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Pathogen profile

Cladosporium fulvum (syn. *Passalora fulva*), a highly specialized plant pathogen as a model for functional studies on plant pathogenic Mycosphaerellaceae

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SUMMARY

Taxonomy: *Cladosporium fulvum* is an asexual fungus for which no sexual stage is currently known. Molecular data, however, support *C. fulvum* as a member of the Mycosphaerellaceae, clustering with other taxa having *Mycosphaerella* teleomorphs. *C. fulvum* has recently been placed in the anamorph genus *Passalora* as *P. fulva*. Its taxonomic disposition is supported by its DNA phylogeny, as well as the distinct scars on its conidial hila, which are typical of *Passalora*, and unlike *Cladosporium s.s.*, which has teleomorphs that reside in *Davidiella*, and not *Mycosphaerella*.

Host range and disease symptoms: The presently known sole host of *C. fulvum* is tomato (members of the genus *Lycopersicon*). *C. fulvum* is mainly a foliar pathogen. Disease symptoms are most obvious on the abaxial side of the leaf and include patches of white mould that turn brown upon sporulation. Due to stomatal clogging, curling of leaves and wilting can occur, leading to defoliation.

***C. fulvum* as a model pathogen:** The interaction between *C. fulvum* and tomato is governed by a gene-for-gene relationship. A total of eight *Avr* and *Ecp* genes, and for four of these also the corresponding plant *Cf* genes, have been cloned. Obtaining conclusive evidence for gene-for-gene relationships is complicated by the poor availability of genetic tools for most Mycosphaerellaceae–plant interactions. Newly developed tools, including *Agrobacterium*-mediated transformation and RNAi, added to the genome sequence of its host tomato, which will be available within a few years, render *C. fulvum* attractive as a model species for plant pathogenic Mycosphaerellaceae.

Useful websites: http://www.sgn.cornell.edu/help/about/index.html; http://cogeme.ex.ac.uk

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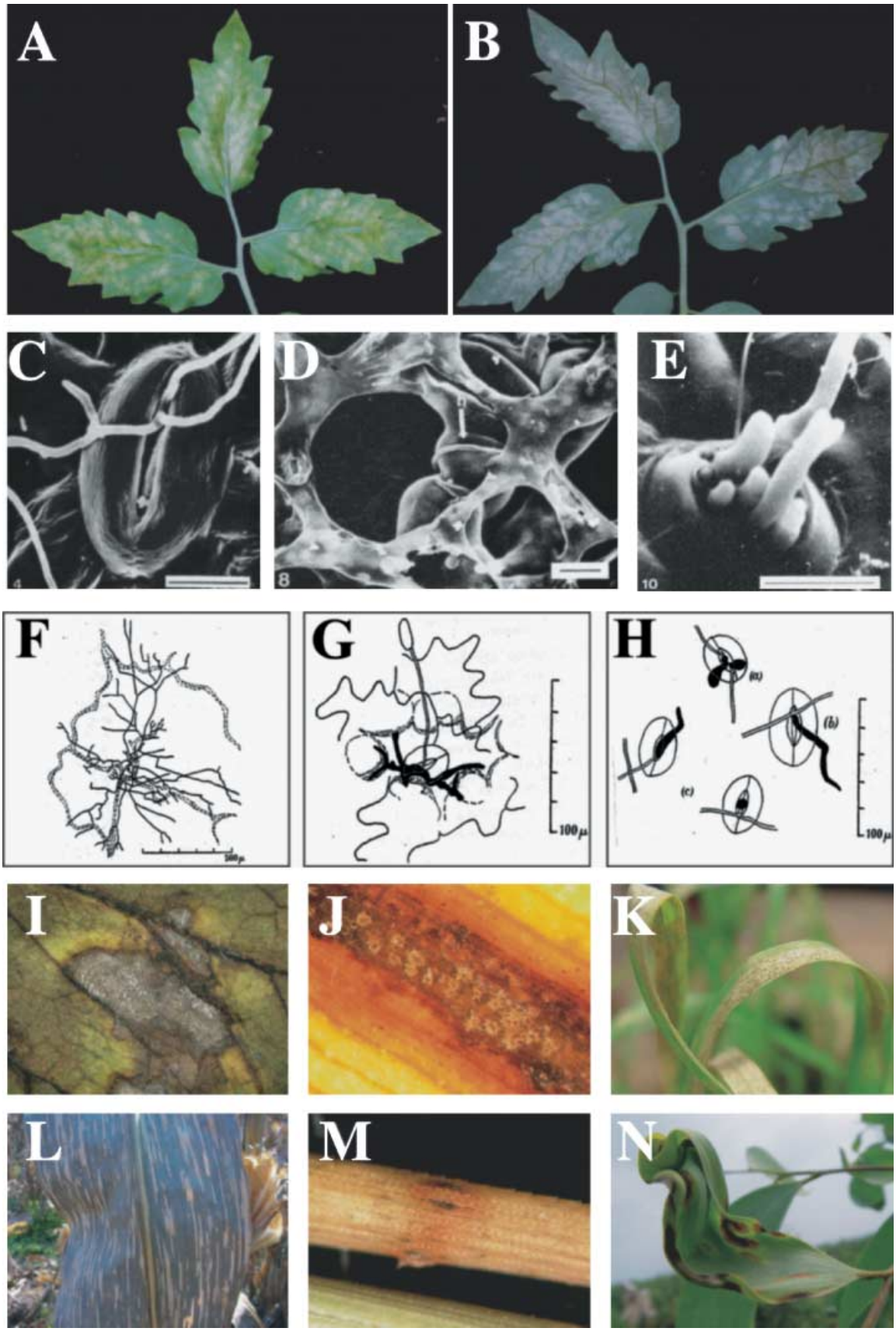
INTRODUCTION

Cladosporium fulvum [syn. *Passalora fulva* (Braun *et al.*, 2003)] is the causal organism of tomato leaf mould, a fungal disease first described by Cooke (1883). Generally, foliage is the only tissue affected by the fungus, although occasionally also stems, blossoms, petioles and fruit are attacked (Butler and Jones, 1949; Jones *et al.*, 1997). Conidia of the fungus can infect successfully if they settle on the abaxial side of a leaf, germinate, and subsequently enter through open stomata. Initial disease symptoms occur at the earliest 1 week after the start of infection as pale green or yellowish diffuse spots on the upper leaf surface, which later enlarge, turning into distinctive yellow spots (Fig. 1A). This appearance is the effect of cell death in the palisade parenchyma. The abaxial side of the leaf shows the most distinct symptoms: patches of white to olive-green mould that turn brown once sporulation commences (Fig. 1B). In advanced stages of disease development stomata do not function properly, because they are blocked by aggregations of conidiophores (Fig. 1E) that use the stomata to exit the leaf and liberate conidia. These subsequently contribute to spread of the disease. As a result of stomatal clogging, plant respiration is severely hampered (Butler and Jones, 1949). This can result in wilting of leaves, partial defoliation and, in severe infections, death of the host (Jones *et al.*, 1997).

