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Cucurbit powdery mildews: methodology for objective determination and denomination of races

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Abstract Cucurbit powdery mildew (CPM), a disease on field and greenhouse cucurbit crops worldwide, is caused most frequently by two obligate erysiphaceous ectoparasites (*Golovinomyces orontii* s.l., *Podosphaera xanthii*) that are highly variable in their pathogenicity and virulence. Various independent systems of CPM race determination and denomination are used worldwide, having to date been differentiated on different cultivars or lines of melon (*Cucumis melo* L.). We briefly review historical perspectives and the current state of understanding of the virulence variation of the two CPM pathogens at the pathogenic race level, their differentiation and their designation. Furthermore, we propose for use by the international CPM research, breeding, seed and production community new tools to enhance research, communication and management of CPM. These tools consist of five components: 1) a set of 21 differential genotypes of *Cucumis melo* L. for the identification of CPM races; 2) a triple-part, septet code for meaningful, concise designation of CPM races; 3) protocols for maintaining CPM isolates and differential

genotypes and for laboratory assays to examine the virulence of CPM isolates; 4) rules and principles of practical application of this system in breeding, seed production and cucurbit growing, including a proposal of a race denomination suitable for practical application; and 5) crucial activities leading to the implementation and running of new tools for CPM research and management. The five components of this package have equal importance, forming a compact system, and none of them can be omitted.

Keywords *Golovinomyces orontii* s.l. · *Podosphaera xanthii* · *Cucumis melo* · Virulence variability · Race-specificity · Septet code

Introduction

Cucurbit powdery mildew (CPM) is a serious disease of field and greenhouse cucurbit crops worldwide (Sitterly 1978; McGrath and Thomas 1996), causing reduction in plant growth, premature desiccation of the leaves and consequent reduction of the quality and marketability of the fruits. Observations from the last 20 years have revealed, that CPM 1) can start to develop on cucurbits early in crop production, often before or by the start of harvest period; 2) occurs in growing areas where it was not previously reported; and 3) host crops can be quickly colonized (Cohen et al. 2004; Lebeda et al. 2009).

The world growing area of cucurbits both in the field and under cover of 8.6 million ha is almost two times higher than the growing area of tomatoes (4.6 million

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ha) (FAOSTAT 2014). The economic and ecologic impacts of disease considered from either the viewpoint of yield losses, or the viewpoint of chemical protection of plants by fungicides are enormous (Sedláková and Lebeda 2008; Lebeda et al. 2010b).

Among three species (fam. Erysiphaceae) known to cause CPM (Braun 1995), *Leveillula taurica* (Lev.) Arnaud is considered to be of minor economic importance, while *Golovinomyces orontii* (Castagne) Heluta (Braun and Cook 2012) and *Podosphaera xanthii* (Castag.) U. Braun & N. Shish. (Shishkoff 2000) can heavily infect cucurbit crops with adverse ecologic and economic consequences (Braun 1995; Pitrat et al. 1998). *Podosphaera xanthii* (*Px*) (Castagne) U. Braun & Shishkoff (previously named *Sphaerotheca* (*Podosphaera*) *fusca* emend. (*s. lat.*) (Braun 1987), *Sphaerotheca fuliginea* f. *cucurbitae* Jacz.) is common in subtropical and tropical areas and greenhouse crops, while *G. orontii* (*Go*) (previously reported as *Golovinomyces cichoracearum* (DC.) V.P. Gelyuta (Vakalounakis and Klironomou 2001) occurs more frequently in temperate and colder areas under field conditions (Křístková et al. 2007). *Podosphaera xanthii* is the dominate pathogen in the USA where the climate is mostly temperate (McCreight et al. 2012). *Go* and *Px* occur singly or together on cucurbits in Central Europe (Lebeda 1983; Křístková et al. 2007; Lebeda et al. 2007b). Both species can be easily identified based on conidia morphology (Lebeda 1983). The broad host ranges of the two species are not completely identical, covering several different families and/or genera (Braun 1995; Braun and Cook 2012), and they differ also by variability in response to fungicides (McGrath 2001; Sedláková and Lebeda 2008; Lebeda et al. 2010b).

