Pathogen profile

The powdery mildew fungus *Podosphaera fusca* (synonym *Podosphaera xanthii*), a constant threat to cucurbits

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SUMMARY

Numerous vegetable crops are susceptible to powdery mildew, but cucurbits are arguably the group most severely affected. *Podosphaera fusca* (synonym *Podosphaera xanthii*) is the main causal agent of cucurbit powdery mildew and one of the most important limiting factors for cucurbit production worldwide. Although great efforts have been invested in disease control, by contrast, many basic aspects of the biology of *P. fusca* remain unknown.

Taxonomy: *Podosphaera fusca* (Fr.) Braun & Shishkoff. Kingdom Fungi; Phylum Ascomycota; Subdivision Pezizomycotina; Class Leotiomycetes; Order Erysiphales; Family Erysiphaceae; genus *Podosphaera*; species *fusca.*

Identification: Superficial persistent mycelium. Conidia in chains, hyaline, ellipsoid to ovoid or doliform, about 24–40 × 15–22 μm, with cylindrical or cone-shaped fibrosin bodies, which often germinate from a lateral face and produce a broad, clavate germ tube and cylindrical foot-cells. Unbranched erect conidiophores. Cleistothecia globose, mostly 70–100 μm in diameter, dark brown/black. One ascus per cleistothecium with eight ascospores.

Host range: Angiosperm species that include several families, such as Asteracea, Cucurbitaceae, Lamiaceae, Scrophulariaceae, Solanaceae and Verbenaceae.

Disease symptoms: White colonies develop on leaf surfaces, petioles and stems. Under favourable environmental conditions, the colonies coalesce and the host tissue becomes chlorotic and usually senesces early.

Control: Chemical control and the use of resistant cultivars. Resistance has been documented in populations of *P. fusca* to some of the chemicals registered for control.

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INTRODUCTION

The Cucurbitaceae or cucurbit family is a medium-sized plant family composed of a diverse group of species grown around the world's warmer regions. It is a major family for economically important species, particularly those with edible fruits, and comprises an important starch resource in many regional diets. The major cultivated types include cucumber, melon, watermelon, squash and pumpkin. Minor cultivated types include chayote, citron, gherkin, gourds, horned cucumber and wild cucumber. They are amongst the earliest cultivated plants in both the Old and New Worlds, and some have medicinal and other uses. Unfortunately, diseases plague the production of cucurbits. There are over 200 known cucurbit diseases of diverse aetiologies (Zitter *et al.*, 1996).

Powdery mildew is probably the most common, conspicuous, widespread and easily recognizable disease of cucurbits. Like other powdery mildew diseases, its symptoms are characterized by the whitish, talcum-like, powdery fungal growth that develops on both leaf surfaces (Fig. 1A,B), petioles and stems (Sitterly, 1978; Zitter *et al.*, 1996), and rarely on fruits. The disease can be caused by either *Golovinomyces cichoracearum* or *Podosphaera fusca*. These obligate biotrophic ectoparasites induce identical symptoms, but can be easily distinguished from each other by light microscopy (Braun *et al.*, 2002). In Spain, as in many other countries around the world, cucurbit powdery mildew is a serious threat, and *P. fusca* is considered to be the main causal agent of powdery mildew on cucurbits and one of the most important limiting factors for cucurbit production (Fernández-Ortuño *et al.*, 2006; del Pino *et al.*, 2002).

