Eur J Plant Pathol (2011) 129:157–192
DOI 10.1007/s10658-010-9658-1

Cucurbit downy mildew (*Pseudoperonospora cubensis*)—biology, ecology, epidemiology, host-pathogen interaction and control

Aleš Lebeda · Yigal Cohen

Accepted: 5 July 2010 / Published online: 25 July 2010

Abstract Cucurbit downy mildew, caused by the oomycete *Pseudoperonospora cubensis*, is a devastating, worldwide-distributed disease of cucurbit crops in the open field and under cover. This review provides recent data on the taxonomy, biology, ecology, host range, geographic distribution and epidemiology of *P. cubensis*. Special attention is given to host-pathogen interactions between *P. cubensis* and its economically-important cucurbit hosts (*Cucumis sativus, C. melo, Cucurbita pepo, C. maxima,* and *Citrullus lanatus*); pathogenic variability in *P. cubensis* at the species, genus, and population levels; and, differentiation of pathotypes and races. Genetics and variability of host resistance and cellular and molecular aspects of such resistance are considered. A focus is given to methods of crop protection, including prevention and agro-technical aspects, breeding for resistance—classical and transgenic approaches, chemical control and fungicide resistance. Novel technologies in biological and integrated control are also discussed. This review also summarizes the most important topics for future research and international collaboration.

Keywords Biological control · Breeding for resistance · Classification · Chemical control · Cisgenic plants · Epidemiology · Fungicide resistance · Genetics of resistance · Geographic distribution · Host range · Disease cycle · Integrated pest management · Life cycle · Migration · Overwintering · Pathotypes · Races · Symptomatology · Systematics · Transgenic plants

A. Lebeda (✉)
Department of Botany, Faculty of Science,
Palacký University in Olomouc,
Šlechtitelů 11,
CZ-783 71 Olomouc, Czech Republic
e-mail: ales.lebeda@upol.cz

Y. Cohen
Bar Ilan University, Faculty of Life Sciences,
52100 Ramat Gan, Israel
e-mail: ycohen@mail.biu.ac.il

Introduction

Pseudoperonospora cubensis (causal agent of cucurbit downy mildew) is one of the most economically important and widespread plant pathogens. It is a biotrophic plant parasite belonging to the kingdom Chromista, class Peronosporomycetes (Thomas 1996; Dick 2001a; Göker et al. 2007; Voglmayr 2008). Although it has been studied by mycologists and plant pathologists for more than 100 years, there is still a lack of information about this pathogen in many specific areas (Lebeda 1990, 1999; Lebeda and Schwinn 1994). Together with *Bremia lactucae* (Lebeda et al. 2002, 2008a, b; Michelmore and Wong 2008) and *Hyaloperonospora parasitica* (Slusarenko and Schlaich 2003; Holub 2008) it is one of the most studied Peronosporomycete biotrophic parasites of plants. The

last comprehensive and internationally-available reviews on this pathogen were published thirty years ago (Palti and Cohen 1980; Cohen 1981). In the present review we discuss a wide range of specific issues related to *P. cubensis* including the biology, diversity, ecology, distribution, host range, epidemiology, host-pathogen interactions, genetics of resistance, breeding and control. *P. cubensis* is used as a "case study" to demonstrate the diverse, complex interactions of downy mildews with their host plants.

Parts of this review were published before in Czech (Lebeda 1990; Lebeda et al. 2006a; Lebeda and Urban 2005). The current review was broadened and supplemented with the literature published in the last twenty years. This literature mainly considers the topics of variability of host-pathogen interactions, variation of pathogenicity, resistance of host plants, breeding for resistance and pathogen control, focusing mainly on efficacy of and resistance to fungicides. The aims of this paper are to summarize the current information on *P. cubensis*, critically discuss the areas in which we still lack basic data, and introduce them into the wider context of biotrophic parasitism within the downy mildews.

Taxonomy

History of nomenclature

Pseudoperonospora cubensis (Berk. et Curt.) Rostov. was first described by Berkeley in 1868 in herbarium plant material originated from Cuba, and hence its species name *cubensis* (Skalický 1961). It was also identified as a new genus, *Pseudoperonospora* Berkeley, together with the description of the species *Pseudoperonospora cubensis*. *P. cubensis*, therefore, served as the type species of the *Pseudoperonospora* genus (Dick 2001b, 2002b). The pathogen was first observed and described on live plants by Rostovzev in The Botanical Gardens of Moscow (Russia) in 1903 (Skalický 1961).

During the last century some misleading synonyms were used to describe *P. cubensis*, such as *Peronospora cubensis* Berk. et Curt., *Peronospora cubensis* Berk. et Curt. var. *atra* Zimmerm., *Plasmopara cubensis* (Berk. et Curt.) Humphrey, and *Peronoplasmopara cubensis* (Berk. et Curt.) Clinton (Dick 2001b).

Recent systematic classification

According to recent taxonomic classification, *Pseudoperonospora cubensis* belongs to kingdom Chromista, subdivision Peronosporomycotina, class Peronosporomycetes (originally described as Oomycetes), order Peronosporales (downy mildews), family Peronosporaceae (Göker et al. 2007; Voglmayr 2008). Peronosporomycetes is comprised of ca 900 (perhaps up to 1500) species, 75 genera and 19 families (Dick 2001a, c), and new genera were recently described (Göker et al. 2007; Voglmayr 2008).

The genus *Pseudoperonospora* belongs to a taxonomic group on the border between genera that regularly produce zoospores (*Pythium* spp.) and genera that never produce zoospores (*Peronospora* spp., *Albugo* spp.) (Göker et al. 2007). *P. cubensis* does produce zoospores but its sporophores resemble those of *Peronospora* spp. It is a species with highly distinct host specificity attacking above-ground parts, mainly leaves, of only the Cucurbitaceae. The formation of zoospores in this species depends on environmental conditions. It occurs in water only and is inhibited at high temperatures. The whole zoosporangium content is cleaved and biflagellate zoospores, 10–13 μm in diameter, are released (Thomas 1996). However, there are also reports on direct germination by germ tubes (Lange et al. 1989a, c). Sporangia easily dislodge from the sporangiophore and are distributed by air or rain splash (similar to other *Pseudoperonospora* spp.) (Lange et al. 1989c). *Pseudoperonospora* spp. has dichotomously-branched sporangiophores with terminal growth; the sporangia of similar age are present at the ends of sterigma (Choi et al. 2005; Voglmayr 2003). The family Peronosporaceae includes at least 17 genera (Göker et al. 2007; Voglmayr 2008). Probably the most widespread genera in Europe and North America are *Bremia, Peronospora, Hyaloperonospora* and *Plasmopara* of which representatives cause serious diseases of cultivated plants (Lebeda and Schwinn 1994; Voglmayr 2008). The individual genera are mainly characterized by the shape and branching of the sporophore/sporangiophores and the ability to discharge zoospores. From this point of view, *Pseudoperonospora* represents a transitional type between *Plasmopara* and *Peronospora* (Dick 2001b, 2002a, b; Voglmayr 2003; Choi et al. 2005; Göker et al. 2007). According to Riethmüller et al. (2002) the genus *Pseudoperonospora* is monophyletic, however, it has

close relationship with *Peronospora*, as they share similar haustoria, conidiosporangiophore morphology, and conidiosporangium colour. Recently, several haustorial types (e.g. clavate-branched, ellipsoid-pyriform, hyphal) were recognized for downy mildews, including *Pseudoperonospora* (clavate-branched), which are considered an important diagnostic feature (Voglmayr et al. 2004).

Based on the variability of the genome size, Voglmayr and Greilhuber (1998) stated that the phylogenetic position, and the definition of individual genera, has not yet been clearly resolved. Recent molecular-phylogenetic studies done with Peronosporales and Peronosporaceae (Göker et al. 2003, 2007; Riethmüller et al. 2002; Voglmayr 2008) demonstrated that the genus *Pseudoperonospora* is a unique monophyletic group. This supported the previous opinion of taxonomists (Constantinescu 2000; Waterhouse and Brothers 1981), and disagreed with Skalický's (1966) concept on the division of the genus. Based on sporangial ultrastructure and the phenetic characters shared by the species of the genus, Constantinescu (2000) suggested that a distinct genus for *Pseudoperonospora* is justified (Riethmüller et al. 2002). Indeed, comparative morphological and molecular (ITS rDNA) studies of *P. cubensis* and *P. humuli* showed that the genus *Pseudoperonospora* is a distinct taxonomical unit. However, recent studies also demonstrated that both species are very similar (Choi et al. 2005; Gent et al. 2009; Sarris et al. 2009), and *P. humuli* was suggested as a synonym of *P. cubensis* (Choi et al. 2005). Previous (Choi et al. 2005) and recent (Sarris et al. 2009) studies of ITS rDNA showed only a limited intraspecific variability of *P. cubensis*. Nevertheless, the limited ITS rDNA intraspecific variability stands in contrast with the very broad pathogenic variability of *P. cubensis* (Lebeda and Widrlechner 2003; Lebeda et al. 2006b). Recently, the mitochondrial cytochrome oxidase (COX) gene cluster, and two nuclear loci, ITS, NADH gene regions and ß-tubulin, were sequenced. Conserved single nucleotide polymorphisms (SNPs) were found that consistently differentiate *P. cubensis* and *P. humuli* (Mitchell et al. 2009). Host range and pathogenicity studies demonstrated that these species have distinct pathogenic capabilities (Gent et al. 2009), *P. cubensis* may infect hop, but caused very little disease, whereas *P. humuli* never infected cucurbits (Mitchell et al. 2009). Current studies are focused on identifying a suite of effector proteins from *P. cubensis*, and characterizing them based on *in planta* localization, putative function, and in part, contribution to overall pathogen virulence (Day and Hausbeck 2009).

All these results unambiguously showed that a detailed understanding of inter and intraspecific variability of *Pseudoperonospora* spp. must be based on more complex experimental approach (e.g. Gent et al. 2009). From a purely phytopathological viewpoint, pathogenicity is a much more significant taxonomic criterion as it enables specification of intraspecific pathogenic variants (pathotypes and races) of *P. cubensis* (Lebeda et al. 2006b).

Biology

Symptoms of infection

P. cubensis is a leaf pathogen, attacking exclusively the leaves of cucurbitaceous plants (Cohen 1981; Thomas 1996). However, the formation of sporangiophores was observed also on stems, leaf petioles, tendrils and peduncles of heavily infected melons. On cucumber, fruit watery spots were recorded followed by pathogen sporulation (Palti and Cohen 1980). Host plants may be infected at all developmental stages (seedlings, young and adult plants) but symptoms on young, newly developing leaves are rather rare (Lebeda 1990). However, cotyledons are actually more susceptible than true leaves. Symptoms differ markedly among cucurbit species (Fig. 1). In some species (cucumber, *Luffa*) *P. cubensis* causes irregular, localized, yellow lesions, restricted by leaf veins whereas in cantaloupe and watermelon, lesions are not restricted by leaf veins and are more circular and irregular. Unlike some other members of the Peronosporaceae (Lebeda and Schwinn 1994), *P. cubensis* does not produce systemic infection of the whole plant (Cohen 1981).

The incubation period, from penetration until visible external symptoms, is 4–12 days under field conditions, depending on the environmental conditions and inoculum load (Cohen 1977), and resistance/susceptibility of the host plant (Lebeda and Widrlechner 2003). The first symptoms of *P. cubensis* infection in Cucurbitaceae are pale-yellow, oily lesions on the upper side of the leaf, sometimes restricted by leaf veins which are described as angular leaf spots (Lebeda 1986b). The size of primary lesions

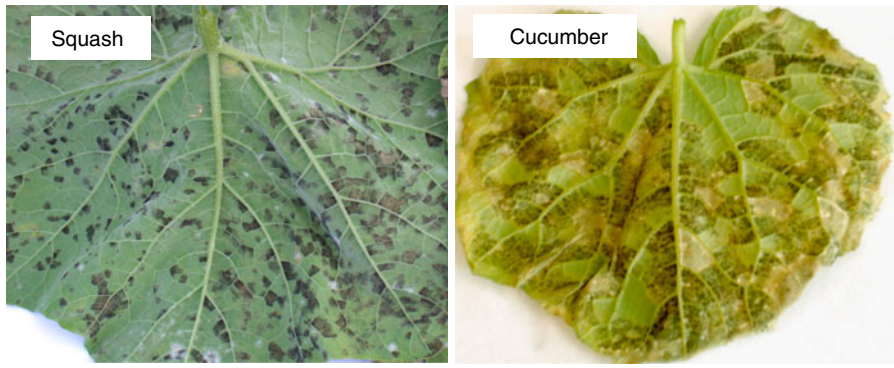

Fig. 1 Symptoms of *Pseudoperonospora cubesis* in leaves of cucurbits taken from the field. Note that the dark sporulating areas in squash are smaller (5–8 mm) than those in cucumber (5–20 mm)

varies from 3–10 mm. During the development of the disease, lesions coalesce and form larger lesions, and may eventually cover the entire leaf. Artificially-inoculated plants or leaf discs may show irregular, chlorotic or even necrotic lesions, especially when inoculum concentration is high and/or the host is highly susceptible (Lebeda 1986b, 1990).

During the reproductive phase of disease development, a thin layer of dark brown, grey or violet-black sporangiophores bearing sporangia appear on the lower (abaxial) surface of the leaves. Under extremely heavy infection, leaves become necrotic followed by death of the whole plant (within 4 to 10 days from first symptoms, depending on weather conditions, inoculum concentration, and host genotype) (Lebeda, 1990). Heavy infection may significantly reduce yield quantity and quality (Lebeda and Urban 2004b).

The symptoms of the disease can be quite variable not only among different species of cucurbits, but also genotypes (cultivars) of the same host species. They could be also influenced by weather conditions, e.g., atypical water-soaked lesions may be seen under extremely humid conditions on some host species or genotypes (Lebeda 1986b, 1990). Genetically-resistant cultivars of melon inoculated in growth chambers produce water-soaked lesions of 1-2 mm in diameter (Thomas et al. 1987; Thomas 1996). Such cultivars produce no symptoms under epiphytothic field conditions.

Parasitism

P. cubensis (like all other members of the family Peronosporaceae (Göker et al. 2007)) is an obligate biotrophic parasite, absolutely dependent on its host plant for growth and survival. It cannot survive outside its host except as oospores. It attacks living host tissues in order to survive and reproduce (Palti and Cohen 1980). Necrosis of the affected plant tissue will lead to death of *P. cubensis*. Like other plant pathogenic Peronosporaceae, *P. cubensis* does not produce toxins (Švábová and Lebeda 2005); it releases, to a limited extent, the enzymes necessary for the primary penetration of the cell wall (Lebeda et al. 2001a). At the initial stage of development, *P. cubensis* can temporarily support the growth of host cells and increase their number of organelles (Lebeda and Schwinn 1994).

An early necrotic reaction of the infected plant tissue (hypersensitive reaction) is typical for resistant hosts (Cohen et al. 1989) whereas in susceptible hosts necrosis appears as a late reaction of the infected tissue. The obligate biotrophic nature of *P. cubensis* is also characterized by the fact that it cannot be cultivated on artificial nutritive media (Cohen and Eyal 1977; Lebeda 1986b).

Life and disease cycle

Asexual reproduction

The primary and the main infective unit is the asexual spore (conidiosporangium, zoosporangium; Fig. 2). Sporangia are ovoid or elliptic in shape, and measure 15 to 25×20 to 35 μm (Skalický 1961). At maturity sporangia are light-grey to deep-purple in colour (Thomas 1996). They easily dislodge from the sporangiophores and are distributed by wind or water splash. After deposition on the leaf surface of a host plant, sporangia require contact with water (e.g., rain or dew) in order to germinate. Germination is indirect: the multinucleate protoplast differentiates into 5 to 15 biflagellate zoospores measuring 8–12 μm, that

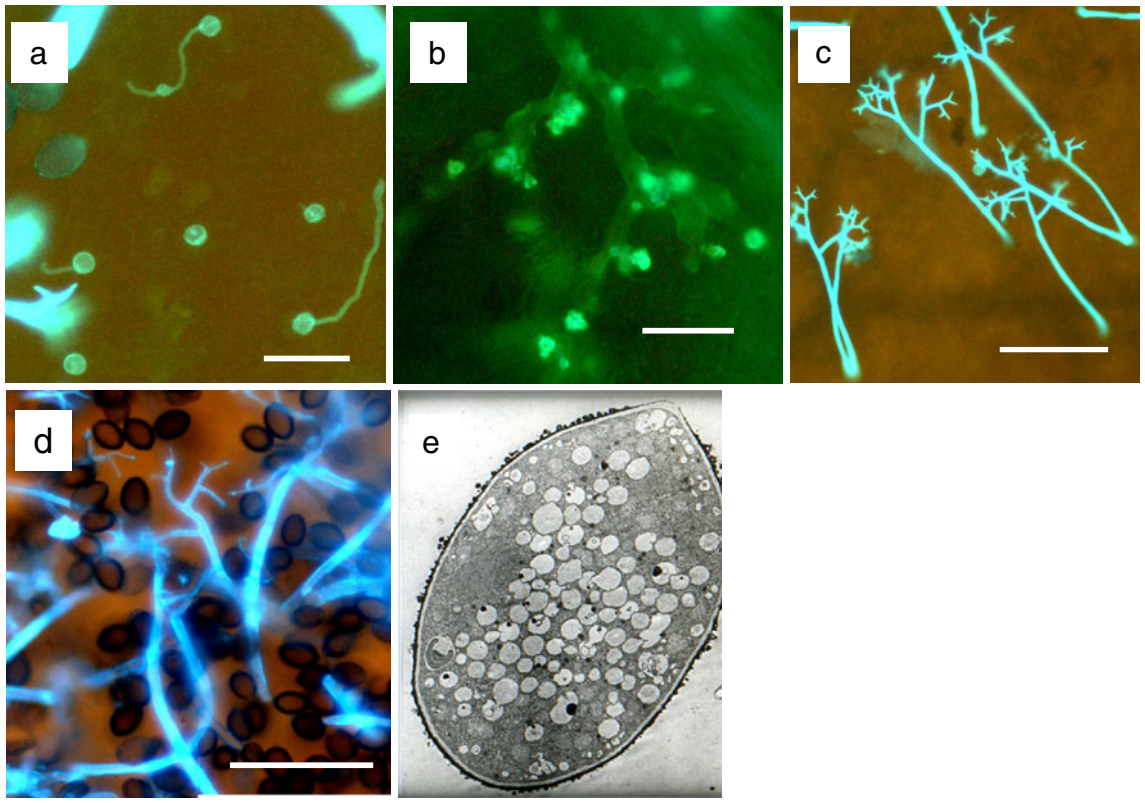

Fig. 2 Developmental stages in the pathogenesis of *Pseudoperonospora cubensis* in cucumber. **a** germinating cystospores on leaf surface. Bar=25 μm. **b** intercellular mycelium with haustoria. The lighter spots are the callose collars surrounding the haustorial neck. Bar=25 μm. **c** sporangiophores emerging from stomata. Bar=100 μm. **d** sporangiophores (blue) and sporangia (dark brown) on leaf surface. Bar=100 μm **e** ultrasructure of a sporangium. ×2000

emerge through a papilum (Palti and Cohen 1980). The zoospores actively swim in the direction of stomatal apertures where they settle, lose their flagella and encyst (Cohen 1981). A germ tube subsequently grows from the cyst (Fig. 2), produces an appressorium from which a penetration hypha develops and penetrates into the stomatal aperture to the substomatal cavity of the leaf tissue. The penetration via stomata is the most frequent mechanism of penetration of *P. cubensis* (Cohen 1981). Rarely, a direct (epidermal) penetration occurs (Lebeda 1990). In *Bremia lactucae*, 95% of penetrations occur directly *via* the epidermis (Lebeda and Reinink 1991). In *P. humuli*, the zoospores swim towards the stomatal apertures by thigmotropism, settle, and produce a germ-tube that penetrates into the stoma (Royle and Kremheller 1981).

Under suitable environmental conditions and in a susceptible host, the colonization of the parasite in tissue proceeds relatively quickly and sporangiophores emerge from stomata within 5 to 7 days (Fig. 2), mainly on the lower side of the leaves where stomata are more frequent (Cohen 1981). On susceptible hosts, a new infection cycle takes place once in 7 to 14 days, depending on the environmental conditions. *P. cubensis* is polycyclic with regards to its disease cycle (Kranz 2003).

Sexual reproduction

Sexual reproduction is rare, and so far has not been proven in most countries where *P. cubensis* prevails. Sexual reproduction of *P. cubensis,* as in other species of the *Peronosporaceae*, proceeds via the production of oospores (Michelmore 1981). It occurs at the end of the season when the infected tissues become necrotic (Bedlan 1989; Lebeda 1990). In Europe, the only unambiguously observed occurrence of oospores came from Austria (Bedlan 1989). The other records of oospore occurrence came from Israel (Cohen et al. 2003), India (Mahrisi and Siradhana 1984; Singh and Sokhi 1989), Iran (Zaker and Ommati 1991) and China

(Zhang et al. 2006). The attempts to find oospores in cucurbit plants in the Czech Republic, including the possibility to produce oospores experimentally, were unsuccessful (Lebeda and Urban 2004a; Lebeda unpubl. data). Similar attempts were made in the USA without success (Kanetis and Holmes, unpubl. data). It is, therefore, unclear whether this pathogen survives in Central Europe or USA by oospores (Lebeda 1986a; Lebeda and Schwinn 1994; Lebeda and Urban 2004a).

Ecology and epidemiology

Environmental and ecological conditions have great impact on progress of the disease cycle, pathogenic processes, symptom expression and epidemiology (Cohen 1981). Current advances on comparative ecology and epidemiology of zoosporic plant pathogens were summarized by Jeger and Pautasso (2008). We focus here on the most important ecological factors that influence the epidemiology of *P. cubensis*.

