Infection Strategies of *Botrytis cinerea*

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Abstract

***Botrytis cinerea* is a ubiquitous filamentous fungal pathogen of a wide range of plant species. The fungus is able to infect all aerial parts of its host plants to a certain extent. Infection may cause enormous damage both during plant growth and in the post-harvest phase (during cold storage or transport). *B. cinerea* is a major cause of economic loss in the production chain of cut flowers, bulb flowers and pot plants. Molecular-genetic studies performed over the past decade have provided a wealth of novel insights into the infection mechanisms utilised by the pathogen. Fungal genes were identified that are important for successful infection by *B. cinerea.* Such knowledge provides perspectives for designing novel, rational plant protection strategies that effectively counteract important fungal virulence factors. In this review I will divide the infection process into different stages and discuss the role of various fungal enzymes and metabolites in the individual stages. Finally some perspectives are addressed for novel control strategies that may reduce and/or delay the damage inflicted by *B. cinerea* infection.**

INTRODUCTION

Botrytis cinerea Persoon: Fries (teleomorph *Botryotinia fuckeliana*, also known as "grey mould fungus") causes serious pre- and post-harvest diseases in at least 200 plant species (Jarvis, 1977), including agronomically important crops and harvested commodities, such as grapevine, tomato, strawberry, cucumber, bulb flowers, cut flowers and ornamental plants. The pathogen is a necrotroph, inducing host cell death resulting in serious damage to plant tissues culminating in rot of the plant or the harvested product. Reviews have been published on the infection mechanisms of *B. cinerea*, with emphasis on microscopic and biochemical studies (Staples and Mayer, 1995) or molecular genetics (Prins et al., 2000b). The application of molecular-genetic tools such as transformation (Hamada et al., 1994), differential gene expression analysis (Benito et al., 1996), gene cloning (van der Vlugt-Bergmans et al., 1997a,b) and targeted mutagenesis (van Kan et al., 1997) has led to novel insight in the structure of *B. cinerea* genes and their role in the infection process. Here our current understanding of the mechanisms that *B. cinerea* exploits to infect its host plants will be summarised.

DISEASE CYCLE

For the purpose of simplification, different stages are distinguished in the disease cycle of *B. cinerea* (Fig. 1). The disease cycle starts with a conidium landing on the host surface. *B. cinerea* conidia are ubiquitous in the air and can be transported by wind over long distances before infecting the next host (Jarvis, 1977). Following attachment, the conidium germinates on the host surface under moist conditions and produces a germ tube that develops into an appressorium that penetrates the host surface. The underlying cells are killed and the fungus establishes a primary lesion, in which necrosis and host defense responses may occur. In some cases this is the onset of a period of quiescence of an undefined length, in which fungal outgrowth is restricted (reviewed by Prusky, 1996). Quiescence is especially an important feature in host species infected at the flowering stage (e.g. strawberry). At a certain stage, the defense barriers are breached and the fungus starts a vigorous outgrowth, resulting in rapid maceration of plant tissue, on which the fungus eventually sporulates to produce inoculum for the next infection. Under

optimal conditions, an infection cycle may be completed in as little as 3-4 days, depending on the type of host tissue attacked. In the following paragraphs I will discuss the different stages in the disease cycle and the role that enzymes and metabolites (mainly of fungal origin) play in these stages. Given the wide host range of the fungus, not all stages or processes may occur in every infection.

Attachment of Conidia

Attachment is thought to be mediated by physical surface interactions on the plant cuticle. Two steps were distinguished in the attachment to host tissue. The first stage, preceding the hydration of conidia, typically involves weak adhesive forces resulting from hydrophobic interactions between host and conidial surfaces (Doss et al., 1993). Stronger binding occurs in the second stage (Doss et al., 1995), several hours after inoculation, when conidia have germinated. The tips of germ tubes are covered with fibrillar-like extracellular matrix material (Doss et al., 1995; Cole et al., 1996; see also Fig. 2 in Prins et al., 2000b), consisting of carbohydrates and proteins (Doss, 1999). The matrix contains fungal enzymes (Gil-Ad et al., 2001). It may act as an adhesive on the host surface (Doss et al. 1995) and protect hyphae from dehydration and defense mechanisms of the host.

