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Abstract

Cucurbit downy mildew (*Pseudoperonospora cubensis*) is characterized by large variation in pathogenicity, specificity and host-parasite interactions. This report reviews the current state of understanding regarding interactions between *P. cubensis* and Cucurbitaceae, the genetic control of host reactions, and overall variation within the pathogen. A well-characterized set of differential Cucurbitaceae taxa and genotypes for the identification of *P. cubensis* pathotypes or races has not yet been designated. A preliminary set designated by Thomas et al. (1987) was, in certain cases, deficient in taxonomic description, determination of membership at the level of accession, uniformity of differential response, and international availability. An improved differential set of cucurbit accessions for characterization of pathogenic variability of *P. cubensis* isolates is proposed in this paper. The proposed set includes 12 taxa from six genera (*Benincasa*, *Citrullus*, *Cucumis*, *Cucurbita*, *Lagenaria* and *Luffa*). These differentials are natural host plants of *P. cubensis*, and basic information on their host-parasite specificity and variability is available. The members of this new set are taxonomically well characterized and available as genebank accessions. The data about host genera and pathogen variation summarized here fully support previous reports about the existence of distinct physiological forms (pathotypes and races) of *P. cubensis*, and a tetrad coding system is offered to designate *P. cubensis* pathotypes. This paper presents evidence that such forms may be delimited based on host genus, species and intraspecific specificities. This proposed differential set of Cucurbitaceae should serve as an appropriate baseline for more detailed research about *P. cubensis* isolate variation and population structure at the pathotype level. Steps to foster international collaboration on this topic are suggested and discussed.

Keywords

Benincasa, Citrullus, Cucumis, Cucurbita, Lagenaria, Luffa, cucurbit downy mildew, pathogen variability, genetic resources, host-parasite specificity, physiological specialization, resistance breeding

Disciplines

Agricultural Science | Agriculture | Horticulture | Plant Sciences

Comments

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A set of Cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes

Ein Testsortiment von Cucurbitaceae-Taxa für die Differenzierung der Pathotypen von *Pseudoperonospora cubensis*

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Summary

Cucurbit downy mildew (*Pseudoperonospora cubensis*) is characterized by large variation in pathogenicity, specificity and host-parasite interactions. This report reviews the current state of understanding regarding interactions between *P. cubensis* and Cucurbitaceae, the genetic control of host reactions, and overall variation within the pathogen. A well-characterized set of differential Cucurbitaceae taxa and genotypes for the identification of *P. cubensis* pathotypes or races has not yet been designated. A preliminary set designated by THOMAS et al. (1987) was, in certain cases, deficient in taxonomic description, determination of membership at the level of accession, uniformity of differential response, and international availability. An improved differential set of cucurbit accessions for characterization of pathogenic variability of *P. cubensis* isolates is proposed in this paper. The proposed set includes 12 taxa from six genera (*Benincasa*, *Citrullus*, *Cucumis*, *Cucurbita*, *Lagenaria* and *Luffa*). These differentials are natural host plants of *P. cubensis*, and basic information on their host-parasite specificity and variability is available. The members of this new set are taxonomically well characterized and available as genebank accessions. The data about host genera and pathogen variation summarized here fully support previous reports about the existence of distinct physiological forms (pathotypes and races) of *P. cubensis*, and a tetrad coding system is offered to designate *P. cubensis* pathotypes. This paper presents evidence that such forms may be delimited based on host genus, species and intraspecific specificities. This proposed differential set of Cucurbitaceae should serve as an appropriate baseline for more detailed research about *P. cubensis* isolate variation and population structure at the pathotype level. Steps to foster international collaboration on this topic are suggested and discussed.

Key words: *Benincasa*; *Citrullus*; *Cucumis*; *Cucurbita*; *Lagenaria*; *Luffa*; cucurbit downy mildew; pathogen variability; genetic resources; host-parasite specificity; physiological specialization; resistance breeding

Zusammenfassung

Der Falsche Mehltau an Gurken (*Pseudoperonospora cubensis*) ist gekennzeichnet durch erhebliche Schwankungen in der Pathogenität, Spezifität und den Wirt-Parasit-Interaktionen. Dieser Bericht fasst den gegenwärtigen Kenntnisstand bezüglich der Wechselwirkungen zwischen *P. cubensis* und den Cucurbitaceae zusammen sowie der genetischen Steuerung der Wirtsreaktionen und aller Variationen innerhalb des Erregers. Ein gut charakterisiertes Sortiment an differenzierenden Cucurbitaceae-Taxa