Although *Lycopersicon esculentum* (tomato) is susceptible to the fungus, many other *Lycopersicon* species are often resistant (Butler and Jones, 1949). About 100 years ago it was discovered that resistance against *C. fulvum* is genetically determined by the presence of *Cf* resistance genes (Lind, 1909; Norton, 1914). Later it was found that the relationship between host and pathogen is governed by a so-called 'gene-for-gene' relationship. The gene-for-gene hypothesis states that each dominant pathogen avirulence (*Avr*) gene confers recognition to a corresponding dominant host resistance (*R*) gene (Flor, 1942, 1946; Oort, 1944). Although *C. fulvum* most likely originates from the natural habitat of *Lycopersicon* species in South America, greenhouse cultivation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has also generated favourable conditions for the pathogen in temperate climate areas. As a result, for decades yearly outbreaks of the disease occurred also in these regions in greenhouses and tomato leaf mould became a persistent disease. However, the introduction of resistance loci from related wild species of tomato (*Cf-1* to *Cf-5*) into cultivated tomato has resulted in efficient containment of the pathogen (Boukema and Garretsen, 1975; Boukema, 1977; Hubbeling, 1978; Kerr *et al.*, 1971; Langford, 1937). Since the introduction of the *Cf-9* resistance gene in the late 1970s in currently grown tomato cultivars in the late 1970s, *C. fulvum* no longer poses a serious threat to commercial tomato cultivation. Despite its limited agronomic importance, the *C. fulvum*–tomato interaction has become a model to study plant–pathogen interactions after intensive studies by the research groups of Drs Higgins (Higgins *et al.*, 1998), Oliver (Oliver *et al.*, 2000) and de Wit (Joosten and de Wit, 1999).

This review will mainly focus on the pathogenic properties of *C. fulvum* and the mechanisms deployed by the fungus to establish pathogenicity. In addition, the properties of this interaction to serve as a model system for the interaction between plants and other members of *Mycosphaerella* will be discussed. As recent advances of the research on the tomato *Cf* resistance genes and homologous genes that act in pathogen defence from other plant species have been extensively reviewed (Kruijt *et al.*, 2005; Rivas and Thomas, 2002), we will not address *Cf*-gene structures and *Cf*-mediated downstream defence signalling.

THE INFECTION CYCLE ON SUSCEPTIBLE PLANTS: THE COMPATIBLE INTERACTION

The conidia of *C. fulvum* are generally spread by wind or water splash. If conidia land on the abaxial side of a leaf, successful infection can occur. At high relative humidity (over 85%) conidia germinate and form thin runner hyphae that grow randomly (undirectional) over the leaf surface (Bond, 1938; de Wit, 1977; Lazarovits and Higgins, 1976a). After approximately 3 days, a main germ tube or a lateral branch of the hyphae enters the tomato leaf upon encountering an open stoma (Fig. 1C). From this stage onward, the diameter of fungal hyphae enlarges at least two-fold. Subsequently, hyphal growth continues from the substomatal cavity into the intercellular space between the spongy mesophyll cells (apoplast) by the formation of long, branched hyphal structures (Bond, 1938; de Wit, 1977; Lazarovits and Higgins, 1976a). Fungal growth appears to be preferentially directed towards the vascular tissues, probably triggered by a sucrose gradient around the phloem (van den Ackerveken *et al.*, 1994; Wubben *et al.*, 1994). Sometimes, but only in later stages of the infection, the palisade parenchyma is invaded (Lazarovits and Higgins, 1976a).

Although no obvious feeding structures such as haustoria can be observed, growth of the fungus appears to depend on maintenance of close contact between fungal hyphae and host cells (Fig. 1D). This can sometimes be observed as slight indentations where fungal hyphae touch host cells (de Wit, 1977). This close contact suggests that the pathogen actively withdraws nutrients from the host (Bond, 1938; Lazarovits and Higgins, 1976b). No visible reaction of the host cells other than occasional callose deposition on the mesophyll cell walls can be observed during these stages of infection (de Wit, 1977; Lazarovits and Higgins, 1976a,b). However, several ultrastructural changes have been described, including the occurrence of endoplasmic reticulum parallel to the plasmamembrane at the site of fungal contact, and cytoplasmic lipid bodies and microbodies containing crystalline inclusions (Lazarovits and Higgins, 1976b). In mature lesions, mesophyll cells display various signs of degeneration of cell organelles, more specifically the mitochondria and chloroplasts (Lazarovits and Higgins, 1976b). Occasionally, the release of cytoplasmic contents due to damage to the plasmamembrane and

Fig. 1 Physiology of the *C. fulvum* infection on host and non-host plants (A–H) and typical symptoms on host plants caused by other plant pathogenic Mycosphaerellaceae as found in nature (I–N). (A) Adaxial side of a tomato leaf (MoneyMaker *Cf-0*) 18 days after inoculation with a compatible race of *C. fulvum*. Distinctive yellow spots can be seen as a result of dead palisade parenchyma cells. (B) Abaxial side of a tomato leaf (MoneyMaker *Cf-0*) 18 days after inoculation with a compatible race of *C. fulvum*. White mould can be seen developing into light brown patches where sporulation takes place. (C–E) SEM images from *C. fulvum*-infected tomato leaves in a compatible interaction at different timepoints after inoculation (pictures are taken from: de Wit, P.J.G.M. Light and scanning-electron microscopic study of infection of tomato plants by virulent and avirulent races of *Cladosporium fulvum*. *Neth. J. Plant Pathol.* (1977) 83, 109–122, with permission). (C) *C. fulvum*-infected tomato leaf in a compatible interaction 2 days post inoculation with fungal hyphae entering a stoma. (D) *C. fulvum*-infected tomato leaf in a compatible interaction 7 days post inoculation. In the spongy mesophyll hyphae (h) grow in close contact with the plant cells. (E) *C. fulvum*-infected tomato leaf in a compatible interaction 12 days post inoculation. Young conidiophores emerging from the stomata are observed. (F–H) Drawings upon microscopic analysis of lactophenol-stained leaf material of several plant species upon inoculation with *C. fulvum* (drawings are reproduced from: Bond, T.E.T. Infection experiments with *Cladosporium fulvum* Cooke and related species. *Ann. Appl. Biol.* (1938) 25, 277–307, by permission of Oxford University Press). (F) Growth of *C. fulvum* mycelium in the tomato cultivar 'Giant red' 7 days after inoculation. The growth is characterized by long runner hyphae that pass between spongy mesophyll cells to send out ascending branches. (G) Limited growth of mycelium in *Hyoscyamus niger* (Solanaceae) 6 days after inoculation. Fungal growth does not go further than the substomatal cavity and a ring of discolored cells is observed. (H) Penetration of *C. fulvum* in so-called inappropriate hosts (or non-hosts) 6 days after inoculation: *Anthirrhinum majus* (a), *Bryonia dioica* (b) and *Callistephus* sp. (c). Mycelium is confined to single peg-like branches. (I) *Cercospora beticola* sporulating on sugarbeet leaves (*Beta vulgaris*). (J) Fasciculate conidiophores of *Pseudocercospora fijiensis* on banana (*Musa*) leaves. (K) Pycnidia of *Mycosphaerella graminicola* on wheat. (L) Angular leaf spots of *Cercospora zeae-maydis* on maize (*Zea mays*). (M) Conidiomata of *Dothistroma septospora*, causing red band needle disease of *Pinus* sp. (N) *Passalora perplexa* causing Crassicarpa leaf blight on *Acacia crassicarpa*.

sometimes even the tonoplast has been observed (Lazarovits and Higgins, 1976b). Nine to 10 days after the onset of the infection, hyphal aggregations (stromatic bodies) are produced by the fungus in the substomatal spaces. Subsequently, aerial mycelium is formed from which conidiophores protrude through the stomata to the exterior where they produce chains of mostly two-celled conidia (Fig. 1E). After dispersal these conidia can contribute to the spread of the disease (Bond, 1938).

