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Grazing preference and utilization of soil fungi by *Folsomia candida* (Isotomidae:Collembola)

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ABSTRACT

Soil fungi are important food resources for soil fauna. Here we ask whether the collembolan *Folsomia candida* shows selectivity in grazing between four saprophytic fungi (*Penicillium chrysogenum*, *Penicillium expansum*, *Absidia glauca*, and *Cladosporium herbarum*), whether grazing preference corresponds to effects on collembolan reproduction, and whether the effects of fungi on grazing and reproduction depends on the fungal substrate, which included three kinds of litter (*Alnus glutinosa*, *Salix caprea*, and *Quercus robur*) and one kind of agar (yeast extract). On agar, *C. herbarum* and *A. glauca* were the most preferred fungi and supported the highest collembolan reproduction. On fungal-colonized litter, grazing preference was more affected by litter type than by fungal species whereas collembolan reproduction was affected by both litter type and fungal species. On fungal-colonized litter, the litter type that was most preferred for grazing did not support the highest reproduction, i.e., there was an inconsistency between food preference and suitability. Alder and willow were preferred over oak for grazing, but alder supported the least reproduction.

1. Introduction

The study of food biology in soil ecosystems is difficult because the soil fauna ingest and utilize various components of soil organic matter usually associated with soil microflora [6,19,20]. Both springtails (collembolans) and fungi are important members of soil decomposer food webs [29,33–36], and soil fungi represent a significant food resource for collembolans and certain other soil invertebrates [2,9,10,14,15].

Fungal grazing by collembolans can alter the composition of the fungal community and thereby alter effects of fungi on litter decomposition and responses of fungi to other stress factors [1,3–5,7,8,15,21–23,38]. On the other hand, the composition of the fungal community and the interaction between fungi and the substrate affect collembolan food choice and reproductive success [19,20,41]. Soil fauna often prefer some species of fungi over others as food [5,16,17,25,26,29–32]. Fungi preferred by fungivorous fauna often include *Cladosporium cladosporoides*, *Cladosporium herbarum*, *Alternaria alternata*, and *Ulocladium* sp. [18,26,27].

Several authors concluded that the most preferred fungi are also the most suitable for growth and development of fungivorous fauna [10,17,28] but Frouz and Nováková [11] found that some highly preferred fungi did not support fungivore development. In addition, the substrate on which the fungi grown can strongly affect their attractiveness as a food source for fungivores and their suitability for fungivores growth and development [10,17]. We expect that substrate used for growing fungi used as a food for *Folsomia candida* may substantially affect fungal preference and suitability. In the current paper, we determined the preference of the collembolan *F. candida* for several species of fungi, whether this preference corresponds with food suitability, and how both preference and food suitability are affected by the substrate supporting fungal growth.

2. Materials and methods

2.1. Collembolans, fungi, and litter

Folsomia candida (Isotomidae:Colembolla) was obtained from the Institute of Soil Biology, České Budějovice, and was reared according to a standard protocol [39,40]. The following fungi were obtained

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from the Collection of Micromycete Strain in the Institute of Soil Biology, České Budějovice: *Penicillium expansum*, *Absidia glauca*, *C. herbarum*, and *Penicillium chrysogenum*. The fungi were cultivated by transferring spores to Petri dishes containing 8%yeast extract agar and incubating the cultures at 20–25 °C. Litter was collected from alder (*Alnus glutinosa*) and oak (*Quercus robur*) plantations and from willow (*Salix caprea*) trees (spontaneous regrowth) on a post-mining heap near Sokolov (Czech Republic) [13]. Litter was collected by placing nylon bags under these trees; the bag openings (0.5 × 0.5 m) were parallel with the soil surface and about 0.5 m above the soil surface, and the bags were deployed for 14 days during leaf drop in November. The litter was air dried, placed in sealed zip-lock bags, and sterilized with gamma radiation (2.5 MG) before use.

