PALACKÝ UNIVERSITY IN OLOMOUC
FACULTY OF SCIENCE
DEPARTMENT OF BOTANY

Bc. Ing. Jana Pavelková
(roz. Hübschová)

Temporal population dynamics of *Pseudoperonospora cubensis*

PH.D. THESIS
Ph.D. program of Biology – Botany

Supervisor: Prof. Ing. Aleš Lebeda, DrSc.

Olomouc
2012

BIBLIOGRAPHICAL IDENTIFICATION

Author's first name and surname: Jana Pavelková (roz. Hübschová)

Title: Temporal population dynamics of *Pseudoperonospora cubensis*

Type of thesis: Ph.D. Thesis

Department: Department of Botany

Supervisor: Prof. Ing. Aleš Lebeda, DrSc.

The year of presentation: 2012

Abstract: The occurrence of cucurbit downy mildew was recorded annually throughout the area of the Czech Republic. Natural infection was observed predominantly on *C. sativus* and only rarely on other cucurbits (*Cucurbita* spp. and *Citrullus lanatus* – since the year 2009). Our long-lasting observations showed the annual disease fluctuations within the recorded localities with *C. sativus* crops that could be probably caused by variability in the macro- and microclimatic conditions in individual years. However the substantial declines of disease damage to the cucumber crop have never been observed during the studied period. The virulence profile of the studied pathogen population was highly variable. Majority of the screened *Pseudoperonospora cubensis* isolates were highly virulent (with 9 to 12 virulence factors /VF/). A total of 67 different pathotypes were determined in the period 2001-2010. Virulence structure showed a temporal shift from 2001 to 2007 to a higher number of VF and a lower number of pathotypes. However, variation in virulence changed and increased again from 2008 to 2010. A broad spectrum of variation in virulence observing in Czech *P. cubensis* population from 2001 to 2010 has not been reported in *P. cubensis* population from another country till now. Results of screening of fungicide tolerance/resistance in Czech *P. cubensis* could be divided into three groups: propamocarb and fosetyl-Al, towards which no resistance has created, however the risk of resistance development still exists; metalaxyl, metalaxyl-M and cymoxanil, toward which a high resistance has developed; and dimethomorph, towards which a shift from resistance to tolerance and/or susceptibility has noted.

Keywords: cucurbit downy mildew, *Pseudoperonospora cubensis,* Cucurbitaceae, host specificity, virulence, pathotype, fungicide efficacy, propamocarb, fosetyl-Al, metalaxyl, metalaxyl-M, cymoxanil, dimethomorph, resistance, tolerance

Number of pages: 124

Language: English

BIBLIOGRAFICKÁ IDENTIFIKACE

Jméno a příjmení autora: Jana Pavelková (roz. Hübschová)

Téma: Populační dynamika plísně okurkové (*Pseudoperonospora cubensis*)

Druh práce: Doktorská disertační

Katedra: Katedra botaniky

Školitel: Prof. Ing. Aleš Lebeda, DrSc.

Rok obhájení práce: 2012

Abstrakt: Výskyt plísně okurkové byl zaznamenán každoročně na celém území České Republiky. Přirozená infekce byla pozorována převážně na *Cucumis sativus* a jen výjimečně na ostatních tykvovitých (*Cucurbita* spp. a *Citrullus lanatus* – od roku 2009). Během našich dlouhodobých pozorování se ukázaly meziroční fluktuace výskytu choroby v rámci sledovaných lokalit s porosty *C. sativus*, které mohou být pravděpodobně způsobeny variabilitou makro- a mikroklimatických podmínek v jednotlivých letech. Nicméně, podstatné snížení dopadu choroby nebylo během studované periody pozorováno. Profil virulence studované populace patogena se ukázal vysoce variabilní. Většina testovaných izolátů *Pseudoperonospora cubensis* bylo vysoce virulentních (s 9-12 faktory virulence /VF/). Během let 2001-2010 bylo determinováno celkem 67 různých patotypů. Takto široké spektrum variability ve virulenci, pozorované v české populaci *P. cubensis*, nebylo dosud zaznamenáno v populaci *P. cubensis* v žádném jiném státě. Výsledky testování tolerance/resistence k fungicidům v české populaci *P. cubensis* mohou být rozděleny do tří skupin: propamocarb a fosetyl-Al, vůči nimž nebyla vyvinuta rezistence, nicméně, stále existuje riziko rozvoje rezistence; metalaxyl, metalaxyl-M a cymoxanil, vůči nimž se vytvořila rezistence; a dimethomorph, vůči němuž byl zaznamenán posun směrem od rezistence k toleranci a/nebo k citlivosti.

Klíčová slova: plíseň okurková, *Pseudoperonospora cubensis*, Cucurbitaceae, hostitelská specificita, virulence, patotyp, fungicidní účinnost, propamocarb, fosetyl-Al, metalaxyl, metalaxyl-M, cymoxanil, dimethomorph, resistence, tolerance

Počet stran: 124

Jazyk: English

CONTENTS

Acknowledgements

I would like to express my great thanks to Prof. Aleš Lebeda for his professional assistance, experiences and advices during all the years.
I also give thanks to all of colleagues from Laboratory of phytopathology in Olomouc because this Ph.D. study has not been able to originate without their assistance and contribution.
As well I would like to offer thanks all of my friends and especially my family for their support for all the years.

Loštice, May 31th 2012 Jana Pavelková

1. INTRODUCTION

The downy mildews are among the most devastating plant diseases caused by pathogens (Gisi, 2002). The cucurbit downy mildew is one of the most important plant pathogens in cucurbits and its causal agent *Pseudoperonospora cubensis* [(Berkeley & MA Curtis) Rostovzev] is one of the most studied Peronosporomycete biotrophic parasites of plants (Lebeda and Cohen, 2011; Savory et al., 2010). Its wide host range and global distribution lead to significant yield loses in cucurbitaceous crops. Because of polycyclic nature of *P. cubensis* the disease spread rapidly in both open fields and protected environments (Lebeda and Cohen, 2011). Cucurbit downy mildew can be found in temperate areas such as Americas, Europe, Japan and Australia; in tropical areas in South Africa; and in some semiarid regions in the Middle East. Within these regions the pathogen is very destructive in all humid areas of the word as well as some temperate areas (Cohen, 1981; Lebeda, 1990; Lebeda and Cohen, 2011). Cucurbit downy mildew is an annual problem on cucurbits in the majority of world producing areas. *P. cubensis* can overwinter in areas with mild winter temperatures or in protected cultivation, as active mycelium in either cultivated or wild species of cucurbits and than is reintroduced via long distance transport of inoculum in areas with hard winters (Lebeda and Cohen, 2011). Survival by oospores is still an ongoing debate: there are reports of oospores formation from Russia, China, Japan, India and Italy (for detail see Cohen and Rubin, 2011; Lebeda and Cohen, 2011). Recently, infectious oospores were produced experimentally under laboratory conditions (Cohen and Rubin, 2011).

Previous studies have shown that *P. cubensis* is a highly variable pathogen from the viewpoint of host-specificity, race-specificity and virulence (Lebeda *et al*., 2006; Lebeda and Cohen, 2011). These findings are also supported by recent molecular studies (Sarris *et al*., 2009; Mitchell *et al*., 2011; Quesada-Ocampo *et al*., 2012; Runge *et al*., 2011). At least 60 cucurbit species are known hosts of *P. cubensis*, but the most economically important host crops are *Cucumis sativus* (cucumber), *Cucumis melo* (cantaloupe and muskmelon), *Cucurbita pepo* (zucchini, pumpkin and winter and summer squash) and *Citrullus lanatus* (watermelon) (Lebeda and Widrlechner, 2003). *C. sativus* is relatively the most susceptible to *P. cubensis*. Palti (1974) reported that the difference in the host species response to the pathogen was likely due to physiological races and/or pathotypes in various countries. A detailed survey of virulence in *P. cubensis* demonstrated the existence of large number pahotypes and potential races around the world (Lebeda et al., 2006).

The disease is often devastating and requires frequent fungicide application for control (Lebeda and Cohen, 2012). However, resistance has rendered several fungicides ineffective.

P. cubensis was the first oomycete to develop resistance to metalaxyl and reduced sensitivity to mancozeb. There is a broad spectrum of fungicides that are ineffective against *P. cubensis* (Lebeda and Cohen, 2011, 2012). The following principles are therefore used as the basis for a system for protection of cucurbits against *P. cubensis*; choice of relatively resistant cultivar, use of a highly effective fungicide rates, alternation and mixtures of fungicides appropriate spray intervals and determination of optimal dates for application (Gisi, and Sierotzki, 2008).

This research reviews the current information about *P. cubensis*, including distribution, host range, disease impact, virulence, pathogenicity and resistance to fungicides. Our results at once provide important knowledge of efficient disease management system creation and highlight unavailable data for future research needed for this pathogen. New extensive knowledge, which extend the previous reviews (Palti and Cohen, 1980; Cohen, 1981; Lebeda, 1990; Lebeda et al., 2006) are needed to the development of sustainable management strategies. Control and prediction of future *P. cubensis* epidemics assure only an integrated research approach that includes all factors affecting disease (pathogen, host and environment).

2. AIMS OF PH.D. THESIS

The main aims of the Ph.D. study could be summarized in the following points:

1. Review on *Pseudoperonospora cubensis*;

2. Distribution, host range, and disease impact of cucurbit downy mildew populations on cucurbitaceous crops in the Czech Republic;

3. Physiological specialization of *Pseudoperonospora cubensis*;

4. Fungicide resistance of *Pseudoperonospora cubensis*;

5. Temporal changes in cucurbit downy mildew populations

3. SURVEY OF RESULTS

3.1 Review on *Pseudoperonospora cubensis*

3.2 Distribution, host range, and disease impact of cucurbit downy mildew populations on cucurbitaceous crops in the Czech Republic

3.3 Physiological specialization of *Pseudoperonospora cubensis*

3.4 Fungicide resistance of *Pseudoperonospora cubensis*

3.5 Temporal changes in cucurbit downy mildew populations

3.1 Review on *Pseudoperonospora cubensis*

3.1.1 Pathogen profile of *Pseudoperonospora cubensis*

Pathogen profile of *Pseudoperonospora cubensis*

Jana Pavelková, Aleš Lebeda

INTRODUCTION

The causal agent of cucurbit downy mildew is *Pseudoperonospora cubensis* (Berk. et Curt.)Rostow, a biotrophic plant parasite which belongs to the most well-known downy mildew pathogens with worldwide distribution (Lebeda and Cohen, 2011; Savory et al., 2010). The occurrence of *P. cubensis* has been reported in over 80 countries, including environments ranging from semi-arid to tropical. Because of polycyclic nature of *P. cubensis* the disease spread rapidly on field as well as on protected (glasshouse, plastic house and shade house) crops (Lebeda and Cohen, 2011). More than 60 cucurbit species are known as hosts of this oomycete (Lebeda and Cohen, 2011; Lebeda and Widrlechner, 2003).

P. cubensis belongs to a group of "the highest risk pathogens" with high evolutionary potential (McDonald and Linde, 2002). Pathogenic and morphological variation of this oomycete appear to be correlated with host and environmental conditions (Lebeda and Widrlechner, 2003), and significant variation has been found at both the individual and population levels. Pathotype and race identification or host-parasite interaction of *P. cubensis* has been conducted by many researchers and various pathotypes of *P. cubensis* have been recorded in cucurbits in different countries (Cohen et al., 2003; Colucci, 2008; Shetty et al., 2002; Thakur and Mathur, 2002; Thomas et al., 1987).

The disease has two major economic impacts: the decreased yields and the lower fruit quality (Lebeda and Widrlechner, 2003). To avoid these losses, disease control is required mainly by using chemical products. Majority of agricultural fungicides were protectants and multi-site inhibitors until about 1960. New and more systemic fungicides with specific activity were introduced after the restrictions on the use of several fungicides with a different mode of action (Fernández-Ortuño et al., 2008; Mitani et al., 2001). Unfortunately, some cases of difficulty of disease control have been detected since the early 1970s. Among others reasons (e.g. poor application, spray timing, rain fastness, misidentification of the pathogen), resistance was the major problem, which the target pathogens have acquired against certain of the fungicides that normally control them well (Cohen and Coffey, 1986; Gisi, 2002; Gisi and Sierotzki, 2008; Holmes and Ojiambo, 2009; Lebeda and Schwinn, 1994; Urban and Lebeda, 2006).

TAXONOMY

Kingdom Chromista, subdivision Peronosporomycotina, class Peronosporomycetes, order Peronosporales, family Peronosporaceae, genus *Pseudoperonospora* (Thomas, 1996; Dick, 2001a; Göker et al., 2007; Voglmayr, 2008). *P. cubensis* is the type species of the genus *Pseudoperonospora*, which includes five accepted species: *P. cubensis*, *P. humuli*, *P. cannabina*, *P. celtidis* and *P. urticae* (Choi et al., 2005, Dick 2001b, 2002b).

NOMENCLATURE

P. cubensis was first recorded by Berkley in herbarium plant material originated from Cuba in 1868 (Skalický, 1961). In 1903, original name *Peronospora cubensis* was reclassified, according to further observations of sporangia germination (Rostovzev, 1903). Although, the genus *Pseudoperonospora* has close relationship with *Peronospora* (they share similar haustoria, sporophores are resemble those of *Peronospora* spp.), *P. cubensis* does produce zoosporangia and *Peronospora* has sporangia that germinate directly via a germ tube (Palti and Cohen, 1980; Rostovzev, 1903, Thomas, 1996). *P. cubensis* also stands between genera that regularly produce zoospores (*Pythium* spp.) and genera that never produce zoospores (*Peronospora* spp., *Albugo* spp.) (Göker et al., 2007). 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). Recently, several haustorial types (e.g. clavate-branched, ellipsoid-pyriform, hyphal) were recognized for downy mildew, including *Pseudoperonospora* (clavate-branched), which are considered an important diagnostic feature (Voglmayr et al., 2004).

According to Runge and Thines (2011), morphological characters may not provide sufficient information. *P. cubensis* sporangiophore morphology can vary with temperature, and sporangia dimensions are influenced by the cucurbit host (Iwata, 1942; Waterhouse and Brothers, 1981). Moreover, the host cell matrix can influence some morphological criteria (Runge and Thines, 2011). These results indicate that it is desirable to include information from genetic markers when resolving phylogenic relationships in species of *Pseudoperonospora* (Savory et al., 2010). Molecular-phylogenetic studies done with Peronosporales and Peronosporaceae (Göker et al., 2003; Riethmüller et al., 2002; Voglmayr, 2008) demonstrated that the genus *Pseudoperonospora* is a unique monophyletic group. This has been supported with some morphological and molecular studies of the species of the

genus, which showed that the genus *Pseudoperonospora* is a distinct taxonomical unit (Constantinescu, 2000; Riethmüller et al., 2002). However, recent work has shown that both species are very similar (Choi et al., 2005; Gent et al., 2009; Sarris et al., 2009), there are no significant morphological differences between *P. cubensis* and *P. humuli*, internal transcribed spacer (ITS) region sequences of both pathogens are highly similar. According to that, *P. humuli* was suggested as a synonym of *P. cubensis* (Choi et al., 2005). Nevertheless, the limited ITS r DNA intraspecific variability stands in contrast with very broad pathogenic variability of *P. cubensis* (Lebeda and Widrlechner, 2003; Lebeda et al., 2006b). Also host range and pathogenicity studies demonstrated that these species have distinct pathogenic capabilities (Gent et al., 2009). There is no evidence that *P. humuli* can infect cucurbits, and limited support for *P. cubensis* pathogenicity on hops. Conserved single nucleotide polymorphisms (SNPs) were found that consistently differentiate *P. cubensis* and *P. humuli* (Mitchell et al., 2009).

LIFE CYCLE

The primary and the main infective unit is the asexual spore (conidosporangium, zoosporangium). Sporangia are ovoid or elliptic in shape, and measure 15 to 25 × 20 to 35 µm (Skalický, 1961). They easily dislodge from the sporangiophores and are distributed by wind or water splash. After deposition on the leaf surface of a host plant, and after contact with water, 5-15 biflagellate zoospores (measuring 8-12 µm) release (Palti and Cohen, 1980) and actively swim to open stomata, where they encyst (Cohen, 1981). A germ tubes grow from the cyst, produce an appressorium. A penetration hypha develops from the appressorium and enters through the stomatal aperture into the leaf tissue. Hyaline coenocytic hyphae subsequently form and grow intercellularly through the mesophyll and palisade tissues. Clavate-branched haustoria are established within mesophyll cells where they invaginate the plant cell membrane (Voglmayr et al., 2004). Intercellular mycelium produced the sporangiophores. At first, they are poorly branched and later, they become dichotomous. Each branch of a sporangiophore terminates with a sporangium (zoosporangium), in which zoospores are produced. 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, mainly on the lower side of the leaves where stomata are more frequent (Cohen, 1981). On susceptible hosts, a new infection cycle takes place one in 7 to 14 days, depending on the environmental conditions (Kranz, 2003).

Sexual reproduction is rare, and so far has not been proven in most countries where *P. cubensis* prevails. The production of oospores occurs at the end of the season when the infected tissues become necrotic (Bedlan, 1989; Lebeda, 1990; Michelmore, 1981). In Europe, the only unambiguously observed occurrence of oospores came from Austria (Bedlan, 1989). The other records of oospore occurrence came from Israel, India, Iran, China, Japan, Russia and Italy (for details see Cohen and Rubin, 2011; Lebeda and Cohen, 2011). But it is unclear whether this pathogen survives in Central Europe or USA by oospores (Lebeda, 1986a; Lebeda and Schwinn, 1994; Lebeda and Urban, 2004a). Nonetheless, although the occurrence of oospores has not been verified in Central Europe, and in the USA (Lebeda and Urban, 2004a; Lebeda unpubl. data; Kanetis and Holmes, unpubl. data), infectious oospores were produced experimentally under laboratory conditions in Israel (Cohen and Rubin, 2011).

OVERWINTERING

The rare occurrence of oospores, limits *P. cubensis* survival in the absence of a living host. *P. cubensis* is like an obligate biotrophic parasite, absolutely dependent on its host plant for growth and survival (Palti and Cohen, 1980). Therefore, the pathogen mostly overwinters in areas with mild winter temperatures that permit cucurbit hosts to be grown year round (Bains and Jhooty, 1976), or in greenhouses (Thomas, 1996). Except for the ´green bridge´, the perennial mycelia can overwinter on some host species (e.g. *Citrullus* spp., *Cucumis* spp.) in areas with a suitable climate, even under field conditions as proved e.g. in India and southern USA (Palti and Cohen, 1980; Holmes et al., 2004). A new possibility for overwintering of *P. cubensis* in Central and Northern Europe, the only perennial cucurbit species *Bryonia dioica*, was suggested recently (Runge and Thines, 2009). However, this has not been supported by observations in the field (Lebeda and Cohen, 2010), and it is unknown whether *B. dioica* plays an important role in the life cycle of *P. cubensis* (Runge and Thines, 2009).

DISPERSION

The pathogen is thought to be reintroduced annually via long distance transport of inoculum in areas, where *P. cubensis* cannot overwinter. It has been suggested, that in northern latitudes (with hard winters), primary infections are the result of annual long-range dispersal of spore from southern and south-eastern regions (from warmer areas) of Europe or USA (Holmes et al., 2004; Lebeda, 1990; Ojiambo et al., 2009). Asexual sporangia are produced on infected foliage, which may be liberated to the air following a reduction in relative humidity when

hygroscopic twisting movements of sporangiophores actively release sporangia into air currents (Lange et al., 1989). Hence, airborne *P. cubensis* sporangia concentrations are greater in the morning and early afternoon, when changes in relative humidity and leaf wetness tend to occur. 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 (Cohen and Rotem, 1971b). Disperse 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, 1999).

SYMPTOMS

Although, disease symptoms are confined to the leaves, adversely are affect the quality and yield of fruit and consequently. 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 and the severe damage of cucurbit crops ending usually with death of the adult plants. But symptoms on young, newly developing leaves are rather rare. However, cotyledons are actually more susceptible than true leaves (Lebeda, 1990; Lebeda and Cohen, 2011).

The pathogen initially induces light-green or yellow angular lesions on the upper side (adaxial) of the leaf. The size of primary lesions varies from 3–10 mm. These lesions become first chlorotic, than coalesce and form larger lesions and may eventually cover the entire leaf and turn into necrotic lesions on the older leaves. Severely infected leaves wither early and are malformed (Lebeda and Cohen, 2011). Sporulation (a thin layer of ark brown, grey or violet-black sporangiophores bearing sporangia) is primarily seen on lower (abaxial) leaf surfaces. Symptomatic plants with yellow lesions have the greatest sporulating capacity. 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). Heavy infection ended with death of the whole plants, within 4 to 10 days from first symptoms, depending on weather conditions inoculum concentration, and host genotype (Lebeda, 1990). Symptoms differ markedly among cucurbit species. In some species (*C. sativus*, *Luffa*) *P. cubensis* causes irregular, localized, yellow lesions, restricted by leaf veins whereas in *C. melo* and *C. lanatus*, lesions are not restricted by leaf veins and are more circular nad 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 symptoms of the disease can be quite variable also among genotype (cultivars) of the same host species. They could be also influenced by weather conditions, e.f., atypical water-soaked lesions may be seen under extremely humid conditions on some host species or genotypes (Lebeda, 1986b, 1990). 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 late reaction of the infected tissue (Lebeda and Cohen, 2011). 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).

ECOLOGY

Like other foliar diseases, *P. cubensis* undergoes many and short disease cycles per season and the dispersal through spores over time and space is high (Lebeda and Cohen, 2011; Pathogen risk list 2005, www.frac.info). Infectivity by *P. cubensis* was shown to be dependent on the host plant and environmental conditions (Iwata, 1942; Palti J, 1974; Palti and Cohen, 1980; Waterhouse and Brother, 1981). Free leaf moisture, essential for germination and for the formation of primary infectious structures, greatly accelerates the development of *P. cubensis* (Lebeda and Cohen, 2011). At 15°C, the optimum temperature for infection, minimal wetting period required for germination and penetration is approximately 2 h when high levels of inoculum are present. The pathogen optimally completes its penetration into the stomata and becomes independent of the presence of free water on the leaf surface, in six hours dew period (Cohen, 1981). Zoospores release does not occur under anaerobic conditions and temperature range is 9-30°C. Light is a factor that may support the development of infection even in a short dew period (Cohen et al., 1971). Leaves colonized by *P. cubensis* undergo changes in temperature and transpiration rates, which vary during the course of infection and over the leaf surface (Lindenthal et al., 2005; Oerke et al., 2006). Low temperatures can delay symptom development whilst still promoting colonization of the leaf tissue, whereas higher temperatures result in faster lesion chlorosis that may inhibit pathogen growth (Cohen, 1977). The incubation period depends on temperature, photoperiod, inoculum concentration and leaf wetness duration, and can range from 4 to 12 days (Cohen, 1977; Thomas, 1996). At the initial phase of the infection process a temperature regime of 25-30°C/10-15°C day/night is favorable (Palti and Cohen, 1980). Low light intensity leads to the

reduction in number and size of lesions due to the weak development of hyphae and haustoria. A period of near-saturated relative humidity must occur for 6 h or more to induce sporulation (Cohen, 1981). Sporulation, as in other downy mildews, is dependent on the diurnal cycle, and is enhanced by longer photoperiods (Cohen and Rotem, 1971b). The differentiation of sporangia requires a minimum dark period of 6 h (Cohen, 1977). The optimum temperature for sporangia production is 15–20°C, but sporangia may form on cucumber at temperatures from 5 to 30°C (Cohen et al., 1971; Thomas, 1996). Other factors, such as the host species, cultivar, host nutritional status and host age, may also affect sporulation. Low humidity and dry leaf surface are optimal for the dispersion of sporangia. Temperature and light have very low influence on dispersal (Cohen, 1981). Asexual spores 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). Detached sporangia were shown to survive better in cloudy 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).

HOST RANGE AND GEOGRAPHIC 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 or protected crops, especially in areas with annual precipitation of > 300 mm (Cohen, 1981; Lebeda, 1990). *P. cubensis* has a broad host range, affects 60 species and 20 genera of the Cucurbitaceae family (Palti and Cohen, 1980; Lebeda, 1992b, 1999; Lebeda and Widrlechner, 2003). Except to cultivated species, various semi-cultivated, weedy and wild genera and species of Cucurbitaceae belong to the host list of *P. cubensis* (Cohen, 1981; Lebeda and Widrlechner, 2003, 2004). The most economically important natural hosts of *P. cubensis* are: *Cucumis sativus* L. (cucumber) and *Cucumis melo* L. (muskmelon) with infection recorded in more then 80 and 50 countries; five abundantly-grown species of the genus *Cucurbita* (*C. argyrosperma* C. Huber, *C. ficifolia* Bouche, *C. maxima* Duchesne, *C. moschata* Duchesne and *C. pepo* L.), in approximately 40 countries; *Citrullus colocynthis* (L.) Schrad. and *Citrullus lanatus* (Thunb.) Matsum. Et Nakai (watermelon) in about 25 countries; *Lagenaria siceraria* (Molina) Standl. (a cultivated crop) and *Lagenaria sphaerica* (Sond.) Naud; *Benincasa hispida* (Thunb.) Cogn., *Luffa acutangula* (L.) Roxb. (angular sponge gourd) and *Luffa cylindrica* (L.) M. J. Roem. (syn. *L. aegyptiaca* Mill.) (Lebeda, 1990; Lebeda and Widrlechner, 2003; Palti and Cohen, 1980; Thakur and Mathur, 2002).

P. cubensis exhibits clear host specialization (Palti and Cohen, 1980; Thomas et al., 1987); nevertheless, divergences in host range have been reported within and among countries (Lebeda, 1990; Lebeda and Cohen, 2011; Shetty et al., 2002). A severe outbreak of cucurbit downy mildew occurred on melons in France in 1984, as the most serious pathogen on melon cultures become in 1992 (Epinat and Pitrat, 1994a,b). In 1985, the disease reached epidemic levels in cucumber grown in Central-Eastern Europe. Reports about variation in the host range originate from many countries: in Italy, cucurbit downy mildew appears on squash (Cappelli et al., 2003). In Israel, other cucurbit species (*C. moschata* and *C. pepo* subsp. *pepo*) were first attacked in 2002 (Cohen et al., 2003). In southern China (Cohen et al., 2003) and in India (Fugro et al., 1997; Mahrishi and Siradhana, 1988), a severe epidemic of downy mildew was observed on *Luffa* spp.. These populations are different from the populations in the USA and Israel, which are incompatible with *Luffa* spp (Lebeda et al., 2006b; Thomas et al., 1987). In the USA, new populations of *P. cubensis* have emerged in 2004, which rendered downy mildew-resistant cultivars no longer resistant (Colucci et al., 2006). The host range was extended to *Lagenaria siceraria* in Korea and to *Sechium edule* in Thaiwan in the year 2005 (Choi and Shin, 2008; Ko et al., 2008). In 2008, *P. cubensis* has been reported on *Sechium edule* in India (Baiswar et al., 2010) and on *Trichosanthes cucumerina* in Malaysia (Salati et al., 2010). In the period 2009-2011, a dramatic change in host range of Czech *P. cubensis* populations was recorded. Pathogen presence was observed on *Cucurbita* spp. (*C. moschata* /2009-2010, Pavelková et al., 2011/ *C. pepo, C. maxima* /2010-2011/, *Cucurbita ficifolia* /2010/) and *Lagenaria siceraria* /2011/ for the first time (Lebeda et al., 2012). Occurrence of *P. cubensis* on *C. melo* and *C. lanatus* has been formerly reported from the Czech Republic (Lebeda et al., 2011). Moreover, *P. cubensis* was also observed on taxonomically distant species *Impatiens irvingii* in Cameroon in 2007 (Voglmayr et al., 2009). This is the first report on *P. cubensis* attacking another family (Balsaminaceae). Global climate changes could be one of the influences on the ability of the pathogen to expand both its geographical and host range (Garrett et al., 2006).

PATHOGENICITY AND VIRULENCE

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). 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 (Lebeda and Schwinn, 1994; Lebeda and Widrlechner, 2003). Specialization in *P. cubensis* is rather diverse and distinct in various pathogen populations (Lebeda et al., 2006b). Palti (1974) reported that the difference in the host species response to the pathogen was likely due to physiological races and/or pathotypes in various countries. A detailed survey of virulence in *P. cubensis* demonstrated the existence of large number pathotypes (c. 100) and potential races around the world (Lebeda et al., 2006b).

Thomas et al. (1987) proposed the first differential set for virulence determination based on three host genera (*Cucumis*, *Cucurbita*, *Citrullus*) and distinguished five different pathotypes of *P. cubensis* (isolates 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. Based on this differential set (including *Luffa cylindrica*) a new pathotype (6) was described in Israel in 2003 (Cohen et al., 2003). Nevertheless, a differential set of Thomas et al. (1987) had 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. An extend differential set was developed for *P. cubensis* pathotype determination based on 12 Cucurbitaceae differential genotypes belonging to the six most important host genera (*Cucumis*, *Cucurbita*, *Citrullus*, *Benincasa*, *Luffa* and *Lagenaria*) (Lebeda and Widrlechner, 2003). 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). Pathotypes are determined by the interactions observed on each of the 12 hosts and assigned unique tetrade codes to describe the interaction (Lebeda and Widrlechner, 2003). This system allowed for characterization of the virulence variability of *P. cubensis* at the individual and population level (Lebeda et al., 2006b; Lebeda and Urban, 2007).

Both differential sets (Lebeda and Widrlechner, 2003; Thomas et al., 1987) were chosen to unify the level of pathotype (Lebeda and Gadasová, 2002; Lebeda and Urban, 2004a). In the US, two isolates were assayed and two pathotypes, 4 and 5, were described.

The sudden increased severity of *P. cubensis* to cucumber suggested the possibility that a new pathopypes of the pathogen might have been introduced into the US. Colucci (2008) investigated 32 *P. cubensis* isolates, using the new set of cucurbit host differentials proposed by Lebeda et al. (2002 and 2003) and she found 32 different host range patterns. According to this US investigation, it can be presumed that US *P. cubensis* populations are more diverse than previously described with respect to their host range.

There are available some reports from the literature, that *P. cubensis* might also vary at the species level, suggesting the occurrence of physiological races. Such races are characterized by specialization to different cultivars of one host species (Caten, 1987; Holliday, 2001). Lebeda and Křístková (1993) noted that host–pathogen specificity between *Cucurbita pepo* and *P. cubensis* is probably controlled by race-specific factors. In contrast, no virulence variation in *P. cubensis* (originating from cucumber) has been detected on *C. sativus* and wild *Cucumis* species (Lebeda, 1992a,b; Lebeda and Prášil, 1994). 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* spp. (Lebeda, 1992a,b). 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., 1987; Lebeda, 1991; Lebeda et al., 2007a). Race-specific interactions were also displayed on *Citrullus* (Thomas et al., 1987). Race-specific factors can serve as a force for microevolutionary changes in pathogen populations (Lebeda et al., 2012).

The cucurbit–*P. cubensis* system is not well known or well defined from a host–pathogen specificity and genetic point of view (Lebeda and Widrlechner, 2003; Lebeda et al., 2006b; Lebeda and Cohen, 2011). The virulence structure of the pathogen population is principally a function of the structure of the host population (i.e. host species, number of resistance genes and their dynamics in time and space) (Müller et al., 1996). However, the population structure is influenced by other factors (Lebeda and Zinkernagel, 2003). In general, among the most important processes that affect the generation and maintenance of genetic diversity within populations of downy mildews include mutation, reproductive system, cytoplasmic factors, migration and gene flow, genetic drift and selection (Drenth and Goodwin, 1999). Similar processes also could influence variation of *P. cubensis* populations. Little is known about mutations and mutation rates in oomycetes (Drenth and Goodwin, 1999), including *P. cubensis* (Lebeda and Cohen, 2011). Nevertheless, this process could

contribute to broad variation because of extremely large population sizes and high asexual reproduction potential (Lebeda and Cohen, 2011). Recent laboratory studies of sexual reproduction showed oospore formation in *P. cubensis* which may play a crucial role in sexual recombination of this pathogen and the appearance of new pathotypes (Cohen and Rubin, 2011). However, detailed studies of this phenomenon need to be conducted (Lebeda and Cohen, 2011). Migration and gene flow is considered a very important aspect of the population genetics of oomycetes (Drenth and Goodwin, 1999). US and European population studies of *P. cubensis*, suggested that transport of the pathogen can occur over long distances via atmospheric wind currents (Holmes et al., 2004; Lebeda and Schwinn, 1994). Recent detailed spatiotemporal study of *P. cubensis* spread in the eastern US clearly showed that infection of cucurbits by *P. cubensis* appears to be an outcome of a contagion process and that factors occurring on a large spatial scale (*c.* 1000 km) facilitate the spread of the pathogen (Ojiambo and Holmes, 2011). Spatial dispersal depends not only on climatic factors (e.g. temperature, humidity, wind currents), but also on the host population density, species and genetic structure. Previous and recent population studies of *P. cubensis* in the Czech Republic showed that the pathogen population is very diverse (pathogenicity variation, fungicide resistance) and dynamic in time and space (Lebeda and Urban, 2007; Urban and Lebeda, 2007; Lebeda et al., 2010). Quesada-Ocampo et al. (2012) recently reported on a detailed study of the genetic structure of worldwide *P. cubensis* populations and identified six different genetic clusters. However, approximately half of the isolates belonged to one of the clusters. There was no direct correspondence between inferred genetic clusters and grouping of isolates by predefined geographic and host categories. Results of genetic studies of Sarris et al. (2009) and Quesada-Ocampo et al. (2012) showed that a high genetic differentiation of *P. cubensis* populations exists in Europe and surrounding countries and this can also contribute to the virulence variation in these countries. Processes of genetic drift and selection could also influence the virulence structure of *P. cubensis* populations. Recent phylogenetic studies showed some genetic similarity between *P. cubensis* and *P. humuli* (Choi et al., 2005; Sarris et al., 2009). Population genetic studies including *P. humuli* isolates could be key to determining the potential extent of gene flow between these sister species, including the contribution of *P. humuli* populations to the genetic and variation in virulence of *P. cubensis* populations (Lebeda et al., 2012). The study of Quesada-Ocampo et al. (2012) showed that there is some genetic diversification between the isolates originating from different host cucurbit species. Higher genetic diversity of resistance in *Cucurbita* spp. and some other Cucurbitaceae may substantially contribute to the selection of a new *P. cubensis* pathotypes

(Lebeda et al., 2006b; Lebeda and Cohen, 2011). These results also suggest that inclusion of isolates from *Cucumis melo* and *Cucurbita* spp., that show a different genetic composition and high genetic diversity and is necessary to capture the genetic variation of *P. cubensis.* These aspects must be also considered from the viewpoint of geography, i.e. regions with high genetic diversity should be of special concern because *P. cubensis* populations with high levels of genetic variation are likely to adapt more rapidly to resistant hosts (Quesada-Ocampo et al., 2012).

DISEASE MANAGEMENT

Management requires a multi-faceted approach including cultural practices to decrease free leaf moisture, avoidance by changing the planting date, using disease resistant or tolerant varieties and applying effective fungicides. Forecasting is an efficient aid in control of *P. cubensis* (Main et al., 2001). 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). Knowledge of the pathogen biology and ecology may serve in preventing the disease. A preventive precaution should lead to actions that encourage airflow and reduce leaf wetness. However, such actions are often insufficient during prolonged, favorable environmental conditions and in the presence of high inoculum levels. 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 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 (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).

RESISTANCE TO CUCURBIT DOWNY MILDEW

Disease resistance can be broadly defined as the host's ability to suppress or inhibit a pathogen's activity. Resistance is named as non-host, when the plant is not infected, because pathogen is not able to establish infection and cause disease. Resistance determined by resistance genes is divided in two groups: race-specific (gene-for-gene relationship) and race-nonspecific (multiple genes of small individual effect) (Ton et al., 2006). The induced

systemic resistance (ISR) is a phenomenon in which a biotic or an abiotic stimulus (e.g. a pathogen infection, activation of plant associated microorganisms or the application of chemicals) causes an elevation of plant resistance to a specific pathogen, or a group of pathogens (Kuc, 2006; Tuzun, 2006). The identification of two types of resistance (single-gene-mediated and polygenically inherited resistance) in the studies of the genetic bases of resistance to *P. cubensis*, could be likely due to use of different plant materials or different parameters to measure resistance (suppression of sporulation, presence or absence of chlorosis/necrosis on infected leaves) (van Vliet and Meysing, 1974; Epinat and Pitrat, 1994a). Most popularly used cultivars of cucumber and cantaloupe and to a lesser extent squash and pumpkin, have some level of downy mildew resistance bred into them. Even though cultivars with downy mildew resistance may become diseased, disease onset may be delayed, disease may be less severe or the pathogen may produce fewer sporangia than on cultivars without resistance (Colucci, 2008). Unfortunately, *P. cubensis*, as "risky" pathogen (according to terminology of McDonald and Linde, 2002) with a high evolutionary potential, is able to overcome new disease resistant host genotypes rapidly (Drenth and Goodwin, 1999).

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). Studies in Japan showed that *Cucurbita pepo* cultivar Soumen displayed a high level of field resistance against the isolates of *P. cubensis* from *Cucumis sativus* and *Cucumis melo* (Inaba et al., 1986). Although, efficient sources of resistance were found in wild and weedy accessions of *Cucurbita* spp. (e.g. *Cucurbita foetidissima*, *C. argyrosperma* var. *palmeria C. argyrosperma* var. *sororia*) (Lebeda and Widrlechner, 2004), there is relatively little effort in breeding for resistance in pumpkin and squash (Ferriol and Picó 2008; Paris 2008). It is evident that currently are not enough data about source of resistance in *Cucurbita* spp. against *P. cubensis*, including characteristics of commercial cultivars (Lebeda and Cohen, 2011). Within the cucurbits, breeding for resistance against *P. cubensis* was most comprehensively elaborated in muskmelon (*Cucumis melo*) (Lebeda et al., 2007a,b; Pitrat, 2008). 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). Both genotypes originated from Calcutta, India and became the background for the resistance breeding of muskmelon against *P. cubensis* in the USA (Cohen, 1981). Rather little information is available on resistance against *P. cubensis* in *Citrullus*, *Benincasa*, *Luffa* and *Lagenaria* (Lebeda and Widrlechner, 2003). Resistance breeding of watermelon (*C. 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).

Resistance to downy mildew is likely to be determined by a recessive gene or genes in *C. sativus*, whereas in *C. melo*, it is likely to be determined by a dominant gene(s) (Olczak-Woltman et al., 2011). Three recessive resistance genes were reported and designated as dm1, dm2, dm3 (Doruchowski and Lachoska-Ryk, 1992; Shimizu et al., 1963). On the other hand, resistance in cultivar Poinsett (selected from PI 197 087) was determined by one recessive gene (dm) (van Vliet and Meysing, 1974). Resistance in *C. melo* was described by Thomas et al. (1988) – two complementary, incompletely dominant genes (Pc-1 and Pc-2). However, several authors: Epinat and Pitrat (1989), Angelov and Krasteva (2000) using different plant material, but reached the same result and reported that resistance in melon was controlled by a single dominant gene designated Pc-3. These findings indicate major differences in the understanding of the genetics of resistance to downy mildew in *C. sativus* and *C. melo*. Resistant phenotypes that do not segregate into discrete categories of resistance are assumed to be under the control of multiple genes for resistance. In general, quantitative resistance conferred by a single dominant gene (Kelly and Vallejo, 2006). However, quantitative inheritance of resistance as measured by disease severity is often characterized by low heritability and is under significant environmental influence (Olczak-Woltman et al., 2009). 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). The original source of host resistance (i.e. the recessive dm1 gene) was identified in cucumber accession PI197087 and first described in India in the year 1954 (Barnes and Epps, 1954). The resistance response governed by dm1 is characterized by sparse pathogen sporulation, small necrotic lesions, tissue browning and rapid cell death, indicative of the classical hypersensitive response (HR)-type resistance. Since the 1950s, resistance conferred by dm1 has been widely used in commercial cultivars for cucumber production in the USA, and was sufficient to prevent losses caused by downy mildew until 2004 (Holmes and Thomas, 2009). Cultivars containing the dm1 gene still show some level of resistance, unfortunately the high level of resistance

once observed has now been lost. In addition, susceptible cultivars without the dm1 gene become infected earlier in the season, and exhibit more severe damage than was observed previously (Holmes et al., 2004). 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). Resistance in cucumber was reported decades ago (e.g. Cohen, 1981), however, in many cultivars (e.g. Palmeto) a relatively rapid breakdown occurred followed by serous infection with *P. cubensis* (Lebeda, 1990). Current breeding research for resistance to downy mildew in cucumber is focused on the identification of resistance germplasm(s) and cultivars via large-scale screening trials (Shetty et al., 2002; Wehner and Shetty, 1997). This screening provided no single genotype displaying complete incompatibility to current pathotypes (Lebeda, 1992a; Lebeda and Prášil, 1994), probably because of limited genetic diversity for *P. cubensis* resistance in cucumber (Lebeda and Widrlechner, 2003; Shetty et al., 2002). Recent achievements in cucumber genome mapping and sequencing (Huang et al., 2009; Ren et al., 2009) provides new opportunities for research, breeding and development of elite cucumber cultivars with new traits, as well as resistance to diseases and pests.

CHEMICAL CONTROL

Downy mildews caused by the Peronosporales, including *P. cubensis*, were not affected by chemical control until modern, systemic compounds became available. Majority of agricultural fungicides were protectants and multi-site inhibitors until about 1960. For many decades, the copper formulations, the dithiocarbamates, fentins, chloronitrines and phthalimides were the only fungicides available for the control of downy mildews. Modern fungicides with specific modes of action affect were introduced with selective, systemic and curative activity. These fungicides have specially target pathogens, so that they are much safer. They protect growing parts or areas which are not covered by applied fungicides and in contrast to contact fungicides, they control pathogens also at later stages. These features have allowed the reduction of application number and application rate required during the growing season (Fernández-Ortuño et al., 2008; Gisi, 2002; Mitani et al., 2001). Unfortunately, some cases of difficulty of disease control have been detected since the early 1970s. Among other

reasons (e.g. poor application, spray timing, rain fastness, misidentification of the pathogen) resistance was the major problem, which the target pathogens have acquired against certain of the fungicides that normally control them well (Cohen and Coffey, 1986; Gisi, 2002; Gisi and Sierotzki, 2008; Holmes and Ojiambo, 2009; Lebeda and Schwinn, 1994; Urban and Lebeda, 2006). The development of fungicide resistance is influenced by complex interactions of factors such as the biology of the pathogen, mode of action of the fungicide, fungicide use pattern, the cropping system and environmental considerations (Gisi and Sierotzki, 2008). Various mechanisms of resistance are known, such as an altered target site, which reduces the binding of the fungicide; the synthesis of an alternative enzyme capable of substituting the target enzyme; the overproduction of the fungicide target; an active efflux of reduced uptake of the fungicide and a metabolic breakdown of the fungicide (Gisi and Sierotzki, 2008; Ma and Michailides, 2005, Urban and Lebeda, 2006). According to McGrath (2001), two types of resistance are described. Qualitative resistance, when resistance results from modification of a single major gene, is seen as complete loss of disease control, pathogens are either resistant or sensitive to the fungicide. In contrast, quantitative resistance results from modification of several interacting genes and pathogens exhibit a range of sensitivity to the fungicide, depending on the number of gene changes. Variation in sensitivity within the population is continuous or unimodal. Resistance in this case can be regained by using higher rates or more frequent applications. However, additional selection in the pathogen may eventually result in complete loss of control.

Quite aggressive programme is essential to make a protective barrier of fungicide prior to sporangium deposition (Savory et al., 2010). The rather “old” multi-site fungicides (including e.g. mancozeb, folpet and copper formulations) are still very important elements in the spray programmes (about 50% of the total oomycete fungicide market). For this aspect, there is surely crucial the fact that resistance to such inhibitors has never developed and is unlikely to evolve and those they improve single-site activity and delay resistance evolution. The major site-specific fungicides are from four chemical classes: the Quinone outside inhibitors (Qols; “strobilurins”, e.g. azoxystrobin, famoxadone, fenamidone), phenylamides (PAs; e.g. metalaxyl-M) carboxylic acid amides (CAAs; e.g. dimethomorph, iprovalicarb, benthiavalicarb, mandipropamid) and cyano-acetamide oximes (e.g. cymoxanil). Smaller market shares are taken by phosphonates (mainly fosetyl-Al), dinitroanilines (fluazinam), carbamates (propamocarb) and plant defence inducers such as the benzothiadiazoles (BTH; acibenzolar-S-methyl/Bion) (Gisi and Sierotzki, 2008). Unfortunately, *P. cubensis*, as one of 10 highest risk pathogens with high evolutionary potential pathogens (McDonald and Linde,

2002; Lebeda and Urban, 2004a,b; Lebeda et al., 2006a,b, 2010; Pathogen risk list 2005, www.frac.info) develop in its populations quite quickly resistance to key fungicides. *P. cubensis* was the first oomycete with documented resistance to metalaxyl and reduced sensitivity to mancozeb (Reuveni et al., 1980; Thomas and Jourdain, 1992). In addition, populations of *P. cubensis* resistant to strobilurin fungicides have been described (Heaney et al., 2000). Also CAA-resistant isolates have recently been detected in a few trial site locations, one each in South Korea, Israel and USA (FRAC CAA working group reports, www.frac.info) and in China (Zhu et al., 2007). In other fungicide classes reduced sensitivity (or resistant) isolates obtained on *Plasmopara viticola* (cymoxanil) (Gullino et al., 1997; Genet and Vincent, 1999) and *Pythium* species (propamocarb) (Moorman and Kim, 2004). Fosetyl-Al resistant isolates in field populations have never been detected (Gisi and Sierotzki, 2008). Chemical control has to be combined with resistant varieties and cultural techniques, to minimize selection of fungicide resistant strains and to decrease the high risk of overcoming resistance genes by pathogen (Lebeda and Cohen, 2011; Savory et al., 2010).

CONCLUSIONS

P. cubensis persistence across much of Europe and Asia and the re-emergence in the USA represent a significant threat to cucurbit production worldwide. New extensive knowledge, which extend the previous reviews (Palti and Cohen, 1980; Cohen, 1981; Lebeda, 1990; Lebeda et al., 2006a,b) are needed to the development of sustainable management strategies, such a durable host resistance. Control and prediction of future *P. cubensis* epidemics assure only an integrated research approach that includes all factors affecting disease (pathogen, host and environment).

REFERENCES

Angelov, D., & Krasteva, L. (2000). Dominant inheritance of downy mildew resistance in melons. *Acta* Horticulturae, *510*, 273–275.

