

The Genome of *Botrytis cinerea*, a Ubiquitous Broad Host Range Necrotroph

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Abbreviations

SM	Secondary metabolism
TF	Transcription factor(s)

2.1 Introduction

The genus *Botrytis*, together with the well-known genera *Sclerotinia* and *Monilinia*, belongs to the family Sclerotiniaceae within the class Ascomycetes. The family Sclerotiniaceae represents a rather recently evolved fungal lineage, and is assumed to consist mainly of necrotrophic plant pathogens. These include the two prominent wide host range species *Botrytis cinerea* and *Sclerotinia sclerotiorum*, many host specialists, but also some species with biotrophic and endophytic lifestyles (Andrew et al. 2012; Shipunov et al. 2008). The genus *Botrytis* currently comprises roughly 25–30 species, all of which are considered to be necrotrophic plant pathogens. Since the inference of a sequenced-based phylogeny of 22 recognized species (Staats et al. 2005), several

new species have been reported: *B. pseudocinerea* (Walker et al. 2011), *B. caroliniana* (Li et al. 2012a, b), *B. fabiopsis* (Zhang et al. 2010a, b), *B. sinoallii* (Zhang et al. 2010a, b), *B. deweyae* (Grant-Downton et al. 2013). Additional *Botrytis* isolates have been described which are phylogenetically distinct from described species but remain unnamed (Shipunov et al. 2008). There is one hybrid, polyploid species, which arose from hybridization between *B. aclada* and *B. byssoides* (Nielsen and Yohalam 2001; Staats et al. 2005). *Botrytis* species were grouped into two clearly distinct phylogenetic clades. The major clade, which is divided into five subclades, contains mainly species with monocot hosts, but also a few dicot pathogens. The other clade contains five species, including *B. cinerea*, that only infect dicot hosts (Staats et al. 2005; Walker et al. 2011).

Botrytis cinerea Pers. Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) is by far the most thoroughly studied species, because of its broad host range (>200 species; Fig. 2.1a, b) and its huge economic impact, causing pre- and postharvest crop losses worldwide (Dean et al. 2012). Its ability to infect many different plant species and tissues under a wide range of environmental conditions, as well as the ability of sclerotia to survive in the soil, contribute to its

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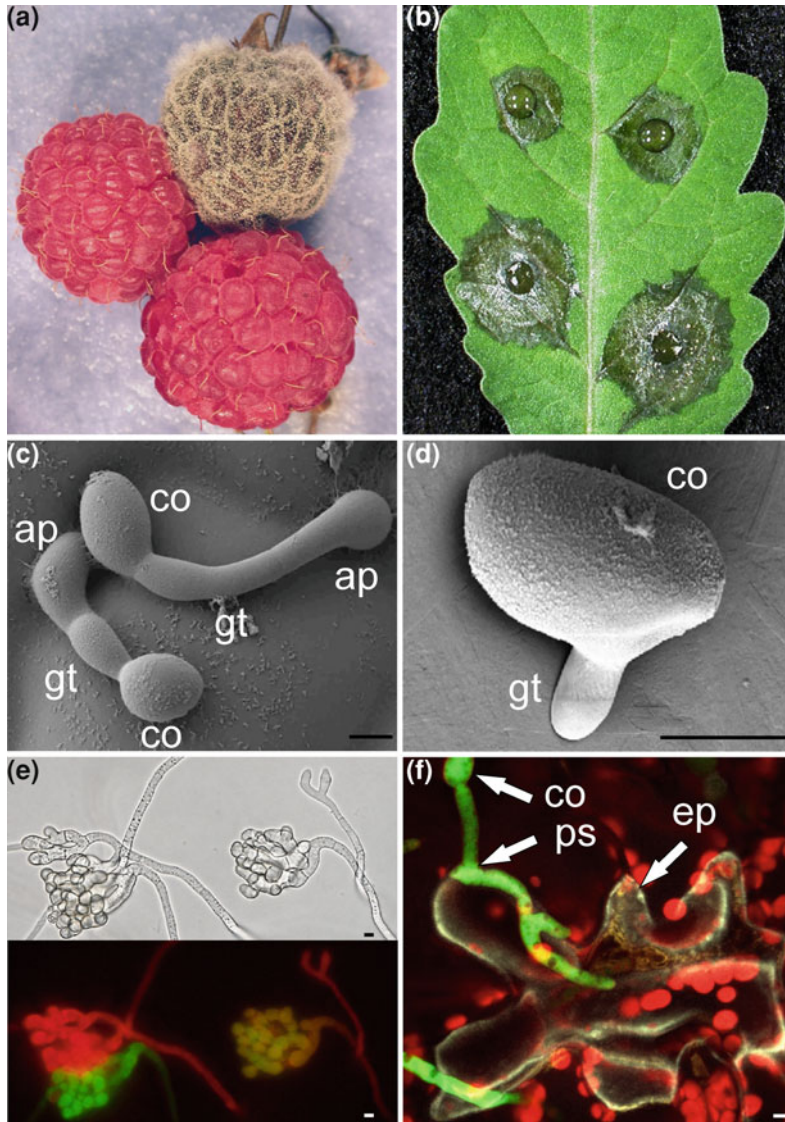


Fig. 2.1 The gray mold fungus *Botrytis cinerea*. **a** Sporulation on raspberry. **b** Expanding lesions on a detached tomato leaf, 72 h after inoculation with 10 μ l droplets containing 10^2 (top) and 10^3 (bottom) conidia. **c** Conidia (co) germinated in 10 mM fructose on a bean leaf surface, with germ tubes (gt) and appressoria (ap) (24 h). **d** Conidium germinated in water on polyethylene. A short germ tube is formed, with no apparent appressoria (48 h). **e** Complex appressoria (infection cushions), formed on a glass surface by a mixture of GFP and

mCherry expressing strains. On the right side, hyphal fusion resulted in hyphae expressing both fluorescent proteins. **f** GFP-marked hyphae during early invasion of *Arabidopsis* leaf cells. From a germinated conidium (co), a hypha penetrated (ps penetration site) into an epidermis cell (ep), which collapsed and showed cell wall autofluorescence. Red autofluorescence is from mesophyll cell chloroplasts. **c, d** Kindly provided by K. Mendgen. **e, f** Modified from Leroch et al. 2013a, with permission

persistent and widespread nature. Dispersal of *B. cinerea* is via airborne conidia, and to lesser extent via ascospores. Although fruiting bodies

(apothecia) are rarely observed, population genetics data suggest that sexual reproduction occurs frequently. The availability of molecular

tools has considerably advanced our understanding of the infection strategies of *B. cinerea*, which we will summarize in the next paragraph. In the remaining paragraphs, we will discuss several topics with special ‘-omics’ relevance.

2.2 Current Knowledge of *B. cinerea* Infection Strategies

Conidia, produced in large quantities in necrotized host tissue, are the main sources of infection by *B. cinerea*. In the presence of free water or high humidity (>93 % RH), germination is triggered by a combination of physical and chemical signals. While the mechanisms of signal perception are largely unknown yet, both a cAMP-dependent pathway and a MAP kinase cascade are involved in signal transduction leading to germination (Schumacher et al. 2008a; Schamber et al. 2010). On the host cuticle, the germlings form nonmelanized appressoria that are morphologically not always distinct, and not separated by a septum from the germ tube (Fig. 2.1c, d). Appressorium formation and function requires the interplay of many regulatory components, as exemplified by various kinds of penetration defects observed in mutants lacking heterotrimeric G protein components, adenylate cyclase, and either of the three MAP kinase cascades (Schumacher et al. 2008a, b, c; see Sect. 2.6.1). A specific role in appressorium-mediated penetration of *B. cinerea* and other plant pathogens could be assigned to the tetraspanin Pls1, a membrane-spanning regulatory protein (Gourgues et al. 2004, Siegmund et al. 2013). Besides appressoria, the fungus is able to form more complex penetration structures called infection cushions (Choquer et al. 2007). Because of their rather low degree of differentiation, the physical penetration ability of *B. cinerea* appressoria into intact host tissue seems to be limited, and probably needs to be supported by secreted lytic enzymes. Moreover, the need of a stable cell wall of the penetration structures may require the activity of specific chitin synthase isoenzymes, several of which were shown to be important for pathogenicity (Arbelet et al. 2010; Morcx et al. 2013).

Penetration is usually accompanied by rapid death of the host cells. *B. cinerea* secretes several toxic compounds that play a role in host killing. The toxic secondary metabolites (SM), botrydial and botcinic acid derivatives, will be described in Sect. 2.4.3. Hydrogen peroxide (H_2O_2) is released by the invading fungus into the host tissue (Tenberge 2004), presumably by an extracellular Cu–Zn superoxide dismutase which is important for virulence (Rolke et al. 2004). However, the situation is complicated by the production of defense-related H_2O_2 from the invaded host cell. Several secreted proteins have been studied for their role in pathogenesis. The role of necrosis-inducing proteins of the NLP family is discussed in Sect. 2.6.5. A xylanase, Xyn11A, was shown to be required for full virulence (Brito et al. 2006). This function was not related to its enzymatic activity, but rather to its necrosis-inducing activity which was retained in a catalytically-impaired Xyn11A variant (Noda et al. 2010). A cerato-platanin family protein, Spl1, was also found to contribute to full virulence. Purified Spl1 induces hypersensitive cell death and salicylic acid-mediated systemic acquired resistance (Frías et al. 2011, 2013). Plant wall degradation is another tool of necrotrophic fungi for efficient colonization of host tissue. Due to the redundancy of lytic enzymes, however, their role is experimentally difficult to assess. The two endopolygalacturonases, PG2 and PG1, have been shown to be involved in primary lesion formation and lesion outgrowth (ten Have et al. 1998; Kars et al. 2005a). In contrast, two pectin methylesterases (PME1 and PME2) were dispensable for pathogenesis, indicating that pectin demethylation is not a prerequisite for PG activity on the host cell wall, despite a previous report which indicated some importance for PME1 (Valette-Collet et al. 2003; Kars et al. 2005b). For cellulases, hemicellulases and proteases, no evidence for their role in the infection process has yet been obtained, although they contribute in decomposition of the killed host tissue.

While oxalic acid is an essential virulence factor for *S. sclerotiorum*, its role in *B. cinerea* is less prominent. A *B. cinerea* deletion mutant

lacking oxaloacetate acetyl hydrolase was unable to produce oxalic acid (Han et al. 2007). On several host plants, this mutant had no effect on virulence, but it was reduced in lesion outgrowth on *Arabidopsis* and cucumber leaves (van Kan unpublished). Other *B. cinerea* mutants have been described that were defective in oxalic acid production and impaired in virulence, but a causal relationship has not been proven (Kunz et al. 2006; Schumacher et al. 2012). Recent studies with *S. sclerotiorum* indicate that oxalic acid, on one hand promotes the apoptotic oxidative burst in the host plant that supports necrotrophic development, and on the other hand suppresses an early, defense-associated oxidative burst during the initial stages of infection (Williams et al. 2011). *B. cinerea* can infect a variety of host tissues that greatly differ in their pH values, and the fungus itself modulates the pH during infection (Manteau et al. 2003; Billon-Grand et al. 2010). Infection of sunflower cotyledons by *B. cinerea* was accompanied by a first phase of acidification and accumulation of several organic acids, and a second phase of alkalinization and ammonium formation in the colonized tissue (Billon-Grand et al. 2010). Because expression and activity of pectinolytic and proteolytic enzymes is pH dependent, *B. cinerea* seems to be able to release different sets of virulence factors, which illustrates its remarkable pathogenic plasticity.

According to a long-standing hypothesis, *B. cinerea* and other necrotrophic fungi induce the plant hypersensitive response (HR) as part of their infection strategy (Govrin and Levine 2000; van Baarlen et al. 2007; Williamson et al. 2007). This is supported by the observation that cell death suppression in transgenic tobacco expressing animal anti-apoptotic genes results in resistance to necrotrophic fungi (Dickman et al. 2001). The role of necrotrophic effector proteins in triggering host plant HR will be discussed in Sect. 2.6.5. How *B. cinerea* overcomes the strong defense reactions that accompany the HR, such as the release of reactive oxygen species (ROS), secretion of PR proteins and production of phytoalexins, is poorly understood. Surprisingly, a TF with a central role in ROS

detoxification, AP-1, was found to be not required for infection, which indicated that the fungus does not seem to suffer ROS stress during host invasion (Temme and Tudzynski, 2009). The situation is further complicated by the discovery that modulation of fungal apoptosis also plays a role in pathogenesis of *B. cinerea* (Shlezinger et al. 2011a).

Detoxification of host defense compounds has been demonstrated to be important for fungal pathogenesis. *B. cinerea* is capable of enzymatic conversion of the phytoalexin resveratrol into ϵ -viniferin by a substrate-inducible laccase, Lcc2. Surprisingly, loss of Lcc2 resulted in higher tolerance to resveratrol in vitro, because the mutant was unable to convert it to ϵ -viniferin which has a higher fungitoxicity than resveratrol (Schouten et al. 2002). Another detoxification mechanism is mediated by drug efflux transporters (De Waard et al. 2006). The *B. cinerea* ABC-type efflux transporter, AtrB, was found to lower the toxicity of camalexin, the major antifungal phytoalexin of *Arabidopsis*. *B. cinerea atrB* mutants are impaired in virulence on *Arabidopsis* wild type, but similarly aggressive as the wild type strain on the camalexin-deficient *Arabidopsis pad3* mutant (Stefanato et al. 2009). In addition to its role in infection, AtrB was found to be involved in a multidrug-resistance phenotype (MDR) in *B. cinerea*, which considerably reduces its sensitivity to several fungicides (see Sect. 2.5.3).

Being known as a devastating necrotroph, the discovery that *B. cinerea* is able to grow as an endophyte was surprising. In several host plants, such as *Lactuca* and *Primula* spp., the fungus has been found to grow in nonsymptomatic plant tissue (Sowley et al. 2010). A population genetic study indicated no differentiation between endophytic and necrotrophic forms of the fungus (Rajaguru and Shaw 2010). Another study recently demonstrated the occurrence of several new putative *Botrytis* spp. from a symptomless plant (Shipunov et al. 2008). It will be of great interest to understand the molecular mechanism of this 'hidden' life form, and transcriptomics would be a tool of choice to decipher the changes in regulatory pathways compared to the

necrotrophic lifestyle. A prerequisite for further investigation, however, would be the establishment of a controlled infection leading to endophytic growth.