Cucurbit isolates of *Go* and *Px* cross-infect all cucurbit species and genera (Lebeda et al. 2007a), and their highly variable pathogenicity and virulence are manifested by the existence of large number of different pathotypes and races (Bertrand et al. 1992; Lebeda et al. 2004, 2007a; Lebeda and Sedláková 2006; McCreight 2006; McCreight et al. 2005; Sedláková et al. 2014). The relatively recent and widespread pathogenicity of *Px* on watermelon (*Citrullus lanatus* L.) worldwide (McGrath and Thomas 1996; McGrath 2001; Davis et al. 2002), increased the number of races of *Px* on melon (Coffey et al. 2006; McCreight 2006)

and *Go* on *Cucurbita* spp. (Lebeda and Sedláková 2006).

The most comprehensive survey of CPM populations was done from 2000 through 2007 in the Czech Republic. Virulence analysis of more than 400 isolates of both species revealed: 1) the species spectrum in Czech Republic differs from other European countries (Křístková et al. 2009); 2) the virulence structure of Czech CPM populations is different from that in France and Spain (comparable data from other European countries are not available), and from that in other countries outside of Europe; 3) a large number of new pathotypes and races are present in Czech Republic; and 4) Czech CPM populations vary temporally and spatially (Lebeda and Sedláková 2004, 2006, 2010; Lebeda et al. 2004, 2007a, 2009; Sedláková et al. 2014).

Breeding of cucurbits for resistance to CPM is hindered by the lack of clear and uniform descriptions of the genetic variation in the virulence of the CPM pathogens (Bardin et al. 1997, 1999; McCreight 2003, 2006; Lebeda and Sedláková 2006; Lebeda et al. 2007a). For melon, this is due in part to the diverse genetic pool from which many sources of genetic resistance have been identified (Pitrat et al. 1998; McCreight 2003, 2006). It has been suggested that a large fraction of the reported races are not relevant to most *Px* resistance breeding, which may have to be done on a regional basis for subsets of races. Only a limited number of races highly harmful to cucurbit crops are distributed over a large area (McCreight et al. 2012).

Knowledge of the cucurbit–powdery mildew pathosystem is very limited (Jahn et al. 2002). Such studies have been very rare (Lebeda et al. 2007a), but are considered important from both the theoretical genetic and the applied resistance breeding viewpoints (Lebeda et al. 2006). We have limited knowledge about the genetics of resistance to *Go* and *Px* in cucurbits (Pitrat et al. 1998; McCreight 2006), and no knowledge about the genetic basis for any trait of *Go* or *Px*, including the molecular basis for physiological races of *Go* and *Px* (Montoro et al. 2004). Adoption of an objective, efficient, uniform, comprehensive system for race designation would create a basis for the application of population biology and genetic studies of *Go* and *Px* (Lebeda 1982).

The increasing international trade of cucurbits (fruit and seed) warrants a coordinated, standardized, uniform system for further development and exchange of information on CPM races to facilitate optimal and strategic

control of CPM. The need to elaborate a sophisticated and functional system to understand genetic background of the CPM pathosystem and to produce elite CPM-resistant germplasm adapted to diverse cucurbit production systems worldwide has been highlighted to scientific and breeder communities (McCreight and Pitrat 1993; Pitrat et al. 1998; McCreight 2006; Lebeda and Sedláková 2006; Lebeda et al. 2007a, 2008, 2010a). This paper focuses on methodology for objective determination and denomination of CPM races exclusively, the topic of pathotypes (in sensu Holliday 1998; Lebeda et al. 2008, 2011) is not treated here.

Scientific platform for CPM

Pathogenic specialization in cucurbit powdery mildew was described by Sitterly (1978) and Thomas (1988). Host plant–powdery mildew interactions very often clearly express the compatibility or incompatibility (Lebeda and Sedláková 2010), which allows classification of CPM races based on patterns of compatible or incompatible reactions on the differential hosts (genotypes). The CPM pathosystem is likely to be similar to other host–powdery mildew interactions that are based on the principles of gene-for-gene interactions (Brown 2002), e.g., *Blumeria graminis* in cereals (Li et al. 2013).

