Adaptation to the Host Environment by Plant-Pathogenic Fungi

van der Does, H.C.; Rep, M.

DOI
10.1146/annurev-phyto-080516-035551
Publication date
2017
Document Version
Final published version
Published in
Annual Review of Phytopathology

ANNUAL REVIEWS

Annual Review of Phytopathology

Adaptation to the Host Environment by Plant-Pathogenic Fungi

H. Charlotte van der Does and Martijn Rep

Molecular Plant Pathology, University of Amsterdam, 1098XH Amsterdam, The Netherlands; email: m.rep@uva.nl

Annu. Rev. Phytopathol. 2017. 55:427–50

First published as a Review in Advance on June 23, 2017

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

https://doi.org/10.1146/annurev-phyto-080516-035551

Keywords

plant-pathogenic fungi, transcriptomics, host sensing, transcription factors

Abstract

Many fungi can live both saprophytically and as endophyte or pathogen inside a living plant. In both environments, complex organic polymers are used as sources of nutrients. Propagation inside a living host also requires the ability to respond to immune responses of the host. We review current knowledge of how plant-pathogenic fungi do this. First, we look at how fungi change their global gene expression upon recognition of the host environment, leading to secretion of effectors, enzymes, and secondary metabolites; changes in metabolism; and defense against toxic compounds. Second, we look at what is known about the various cues that enable fungi to sense the presence of living plant cells. Finally, we review literature on transcription factors that participate in gene expression in planta or are suspected to be involved in that process because they are required for the ability to cause disease.

INTRODUCTION

Fungi are prominent among plant-pathogenic microbes, posing a persistent threat to agriculture, horticulture, and forests (38). All crops must be protected from this menace by antifungal compounds, biological control agents, agricultural practices, development of resistant cultivars, or any combination of these measures (24, 115, 195). Only a limited number of fungal species, or even strains within a fungal species, can cause disease on any crop plant. This attests to the general effectiveness of the plant immune system in limiting the growth or development of invading microorganisms. It also implies that pathogenic fungi are highly adapted to a limited set of hosts and are able to reduce the effectiveness of the defenses of only those plant species. The key to pathogenicity of fungi, therefore, lies in responding to the host environment in a way that promotes growth inside the host despite the presence of surveillance and defense mechanisms. This response involves sensing of the proper cues from the host and then adapting gene expression such that infection structures are formed, host defenses are either not induced or rendered ineffective, and all nutrients required for growth are obtained from the host.

With the emergence of transcriptomics technology, especially RNA sequencing (RNA-seq[1]), changes in fungal transcriptomes upon invasion of living plants by a variety of plant-pathogenic fungi have been documented. This has yielded a rich overview of how fungi change their gene expression upon host invasion. We summarize here what has been learned to date using transcriptomics about how plant-pathogenic fungi adapt to the host environment. The more difficult challenges are to identify the cues from the host that trigger these changes in gene expression and uncover how sensing of these cues leads to changes in gene expression. We review here the most important discoveries that contributed to answering these questions. With regard to mechanisms underlying changes in gene expression upon sensing of living plant tissue, we focus on the transcription factors (TFs) involved. Because we focus on the shift from saprophytic to invasive growth, we do not cover obligate biotrophic fungi in this review.

PLANT-PATHOGENIC FUNGI ADAPT TO THE HOST ENVIRONMENT

When attempting to discover how fungi adapt their gene expression to the living plant environment, a crucial issue is which conditions to compare. Several approaches have been taken. One basic approach is to compare fungal transcriptomes of colonized plant tissue with transcriptomes of in vitro cultures of the same fungus. In this approach, the choices of plant tissue, stage of infection and in vitro condition are crucial because these determine to a large extent which genes are identified as plant induced. More sophisticated analyses involve multiple time points of infection, combined with in vitro conditions that are known or suspected to partially mimic in planta conditions (6, 96, 103) or specific stages thereof, e.g., the plant surface (118), different tissues colonized by the same fungus (3, 47, 143), or comparisons between a wild-type and mutant strain (87, 145, 162). In addition, host-specific induction of fungal gene expression has been investigated by comparing fungal transcriptomes upon colonization of different hosts (56) or a compatible versus an incompatible interaction (71, 72, 158). An elegant approach for identification of genes that respond specifically to the living plant host is to compare gene expression between growth in living tissue and growth in the same tissue killed by freezing (7). The major transcriptomics studies we reviewed are summarized in **Supplemental Table 1**. It is important to realize that such studies do not establish causal relationships; genes induced upon colonization of the host or at certain stages of development are not necessarily required for growth under those circumstances. Nevertheless,

Supplemental Material

[1] In fact, cDNA is sequenced in most current RNA-seq methods.

these studies help to formulate hypotheses on metabolic processes, cell wall modifications, defense against host compounds, and other processes required for host colonization and also help identify candidate genes required for certain stages of pathogenicity, such as effector genes, secondary metabolite gene clusters, and genes encoding nutrient importers or secreted enzymes.

Effectors

Pathogen-secreted proteins are of primary interest in transcriptomics studies of plant-fungus interactions, as they play crucial roles in adaptation to the host environment by plant-pathogenic fungi. Two major classes of secreted proteins are commonly distinguished: enzymes and small (nonenzymatic) secreted proteins that are commonly called effectors. Effectors can be defined functionally as secreted proteins that in some way promote host colonization and/or the manifestation of disease symptoms, either by protecting the fungus from host defensive compounds such as cell wall–degrading enzymes (CWDEs) or by interfering with the host's immune system (33, 78, 122). Apart from being small (generally fewer than 300 amino acids) and commonly, but not necessarily, having high cysteine content, effectors are often fast evolving because of their participation in the molecular arms race with host plants (140, 163).

Effectors are generally associated with the biotrophic growth phase, where they play a crucial role in keeping host cells alive and the immune system suppressed. Indeed, in virtually all pathosystems investigated, expression of effector genes is induced early in host invasion and declines after a switch to necrotrophic growth. This has been observed in transcriptomics studies with *Colletotrichum higginsianum* (77, 118), *Colletotrichum orbiculare* (42), *Colletotrichum graminicola* (162), *Fusarium oxysporum* (158), *Magnaporthe oryzae* (71, 103), *Zymoseptoria tritici* (132), *Leptosphaeria maculans* (47, 54), and *Ustilago maydis* (70).

Some classes of small secreted proteins are associated with necrotrophy, e.g., members of the necrosis and ethylene-inducing peptide-1 (Nep-1)-like protein (NLP) family (50, 128, 193). In the transcriptomics studies under review here, expression of genes for such toxins, sometimes also called effectors (86), was observed for *L. maculans* (54), *C. higginsianum* (77), and *C. graminicola* (162). Interestingly, in *U. maydis*, biotrophic colonization of seedlings, adult leaves, or tassels is associated with expression of only partially overlapping sets of effector genes (143). This suggests that *U. maydis* senses not only plant tissue in general but also organ-specific cues. Nine effector genes expressed in an organ-specific manner were later shown to be required for virulence specifically in the organ in which they are expressed (137).

Secondary Metabolites

For many plant-pathogenic fungi, not only effectors but also secreted secondary metabolites (SMs) are key determinants for facilitation of plant colonization. Fungal SMs are commonly grouped based on the basic skeleton such as polyketides, synthesized by polyketide synthases (PKSs), and nonribosomal peptides, synthesized by nonribosomal peptide synthases (NRPSs). Many PKS and NRPS genes reside in gene clusters for the synthesis of a particular compound (11, 169). Well-known examples of SMs that contribute to plant diseases or food and feed contamination are trichothecenes produced by *Fusarium* (31, 98) and host-specific toxins produced by *Alternaria* (40, 126), but all filamentous fungi produce SMs and, in many cases, particular gene clusters are activated during plant invasion. Examples of fungal plant pathogens in which genes for SMs are in induced during host colonization are *Z. tritici* (72, 132), *L. maculans* (54), *Dothistroma septosporum* (10), *Botrytis cinerea* (87), and *Fusarium graminearum* (7, 56, 96, 198, 199). In most of these cases, SM gene expression is associated with necrotrophy, but in some cases expression of particular

SM gene clusters is associated with biotrophy, notably in *Colletotrichum* (42, 118, 162) and/or development of appressoria as in *M. oryzae* (145).

Secreted Enzymes

In most transcriptomics studies, a large proportion of genes whose expression is upregulated upon host invasion relative to axenic growth encode secreted enzymes, in particular, plant CWDEs (PCWDEs) and proteases. Generally, the highest number of such genes is expressed during necrotrophic growth, such as in *C. orbiculare* (42), *Colletotrichum gloeosporioides* (3), *C. graminicola* (162), *Z. tritici* (132), *D. septosporum* (10), *L. maculans* (47), and *F. graminearum* (198), signifying large-scale breakdown of plant cell walls, presumably for nutrient acquisition as fungal mass increases rapidly in this phase. In some cases, particular PCWDEs, such as pectin-degrading enzymes in *L. maculans*, are also expressed in early/biotrophic phases (54). Other fungi, such as *B. cinerea* (87) and *Penicillium expansum* (6), grow necrotrophically from the outset of infection and immediately start to express many PCWDE genes.

Despite the widespread consensus that pathogenic fungi induce expression of secreted enzymes during invasion, from most studies it cannot be concluded that this is a specific response to the living host environment. Comparisons are mostly done with axenic growth in the presence of a simple carbon source such as glucose. The absence of a sugar and the presence of complex polysaccharides already induce expression of many CWDEs (80). In an interesting approach to differentiate this type of induction with changes in gene expression that are specific to a living host environment, Boedi et al. (7) compared global gene expression in *F. graminearum* between colonization of living and cold snap–killed wheat heads. As expected, most PCWDE genes were induced in both living and dead tissue.

Nutrient Uptake

Plant colonization is commonly associated with upregulation of genes encoding membrane transporters that likely function as importers of nutrients such as sugars and amino acids. Induced expression of nutrient importers is especially prominent during the necrotrophic phase, concomitantly with secretion of hydrolases, such as in *C. graminicola* (162), *C. higginsianum* (118), *F. graminearum* (198), *F. oxysporum* (158), *D. septosporum* (10), *Z. tritici* (132), *L. maculans* (54), and *M. oryzae* (145). That this general response is not related to the presence of living plant cells is borne out by the fact that *F. graminearum* strongly induces genes for transport of ions, sugars, amino acids, and di- and tripeptides both in living and cold snap–killed wheat heads (7). For *M. oryzae* and *C. orbiculare*, induced expression of genes required for quinate uptake and utilization was noted (42, 145). Quinate is a cyclic polyol that can be used as the sole carbon source by fungi and is abundant in decaying leaf litter (59). In *C. orbiculare*, genes for quinate utilization had the highest expression during late necrotrophy (42). In *M. oryzae*, quinate permease genes are expressed at all stages of appressoria formation (145). In *F. graminearum*, members of the TPO family of polyamine transporters are highly expressed in maize, possibly to import polyamines such as spermine, putrescine, and spermidine (56), some of which induce expression of genes involved in trichothecene production (see below). Also in *F. graminearum*, genes for allantoin and allantoate transporters were observed to be enriched among genes expressed in wheat but not barley (96). A remarkable mechanism in *U. maydis*, which grows biotrophically throughout its colonization of maize, is the infection-specific expression of the sucrose-specific transporter Srt. This may help to avoid eliciting the maize immune system because it obviates the need to secrete invertase for sugar uptake. Significantly, Srt1 is required for full virulence of *U. maydis* (176).

When certain nutrients are limiting during growth in planta, fungi may also reduce the amounts needed for growth. When infecting maize stalks, *F. graminearum* shows reduced expression, compared to in vitro growth, of 36 genes predicted to be involved in the metabolism of glycerophospholipids, which are structural components of membranes (200). Further investigation revealed a connection with low phosphate availability during maize stalk colonization: Expression of the *BTA1* (betaine lipid synthase) gene, which is responsible for the synthesis of the phosphorus-free lipid DGTS (diacylglyceryl-N,N,N-trimethylhomoserine), was much higher in planta than in vitro. Expression of genes encoding putative phosphate transporters was also enhanced. The phosphate concentration in the apoplastic space was estimated to be only 0.3 mM, and deletion of *BTA1* prevented intercellular growth of *F. graminearum* in maize stalk and reduced virulence, which was restored by the addition of phosphate at the infection site (200). Enhanced expression of genes for phosphate transporters in *C. graminicola* during colonization of detached maize leaf sheaths also suggests that available phosphate levels are relatively low under those conditions (162).