2.3 Technical Advances in Molecular Research on *Botrytis*

In the pregenomic era, the identification of putative virulence genes was tedious, requiring their cloning from genomic or cDNA libraries usually based on heterologous hybridization, or by PCR using gene-specific, degenerate primers. This changed rapidly with the availability of the genome sequence, which allowed easy access to genes encoding protein families, such as aspartic peptidases (ten Have et al. 2010), chitin synthases (Morcx et al. 2013), or to proteins involved in a common regulatory process, such as the BMP1 MAP kinase cascade (see below) or regulators of fungal apoptosis (Shlezinger et al. 2011b). Transformation of *B. cinerea*, either via protoplasts or by using *Agrobacterium tumefaciens*, is a standard tool, but methods for functional analysis of multiple genes remain to be optimized. At present, only double or, with some effort, triple mutants can be generated in *B. cinerea*, due to the limitation of commonly available selection markers (for hygromycin, nourseothricin, and phleomycin resistance). For example, a double knock-out mutant lacking cutinase A and the cutinolytic lipase 1 has been constructed, and was found to be fully virulent on several hosts (Reis et al. 2005). This result, however, did not exclude a role of cutinases in penetration because the *B. cinerea* genome contains several additional genes encoding cutin degrading enzymes. In another study, a mutant lacking all three hydrophobin encoding genes was constructed, to evaluate their role in the formation of hydrophobic surface layers in conidia and hyphae. The triple mutant did not show any differences in the hydrophobicity of conidia and aerial hyphae, which led to the

conclusion that in *B. cinerea*, hydrophobins do not contribute to surface coating of these structures (Mosbach et al. 2011). For simultaneous mutational studies on multiple genes, serial gene replacement strategies by iterative use of a single resistance marker are now available for some filamentous fungi such as *Aspergillus fumigatus* (Krappmann et al. 2005), but remain to be developed for *B. cinerea*. Another strategy for targeting multiple genes, simultaneous RNA silencing, has been successfully applied in *Magnaporthe oryzae*. Simultaneous silencing of ten xylanase genes (Nguyen et al. 2011) and nine cellulase genes (van Vu et al. 2012) resulted in significant reduction of lesion formation, supporting a role of xylan and cellulose degradation in infection of the rice blast fungus. Although this approach suffers from the fact that complete suppression of the target genes is not achieved, it could be used to analyze the function of similar gene families in *B. cinerea*. A comprehensive, unbiased and genome-wide approach for analyzing gene function is random insertion mutagenesis. In a recent study, 2367 *A. tumefaciens*-induced *B. cinerea* insertion mutants were generated, of which 68 mutants showed a significant reduction in virulence (Giesbert et al. 2012). This mutant collection, although as yet not saturated, provides a valuable source for identification of novel genes involved in *B. cinerea* pathogenicity.

To improve the efficiency and versatility of constructs used for manipulation of *B. cinerea*, a set of vectors has been generated (Schumacher 2012). These vectors allow targeted integration of constructs into two nonessential gene loci (*niaD* encoding nitrate reductase, *niiA* encoding nitrite reductase), using three selection markers, and the integration of coding sequences into suitable expression cassettes. They simplify the fusion of proteins with codon-optimized reporters such as GFP for analysis of subcellular localization and in vivo protein-protein interactions. Using one of these vectors, a redox-sensitive GFP has been demonstrated to be functional in *B. cinerea*, which is useful for

investigating the role of infection-related redox processes (Heller et al. 2012a). A valuable addition for this toolbox would be a chemically inducible promoter system, such as a tetracyclin-inducible promoter that has been developed for *Aspergillus fumigatus* (Vogt et al. 2005).

2.4 Lessons Learned from the *B. cinerea* Genome

2.4.1 Genome Organization

Two *B. cinerea* strains were sequenced, with approximately five-year intervals. Strain B05.10 was among the first filamentous fungi to be sequenced, in the mid 1990s, whereas strain T4 was sequenced in the early years of the twenty-first century, when methods were more established, affordable and reliable, and more fungal genomes were available for comparison. This time lapse between the two genome projects had implications for the sequence strategy, methodology, and data quality, eventually leading to sequence assemblies of overall distinct quality (Amselem et al. 2011). While the assembly of B05.10 had low (4.5x) genome coverage and consisted of >4500 contigs that were merged into 588 scaffolds, the assembly of T4 (10x coverage) had 679 contigs merging into 118 scaffolds. In the case of T4, a genetic map allowed to further group the 118 scaffolds in 36 superscaffolds. A recent effort to resequence and reassemble the genomes of both these strains using Illumina technology (Staats and van Kan 2012) led to a much higher coverage (>100x), lower numbers of scaffolds (82 and 56 in B05.10 and T4, respectively) and concomitantly higher N50 (970 and 1710 kb, respectively). More importantly, the resequencing corrected many tens of thousands of wrong base calls and indels, closed hundreds of gaps and reduced the number of Ns from 3.9 to 1.6 million (version 1) to <0.5 million for both genomes (version 2; Staats and van Kan 2012; Table 2.1). The numbers of predicted protein-coding genes in version 1 genome assemblies were in the order of 16,400,

of which some 2,100–2,800 were considered dubious (Amselem et al. 2011) and many hundreds were split gene models. The version 2 genomes resulting from the resequencing contain an estimate of 10,400 predicted protein-coding genes (Staats and van Kan 2012), however, it was noted that the gene prediction tool had failed to predict roughly 1,100 bonafide gene models for various technical (bioinformatics) reasons (van Kan, unpublished). The number of protein-coding genes in *B. cinerea* is thus estimated to amount to approximately 11,500 (Table 2.1). Further optimization of the genome assembly of strain B05.10 and the gene models and annotations is ongoing (Stassen and van Kan, unpublished).

The average GC contents of the *B. cinerea* and *S. sclerotiorum* genomes (~42 %), are remarkably low compared to the GC contents from other filamentous ascomycetes, which are above 50–52 %. Interestingly, they are similar to the GC content of another Leotiomycete, the obligate biotroph *Blumeria graminis*, indicating that this is a general character of this class within the ascomycetes. The evenly distributed, low GC content might have consequences for chromosome organization and gene expression. For example, poor expression of the commonly used eGFP gene in *B. cinerea* was found to be due to its high GC content. Synthesis of a gene with lower GC content and a codon usage adapted to that of *B. cinerea* resulted in strongly increased expression and fluorescence of eGFP (Leroch et al. 2011; Fig. 2.1e, f).

Despite their close relatedness, the *B. cinerea* and *S. sclerotiorum* genomes differ remarkably in their content of transposable elements. While 7 % of the genomic DNA in *S. sclerotiorum* is made up of transposable elements, *B. cinerea* strains T4 and B05.10 contain <1 % transposable elements. Phylogenetic analysis indicates that this difference was caused by recent bursts of transposition and amplification events in *S. sclerotiorum*. Despite the similar total number of predicted genes in the two species, *B. cinerea* tends to have higher numbers of genes than *S. sclerotiorum* for several classes of proteins

Table 2.1 Assembly and gene statistics of *B. cinerea* and *S. sclerotiorum* genomes

	<i>B. cinerea</i>		<i>S. sclerotiorum</i>
	Strain T4	Strain B05.10	Strain 1980
Coverage	10x (>100x)	4.5x (>100x)	9.1x
Assembly size (Mb)	39.5 (41.6)	42.3 (41.2)	38.3
Scaffolds	118 (56)	588 (82)	36
Scaffold N50 (kb)	562 (1,710)	257 (970)	1,630
GC%	43.2 (42.4)	43.1 (42.8)	41.8
GC exons (%)	46.2	46.2	45.7
GC introns (%)	40.0	40.9	39.9
Transposable elements (% of total genome size)	0.7	0.9	7.0
Predicted protein coding regions	13,664	14,270	11,860
Median exon length (nt)	208	190	182
Median intron length (nt)	62	74	78
Carbohydrate Active Enzymes (CAZymes)	367		346
Plant cell wall (PCW) degrading CAZymes	118		106
Pectin degrading enzymes (percent of PCW CAZymes)	44 (37 %)		33 (31 %)
Total secondary metabolism key enzymes	43		28
PKS	16		15
NRPS	9		6
PKS-NRPS	5		3
Terpene synthases	11		2
Total transporters	432		377
ABC transporters	40		33
MFS transporters	286		218
P-type transporters	20		17
Total transcription factors	419		335
Zn(II) ₂ Cys ₆ TF	222		155
C ₂ H ₂ TF	116		98
Secreted proteins (minus CAZymes and peptidases)	879		603
Secreted proteins >300 aa	521		363

Data from Amselem et al. (2011), bold numbers in parenthesis are data from resequencing by Staats and van Kan (2012). Data for *B. cinerea* strain T4 (version 1, as published by Amselem et al. 2011) are accessible via <http://urgi.versailles.inra.fr/Species/Botrytis/Sequences-Databases>. Data for strain T4 (version 2, as published by Staats and van Kan 2012) and both versions of B05.10 (as published by Amselem et al. 2011; Staats and van Kan 2012) are accessible via http://www.broadinstitute.org/annotation/genome/botrytis_cinerea/MultiHome.html

(Table 2.1). The content of carbohydrate-active enzymes (CAZymes; Cantarel et al. 2009) encoding genes is of special interest because of their potential role in host cell wall penetration and plant tissue breakdown. Overall, the CAZome of *B. cinerea* and *S. sclerotiorum* is not exceptionally large as compared to other necrotrophic and hemibiotrophic ascomycetes. However, the proportion of pectin-degrading enzymes is high (37 and 31 %, respectively, of

B. cinerea and *S. sclerotiorum* plant cell wall CAZymes), being exceeded currently only by *Aspergillus niger* (38 %), a saprophyte that also develops well on fruits.

Analyses of fungal genomes for the content of genes encoding membrane transporters did not reveal major differences between saprotrophs and pathogens. Both, ABC transporters (40 in *B. cinerea*, 33 in *S. sclerotiorum*) and MFS transporters (286 in *B. cinerea*, 218 in *S.*

sclerotiorum) occur in higher numbers in *B. cinerea* than in *S. sclerotiorum*. In contrast, no pronounced differences were observed for P-type ATPases, amino acid transporters, mitochondrial carriers, and aquaporins (Amselem et al. 2011). Since the majority of *B. cinerea* transporter genes that are absent in *S. sclerotiorum* do not have orthologs in other fungi, gene expansion in *B. cinerea* rather than gene loss in *S. sclerotiorum* has probably led to the observed differences.

Another striking difference between both species is in their content of transcription factors (TFs). A total of (approximately) 419 predicted TF-encoding genes were identified in *B. cinerea*, but only 330 in *S. sclerotiorum*. This difference is mostly due to the number of genes of the Zn(II)₂Cys₆ binuclear cluster family (*B. cinerea* 222; *S. sclerotiorum* 155) and the Zn(II)-coordinating C₂H₂ family (*B. cinerea* 116; *S. sclerotiorum* 98). *B. cinerea* has 96 TFs that are absent in *S. sclerotiorum*, while only 12 TFs are unique to *S. sclerotiorum*. Among the 96 *B. cinerea* TFs that have no counterpart in *S. sclerotiorum*, 57 have orthologs in other fungal genomes. This suggests that these 57 TFs have been lost in *S. sclerotiorum*, while the remaining 39 TFs resulted from gene duplication or horizontal gene transfer in *B. cinerea* after separation between the two species. Nine of these *B. cinerea*-specific TF genes are located in SM gene clusters that are not present in *S. sclerotiorum* (Sect. 2.4.3).

Effector proteins that are translocated into plant host cells are typically major determinants of pathogenesis in biotrophic and hemibiotrophic fungi. The potential role of necrotrophic effectors for *Botrytis* spp. is discussed in Sect. 2.6.5. The size of the secretome (secreted proteins without CAZymes and peptidases) of *B. cinerea* is considerably larger (879) than that of *S. sclerotiorum* (603). So far, the role of most of these secreted proteins in *B. cinerea* remains unclear.

Overall, the genome sequences and their gene contents of *B. cinerea* and *S. sclerotiorum* did not reveal unique features that could explain their lifestyles as wide host range necrotrophs,

and set them apart from other pathogenic of saprotrophic fungi.

2.4.2 Mating Type Genes

B. cinerea is considered to be a heterothallic (obligate outcrossing) species with two distinct mating types, designated MAT1-1 and MAT1-2. The two sequenced *B. cinerea* strains are of opposite mating type (B05.10 is of MAT1-1 identity and T4 is MAT1-2), and have the typical *MAT* organization of heterothallic Pezizomycotina, with the presence of dissimilar idiomorph sequences at a single locus. The *MAT1-1* idiomorph of B05.10 contains a *MAT1-1-1* alpha-domain encoding gene, whereas the *MAT1-2* idiomorph of T4 contains a *MAT1-2-1* HMG-domain encoding gene. Besides these archetypal genes, two novel open reading frames (ORFs) were detected that have no known PFAM domain and were not previously reported from other fungi. The first novel ORF is located in the *MAT1-1* idiomorph of B05.10 and was designated *MAT1-1-5*. The second novel ORF is located in the *MAT1-2* idiomorph of T4 and named *MAT1-2-4*. Gene knock-out mutants in the *MAT1-1-5* and *MAT1-2-4* genes showed identical phenotypes, i.e., they produced apothecial stipes of normal appearance, but the stipes were arrested in the transition to apothecial disk development (Terhem and van Kan, unpublished). These observations suggest that *MAT1-1-5* and the *MAT1-2-4* gene might be transcriptional regulators that jointly control differentiation of the apothecial disk. Transcriptome analysis of *B. cinerea* apothecia in different developmental stages is ongoing (van Kan, unpublished).

