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SHORT COMMUNICATION

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Abundance of Collembola (Insecta) inhabiting the hyphal mat of an ectomycorrhizal fungus, *Sarcodon scabrosus*, in a *Pinus densiflora* forest

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Abstract The total number of collembolans collected from the hyphal mat of the ectomycorrhizal fungus *Sarcodon scabrosus* was less than half of that collected from the adjacent non-mat soil. The same was true of all families of collembolans except one, although not all differences were significant. The exception was the Hypogastruridae, with more individuals in the hyphal mat on the sampling day in the fruiting season; these were also the most abundant collembolans on the fruiting bodies. These results indicate that most collembolans avoid the hyphal mat of *S. scabrosus* in a Japanese red pine forest.

Key words Collembola · Ectomycorrhizal fungi · Mycophagy · *Sarcodon* · Springtail

Mycorrhizal fungi interact with a wide range of other organisms at all stages of their life cycles (Fitter and Garbaye 1994). Disturbance of the mycorrhizal hyphal network by soil animals through grazing can reduce the efficiency of the mutualistic association between mycorrhizal fungi and plant roots, mainly by reducing the transport of mineral nutrients to roots (Fitter and Garbaye 1994). Collembolans differ in their preferences for fungal species, but collembolans have nonetheless been found to feed on many kinds of ectomycorrhizal fungi in laboratory experiments (Shaw 1988; Schultz 1991; Ek et al. 1994; Hiol Hiol et al. 1994). The few microcosm experiments conducted have shown that collembolans avoid mycorrhizal fungi associated with living roots (Klironomos and Kendrick 1996; Klironomos and Hart 2001; Kaneda and Kaneko 2004). In microcosm experiments, Klironomos and Hart (2001) found that fewer than 5% of collembolan individuals survived after 2 weeks exposure to the ectomycorrhizal fungus *Laccaria bicolor* (Marie) P.D. Orton associated with *Pinus strobus* L., but all individuals survived after 2 weeks exposure to *Cenococcum geophilum* Fr. associated with the same species. They suggested that some ectomycorrhizal fungi have evolved to deter grazing by collembolans. In field surveys, Cromack et al. (1988) found that hyphal mats of *Hysterangium setchellii* Fisher housed more collembolans than did the adjacent non-mat soil. However, Sawahata and Narimatsu (2006) reported that the number of collembolans collected from the hyphal mat of *Tricholoma matsutake* (S. Ito et Imai) Sing. did not differ significantly from that on the adjacent non-mat soil. These studies suggest the existence of various relationships between collembolans and ectomycorrhizal fungi in the field.

Sarcodon scabrosus (Fr.) Karst. is an ectomycorrhizal fungus that forms distinct hyphal mats about 5 cm in thickness in the lower part of the A_0 layer on the forest floor (Ogawa 1978). *Sarcodon scabrosus* forms bitter fruiting bodies and produces antibiotics (Shibata et al. 1998). Sawahata and Narimatsu (2005) found that the hyphal mat of this fungus housed fewer proturan insects than the adjacent non-mat soil. Thus, it is likely that *S. scabrosus* has evolved defenses to deter grazing by microarthropods, but its influence on collembolans has not yet been studied. In field surveys, we counted collembolans on the hyphal mat formed by *S. scabrosus*. In addition, we also observed collembolans on fruiting bodies occurring from the hyphal mat for reference.

The study area was in a Japanese red pine (*Pinus densiflora* Sieb et Zucc.) forest in Iwate Prefecture (380 m above sea level; 39°56′N, 141°13′E), in northern Japan. *Sarcodon scabrosus* fruits in this forest every autumn. The tree layer of the forest consists of *P. densiflora* from 10 to 85 years old and 3.8 to 79.3 cm in diameter at breast height, with a density of 1650 ha^{-1}. The shrub layer and herb layer are poorly developed. The soil is a brown forest soil with a well-developed 4-cm-thick A_0 layer.

