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Pathogen profile

Tobacco blue mould disease caused by *Peronospora hyoscyami* f. sp. *tabacina*

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SUMMARY

Blue mould [*Peronospora hyoscyami* f. sp. *tabacina* (Adam) Skalicky 1964] is one of the most important foliar diseases of tobacco that causes significant losses in the Americas, south-eastern Europe and the Middle East. This review summarizes the current knowledge of the mechanisms employed by this oomycete pathogen to colonize its host, with emphasis on molecular aspects of pathogenicity. In addition, key biochemical and molecular mechanisms involved in tobacco resistance to blue mould are discussed.

Taxonomy: Kingdom: Chromista (Straminipila); Phylum: Heterokontophyta; Class: Oomycete; Order: Peronosporales; Family: Peronosporaceae; Genus: *Peronospora*; Species: *Peronospora hyoscyami* f. sp. *tabacina*.

Disease symptoms: The pathogen typically causes localized lesions on tobacco leaves that appear as single, or groups of, yellow spots that often coalesce to form light-brown necrotic areas. Some of the leaves exhibit grey to bluish downy mould on their lower surfaces. Diseased leaves can become twisted, such that the lower surfaces turn upwards. In such cases, the bluish colour of the diseased plants becomes quite conspicuous, especially under moist conditions when sporulation is abundant. Hence the name of the disease: tobacco blue mould.

Infection process: The pathogen develops haustoria within plant cells that are thought to establish the transfer of nutrients from the host cell, and may also act in the delivery of effector proteins during infection.

Resistance: Several defence responses have been reported to occur in the *Nicotiana tabacum*–*P. hyoscyami* f. sp. *tabacina* interaction. These include the induction of pathogenesis-related genes, and a correlated increase in the activities of typical pathogenesis-related proteins, such as peroxidases, chitinases, β-1,3-glucanases and lipoxygenases. Systemic acquired resistance is one of the best characterized tobacco defence responses activated on pathogen infection.

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INTRODUCTION

Blue mould (*Peronospora hyoscyami* f. sp. *tabacina*) is one of the most important foliar diseases of tobacco that causes significant losses in the Americas, south-eastern Europe and the Middle East. It was first reported in tobacco-growing areas of Australia during the 1800s (Cooke, 1891). Blue mould epidemics have resulted in annual losses exceeding $200 million in North America (Heist *et al*., 2002; Lucas, 1980; Nesmith, 1984). In Cuba, the disease caused severe losses between 1978 and 1980 (Pérez *et al*., 2003).

The development of biotechnological tools has permitted the identification of tobacco genes that are involved in resistance against blue mould (Alexander *et al*., 1993; Borrás-Hidalgo *et al*., 2006; Kroumova *et al*., 2007; Lusso and Kuc, 1996; Salt *et al*., 1986; Schiltz, 1974). Furthermore, the identification of molecular markers linked to genetic factors controlling resistance has been improved (Julio *et al*., 2006; Milla *et al*., 2005). This may also facilitate the development of resistant cultivars. This review summarizes the current knowledge of the pathogenicity mechanisms employed by the oomycete to colonize its host. In addition, the biochemistry and molecular events involved in tobacco resistance to blue mould are discussed.

SYMPTOMS OF TOBACCO BLUE MOULD DISEASE

The pathogen is capable of infecting tobacco plants in growing regions worldwide throughout the growing season (including

