Sabine Fillinger · Yigal Elad *Editors*

Botrytis – the Fungus, the Pathogen and its Management in Agricultural Systems

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Preface

Botrytis, a fungal genus, is the focus of intensive scientific research worldwide. The complex interactions between this pathogen and the plants it infects (referred to collectively as the pathosystem) and the economic importance of the diseases caused by *Botrytis* on more than 1400 species of cultivated plants, many of which are important agricultural crops, render this pathogen of particular interest to farmers, agriculture experts, advisers, extension staff, students and researchers in many fields worldwide.

The idea for this book arose from the *Botrytis* symposium that took place in June 2013 near Bari, Italy. A book published on *Botrytis* in 2004 (*Botrytis*: *Biology, Pathology and Control*, Elad Y, Williamson B, Tudzynski P. Delen N (eds), Kluwer Academic Publishers (Springer)) has become the reference book on this subject. However, new aspects of the biology of *Botrytis* have since come to light, making it essential to publish a new edition.

This book is the product of intensive work by 41 authors, all of whom are leading scientists from various scientific disciplines studying *Botrytis* as a fungus and as a pathogen. The authors of this book have amassed state-of-the-art knowledge on diverse topics, including *Botrytis* epidemiology, disease management, biological and chemical control and aspects of the plant-pathogen interactome, including virulence factors and defence processes, signalling cascades, the oxidative burst and general biological aspects, such as vegetative incompatibility, mycoviruses and the revised *Botrytis* species concept. This book also provides reviews of the genetic and postgenomic analyses, such as transcriptomics and proteomics, used to study *Botrytis* biology and pathogenicity.

This 20-chapter book is a comprehensive treatise covering the rapidly developing science of *Botrytis* and reflecting the major developments in studies of this fungus.

It will serve as a source of general information for specialists in agriculture and horticulture, but also for students and scientists interested in the biology of this fascinating, multifaceted phytopathogenic fungal species.

Thiverval-Grignon, France — Sabine Fillinger
Bet Dagan, Israel — Yigal Elad

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The editors thank Professor Melané Vivier from Stellenbosch University for her critical reading and constructive correction of Chapters 17–19. We also thank all the recognised and anonymous authors and reviewers for their contributions to this new *Botrytis* book, the production of which was an exciting and rich experience.

Contents

Chapter 1
Botrytis, the Good, the Bad and the Ugly

Yigal Elad, Melané Vivier, and Sabine Fillinger

Abstract *Botrytis* spp. are efficient pathogens, causing devastating diseases and significant crop losses in a wide variety of plant species. Here we outline our review of these pathogens, as well as highlight the major advances of the past 10 years in studying *Botrytis* in interaction with its hosts. Progress in molecular genetics and the development of relevant phylogenetic markers in particular, has resulted in the characterisation of approximately 30 species. The host range of *Botrytis* spp. includes plant species that are members of 170 families of cultivated plants.

Keywords Host range • Control strategies • Genomics • Fungus-plant interaction

1.1 What Makes *Botrytis* Species Such Threats?

The genus *Botrytis* is highly diverse, with numerous species identified that differ in terms of their biology, ecology, morphological features and host range. Intensive research has been carried out since the publication of the previous Botrytis book (Elad et al. 2004). Progress in molecular genetics and the development of relevant phylogenetic markers in particular, has resulted in the characterisation of approximately 30 species, of which 7 new species, a hybrid and a species complex have been identified in the last decade (Chap. 6).

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Fig. 1.1 *Botrytis cinerea* on a lisianthus cotyledon (Photo: Yigal Elad)

Species of *Botrytis* are responsible for heavy losses in a number of economically important horticultural and floral crops. Most species have a limited host range attacking either monocotyledonous or dicotyledonous plants. A notable exception is *B. cinerea* with a very wide host range (Chap. 20), but *B. pseudocinerea* was found on diverse plant hosts as well and may be suspected to have also a broad host range as well (Walker et al. 2011; Plesken et al. 2015). The plant hosts of *Botrytis* spp. include species of 170 families of cultivated plants (Chap. 20).

The pathogen displays an extraordinary variability in phenotypic traits, making it a model for studying sources of variation in filamentous fungi. Moreover, evidence that some species can be present as (non-symptomatic) endophytes is increasing (Chap. 2). Epidemics of *Botrytis*-incited grey mould are common in open fields, orchards and greenhouses (Fig. 1.1). These infections are promoted by high humidity. A *Botrytis* epidemic comprises a sequence of processes, each of which is influenced by the host and the surrounding environment (Chap. 7). For most *Botrytis*-induced diseases, the pathogen disperses by large amounts of air-dispersed conidia. Hence aerobiology plays a key role in understanding the epidemiology of *Botrytis* spp., since they have developed a variety of strategies to infect and colonise their host (Chap. 7).

With regard to control, different cultural methods aiming to reduce humidity can be combined for improved disease suppression (Chap. 8), in addition to application of chemical botryticides or biocontrol treatments. Recent years have seen the development of many biological control agents and other biopesticides, such as plant extracts, minerals and organic compounds, against *Botrytis*-incited diseases (Chap. 9). Chemical control based on the application of synthetic fungicides, however still constitutes the principal means of efficient and reliable protection of

many crops (Chap. 10). In addition, integrated management programs adopting a holistic approach are the key for minimizing postharvest losses caused by *B. cinerea* (Chap. 11).

