BIOLOGICAL CHARACTERISTICS OF SLOVAK ISOLATES OF ENTOMOPATHOGENIC FUNGUS PANDORA NEOAPHIDIS (REMAUDIÈRE ET HENNEBERT) HUMBER (ZYGOMYCETES, ENTOMOPHTHORALES)

BIOLOGICKÉ VLASTNOSTI SLOVENSKÝCH IZOLÁTOV ENTOMOPATOGÉNNEJ HUBY PANDORA NEOAPHIDIS (REMAUDIÈRE ET HENNEBERT) HUMBER (ZYGOMYCETES, ENTOMOPHTHORALES)

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ABSTRACT

Intraspecific variability of biological characteristics within entomopathogenic fungus Pandora neoaphidis was evaluated. Fifteen isolates of the fungus were obtained from 5 aphid species in Slovakia. Size of conidia, conidial germination, virulence, radial growth, and biomass production were evaluated. Conidial size varied considerably with exception of isolates originating from the same host population. Conidial germination was observed on all the surfaces tested and it was greatest at saturated humidity. Virulence, daily rate of radial growth and biomass production varied depending on isolates. Isolates obtained from the same host colonies during fungal epizootics shoved also significant differences in the characteristics, what may suggest that epizootics in aphid populations are caused by associations of strains and not by prevalence of a single virulent strain.

Keywords: Pandora neoaphidis; Acyrthosiphon pisum; intraspecific variability; isolate variability

ABSTRAKT

V práci sa hodnotila vnútrodruhová variabilita biologických vlastností entomopatogénnej huby Pandora neoaphidis. Hodnotilo sa 15 izolátov získaných z 5 druhov vošiek. Posudzovali sa nasledovné vlastnosti: veľkosť a klíčivosť konídií, virulencia, radiálny rast a produkcia biomasy izolátov. Zistili sa preukazné rozdiely vo veľkosti konídií, s výnimkou izolátov získaných z vošiek pochádzajúcich z rovnakých populácií. Konídie klíčili na všetkých hodnotených povrchoch, najvyššia klíčivosť bola pri 100% relatívnej vlhkosti. Virulencia izolátov, denná rýchlosť rastu a produkcia biomasy varírovala v závislosti od izolátu. Izoláty, ktoré boli získané z rovnakých kolónií vošiek počas epizoócií patogéna preukázali tiež významné rozdiely v hodnotených vlastnostiach. Toto poukazuje na skutočnosť, že epizoócie v populáciách vošiek sú vyvolávané asociáciou viacerých a nie prevalenciou jedného virulentného kmeňa patogéna.

Kľúčové slová: Pandora neoaphidis; Acyrthosiphon pisum; vnútrodruhová variabilita, variabilita izolátov

DETAILED ABSTRACT

V práci sa hodnotila vnútrodruhová variabilita biologických vlastností entomopatogénnej huby Pandora neoaphidis (Remaudière et Hennebert) Humber. Hodnotilo sa 15 izolátov získaných z 5 druhov vošiek: Myzus persicae (Sulzer), Microlophium carnosum (Buckton), Sitobion avenae (Fabricius), Uroleucon aeneum (Hille Ris Lambers) a Rhopalosiphum padi (Linnaeus). Mŕtve vošky boli nazbierané na viacerých lokalitách západného a stredného Slovenska. Posudzovali sa nasledovné vlastnosti izolátov: veľkosť a klíčivosť konídií, virulencia k Acyrthosiphon pisum Harris, radiálny rast a produkcia biomasy izolátov. Testovanie uvedených parametrov poukázalo na významné rozdiely medzi získanými izolátmi. Zistili sa preukazné rozdiely vo veľkosti konídií, s výnimkou izolátov získaných z vošiek pochádzajúcich z rovnakých populácií. Konídie klíčili na všetkých hodnotených povrchoch (sklo, vodný agar, živinový agar) a najvyššia klíčivosť bola pri 100% relatívnej vlhkosti. V závislosti od izolátu sa virulencia vyjadrená hodnotou bola LC_{50} pohybovala od 24 do 233 konídií/mm^2, denná rýchlosť rastu bola 1,03-2,00 mm a produkcia biomasy varírovala medzi 1,63 a 8,72 g.l^{-1}. Izoláty, ktoré boli získané z rovnakých kolónií vošiek počas epizoócií patogéna preukázali tiež významné rozdiely v hodnotených vlastnostiach. Toto poukazuje na skutočnosť, že epizoócie v populáciách vošiek sú pravdepodobne vyvolávané asociáciou viacerých a nie prevalenciou jedného virulentného kmeňa patogéna.

