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Significance and feeding of psocids (*Liposcelididae*, *Psocoptera*) with microorganisms

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Abstract

After our investigations of psocids in the grain storages in Croatia, the most abundant were representatives of genus *Liposcelis*, as following: *Liposcelis decolor* (Pearman), *L. corrodens* (Heymons), *L. entomophila* (Enderlein), *L. pearmani* Lienhard, *L. bostrichophila* Badonnel, *L. paeta* Pearman, *L. mendax* Pearman, *L. brunnea* Motschulsky, *L. pubescens* Broadhead, *L. tricolor* Badonnel. Feeding and food utilisation of *L. corrodens* and *L. bostrichophila* with pure culture of microorganisms (fungi – *Mucor spp.*, *Apergillus niger* Tiegh., *A. flavus* Link ex Gray, *Penicillium spp.*, *Cladosporium spp.*, *Fusarium spp.* and bacteria – *Esherichia coli*, *Bacillus subtilis*, as well cellulolytic microorganisms of genera *Cytophaga*, *Cellvibrio*) were investigated. Also feeding with microorganisms causing plant diseases in the herbarium plant material (*Uromyces pisi* (Pers.) de Bary, *Helminthosporium turcicum* Pass., *Ustilago maydis* (DC) Corda, *U. levis* (Kell.et Sw.) Magn., *U. nuda* (Jens.) Kell. et Sw., *Tilletia tritici* (Bjerk.) Winter, *Pseudoperonospora humuli* (Miyabe et Takah.) G. Wilson, *Peronospora schleideni* Ung., *Cercospora beticola* Sacc.), was studied. Transporting of harmful microorganisms of stored wheat by bodies, hairs and in the faecal matter of *Liposcelididae* was investigated too.

Key words: *Liposcelididae*; microorganisms; feeding; food utilisation; harmful microorganisms,

transporting.

Introduction

Psocids do not have economic importance for stored commercial wheat and corn in Croatia. Psocids are considered to be insect pests of seed products since they usually feed on the germ part of a whole seed and even the complete embryo (Rees, 2004). Psocids appear to cause a significant problem for packaged foodstuffs. According to our previous research, psocids were found in packaged pasta, rice, flour, wheat and corn grits, as well as in different kinds of tea (Kalinovic et al., 1981). The highest number of psocid species was found in stored wheat and corn (Kalinovic, 1979; 1995; Kalinovic et al., 2006). Psocids were found in a small number in empty storehouses in Croatia (Kalinovic and Rozman, 2000; Kalinovic et al. 2006), whereas in Australia psocid species *Lachesilla quercus* Kolbe are considered to be harmful pests in coastal grain handling facilities (Rees, 2004). Moreover, *Liposcelis spp.* are known to cause allergic responses in sensitized people (Rees, 2004). There are still no extensive data published on feeding activities and food selection of *Liposcelididae*. Sinha and Srivastava (1970) identified *L. entomophila* in packaged rice and moldy rice stems in the fields, which indicates that they feed on fungi and bacteria that hydrolyze cellulose. Furthermore, Srivastava and Sinha

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(1975) found that *L. entomophila* showed a preference for flour, dead insects and bark when compared with wood fungi, straw and paper. *Liposcelididae* that live outdoors feed on mycelium of *Ascomyceta* fungi, organic matter and pollen of various plants (Günther, 1974). According to the research on *L. bostrichophila* food selection such as buckwheat, pot barley, brown rice flour, yellow millet and wheatgerm, the results show that their most preferred choice were ground buckwheat and yellow millet impregnated with glucose and fructose.