Germination

Several factors influence the germination of a conidium. Free surface water or high relative humidity (>93% RH) is essential to germinate and penetrate the host epidermis (Williamson et al., 1995). When dry conidia are inoculated on plant surfaces and incubated in the absence of free surface water, the emerging germ tube usually remains short before it penetrates the surface (Salinas and Verhoeff, 1995; Williamson et al., 1995; Cole et al., 1996). Inoculation with conidia in an aqueous suspension requires the addition of nutrients (Harper et al., 1981; van den Heuvel, 1981), which might mimic the situation in a wound on the plant epidermis, from which nutrients leach. A highly efficient germination and synchronous infection of tomato leaves was obtained, when conidia were pre-incubated for 2-4 hours in liquid medium supplemented with phosphate and sugar, prior to inoculation (Benito et al., 1998).

Gaseous compounds may stimulate germination. Elad and Volpin (1988) found a correlation between the level of ethylene production by rose cultivars and the severity of grey mould symptoms. Stimulation of grey mould development by ethylene was also found in strawberry, tomato, cucumber and pepper (e.g. Elad and Volpin, 1988; McNicol et al., 1989). This observation is usually ascribed to host tissue senescence that coincides with ethylene production. The influence of ethylene on *B. cinerea* itself has not been studied in detail. Analogous to *Colletotrichum gloeosporioides* (Flaishman and Kolattukudy, 1994), germination of *B. cinerea* seems to be influenced by ethylene. Germination of conidia on a hydrophobic surface was stimulated by exogenous ethylene, but the germ tube length was unaffected (Kepczynski and Kepczynska, 1977). Application of 2,5-norbornadiene, a competitive inhibitor of ethylene perception in plants, inhibited germination in a reversible manner (Kepczynska, 1993). In a hydrophilic environment, however, ethylene stimulated germ tube elongation without affecting the percentage of germination (Barkai-Golan et al., 1989). Ethylene produced by the plant during tissue senescence or fruit ripening might function as a signal for the conidia on the (hydrophobic) plant surface to germinate and initiate the infection. Germ tube elongation might subsequently be stimulated by ethylene in the more hydrophilic environment of the invaded plant tissue. Thus, ethylene might favour grey mould development by weakening the host, as well as by stimulating germination of *B. cinerea* conidia and outgrowth of hyphae. Molecular and biochemical approaches are required to further elucidate the effects of ethylene on *B. cinerea*.

Differentiation of Infection Structures on the Host Surface

B. cinerea forms appressoria during penetration, albeit not the highly organized

appressoria that are typical for many plant pathogenic fungi (reviewed by Mendgen et al., 1996). Several authors observed the swelling of hyphal tips of germ tubes and interpreted these as an appressorium-like structure (Akutsu et al., 1981; van den Heuvel and Waterreus, 1983; Cole et al., 1996). Recent microscopic and histochemical studies (Tenberge et al., 2002) and gene function analysis (Gourgues et al., 2003) indicate that these structures act as functional appressoria. The swelling of the hyphal tip may be the consequence of a rise in osmotic value in the hyphal tip, resulting in water absorption. In the absence of a rigid layer in the outer wall, swelling cannot result in an equally high turgor as in the appressoria of *Magnaporthe grisea* (Howard et al., 1991; de Jong et al., 1997). The extracellular matrix may contribute to the swelling by retaining water, as its major polysaccharide component (cinerean), is extremely hygroscopic.

Penetration of the Host Surface

Invasion of host tissue can be achieved by active penetration or passive ingress. *B. cinerea* is an opportunist that can initiate infection at wound sites, or at sites previously infected by other pathogens. *B. cinerea* can also enter the substomatal cavity via an open stoma. Nevertheless the pathogen is perfectly able to penetrate intact host surfaces. Only direct penetration of the epidermal surface is discussed in this paragraph. For reasons of simplicity the penetration of dead or wounded tissue, as well as via stomata, is regarded as an expansion rather then a penetration process, and is dealt with in later paragraphs.

