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TO THE STUDY OF BIOCENOTIC RELATIONSHIPS BETWEEN HOUSE DUST MITES (ACARIFORMES: PYROGLYPHIDAE) AND MOULD FUNGI

К ИЗУЧЕНИЮ БИОЦЕНОТИЧЕСКИХ ОТНОШЕНИЙ ПИРОГЛИФИДНЫХ КЛЕЩЕЙ (ACARIFORMES: PYROGLYPHIDAE) И ПЛЕСНЕВЫХ ГРИБОВ

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ABSTRACT

The population dynamics of two house dust mites species, *Dermatophagoides pteronyssinus* and *D. farinae*, and the mould fungi were studied during long-term culturing of mites (30 weeks) in the simple periodic cultures (SPC) without food supply added. Beard shavings (BS), were used as the food substrate. The population dynamics of *D. pteronyssinus* and *D. farinae* in SPC were similar in general and differed by the longevity of the lag-phase, exponential growth, plateau, and decline, as well as by the maximum abundance and the relative speed of the population growth.

The species diversity and abundance of micromycetes in the primary food substrate for mites (control) were low. In the following experimental period these species were not recorded anymore. In SPC of *D. pteronyssinus* and *D. farinae* 14 species of mould fungi from 6 genera were revealed. Ten species of micromycetes from 4 genera were recorded in the culture of *D. pteronyssinus*, and 11 species from 5 genera were found in the culture of *D. farinae*. The Jaccard similarity index constituted 50%. In the cultures of both species *Aspergillus penicillioides* predominated with the frequency value 100% and the maximum number more than 10^8 CFU/g of substrate. *A. repens, Wallemia sebi* and *Chrysosporium* sp. had subdominant positions. An increase in number of mites and mould in cultures was synchronous. However after reaching the plateau there were no decrease in number of micromycetes observed like in the populations of mites. These data should be taken into account when preparing mite allergens. The relationships between mites and mould can be regarded in this experiment as *protocooperation*.

РЕЗЮМЕ

Изучена динамика численности двух видов аллергенных клещей домашней пыли *Dermatophagoides pteronyssinus, D. farinae* и плесневых грибов при длительном культивировании клещей (30 недель) в простых периодических культурах (ППК) (без добавления корма). Пищевой субстрат — утильные волосы из электробритв. Характер динамики численности популяций *D. pteronyssinus* и *D. farinae* в ППК был сходен в общих чертах, но отличался по длительности лаг-фазы, экспоненциального роста, плато и снижения численности, а также уровню максимальной численности и удельной скорости роста численности популяций.

Видовое разнообразие и численность микромицетов в исходном пищевом субстрате (контроль) для клещей были низки, и в даль-

нейшем ходе эксперимента эти грибы практически не выявлялись. В ППК *D. pteronyssinus* и *D.farinae* выявлено в общей сложности 14 видов плесневых грибов из 6 родов: в культуре *D. pteronyssinus* — 10 видов из 4 родов, а в культуре *D. farinae* — 11 видов из 5 родов. Коэффициент сходства Жаккара составил 50%. В культурах обоих видов клещей супердоминирующее положение занимал *Aspergillus penicillioides,* частота обнаружения которого составляла 100%, а численность достигала порядка 10^8 КОЕ/г субстрата. Субдоминирующее положение занимали *A. repens, Wallemia sebi* и *Chrysosporium* sp. Нарастание численности клещей и грибов в культурах происходило синхронно. Однако после достижения плато снижение численности микромицетов, в отличие от клещей, не наблюдалось. Полученные данные следует учитывать при изготовлении клещевых аллергенов. Взаимоотношения клещей и грибов, в первую очередь *A. penicillioides*, в данном эксперименте можно рассматривать как протокооперацию.

INTRODUCTION

The house dust mites of the family Pyroglyphidae and mould fungi are of special interest as the allergens sources. The biocenotic relationships between these two groups influence the complex indoor allergens exposure.

It is known that mould fungi play an important role in the diet of various mites. For example, the mould effects the food substrate to be utilized by storage mites [Zakhvatkin, 1941]. Experiencing shortage of the major food source the mites can shift completely to the mould diet [Griffith et al., 1959; Sinha, 1964, 1966; Farahat, 1966; Abdel-Sater, Eraky, 2002].

The biocenotic relationships between pyroglyphid mites and fungi were first studied in 1970s [Sinha et al., 1970; Bronswijk, Sinha, 1971, 1973]. The research in this field was focused mostly on trophic relations of these mites with mould fungi, which were used as additives to the food substrates for culturing house dust mites as these cultures were used for preparing mite allergens [Wharton, 1976, etc.].