Carbon Metabolism

In several plant-pathogenic fungi, the glyoxylate cycle, which is required when carbon needed for anabolism is derived from fatty acids instead of sugars, has been implicated in the ability to colonize living plant tissue, because mutants in key enzymes needed for the glyoxylate cycle, such as isocitrate lyase, are less virulent (35). In *F. graminearum*, expression of fatty acid oxidation and glyoxylate cycle genes was higher during coleoptile infection than in an in vitro culture, whereas the reverse was true for genes required for glycolysis (198). It may be that carbon is to a large extent derived from stored lipids, especially in early stages of infection. This seems to clearly be the case in *C. gloeosporioides*, which forms appressoria before penetration that accumulate transcripts for β-oxidation of fatty acids. During necrotrophic growth in mature tomato fruits, genes for glycolysis are induced instead, suggesting the use of carbon in the form of sugars derived from the plant (3). In a mutant of *C. graminicola* that cannot (fully) establish biotrophy, key enzymes of the glyoxylate cycle are expressed more highly than in the wild type, which could reflect stalling at the appressorial or early biotrophic stage (162). Also for *M. oryzae*, the formation of appressoria is associated with both β-oxidation of fatty acids and the glyoxylate cycle (145). Fatty acid generation from lipids and their β-oxidation, as well as the glyoxylate cycle, are also prominent during early infection by *Z. tritici* (132).

Detoxification and Reactive Oxygen Species Metabolism

Production of reactive oxygen species (ROS) is a universal response of plant cells to the perception of microbe-associated molecular patterns (117), and it has been shown that the ability to respond to ROS is required for the virulence of some plant-pathogenic fungi (53, 91, 111, 190) but not others (88, 113). Monitoring the expression of 206 TF genes in *M. oryzae* under various conditions, Park et al. (123) found that expression patterns during colonization of rice (78 and 150 hpi) are similar to expression patterns under oxidative stress conditions. Wheat coleoptiles accumulate more ROS at sites infected with *F. graminearum* at 16 hpi than at mock-inoculated sites (198). At that stage of infection the expression of genes for catalases and superoxide dismutases, which convert the ROS hydrogen peroxide and superoxide, respectively, has increased in *F. graminearum*. Later during coleoptile infection, at 64 hpi, expression of genes increased for extracellular ROS-producing enzymes, such as NADPH oxidases, suggesting that at that time the fungus produces ROS, coinciding with the secretion of many PCWDEs (198). Also, in *C. graminicola*, genes for proteins involved in peroxide detoxification are expressed early, during the biotrophic phase of

infection (162). Likewise, symptomless wheat leaf colonization by *Z. tritici* is associated with enrichment of expression of genes for peroxidase activity, oxidoreductase activity, and antioxidant activity, possibly to reduce ROS produced by plant cells (47, 72). The importance of protection against plant-produced compounds during infection is further suggested by increased expression of genes associated with detoxification, encoding, for example, cytochrome P450s, MDR (multi-drug resistance) proteins, and ABC transporters in *Z. tritici* (72) and *F. graminearum* (7, 199). An interesting case of interference with the plant ROS response is the effector Pep1 of *U. maydis*. This secreted protein is required for virulence and was found to inhibit peroxidases secreted by maize cells, thereby blocking the oxidative burst (63).

Siderophores

In planta upregulation of genes required for siderophores, molecules that facilitate uptake of iron, has been noted in *C. graminicola* during the transition to biotrophic growth (162) and in *C. gloeosporioides* at the appressorial stage along with the gene for the iron transport multicopper oxidase Fet3 (3). In *F. graminearum*, siderophore genes and a siderophore permease were highly expressed during maize stalk infection (199). Also in *F. graminearum*, the NRPS-encoding genes of the ferricrocin, malonichrome, and triacetylfusarinine siderophore clusters, as well as iron transporters, are specifically expressed in the living (as opposed to cold snap–killed) wheat head (7), and these gene clusters are also activated during maize stalk infection (199). Expression of the NRPS genes of the ferricrocin and triacetylfusarinine clusters was also detected early by Harris et al. (56) during colonization of wheat, barley, and maize, whereas expression of the NRPS gene of the malonichrome cluster was detected in wheat and barley but not maize. Genes for proteins related to the major facilitator MirA and a ferric reductase, which is involved in siderophore uptake, were found to be coexpressed in that study.

The Pth11 Family of G Protein–Coupled Receptors

Pth11 is a G protein–coupled receptor required for appressorium differentiation in *M. oryzae* (32). It contains an extracellular CFEM domain that is unique to filamentous ascomycetes, and it is presumed to be involved in sensing and transducing external signals that stimulate appressorium development (81, 82). In *C. graminicola*, 10 Pth11-family genes are expressed differentially in wheat: eight early and two late during infection (162). *F. graminearum* expresses six Pth11-family members at different times after infection of wheat coleoptiles (198), whereas Harris et al. (56) detected no less than 30 Pth11-family genes expressed by *F. graminearum* during colonization of wheat, barley, and/or maize, with 27 exhibiting host-preferential expression in wheat, barley, or both.

HOW DO FUNGI SENSE THE HOST?

Fungi continually sense their environment through light, chemical, and physical cues. Sensing of these cues activates signal transduction processes that induce changes in metabolism, cellular organization, and gene expression. These changes, in turn, lead to developmental processes such as sporulation, morphological changes such as redirection of growth, the ability to degrade complex organic compounds and import nutrients, and the ability to survive stressful conditions such as osmotic and oxidative environments, heat stress, and the presence of toxic compounds. Pathogenic fungi may be seen to have tuned these basic processes, present in all fungi, to sense the proximity of living plant tissue and respond in a way that promotes colonization. Beyond this, differences in

host species or different organs of the same host species can provoke different responses (56, 72, 143).

Sensing the Proximity of Plant Roots

In the soil, some fungi can sense the proximity of plant roots through compounds released by living roots and respond by growing toward the source of those compounds, a process called chemotropism (135, 167). A well-known example of a fungal response to root-released compounds is the hyphal branching response of arbuscular mycorrhizal fungi to strigolactones (2, 44). As for plant-pathogenic fungi, a remarkable recent discovery is the response of the vascular wilt pathogen *F. oxysporum* to peroxidases released by roots (168). *F. oxysporum* was found to reorient growth toward nutrients but also to active plant peroxidases, which are released by (wounded) tomato roots. The latter response requires a functional homolog of a yeast pheromone receptor, and the corresponding MAP kinase signal transduction pathway (168). Much remains to be learned about plant-released compounds that are sensed by pathogenic or symbiotic fungi and the chemotropic response that they elicit.

Sensing the Plant Surface

Pathogenic fungi that invade aboveground tissues recognize the plant surface, the cuticle, through its hydrophobicity and chemical composition. The cuticle is composed of cutin, a polymer of hydroxy fatty acids, and intracuticular and epicuticular waxes with complex compositions (14). Sensing of the rice cuticle by *M. oryzae*, which leads to the formation of appressoria, requires the membrane proteins Msb2 and Sho1. Msb2 is required for sensing hydrophobicity and cutin monomers, and Sho1 is more important for responding to primary alcohols, which are components of wax (93). The basidiomycete *U. maydis* likewise depends on Msb2 and Sho1 for the development of appressoria on the maize leaf surface, requiring both proteins for responding to a hydrophobic surface but not to the cutin monomer 16-hydroxy hexadecanoic acid (85). Next to induction of appressoria, these surface cues induce expression of effector genes associated with biotrophic growth (84). This would explain the secretion of effectors from appressorial penetration pores by *C. higginsianum* (77) and the appressorial expression of effector genes in *M. oryzae* (41, 147). Wang et al. (179) showed that in *M. oryzae*, Msb2 has overlapping functions with another mucin, Cbp1, in the formation of appressoria, and that extracellular and cytoplasmic domains of Msb2 have distinct roles in appressorium formation and invasive growth (179). *B. cinerea* requires Msb2 for the formation of appressoria or infection cushions on hard surfaces, even though *msb2* mutants of this fungus are still virulent in various plant species (87). The root-invading fungus *F. oxysporum* does not encounter a cuticle but nevertheless requires both Msb2 and Sho1 for invasive growth, root colonization, and secretion of pectinolytic activity, apparently acting through the Fmk1 MAP kinase (125). Another root pathogen, *Fusarium solani*, responds to cutin monomers by induced expression of a cutinase gene (4, 89, 90).

As mentioned above, Pth11 is required for the development of appressoria in *M. oryzae* and is suspected to be involved in host surface recognition, and expression of several Pth11 homologs is induced during colonization of wheat by *C. graminicola* and *F. graminearum* (see above). Deletion of a gene for a homolog of Pth11 in *B. cinerea* slightly reduces virulence and affects expression of genes encoding glutathione S-transferases (51). It is still unknown what signal Pth11, or any of its homologs, may sense, but there may be a connection between Pth11 and ROS homeostasis in *M. oryzae* (79).

Sensing Living Cells

As already mentioned, global gene expression in *F. graminearum* is very different between colonization of live and cold snap–killed wheat heads (7). This suggests that, apart from relatively stable chemical or physical characteristics shared between living and dead plant tissue, pathogenic fungi are able to sense living plant cells specifically. This was also suggested by studies with the effector gene *SIX1* of *F. oxysporum*, which was induced upon invasion of roots and in a plant cell culture but not in dead roots or by root exudate or root extracts (170). Possibly, living plant cells produce unstable compounds that are quickly depleted upon cell death and can be sensed by fungi. Another possibility is that living plant cells produce specific compounds only when they themselves sense the presence of microbes. An interesting example of the latter is the accumulation of polyamine putrescine, a compound that, in turn, can induce expression of genes such as *TRI5* that lead to the production of the mycotoxin deoxynivalenol (DON) in wheat heads upon infection by *F. graminearum* (45, 46). If a polyamine is indeed the key trigger for *TRI5* activation, it must be a very early host response because *TRI5* is already induced in infection cushions (8). Flavonoids such as the antimicrobial compound pisatin can induce expression of pisatin demethylase in *F. solani* (152, 153). Induced production or release of such compounds may also be a specific response of living plant cells to the presence of microbes and thereby allows fungi to sense the presence of living plant cells (58).

TRANSCRIPTION FACTORS REQUIRED FOR VIRULENCE

Signal transduction processes connect sensing of host cues to transcriptional reprogramming to adapt to the host environment. This is a vast field of research, and we mainly focus here on TFs that are required for virulence of plant-pathogenic fungi. These TFs are listed in **Supplemental Table 2**. It is important to note that some of the TFs that are required for virulence may not regulate infection-specific processes. Conversely, some TFs, such as Mzr1 and Bot6, regulating transcriptional changes that occur upon switching to an invasive lifestyle are not required for virulence (127, 201). Mzr1 from *U. maydis* is required for expression of some of the effector genes of the *MIG2* cluster but is not required for virulence. To activate *MIG* gene expression, Mzr1 requires the presence of Biz1 (201). Biz1 is a C2H2 zinc finger TF that is activated upon developmental changes (mating) preceding infection by *U. maydis* and is required for pathogenicity (39). Overexpression of *BIZ1* alone, in an *mzr1* deletion mutant, is sufficient to induce *MIG* expression (201). Bot6 is a Zn(2)Cys(6)-type TF in *B. cinerea* that is part of the *BOT* gene cluster that produces the SM botrydial, a phytotoxic sesquiterpene. Bot6 is required for the expression of the other *BOT* genes. Botrydial is, however, dispensable for pathogenicity, as is *BOT6* (127). Also, the C2H2 TF Yoh1, which regulates expression of multiple virulence-associated processes, such as phytotoxin biosynthesis, detoxification, and SM gene cluster expression, including the *BOT* cluster, is not required for virulence of *B. cinerea* (142). However, when both botrydial and botcinic acid production are disrupted, the fungus is reduced in virulence, suggesting that these two SMs are functionally redundant and contribute to virulence (28).