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.

Baiswar, P., Chandra, S., & Ngachan, S. V. (2010). *Pseudoperonospora cubensis* on *Sechium edule* in India. *Australasian Plant Disease Notes*, *5*, 3–4.

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). Temperaturedependent resistance to downy mildew in muskmelon: structural responses. *Physiological and Molecular Plant Pathology*, *43*, 11–20.

Barnes, W.-C., & Epps, W.-M. (1954). An unreported type of resistance to cucumber downy mildew. *Plant Disease Reporter*, *38*, 620.

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

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., 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.

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

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., & Rubin, A. E. (2011). Mating type and sexual reproduction of *Pseudoperonospora cubensis*, the downy mildew agent of cucurbits. *European Journal of Plant Pathology*, *132*, 577–92.

Cohen, Y. & Rotem, J. (1971a). Dispersal and viability of sporangia of *Pseudoperonospora cubensis*. *Transactions of the 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., & Coffey, M. D. (1986). Systemic fungicides and the control of Oomycetes. *Annual Review of Phytopathology*, *24*, 311–338.

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

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

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

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

Colucci, S. J., Wehner, T. C., & Holmes, G. J. (2006). The downy mildew epidemic of 2004 and 2005 in the eastern United States. In G. J. Holmes (Ed.), *Proceedings Cucurbitaceae 2006* (pp. 403–411). Raleigh: Universal.

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

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.

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.

Doruchowski, R., & Lakowska-Ryk, E. (1992) Inheritance of resistence to downy mildew (*Pseudoperonospora cubensis* Berk. & Court.) in *Cucumis sativus*. In R. W. Doruchowski, E. Kozik, & K. Niemirowicz-Szczytt (Eds.), *Proceedings of Cucurbitaceae 1992. The 5th EUCARPIA Cucurbitaceae Symposium* (pp 66–69). Warsaw, PL.

Doruchowski, R. W., & Lakowska-Ryk, E. (2000). F1 hybrid pickling cucumbers developed for increased yield, earliness and resistance to downy mildew. *Acta Horticulturae*, *510*, 45–46.

Drenth, A., & Goodwin, S.-B. (1999). Population structure of oomycetes. In J. J. Worrall (Ed.), *Structure and Dynamics of Fungal Populations* (pp. 195–224). Dordrecht: Kluwer Academic Publishers,.

Epinat, C., & Pitrat, M. (1989). Inheritance of resistance of three lines of muskmelon (*Cucumis melo*) to downy mildew (*Pseudoperonospora cubensis*). In C. E. Thomas (Ed.), *Evaluation and enhancement of cucurbit germplasm* (pp. 133–135). Charleston, SC: Cucurbitaceae Proceedings.

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 resistence of cucumber to downymildew. *Bulletin Tokai-Kinki National Agricultural Experiment Station*, *27*, 42–45.

Fernández-Ortuño, D., Torés, J. A., de Vincente, A. & Pérez-García, A. (2008). Mechanisms of resistance to Qol fungicides in phytopathogenic fungi. *International Mikrobiology*, *11*,1-9.

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.

Fugro, P. A., Rajput, J. C., & Mandokhot, A. M. (1997). Sources of resistence to downy mildew in ridge gourd and chemical control. *Indian Phytopathology*, *50*, 125–126.

Garrett, K. A., Dendy, S. P., Frank, E. E., Rouse, M. N., & Travers, S. E. (2006). Climate change effects on plant disease: genomes to ecosystems. *Annual review of phytopathology*, *44*, 489–509.

Genet, J. L. & Vincent, O. (1999). Sensitivity of European *Plasmopara viticola* populations to cymoxanil. *Pesticide Science*, *55*, 129–136.

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*, 171.

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 Patology*, *122*, 157-167.

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

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.

Gullino, M. L., Mescalchin, E., & Mezzalama, M. (1997). Sensitivity to cymoxanil in populations of *Plasmopara viticola* in northern Italy, *Plant Pathology*, 46, 729-736.

Heaney, S. P., Hall, A. A., Davis, S. A., & Olaya, G. (2000). Resistance to fungicides in the QoI-STAR cross resistance group: current perspectives. *Proceedings Brighton Crop Protection Konference* (pp. 755-762). Croydon: BCPC Publications.

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

Holmes, G. J., & Thomas, C. (2009). The history and re-emergence of cucurbit downy mildew. *Phytopathology*, *99*, 171.

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*.

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

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

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.

Iwata, Y. (1942). Specialization of *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. II. Comparative studies of the morphologies of the fungi from *Cucumis sativus* L. and *Cucurbita moschata* Duchesne. *Annals of the Phytopathological Society of Japan*, *11*, 172-185.

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

Kelly, J. D., & Vallejo, V. (2006). QTL analysis of multigenic disease resistance in plant breeding. In S. Tuzun, & E. Bent (Eds.), *Multigenic and induced systemic resistance in plants* (pp. 21–48). New York: Springer Science.

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

Kenigsbuch, D., & Cohen, Y. (1992a). Inheritance of resistence 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.
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.
Kranz, J. (2003). *Comparative epidemiology of plant diseases*. Berlin: Springer.
Kuc, J. (2006). What's old and what; new in concepts of induced systemic resistance in plants, and its applications. In S. Tuzun & E. Bent. (Eds.), *Multigenic and Induced Resistance in Plants*. New York: Springer.
Lange, L., Eden, U., & Olson, L.W. (1989). Zoosporogenesis in *Pseudoperonospora cubensis*. The causal agent of cucurbit downy mildew. *Nordic Journal of Botany*, *8*, 497–504.
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 resistence 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., & Cohen, Y. (2011). Cucurbit downy mildew (*Pseudoperonospora cubensis*) – biology, ecology, epidemiology, host-pathogen interaction and control. *European Journal of Plant Patology*, *129*, 157-192.
Lebeda, A. & Schwinn, F.-J. (1994). The downy mildews – an overview of recent research progress. *Journal of Plant Diseases and Plant Protection*, *101*, 225-254.
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., Hübschová, J., & Urban. J. (2010). Temporal population dynamics of *Pseudoperonospora cubensis*. In J. A. Thies, S. Kousik, & A. Levi (Eds.), *Cucurbitaceae 2010 Proceedings* (pp. 240–243). Alexandria: American Society for Horticultural Science.
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., Pavelková, J., Sedláková, B., Urban, J. (2012). Structure and temporal shifts virulence of *Pseudoperonospora cubensis* populations in Czech Republic. *Plant Pathology* 2012 (in press)
Lebeda, A., Pavelková, J., Urban, J., & Sedláková, B. (2011) Distribution, host range and disease severity of *Pseudoperonospora cubensis* on cucurbits in the Czech Republic. *Journal of Phytopathology*, *159*, 589–596.
Lebeda, A., & Prášil, J. (1994). Susceptibility of *Cucumis sativus* cultivars to *Pseudoperonospora cubensis*. *Acta Phytopathologica et Entomologica Hungarica*, *29*, 89–94.
Lebeda, A., & Urban, J. (2004a). Disease impact and pathogenicity variation in Czech populations of *Pseudoperonospora cubensis*. In: A. Lebeda & H. S. Paris (Eds.), *Proceedings of 30 Cucurbitaceae 2004, the 8th Eucarpia Meeting on Cucurbit Genetics and Breeding* (pp. 267-273). Olomouc.
Lebeda, A., & Urban, J. (2004b). Distribution, harmfulness and pathogenic variation of cucurbit downy mildew in the Czech Republic. Proceedings of the XVI. Slovak and Czech Plant Protection Conference. *Acta fytotechnica et zootechnica*, *7*,.
Lebeda, A., & Urban, J. (2007). Temporal changes in pathogenicity and fungicide resistance in *Pseudoperonospora cubensis* populations. *Acta Horticulturae*, *731*, 327–336.
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., & 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., Štěpánková, J., & Urban, J. (2006a). Plíseň okurky (*Pseudoperonospora cubensis*)—taxonomie, biologie, ekologie, interakce hostitel-patogen a možnosti ochrany (Cucurbita 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). *Cucurbita* (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., & Zinkernagel, V. (2003). Evolution and distribution of virulence in the German population of *Bremia lactucae*. *Plant Pathology*, *52*, 1–51.

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.

Ma, Z., & Michailides T. J. (2005). Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Protection*, *24*, 853-863.

Mahrishi, R. P., & Siradhana B. S. (1988). Studies on downy mildew of cucurbits in Rajasthan: incidence distribution, host range and field losses in muskmelon. A*nnals of arid zone*, *27*, 67–70.

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 Apríl 25 through May 31. http://www.apsnet.org/ online/future/ forecast/

McDonald, B. A. & Linde, C. (2002). Pathogen population genetics, evolutionary potential and durable resistence. *Annual Review of Phytopathology*, *40*, 349-379.

McGrath, M. T. (2001). Fungicide resistance in cucurbit powdery mildew: Experiences and challenges. *Plant Disease*, *85*, 236-245.

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.

Mitani, S., Araki, S., Yamaguchi, T., Takii, Y., Ohshima, T., & Matsuo, N. (2001). Biological properties of the novel fungicide cyazofamid against *Phytophthora infestans* on tomato and *Pseudoperonospora cubensis* on cucumber. *Pest management science*, *58*, 139-145.

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*, 87.

Moorman, G. W. & Kim, S. H. (2004). Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. *Plant Disease*, *88*, 630-632.

Müller, K., McDermott, J. M., Wolfe, M. S., & Limpert, E. (1996). Analysis of diversity in populations of plant pathogens: the barley powdery mildew pathogen across Europe. *European Journal of Plant Pathology*, *102*, 385–95.

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.-S., & Holmes, G.-J. (2011). Spatiotemporal spread of cucurbit downy mildew in the Eastern United States. *Phytopathology*, *101*, 451–61.

Ojiambo, P., Kanetis, L., & Holmes, G. (2009). Forecasting long distance movement of *Pseudoperonospora cubensis* and the Cucurbit ipmPIPE. *Phytopathology*, *99*, 171

Olczak-Woltman, H., Bartoszewski, G., Mądry, W., & Niemirowicz-Szczytt, K. (2009). Inheritance of resistance to angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*) in cucumber and identification of molecular markers linked to resistance. *Plant Pathology*, *58*, 145–151.

Olczak-Woltman, H., Marcinkowska, J., & Niemirowicz-Szczytt K. (2011). The genetic basis of resistance to downy mildew in *Cucumis* spp.—latest developments and prospects. *Journal of Applied Genetics*, *52*, 249–255.

Palti, J. (1974). The significance of pronounced divergences in the distribution of *Pseudoperonospora cubensis* on its crop hosts. *Phytoparasitica*, *2*, 109–115.

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.

Pavelková, J., Lebeda, A., & Sedláková, B. (2011). First report of *Pseudoperonospora cubensis* on *Cucurbita moschata* in the Czech Republic. *Plant Disease, 95*, 878–879.

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.

Quesada-Ocampo, L., Granke, L., Olsen, J. et al. (2012). The genetic structure of *Pseudoperonospora cubensis* populations. *Plant Disease* ???, ????–????, in press).

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.

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.

Rostovzev, S. I. (1903). Beiträge zur Kenntnis der Peronosporeen. *Flora*, *92*, 405-430.

Runge, F., & Thines, M. (2011). Host matrix has major impact on the morphology of *Pseudoperonospora cubensis*. *European Journal of Plant Pathology*, *129*, 147-156.

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

Salati, 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), 140.

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.

Shimizu, S., Kanazawa, K., & Kato, A. (1963). Studies on the breeding of cucumber for resistance to downy mildew. Part 2. Difference of resistance to downy mildew among the cucumber varieties and the utility of the cucumber variety resistance to downy mildew. *Bulletin of the Horticultural Research Station, Japan*, *2*, 80–81.

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.

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.

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

Thomas, C. E., & Jourdain, E. L. (1992). Host effect on selection of virulence factors affecting sporulation by *Pseudoperonospora cubensis. Plant Disease*, *76*, 905-907.

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

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., Cohen, Y., McCreight, J. D., Jourdain, E. L., & Cohen, S. (1988). Inheritance of resistance to downy mildew in *Cucumis melo*. *Plant Disease*, *72*, 33–35.

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.

Ton, J., Pieterse, C. M. J., & Van Loon, L. C. (2006). The relationship between basal and induced resistance in *Arabidopsis*. In S. Tuzun & E. Bent (Eds.), *Multigenic and Induced Systemic Resistance in Plants* (pp. 197-224). New York: Springer, Science+Business Media.

Tuzun, S. (2006). Terminology related to induced systemic resistance: incorrect use of synonyms may lead to a scientific dilemma by misleading interpretation of results. In S. Tuzun, & E. Bent (Eds.), *Multigenic and Induced systemic Resistance in Plants* (pp. 1-9). Berlin: Springer.

Urban, J., & Lebeda, A. (2006). Fungicide resistance in cucurbit downy mildew – methological, biological and population aspects. *Annual Review of Phytopathology*, *149*, 63-75.

Urban, J., & Lebeda, A. (2007). Variation for fungicide resistance in Czech populations of *Pseudoperonospora cubensis*. *Journal of Phytopathology*, *155*, 143–51.

Van Vliet, G. J. A. & Meysing, W. D. (1974). Inheritance of resistance to *Pseudoperonospora cubensis* Rost. in cucumber (*Cucumis sativus* L.). *Euphytica*, *23*, 251-255.

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., Piatek, M., & Mossebo, D. C. (2009) *Pseudoperonospora cubensis* causing downy mildew disease on *Impatiens irvingii* in Cameroon: a new host for the pathogen. *Plant Pathology*, *58*, 394.

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.

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.

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*, 527–532.

Zhu, S. S., Liu, X. L,, Wang, Y., Wu, X. H,, Liu, P. F., Li, J. Q., Yuan, S. K., & Si, N. G. (2007). Resistance of *Pseudoperonospora cubensis* to flumorph on cucumber in plastic houses. *Plant Pathology*, *56*, 967-975.

3.2 Distribution, host range, and disease impact of cucurbit downy mildew populations on cucurbitaceous crops in the Czech Republic

3.2.1 Lebeda, A., Hübschová, J. 2010. Distribution and harmfulness of *Pseudoperonospora cubensis* populations in the Czech Republic. Acta Horticulturae, 871, 251-258. (ISSN 0567-7572)

3.2.2 Pavelková, J., Lebeda, A., Sedláková, B. 2011. First report of *Pseudoperonospora cubensis* on *Cucurbita moschata* in the Czech Republic. Plant Disease, 95, 878-879.

3.2.3 Lebeda, A., Pavelková, J., Urban, J., Sedláková, B. 2011. Distribution, host range and disease severity of *Pseudoperonospora cubensis* on cucurbits in the Czech Republic. Journal of Phytopathology 159, 589-596.

3.2.4 Lebeda, A., Sedláková, B., Pavelková, J. 2012. New hosts of *Pseudoperonospora cubensis* in the Czech Republic and pathogen virulence variation. Acta Horticulturae.(in press)

Distribution and Harmfulness of *Pseudoperonospora cubensis* on Cucurbits in the Czech Republic in 2005-2007

A. Lebeda [a] and J. Hübschová
Faculty of Science
Department of Botany
Palacký University in Olomouc
Šlechtitelů 11, 783 71 Olomouc-Holice
Czech Republic

Keywords: cucurbit downy mildew, disease prevalence, disease incidence, epidemiology, cucumber, *Cucumis sativus*, melon, *Cucumis melo*, squash, *Cucurbita pepo*, pumpkin, *Cucurbita maxima*, *Cucurbita moschata*, *Luffa cylindrica*, *Luffa acutangula*

Abstract

The oomycete *Pseudoperonospora cubensis* causes downy mildew and is a highly destructive pathogen of cucurbitaceous plants. The distribution of *P. cubensis* on cucurbits, and the damage to crops caused by it, were evaluated at 115 localities (96 in 2005, 105 in 2006, 91 in 2007) in the Czech Republic (Central Europe) from 2005 to 2007. In the Czech Republic there are annual epidemics of *P. cubensis* since 1984. The first symptoms are usually observed in southern Moravia at the end of June and epidemics usually start by the second half of July or the beginning of August. Natural infection has been observed only on cucumber (*Cucumis sativus*), other cucurbits such as *Cucurbita pepo*, *C. maxima*, *Cucumis melo* remaining free of infection. Symptoms appear only on the leaf lamina. Most cucumber fields are destroyed by the end of growing season (second half of August). The loss of foliage has two major economic impacts: decreased yields and lower fruit quality. Infection was detected at the majority of localities (70-94%). Disease prevalence was usually high or very high (e.g. 63% in 2005 and 72% in 2007). Fields with high or very high disease prevalence were not as widespread in the year 2006, and 30% of the fields were free of infection. Our long-term observations indicated that there has been no substantial decline in the incidence and prevalence of the disease.

INTRODUCTION

The influence of harmful agents on the fitness of cultivated plants and the search for efficient disease control measures has been a major preoccupation of phyto-pathologists, plant breeders and growers for more than a century. Throughout their life plants are exposed to a wide range of potential pathogens and pests (Parlevliet, 1992). The study of the epidemiology of oomycetes has a special place in the history of plant pathology (Jegger and Pautasso, 2008). Today we know that plant pathogens from this group cause many of the world's most serious plant diseases and they are unique among microbial pathogens in being able to breach the intact surfaces of their host plants and rapidly establish infections (Soanes et al., 2007).

In recent years there has been a dramatic increase in the occurrence of *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev (1903), a causal agent of downy mildew on cucurbitaceous plants (Colucci et al., 2006; Holmes et al., 2004; Lebeda and Cohen, 2010). Like many other downy mildew pathogens, *P. cubensis* is an zoosporic and obligate biotrophic parasite. Primarily, it is a foliar disease. Damage of leaves caused by the pathogen has several major economic impacts, particularly decreased harvest time and yields and lower fruit quality (Cohen, 1981; Colucci et al., 2006; Palti and Cohen, 1980).

Apparently, *P. cubensis* is a pathogen with a high capacity to adapt to changing environmental conditions and new geographical areas. It is common in temperate and

[a]Corresponding author: ales.lebeda@upol.cz

humid areas of the world, but it is also known in cooler regions, such as Scandinavia (Lebeda and Schwinn, 1994). The occurrence of this pathogen in Central Europe (Austria, Czech Republic, Slovak Republic, Poland) had been known since the beginning of the last century (Hecke, 1904), but mostly without any epidemic and destructive effect. The European population of *P. cubensis* has been in epidemic progress since 1984, with a deleterious impact on cucumber crops, but almost no epidemics have been recorded on *Cucumis melo* and *Cucurbita* spp. under either field or glasshouse conditions in Central Europe (Lebeda, 1999; Lebeda and Cohen, 2010).

The aim of the research described here was to survey the impact of cucurbit downy mildew disease on cucurbitaceous vegetables in the territory of the Czech Republic, focusing on its distribution and harmfulness. This paper summarizes the results from the period 2005-2007 and present a continuation of our long-term field and experimental studies of *P. cubensis* (e.g. Lebeda and Urban, 2004, 2007; Lebeda et al., 2006; Sarris et al., 2009).

MATERIALS AND METHODS

Area and Period of Surveying

Distribution and harmfulness (expressed as disease incidence and disease prevalence) of *P. cubensis* on cucurbitaceous plants were evaluated annually in the Czech Republic during the period 2005-2007. Monitoring visits were undertaken during harvest time (end of July and August) and included domestic vegetable gardens, small private fields and larger production areas. The distribution of *P. cubensis* was studied in the two main geographical areas of the Czech Republic, namely Moravia (its central and southern parts) and Bohemia (eastern and central parts) (Fig. 1). The main cucurbitaceous vegetable production areas (South and Central Moravia, East and Central Bohemia, and Polabí (the lowland region of Bohemia along the River Labe)) were included in the survey, as well as some areas that are marginal for cucurbit production (e.g. hilly and mountainous areas of Jeseníky, Beskydy, Českomoravská vrchovina, Podkrkonoší). The surveying expeditions were rather extensive (Table 1 and Fig. 1) and altogether, the occurrence of *P. cubensis* was monitored in 115 localities (96 in 2005, 105 in 2006, and 91 in 2007).

Recorded Characteristics

Several characteristics were recorded at each locality: the date of observation, geographic, regional and ecological parameters of the locality, character of growing area, disease incidence and prevalence, occurrence of other harmful organisms and, when data were available, records of any fungicide treatment. Two epidemiological parameters were used to assess the occurrence of *P. cubensis*. Disease incidence was expressed as a percentage of surveyed localities and in host plants crops at which *P. cubensis* occurred. Disease prevalence (disease intensity) was assessed visually by using a 0-4 scale, modified for *P. cubensis* (Lebeda and Křístková, 1994; Table 2). Leaf samples were taken from crops with infected plants for subsequent isolation of pure cultures of *P. cubensis* for further investigation (e.g. pathotyping, sensitivity to fungicides, molecular variation).

RESULTS

Host Range of *P. cubensis*

The occurrence of *P. cubensis* in the Czech Republic has been most frequently observed on cucumber (*Cucumis sativus*), pumpkin (*Cucurbita maxima*), squash (*Cucurbita pepo*), watermelon (*Citrullus lanatus*); rarely also on melon (*Cucumis melo*), *Lagenaria siceraria*, and wild species (*Bryonia alba* and *Echinocystis lobata*). During the period of our study (2005-2007), natural infection was recorded only on *C. sativus*, other cucurbitaceous plants and wild cucurbits were without disease symptoms. On cucumber, infection was recorded only on leaf laminas, not on fruits, flowers or stems.

Natural Distribution, Seasonal and Spatial Dynamics of *P. cubensis* in *C. sativus* Crops

Disease incidence and prevalence were assessed in the visited localities with *C. sativus* crops during 2005-2007 (Tables 3 and 4, Fig. 2). *P. cubensis* was widespread across the whole area of the Czech Republic studied and in total 84% of visited localities. The percentage of localities with infected *C. sativus* crops was almost identical in the years 2005 and 2007, but was slightly lower in 2006 (Table 3). This is because of the higher occurrence of healthy crops in the Moravian area that year (Table 4), the most likely reason for which was a dry summer. The level of disease prevalence was assessed in all localities with infected *C. sativus* crops (Table 3, Fig. 2). Serious infection (disease prevalence rated as 3 or 4) was detected in the majority (70%) of surveyed localities in successive years.

Coincidence of Other Harmful Organisms in the Crops of *C. sativus*

Two ascomycete pathogens, *Golovinomyces cichoracearum* and *Podosphaera xanthii*, causal agents of cucurbit powdery mildew, were the most commonly recorded natural infections. Only rarely, other fungal pathogens occurred, including *Colletotrichum orbiculare*, *Cladosporium cucumerinum* and *Didymella bryoniae.* A frequently observed bacterial pathogen was *Pseudomonas syringae* pv. *Lachrymans*. In some localities, symptoms of viral infections were also noted. Among the most frequently occurring pests were two-spotted spider mite (*Tetranychus urticae*), various species of aphids (Aphidoidea), whitefly (*Trialeurodes vaporianum*), and in polytunnels and greenhouses, the thrips (*Frankliniella occidentalis*).

DISCUSSION

Cucurbits, in particular cucumbers are among the most traditional and favorite vegetables grown in the Czech Republic (Moravec et al., 2004). The devastating epidemics of cucurbit downy mildew which have occurred annually since the half of the 1980s (Lebeda, 1986) have resulted in heavy economic losses in cucumber production, and this has led to a serious reduction of the production area (CMVU, 2008; Moravec et al., 2004). Despite this, detailed information on the distribution, severity and epidemiology of the disease is still very limited. The results reported here contribute to an understanding of the distribution and harmfulness of *P. cubensis* in the Czech Republic over the period 2005-2007 and supplement data from our previous reports covering the years 2001-2004 (Lebeda and Urban, 2004).

Our recent survey over the period 2005-2007 showed that the pathogen is very damaging, especially for cucumbers. It was distributed across the whole area studied, a disease impact was evident both in the main and marginal production areas. It caused serious epidemics each year and the majority of the monitored cucumber fields were severely infected. The first occurrence of *P. cubensis* was repeatedly observed in the lowlands of South Moravia at the end of June or at the beginning of July; however, serious epidemics usually began in the second half of July (Lebeda and Urban, 2004). Our field observations confirmed that, because of its polycyclic nature, *P. cubensis* spreads very rapidly within cucumber fields in the Czech Republic. Some records of the epidemic occurrence of *P. cubensis* are also available from other European countries (Lebeda, 1990; Lebeda and Cohen, 2010). In Central Europe, the occurrence of *P. cubensis* is very unpredictable and outbreaks can be long-lasting and extremely destructive (Lebeda, 1999; Lebeda and Cohen, 2010). However, with the exception of the Czech Republic, there is no detailed information on the distribution of *P. cubensis* in others parts of Europe. Nevertheless, *P. cubensis* is definitely a very invasive pathogen and it occurs in all the most important growing areas of the world and there is some evidence to indicate that the frequency and severity of outbreaks of the disease are increasing (Holmes et al., 2004, 2006; Lebeda and Cohen, 2010).

The spatial and temporal dynamics of the distribution of *P. cubensis* on cucumber in the Czech Republic has been regularly assessed during the last ten years (Lebeda and Urban, 2004, 2007). Our results on the geographical distribution of *P. cubensis* revealed

some differences between the two main regions of Czech Republic (i.e. Moravia and Bohemia) over the years. A relatively high fluctuation in disease severity in particular years and localities were recorded in both areas. However, during the period 2005-2007, we did not observe any significant increase of prevalence or harmfulness of cucurbit downy mildew in the Czech Republic. Recorded differences in disease incidence and prevalence (Tables 3 and 4) were more likely the result of among-years-fluctuations, which agrees with our previous data obtained since 2001 (Lebeda and Urban, 2004; Lebeda, unpublished data).

P. cubensis exhibits clear host specialization (Palti and Cohen, 1980; Thomas et al., 1987); nevertheless, variation in host range has been reported from different countries (Lebeda and Cohen, 2010; Shetty et al., 2002). During our surveys the recorded and evaluated potential hosts were mainly cucumber (*Cucumis sativus*), squash (*Cucurbita pepo*), pumpkin (*Cucurbita maxima*), but very rarely also melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*). Nevertheless, repeating natural infections were found only on cucumbers; macroscopical symptoms of infection were not observed on other cucurbit crops, including wild growing Cucurbitaceae (e.g. *Bryonia alba*). However, from previous observations in the Czech Republic it is evident that infections were occasionally observed on *Cucumis melo* in 1984 (Křístková et al., 2007), 2003 and 2004 (Urban and Lebeda, 2007). Microscopically, sporangia of *P. cubensis* were also detected on *Cucurbita maxima* in 1996 and *Cucurbita pepo* in 2000 (Křístková et al., 2007). Limited development of symptoms was also recorded on *Citrullus lanatus* and *Cucurbita pepo* in 1985 and 1997, but those observations were from glasshouses where the plants were under a strong infection pressure (Lebeda et al., 2007).

Because it is an obligate biotrophic parasite, the survival of *P. cubensis* is dependent on the availability of living host tissues. It has therefore been suggested (Holmes et al., 2004; Lebeda, 1990; Lebeda and Cohen, 2010) that in northern latitudes, primary infections are the result of annual long-range dispersal of spores from more southerly regions, because in northern areas cucurbit plants die back in autumn and are not available for pathogen overwintering (as active mycelium in host tissues). This possibility is supported by the recorded ability of Czech isolates of *P. cubensis* to infect cucurbit species, which are not common in Central Europe and the Czech Republic (e.g. wild *Cucurbita* spp., *Citrullus lanatus*, *Lagenaria siceraria*; Lebeda and Widerlechner, 2003, 2004). From the results presented in this paper and from previously published data it is evident that *P. cubensis* is a very aggressive pathogen with a high epidemiological potential (Lebeda and Urban, 2007). The existence of various pathotypes was confirmed (Lebeda et al., 2006) and the spectrum of new host species is increasing (e.g. Runge and Thines, 2009; Voglmayr et al., 2009).

With regard to the harmfulness of *P. cubensis*, the high number of localities and crops included in our study enabled us to obtain a clear picture of the geographic distribution, spatial dynamics and epidemiology of *P. cubensis*, at least for the Czech Republic. Our extensive data collected over many years (Lebeda and Urban, 2004) are probably the most comprehensive reports available about the spatial distribution and dynamics of *P. cubensis* in Europe. These results also support previous assumptions (Lebeda and Schwinn, 1994) about the highly negative economic impact of *P. cubensis* on cucumber production in the Czech Republic (in comparison with 1980s the reduction of production area at the beginning of 2000 reached ca 75% (CMVU, 2008; Moravec et al., 2004)). Further detailed research on this pathogen is urgently required, together with broad international cooperation focused on various aspects its epidemiology (Lebeda and Cohen, 2010; Lebeda et al., 2006).

ACKNOWLEDGEMENTS

The authors would like to thank Prof. David A. Morris (Southampton University, U.K.) for reading and remarks on the first draft of the manuscript. This research was supported by grant MSM 6198959215 (Czech Ministry of Education, Youth and Sports), QH 71229 (Czech Ministry of Agriculture) and National Programme of Genepool

Conservation of Microorganisms and Small Animals of Economic Importance (NPGZ-M/03-023, Czech Ministry of Agriculture).

Literature Cited

CMVU - Czech and Moravian Vegetable Union. Vegetable farming in CR and Most common vegetables in CZ. http://www.zelinarskaunie.cz/ZU%C4%8CMhlavn%C3%ADstr% C3% A1nka/P%C4%9Bstov%C3%A1n%C3%ADzeleninyv%C4%8CR/tabid/76/Default.aspx. Accessed October 2008.

Cohen, Y. 1981. Downy mildew of cucurbits. p.341-354. In: D.M. Spencer (ed.), The Downy Mildews. Academic Press, London, UK.

Colucci, S.J., Wehner, T.C. and Holmes, G.J. 2006. The downy mildew epidemic of 2004 and 2005 in the eastern United States. p.403-411. In: G.J. Holmes (ed.), Proceedings of Cucurbitaceae 2006. Universal Press, Raleigh, North Carolina, USA.

Hecke, L. 1904. Über das Auftreten von *Plasmopara cubensis* in Österreich. Ann. Mycol. 2:356-358.

Holmes, G., Wehner, T. and Thornton, A. 2006. An old enemy re-emerges. American Vegetable Grower, 14-15.

Holmes, G.J., Main, C.E. and Keever III, Z.T. 2004. Cucurbit downy mildew: A unique pathosystem for disease forecasting. p.69-80. In: P.T.N. Spencer-Phillips and M. Jeger (eds.), Advances in Downy Mildew Research, Vol. 2. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Jeger, M.J. and Pautasso, M. 2008. Comparative epidemiology of zoosporic plant pathogens. Europ. J. Plant Pathol. 122:111-126.

Křístková, E., Lebeda, A. and Sedláková, B. 2007. Temporal and spatial dynamics of powdery mildew species on cucurbits in the Czech Republic. Acta Hort. 731:381-388.

Lebeda, A. 1986. Epidemic occurrence of *Pseudoperonospora cubensis* in Czechoslovakia. Temperate Downy Mildews Newsl. 4:15-17.

Lebeda, A. 1990. Biology and ecology of cucurbit downy mildew. p.13-46. In: A. Lebeda (ed.), Cucurbit downy mildew. Czechoslovak Scientific Society for Mycology by Czechoslovak Academy of Sciences, Praha, Czech Republic.

Lebeda, A. 1999. *Pseudoperonospora cubensis* on *Cucumis* spp. and *Cucurbita* spp. – resistance breeding aspects. Acta Hort. 492:363-370.

Lebeda, A. and Cohen, Y. 2010. Cucurbit downy mildew (*Pseudoperonospora cubensis*) – biology, ecology, epidemiology, host-pathogen interactions and control. Europ. J. Plant Pathol. (in press)

Lebeda, A. and Křístková, E. 1994. Field resistance of *Cucurbita* species to powdery mildew (*Erysiphe cichoracearum*). J. Plant Dis. Protect. 101:598-603.

Lebeda, A. and Schwinn, F.J. 1994. The downy mildews – an overview of recent research progress. J. Plant Dis. Protect. 101:225-254.

Lebeda, A. and Urban, J. 2004. Distribution, harmfulness and pathogenic variability of cucurbit downy mildew in the Czech Republic. Acta Fytotech. et Zootech. 7:170-173.

Lebeda, A. and Urban, J. 2007. Temporal changes in pathogenicity and fungicide resistance in *Pseudoperonospora cubensis* populations. Acta Hort. 731:327-336.

Lebeda, A. and Widrlechner, M.P. 2003. A set of *Cucurbitaceae* taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. J. Plant Dis. Protect. 110:337-349.

Lebeda, A. and Widrlechner, M.P. 2004. Response of wild and weedy *Cucurbita* L. to pathotypes of *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov. (cucurbit downy mildew). Advances in Downy Mildew Research 2:203-210.

Lebeda, A., Sedláková, B. and Křístková, E. 2007. Temporal changes in pathogenicity structure of cucurbit powdery mildew populations. Acta Hort. 731:381-388.

Lebeda, A., Widrlechner, M.P. and Urban, J. 2006. Individual and population aspects of interactions between cucurbits and *Pseudoperonospora cubensis*: pathotypes and races. p.453-467. In: G.J. Holmes (ed.), Proceedings of Cucurbitaceae 2006, Universal Press, Raleigh, North Carolina, USA.

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

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

Parlevliet, J.E. 1992. Selecting components of partial resistance. In: H.T. Stalker and J.P. Murphy (eds.), Plant Breeding in the 1990s. Proceedings of the symposium on plant breeding in the 1990s, CAB International Wallingford.

Runge, F. and Thines, M. 2009. A potential perennial host for *Pseudoperonospora cubensis* in temperate regions. Eur. J. Plant. Pathol. 123:483-486.

Sarris, P.F., Abdelhalim, M., Kitner, M., Skandalis, N., Panopoulos, N.J., Doulis, A.G. and Lebeda, A. 2009. Molecular polymorphism between populations of *Pseudoperonospora cubensis* from Greece and the Czech Republic and their phytopathological and phylogenetic implications. Plant Pathol. 58:933-943.

Shetty, N.V., Wehner, T.C., Thomas, C.E., Doruchowski, R.W. and Shetty, K.P. V. 2002. Evidence for downy mildew races in cucumber tested in Asia, Europe, and North America. Sci. Hort. 94:231-239.

Soanes, D.M., Richards, T.A. and Talbot, N.J. 2007. Insights from sequencing fungal and oomycete genomes: what can we learn about plant disease and the evolution of pathogenicity? Plant Cell 19:3318-3326.

Thomas, C.E., Inabana, T. and Cohen, Y. 1987. Physiological specialization of *Pseudoperonospora cubensis*. Phytopathology 77:1621-1624.

Voglmayr, H., Piatek, M. and Mossebo, D.C. 2009. *Pseudoperonospora cubensis* causing downy mildew disease on *Impatiens irvingii* in Cameroon: a new host for the pathogen. Plant Pathol. 58:394.

Tables

Table 1. Regions of the Czech Republic (see Fig. 1) surveyed for distribution and harmfulness of cucurbit downy mildew (2005-2007).

Moravia/Region	Abbr.	Bohemia/Region	Abbr.
South Moravia	JM	Hradec Králové	KH
Olomouc	OL	Pardubice	PA
Zlín	ZL	Central Bohemia	SC
Moravia–Silesia	MS	South Bohemia	JC

Table 2. Visual scale for expressing different levels of disease prevalence (according to Lebeda and Křístková, 1994).

Degree of scale	Percentage of the total leaf surface in the crop infected	Disease prevalence
0	0	No disease symptoms
1	0 ≤25	Low
2	>25 ≤50	Medium
3	>50 ≤75	High
4	>75	Very high

Table 3. Incidence and harmfulness of cucurbit downy mildew in the Czech Republic over the period 2005-2007.

Year	No. of evaluated localities with *Cucumis sativus* crops	Disease incidence		Disease prevalence			
		% of localities with		Degree of scale/% of localities with recorded infection			
		healthy crops	infected crops	1	2	3	4
2005	83	7	93	21	12	27	40
2006	93	30	70	22	12	20	46
2007	66	6	94	7	16	29	48

Table 4. Differences in incidence and harmfulness of cucurbit downy mildew in two major parts of the Czech Republic (Moravia and Bohemia).

Year/area of the Czech Republic	No. of evaluated localities with *Cucumis sativus* crops	Disease incidence		Disease prevalence			
		% of localities with		Degree of scale/% of localities with recorded infection			
		healthy crops	infected crops	1	2	3	4
2005							
Moravia	53	6	94	10	8	32	50
Bohemia	30	10	90	41	18	19	22
2006							
Moravia	59	41	59	34	20	32	14
Bohemia	34	12	88	7	3	7	83
2007							
Moravia	50	8	92	9	17	33	41
Bohemia	16	0	100	0	12	19	69

Figures

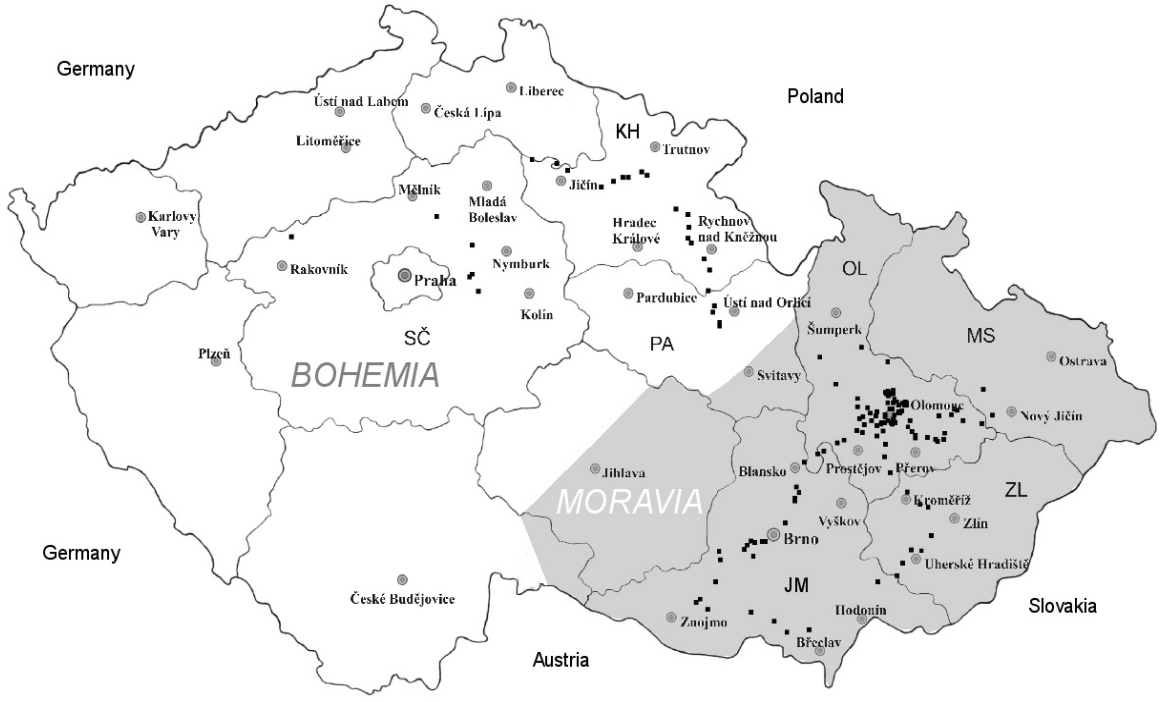


Fig. 1. Localities (by dots) surveyed for the *Pseudoperonospora cubensis* incidence in the Czech Republic in period 2005-2007.

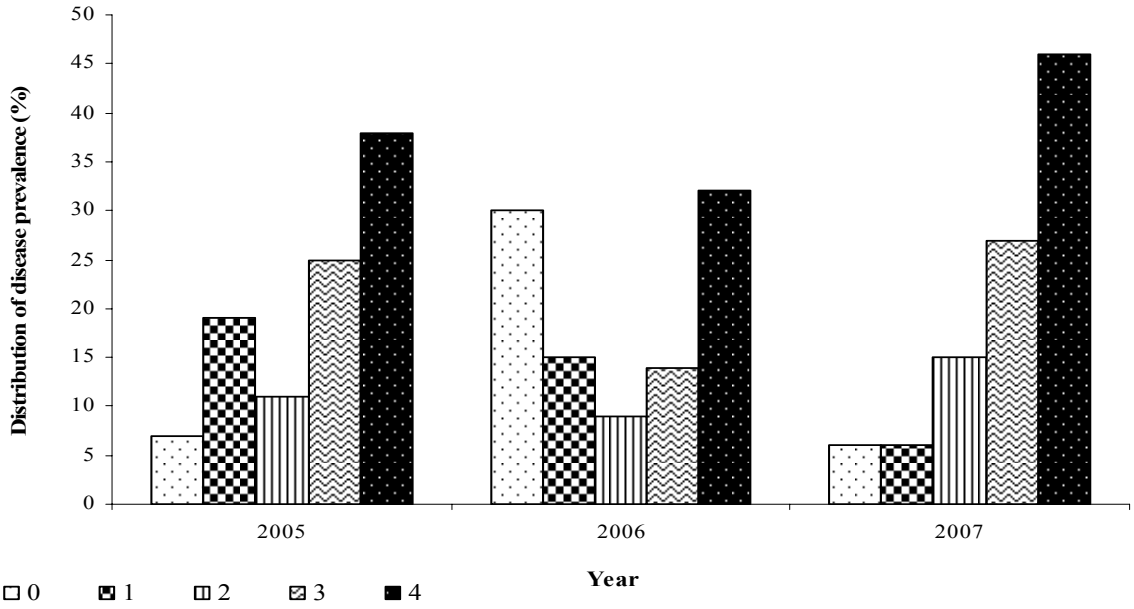
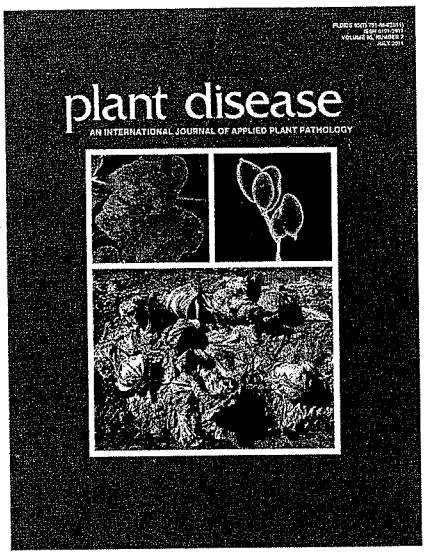


Fig. 2. Distribution of disease prevalence among years. Figure shows among-year variation in localities with *Cucumis sativus* crops differing in disease prevalence. Different levels of disease prevalence were expressed on a scale of 0-4 (Lebeda and Křístková, 1994): 0 – no symptoms of *P. cubensis* infection on a surveyed locality; 1 – low disease prevalence; 2 – medium disease prevalence; 3 – high disease prevalence; 4 – very high disease prevalence (for details see Table 2).



COVER

(*Clockwise from upper left*): Symptoms of downy mildew (*Pseudoperonospora cubensis*) on upper leaf surface of *Cucurbita moschata* (courtesy J. Pavelková et al., see page 878); scanning electron photomicrograph of the sporangia produced by *Phytophthora infestans* (courtesy L. M. Kawchuk et al., see page 873); symptoms of Verticillium wilt caused by *Verticillium dahliae* on crisphead lettuce (courtesy Z. K. Atallah et al., see page 784).

The American Phytopathological Society
3340 Pilot Knob Road
St. Paul, MN 55121
+1.651.454.7250
http://apsjournals.apsnet.org

plant disease

AN INTERNATIONAL JOURNAL OF APPLIED PLANT PATHOLOGY

An Official Journal of The American Phytopathological Society

July 2011 **Volume 95, Number 7**

FOCUS

FEATURE

RESEARCH

DISEASE NOTES

PLANT DISEASE replaces *Plant Disease Reporter*, published by the United States Department of Agriculture from 1917 through 1979. Volume numbers of PLANT DISEASE are continuous with those of *Plant Disease Reporter*.

2011 PLANT DISEASE Subscription Rates and Access Information

Print Subscription

$726 USA; $827 elsewhere per year (includes expedited delivery outside the USA). Canadian subscribers must add 5% GST/HST to the elsewhere price (Canadian GST #R129888129).

Online Subscriptions

To request a price quote for electronic subscriptions via I.P. access, to read Terms of Use and FAQs for APS Online journal subscriptions, or to view a sample issue online, go to:

http://apsjournals.apsnet.org/

Single Issue Purchases

Current volume year: $69 per issue USA; $75 per issue elsewhere. Prior volume years: $74 per issue USA; $80 per issue elsewhere.

Single Article Purchases

PDFs of articles published within the last 12 months may be purchased online. Go to:

http://apsjournals.apsnet.org/loi/pdis

to locate the article and then click the PDF link. Articles published more than 12 months ago may be accessed online without charge when available. APS retains full copyright to any article that is purchased or retrieved with or without payment, and all copyright laws apply to any subsequent usage.

Payment Information

Send subscription payments to APS Headquarters in the USA. All prices shown are US$, and payments must be made in US funds through a member bank of the USA Federal Reserve System. VISA, MasterCard, and American Express are also accepted.

Change of Subscription Address

Provide your current mailing label to APS USA Headquarters with forwarding address information 6 weeks before your address change.

Personal Subscriptions

Personal subscriptions are available only to APS members at a discount. Members may subscribe at any time by contacting Denise Kessler, e-mail: dkessler@scisoc.org, phone: +1.800.481.2698 or +1.651.454.7250.

PLANT DISEASE (ISSN 0191-2917) is published monthly by The American Phytopathological Society, 3340 Pilot Knob Road, St. Paul, MN 55121. Periodicals postage paid at St. Paul, MN, and additional mailing offices. USPS 522-750. POSTMASTER: Send address changes to PLANT DISEASE, 3340 Pilot Knob Road, St. Paul, MN 55121.

June 2011 PLANT DISEASE issued 9 May 2011.

First Report of Verticillium Wilt Caused by *Verticillium dahliae* on *Coleus verschaffeltii* in Italy. A. Garibaldi, D. Bertetti, A. Poli, and M. L. Gullino, Center of Competence AGROINNOVA, University of Torino, Via Leonardo da Vinci, 44, 10095 Grugliasco, Italy. Plant Dis. 95:878, 2011; published online as doi:10.1094/PDIS-03-11-0247. Accepted for publication 23 April 2011.