THE INFECTION ON RESISTANT PLANTS: THE INCOMPATIBLE INTERACTION

No differences are generally observed between compatible and incompatible interactions with regard to conidial germination, formation of runner hyphae and stomatal penetration (de Wit, 1977; Lazarovits and Higgins, 1976a). Although the initial stages of incompatible and compatible interactions are very similar, occasionally in incompatible interactions the fungus grows out of the stoma again after having entered it (de Wit, 1977). This suggests that runner hyphae entering the apoplast through an open stoma elicit host defence responses that successfully repel the fungus. However, the majority of hyphae do not grow out of the stomata, and host defence results in arrest of fungal growth 1 or 2 days after penetration (de Wit, 1977). By then, the fungus has hardly grown from the stomatal cavity into the apoplast and hyphae appear swollen and curled. Hyphal cells that are in close contact with host mesophyll cells often collapse. Cell wall depositions containing callose are formed, leading to increased cell wall thickness, and deposits of extracellular material in the vicinity of fungal hyphae (de Wit, 1977; Lazarovits and Higgins, 1976a). At the molecular level, phytoalexins as well as pathogenesis-related (PR)-proteins accumulate (de Wit and Flach, 1979; de Wit and Kodde, 1981; de Wit and van der Meer, 1986; Joosten and de Wit, 1989). Although the accumulation of PR-proteins also occurs in compatible interactions, in incompatible interactions the accumulation usually is faster. Chitinases and β-1,3-glucanases were found to accumulate in vacuolar protein aggregates and in extracellular material surrounding mesophyll cells (Wubben *et al.*, 1992). In addition, accumulation of PR-proteins near the stomata is observed, although this phenomenon also occurs in compatible interactions (Wubben *et al.*, 1993). Therefore, it can be concluded that the accumulation of PR-proteins by itself does not contain the fungus, although the speed at which the accumulation takes place might influence the outcome of the interaction (Wubben *et al.*, 1993).

The most striking feature of host defence in the incompatible interaction is the hypersensitive response (HR), in which mesophyll cells adjacent to the intracellular hyphae (and in addition occasionally guard cells and some epidermal cells) collapse in a manner that is reminiscent of apoptosis. As a result of this defence response the fungus is contained in a limited area of infection sites, exposed to components that are released upon host cell disruption, and thus cannot establish a successful infection (Joosten and de Wit, 1999).

THE INFECTION ON NON-HOST PLANTS: BASIC INCOMPATIBILITY

As mentioned before, the host range of *C. fulvum* is restricted to *Lycopersicon* species and thus species from other plants are non-hosts (Bond, 1938). As early as 1938, experiments were described measuring the growth of *C. fulvum* on host plants, resistant plants and diverse 'inappropriate hosts' (Fig. 1F–H). Although no visual symptoms were recorded upon inoculation of non-host plants, stomatal penetration occurred in almost all species, although usually less frequent than on tomato. Maximal growth was recorded in a number of solanaceous species that allowed some growth on young tissues, whereas mature tissues allowed almost no fungal growth outside substomatal cavities, and often necrosis was observed (Fig. 1G). The most restricted fungal growth was reported in *Callistephus* sp. (aster), *Antirrhinium majus* (snapdragon) and *Bryonia dioica* (white bryony) where hyphae were hardly able even to enter the substomatal cavity (Fig. 1H). It was noted that in those interactions, fungal mycelium was confined to single peg-like branches and that host cell death did not occur (Bond, 1938).

Even at present, non-host resistance is a poorly understood defence mechanism (Mysore and Ryu, 2004). When assessing HR-associated recognition of extracellular *C. fulvum* components in non-host plants it was noted that ECP2 displayed elicitor activity in several *Nicotiana* species (de Kock *et al.*, 2004; Laugé *et al.*, 2000). This observation justifies the question of whether ECP2 recognition establishes non-host resistance in those species. This appeared not to be the case. On non-host *Nicotiana* species *C. fulvum* conidia did germinate and produce runner hyphae. Subsequent stomatal penetration was observed in rare cases, but hyphal growth always arrested very soon thereafter; by no means was the fungus able to grow further than the substomatal cavity. No differences were observed between ECP2-recognizing and non-recognizing accessions and it is unclear what controls fungal arrest (de Kock *et al.*, 2004). It is speculated that this is due to lack of production of the essential pathogenicity factors by the pathogen or due to the production and accumulation of effective defence components by the plant (Bond, 1938; de Kock *et al.*, 2004).

TAXOMONY OF *CLADOSPORIUM FULVUM*

C. fulvum is an asexual fungal species. The genus *Cladosporium* is extremely heterogeneous, containing more than 700 names, and consisting of close to 20 distinct, as yet undescribed genera (P. W. Crous, unpublished data). The genus *Cladosporium s.s.*,

which has teleomorphs in *Davidiella* (Mycosphaerellaceae), contains saprobic as well as pathogenic taxa. *C. fulvum* (syn. *P. fulva*) is a typical species of *Passalora*, belonging to *Mycosphaerella s.s.* As *C. fulvum* is a biotrophic fungus of the non-obligate type, it can be cultured *in vitro* on simple media. The colonies that appear are strongly pigmented, greenish to black, and relatively slow-growing. The one- or two-celled, pigmented conidia are present in long, branched chains, arising from pigmented conidiophores. The superficial mycelium of *C. fulvum* is well developed, and consists of branched, septate hyphae, with cell walls consisting mainly of glucan and chitin polysaccharides (Joosten and de Wit, 1999).

