



Role of Light in the Life Cycle of *Botrytis cinerea* 14

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Abstract

The fungus *Botrytis cinerea* (*Botryotinia fuckeliana*) infects more than 500 plant species and causes a wide range of symptoms: soft rots, accompanied by collapse and water-soaking of tissues followed by the appearance of gray masses of conidia on leaves and soft fruits (gray mold), and spots that may turn brown to full-scale soft rotting on flower petals (Botrytis blight). In general, *B. cinerea* is responsible for severe economic losses that are either due to the damage of growing plants in the field or the rot of harvested fruits, flowers, and vegetables during storage under cold and humid conditions. *B. cinerea* has adapted to the plant host and its environment by evolving strategies to use plant tissues for proliferation in terms of a necrotrophic lifestyle and to survive biotic (host responses) as well as abiotic stresses such as sunlight. *B. cinerea* maintains a complex regulatory network of light-sensitive proteins and signal transduction pathways to use light for coordinating stress responses, virulence, and reproduction. Different light-controlled reproduction cycles enable *B. cinerea* to live in moderate climate zones by infecting and

propagating in summer and remaining dormant in winter when green host tissues are unavailable as a nutrient source.

Keywords

Gray mold fungus · Plant pathogen · Light · Photoreceptors · Development

14.1 Adaptations to the Plant Host

The interaction between *B. cinerea* and its host plants cover a spectrum of outcomes, ranging from full immunity (no infection) to full susceptibility (fast infection) and all the intermediate stages in between. Another lifestyle option, although not yet that well understood, is the asymptomatic colonization of the host (Williamson et al. 2007; Shaw et al. 2016; Veloso and van Kan 2018). The outcome of the interaction with the host depends on the host species and its capacity to prevent fungal growth, the *B. cinerea* strain and its growth characteristics, and the climate conditions which can be more favorable for the host or the pathogen. Notably, *B. cinerea* field populations are characterized by high levels of variability in morphology and preferred mode of reproduction, virulence, spectra of secondary metabolites produced, and fungicide resistance. The genetic variability can be achieved through sexual reproduction, hyphal anastomosis, transposable elements,

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heterokaryosis, heteroploidy, or viruses (Coley-Smith et al. 1980; Elad et al. 2004; Fillinger and Elad 2016). In this regard, it is important to use reference host plants and *B. cinerea* strains in the laboratory for elucidating the mechanisms and regulation of pathogen attack, host immune response, and fungal reproduction. *Phaseolus vulgaris* (French bean) and *Arabidopsis thaliana* are valuable model experimental hosts that exhibit high and low susceptibility to aggressive *B. cinerea* strains such as B05.10 (Fig. 14.1). Thus, the use of the highly sensitive host, *P. vulgaris*, enables the study of the interaction from the fungal perspective by revealing differences between the capacity of *B. cinerea* mutants to enter and colonize plant tissues (Giesbert et al. 2012; de Vallee et al. 2019). In contrast, the role of plant immune responses can be studied in (genetically modified) *A. thaliana*, which represents a “poor host” because of its potent phytoalexin camalexin (Denby et al. 2004; Rowe and Kliebenstein 2008; Mengiste et al. 2009).

The life cycle of *B. cinerea* includes vegetative mycelia for substrate colonization, (macro)-conidia for dispersal, sclerotia for survival and as a prerequisite for sexual development, microconidia as male gametes, and fruiting bodies bearing the sexual spores (Fig. 14.2). The relationship with the host further includes the development of penetration structures and the active secretion of factors for killing host cells and for obtaining nutrition from the dead tissue.

14.1.1 Penetration Structures

B. cinerea enters the host by using two different pre-penetration structures (Backhouse and Willets 1987; Choquer et al. 2007). The conidia that are ubiquitously distributed in the air germinate after landing on the plant surface under favorable conditions and form short germ tubes with apical swellings (appressorium-like structures), which directly penetrate the plant surface. Alternatively, hyphae derived from already established mycelia or older elongated germ tubes

form multicellular penetration structures, the so-called infection cushions. The penetration process by germ tubes and infection cushions can be easily monitored on onion epidermis by using lactophenol blue staining for distinguishing structures inside and outside of the onion cells (Fig. 14.1).

Several signaling pathways are implicated in the early stages of plant infection. Deletion of the genes encoding the mitogen-activated protein (MAP) kinases, BMP1 and BcSAK1, results in avirulent mutants (Zheng et al. 2000; Segmüller et al. 2007; Schamber et al. 2010). In contrast, deletion of the genes encoding the MAP kinase BMP1 and components of the cAMP cascade leads to mutants with retarded infection due to reduced penetration efficiency (Doehlemann et al. 2006b; Rui and Hahn 2007; Schumacher et al. 2008b). Deletion of genes encoding members of the NoxB (NADPH oxidase) complex results in another interesting phenotype: conidia germinate, form germ tubes and appressorium-like structures, which initiate penetration but without success. New outgrowths appear that are followed by further attempts to penetrate. Components of the NoxA complex are specifically involved in the formation of infection cushions. However, all NOX mutants eventually colonize the host tissue (Segmüller et al. 2008; Marschall et al. 2016). An intact fungal wall cell is critical for the differentiation of functional infection cushions as highlighted by phenotype analysis of $\Delta bcchs3a$, $\Delta bccrz1$, and $\Delta bcskn7$ mutants that are more strongly impaired in mycelia-derived than in conidia-derived infection processes (Schumacher et al. 2008a; Arbelet et al. 2010; Viefhues et al. 2015).

Taken together, conidia and mycelia of the same strain may have different penetration capabilities due to the two different kinds of infection structures whose formation is regulated by independent pathways. A recent study identified genes that are upregulated in mature infection cushions including two that encode secreted fasciclin-like proteins that are required for full virulence on *P. vulgaris* (Choquer et al. 2021).

