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Comparative Morphological Studies on Tomato Powdery Mildew *(Oidium neolycopersici)*

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Fourteen isolates of tomato powdery mildew *(Oidium neolycopersici)* and one isolate of the following species: *Podosphaera fusca (= Sphaerotheca fusca), Erysiphe orontii* (cucumber powdery mildews), *Erysiphe cichoracearum* (lettuce powdery mildew) and *Erysiphe aquilegiae* var. *ranunculi* (*Ranunculus lingua* powdery mildew) were used for comparative morphological studies. Basic characteristics of the anamorphs, including outer conidial wall patterns, were compared using light and scanning electron microscopy (SEM). In main morphological features, *O. neolycopersici* was strongly differentiated from *E. cichoracearum, E. orontii* and *P. fusca.* However, based on morphological features (e.g. germination type; appressorium shape; morphology of conidiophores) *O. neolycopersici* was close to *E. aquilegiae* var. *ranunculi* (both belong to *Oidium* subgen. *Pseudoidium*) and it probably could be placed to *Erysiphe* sect. *Erysiphe* (= *Erysiphe* s. str.)

Keywords: Erysiphales, *Erysiphe* spp., anamorph, morphology, cluster analysis, scanning electron microscopy.

Several powdery mildew species have been reported on tomato (cf. Mieslerová and Lebeda, 1999; Mieslerová et al., 2000): *Leveillula taurica* (Lév.) Arnaud, 1921 [*Oidiopsis taurica* (Lév.) Salmon] occurs only in warmer regions (Palti, 1988), and it is easily distinguished from the other powdery mildews by the presence of branched conidiophores growing through the stomata. *Podosphaera fusca* (Fr.) U. Braun and N. Shishkoff, 2000 [*Sphaerotheca fusca* (Fr.) Blumer, 1933, emend. Braun, 1985] (Braun and Takamatsu, 2000), one of the main powdery mildews of the Cucurbitaceae, has also been found on tomatoes in the Netherlands (Stolk and Cools, 1983) and Bulgaria (Angelov and Georgiev, 1993; Georgiev and Angelov, 1993). This species is distinguished from other powdery mildews by the presence of fibrosin bodies in the conidia. Species of *Oidium* (including *O. neolycopersici*), different both morphologically and biologically. Detailed study of Kiss et al. (1999, 2001) using morphological and molecular phylogenetic analyses revealed the existence of two tomato powdery mildew species of the anamorphic genus *Oidium. Oidium lycopersici* Cooke and Massee (Cooke and Massee, 1888, emend. Noordeloos and Loerakker, 1989), with catenate conidia (euoidium type;

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Oidium subgen. *Reticuloidium*) was recorded in Australia (Cooke and Massee, 1888) and is probably limited to this continent. *Oidium neolycopersici* L. Kiss (Kiss et al., 2001), producing conidia singly (pseudoidium type) is widespread in Europe, Africa, North and South America and Asia. Reports of ability of *O. neolycopersici* to infect cucurbitaceous species (Fletcher et al., 1988; Corbaz, 1993; Lebeda and Mieslerová, 1999a) and wide host range of *Erysiphe orontii* Cast. 1851 emend. Braun, 1987, including members of the family Solanaceae, has led to previous hypothesis that *O. neolycopersici* is related to *E. orontii* (Fletcher et al., 1988; Corbaz, 1993). However, recent molecular analyses (Jones et al., 1999, 2000; Kiss et al., 2001) have shown that *O. neolycopersici* is phylogenetically close to *Erysiphe aquilegiae* var. *ranunculi* (Grev.) Zeng and Chen, 1981.

The objective of the study reported here was to determine differences between *O. neolycopersici, Erysiphe aquilegiae* var. *ranunculi, E. cichoracearum* DC., 1805, *E. orontii* and *Podosphaera fusca* based on morphology of the anamorph, including scanning electron microscopy (Cook et al., 1997; Cook and Inman, 1999).

Materials and Methods

Fungal material

Fourteen isolates of *O. neolycopersici* were used for comparative morphological studies. These were collected in the Czech Republic, Germany, the Netherlands, Poland and the UK *(Table 1).* Two powdery mildew species (*P. fusca* and *E. orontii*) commonly occurring on Cucurbitaceae family and powdery mildew of lettuce *(E. cichoracearum)* and *Ranunculus lingua* (*E. aquilegiae* var. *ranunculi*), were included in the study.

Light microscope evaluation

Fresh and dried materials were used for light microscope evaluation. Infected pieces of leaf materials were mounted in glacial acetic acid (99.7%) for 24 h, before being transferred to chloral hydrate (1.7 g/ml). Pieces were mounted in 100% glycerol before observation (Lebeda and Mieslerová, 1999a). Morphological characteristics were used to determine the relationships between species and isolates as previously described by Braun (1987 and 1995) and Zeller (1995). The dimensions of conidia [length, width and shape index (l/w)], length of conidiophores, length and width of conidiophore foot-cell, the number of distal conidial cells, and the length of mycelial cells were measured in each specimen. Germination type and appressorium shape of all specimens were noted. Fibrosin bodies were visualized by using a 3% solution of potassium hydroxide. One hundred conidia and 50 conidiophores and mycelial cells were evaluated from each specimen. For dried samples, only the size of conidia was measured.

Scanning electron microscope (SEM) evaluation

One isolate of the following powdery mildew species: *O. neo1ycopersici* (C-1), *E. aquilegiae* var. *ranunculi* (2/99), *E. cichoracearum* (1/99), *E. orontii* (2/98) and *P. fusca* (28/97) *(Table 1),* were examined by two methods (high and low vacuum SEM).

Table 1

List of isolates of *O. neolycopersici, E. aquilegiae* var. *ranunculi, E. cichoracearum, E.orontii* and *P. fusca* used in the comparative morphological study

Powdery mildew species	Isolate	Origin (site/county/host plant)	Year of collection
Oidium neolycopersici	C-O*	Olomouc, RIVGB[1], Czech Republic, *Lycopersicon esculentum* cv. Lucy	1990
	C-1	Olomouc, SPA[2], Czech Republic, *L. esculentum* cv. Lucy	1996
	C-KV	Vřesová, SPA[2] Karlovy Vary, Czech Republic, *L. esculentum* cv. Aromata	1997
	E-1	Wellesbourne, HRI[3], England, *L. esculentum*	1998
	G-1*	Freising, TU[4], Germany, *L. esculentum* cv. Ildi	1996
	G-2	Freising, TU[4], Germany, *L. esculentum* cv. Idyll	1996
	G-3	Freising, TU[4], Germany, *L. esculentum* cv. Intakt	1996
	G-4	Freising, TU[4], Germany, *L. esculentum* cv. Isnova	1996
	G-5	Freising, TU[4], Germany, *L. esculentum* cv. Harzfeuer	1996
	P-1*	Skierniewice, RIVG[5], Poland, *L. esculentum* cv. M 1514	1990
	P-2*	Skierniewice, RIVG[5], Poland, *L. esculentum* cv. M 1586	1990
	W-1	Wageningen, AU[6], The Netherlands, *L. esculentum*	1997
	W-2	Wageningen, AU[6], The Netherlands, *L. esculentum*	1997
	RZ-1	De Lier, Rijk Zwaan, The Netherlands, *L. esculentum*	1997
Erysiphe aquilegiae var. ranunculi	2/99	Olomouc, DB[7], Czech Republic, *Ranunculus lingua*	1999
Erysiphe cichoracearum	1/99	Olomouc, DB[7], Czech Republic, *Lactuca serriola*	1999
Erysiphe orontii	2/98	Olomouc, DB[7], Czech Republic, *Cucumis sativus*	1998
Podosphaera fusca	28/97	Olomouc, DB[7], Czech Republic, *Cucumis sativus*	1997

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*Dried material.