Supplemental Material

Morphological Changes

For many plant-pathogenic fungi, invasion of plant tissue is preceded by the production of specialized infection structures, such as appressoria or infection cushions (8, 101). However, this does not necessarily mean that these structures are required for invasive growth inside the plant. For example, when plants are directly inoculated with mycelium of an *M. oryzae HOX7* deletion

mutant, which is unable to produce appressoria, the fungus is fully able to cause disease (75). Likewise, infections of aboveground parts of plants usually start from conidia, but the ability to produce conidia is not a requirement to grow invasively into plant tissue. For example, *MoHOX2* and *FgFlbD* deletion mutants do not produce conidia but are nevertheless virulent (75, 146). Still, in a number of TF deletion mutants, reduced virulence coincides with defects in conidia production. In *M. oryzae*, many such TF genes have been identified: *CON7*, *CDTF1*, *COD1*, *COD2*, *COM1*, *COS1*, *RFX1*, *SOM1*, *TUP1*, *WOR1*, and *YCP4* are all required for full virulence as well as normal conidia production (18–20, 23, 119, 154, 184, 186, 202). Homologs of Con7 and Wor1 also function in virulence and conidiation in other fungal species (12, 68, 106–109, 121, 133, 134, 161, 164). Other TFs involved in both processes are Spt3, Spt8, Skn7, Atf1, and Swi6 in *F. graminearum*, Ste12 in *Penicillium digitatum* and *Setosphaeria turcica*, and StuA in *L. maculans* and *Stagonospora nodorum* (43, 52, 66, 67, 92, 149, 174). A reverse situation was observed for Znf1 in *M. oryzae*: Its deletion increases conidia production while reducing virulence (191).

This repeated co-occurrence of phenotypes suggests a transcriptional connection between processes required for virulence and the production of conidia. Possible connections could be a generally reduced metabolic or regulatory capacity of the deletion mutant. Indeed, some of the deletion mutants (*CON7*, *CDTF1*, *RFX1*, *SOM1*, and *TUP1*) are also impaired in vegetative growth (19, 119, 154, 184). Alternatively, it is known that Wor1 homologs and Con7 can affect cell wall composition. It is, however, unknown whether changes in cell wall composition cause the problems in conidiation (20, 119).

Secondary Metabolite Gene Clusters

Secondary metabolism in fungi is influenced by many factors [reviewed by Brakhage (11) and Tudzynski (166)], but we restrict ourselves to TFs implied in virulence. Among the clearest examples of SMs that fulfill an indispensable role in virulence are the host selective toxins (HSTs) of *Alternaria alternata*, which determine host-specific virulence (165). Although putative TF genes in several of the *A. alternata* HST gene clusters have been identified, there is currently no genetic evidence for their requirement for HST production (180). For other SMs required for (full) virulence, the effect of pathway-specific TFs, such as for *Helminthosporium carbonum* (HC) toxin, depudecin, trichothecenes, and fumonisin, has been determined (1, 13, 141, 182).

HC toxin is a cyclic tetrapeptide that is produced by *Cochliobolus carbonum* and inhibits histone deacetylases. Its production relies on the *TOX* genes at the *TOX2* locus. ToxE is the pathway-specific bZIP TF required for the expression of the other *TOX* genes (1). Depudecin, which also exhibits histone deacetylase inhibitor activity, is a polyketide produced by the *DEP* gene cluster in *Alternaria brassicicola*. *DEP6*, part of the cluster, encodes a TF that regulates expression of the five other *DEP* genes. The contribution of depudecin to virulence is, however, very small (182).

Fumonisin is a *Fusarium* mycotoxin with structural similarity to sphingolipids that interferes with sphingolipid metabolism (178). Fumonisin production is governed by the *FUM* cluster and regulated by the pathway-specific TF Fum21. Deletion of *FUM21* results in the loss of fumonisin production and loss of pathogenicity in *Fusarium fujikuroi* and *Fusarium verticillioides* (130). Production of fumonisins is also regulated on other levels. Deletion of any one of the TF genes *AreA*, *AreB*, *ART1*, *FUG1*, or *SGE1* reduces both fumonisin production and virulence (12, 74, 120, 129, 130). AreA and AreB are key TFs in nitrogen catabolite repression (166), and Art1 is required for starch hydrolysis (120). Sge1 is a homolog of Wor1, a regulator of lifestyle switching, effector gene expression, and SM production in different fungi (12).

F. graminearum produces the trichothecene DON. Trichothecenes are a group of sesquiterpenes that are potent inhibitors of protein synthesis in mammalian cells. DON production is

regulated by two pathway-specific TFs: Tri6 and Tri10. Deletion of *TRI6* or *TRI10* abolishes DON production in *F. graminearum* and severely reduces pathogenicity (141). Surprisingly, Tri10 and Tri6 control more genes than just the *TRI* cluster, and loss of DON production only partially explains the loss of pathogenicity of the deletion mutants (27, 114, 124). Tri10 affects only a few other genes, but one is a homolog of *PTH11* from *M. oryzae*, which encodes a transmembrane protein suggested to function in host sensing (see above) (124). Tri6 controls many more genes, including genes involved in lipid and nitrogen metabolism. Two of these are TFs with an NmrA domain, a domain that can act as a negative regulator of nitrogen catabolite repression (114). In a systematic study by Son and coworkers, the inability to produce DON was not coupled to a reduction in virulence (146). Deletion mutants of five uncharacterized TFs and, remarkably, Tri6 itself were still virulent, despite a complete lack of DON production. A possible explanation given is the use of a highly susceptible wheat cultivar and a growth chamber environment that favors disease. Interestingly, in all deletion mutants the production of another major SM of *F. graminearum*, zearalenone, was also abolished or reduced (146).

The production of DON is also influenced by several other factors, among which are the TFs AreA, Atf1, Art1, Fgp1 (Wor1 homolog), Myt3, Skn7, Zif1, and Swi6 (49, 68, 76, 92, 120, 172, 181). The nitrogen regulator AreA physically interacts with the Tri10 protein (64). Atf1 and Skn7 have a role in the oxidative stress response, and Myt3 affects nitrogen metabolism and conidiation (67, 76). PacC and Ap1, regulators of the pH response and oxidative stress, respectively, affect DON production, but not pathogenicity, in *F. graminearum* (105, 112). Homologs of PacC and Ap1 in other fungi are required for pathogenicity toward plants, and PacC is also required for the production of SMs in *P. expansum* (5, 53, 83, 91, 110, 111, 155, 188–190, 196). Other TFs that affect SM production are Con7, Cdtf1, and Asd4 in *M. oryzae*. Con7 and Cdtf1 are also required for conidiation, whereas Asd4 is required for nitrogen utilization (100, 119, 184). In *Verticillium dahliae* and *F. graminearum*, Mcm1, a MADS box TF, is required for normal conidiation, sclerotia formation, and SM production (183, 185).

Effector Gene Expression

Reminiscent of the pathway-specific TFs of secondary metabolism gene clusters, the TF gene *FTF1* from *F. oxysporum* is located close to small groups of *SIX* effector genes (138). Ftf1 strongly induces *SIX* effector gene expression upon overexpression, likely via direct binding to a motif found in the promoter of these effector genes (171). *FTF1* has multiple homologs in *F. oxysporum*, and a reduction of mRNA levels of the entire gene family via silencing resulted in a slight reduction of virulence (116). Expression of effector genes in *F. oxysporum* requires the presence of Sge1 (Wor1 homolog), even in strains that overexpress *FTF1* (171). As in other fungi, deletion of *SGE1* results in complete loss of pathogenicity (108). Homologs of Wor1 regulate SM production and/or effector gene expression in fungi with different infection strategies, such as *Fusarium* species, *B. cinerea*, *M. oryzae*, and *U. maydis* (12, 20, 68, 106–109, 121, 134, 161). Other regulators of effector gene expression are Vta2, the Con7 homolog of *V. dahliae*, which also affects expression of a *PTH11*-like gene, Pf2 in *A. brassicicola*, and the APSES TF StuA in *L. maculans* and *S. nodorum* (21, 66, 149, 164). Rbf1, Biz1, Mzr1, Hdp2, and Cib1 play a role in effector gene expression in *U. maydis* (see below) (39, 55, 62, 201).

Mating in *Ustilago*

In *U. maydis*, pathogenicity is intimately linked to mating. When two haploid spores of opposite mating type land on the leaf surface and fuse, subsequent signaling leads to (*a*) expression of the

PRF1 TF gene, in which the TF Tup1 is involved, and (*b*) activation of Prf1 (36, 57, 104, 194). Prf1 then incudes expression of the b mating-type loci of both genomes, which together encode the bE/bW heterodimer. bE/bW is the transcriptional regulator that initiates filamentous growth and invasion into the plant. Disruption of the mating type response by deletion of *TUP1* or *PRF1* or production of pheromones (regulated by the TF Med1) results in loss of pathogenicity in wild-type strains (17, 36, 57). If the bE/bW heterodimer is expressed in a haploid strain, said strain is able to infect without mating (70).

Downstream of bE/bW, the TF Rbf1 takes care of a large portion of the bE/bW response; downstream of Rbf1, the TFs Biz1, Hdp1, and Hdp2 are activated (62). Hdp1 is involved in filamentation, and Rbf1, Biz1, and Hdp2 are each required for pathogenicity. From the b-dependent differentially expressed genes identified, two-thirds are induced by leaf surface cues, including all TF genes of the b-cascade (bE, bW, Rbf1, Hdp1, Hdp2, and Biz1) (62, 84). Biz1 and Hdp2 are required for the expression of effector genes, and Biz1 also collaborates with Mzr1, a TF that regulates expression of some of the *MIG2* effector genes (39, 84, 201). Interestingly, two of the downstream TFs, Biz1 and Hdp2, are Sho1- and Msb2-dependently expressed, and some of the downstream responses of Biz1 and Hdp2 after exposure to surface cues from the leaf also require the proposed surface sensors Msb2 and Sho1 (84).

Activation of the bE/bW dimer also results in cell cycle arrest. This arrest is relieved after the fungus has penetrated the leaf surface, and this relief requires Clp1 (Clampless 1), which is required for pathogenicity. The *CLP1* gene is upregulated by bE/bW, but production of the protein only starts after penetration of the plant surface and may require a signal from the plant (61). Clp1 interacts with bW and Rbf, and these protein complexes inhibit bE/bW signaling and the mating response and relieve the cell cycle arrest. Clp1 also interacts with Cib1, the *U. maydis* homolog of Hac1, which is the TF regulating the unfolded protein response (UPR) that is activated upon ER stress (61). The interaction between Clp1 and Cib1 stabilizes Clp1 and is required for resistance to ER stress–inducing agents. Deletion of Cib1 also results in loss of pathogenicity. The UPR is thought to be important for the secretion of effector proteins, as their strong upregulation may cause significant stress to the ER and the secretion machinery (60, 61). Additionally, Cib1 has been shown to bind to some effector promoters directly via UPR elements and induce their expression (55). In *A. brassicicola*, the homolog of Cib1 (AbHacA) is also required for pathogenicity (69).

Nitrogen Metabolism

For the production of SMs, the form of nitrogen available is an important cue, as reviewed by Tudzynski (166). Expression of some effector genes is also influenced by nitrogen or nitrogen-responsive TFs (159). Indeed, several regulators of nitrogen metabolism are required for virulence in different fungi. Nitrogen metabolism in fungi is regulated via nitrogen catabolite repression. In the presence of a preferred nitrogen source, such as NH_4^+ or glutamine (Gln), expression of genes required for assimilation of other nitrogen sources is repressed. For these metabolic pathways to be expressed, repression needs to be relieved by removal of the preferred nitrogen source and a pathway-specific regulator needs to be activated. The latter usually occurs after perception of the compound that can be assimilated through the particular pathway. For example, the nitrate reductase gene is only expressed in the absence of NH_4^+ or Gln and the presence of nitrate. The TFs that mediate repression in the presence of a preferred nitrogen source are AreA/Nit2 and AreB. The transcriptional repressors Nmr and MeaB are part of the AreA network. Nmr can repress AreA itself, and MeaB represses some of the SM clusters (166).

The requirement of nitrogen-responsive TFs in SM gene expression and virulence raises several questions: (*a*) What is the nitrogen status in the plant? (*b*) Do nitrogen regulators play a role

in transcriptional regulation of SM and effector genes in planta? (*c*) Is a requirement for SM production and/or effector gene expression the main reason for the virulence reduction caused by the absence of nitrogen regulators?