und Genotypen zur Identifizierung von *P. cubensis*-Pathotypen oder -Rassen wurde bis jetzt nicht beschrieben. Ein vorläufiges Sortiment hatten THOMAS et al. (1987) vorgestellt, aber in gewissen Fällen mangelte es diesen an taxonomischer Beschreibung, an einer Bestimmung der Zugehörigkeit auf dem Niveau der Herkünfte, an der Uniformität der differenzierten Reaktion und an der internationalen Verfügbarkeit. In der vorliegenden Arbeit wird ein verbessertes Sortiment zur Charakterisierung der Variabilität von *P. cubensis* vorgestellt. Das Sortiment besteht aus 12 Taxa, aus sechs Gattungen (*Benincasa*, *Citrullus*, *Cucumis*, *Cucurbita*, *Lagenaria* und *Luffa*). Alle sind natürliche Wirte von *P. cubensis* und grundlegende Informationen über ihre Wirt-Parasit-Spezifitäten und Variabilitäten sind verfügbar. Außerdem sind sie taxonomisch gut charakterisiert und in Genbanken erhältlich, Die Daten über die Wirtsgattungen und über die Variabilität des Erregers, die hier zusammengefasst sind, unterstützen frühere Berichte über die Existenz unterschiedlicher physiologischer Formen (Pathotypen und Rassen) von *P. cubensis*. Es wird ein Viererschlüssel-System zur Bestimmung von *P. cubensis*-Pathotypen beschrieben. In dieser Arbeit wird der Beweis erbracht, dass solche Formen auf der Basis von Wirtsgattung, Art und intraspezifischer Besonderheiten abgegrenzt werden können. Dieses Differential-sortiment von Cucurbitaceae könnte eine geeignete Basis für detailliertere Untersuchungen über die Variation von *P. cubensis*-Isolaten und über die Populationsstruktur auf der Pathotyp-Ebene dienen. Es werden Schritte zur Förderung der internationalen Zusammenarbeit auf diesem Gebiet vorgeschlagen und diskutiert.

Stichwörter: *Benincasa*; *Citrullus*; *Cucumis*; *Cucurbita*; *Lagenaria*; *Luffa*; Gurkenmehltau; *Pseudoperonospora cubensis*; Erregervariabilität; genetische Ressourcen; Wirt-Parasit-Spezifität; physiologische Spezialisierung; Resistenzzüchtung

1 Introduction

Downy mildew of cucurbits, *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov., has been a serious problem world-wide on various Cucurbitaceae crops (LEBEDA 1990; PALT and COHEN 1980; THOMAS 1996). There are more than 50 cucurbit species known as hosts of *P. cubensis* (LEBEDA 1990, 1992a; PALT and COHEN 1980). Most published information on host specificity originates from Asia and USA (BAINS and SHARMA 1986; THOMAS et al. 1987), with very little derived from Europe (LEBEDA 1999; LEBEDA and GADASOVÁ 2002). Our understanding of intraspecific variation and genetics in the Cucurbitaceae – *P. cubensis* pathosystem is limited, but extremely important from the viewpoint of theoretical knowledge and practical resistance breeding (LEBEDA 1999; LEBEDA and GADASOVÁ 2002).

The aims of this paper are to review and apply information data about Cucurbitaceae – *P. cubensis* interactions to the development of well specified differential set of Cucurbitaceae for the characterization of *P. cubensis* pathogenic variability.

2 Framework for the development of differential set for *P. cubensis*

Pathogenic variability, as expressed in differential virulence, has been described for several downy mildew species. In host plant – downy mildew interactions, there is often a very clear expression of compatibility or incompatibility. This allows a classification of pathotypes or races based on the pattern of compatible and incompatible reactions on the differential hosts. Each differential-host genotype may carry different resistance alleles, which interact with corresponding pathogen avirulence genes. In investigating downy mildew pathogenic variability, the following issues are of importance (LEBEDA and SCHWINN 1994):

1. General knowledge about host-pathogen variability and specificity of interaction;
2. Knowledge about host-pathogen genetics; and
3. Availability of a theoretical genetic model explaining host-pathogen interactions.

To date, the above-mentioned criteria have been applied to only a limited number of host plant – downy mildew interactions, and there are only a few downy mildew species (*Bremia lactucae* Regel, *Peronospora halstedii* Farlow, *P. parasitica* (Pers.) Tul. and *P. viciae* (Berk.) Caspary) where the phe-

nomenon of pathogenic variability is more clearly described (LEBEDA and SCHWINN 1994; SPENCER-PHILLIPS et al. 2002).

The differentiation of pathotypes and races of *P. cubensis* is a work in progress. A study of *P. cubensis* isolates originating from USA, Israel and Japan on 26 plant genotypes representing the seven cucurbit genera *Benincasa* Savi, *Citrullus* Schrad. ex Eckl. & Zeyh., *Cucumis* L., *Cucurbita* L., *Lagenaria* Ser., *Luffa* Mill., and *Momordica* L., revealed that distinct pathotypes exist among isolates (THOMAS et al. 1987). In that study, representatives of three cucurbit genera, *Citrullus*, *Cucumis*, and *Cucurbita*, were used to differentiate among *P. cubensis* pathotypes. Their research also revealed that five distinct pathotypes of *P. cubensis* exist among isolates originating from the USA, Israel and Japan (THOMAS et al. 1987). In general, this study serves as a crucial starting point for a more detailed understanding of host – parasite interactions in the Cucurbitaceae – *P. cubensis* pathosystem. Their results also provided the first opportunities for detailed characterization of *P. cubensis* isolates, studies of pathogen population variability in different countries, and evaluation of variation in resistance in cucurbit germplasm to defined isolates. Ultimately, this can lead to the development of improved, resistant cultivars for these crops. However, each step forward creates new topics for future investigation, as noted by THOMAS et al. (1987) at the close of their paper “The pathotype concept discussed above for this pathosystem is not presented as the entire, definite picture of physiological specialization in *P. cubensis*, but as a framework for continued study.”