Powdery mildew fungi are biotrophic parasites that usually grow on the plant surface, obtaining nutrients from the host epidermal cells by means of haustoria (Green *et al.*, 2002). Owing to their nature as obligate parasites, they cannot be cultured on nutrient medium, a fact that has significantly hampered research from genetic and molecular points of view. Appropriate disease
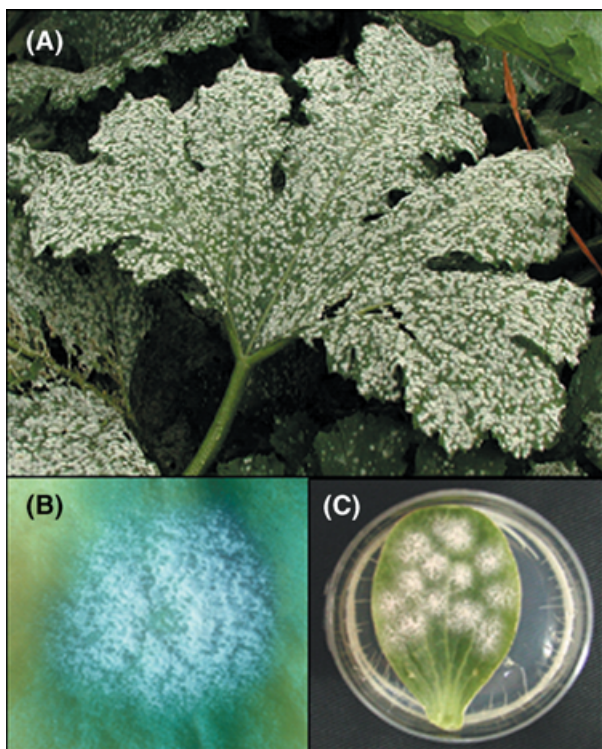
Fig. 1 Cucurbit powdery mildew symptoms caused by *Podosphaera fusca*. (A) Symptoms on a zucchini leaf. (B) Detail of a powdery mildew colony. (C) Zucchini cotyledon maintained *in vitro* and infected with *P. fusca* showing typical powdery mildew colonies.

management programmes require a good understanding of the biology of the responsible pathogen. This review summarizes our current knowledge of the biology of *P. fusca*, focusing on the molecular aspects of host–pathogen interactions, fungicide resistance and multitrophic interactions between host, *P. fusca* and biological control agents.

TAXONOMY

The nomenclature of the main causal agent of cucurbit powdery mildew is not yet completely standardized in the literature. The fungus has been designated *Sphaerotheca fuliginea*, *Sphaerotheca fusca*, *Podosphaera fusca* or *Podosphaera xanthii*. Based on scanning electron microscopy and molecular results, the reduction of the genus *Sphaerotheca* to synonymy with *Podosphaera* is now widely accepted (Braun *et al.*, 2002). The separation of *P. xanthii* from the *P. fusca* group was proposed according to a morphological species concept based on the teleomorph, by which the organism on cucurbits seems to have large ascomata and an ascus with a large oculus (Braun and Takamatsu, 2000; Braun *et al.*, 2001). In fungi, many morphological characters are plastic, and the natural variation of these characters within a

species is difficult to assess. It has therefore become evident for many mycologists that the morphological species concept is largely unsatisfactory (Moncalvo, 2005). This seems to be the case for *P. xanthii*, because the morphological features proposed to define this species represent doubtful criteria for two main reasons. First, chasmothecia are rarely or never observed in the field (McGrath, 1994), and second, although the production of chasmothecia can be induced in the laboratory (Bardin *et al.*, 1997), these are nonfertile as the ascospores obtained are not able to cause infection on cucurbits (McGrath, 1994). In addition, although some fragmentary molecular data based on internal transcribed spacer (ITS) sequences have become available (Hirata *et al.*, 2000), these data are not sufficient to make a decision because, as convincingly argued by Taylor *et al.* (2000), a phylogenetic approach for recognizing fungal species should not be based on a single gene phylogeny, but on the concordance of multiple gene genealogies. Thus, this division remains controversial and many authors have continued to consider *P. xanthii* as synonymous with *P. fusca*.