As is often the case for asexual fungal species, classification is ambiguous, as it has in the past mostly been based on the phenotype, which was rarely supported by DNA phylogeny, or links to known teleomorph states. Earlier attempts to reduce heterogeneity of the genus *Cladosporium* by placing taxa in genera such as *Fulvia* or *Mycovellosiela* (Ciferri, 1952; von Arx, 1983) never gained broad acceptance. The introduction of a more phylogenetic approach has resulted in a simplification in many of these anamorph generic concepts in the Mycosphaerellaceae (Crous *et al.*, 2000, 2001). Based on phylogenetic analysis of internal transcribed spacer (ITS) regions from ribosomal DNA (rDNA) it was anticipated only a decade ago that *Cladosporium* species, inluding *C. fulvum*, comprised a monophyletic group (Curtis *et al.*, 1994). In addition, *C. fulvum* was found by molecular data to belong to the genus *Mycosphaerella*, the most numerous genus of the Ascomycetes with more than 2000 described species (Crous *et al.*, 2001; Goodwin *et al.*, 2001). Recently, it has again been questioned whether *C. fulvum* should indeed be assigned to *Cladosporium*, as morphological and molecular data did not clearly support this link (Wirsel *et al.*, 2002). Furthermore, as part of a taxonomic revision of *Cladosporium*, Braun *et al.* (2003) restricted *Cladosporium* to *C. herbarum* and its allies, and placed their teleomorphs in the newly formed teleomorph genus *Davidiella*. The genus *Passalora* is distinguished from *Cladosporium* by having conidial hila that are darkened, thickened and refractive, but not protuberant as in the case of *Cladosporium*, and having *Mycosphaerella* teleomorphs, while those of *Cladosporium* belong to *Davidiella* (Braun *et al.*, 2003; Crous & Braun 2003). By resolving *C. fulvum* to be a species of *Passalora*, and thus a true *Mycosphaerella* anamorph, it also suggests that many of the host-pathogen mechanisms resolved in this pathosystem should also be active in other *Mycosphaerella* pathosystems. An extremely high percentage of DNA similarity (ITS1, 5.8S, ITS2) is observed between the DNA sequences of *C. fulvum* and other well-known *Mycosphaerella* pathogens lodged in GenBank, such as those causing *Mycosphaerella* leaf blotch of *Eucalyptus* (*M. aurantia*, *M. ellipsoidea*, *M. kensiensis*, 96–98%) (Crous *et al.*, 2004a), leaf spot of grapevines (*P. dissiliens*, 97%), red band needle disease of pines (*Dothistroma* spp., 96%) (Barnes *et al.*, 2004; see Fig. 1M), leaf and sheath red spot of sugarcane (*P. vaginae*, 97%), purple seed stain and leaf blight of soybean (*Cercospora kikuchii*, 92%), crassicarpa leaf blight of acacia (*P. perplexa*, 95%) (Beilharz *et al.*, 2004; see Fig. 1N), leaf spot of cassava (*P. henningsii*, 97%), ivy (*M. hedericola*, 97%), lupin (*M. lupini*, 95%) (Kaiser and Crous, 1998), peanuts (*P. arachidicola*, 94%), sugarbeet (*Cercospora beticola*, 92%) and *Acacia* (*C. acaciae–mangii*, 92%) (Crous *et al.*, 2004b).

The genus *Mycosphaerella* contains numerous economically important non-obligate hemi-biotrophic plant pathogens. These include *M. fijiensis*, *Cercospora zeae–maydis* and *M. graminicola*, the causal agents of black Sigatoka on banana (Fig. 1J), grey leaf spot disease on maize (Fig. 1L) and Septoria leaf blotch on wheat (Fig. 1K), respectively (Balint-Kurti *et al.*, 2001; Palmer and Skinner, 2002; Ward *et al.*, 1999) to name but a few. Plant pathogenic Mycosphaerellaceae species seem to share a number of characteristics: penetration through natural openings like stomata, extracellular growth between mesophyll cells without forming obvious feeding structures, and lack of obvious disease symptoms until re-emergence of conidiophores from stomata to release conidia. In all cases active penetration by appressoria and formation of haustoria has never been observed; colonization is strictly intercellular and mainly restricted to the mesophyll. Intriguingly, plant pathogenic Mycosphaerellaceae species have narrow host ranges, their hosts are highly divergent plant species, and they are found on all continents. The genetic relationship between different Mycosphaerellaceae species and the high degree of host specialization suggests an evolutional lineage from a common fungal ancestor. This ancestor might have been a pathogen of an ancestral plant species that existed before the divergence into the many different plant species that are attacked by the different Mycosphaerellaceae species today. Co-evolution between host and pathogen has since then resulted in the high degree of specialization among species that all have a narrow host range.

UPTAKE OF NUTRIENTS BY *C. FULVUM*

C. fulvum prefers to colonize a well-nourished host. On weak, starved, chlorotic or senescent plants growth of the fungus is severely limited (Butler and Jones, 1949). In advanced infections, most fungal biomass is concentrated around vascular tissues (van den Ackerveken *et al.*, 1994; Wubben *et al.*, 1994). In most plant species sucrose is the major sugar translocated in the phloem, and thus the concentration of fungal biomass around the vascular tissues is most likely caused by the gradient of apoplastic sucrose, of which the highest concentrations can be found near the phloem cells. *C. fulvum* is able to convert apoplastic sucrose into the hexose monomers glucose and fructose using a cell-wall-bound invertase and store it as mannitol (Joosten *et al.*, 1990). Furthermore, it has been demonstrated that during colonization of the leaf the concentration of apoplastic sucrose decreases (Joosten

et al., 1990; Noeldner *et al.*, 1994), indicating that there is an increase of invertase activity. It is not known whether the increased invertase activity in the *C. fulvum*–tomato interaction is solely due to fungal invertases because host cell invertase activity is also generally found to increase upon pathogen infections (Berger *et al.*, 2004; Roitsch *et al.*, 2003; Sturm and Chrispeels, 1990). The increase in extracellular invertase activity by the host is a common response to pathogen challenge (Hall and Williams, 2000; Roitsch *et al.*, 2003). Because the activation of plant defence responses triggered upon pathogen detection requires energy, the local increase of invertase activity could meet the increased demand for carbohydrates in tissues invaded by pathogens (Roitsch *et al.*, 2003). Furthermore, an increase in carbohydrates generates a metabolic signal for the expression of defence-related genes (Roitsch *et al.*, 2003). In turn, fungi can take up and convert hexose monomers such as glucose and fructose into polyhydroxy alcohols (polyols) such as mannitol (the predominant polyol stored by *C. fulvum*), glycerol or sorbitol. As many plants (including tomato) are not able to metabolize sugar alcohols, the accumulation of polyols allows fungi to store carbon in such a way that it is inaccessible to the host (Lewis and Smith, 1967). *C. fulvum* displays mannitol dehydrogenase activity, leading to a significant increase of mannitol concentrations during infection (Joosten *et al.*, 1990; Noeldner *et al.*, 1994). Polyols have been implicated in diverse roles in fungi, including contribution to the osmotic balance, antioxidants (quenchers of host-produced reactive oxygen species), facilitation of carbon transportation through the hyphae and storage (Jennings, 1984; Lewis and Smith, 1967). The observation that mannitol is found in fungal conidia where it is metabolized at a very early stage of germination and the finding that polyols are metabolized under starvation conditions has strengthened the view that polyols are indeed used as storage compounds (Dijkema *et al.*, 1985; Horikoshi *et al.*, 1965; Witteveen and Visser, 1995). In some cases a role in fungal virulence has been shown for polyols. For instance, in *Magnaporthe grisea*, the polyol glycerol is required to build up the osmotic pressure in the appressorium that is required for epidermal penetration (de Jong *et al.*, 1997). In *C. fulvum* mannitol most likely accumulates as a carbon storage compound and a role in fungal virulence has not been established yet.