Sources of inoculum

In some oomycetes e.g., *Bremia lactucae, Sclerospora sorghi* and *Phytophthora infestans* (in some regions) the pathogen emerges in the new season from oospores harboring in plant debris in soil. Others might emerge from oospores carried by seeds, e.g., *P. infestans* in tomato seeds and *Plasmopara halstedii* in sunflower seeds. In *P. infestans*, inoculum often emerges from mycelia harboring in potato tubers or tomato seeds. In *P. cubensis* none of these mechanisms is known. The pathogen produces oospores very rarely; mycelia, sporangia or zoospores do not occur in seeds or fruits, nor survive in soil or in plant debris. Therefore, an overwintering mechanism is unknown. The survival of *P. cubensis* on wild cucurbits is discussed below. In Israel, downy mildew occurs every year since records have been taken (about 70 years), but the source of initial inoculum was never recorded. Nowadays, the fact that cucurbits are grown year around in the open field, net-houses or greenhouses, assures that inoculum will occur year around.

Factors influencing early stages of infection

The lifespan of sporangia is very short. Normally, it does not exceed 48 h and in many cases not more than several hours after the dislodge of the sporangium from the sporagiophore (Cohen and Rotem 1971a). During this short period, the sporangia must land on the leaf surface of a susceptible host and germinate. Dispersed sporangia laying on cucumber plants lose infectivity as temperature rises. However, infectivity was better retained when plants were incubated at low RH (5–28%) than at high RH (84–90%) (Cohen and Rotem 1971a). The presense of free water on the leaves is essential for germination and for the formation of primary infectious structures. The minimal wetting period required for germination and penetration is approximately 2 h (Cohen 1981). Germination occurs in different frequencies on susceptible and resistant host genotypes, as well as on nonhost plants (Cohen 1976). The optimum temperature for germination is 10–20°C (Cohen 1977).

A short drying period (ca 10–15 min) applied to sporangia during germination leads to disruption of integrity of the internal mitochondrial membranes of sporangia, thus, preventing the formation of zoospores and hence cessation of the entire infection process (Cohen 1977). Six hours of dew period are considered optimal for infection. During this period the pathogen completes its penetration into the stomata and becomes independent of the presence of free water on the leaf surface (Cohen 1981). *P. cubensis* can also use guttation droplets for its germination. This event appears later as symptoms developing on the periphery of leaf lamina blades, especially in greenhouses. Production of sporangia can occur at temperatures from 5 to 30°C, with optimum production at 15–20°C (Cohen et al. 1971).

The release of zoospores from the zoosporangium does not occur under anaerobic conditions or in the presence of respiration inhibitors. This process is temperature-dependent within the range of 9 to 30°C. Zoospores may persist in a water environment for 18 h at low temperatures, but they immediately encyst at higher temperatures. Optimal temperature for cyst germination is 25°C (Cohen 1981). In *P. humuli* on hop, zoospores swim towards the stomatal openings, land on stomatal apertures, encyst, and produce a germ-tube which grows into the meshophyll (Royle and Kremheller 1981). The first penetrations are observable approximately 5 h after the adhesion of the sporangia to the leaf surface (Lebeda 1990). Light is a factor that may support the development of infection even in a short dew period (Cohen et al.

1971). The initial stages of the disease cycle of *P. cubensis* (from the release of zoospores to the formation of the first hyphae) take place in both susceptible and resistant hosts (Cohen 1981). In resistant hosts, the growth stops after the formation of the first haustorium. The formation of haustoria was not observed in non-hosts (Cohen 1981), neither in a resistant melon Cohen et al. 1989). This fact does not correspond to the observations made with other downy mildews (e.g., *Lactuca–Bremia lactucae*) in which haustoria were seen also in nonhost plants (Lebeda et al. 2001b, 2002, 2008b). Ultrastructure of *P. cubensis* in leaves of *C. melo* is summarized in Fig. 3.

Factors influencing colonisation and symptom development

During the incubation period, while the colonization of host tissue is in progress, mycelia of *P. cubensis* grow in intercellular spaces and haustoria develop inside the mesophyll cells (Lange et al. 1989b). Under natural (field) conditions, the incubation period lasts for 4–12 days, depending on climatic conditions and host genotype (Lebeda 1986b). At the initial phase of the infectious process a temperature regime of 25–30° C during the day and 10–15°C during the night, is favourable (Palti and Cohen 1980). Better illumination in the incubation period supports the development of hypha and haustoria in the tissue leading to the formation of larger lesions on leaves. Low light intensity leads to the reduction in number and size of lesions due to the weak development of hypha and haustoria (Cohen 1981). The incubation period (the period lapsed between inoculation and the appearance of first symptoms) is significantly influenced by inoculum concentration. The first symptoms may appear 3 to 4 days after inoculation at high inoculum concentration (ca 1000 sporangia/cm^2 leaf), as compared to seven or more days at low doses (ca 10 sporangia/cm^2 per leaf) (Cohen and Eyal 1977).

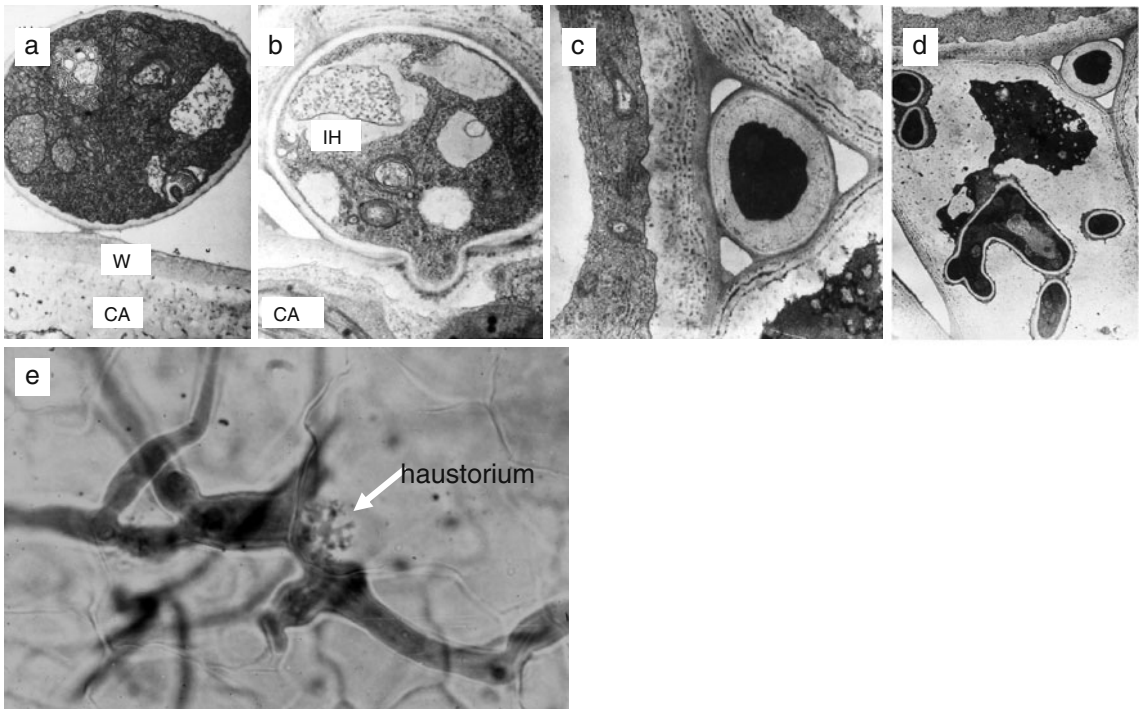

Fig. 3 Ultrastructure of *Pseudoperonosapora cubensis* in leaves of *Cucumis melo* PI124111F (**a**–**d**) and bright field microscopy in Ananas Yokneam /AY/ (**e**) (modified from Cohen et al. 1989). **a** Interface between hypha and host cell at 20 h post inoculation (hpi). Note heavy callose in the host cell (×13,500); W—host cell wall, CA—callose like material. **b** Attempted penetration, 96 hpi (×8250); IH—intercellular hypha. **c** Intercellular hypha surrounded by three host cells, 96 hpi. Note—heavy callose in both the host and pathogen (×20,250); **d** Necrotic lobbed haustorium in a necrotic host cell heavily embedded with callose, 96 hpi (×6175); **e** Hyphae and haustorium in the susceptible AY, 96 hpi, aniline blue staining (×160)

The rate of tissue colonization and symptom development are significantly influenced by temperature. Lower temperatures, although allowing colonization, delay symptom development while higher temperatures enhance symptom development, speed up the progress of lesions from chlorotic to necrotic and terminate the development of the pathogen earlier (Cohen 1977). The sporulation potential of necrotic lesions is very low and the vitality of the formed spores decreases quickly (Lebeda 1990). Hot and dry spells in the field enhance the necrotization of the lesions and terminate the survival of *P. cubensis* in the leaves, thus bringing to an end the development of the disease and its spread (Cohen 1981).

Factors influencing sporulation

Asexual sporulation is a process in which sporangiophores emerge from stomatal openings, ramify dichotomously, and produce sporangia on sterigmata. In a conducive environment, the whole process lasts ca 6 h (Cohen 1977). Sporangiophores emerge from stomatal openings only when relative humidity is 90% or above, regardless of whether the leaves are incubated in light or darkness. In contrast, differentiation of sporangia on sterigmata takes place in darkness only. Infected cotyledon leaves placed on wet filter paper in Petri dishes under continuous light conditions produce abundant white sporangiophores (Cohen 1977). Sporulation occurs on lesions of certain physiological age only. Normally it will happen on chlorotic lesions but not necrotic ones. The lesions should contain enough photosynthetic carbohydrates to support the process, as sporangiophores and sporangia are rich in glucans (Perl et al. 1972). Photosynthate accumulation occurs when plants are exposed to a continous strong light, preferably blue or red, which enhances photosynthesis. The glucan polymers accumulated in the leaf degrade during darkness to hexoses (a monosaccharide with six carbon atoms) which are consumed by the mycelia of *P. cubensis* for the production of sporangia (Ibid). Hexose formation occurs faster at higher temperature and is not dependent on relative humidity. Therefore, the first part of the dew period required for sporulation may be replaced by a dry dark period without affecting sporulation (Cohen 1977). Under optimal environmental conditions during incubation (temperature and humidity as mentioned above), the sporulation takes place as early as 4 to 5 days after inoculation (Palti and Cohen 1980; Lebeda 1986b). The quality and intensity of light significantly influence the formation of sporangia. Maximal production of sporangia occurs at night and is greatly inhibited by light. It was found experimentally that blue light is most inhibitory to sporulation whereas green and red light had a smaller influence. The inhibitory effect of light on the formation of spores is strongly dependent on temperature (increased inhibition with higher temperature) (Cohen and Eyal 1977). Light is desirable for *P. cubensis* before spore formation starts; then practically every factor stimulating photosynthesis of the host enhances spore production in the following dark period (e.g. long light period, more intensive illumination, adequete light spectrum, higher temperature) (Cohen 1981). Cohen et al. (1971) suggested that at least 6 h of darkness period is required for the formation of sporangia because this is the period needed for conversion of accumulated assimilates into compounds required by the pathogen for sporangial formation.

Experiments with *C. melo* showed that plant nutrition also plays an important role in disease development (Bains and Jhooty 1978; Mahrisi and Siradhana 1988). In general, it was shown that unbalanced treatments by N, P and K reduced *P. cubensis* infection. However, balanced treatments with the most suitable level of nutrients for *C. melo* development and fruit yield were also the best for disease development.

A continual layer of free water on the leaves (water film) allows the growth of sporangiophores (Cohen 1981). Moreover, the formation of sporangiophores is induced by high humidity and is often related to dew on the leaves. An increase of temperature and humidity causes rapid maturation and intensive liberation of sporangia (Cohen et al. 1971; Cohen and Eyal 1977).

The age of the infected leaf and the host (variety, genotype) also affect symptom development and sporulation intensity. Symptoms emerge earlier on young leaves (first to third true leaf), develop faster and sporulation potential is higher compared to older leaves. Under optimal conditions, the number of sporangia in susceptible varieties of *Citrullus lanatus* reaches $4\times10^3/cm^2$, in *Cucumis sativus* $7\times10^4/cm^2$ and in *Cucumis melo* up to $1\times10^5/cm^2$ of leaf area (Cohen 1981). The density of sporangiophores is usually higher on small-area lesions then on large-area lesions. This fact can be explained by better

supply of nutrients from the surrounding healthy tissue. Sporulation is much more intense on chlorotic lesions than in necrotic lesions. Physical and biotic factors causing necrosis simultaneously limit sporulation. For example, at lower temperatures, sporulation occurs later but lasts longer (e.g. lesions can be fertile for at least 16 days) (Cohen 1977; Cohen and Eyal 1977).

Factors influencing dispersion

Low humidity and dry leaf surface are optimal for the dispersion of sporangia. Temperature and light have very low influence on dispersal (Cohen 1981). The most significant mechanisms of *P. cubensis* spores disperse autonomously by wind and water. Dispersal by wind (anemochory) is considered as the primary and most effective way of dispersion by which the spores are transferred by wind to distances of several hundred kilometers (Lebeda 1990). Wind dispersal caused intensive infections of cucumbers with *P. cubensis* in southern Sweden and Finland (Forsberg 1986; Tahvonen 1985) in the second half of the 80 s, due to migration from Central Europe where only one year earlier strong epidemics of *P. cubensis* were first recorded (Lebeda 1986a, 1990; Lebeda and Schwinn 1994). Similar long-distance distribution from the south to the north occurs in the eastern USA (Holmes et al. 2004, Ojiambo et al. 2009). Due to such long-distance transport, infection can progress in the areas where *P. cubensis* can not overwinter (Lebeda 1990). Dispersal by water (hydrochory), is a secondary mechanism of spore distribution over short distances (from leaf to leaf and plant to plant) within cucurbit fields (Lebeda 1990).

Factors influencing sporangial viability and overwintering of *P. cubensis*

Asexual spores (conidiosporangia) do not survive for a long time under common environmental conditions. When detached from sporangiophores, or when positioned on non-living or necrotic leaves, they lose viability and infection ability rather quickly (i.e., 24–72 h) (Cohen and Rotem 1971a). Their long-lasting maintenance or preservation is possible only under low (−18°C) or ultralow (−80°C) temperatures (Lebeda 1986b). Because of the obligate biotrophic nature of *P. cubensis* survival of mycelium in dead leaves is not possible (Lebeda 1990).

Detached sporangia were shown to survive better in cloudy than in sunny days and to withstand up to 23.5 MJ/m^2 and 1.2 MJ/m^2 of solar and UV irradiance, respectively (Kanetis et al. 2010; Ojiambo et al. 2009).

The main way of survival under inconvenient conditions (overwintering) is the formation of thick-walled resting oospores. Their occurrence was recorded in Japan, China, India, and Asian part of former USSR, Israel and Italy (Bedlan 1989; Cohen et al. 2003; Lebeda 1990; Palti and Cohen 1980; Cohen et al. 2003; Zhang et al. 2006). In Central Europe, the occurrence of oospores has not been verified so far, suggesting that *P. cubensis* may not overwinter in this region (Bedlan 1989; Lebeda 1990). Oospores were detected in Austria only, in older leaves of greenhouse cucumbers (Bedlan 1989). In the Czech Republic, oospores have not been unambiguously detected so far despite the fact that their occurrence is probable (Lebeda and Urban 2004a). In Central Europe, the main source of inoculum emigrates every year by air streams from Southeast Europe, where the pathogen can overwinter on living plants (Lebeda 1990; Lebeda and Schwinn 1994).

P. cubensis can also survive the winter *via* so-called green bridge, on protected cultures of cucurbit plants (e.g., in greenhouses). In Michigan USA, the early attacks in field-grown cucumber are thought to originate from greenhouses in Ontario, Canada (Day and Hausbeck 2009). In areas with a suitable climate, the perennial mycelia can overwinter on some host species (e.g. *Citrullus* spp., *Cucumis* spp.) even under field conditions as proved e.g. in India and southern USA (Palti and Cohen 1980; Holmes et al. 2004).

Long distance migration of sporangia

Spore trapping studies conducted in Israel (Cohen and Rotem 1971a, b) showed that in a regular summer day the peak of sporangial dispersal occurred at 8am. It starts at sunrise (6am), when the temperature rises, and the RH decreases. Dispersal (as indicated by the spore trap) continues until 4pm at very low rates. These late-dispersed sporangia have a better chance, compared to the early-dispersed sporangia, to remain viable and infect when the sun sets and dew accumulates. Sporangia of *P. cubensis* dislodge from sporophores by a twisting mechanism of the sporophore. This twisting occurs when RH decreases, and

is responsible for dislodging the sporangium from its sporophore. Sporangia can dislodge by water splash also, which ensures their rapid germination and infection. However, if splash dispersal happens with the aid of sprinkling irrigation, sporangial viability may be hampered if penetration was not completed before the end of the irrigation. The dispersed sporangia were shown to withstand high temperatures, provided that air relative humidity is low (Cohen and Rotem 1971a).

The best data on sporangial migration came from the USA where Nusbaum (1944, 1948) showed that infected cucurbits grown in the southeast in the winter time served as a source of air-borne sporangia to cucurbits crops grown along the East coast of the USA and Canada. He showed, on the basis of visual observations of disease outbreaks, that sporangia migrate in air trajectories, progressively reaching northern states in the spring and summer, and finally reaching Canada in late summer.

Recently, a very detailed migration, forecasting and epidemiological study showed the spatial and temporal movement of *P. cubensis* sporangia from southern Florida or Mexico to the north East coast of the USA. For this purpose a network of ca 40 representatives from the USA and Mexico was established (Holmes et al. 2004; Ojiambo et al. 2009). In comparison with Nusbaum (1944, 1948), the current system is aided by prognosis, it is rapid and more precise and availability of the forecasts to farmers is via the Internet. The precision resulted from two improvements: 1) use of meteorological models to actually track spore movement; and 2) a large network of collaborators who report disease outbreaks. A unique feature of this forecasting system is that growers can sign up on the forecasting web site to receive alerts of new disease outbreaks or risk of disease outbreak, via email and text messages on their cell phones (Holmes et al. 2004; Ojiambo et al. 2009). Recently, molecular and bioinformatic approaches are also introduced to the epidemiological studies of *P. cubensis* (Day and Hausbeck 2009).

Another significant example of long-distance travel of *P. cubensis* occured in 1985 when inoculum was distributed via air streams from Central Europe and Poland to Finland and Sweden causing extensive damage to cucurbits (Forsberg 1986; Tahvonen 1985). In Czechoslovakia, the pathogen spreads from Hungary via South Slovakia to South and Central Moravia, and later to East and Central Bohemia, i.e., the pathogen movement was from south-east to the western parts of Czechoslovakia (Lebeda 1990).

In Israel, *Cucurbita* spp. did not serve as hosts of *P. cubensis* until 2002 (Cohen et al. 2003). Thus, infection of *Cucurbita* spp. since 2002 could have been due to sporangia belonging to a pathotype capable of infecting *Cucurbita* spp. that may have emigrated from Southern or Central Europe (possibly Poland). Polish genotypes of *P. infestans* were identified in Israel on potato crops (Cohen 2002b).

Disease in protected crops (plastic and glasshouses)

Plastic houses, glass houses and net houses are widely used for growing cucurbits. In Israel, cucumbers are grown under plastic during the winter season assuring fruit supply year around. Watermelon and melon are grown under cover in the Arava valley during the winter allowing export of fruits to Europe during Christmas. Farmers face severe problems of downy mildew attacks during the winter, especially in melon and cucumber, and are forced to use frequent fungicide applications. The higher temperatures and relative humidity occurring under such covers in winter normally favour the development of downy mildew. The formation of guttation droplets, along the leaf boundries due to the reduced water potential in the xylem vessels at night, further increases the infection frequency. The closed environment enables the sporangia produced to stay inside the house so as to cause more infection. However, a closed plastic house may be devastating to the disease. This might happen under sunny skies when no ventilation takes place. The temperature may than elevate to 45°C or more with RH of >90%, a combination lethal to the pathogen.

Severe infection of *P. cubensis* on oriental melon (*C. melo* var. *makuwa*), grown in plastic greenhouses, was reported in Korea (Yeon et al. 2002). Downy mildew prevailed until the 2nd harvest and caused 16–34% yield reduction. Melon yield and downy mildew incidence were negatively correlated, and appropriate control methods had be taken at initial stage of the disease (Yeon 2007). As a part of appropriate control measures, an early warning model for occurrence of cucurbit downy mildew in non-heated greenhouses was developed based on disease records and microclimatic parameter analysis (Yang et al. 2007).

The microclimate manipulation by the polyethylene mulch could be one of the solutions for reducing *P. cubensis* infection in greenhouses. The ability of the mulch to suppress *Phytophthora infestans* in tomato and *P. cubensis* in cucumber was studied successfully (Shtienberg et al. 2010). The disease-suppressing effect of mulch appeared to come from a reduction in both the frequency of nights when dew formed and the number of dew hours per night when it formed. Mulching also reduced RH in the canopy, which may have reduced sporulation (Shtienberg et al. 2010).

Host range

P. cubensis affects the Cucurbitaceae family only (Palti and Cohen 1980; Lebeda and Widrlechner 2003). This family is relatively large and very heterogeneous. Currently, it includes more than 118 genera with 825 species (Lebeda et al. 2007b). Cohen (1981) reported that approximately 12 species of Cucurbitaceae are cultivated and nine of them are affected by *P. cubensis* under natural conditions. In fact, there are probably more cultivated hosts with economic significance (Lebeda 1990; Lebeda et al. 2007b). In addition to cultivated species, *P. cubensis* also attacks various semi-cultivated, weedy and wild genera and species of Cucurbitaceae (Cohen 1981; Lebeda and Widrlechner 2003, 2004). Artificial inoculations and observations made in nature, demonstrated that ca 60 species and 20 genera belong to the host list of *P. cubensis* (Lebeda 1992b, 1999).