Coleus verschaffeltii Lem. (synonym *C. blumei* Benth., *Plectranthus scutellaroides* (L.) R. Br., and *Solenostemon scutellarioides* (L.) Codd), a perennial plant belonging to the Lamiaceae family, is used as a bedding plant for public gardens. The most popular cultivars produce speckled leaves of various colors. In October 2010, severe outbreaks of a previously unknown wilt were observed in a public garden at Torino (northern Italy) on 50 8-month-old plants. Plants were sprinkle irrigated. Initial symptoms were withering of leaves starting from the collar and brown streaks in the vascular tissue of roots, crown, and stem. Subsequently, infected tissues wilted and plants became stunted. Early leaf drop was observed and plants appeared bare, keeping few leaves only at the end of stems. Infected plants did not die but they lost the original ornamental aspect. Seventy percent of the plants were affected. Stems of 10 plants were disinfected with 1% sodium hypochlorite. Cross-sections through symptomatic vascular tissues were plated on potato dextrose agar amended with 25 ppm of streptomycin sulfate. After 10 days at 20 to 23°C, a fungus was consistently recovered from 90% of stems. Irregular, black microsclerotia, 29 to 76 × 14 to 52 (average 49 × 28) µm, developed in hyaline hyphae after 15 days of growth. Hyaline, elliptical, single-celled conidia, 3.9 to 7.2 × 1.7 to 2.8 (average 5.1 × 2.2) µm, developed on verticillate conidiophores with three phialides at each node. On the basis of these morphological characteristics, the fungus was identified as *Verticillium dahliae* (3). The internal transcribed spacer (ITS) region of rDNA was amplified using the primers ITS1/ITS4 (4) and sequenced. BLASTn analysis (1) of the 491-bp segment showed a 99% homology with the sequence of *V. dahliae* (Accession No. GU461634). The ITS nucleotide sequence of our isolate has been assigned the GenBank Accession No. JF704205. Pathogenicity tests were performed twice using 45-day-old plants obtained from seeds of *C. verschaffeltii* grown in 1-liter pots containing a 50:20:20:10 steamed mix of peat moss/pumice/pine bark/clay. Roots of 10 healthy plants were immersed in a conidial suspension (1.7×10^7 ml^{-1}) of one culture of *V. dahliae* isolated from infected plants. Ten plants immersed in sterile water served as controls. Plants were maintained in a glasshouse at daily average temperatures between 20 and 28°C and relative humidity between 50 and 80%. First wilt symptoms and vascular discoloration in the roots, crown, and stems developed 20 days after inoculation. *V. dahliae* was consistently reisolated from infected vascular tissues of crown and stems of symptomatic plants. Noninoculated plants remained healthy. To our knowledge, this is the first report of Verticillium wilt on *C. verschaffeltii* in Italy. Verticillium wilt had been previously reported on *S. scutellaroides* in the United States (2). At this time, the economic importance of Verticillium wilt on *C. verschaffeltii* in Italy is limited.

References: (1) S. F. Altschul et al. Nucleic Acids Res. 25:3389, 1997. (2) D. Farr et al. Fungi on Plants and Plant Products in the United States. The American Phytopathological Society. St Paul, MN, 1989. (3) G. F. Pegg and B. L. Brady. Verticillium Wilts. CABI Publishing, Wallingford, UK, 2002. (4) T. J. White et al. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, 1990.

First Report of Frogeye Leaf Spot of Soybean Caused by *Cercospora sojina* Race 11 in Virginia. M. L. Rosso, A. Vazquez, and K. M. Rainey, Virginia Polytechnic Institute and State University, Blacksburg 24061. Plant Dis. 95:878, 2011; published online as doi:10.1094/PDIS-03-11-0151. Accepted for publication 18 April 2011.

Frogeye leaf spot of soybean (FLS) (*Glycine max* (L.) Merr.), caused by *Cercospora sojina* Hara, was first detected in Virginia in 1942 (1). During the 2008 growing season, a FLS survey was conducted in soybean fields in Virginia. This was the first FLS race survey conducted in Virginia. Typical frogeye leaf spot symptoms, as reported by Phillips (4), were observed on soybean leaves in Westmoreland County. During 2008, Westmoreland County planted 7,365 ha of soybean. Symptomatic leaves were collected from V06-1891, V06-1365, V05-4394, V04-8405, and Hutcheson cultivars from plants in growth stages R5 to R6. Leaves were placed in a moist chamber for 24 h at 21°C with 12-h light to induce sporulation. *C. sojina* was only recovered from V06-1365. Conidia were removed from the leaves, placed into V8 juice agar amended with rifampicin (10 mg ml^{-1}) and ampicillin (0.25 g liter^{-1}) and incubated at 21°C with 12-h light. Cultures with dark pigmentation and presence of conidia were observed after 3 weeks. Conidia matched the description of *C. sojina* (4). Conidia had three to nine septa, were hyaline, elongate to fusiform, and measured 3 to 6 × 25 to 40 µm. Race identification was conducted using the set of differentials reported by Mian et al. (3). Spores for inoculation were produced on soybean stem lima bean agar (SSLBA) media. Ten-centimeter-diameter pots, each containing four plants, were used. The test was conducted twice in a complete randomized design with three replications. Seedlings were inoculated at the V3 growth stage with a spore suspension of 6×10^4 spores/ml. Control plants were sprayed with sterile distilled water. Plants were placed in a greenhouse bench humidity chamber at 21°C for 72 h. Disease rating was conducted 14 days after inoculation. Since the resistance to FLS is known to be controlled by single dominant genes, the FLS was scored as a qualitative trait (i.e., resistant versus susceptible) as previously done by Mian et al. (2). Plants that showed numerous, large, spreading lesions were classified as susceptible and each plant was given a score of 1. Plants that showed no lesions or only small lesions or flecks were classified as resistant and each plant was given a score of 0. Control plants remained healthy. On the basis of the reaction response of the isolate on the set of differentials and comparison with the proposed race designations of Mian et al. (3), the isolate was classified as race 11. Race 11 shows compatible reaction (susceptibility) on the soybean cv. Lincoln, which is the source of *Rcs1* resistance gene, and incompatible reactions (resistance) on cvs. Peking, Davis, and Kent. The latter two cultivars are sources of the *Rcs3* and *Rcs2* genes, respectively. Successful development of soybean cultivars with FLS resistance not only depends on knowledge of the presence of resistance genes, but also on the understanding of the pathogen population structure. To our knowledge this is the first report of *C. sojina* race 11 from soybean in Virginia. Resistance to this race is conditioned by *Rcs2*, *Rcs3*, and the single dominant gene in Peking (3). We recommend use of *Rcs3* and *Rcs2* genes and the single dominant gene in Peking for resistance to FLS in Virginia.

References: (1) S. B. Fenn. Plant Dis. Rep. 26:383, 1942. (2) M. A. R. Mian et al. Crop Sci. 39:1687, 1999. (3) M. A. R. Mian et al. Crop Sci. 48:14, 2008. (4) D. V. Phillips. Page 20 in: Compendium of Soybean Diseases. 4th ed. The American Phytopathological Society. St. Paul, MN, 1999.

e-Xtra*

First Report of *Pseudoperonospora cubensis* on *Cucurbita moschata* in the Czech Republic. J. Pavelková, A. Lebeda, and B. Sedláková, Department of Botany, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 11, 783 71 Olomouc-Holice, Czech Republic. This research was supported by grants QH 71229, MSM 6198959215, and internal grant of Palacký University in Olomouc IGA PrF_ 2011. Plant Dis. 95:878, 2011; published online as doi:10.1094/PDIS-01-11-0055. Accepted for publication 1 April 2011.

Pseudoperonospora cubensis (Berk. & M.A. Curtis) Rostovzev, the causal agent of cucurbit downy mildew, was observed for the first time on *Cucurbita moschata* Duchesne in the Czech Republic (CR) in August 2009 and repeatedly in September 2010. Recently, *C. moschata* has not been an economically important crop in the CR; however, related crops *C. pepo* and *C. maxima* have increased in importance. Infected plants with *P. cubensis* were found in two locations: in a hobby garden in north Moravia (Nový Jičín – Kojetín [49°33′48.088″N, 17°59′16.632″E], 2009 and 2010) and in a commercial field in central Moravia (Olomouc-Holice [49°34′31.95″N, 17°17′35.462″E], 2010). The pathogen caused small, angular, yellowish or pale green lesions on the upper leaf surfaces and produced sporangiophores and sporangia on the lower leaf surfaces. The lesions were delimited by leaf veins and later turned necrotic. Sporangiophores were hyaline, branched, and emerged in groups from stomata. Olive brown-to-dark brown sporangia were ellipsoidal to oblong. Our morphological observations confirmed that the pathogen was *P. cubensis* (2). No previous reports are available of *P. cubensis* on *C. moschata* in CR or anywhere in Central Europe. However, *P. cubensis* is common on *C. moschata* in some parts of Asia and the United States (1,2). *P. cubensis* exhibiting clear host specialization has been reported in different countries and geographic areas (2). A *C. moschata* isolate (PC 88/2009) originating from the naturally infected plants was inoculated (1×10^5 spores per ml and

incubation temperature of 18/15°C during light/dark cycles) according to the methodology described by Lebeda and Urban (3) onto the abaxial surface of leaf discs of all genotypes of a differential set of cucurbits for *P. cubensis* pathotype determination (4). *C. moschata* (line Novo5, Nohel-Garden, CR) was added to this set. The isolate PC 88/2009 was highly pathogenic to all screened *Cucurbita* spp. genotypes (*C. pepo*, *C. maxima*, and *C. moschata*). However, no infection was detected on most of the *Cucumis* accessions; only *Cucumis melo* subsp. *agrestis* var. *conomon* was susceptible. Also, no infection was observed on other differentials (*Citrullus*, *Benincasa*, *Luffa*, and *Lagenaria*). The pathotype was classified as Pc 4/15/0. This pathotype had not been previously detected in CR.

References: (1) D. F. Farr and A. Y. Rossman. Fungal Databases. Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved from http://nt.ars-grin.gov/fungaldatabases/. December 16, 2010. (2) A. Lebeda and Y. Cohen. Eur. J. Plant Pathol. 129:157, 2011. (3) A. Lebeda and J. Urban. Page 285 in: Mass Screening Techniques for Selecting Crops Resistant to Disease. M. M. Spencer and A. Lebeda, eds. International Atomic Energy Agency (IAEA), Vienna, Austria, 2010 (4) A. Lebeda and M. P. Widrlechner. J. Plant Dis. Protect. 110:337, 2003.

*The *e*-Xtra logo stands for "electronic extra" and indicates this Disease Note online contains supplemental material not included in the print edition.

e-Xtra*

First Report of Powdery Mildew of *Hexinia polydichotoma* Caused by *Leveillula lactucae-serriolae* in China. B. Xu, J. G. Song, G. He, M. F. Lv, L. L. Zhang, and Z. Y. Zhao, College of Life Sciences, Tarim University, and Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin affiliated to Xinjiang Production and Construction Groups, Tarim University, Alar 843300, China. Plant Dis. 95:879, 2011; published online as doi:10.1094/PDIS-03-11-0249. Accepted for publication 29 April 2011.

Hexinia polydichotoma (Ostenf) H.L. Yang (synonym *Chondrilla polydichotoma* Ostenf.) is an indigenous sand-binding plant that is widely distributed only in the desert regions of Northwest China. During the summer of 2007, severe outbreaks of a previously unknown powdery mildew were observed in the Taklimakan Desert in Xinjiang, China. Almost 95% of the plants surveyed were affected in this area. The upper surfaces of the stem were covered with white mycelia and the corresponding abaxial surfaces of infected leaves were chlorotic. Affected young, green stems also showed extended chlorosis. As the disease progressed, the infected stems turned yellow and necrotic. Heavy infection resulted in death of the plants. The primary conidia of the fungus were lanceolate with apical pointed, rarely cylindrical or subcylindrical with attenuated apex. They measured 53 to 73 × 15 to 21 µm and had a surface with a net of irregular rides and warts. Subcylindrical or subclavate secondary conidia with rounded ends measuring 50 to 77 × 13 to 20 µm were observed. The ascomata are subgregarious to scattered, globose, and 165 to 200 µm in diameter that are immersed in the dense mycelial tomentum. Numerous and well-developed appendages on the lower half of the ascomata are irregularly branched and can be as long as up to the ascomata diameter. The appendages measure 79 to 106 × 5 to 10 µm and are aseptate, thin walled, and smooth. Asci are numerous (usually more than 20 per ascoma), stalked, clavate-ovoid to nearly cylindrical, and contain two spores (rarely one or three). Ascospores are ellipsoid, hyaline, and measure 25 to 35 × 14 to 20 µm. On the basis of these characteristics, the fungus was identified as *Leveillula lactucae-serriolae* (2). A voucher specimen was deposited in the Herbarium of Martin Luther University, Halle, Germany (Accession No. HAL 2439F). To confirm the identification, the internal transcribed spacer (ITS) rDNA was amplified and sequenced, and deposited in GenBank (Accession No. HQ821500). Comparison with sequences available in the GenBank database revealed that the ITS sequence shares 99% similarity with that of *L. lactucae-serriolae* on *Lactuca serriola* from Iran (Accession No. AB044375.1) (1). Thus, the pathogen was identified as *L. lactucae-serriolae* based on the host plant species, anamorph morphology, and ITS sequence. Pathogenicity was confirmed through inoculation by gently pressing a diseased stem onto the stem of healthy *H. polydichotoma* plants. Five inoculated plants were kept under a plastic humid chamber, whereas the same number of noninoculated plants served as the control. The plants were placed under natural conditions (25 to 28°C) with 80 to 90% humidity. At 15 days after inoculation, typical symptoms of powdery mildew developed on the inoculated plants. No symptoms were seen on the control plants. To our knowledge, this is the first report of *L. lactucae-serriolae* in China and the first record of *L. lactucae-serriolae* on *H. polydichotoma* in the world (http://nt.ars-grin.gov/fungaldatabases/index.cfm). Because the plant is becoming widely cultivated in the Taklimakan Desert for use in sand-binding, the powdery mildew poses a serious threat to desertification control.

References: (1) S. A. Khodaparast et al. Mycol Res. 105:909. 2001. (2) S. A. Khodaparast et al. Mycoscience 43:459, 2002.

*The *e*-Xtra logo stands for "electronic extra" and indicates this Disease Note online contains supplemental material not included in the print edition.

e-Xtra*

First Report of Powdery Mildew on Ruth's Golden Aster (*Pityopsis ruthii*) Caused by *Golovinomyces cichoracearum* (*Erysiphe cichoracearum*). R. N. Trigiano, Department of Entomology and Plant Pathology, University of Tennessee, Knoxville 37996-4560; A. J. Dattilo, Biological Compliance, Tennessee Valley Authority, Knoxville 37902; and P. A. Wadl, Department of Entomology and Plant Pathology, University of Tennessee, Knoxville 37996-4560. Plant Dis. 95:879, 2011; published online as doi:10.1094/PDIS-04-11-0289. Accepted for publication 18 April 2011.

Ruth's golden aster (*Pityopsis ruthii* (Small) Small: Asteraceae) is an endangered, herbaceous perennial that occurs only at a few sites along small reaches of the Hiwassee and Ocoee rivers in Polk County, Tennessee. As part of a planned restoration program, Ruth's golden aster has been micropropagated in vitro and acclimatized to greenhouse conditions. In February 2011, several established plants in a greenhouse in Knoxville, TN exhibited signs and symptoms of powdery mildew including growth of white mycelium and conidiophores on the adaxial surface of leaves and slight curling upward of leaf margins. Mycelium was superficial and nipple-shaped appressoria were present. Mycelia, conidiophores, and conidia were removed from several leaves, mounted in water, and examined microscopically. Cylindrical to ovoid conidia (n = 100) lacking fibrosin bodies were borne in chains and had a mean length of 32.0 µm (19.2 to 38.7 µm) and width of 14.9 µm (6.3 to 21.2 µm). The description and dimension of the conidia agreed well with that provided for *Golovinomyces cichoracearum* (*Erysiphe cichoracearum*) reported on *Coreopsis* spp. (1,3) and *Cirsium arvense* (creeping thistle) (2). The teleomorph was not observed. Total genomic DNA was extracted from infected leaves, amplified with ITS1 and ITS4 primers for the 18S rRNA subunit (4), and visualized on a 2% ethidium bromide agarose gel. An amplicon of fungal origin, approximately 550 bp and smaller than the approximately 700-bp plant ITS amplicon, was excised, purified, and then sequenced. This sequence was deposited in GenBank (Accession No. JF779687) and was 99% identical to two *G. cichoracearum* accessions (Nos. AB77627 and AB77625). Infected leaves were rubbed on leaves of four healthy plants and healthy leaves were rubbed onto other healthy leaves of two additional plants as controls in the greenhouse. Signs of powdery mildew developed on those plants inoculated with infected leaves after 7 to 10 days and the morphology of the fungus was identical to our previous description. To our knowledge, this is the first report of *G. cichoracearum* (*E. cichoracearum*) infecting Ruth's golden aster. We are not aware of the disease occurring in wild populations of the plant, but it does impact the production of micropropagated plants in the greenhouse.

References: (1) D. A. Glawe et al. Online publication. doi:10.1094/PHP-2006-0405-01-BR. Plant Health Progress, 2006. (2) G. Newcombe and C. Nischwitz. Plant Dis. 88:312, 2004. (3) T. E. Seijo et al. Online publication. doi: 10.1094/PHP-2006-1214-01-BR. Plant Health Progress, 2006. (4) T. J. White et al. Page 315 in: PCR Protocols: A Guide to Methods and Applications. M. A. Innis et al., eds. Academic Press Inc, New York, 1990.

*The *e*-Xtra logo stands for "electronic extra" and indicates this Disease Note online contains supplemental material not included in the print edition.

(*Disease Notes* continued on next page)

Journal of Phytopathology

J Phytopathol **159**:589–596 (2011)
doi: 10.1111/j.1439-0434.2011.01811.x

Palacký University in Olomouc, Olomouc-Holice, Czech Republic

Distribution, Host Range and Disease Severity of *Pseudoperonospora cubensis* on Cucurbits in the Czech Republic

Aleš Lebeda, Jana Pavelková, Jiří Urban and Božena Sedláková

Authors' address: Department of Botany, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 11, 783 71, Olomouc-Holice, Czech Republic (correspondence to A. Lebeda. E-mail: ales.lebeda@upol.cz)

Received December 27, 2010; accepted April 19, 2011

Keywords: cucurbit downy mildew, *Pseudoperonospora cubensis*, cucurbits, disease prevalence, disease severity, Czech Republic

Abstract

Cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, is a major cucumber disease in the Czech Republic. Disease prevalence, host range and disease severity were evaluated from 2001 to 2009. The geographical distribution of *P. cubensis* was assessed on ca 80–100 locations per year in two main regions of the Czech Republic (central and southern Moravia, and eastern, northern and central Bohemia). Infection by *P. cubensis* was observed primarily on cucumber (*Cucumis sativus*) but only on the leaves. During the study, disease prevalence ranged from 66 to 100%. The majority of *C. sativus* crops were heavily infected at the end of the growing season (second half of August). Generally, *P. cubensis* was present at high or very high disease severity. The loss of foliage results in the reduction in the quality and quantity of marketable yield of fruit. *Pseudoperonospora cubensis* was widespread across the whole area of the Czech Republic studied. Very rarely, infection was recorded in muskmelon (*Cucumis melo*) and *Cucurbita moschata*. Of other pathogens, the most frequently recorded was the cucurbit powdery mildew (*Golovinomyces cichoracearum* and *Podosphaera xanthii*).

Introduction

The Cucurbitaceae, a large family and heterogeneous group of plants, originated from America, Africa and Asia (Bates et al. 1990; Lebeda et al. 2007b) but are now grown in most countries of the world, primarily in the warmer and temperate regions. It is a family of many economically important species, particularly those with edible fruits (Robinson and Decker-Walters 1997). In the Czech Republic, cucumber (*Cucumis sativus*) is among the traditional, favourite and most frequently grown vegetable crops (Moravec et al. 2004). However, since 1984, cucumber production has been seriously limited by the occurrence of epidemics of downy mildew caused by the oomycete *Pseudoperonospora cubensis* (Lebeda 1986). These outbreaks cause significant crop damage and lead to death of the plants at the adult stage. The disease therefore plays a crucial role in determining the quantity and quality of cucurbit production (Lebeda and Cohen 2011; Perchepied et al. 2005; Velichi 2009).

In Europe, severe outbreaks of cucurbit downy mildew, caused by *P. cubensis*, have been reported repeatedly (Lebeda and Cohen 2011) with frequent yield reductions recorded in field-grown cucumbers during the past 20 years (Doruchowski and Lakowska-Ryk 1992). Unlike as in other countries (e.g. USA), no epidemics have been observed in *Cucumis melo* L. and *Cucurbita* spp. under either field or glasshouse conditions (Lebeda 1999; Lebeda and Cohen 2011). Nevertheless, *P. cubensis* is potentially a dangerous and devastating pathogen and annually causes serious threats to cucurbit crops (e.g. melon, cucumber, squash, watermelon and luffa) grown around the world (Cohen 1981; Lebeda and Cohen 2011).

Pseudoperonospora cubensis was first recorded in Cuba in 1868 (Berkeley and Curtis 1868). It spread rapidly throughout the majority of the European countries; it was recorded in Austria and Hungary from 1904, in Yugoslavia from 1952, in Russia from 1963 and in Bulgaria, Romania, Switzerland, Germany, the Netherlands, Greece, France and Great Britain after 1970 (Lebeda 1990). Since 1984, cucurbit downy mildew has been considered as a disease of high economic impact in the former Czechoslovakia and in Central Europe (Lebeda 1990). *Pseudoperonospora cubensis* spread from Czechoslovakia (Lebeda 1986) to Poland in 1985 (Rondomanski 1988) and spread by windblown inoculum to Sweden and Finland (Forsberg 1986; Tahvonen 1985). Recently, more than 60 species were reported to be affected by *P. cubensis* (Lebeda and Cohen 2011). In addition to cucumber, the most frequently affected hosts are muskmelon (*Cucumis melo*), watermelon (*Citrullus lanatus*), pumpkin (*Cucurbita maxima*) and squash (*Cucurbita pepo*) (Lebeda and Widrlechner 2003).

Symptoms are usually confined to leaves, which influence the quality and yield of fruits. Infectivity by *P. cubensis* was shown to be dependent on the host plant and environmental conditions (Iwata 1942, 1953a,b; Palti 1974; Palti and Cohen 1980; Waterhouse and Brothers 1981). Free leaf moisture greatly accelerates the development of *P. cubensis* (Lebeda and Cohen 2011).

The pathogen initially induces foliar chlorotic lesions that later turn into necrotic lesions on the older leaves. Sporulation is primarily seen on abaxial leaf surfaces. Severely infected leaves wither early and are malformed (Lebeda and Cohen 2011).

Despite its significance, population and quantitative studies of the disease are still very rare (Lebeda et al. 2010). In Europe, there is limited information about spatial distribution, host range and temporal changes in the prevalence of *P. cubensis* in specific geographical regions. We have focused on the temporal and spatial changes in distribution, host range and disease severity of *P. cubensis* in the Czech Republic. The study included both preliminary (2001–2003) and previously published data (Lebeda and Urban 2004) and new data collected from 2004 to 2009. Our aim was to examine in detail the spatial distribution and disease severity of downy mildew in cucumber, the main host plant species of *P. cubensis* in Central Europe. The occurrence of powdery mildew was also recorded.

Materials and Methods

Area and period of surveying

The distribution, occurrence and damage were evaluated in 12 regions (from 14) and 37 districts (from 77) of the Czech Republic (Tables 1 and 2). In most years, three main surveys were made per year (end of July till end of August) in two main areas of the Czech Republic (central and southern Moravia and eastern and central Bohemia) from 2001 to 2009. The main cucurbitaceous vegetable production areas were visited (e.g. southern and central Moravia, eastern Bohemia and Polabí), but some marginal areas for cucurbit cultivation (e.g. areas of Jeseníky, Beskydy, Českomoravská Vrchovina, Podkrkonoší) were also considered (Fig. 1). The occurrence of *P. cubensis* was monitored during the main harvest period on hobby gardens, small private fields and large production fields. The prevalence and severity was evaluated annually at ca 80–100 locations (Table 2).

Table 1
Regions surveyed in Czech Republic from 2001 to 2009

Moravia/region	Bohemia/region
South Moravia (SM)	Hradec Králové (HK)
Olomouc (OL)	Pardubice (PA)
Zlín (ZL)	Central Bohemia (CB)
Moravia–Silesia (MS)	South Bohemia (SB)
	Liberec (LI)
	Praque (P)
	Ústí nad Labem (ÚL)
	Vysočina (VY)

Recorded characteristics

During each survey, in addition to epidemiological data (disease prevalence and disease severity), geographical, regional and ecological characteristics were also recorded for each site, including the date of observation, type of growing area, size of crop, fungicide treatment (if data available) and presence of other harmful organisms.

Downy mildew severity in cucumber was assessed using two epidemiological parameters (disease prevalence and disease severity). Disease prevalence was expressed as the percentage of surveyed locations at or crops on which *P. cubensis* occurred. Disease severity was assessed visually by using a 0–4 scale (0 = no disease symptoms; 1 = 0 ≤ 25 percentage of the total leaf surface in the fields infected, low severity; 2 = > 25 ≤ 50, medium severity; 3 = > 50 ≤ 75, high severity; 4 = > 75, very high severity), modified for *P. cubensis* (Lebeda and Křístková 1994).

Results

Host range of *Pseudoperonospora cubensis*

A relatively small range of cucurbit vegetables are grown in the Czech Republic. The most frequently grown are cucumbers (*C. sativus*), squashes (*Cucurbita pepo*) and pumpkins (*Cucurbita maxima*); less frequently grown are melon (*C. melo*) and watermelon (*Citrullus lanatus*); and only rarely *Cucurbita foetidissima*, *Cucurbita moschata* and *Lagenaria siceraria*. The occurrence of *P. cubensis* in the Czech Republic was most frequently recorded in *C. sativus* (Table 3). Infection of *C. melo* was recorded only at two locations (2003 in Oplocany, Olomouc region; 2004 and 2009 in Olomouc-Holice, Olomouc region). Infection of *C. moschata* was first observed in the Czech Republic in Nový Jičín-Kojetín (North Moravia – Silesia region) in 2009 (Pavelková et al. 2011). Infection in other cucurbitaceous species was found during any of the nine survey years (Table 3).

Expression of *Pseudoperonospora cubensis* infection in *Cucumis sativus* crops

During the survey period, infection was recorded only in leaf laminas, but not in fruits, flowers, stems or

Table 2
Number of surveyed regions, districts and locations from 2001 to 2009

	Historical regions				Locations	
Year	Σ	Bohemia	Moravia	District	Σ	With *C. sativus* crops
2001	10	6	4	26	130	102
2002	6	2	4	23	109	91
2003	7	3	4	19	107	87
2004	8	4	4	21	110	96
2005	8	4	4	22	96	83
2006	7	4	3	21	105	93
2007	7	3	4	20	91	66
2008	6	3	3	14	76	65
2009	7	3	4	22	106	92

Fig. 1 Localities (*by dots*) surveyed for the occurrence of *Pseudoperonospora cubensis* in the Czech Republic in the period 2001–2009

Table 3
Host range of cucurbit downy mildew in the Czech Republic from 2001 to 2009

	Year no. of monitored locations/locations with infected crops								
Host plant	2001	2002	2003	2004	2005	2006	2007	2008	2009
Cucumis sativus	102/98	91/90	87/87	96/63	83/77	93/65	66/62	65/63	92/91
Cucumis melo	0/0	0/0	7/1	2/1	2/0	1/0	1/0	1/0	2/1
Cucurbita pepo	71/0	91/0	68/0	100/0	60/0	82/0	69/0	49/0	77/0
Cucurbita maxima	27/0	34/0	38/0	36/0	36/0	23/0	38/0	32/0	41/0
Cucurbita moschata	0/0	0/0	0/0	0/0	0/0	1/0	1/0	2/0	3/1
Citrullus lanatus	0/0	2/0	3/0	7/0	5/0	4/0	12/0	2/0	4/0
Lagenaria siceraria	0/0	0/0	1/0	1/0	1/0	0/0	0/0	0/0	0/0
Total	130/98	105/90	91/87	110/63	96/77	105/65	91/62	76/63	106/91

petioles. The disease progress was frequently connected with the increase in leaf tissue necrosis. Yellow and angular lesions (delineated by leaf veins) become dark brown to almost black. Diseased leaves soon withered and cup upward. Symptoms first appeared on the older leaves and progressively spread on the younger.

Natural distribution and severity of *Pseudoperonospora cubensis* in *Cucumis sativus* crops

The distribution of *P. cubensis* in *C. sativus* crops was assessed from 2001 to 2009 (Table 4). The proportion of healthy (non-infected) and *P. cubensis*-infected *C. sativus* crops varied from year to year within all surveyed locations. Disease prevalence ranged from 66 to 100%. The lowest values were recorded in 2004 (66%) and 2006 (70%); by contrast, the highest values were in 2003 (100%), 2002 and 2009 (99%). Fluctuations were also recorded in the distribution of the pathogen between two main geographical areas, Moravia and Bohemia, and between the years. Substantial differences between values of disease prevalence were demonstrated in 2004 and 2006 (Table 4).

All infected *C. sativus* crops were also evaluated for the degree of disease severity from 2001 to 2009 (Fig. 2). The proportion of different disease severity levels differed among individual years. Generally, *P. cubensis* was most frequently present at very high infection levels (DI = 3–4, high or the highest disease severity), and occurrence at low infection levels (DI = 1–2, low or medium disease severity) was rather rare (Fig. 2). Fields with healthy or slightly infected *C. sativus* plants predominated only sporadically during the surveyed period, particularly in 2004 and 2006. Some substantial differences were also recorded by assessing disease severity on surveyed localities in Moravia and Bohemia as well as within individual years. A greater variation in the degree of disease prevalence was observed in Bohemia than in Moravia.

Table 4
Fluctuation in the prevalence (%) of *Pseudoperonospora cubensis* infection in *Cucumis sativus* crops in the Czech Republic (Bohemia and Moravia)

	Bohemia		Moravia		Total	
Year	Healthy	Infected	Healthy	Infected	Healthy	Infected
2001	9	91	0	100	4	96
2002	0	100	1	99	1	99
2003	0	100	0	100	0	100
2004	88	12	5	95	34	66
2005	10	90	6	94	7	93
2006	12	88	41	59	30	70
2007	0	100	8	92	6	94
2008	6	94	0	100	3	97
2009	2	98	0	100	1	99

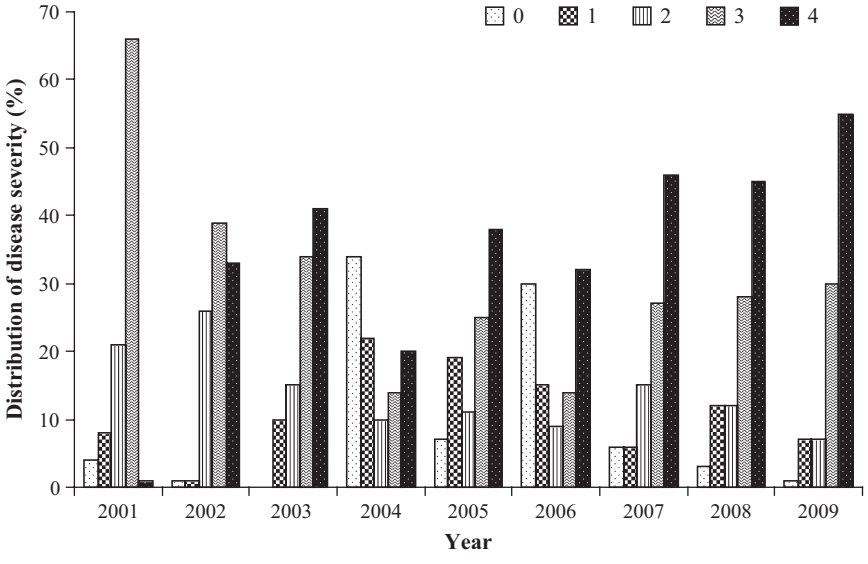


Fig. 2 *Pseudoperonospora cubensis* severity in 2001–2009 in the Czech Republic (Bohemia and Moravia), values 0–4 represents degree of scale for disease severity

Occurrence of *Pseudoperonospora cubensis* with other harmful organisms

The natural occurrence of other fungal pathogens on *C. sativus* plants was also recorded. The most common were two ascomycete pathogens, *Golovinomyces cichoracearum* and *Podosphaera xanthii*, the causal agents of cucurbit powdery mildew (Table 5).

Discussion

We here summarize information about the occurrence, distribution and disease severity of cucurbit downy mildew on cucurbitaceous crops in the Czech Republic from 2001 to 2009. The occurrence of cucurbit downy mildew was observed every year throughout the Czech Republic. Natural infection was recorded frequently in *C. sativus* and only rarely in other cucurbits (*Cucumis melo* and *Cucurbita moschata*) (Table 3). Disease prevalence varied during the period studied (Table 4). Differences in prevalence within the recorded localities with *C. sativus* crops and within particular years were probably caused by both the quality of long-distance-transported primary inoculum and variability in the macro- and microclimatic conditions in individual years. This supposition is supported by our studies of spatial and temporal changes of pathogenic variability of *P. cubensis* populations in the Czech Republic (Lebeda and Urban 2007). Also, our comparative molecular study showed that *P. cubensis* populations from the southern Europe are completely different from those in Central Europe (Sarris et al. 2009). Similar results of species spectra and pathogenic variability were recently reported for cucurbit powdery mildew (Křístková et al. 2009; Lebeda et al. 2009).

During our surveys, the first occurrence of *P. cubensis* was repeatedly observed in lowlands of South Moravia (Czech Republic) at the end of June or at the first half of July. Widespread epidemics usually began in the second half of July (Lebeda and Urban 2004). Disease impact was evident in the main as well as marginal production areas; the majority of monitored fields with cucumbers were repeatedly seriously infected (Fig. 2). Some differences in the geographical distribution of *P. cubensis* were revealed between the two main regions of Czech Republic (i.e. Moravia and Bohemia) (Table 4), as well as relatively high fluctuation in disease severity within localities in the given years in both areas. Recorded differences in disease severity were more probably the result of weather fluctuations among the years. The presence of crops with serious infection was slightly higher in the years 2007, 2008 and 2009 than in the other years. Serious regular infections and reduction in cucumber yields have been the main reasons for the continued reduction (ca 80% until now) in the growing area since the 1990s (CMVU – Czech and Moravian Vegetable Union

Table 5
Disease impact and species spectrum of cucurbit powdery mildew on location with *Cucumis sativus*-infected plants monitored under field or covered conditions in the Czech Republic during the years 2001–2009

Year	No. of monitored locations	Infection degree ID[a]/No. of monitored locations					Species spectrum/No. of locations		
		0	1	2	3	4	*Gc*	*Px*	*Gc* + *Px*
2001	102	99	2	1	0	0	3	0	0
2002	91	88	2	1	0	0	3	0	0
2003	87	82	5	0	0	0	2	2	2
2004	96	83	11	1	1	0	10	2	1
2005	83	79	1	2	1	0	2	1	1
2006	93	81	8	2	0	2[b]	8	1	2
2007	66	57	5	1	2	1[b]	3	3	2
2008	65	61	3	1	0	0	1	1	2
2009	92	91	1	0	0	0	1	0	0
Σ	775	721	38	9	4	3	33	8	10

Gc, *Golovinomyces cichoracearum*; *Px*, *Podosphaera xanthii*; *Gc* + *Px*, *Golovinomyces cichoracearum* + *Podosphaera xanthii*.
[a]Infection degree (ID) 0–4 visual scale (Lebeda and Křístková 1994).
[b]One location from 2006 and one from 2007 – only cucurbit powdery mildew infection was observed; no data about cucurbit powdery mildew species spectra on *Cucumis sativus* from these two locations.

2004; Moravec et al. 2004). On the other hand, there is an increasing area of *Cucurbita pepo* and *Cucurbita maxima* production (Moravec et al. 2004) because of their resistance against local *P. cubensis* populations (Table 3).

The survival of *P. cubensis* is primarily dependent on availability of the living host tissues. It has been suggested (Holmes et al. 2004; Lebeda 1990) that in northern latitudes, primary infections are the result of annual long-range dispersal of spores from southern Europe, where the pathogen can overwinter as active mycelium in living host tissue. This is also supported by the repeatedly recorded ability of *P. cubensis* isolates (established experimentally in laboratory conditions) to infect cucurbit species (e.g. *Citrullus lanatus*, *Benincasa hispida* and *Lagenaria siceraria*), which are not commonly grown in the Central Europe (Table 3; Lebeda et al. 2006; Lebeda and Urban 2007). It seems that at least a portion of the pathogen population occurring in Central Europe might have been transported from southern and south-eastern regions of Europe, where highly pathogenic isolates (pathotypes) are known (Cohen et al. 2003; Lebeda and Urban 2004; Lebeda et al. 2006, 2010). Nevertheless, this survey has shown that species like *Cucumis melo*, *Cucurbita* spp., *Citrullus lanatus* and *Lagenaria siceraria* are rarely infected by *P. cubensis* when grown in natural field conditions (Table 3). This means that in the pathogen population, isolates can overcome resistance of these species (Lebeda and Urban 2007; Lebeda et al. 2010). However, the environmental conditions and other circumstances are unfavourable for the establishment of infection.

Except for the 'green bridge' discussed elsewhere, the overwintering of *P. cubensis* is due to the formation of oospores (Lebeda and Cohen 2011). There are some reports of the occurrence of oospores in Europe (e.g. Austria and Italy), Russia, China, Japan and India (Bains et al. 1977; Bedlan 1989; D'Ercole 1975; Hiura and Kawada 1933; Lange et al. 1989a,b; Palti and Cohen 1980; Waterhouse and Brothers 1981). However, oospores have not yet been recorded in the Czech Republic (Lebeda and Urban 2007). A new possibility for overwintering of *P. cubensis* was suggested recently (Runge and Thines 2009). The only perennial cucurbit species that is native to temperate Europe is climbing *Bryonia dioica* (bryony or wild hops). It is widely distributed throughout Europe and in the Mediterranean region (Jeffrey 2001) and could, theoretically, serve as an overwintering host of *P. cubensis* in Europe (Runge and Thines 2009). So far, there has been no record of cucurbit downy mildew disease (A. Lebeda unpubl. data) on either *Bryonia* species (*B. alba* and *B. dioica*) occurring in the Czech Republic (Kubát et al. 2002), although *B. dioica* has been shown to be a host of *P. cubensis* under laboratory conditions (Runge and Thines 2009; Waterhouse and Brothers 1981). Nevertheless, no direct role of this species in the field epidemiology of *P. cubensis* has been shown. The epidemiology of *P. cubensis* in Central Europe and its economic impact on cucurbit crops could be largely influenced by variation in diverse factors such as microclimatic conditions, fungicide treatment, growing of different species and resistant cultivars (Lebeda et al. 2006).

A relatively narrow range of potential hosts for *P. cubensis* are grown in the Czech Republic (Moravec et al. 2004); mainly cucumber (*C. sativus*), squash (*Cucurbita pepo*), pumpkin (*Cucurbita maxima*) and more rarely melon (*Cucumis melo*) and watermelon (*Citrullus lanatus*) were recorded and evaluated during our surveys (Table 3). Although recent laboratory studies on variation in *P. cubensis* pathogenicity showed that most isolates are compatible with cucumber, melon, squash, pumpkin, watermelon and *Benincasa* (Lebeda and Gadasová 2002; Lebeda and Urban 2007; Lebeda et al. 2010), repeated natural and serious infections were found only in cucumbers (Table 3). Occasional infections were observed in *Cucumis melo* in 1984 (Křístková et al. 2007), in 2003 and 2004 (Lebeda and Urban 2007) and in 2009. Moreover, sporangia of *P. cubensis* were microscopically detected also in *Cucurbita maxima* in 1996 and *Cucurbita pepo* in 2000 (Křístková et al. 2007). Limited development of symptoms was also recorded in *Citrullus lanatus* and *Cucurbita pepo* in 1985 and 1997, but these observations were in the glasshouses where plants were under strong infection pressure (Lebeda et al. 2007a). Macroscopic symptoms of infection were not observed in the period 1985–2009 on wild-growing Cucurbitaceae (e.g. *Bryonia alba*) (Lebeda unpublished results). *Pseudoperonospora cubensis* exhibits clear host specialization (Palti and Cohen 1980; Thomas et al. 1987); nevertheless, variation in host range has been reported from different countries (Lebeda and Cohen 2011; Shetty et al. 2002). In Italy, cucurbit downy mildew appears on cucumber and melon and recently on squash (Cappelli et al. 2003). In France, the disease appeared in melon in 1984 and has since been commonly found in the main French production areas; in 1992, downy mildew was the most serious pathogen on melon cultures (Epinat and Pitrat 1994).

Since the early 1960s, downy mildew in Israel occurred naturally in only cucumber and melon (Cohen 1981; Palti and Cohen 1980). Other cucurbit species (*Cucurbita moschata* and *C. pepo* subsp. *pepo*) were first attacked in 2002 (Cohen et al. 2003). In southern China, a severe epidemic of downy mildew on cultivated *Luffa acutangula* (angular sponge gourd) was observed in 1999 (Cohen et al. 2003). Reports from India (Fugro et al. 1997; Mahrishi and Siradhana 1988) indicate that *Luffa* spp. may be severely attacked under field conditions. These Chinese and Indian populations may belong to a distant pathotype different from the populations in the USA and Israel, which are incompatible with *Luffa* spp. (Lebeda et al. 2006; Thomas et al. 1987). In the USA, *P. cubensis* was considered as the most important pathogen of cucumber in the 1940s. During the 1950s and 1960s, downy mildew–resistant cucumber cultivars were produced,

which were responsible for disease control for 40 years (Colucci et al. 2006). New population of *P. cubensis* has emerged in 2004, which rendered these cultivars no longer resistant (Colucci et al. 2006). First records of *P. cubensis* in new host plants have been reported. In Korea, the host range of *P. cubensis* was extended to *Lagenaria siceraria* in 2005 (Choi and Shin 2008) than to *Sechium edule* in Taiwan in 2005 (Ko et al. 2008) and in India in 2008 (Baiswar et al. 2010). Symptoms of cucurbit downy mildew were first reported on *Trichosanthes cucumerina* in Malaysia in 2008 (Salati et al. 2010). The first detection of *P. cubensis* in *Cucurbita moschata* in the Czech Republic was in 2009 (Table 3; Pavelková et al. 2011). Moreover, *P. cubensis* was also observed on taxonomically distant species *Impatiens irvingii* in Cameroon in 2007 (Voglmayr et al. 2009). This is the first report on *P. cubensis* attacking another family (Balsaminaceae). Global climate changes could be one of the influences on the ability of the pathogen to expand both its geographical and host range (Garrett et al. 2006).

Like other members of Peronosporaceae, *P. cubensis* has specific requirements for general environmental and microclimatic conditions on individual sites (Cohen 1981). Diverse optimal values have been reported for the microclimatic factors regarding the dependence of *P. cubensis* occurrence and distribution on environmental conditions (Bedlan 1987; Bulovienė and Survilienė 2006; Cohen 1981; Lindenthal et al. 2005). It was found that the length of a latent period and intensity of infection were dependent upon the value of each individual factor (Cohen 1981). Temperature and leaf moisture are probably dominant factors affecting the initiation of infection and symptom appearance (Li 2006). Our field observations revealed that different microclimatic conditions on individual localities, even within a very fine spatial scale, may modify disease severity, and in extreme cases, they may lead to the total absence of a disease (see Fig. 2).

Coincidence with other plant pathogenic fungi on *C. sativus* populations was also recorded. Powdery mildew (causal agents, *Golovinomyces cichoracearum* and *Podosphaera xanthii*) is a rather common disease affecting cucumbers in Central Europe (Křístková et al. 2009) and the Czech Republic (Lebeda et al. 2009). It was suggested that the very low powdery mildew frequency and infection intensity on *C. sativus* are caused by early and serious infection by *P. cubensis* (Křístková et al. 2007). This is the main explanation why in our monitoring, the co-occurrence of cucurbit downy and powdery mildew was observed only in a few cases. Higher frequency of localities with these two diseases was recorded only in the years 2004 and 2006, when the macroclimatic conditions were unfavourable for *P. cubensis* development (Fig. 2). The occurrence of powdery mildew alone was higher in the same years, in the area without occurrence of downy mildew; it was observed in Bohemia in 2004 and in Moravia in 2006. More research is needed to explore this phenomenon. Although *P. cubensis* first appeared in Europe approximately one hundred years ago, information about the geographical distribution of the pathogen is still limited. From previous studies, it is obvious that in the Czech Republic, the main damage of this disease is caused to field-grown cucumbers, but some rare records on the occurrence of the fungus on other cucurbits (*Cucumis melo* and *Cucurbita moschata*) have started to appear. One of the aims of the future research should be focused, therefore, on detailed screening of potential host species for natural infection of this pathogen, to reveal its increasing infectious potential. However, the field observations must be followed by the study of multiple pathotypes of the pathogen. This information could help the breeders and growers to better predict the occurrence of this disease and in time provide the best protection of their crop.

Acknowledgements

The authors thank Prof. D.A. Morris (formerly of Southampton University, UK) for remarks on the first draft of the manuscript. This research was supported by grants from 1) QD 1357, 2) QH 71229 (both Ministry of Agriculture of the Czech Republic), 3) MSM 6198959215 (Ministry of Education, Youth and Sports of the Czech Republic) and 4) Internal grant of Palacký University in Olomouc IGA PrF_ 2010_ 001 and 2011.

References

Bains SS, Sokhi SS, Jhooty JS. (1977) *Melothria maderaspatana* – a new host of *Pseudoperonospora cubensis*. *Indian J Mycol Plant Pathol* **7**:86.

Baiswar P, Chandra S, Ngachan SV. (2010) *Pseudoperonospora cubensis* on *Sechium edule* in India. *Australas Plant Dis Notes* **5**: 3–4.

Bates DM, Robinson RW, Jeffrey C. (1990) *Biology and Utilization of the Cucurbitaceae*. Ithaca, NY, USA, Comstock.

Bedlan G. (1987) Studies for optimization of spraying dates to control *Pseudoperonospora cubensis* in cucumbers in Austria. *Pflanzenschutzberichte* **48**:1–11.

Bedlan G. (1989) Erstmaliger Nachweis von Oosporen von *Pseudoperonospora cubensis* (Berk. et Curt.) Rost. an Gewächshausgurken in Österreich. *Pflanzenschutzberichte* **50**:119–120.

Berkeley MS, Curtis A. (1868) *Peronospora cubensis*. *J Linnean Soc Botany* **10**:363.

Bulovienė V, Survilienė E. (2006) Effect of meteorological condition on spread and intensity of *Pseudoperonospora cubensis* on cucumber. *Sodininkysté ir Daržininkysté*. *Mokslo Darbai* **25**:186–191.