Important issues when investigating host-parasite interactions postulated by Lebeda and Widrlechner (2003) are: 1) general knowledge about host-pathogen variability and specificity of the interaction; 2) knowledge about host-pathogen genetics; and 3) availability of a theoretical model explaining the genetics of host-pathogen interaction.

The details about the most important host genera (*Cucumis*, *Cucurbita* and *Citrullus*), including their genetic variation were summarized recently by Lebeda et al. (2007b). Current knowledge about the genetics of CPM host-pathogen interactions is limited and unclear (Jahn et al. 2002), with the exception of *C. melo* and *Px* (McCreight et al. 1987; McCreight 2006; McCreight and Coffey 2011).

The most recent review of sources of resistance and their genetic control to CPM was published by Jahn et al. (2002). Gene lists for economically important Cucurbitaceae including the known genes for resistance to *Go* and *Px* are maintained and periodically updated under the auspices of the Cucurbit Genetics Cooperative

(USA): cucumber (*Cucumis sativus* L.) (Call and Wehner 2010–2011), melon (*Cucumis melo* L.) (Dogimont 2010–2011), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) (Wehner 2012–2013), *Cucurbita* spp. (Paris and Kabelka 2008–2009), and other genera of Cucurbitaceae (Van den Langenberg and Wehner 2012–2013).

Virulence differentiation and race concepts

Powdery mildew pathogenicity, including for CPM, is most frequently expressed as virulence using a binary system (compatible vs. incompatible reaction) (Bardin et al. 1999; Brown 2002; McCreight 2006; Lebeda and Sedláková 2006; Lebeda et al. 2008). Interactions between most cucurbits and *Go* or *Px* are pathotype- and/or race-specific (Bardin et al. 1999; Křístková and Lebeda 1999; Jahn et al. 2002; Lebeda et al. 2007a, 2008). Patterns of virulence reactions on differential genotypes are the basis for identification and denomination of *Go* and *Px* races of melon (Pitrat et al. 1998; Bardin et al. 1999; Lebeda and Sedláková 2006; Lebeda et al. 2007a, 2008).

Description of the CPM pathosystem began with a simple number system and three differentials in the 1920s in response to the appearance of *Px* race 2 (Jagger et al. 1938; McCreight 2004). A new melon differential and an accompanying numbered race (race 3) were described 50 years later (Thomas 1978). Various, independent systems of CPM race determinations and denominations are in use worldwide (Lebeda et al. 2011). The six races known on melon by 1998 had simple numerical designations that reflected their order of discovery, or as in the case of race 0, their unique reaction on the known melon differentials (Pitrat et al. 1998). Numeric denomination of races was used by Thomas (1988), Bertrand (1991), Bertrand et al. (1992) Genetic variation in CPM races on melon reported from the 1980s through the present has been designated with alphabetic (Křístková and Lebeda 1999; Lebeda and Sedláková 2006), or alphanumeric (McCreight et al. 1987; Floris and Alvarez 1995; Alvarez et al. 2000; Hosoya et al. 2000; Bertrand 2002; Cohen et al. 2004) denominations (Table 1).

The most frequently cited set of melon differentials includes 11 genotypes (Bertrand 1991; Bertrand et al. 1992; Lebeda and Sedláková 2006; McCreight 2006; Lebeda et al. 2011) that can differentiate CPM races

Table 1 Race differential sets (in chronological order) used for cucurbit powdery mildew incited by *Podosphaera xanthii* (*Px*) and *Golovinomyces orontii* (*Go*) on melon (*Cucumis melo*) (modified according to Lebeda et al. 2011)