DISEASE SYMPTOMS AND LIFE CYCLE

The disease caused by *P. fusca* (similar to other powdery mildews) is easily recognized by the presence of a visual white powdery mass, mainly composed of mycelia and conidia, on leaf surfaces (Fig. 1A,B), petioles and young stems (Zitter *et al.*, 1996). Under favourable environmental conditions, fungal colonies may coalesce, covering the entire top surface of the leaves. The fungus feeds the plant nutrients, reduces photosynthesis and causes yellowing, and sometimes the death of leaves. A severe infection may kill the plant. Crop yields can be reduced because of reduced size or number of fruits. Fruit from affected plants can have low quality (Zitter *et al.*, 1996). Although cucurbit fruits are not directly or rarely attacked by powdery mildew fungi, they may be malformed, sunburned and ripened prematurely or incompletely because of a loss of foliage cover caused by premature senescence of infected leaves (Sitterly, 1978).

The asexual life cycle of *P. fusca* is very similar to that of other powdery mildew fungi (Fig. 2). Typically, after landing on a susceptible host, conidia produce a short germ tube, ending in a primary differentiated appressorium, from which a primary haustorium forms in an epidermal cell. From the primary appressorium, or from another pole of the conidium, a first hypha (primary hypha) arises that forms secondary appressoria from which secondary haustoria are formed. Later, the primary hypha branches to form secondary hyphae. Conidiophores emerge vertically from some of the secondary hyphae as morphologically distinct structures. At the tip of each conidiophore, 5–10 ovoid-shaped conidia are produced in chains. The mat of secondary hyphae and conidia forms the white mycelium on the surface of the plant, the typical visible symptom of powdery mildews (Pérez-García *et al.*, 2001).
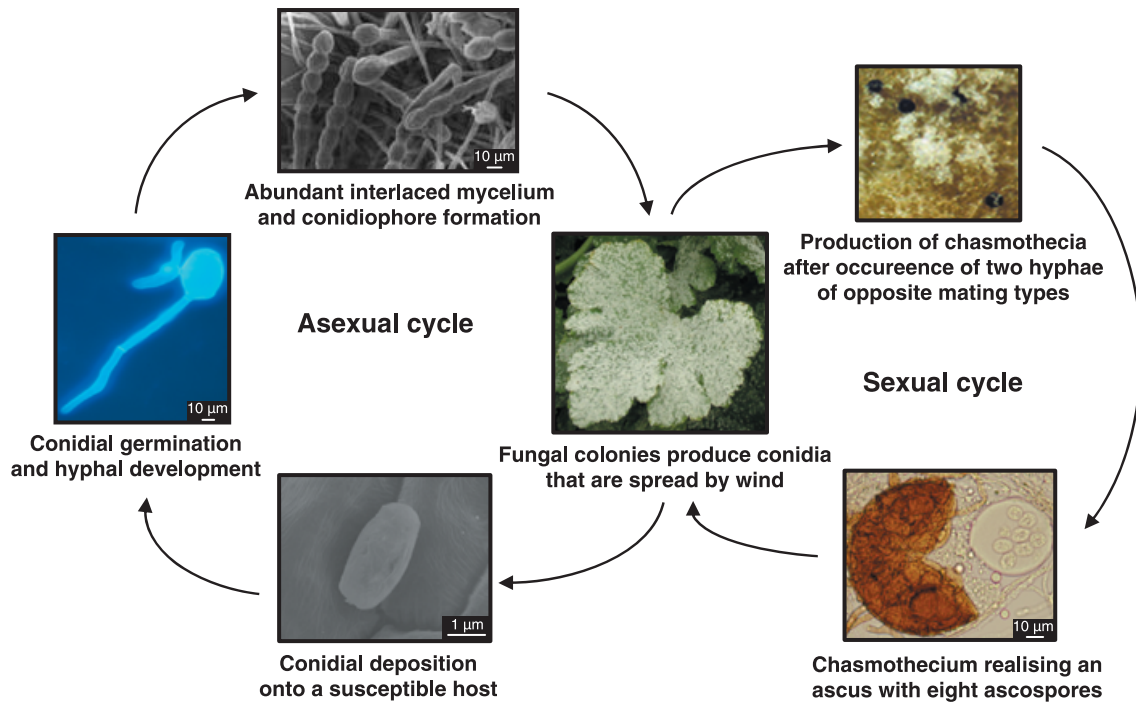
Fig. 2 Diagram depicting the life cycle of *Podosphaera fusca*.