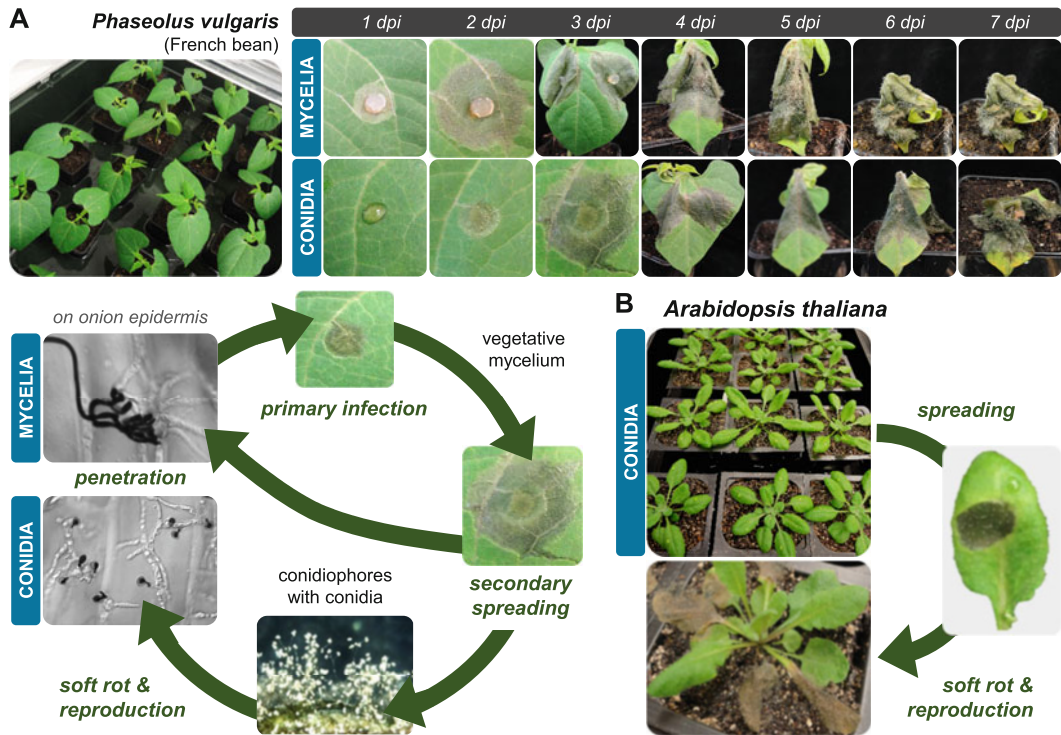


Fig. 14.1 Infection cycle of *B. cinerea* B05.10 under laboratory conditions. **(a)** On *Phaseolus vulgaris* (French bean) as a highly susceptible host. Primary leaves of living plants are inoculated with plugs of vegetative mycelia (two-day-old complete medium (CM) cultures) or conidial suspensions (2×10^5 conidia/ml Gamborg B5 + 2% glucose) and are incubated under humid conditions and natural light conditions at room temperature. The fungus establishes primary (restricted) infections that spread quickly. After one week the whole leaf is infected: the plant tissue collapses and becomes watery (soft rot), which is accompanied by the formation of gray conidiophores and conidia. The penetration process is monitored on isolated onion epidermis. Hyphae deriving from

vegetative mycelia produce infection cushions; conidia form short germ tubes that directly penetrate the epidermal cells (hyphae growing inside the onion epidermis are colorless while fungal structures outside the host are stained with lactophenol blue). Dpi—days post-inoculation. **(b)** On the model plant *Arabidopsis thaliana* as a lowly susceptible host. Leaves of living plants can be infected by conidia as described above, resulting in fast spreading and soft rot formation. In comparison to French bean, no defined stages (i.e., primary and secondary infections) can be distinguished. Due to the high level of basal resistance, the outcomes of different assays may vary significantly. *A. thaliana* is likely not a (natural) host for *B. cinerea* outside the laboratory

14.1.2 Virulence Determinants

After penetration, the epidermal and underlying cells die and *B. cinerea* establishes a primary infection that is characterized by collapsed brownish tissue and defined margins. At this stage, hyphae are probably restricted to this region. The subcuticular mycelia may remain inactive in this restricted lesion for an indefinite duration (quiescence) and become active again under specific circumstances, such as during the

ripening processes in which fungitoxic compounds decline in concentration, levels of sugars increase and the chemical composition of the cell wall changes (Prusky et al. 2013). Finally, *B. cinerea* overcomes the plant defense barriers and initiates vigorous secondary growth; hyphae grow invasively and decompose the plant tissue very rapidly resulting in soft rot and formation of conidiophores and conidia (Fig. 14.1).

B. cinerea secretes several non-host-specific cell death-inducing factors, which are low

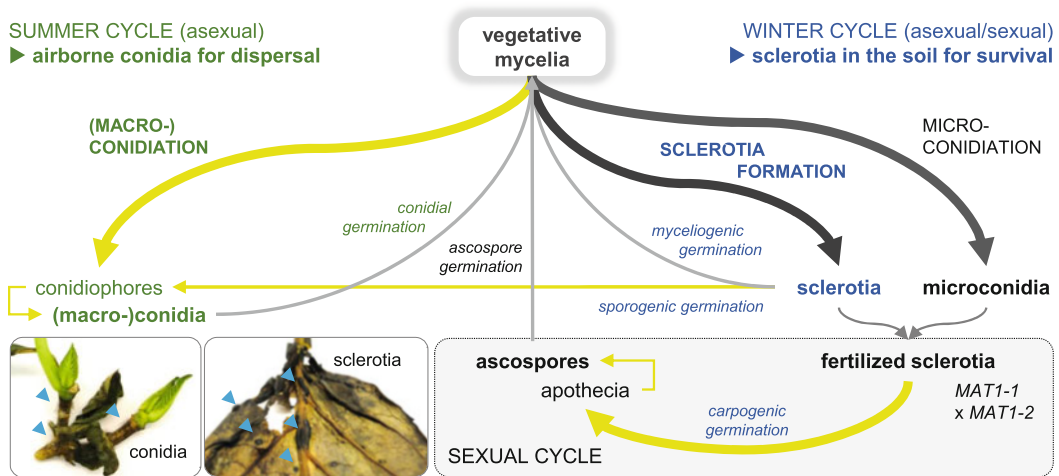


Fig. 14.2 Reproduction cycles of light-responsive *B. cinerea* strains. Young vegetative mycelia may develop either macro-conidia for dispersal (summer cycle, green) or sclerotia for survival (winter cycle, blue). Both conidia and sclerotia germinate asexually under favorable conditions. Fertilization of the sclerotia by microconidia of the opposite mating type is part of the sexual cycle that results in the formation of apothecia as fruiting bodies in