Data on biocenotic relationships between pyroglyphid mites and mould fungi are not complete and often contradictory [Lustgraaf, 1978; Douglas, Hart, 1989; Hart, Douglas, 1991; Hay et al., 1993]. Correspondingly, the main purpose of the present research was the experimental study of population dynamics of *Dermatophagoides pteronyssinus, D. farinae* and mould fungi in the simple periodic cultures (SPC).

MATERIAL AND METHODS

The initial laboratory cultures of *Dermatophagoides pteronyssinus* and *D. farinae* were used in the experiment being kept on beard shavings (BS). Two grams of the food substrate (BS) that was not previously sterilized were placed in the culturing container. Mites were placed in the containers in the proportion 100 individuals per 1 g of BS (50 males and 50 females). The cultures were kept in a thermostat (no light, temperature 25±2°C and the relative humidity (RH) of 75±3%). During the experiment (30 weeks) the food was not added to the cultures, thus making the experiment to be done in a simple periodic culture (SPC). Each experiment variant was performed in four replications. BS served as a control and was kept at the temperature 25±2°C and 75±3% RH. The samples were taken from the culturing containers at 3rd, 5th, 8th, 11th, 18th, 21st, 25th, 28th and 30th week from the day the cultures were established. Micromycetes were revealed by the method of serial dilutions on xerophilic (600 ml wort, 400 ml H_2O, 100 g NaCl, 20 g agar) and Czapek's agar. The number of colony-forming units (CFU) of micromycetes were extrapolated on 1 g of the substrate.

MS Excel 6.0 was used to prepare tables, graphs and for statistical processing.

RESULTS

The experimental populations of *Dermatophagoides pteronyssinus* and *D. farinae* in SPC undergo the following phases: lag-phase, exponential (logarithmic) growth, slow growth, plateau and decline (abundance decrease) [Zheltikova, 1998]. Not all phases were clearly observed in two species. For example, the plateau phase was not well expressed in *D. farinae* comparing with *D. pteronyssinus* (Fig. 1).

In *D.pteronyssinus* the lag-phase ended from 3rd to 5th week since the culture was established, followed by the exponential growth phase (Fig. 1). The middle of the latter phase was reached on 11th week and was characterized by a maximum value of the relative growth speed [Odum, 1986] of the mites population (0.69 mites per day). The number of *D.pteronyssinus* reached maximum at 18th week of culturing (6810 mites/g of substrate) exceeding the primary number more than 68 times. After 18th week the number of mites went down and was only 4165 mites/g at the end of the experiment, that was