A model was proposed for the evolution of the *B. cinerea* mating system from a homothallic ancestral locus, much like the locus present in contemporary *Sclerotinia sclerotiorum*, following (hypothetical) occurrence of two simple inversions and two distinct deletion events, yielding the two separate (MAT1-1 and MAT1-2) heterothallic configurations of the locus

(Amselem et al. 2011). This model was recently challenged by Chitrampalam et al. (2013), who reported the occurrence of an inversion of a 3.6 kb segment of the *S. sclerotiorum* MAT locus in every meiotic generation in half the progeny, due to a recombination event between two short inverted repeats within the MAT locus. Based on these observations, Chitrampalam et al. (2013) postulated that the homothallic behavior of *S. sclerotiorum* evolved from a heterothallic ancestor by a recombination event that brought both idiomorphs together in a single genome.

2.4.3 Secondary Metabolism

Before sequencing of its genome, *B. cinerea* was known to produce three SM with potential or confirmed role in the interaction with host plants: two families of sesquiterpenes, i.e., botrydial (Colmenares et al. 2002) and abscisic acid (ABA; Siewers et al. 2006) and one family of polyketides, i.e., botcinic acid (Tani et al. 2006). While ABA is a plant hormone, botrydial and botcinic acid are nonspecific toxins that have been only isolated in *Botrytis* so far. Identification of the genes involved in the biosynthesis of these particular compounds was initiated by pregenomics approaches such as the use of ESTs for library screening (*bot1*; Siewers et al. 2005) but the sequence of the whole genome revealed that *B. cinerea* has a significantly higher potential for SM biosynthesis. In contrast to genes involved in primary metabolism, genes that contribute to the biosynthesis of a single SM or multiple structurally closely-related SMs are usually clustered at one genomic locus (Hoffmeister and Keller 2007; Brakhage 2013) which helps to identify them on the genome sequence. The enzyme responsible for the committed biosynthetic step, often referred to as the “key” enzyme, can be a PolyKetide Synthase (PKS), a Non-Ribosomal Peptide Synthetase (NRPS), a hybrid PKS/NRPS, a DiMethylAllylTryptophan Synthase (DMATS), or a terpene synthase. The *B. cinerea* genome contains 43 genes that were predicted to

encode key enzymes: 16 type I iterative PKS, nine NRPS, five PKS/NRPS, one DMATS, one type III PKS (chalcone synthase), and 11 terpene synthases. Most of these genes (37) are clustered with other genes encoding biosynthesis enzymes such as P450 monooxygenases. About one-third of the clusters also encodes an ABC or MFS transporter and a similar proportion encodes a Zn(II)₂Cys₆ zinc finger TF.

The total number of SM clusters is similar to the average in ascomycetes but strikingly, *B. cinerea* has the highest number (i.e., six) of terpene synthases from the sesquiterpene cyclase (STC) subfamily, although similar numbers of STC genes were identified in basidiomycetes (Agger et al. 2009). One of the six *B. cinerea* STC, BOT2, is the first presilphiperfolan-8 β -ol synthase identified in fungi and the key enzyme for botrydial synthesis (Pinedo et al. 2008). The gene encoding BOT2 is clustered with four other genes (Fig. 2.2), all required for the successive steps of botrydial synthesis (Siewers et al. 2005; I. G. Collado, *unpublished*). Due to AT-rich regions upstream and downstream of these five *bot* genes, the surrounding genomic regions and genes could not be investigated through the different sequencing projects or PCR approaches. Although the five *bot* genes may represent the complete toolbox for botrydial synthesis, additional (unidentified) genes are likely required for regulation of the cluster and toxin export. The terpenes produced by the five other STC and the three DiTerpene Cyclases (DTC) are unknown, although one of the STC is probably the key enzyme for ABA biosynthesis. Finally, two other clusters involved in the biosynthesis of terpenes were predicted. The first one is a carotenoid gene cluster with genes encoding a phytoene synthase, a putative oxidase, and an opsin photoreceptor with a conserved retinal-binding domain. This cluster could be involved in retinal biosynthesis as described in *Fusarium fujikuroi* (Prado-Cabrero et al. 2007). The second cluster contains a terpene synthase encoding gene orthologous to the *Penicillium paxilli* *PaxC* gene, involved in biosynthesis of the indole-diterpene paxillin (Saikia et al. 2008).

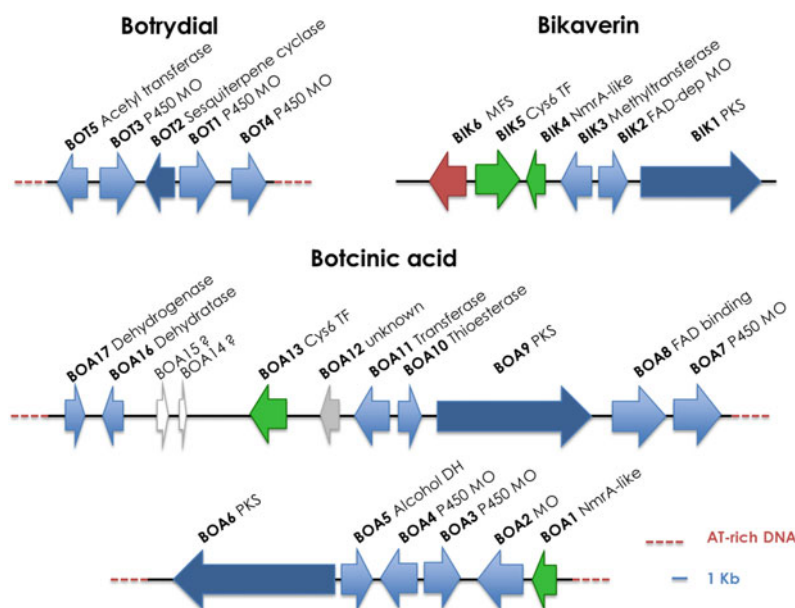


Fig. 2.2 Examples of secondary metabolism gene clusters in *B. cinerea*. Genes encoding key enzymes (sesquiterpene cyclase and polyketide synthases) are in **dark blue** while genes encoding other biosynthetic enzymes (P450 monooxygenases, dehydrogenases, transferases...) are in **light blue**. Genes encoding regulators (Zn(II)₂Cys₆ transcription factors, NmrA-like regulators) are in **green**,

and a Major Facilitator Superfamily transporter gene is in **red**. Dotted lines indicate AT-rich regions exhibiting AT contents higher than 70 % that could not be sequenced. The cluster responsible for the synthesis of the pigment bikaverin is from the *pinkish strain* 1750. Note that in *gray strains* such as B05.10 and T4, *bik1* is missing

The repertoire of *B. cinerea* PKS genes predicted from the genome of B05.10 (Kroken et al. 2003) enabled identification through mutagenesis of the genes responsible for the production of the reduced polyketide botcinic acid. This biosynthesis requires two PKSs, BOA6, and BOA9 (Fig. 2.2; Dalmais et al. 2011). The iterative nature of fungal PKSs means that, in the majority of cases, only a single PKS is involved in the synthesis of one particular polyketide. However, a few fungal polyketides such as lovastatin in *Aspergillus terreus* (Sutherland et al. 2001) are known to be assembled by the action of two PKSs. Isotopic labeling experiments showed that BOA6 produces the pentaketide core of botcinic acid, while BOA9 was responsible for the tetraketide side chain (Massaroli et al. 2013). In the successive T4 and B05.10 genome assemblies (Amselem et al. 2011), the genes encoding BOA6 and BOA9

were on different small contigs, each being adjacent to other putative botcinic acid biosynthetic genes (*boa1-17*). The compounds produced by the other PKS genes in the genomes of B05.10 and T4 remain to be identified. Among the nonreducing PKSs, phylogenetic analysis suggested that PKS12 and PKS13, however, are candidates for melanin biosynthesis (Kroken et al. 2003). Two other genes encoding enzymes involved in DHN melanin synthesis (scytalone dehydratase and hydroxynaphthalene reductase; Pihet et al. 2009) are adjacent to the *pks13* gene.

The *B. cinerea* genome contains nine mono- or multimodular NRPS encoding genes. Recent phylogenetic analysis of fungal NRPS by Bushley and Turgeon (2010) indicates that NRPS6 is involved in the biosynthesis of coprogen siderophore, while NRPS2 and NRPS3 belong to the intracellular siderophore synthetases subfamily.

In conclusion, sequencing the *B. cinerea* genome revealed that, in addition to the two previously known phytotoxins i.e. botrydial and botcinic acid, the fungus has the capacity to produce about 38 other SM. Several of them are shared by many ascomycetes i.e. melanin and siderophores, while the majority represent compounds with unknown structures and functions.

The role of the SM in pathogenicity of *B. cinerea* was investigated by inactivation of genes encoding SM biosynthesis enzymes. This approach allowed the identification of the botrydial (Siewers et al. 2005; Pinedo et al. 2008) and the botcinic acid gene clusters (Dalmais et al. 2011) described in Fig. 2.2. Deletion of the key enzyme BOA6 or BOT2 encoding genes did not modify significantly the virulence of B05.10, while *boa6-bot2* double mutants that do not produce either of the two toxins were severely impaired in virulence on several hosts suggesting that the compounds have a redundant function in plant tissue colonization (Dalmais et al. 2011).

Transcriptomic studies based on microarrays (see Sect. 2.5) indicate that the SM biosynthesis clusters have different expression patterns. Some, like the botrydial and botcinic acid clusters, are expressed on several media as well as during plant infection, while other clusters are more specifically expressed at one specific stage of the life cycle or under specific nutritional conditions (e.g., nitrogen starvation). Of particular interest were the clusters that are upregulated during appressorium formation and/or host colonization. Out of seven PKS- and STC-encoding genes inactivated so far, three resulted in mutants that were altered in virulence and sporulation (Viaud unpublished). The polyketide and sesquiterpenes produced by the three corresponding clusters remain to be identified as well as their mode of action. In conclusion, although botrydial and botcinic acid toxins play together a significant role in virulence, other SM seems to be crucial in *B. cinerea* lifestyle. The current hypothesis is that some compounds have both a role in necrotrophy and in fungal development.

2.5 Genome-Enabled Approaches/Applications of the Genome

2.5.1 Transcriptomics

Before the advent of high-throughput sequencing, approaches to identify genes expressed in a stage-specific manner were tedious. For example, suppression subtractive hybridization was used to identify genes controlled by the G α 1 subunit of the heterotrimeric G protein, BCG1 (Schulze Gronover et al. 2004). Transcriptomic approaches started before the availability of the *B. cinerea* genome sequence, using EST sequences bound to Nylon filters as macroarrays. They were used for the discovery of genes that were upregulated during infection of *Arabidopsis* leaves (Gioti et al. 2006), genes that were regulated by BCG1 and the calcineurin phosphatase (Viaud et al. 2003; Schumacher et al. 2008a), and genes controlled by the ROS regulator AP-1 (Temme and Tudzynski 2009).

After the annotated *B. cinerea* genome sequences became available, Nimblegen microarrays were designed, containing 21,200 gene models identified originally in the B05.10 and T4 genomes (Amselem et al. 2011). RNA isolated either from mycelium grown on agar or from inoculated sunflower cotyledons (48 hpi) was used as labeled hybridization probes for a comparative transcriptome analysis of saprotrophic and necrotrophic growth. A total of 253 genes were upregulated, and 247 genes were downregulated in leaves. The upregulated genes were enriched in genes encoding CAZymes, P-type ATPases, MFS-type sugar transporters and peptidases. When compared to the results of a parallel experiment performed with *S. sclerotiorum*, an overlap in patterns of *in planta* upregulated genes was observed only for the CAZyme-encoding genes. The transcriptome data provided no evidence for a major role of small secreted proteins during infection. Among the 521 genes encoding secreted proteins smaller than 300 amino acids in *B. cinerea*, only 20 (4 %) were significantly upregulated *in planta*

(Amselem et al. 2011). This is in contrast to the related biotroph, *Blumeria graminis*, which was found to encode 195 candidate secreted effector proteins (with no homologs to nonpowdery mildew fungi) that were strongly upregulated in haustoria (Spanu et al. 2010).

In another study, microarrays were used for transcriptome profiling during germination of *B. cinerea* conidia (Leroch et al. 2013a). On apple wax-coated surfaces, which mimicked the hydrophobic plant cuticle and allowed rapid germination in a minimal medium containing fructose, massive changes in gene expression were observed within the first hour of incubation, before germ tube emergence, with 2,864 genes (23.7 % of the expressed genes) being up- or downregulated. Smaller changes were observed in the time window between 1 h and 4 h, when the germ tubes grew out and appressoria were formed. Among the genes that were induced during germination, a significant enrichment was observed of genes encoding secreted proteins, including many CAZymes. A comparison between the wild type and a mutant deficient in the MAP kinase BMP1 revealed that a high proportion of the germination-induced genes encoding secreted proteins were not upregulated in the *bmp1* mutant. These data indicated that germination is accompanied by the production of many secreted proteins, and the BMP1 MAP kinase cascade plays an important role in this process (Leroch et al. 2013a; Sect. 2.6.1).

2.5.2 Proteomics

Proteomics approaches were launched by several groups soon after the annotated *B. cinerea* genome sequences were available (Fernandez—Acero et al. 2009; Shah et al. 2009; Espino et al. 2010; Fernandez—Acero et al. 2010). They primarily focused on the fungal secretome, as secreted proteins are often considered to be crucial determinants in host plant invasion. In some of these studies, a host environment was simulated by culturing *B. cinerea* in the presence

of plant extracts (Shah et al. 2009; Espino et al. 2010; Shah et al. 2012). The observed protein profiles demonstrated that the *B. cinerea* secretome consists of a common set of proteins, but varies in response to different nutrients. In several studies, proteins which have been previously characterized by mutant analysis as virulence factors, including the polygalacturonases PG1 and PG2, the xylanase Xyn11, and the ceratoplatanin family protein Spl1 were detected. A recent study investigated the response of the *B. cinerea* secretome to cultivation at different pH values, and revealed distinct profiles between pH 4 and pH 6. Among the 21 proteins that were uniquely detected at only one pH value, proteins involved in proteolysis were found to be induced at pH 4, while most of the proteins produced at pH 6 were cell wall degrading enzymes (Li et al. 2012a). Most of these proteome investigations were, however, performed when only the first version of the B05.10 genome was publicly accessible. Several proteins may have remained undetected of which the peptide mass peaks could not be assigned because of incorrect or incomplete gene models resulting from the low genome coverage. Future studies employing new versions of the genome assemblies will increase sensitivity of proteomics approaches.