The need for expanded research in this area has been stressed many times (LEBEDA 1990, 1992a, 1999; LEBEDA and WIDRLECHNER 2003a). The most compelling reasons can be outlined as follows.

1. Host plant (Cucurbitaceae genera and species)
 - a. Establish a more clearly described set of differential host genotypes in relation to taxonomic identity, geographic origin, and genetics (resistance factors/genes, markers, other genetic characters);
 - b. Conserve these genotypes (accessions) in a germplasm collection under standard genebank conditions; and
 - c. Share them freely and encourage their use throughout the research community.
2. Pathogen (*Pseudoperonospora cubensis*)
 - a. Establish a standard collection of well-characterized (geographic origin, country and place of origin, host species and genotype, pathogenic variation, etc.) isolates of *P. cubensis*;
 - b. Conserve these isolates under standard and controlled conditions; and
 - c. Share them freely, within the limits of phytosanitary control and appropriate restrictions to reduce the risk of the evolution and spread of new pathotypes, and encourage their use throughout the research community.

3 Current status of knowledge

3.1 Host plant

The family Cucurbitaceae is a very large and heterogeneous group of plants, represented by 118 genera and more than 825 species (BATES et al. 1990; JEFFREY 2001). However, only about 20 genera and 50 species are known as hosts of *P. cubensis* (PALTÍ and COHEN 1980; LEBEDA 1990, 1992a). During the 1990s, many experimental studies following standard methods of controlled inoculation and incubation (LEBEDA 1986) were conducted to help elucidate interactions between Cucurbitaceae and *P. cubensis*. These studies are briefly characterized and summarized below according to the most important crop hosts.

3.1.1 *Cucumis*

3.1.1.1 *C. sativus* L.

Contrary to earlier reports (BARCZYNSKA et al. 1988; FANOURAKIS and SIMON 1987), relatively comprehensive screening studies (LEBEDA 1992b; LEBEDA and PRÁŠIL 1994) showed that available *C. sativus* germplasm accessions and recent commercial cucumber cultivars do not possess effective sources of resistance, and race-specific interactions are unknown (LEBEDA 1999). Resistance reported in some

C. sativus accessions (PI 179676, PI 197085, PI 197086, PI 197088) and their race-specific background (ANGELOV et al. 2000; SHETTY et al. 2002) is questionable because of methodology used (poorly specified and possible mixtures of isolates) and thus require verification. Limited variation in the *C. sativus*-*P. cubensis* interaction supports previous reports that the genetic diversity in this crop is rather low (KNERR et al. 1989; KUPPER and STAUB 1988; STAUB et al. 1987), including diversity for some disease traits (BARCZYNSKA et al. 1988). Thus, we can conclude that *C. sativus* genotypes could be used in a differential set only as a susceptible control (Table 1). Nevertheless, it is important to continue investigations of this host-parasite interaction because there may still be opportunities to discover race-specific resistance in this species, as new sources of diversity in *C. sativus* become available (MLIKI et al. 2002; SHETTY et al. 2002; STAUB et al. 1997, 1999). For example, there is a lack of information on host response to *P. cubensis* pathotypes in the wild relatives, *C. sativus* var. *hardwickii* (Royle) Gabaev and var. *sikkimensis* Hook. There are, however, recent reports of germplasm and selected cultivars of *C. sativus* with higher levels of field resistance (BJOERN and KAMPMANN 2000; DHILLON et al. 1999; DORUCHOWSKI and LAKOWSKA-RYK 2000; HOREJSI et al. 2000; LEBEDA 1999; LEBEDA and DOLEŽAL 1995; LEBEDA and PRÁŠIL 1994; PETROV et al. 2000; SHETTY et al. 2002; WEHNER and SHETTY 1997). Well-defined pathotypes would also be valuable in verifying levels of this type of resistance to *P. cubensis*. Strategies based on the use of known pathotypes could also help improve the efficiency of marker-assisted selection (MAS) methods recently developed (HOREJSI et al. 2000) for breeding cucumber cultivars with higher level of downy mildew field resistance.

3.1.1.2 *C. melo* L.

Recent reports characterize *Cucumis melo* as displaying considerable intraspecific variation at various levels, i. e., morphological, genetic and molecular (PITRAT et al. 2000; STEPANSKY et al. 1999). *Cucumis melo* is the only species in the genus wherein the phenomenon of race-specificity of *P. cubensis* is relatively well understood (BAINS and SHARMA 1986; LEBEDA 1990, 1991, 1999; PALTÍ and COHEN 1980; THOMAS et al. 1987), and some effective sources of resistance are available (LEBEDA 1999; PITRAT et al. 1998). Characterized sources of resistance are under di- or oligogenic control and are partially dominant (PITRAT et al. 1998). *Cucumis melo* has recently been considered as an important source of genetic variation, including resistance to *P. cubensis*, for incorporation into *C. sativus* through interspecific

Table 1. Characteristics of a new Cucurbitaceae differential set for the determination of pathogenic variability in *Pseudoperonospora cubensis*