2.2. Grazing preference experiments

For the first grazing preference experiment, the four fungi were grown on yeast extract agar for 5 days as described above before fungal-colonized agar disc (1 cm diameter) were cut from the colony. One agar disc of each of the four fungal species was placed in random order around the periphery of an empty, sterile 9-cm-diameter Petri dish [39]. Thirty *F. candida* were then placed in the middle of the Petri dish, which was then covered and kept at 20 °C in the dark. The *F. candida* individuals on each agar disc were counted at the same time of day (14.00 h) during 8 days. The experiment included four replicate Petri dishes. Cumulative occurrence used for the comparison of multiple reading done in one dish was assumed as one event [18].

A similar experiment was conducted with pieces of litter that were about 1 cm^2 in surface area and that had been colonized by one of the three fungi. Each of litter pieces (*A. glutinosa, Q. robur, S. caprea*) were colonized by one of three stems of fungi (*A. glauca, C. herbarum, P. chrysogenum*). Nine fungal-colonized pieces of litter (one piece for each combination of fungal species and litter type) were placed on the periphery of an empty 9-cm diameter Petri dish, and 30 *F. candida* were added. The Petri dish was covered and kept at 20 °C in the dark. After the *F. candida* on each piece of litter were counted. The experiment with litter was shorter than that with agar disc because the small litter pieces deteriorated after only a few days. This second experiment also included three replicate Petri dishes.

2.3. Reproduction experiments

For the first reproduction experiment, Petri dishes containing yeast extract agar were inoculated with one of the four species of fungi. After the fungus had completely colonized the dish, 10 *F. candida* from a synchronized culture [39] were added to each dish. There were three replicate dishes, and temperature and light were as described in the grazing experiments. After 30 days, 70% ethanol was added to each dish, and the *F. candida* individuals in each dish were counted. The second reproductive experiment was similar to the first except that each dish contained 2 g of litter (one of three kinds of litter) that had been colonized by one of three species of fungi. In the second reproductive experiment, there were three replicate plates for each combination of litter type (*A. glutinosa, Q. robur, S. caprea*) and fungal species (*A. glauca, C. herbarum, P. chrysogenum*).

2.4. Statistical analysis

Data were subjected to a one-way ANOVA (for the first grazing preference experiment and the first reproduction experiment) or a two-way ANOVA (for the second grazing preference experiment and the second reproduction experiment). In case preference tests when several observations were done in one dish, all observations made in one dish was pooled and assumed as one observation for future statistical analysis [42]. If an ANOVA was significant, means were compared with the Tukey–Kramer Multiple Comparison Test. The R programme was used for statistical analyses [42].

3. Results

3.1. Grazing preference experiments

In the experiment concerning collembolan preference among fungi growing on discs of yeast extract agar, *F. candida* preferred *C. herbarum* and *A. glauca* over *P. chrysogenum* and *P. expansum*-see Fig. 1 ($F_{3,12} = 28.530$, $p = 0.0004$). In the experiment concerning collembolan preference among fungi growing on pieces of litter, the number of *F. candida* on the litter pieces was significantly affected by litter type (*F. candida* preferred alder and willow litter over oak) but was not significantly affected by which fungus had colonized the litter (Fig. 2). According to a two-way ANOVA, the effect of litter was significant ($F_{2,6} = 6.3$, $p < 0.005$) but the effects of fungal species ($F_{2,6} = 1.1$, $p = 0.346$) and the interaction between litter type and fungal species ($F_{8,24} = 1.1$, $p = 0.349$) were not significant.

3.2. Reproduction experiments

In the first reproduction experiment, in which the fungal-colonized agar discs were offered to the collembolans, *F. candida*