INTRODUCTION

The entomopathogenic fungus Pandora neoaphidis (Remaudière et Hennebert) Humber has been often considered the most important natural regulation agent of aphid colonies and has been long investigated as a potential biological control agent [23]. Augmentation of P. neoaphidis in field and greenhouse bioassays has been attempted but with limited success [27,35,36]. In general, a strain with high virulence is necessary to select for inoculative or inundative augmentation biocontrol strategies [23,29]. Conservation biocontrol strategies do not rely on inoculum release, but on providing of favourable conditions within agroecosystems to enhance entomopathogen activity [7]. For conservation biocontrol it is likely that a diverse range of fungal strains needs to be encouraged and these strains should be able to infect target or alternative hosts [23]. Recently, a three-tiered assay system has been devised for assessment of P. neoaphidis isolates against aphids within the program of biological control [28]. From practical point of view, both augmentation and conservation programs necessarily require information on intraspecific variability in pathogen population since the variability of fungal strains in agroecosystems may enhance its impact. In Slovakia, P. neoaphidis is the most common aphid pathogen in agroecosystems with ability to produce epizootics in host populations [2,3,6]. Variability of Slovak strains of this important aphid pathogen has not yet been tested. We obtained 15 isolates of P. neoaphidis from different aphid hosts and localities during our previous study of the fungal prevalence and effectiveness in aphid population. This paper presents information about intraspecific variability within selected phenotypic and physiological characteristics of the fungal isolates.

MATERIAL AND METHODS

Fifteen isolates of P. neoaphidis (Table 1) obtained from various aphid species in Slovakia were involved in the bioassay. The small group of Slovak isolates originated from fungus-killed aphids collected in different parts of the country, mostly in the south-western Slovakia. Eight isolates were originally obtained from pest aphid species and 7 isolates originated from non-pest aphids. Majority of the isolates had a different origin, but there were also isolates obtained from aphid cadavers collected in the same colony during fungal epizootics. Altogether five characteristics of the isolates were evaluated: size of primary conidia, conidial germination on different surfaces, virulence against pea aphid, radial growth and biomass production of isolates.

Size of primary conidia. A piece of sporulating fungal mat taken from a fast growing margin of a culture was placed in the bottom of sterile Petri dish lined with water-soaked filter paper. Above the mycelium a cover glass was fixed in a distance of 2-3 mm. Conidia actively discharged from the mycelial piece were collected on the cover glass for 15 minutes. Two morphological characteristics, the conidial length (L) and the conidial diameter (D), were measured and a ratio of the length to the diameter (L/D) was calculated. These parameters were measured for 4 x 25 conidia for each isolate using microscope (at magnification of 16 x 45) fitted with a scaled eyepiece. The same procedure of conidial measurement was also performed for primary spores collected directly from naturally killed aphids before a particular isolates were obtained. An average values were counted and obtained data were analysed by one-way ANOVA.