Cappelli C, Buonaurio R, Stravato VM. (2003) Occurrence of *Pseudoperonospora cubensis* Pathotype 5 on Squash in Italy. *Plant Dis* **87**:449.

Choi YJ, Shin HD. (2008) First record of downy mildew caused by *Pseudoperonospora cubensis* on bottle gourd in Korea. *Plant Pathol* **57**:371.

CMVU – Czech and Moravian Vegetable Union (2004) Vegetable farming in CR and Most common vegetables in CZ. http://www.zelinarskaunie.cz/ZU%C4%8CMhlavn%C3%ADstr%C3%A1nka/P%C4%9Bstov%C3%A1n%C3%ADzeleninyv%C4%8CR/tabid/76/Default.aspx. Accessed October 15, 2008.

Cohen Y. (1981) Downy mildew of cucurbits. In: Spencer DM. (ed) *The Downy Mildews*. London, Academic Press, pp 341–354.

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 SJ, Wehner TC, Holmes GJ (2006) The downy mildew epidemic of 2004 and 2005 in the eastern United States. In: Holmes GJ. (ed) *Proceedings Cucurbitaceae 2006*. Raleigh, NC, USA, North Carolina State University, pp 403–411.

D'Ercole N. (1975) La peronoospora del cetriolo in coltura protetta. *Inform Fitopathol* **25**:11–13.

Doruchowski R, Lakowska-Ryk E. (1992) Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis* Berk. & Court.) in *Cucumis sativus*. In: Doruchowski RW, Kozik E, Niemirowicz-Szczytt K. (eds) *Proceedings of Cucurbitaceae 1992*. The 5th EUCARPIA Cucurbitaceae Symposium, Warsaw, PL, pp 66–69.

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

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

Fugro PA, Rajput JC, Mandokhot AM. (1997) Sources of resistance to downy mildew in ridge gourd and chemical control. *Indian Phytopathol* **50**:125–126.

Garrett KA, Dendy SP, Frank EE, Rouse MN, Travers SE. (2006) Climate change effects on plant disease: genomes to ecosystems. *Annu Rev Phytopathol* **44**:489–509.

Hiura M, Kawada S. (1933) On the overwintering of *Peronoplasmopara cubensis*. *Japanese J Botany* **6**:507–513.

Holmes GJ, Main CE, Keever ZT III. (2004) Cucurbit downy mildew: a unique pathosystem for disease forecasting. In: Spencer-Phillips PTN, Jeger M. (eds) *Advances in Downy Mildew Research. Vol. 2*. Dordrecht, The Netherlands, Kluwer Academic Publishers, pp 69–80.

Iwata Y. (1942) Specialization of *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. II. Comparative studies of the morphologies of the fungi from *Cucumis sativus* L. and *Cucurbita moschata* Duchesne. *Ann Phytopathol Soc Japan* **11**:172–185 [in Japanese].

Iwata Y. (1953a) Specialization in *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. IV. Studies on the fungus from Oriental pickling melon (*Cucumis melo* var. *conomon* Makino). *Bull Faculty Agriculture, Mie University* **6**:30–35.

Iwata Y. (1953b) Specialization in *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. V. on the fungus from Calabash gourd *Lagenaria vulgaris* Ser. var. *clavata* Ser. *Bull Faculty Agriculture, Mie University* **6**:32–36.

Jeffrey C. (2001) *Bryonia* L. In: Hanelt P. (ed) *Mansfeld*'s Encyclopedia of Agricultural and Horticultural Crops. Vol. 3. Berlin, Heidelberg, New York, Springer-Verlag, pp 1537–1538.

Ko Y, Chen CY, Liu CW, Chen SS, Maruthasalam S, Lin CH. (2008) First report of downy mildew by *Pseudoperonospora cubensis* on chayote (*Sechium edule*) in Taiwan. *Plant Dis* **92**:1706.

Křístková E, Lebeda A, Sedláková B. (2007) Temporal and spatial dynamics of powdery mildew species on cucurbits in the Czech Republic. *Acta Hort* **731**:381–388.

Křístková E, Lebeda A, Sedláková B. (2009) Species spectra, distribution and host range of cucurbit powdery mildews in the Czech Republic, and in some other European and Middle Eastern countries. *Phytoparasitica* **37**:337–350.

Kubát K, Hrouda L, Chrtek J jun, Kaplan Z, Kirschner J, Štěpánek J. (eds) (2002) *Klíč ke květeně České republiky (Key to the Flora of the Czech Republic)*. Praha, Czech Republic, Academia [in Czech].

Lange L, Eden U, Olson LW. (1989a) Zoosporogenesis of *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew. *Nord J Bot* **8**:497–504.

Lange L, Eden U, Olson LW. (1989b) The zoospore of *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew. *Nord J Bot* **8**:511–516.

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

Lebeda A. (1990) Biology and ecology of cucurbit downy mildew. In: Lebeda A. (ed) *Cucurbit Downy Mildew*. Praha, Czech Republic, Czechoslovak Scientific Society for Mycology by Czechoslovak Academy of Sciences, pp 13–46.

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

Lebeda A, Cohen Y. (2011) Cucurbit downy mildew (*Pseudoperonospora cubensis*)–biology, ecology, epidemiology, host-pathogen interactions and control. *Eur J Plant Pathol* **129**:157–192. (Doi: 10.1007/s10658-010-9658-1).

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

Lebeda A, Křístková E. (1994) Field resistance of *Cucurbita* species to powdery mildew (*Erysiphe cichoracearum*). *J Plant Dis Protection* **101**:598–603.

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

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

Lebeda A, Widrlechner MP. (2003) A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *J Plant Dis Protection* **110**:337–349.

Lebeda A, Widrlechner MP, Urban J. (2006) Individual and population aspects of interactions between cucurbits and *Pseudoperonospora cubensis*: pathotypes and races. In: Holmes GJ. (ed) *Proceedings of Cucurbitaceae 2006*. Raleigh, NC, USA, Universal Press, pp 453–467.

Lebeda A, Sedláková B, Křístková E. (2007a) Temporal changes in pathogenicity structure of cucurbit powdery mildew populations. *Acta Hort* **731**:381–388.

Lebeda A, Widrlechner MP, Staub J, Ezura H, Zalapa J, Křístková E. (2007b) Cucurbits (Cucurbitaceae; *Cucumis* spp., *Cucurbita* spp., *Citrullus* spp.), Chapter 8. In: Singh R. (ed) *Genetic Resources, Chromosome Engineering, and Crop Improvement Series, Volume 3 – Vegetable Crops*. Boca Raton, FL, USA, CRC Press, pp 271–376.

Lebeda A, Sedláková B, Křístková E, Vysoudil M (2009) Long-lasting changes in the species spectrum of cucurbit powdery mildew in the Czech Republic – influence of air temperature changes or random effect? *Plant Prot Sci*, **45** (Special Issue):S41–S47.

Lebeda A, Hübschová J, Urban J. (2010) Temporal population dynamics of *Pseudoperonospora cubensis*. In: Thies JA, Kousik S, Levi A. (eds) *Cucurbitaceae 2010 Proceedings*. Alexandria, VA, USA, American Society for Horticultural Science, pp 240–243.

Li HM. (2006) *The Survey Criterion for Diseases and Insect Pest Forecast in Vegetables*. Shanghai, Shanghai Scientific Technical Publishers, pp 88–94.

Lindenthal M, Steiner U, Dehne HW, Oerke EC. (2005) Effect of downy mildew development on transpiration of cucumber leaves visualized by digital infrared thermography. *Phytopathology* **95**:233–240.

Mahrishi RP, Siradhana BS. (1988) Studies on downy mildew of cucurbits in Rajasthan: incidence distribution, host range and yield losses in muskmelon. *Ann Arid Zone* **27**:67–70.

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

Palti J. (1974) The significance of pronounced divergences in the distribution of *Pseudoperonospora cubensis* on its crop hosts. *Phytoparasitica* **2**:109–115.

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

Pavelková J, Lebeda A, Sedláková B. (2011) First report of *Pseudoperonospora cubensis* on *Cucurbita moschata* in the Czech Republic. *Plant Dis* **95**: (doi: 10:1094/PDIS-00-00-0000); (in press).

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

Robinson RW, Decker-Walters DS. (1997) *Cucurbits*. Crop Production Science in Horticulture Series. Wallingford, UK, CAB International.

Rondomanski W. (1988) Downy mildew on cucumber – a serious problem in Poland. Abstracts of Papers 5th International Congress of Plant Pathology, Kyoto, Japan 1988. Poster P.VIII-2-48.

Runge F, Thines M. (2009) A potential perennial host for *Pseudoperonospora cubensis* in temperate regions. *Eur J Plant Pathol* **123**:483–486.

Salati M, Wong MY, Sariah M, Nik Masdek H. (2010) First Report of *Pseudoperonospora cubensis* causing downy mildew of *Trichosanthes cucumerina* in Malaysia. *Plant Dis* **94**:642.

Sarris PF, Abdelhalim M, Kitner M, Skandalis N, Panopoulos NJ, Doulis AG, Lebeda A. (2009) Molecular polymorphisms between populations of *Pseudoperonospora cubensis* from Greece and the Czech Republic and the phytopathological and phylogenetic implications. *Plant Pathol* **58**:933–943. (Doi: 10.1111/j.1365-3059.2009.02093.x).

Shetty NV, Wehner TC, Thomas CE, Doruchowski RW, Shetty KPV. (2002) Evidence for downy mildew races in cucumber tested in Asia, Europe, and North America. *Sci Hort* **94**:231–239.

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

Thomas CE, Inaba T, Cohen Y. (1987) Physiological specialization of *Pseudoperonospora cubensis*. *Phytopathology* **77**:1621–1624.

Velichi E. (2009) Dynamics of appearance and evolution to the watermelon (*Citrullus lanatus* L.), of downy mildew [*Pseudoperonospora cubensis* (Berk. et Curt.) Rostow.], in the rainy years 2004, 2005, in Baragan field, (Braila area). *Research J Agric Sci* **41**:345–350.

Voglmayr H, Piatek M, Mossebo DC. (2009) *Pseudoperonospora cubensis* causing downy mildew disease on *Impatiens irvingii* in Cameroon: a new host for the pathogen. *Plant Pathol* **58**:394.

Waterhouse GM, Brothers MP. (1981) The taxonomy of *Pseudoperonospora*. *Mycol Papers* **148**:1–28.

New hosts of *Pseudoperonospora cubensis* in the Czech Republic and pathogen virulence variation

A. Lebeda*, B. Sedláková, and J. Pavelková
Palacký University in Olomouc, Faculty of Science, Department of Botany, Šlechtitelů 11, Olomouc-Holice, Czech Republic (*corresponding author e-mail: ales.lebeda@upol.cz)

Key words: *Cucumis sativus*, *C. melo*, *Cucurbita* spp., *Citrullus lanatus*, cucurbit downy mildew, disease prevalence and severity, pathotypes, virulence factors

Abstract

During the 2009, 2010, and 2011 growing seasons, disease prevalence and severity and the host range of *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew, were evaluated at more than 70 locations in two main regions of the Czech Republic. Infection by *P. cubensis* was observed primarily on *Cucumis sativus*, rarely on other cucurbits. Medium to high disease-severity levels were most frequently recorded on *C. sativus*. During the years 2010 and 2011, *P. cubensis* infection was also recorded on *Cucumis melo*, *Citrullus lanatus* and *Cucurbita moschata*. Occurrence of *P. cubensis* on *C. melo* and *C. lanatus* has been formerly reported from the Czech Republic, however, infection on *C. moschata* was observed for the first time in the Czech Republic in 2009. In the years 2010 and 2011, four new hosts (*Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita ficifolia* and *Lagenaria siceraria*) of *P. cubensis* were found in the Czech Republic.

Virulence structure and its temporal changes (2010 to 2011) were studied in populations of *P. cubensis* in the Czech Republic. Seventy *P. cubensis* isolates, collected from *Cucumis sativus* and *melo, Cucurbita maxima, pepo,* and *moschata*, and *Citrullus lanatus*, were analyzed for virulence variation. The variation of pathogen populations was expressed by the designation of pathotypes using tetrad numerical codes. The most susceptible group of differentials were *Cucumis* species; in contrast, the lowest frequency of pathogenicity was recorded on *Cucurbita pepo* subsp. *pepo*, *Citrullus lanatus* and *Luffa cylindrica*. A high proportion of Czech *P. cubensis* isolates were able to infect two cucurbit species, *Benincasa hispida* and *Lagenaria siceraria*, that are not commonly cultivated in the Czech Republic or elsewhere in Central Europe. In this study period (2009-2011), there were substantial changes to the pathogen virulence structure in comparison with the period 2001-2008.

INTRODUCTION

The Cucurbitaceae, a large family and heterogeneous group of plants are most diverse in America, Africa and Asia (Bates et al., 1990; Lebeda et al., 2007) but are now grown in most countries of the world, primarily in the warmer and temperate regions. It is a family of many economically important species, particularly those with edible fruits (Robinson and Decker-Walters, 1997). In the Czech Republic, cucumber (*Cucumis sativus*) is among the traditional, favorite and most frequently grown vegetable crops (Moravec et al., 2004). However, since 1984 cucumber production has been seriously limited by the occurrence of epidemics of cucurbit downy mildew caused by the oomycete, *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev (1903), (Lebeda, 1986). These outbreaks cause significant crop damage and lead to death of the plants at the adult stage (Lebeda and Schwinn, 1994). During last decade in the Czech Republic, other cucurbits, such as *Cucurbita pepo*, *C. maxima* and *C. moschata* have been grown more frequently (Lebeda et al., 2011).

Cucurbit downy mildew is the most important foliar disease of cucurbit crops worldwide. Currently, the pathogen is very destructive in all humid areas of the world as well as some temperate areas, and the disease, therefore, plays a crucial role in determining the

quantity and quality of cucurbit production. Recently, more than 60 species were reported to be affected by *P. cubensis* (Lebeda and Cohen, 2011). In the Czech Republic, disease prevalence, host range and disease severity were evaluated from 2001-2009. The geographical distribution of *P. cubensis* was assessed on ca. 80 -100 locations per year in two main regions of the Czech Republic (central and southern Moravia, and eastern, northern, and central Bohemia) (Lebeda et al., 2011). Infection by *P. cubensis* was observed primarily on cucumber (*Cucumis sativus*) but only on the leaves. The majority of *C. sativus* crops were heavily infected at the end of growing season (second half of August). Generally, *P. cubensis* was present at high or very high disease severity across the whole area of the Czech Republic studied. The loss of foliage from such severe infections results in the reduction of the quality and quantity of marketable fruits. Very rarely, in past years, we had also recorded infections on muskmelon (*Cucumis melo*) and *Cucurbita moschata* (Lebeda et al., 2011). However, beginning in 2009, the pathogen population changed dramatically, and new hosts were recognized by new pathotypes that were able to establish serious infection of *Cucurbita* spp. (Pavelková et al., 2011) and *Citrullus lanatus*, not observed in 2001 to 2008 (Lebeda et al., 2011).

The objectives of this study were: 1) to address the existence of new host species for *P. cubensis*; 2) to measure variation in virulence (at the level of a pathotype) of *P. cubensis* in the Czech Republic in the period 2009-2011.

MATERIALS AND METHODS

The distribution, disease prevalence and severity caused by *P. cubensis* on cucurbitaceous vegetables were evaluated in 2009-2011 in the Czech Republic, in a similar fashion to our earlier surveys (Lebeda et al., 2011). Three surveys were made per year (late July to late August) in two main areas of the Czech Republic (Moravia, central and southern parts; and Bohemia, eastern and central parts; Fig 1) from 2009 to 2011 (Lebeda *et al*., 2011). The main cucurbitaceous vegetable production areas were visited (e.g., South and Central Moravia, East Bohemia and Polabí). However, some marginal areas for cucurbit cultivation (e.g., areas of Jeseníky, Beskydy, Českomoravská Vrchovina, Podkrkonoší) were also surveyed. The occurrence of *P. cubensis* was monitored during the main harvest period in hobby gardens, small private fields and large production fields. Disease prevalence and severity were evaluated annually at more than 70 locations following the methodology of Lebeda *et al*. (2011).

The virulence of 70 isolates (collected in 2010 /37/ and 2011 /33/) was screened on a differential set of 12 cucurbit taxa (Lebeda and Widrlechner, 2003). A leaf-disc method was used (Lebeda and Urban, 2010), with a visual 0-4 scale (Lebeda, 1991) used to evaluate sporulation intensity over a two-day period from 6 to 14 days after inoculation. The sporulation intensity was expressed as the percentage of maximum sporulation intensity (Lebeda and Urban, 2010). Leaf discs with no, or only a low level of sporulation (≤ 35%), were considered to show an incompatible response; those with a medium or high level of sporulation were considered compatible genotypes (Lebeda and Urban, 2010). The virulence level of isolates was determined on the basis of the number of virulence factors, i.e. number of compatible reactions within the differential set of cucurbitaceous taxa. Pathotypes were designated with tetrad numerical codes (Lebeda and Widrlechner, 2003).

RESULTS AND DISCUSSION

During the 2009, 2010, and 2011 growing seasons, disease prevalence and severity and the host range of *P. cubensis*, were evaluated at more than 70 locations in two main regions of the Czech Republic (central and southern Moravia, and eastern, northern and central Bohemia). Infection was observed primarily on cucumber (*Cucumis sativus*), rarely on other

cucurbits. Disease prevalence of *P. cubensis* on *C. sativus* ranged from 91-97%. Disease severity was assessed visually by using a 0-4 scale (Lebeda et al., 2011). The low (infection degree ID = 1), or medium to high (ID = 2-3) severity levels were most frequently recorded on *C. sativus.* During the years 2010 and 2011, *P. cubensis* infection was also recorded also on *Cucumis melo* (2011), *Citrullus lanatus* (both years) and *Cucurbita moschata* (2010) (Table 1). The occurrence of *P. cubensis* on *C. melo* and *C. lanatus* has been formerly reported from the Czech Republic (Lebeda et al., 2011), however, infection on *C. moschata* was only reported for the first time in the Czech Republic in 2009 (Pavelková et al., 2011). During the years 2010 and 2011, four new hosts (*Cucurbita pepo* and *Cucurbita maxima* /2010-2011/, *Cucurbita ficifolia* /2010/ and *Lagenaria siceraria* /2011/) were found (Table 1).

The structure of, and temporal changes in, virulence in *Pseudoperonospora cubensis* have been studied in the Czech Republic from 2001 to 2010 (Lebeda et al., in press). Nearly 400 *P. cubensis* isolates collected mostly from *Cucumis sativus* (ca 96%), but also from *Cucumis melo, Cucurbita maxima, Cucurbita pepo, Cucurbita moschata*, and *Citrullus lanatus*, were analyzed for variation in virulence (at the pathotype level). The structure of, and changes in, virulence in the pathogen populations were expressed by the designation of pathotypes using tetrad numerical codes, based on a differential set of 12 genotypes of cucurbitaceous taxa (Lebeda and Widrlechner, 2003). The differential set identified 67 different pathotypes of *P. cubensis*. In these pathogen populations, 70 to 100% of the isolates expressed a high number (9-12) of virulence factors. "Super pathotype" 15.15.15 was often observed in our study and was one of the four most frequently recorded pathotypes (Lebeda et al., in press). Recently two new pathotypes were determined (15.7.9., 14.0.0.) from the new host plants (Table 2), however, we are expecting more new pathotypes from cucumbers (data not elaborated yet).

The most susceptible group of differentials were *Cucumis* species, in contrast the lowest frequencies of virulence were recorded on *Cucurbita pepo* subsp. *pepo*, *Citrullus lanatus* and *Luffa cylindrica*. Notably, many Czech *P. cubensis* isolates were also able to infect two cucurbit species (*Benincasa hispida* and *Lagenaria siceraria*; Table 2) that are not commonly cultivated in the Czech Republic or elsewhere in Central Europe.

Over time, clearly *P. cubensis* populations have been evolving toward higher levels of virulence, with substantial changes when compared to the period 2001-2009 (Lebeda et al., 2010). Since 2009, the pathogen population has changed dramatically, and new pathotypes are now able to establish serious infections on *Cucurbita* spp. and *Citrullus lanatus* (Lebeda et al., 2011; Tables 1 and 2), which was not observed between 2001 and 2008 (Lebeda et al., in press).

Previous studies have shown that *P. cubensis* is a highly variable pathogen from the viewpoints of host-specificity, race-specificity and virulence (Lebeda et al., 2006, in press, Fig. 1.; Lebeda & Cohen, 2011), findings also supported by recent molecular studies (Sarris et al., 2009; Mitchell et al., 2011; Quesada-Ocampo *et al*., in press; Runge et al., 2011). The comparison of *P. cubensis* isolates collected from 2001 to 2010 in the Czech Republic confirms that the virulence structure of Czech pathogen populations is very broad and dynamic in time and space (Lebeda et al., in press). However, from this study it is clearly evident that during these most recent surveys the host range and variation in virulence changed substantially (Tables 1 and 2). This supports previous hypotheses (Lebeda et al., 2006; Lebeda and Cohen, 2011) that higher genetic diversity of resistance in *Cucurbita* spp. and some other Cucurbitaceae may indirectly, but significantly contribute to the selection of new *P. cubensis* pathotypes (Lebeda et al., in press). These changes must be seriously considered in the development of effective disease management, including the evolutionary dynamics of fungicide resistance (Lebeda and Cohen, 2012)

ACKNOWLEDGEMENTS

Critical comments by Dr. M.P. Widrlechner (Iowa State University, Ames, USA) to the first draft of manuscript are acknowledged. This research was supported by the following grants: MSM 6198959215, QH 71229 and Internal Grant Agency (Prf-2011-3, PřF 2012-001).

Literature Cited

Bates DM, Robinson RW, Jeffrey C (Eds.) (1990) Biology and Utilization of the Cucurbitaceae. Comstock Publ. Assoc., Ithaca, NY

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

Lebeda A (1991) Resistance in muskmelons to Czechoslovak isolates of *Pseudoperonospora cubensis* from cucumbers. Sci Hortic 45: 255-260

Lebeda A, Cohen Y (2011) Cucurbit downy mildew (*Pseudoperonospora cubensis*) – biology, ecology, epidemiology, host-pathogen interaction and control. Europ J Plant Pathol 129: 157–192

Lebeda A, Cohen Y (2012) Fungicide resistance in *Pseudoperonospora cubensis*, the causal pathogen of cucurbit downy mildew, Chapter 4. In: Singh T (Ed.) Fungicide Resistance in Crop Protection. Risk and Management. Wallingford, UK: CABI, pp. 44–63

Lebeda A, Hübschová J, Urban J (2010) Temporal population dynamics of *Pseudoperonospora cubensis*. In: Thies JA, Kousik S, Levi A (Eds.) Cucurbitaceae 2010 Proceedings. American Society for Horticultural Science, Alexandria, VA, USA, pp. 240–243

Lebeda A, Křístková E (1994) Field resistance of *Cucurbita* species to powdery mildew (*Erysiphe cichoracearum*). J Plant Dis Protect 101: 598-603

Lebeda A, Pavelková J, Sedláková B, Urban J (2012) Structure and temporal shift in virulence of *Pseudoperonospora cubensis* populations in Czech Republic. Plant Pathol (in press)

Lebeda A, Pavelková J, Urban J, Sedláková B (2011) Distribution, host range and disease severity of *Pseudoperonospora cubensis* on cucurbits in the Czech Republic. J Phytopathol 159: 589–596

Lebeda A, Schwinn FJ (1994) The downy mildews – an overview of recent research progress. J Plant Dis Protect 101: 225–254

Lebeda A, Urban J (2010) Screening for resistance to cucurbit downy mildew (*Pseudoperonospora cubensis*); Chapter 18. In: Spencer MM, Lebeda A, eds. *Mass Screening Techniques for Selecting Crops Resistant to Disease*. Vienna, Austria: International Atomic Energy Agency (IAEA), pp. 285–294

Lebeda A, Widrlechner MP (2003) A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. J Plant Dis Protect 110: 337–349

Lebeda A, Widrlechner MP, Staub J, Ezura H, Zalapa J, Křístková E (2007) Cucurbits (Cucurbitaceae; *Cucumis* spp., *Cucurbita* spp., *Citrullus* spp.), Chapter 8, In: Singh R. (Ed.) Genetic Resources, Chromosome Engineering, and Crop Improvement Series, Volume 3 – Vegetable Crops. CRC Press, Boca Raton, FL, USA, pp 271-376

Lebeda A, Widrlechner MP, Urban J (2006) Individual and population aspects of interactions between cucurbits and *Pseudoperonospora cubensis*: pathotypes and races. In: Holmes GJ (Ed.) Proceedings of Cucurbitaceae 2006. Raleigh, NC, USA: Universal Press, pp. 453–67

Mitchell MN, Ocamb CM, Grunwald NJ, Mancino LE, Gent DH (2011) Genetic and pathogenic relatedness of *Pseudoperonospora cubensis* and *P. humuli*. Phytopathology 101: 805–818

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

Pavelková J, Lebeda A, Sedláková B (2011) First report of *Pseudoperonospora cubensis* on *Cucurbita moschata* in the Czech Republic. Plant Disease 95: 878–879

Quesada-Ocampo L, Granke L, Olsen J, Gutting H, Runge F, Thines M, Lebeda A, Hausbeck M (2012) The genetic structure of *Pseudoperonospora cubensis* populations. Plant Disease (in press)

Robinson RW, Decker-Walters DS (1997) Cucurbits. Wallingford, UK: CAB International

Runge F, Choi YJ, Thines M (2011) Phylogenetic investigations in the genus *Pseudoperonospora* reveal overlooked species and cryptic diversity in the *P. cubensis* species cluster. Europ J Plant Pathol 129: 3–14

Sarris PF, Abdelhalim M, Kitner M, Skandalis N, Panopoulos NJ, Doulis AG, Lebeda A, 2009. Molecular polymorphisms between populations of *Pseudoperonospora cubensis* from Greece and the Czech Republic and the phytopathological and phylogenetic implications. Plant Pathology 58: 933–943

Table 1. *Pseudoperonospora cubensis* infection of cucurbitaceaous vegetables, other than cucumber (*Cucumis sativus*), recorded in the Czech Republic in 2009-2011

Host species	Number of monitored localities	DI (degree of infection)*/ [number of monitored localities /frequency of localities (%)]				
Year		0	1	2	3	4
2009						
Cucumis melo	2	1/50	1/50	0/0	0/0	0/0
Cucurbita moschata	3	2/75	1/25	0/0	0/0	0/0
2010						
Cucurbita pepo	66	56/85	8/12	2/3	0/0	0/0
Cucurbita maxima	38	32/85	4/10	2/5	0/0	0/0
Cucurbita moschata	2	0/0	2/100	0/0	0/0	0/0
Cucurbita ficifolia	1	0/0	1/100	0/0	0/0	0/0
Citrullus lanatus	6	5/83	0/0	0/0	1/17	0/0
2011						
Cucumis melo	2	1/50	0/0	0/0	0/0	1/50
Cucurbita pepo	58	48/83	7/12	3/5	0/0	0/0
Cucurbita maxima	29	18/62	9/31	2/7	0/0	0/0
Cucurbita moschata	2	1/50	1/50	0/0	0/0	0/0
Citrullus lanatus	10	8/80	0/0	1/10	1/10	0/0
Lagenaria siceraria	2	0/0	2/100	0/0	0/0	0/0

*Degrese of infection (DI) measured on a 0-4 scale (Lebeda and Křístková, 1994)

Table 2. Characterization of virulence of *P. cubensis* isolates originating from a new host species (sampled in the period 2009-2011) (added and adapted according to Lebeda et al., in press)

Host plant	Isolate	Origin Region/District/Location	Pathotype	No. of differential genotype												VF
				1	2	3	4	5	6	7	8	9	10	11	12	
CMe	89/09	OL/OL/ Olomouc-Holice	15.14.11	+	+	+	+	-	+	+	+	+	+	-	+	10
CMe	19/11	OL/OL/Olomouc-Holice	15.14.15	+	+	+	+	-	+	+	+	+	+	+	+	11
CMe	20/11	OL/OL/Olomouc-Holice	15.7.9	+	+	+	+	+	+	+	-	+	-	-	+	9
CP	58/10	JM/HO/Mutěnice	15.15.3	+	+	+	+	+	+	+	+	+	+	-	-	10
CP	61/10	JM/HO/Ratiškovice	15.15.11	+	+	+	+	+	+	+	+	+	+	-	+	11
CP	72/10	ZL/ZL/Napajedla	15.15.3	+	+	+	+	+	+	+	+	+	+	-	-	10
CP	73/10	ZL/ZL/Napajedla	15.15.3	+	+	+	+	+	+	+	+	+	+	-	-	10
CP	59/11	ZL/KM/Postoupky	15.15.10	+	+	+	+	+	+	+	+	-	+	-	+	10
CP	66/11	ZL/KM/Napajedla	15.15.11	+	+	+	+	+	+	+	+	+	+	-	+	11
CP	87/11	MS/NJ/Nový Jičín-Kojetín	14.0.0.	-	+	+	+	-	-	-	-	-	-	-	-	3
CP	89/11	MS/NJ/Nový Jičín-Kojetín	15.15.3	+	+	+	+	+	+	+	+	+	+	-	-	10
CM	12/10	JM/BO/Moravské Bránice	15.15.7	+	+	+	+	+	+	+	+	+	+	+	-	11
CM	67/10	JM/HO/Veselí nad Moravou	15.15.11	+	+	+	+	+	+	+	+	+	+	-	+	11
CM	81/10	OL/OL/Olomouc-Holice	15.6.0	+	+	+	+	-	+	+	-	-	-	-	-	6
CM	45/11	OL/OL/Olomouc-Holice	15.15.3	+	+	+	+	+	+	+	+	+	+	-	-	10
CM	54/11	OL/PR/Polokovice	15.15.10	+	+	+	+	+	+	+	+	-	+	-	+	10
CMo	86/10	MS/NJ/Nový Jičín-Kojetín	15.15.15	+	+	+	+	+	+	+	+	+	+	+	+	12
CMo	88/09	MS/NJ/Nový Jičín-Kojetín	4.15.0	-	-	+	-	+	+	+	+	-	-	-	-	5
CL	83/10	OL/OL/Olomouc-Holice	15.15.11	+	+	+	+	+	+	+	+	+	+	-	+	11

CMe - *Cucumis melo;* CP - *Cucurbita pepo;* CM - *Cucurbita maxima;* CMo - *Cucurbita moschata;* CL - *Citrullus lanatus*
1 – 12, for details see Lebeda and Widrlechner (2003).
+, compatible reaction of *P. cubensis* isolates on cucurbit differential genotypes; -, incompatible reaction of *P. cubensis* isolates on cucurbit differential genotypes.
VF, number of virulence factors.

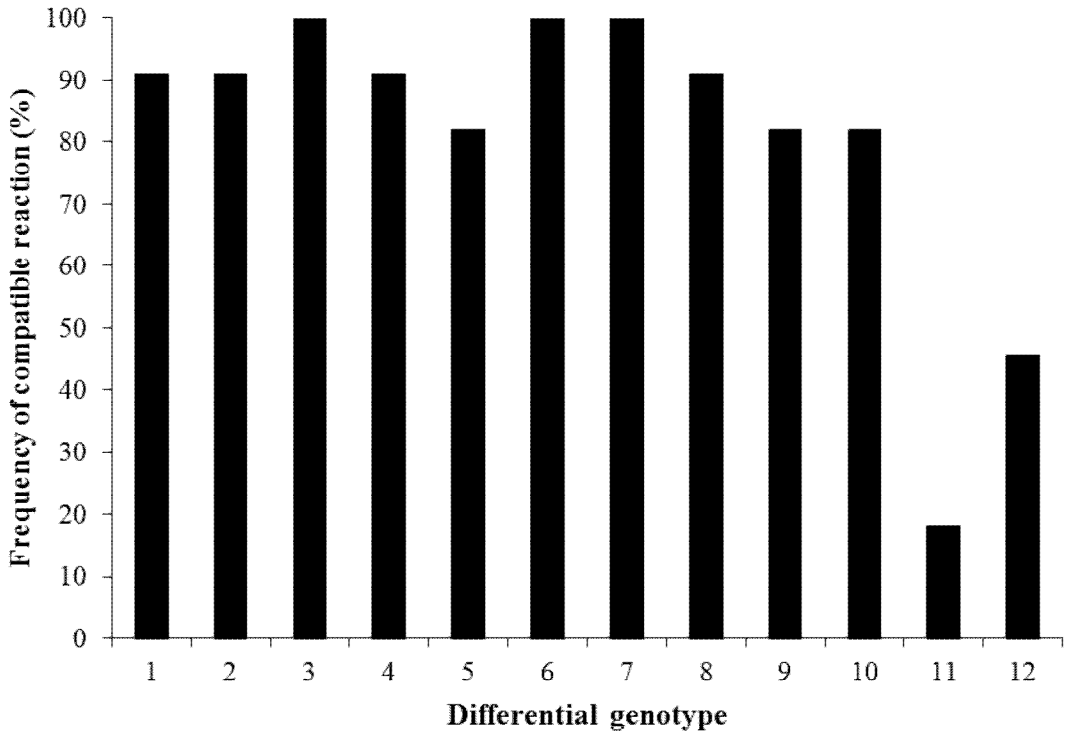


Fig. 1. Variation in susceptibility of cucurbit differential genotypes (1-12, see Lebeda and Widrlechner (2003)) to *P. cubensis* isolates (N=11) from new host species (origin from the years 2009 and 2010, see Tables 1 and 2) (adapted according to Lebeda et al., in press)

3.3 Physiological specialization of *Pseudoperonospora cubensis*

3.3.1 Lebeda, A., Pavelková, J., Sedláková, B., Urban, J. 2012. Structure and temporal shift virulence of *Pseudoperonospora cubensis* populations in Czech Republic. Plant Pathology 2012 (in press)

Plant Pathology (2012) Doi: 10.1111/j.1365-3059.2012.02642.x

Structure and temporal shifts in virulence of *Pseudoperonospora cubensis* populations in the Czech Republic

A. Lebeda*, J. Pavelková, B. Sedláková and J. Urban

Department of Botany, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 11, 783 71 Olomouc, Czech Republic

The structure and temporal dynamics of the virulence of *Pseudoperonospora cubensis* (causal agent of cucurbit downy mildew) were studied in pathogen populations in the Czech Republic from 2001 to 2010. A total of 398 *P. cubensis* isolates collected from *Cucumis* (*Cm.*) *sativus*, *Cm. melo*, *Cucurbita* (*Cr.*) *maxima*, *Cr. pepo*, *Cr. moschata* and *Citrullus lanatus* were analysed for variation in virulence (pathotypes). Virulence was evaluated on a differential set of 12 genotypes of cucurbitaceous plants. All isolates of *P. cubensis* were characterized by their level of virulence (classified according the number of virulence factors, VF; low VF = 1–4, medium VF = 5–8, high VF = 9–12): high (75%), medium (24%) and low (1%). The structure and dynamics of virulence in the pathogen populations were expressed by pathotypes using tetrad numerical codes and a total of 67 different pathotypes of *P. cubensis* were determined. The most susceptible group of differentials was *Cucumis* spp., while the lowest frequency of virulence was recorded on *Cr. pepo* ssp. *pepo*, *Ci. lanatus* and *Luffa cylindrica*. A high proportion (*c.* 90%) of isolates were able to infect cucurbit species *Benincasa hispida* and *Lagenaria siceraria*, which are not commonly cultivated in the Czech Republic or elsewhere in central Europe. In the recent pathogen populations (2008–2010) there was prevailing frequency (70–100%) of isolates with high numbers (9–12) of virulence factors. 'Super pathotype' 15.15.15 was often observed in the study within the pathogen populations and was one of the four most frequently recorded pathotypes. *Pseudoperonospora cubensis* populations shifted to a higher virulence over time. From 2009 the pathogen population changed dramatically and new pathotypes appeared able to establish natural and serious infection of *Cucurbita* spp. and *Ci. lanatus*, which was not observed in 2001–2008. Generally, virulence structure and dynamics of *P. cubensis* populations are extremely variable in the Czech Republic.

Keywords: cucumber, cucurbit downy mildew, cucurbits, pathotypes, tetrad numerical codes, virulence differentiation

Introduction

Cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, is the most important foliar disease of cucurbit crops worldwide (Lebeda & Cohen, 2011). The disease is most often recorded in tropical, subtropical and warm temperate areas of the world. However, in the 1980s it was also observed in cooler areas, such as Scandinavia (Tahvonen, 1985; Forsberg, 1986). Currently, the pathogen is very destructive in all humid areas of the world, as well as some temperate areas (Lebeda & Cohen, 2011). *Pseudoperonospora cubensis* can overwinter in areas with mild winter temperatures or in protected cultivation, as active mycelium in either cultivated or wild species of cucurbits (Lebeda & Cohen, 2011). In the USA, the pathogen is thought to be reintroduced annually in areas with hard winters via long-distance transport of inoculum from warmer areas (Holmes *et al.*, 2004; Ojiambo & Holmes, 2011). Survival by oospores is the subject of ongoing debate; there are reports of oospore formation from Russia, China, Japan, India and Italy (for details Cohen & Rubin, 2011; Lebeda & Cohen, 2011), and recently, infectious oospores were produced experimentally under laboratory conditions (Cohen & Rubin, 2011). Because of the polycyclic nature of *P. cubensis*, the disease spreads rapidly both in open fields and protected environments (Lebeda & Cohen, 2011). Infection is caused by zoospores, which requires free leaf moisture, with sporulation occurring at relative humidity >90% (Cohen & Rotem, 1971; Cohen, 1977). The disease is often devastating and requires frequent fungicide application for control (Lebeda & Cohen, 2012). However, resistance has rendered several fungicides ineffective. *Pseudoperonospora cubensis* was the first oomycete to develop resistance to metalaxyl and reduced sensitivity to mancozeb, and there are now a broad spectrum of fungicides that are ineffective against *P. cubensis* (Lebeda & Cohen, 2011, 2012).

*E-mail: ales.lebeda@upol.cz

PPA 2642 B | Dispatch: 15.5.12 | Journal: PPA | CE: Sangeeta | Journal Name | Manuscript No. | Author Received: | No. of pages: 10 | PE: Nagappan

Pseudoperonospora cubensis is considered a 'risky' pathogen (*sensu* McDonald & Linde, 2002) with a high evolutionary potential and broad adaptability (Lebeda & Urban, 2007). The spectrum of known host species is enlarging (Lebeda & Widrlechner, 2003; Lebeda & Cohen, 2011) and there have been reports of new hosts of *P. cubensis* in many countries (e.g. Cohen *et al.*, 2003; Colucci *et al.*, 2006; Choi & Shin, 2008; Ko *et al.*, 2008; Baiswar *et al.*, 2010; Salati *et al.*, 2010a; Pavelková *et al.*, 2011). Palti (1974) reported that differences in host species' responses to the pathogen were probably the result of different physiological races and/or pathotypes in various countries. A detailed survey of virulence in *P. cubensis* demonstrated the existence of a large number of pathotypes (*c.* 100) and potential races around the world (Lebeda *et al.*, 2006).

Thomas *et al.* (1987) proposed the first differential set for virulence determination based on three host genera (*Cucumis* [*Cm.*], *Cucurbita* [*Cr.*], *Citrullus*) and distinguished five different pathotypes of *P. cubensis*. Lebeda & Křístková (1993) noted that host–pathogen specificity between *Cr. pepo* and *P. cubensis* is probably controlled by race-specific factors. In contrast, no virulence variation in *P. cubensis* (originating from cucumber) was detected on *Cm. sativus* and wild *Cucumis* species (Lebeda, 1992a,b; Lebeda & Prášil, 1994). In 2003 a new pathotype of *P. cubensis* was described in Israel (Cohen *et al.*, 2003). A differential set was developed for *P. cubensis* pathotype determination based on 12 cucurbitaceous differential genotypes (Lebeda & Widrlechner, 2003). Pathotypes are determined by the interactions observed on each of the 12 hosts and assigned unique tetrad codes to describe the interaction (Lebeda & Widrlechner, 2003). This system allowed characterization of the virulence variability of *P. cubensis* at the individual and population levels (Lebeda *et al.*, 2006; Lebeda & Urban, 2007). Recently, studies on the genetic variation of *P. cubensis* (Sarris *et al.*, 2009; Mitchell *et al.*, 2011) and its populations (Runge *et al.*, 2011; Quesada-Ocampo *et al.*, 2012) were published. However, detailed studies focusing on virulence variation of this pathogen in areas of its natural distribution are lacking. This paper is a contribution to this knowledge gap. The objectives of this study were: (i) to measure variation in virulence (at the level of a pathotype) of *P. cubensis* in the Czech Republic in the period 2001–2010; and (ii) to illustrate the dynamics of pathogen populations from the viewpoint of temporal and spatial changes in virulence structure.

Materials and methods

Study area and survey period

The distribution, occurrence and damage caused by *P. cubensis* on cucurbitaceous vegetables were evaluated in 12 of 14 regions and 37 of 77 districts of the Czech Republic. Three main surveys were conducted per year (late July to late August) in two main areas of the Czech Republic (Moravia, central and southern parts; and Bohemia, eastern and central parts; Fig. 1) from 2001 to 2010 (Lebeda *et al.*, 2011). The main cucurbitaceous vegetable production areas were visited (e.g. south and central Moravia, east Bohemia and Polabí) but some marginal areas for cucurbit cultivation (e.g. areas of Jeseníky, Beskydy, Českomoravská Vrchovina and Podkrkonoší) were also surveyed (Fig. 1). The occurrence of *P. cubensis* was monitored during the main harvest period in hobby gardens, small private fields and large production fields. Disease prevalence and severity were evaluated annually at *c.* 80–100 locations (Lebeda *et al.*, 2011).

Source of *Pseudoperonospora cubensis* isolates

Extensive monitoring carried out from 2001 to 2010 confirmed that *P. cubensis* is widespread and occurs annually

Figure 1 Main collection areas of cucurbit downy mildew (*Pseudoperonospora cubensis*) isolates in the Czech Republic and origin of the isolates collected on new host plants (*Cucumis melo*, *Cucurbita* spp., *Citrullus lanatus*).

Table 1 Survey of screened *Pseudoperonospora cubensis* isolates in the period 2001–2010

	No. of screened isolates			Original host plant					
Year	Total	Bohemia	Moravia	CS	CMe	CP	CM	CMo	CL
2001	42	19	23	42	0	0	0	0	0
2002	54	11	43	54	0	0	0	0	0
2003	56	14	42	56	0	0	0	0	0
2004	40	4	36	40	0	0	0	0	0
2005	25	7	18	25	0	0	0	0	0
2006	29	12	17	29	0	0	0	0	0
2007	39	12	27	39	0	0	0	0	0
2008	32	14	18	32	0	0	0	0	0
2009	44	16	28	42	1	0	0	1	0
2010	37	13	24	28	0	4	3	1	1
Total	398	122	276	387	1	4	3	2	1

CS: *Cucumis sativus*; CMe: *Cucumis melo*; CP: *Cucurbita pepo*; CM: *Cucurbita maxima*; CMo: *Cucurbita moschata*; CL: *Citrullus lanatus*.

across the whole studied area of the Czech Republic. The plant material infected by *P. cubensis* was most frequently collected from *Cm. sativus* (2001–2010) (Table 1). Infection of *Cm. melo* was recorded only at two locations in 3 years (2003: Oplocany, Olomouc region; 2004 and 2009: Olomouc-Holice, Olomouc region; Lebeda *et al.*, 2011). From 2009, rare infection of *Cr. moschata* was recorded at two locations in the Czech Republic (Nový Jičín-Kojetín, Silesia region of Moravia, 2009–2011; Olomouc-Holice, central Moravia, 2010) (Lebeda *et al.*, 2011; Pavelková *et al.*, 2011; A. Lebeda & B. Sedláková, unpublished results). Other cucurbitaceous plants were without natural infection of *P. cubensis* until 2010. From 2010 *P. cubensis* infection was also recorded on *Cr. pepo*, *Cr. maxima*, *Cr. ficifolia*, *Citrullus lanatus* and *Lagenaria siceraria* (A. Lebeda *et al.*, unpublished results), mostly in south Moravia (Fig. 1). *Pseudoperonospora cubensis* isolates originating from these crops were also subjected to pathotyping.

Isolation and maintenance of *P. cubensis*

The collected infected leaf samples were incubated on wet filter paper in plastic pots (110 × 85 × 45 mm) (Lebeda & Urban, 2010). Inoculum was prepared by cutting out one lesion from each infected leaf and agitating in distilled water to detach the spores. This water was then sprayed over the abaxial surface of a leaf of the highly susceptible cucumber cv. Marketer 430 and placed in a Petri dish (100 mm in diameter) on wet filter paper. Inoculated leaves were incubated in a growth chamber under standard conditions as previously described (Lebeda & Urban, 2010). The pathogen usually produced spore-bearing sporangiophores 7–8 days after inoculation.

Cultures of *P. cubensis* on cucumber leaf discs were stored in Petri dishes at −80°C. The spores were viable for about 6 months, after which it was necessary to inoculate a new set of plants to maintain pathogen viability. Some of the *P. cubensis* isolates used in this research are deposited in the Czech National Collection of Microorganisms at Palacký University in Olomouc.

Plant material

Cucumis sativus cv. Marketer 430 was used for multiplication of the pathogen isolates. The virulence of isolates was established on a differential set of 12 cucurbit taxa (Lebeda & Widrlechner, 2003) (Table 2). Plants were grown in the greenhouse at 25/15°C day/night temperature, under natural lighting with daily watering and the addition of fertilizer (Kristalon Start, applied by watering) once per week. Plants were not treated with any pesticides. The plants (leaves) were used for testing when 5–8 weeks old (three to six true leaves present).

Determination of variation in virulence

A total of 398 isolates of *P. cubensis* were recovered and screened for virulence variation (pathotypes) from 2001 to 2010 (Table 1). A leaf-disc method (Lebeda & Urban,

Table 2 Differential set of Cucurbitaceae taxa for the determination of pathogenic variability in *Pseudoperonospora cubensis* (Lebeda & Widrlechner, 2003)

		Accession number			
No.	Taxon	Donor	EVIGEZ[a]	Cultivar name	Country of origin
1	*Cucumis sativus*		H39-0121	Marketer 430	USA
2	*Cucumis melo* ssp. *melo*	PI 292008	H40-1117	Ananas Yoqne′am	Israel
3	*Cucumis melo* ssp. *agrestis* var. *conomon*	CUM 238/1974	H40-0625	Baj-Gua	Japan
4	*Cucumis melo* ssp. *agrestis* var. *acidulus*	PI 200819	H40-0611		Myanmar
5	*Cucurbita pepo* ssp. *pepo*	PI 171622	H42-0117	Dolmalik	Turkey
6	*Cucurbita pepo* ssp. *texana*	PI 614687	H42-0130		USA
7	*Cucurbita 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		Unknown
12	*Lagenaria siceraria*		H59-0009		Unknown

[a]EVIGEZ – Czech genebank number.