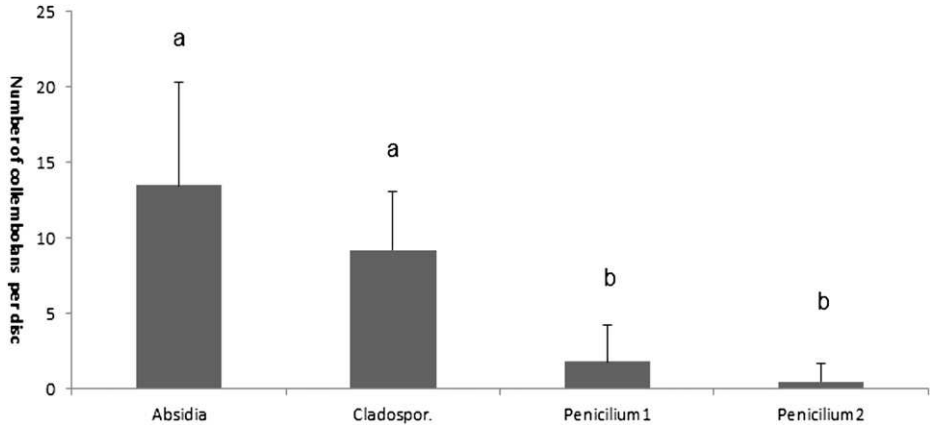

Fig. 1. Numbers of *F. candida* on fungal-colonized agar disks as affected by fungal species; the collembolans were counted 12 days at same time 30 individuals were added per dish. Values are means + SD of all sampling dates. Means with the same letter are not significantly different according to an Tukey–Kramer Multiple Comparison Test ($p > 0.05$). Absidia (*Absidia glauca*), Cladospor, (*Cladosporium herbarum*), Penicilium1 (*Penicillium chrysogenum*), Penicilium2 (*Penicillium expansum*).

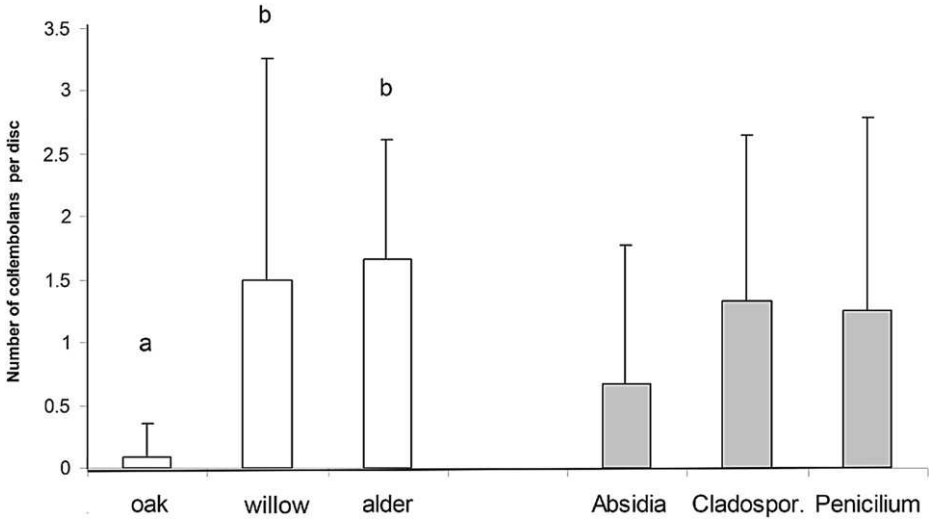

Fig. 2. Numbers of *F. candida* on fungal-colonized litter pieces as affected by species of fungi and litter source; the collembolans were counted 1 and 2 days after 30 were added per dish. Values are means + SD of both sampling dates. Data for litter type were averaged across fungi, and data for fungi were averaged across litter types. Means with the same letter are not significantly different according to an Tukey–Kramer Multiple Comparison Test ($p > 0.05$). Absidia (*Absidia glauca*), Cladospor. (*Cladosporium herbarum*), Penicilium (*Penicillium chrysogenum*).

