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Dispersal of entomopathogenic fungi by collembolans

Karsten M. Dromph*

Department of Ecology, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

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Abstract

The aim of the study was to investigate the possibility of and mechanisms involved in dispersal of entomopathogenic fungi by collembolans. It was found that the collembolan species: *Folsomia fimetaria*, *Hypogastrura assimilis* and *Proisotoma minuta* are able to carry viable conidia of the three entomopathogenic fungi: *Beauveria bassiana*, *B. brongniartii* and *Metarhizium anisopliae* both on the cuticle and in the gut. Between 38.3 and 96.1% of the collembolans exposed to sporulating cultures for 1 h gave rise to colonies of the three fungi through conidia carried on the cuticle. After exposure for 1 h in a sphagnum based growth medium containing 10^7 conidia g^{-1} wet weight, between 8.2 and 78.8% of the collembolans gave rise to colonies of the three fungi through conidia carried on the cuticle. There were no clear differences between the ability of the three collembolan species to carry conidia on the cuticle, or the ability of the conidia of three fungi to attach to the collembolans. Ingestion by the collembolans had no effect on the viability of conidia of *B. bassiana* and *B. brongniartii*, while ingestion of *M. anisopliae* by *F. fimetaria* and *P. minuta* reduced the viability of conidia significantly to 24.3 and 54.0% compared with a germination of 98.8% of the conidia in the uningested control. After feeding on sporulating fungal cultures for 1 h, between 52.2 and 100% of the faecal pellets formed by each of the three collembolan species formed colonies of the three entomopathogenic fungi. While between 6.2 and 70.8% of the faecal pellets produced by each of the three collembolan species exposed to inoculated sphagnum formed colonies of the three entomopathogenic fungi. There were no systematic differences between the abilities of the three collembolan species to disperse the fungi nor between the dispersal rates of the three fungi.

Keywords: *Metarhizium anisopliae*; *Beauveria brongniartii*; *Beauveria bassiana*; Entomopathogenic fungi; Spore dispersal; Collembola

1. Introduction

Collembolans constitute a very large proportion of the soil fauna (Hale, 1967). They are mainly fungivores and interact with soil fungi in several ways (Christiansen, 1964; Hale, 1967). They can either reduce the amount of fungal inoculum by selectively grazing on the mycelium and digesting the spores (Curl, 1988; Lartey et al., 1991), or disperse the fungi by carrying viable spores on the cuticle or in the gut (Wiggins and Curl, 1979; Whipps and Budge, 1993; Williams et al., 1998).

The entomopathogenic hyphomycete fungi *Metarhizium anisopliae* (Metch.) Sorokin and *Beauveria bassiana* (Bals.) Vuill. are commonly found in soil (Klingen et al., 1998; Mietkiewski and Tkaczuk, 1998). They have a very broad host range, infecting several insect orders (Leatherdale, 1970) and are being developed as biocontrol agents against soil dwelling coleopteran pests like scarabs and weevils (Keller, 1992; Moorhouse et al., 1993; Rath et al., 1995).

Very few studies have focused on the interaction between collembolans and entomopathogenic fungi in soil. It has been demonstrated that collembolans are able to transport viable conidia of several entomopathogenic fungi including *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces farinosus* (Holm ex Gray) A.H.S. Brown & G. Sm. and *Verticillium lecanii* (Zimm.) Viegas (Zimmermann and Bode, 1983; Visser et al., 1987). However, the mechanisms involved in the dispersal have not been elucidated.

The aim of the present study was to investigate the rate of transmission and the mechanisms involved in the transport of the three entomopathogenic fungi *B. bassiana*, *B. brongniartii* (Saccardo) Petch and *M. anisopliae* in the soil by the three collembolan species *Folsomia fimetaria* (L.), *Hypogastrura assimilis* (Krausbauer) and *Proisotoma minuta* (Tullberg), when exposed to the fungi in the form of sporulating cultures or as conidia dispersed in soil.

* Present address: Lancaster University, Institute of Environmental & Natural Science, Department of Biological Sciences, Lancaster LA1 4YQ, UK. Tel.: +44 1524 592 278; fax: +44 1524 843 854.

E-mail address: k.dromph@lancaster.ac.uk (K.M. Dromph).