<i>Px</i> and <i>Go</i> races differentiated	No. geno-types	<i>C. melo</i> genotype(s)	References
<i>Px</i> races 1 and 2	1	PMR 45	Jagger et al. (1938)
<i>Px</i> races 1, 2, and 3	5	Hale's Best Jumbo, PMR 45, PMR 5, PMR 6, Edisto 47	Thomas (1978, 1988)
<i>Px</i> races 1, 2, and 3	10	Delicious 51, Top Mark, Védrañtais, PMR 45, PMR 450, PMR 6, Perlita, PI 124111, PI 124112, Seminole	McCreight et al. (1987)
<i>Px</i> races 1 and 2	4	Piel de Sapo, PMR 45, PMR 5, PI 124112	Vakalounakis and Klironomou (1995)
<i>Px</i> races 0, 1, 2U.S., 2F, 3, 4, 5	11	Iran H, Védrañtais, Top Mark, Ananas, PMR 45, PMR 5, WMR 29, Edisto 47, PI 414723, MR-1, PI 124112	Bardin et al. (1999); Bertrand (1991); Bertrand et al. (1992); Jahn et al. (2002); Lebeda and Sedláková (2010); Pitrat et al. (1998)
<i>Go</i> races N, N ^S , O, P, R, R ^S , S, V, Y, Z, a, and <i>Px</i> races 2US, B, C, F, G, H	11	Iran H, Védrañtais, Solartur, PMR 45, PMR 5, WMR 29, Edisto 47, PI 414723, MR-1, PI 124112, Nantais Oblong	Křístková and Lebeda (1999); Lebeda and Sedláková (2004, 2006); Lebeda et al. (2004, 2007a)
<i>Px</i> races 2F, 2Z	11	Doublon, Rochet, PMR 45, PMR 5, Edisto 47, WMR 29, PI 124112, PI 414723, Negro, BG 6011, BG 6016	Alvarez et al. (2000)
<i>Px</i> races 1, 2F, 2U.S., 3, 4, 5, N1, N2, N3	10	Fuyu 3, PMR 45, PMR 5, WMR 29, Edisto 47, PI 414723, Hainan 21, Quincy, Earl's Knight Natsu 2, Earl's Miyabi Natsu 2	Hosoya et al. (2000)
<i>Px</i> races 1 and 2	8	PMR 45, PMR 5, WMR 29, Edisto 47, PI 313970, PI 124111, PI 124112, PI 414723	McCreight (2003)
<i>Px</i> races 1, 2 and 5	8	Fuyu 3, PMR 45, PMR 5, WMR 29, Edisto 47, MR-1, PI 124112, PI 414723	Kuzuya et al. (2006)
<i>Px</i> races 1 and 2	11	Iran H, Védrañtais, Top Mark, PMR 45, PMR 5, WMR 29, Edisto 47, PI 414723, MR-1, PI 124111, PI 124112	McCreight (2006)

originating from melon (McCreight 2006) and other cucurbits, e.g., cucumber, *Cucurbita* spp. and watermelon (Lebeda and Sedláková 2006, 2010; Lebeda et al. 2004, 2007a, 2008, 2011) (Table 1).

Proposed system for race identification and denomination

We propose, therefore, a CPM race system based on the intraspecific genetic variability of melon (race-specific resistance). This system of CPM race determination and denomination is based upon three components: 1) standard set of race differentials (Lebeda et al. 2008); 2)

uniform code for the host-CPM interactions/scores (Lebeda et al. 2008, 2011; Sedláková et al. 2014); and 3) a uniform screening methodology (Lebeda and Sedláková 2010). Guidelines for more complete denominations of races are available in the phytopathological literature, e.g., Limpert et al. (1994).

Set of CPM race differentials

A set of 21 differential melon genotypes (Table 2) is proposed for differentiation of *Go* and *Px* races because they show enough variation and differentiation capacity for both CPM species (Lebeda and Sedláková 2006; Lebeda et al. 2007a, 2008, 2012; McCreight 2006;

Sedláková et al. 2014). This enlarged set is based on the set of melon CPM differentials developed by French investigators (Bertrand 1991; Bertrand et al. 1992) (Table 1), and is supplemented with 10 additional melon genotypes that revealed new *Px* and *Go* races (Hosoya et al. 2000; Bertrand 2002; Lebeda and Sedláková 2006; Longzhou et al. 2008; McCreight 2006; Lebeda et al. 2007a, 2008, 2011; McCreight et al. 2012; Sedláková et al. 2014).

