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Pathogen profile

The cucurbit downy mildew pathogen *Pseudoperonospora cubensis*

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SUMMARY

Pseudoperonospora cubensis [(Berkeley & M. A. Curtis) Rostovzev], the causal agent of cucurbit downy mildew, is responsible for devastating losses worldwide of cucumber, cantaloupe, pumpkin, watermelon and squash. Although downy mildew has been a major issue in Europe since the mid-1980s, in the USA, downy mildew on cucumber has been successfully controlled for many years through host resistance. However, since the 2004 growing season, host resistance has been effective no longer and, as a result, the control of downy mildew on cucurbits now requires an intensive fungicide programme. Chemical control is not always feasible because of the high costs associated with fungicides and their application. Moreover, the presence of pathogen populations resistant to commonly used fungicides limits the long-term viability of chemical control. This review summarizes the current knowledge of taxonomy, disease development, virulence, pathogenicity and control of *Ps. cubensis*. In addition, topics for future research that aim to develop both short- and long-term control measures of cucurbit downy mildew are discussed.

Taxonomy: Kingdom Straminipila; Phylum Oomycota; Class Oomycetes; Order Peronosporales; Family Peronosporaceae; Genus *Pseudoperonospora*; Species *Pseudoperonospora cubensis*.

Disease symptoms: Angular chlorotic lesions bound by leaf veins on the foliage of cucumber. Symptoms vary on different cucurbit species and varieties, specifically in terms of lesion development, shape and size. Infection of cucurbits by *Ps. cubensis* impacts fruit yield and overall plant health.

Infection process: Sporulation on the underside of leaves results in the production of sporangia that are dispersed by wind. On arrival on a susceptible host, sporangia germinate in free water on the leaf surface, producing biflagellate zoospores that swim to and encyst on stomata, where they form germ tubes. An appressorium is produced and forms a penetration hypha, which enters the leaf tissue through the stomata. Hyphae grow through the mesophyll and establish haustoria, specialized structures for the transfer of nutrients and signals between host and pathogen.

Control: Management of downy mildew in Europe requires the use of tolerant cucurbit cultivars in conjunction with fungicide applications. In the USA, an aggressive fungicide programme, with sprays every 5–7 days for cucumber and every 7–10 days for other cucurbits, has been necessary to control outbreaks and to prevent crop loss.

Useful websites: http://www.daylab.plp.msu.edu/pseudoperonospora-cubensis/ (Day Laboratory website with research advances in downy mildew); http://veggies.msu.edu/ (Hausbeck Laboratory website with downy mildew news for growers); http://cdm.ipmpipe.org/ (Cucurbit downy mildew forecasting homepage); http://ipm.msu.edu/downymildew.htm (Downy mildew information for Michigan's vegetable growers).

INTRODUCTION

Cucurbit downy mildew (caused by *Pseudoperonospora cubensis*) is one of the most important foliar diseases of cucurbits, causing significant yield losses in the USA, Europe, China and Israel (Thomas, 1996). The pathogen has a wide geographical distribution and has been reported in over 70 countries, including environments ranging from semi-arid to tropical. In addition, *Ps. cubensis* has a wide host range, infecting approximately 20 different genera of cucurbits (Lebeda and Urban, 2007; Palti and Cohen, 1980). The cucurbit crops grown in the USA that are susceptible to this aerially dispersed oomycete pathogen are valued at more than $246.2 million per annum (Anonymous, 2009).

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The control of downy mildew relies on the application of fungicides and the use of host resistance. Nonetheless, fungicide-resistant *Ps. cubensis* populations have been documented throughout the world (Colucci and Holmes, 2007; Hausbeck and Cortright, 2009; Lebeda and Urban, 2007; Mitani *et al*., 2001; Zhu *et al*., 2008), and host resistance is no longer sufficient to control the disease as it once was in the USA (Holmes and Thomas, 2006). The additional cost of fungicides, coupled with potential yield losses of up to 100% caused by downy mildew, threaten the long-term viability of cucurbit crop production (Hausbeck and Cortright, 2009; Holmes *et al*., 2006; Lebeda and Urban, 2007).

A detailed knowledge of *Ps. cubensis* epidemiology, infection processes and population genetics is currently lacking, but is necessary to guide future efforts for the development of new resistant varieties and fungicides, and for the prevention of pathogen populations from overcoming host resistance and chemical control. Studies of pathogen epidemiology and global population genetics, the evaluation of fungicides for disease control, and the development of a high-coverage draft genome sequence will assist in our understanding of the pathogen, as well as in developing effective diagnostics and control measures for *Ps. cubensis*. The aim of this review is to briefly summarize what is currently known about the cucurbit downy mildew pathogen, *Ps. cubensis*, including taxonomy, disease development, virulence, pathogenicity and management.

