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# FUNGAL ODOUR ATTRACTS SOIL COLLEMBOLA

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**Summary**—A soil-inhabiting species of Collembola, *Onychiurus armatus* (Tullb.), locates its food source, hyphomycetous fungi, by volatile compounds released from the mycelium. Attractivity was ranked based on the odour of the fungal species, represented by compounds in the range $C_5$–$C_{18}$ and typically released in olfactometer experiments at a rate of 250 pg $h^{-1}$ from a 175 $mm^2$ fungal patch representing about 400 $\mu g$ mycelium. Among fungi grown in agar *Verticillium bulbillosum* was most attractive; the preference of *O. armatus* switched to other species, however, when these were cultured in soil.

## INTRODUCTION

In temperate forest soils Collembola form one of the most abundant soil arthropod group with densities ranging fom 10,000 to 100,000 $m^{-2}$ (Persson and Lohm, 1977). Most species feed on detritus, fungi or bacteria and can be characterized as secondary decomposers. Whether Collembola show food specialization or not is controversial. Anderson and Healey (1972) have argued that their feeding is mainly unspecific, but species used in laboratory experiments tend to rank food items (Singh, 1969; Visser and Whittaker, 1977; Bengtsson *et al.*, 1985). The conventional optimal foraging theory, as reviewed by Pyke *et al.* (1977), specifies that animals should rank alternative food items based on their profitability and forage randomly until they encounter food items or food patches that increase their rate of energy acquisition. Hence, the theory predicts that the animals will discriminate only when foraging in a productive habitat and become progressively more indiscriminate as the habitat becomes poor. The theory has been criticized as being incomplete and illogical (Rapport, 1981; Glasser, 1982), and alternative theories that allow foraging efficiencies to reflect previous encounters have been described (Schluter, 1981; Glasser, 1982). In essence, these theories suggest, that the foraging strategies depend on habitat conditions with obligate strategies under constant conditions and facultative strategies under variable conditions. In any case, both obligate and facultative consumers should specialize when food is abundant. Precise visual or olfactorial identification cues may then be helpful in productive habitats to increase the rates of energy acquisition of the animals.

There are both direct and indirect ways to estimate the food supply for Collembola in soils. Bengtsson *et al.* (1985) showed that survival and growth of *Onychiurus armatus* (Tullb.) were independent of enrichment of the fungal biomass greater than the amounts found in a common spruce forest soil but were increased when the fungal biomass was enhanced in a metal-polluted soil. Alternatively, one can calculate the fungal biomass production in an average soil and the part consumed by Collembola. Given an average fungal biomass of 2 g $m^{-2}$ (Nordgren *et al.*, 1983), 30 generations of biomass $yr^{-1}$ (calculated from Flanagan and Van Cleve, 1977) and 21 kJ $g^{-1}$ of mycelium, we would arrive at a net production of 1260 kJ $m^{-2}$ $yr^{-1}$. Assuming that only 10% of the net production is available for Collembola, about 126 kJ $m^{-2}$ $yr^{-1}$ would be the net influx of energy for Collembola.

The total biomass of Collembola in a soil is about 140 mg $m^{-2}$ (Persson and Lohm, 1977), and two generations $yr^{-1}$ produce about 8 kJ $m^{-2}$ of tissues. The annual respiration, 42 kJ $m^{-2}$ (Persson and Lohm, 1977 and references therein), gives a total demand of 50 kJ $m^{-2}$ $yr^{-1}$. This is less than half of the available mycelial biomass as given above, and the assumption that the habitat of Collembola is productive enough to sustain precise food recognition cues seems to be supported. Moreover, if the excess mycelium is patchily rather than uniformly distributed, which is indirectly indicated (see e.g. Bååth and Söderström, 1982), foraging would be more irregular but also more rewarding when a patch is located. A specialization for locating food items by olfactorial sensory organs at a distance would seem to be advantageous.