1.2 Sex, Drugs and Viruses

Botrytis cinerea is an interesting model system for necrotrophic pathogens, which is not simple to deal with. Among natural strains, variations in karyotypes are frequent and some isolates of this heterothallic fungus behave as dual-maters without changes in the mating-type genes (Chap. 3). Genome plasticity and evolution may be due to mobile genetic elements, such as transposons (Biémont 2010) or inteins, these latter elements representing local hotspots for meiotic recombination in fungi (Liu and Yang 2004). The presence of the active PRP8 intein varies among *Botrytis* spp. and *B. cinerea* can display high rates of meiotic gene conversion in crosses between an isolate that possesses an intein element and an isolate that doesn't (Bokor et al. 2010, 2012). Compared to other fungal species, the *B. cinerea* genome seems to be rather poor in transposable elements (0.7–4 %). Repeat induced point mutation (RIP) of duplicated sequences constitutes another fungal meiotic gene modification mechanism involved in genome evolution (Galagan and Selker 2004) and might be responsible for reduced expansion of transposable elements in this species (Amselem et al. 2011, 2015). Crosses, gene knockouts, site-directed mutagenesis and gene-fusions are current genetic tools, which can be used in addition to functional genomics to address important biological questions (Chaps. 3, 12, 13, 14, 15, 16, and 17).

The genetic basis of vegetative incompatibility in *B. cinerea* is not known, but is presumed to conform to the system found in other ascomycetes. The large number of vegetative compatibility groups (VCGs) and limited occurrence of isolates displaying the same VCG suggest that sexual recombination occurs in field populations of *B. cinerea* (Chap. 4). Evidence of recombination and gene flow, between and within populations, has also been obtained by population genetics as indirect evidence for sexual recombination (Chap. 6). Interestingly, RNA mycoviruses have been found to be widespread in *Botrytis* and some can even attenuate pathogenicity of their fungal host (Chap. 5). The identification of mycoviruses capable of overcoming vegetative incompatibility in horizontal transmission could be useful to establish mycoviruses as a control mechanism against plant diseases caused by *Botrytis* (Chaps. 4 and 5).

1.3 Communicating with the Plant

Molecular research, including the complete genome sequencing and analyses of two *B. cinerea* strains, has led to a working model outlining the molecular strategy used by *B. cinerea* in the infection process. In Chap. 12, the significant advances

made in understanding the molecular mechanisms underlying *B. cinerea* attack on susceptible hosts are highlighted. In the last decade, much new knowledge has been gained about the signalling cascades that mediate the communication between environment and the cellular machinery regulating developmental programs in *B. cinerea* (Chap. 13). Genomic analyses have been complemented by proteomics and led to the identification of new virulence factors (Chap. 16).

During host-pathogen interactions reactive oxygen species (ROS) are of key importance for plant defence, but also for fungal attack. Since *Botrytis* exploits this plant defence reaction and even contributes to the oxidative burst, the fungus needs a robust oxidative stress responsive system to cope with ROS. Chapter 14 deals with the role of ROS in *Botrytis* – host interaction and both ROS generating and detoxifying systems and their importance for pathogenicity. *B. cinerea* has the potential to produce many secondary metabolites (SMs) including botrydial and botcinic acid, two unspecific phytotoxins contributing to the necrotrophic and polyphagous lifestyle of the fungus (Chap. 15).

A key challenge to understand the pathology of *Botrytis* spp. involves unravelling host responses. Chapter 17 updates recent knowledge of the complex regulatory mechanisms and multiple downstream defence processes achieved by high-throughput 'omics technologies'. Chapter 18 focuses on the cell walls of host plant tissues during infections by *Botrytis* and on fungal enzymes that could modify plant cell walls as a consequence of infection. Chapter 19 describes how fruit ripening gradually reduces host tissue resistance, and how *Botrytis* modifies its infection strategy accordingly.

1.4 The Good Side of *Botrytis*

Botrytis cinerea not only provokes damaging plant diseases but it is also at the origin of noble rot used for sweet dessert wines (e.g. Sauternes). The incidence of noble rot on the grape berries can be extremely variable according to weather conditions. The grapes are dehydrated, concentrating the juice, and the original composition of botrytized grapes is not only due to the *B. cinerea* metabolism, but also to desiccation, known as *passerillage* (Magyar 2011).

To increase the ability to control the noble rot development, artificial induction of *B. cinerea* strains in harvested grapes has been tested since the middle of the twentieth century and incited researchers to select *B. cinerea* strains with higher noble rot efficacy (Azzolini et al. 2013). However, it was shown recently by population genetics that *B. cinerea* populations isolated from grey mould and noble rot are genetically not different (Fournier et al. 2013). Noble rot symptoms therefore do not seem to be caused by a specific *B. cinerea* population, but instead seem to depend essentially on microclimatic conditions, which infer that continuing research on climate conditions that promote the production of sweet wines is still necessary.

1.5 What Has Changed Since the Last *Botrytis* Book?

1.5.1 One Fungus: One Name

Pleomorphism is encountered in many of the most important ascomycete plant pathogens such as the different morphological forms and different sexual stages. A dual system of fungal nomenclature was proposed in 1905, recommending both asexual and sexual names for fungi. In that sense, *Botrytis cinerea* is the name of the asexual stage (anamorph) and *Botryotinia fuckeliana* that of the sexual stage (teleomorph) (Faretra et al. 1988). DNA sequence comparisons have made it possible to reliably connect asexual states of fungi to their sexual states. A community effort of the mycologists was launched in 2011 to simplify the taxonomy and nomenclature of fungi, known as the 'one fungus – one name' initiative (reviewed in Wingfield et al. 2012). The *Botrytis* community agreed in 2013 at the Botrytis-Symposium in Bari to use *Botrytis cinerea* as generic name. For all other species of the same genus, the species prefix *Botrytis* is adopted.