Germination of primary conidia. Conidia for the experiment were obtained from the sporulating fungal mat as described above. The conidia were collected on three surfaces: a sterile cover glass, a square piece (10 x 10 mm) of pure water agar (WA) (2% agar), and on a

Table 1. List of *P. neoaphidis* isolates included into study with information on their origin (source, date and place of isolation with coordinates of locality (North latitude and East longitude))
Tabuľka 1. Zoznam izolátov *P. neoaphidis* použitých v experimentoch s informáciou o ich pôvode (hostiteľ, dátum a miesto nálezu so súradnicami lokality (severná šírka a východná dĺžka)

Isolate number	Source of isolation: (Host aphid, host plant)	Date of isolation, Locality with coordinates:
1	*Myzus persicae* (Sulzer), *Nicotiana tabacum* L.	7.11. 2000, Veľký Kýr, 48°11'00" 18°09'30"
2	*Myzus persicae* (Sulzer), *Nicotiana tabacum* L.	13.11. 2000, Komjatice, 48°09'20" 18°10'00"
3	*Myzus persicae* (Sulzer), *Nicotiana tabacum* L.	17.11. 2000, Veľký Kýr, 48°11'00" 18°09'30"
4	*Myzus persicae* (Sulzer), *Brassica oleracea* L.	5.12. 2000, Komjatice, 48°09'20" 18°10'00"
5	*Microlophium carnosum* (Buckton), *Urtica dioica* L.	18.4. 2001, Dolná Malanta, 48°19'00" 18°07'00"
6	*Microlophium carnosum* (Buckton), *Urtica dioica* L.	28.4. 2001, Dolná Malanta, 48°19'00" 18°07'00"
7	*Microlophium carnosum* (Buckton), *Urtica dioica* L.	28.4. 2001, Dolná Malanta, 48°19'00" 18°07'00"
8	*Sitobion avenae* (Fabricius), *Triticum aestivum* L.	21.5. 2001, Komjatice, 48°09'20" 18°10'00"
9	*Sitobion avenae* (Fabricius), *Triticum aestivum* L.	21.5. 2001, Komjatice, 48°09'20" 18°10'00"
10	*Uroleucon aeneum* (Hille Ris Lambers), *Carduus nutans* L.	13.6. 2002, Komjatice, 48°09'20" 18°10'00"
11	*Uroleucon aeneum* (Hille Ris Lambers), *Carduus nutans* L.	22.6. 2002, Veľký Cetín, 48°13'00" 18°11'30"
12	*Microlophium carnosum* (Buckton), *Urtica dioica* L.	17.6. 2002, Námestovo, 49°24'30" 19°29'00"
13	*Microlophium carnosum* (Buckton), *Urtica dioica* L.	18.6. 2002, Sliač, 48°36'50" 19°08'00"
14	*Rhopalosiphum padi* (L.), *Zea mays* L.	15.10. 2002, Vlkas, 48°07'30" 18°16'30"
15	*Rhopalosiphum padi* (L.), *Zea mays* L.	21. 10. 2002, Komjatice, 48°09'20" 18°10'00"

square piece of YG agar (2% yeast extract, 3% glucose, 1.5% agar) [30]. The conidia were incubated for 4 h at 100% or 75% of relative humidity, at 20 ± 2°C, under constant illumination. The humidity was controlled by a method of saturated water solutions proposed by [37]. The germination experiment was carried out at five replicates (20 conidia per replicate were evaluated) for each isolate, surface and relative humidity regime. A type of germination, germ-tube formation or secondary spore formation, were recorded separately for each isolate.

Radial growth rate of the isolates. Sabouraud dextrose agar with egg yolk and milk [22] was used when radial growth of isolates was measured. Five-mm diameter discs of unsporulated mycelium were cut from the plates using a sterile cork borer and individually placed upside down in the centre of 70 mm Petri dishes containing culture medium. Five replicates per each isolate were incubated at 20 ± 2°C under constant illumination for 30 days and examined within 5-day intervals to measure a diameter of fungal colonies. The daily rate of radial growth was calculated as (x – 5) / t, where x is a diameter of fungal colony (in mm) measured at the time t expressed in days and the parameter of 5 is a diameter (in mm) of the disk used for inoculation. The radial growth rate was compared among the isolates by one-way ANOVA.