These questions are not so easy to answer, as illustrated by the effect of nitrogen source on effector gene expression in *Cladosporium fulvum*. Expression of several effector genes is influenced by nitrogen availability in different ways. In some cases, Nrf1, the *C. fulvum* AreA homolog, is required for expression. However, during infection all effector genes but one are expressed in an Nrf1-independent manner (159). Only *AVR9* requires Nrf1 for in planta expression, and for this the AreA boxes in the *AVR9* promoter are necessary, suggesting direct binding of Nrf1 to the *AVR9* promoter (144, 159). Even in the case of *AVR9*, however, it cannot be excluded that during infection, signals other than nitrogen availability feed into the Nrf1 regulatory network, leading to nitrogen-independent recruitment of Nrf1. *AVR9* deletion has no effect on pathogenicity, whereas deletion of Nrf1 leads to reduced virulence, suggesting that Nrf1 has more in planta targets than *AVR9*.

Nitrogen availability also affects intracellular levels of Gln, and high levels of intracellular Gln activate the TOR pathway, playing a role in regulation of the cell cycle and the autophagy response, which is known to be important for appressoria formation in *M. oryzae*, and represses certain SM gene clusters (94, 99, 156). Some TFs, such as MeaB and Asd4, which are required for virulence, are also connected to TOR signaling. Asd4 affects intracellular Gln levels and, as such, affects the TOR pathway (95, 100). Among the TFs required for virulence, we find homologs of Cpc1 in *F. fujikuroi*, *Verticillium* spp., and *L. maculans* (37, 139, 160). This TF is important for responding to amino acid starvation and is upregulated in *F. fujikuroi* upon deletion of the glutamine synthetase gene, which affects TOR signaling (139).

pH

Ambient pH can be an important factor in disease development, as some fungi acidify or alkalize plant tissue as part of their infection strategy. *Penicillium* spp., *B. cinerea*, and *Sclerotinia sclerotiorum* secrete organic acids that acidify the environment. *Colletotrichum* spp., *A. alternata*, and *F. oxysporum* secrete ammonia, which alkalizes the environment (30). *F. oxysporum* also produces rapid alkalization factors (RALFs), which trigger alkalization by plant cells (102).

The pH regulator PacC is required for virulence in several fungi. PacC is activated at ambient alkaline pH and then induces expression of alkaline-activated genes and represses genes expressed under acidic conditions (30). In *PacC* deletion mutants, lytic enzymes are often misregulated. Lower cellulase, cutinase, xylanase, pectate lyase, pectin lyase, polygalacturonase, and catalase activity has been reported (83, 110, 189, 196). An increase in polygalacturonase gene expression and pectinolytic activity has also been observed in *pacc* mutants (16, 189). In *F. graminearum* and *P. expansum*, production of the SMs DON and patulin, respectively, is reduced in the absence of PacC (5, 105).

Detoxification of Host Compounds

As mentioned above, the rapid production of ROS by plant cells, the so-called oxidative burst, is one of the earliest cellular responses following pathogen recognition (15). In line with this, several different TFs required for ROS tolerance have also been found to be required for virulence. Among these are Ap1, Skn7, Vta2, Crz1, Fug1, Nuc-2, and Spt3 (29, 48, 53, 91, 111, 129, 155, 164, 173, 187, 190, 197). Some of the mutants deleted for one of these TFs also have other defects, for example, in conidiation or SM production. Ap1 is the classical example of a TF that responds

to changes in oxidative state, as it is a redox sensor and localizes to the nucleus upon oxidation (9). Indeed, in several fungi, *ap1* deletion mutants are more sensitive to oxidative stress (e.g., the *bap1* deletion mutant in *B. cinerea*) (88, 112, 157). Challenging *B. cinerea* with H_2O_2 induces expression of Bap1-dependent ROS detoxification pathways. Surprisingly, two days post inoculation, when H_2O_2 is detectable in the infected leaves, the Bap1 target genes involved in ROS detoxification are not upregulated, and the *bap1* deletion mutant is not less virulent: The fungus apparently does not suffer H_2O_2-induced oxidative stress in planta. This questions the role of the oxidative burst in the infection process and also highlights the still poorly understood differences between growth in vitro and in planta (157).

Examples of detoxification of host compounds, other than ROS, are the production by *F. solani* f. sp. *pisii* of Pda1, a demethylase that detoxifies pisatin, a compound secreted by pea plants, and requires the TF Prf1. Deletion of *PRF1* results in loss of virulence (73). A similar case is Bdtf, a TF that is essential for detoxification of brassinin and is required for virulence of *A. brassicicola* (151).

Chromatin Remodeling

An interesting feature of many plant-pathogenic fungi is their bipartite genome. Housekeeping genes reside in conserved genomic regions, often referred to as the core genome, whereas conditionally dispensable genes, such as genes required only for infection, are subtelomeric or reside in accessory chromosomes or regions. These accessory regions are often characterized by a high repeat content and a different histone code. Effector genes and SM gene clusters are often found in such regions (97, 131, 150). Compartmentalization of conditionally dispensable genes may facilitate accelerated evolution of these parts of the genome, which is necessary to stay adapted to an evolving host (25, 26).

The chromatin state of accessory genomic regions may provide another level of transcriptional control for the genes encoded there. Indeed, epigenetic regulation of SM gene clusters and of effector genes has been demonstrated (148, 175). Also, some genes required for virulence have a role in chromatin remodeling. For the nitrogen catabolite repressor AreA and for LeaA, a methyltransferase thought to have an epigenetic control function and a regulator of secondary metabolism and pathogenicity in several fungi, chromatin-modifying activity has been reported (11, 136). Finally, deletion of *SPT3* and/or *SPT8* results in strongly reduced virulence in *F. graminearum* and *B. cinerea*. Spt3 and Spt8 are components of the SAGA complex, a chromatin-acetylating transcriptional coactivator with histone acetyl transferase activity. SAGA opens up chromatin, which can allow binding of additional TFs and the transcription preinitiation complex (43, 48).

CONCLUDING REMARKS

Some of the major findings regarding adaptation to the host environment by (nonobligate) plant-pathogenic fungi are summarized in **Figure 1**. Colonization of living plant tissue requires fungi to be able to respond to a combination of developmental and environmental cues by employing sensors, signal transduction pathways, and a multitude of TFs that regulate genes required for adaptation to the host environment. Sensing cues and responding appropriately are fine-tuned processes, as borne out by the fact that all plant-pathogenic fungi are host specific, albeit with variations in broadness. However, not every gene for which expression is activated upon host invasion is required for pathogenicity, and even some TFs that are required for gene expression in planta are not required for pathogenicity. For instance, upregulation of effector genes and SM gene clusters is a hallmark of the switch from a saprotrophic to an invasive lifestyle, but individual effector genes or SM clusters are not always essential for virulence, at least not under laboratory

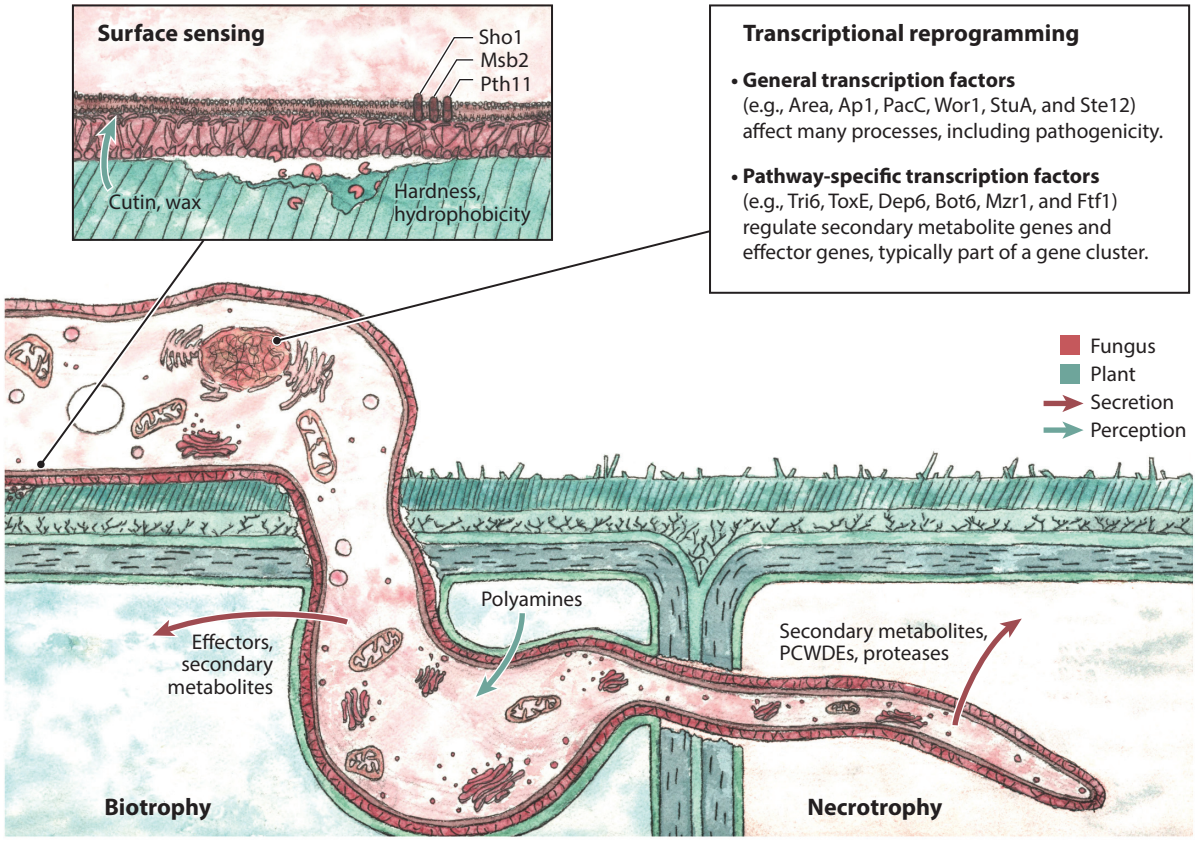

Figure 1

Adaptation to the host environment by plant-pathogenic fungi. This graphic represents a fungus that senses the plant surface, penetrates a plant cell, develops a biotrophic hypha, invades the next cell, and there switches to necrotrophic growth. Sho1, Msb2, and Pth11 are putative sensors of host signals. Abbreviation: PCWDEs, plant cell wall–degrading enzymes.

conditions. Strict assessment of the requirement of a gene for fitness, such as competition assays between mutant and wild-type strains in natural or agricultural settings, may still reveal roles for such genes.

It is also clear that there is no class of genes that can be considered to be unique for pathogenic fungi. All fungi have conserved systems to sense cues from the environment, to transduce signals, and to activate transcription, and all secrete enzymes, small proteins, and SMs. It appears that the key to pathogenicity lies in expanding the diversity of, for example, secreted proteins and by adapting conserved systems, such as a pheromone sensor (168) or a TF conserved in all ascomycetes (108), to respond to the host environment. This explains why most TFs required for virulence also have other functions. There are a few TFs that do seem to have no other important functions besides being required for pathogenicity (22, 34, 65, 116, 192). Further research is required to see whether these are really exclusively dedicated to the ability to colonize a living plant.

The study of the role of TFs required for host colonization is difficult because a mutant in such a TF cannot be studied during infection. The surprising effect of Nrf1 on effector expression in vitro and in planta and the role of Bap1 in ROS detoxification in vitro and in planta, as discussed above,

underline the importance of testing ideas in an infection situation. Conditional expression of TFs can be a solution to this problem, but the technological requirements for conditional expression are not always easy to meet and we know of no example of this in literature. Another option to study the role of TFs required for pathogenicity during infection is the use of temperature sensitive alleles, which has been used successfully to study the role of bE/bW during infectious growth of *U. maydis* in maize (177).

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Machiel Beijaert for providing the artwork for **Figure 1**.