spring. The apothecia contain asci with eight ascospores in ordered tetrads. Light promotes conidiation and represses sclerotial development. Sclerotia and microconidia are formed under the same conditions that is the absence of light. Left bottom corner: conidiophores and sclerotia (blue arrows) formed by *B. cinerea* on *Hydrangea* plants from the greenhouse (left) and a dark cold storage (right)

molecular weight metabolites or proteins (van Kan 2006; Amselem et al. 2011). The expression of these factors may be induced during the fungus-host interaction but synthesis is not restricted to this state. Two families of phytotoxic secondary metabolites, the botryanes (BOT) and the botcinins (BOA) contribute to full virulence (Dalmais et al. 2011; Viaud et al. 2016). Reactive oxygen species (ROS) play a dual role in the relationship as both interacting partners produce ROS. H_2O_2 generation is observed in the penetration structures, in and around the penetrated host cell wall in the early stages and in colonized tissues (Choquer et al. 2007; Heller and Tudzynski 2011). Several secreted necrosis-inducing proteins have been identified of but only a few contribute significantly to virulence. Cell wall-degrading enzymes (CWDEs) may be involved in different stages of plant infection: in penetration by lysing the cuticle or epidermal cell walls, in the manifestation of infection by degrading components of the plant defense, or in nutrition by effective decomposition of dead

tissues. Some CWDEs also exhibit necrotizing activity (González et al. 2016). The redundancy of these groups of virulence determinates has been demonstrated by Hahn & co-workers who generated mutants using CRISPR/Cas9 technology where multiple genes encoding cell death-inducing proteins and key enzymes for the synthesis of BOT and BOA were deleted. On all tested tissues, infection efficiency of the mutants decreased with increasing number of deleted genes. However, even mutants with twelve genes deleted were eventually able to colonize the host tissue and to form conidia (Leisen et al. 2021).

A relatively new class of secreted effectors produced by *B. cinerea* are small RNAs (sRNAs) that derive from retrotransposons. They are taken up and recognized by the host resulting in the suppression of defense-related gene expression due to RNA interference (pathogen-induced gene silencing). Deletion of the genes encoding fungal Dicer-like proteins, BcDCL1 and BcDCL2, abolishes the generation

of sRNAs and consequently results in reduced virulence. On the other hand, the mutation of AGO1 in *A. thaliana* that is required for active gene silencing leads to decreased susceptibility, indicating that cellular components of both interaction partners are involved in the process (Weiberg et al. 2013; Wang et al. 2016; Porquier et al. 2021).

Like other necrotrophs, *B. cinerea* induces plant defense responses by pathogen-associated molecular pattern (PAMP) recognition, such as cell wall fortification and production of antifungal compounds that activate JA/ET (jasmonate/ethylene)-dependent signaling pathways. Mutations that abolish JA and ET signaling result in increased susceptibility to *B. cinerea* (Mengiste et al. 2009). In addition, *B. cinerea* triggers SA (salicylic acid) signaling to induce the hypersensitive response (HR), a localized cell death reaction that is effective against biotrophic but supports necrotrophic pathogens (El Oirdi et al. 2011; Rossi et al. 2011). As JA/ET and SA pathways are antagonistic, the induction of SA signaling concurrently results in the suppression of JA/ET-associated defense responses (Robert-Seilaniantz et al. 2007; Mengiste 2012). Furthermore, *B. cinerea* may affect the hormone balance by fungal production of ethylene, auxins, and abscisic acid (ABA) (Sharon et al. 2004). However, the impact of fungal-derived phytohormones in the interaction is still unclear. JA-dependent defense responses against necrotrophs are downregulated in plants in which the shade avoidance response has been initiated. This program is induced by a low red/far-red light ratio which indicates the presence of competitors and the concomitant decrease of photosynthetic yields. The shade avoidance response consequently redirects more carbon resources to promote growth for optimizing photosynthetic yields and allows the plants to finish the life cycle at the expense of defense (Ballaré 2014; Ballaré and Pierik 2017; Liu et al. 2021). Because of its short repetitive infection cycles that yield high numbers of airborne conidia for consecutive infections of “immunocompromised” high-yielding host plant varieties planted in high

densities, *B. cinerea* is a highly destructive pathogen in industrial monocultures.

14.2 Adaptations to Light

Sunlight is an important environmental factor in almost all ecosystems as it represents a source of energy, information, and stress. Cyanobacteria, algae and plants all use light for energy generation (photosynthesis). Less apparent is the utilization of light for repair of photodamaged DNA or for establishing proton gradients by microbial opsins. All organisms must protect themselves from the harmful effects of light such as UV radiation, ROS accumulation, heat, and desiccation. Finally, light quality and quantity can be used for signaling (photoinduction, photomorphogenesis), as a guide for directed growth (phototropism) and timing (photoentrainment), processes that include sensing and transduction into intracellular signals.

B. cinerea and other plant pathogens infecting the sun-exposed parts of the plant must cope with the high (UV, blue) light conditions the host plant seeks. Furthermore, the fungus experiences an altered light spectrum (‘green gap’) when it colonizes shaded parts of the plant. It is depleted for blue and red light that is absorbed by the plant chlorophyll and enriched for green and far-red light that is reflected or transmitted by the plant tissue. As these ambient light conditions trigger the shade avoidance response in the plant, the pathogens may trigger their own “shade response” such as the upregulation of virulence determinants and inoculum production assuming that new host tissues are close and highly susceptible.