Materials and methods

Booklice were collected from the samples of stored grains (wheat, barley, corn, oat, soybeans, sugar beet, sunflower, oil seed rape) and packaged foodstuffs (flour, rice, wheat and corn grits, different kinds of tea, dried fruit and vegetables) in the period between 1975 and 2005. The samples weighed 100g. They were sieved with automatic sieves that ranged in diameter from 0.2 - 2.5 mm (standard method of sample analysis). Booklice obtained from the sieved material were collected with camel hair brushes and were put in test tubes with 70 % alcohol (Günther, 1974, Smithers, 1981). Booklice from empty storehouses were collected with camel hair brushes at the area of 1 m². Also, booklice from herbarium material of plant species that cause plant diseases during vegetation period (*Uromyces pisi*, *Helminthosporium turcicum*, *Ustilago maydis*, *U. nuda*, *Tilletia tritici*, *Pseudoperonospora humuli*, *Peronospora schleideni*, *Cercospora beticola*) were collected with camel hair brushes and were put in 70 % alcohol. Laboratory reared species *L. corrodens* and *L. bostrichophila* were placed on herbarium plant material to feed for 48 hours. *Liposcelididae* were macerated by Günther method (1974). Specimens obtained were identified using the keys of Günther (1974), Lienhard (1990), Lienhard and Smithers (2002) and Mockford (1993).

Laboratory rearing of *Liposcelis*

bostrichophila (Figure 1) and *L. corrodens* for the study of feeding activity of booklice was performed by Wyniger method (1974).

The surface microflora from grain products as well as microorganisms from the bodies of booklice were isolated in the Petri dishes on the Czapek's agar plate. The incubation was performed in the thermostat at 25°C for the 7-day-period (standard method).

Feeding activity on pure fungi cultures

Feeding activity of *L. bostrichophila* and *L. corrodens* was studied with pure fungi cultures (*Mucor spp.*, *Aspergillus niger*, *A. flavus*, *Penicillium spp.*, *Cladosporium spp.* and *Fusarium spp.*) developed in the thermostat on the Czapek's agar plate. Laboratory reared booklice were isolated in the sterile Petri dishes for 48-72 hours in order to cleanse their digestive system, as well as to clean their bodies, heads, legs and antennae from the agar i.e. from the fungi on their hair, which was examined under the stereomicroscope. Finally, starved and cleaned booklice (5 adult *Liposcelididae*) were placed in the Petri dishes with the prepared pure fungi cultures substrate for their feeding which was examined under the stereomicroscope. The Petri dishes with booklice were placed in the thermostat at 28 °C with the relative humidity of



Figure 1. *Liposcelis bostrichophila* (photo E.C.N. Leong, S.H.Ho).

80 % which was accomplished with damp sterile filter paper inside the lid of each Petri dish. *Liposcelididae* were left to feed on pure fungi cultures (mycelium parts) for 72 hours. After 3 days of feeding, psocids were placed in an empty sterile Petri dish to examine removal of vegetative organs of fungi (spores or conidia) from their bodies under stereomicroscope. Next, *Liposcelididae* (3-5 adult specimens) were placed in sterile Petri dishes in order to perform mechanical maceration with sterile microbiological and Platinum needles and were poured over with nutrient Czapek's plate. Developed fungi in the digestive system of *Liposcelididae* were studied after the incubation period in the thermostat at 25 °C during 7-20 days.

***Liposcelididae* feeding activity on bacteria**

Having determined the presence of bacteria on the bodies and in the digestive system of psocids species in stored wheat and corn, the isolates of pure cultures of the dominant bacteria *Escherichia coli* and *Bacillus subtilis* were prepared. They were grown on 2 % meat peptone agar slant in order to gain higher biomass. Bacterial biomass was removed from the agar slant with Platinum needle and placed into the sterile Petri dishes together with the observed *Liposcelididae* species (starved for 72 hours). The period of biomass feeding of psocids on pure bacteria cultures lasted 48 hours, after which they were chemically macerated (surface sterilization), rinsed with 10 % KOH, distilled water and 45 %, 70 %, 85 % and 96 % alcohol (Günther, 1974) and smeared onto a glass slide. The smear was dried and stained with base Fuchsin solution. Evaluation of bacterial cells consumption in the digestive system of psocids was carried out after 48 and 72 hours of their starvation. The same psocids were macerated mechanically in the sterile Petri dishes where they were poured over with 2 % meat peptone agar cooled at 45 °C. Examination of the bacterial development was conducted after 72 hours of incubation at 24 °C.