LITERATURE CITED

1. Ahn JH, Walton JD. 1998. Regulation of cyclic peptide biosynthesis and pathogenicity in *Cochliobolus carbonum* by TOXEp, a novel protein with a bZIP basic DNA-binding motif and four ankyrin repeats. *Mol. Gen. Genet.* 260:462–69
2. Akiyama K, Hayashi H. 2006. Strigolactones: chemical signals for fungal symbionts and parasitic weeds in plant roots. *Ann. Bot.* 97:925–31
3. Alkan N, Friedlander G, Ment D, Prusky D, Fluhr R. 2015. Simultaneous transcriptome analysis of *Colletotrichum gloeosporioides* and tomato fruit pathosystem reveals novel fungal pathogenicity and fruit defense strategies. *New Phytol.* 205:801–15
4. Bajar A, Podila GK, Kolattukudy PE. 1991. Identification of a fungal cutinase promoter that is inducible by a plant signal via a phosphorylated *trans*-acting factor. *PNAS* 88:8208–12
5. Barad S, Horowitz SB, Kobiler I, Sherman A, Prusky D. 2014. Accumulation of the mycotoxin patulin in the presence of gluconic acid contributes to pathogenicity of *Penicillium expansum*. *Mol. Plant-Microbe Interact.* 27:66–77
6. Barad S, Sela N, Kumar D, Kumar-Dubey A, Glam-Matana N, et al. 2016. Fungal and host transcriptome analysis of pH-regulated genes during colonization of apple fruits by *Penicillium expansum*. *BMC Genom.* 17:330
7. Boedi S, Berger H, Sieber C, Munsterkotter M, Maloku I, et al. 2016. Comparison of *Fusarium graminearum* transcriptomes on living or dead wheat differentiates substrate-responsive and defense-responsive genes. *Front. Microbiol.* 7:1113
8. Boenisch MJ, Schafer W. 2011. *Fusarium graminearum* forms mycotoxin producing infection structures on wheat. *BMC Plant Biol.* 11:110
9. Boronat S, Domenech A, Paulo E, Calvo IA, Garcia-Santamarina S, et al. 2014. Thiol-based H_2O_2 signalling in microbial systems. *Redox Biol.* 2:395–99
10. Bradshaw RE, Guo Y, Sim AD, Kabir MS, Chettri P, et al. 2016. Genome-wide gene expression dynamics of the fungal pathogen *Dothistroma septosporum* throughout its infection cycle of the gymnosperm host *Pinus radiata*. *Mol. Plant Pathol.* 17:210–24
11. Brakhage AA. 2013. Regulation of fungal secondary metabolism. *Nat. Rev. Microbiol.* 11:21–32
12. Brown DW, Busman M, Proctor RH. 2014. *Fusarium verticillioides* SGE1 is required for full virulence and regulates expression of protein effector and secondary metabolite biosynthetic genes. *Mol. Plant-Microbe Interact.* 27:809–23
13. Brown DW, Butchko RA, Busman M, Proctor RH. 2007. The *Fusarium verticillioides FUM* gene cluster encodes a Zn(II)2Cys6 protein that affects *FUM* gene expression and fumonisin production. *Eukaryot. Cell* 6:1210–18

14. Buschhaus C, Jetter R. 2011. Composition differences between epicuticular and intracuticular wax substructures: How do plants seal their epidermal surfaces? *J. Exp. Bot.* 62:841–53
15. Camejo D, Guzman-Cedeno A, Moreno A. 2016. Reactive oxygen species, essential molecules, during plant-pathogen interactions. *Plant Physiol. Biochem.* 103:10–23
16. Caracuel Z, Roncero MI, Espeso EA, Gonzalez-Verdejo CI, Garcia-Maceira FI, Di Pietro A. 2003. The pH signalling transcription factor PacC controls virulence in the plant pathogen *Fusarium oxysporum*. *Mol. Microbiol.* 48:765–79
17. Chacko N, Gold S. 2012. Deletion of the *Ustilago maydis* ortholog of the *Aspergillus* sporulation regulator medA affects mating and virulence through pheromone response. *Fungal Genet. Biol.* 49:426–32
18. Chen Y, Le X, Sun Y, Li M, Zhang H, et al. 2016. MoYcp4 is required for growth, conidiogenesis and pathogenicity in *Magnaporthe oryzae*. *Mol. Plant Pathol.* **https://doi.org/10.1111/mpp.12455**
19. Chen Y, Zhai S, Sun Y, Li M, Dong Y, et al. 2015. MoTup1 is required for growth, conidiogenesis and pathogenicity of *Magnaporthe oryzae*. *Mol. Plant Pathol.* 16:799–810
20. Chen Y, Zhai S, Zhang H, Zuo R, Wang J, et al. 2014. Shared and distinct functions of two Gti1/Pac2 family proteins in growth, morphogenesis and pathogenicity of *Magnaporthe oryzae*. *Environ. Microbiol.* 16:788–801
21. Cho Y, Ohm RA, Grigoriev IV, Srivastava A. 2013. Fungal-specific transcription factor AbPf2 activates pathogenicity in *Alternaria brassicicola*. *Plant J.* 75:498–514
22. Cho Y, Srivastava A, Ohm RA, Lawrence CB, Wang KH, et al. 2012. Transcription factor Amr1 induces melanin biosynthesis and suppresses virulence in *Alternaria brassicicola*. *PLOS Pathog.* 8:e1002974
23. Chung H, Choi J, Park SY, Jeon J, Lee YH. 2013. Two conidiation-related Zn(II)2Cys6 transcription factor genes in the rice blast fungus. *Fungal Genet. Biol.* 61:133–41
24. Consort. REX. 2016. Combining selective pressures to enhance the durability of disease resistance genes. *Front. Plant Sci.* 7:1916
25. Croll D, McDonald BA. 2012. The accessory genome as a cradle for adaptive evolution in pathogens. *PLOS Pathog*. 8:e1002608
26. Croll D, Zala M, McDonald BA. 2013. Breakage-fusion-bridge cycles and large insertions contribute to the rapid evolution of accessory chromosomes in a fungal pathogen. *PLOS Genet*. 9:e1003567
27. Cuzick A, Urban M, Hammond-Kosack K. 2008. *Fusarium graminearum* gene deletion mutants *map1* and *tri5* reveal similarities and differences in the pathogenicity requirements to cause disease on *Arabidopsis* and wheat floral tissue. *New Phytol*. 177:990–1000
28. Dalmais B, Schumacher J, Moraga J, Le Pêcheur P, Tudzynski B, et al. 2011. The *Botrytis cinerea* phytotoxin botcinic acid requires two polyketide synthases for production and has a redundant role in virulence with botrydial. *Mol. Plant Pathol.* 12:564–79
29. Deng S, Wang CY, Zhang X, Wang Q, Lin L. 2015. VdNUC-2, the key regulator of phosphate responsive signaling pathway, is required for *Verticillium dahliae* infection. *PLOS ONE* 10:e0145190
30. Denison SH. 2000. pH regulation of gene expression in fungi. *Fungal Genet. Biol.* 29:61–71
31. Desjardins AE, Proctor RH. 2007. Molecular biology of *Fusarium* mycotoxins. *Int. J. Food Microbiol.* 119:47–50
32. DeZwaan TM, Carroll AM, Valent B, Sweigard JA. 1999. *Magnaporthe grisea* pth11p is a novel plasma membrane protein that mediates appressorium differentiation in response to inductive substrate cues. *Plant Cell* 11:2013–30
33. Doehlemann G, Hemetsberger C. 2013. Apoplastic immunity and its suppression by filamentous plant pathogens. *New Phytol*. 198:1001–16
34. Dufresne M, Perfect S, Pellier AL, Bailey JA, Langin T. 2000. A GAL4-like protein is involved in the switch between biotrophic and necrotrophic phases of the infection process of *Colletotrichum lindemuthianum* on common bean. *Plant Cell* 12:1579–90
35. Dunn MF, Ramirez-Trujillo JA, Hernandez-Lucas I. 2009. Major roles of isocitrate lyase and malate synthase in bacterial and fungal pathogenesis. *Microbiology* 155:3166–75
36. Elias-Villalobos A, Fernandez-Alvarez A, Ibeas JI. 2011. The general transcriptional repressor Tup1 is required for dimorphism and virulence in a fungal plant pathogen. *PLOS Pathog*. 7:e1002235

37. Elliott CE, Fox EM, Jarvis RS, Howlett BJ. 2011. The cross-pathway control system regulates production of the secondary metabolite toxin, sirodesmin PL, in the ascomycete, *Leptosphaeria maculans. BMC Microbiol.* 11:169
38. Fisher MC, Gow NA, Gurr SJ. 2016. Tackling emerging fungal threats to animal health, food security and ecosystem resilience. *Philos. Trans. R. Soc. Lond. Ser. B* 371:20160332
39. Flor-Parra I, Vranes M, Kamper J, Perez-Martin J. 2006. Biz1, a zinc finger protein required for plant invasion by *Ustilago maydis*, regulates the levels of a mitotic cyclin. *Plant Cell* 18:2369–87
40. Friesen TL, Faris JD, Solomon PS, Oliver RP. 2008. Host-specific toxins: effectors of necrotrophic pathogenicity. *Cell. Microbiol.* 10:1421–28
41. Fudal I, Collemare J, Bohnert HU, Melayah D, Lebrun MH. 2007. Expression of *Magnaporthe grisea* avirulence gene *ACE1* is connected to the initiation of appressorium-mediated penetration. *Eukaryot. Cell* 6:546–54
42. Gan P, Ikeda K, Irieda H, Narusaka M, O'Connell RJ, et al. 2013. Comparative genomic and transcriptomic analyses reveal the hemibiotrophic stage shift of *Colletotrichum* fungi. *New Phytol.* 197:1236–49
43. Gao T, Zheng Z, Hou Y, Zhou M. 2014. Transcription factors spt3 and spt8 are associated with conidiation, mycelium growth, and pathogenicity in *Fusarium graminearum. FEMS Microbiol. Lett.* 351:42–50
44. Garcia-Garrido JM, Lendzemo V, Castellanos-Morales V, Steinkellner S, Vierheilig H. 2009. Strigolactones, signals for parasitic plants and arbuscular mycorrhizal fungi. *Mycorrhiza* 19:449–59
45. Gardiner DM, Kazan K, Manners JM. 2009. Nutrient profiling reveals potent inducers of trichothecene biosynthesis in *Fusarium graminearum. Fungal Genet. Biol.* 46:604–13
46. Gardiner DM, Kazan K, Praud S, Torney FJ, Rusu A, Manners JM. 2010. Early activation of wheat polyamine biosynthesis during Fusarium head blight implicates putrescine as an inducer of trichothecene mycotoxin production. *BMC Plant Biol.* 10:289
47. Gervais J, Plissonneau C, Linglin J, Meyer M, Labadie K, et al. 2016. Different waves of effector genes with contrasted genomic location are expressed by *Leptosphaeria maculans* during cotyledon and stem colonization of oilseed rape. *Mol. Plant Pathol.* **https://doi.org/10.1111/mpp.12464**
48. Giesbert S, Schumacher J, Kupas V, Espino J, Segmuller N, et al. 2012. Identification of pathogenesis-associated genes by T-DNA-mediated insertional mutagenesis in *Botrytis cinerea*: a type 2A phosphoprotein phosphatase and an SPT3 transcription factor have significant impact on virulence. *Mol. Plant-Microbe Interact.* 25:481–95
49. Giese H, Sondergaard TE, Sorensen JL. 2013. The AreA transcription factor in *Fusarium graminearum* regulates the use of some nonpreferred nitrogen sources and secondary metabolite production. *Fungal Biol.* 117:814–21
50. Gijzen M, Nurnberger T. 2006. Nep1-like proteins from plant pathogens: recruitment and diversification of the NPP1 domain across taxa. *Phytochemistry* 67:1800–7
51. Gronover CS, Schumacher J, Hantsch P, Tudzynski B. 2005. A novel seven-helix transmembrane protein BTP1 of *Botrytis cinerea* controls the expression of GST-encoding genes, but is not essential for pathogenicity. *Mol. Plant Pathol.* 6:243–56
52. Gu SQ, Li P, Wu M, Hao ZM, Gong XD, et al. 2014. StSTE12 is required for the pathogenicity of *Setosphaeria turcica* by regulating appressorium development and penetration. *Microbiol. Res.* 169:817–23
53. Guo M, Chen Y, Du Y, Dong Y, Guo W, et al. 2011. The bZIP transcription factor MoAP1 mediates the oxidative stress response and is critical for pathogenicity of the rice blast fungus *Magnaporthe oryzae*. *PLOS Pathog.* 7:e1001302
54. Haddadi P, Ma L, Wang H, Borhan MH. 2016. Genome-wide transcriptomic analyses provide insights into the lifestyle transition and effector repertoire of *Leptosphaeria maculans* during the colonization of *Brassica napus* seedlings. *Mol. Plant Pathol.* 17:1196–210
55. Hampel M, Jakobi M, Schmitz L, Meyer U, Finkernagel F, et al. 2016. Unfolded protein response (UPR) regulator Cib1 controls expression of genes encoding secreted virulence factors in *Ustilago maydis*. *PLOS ONE* 11:e0153861
56. Harris LJ, Balcerzak M, Johnston A, Schneiderman D, Ouellet T. 2016. Host-preferential *Fusarium graminearum* gene expression during infection of wheat, barley, and maize. *Fungal Biol.* 120:111–23