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2. Materials and methods

2.1. Fungal isolates

The fungal isolates used in this study were *B. bassiana* (KVL 98-22), isolated in Denmark from *Hepialus* sp. (Lepidoptera: Hepialidae), *B. brongniartii* (FAL 546), isolated in Switzerland from *Melolontha melolontha* (L.) (Coleoptera: Scarabaeidae) and *M. anisopliae* (Ma 275), isolated in Germany from *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). The fungi were grown on Sabourad dextrose agar (SDA) plates for 2 weeks at 20°C before they were used in the experiments. Stock cultures were stored on SDA plates at 5°C.

2.2. Collembolans

The collembolans used in the present study originated from laboratory stock cultures derived from specimens collected in a clover-grass field at Højbakkegaard, an experimental farm situated 20 km west of Copenhagen, Denmark. The cultures were maintained at 20°C in a mix of sphagnum and finely chopped barley straw (v:v 4:1) with addition of baker's yeast as a food source. Samples were taken from the cultures at several times and identified under a light microscope according to Gisin (1960) and Fjellberg (1980) in order to confirm the purity of the cultures. For the present experiments, reproductive adults were selected based on size.

2.3. Transmission of conidia on the cuticle

To test the possibility of transmission of viable conidia on the cuticle of the collembolans, ten adults of either *F. fimetaria*, *H. assimilis* or *P. minuta* were exposed for 1 h at 20°C to sporulating cultures of the three fungal isolates growing on SDA plates. The short exposure time used in this experiment enabled the collembolans to move around on the cultures, but not to start defecating fungal material ingested during the exposure. After the feeding period the collembolans were transferred singly to 50 μl of 0.05% Tween-20 and stirred twice for 30 s each at 2000 rev min^{-1}, in order to wash any fungal spores off the cuticle while leaving the collembolan intact. The suspensions were plated on a semi-selective SDA medium (SDA with 0.5 μg dodine/Radspor FL, 50.0 μg Chloramphenicol, 50.0 μg Streptomycin sulphate l^{-1}) and incubated at 20°C. After 2 weeks fungal colonies were identified under a light microscope (Barnet and Hunter, 1998), and the number of *B. bassiana*, *B. brongniartii* or *M. anisopliae* colonies was recorded. The experiment was replicated six times.

To test if transmission of viable conidia on the cuticle was possible from inoculated soil, collembolans were incubated in sphagnum based growth medium inoculated with conidia suspensions. A commercial fine-grained sphagnum based growth medium, pH 5.6 6.6, produced by Pinstrup Mosebrug A/S, Denmark, was sterilised by heating to 150°C for 2 days, and thereafter wetted to reach 60% water content (w:w). Moist sphagnum (1.5 g) was transferred to 30-ml plastic vials and inoculated with 1 ml of suspension of one of the three fungal species. Thereby the sphagnum reached a water content of 70% and a concentration of 10^7 conidia g^{-1} wet sphagnum. Ten adults of each of the three species were added and the vials were incubated in darkness at 20°C for 1 h, after which the collembolans were extracted by heat and immediately transferred singly to Eppendorff tubes containing 50 μl of 0.05% Tween-20 and treated as described above. The experiment was replicated six times.

2.4. Effects of ingestion on conidia viability

To test the effects of ingestion on the viability of the conidia, 20 adult collembolans were allowed to feed for 24 h at 20°C on sporulating fungal cultures grown on SDA plates. After the feeding period the collembolans were transferred to 30-ml plastic vials with 5 ml of 3% water agar at the bottom. After 10 min the collembolans were transferred to vials with clean water agar in order to reduce contamination from spores carried on the cuticle. Faecal pellets were collected after 3 h incubation at 20°C and 30 faecal pellets were suspended in 50 μl of 0.05% Tween-20. The suspensions were plated on semi-selective SDA medium and incubated for 24, 48 or 72 h at 20°C in darkness. The agar was then stained with lactophenol-cotton blue and the spore viability was assessed by examining 2×100 spores per replicate under a light microscope. As control a 10^6 spores ml^{-1} 0.05% Tween-20 suspension was plated on semi-selective SDA and incubated the same way as described above. The experiment was replicated six times.