The longest list of host species belongs to the genus *Cucumis*. It includes over 30 wild species, occurring mainly in arid and semi-arid areas of Africa, and two commonly cultivated species, *C. sativus* L. (cucumber) and *C. melo* L. (muskmelon) that have probably originated from the Indian gene centre (Kirkbride 1993; Lebeda et al. 2007b). Both species and ca eight wild *Cucumis* species are known as the natural hosts of *P. cubensis* (Lebeda and Widrlechner 2003).

Among the frequent hosts of *P. cubensis* are also representatives of genus *Cucurbita*. It includes approximately 14 species native to the area from the USA to Argentina (Lebeda et al. 2007b). Five species (*C. argyrosperma* C. Huber, *C. ficifolia* Bouche, *C. maxima* Duchesne, *C. moschata* Duchesne and *C. pepo* L.), domesticated in these areas before the arrival of Europeans, are currently abundantly-grown in many parts of the world (Lebeda et al. 2007b). All these species are hosts of *P. cubensis* (Lebeda and Widrlechner 2003, 2004). Artificial inoculations under laboratory conditions proved that *P. cubensis* might infect a number of wild and weedy genotypes of *Cucurbita* spp. (Lebeda and Widrlechner 2004).

Citrullus, *Lagenaria*, *Benincasa* and *Luffa* are important host genera (Robinson and Decker-Walters 1997; Rubatzky and Yamaguchi 1997). The genus *Citrullus* includes four species, of which *C. colocynthis* (L.) Schrad. and *C. lanatus* (Thunb.) Matsum. et Nakai (watermelon) were reported as hosts of *P. cubensis* (Lebeda and Widrlechner 2003). The genus *Lagenaria* which originated from Africa includes six species of which only *L. siceraria* is a cultivated crop. Natural hosts of *P. cubensis* are *L. siceraria* (Molina) Standl. and *L. sphaerica* (Sond.) Naud (Lebeda and Widrlechner 2003). *Benincasa* is a monotypic genus represented only by the species *B. hispida* (Thunb.) Cogn., originating from Southeast China. This species is a natural host of *P. cubensis*. Two cultivated species out of seven species of genus *Luffa* originating from tropic Asia, *L. acutangula* (L.) Roxb. and *L. cylindrica* (L.) M. J. Roem. (syn. *L. aegyptiaca* Mill.), are also natural hosts of *P. cubensis* (Lebeda and Widrlechner 2003).

A limited number of cucurbit vegetables are cultivated in Central Europe, mainly *Cucumis sativus*, *C. melo*, *Cucurbita maxima*, *C. pepo* and *Citrullus lanatus*. *Cucurbita foetidissima* and *Lagenaria siceraria* are also marginally grown in Central Europe (Moravec et al. 2004). The major natural host of *P. cubensis* in the Czech Republic is cucumber (*Cucumis sativus*), on which the pathogen causes extensive damage (ca 90% of the available host tissue was infected and necrotic during the second half of growing season in 1986–1990) (Lebeda and Schwinn 1994). In 2003, natural infection was recorded on *C. melo* (Lebeda and Urban 2004a, b). Lebeda (1986a) reported the first infection of *C. melo* in 1984 and the sporadic occurrence of the pathogen on *Citrullus lanatus* in 1985. Wild cucurbits probably do not serve as hosts of *P. cubensis* in the Czech Republic (Lebeda and Urban 2004a). *Cucumis* was the only genus for which specific physiological specialization of the local population of *P. cubensis* could be distinghished. This specialization predominated in Central Europe (Lebeda 1986a). More details regarding this topic are discussed below under "pathogenic variability."

Recently, the occurrence of *P. cubensis* on some weedy and cultivated Cucurbitaceae was published (Choi and Shin 2008; Ko et al. 2008; Salti et al. 2010). Laboratory experiments demonstrated that *P. cubensis* can infect *Bryonia dioica*, the only perennial plant of the Cucurbitaceae in Central and Northern Europe. Based on this finding, it was hypothesised, that *P. cubensis* may overwinter on this host species and serve as a primary source of inoculum (Runge and Thines 2009). However, long-lasting field observations of *B. dioica* in the Czech Republic (Lebeda 1990, unpublished results; Lebeda and Urban 2004a, b), failed to confirm this hypothesis. From the viewpoint of exclusive alignment of *P. cubensis* with Cucurbitaceae it is interesting to refer to Dick (2001a) who reported on the susceptibility of beans to *P. cubensis* when exposed to *Uromyces* sp. (Uredinales).

Geographic distribution

Worldwide distribution

P. cubensis is widely distributed in all continents of the north and south hemispheres where cucurbit plants are cultivated. It mainly occurs in warm, temperate, subtropic and tropic areas on field cultures as well as on protected (glasshouse, plastichouse and shadehouse) crops (Cohen 1981; Lebeda 1990), especially in areas with annual precipitation of >300 mm (Lebeda 1990). Although distributed worldwide, essential differences among geographic areas may be observed in the occurrence of *P. cubensis*, and the damage it causes to various host species (Lebeda 1990; Palti and Cohen 1980; Thakur and Mathur 2002).

The highest frequency of *P. cubensis* is apparent on the genus *Cucumis*. It currently occurs in more then 80 countries on *C. sativus* and in more then 50 countries on *C. melo* (Palti and Cohen 1980; Lebeda 1990; Thakur and Mathur 2002). In the eastern U.S. A. *P. cubensis* has re-surged as a major problem on cucumbers beginning in 2004 (Holmes et al. 2004; Holmes and Ojiambo 2009; Holmes and Thomas 2009). In Europe, *P. cubensis* was originally common in the Mediterranean region. Recently, it has quickly spread to most European countries, reaching Scandinavia (Lebeda 1990). This indicates the high adaptative capability of *P. cubensis* which enabled it to inhabit new geographic regions with very diverse ecological conditions (Lebeda and Schwinn 1994).

The distribution of *P. cubensis* on *Cucurbita* spp. is relatively limited in comparison with *Cucumis*. It was recorded in approximately 40 countries around the world and the main centre of this distribution is Central America and the Caribbean region (Palti and Cohen 1980). In Europe, the documented occurrence of *P. cubensis* comes from Yugoslavia and former USSR (Lebeda 1990).

The distribution of the pathogen on the genus *Citrullus* is even more limited, in about 25 countries, with the main centre of occurrence in Central America. The natural occurrence of *P. cubensis* on *Citrullus lanatus* is common in Florida, USA but not in Europe or the Middle East (Lebeda 1990; Cohen et al. 2003).

Distribution in Europe

P. cubensis is known in Central Europe since the beginning of 20th century (Lebeda 1991; Skalický 1961). During the first half of the 20th century, it was recorded several times, but as sporadic events. In Czechoslovakia, *P. cubensis* was also observed at the beginning of 20th century and then, in 1924 and 1925 (Zacha et al. 1985; Lebeda 1986a; Ackermann 1990).

Broad and epidemic distribution of the pathogen in Central Europe occurred in the second half of the 1980s (Lebeda 1986a; Lebeda and Schwinn 1994). The year 1985 is considered crucial, during which strong epidemics of downy mildew occured in cucumber (*Cucumis sativus* only) all over Central Europe, parts of Western Europe (Czechoslovakia, Poland, Germany, Austria, Switzerland, Hungary), and even in some Eastern and Southern European states (Yugoslavia, Bulgaria, Romania, Moldova, The Ukraine, Byelorussia) (Lebeda 1990). In that period, inoculum distributed via air streams from Poland to Finland and Sweden where it caused, for the first time, extensive damage to cucurbits (Tahvonen 1985; Forsberg 1986). In the same period *P. cubensis* did not occur in Central Europe on *C. melo* or *Cucurbita* spp. in the field or the greenhouse (Lebeda and Gadasová 2002). The repeated occurrence of heavy infections on cucumbers in the following two decades indicated that *P. cubensis* acquired an epidemic character in Central Europe, comparable to many other areas of the world (e.g. Israel, Japan, India) (Lebeda and Schwinn 1994; Cohen et al. 2003; Holmes et al. 2004).

However, in these areas, the epidemics also occur on other crops (e.g. *Cucumis melo*, *Cucurbita* spp., *Citrullus lanatus*).

In the former Czechoslovakia, the first epidemic of cucurbit downy mildew occurred in 1984 on cucumbers (mainly in Southern Slovakia, South, Central and North Moravia and in areas surrounding Prague). The damage in cucumbers was not very high due to the late appearance of the disease (end of July–August) (Zacha et al. 1985; Lebeda 1986a). A year later, 1985, the epidemics were extremely devastating, with an estimated yield loss of 80–90% in cucumbers (Lebeda 1991). The epidemics in the following years 1986–1988 coresponded (by level) to the epidemics in 1985, but due to better crop protection measures losses were lower (Rod 1990). In 1989, losses again went to over 80% (Lebeda 1991). In all these years the disease in Czechoslovakia was a consequence of pathogen spread from Hungary *via* South Slovakia to South and Central Moravia, and later to East and Central Bohemia. The cucumbers in South and West Bohemia (areas of recent Czech Republic near the Austrian and German border) were always affected later (Lebeda 1986a, 1990).

Detailed observations showed that during the last decade, *P. cubensis* occurred every year all over the Czech Republic, not only in the main host growing areas, but also in marginal areas such as foothills and hills, where cucurbit crops are rarely cultivated. Generally, the first infection symptoms on cucumber, not the other Cucurbitaceae, appeared in the second half of July or at the beginning of August. The fluctuation of the distribution and the intensity of infection was significantly influenced by environmental conditions (Lebeda and Urban 2004a,b). Disease index in cucumber crops ranges from strong to very strong severities (DI 3-4, Fig. 4) at harvest time (Lebeda and Urban 2005, 2007).

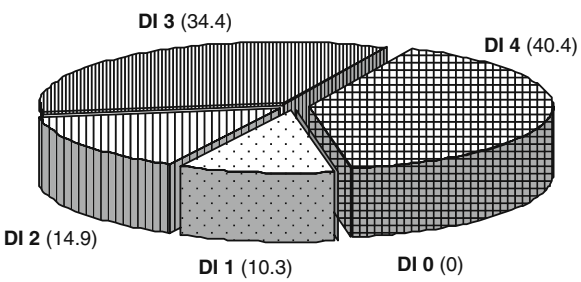

Fig. 4 Frequency (per field) of *P. cubensis* disease intensity / severity/ (DI, %) on cucumber (*Cucumis sativus*) in the Czech Republic in 2003

Host-pathogen interactions Cucurbitaceae–*P. cubensis*

Specificity of relationships between Cucurbitaceae and *P. cubensis*

Species of the Peronosporaceae are characterized by their complicated relationships with their hosts on various levels of biological organization (Lebeda and Schwinn 1994; Göker et al. 2007). Their biotrophic obligate parasitic nature dictates strict host specificity (Crute 1981; Dick 2002a). However, individual species of Peronosporaceae differ in the level of their host specificity, from a single plant species to a relatively large number of species and genera (Lebeda and Schwinn 1994). *P. cubensis* affects a limited number of genera and species within the Cucurbitaceae (Cohen 1981; Lebeda 1999). Specialization in *P. cubensis* is rather diverse and distinct in various pathogen populations (Lebeda et al. 2006b).

P. cubensis is compatible (host-pathogen interaction) with some, but not all, species and genera of Cucurbitaceae (see Host range section). However, certain genotypes (varieties, lines) of host species assign race-specific resistance (Lebeda et al. 2006b). This resistance is a result of highly specific and concurrently relatively simple metabolic and genetic adaptations for the formation of defence mechanisms (Mauch-Mani 2002). It is, therefore, relatively unstable because of the changes that take place in the pathogen from one to another (Lebeda and Schwinn 1994). On the other hand, *P. cubensis* forms physiologically specialized entities (pathotypes and races) characterized by certain types of pathogenicity that enables them to overcome the race-specific resistance of certain host genotypes (Lebeda and Widrlechner 2003; Lebeda et al. 2006b).

The display of compatibility/incompatibility in the interactions between oomycetes and their hosts is well differentiated and has a discontinuous character. For this reason, the classification of pathotypes and physiological races is based on the display of compatible/incompatible reactions on differential host species and genotypes (for details see below) (Lebeda and Schwinn 1994; Lebeda and Widrlechner 2003).

Pathogenic variability of *P. cubensis*

P. cubensis shows an extensive intraspecies variability in pathogenicity (Thomas et al. 1987c; Lebeda et al.

2006b) as with other oomycetes (Lebeda and Schwinn 1994). The first information about the pathogenic variability of *P. cubensis* came from Japan in the 1940s (Iwata 1941). This topic was later elaborated in detail in India, Israel, Japan and USA (Hughes and van Halteren 1952; Cohen 1976; Bains and Sharma 1986; Inaba et al. 1986; Thomas et al. 1987c). Pathogenic variability appears when host genera, species or their lower taxonomic and genetic units interact differentialy with *P. cubensis*. The interactions between the pathogen populations and the host populations can take place at various levels of specifity that are (on the side of pathogen) expressed in the differentiation of different pathogenic groups (Caten 1987). In *P. cubensis*, physiological races and/or phenotypes of virulence, pathotypes and possibly formae speciales were reported (see below).

Formae speciales

A special form (*forma specialis,* f. sp.) is an intraspecific taxonomic unit used only for phytopathogenic fungi. The populations and isolates without morphological differences that are distinguished physiologically are classified as special forms by their parasitic adaptation on various host genera. The main criterion of this specialization is often a certain genus (or other group such as tribes) within the frame of a complete host range (Holliday 2001). The special forms can be distinguished by cross inoculations, where the widest spectrum of host genera known for a certain pathogen is inoculated by the isolates from certain host genera and species.

Such cross inoculations conducted with several representatives of Peronosporaceae showed that their isolates were often compatible only with that host genera or species from which they were isolated. A characteristic example is *Bremia lactucae* where the isolates assigning the specificity to various genera of the family Asteraceae were distinguished as special forms (Skidmore and Ingram 1985). *P. cubensis* isolates from *Cucumis sativus* were compatible with five different genera of the Cucurbitaceae (Lebeda and Widrlechner 2003, 2004), however, they expressed also incomplete compatibility on some other genera (Lebeda and Widrlechner 2003).

Five special forms were originally considered for *P. cubensis*: f.sp. *cucumae*, f.sp. *cucurbitae*, f.sp. *lagenariae*, f.sp. *benincasae* and f.sp. *luffae*, which coresponded to the isolates collected from the genera *Cucumis*, *Cucurbita*, *Lagenaria*, *Benincasa* and *Luffa* (Lebeda 1990). According to the results of compared cross inoculations published by various authors, we found significant differences in pathogenicity among the given isolates (Lebeda et al. 2006b). New experiments with the isolates originated from *Cucumis sativus* and *Cucumis melo* showed that the classification of pathogenic variability of *P. cubensis* on the level of special forms might be misleading (Lebeda and Gadasová 2002), as was also considered by Thomas et al. (1987c). Recent molecular studies showed that isolates of *P. cubensis* from various hosts were almost identical in terms of sequence analysis of ITS rDNA, which was interpreted as *P. cubensis* being a homogeneous taxon (Choi et al. 2005).

To this date, the existence of special forms of *P. cubensis* is neither confirmed nor negated. Additionally classical, genetic and molecular research (focused on host-pathogen specificity and cross-infection ability), based on broad international co-operation (esp. exchange of isolates originating from different Cucurbitaceae), might contribute more knowledge in this area (Lebeda et al. 2006b).

Pathotypes

Differentiation of pathotypes

Pathotypes and physiological races are very common in plant pathogens. They are morphologically identical but differ in their ability to attack different species within a genus, and/or among different cultivars within a single species (Holliday 2001). Pathotypes of *P. cubensis* represent the variability in the pathogen from the viewpoint of host range within the family Cucurbitaceae (Thomas et al. 1987c; Lebeda and Widrlechner 2003; Lebeda et al. 2006b). In principle, pathotypes are physiological forms that differ in host specifity on the level of genera, species or subspecies of various Cucurbitaceae (Lebeda and Gadasová 2002; Lebeda and Widrlechner 2003). Pathotypes may also represent pathogen variability previously considered as formae speciales of *P. cubensis* (see section on formae speciales).

Thomas et al. (1987c) were the first to establish a method to identify pathotypes of *P. cubensis*. They used a set of differential species which included *Cucumis sativus, C. melo reticulatus,*

C. melo conommon, C. melo acidullus, Citrullus lanatus and *Cucurbita pepo*. This set was selected based on the results obtained from inoculation of 26 genotypes of seven genera of Cucurbitaceae from which, for differentiation of pathotypes, they chose the most susceptible genotypes enabling a clear differentiation of compatibility/incompatibility (Thomas et al. 1987c).

Using this set, five pathotypes were distinguished according to the different reaction patterns of eight tested isolates of *P. cubensis* originating from the USA, Israel and Japan. The authors described them as "pathotypes 1 to 5" according to the increasing number of hosts on which a virulent (compatible) reaction occurred (Thomas et al. 1987c). Pathotype 1 was compatible with only one differential genotype, while pathotype 5 was compatible with all six differential genotypes. Based on a similar differential set (including *Luffa cylindrica*) pathotype 6 from Israel was described (Cohen et al. 2003).

The differential set of Thomas et al. (1987c) had, nevertheless, several limitations (Lebeda and Widrlechner 2003): it did not include important host genera (e.g. *Benincasa*, *Luffa*, *Lagenaria*); differential genotypes were not precisely taxonomically-defined (on species, subspecies and genotype/accession level); and were not maintained as a complete unit, by any responsible institution. Lebeda and Widrlechner (2003) used an extended set of differential genotypes which included also *Lagenaria siceraria*, *Benincasa hispida*, and *Cucurbita maxima* (Table 1).

This new differential set was developed for the differentiation of *P. cubensis* pathotypes according to a new denomination system (Lebeda and Widrlechner 2003). It was based on the set of Thomas et al. (1987c), but was expanded to include taxonomically-defined genotypes. The new set consists of 12 genotypes belonging to the six most important host genera of the Cucurbitaceae: *Cucumis*, *Cucurbita*, *Citrullus*, *Benincasa*, *Luffa* and *Lagenaria* (Table 1). The basic data on specificity and variability of the interactions between *P. cubensis* and these taxons are available. All taxa are well defined on the level of species, sub-species and genotype, and are maintained as accessions in several international gene bank collections (e.g. Plant Introduction Station, USDA, Ames, Iowa, USA). This adjusted differential set enables the characterization of *P. cubensis* pathotypes by distinguishing between 12 "pathogenicity factors" and their combinations. A new system of description and denomination of pathotypes was developed in parallel (Lebeda and Widrlechner 2003). This system is based on numerical tetrade codes (Limpert et al. 1994). Based on a binary evaluation of compatible/incompatible reaction pattern (+ or −) of a certain isolate, a numeric tetrade code was created for this isolate. Numeric composition of the code gives a clear picture

Table 1 Differentials set of cucurbit taxa for determination of *P. cubensis* pathotypes (modified according to Lebeda and Widrlechner (2003))

Number	Differential genotype	Genotype code		Cultivar	Country of origin
		Donor	EVIGEZ		
1	*Cucumis sativus*		H39-0121	Marketer 430	USA
2	*C. melo* subsp. *melo*	PI 292008	H40-1117	Ananas Yoqne´am	Israel
3	*C. melo* subsp. *agrestis* var. *conomon*	CUM 238/1974	H40-0625	Baj-Gua	Japan
4	*C. melo* subsp. *agrestis* var. *acidulus*	PI 200819	H40-0611		Myanmar
5	*Cucurbita pepo* subsp. *pepo*	PI 171622	H42-0117	Dolmalik	Turkey
6	*C. pepo* subsp. *texana*	PI 614687	H42-0130		USA
7	*C. pepo* subsp. *fraterna*	PI 532355	H42-0136		Mexico
8	*Cucurbita maxima*		H42-0137	Goliáš	Czechoslovakia
9	*Citrullus lanatus*		H37-0008	Malali	Israel
10	*Benincasa hispida*	BEN 485	H15-0001		USA
11	*Luffa cylindrica*		H63-0010		?
12	*Lagenaria siceraria*		H63-0009		?

EVIGEZ—the information system on plant genetic resources (Gene Bank VURV Olomouc, Czech Republic)
Taxonomy of genus *Cucurbita* adjusted according to Lebeda et al. (2006b, 2007b)

on the pathogenicity of an isolate and concurrently identifies (numerically describes) its relevant pathotype (Table 2). One can deduce the pathogenicity of an isolate to each differential from its tetrade code.

This set might be extended by the incorporation of new taxa or genotypes of Cucurbitaceae. It will, thus, be possible to make pathotype differentiation a flexible process which continuously develops (Lebeda and Widrlechner 2003; Lebeda et al. 2006b). Current attempts are aimed to also differentiate races of *P. cubensis* (Lebeda et al. 2006b).