There were two objectives for our work: (1) To investigate the possible production of volatile substances by three common species of soil fungi. (2) To determine whether the collembolan species *Onychiurus armatus* can discriminate between the same species of fungi in olfactorial experiments. It was assumed that differences in response to the fungi corresponding to differences in composition of volatile compounds would be evidence for a food preference based on kairomones, compounds that benefit the organism receiving them (Brown *et al.*, 1970).

## MATERIALS AND METHODS

### *Test organisms*

*Onychiurus armatus*, sensu Gisin (1960), was extracted from the litter and mor horizons of an alder-spruce forest using a modified high-gradient extractor (Macfadyen, 1961). The Collembola were cultured in Petri dishes with a bottom layer of a mixture of plaster of Paris and activated carbon (9:1, vol:vol; Goto, 1960). The bottom had two holes fitting plastic feeding cups (dia—15 mm; depth—

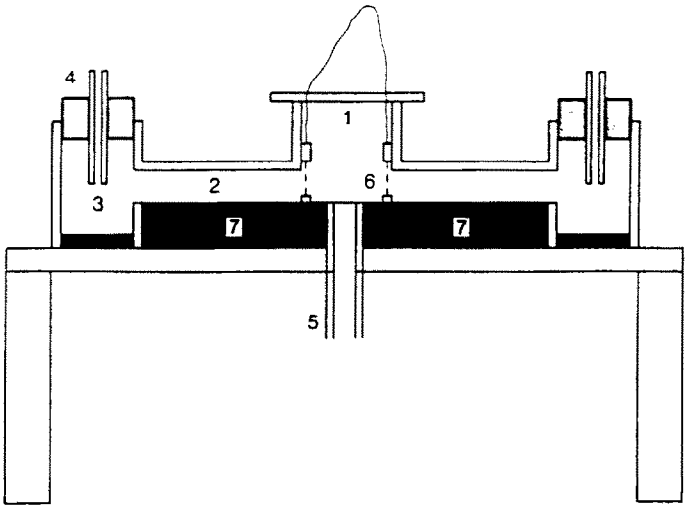


Fig. 1. Olfactometer design. 1. Central chamber. 2. Side arm. 3. Side-chamber. 4. Air inlet. 5. Air outlet to suction pump. 6. Inner ring with plastic net. 7. Plaster of Paris layer.

6 mm) and was kept moist with distilled water. The Collembola were raised on the soil hyphomycete *Mortierella isabellina* (Oudem).

Three species of soil hyphomycetes were used, viz. *Verticillium bulbillosum* (W. Gams and Malla), *Penicillium spinulosum* (Thom) and *M. isabellina*. They are all common in mor of spruce forest soils (Nordgren *et al.*, 1983), where *O. armatus* is also found. Earlier food preference tests had shown that *V. bulbillosum* was highly preferred by *O. armatus*, whereas *P. spinulosum* was less preferred than *M. isabellina* (Bengtsson *et al.*, 1983, 1985).

*Fungal cultures*

The three species of fungi presented to *O. armatus* were grown either in agar or in soil. They were aseptically inoculated on agar (3% agar, 1.5% malt extract, 30 $\mu$g ml$^{-1}$ chlortetracycline) in sterilized cups (cf. feeding cups above) and held at 20°C until the hyphae covered the surface. The soil was obtained from a mor of a spruce forest soil. It was sterilized in packets of 10 g in a 10 MeV electron accelerator with a dose of 45 kGy, aseptically inoculated with a fungal spore suspension and kept at 20°C in sterile glass vials. When hyphal growth was observed on the soil surface, soil and hyphae were aseptically tranferred to cups and immediately used in the experiments.

*Olfactometer*

The olfactometer modified from Pettersson (1970) and Vet *et al.* (1983) was made of plexiglass and PVC and had a layer of plaster of Paris and activated carbon at the bottom of a central chamber and the two arms leading to the odour sources in the side-chambers which were separated from the arms by a fine mesh net (Fig. 1). A suction pump produced a continuous airflow of 3 ml min$^{-1}$ through the olfactometer.