2010) was used to determine *P. cubensis* pathotypes (Lebeda & Widrlechner, 2003). Each genotype of a differential set (Lebeda & Widrlechner, 2003) was represented by five leaf discs (15 mm diameter) in three replicates (one replicate per plant, i.e. altogether 15 leaf discs were screened); replicates were carried out at the same time. A highly susceptible cucumber (*Cm. sativus*) cv. Marketer 430 was used as a control. Discs were inoculated with a spore suspension (10^5 spores mL^{-1}) using a glass sprayer and incubated in a growth chamber under standard conditions (Lebeda & Urban, 2010). A visual 0–4 scale (Lebeda, 1991; Lebeda & Urban, 2010) was used to evaluate sporulation intensity over a 2-day period from 6 to 14 days after inoculation as follows: 0 = no sporulation, 1 = 0 to ≤25% of the leaf disc covered by sporulation, 2 = >25% to ≤50% of the leaf disc covered by sporulation, 3 = >50% to ≤75% of the leaf disc covered by sporulation, and 4 = >75% of the leaf disc covered by sporulation.

Sporulation intensity (SP) was expressed as the percentage of maximum sporulation intensity (Lebeda, 1992a):

$$SP = \sum \frac{(n \times v) \times 100}{x \times N}$$

where n = the number of discs in every category of infection, v = the category of infection, x = the maximum level of sporulation and N = the total number of evaluated discs.

Sporulation was used as a measure of virulence of individual isolates. Leaf discs with no, or only a low level of sporulation (SP ≤ 35%), were considered to show a resistant response (usually a resistant reaction (−); values 0 or 1 on the above scale); and those with a medium or high level of sporulation (SP > 35%) to be from susceptible genotypes (susceptible reaction (+); values 2–4 on the scale). The reproducibility of results was very high (i.e. 95%), there were no or only small differences (mostly only one degree on the evaluation scale) between individual discs (of which there were 15 for each differential genotype) in each assay. The virulence level of isolates was determined on the basis of the number of virulence factors (VF 1–12, numbered according the differential genotypes, Table 2), i.e. the number of susceptible reactions within the differential set of cucurbitaceous taxa (Table 2). Based on a binary evaluation of susceptible/resistant reaction patterns (+ or −) of a certain isolate of *P. cubensis*, a numeric tetrad code was created for each isolate, on the basis of differential set groupings. The code comprised three parts, each corresponding to one of three groups of four differentials (Table 2, nos 1–4, 5–8, 9–12). Within each group, numerical values of 1, 2, 4 or 8 were assigned to + results and then summed. The three sums were then presented as a code, in the format (sum of group 1).(sum of group 2).(sum of group 3), which served as a identifier for each pathotype (Tables 3–5). The numeric composition of the code gives a clear picture of the virulence of an isolate (Lebeda & Widrlechner, 2003).

Results

Host–pathogen specificity

The development of infection on individual differential genotypes differed substantially during the studied period (Fig. 2a). From the screening, *Cucumis* spp. genotypes were most frequently susceptible, i.e. a large number of isolates were virulent on them. Genotypes of *Cucurbita* spp. exhibited a large race-specific-like variation in susceptibility to different *P. cubensis* isolates. *Benincasa hispida* and *La. siceraria* were frequently susceptible to

Table 3 Variability of the most frequently recorded pathotypes of *Pseudoperonospora cubensis* in the period 2001–2010

Pathotype	No. of differential genotypes/pathotype reaction pattern												Nor.[a]	Frequency (% of total)
	1	2	3	4	5	6	7	8	9	10	11	12		
15.14.10[b]	+	+	+	+	−	+	+	+	−	+	−	+	76	19·1
15.14.11[b]	+	+	+	+	−	+	+	+	+	+	−	+	51	12·8
15.15.11	+	+	+	+	+	+	+	+	+	+	−	+	29	7·3
15.15.15	+	+	+	+	+	+	+	+	+	+	+	+	26	6·5
15.14.14	+	+	+	+	−	+	+	+	−	+	+	+	21	5·3
15.15.10	+	+	+	+	+	+	+	+	−	+	−	+	19	4·8
15.10.10	+	+	+	+	−	+	−	+	−	+	−	+	16	4·0
15.10.14	+	+	+	+	−	+	−	+	−	+	+	+	15	3·8
15.15.14	+	+	+	+	+	+	+	+	−	+	+	+	15	3·8
15.2.10	+	+	+	+	−	+	−	−	−	+	−	+	12	3·0
7.14.10	+	+	+	−	−	+	+	+	−	+	−	+	12	3·0
Total													292	73·4

[a]Nor., number of records (total of 398 isolates of *P. cubensis*, Table 1).
[b]The two most frequently observed pathotypes in the period 2001–2010.
+, compatible/virulent reaction of *P. cubensis* isolates on cucurbit differential genotypes.
−, incompatible/avirulent reaction of *P. cubensis* isolates on cucurbit differential genotypes.

Table 4 Fluctuation of the most frequent pathotypes in the *Pseudoperonospora cubensis* population in the period 2001–2010

	Year/Frequency of pathotype (%)[a]									
Pathotype	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
15.14.10[b]		14·8			36·0	34·5	28·2	25·0	45·5	
15.14.11[b]			19·6		12·0	13·8	53·8			
15.15.11				25·0						18·9
15.15.15			10·7					21·9		27·0
15.14.14	9·5		10·7							
15.15.10				20·0						
15.10.10		18·5								
15.10.14	7·1	14·8								
15.15.14	4·8		10·7							
15.2.10									13·6	
7.14.10						13·8				

[a]Frequency was calculated from the total number of collected and analysed (for pathotypes) isolates of *P. cubensis* in individual years (Table 1).
[b]The two most frequently recorded pathotypes in the period 2001–2010.

Table 5 Characterization of *Pseudoperonospora cubensis* isolates originating from new host species (sampled in the period 2009–2010)

				No. of differential genotypes												
Host plant	Isolate	Origin	Pathotype	1	2	3	4	5	6	7	8	9	10	11	12	VF
CMe	89/09	OL/OL Olomouc-Holice	15.14.11	+	+	+	+	−	+	+	+	+	+	−	+	10
CP	58/10	JM/HO Mutěnice	15.15.3	+	+	+	+	+	+	+	+	+	+	−	−	10
CP	61/10	JM/HO Ratiškovice	15.15.11	+	+	+	+	+	+	+	+	+	+	−	+	11
CP	72/10	ZL/ZL Napajedla	15.15.3	+	+	+	+	+	+	+	+	+	+	−	−	10
CP	73/10	ZL/ZL Napajedla	15.15.3	+	+	+	+	+	+	+	+	+	+	−	−	10
CM	12/10	JM/BO Moravské Bránice	15.15.7	+	+	+	+	+	+	+	+	+	+	+	−	11
CM	67/10	JM/HO Veselí nad Moravou	15.15.11	+	+	+	+	+	+	+	+	+	+	−	+	11
CM	81/10	OL/OL Olomouc-Holice	15.6.0	+	+	+	+	−	+	+	−	−	−	−	−	6
CMo	86/10	MS/NJ Nový Jičín-Kojetín	15.15.15	+	+	+	+	+	+	+	+	+	+	+	+	12
CMo	88/09	MS/NJ Nový Jičín-Kojetín	4.15.0	−	−	+	−	+	+	+	+	−	−	−	−	5
CL	83/10	OL/OL Olomouc-Holice	15.15.11	+	+	+	+	+	+	+	+	+	+	−	+	11

CMe: *Cucumis melo*; CP: *Cucurbita pepo*; CM: *Cucurbita maxima*; CMo: *Cucurbita moschata*; CL: *Citrullus lanatus*; VF: number of virulence factors.
+, compatible/virulent reaction of *P. cubensis* isolates on cucurbit differential genotypes.
−, incompatible/avirulent reaction of *P. cubensis* isolates on cucurbit differential genotypes.

the Czech isolates of *P. cubensis* (except for an isolate from 2010, for which the frequency of compatible reactions on *La. siceraria* was lower – only 69%). *Luffa cylindrica* expressed high variability in susceptibility/resistance (over 60% susceptible reactions in 2001 and 2010 and only about 3% in 2006 and 2007). *Citrullus lanatus* showed a high frequency of resistant reactions. However, compatible interactions with more than 40% of all tested isolates were recorded in 2003, 2004, 2007 and 2008, and 74% in 2010.

The reaction of 11 *P. cubensis* isolates (originating from *Cucurbita* spp. and *Ci. lanatus*; Table 1) on differential genotypes 1–10 (Table 2) were almost identical (Fig. 2b). However, there were only two susceptible reactions with *Lu. cylindrica* and five with *La. siceraria*, which was the lowest frequency of susceptible reactions detected. A high frequency of susceptibility to these isolates was recorded on *Cr. pepo* ssp. *pepo* and *Ci. lanatus*.

Variation in virulence at the isolate level

In total, the 398 isolates were classified as 67 different pathotypes. The number of pathotypes recorded ranged from 33 in 2001 to five in 2007 (Fig. 3). The most frequent pathotypes detected varied by year, but overall, pathotypes 15.14.10 and 15.14.11 were the most frequently recorded (Table 3). One pathotype (15.15.15; i.e. virulent to every differential genotype) was named 'super pathotype' and detected frequently in 2001, 2003, 2004, 2008 and 2010, and belonged to the four most frequently recorded pathotypes (Table 3), especially in the years 2003, 2008 and 2010 (Table 4). At the isolate level, 73·4% of isolates were classified into 11 *P. cubensis* pathotypes (Table 3).

Eleven isolates, originating from *Cm. melo*, *Cr. pepo*, *Cr. maxima*, *Cr. moschata* and *Ci. lanatus* (Table 1), sampled in 2009 and 2010, were classified as seven different

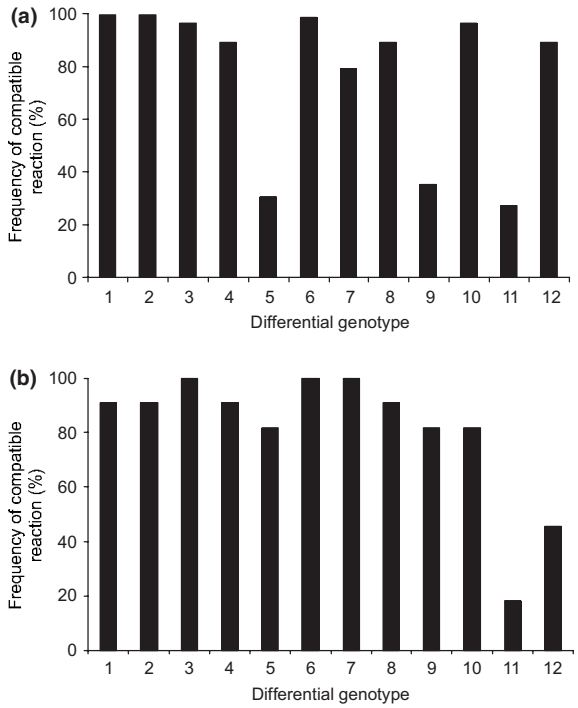


Figure 2 Variation in susceptibility of cucurbit differential genotypes (1–12, Table 2) to (a) 398 *Pseudoperonospora cubensis* isolates (Table 1; mean for the period 2001–2010) and (b) 11 *P. cubensis* isolates from new hosts from 2009 to 2010 (Table 5 and Fig. 1).

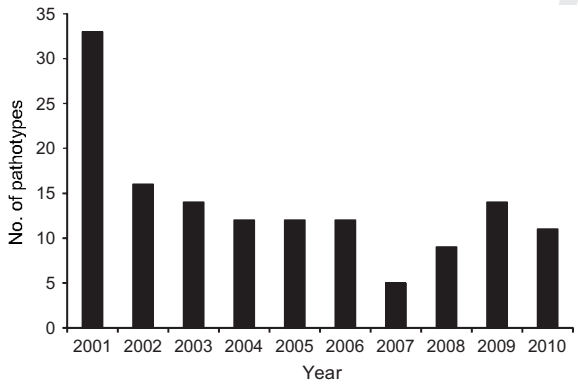


Figure 3 Temporal shift of virulence variation (number of pathotypes/year) of *Pseudoperonospora cubensis* in the Czech Republic in the period 2001–2010.

pathotypes (Table 5). Two of the pathotype designations (4.15.0 and 15.6.0) were unique in the whole period of study.

Variation in virulence at the population level

The virulence profiles of the studied pathogen populations were highly variable. At the population level, a large proportion of the screened isolates could be considered as highly virulent (i.e. 9–12 VF; Fig. 4). Only pathotypes

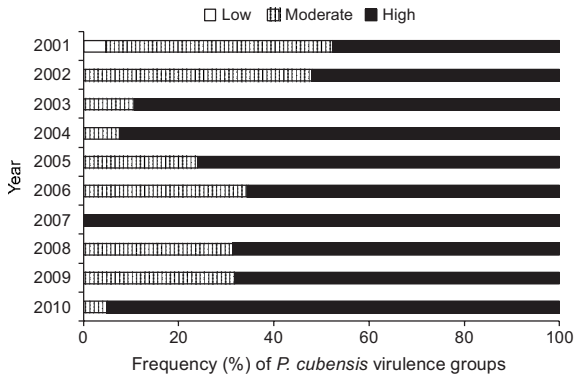


Figure 4 Structure of *Pseudoperonospora cubensis* populations according to their virulence level (i.e. total number of virulence factors (VF) able to overcome differential genotypes, Table 2). Three basic categories were distinguished: low virulence level (1–4 VF), moderate (5–8 VF) and high (9–12 VF).

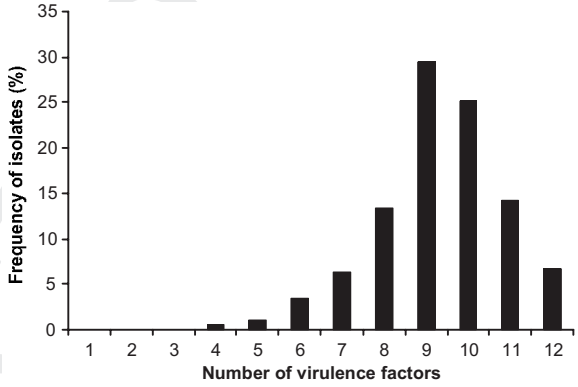


Figure 5 Frequency of *Pseudoperonospora cubensis* isolates by number of virulence factors (mean for the period 2001–2010).

with high virulence were detected in 2007. In contrast, isolates with low virulence (1–4 VF) were recorded only in 2001. Isolates with 9–10 VF had a considerably higher frequency than isolates with other numbers of VF (Fig. 5). Although isolates with moderate and high virulence predominated in the pathogen populations from 2001 to 2010, the ratio between these two pathogenic groups varied. It was about 1:1–1:3 in 2001, 2002, 2005, 2006, 2008 and 2009, but changed to 1:8 in 2003, 1:12 in 2004 and 1:17 in 2010 (Fig. 4).

Of the isolates originating from a new host species (*Cm. melo*, *Cucurbita* spp., *Ci. lanatus*), 82% were highly virulent pathotypes (9–12 VF) and only 18% expressed moderate virulence (5–8 VF).

Temporal and spatial variation in virulence

Temporal changes in the virulence of *P. cubensis* populations in the Czech Republic during the 10-year period were observed (Fig. 3). This phenomenon was demonstrated by the frequency with which specific pathotypes

were detected in individual years (Table 4). The highest number of pathotypes (33) was detected in the first year of the study (2001), decreasing dramatically to 17 pathotypes in 2002, and then shifting to between 5 and 14 (Fig. 3) from 2003 to 2010. The distinct temporal shift to reduced variation in virulence was most apparent from 2001 to 2007, after which the number of pathotypes increased to 10–15, as before (Fig. 3). Virulence of isolates varied by location, but this was not substantial and it is difficult to make any concrete conclusions regarding this phenomenon. Starting in 2009, new *P. cubensis* pathotypes were found annually and it seems that the virulence structure of the pathogen population has been changing substantially during the last 3 years (Table 4, Fig. 2b; A. Lebeda & J. Pavelková, unpublished results).

Discussion

Previous studies have shown that *P. cubensis* is a highly variable pathogen from the viewpoint of host-specificity, race-specificity and virulence (Lebeda *et al.*, 2006; Lebeda & Cohen, 2011). These findings are also supported by recent molecular studies (Sarris *et al.*, 2009; Mitchell *et al.*, 2011; Runge *et al.*, 2011; Quesada-Ocampo *et al.*, 2012). Nevertheless, understanding the variation in virulence of populations at the scale of country or continent, including spatiotemporal shifts, is still very poor. This paper may be the first comprehensive contribution to this topic from the viewpoint of pathotypes.

The comparison of *P. cubensis* isolates collected from 2001 to 2010 in the Czech Republic confirms that the virulence structure of Czech pathogen populations is very broad and dynamic in time and space. It is evident that *P. cubensis* populations are extremely variable in host specificity and virulence. Variation in virulence within Czech populations is even broader than was previously reported in 2002 (Lebeda & Gadasová, 2002). Virulence structure showed a temporal shift from 2001 to 2007 to a higher number of virulence factors and a lower number of pathotypes (Lebeda & Urban, 2007). However, variation in virulence changed and increased again in the few years after that (2008–2010) (Table 4; Fig. 4).

Although this variation is consistent with some other downy mildews (e.g. Lebeda & Schwinn, 1994), such a broad spectrum of variation in virulence has not been reported before for *P. cubensis* from any other country (e.g. Bains & Sharma, 1986; Thomas *et al.*, 1987; Cohen *et al.*, 2003; Salati *et al.*, 2010b) where mostly only a few pathotypes were recorded. Highly virulent isolates were observed in Israel (Cohen *et al.*, 2003) originating from *Cr. moschata* and *Cr. pepo* ssp. *pepo*. European isolates (mostly originating from the Czech Republic) are highly variable and differ substantially (Lebeda & Gadasová, 2002; Lebeda *et al.*, 2006, 2010) in comparison to the five pathotypes described previously from Japan, Israel and the USA (Thomas *et al.*, 1987; Cohen *et al.*, 2003). However, the previously reported pathotypes were described based on a different set of differential host genotypes, i.e. it is likely that the variability was underestimated. Nevertheless, the high variability in European isolates was hypothesized and expected (Lebeda, 1991), and agrees with previous experimental results that demonstrated substantial differences in pathotype structure among various European locations (Lebeda & Gadasová, 2002; Lebeda & Urban, 2007). Unfortunately, these results cannot be compared directly with the data from other countries/continents (e.g. USA, Asia), where mostly only molecular polymorphism of *P. cubensis* populations has been studied (e.g. Quesada-Ocampo *et al.*, 2012). Nevertheless, the results of the present work indicate that the virulence structure of Czech *P. cubensis* populations is very variable and probably also different from that of other parts of the world (Lebeda & Gadasová, 2002; Lebeda *et al.*, 2006; Colucci, 2008; Lebeda & Cohen, 2011). This is also supported by recent molecular studies (Quesada-Ocampo *et al.*, 2012). In addition, *P. cubensis* was shown to be quite variable in time and space (Tables 4 & 5; Fig. 3). This explains in part why it is not possible to predict reliably its development and structure.

Downy mildew pathogens are variable and able to overcome new disease-resistant host genotypes rapidly (Drenth & Goodwin, 1999). There are good examples (e.g. *Bremia lactucae*) that show a temporal shift in virulence (Lebeda & Zinkernagel, 2003). McDonald & Linde (2002) hypothesized that the evolutionary potential of a pathogen population is reflected in its population genetic structure, and pathogen populations with a high evolutionary potential are more likely to overcome genetic resistance than those with a low evolutionary potential. The findings of the present study indicate that *P. cubensis* has a high evolutionary potential and, according to the terminology of McDonald & Linde (2002), it should be considered a 'risky' pathogen (Lebeda & Urban, 2007). The evolutionary forces operating in cucurbit–*P. cubensis* interactions and responsible for pathogen population diversity and breakdown of host resistance are not well known (Lebeda & Widrlechner, 2003). The high potential for genetic variation could depend on many variables which are the subject of the following discussion.

Populations of *P. cubensis* have not been sufficiently studied at the pathotype level, using a settled differential set, for there to be enough data to understand spatiotemporal virulence variation (Lebeda *et al.*, 2006). The differential set used by Thomas *et al.* (1987) for pathotype determination was expanded (Lebeda & Gadasová, 2002; Lebeda & Widrlechner, 2003) to comprehensively unify the effort for characterizing populations of *P. cubensis* at the level of pathotypes (Lebeda *et al.*, 2006). While the majority of available studies solely define pathotypes (Lebeda & Widrlechner, 2003), the lack of uniform and comparable differential genotypes does not allow for pathotype determinations to be drawn from the pronounced divergences in host-range reactions reported both within and between countries. The situation is further complicated by the lack of differentials to distinguish among races on the most important Cucurbitaceae host taxa (e.g. *Cucumis*, *Cucurbita*, *Citrullus*) (Lebeda *et al.*,

2006). However, different races have been postulated and reported (e.g. Shetty *et al.*, 2002; Lebeda *et al.*, 2006) on various cucurbits, and there are probably different genes involved in resistance to different races (Lebeda *et al.*, 2006), if a gene-for-gene interaction is assumed. Other data indicate that the host–pathogen specificity between *Cm. melo–P. cubensis* and *Cucurbita* spp.–*P. cubensis* is controlled by race-specific factors (Lebeda, 1991, 1999; Lebeda & Křístková, 1993, 2000; Lebeda & Gadasová, 2002; Lebeda & Widrlechner, 2003, 2004) which can serve as a force for microevolutionary changes in pathogen populations.

The cucurbit–*P. cubensis* system is not well known or well defined from the viewpoint of host–pathogen specificity and genetics (Lebeda & Widrlechner, 2003; Lebeda *et al.*, 2006; Lebeda & Cohen, 2011). The virulence structure of the pathogen population is principally a function of the structure of the host population (i.e. host species, number of resistance genes and their dynamics in time and space) (Müller *et al.*, 1996). However, a study on coevolution in various pathosystems showed that this is not totally correct (Lebeda & Zinkernagel, 2003) and population structure is influenced by other factors. In general, the most important processes that affect the generation and maintenance of genetic diversity within populations of downy mildews include mutation, the reproductive system, cytoplasmic factors, migration and gene flow, genetic drift and selection (Drenth & Goodwin, 1999). Similar processes could also influence variation of *P. cubensis* populations. Little is known about mutations and mutation rates in oomycetes (Drenth & Goodwin, 1999), including *P. cubensis* (Lebeda & Cohen, 2011). Nevertheless, this process could contribute to broad variation because of extremely large population sizes and high asexual reproduction potential (Lebeda & Cohen, 2011). Recent laboratory studies of sexual reproduction demonstrated oospore formation in *P. cubensis*, which may play a crucial role in sexual recombination of this pathogen and the appearance of new pathotypes (Cohen & Rubin, 2011). However, detailed studies of this phenomenon need to be conducted (Lebeda & Cohen, 2011).

Migration and gene flow are considered very important aspects of the population genetics of oomycetes (Drenth & Goodwin, 1999). In US population studies of *P. cubensis*, it was hypothesized that transport of the pathogen can occur over long distances via atmospheric wind currents (Holmes *et al.*, 2004). A recent detailed spatiotemporal study of *P. cubensis* spread in the eastern USA clearly showed that infection of cucurbits by *P. cubensis* appears to be an outcome of a contagion process and that factors occurring on a large spatial scale (*c.* 1000 km) facilitate the spread of the pathogen (Ojiambo & Holmes, 2011). The study demonstrated that the median nearest-neighbour distance of a spread of new disease cases was *c.* 110 km, and the epidemic expanded at a rate of 9·2 and 10·5 km day^{-1} (Ojiambo & Holmes, 2011). In Europe, transport of sporangia from southern to central Europe and to Scandinavia has also been suggested (Lebeda & Schwinn, 1994). However, spatial dispersal depends not only on climatic factors (e.g. temperature, humidity, wind currents), but also on host population density, species and genetic structure. Previous and recent population studies of *P. cubensis* in the Czech Republic showed that the pathogen population is very diverse (pathogenicity variation, fungicide resistance) and dynamic in time and space (Lebeda & Urban, 2007; Urban & Lebeda, 2007; Lebeda *et al.*, 2010). The data from the present study showed the occurrence of a large number of different pathotypes (67 in total) in the pathogen population over the studied period and their spatiotemporal fluctuations. This rather unique and changeable virulence structure could be the result of pathogen migration in Europe. Because the Czech Republic is located in central Europe and sporangia of *P. cubensis* are wind-dispersed over a long distance (Ojiambo & Holmes, 2011), the population structure could be primarily (at the beginning of the cucurbit growing season, i.e. May–June) formed by pathogen propagules coming from southern Europe, and secondarily (i.e. during the main growing season, July–August) by propagules from all neighbouring and some other European countries. The main reason for this expectation could be relatively short distances and a high population turnover in countries in Europe, very frequently changing wind currents, and differences in the cucurbits grown, which could contribute to the selection of various pathotypes. These factors could also substantially contribute to the very changeable virulence structure of *P. cubensis* populations from a temporal viewpoint. On the other hand, infrequent human-mediated transport of *P. cubensis* is also expected (Quesada-Ocampo *et al.*, 2012).

Quesada-Ocampo *et al.* (2012) recently reported on a detailed study of the genetic structure of worldwide *P. cubensis* populations and identified six different genetic clusters. However, approximately half of the isolates belonged to one of the clusters. There was no direct correspondence between inferred genetic clusters and grouping of isolates by predefined geographic and host categories. Multilocus genotypes were shared across continental, host-of-origin and temporal scales, indicating that some genotypes are widely dispersed and persistent (Quesada-Ocampo *et al.*, 2012). Previous genetic studies found that isolates from the Czech Republic, the Netherlands and France were significantly different from isolates from Greece (Sarris *et al.*, 2009). Also, the study by Quesada-Ocampo *et al.* (2012) showed that countries in western Europe (France, the Netherlands, Germany) had a similar cluster composition, as did Greece, Italy and Spain. However, the Czech Republic, Turkey and Israel each displayed a different cluster composition. These results show that a high genetic differentiation of *P. cubensis* populations exists in Europe and surrounding countries and this can also contribute to the virulence variation in these countries, including the Czech Republic. This conclusion is supported by the high genetic diversity estimates for isolates from Greece, Italy and the Czech Republic. In contrast, Quesada-Ocampo *et al.* (2012)

observed that, compared with this, California had low diversity estimates. This may be because of its geographic separation from eastern US states with severe downy mildew outbreaks. Recently, Runge *et al.* (2011) found potential evidence for a spatiotemporal split between the two major lineages/subspecies of *P. cubensis* between east Asia and western countries, which seems to have equalized over time through the worldwide spread of both groups of pathogens.

Processes of genetic drift and selection could also influence the virulence structure of *P. cubensis* populations. Recent phylogenetic studies showed some genetic similarity between *P. cubensis* and *P. humuli* (Choi *et al.*, 2005; Sarris *et al.*, 2009). There were also reports (Mitchell *et al.*, 2011; Runge *et al.*, 2011) that there is some limited infectivity of *P. cubensis* on hop and *P. humuli* on cucumbers. According to Mitchell *et al.* (2011), *P. humuli* is nested within *P. cubensis*; however, Runge *et al.* (2011) demonstrated that *P. humuli* is separate and most likely basal to *P. cubensis*. Population genetic studies including *P. humuli* isolates could be key to determining the potential extent of gene flow between these sister species, including the contribution of *P. humuli* populations to the genetics and variation in virulence of *P. cubensis* populations. The study of Quesada-Ocampo *et al.* (2012) showed that there is some genetic diversification between the isolates originating from different host cucurbit species. The diversity estimates were also higher for isolates collected from non-cucumber hosts than from cucumber. This supports a previous idea (Lebeda *et al.*, 2006; Lebeda & Cohen, 2011) that higher genetic diversity of resistance in *Cucurbita* spp. and some other Cucurbitaceae may substantially contribute to the selection of new *P. cubensis* pathotypes. These results also suggest that inclusion of isolates from *Cm. melo* and *Cucurbita* spp. that show different genetic composition and high genetic diversity is necessary to capture the genetic variation of *P. cubensis*. These aspects must be also considered from the viewpoint of geography, i.e. regions with high genetic diversity should be of special concern because *P. cubensis* populations with high levels of genetic variation are likely to adapt more rapidly to resistant hosts (Quesada-Ocampo *et al.*, 2012). This process was recently demonstrated in the Czech Republic by repeated severe infections of *Cucurbita* spp. (Table 5; Lebeda *et al.*, 2011; Pavelková *et al.*, 2011). There is an alternative possibility that a new pathotype(s) evolved somewhere else and migrated into the Czech Republic. Future detailed studies may contribute to the understanding of the evolutionary forces responsible for spatiotemporal shifts in pathogenicity and genetic variation within and among populations of *P. cubensis*.

Acknowledgements

The authors thank G. J. Holmes (Valent USA Corporation, Cary, North Carolina) for his valuable critique of this manuscript. This research was supported by grants MSM 6198959215 (Czech Ministry of Education, Youth and Sports) and QH 71229 (Czech Ministry of Agriculture), and the Internal Grant Agency (Prf-2010-001 and Prf-2011-3).

References

Bains SS, Sharma NK, 1986. Differential response of certain cucurbits to isolates of *Pseudoperonospora cubensis* and characteristics of identified races. *Phytoparasitica* **18**, 31–3.

Baiswar P, Chandra S, Ngachan SV, 2010. *Pseudoperonospora cubensis* on *Sechium edule* in India. *Australasian Plant Disease Notes* **5**, 3–4.

Choi YJ, Shin HD, 2008. First record of downy mildew caused by *Pseudoperonospora cubensis* on bottle gourd in Korea. *Plant Pathology* **57**, 371.

Choi YJ, Hong SB, Shin HD, 2005. A re-consideration of *Pseudoperonospora cubensis* and *P. humuli* based on molecular and morphological data. *Mycological Research* **109**, 841–8.

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**, 1478–87.

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

Cohen Y, Rubin AE, 2011. Mating type and sexual reproduction of *Pseudoperonospora cubensis*, the downy mildew agent of cucurbits. *European Journal of Plant Pathology* **132**, 577–92.

Cohen Y, Meron I, Mor N, Zuriel S, 2003. A new pathotype of *Pseudoperonospora cubensis* causing downy mildew in cucurbits in Israel. *Phytoparasitica* **31**, 458–66.

Colucci SJ, 2008. *Host Range, Fungicide Resistance and Management of Pseudoperonospora cubensis, Causal Agent of Cucurbit Downy Mildew*. Raleigh, NC, USA: Department of Plant Pathology, North Carolina State University, MSc thesis.

Colucci SJ, Wehner TC, Holmes GJ, 2006. The downy mildew epidemic of 2004 and 2005 in the eastern United States. In: Holmes GJ, ed. *Cucurbitaceae 2006 Proceedings*. Raleigh, NC, USA: Universal Press, 403–11.

Drenth A, Goodwin SB, 1999. Population structure of oomycetes. In: Worrall JJ, ed. *Structure and Dynamics of Fungal Populations*. Dordrecht, Netherlands: Kluwer Academic Publishers, 195–224.

Forsberg AS, 1986. Downy mildew – *Pseudoperonospora cubensis* in Swedish cucumber fields. *Växtskyddsnotiser* **50**, 17–19.

Holmes GJ, Main CE, Keever ZT III, 2004. Cucurbit downy mildew: a unique pathosystem for disease forecasting. In: Spencer-Phillips PTN, Jeger M, eds. *Advances in Downy Mildew Research*, Vol. **2**. Dordrecht, Netherlands: Kluwer Academic Publishers, 69–80.

Ko Y, Chen CY, Liu CW, Chen SS, Maruthasalam S, Lin CH, 2008. First report of downy mildew by *Pseudoperonospora cubensis* on chayote (*Sechium edule*) in Taiwan. *Plant Disease* **92**, 1706.

Lebeda A, 1991. Resistance in muskmelons to Czechoslovak isolates of *Pseudoperonospora cubensis* from cucumbers. *Scientia Horticulturae* **45**, 255–60.

Lebeda A, 1992a. Screening of wild *Cucumis* species against downy mildew (*Pseudoperonospora cubensis*) isolates from cucumbers. *Phytoparasitica* **20**, 203–10.

Lebeda A, 1992b. Susceptibility of accessions of *Cucumis sativus* to *Pseudoperonospora cubensis*. *Tests of Agrochemicals and Cultivars* **13**, 102–3.

Lebeda A, 1999. *Pseudoperonospora cubensis* on *Cucumis* spp. and *Cucurbita* spp. – resistance breeding aspects. *Acta Horticulturae* **492**, 363–70.

Lebeda A, Cohen Y, 2011. Cucurbit downy mildew (*Pseudoperonospora cubensis*) – biology, ecology, epidemiology, host–pathogen interaction and control. *European Journal of Plant Pathology* **129**, 157–92.

Lebeda A, Cohen Y, 2012. Fungicide resistance in *Pseudoperonospora cubensis*, the causal pathogen of cucurbit downy mildew. In: Thind TS, ed. *Fungicide Resistance in Crop Protection: Risk and Management*. Wallingford, UK: CABI, 44–63.

Lebeda A, Gadasová V, 2002. Pathogenic variation of *Pseudoperonospora cubensis* in the Czech Republic and some other European countries. *Acta Horticulturae* **588**, 137–41.

Lebeda A, Křístková E, 1993. Resistance in *Cucurbita pepo* and *Cucurbita moschata* varieties to cucurbit downy mildew. *Plant Varieties and Seeds* **6**, 109–14.

Lebeda A, Křístková E, 2000. Interactions between morphotypes *Cucurbita pepo* and obligate biotrophs (*Pseudoperonospora cubensis*, *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*). *Acta Horticulturae* **510**, 219–25.

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

Lebeda A, Schwinn FJ, 1994. The downy mildews – an overview of recent research progress. *Journal of Plant Diseases and Protection* **101**, 225–54.

Lebeda A, Urban J, 2007. Temporal changes in pathogenicity and fungicide resistance in *Pseudoperonospora cubensis* populations. *Acta Horticulturae* **731**, 327–37.

Lebeda A, Urban J, 2010. Screening for resistance to cucurbit downy mildew (*Pseudoperonospora cubensis*). In: Spencer MM, Lebeda A, eds. *Mass Screening Techniques for Selecting Crops Resistant to Disease*. Vienna, Austria: International Atomic Energy Agency (IAEA), 285–94.

Lebeda A, Widrlechner MP, 2003. A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *Journal of Plant Diseases and Protection* **110**, 337–49.

Lebeda A, Widrlechner MP, 2004. Response of wild and weedy *Cucurbita* L. to pathotypes of *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov. (cucurbit downy mildew). In: Spencer-Phillips PTN, Jeger M, eds. *Advances in Downy Mildew Research*. Dordrecht, Netherlands: Kluwer Academic Publishers, 203–10.

Lebeda A, Zinkernagel V, 2003. Evolution and distribution of virulence in the German population of *Bremia lactucae*. *Plant Pathology* **52**, 41–51.

Lebeda A, Widrlechner MP, Urban J, 2006. Individual and population aspects of interactions between cucurbits and *Pseudoperonospora cubensis*: pathotypes and races. In: Holmes GJ, ed. *Cucurbitaceae 2006 Proceedings*. Raleigh, NC, USA: Universal Press, 453–67.

Lebeda A, Hübschová J, Urban J, 2010. Temporal population dynamics of *Pseudoperonospora cubensis*. In: Thies JA, Kousik S, Levi A, eds. *Cucurbitaceae 2010 Proceedings*. Alexandria, VA, USA: American Society for Horticultural Science, 240–3.

Lebeda A, Pavelková J, Urban J, Sedláková B, 2011. Distribution, host range and disease severity of *Pseudoperonospora cubensis* on cucurbits in the Czech Republic. *Journal of Phytopathology* **159**, 589–96.

McDonald BA, Linde C, 2002. Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology* **40**, 349–79.

Mitchell MN, Ocamb CM, Grunwald NJ, Mancino LE, Gent DH, 2011. Genetic and pathogenic relatedness of *Pseudoperonospora cubensis* and *P. humuli*. *Phytopathology* **101**, 805–18.

Müller K, McDermott JM, Wolfe MS, Limpert E, 1996. Analysis of diversity in populations of plant pathogens: the barley powdery mildew pathogen across Europe. *European Journal of Plant Pathology* **102**, 385–95.

Ojiambo PS, Holmes GJ, 2011. Spatiotemporal spread of cucurbit downy mildew in the Eastern United States. *Phytopathology* **101**, 451–61.

Palti J, 1974. The significance of pronounced divergences in the distribution of *Pseudoperonospora cubensis* on its crop hosts. *Phytoparasitica* **2**, 109–15.

Pavelková J, Lebeda A, Sedláková B, 2011. First report of *Pseudoperonospora cubensis* on *Cucurbita moschata* in the Czech Republic. *Plant Disease* **95**, 878–9.

Quesada-Ocampo L, Granke L, Olsen J *et al.*, 2012. The genetic structure of *Pseudoperonospora cubensis* populations. *Plant Disease* ???, ????–????, in press).

Runge F, Choi YJ, Thines M, 2011. Phylogenetic investigations in the genus *Pseudoperonospora* reveal overlooked species and cryptic diversity in the *P. cubensis* species cluster. *European Journal of Plant Pathology* **129**, 3–14.

Salati M, Wong MY, Sariah M, Nik Masdek H, 2010a. First report of *Pseudoperonospora cubensis* causing downy mildew of *Trichosanthes cucumerina* in Malaysia. *Plant Disease* **94**, 642.

Salati M, Yun WM, Meon S, Masdek HN, 2010b. Host range evaluation and morphological characterization of *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew in Malaysia. *African Journal of Biotechnology* **9**, 4897–903.

Sarris PF, Abdelhalim M, Kitner M *et al.*, 2009. Molecular polymorphisms between populations of *Pseudoperonospora cubensis* from Greece and the Czech Republic and the phytopathological and phylogenetic implications. *Plant Pathology* **58**, 933–43.

Shetty NV, Wehner TC, Thomas CE, Doruchowski RW, Shetty VKP, 2002. Evidence for downy mildew races in cucumber tested in Asia, Europe, and North America. *Scientia Horticulturae* **94**, 231–9.

Tahvonen R, 1985. Downy mildew of cucurbits found for the first time in Finland. *Växtskyddsnotiser* **49**, 42–4.

Thomas CE, Inaba T, Cohen Y, 1987. Physiological specialization in *Pseudoperonospora cubensis*. *Phytopathology* **77**, 1621–4.

Urban J, Lebeda A, 2007. Variation for fungicide resistance in Czech populations of *Pseudoperonospora cubensis*. *Journal of Phytopathology* **155**, 143–51.

3.4 Fungicide resistance of *Pseudoperonospora cubensis*

3.4.1 Hübschová, J., Lebeda, A. 2010. Fungicide effectiveness on Czech populations of *Pseudoperonospora cubensis.* Acta Horticulturae, 871, 457-463. (ISSN 0567-7572).

3.4.2 Pavelková, J., Lebeda, A., Sedláková, B. 2012. Efficacy of fosetyl-Al, propamocarb, dimethomorph, cymoxanil, metalaxyl and metalaxyl-M in Czech cucurbit downy mildew populations during the years 2005-2010 (manuscript).

Fungicide Effectiveness on Czech Populations of *Pseudoperonospora cubensis*

J. Hübschová and A. Lebeda[a]
Faculty of Science
Department of Botany
Palacký University in Olomouc
Šlechtitelů 11, 783 71 Olomouc-Holice
Czech Republic

Keywords: cucurbit downy mildew, chemical control, metalaxyl, metalaxyl-M, cymoxanil, dimethomorph, fosetyl-Al, propamocarb, fungicide resistance, tolerance, sensitivity, oomycetes

Abstract

Application of fungicides is still the principal method for managing harmfulness of *Pseudoperonospora cubensis*, a causal agent of cucurbit downy mildew, which is one of the most important diseases on cucumber. There are non-systemic (contact) fungicides as well as systemic fungicides (single-site inhibitors). The latter ones are more efficient but, unfortunately, their repeated application highly increases a risk of development of resistant pathogen strains. Therefore, effective chemical control strategies rely mostly upon weekly applications of contact fungicides. Totally 63 isolates of *P. cubensis* collected in 8 regions of the Czech Republic (25 in 2005, 18 in 2006 and 20 in 2007) were assessed for a degree of their resistance to fungicides. Six fungicide agents were chosen for testing their effectiveness, using five concentrations, in a floating disc bioassay. The active ingredient of two fungicide agents used was metalaxyl. As one of these agents has been so far the most widely used against downy mildew on cucumber in the Czech Republic in the past few years, it enabled us to monitor the distribution and development of resistant strains in Czech *P. cubensis* populations. Significant differences in effectiveness among fungicides were observed. Metalaxyl, metalaxyl-M, cymoxanil and dimethomorph were found ineffective; nearly all pathogen isolates expressed a resistant reaction to the recommended concentration of these fungicides. In contrast, propamocarb and fosetyl-Al were found effective at the recommended concentrations. Nevertheless, a few strains resistant or tolerant to propamocarb were observed in 2005 and 2006, indicating that selection for resistance strains is in progress in the Czech *P. cubensis* populations, and thus more screening is necessary. The results indicate that generally, the effectiveness of the studied fungicides against *P. cubensis* has progressively declined.

INTRODUCTION

Usage of fungicides is the most effective and most economic approach currently used to protect cucurbit crops (Urban and Lebeda, 2006). Externally operating protectants and multi-site fungicides were the only possibilities how to control downy mildews during the last century until about 1960 (Fernández-Ortuño et al., 2008). The restrictions on the use of several fungicides with a different mode of action have resulted in a growing need for new products (Mitani et al., 2001). The detection and introduction of new and more systemic fungicides with specific activity against oomycete plant pathogens offered the benefit of internal plant therapy (Fernández-Ortuño et al., 2008). Unfortunately, the occurrence of pathogen resistance is not rare, especially in agricultural practice. Particularly under the selection pressure when the fungicides were used over time (Gisi and Sietrotzki, 2008; Ma and Michailides, 2005). If the initial inoculum density is low, the probability of surviving of resistant individuals is smaller. Therefore all agronomic measures reducing disease pressure are very important for a successful resistance

[a]ales.lebeda@upol.cz

management (Gisi and Sietrotzki, 2008). Persistence of fungicide-resistant subpopulations involves theirs ecological fitness. If they are fit as sensitive isolates, they persist for a long time even without any use of the fungicides (Ma and Michailides, 2005).

Since 1984, cucurbit downy mildew has been the most devastating foliar disease of cucumber crops grown both on fields and greenhouses in the Czech Republic (Lebeda and Urban, 2004a, b), as well as in the whole Central Europe (Lebeda, 1990; Lebeda and Schwinn, 1994). Control of this disease in the fields was mostly based on the use of phenylamides, such as metalaxyl, which have been shown very effective since their registration in 1980 (O'Brien and Weinert, 1995). However, the appearance of *P. cubensis* strains resistant and/or tolerant to this fungicide has raised serious concern for more detailed research of this problematics (Lebeda and Schwinn, 1994).

The current paper summarizes the results of our research on the dynamics of temporal changes in *P. cubensis* populations on cucumber in the Czech Republic during the period 2005-2007. Pathogen populations studied were monitored for the possible occurrence of resistant or tolerant reaction to several commonly used fungicides.

MATERIALS AND METHODS

Origin and Characterization of *Pseudoperonospora cubensis* Isolates

The extensive screening of *P. cubensis* distribution, harmfulness and host range was carried out in the Czech Republic during the growing seasons of 2005, 2006, and 2007. The timing of expeditions was focused on the main harvest time (August and September). During the studied period, 115 locations (96 in 2005, 105 in 2006, and 91 in 2007) were visited in the main growing areas, as well as in some areas with climatic conditions not common for growing cucurbits. Infection was recorded only on cucumber (*C. sativus*), other cucurbitaceous crops frequently grown in the Czech Republic were free of infection. Many leaf samples were taken from infected plants of *C. sativus* for subsequent isolation of pure cultures of *P. cubensis*. Altogether, 63 of obtained isolates (25 in 2005, 18 in 2006, 20 in 2007) were used for a fungicide resistance bioassay. All screened isolates belonged to various pathotype groups and expressed mostly either medium or high pathogenicity.

Pathogen Isolation and Maintenance

The leaf samples taken from infected plants were placed on wet filter paper in plastic pots (110×85×45 mm) and incubated in humid conditions at approximately 18°C for 1-2 days until sporangiophores with sporangia occurred (Lebeda, 1986). Inoculum was prepared by shaking small pieces of leaves with visible sporulation in distilled water. Leaves of susceptible *C. sativus* were placed in a Petri dish on wet filter paper and inoculated by atomizing prepared spore suspension by means of a glass sprayer over their abaxial surface of leaves. Inoculated leaves were incubated in a growth chamber under standard conditions (Lebeda, 1986, 1991). Pathogen sporulation occurred usually after 7-8 days of incubation. Obtained pure cultures of *P. cubensis* were stored in Petri dishes at -80°C and later used for fungicide bioassay. In the freezer, the spores stayed viable for about 6 months, after which it was necessary to refresh the stored cultures by new inoculations.

Plant Material

A highly susceptible cultivar of cucumber ('Marketer 430') was used for pathogen multiplication and floating leaf-disc bioassays. Plants were grown under optimal conditions (25°C/15°C day/night, daily watering, and weekly fertilization) in a glasshouse and were not treated chemically. The leaves from 6-8-week-old plants (3-6-true-leaf stage) were used for multiplication and for discs cutting as well (Lebeda, 1986; Lebeda and Widrlechner, 2003).

Fungicides and a Floating Leaf Disc Bioassay

Six commonly used agents were chosen for screening *P. cubensis* resistance to fungicides; Ridomil Plus 48 WP (active ingredients: 40% Cu-oxychloride, 8% metalaxyl), Ridomil Gold MZ 68 WP (a.i.: 64% mancozeb, 4% metalaxyl-M), Acrobat MZ (a.i.: 600 g/l mancozeb, 90 g/l dimethomorph), Aliette 80 WP (a.i.: 80% fosetyl-Al), Previcur 607 SL (a.i.: 607 g/L propamocarb), Curzate K (a.i.: 77.3% Cu-oxychloride, 4% cymoxanil). Five concentrations of each fungicide were tested, one recommended by the producer (i.e. optimal), two others bellow and two above the optimum (Table 1). Leaf discs treated with distilled water served as a positive control. Leaf discs (15 mm in diameter) were floated, abaxial surface up, on fungicide solutions in multiwell plates (Anonymous, 1982). Four leaf discs were tested from each concentration of fungicides used, each test made in three replicates. After 24h, leaf discs were inoculated with spore suspensions (1×10^5 spores/ml) and incubated as described above.