2.5. Dispersal through faecal pellets

To assess the proportion of faecal pellets containing viable conidia, 15 adults of the three collembolan species were exposed for 24 h either to sporulating cultures or inoculated sphagnum (Section 2.4). After the feeding period, the collembolans were extracted and treated as described in Section 2.3. The collembolans were incubated for 3 h at 20°C in 30-ml plastic vials containing 5 ml of 3% water agar, after which ten faecal pellets were collected and smeared singly onto semi-selective SDA. After incubation for 2 weeks at 20°C fungal colonies were examined under a light microscope and the number of faecal pellets giving rise to colonies of either *B. bassiana*, *B. brongniartii* or *M. anisopliae* were recorded. The experiment was replicated six times.

2.6. Statistical analysis

The data were logit transformed and analysed using the GENMOD procedure in SAS 6.12. When the effects of treatments were found to be significant ($P < 0.05$), the mean treatment differences between all combinations of

Table 1
Percentage of collembolans giving rise to colonies of entomopathogenic fungi through conidia carried on the cuticle. Numbers are means of six replicates ± SE, back transformed after logit transformation. (a) After incubation for 1 h on sporulating cultures, (b) After incubation for 1 h in sphagnum containing 10^7 conidia g^{-1} wet weight. Rows followed by different letters are significantly different from each other ($P < 0.05$)

Fungus	Collembola	% collembolans giving rise to colonies	% colonies − SE % colonies + SE	Pairwise comparison
(a)				
B. bassiana	*P. minuta*	81.9	77.7 85.5	A, D
B. bassiana	*F. fimetaria*	83.6	79.5 87.0	A, E
B. bassiana	*H. assimilis*	63.5	58.5 68.2	B, D, E
B. brongniartii	*P. minuta*	38.3	33.1 43.7	E, F
B. brongniartii	*F. fimetaria*	96.1	94.0 97.4	A, E
B. brongniartii	*H. assimilis*	44.8	40.2 49.5	F
M. anisopliae	*P. minuta*	51.7	46.6 56.7	B, G
M. anisopliae	*F. fimetaria*	71.9	67.1 76.2	A, E, G
M. anisopliae	*H. assimilis*	85.2	81.3 88.5	A, B
(b)				
B. bassiana	*P. minuta*	45.0	39.6 50.5	A, B, C
B. bassiana	*F. fimetaria*	29.9	25.2 35.2	A, B, C
B. bassiana	*H. assimilis*	31.6	26.8 36.9	A, B, C
B. brongniartii	*P. minuta*	8.2	5.8 11.4	A, B, C
B. brongniartii	*F. fimetaria*	18.3	14.4 22.9	B
B. brongniartii	*H. assimilis*	53.3	47.9 58.7	C
M. anisopliae	*P. minuta*	19.9	15.9 24.7	A, B
M. anisopliae	*F. fimetaria*	78.8	70.9 84.6	A, B, C
M. anisopliae	*H. assimilis*	47.1	42.7 51.5	A, B, C

collembolan and fungal species were compared using the CONTRAST procedure for pairwise comparisons ($P < 0.05$) (SAS Institute, 1990).

3. Results

3.1. Transmission of conidia on the cuticle

The proportions of collembolans of the three species carrying viable conidia of the three entomopathogenic fungi on the cuticle are shown in Table 1. All three collembolan species carried enough viable conidia on the cuticle to form colonies of *B. bassiana*, *B. brongniartii* or *M. anisopliae* both after exposure to sporulating cultures or to inoculated soil. There were no clear differences between the three collembolan species in the ability to transmit the fungi through conidia carried on the cuticle.

3.2. Effects of ingestion on spore viability

The effects of ingestion by collembolans on the viability of the conidia are shown in Fig. 1. Ingestion by the three collembolan species lowered the germination rate of *B. bassiana* at 24 h incubation time, but had no effect on the total viability of conidia of *B. bassiana* and *B. brongniartii*. Ingestion of *M. anisopliae* by *F. fimetaria* and *P. minuta* significantly reduced the total viability of conidia, while ingestion by *H. assimilis* only reduced the germination rate at 24 h incubation time.

3.3. Dispersal through faecal pellets

The proportion of faecal pellets forming colonies of the three entomopathogenic fungi are shown in Table 2. All three collembolan species carried enough viable conidia in the gut to form colonies of *B. bassiana*, *B. brongniartii* or *M. anisopliae* exposure to either sporulating cultures or inoculated soil.