Podosphaera fusca is a heterothallic fungus, and only after the encounter of two hyphae of opposite mating types does sexual reproduction occur (Fig. 2). As a consequence, a type of fruiting body, termed a chasmothecium, is formed which, in the case of *P. fusca*, contains only one ascus bearing eight ascospores or sexual spores (McGrath, 1994). Chasmothecia are, in general, considered to be overwintering and oversummering sources of inoculum. Although poorly investigated, the outbreak of the disease caused by ascospores is thought to resemble that of asexual conidia (Butt, 1978; Jarvis *et al.*, 2002). In the case of cucurbit powdery mildew, chasmothecia have rarely or never been observed in several of the world's most important cucurbit growing areas (McGrath, 1994). For this reason, the question of the prevalence and epidemiological relevance of the sexual stage of the pathogen remains largely unanswered.

RESEARCH TOOLS

Powdery mildews are comparatively difficult fungi to work with. Their obligate, biotrophic, parasitic nature and consequent inability to grow on artificial culture medium significantly hamper research. *Podosphaera fusca* has been traditionally cultured and conserved by periodical transfers of conidia to fresh plant material. In the laboratory, *P. fusca* is usually grown on detached cotyledons of several cucurbit species, such as *Lagenaria*, melon or zucchini, which are maintained *in vitro* on agarized medium

(Fig. 1C) (Álvarez and Torés, 1997; Bardin *et al.*, 2007). As a conservation system, this method is not very practical for maintaining large numbers of isolates and does not prevent genetic or physiological changes during long-term and frequent subculturing (Nicot *et al.*, 2002). Cryopreservation in liquid nitrogen is considered to be the best and most widely applicable preservation technique available for filamentous fungi, and has also been reported for the long-term storage of *P. fusca* (Bardin *et al.*, 2007; O'Brien and Weinert, 1994). Briefly, conidia are dried in air or using a desiccating agent such as $CaCl_2$, and stored at $-196\ ^{\circ}C$ in liquid nitrogen. However, freezing in ultralow freezers is the most commonly used method to preserve microbial culture collections, and the method that adjusts better to the requirements of any standard laboratory (Pérez-García *et al.*, 2006). Recently, a storage technique for *P. fusca* has been developed, which has made possible the long-term preservation of the pathogen in ultralow freezers. Basically, conidia are desiccated in the presence of anhydrous silica gel for 8 h in the dark at 22 $^{\circ}C$ and then stored at $-80\ ^{\circ}C$ (Pérez-García *et al.*, 2006). The method has been tried for up to 8 years (F. López-Ruiz, unpublished data). Currently, more than 1500 isolates of *P. fusca* have been stored at $-80\ ^{\circ}C$ using this method in our laboratory. This preservation method also seems to work for other powdery mildew species, such us *Blumeria graminis* f. sp. *tritici* (J. Brown, John Innes Centre, Norwich, UK; personal communication).

The genetics of powdery mildew fungi represents a serious challenge to researchers. The range of characters that can be

studied is restricted to those that are most important for the adaptation of the fungus to its host, namely host range and race-specific avirulence, and fungicide resistance (Brown, 2002). In contrast with other powdery mildew fungi, such as *B. graminis*, the genetics of *P. fusca* remains virtually unexplored because we lack a system to perform *P. fusca* meiotic crosses. Although the production of chasmothecia can be induced in the laboratory on detached cotyledons when two strains of opposite mating types are co-inoculated (McGrath, 1994), the special conditions for chasmothecial maturation and the appropriate development of ascospores have yet to be determined. Thus, this is without doubt the main bottleneck to the performance of genetic analysis in *P. fusca*.