Biomass production of the isolates. Fungal biomass was grown in a liquid medium [30] containing 1% yeast extract, 10% milk, and 1.6% glucose. Before the biomass production experiment was established a preculture had been grown. Five pieces were cut from fast growing margins of culture and added to 25 ml of medium in a 100 ml flask. The submerge cultures were incubated at 20

Table 2. Dimensions of primary conidia, virulence expressed by LC_{50} and biomass production of *P. neoaphidis* isolates
Tabuľka 2. Rozmery primárnych konídií, virulencia vyjadrená v LC_{50} a produkcia biomasy izolátov *P. neoaphidis*

Isolate number	Size of *in vitro*-primary conidia (µm)			Size of *in vivo*-primary conidia (µm)			LC_{50} (conidia/mm^2)	Average biomass production (g.l^{-1})
	Length (L)	Diameter (D)	Ratio L/D	Length (L)	Diameter (D)	Ratio L/D		
1	36.53 i*	17.92 ef*	2.04 ef*	26.73 c*	12.97 ef*	2.05 de*	24 a*	8.40 d*
2	31.87 fg	16.29 d	1.96 de	23.36 ab	11.82 bc	1.98 cd	203 cd	3.42 b
3	35.42 i	19.97 g	1.78 bc	24.73 abc	13.74 g	1.79 ab	60 ab	3.78 b
4	29.17 bc	18.12 f	1.61 a	22.72 a	14.59 h	1.66 a	44 ab	3.95 b
5	25.06 a	11.95 a	2.09 fgh	22.98 a	11.30 ab	1.99 cd	213 cd	1.82 a
6	31.44 ef	14.65 c	2.15 h	24.46 abc	11.86 bc	2.06 dc	113 abc	8.32 d
7	30.57 de	16.84 d	1.82 c	24.44 abc	11.84 bc	2.08 de	30 ab	5.80 c
8	30.42 de	14.10 bc	2.13 gh	22.92 a	11.07 a	2,07 de	214 cd	1.83 a
9	29.58 cd	14.07 bc	2.10 fgh	22.39 a	10.90 a	2.06 de	35 ab	5.32 c
10	24.47 a	13.40 b	1.83 c	23.50 ab	12.54 de	1.88 bc	131 bcd	7.93 d
11	32.83 gh	16.99 de	1.93 d	25.72 bc	13.89 g	1.85 bc	173 cd	3.23 b
12	36.09 i	18.34 f	1.97 de	25.99 c	13.49 fg	1.94 cd	233 d	3.25 b
13	33.32 h	19.31 g	1.73 b	25.76 bc	13.95 g	1.86 bc	194 cd	1.63 a
14	28.08 b	12.04 a	2.34 i	23.47 ab	11.24 ab	2.17 d	200 cd	8.72 d
15	29.69 cd	14.43 c	2.06 fg	25.44 bc	12.24 cd	2.07 de	207 cd	6.57 c

* Means followed by the same letter within each column are not significantly different (Tukey's HSD test, $p > 0.05$)
*Hodnoty s rovnakým písmenom v stĺpci nie sú významne rozdielne (Tukeyov HSD test, $p > 0.05$)

± 2°C in the dark and agitated on rotary shaking machine (at 170 rpm) for 5 days. To establish a production culture 7 ml of the preculture was added to 40 ml of medium in a 100 ml flask. The cultures were incubated under the same conditions as the precultures for 5 days. The biomass was harvested by filtration and desiccated on filter paper at 50°C for 60 minutes. The dry mass of mycelium was evaluated. The experiment was carried out at 6 replicates for each isolate and the means of harvested biomass were compared among the isolates by one-way ANOVA.

Virulence of the isolates. The bioassay for assessment of virulence against pea aphid, Acyrthosiphon pisum Harris, was carried out following the procedure originally designated by [15,21]. Colonies of single pea aphid clone were maintained on potted pea plants, Pisum sativum L., at 20 ± 2°C, 40-60% of relative humidity and under a photoperiod of L:D 16:8 h. Plates of tested isolates were used as a source of inoculum. Altogether 3 replicates of 20 nymphs per isolate were used. Control variants (20 nymphs) were treated similarly, but aphids were not exposed to conidia. The control was used to check potential presence of naturally occurring fungal pathogens. A value of LC_{50} (a concentration of conidia killing 50% of the treated insects), which characterized the virulence of an isolate at the time of experiment, was estimated from regression line of probit mortality versus log-dose [9]. One-way ANOVA was performed on the LC_{50} values to determine if there were significant differences between isolates. Comparisons of means were performed using Tukey's honestly significant different (HSD) test at the 5% level.