Coding system for CPM races designation and denomination

The proposed numerical system for designation of races is derived from “coded triplets,” a system for designation of pathotypes of plant pathogens (Limpert et al. 1994; Limpert and Müller 1994). The binary results of CPM assays, i. e., compatible (+) and incompatible (–) reactions, are translated into unique triplet codes that serve as identifiers for races of *Go* and *Px*.

CPM race differentials within this system are arbitrarily divided into three groups, called triplets, each containing seven differentials (Table 3). The differentials are assigned an arbitrary, permanent order within a respective group. A value of 1, 2, 4, 8, 16, 32, or 64 is attributed to the differentials in relation to their order within each group (Table 3). The binary results of any CPM assay are then translated into a triplet-septet code. Each differential that is susceptible (compatible) to a given CPM isolate contributes to this code by defined value of 1, 2, 4, 8, 16, 32, or 64, according to its position in the group, and resistant (incompatible) differentials are assigned a score of zero (0). The three sums are then combined to form a unique triplet code in the format: group 1 sum.group 2 sum.group 3 sum. Examples of the translation of disease reactions (compatible and incompatible) to triplet values composition (construction) of triplet-septet codes for four hypothetical CPM isolates are presented in Table 3.

Flexibility of the system

The proposed system for CPM race determination and denomination is open and flexible to reveal unique virulence patterns and designation of new races of each pathogen in the future. Addition of new differential genotypes should be approved by the international research and breeding community. New differential genotypes can be easily added to the current accepted

differential set. Actually, they would compose the fourth (fifth, etc.) septet group, and addition of new differential genotypes is theoretically unlimited. The only principle that must be strictly kept is, that once a differential is accepted into the differential set, the composition of differentials and their positions within septets are fixed. This rule is essential in order to compare virulence patterns over time. Such a system is dynamic and open, and avoids the current, chaotic situation that resulted from non-managed changes in differential genotypes and approaches for CPM race determination and denomination.

Methods for maintenance of CPM isolates

Long-term cryo-preservation of CPM isolates has been successful for *Px*, but not for *Go* (O'Brien and Weinert 1994; Pérez-García et al. 2006; Bardin et al. 2007). Short-term conservation methods for both species require maintenance of isolates on the primary leaves of susceptible host seedlings and serial transfers at 14-day intervals (Lebeda 1986; Lebeda and Sedláková 2010). Maintenance of a large number of isolates is time- and space-intensive.

Methods for maintenance of differentials

Regeneration of melon race differential genotypes should respect the floral biology of the species. Melon plants are usually monoecious or andromonoecious, bearing staminate and pistillate flowers, pollinated by insects. Differentials should not be hybrids to ensure their ease of seed production and continued availability. Basic rules and regeneration protocol of melon given by George (1999) include appropriate technical isolation of each genotype to avoid cross-pollination. Flowers are pollinated manually (sib-pollination rather than self-pollination), or by insects (honey-bees, bumble-bees). It is recommended to use at least 10–15 plants per accession for regeneration to minimize the genetic drift (Lebeda et al. 2007b; Anonyme 2011). Harvested seeds are dried, cleaned and stored in hermetically sealed bags or glass jars at a temperature of -18°C . The ultra-dry seed method elaborated by Gómez-Campo (2006) is also recommended. The genetic identity of differentials should be characterized and confirmed after regeneration by molecular marker analysis (Fernandez-Silva et al. 2008; Esteras et al. 2013).