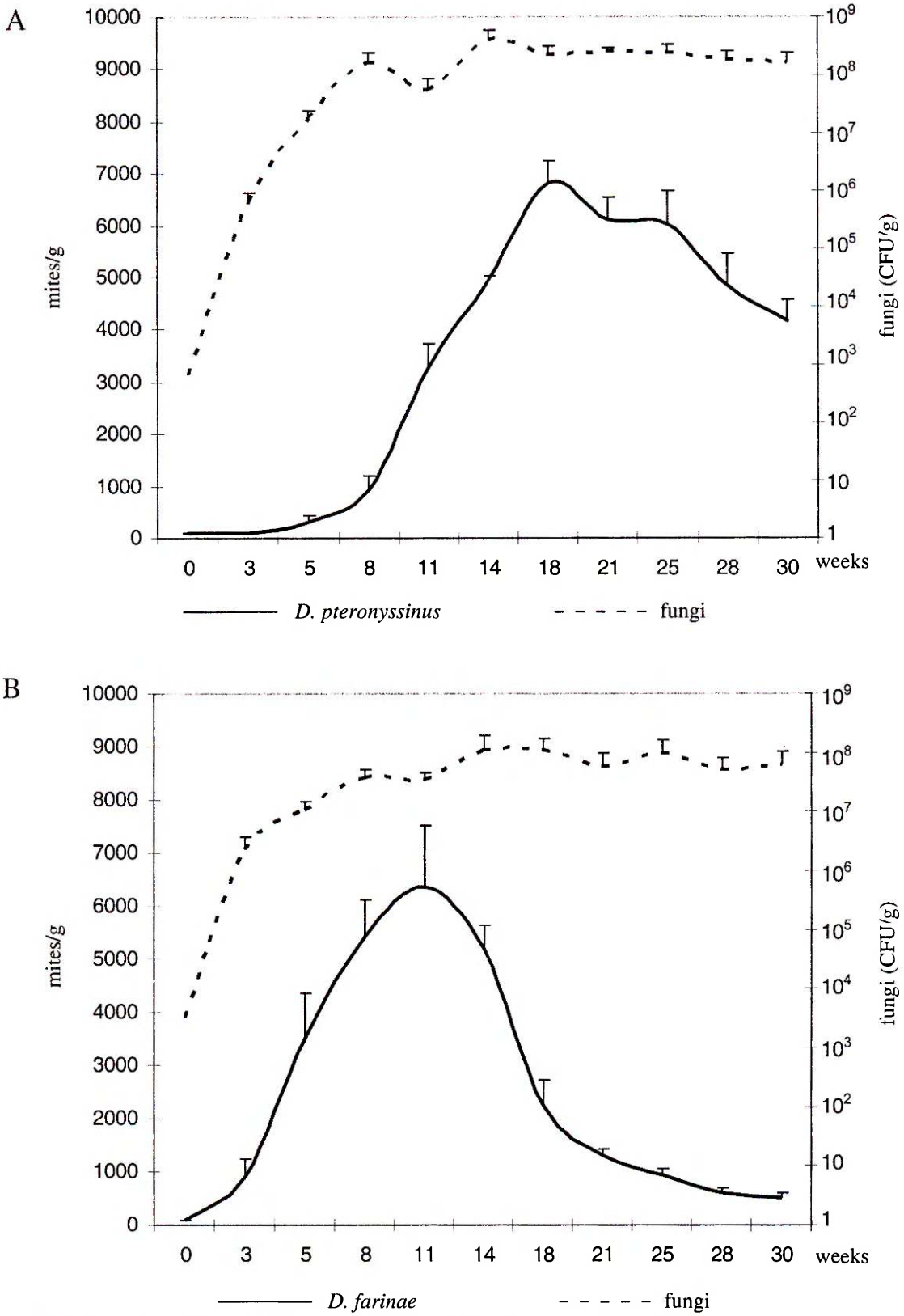


Fig. 1. The population dynamics of house dust mites and fungi in the simple periodic cultures. A — *Dermatophagoides pteronyssinus*, B — *D. farinae*.

1.6 times less than the maximum abundance of mites reached.

In *D. farinae* the lag-phase presumably ended up before the 3rd week since the time the culture was established (Fig. 1). The middle of the exponential growth phase (5th week) was characterized by the maximum relative growth speed of the population (0.91 mites per day). The number of mites reached its

maximum at 11th week constituting 6353 mites/g of substrate, that exceeded the primary mite abundance 63.5 times. After 11th week the number of mites went down, being 3 times less than the maximum abundance at 18th week. At 30th week the number of mites constituted only 528 mites/g of substrate.

Three species of micromycetes from 2 genera and 2 species of micromycetes from 2 genera, respectively, were revealed in the initial cultures of *D. pteronyssinus* and *D. farinae*. *A. penicillioides* predominated in the initial cultures of both species of mites outnumbering other species of fungi 10^5 times (Table 1, 2). The initial culture of *D. pteronyssinus* was characterized by presence of *A. repens* and *Wallemia sebi*, which were frequently encountered lately in the experimental cultures of these mites (Table 1).

Two species of micromycetes from 2 genera were revealed in the food substrate (BS, control) where mites were not present during the experiment. Both species of fungi were recorded only after the first isolation. Lately the micromycetes were not encountered (Table 1, 2).

Eight species of micromycetes from 3 genera were revealed in the food substrate (BS), which was lately inhabited by mites. The number of fungal propagules was 10^2–10^3 CFU/g of substrate. In the following period of time these fungi were not recorded anymore with the exception of *A. ustus* and *P .citrinum* (Table 1, 2).

In the culture of *D. pteronyssinus* 10 fungal species from 4 genera were revealed (Table 3). *A. penicillioides* predominated, its relative abundance constituted 99.8% with 100% frequency. The frequency of *A. repens* and *W. sebi* was 90% and 70%, correspondingly. However their relative abundance was less than 0.1%. The frequency and relative abundance of other micromycetes did not exceed 30% and 0.1%, correspondingly (Table 3). This fact suggests that these species are "accidental" in the cultures.

Eleven fungal species from 5 genera were revealed in the culture of *D. farinae* (Table 3). As in the case with the culture of *D. pteronyssinus, A. penicillioides* predominated. Its relative abundance constituted 99.4% with the frequency being 100%. The frequency for *A. repens* was 40%, and that for *Chrysosporium* sp. was 30%. The relative abundances of the latter fungal species were less than 0.1% and 0.4%, correspondingly (Table 3). *W. sebi* was not found in the culture of *D. farinae*, despite the fact it was regularly revealed in the culture of *D. pteronyssinus*.

Thus, in total 14 species of micromycetes from 6 genera were revealed in the cultures of *D. pteronyssinus* and *D. farinae* (Table 3). Seven species of micromycetes were identical in the cultures of both mites species. The Jaccard similarity index equaled 50%.