An in silico approach was followed to predict secreted proteins with O-glycosylation based on their content of serine/threonine-rich regions. According to prediction by NetOGlyc, most secretory proteins in filamentous fungi are O-glycosylated (González et al. 2012). The importance of O-glycosylation in fungi was established by the analysis of mutants defective in genes encoding protein O-mannosyltransferases (PMT), which showed reduced ability to add mannoses to Ser/Thr residues during the secretion pathway. O-glycosylation of secreted proteins seems to increase their stability and solubility. In *Ustilago maydis*, deletion of a singly PMT (Pmt4) resulted in complete loss of pathogenicity, without affecting growth rate and morphology (Fernández—Álvarez et al. 2009). A substrate of *U. maydis* Pmt4 that was partly responsible for the defect in penetration, was identified as the

signaling mucin Msb2 (Fernández-Álvarez et al. 2012; Sect. 2.6.1). Individual inactivation of the three *pmt* genes in *B. cinerea* revealed that they are important for cell wall stability, extracellular matrix formation, adherence to plant surface, and host invasion. These data support an important role of O-glycosylated secreted proteins in the infection process (González et al. 2013).

Protein phosphorylation is another important posttranslational modification. In particular, signal transduction cascades modulate cellular responses to environmental stimuli through phosphorylation and dephosphorylation. Tracing protein phosphorylation profiles through proteomics has been developed for model yeasts (Amoutzias et al. 2012), but only few data are available for filamentous fungi (Rampitsch et al. 2012). Off-gel phosphoproteomics, which is more sensitive than 2D-gel phosphoprotein detection (Villen and Gygi 2008), were applied to *B. cinerea* proteins extracted from exponentially grown mycelia and resulted in a set of 1361 phosphopeptides representing 818 phosphoproteins (82.7 % phosphoserine, 15.8 % phosphothreonine, and 1.5 % phosphotyrosine) (S. Fillinger, pers. comm.). Comparative phosphoproteomics of different growth conditions coupled with signal-transduction mutants will reveal further insight into the precise signaling pathways and their interactions.

2.5.3 Map-Based Cloning

The availability of genome sequences and the possibility to perform sexual crosses with *B. cinerea* under controlled laboratory conditions has allowed the application of map-based-cloning approaches for interesting genes. Two examples are presented here: The first example concerns the TF Mrr1, the determinant of a multidrug-resistance phenotype called MDR1. *B. cinerea* strains showing low-to-medium levels of resistance to several fungicides, were isolated for the first time in Champagne vineyards in the mid 1990s, and are now widely distributed in French and German wine producing regions. In contrast to the common type of fungicide resistance caused by

mutations that alter the fungicide target protein, MDR strains show increased fungicide efflux (Chapeland et al. 1999). This was confirmed by the observation that *B. cinerea* strains with MDR1 showed a massive constitutive overexpression of the gene encoding the ABC-type efflux transporter AtrB. Crosses between MDR1 and sensitive strains revealed that MDR1 is controlled by a single dominant gene. Microsatellite-assisted mapping was able to pinpoint the gene down to a region of more than 100 kb. Based on the knowledge from *Candida albicans* MDR phenotypes, previously described to be caused by gain-of-function mutations in TF-encoding genes (Morschhäuser 2010), it was hypothesized that similar mutations could be responsible for *B. cinerea* MDR1. Among the few candidate genes in the mapped region, one gene encoding a Zn(II)₂Cys₆ zinc cluster TF, called Mrr1 (MDR1-related regulator), was found to carry MDR1-related point mutations. Indeed, all *B. cinerea* field isolates with MDR1 phenotypes contained at least one mutation that resulted in an amino acid substitution in Mrr1, compared to the Mrr1 sequences of sensitive strains T4 and B05.10. Knock-out mutants in *mrr1* failed to activate *atrB*, even in the presence of inducing fungicides, and confirmed the regulatory role of Mrr1 in efflux-based fungicide tolerance (Kretschmer et al. 2009).

Another example of map-based cloning assisted by genome sequence data pertains to the phenotypic differences between the two sequenced *B. cinerea* strains, B05.10 and T4. While B05.10 is highly virulent and conidiates in a light-dependent manner, T4 shows weak virulence, lack of sclerotia formation and oxalic acid production, and light-independent conidia formation. All these phenotypes co-segregated in the progeny of a cross, in a 1:1 ratio, indicating that they are all collectively controlled by a single gene locus. Polymorphic microsatellite markers were generated that co-segregated with the locus, and allowed the locus to be narrowed down to a 115-kb genomic region which included 44 predicted genes in the T4 genome sequence. A single nucleotide polymorphism (SNP) between the two strains was found in a gene encoding the velvet

protein, VEL1. This SNP resulted in a truncation of the protein (184 amino acids) in T4 instead of the 575 amino acids of the full-length protein (Schumacher et al. 2012). As described below, VEL1 is also involved in the regulation of SM synthesis. With the decreasing costs of generating genome sequences, we expect that map-based cloning, or similar approaches that allow the rapid correlation of point mutations with phenotype alterations, will be used more and more frequently as a tool in fungal biology.

2.6 The Impact of ‘-Omics’ Approaches in Our Understanding of *Botrytis* Biology

2.6.1 MAP Kinase Signal Transduction Pathways

Fungi contain highly conserved mitogen-activated protein kinase (MAPK) cascades that serve as key signal transducers in a variety of cellular processes. Because of their conservation, most MAPK components in filamentous fungi have been identified by comparative genomics, using yeast as a reference. MAPK cascades consist of three interlinked protein kinases that are sequentially activated by phosphorylation, namely MAPK kinase kinases (MAP3 K), MAPK kinase (MAP2 K), and MAPK. In filamentous fungi, three major MAPK pathways exist. In addition to their crucial roles in the regulation of basic developmental processes, they control various aspects of infectious growth in plant pathogens. The MAPK cascade controlling filamentous and invasive growth in yeast, Sc-Ste11 (MAP3 K)- Sc-Ste7 (MAP2 K)- Sc-Kss1 (MAPK), is mirrored in *B. cinerea* by the orthologous Ste11-Ste7-BMP1 cascade. Mutants in each of these components, and in the associated adapter protein Ste50, are unable to differentiate appressoria, do not penetrate into host cells, and do not even colonize wounded host tissue. In addition, such mutants show reduced growth, impaired germination on hydrophobic surfaces, lack of

sclerotium formation and sexual development, and inability to form hyphal fusions (Doehlemann et al. 2006; Schamber et al. 2010; U. Siegmund, personal communication). The similarity of phenotypes of the *ste11*, *ste7*, *bmp1*, and *ste50* mutants indicates that there are no branch points in the cascade upstream of BMP1, and that, in contrast to yeast, Ste11 displays no cross talk with the high osmolarity MAP kinase pathway (see below). The TF Ste12, one of the major targets of yeast Kss1, also seems to be under MAPK control in filamentous fungi. In *B. cinerea*, *ste12* mutants have defects in host penetration, and show reduced virulence (Wong Sak Hoi and Dumas 2010; Schamber et al. 2010). Recent data obtained with *Aspergillus nidulans* impressively demonstrate in vivo interaction of all four MAPK components at the plasma membrane during initiation of sexual development, and the interaction of AnFus3 with AnSte12 and VeA (the homolog of VEL1, see Sects. 2.5.3 and 2.6.2) in the nucleus (Bayram et al. 2012). It can be expected that similar dynamic interactions involving MAPK modules occur in *B. cinerea* during pathogenic differentiation.

To identify target genes of the BMP1 MAPK pathway that play a role in the infection process, a microarray-based transcriptomics approach was followed (see Sect. 2.5.1). The sensory mechanisms and the upstream components that activate the BMP1 MAPK pathway are still poorly understood. In yeast, *U. maydis* and *M. oryzae*, the sensory mucin Msb2 has been shown to be involved in surface sensing which is transduced via the filamentous and invasive growth MAPK pathway (Lanver et al. 2010; Liu et al. 2011a). In *B. cinerea*, a similar function for Msb2 was indicated by the phenotype of an *msb2* mutant, which failed to form appressoria on artificial or plant surfaces, and showed reduced phosphorylation of BMP1 during germination on hard surfaces (Leroch and Hahn, unpublished).

The fungal high osmolarity MAPK pathway of fungi has attracted particular attention because of its involvement in the sensitivity and resistance to phenylpyrrole (e.g., fludioxonil)

and dicarboximide (e.g., iprodione) fungicides. The homolog of the high osmolarity pathway MAPK in yeast, *Sc-Hog1*, was called *Sak1* ('stress-activated kinase'). Mutants in *Sak1* are hypersensitive to hyperosmotic and oxidative stress, they do not form conidiospores, are unable to differentiate appressoria, and fail to invade intact host tissue. Nevertheless, they retain the ability to form primary and secondary lesions if inoculated on wounded tissue (Segmüller et al. 2007). Similar phenotypes were observed for mutants defective in the kinases upstream of *Sak1*, *BOS5* (MAP2 K) and *Os4* (MAP3 K) (Yan et al. 2010; Yang et al. 2012). The *bos5* and *os4* mutants were unable to phosphorylate *Sak1* in response to osmotic stress, which confirmed their upstream position in the pathway. The *Bos1* histidine kinase, which is believed to be involved in osmosensing, has also been shown to be required for full virulence in *B. cinerea* (Viaud et al. 2006; Liu et al. 2008). In contrast to the situation in other filamentous fungi, *Bos1* seems to negatively regulate the activity of *Sak1*, because *Sak1* phosphorylation levels were increased in the *bos1* mutant (Liu et al. 2008). In addition to its role in osmoregulation, *Bos1* is also involved in the sensitivity to fludioxonil and iprodione. Several mutations in *bos1* were mapped in mutants that were selected for resistance to fludioxonil and iprodione. Site-directed mutagenesis confirmed that these mutations lead to resistance to the fungicides and to hypersensitivity to osmotic stress (Fillinger et al. 2012). Another component of this pathway is the putative response regulator *Brrg-1* (Yan et al. 2011). Transcriptomic studies with microarrays with the *sak1* mutant revealed that the majority of *Sak1*-regulated genes are unrelated to stress responses, but rather involved in general metabolic functions (Heller et al. 2012b).

The 'cell wall integrity' pathway, represented by the (Stl2-like) *Bmp3* MAPK, is poorly analyzed in *B. cinerea*. Mutant analysis revealed an involvement of *Bmp3* in vegetative growth, penetration, and lesion development, but surprisingly, no role in cell wall integrity (Rui and Hahn 2007). Instead of *Bmp3*, *Sak1* was

identified as the MAPK mainly involved in cell wall integrity maintenance, as indicated by aberrations in the cell wall structure in *sak1* mutants, and the observation that *Sak1* was found to be phosphorylated in response to cell wall stress (Liu et al. 2011b). The three MAPK cascades show several interconnections. All are involved in surface sensing by germ tubes or hyphae leading to the appressorium formation, and in the regulation of melanin biosynthesis genes. *Sak1* was found to interfere with the phosphorylation status of *BMP3* during cell wall stress, hinting to cross talk between both MAPK pathways (Liu et al. 2008; Liu et al., 2011b). Both *Sak1* and *Bmp3* are required for normal expression of *Reg1*, a transcriptional regulator involved in secondary metabolism (SM) and pathogenicity (Michiels et al. 2011; see Sect. 2.6.1).

2.6.2 Regulation of Secondary Metabolism

In recent years, important progress has been made in the identification of signal transduction pathways that regulate the production of the two identified phytochemicals botrydial and botcinic acid. Expression of the *bcbot* and *bchoa* genes has been shown to depend on two pathways. The first involves the α subunit *BCG1* of a heterotrimeric G protein, phospholipase C (*PLC1*), calcineurin phosphatase (Viaud et al. 2003; Pinedo et al. 2008; Schumacher et al. 2008a; Dalmais et al. 2011) and the downstream Cys2His2 zinc finger TF *CRZ1* (Schumacher et al. 2008c). The second transduction pathway is the stress-activated MAPK cascade (Sect. 2.6.1) which controls the basic region leucine zipper (bZIP) TF *Atf1* (Temme et al. 2012) and the transcriptional regulator *Reg1* (Michiels et al. 2011). The pleiotropic phenotypes of the *crz1* Δ , *atf1* Δ and *reg1* Δ mutants and analyses of the *CRZ1*- and *Atf1*-dependent genes has confirmed that these three proteins regulate broad sets of genes involved in different cellular processes (Schumacher et al. 2008b; Temme et al. 2012; Michiels et al. 2011), suggesting that they play only an indirect role in

bcbot and *bchoa* genes expression. Indeed, in fungi, pathway-specific TF (often of the Zn(II)₂Cys₆ family) and broad-domain TF responsive to general environmental factors seem to act jointly to regulate SM biosynthesis (Yin and Keller 2011). In *B. cinerea*, 11 SM clusters, including the botcinic acid cluster (Fig. 2.2), contain a gene encoding a putative pathway-specific Zn(II)₂Cys₆ TF, whereas the remaining 29 SM clusters contain no identifiable TF-encoding gene. Gene inactivation of *boa13* encoding a Zn(II)₂Cys₆ zinc finger TF showed that it indeed regulates all the *boa* genes (Viaud, unpublished). Unlike the botcinic acid cluster, the botrydial biosynthesis gene cluster contains no TF gene that could specifically co-regulate the expression of *bcbot* genes. To identify which TF(s) interact with the promoters of this cluster, a postgenomic strategy based on the Yeast One-Hybrid (Y1H) method was developed (Simon et al. 2013). A Y1H library consisting of 393 TF DNA-binding sites out of the 406 predicted from the *B. cinerea* genome was screened with the promoter of *bot2* which allowed to identify a Cys2His2 TF called YOH1. Inactivation of *yoh1* confirmed that this TF positively regulates *bot* genes but also revealed that it is not a pathway-specific TF. Indeed, microarray data showed that YOH1 regulates the expression of genes from more than half (22 of 40) the SM clusters of *B. cinerea* including the botrydial and botcinic acid clusters, suggesting that this TF is a global regulator of SM. In addition, the list of genes regulated by YOH1 is enriched in two other main categories of genes encoding proteins thought to be associated with the necrotrophic processes of *B. cinerea*: CAZymes and MFS transporters, involved in carbohydrate uptake or in efflux of SM from the hyphae into the host.

