disease is limited and virulence does not appear to be due to the presence of the toxin (Jansen et al., 2005). A detailed understanding of the interactions of *F. graminearum* with maize and barley is lacking.

F. graminearum can readily complete its life cycle in culture or in association with its host. As with the vast majority of fungi, *F. graminearum* is haploid for most of its life cycle. For this fungus, sexual development begins with the formation of hyphae with binucleate cells. An extended binucleate phase (called the dikaryotic phase when two genetically distinct nuclei remain paired as new cells form) is a hallmark of the phylum Ascomycota (to which *F. graminearum* belongs). In the Ascomycota, this phase is the initial step in sexual development. *F. graminearum* is homothallic, meaning that it does not need a sexually distinct partner to develop sexual spores (ascospores), and as a result, the two nuclei of the binucleate cells are genetically identical. Homothallism in *F. graminearum* is due to the presence of genes associated with both mating types (*Mat1-1* and *Mat1-2*) in the haploid genome (Yun et al., 2000). The binucleate cells of *F. graminearum* develop

small coiled cells, which are the fruiting body initials (Trail and Common, 2000). In culture, the initials develop uninterrupted into flask-shaped perithecia that are filled with asci. Asci are tubular sacs containing the ascospores, which are the products of meiosis. Asci extend up to the mouth of the perithecium and forcibly discharge their ascospores into the air (Fig. 1). The mechanism of ascospore discharge is under study (Trail et al., 2002, 2005; Hallen and Trail, 2008). In the laboratory, the entire life cycle takes about 2 weeks, with asci maturing and firing sequentially for about the last 4 d (Bowden and Leslie, 1999; Trail et al., 2002).

Sexual development is a critical part of the disease cycle. In infected wheat, perithecium initials develop in association with the plant's stomates and silica cells, and, together with the binucleate hyphae from which they arise, are the overwintering structures (Guenther and Trail, 2005). In the field, the perithecia are ephemeral. The airborne ascospores are the primary inoculum of the disease, which is considered to be a monocyclic disease. In one study, elimination of the sexual stage by deletion of the mating-type locus resulted in substantial disease reduction in field trials compared with plots inoculated with a strain complemented back to wild-type ascospore production (Desjardin et al., 2006).

Copious asexual spores (conidia) are produced on the surface of infected plants or on crop residue during damp periods. Conidia are produced in slimy masses borne on sporodochia (cushion-shaped hyphal structures). The fusiform shape of the conidia and their formation in slimy masses have been associated with rain-splash dispersal (Deacon, 2006). Conidia are considered to serve predominantly in short-distance dispersal (Shaner, 2003). However, the relative contributions of conidia versus ascospores to disease epidemiology remain unresolved.

We hypothesize that during vegetative growth within the host, the acquisition of carbon resources is the most important activity. Lipid bodies can be seen in the binucleate hyphae associated with the perithecium initials. These resources would be necessary for overwinter survival and sexual reproduction, and their acquisition appears to occur during the initial colonization of the host's stalk (Guenther and Trail, 2005). Through gene expression analysis of colonized stalk tissue and functional analysis of individual genes involved in lipid biosynthesis and degradation, we are exploring these aspects of the life cycle. Once the stalk senesces, the resources must be protected from other microorganisms that may compete for host residues in the soil or may attack the fungus. Little is known of the microbial interactions in the crop debris and soil that affect the overwinter survival of this pathogen.