The cuticle consists of cutin, a polyester of hydroxylated and epoxidized C_{16}- and C_{18}-fatty acids, covered with wax. Physical damage or brute mechanical penetration of the cuticle by *B. cinerea* is not usually observed (Williamson et al., 1995; Cole et al., 1996). Hence, it was considered that enzymatic (cutinolytic) activity is required for penetrating intact host surfaces (Salinas and Verhoeff, 1995; van der Vlugt-Bergmans, 1997a). Salinas (1992) raised monoclonal antibodies against a 18 kDa cutinase. Application of the antibody to gerbera flowers prior to inoculation reduced the number of lesions formed. The gene encoding this cutinase enzyme was cloned (van der Vlugt-Bergmans et al., 1997a) and a gene replacement mutant was made that was entirely devoid of this cutinase activity (van Kan et al., 1997). The mutant was equally virulent as the wild type isolate, both on gerbera flowers and tomato fruits and the fungus remained able to penetrate intact cuticle surfaces (van Kan et al., 1997). Although the observations of Salinas (1992) remain to be explained, it can be concluded that this 18 kDa cutinase is not essential in penetration.

A different enzyme that may mediate host penetration is a 60 kDa lipase (Comménil et al., 1995), inducible by apple cutin (Comménil et al., 1998) as well as grape berry cuticle components (Comménil et al., 1999). The lipase possesses cutinolytic activity although with clearly distinct kinetic properties than the 'typical' cutinase mentioned above (Comménil et al., 1998). When polyclonal antibodies raised against this lipase were applied prior to inoculation with *B. cinerea* conidia, germ tubes were prevented from penetrating the cuticle. The antibodies did not affect germination (Comménil et al., 1998). The protein has been partially sequenced (Commenil et al., 1999). Constructing a targeted lipase-deficient mutant and determining its virulence should assess the role of the lipase in host tissue penetration.

Killing the Host

B. cinerea kills host cells before they are invaded by hyphae (Clark and Lorbeer, 1976). Invasion of plant tissue by *B. cinerea* triggers nuclear condensation and plant membrane damage, indicators for programmed cell death, in a ring of cells around the hyphae (Govrin and Levine, 2000). These results imply that diffusible factors have a direct or indirect phytotoxic activity. These factors may be proteins or low molecular weight compounds secreted by the fungus into its environment. The induction of programmed cell death facilitates *B. cinerea* invasion and may in fact be essential for successful infection (Govrin and Levine, 2000).

1. Toxins. Culture filtrates of *B. cinerea* may induce toxic effects when applied to plant

tissue (Rebordinos et al., 1996). Phytotoxic compounds were identified as botcinolide, a highly substituted lactone (Cutler et al., 1993) and botrydial, a tricyclic sesquiterpene (Colmenares et al., 2002). The observation that both secondary metabolites were only secreted by *B. cinerea* in medium with high glucose levels initially raised doubts about their physiological relevance in planta. Recent analytical chemistry studies have, however, demonstrated that botrydial accumulates in infected tissue at physiologically relevant concentrations (Deighton et al., 2001; Muckenschnabel et al., 2003). The production of botrydial may be an important factor in the infection of (some) host plants, but it needs to be evaluated by constructing mutants in the botrydial synthetic pathway. Resolution of this pathway is in progress (Duran et al., 2001) but the relevant botrydial biosynthetic genes have not yet been identified and mutants are not available.

2. Oxalic Acid. Secretion of oxalic acid (OA) occurs in fungi from various taxonomic classes (reviewed by Dutton and Evans, 1996). A key role for OA in pathogenesis has been postulated for *Sclerotinia sclerotiorum* (Godoy et al., 1990), a close relative of *B. cinerea*. Mutants of *S. sclerotiorum*, deficient in OA production, were unable to infect *Arabidopsis* plants (Dickman and Mitra, 1992) and the deficiency could be restored by supplementing the inoculum with OA. *B. cinerea* produces OA both in vitro (Gentile, 1954; Germeier et al., 1994) and in planta (Verhoeff et al., 1988). OA may form calcium oxalate crystals within the host tissue (see Figure 3 in Prins et al., 2000b). The sizes of lesions induced by several strains of *B. cinerea* on grapevine and bean leaves correlated with the amount of OA that these strains secreted in vitro (Germeier et al., 1994). It remains unclear whether the amounts of OA produced in planta are sufficient to cause host cells to collapse. OA may in fact be a co-factor in pathogenesis rather than the primary phytotoxic agent. Ten Have et al. (2002) postulated that OA might act in synergy with endoPGs during tissue maceration. Fungal endoPGs have an activity optimum at low pH and may therefore be stimulated by the simultaneous secretion of OA. Moreover, OA may stimulate pectin degradation resulting from endoPG action by sequestering the Ca^{2+} ions from (intact or partially hydrolyzed) Ca-pectates in the cell walls. The removal of Ca^{2+} ions disturbs intermolecular interactions between pectic polymers and disrupts the integrity of the pectic backbone structure. Consequently, the pectic structure absorbs water and swells, as described by Mansfield and Richardson (1981).