Before viewing under high vacuum SEM, samples were immersed in mixture of 2% glutaraldehyde and 1% formaldehyde each in 0.1 M phosphate buffer pH 7.2 for 2 hours. Samples were dehydrated in acetone and dried in a critical point drier (CPD 040, Balzers Unione, Lichtenstein) with carbon dioxide. Samples were mounted on an aluminium mount and coated with gold palladium (Coating Unit ES 5100 Polaron, England) before examination using a Tesla BS 340 scanning electron microscope. Fresh samples were examined using a low vacuum Philips SEM with a Peltier table at pressures of 5 – 6.5 Torr.

Statistical analyses

The mean, standard deviation, minimum, maximum and range of all tested characteristics were determined. The data were subjected to a one-way analysis of variance and a multiple range test (LSD) using Statgraphics 5.0 version (Koschin, 1992). Scatter plots of mean conidial length vs mean conidial width; mean conidiophore length vs number of distal conidial cells and mean foot-cell length vs mean foot-cell width summarized the data. Proposed relationships between species were based on all morphological data (length, width and shape index of conidia; presence of fibrosin bodies; length of conidiophores; length and width of conidiophore foot-cell; the number of distal conidial cells; germination type and appressorium shape). An Unweighted Pair-Group Method of Arithmetic Averages (programme NCSS) (Lepš, 1996) was used to separate different isolates or tested species. Dried samples of *O. neolycopersici* (C-O, G-1, P-1, P-2), in which only size of conidia were measured, were excluded from cluster analysis.

Results

Morphology of conidia

The morphological characteristics of powdery mildew isolates are summarized in *Tables 2 and 3*. Differences in conidial size were observed within *O. neolycopersici* isolates and between different powdery mildew species. Average conidial length of *O.*

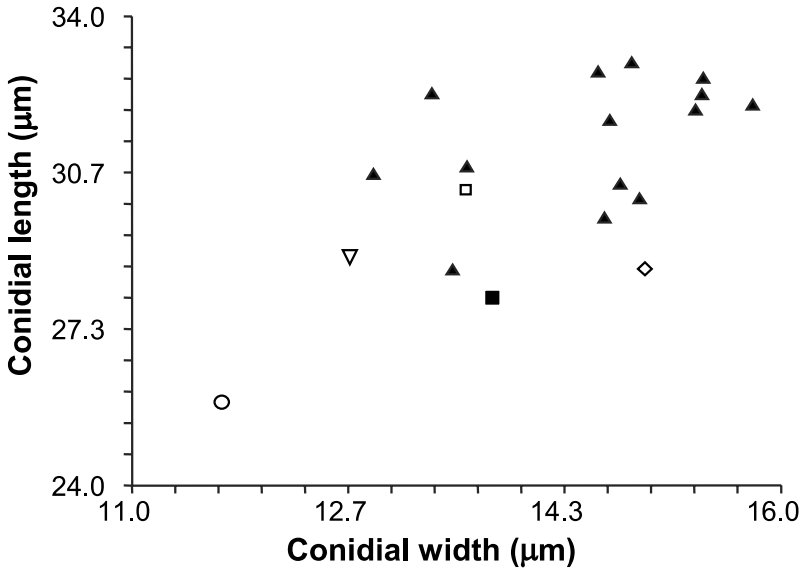

Fig. 1. Scatter plot of mean conidial length vs mean conidial width of isolates of *O. neolycopersici* (all isolates) on *L. esculentum* (▲), *O. neolycopersici* (C-1/CS) on *C. sativus* (❑), *E. aquilegiae* var. *ranunculi* (■), *E. cichoracearum* (▽), *E. orontii* (❍) and *P. fusca* (◊)

Table 2

Basic conidial characteristics of powdery mildew species (*O. neolycopersici, E. aquilegiae* var. *ranunculi, E. cichoracearum, E. orontii* and P. *fusca*) – ranged on similarity in shape index

Species	Isolate	Conidia			Fibrosin bosies	Germination type
		Shape index (mean ± SD)	Length (µm) (mean ± SD)	Width (µm) (mean ± SD)		
P. fusca	28/97	$1.87^{a**} \pm 0.28$	$27.62^{b} \pm 2.66$	$14.95^{de} \pm 1.74$	Yes	Fuliginea
E. aquilegiae var. *ranunculi*	2/99	$2.01^{a} \pm 0.27$	$27.49^{b} \pm 3.31$	$13.78^{c} \pm 0.96$	No	Polygoni
O. neolycopersici	P-2	$2.03^{b} \pm 0.26$	$30.09^{de} \pm 3.24$	$14.92^{d} \pm 1.40$	No	nd
O. neolycopersici	G-4	$2.04^{b} \pm 0.23$	$29.68^{cd} \pm 3.14$	$14.64^{d} \pm 1.23$	No	Polygoni
O. neolycopersici	G-1	$2.05^{bc} \pm 0.26$	$32.09^{gh} \pm 3.77$	$15.79^{f} \pm 1.68$	No	nd
O. neolycopersici	G-5	$2.08^{bcd} \pm 0.37$	$30.40^{de} \pm 3.07$	$14.76^{d} \pm 1.49$	No	Polygoni
O. neolycopersici	G-3	$2.11^{bcde} \pm 0.08$	$31.99^{gh} \pm 3.66$	$15.34^{e} \pm 1.85$	No	Polygoni
O. neolycopersici	P-1	$2.13^{cde} + 0.36$	$32.31^{gh} \pm 4.09$	$15.40^{ef} \pm 2.09$	No	nd
O. neolycopersici	G-2	$2.14^{def} \pm 0.28$	$32.66^{gh} \pm 3.40$	$15.40^{ef} \pm 1.63$	No	Polygoni
O. neolycopersici	E-1	$2.14^{def} \pm 0.33$	$28.57^{b} \pm 3.44$	$13.46^{c} \pm 1.28$	No	Polygoni
O. neolycopersici	C-O	$2.18^{efg} \pm 0.27$	$31.76^{fg} \pm 3.29$	$14.68^{d} \pm 1.42$	No	nd
E. orontii	2/98	$2.22^{fgh} \pm 0.25$	$25.78^{a} \pm 1.91$	$11.69^{a} \pm 1.19$	No	Cichorac.
O. neolycopersici	C-1	$2.25^{gh} \pm 0.32$	$32.99^{h} \pm 4.07$	$14.85^{d} \pm 2.00$	No	Polygoni
E. cichoracearum	1/99	$2.25^{gh} \pm 0.27$	$28.60^{bc} \pm 2.08$	$12.78^{b} \pm 1.02$	No	Cichorac.
O. neolycopersici	C-1/CS*	$2.26^{gh} \pm 0.34$	$30.31^{de} \pm 3.03$	$13.56^{c} \pm 1.24$	No	Polygoni
O. neolycopersici	W-1	$2.27^{gh} \pm 0.39$	$32.79^{gh} \pm 4.60$	$14.59^{d} \pm 1.63$	No	Polygoni
O. neolycopersici	RZ-1	$2.29^{gh} \pm 0.38$	$30.77^{ef} \pm 4.03$	$13.58^{c} \pm 1.29$	No	Polygoni
O. neolycopersici	C-KV	$2.39^{h} \pm 0.34$	$30.61^{de} \pm 3.59$	$12.86^{b} \pm 0.90$	No	nd
O. neolycopersici	W-2	$2.44^{h} \pm 0.39$	$32.33^{gh} \pm 4.69$	$13.31^{c} \pm 1.45$	No	Polygoni