VIRULENCE OF *CLADOSPORIUM FULVUM*

C. fulvum is a pathogen that does not penetrate host cells at any stage of its life cycle. Although hyphae are observed to grow in close contact with mesophyll cells, all communication and exchange of components between pathogen and host occurs in the apoplastic space and the extracellular matrices of both pathogen and host. Because the apoplastic fluids can be harvested by vacuum infiltration of infected tomato leaves with water or buffer followed by low-speed centrifugation, these components can be identified fairly easily (de Wit and Spikman, 1982). Considerable efforts have been made to isolate fungal components that contribute to virulence in this way.

THE ROLE OF NITROGEN IN *CLADOSPORIUM FULVUM* PATHOGENICITY

Although knowledge of nitrogen metabolism of plant pathogens is limited, nitrogen seems to play an important role in pathogenesis (Snoeijers *et al.*, 2000). A large proportion of genes that exhibit *in planta*-induced expression are also expressed *in vitro* under nutrient-deprived conditions both in *C. fulvum* and in other fungi (Coleman *et al.*, 1997; Pieterse *et al.*, 1994; Talbot *et al.*, 1993; van den Ackerveken *et al.*, 1993b). For instance, the race-specific elicitor gene *Avr9* is highly induced *in planta* and was found to be induced by nitrogen limitation *in vitro* (van den Ackerveken *et al.*, 1993b). This suggests that during *in planta* growth, limited nitrogen is available for the colonizing pathogen (Snoeijers *et al.*, 2000). Several studies have shown that plant pathogens have found ways to alter their host's nitrogen metabolism to their own benefit (Hall and Williams, 2000; Snoeijers *et al.*, 2000). In *C. fulvum* there are indications for such a mechanism with respect to production of γ-aminobutyric acid (GABA), a non-protein-type amino acid that is produced in organisms ranging from microbes to plants and mammals (Bouché and Fromm, 2004). It is a predominant metabolite in plants and is expected to be involved in many processes. It is suggested that GABA, similar to its neurotransmitter role in animals, acts as a signalling molecule. In addition, GABA has been suggested to play a role in osmoregulation, pH regulation, nitrogen metabolism, and in defence against insects and oxidatve stress (Bouché and Fromm, 2004). In uninfected plants GABA is already the most abundant non-protein amino acid in the tomato apoplast, and during infection its concentration rises three- to four-fold (Solomon and Oliver, 2001). A GABA transaminase involved in metabolizing GABA has been isolated from *C. fulvum* and was found to be induced by the addition of GABA *in vitro*. In addition, the tomato GABA biosynthetic enzyme glutamate decarboxylase is induced during infection (Solomon and Oliver, 2002). It was suggested that *C. fulvum* manipulates the host metabolism to release nutrients because the presence of *C. fulvum* in the apoplast leads to enhanced GABA production by the plant and *in vitro* assays indicate that *C. fulvum* can utilize GABA both as a nitrogen and as a carbon source (Oliver and Solomon, 2004; Solomon and Oliver, 2002). In addition, both GABA and mannitol can act as a protection agent for plant cells against oxidative damage caused by the oxidative burst that is elicited as a defence response against the invading pathogen (Bouché *et al.*, 2003; Coleman *et al.*, 2001). In a compatible interaction, however, the oxidative burst is not effective as a defence response and the fungus may have developed means to utilize the secreted GABA as a nutritional source.

NITROGEN-CONTROLLED PATHOGENICITY GENES

As mentioned above, the avirulence gene *Avr9* is induced both *in planta* and *in vitro* during nitrogen starvation (Snoeijers *et al*., 1999; van den Ackerveken *et al*., 1994). Analysis of the *Avr9* promoter showed the presence of 12 (TA)GATA boxes (Snoeijers *et al*., 1999). These are known to act as binding sites for GATA-type regulators such as AREA in *Aspergillus nidulans* or NIT2 in *Neurospora crassae* (Chiang and Marzluf, 1995; Punt *et al*., 1995). Indeed, it has been shown using a reporter construct in *A. nidulans* that the *C. fulvum Avr9* promoter is induced during nitrogen starvation, but remains inactivated in an *areA* null mutant (Snoeijers *et al*., 1999; van den Ackerveken *et al*., 1994). Subsequently, from *C. fulvum* the *AREA/NIT2* homologue *Nrf1* (for Nitrogen response factor 1) was isolated (Pérez-García *et al*., 2001). As expected, this transcription factor was found to regulate *Avr9* transcription as *C. fulvum* transgenes deleted for *Nrf1* show severely reduced *Avr9* induction *in vitro* under nitrogen limitation and *in planta* during infection. Nevertheless, residual production of Avr9 in the *Nrf1* knockout line suggests that additional regulators of *Avr9* exist (Pérez-García *et al*., 2001). Although initial data suggested that deletion of *Nrf1* did not affect pathogenic capacity (Pérez-García *et al*., 2001), recent results indicate that virulence of *Nrf1* knockout strains is actually decreased (B.P.H.J.T., unpublished data). In addition, the virulence of *Nrf1* knockout strains was compared with a strain in which only the *Avr9* gene is deleted. The results show that *Avr9* deletion lines, in contrast to the *Nrf1* knockouts, show a level of virulence that is similar to the parental lines (B.P.H.J.T., unpublished data). This leads to the conclusion that *Nrf1* is a virulence factor that controls, in addition to *Avr9*, other fungal components that are involved in the establishment of successful colonization.

Seven (TA)GATA consensus sequences are present in the promoter of the avirulence gene *Avr4E* (Westerink *et al*., 2004), suggesting that *Avr4E* expression might be controlled by *Nrf1* in a similar fashion. It has been noted that overlapping TAGATA sequences contribute to the inducibility of the *Avr9* promoter (Snoeijers *et al*., 2003). However, in contrast to the two overlapping TAGATA boxes present in the *Avr9* promoter, the *Avr4E* promoter lacks overlapping boxes. At present is unclear whether the *Avr4E* promoter is induced under low nitrogen conditions. None of the promoters of other known genes encoding secreted elicitor peptides carries (TA)GATA boxes. *Avr9* is the only elicitor gene for which there is evidence that it is induced by nitrogen starvation. This also suggests that factors other than nitrogen depletion are involved in regulation of *C. fulvum* pathogenicity.

Several other starvation-induced genes, in addition to *Avr9*, include an alcohol dehydrogenase (*Adh1*), an alcohol oxidase (*Aox1*) and an acetaldehyde dehydrogenase (*Aldh1*) (Coleman *et al*., 1997; Oliver and Solomon, 2004). *Aox1* was found to be inducible by carbon starvation but repressed by nitrogen starvation *in vitro* (Segers *et al*., 2001). Remarkably, *in planta Aox1* is highly expressed, which could mean that either sucrose levels are depleted at sites of fungal growth or that factors other than carbon starvation trigger expression of *Aox1*. Targeted disruption of *Aox1* resulted in decreased growth *in planta* and reduced sporulation. Currently, the role of alcohol oxidases in pathogenicity is not clear. In general these enzymes catalyse the conversion of ethanol or methanol to hydrogen peroxide and acetaldehyde or formaldehyde, respectively. Contribution to pathogenicity could be due to its contribution to carbon metabolism, the removal of (m)ethanol present in tomato leaves, or the production of H_2O_2 (Segers *et al*., 2001). Although disruption of the acetaldehyde dehydrogenase 1 gene (*Aldh1*) was not found to affect pathogenicity, its expression was also found to be highly induced *in planta* (Segers *et al*., 2001). Possibly, the acetaldehyde that is generated by *Aox1*-mediated oxidation of ethanol is oxidized to acetate by the ALDH1-enzyme or, alternatively, reduced to ethanol by alchohol dehydrogenase (ADH1).