The mould fungi underwent the same phases in their development in SPC as the mites: lag-phase, exponential (geometric) growth, slow growth, and plateau. Distinctly from mites the number of fungi did not decrease after reaching the maximum and the plateau phase until the end of the experiment (Fig. 1, Table 1, 2). The dynamics of the mould fungi total number in the cultures of *D. pteronyssinus* and *D. farinae* were similar and were found to be determined by the dynamics of *A. penicillioides*. The abundance of the latter species grew constantly since it was first found and reached the plateau at 8th and 5th week for *D. pteronyssinus* and *D. farinae*, respectively. The total number of mould fungi in cultures of both *D. pteronyssinus* and *D. farinae* grew parallel to the number of mites (Fig. 1). The maximum concentration of micromycetes recorded in the culture of *D. pteronyssinus* and *D. farinae* was 4×10^8 CFU/g of substrate and 1.7×10^8 CFU/g of substrate, respectively.

DISCUSSION

The species diversity of micromycetes in SPC of pyroglyphid mites *D. pteronyssinus* and *D. farinae* embraces only 14 species belonging to 6 genera (not counting control) (Table 3). Most species of fungi were revealed only once and their number did not exceed 10^3 CFU/g of substrate, the fact suggesting that the fungi propagules were brought accidentally, probably, from the indoor air. Presumably the low taxonomic diversity of micromycetes was associated with the fact that BS as a food source were available only for a limited spectrum of the fungal species.

The structural organization of mould fungi complex in pyroglyphid mites cultures can be influenced by several factors. 75% RH that was applied in the experiment has affected development of most micromycetes but appeared to be an optimal one for the growth of the xerophilic species *A. penicillioides, A. repens, Chrysosporium* sp. and *W. sebi*. The superdominant *A. penicillioides* as well as *A. repens* and *W. sebi* were found in the initial cultures of mites in similar concentrations (Table 1, 2). Two latter species were found regularly in experimental cultures. Obviously, the mites brought the spores of these fungi on the cuticle surface as well as in the

Table 1

Fungal species and their concentration (CFU/g) in the simple periodic cultures of the house dust mite *D. pteronyssinus*

Таблица 1

Виды и концентрация плесневых грибов (КОЕ/г), выделенных из простых периодических культур синантропных клещей *D. pteronyssinus*

Species	Control				Weeks									
	I*	BS1*	BS2−0*	BS2−1*	3	5	8	11	14	18	21	25	28	30
Aspergillus fumigatus Fresen.	–	1.0×10^3	–	–	–	–	–	–	–	–	–	–	–	–
A. niger v.Thiegh.	–	–	2.8×10^2	–	–	–	–	–	9.9×10^3	–	–	–	–	5.6×10^2
A. penicillioides Speg.	10^8	–	–	–	4.2×10^5	1.8×10^7	1.7×10^8	5.7×10^7	4.0×10^8	2.3×10^8	2.6×10^8	2.5×10^8	1.9×10^8	1.7×10^8
A. repens De Bary	10^3	–	–	–	–	1.9×10^3	3.1×10^3	4.2×10^3	9.2×10^3	2.7×10^4	7.2×10^3	2.9×10^3	7.6×10^3	2.2×10^3
A. ustus (Bain.) Thom & Church	–	4.2×10^2	–	–	–	–	–	–	–	2.3×10^4	1.4×10^5	–	–	1.4×10^5
Aureobasidium pullulans (d By.) Arnaud	–	–	2.8×10^2	–	–	–	–	–	–	–	–	–	–	–
Cladosporium cladosporioides (Fresen.) de Vries	–	1.0×10^3	–	–	–	–	–	–	–	–	–	–	–	–
C. herbarum (Pers.: Fr.) Lk.	–	–	–	–	–	–	2.8×10^3	–	–	–	–	–	–	–
Penicillium chrysogenum Thom	–	–	–	–	–	–	2.9×10^3	–	1.9×10^4	–	–	1.4×10^5	–	–
P. citrinum Thom	–	2.8×10^3	–	–	–	–	–	–	–	–	–	–	–	2.8×10^3
P. glabrum (Wehmer) Westl.	–	1.2×10^3	–	–	–	–	–	–	–	–	–	–	–	–
P. lanosum Westl.	–	–	–	–	–	–	1.4×10^5	–	–	–	–	–	–	1.4×10^5
P. janczewskii Zaleski	–	–	–	–	–	–	3.5×10^3	–	–	–	–	–	–	–
P. verrucosum Dierckx	–	4.2×10^2	–	–	–	–	–	–	–	–	–	–	–	–
Penicillium sp.	–	–	–	–	2.0×10^5	2.4×10^4	1.3×10^6	–	–	–	–	–	–	–
Wallemia sebi (Fr.) v.Arx	10^3	–	–	–	–	4.2×10^3	4.8×10^4	1.5×10^4	1.9×10^4	1.7×10^4	2.2×10^4	–	1.4×10^3	–