**O. neolycopersici* transferred to *Cucumis sativus;* **homogeneous groups; nd – not determined

neolycopersici conidia ranged from 28.57 to 32.99 µm (mean = 31.28 µm), and it was 27.62 µm in *P. fusca,* 25.78 µm in *E. orontii,* 27.49 µm in *E. aquilegiae* var. *ranunculi* and 28.60 µm in *E. cichoracearum;* average conidial width ranged in *O. neolycopersici* isolates from 12.86 to 15.79 µm (mean = 14.47 µm), and it was 14.95 µm in *P. fusca,* 11.69 µm in *E. orontii,* 13.78 µm in *E. aquilegiae* var. *ranunculi* and 12.78 µm in *E. cichoracearum.* Shape index ranged from 2.02 to 2.44 (mean = 2.18) in *O. neolycopersici* isolates, and it was 1.87 in *P. fusca,* 2.22 in *E. orontii,* 2.01 in *E. aquilegiae* var. *ranunculi* and 2.25. in *E. cichoracearum.* Length, width and shape index of conidia were significantly different between species ($P < 0.05$). Shape of *P. fusca* conidia was elipsoid-ovoid, however *Erysiphe* species produced a more doliiform-cylindric conidia and all *O. neolycopersici* isolates produced ovoid-doliiform-cylindric types of conidia. Scatter plot of mean conidial length vs mean conidial width *(Fig. 1)* shows a heterogeneous group consisting of all *O. neolycopersici* isolates, *E. aquilegiae* var. *ranunculi, E. cichoracearum* and *P. fusca,* but not *E. orontii.*

Table 3

Basic conidiophore and mycelial characteristics of powdery mildew species (*O. neolycopersici, E. aquilegiae* var. *ranunculi, E. cichoracearum, E. orontii* and *P. fusca*) – ranged on similarity in number of distal conidial cells

Species	Isolate	Number of distal conidial cells (mean ± SD) (min – max)	Conidiophore length (µm) (mean ± SD) (min – max)	Conidio-genesis	Foot-cell length (µm) (mean ± SD)	Foot-cell width (µm) (mean ± SD)	Mycelial cell length (µm) (mean ± SD)	Shape of appressoria
O. neolycopersici	W-1	$2.08^{a**} \pm 0.63$ (1 – 4)	$76.32^{b} \pm 10.21$ (61 – 114.68)	Singly	$42.13^{ef} \pm 10.79$	$7.44^{abcd} \pm 0.50$	$50.46^{a} \pm 6.78$	SL
O. neolycopersici	G-2	$2.42^{b} \pm 0.67$ (1 – 4)	nm	Singly	$39.36^{de} \pm 7.79$	$7.58^{bcd} \pm 1.13$	$58.63^{d} \pm 11.52$	SL
O. neolycopersici	W-2	$2.48^{bc} \pm 0.51$ (2 – 3)	$75.88^{b} \pm 8.63$ (61 – 97.6)	Singly	$45.11^{f} \pm 6.56$	$7.96^{e} \pm 0.85$	$53.17^{ab} \pm 6.98$	SL
O. neolycopersici	C-1	$2.56^{bcd} \pm 0.78$ (1 – 4)	$107.70^{d} \pm 14.39$ (82.96 – 141.52)	Singly	$31.65^{b} \pm 6.14$	$7.68^{de} \pm 1.50$	$51.02^{a} \pm 11.22$	SL
O. neolycopersici	G-3	$2.60^{bcd} \pm 0.83$ (1 – 5)	$83.91^{c} \pm 12.03$ (61 – 114.68)	Singly	$44.38^{f} \pm 8.88$	$7.32^{abc} \pm 1.04$	$56.92^{cd} \pm 12.65$	SL
O. neolycopersici	RZ-1	$2.62^{bcd} \pm 0.56$ (1 – 4)	$79.68^{bc} \pm 10.69$ (61 – 100.04)	Singly	$42.31^{ef} \pm 7.85$	$7.91^{e} \pm 0.97$	$55.33^{bcd} \pm 7.44$	SL
O. neolycopersici	C-KV	$2.66^{bcde} \pm 1.00$ (1 – 6)	nm	Singly	$37.63^{cd} \pm 8.48$	$7.88^{e} \pm 0.80$	$56.01^{bcd} \pm 9.03$	SL
E. aquilegiae var. *ranunculi*	2/99	$2.72^{bcde} \pm 0.45$ (2 – 3)	$54.94^{a} \pm 9.61$ (41.48 – 80.52)	Singly	$20.99^{a} \pm 5.19$	$8.02^{e} \pm 0.82$	nm	SL, L
O. neolycopersici	G-4	$2.72^{bcde} \pm 0.80$ (2 – 6)	nm	Singly	$43.79^{f} \pm 8.63$	$7.17^{a} \pm 0.80$	$56.35^{bcd} \pm 9.82$	SL
O. neolycopersici	E-1	$2.77^{cde} \pm 0.53$ (2 – 4)	$84.85^{c} \pm 12.26$ (53.68 – 109.8)	Singly	$36.87^{cd} \pm 8.55$	$7.26^{ab} \pm 0.38$	nm	SL

Table 3 (cont)

Basic conidiophore and mycelial characteristics of powdery mildew species

Species	Isolate	Number of distal conidial cells (mean ± SD) (min – max)	Conidiophore length (µm) (mean ± SD) (min – max)	Conidio-genesis	Foot-cell length (µm) (mean ± SD)	Foot-cell width (µm) (mean ± SD)	Mycelial cell length (µm) (mean ± SD)	Shape of appressoria
O. neolycopersici	G-5	2.84^{de} ± 0.65 (1 – 5)	nm	Singly	35.16^{bc} ± 8.62	7.12^{a} ± 0.83	53.58^{abc} ± 9.26	SL
O. neolycopersici	C-1/CS*	2.94^{e} ± 0.31 (2 – 4)	76.64^{b} ± 12.14 (48.8 – 114.68)	Singly	34.36^{bc} ± 7.67	7.63^{cd} ± 0.65	nm	SL
E. cichoracearum	1/99	5.00^{f} ± 0.59 (4 – 6)	132.60^{e} ± 13.69 (102.48 – 153.72)	Often in chains	50.06^{g} ± 10.09	9.59^{fg} ± 0.90	nm	SN
E. orontii	2/98	6.06^{g} ± 1.23 (3 – 8)	177.25^{f} ± 31.05 (107.32 – 231.8)	Often in chains	68.95^{h} ± 17.73	9.44^{f} ± 0.77	56.60^{bcd} ± 9.18	SN
P. fusca	28/97	8.22^{h} ± 1.09 (4 – 10)	228.57^{g} ± 28.62 (148.84 – 278.16)	Often in chains	51.38^{g} ± 10.08	9.97^{g} ± 0.80	nm	I, SN