numbers were significant higher ($F_{3,9} = 6.269$, $p = 0.017$) with *A. glauca* and *C. herbarum* than with *P. chrysogenum* or *P. expansum* (Fig. 3). In the second reproduction experiment, in which the fungi were grown on different types of litter, *F. candida* numbers were significantly affected by fungal species and litter type but not by their interaction (Fig. 4). As in the second grazing preference experiment, more of the variation was explained by litter type than by fungal species. *F. candida* numbers were largest on willow litter, intermediate on oak litter, and smallest on alder litter, which differs from the order obtained in the second grazing preference experiment (Fig. 3). According to a two-way ANOVA, the effects of both litter and fungal species were significant ($F_{2,6} = 57.3$, $p < 0.001$ and $F_{2,6} = 7.1$ and $p = 0.005$ respectively) but the interaction was not significant ($F_{8,24} = 1.9$, $p = 0.150$). With respect to fungi in the second reproductive experiment, *F. candida* numbers were larger with *A. glauca* and *P. chrysogenum* than with *C. herbarum* (Fig. 4).

4. Discussion

When offered fungi growing on agar disc in the current study, *Folsomia candida* preferred some fungal species and also increased to higher number on some species than on others. When offered fungi grown on different litter types (alder, willow, and oak), however, grazing preference and reproduction were more affected by litter type than by fungal species. In agreement with other authors, this indicates that the substrate on which fungi grow is largely responsible for fungal attractiveness and nutritional quality for fungivores [11,12,21]. None of previous studies deal with litter which is more field realistic that any growing media, basic novelty of our study is that litter is more important than fungal species itself. This finding also supports the conclusion of Jørgensen et al. [20] that natural substrates should be used in studies of fungivore–fungus interactions.

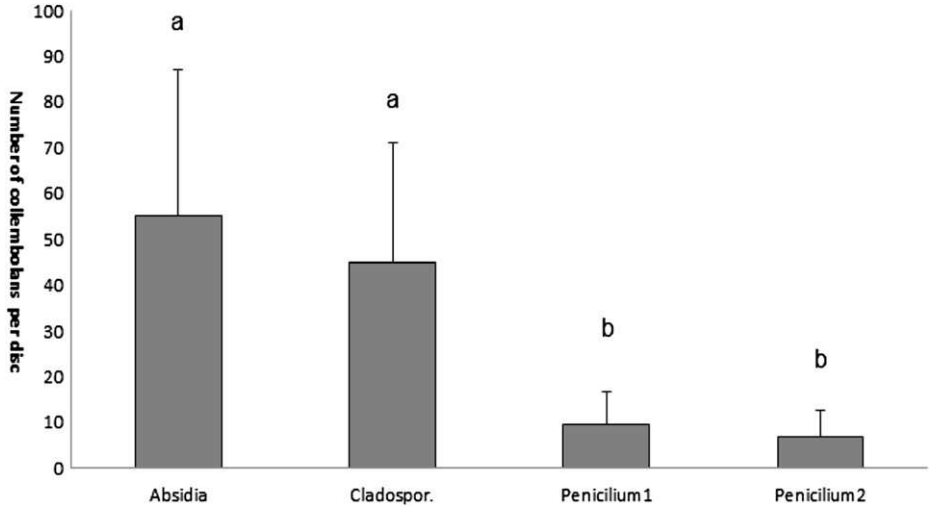

Fig. 3. Numbers of *F. candida* in Petri dishes containing fungi growing on yeast extract agar; the collembolans were counted 30 days after 10 were added per dish. Values are means + SD, and means with the same letter are not significantly different based on an Tukey–Kramer Multiple Comparison Test ($p > 0.05$). Cladospor. (*Cladosporium herbarum*), Penicilium1 (*Penicillium chrysogenum*), Penicilium2 (*Penicillium expansum*).