Table 2 Proposed CPM race differential genotypes (*Cucumis melo* L.) by group number

Differential genotype			Origin	
Group.No	Cultigen/Accession	Other designation(s) ^a	Source	Country
1.1	Iran H	–	INRA	Iran
1.2	Védrantais	M 319	INRA	France
1.3	PI 179901	Teti	USDA	India
1.4	PI 234607	Sweet Melon	USDA	South Africa
1.5	AR HBJ	AR Hale's Best Jumbo	USDA	USA
1.6	PMR 45	M 321	USDA	USA
1.7	PMR 6	Ames 26810	USDA	USA
2.1	WMR 29	M 322	USDA	USA
2.2	Edisto 47	NSL 34600	Clemson Univ.	USA
2.3	PI 414723	LJ 90234	USDA	India
2.4	PMR 5	Ames 26809	USDA	USA
2.5	PI 124112	Koelz 2564	USDA	India
2.6	MR-1	Ames 8578	USDA	USA
2.7	PI 124111	Koelz 2563	USDA	India
3.1	PI 313970	90625 VIR 5682 PI 315410	USDA	India
3.2	Noy Yizre'el	–	Bar Ilan Univ.	Israel
3.3	PI 236355	–	USDA	England
3.4	Negro	–	Univ. Zaragoza	Spain
3.5	Amarillo	–	Univ. Zaragoza	Spain
3.6	Nantais Oblong	M 320	INRA	France
3.7	Ames 31282	PI 134198	USDA	China

Differential genotypes of *C. melo* listed are maintained by Dept. Botany, Palacký University, Olomouc (Czech Republic), and by ARS, USDA, Salinas, California (USA)

M 319 – 322 original designation by M. Pitrat, INRA, Montfavet, France; provided to A. Lebeda in 1997, *INRA* L'Institut National de la Recherche Agronomique, Montfavet (France), *USDA* United States Department of Agriculture, Agricultural Research Service; info. on USDA accessions available on the website of the National Genetic Resources Program, Germplasm Resource Information Network (GRIN), http://www.ars-grin.gov/ngs/acc/acc_queries.html

^a Name or additional accession number in the germplasm database or in working collection

Screening and evaluation protocols

Screening and evaluation protocols have been described in detail (Lebeda 1984; Lebeda and Sedláková 2010), and followed in the development of this new differential set. The most essential points related to the screening methodology are summarized herein.

Race differentials (Lebeda et al. 2008, 2011) and the universally susceptible cucumber 'Marketer 430' (Lebeda 1984), or another highly susceptible genotype of cucumber or melon, are grown in pots filled with substrate suitable for healthy growth and development,

and watered and fertilized accordingly (Lebeda and Sedláková 2010). Plants are grown in a location free of CPM inoculum.

Virulence of isolates is determined by using the leaf-disc method (Lebeda and Sedláková 2004, 2010). Leaf discs, 15 mm in diam., are taken from 6 to 8 week-old, CPM-free plants of the differentials; 3–5 leaf discs per plant per genotype; three plants per differential per test. The leaf discs are placed on moist filter paper in clear plastic boxes and inoculated by tapping conidia, produced on a highly susceptible genotype e.g., 'Marketer 430', onto their adaxial surfaces (Lebeda 1984).

Table 3 Examples of triplet-septet codes for four CPM (*G. orontii*, *P. xanthii*) hypothetical isolates based on their reactions of CPM race differentials in three septet groups

Hypothetical isolate	Triplet																					Triplet-septet code
	1							2							3							
	Differential							Differential							Differential							
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	
Triplet value							Triplet value							Triplet value								
	1	2	4	8	16	32	64	1	2	4	8	16	32	64	1	2	4	8	16	32	64	
#1																						
Reaction	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Score	1	2	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.0.0
#2																						
Reaction	+	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	
Score	1	2	4	8	16	0	0	0	0	0	0	0	0	64	1	0	0	0	0	32	0	31.64.33
#3																						
Reaction	+	+	-	-	+	-	-	-	-	-	-	+	-	-	+	-	+	+	+	+	+	
Score	1	2	0	0	16	0	0	0	0	0	0	16	0	0	1	0	4	8	16	32	64	19.16.125
#4																						
Reaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Score	1	2	4	8	16	32	64	1	2	4	8	16	32	64	1	2	4	8	16	32	64	127.127.127