1.5.2 Agreement on a Common Gene Nomenclature

The completion of the *B. cinerea* genome sequences and the automatic annotation of more than 10.000 genes have increased the frequency of newly identified genes, albeit still different formats of *Botrytis* gene and protein names exist in literature. The *Botrytis* community involved in the genome project agreed at the 'Botrytis-Sclerotinia genome workshop' in Versailles in 2006 on a common gene nomenclature that is summarized in Table 1.1. Gene names should be written in a three-letters-one-digit code. A prefix "*Bc*" can be used for *B. cinerea* genes if the species is ambiguous. Genes and alleles are to be written in lower case italics (*abc1*), while the unified format of protein names is standard characters with the first letter in upper case (Abc1). The mutant alleles are in superscript (numbers, known mutation or phenotype abbreviation) following the gene name, ex: $abc1^{T324S}$, knockouts: Δ*abc1*. Genotype symbols are italicised, phenotype symbols are not.

Table 1.1 Proposed nomenclature for *Botrytis* genes and proteins

Gene	Protein	Allele	Comment
Bcpde1	BcPde1	Δ*Bcpde1*	Gene name preceded by *B. cinerea*'s initials; the mutant allele is the gene deletion
bos1	Bos1	$bos1^{I365S}$	The amino-acid replacement of the mutant allele is indicated in superscript
cyp684	Cyp684	$cyp684^{HydR2}$	The phenotype associated to the mutant allele is indicated in superscript

The following exceptions may apply: (i) for gene families which have ubiquitous nomenclature (e.g. ABC-transporters; extracellular polysaccharide degrading enzymes, cytochrome P540s, etc.) this general nomenclature should be adapted accordingly; (ii) gene names published before 2015 shall not be renamed. This convention may be used for publication purposes.

1.5.3 Botrytis *Infection: Which Way to Choose?*

Botrytis spp. have long been considered exclusively as necrotrophic pathogens, but evidence is accumulating that some *Botrytis* species can be present inside plant tissues without triggering disease symptoms and should therefore be considered as endophytes. These infections may in some cases induce symptoms at a later stage or be transferred by seed propagation. Numerous dicotyledonous plants have been found to harbour endophytic *B. cinerea* infestation, although the full implications of this type of interaction remain unknown (reviewed in Chap. 2 and Van Kan et al. 2014). The newly identified species *B. deweyae* is suspected to have emerged as pathogen from endophytic origin (Grant-Downton et al. 2014). The capacity of endophytic development before switching to disease (necrotrophy) makes the infection cycle more complex and renders *Botrytis* disease management even more challenging. Identifying the factors that determine the choices in lifestyles of the fungus and understanding the underlying regulatory patterns constitute interesting future research *foci*.

1.5.4 The Host Range of Botrytis

This book comprises the most complete inventory of *Botrytis* diseases ever reported, increasing the reported number of hosts for *B. cinerea* from over 200 (Jarvis 1977) to more than 1,400 (Chap. 20). Fungi belonging to the *Botrytis* genus infect all plant parts including seeds, and other planting material, seedlings, stems, leaves, flowers and fruits at pre-harvest and postharvest stages. It is now clear that 596 plant genera are known to be affected by *Botrytis* spp. and 586 genera are affected by *B. cinerea* (Chap. 20). Of the 596 genera, the majority belongs to the division of seed plants, few (15) belong to the division of flowerless plants, and only one to the division of spore-bearing vascular plants. *Botrytis* host plants include species that grow in a variety of climate regions spanning from the tropics to cold regions, in humid as well as in dry places, in open fields, in greenhouses, in closed environments and even during cold storage. Host plants affected by *Botrytis* spp. are native to most continents, i.e., the Americas, Africa, Europe, Asia, Australia and various islands (Chap. 20).

1.5.5 Light Regulation

In the past it was demonstrated that *B. cinerea* responds to near-UV, blue, red and far-red light. A "two-receptor-model" was postulated in which near-UV/ blue- and red/ far-red-reversible photoreceptors are closely interacting to regulate asexual reproduction (Epton and Richmond 1980). Later it was demonstrated that *B. cinerea* possesses near-UV-sensing cryptochromes (BcCry1, BcCry2), potential blue light sensors (BcWcl1, BcVvd1, BcLov3, BcRgs1), opsins (Bop1, Bop2) as well as red/ far-red light-sensing phytochromes (BcPhy1-3) (Schumacher and Tudzynski 2012). Exposure of a *B. cinerea* isolate to white light resulted in induction of expression of 249 light-induced genes, among which genes involved in photoperception, oxidative stress response and transcription (Schumacher et al. 2014). Chapter 13 gives an overview about light-regulation in *B. cinerea*.

1.5.6 Secondary Metabolites

Sequencing and annotation of the complete *B. cinerea* genome (Amselem et al. 2011) revealed more than 40 clusters of genes dedicated to the synthesis of polyketides, terpenes, non-ribosomal peptides and alkaloids which indicates that *B. cinerea* has the potential to produce many metabolites that have not been described so far (Chap. 15). It is suggested that toxins and secondary metabolites (SMs) play a significant role in plant tissue colonization and some of these metabolites seem to be crucial for necrotrophic behavior (Chap. 13). Interestingly, the *Velvet* gene complex was shown to link light-dependent development and secondary metabolism in *B. cinerea* (Schumacher et al. 2012). Many *B. cinerea* SMs are detailed in Chap. 13. However, besides botcinic acid and botrydial that were shown to be required for necrotrophy, the biological role of several other SMs needs to be investigated. The role of many SMs was not previously linked to disease development in *Botrytis* and still awaits elucidation (Chap. 13). Findings so far are just a tip of the iceberg of necrotrophic fungal effectors.