***Liposcelididae* feeding activity on aerobic cellulose degraders**

Studying of *Liposcelididae* feeding on aerobic cellulose degraders was performed with genera *Cytophaga* and *Cellvibrio*. Starved *Liposcelididae* were placed on the developed colonies of cellulolytic microorganisms to feed for 48 hours in the thermostat at 28 °C. The testing procedure was the same as with bacteria but the slide was stained with Methylene Blue solution. Likewise, cellulolytic microorganisms in the digestive system were determined with the same procedure as with bacteria after 72 hours, but for the development of bacteria, after maceration of psocids, liquid Waksman-Carey plate in the test tubes with filter paper was used. The incubation lasted 30 days at 30 °C.

Testing of *Liposcelididae* feces

Testing of *Liposcelididae* feces was carried out after their feeding on fungi, bacteria and cellulolytic microorganisms. Psocids were placed into empty sterile Petri dishes to examine their fecal excretion under the stereomicroscope. They were transferred into the sterile Petri dishes and poured over with the appropriate medium for the development of certain groups of microorganisms. The plates with the feces of psocids were placed in incubation for 7-30 days in the thermostat at an appropriate temperature, depending on the time needed for the complete development of the microorganisms. Adult specimens of *Liposcelididae* isolated from the wheat in silos were placed on the nutrient plate in the Petri dishes for the study of microorganisms from their bodies and hair.

Results

Relatively high incidence of order *Psocoptera* observed during this multiyear research was shown in Figure 2. Obviously, the most frequently encountered suborder was *Troctomorpha*, infraorder *Nanopsocetae* – *Liposcelididae*, with

the prevalence of 87 %, suborder *Trogiomorpha*, infraorder *Atropetae – Trogiidae*, with the prevalence of 8 %, followed by the same suborder, infraorder Psocoathropetae – *Psyllipsocidae*, with 4 %, and suborder *Psocomorpha*, infraorder *Homilopsocidea – Lachesillidae* with the 1 % prevalence. Figure 3 shows incidence of Psocoptera species in the analyzed samples of grain products and foodstuffs. There were 17 Psocoptera species identified with the most dominant genus *Liposcelis* and its following 10 species: *Liposcelis decolor* (Pearman) 20 %, *L. corrodens* (Heymons) 18 %, *L. entomophila* (Enderlein) 16 %, *L. pearmani* Lienhard 13%, *L. bostrichophila* Badonnel 8 %, *L. paeta* Pearman 4 %, *L. mendax* Pearman 3 %, *L. brunnea* Motschulsky 2 %, *L. pubescens* Broadhead 2%, and *L. tricolor* Badonnel 1 %. The genus *Lepinotus* was identified with its 2 species *Lepinotus reticulatus* Enderlein and *L. inquilinus* Heyden with the prevalence of 6 % and 1 % respectively. The genus *Psyllipsocus* was identified with 2 forms of 1 species *Psyllipsocus ramburi* Selys-Longchamps f. *destructor*, *P. ramburi* f. *troglodytes* with the prevalence of 2 % and 1 % respectively. The genus *Dorypteryx* was encountered with species *Dorypteryx domestica* (Smithers) and genus *Trogium* with its 1 species *Trogium pulsatorium* (Linneaus) both with the

prevalence rate of 1 %. Finally, the genus *Lachesilla* was observed with the prevalence of 1 % of its species *Lachesilla pedicularia* (Linneaus).