*Experimental design*

All experiments were run in darkness and at constant temperature and humidity (19°C, 70% r.h.). Seven adult *O. armatus* starved for 5–7 days were used in each test. The animals were released in the central chamber. An inner ring prevented them from entering the arms in advance. The odour source was placed in a side chamber, the airflow was opened and the animals were left for 2 h to acclimatize. The inner ring was removed and the animals allowed to the side arms. The numbers entering an arm and approaching the net of its side chamber were determined after 10 min under red light. At least six replicates of each combination of odour sources were run. Fungi were placed in different side chambers from one test to another, and the colonies used were always of the same age and never more than 3–4 weeks old.

The olfactometer was rinsed with distilled water after each test and used only once a day to ensure no carry-over of effects.

*Collecting odours*

Volatile substances were trapped by a 2 mm activated carbon filter in a 7 mm o.d. glass tube. Samples of agar and soil with and without fungi were left on a metal screen in a 25 mm o.d. glass tube. The tube exit was connected to the carbon filter and an air pump. Traces of contaminants in the air were removed by an activated carbon filter ahead of the exposed sample. The trapped volatiles were extracted with 3 × 20 $\mu$l methylene chloride. The samples were kept in 3 mm o.d. glass capillaries at −20°C until analyzed.

The carbon filter in the glass tube was washed in methylene chloride and glass in acetone. The equipment was sterilized by autoclaving at 120°C for 15 min.

*Gas chromatography*

A Varian model 3700 gas chromatograph with splitless injection, a flame ionization detector and a 25 m SE-54 fused silica column was used to analyze the samples. The carrier gas, $N_2$, had a flow rate of 1.5 ml min$^{-1}$. The column temperature was raised from 55 to 230°C at a rate of 8°C min$^{-1}$ and held at 230°C for 12 min. The chromatograms were compared with respect to retention times.

Table 1. Distribution of *O. armatus* in olfactometer performance tests. Each combination was run with 6 replicates and 7 starved adults. Mean and standard deviation are given

| Side chamber combinaton | Number of specimens in side arm | $\chi^2$ | Central chamber (not moved) |
|---|---|---|---|
| Empty, no airflow | 2.0 ± 1.7 | | |
| $\chi^2$ | NS | NS | 2.3 ± 0.5 |
| Empty and airflow | 2.7 ± 1.2 | | |
| Empty and airflow | 2.0 ± 1.1 | | |
| $\chi^2$ | NS | $P < 0.05$ | 3.0 ± 1.1 |
| Agar and airflow | 2.0 ± 0.9 | | |
| Empty and airflow | 2.0 ± 2.6 | | |
| $\chi^2$ | NS | $P < 0.05$ | 3.0 ± 1.0 |
| Soil and airflow | 2.0 ± 2.0 | | |
| Empty and airflow | 1.3 ± 1.0 | | |
| $\chi^2$ | $P < 0.05$ | $P < 0.05$ | 1.5 ± 0.8 |
| *V. bulbillosum* in agar and airflow | 3.3 ± 1.0 | | |

NS = no significance.

*Statistical method*

The observed proportions of individuals in side arm and central chamber were compared with an expected distribution by a $\chi^2$-test. The first comparison was made using the null hypothesis that animals were equally distributed in side arms and central chamber. The side chamber sources, whether air, agar, soil or fungi, were classified as attractive if a significantly higher number of animals had moved into the side arms than stayed in the central chamber. Next the distribution in side arms only was compared, and a side arm source was assumed to be more attractive than another if the number of individuals there was significantly higher than expected. All comparisons were made from at least 6 replicate observations.

## RESULTS

The test animals showed no behaviour to aggregate in response to air flow, pure agar or pure soil (Table 1). With *V. bulbillosum* in one of the side chambers twice as many animals were found in the adjacent side arm as in the central chamber or in the other side arm. From these tests we conclude that *V. bulbillosum* growing in agar attracted *O. armatus*.