Molecular studies of powdery mildew fungi are plagued by the intrinsic difficulty of extracting DNA or RNA of suitable quality and sufficient quantity to perform large-scale molecular analyses. However, the technologies generally termed whole genome amplification (WGA) and whole transcriptome amplification (WTA) have the potential to overcome these limitations. Recently, the WGA method called multiple displacement amplification (MDA) has been used to amplify the whole genome of *P. fusca* (Fernández-Ortuño *et al.*, 2007, 2008a). MDA is a technique capable of generating microgram quantities of high-molecular-weight DNA from a few nanograms of input DNA, the MDA-synthesized DNA being highly accurate and representative of the amplified genome and suitable for a diverse set of downstream applications. The MDA method is especially useful in population biology studies, when a large number of samples or isolates need to be analysed, in fields such as molecular epidemiology (e.g. detection of fungicide resistance alleles) or population genetics (e.g. determination of the genetic structure of pathogen populations using polymerase chain reaction-based approaches, such as multilocus sequence typing) (Fernández-Ortuño *et al.*, 2007, 2008a). MDA technology can also be very useful in systematics, contributing to overcome the most limiting factor in the advancement of fungal molecular phylogenetics, i.e. the relatively small number of genes that are readily accessible for systematics, especially from fungi recalcitrant to study, such as powdery mildews (Fernández-Ortuño *et al.*, 2007). Similar to WGA, WTA methods amplify the whole transcriptome of a given organism from a few nanograms of starting RNA (Tomlins *et al.*, 2006). Although WTA methods have yet to be applied to *P. fusca*, it seems probable that both WGA and WTA technologies will facilitate molecular studies in this and other obligate fungal pathogens.

HOST–PATHOGEN INTERACTIONS

Genetic resistance to powdery mildew in cucurbits is a very important attribute, in that it contributes to higher yields of better quality crops and low fungicide applications, hence increasing environmental and health benefits. Breeding for resistance to

powdery mildew in the Cucurbitaceae family has a long and successful history (Jahn *et al.*, 2002), as the use of resistant cultivars represents one of the main means of disease control, although with variable success (Zitter *et al.*, 1996). Many commercial varieties and breeding lines of cucumber, melon and squash have been released with resistance to *P. fusca*. Several resistance genes (*Pm*) have been reported, especially in melon (Pitrat, 2002). Resistance is usually conferred by single dominant genes in most cases, although recessive genes have also been described (Jahn *et al.*, 2002). The fourth major crop, watermelon, has been traditionally considered to be resistant to powdery mildew. Unfortunately, in recent years, outbreaks of powdery mildew in watermelon have been reported that appear to be elicited by highly aggressive isolates of *P. fusca* (Cohen *et al.*, 2000; Jahn *et al.*, 2002; del Pino *et al.*, 2002). Although, as previously mentioned, many commercial varieties have been released with resistance to *P. fusca*, the development of new races of the pathogen hinders disease management through resistance breeding. This problem is especially serious in melon, where there have been more than 30 reported sources of resistance to the 28 putative races of *P. fusca* described so far (McCreight, 2006).

Various resistance mechanisms to powdery mildews have been reported, and can be roughly classified as pre- and post-haustorial resistance (Huang *et al.*, 1998). With pre-haustorial resistance, the formation of haustoria is prevented or reduced by papillae and is not associated with plant cell necrosis (hypersensitive reaction). This mechanism has been reported in quantitative non-race-specific types of resistance and also in non-host resistance to powdery mildew species. To our knowledge, this resistance mechanism has not been reported in cucurbits against cucurbit powdery mildew. Post-haustorial resistance is usually associated with a hypersensitive reaction. This is the typical mechanism of the major genic race-specific resistance to powdery mildews. Resistance to races 1 and 2 of *P. fusca* has been reported as post-haustorial in several melon cultivars, such as PMR-6 (Cohen and Eyal, 1988; Cohen *et al.*, 1990; Pérez-García *et al.*, 2001), and in some resistant cucumber and melon lines, but switched by temperature variations (Munger, 1979; Pérez-García *et al.*, 2001). Although the interaction between melon and *P. fusca* is assumed to comply with the gene-for-gene concept, DNA sequences encoding *Pm* genes have not been isolated, nor have avirulence genes been identified.