14.2.1 Genetic Make-up: Photoperception

Light is perceived by chromophore-containing proteins called photoreceptors (PRs). FAD (flavin adenine dinucleotide), MTHF (5,10-methyltetrahydrofolate), retinal and bilin are

bound by conserved input domains allowing for the distinction of four major PR classes: (i) near-UV/blue-sensing cryptochrome/photolyase-family proteins (CPFs), (ii) blue-sensing light-oxygen-voltage domain-containing proteins (LOVs), green-sensing membrane-associated opsins (OPs) and red/far-red-sensing phytochromes (PHYs) (Heintzen 2012). Great numbers of PR-encoding genes are found in phototroph-associated microbes—including plant-pathogenic and rock-inhabiting fungi—pointing to the relevance of light in their life cycles (Schumacher and Gorbushina 2020; Losi and Gärtner 2021). *B. cinerea* possesses substantially more PRs than the long-established models in fungal photobiology, including *Aspergillus nidulans* which has three and *Neurospora crassa* which has eight PRs (Dasgupta et al. 2016). Striking is the expansion of PHYs (at least two) in the Leotiomycetes. The presence of two CPFs (BcCRY1, 2), four LOVs (BcWCL1, VVD1, LOV3, 4), two OPs (BOP1, 2), and three PHYs (BcPHY1, 2, 3) enables *B. cinerea* to sense near-UV, blue, green, red and far-red light. Yet the mechanism and proteins involved for sensing UV (280–315 nm) are unknown and thus the total number of PRs of *B. cinerea* may be even larger. The eleven PRs of *B. cinerea* possess the conserved chromophore binding sites, and all are expressed in response to light. While deletion and overexpression mutants have been generated, characterization of these mutants is just beginning (Schumacher 2017).

14.2.2 Gene Expression: Photoregulation

Changes at the transcriptional level are prerequisites for all photoresponses and indicate that the organisms perceive and respond to light. But not all changes in gene expression will result in obvious phenotypes. Thus, PRs and light-responsive transcription factors (LTFs), which may control the expression of several genes have been studied to elucidate the regulatory networks and genes involved in the photoresponses.

The exposure of vegetative mycelia of *B. cinerea* B05.10 to full-spectrum light significantly alters gene expression (Fig. 14.3). The group of light-induced genes (LIGs) comprises genes involved in photoperception, stress responses, and transcriptional regulation. The group of light-repressed genes (LRGs) is smaller and enriched for genes encoding amino acid transporters (Schumacher et al. 2014). LIG expression occurs with different kinetics: genes are either subjected to photoadaptation or not. LIGs of the first group (early, late) mediate short-term (adaptive) responses. LIGs of the second group exhibit maximal expression after longer exposure to light and may be critical for development (*dev* LIGs). The expression levels of all PR-encoding genes are influenced by light: *bccry1*, *bccry2*, *bcvvd1*, and *bop1* are early LIGs, *bclov3*, *bcphy2* and *bop2* are late LIGs, and *bcphy1*, *bcphy3*, *bclov4*, and *bcwcl1* belong to the group of *dev* LIGs. Importantly, the blue light-sensing BcWCL1 as part of the White Collar complex (WCC) acts as the primary PR by modulating the expression levels of the remaining PR-encoding genes within a short period of time (Schumacher 2017). To date, eight LTFs out of the identified 30 LTFs (Schumacher et al. 2019) have been functionally characterized including the early LIGs that encode BcLTF3 and BcREG1, the late LIGs that encode BcLTF1, BcATF1 and BcLTF4, 5, 6, and the only studied *dev* LIG that encodes BcLTF2 (Michielse et al. 2011; Temme et al. 2012; Schumacher et al. 2014; Cohrs et al. 2016; Brandhoff et al. 2017).

14.2.3 Reproduction: Photomorphogenesis and Phototropism

B. cinerea can reproduce asexually via macroconidia and sclerotia, and sexually via ascospores. The reproduction cycles depend on the availability of a host and the environmental conditions—mainly light and its absence, temperature, substrate—and are therefore linked to the seasons. Macroconidia are predominantly formed in the summer as inoculum for new infections

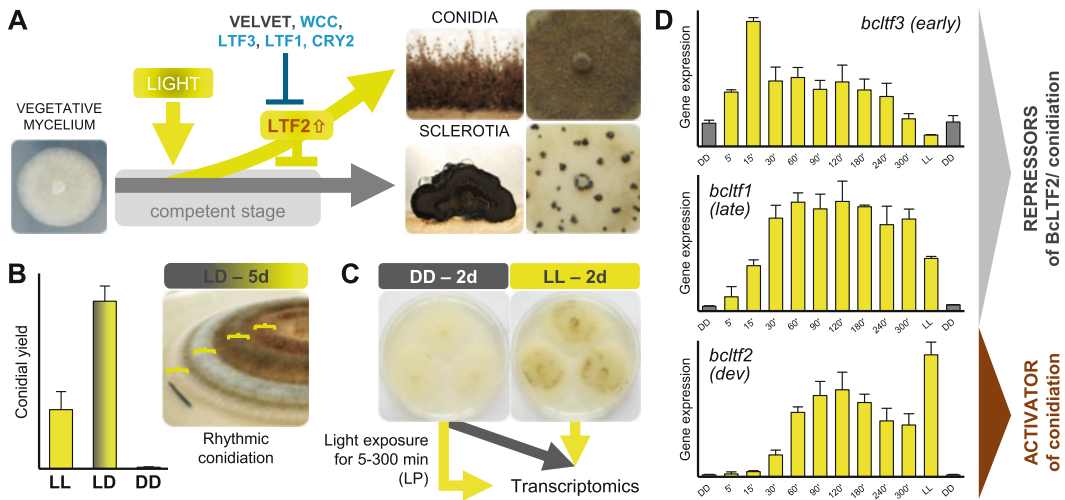


Fig. 14.3 Prominent light responses of *B. cinerea* WT: B05.10—morphogenesis and gene expression. (a) Light induces conidiation and represses sclerotium formation via transcriptional activation of BcLTF2. Low light dosages received in the competent stage of vegetative mycelia prevent the formation of sclerotia. Conidia and sclerotia as observed after two weeks of cultivation on solidified CM in 12 h light/12 h dark (LD) or constant darkness (DD) at 20 °C; vegetative mycelium as formed from a mycelial plug after two days of incubation in darkness. (b) Light-dark cycles are more effective than constant light

(LL) and cause rhythmic conidiation (“rings”). Conidiophores with conidia are formed overnight on aerial mycelia that have been grown during the day (yellow brackets). (c) Transcriptional light responses are studied in surface-grown mycelia obtained from cellophane-covered solidified CM. Dark-grown mycelia are exposed to light pulses (LPs) before harvest. (d) Gene expression profiles determined by RT-qPCR of three light-responsive transcription factors (LTFs) exhibiting different kinetics. *Bcltf3*, *ltf1*, and *ltf2* represent early, late, and developmental light-induced genes (LIGs) and affect conidiation