TAXONOMY AND MORPHOLOGY

Pseudoperonospora cubensis is the type species of the genus *Pseudoperonospora*, which includes five accepted species: *Ps. cubensis*, *Ps. humuli*, *Ps. cannabina*, *Ps. celtidis* and *Ps. urticae* (Choi *et al*., 2005). In addition, there are reports of a sixth species, *Ps. cassiae*, which, although rare, may also be a true species of *Pseudoperonospor*a (Waterhouse and Brothers, 1981). Originally named '*Peronospora cubensis*' when discovered in Cuba by Berkeley and Curtis (1868), *Ps. cubensis* was reclassified in 1903 after further observations of sporangia germination (Rostovzev, 1903). *Pseudoperonospora* species have true sporangia that germinate via cytoplasmic cleavage to produce zoospores (Fig. 2c), whereas species of *Peronospora* have sporangia that germinate directly via a germ tube (Palti and Cohen, 1980; Rostovzev, 1903).

Recent work has shown that there are no significant morphological differences between *Ps. cubensis* and the hop (*Humulus* spp.) pathogen *Ps. humuli*; nonetheless, there is no evidence that *Ps. humuli* can infect cucurbits, and limited support for *Ps. cubensis* pathogenicity on hops (Mitchell *et al*., 2009). Molecular evidence also shows conflicting results. Internal transcribed spacer (ITS) region sequences of both pathogens are highly similar, which suggests that *Ps. humuli* could be a taxonomic synonym of *Ps. cubensis* (Choi *et al*., 2005). However, a recent study using single nucleotide polymorphisms (SNPs) indicates that two nuclear and one mitochondrial gene support the separation of *Ps. cubensis* from *Ps. humuli* (Mitchell *et al*., 2009). In addition, host range studies have demonstrated pathogenic differences between *Ps. cubensis* and *Ps. humuli* that further support the separation of these species (Gent *et al*., 2009). Overall, these genetic, phenotypic and physiological characterizations of *Ps. cubensis* and *Ps. humuli* provide support for the distinction between these species. Further studies, including evidence from hundreds of loci, would be helpful to fully resolve the phylogeny of these closely related species.

Morphological characters may not provide sufficient information for the characterization of *Ps. cubensis* isolates, or even for differentiation between species of *Pseudoperonospora* (Runge and Thines, 2010). *Pseudoperonospora cubensis* sporangiophore morphology can vary with temperature, and sporangia dimensions are influenced by the cucurbit host (Iwata, 1942; Waterhouse and Brothers, 1981). Recent work with a single isolate of *Ps. cubensis* inoculated onto six different cucurbit species has shown that the host cell matrix plays a role in influencing five morphological criteria, including sporangiophore length, length of ultimate branchlets, sporangial length and width, and the ratio between sporangial length and width (Runge and Thines, 2010). The differences among these morphological characteristics were more obvious in phylogenetically unrelated hosts. These results indicate that it is desirable to include information from genetic markers when resolving phylogenetic relationships in species of *Pseudoperonospora*.

SYMPTOMS AND SIGNS

Downy mildew of cucurbits is a foliar disease, and is easily recognizable by the development of chlorotic lesions on the adaxial leaf surface, sometimes with necrotic centres. These lesions can be restricted by the leaf vein, as in cucumber, giving them an angular appearance (Fig. 1). In other cucurbits, the symptoms may vary slightly in terms of shape and colour. For example, in both melon and watermelon, foliar lesions are less defined than those on cucumber, and are not always bound by leaf veins (Thomas, 1996). As infection progresses, the chlorotic lesions expand and may become necrotic (Oerke *et al*., 2006), with necrosis occurring more quickly in hot, dry weather (Cohen and Rotem, 1971c). Leaves colonized by *Ps. cubensis* undergo changes in temperature and transpiration rates, which vary during the course of infection and over the leaf surface (Lindenthal *et al*., 2005; Oerke *et al*., 2006). Low temperatures can delay symptom development whilst still promoting colonization of the leaf tissue, whereas higher temperatures result in faster