Evaluation of a Fungicide Bioassay

The evaluation was made in two-days intervals, 6^{th}–14^{th} day after inoculation using the 0-4 scale (Lebeda, 1986; Lebeda and Widrlechner, 2003). On control leaf discs, first symptoms of sporulation occurred most frequently 5-6 days after inoculation. The total degree of infection (sporulation intensity) was expressed as a percentage of the maximum scores according to Towsend and Heuberger (1943):

$$P = \sum \frac{(n \times v) \times 100}{x \times N},$$

where P is the total degree of infection (%), n the number of discs in each (0-4) infection degree category, v infection degree, x the extent of a used scale and N the total number of leaf discs evaluated in three replications.

Three types of reactions were assigned according to degree of infection obtained under each fungicide concentration tested: sensitive (the total degree of infection P $\leq$10%), tolerant (10< P $\leq$35%) and resistant (P >35%).

The values of ED50 (a fungicide concentration, which inhibits fungal growth by 50%) were determined for each isolate screened and expressed in the ranges of fungicide concentrations.

RESULTS AND DISCUSSION

Altogether, 63 isolates of *P. cubensis* were screened for growth characteristic after treatment with 6 agents used. The studied isolates were uniform in the sensitivity to fosetyl-Al. Highly sensitive reactions were recorded to all tested concentrations of this fungicide. Generally, propamocarb also showed a high level of effectiveness and the majority of tested isolates were controlled even by the lowest tested concentration (607 µg a.i./ml). However, sensitive isolates with some very limited sporulation were recorded during the whole studied period (Table 2). Furthermore, in 2005 and 2006 a few isolates exhibited tolerant or resistant reaction to lower (607 and 1214 µg a.i./ml) and optimal (2428 µg a.i./ml) concentrations (Table 2).

Metalaxyl, metalaxyl-M and cymoxanil were generally ineffective and 81.5% of the screened isolates showed resistance even to the optimal concentration. Moreover, temporal shift towards higher resistance to these fungicides was observed in the Czech *P. cubensis* populations during the monitored period (Fig. 1).

Reaction of *P. cubensis* isolates was eminently different for dimethomorph. This fungicide was practically ineffective, and the majority of isolates showed resistance or tolerance to the optimal concentration (450 µg a.i./ml). Surprisingly, the number of pathogen isolates tolerant to all concentrations tested increased intensely from 2005 to 2007 (Table 3).

New fungicides effective against cucurbit downy mildew, which have been introduced to the market during the last few decades, replaced the contact fungicides used

earlier. Unfortunately, some reports are available about failures in their effectivity against *P. cubensis* and a rapid increase of fungicide-resistant subpopulations of a pathogen, especially under selection pressure from different fungicides (Gisi, 2002; Urban and Lebeda, 2006). This phenomenon is known mostly from countries outside Europe (Australia, Israel, Japan, Taiwan, USA), only a few data are available for European countries (Greece, Italy, Russia), the majority of them from 1980s and 1990s (Urban and Lebeda, 2006). Furthermore, the mechanisms of pathogen's resistance to fungicides are not well known, with the exception of the strobilurin fungicides (Takeda et al., 1999; Ishii et al., 2002). Our research confirms the decreased sensitivity or temporal shift to higher level of resistance to common used fungicides. This trend is probably typical in the whole Central Europe. Nevertheless, chemical control is the most effective measure currently used to protect cucumber crops from cucurbit downy mildew. More detailed research should focus on the geographic distribution and the dynamics of spatial and temporal aspects of fungicide resistance in *P. cubensis*. Intensive international collaboration in this area is required.

ACKNOWLEDGEMENTS

This research was supported by grants: QD 1357; MSM 153100010 and MSM 6198959215; National Programme of Genepool Conservation of Microorganisms and Small Animals of Economic Importance.

Literature Cited

Anonymous, 1982. FAO Method No. 30. In: FAO Plant Protect. Bull. Vol. 30/2.

Fernández-Ortuño, D., Torés, J.A., de Vincente, A. and Pérez-García, A. 2008. Mechanisms of resistance to QoI fungicides in phytopathogenic fungi. International Mikrobiology 11:1-9.

Gisi, U. 2002. Chemical control of downy mildews. p.119-159. In: P.T.N. Spencer-Phillips, U. Gisi and A. Lebeda (eds.), Advances in downy mildew research, Kluwer Academic Publishers, Dordrecht.

Gisi, U. and Sierotzki, H. 2008. Fungicide modes of action and resistance in downy mildews. Eur. J. Plant Pathol. 122(1):157-167.

Ishii, H., Sugiyama, T., Nishimura, K. and Ishikawa, Y. 2002. Strobilurin resistance in cucumber pathogens: persistence and molecular diagnosis of resistance. p.149-159. In: H.W. Dehne, U. Gisi, K.H. Kuck, P.E. Russell and H. Lyr (eds.), Modern Fungicides and Antifungal Compounds III. The 13th International Reinhardsbrunn Symposium Germany: AgroConcept., Bonn.

Kužma, Š. (ed.) 2005. Seznam registrovaných přípravků na ochranu rostlin 2005 (List of registered preparations for plant protection 2005). Státní rostlinolékařská správa, Praha.

Lebeda, A. 1986. *Pseudoperonospora cubensis*. p.81-85. In: A. Lebeda, (ed.), Methods of Testing Vegetable Crops for Resistance to Plant Pathogens, VHJ Sempra, Research Institute of Vegetable Crops, Olomouc.

Lebeda, A. 1990. Biology and ecology of cucurbit downy mildew. p.13-46. In: A. Lebeda (ed.), Cucurbit Downy Mildew, Czechoslovak Scientific Society for Mycology by Czechoslovak Academy of Sciences, Praha.

Lebeda, A. 1991. Resistance in muskmelons to Czechoslovak isolates of *Pseudo-peronospora cubensis* from cucumbers. Sci. Hort. 45:255-260.

Lebeda, A. and Schwinn, F.J. 1994. The downy mildews – an overview of recent research progress. J. Plant Dis. Protect. 101:225-254.

Lebeda, A. and Urban, J. 2004a. Disease impact and pathogenicity variation in Czech populations of *Pseudoperonospora cubensis*. p.267-273. In: A. Lebeda and H.S. Paris (eds.), Progress in Cucurbit Genetics and Breeding Research, Proceedings of Cucurbitaceae 2004, the 8th Eucarpia, Meeting on Cucurbit Genetics and Breeding, Palacký University in Olomouc, Olomouc, Czech Republic.

Lebeda, A. and 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. and Widrlechner, M.P. 2003. A set of *Cucurbitaceae* taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. J. Plant Dis. Protect. 110:337-349.
Ma, Z. and Michailides, T.J. 2005. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phyto-pathogenic fungi. Crop Protection 24:853-863.
Minář, P. (ed.) 2006. Seznam registrovaných přípravků na ochranu rostlin 2006 (List of registered preparations for plant protection 2006). Státní rostlinolékařská správa, Praha.
Minář, P. (ed.) 2007. Seznam registrovaných přípravků na ochranu rostlin 2007 (List of registered preparations for plant protection 2007). Státní rostlinolékařská správa, Praha.
Mitani, S., Araki, S., Yamaguchi, T., Takii, Y., Ohshima, T. and Matsuo, N. 2001. Biological properties of the novel fungicide cyazofamid against *Phytophthora infestans* on tomato and *Pseudoperonospora cubensis* on cucumber. Pest management science 58:139-145.
O´Brien, R.G.O. and Weinert, M.P. 1995. Three metalaxyl sensitivity levels in Australian isolates of *Pseudoperonospora cubensis* (Berk. et Curt.) Rost. Austr. J. Exp. Agr. 35:543-546.
Takeda, T., Kawagoe, Y., Uchida, K., Fuji, M. and Amano, T. 1999. The appearance of resistant isolates to strobilurins. Ann. Phytopathol. Soc. Jap. 65:655 (Abstr.).
Towsend, G.R. and Heuberger, W. 1943. Methods for estimating losses caused by diseases in fungicide experiments. Plant Dis. Rep. 27:340-343.
Urban, J. and Lebeda, A. 2006. Fungicide resistance in cucurbit downy mildew - methodological, biological and population aspects. Ann. Appl. Biol. 149:63-75.

Tables

Table 1. Tested concentrations of the fungicides.

Fungicide	Concentration of fungicide (µg a.i./ml)/concentration of source agent (%)				
	1	2	3*	4	5
Metalaxyl	50/0.0630	100/0.125	200/0.250	400/0.500	800/1.000
Metalaxyl-M	25/0.0630	50/0.125	100/0.250	200/0.500	400/1.000
Propamocarb	607/0.1000	1214/0.200	2428/0.400	4856/0.800	9712/1.600
Fosetyl-Al	400/0.0500	800/0.100	1600/0.200	3200/0.400	6400/0.800
Cymoxanil	30/0.0750	60/0.150	120/0.300	240/0.600	480/1.200
Dimethomorph	112.5/0.125	225/0.250	450/0.500	900/1.000	1800/2.000

*The concentration recommended by the producer (Kužma, 2005; Minář, 2006, 2007).

Table 2. Response of *P. cubensis* isolates to different concentrations of propamocarb (finer scale was used, because of shift in sensitive reaction of some isolates).

Year	Type of reaction	Propamocarb concentration (µg a.i./ml)/frequency of isolates (%)					
		0	607	1214	2428	4856	9712
2005	S_{sp-}	0.0	48.0	56.0	56.0	92.0	100.0
	S_{sp+}	0.0	44.0	40.0	40.0	8.0	0.0
	T	0.0	8.0	4.0	4.0	0.0	0.0
	R	100.0	0.0	0.0	0.0	0.0	0.0
2006	S_{sp-}	0.0	61.0	89.0	89.0	94.5	94.5
	S_{sp+}	0.0	28.0	0.0	0.0	0.0	0.0
	T	0.0	5.5	5.5	11.0	5.5	5.5
	R	100.0	5.5	5.5	0.0	0.0	0.0
2007	S_{sp-}	0.0	70.0	90.0	100.0	100.0	100.0
	S_{sp+}	0.0	30.0	10.0	0.0	0.0	0.0
	T	0.0	0.0	0.0	0.0	0.0	0.0
	R	100.0	0.0	0.0	0.0	0.0	0.0

S_{sp-}: Sensitive reaction without sporulation (P = 0%).
S_{sp+}: Sensitive reaction with limited sporulation (0 < P ≤ 10%).
T: Tolerant reaction (10 < P ≤ 35%).
R: Resistant reaction (P > 35%).

Table 3. Response of *P. cubensis* isolates to different concentrations of dimethomorph.

Year	Type of reaction	Dimethomorph concentration (µg a.i./ml)/frequency of isolates (%)					
		0	112.5	225	450	900	1800
2005	S	0.0	8.0	12.0	28.0	36.0	100.0
	T	0.0	4.0	8.0	4.0	32.0	0.0
	R	100.0	88.0	80.0	68.0	32.0	0.0
2006	S	0.0	0.0	0.0	5.5	45.0	83.5
	T	0.0	16.5	27.5	49.5	49.5	16.5
	R	100.0	83.5	72.5	45.0	5.5	0.0
2007	S	0.0	0.0	0.0	0.0	30.0	80.0
	T	0.0	35.0	70.0	85.0	70.0	20.0
	R	100.0	65.0	30.0	15.0	0.0	0.0

S: Sensitive reaction (0 < P ≤ 10%).
T: Tolerant reaction (10 < P ≤ 35%).
R: Resistant reaction (P > 35%).

Figures

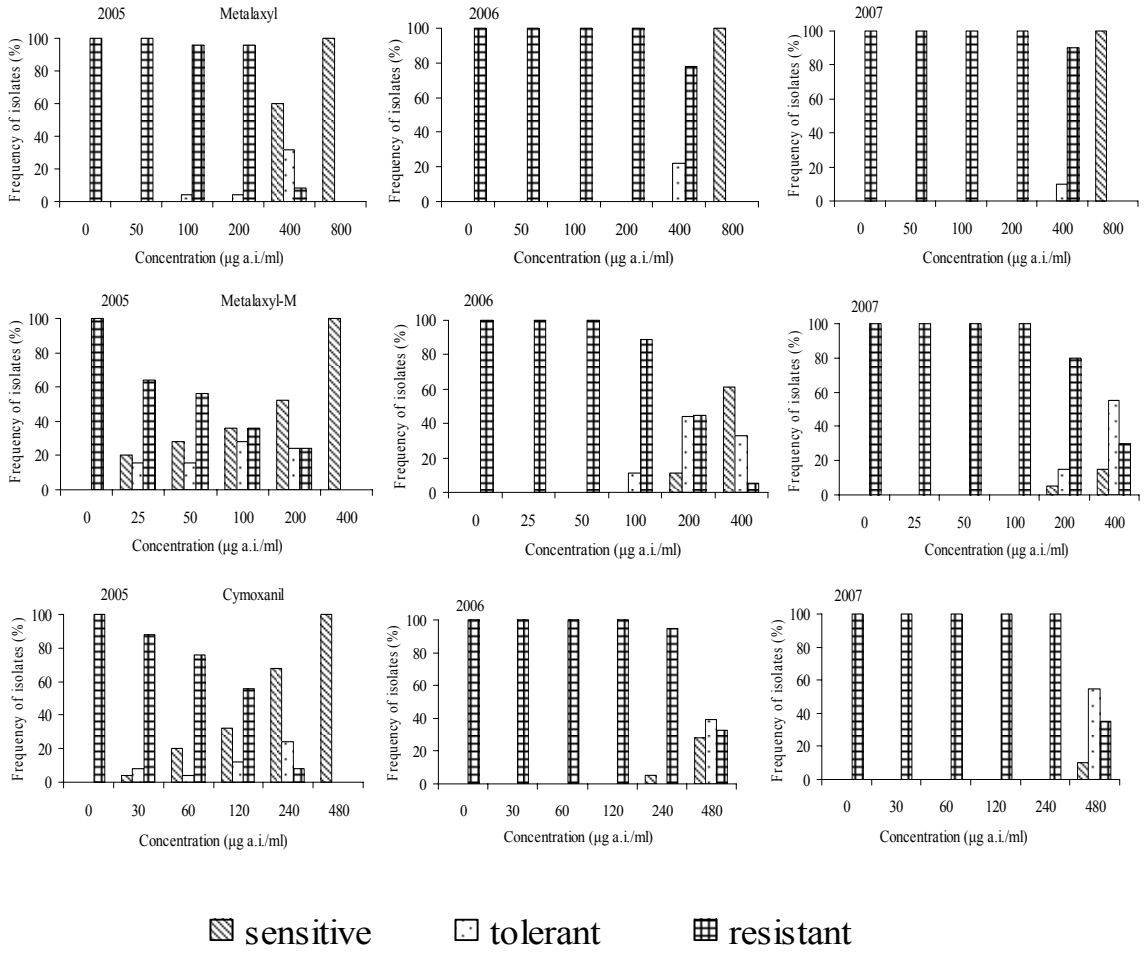


Fig. 1. Responses of *P. cubensis* populations to different concentrations of metalaxyl and metalaxyl-M and cymoxanil during 2005-2007.

Efficacy of fosetyl-Al, propamocarb, dimethomorph, cymoxanil, metalaxyl and metalaxyl-M in Czech cucurbit downy mildew populations during the years 2005 and 2010

Jana Pavelková, Aleš Lebeda and Božena Sedláková

Authors' addresses: Department of Botany, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 11, 783 71 Olomouc, Czech Republic (correspondence to Aleš Lebeda: *ales.lebeda@upol.cz*)

Abstract

BACKGROUND: Cucurbit downy mildew, major disease of cucurbit crops worldwide, causes substantial economic losses. Disease control is mainly managed by fungicide applications. In the Czech Republic, *Pseudoperonospora cubensis* is responsible for enormous yield losses in the past 25 years. Severe outbreaks of cucurbit downy mildew have been reported repeatedly on cucumbers.

RESULTS: A total of 142 Czech *P. cubensis* isolates were used for fungicide resistance/tolerance screening. Significant differences were proved among tested fungicides and even during the six-year period of study (2005-2010). Six commonly used and registered fungicides (except Ridomil Plus 48 WP that served as control fungicide) were screened for chemical protection of cucurbit plants against *P. cubensis* in the Czech Republic. Majority of isolates (134) originated from *C. sativus* and 8 isolates from new hosts (*Cucurbita* spp. and *Citrullus lanatus*. The frequency of occurrence of sensitive/tolerant/resistant isolates to all tested concentrations of individual fungicides was investigated. Fosetyl-Al (Aliette 80 W) and propamocarb (Previcur 607 SL) were the most effective fungicides in the studied period. All tested isolates were sensitive on all tested concentrations of fosetyl-Al. However, some isolates expressed resistance (profuse sporulation) or tolerance (limited sporulation) to lower and/or even to recommended concentrations of propamocarb in the years 2006 and 2008-2010. Metalaxyl (Ridomil PLUS 48 WP) and metalaxyl-M (Ridomil Gold MZ 68 WP) were ineffective. There was recorded higher variability of isolates with tolerant or resistant response at higher concentrations in the years 2008-2009. However, this trend was not confirmed in the year 2010, when effectivity of these fungicides slightly increased and 69% and 43% of isolates were controlled at recommended concentration of metalaxyl-M and metalaxyl respectively and limited or profuse sporulation was observed only sporadically at higher concentrations. Sensitivity of isolates to cymoxanil (Curzate K) differed also among the studied years. While cymoxanil was ineffective in the years 2005-2008 and in 2010, there was 68% of isolates controlled at recommended concentration in 2009. In Czech *P. cubensis* populations during the years 2005-2010, there was recorded the temporal shift towards higher sensitivity to dimethomorph on all screened concentrations.

CONCLUSION: The efficacy of screened fungicides varied significantly, but it corresponded with the results of previous years. The variability in fungicide efficacy could be the result of pathogen migration in Europe. It could be expected, that the efficacy of disease control measures will be very variable in the future according to *P. cubensis* character.

Keywords: *Pseudoperonospora cubensis*, cucumber, *Cucurbita* spp., *Citrullus lanatus*, fungicide resistance, ED_{50} value

1 INTRODUCTION

Fungicides have been used for over 200 years to successful managing crop diseases (Brent KJ, www.frac.info). Majority of agricultural fungicides were protectants and multi-site inhibitors until about 1960. Inorganic Bordeaux mixture, sulfur and copper compound and organic such as phthalimides, dithiocarbamates, dinitrophenols, and aromatic hydrocarbons were employed most frequently. New and more systemic fungicides with specific activity have been introduced after the restrictions on the use of several fungicides with a different mode of action (Fernández-Ortuño et al., 2008; Mitani et al., 2001). These site-specific fungicides significantly increased the efficiency of plant protection, mainly because of some curative effects and quickly translocation in the plant even to the parts not directly treated. The major problem of disease control that has been detected since the early 1970s was resistance of pathogens that have acquired against certain of the fungicides that normally control them well (Cohen and Coffey, 1986; Gisi, 2002; Gisi and Sierotzki, 2008; Holmes and Ojiambo, 2009; Lebeda and Schwinn, 1994; Urban and Lebeda, 2006). The development of fungicide resistance is influenced by complex interactions of factors such as the biology of the pathogen, mode of action of the fungicide, fungicide use pattern, the cropping system and environmental considerations (Gisi and Sierotzki, 2008).

For determination of the risk of a pathogen to develop resistance to fungicides pathogens were classificated in some groups (McDonald and Linde, 2002; Pathogen risk list 2005, (www.frac.info)). Pathogens have evolved resistance to fungicides already after few years of product use and have shown a high risk of development of resistance to fungicides. *Pseudoperonospora cubensis* [(Berkeley & MA Curtis) Rostovzev], a causal agent of cucurbit downy mildew, is typical member of this high risk class (Lebeda and Cohen, 2011; McDonald and Linde, 2002; Pathogen risk list 2005, www.frac.info). Like other foliar diseases, *P. cubensis* undergoes many and short disease cycles per season and the dispersal through spores over time and space is high (Lebeda and Cohen, 2011; Pathogen risk list 2005, www.frac.info). Although, disease symptoms are confined to the leaves, adversely are affecting the quality and consequently yield of fruit. According to severe damage of cucurbit crops, which ending with death of the adult plants, *P. cubensis* is determining element in cucurbit production in certain regions (Cohen, 1981; Perchepied et al., 2005; Velichi, 2009; Lebeda and Cohen, 2011). Recently, cucurbit downy mildew has been reported in more than 80 countries on more than 60 species (Lebeda and Cohen, 2011). Although *P. cubensis* is distributed worldwide, the occurrence and damage of cucurbitaceous crops by this pathogen may differ among various geographic regions and host plants (Palti and Cohen, 1980; Cohen,

1981; Lebeda, 1990). Development of *P. cubensis* is dependent on host susceptibility and favorable environmental conditions (Iwata, 1942, 1953a,b; Palti, 1974; Palti and Cohen, 1980). In Europe as well as in the Czech Republic, severe outbreaks of cucurbit downy mildew have been reported repeatedly on cucumbers (*Cucumis sativus* L.) since approximately 1985 (Doruchowski and Lachowska-Ryk, 1992; Lebeda and Cohen, 2011; Urban and Lebeda, 2004a,b, 2006, 2007). However recent observations from the Czech Republic from the years 2009-2011 showed that the host range of *P. cubensis* has been changing. In the Czech Republic, *P. cubensis* infections have been newly recorded on muskmelon *(Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai), pumpkin (*Cucurbita maxima* Duch.), squash (*Cucurbita pepo* L.), *Cucurbita moschata* Duch., *Cucurbita ficifolia* Bouché and *Lagenaria siceraria* (Mol.) Standl., (Lebeda et al., 2012; Pavelková et al., 2011). As well as from other countries, this phenomenon has been reported, namely from Israel in 2002 (Cohen et al., 2003) and from the USA in 2004 (Holmes et al., 2004; Colucci et al., 2006; Holmes and Ojiambo, 2009; Holmes and Thomas, 2009). It confirms that *P. cubensis* belongs currently to the most dangerous and destructive pathogen of cucurbit crops around the world (Lebeda and Cohen, 2011; Lebeda and Schwinn, 1994; Lebeda and Urban, 2007).

P. cubensis is highly variable in its pathogenicity (Lebeda and Widrlechner, 2003, 2004; Lebeda et al., 2012). The continual changes in pathogen populations worldwide reported make difficult to manage cucurbit downy mildew. Chemical control has being combined with resistant varieties and cultural techniques to minimize selection of fungicide resistant strains and to decrease the high risk of overcoming resistance genes by pathogen (Lebeda and Cohen, 2011; Savory et al., 2010). Quite aggressive programme is essential to make a protective barrier of fungicide prior to sporangium deposition (Savory et al., 2010). The rather "old" multi-site fungicides (including e.g. mancozeb, folpet and copper formulations) are still very important elements in the spray programmes (about 50% of the total oomycete fungicide market). Crucial step for this aspect is surely fact that resistance to such inhibitors has never developed and is unlikely to evolve, and that they improve single-site activity and delay resistance evolution. The major site-specific fungicides used in chemical control of *P. cubensis* are from four chemical classes: the Quinone outsie inhibitors (Qols; "strobilurins", e.g. azoxystrobin, famoxadone, fenamidone), phenylamides (PAs; e.g. metalaxyl-M) carboxylic acid amides (CAAs; e.g. dimethomorph, iprovalicarb, benthiavalicarb, mandipropamid) and cyano-acetamide oximes (e.g. cymoxanil). Smaller market shares are taken by phosphonates (mainly fosetyl-Al), dinitroanilines (fluazinam),

carbamates (propamocarb) and plant defence inducers such as the benzothiadiazoles (BTH; acibenzolar-S-methyl/Bion) (Gisi and Sierotzki, 2008). Unfortunately, *P. cubensis*, as one of 10 highest risk pathogens with high evolutionary potential pathogens has developed in its populations quite quickly resistance to key fungicides (McDonald and Linde, 2002; Lebeda and Urban, 2004a,b; Lebeda et al., 2006, 2010; Pathogen risk list 2005, www.frac.info). *P. cubensis* was the first oomycete with documented resistance to metalaxyl and reduced sensitivity to mancozeb (Reuveni et al., 1980; Thomas and Jourdain, 1992). Occurrence of resistant strains of this pathogen to some other fungicide classes are also reported, such to strobilurins (Heaney et al., 2000), CAAs in South Korea, Israel, USA (FRAC CAA working group reports, www.frac.info) and in China (Zhu et al., 2007). In other fungicide classes, reduced sensitivity (or occurrence of resistant isolates) has been reported from other oomycete fungi, e.c. *Plasmopara viticola* (cymoxanil) (Gullino et al., 1997; Genet and Vincent, 1999), *Phytophthora infestans* (Cohen et al., 2007; Gisi and Sierotzki, 2008; Grünwald et al., 2006; Pérez et al., 2009) and *Pythium* species (propamocarb) (Moorman and Kim, 2004). Nevertheless Fosetyl-Al resistant isolates in field populations have never been detected (Gisi and Sierotzki, 2008).

Cucurbit downy mildew is one of the most economically important and prevalent diseases of cucurbitaceous crops with worldwide distribution (Lebeda and Cohen, 2011; Palti and Cohen, 1980). Despite of importance of *P. cubensis* (one of the most surveyed Peronosporomycete biotrophic plant parasite, studied by mycologist and plant pathologist more than 100 years), progress in resistance breeding, fungicide control and disease management is rather slow (Lebeda and Cohen, 2011). The present work describes dynamics in efficacy of six screened fungicides and provides information about temporal changes in populations of *P. cubensis* in the Czech Republic using experimental data obtained during the period 2005-2010.

2 MATERIALS AND METHODS

2.1 Origin and Characterization of *Pseudoperonospora cubensis* Isolates

Occurrence, host range and disease severity of *P. cubensis* were monitored on cucurbits in the Czech Republic during the period 2005-2010. Over 100 locations per year were evaluated in the main and in some marginal cucurbitaceous vegetable production areas, especially at the end of harvest time (August and first half of September). Infected host plants were visually evaluated using a 0-4 scale (Lebeda and Křístková, 1994), modified for *P. cubensis*. During the growing seasons between the years 2005-2008, only infected leaves of cucumber (*C.*

sativus) were collected, but in the years 2009 and 2010 other cucurbits (Table 1) were also infected. Most of the leaf samples were taken from infected cucurbitaceous plants for subsequent isolation of pure cultures of *P. cubensis*. A total of 142 obtained isolates (25 in 2005, 18 in 2006, 20 in 2007, 22 in 2008 and 2009, 35 in 2010) were used for a fungicide resistance bioassay, but they were firstly screened on pathogenic variation (detection of pathotypes). Tested isolates belonged to various pathotype groups and expressed mostly either medium or high pathogenicity. 8 of screened isolates originated from *Cucurbita* spp. and *Citrullus lanatus* (Table 2).

2.2 Pathogen Isolation and Maintenance

The leaf samples taken from infected plants were placed on wet filter paper in plastic pots (110 × 85 × 45 mm) and incubated in humid conditions at approximately 18°C for 1-2 days until sporangiophores with sporangia occurred (Lebeda, 1986). Inoculum was prepared by shaking small pieces of leaves with visible sporulation in distilled water. Leaves of susceptible *C. sativus* were placed in a Petri dish on wet filter paper and inoculated by atomizing prepared spore suspension by means of a glass sprayer over their abaxial surface of leaves. Inoculated leaves were incubated in a growth chamber under standard conditions (Lebeda, 1986, 1991). Pathogen sporulation occurred usually after 7-8 days of incubation. Obtained pure cultures of *P. cubensis* were stored in Petri dishes at -80°C and later used for fungicide bioassay. In the freezer, the spores stayed viable for about 6 months, after that it was necessary to refresh the stored cultures by new inoculations.

2.3 Plant Material

A highly susceptible cultivar of cucumber (‘Marketer 430’) was used for pathogen multiplication and floating leaf-disc bioassays. Plants were grown under optimal conditions (25°C/15°C day/night, daily watering, and weekly fertilization) in a glasshouse and were not treated chemically. The leaves from 6-8-week-old plants (3-6-true-leaf stage) were used for multiplication and as well for discs cutting (Lebeda, 1986; Lebeda and Widrlechner, 2003).

2.4 Fungicides and a Floating Leaf Disc Bioassay

Six agents commonly used and registered (except Ridomil Plus 48 WP*) for chemical protection of cucurbit plants against *P. cubensis* in the Czech Republic were chosen for screening *P. cubensis* resistance to fungicides. These fungicides belonged to different chemical classes and were with specific features (Tomlin, 1997; Table 3): Ridomil Plus 48 WP (active ingredients: 40% Cu-oxychloride, 8% metalaxyl, producer: Novartis Crop Protection AG, Basel, Switzerland), Ridomil Gold MZ 68 WP (a.i.: 64% mancozeb, 4% metalaxyl-M, producer: Syngenta Crop Protection AG, Basel, Switzerland), Acrobat MZ (a.i.:

600 g/l mancozeb, 90 g/l dimethomorph, BASF SE, Ludwigshafen Germany), Aliette 80 WP (a.i.: 80% fosetyl-Al, producer: Aventis CropScience SA, Lyon, France), Previcur 607 SL (a.i.: 607 g/l propamocarb, producer: Aventis CropScience GmbH, Frankfurt/Main, Germany), Curzate K (a.i.: 77.3% Cu-oxychloride, 4% cymoxanil, producer: SPOLANA a.s., Neratovice, Czech Republic).). *Ridomil Plus 48 served as a control fungicide because its registration validity has already stopped in the CR and it is admitted to expend it (Kužma, 2004, 2005). These screened agents are often worldwide used in chemical control of many oomycete fungi from the families Peronosporaceae and Pythiaceae (Gisi, 2002; Gisi and Sierotzki, 2008)

Five concentrations of each fungicide were tested, one recommended by the producer (i.e. optimal), two others bellow and two above the optimum (Table 4). Leaf discs treated with distilled water served as a positive control. Leaf discs (15 mm in diameter) were floated, abaxial surface up, on fungicide solutions in multiwell plates (Anonymous, 1982). Four leaf discs were tested from each concentration of fungicides used, each test made in three replicates. After 24h, leaf discs were inoculated with spore suspensions (1 x 10^5 spores/ml) and incubated as described above.

2.5 Evaluation of a Fungicide Bioassay

The evaluation was made in two-day intervals, 6^{th} – 14^{th} day after inoculation using the 0-4 scale (Lebeda, 1986; Lebeda and Widrlechner, 2003). On control leaf discs, first symptoms of sporulation occurred most frequently 5-6 days after inoculation. The total degree of infection (sporulation intensity) was expressed as a percentage of the maximum scores according to Towsend and Heuberger (1943):

$$P = \sum \frac{(n \times v) \times 100}{x \times N},$$

where P is the total degree of infection (%), n the number of discs in each (0-4) infection degree category, v infection degree, x the maximum level of sporulation and N the total number of leaf discs evaluated in three replications.

Three types of reactions were assigned according to degree of infection obtained under each fungicide concentration tested: sensitive (the total degree of infection P ≤10%), tolerant (10< P ≤35%) and resistant (P >35%).

The values of ED_{50} (a fungicide concentration, which inhibits fungal growth by 50%) were determined for each isolate screened and expressed in the ranges of fungicide concentrations.

3 RESULTS

3.1 General characteristics of investigated pathogen populations

During the growing seasons between the years 2005 and 2010, infection of *P. cubensis* was observed primarily on *C. sativus* (93%), other cucurbitaceous crops were rarely infected (*Cucumis melo* 0,6%; *Cucurbita pepo* 2,4%; *C. maxima* 1,8%; *Cucurbita moschata* 1,2%; and *Citrullus lanatus* 0,6%) and only during the years 2009 - 2010 (Table 1). In generally, majority of *C. sativus* crops were heavily infected at the end of the growing season (second half of August) and *P. cubensis* was present at high or very high disease severity. *P. cubensis* was widespread across the whole area of the Czech Republic studied (central and southern Moravia and eastern, northern and central Bohemia) (Lebeda et al., 2011; unpubl. data).

Pathogenicity structure and their temporal changes were studied in the populations of *P. cubensis* in the period 2005-2010. About 200 isolates were analyzed for pathogenic variation (pathotypes) (Table 1). The substantial part of *P. cubensis* isolates (80%) belonged to the group of highly pathogenic isolates, and the rest was detected as medium pathogenicity pathotypes. The data on structure and changes in pathogen populations were expressed by pathotypes. More than 60 different pathotypes have been determined. "Super pathotype" 15.15.15 was recorded repeatedly. Since the year 2009 the pathogen population has changed dramatically, a new pathotypes appeared able to establish the natural infection of *Cucurbita* spp. and *Citrullus lanatus*, this phenomenon was not observed before. In Czech *P. cubensis* populations, there was prevailing frequency of isolates with high number (8-11) of pathogenicity factors (Lebeda et al., 2012). A total of 142 *P. cubensis* isolates were used for fungicide resistance/tolerance screening.

3.2 Fungicide efficacy

Effectiveness of screened fungicides against *P. cubensis* isolates was investigated among the years 2005-2010 (Table 1-7, Figs 1-5). Significant differences among tested fungicides and even during the six-year period of study were proved. The range of reaction types of *P. cubensis* isolates was very wide in the studied period, altogether 20 patterns of reaction types were detected (14 types were determined in cymoxanil, 18 in dimethomorph, 13 in metalaxyl, 16 in metalaxyl-M). Fosetyl-Al and propamocarb showed to be highly effective for the field

control of the disease. Effectiveness of dimethomorph has changed from resistance to tolerance and sensitivity during the six-year period of study. Czech *P. cubensis* populations appeared to be insensitive to metalaxyl, metalaxyl-M and cymoxanil.

3.2.1 Fosetyl-Al

P. cubensis isolates were uniform in the sensitivity to fosetyl-Al in the period 2005-2010 (Table 5). Sensitive reactions were recorded on all tested concentrations. All screened *P. cubensis* isolates were controlled by the optimal concentration (1600 μg a.i./ml) and ED_{50} values were lower than 400 μg a.i./ml. However, some isolates from the year 2009 expressed sensitive reaction with limited sporulation (Ssp+). Moreover, this type of reaction appeared also at same *P. cubensis* isolates at concentrations 400 and 800 μg a.i./ml in the years 2009 and 2010. Despite the risk of potential selection of more insensitive strains to this fungicide (in the years 2009 and 2010), fosetyl-Al remains a highly effective as a control agent of Czech cucurbit downy mildew populations.

3.2.2 Propamocarb

Sensitive reactions of tested isolates predominated on all concentrations of propamocarb. ED_{50} values were lower than 607 μg a.i./ml in the period 2005-2010 (Table 6). Despite this, Czech populations of *P. cubensis* appeared to be very variable in responses to this fungicide. The proportion of the isolates with sensitive reaction with limited sporulation (Ssp+) in optimal (2428 μg a.i./ml) and lower concentrations (607 and 1214 μg a.i./ml) was quite high (especially in the years 2005 and 2008). Moreover, strains with increased insensitivity (tolerant or resistant response) to this fungicide were also detected. Some analyzed isolates were tolerant/resistant to the optimal and lower concentrations. Additionally, in 2006, tolerant response were detected also in higher concentrations (4856 and 9712 μg a.i./ml). Profuse sporulation (resistant reaction) was observed only on lower concentrations of some isolates from the years 2006 and 2009. Despite the variation in occurrence of propamocarb-tolerant or propamocarb-resistant strains, the recent efficacy of propamocarb is still high for the field control of the disease.

3.2.3 Dimethomorph

Frequency of sensitive/tolerant/resistant strains to dimethomorph varied significantly during six-years period of study (Fig. 1). Till the year 2007, when the majority of isolates showed resistance or tolerance to the optimal concentration (450 μg a.i./ml), this fungicide was

practically ineffective. However, isolates with higher values of ED_{50} (450-1800 μg a.i./ml) predominated only in the year 2005 (Fig. 2). Sensitive/tolerant responses prevailed on recommended concentration from 2008 to 2009 and ED_{50} values were frequently lower than 112.5 μg a.i./ml (75% in 2007, 100% in 2008, 73% in 2009 and 100% in 2010). Surprisingly in 2010, the number of isolates expressed sensitive reaction reached nearly 100% on all tested concentrations. From 2005 to 2010, temporal shift towards higher sensitivity on all screened concentrations was recorded in Czech *P. cubensis* populations.

3.2.4 Cymoxanil

Except the year 2009, the majority of isolates were resistant to the recommended concentration of cymoxanil (120 μg a.i./ml), and also to lower concentrations (30 and 60 μg a.i./ml) during 2005-2010 (Fig. 3). According to this fact, highly resistant strains being characterized with ED_{50} values higher than 120 μg a.i./ml prevailed in these years (Fig. 2). From the year 2006 to the year 2008, *P. cubensis* populations were highly resistant to concentration 240 μg a.i./ml and also to lower tested concentrations and isolates which belonged to the most resistant category (ED_{50} values higher than 480 μg a.i./ml) were detected during this three-years period of study. In the year 2009, cymoxanil showed increasing efficacy when resistant strains prevailed only on lower tested concentrations and the majority of the populations was controlled by the recommended concentration. ED_{50} values of the majority isolates (91%) were lower than 120 μg a.i./ml in 2009. Sensitive reactions predominated only on concentrations 2x and 4x higher than optimal (240 and 480 μg a.i./ml) in the years 2005, 2009 and 2010. Cymoxanil showed very low efficacy during studied period (except the year 2009).

3.2.5 Metalaxyl

Metalaxyl showed to be ineffective in the years 2005-2010, practically all of the screened isolates expressed resistance even to the optimum concentration (200 μg a.i./ml) and the highest proportion of isolates with ED_{50} values between 200-800 μg a.i./ml was noted during this six-years period of study. (Fig 2 and 4). In the years 2007 and 2008, the majority of screened isolates was also resistant to concentration 400 μg a.i./ml (2x higher than optimal). However, the isolates which belonged to the most resistant category (ED_{50} values higher than 800 μg a.i./ml) were not detected during this two-years screened period. In the period 2005-2009, sensitive response appeared markedly on concentrations 400 and 800 μg a.i./ml and it was also observed in all concentrations in 2010. ED_{50} values lower than 50 μg a.i./ml were

detected only in the year 2010. Isolates with ED_{50} values between 50-200 μg a.i./ml were noted during all studied period, but they prevailed only in the year 2010. Monitored *P. cubensis* populations were characterized by a high level of resistance to metalaxyl and remained ineffective for disease control.

3.2.6 Metalaxyl-M

The majority of screened isolates (61%) belonged to the group of highly resistant strains with resistant reaction at the recommended concentration (100 μg a.i./ml) of metalaxyl-M (Fig. 5). Isolates with higher values of ED_{50} (100-400 μg a.i./ml) predominated in the period 2006-2009 (Fig. 2). And during this four-years period, *P. cubensis* isolates showed extremely high resistance (resistance exhibited on all tested concentrations of the fungicide) were detected. However, the most resistant category of isolates with ED_{50} values higher than 400 μg a.i./ml was determined only in the years 2007 and 2009. Proportion of responses of screened *P. cubensis* isolates to metalaxyl-M showed to be more heterogenous as compared with metalaxyl. Occurrence of sensitive/tolerant strains on all screened concentrations and isolates with ED_{50} values lower than 25 μg a.i./ml was observed only in the years 2005, 2008 and 2010. In the year 2010, metalaxyl-M showed similar increasing efficacy as metalaxyl in 2010. Sensitive strains prevailed on optimal (100 μg a.i./ml) and higher concentrations (200 and 400 μg a.i./ml). In addition, 94% of isolates expressed ED_{50} values between 25-100 μg a.i./ml. This phenomenon was not observed in previous years (2005-2009). Despite the increasing occurrence of metalaxyl-M-sensitive/tolerant strains, the recent efficacy of metalaxyl-M is still low for the field control of the disease, as well as in the case of metalaxyl.

3.3 Detailed characterization of *P. cubensis* isolates originated from *Cucurbita* spp. and *Citrullus lanatus*

P. cubensis isolates collected from *Cucurbita maxima, Cucurbita pepo, Cucurbita moschata*, *Cucurbita ficifolia* and *Citrullus lanatus* were also used for fungicide resistance/tolerance screening. (Table 7). Although, majority of these tested isolates (62%) showed sensitive responses on recommended concentration of all screened fungicides, significant differences in efficacy of tested fungicides among these isolates were detected. Whereas cymoxanil and metalaxyl found to be ineffective, *P. cubensis* isolates appeared to be sensitive to metalaxyl-M, dimethomorph, propamocarb and fosetyl-Al.

These *P. cubensis* isolates showed tolerant or resistant response to the recommended and lower concentrations of cymoxanil (30, 60, and 120 μg a.i./ml). 62.5% of isolates was

highly resistant with ED_{50} values between 120-480 µg a.i./ml. Metalaxyl-resistant and tolerant strains showed similar proportion as cymoxanil on recommended and lower concentrations (50, 100 and 200 µg a.i./ml), and ED_{50} values were recorded between 200-400 µg a.i./ml. However some sensitive strains with ED_{50} values were lower than 50 µg a.i./ml were also detected on this concentrations.

Sensitive response to metalaxyl-M and dimethomorph predominated on recommended concentrations at these tested fungicides. Nevertheless, differences in sensitivity were found between these two fungicides on lower concentrations. Whereas sensitive response to dimethomorph prevailed above tolerant, and ED_{50} values were lower than 112.5 µg a.i./ml, most of *P. cubensis* isolates (62.5%) showed resistant/tolerant reactions to metalaxyl-M and ED_{50} values expressed broader range (25-100 µg a.i./ml).

Fosetyl-Al and propamocarb were found effective. All screened *P. cubensis* isolates belonged to highly sensitive strains with ED_{50} values lower than 400 µg a.i./ml for fosetyl-Al and 607 µg a.i./ml for propamocarb. Unfortunately, some sensitive strains with limited sporulation (25%) and limited sporulation (tolerant reaction, 25%) on lowest concentration of propamocarb (607 µg a.i./ml) appeared.

There were noted significant differences in responses of *P. cubensis* isolates collected from other infected cucurbit crops (*Cucurbita spp., Citrullus lanatus*) to screened fungicides. The most sensitive to fungicides were *P. cubensis* isolates originated from *Cucurbita moschata* and *Cucurbita ficifolia*, on contrary to the lowest efficacy to fungicides of *P. cubensis* isolates from *Citrullus lanatus* and *Cucurbita maxima*.

4. DISCUSSION

Chemical control still constitutes the predominant part of the control measures used against oomycetes (Cohen and Coffey, 1986; Lebeda and Schwinn, 1994; Gisi, 2002; Gisi and Sierotzki, 2008). Unfortunately, there are some reports about failures in their effectivity against *P. cubensis* and a rapid increase of fungicide-resistant subpopulations of a pathogen, especially under selection pressure from different fungicides (Gisi, 2002; Gisi et al., 2007a,b; Lebeda and Cohen, 2012; Urban and Lebeda, 2004a,b, 2006, 2007; Savory et al., 2011). This phenomenon is known mostly from countries outside Europe (Australia, Israel, Japan, Taiwan, USA), only a few data are available for European countries (Greece, Italy, Poland, Russia), the majority of them from 1980s and 1990s (Lebeda and Cohen, 2012; Urban and Lebeda, 2004a,b, 2006, 2007) with exception of investigations in Poland /data from 1997-1998/ (Robak, 2001). Furthermore, the mechanisms of pathogen's resistance to fungicides are

not well known, with the exception of some groups of fungicides, namely strobilurins (Takeda et al., 1999; Ishii et al., 2002) and recently CAA fungicides (Blum et al, 2011; Gisi et al., 2007a,b, Gisi and Sierotzki, 2008; Zhu et al., 2008). Our research on this field presented here showed significant differences among tested fungicides and even during the six-year period of study (2005-2010). Our results of screened fungicides could be divided into three groups: propamocarb and fosetyl-Al, which are still effective in disease management; metalaxyl, metalaxyl-M and cymoxanil, towards which a high resistance has developed; and dimethomorph, towards which a shift to higher sensitivity has occurred. As well as they verified our previous experiments in some fungicides (fosetyl-Al, propamocarb, methalaxyl) from 2001-2004 (Urban and Lebeda, 2004a,b, 2006, 2007) from the Czech Republic and confirm the trend noted in *P. cubensis* populations in the whole Central Europe. The issue is that the decreased sensitivity or temporal shift to higher level of resistance to common used fungicides has been often observed in pathogen populations. Some preliminary studies have already been published about this fungicides (fosetyl-Al, propamocarb, methalaxyl) from 2005 to 2007, as well as about new groups of fungicides (cymoxanil, dimethomorph, metalaxyl-M) (Hübschová and Lebeda, 2010). Nevertheless, this paper is the first comprehensive composed contribution about resistance/tolerance of 142 Czech *P. cubensis* isolates from 2005-2010 to six commonly used and registered fungicides (except Ridomil Plus 48 WP that served as control fungicide, for details see section Material and Methods) for chemical protection of cucurbit plants against *P. cubensis* in the Czech Republic. Majority of isolates originated from *C. sativus* and only eight isolates from new hosts (*Cucurbita* spp. and *Citrullus lanatus* (Table 1,2). The frequency of sensitive/tolerant/resistant isolates to all tested concentrations of individual fungicides was investigated.

Fosetyl-Al

Despite the fact that fosetyl-Al appeared to be the most effective control agent for Czech *P. cubensis* populations, data in this paper demonstrate the risk of potential selection of more fosetyl-Al insensitive strains. Sensitive reaction with limited sporulation on optimal and lower concentrations was recorded in *P. cubensis* populations in 2009 and 2010. This phenomenon was also recorded during the previous three-years period of study /2001-2003/ (Lebeda and Urban, 2007; Urban and Lebeda, 2004a,b, 2006, 2007). Even a high proportion of isolates had a certain level of resistance/tolerance to fosetyl-Al in 2001. However, this type of insensitivity was not observed in the following years in the Czech Republic. The nature of *P. cubensis* populations could be one of the possible explanations for the extinction and restoration of *P.*

cubensis strains with increased insensitivity to the fosetyl-Al. The structure (genetic, pathogenicity and fungicides resistance) of Czech pathogen populations is strongly affected by its localization on the edge of *P. cubensis* distribution area, however, by location in Central Europe (Lebeda, 1990) and is annually renovated with the primary inoculum as nature of source areas. Because overwintering through oospores is very rare in *P. cubensis* populations and it has been observed not yet in many countries with *P. cubensis* occurrence (Cohen and Rubin, 2011; Lebeda and Schwinn, 1994). Reduced fitness of the resistant subpopulations compared with the wild-type population could be another possible explanations for the extinction and restoration of *P. cubensis* strains with increased insensitivity to the fosetyl-Al (Lebeda and Cohen, 2012; Urban and Lebeda, 2007). Occurrence of fosetyl-Al resistant *P. cubensis* strains is very rarely reported (Gisi and Sierotzki, 2008) and has been noted only in Israel (Cohen and Samoucha, 1984) and China (Sun, 1996; Wang et al., 1996a,b). Resistance to fosetyl-Al is also known in some other oomycete fungi, e.g. *Phytophthora infestans* in Israel (Cohen and Samoucha, 1984), *Phytophthora cinnamoni* in France (Vegh et al., 1985) or *Bremia lactucae* in California (Brown et al., 2004). However, according to Gisi and Sierotzki (2008) resistant isolates in field populations have never been detected till this time.