Geographic distribution of pathotypes

Thomas et al. (1987c) and other authors (for references see Lebeda et al. (2006b)) studied pathotype variability of *P. cubensis* with a limited number of isolates from Japan, Israel and the USA. Data collected by Lebeda and Widrlechner (2003) and Lebeda et al. (2006b) showed substantial differences in virulence of *P. cubensis* in different geographical regions of the world (Table 3). Pathotypes were not determined for European isolates until the late 1990s (Lebeda and Widrlechner 2003). In 2002, Lebeda and Gadasová (2002) used the above new differential set of cucurbits to identify pathogenic variability among 22 isolates of *P. cubensis* originating from four European countries (mostly from Czech Republic). They distinguished 13 different pathotypes that differed from pathotypes 1 to 5 described by Thomas et al. (1987c). Only one isolate coresponded to pathotype 1. The newly distinguished pathotypes produced between 2 to 9 susceptible reactions on the 12 differential genotypes, suggesting that they each carry 2 to 9 pathogenicity factors (PF, i.e. factors able to overcome resistance of individual differential genotypes). The European population of *P. cubensis* is, therefore, significantly variable in pathotype structure and generally does not resemble the model of Thomas et al. (1987c) which cannot detect such variability (Lebeda and Gadasová 2002) due to its limited range. The pathotype structure of *P. cubensis* population differed significantly across various areas of Europe (Lebeda and Gadasová 2002; Sarris et al. 2009). We assume that similar variabilities might also occur in other countries and continents (Lebeda et al. 2006b; Lebeda and Widrlechner 2003) like it is evident on recent data from USA (Colucci 2008).

Until now, pathogenic variation in *P. cubensis* was studied in detail only in the Czech Republic (Lebeda et al. 2006b), and more recently in USA (Colucci 2008). The pathotype structure of the pathogen in the Czech Republic is quite variable and highly pathogenic, i.e. with isolates having a high number of pathogenicity factors (Fig. 5). Over 40 pathotypes were distinguished among 198 isolates collected during 2001 to 2004 and ca 70% of these isolates

Table 2 Some examples of tetrade numeric codes of pathotypes of *P. cubensis*. Codes were established on the basis of the reaction of selected Czech isolates with cucurbit plants (differential set, see Table 1) (Lebeda and Widrlechner 2003)

Groups of differentials*	1. *Cucumis* spp.				2. *Cucurbita* spp.				3. Other Cucurbitaceae				Code of pathotype
Differential genotype*	1	2	3	4	5	6	7	8	9	10	11	12	
Value	1	2	4	8	1	2	4	8	1	2	4	8	
P. cubensis isolates													
PC 3/00	1	2	0	0	0	0	0	0	0	0	0	0	3.0.0
PC 13/00	1	0	0	8	0	2	0	8	0	0	0	0	9.10.0
PC 1/88	1	2	0	0	0	2	0	0	0	2	0	8	3.2.10
PC 3/98	1	0	0	0	0	2	0	8	0	2	0	8	1.10.10
PC 1/98	1	2	0	0	0	2	0	8	0	2	0	8	3.10.10
PC 4/00	1	0	4	0	0	2	4	8	0	2	0	8	5.14.10
PC 12/00	1	2	0	8	0	2	0	8	0	2	4	8	11.10.14
PC 1/97	1	2	4	8	0	2	0	8	1	2	0	8	15.10.11
PC 24/01	1	2	4	8	1	2	4	8	0	2	4	8	15.15.14
PC 39/01	1	2	4	8	1	2	4	8	1	2	4	8	15.15.15

* see Table 1; 0 = resistant reaction; 1, 2, 4, 8: susceptible reaction

Table 3 Availability of data on pathotypes and races of *P. cubensis* in various countries (modified according to Lebeda et al. (2006b))

Pathogenicity category	Pathotype		Race	
Country	Data available	References	Data available	References
China	?		+?	11
Czech Republic	+	6,7,8,9,10	+	6,7,8,9,10
Bulgaria	+?	1	+?	1
India	+	2	+?	2,11
Israel	+	3,12	+	3,12
Japan	+	12	+?	12
Poland	?		+?	11
USA	+	11	+	4,5,11,12
Others (FR, NL, SP) *	+	7	+	7,9

− = pathotype or race absent; + = pathotype or race present; ? = data not available or not experimentally confirmed; * only one isolate determined

FR France, *NL* the Netherlandsm, *SP* Spain

References (full citations are in References): (1) Angelov et al. (2000); (2) Bains and Sharma (1986); (3) Cohen et al. (2003); (4) Colucci (2008); (5) Horejsi et al. (2000); (6) Lebeda (1999); (7) Lebeda and Gadasová (2002); (8) Lebeda and Urban (2004a,b, 2006); (9) Lebeda and Widrlechner (2003); (10) Lebeda and Widrlechner (2004); (11) Shetty et al. (2002); (12) Thomas et al. (1987c)

carried 9 to 12 pathogenicity factors/PF/) (Lebeda and Urban 2004a,b, 2005, 2007).

A shift towards higher pathogenicity was evident during the evaluation period (Lebeda and Gadasová 2002; Lebeda and Urban 2004a, b, 2007). Pathotypes with low pathogenicity (total number of PF up to 4) were detected only in 2001. Isolates with moderate pathogenicity (total number of PF betwen 5 to 8) and high pathogenicity (total number of PF betwen 9 to 12) predominated in the pathogen populations during 2001–2004. The ratio between the last two pathogenic groups was about 1:1 in 2001 and 2002, but changed to 1:7 in 2003 and 2004 when 87.5–93% of isolates respectively, belonged to the group of highly pathogenic pathotypes (Fig. 5). The data show that PF 5, 9 and 11 were least common. The frequency of PF 5 (squash) and 9 (watermelon) tended to increase during the sampling period, whereas that of PF 11 (*Luffa*) tended to decrease.

Increased pathogenicity of *P. cubensis* was recently observed in Israel and USA. In Israel, pathotype 3 (sensu Thomas et al 1987c, attacking cucumber and melons only) was common since pathotype

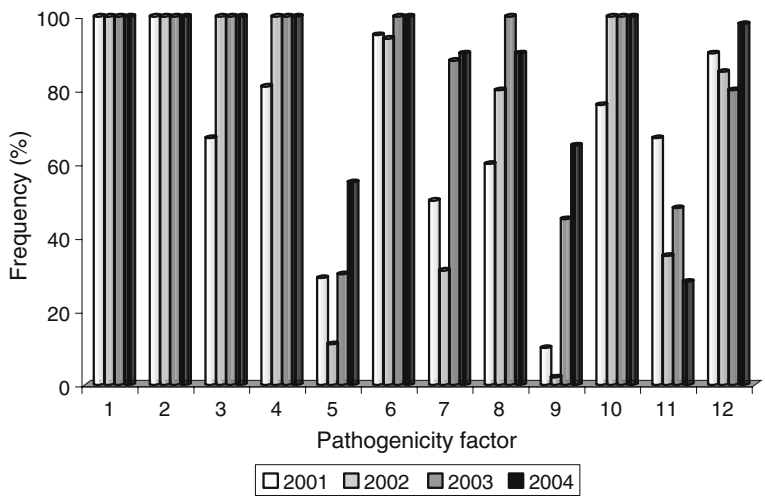

Fig. 5 The frequency of pathogenicity factors (see Tables 1 and 2) in 198 isolates of *P. cubensis* collected in the Czech Republic between the years 2001 and 2004 (Lebeda et al. 2006b)

determination studies were done in 1965 (Y. Cohen, unpublished). In 2003, a new pathotype (number 6) which can also attack squash and watermelon appeared in Israel (Cohen et al. 2003). In the USA, increased virulence to cucumber was observed since 2004 (Holmes et al. 2004; Holmes and Thomas, 2009).

Physiological races

There are indications in the literature (Table 3) that *P. cubensis* might also vary at the species level, suggesting the occurrence of physiological races. Such races (particularly in oomycetes and fungi) are characterized by specialization to different cultivars of one host species (Caten 1987; Holliday 2001). This phenomenon was also described for the interactions of some cucurbits with *P. cubensis* (Lebeda et al. 2006b; Lebeda and Widrlechner 2003) (Table 3).

In 1932, cucumbers that were resistant to downy mildew in Massachusetts, were found to be susceptible in other parts of the USA (Cohen 1981). Another famous example is the sudden breakdown of resistance of the cultivar Palmetto (in South Carolina, 1950) causing severe crop losses due to the selection of a new race of *P. cubensis* (Cohen 1981). A major problem in the last 30 years is the evolution of new field populations of *P. cubensis* resistant to a number of commonly-used fungicides. The first appearance of such a new strain was reported by Reuveni et al. (1980), who detected metalaxyl-resistance in *P. cubensis* from cucumber greenhouses where this fungicide was used repeatedly. Under the influence of repeated applications of different fungicides, a fast selection of resistant strains has occurred (Lebeda and Urban 2004a, b; Urban and Lebeda 2004, 2006, 2007). Recently, Cohen and co-workers (unpublished) have detected strains of *P. cubensis* in Israel which carry double resistance to metalaxyl/mefenoxam and CAA (carboxylic acid amide) fungicides (dimethomorph, iprovalicarb, etc). The same is true in the USA where failures with CAA fungicides have been shown repeatedly in field studies (Colucci 2008).

Lebeda and Schwinn (1994) reported that the differentiation of *P. cubensis* races was not fully unambiguous because the pathogen did not show any significant differences in virulence on *Cucumis sativus* and wild *Cucumis* species (Lebeda 1992a, b). The existence of differential responses (compatibility/incompatibility) of various cucumber cultivars has not been experimentally demonstrated (Lebeda 1992a, 1999; Lebeda and Prášil 1994; Lebeda and Urban 2005), although differences in sporulation intensity are documented (Lebeda and Doležal 1995; Lebeda 1999). Thus, so it is clear that levels of resistance from high to low exist in cucumber, but that no cucumber cultivar has been shown to be completely resistant to *P. cubensis* infection. Nevertheless, the existence of physiological races was proved on *Cucumis melo* (Thomas et al. 1987c; Lebeda 1991; Lebeda et al. 2007a), *Cucurbita pepo* and other *Cucurbita* spp. (Thomas et al. 1987c; Lebeda and Křístková 1992, 1993; Lebeda and Widrlechner 2004). Race-specific interactions also were displayed on *Citrullus* (Thomas et al. 1987c). The recent knowledge about *P. cubensis* races mostly refers to isolates originating from *Cucumis sativus* and *C. melo* (Lebeda 1990; Lebeda et al. 2006b). The problems related to the topic of *P. cubensis* races were discussed in detail by Lebeda et al. (2006b). Our current understanding suggests that *P. cubensis* races do exist. Unfortunately, no suitable differential sets are yet available for the most important host genera, *Cucumis*, *Cucurbita* and *Citrullus* (Lebeda at al. 2006b).

Genetic diversity of P. cubensis

Only limited information is available on the genetic diversity of *P. cubensis* in relationship to geographic distribution, pathogenicity variation of isolates and populations. Amplified Fragment Length Polymorphisms (AFLP) and the nucleotide sequence of the *ITS1-5.8S-ITS2* subunit of ribosomal DNA (*rDNA-ITS*) have been used for studying genetic diversity in *Phytophthora infestans* (Cooke and Lees 2004) and for taxonomic and phylogenetic studies of downy mildew pathogens (e.g. Voglmayr 2008), but not for intra-species population studies of isolates from geographically distant areas (Sarris et al. 2009). In a recent molecular investigation the genetic diversity of *P. cubensis* was compared in populations originating from Crete; Czech Republic and Central Europe; the Western European countries France and the Netherlands (Sarris et al. 2009). All studied *P. cubensis* isolates originated from cucumber (*Cucumis sativus*). AFLP fingerprinting produced ample polymorphisms and isolates were grouped into two separate clusters; one included the Czech (Central Europe) and West

European (the Netherlands, France) isolates, and the other included the isolates of Crete. Significant differences were found between these two populations. Within each group some variations found were attributed to geographic origin, host cultivar, pathogenicity and fungicide resistance. *rDNA ITS* analysis showed no variability among isolates in *ITS1;* however, all *ITS2 rDNA* sequences of Crete and Czech isolates clustered together with isolates from Austria, forming a large cluster together with *P. humuli*, indicating their close taxonomic relationship (see part Taxonomy) (Sarris et al. 2009). These results need to be validated with a larger number of isolates of *P. cubensis* originating from largely distinct areas and well characterized in their phytopathological attributes (pathotype, race and fungicide resistance). This will provide the basis for investigating the sources and shifts in genetic diversity within and between *P. cubensis* populations (Choi et al. 2005; Gent et al. 2009; Sarris et al. 2009), as well as a better background for diseases management. In a most recent study (H. Sierotzki, M. Blum, G. Olaya, M. Waldner-Zulauf, J. Wullschleger, Y. Cohen and U. Gisi, unpublished data) resistance of *P. cubensis* isolates towards CAA fungicides was related to the cellulose synthase A3 (cesA3) gene structure: isolates obtained from US or Israel displayed a different mutation at position 1105 of cesA3: US-Gly ggg to Trp tgg and Israel-Gly ggg to Val gtg.

Host variability in interactions with *P. cubensis*

The extensive intraspecific variability of *P. cubensis* host specificity derives from the large taxonomic and genetic diversity of the Cucurbitaceae. Although host genotypes display clear pathotype or race-specificity (Lebeda et al. 2006b, 2007a), heterogeneous reactions and incomplete resistance/compatibility are also observed (Lebeda and Widrlechner 2003).

Optimal laboratory (growth chamber) conditions for inoculation and disease development enable the interactions between host plants and *P. cubensis* to be precisely described. In contrast, the reaction of the same host genotypes under natural epiphytotic conditions in the field can differ markedly. This is why the phenomenon of field resistance (Lebeda and Jendrulek 1988) to *P. cubensis* was also studied in some cucurbits (Cohen and Rotem 1971b; Lebeda and Doležal 1995; Wehner and Shetty 1997; Lebeda 1999). Field resistance is defined as the interaction of a plant population (cultivar, accession) with a pathogen population during the cultivation period (Lebeda and Jendrulek 1988). Field resistance is therefore a complex epidemiological phenomenon characterized by many different features, such as timing of disease onset, length of latent period, rate of disease progress (low epidemic rate, r), infection frequency, disease incidence (leaf or plant basis), degree of sporulation (Lebeda and Jendrulek 1988; Lebeda and Schwinn 1994). This type of resistance which may provide effective protection of the crop in the field, may not be easily detected or characterized in greenhouse or laboratory tests (Lebeda and Reinink 1991). Field resistance appears with low inoculum levels, is markedly dependent on environmental conditions (Lebeda 1990), and is not directly dependent on the composition of pathogen population(s) (Lebeda 1991b).

The following section of this review provides details on the variation of host-pathogen interactions between the most economically important Cucurbitaceae and *P. cubensis*. They are primarily based on experimental studies done under controlled conditions.

Cucumis spp.

Cucumis sativus (cucumber) is genetically fairly homogenous, and therefore exhibits low variability in its interactions with *P. cubensis* under laboratory conditions (Lebeda and Widrlechner 2003; Lebeda and Urban 2004a). *C. sativus* is highly susceptibile to *P. cubensis*. The available genotypes (including commercial cultivars) do not contain reliable sources of resistance and no unambiguously proved race-specific interactions were detected by Lebeda et al. (Lebeda 1991, 1992a, b; Lebeda and Widrlechner 2003; Lebeda and Urban 2004a, b; Lebeda et al. 2006b). Nevertheless, Shetty et al. (2002) discussed the possibility of race-specificity in cucumbers. *C. sativus* serves mostly as a susceptible control in differential sets for distinguishing pathotypes and races (Lebeda and Widrlechner 2003). Cultivars of *C. sativus* with a high level of field resistance (Table 4) are also known (Lebeda 1999; Lebeda and Doležal 1995; Wehner and Shetty 1997; Bjoern and Kampmann 2000; Doruchowski and Lakowska-Ryk

Table 4 The variability of field resistance in *Cucumis sativus* germplasm against *P. cubensis* (modified according to Lebeda 1999)

Cultivar/accession *C. sativus*	Origin	AUDPC
Commercial cultivars		
Santana F1	The Netherlands	1500
Regina F1	Czech Republic	1297
Niva F1	Israel	1203
Anuschka F1	The Netherlands	1044
Admira F1	Czech Republic	950
Nora F1	Czech Republic	919
Poinsett 76	USA	631
Dalnevostočnyj-6	USSR	528
Germplasm		
PI 169395[a]	Turkey	2162
PI 267741	Japan	1225
PI 263083	China	738
PI 288238	Egypt	441
PI 197085	India	288
C. melo[b]		
PI 124111	India	13

AUDPC area under disease progress curve. Lower values represent higher field resistance

[a] Susceptible control characterized by very low level of field resistance

[b] Resistant control characterized by high level of field resistance

2000; Petrov et al. 2000; Lebeda and Widrlechner 2003). Recently, the development of *Cucumis sativus-hystrix* introgression lines exhibiting resistance to downy mildew was reported (Zhou et al. 2008).

Unlike cucumber, muskmelon (*Cucumis melo*) is a very variable species from morphological, genetic and molecular viewpoints (Lebeda et al. 2007b). Despite this fact, all its forms are easily crossable (Thomas et al. 1987a, b, c). Within the genus *Cucumis*, *C. melo* is the only species with relatively well-investigated race-specificity (Lebeda et al. 2007a) and available effective sources of resistance (Thomas 1982, 1986; Cohen and Eyal 1987; Lebeda and Widrlechner 2003; Lebeda et al. 2006b). Its intraspecific taxonomic units and genotypes display the basic differences in resistance/susceptibility to *P. cubensis* and are therefore used for differentiation of pathotypes (and races) (Lebeda et al. 2006b, 2007a). Thomas et al. (1987a, b) included three subspecies of *C. melo* in their differential set and distinguished three pathotypes of *P. cubensis* on these genotypes.

In previous (Thomas et al. 1987c) and current (Lebeda and Widrlechner 2003) differential sets for identifying pathotypes of *P. cubensis* three genotypes of three taxons of *C. melo* (*C. melo* subsp. *melo*, *C. melo* var. *conomon*, *C. melo* var. *acidulus*) are included. With these three genotypes, Lebeda and Gadasová (2002) discovered significant variability in the interactions with European isolates of *P. cubensis*. In *C. melo*, there are also cultivars with field resistance (Table 4) (Thomas et al. 1987a, b; Lebeda and Schwinn 1994; Lebeda 1999).

The screening of 20 wild *Cucumis* species did not show any significant differences in reaction patterns, most of the accessions exhibited susceptibility, only in some cases was race-specific resistance recorded (Lebeda 1992b; Lebeda and Widrlechner 2003). The elaboration of a differential set for *Cucumis melo* is currently being investigated (Lebeda et al. 2006a, b, 2007a).

Cucurbita spp.

The genus *Cucurbita* is genetically extremely variable (Lebeda et al. 2007b) with the frequent occurrence of race-specific resistance (Lebeda and Widrlechner 2003; Lebeda et al. 2006b) against *P. cubensis*. In a number of genotypes, a high level of resistance or susceptibility with clear expression of race-specificity was observed (Lebeda and Křístková 1992, 1993, 2000; Lebeda and Widrlechner 2004).

C. pepo represents agriculturally the most significant and polymorphic species, and from the viewpoint of interaction with *P. cubensis*, is the most studied species of the genus. It expresses significant race-specificity as seen by the different reaction patterns of subspecies, botanical varieties and cultivars inoculated with different isolates of *P. cubensis* (Lebeda and Widrlechner 2003, 2004). The differences in the resistance/susceptibility to *P. cubensis* were discovered for example among individual morphotypes of *C. pepo* (i.e., the groups of genotypes with a certain characteristic shape of the fruits (Paris 2008)) (Lebeda and Křístková 2000). Certain morphotypes are highly resistant while others are highly susceptible. The variability in the morphotype reactions is influenced by their origin, genetic relationships and cultivation methods (Lebeda and Křístková 2000).

Pathotype specificity was described for *Cucurbita pepo, C. maxima* and *C. moschata*. Three accessions of *C. pepo* (*C. pepo* subsp. *pepo, C. pepo* subsp.

texana, C. pepo subsp. *fraterna*) and one accession of *C. maxima* are therefore included in the differential set for pathotype determination (Lebeda and Widrlechner 2003).

Wide variability in the reaction with isolates of *P. cubensis* was also shown among wild and weedy *Cucurbita* species ranging from highly resistant to susceptible with the majority of genotypes showing pathotype- or race-specific resistance/susceptibility. The phenomenon of incomplete resistance was also recorded (Lebeda and Widrlechner 2004).

Other important host genera

We have a very limited knowledge of the specificity of interaction between *Citrullus* spp. and *P. cubensis* (Thomas 1970; Thomas et al. 1987c; Cohen et al. 2003; Lebeda and Widrlechner 2003). Within the genus *Citrullus*, significant cultivar variability in the reaction to isolates of *P. cubensis* was observed, with pathotype and race-specificity for *C. lanatus* (Lebeda et al. 2006b). Therefore, this species is included in the original (Thomas et al. 1987c), as well as in the new set of differentials for *P. cubensis* pathotype identification (Lebeda and Widrlechner 2003; Lebeda et al. 2006b).

The interaction of *P. cubensis* with the representatives of the genera *Benincasa*, *Luffa* and *Lagenaria* were studied to a limited extent. In *Benincasa hispida* (the only species of the genus) pathotype-specific resistance to *P. cubensis* was shown (Thomas et al. 1987c; Lebeda and Widrlechner 2003; Lebeda et al. 2006b). A genotype used in the inoculation experiments of Thomas et al. (1987c) was highly resistant to the isolates from Japan, Israel and the USA. In contrast, the genotype included in the new differential set was very susceptible to the European isolates of pathogen (Lebeda and Gadasová 2002; Lebeda et al. 2006b).

