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INFLUENCE OF MICROHABITAT ON THE SELECTION OF COLLEMBOLAN SPECIES *TOMOCERUS LONGICORNIS* (MÜLL.) AMONG FUNGI AVAILABLE IN MOSS USHION

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Вплив мікросередовища на вибірковість колембол виду Tomocerus longicornis (Müll.) до грибів, що мешкають в подушці моху. – Я.Ворго, З.Ноар, В.Рошко. *– Нами досліджувався склад вмісту кишечника ногохвісток (Collembola) виду Tomocerus longicornis (Müll.), зібраних на рослинах моху Tortella tortuosa (Hedw.) Limp. з двох місць зростання. Вміст кишечника складався з детриту (50%), частинок моху (20%) і грибних пропагул (10%). Отримані культури грибів були ізольовані та ідентифіковані для визначення селективності харчування колембол грибами, що зустрічаються на мохах. Різноманіття мікобіоти, виділеної із колембол на обох місцях відбору проб, було схоже. Але чисельність окремих видів грибів у вмісті кишечника істотно відрізнялась від їх чисельності на мохові. На вибірковість колембол до специфічних грибів впливає як видова належність комах, так і місце відбору проб.*

Ключові слова*: колемболи, гриби, мохи, селективне харчування*

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Influence of microhabitat on the selection of Collembolan species Tomocerus longicornis (Müll.) among fungi available in moss cushion. – Varga J., Naár Z., Rohsko V. *The composition of intestine content of Collembolan species, Tomocerus longicornis (Müll.) collected from Tortella tortuosa (Hedw.) Limp. moss samples, was recorded at two sites. The intestine content consisted of detritus (55 %), moss particles (20 %) and fungal propagules (10 %).. The culturable fungi was isolated and identified to reveal the selective feeding of collembolans on moss inhabiting fungi. The difference in diversity of mycobiota found both in moss and in collembolans was similar at the two collection sites; however, the rate of some fungi in the intestine content markedly differed from their frequency on moss. The preference of collembolans for particular fungi was influenced both by the insect species and the collecting site.*

Key words*: collembolans, fungi, moss, selective feeding*

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Introduction

Numerous studies confirmed that collembolans are opportunists regarding their feeding: they select among various food sources but fungi are among the most often consumed [8]. However, they consume the soil fungi at widely different rates; a definite selection among them was revealed by researchers [1,5,6]. The moss cushions give a characteristic accommodation to a wide variety of insects, among them springtails (Collembola): they are different from soil both as a poor food source and as a special physico-chemical environment [4]. Mosses have a complex mycobiota which range from specialized ascomycetes (more than 300 species) occurring exclusively on bryophytes [3] to common soil fungi which colonise and decompose the living or dead plant parts [9]. Thus, it is uncertain that observations achieved for soil are valid for the environmental conditions of moss cushions. To our knowledge, there are no data regarding what components of moss mycobiota are consumed by collembolans. The aim of this study was to show whether the differences between microhabitats influence the selection in fungal diet of the investigated Collembolan species.

Materials and Methods

Collection sites and methods

The plant associations with moderately different ecological features were chosen in Szarvaskő (Bükk National Park, Bükk mountains, Hungary). Collection

site I.: a rocky area with southern exposition, *Pulsatillo-Festucetum rupicolae* community. Collection site II.: a dry area interspersed with rocks growing out of the surface which has a south-western exposition, *Potentillo-Festucetum pseudodalmaticae* community.

Pieces of moss cushions of 10x10 cm size from both sites were collected on 18 September, 2000, according to the statement that the size of the moss cushions must be at least 20 times larger than the animals collected in them [2]. The collection of moss fauna was made by prespan funnel (Berlese system) at room temperature. Berlese–Balogh salting method was applied for the separation of invertebrata from debris. Adult sized Collembola (about 4-5 mm) were picked up and identified in open preparations.

Microscopic examinations on the intestine content

Those mature individuals of *Tomocerus longicornis* (Müll.) were chosen under preparative microscope for the study, which showed dark coloured intestines full of consumed feed. The whole intestine of collembolans (10-10 individuals/species/collection site) were prepared with needles in three replicates. The intestines were homogenized in 5 cm^3 of distilled water, dropped with lactophenol-anilin blue stain and spread on microscopic slides. Three types of intestine content particles were differentiated: moss fragments, fungal propagules, and amorphous detritus. We used the method of Hodkinson et al. [7] by which the particles are counted on the examined surface along linear transects. Percent value data were lg transformed before statistical analysis. The mean rate of food types by Collembola species or collection sites were compared pairwise with Students t-test.

Assessment of mycobiota of Collembolan intestine and the moss cushion.

Collembolans collected alive from the moss samples (five adults with full gut per site) were washed 10 times with sterile distilled water containing Tween 80 to remove particles from their bodies. During aseptical exploration, the intestines were collected from the same species from the same site and ground together in 3 cm^3 sterile tapwater with a tissue homogenizer. Aliquots (0.2 cm^3) of suspensions were spread on Martin-agar and glucose containing Czapek-Dox agar plates in three replicates. To detect the rarer fungi, the remaining inoculum was plated out in aliquots of 0.3 cm^3. Three parallels of both media were in this case, too. Inoculated plates were cultured at 27-28 C° in darkness. Each developing fungal colony was picked up and transferred on glucose-pepton agar slants. Isolates were identified microscopically in lactophenol-anilin blue stained preparations.