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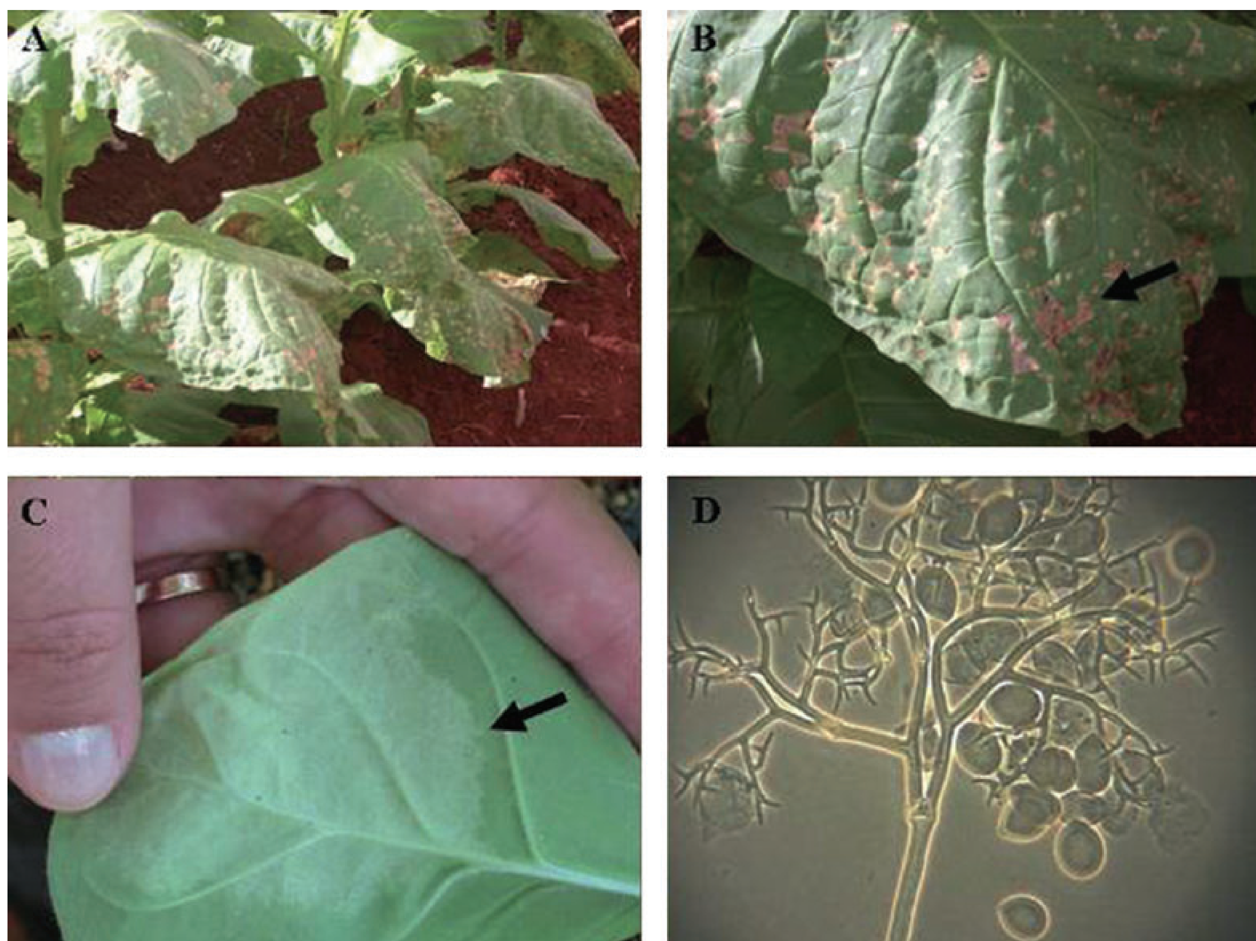

Fig. 1 Symptoms and conidiophores of *Peronospora hyoscyami* f. sp. *tabacina*: (A) leaf lesions on field tobacco produced by tobacco blue mould; (B) typical symptoms of blue mould; (C) *P. hyoscyami* f. sp. *tabacina* sporulation on the lower side of the leaf; (D) microscopic observation of conidiophores produced by *P. hyoscyami* f. sp. *tabacina*.

transplant production) and can spread rapidly under favourable weather conditions (Main, 1991). If the weather is cloudy and cool, the disease can result in complete crop destruction (Lucas, 1975). On seedbeds of small seedlings with leaves of less than 2 cm in diameter, small patches of dead or dying seedlings with erect leaves provide evidence of the disease (Lucas, 1975; Wolf *et al*., 1934). After 7–10 days, when sufficient secondary inoculum has been produced, a general epidemic occurs and the entire seedbed may be affected. Older plants with leaves up to 4 cm exhibit a grey or bluish downy mould on the lower surface (Fig. 1). The upper surfaces of infected leaves can remain almost normal in appearance for 1–2 days before the plants begin to die and turn light brown, especially under wet conditions, where sporulation is abundant. In addition, vascular discoloration inside the stems caused by systemic stem infection may occur, resulting in partial or overall stunting of the plant (Lucas, 1975; Reuveni *et al*., 1986). If this occurs near the base of stems, the plants often lodge or snap off.