OTHER PUTATIVE VIRULENCE PROTEINS: AVRS AND ECPS

A number of proteins have been identified that are secreted by *C. fulvum* in the apoplast of susceptible tomato leaves. Apparently, tomato has built at least part of its surveillance system on recognizing these peptides as resistance depends on the perception of the presence or activity of these proteins mediated by the *Cf* resistance genes (Kruijt *et al*., 2005). The proteins secreted by *C. fulvum* are divided into extracellular proteins (Ecps) and avirulence proteins (Avrs) based on the observation that some of them are produced by all strains (Ecps) whereas others are race-specific (Avrs). However, this largely is a matter of semantics as Ecps, like Avrs, are specific elicitors that are recognized only by a few plants (Laugé *et al*., 1998). All currently known *Avr* and *Ecp* genes are highly expressed *in planta* but hardly any expression is detected *in vitro*. This has led to the idea that these proteins play a central role in the establishment of disease and all have been recognized by some genotypes that occurred during the tomato population evolution.

Although the degree of sequence conservation is very limited between individual Avrs and Ecps, the proteins are small (varying between 3 and 15 kDa) and contain an even number of cysteines (varying between four and eight). These cysteines are connected by disulphide bridges that contribute to the stability and activity of these proteins in the harsh protease-rich environment of the host apoplast (Kooman-Gersmann *et al*., 1997; Luderer *et al*., 2002a; van den Burg *et al*., 2003; van den Hooven *et al*., 2001). For Avr9 it has indeed been shown that the three-dimensional structure of the 28 amino acid peptide contains three anti-parallel beta-sheets with two solvent-exposed loops, which are stabilized by three

disulphide bridges (Mahé *et al.*, 1998; van den Hooven *et al.*, 2001; Vervoort *et al.*, 1997). This overall structure is typical for cystine-knotted peptides, which, although structurally related, share very little sequence homology and display very diverse biological functions (Pallaghy *et al.*, 1994).

Despite the absence of clear homology between *Avr* and *Ecp* genes and absence of sequence homology with other proteins in public databases, some of their properties point towards putative intrinsic functions. *Avr9* encodes a 63 amino acid protein that is C- and N-terminally processed by fungal as well as plant proteases, leading to a 28 amino acid peptide containing six cysteine residues (van den Ackerveken *et al.*, 1993b; van Kan *et al.*, 1991). Based on length, cysteine spacing and beta-sheet character, homology of Avr9 with peptidase inhibitors was suggested and indeed a high structural homology to a carboxypeptidase inhibitor was found (van den Hooven *et al.*, 2001; Vervoort *et al.*, 1997). Functional assays, however, could not show inhibition of carboxypeptidases by Avr9 (van den Hooven *et al.*, 2001). Nevertheless, it was demonstrated that Avr9 can bind to a component that is present in the plasma membrane of tomato and other *Solanaceous* plants (Kooman-Gersmann *et al.*, 1996). The binding is probably independent of expression of the *Cf-9* resistance gene, as experiments to establish binding between Cf-9 and Avr9 were unsuccessful (Luderer *et al.*, 2001). Despite many efforts, the nature of this binding site is not known yet. Possibly, Avr9 acts as a blocker of specific membrane channels as has been found for other cystine-knotted peptides.

Like Avr9, Avr4 was found to attach to membrane components. However, unlike Avr9, Avr4 binds to those of fungal rather than of plant origin (Westerink *et al.*, 2002). Nevertheless, because Avr4 triggers an HR in *Cf-4*-carrying plants, it can be anticipated that Avr4 also binds to a component of plant origin. *Avr4* encodes a 135 amino acid pre-pro-protein, which is C- and N-terminally processed upon secretion in the apoplast, resulting in an 86 amino acid mature protein carrying eight cysteine residues (Joosten *et al.*, 1994, 1997; Laugé *et al.*, 1997; Vervoort *et al.*, 1997). Based on the disulphide pattern of Avr4, a homologous sequence designated as an invertebrate chitin-binding domain (inv ChBD, Shen and Jacobs-Lorena, 1999) was identified. Binding of Avr4 to chitin was confirmed experimentally (van den Burg *et al.*, 2003, 2004). Interestingly, Avr4 was found to protect effectively the cell wall of the fungi *Trichoderma viride* and *Fusarium solani* against antifungal activity by basic chitinases *in vitro* (van den Burg *et al.*, 2003). Although the chitin-binding domain of plant chitinases (also called the Hevein domain) and the inv ChBD are sequentially unrelated, they do show strong structural homology. Remarkably, and in contrast to plant chitin-binding proteins, positive allosteric interactions were observed between chitin-binding Avr4 molecules (van den Burg *et al.*, 2004). During growth *in vitro* *C. fulvum* does not produce Avr4 and its chitin is inaccessible. However, during infection of tomato, chitin in the fungal cell walls is accessible and AVR4 is produced (H. A. van den Burg and P. J. G. M. de Wit, unpublished data). This all suggests that Avr4 shields fungal cell walls against activated host enzymes during infection. Apparently, some tomato plants have developed means (i.e. *Cf-4*) to recognize Avr4, recognition of which results in HR. Natural isoforms of Avr4 that are no longer recognized by plants carrying the resistance gene *Cf-4* exist that are still able to bind chitin. This shows that in some mutant alleles the intrinsic function of Avr4 seems to be preserved while unstable and protease-sensitive Avr4 variants still show chitin binding capability (van den Burg *et al.*, 2003). Despite this, the absence of functional AVR4 in a mutant carrying a single nucleotide deletion does not lead to a compromised virulence phenotype, indicating that Avr4 is dispensable for full virulence (Joosten *et al.*, 1997).

Dispensability for full fungal virulence also holds true for AVR9, as fungal strains in which the *Avr9* gene is either absent or replaced do not display markedly decreased virulence (Marmeisse *et al.*, 1993; van Kan *et al.*, 1991). It is therefore not unlikely that functional redundancy occurs for *Avr* genes as they are not uniformly present throughout all *C. fulvum* strains.