**O. neolycopersici* transferred on *Cucumis sativus;* **homogeneous groups;
nm – not measured; I – Indistinct; SN – Slightly nipple-shaped; SL – Slightly lobed; L – Lobed

Conidial germination

There were substantial differences in the type of conidial germination between the species studied. *P. fusca* germinated with short germ tubes from the lateral side of conidia which were often forked (fuliginea type; Braun, 1987). These were not observed in the other powdery mildew species. *E. cichoracearum* and *E. orontii* had long germ tubes approximately 5 µm wide which arose from the apical side of conidia without lobed appressoria (cichoracearum type). *O. neolycopersici* and *E. aquilegiae* var. *ranunculi* germinated from the apical side of conidium with germ tubes approximately 2.5 µm wide, and these often terminated in a lobed appressorium (polygoni type). Fibrosin bodies were detected only in conidia of *P. fusca.*

Morphology of conidiophores, mycelia and appressoria

There were differences in conidiophore morphology and the number of distal conidial cells between all isolates of *O. neolycopersici* (average number ranged from 2.08 to 2.94 cells (mean = 2.60)) and the isolate of *E. aquilegiae* var. *ranunculi* (2.72) as compared with *E. cichoracearum* (5.0), *E. orontii* (6.06) and *P. fusca* (8.22) ($P < 0.05$).

Conidiophore length of *O. neolycopersici* isolates (75.88–107.70 µm; mean = 83.57 mm) was significantly ($P < 0.05$) less than that of *E. orontii* (177.25 µm), *E. cichoracearum* (132.60 µm) and *P. fusca* (228.57 µm), but significantly greater than the conidiophore length of *E. aquilegiae* var. *ranunculi* (54.94 µm). The Czech isolate of *O. neolycopersici* (C-1; 107.70 µm) significantly differed ($P < 0.05$) from the rest of *O. neolycopersici* isolates and from other tested powdery mildew species *(Fig. 2).*

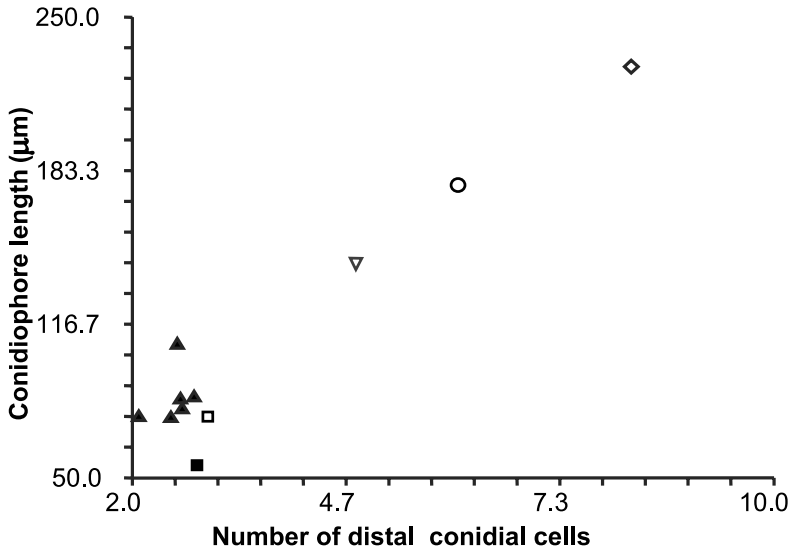

Fig. 2. Scatter plot of mean conidiophore length vs number of distal conidial cells of isolates of *O. neolycopersici* (all isolates) on *L. esculentum* (▲), *O. neolycopersici* (C-1/CS) on *C. sativus* (❑), *E. aquilegiae* var. *ranunculi* (■), *E. cichoracearum* (▽), *E. orontii* (○) and *P. fusca* (◊)

Conidia of *O. neolycopersici* and *E. aquilegiae* var. *ranunculi* were not observed in chains on conidiophores (Pseudoidium type), (except in high humidity) in contrast to *P. fusca, E. cichoracearum* and *E. orontii* [all classified as Euoidium type (Braun, 1995)].

There were significant differences in foot-cell length and width ($P < 0.05$). Average foot-cell length of *O. neolycopersici* isolates ranged from 31.65 to 45.11 µm (mean = 39.34 µm), and it was distinct from *E. aquilegiae* var. *ranunculi* (20.99 µm), *E. cichoracearum* (50.06 µm), *E. orontii* (68.95 µm) and *P. fusca* (51.38 µm). However, isolates of *O. neolycopersici* originating from the Netherlands (W-1, W-2, RZ-1) and some German isolates (G-3, G-4) differed significantly ($P < 0.05$) in foot-cell length (means from 42.13 to 45.11 µm) from the rest of *O. neolycopersici* isolates (means from 31.65 to 37.63 µm). Foot-cell width of isolates of *O. neolycopersici* and *E. aquilegiae* var. *ranunculi* was different from *P. fusca, E. cichoracearum* and *E. orontii (Fig. 3).* Length of mycelial cells was not significantly different ($P > 0.05$) within and between powdery mildew species.

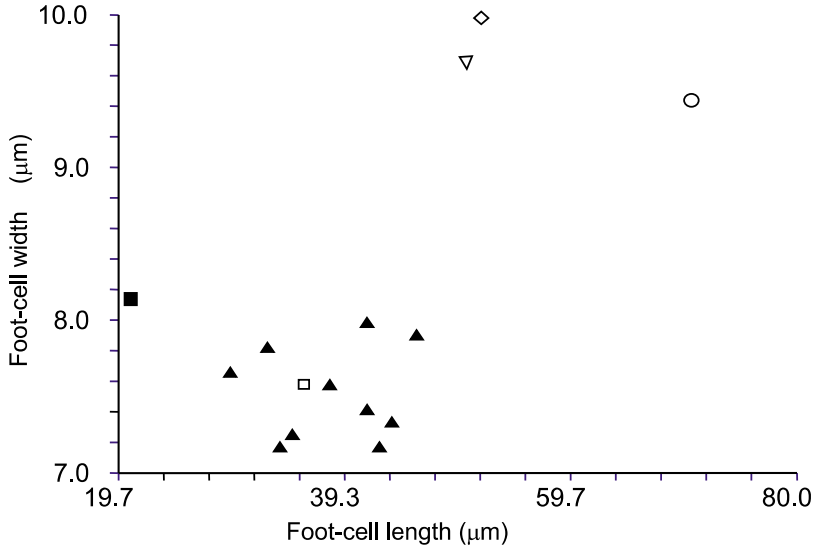

Fig. 3. Scatter plot of mean foot-cell length vs mean foot-cell width of isolates of *O. neolycopersici* (all isolates) on *L. esculentum* (▲), *O. neolycopersici* (C-1/CS) on *C. sativus* (❑), *E. aquilegiae* var. *ranunculi* (■), *E. cichoracearum* (▽), *E. orontii* (○) and *P. fusca* (◊)

Differences were recorded in the shape of appressoria. *P. fusca* had indistinct appressoria, which were sometimes slightly nipple-shaped, similar to *E. cichoracearum* and *E. orontii.* In contrast, *O. neolycopersici* and *E. aquilegiae* var. *ranunculi,* had slightly lobed appressoria.