I* — initial culture

BS1* — food substrate before mites inoculation

BS2* — food substrate not inhabited by mites during the whole experiment (BS2-0 — at the beginning of the experiment, BS2-1 — in 1 and 2 months)

Table 2

Fungal species and their concentration (CFU/g) in the simple periodic cultures of the house dust mite *D. farinae*

Таблица 2

Виды и концентрация плесневых грибов (КОЕ/г), выделенных из простых периодических культур синантропных клещей *D. farinae*

Species	Control				Weeks									
	I*	BS1*	BS2−0*	BS2−1*	3	5	8	11	14	18	21	25	28	30
Aspergillus niger v.Thiegh.	–	–	2.8×10^2	–	–	2.9×10^3	–	–	–	–	–	2.8×10^3	–	–
A. penicillioides Speg.	10^8	–	–	–	1.2×10^6	1.0×10^7	3.7×10^7	3.5×10^7	1.1×10^8	1.7×10^8	5.8×10^7	9.5×10^7	5.2×10^7	6.2×10^7
A. repens De Bary	–	–	–	–	1.4×10^5	–	1.4×10^5	4.2×10^2	–	–	2.8×10^2	–	–	–
A. ustus (Bain.) Thom & Church	–	–	–	–	–	–	–	–	4.4×10^5	–	–	–	–	–
Aureobasidium pullulans (d By.) Arnaud	–	–	2.8×10^2	–	–	–	–	–	–	–	–	–	–	–
Chrysosporium sp.	–	–	--	–	1.0×10^6	5.9×10^5	4.7×10^5	–	–	–	–	–	–	–
Cladosporium cladosporioides (Fresen.) de Vries	–	1.0×10^3	–	–	–	–	–	–	–	–	–	–	–	–
C. sphaerospermum Penz.	–		–	–	2.8×10^2	–	–	–	–	–	–	–	–	–
Paecilomyces variotii Bain.	–	–	–	–	–	–	–	–	--	–	–	3.3×10^5	–	–
Penicillium brevi-compactum Dierckx	–	1.0×10^3	–	---	–	–	–	–	–	–	–	–	–	–
P. chrysogenum Thom	–	–	–	–	–	–	1.5×10^5	–	7.6×10^4	–	–	–	–	–
P. citrinum Thom	–	–	–	---	–	–	–	–	–	–	1.9×10^4	–	–	–
P. glabrum (Wehmer) Westl.	10^3	1.4×10^3	–	–	–	–	–	–	–	–	–	–	–	–
P. lanosum Westl.	–	--	–	–	–	3.7×10^4	–	–	–	–	–	–	–	1.9×10^4
P. variabile Sopp	–	–	–	–	–	–	–	–	5.6×10^4	–	–	–	–	–
Penicillium sp.	–	–	–	–	–	–	5.7×10^3	–	–	–	–	–	–	–

I* — initial culture

BS1* — food substrate before mites inoculation

BS2* — food substrate not inhabited by mites during the whole experiment (BS2-0 — at the beginning of the experiment, BS2-1 — in 1 and 2 months)

Table 3

Frequency and abundance of mould fungi in the laboratory cultures of house dust mites

Таблица 3

Встречаемость и обилие плесневых грибов в лабораторных культурах клещей сем. Pyroglyphidae

