Review

Exploitation of genomics in fungicide research: current status and future perspectives

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SUMMARY

Every year, fungicide use to control plant disease caused by pathogenic fungi increases. The global fungicide market is now worth more than £5.3 billion, second only to the herbicide market in importance. In the UK, over 5500 tonnes of fungicide were applied to crops in 2010 (The Food and Environment Research Agency, Pesticide Usage Statistics), with 95.5% of the wheat-growing area receiving three fungicide sprays. Although dependence on fungicides to produce food securely, reliably and cheaply may be moderated in the future by further developments in crop biotechnology, modern crop protection will continue to require a diversity of solutions, including effective and safe chemical control. Therefore, investment in exploiting the increasingly available genome sequences of the most devastating fungal and oomycete phytopathogenic species should bring an array of new opportunities for chemical intervention. To date, the impact of whole genome research on the development, introduction and stewardship of fungicides has been limited, but ongoing improvements in computational analysis, molecular biology, chemical genetics, genome sequencing and transcriptomics will facilitate the development and registration of the future suite of crop protection chemicals.

INTRODUCTION

Agricultural productivity in the second half of the 20th century has continued to advance through a combination of crop genetic improvement, innovations in crop cultivation and farming practice, and advances in the effectiveness and consistency of crop protection chemicals. In the future, the demands on food production systems will be even higher. Certainly, advances in plant

biotechnology, plant breeding and agronomy are central to meeting these demands. However, successfully combating pathogen-driven diseases will require a combined approach, including the use of chemicals (Lucas, 2011).

Fungicides marketed for the control of disease on food crops are under pressure, the most predictable being the development of pathogen resistance, the consequence of a basic evolutionary process. The application of a fungicide can lead to the selection of a proportion of the population that survives, is able to resist the compound and passes this characteristic to its offspring. This phenomenon affects all fungicides, including certain protectant multisite inhibitors. Resistance to modern, systemic and highly efficacious fungicides, which have a single biochemical target, readily occurs, as a mutation in a single gene is usually sufficient to confer a highly resistant phenotype (Brent and Hollomon, 2007). Consequently, populations of some of the most destructive pathogen species are resistant to many of the currently registered antifungal chemicals (Table 1). In addition, the diversity of compounds available to growers for the control of crop diseases could be restricted in the future by European pesticide legislation, aiming to minimize the risks to human health and the environment from the use of pesticides [Sustainable Use Directive 2009/128/EC and Plant Protection Products Regulation (EC) 1107/2009]. Therefore, to be able to maintain chemical diversity, there is a pressing requirement to identify and develop new crop protection chemicals.

The expansion of genomic information and the advancement of technologies to exploit this information provide a novel platform from which new routes can be developed to obtain the future suite of crop protection chemicals. In 2002, after the release of the genomes of important human fungal pathogens, including *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*, several genomics strategies were initially suggested to permit the rational design of antifungal compounds. These included transcript profiling, fitness tests and proteomics. However, the authors at the time predicted that these new research approaches might take a decade to bring a novel bioactive to the marketplace (Jiang *et al.*, 2002). Since then, the genomes of many of the most important fungal and oomycete

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Useful websites

FRAC—Fungicide Resistance Action Committee (<http://www.frac.info/frac/index.htm>). GOLD—Genomes On Line Database (<http://www.genomesonline.org>). PHI-base—The Pathogen–Host Interactions Database (<http://www.phi-base.org>). Phytopath—an integrated resource for comparative phytopathogen genomics (<http://www.phytopathdb.org>).

Table 1 Examples of fungicide resistance reported in globally important plant pathogenic fungi and oomycetes with genomes sequenced.

Pathogen	Crop	Example(s) of resistance (reference)	Genome sequence status (strain/current version/reference)	Genome centre/laboratory*
<i>Blumeria graminis</i> f. sp. <i>hordei</i>	Barley	MBCs (Vargas, 1973) DMIs (De Waard <i>et al.</i> , 1986) Azanaphthalenes (Hollomon <i>et al.</i> , 1997) QoIs (Heaney <i>et al.</i> , 2000) MBCs (Leroux and Clerjeau, 1985) DMIs (Eld, 1992) QoIs (Ishii <i>et al.</i> , 2009) SDHs (Avenot and Michailides, 2010) MBCs (Chen <i>et al.</i> , 2009) Phenylpyrroles (Peters <i>et al.</i> , 2008) DMIs (Yin <i>et al.</i> , 2009)	Partially assembled (DH14/version 1/Spanu <i>et al.</i> , 2010) Draft complete (B05.10/version 2/Amselem <i>et al.</i> , 2011) Assembled (Cuomo <i>et al.</i> , 2007; Ma <i>et al.</i> , 2010) Finished (70-15/version 8/Dean <i>et al.</i> , 2005) Draft complete (CIRAD86/version 2) Finished (IP0323/version 2/Goodwin <i>et al.</i> , 2011) Draft complete Partially assembled (T30-4/version 3/Haas <i>et al.</i> , 2009) Finished (CRL 75-36-700-3/version 1/Cantu <i>et al.</i> , 2011; Duplessis <i>et al.</i> , 2011) Draft complete (Elwood <i>et al.</i> , 2010) Draft complete Ongoing	Blumeria Genome Sequencing Consortium BROAD Institute BROAD Institute BROAD Institute DOE Joint Genome Institute DOE Joint Genome Institute DOE Joint Genome Institute BROAD Institute BROAD Institute DOE Joint Genome Institute BROAD Institute Wolfgang Knogge, Leibniz Institute of Plant Biochemistry, Halle, Germany† BROAD Institute
<i>Botrytis cinerea</i>	Various, particularly grapevine			
<i>Fusarium</i> spp.	Various			
<i>F. graminearum</i>	Cereal/noncereal			
<i>F. oxysporum</i> f. sp. <i>tomato</i>	Tomato			
<i>F. verticillioides</i>	Cereal			
<i>Magnaporthe oryzae</i>	Rice			
<i>Mycosphaerella fijiensis</i>	Banana			
<i>Mycosphaerella graminicola</i>	Wheat			
<i>Phakopsora pachyrhizi</i>	Soya bean			
<i>Phytophthora infestans</i>	Potato/tomato			
<i>Puccinia graminis</i> f. sp. <i>tritici</i>	Wheat			
<i>Pyrenophora teres</i>	Barley			
<i>Pyrenophora tritici-repentis</i>	Wheat			
<i>Rhynchosporium commune</i>	Barley			
<i>Stagnospora nodorum</i>	Wheat			

CAA, carboxylic acid amide; DMI, demethylation inhibitor; MBC, methylbenzimidazole carbamate; MBI-D, melanin biosynthesis dehydratase inhibitor; MBI-R, melanin biosynthesis reductase inhibitor; PA, phenylamide; QoI, quinone outside inhibitor; SDHI, succinate dehydrogenase inhibitor.

*BROAD Institute fungal genome initiative (<http://www.broadinstitute.org/scientific-community/science/projects/fungal-genome-initiative>); JGI genome sequencing project list (<http://genome.jgi.doe.gov/genome-projects/>); Blumeria Genome Sequencing Consortium (<http://www.blugen.org>).

†Not publicly available.

Fig. 1 Current impact of genomics on the fungicide development pipeline.

pathogens of crops have been sequenced. These are now available as advanced drafts, partial assemblies or fully assembled formats (Table 1). Typically, the genome of a selected reference strain for each pathogenic species has been assembled, annotated by the research community and is available within a genome browser and fully accessible through downloads. In addition, for some species, the genomic sequences have been aligned to the available chromosome information.

Comparative bioinformatic analysis of these sequenced fungal and oomycete genomes has already led to the identification of specific genes and/or gene families important for the lifestyle of priority pathogens and, in some species, the elucidation of the genetic landscape underpinning the evolutionary arms race with their hosts (Goodwin *et al.*, 2011; Haas *et al.*, 2009; Ma *et al.*, 2010; Raffaele and Kamoun, 2012; Rouxel *et al.*, 2011; Spanu *et al.*, 2010). Since 2010, whole or partial genome sequence information of additional pathogen strains, isolates and/or races with specific biological properties has emerged. By exploring sequence variation within a species, further biological insights can be gained. To date, this latter approach has been used to study pathogen effectors, but could readily be applied to determine intrinsic or acquired variation in response to novel and/or existing chemistry.

We discuss the current and potential use of large-scale genomic resources to develop new fungicides, including the identification of prospective fungicide targets and the tools available for their validation (Fig. 1). We explore the types of studies being carried out to determine the precise mode of action of existing chemistry and the benefits for future chemical design and/or the editing of existing designs. We also consider how genome-wide analyses can be used to discover the genes and metabolic processes involved in fungicide adaptation and resistance. Finally, we explore some of the prospective uses to

which multiple fungal genome information can be applied to enhance chemical crop protection in the future.

IMPACT OF GENOMICS ON FUNGICIDE DEVELOPMENT

Most fungicides currently marketed were discovered by empirical screening—the testing of a diverse library of chemicals against target organisms, often in glasshouse tests on whole plants. This approach has the advantage of identifying chemicals with good activity, and good mobility, able to cross the cuticle, enter the plant tissue and act within the plant. Whether the traditional approach will be superseded in the near future by genome-based technologies is unknown, although the targeting of conserved gene products essential for life in the most important pathogens is now simpler (Wang *et al.*, 2011a).

Identification of potential fungicide targets

Various genome sequencing projects have revealed the genetic complement required for fungi to exploit different ecological niches: for example, the expansion of genes encoding proteins involved in plant cell wall degradation in the necrotrophs *Botrytis cinerea* and *Sclerotinia sclerotiorum* (Amselem *et al.*, 2011) and the contraction of gene families encoding these enzymes in the exclusively biotrophic mildews and rusts (Duplessis *et al.*, 2011; Spanu *et al.*, 2010) and in some hemibiotrophs, including *Mycosphaerella graminicola* (Goodwin *et al.*, 2011). Allied with genome-wide transcriptional studies using microarrays, serial analysis of gene expression (SAGE) and, more recently, next-generation sequencing technologies (Tan *et al.*, 2009), genes potentially important for differentiation and pathogenicity in

individual species can easily be identified and their *in planta* expression followed. For example, the genes required for appressorium formation in *Magnaporthe oryzae* (Oh *et al.*, 2008) and for epidermal penetration and colonization in *Blumeria graminis* (Both *et al.*, 2005) were identified by differential gene expression. However, the primary criterion for selection of a fungicide or antifungal compound is activity against a number of target species (so-called broad-spectrum activity), with no (or, at worst, minimal) effect on the host. Consequently, comparative genomics is a useful tool to identify potential targets shared by different plant pathogenic species, particularly as the number of available fungal genomes continues to increase (Table 1) and is tracked in the Genomes On Line Database (GOLD) (Liolios *et al.*, 2010).

Comparative genomics has been used to identify novel antibacterial targets (White and Kell, 2004) and novel targets against nematodes (Kumar *et al.*, 2007) and flatworms (Caffrey *et al.*, 2009). In human pathogenic species, by using homologues of genes previously confirmed as essential for life in *C. albicans* and *A. fumigatus*, the sequenced genomes of eight fungi were manually mined for potential new antifungal therapies. This approach identified 10 genes present in all pathogenic fungi which are absent in humans, not auxotrophic and with accessible cellular locations. Of these, four single-copy genes were investigated further as potential drug targets, with two (*trr1*, encoding thioredoxin reductase, and *kre2*, encoding an α -1,2-mannosyltransferase) sufficiently similar to Protein Data Bank templates for homology modelling. These models are now used to screen virtual chemical libraries (Abadio *et al.*, 2011).