A dendrogram *(Fig. 4)* clustering powdery mildew isolates and species on their basic morphological characteristics, revealed that all isolates of *O. neolycopersici* and *E. aquilegiae* var. *ranunculi* formed one relative uniform group and *P. fusca, E. cichoracearum* and *E. orontii* formed a distinct group.

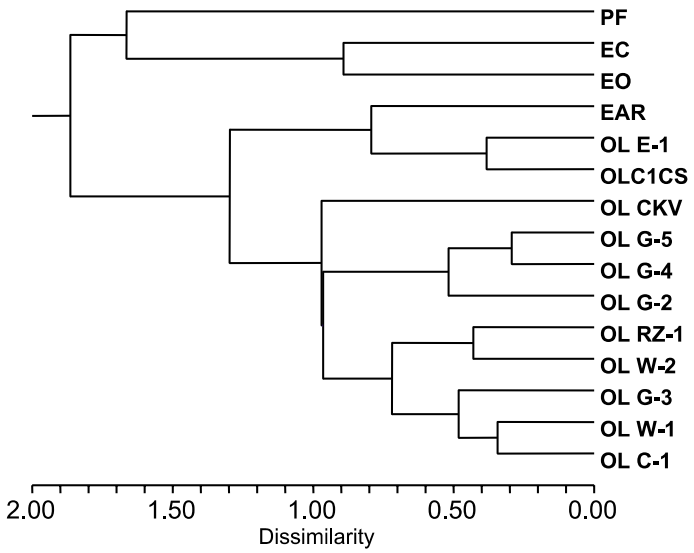

Fig. 4. Dendrogram constructed on morphological data (length, width and shape index of conidia; presence of fibrosin bodies; length of conidiophores; length and width of conidiophore foot-cell; the number of distal conidial cells; germination type and appressorium shape) showing similarity between isolates of *O. neolycopersici* (OL), *E. aquilegiae* var. *ranunculi* (EAR), *E. cichoracearum* (EC), *E. orontii* (EO) and *P. fusca* (PF)

Scanning electron microscope observations

FRESH SAMPLES (HIGH VACUUM)

There was loss of turgor in fresh leaf samples infected with powdery mildews after chemical treatment prior to SEM observation. Visible patterns on conidial walls of these samples corresponded with those described previously (Cook et al., 1997) on wrinkled conidia. The outer walls of *P. fusca* conidia looked very smooth *(Fig. 5a),* though they had reduced turgidity, in contrast to the other species. *E. cichoracearum (Fig. 6a)* and *E. orontii* had very similar conidial wall patterns, which looked like a net or reticulum. *E. aquilegiae* var. *ranunculi* conidia lost turgidity, showing broad rectangular pattern. Isolates of *O. neolycopersici* showed similar conidial patterns *(Fig. 7a),* with broad rectangular wrinkling.

FRESH SAMPLES (LOW VACUUM)

In low vacuum SEM, conidia of *O. neolycopersici, P. fusca* and *E. cichoracearum* showed relatively slight and indistinct outer wall patterns. *O. neolycopersici (Fig. 7b)* had smooth walls, while some structures on outer conidial walls were detected in both *P. fusca (Fig. 5b)* and *E. cichoracearum (Fig. 6b).*

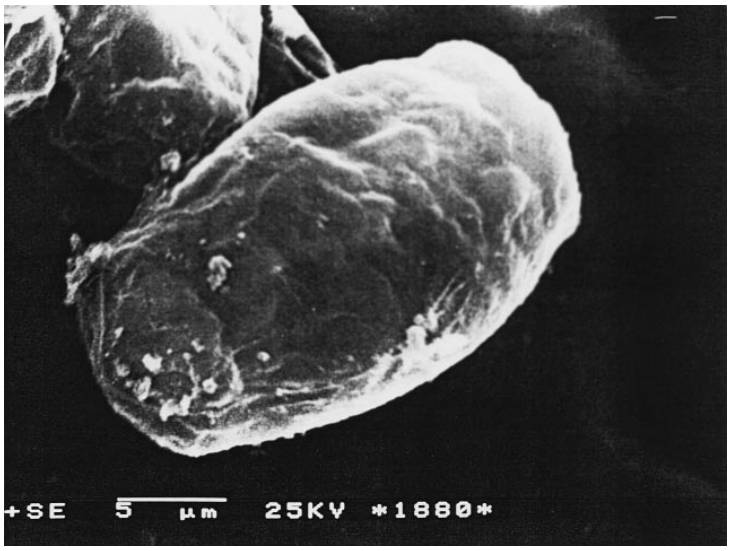

Fig. 5a. Outer conidial wall patterns of the studied powdery mildew species. High vacuum Tesla SEM. *Podosphaera fusca*

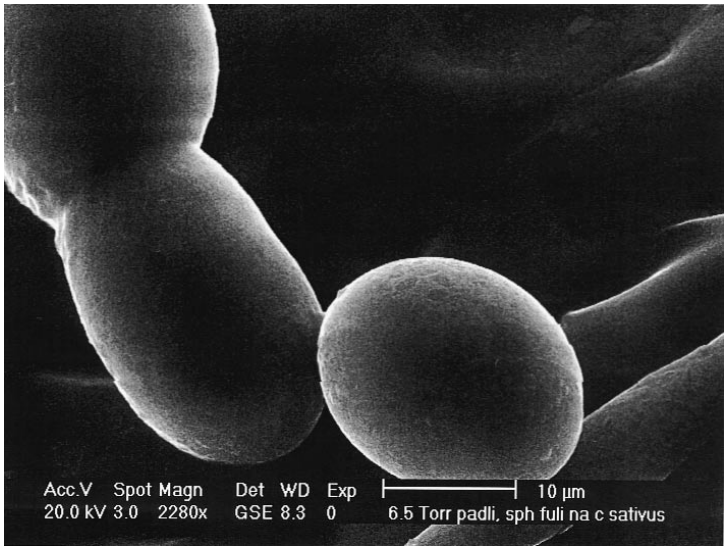

Fig. 5b. Outer conidial wall patterns of the studied powdery mildew species. Low vacuum Philips SEM. *Podosphaera fusca*

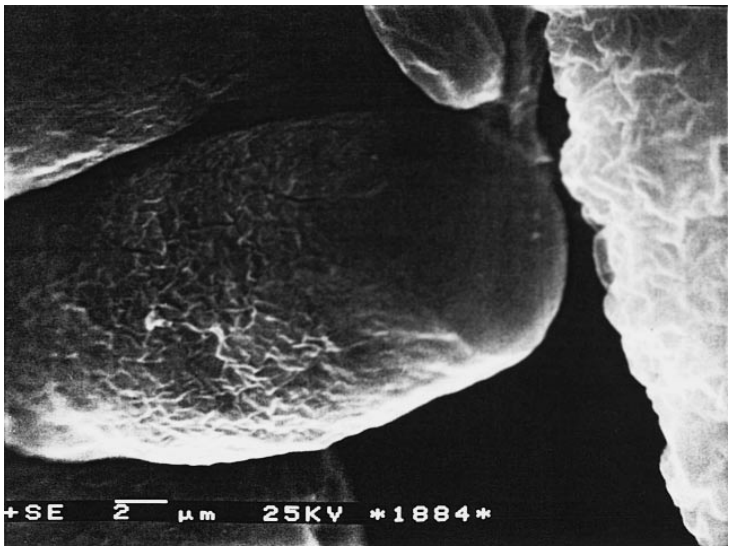

Fig. 6a. Outer conidial wall patterns of the studied powdery mildew species. High vacuum Tesla SEM. *Erysiphe cichoracearum*