CAUSAL ORGANISM AND DISEASE CYCLE

Oomycetes are traditionally treated within mycology; however, ultrastructural, biochemical and molecular phylogenetic data demonstrate that they are not related to true fungi (Kingdom Fungi), but belong to the Kingdom Chromista (Straminipila), which also contains the chromistan (heterokont) algae (Dick, 2002; Kirk *et al*., 2001; Voglmayr, 2008).

The blue mould pathogen belongs to the Class Oomycetes, Order Peronosporales and Family Peronosporaceae (Voglmayr, 2008). Molecular phylogenies using internal transcribed spacer (ITS) data not only confirm a rather narrow species concept in *Peronospora*, but also help to clarify species attribution (Voglmayr, 2008). This downy mildew pathogen of the Solanaceae was originally described from a *Hyoscyamus* sp. in Czechoslovakia in 1859, as *Peronospora effusa* var. *hyoscyami*. In 1863, de Bary elevated it to the rank of species, as *P. hyoscyami* de Bary 1863. In 1933, Adam proposed the name *P. tabacina* for

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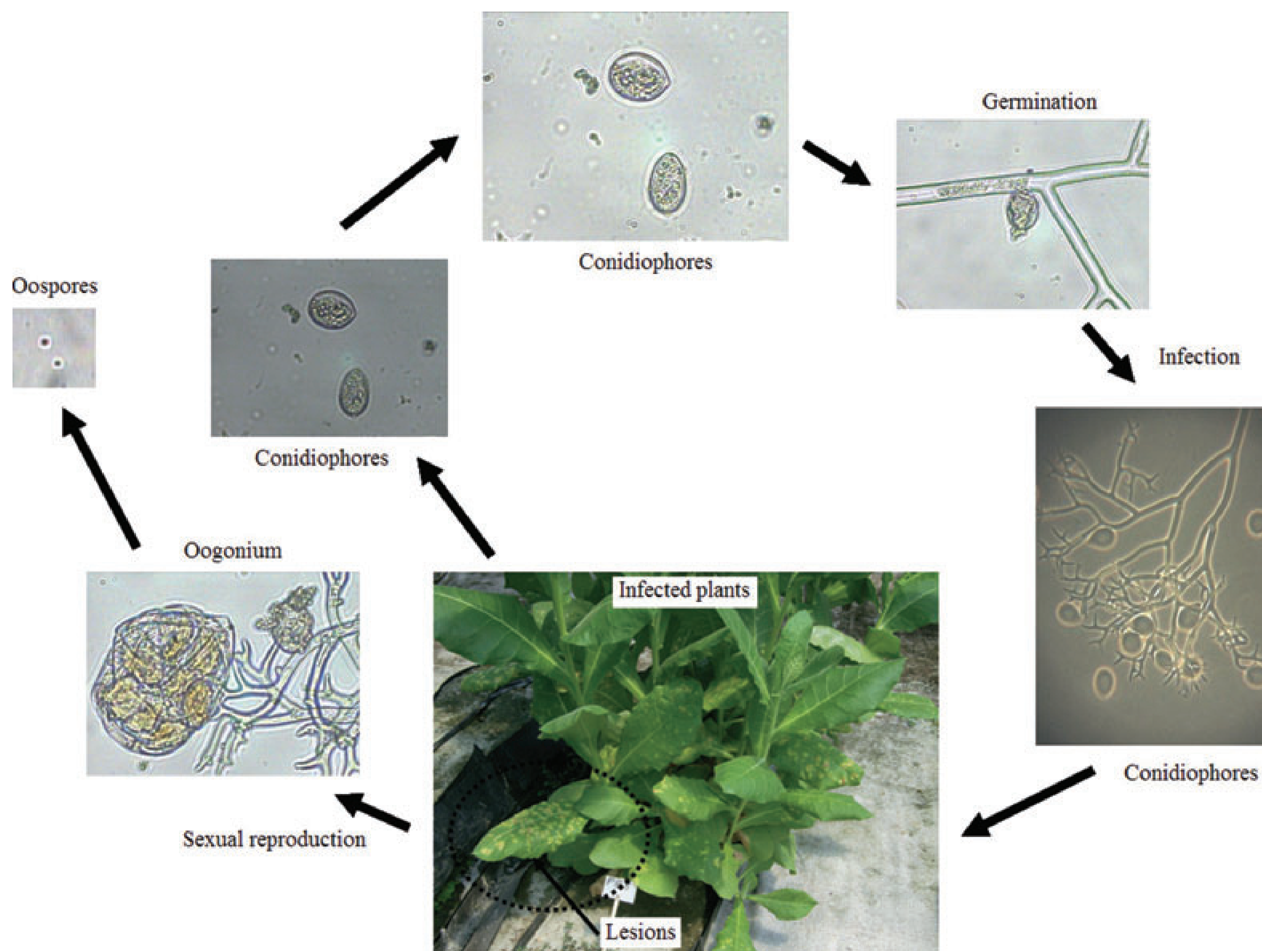