(host tissue is available) and sclerotia in autumn for surviving the winter in the absence of a host. Sexual reproduction—fertilization of sclerotia with microconidia and subsequent apothecial development—is promoted by nutrient starvation and lower temperatures and happens in winter, resulting in the appearance of the fruiting bodies (apothecia) the following spring. Non-fertilized sclerotia and ascospores germinate with mycelia and conidia to initiate the summer cycle (Williamson et al. 2007; Amselem et al. 2011) (Fig. 14.2). Field populations of *B. cinerea* are highly diverse regarding the formation of these reproduction structures. Strains may or may not differentiate macroconidia, microconidia, sclerotia, and apothecia with ascospores during their life cycles. In general, *B. cinerea* strains can be classified into light-responsive strains that undergo photomorphogenesis, and blind strains that exhibit the same phenotypes in light and

constant darkness (*always mycelia*, *always sclerotia* or *always conidia*). Light responsiveness allows for the formation of all structures—as found in strain B05.10 that is used in most laboratories as the host for genetic modification (Fig. 14.3). Photomorphogenesis is considered a characteristic feature of *B. cinerea* and its absence as the result of mutated regulatory genes.

Vegetative mycelia of *B. cinerea* B05.10 quickly colonize the host tissue (invasive growth) or the agar medium (saprophytic growth). For example, the growth rates on solidified complete medium are 10–15 mm per day so that the area of a standard Petri dish is colonized after three days of incubation under optimal temperature conditions (~20 °C). Little aerial hyphae and biomass accumulates as mycelia consist of thin layers of hyphae. Young vegetative mycelia are fully competent and can develop conidiophores

with macroconidia or sclerotia. Light responsiveness depends on the physiological age and is less effective in aged colonies that have completely colonized the substrate or have already initiated one of the reproduction programs.

The (macro)conidia are short-lived asexual dispersal units. They are multinucleate, contain a two-layered cell wall—the outer wall is melanized—and are formed at denticles that arise from the spherical ampulla located at the tip of a conidiophore (synchronous holoblastic conidiogenesis) (Cole 1981). The development of conidia (conidiation) starting from initial conidiophores through to the presence of mature conidia may be completed within eight hours and requires light. Alternation of black light (near-UV) and white light, or alternation of full-spectrum light and darkness (Fig. 14.3) are most effective in promoting conidiation, while blue light alone inhibits conidiation and causes morphological changes in the various stages of conidiogenesis as mature conidiophores, ampullae at the tips of conidiophores, denticles, and conidial initials de-differentiate into sterile hyphae. Inhibition by blue light can be overcome by subsequent exposure to near-UV or far-red light. Red light does not promote conidiation after blue light inhibition and reduces conidiation in far-red light-treated colonies (Tan 1975; Suzuki et al. 1977).

Conidiophores positively respond to blue light after induction of conidiation by black light (positive phototropism)—most likely mediated through the blue light-sensing WCC—while green, yellow, and red light are ineffective (Jarvis 1972). Three TFs are known to be required for conidiogenesis: *Bcreg1* mutants form conidiophores with ampullae and denticles but fail to form mature conidia, the mutation of *bclt3* phenocopies the blue light effect as the conidial initials grow out to sterile hyphae, and BcHOX8 is crucial for both proper conidiophore development and conidiogenesis (Michielse et al. 2011; Antal et al. 2012; Brandhoff et al. 2017). Initiation of conidiophore development requires BcLTF2 (sufficient and essential for conidiation),

the stress-activated MAPK module, the response regulator BcSKN7, and the cell wall integrity-related MAPK module (Rui and Hahn 2007; Segmüller et al. 2007; Yan et al. 2010; Liu et al. 2011; Yang et al. 2012, 2015; Viefhues et al. 2015; Cohrs et al. 2016; Wang et al. 2018). Conidiation is repressed by the transcription factors BcLTF1, BcWCL1, BcMADS1, BcSTE12, the hybrid-histidine kinases BOS1 and BcSLN1 acting upstream of the BcSAK1 module, and the VELVET complex (Liu et al. 2008; Schamber et al. 2010; Schumacher et al. 2012, 2014, 2015; Canessa et al. 2013; Yang et al. 2013; Zhang et al. 2016; Müller et al. 2018; Ren et al. 2019).

The conidia may stay dormant for long periods, held in check by lack of moisture and nutrients. Germination starts with the swelling of the conidium, followed by the emergence of the germ tube. This is accompanied by nuclear divisions, the induction of genes encoding secreted proteins and lytic enzymes, and the production of an extracellular polysaccharide (EPS) matrix (Gull and Trinci 1971; Doss 1999; Leroch et al. 2013). Differences between isolates were observed regarding the induction of this process; some *B. cinerea* isolates germinate readily in distilled water, while others require sugars and amino acids (Blakeman 1975). Conidial germination on hydrophilic surfaces is stimulated by carbon sources and requires the MAP kinase BMP1, the G α subunit BCG3 and the adenylate cyclase BAC, but not by the cAMP-dependent protein kinase (PKA), and is accompanied by the rapid degradation of trehalose, an important carbon storage compound and stress protectant (Doehlemann et al. 2006a, 2006b; Schumacher et al. 2008b). Germination on hydrophobic surfaces in the absence of nutrients is controlled by the MAP kinase BMP1 in a cAMP-independent manner (Doehlemann et al. 2006b). Other mutations result in multiple or elongated germ tubes with abnormal shapes, possibly due to defects in surface sensing (Rui and Hahn 2007; Kokkelink et al. 2011; Leroch et al. 2015). Experimental evidence for the secretion of self-inhibitors of germination by *B. cinerea* exist

(Carlile and Sellin 1963; Kritzman et al. 1980); yet the compounds and regulatory networks remain elusive.