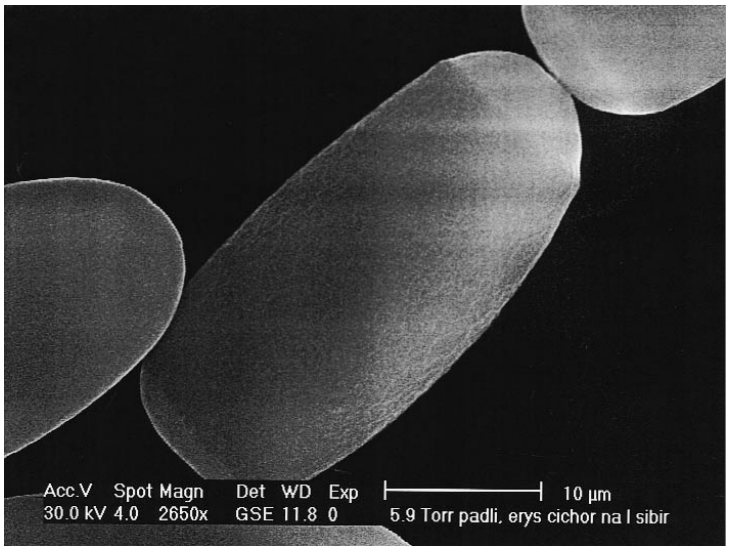

Fig. 6b. Outer conidial wall patterns of the studied powdery mildew species. Low vacuum Philips SEM. *Erysiphe cichoracearum*

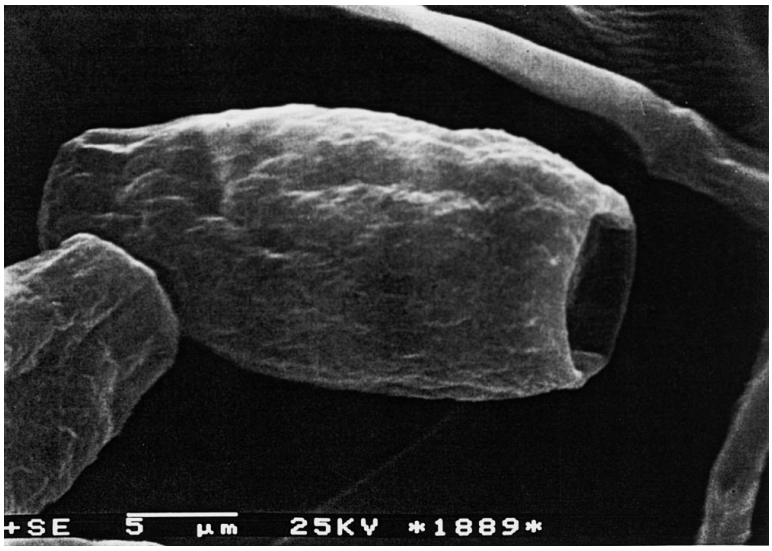

Fig. 7a. Outer conidial wall patterns of the studied powdery mildew species. High vacuum Tesla SEM. *Oidium neolycopersici*

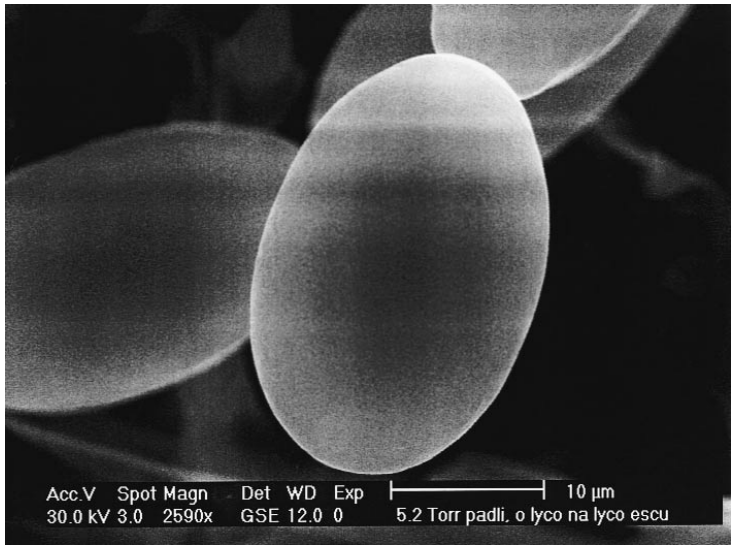

Fig. 7b. Outer conidial wall patterns of the studied powdery mildew species. Low vacuum Philips SEM. *Oidium neolycopersici*

Concluding remarks on morphological features

Based on morphological features we can conclude that *O. neolycopersici* is clearly separated from *P. fusca,* as well as from *E. cichoracearum* and *E. orontii* (type of germination, size of conidiophores, number of distal conidial cells, shape of appressoria). However, the type of germination, conidial arrangement, number of distal conidial cells and shape of appressoria were similar to those observed in *E. aquilegiae* var. *ranunculi,* (both belong to *Oidium* subgen. *Pseudoidium*), which suggests that *O. neolycopersici* could be placed to *Erysiphe* sect. *Erysiphe* (= *Erysiphe* s. str.).

Discussion

Previously, taxonomy and identification of powdery mildews was based primarily on the characteristics of the teleomorphs [shape of the appendages and number of asci in cleistothecium (Braun, 1995)]. Based on comprehensive molecular examinations of powdery mildews (Takamatsu et al., 1998, 1999; Mori et al., 2000), it is evident, that the value of these characters is of secondary importance for the taxonomy. On the other hand, anamorphs play an increasing important role, which has recently been confirmed by SEM examinations (Cook et al., 1997).

In our study (Lebeda and Mieslerová, 2000, unpubl.) formation of cleistothecia under laboratory conditions using different isolates of *O. neolycopersici* and various temperature conditions, failed. In previous studies, Blumer (1967) reported that production of cleistothecia is dependent on age of host, presence of other parasitic fungi or insects and suitable environmental conditions. Also production of cleistothecia on non-host species, or distant host species is problematical and rare (Braun, 1987).

Thus, only anamorphic, host range and molecular criteria are available to distinguish various tomato powdery mildew isolates and for comparison with other, potentially related, powdery mildews.