USE OF OMICS TOOLS FOR STUDYING *F. GRAMINEARUM*

The head blight pathogen has been studied for well over a century, beginning with the first report of the disease in England by Smith (1884). As in many organisms, research has been revitalized by the availability of genome sequence. The focus has largely shifted to gene function analyses, which can be performed using

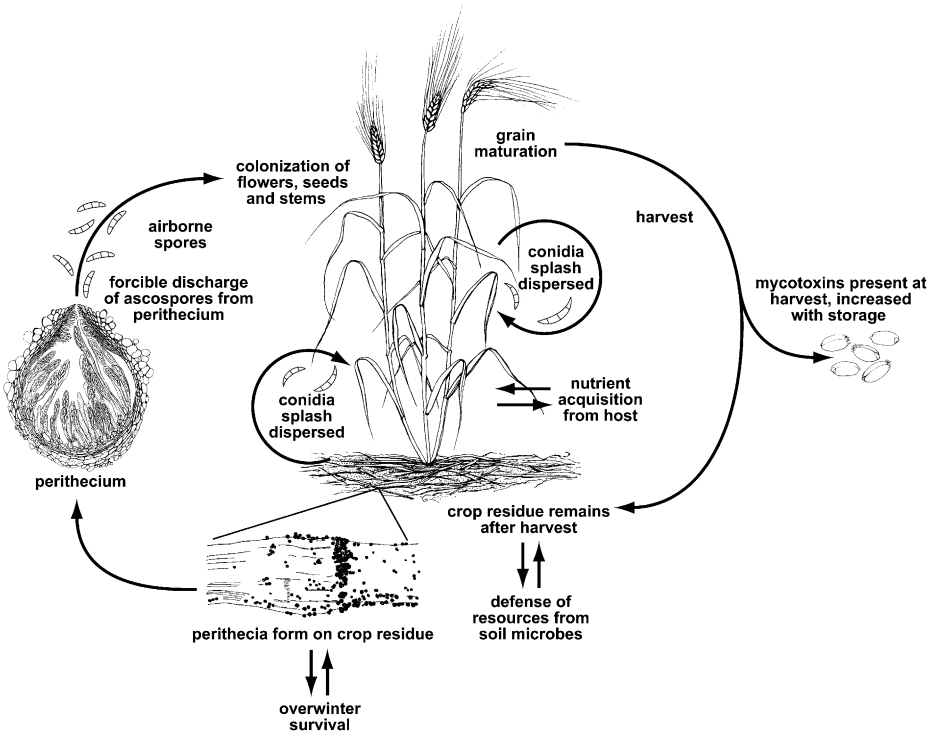

Figure 1. The life cycle of *F. graminearum* (sexual phase, *G. zeae*), causal agent of *Fusarium* head blight on wheat. Details of specific aspects of the cycle are discussed in the text.

a variety of "omics" approaches. Following the sequencing of the genome, assigning function to the predicted proteins has been a challenge. Yet, the efforts of many laboratories worldwide have made the *F. graminearum* genome one of the best annotated of the genome sequences of filamentous fungi. The hand annotation effort has been spearheaded by the Munich Information Center for Protein Sequences (mips.gsf.de/genre/proj/FGDB). Following the release of the genome, an Affymetrix GeneChip was designed and produced for expression analyses (Gueldener et al., 2006). To date, data from over 100 chip analyses have been posted on www.plexdb.org, including time courses of inoculated barley heads (Gueldener et al., 2006), sexual development (Hallen et al., 2007), and conidial germination (Seong et al., 2008), as well as analyses of mutants (Hallen and Trail, 2008) and mycelia in culture under starvation conditions (Gueldener et al., 2006). Coexpression analyses based on these arrays can be used for assignment of gene function based on the association of expression patterns across a variety of conditions. Coexpression of sets of homologous genes in multiple filamentous fungi will be an even more powerful tool for assigning functions to genes whose roles are not clear.