No.	Taxon	Accession number		Cultivar name	Country of origin
		Donor	EVIGEZ		
1	<i>Cucumis sativus</i>		H39-0121	'Marketer 430'	USA
2	<i>C. melo</i> subsp. <i>melo</i>	PI 292008	H40-1117	'Ananas Yokneam'	Israel
3	<i>C. melo</i> var. <i>conomon</i>	CUM 238/1974	H40-0625	'Baj-Gua'	Japan
4	<i>C. melo</i> var. <i>acidulus</i>	PI 200819	H40-0611		Myanmar
5	<i>Cucurbita pepo</i> var. <i>pepo</i>	PI 171622	H42-0117	'Dolmalik'	Turkey
6	<i>C. pepo</i> var. <i>texana</i>	PI 614687	H42-0130		USA
7	<i>C. pepo</i> var. <i>fraterna</i> *	PI 532355	H42-0136		Mexico
8	<i>C. maxima</i>		H42-0137	'Goliáš'	Czechoslovakia
9	<i>Citrullus lanatus</i>		H37-0008	'Malali'	Israel
10	<i>Benincasa hispida</i>	BEN 485	H15-0001		USA
11	<i>Luffa cylindrica</i>		H63-0010		?
12	<i>Lagenaria siceraria</i>		H63-0009		?

EVIGEZ – Czech genebank number (accessions are maintained in RICP in Prague, Department of Gene Bank, Workplace Olomouc, Czech Republic; working collection is maintained in Laboratory of Plant Pathology, Department of Botany, Faculty of Science, Palacký University, Olomouc, Czech Republic)

* originally described as *Cucurbita fraterna* (LEBEDA and GADASOVÁ 2002)

? unknown

hybridization (CHEN and ADELBERG 2000; LEBEDA et al. 1996, 1999). The analysis of intraspecific variation in *C. melo* uncovered basic differences in resistance to *P. cubensis* and established a system to differentiate pathogen isolates originating from Japan and Israel (THOMAS et al. 1987). Recent studies (LEBEDA and GADASOVÁ 2002) demonstrated that *P. cubensis* isolates originating from Europe are broadly variable in virulence on three *C. melo* genotypes/accessions (Table 2). More detailed research of *C. melo* accessions maintained in world genebank collections might yield new information on host-pathogen interactions and the evolution of this pathosystem, leading to a more precise differentiation of *P. cubensis* and a better understanding of the genetics of resistance based on well-defined host genotypes and pathogen isolates.

3.1.1.3 Other *Cucumis* species

In addition to the two widely cultivated species discussed above, the most recent taxonomic monograph of the genus *Cucumis* recognizes about 30 wild species, most native to Africa (KIRKBRIDE 1993). However, only few of them (ca. eight species) are known as natural hosts of *P. cubensis* (PALTÍ and COHEN 1980). To date, only limited information has been published (LEBEDA 1992a, 1999) on the specificity of interactions between wild *Cucumis* taxa and *P. cubensis*. Recent screening with controlled inoculation of a set of about 100 accessions representing more than 20 wild species with six *P. cubensis* isolates demonstrated no clear differential patterns (LEBEDA, unpubl. results). Thus, it can be concluded that wild *Cucumis* species are unlikely to have played an important role in the differentiation of *P. cubensis* pathotypes. However, more intensive research with additional, well specified *P. cubensis* isolates may bring new and interesting results.

3.1.2 *Citrullus*

The genus *Citrullus* is represented by four taxonomically valid species, *C. colocynthis* (L.) Schrad., *C. ecirrhosus* Cogn., *C. lanatus* (Thunb.) Matsum. & Nakai and *C. rehmii* De Winter (ROBINSON and DECKER-WALTERS 1997). Two of them, *C. colocynthis* and *C. lanatus* (syn. *C. vulgaris* Schrad.), are known as natural hosts of *P. cubensis* (PALTÍ and COHEN 1980). Two botanical varieties are recognized in *C. lanatus*: var. *lanatus*, the cultivated types, the "egusi" melon; and var. *citroides* (L. H. Bailey) Mansf., known as the citron, preserving melon, or "tsamma" (JARRET et al. 1997). Both types have been

Table 2. Examples of reaction patterns of some *Pseudoperonospora cubensis* isolates originating in Europe and their degree of pathogenicity (modified from LEBEDA and GADASOVÁ 2002; LEBEDA unpubl. results)

No.	Taxon	<i>P. cubensis</i> /isolate PC									
		3/00 ¹⁾	13/00 ²⁾	1/88	3/98	1/98	4/00	12/00	1/97	24/01	39/01
1	<i>Cucumis sativus</i>	+	+	+	+	+	+	+	+	+	+
2	<i>C. melo</i> subsp. <i>melo</i>	+	-	+	-	+	-	+	+	+	+
3	<i>C. melo</i> var. <i>conomon</i>	-	-	-	-	-	+	-	+	+	+
4	<i>C. melo</i> var. <i>acidulus</i>	-	+	-	-	-	-	+	+	+	+
5	<i>Cucurbita pepo</i> var. <i>pepo</i>	-	-	-	-	-	-	-	-	+	+
6	<i>C. pepo</i> var. <i>texana</i>	-	+	+	+	+	+	+	+	+	+
7	<i>C. pepo</i> var. <i>fraterna</i>	-	-	-	-	-	+	-	-	+	+
8	<i>C. maxima</i>	-	+	-	+	+	+	+	+	+	+
9	<i>Citrullus lanatus</i>	-	-	-	-	-	-	-	+	-	+
10	<i>Benincasa hispida</i>	-	-	+	+	+	+	+	+	+	+
11	<i>Luffa cylindrica</i>	-	-	-	-	-	-	+	-	+	+
12	<i>Lagenaria siceraria</i>	-	-	+	+	+	+	+	+	+	+