Race-specificity is also known in *Luffa cylindrica* and *L. acutangula* (Thomas et al. 1987c; Lebeda et al. 2006b). The genotype of *L. cylindrica* which is part of the differential set for *P. cubensis* pathotype identification (Lebeda and Widrlechner 2003) is resistant to a number of European isolates (Lebeda and Gadasová 2002; Lebeda and Urban 2004a,b; Lebeda et al. 2006b). Thomas et al. (1987c) also reported resistance of this species. However, strong epidemics of downy mildew were recorded in field-grown *Luffa* spp. in China and India (Singh and Singh 1998; Jamadar and Desai 1999; Lebeda and Widrlechner 2003).

Within the genus *Lagenaria*, there is only information about the reactions of *L. siceraria*, for which race-specificity to *P. cubensis* was demonstrated (Lebeda and Widrlechner 2003). The genotype included in the differential set (Lebeda and Widrlechner 2003) showed some susceptibility to European isolates (Lebeda and Gadasová 2002). Thomas et al. (1987c) reported frequent resistance to their isolates.

Genetic aspects in the interaction between Cucurbitaceae and *P. cubensis*

The basic prerequisite for the understanding of the genetic relationships between a host and its pathogen is a parallel genetic study of host resistance and pathogen virulence (Crute 1986). While such detailed studies are available for *Lactuca/Bremia* (Lebeda et al. 2002; Michelmore and Wong 2008), and *Arabidopsis*/*Hyaloperonospora*, only limited information is avilable for other Peronosporaceae, including cucurbits/*P. cubensis* (Göker et al. 2007; Hardham 2007; Lebeda et al. 2008b).

The resistance of plants against oomycetes follows, in general, the heredity rules of Mendel (Lebeda and Schwinn 1994). The pathotype/race specificity in the interactions between cucurbits and *P. cubensis* follow the "gene-for-gene" theory of Flor (Lebeda and Schwinn 1994; Lebeda 1999; Lebeda and Widrlechner 2004). Pathotype/race specific resistance is usually controlled by one or a few major genes (monogenic up to oligogenic heritability). Field resistance against *P. cubensis* which has also been reported in cucurbits (Lebeda and Doležal 1995) might be of polygenic nature. For example, two recessive genes were reported to control a high level of field resistance against *P. cubensis* an F_1 cucumber cultivar (Doruchowski and Lakowska-Ryk 2000).

Basic information on the genetic control of resistance against *P. cubensis* is available for only a few host species (Lebeda and Widrlechner 2003, 2004; Lebeda et al. 2006b). Monogenic (or oligogenic) resistance occurs in *Cucumis sativus* and *C. melo* (Palti and Cohen 1980; Cohen 1981; Lebeda 1999; etc.). The major genes for specific resistance in *C. sativus* probably have a recessive effect (Cohen 1981). The described sources of resistance of *C. melo* have

monogenic or oligogenic character (Cohen 1981; Pitrat 1990; Cohen 1992a; Kenigsbuch and Cohen 1992a, b; Lebeda and Widrlechner 2003). The genetic background of resistance was studied in detail in the accession PI 124111 (*C. melo* var. *reticulatus*), where resistance is probably based on two partially-dominant complimentary genes Pc-1 and Pc-2 (Thomas 1986; Cohen and Eyal 1987). Balass et al. (1993) reported on a high level of resistance in two lines (PI 124111F and 31-10), which were derived from PI 124111. These authors also reported that the genes for resistance were specifically inactivated by low temperature (see below for more details). Kenigsbuch and Cohen (1992a) reported that resistance in another accession PI 124112 (*C. melo* var. *reticulatus*) is controlled by two partially-dominant complementary genes Pc-1 and Pc-3. However, a polygenic control was described for this accession by others (Epinat and Pitrat 1994a,b; Perchepied et al. 2005). Generation mean analysis of resistance to *P. cubensis* in adult *C. melo* plants showed that genetic dominance has a greater importance for expression of resistance. There were observed expression of significant and positive additive gene effects, estimates of heritability were high. Therefore some inbred lines could be used to exploit heterotic effects (Shashikumar et al. 2010). The genetics of host resistance to *P. cubensis* in other cucurbits (e.g. *Cucurbita* spp., *Citrullus* spp.) is still unknown (Lebeda et al. 2007b; Paris 2008).

The genetic basis of virulence variability of *P. cubensis* is known to a very limited extent (Lebeda et al. 2006b). From this aspect, the best known oomycete pathogen is *Bremia lactucae* (Michelmore and Wong 2008). In general, virulence to each host genotype/cultivar is usually determined by one recessive independent allele on one locus (Lebeda and Schwinn 1994). Thomas et al. (1987c) suggested that the significant genetic variability in *P. cubensis* derives from its diploid nature. The double set of genes enables a wider range of responses of *P. cubensis* to selective stresses and therefore increases the ability of adaptation to specific hosts. So far, the contribution of oospores to genetic recombinations of *P. cubensis* is not known because of their rare occurrence.

Currently, a three-fold integrative approach has been undertaken to identify the genetic basis for enhanced virulence observed in recent isolates of *P. cubensis*. This approach involves the following steps: 1) phylogenetic characterization and sequencing of *P. cubensis* isolates, and determination of the genetic basis of virulence; 2) identification of genes which are differentially expressed following infection of *P. cubensis*; 3) isolation of single host cells at various stages of pathogen infection and profiling gene expression during pathogen invasion. It is expected that this approach will provide a framework for determining the basis of pathogenicity and susceptibility in the cucumber/*P. cubensis* interaction (Savory et al. 2008).

Cellular and molecular aspects of interaction

The recognition (compatibility/incompatibility) between a host plant and its oomycete pathogen is determined shortly after infection structures have developed in the plant cell (Lebeda et al. 2008b; Bouwmeester et al. 2009). Recognition occurs when some individual structural constituents of an incompatible pathogen (Pathogen Molecular Patterns, or PAMP) are detected by specific diagnostic molecules of a resistant plant. The detection of "foreign structures (molecules)" induces a sequence of quick biochemical processes that lead to the termination of further growth of the pathogen. If a pathogen is not recognized as "foreign", it results in a compatible relationship with the host, colonizes its tissues and reproduces (Lebeda et al. 2008b; de Jong and van den Ackerveken 2009).

As with *Bremia lactucae*, in which the recognition occurs during the interaction between host plasmalemma and the primary infecting structures of the pathogen (primary and secondary vesicules, haustoria) (Lebeda et al. 2008b; de Jong and van den Ackerveken 2009) so is the recognition between *P. cubensis* and its hosts.

The response of resistant genotypes of cucurbit plants to penetration by *P. cubensis* is often characterized by a hypersensitive response (Cohen et al. 1989; Balass et al. 1993; Eckardt 2004; Taler et al. 2004). Cohen et al. (1989) followed the development of *P. cubensis* in suceptible and resistant *C. melo* by using electron microscopy (Fig. 3). These authors found that the pathogen failed to penetrate into the mesophyll cells of the resistant host due to massive accumulation of callose, not only along the host cell walls but also along the inner wall surface of pathogen mycelia. This failure was also accompanied by a massive accumulation of dark, dense material in the cytoplasm of host cells which leads to necrosis. The structural and biochemical responses were studied in the interaction of *Cucumis*

melo with *P. cubensis* (Balass et al. 1993). These authors reported that resistance was accompanied by extensive accumulation of phenolics, callose (Fig. 6) and lignin in the infected sites which probably limit the growth of *P. cubensis* mycelium. These changes were accompanied by a rapid increase in peroxidase activity (Balass et al. 1993). Peroxidase activity was used in melon as a marker for resistance to *P. cubensis* (Reuveni et al. 1990; Lebeda and Doležal 1995). No such changes occurred in susceptible genotypes of *C. melo* upon inoculation with *P. cubensis* (Balass et al. 1993).

Resistance of *Cucumis melo* against *P. cubensis* was temperature-dependent (Balass et al. 1993), a phenomenon quite rare in cultivated plants. The resistance was fully expressed at higher temperatures (21–25°C) but was nullified at lower temperatures (12–15°C), perhaps as a result of specific inactivation of transcription of the resistance genes (Pc-1 and Pc-2) at low temperatures (Balass et al. 1993). It should be noted that the effect of temperature on resistance occurred during the first half of the incubation period when the above-mentioned defense mechanisms were launched.

These results gave rise to the idea that resistance was controlled by the metabolic activity of the host. Indeed, in a later study, Taler et al. (2004) showed that resistance in *C. melo* PI124111F, which carries the resistance genes Pc-1 and Pc-2, is metabolic, resulting from enhanced activity of peroxisomal glyoxylate aminotransferase encoded by the genes *At1* and *At2*. When either gene was transformed into a susceptible plant it became resistant (Fig. 7). These authors speculated that Pc-1 and Pc-2 are in fact *At1* and *At2*. Such resistance, which is primarily conferred by enzymatic activity (Lebeda et al. 2001a), was described as "enzymatic resistance" to downy mildew (Eckardt 2004; Taler et al. 2004). In a more recent study, Benjamin et al. (2009) showed that in susceptible genotypes of *C. melo*, *At1* and *At2* are present but not expressed. Over-expression of these glyoxylate-aminotransferase genes in cisgenic melons makes them resistant to downy mildew (Benjamin et al. 2009).

Pathogenesis of *P. cubensis* on cucumber leaves resulted in metabolic changes, including transpiration rate and increased leaf temperature, depending on the stage of pathogen development and disease severity. Spatial and temporal changes in the transpiration rate

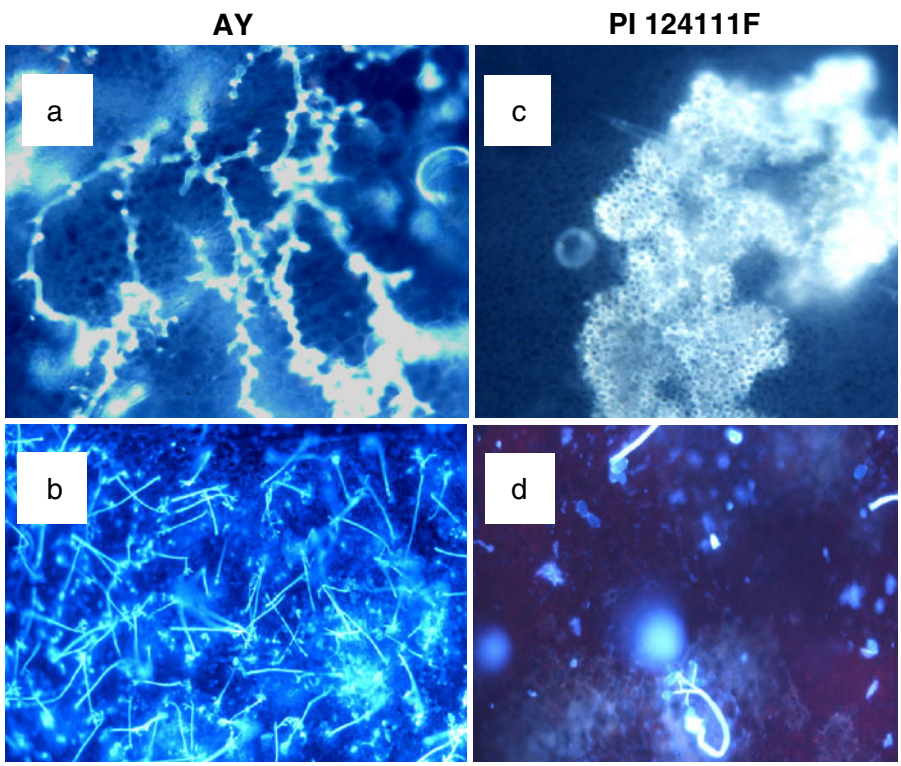

Fig. 6 Epifluorescence micrographs of the development of *Pseudoperonospora cubensis* in *Cucumis melo*: AY (Ananas Yokneam), susceptible and PI124111F, resistant (modified from Cohen et al. 1989). **a** and **c** at 4 days post inoculation (dpi), **b** and **d**, at 7 dpi. Leaves were clarified with ethanol and placed in basic aniline blue and then calcofluor. Note mycelium and sporulation in AY. Note the hypersensitive response (HR) with callose and no sporulation in PI124111F

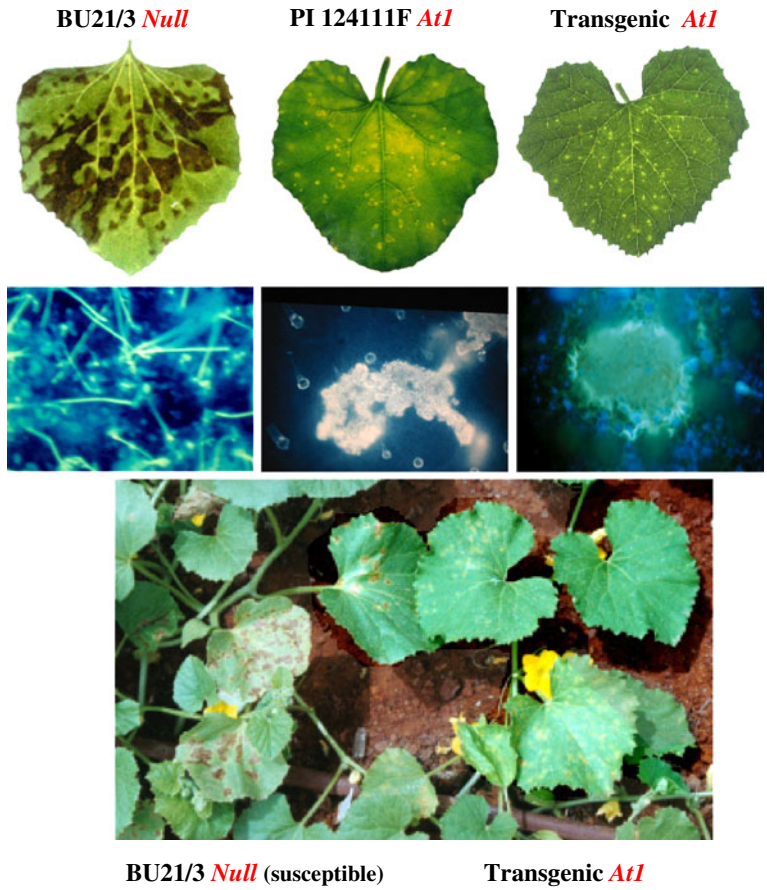

Fig. 7 Symptomology of *Pseudoperonospora cubensis* on susceptible, resistant and transgenic genotypes of *Cucumis melo* (modified from Taler et al. 2004). Upper panel: symptom of downy mildew on melon leaves of susceptible BU21/3, resistant PI124111F and transgenic line. The resistance gene *At1* was transferred from PI124111F to the transgenic line. Middle panel: epifluorescent micrographs of respective leaves after staining with calcofluor for sporangiophores and basic aniline blue for callose. Bottom panel: natural infection with downy mildew of the susceptible and the transgenic line in the field

of infected and noninfected leaf tissue could be visualized by digital infrared thermography (DIT) (Lindenthal et al. 2005). Due to the negative correlation between transpiration rate and leaf temperature (quantified by the maximum temperature difference, MTD), DIT permits a non-invasive monitoring and an indirect visualization of downy mildew development. However, MTD alone is not suitable for quantification of downy mildew severity in the field (Oerke et al. 2006).

Methods of crop protection

Forecasting is an efficient aid in control of *P. cubensis* (Main et al. 2001). The parameters used in forecasting and their integration in protection of cucurbits against *P. cubensis* are summarized in a few papers (Holmes et al. 2004; Lebeda and Urban 2005; Urban and Lebeda 2006). Monitoring the occurrence and movement of the pathogen enables the prediction of disease outbreaks in specific areas and the application of suitable control measures prior to infection (Holmes et al. 2004; Ojiambo et al 2009; Zhao et al. 2007).

Prevention and agrotechnical aspects

Knowing the biology and ecology of the pathogen may serve in preventing the disease (see above). The basic abiotic factor influencing infection with *P. cubensis* is free leaf moisture. A preventive precaution therefore should lead to elimination of free water from the leaves. Cohen (1977) showed that as the inoculum load increases and temperature approaches optimum, the shorter the leaf wetness duration required for infection. The surface of leaves should not be wet for more than 2–3 h (Cohen 1981). Leaf wetness can be partially controlled in sheltered vegetation by using drip irrigation instead of overhead irrigation, frequent ventilation, and heating before sunrise. Also, under field conditions, drip irrigation is preferable, but dew formation and rain cannot be

avoided. Earlier sowing of the crop and decreased plant density can also contribute to the reduction of infection to the extent that it reduces leaf wetness duration (Palti and Cohen 1980). High vegetation density increases the risk of infection as it increases humidity for prolonged periods, stimulates sporulation of *P. cubensis*, and facilitates transfer of sporangia among plants (Lebeda 1990).

Breeding for resistance, classical and transgenic approaches

Availability of sources of resistance and using appropriate methods for testing resistance are among the basic requirements for successful breeding for resistance. There are significant differences in the availability of resistance sources among the most important cucurbits. Most sources are available for *Cucurbita pepo* and *Cucumis melo* (Lebeda 1999; Lebeda et al. 2007a, b; Pitrat 2008; Staub et al. 2008). Recently, great progress has been made in the knowledge of resistance in *Cucurbita* spp. (Lebeda and Křístková 2000; Lebeda and Widrlechner 2004; Lebeda et al. 2006b), although the availability of genotypes with effective and broad levels of resistance is still limited (Ferriol and Picó 2008; Paris 2008). Rather little information is available on resistance against *P. cubensis* in *Citrullus*, *Benincasa*, *Luffa* and *Lagenaria* (Lebeda and Widrlechner 2003). Resistance breeding of watermelon (*Citrullus lanatus*) is still not very well developed (Wehner 2008). The breeding of cucurbits for resistance against *P. cubensis* is further complicated due to the great variability in the pathogen population, pathotypes and races (Lebeda et al. 2006b). In response to recent epidemics, there is an intensified cucumber breeding effort in the USA to develop resistance to downy mildew (Holmes et al. 2006).

Cucumis sativus

Breeding of cucumber for resistance against *P. cubensis* was initiated in Puerto Rico in the 1930s to 1940s by systematic searching for resistance sources (Sitterly 1972). Some resistant/tolerant plants were discovered under field conditions in the materials originating from China and India: Chinese Long from China and Bangalore and accession PI197087 from India (Sitterly 1972; Peterson 1975). These genotypes served, mainly in USA, as the background of cucumber resistance breeding to *P. cubensis* (Staub et al. 2008).

Besides the USA, breeding for resistance also took place in Japan (Ezuka and Komada 1974), Cuba (Pivovarov 1984; Pivovarov and Kudelich 1985), USSR (Medvedeva and Medvedev 1983), and since 1985 also in Czechoslovakia (Lebeda 1990, 1999; Lebeda and Prášil 1994) and in Poland (Doruchowski and Lakowska-Ryk 2000). Unfortunately, no reliable sources of resistance were found in *C. sativus*, and therefore, cucumber cultivars with genetically fixed and efficient resistance were not produced (Lebeda 1991, 1992a; Lebeda and Prášil 1994; Lebeda and Widrlechner 2003; Lebeda et al. 2006b).

The available commercial cultivars do not posses the character of complete incompatibility (resistance). They only allow for a limited level of pathogen sporulation (Lebeda 1999). Resistance in cucumber was reported decades ago (e.g. Cohen 1981), however, in many cultivars (e.g., Palmetto) a relatively rapid breakdown occurred followed by serious infection with *P. cubensis* (Lebeda 1990). One notable exception to this was the durability of resistance in cucumber in the USA. Downy mildew resistant cultivars developed in the late 1969s remained sufficiently resistant to downy mildew that fungicides were not necessary to control the disease until 2004 (Holmes and Thomas 2009). The screening of large sets of *C. sativus* germplasm and cultivars provided no single genotype displaying complete incompatibility to current pathotypes (Lebeda 1992a; Lebeda and Prášil 1994). Differences in field resistance were, nevertheless, found (Table 4), characterized by a delay (7–14 days) in the onset and a slower rate of disease progress under strong infection pressure (Lebeda 1999; Lebeda and Doležal 1995).

Recent achievements in cucumber genome mapping and sequencing (Huang et al. 2009; Ren et al. 2009) provides the new opportunities for research, breeding and development of elite cucumber cultivars with new traits, as well as resistance to diseases and pests.

Cucumis melo

Within the cucurbits, breeding for resistance against *P. cubensis* was most comprehensively elaborated in muskmelon (*Cucumis melo*) (Lebeda et al. 2007a, b; Pitrat 2008). The first resistance research was realized

in the 1940s in the USA, where four cultivars (Cuban Castilian, Green Fleshed Rocky Dew, Orange Fleshed Rocky Dew and Smith´s Perfect) with high levels of resistance against *P. cubensis* were described (Ivanoff 1944). A highly resistant cultivar (Tainan 2 /PI 321005/) was further released in Taiwan (Sowell and Corley 1974). Currently, the most significant two sources of resistance are the accessions of *C. melo* var. *reticulatus* PI 124111 (Balass et al. 1992, 1993; Cohen 1981; Thomas 1982, 1986; Cohen and Eyal 1987; Kenigsbuch and Cohen 1989; Lebeda 1991, 1999; Lebeda et al. 2007a) and PI 124112 (Lebeda 1991; Kenigsbuch and Cohen 1992a, b; Table 5). Both genotypes originated from Calcutta, India and became the background for the resistance breeding of muskmelon against *P. cubensis* in the USA (Cohen 1981). The cultivars derived from PI 124111 (e.g. the line MR-1) (Table 5) displayed high levels of resistance under natural infection conditions, with attacked plants showing only minute yellow lesions (1–2 mm) with no sporulation (Lebeda and Doležal 1995). However, experiments with artificial inoculations proved also to have race-specific resistance against *P. cubensis* in PI 124111 and MR-1 (Lebeda 1991; Lebeda et al. 2007a).