In post-haustorial resistance, the cascade of mechanisms identified in melon plants resistant to *P. fusca* proceeds as follows. *Podosphaera fusca* conidia are hypothesized to release cell wall monomers, as described for *B. graminis*. After recognition, the resistant plant triggers a sequence of physiological changes initiated by the rapid accumulation of reactive oxygen species, such as hydrogen peroxide and superoxide anions. These changes take place a few hours after pathogen inoculation and before

the formation of the first haustorium (Romero *et al.*, 2008). This oxidative burst is followed by a strong reinforcement of plant cell walls of *P. fusca*-invaded and surrounding cells. The reinforcement is caused by the deposition of lignin and callose polymers, which may slow down pathogen ingress into the cell and disrupt nutrient flow (Cohen *et al.*, 1990; Romero *et al.*, 2008). The coordinated spatial–temporal accumulation pattern of cell wall deposits and the production of hydrogen peroxide, combined with the increase in peroxidase activity found in diverse cucurbit species in response to *P. fusca*, also suggest the involvement of this reactive oxygen species in cell wall strengthening (Lebeda *et al.*, 1999; Reuveni and Bothma, 1985; Romero *et al.*, 2008). Interestingly, the transcriptional levels of phenylalanine ammonia-lyase, an important enzyme for phenylpropanoid metabolism, do not seem to change in response to *P. fusca* inoculation (Romero *et al.*, 2008). The additional accurate deployment of pathogenesis-related proteins and phytoalexins in the zone of penetration has been suggested to arrest *P. fusca* development directly. A differential expression of β-glucanase has been reported to occur in susceptible and resistant cultivars in response to infection by *P. fusca* (Rivera *et al.*, 2002); however, its precise role in *P. fusca* resistance has not been conclusively established, as immunocytolocalization studies have yet to be undertaken. In any case, the manifestation of the response mechanism, including plant cell necrosis, is typically post-haustorial (Cohen *et al.*, 1990; Rivera *et al.*, 2002). Recent studies have demonstrated, however, that, in response to inoculation with the same *P. fusca* race, some resistant lines afford a post-haustorial resistance pattern slightly modified from that mentioned previously. In this case, the accumulation of callose appears to be the most relevant physiological modification, whereas the typical hypersensitive reaction seems to play a complementary role (Kuzuya *et al.*, 2006).

FUNGICIDE RESISTANCE

Although great efforts have been invested in plant breeding programmes, growers still have important concerns about disease control, and the application of fungicides continues to be the principal practice for the management of powdery mildew in most cucurbit crops (McGrath, 2001). The impact of chemical control, however, has been very much tempered by the ease with which *P. fusca* develops resistance, quickly rendering many systemic fungicides ineffective, perhaps because high disease pressures require repeated fungicide treatments (Hollomon and Wheeler, 2002). The phenomenon of fungicide resistance in cucurbit powdery mildew traces back to 1967, when benomyl-resistant strains were detected in an experimental glasshouse in the USA (Schroeder and Provvidenti, 1969). Since then, the cucurbit powdery mildew fungus has exhibited a high potential for developing resistance in many areas of the world to several

fungicide classes, including methyl benzimidazole carbamates, sterol demethylation inhibitors (DMIs), morpholines, organo-phosphates, hydroxypyrimidines, Qo inhibitors (QoIs) and quinoxalines (McGrath, 2001).