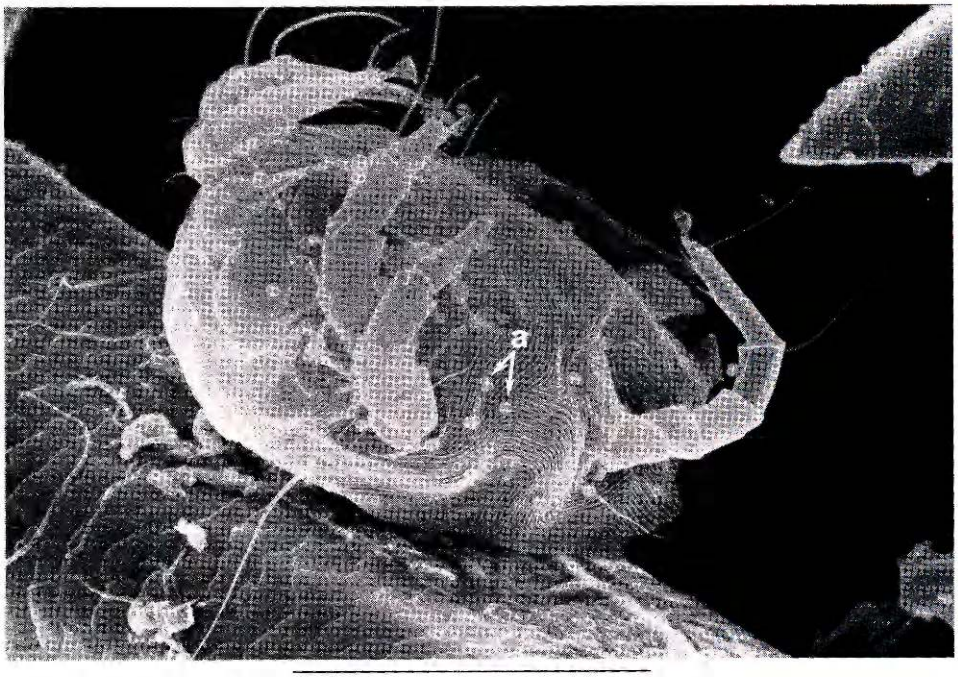
Species	*Dermatophagoides pteronyssinus*			*Dermatophagoides farinae*		
	Frequency (%)	Abundance		Frequency (%)	Abundance	
		absolute (CFU/g)	relative (%)		absolute (CFU/g)	relative (%)
Aspergillus penicillioides	100	1.8×10^9	99.8	100	5.7×10^8	99.4
A. repens	90	6.5×10^4	<0.1	40	2.8×10^5	<0.1
Wallemia sebi	70	1.3×10^5	<0.1	–	–	–
Chrysosporium sp.	–	–	–	30	2.1×10^6	0.4
Penicillium chrysogenum	30	1.6×10^5	<0.1	20	2.3×10^5	<0.1
Aspergillus ustus	30	3×10^5	<0.1	10	4.4×10^5	0.1
Penicillium lanosum	20	2.8×10^5	<0.1	20	5.6×10^4	<0.1
Aspergillus niger	20	1.1×10^4	<0.1	20	5.7×10^3	<0.1
Penicillium citrinum	10	2.8×10^3	<0.1	10	1.9×10^4	<0.1
P. janczewskii	10	3.5×10^5	<0.1	–	–	–
P. variabile	–	–	–	10	5.6×10^4	<0.1
Paecilomyces variotii	–	–	–	10	3.3×10^5	<0.1
Cladosporium herbarum	10	2.8×10^3	<0.1	–	–	–
C. sphaerospermum	–	–	–	10	2.8×10^2	<0.1

gut that resulted in a mass colonization of the new food substrate by these fungi (Fig. 2). The high abundance of *A. penicillioides* in cultures of both mites species makes it possible to conclude that BS is the most suitable substrate for this fungus (Fig. 3, 4). This makes *A. penicillioides* more competitive comparing to other species of fungi. The association of pyroglyphid mites namely with *A. penicillioides* in laboratory cultures were mentioned by other researchers [Douglas, Hart, 1989; Hart, Douglas, 1991]. As it is known some insects as well as the storage mites and the pyroglyphid mite *D. farinae* can produce the alarm pheromones that have strong fungistatic effect and suppress the development of fungi in the cultures of these arthropods [Cole et al., 1975; Matsumoto et al., 1979]. Our data suggest that *A. penicillioides* probably is not sensitive to alarm pheromones of pyroglyphid mites.

The synchronous population increase observed in mites and mould fungi, the one-time reaching plateau in the cultures of both species as well as the very high fungal number suggest that the main limiting factor for mites and *A. penicillioides* was the food substrate depletion.