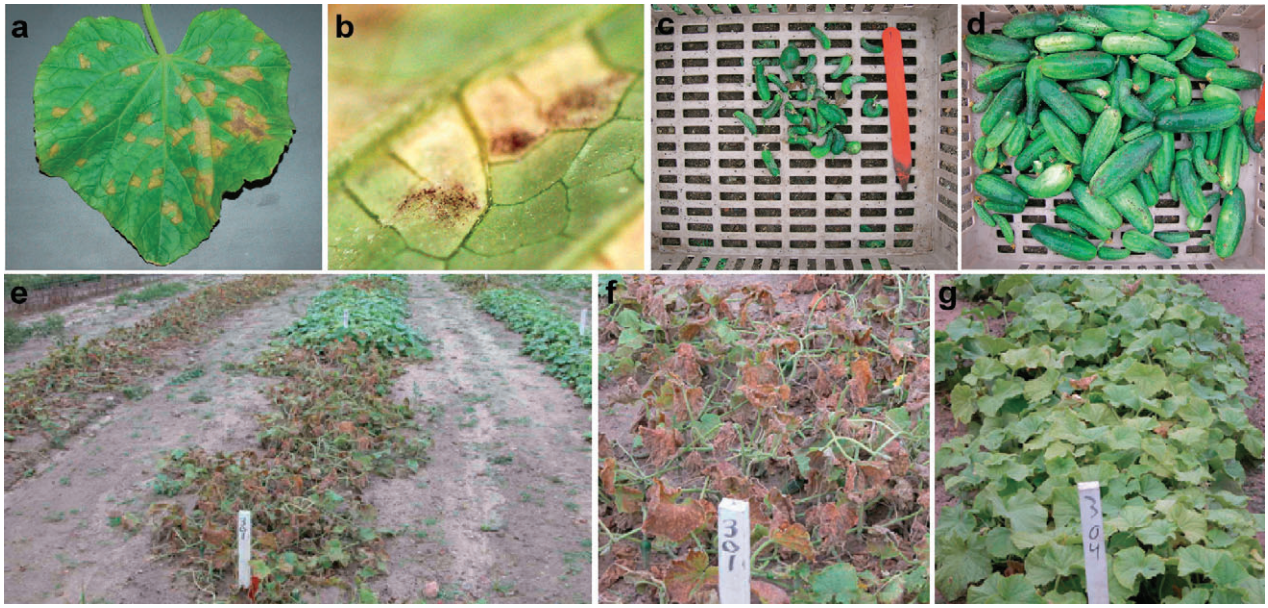

Fig. 1 Symptoms caused by *Pseudoperonospora cubensis*. (a) Yellow angular lesions on a cucumber leaf. (b) Typical 'downy' appearance on abaxial leaf surface caused by sporulation. Yields from untreated (c) and treated (d) cucumbers infected with *Ps. cubensis*. (e) Field showing typical disease symptoms. Severe symptoms on untreated (f) versus treated (g) cucumbers.

lesion chlorosis that may inhibit pathogen growth (Cohen, 1977b). As the downy mildew disease progresses, entire leaves may die within days following the initial infection, as lesions expand and coalesce (Thomas, 1996). A reduced canopy leads to cessation of fruit development and increased sun exposure of the fruit, allowing for sun scald and secondary rots (Keinath *et al*., 2007). Ultimately, crop yield and fruit quality are affected (Fig. 1).

When temperatures are below those that allow lesion formation and relative humidity is 90% or more, sporulation, the eponymous 'downy' appearance on the lower leaf surface, may be the first sign of disease (Fig. 1) (Rotem *et al*., 1978). Hyaline sporangiophores (180–400 μm) bearing papillate, lemon-shaped, grey–purple sporangia (20–40 × 14–25 μm) on sterigmata emerge in groups of one to six from stomata on the abaxial surface of infected leaves (Fig. 2) (Choi *et al*., 2005; Palti, 1975). Although leaf wetness is prohibitory for sporangium production, a period of near-saturated relative humidity must occur for 6 h or more to induce sporulation (Cohen, 1981). Sporulation, as in other downy mildews, is dependent on the diurnal cycle, and is enhanced by longer photoperiods (Cohen and Rotem, 1971b). The differentiation of sporangia requires a minimum dark period of 6 h (Cohen, 1977a). The optimum temperature for sporangia production is 15–20 °C, but sporangia may form on cucumber at temperatures from 5 to 30 °C (Thomas, 1996). Other factors, such as the host species, cultivar, host nutritional status and host age, may also affect sporulation (Cohen, 1981).

DISPERSAL AND SURVIVAL

Pseudoperonospora cubensis cannot overwinter in geographical locations with killing frosts. Instead, the pathogen overwinters in areas with mild winter temperatures that permit cucurbit hosts to be grown year round (Bains and Jhooty, 1976), or in greenhouses (Thomas, 1996). It has been demonstrated recently that *Ps. cubensis* could infect a perennial member of the Cucurbitaceae, *Bryonia dioica*, in the laboratory, and the pathogen could potentially overwinter on this host in Central and Northern Europe (Runge and Thines, 2009). However, this has not been supported by observations in the field (Lebeda and Cohen, 2010), and it is unknown whether *B. dioica* plays an important role in the life cycle of *Ps. cubensis* (Runge and Thines, 2009). Although oospores have been observed in both temperate and tropical regions, including India, Japan, Austria, Russia, China, Italy and Israel (Bains *et al*., 1977; Bedlan, 1989; Cohen *et al*., 2003; D'Ercole, 1975; Hiura and Kawada, 1933; Palti and Cohen, 1980), the production of oospores is very rare (Palti and Cohen, 1980; Thomas, 1996). The rare occurrence of thick-walled resting structures, i.e. oospores, limits *Ps. cubensis* survival in the absence of a living host. It is currently unknown whether oospores play an important role in the disease cycle (Fig. 3).