The Psocoptera prevalence rate was 77.2 % in the stored grain products with the most dominant genus *Liposcelis* with the highest infestation of stored wheat and corn (Kalinovic, 1979, 1995). Likewise, the multiyear research revealed the Psocoptera incidence of 22.8 % in packaged foodstuffs also with the dominant genus *Liposcelis*. The highest infestation was detected in packaged pasta, rice and flour and less in wheat and corn grits and tea (Kalinovic et al., 1981). Inspection of empty storehouses, mostly in silos basements identified the following psocids species *Lachesilla pedicularia*, *L. quercus*, *Lepinotus raticulatus* and *Liposcelis bostrichophila*. The relative humidity and air temperatures varied from 65–68 % and 22–25 °C respectively. *Psocoptera* were mostly found on moldy basement walls feeding on fungi and bacteria (Kalinovic and Rozman, 2000).

Liposcelididae – Liposcelis corrodens and *Liposcelis bostrichophila* were identified on the herbarium material of plant species that cause the following plant diseases *Uromyces pisi*, *Helminthosporium turcicum*, *Ustilago maydis*, *U. levis*, *U. nuda*, *Tilletia tritici*, *Pseudoperonospora*

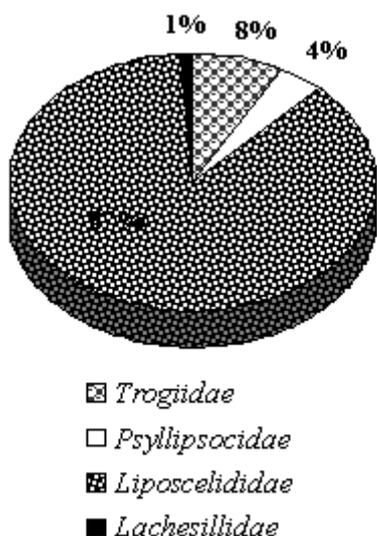


Figure 2. Relatively number of Psocoptera by family affiliation.

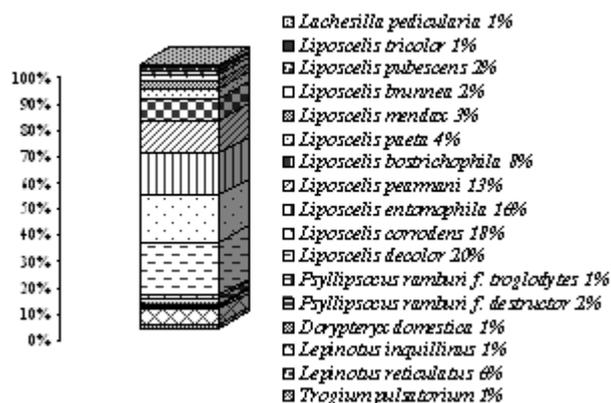


Figure 3. Relatively number of Psocoptera species in analyzed samples.

humuli, *Peronospora shleideni* and *Cercospora beticola*. Chemical maceration of the digestive system of laboratory reared Liposcelididae that were feeding on the herbarium plant material revealed mycelium and spores of plant diseases proving that those plant diseases were perfect source for their feeding (Figure 4 and 5).

For the purpose of studying psocids feeding activity, first the surface microflora of the stored wheat and corn grains from the samples taken from silos was tested. Next, the isolation of the surface microflora determined that the most prevalent fungi species were that of the genera *Fusarium*, *Alternaria*, *Penicillium*, *Cladosporium*, *Mucor* and *Rhizopus*, and bacteria *Escherichia coli* and *Bacillus subtilis*, as well as aerobic cellulose degraders – cellulolytic microorganisms of the genera *Cytophaga* and *Cellvibrio*. Pure fungi cultures were prepared for the laboratory reared *Psocoptera* species *L. corrodens* and *L. bostrichophila* that were starved in the Petri dishes. After a rather short time, psocids found their way to fungi colonies and started feeding on them. Their behavior and feeding activity were examined under stereomicroscope. It showed that the adult specimen moved rather quickly to the very dense but widespread fungi colony of *Mucor* spp. It bit sporangiophore with its mouthparts all the way to sporangium which, according to its maturity phase, broke open. It was greedily