Comparative genomics has been used to explore the evolution of eukaryotic microbial pathogenesis, identifying sets of genes expanded in plant pathogens in comparison with free-living fungal species, which may represent gene families with important roles in plant disease (Soanes *et al.*, 2008), and therefore potential fungicide targets. The creation of multispecies databases, for example the Pathogen–Host Interactions Database (PHI-base, <http://www.phi-base.org>), to catalogue genes experimentally confirmed as pathogenicity genes, virulence genes, effectors or those not involved in pathogenicity, as well as fungicide targets, has greatly simplified cross-species comparison, further enriching the comparative genomics approaches for the identification of novel targets (Winnenburg *et al.*, 2008). For example, in an early study, 61 *Fusarium graminearum* genes in PHI-base, verified as having a role in pathogenicity or virulence, were combined with gene function data from other plant- and animal-infecting species. Through comparative analysis, this identified 211 homologues of pathogenicity and virulence genes in the *F. graminearum* genome that could be further explored experimentally as a prioritized list (Antoniw *et al.*, 2011). The recent proposal to catalogue the properties of bioactive compounds generated by commercial and academic groups in a database, so-called Minimum Information About a Bioactive Entity (MIABE; Orchard *et al.*, 2011), in a format

that is amenable to efficient data mining, provides a new pathway to understand how current chemistry can be improved, and, when combined with gene sequence to phenotype databases, for example PHI-base, should facilitate the re-optimization of the biological properties of existing fungicides, and lead to the identification of new activities. These resources will also facilitate the design of molecules active against resistant individuals (Frey *et al.*, 2010).

Target validation

Concomitant with the increase in the available sequenced genomes of plant pathogens, there have been advances in technologies to experimentally validate genes considered as fungicide targets. These reverse genetics approaches have become higher throughput, benefiting from the immediate availability of flanking sequences and information on gene copy number, combined with advances in binary vector design and construction (for a review, see Frandsen, 2011). In addition, targeting efficiency has improved in several plant pathogenic species by the use of mutants with defects in the nonhomologous end-joining (NEHJ) system, such as *ku70* and *ku80* mutants (van Attikum *et al.*, 2001), enabling the rapid validation of gene function through gene deletion and replacement. Fungi for which NEHJ mutants are now available include the important plant pathogens *B. cinerea* (Choquer *et al.*, 2008), *M. oryzae* (Villalba *et al.*, 2008), *S. sclerotiorum* (Levy *et al.*, 2008), *My. graminicola* (Bowler *et al.*, 2010) and *Alternaria alternata* (Wang *et al.*, 2011b), and the biocontrol fungus *Trichoderma virens* (Catalano *et al.*, 2011). Therefore, the number of genes confirmed in different pathosystems as being crucial in infection and, in the case of *T. virens*, antifungal activity, now runs in the range of hundreds to thousands for any given species.

Recently, a new genomic resource available at <http://www.phytopathdb.org> has been developed that displays directly, within the genome browser, the exact experimentally determined *in planta* phenotypes for specific gene sequences when deleted or disrupted. This new resource is specifically focused on plant pathogenic species that regularly inflict serious levels of disease on globally important crop plant species, such as rice, wheat, maize, potato and oilseed rape. This gene-centric approach could be used to link different information potentially important for fungicide target validation. However, it should be noted that the perturbation of an essential biosynthetic pathway leading to minimal growth *in vitro*, can sometimes be overcome during host infection if the missing metabolite(s) can be supplied from the host tissue. The latter type of failure has been noted for human- and plant-infecting pathogens. For example, the enzyme ornithine decarboxylase (ODC) is essential for polyamine biosynthesis, and hence fungal growth, *in vitro*. However, when *odc* mutants of *C. albicans* (Herrero *et al.*, 1999) or *Tapesia yallundae* (Mueller *et al.*, 2001) infect, the host tissue supplies this missing metabolite and growth

is no longer compromised, whereas, for other pathogenic fungal species, this fungicide target is readily exploitable because only low levels of host-derived polyamines are present in the infected tissues. For example, *odc* mutants of the toxin-producing necrotroph *Stagonospora nodorum*, which infects wheat (Bailey *et al.*, 2000), and the biotroph *Ustilago maydis*, which infects maize (Valdés-Santiago *et al.*, 2010), cause only minimal disease.

USE OF GENOMIC TOOLS TO DETERMINE THE MODE OF ACTION OF THE FUNGICIDE

A consequence of the success of *in vivo* empirical screening of libraries for antifungal discovery is the unknown mode of action of many commercial chemicals. Although not a requirement for registration of a novel compound, the determination of the biochemical target is desirable as it enables structure–activity studies, and therefore the optimization of a compound's affinity and selectivity, as well as the use of modern polymerase chain reaction (PCR)-based tools to monitor resistance (Fraaije *et al.*, 2005, 2007).

Determination of the primary mode of action

Genome-wide gene expression studies have been particularly successful in determining the physiological and metabolic consequences of antifungal chemicals. These so-called biological spectral analyses are a useful approach for defining the similarities of the temporal responses to compounds of unknown and known activity (Fliri *et al.*, 2005) and therefore for identifying potential new modes of action. However, the specific biochemical targets of novel compounds are more elusive. For example, the specific up-regulation of genes involved in iron uptake and metabolism, together with *in vitro* phenotyping, demonstrated that exogenous iron levels are critical for ciclopirox inhibition of *C. albicans* (Sigle *et al.*, 2005). Transcriptome profiling of the response of *Phytophthora infestans* to the novel oomycete fungicide fluopicolide, a benzamide (acylpicolide), identified genes linked to the endoplasmic reticulum and Golgi function, and, in accordance with microscopy studies, effects on vesicle transport and interactions with spectrin-like proteins are suggested as modes of inhibition (Toquin *et al.*, 2010). However, despite numerous transcriptomic studies, the ciclopirox (Almeida *et al.*, 2007) and fluopicolide targets remain unknown.

Chemogenomic and chemical genetic screens are both powerful ways to determine the bioactivity of small molecules, including antifungal drugs and commercially available fungicides (Hoon *et al.*, 2008). The approach most commonly employed in antifungal target identification and/or verification is haploid insufficiency profiling (HIP; also referred to as the 'Fitness Test'). This exploits the diploid state of the model yeast *Saccharomyces cerevisiae* to determine the activity of small molecules by screening against a large collection, often genome-wide, of heterozygous deletion

strains. Strains affected are sensitized to compounds that inhibit the heterozygous locus (Giaever *et al.*, 2004). This can identify specific small-molecule protein interactions and, as the approach is unbiased, can capture interactions with other proteins, so-called secondary or off-target activity. For the human-infecting pathogen *C. albicans*, a HIP or Fitness Test (CaFT), adapted from the yeast system, has been developed and used to determine the mechanism of action of novel inhibitors of microtubule formation (Xu *et al.*, 2007) and purine metabolism (Rodriguez-Suarez *et al.*, 2007). The latter study identified guanine 5'-monophosphate synthetase as the protein target. More recently, a similar approach has uncovered a new *C. albicans* specific antifungal target, Tfp1p, a regulator of vesicle pH (Oh *et al.*, 2010).

In plant pathogenic fungal species, HIP studies could also be used to investigate the mode of action of fungicides. Although this approach would require a predominantly diploid species with a genome-wide gene deletion library available, for some fungi, for example *U. maydis*, and oomycetes, such as *P. infestans*, if the appropriate investments were made, an *in vitro* chemical screen could be particularly informative. *In vitro* mutagenesis studies offer a powerful method to identify and/or further define the primary biochemical target of a compound. For unknown targets, next-generation sequencing technologies are currently being exploited to screen resistant mutants and to define the mode of action of fungicides, although, again, this approach is dependent on a tractable sexual stage (Box 1). In addition, the generation of mutants resistant to compounds with known targets can clarify ligand–protein interactions (Fraaije *et al.*, 2012), and thereby provide valuable information for the rational design of more active chemicals.

In haploid filamentous fungi, the systematic identification of antifungal targets is difficult as they are, by definition, essential genes. In *A. fumigatus*, for example, transposon mutagenesis, together with parasexual genetics, has been used for unbiased searches for essential genes (Firon *et al.*, 2003). In addition, the predicted orthologues of known essential genes or antifungal targets, identified by HIP of *C. albicans* and *Sa. cerevisiae*, have been studied by promoter replacement strategies (Hu *et al.*, 2007; Romero *et al.*, 2003) or RNA interference (RNAi) (Mouyna *et al.*,

Box 1

'We mapped a factor involved in decreased sensitivity to a novel research antifungal using forward genetics in *Mycosphaerella graminicola*. From a cross between wild-type and a less sensitive mutant, the trait was determined as monogenic. We then performed genomic high throughput sequencing – bulk segregation analysis using 30 sensitive and 30 resistant progeny which led us to a rough identification of the locus (~340 kb). Fine mapping using CAPS markers on the whole progeny (239 isolates) identified a 12 kb mapping window where the gene responsible for the phenotype was identified. We then validated the mechanism using reverse genetics. This overall process took about a year, we are now much quicker.' *Gabriel Scalliet, Syngenta.*

2004). Most ascomycete fungi have the molecular machinery required for RNA silencing, with 'knockdown' of pathogenicity-related genes successfully demonstrated in *M. oryzae* (Nakayashiki, 2005). Screens for essential for life genes in plant pathogenic fungi have not been reported, although these studies are underway. Alternatively, large-scale reverse genetics experiments targeted to explore the function of a protein type can lead indirectly to the identification of essential for life genes. For example, in *F. graminearum*, the individual deletion of 116 predicted protein kinase genes identified eight causing a reduction by more than 20% in transformant growth compared with the wild-type, whereas, for 20 genes, no transformants were recovered, suggesting that these are essential for life (Wang *et al.*, 2011a).