1.5.7 The Plant-Botrytis *Interactome*

The paradigm of *B. cinerea* infection has been revisited during the past years. It is now clear that the pathogenic development of *B. cinerea* is far more complex than previously appreciated. Disease progression is tightly regulated during the entire infection process, and the fungus makes developmental adaptations that match the different infection stages. *Botrytis* infections are "dynamic events" that involve significant crosstalk and even modulation of the reaction of the host by the pathogen. An exciting example of such modulation is the uncovering of the role of

B. cinerea small interfering RNAs (Bc-siRNAs) as effector molecules. Protein effectors are known to be delivered into host cells by plant and animal pathogens, but the delivery of Bc-siRNAs into plant hosts provides an example of active trans-kingdom movement of siRNAs (Knip et al. 2014). siRNAs could be exchanged between cells of an organism, typically leading to gene silencing events in cells and tissues. Moreover, pathogen genes can be silenced by plant-produced siRNAs through RNA interference (RNAi) when these specific genes are targeted through a process known as Host-Induced Genes Silencing (HIGS). Interestingly, Bc-siRNAs have been shown to utilise the RNAi machinery of the host cell to target and silence defense genes in the hosts. The pathogen thus hijacks an inherent host defence component to enhance its own pathogenicity (Weiberg et al. 2013; Knip et al. 2014). Both plant (host) machinery and pathogen factors are essential to this sophisticated mechanism of modulating the host response (Weiberg et al. 2013).

Other equally subtle examples of the crosstalk between pathogens and hosts include the very interesting observation that *Botrytis* infection can enhance ripening in unripe tomatoes (Chap. 19). On the other hand, ripeness level in tomatoes also influences the success of infections: green tomatoes are resistant to *Botrytis*, whereas ripening tomatoes become susceptible (Fig. 1.2). Therefore, the inherent developmental processes and ripening-specific changes in organs encountered by *Botrytis* cause subtle changes to these "substrates" that either make them easy to infect or perhaps more resistant. The host cell walls are particularly important in this scenario, since these are the barriers that *Botrytis* needs to breach to gain access to the

Fig. 1.2 ***Botrytis cinerea*** **infection of ripe tomato fruit**. Close-up photo of *B. cinerea* growing on a droplet of juice released by a wounded ripe tomato (Photo by Dario Cantu; reproduced with permission from PNAS (2008) 105: 827; Copyright National Academy of Sciences, U.S.A.)

plant's sub-tissue to utilise its resources. Genome-wide transcriptional profiling of *B. cinerea* infecting different hosts has led to important insights regarding genes targeting plant cell walls to facilitate infection (Blanco-Ulate et al. 2014). A large number (275) of putatively secreted Carbohydrate-Active enZymes (CAZymes) were annotated in the *B. cinerea* genome and it is tempting to speculate that the diversity of *Botrytis* CAZymes could contribute to its wide host range (Blanco-Ulate et al. 2014). This study also shows that *Botrytis* probably modulates its enzymatic arsenal depending on the composition of the specific host cell walls, clearly showing the ability to adapt its infection strategy as per host encountered.

These examples highlight the importance of studying the infection process as a dynamic interaction. Indeed, this is not a new idea; it has been acknowledged and appreciated for a while, but implementing strategies to achieve this was particularly challenging from an experimental design and technology point of view. The availability of sequenced genomes of *Botrytis* species, as well as an increasing availability of sequenced plant genomes provide essential baseline information to support experiments where the interactome of pathogens and hosts can be studied. The importance to "follow" an infection cycle with high-resolution using systems-biology was recently shown in a study of the *Botrytis* transcriptome in interaction with *Arabidopsis* and the evaluation of the data using network-modelling approaches (Windram et al. 2012).

1.5.8 Control

Although chemical control of *Botrytis* still largely relies on the use of chemical pesticides, suppression of diseases can significantly benefit from cultural management of crops mainly by restricting the humidity around the plant and by limiting plant surface wetness. Plant and microbial extracts, organic compounds, and biocontrol agents have become available in some countries for *Botrytis* suppression either for use at pre- or postharvest. In the last few years, the definition of biocontrol has been broadened to include a range of alternative means of control (Chap. 9). Biocontrol relies on various modes of action including induced resistance (IR) i.e., application of natural compounds or their derivatives that are able to upregulate plant genes contributing to systemic resistance. A major advancement in this field is the understanding of the plant responses to the resistance inducers. For instance, *Trichoderma* that induces systemic resistance against *B. cinerea* primes salicylic acid (SA) and ethylene (ET)-related genes in strawberry (Meller Harel et al. 2014). Biocontrol induced by biochar primes defence genes in ET and jasmonic acid (JA)-related pathways in tomato, but biochar-mediated IR in the *B. cinerea*-tomato pathosystem exclusively involves the JA pathway (Mehari et al. 2015). More examples regarding the advancement in IR research are given in Chap. 9.

New developments based on the combination of biocontrol agents, physical means, natural antimicrobials, and decontaminating agents have also been achieved with post harvest *Botrytis* management (Chap. 11). These alternative methods are

effective in small-scale experiments, as standalone treatments, whereas commercial applications are less effective. It is recommended to combine alternative methods at postharvest (Romanazzi et al. 2012; Mlikota Gabler et al. 2010) along with preharvest disease and inoculum suppression.

During the last decades, restriction in fungicide application have become necessary to reduce their impact on the environment (Fenner et al. 2013) and to limit fungicide residues on harvest (Verger and Boobis 2013), requiring optimized protection strategies. At the same time, acquired resistance to most botryticides arose in many agronomical situations, sometimes impeding fungicide efficacy and leading to additional sprays. Not only are the frequently found target-site modifications responsible for *Botrytis*' strong capacity to cope with most fungicides, but active fungicide efflux leading to multiple drug resistance (MDR) is increasingly observed in *B. cinerea* (Kretschmer et al. 2009; Leroch et al. 2013). Chapter 10 gives a detailed overview of the current state of knowledge of fungicide resistance to end up with strategy proposals combining cropping practices for optimal resistance management.