The first molecular data on fungicide resistance in *P. fusca* were related to resistance to strobilurins (QoI fungicides). The main mechanism conferring resistance to QoIs in phytopathogenic fungi is a target site modification that involves mutations in the cytochrome *b* gene *CYTB*, such as the substitution of glycine by alanine at position 143 (G143A) (Fernández-Ortuño *et al.*, 2008b). The occurrence of the G143A amino acid change in isolates of *P. fusca* resistant to QoI fungicides was first documented in Spain, where it was reportedly widespread in cucumber (Heaney *et al.*, 2000), and also in Japan (Ishii *et al.*, 2001). However, in a recent study with resistant isolates obtained from melon and other cucurbit crops in south-central Spain, the mechanism responsible for QoI resistance in *P. fusca* was not shown to be linked to typical mutations in *CYTB* (Fernández-Ortuño *et al.*, 2008a). In that report, neither G143A nor other consistent amino acid substitutions in cytochrome *b* QoI domains were found to correlate with resistance. In addition, the absence of the G143A substitution could not be explained by an intron following codon 143, as found in several rust fungi. In the same study, the role of alternative respiration in QoI resistance was also ruled out. Considering the pattern of cross-resistance to different QoIs, the high levels of resistance of the resistant *P. fusca* isolates and the absence of consistent mutations in *CYTB*, a structural change in the Rieske-FeS protein (ISP), the other protein component of the target site of QoI fungicides, may well be responsible for QoI resistance (Fernández-Ortuño *et al.*, 2008a). Experimental evidence regarding the role of the Rieske protein in resistance to QoI fungicides in *P. fusca* has yet to be obtained.

Sterol DMI fungicides are one of the most important classes of agricultural fungicides and the current leading class against powdery mildews. Unfortunately, resistance to DMI fungicides in *P. fusca* has been documented (McGrath, 2001). In contrast with QoI resistance, DMI resistance seems to be quantitative, with resistance resulting from the modification of several interacting genes. Several mechanisms of DMI resistance seem to be operating in plant pathogens, including mutations and overexpression of the target C14α-demethylase (*CYP51*) gene (Ma and Michailides, 2005). The molecular basis of resistance to DMI fungicides in *P. fusca* is being elucidated currently. Recent data on the molecular analysis of the *CYP51* gene from azole-resistant and azole-sensitive *P. fusca* isolates showed a correlation between certain amino acid substitutions and the different resistance phenotypes observed (López-Ruiz *et al.*, 2008), but conclusive data about the precise role of these amino acid changes in DMI resistance have yet to be provided. Furthermore, differences in *CYP51* expression do not appear to play a significant role in DMI resistance (F. López-Ruiz, unpublished data).

MULTITROPHIC INTERACTIONS BETWEEN HOST, PATHOGEN AND BIOLOGICAL CONTROL AGENTS

The increasing problem of fungicide resistance and public concerns about the hazardous effects of chemicals on the environment have led researchers to explore suitable environmentally friendly alternatives or complements to chemicals for the management of cucurbit powdery mildew. Although certain natural products, such as plants and compost extracts, detergents and mineral oils, micronutrient solutions, silicon and even animal-based products, such as unpasteurized milk, have been proposed in the literature as less harmful alternatives to traditional chemical practices, management approaches based on the use of natural enemies of pathogens (referred to as biological control) are by far the most investigated of such alternatives (Bélanger and Labbé, 2002).

Several filamentous fungi, yeasts and bacteria have been proposed as biocontrol agents of cucurbit powdery mildew, but only a few have shown significant effectiveness. Much of the work has been performed with the mycoparasites *Acremonium alternatum* and *Ampelomyces quisqualis*, and the entomopathogenic fungus *Lecanicillium lecanii* (Romero *et al.*, 2003). Two main effects on *P. fusca* development have been attributed to mycoparasites: (i) reduction in nutrient uptake as a result of the limitation of the number of haustoria, leading to a reduction in the growth and size of the colony; (ii) limitation of the number of conidiophores and conidia, essential structures for pathogen multiplication, resulting in a reduction in disease spread (Romero *et al.*, 2003, 2007c). Two different interaction strategies between these mycoparasites and *P. fusca* have been recognized. The endoparasitic behaviour of *A. quisqualis* has been well documented by fluorescence microscopy (Romero *et al.*, 2003; Wilson and Backman, 1999). Scanning electron microscopy (SEM) analysis revealed its penetration into *P. fusca* (Romero *et al.*, 2003; Sztejnberg *et al.*, 1989), which seems to be favoured by the secretion of β-1,3-glucanases (Rotem *et al.*, 1999). *Acremonium alternatum* and *L. lecanii*, unlike *A. quisqualis*, behave as ectoparasites (Askary *et al.*, 1997; Malathrakis, 1985). SEM analysis has also revealed the penetration of *L. lecanii* into *P. fusca* which, in this case, seems to be favoured by different enzymatic activities, such as proteases and lipases (Askary *et al.*, 1997).