Identification of multisite or nonspecific effects

In fungi, genomic studies of the fungicide response often suggest oxidative stress and the accumulation of reactive oxygen species (ROS) as a common mechanism of action. In the yeasts, *Sa. cerevisiae* transcripts of oxidative stress response genes are more abundant after azole treatment (Bammert and Fostel, 2000), and, in *C. albicans* the oxidative stress response appears to be integral to azole adaptation and, eventually, resistance (see 'Determination of the primary mode of action' section) (Rogers and Barker, 2003). Treatment with caspofungin, a β -1,3-glucan synthesis inhibitor, and fenpropimorph, an ergosterol biosynthesis inhibitor, induces the expression of genes involved in ROS scavenging and oxidative stress in the filamentous fungus *Aspergillus niger*, although the authors suggest that this is part of a response to morphological changes caused by the fungicides (Meyer *et al.*, 2007). The analysis of proteome changes in *C. albicans* after treatment with three unrelated antifungal classes, namely polyene, azole and enchinocandin, identified a group of genes involved in the tricarboxylic acid (TCA) cycle as responsive (Hoe-hamer *et al.*, 2010). A similar response after epoxiconazole treatment has been reported in the plant pathogen *My. graminicola* (Cools *et al.*, 2007), with the specific up-regulation of genes involved in mitochondrial electron transport. Epoxiconazole treatment also increases hydrogen peroxide (H_2O_2) production in healthy wheat plants, thereby enhancing the anti-oxidative potential of plants, whilst delaying senescence (Wu and von Tie-demann, 2001). In contrast, treatment with sublethal concentrations of the triazole, prothioconazole, induced deoxynivalenol (DON) mycotoxin production in the wheat pathogen *F. graminearum*. The enhanced production of DON was preceded directly by a hyperinduction of H_2O_2 (Audenaert *et al.*, 2010). These authors suggest that the inhibition of ergosterol biosynthesis increases membrane permeability, releasing H_2O_2 , which, in turn, activates DON production. However, considering the evidence accumulated from global analyses of the response to azoles and other

fungicides, together with the rapid induction of H_2O_2 production (4 h after treatment), in the latter *F. graminearum* study, a secondary nonspecific activity seems more likely.

Impact on nontarget organisms

The combination of genomics and environmental toxicology has led to the emergence of a new scientific field, termed 'toxicogenomics' (Nuwaysir *et al.*, 1999). As concerns over the environmental impact of agricultural fungicide use grow, the value of genomics approaches in defining pathways of toxicity common to different nontarget beneficial organisms has also increased. Model nontarget organisms used in these studies include bacteria (Kim and Gu, 2007), yeasts (Yasokawa and Iwahashi, 2010), fish (Skolness *et al.*, 2011) and rodents (Goetz and Dix, 2009). Recent toxicogenomics studies focusing on commercially available azole fungicides have identified common pathways affected *in vitro*, including genes involved in androgen and oestrogen metabolism, suggesting potential endocrine disruptor activity (Goetz and Dix, 2009). As a regulatory framework is put in place to provide guidance for the interpretation of these genome-wide data (Goetz *et al.*, 2011), these studies are likely to become an increasingly important component of European pesticide legislation.

USE OF GENOMICS TO EXPLORE FUNGICIDE RESISTANCE

Before the era of genomics, studies of the genetic mechanisms of adaptation and resistance to fungicides in fungal pathogens were based on classical genetics allied with biochemical and candidate gene approaches. High-efficiency transformation protocols to confirm the impact of genes suspected to confer resistance have been available for a few plant pathogens and saprophytes for some time (Steffens *et al.*, 1996). Through the use of plasmids for homologous gene replacement and/or heterologous expression (Gems *et al.*, 1991; Tsukuda *et al.*, 1988), early descriptions of the molecular mechanisms underlying resistance were forthcoming. For example, resistance mechanisms were reported for the methylbenzimidazole carbamates (MBCs) (Jung *et al.*, 1992) and the succinate dehydrogenase inhibitor carboxin (Keon *et al.*, 1991). These early studies were dominated by the tractable species *Aspergillus nidulans* and *U. maydis*.

Recent studies of resistance to novel fungicides use the same principles, although the newer next-generation sequencing technologies can substantially enhance the process. For most fungicide classes, field resistance is conferred by point mutations in the target gene encoding an amino acid change that prevents fungicide inhibition without perturbing the activity of the target protein (Brent and Hollomon, 2007). Consequently, sequencing of target genes has proved to be successful in identifying the variant sequences responsible for resistance when a clear relationship to

phenotype can be established: for example, by revealing the mutations in the β -tubulin gene causing resistance to MBCs (for a review, see Ma and Michailides, 2005) and in the *cytochrome b* gene causing resistance to the quinone outside inhibitors (Qols; for a review, see Fernandez-Ortuno *et al.*, 2008).

When target site changes are absent or their effect on phenotype is uncertain, or classical genetics has identified more than one gene associated with a resistance phenotype, the advantages of using an unbiased genome-wide experimental approach to identify altered genes, transcripts, proteins and/or metabolites in a fungicide-resistant individual are clear, for example in the analysis of azole resistance mechanisms in plant and human pathogenic fungi (Becher *et al.*, 2011; Cools *et al.*, 2007; De Backer *et al.*, 2001; Ferreira *et al.*, 2006; Silva *et al.*, 2011). Perhaps not so apparent, however, is the power of using genomics to reveal both the conserved and divergent molecular response pathways involved in the adaptation and development of resistance to different fungicide classes. These types of studies will, over time, reveal whether a conserved response to synthetically generated xenobiotics exists, and whether this is the same or different to common responses induced by a range of natural xenobiotics.

Use of genomics to identify the mechanisms of resistance to fungicides

For several human pathogenic fungi, the analysis of transcriptional changes underlying their adaptation to unrelated fungicides, eventually leading to stable resistance, has permitted the characterization of common programmes of evolution (Cowen *et al.*, 2002). These changes are dependent on a set of co-regulated genes (Sanglard *et al.*, 2009). For example, the ATP binding cassette (ABC) transporters *CDR1* and *CDR2* (*Candida* drug resistance 1 and 2) and the major facilitator gene *MDR1* (multidrug resistance 1) are commonly overexpressed in clinical and experimentally evolved azole-resistant strains of *C. albicans* (Cowen *et al.*, 2002; Sanglard *et al.*, 1995; White, 1997). Several genes coordinately regulated with both *CDR* genes and *MDR1* are also responsive to the β -tubulin inhibitor benomyl, and are up-regulated by H_2O_2 (Enjalbert *et al.*, 2003). As mentioned previously, genome-wide transcriptional profiling has suggested that oxidative stress is a general mechanism of fungicide action. However, the discovery of a transcriptional regulator of these genes, *MRR1* (multidrug resistance regulator), provides a mechanism for concurrent responses to oxidative stress and fungicides (Cowen *et al.*, 2002) which can mediate the development of fungicide resistance (Rogers and Barker, 2003). Indeed, clinical isolates of *C. albicans* resistant to multiple unrelated antifungals have been shown to carry gain-of-function mutations in *MRR1* that result in constitutive overexpression of *MDR1*, as well as genes involved in the regulation of the cellular redox state (Karababa *et al.*, 2004; Morschhauser *et al.*, 2007). Transcriptional profiling of *Candida parapsilosis* strains cor-

related resistance to fluconazole and voriconazole with *MDR1* overexpression. The resistance phenotype was again caused by point mutations in *MRR1* (Silva *et al.*, 2011).

Similarly, recent studies of strains of the plant pathogen *B. cinerea*, resistant to a variety of unrelated fungicides, identified activating mutations in the orthologous transcription factor (*MRR1*), controlling the expression of the ABC transporter *AtrB* (Kretschmer *et al.*, 2009). ABC transporters have also been implicated in resistance to unrelated fungicides in *M. oryzae* (Lee *et al.*, 2005), *Penicillium digitatum* (Nakaune *et al.*, 1998), *Oculimacula yallundae* and *My. graminicola* (Leroux and Walker, 2011). Although a multidrug-resistant phenotype in *My. graminicola* has not been reported in other studies, and microarray analysis failed to identify an ABC transporter responsive to fungicide treatment (Cools *et al.*, 2007).

Use of genomics to explore intrinsic resistance to fungicides

Several important human and plant pathogenic fungi are unaffected by some of the most widely used broad-spectrum fungicides. For example, *A. fumigatus* is intrinsically resistant to the azole fungicides fluconazole and ketoconazole, although inhibited by voriconazole and itraconazole. Strains of the globally important soil-dwelling pathogen, *Gaeumannomyces graminis*, which causes take-all disease in many small-grain cereal species, including wheat and barley, can exhibit intrinsic resistance to silthiofam, which leads to the inhibition of mitochondrial ATP transport (Joseph-Horne *et al.*, 2000). Several species of *Fusarium* are naturally resistant to some azoles. *Fusarium solani* (syn. *Nectria haematococca*), for example, an opportunistic pathogen of both humans and plants, is resistant to all commonly used medical azoles, including fluconazole, itraconazole, voriconazole and the recently introduced posaconazole (Nucci and Anaissie, 2007). The analysis of genome sequences now publicly available for four *Fusarium* species has identified multiple copies of the *CYP51* gene encoding the azole target sterol 14 α -demethylase (Becher *et al.*, 2011), contradicting the long-held view that *CYP51* exists as a single gene in all phyla. Indeed, genome information has revealed that paralogous *CYP51* genes are common in ascomycete fungi, including species of *Aspergillus*, the rice blast fungus *M. oryzae* and the wheat pathogen *Pyrenophora tritici-repentis*. In microarray experiments, the *CYP51A* gene of *A. fumigatus* and *F. graminearum* is consistently the most transcriptionally responsive gene to disruption of ergosterol function (Becher *et al.*, 2011; Gautam *et al.*, 2008; Liu *et al.*, 2010). In addition, *CYP51A* disruptants in *A. fumigatus* (Mellado *et al.*, 2005), *M. oryzae* (Yan *et al.*, 2011) and *F. graminearum* (Liu *et al.*, 2011a) are more sensitive to azoles, confirming the importance of *CYP51A* in intrinsic resistance. Unlike other plant pathogenic Ascomycete species, *F. graminearum* is also intrinsically resistant to amine fungicides.

These are ergosterol biosynthesis inhibitors that inhibit at C-14 reductase, encoded by *ERG24*. Genomic analysis has identified two *ERG24* genes (*ERG24A* and *ERG24B*; Liu *et al.*, 2011b) on different chromosomes. Therefore, recent genome information has provided a rationale for the ineffectiveness of some broad-spectrum fungicide classes against important pathogens. This is vital information for the development of new agrochemicals. Conversely, gene sequence information can indicate potential limitations to resistance development in particular pathogens. For example, a number of species of rust and *Alternaria* have a self-splicing type 1 intron in the mitochondrial *cytochrome b* sequence located directly after codon 143. This prevents the evolution of Qol resistance conferring G134A substitution, as interruption of intron splicing would inactivate the protein. Therefore, widespread Qol resistance is unlikely to occur in rusts or *Alternaria* spp. (Grasso *et al.*, 2006).

Use of genomics to identify chromosomal changes conferring fungicide resistance

Comparative genomic hybridization, a microarray-based technique to analyse gene copy number across the genome, has identified karyotype variability as a common mechanism of antifungal resistance in *C. albicans*, with aneuploidy, primarily trisomy of chromosome 5, more common in fluconazole-resistant strains (Selmecki *et al.*, 2006) than in sensitive strains. The phenotype is conferred by the amplification of *ERG11* (*CYP51*) and transcriptional activator of *CDR* genes 1 (*TAC1*), a regulator of ABC transporter expression, located on the long arm of chromosome 5 (Selmecki *et al.*, 2008). Similar chromosomal rearrangements are correlated with azole resistance in *Cryptococcus neoformans*, with duplication of chromosome 1, on which the *CYP51* and an ABC transporter gene antifungal resistance 1 (*AFR1*) reside, associated with fluconazole adaptation (Sionov *et al.*, 2010). The contribution of karyotype variation to resistance to synthetic fungicides in plant pathogens has not been reported, although, in *F. solani* isolates, resistance to the plant-derived antimicrobial phytoalexin pisatin was mapped to a mitotically unstable chromosome dispensable for normal growth (Coleman *et al.*, 2009; Miao *et al.*, 1991). Indeed, a number of plant pathogens have dispensable chromosomes (Covert, 1998), some of which are known to cause phenotypic variation (Hatta *et al.*, 2002), whereas others, for example in *My. graminicola*, have no clear function (Goodwin *et al.*, 2011). In the future, genome-wide analyses will undoubtedly elucidate a role for chromosome variation in fungicide resistance.