The method used for mycobiota of moss cushion was the same with the exception of inoculum preparation. 0.5 g (dry weight) of each bryophyte sample were separated before the collection of insects and washed in 100 cm^3 of distilled water. Washing water was used as inoculum for the isolation of fungi that are easily removable from the surface of moss. Tissue suspensions were used for isolation of the fungi firmly in contact with the moss. To prepare this inoculum, the once washed moss samples were washed four times again in sterile water. The excess water was removed with sterile filter paper, and the moss pieces were ground in a tissue homogenizer. Serial dilutions (up to 10^4) of the homogenizate were used as inoculum with the same method as intestine homogenizates.

Results

Composition of the intestinal content

The intestine content of collembolans was composed mainly of detritus, the rate of which did not differ significantly between sites. Moss fragments and fungal particles were found less densely, but marked differences were observed between their rates. *T. longicornis* consumed moss particles at 2.75 times higher rate than fungi on site I, whereas this rate was 1.11 on site II. Also differences were observed in the rate of fungal species between sites. During the microscopic examinations, some fungi with characteristic morphology were directly identifiable such as a typical colony (sporulating hyphae) of *Alternaria* sp. on an eaten moss fragment. Sometimes, fragments of animal origin could be well recognized in the intestinal content. There were scales from wing fragments, which were not determined for higher taxa but on the basis of their nervature they were of dipteran or hymenopteran origin, then leg fragments of insects and fragments from moulten larval skin. Some moss fragments seemed to be degraded, suggesting that its biomass may be utilizable by collembolans.

Mycobiota of moss and the intestinal content of collembolans

In strong accordance with microscopic observations, a three times higher quantity of fungal propagules was cultured from collembolans collected from site II. than from site I. Ten fungal taxa were identified from moss samples: *Alternaria alternata* (Fries: Fries) von Keissler, *Aspergillus* Micheli ex Fries sp., *Fusarium* Link ex Fries sp., *Gliocladium roseum* Bainier, *Gliocladium* Corda sp., *Mucor* Micheli ex Fries sp., *Penicillium* Link ex Fries sp., *Rhizopus* Ehrenberg ex Corda sp., *Trichoderma atroviride* Bisett, *Trichoderma harzianum* Rifai. Any of them could be cultured from collembolans with the exception of *Rhizopus* sp. Further thirteen fungal taxa were identified only from the intestinal contents: *Absidia spinosa* Lendner, *Acrenomium terricola* (Miller et al.) Gams, *Chaetomium murorum* Corda, *Cladosporium herbarum* (Person: Fries) Link, *Coniochaeta malacotricha* (Niessl) Traverso, *Isaria arachnophila* Ditmar, *Papulaspora* Preuss sp.,

Paecilomyces Bainier sp., *Stachybotris alternans* Bonorden, *Stachybotris lobulata* Berkeley, *Trichoderma koningii* Oudemans, *Trichoderma longipilis* Bissett, *Verticillium lateritium* Berkeley. *T. longicornis* did host six and eight taxa on collection sites I and II, respectively.

Table 1 shows that *A. alternata* was the most preferred fungus by *T. longicornis* at site I. It had twice as many propagules in moss at site II than site I but this difference did not appear in the intestine of *T. longicornis*. *Aspergillus* sp. detected in moss only from site II was consumed by collembolans. The density of *Fusarium* sp., *Gliocladium* sp., *G. roseum*, and *T. harzianum* in moss did not differ between the collection sites. The propagule number of *Mucor* sp. was under the threshold of detection in moss from site I, but it was high on site II. *T. longicornis* did not eat it on site I. On site II, the collembolans consumed *Mucor* sp. at a medium level. The relation of collembolans to *Penicillium* sp. was similar to *A. alternata*, the fungus of which was more frequent in moss at site II; however, this rate did not appear in fungal composition of intestine content. The case of *T. atroviride* was very similar to that of *Mucor* sp.: it was not isolated from moss from site I. The moss propagule density of *T. harzianum* differed not between sites, its density was similar in collembolans at site I.

The culturable mycobiota of collembolans' intestine proved to be more diverse on both sites than that of moss samples. The diversity was higher on site II. These data suggested a strong selection among fungi during grazing on the moss, but it could be more obvious with mathematical analysis of preference that is under process and will be published later.

Table 1. Fungal taxa isolated and identified both from moss *Tortella tortuosa* and the intestinal content of Collembola species

	Mean number of colony-forming units (CFU)*			
	Site I		Site II	
	Moss	Gut content	Moss	Gut content
Alternaria alternata	2.0 ±0.41	1.67 ±0.48	4.0 ±1.20	1.33 ±0.23
Aspergillus sp.	n.f.	n.f.	1.67 ±0.66	1.33 ±0.23
Fusarium sp.	25.0 ±3.34	0.33 ±0.23	22.67 ±2.75	1.67 ±0.23
Gliocladium roseum	33.67 ±4.91	0.33 ±0.23	34.67 ±6.13	1.33 ±0.47
Gliocladium sp.	29.67 ±4.04	1.0 ±0.41	29.0 ±5.10	1.00 ±0.71
Mucor sp.	n.f.	n.f.	20.0 ±2.65	1.67 ±0.66
Penicillium sp.	11.67 ±1.93	2.33 ±0.66	27.33 ±2.32	4.00 ±0.85
Trichoderma atroviride	n.f.	n.f.	14.67 ±1.54	1.33 ±0.23
Trichoderma harzianum	18.67 ±1.02	1.33 ±0.47	15.00 ±3.26	1.00 ±0.71
Total CFU**	362	31	510	99
Shannon diversity index**	1.61	2.31	2.41	2.51

* Colony forming unit (CFU) data were obtained from medium of Czapek-Dox or Martin-agar that gave a higher number. CFU data from washing and suspension of moss samples were added before estimating the standard error.

** Including fungi that were observed only in collembolans.

n.f. Not found in total of 1.5 g moss sample or 15 Collembola individuals.

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