RESULTS

Size of primary spores of P. neoaphidis isolates and dimensions of in vivo-primary spores collected from killed aphids prior the fungus isolation are presented in the Table 2. Size of in vitro-primary spores varied among the isolates indicating a great morphological heterogeneity. One-way ANOVA showed significant difference among the morphological characteristics of in vitro produced primary spores at 99% confidence level ($F_{2.51} = 76.35$, $P < 0.01$ for length; $F_{2.51} = 57.30$, $P < 0.01$ for diameter; and $F_{2.51} = 40.45$, $P < 0.01$ for the ratio of L/D). A multiple comparison procedure to determine which values were significantly different from which others revealed 9 homogenous groups for the length and the L/D ratio, and 7 homogenous groups for the spore diameter. These results confirmed a relevant biometrical heterogeneity among the isolates. Conidial dimensions of isolates that originated from the same host, site and date (isolates No. 6, 7 and 8, 9) were not significantly different. Certain degree of variability was detected in biometrics of conidia collected from aphid cadavers, as well ($F_{2.04} = 2.69$, $P < 0.05$ for length; $F_{2.04} = 29.62$, $P < 0.05$ for diameter; and $F_{2.04} = 7.45$, $P < 0.05$ for the ratio of L/D).

Estimates of lethal concentrations of conidia (LC_{50}) of Slovak P. neoaphidis isolates for the pea aphid are presented in the Table 2. The estimated LC_{50} values ranged from 24 to 233 conidia per mm^2. This range indicates a rather great variability among the isolates and one-way ANOVA showed significant differences in the LC_{50} values among the isolates at 99% confidence level ($F_{2.74} = 4.91$, $P < 0.01$). Multiple range tests detected 4 homogenous groups at the confidence level of 95%.

Results on conidial germination experiment are shown in the Table 3. Generally, primary conidia of isolates could germinate on all surfaces and in both relative humidities being tested. However, a character of germination varied between surface types and relative humidity regimes. As for relative humidity it was evident that higher germination appeared at saturated atmosphere. At this level of humidity conidia of all the isolates germinated on each surface type, but no germination was recorded on nutrient agar (YG) for isolates 7, 13 and 15. At 75% relative humidity conidia did not germinate on a glass surface, or only few conidia germinated. On the contrary, conidia germinated well at 75% relative humidity on water agar and YG agar.

P. neoaphidis isolates showed a good growth on the artificial culture medium. Daily rate of the radial growth ranged from 1.03 to 2.00 mm (Table 4). One-way ANOVA showed significant difference in radial growth rate among the isolates tested at 99% confidence level ($F_{2.39} = 22.11$, $P < 0.01$). Multiple range tests showed heterogeneity in the results and 9 homogenous groups were detected. Isolates of the same origin have also significantly different growth rate.

Laboratory-scale bioassay was carried out to evaluate and quantitatively compare a fungal mass production of the isolates. Results of the experiment are shown in the Table 2. Average biomass production of P. neoaphidis isolates ranged between 1.63 and 8.72 $g.l^{-1}$. The biomass production was significantly different among the isolates ($F_{2.32} = 29.86$, $P < 0.01$). Tukey's HSD test identified heterogeneity in the results and 4 homogenous groups were determined. We found a strong positive correlation (+0.737; $t_{3.67}=3.93$, P=0.001, df=28) between the average biomass production and the daily rate of radial growth.