OUTLOOK

Within less than 10 years, the fungal research community has moved from a position in which a reference genome was available

for just a handful of human and plant pathogenic fungal species to a position today in which a reference and annotated genome sequence is available for hundreds of pathogenic and nonpathogenic species across the fungal tree of life. This new knowledge is set to have an impact on every part of agrochemical development (Fig. 1).

Impact on discovery

Where the use of genomic information is currently not part of the discovery toolkit, it soon will be. For example, the percentage of proteins annotated from the genome as hypothetical proteins or conserved hypothetical proteins in a given species is typically in the range 40%–55%, although in some of the newly sequenced obligate biotrophs, e.g. rusts and mildews, this figure can rise to over 80% (Cantu *et al.*, 2011; Duplessis *et al.*, 2011; Spanu *et al.*, 2010). This large subset of predicted proteins represents the untapped potential for chemical intervention. By exploring their expression and abundance patterns in a range of different pathogenic species in response to the same chemical class, and thereby linking the subsets of conserved hypothetical sequences across species, novel chemical targets can be derived. Currently, most fungicides target either cytoplasmically localized or membrane-bound proteins. For the genes annotated as coding for conserved hypothetical or hypothetical proteins, various bioinformatic software can be used to predict their potential cellular location [for example, ProtCom (ProtComp v8.0; <http://www.softberry.com>) or WolfPsort (Horton *et al.*, 2007)], and this will provide an initial layer of annotation. Second, some predicted hypothetical proteins are now recognized to contain a specific domain of unknown function (DUF) or computationally derived motifs. These new types of sequence-derived information can be used to further annotate existing pathogen chemical induction transcriptomics and proteomics datasets based on the putative cellular location of the predicted hypothetical protein. A further refinement of the list of hypothetical proteins that could be explored for fungicide targeting is to determine the gene copy number across multiple species. For example, a single-copy highly conserved sequence across species could be ideal as a potential multispecies target, whereas the discovery of variable copy numbers and high copy numbers across species may immediately raise concerns.

An emerging new technical advance is host-induced gene silencing (HIGS). In HIGS, gene silencing is triggered directly from the host plant and is used to target genes in the plant pathogen or pest. Already, HIGS has been successfully applied to study gene function in two cereal-infecting obligate biotrophs: *Blumeria graminis* f. sp. *hordei* during the infection of barley leaves (Nowara *et al.*, 2010) and *Puccinia striiformis* f. sp. *tritici* during the infection of wheat leaves (Yin *et al.*, 2011). The triggers for these trans-species gene silencing events are short double-stranded

RNAs (dsRNAs); however, the mechanism(s) involved in delivering the RNA silencing signals from the plant cell into fungal cells is not yet known (reviewed in Lee *et al.* (2012). For obligate biotrophic fungal species, the availability of this exciting new tool should permit the development of a novel *in planta* route for the discovery of suitable intervention targets. In theory, HIGS could also be used directly to deliver RNA silencing-based products against the target gene, and thereby to directly control pathogen and pest infection. This approach would require HIGS to be highly efficient within the growing crop. A control strategy based on RNA silencing in the pathogen would obviate the need to identify chemicals that interfere with either a newly discovered target or an existing intervention target.

Impact on lead optimization

Screening of activities within libraries of newly discovered agrochemicals using 'omic' (transcriptomic, proteomic, metabolomics) technologies, together with the improvement in *in silico* modelling tools, will be facilitated by the ever-increasing numbers of different plant pathogen genomes available and, moreover, the genomes of specific individuals within a species which exhibit distinct biological properties. Collectively, these data will greatly enhance the detection and optimization of broad-spectrum inter- and intraspecific activity. The integration of genome information from different pathogens and individuals within populations can be used to select additional common target sites present in a broad range of problem species. In addition, with the definition of potential detoxification pathways, for example expanded P450-omes of ascomycete fungi (Cresnar and Petric, 2011), our understanding of fungicide degradation will be enhanced, enabling the modification of new chemicals that would previously have been discarded. These attractive new targets for chemical intervention could improve overall existing product efficacy and longevity, or could be used to start the discovery pipeline afresh.

Impact on registration

Once a regulatory framework is in place, toxicogenomic profiling of a newly discovered and optimized fungicide chemistry will rapidly provide information on the environmental consequences of the use of a fungicide, thereby circumventing the need for animal models, and speeding up the registration process. An assessment of resistance risk would also be enriched by profiling the genomic response of a target pathogen.

Impact on mode of action studies

To date, the impact of genomics on novel mode of action studies has been minimal, primarily because of the difficulty in identifying the primary biochemical target. Most detected biological effects

are the secondary consequences of fungicide application leading to restricted pathogen growth. However, in the future, by combining labelled chemistry with transcriptomics and/or proteomics, early cellular distribution and response can be explored simultaneously, thereby potentially providing the subcellular locations of targets. In addition, the screening of mutagenized pathogen populations for strains with small shifts in sensitivity, followed by genome resequencing of multiple strains, may reveal the target sequences of potential interest.

Impact on stewardship

For the commercial launch of a novel chemistry, genomic information will increasingly be used to assist in prolonging the longer term efficacy of each chemical class. Advances in sequencing technologies and the reduction in costs mean that, in the very near future, genome-wide information for a species or isolate found at a new location will be available within days or even hours of initial discovery. Shortly afterwards, the sequence variation will have been computationally predicted and available for scrutiny via a genome browser. By monitoring simultaneously the global changes to a genome, i.e. the shifts in the entire 'genome landscape', as well as the local target site-specific changes occurring in problematic pathogenic species within intensive production systems, it should be possible to re-use existing target sites by modelling the changed chemical sites, and thereby to design novel chemistry that can intervene.

CONCLUSIONS

The availability of genomic sequence information for an increasing number of pathogenic species and isolates with distinct lifestyles and unique biological properties now provides all aspects of fungicide research with a comprehensive and informative starting point. With the financial return on the efficient production of healthy food predicted to rise, there is a keen interest in the development of innovative ways to combine the plethora of genomic information with advances in modelling and chemical synthesis, and to align this with our ever-increasing understanding of pathogen biology and the pathogenicity process. Undoubtedly, the future rewards to fungicide research achievable through the application of genomic and post-genomic technologies will be both numerous and exciting. However, although full genome sequencing and transcriptomic studies will be able to provide, within a few days, deep insight into the evolutionary changes that have recently occurred in important pathogen populations and, via modelling, potential ways to modify chemistry to regain the upper hand, some obstacles remain. New chemistry tailored to novel targets will still have to pass the acid tests of activity

in planta and efficacy in the field. Furthermore, as it stands, both new and modified chemicals are required to complete the full registration process that normally takes several years. Potential markets and new chemical solutions for problem diseases will not be accessed immediately. Therefore, unless a faster route to register chemistry from a preranked list of the potential safest chemical compounds can occur, possibly based on prior knowledge, the full effects of genomic devised solutions to intervention and pathogen control in a commercial crop setting and/or clinical practice will, at best, remain slow. Nonetheless, these new approaches and technologies promise to accelerate the rate at which novel candidates are identified and to improve the breadth of the product pipeline.

ACKNOWLEDGEMENTS

The authors would like to thank John Lucas, Martin Urban and Jason Rudd at Rothamsted Research for their comments on the manuscript, and Gabriel Scalliet of Syngenta for his input. Rothamsted Research receives grant-aided support from the Biological and Biochemical Research Council (BBSRC) of the UK. The Phytopath and PHI-base projects are supported by a BBSRC grant (BB/I000488/1). PHI-base also receives National Capability funding from the BBSRC.