Resistance of MR-1 was differentially expressed in whole leaves as against leaf discs. Detached whole leaves showed resistance against all tested isolates of *P. cubensis*, whereas the reactions of leaf discs were heterogeneous (some plants displayed resistance while others displayed susceptibility) (Lebeda 1991).

In *C. melo*, there are cultivars with significant levels of field resistance. In Israel, valuable resistance was found under field conditions in three genotypes, including PI 124111, among 19 genotypes tested (Cohen 1981). Nevertheless, the breeding for field resistance in muskmelon is not well developed (Paris 2008).

Attempts to transfer the resistance from *C. melo*, mainly that derived from MR-1, into cucumber was done by using biotechnological approaches (embryo-cultures, protoplast fusion) (Fellner et al. 1996; Lebeda et al. 1996, 1999; Gajdová et al. 2004; Skálová et al. 2004), however, without substantial success.

A successor of PI 124111 that carries resistance to Fusarium wilt races 0, 1 and 2 was named PI 124111 F (Cohen and Eyal 1987). The ultrastructure of resistance against *P. cubensis* in this line revealed (Cohen et al. 1989) intensive callose and lignin deposition in the inoculated sites, which prohibited any growth of the pathogen. Resistance of this line to *P. cubensis* was found to be temperature-dependent: it operates at ≥15°C (Balass et al. 1993). PI 124111 F and its descendent 31/10 (F_{10} from a cross between Hemed and PI 124111 F) carries a specific protein, P45. This protein co-segragated with resistance (Balass et al. 1993) and does not show up in susceptible melons. Taler et al. (2004) discovered that P45 is a peroxisomal glyoxylate aminotransferase. They succeeded in cloning the genes *At1* and *At2* which are responsible for the synthesis of P45 and to transfer them into a susceptible melon under an S35 promoter. The transgenic plants, carrying either *At1* or *At2*, were completely resistant to *P. cubensis*. Recently, Benjamin et al. (2009) showed that *At1* or *At2* occur in susceptible melons but are not

Table 5 Germplasm of *Cucumis melo* with resistance against *P. cubensis* and some other diseases (modified according to Lebeda 1999)

Cucumis melo (accession)	Country of origin	Resistance against disease	Genes of resistance	References
C. melo (PI 124111 /PI 124111F)	India	DM, PM, F	*Pc-1, Pc-2, Pm-3, Pm-6*	Thomas (1986), Cohen and Eyal (1987)
C. melo (PI 124112)	India	DM, PM	*Pc-1, Pc-3, Pm-4, Pm-5*	Pitrat (1990), Cohen (1992a, b), Kenigsbuch and Cohen (1992a)
C. melo var. *acidulus* (PI 200819)	Burma	DM, F	?	Lebeda (1999), Widrlechner (1999, pers. comm.)
C. melo (PI 321005)	Thaiwan	DM, GSB	?	Lebeda (1999), Widrlechner (1999, pers. comm.)
C. melo (line MR-1 derived from PI 124111)	USA	DM, PM, F, A	*Pc-1, Pc-2, Pm-3, Pm-6, Fom-1, Fom-2, Ac*	Thomas (1986), Cohen and Eyal (1987), Pitrat (1990), Lebeda (1991), Cohen (1992a, b), Kenigsbuch and Cohen (1992b)
C. melo var. *agrestis* (CGN 2365)	Kenya	DM	?	Lebeda (1999)

DM, *Pc Pseudoperonospora cubensis*, PM, *Pm Podosphaera xanthii*, F, *Fom Fusarium oxysporum* f. sp. *melonis*, A, *Ac Alternaria cucumerina*, GSB = *Didymella bryoniae*

transcribed. When overexpressed in cisgenic plants under a strong promoter plants become resistant (Fig. 7).

In spite of this enormous progress in understanding the resistance of *C. melo* to *P. cubensis*, there is no germplasm or cultivars of *C. melo* with complete resistance to all known pathotypes/races of *P. cubensis* (Lebeda et al. 2006b, 2007a; Pitrat 2008).

Cucurbita spp.

The most important information related the host-pathogen interaction and resistance of *Cucurbita* spp. to *P. cubensis* was summarized above. It is evident that currently there are not enough data about sources of resistance in *Cucurbita* spp. against *P. cubensis*, including characteristics of commercial cultivars. Studies in Japan showed that *C. pepo* cultivar Soumen displayed a high level of field resistance against the isolates of *P. cubensis* from *Cucumis sativus* and *C. melo* (Inaba et al. 1986). Studies in Czechoslovakia showed the occurrence of incomplete resistance and frequent display of race-specific resistance in zucchini and squash cultivars (Lebeda and Křístková 1992, 1993, 2000). Efficient sources of resistance were found in wild and weedy accessions of *Cucurbita* spp., e.g. *Cucurbita foetidissima*, *C. argyrosperma* var. *palmeri* a *C. argyrosperma* var. *sororia* (Lebeda and Widrlechner 2004). However, there is relatively little effort in breeding for resistance in pumpkin and squash (Ferriol and Picó 2008; Paris 2008).

Other cucurbitaceae

Our knowledge on resistance of *Citrullus* spp. to *P. cubensis* is rather limited (Lebeda and Widrlechner 2003). The most important and comprehensive data were reported by Winstead et al. (1957) who found two highly resistant accessions (PI 179660 and PI 179875) amongst a collection of 300 genotypes of *C. lanatus*. A high level of field resistance was reported by Thomas (1970) in cv. Charleston. Currently, breeding of watermelon for resistance to *P. cubensis* is not considered an important goal because of the limited occurrence of the disease (Wehner 2008). This is the main reason why resistant cultivars are not used as a measure in disease control. This conclusion is true also for *Benincasa*, *Luffa* and *Lagenaria* (Lebeda and Widrlechner 2003).

Chemical control

Although chemical control by fungicides may have negative environmental effects and limitations (de Waard et al. 1993), fungicides still constitute the predominant part of the control measures used against oomycetes (Cohen and Coffey 1986; Lebeda and Schwinn 1994; Gisi 2002; Gisi and Sierotzki 2008). According to Gisi (2002) the sales value of fungicides against downy mildews (Table 6) amounted to 1.2 billion SFr in 1996, of which 10% were used to fight mainly *P. cubensis* on cucurbit crops (Urban and Lebeda 2006). Chemical control of *P. cubensis* was done for many decades by contact fungicides, copper formulations at early times, and dithiocarbamates more recently (Palti and Cohen 1980; Cohen 1981). These fungicides prevent zoospore release and cystospore germination. Their application is effective only if done before infection, possibly before sporangial deposition (Urban and Lebeda 2006). Streptomycin, that suppressed tissue colonization by *P. cubensis* (Cohen 1981), is not used in practice.

During the last decades, new, mostly systemic, fungicides have been developed and widely used for disease control (e.g., cymoxanil in 1976, phosetyl-Al in 1977, phenylamides in 1977–1983, propamocarb in 1978, dimethomorph in 1988, cyazofamid in 2001, zoxamide in 2001, mandipropamid in 2005, fluopicolide in 2006). Most systemic fungicides have a specific, single-site mode of action, which means that they are active at one point in one metabolic pathway of the pathogen (Gisi 2002; Urban and Lebeda 2006). The introduction of these systemic fungicides significantly increased the efficiency of plant protection against downy mildews (Cohen and Coffey 1986; Lebeda and Schwinn 1994; Gisi 2002; Cohen et al. 1995; Holmes and Ojiambo 2009; Wang et al. 2009). Systemic fungicides also have some curative effects, as they can stop the development of disease for a certain time after infection. Systemic fungicides quickly translocate in the plant even to the parts not directly treated (Urban and Lebeda 2006). Metalaxyl (and its active enantiomer mefenoxam), oxychloride Cu, propamocarb, prothiocarb and fosetyl-Al are among the most effective substances (Gisi 2002; Tomlin 2003; Urban and Lebeda 2004, 2006, 2007). Fluopicolide is currently the most effective product in the USA (Holmes and Ojiambo 2009).

Systemic fungicides bear a high risk of resistance development in the pathogen. Furthermore, *P. cubensis*

Table 6 Fungicides used against *P. cubensis* and several other oomycete pathogens (modified according to Urban and Lebeda 2006)

Level of systemicity	Cross-resistance group	Common name of compound	Type of activity/ translocation behavior within plants	Biochemical and physiological mode of action
Fully systemic	Phenylamides	Metalaxyl Mefenoxam Benalaxyl Ofurace	Preventive, curative, eradicative/apoplastic, symplastic, translaminar	Inhibition of rRNA synthesis
	Phosphonates	Phosetyl-Al	Preventative, curative/ apoplastic, symplastic	Inhibition of spore germination, retardation of mycelia development and sporulation, induction of host resistance
	Carbamates	Propamocarb Prothiocarb	Preventative, eradicative/ apoplastic	Multisite inhibitor, affect membrane permeability
	Cyano-acetamide oximes	Cymoxanil	Preventative, curative/ apoplastic, symplastic, translaminar	(?)
	Benzamide*	Fluopicolide	Preventative, curative/ apoplastic, symplastic, translaminar	Delocalisation of spectrin-like proteins
Partially systemic	Cinnamic acids	Dimethomorph	Preventative, curative, eradicative/mainly translaminar	Inhibits cell wall synthesis
	Complex III respiration inhibit. (QoI)	Azoxystrobin Fenamidone Trifloxystrobin Kresoxim-methyl Pyraclostrobin	Preventative/translaminar apoplastic (azoxystrobin, fenamid.), "mesostemic" (trifloxystrobin), "quasi-systemic" (kresoxim-methyl)	Inhibit mitochondrial respiration at the enzyme complex III (Qo site)
	Complex III respiration inhibit. (QiI)	Cyazofamid	Preventative, curative, eradicative/translaminar	Inhibit mitochondrial respiration at the enzyme complex III (Qi site)
	Amino acid amide carbamates	Iprovalicarb	Preventative, curative, eradicative/apoplastic, symplastic	Affect cell wall deposition (?)
Non-systemic	Dinitroanilines	Fluazinam	Preventative /-	Inhibit ATP production
	Miscellaneous	Zoxamide	Preventative /-	(?)
	Multisites inhibitors	Inorganic copper fungicides (Cu-oxychloride, Cu-hydroxide)	Preventative /-	Multisite inhibitor
		Organic dithiocarbamate fungicides (e.g. mancozeb)	Preventative /-	Multisite inhibitor
		Chlorothalonil	Preventative /-	Multisite inhibitor
		Folpet	Preventivní /-	Multisite inhibitor
		Other multisites		

(?) not well and/or exactly known

* Anonymous (2006)

is considered by FRAC as one of 10 plant pathogens accepted as showing a high risk of resistance development to fungicides (Pathogen risk list 2005, http://FRAC.info). The introduction of systemic fungicides evoked selection of strains resistant against that fungicide in field pathogen populations (Urban and Lebeda 2006). This occurred first with phenylamide-based products (e.g. Ridomil which contains metalaxyl) against which resistance was developed very shortly after their introduction to the market (Lebeda and Schwinn 1994). The first report of resistance in oomycetes against phenylamides was reported in Israel in 1979, just two years after the introduction of metalaxyl for the control of *P. cubensis* (Reuveni et al. 1980).

During the last 20 years, resistance of *P. cubensis* against other fungicides was also recorded (Table 7) (Urban and Lebeda 2006, 2007; Zhu et al. 2008; Okada and Furukawa 2008; Zhang et al. 2008; Olaya et al. 2009).

Biological control

There is only one report on biological control of downy mildew in cucurbits. Korbel (1990) showed that the treatment of cucumber seeds and spraying the leaves with the mycoparasitic fungus *Pythium oligandrum* (Veselý 1977) delayed the primary infection of the leaves and leaves remained viable longer (Korbel 1990). However, efficiency of protection was relatively low under high infection pressure and thus, this practice is of limited commercial application.

Extracts made from dry leaves of the perennial composite *Inula viscosa* were shown to be effective against several foliar fungal pathogens, including *P. cubensis* (Wang et al. 2004). These extracts are antifungal, inhibiting zoospore release and cystospore germination (Cohen, unpublished). Such extracts are in the process of registration for organic farming. Also the volatile antimicrobial substance allicin (diallylthiosulphinate) from garlic (*Allium sativum*), at concentrations 50–1000 $\mu g ml^{-1}$, reduced the severity of *P. cubensis* on cucumber by approximately 50–100% (Portz et al. 2008).

The non-protein amino acid BABA (β-aminibutyric acid) induces resistance against many diseases in various crops (Cohen 2002a) including *P. cubensis* in cucumber (Ovadia et al. 2000; Walz and Simon 2008). BABA has no direct effect on the pathogen. Rather, it activates host defense (Cohen 2002a; Walz and Simon 2008). BABA was shown to synergize with mancozeb in controlling *P. cubensis* in cucumber (Baider and Cohen 2003).

In the future, it seems that chemically and biologically mediated systemic resistance in cucurbits against *P. cubensis* and some other fungal pathogens (Anand et al. 2007) could be efficient part of Integrated Pest Management (IPM) which is currently implemented for plant disease control to reduce use of fungicides (Lebeda and Schwinn 1994; Urban and Lebeda 2006).

Table 7 Occurrence of strains resistant/tolerant to fungicides in *Pseudoperonospora cubensis* (modified according to Urban and Lebeda 2006; Olaya et al. 2009; Cohen unpublished)

Chemical group/ chemical class	Common name	Countries where resistant/ tolerant strains occurred
Phenylamides	Metalaxyl	Israel (1980)*
		Greece (1981)
		Italy (1985)
		USA (1987)
		USSR (1992)
		Australia (1995)
		Czech Republic (1990 /2004/)
Strobilurins	Azoxystrobin, Kresoxim-methyl, Pyraclostrobin	Japan (1999)
		Taiwan (2001)
		USA (2004)
Phosphonates	Fosetyl-Al	Israel (1984)
		Czech Republic (2004)
Carbamates	Propamocarb	Israel (1984)
Phthalimides	Folpet	Israel (1984)
Dithiocarbamates	Mancozeb	Israel (1984)
Carboxyic acid amides	Dimethomorph	Israel (2006)
	Mandipropamid Iprovalicarb	USA (2007)
	Benthiavalicarb	

* = the year of first described occurrence

Conclusions

Since the publication of the previous reviews (Palti and Cohen 1980; Cohen 1981; Lebeda 1990; Lebeda et al. 2006a, b) extensive knowledge has been gained on the biology and ecology of *P. cubensis*, the variability of interactions between *P. cubensis* and its hosts, sources of resistance and resistance mechanisms, breeding for resistance, and disaese control. One area that has not been well addressed for *P. cubensis* and very little is available in the literature (Sarris et al., 2009) is the genetic diversity of *P. cubensis* and to what extent our understanding of this part of the biology has shaped our effort to effectively manage cucurbit downy mildew. This should be considered as an area that research efforts needs to be concentrated.

Further research should be directed to the following areas:

1) Genome sequencing of *P. cubensis*;
2) Sexual reproduction and strategies of overwintering of *P. cubensis*;
3) Host range of *P. cubensis* on cultivated and wild species of Cucurbitaceae;

4) Development of an internationally-accepted system for pathotype and race determination and denomination;
5) Genetics of pathotype-specific and race-specific resistance/susceptibility;
6) Mechanisms of host resistance: histological, cytological, physiological, biochemical and molecular;
7) Discovery of effective sources of resistance, including their transfer to breeding materials;
8) Understanding the pathogenic variability and resistance to fungicides;
9) Comparative epidemiology and forecasting of downy mildew in cucurbits;
10) IPM of downy mildew in cucurbits including effective fungicides and alternative control measures.

Acknowledgements The authors thank G. J. Holmes (North Carolina, USA) for his valuable critiques of our report. Preparation of this review was supported by grants MSM 6198959215 and QH 71229. Some *P. cubensis* isolates used in this research are deposited in the Czech National Collection of Microorganisms (http://www.vurv.cz/collections/vurv.exe/) at Palacký University in Olomouc, Department of Botany (http://botany.upol.cz).

References

Ackermann, P. (1990). Výskyt a ochrana proti plísni okurkové v Československu (Occurrence and protection against cucurbit downy mildew in Czechoslovakia). In A. Lebeda (Ed.) *Plíseň okurková* (*Cucurbit downy mildew*) (pp. 51–61). Praha, Czechoslovakia: Československá vědecká společnost pro mykologii při ČSAV (Czechoslovak Scientific Society for Mycology by Czechoslovak Academy of Sciences).

Anand, T., Raguchander, T., Karthikeyan, G., Prakasam, V., & Samiyappan, R. (2007). Chemically and biologically mediated systemic resistance in cucumber (*Cucumis sativus* L.) against *Pseudoperonospora cubensis* and *Erysiphe cichoracearum*. *Phytopathologia Mediterranea, 46*, 259–271.

Angelov, D., Georgiev, P., & Krasteva, L. (2000). Two races of *Pseudoperonospora cubensis* on cucumbers in Bulgaria. *Acta Horticulturae, 510*, 81–83.

Anonymous (2006). Pflanzeschutz Nachrichten Bayer, 59, 2–3 (http://www.bayercropscience.com/BCSWeb/ CropProtection.nsf/id/EN1_Journal).

Baider, A., & Cohen, Y. (2003). Synergistic interaction between BABA and Mancozeb in controlling *Phytophthora infestans* in potato and tomato and *Pseudoperonospora cubensis* in cucumber. *Phytoparasitica, 31*, 399–409.

Bains, S. S., & Jhooty, J. S. (1978). Relationships between mineral nutrition of muskmelon and development of downy mildew caused by *Pseudoperonospora cubensis*. *Plant and Soil, 49*, 85–90.

Bains, S. S., & Sharma, N. K. (1986). Differential response of certain cucurbits to isolates of *Pseudoperonospora cubensis* and characteristics of identified races. *Phytophylactica, 18*, 31–33.

Balass, M., Cohen, Y., & Bar-Joseph, M. (1992). Identification of a constitutive 45 kD soluble protein associated with resistance to downy mildew in muskmelon (*Cucumis melo* L.) line PI 124111F. *Physiological and Molecular Plant Pathology, 41*, 387–396.

Balass, M., Cohen, Y., & Bar-Joseph, M. (1993). Temperature-dependent resistance to downy mildew in muskmelon: structural responses. *Physiological and Molecular Plant Pathology, 43*, 11–20.

Bedlan, G. (1989). Erstmaliger Nachweis von Oosporen von *Pseudoperonospora cubensis* (Berk. et Curt.) Rost. an Gewächshausgurken in Österreich. *Pflanzenschutzberichte, 3*, 119–120.

Benjamin, I., Kenigsbuch, D., Galperin, M., Abrameto, J. A., & Cohen, Y. (2009). Cisgenic melons over expressing glyoxylate-aminotransferase are resistant to downy mildew. *European Journal of Plant Pathology, 125*, 355–365.

Bjoern, G. K., & Kampmann, H. H. (2000). Screening field Riesenchäel cucumbers for resistance to downy mildew: management of interplot interference problems. *Acta Horticulturae, 510*, 77–80.

Bouwmeester, K., van Poppel, P. M. J. A., & Govers, F. (2009). Genome biology cracks enigmas of oomycete plant pathogens. *Annual Plant Reviews*, *34*, 102–133. Oxford, UK: Wiley-Blackwell.

Caten, C. E. (1987). The concept of race in plant pathology. In M. S. Wolfe & C. E. Caten (Eds.), *Populations and plant pathogens: Their dynamics and genetics* (pp. 21–37). Oxford, UK: Blackwell Scientific Publications.

Choi, Y.-J., & Shin, H.-D. (2008). First record of downy mildews caused by *Pseudoperonospora cubensis* on bottle gourd in Korea. *Plant Pathology, 57*, 371.

Choi, Y.-J., Hong, S.-B., & Shin, H.-D. (2005). A re-consideration of *Pseudoperonospora cubensis* and *P. humuli* based on molecular and morphological data. *Mycological Research, 109*, 841–848.

Cohen, Y. (1976). Quantitation of resistance of cucumbers and cantaloups to *Pseudoperonospora cubensis*. *Phytoparasitica, 4*, 25–31.

Cohen, Y. (1977). The combined effects of temperature, leaf wetness, and inoculum concentration on infection of cucumbers with *Pseudoperonospora cubensis*. *Canadian Journal of Botany, 55*, 1478–1487.

Cohen, Y. (1981). Downy mildew of cucurbits. In D. M. Spencer (Ed.), *The downy mildews* (pp. 341–354). London: Academic.

Cohen, Y. (1992a). Genes and mechanisms of resistance againts fungal pathogens breeding lines of muskmelon. In R. W. Doruchowski, E. Kozik, & K. Niemirowicz-Suczytt (Eds.), *Proceedings of Fifth Eucarpia Cucurbitaceae Symposium* (pp. 139–141). Poland: Skierniewice.

Cohen, Y. (1992b). Determination of the sensitivity of *Pseudoperonospora cubensis* to phenylamides. *EPPO Bulletin, 22*, 318–320.

Cohen, Y. (2002a). β-aminobutyric acid-induced resistance against plant pathogens. (Feature Article). *Plant Disease, 86*, 448–457.

Cohen, Y. (2002b). Populations of *Phytophthora infestans* in Israel underwent three major genetic changes during 1983–2000. *Phytopathology, 92*, 300–307.

Cohen, Y., & Coffey, M. D. (1986). Systemic fungicides and the control of Oomycetes. *Annual Review of Phytopathology, 24*, 311–338.

Cohen, Y., & Eyal, H. (1977). Growth and differentiation of sporangia and sporangiophores of *Pseudoperonospora cubensis* on cucumber cotyledons under various combinations of light and temperature. *Physiological Plant Pathology, 10*, 93–103.