The global regulator *velvet* also takes part in regulation of SM production in *B. cinerea*. This protein complex is conserved in filamentous fungi and coordinates SM and light-dependent development (Bayram and Braus 2012). The *velvet* complex of *A. nidulans* consists of at least three proteins: LaeA, VeA, and VelB; LaeA links light regulation to chromatin modification (Brakhage 2013). The VeA ortholog in *B.*

cinerea (*vell1*) was identified by a map-based cloning approach (Sect. 2.5.3). The T4 strain harbors a truncated BcVEL1 protein (184 aa instead of 575 aa), which accounts for the lack of sclerotia development and oxalic acid formation, as well as the reduced virulence (Schumacher et al. 2012). Strikingly, a *B. cinerea* strain producing a red pigment, bikaverin, which has been previously characterized in *Fusarium* spp., was recently shown to be another natural *velvet* mutant in which the VEL1 protein is even shorter (100 aa). By comparing the phenotypes due to the different truncated BcVEL1 proteins or to the complete KO, it was concluded that the presence of the C-terminal part (aa 185–575) of BcVEL1 is a prerequisite for nuclear localization, sclerotia formation, oxalic acid formation, and full virulence while only the first 100 aa of BcVEL1 are required for regulation of pigment formation, i.e., repression of melanin formation and stimulation of bikaverin formation (Schumacher et al. 2013). Further knowledge on the transcriptional and epigenetic regulation mechanisms that control SM biosynthesis is crucial to understand the development and virulence of the gray mold fungus. This knowledge could also provide approaches to “wake up” SM clusters during in vitro growth, so that the compounds could be characterized by chemists. Possible approaches include genetic engineering (e.g., overexpression of key enzymes encoding genes or pathway-specific TFs), modification of the chromatin structure or stimulation by coculture with other microorganisms (Brakhage 2013).

2.6.3 Intra- and Interspecific Diversity of *B. Cinerea* and Related Species

Rowe and Kliebenstein (2010) illustrated that intraspecific diversity is often ignored in studies with necrotrophic pathogens. In a survey of literature from 2000–2010 about plant defense against *B. cinerea*, only 12 % of the studies reported experiments with more than one isolate. Studies on biotrophic pathogens are more often

performed with multiple isolates to study gene-for-gene interactions of pathogen isolates with host genotypes. However, this is usually not the case in studies with necrotrophs such as *B. cinerea*, for which the genetic basis of (low) host specificity is unknown. Indeed, it remains to be clarified whether the reported host range of more than 200 plant species could be attributed to a single isolate of *B. cinerea*. Isolate-specific interactions between *Arabidopsis thaliana* and *B. cinerea* were observed upon infection of *Arabidopsis* mutants in jasmonate-dependent defense with diverse isolates of *B. cinerea*. Pathogen isolates were inhibited to variable extent by jasmonate-mediated defense responses. Transcriptome analysis of plants infected by two distinct *B. cinerea* isolates showed only minor differences in transcriptional responses of wild-type *Arabidopsis*, but notable isolate-specific transcript differences in jasmonate-insensitive mutants (Rowe et al. 2010).

Field isolates of *B. cinerea* have been shown to exhibit considerable phenotypic variability in vegetative growth, conidiation, sclerotium formation, SM, and virulence (Kerssies and Bosker-van Zessen 1997; Martinez et al. 2003; Schumacher et al. 2013). This is reflected by a similar variability in genotypic characters, which have been analyzed by PCR-assisted techniques such as random amplification of polymorphic DNA, amplified restriction length polymorphism, detection of transposable elements and microsatellite heterogeneity (Kerssies and Bosker-van Zessen 1997; Giraud et al. 1997; Fournier et al. 2002; Moyano et al. 2003). In some of these studies, the majority of field isolates could be assigned to different haplotypes (Giraud et al. 1999; Kretschmer and Hahn 2008). However, a subdivision of *B. cinerea* into distinct subgroups turned out to be difficult. Studies performed with isolates from French vineyards, initially indicated the occurrence of genetically distinct groups, which could be distinguished by the presence (*transposa* strains) or absence (*vacuma* strains) of two transposable elements called Boty and Flipper (Giraud et al. 1997, 1999). This distinction between *transposa* and *vacuma* isolates, although widely used,

turned out to be inadequate for subdivision of *B. cinerea* populations into genetically distinct groups. Indeed, gene sequencing demonstrated that only a subgroup (called 'group I') mostly of the *vacuma* strains represented a species distinct from *B. cinerea*, which has recently been named *B. pseudocinerea* (Fournier et al. 2005; Walker et al. 2011). *B. pseudocinerea* has been found to occur as a minor gray mold species in French vineyards, and on several other plants. Seasonal variations in the frequency of *B. pseudocinerea* in vineyards, and its rare occurrence on molded grape berries suggests that it has a lower pathogenic performance than *B. cinerea*, but might survive better as a saprophyte (Martinez et al. 2005). *B. pseudocinerea* is morphologically indistinguishable from *B. cinerea*, but shows intrinsic resistance to the fungicide fenhexamid. This resistance is correlated with a reduced affinity of the fenhexamide target enzyme, 3-ketoreductase, compared to the target enzyme in *B. cinerea* (Debieu et al. 2013). Furthermore, a fenhexamid-induced cytochrome P450 monooxygenase, presumably is involved in detoxification, has been found to contribute to fenhexamid resistance in *B. pseudocinerea* (S. Azeddine and S. Fillinger, personal communication). Together with the host-specific *B. pelargonii*, *B. fabae* and *B. calthae*, *B. cinerea* and *B. pseudocinerea* represent the so-called dicot clade of *Botrytis* spp., comprising species that only infect dicot plants (Staats et al. 2005; Walker et al. 2011).

Although the genetic variability of *B. cinerea* is well documented, correlations between biological or pathogenic traits and genetic patterns have rarely been identified. Nevertheless, evidence of genetic differentiation within populations has been obtained. Populations of *B. cinerea*, sampled from grapes and blackberries, growing side-by-side in six regions of France, were shown by microsatellite-based population genetic studies to be significantly differentiated, indicating restricted gene flow between strains that infect these different hosts (Fournier and Giraudn 2008). A recent study on gray mold isolates from intensively fungicide-treated strawberry fields revealed a hitherto unknown

genotype that is closely related but distinct from *B. cinerea*. It was detected during analysis of the molecular basis of a stronger variant of the multidrug-resistance phenotype 1 (MDR1), called MDR1h. Sequencing of the *mrr1* gene of MDR1 h-isolates, and of some non-MDR1h isolates from strawberries, revealed considerable sequence divergence from the *mrr1* genes from *B. cinerea*. A phylogenetic tree based on the combined sequence analysis of five genes placed *Botrytis* group S between *B. cinerea* and the host-specific *B. fabae* (Leroch et al. 2013b). Isolates with sequence signatures of *Botrytis* group S were found in different European countries, which indicated that little genetic exchange occurs between *B. cinerea* and *Botrytis* group S. To clarify the taxonomic status of *Botrytis* group S, genome sequencing and sexual crosses are underway. A population study revealed distinct patterns in the prevalence of *Botrytis* genotypes: while *Botrytis* group S was more abundant than *B. cinerea* in fungicide-treated strawberry fields, wine grapes were infected almost exclusively by *B. cinerea*. These data, together with those of putative new endophytic *Botrytis* species that were grouped close to *B. cinerea* (Shipunov et al. 2008), strongly indicate that the gray mold fungus indeed is not represented by a single species but rather a species complex, comprising genetically distinct taxa that are differentiated by host preference, virulence, accumulation of fungicide resistance mutations and other, as yet unknown physiological properties.

2.6.4 Genes under Positive Selection

It has often been observed that genes which are involved in interactions with an organism's (biotic) environment display an increased evolutionary rate, and are under positive (also called 'diversifying') selection. When coding sequences of such genes are compared between individuals within a population of a species, or between closely related species, positive selection is reflected in a high overall ratio of

nonsynonymous nucleotide substitutions versus synonymous substitutions (dN/dS), often at positions within the protein that are potentially important for its interactions with receptors or ligands. In *B. cinerea*, two endopolygalacturonase genes, *Bcpg1* and *Bcpg2*, both of which contribute to full virulence (ten Have et al. 1998; Kars et al. 2005a, b) are under positive selection while other endopolygalacturonase genes are not (Rowe and Kliebenstein 2007; Cettul et al. 2008). Similarly, a study on NLP genes in the entire genus *Botrytis*, showed that the phytotoxic protein-encoding genes NEP1 and NEP2 are under positive selection (Staats et al. 2007a), however, functional analyses using mutants in *B. cinerea* and *B. elliptica* showed that these NLP genes do not make any detectable contribution to virulence (Staats et al. 2007b; Cuesta Arenas et al. 2010).

In other necrotrophic fungi, it was observed that genes that encode important virulence determinants are under positive selection. Building on such concepts, the availability of *Botrytis* genome or transcriptome sequences enables genome-wide studies to identify genes under positive selection, and to mark those genes as candidate virulence determinants. Using such an approach, Aguileta et al. (2012) compared the sequences of ESTs from *B. tulipae* and *B. ficariarum* with gene models of *B. cinerea* and *S. sclerotiorum*, and detected 21 genes under positive selection. For nine of these 21 genes, additional sequencing was done in 12 more *Botrytis* species and data confirmed the conclusion that these genes are under positive selection. Functional analysis of four of these genes by targeted knockout in *B. cinerea*, indicated that mutations in these genes did not significantly affect virulence on bean and tomato leaves (Aguileta et al. 2012). One of these genes, however, encoding the cerato-platanin family protein Spl1 was also analyzed by Frías et al. (2011) and found to be involved in virulence and induction of necrosis on tobacco leaves (Sect. 2.2). Interestingly the two sites found to be under positive selection in the Spl1 protein were situated precisely in the region with necrosis-inducing activity on tobacco leaves.

2.6.5 Evidence for Necrotrophic Effector Proteins

There are several lines of evidence for the ability of necrotrophs to subvert plants by co-opting the programmed cell death machinery of the host (Dickman et al. 2001; van Kan 2006; Oliver and Solomon 2010). The induction of host cell death provides an entry point for infection, and is essential for a necrotrophic fungus to successfully infect a host. Host cell death induction is achieved by releasing metabolites or proteins with phytotoxic action into a host plant (Stergiopoulos et al. 2013). Phytotoxic proteins of necrotrophic fungi are recently referred to as ‘necrotrophic effectors,’ by analogy to molecules that biotrophic pathogens secrete to promote virulence (Tan et al. 2010; Ciuffetti et al. 2010). There are several well-studied, host-specific necrotrophic effectors, e.g., the ToxA protein of *Pyrenophora tritici-repentis* (Ciuffetti et al. 2010). The wheat gene *Tsn1*, encoding an NBS-LRR gene (similar to resistance genes effective against biotrophs), governs sensitivity to ToxA, and thereby confers susceptibility to *P. tritici-repentis* as a dominant trait (Faris et al. 2010). Thus, recognition of necrotrophic effectors often relies on similar mechanisms as the recognition of effectors from biotrophs (‘avirulence proteins’) by plant receptor proteins, leading to disease resistance. However, the outcome of necrotrophic effector recognition is inverse to classical gene-for-gene-based pathosystems, i.e., effector recognition triggers susceptibility (Tan et al. 2010, 2012).

While *B. cinerea* has a wide host range, all other *Botrytis* species are considered to be much more restricted to rather few related host species, or to a single host species. One working model for their host specificity is that each species secretes effector proteins that are toxic to its host plant, but not to nonhosts. Several independent host jumps occurred during speciation in the genus *Botrytis* (Staats et al. 2005), which may have been caused by the acquisition or evolution of effector genes. An important aspect of genome projects in related necrotrophic fungi could be to

identify unique effector genes, encoding proteins which only kill a specific host species. Such effector genes may be identified by a combination of comparative genomics and proteomics approaches. A limitation of this working model is the difficulty to explain the wide host range of *B. cinerea*, which includes hosts infected by the host-specific *Botrytis* spp.. For example, *B. cinerea* also infects *Vicia faba* (broad bean) and *Caltha palustris*, the preferred or exclusive host plants of *B. fabae* and *B. calthae*, respectively (Hahn, unpublished). *B. cinerea* is closely related to *B. fabae*, which raises the question as to the genomic differences that are responsible for their strikingly different host ranges.

B. cinerea produces two known phytotoxic SM, and several phytotoxic proteins, all of which can induce cell death in a range of dicot plants. The multiplicity of cell death-inducing compounds implies that they perform redundant functions in pathogenesis (Cuesta Arenas et al. 2010; Dalmis et al. 2011; Frías et al. 2011). Transcriptome studies did not provide strong evidence for the existence of effector proteins similar to those discovered in other necrotrophic fungi (Sect. 2.5.1). Some of the host-specific *Botrytis* species are known to produce phytotoxic botrydial and/or botcinic acid related compounds (Collado et al. 2000), and there are also some reports of phytotoxic effector proteins. Functional analysis of proteins of the NLP family from the lily pathogen *B. elliptica* (BeNEP1 and BeNEP2) showed that remarkably, these proteins were phytotoxic to all dicots tested, but not to lily, and the *BeNEP1* or *BeNEP2* genes were not required for pathogenicity on lily (Staats et al. 2007a, b). Instead, there is evidence for the production of proteinaceous host-specific effectors by *B. elliptica* (van Baarlen et al. 2004) and by *B. fabae* (Harrison 1980). Proteins secreted by *B. elliptica* specifically caused programmed cell death in lily (van Baarlen et al. 2004), but not in other plants (a.o. onion, tulip, rice, maize, tobacco, Arabidopsis; van Kan, unpublished). Furthermore, prior infiltration of the *B. elliptica* protein sample into lily leaves enabled the otherwise avirulent species *B. cinerea* and *B. tulipae* to infect lily, thereby

proving the importance of host-specific effector protein activity for infecting lily (van Kan, unpublished). The *B. elliptica* protein sample was analyzed by LC-MS/MS and mass peaks were matched to the proteins predicted to be encoded in the *B. elliptica* genome. This resulted in identification of 10 candidate effector proteins, which remain to be further analyzed (van Kan, unpublished).