When the animals were given a choice between two different fungal species growing in agar, only *V. bulbillosum* attracted them (Table 2). *M. isabellina*, which the animals were successfully raised on, did not evoke any response until grown in sterilized soil (Table 2). It was then more attractive than *V. bulbillosum*, which was ignored by the majority of the animals also when *M. isabellina* was the alternative odour source (Table 2). Between 22 and 33% of the animals remained in the central chamber in all experiments reflecting the possibility that some individuals were in a non-feeding moulting stage (Joosse, 1981).

A fungus grown in agar produced fewer (≤50%) volatile compounds than when grown in soil (Fig. 2), and the lowest number of compounds was recorded from the only attractive species, *V. bulbillosum*. When grown in soil this species emitted most volatiles. Many of the compounds from one fungus were shared by at least one other species, and the chain length of the trapped compounds covered the range between 5 carbon atoms up to 18, as justified from a pure alkane standard. Preliminary structure identification indicates that short-chained alcohols and ketones were present in the odour.

The odour differences between fungal species were both qualitative and quantitative, but the present data were not sufficiently precise to determine the origin of the behaviour differences. If single compounds are indicative of odour differences, then only one compound from *V. bulbillosum* growing in agar (peak 17) and one from *M. isabellina* growing in soil (peak 8) would be considered, since these were the

Table 2. Distribution of *O. armatus* in experiments with fungi grown in agar or in soil. Six parallels with 7–8 adult Collembola were run. Mean and SD are given

| Side chamber combination | No. of animals | Number of specimens attracted by | | | | $\chi^2$-test side arms | $\chi^2$-test side arms + chamber |
|---|---|---|---|---|---|---|---|
| | | *V. bulbillosum* | *M. isabellina* | *P. spinulosum* | Not removed | | |
| | | | In agar | | | | |
| *M. isabellina* vs *P. spinulosum* | 46 | — | 2.1 ± 0.9 | 2.3 ± 0.9 | 2.1 ± 0.4 | NS | NS |
| *V. bulbillosum* vs *M. isabellina* | 43 | 3.8 ± 0.7 | 1.3 ± 0.5 | — | 2.0 ± 0.9 | $P < 0.01$ | $P < 0.01$ |
| *V. bulbillosum* vs *P. spinulosum* | 42 | 3.3 ± 1.0 | — | 1.3 ± 1.0 | 2.3 ± 1.0 | $P < 0.05$ | $P < 0.05$ |
| | | | In soil | | | | |
| *M. isabellina* vs *P. spinulosum* | 40 | — | 2.5 ± 1.4 | 2.7 ± 0.8 | 1.5 ± 1.2 | NS | $P < 0.05$ |
| *V. bulbillosum* vs *M. isabellina* | 42 | 1.7 ± 1.2 | 3.7 ± 1.5 | — | 1.7 ± 0.5 | $P < 0.05$ | $P < 0.05$ |
| *V. bulbillosum* vs *P. spinulosum* | 40 | 2.2 ± 1.2 | — | 2.3 ± 0.8 | 2.2 ± 0.8 | NS | NS |