Cohen, Y., & Eyal, H. (1987). Downy mildew-, powdery mildew- and Fusarium wilt-resistant muskmelon reeding line PI-124111F. *Phytoparasitica, 15*, 187–195.

Cohen, Y., & Rotem, J. (1971a). Dispersal and viability of sporangia of *Pseudoperonospora cubensis*. *Transactions of British Mycological Society, 57*, 67–74.

Cohen, Y., & Rotem, J. (1971b). Field and growth chamber approach to epidemiology of *Pseudoperonospora cubensis* on cucumbers. *Phytopathology, 61*, 736–737.

Cohen, Y., Perl, M., & Rotem, J. (1971). The effect of darkness and moisture on sporulation of *Pseudoperonospora cubensis* in cucumbers. *Phytopathology, 61*, 594–595.

Cohen, Y., Eyal, H., Hanania, J., & Malik, Z. (1989). Ultrastructure of *Pseudoperonospora cubensis* in muskmelon genotypes susceptible and resistant to downy mildew. *Physiological and Molecular Plant Pathology, 34*, 27–40.

Cohen, Y., Baider, A., & Cohen, B. H. (1995). Dimethomorph activity against oomycete fungal pathogens. *Phytopathology, 85*, 1500–1506.

Cohen, Y., Meron, I., Mor, N., & Zuriel, S. (2003). A new pathotype of *Pseudoperonospora cubensis* causing downy mildew in cucurbits in Israel. *Phytoparasitica, 31*, 458–466.

Colucci, S. J. (2008). Host range, fungicide resistance and management of *Pseudoperonospora cubensis*, causal agent of cucurbit downy mildew. MS Thesis, North Carolina State University, Raleigh, USA

Constantinescu, O. (2000). The fine structure of the sporangium in *Pseudoperonospora humuli* (Chromista, Oomycota, Peronosporales). *Cryptogamic Mycology, 21*, 93–101.

Cooke, O. P. R., & Lees, A. K. (2004). Markers, old and new, for examining *Phytophthora infestans* diversity. *Plant Pathology, 53*, 692–704.

Crute, I. R. (1981). The host specificity of peronosporaceous fungi and the genetics of the relationship between host and parasite. In D. M. Spencer (Ed.), *The downy mildews* (pp. 45–56). London: Academic.

Crute, I. R. (1986). Investigations of gene-for-gene relationships: the need for genetic analyses of both host and parasite. *Plant Pathology, 35*, 15–17.

Day, B., & Hausbeck, M. (2009). Epidemiology of downy mildew: a regional and molecular approach. *Phytopathology, 99*, S172.

de Jong, M., & Van den Ackerveken, G. (2009). Fungal and oomycete biotrophy. *Annual Plant Reviews*, *34*, 2009, 102–133. Oxford, UK: Wiley-Blackwell.

de Waard, M. A., Georgopoulos, S. G., Hollomon, D. W., Ishii, H., Leroux, P., Ragsdale, N. N., et al. (1993). Chemical control of plant diseases: Problems and prospects. *Annual Review of Phytopathology, 31*, 403–421.

Dick, M. W. (2001a). The peronosporomycetes. In D. J. McLaughlin, E. G. McLaughlin, & P. A. Lemke (Eds.), *The mycota VII Part A: Systematics and evolution* (pp. 39–72). Berlin: Springer-Verlag.

Dick, M. W. (2001b). *Straminipilous fungi: Systematics of the Peronosporomycetes, including accounts of the marine Straminipilous Protists, the Plasmodiophorids, and similar organisms*. Dordrecht: Kluwer Academic Publishers.

Dick, M. W. (2002a). Towards an understanding of the evolution of the downy mildews. In P. T. N. Spencer-Phillips, U. Gisi, & A. Lebeda (Eds.), *Advances in downy mildew research* (pp. 1–57). Dordrecht: Kluwer Academic Publishers.

Dick, M. W. (2002b). Binomials in the peronosporales, sclerosporales and pythiales. In P. T. N. Spencer-Phillips, U. Gisi, & A. Lebeda (Eds.), *Advances in downy mildew research* (pp. 225–265). Dordrecht: Kluwer Academic.

Dick, M. W. (2002c). The Peronosporomycetes and other flagellate fungi. In D. H. Howard (Ed.), *Fungi pathogenic for humans and animals: Second edition, revised and expanded* (pp. 17–66). Washington: American Society for Microbiology, Marcel Dekker, Inc.

Doruchowski, R. W., & Lakowska-Ryk, E. (2000). F_1 hybrid pickling cucumbers developed for increased yield, earliness and resistance to downy mildew. *Acta Horticulturae, 510*, 45–46.

Eckardt, N. A. (2004). Aminotransferases confer "Enzymatic resistance" to downy mildew in melon. *Plant Cell, 15*, 1–4.

Epinat, C., & Pitrat, M. (1994a). Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis*) in muskmelon (*Cucumis melo*). I. Analysis of a 8×8 diallel table. *Agronomie, 14*, 239–248.

Epinat, C., & Pitrat, M. (1994b). Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis*) in muskmelon (*Cucumis melo*). II. Generation means analysis of 5 genitors. *Agronomie, 14*, 249–257.

Ezuka, A., & Komada, H. (1974). Varietal difference in resistance of cucumber to downy mildew. *Bulletin Tokai-Kinki National Agricultural Experiment Station, 27*, 42–45.

Fellner, M., Binarová, P., & Lebeda, A. (1996). Isolation and fusion of *Cucumis sativus* and *Cucumis melo* protoplasts. In M. L. Gómez-Guillamón, C. Soria, J. Cuartero, J. A. Torés, & R. Fernández-Muñoz (Eds.), *Cucurbits towards 2000. Proceedings of the VIth Eucarpia Meeting on Cucurbit Genetics and Breeding* (pp. 202–209). May 28–30, 1996, Malaga, Spain.

Ferriol, M., & Picó, B. (2008). Pumpkin and winter squash. In J. Prohens & F. Nuez (Eds.), *Vegetables I. Asteraceae, brassicaceae, chenopodiaceae, and cucurbitaceae* (pp. 317–349). New York: Springer.

Forsberg, A. S. (1986). Downy mildew-*Pseudoperonospora cubensis* in Swedish cucumber fields. *Växtskyddsnotiser, 50*, 17–19.

Gajdová, J., Lebeda, A., & Navrátilová, B. (2004). Protoplast cultures of *Cucumis* and *Cucurbita* spp. In A. Lebeda, & H. S. Paris (Eds.) *Progress in cucurbit genetics and breeding research*. Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding (pp. 441–454). Olomouc, Czech Republic: Palacký University in Olomouc.

Gent, D. H., Mitchell, M. N., & Holmes, G. J. (2009). Genetic and pathogenic relatedness of *Pseudoperonospora cubensis* and *P. humuli*: Implications for detection and management. *Phytopathology, 99*, S171.

Gisi, U. (2002). Chemical control of downy mildews. In P. T. N. Spencer-Phillips, U. Gisi, & A. Lebeda (Eds.), *Advances in downy mildew research* ((pp, Vol. 1, pp. 119–159). Dordrecht: Kluwer Academic Publishers.

Gisi, U., & Sierotzki, H. (2008). Fungicide modes of action and resistance in downy mildews. *European Journal of Plant Pathology, 122*, 157–167.

Göker, M., Voglmayr, H., Riethmüller, A., Weiß, M., & Oberwinkler, F. (2003). Taxonomic aspects of Peronosporaceae inferred from Bayesian molecular phylogenetics. *Canadian Journal of Botany, 81*, 672–683.

Göker, M., Voglmayr, H., Riethmüller, A., & Oberwinkler, F. (2007). How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews. *Fungal Genetics and Biology, 44*, 105–122.

Hardham, A. R. (2007). Cell biology of plant-oomycete interactions. *Cellular Microbiology, 9*, 31–39.

Holliday, P. (2001). *A dictionary of plant pathology* (2nd ed.). Cambridge: Cambridge University Press.

Holmes, G. J., & Ojiambo, P. (2009). Chemical control of cucurbit downy mildew: a summary of field experiments in the U.S. *Phytopathology, 99*, S171.

Holmes, G. J., & Thomas, C. (2009). The history and re-emergence of cucurbit downy mildew. *Phytopathology, 99*, S171.

Holmes, G. J., Main, C. E., & Keever, Z. T., III. (2004). Cucurbit downy mildew: a unique pathosystem for disease forecasting. In P. T. N. Spencer-Phillips & M. Jeger (Eds.), *Advances in downy mildew research, vol. 2* (pp. 69–80). Dordrecht: Kluwer Academic Publishers.

Holmes, G., Wehner, T., & Thornton, A. (2006). An old enemy re-emerges: downy mildew rears its ugly head on cucumber, impacting growers up and down the Eastern U.S. *American Vegetable Grower*, February, 14–15.

Holub, E. B. (2008). Natural history of *Arabidopsis thaliana* and oomycete symbioses. *European Journal of Plant Pathology, 122*, 91–109.

Horejsi, T., Staub, J. E., & Thomas, C. (2000). Linkage of random amplified polymorphic DNA markers to downy mildew resistance in cucumber (*Cucumis sativus* L.). *Euphytica, 115*, 105–113.

Huang, S., et al. (2009). The genome of the cucumber, *Cucumis sativus* L. *Nature Genetics, 41*, 1275–1281.

Hughes, M. B., & van Halteren, F. (1952). Two biological forms of *Pseudoperonospora cubensis*. *Plant Disease Reporter, 36*, 365–367.

Inaba, T., Morinaka, T., & Hamaya, E. (1986). Physiological races of *Pseudoperonospora cubensis* isolated from cucumber and muskmelon in Japan. *Bulletin National Institute of Agro-Environmental Science, 2*, 35–43.

Ivanoff, S. C. (1944). Resistance of cantaloupes to downy mildew and the melon aphid. *Journal of Heredity, 35*, 35–39.

Iwata, Y. (1941). Specialisation in *Pseudoperonospora cubensis* (Berk. Et Curt.) Rostow. I. Comparative studies on the pathogenicities on the fungi from *Cucumis sativus* L. and *Cucurbita moschata* Duch. *Annales of Phytopathological Society of Japan, 11*, 101–113.

Jamadar, M. M., & Desai, S. A. (1999). Reaction of ridgegourd local cultivars against downy mildew caused by *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. *Karnataka Journal of Agricultural Sciences, 12*, 204–205.

Jeger, M. J., & Pautasso, M. (2008). Comparative epidemiology of zoosporic plant pathogens. *European Journal of Plant Pathology, 122*, 111–126.

Kanetis, L., Holmes, G. J., & Ojiambo, P. S. (2010). Survival of *Pseudoperonospora cubensis* sporangia exposed to solar radiation. *Plant Pathology, 59*, 313–323.

Kenigsbuch, D., & Cohen, Y. (1989). Inheritance of resistance to downy mildew in a gynoecious muskmelon. *Plant Disease, 73*, 994–996.

Kenigsbuch, D., & Cohen, Y. (1992a). Inheritance of resistance to downy mildew in *Cucumis melo* PI 124112 and commonality of resistance genes with PI 124111F. *Plant Disease, 76*, 615–617.

Kenigsbuch, D., & Cohen, Y. (1992b). Inheritance and allelism of genes for resistance against races 1 and 2 of *Sphaerotheca fuliginea* in muskmelons. *Plant Disease, 76*, 626–629.

Kirkbride, J. H., Jr. (1993). *Biosystematic monograph of the genus Cucumis (Cucurbitaceae)*. Boone: Parkway.

Ko, Y., Chen, C. Y., Liu, C. W., Chen, S. S., Maruthasalam, S., & Lin, C. H. (2008). First report of downy mildew by *Pseudoperonospora cubensis* on chayote (*Sechium edule*) in Taiwan. *Plant Disease, 92*, 1706.

Korbel, E. (1990). Možnost použití biologické ochrany proti plísni okurkové (Possibility of biological control utilisation against cucurbit downy mildew). In A. Lebeda (Ed.), *Plíseň okurková (Cucurbit downy mildew)* (pp. 62–64). Praha, Czechoslovakia: Československá vědecká společnost pro mykologii při ČSAV (Czechoslovak Scientific Society for Mycology by Czechoslovak Academy of Sciences).

Kranz, J. (2003). *Comparative epidemiology of plant diseases*. Berlin: Springer.

Lange, L., Eden, U., & Olson, L. W. (1989a). Zoosporogenesis in *Pseudoperonospora cubensis.* The causal agent of cucurbit downy mildew. *Nordic Journal of Botany, 8*, 497–504.

Lange, L., Eden, U., & Olson, L. W. (1989b). Internal mycelium of *Pseudoperonospora cubensis.* The causal agent of cucurbit downy mildew. *Nordic Journal of Botany, 8*, 505–510.

Lange, L., Eden, U., & Olson, L. W. (1989c). The zoospore of *Pseudoperonospora cubensis.* The causal agent of cucurbit downy mildew. *Nordic Journal of Botany, 8*, 511–516.

Lebeda, A. (1986a). Epidemic occurrence of *Pseudoperonospora cubensis* in Czechoslovakia. *Temperate Downy Mildews Newsletter, 4*, 15–17.

Lebeda, A. (1986b). *Pseudoperonospora cubensis*. In A. Lebeda (Ed.) *Metody testování rezistence zelenin vůči rostlinným patogenům* (*Methods of vegetable resistance screening against pathogens)* (pp. 81–85). Olomouc, Czechoslovakia: VHJ Sempra, Research and Breeding Institute for Vegetable Crops.

Lebeda, A. (1990). Biologie a ekologie plísně okurkové (Biology and ecology of cucurbit downy mildew). In A. Lebeda (Ed.) *Plíseň okurková* (*Cucurbit downy mildew*) (pp. 13–45). Praha, Czechoslovakia: Československá vědecká společnost pro mykologii při ČSAV (Czechoslovak Scientific Society for Mycology by Czechoslovak Academy of Sciences).

Lebeda, A. (1991). Resistance in muskmelons to Czechoslovak isolates of *Pseudoperonospora cubensis* from cucumbers. *Scientia Horticulturae, 45*, 255–260.

Lebeda, A. (1992a). Susceptibility of accessions of *Cucumis sativus* to *Pseudoperonospora cubensis. Tests of Agrochemicals and Cultivars No. 13 (Annals of Applied Biology*, *120 Suppl.)*, 102–103.

Lebeda, A. (1992b). Screening of wild *Cucumis* species against downy mildew (*Pseudoperonospora cubensis*) isolates from cucumbers. *Phytoparasitica, 20*, 203–210.

Lebeda, A. (1999). *Pseudoperonospora cubensis* on *Cucumis* spp. and *Cucurbita* spp.–resistance breeding aspects. *Acta Horticulturae, 492*, 363–370.

Lebeda, A., & Jendrulek, T. (1988). Application of methods of multivariate analysis in comparative epidemiology and research into field resistance. *Journal of Plant Diseases and Protection, 95*, 495–505.

Lebeda, A., & Reinink, K. (1991). Variation in the early development of *Bremia lactucae* on lettuce cultivars with different levels of field resistance. *Plant Pathology, 40*, 232–237.

Lebeda, A., & Křístková, E. (1992). Differences in resistance to *Pseudoperonospora cubensis* in *Cucurbita pepo*. In R. W. Doruchowski, E. Kozik, & K. Niemirowicz-Szczytt (Eds.) *Proceedings of Fifth Eucarpia Cucurbitaceae Symposium* (pp. 175–179). July 27–31, 1992, Skierniewice, Poland: Research Institute for Vegetable Crops

Lebeda, A., & Křístková, E. (1993). Resistance of *Cucurbita pepo* and *Cucurbita moschata* varieties to cucurbit downy mildew (*Pseudoperonospora cubensis*). *Plant Varieties and Seeds, 6*, 109–114.

Lebeda, A., & Prášil, J. (1994). Susceptibility of *Cucumis sativus* cultivars to *Pseudoperonospora cubensis*. *Acta Phytopathologica et Entomologica Hungarica, 29*, 89–94.

Lebeda, A., & Schwinn, F. J. (1994). The downy mildews—an overview of recent research progress. *Journal of Plant Diseases and Protection, 101*, 225–254.

Lebeda, A., & Doležal, K. (1995). Peroxidase isozyme polymorphism as a potential marker for detection of field resistance in *Cucumis sativus* to cucumber downy mildew (*Pseudoperonospora cubensis* (Berk. et Curt.) Rostov.). *Journal of Plant Diseases and Protection, 102*, 467–471.

Lebeda, A., & Křístková, E. (2000). Interactions between morphotypes of *Cucurbita pepo* and obligate biotrophs (*Pseudoperonospora cubensis*, *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*). *Acta Horticulturae, 510*, 219–225.

Lebeda, A., & Gadasová, V. (2002). Pathogenic variation of *Pseudoperonospora cubensis* in the Czech Republic and some other european countries. *Acta Horticulturae, 588*, 137–141.

Lebeda, A., & Widrlechner, M. P. (2003). A set of Cucurbitaceae taxa for differentiation of *P. cubensis* pathotypes. *Journal of Plant Diseases and Protection, 110*, 337–349.

Lebeda, A., & Urban, J. (2004a). Disease impact and pathogenicity variation in Czech populations of *Pseudoperonospora cubensis.* In A. Lebeda, & H. S. Paris (Eds.), *Progress in cucurbit genetics and breeding research. Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding* (pp. 267–273). Olomouc, Czech Republic: Palacký University in Olomouc.

Lebeda, A., & Urban, J. (2004b). Distribution, harmfulness and pathogenic variability of cucurbit downy mildew in the Czech Republic. *Acta Fytotechnica et Zootechnica, 7*, 170–173.

Lebeda, A., & Widrlechner, M. P. (2004). Response of wild and weedy *Cucurbita* L. to pathotypes of *Pseudoperonospora cubensis* (Berk. et Curt.) Rostov. (Cucurbit downy mildew). In P. Spencer-Phillips & M. Jeger (Eds.), *Advances in downy mildew research, Vol. 2* (pp. 203–210). Dordrecht: Kluwer Academic Publishers.

Lebeda, A., & Urban, J. (2005). Ochrana okurek a dalších tykvovitých zelenin vůči plísni okurkové (Protection of cucumbers and other cucurbitaceous vegetables to cucurbit downy mildew). In F. Kocourek et al. (Eds.) Metodika pro integrovaný systém ochrany polní zeleniny vůči škodlivým organismům (Methods for integrated system of field vegetables protection to dangerous organisms) (pp. 24–38). Olomouc, Czech Republic: Zelinářská unie Čech a Moravy, Olomouc (Vegetables Grower Union of Bohemia and Moravia).

Lebeda, A., & Urban, J. (2007). Temporal changes in pathogenicity and fungicide resistance in *Pseudoperonospora cubensis* populations. *Acta Horticulturae, 731*, 327–336.

Lebeda, A., Křístková, E., & Kubaláková, M. (1996). Interspecific hybridization of *Cucumis sativus* × *Cucumis melo* as potential way to transfer resistance to *Pseudoperonospora cubensis*. In M. L. Gómez-Guillamón, C. Soria, J. Cuartero, J. A. Torés, & R. Fernández-Muñoz (Eds.) *Cucurbits towards 2000. Proceedings of the VIth Eucarpia Meeting on Cucurbit Genetics and Breeding* (pp. 31–37), May 28–30, 1996, Malaga, Spain.

Lebeda, A., Kubaláková, M., Křístková, E., Navrátilová, B., Doležal, K., Doležel, J., et al. (1999). Morphological and physiological characteristics of plants issued from an interspecific hybridization of *Cucumis sativus*×*Cucumis melo*. *Acta Horticulturae, 492*, 149–155.

Lebeda, A., Luhová, L., Sedlářová, M., & Jančová, D. (2001a). The role of enzymes in plant—fungal pathogens interactions. *Journal of Plant Diseases and Protection, 108*, 89–111.

Lebeda, A., Pink, D. A. C., & Mieslerová, B. (2001b). Host-parasite specificity and defense variability in the *Lactuca* spp.-*Bremia lactucae* pathosystem. *Journal of Plant Pathology, 83*, 25–35.

Lebeda, A., Pink, D. A. C., & Astley, D. (2002). Aspects of the interactions between wild *Lactuca* spp. and related genera and lettuce downy mildew (*Bremia lactucae*). In P. T. N. Spencer-Phillips, U. Gisi, & A. Lebeda (Eds.), *Advances in downy mildew research* (pp. 85–118). Dordrecht: Kluwer Academic Publishers.

Lebeda, A., Štěpánková, J., & Urban, J. (2006a). Plíseň okurky (*Pseudoperonospora cubensis*)—taxonomie, biologie, ekologie, interakce hostitel-patogen a možnosti ochrany (Cucurbit downy mildew (*Pseudoperonospora cubensis*)—taxonomy, biology, ecology, host-pathogen interactions and possibilities of protection). In A. Lebeda, J. Mazáková, & V. Táborský (Eds.) *Protozoa a Chromista. Taxonomie, biologie a hospodářský význam* (*Protozoa and Chromista. Taxonomy, biology and economical impact*) (pp. 47–78). Praha, Czech Republic: Česká fytopatologická společnost (Czech Phytopathological Society).

Lebeda, A., Widrlechner, M. P., & Urban, J. (2006b). Individual and population aspects of interactions between cucurbits and *Pseudoperonospora cubensis*: pathotypes and races. In G. J. Holmes (Ed.), *Proceedings of Cucurbitaceae 2006* (pp. 453–467). Raleigh: Universal.

Lebeda, A., Štěpánková, J., Kršková, M., & Widrlechner, M.P. (2007a) Resistance in *Cucumis melo* germplasm to *Pseudoperonospora cubensis* pathotypes. In A. Lebeda, & P. T. N. Spencer-Phillips (Eds.) *Advances in Downy Mildew Research, Vol. 3. Proceedings of The 2nd International Downy Mildews Symposium* (pp. 157–167.). Olomouc and Kostelec na Hané, Czech Republic: Palacký University in Olomouc and JOLA, v.o.s.