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Table 1. The number of collembolans in different families collected from the hyphal mat of *Sarcodon scabrosus* and the adjacent non-mat soil (number per 25 cm^2: $n = 7$)

Collembolan family	September 22, 2003		*P* value	June 4, 2004		*P* value
	Hyphal mat Means ± SD	Non-mat soil Means ± SD		Hyphal mat Means ± SD	Non-mat soil Means ± SD	
Onychiuridae	7.8 ± 6.8	13.8 ± 8.4	n	7.6 ± 5.1	11.0 ± 8.7	n
Isotomidae	8.8 ± 8.4	25.2 ± 15.1	**	3.3 ± 3.8	11.3 ± 5.1	*
Hypogastruridae	6.5 ± 7.3	1.9 ± 4.0	**	0.3 ± 0.5	2.0 ± 1.7	*n*
Tomoceridae	1.1 ± 1.2	10.5 ± 11.8	**	0	3.0 ± 2.6	–
Entomobryidae	0.1 ± 0.3	1.0 ± 1.2	n	0	0.3 ± 0.6	–
Pseudachorutidae	0.4 ± 0.8	1.7 ± 1.3	**	0.3 ± 0.5	0.7 ± 0.6	n
Neanuridae	0.1 ± 0.2	1.1 ± 1.2	*	0.1 ± 0.4	0.3 ± 0.6	n
Sminthuridae	0	0.1 ± 0.3	–	0	0.3 ± 0.6	–
Neelidae	0	0.2 ± 0.4	–	0	0	–
Total	24.7 ± 19.7	55.5 ± 31.1	**	11.6 ± 6.7	29.0 ± 10.5	**

* Significant difference, $P < 0.05$ (*U* test); ** significant difference, $P < 0.01$ (*U* test); n, no difference was observed; –, statistical analysis was not performed

On September 22, 2003, we collected seven fresh fruit bodies (2.4 g in dry weight on average) in which the pileus had expanded and immediately put them into separate paper bags. Using a stainless steel sampler, we took seven soil cores (5 cm × 5 cm and 12 cm deep) from the points where the *S. scabrosus* had fruited (the hyphal mat) and another from the position 50 cm to the right or left away from the points (the adjacent non-mat soil). These seven pairs (14 samples in total) sampling points were marked, and on June 4, 2004, new cores were taken from a position 5 cm from the originals. The samples were brought to the laboratory and put into a Tullgren funnel (I. Field, Gunma, Japan) to collect collembolans, which were preserved in 100% ethanol. The collembolans were mounted with Hoyer's solution on slides. Under an optical microscope, they were identified to the family level (Itoh et al. 1999) and counted. The core samples taken from the sites of the fruiting bodies were gray owing to large amounts of extraradical mycelium but those taken 50 cm away were not.

Table 1 shows the number of collembolans collected from the hyphal mat and adjacent non-mat soil. In contrast to the results of Cromack et al. (1988), significantly fewer collembolans were collected from the hyphal mat than from the adjacent non-mat soil on both sampling days (*U* test: $P < 0.01$). In all families except the Hypogastruridae (= *Hypogastrura horrida* Yosii and *Hypogastrura* sp.), consistently fewer collembolans were collected from the mat than from the adjacent non-mat soil. Members of the Sminthuridae and Neelidae were not collected from the hyphal mat on September 22, 2003, and members of the Tomoceridae, Entomobryidae, Neelidae, and Sminthuridae were not collected from the hyphal mat on June 4, 2004. Given that fewer collembolans except hypogastrurids were collected from the hyphal mat, *S. scabrosus* seems to exclude collembolans. This is the first report of a negative effect by ectomycorrhizal fungi on collembolans in the field. We anticipate that ectomycorrhizal fungi would have effects ranging from positive to negative on collembolans in the field.

Significantly more hypogastrurids were collected from the hyphal mat than from the adjacent non-mat soil on September 22, 2003, but the numbers did not differ statistically from these on June 4, 2004 ($P = 0.53$). It is difficult to explain why only hypogastrurid numbers changed greatly between the two sampling days. Some species of *Hypogastrura* active in spring and autumn and inactive in summer and winter (Cassagnau and Ferrero 1966; Cassagnau 1986; Sawahata et al. 2002), and Sawahata et al. (2002) found large numbers of *Hypogastrura denisana* Yosii aggregating on fungal fruiting bodies in the second active season (= autumn). Thus, hypogastrurids might cease feeding in June at our study site, and resume feeding on *S. scabrosus* in September. We saw many hypogastrurids on *S. scabrosus* fruit bodies (270 per fruit body on average). These insects might bolster the hypogastrurid population in the hyphal mat below the fruit bodies.

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