As an obligate biotroph, *Ps. cubensis* requires live host tissue for reproduction and dispersal. Copious asexual sporangia are produced on infected foliage, which may be liberated to the air following a reduction in relative humidity when hygroscopic

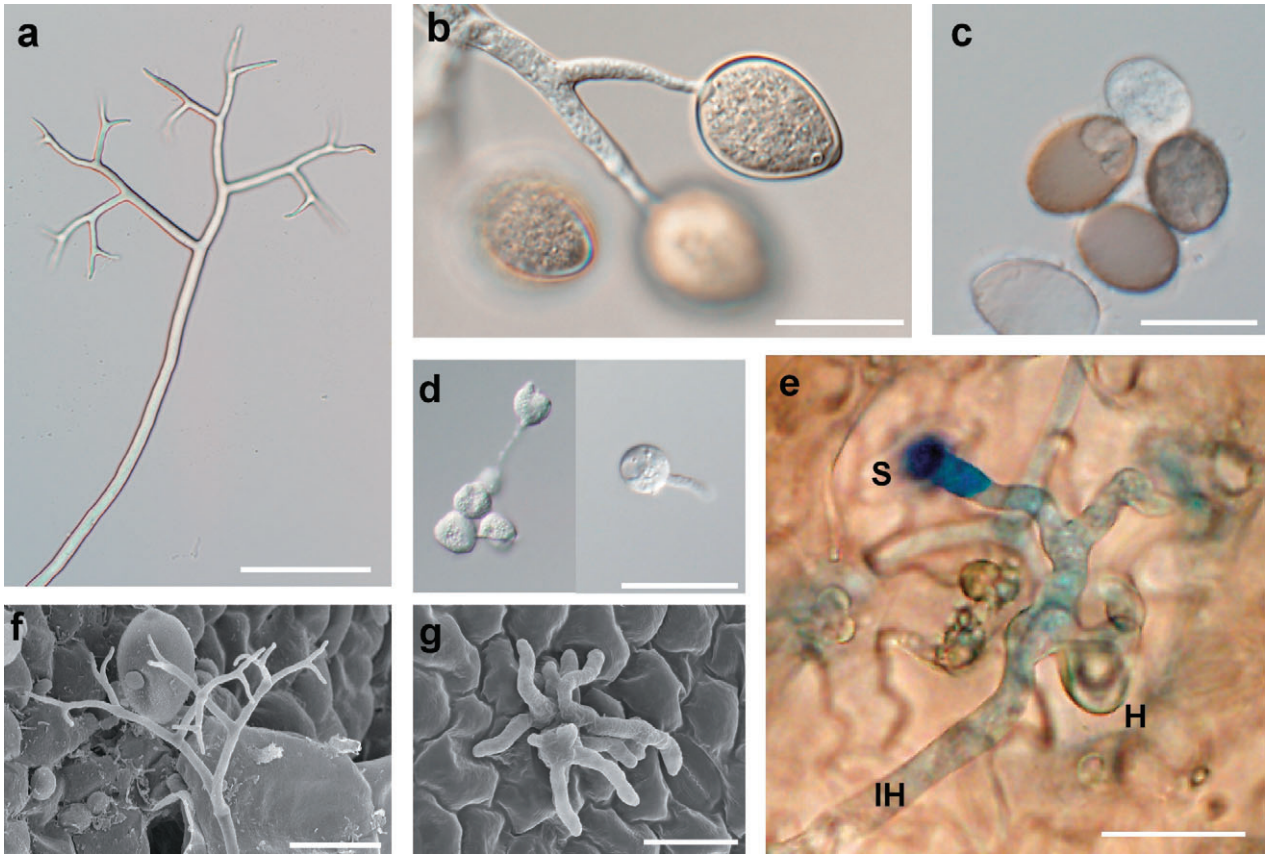

Fig. 2 Morphology of *Pseudoperonospora cubensis*. (a) Sporangiophore (bar, 50 μm). (b) Sporangia attached to distal end of sporangiophores. (c) Sporangia germinating via cytoplasmic cleavage. (d) Left panel: zoospores. Right panel: encysted zoospore with germ tube. (e) Intercellular growth: H, haustorium; IH, intercellular hyphae; S, stomata. (f) Scanning electron micrograph of sporangiophore (bar, 20 μm). (g) Scanning electron micrograph of multiple sporangiophores emerging through stomata (bar, 20 μm). Bars, 25 μm, except where noted.

twisting movements of sporangiophores actively release sporangia into air currents (Lange *et al*., 1989). Hence, airborne *Ps. cubensis* sporangia concentrations are greater in the morning and early afternoon, when changes in relative humidity and leaf wetness tend to occur (Cohen and Rotem, 1971b). The distance sporangia travel depends on where in the canopy the sporangia are produced, as well as the wind conditions as they become airborne (Aylor, 1990). Like other downy mildews (Aylor and Taylor, 1982; Wu *et al*., 2001), *Ps. cubensis* sporangia may be dispersed by wind over long distances (Thomas, 1996). As such, it has been proposed that, in the USA, *Ps. cubensis* overwinters in frost-free areas of the southern states (e.g. Florida and Texas) and spreads northwards each growing season via wind currents (Doran, 1932; Holmes *et al*., 2004; Van Haltern, 1933). Likewise, sporangia that infect cucurbits in Central Europe originate from year-round production areas in south-east Europe, and are transported via wind currents (Lebeda and Schwinn, 1994). Subsequent local transport of secondary inoculum occurs primarily via wind, but sporangia may also be dispersed by rain splash or physical transfer on equipment within a field (Thomas, 1996).