57. Hartmann HA, Kruger J, Lottspeich F, Kahmann R. 1999. Environmental signals controlling sexual development of the corn smut fungus *Ustilago maydis* through the transcriptional regulator Prf1. *Plant Cell* 11:1293–306
58. Hassan S, Mathesius U. 2012. The role of flavonoids in root-rhizosphere signalling: opportunities and challenges for improving plant-microbe interactions. *J. Exp. Bot.* 63:3429–44
59. Hawkins AR, Lamb HK, Moore JD, Charles IG, Roberts CF. 1993. The pre-chorismate (shikimate) and quinate pathways in filamentous fungi: theoretical and practical aspects. *J. Gen. Microbiol.* 139:2891–99
60. Heimel K, Freitag J, Hampel M, Ast J, Bolker M, Kamper J. 2013. Crosstalk between the unfolded protein response and pathways that regulate pathogenic development in *Ustilago maydis*. *Plant Cell* 25:4262–77
61. Heimel K, Scherer M, Schuler D, Kamper J. 2010. The *Ustilago maydis* Clp1 protein orchestrates pheromone and b-dependent signaling pathways to coordinate the cell cycle and pathogenic development. *Plant Cell* 22:2908–22
62. Heimel K, Scherer M, Vranes M, Wahl R, Pothiratana C, et al. 2010. The transcription factor Rbf1 is the master regulator for b-mating type controlled pathogenic development in *Ustilago maydis*. *PLOS Pathog.* 6:e1001035
63. Hemetsberger C, Herrberger C, Zechmann B, Hillmer M, Doehlemann G. 2012. The *Ustilago maydis* effector Pep1 suppresses plant immunity by inhibition of host peroxidase activity. *PLOS Pathog.* 8:e1002684
64. Hou R, Jiang C, Zheng Q, Wang C, Xu JR. 2015. The AreA transcription factor mediates the regulation of deoxynivalenol (DON) synthesis by ammonium and cyclic adenosine monophosphate (cAMP) signalling in *Fusarium graminearum*. *Mol. Plant Pathol.* 16:987–99
65. Imazaki I, Kurahashi M, Iida Y, Tsuge T. 2007. Fow2, a Zn(II)2Cys6-type transcription regulator, controls plant infection of the vascular wilt fungus *Fusarium oxysporum*. *Mol. Microbiol.* 63:737–53
66. IpCho SVS, Tan KC, Koh G, Gummer J, Oliver RP, et al. 2010. The transcription factor StuA regulates central carbon metabolism, mycotoxin production, and effector gene expression in the wheat pathogen *Stagonospora nodorum*. *Eukaryot. Cell* 9:1100–8
67. Jiang C, Zhang S, Zhang Q, Tao Y, Wang C, Xu JR. 2015. FgSKN7 and FgATF1 have overlapping functions in ascosporogenesis, pathogenesis and stress responses in *Fusarium graminearum*. *Environ. Microbiol.* 17:1245–60
68. Jonkers W, Dong Y, Broz K, Kistler HC. 2012. The Wor1-like protein Fgp1 regulates pathogenicity, toxin synthesis and reproduction in the phytopathogenic fungus *Fusarium graminearum*. *PLOS Pathog.* 8:e1002724
69. Joubert A, Simoneau P, Campion C, Bataille-Simoneau N, Iacomi-Vasilescu B, et al. 2011. Impact of the unfolded protein response on the pathogenicity of the necrotrophic fungus *Alternaria brassicicola*. *Mol. Microbiol.* 79:1305–24
70. Kamper J, Kahmann R, Bolker M, Ma LJ, Brefort T, et al. 2006. Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature* 444:97–101
71. Kawahara Y, Oono Y, Kanamori H, Matsumoto T, Itoh T, Minami E. 2012. Simultaneous RNA-seq analysis of a mixed transcriptome of rice and blast fungus interaction. *PLOS ONE* 7:e49423
72. Kellner R, Bhattacharyya A, Poppe S, Hsu TY, Brem RB, Stukenbrock EH. 2014. Expression profiling of the wheat pathogen *Zymoseptoria tritici* reveals genomic patterns of transcription and host-specific regulatory programs. *Genome Biol. Evol.* 6:1353–65
73. Khan R, Tan R, Mariscal AG, Straney D. 2003. A binuclear zinc transcription factor binds the host isoflavonoid-responsive element in a fungal cytochrome p450 gene responsible for detoxification. *Mol. Microbiol.* 49:117–30
74. Kim H, Woloshuk CP. 2008. Role of AREA, a regulator of nitrogen metabolism, during colonization of maize kernels and fumonisin biosynthesis in *Fusarium verticillioides*. *Fungal Genet. Biol.* 45:947–53
75. Kim S, Park SY, Kim KS, Rho HS, Chi MH, et al. 2009. Homeobox transcription factors are required for conidiation and appressorium development in the rice blast fungus *Magnaporthe oryzae*. *PLOS Genet.* 5:e1000757
76. Kim Y, Kim H, Son H, Choi GJ, Kim JC, Lee YW. 2014. MYT3, a Myb-like transcription factor, affects fungal development and pathogenicity of *Fusarium graminearum*. *PLOS ONE* 9:e94359

77. Kleemann J, Rincon-Rivera LJ, Takahara H, Neumann U, Ver Loren van Themaat E, et al. 2012. Sequential delivery of host-induced virulence effectors by appressoria and intracellular hyphae of the phytopathogen *Colletotrichum higginsianum*. *PLOS Pathog.* 8:e1002643
78. Koeck M, Hardham AR, Dodds PN. 2011. The role of effectors of biotrophic and hemibiotrophic fungi in infection. *Cell. Microbiol.* 13:1849–57
79. Kou Y, Tan YH, Ramanujam R, Naqvi NI. 2017. Structure-function analyses of the Pth11 receptor reveal an important role for CFEM motif and redox regulation in rice blast. *New Phytol.* 214:330–42
80. Kubicek CP, Starr TL, Glass NL. 2014. Plant cell wall–degrading enzymes and their secretion in plant-pathogenic fungi. *Annu. Rev. Phytopathol.* 52:427–51
81. Kulkarni RD, Kelkar HS, Dean RA. 2003. An eight-cysteine-containing CFEM domain unique to a group of fungal membrane proteins. *Trends Biochem. Sci.* 28:118–21
82. Kulkarni RD, Thon MR, Pan H, Dean RA. 2005. Novel G-protein-coupled receptor-like proteins in the plant pathogenic fungus *Magnaporthe grisea*. *Genome Biol.* 6:R24
83. Landraud P, Chuzeville S, Billon-Grande G, Poussereau N, Bruel C. 2013. Adaptation to pH and role of PacC in the rice blast fungus *Magnaporthe oryzae*. *PLOS ONE* 8:e69236
84. Lanver D, Berndt P, Tollot M, Naik V, Vranes M, et al. 2014. Plant surface cues prime *Ustilago maydis* for biotrophic development. *PLOS Pathog.* 10:e1004272
85. Lanver D, Mendoza-Mendoza A, Brachmann A, Kahmann R. 2010. Sho1 and Msb2-related proteins regulate appressorium development in the smut fungus *Ustilago maydis*. *Plant Cell* 22:2085–101
86. Lee SJ, Rose JK. 2010. Mediation of the transition from biotrophy to necrotrophy in hemibiotrophic plant pathogens by secreted effector proteins. *Plant Signal. Behav.* 5:769–72
87. Leroch M, Mueller N, Hinsenkamp I, Hahn M. 2015. The signalling mucin Msb2 regulates surface sensing and host penetration via BMP1 MAP kinase signalling in *Botrytis cinerea*. *Mol. Plant Pathol.* 16:787–98
88. Lev S, Hadar R, Amedeo P, Baker SE, Yoder OC, Horwitz BA. 2005. Activation of an AP1-like transcription factor of the maize pathogen *Cochliobolus heterostrophus* in response to oxidative stress and plant signals. *Eukaryot. Cell* 4:443–54
89. Li D, Kolattukudy PE. 1995. Cloning and expression of cDNA encoding a protein that binds a palindromic promoter element essential for induction of fungal cutinase by plant cutin. *J. Biol. Chem.* 270:11753–56
90. Li D, Sirakova T, Rogers L, Ettinger WF, Kolattukudy PE. 2002. Regulation of constitutively expressed and induced cutinase genes by different zinc finger transcription factors in *Fusarium solani* f. sp. *pisi* (*Nectria haematococca*). *J. Biol. Chem.* 277:7905–12
91. Lin CH, Yang SL, Chung KR. 2009. The YAP1 homolog-mediated oxidative stress tolerance is crucial for pathogenicity of the necrotrophic fungus *Alternaria alternata* in citrus. *Mol. Plant-Microbe Interact.* 22:942–52
92. Liu N, Fan F, Qiu D, Jiang L. 2013. The transcription cofactor FgSwi6 plays a role in growth and development, carbendazim sensitivity, cellulose utilization, lithium tolerance, deoxynivalenol production and virulence in the filamentous fungus *Fusarium graminearum*. *Fungal Genet. Biol.* 58–59:42–52
93. Liu W, Zhou X, Li G, Li L, Kong L, et al. 2011. Multiple plant surface signals are sensed by different mechanisms in the rice blast fungus for appressorium formation. *PLOS Pathog.* 7:e1001261
94. Loewith R, Hall MN. 2011. Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* 189:1177–201
95. Lopez-Berges MS, Rispail N, Prados-Rosales RC, Di Pietro A. 2010. A nitrogen response pathway regulates virulence functions in *Fusarium oxysporum* via the protein kinase TOR and the bZIP protein MeaB. *Plant Cell* 22:2459–75
96. Lysoe E, Seong KY, Kistler HC. 2011. The transcriptome of *Fusarium graminearum* during the infection of wheat. *Mol. Plant-Microbe Interact.* 24:995–1000
97. Ma LJ, van der Does HC, Borkovich KA, Coleman JJ, Daboussi MJ, et al. 2010. Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* 464:367–73
98. Maier FJ, Miedaner T, Hadeler B, Felk A, Salomon S, et al. 2006. Involvement of trichothecenes in fusarioses of wheat, barley and maize evaluated by gene disruption of the trichodiene synthase (*Tri5*) gene in three field isolates of different chemotype and virulence. *Mol. Plant Pathol.* 7:449–61