Propamocarb

During our six-years period of study, there was noted a detectable shift to higher insensitivity, especially on suboptimal propamocarb concentrations in the *P. cubensis* populations (mainly in 2006 and 2009). Our obtained recent data also confirmed previous shift, when the sensitive isolates with limited sporulation (S_{sp+}) on the lower and optimal concentrations of the fungicide have been detected firstly since the year 2001 and when the strains with tolerant reaction (T) on these concentrations appeared in 2004 for the first time (Urban and Lebeda, 2004a,b, 2006, 2007). Even this fact, propamocarb is still effective against cucurbit downy mildew in field conditions in the Czech Republic. The fact that *P. cubensis* strains with increased insensitivity to propamocarb are persistently incorporated into the populations (probably via mutation, migration, gene flow) could be the reason for their repeatedly reported occurrence in the Czech Republic during the period 2001-2009 (Drenth and Goodwinn, 1999; Urban and Lebeda, 2004a,b, 2006, 2007, McDonald and Linde, 2002). The occurrence of propamocarb-resistant strains is world-wide reported very rarely and was described in *P. cubensis* only in Israel (Cohen and Samoucha, 1984). Field resistance to propamocarb has been detected in *Pythium* species (Gisi and Sierotzki, 2008).

Dimethomorph

In Czech *P. cubensis* populations during the years 2005 - 2010, there was recorded the temporal shift towards higher sensitivity to dimethomorph on all screened concentrations. Dimethomorph was discovered as a specific Oomycete fungicide in the early 1980s (Gisi et al., 2007a,b) and has been widely used for oomycete disease control and could replace other fungicides to manage resistance of this pathogen (Wang et al., 2009; Zhu et al., 2007). Recently, Blum et al. (2011) reported some information about the mechanisms of *P. cubensis* resistance to fungicides. The four putative CeA genes were identified in *P. cubensis*. The CesA3gene of CAA-resistant and CAA-sensitive isolates was sequenced, and mutations were identified at position 1105 of this gene, conferring CAA resistance. CAA fungicides fully control sensitive *P. cubensis* populations. In *P. cubensis*, dimethomorph-resistant isolates have been recently found in a few trial site locations, in South Korea, Israel, USA (www.frac.info) and China (Zhu et al., 2007). However, CAA-resistant isolates have been detected in some other oomycete fungi, e.g. in *Plasmopara viticola* populations firstly for more than 15 years (Olaya et al., 2009) and have been reported repeatedly mainly from some of the grape growing regions in France and Germany, but with reported no serious product failures (Gisi et al., 2007b; Chabane et al., 1996). Gisi et al. (2007a,b) and also Zhu et al. (2008) mentioned the fact that because CAAs express different intrinsic activities, resistance factors can vary significantly. The segregation of pattern suggests that resistance to CAA may be controlled by more than one (probably two) recessive nuclear genes. And hence resistance is expressed only in homozygous offspring, which may require several cycles of sexual reproduction to become fixed and expressed in phenotypically aggressive isolates. As well as Blum et al. (2010, 2011) reported recently that the resistance mechanism in *P. viticola* has been elucidated and linked to a single point mutation in the CesA3 gene. According to the observations by Gisi and Sierotzki (2008), CAA-resistant isolates of *P. viticola* may be less fit in the absence of selection pressure than sensitive isolates. Therefore, the risk and extent of resistance in *P. viticola* is moderate for CAA fungicides. Nevertheless no dimethomorph-resistant isolates have been detected in other oomycete fungi, *Phytophthora infestans,* even the fact that CAA fungicides (dimethomorph) have been used commercially for more than 10 years against *P. infestans* (Cohen et al., 2007; Gisi and Sierotzki, 2008). Also, enforced selection experiments and mutagenesis did not yield isolates in *P. infestans* with stable resistance to CAAs (Bagirova et al., 2001; Stein and Kirk, 2004; Yuan et al., 2006; Cohen et al., 2007; Rubin et al., 2008). Cross-resistance among all CAAs was reported (Gisi et al., 2007a,b). However, isolates were found at low frequency which showed resistance only to

iprovalicarb but not to dimethomorph and *vice versa*. Nevertheless no cross resistance was found between CAAs and other modes of action such as phenylamides, QoI fungicides and zoxamide (Gisi et al., 2007b).

Cymoxanil

Data in this paper show a high level of resistance of the monitored *P. cubensis* populations to cymoxanil during the six-years period of study. Although, some cymoxanil-sensitive strains were found on optimal (120 µg a.i./ml) and higher concentrations (240 and 480 µg a.i./ml) in 2009 and 2010. Therefore cymoxanil showed very low efficacy for disease control in the Czech Republic during the years 2005-2010. Unfortunately, data are lacking about this phenomenon from Czech Republic till the year 2004, because screening of cymoxanil resistance/tolerance in Czech cucurbit downy mildew populations has not been investigated until the year 2004. As well as also from other countries there is available only limited information about efficacy of cymoxanil that originated from Israel (Cohen, 1986; Cohen and Grinberger, 1987; Cohen et al., 1985; Samoucha and Cohen, 1988a, b) and Poland (Robak, 2001). In Israel, Cohen and Grinberger (1987) reported that the cymoxanil dose required to achieve 90% control of the disease (ED_{90}) ranged between 197-647 and 201-878 µg a.i./ml for metalaxyl-sensitive and metalaxyl-resistant isolates. Samoucha and Cohen (1988a,b) discovered that fungicidal mixtures, especially those containing cymoxanil, were highly synergistic in controlling downy mildew in intact cucumber plants. This phenomenon was also verified by Robak (2001) in Poland who reported that the tank mixture of two fungicide products, Curzate M 72,5WP (cymoxanil/mancozeb) and Bravo Plus 500 SC (chlorothalonil/zinc) has belonged among the most effective products for disease control in Poland during the two-years field investigations (1997-1998). Cymoxanil was introduced 30 years ago and has been used also for control to other Oomycete fungi, mainly *Phytophthora infestans* and *Plasmopara viticola* in many European countries and as well as worldwide (Gisi and Sierotzki, 2008; /this citation related to *P. viticola/*; Hamlen and Power, 1998; Pérez et al., 2009; Power et al., 1995; Samoucha and Cohen, 1989 /*P. infestans/*). For more than 15 years, there was no or little evidence for resistance to cymoxanil in *P. infestans* populations in some European countries (Pérez et al., 2009; Power et al., 1995; Reis et al., 2005; Sujkowski et al., 1995) that was also verified by Hamlen and Power (1998) with results of *in vitro* and *in vivo* study of distribution of sensitivity responses to cymoxanil within global populations of *P. infestans.* These facts could be supported by the multi-site mechanism of action of cymoxanil-based fungicides, use of this fungicide in mixture with fungicides from other different

chemical classes and by no evidence of field control failures of *P. infestans* (Hamlen and Power, 1998) Nevertheless recently, Grünwald et al. (2006) demonstrated directional selection for resistance to cymoxanil after repeated field applications of the compound and indicated the potential for resistance within *P. infestans* in Mexico, the putative center of origin of this pathogen. This phenomenon was also reported in field populations of *Plasmopara viticola* in several vineyards of Italy and France where reduced sensitive (or resistant) *P. viticola* isolates have been occurred (Gisi and Sierotzki, 2008; Guillino et al., 1997; Genet and Vincent, 1999; Klinkenberg et al., 1998).

Metalaxyl, Metalaxyl-M

Data in this paper clearly demonstrated that monitored *P. cubensis* populations showed a high level of resistance to metalaxyl and remained ineffective for disease control and correspond with our previous published results from Czech Republic during the years 2001-2004 (Urban and Lebeda, 2004a,b, 2006, 2007). However, different situation has been found in Czech *P. cubensis* populations until the year 2000 where the metalaxyl-sensitive strains have predominated probably (Ackermann, 1990), although the existence of some resistant strains was proved already for 1995 (Urban and Lebeda, 2006). Our results related to metalaxyl-M showed that the recent efficacy of metalaxyl-M is still low for the field control of the disease despite the increasing occurrence of metalaxyl-M-sensitive/tolerant strains in pathogen populations. However previous experimental data about resistance/tolerance of Czech *P. cubensis* populations to metalaxyl-M is not available till the year 2004, therefore our results related to metalaxyl-M are for the first time presented in details here. Due to the continuous and intensive usage of metalaxyl and other fungicides of the same cross-resistance group (especially metalaxyl-M) during the monitoring period, it is impossible to compare the fitness of resistant and sensitive *P. cubensis* subpopulations without the presence of selection pressure of the fungicides (Urban and Lebeda, 2007). Nevertheless, (Cohen et al., 1983) reported that resistant (R) strains had a high competitive capacity compared to sensitive (S) ones in the absence of metalaxyl. In the later studies, Samoucha and Cohen (1984) showed that S strains increased the infectivity of R strains when mixtures of the two were inoculated into cucumber plants treated with metalaxyl. And the same authors in the next year (1985) showed that R strains diminished in sites where metalaxyl was abandoned, but they rapidly built up when it was reintroduced. Furthermore, recent data indicate that highly resistant strains probably predominate in Central Europe, where the expected great mixing of populations occurs due to migration, i.e. long-distance transport of spores (Lebeda, 1990).

However, limited information is available from surrounding countries (Austria, Germany, Slovakia), that's the main reason why the experimental confirmation is still missing. The low efficacy of metalaxyl has been reported during the field investigations only from Poland, the only one neighbor of Czech Republic (Robak, 2001). In scientific literature, occurrence of metalaxyl-resistant/tolerant strains in *P. cubensis* populations has been very often mentioned and has been found in Israel, Greece, Italy, USA, Russia and Australia (reviewed by Urban and Lebeda, 2006, Lebeda and Cohen, 2012), as well as Poland (Robak, 2001) and in Serbia (Bagi et al., 2009). This phenomenon has been also known from other Oomycete fungi (Gisi, 2002), e.g. *Phytophthora infestans* (Mukalazi et al., 2001; Pérez et al., 2009; Sujkowski et al., 1995) and *Pythium* (Wynn and Crute, 1983). There is well known the monogenic, semi-dominant nature of resistance to phenylamide fungicides. Therefore, the risk and extent of resistance in all oomycetes is classified as high for phenylamides and as moderate for CAA fungicides (Gisi et al., 2007b). Meanwile, metalaxyl as major fungicide for controlling cucurbit downy mildew has been gradually replaced by the cinnamic acids fungicide dimethomorph (Wang et al., 2009) because there is no cross-resistance between dimethomorph and phenylamides (Cohen and Samoucha, 1984).

Detailed characterization of *P. cubensis* isolates originated from *Cucurbita* spp. and *Citrullus lanatus*

Although, majority of tested *P. cubensis* isolates collected from *Cucurbita maxima, C. pepo, C. moschata*, *C. ficifolia* and *Citrullus lanatus* showed sensitive responses on recommended concentrations of all screened fungicides. However the significant differences in efficacy of tested fungicides among these isolates were found. There's no available previous data about resistance/tolerance of *P. cubensis* isolates originated from other cucurbit hosts to fungicides. That's why our results related to this phenomenon are presented for the first time in detail on this article. In Europe, severe outbreaks of cucurbit downy mildew have been reported repeatedly on cucumbers (*Cucumis sativus* L.) since approximately 1985 (Doruchowski and Lachowska-Ryk, 1992; Lebeda and Cohen, 2011). Newly (2009-2011), *P. cubensis* infections have been observed on other cucurbit plants *Cucumis melo, Citrullus lanatus, Cucurbita maxima, C. pepo, C. moschata*, *C. ficifolia* and *Lagenaria siceraria* in the Czech Republic (Lebeda et al., 2012; Pavelková et al., 2011). Such a dramatic change in the population structure of the pathogen appeared in Israel in the year 2002 (Cohen et al., 2003), USA in 2004 (Holmes et al., 2004; Colucci et al., 2006; Holmes and Ojiambo, 2009; Holmes and Thomas, 2009) and in Italy (Cappelli et al., 2003). The source/reason for the changes in

P. cubensis populations has been known not yet. Nevertheless possible reasons are mutation, migration and/or sexual recombination. That's why further detailed research of *P. cubensis* isolates originated from newly observed cucurbit hosts will be very useful and should be included also screening of resistance/tolerance of these isolates to fungicides. As well as the international collaboration will be necessary on this field in the future.

REFERENCES

1. Anonymous, FAO Method No. 30. In: FAO Plant Protect. Bull. Vol. 30/2 (1982).
2. Bagi FF, Balaž FF, Stojšin VB, Budakov DB, Sokolovski TV and Radonić BK, Susceptibility level of cucumber downy mildew (*Pseudoperonospora cubensis*) to metalaxyl. *Proceedings for Natural Sciences, Matica Srpska, Novi Sad* **116**:141-147 (2009).
3. Bagirova SF, Li AZ, Dolgova AV, Elansky SN, Shaw DS and Dyakov T, Mutants of *Phytophthora infestans* resistant to dimethomorph fungicide. *Journal of Russian Phytopathological Society* **2**:19-24 (2001).
4. Blum M, Waldner M and Gisi U, A single point mutation in the novel PvCesA3 gene confers resistance to the carboxylic acid amide fungicide mandipropamid in *Plasmopara viticola*. *Fungal Genetics and Biology* **47**:499–510 (2010).
5. Blum M, Waldner M, Olaya G, Cohen Y, Gisi U and Sierotzki H. Resistance mechanism to carboxylic acid amide fungicides in the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. *Pest Management Science*, **67**:1211–1214 (2011).
6. Brown S, Koike ST, Ochoa OE, Laemmlen F and Michelmore RW, Insensitivity to the fungicide fosetyl-aluminum in California isolates of the lettuce downy mildew pathogen, *Bremia lactucae*. *Plant Disease* **88**:502–508 (2004).
7. Cappelli C, Buonaurio R and Stravato VM, Occurrence of *Pseudoperonospora cubensis* pathotype 5 on squash in Italy. *Plant Disease*, **87**:449 (2003).
8. Chabane K, Leroux P, Maia N and Bompeix G, Resistance to dimethomorph in laboratory mutants of *Phytophthora parasitica*, in *Modern Fungicides and Antifungal Compounds*, ed. by Lyr H, Russell PE and Sisler HD. Intercept, Andover, UK, pp. 387-391 (1996).
9. Cohen Y, Downy mildew of cucurbits, in *The Downy Mildews*, ed. by Spencer DM. Academic Press, NY pp 636 (1981).
10. Cohen Y, Fungicide mixtures controlling late blight in potatoes. *Mededelingen van de Facultheit Landbouwwetenschappen Rijksuniversiteit Gent* **51/2b**:715-718 (1986).
11. Cohen Y and MD Coffey, Systemic Fungicides and the Control of Oomycetes. *Annual Review of Phytopathology* **24**:311-338 (1986).
12. Cohen Y and Grinberger M, Control of metalaxyl-resistant causal agents of late blight in potato and tomato and downy mildew in cucumber by cymoxanil. *Phytopathology* **77**:1283-1288 (1987).
13. Cohen Y, Meron I, Mor N and Zuriel S, A new pathotype of *Pseudoperonospora cubensis* causing downy mildew in cucurbits in Israel. *Phytoparasitica* **31**:458-466 (2003).
14. Cohen Y and Rubin AE, Mating type and sexual reproduction of *Pseudoperonospora cubensis*, the downy mildew agent of cucurbits. *European Journal of Plant Pathology* **132**: 577–92 (2011).

15. Cohen Y and Samoucha Y, Cross-resistance to four systemic fungicides in metalaxyl-resistant strains of *Phytophthora infestans* and *Pseudoperonospora cubensis*. Plant *Disease* **68**:137–139 (1984).
16. Cohen Y, Reuveni M, and Samoucha Y, Competition between metalaxyl-resistant and sensitive strains of *Pseudoperonospora cubensis* on cucumber plants. *Phytopathology* **73**:1516-1520 (1983).
17. Cohen Y, Eyal H and Sheinboim Y, Efficacy of cymoxanil in controlling metalaxyl-resistant isolates of *Phytophthora infestans* and *Pseudoperonospora cubensis*, in *Fungicides for Crop Protection, 100 Years of Progress, Monogr. 31*, ed. by Smith M. British Crop Protection Council, Publ., Croydon, England, pp. 307-310 (1985).
18. Cohen, Y, Rubin, E, Hadad, T, Gotlieb, D, Sierotzki, H and Gisi, U Sensitivity of *Phytophthora infestans* to mandipropamid and the effect of enforced selection pressure in the field. *Plant Pathology* **56**:836-842 (2007).
19. Colucci SJ, Wehner TC and Holmes GJ, The downy mildew epidemic of 2004 and 2005 in the eastern United States, in *Proceedings of Cucurbitaceae 2006*, ed. by Holmes GJ. Universal Press, Raleigh, North Carolina, USA, pp. 403-411 (2006).
20. Doruchowski R and Lakowska-Ryk E, Inheritance of resistance to downy mildew (*Pseudoperonospora cubensis* Berk. & Court.) in *Cucumis sativus*, in *Proceedings of Cucurbitaceae 1992*, the 5 th EUCARPIA Cucurbitaceae Symposium, ed. by Doruchowsky RW, Kozik E, and Niemirowicz-Szczytt K. Warsaw, Poland, pp. 66-69 (1992).
21. Drenth A and Goodwin SB, Population structure of oomycetes, in *Structure and Dynamics of Fungal Populations*, ed by Worrall JJ. Dordrecht, Kluwer Academic Publishers, pp. 195–224, (1999).
22. Fernández-Ortuño D, Torés JA, de Vincente A and Pérez-García A, Mechanisms of resistance to Qol fungicides in phytopathogenic fungi. *International Microbiology* **11**:1-9 (2008).
23. Gisi U, Chemical control of downy mildews, in *Advances in Downy Mildew Research*, ed. by Spencer-Phillips PTN, Gisi U and Lebeda A. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 119-159 (2002).
24. Gisi U, Waldner M, Kraus N, Dubuis PH and Sierotzki H, Inheritance of resistance to carboxylic acid amide (CAA) fungicides in *Plasmopara viticola*. *Plant Pathology* **56**:199–208 (2007a).
25. Gisi U, Lamberth C, Mehl A, Seitz T, Carboxylic Acid Amide (CAA) fungicides, in *Modern Crop Protection Compounds*, ed. by Krämer W and Schirmer U. Wiley-VCH, Weinheim, Germany, pp. 651-674 (2007b).
26. Gisi U and Sierotzki H, Fungicide modes of action and resistance in downy mildews. *European Journal of Plant Patology* **122**:157-167 (2008).
27. Genet JL and Vincent O, Sensitivity of European *Plasmopara viticola* populations to cymoxanil. *Pesticide Science* **55**:129–136 (1999).
28. Grünwald NJ, Sturbaum AK, Montes GR, Serrano EG, Lozoya-Saldaña H and Fry WE, Selection for fungicide resistance within a growing season in field populations of *Phytophthora infestans* at the center of origin. *Phytopathology* **96**:1397-1402 (2006).
29. Gullino ML, Mescalchin E and Mezzalama M, Sensitivity to cymoxanil in populations of *Plasmopara viticola* in northern Italy, *Plant Pathology* **46**:729-736 (1997).
30. Hamlen RA and Power RJ, Distribution of sensitivity responses to cymoxanil within global populations of *Phytophthora infestans*. *Pesticide Science* **53**:101-103 (1998)
31. Heaney SP, Hall AA, Davis SA and Olaya G, Resistance to fungicides in the QoI-STAR cross resistance group: current perspectives, in *Proceedings Brighton Crop Protection Konference*. BCPC Publications, Croydon, UK, pp. 755-762 (2000).

32. Holmes GJ and Ojiambo P, Chemical control of cucurbit downy mildew: a summary of field experiments in the U.S. *Phytopathology* **99**:171 (2009).
33. Holmes GJ and Thomas C, The history and re-emergence of cucurbit downy mildew. *Phytopathology* **99**:171 (2009).
34. Holmes GJ, Main CE and Keever III ZT, Cucurbit downy mildew: A unique pathosystem for disease forecasting, in *Advances in Downy Mildew Research*, ed. by Spencer-Phillips PTN and Jeger M. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 69-80 (2004).
35. Hübschová J and Lebeda A, Fungicide effectiveness on Czech populations of *Pseudoperonospora cubensis*, in *The 4th International Cucurbitaceae Symposium*, September 20-24, 2009, Changsha, Hunan, China, Abstracts. Published by Hunan Agricultural University and International Society for Horticultural Science (ISHS), Changsha, Hunan, China, p. 116 (2009).
36. Ishii H, Sugiyama T, Nishimura K and Ishikawa Y, Strobilurin resistance in cucumber pathogens: persistence and molecular diagnosis of resistance, in *Modern Fungicides and Antifungal Compounds III*, ed. by Dehne HW, Gisi U and Kuck KH. AgroConcept, Bonn, Germany, pp. 149–159 (2002).
37. Iwata Y, Specialization in *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. IV. Studies on the fungus from Oriental pickling melon (*Cucumis melo* var. *conomon* Makino). *Bulletin of the Faculty of Agriculture*, Mie University 6:30-35 (1953a).
38. Iwata Y, Specialization in *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. V. on the fungus from Calabash gourd *Lagenaria vulgaris* Ser. var. *clavata* Ser. *Bulletin of the Faculty of Agriculture*, Mie University 6:32-36 (1953b).
39. Iwata Y, Specialization of *Pseudoperonospora cubensis* (Berk. et Curt.) Rostow. II. Comparative studies of the morphologies of the fungi from *Cucumis sativus* L. and *Cucurbita moschata* Duchesne. *Annals of the Phytopathological Society of Japan* **11**: 172-185 (1942).
40. Klinkenberg HJ, Stierl R and Dehne HW, Investigations on fungicide resistance in oomycetes. *Mededelingen van de Faculteit Landbouwwetenschappen Universiteit Gent*, **63(3b)**:1009–1015 (1998)
41. Kužma, Š. (ed.). Seznam registrovaných přípravků na ochranu rostlin 2004 (List of the Registered Plant Protection Products 2004). Státní rostlinolékařská správa (The State Phytosanitary Administration), Praha, Czech Republic (2004). (in Czech, with English summary).
42. Kužma, Š. (ed.). Seznam registrovaných přípravků na ochranu rostlin 2005 (List of the Registered Plant Protection Products 2005). Státní rostlinolékařská správa (The State Phytosanitary Administration), Praha, Czech Republic (2005). (in Czech, with English summary)
43. Lebeda A, *Pseudoperonospora cubensis*, in *Methods of Testing Vegetable Crops for Resistance to Plant Pathogens*, ed. by Lebeda A. VHJ Sempra, Research Institute of Vegetable Crops, Olomouc, Czech Republic, pp.81-85 (1986).
44. Lebeda A, Biology and ecology of cucurbit downy mildew, in *Cucurbit downy mildew*, ed. by Lebeda A. Czechoslovak Scientific Society for Mycology by Czechoslovak Academy of Sciences, Praha pp 13-46 (1990).
45. Lebeda A, Resistance in muskmelons to Czechoslovak isolates of *Pseudoperonospora cubensis* from cucumbers. *Scientia Horticulturae* **45**:255-260 (1991).
46. Lebeda A and Cohen Y, Cucurbit downy mildew (*Pseudoperonospora cubensis*) – biology, ecology, epidemiology, host-pathogen interaction and control. *European Journal of Plant Patology* **129**:157-192 (2011).

47. Lebeda A and Cohen Y, Fungicide resistance in *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew, in *Fungicide Resistance in Crop Protection: Risk and Management*, ed. by Thind TS. CAB International, pp. 45-63 (2012).
48. Lebeda A and Křístková E, Field resistance of Cucurbita species to powdery mildew (Erysiphe cichoracearum). *Journal of Plant Diseases and Plant Protection* **101**:598-603 (1994).
49. Lebeda A and Schwinn FJ, The downy mildews – an overview of recent research progress. *Journal of Plant Diseases and Plant Protection* **101**:225-254 (1994).
50. Lebeda A and Urban J, Disease impact and pathogenicity variation in Czech populations of *Pseudoperonospora cubensis*, in *Progress in Cucurbit Genetics and Breeding Research, Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA, Meeting on Cucurbit Genetics and Breeding, Palacký University in Olomouc*, ed. by Lebeda A and Paris HS. Olomouc, Czech Republic, pp. 267-273 (2004a).
51. Lebeda A and Urban J, Distribution, harmfulness and pathogenic variability of cucurbit downy mildew in the Czech Republic. *Acta fytotechnica et zootechnica* **7**:170-173 (2004b).
52. Lebeda A and Urban J, Temporal changes in pathogenicity and fungicide resistance in *Pseudoperonospora cubensis* populations. *Acta Horticulturae* **731**: 327-336 (2007).
53. Lebeda A and Widrlechner MP, A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *Journal of Plant Diseases and Plant Protection* **110**:337-349 (2003).
54. Lebeda A, Widrlechner MP, Response of wild and weedy *Cucurbita* L. to pathotypes of *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov. (cucurbit downy mildew), in *Advances in Downy Mildew Research*, ed. by Spencer-Phillips PTN and Jeger M. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 203-210 (2004).
55. Lebeda A, Widrlechner MP and Urban J, Individual and population aspects of interactions between cucurbits and *Pseudoperonospora cubensis*: pathotypes and races, in *Proceedings of Cucurbitaceae 2006*, ed. by Holmes GJ. Universal Press, Raleigh, North Carolina, USA, pp. 453-467 (2006).
56. Lebeda A, Hübschová J and Urban J, Temporal population dynamics of *Pseudoperonospora cubensis*, in *Cucurbitaceae 2010 Proceedings, American Society for Horticultural Science*, ed. by Thies JA, Kousik S and Levi A. Alexandria, Virginia, USA, pp. 240-243 (2010).
57. Lebeda A, Pavelková J, Urban J and Sedláková B, Distribution, host range and disease severity of *Pseudoperonospora cubensis* on cucurbits in the Czech Republic. *Journal of Phytopathology* **159**:589-596 (2011).
58. Lebeda A, Pavelková J, Sedláková B and Urban J, Structure and temporal shifts in virulence of *Pseudoperonospora cubensis* populations in the Czech Republic. *Plant Pathology* 2012 (in press) (2012).
59. Lebeda A., Sedláková, B. and Pavelková J, New hosts of *Pseudoperonospora cubensis* in the Czech Republic and pathogen virulence variation. *Acta Horticulturae*.(in press) (2012).
60. McDonald BA and Linde C, Pathogen population genetics, evolutionary potential and durable resistance. *Annual Review of Phytopathology* **40**:349-379 (2002).
61. Minář P (ed.), Seznam registrovaných přípravků na ochranu rostlin 2006 (List of the Registered Plant Protection Products 2006). Státní rostlinolékařská správa (The State Phytosanitary Administration), Praha, Czech Republic (2006). (in Czech, with English summary)
62. Minář P (ed.), Seznam registrovaných přípravků na ochranu rostlin 2007 (List of the Registered Plant Protection Products 2007). Státní rostlinolékařská správa (The State

Phytosanitary Administration), Praha, Czech Republic (2007). (in Czech, with English summary)
63. Minář P (ed.), Seznam registrovaných přípravků na ochranu rostlin 2008 (List of the Registered Plant Protection Products 2008). Státní rostlinolékařská správa (The State Phytosanitary Administration), Praha, Czech Republic (2008). (in Czech, with English summary)
64. Minář P (ed.), Seznam registrovaných přípravků na ochranu rostlin 2009 (List of the Registered Plant Protection Products 2009). Státní rostlinolékařská správa (The State Phytosanitary Administration), Praha, Czech Republic (2009). (in Czech, with English summary)
65. Minář P (ed.), Seznam registrovaných přípravků na ochranu rostlin 2010 (List of the Registered Plant Protection Products 2010). Státní rostlinolékařská správa (The State Phytosanitary Administration), Praha, Czech Republic (2010). (in Czech, with English summary)
66. Mitani S, Araki S, Yamaguchi T, Takii Y, Ohshima T and Matsuo N, Biological properties of the novel fungicide cyazofamid against *Phytophthora infestans* on tomato and *Pseudoperonospora cubensis* on cucumber. *Pest Management Science* **58**:139-145 (2001).
67. Moorman GW and Kim SH, Species of *Pythium* from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam. *Plant Disease* **88**:630-632 (2004).
68. Mukalazi J, Adipala E, Sengooba T, Hakiza JJ, Olanya M and Kidanemariam HM, Metalaxyl resistance, mating type and pathogenicity of *Phytophthora infestans* in Uganda. *Crop Protection* **20**:379-388 (2001).
69. Olaya G, Kuhn P, Hert A, Holmes G and Colucci S, Fungicide resistance in cucurbit downy mildew. *Phytopathology* **99**:171 (2009).
70. Palti J, The significance of pronounced divergences in the distribution of *Pseudoperonospora cubensis* on its crop hosts. *Phytoparasitica* **2**:109-115 (1974).
71. Palti J and Cohen Y, Downy mildew of cucurbits (*Pseudoperonospora cubensis*). The fungus and its hosts, distribution, epidemiology, and control. *Phytoparasitica* **8**:109-147 (1980).
72. Pavelková J, Lebeda A, Sedláková, B. First Report of *Pseudoperonospora cubensis* on *Cucurbita moschata* in the Czech Republic. *Plant Disease* **95**:878-879. publ. on line DOI: 10.1094/PDIS -01-11-0055. July 2011 (2011).
73. Perchepied L, Bardin M, Dogimont C and Pitrat M, Relationship between loci conferring downy mildew and powdery mildew resistance in melon assessed by quantitative trait loci mapping. *Phytopathology* **95**:556-565 (2005).
74. Pérez W, Lara J and Forbes GA, Resistance to metalaxyl-M and cymoxanil in a dominant clonal lineage of *Phytophthora infestans* in Huánuco, Peru, an area of continuous potato production. *European Journal of Plant Pathology* **125**:87-95 (2009).
75. Power RJ, Hamlen RA and Morehart LA, Variation in sensitivity of *Phytophthora infestans* field isolates to cymoxanil, chlorothalonil and metalaxyl, in *European Association for Potato Research. Phytophthora infestans. 1845-1995*, ed. by Dowley LJ, Bannon E, Cooke LR, Keane T and O'Sullivan E, Boole Press Ltd., Dublin, Ireland, pp. 154-159 (1995).
76. Reis A, Ribeiro FHS, Maffia LA, and Mizubuti ESG, Sensitivity of Brazilian isolates of *Phytophthora infestans* to commonly used fungicides in tomato and potato crops. *Plant Disease* **89**:1279-1284 (2005).
77. Reuveni M, Eyal H and Cohen Y, Development of resistance to metalaxyl in *Pseudoperonospora cubensis*. *Plant Disease* **64**:1108-1109 (1980).

78. Robak J, An attempt at integrated control of cucumber downy mildew (*Pseudoperonospora cubensis*). *Journal of Vegetable Crop Production* **7**:21-32 (2001).
79. Rubin E, Gotlieb D, Gisi U and Cohen Y, Mutagenesis of *Phytophthora infestans* for resistance against carboxylic acid amide (CAA) and phenylamide fungicides. *Plant Disease* **92**:675-683 (2008).
80. Samoucha Y and Cohen Y, Synergistic interactions of cymoxanil mixtures in the control of metalaxyl-resistant *Phytophthora infestans* of potato. *Phytopathology* **78**:636-640 (1988a).
81. Samoucha Y and Cohen Y, Synergism in fungicide mixtures against *Pseudoperonospora cubensis*. *Phytoparasitica* **16**:337-342 (1988b).
82. Samoucha Y and Cohen Y, Field control of potato late blight by synergistic fungicidal mixtures. *Plant Disease* **73**: 751-753 (1989).
83. Savory EA, Granke LL, Quesada-Ocampo LM, Varbanova M, Hausbeck M and Day B, The cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. *Molecular Plant Pathology* **12(3)**:217-226 (2011).
84. Stein JM and Kirk WW, The generation and quantification of resistance to dimethomorph in *Phytophthora infestans*. *Plant Disease* **88**:930-934 (2004).
85. Sujkowski LS, Fry BA, Power RJ, Goodwin SB, Peever TL, Hamlen RA and Fry WE, Sensitivities of Mexican isolates of *Phytophthora infestans* to chlorothalonil, cymoxanil, and metalaxyl. *Plant Disease* **79**:1117-1120 (1995).
86. Sun XQ, Preliminary report on the sensitivity of *Pseudoperonospora cubensis* to metalaxyl and fosetyl-Al. *Plant Protection Technology and Extension* **16**:18-19. (1996) (in Chinese).
87. Takeda T, Kawagoe Y, Uchida K, Fuji M and Amano T, The appearance of resistant isolates to strobilurins. (Abstr.) *Annales Phytopathological Society of Japan* **65**:655 (1999).
88. Thomas CE and Jourdain EL, Host effect on selection of virulence factors affecting sporulation by *Pseudoperonospora cubensis. Plant Disease* **76**:905-907 (1992).
89. Tomlin CDS (ed.), The Pesticide Manual. Eleven Edition. British Crop Protection Council, Farnham, UK (1997).
90. Towsend GR and Heuberger W, Methods for estimating losses caused by diseases in fungicide experiments. *Plant Disease Reporter* **27**:340-343 (1943).
91. Urban J and Lebeda A, Differential sensitivity to fungicides in Czech populations of *Pseudoperonospora cubensis*, in *Progress in Cucurbit Genetics and Breeding Research. Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding. Palacký University in Olomouc*, ed. by Lebeda A and Paris HS. Olomouc, Czech Republic, pp. 275-280 (2004a).
92. Urban J and Lebeda A, Resistance to fungicides in population of cucurbit downy mildew in the Czech Republic. *Acta fytotechnica et zootechnica* 7:327-329 (2004b).
93. Urban J and Lebeda A, Fungicide resistance in cucurbit downy mildew – methological, biological and population aspects. *Annual Review of Phytopathology* **149**:63-75 (2006).
94. Urban J and Lebeda A, Variation for fungicide resistance in Czech populations of *Pseudoperonospora cubensis*. *Journal of Phytopathology* **155**:143-151 (2007).
95. Van Den Bosch F and Gilligan CA, Models of Fungicide Resistance Dynamics. *Annual Review of Phytopathology*, **46**:123-147 (2008).
96. Vegh I, Leruox P, LeBerre A and Lanen C. Detection sur *Chamaecyparis lawsoniana* Ellwoodii d'une souche de *Phytophthora cinnamomi* Rands re´sistante au phosethyl-Al. *PHM-Rev Hort* **262**:19–21 (1985).

97. Velichi E, Dynamics of appearance and evolution to the watermelon (*Citrullus lanatus* L.), of downy mildew [*Pseudoperonospora cubensis* (Berk. et Curt.) Rostow.], in the rainy years 2004, 2005, in Baragan field, (Braila area). *Research Journal of Agricultural Science* **41**: 345-350 (2009).
98. Wang WQ, Liu GR, Yan LE, Zhang XF, Ma ZQ, Han XY, Cross-resistance in *Pseudoperonospora cubensis* and *Plasmopara viticola* against different systemic fungicides. *Acta Phytopathologica Sinica* **23:**84-88 (in Chinese with English abstract) (1996a).
99. Wang WQ, Liu GR, Yan LE, Zhang XF, Ma ZQ, Han XY, Monitoring of resistance to three fungicides in downy mildews on cucumber and grape. *Journal of Nanjing Agricultural University* **19 (Supplement):**127-131 (in Chinese with English Abstract) (1996b)
100. Wang HC, Zhou MG, Wang JX, Chen CJ, Li HX and Sun HY, Biological mode of action of dimethomorph on *Pseudoperonospora cubensis* and its systemic activity in cucumber. *Agricultural Sciences in China* **8**:172–181 (2009).
101. Wynn EC and Crute IR, Bioassay of metalaxyl in plant tissue. *Annals of Applied Biology* **102**:117–121 (1983).
102. Yuan SK, Liu XL, Si NG, Dong J, Gu BG and Jiang H, Sensitivity of *Phytophthora infestans* to flumorph: in vitro determination of baseline sensitivity and the risk of resistance. *Plant Pathology* **55**:258-263 (2006).
103. Zhu SS, Liu XL, Wang Y, Wu XH, Liu PF, Li JQ, Yuan SK and Si NG, Resistance of *Pseudoperonospora cubensis* to flumorph on cucumber in plastic houses. *Plant Pathology* **56**: 967-975 (2007).
104. Zhu SS, Liu PF, Liu XL, Li JQ, Yuan SK and Sil NG, Assessing the risk of resistance in *Pseudoperonospora cubensis* to the fungicide flumorph *in vitro*. *Pest Management Science* **64**:255–261 (2008).

TABLES

Table 1
Origin and characterization of *Pseudoperonospora cubensis* isolates

Year	No. of isolates	Origin of isolates			No. of pathotypes
		No. of isolates in Bohemia	No. of isolates in Moravia	Host plant	
2005	25	7	18	*CS*	12
2006	29	12	17	*CS*	12
2007	39	12	27	*CS*	5
2008	32	14	18	*CS*	9
2009	44	16	28	*CS, CMe, CMo*	14
2010	37	13	24	*CS, CP, CM, CMo, CF*, *CL*	11
In total	206	74	132	-	63

CS – *Cucumis sativus,* CMe – *Cucumis melo*, CP – *Cucurbita pepo*, CM – *Cucurbita maxima*, CMo – *Cucurbita moschata*, CF- *Cucurbita ficifolia,* CL – *Citrullus lanatus*

Table 2
Origin and characterization of new hosts isolates

Host	Isolate	Origin		Pathotype
		Region/District	Location	
CP	58/10	South Moravia Hodonín	Mutěnice	15.15.3
CP	61/10	South Moravia Hodonín	Ratiškovice	15.15.11
CP	72/10	Zlín Zlín	Napajedla	15.15.3
CM	67/10	South Moravia Hodonín	Veselí nad Moravou	15.15.11
CM	81/10	Olomouc Olomouc	Olomouc-Holice	15.6.0
CMo	86/10	Moravia-Silesia Nový Jičín	Nový Jičín-Kojetín	15.15.15
CF	87/10	Olomouc Olomouc	Olomouc-Holice	X
CL	83/10	Olomouc Olomouc	Olomouc-Holice	15.15.11

CP – *Cucurbita pepo*, CM – *Cucurbita maxima*, CMo – *Cucurbita moschata*, CF- *Cucurbita ficifolia,* CL – *Citrullus lanatus*

Table 3
Characterization of the fungicides used for resistance screening (modified according to Urban and Lebeda, 2006; Gisi, 2002)

Fungicide/ Group name/ Chemical group	Mode of action – target site/ systemicity/Transportation/Translocation behavior within plants	Resistance risk
Metalaxyl, Metalaxyl-M/ Phenylamides (PA)/ acylalanines	Nucleic acids synthesis – RNA polymerase I/ Systemic with protective, curative and eradicative action/ Apoplastic symplasic, translaminar	Resistance and cross resistance well known in various Oomycetes but mechanism unknown. High risk.
Propamocarb/ Carbamates/ Carbamates	Lipid synthesis and membrane integrity – multi-site inhibitor, affect membrane permeability, fatty acids (proposed)/ Systemic with protective action/ acropettally/apoplastic	Low to medium risk.
Fosetyl-Al/ Phosphonates/ Ethyl phosphonates	Combination of direct antifungal activity (inhibits spore germination or blocks mycelium development and sporulation) and induction of host plant defense (higher-than-normal levels of phytoalexin and others metabolites in treated and infected plants) – primary and direct site of action is not elucidated/Systemic with protective and curative action/Acropetally and basipetally/Apoplastic and symplastic	Few resistance cases reported in few pathogens. Low risk.
Cymoxanil/ Cyanoacetamide-oxime/ Cyanoacetamide-oxime	Affects growth of intercellular hyphae and formation of haustoria as well as production of sporangia – primary and direct site of action is not elucidated (it has been arguet inhibition RNA and acid synthesisi (Leroux et al., 1987)/Systemic, curative action/rapidly translocated – acropetally (in the shoot and leaves, after foliar and stem application), basipetally and translaminar (within leaves)	Resistance claims described. Low to medium risk.
Dimethomorph /Carboxylic Acid Amides (CAA)/ cinnamic acid amides	Cell wall biosynthesis – cellulose synthase/ Locally systemic, preventive and curative action (strong antisporulant activity)/Translaminar and weak acropetal in leaves (but not from leaf to leaf)	Resistance known in *Plasmopara viticola* but not in *Phytophthora infestans*. Cross resistance between all members of the CAA group Low to medium risk

Table 4
Tested concentrations of the fungicides

Fungicide	Concentration of fungicide (µg a.i./ml)/concentration of source preparation* (%)				
	1	2	3**	4	5
Metalaxyl	50/0.063	100/0.125	200/0.25	400/0.5	800/1.0
Metalaxyl-M	25/0.063	50/0.125	100/0.25	200/0.5	400/1.0
Propamocarb	607/0.1	1214/0.2	2428/0.4	4856/0.8	9712/1.6
Fosetyl-Al	400/0.05	800/0.1	1600/0.2	3200/0.4	6400/0.8
Cymoxanil	30/0.075	60/0.150	120/0.300	240/0.600	480/1.200
Dimethomorph	112.5/0.125	225.0/0.250	450.0/0.500	900/1.000	1800/2.000

* Metalaxyl – source of preparation Ridomil Plus 48 WP, metalaxyl-M – s.p. Ridomil Gold MZ 68 WP, propamocarb – s.p. Previcur 607 SL, fosetyl-Al – s.p. Aliette 80 WP, cymoxanil – s.p. Curzate K, dimethomorph – s.p. Acrobat MZ
** The concentration recommended by the producer (Kužma, 2005; Minář, 2006, 2007, 2008 ,2009 and 2010)

Table 5
Response of *P. cubensis* isolates to different concentrations of fosetyl-Al

Year	Type of reaction	Fosetyl-Al concentration (µg a.i./ml)/frequency of isolates (%)				
		400	**800**	**1600***	**3200**	**6400**
2005	Ssp-	100.0	100.0	100.0	100.0	100.0
	Ssp+	0.0	0.0	0.0	0.0	0.0
	T	0.0	0.0	0.0	0.0	0.0
	R	0.0	0.0	0.0	0.0	0.0
2006	Ssp-	100.0	100.0	100.0	100.0	100.0
	Ssp+	0.0	0.0	0.0	0.0	0.0
	T	0.0	0.0	0.0	0.0	0.0
	R	0.0	0.0	0.0	0.0	0.0
2007	Ssp-	100.0	100.0	100.0	100.0	100.0
	Ssp+	0.0	0.0	0.0	0.0	0.0
	T	0.0	0.0	0.0	0.0	0.0
	R	0.0	0.0	0.0	0.0	0.0
2008	Ssp-	100.0	100.0	100.0	100.0	100.0
	Ssp+	0.0	0.0	0.0	0.0	0.0
	T	0.0	0.0	0.0	0.0	0.0
	R	0.0	0.0	0.0	0.0	0.0
2009	Ssp-	68.2	90.9	95.5	100.0	100.0
	Ssp+	**31.8**	**9.1**	**4.5**	0.0	0.0
	T	0.0	0.0	0.0	0.0	0.0
	R	0.0	0.0	0.0	0.0	0.0
2010	Ssp-	97.0	100.0	100.0	100.0	100.0
	Ssp+	**3.0**	0.0	0.0	0.0	0.0
	T	0.0	0.0	0.0	0.0	0.0
	R	0.0	0.0	0.0	0.0	0.0

Ssp- = sensitive reaction without sporulation (P = 0 %); Ssp+ = sensitive reaction with limited sporulation (0 < P ≤ 10%); T = tolerant reaction (10< P ≤35%); R = resistant reaction (P >35%)
* The concentration recommended by the producer (Kužma. 2005; Minář. 2006. 2007. 2008 .2009 and 2010)

Table 6
Response of *P. cubensis* isolates to different concentrations of propamocarb

Year	Type of reaction	Propamocarb concentration (µg a.i./ml)/frequency of isolates (%)				
		607	1214	2428*	4856	9712
2005	Ssp-	48.0	56.0	56.0	92.0	100.0
	Ssp+	**44.0**	**40.0**	**40.0**	**8.0**	0.0
	T	**8.0**	**4.0**	**4.0**	0.0	0.0
	R	0.0	0.0	0.0	0.0	0.0
2006	Ssp-	61.0	89.0	89.0	94.5	94.5
	Ssp+	**28.0**	0.0	0.0	0.0	0.0
	T	**5.5**	**5.5**	**11.0**	**5.5**	**5.5**
	R	**5.5**	**5.5**	0.0	0.0	0.0
2007	Ssp-	70.0	90.0	100.0	100.0	100.0
	Ssp+	**30.0**	**10.0**	0.0	0.0	0.0
	T	0.0	0.0	0.0	0.0	0.0
	R	0.0	0.0	0.0	0.0	0.0
2008	Ssp-	32.0	54.5	82.0	100.0	100.0
	Ssp+	**59.0**	**45.5**	**18.0**	0.0	0.0
	T	**9.0**	0.0	0.0	0.0	0.0
	R	0.0	0.0	0.0	0.0	0.0
2009	Ssp-	40.9	77.3	90.9	100.0	100.0
	Ssp+	**27.3**	**13.6**	**9.1**	0.0	0.0
	T	**18.2**	**9.1**	0.0	0.0	0.0
	R	**13.6**	0.0	0.0	0.0	0.0
2010	Ssp-	55.0	89.0	97.0	100.0	100.0
	Ssp+	**34.0**	**11.0**	**3.0**	0.0	0.0
	T	**11.0**	0.0	0.0	0.0	0.0
	R	0.0	0.0	0.0	0.0	0.0

Ssp- = sensitive reaction without sporulation (P = 0 %); Ssp+ = sensitive reaction with limited sporulation (0 < P ≤ 10%); T = tolerant reaction (10< P ≤35%); R = resistant reaction (P >35%)
* The concentration recommended by the producer (Kužma. 2005; Minář. 2006. 2007. 2008 .2009 and 2010)