PC = general designation of *P. cubensis* isolates, coding = isolate number/year of collecting; origin of isolates: all from *Cucumis sativus*; ¹⁾ = France, ²⁾ = others Czech Republic; - = resistant reaction, no visible symptoms of sporulation or very sparse sporulation; + = susceptible reaction (moderate or abundant sporulation).

found to exhibit relatively high levels of resistance or tolerance to certain fungal and virus diseases (JARRET et al. 1997); however, there is limited information about the specificity of interactions between *Citrullus* spp.-*P. cubensis*. Available data were summarized by BAINS and SHARMA (1986), LEBEDA (1990, 1999), PALT and COHEN (1980) and THOMAS et al. (1987). The first clear pathotype-specific reaction pattern was identified in *C. lanatus* (THOMAS et al. 1987). The existence of this phenomenon was verified by inoculation with some European (LEBEDA and GADASOVÁ 2002) and Israeli (COHEN et al. 2003) isolates. For other *Citrullus* species, we lack information on interactions with various *P. cubensis* isolates. A broader screening of *Citrullus* species germplasm collections should be conducted to obtain information about patterns of variation for resistance. Presently, there are no data available on the genetics of resistance to this pathogen.

3.1.3 *Cucurbita*

The genus *Cucurbita* includes a relatively small group of species with the greatest taxonomic diversity in Mexico (LIRA-SAADE 1995; MERRICK 1995; SANJUR et al. 2002; WHITAKER and BEMIS 1964). Five species, *C. argyrosperma* C. Huber, *C. ficifolia* Bouche, *C. maxima* Duchesne, *C. moschata* Duchesne and *C. pepo* L., are used as horticultural crops; others are wild species (LIRA-SAADE 1995). Wild *Cucurbita* species are ecologically variable and form two groups, mesophytic and xerophytic species, with different geographic distributions (WHITAKER and BEMIS 1964). Only three species, *C. maxima*, *C. moschata* and *C. pepo*, have been reported as natural hosts of *P. cubensis* (PALT and COHEN 1980). *Cucurbita pepo*, native in North America and cultivated extensively in the Old World, is the most widely grown and polymorphic of the *Cucurbita* species. However, *C. pepo* germplasm generally is considered poor in genes for disease resistance (PARIS 2001). Limited research and breeding of *Cucurbita* species for resistance to *P. cubensis* have been conducted (LEBEDA 1992c; WHITAKER and ROBINSON 1986). A detailed analysis of the responses of 60 cultivars of *C. pepo* to three isolates of *P. cubensis* demonstrated large variation in compatibility, characterized differences among subspecies and botanical varieties, and suggested that host-parasite specificity between *C. pepo* and *P. cubensis* is controlled by race-specific factors (LEBEDA and KRÍSTKOVÁ 1993), but the genetics of resistance remains unknown.

Significant differences in resistance/susceptibility to downy and powdery mildews (*Erysiphe cichoracearum* DC., *Sphaerotheca fuliginea* (Schlecht.) Pollacci) among fruit morphotypes (PARIS 1989) have been reported. Although zucchini, cocozelle and vegetable marrow expressed high resistance to *P. cubensis*, they also had relatively high powdery mildew sporulation. Cultivars with the fruit types acorn, straightneck and ornamental gourd were quite susceptible to *P. cubensis*, but they were scored as resistant to powdery mildew in laboratory and field evaluations (LEBEDA and KRÍSTKOVÁ 2000). Pathotype-specificity has also been described for *C. maxima* and *C. moschata* (BAINS and SHARMA 1986; COHEN et al. 2003; THOMAS et al. 1987) and for *C. pepo* var. *fraterna* (L. H. Bailey) Filov (LEBEDA, unpubl. results). Some of these species (*C. maxima*, *C. pepo* and *C. pepo* var. *fraterna*) are included in the new version of the differential set (Table 1). Selected accessions of *C. moschata* have also been characterized for greenhouse and field susceptibility to *P. cubensis* (WESSEL-BEAVER 1993). Based on WESSEL-BEAVER'S (1993) results, it would be worthwhile to determine whether selected accessions of *C. moschata* might contribute new information to the expanded differential set. For a more detailed understanding of *Cucurbita*-*P. cubensis* interactions, more species and germplasm accessions must be screened. The results from screening of wild and weedy *Cucurbita* species suggest that the phenomenon of race-specificity is fairly widespread (LEBEDA and WIDRLECHNER 2003b).

3.1.4 *Benincasa*

There is only one species in the genus *Benincasa*, *B. hispida* (Thunb.) Cogn., native to southeastern China (ROBINSON and DECKER-WALTERS 1997). It has been described as a natural host of *P. cubensis* (PALT and COHEN 1980). Relatively few disease-resistance studies have been conducted with this species (DECKER-WALTERS 1998), and only limited experimental data are available on resistance of *B. hispida* to *P. cubensis*. Pathotype-specific resistance of *B. hispida* against *P. cubensis* was demonstrated by BAINS and SHARMA (1986) and confirmed by THOMAS et al. (1987), but basic information about the genetics of resistance is lacking.