Another avirulence protein for which there are leads towards a function is Avr2. The corresponding *Avr2* gene was cloned and found to encode a 58 amino acid mature protein that contains eight cysteine residues (Luderer *et al.*, 2002b). The expression of Avr2 leads to an HR in plants carrying the resistance gene *Cf-2* (Dixon *et al.*, 1996; Luderer *et al.*, 2002b). In addition, a gene has been identified that is required for *Cf-2*-mediated resistance called *Rcr3* (Dixon *et al.*, 2000; Krüger *et al.*, 2002). As *Rcr3* only plays a role in *Cf-2*-mediated resistance, and not in resistance mediated by other *Cf* genes, it is anticipated that this component functions upstream of the signalling cascade that leads to the HR (Dixon *et al.*, 2000). Moreover, the predicted apoplastic localization of Rcr3 suggests that this protein is involved in the interaction between Avr2 and Cf-2, perhaps mediating the actual perception of the AVR protein by the Cf protein (Krüger *et al.*, 2002; Luderer *et al.*, 2002b). This would be in agreement with the 'guard hypothesis'. This hypothesis suggests that *R* gene products can act as guards that sense the modification of specific plant components that are targets of pathogen virulence components (van der Biezen and Jones, 1998). *Rcr3* was cloned and it was found to encode a cysteine protease (Krüger *et al.*, 2002). Recent evidence indeed points towards a function of AVR2 as a cysteine protease inhibitor (Rooney *et al.*, 2005). How AVR2 enhances virulence of the fungus in susceptible tomato plants that do not carry the *Cf-2* resistance gene still remains to be determined.

Five Ecps have been isolated from the apoplast of *C. fulvum*-colonized tomato leaves and four of the corresponding genes have been cloned (Laugé *et al.*, 2000; van den Ackerveken *et al.*, 1993a). In contrast to *Avr* genes, all *Ecp* genes are consistently present throughout the *C. fulvum* isolates. This observation, in addition to the finding that these genes are highly expressed *in*

planta (Wubben *et al*., 1994), has led to the idea that *Ecp* genes are essential for virulence. This has indeed been shown for *Ecp1* and *Ecp2* as virulence assays on 6-week-old soil-grown plants showed a significant decrease in fungal growth of *Ecp1*- and *Ecp2*-disruptants (Laugé *et al*., 1997; Marmeisse *et al*., 1994). For *Ecp4* and *Ecp5* a contribution to virulence needs yet to be established.

Structural analysis showed that the cysteine spacing of Ecp1 has remarkable similarity to the cysteine spacing of tumour necrosis factor receptors (TNFRs) (Bazan, 1993). One of the functions of TNFR family proteins is to initiate programmed cell death (Itoh *et al*., 1991). This is typically achieved by signalling through a ligand passing mechanism, meaning that a first accessory receptor recruits the ligand and regulates the association with the second receptor (Tartaglia *et al*., 1993). Pathogen-derived TNFRs that have been found in mammalian viruses interfere in the function of mammalian cytokines by mimicking their receptors (the endogenous TNFRs) and thus preventing the cytokines from reaching their endogenous targets and eliciting defence (Alcami and Smith, 1992). Interestingly, receptor molecules that share homology with mammalian TNFR molecules have also been identified in plants (Becraft *et al*., 1996). Experimental evidence establishing this particular function for ECP1 is still lacking.

Intriguingly, in contrast to *Avr* encoding genes, no significant sequence variation has been found in *Ecp* genes of *C. fulvum* isolates gathered from tomato fields and greenhouses. The high mutation frequency for *Avr* genes is thought to be due to selection pressure imposed by the use of *Cf* resistance genes in commercial tomato cultivation. Although *Cf-Ecp* resistance genes have been identified (Laugé *et al*., 2000), they have not been used on a large scale in tomato cultivars, resulting in absence of selection pressure on *Ecp* genes.

Because *Avr* genes are not ubiquitously represented throughout the *C. fulvum* species, it can be argued that none of the individual *Avr* genes is absolutely required for the establishment of disease. It is likely that redundancy occurs within the total pool of *Avr* genes present in the fungal genome, making individual *Avr* genes dispensable. As a consequence, fungal pathogenicity could rely on a set of virulence factors that are partially dispensable, although their combination is required for full virulence. Possible intrinsic functions could be the induction of nutrient leakage, the suppression of defence responses or the establishment of protection against host defence. In addition, it cannot be excluded that some of these factors have an important function for survival or competition in a specific habitat outside the natural host, although the specific plant-induced expression patterns suggest differently. This plant-induced expression could, however, also be explained as an induction that is caused upon monitoring the presence of specific antagonists of *C. fulvum* in the apoplast of tomato leaves, a phenomenon that has not yet been studied. It is expected that the use of *Arabidopsis thaliana* can greatly facilitate investigations into the intrinsic function of these secreted proteins and can help to determine the effects of these proteins on plants that do not carry corresponding *Cf* resistance genes. This would be facilitated even more with the availability of *Mycosphaerella* species that are able to infect Arabidopsis.

OTHER PUTATIVE VIRULENCE PROTEINS: HYDROPHOBINS

Many if not all filamentous fungi produce cell wall proteins that confer a water repellent nature to conidia and mycelium called hydrophobins. They are relatively small proteins that display a low level of sequence conservation but share similar hydropathic profiles and contain eight cysteine residues arranged in a strictly conserved manner (Whiteford and Spanu, 2002). They cover the surface of fungal structures by spontaneous polymerization into amphipathic bilayers. Hydrophobins are involved in various developmental processes such as the formation of aerial mycelium, sporulation, formation of infection structures, formation of fruit bodies and dispersal of conidia (Whiteford and Spanu, 2002; Wösten, 2001). In some cases it has also been demonstrated that hydrophobins contribute to fungal virulence. The rice blast fungus *Magnaporthe grisea*, for instance, requires the hydrophobin MPG1 for attachment and appressorium formation (Talbot *et al*., 1993, 1996). In addition, a hydrophobin appears to act as a virulence factor by increasing pathogen fitness in the causal agents of Dutch elm disease, *Ophiostoma ulmi* and *O. novo-ulmi* (del Sorbo *et al*., 2000; Temple and Horgen, 2000).

In *C. fulvum* six hydrophobin genes (*HCf-1* to *-6*) have been identified, each showing different expression profiles (Nielsen *et al*., 2001; Segers *et al*., 1999; Spanu, 1997). *HCf-1* appears to be specifically expressed after emergence of the conidiophores from the plant and during the start of the production of conidia, and was found to play a role in water-mediated dispersal of conidia (Whiteford and Spanu, 2001; Whiteford *et al*., 2004). *HCf-6* is specifically expressed in runner hyphae that enter stomata and it is speculated that HCf-6 may act as a primer for hydrophilic molecules that help the fungus to attach to the leaf surface. Alternatively, HCf-6 could be involved in preventing elicitation of host defence responses by helping to mask the presence of the pathogen (Whiteford *et al*., 2004). Single deletion mutants of the *C. fulvum* hydrophobins *HCf-1*, *HCf-2* or *HCf-6* did not display a reduction in virulence (Spanu, 1998; Whiteford and Spanu, 2001; Whiteford *et al*., 2004). This suggests that a high degree of functional redundancy exists between different hydrophobins although a double knock-out of *HCf-1* and *HCf-2* did not show altered virulence (Whiteford and Spanu, 2001). Nevertheless, redundancy with other hydrophobin genes or functional homologues can occur or the role of hydrophobins in virulence is indeed not imperative.