1.6 New Knowledge, New Tools

1.6.1 Botrytis *Genomic Resources*

One of the prominent changes since the previous *Botrytis* book (Elad et al. 2004) is the determination of the genome sequences of three *B. cinerea* strains, as alluded to in many of the book chapters. The first sequencing initiative was launched already in the late 1990s by Syngenta, but the sequence of strain B05.10 was only published at the same time as the sequence of strain T4, resulting from an international community effort (Amselem et al. 2011). Thanks to the improvement of sequencing technologies and the extreme reduction of sequencing costs, improved genome sequences and gene annotations of both strains were published only 1 year later (Staats and Van Kan 2012). The genomes and annotations can be accessed and searched at the website of URGI.[1]

A new, near-complete assembly of the B05.10 genome, based on PacBio sequence data and confirmed by a linkage map and optical map can be accessed through the EBI webserver[2] (J. van Kan, *pers. comm.*). The assembly consists of 18 chromosomes (including two mini-chromosomes of 208 and 247 kb, respectively) without any internal gaps, of which 10 are full length from telomere to telomere. In addition, the genome sequence of the noble rot isolate BcWD1 (Blanco-Ulate et al. 2013) is accessible in public databases.[3] The three strains differ by roughly 162,000 SNPs (≈4 SNPs/kb).

[1] http://urgi.versailles.inra.fr/Species/Botrytis

[2] http://fungi.ensembl.org/Botrytis_cinerea/Info/Index

[3] DBJ/EMBL/GenBank accession no.AORW00000000.

RNA sequencing considerably improved the quality of genome annotation including gene prediction for *B. cinerea*, but also constitutes the current technology for global expression studies (see below). 11,701 genes have been predicted with substantial cDNA support for the last version of the B05.10 genome, while there were 16,448 predicted genes in the first genome version. The noble rot strain does not differ significantly either in genome size or in predicted gene number (11,073). Whether differences in secreted proteins or CAZymes (Blanco-Ulate et al. 2013) constitute a general feature of noble rot strains, remains to be investigated. A community effort has been organized with support from EBI in the framework of the Ensembl Fungi platform for manual annotation of all genes of the *B. cinerea* B05.10 strain.

1.6.2 Functional Genomics

The establishment of the *B. cinerea* genome sequences paved the way to "post-genomic" analyses, such as transcriptomics[4] (Chap. 17), proteomics and phosphoproteomics (Chap. 16), which allowed the identification of new virulence factors (e.g., Frias et al. 2011). In addition, high-throughput functional genomics, such as random mutagenesis (insertion-library) or site-directed mutagenesis with optimized tools (Schumacher 2012, Fig. 1.3) have become possible, but also the identification of transcription-factor binding sites through a yeast-one hybrid approach (Simon et al. 2013). All published gene knockout mutants for *B. cinerea* can be retrieved on

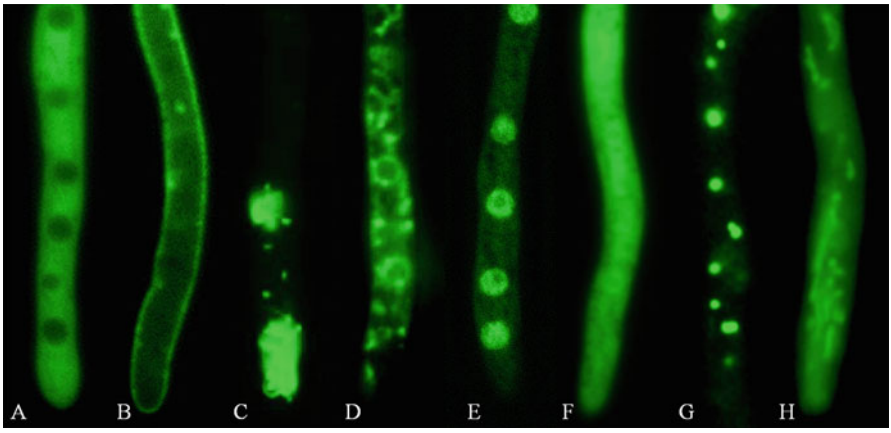

Fig. 1.3 GFP-tagged *Botrytis cinerea* hyphae. The observed green fluorescence is due to the following localisations: (**a**) cytosolic (not nuclear); (**b**) membrane; (**c**) hyphal tip and septum (F-actin); (**d**) endoplasmic reticulum; (**e**) nuclei; (**f**) cytosolic and nuclear; (**g**) peroxisomes; (**h**) mitochondria (Microscopic images kindly provided by R. Marschall and J. Schumacher)

[4] http://www.ncbi.nlm.nih.gov/gds/?term=Botrytis+cinerea[Organism]

a dedicated web site.[5] Genetic and genomic tools are important resources to get deeper insights into the fungal biology, to identify pathogenicity factors and new fungicide targets. They also accelerate the identification of naturally occurring mutations such as those involved in fungicide resistance.

1.6.3 Genome Perspectives

The genomes of six additional *B. cinerea* strains and of 11 different *Botrytis* species (*B. calthae*, *B. convoluta*, *B. elliptica*, *B. galanthina*, *B. hyacinthi*, *B. paeoniae*, *B. porri*, *B. narcissicola*, *B. tulipae*, *B. fabae*, *B. pseudocinerea*) have been sequenced (Staats and Van Kan, unpublished data; M. Hahn, *pers. comm.*). Large scale intra- and inter-species comparison will certainly help to understand some aspects of genome plasticity in *Botrytis*, such as variation in karyotypes (Chap. 3) or infection development (endophytic versus necrotrophic behaviour; grey mould versus noble rot), but also factors involved in the adaptation to the biotic and abiotic environment (plant hosts, fungicides, microclimate conditions). Interspecies comparison may allow to trace the evolutionary history of dispensable genomic elements, e.g., the bikaverin gene cluster (Campbell et al. 2013, Chap. 15) and facilitate our understanding of host adaptation.