A recent study reported on the presence of 1-phenylethanol and 3-phenylpropanol and the absence of farnesol and tyrosol—four commonly involved compounds in quorum sensing in bacteria and fungi—in culture extracts of *B. cinerea* (Rosero-Hernandez and Echeverri 2020). Three forms of conidial fusions can be distinguished: fusions between conidia via a short connective hypha (conidial anastomosis tubes, CATs) (dominant type), fusions between germ tubes and conidia, and fusions between germ tubes directly or indirectly via CATs (Akutsu et al. 1981). Conidial/germling fusions are induced by a lack of nutrients and require the NoxA complex (Roca et al. 2012; Siegmund et al. 2015). Another factor influencing the germination process is light. Conidia germinate less well under green light conditions (Zhu et al. 2013), and germ tubes develop more frequently at the un-illuminated site of the conidium and grow away from the white light source (negative phototropism) (Robinson 1914; Gettkandt 1954; Islam et al. 1998). These observations suggest that light guides the germ tubes to the host surface and prevents conidial germination during the day.

Sclerotia are dark pigmented structures of limited growth, which allow the fungus to survive many years in the absence of suitable hosts or conditions favoring active growth. Sclerotial development consists of the three stages (i) initiation: formation of dichotomous branches, (ii) development: growth to the full size, and (iii) maturation: surface delimitation, internal changes, pigmentation of the peripheral hyphae, and exudation of water. The mature sclerotium has three distinct layers consisting of a melanized outer rind, a cortex of thin-walled pseudoparenchymatous cells, and a large medulla of loosely arranged filamentous hyphae. The formation of sclerotia is influenced by different factors, such as temperature, nutrition, pH, physical damage and growth against mechanical barriers, and is generally promoted by lower temperatures, high humidity, and the absence of light (Coley-Smith et al. 1980). The NoxA complex is essential for

sclerotial development, which might be due to the inability of the mutants to form anastomoses (hyphal fusions) as common features of infection cushions and sclerotia (Segmüller et al. 2008; Siegmund et al. 2015). Several other mutants do not produce sclerotia, but this is due to the deregulation of morphogenetic programs rather than due to defects in sclerotial development. For example, inappropriate induction of vegetative growth resulting in *always mycelia* is caused by the overexpression of WCC components or deletion of the G $\beta\gamma$ dimer of heterotrimeric G proteins (Canessa et al. 2013; Tang et al. 2021). Conidiation in the dark by *always conidia* mutants has been described above. Only a few mutations are known to cause the *always sclerotia* phenotype; for example the deletion of *bckdm1* encoding a putative histone 3 lysine 36 (H3K36) demethylase (Schumacher et al. 2019). The sclerotia may remain quiescent for long periods and can germinate in the presence of nutrients and after low temperature activation in three different ways: myceliogenically (by hyphae), sporogenically (by conidiophores), or carpogenically (by apothecia) (Coley-Smith and Cooke 1971) (Fig. 14.2).

Microconidia are the male gametes (spermatia). They are uninucleate, contain a lightly melanized cell wall, and are formed by phialides on conidial germ tubes, more mature hyphae, infection cushions or sclerotia under nutrient limitation conditions. They germinate exclusively in the presence of a female parent of the opposite mating type (Faretra and Antonacci 1987; Fukumori et al. 2004). The process of microconidiation is rather poorly studied. Nevertheless, microconidia and sclerotia are formed simultaneously in the laboratory—that is after two weeks in the absence of light under nutrient-rich conditions—suggesting that both production structures are formed at the same time in the field so that mating can occur to initiate the sexual cycle.

Apothecia (fruiting bodies) contain sexual spores. They arise from sclerotia after the sexual process (spermatization by microconidia) and are 2–20 mm in size. The upper part (disc) consists of a hymenial layer composed of paraphyses and

asci containing the eight uninucleate ascospores in ordered tetrads. *B. cinerea* is self-sterile/heterothallic with the two mating types *MATI-1* and *MATI-2*. Strains of the opposite mating types may be reciprocally crossed by using either sclerotia or microconidia. Derivatives of strain B05.10 and SAS56 (*MATI-1*) can be crossed with the wild strain SAS405 (*MATI-2*) (Faretra et al. 1988b; Amselem et al. 2011). There is little information about the physiology of the apothecia. Carpogenic germination of sclerotia requires pre-conditioning in cold, moist conditions, moderate temperatures, sufficient air, and light. Thereby, apothecial initials grow toward the light source (positive phototropism). Besides, the origin of the sclerotia plays a role as smaller sclerotia from minimal medium are unable to form apothecia likely due to the shortage of storage substances (Faretra and Antonacci 1987; Faretra et al. 1988a). Mutants may be female-sterile because they are unable to form sclerotia or the sclerotia are non-functional, but this does not imply male sterility (Segmüller et al. 2008; Jonkers et al. 2011; Siegmund et al. 2013). Genes expressed in different stages of apothecial development have been identified. Hydrophobins in maternal tissues and the mating type genes are essential for the regular development of apothecia (Terhem and van Kan 2014; Rodenburg et al. 2018).

The response of vegetative mycelia of *B. cinerea* to light is exceptional, and the switch to conidiation or sclerotial development can be easily monitored to identify the regulatory networks. Sclerotial development is the morphogenetic program that the fungus usually pursues (in the dark). Light prevents further sclerotial development in a competent stage by inducing the formation of aerial hyphae that develop into conidiophores and conidia. As a result, the upregulation of *bcltf2* is sufficient for conidiation even in the dark (*always conidia*). The accumulation of vegetative mycelia (*always mycelia*)—optionally with aerial hyphae—suggests that the conidiation program is turned on and prevents sclerotial development but cannot be completed because of defects in conidiophore differentiation. Importantly, these regulators can be essential

for full virulence. Thus, mutations of the BcVEL1-encoding gene were found to cause hyperconidiation (*always conidia*) and reduced virulence in wild strains T4 and 1750 (Schumacher et al. 2012, 2013). Recently, mutations in *Bcin04g03490* encoding a putative transcriptional regulator with Zn₂Cys₆-type DNA binding and acetyltransferase domains were demonstrated to cause a mycelial morphotype and avirulence in *B. cinerea* field isolates (Acosta Morel et al. 2021). In these studies, mutated genes were identified in blind wild isolates. Their deletion in light-responsive B05.10, phenocopied blindness and reduced virulence, while addition of the intact genes from B05.10 rescued the phenotypes in the blind wild strains.