An alternative, or complementary, mechanism that could determine host specificity in *Botrytis* spp. is their differential sensitivity to plant defense metabolites. *Vicia faba* leaves respond to infection by *Botrytis* spp. with the synthesis of wyerone and related phytoalexins (Hargreaves et al. 1977). *B. fabae*, which is highly aggressive on *V. fabae*, was found to be significantly more tolerant to wyerone than *B. cinerea* (Rossall et al. 1980). In another study, the ability to detoxify α -tomatine was found in most natural isolates of *B. cinerea*. However, one isolate from grapevine was found to lack tomatinase activity, and this isolate was highly sensitive to α -tomatine and strongly impaired in virulence on tomato leaves, but normally virulent on bean leaves (Quidde et al. 1998).

2.7 Future Perspectives

Research in the last few years has revealed that the pathogenic mechanisms of *B. cinerea* are more complex than previously imagined, and still far from being understood. Recent studies provide evidence for the ability of *Botrytis* to suppress host defense by several mechanisms. These include the manipulation of plant hormone pathways, in particular those that are involved in defense responses, namely salicylic acid (which generally promotes infection by necrotrophs), jasmonic acid and ethylene (which both impair infection) (reviewed by Mengiste 2012). Consequently, ‘-omics’ approaches that include the host plant will be increasingly applied to decipher the pathogen-induced changes in host metabolism. Examples are a recently performed, high resolution expression temporal transcriptomic analysis with *Arabidopsis* leaves

during infection with *B. cinerea* (Windram et al. 2012), and a combined proteomic analysis of pathogen and host proteins in *B. cinerea*-infected ripening tomato fruits (Shah et al. 2012). An additional layer of complexity in our understanding of the infection mechanisms of *B. cinerea* has been recently uncovered by the discovery of small RNAs that suppress plant immunity by means of hijacking the host RNA interference pathway (Weiberg et al. 2013).

It is only a matter of time until the genome sequences of all known *Botrytis* species will be determined. Besides *B. cinerea*, genome assemblies have been generated for nine other *Botrytis* species (Staats and van Kan, unpublished), but remain to be annotated and analyzed in detail. For species of greater economic interest, multiple strains will be sequenced to explore intraspecific genetic diversity. An effort is in progress to sequence a large collection of *B. cinerea* field isolates and perform a genome-wide association analysis of phenotypic traits (Kliebenstein, pers. comm.). The genome sequences, together with transcriptome and proteome data will provide valuable resources for further studies, aimed at unraveling the biology of *Botrytis* spp..

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References

- Agger S, Lopez-Gallego F, Schmidt-Dannert C (2009) Diversity of sesquiterpene synthases in the basidiomycete *Coprinus cinereus*. *Mol Microbiol* 72:1181–1195
- Aguileta G, Lengelle J, Chiapello H, Giraud T, Viaud M, Fournier E, Rodolphe F, Marthey S, Ducasse A, Gendault A, Poulain J, Wincker P, Gout L (2012) Genes under positive selection in a model plant pathogenic fungus, *Botrytis*. *Infect Genet Evol* 12:987–996
- Amoutzias GD, He Y, Lilley KS, van de Peer Y, Oliver SG (2012) Evaluation and properties of the budding yeast phosphoproteome. *Mol Cell Proteom* 11(M111):009555
- Amselem J, Cuomo CA, van Kan JA, Viaud M, Benito EP, Couloux A, Coutinho PM, de Vries RP, Dyer PS, Fillinger S, Fournier E, Gout L, Hahn M, Kohn L, Lapalu N, Plummer KM, Pradier JM, Quévillon E,

- Sharon A, Simon A, ten Have A, Tudzynski B, Tudzynski P, Wincker P, Andrew M, Anthouard V, Beever RE, Boffa R, Benoit I, Bouzid O, Brault B, Chen Z, Choquer M, Collémare J, Cotton P, Danchin EG, Da Silva C, Gautier A, Giraud C, Giraud T, Gonzalez C, Grossetete S, Güldener U, Henrissat B, Howlett BJ, Kodira C, Kretschmer M, Lappartient A, Leroch M, Levis C, Mauceli E, Neuvéglise C, Oeser B, Pearson M, Poulain J, Poussereau N, Quesneville H, Rasclé C, Schumacher J, Séguens B, Sexton A, Silva E, Sirven C, Soanes DM, Talbot NJ, Templeton M, Yandava C, Yarden O, Zeng Q, Rollins JA, Lebrun MH, Dickman M (2011) Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *PLoS Genet* 7:e1002230
- Andrew M, Barua R, Short SM, Kohn LM (2012) Evidence for a common toolbox based on necrotrophy in a fungal lineage spanning necrotrophs, biotrophs, endophytes, host generalists and specialists. *PLoS ONE* 7:e29943
- Arbelet D, Malfatti P, Simond-Côte E, Fontaine T, Desquilbet L, Expert D, Kunz C, Soulié MC (2010) Disruption of the Bcchs3a chitin synthase gene in *Botrytis cinerea* is responsible for altered adhesion and overstimulation of host plant immunity. *Mol Plant Microbe Interact* 23:1324–1334
- Bayram O, Braus GH (2012) Coordination of secondary metabolism and development in fungi: the velvet family of regulatory proteins. *FEMS Microbiol Rev* 36:1–24
- Bayram Ö, Bayram ÖS, Ahmed YL, Maruyama J, Valerius O, Rizzoli SO, Ficner R, Irniger S, Braus GH (2012) The *Aspergillus nidulans* MAPK module AnSte11-Ste50-Ste7-Fus3 controls development and secondary metabolism. *PLoS Genet* 8:e1002816
- Billon-Grand G, Rasclé C, Droux M, Rollins JA, Poussereau N (2010) pH modulation differs during sunflower cotyledon colonization by the two closely related necrotrophic fungi *Botrytis cinerea* and *Sclerotinia sclerotiorum*. *Mol Plant Pathol* 13:568–578
- Brakhage AA (2013) Regulation of fungal secondary metabolism. *Nat Rev Microbiol* 11:21–32
- Brito N, Espino JJ, González C (2006) The endo-beta-1,4-xylanase Xyn11A is required for virulence in *Botrytis cinerea*. *Mol Plant-Microbe Interact* 19:25–32
- Bushley KE, Turgeon BG (2010) Phylogenomics reveals subfamilies of fungal nonribosomal peptide synthetases and their evolutionary relationships. *BMC Evol Biol* 10:26
- Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B (2009) The Carbohydrate-active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucl Acids Res* 37:D233–D238
- Cettul E, Rekab D, Locci R, Firrao G (2008) Evolutionary analysis of endopolygalacturonase-encoding genes of *Botrytis cinerea*. *Mol Plant Pathol* 9:675–685
- Chapeland F, Fritz R, Lanen C, Gredt M, Leroux P (1999) Inheritance and mechanisms of resistance to anilinopyrimidine fungicides in *Botrytis cinerea* (*Botryotinia fuckeliana*). *Pestic Biochem Physiol* 64:85–100
- Chitrampalam P, Inderbitzin P, Maruthachalam K, Wu BM, Subbarao KV (2013) The *Sclerotinia sclerotiorum* mating type locus (*MAT*) contains a 3.6-kb region that is inverted in every meiotic generation. *PLoS ONE* 8:e56895
- Choquer M, Fournier E, Kunz C, Levis C, Pradier JM, Simon A, Viaud M (2007) *Botrytis cinerea* virulence factors: new insights into a necrotrophic and polyphagous pathogen. *FEMS Microbiol Lett* 277:1–10
- Ciuffetti LM, Manning VA, Pandelova I, Betts MF, Martinez JP (2010) Host-selective toxins, Ptr ToxA and Ptr ToxB, as necrotrophic effectors in the *Pyrenophora tritici-repentis*-wheat interaction. *New Phytol* 187:911–919
- Collado G, Aleu J, Hernandez-Galan R, Duran-Patron R (2000) *Botrytis* species: an intriguing source of metabolites with a wide range of biological activities. Structure, chemistry and bioactivity of metabolites isolated from *Botrytis* species. *Curr Organ Chem* 4:1261–1286
- Colmenares AJ, Aleu J, Duran-Patron R, Collado IG, Hernandez-Galan R (2002) The putative role of botrydial and related metabolites in the infection mechanism of *Botrytis cinerea*. *J Chem Ecol* 28:997–1005
- Cuesta Arenas Y, Kalkman E, Schouten A, Dieho M, Vredenbregt P, Uwumukiza B, Osés Ruiz M, van Kan JAL (2010) Functional analysis and mode of action of phytotoxic Nep1-like proteins of *Botrytis cinerea*. *Physiol Mol Plant Pathol* 74:376–386
- Dalmats B, Schumacher J, Moraga J, Le Pêcheur P, Tudzynski B, Collado IG, Viaud M (2011) The *Botrytis cinerea* phytotoxin botcinic acid requires two polyketide synthases for production and has a redundant role in virulence. *Mol Plant Pathol* 12:564–579
- Dean R, Van Kan JA, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD (2012) The top 10 fungal pathogens in molecular plant pathology. *Mol Plant Pathol* 13:414–430
- Debieu D, Bach J, Montesinos E, Fillinger S, Leroux P (2013) Role of sterol 3-ketoreductase sensitivity in susceptibility to the fungicide fenhexamid in *Botrytis cinerea* and other phytopathogenic fungi. *Pest Manag Sci* 69:642–651
- Dickman MB, Park YK, Oltersdorf T, Li W, Clemente T, French R (2001) Abrogation of disease development in plants expressing animal antiapoptotic genes. *Proc Natl Acad Sci USA* 98:6957–6962
- Doehlemann G, Berndt P, Hahn M (2006) Different signalling pathways involving a Galpha protein, cAMP and a MAP kinase control germination of *Botrytis cinerea* conidia. *Mol Microbiol* 59:821–835
- Espino JJ, Gutierrez-Sanchez G, Brito N, Shah P, Orlando R, Gonzalez C (2010) The *Botrytis cinerea* early secretome. *Proteomics* 10:3020–3034

- Faris JD, Zhang Z, Lu H, Lu SW, Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen JB, Xu SS, Oliver RP, Simons KJ, Friesen TL (2010) A unique wheat disease resistance-like gene governs effector-triggered susceptibility to necrotrophic pathogens. *Proc Natl Acad Sci USA* 107:13544–13549
- Fernandez-Acero JF, Colby T, Harzen A, Cantoral JM, Schmidt J (2009) Proteomic analysis of the phyto-pathogenic fungus *Botrytis cinerea* during cellulose degradation. *Proteomics* 9:2892–2902
- Fernandez-Acero JF, Colby T, Harzen A, Carbu M, Wieneke U, Cantoral JM, Schmidt J (2010) 2-DE proteomic approach to the *Botrytis cinerea* secretome induced with different carbon sources and plant-based elicitors. *Proteomics* 10:2270–2280
- Fernández-Álvarez A, Elías-Villalobos A, Ibeas JI (2009) The O-mannosyltransferase PMT4 Is essential for normal appressorium formation and penetration in *Ustilago maydis*. *Plant Cell* 21:3397–3412
- Fernández-Álvarez A, Marín-Menguiano M, Lanver D, Jiménez-Martín A, Elías-Villalobos A, Pérez-Pulido AJ, Kahmann R, Ibeas JI (2012) Identification of O-mannosylated virulence factors in *Ustilago maydis*. *PLoS Pathog* 8:e1002563
- Fillinger S, Ajouz S, Nicot PC, Leroux P, Bardin M (2012) Functional and structural comparison of pyrrolnitrin- and iprodione-induced modifications in the class III histidine-kinase Bos1 of *Botrytis cinerea*. *PLoS One* 7(8):e42520
- Fournier E, Giraud T, Loiseau A, Vautrin D, Estoup A, Solignac M, Cornuet JM, Brygoo Y (2002) Characterization of nine polymorphic microsatellite loci in the fungus *Botrytis cinerea* (Ascomycota). *Mol Ecol Notes* 2:253–255
- Fournier E, Giraud T, Albertini C, Brygoo Y (2005) Partition of the *Botrytis cinerea* complex in France using multiple gene genealogies. *Mycologia* 97:1251–1267
- Fournier E, Giraud T (2008) Sympatric genetic differentiation of a generalist pathogenic fungus, *Botrytis cinerea*, on two different host plants, grapevine and bramble. *J Evol Biol* 21:122–132
- Frías M, González C, Brito N (2011) BcSpl1, a ceratoplatenin family protein, contributes to *Botrytis cinerea* virulence and elicits the hypersensitive response in the host. *New Phytol* 192:483–495
- Frías M, Brito N, González C (2013) The *Botrytis cinerea* cerato-platanin BcSpl1 is a potent inducer of systemic acquired resistance (SAR) in tobacco and generates a wave of salicylic acid expanding from the site of application. *Mol Plant Pathol* 14:191–196
- Giesbert S, Schumacher J, Kupas V, Espino J, Segmüller N, Haeuser-Hahn I, Schreier PH, Tudzynski P (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* 25:481–495
- Gioti A, Simon A, Le Pêcheur P, Giraud C, Pradier JM, Viaud M, Levis C (2006) Expression profiling of *Botrytis cinerea* genes identifies three patterns of up-regulation in planta and an FKBP12 protein affecting pathogenicity. *J Mol Biol* 358:372–386
- Giraud T, Fortini D, Levis C, Leroux P, Brygoo Y (1997) RFLP markers show genetic recombination in *Botryotinia fuckeliana* (*Botrytis cinerea*) and transposable elements reveal two sympatric species. *Mol Biol Evol* 14:1177–1185
- Giraud T, Fortini D, Levis C, Lamarque C, Leroux P, LoBuglio K, Brygoo Y (1999) Two sibling species of the *Botrytis cinerea* complex, *transposa* and *vacuina*, are found in sympatry on numerous host plants. *Phytopathology* 89:967–973
- González M, Brito N, González C (2012) High abundance of Serine/Threonine-rich regions predicted to be hyper-O-glycosylated in the secretory proteins coded by eight fungal genomes. *BMC Microbiol* 12:213
- Govrin EM, Levine A (2000) The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Curr Biol* 10:751–757
- González M, Brito N, Frías M, Gonzalez C (2013) *Botrytis cinerea* protein O-mannosyltransferases play critical roles in morphogenesis, growth, and virulence. *PLoS One* 8(6):e65924
- Gourgues M, Brunet-Simon A, Lebrun MH, Levis C (2004) The tetraspanin BcPls1 is required for appressorium-mediated penetration of *Botrytis cinerea* into host plant leaves. *Mol Microbiol* 51:619–629
- Grant-Downton R, Terhem RA, Kapralov MV, Mehdi S, Rodriguez-Enriquez MJ, Gurr SJ, van Kan JAL, Dewey FM (2013) A novel *Botrytis* species is associated with a newly emergent foliar disease in cultivated *Hemerocallis* (Submitted for publication)
- Han Y, Joosten HJ, Niu W, Zhao Z, Mariano PS, McCalman M, van Kan J, Schaap PJ, Dunaway-Mariano D (2007) Oxaloacetate hydrolase, the C-C bond lyase of oxalate secreting fungi. *J Biol Chem* 282:9581–9590
- Hargreaves JA, Mansfield JW, Rossall S (1977) Changes in phytoalexin concentrations in tissues of the broad bean plant (*Vicia faba* L.) following inoculation with species of *Botrytis*. *Physiol Plant Pathol* 11:227–242
- Harrison JG (1980) The production of toxins by *Botrytis fabae* in relation to growth of lesions on bean leaves at different humidities. *Ann Appl Biol* 95:63–72
- ten Have A, Mulder W, Visser J, van Kan JAL (1998) The endopolygalacturonase gene Bcpg1 is required for full virulence of *Botrytis cinerea*. *Mol Plant-Microbe Interact* 11:1009–1016
- ten Have A, Espino JJ, Dekkers E, van Sluyter SC, Brito N, Kay J, González C, van Kan JA (2010) The *Botrytis cinerea* aspartic proteinase family. *Fungal Genet Biol* 47:53–65
- Heller J, Meyer AJ, Tudzynski P (2012a) Redox-sensitive GFP2: use of the genetically encoded biosensor of the redox status in the filamentous fungus *Botrytis cinerea*. *Mol Plant Pathol* 13:935–947
- Heller J, Ruhnke N, Espino JJ, Massaroli M, Collado IG, Tudzynski P (2012b) The mitogen-activated protein