Compatible (+) reactions result in a score equal to the code value for the respective differential in each septet group; incompatible (-) reactions are scored zero. The sum of the seven scores in each group is the code for the respective triplet, and the septet scores are combined to form a triplet code for the isolate

Based on a binary evaluation of susceptible/resistant reaction patterns (+ or -) of a certain isolate of cucurbit powdery mildew (*Go*, *Px*), a numeric triplet-septet code is created for each isolate (see Table 3, column Triplet-septet code), on the basis of differential set groupings (Groups 1 - 3). The code comprises three parts, each corresponding to one of three groups of seven differentials (Table 3, nos 1-7 in each group). Within each group, numerical values of 1, 2, 4, 8, 16, 32 and 64 are assigned to + results and then summed. The three sums are then presented as a code, in the format (sum of group 1).(sum of group 2).(sum of group 3), which serve as a identifier for each isolate/race (Table 3)

Inoculated discs are incubated in growth chambers set at 24 °C/18 °C day/night and 12 h photoperiod (McGrath 1994; Lebeda and Sedláková 2010).

Sporulation intensity (also called degree of infection, DI) is evaluated 6, 8, 10, 12 and 14 days post-inoculation using a 0 to 4 scale (Lebeda 1984; Lebeda and Sedláková 2010). Genotypes with no or low sporulation (DI=0-1) are considered to be resistant, whereas genotypes with a DI=2-4 are scored as susceptible. Details of this methodology were described by Lebeda and Sedláková (2010).

An important feature of the leaf disc-based protocol is the uniformity of the test conditions and, therefore, repeatability of results. Whole plant assays should always be done where possible to confirm leaf disc assays, and include complete sets of the race differentials.

Practical application of proposed system in cucurbit breeding, and seed and crop production

A case study for the application of the newly proposed approach for characterization and denomination of races of cucurbit powdery mildews that was done in the Czech Republic revealed an enormous number of different reaction patterns and spatio-temporal fluctuations (Lebeda et al. 2012; Sedláková et al. 2014). Within the large number of *Px* races reported from 2002 through 2011 from California and Arizona (USA), or races of *Go* reported from Central Europe/Czech Republic (Lebeda and Sedláková 2006; Lebeda et al. 2007a; Sedláková et al. 2014), only selected races should be relevant for resistance breeding (McCreight et al. 2012).

Practical application of the proposed system to breeders, seed producers and growers has been inspired by the approach of the International *Bremia* Evaluation Board (<http://www.worldseed.org/isf/ibeb.html>). CPM isolates being collected each year in the most important cucurbit production areas should be tested on the differential set in order to confirm the novelty of their reaction patterns. When a specific and novel reaction pattern appears repeatedly in several countries and over several years the international board (committee) for CPM (to be established) will: (i) identify isolates with this pattern as a threat to the cucurbit industry, and (ii) define a new race and denominate an isolate for this new race. The “type isolate” for the new race should at that time be increased for long-term cryopreservation to ensure its availability for future reference and research.

Denomination of cucurbit powdery mildews (CPM) races for practical purposes

When we were thinking of the most suitable approach to construct the formulas for CPM race denomination for the praxis, we considered the following three criteria: i) the name of pathogen species; ii) the name of the cucurbitaceae host plant species; and iii) “historical” or traditional indications of CPM races (see Table 1).

This proposal to construct the formulas for CPM races is based on combining three components (abbreviations):

- i) abbreviations **Px** for *Podosphaera xanthii* and **Go** for *Golovinomyces orontii*, regardless of possible changes in CPM taxonomy and nomenclature in the future.
- ii) abbreviation **mel** for differential plant species *Cucumis melo*. We can suppose that in the future a similar system for race determination will be elaborated for other economically important cucurbits, at least for *Cucumis sativus*, *Cucurbita* spp., *Citrullus lanatus*; for these systems the abbreviations **sat**, **crb** (eventually **pep**, **max**, **mos**), **lan** can be used, respectively. The proposed abbreviations will likely be relatively stable after potential taxonomical changes e.g., *Cucumis melo* versus *Melo sativus* for melon.
- iii) numbers of **1, 2, 3, etc.** given chronologically to internationally accepted (acknowledged) races

identified by the differential set given in Table 2. It is impossible to simply adopt previously used race denominations because various differential sets were used (Table 1). This new numbering (1, 2, 3, ...) of CPM races has no relationship to previously used designations (Table 1).