4. Discussion

The present study shows that collembolans are able to carry viable conidia of the three entomopathogenic fungi *B. bassiana*, *B. brongniartii* and *M. anisopliae* both on the cuticle and in the gut, after exposure to the fungi both in the form of sporulating colonies and conidia dispersed in the soil. There were no systematic differences in the ability of the three collembolan species to transmit the fungi. Although both *F. fimetaria* and *P. minuta* significantly reduced the viability of the conidia of *M. anisopliae* compared to the control (Fig. 1), this did not result in significant differences in the proportions of faecal pellets giving rise to colonies (Table 2). In field entomopathogenic hyphomycete fungi are usually found as concentrated spots around sporulating cadavers in the soil (T. Steenberg, unpublished). The exposure of the collembolans in the present experiments to either high concentrations of conidia dispersed in the soil or directly to sporulating material simulates therefore the way they would come into contact with these fungi under natural conditions.

Collembolans form a substantial part of the soil fauna,

Table 2
Percentage of faecal pellets forming colonies of entomopathogenic fungi. Numbers are means of six replicates ± SE, back transformed after logit transforma tion. (a) After incubation for 24 h on sporulating cultures, (b) After incubation for 24 h in sphagnum containing 10^7 conidia g^{-1} wet weight. Rows followed by different letters are significantly different from each other ($P < 0.05$)

Fungus	Collembola	% faecal pellets giving rise to colonies	% colonies − SE % colonies + SE	Pairwise comparison
(a)				
B. bassiana	*P. minuta*	95.6	94.1 96.7	A
B. bassiana	*F. fimetaria*	100.0	100.0	B, C
B. bassiana	*H. assimilis*	98.8	97.6 99.1	A, C
B. brongniartii	*P. minuta*	94.1	92.5 95.4	A
B. brongniartii	*F. fimetaria*	85.5	76.5 91.5	A
B. brongniartii	*H. assimilis*	52.2	46.2 58.0	A
M. anisopliae	*P. minuta*	92.6	90.8 94.1	A
M. anisopliae	*F. fimetaria*	97.1	95.9 97.9	A
M. anisopliae	*H. assimilis*	94.1	92.5 95.4	A
(b)				
B. bassiana	*P. minuta*	15.9	13.8 18.2	A, B, C
B. bassiana	*F. fimetaria*	14.2	12.3 16.5	A, B, C
B. bassiana	*H. assimilis*	11.0	9.3 13.0	A, B, C
B. brongniartii	*P. minuta*	21.0	18.5 23.7	A, C
B. brongniartii	*F. fimetaria*	24.0	21.7 27.2	A, B, C
B. brongniartii	*H. assimilis*	12.6	10.7 14.7	A, B
M. anisopliae	*P. minuta*	6.2	4.9 7.8	B
M. anisopliae	*F. fimetaria*	70.8	64.6 76.3	C
M. anisopliae	*H. assimilis*	65.9	62.2 69.4	A, B, C

with 10 20,000 collembolans m^{-2} in cultivated fields (Lagerlöf and Andrén, 1991; P.H. Krogh, unpublished) and more than 100,000 per m^2 in permanent grasslands (Persson and Lohm, 1977). Although dispersal rates of collembolans usually are less than 10 cm d^{-1}, some individuals may move up to 16 cm h^{-1} on a surface of plaster of Paris (Johnson and Wellington, 1983) and up to 40 cm d^{-1} through soil (Bengtsson et al., 1994). The present results