14.2.4 Metabolism: Photoprotection

Pigments are versatile secondary metabolites that protect cells from the detrimental effects of light—especially from UV-induced damage. Often, they have further beneficial functions such as the strengthening of reproduction structures or antimicrobial activities. Common in Ascomycota are the dark 1,8-dihydroxynaphthalene (DHN) melanin and the orange to yellow carotenoids. However, certain species evolved other pigment synthesis pathways leading to their characteristic pigmentations. While the genes for DHN melanogenesis and carotenogenesis are highly conserved in several species, their differential expression profiles reflect the adaptation to environmental light conditions.

Thus, fungi such as *B. cinerea* produce DHN melanin and carotenoids in a spatial and temporal manner while fungi in more extreme sun-exposed habitats usually exhibit constitutive melanogenesis and carotenogenesis leading to the term “black fungi” (Schumacher and Gorbushina 2020). Likewise, the designation “gray mold fungus” for *B. cinerea* is based on the production of DHN melanin during the latter stages of host infection resulting in plant debris covered with a layer of grayish conidiophores and conidia. The sclerotia of *B. cinerea* appear blackish and resemble the

dense microcolonies of black fungi. DHN melanogenesis can be divided into three phases: (i) the *de novo* synthesis of the pentaketide T4HN (1,3,6,8-tetrahydroxynaphthalene), (ii) its modification to DHN, and (iii) the polymerization of DHN resulting in the highly complex DHN melanin (Butler and Day 1998). The genes involved in the first two phases are highly conserved in all fungi and are tightly, partially, or not clustered in the genome. In contrast, different multicopper oxidases (laccases) may carry out the cross-linking of DHN in distantly related Ascomycetes (Jia et al. 2021). Remarkably, *B. cinerea* possesses two different types of melanin polyketide synthases (phase I) mediating DHN melanogenesis together with a shared set of downstream-acting enzymes (phase II), and most likely again with different multicopper oxidases (phase III) in different developmental stages. BcPKS12 directly and BcPKS13 via BcYGH1 provide the precursor T4HN in either sclerotia or conidia and light-stressed mycelia; then T4HN is further converted by the same enzymes in both structures (Schumacher 2016). Melanogenesis is coupled with developmental programs because the genes involved are under the control of the respective master regulators. BcLTF2 as an activator of conidiation positively affects the expression of *bcpks13* (*dev* LIG) and the core genes (conidial DHN melanogenesis), and *bcltf2* expression is repressed by the WCC, BcLTF1, and the Velvet complex. Expression of *bcpks12* and the core genes, and in turn sclerotial melanogenesis, depends on BcSMR1 (sclerotial melanin regulator) whose expression is predicted to be repressed by a yet unknown WCC-dependent LTF (Cohrs et al. 2016; Zhou et al. 2017). The upregulated expression of *bcltf2*, *bcpks13* and the other melanogenic genes in the multicellular infection cushions suggests that the melanin is of the “conidial/BcPKS13 type” (Choquer et al. 2021). A recent study reported on the compartmentalization of melanogenic enzymes to avoid poisoning by pathway intermediates (Chen et al. 2021). Melanin is dispensable for virulence and the differentiation of the reproduction structures but contributes to their stability and longevity

(Schumacher 2016; Zhu et al. 2017; Zhou et al. 2018).

B. cinerea possesses the highly conserved cluster of four genes required for carotenogenesis (*bcpks1*, *bcpkd1*), retinal formation (*bccaol*), and green light sensing (*bop2*). The bifunctional BcPHS1 (phytoene synthase and lycopene cyclase) and the phytoene dehydrogenase (BcPHD1) may catalyze consecutive reactions leading to β -carotene and torulene. Retinal—the chromophore for green light absorption by opsins—can be formed by cleavage of β -carotene by the carotenoid oxygenase. The genes are induced by light and in a blue light/WCC- and BcLTF1-dependent manner (Schumacher 2017). In summary, carotenoids may help *B. cinerea* to resist light-induced stresses, for example, by scavenging of singlet molecular oxygen and free radicals and by protecting the integrity of the membranes. Furthermore, they represent the basis for the formation of retinal for the green light-absorbing opsins. In *B. cinerea*, the carotenoids are almost invisible as the faint orange pigmentation is masked by the dark melanin.

A few *B. cinerea* strains exhibit a remarkable red pigmentation due to the synthesis of bikaverin, a polyketide with antimicrobial properties that is typically produced by different *Fusarium* species (Dos Santos and Bicas 2021). The reddish *B. cinerea* strains possess the entire bikaverin gene cluster consisting of six genes, while grayish *B. cinerea* strains such as B05.10 lack the key enzyme-coding gene (Campbell et al. 2012; Schumacher et al. 2013). Bikaverin production by *B. cinerea* is relevant for interactions with bacteria and certain host species rather than for photoprotection (Spraker et al. 2018; Plesken et al. 2021). However, bikaverin synthesis is regulated by the VELVET complex (Schumacher et al. 2013).

B. cinerea also produces mycosporine-like amino acids (MAAs) for UV absorption and as compatible solutes and antioxidants (Volkman and Gorbushina 2006). Mycosporine-glutamicol—called mycosporine-2—was first isolated as a UV-absorbing compound (P310)

from near-UV-treated cultures that had initiated conidiation. This compound was not detected in dark-grown vegetative cultures leading to the hypothesis that it may have a regulatory effect on conidiation (Tan and Epton 1974; Arpin et al. 1977).

The extracellular polysaccharide (EPS) matrix of *B. cinerea* consists mostly of cinerean [β -(1,3) (1,6)-D-glucan]. The polymer forms a capsule strongly adhering to the germinating conidia and hyphae and contributes to the adhesion to (host) surfaces, protection from light and concomitant heat and desiccation stresses, creation of a micro-environment for extracellular enzymes and as a nutrient reservoir. Thus, *B. cinerea* synthesizes cinerean under carbon surplus conditions and may degrade it by extracellular β -1,3 glucanases under carbon starvation conditions (Dubourdiou et al. 1981; Stahmann et al. 1992; Doss 1999).