Denomination of races (construction of formulas) We propose the following formula for both pathogens, starting by **Pxmel 1** and **Gomel 1**. It is evident that reaction patterns expressed by triple-part septet code of **Pxmel 1** will differ from **Gomel 1**, and both series of CPM races, i.e., **Pxmel** and **Gomel** will be developed independently of each other.

Urgent requirements and conclusion

The adoption of the proposed system is based on the following three, equally important components that form a complete system, none of which can be omitted.

1. Differential hosts
 - a. Establish a clearly described set of differential host genotypes.

The current set of melon race differentials expanded in an ad hoc manner since the appearance of race 3 and subsequent new races were observed. This set of melon differentials is imperfect in that many of them possess more than one resistance gene (Pitrat et al. 1998). A set of monogenic differentials would be ideal but is not currently available. However, all recently involved differentials (Table 2) showed, in extensive screening by both pathogens (*Go* and *Px*), various and clearly distinct race-specific reaction patterns.
 - b. Conserve the differentials in an active germplasm collection or repository.

The U.S. Dept. of Agriculture, Agricultural Research Service, Genetic Resources Information Network (GRIN) maintains extensive working and backup collections of most crop species and their “wild” relatives under standard storage and operating conditions (Clark et al. 1991).

- c. Freely distribute the differentials upon request to Cucurbit researchers, breeders (to include seed production specialists) and diagnosticians for their multiplication and use.
2. Pathogen (*Podosphaera xanthii*, *Golovinomyces orontii*)
 - a. Accept the unified system of determination, description and denomination of races for each CPM species; including standardization of screening methodology. Except for detailed, scientific denomination of races (Table 3), the simple race coding system described above (Denomination of CPM races for practical purposes) could be used in breeding, seed industry and fruit production. Only the most important isolates of each pathogen will be selected to receive the simple race designation; these must be agreed by board of breeding and seed producing companies, as it is done by the IBEB for *Bremia lactucae* (Anonyme 2014).
 - b. Establish a standard collection of well-characterized (geographic origin, country and place of origin, host species and genotype, pathogenic variation, etc.) isolates of each CPM species. Molecular markers should, where possible, be used to confirm species identity of every isolate.
 - c. Conserve these isolates under monitored, standardized and controlled, if possible cryogenic conditions.
 - d. Share race isolates freely, within the limits of international and national phytosanitary controls in order to reduce the risk of the evolution and spread of new pathotypes/races for their examination by researchers and breeders (to include seed production specialists), and diagnosticians.
 3. Cucurbit experts
 - a. Establish a network of public and commercial researchers and breeders to coordinate the maintenance, characterization and distribution of race differentials and, subject to regulatory constraints, pathogen isolates.

- b. Investigate this pathosystem and related topics, for cucurbit breeding, and seed and crop production.
- c. Advance our understanding, additional information based on extensive testing of the widest possible array of host and pathogen genotypes.

There remains a need for additional discussion among the international community of CPM researchers and cucurbit breeders about these proposals. Moreover, the focus of this paper has been influenced or restricted by the authors' knowledge of the cucurbit production systems in Europe and the U.S. In addition, it is our intent to address in the future the many aspects of CPM assay protocols, international standardization, cooperation and coordination of activities related to CPM pathogenicity variation.

The crucial questions to be answered are, who (which institution) can take the responsibility to coordinate the activities of the CPM network, and what will be its *modus operandi*?

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Compliance with ethical standards

Conflict of interest There are no potential conflicts (financial or non-financial) of interest.

Human and animal rights Research did not involve Human Participants and/or Animals

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