consuming released sporangiospores with its mouthpart, settled down to chew and subsequently swallowed its food. It remained at the bottom of the colony probably due to the less light exposure and higher influence of humidity, where it continued to feed. Chemical maceration showed the digestive system of *Psocoptera* (Figure 6). Observation of feeding activity of *Psocoptera* on *Aspergillus niger* and *A. flavus* fungi that were quite widespread, revealed that the adult specimen climbed on their conidiophores and bit them in two in their middle part where they are softer, continued biting it towards conidiophore head with conidia which it swallowed after 3-4 bites (content of the digestive system was shown in Figure 8). In fungi species with more dense mycelium (*Penicillium* spp., *Cladosporium* spp., *Fusarium* spp.), adult specimens fed on conidia from the upper surface of the colony and then due to its density easily climb on its top and continued to feed on conidia and sterigmata (Figure 7, 9). Feeding of Liposcelididae on pure fungi cultures of *E. coli* and *B. subtilis* did not reveal any significant difference in bacterial intake into their digestive system. Chemical maceration and microscopic examination of the permanent slide determined the existence of big vegetative bacilli and coccoidal forms of *Bacillus* spp. spores (Figure 10). Evaluation of bacteria consumption in the digestive system of Liposcelididae, maceration of adult specimens and pouring over with meat peptone agar

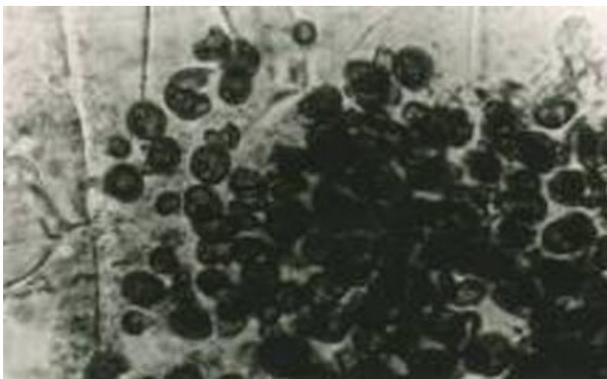


Figure 4. Spores of *Ustilago maydis* in digestive system of *Liposcelis corrodens* ($\pm 600x$, photo I. Kalinovic).

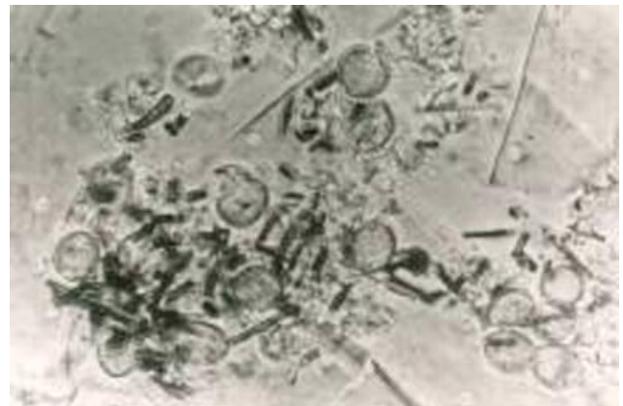


Figure 5. Spores and parts of conidiophore of *Uromyces pisi* in digestive system of *Liposcelis bostrichophila* ($\pm 600x$, photo I. Kalinovic).

after the incubation determined only the development of pure cultures of *Bacillus subtilis*, whereas other bacteria did not develop. This proves that the adult digestive system completely absorbs asporogenous and vegetative bacteria forms, whereas conservation forms (spores) of sporogenous bacteria are excreted.

