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SHORT COMMUNICATION

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Hymenial area of agaric fruit bodies consumed by Collembola

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Abstract On the gill surfaces of agaric fruit bodies fed on by Collembola, the hymenium layer was consumed and many fecal pellets containing many basidiospores were observed. The hymenial area consumed by a collembolan (=*Hypogatsrura denisana*) varied from 1% to 92%, correlating with the density of collembolans on each fruit body among 11 agaric species. In *Lactarius quietus*, the hymenial area consumed by collembolans varied from 30% to 69% during the 5 days during which sampling took place. A weak correlation was found between the ratio of the hymenial area and the density of collembolans on the fruit bodies, and this fact suggested that other factors influenced the ratio of the hymenial area consumed by collembolans.

Key words Agaricales · Collembola · Fungivores · Insect pest · Spore dispersal

Most studies on insect–fungus relationships have treated fruit bodies as insect habitats and resources from the point of view of entomology (Hanski 1989), while the effects of fungivores on the ability of the host to disperse its spores have been little studied except for a few cases (Tsuda et al. 1996; Tuno 1998, 1999).

Collembolans are the most abundant insects found on agaric mushrooms (Yamashita and Hijii 2003); they feed on the basidiospores and hyphae between the hymenial surfaces (Sawahata et al. 2000, 2001). Hundreds and thousands of collembolans have been collected from agaric fruit bodies in autumn (Sawahata et al. 2000, 2001; Nakamori and Suzuki 2005). Hence, the collembolans may feed on a considerable area of the hymenia and may affect the ability of the fruit bodies to disperse their basidiospores by wind. Up to now, the width of the gill surface grazed on by collembolans has not been studied. In this study, the correlation between hymenial loss and the density of collembolans was examined.

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Sampling of the agaric fruit body was performed in an area approximately 30 m × 30 m located in a mixed forest composed of *Pinus densiflora* Sieb. et Zucc. and *Quercus serrata* Murray at the campus of the Faculty of Agriculture of Shinshu University in southern Nagano Prefecture (760 m asl, 35°51.6′ N, 137°56.5′ E), central Japan (Sawahata et al. 2000). The fruit bodies of 11 species of Agaricales were collected on October 28, 1999, 2 days after a rainfall (Table 1). Additional fruit bodies of *Lactarius quietus* (Fr.) Fr, which is the species most preferred by collembolans (Sawahata et al. 2000), were collected in August and October 2001 (Table 2). Fresh fruit bodies of which pilei had expanded were collected, and each was put into a separate paper bag. The fruit bodies and the collembolans found on the fruit bodies were then transfered into plastic bottles with 100% ethanol for fixation and preservation. Each fruit body preserved in ethanol was removed from the bottle and ten gills were randomly picked from the fruit body using sharp tweezers. Then, the gills were stained using Melzer's solution and mounted in Hoyer's solution.

Traces of grazing on the hymenial area by collembolans were observed with an optical microscope with the eyepiece equipped with an optical micrometer (0.1 mm^2). One hundred points for each of the ten gills were chosen at random, and the number of grid spaces of the eyepiece that the trace overlapped for each of the 100 points was used to calculate the ratio of the hymenial area consumed by collembolans for each fruit body.

Collembolans preserved in ethanol were collected with a syringe and mounted using Hoyer's solution after staining with Melzer's solution. The numbers of Collembola were counted with a stereoscopic dissecting microscope and their gut contents were observed with an optical microscope. The density of the collembolans on fruit bodies was calculated using the following equation: $D = N/\pi[(a + b)/2]^2$, where D is the density of collembolans on the fruit body, N is the number of collembolans on the fruit body, a is the maximum diameter (cm), and b is the minimum diameter (cm) of the pileus of the fruit body (Sawahata et al. 2000). To iden-

Table 1. Density of *Hypogastrura denisana* on the fruit body of mushrooms and the ratio of hymenial area consumed by *H. denisana*[a]