Sporangial survival during transport is limited to 1–16 days after dispersal (Cohen, 1981; Cohen and Rotem, 1971a) depending on temperature, relative humidity (Thomas, 1996) and solar radiation (Kanetis *et al*., 2009). Once a sporangium lands on a host plant, that sporangium must survive until environmental conditions are favourable for infection. Sporangial survival is favoured by conditions of low relative humidity, lower temperature and cloudy days (Cohen and Rotem, 1971a; Kanetis *et al*., 2009).

INFECTION MECHANISMS

Although the liberation and dispersal of sporangia occur under conditions of low leaf moisture, leaf wetness is necessary for the pathogen to successfully germinate and infect the host plant. At 15 °C, the optimum temperature for infection, at least 2 h of leaf wetness are required for infection when high levels of inoculum

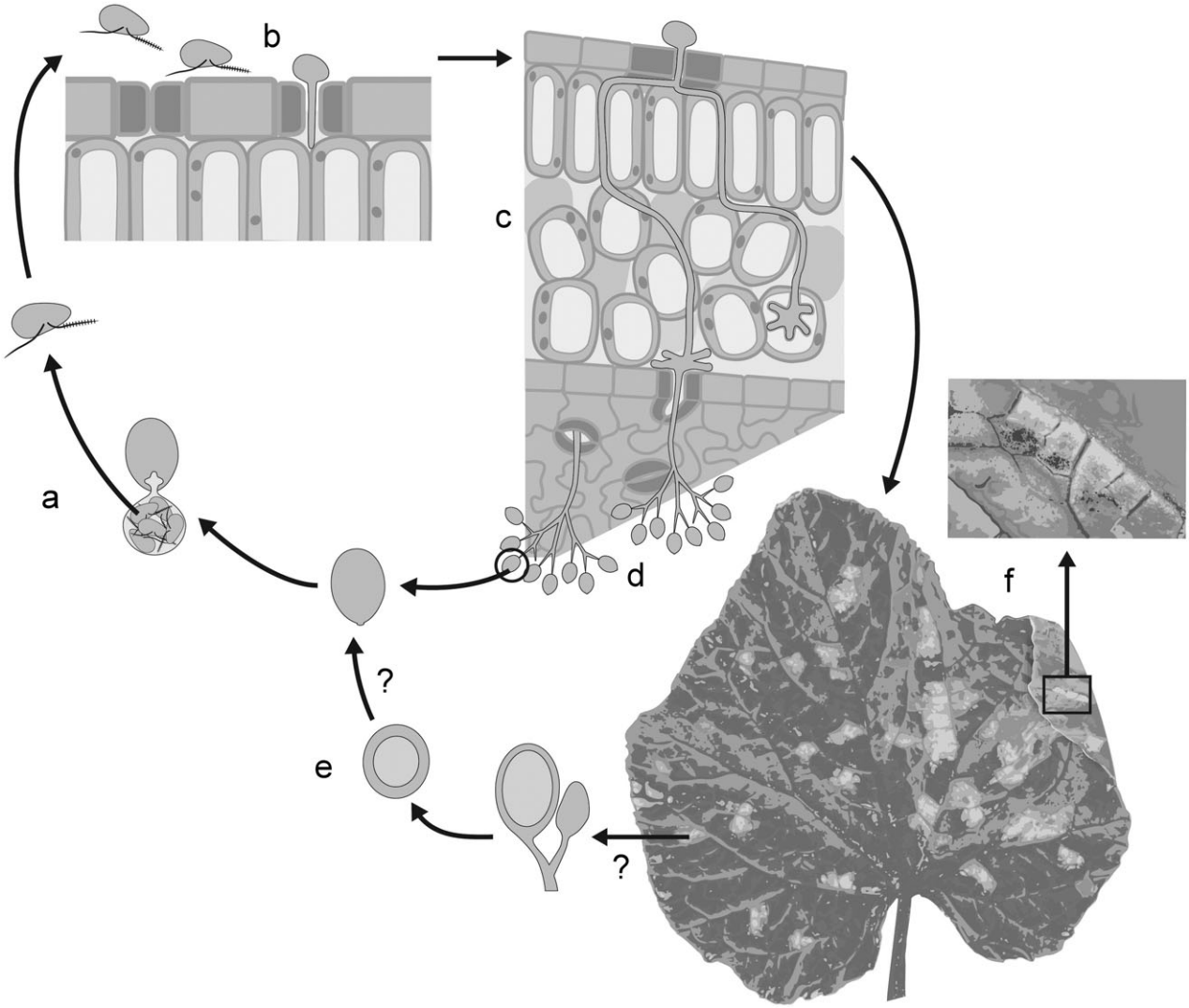

Fig. 3 Life cycle of *Pseudoperonospora cubensis*. (a) Aerially dispersed, lemon-shaped, grey–purple sporangia land on the leaf surface and germinate in free moisture to form biflagellate zoospores. (b) Zoospores swim to and encyst in stomata, and then penetrate the leaf surface via a germ tube. (c) Hyphae colonize the mesophyll layer, establishing clavate-branched haustoria within plant cells. (d) The diurnal cycle triggers sporulation and up to six sporangiophores emerge through each stomate, bearing sporangia at their tips. Sporangia are dislodged from sporangiophores by changes in hydrostatic pressure and are picked up by wind currents that carry them to their next host. (e) Chlorotic, angular lesions bound by leaf veins are a symptom of *Ps. cubensis* infection visible on the adaxial leaf surface. On the lower leaf surface, sporulation is visible (inset). (f) The role of the sexual stage of *Ps. cubensis* is unknown.

are present. Sporangia may also germinate and cause infection at temperatures ranging from 5 to 28 °C (Cohen, 1981), but longer periods of leaf wetness are required (Cohen, 1977b; Rotem *et al*., 1978) under these conditions or when less inoculum is present (Cohen, 1981). The incubation period depends on temperature, photoperiod, inoculum concentration and leaf wetness duration, and can range from 4 to 12 days (Cohen, 1977b; Thomas, 1996).

Sporangia germinate via cytoplasmic cleavage, resulting in the release of 2–15 motile, biflagellate zoospores (Palti and Cohen, 1980), which preferentially swim to open stomata, where they encyst (Iwata, 1949) (Figs 2 and 3). Germ tubes form from encysted zoospores and produce appressoria. A penetration hypha develops from the appressorium and enters through the stomatal aperture into the leaf tissue. Hyaline coenocytic hyphae subsequently form and grow intercellularly through the mesophyll and palisade tissues. Clavate-branched haustoria are established within mesophyll cells where they invaginate the plant cell membrane (Fraymouth, 1956; Voglmayr *et al*., 2004) (Figs 2 and 3). These specialized structures are the site of nutrient uptake by the pathogen and the delivery of effector proteins that could potentially function to redirect host metabolism and suppress