THE *C. FULVUM*–TOMATO PATHOSYSTEM AS A FUTURE EXPERIMENTAL MODEL FOR STUDYING THE MYCOSPHAERELLACEAE

Despite the study of many pathosystems, there is still little insight into what determines pathogenicity of a filamentous fungus, which are the required virulence factors and what determines its host range. *C. fulvum* is no exception. Through selection pressure new *C. fulvum* strains have emerged that have overcome the introgressed resistance traits and thus regained virulence by modification of *Avr* genes (Day, 1957). Although in these virulent *C. fulvum* strains *Avr* genes were sometimes found to be absent (van Kan *et al*., 1991), others contained point mutations (Joosten *et al*., 1994) or transposon insertions (Luderer *et al*., 2002b). In addition, mutagenesis experiments have yielded large sets of *C. fulvum* mutants that display reduced virulence, but the affected genes have not been characterized (Kenyon *et al*., 1993). The study of virulence mechanisms of fungal pathogens should greatly be facilitated with the increasing availability of fungal genome sequences. With sequencing and annotation of microbial genomes becoming more and more common practice, establishment of the *C. fulvum* genome sequence will also become more feasible. In the meantime, research on *C. fulvum* will benefit from genome sequences that are currently generated for the Mycosphaerellaceae species *M. graminicola* and *M. fijiensis* and vice versa when functional analysis of the latter species will have to be carried out.

Based on phylogenetic data, *C. fulvum* is found to be closely related to a number of economically important *Mycosphaerella* pathogens (Braun *et al*., 2003; Crous *et al*., 2001; Goodwin *et al*., 2001). This phylogenetic relationship is supported by morphological observations on the interactions of these pathogens with their respective host plants. For instance, cytological studies of the interaction between *M. fijiensis* (the causal agent of the devastating black Sigatoka disease; Fig. 1J) and *Musa* spp. (banana and plantain) revealed that *M. fijiensis*, like *C. fulvum*, behaves as a biotrophic pathogen, entering the leaf through open stomata and exclusively colonizing the intercellular space between mesophyll cells without forming haustoria (Beveraggi *et al*., 1995). In susceptible cultivars the interaction is characterized by a long biotrophic stage before morphological distortions are observed; in resistant cultivars depositions of fluorescent materials near the entry sites of the fungus are observed as early as 7 days post inoculation and early necrosis of guard cells also occurs, reminiscent of an HR mediated by a gene-for-gene relationship (Beveraggi *et al*., 1995). Another example is the Septoria wheat blotch pathogen, *M. graminicola*, which is a major foliar wheat pathogen (Fig. 1K) in temperate and subtropical regions, and employs similar infection mechanisms: no active penetration, purely extracellular growth, a lack of feeding structures and eventually the fungus causes, like *C. fulvum*, wilting as a consequence of non-functioning stomata (Palmer and Skinner, 2002).

Cercospora leaf spot disease (Fig. 1I) is considered to be the most important foliar disease of sugar beet (*Beta vulgaris*) worldwide (Weiland and Koch, 2004). The disease is caused by the asexual fungus *Cercospora beticola* that, apart from species of the genus *Beta*, also infects a number of Chenopodiaceae species. Although this fungal species appears to have a less narrow host range than many of the other *Mycosphaerella* pathogens, again, in addition to the taxonomic relationship, the cytology of infection of *C. beticola* resembles that of *C. fulvum*. The fungus penetrates the abaxial side of the leaf through stomata and grows within the intercellular space of the leaf during the biotrophic stage of its infection cycle. After intense colonization of the leaf tissue, the parenchyma and epidermal cells collapse in the vicinity of the fungal hyphae and the final necrotic zone appears, causing typical sporulating leaf spots (Feindt *et al*., 1981; Steinkamp *et al*., 1979).

Another interesting feature that many of these pathogens have in common is their appearance as epi- or endophytes that become pathogenic only under certain conditions. Endophytic growth of *C. beticola* has been reported upon root-inoculation of sugarbeet seedlings prior to the pathogenic stages (Vereijsen *et al*., 2004). In addition, such an endophytic lifestyle has been demonstrated for *M. buna*, which colonizes foliage of Japanese beech (*Fagus crenata*), and also for the type species of *Mycosphaerella*, *M. punctiformis*, which was isolated from asymptomatic living oak (*Quercus robur*) leaves (Kaneko and Kakishima, 2001; Verkley *et al*., 2004).

Despite the lack of a genome sequence, *C. fulvum* is a baseline *Mycosphaerella* pathogen that provides an ideal model to investigate basic pathogenicity mechanisms. As a result of the limited contact between pathogen and host, the lack of complicated feeding structures, and because host cells stay intact during the major part of the interaction, communication signals of the two interacting organisms present in the apoplast can easily be isolated by harvesting intercellular washing fluids. This has led to the identification of many secreted proteins and the corresponding genes as discussed above.

Although one major disadvantage of *C. fulvum* is the lack of a sexual stage and thus the inability to generate the crossings that are imperative for gene mapping studies, a number of important genomics tools have been developed in recent years. Although genomic transformation has long been possible in *C. fulvum*, recently an *Agrobacterium tumefaciens*-mediated transformation protocol was established facilitating transformation procedures and reducing artefacts as protoplasting is no longer required (B. F. Brandwagt, B. P. H. J. Thomma and P. J. G. M. de Wit, unpublished data). In addition, RNAi-technology has been established which, in combination with *Agrobacterium*-mediated transformation, should facilitate the study of putative pathogenicity genes (B. F. Brandwagt, B. P. H. J. Thomma and P. J. G. M. de Wit, unpublished data).

Another important advantage is the considerable effort that has been made to unravel disease resistance signalling in the interaction between *C. fulvum* and its host (Rivas *et al*., 2004; Rowland *et al*., 2005). *C. fulvum* was the first biotrophic fungus for which not only the first *Avr* genes were isolated but also the first corresponding *R* gene was cloned (Jones *et al*., 1994) and by now quite a number of *Cf* genes, and even complete *Cf*-clusters of genes, have been isolated (Kruijt *et al*., 2005). In total, four *Avr* genes and their corresponding plant *Cf* genes have been cloned. Apart from the *C. fulvum*–tomato interaction, for most other interactions between Mycosphaerellaceae and their hosts, conclusive evidence for gene-for-gene relationships is lacking. Nevertheless, recently such an interaction was demonstrated for resistance of wheat against a specific isolate of *M. graminicola* (Brading *et al*., 2002). For the other interactions, although suggested, such a relationship has never been proven, probably because of the poor availability of genetic tools for these plant–pathogen interactions (Harelimana *et al*., 1997; Lewellen and Whitney, 1976; Weiland and Koch, 2004).

More recent efforts on the *C. fulvum*–tomato interaction are directed towards downstream signalling that establishes the final resistance. The sequencing of the tomato genome by an international consortium (http://www.sgn.cornell.edu/help/about/index.html) will greatly facilitate this research. Tomato will most likely be the first dicotyledenous crop plant for which a genome sequence is available and is therefore likely to develop even more into a model plant for the Solanaceae than it is today.

In light of these advancements, we anticipate that *C. fulvum* can act as a model for many fungus–pathogen interactions in general and *Mycosphaerella*–plant interactions more specifically.

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