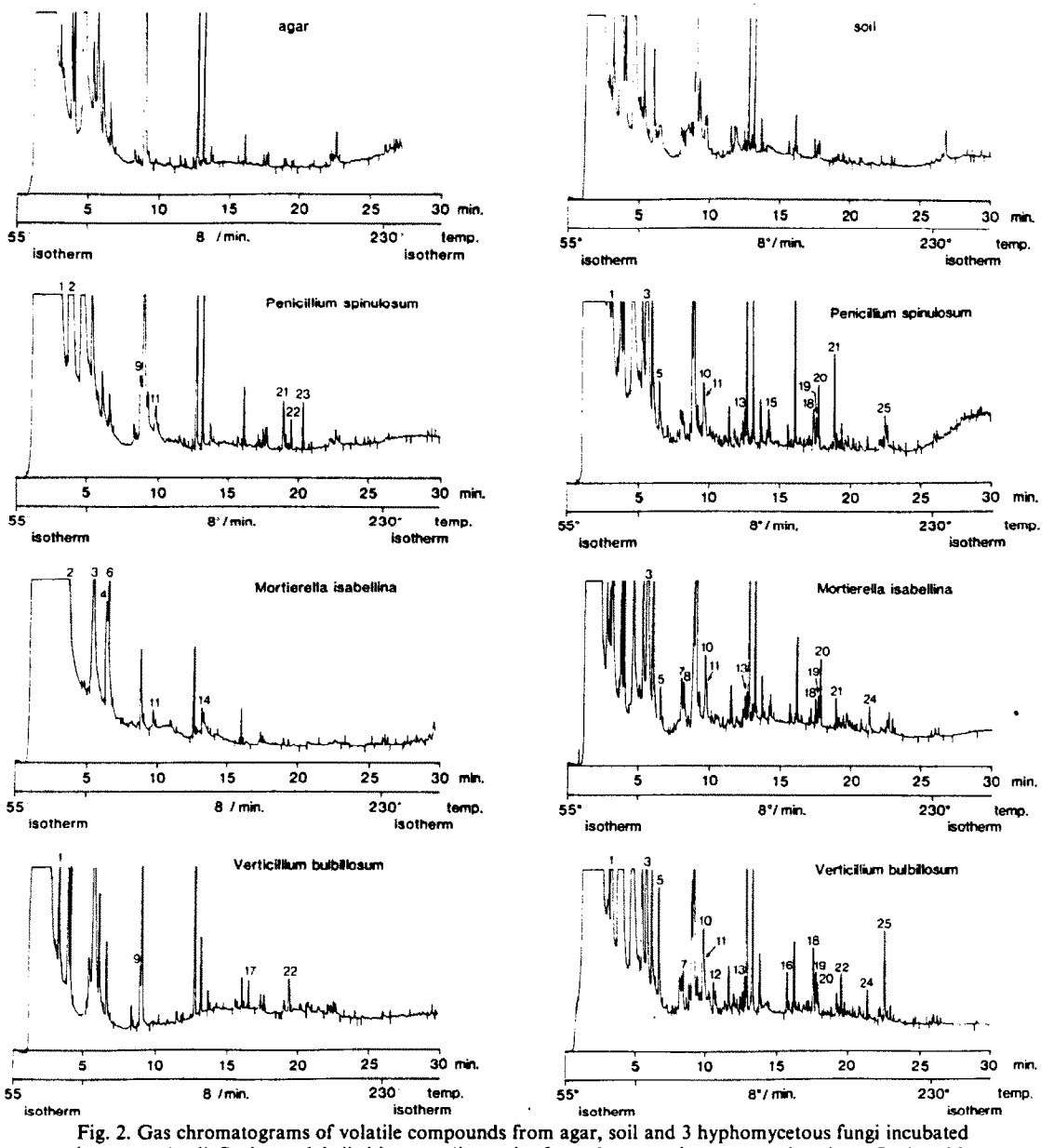


Fig. 2. Gas chromatograms of volatile compounds from agar, soil and 3 hyphomycetous fungi incubated in agar and soil. Peaks are labelled in ascending order from shorter to longer retention times. Peaks with identical retention times (±3 s) were given the same numbers.

only two not shared by other species. The possibility that a combination of compounds produced the attractive behaviour cannot be ruled out.

A typical peak in the chromatograms represented about 2 ng of substance. Considering extraction dilution and sampling procedure we find that about 40 ng of a compound was produced during a week, which is equivalent to about 250 pg $h^{-1}$. The animals were exposed to the odour for 10 min and consequently may have responded to 40 pg of less of the individual compounds. Each patch of mycelium covered 175 $mm^2$ and represented a biomass of about 400 $\mu$g.