3.1.5 *Luffa*

The genus *Luffa* includes seven species, four well-differentiated species of the Old World tropics and three rather similar Neotropical species (HEISER and SCHILLING 1990). Both domesticated species, *L. acutangula* (L.) Roxb. and *L. aegyptiaca* Mill. (syn. *L. cylindrica* M. Roem.), and two wild species, *L. echinata* Roxb. and *L. graveolens* Roxb., have their origins in Tropical Asia (RUBATZKY and YAMAGUCHI 1997); the other three wild species (*L. astorii* Svenson, *L. operculata* (L.) Cogn. and *L. quinquefida* (Hook & Arn.) Seem), are indigenous to South America (HEISER and SCHILLING 1990; ROBINSON and DECKER-WALTERS 1997). *Luffa acutangula* and *L. aegyptiaca* are described as natural hosts of *P. cubensis* (PALTÍ and COHEN 1980). Recent reports indicate severe attacks under field conditions by *P. cubensis* on *L. acutangula* and *Luffa* spp. from China (COHEN et al. 2003) and India (FUGRO et al. 1997). However, data on resistance of *Luffa* species to diseases, including *P. cubensis*, are limited (SINGH and SINGH 1998). JAMADAR and DESAI (1999) reported that none of the *L. acutangula* cultivars evaluated was resistant to *P. cubensis*. The first experimental studies demonstrating that *L. cylindrica* expressed pathotype(race?)-specificity to *P. cubensis* isolates were reported by BAINS and SHARMA (1986). This phenomenon was confirmed by THOMAS et al. (1987) for *L. acutangula* and *L. cylindrica*, and, more recently, has also been supported by LEBEDA and GADASOVÁ (2002). Data from more thorough screening of these two domesticated species are not available, nor is basic knowledge on resistance genetics.

3.1.6 *Lagenaria*

The genus *Lagenaria* was previously considered to be monotypic, but recently six species were recognized, including five wild species *L. abyssinica* (Hook. f.) C. Jeffrey, *L. breviflora* (Benth.) Roberty, *L. guineensis* (G. Don) C. Jeffrey, *L. rufa* (Gilg) C. Jeffrey and *L. sphaerica* (Sond.) Naud., native to Africa (ROBINSON and DECKER-WALTERS 1997). *Lagenaria siceraria* (Molina) Standl. (syn. *L. vulgaris* Ser. and *L. leucantha* Rusby) is used as a crop known as bottle gourd. There are two reasonably well-defined subspecies of *L. siceraria* (DECKER-WALTERS et al. 2001), showing intraspecific variation. *Lagenaria siceraria* and *L. sphaerica* are natural hosts of *P. cubensis* (PALTÍ and COHEN 1980), yet experimental data on the resistance of *Lagenaria* species to *P. cubensis* are limited. BAINS and SHARMA (1986) found only susceptible reactions to *P. cubensis*; in contrast, THOMAS et al. (1987) noted a high frequency of resistance or incomplete resistance after inoculation with six *P. cubensis* isolates that originated in Japan and Israel. Recent results by LEBEDA and GADASOVÁ (2002) and COHEN et al. (2003) showed a well defined, race-specific response. Although *L. siceraria* displays race-specific resistance, we do not know its genetic basis nor how other *Lagenaria* species might react to various *P. cubensis* isolates.

3.2 Characterization of a set of Cucurbitaceae taxa for differentiation of *P. cubensis* pathotypes

An improved set of differential cucurbit genotypes (Table 1) for use in the characterization of *P. cubensis* virulence variation was created from an existing set proposed by THOMAS et al. (1987) through the addition of more species and a clearer specification of membership through the identification of specific genebank accessions. The utility of the existing set was limited because of the following points: a) not all of the most important host genera of *P. cubensis* were included; b) not all genotypes were defined to the level of species (e. g., *Cucurbita*); c) specific accessions were not defined; and d) the set has not been maintained as a unit by at least one responsible institution making acquisition problematic.

The improved set meets all of the limitations noted above. It includes 12 taxa from the five most important host genera of Cucurbitaceae (Table 1), allowing a more precise specification of *P. cubensis* pathotypes. The new set was based on previously published results (BAINS and SHARMA 1986; LEBEDA 1992a,b, 1999; LEBEDA and KRÍSTKOVÁ 1993; LEBEDA and PRÁŠIL 1994; THOMAS et al. 1987) that demonstrated various details of host-parasite specificity and pathogen variation.

A preliminary validation of this set was conducted by testing 22 *P. cubensis* isolates collected in four European countries (LEBEDA and GADASOVÁ 2002). Ideally, we would also prefer to validate this set by using the pathotypes enumerated by THOMAS et al. (1987), but this has not been possible.

By using this new differential set (Table 1), at least 12 pathogenicity factors and their combinations could be determined. Some of these combinations and/or reaction patterns are shown in Table 2

(10 distinct patterns). On the basis of the data presented in Tables 1 and 2, the theoretical maximum number of pathotypes is given by 2^n (n = number of differential genotypes), i. e., 2^{12} pathotypes of *P. cubensis* could be recognized (LEBEDA and SCHWINN 1994).