14.2.5 Enzyme Systems: Photodamage Repair

The EPS matrix represents the first layer for protecting the cellular contents from environmental stresses, with the two-layered cell walls fortified with DHN melanin, and membranes with incorporated carotenoids providing second and third layers. The cellular lumen contains several primary metabolites functioning as UV screens (e.g., mycosporines), osmoprotectants (e.g., polyols, sugars, amino acids), and/or antioxidants (e.g., tocopherols, ascorbic acid, and carotenoids). Nonetheless, excessive sunlight (UV) may pass these lines of defense and cause mutagenic lesions in the DNA, disadvantageous conformational changes of proteins and the accumulation of harmful ROS. Therefore, enzymatic mechanisms must accomplish the repair of damaged DNA, misfolded proteins, and the detoxification of ROS. In fact, these genes are often upregulated upon different stresses via the stress-activated MAPK module and/or by light in a WCC-dependent fashion.

Photo-damaged DNA is repaired by the CPF protein BcCRY1 using visible light as a source of energy. The second related protein (BcCRY2)

exerts a regulatory role in photomorphogenesis and does not exhibit photoreactivation activity (Cohrs and Schumacher 2017). With BcCRY1, a cyclobutane pyrimidine dimer (CPD) photolyase, *B. cinerea* possesses a single photolyase only. Other Leotiomycetes contain an additional (6-4)-pyrimidine-pyrimidone photoproduct (6-4PP) photolyase, and several Chaetothyriales (Eurotiomycetes) and Dothideomycetes even possess a second CPD photolyase (Schumacher and Gorbushina 2020). Expression of *bccry1*, *bccry2*, and *bcuve1* encoding a conserved UV endonuclease for UVDE-dependent excision repair is induced by light in a WCC-dependent manner. Consequently, the $\Delta bcwcl1$ mutant exhibits a decreased UV tolerance (Cohrs and Schumacher 2017).

Refolding of partially denatured proteins in *B. cinerea* can be mediated by molecular co-chaperones including small heat shock proteins (HSPs). The expression of several genes encoding HSPs, dehydration-induced protein-like (dehydrins), and other stress-related proteins is induced upon exposure to light, as well as to temperature, osmotic and oxidative stress (Heller et al., 2012).

The oxidative stress response (OSR) system of *B. cinerea* comprises detoxifying enzymes, such as superoxide dismutases, catalases, peroxidases, peroxiredoxins, glutaredoxin, and thioredoxins and enzymes reducing glutathione and the thioredoxins (Viefhues et al. 2014). The corresponding genes are upregulated in response to oxidative stress by the transcriptional regulators BAP1 and BcSKN7 (Temme and Tudzynski 2009; Viefhues et al. 2015; Yang et al. 2015). The OSR system has a moderate basal activity that ensures that ROS produced as by-products of various metabolite pathways do not accumulate to toxic amounts. Simultaneous exposure to light and oxidative stress exceeds the buffer capacity, explaining the stronger effect of oxidants on *B. cinerea* in light compared to constant darkness. Both $\Delta bclt1$ and $\Delta bcwcl1$ mutants affected in photoinduction of gene expression are more sensitive to oxidative stress in light (Canessa et al. 2013; Schumacher et al. 2014).

14.2.6 Circadian Clock: Photoentrainment

Several genes involved in coping with light-associated stress in *B. cinerea* are subject to photoregulation by the transcriptional WCC complex and BcLTF1 as a co-activator, as well as by the stress-activated MAP kinase BcSAK1 (Canessa et al. 2013; Schumacher et al. 2014) (unpublished). By this mechanism, the cell activates the needed stress protection measures in a timely fashion and saves resources under non-stressed conditions. Almost all organisms on earth are exposed to a 24 h day/night cycle of changing light and temperature conditions, leading to independent evolution of circadian clocks in the different kingdoms. As molecular machinery entrained by light and temperature cues, they can keep time and enable the organism to anticipate daily changing conditions and to adapt their metabolism in advance. The molecular basis of the fungal circadian clock has been intensively studied in *N. crassa* (Baker et al. 2012), but little is known about the presence and biological functions of the circadian clock in other fungi (Hevia et al. 2016). However, the maintenance of a clock (gain vs. loss) may reflect the relevance of light in an ecological niche. A functional circadian clock exists in *B. cinerea* that shares similarity with the *N. crassa* system, comprising the blue light-sensing WCC as the input pathway, and BcFRQ1 (frequency) as the oscillator. *Bcfrq1* transcript and protein levels oscillate in constant darkness, which requires BcWCL1 as part of the WCC. Likewise, the entrainment of the clock by light requires the WCC. The output of the clock is visible on the host *A. thaliana*: larger lesions are formed when leaves are inoculated at dusk than at dawn which is in accordance with increased susceptibility of the host during the night. $\Delta bcfrq1$ mutants exhibit decreased conidiation accompanied by sclerotial development in the light (*always sclerotia*), suggesting that BcFRQ1 modulates photomorphogenesis in a nutrition-dependent manner (Canessa et al. 2013; Hevia et al. 2015).

14.3 Conclusion: *Botrytis* Exploits the Host for Nutrition and Sun Protection

B. cinerea maintains a highly sophisticated light signaling machinery that senses different light qualities to trigger a variety of responses, that are photoprotection, photomorphogenesis, positive and negative phototropism and photoentrainment. These processes render *B. cinerea* a valuable model to enlighten the role of light in parasitic fungus-plant interactions.

The vegetative mycelium of *B. cinerea* is the core of all infection and developmental programs in that it mediates the rapid colonization of dead plant tissues in terms of necrotrophic (active killing and colonization) and saprophytic growth (colonization of already dead tissues). The vegetative mycelium is not visibly pigmented and thus considered to be sensitive to different biotic and abiotic stresses. However, the vegetative hyphae have a very limited half-life and are usually restricted to the invasive growth phase in which the hyphae are protected from light by the plant tissue. Fast colonization of fresh tissues and proper nutrient acquisition enables the rapid formation of well-protected and long-lasting reproduction structures (conidiophores with conidia and sclerotia) on the surfaces of rotted plant tissues. Depending on the light and temperature conditions, conidiation or sclerotial development is initiated for spreading of disease (summer cycle) or fungal survival (winter cycle). Taken together, *B. cinerea* uses the light regulatory networks to avoid light whenever possible; for example, by minimizing the half-life of sensitive cells that are hiding in plant tissues and by scheduling critical steps for the night including conidiogenesis, conidial germination, and penetration of plant tissues. Not least, the multinucleate nature of hyphae and conidia helps *B. cinerea* to survive in light-flooded habitats by compensating for detrimental mutations in single nuclei.

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