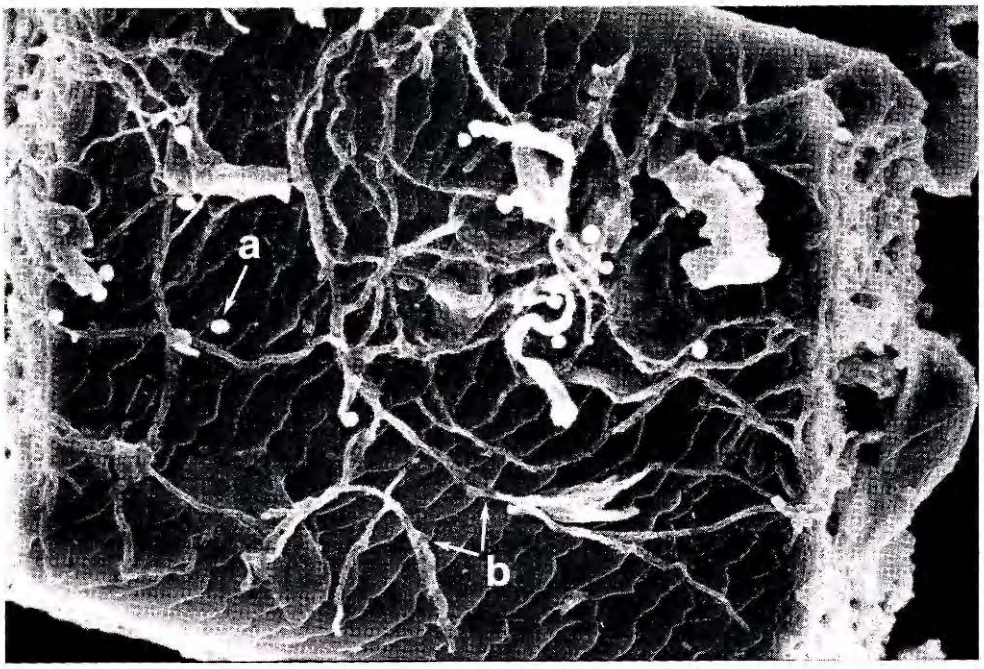
The fungal spores and mycelia were reported many times from the gut and faecal pellets of pyroglyphid mites [Sinha et al., 1970; Douglas, Hart, 1989; Hart, Douglas, 1991, personal data]. Hart and Douglas [1991] found the spores of only two species, *A. penicillioides* and *W. sebi,* in the gut of *D. pteronyssinus*. The authors hypothesized that such a high selectivity in swallowing spores can be explained by the fact that mites avoided most fungal species except *A. penicillioides* and *W.sebi*, or these fungi were not digested by *D. pteronyssinus*. However Lustgraaf [1978] did not exclude that the fungal spores could be swallowed by mites accidentally and were not a food source.

Several researchers revealed indirect trophic relationships between pyroglyphids and mould fungi. The pilled off scales of epidermis, which are the main food source for the house dust mites, are not rich with water- and oil-soluble vitamins to provide sufficient supply for mites [Marples, 1965, cited by Bronswijk, Sinha, 1973]. This makes it possible to suggest that namely mould fungi can compensate shortage of vitamins and sterols [Bronswijk, Sinha, 1973; Saint Georges-Gridelet, 1987; Douglas, Hart, 1989; Hay et al., 1993]. Fungi may supply mites with sterols in the form of ergosterols, which are the precursors of the vitamin D [Cantone et al., 1983, cited by Saint Georges-Gridelet, 1987]. The function of fungi as sources of vitamins and microelements can possibly explain comparatively fast growth of populations of mites on culture medium that was preliminary inoculated with mould fungi



100 μm

Fig. 2. Conidia of *Aspergillus penicillioides* on *D. farinae*: a — non-germinated conidia (SEM).



10 μm

Fig. 3. Conidia and vegetative hyphae of A. penicillioides on the human hair fragment: a — conidia, b — vegetative hyphae (SEM).

than on sterile culture medium [Bronswijk, Sinha, 1973; Hay et al., 1993].

As in *D. pteronyssinus* and *D. farinae* the keratinase is revealed in trace quantities [Barabanova, Zheltikova, 1985], it is not excluded that micromycetes play the role of primary destructors of such components of the food substrate as the hair and human skin scales (Fig. 3). There is an opinion that micromycetes (*A. penicillioides* as well as the representatives of the *A. glaucus* group) assist destruction of the lipid component of epidermis that makes it easy to utilize the food substrate by pyroglyphid mites [Bronswijk, Sinha, 1973; Lustgraaf, 1978]. Probably the primary processing of the food