- kinase BcSak1 of *Botrytis cinerea* is required for pathogenic development and has broad regulatory functions beyond stress response. *Mol Plant Microbe Interact* 25:802–816
- Hoffmeister D, Keller NP (2007) Natural products of filamentous fungi: enzymes, genes, and their regulation. *Nat Prod Rep* 24:393–416
- Kars I, McCalman M, Wagemakers L, van Kan JA (2005a) Functional analysis of *Botrytis cinerea* pectin methylesterase genes by PCR-based targeted mutagenesis: Bcpme1 and Bcpme2 are dispensable for virulence of strain B05.10. *Mol Plant Pathol* 6:641–652
- Kars I, Krooshof G, Wagemakers CAM, Joosten R, Benen JAE, van Kan JAL (2005b) Necrotising activity of five *Botrytis cinerea* endopolygalacturonases produced in *Pichia pastoris*. *Plant J* 43:213–225
- Kerssies A, Bosker-van Zessen AI (1997) Variation in pathogenicity and DNA polymorphism among *Botrytis cinerea* isolates sampled inside and outside a glasshouse. *Plant Dis* 81:781–786
- Krappmann S, Bayram Ö, Braus GH (2005) Deletion and allelic exchange of the *Aspergillus fumigatus* *veA* locus via a novel recyclable marker module. *Eukaryot Cell* 4:1298–1307
- Kretschmer M, Hahn M (2008) Fungicide resistance and genetic diversity of *Botrytis cinerea* isolates from a vineyard in Germany. *J Plant Dis Protect* 115:214–219
- Kretschmer M, Leroch M, Mosbach A, Walker AS, Fillinger S, Mernke D, Schoonbeek HJ, Pradier JM, Leroux P, De Waard MA, Hahn M (2009) Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus *Botrytis cinerea*. *PLoS Pathog* 5:e1000696
- Kroken S, Glass NL, Taylor JW, Yoder OC, Turgeon BG (2003) Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. *Proc Natl Acad Sci USA* 100:15670–15675
- Kunz C, Vandelle E, Rolland S, Poinssot B, Bruel C, Cimerman A, Zotti C, Moreau E, Vedel R, Pugin A, Boccara M (2006) Characterization of a new, non-pathogenic mutant of *Botrytis cinerea* with impaired plant colonization capacity. *New Phytol* 170:537–550
- 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* 22:2085–2101
- Leroch M, Kleber A, Silva E, Coenen T, Koppenhöfer D, Shmaryahu A, Valenzuela PD, Hahn M (2013a) Transcriptome profiling of *Botrytis cinerea* conidial germination reveals upregulation of infection-related genes during the pre-penetration stage. *Eukaryot Cell* 12:614–626
- Leroch M, Plesken C, Weber RW, Kauff F, Scalliet G, Hahn M (2013b) Gray mold populations in German strawberry fields are resistant to multiple fungicides and dominated by a novel clade closely related to *Botrytis cinerea*. *Appl Environ Microbiol* 79:159–167
- Leroch M, Mernke D, Koppenhoefer D, Schneider P, Mosbach A, Doehlemann G, Hahn M (2011) Living colors in the gray mold pathogen *Botrytis cinerea*: codon-optimized genes encoding green fluorescent protein and mCherry, which exhibit bright fluorescence. *Appl Environ Microbiol* 77:2887–2897
- Li X, Kerrigan J, Chai W, Schnabel G (2012a) *Botrytis caroliniana*, a new species isolated from blackberry in South Carolina. *Mycologia* 104:650–658
- Li B, Wang W, Zong Y, Qin G, Tian S (2012b) Exploring pathogenic mechanisms of *Botrytis cinerea* Secretome under different ambient pH based on comparative proteomic analysis. *J Proteome Res* 11:4249–4260
- Liu W, Zhou X, Li G, Li L, Kong L, Wang C, Zhang H, Xu JR (2011a) Multiple plant surface signals are sensed by different mechanisms in the rice blast fungus for appressorium formation. *PLoS Pathog* 7:e1001261
- Liu W, Leroux P, Fillinger S (2008) The HOG1-like MAP kinase Sak1 of *Botrytis cinerea* is negatively regulated by the upstream histidine kinase Bos1 and is not involved in dicarboximide- and phenylpyrrole-resistance. *Fungal Genet Biol* 45:1062–1074
- Liu W, Soulié MC, Perrino C, Fillinger S (2011b) The osmosensing signal transduction pathway from *Botrytis cinerea* regulates cell wall integrity and MAP kinase pathways control melanin biosynthesis with influence of light. *Fungal Genet Biol* 48:377–387
- Manteau S, Abouna S, Lambert B, Legendre L (2003) Differential regulation by ambient pH of putative virulence factor secretion by the phytopathogenic fungus *Botrytis cinerea*. *FEMS Microbiol Ecol* 43:359–366
- Martinez F, Blancard D, Lecomte P, Levis C, Dubos B, Fermaud M (2003) Phenotypic differences between vacuole and transposon subpopulations of *Botrytis cinerea*. *Eur J Plant Pathol* 109:479–488
- Martinez F, Dubos B, Fermaud M (2005) The role of saprotrophy and virulence in the population dynamics of *Botrytis cinerea* in vineyards. *Phytopathology* 95:692–700
- Massaroli M, Moraga J, Bastos Borges K, Ramirez-Fernández J, Viaud M, González Collado I, Durán-Patrón R, Hernández-Galán R (2013) A shared biosynthetic pathway for botcinins and botrylactones revealed through gene deletions. *Chem Bio* 14:132–136
- Mengiste T (2012) Plant immunity to necrotrophs. *Annu Rev Phytopathol* 50:267–294
- 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* 24:1074–1085
- Morcx S, Kunz C, Choquer M, Assie S, Blondet E, Simond-Côte E, Gajek K, Chapeland-Leclerc F, Expert D, Soulié MC (2013) Disruption of Bcchs4, Bcchs6 or Bcchs7 chitin synthase genes in *Botrytis*

- cinerea* and the essential role of class VI chitin synthase (Bcchs6). *Fungal Genet Biol* 52:1–8
- Morschhäuser J (2010) Regulation of multidrug resistance in pathogenic fungi. *Fungal Genet Biol* 47:94–106
- Mosbach A, Leroch M, Mendgen KW, Hahn M (2011) Lack of evidence for a role of hydrophobins in conferring surface hydrophobicity to conidia and hyphae of *Botrytis cinerea*. *BMC Microbiol* 11:10
- Moyano C, Alfonso C, Gallegro J, Raposo R, Melgarejo P (2003) Comparison of RAPD and AFLP marker analysis as a means to study the genetic structure of *Botrytis cinerea* populations. *Eur J Plant Pathol* 109:515–522
- Nielsen K, Yohalam DS (2001) Origin of a polyploidy *Botrytis* pathogen through interspecific hybridization between *Botrytis aclada* and *Botrytis byssoides*. *Mycologie* 93:1064–1071
- Nguyen QB, Itoh K, Van Vu B, Tosa Y, Nakayashiki H (2011) Simultaneous silencing of endo- β -1,4 xylanase genes reveals their roles in the virulence of *Magnaporthe oryzae*. *Mol Microbiol* 81:1008–1019
- Noda J, Brito N, González C (2010) The *Botrytis cinerea* xylanase Xyn11A contributes to virulence with its necrotizing activity, not with its catalytic activity. *BMC Plant Biol* 10:38
- Oliver RP, Solomon PS (2010) New developments in pathogenicity and virulence of necrotrophs. *Curr Opin Plant Biol* 13:415–419
- Pihet M, Vandeputte P, Tronchin G, Renier G, Saulnier P, Georgeault S, Mallet R, Chabasse D, Symoens F, Bouchara JP (2009) Melanin is an essential component for the integrity of the cell wall of *Aspergillus fumigatus* conidia. *BMC Microbiol* 9:177
- Pinedo C, Wang CM, Pradier JM, Dalmais B, Choquer M, Le Pêcheur P, Morgant G, Collado IG, Cane DE, Viaud M (2008) Sesquiterpene synthase from the botrydial biosynthetic gene cluster of the phytopathogen *Botrytis cinerea*. *ACS Chem Biol* 3:791–801
- Prado-Cabrero A, Scherzinger D, Avalos J, Al-Babili S (2007) Retinal biosynthesis in fungi: characterization of the carotenoid oxygenase CarX from *Fusarium fujikuroi*. *Eukaryot Cell* 6:650–657
- Quidde T, Osbourn AE, Tudzynski P (1998) Detoxification of α -tomatine by *Botrytis cinerea*. *Physiol Mol Plant Pathol* 52:151–165
- RajaguruShaw P, Shaw M (2010) Genetic differentiation between hosts and locations in populations of latent *Botrytis cinerea* in southern England. *Plant Pathol* 59:1081–1090
- Rampitsch C, Tinker NA, Subramaniam R, Barkow-Oesterreicher S, Laczkó E (2012) Phosphoproteome profile of *Fusarium graminearum* grown in vitro under nonlimiting conditions. *Proteomics* 12:1002–1005
- Reis H, Pfiffi S, Hahn M (2005) Molecular and functional characterization of a secreted lipase from *Botrytis cinerea*. *Mol Plant Pathol* 6:257–267
- Rolke Y, Liu S, Quidde T, Williamson B, Schouten A, Weltring KM, Siewers V, Tenberge KB, Tudzynski B, Tudzynski P (2004) Functional analysis of H₂O₂-generating systems in *Botrytis cinerea*: the major Cu-Zn-superoxide dismutase (BCSOD1) contributes to virulence on French bean, whereas a glucose oxidase (BCGOD1) is dispensable. *Mol Plant Pathol* 5:17–27
- Rossall S, Mansfield JW, Hutson RA (1980) Death of *Botrytis cinerea* and *B. fabae* following exposure to wyerone derivatives *in vitro* and during infection development in broad bean leaves. *Physiol Plant Pathol* 16:135–146
- Rowe HC, Kliebenstein DJ (2007) Elevated genetic variation within virulence-associated *Botrytis cinerea* polygalacturonase loci. *Mol Plant-Microbe Interact* 20:1126–1137
- Rowe HC, Walley JW, Corwin J, Chan EK, Dehesh K, Kliebenstein DJ (2010) Deficiencies in jasmonate-mediated plant defense reveal quantitative variation in *Botrytis cinerea* pathogenesis. *PLoS Pathog* 6:e1000861
- Rowe HC, Kliebenstein DJ (2010) All mold is not alike: the importance of intraspecific diversity in necrotrophic plant pathogens. *PLOS Pathog* 6:e1000759
- Rui O, Hahn M (2007) The Slr2-type MAP kinase Bmp3 of *Botrytis cinerea* is required for normal saprotrophic growth, conidiation, plant surface sensing and host tissue colonization. *Mol Plant Pathol* 8:173–184
- Saikia S, Nicholson MJ, Young C, Parker EJ, Scott B (2008) The genetic basis for indole-diterpene chemical diversity in filamentous fungi. *Mycol Res* 112:184–199
- Schamber A, Leroch M, Diwo J, Mendgen K, Hahn M (2010) The role of mitogen-activated protein (MAP) kinase signalling components and the Ste12 transcription factor in germination and pathogenicity of *Botrytis cinerea*. *Mol Plant Pathol* 11:105–119
- Schouten A, Wagemakers L, Stefanato FL, van der Kaaij RM, van Kan JA (2002) Resveratrol acts as a natural profungicide and induces self-intoxication by a specific laccase. *Mol Microbiol* 43:883–894
- Schumacher J, Kokkelink L, Huesmann C, Jimenez-Teja D, Collado IG, Barakat R, Tudzynski P, Tudzynski B (2008a) The cAMP-dependent signaling pathway and its role in conidial germination, growth, and virulence of the gray mold *Botrytis cinerea*. *Mol Plant-Microbe Interact* 21:1443–1459
- Schumacher J, Viaud M, Simon A, Tudzynski B (2008b) The G α subunit BCG1, the phospholipase C (BcPLC1), and the calcineurin phosphatase coordinately regulate gene expression in the grey mold fungus *Botrytis cinerea*. *Mol Microbiol* 67:1027–1050
- Schumacher J, de Larrinoa IF, Tudzynski B (2008c) Calcineurin-responsive zinc finger transcription factor CRZ1 of *Botrytis cinerea* is required for growth, development, and full virulence on bean plants. *Eukaryot Cell* 7:584–601
- Schumacher J, Pradier JM, Simon A, Traeger S, Moraga J, González Collado I, Viaud M, Tudzynski B (2012) Natural variation in the VELVET gene *bceV1* affects virulence and light-dependent differentiation in *Botrytis cinerea*. *PLoS One* 7:e47840