Some morphological characteristics of *O. neolycopersici* determined in our studies were similar to those recorded by other authors (Fletcher et al., 1988; Vakalounakis and Papadakis, 1992; Arredondo et al., 1996; Whipps et al., 1998). The number of distal conidial cells, conidiophore length and foot-cell length were constant characteristics. However, conidial sizes observed by many authors (Aloi and Garibaldi, 1990; Olalla and Torés, 1998; Lemaire et al., 1999) differed from present and previous data (Lebeda and Mieslerová, 1999a, b). Reported mean conidial widths varied from 12.0 mm to 19.0 mm. These differences probably resulted from the differences in conidial state (fresh or dry), or using of reagents which restore the turgidity of conidium. However, conidial lengths differed considerably in all observations. Zeller (1995) reported that size of conidia and shape index show a high degree of polymorphism and are not very valuable in taxonomic studies. Other studies reported (Braun, 1995) that conidia which develop on senescent leaf surfaces could be smaller. Whipps and Helyer (1994) stated that size of conidiophores and conidia were also affected by environmental conditions and hosts. However, in the present study the transfer of *O. neolycopersici* to *Cucumis sativus* did not result in any substantial

change of morphological characteristics. Nevertheless, these results revealed significant variation in conidial morphology between different powdery mildew species as well as between isolates of *O. neolycopersici (Table 2).*

The type of germination is a very constant characteristic which could clearly distinguish powdery mildew species. Also, the presence or absence of conidial chains is a criterium which can be used to distinguish *O. neolycopersici* and *E. aquilegiae* var. *ranunculi* (Pseudoidium group) from *E. orontii, E. cichoracearum* and *P. fusca* (Euoidium group). However, this characteristic could be strongly influenced by environmental conditions (e.g. humidity). In high humidity, conidia produced singly may adhere together to produce pseudochains. Kiss et al. (2001) in their detailed study based on morphological characteristics (and molecular phylogenetic analysis) identified two *Oidium* species on tomato. Anamorphic specimens obtained from Australia represented the euoidium type [*O.* subgen. *Reticuloidium;* teleomorph *Golovinomyces* sp. (formerly *Erysiphe* sect. *Golovinomyces*)] and were neotypified as *O. lycopersici.* In contrast, *O. neolycopersici,* widespread in Europe, Africa, North and South America and Asia, produces conidia singly and belongs to *Oidium* subgen. *Pseudoidium* (teleomorph: *Erysiphe* sect. *Erysiphe*) (Kiss et al., 2001). As only European isolates of *O. neolycopersici* were used in the present study it is not surprising that only the Pseudoidium type was observed.

Scanning electron microscope (SEM) evaluation of distinctive patterns on surfaces of conidial outer and end walls has been shown to aid the identification of powdery mildews in the absence of cleistothecia (Cook et al., 1997; Cook and Inman, 1999). Because patterns on septa (end walls separating conidia from conidiophore or from other conidia) were only observed at relatively high magnifications (7–12,000), they were not reliably detected in our SEM studies. Only patterns on the outer walls, which were visible even at lower magnifications, were used in our comparative study.

Using high vacuum SEM with fresh infected leaf material resulted in a loss of conidial turgidity. Wall patterns observed were close to those described on wrinkled conidia. Relatively smooth patterns on outer wall surfaces were observed on conidia of *P. fusca* (which did not lose turgidity so drastically) as in previous studies (Cook et al., 1997) and differed from those observed in *E. cichoracearum* and *E. orontii,* described as polygonal reticulate (*Oidium* subgen. *Reticuloidium*). However, in the present study rectangular wrinkling pattern was observed on conidia of *O. neolycopersici* and *E. aquilegiae* var. *ranunculi.* Evaluation using low vacuum SEM of fresh leaf material showed that the conidial wall patterns were generally indistinct. The observed wall patterns were smooth for *O. neolycopersici* and with a netted appearance for *E. cichoracearum.*

Finally, based on present morphological studies, position of *O. neolycopersici* is undoubtely in *Erysiphe* sect. *Erysiphe* (= *Erysiphe* s. str.; defined by Pseudoidium type of anamorph and rectangular wrinkled outer conidial wall patern) in contrast with *E. cichoracearum* and *E. orontii* (placed in *Erysiphe* sect. *Golovinomyces* with Euoidium type of anamorph and reticulate outer conidial wall pattern). Thus, although the successful transfer of *O. neolycopersici* to Cucurbitaceae was verified (Fletcher et al., 1988; Corbaz, 1993; Whipps et al., 1998; Lebeda and Mieslerová, 1999a, b, 2000), the hypothesis of these taxons relationship was not confirmed.

Recently, Braun (1999) stressed that the genus *Erysiphe* s. lat. has a heterogeneous, paraphyletic character and should be divided into three smaller units, i.e. genus *Erysiphe* s. str. (= *Erysiphe* sect. *Erysiphe*), *Golovinomyces* (= *Erysiphe* sect. *Golovinomyces*) and *Neoerysiphe* (= *Erysiphe* sect. *Galeopsidis*) and introduced some possible new combinations. Moreover, some molecular data (Saenz and Taylor, 1999; Takamatsu et al., 1999) showed that relationships between *Erysiphe* sect. *Erysiphe* and *Microsphaera, Uncinula* are much closer than between *Erysiphe* s. str. and *Erysiphe* sect. *Golovinomyces.*

The results of our morphological study are in good agreement with recent molecular data. Huang et al. (1998) studied *O. neolycopersici* variability by AFLP analysis of four Dutch isolates and reported only little genetic variability and large differences from *E. orontii* and *Sphaerotheca* (= *Podosphaera*) *fusca* isolates. Studies of Jones et al. (1999; 2000) comparing the rDNA ITS sequence of *O. neolycopersici* with the sequences of other powdery mildew species, showed that *O. neolycopersici* is essentially identical to *E. aquilegiae* var. *ranunculi,* and is clearly distinct from *E. cichoracearum* and *E. orontii.* In agreement with these results, Takamatsu et al. (1998) in their work using ITS sequence analysis clearly separated *E. aquilegiae* from *E. cichoracearum.* However, considerable variability in pathogenicity on *Lycopersicon* spp. was revealed within *O. neolycopersici* isolates originating from different European countries (Lebeda and Mieslerová, 2000; 2001). This fact open a broad scale of questions for further research regarding the instraspecific variability of *O. neolycopersici,* its host range, genetics and nature of host-pathogen interactions.

Although the transfer of *E. aquilegiae* var. *ranunculi* from *Ranunculus lingua* and *Ranunculus repens* to *L. esculentum* was not successful (Mieslerová and Lebeda, unpubl.), the present morphological studies confirmed similarities between *O. neolycopersici* and *E. aquilegiae* var. *ranunculi* (both belong to *Oidium* subgen. *Pseudoidium*). Thus, the importance and value of these approaches (molecular, biological and morphological) in taxonomy and plant pathology must be considered.

Further studies based on morphology, host range, biological studies, biochemical and molecular markers are required to investigate the relationships between and within some powdery mildew species included in this study.

Acknowledgement

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Literature

Aloi, C. and Garibaldi, A. (1990): Un mal bianco del pomodoro causato da *Erysiphe* sp., nuovo per l'Italia. Informatore Fitopatologico 40, 57–58.

Angelov, D. and Georgiev, P. (1993): Identification of the new powdery mildew agent on tomatoes in Bulgaria. Proceedings of the XIIth EUCARPIA Meeting on Tomato Genetics and Breeding, Plovdiv, Bulgaria, 51–54.

Arredondo, C. R., Davis, R. M., Rizzo, D. M. and Stahmer, R. (1996): First report of powdery mildew of tomato in California caused by an *Oidium* sp. Plant Disease 80, 1303.

Blumer, S. (1967): Echte Mehltaupilze (Erysiphaceae). Jena, Germany, VEB Gustav Fischer Verlag, pp. 436.

Braun, U. (1987): The monograph of the Erysiphales (powdery mildews). Beihefte zur Nova Hedwigia 89, 1–700.

Braun, U. (1995): The powdery mildews (Erysiphales) of Europe. Jena, Germany, Gustav Fisher Verlag, pp. 337.