1.7 Conclusions

Fungi of the genus *Botrytis* are important pathogens of many economically important crops, such as grapevine and many other crops, at pre- and post-harvest stages (Chap. 20). Intensive research has led to accumulation of important information on *Botrytis* spp. and their establishment in host tissues. The recent advances in *Botrytis* research surely will all have a major impact on disease prevention and control. However, further research efforts are needed on these fungi and their interactions with plants. For instance, it has been clear for a very long time that *Botrytis* spp., particularly *B. cinerea*, are necrotrophs, until recent studies have demonstrated that some species can act as endophytes in plants. The study of the endophtyic processes, the host reactions to the endophytic phase, as well as the switch from an endophytic to an aggressive stage needs to be prioritised to improve *Botrytis* management.

There is a significant importance in genetic analysis of *Botrytis* (Chap. 3). The large number of vegetative compatibility groups (VCGs), the limited occurrence of isolates displaying the same VCG (Chap. 4) and population genetics (Chap. 6) indicate that sexual recombination occurs in *B. cinerea* populations. Despite this, only limited reports of actual apothecia present in natural field settings are available. This calls for further research into the role of the sexual stage in shaping the variability of *Botrytis* spp. There is evidence for a high proportion of *B. cinerea* isolates

[5] http://botbioger.versailles.inra.fr/botmut/

containing mycoviruses (Chap. 5). In view of the vegetative incompatibility abundance in *Botrytis* forming barriers for movement of mycoviruses in fungal populations, it is crucial to discover and to characterize novel mycoviral species in *Botrytis*, but also to test their potential in biocontrol of the pathogen. Furthermore, genetical and phenotypical variation in *Botrytis* populations is immense (Chaps. 3 and 6).

Improved knowledge of the components of variability and of epidemiological (Chap. 7) behaviour like spatial distribution of disease and inoculum has been achieved and needs to be pursued. This may also help to reduce *Botrytis*-incited losses to a level that is acceptable at pre- or post-harvest stages, which still pose a great challenge for producers and handlers. Different cultural (Chap. 8), chemical (Chap. 10) and biological (Chap. 9) methods in the field and various compounds and methods after harvest (Chap. 11) can be used for improved disease suppression. Yet, the combination of all types of disease management in holistic systems needs further attention for effective *Botrytis* suppression along with strategies to minimize the development of resistance to chemical botryticides and of chemical fungicides residues in the produce. Such combination of control methods needs to take advantage of the right fungicide and of epidemiological considerations for the achievement of long lasting efficient disease suppression systems.

Studying the infection process still holds potential for further understanding of the plant pathogen interaction (Chap. 12). Future discoveries of the fungal factors, the identification of their plant targets and response processes are promising for improved plant defense tools. Furthermore, the identification of signal transduction pathways in the pathogen, those recognizing the plant host or the fungal specific light signaling pathways can be a future gateway to understand processes that govern the pathogen (Chap. 13). Oxidative burst is part of the successful infection of *B. cinerea* (Chap. 14). However, ROS scavenging and detoxification during infection is not clear and needs thorough clarification in order to understand how the fungus produces and copes with large amounts of ROS. Part of the infection complex involves production of SMs by the pathogen (Chap. 15). There is much awaiting elucidation as for the mode of action and the actual role of SM in the *Botrytis* infection process; nevertheless, their activities toward plants could have interesting toxic activities and requires further research.

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Chapter 2
Botrytis-Biology, Detection and Quantification

Frances M. Dewey (Molly) and Robert Grant-Downton

Abstract Species of *Botrytis* are responsible for heavy losses in a number of economically important horticultural and floral crops, the most important being *Botrytis cinerea*. Most species, except *B. cinerea*, have a limited host range attacking either monocotyledonous or dicotyledonous plants. Within the genus there are about 28 well-described species but new species continue to be isolated. Most species are opportunist growing as saprophytes on dead and decaying matter but have the ability to become aggressive pathogens under environmental conditions adverse to their hosts. Evidence that some species can be present as endophtyes (non-symptomatic) is increasing, as is evidence that such infections may either become aggressive at a later stage, notably at flowering time, or be transferred non-symptomatically by clonal or seed propagation of the host. Both the sexual and asexual stages are known for *B. cinerea*. The common method of dispersal of nearly all species is the production of asexual spores (macroconidia, common name conidia) dispersed by wind or water. Survival from one season to the next is generally by the production of sclerotia. Infections are most easily recognized by the appearance of characteristic grey conidial clusters on the surfaces of infected material but early detection, preconidiation or in non-symptomatically infected material is difficult; commonly surface sterilization or freezing of material followed by plating out on selective media is used. Detection at the species level requires molecular methods with species-specific probes. Detection and quantification, at the genus level, in extracts from infected tissues, juice and wines, is relatively easy using commercially produced rapid Lateral Flow immunological devices.

Keywords Botrytis species • Conidia • PCR • Monoclonal antibodies • ELISA

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2.1 Introduction

The genus *Botrytis* comprises about 28 well-described species (Beever and Weeds 2004). Each year new species continue to be identified such as *B. caroliniana*, pathogenic on blackberries (Li et al. 2012), *B. fabiopsis* on broad beans in Central China (Zhang et al. 2010a), *B. deweyae* on daylilies (*Hemerocallis*) (Grant-Downton et al. 2014) and *B. sinoallii* on *Allium* crops in China (Zhang et al. 2010b; Chap. 6). Most species, with the exception of *B. cinerea*, have a limited host range attacking only a few species of either monocotyledonous plants or dicotyledonous plants while some, such as the snowdrop fungus, *Botrytis galanthina*, appear to be host-specific (Beever and Weeds 2004). Undoubtedly, the most common and the most important species of *Botrytis* is *B. cinerea*; it is responsible for considerable losses in crops, notably grape vines, and post harvest spoilage of many fruits (Droby and Lichter 2004). *B. cinerea* can infect over 200 species of plants both monocots and dicots, gymnosperms, pteridophytes, bryophytes and macroalgae (Anderson 1924; Beever and Weeds 2004; Capieau et al. 2004; Choquer et al. 2007; Mirzaei et al. 2007; Mittal et al. 1987; Chap. 20). Other species of *Botrytis* that are known to cause significant losses in horticulture, agriculture and floriculture are listed in Table 2.1.