- Schumacher J (2012) Tools for *Botrytis cinerea*: New expression vectors make the gray mold fungus more accessible to cell biology approaches. *Fungal Genet Biol* 49:483–497
- Schumacher J, Gautier A, Morgant G, Le Pêcheur P, Azeddine S, Fillinger S, Leroux P, Tudzynski B, Viaud M (2013) A functional bikaverin biosynthetic gene cluster in rare isolates of *Botrytis cinerea* is positively regulated by the Velvet complex. *PLoS One* 8:e53729
- Schulze Gronover C, Schorn C, Tudzynski B (2004) Identification of *Botrytis cinerea* genes up-regulated during infection and controlled by the Galpha subunit BCG1 using suppression subtractive hybridization (SSH). *Mol Plant-Microbe Interact* 17:537–546
- Shah P, Gutierrez-Sanchez G, Orlando R, Bergmann C (2009) A proteomic study of pectin-degrading enzymes secreted by *Botrytis cinerea* grown in liquid culture. *Proteomics* 9:3126–3135
- Shah P, Powell ALT, Orlando R, Bergmann C, Gutierrez-Sanchez G (2012) Proteomic analysis of ripening tomato fruit infected by *Botrytis cinerea*. *J Proteome Res* 11:2178–2192
- Shipunov A, Newcombe G, Raghavendra AK, Anderson CL (2008) Hidden diversity of endophytic fungi in an invasive plant. *Am J Bot* 95:1096–1108
- Siegmund U, Heller J, van Kan JA, Tudzynski P (2013) The NADPH oxidase complexes in *Botrytis cinerea*: Evidence for a close association with the ER and the tetraspanin Pls1. *PLoS One* 8:e55879
- Shlezinger N, Minz A, Gur Y, Hatam I, Dagdas YF, Talbot NJ, Sharon A (2011a) Anti-apoptotic machinery protects the necrotrophic fungus *Botrytis cinerea* from host-induced apoptotic-like cell death during plant infection. *PLoS Pathog* 7:e1002185
- Shlezinger N, Doron A, Sharon A (2011b) Apoptosis-like programmed cell death in the grey mould fungus *Botrytis cinerea*: genes and their role in pathogenicity. *Biochem Soc Trans* 39:1493–1498
- Siewers V, Viaud M, Jimenez-Teja D, Collado IG, Gronover CS, Pradier JM, Tudzynski B, Tudzynski P (2005) Functional analysis of the cytochrome P450 monooxygenase gene *bcbot1* of *Botrytis cinerea* indicates that botrydial is a strain-specific virulence factor. *Mol Plant-Microbe Interact* 18:602–612
- Siewers V, Kokkelink L, Smedsgaard J, Tudzynski P (2006) Identification of an abscisic acid gene cluster in the grey mold *Botrytis cinerea*. *Appl Environ Microbiol* 72:4619–4626
- Simon A, Dalmats 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. *Fung Genet Biol* 52:9–19
- Sowley ENK, Dewey FM, Shaw MW (2010) Persistent, symptomless, systemic, and seed-borne infection of lettuce by *Botrytis cinerea*. *Eur J Plant Pathol* 126:61–71
- Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, Stüber K, Loren Ver, van Themaat E, Brown JK, Butcher SA, Gurr SJ, Lebrun MH, Ridout CJ, Schulze-Lefert P, Talbot NJ, Ahmadinejad N, Ametz C, Barton GR, Benjdia M, Bidzinski P, Bindschedler LV, Both M, Brewer MT, Cadle-Davidson L, Cadle-Davidson MM, Collemare J, Cramer R, Frenkel O, Godfrey D, Harriman J, Hoede C, King BC, Klages S, Kleemann J, Knoll D, Koti PS, Kreplak J, López-Ruiz FJ, Lu X, Maekawa T, Mahanil S, Micali C, Milgroom MG, Montana G, Noir S, O'Connell RJ, Oberhaensli S, Parlange F, Pedersen C, Quesneville H, Reinhardt R, Rott M, Sacristán S, Schmidt SM, Schön M, Skamnioti P, Sommer H, Stephens A, Takahara H, Thordal-Christensen H, Vigouroux M, Wessling R, Wicker T, Panstruga R (2010) Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. *Science* 330:1543–1546
- Staats M, van Baarlen P, van Kan JA (2005) Molecular phylogeny of the plant pathogenic genus *Botrytis* and the evolution of host specificity. *Mol Biol Evol* 22:333–346
- Staats M, van Baarlen P, Schouten A, van Kan JA, Bakker FT (2007a) Positive selection in phytotoxic protein-encoding genes of *Botrytis* species. *Fungal Genet Biol* 44:52–63
- Staats M, van Baarlen P, Schouten A, van Kan JA (2007b) Functional analysis of NLP genes from *Botrytis elliptica*. *Mol Plant Pathol* 8:209–214
- Staats M, van Kan JA (2012) Genome update of *Botrytis cinerea* strains B05.10 and T4. *Eukaryot Cell* 11:1413–1414
- Stefanato FL, Abou-Mansour E, Buchala A, Kretschmer M, Mosbach A, Hahn M, Bochet CG, Métraux JP, Schoonbeek HJ (2009) The ABC transporter BcatB from *Botrytis cinerea* exports camalexin and is a virulence factor on *Arabidopsis thaliana*. *Plant J* 58:499–510
- Stergiopoulos I, Collemare J, Mehrabi R, de Wit PJGM (2013) Phytotoxic secondary metabolites and peptides produced by plant pathogenic Dothideomycete fungi. *FEMS Microbiol Rev* 37:67–93
- Sutherland A, Auclair K, Vederas JC (2001) Recent advances in the biosynthetic studies of lovastatin. *Curr Opin Drug Discov Devel* 4:229–236
- Tan K-C, Oliver RP, Solomon PS, Moffat CS (2010) Proteinaceous necrotrophic effectors in fungal virulence. *Funct Plant Biol* 37:907–912
- Tan K-C, Ferguson-Hunt M, Rybak K, Waters ODC, Bond CS, Stukenbrock EH, Friesen TL, Faris JD, McDonald BA, Oliver RP (2012) Quantitative variation in effector activity of ToxA isoforms from *Stagonospora nodorum* and *Pyrenophora tritici-repentis*. *Mol Plant-Microbe Interact* 25:515–522
- Tani H, Koshino H, Sakuno E, Cutler HG, Nakajima H (2006) Botcinins E and F and botcinolide from *Botrytis cinerea* and structural revision of botcinolides. *J Nat Prod* 69:722–725
- Temme N, Tudzynski P (2009) Does *Botrytis cinerea* ignore H₂O₂-induced oxidative stress during infection? Characterization of botrytis activator protein 1. *Mol Plant Microbe Interact*. 22:987–998

- Temme N, Oeser B, Massaroli M, Heller J, Simon A, González Collado I, Viaud M, Tudzynski P (2012) BcAtf1- A global regulator controls various differentiation processes and phytotoxin production in *Botrytis cinerea*. *Mol Plant Pathol* 3:704–718
- Tenberge KB (2004) Morphology and cellular organization in *Botrytis* interaction with plants. In: Elad Y, Williamson B, Tudzynski P, Delen N (eds) *Botrytis: biology, pathology and control*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 67–84
- Valette-Collet O, Cimerman A, Reignault P, Levis C, Boccara M (2003) Disruption of *Botrytis cinerea* pectin methylesterase gene Bcme1 reduces virulence on several host plants. *Mol Plant-Microbe Interact* 16:360–367
- van Baarlen P, Staats M, van Kan JAL (2004) Induction of programmed cell death in lily by the fungal pathogen *Botrytis elliptica*. *Mol Plant Pathol* 5:559–574
- van Baarlen P, Woltering EJ, Staats M, van Kan JAL (2007) Histochemical and genetic analysis of host and non-host interactions of *Arabidopsis* with three *Botrytis* species: an important role for cell death control. *Mol Plant Pathol* 8:41–54
- van Kan JA (2006) Licensed to kill: the lifestyle of a necrotrophic plant pathogen. *Trends Plant Sci* 11:247–253
- Van Vu B, Itoh K, Nguyen QB, Tosa Y, Nakayashiki H (2012) Cellulases belonging to glycoside hydrolase families 6 and 7 contribute to the virulence of *Magnaporthe oryzae*. *Mol Plant-Microbe Interact* 25:1135–1141
- Viaud M, Brunet-Simon A, Brygoo Y, Pradier JM, Levis C (2003) Cyclophilin A and calcineurin functions investigated by gene inactivation, cyclosporin A inhibition and cDNA arrays approaches in the phytopathogenic fungus *Botrytis cinerea*. *Mol Microbiol* 50:1451–1465
- Viaud M, Fillinger S, Liu W, Polepalli JS, Le Pêcheur P, Kunduru AR, Leroux P, Legendre L (2006) A class III histidine kinase acts as a novel virulence factor in *Botrytis cinerea*. *Mol Plant-Microbe Interact* 19:1042–1050
- Villen J, Gygi SP (2008) The SCX/IMAC enrichment approach for global phosphorylation analysis by mass spectrometry. *Nat Protoc* 3:1630–1638
- Vogt K, Bhabhra R, Rhodes JC, Askew DS (2005) Doxycycline-regulated gene expression in the opportunistic fungal pathogen *Aspergillus fumigatus*. *BMC Microbiol* 5:1
- Walker AS, Gautier AL, Confais J, Martinho D, Viaud M, Le P, Cheur P, Dupont J, Fournier E (2011) *Botrytis pseudocinerea*, a new cryptic species causing gray mold in French vineyards in sympatry with *Botrytis cinerea*. *Phytopathology* 101:1433–1445
- De Waard MA, Andrade AC, Hayashi K, Schoonbeek HJ, Stergiopoulos I, Zwiers LH (2006) Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. *Pest Manag Sci* 62:195–207
- Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, Huang HD, Jin H (2013) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science* 342:118–123
- Williamson B, Tudzynski B, Tudzynski P, van Kan JAL (2007) Pathogen profile—*Botrytis cinerea*: the cause of grey mould disease. *Mol Plant Pathol* 8:561–580
- Williams B, Kabbage M, Kim HJ, Britt R, Dickman MB (2011) Tipping the balance: *Sclerotinia sclerotiorum* secreted oxalic acid suppresses host defenses by manipulating the host redox environment. *PLoS Pathog* 7(6):e1002107
- Windram O, Madhou P, McHattie S, Hill C, Hickman R, Cooke E, Jenkins DJ, Penfold CA, Baxter L, Breeze E, Kiddle SJ, Rhodes J, Atwell S, Kliebenstein DJ, Kim YS, Stegle O, Borgwardt K, Zhang C, Tabrett A, Legaie R, Moore J, Finkenshtadt B, Wild DL, Mead A, Rand D, Beynon J, Ott S, Buchanan-Wollaston V, Denby KJ (2012) *Arabidopsis* defense against *Botrytis cinerea*: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis. *Plant Cell* 24:3530–3537
- Wong Sak Hoi J, Dumas B (2010) Ste12 and Ste12-like proteins, fungal transcription factors regulating development and pathogenicity. *Eukaryot Cell* 9:480–485
- Yin W, Keller NP (2011) Transcriptional regulatory elements in fungal secondary metabolism. *J Microbiol* 49:329–339
- Yang Q, Yan L, Gu Q, Ma Z (2012) The mitogen-activated protein kinase kinase BcOs4 is required for vegetative differentiation and pathogenicity in *Botrytis cinerea*. *Appl Microbiol Biotechnol* 96:481–492
- Yan L, Yang Q, Jiang J, Michailides TJ, Ma Z (2011) Involvement of a putative response regulator Brrg-1 in the regulation of sporulation, sensitivity to fungicides, and osmotic stress in *Botrytis cinerea*. *Appl Microbiol Biotechnol* 90:215–226
- Yan L, Yang Q, Sundin GW, Li H, Ma Z (2010) The mitogen-activated protein kinase kinase BOS5 is involved in regulating vegetative differentiation and virulence in *Botrytis cinerea*. *Fungal Genet Biol* 47:753–760
- Zhang J, Wu MD, Li GQ, Yang L, Yu L, Jiang DH, Huang HC, Zhuang WY (2010a) *Botrytis fabiopsis*, a new species causing chocolate spot of broad bean in central China. *Mycologia* 102:1114–1126
- Zhang J, Zhang L, Li GQ, Yang L, Jiang DH, Zhuang WY, Huang HC (2010b) *Botrytis sinoallii*: a new species of the grey mould pathogen on *Allium* crops in China. *Mycoscience* 5:421–431

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