An important feature of any differential set is the repeatability of test results. Standardized screening methodology is a key aspect of repeatability. Screening and evaluation protocols have fortunately been described in detail in earlier reports (LEBEDA 1986, 1991, 1992a,b; LEBEDA and GADASOVÁ 2002; LEBEDA and KRÍSTKOVÁ 1993) and have been followed in the development of this new differential set.

The plants were grown in a growth chamber in plastic pots filled with garden soil. Evaluations were conducted on leaf disks (20 mm in diameter) removed from adult plants that were about 6 to 8 weeks old. Five disks from one leaf and three plants per test were used. The leaf disks were placed on moist filter paper in Petri dishes or in plastic boxes of various sizes (e. g., 340×265 mm) and inoculated on their abaxial surface (LEBEDA 1991). The evaluation of sporulation intensity was rated 6, 8, 10, 12 and 14 days after inoculation following a 0–4 scale (LEBEDA 1986, 1992a). Recently, a similar scale and methodology were used by COHEN et al. (2003) to characterize a new Israeli pathotype. The reliability of this leaf disk inoculation bioassay for *Cucumis sativus* has also been recently confirmed by comparison with the results of field experiments (PETROV et al. 2000).

This host-differential set can be expanded to include new members, which may be discovered through future research on host-pathogen specificity and genetic control. Research in this area should lead to a comprehensive, well specified, host-differential set, not only for differentiation of pathotypes (variability of pathogenicity among different genera of Cucurbitaceae), but also of races (variability of pathogenicity within a single genus) of *P. cubensis*, such as made recently available for *Bremia lactucae* (ETTEKOVEN and AREND VAN DER 1999; LEBEDA and ZINKERNAGEL 2003).

3.3 Pathogen (*Pseudoperonospora cubensis*)

To date, few studies have focused on pathogenicity variation in *P. cubensis*. IWATA (1942) was probably the first who recognized the host specificity exhibited by different isolates of *P. cubensis*, conclusions supported by observations of HUGHER and VAN HALTEREN (1952). Later, PALTÍ (1974) analyzed the significance of divergence of the pathogen on various cucurbit hosts. THOMAS (1982) emphasized the need for international research to determine relationships among host specificities for downy mildew on cucurbits. BAINS and SHARMA (1986) used 18 genotypes from six genera of Cucurbitaceae as differential hosts to characterize races of *P. cubensis*. THOMAS et al. (1987) used these past results to develop the best characterized differential set at that time. This set allowed the first clear description of *P. cubensis* pathotypes based on host compatibilities between various isolates and different cucurbit taxa. Recently, COHEN et al. (2003) expanded this set by including two additional taxa (*Lagenaria* spp., *Luffa* spp.). All these studies relied upon a limited number of pathogen isolates from Japan, India, Israel and USA. Until recent reports by Lebeda and colleagues (LEBEDA 1999; LEBEDA and GADASOVÁ 2002), no European isolates of *P. cubensis* had ever been analyzed for pathotype variation.

Recent data, obtained with *P. cubensis* isolates originating from *Cucumis sativus* and *C. melo* showed that assigning variation to *formae speciales* could be misleading (LEBEDA and GADASOVÁ 2002), a limitation also noted by THOMAS et al. (1987). This limitation notwithstanding, our data do fully support initial reports about the existence of distinct physiological forms of *P. cubensis* (BAINS and SHARMA 1986; LEBEDA and KRÍSTKOVÁ 1993; THOMAS et al. 1987) that may be delimited based on host genus, species and infraspecific specificities. THOMAS et al. (1987) proposed six differentials to designate five *P. cubensis* pathotypes (1–5) with increasing virulence patterns. Twenty-two European isolates were recently divided into 13 groups according to their reaction patterns; i.e., at least 13 different pathotypes could be distinguished (LEBEDA and GADASOVÁ 2002). However, some new data demonstrate much more broader variability in pathogenicity of *P. cubensis* (e. g. isolates 24/01 and 39/01; LEBEDA, unpubl. results). Examples of some of these reaction patterns are presented in Table 2, demonstrating that European (mostly originating from the Czech Republic) isolates of *P. cubensis* are highly variable and differ from the five pathotypes described from Japan, Israel and the United States (THOMAS et al. 1987). Recently, LEBEDA and GADASOVÁ (2002) were unable to compare European isolates with the five pathotypes described by THOMAS et al. (1987). Those pathotypes could not serve

as controls because they were no longer available. Nevertheless, isolate PC 3/00 (Table 2) can be considered equivalent in its reaction pattern to Pathotype 1 of THOMAS et al. (1987).

On the basis of field evaluations of cucumbers, HOREJSI et al. (2000) hypothesized that Europe and the United States may harbor different races of *P. cubensis*; however, they found no conclusive evidence that races in these two continents differ. These authors noted that differences in downy mildew scores between locations may have been due to environmental and/or pathogen factors. But recently a set of cucumber cultivars and breeding lines (cultigens) was tested under field or greenhouse conditions for resistance to *P. cubensis* in sites in the USA, Poland, China, and India thought to differ in *P. cubensis* virulence. Individual cultigens differed significantly in their field or glasshouse resistance to downy mildew at different locations (SHETTY et al. 2002), providing good evidence for variation in the level of aggressivity of pathogen populations in different locations and for the probable existence of *P. cubensis* races on cucumbers. However, these results will need to be verified by standardized screening methodology (see discussion above). In addition, recent experimental results (LEBEDA and GADASOVÁ 2002; LEBEDA unpubl. results) have demonstrated that there are substantial differences in pathotype structure among various European locations (Czech Republic, France, the Netherlands, Spain), and we expect that must also be true for differences between continents.