Proteomics is the large-scale study of proteins. Proteomics can be used to enhance the results of expression studies and to differentiate proteins present in specific compartments or cell types. Proteomics analyses have been conducted in *F. graminearum* to identify proteins regulated by the mating-type gene in a mutant of *Mat1-2* (Lee et al., 2008) and those expressed in culture growing on plant cell walls (Phalip et al., 2005). To identify genes associated with mycotoxin production, proteomic analyses were also performed for *F. graminearum* grown under mycotoxin-inducing conditions (Taylor et al., 2008). Paper et al. (2007) conducted an extensive analysis of extracellular proteins secreted by *F. graminearum* cultured on a variety of media, including isolated plant cell walls, to identify proteins important to the host-pathogen interaction. The in vitro proteome was compared with *F. graminearum* proteins isolated from the apoplast of infected plants. Interestingly, the proteins identified from the apoplast differed from those identified in in vitro conditions, with the majority of the genes expressed in the apoplast corresponding to regions of the genome containing a high quantity of SNPs. Surprisingly, the genes of a significant fraction of secreted proteins identified in the apoplast contained no identifiable signal peptide sequence, which suggests that this signal may not exist in all secreted proteins. The genes identified in this study are expressed during a period of extracellular growth that the fungus undergoes prior to intracellular invasion and demonstrate a unique contribution of proteomics to our understanding of the pathogenicity of *F. graminearum*.

The term "metabolomics" refers to the large-scale study of metabolites produced by an organism grown under a particular set of conditions or over a period of time. Metabolomic studies of *F. graminearum* are just being initiated. Understanding the breadth of metabolites produced during the various phases of the life cycle will contribute to our understanding of disease. In addition, knowledge of the chemical diversity, especially of secondary metabolites, will be useful for ecological studies (e.g. what role these metabolites play in the interaction of *F. graminearum* with its environment) and for pharmaceutical research. Historically, secondary metabolism has been the object of intense studies in mycotoxigenic fungi but has been limited to individual pathways and products (primarily mycotoxins). A comprehensive metabolomics effort focused on understanding the metabolic characteristics associated with DON biosynthesis is ongoing (K. Hammond-Kosack, personal communication). Similar studies of the range of metabolic products produced by *F. graminearum* will greatly expand our current knowledge of these processes in this fungus.

PARSING THE GENOME TO ELUCIDATE THE *F. GRAMINEARUM* LIFE CYCLE

The genome sequence provides a tableau from which to select individual genes or groups of genes for functional exploration. Techniques for genetic manipulation are well developed for *F. graminearum* and have greatly enhanced the ability to use the genome for functional analyses. *F. graminearum* has highly efficient homologous recombination that can be used for targeted gene replacement. Although it is homothallic, *F. graminearum* can outcross, which makes it possible to perform genetic analyses (Bowden and Leslie, 1999). Random insertional mutagenesis has been used to generate tagged mutants, which can then be screened for phenotypes such as loss of virulence (Seong et al., 2006; Kim et al., 2007), loss of mycotoxin production, or arrested perithecium development (Kim et al., 2007). The availability of the whole genome streamlines the process of gene identification by providing regional sequences, which enables quick identification of a gene from a DNA sequence flanking the inserted tag. A gene replacement construct can then be generated easily to confirm the mutation. Recently, RNA interference was successfully adapted to silence genes in *F. graminearum* (McDonald et al., 2005). This technique will be particularly useful for reducing the expression of genes that may cause lethal phenotypes when deleted. In addition, several genes can be included in series, allowing one vector to target several genes that might have redundant functions. Inducible promoters add another dimension of control for these experiments, and several of them are available for use in fungi (Romero et al., 2003; Shoji et al., 2005).

Since the genome sequence became available, much of the focus of research has been on identifying genes that function in pathogenicity. Several approaches have been taken to identify these genes, including targeting major signal transduction proteins (Hou et al., 2002;

Jenczmionka et al., 2003; Urban et al., 2003, Yu et al., 2008), screening random insertional mutants for loss of the ability to induce disease (Seong et al., 2006; Han et al., 2007), and identifying genes orthologous to pathogenicity genes from other fungi (Lu et al., 2003; Oide et al., 2006; Shim et al., 2006). Disruption of these orthologs can also demonstrate that genes required for pathogenicity in one fungus are not required for pathogenicity in other fungi. Homologous genes may serve different purposes in different fungi, aiding a pathogen to induce disease while affecting other processes in a saprotroph. In a single organism, the same gene may be required for basic growth both in vitro and in planta, blurring the line between what is truly a pathogenicity factor and what is important to basic cell function. The very specific relationship between host and pathogen genotypes known as "gene-for-gene" has not been identified in the *F. graminearum* interaction with its hosts.