99. Marroquin-Guzman M, Sun G, Wilson RA. 2017. Glucose-ABL1-TOR signaling modulates cell cycle tuning to control terminal appressorial cell differentiation. *PLOS Genet*. 13:e1006557
100. Marroquin-Guzman M, Wilson RA. 2015. GATA-dependent glutaminolysis drives appressorium formation in *Magnaporthe oryzae* by suppressing TOR inhibition of cAMP/PKA signaling. *PLOS Pathog*. 11:e1004851
101. Martin-Urdiroz M, Oses-Ruiz M, Ryder LS, Talbot NJ. 2016. Investigating the biology of plant infection by the rice blast fungus *Magnaporthe oryzae*. *Fungal Genet. Biol.* 90:61–68
102. Masachis S, Segorbe D, Turra D, Leon-Ruiz M, Furst U, et al. 2016. A fungal pathogen secretes plant alkalinizing peptides to increase infection. *Nat. Microbiol.* 1:16043
103. Mathioni SM, Belo A, Rizzo CJ, Dean RA, Donofrio NM. 2011. Transcriptome profiling of the rice blast fungus during invasive plant infection and in vitro stresses. *BMC Genom*. 12:49
104. Mendoza-Mendoza A, Eskova A, Weise C, Czajkowski R, Kahmann R. 2009. Hap2 regulates the pheromone response transcription factor prf1 in *Ustilago maydis*. *Mol. Microbiol.* 72:683–98
105. Merhej J, Richard-Forget F, Barreau C. 2011. The pH regulatory factor Pac1 regulates *Tri* gene expression and trichothecene production in *Fusarium graminearum*. *Fungal Genet. Biol.* 48:275–84
106. Michielse CB, Becker M, Heller J, Moraga J, Collado IG, Tudzynski P. 2011. The *Botrytis cinerea* Reg1 protein, a putative transcriptional regulator, is required for pathogenicity, conidiogenesis, and the production of secondary metabolites. *Mol. Plant-Microbe Interact.* 24:1074–85
107. Michielse CB, Studt L, Janevska S, Sieber CM, Arndt B, et al. 2015. The global regulator FfSge1 is required for expression of secondary metabolite gene clusters but not for pathogenicity in *Fusarium fujikuroi*. *Environ. Microbiol.* 17:2690–708
108. Michielse CB, van Wijk R, Reijnen L, Manders EM, Boas S, et al. 2009. The nuclear protein Sge1 of *Fusarium oxysporum* is required for parasitic growth. *PLOS Pathog*. 5:e1000637
109. Mirzadi Gohari A, Mehrabi R, Robert O, Ince IA, Boeren S, et al. 2014. Molecular characterization and functional analyses of ZtWor1, a transcriptional regulator of the fungal wheat pathogen *Zymoseptoria tritici*. *Mol. Plant Pathol.* 15:394–405
110. Miyara I, Shafran H, Kramer Haimovich H, Rollins J, Sherman A, Prusky D. 2008. Multi-factor regulation of pectate lyase secretion by *Colletotrichum gloeosporioides* pathogenic on avocado fruits. *Mol. Plant Pathol.* 9:281–91
111. Molina L, Kahmann R. 2007. An *Ustilago maydis* gene involved in H_2O_2 detoxification is required for virulence. *Plant Cell* 19:2293–309
112. Montibus M, Ducos C, Bonnin-Verdal MN, Bormann J, Ponts N, et al. 2013. The bZIP transcription factor Fgap1 mediates oxidative stress response and trichothecene biosynthesis but not virulence in *Fusarium graminearum*. *PLOS ONE* 8:e83377
113. Montibus M, Khosravi C, Zehraoui E, Verdal-Bonnin MN, Richard-Forget F, Barreau C. 2016. Is the Fgap1 mediated response to oxidative stress chemotype dependent in *Fusarium graminearum*? *FEMS Microbiol. Lett.* 363:fnv232
114. Nasmith CG, Walkowiak S, Wang L, Leung WW, Gong Y, et al. 2011. Tri6 is a global transcription regulator in the phytopathogen *Fusarium graminearum*. *PLOS Pathog.* 7:e1002266
115. Nejat N, Rookes J, Mantri NL, Cahill DM. 2017. Plant-pathogen interactions: toward development of next-generation disease-resistant plants. *Crit. Rev. Biotechnol.* 37:229–37
116. Nino-Sanchez J, Casado-Del Castillo V, Tello V, De Vega-Bartol JJ, Ramos B, et al. 2016. The FTF gene family regulates virulence and expression of SIX effectors in *Fusarium oxysporum*. *Mol. Plant Pathol.* 17:1124–39
117. O'Brien JA, Daudi A, Butt VS, Bolwell GP. 2012. Reactive oxygen species and their role in plant defence and cell wall metabolism. *Planta* 236:765–79
118. O'Connell RJ, Thon MR, Hacquard S, Amyotte SG, Kleemann J, et al. 2012. Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. *Nat. Genet.* 44:1060–65
119. Odenbach D, Breth B, Thines E, Weber RW, Anke H, Foster AJ. 2007. The transcription factor Con7p is a central regulator of infection-related morphogenesis in the rice blast fungus *Magnaporthe grisea*. *Mol. Microbiol.* 64:293–307

120. Oh M, Son H, Choi GJ, Lee C, Kim JC, et al. 2016. Transcription factor ART1 mediates starch hydrolysis and mycotoxin production in *Fusarium graminearum* and *F. verticillioides*. *Mol. Plant Pathol.* 17:755–68
121. Okmen B, Collemare J, Griffiths S, van der Burgt A, Cox R, de Wit PJ. 2014. Functional analysis of the conserved transcriptional regulator CfWor1 in *Cladosporium fulvum* reveals diverse roles in the virulence of plant pathogenic fungi. *Mol. Microbiol.* 92:10–27
122. Okmen B, Doehlemann G. 2014. Inside plant: biotrophic strategies to modulate host immunity and metabolism. *Curr. Opin. Plant Biol.* 20:19–25
123. Park SY, Choi J, Lim SE, Lee GW, Park J, et al. 2013. Global expression profiling of transcription factor genes provides new insights into pathogenicity and stress responses in the rice blast fungus. *PLOS Pathog.* 9:e1003350
124. Peplow AW, Tag AG, Garifullina GF, Beremand MN. 2003. Identification of new genes positively regulated by Tri10 and a regulatory network for trichothecene mycotoxin production. *Appl. Environ. Microbiol.* 69:2731–36
125. Perez-Nadales E, Di Pietro A. 2015. The transmembrane protein Sho1 cooperates with the mucin Msb2 to regulate invasive growth and plant infection in *Fusarium oxysporum*. *Mol. Plant Pathol.* 16:593–603
126. Pinto VE, Patriarca A. 2017. *Alternaria* species and their associated mycotoxins. *Methods Mol. Biol.* 1542:13–32
127. Porquier A, Morgant G, Moraga J, Dalmais B, Luyten I, et al. 2016. The botrydial biosynthetic gene cluster of *Botrytis cinerea* displays a bipartite genomic structure and is positively regulated by the putative Zn(II)2Cys6 transcription factor BcBot6. *Fungal Genet. Biol.* 96:33–46
128. Qutob D, Kemmerling B, Brunner F, Kufner I, Engelhardt S, et al. 2006. Phytotoxicity and innate immune responses induced by Nep1-like proteins. *Plant Cell* 18:3721–44
129. Ridenour JB, Bluhm BH. 2017. The novel fungal-specific gene *FUG1* has a role in pathogenicity and fumonisin biosynthesis in *Fusarium verticillioides*. *Mol. Plant Pathol.* 18(4):513–28
130. Rosler SM, Sieber CM, Humpf HU, Tudzynski B. 2016. Interplay between pathway-specific and global regulation of the fumonisin gene cluster in the rice pathogen *Fusarium fujikuroi*. *Appl. Microbiol. Biotechnol.* 100:5869–82
131. Rouxel T, Balesdent MH. 2017. Life, death and rebirth of avirulence effectors in a fungal pathogen of *Brassica* crops, *Leptosphaeria maculans*. *New Phytol.* 214(2):526–32
132. Rudd JJ, Kanyuka K, Hassani-Pak K, Derbyshire M, Andongabo A, et al. 2015. Transcriptome and metabolite profiling of the infection cycle of *Zymoseptoria tritici* on wheat reveals a biphasic interaction with plant immunity involving differential pathogen chromosomal contributions and a variation on the hemibiotrophic lifestyle definition. *Plant Physiol.* 167:1158–85
133. Ruiz-Roldan C, Pareja-Jaime Y, Gonzalez-Reyes JA, Roncero MI. 2015. The transcription factor Con7-1 is a master regulator of morphogenesis and virulence in *Fusarium oxysporum*. *Mol. Plant-Microbe Interact.* 28:55–68
134. Santhanam P, Thomma BP. 2013. *Verticillium dahliae* Sge1 differentially regulates expression of candidate effector genes. *Mol. Plant-Microbe Interact.* 26:249–56
135. Sbrana C, Giovannetti M. 2005. Chemotropism in the arbuscular mycorrhizal fungus *Glomus mosseae*. *Mycorrhiza* 15:539–45
136. Scazzocchio C. 2000. The fungal GATA factors. *Curr. Opin. Microbiol.* 3:126–31
137. Schilling L, Matei A, Redkar A, Walbot V, Doehlemann G. 2014. Virulence of the maize smut *Ustilago maydis* is shaped by organ-specific effectors. *Mol. Plant Pathol.* 15:780–89
138. Schmidt SM, Houterman PM, Schreiver I, Ma L, Amyotte S, et al. 2013. MITEs in the promoters of effector genes allow prediction of novel virulence genes in *Fusarium oxysporum*. *BMC Genom.* 14:119
139. Schonig B, Vogel S, Tudzynski B. 2009. Cpc1 mediates cross-pathway control independently of Mbf1 in *Fusarium fujikuroi*. *Fungal Genet. Biol.* 46:898–908
140. Selin C, de Kievit TR, Belmonte MF, Fernando WG. 2016. Elucidating the role of effectors in plant-fungal interactions: progress and challenges. *Front. Microbiol.* 7:600
141. Seong KY, Pasquali M, Zhou X, Song J, Hilburn K, et al. 2009. Global gene regulation by *Fusarium* transcription factors Tri6 and Tri10 reveals adaptations for toxin biosynthesis. *Mol. Microbiol.* 72:354–67

142. Simon A, Dalmais B, Morgant G, Viaud M. 2013. Screening of a *Botrytis cinerea* one-hybrid library reveals a Cys2His2 transcription factor involved in the regulation of secondary metabolism gene clusters. *Fungal Genet. Biol.* 52:9–19
143. Skibbe DS, Doehlemann G, Fernandes J, Walbot V. 2010. Maize tumors caused by *Ustilago maydis* require organ-specific genes in host and pathogen. *Science* 328:89–92
144. Snoeijers SS, Vossen P, Goosen T, Van den Broek HW, De Wit PJ. 1999. Transcription of the avirulence gene *Avr9* of the fungal tomato pathogen *Cladosporium fulvum* is regulated by a GATA-type transcription factor in *Aspergillus nidulans*. *Mol. Gen. Genet*. 261:653–59
145. Soanes DM, Chakrabarti A, Paszkiewicz KH, Dawe AL, Talbot NJ. 2012. Genome-wide transcriptional profiling of appressorium development by the rice blast fungus *Magnaporthe oryzae*. *PLOS Pathog.* 8:e1002514
146. Son H, Seo YS, Min K, Park AR, Lee J, et al. 2011. A phenome-based functional analysis of transcription factors in the cereal head blight fungus, *Fusarium graminearum*. *PLOS Pathog.* 7:e1002310
147. Sornkom W, Miki S, Takeuchi S, Abe A, Asano K, Sone T. 2016. Fluorescent reporter analysis revealed the timing and localization of AVR-Pia expression, an avirulence effector of *Magnaporthe oryzae*. *Mol. Plant Pathol.* **https://doi.org/10.1111/mpp.12468**
148. Soyer JL, El Ghalid M, Glaser N, Ollivier B, Linglin J, et al. 2014. Epigenetic control of effector gene expression in the plant pathogenic fungus *Leptosphaeria maculans*. *PLOS Genet.* 10:e1004227
149. Soyer JL, Hamiot A, Ollivier B, Balesdent MH, Rouxel T, Fudal I. 2015. The APSES transcription factor LmStuA is required for sporulation, pathogenic development and effector gene expression in *Leptosphaeria maculans*. *Mol. Plant Pathol.* 16:1000–5
150. Soyer JL, Moller M, Schotanus K, Connolly LR, Galazka JM, et al. 2015. Chromatin analyses of *Zymoseptoria tritici*: methods for chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq). *Fungal Genet. Biol.* 79:63–70
151. Srivastava A, Cho IK, Cho Y. 2013. The *Bdtf1* gene in *Alternaria brassicicola* is important in detoxifying brassinin and maintaining virulence on *Brassica* species. *Mol. Plant-Microbe Interact*. 26:1429–40
152. Straney D, Khan R, Tan R, Bagga S. 2002. Host recognition by pathogenic fungi through plant flavonoids. *Adv. Exp. Med. Biol.* 505:9–22
153. Straney D, Ruan Y, He J. 1994. In vitro transcription and binding analysis of promoter regulation by a host-specific signal in a phytopathogenic fungus. *Antonie Van Leeuwenhoek* 65:183–89
154. Sun D, Cao H, Shi Y, Huang P, Dong B, et al. 2016. The regulatory factor X protein MoRfx1 is required for development and pathogenicity in the rice blast fungus *Magnaporthe oryzae*. *Mol. Plant Pathol.* **https://doi.org/10.1111/mpp.12461**
155. Sun Y, Wang Y, Tian C. 2016. bZIP transcription factor CgAP1 is essential for oxidative stress tolerance and full virulence of the poplar anthracnose fungus *Colletotrichum gloeosporioides*. *Fungal Genet. Biol.* 95:58–66
156. Teichert S, Wottawa M, Schonig B, Tudzynski B. 2006. Role of the *Fusarium fujikuroi* TOR kinase in nitrogen regulation and secondary metabolism. *Eukaryot. Cell* 5:1807–19
157. Temme N, Tudzynski P. 2009. Does *Botrytis cinerea* ignore H_2O_2-induced oxidative stress during infection? Characterization of botrytis activator protein 1. *Mol. Plant-Microbe Interact.* 22:987–98
158. Thatcher LF, Williams AH, Garg G, Buck SG, Singh KB. 2016. Transcriptome analysis of the fungal pathogen *Fusarium oxysporum* f. sp. *medicaginis* during colonisation of resistant and susceptible *Medicago truncatula* hosts identifies differential pathogenicity profiles and novel candidate effectors. *BMC Genom*. 17:860
159. Thomma BP, Bolton MD, Clergeot PH, PJ DEW. 2006. Nitrogen controls in planta expression of *Cladosporium fulvum Avr9* but no other effector genes. *Mol. Plant Pathol.* 7:125–30
160. Timpner C, Braus-Stromeyer SA, Tran VT, Braus GH. 2013. The Cpc1 regulator of the cross-pathway control of amino acid biosynthesis is required for pathogenicity of the vascular pathogen *Verticillium longisporum*. *Mol. Plant-Microbe Interact.* 26:1312–24
161. Tollot M, Assmann D, Becker C, Altmuller J, Dutheil JY, et al. 2016. The WOPR protein Ros1 is a master regulator of sporogenesis and late effector gene expression in the maize pathogen *Ustilago maydis*. *PLOS Pathog*. 12:e1005697