DISCUSSION

The size of conidia produced in vitro generally exceeded

Table 3. Germination of *P. neoaphidis* primary spores on three surfaces and at two humidity regimes
Tabuľka 3. Klíčenie primárnych konídií *P. neoaphidis* na rôznych povrchoch a vlhkostných režimoch

Isolate number	Germination type	100% relative humidity			75% relative humidity		
		Glass	WA	YG	Glass	WA	YG
	Germ tube	76%	0%	0%	0%	0%	0%
1	Secondary conidium	23%	93%	85%	0%	100%	96%
	Total germination	**99%**	**93%**	**85%**	**0%**	**100%**	**96%**
	Germ tube	3%	1%	0%	0%	0%	0%
2	Secondary conidium	1%	96%	10%	0%	90%	0%
	Total germination	**4%**	**97%**	**10%**	**0%**	**90%**	**0%**
	Germ tube	50%	0%	0%	0%	0%	0%
3	Secondary conidium	0%	14%	27%	0%	83%	15%
	Total germination	**50%**	**14%**	**27%**	**0%**	**83%**	**15%**
	Germ tube	83%	0%	0%	0%	0%	0%
4	Secondary conidium	0%	91%	84%	0%	97%	91%
	Total germination	**83**	**91%**	**84%**	**0%**	**97%**	**91%**
	Germ tube	4%	1%	0%	0%	0%	0%
5	Secondary conidium	0%	43%	63%	0%	90%	51%
	Total germination	**4%**	**44%**	**63%**	**0%**	**90%**	**51%**
	Germ tube	54%	0%	1%	0%	0%	0%
6	Secondary conidium	0%	96%	0%	0%	100%	0%
	Total germination	**54%**	**96%**	**1%**	**0%**	**100%**	**0%**
	Germ tube	19%	0%	0%	0%	0%	0%
7	Secondary conidium	75%	63%	0%	0%	100%	58%
	Total germination	**94%**	**63%**	**0%**	**0%**	**100%**	**58%**
	Germ tube	28%	0%	0%	0%	0%	0%
8	Secondary conidium	2%	81%	61%	7%	89%	0%
	Total germination	**30%**	**81%**	**61%**	**7%**	**89%**	**0%**
	Germ tube	35%	0%	0%	1%	0%	0%
9	Secondary conidium	48%	95%	98%	0%	96%	92%
	Total germination	**83%**	**95%**	**98%**	**1%**	**96%**	**92%**
	Germ tube	5%	8%	0%	0%	2%	0%
10	Secondary conidium	60%	63%	34%	0%	81%	58%
	Total germination	**65%**	**71%**	**34%**	**0%**	**83%**	**58%**
	Germ tube	10%	0%	0%	0%	0%	0%
11	Secondary conidium	16%	26%	10%	0%	58%	41%
	Total germination	**26%**	**26%**	**10%**	**0%**	**58%**	**41%**
	Germ tube	8%	5%	0%	1%	0%	0%
12	Secondary conidium	7%	74%	59%	0%	92%	85%
	Total germination	**15%**	**79%**	**59%**	**1%**	**92%**	**85%**
	Germ tube	42%	0%	0%	2%	2%	0%
13	Secondary conidium	0%	96%	0%	0%	92%	0%
	Total germination	**42%**	**96%**	**0%**	**2%**	**94%**	**0%**
	Germ tube	19%	0%	0%	9%	0%	0%
14	Secondary conidium	0%	88%	1%	0%	87%	4%
	Total germination	**19%**	**88%**	**1%**	**9%**	**87%**	**4%**
	Germ tube	21%	0%	0%	0%	0%	0%
15	Secondary conidium	0%	89%	0%	0%	90%	1%
	Total germination	**21%**	**89%**	**0%**	**0%**	**90%**	**1%**

the ranges for conidia from aphids described by Keller [14] 22.7 – 25.0 x 8.5 – 15.0 µm, or the ranges by Bałazy [1] 21.0 – 32.0 x 11.0 – 14.0 µm. However, the L/D ratios corresponded with that published by Keller [14]. In other studies on intraspecific variation in P. neoaphidis strains the differences in conidial size were examined and very little variation was found [16]. On the other hand it is usually observed that primary conidia from cultures are obviously larger than those from insects [17]. We also found that in vivo-produced conidia are significantly smaller than in vitro produced conidia ($F_{7.64}$ = 45.19, P < 0.01 for length; $F_{7.64}$ = 21.52, P < 0.01 for diameter). Measurements of the in vivo-produced conidia were consistent with those presented by Keller [14] and Bałazy [1]. It is necessary to emphasize that variation in conidial morphology is typical for this species [1,14,24] and even it is considered as a complex of species [1,13].