A different approach was taken to identify a lipase involved in virulence. The gene (FGSG_05906) was identified from cDNA analysis of a culture grown on wheat germ oil (Voigt et al., 2005). When this gene was disrupted, the resulting mutant lost both its ability to grow on wheat germ oil and, more significantly, its ability to cause disease on wheat. However, lipolytic activity of the mutant increased after extended incubation in culture, implying that additional lipases were secreted. An examination of the annotated genome sequence revealed 67 putative lipases, 27 of which are annotated as triacylglycerol lipases. Use of expression data in this case allowed researchers to narrow the pool of candidate genes to identify the relevant gene.

Before the genome sequence was available, the chemical potential of *F. graminearum* was poorly understood. In fungi, genes for secondary metabolism and some genes for primary metabolism are clustered (Stadler and Keller, 2008). These clusters differ from bacterial operons, in that the genes within the cluster have independent regulatory sequences and can be expressed differentially. Clusters consist of genes involved in metabolite biosynthesis, together with regulatory genes and genes for self-protection or secretion of the metabolite. The three largest gene families for fungal secondary metabolites encode genes for polyketide synthases, terpene synthases, and nonribosomal peptide synthetases (NRPSs). These classes of compounds are commonly bioactive and appear to be present in most ascomycetes, both saprotrophic and pathogenic, but they are associated in many fungi with virulence and with toxicity to humans.

Prior to sequencing of the genome, *F. graminearum* was known to produce four polyketide mycotoxins: fusarin C, a red pigment (now identified as aurofusarin), fusaric acid, and zearalenone. The sequence revealed an abundance of polyketide synthase (PKS) genes not originally known to exist, and the availability of the genome sequence allowed the entire set of PKS genes to be identified (Kroken et al., 2003; Gaffoor et al., 2005). Specific disruption of each PKS gene independently in *F. graminearum* was accomplished and the mutants were characterized (Gaffoor et al., 2005). Of the 15 PKS genes disrupted, functions were assigned to five; products of the others remain obscure. Two PKS genes, which were divergently transcribed, encoded the zearalenone biosynthetic pathway (Kim et al., 2005b; Gaffoor and Trail, 2006; Lysøe et al., 2006). In addition, genes for the synthesis of aurofusarin (Gaffoor et al., 2005; Kim et al., 2005a; Malz et al., 2005), the black pigment of the perithecium wall, and fusarin C were identified (Gaffoor et al., 2005). Interestingly, disruption of the PKS genes had no obvious effects on pathogenicity (Gaffoor et al., 2005).

Of the 19 predicted NRPS genes, three have been functionally analyzed (NPS1, NPS2, and NPS6) and determined to be involved in the synthesis of siderophores, which are involved in sexual development and pathogenicity (Oide et al., 2007; Tobiasen et al., 2007; Turgeon et al., 2008). The functions of the remaining NRPS genes have not been determined. Functional studies of the 17 predicted terpene synthases have not been reported.

The role of the mycotoxins other than DON in the life cycle is not clear. It is possible that the expression of these genes is important for environmental interactions with other organisms. For instance, evidence suggests that zearalenone affects sexual development (Nelson, 1971) and vegetative growth of other fungi (Utermark and Karlovsky, 2007). In addition, rubrofusarin, a precursor of aurofusarin, is a well-known antimicrobial agent (Graham et al., 2004; Frandsen et al., 2006), although aurofusarin has not been tested for similar activity.

Expression array analyses have been performed on five stages of development during the maturation of the perithecia (Hallen et al., 2007). These are the first comprehensive expression analyses of sexual development in any filamentous fungus. The strain used for genomic sequencing was chosen because of its capacity to produce a lawn of synchronously developing perithecia on the surface of carrot (*Daucus carota*) agar in a petri dish. The developmental stages corresponded to the generation of each tissue type in the perithecium: binucleate hyphae, perithecium wall, paraphyses (internal sterile hyphae that define the central cavity), asci, and ascospores. Approximately 2,000 genes were identified as expressed only during sexual development; of these, 175 were orphan genes, unique to *F. graminearum* (Hallen et al., 2007). Functional analysis of these genes will contribute to an understanding of the process of sexual development in the ascomycetous fungi.