3. Induction of Reactive Oxygen Species. Recent studies have focussed on Reactive Oxygen Species (ROS) production in relation to *B. cinerea* pathogenicity. ROS is the joint term for the superoxide anion, hydroxyl radical and hydrogen peroxide. The level of H_2O_2 released from bean leaf discs inoculated with different *B. cinerea* isolates, correlated with the aggressiveness of the isolate on such leaf tissue (Tiedemann, 1997). The sensitivity of genotypes of bean (*Phaseolus vulgaris*) to oxidative stress is correlated to their susceptibility to *B. cinerea* (Tiedemann, 1997). In *B. cinerea*-infected *Arabidopsis thaliana* leaves, H_2O_2 was detected in the apoplastic space several cell layers away from the fungal hyphae (Govrin and Levine, 2000). Enormous perturbances in redox status are observed at the host-fungal interface, even in plant tissue at some distance from the infection front (Muckenschnabel et al., 2001a,b; 2002). Also lipid peroxidation was observed (Weigend and Lyr, 1996; Muckenschnabel et al., 2001a; 2002). Antioxidants reduce grey mould disease development (Elad, 1992; Tiedemann, 1997).

Generation of ROS occurs at the host-fungal interface. Fungal as well as host enzymes presumably contribute to the process (Schouten et al., 2002a; see also Fig. 4 in Prins et al., 2000b). Oligogalacturonides released from the plant cell wall by pectinases of *B. cinerea* are potential elicitors of an oxidative burst (Legendre et al., 1993), presumably mediated by a plasmamembrane-bound NADPH oxidase, inducible by fungal elicitors and requiring extracellular Ca^{2+} (Schwacke and Hager, 1992). Indeed Schouten et al. (2002a) demonstrated the production of H_2O_2 in the plasmamembrane of host cells adjacent to a fungal hypha. Infiltration of the NADPH oxidase inhibitor DPI into leaves of *Arabidopsis thaliana* prior to inoculation with *B. cinerea* resulted in a reduction of ROS production and slower colonization of host tissue by the fungus (Govrin and Levine, 2000). The precise mechanism by which *B. cinerea* induces oxidative stress in its hosts is

still unclear. Fungal extracellular sugar oxidases (Liu et al., 1998; Edlich et al., 1989) or superoxide dismutase (Tenberge et al., 2002) may be responsible for generating the H_2O_2. The identification of the fungal enzymes that are important for H_2O_2 accumulation at the host-fungus interface awaits molecular-genetic studies with targeted mutants.

Formation of Primary Lesions, Defense Responses in the Host

Host surface penetration and the rupture of plant cell walls by enzymes of *B. cinerea* triggers a cascade of processes in the fungus as well as the host. This paragraph deals with defense responses at the host-fungus interface and their impact on the progress of infection.

1. Induction of Necrosis. The initial establishment of primary necrotic lesions coincides with (and is in fact the result of) host defense activation in the neighbouring tissue in response to the death of an invaded cell. It is as yet unclear whether cell death caused by a necrotroph, such as *B. cinerea*, is equivalent to cell death during a hypersensitive response (HR) to a biotrophic pathogen (reviewed by Lamb and Dixon, 1997). Govrin and Levine (2000) showed that an oxidative burst occurs in plant tissue several cell layers away from the fungal hyphae. Cytological staining provided evidence for rapid nuclear condensation and irreversible membrane damage, indicative of a programmed cell death process (Govrin and Levine, 2000). Largely the same defense responses are activated during an infection by *B. cinerea* as during HR to avirulent races of a biotrophic pathogen: lignification (Maule and Ride, 1976; Heale and Sharman, 1977), biosynthesis of phytoalexins (e.g. Bennett et al., 1994) and accumulation of PR proteins (e.g. Benito et al., 1998; Diaz et al., 2002). The total spectrum of defense responses results in a primary necrotic lesion in which the fungus is effectively restricted. Depending on the type of host tissue and yet unidentified physiological aspects of the host, the lesions enter a lag phase in which they do not expand. A proportion of the primary lesions eventually develops into aggressive, expanding lesions (van den Heuvel, 1981; De Meyer and Höfte, 1997; Benito et al., 1998; Diaz et al., 2002). In the non-expanding lesions the fungus is not killed, since viable fungal mycelium could be recovered from all lesions (Benito and van Kan, unpublished results). Thus, an active defense contributes to (temporarily) restricting the fungus within the primary lesions, giving rise to a period of quiescence.