Another successful biocontrol strategy has been the use of antibiotic-producing microorganisms, such as some bacteria and yeasts. To date, the yeast *Pseudozima flocculosa* is probably the most promising anti-powdery mildew biological agent currently available (Avis and Bélanger, 2001). This biocontrol agent produces antifungal compounds closely related to fatty acids, which induce plasmolysis of the target cells in different growth stages (Hajlaoui *et al.*, 1992). With regard to bacterial agents, species belonging to the genus *Bacillus* deserve special attention. Recent work has demonstrated that strains of *Bacillus subtilis*,

producers of lipopeptide antibiotics, may be suitably applied in glasshouse cropping conditions against *P. fusca* (Romero *et al.*, 2004, 2007c). Among these compounds, iturin and fengycin lipopeptides have been shown to be key factors in the antagonism of *B. subtilis* towards *P. fusca* (Romero *et al*, 2007b). These lipopeptides, similar to the fatty acids of *P. flocculosa*, target biological membranes and induce ultrastructural and morphological damage, leading to the failure of germination in *P. fusca* conidia (Romero *et al.*, 2007a).

Mycoparasitism and antibiosis imply direct interactions between *P. fusca* and biocontrol agents, and generally require a high relative humidity for optimal disease-suppressive activity (Romero *et al.*, 2007c). Therefore, their effectiveness may be easier to control in the protected microclimatic environments of glasshouse-grown crops than in the field. An interesting approach to overcoming this environmental restriction relies on the use of microorganisms that are able to colonize the most buffered plant rhizosphere and promote the induction of natural plant defences, named induced systemic resistance (van Loon *et al.*, 1998). Strains belonging to the genera *Pseudomonas* and *Bacillus*, which are widely distributed in nature, have attracted most attention, and have been tested in biocontrol trials against diverse aerial diseases (Ji *et al.*, 2006; Kloepper *et al.*, 2004). The small number of reports dealing with the use of microorganisms as inducers of systemic resistance against *P. fusca* in cucurbits (García-Gutiérrez, 2007; Reuveni and Reuveni, 2000) and the gaps in our knowledge with regard to the complex signalling network underlying these beneficial interactions should stimulate more intensive research.

CONCLUSIONS AND FUTURE PROSPECTS

Powdery mildew caused by *P. fusca* represents one of the most serious threats to cucurbit production worldwide. Despite significant breeding efforts and the development of new fungicides, consistently effective disease control remains elusive to growers. The deployment of resistant varieties, combined with fungicide rotation and the progressive introduction of safer alternatives to chemicals, including inorganic, organic and biological control products, will be used in disease control for several years to come. Although the phase of chasmothecial maturation to the production of viable sexual progeny for genetic analysis is still irresolute, the emerging molecular approaches developed for the pathogen and the forthcoming genome sequences of *B. graminis* f. sp. *hordei* and other powdery mildew fungi will be very useful for further research. In particular, these developments will facilitate the analysis of the physiological and molecular processes involved in *P. fusca* pathogenicity and biology, and an understanding of the intimate molecular dialogue between host and pathogen. This will provide the basis for the design of novel chemicals effective against this powdery mildew fungus, enabling us to face the disease and the design of disease control programmes in a more rational manner.

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