Fig. 2 Disease cycle of tobacco blue mould caused by *Peronospora hyoscyami* f. sp. *tabacina*.

the blue mould of *Nicotiana tabacum* (tobacco) and other *Nicotiana* spp., but acknowledged that there were few morphological differences from *P. hyoscyami*. Analysis of a wider sample of isolates revealed no consistent morphological differences between *P. hyoscyami* and *P. tabacina*, and therefore *P. tabacina* was considered to be a synonym of the earlier name *P. hyoscyami* (Shepherd, 1970). Nevertheless, the name *P. tabacina* has continued to appear in the literature (Schiltz, 1981; Voglmayr, 2008).

The pathogen is an obligate biotrophic parasite and produces both conidiophores and oospores (Fig. 2). The hyaline and lemon-shaped conidiophores (15×25 μm) are borne on tree-like and dichotomously branched, which terminate in curved, acute apices (Fig. 1D). The conidiophores emerge through the stomates on the underside of the leaf in great numbers and vary from 400 to 750 μm in length (Fig. 1D). The conidiophores are fragile and short-lived. They are sensitive to UV light and, when released, exposure to direct sunlight kills most within 1 h (Aylor, 1986; Bashi and Aylor, 1983; Rotem *et al*., 1985). They seldom are found viable on leaf lesions more than 72–96 h old.

There are two basic ways in which the pathogen moves around. The first is by spores carried on the wind (Davis and Monahan, 1991; Lucas, 1980) and the second is by the movement, by people, of infected tobacco transplants (Aylor, 1986). Epidemics are usually cyclic and progressive; once established, they advance as a more or less definable front via wind-borne spores. The difference between continuous and discontinuous epidemic fronts is related to inoculum dispersal patterns, localized weather, density and spatial aggregation of tobacco fields within a production region, and planting schedules. The pathogen is a prolific producer of spores; it has been estimated that 500 ha of heavily diseased tobacco can produce about 6.44×10^{13} spores per day (Aylor, 1986).

Once the air-borne conidiophores land during the morning hours on a leaf surface, in the presence of free water germination and infection can occur in as little as 2–4 h. A 5- to 7-day, symptom-free incubation period takes place before the appearance of the first visible symptoms (yellow lesions) and sporulation. For conidiophores to appear, the relative humidity must exceed 95% for 3 h and darkness must last for a

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minimum of 1.5 h. Maximum sporulation occurs at 15–23 °C.

The oospores are sexual spores and have been suggested as another method of dissemination (Hall, 1989). However, it is unclear whether the pathogen is capable of overwintering in infected debris, and the role of oospores in disease is not clearly understood (Ristaino *et al*., 2007). They are sometimes produced in the mesophyll of dead parts of the infected plant (Lucas, 1975; Milholland *et al*., 1980). Mature oospores are usually reddish brown and 20–60 μm in diameter; their size varies under different conditions. Heist *et al*. (2002) observed that oospores of *P. tabacina* were produced on hyphae emerging from roots of *N. repanda*. This finding appears to be relevant to future studies of this pathosystem from the standpoint of potential secondary spread in commercial tobacco production systems and to address questions pertaining to the life cycle of *P. tabacina* in both wild and commercial *Nicotiana* species. The disease cycle of tobacco blue mould is shown in Fig. 2.