REFERENCES

- Abadio, A.K.R., Kioshima, E.S., Teixeira, M.M., Martins, N.F., Maigret, B. and Felipe, M.S.S. (2011) Comparative genomics allowed the identification of drug targets against human fungal pathogens. *BMC Genomics*, **12**, 75.
- Almeida, B., Sampaio-Marques, B., Carvalho, J., Silva, M.T., Leao, C., Rodrigues, F. and Ludovico, P. (2007) An atypical active cell death process underlies the fungicidal activity of ciclopirox olamine against the yeast *Saccharomyces cerevisiae*. *FEMS Yeast Res.* **7**, 404–412.
- Amselem, J., Cuomo, C.A., van Kan, J.A.L., Viaud, M., Benito, E.P., Couloux, A., Coutinho, P.M., de Vries, R.P., Dyer, P.S., Fillinger, S., Fournier, E., Gout, L., Hahn, M., Kohn, L., Lapalu, N., Plummer, K.M., Pradier, J.M., Quevillon, E., Sharon, A., Simon, A., ten Have, A., Tudzynski, B., Tudzynski, P., Wincker, P., Andrew, M., Anthouard, V., Beever, R.E., Beffa, R., Benoit, I., Bouzid, O., Brault, B., Chen, Z.H., Choquer, M., Collemare, J., Cotton, P., Danchin, E.G., Da Silva, C., Gautier, A., Giraud, C.T., Gonzalez, C., Grossetete, S., Guldener, U., Henrissat, B., Howlett, B.J., Kodira, C., Kretschmer, M., Lappartient, A., Leroch, M., Levis, C., Mauceli, E., Neuveglise, C., Oeser, B., Pearson, M., Poulain, J., Poussereau, N., Quesneville, H., Rascle, C., Schumacher, J., Segurens, B., Sexton, A., Silva, E., Sirven, C., Soanes, D.M., Talbot, N.J., Templeton, M., Yandava, C., Yarden, O., Zeng, Q.D., Rollins, J.A., Lebrun, M.H. and Dickman, M. (2011) Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *PLoS Genet.* **7**, e1002230.
- Antoniw, J., Beacham, A.M., Baldwin, T.K., Urban, M., Rudd, J.J. and Hammond-Kosack, K.E. (2011) OmniMapFree: a unified tool to visualise and explore sequenced genomes. *BMC Bioinformatics*, **12**, 447.
- van Attikum, H., Bundock, P. and Hooykaas, P.J.J. (2001) Non-homologous end-joining proteins are required for Agrobacterium T-DNA integration. *EMBO J.* **20**, 6550–6558.
- Audenaert, K., Callewaert, E., Hofte, 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.
- Avenot, H.F. and Michailides, T.J. (2010) Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Prot.* **29**, 643–651.
- Bailey, A., Mueller, E. and Bowyer, P. (2000) Ornithine decarboxylase of *Stagonospora* (*Septoria*) *nodorum* is required for virulence toward wheat. *J. Biol. Chem.* **275**, 14 242–14 247.
- Bammert, G.F. and Fostel, J.M. (2000) Genome-wide expression patterns in *Saccharomyces cerevisiae*: comparison of drug treatments and genetic alterations affecting biosynthesis of ergosterol. *Antimicrob. Agents Chemother.* **44**, 1255–1265.
- Becher, R., Weihmann, F., Deising, H.B. and Wirsal, S.G.R. (2011) Development of a novel multiplex DNA microarray for *Fusarium graminearum* and analysis of azole fungicide responses. *BMC Genomics*, **12**, 52.
- Blixt, E., Djurle, A., Yuen, J. and Olson, A. (2009) Fungicide sensitivity in Swedish isolates of *Phaeosphaeria nodorum*. *Plant Pathol.* **58**, 655–664.
- Both, M., Csukai, M., Stumpf, M.P.H. and Spanu, P.D. (2005) Gene expression profiles of *Blumeria graminis* indicate dynamic changes to primary metabolism during development of an obligate biotrophic pathogen. *Plant Cell*, **17**, 2107–2122.
- Bowler, J., Scott, E., Taylor, R., Scalliet, G., Ray, J. and Csukai, M. (2010) New capabilities for *Mycosphaerella graminicola* research. *Mol. Plant Pathol.* **11**, 691–704.
- Brent, K.J. and Hollomon, D.W. (2007) Fungicide resistance in crop pathogens: how can it be managed. *FRAC Monograph No. 1*, 2nd revised edition. Brussels: CropLife International.
- Caffrey, C.R., Rohwer, A., Oellien, F., Marhofer, R.J., Braschi, S., Oliveira, G., McKerrrow, J.H. and Selzer, P.M. (2009) A comparative chemogenomics strategy to predict potential drug targets in the metazoan pathogen, *Schistosoma mansoni*. *PLoS ONE*, **4**, e4413.
- Canas-Gutierrez, G.P., Patino, L.F., Rodriguez-Arango, E. and Arango, R. (2006) Molecular characterization of benomyl-resistant isolates of *Mycosphaerella fijiensis*, collected in Colombia. *J. Phytopathol.* **154**, 403–409.
- Canas-Gutierrez, G.P., Angarita-Velasquez, M.J., Restrepo-Florez, J.M., Rodriguez, P., Moreno, C.X. and Arango, R. (2009) Analysis of the CYP51 gene and encoded protein in propiconazole-resistant isolates of *Mycosphaerella fijiensis*. *Pest Manag. Sci.* **65**, 892–899.
- Cantu, D., Govindarajulu, M., Kozik, A., Wang, M.N., Chen, X.M., Kojima, K.K., Jurka, J., Michelmore, R.W. and Dubcovsky, J. (2011) Next generation sequencing provides rapid access to the genome of *Puccinia striiformis* f. sp. *tritici*, the causal agent of wheat stripe rust. *PLoS ONE*, **6**, e24230.
- Catalano, V., Vergara, M., Hauzenberger, J.R., Seiboth, B., Sarrocco, S., Vannacci, G., Kubicek, C.P. and Seidl-Seiboth, V. (2011) Use of a non-homologous end-joining-deficient strain (*delta-ku70*) of the biocontrol fungus *Trichoderma virens* to investigate the function of the laccase gene *lcc1* in sclerotia degradation. *Curr. Genet.* **57**, 13–23.
- Chen, C.J., Yu, J.J., Bi, C.W., Zhang, Y.N., Xu, J.Q., Wang, J.X. and Zhou, M.G. (2009) Mutations in a beta-tubulin confer resistance of *Gibberella zeae* to benzimidazole fungicides. *Phytopathology*, **99**, 1403–1411.
- Choquer, M., Robin, G., Le Pecqueur, P., Giraud, C., Levis, C. and Viaud, M. (2008) *Ku70* or *Ku80* deficiencies in the fungus *Botrytis cinerea* facilitate targeting of genes that are hard to knock out in a wild-type context. *FEMS Microbiol. Lett.* **289**, 225–232.
- Coleman, J.J., Rounsley, S.D., Rodriguez-Carres, M., Kuo, A., Wasmann, C.C., Grimwood, J., Schmutz, J., Taga, M., White, G.J., Zhou, S.G., Schwartz, D.C., Freitag, M., Ma, L.J., Danchin, E.G.J., Henrissat, B., Coutinho, P.M., Nelson, D.R., Straney, D., Napoli, C.A., Barker, B.M., Gribskov, M., Rep, M., Kroken, S., Molnar, I., Rensing, C., Kennell, J.C., Zamora, J., Farman, M.L., Selker, E.U., Salamov, A., Shapiro, H., Panglinian, J., Lindquist, E., Lamers, C., Grigoriev, I.V., Geiser, D.M., Covert, S.F., Temporalini, E. and VanEtten, H.D. (2009) The genome of *Nectria haematococca*: contribution of supernumerary chromosomes to gene expansion. *PLoS Genet.* **5**, e1000618.
- Cools, H.J. and Fraaije, B.A. (2008) Spotlight: are azole fungicides losing ground against Septoria wheat disease? Resistance mechanisms in *Mycosphaerella graminicola*. *Pest Manag. Sci.* **64**, 681–684.
- Cools, H.J., Fraaije, B.A., Bean, T.P., Antoniow, J. and Lucas, J.A. (2007) Transcriptome profiling of the response of *Mycosphaerella graminicola* isolates to an azole fungicide using cDNA microarrays. *Mol. Plant Pathol.* **8**, 639–651.
- Covert, S.F. (1998) Supernumerary chromosomes in filamentous fungi. *Curr. Genet.* **33**, 311–319.
- Cowen, L.E., Nantel, A., Whiteway, M.S., Thomas, D.Y., Tessier, D.C., Kohn, L.M. and Anderson, J.B. (2002) Population genomics of drug resistance in *Candida albicans*. *Proc. Natl. Acad. Sci. USA*, **99**, 9284–9289.
- Cresnar, B. and Petric, S. (2011) Cytochrome P450 enzymes in the fungal kingdom. *Biochim. Biophys. Acta*, **1814**, 29–35.
- 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., Antoniow, 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.,