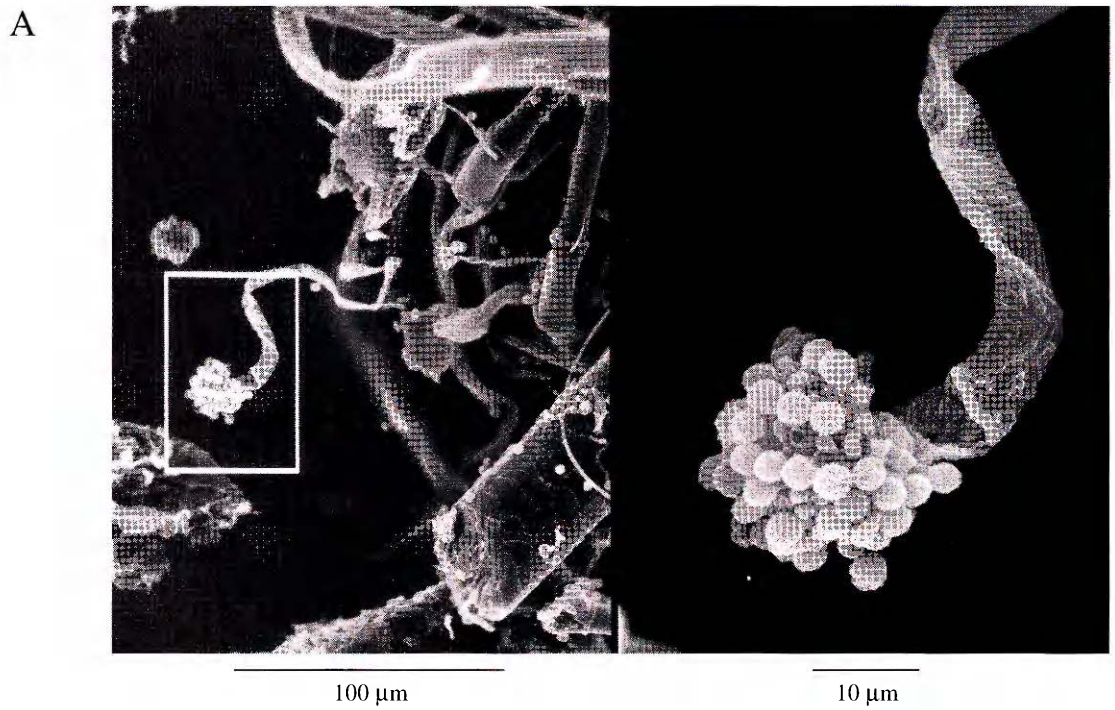


Fig. 4. A — Growth of *A. penicillioides* in the culture of pyroglyphid mites. B — Conidiophore with conidial head of *A. penicillioides* (SEM).

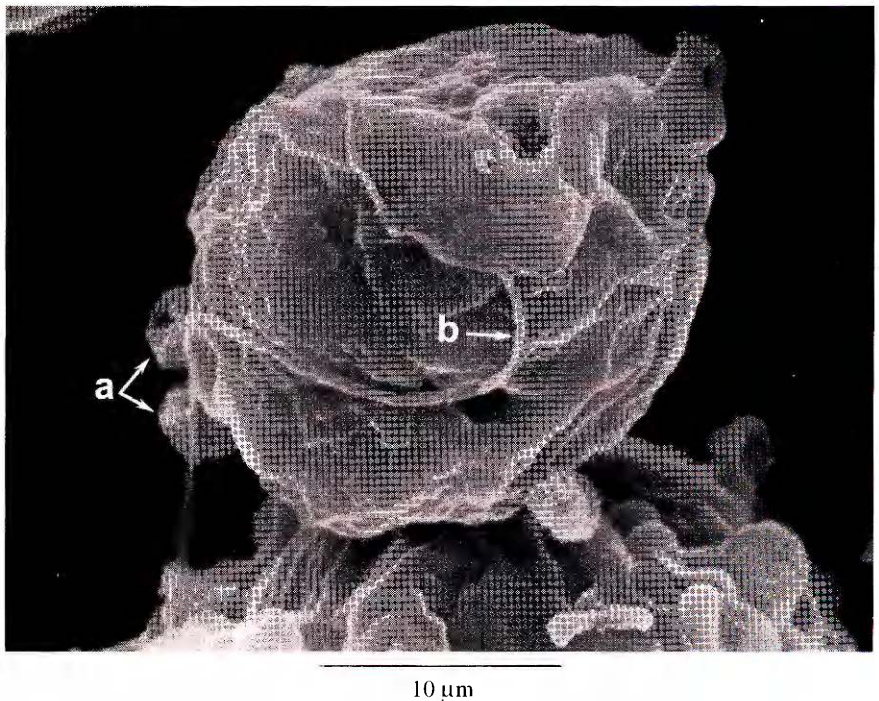


Fig. 5. Conidia and vegetative mycelium of *A. penicillioides* on house dust mite faecal pellet: a — conidia, b — vegetative hyphae (SEM).

source of mites by fungi is not obligatory. However, if taken place it leads to fast and easy utilization of food by mites and the following mite population growth. On the other turn, the faeces of mites serve as the food source for fungi (Fig. 5).

Thus, the experimental data obtained suggest that pyroglyphid mites and mould are associated by direct topical, direct phoric and, probably, direct and indirect trophic relationships. The relationships which are formed between pyroglyphid mites *D. pteronyssinus* and *D. farinae*, and the mould fungi *A. penicillioides, A. repens* and *Wallemia sebi*, can be regarded as protocooperation, thus they are mutually beneficial but not obligatory. The

biocenotic relationships between pyroglyphid mites and mould are very complex and can change from mutualistic to antagonistic thus making necessary further research. The data obtained should be taken into account when the mite allergens are prepared from the cultures of pyroglyphid mites with a potential presence of high micromycetes concentrations.

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