THE INFECTION PROCESS

The infection process of *P. hyoscyami* f. sp. *tabacina* typically begins with the germination of spores on the leaf surface, followed by the development of an appressorium (Lucas, 1975). The development of the appressorium depends on a thigmotrophic signal triggered by the specific topography of the host plant leaf surface (Lucas, 1975). An infection peg formed by the appressorium enters the leaf through a stomata, followed by the development of a substomatal vesicle, an infection hypha, a haustorial mother cell, penetration of a photosynthetic mesophyll cell by a peg and the establishment of a haustorium (Lucas, 1975). The development of the haustorium is the final step of an infection pathway in which the plant host plays a major role (Lucas, 1975).

The oomycete pathogen establishes intimate relations with its hosts by forming haustoria during the infection, which are well-known structures that are thought to be used to obtain nutrients from the plant, redirecting host metabolism and suppressing host defences through the delivery of effector proteins into the host cytoplasm (Hahn and Mendgen, 2001; O'Connell and Panstruga, 2006; Voegele and Mendgen, 2003; Whisson *et al*., 2007). However, little is known about the biochemical and molecular events involved in this process during *P. hyoscyami* f. sp. *tabacina*–tobacco interaction. It would be interesting to determine the effectors mediating the compatible and incompatible interaction.

TOBACCO DEFENCE AGAINST BLUE MOULD INFECTION

Several defence responses have been reported to occur in the *N. tabacum*–*P. hyoscyami* f. sp. *tabacina* interaction (Alexander *et al*., 1993; Borrás-Hidalgo *et al*., 2006; Kroumova *et al*., 2007; Lusso and Kuc, 1996; Salt *et al*., 1986; Schiltz, 1974). These include the activation of pathogenesis-related (PR) genes, and an increase in the activities of typical PR proteins, such as peroxidases, chitinases, β-1,3-glucanases, lipoxygenases and T-phylloplanin (Alexander *et al*., 1993; Kroumova *et al*., 2007; Lusso and Kuc, 1996). In addition, systemic acquired resistance is one of the best characterized tobacco defence responses activated on pathogen infection (McIntyre *et al*., 1981) (Table 1).

The sporulation and growth of *P. hyoscyami* f. sp. *tabacina* are inhibited by β-ionone (Salt *et al*., 1986; Schiltz, 1974). β-Ionone is a terpenoid volatile synthesized from carotenoids. It has been shown that T-phylloplanin proteins secreted on the aerial surfaces of tobacco (*N. tabacum*) by short procumbent trichomes inhibit spore germination and blue mould disease caused by the oomycete pathogen *P. hyoscyami* f. sp. *tabacina* (Kroumova *et al*., 2007) (Table 1).

Tobacco plants with an *N* gene (resistance gene) inoculated on the lower leaves with tobacco mosaic virus (TMV) mount a systemic acquired resistance, which protects against *P. hyoscyami* f. sp. *tabacina* (McIntyre *et al*., 1981). Using a cultivar of tobacco (KY14) carrying the *N* gene, assays have shown that plants initially inoculated with TMV or with *P. hyoscyami* f. sp. *tabacina* exhibit increased activity of PR proteins, including β-1,3-glucanase (PR-2), and this increased activity is associated with resistance to challenge inoculation with *P. hyoscyami* f. sp. *tabacina* (Ye *et al*., 1990). Evidence provided by transgenic

Table 1 Tobacco genes involved in blue mould defence.

Gene	Effect on disease development	Reference
β-Ionone	Inhibits the sporulation and growth of *P. hyoscyami* f. sp. *tabacina*	Schiltz (1974) Salt *et al*. (1986)
PR-1a	Overexpression in transgenic plants reduces rate and final disease	Alexander *et al*. (1993)
Glucanase	Overexpression in transgenic plants reduces disease symptoms	Lusso and Kuc (1996)
Glutathione synthetase and the EIL2 transcription factor	Knockdown of these genes in *Nicotiana megalosiphon* compromises disease resistance against *P. hyoscyami* f. sp. *tabacina*	Borrás-Hidalgo *et al*. (2006)
T-phylloplanin	Inhibits spore germination and disease	Kroumova *et al*. (2007)

plants suggests that PR-2 has an important role in protecting plants against *P. hyoscyami* f. sp. *tabacina* (Ryals *et al*., 1996).