- Munsterkotter, M., Nelson, D., O'Donnell, K., Ouellet, T., Qi, W.H., Quesneville, H., Roncero, M.I.G., Seong, K.Y., Tetko, I.V., Urban, M., Waalwijk, C., Ward, T.J., Yao, J.Q., 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.
- Davidse, L.C., Looijen, D., Turkensteen, L.J. and Vanderwal, D. (1981) Occurrence of metalaxyl resistant strains of *Phytophthora infestans* in Dutch potato fields. *Neth. J. Plant Pathol.* **87**, 65–68.
- Dean, R.A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., Orbach, M.J., Thon, M., Kulkarni, R., Xu, J.R., Pan, H.Q., Read, N.D., Lee, Y.H., Carbone, I., Brown, D., Oh, Y.Y., Donofrio, N., Jeong, J.S., Soanes, D.M., Djonovic, S., Kolomietis, E., Rehmeier, C., Li, W.X., Harding, M., Kim, S., Lebrun, M.H., Bohnert, H., Coughlan, S., Butler, J., Calvo, S., Ma, L.J., Nicol, R., Purcell, S., Nusbaum, C., Galagan, J.E. and Birren, B.W. (2005) The genome sequence of the rice blast fungus *Magnaporthe oryzae*. *Nature*, **434**, 980–986.
- De Backer, M.D., Ilyina, T., Ma, X.-J., Vandoninck, S., Luyten, W.H.M. and Bossche, H.V. (2001) Genomic profiling of the response of *Candida albicans* to itraconazole treatment using a DNA microarray. *Antimicrob. Agents Chemother.* **45**, 1660–1670.
- De Waard, M.A., Kipp, E.M.C., Horn, N.M. and Van Nistelrooy, J.G.M. (1986) Variation in sensitivity to fungicides which inhibit ergosterol biosynthesis in wheat powdery mildew. *Neth. J. Plant Pathol.* **92**, 21–32.
- Dereviagina, M.K., Elanskij, S.N. and Diakov, Y.T. (1999) Resistance of *Phytophthora infestans* to the dimethomorph fungicide. *Mikol. Fitopatol.* **33**, 208–213.
- Duplessis, S., Cuomo, C.A., Lin, Y.C., Aerts, A., Tisserant, E., Veneault-Fourrey, C., Joly, D.L., Hacquard, S., Amselem, J., Cantarel, B.L., Chiu, R., Coutinho, P.M., Feau, N., Field, M., Frey, P., Goldhage, E., Goldberg, J., Grabherr, G., Kodira, C.D., Kohler, A., Kues, U., Lindquist, E.A., Lucas, S.M., Mago, R., Mauceli, E., Morin, E., Murat, C., Pangilinan, J.L., Park, R., Pearson, M., Quesneville, H., Rouhier, N., Sakthikumar, S., Salamov, A.A., Schmutz, J., Selles, B., Shapiro, H., Tanguay, P., Tuskan, G.A., Henrissat, B., Van de Peer, Y., Rouze, P., Ellis, J.G., Dodds, P.N., Schein, J.E., Zhong, S.B., Hamelin, R.C., Grigoriev, I.V., Szabo, L.J. and Martin, F. (2011) Obligate biotrophy features unraveled by the genomic analysis of rust fungi. *Proc. Natl. Acad. Sci. USA*, **108**, 9166–9171.
- Elad, Y. (1992) Reduced sensitivity of *Botrytis cinerea* to two sterol biosynthesis inhibiting fungicides fenetrazole and fenethanil. *Plant Pathol.* **41**, 47–54.
- Ellwood, S.R., Liu, Z.H., Syme, R.A., Lai, Z.B., Hane, J.K., Keiper, F., Moffat, C.S., Oliver, R.P. and Friesen, T.L. (2010) A first genome assembly of the barley fungal pathogen *Pyrenophora teres* f. *terres*. *Genome Biol.* **11**, R109.
- Enjalbert, B., Nantel, A. and Whiteway, M. (2003) Stress-induced gene expression in *Candida albicans*: absence of a general stress response. *Mol. Biol. Cell* **14**, 1460–1467.
- Fernandez-Ortuno, D., Tores, J.A., De Vicente, A. and Perez-Garcia, A. (2008) Mechanisms of resistance to Qo fungicides in phytopathogenic fungi. *Int. Microbiol.* **11**, 1–9.
- Ferreira, M.E., Malavazi, I., Savoldi, M., Brakhage, A.A., Goldman, M.H.S., Kim, H.S., Nierman, W.C. and Goldman, G.H. (2006) Transcriptome analysis of *Aspergillus fumigatus* exposed to voriconazole. *Curr. Genet.* **50**, 32–44.
- Firon, A., Villalba, F., Beffa, R. and d'Enfert, C. (2003) Identification of essential genes in the human fungal pathogen *Aspergillus fumigatus* by transposon mutagenesis. *Eukaryot. Cell*, **2**, 247–255.
- Fliri, A.F., Loging, W.T., Thadeio, P.F. and Volkmann, R.A. (2005) Biological spectra analysis: linking biological activity profiles to molecular structure. *Proc. Natl. Acad. Sci. USA*, **102**, 261–266.
- Fraaije, B.A., Cools, H.J., Fountaine, J., Lovell, D.J., Motteram, J., West, J.S. and Lucas, J.A. (2005) The role of ascospores in further spread of Qo-resistant cytochrome *b* alleles (G143A) in field populations of *Mycosphaerella graminicola*. *Phytopathology*, **95**, 933–941.
- Fraaije, B.A., Cools, H.J., Kim, S.-H., Motteram, J., Clark, W.S. and Lucas, J.A. (2007) A novel substitution I381V in the sterol 14 α -demethylase (CYP51) of *Mycosphaerella graminicola* is differentially selected by azole fungicides. *Mol. Plant Pathol.* **8**, 245–254.
- Fraaije, B.A., Bayon, C., Atkins, S., Cools, H.J., Lucas, J.A. and Fraaije, M.W. (2012) Risk assessment studies on Succinate Dehydrogenase Inhibitors, the new weapons in the battle to control *Septoria* leaf blotch in wheat. *Mol. Plant Pathol.* **13**, 263–275.
- Frandsen, R.J.N. (2011) A guide to binary vectors and strategies for targeted genome modification in fungi using *Agrobacterium tumefaciens*-mediated transformation. *J. Microbiol. Methods*, **87**, 247–262.
- Frey, K.M., Georgiev, I., Donald, B.R. and Anderson, A.C. (2010) Predicting resistance mutations using protein design algorithms. *Proc. Natl. Acad. Sci. USA*, **107**, 13 707–13 712.
- Gautam, P., Shankar, J., Madan, T., Sirdeshmukh, R., Sundaram, C.S., Gade, W.N., Basir, S.F. and Sarma, P.U. (2008) Proteomic and transcriptomic analysis of *Aspergillus fumigatus* on exposure to amphotericin B. *Antimicrob. Agents Chemother.* **52**, 4220–4227.
- Gems, D., Johnstone, I.L. and Clutterbuck, A.J. (1991) An autonomously replicating plasmid transforms *Aspergillus-nidulans* at high frequency. *Gene*, **98**, 61–67.
- Giaever, G., Flaherty, P., Kumm, J., Proctor, M., Nislow, C., Jaramillo, D.F., Chu, A.M., Jordan, M.I., Arkin, A.P. and Davis, R.W. (2004) Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. *Proc. Natl. Acad. Sci. USA*, **101**, 793–798.
- Goetz, A.K. and Dix, D.J. (2009) Mode of action for reproductive and hepatic toxicity inferred from a genomic study of triazole antifungals. *Toxicol. Sci.* **110**, 449–462.
- Goetz, A.K., Singh, B.P., Battalora, M., Breier, J.M., Bailey, J.P., Chukwudebe, A.C. and Janus, E.R. (2011) Current and future use of genomics data in toxicology: opportunities and challenges for regulatory applications. *Regul. Toxicol. Pharmacol.* **61**, 141–153.
- Goodwin, S.B., Ben M'Barek, S.B., Dhillon, B., Wittenberg, A.H.J., Crane, C.F., Hane, J.K., Foster, A.J., Van der Lee, T.A.J., Grimwood, J., Aerts, A., Antoniw, J., Bailey, A., Bluhm, B., Bowler, J., Bristow, J., van der Burgt, A., Canto-Canche, B., Churchill, A.C.L., Conde-Ferraz, L., Cools, H.J., Coutinho, P.M., Csukai, M., Dehal, P., De Wit, P., Donzelli, B., van de Geest, H.C., van Ham, R.C.H.J., Hammond-Kosack, K.E., Henrissat, B., Kilian, A., Kobayashi, A.K., Koopmann, E., Kourmpetis, Y., Kuzniar, A., Lindquist, E., Lombard, V., Maliepaard, C., Martins, N., Mehrabi, R., Nao, J.P.H., Ponomarenko, A., Rudd, J.J., Salamov, A., Schmutz, J., Schouten, H.J., Shapiro, H., Stergiopoulos, I., Torriani, S.F.F., Tu, H., de Vries, R.P., Waalwijk, C., Ware, S.B., Wiebenga, A., Zwiers, L.-H., Oliver, R.P., Grigoriev, V. and Kema, G.H.J. (2011) Finished genome of *Mycosphaerella graminicola* reveals dispensome structure, chromosome plasticity and stealth pathogenesis. *PLoS Genet.* **7**, e1002070.
- Grasso, V., Palermo, S., Sierotzki, H., Garibaldi, A. and Gisi, U. (2006) Cytochrome *b* gene structure and consequences for resistance to Qo inhibitor fungicides in plant pathogens. *Pest Manag. Sci.* **62**, 465–472.
- Griffin, M.J. and Fisher, N. (1985) Laboratory studies on benzimidazole resistance in *Septoria tritici*. *EPPO Bull.* **15**, 505–511.
- Haas, B.J., Kamoun, S., Zody, M.C., Jiang, R.H.Y., Handsaker, R.E., Cano, L.M., Grabherr, M., Kodira, C.D., Raffaele, S., Torto-Alalibo, T., Bozkurt, T.O., Ah-Fong, A.M.V., Alvarado, L., Anderson, V.L., Armstrong, M.R., Avrova, A., Baxter, L., Beynon, J., Boevink, P.C., Bollmann, S.R., Bos, J.I.B., Bulone, V., Cai, G.H., Cakir, C., Carrington, J.C., Chawner, M., Conti, L., Costanzo, S., Ewan, R., Fahlgren, N., Fischbach, M.A., Fugelstad, J., Gilroy, E.M., Gnerre, S., Green, P.J., Grenville-Briggs, L.J., Griffith, J., Grunwald, N.J., Horn, K., Horner, N.R., Hu, C.H., Huitema, E., Jeong, D.H., Jones, A.M.E., Jones, J.D.G., Jones, R.W., Karlsson, E.K., Kunjeti, S.G., Lamour, K., Liu, Z.Y., Ma, L.J., MacLean, D., Chibucos, M.C., McDonald, H., McWalters, J., Meijer, H.J.G., Morgan, W., Morris, P.F., Munro, C.A., O'Neill, K., Ospina-Giraldo, M., Pinzon, A., Pritchard, L., Ramsahoye, B., Ren, Q.H., Restrepo, S., Roy, S., Sadanandom, A., Savidor, A., Schornack, S., Schwartz, D.C., Schumann, U.D., Schwessinger, B., Seyer, L., Sharpe, T., Silvar, C., Song, J., Studholme, D.J., Sykes, S., Thines, M., van de Vondervoort, P.J.I., Phuntumart, V., Wawra, S., Weide, R., Win, J., Young, C., Zhou, S.G., Fry, W., Meyers, B.C., van West, P., Ristaino, J., Govers, F., Birch, P.R.J., Whisson, S.C., Judelson, H.S. and Nusbaum, C. (2009) Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature*, **461**, 393–398.
- Hane, J.K., Lowe, R.G.T., Solomon, P.S., Tan, K.C., Schoch, C.L., Spatafora, J.W., Crous, P.W., Kodira, C., Birren, B.W., Galagan, J.E., Torriani, S.F.F., McDonald, B.A. and Oliver, R.P. (2007) Dothideomycete-plant interactions illuminated by genome sequencing and EST analysis of the wheat pathogen *Stagonospora nodorum*. *Plant Cell*, **19**, 3347–3368.
- Hatta, R., Ito, K., Hosaki, Y., Tanaka, T., Tanaka, A., Yamamoto, M., Akimitsu, K. and Tsuge, T. (2002) A conditionally dispensable chromosome controls host-specific pathogenicity in the fungal plant pathogen *Alternaria alternata*. *Genetics*, **161**, 59–70.
- Heaney, S.P., Hall, A.A., Davies, S.A. and Olaya, G. (2000) Resistance to fungicides in the Qo-STAR cross-resistance group: current perspectives. In: *Proceedings of the BCPC conference—Pests and Diseases* pp. 1–3, 755–762. Alton, UK: BCPC.
- Herrero, A.B., López, M.C., García, S., Schmidt, A., Spaltmann, F., Ruiz-Herrera, J. and Dominguez, A. (1999) Control of filament formation in *Candida albicans* by polyamine levels. *Infect. Immun.* **67**, 4870–4878.
- Hoehamer, C.F., Cummings, E.D., Hilliard, G.M. and Rogers, P.D. (2010) Changes in the proteome of *Candida albicans* in response to azole, polyene, and echinocandin antifungal agents. *Antimicrob. Agents Chemother.* **54**, 1655–1664.