2. Quiescence. In some tissues, *B. cinerea* causes long-lasting quiescent infections (reviewed by Prusky, 1996), in which no symptoms are discernible at first. Prominent examples are described in soft fruit such as strawberry, raspberry and grape. In these hosts, *B. cinerea* predominantly infects the host flowers and resides quiescent in the developing fruit tissue, often for several weeks. Fungal growth resumes at the onset of fruit ripening. It has been postulated that high levels of fungitoxic or fungistatic compounds (phytoalexins) in immature fruits contribute to grey mould quiescence. The level of these compounds decreases during the ripening process concomitant with fungal outgrowth. Attempts have been undertaken to increase the levels of antifungal compounds or to prevent their degradation during ripening. The level of the stilbene phytoalexin resveratrol in grapes is correlated with grey mould resistance (Langcake, 1981; Bavaresco et al., 1997). The effect of over-expressing stilbene synthase genes from *Vitis* in transgenic plants on resistance towards *B. cinerea* was evaluated. A significant, partial resistance was obtained in tobacco (Hain et al., 1993) but not in tomato (Thomzik et al., 1997).

Besides phytoalexins, immature fruits usually contain high levels of proteinaceous inhibitors of fungal cell wall degrading enzymes, the PolyGalacturonase Inhibiting Proteins (PGIPs) and their level decreases during ripening (De Lorenzo et al., 2001). In view of the significant role that polygalacturonases play in the infection (see below), efforts to produce transgenic plants overexpressing PGIPs have been undertaken to obtain resistance towards *B. cinerea*. Indeed high constitutive expression of a heterologous PGIP gene in tomato and an endogenous PGIP gene in *Arabidopsis* resulted in an increased resistance to *B. cinerea* (Powell et al., 2000; Ferrari et al., 2003). One of the considerations in this strategy is that PGIPs have a differential activity towards individual

fungal endoPGs (Desiderio et al., 1997; De Lorenzo et al., 2001). This makes it relevant to chose PGIPs that are most potent against the *B. cinerea* endoPG isozymes that are important in virulence (Ten Have et al., 2002).

Evasion of Chemical Defense

Pathogenic fungi have developed mechanisms to overcome deleterious effect of preformed (phytoanticipins) or induced (phytoalexins) plant defense compounds. One such mechanism involves an energy-dependent secretion by ABC-transporters that confers some degree of tolerance to the fungitoxic effects of such compounds (de Waard, 1997). About a dozen ABC transporters have been cloned from *B. cinerea* (de Waard, unpublished results) and their role in resistance towards plant defense compounds and virulence on various hosts is being studied (Schoonbeek et al., 2001; 2002).

The major strategy of plant pathogenic fungi to deal with antifungal plant compounds is an enzymatic detoxification (reviewed by Osbourn et al., 1998). Because of its broad host range, *B. cinerea* encounters a wide spectrum of antimicrobial compounds, depending on the host species that it attempts to infect. The ability of *B. cinerea* to degrade or detoxify phytoalexins was already intensively studied two decades ago (reviewed by Mansfield, 1980). The best studied example for phytoalexin detoxification by *B. cinerea* is the detoxification of the *Vitis* phytoalexins pterostilbene and resveratrol. The ability of fungal isolates to detoxify these phytoalexins was correlated with their virulence (Sbaghi et al., 1996). Conversely, the resistance level of *Vitis* genotypes against grey mould is correlated with their phytoalexin content (Langcake, 1981; Jeandet et al., 1992). *B. cinerea* produces a substrate-specific laccase (stilbene oxidase) that is able to oxidize both compounds to non-toxic derivatives (Pezet et al., 1991). The enzyme was purified and characterized (Pezet, 1998) and the structure of stilbene degradation products elucidated (Breuil et al., 1998). Schouten et al. (2002b) cloned a laccase gene responsible for resveratrol conversion and generated targeted mutants that lost the ability to convert resveratrol. The mutants did not show reduced virulence on *Vitis* or any other host tested (Schouten et al., 2002b), indicating resveratrol conversion is not essential for virulence.