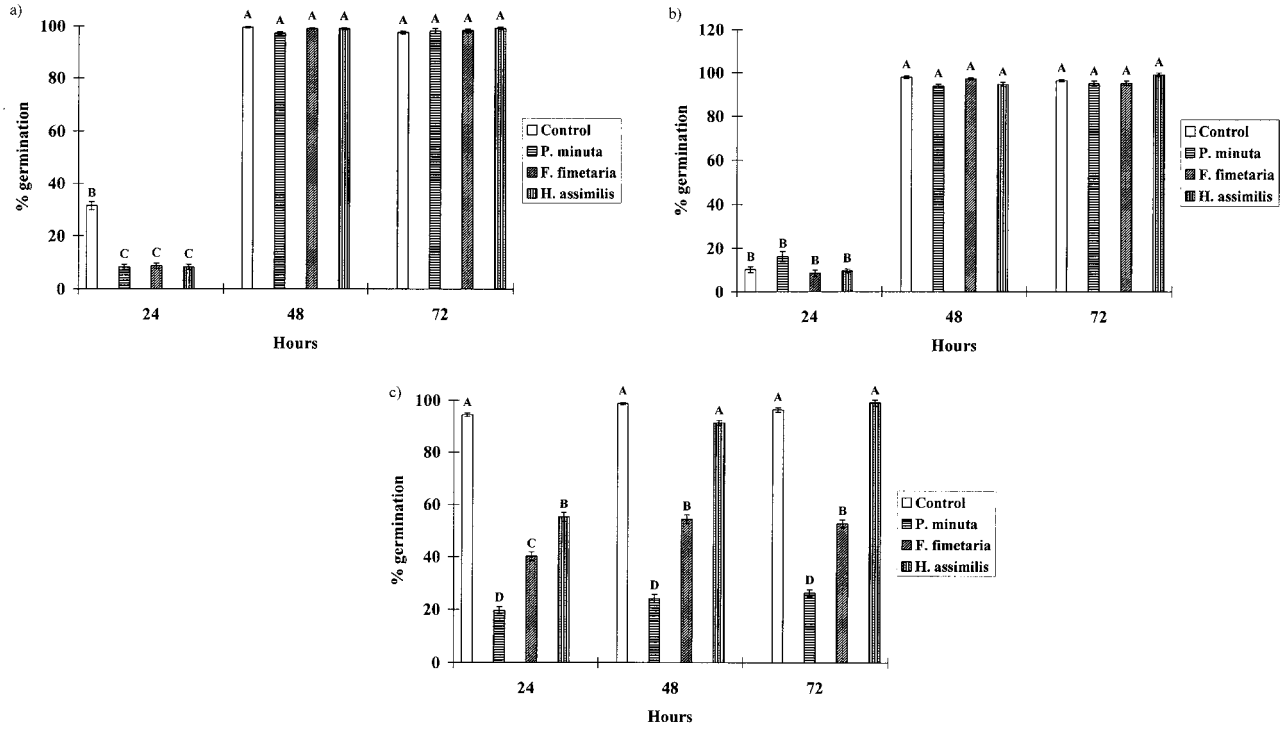

Fig. 1. Germination percentage of conidia after ingestion by collembolans. (a) *B. bassiana*, (b) *B. brongniartii* and (c) *M. anisopliae*. Columns are means of six replicates back transformed after logit transformation, error bars indicate S.E. columns headed by different letters are significantly different ($P < 0.05$).

show that even a short exposure to either soil containing conidia or sporulating colonies is enough to enable transmission of these fungi. This, combined with the dispersal rates of collembolans, indicates that collembolans can play an important role in the dispersal of entomopathogenic fungi, by transporting inoculum both horizontally and vertically in the soil. The increased dispersal rate of the fungi by collembolans is likely to be of great importance, as theoretical studies recently have indicated that pathogen-bearing agents, like collembolans, may play a crucial role in the dispersal of entomopathogens and that the dispersal rate of the pathogen is one of the main factors regulating the infection level in a susceptible host population (White et al., 2000). It is therefore highly possible that the dispersal of entomopathogenic hyphomycete fungi in the soil by collembolans will affect the regulation of susceptible soil invertebrates by entomopathogenic fungi by transporting the fungi to uncolonised parts of the soil, where they either can establish by either growing saprophytically or directly cause infection in susceptible hosts. The latter suggestion is supported by previous work demonstrating that transmission of the mycoparasitic fungus *Coniothyrium minitans* Campell by collembolans between inoculated and non-inoculated sclerotia of *Sclerotinia sclerotiorum* (Lib.) in the soil is possible (Whipps and Budge, 1993).

The dispersal mechanism described in the present paper may therefore be considered to be an ecological advantage for the entomopathogenic fungi occurring naturally in the soil. Additionally, it may increase the effectiveness of these fungi as microbial bio-control agents of soil dwelling pests. This effect is likely to be most pronounced for the use of *B. brongniartii* against scarab larvae as this, in several countries, is carried out by applying the fungus to the soil as granules (Keller, 2000) from which further dispersal of inoculum is necessary in order to obtain a sufficient level of infection on the pest population.

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