defence responses (Hahn and Mendgen, 2001; Whisson *et al*., 2007).

PATHOGENICITY AND VIRULENCE

Pseudoperonospora cubensis has a broad host range, infecting over 49 species in 20 genera within the Cucurbitaceae, including 19 species in the genus *Cucumis* (Lebeda and Widrlechner, 2003; Palti and Cohen, 1980). Cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* Matsum. and Nakai) and squash (*Cucurbita* spp.) are the four major food crops that are hosts to *Ps. cubensis*. Other cucurbits infected by *Ps. cubensis* include loofa [*Luffa acutangula* (L.) Roxb.], bottle gourd [*Lagenaria siceraria* (Molina) Stand.], wax gourd [*Benincasa hispida* (Thunb.) Cogn.] and bitter melon (*Momordica charantia* L.) (Palti and Cohen, 1980).

Pseudoperonospora cubensis isolates show differences in virulence and pathogenicity depending on the cucurbit variety. To date, six physiological races have been identified in the USA, Israel and Japan, and additional evidence suggests that many more exist in Europe (Cohen *et al*., 2003; Lebeda and Gadasova, 2002; Sarris *et al*., 2009). Using *Citrullus*, *Cucumis* and *Cucurbita* spp., Thomas *et al*. (1987) identified five distinct physiological races of *Ps. cubensis*: 1 and 2 from Japan, 3 from Israel, and 4 and 5 from the USA. In 2003, Cohen *et al*. identified a sixth physiological race in Israel based on its pathogenicity to a wider range of susceptible cucurbits compared with race 3. All six physiological races that have been described are pathogenic on cucumber and muskmelon (*C. melo* var. *reticulatus*), but show differences in pathogenicity on watermelon, squash or pumpkin. Subsequently, Lebeda and Widrlechner (2003) developed a set of differential taxa that included 12 representatives from six genera, *Benincasa*, *Citrullus*, *Cucumis*, *Cucurbita*, *Lagenaria* and *Luffa*, which represent natural hosts of *Ps. cubensis*. Using this set of hosts, the authors evaluated the differences in pathogenicity of 22 additional isolates from the Czech Republic, Spain, France and the Netherlands, which were classified as 13 physiological races based on their virulence (Lebeda and Gadasova, 2002; Lebeda and Widrlechner, 2003). Collectively, these studies were the first to describe differences in the virulence and pathogenicity of *Ps. cubensis* in Europe in detail; however, the genetic basis for differences among physiological races has not been established.