According to existing literature, Liposcelididae live in the nature in the places rich in cellulose i.e. under the tree bark, stumps, old paper and in rice straw (Sinha and Srivastava, 1970), which proves their ability to digest cellulose during their nutrition process. In natural conditions, cellulolytic microorganisms of the genera *Cytophaga* and *Cellvibrio* play the dominant role in cellulose transformation. Liposcelididae were very active on

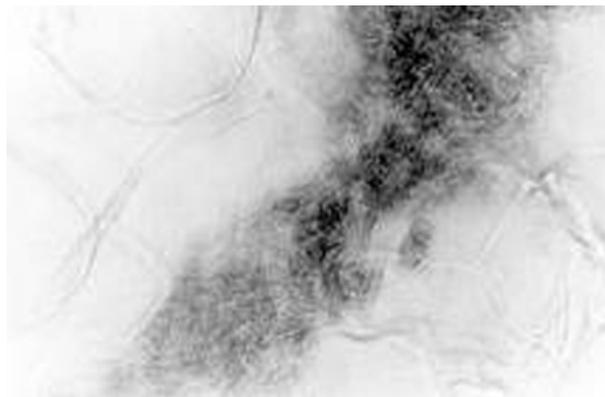


Figure 6. Digestive system content of *L. corrodens* fed by clean culture of *Alternaria* spp. ($\pm 600x$, photo I. Kalinovic).

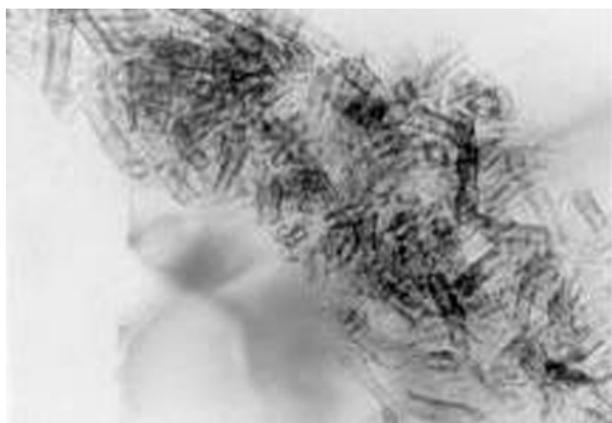


Figure 7. Digestive system content of *L. corrodens* fed by clean culture of *Cladosporium* spp. ($\pm 600x$ photo I. Kalinovic).

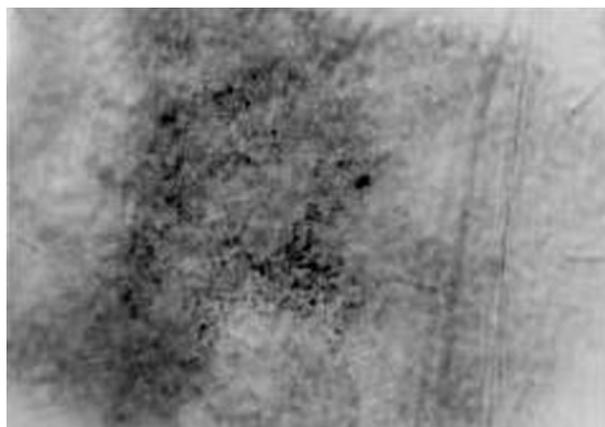


Figure 8. Digestive system content of *L. bostrichophila* fed by clean culture of *Aspergillus niger* ($\pm 600x$ photo I. Kalinovic).

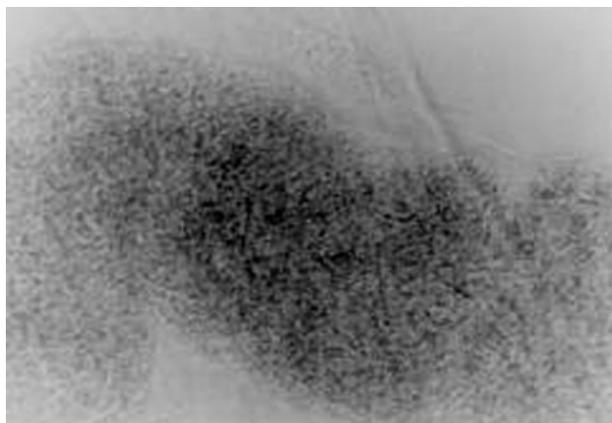


Figure 9. Digestive system content of *L. corrodens* fed by clean culture of *Penicillium* spp. ($\pm 600x$ photo I. Kalinovic).