B. cinerea is also able to detoxify preformed antimicrobial compounds such as the tomato saponin α-tomatine (Verhoeff and Liem, 1975). Quidde et al. (1998) showed that α-tomatine is only partly deglycosylated by removal of the terminal xylose, yielding β_1-tomatine which is less toxic than α-tomatine. A field survey showed that *B. cinerea* isolates from various host plants and geographic origin all possessed tomatinase activity, with one exception. Interestingly, the strain lacking tomatinase activity was highly sensitive to α-tomatine, completely non-pathogenic on tomato, yet highly aggressive on *Phaseolus vulgaris* (Quidde et al., 1998). These data suggest that the ability to detoxify α-tomatine is correlated with virulence of *B. cinerea* on tomato. However, this strain might have further defects that cause the specific loss of virulence on tomato; the cloning and targeted mutagenesis of the tomatinase gene in a wild type strain will be necessary for final evaluation of the role of tomatinase in the infection of tomato.

The oxidative burst at the host-pathogen interface imposes stress on the host as well as the pathogen (Schouten et al., 2002a). *B. cinerea* is able to cope with external oxidative stress in order to survive in the necrotic tissue. Successful detoxification of H_2O_2 is presumably mediated by an extracellular catalase (Schouten et al., 2002a) with a Glutathione S-Transferase functioning as intracellular back-up (Prins et al., 2000a; Schouten et al., 2002a). Targeted mutagenesis of the extracellular catalase gene did not negatively affect the survival of the mutant within the oxidative environment of a necrotic lesion. Virulence of the mutant on several hosts was indistinguishable from that of the wild type (Schouten et al., 2002a).

Disease Expansion and Tissue Maceration

B. cinerea must be able to macerate plant tissue and convert it into fungal biomass. The initial step in expansion of primary lesions is presumably the killing of neighbouring cells by mechanisms similar to the ones described above. In order to grow

from the primary lesion into neighbouring tissue, *B. cinerea* must actively degrade plant cells. The major barrier that the pathogen encounters is the host cell wall. Cell wall degradation facilitates the entry of the pathogen and it provides nutrients for growth (reviewed by Ten Have et al., 2002).

Microscopic studies showed that after penetration of the cuticle, hyphae of *B. cinerea* frequently invade the anticlinal wall between two epidermal cells. The concomitant swelling of the epidermal cell wall (Mansfield and Richardson, 1981) is indicative for the degradation of pectin in the matrix of the epidermal wall, resulting in water absorption. Biochemical evidence suggested that pectinases are involved in primary infection. *B. cinerea* possesses a set of cell wall degrading enzymes (CWDEs) including pectin lyase (Movahedi and Heale, 1990), pectin methylesterase (Reignault et al., 1994; Valette-Collet and Boccara, 2003), exo- and endo-polygalacturonase (Johnston and Williamson, 1992) and cellulase (Barkai-Golan et al., 1988). *B. cinerea* genes have been cloned that encode CWDEs: pectin and pectate lyase (Mulder, unpublished), rhamnogalacturonan-hydrolase (Chen et al., 1997) six endoPGs (Ten Have et al., 1998, Wubben et al., 1999), pectin methylesterase (Valette-Collet et al., 2003) and cellulases (van Kan et al., unpublished). The endoPG genes, denoted *Bcpg*1-6, constitute a well studied and, most probably, complete gene family. The expression patterns of the individual endoPG genes in planta depend on the host species that is infected, on the stage of the infection, as well as on the external conditions during which infection occurs (Ten Have et al., 2001).

Targeted deletion mutants were made in *Bcpg*1 by gene replacement. The lesion expansion rate of such mutants was reduced by about 25% as compared to the wild type (Ten Have et al., 1998). The BcPG1 protein might be important in facilitating intercellular growth at the periphery of the invading hyphae. Another explanation for the reduction in virulence may be purely nutritional. The absence of BcPG1 reduces the release of pectin degradation products that serve as nutrients, hence resulting in slower growth of the fungus through the tissue. Mutagenesis of four additional endoPG genes is in progress (van Kan et al., unpublished results). The distinction between a “pathogenic function” and a “nutritional function” of *B. cinerea* endoPGs (Ten Have et al., 2002) is difficult to make.