For entomophthoralean fungi the bioassay procedures using sporulating cadavers or in vitro cultures as an inoculum source have been described in a number of studies [e.g. 15,20,21,22]. Generally, values of LC_{50} for Entomophthorales range from a few to tens conidia per mm² [21]. Average virulence for all the Slovak isolates was 138 conidia per mm². Several previous studies reported much lower LC_{50} values for P. neoaphidis than that presented in this work [e.g. 8,28,30]. Sierotzki et al. [30] state a value of 16 conidia per mm² as a mean LC_{50} for 4 Swiss P. neoaphidis isolates. Out of the Slovak isolates 4 isolates had virulence to the pea aphid with LC_{50} below a level of 50 conidia per mm². On the contrary, 6 isolates had the estimated LC_{50} of about 200 conidia per mm². The relatively low virulence of the Slovak isolates could be attributed to the fact that neither of the isolates was originally obtained from A. pisum, the aphid used in the bioassay. However, Sierotzki et al. [30] found no significant difference in virulence among strains isolated from aphid species different or the same to the species used in bioassay. Shah et al. [28] presented results where isolate of P. neoaphidis obtained from Myzus persicae (Sulzer) was approximately 3-10 times more virulent to A. pisum than to its original host. The relatively low virulence in our study could also be a result of using nymphs in the bioassay. Nymphs can be less susceptible to the pathogens than adults because the invading conidia

Table 4. Radial growth rate of the *P. neoaphidis* isolates
Tabuľka 4. Radiálny rast izolátov *P. neopahidis*

Isolate number	Mean radial growth (a diameter of fungal colony in mm)						Daily rate of growth
	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	
1	15	25	35	45	54	60	**1.84 gh***
2	7	13	18	25	30	39	**1.13 ab**
3	8	11	17	30	39	45	**1.32 bc**
4	8	13	21	27	40	49	**1.47 def**
5	8	13	17	24	29	36	**1.03 a**
6	12	23	33	42	51	61	**1.86 h**
7	8	13	25	35	45	52	**1.56 def**
8	13	18	24	33	43	54	**1.62 fgh**
9	10	17	28	40	52	61	**1.87 gh**
10	12	22	32	38	47	55	**1.66 efg**
11	10	15	21	25	30	37	**1.05 a**
12	11	19	27	37	47	30	**1.50 cde**
13	10	14	22	27	34	40	**1.16 a**
14	21	30	43	51	61	65	**2.00 i**
15	8	12	21	30	38	46	**1.38 cd**

* Means within column followed by the same letter are not significantly different (Tukey's HSD test, $p > 0.05$)
*Hodnoty s rovnakým písmenom v stĺpci nie sú významne rozdielne (Tukeyov HSD test, $p > 0.05$)

could be shed with the old cuticle during ecdysis [15]. Virulence of isolates may also decrease significantly during long-term maintenance of cultures in vitro [16,21]. Each isolate used in our experiments was passaged through specimen of pea aphid and re-isolated not longer than 6 months prior to the bioassay. A great variation in virulence among the Slovak isolates was obvious. We even determined significant differences among isolates of the same provenance. This is for example the case of isolates 1 and 3 obtained from M. persicae during fungal epizootic. Virulence was also significantly different between isolates 6 and 7 obtained from Microlophium carnosum on Urtica dioica L. and isolates 8 and 9 obtained from a single colony of Sitobion avenae on a wheat ear. Apparent differences between isolates originating from the same host and collection site was detected using molecular analysis by Sierotzki et al. [30] and Rohel et al. [25]. Some degree of variation can be observed between values of LC_{50} for one strain with one test-insect species evaluated at different times [21,22]. A source of variability in the results can also come from heterogeneity in the groups of aphids submitted to given amounts of inoculum. The degree of aphid susceptibility to fungi can depend on numerous parameters, e.g. developmental stage and physiological state of the insects [15]. We tried to use as homogenous population of aphid as possible using a progeny of single mother aphid and a maximal standardization of rearing conditions.