Differences in effector content could be a potential explanation for differences among physiological races. Effector proteins have been shown to play roles as both virulence and avirulence determinants in other oomycetes (Hogenhout *et al*., 2009; Oliva *et al*., 2010; Schornack *et al*., 2009; Thines and Kamoun, 2010). Oomycete effector proteins contain the RXLR motif that is located downstream of the signal peptide, and are under diversifying selection at the C-terminal domain (Win *et al*., 2007). Preliminary sequence data of the *Ps. cubensis* genome have yielded 61 putative effector proteins (Savory *et al*., 2009; Tian *et al*., 2010). Of these, 32 were secreted proteins containing the RXLR motif, typical of previously identified oomycete effector proteins, whereas the remaining 29 had an R to Q substitution at the first amino acid (i.e. QXLR). A family of QXLR-containing effectors, *PcQNE* (*Ps. cubensis* QXLR nuclear localized effectors), has been shown to localize to the nucleus, and the C-terminal domain was under diversifying selection, as observed for RXLR effectors (Tian *et al*., 2010). Understanding the diversity and role of effector proteins is key to understanding the pathogenicity and genetic basis for virulence differences between isolates.

The pathogenic and genetic diversity of *Ps. cubensis* has been shown to vary temporally and geographically (Palti and Cohen, 1980; Thomas *et al*., 1987; Thomas and Jourdain, 1992). In a study by Lebeda and Urban (2007), *Ps. cubensis* isolates were collected over a 3-year period in the Czech Republic, and a general population shift from isolates with low pathogenicity to those with high pathogenicity was observed. This work also demonstrated that the variability of the population decreased from 33 different physiological races in 2001 to only 13 physiological races in 2003 (Lebeda and Urban, 2007). An increased representation of a highly pathogenic physiological race, capable of causing infection on all cucurbits studied, was also observed over the course of the experiments, indicating a population shift to high pathogenicity isolates (Lebeda and Urban, 2007). Recently, using a combination of amplified fragment length polymorphism (AFLP) and ITS sequence analyses, molecular polymorphisms were identified among populations of *Ps. cubensis* from Greece, the Czech Republic, the Netherlands and France (Sarris *et al*., 2009). Although there was no clear grouping of isolates based on their pathogenicity, AFLP analysis indicated genetic differentiation between the Greek isolates and those from central and western Europe (Sarris *et al*., 2009). The work by Sarris *et al*. (2009) suggested that the clustering of *Ps. cubensis* isolates from Greece corresponded to their geographical distribution rather than their pathogenicity or virulence on cucurbit hosts. Nevertheless, this study was supported by SNP evidence from one single locus and AFLP evidence with error rates of 1–4% as a result of band scoring. A similar study with information from several loci, a reliable polymorphism scoring system and a more extensive sampling of regions of interest is needed to support or disprove the proposals of Sarris *et al*. (2009).

DISEASE MANAGEMENT

Pseudoperonospora cubensis outbreaks over the past several decades have been responsible for annual yield losses of up to 80% and, as a result, cucurbit downy mildew is currently the most destructive disease of cucumbers for both field and glass-

house production in Europe (Lebeda and Schwinn, 1994; Lebeda and Urban, 2007). Before 1984, downy mildew was not a major issue for Central (Lebeda, 1986; Lebeda and Schwinn, 1994) and Northern (Forsberg, 1986; Tahvonen, 1985) Europe. However, from approximately 1985 onwards, epidemics of downy mildew have been a challenge for cucurbit production in Europe (Lebeda and Schwinn, 1994). In Europe, it has been suggested that tolerant cultivars should be used in conjunction with fungicide applications when conditions are favourable for downy mildew (Chaban *et al*., 2000; Urban and Lebeda, 2006). In the USA, host resistance introduced in the 1950s was effective in limiting losses caused by downy mildew without the use of fungicides. A resurgence of the disease was observed in many states along the Eastern Seaboard and the upper Midwest in 2004 and 2005, respectively (Holmes and Thomas, 2006). This loss in resistance has led to the management of downy mildew through a spray programme, with recommended fungicides applied every 5–7 days on cucumbers (Gevens and Hausbeck, 2006; Hausbeck and Cortright, 2009) and every 7–10 days on other cucurbits (Hausbeck, 2009; M. K. Hausbeck, unpublished data).

An aggressive spray programme is essential, as plants must have a protective barrier of fungicide prior to sporangium deposition to avoid yield losses. However, additional fungicide applications to control downy mildew greatly increase the cost of production. In Michigan, for example, major outbreaks of the disease have been observed since 2005, and the cost of additional fungicide sprays is over 6 million dollars annually (M. K. Hausbeck, unpublished data). As fungicide applications are expensive, downy mildew sporangia trapping and forecasts can be useful tools to alert growers to when airborne sporangia are present or likely to be present, so that they can make an informed decision about when to initiate fungicide applications (Holmes *et al*., 2004). Delaying the initiation of a fungicide spray programme may reduce the management costs for growers and the amount of fungicides in the environment.