- Hollomon, D.W., Wheeler, I., Dixon, K., Longhurst, C. and Skylakakis, G. (1997) Defining the resistance risk of the new powdery mildew fungicide quinoxifen. *Pestic. Sci.* **51**, 347–351.
- Hoon, S., St, Onge, R.P., Giaever, G. and Nislow, C. (2008) Yeast chemical genomics and drug discovery: an update. *Trends Pharmacol. Sci.* **29**, 499–504.
- Horton, P., Park, K.J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C.J. and Nakai, K. (2007) WoLF PSORT: protein localization predictor. *Nucleic Acids Res.* **35**, W585–W587.
- Hu, W.Q., Sillaots, S., Lemieux, S., Davison, J., Kauffman, S., Breton, A., Linteau, A., Xin, C.L., Bowman, J., Becker, J., Jiang, B. and Roemer, T. (2007) Essential gene identification and drug target prioritization in *Aspergillus fumigatus*. *PLoS Pathog.* **3**, e24.
- Ishii, H., Fountaine, J., Chung, W.H., Kansako, M., Nishimura, K., Takahashi, K. and Oshima, M. (2009) Characterisation of Qol-resistant field isolates of *Botrytis cinerea* from citrus and strawberry. *Pest Manag. Sci.* **65**, 916–922.
- Jiang, B., Bussey, H. and Roemer, T. (2002) Novel strategies in antifungal lead discovery. *Curr. Opin. Microbiol.* **5**, 466–471.
- Joseph-Horne, T., Heppner, C., Headrick, J. and Hollomon, D.W. (2000) Identification and characterization of the mode of action of MON 65500: a novel inhibitor of ATP export from mitochondria of the wheat 'take-all' fungus, *Gaeumannomyces graminis* var. *tritici*. *Pest. Biochem. Physiol.* **67**, 168–186.
- Jung, M.K., Wilder, I.B. and Oakley, B.R. (1992) Amino-acid alterations in the *BenA* (β -tubulin) gene of *Aspergillus nidulans* that confer benomyl resistance. *Cell Motil. Cytoskeleton*, **22**, 170–174.
- Karababa, M., Coste, A.T., Rognon, B., Bille, J. and Sanglard, D. (2004) Comparison of gene expression profiles of *Candida albicans* azole-resistant clinical isolates and laboratory strains exposed to drugs inducing multidrug transporters. *Antimicrob. Agents Chemother.* **48**, 3064–3079.
- Kendall, S.J., Hollomon, D.W., Cooke, L.R. and Jones, D.R. (1993) Changes in sensitivity to DMI fungicides in *Rhynchosporium secalis*. *Crop Prot.* **12**, 357–362.
- Keon, J.P.R., White, G.A. and Hargreaves, J.A. (1991) Isolation, characterization and sequence of a gene conferring resistance to the systemic fungicide carboxin from the maize smut pathogen, *Ustilago maydis*. *Curr. Genet.* **19**, 475–481.
- Kim, B.C. and Gu, M.B. (2007) Discrimination of toxic impacts of various chemicals using chemical-gene expression profiling of *Escherichia coli* DNA microarray. *Process Biochem.* **42**, 392–400.
- Kretschmer, M., Leroch, A., Mosbach, A., Walker, A.S., Fillinger, S., Mernke, D., Schoonbeek, H.J., Pradier, J.M., Leroux, P., De Waard, M.A. and Hahn, M. (2009) Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus *Botrytis cinerea*. *PLoS Pathog.* **5**, e1000696.
- Kumar, S., Chaudhary, K., Foster, J.M., Novelli, J.F., Zhang, Y.H., Wang, S.L., Spiro, D., Ghedin, E. and Carlow, C. (2007) Mining predicted essential genes of *Brugia malayi* for nematode drug targets. *PLoS ONE*, **2**, e1189.
- Lee, W.-S., Hammond-Kosack, K.E. and Kanyuka, K. (2012) Barley stripe mosaic virus-mediated tools for investigating gene function in cereal plants and their pathogens: VIGS, HIGS and VOX. *Plant Physiol.* **160**, 582–590.
- Lee, Y.J., Yamamoto, K., Hamamoto, H., Nakaune, R. and Hibi, T. (2005) A novel ABC transporter gene *ABC2* involved in multidrug susceptibility but not pathogenicity in rice blast fungus, *Magnaporthe oryzae*. *Pest. Biochem. Physiol.* **81**, 13–23.
- Leroux, P. and Clerjeau, M. (1985) Resistance to *Botrytis cinerea* and *Plasmopara viticola* to fungicides in French vineyards. *Crop Prot.* **4**, 137–160.
- Leroux, P. and Walker, A.S. (2011) Multiple mechanisms account for resistance to sterol 14 alpha-demethylation inhibitors in field isolates of *Mycosphaerella graminicola*. *Pest Manag. Sci.* **67**, 44–59.
- Levy, M., Erental, A. and Yarden, O. (2008) Efficient gene replacement and direct hyphal transformation in *Sclerotinia sclerotiorum*. *Mol. Plant Pathol.* **9**, 719–725.
- Liolios, K., Chen, I.-M.A., Mavromatis, K., Tavernarakis, N., Hugenoltz, P., Markowitz, V.M. and Kyripides, N.C. (2010) The Genomes On Line Database (GOLD) in 2009: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res.* **38**, D346–D354.
- Liu, X., Jiang, J.H., Shao, J.F., Yin, Y.N. and Ma, Z.H. (2010) Gene transcription profiling of *Fusarium graminearum* treated with an azole fungicide tebuconazole. *Appl. Microbiol. Biotechnol.* **85**, 1105–1114.
- Liu, X., Yu, F., Schnabel, G., Wu, J.B., Wang, Z.Y. and Ma, Z.H. (2011a) Paralogous *cyp51* genes in *Fusarium graminearum* mediate differential sensitivity to sterol demethylation inhibitors. *Fungal Genet. Biol.* **48**, 113–123.
- Liu, X., Fu, J., Yun, Y.Z., Yin, Y.N. and Ma, Z.H. (2011b) A sterol C-14 reductase encoded by *FgERG24B* is responsible for the intrinsic resistance of *Fusarium graminearum* to amine fungicides. *Microbiology*, **157**, 1665–1675.
- Locke, T. and Phillips, A.N. (1995) The occurrence of benzimidazole resistance in *Rhynchosporium secalis* on winter barley in England and Wales in 1992 and 1993. *Plant Pathol.* **44**, 294–300.
- Lucas, J.A. (2011) Foresight project on global food and farming futures: advances in plant disease and pest management. *J. Agric. Sci.* **149**, 91–114.
- Ma, B. and Uddin, W. (2009) Fitness and competitive ability of an azoxystrobin-resistant G143A mutant of *Magnaporthe oryzae* from perennial ryegrass. *Plant Dis.* **93**, 1044–1049.
- 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.H., Xu, J.R., Antoniwi, 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, Y.H., Li, L.D., Manners, J.M., Miranda-Saavedra, D., Mukherjee, M., Park, G., Park, J., Park, S.Y., Proctor, R.H., Regev, 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.D., Zhou, S.G., Galagan, J., Cuomo, C.A., Kistler, H.C. and Rep, M. (2010) Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature*, **464**, 367–373.
- Ma, Z.H. and Michailides, T.J. (2005) Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Prot.* **24**, 853–863.
- Mellado, E., Garcia-Effron, G., Buitrago, M.J., Alcazar-Fuoli, L., Cuenca-Estrella, A. and Rodriguez-Tudela, J.L. (2005) Targeted gene disruption of the 14-alpha sterol demethylase (*cyp51A*) in *Aspergillus fumigatus* and its role in azole drug susceptibility. *Antimicrob. Agents Chemother.* **49**, 2536–2538.
- Meyer, V., Damveld, R.A., Arentshorst, M., Stahl, U., van den Hondel, C.A.M.J.J. and Ram, A.F.J. (2007) Survival in the presence of antifungals—genome-wide expression profiling of *Aspergillus niger* in response to sublethal concentrations of caspofungin and fenpropimorph. *J. Biol. Chem.* **282**, 32 935–32 948.
- Miao, V.P., Covert, S.F. and Vanetten, H.D. (1991) A fungal gene for antibiotic-resistance on a dispensable (B) chromosome. *Science*, **254**, 1773–1776.
- Morschhauser, J., Barker, K.S., Liu, T.T., Blass-Warmuth, J., Homayouni, R. and Rogers, P.D. (2007) The transcription factor Mrf1p controls expression of the *MDR1* efflux pump and mediates multidrug resistance in *Candida albicans*. *PLoS Pathog.* **3**, 1603–1616.
- Mouyna, I., Henry, C., Doering, T.L. and Latge, J.P. (2004) Gene silencing with RNA interference in the human pathogenic fungus *Aspergillus fumigatus*. *FEMS Microbiol. Lett.* **237**, 317–324.
- Mueller, E., Bailey, A., Corran, A., Michael, A.J. and Bowyer, P. (2001) Ornithine decarboxylase knockout in *Tapesia yellundae* abolishes infection plaque formation *in vitro* but does not reduce virulence toward wheat. *Mol. Plant-Microbe Interact.* **14**, 1303–1311.
- Nakaune, R., Adachi, K., Nawata, O., Tomiyama, M., Akutsu, K. and Hibi, H. (1998) A novel ATP-binding cassette transporter involved in multidrug resistance in the phytopathogenic fungus *Penicillium digitatum*. *Appl. Environ. Microbiol.* **64**, 3983–3988.
- Nakayashiki, H. (2005) RNA silencing in fungi: mechanisms and applications. *FEBS Lett.* **579**, 5950–5957.
- Napier, B.A.S., Bayles, R.A., Stigwood, P.L. and Burnett, F.J. (2000) Sensitivity of powdery mildew and yellow rust to DMI, morpholine and strobilurin fungicides in England and Scotland. In: *Proceedings of the BCPC Conference—Pests and Diseases* pp. 1–3, 427–434. Alton, UK: BCPC.
- Nowara, D., Gay, A., Lacomme, C., Shaw, J., Ridout, C., Douchkov, D., Hensel, G., Kumlehn, J. and Schweizer, P. (2010) HIGS: host-induced gene silencing in the obligate biotrophic fungal pathogen *Blumeria graminis*. *Plant Cell*, **22**, 3130–3141.
- Nucci, M. and Anaissie, E. (2007) *Fusarium* infections in immunocompromised patients. *Clin. Microbiol. Rev.* **20**, 695–704.
- Nuwaysir, E.F., Bittner, M., Trent, J., Barrett, J.C. and Afshari, C.A. (1999) Microarrays and toxicology: the advent of toxicogenomics. *Mol. Carcinogen.* **24**, 153–159.
- Oh, J., Fung, E., Schlecht, U., Davis, R.W., Giaever, G., St Onge, R.P., Deutschbauer, A. and Nislow, C. (2010) Gene annotation and drug target discovery in *Candida albicans* with a tagged transposon mutant collection. *PLoS Pathog.* **6**, e1001140.
- Oh, Y., Donofrio, N., Pan, H.Q., Coughlan, S., Brown, D.E., Meng, S.W., Mitchell, T. and Dean, R.A. (2008) Transcriptome analysis reveals new insight into appressorium formation and function in the rice blast fungus *Magnaporthe oryzae*. *Genome Biol.* **9**, R85.