Dependence of sporulation [34] and spore germination [15,30,31] on relative humidity near to or at saturation level was reported for P. neoaphidis. Our results confirmed the necessity of saturation for conidial germination. We assume that the higher proportion of germinating conidia recorded at 75% relative humidity on the surface of water agar and artificial medium was due to water present in the media, which compensated the low air humidity in the vicinity of conidia. While glass did not offered the alternative source of water the germination was absent or low. Airborne conidia are considered very short-lived propagules [12]. For infection to start, after conidia are produced and discharged, they must survive until contacting a new host. We observed that conidia deposited on glass and exposed to low humidity (75%) for 4 hours did not germinate. The germination did not occur although the conidia were subsequently exposed to 100% relative humidity. Uziel & Kenneth [32] also found that primary conidia could not tolerate prolonged exposure to low relative humidity. However, Brobyn et al. [5] demonstrated that P. neoaphidis conidia could survive a period of several days at relatively low humidity.

Our results indicate that character of surface can influence a type of conidial germination. On the glass surface the germ-tube formation prevailed over the secondary conidium formation at saturated atmosphere, whereas secondary conidia were almost exclusively produced on the agarized surfaces at both humidity regimes. Recently, similar experiment has been carried out by Sierotzki et al. [30]. They found out secondary conidium production on agar substrate and germ-tube production on polystyrene surface. They observed a formation of secondary conidia on glass slides when water condensated on the slides and contacted the conidia. It was hypothesized that the presence or absence of free water, rather than physico-chemical properties of the surface, was the trigger for differentiation into secondary conidia or germ-tubes. However, in our experiments water condensed on glass slides, but mostly germ-tubes were produced. Hajek et al. [11] observed that rather chemical stimuli and not changes in hydrophobicity of surface affected germination of conidia. Germination of conidia on various surfaces has been studied for a number of entomopathogenic fungi [e.g. 10,11,30,31,33] and influence of diverse physico-chemical and nutritional parameters on stimulation and differentiation of conidial germination were evaluated. At optimal conditions (temperature and humidity) the process of germination can be affected by nutrients or chemical stimuli [4,19,26,33], by conidial density on the surface [10], or by rigidity of surface [11].

The evaluated growth parameters of Slovak isolates are close properties, what indicates the strong correlation between both of them. It is of interest that Slovak isolates of the same origin (isolates of numbers 6, 7 and 8, 9) produce significantly different amount of biomass at standard conditions. Isolates 6 and 7 also displayed significantly different parameters of radial growth, but variability of the radial growth between isolates 8 and 9 was not significant. Sierotzki et al. [30] published that biomass production of Swiss isolates ranged between 7.8 and 14.9 $g.l^{-1}$. We used liquid medium of the same composition in the experiment, but the Slovak isolates yielded fewer amount of biomass. The Swiss isolates also showed significant differences in biomass production irrespective of isolate origin [30].

We evaluated phenotypic and physiological characters of P. neoaphidis isolates and the results revealed a significant intraspecific variation within this species. Intraspecific variability of entomophthoralean fungi used to be principally characterised by differences in pathogenicity [21]. Recently, molecular techniques have also been involved to analyse intraspecific variation in the fungal isolates [25,30]. The Slovak isolates varied significantly in all characteristics evaluated. Even, isolates obtained from single colonies during epizootics showed significant differences in the characteristics investigated, except

that of conidial size. This may indicate that epizootics in the field are caused by consortia of strains and not by prevalence of a single virulent strain. Within-field variability of P. neoaphidis strains has been discussed by Shah et al. [28]. Results of molecular analyses by Rohel et al. [25] and Sierotzki et al. [30] displaying variability in strains of different geographic origin and also isolates originating from the same field and host, support the supposition of association of more fungus strains during epizootics. The knowledge of different fungal strain co-existence with variable physiological characteristics, including their virulence, during epizootics in host populations is important to understand epizoological processes of this fungus.

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