The efficacy of chemical control measures may be diminished if *Ps. cubensis* populations develop resistance to key fungicides; *Ps. cubensis* was the first oomycete with documented resistance to metalaxyl and reduced sensitivity to mancozeb (Reuveni *et al*., 1980; Thomas and Jourdain, 1992). In addition, populations of *Ps. cubensis* resistant to strobulurin fungicides have been described (Heaney *et al*., 2000). In the Eastern US field and glasshouse fungicide trials, products containing mefenoxam and strobilurins as the active ingredients failed to provide adequate downy mildew disease control, indicating that resistance is widespread in this region (Colucci and Holmes, 2007; Gevens and Hausbeck, 2006; Hausbeck and Cortright, 2009; Keinath *et al*., 2008). As *Ps. cubensis* has a high potential to develop resistance to fungicides (Russell, 2004), it is important that populations are carefully monitored for resistance to currently registered products and that new active ingredients are tested.

Although fungicide applications are currently necessary for adequate disease control (Gisi, 2002), resistant varieties and cultural techniques are important components of a management strategy. The full genetic parameters controlling resistance to downy mildew in cucumber are unknown. The original source of host resistance (i.e. the recessive *dm1* gene) was identified in cucumber accession PI 197087 and first described in India in 1954 (Barnes and Epps, 1954). The resistance response governed by *dm1* is characterized by sparse pathogen sporulation, small necrotic lesions, tissue browning and rapid cell death, indicative of the classical hypersensitive response (HR)-type resistance. Since the 1950s, resistance conferred by *dm1* has been widely used in commercial cultivars for cucumber production in the USA, and was sufficient to prevent losses caused by downy mildew until 2004 (Holmes and Thomas, 2006). Cultivars containing the *dm1* gene still show some level of resistance; unfortunately, the high level of resistance once observed has now been lost. In addition, susceptible cultivars without the *dm1* gene become infected earlier in the season, and exhibit more severe damage than was observed previously (Holmes *et al*., 2004).

Although *dm1* remains useful in a disease management programme, a robust source of resistance is highly desirable. Current breeding research for resistance to downy mildew in cucumber is focused on the identification of resistant germplasm(s) and cultivars via large-scale screening trials (Shetty *et al*., 2002; Wehner and Shetty, 1997). Tolerant and high-yielding germplasm has been identified in these studies, but a source of complete resistance to downy mildew in cucumber has been elusive, probably because of limited genetic diversity for *Ps. cubensis* resistance in cucumber (Lebeda and Widrlechner, 2003; Shetty *et al*., 2002). Other *Cucumis* spp., such as melon, may be more relevant for the identification of effective sources of resistance (Lebeda and Widrlechner, 2003). The wild melon line PI 124111F [PI], for example, has been shown to be resistant to the six physiological races of *Ps. cubensis* via two constitutively expressed glyoxylate aminotransferase-encoding genes, *At1* and *At2* (Taler *et al*., 2004). These two genes are known as enzymatic resistance (eR) genes which, when expressed at high levels in either wild-type or transgenic plants, confer complete resistance to infection by *Ps. cubensis*.

FUTURE PROSPECTS

The re-emergence of cucurbit downy mildew in the USA and its persistence across much of Europe and Asia represent a significant threat to cucurbit production worldwide. Although the disease was successfully managed for decades within the USA using host resistance (i.e. the *dm1* locus), severe epidemics have

occurred since 2004. Whether this is a result of a change in pathogen populations or a change in the environment is currently unknown. To this end, we need to investigate the changes in pathogen populations, environmental factors and how the pathogen–environment interaction affects the host–pathogen interaction and disease development.

Studies have investigated some aspects of the basic biology of *Ps. cubensis* and its interactions with various cucurbit hosts. However, additional research is needed to further clarify the taxonomy, variations in virulence and pathogenicity among physiological races, and the pathogenicity determinants of the pathogen. A better understanding of each of these components will ultimately facilitate the development of durable host resistance. To this end, the forthcoming genome sequence will provide molecular tools for gene discovery and the development of molecular markers, which may then be used to investigate the population and evolutionary biology of the pathogen. Such studies will yield information about possible migration events or evolutionary changes within pre-existing US pathogen populations that have resulted in strains with increased virulence.

An integrated research approach that includes all factors affecting disease development (pathogen, host and environment) is essential to control and predict future cucurbit downy mildew epidemics. First, it would benefit growers if new fungicides that are more economical and provide effective control were identified. Second, screening of breeding lines and wild germplasm will help to identify durable sources of genetic resistance that would be preferable to an aggressive spray programme. Finally, studies to determine the effects of environment on inocula and disease development will serve as a first step in the development of rapid and highly specific forecasting systems. In summary, research efforts contributing to the development of sustainable management strategies, such as durable host resistance, are a priority to ensure the long-term viability of the cucurbit production industry.

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