Lebeda, A., Widrlechner, M. P., Staub, J., Ezura, H., Zalapa, J., & Křístková, E. (2007b). Cucurbits (Cucurbitaceae; *Cucumis* spp., *Cucurbita* spp., *Citrullus* spp.), Chapter 8. In R. Singh (Ed.), *Genetic resources, chromosome engineering, and crop improvement series, volume 3—vegetable crops* (pp. 273–377). Boca Raton: CRC.

Lebeda, A., Petrželová, I., & Maryška, Z. (2008a). Structure and variation in the wild-plant pathosystem: *Lactuca serriola-Bremia lactucae*. *European Journal of Plant Pathology, 122*, 127–146.

Lebeda, A., Sedlářová, M., Petřivalský, M., & Prokopová, J. (2008b). Diversity of defence mechanisms in plant-oomycete interactions: a case study of *Lactuca* spp. and *Bremia lactucae*. *European Journal of Plant Pathology, 122*, 71–89.

Limpert, E., Clifford, B., Dreiseitl, A., Johnson, R., Müller, K., Roelfs, A., et al. (1994). Systems of designation of pathotypes of plant pathogens. *Journal of Phytopathology, 140*, 359–362.

Lindenthal, M., Steiner, U., Dehne, H. W., & Oerke, E. C. (2005). Effect of downy mildew development on transpiration of cucumber leaves visualized by digital infrared thermography. *Phytopathology, 95*, 233–240.

Mahrisi, R. P., & Siradhana, B. S. (1984). On the occurrence of oospores of *Pseudoperonospora cubensis* in Rajasthan, India. *Indian Phytopathology, 37*, 323–325.

Mahrisi, R. P., & Siradhana, B. S. (1988). Effect of nutrition on downy mildew disease caused by *Pseudoperonospora cubensis* (Berk. and Curt.) Rostow. on muskmelon. *Annals of Arid Zone, 27*, 153–155.

Main, C. E., Keever, T., Holmes, G. V., & Davis, J. M. (2001). Forecasting long-range transport of downy mildew spores and plant disease epidemics. APS net Feature Story April 25 through May 31. http://www.apsnet.org/ online/future/forecast/

Mauch-Mani, B. (2002). Host resistance to downy mildew diseases. In P. T. N. Spencer-Phillips, U. Gisi, & A. Lebeda (Eds.), *Advances in downy mildew research* (pp. 59–83). Dordrecht: Kluwer Academic.

Medvedeva, N. I., & Medvedev, A. V. (1983). Agronomic and biological assessment of cucumber varieties with potential for breeding for resistance to downy mildew. *Trudy po Prikladnoj Botanike, Genetike i Selekcii, 77*, 25–28.

Michelmore, R. W. (1981). Sexual and asexual sporulation in the downy mildews. In D. M. Spencer (Ed.), *The downy mildews* (pp. 165–181). London: Academic.

Michelmore, R., & Wong, J. (2008). Classical and molecular genetics of *Bremia lactucae*, cause of lettuce downy mildew. *European Journal of Plant Pathology, 122*, 19–30.

Mitchell, M. N., Ocamb, C., & Gent, D. (2009). Addressing the relationship between *Pseudoperonospora cubensis* and *P. humuli* by multigenic characterization and host specificity. *Phytopathology, 99*, S87.

Moravec, J., Lebeda, A., & Křístková, E. (2004). History of growing and breeding of cucurbitaceaous vegetables in Czech Lands. In A. Lebeda, & H. S. Paris (Eds.), *Progress in cucurbit genetics and breeding research. Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding* (pp. 21–38). Olomouc, Czech Republic: Palacký University in Olomouc.

Nusbaum, C. J. (1944). The seasonal spread and development of cucurbit downy mildew in the Atlantic coastal states. *Plant Disease, 28*, 82–85.

Nusbaum, C. J. (1948). A summary of cucurbit downy mildew reports from Atlantic coastal states in 1947. *Plant Disease, 32*, 44–48.

Oerke, E. C., Steiner, U., Dehne, H. W., & Lindenthal, M. (2006). Thermal imaging of cucumber leaves affected by downy mildew and environmental conditions. *Journal of Experimental Botany, 57*, 2121–2132.

Ojiambo, P., Kanetis, L., & Holmes, G. (2009). Forecasting long distance movement of *Pseudoperonospora cubensis* and the Cucurbit ipmPIPE. *Phytopathology, 99*, S171.

Okada, K., & Furukawa, M. (2008). Occurrence and countermeasure of fungicide-resistant pathogens in vegetable field of Osaka prefecture. *Journal of Pesticide Science, 33*, 326–329.

Olaya, G., Kuhn, P., Hert, A., Holmes, G., & Colucci, S. (2009). Fungicide resistance in cucurbit downy mildew. *Phytopathology, 99*, S171.

Ovadia, A., Biton, R., & Cohen, Y. (2000). Induced resistance to downy mildew and Fusarium wilt in cucurbits. In N. Katzir, & H. S. Paris (Eds.) "*Proceedings of 7th EUCARPIA Meeting on Cucurbit Genetics and Breeding*". *Acta Horticulture*, *510*, 55–59.

Palti, J., & Cohen, Y. (1980). Downy mildew of cucurbits (*Pseudoperonospora cubensis*): The fungus and its hosts, distribution, epidemiology and control. *Phytoparasitica, 8*, 109–147.

Paris, H. S. (2008). Summer squash. In J. Prohens & F. Nuez (Eds.), *Vegetables I. Asteraceae, brassicaceae, chenopodiaceae, and cucurbitaceae* (pp. 351–379). New York: Springer.

Perchepied, L., Bardin, M., Dogimont, C., & Pitrat, M. (2005). Relationship between loci conferring downy mildew and powdery mildew resistance in melon assessed by quantitative trait loci mapping. *Phytopathology, 95*, 556–565.

Perl, M., Cohen, Y., & Rotem, J. (1972). The effect of humidity during darkness on the transfer of assimilates from cucumber leaves to sporangia of *Pseudoperonospora cubensis*. *Physiological Plant Pathology, 2*, 113–122.

Peterson, C. E. (1975). Plant introduction in the improvement of vegetable cultivars. *HortScience, 10*, 575–579.

Petrov, L., Boogert, K., Sheck, L., Baider, A., Rubin, E., & Cohen, Y. (2000). Resistance to downy mildew, *Pseudoperonospora cubensis,* in cucumbers. *Acta Horticulturae, 510*, 203–209.

Pitrat, M. (1990). Gene list for *Cucumis melo* L. *Cucurbit Genetics Cooperative, 13*, 58–68.

Pitrat, M. (2008). Melon. In J. Prohens & F. Nuez (Eds.), *Vegetables I. Asteraceae, brassicaceae, chenopodiaceae, and cucurbitaceae* (pp. 283–315). New York: Springer.

Pivovarov, V. F. (1984). Breeding cucumber for disease resistance using different ecological conditions. *Selekcia i Semenovodstvo USSR, 10*, 20–22 (in Russian).

Pivovarov, V. F., & Kudelich, V. S. (1985). Using the VIR collection in breeding cucumber for downy mildew resistance in the Republic of Cuba. *Trudy po Prikladnoj Botanike, Genetike i Selekcii, 92*, 97–102.

Portz, D., Koch, E., & Slusarenko, A. J. (2008). Effects of garlic (*Allium sativum*) juice containing allicin on *Phytophthora infestans* and downy mildew of cucumber caused by *Pseudoperonospora cubensis*. *European Journal of Plant Pathology, 122*, 197–206.

Ren, Y., et al. (2009). An integrated genetic and cytogenetic map of the cucumber genome. *PloS ONE, 4*, e5795.

Reuveni, M., Eyal, H., & Cohen, Y. (1980). Development of resistance to metalaxyl in *Pseudoperonospora cubensis*. *Plant Disease, 64*, 1108–1109.

Reuveni, R., Shimoni, M., & Karchi, Z. (1990). A rapid assay for monitoring peroxidase-activity in melon as a marker for resistance to *Pseudoperonospora cubensis*. *Journal of Phytopathology, 129*, 333–338.

Riethmüller, A., Voglmayr, A., Göker, M., Weiß, M., & Oberwinkler, F. (2002). Phylogenetic relationships of the downy mildews (Peronosporales) and related groups based on nuclear large subunit ribosomal DNA sequences. *Mycologia, 94*, 834–849.

Robinson, R. W., & Decker-Walters, D. S. (1997). *Cucurbits*. Wallingford: CAB International.

Rod, J. (1990). Principy chemické ochrany (Principles of chemical control). In A. Lebeda (Ed.) *Plíseň okurková (Cucurbit downy mildew)* (pp. 46–50). Praha, Czechoslovakia: Československá vědecká společnost pro mykologii při ČSAV.

Royle, D. J., & Kremheller, H Th. (1981). Downy mildew of the hop. In D. M. Spencer (Ed.), *The downy mildews* (pp. 395–419). London: Academic.

Rubatzky, V. E., & Yamaguchi, M. (1997). *World vegetables. Principles, production and nutritive values* (2nd ed.). New York: Chapman & Hall.

Runge, F., & Thines, M. (2009). A potential perennial host for *Pseudoperonospora cubensis* in temperate regions. *European Journal of Plant Pathology, 123*, 483–486.

Salti, M., Wong, M. Y., Sariah, M., & Masdek, H. N. (2010). First report of *Pseudoperonospora cubensis* causing downy mildew of *Trichosanthes cucumerina* in Malaysia. *Plant Disease, 94*, 642.

Sarris, P.F., Abdelhalim, M., Kitner, M., Skandalis, N., Panopoulos, N.J., Doulis, A.G., & Lebeda, A. (2009). Molecular polymorphism between populations of *Pseudoperonospora cubensis* from Greece and the Czech Republic and their phytopathological and phylogenetic implications. *Plant Pathology* (doi:10.1111/j.1365-3059.2009.02093.x)

Savory, E. A., Tian, M., Erhardt, C., Hausbeck, M., Hammerschmidt, R., & Day, B. (2008). An integrative approach to characterizing the cucumber–*Pseudoperonospora cubensis* interaction. *Phytopathology, 98* (*June, Supplement*), S140.

Shashikumar, K. T., Pitchaimuthu, M., & Rawal, R. D. (2010). Generation mean analysis of resistance to downy mildew in adult muskmelon plants. *Euphytica, 173*, 121–127.

Shetty, N. V., Wehner, T. C., Thomas, C. E., Doruchowski, R. W., & Shetty, K. P. W. (2002). Evidence for downy mildew races in cucumber tested in Asia, Europe, and North America. *Scientia Horticulturae, 94*, 231–239.

Shtienberg, D., Elad, Y., Bornstein, M., Ziv, G., Grava, A., & Cohen, S. (2010). Polyethylene mulch modifies greenhouse microclimate and reduces infection of *Phytophthora infestans* in tomato and *Pseudoperonospora cubensis* in cucumber. *Phytopathology, 100*, 97–104.

Singh, P. P., & Sokhi, S. S. (1989). 1st report of occurrence of oospores of *Pseudoperonospora cubensis* on 2 cucurbitaceous hosts. *Current Science, 58*, 1330–1331.

Singh, R. D., & Singh, J. P. (1998). Improvement and cultivation: *Lagenaria* and *Luffa*. In N. M. Naynar & T. A. More (Eds.), *Cucurbits* (pp. 199–204). Enfield: Science Publishers.

Sitterly, W. R. (1972). Breeding of disease resistance in cucurbits. *Annual Review of Phytopathology, 10*, 471–490.

Skalický, V. (1961). Plíseň okurková–*Peronoplasmopara cubensis*. In J. Benada, & J. Špaček (Eds.) *Zemědělská fytopatologie, díl III-Choroby zeleniny* (*Agricultural phytopathology, vol. III–diseases of vegetable crops*) (pp. 390–393). Praha, Czechoslovakia: Státní zemědělské nakladatelství.

Skalický, V. (1966). Taxonomie der Gattungen der Familie Peronosporaceae. *Preslia, 38*, 117–129.

Skálová, D., Lebeda, A., & Navrátilová, B. (2004). Embryo and ovule cultures in *Cucumis* species and their utilization in interspecific hybridization. In A. Lebeda, & H. S. Paris (Eds.), *Progress in cucurbit genetics and breeding research*. Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding (pp. 415–430). Olomouc, Czech Republic: Palacký University in Olomouc.

Skidmore, D. I., & Ingram, D. S. (1985). Conidial morphology and the specialization of *Bremia lactucae* Regel (Peronosporaceae) on hosts in the family Compositae. *Botanical Journal of the Linnean Society, 91*, 503–522.

Slusarenko, A. J., & Schlaich, N. L. (2003). Downy mildew of *Arabidopsis thaliana* caused by *Hyaloperonospora parasitica* (formerly *Peronospora parasitica*). *Molecular Plant Pathology, 4*, 159–170.

Sowell, G., & Corley, W. L. (1974). PI 321005 (Tainan 2), a high quality source of resistance to three cantaloupe diseases. *Plant Disease Reporter, 58*, 899–902.

Staub, J. E., Robbins, M. D., & Wehner, T. C. (2008). Cucumber. In J. Prohens & F. Nuez (Eds.), *Vegetables I. Asteraceae, brassicaceae, chenopodiaceae, and cucurbitaceae* (pp. 241–282). New York: Springer.

Švábová, L., & Lebeda, A. (2005). *In vitro* selection for improved plant resistance to toxin-producing pathogens. *Journal of Phytopathology, 153*, 52–64.

Tahvonen, R. (1985). Downy mildew of cucurbits found for the first time in Finland. *Växtskyddsnotiser, 49*, 42–44.

Taler, D., Galperin, M., Benjamin, I., Cohen, Y., & Kenigsbuch, D. (2004). Plant *eR* genes that encode photorespiratory enzymes confer resistance against disease. *Plant Cell, 16*, 172–184.

Thakur, R. P., & Mathur, K. (2002). Downy mildews of India. *Crop Protection, 21*, 333–345.

Thomas, C. E. (1970). Sporulation of *Pseudoperonospora cubensis* on watermelon. *Plant Disease Reporter, 54*, 108–111.

Thomas, C. E. (1982). Resistance to downy mildew in *Cucumis melo* plant introductions and American cultivars. *Plant Disease, 66*, 500–502.

Thomas, C. E. (1986). Downy and powdery mildew resistant muskmelon breeding line MR-1. *HortScience, 21*, 329.

Thomas, C. E. (1996). Downy mildew. In T. A. Zitter, D. L. Hopkins, & C. E. Thomas (Eds.), *Compendium of cucurbit diseases* (pp. 25–27). St. Paul: American Phytopathological Society Press.

Thomas, C. E., Cohen, Y., Jourdain, E. L., & Eyal, H. (1987a). Use of reaction types to identify downy mildew resistance in muskmelons. *HortScience, 22*, 638–640.

Thomas, C. E., Cohen, Y., Mc Creight, Y. D., Jourdion, E. L., & Cohen, S. (1987b). Inheritance of resistance to downy mildew in *Cucumis melo*. *Plant Disease, 72*, 33–35.

Thomas, C. E., Inaba, T., & Cohen, Y. (1987c). Physiological specialization in *Pseudoperonospora cubensis*. *Phytopathology, 77*, 1621–1624.

Tomlin, C. D. S. (2003). *The pesticide manual* (thirteenthth ed.). Alton: British Crop Protection Council.

Urban, J., & Lebeda, A. (2004). Differential sensitivity to fungicides in Czech populations of *Pseudoperonospora cubensis.* In A. Lebeda, & H. S. Paris (Eds.) *Progress in cucurbit genetics and breeding research*. Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding (pp. 275–280). Olomouc, Czech Republic: Palacký University in Olomouc.

Urban, J., & Lebeda, A. (2006). Fungicide resistance in cucurbit downy mildew—methodological, biological and population aspects. *Annals of Applied Biology, 149*, 63–75.

Urban, J., & Lebeda, A. (2007). Variation for fungicide resistance in Czech populations of *Pseudoperonospora cubensis*. *Journal of Phytopathology, 155*, 143–151.

Veselý, D. (1977). Potential biological control of damping-off pathogens in emerging sugar beet by *Pythium oligandrum* Drechsler. *Phytopathologische Zeitschrift, 94*, 113–115.

Voglmayr, H. (2003). Phylogenetic study of *Peronospora* and related genera based on nuclear ribosomal ITS sequences. *Mycological Research, 107*, 1132–1142.

Voglmayr, H. (2008). Progress and challenges in systematics of downy mildews and white blister rusts: new insights from genes and morphology. *European Journal of Plant Pathology, 122*, 3–18.

Voglmayr, H., & Greilhuber, J. (1998). Genome size determination in Peronosporales (Oomycota) by Feulgen Image Analysis. *Fungal Genetics and Biology, 25*, 181–195.

Voglmayr, H., Riethmüller, A., Göker, M., Weiß, M., & Oberwinkler, F. (2004). Phylogenetic relationships of *Plasmopara*, *Bremia* and other genera of downy mildews with pyriform haustoria based on Bayesan analysis of partial LSU rDNA sequence data. *Mycological Research, 108*, 1011–1024.

Walz, A., & Simon, O. (2008). β-aminobutyric acid-induced resistance in cucumber against biotrophic and necrotrophic pathogens. *Journal of Phytopathology, 157*, 356–361.

Wang, W., Ben-Daniel, B. H., & Cohen, Y. (2004). Control of plant diseases by extracts of *Inula viscosa*. *Phytopathology, 94*, 1042–1047.

Wang, H. C., Zhou, M. G., Wang, J. X., Chen, C. J., Li, H. X., & Sun, H. Y. (2009). Biological mode of action of dimethomorph on *Pseudoperonospora cubensis* and its systemic activity in cucumber. *Agricultural Sciences in China, 8*, 172–181.

Waterhouse, G. M., & Brothers, M. P. (1981). The taxonomy of *Pseudoperonospora*. *Mycological Papers, 148*, 1–28.

Wehner, T. C. (2008). Watermelon. In J. Prohens & F. Nuez (Eds.), *Vegetables I. Asteraceae, brassicaceae, chenopodiaceae, and cucurbitaceae* (pp. 381–418). New York: Springer.

Wehner, T. C., & Shetty, N. V. (1997). Downy mildew resistance of the cucumber germplasm collection in North Carolina field tests. *Crop Science, 37*, 1331–1340.

Winstead, N. N., Aycock, R., & Jenkins, J. M. (1957). Reactions of watermelon plant introductions and varieties to downy mildew in North Carolina. *Plant Disease Reporter, 41*, 620–622.

Yang, X. T., Li, M., Zhao, Ch, Zhang, Z., & Hou, Y. L. (2007). Early warning model for cucumber downy mildew in unheated greenhouses. *New Zealand Journal of Agricultural Research, 50*, 1261–1268.

Yeon, I. K. (2007). Incidence of *Pseudoperonospora cubensis* on winter grown oriental melon (*Cucumis melo*) in vinyl houses in Korea. In A. Lebeda, & P. T. N. Spencer-Phillips (Eds.), *Advances in Downy Mildew Research, Vol. 3. Proceedings of The 2nd International Downy Mildews Symposium* (pp. 217–222.). Olomouc and Kostelec na Hané, Czech Republic: Palacký University in Olomouc and JOLA, v.o.s.

Yeon, I. K., Shin, Y. S., Do, H. W., Bae, S. G., & Park, S. D. (2002). Occurrence and chemical control of downy mildew (*Pseudoperonospora cubensis* Rostovzev) infecting oriental melon (*Cucumis melo* L. var. *makuwa* Makino) in plastic greenhouse. *Korean Journal of Horticultural Science & Technology, 20*, 25–28.

Zacha, V., Janýška, A., & Holman, B. (1985). Epifytócia plesne uhorkovej (*Pseudoperonospora cubensis* (Berk. et Curt.) Rost.) v ČSSR v roku 1984. *Sborník ÚVTIZ-Ochrana rostlin*, *21*, 226.

Zaker, M., & Ommati, F. (1991). Observation of oospores of *Pseudoperonospora cubensis* on cucumber leaves in Iran. *Iran Journal of Plant Pathology, 27*, 62–63.

Zhang, Yan-ju, Qin, Zhi-wei, & Zhou, Xiu-yan (2006). Study on the over-wintering of cucumber downy mildew in Heilongjiang province of China. Abstracts, 27th International Horticultural Congress & Exhibition, August 13–19, 2006, COEX (Convention & Exhibition), Seoul, Korea; ISHS and KSHS, S11-P-51, p. 376.

Zhang, X., Chen, Y., Zhang, Y. J., & Zhou, M. G. (2008). Occurrence and molecular characterization of azoxystrobin resistance in cucumber downy mildew in Shandong province in China. *Phytoparasitica, 36*, 136–143.

Zhao, C. J., Li, M., Han, X., Zhang, Z., & Wang, Y. (2007). Analysis and monitoring for epidemic system as a basis for cucumber downy mildew warning in greenhouse. *Progress of Information Technology in Agriculture*, pp. 527–532.

Zhou, X. H., Wan, H. J., Qian, C. T., & Chen, J. F. (2008). Development and characterization of *Cucumis sativus-hystrix* introgression lines exhibiting resistance to downy mildew. In M. Pitrat (Ed.), *Cucurbitaceae 2008. Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae* (pp. 353–358). Avignon: NRA.

Zhu, S. S., Liu, P. F., Liu, X. L., Li, J. Q., Yuan, S. K., & Sil, N. G. (2008). Assessing the risk of resistance in *Pseudoperonospora cubensis* to the fungicide flumorph in vitro. *Pest Management Science, 64*, 255–261.