Conidial germination of *F. graminearum* was followed both microscopically and through gene expression changes using the Affymetrix GeneChip (Seong et al., 2008). The study revealed genes associated with specific stages of development during the germination process. Expression analysis also revealed genes associated with the developmental processes observed microscopically. For example, genes associated with peroxisome development were identified as the devel-

opment occurred. Shifts in metabolic processes and the coordination of differentiation of cell types identified in the studies of conidial germination and perithecium development provide basic information about molecular mechanisms that can be used to understand developmental processes in fungi in general.

APPLYING GENOMICS TO THE CONTROL OF HEAD BLIGHT

In studies of plant disease, the emphasis frequently is on the host, in the belief that understanding the host response will lead to the greatest impact on disease control. This approach has been influenced largely by the traditional approach to breeding for resistance and has, in many cases, been very effective. Another approach to the control of disease has been to use broad-spectrum pesticides, but often with only superficial knowledge of the specific life cycles of the pathogens. The pathogen *F. graminearum* exemplifies the difficulty of developing effective control by either of these means. Breeding for strong resistance has not been particularly effective in wheat. Moderate resistance has been associated with the Sumai 3 genotype (for review, see Mesterhazy, 2003), which breaks down under high disease pressure. Fungicides have not been economically justifiable. Adequate coverage of the grain head also has been problematic for fungicide application. These circumstances provide an incentive to develop alternative controls. The effective approach is to interrupt the pathogen life cycle. An understanding of that life cycle forms the basis of a solid approach to long-term control.

The approaches outlined above have begun to identify those genes that can be used to arrest the life cycle. Strikingly, genomic sequencing has revealed that approximately 30% of the predicted genes in filamentous fungi are unique to fungi. This large number of genes provides the greatest potential for identifying controls that uniquely target fungi. These genes need to be examined functionally to determine each of their roles in the life cycle. This knowledge can be used to develop chemical control, modify culture practices, or genetically engineer crops for disease resistance.

FUTURE DIRECTIONS

The agricultural field is an ecosystem dominated by one plant species and manipulated by chemicals and physical disturbance. However, a microbial community as well as other flora and fauna are parts of this ecosystem. Understanding ecosystem interactions is important for the long-term sustainability of agriculture. The genome sequence of *F. graminearum* is one tool that can help us elucidate the ecological potential of this species. Genomes of several other *Fusarium* species (*F. verticillioides* [sexual stage, *Gibberella moniliformis*], *F. oxysporum* [asexual], and *F. solani* [sexual stage, *Nectria haematococca*]) have recently also been sequenced. These species illustrate the diversity of lifestyles within this group of phytopathogenic species. To understand what shapes the genomes of the pathogenic fungi, they must also be studied in the context of genomes of the host plants, endophytic and saprobic fungi and bacteria that may also colonize the host tissue, and soil microbes that will be encountered in crop debris. The release of the maize and wheat genome sequences in the future will be a critical step toward this goal. Expression studies of the response of wheat and barley to *F. graminearum* have already been initiated (Kruger et al., 2002; Boddu et al., 2006).

Genetic changes in one species in an agricultural ecosystem can affect other species in the system. In the future, integration of transcriptome, proteome, metabolome, and functional analyses for fungi of different lifestyles (e.g. saprotrophs, biotrophs, necrotrophs, plant pathogens, and animal pathogens) will affect our understanding of the commonalities and differences among all of these niches (Raes and Bork, 2008) and of what makes organisms such as *F. graminearum* able to thrive among them. Genes that affect host-fungus interactions, interactions with other fungi (endophytes, pathogens, and saprotrophs), microbes, and insects are important goals for the future.

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