Table 7
Response of new hosts *P. cubensis* populations* to different concentrations of screened fungicides in the year 2010

P	Fungicide concentration**					Fungicide/Total no. of isolates (%)					
	1	2	3***	4	5	F-Al	Pr	C	D	M	M-M
I	-	-	-	-	-	100	75	0	62.5	12.5	37.5
II	(-)	-	-	-	-	0	25	0	25	0	0
III	(-)	(-)	-	-	-	0	0	0	0	25	0
IV	(-)	(-)	(-)	-	-	0	0	12.5	12.5	0	0
V	+	(-)	-	-	-	0	0	0	0	0	37.5
VI	+	(-)	(-)	-	-	0	0	12.5	0	0	0
VII	+	+	(-)	-	-	0	0	12.5	0	0	0
VIII	+	+	+	-	-	0	0	0	0	62.5	0
IX	+	+	+	(-)	-	0	0	12.5	0	0	25
X	+	+	+	+	-	0	0	50	0	0	0

*-, sensitive reaction (no sporulation); (-), tolerant reaction (limited sporulation); +, resistant reaction (profuse sporulation); ** 1-5 fungicide concentration, see Table 3; *** concentration as recommended by the producer; P, patterns of reaction types I-X; F-Al, fosetyl-Al; Pr, propamocarb; C, cymoxanil; D, dimethomorph; M, metalaxyl; M-M, metalaxyl-M

FIGURES

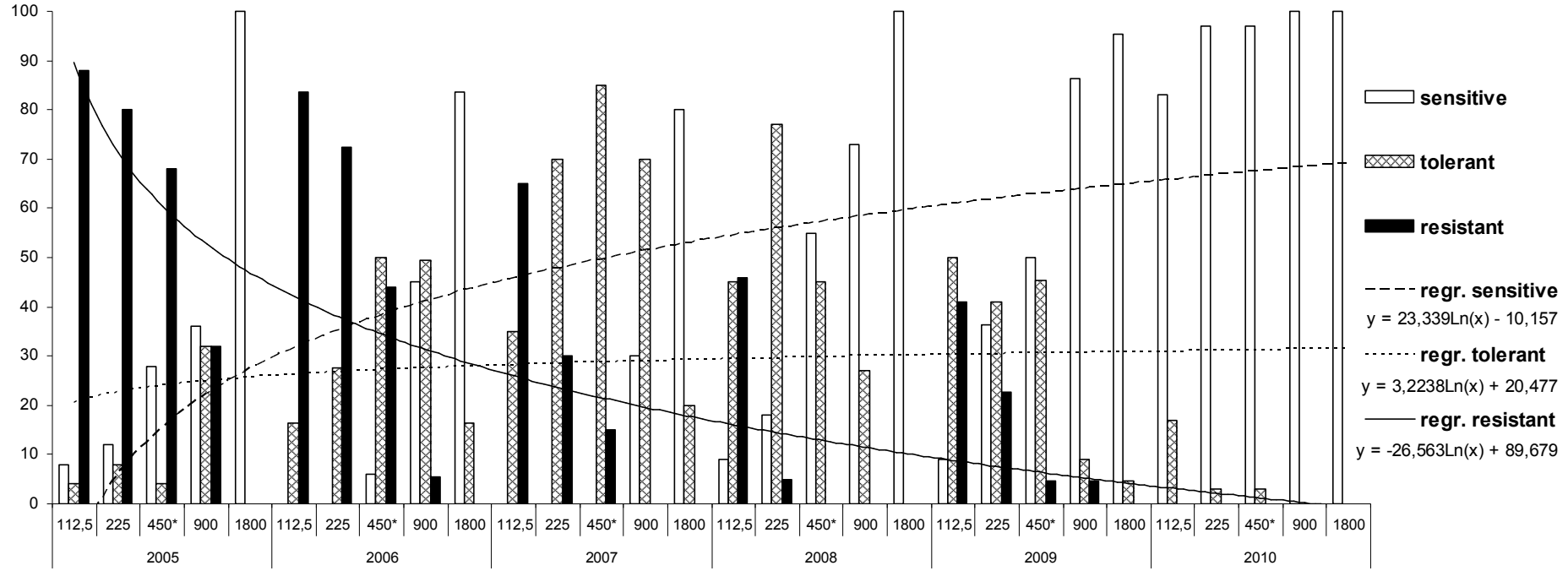


Fig. 1 Responses of *P. cubensis* population to different concentrations of dimethomorph in the period 2005-2010

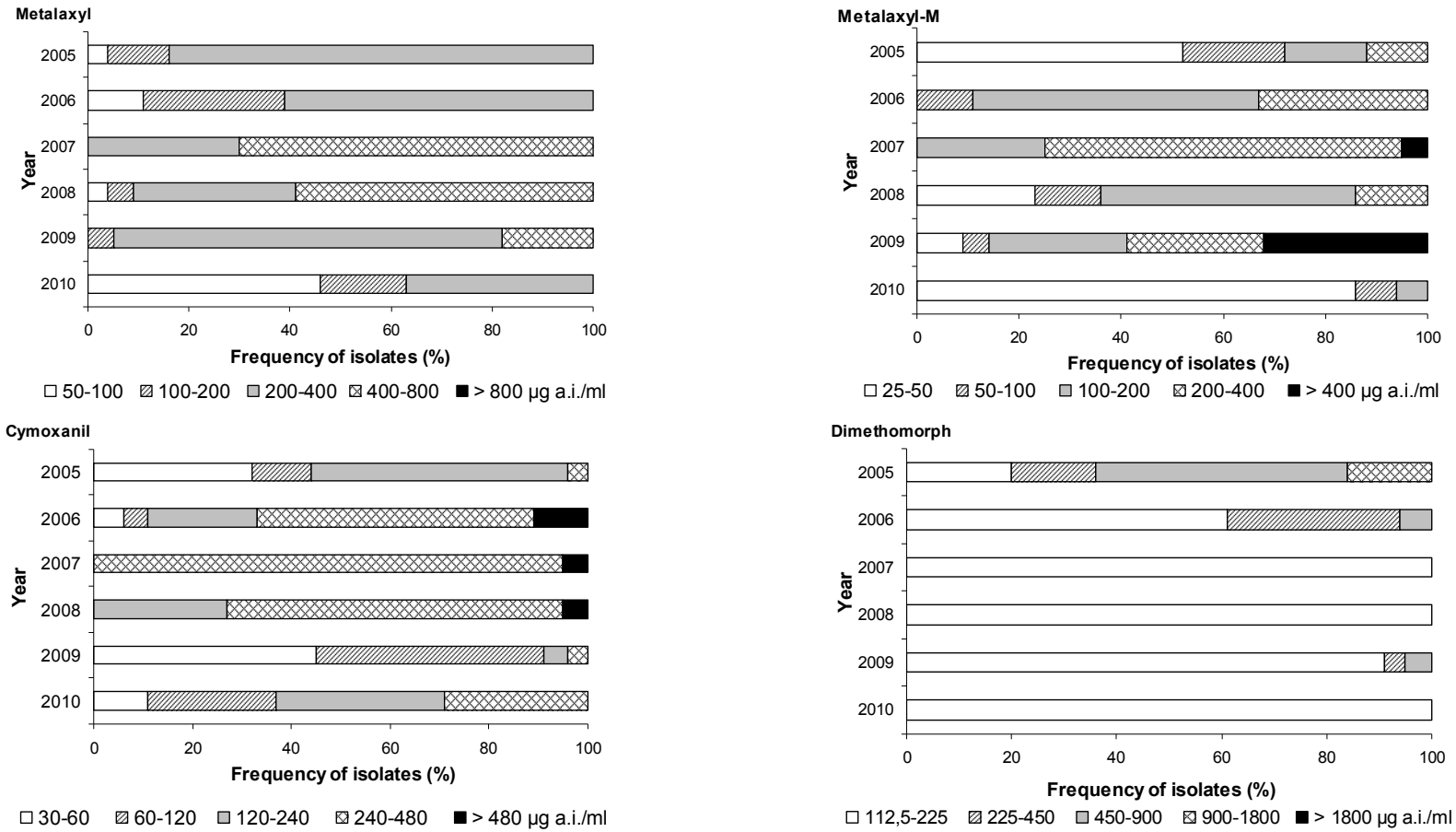


Fig. 2 Structure of *P. cubensis* populations in relation to frequency of ED_{50} values for metalaxyl. metalaxyl-M. cymoxanil and dimethomorph in 2005-2010 in the Czech Republic (Information is modified according to Lebeda and Cohen, 2012).

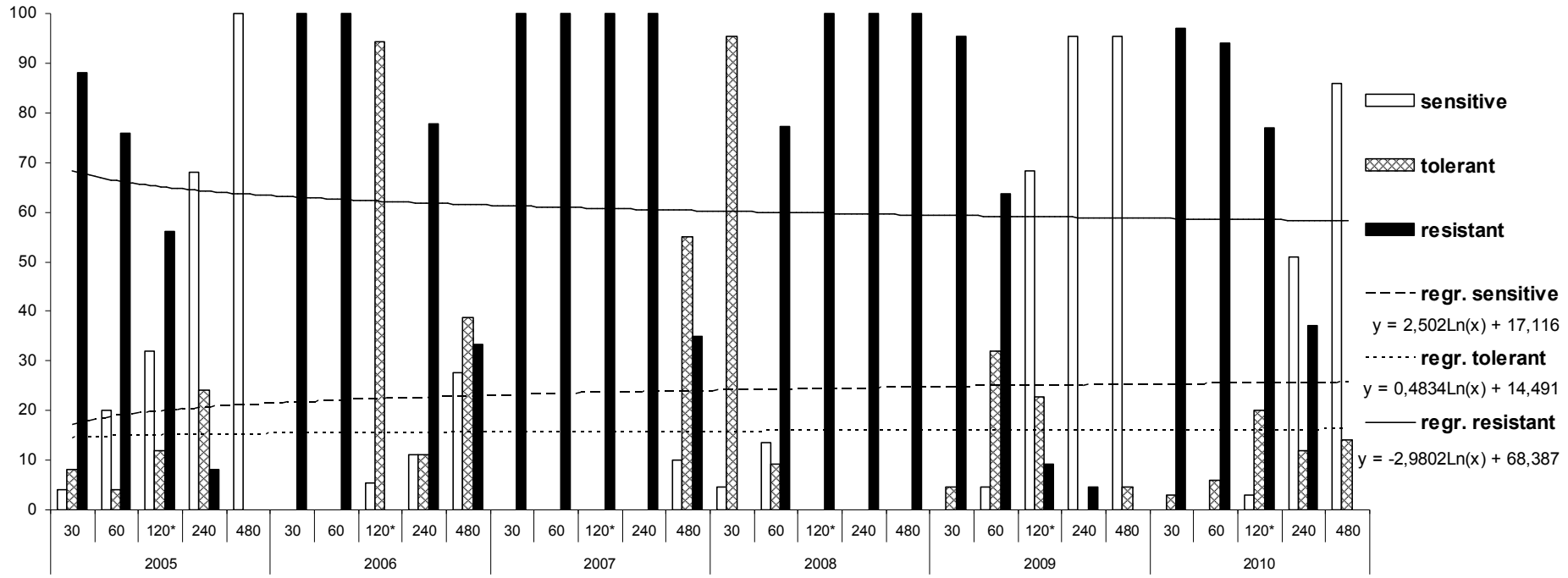


Fig. 3 Responses of *P. cubensis* population to different concentrations of cymoxanil in the period 2005-2010

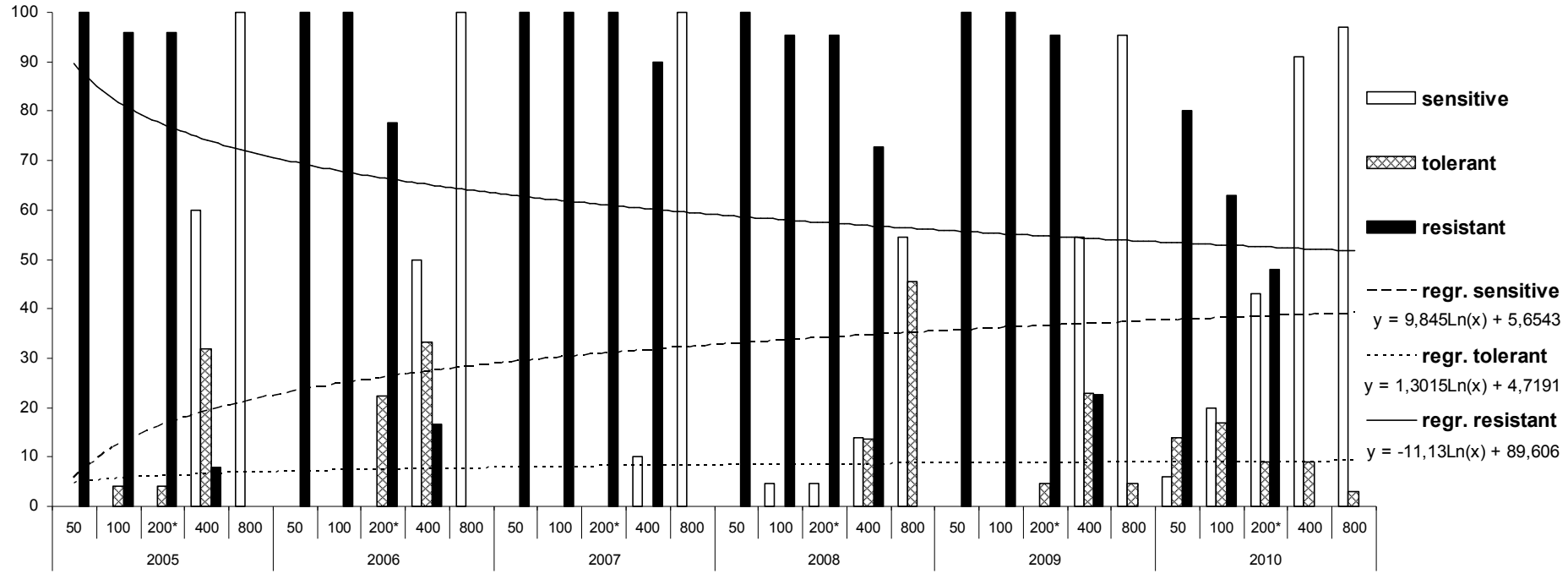


Fig. 4 Responses of *P. cubensis* population to different concentrations of metalaxyl in the period 2005-2010

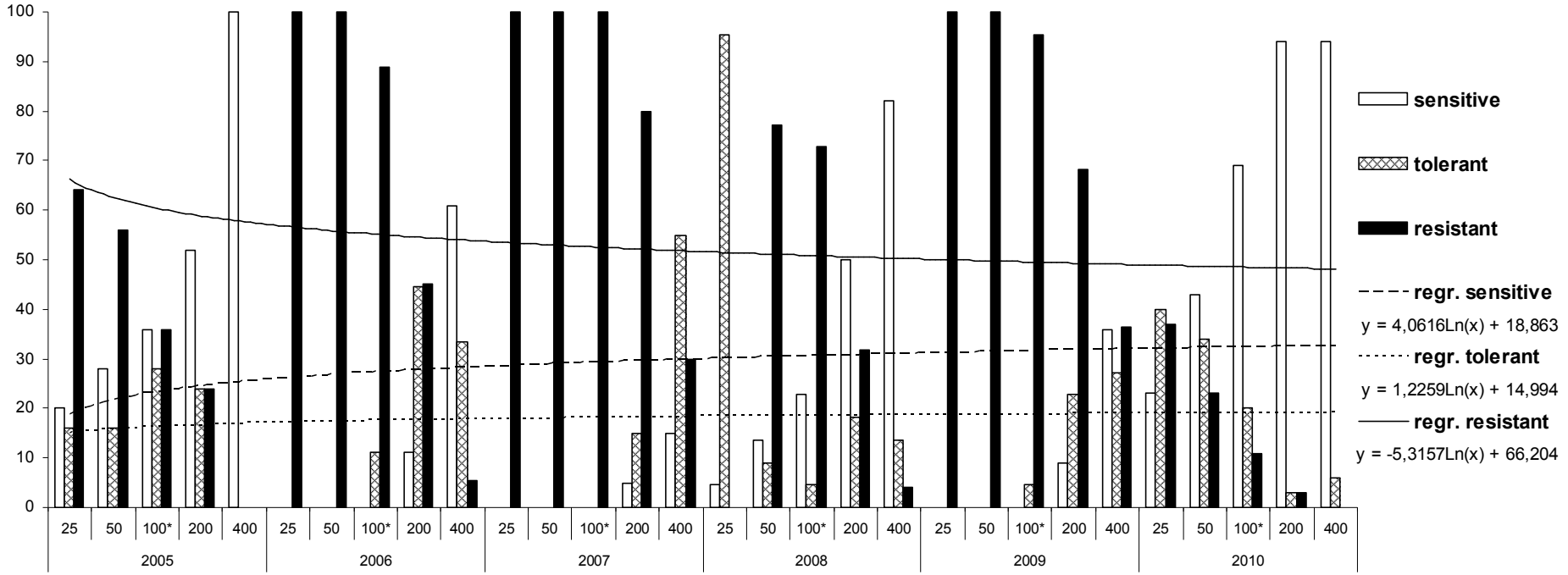


Fig. 5 Responses of *P. cubensis* population to different concentrations of metalaxyl-M in the period 2005-2010

3.5 Temporal changes in cucurbit downy mildew populations

3.5.1 Lebeda, A., Hübschová, J., Urban, J. 2010. Temporal population dynamics of *Pseudoperonospora cubensis*. In: Thies, J.A., Kousik, S., Levi, A. (Eds.): Cucurbitaceae 2010 Proceedings, pp. 240-243. American Society for Horticultural Science, Alexandria, VA, USA. (ISBN 978-0-9830932-0-6)

Temporal Population Dynamics of *Pseudoperonospora cubensis*

A. Lebeda*, J. Hübschová, and J. Urban

Palacký University in Olomouc, Faculty of Science, Department of Botany, Olomouc, Czech Republic

Additional index words. cucurbit downy mildew, Czech Republic, disease incidence, cucurbits, pathogenicity, fungicide efficacy

Abstract. **Since 1984, cucurbit downy mildew, caused by *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev, is economically the most important disease of cucurbit vegetables in the Czech Republic and in central Europe. Between 2001 and 2008 the disease was recorded annually in all growing areas of the Czech Republic. Natural infection has been regularly observed only on *Cucumis sativus*. Sporadic infection was recorded on *Cucumis melo* (2003, 2004), while other cucurbits, e.g., *Cucurbita pepo*, *C. maxima*, *Citrullus lanatus*, were free of infection. The majority of cucumber fields were heavily damaged. Czech populations of *P. cubensis* are found to be characterized by a high level of pathogenicity (expressed on pathotype level). Over the years 2001–08, significant year-to-year differences in the effectiveness of different fungicides have been observed in the Czech Republic.**

In recent years a dramatic increase in the occurrence of downy mildew has been recorded in Europe and the U.S. (Colucci et al., 2006; Holmes et al., 2004; Lebeda and Cohen, 2010). Cucurbitaceous crops worldwide are susceptible to *Pseudoperonospora cubensis*. This pathogen has a high capacity to adapt to changing environmental conditions and new geographical areas (Lebeda and Schwinn, 1994). *P. cubensis* attacks a broad spectrum of cucurbitaceous plants including field-grown and protected crops as well as wild plants. The most important host species include cucumber and melon (*Cucumis sativus* and *C. melo*), and pumpkin and squash (*Cucurbita pepo* and *C. maxima*) (Lebeda and Widrlechner, 2003). The disease has two major economic impacts: decreased yields and lower fruit quality (Lebeda and Widrlechner, 2003). The pathogen is characterized by large variation both in its pathogenicity and in its resistance to fungicides (Lebeda et al., 2006; Urban and Lebeda, 2007). Moreover, according to the classification of pathogens by McDonald and Linde (2002), *P. cubensis* belongs to the group of "the highest risk pathogens," namely, those which can easily break down the resistance of some host cultivars and which also exhibit high fungicide resistance. This paper summarizes population studies of *P. cubensis* in the Czech Republic during last decade (2001–08).

Materials and Methods

Occurrence of P. cubensis. Distribution, host range and damage caused by *P. cubensis* to cucurbits were evaluated repeatedly (at yearly intervals) at about 90 locations per year in the Czech Republic over the period 2001–08 (in 2001, 130 locations; in 2002, 109; in 2003, 107; in 2004, 110; in 2005, 96; in 2006, 105; in 2007, 91; and in 2008, 76). Monitoring was undertaken when plants reached maturity and at harvest time (end of July and August) and sites included domestic vegetable gardens, small private fields and larger commercial production areas in the main cucurbitaceous vegetable production areas (for details see Urban and Lebeda, 2007). Some marginal cucurbit production areas were also surveyed.

To assess damage caused by *P. cubensis* disease incidence and disease prevalence were determined. Disease incidence was expressed as the percentage of surveyed localities and host crops at which *P. cubensis* occurred. Disease prevalence (intensity) was assessed visually by using a graded 0–4 scale, modified for *P. cubensis* (Lebeda and Křístková, 1994).

Isolation and maintenance of P. cubensis. The collected infected leaf samples were incubated on wet filter paper in plastic pots (110 × 85 × 45 mm) (Lebeda, 1986). Leaves of the highly susceptible cucumber cultivar 'Marketer 430' were inoculated and then incubated in a growth chamber under standard conditions (Lebeda, 1986).

Pure cultures of *P. cubensis* were stored in petri dishes at –80 °C. The spores were viable for about 6 months. Some of the *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).

Plant material. Cucumis sativus 'Marketer 430' was used for multiplication of the pathogen isolates and for fungicide-resistance screening. Plants were grown in the glasshouse at 25 °C/15 °C day/night temperature, with daily watering and the addition of fertilizer (Kristalon Start, applied by a watering) once a week. Plants were not treated with pesticides and fungicides. The plants were used for testing when 5–8 weeks old (3–6 true leaves present).

Determination of pathogenic variation. The pathogenicity of 317 isolates was screened on a differential set of 12 cucurbit taxa (Lebeda and Widrlechner, 2003). A leaf-disc method was used (Lebeda, 1986; Urban and Lebeda, 2007). A visual 0–4 scale (Lebeda, 1991) was used to evaluate sporulation intensity over a 2-d period from 6 to 14 d after inoculation. The sporulation

Acknowledgements. The authors thank Prof. D.A. Morris (formerly of Southampton University, U.K.) for remarks on the first draft of the manuscript. This research was supported by grants QH 71229, MSM 6198959215 and PrF 2010 001.
*Corresponding author; phone: +420 585634800; fax: +420 585634824; email: ales.lebeda@upol.cz

Table 1. Tested concentrations of the fungicides [one recommended by the producer (i.e. optimal), and two others below and two above the optimum].

	Concn of fungicide (μg a.i./mL)/concn of source agent (%)				
Fungicide	1	2	3[z]	4	5
Metalaxyl	50/0.0630	100/0.125	200/0.250	400/0.500	800/1.000
Metalaxyl-M	25/0.0630	50/0.125	100/0.250	200/0.500	400/1.000
Propamocarb	607/0.1000	1214/0.200	2428/0.400	4856/0.800	9712/1.600
Fosetyl-Al	400/0.0500	800/0.100	1600/0.200	3200/0.400	6400/0.800
Cymoxanil	30/0.0750	60/0.150	120/0.300	240/0.600	480/1.200
Dimethomorph	112.5/0.125	225/0.250	450/0.500	900/1.000	1800/2.000

[z]The fungicide concentration as recommended by the producer (Minář, 2008).

intensity was expressed as the percentage of maximum sporulation intensity (Lebeda, 1992). Leaf discs with no, or only a low level of sporulation, were considered to show an incompatible response; those with a medium or high level of sporulation, to be from compatible genotypes. The pathogenicity level of isolates was determined on the basis of a number of pathogenicity factors, i.e. number of compatible reactions within the differential set of cucurbitaceous taxa. Pathotypes were designated with tetrade numerical codes (Lebeda and Widrlechner, 2003).

Fungicide efficacy assays. A floating leaf disc bioassay was used to screen for 183 *P. cubensis* isolates for tolerance or resistance to the following fungicides: Ridomil Plus 48 WP (active ingredients: 40% Cu-oxychloride, 8% metalaxyl), Ridomil Gold MZ 68 WP (a.i.: 64% mancozeb, 4% metalaxyl-M), Acrobat MZ (a.i.: 600 g/l mancozeb, 90 g/l dimethomorph), Aliette 80 WP (a.i.: 80% fosetyl-Al), Previcur 607 SL (a.i.: 607 g/l propamocarb) and Curzate K (a.i.: 77.3% Cu-oxychloride, 4% cymoxanil). Ridomil Gold MZ 68 WP, Curzate K, and Acrobat MZ were tested from 2005. Five concentrations of each fungicide were tested (Table 1). Leaf discs (15 mm in diameter) were prepared (Anonymous, 1982) and than inoculated after 24h. Incubation and evaluation were carried out as described above. Three types of reactions were assigned: the total degree of infection $P \leq 10\%$, fungicide tolerant ($10 < P \leq 35\%$) and resistant ($P > 35\%$) (Urban and Lebeda, 2007).

Results and Discussion

Distribution, host range and disease impact of P. cubensis. Extensive monitoring of the occurrence of *P. cubensis* was conducted in the Czech Republic during 2001-2008. It was confirmed that *P. cubensis* is widespread and occurs annually across the whole area of the Czech Republic studied. Periodic natural infection was observed only on the leaf laminae of *Cucumis sativus*. Infection of *C. melo* was recorded only twice (2003: Oplocany, Olomouc region; 2004: Olomouc-Holice, Olomouc region). The majority of the *C. sativus* crops evaluated were infected (Fig. 1). Disease prevalence on these crops was usually high or very high (Fig. 2). From our prolonged observations it is evident that there are annual (year to year) disease fluctuations; however, substantial declines of disease damage to the cucumber crop were never observed during the study period.

Pathogenic variation of P. cubensis populations and its temporal shift. In total, the 317 analyzed isolates belonged to 65 different pathotypes. From both a temporal and spatial viewpoint, substantial fluctuations in pathotype structure were recorded during the study period. The greatest pathogenic variability was recorded in the year 2001 (33 different pathotypes); in contrast the lowest variability was in 2007, when only five different pathotypes were recorded. A large proportion of the screened isolates could be

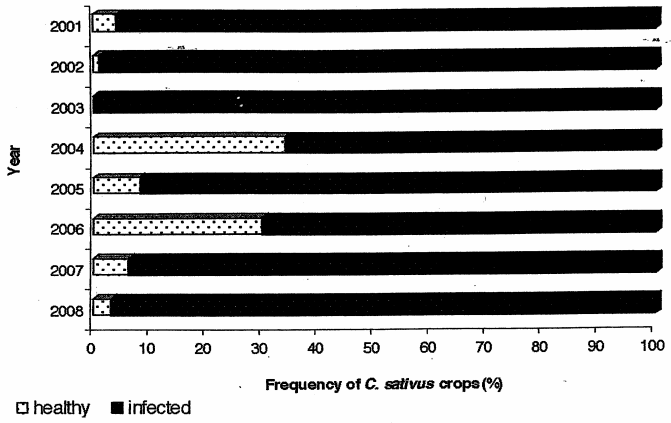


Fig. 1. Temporal fluctuation of *Pseudoperonospora cubensis* incidence in Czech cucumber crops.

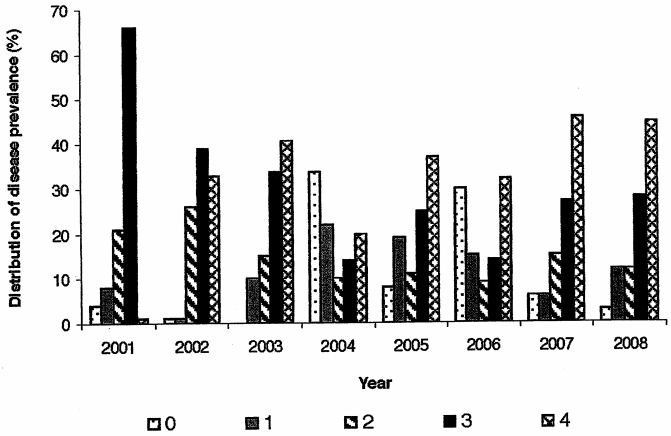


Fig. 2. Temporal fluctuation of *Pseudoperonospora cubensis* prevalence in Czech cucumber crops.

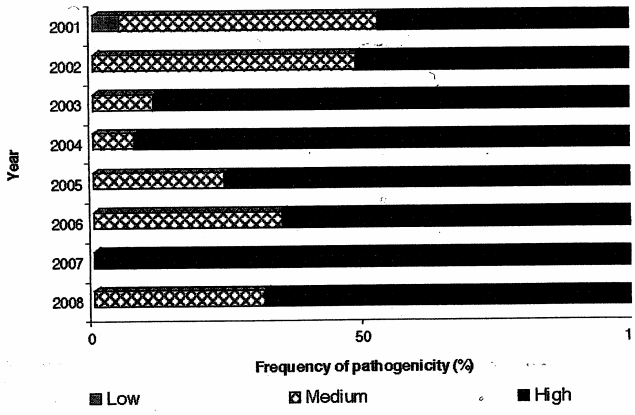


Fig. 3. Structure of *P. cubensis* populations according to their pathogenicity level. Three basic categories were designated: low pathogenicity level (1-4 PF), medium (5-8 PF), and high (9-12 PF).

considered as highly pathogenic (Fig. 3). The unique pathotype ("super pathotype") 15.15.15 was detected repeatedly in 2001, 2003, 2004, and 2008. It was one of the most frequent pathotypes observed in 2003 and 2008.

According to the differential screening tests, *Cucumis* spp. genotypes were confirmed as being highly susceptible. Tested genotypes of *Cucurbita* spp. exhibited a large race-specific variation in susceptibility to different *P. cubensis* isolates. *Benincasa hispida* and *Lagenaria siceraria* were highly susceptible to Czech isolates of *P. cubensis*. *Luffa cylindrica* expressed a general trend of decreasing level of susceptibility. *Citrullus lanatus* showed a high level of resistance; however, compatible interactions with more than 40% of the isolates were recorded in 2003, 2004, 2007, and 2008. Recent data demonstrate a higher pathogenic variability as was reported before (Lebeda and Gadasová, 2002). The pathogenicity structure of the Czech *P. cubensis* populations is not stable and shows a temporal shift to higher pathogenicity levels and to lower pathogenic variability. Various *P. cubensis* pathotypes have been observed in different countries around the world (Cohen et al., 2003; Lebeda et al., 2006). European isolates (mostly from the Czech Republic) are highly variable and differ (Lebeda and Gadasová, 2002; Lebeda et al., 2006) from the five pathotypes described previously from Japan, Israel and the United States (Thomas et al., 1987; Thomas and Jourdain, 1992). Substantial differences in pathotype structure among various European countries are evident (Lebeda and Gadasová, 2002).

Variation in fungicide resistance. A total of 183 isolates of *P. cubensis* were screened for tolerance/resistance to six frequently used fungicides. These fungicides were then compared according to frequency of fungicide tolerant/resistant strains that occurred.

Propamocarb (Previcur 607 SL) and fosetyl-Al (Aliette 80 WP) appeared to be effective at the recommended concentrations.

However, the occurrence of fungus isolates that sporulated at low concentrations of this fungicide indicated that the selection for tolerance occurs in the pathogen population. While a continuous temporal shift to higher resistance was caused by the ineffective fungicides metalaxyl, metalaxyl-M and cymoxanil (Ridomil Gold MZ 68 WP, Ridomil Plus 48 WP, and Curzate K, respectively) the ineffective dimethomorph (Acrobat MZ) was found to cause a temporal shift from resistant to tolerant isolates in the Czech populations. Recently, failures in fungicide efficacy against *P. cubensis* and a rapid increase in the occurrence of fungicide-resistant subpopulations of a pathogen have been found, especially under selection pressure from different fungicides (Urban and Lebeda 2006). *P. cubensis* is capable of quickly overcoming efficacy of some fungicides. It belongs to a group of 'the highest risk pathogens' with high evolutionary potential.

Conclusions

The occurrence of cucurbit downy mildew was observed annually (2001–08) throughout the Czech Republic. Natural infection caused by *P. cubensis* was recorded frequently on *Cucumis sativus* but only rarely on *Cucumis melo*. Disease intensity varied during the study period. Differences in severity within the recorded localities with *C. sativus* crops were probably primarily caused by variability in the macro- and microclimatic conditions in individual years. *P. cubensis* exhibits clear host specialization. However, the host range is known to vary between different countries (Lebeda and Cohen, 2010; Lebeda et al., 2006). Generally the Czech *P. cubensis* populations were characterized by high pathogenicity, but the structure is not stable from the temporal viewpoint. A high level of resistance of *P. cubensis* populations to metalaxyl, confirmed in our study, is commonly known in many Oomycetes including *P. cubensis* (Urban and Lebeda, 2006, 2007). Sensitive strains seem to be absolutely substituted by those with high resistance in Central Europe, where we can expect a great effect of populations mixing, which occurs due to migration, i.e. long-distance transport of spores (Lebeda, 1990). The future development of insensitivity to fosetyl-Al and propamocarb is unpredictable. It is evident that *P. cubensis* is a very aggressive pathogen with a high epidemiological potential, enormous pathogenic variability and broad variation in fungicide resistance.

Literature Cited

Anonymous. 1982. FAO Method No. 30. FAO Plant Protect. Bul. 30:2.

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

Colucci, S.J., T.C. Wehner, and G.J. Holmes. 2006. The downy mildew epidemic of 2004 and 2005 in the eastern United States, p. 403–411. In: Holmes (ed.). Proc. Cucurbitaceae 2006. Universal Press, Raleigh, NC.

Holmes, G.J., C.E Main, and Z.T. Keever, III. 2004. Cucurbit downy mildew: A unique pathosystem for disease forecasting, p. 69–80. In: P.T.N. Spencer-Phillips and M. Jeger (eds.). Advances in downy mildew research, Vol. 2. Kluwer Academic Publ., Dordrecht, The Netherlands.

Lebeda, A. 1986. *Pseudoperonospora cubensis*, p. 81–85. In: A. Lebeda (ed.). Methods of testing vegetable crops for resistance to plant pathogens. VHJ Sempra, Research Institute of Vegetable Crops, Olomouc.

Lebeda, A. 1990. Biology and ecology of cucurbit downy mildew, p. 13–46. In: A. Lebeda (ed.). Cucurbit downy mildew. Czechoslovak Sci. Soc. for Mycol. by Czechoslovak Academy of Sciences, Praha, Czech Republic.

Lebeda, A. 1991. Resistance in muskmelons to Czechoslovak isolates of *Pseudoperonospora cubensis* from cucumbers. Scie. Hort. 45:255–260.

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

Lebeda, A. and Y. Cohen. 2010. Cucurbit downy mildew (*Pseudoperonospora cubensis*)—Biology, ecology, epidemiology, host–pathogen interactions and control. Europ. J. Plant Pathol. (In press.)

Lebeda, A. and V. Gadasová. 2002. Pathogenic variation of *Pseudoperonospora cubensis* in the Czech Republic and some other European countries. Acta Hort. 588:137–141.

Lebeda, A. and E. Křístková. 1994. Field resistance of *Cucurbita* species to powdery mildew (*Erysiphe cichoracearum*). J. Plant Dis. Protect. 101:598–603.

Lebeda, A. and F.J. Schwinn. 1994. The downy mildews—An overview of recent research progress. J. Plant Dis. Protect. 101:225–254.

Lebeda, A. and M.P. Widrlechner. 2003. A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. J. Plant Dis. Protect. 110:337–349.

Lebeda, A., M.P. Widrlechner, and J. Urban. 2006. Individual and population aspects of interactions between cucurbits and *Pseudoperonospora cubensis*: pathotypes and races, p. 453–467. In: G.J. Holmes (ed.). Proc. Cucurbitaceae 2006, Universal Press, Raleigh, NC.

McDonald, B.A. and C. Linde. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40:349–379.

Minář, P. (ed.). 2008. Seznam registrovaných přípravků na ochranu rostlin 2008 (List of registered preparations for plant protection 2008). Státní rostlinolékařská správa, Praha.

Thomas, C.E., T. Inabana, and Y. Cohen. 1987. Physiological specialization of *Pseudoperonospora cubensis*. Phytopathology 77:1621–1624.

Thomas, C.E. and E.L. Jourdain. 1992. Host effect on selection of virulence factors affecting sporulation by *Pseudoperonospora cubensis*. Plant Dis. 76:905–907.

Urban, J. and A. Lebeda. 2006. Fungicide resistance in cucurbit downy mildew—Methodological, biological and population aspects. Ann. Appl. Biol. 149:63–75.

Urban, J. and A. Lebeda. 2007. Variation for fungicide resistance in Czech populations of *Pseudoperonospora cubensis*. J. Phytopathol. 155:143–151.

Cucurbitaceae 2010 Proceedings

14–18 November 2010

Charleston, South Carolina, USA

Organizing Committee

JUDY A. THIES, CHAIR
SHAKER KOUSIK
AMNON LEVI
U.S. Vegetable Laboratory, USDA, ARS, Charleston, South Carolina

American Society for Horticultural Science, 1018 Duke Street, Alexandria, VA 22314-3512
ISBN 978-0-9830932-0-6

4. CONCLUSIONS

The data obtained in this Ph.D. thesis could be summarized as follows.

- The occurrence of cucurbit downy mildew was observed annually throughout the area of the Czech Republic. Natural infection was recorded predominantly on *C. sativus* and only rarely on other cucurbits (*Cucurbita* spp. and *Citrullus lanatus* – since the year 2009). Our long-lasting observations showed the annual disease fluctuations; however substantial declines of disease damage to the cucumber crop have never been observed during the studied period.
- The virulence profile of the studied pathogen population was highly variable. At the population level, majority of the screened *Pseudoperonospora cubensis* isolates were highly virulent (with 9 to 12 virulence factors /VF/). A total of 67 different pathotypes were determined in the period 2001-2010. A broad spectrum of variation in virulence observing in Czech *P. cubensis* population has not been reported in *P. cubensis* from another country till now.
- Results of screening of fungicide tolerance/resistance in Czech *P. cubensis* population could be divided into three groups: propamocarb and fosetyl-Al, towards which no resistance has developed, however the risk of resistance development still exists; metalaxyl, metalaxyl-M and cymoxanil, towards which a high resistance has developed; and dimethomorph, towards which a shift from resistance to tolerance and/or susceptibility has developed.
- Temporal population dynamics of *P. cubensis* were studied. Fluctuation in disease severity within the recorded localities with *C. sativus* crops were probably caused by variability in the macro- and microclimatic conditions in individual years. Virulence structure showed a temporal shift from 2001 to 2007 to a higher number of virulence factors and a lower number of pathotypes. However, variation in virulence changed and increased again from 2008 to 2010. Although fosetyl-Al and propamocarb seems to be highly effective for the field control of the disease, less significant changes in sensitivity were also detected. Despite the increasing occurrence of metalaxyl-M-sensitive/tolerant strains, the recent efficacy of metalaxyl-M is still low for the field control of the disease, as well as for metalaxyl. *P. cubensis* populations appear to be insensitive to cymoxanil. Effectiveness of dimethomorph varied from resistance to tolerance and sensitivity during a period of six consecutive years.

5. SOUHRN (SUMMARY, in Czech)

Název: Populační dynamika plísně okurkové (*Pseudoperonospora cubensis*)

P. cubensis je celosvětově považována za nejvýznamnějšího patogena porostů tykvovitých rostlin. Ačkoli se symptomy choroby objevují pouze na listech, infekce způsobuje ekonomicky významné snížení kvality i kvantity sklizně. V České republice jsou epidemické výskyty na okurkách zaznamenávány každoročně již od roku 1984. Tato práce rozvíjí již získané znalosti a přináší nové poznatky o tomto patogenu, které přispívají k vytvoření vysoce efektivního systému ochrany porostů.

Většina sledovaných porostů *C. sativus* byla v letech 2007-2009 napadena silně až velmi silně (stupeň 3-4), na rozdíl od následující dvouleté periody (2010-2011), kdy na většině porostů *C. sativus* bylo nejčastěji pozorováno buď slabé napadení (stupeň 1) nebo střední až silné (stupeň 2-3). Od roku 2009 byla infekce *P. cubensis* opakovaně zaznamenána rovněž na melounu cukrovém (*Cucumis melo;* 2009, 2011) a na melounu vodním (*Citrullus lanatus;* 2010-2011). Poprvé v historii dlouhodobého sledování byla infekce zjištěna na tykvi muškátové (*Cucurbita moschata;* 2009-2010), tykvi obecné (*Cucurbita pepo*; 2010-2011), tykvi velkoplodé (*Cucurbita maxima;* 2010-2011), tykvi fíkolisté (*Cucurbita ficifolia;* 2010) a na lagenárii (*Lagenaria siceraria;* 2011).

V populaci *P. cubensis* bylo detekováno velké množství patotypů (celkem 67 – za období 2001-2010), převažovaly středně až vysoce virulentní kmeny. Nejvyšší frekvence kompatibilních reakcí mezi izoláty *P. cubensis* a genotypy diferenciačního souboru pro detekci patotypů byla zaznamenána u skupiny genotypů rodu *Cucumis*, naopak nejnižší na *Cucurbita pepo* subsp. *pepo*, *Citrullus lanatus* a *Luffa cylindrica*. Vysoký podíl izolátů byl dokonce schopen infekce na *Benincasa hispida* a *Lagenaria siceraria*, které nejsou běžně pěstovány v České republice, nebo jinde ve Střední Evropě. Od roku 2009 dochází v patogenní populaci *P. cubensis* v České republice k výrazným změnám, kdy se objevily nové vysoce virulentní kmeny patogena schopné způsobit přirozenou a závažnou infekci na *Cucurbita* spp. a na *Citrullus lanatus*, která nebyla v letech 2001-2008 pozorována. „Super patotyp“ 15.15.15 byl zjištěn opakovaně v populaci patogenu a je dokonce čtvrtým nejčastěji zaznamenaným patotypem za celé desetileté období.

Nejúčinnějšími přípravky vůči izolátům *P. cubensis* byly ve sledovaném období Aliette 80 WP a Previcur 607 SL. Všechny testované izoláty byly citlivé vůči všem testovaným koncentracím přípravku Aliette 80 WP. Bohužel u přípravku Previcur 607 SL byl v populaci

patogenu v letech 2008-2010 pozorován výskyt kmenů s rezistencí nebo tolerancí nižších koncentrací. V roce 2008 byla zaznamenána tolerantní reakce dokonce i v doporučené koncentraci. Přípravky, které obsahovaly účinnou látku metalaxyl (Ridomil Gold MZ 68 WP a Ridomil PLUS 48 WP) byly v letech 2005-2009 neúčinné, přičemž v letech 2008-2009 byla zjištěna vyšší heterogenita izolátů s tolerancí/rezistencí koncentrací vyšších než optimální u obou fungicidů, avšak tento trend se v následujícím roce (2010) nepotvrdil, naopak 69% izolátů v případě Ridomilu Gold MZ 68 WP a 43% izolátů u Ridomilu PLUS 48 WP bylo kontrolováno doporučenou koncentrací obou přípravků a omezená nebo výrazná sporulace izolátů na koncentracích vyšších než optimální byla ojedinělá a lze tedy říci, že jeho účinnost v roce 2010 mírně vzrostla. Efektivita přípravku Curzate K se během sledovaného období rovněž měnila, zatímco v letech 2007-2008, a také v roce 2010 byl tento přípravek neúčinný, výjimkou byl rok 2009, kdy 68% izolátů bylo kontrolováno doporučenou koncentrací a pouze 32% populace patogenu vykazovalo toleranci či rezistenci na této koncentraci. Účinnost přípravku Acrobat MZ se ve sledovaném období lišila velmi významně, zatímco v roce 2007 byl tento přípravek neúčinný (70% populace tolerovalo a 20% bylo rezistentní k doporučené koncentraci, a také na vyšších koncentracích převažovaly tolerantní/rezistentní reakce), tak v letech 2008-2009 byla pozorována rostoucí tendence zastoupení tolerantních kmenů ke všem testovaným koncentracím, a v roce 2010 bylo už 83% populace patogenu kontrolováno všemi testovanými koncentracemi tohoto přípravku a pouze 14% tolerovalo nejnižší testovanou koncentraci a 3% také koncentraci 1x vyšší.

P. cubensis se v České Republice vyznačuje každoročními epidemickými výskyty. Snížení intenzity napadení porostů tykvovitých zelenin tímto patogenem byl pozorován v roce 2004 a v letech 2010-2011. V průběhu dlouholetého pozorování se tedy ukázala určitá meziroční fluktuace, ovšem podstatné snížení dopadu choroby na sledované porosty tykvovitých zelenin se nikdy neobjevilo. Nejčastějším a nejcitlivějším hostitelským druhem je *Cucumis sativus*. Od roku 2009 se plíseň tykvovitých objevuje i na druzích rodu *Cucurbita* a na druhu *Citrullus lanatus*. Uvedená skutečnost poukazuje na to, že ve středoevropské populaci *P. cubensis* dochází v posledních letech k zásadním změnám. Dynamika a struktura virulence populací *P. cubensis* v České Republice se ukázala jako extrémně proměnlivá. Výskyt vysokého počtu různých patotypů a jejich značná proměnlivost zastoupení v čase a prostoru se dosud nevyskytla v populacích *P. cubensis* v jiných státech. Tato do značné míry unikátní a nestabilní struktura virulence může souviset s migrací tohoto patogenu a polohou České republiky v Evropě. Účinnost vybraných fungicidů se také významně lišila a

korespondovala s výsledky z minulých let, kdy došlo ke zvýšení rezistence vůči některým přípravkům. V minulosti zjištěné riziko selekce rezistentních/tolerantních kmenů bylo prokázáno u dvou nejúčinnějších přípravků (Aliette 80 WP a Previcur 607 SL), výrazněji u přípravku Previcur 607 SL. Na základě výsledků dlouhodobého sledování v letech 2001-2010 lze říci, že česká populace plísně okurkové je rezistentní vůči přípravkům na bázi metalaxylu. Rezistence k přípravkům Acrobat MZ a Curzate K byla v české populaci tohoto patogenu zaznamenána už v letech 2005-2006. Protože se *P. cubensis* ukázala jako velmi variabilní v čase i prostoru, její další vývoj nelze zcela spolehlivě určit. Ale lze předpokládat, že na základě vysokého evolučního potenciálu a adaptability na nové klimatické podmínky, bude rozšíření, virulence a účinnost kontrolních opatření vůči *P. cubensis* značně kolísat.