- Orchard, S., Al-Lazikani, B., Bryant, S., Clark, D., Calder, E., Dix, I., Engkvist, O., Forster, M., Gaulton, A., Gilson, M., Glen, R., Grigorov, M., Hammond-Kosack, K., Harland, L., Hopkins, A., Larmine, C., Lynch, N., Mann, R.K., Murray-Rust, P., Lo Piparo, E., Southan, C., Steinbeck, C., Wishart, D., Hermjakob, H., Overington, J. and Thornton, J. (2011) Minimum information about a bioactive entity (MIBAE). *Nat. Rev. Drug Discov.* **10**, 661–669.
- Peever, T.L. and Milgroom, M.G. (1992) Inheritance of triadimenol resistance in *Pyrenophora teres*. *Phytopathology*, **82**, 821–828.
- Peters, R.D., Platt, H.W., Drake, K.A., Coffin, R.H., Moorehead, S., Clark, M.M., Al-Mughrabi, K.I. and Howard, R.J. (2008) First report of fludioxonil-resistant isolates of *Fusarium* spp. causing potato seed-piece decay. *Plant Dis.* **92**, 172–172.
- Raffaele, S. and Kamoun, S. (2012) Genome evolution in filamentous plant pathogens: why bigger can be better. *Nat. Rev. Microbiol.* **10**, 417–430.
- Reimann, S. and Deising, H.B. (2005) Inhibition of efflux mediated fungicide resistance in *Pyrenophora tritici-repentis* by a derivative of 4'-hydroxyflavone and enhancement of fungicide activity. *Appl. Environ. Microbiol.* **71**, 3269–3275.
- Rodriguez-Suarez, R., Xu, D.M., Veillette, K., Davison, J., Sillaots, S., Kauffman, S., Hu, W.Q., Bowman, J., Martel, N., Trosok, S., Wang, H., Zhang, L., Huang, L.Y., Li, Y., Rahkhoodae, F., Ransom, T., Gauvin, D., Douglas, C., Youngman, P., Becker, J., Jiang, B. and Roemer, T. (2007) Mechanism-of-action determination of GMP synthase inhibitors and target validation in *Candida albicans* and *Aspergillus fumigatus*. *Chem. Biol.* **14**, 1163–1175.
- Rogers, P.D. and Barker, K.S. (2003) Genome-wide expression profile analysis reveals coordinately regulated genes associated with stepwise acquisition of azole resistance in *Candida albicans* clinical isolates. *Antimicrob. Agents Chemother.* **47**, 1220–1227.
- Romero, B., Turner, G., Olivas, I., Laborda, F. and De Lucas, J.R. (2003) The *Aspergillus nidulans* alcA promoter drives tightly regulated conditional gene expression in *Aspergillus fumigatus* permitting validation of essential genes in this human pathogen. *Fungal Genet. Biol.* **40**, 103–114.
- Rouxel, T., Grandaubert, J., Hane, J.K., Hoede, C., van de Wouw, A.P., Couloux, A., Dominguez, V., Anthouard, V., Bally, P., Bourras, S., Cozijnsen, A.J., Ciuffetti, L.M., Degraeve, A., Dilmaghani, A., Duret, L., Fudal, I., Goodwin, S.B., Gout, L., Glaser, N., Linglin, J., Kema, G.H.J., Lapalu, N., Lawrence, C.B., May, K., Meyer, M., Ollivier, B., Poulain, J., Schoch, C.L., Simon, A., Spatafora, J.W., Stachowiak, A., Turgeon, B.G., Tyler, B.M., Vincent, D., Weissenbach, J., Amselem, J., Quesneville, H., Oliver, R.P., Wincker, P., Balesdent, M.H. and Howlett, B.J. (2011) Effector diversification within compartments of the *Leptosphaeria maculans* genome affected by Repeat-Induced Point mutations. *Nat. Commun.* **2**, 202. doi:10.1038/ncomms1189.
- Sanglard, D., Kuchler, K., Ischer, F., Pagani, J.L., Monond, M. and Bille, J. (1995) Mechanisms of resistance to antifungal agents in *Candida albicans* isolates from AIDS patients involves specific multidrug transporters. *Antimicrob. Agents Chemother.* **39**, 2378–2386.
- Sanglard, D., Coste, A. and Ferrari, S. (2009) Antifungal drug resistance mechanisms in fungal pathogens from the perspective of transcriptional gene regulation. *FEMS Yeast Res.* **9**, 1029–1050.
- Selmecki, A., Forche, A. and Berman, J. (2006) Aneuploidy and isochromosome formation in drug-resistant *Candida albicans*. *Science*, **313**, 367–370.
- Selmecki, A., Gerami-Nejad, M., Paulson, C., Forche, A. and Berman, J. (2008) An isochromosome confers drug resistance in vivo by amplification of two genes, *ERG11* and *TAC1*. *Mol. Microbiol.* **68**, 624–641.
- Semar, M., Strobel, D., Koch, A., Klappach, K. and Stammer, G. (2007) Field efficacy of pyraclostrobin against populations of *Pyrenophora teres* containing the F129L mutation in the cytochrome b gene. *J. Plant Dis. Prot.* **114**, 117–119.
- Sierotzki, H., Parisi, S., Steinfeld, U., Tenzer, I., Poiry, S. and Gisi, U. (2000) Mode of resistance to respiration inhibitors at the cytochrome bc(1) enzyme complex of *Mycosphaerella fijiensis* field isolates. *Pest Manag. Sci.* **56**, 833–841.
- Sigle, H.C., Thewes, S., Niewerth, M., Korting, H.C., Schafer-Korting, M. and Hube, B. (2005) Oxygen accessibility and iron levels are critical factors for the antifungal action of ciclopirox against *Candida albicans*. *J. Antimicrob. Chemother.* **55**, 663–673.
- Silva, A.P., Miranda, I.M., Guida, A., Synnott, J., Rocha, R., Silva, R., Amorim, A., Pina-Vaz, C., Butler, G. and Rodrigues, A. (2011) Transcriptional profiling of azole-resistant *Candida parapsilosis* strains. *Antimicrob. Agents Chemother.* **55**, 3546–3556.
- Sionov, E., Lee, H., Chang, Y.C. and Kwon-Chung, K.J. (2010) *Cryptococcus neoformans* overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. *PLoS Pathog.* **6**, e1000848.
- Skolness, S.Y., Durhan, E.J., Garcia-Reyero, N., Jensen, K.M., Kahl, M.D., Makynen, E.A., Martinovic-Weigelt, D., Perkins, E., Villeneuve, D.L. and Ankley, G.T. (2011) Effects of a short-term exposure to the fungicide prochloraz on endocrine function and gene expression in female fathead minnows (*Pimephales promelas*). *Aquat. Toxicol.* **103**, 170–178.
- Soanes, D.M., Alam, I., Cornell, M., Wong, H.M., Hedeler, C., Paton, N.W., Ratray, M., Hubbard, S.J., Oliver, S.G. and Talbot, N.J. (2008) Comparative genome analysis of filamentous fungi reveals gene family expansions associated with fungal pathogenesis. *PLoS ONE* **3**, e2300.
- Spanu, P.D., Abbott, J.C., Amselem, J., Burgis, T.A., Soanes, D.M., Stuber, K., van Themaat, E.V.L., Brown, J.K.M., Butcher, S.A., Gurr, S.J., Lebrun, M.H., Ridout, C.J., Schulze-Lefert, P., Talbot, N.J., Ahmadinejad, N., Ametz, C., Barton, G.R., Benjdia, M., Bidzinski, P., Bindaschledler, L.V., Both, M., Brewer, M.T., Cadle-Davidson, L., Cadle-Davidson, M.M., Collemare, J., Cramer, R., Frenkel, O., Godfrey, D., Harriman, J., Hoede, C., King, B.C., Klages, S., Kleemann, J., Knoll, D., Koti, P.S., Kreplak, J., Lopez-Ruiz, F.J., Lu, X.L., Maekawa, T., Mahanil, S., Micali, C., Milgroom, M.G., Montana, G., Noir, S., O'Connell, R.J., Oberhaensli, S., Parlange, F., Pedersen, C., Quesneville, H., Reinhardt, R., Rott, M., Sacristan, S., Schmidt, S.M., Schon, M., Skamnioti, P., Sommer, H., Stephens, A., Takahara, H., Thordal-Christensen, H., Vigouroux, M., Wessling, R., Wicker, T. and Panstruga, R. (2010) Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. *Science*, **330**, 1543–1546.
- Steffens, J.J., Pell, E.J. and Tien, M. (1996) Mechanisms of fungicide resistance in phytopathogenic fungi. *Curr. Opin. Biotechnol.* **7**, 348–355.
- Tan, K.C., Ipcho, S.V.S., Trengove, R.D., Oliver, R.P. and Solomon, P.S. (2009) Assessing the impact of transcriptomics, proteomics and metabolomics on fungal phytopathology. *Mol. Plant Pathol.* **10**, 703–715.
- Toquin, V., Barja, F., Sirven, C., Gamet, S., Mauprivez, L., Peret, P., Latorse, M.P., Zundel, J.L., Schmitt, F., Lebrun, M.H. and Beffa, R. (2010) Novel tools to identify the mode of action of fungicides as exemplified with fluopicolide. In: *Recent Developments in the Management of Plant Diseases 1* (Gisi, U. and Chet, I., eds), pp. 19–36. Berlin: Springer-Verlag.
- Tsukuda, T., Carleton, S., Fotheringham, S. and Holloman, W.K. (1988) Isolation and characterisation of an autonomously replicating sequence from *Ustilago maydis*. *Mol. Cell. Biol.* **8**, 3703–3709.
- Valdés-Santiago, L., Guzmán-de-Peña, D. and Ruiz-Herrera, J. (2010) Life without putrescine: disruption of the gene-encoding polyamine oxidase in *Ustilago maydis* odc mutants. *FEMS Yeast Res.* **10**, 928–940.
- Vargas, J.M. (1973) A benzimidazole resistant strain of *Erysiphe graminis*. *Phytopathology*, **63**, 1366–1368.
- Villalba, F., Collemare, J., Landraud, P., Lambou, K., Brozek, V., Cirer, B., Morin, D., Bruel, C., Beffa, R. and Lebrun, M.H. (2008) Improved gene targeting in *Magnaporthe oryzae* by inactivation of *MgKU80* required for non-homologous end joining. *Fungal Genet. Biol.* **45**, 68–75.
- Wang, C.F., Zhang, S.J., Hou, R., Zhao, Z.T., Zheng, Q., Xu, Q.J., Zheng, D.W., Wang, G.H., Liu, H.Q., Gao, X.L., Ma, J.W., Kistler, H.C., Kang, Z.S. and Xu, J.R. (2011a) Functional analysis of the kinome of the wheat scab fungus *Fusarium graminearum*. *PLoS Pathog.* **7**, e1002460.
- Wang, N.Y., Yang, S.L., Lin, C.H. and Chung, K.R. (2011b) Gene inactivation in the citrus pathogenic fungus *Alternaria alternata* defect at the *Ku70* locus associated with non-homologous end joining. *World J. Microbiol. Biotechnol.* **27**, 1817–1826.
- White, T.C. (1997) Increased mRNA levels of *ERG16*, *CDR*, and *MDR1* correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. *Antimicrob. Agents Chemother.* **41**, 1482–1487.
- White, T.A. and Kell, D.B. (2004) Comparative genomic assessment of novel broad-spectrum targets for antibacterial drugs. *Comp. Funct. Genomics* **5**, 304–327.
- Winnenburg, R., Urban, M., Beacham, A., Baldwin, T.K., Holland, S., Lindeberg, M., Hansen, H., Rawlings, C.J., Hammond-Kosack, K.E. and Kohler, J. (2008) PHI-base update: additions to the pathogen–host interaction database. *Nucleic Acids Res.* **36**, D572–D576.
- Wu, Y.X. and von Tiedemann, A. (2001) Physiological effects of azoxystrobin and epoxiconazole on senescence and the oxidative status of wheat. *Pest. Biochem. Physiol.* **71**, 1–10.
- Xu, D., Jiang, B., Ketela, T., Lemieux, S., Veillette, K., Martel, N., Davison, J., Sillaots, S., Trosok, S., Bachewich, C., Bussey, H., Youngman, P. and Roemer, T. (2007) Genome-wide fitness test and mechanism-of-action studies of inhibitory compounds in *Candida albicans*. *PLoS Pathog.* **3**, e92.

- Yamada, N., Motoyama, T., Nakasako, M., Kagabu, S., Kudo, T. and Yamaguchi, I. (2004) Enzymatic characterization of scytalone dehydratase Val75Met variant found in melanin biosynthesis dehydratase inhibitor (MBI-D) resistant strains of the rice blast fungus. *Biosci. Biotechnol. Biochem.* **68**, 615–621.
- Yan, X., Ma, W.B., Li, Y., Wang, H., Que, Y.W., Ma, Z.H., Talbot, N.J. and Wang, Z.Y. (2011) A sterol 14 α -demethylase is required for conidiation, virulence and for mediating sensitivity to sterol demethylation inhibitors by the rice blast fungus *Magnaporthe oryzae*. *Fungal Genet. Biol.* **48**, 144–153.
- Yasokawa, D. and Iwahashi, H. (2010) Toxicogenomics using yeast DNA microarrays. *J. Biosci. Bioeng.* **110**, 511–522.
- Yin, C., Jurgenson, J.E. and Hulbert, S.H. (2011) Development of a host-induced RNAi system in the wheat stripe rust fungus *Puccinia striiformis* f. sp. *tritici*. *Mol. Plant-Microbe Interact.* **24**, 554–561.
- Yin, Y., Liu, X., Li, B. and Ma, Z. (2009) Characterization of sterol demethylation inhibitor-resistant isolates of *Fusarium asiaticum* and *F. graminearum* collected from wheat in China. *Phytopathology*, **99**, 487–497.
- Zhang, C.Q., Zhu, G.N., Ma, Z.H. and Zhou, M.G. (2006) Isolation, characterization and preliminary genetic analysis of laboratory tricyclazole-resistant mutants of the rice blast fungus, *Magnaporthe grisea*. *J. Phytopathol.* **154**, 392–397.