Further attention should be directed to an examination of the world-wide distribution of downy mildew to determine where the pathogen is most diverse. For example, given our knowledge of natural host ranges, Central America, Africa, and China deserve close scrutiny. Are they centers of origin and diversity for host – parasite interactions? This question has only partly been answered for *Cucumis* spp. (LEPPIK 1966; LEBEDA 1992a). The study of population structure and genetics of Oomycetes is still in its infancy, and the centers of origin and diversity of only a few species are known (e. g., *Phytophthora infestans* (Montagne) de Bary, *Bremia lactucae*) (DRENTH and GOODWIN 1999). But a full understanding of the Cucurbitaceae – *P. cubensis* pathosystem will require such information, collected broadly throughout the world.

The description of pathotypes by THOMAS et al. (1987) serves as a starting point for a more formal, detailed description. General guidelines upon which more complete descriptions can be based are available (CATEN 1987; HERRMANN et al. 1999; JOHNSON 1999; LEBEDA and SCHWINN 1994; LIMPET et al. 1994; LIMPET and MÜLLER 1994; THOMAS et al. 1987). In this paper, we propose a new method for the designation of *P. cubensis* pathotypes, in the form of a mathematical code derived from a system developed by LIMPET and MÜLLER (1994) and LIMPET et al. (1994). The binary (–, +; see Table 2) results of leaf-disc bioassays are translated into unique numerical (tetrade) codes, on the basis of differential set groupings. These codes are comprised of three parts, each corresponding to the pattern resulting from three unique groups of four differentials (see Table 3). Within each group, numerical values of 1, 2, 4, and 8 are assigned to + results and then summed. The three sums are then presented

Table 3. Unique (tetrade) numerical codes for *Pseudoperonospora cubensis* pathotypes (Table 2) following the proposed differential set of Cucurbitaceae (Table 1)

Groups of differentials Differential number (Table 2) Value	1. <i>Cucumis</i> spp.				2. <i>Cucurbita</i> spp.				3. Other Cucurbitaceae				Unique code
	1	2	3	4	5	6	7	8	9	10	11	12	
Isolates (Test results)													
PC 3/00	1	2	0	0	0	0	0	0	0	0	0	0	3.0.0
PC 13/00	1	0	0	8	0	2	0	8	0	0	0	0	9.10.0
PC 1/88	1	2	0	0	0	2	0	0	0	2	0	8	3.2.10
PC 3/98	1	0	0	0	0	2	0	8	0	2	0	8	1.10.10
PC 1/98	1	2	0	0	0	2	0	8	0	2	0	8	3.10.10
PC 4/00	1	0	4	0	0	2	4	8	0	2	0	8	5.14.10
PC 12/00	1	2	0	8	0	2	0	8	0	2	4	8	11.10.14
PC 1/97	1	2	4	8	0	2	0	8	1	2	0	8	15.10.11
PC 24/01	1	2	4	8	1	2	4	8	0	2	4	8	15.15.14
PC 39/01	1	2	4	8	1	2	4	8	1	2	4	8	15.15.15

as a unique code, in the format (sum of group 1).(sum of group 2).(sum of group 3), which can serve as a identifier for each pathotype (Table 3). This coding system can be used to standardize communication. Of course, the addition of new differentials must be expected, which will result in changes to our coding system. Given this starting point, additional research and discussion will be required to establish an international standardization of *P. cubensis* pathotypes.

To help accomplish this, since 1996, the Laboratory of Plant Pathology (Department of Botany, Palacký University in Olomouc, Czech Republic), under the umbrella of the "National Programme of Genepool Conservation of Microorganisms and Small Animals of Economic Importance", has maintained a standard collection of well-characterized (country and place of origin, host species and genotype, pathogenic variation, etc.) isolates of *P. cubensis* (examples in Table 2). This collection is maintained under standardized, controlled conditions (LEBEDA 1986), and a reference set of well-characterized isolates is now being made freely available. The laboratory is monitoring and investigating the variability of pathogenicity in Czech and other European pathogen populations. It would be worthwhile to expand this collection to include all new pathotypes, along with those already defined by THOMAS et al. (1987), if possible.

Our newly proposed differential set of Cucurbitaceae (Table 1) should serve as an appropriate baseline for more detailed research of *P. cubensis* population structure and variation at the level of pathotypes throughout the world. It is evident that physiological specialization in European and other populations of *P. cubensis* is widespread and must be seriously considered in plant pathology research and in resistance breeding of cucurbitaceous crops around the world.

4 Recommendations

1. To advance our understanding, additional information based on extensive testing of the widest possible array of host and pathogen genotypes is required as a foundation for the continued improvement of the differential set. This information should also be of practical importance in disease-resistance breeding.
2. There is a need to establish efficient linkages and information exchange among research groups involved in this topic.
3. To this end, a network should be established among public and commercial institutions responsible:
 - a. for maintaining collections of host-differential genotypes and pathogen isolates;
 - b. for investigating this pathosystem and related topics; and
 - c. for Cucurbitaceae breeding and seed production.

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