Fruit body	Density of *H. denisana* on a fruit body (individuals/cm^2)	Ratio of hymenial area consumed by *H. denisana* (%)
Clitocybe clavipes (Pers.: Fr.) Kummer	3	1 ± 17
Naematoloma sublateritium (Fr.) Karst.	12	37 ± 34
Inocybe sp.	60	79 ± 30
Russula sp.	180	25 ± 30
Naematoloma sublateritium	280	56 ± 22
Russula sp.	310	28 ± 32
Cortinarius sp.	320	90 ± 19
Russula sp.	330	40 ± 33
Naematoloma sublateritium	380	51 ± 27
Russula sp.	410	64 ± 34
Amanita porphyria (Alb. & Schw.: Fr.) Secr.	450	81 ± 19
Amanita citrina var. *citirina* (Schaeff.) Pers.	490	56 ± 41
Russula veternosa Fr. (sensu J. Schaffer)	610	84 ± 15
Lactarius quites Fr.	640	80 ± 19
Pholiota spumosa (Fr.) Sing.	660	92 ± 11
Cortinarius tenuipes (Hongo) Hongo	1000	87 ± 20
Russula veternosa Fr. (sensu J. Schaffer)	1020	79 ± 26
Lactarius quites Fr.	1360	91 ± 14
The average of all fruit bodies	450	66 ± 38

[a] Collected on October 28, 1999

Table 2. Density of *Hypogastrura denisana* on the fruit bodies of *Lactarius quietus* and the ratio of the hymenium consumed by *H. denisana*

Sampling date	Samples examined (*n*)	Grazed area in hymenia	Density of *H. denisana* (individuals/cm^2)
		Mean ± SD (%)	Mean ± SD
September 23, 2001	9	30 ± 17	864 ± 359a
September 25, 2001	15	49 ± 19	664 ± 409a
September 27, 2001	8	43 ± 28	348 ± 356b
October 2, 2001	12	69 ± 11	769 ± 252a
October 9, 2001	3	54 ± 46	88 ± 62[a]

Values followed by different letters indicate a significant difference ($P < 0.05$)
[a] This data set was not included in statistical analysis because it is composed of only three data

tify the fecal pellets attached to the gill surfaces, collembolans were collected from two fruit bodies of *Pholiota spumosa* (Fr.) Sing., *L. quietus*, and *Russula* sp. on October 28, 1999, in this forest and placed on malt extract agar (MEA) (3% Difco malt extract, 3% agar) plates in petri dishes. After 3h, the morphological features and contents of the fecal pellets on the plate were observed and compared with those of the fecal pellets on the surface of the hymenium under both a stereoscopic dissecting microscope and an optical microscope. In addition, fungal materials consisting of the fecal materials collected from the plate and from the gill surfaces were compared with those found on the hymenium layer.

In most cases, a species of hypogastrurid collembolan, *Hypogastrura denisana* Yosii, was the most abundant between the gills of the fruit body. This result was the same as that reported previously (Sawahata et al. 2000, 2001; Greenslade et al. 2002; Nakamori and Suzuki 2005). In the gill surfaces of the fruit bodies from which considerable numbers of collembolans were collected, the hymenial layer was consumed and the tramal hyphal layer was exposed (Fig. 1A). Many holes with fecal pellets were observed on the gill surface (Fig. 1B). The fecal pellets on the gill surface contained the basidiospores of the host fungal species (Fig. 1C). The morphological features (cylindrical, 80–140μm in diameter) of the pellets and basidiospores contained in the pellets were the same as those of collembolans on the petri plates (Fig. 2). Thus, fungivores, which ate the hymenium layers of the gill surfaces and left fecal pellets on the gill surfaces, were identified as collembolans.

More than half of the hymenial area was consumed by collembolans in 13 of 18 fruit bodies, and the average consumed proportion of all the fruit bodies collected on October 28, 1999, was 66% ± 38% (see Table 1). The ratio of the hymenial area consumed by Collembola for each fruit body correlated significantly with the density of collembolans on the fruit bodies (Spearman's rank coefficient of correlation: $r = 0.68$, $P < 0.01$), although the extent varied among the gills of a fruit body (Table 1). In *L. quietus*, the ratio of the hymenial area consumed by collembolans varied from 30% to 69% among 5 sampling days (see Table 2). The ratio of the area of the hymenium consumed weakly correlated with the density of collembolans on the *L. quietus* fruit bodies (Speaman's rank coefficient of correlation: $r = 0.52$,