In addition, it has been reported that some genotypes of the desert tobacco *N. obtusifolia* respond to foliar *P. hyoscyami* f. sp. *tabacina* infections by developing necrotic lesions 5–6 days post-inoculation, and that subsequent sporulation of the pathogen is drastically reduced or eliminated in this interaction compared with a compatible interaction. In addition, there is evidence that this resistance in *N. obtusifolia* results from the expression of the hypersensitive response (HR), and is caused by the action of a single, partially dominant gene named *Rpt1* (Heist *et al*., 2004).

Finally, *Nicotiana megalosiphon* genes, which are involved in broad-spectrum resistance to tobacco blue mould, have been identified in the incompatible interaction using suppression subtractive hybridization (Borrás-Hidalgo *et al*., 2006). One hundred and eighty-two clones were differentially expressed and 16% showed homology to known genes, some of which were implicated in disease resistance. Based on their expression profiles, some genes were chosen for functional analysis using virus-induced gene silencing. Each was found to be quickly induced in the incompatible interaction on *N. megalosiphon*, whereas no induction was observed in the compatible interaction on *N. tabacum*. It was shown that a knockdown of the glutathione synthetase gene and the EIL2 transcription factor gene in *N. megalosiphon* compromised disease resistance against *P. hyoscyami* f. sp. *tabacina* (Borrás-Hidalgo *et al*., 2006). This demonstrated that both components are required for *P. hyoscyami* defence, and could provide the basis for the disease-resistant phenotype of *N. megalosiphon*.

DISEASE MANAGEMENT

Some important practices could be introduced in order to control tobacco blue mould. These include making the environment less favourable for the pathogen to survive and infect tobacco, keeping the pathogen out of tobacco and the area for as long as possible, protecting tobacco plants with fungicides when they are most vulnerable and managing the crop to harvest quickly.

The employment of host plant resistance is the most economic and environmentally sustainable method for controlling blue mould. However, naturally occurring resistance within *N. tabacum* is generally very low (Rufty, 1989). Resistance can be found within several *Nicotiana* species of Australian origin where blue mould is endemic, and the resistance has been introgressed into cultivated tobacco from *N. debneyi* (Clayton *et al*., 1967; Lea, 1963), *N. goodspeedii* Wheeler and *N. velutina* Wheeler (Wark, 1970). In Cuba, the non-cultivated tobacco species *N. megalosiphon* has been shown to be highly resistant to *P. hyoscyami* f. sp. *tabacina* (Espino and Rey, 1987).

The incorporation of blue mould resistance into new cultivars has been complicated by the complex interaction between *P. hyoscyami* f. sp. *tabacina* and the tobacco host plant. *Peronospora hyoscyami* f. sp. *tabacina* is an obligate parasite, and selection for resistance is best performed under natural or induced field epidemics. Disease reactions are highly dependent, however, on factors such as plant age, physiological status of the plant and environmental conditions. These factors can cause field experiments to be highly variable and unpredictable (Rufty, 1989). Although DNA polymorphism, as revealed by molecular markers, is generally low for *N. tabacum* (Nishi *et al*., 2003), the identification of markers linked to resistance genes transferred to tobacco from wild *Nicotiana* relatives has been successful (Bai *et al*., 1995; Johnson *et al*., 2002; Lewis, 2005; Yi *et al*., 1998).

FUTURE PERSPECTIVES

In conclusion, the identification of molecular markers linked to genes contributing to blue mould resistance is a valuable tool to increase the capacity for the elimination of susceptible individuals and lines during early stages of breeding programmes. Moreover, knowledge about resistance genes to blue mould and the molecular characterization of *P. hyoscyami* f. sp. *tabacina* isolates will enhance our understanding of the resistance and pathogenicity process, respectively, allowing better strategies to be created to control blue mould disease.

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