SUMMARY AND CONCLUDING REMARKS

Molecular-genetic tools are available to validate microscopic and biochemical observations of the infection strategy of *B. cinerea*. Dozens of genes have been cloned and their expression in planta or in vitro studied. The role(s) of individual genes in the infection process can be analysed by targeted mutagenesis and studying the behaviour of mutants on various hosts. This overview demonstrates that the pathogen is versatile and uses a combination of factors during pathogenesis. We are beginning to understand the roles of various factors in the different stages of the disease cycle and the ways in which some of these factors interact. There seems to be a delicate balance between the attack mechanisms of the fungus and the defense of the host. Such knowledge will contribute to new, rational disease control strategies. The future challenge lies in the design of methods that alter the balance in the interaction in favor of the host. Such a strategy will likely consist of a sophisticated combination of biological control agents, appropriate (partially) resistant plant genotypes and chemicals that either enhance the plant defense response or interfere with crucial steps in the infection process.

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Figure

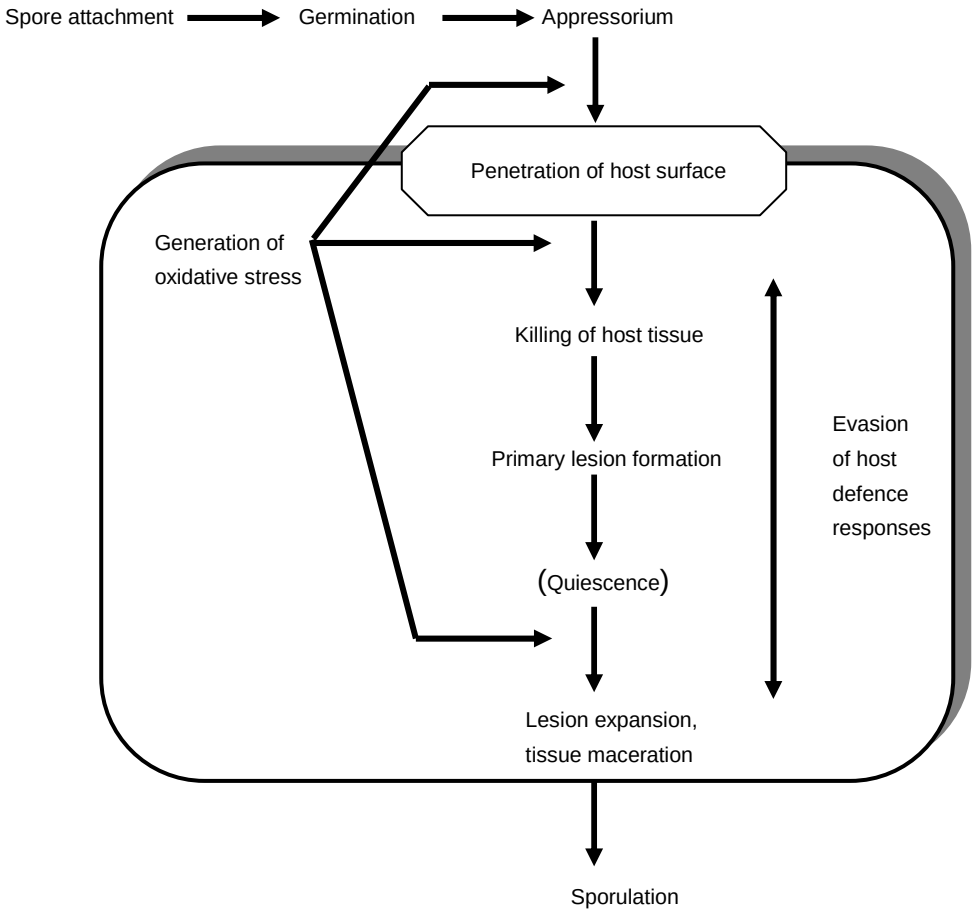

Fig. 1. Different stages in the infection process of *B. cinerea*. The shaded box represents the host tissue.