In the wine industry *Botrytis*-infections in grape berries used to make table wines are undesirable because the fungus is thought to be responsible for 'off' odours and

Table 2.1 A list of *Botrytis* species with importance in agriculture and horticulture

Name	Date of description	Major plant host genus/genera in agriculture and horticulture
Botrytis aclada	1850	*Allium*
Botrytis allii	1917	*Allium*
Botrytis byssoidea	1925	*Allium*
Botrytis caroliniana	2012	*Rubus*, *Fragaria*
Botrytis cinerea	1794	Multiple host genera
Botrytis convoluta	1932	*Iris*
Botrytis deweyae	2014	*Hemerocallis*
Botrytis elliptica	1881	*Lilium*
Botrytis fabae	1929	*Vicia*
Botrytis fabiopsis	2010	*Vicia*
Botrytis gladiolorum	1941	*Gladiolus*
Botrytis globosa	1938	*Allium*
Botrytis hyacinthi	1928	*Hyacinthus*
Botrytis narcissicola	1906	*Narcissus*
Botrytis paeoniae	1897	*Paeonia*
Botrytis pelargonii	1949	*Pelargonium*
Botrytis polyblastis	1926	*Narcissus*
Botrytis porri	1949	*Allium*

(continued)

Table 2.1 (continued)

Name	Date of description	Major plant host genus/genera in agriculture and horticulture
Botrytis pseudocinerea	2011	*Brassica*, *Fragaria*, *Vitis*, likely multiple others?
Botrytis sinoallii	2010	*Allium*
Botrytis sinoviticola	2014	*Vitis*
Botrytis sphaerosperma	1949	*Allium*
Botrytis squamosa	1925	*Allium*
Botrytis tulipae	1913	*Tulipa*
Botrytis sp. Group S	2013	*Fragaria*, *Vitis*, likely multiple others?

poor keeping qualities of the resulting wines but this may be in part due to the presence of other fungi commonly associated with *B. cinerea* infections, notably species of *Aspergillus* and *Penicillium* (Dewey and Meyer 2004; Rousseaux et al. 2014). In contrast *Botrytis* infections of grape berries late in the season are highly desirable, they are used for the production of much-prized dessert wines (Dewey et al. 2008, 2013; Sivertsen et al. 2005).

2.2 Life Cycle

2.2.1 Asexual Stage

Most species and isolates of *Botrytis* produce numerous conidia (macroconidia) that are asexual conidia borne at the tips of the branching conidiophores (Fig. 2.1). The size of the conidia is species related (Beever and Weeds 2004). In general, conidia are short-lived their survival time depending on temperature, moisture, microbial activity and exposure to sunlight (Kerssies et al. 1995; Blanco et al. 2006; Carisse et al. 2012; Nassr and Bakarat 2013).

Overwintering of *Botrytis* species is generally brought about by the production of melanized resting bodies, sclerotia, which are resistant to adverse environmental conditions (Holtz et al. 2004). Some species, notably *B. cinerea*, produce quite large sclerotia (~4 mm), whereas others for example *B. tulipae* are quite small (~0.8 mm). Under favourable conditions, such as interrupted wet periods in Spring, sclerotia will 'germinate' to produce mycelia and conidia. Sclerotia are believed to be the source of early infections at the start of the growing season (Hsiang and Chastagner 1992).

Botrytis spp. can also produce temporary resting structures known as chlamydospores that have thickened, hyaline, walls. These structures can vary considerably in size and shape; they can survive periods of drought of up to 3 months (Urbasch 1983). They are often found in ageing cultures either within hyphae or at the ends of hyphae. In favourable conditions they 'germinate' to produce hyphae or microconidia.

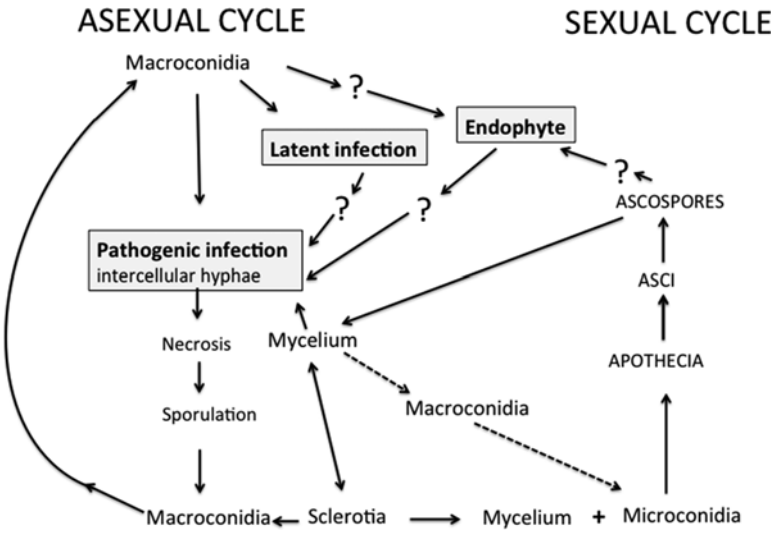

Fig. 2.1 Diagram of life cycle of *Botrytis cinerea* showing known and possible interactions between different phases of growth. Note: Macroconidia common name is conidia

2.2.2 Sexual Stage

The sexual stage or teleomorph of *Botrytis* spp., formerly known as *Botryotinia* spp., is not commonly seen in nature. This may in part be due to lack of recognition of the fruiting structures. Microconidia, which can be produced both from post-germination development of macroconidia and from endogenous formation within old hyphae (Brierley 1918; Fukumori et al. 2004), are uninucleate and have now been confirmed to act as spermatia (Fukumori et al. 2004). The 'fertilisation' of receptive sclerotial structures by spermatia induce the production of apothecia, the sexual structures (Urbasch 1983), where asci and ascospores are generated. Faretra and Antonacci (1987) were the first to induce the sexual stage in *B. cinerea* under laboratory conditions. The asci, containing ascospores, are borne on the upper surface of apothecia (Fig. 2.1). Sexual compatibility is controlled by a single mating type locus with two alleles, MAT-1 and MAT-2. Both mating types are widespread in nature and most isolates are heterothallic i.e. can only produce ascospores when crossed with the opposite mating type (Faretra et al. 1988).