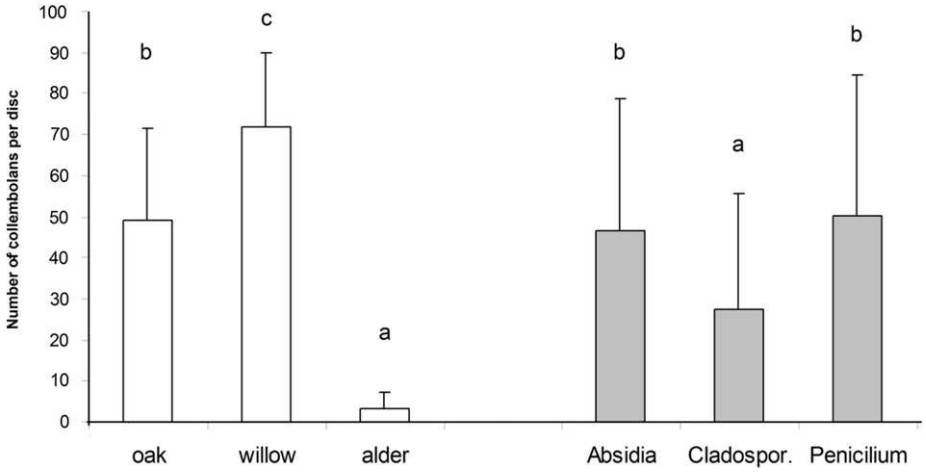

Fig. 4. Numbers of *F. candida* in Petri dishes containing fungal-colonized litter; the collembolans were counted 30 days after 10 were added per dish. Values are means + SD. Data for litter type were averaged across fungi, and data for fungi were averaged across fungal species. Means followed by the same letter are not significantly different based on an Tukey–Kramer Multiple Comparison Test ($p > 0.05$). Absidia (*Absidia glauca*), Cladospor. (*Cladosporium herbarum*), Penicilium (*Penicillium chrysogenum*).

In the grazing preference experiment with fungal-colonized agar disks, the two species of *Penicillium* were the least preferred. In this case, food preference may have been affected by the production of patulin, citrinin, or other mycotoxins. Dowd [8] reported that patulin and ochratoxin caused arthropod mortality. According to Staaden et al. [37], olfactory cues affect the food preference of collembolans because volatiles indicate which secondary metabolites are in the food source.

In the reproduction experiment on agar, *F. candida* population growth was greater with *A. glauca* and *C. herbarum* than *P. chrysogenum* and *P. expansum* These results correspond with those of Hubert et al. [17], who reported that, when the substrate supporting fungal growth was agar, those oribatid mites that preferred *C. cladosporoides* also had the greatest reproduction on *C. cladosporoides*. Tordoff et al. [39], who studied the reproduction of several species of collembolans (*Folsomia candida, Proisotoma minuta, Protaphorura armata*) on four species of Basidiomycetes (*Phanerochaete velutina, Hypholoma fasciculare, Resinicium bicolor*, and *Phallus impudicus*), reported that *P. minuta* survived only on *P. velutina* mycelia. In contrast, *F. candida* was found to be a dietary generalist that was able to increase its abundance on the mycelium of all four species of Basidiomycetes. *P. armata* could survive but not reproduce well on *P. velutina* mycelium. Frouz and Nováková [12] showed that the fungi most preferred by the dipteran *Lycoriella ingenua* are most suitable for the growth and development of its larvae. *Folsomia candida* is able to survive and reproduce on more food resources than other collembolans on various substrates [39].

As noted earlier, collembolan food preference in the current study was more affected by the substrate on which the fungi grew than on the identity of the fungi. Jørgensen et al. [19] documented significant differences in food preference when collembolans were offered eight species of soil fungi growing on a natural soil substrate. In agreement with Kaneko et al. [24], we found that which fungi were preferred by collembolans differed depending on the substrate on which the fungi were growing.

5. Conclusions

Litter type had an inconsistent effect on *F. candida* grazing preference and *F. candida* reproduction. Thus, alder was preferred to oak in the grazing preference experiment but oak supported greater numbers than alder in the reproduction experiment. The reason for this inconsistency is not clear but perhaps can be explained by the short duration of the preference test (data were collected after 12 days) in which sterile alder supported better fungal growth than sterile oak litter. In the reproduction test, which lasted for 30 days, addition of collembola undoubtedly resulted in bacterial contamination of the litter, and the bacteria may have reduced fungal growth to a greater degree on alder than on oak, resulting in greater collembolan reproduction on oak than on alder. This result, which is to some extent contrary to that of Jørgensen et al. [20], indicates that discrepancies between food choice and food suitability may occur even on natural substrates.

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