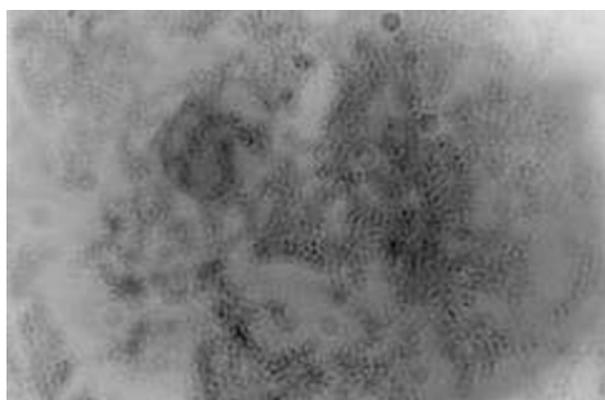


Figure 10. Digestive system content of *L. bostrichophila* fed by clean culture of bacteria biomass *Bacillus* spp. ($\pm 600x$ photo I. Kalinovic).

the grown pure cellulolytic microorganisms colonies, remained on mucilaginous colonies (*Cytophaga*) and had a longer lifespan as a result of proper nutrition (cellulose and cellulolytic microorganisms) and humidity level. Celluloid parts (pieces of filter paper) as well as cellulolytic microorganism cells were found in the digestive system of the dissected adult specimens. We believe that the majority of cellulose is digested during nutrition under the influence of cellulase ferment - aerobic cellulolytic microorganisms (*Cytophaga* and *Cellvibrio*) and transformed into glucoses, which attracts Liposcelididae to feed intensively.

Examination of Liposcelididae feces regarding the consumption of fungi and bacteria in the digestive system showed that sporangiospore or conidia in feces of psocids that were fed on fungi redeveloped on the nutrient plate. On the other hand, feces of psocids that were fed on bacterial flora showed that their digestive system completely absorbed asporogenous and vegetative bacterial forms, and even cellulolytic microorganisms, whereas conservation forms (spores) of sporogenous bacteria were excreted. Regeneration on the nutrient plate was observed only with *Bacillus subtilis* from the spores that redevelop.

Discussion

The research proved that Liposcelididae feed on fungi (*Mucor spp.*, *Aspergillus niger*, *A. flavus*, *Penicillium spp.*, *Cladosporium spp.* and *Fusarium spp.*) and bacteria (*Escherichia coli* and *Bacillus subtilis*), as well as on cellulolytic microorganisms of genera *Cytophaga* and *Cellvibrio*, without preference for any particular specimen. Examination of undigested food content in the digestive system, as well as excreted feces of Liposcelididae revealed that all fungi species redevelop by germination of sporangiospores or conidia from feces on the artificial medium. Only sporogenous specimen of *B. subtilis* has the ability to regenerate, whereas the development of asporogenous bacteria of genus *Chromopseudomonas* did not occur. In

other words, this proves that only sporogenous conservation forms regenerate since they are more resistant to fermentation activity of the Liposcelididae digestive system. Cellulolytic microorganisms are completely consumed under the influence of cellulase ferment so there is no regeneration. To sum up, Liposcelididae are considered to be insect pests not only since their presence, excrements and toxins infest foodstuff but also because they transfer microflora with their bodies. Timely preventive measures are necessary to avoid their rapid increase in number and the possible heavy infestation and damage of packaged products. Incidence of Liposcelididae in certain products indicates increased humidity and the presence of microflora.

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