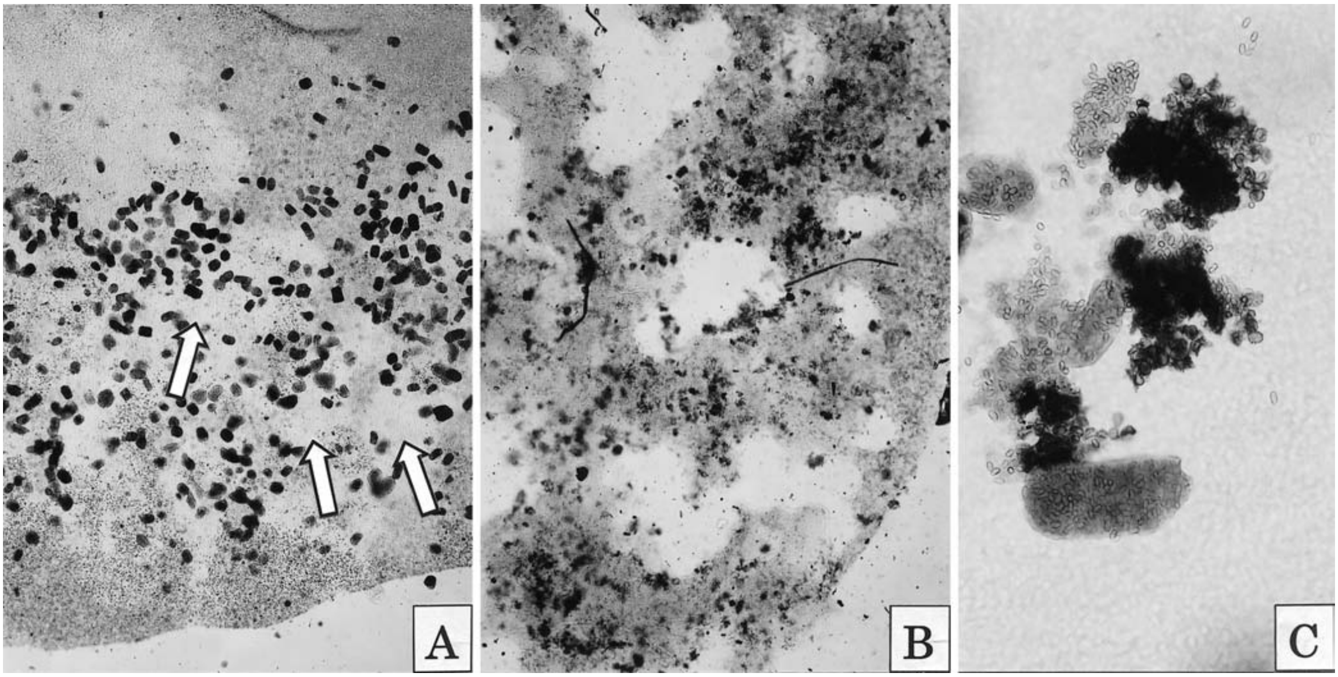

Fig. 1. Gill surfaces of fruit bodies consumed by collembolans. **A** Gill surface of a fruit body of *Pholiota spumosa* consumed by collembolans. Many fecal pellets of collembolans are scattered on the gill surface. The hymenial layer was consumed, and the tramal hyphal layer was exposed (*arrows*). **B** Holes in the pieces of a gill of a fruit body of *Lactarius quietus* made by collembolans. **C** The fecal pellets of collembolans containing many basidiospores. Small spores in fecal pellets are basidiospores of *Pholiota spumosa*. Black fecal pellets contain basidiospores of Russulaceae

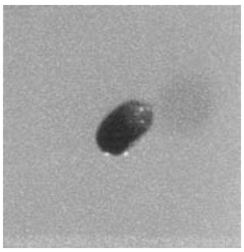

Fig. 2. Fecal pellet of collembolans on agar plate

$P < 0.01$). Therefore, other factors, such as the period during which the collembolans grazed on the hymenium layer and body size of the collembolans, would also influence on width of the hymenium area consumed.

In this study, 50% or more of the hymenial area of fruit bodies observed had been consumed by collembolans (see Tables 1, 2). Collembolans fed on considerable amounts of the basidiospores, excreting them in the form of fecal pellets. The basidiospores in the pellets attached to the gill surface could not be carried away by the wind and so remained until the fruit body rotted away. It can therefore be said that collembolan consumption and excretion may sometimes negatively affect the ability of wind dispersal of basidiospores and thus significantly decrease the amount of wind-carried basidiospores.

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