2.3 Diversity of the Genus

At present, it remains difficult to estimate the number of species within the genus *Botrytis*. Simply, this is because numerous undescribed species are likely to exist. However, a selected list of the pathogenic taxa with importance in horticulture and

agriculture are listed in Table 2.1, along with their major host genus. Morphological circumscription, primarily using features such as macroconidia size, shape and ornamentation, as well as host preference, agrees very well with molecular data (see Table 2.1).

A significant number of species might appear to be specific to certain genera (Table 2.1). However, it is now evident that such rigid host specificity may not be always the true case, especially in man-made environments. For instance, *B. elliptica* has been reported from several genera of distantly related monocots in addition to its classical host *Lilium* – for example daylily, *Hemerocallis* (Chang et al. 2001), tuberose, *Polianthes* (Horst 2013), toad lily, *Tricyrtis* (Furukawa et al. 2005) – as well as the dicot *Stephanotis* (Tompkins and Hansen 1950). Hence, the distinction between polyphagous species – typified by *B. cinerea* – and highly specialised pathogens may not be quite as clear-cut as previously thought.

New pathogenic taxa are still being discovered at a regular frequency. For example, the most recent discoveries are *B. sinoviticola* (Zhou et al. 2014) and *B. deweyae* (Grant-Downton et al. 2014). The former is a cryptic species associated with *Vitis vinifera* in China that, unlike its close relatives, *B. cinerea* and *B. pseudocinerea*, requires injury of the host to permit infection. The latter species has been identified only from cultivated *Hemerocallis* (daylily) and so far appears to be pathogenic only on this genus.

2.4 Plasticity in *Botrytis* Interactions with Plants

Most species of *Botrytis*, including *B. cinerea* only become pathogenic when growing conditions for their hosts are limiting and microclimate is suitable, for example on tomato plants in poorly ventilated, humid greenhouses, or plants suffering from physical damage such as pruning wounds and wind or through insect damage (Fermaud and Le Menn 1992). However, some species such as *B. squamosa* the cause of *Botrytis*-leaf blight of onions, (Carisse et al. 2012) and *B. elliptica* on *Lilium* can rapidly destroy whole crops (Hsieh and Huang 2001). Germination of the conidia on plant surfaces is greatly encouraged by the presence of nutrients such as simple sugars and pollen (Chou and Preece 2008).

2.4.1 Quiescent/Latent Phases

Various workers have shown that, although conidia of *Botrytis* spp. commonly germinate on plant tissues particularly, petals and sepals of their host, invasion of inner tissues does not always follow (Holtz et al. 2004). Such infections are often described as quiescent or latent. In such cases spread of the fungus within host tissue is arrested until later in the season when either host tissues become senescent or the sugar levels change as in ripening of grape berries or in storage (Meyer et al. 2000; Sanzani et al. 2012).

2.4.2 *Non-symptomatic Infections and Endophytic Interactions*

Evidence is increasing, that some *Botrytis* species can be present as endophtyes i.e. grow within host plant tissues without generating disease symptoms and also eliciting little or no response from the host. This was first reported from *B. cinerea* where it was evident that even systemic, endophytic colonization of host plants was possible (Barnes and Shaw 2003; Sowley et al. 2010). Such infections may either become aggressive at a later stage, notably at flowering time, or in storage or, be transferred non-symptomatically by clonal or seed propagation of the host (Barnes and Shaw 2003). Endophytic colonization of plant tissues by *B. cinerea* is now known from a range of unrelated dicot plants (M. Shaw *pers. comm.*). Remarkably, there is evidence that *B. cinerea* may also be able to interact with tissues of the seaweed *Fucus serratus* in this manner (Zuccaro et al. 2008). The extent of endophytic interactions between *Botrytis* and various taxonomic groups may well have been underestimated. A number of undescribed endophytic *Botrytis* have been isolated from pteridophyte, gymnosperm and angiosperm hosts (Shipunov et al. 2008; Zhao et al. 2010; Zhou et al. 2010). The full extent of *Botrytis* diversity as endophytic fungi remains unknown. Indeed, the recently identified *B. deweyae* (Grant-Downton et al. 2014), which causes a disease of emerging spring foliage of cultivated *Hemerocallis* (daylily), is suspected to have endophytic origins. Its sudden emergence as a pathogen may be in part due to physiological changes in the host as a result of their recent intense selection and in-breeding in cultivation.

2.5 Dispersal

The common method of dispersal of nearly all species of *Botrytis* is by transmission of conidia in air currents. Water splash (Blanco et al. 2006), insects (Fermaud and le Menn 1992), agricultural machinery and pruning shears are also important means of transmitting conidia. Survival times and distances travelled by air-borne conidia are debatable; within greenhouses conidia are known to travel 1.8 m (Kerssies et al. 1995) but outdoors it is highly likely that they are carried much further in air currents (Chap. 7).

2.6 Detection and Quantification

2.6.1 *Classical Methods*

Late stage infections are easily recognized in symptomatic material by the appearance of grey/brown conidial clusters on the surfaces of infected material (Khazeli et al. 2010). Where proof of identity at the species level is required, such as infections