Braun, U. (1999): Some critical notes on the classification and the generic concept of the Erysiphaceae. Schlechtendalia 3, 48–54.

Braun, U. and Takamatsu, S. (2000): Phylogeny of the *Erysiphe, Microsphaera, Uncinula* (Erysipheae) and *Cystotheca, Podosphaera, Sphaerotheca* (Cystotheceae) inferred from rDNA ITS sequences – some taxonomic consequences. Schlechtendalia 4, 1–33.

Cook, R. T. A. and Inman, A. J. (1999): How conidial surface patterns help characterise anamorphs of unnamed powdery mildews. The First International Powdery Mildew Conference, Avignon, Programme and Abstracts, 13.

Cook, R. T. A., Inman, A. J. and Billings, C. (1997): Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range data. Mycological Research 101, 975-–1002.

Cooke, M. C. and Massee, G. (1888): Australasian fungi. Grevillea 16, 114.

Corbaz, R. (1993): Extension d un oidium des Cucubitacées *(Erysiphe cichoracearum)* á la tomate. Revue suisse Viticulture, Arboriculture, Horticulture 25, 389–391.

Fletcher, J. T., Smewin, B. J. and Cook, R. T. A. (1988): Tomato powdery mildew. Plant Pathology 37, 594–598.

Georgiev, P. and Angelov, D. (1993): Reaction of different tomato varieties to powdery mildew causal agent – *Sphaerotheca fuliginea* f. *lycopersicum* (Cooke and Massee). Proceedings of the XIIth EUCARPIA Meeting on Tomato Genetics and Breeding, Plovdiv, Bulgaria, 55–58.

Huang, C. C., Lindhout, P. and Niks, R. E. (1998): Genetic differences in powdery mildews prevailing recently on tomato. 7th International Congress of Plant Pathology, Edinburg, Offered Papers, Abstracts – Vol. 2 (Themes 1 and 2), 2.2.18.

Jones, H. E., Whipps, J. M. and Gurr, S. J. (1999): *Oidium lycopersicon:* investigation into conidial germination and host penetration. The First International Powdery Mildew Conference, Avignon, Programme and Abstracts, 22.

Jones, H. E., Whipps, J. M., Thomas, B. J., Carver, T. L. W. and Gurr, S. J. (2000): Initial events in the colonisation of tomatoes by *Oidium lycopersici,* a distinct powdery mildew fungus of *Lycopersicon* species. Canadian Journal of Botany 78, 1361–1366.

Kiss, L., Cook, R. T. A., Saenz, G. S., Cunnington, J. H., Takamatsu, S., Pascoe, I., Bardin, M., Nicot, P. C., Sato, Y. and Rossman, A. Y. (2001): Identification of two powdery mildew fungi, *Oidium neolycopersici* sp. nov. and *O. lycopersici,* infecting tomato in different parts of the world. Mycological Research 105, 684–697.

Kiss, L., Cook, R. T. A., Saenz, G. S., Pascoe, I., Bardin, M., Nicot, P. C., Hughes, K. and Rossman, A. Y. (1999): How many *Erysiphe*-like anamorphs are responsible for the recent outbreak of tomato powdery mildew? The First International Powdery Mildew Conference, Avignon, Programme and Abstracts, 4.

Koschin, F. (1992) (ed.): Statgraphics. Praha, Czech Republic, Grada, pp. 340.

Lebeda, A. and Mieslerová, B. (1999a): Identification, occurrence and host range of tomato powdery mildew *(Oidium lycopersici)* in the Czech Republic. Acta Phytopathologica and Entomologica Hungarica 34, 15–27.

Lebeda, A. and Mieslerová, B. (1999b): Morphological characterization and host range of tomato powdery mildew *(Oidium lycopersici)* originating from the Czech Republic. The First International Powdery Mildew Conference, Avignon, Programme and Abstracts, 15.

Lebeda, A. and Mieslerová, B. (2000): Case study of host-pathogen interaction: Tomato (*Lycopersicon* spp.) – tomato powdery mildew *(Oidium lycopersici).* Plant Protection Science 36, 156–162.

Lebeda, A. and Mieslerová, B. (2001): Variability in pathogenicity of *Oidium neolycopersici* on *Lycopersicon* species. Journal of Plant Diseases and Protection (in press).

Lemaire, J. M., Conus, M., Burgerjon, A. and Mas, P. (1999): *Oidium lycopersicum,* un nouvel oidium de la tomate. PHM Revue Horticole 4, 21–24.

Lepš, J. (1996): Biostatistika. České Budějovice, Czech Republic, South Bohemian University, Biological Faculty, pp. 166.

Mieslerová, B. and Lebeda, A. (1999): Taxonomy, distribution and biology of the tomato powdery mildew. Journal of Plant Diseases and Protection 106, 140–157.

Mieslerová, B., Lebeda, A. and Chetelat, R. T. (2000): Variation in response of wild *Lycopersicon* and *Solanum* spp. against tomato powdery mildew *(Oidium lycopersici).* Journal of Phytopathology 148, 303–311.

Mori, Y., Sato, Y. and Takamatsu, S. (2000): Evolutionary analysis of the powdery mildew fungi using nucleotide sequences of the nuclear ribosomal DNA. Mycologia 92, 74–93.

Noordeloos, M. E. and Loerakker, W. M. (1989): Studies in plant pathogenic fungi – II: On some powdery mildews (Erysiphales) recently recorded from the Netherlands. Persoonia 14, 51–60.

Olalla, L. and Torés, J. A. (1998): First report of powdery mildew of tomato caused by an *Erysiphe* sp. in Spain. Plant Disease 82, 592.

Palti, J. (1988): The *Leveillula* mildews. Botanical Review 54, 423–535.

Saenz, G. S. and Taylor, J. W. (1999): Phylogeny of the Erysiphales (powdery mildews) inferred from internal transscribed spacer (ITS) ribosomal DNA sequences. Canadian Journal of Botany 77, 150–169.

Stolk, J. A. and Cools, M. M. (1983): Early hothouse tomato cultivars: resistance to powdery mildew is definite. Groenten en Fruit 39, 37–39.

Takamatsu, S., Hirata, T. and Sato, Y. (1998): Phylogenetic analysis and predicted secondary structures of the rDNA internal transcribed spacers of the powdery mildew fungi (Erysiphaceae). Mycoscience 39, 441–453.

Takamatsu, S., Hirata, T., Sato, Y. and Nomura, Y. (1999): Phylogenetic relationships of *Microsphaera* and *Erysiphe* section *Erysiphe* (powdery mildews) inferred from the rDNA ITS sequences. Mycoscience 40, 259–268.

Vakalounakis, D. J. and Papadakis, A. (1992): Occurrence of a new powdery mildew of greenhouse tomato in Greece, caused by *Erysiphe* sp. Plant Pathology 41, 372–373.

Whipps, J. M., Budge, S. P. and Fenlon, J. S. (1998): Characteristics and host range of tomato powdery mildew. Plant Pathology 47, 36–48.

Whipps, J. M. and Helyer, N. L. (1994): Occurrence of powdery mildew on aubergine in West Sussex. Plant Pathology 43, 230–233.

Zeller, K. A. (1995): Phylogenetic relatedness within the genus *Erysiphe* estimated with morphological characteristics. Mycologia 87, 525–531.