162. Torres MF, Ghaffari N, Buiate EA, Moore N, Schwartz S, et al. 2016. A *Colletotrichum graminicola* mutant deficient in the establishment of biotrophy reveals early transcriptional events in the maize anthracnose disease interaction. *BMC Genom*. 17:202
163. Toruño TY, Stergiopoulos I, Coaker G. 2016. Plant-pathogen effectors: cellular probes interfering with plant defenses in spatial and temporal manners. *Annu. Rev. Phytopathol.* 54:419–41
164. Tran VT, Braus-Stromeyer SA, Kusch H, Reusche M, Kaever A, et al. 2014. *Verticillium* transcription activator of adhesion Vta2 suppresses microsclerotia formation and is required for systemic infection of plant roots. *New Phytol.* 202:565–81
165. Tsuge T, Harimoto Y, Akimitsu K, Ohtani K, Kodama M, et al. 2013. Host-selective toxins produced by the plant pathogenic fungus *Alternaria alternata*. *FEMS Microbiol. Rev.* 37:44–66
166. Tudzynski B. 2014. Nitrogen regulation of fungal secondary metabolism in fungi. *Front. Microbiol.* 5:656
167. Turra D, Di Pietro A. 2015. Chemotropic sensing in fungus-plant interactions. *Curr. Opin. Plant Biol.* 26:135–40
168. Turra D, El Ghalid M, Rossi F, Di Pietro A. 2015. Fungal pathogen uses sex pheromone receptor for chemotropic sensing of host plant signals. *Nature* 527:521–24
169. Umemura M, Koike H, Machida M. 2015. Motif-independent de novo detection of secondary metabolite gene clusters: toward identification from filamentous fungi. *Front. Microbiol.* 6:371
170. van der Does HC, Duyvesteijn RG, Goltstein PM, van Schie CC, Manders EM, et al. 2008. Expression of effector gene *SIX1* of *Fusarium oxysporum* requires living plant cells. *Fungal Genet. Biol.* 45:1257–64
171. van der Does HC, Fokkens L, Yang A, Schmidt SM, Langereis L, et al. 2016. Transcription factors encoded on core and accessory chromosomes of *Fusarium oxysporum* induce expression of effector genes. *PLOS Genet.* 12:e1006401
172. Van Nguyen T, Kroger C, Bonnighausen J, Schafer W, Bormann J. 2013. The ATF/CREB transcription factor Atf1 is essential for full virulence, deoxynivalenol production, and stress tolerance in the cereal pathogen *Fusarium graminearum*. *Mol. Plant-Microbe Interact.* 26:1378–94
173. Viefhues A, Schlathoelter I, Simon A, Viaud M, Tudzynski P. 2015. Unraveling the function of the response regulator BcSkn7 in the stress signaling network of *Botrytis cinerea*. *Eukaryot. Cell* 14:636–51
174. Vilanova L, Teixido N, Torres R, Usall J, Vinas I, Sanchez-Torres P. 2016. Relevance of the transcription factor PdSte12 in *Penicillium digitatum* conidiation and virulence during citrus fruit infection. *Int. J. Food Microbiol.* 235:93–102
175. Visentin I, Montis V, Doll K, Alabouvette C, Tamietti G, et al. 2012. Transcription of genes in the biosynthetic pathway for fumonisin mycotoxins is epigenetically and differentially regulated in the fungal maize pathogen *Fusarium verticillioides*. *Eukaryot. Cell* 11:252–59
176. Wahl R, Wippel K, Goos S, Kamper J, Sauer N. 2010. A novel high-affinity sucrose transporter is required for virulence of the plant pathogen *Ustilago maydis*. *PLOS Biol.* 8:e1000303
177. Wahl R, Zahiri A, Kamper J. 2010. The *Ustilago maydis* b mating type locus controls hyphal proliferation and expression of secreted virulence factors in planta. *Mol. Microbiol.* 75:208–20
178. Wang E, Norred WP, Bacon CW, Riley RT, Merrill AH Jr. 1991. Inhibition of sphingolipid biosynthesis by fumonisins. Implications for diseases associated with *Fusarium moniliforme*. *J. Biol. Chem.* 266:14486–90
179. Wang G, Li G, Zhang S, Jiang C, Qin J, Xu JR. 2015. Activation of the signalling mucin MoMsb2 and its functional relationship with Cbp1 in *Magnaporthe oryzae*. *Environ. Microbiol.* 17:2969–81
180. Wang M, Sun X, Yu D, Xu J, Chung K, Li H. 2016. Genomic and transcriptomic analyses of the tangerine pathotype of *Alternaria alternata* in response to oxidative stress. *Sci. Rep.* 6:32437
181. Wang Y, Liu W, Hou Z, Wang C, Zhou X, et al. 2011. A novel transcriptional factor important for pathogenesis and ascosporogenesis in *Fusarium graminearum*. *Mol. Plant-Microbe Interact.* 24:118–28
182. Wight WD, Kim KH, Lawrence CB, Walton JD. 2009. Biosynthesis and role in virulence of the histone deacetylase inhibitor depudecin from *Alternaria brassicicola*. *Mol. Plant-Microbe Interact.* 22:1258–67
183. Xiong D, Wang Y, Tian L, Tian C. 2016. MADS-box transcription factor VdMcm1 regulates conidiation, microsclerotia formation, pathogenicity, and secondary metabolism of *Verticillium dahliae*. *Front. Microbiol.* 7:1192

184. Yan X, Li Y, Yue X, Wang C, Que Y, et al. 2011. Two novel transcriptional regulators are essential for infection-related morphogenesis and pathogenicity of the rice blast fungus *Magnaporthe oryzae*. *PLOS Pathog.* 7:e1002385
185. Yang C, Liu H, Li G, Liu M, Yun Y, et al. 2015. The MADS-box transcription factor FgMcm1 regulates cell identity and fungal development in *Fusarium graminearum*. *Environ. Microbiol.* 17:2762–76
186. Yang J, Zhao X, Sun J, Kang Z, Ding S, et al. 2010. A novel protein Com1 is required for normal conidium morphology and full virulence in *Magnaporthe oryzae*. *Mol. Plant-Microbe Interact.* 23:112–23
187. Yang Q, Yin D, Yin Y, Cao Y, Ma Z. 2015. The response regulator BcSkn7 is required for vegetative differentiation and adaptation to oxidative and osmotic stresses in *Botrytis cinerea*. *Mol. Plant Pathol.* 16:276–87
188. You BJ, Choquer M, Chung KR. 2007. The *Colletotrichum acutatum* gene encoding a putative pH-responsive transcription regulator is a key virulence determinant during fungal pathogenesis on citrus. *Mol. Plant-Microbe Interact.* 20:1149–60
189. You BJ, Chung KR. 2007. Phenotypic characterization of mutants of the citrus pathogen *Colletotrichum acutatum* defective in a PacC-mediated pH regulatory pathway. *FEMS Microbiol. Lett.* 277:107–14
190. Yu PL, Wang CL, Chen PY, Lee MH. 2016. YAP1 homologue-mediated redox sensing is crucial for a successful infection by *Monilinia fructicola*. *Mol. Plant Pathol.* **https://doi.org/10.1111/mpp.12438**
191. Yue X, Que Y, Xu L, Deng S, Peng Y, et al. 2016. ZNF1 encodes a putative C2H2 zinc-finger protein essential for appressorium differentiation by the rice blast fungus *Magnaporthe oryzae*. *Mol. Plant-Microbe Interact.* 29:22–35
192. Zahiri A, Heimel K, Wahl R, Rath M, Kamper J. 2010. The *Ustilago maydis* forkhead transcription factor Fox1 is involved in the regulation of genes required for the attenuation of plant defenses during pathogenic development. *Mol. Plant-Microbe Interact.* 23:1118–29
193. Zaparoli G, Barsottini MR, de Oliveira JF, Dyszy F, Teixeira PJ, et al. 2011. The crystal structure of necrosis- and ethylene-inducing protein 2 from the causal agent of cacao's Witches' Broom disease reveals key elements for its activity. *Biochemistry* 50:9901–10
194. Zarnack K, Eichhorn H, Kahmann R, Feldbrugge M. 2008. Pheromone-regulated target genes respond differentially to MAPK phosphorylation of transcription factor Prf1. *Mol. Microbiol.* 69:1041–53
195. Zhan J, Thrall PH, Papaïx J, Xie L, Burdon JJ. 2015. Playing on a pathogen's weakness: using evolution to guide sustainable plant disease control strategies. *Annu. Rev. Phytopathol.* 53:19–43
196. Zhang T, Sun X, Xu Q, Candelas LG, Li H. 2013. The pH signaling transcription factor PacC is required for full virulence in *Penicillium digitatum*. *Appl. Microbiol. Biotechnol.* 97:9087–98
197. Zhang T, Xu Q, Sun X, Li H. 2013. The calcineurin-responsive transcription factor Crz1 is required for conidation, full virulence and DMI resistance in *Penicillium digitatum*. *Microbiol. Res.* 168:211–22
198. Zhang XW, Jia LJ, Zhang Y, Jiang G, Li X, et al. 2012. In planta stage-specific fungal gene profiling elucidates the molecular strategies of *Fusarium graminearum* growing inside wheat coleoptiles. *Plant Cell* 24:5159–76
199. Zhang Y, He J, Jia LJ, Yuan TL, Zhang D, et al. 2016. Cellular tracking and gene profiling of *Fusarium graminearum* during maize stalk rot disease development elucidates its strategies in confronting phosphorus limitation in the host apoplast. *PLOS Pathog.* 12:e1005485
200. Zhang Z, Li H, Qin G, He C, Li B, Tian S. 2016. The MADS-Box transcription factor Bcmads1 is required for growth, sclerotia production and pathogenicity of *Botrytis cinerea*. *Sci. Rep.* 6:33901
201. Zheng Y, Kief J, Auffarth K, Farfsing JW, Mahlert M, et al. 2008. The *Ustilago maydis* Cys2His2-type zinc finger transcription factor Mzr1 regulates fungal gene expression during the biotrophic growth stage. *Mol. Microbiol.* 68:1450–701
202. Zhou Z, Li G, Lin C, He C. 2009. Conidiophore stalk-less1 encodes a putative zinc-finger protein involved in the early stage of conidiation and mycelial infection in *Magnaporthe oryzae*. *Mol. Plant-Microbe Interact.* 22:402–10

Annual Review of Phytopathology

Volume 55, 2017

Contents

Errata

An online log of corrections to *Annual Review of Phytopathology* articles may be found at http://www.annualreviews.org/errata/phyto