## DISCUSSION

Experiments on interactions between microorganisms and soil arthropods have indicated that the animals are not entirely indiscriminate feeders (e.g. Parkinson *et al.*, 1979). How the preference for specific fungal species is maintained has been unclear but most likely the ability of a fungus to attract would be related to its odour, flavour, morphology, nutrient quality etc.

Yeasts and hyphomycetes may produce volatile compounds, including short-chain alcohols and esters, lower terpenes and terpenoids, and acetates (Labows *et al.*, 1979; Saito *et al.*, 1979; Sprecher and Hanssen, 1983; Karahadian *et al.*, 1985), that are potentially attractive or repellent to Collembola. Differences in attractivity may arise from a combination of properties such as the odour concentration gradient and the taste of mycelia or conidia.

Our study shows that at least the odour of fungi is used in food recognition by *O. armatus*. The volatiles

released attract the Collembola, but the attractivity of a fungus is not entirely determined by its odour. The odours from *M. isabellina* and *P. spinulosum* grown in soil were apparently attractive but indistinguishable for *O. armatus*, though the gas chromatograms were not identical. However, given an opportunity to taste, *O. armatus* favours *M. isabellina* over *P. spinulosum* (Bengtsson *et al.*, 1985), although *V. bulbillosum* still remains the most preferred. It is possible that most soil fungi release at least some species specific or quantitatively prominent volatiles, and that the number of combinations of compounds that elicit a quantitative response by the collembola are limited.

The odour is influenced by the growth substrate and a fungal species may be more or less attractive from one soil to another, as illustrated by the movement of *V. bulbillosum* and *M. isabellina* on the ranking scale when a different substrate was used, and the observation that the fewer compounds released from *M. isabellina* in agar were more attractive than the greater number released from the fungus in soil. This indicates that *O. armatus* is sensitive to more than one superior key mixture of volatiles, and that the ranking of food items may vary from one environment to another. This shift in attractivity did not follow from an exchange of one compound or another but rather from an introduction of a greater number of partly shared and partly species specific compounds. *V. bulbillosum* and *M. isabellina* had only one compound each that was unique for growth in agar and soil respectively, so attraction could be determined either solely from these two compounds or from a combination of two or more compounds.

With many key components in the recognition pattern the specificity of the food search behaviour would increase. But extreme food specialization would only pay off when the food concentration is high, and a homogeneously high abundance of one fungal species is rarely found. It seems advantageous to have a general recognition response to palatable fungi, which would reduce the search time in the soil–pore system, in addition to a highly specific response to certain food items. This hypothesis was supported by the observations on *M. isabellina* and *P. spinulosum*, which both became more odoriferous and attractive in soil.

No fungus studied repelled the animals. Rating attractivity purely from the average distribution of animals in the olfactometer may obscure the possibility that some individuals were not receptive to odour from a fungus that attracted the population mean and those that are not significantly attractive. It is clearly advantageous for a fungus to attract animals that can disperse its conidia or mycelium, as long as the mycelium is not overgrazed but the extent of intraspecific variation in host reception of Collembola still remains unknown.

Conflicting reports on the effects of grazing by Collembola on fungal growth have been reported (Addison and Parkinson, 1978; Hanlon and Anderson, 1979; Bengtsson and Rundgren, 1983; Newell, 1984). The balance between grazing and fungal growth may be determined by nutrient supply (Park, 1976; Hanlon, 1981), quality of the fungus (Booth and Anderson, 1979), collembolan population densities (Hanlon and Anderson, 1979), patchy distribution of the fungi (Bengtsson and Rundgren, 1983) and, possibly, by odour composition. Clearly, a better knowledge of the dispersion of attractive compounds in the soil, the arthropod receptor system and the response thresholds would improve the understanding of the foraging behaviour of soil-living Collembola.

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