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SHORT NOTE

# *Tricholoma matsutake* 1-Ocen-3-ol and methyl cinnamate repel mycophagous *Proisotoma minuta* (Collembola: Insecta)

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**Abstract** Two major volatiles produced by the mycelia and fruiting bodies of *Tricholoma matsutake* (1-octen-3-ol and methyl cinnamate) repel a mycophagous collembolan, *Proisotoma minuta*. Aggregation of the collembolans on their diet was significantly inhibited by exposure to 1 ppm methyl cinnamate or 10 to 100 ppm 1-octen-3-ol. The aggregation activity decreased dose-dependently upon exposure to 1-octen-3-ol at concentrations higher than 0.01 ppm. Aggregation in the presence of methyl cinnamate exhibited three phases: no significant effect at concentrations ranging from 0.001 to 0.1 ppm, significant inhibition from 1 to 100 ppm, and strong inhibition at 1,000 ppm. These results may explain why certain collembolan species do not prefer *T. matsutake* fruiting bodies.

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## Introduction

Fungal fruiting bodies are more nutritious than their mycelia (Stark 1972; Cromack et al. 1975; Vogt and Edmonds 1980) and are exploited by various arthropods, including dipteran larvae, collembolans, and acarine and oribatid mites (Hammond and Lawrence 1989). Collembolans are the most abundant group of insects found on agaric fruiting bodies (Yamashita and Hijii 2003), and numbers often reach hundreds to thousands on a suitable fruiting body (Sawahata et al. 2000, 2001, 2002; Nakamori and Suzuki 2005a). Collembolans attack the hymenial area, often consuming 50% or more of it in suitable fruiting bodies (Sawahata 2006). Additionally, collembolans are able to break the hyaline and thin-walled basidiospores of some fungi (Ponge and Charpentie 1981; Sawahata et al. 2001; Nakamori and Suzuki 2005b). Therefore, collembolans are likely to adversely affect spore production by fungal species with these types of spores.

The fruiting body of the ectomycorrhizal fungus *Tricholoma matsutake* (S. Ito and Imai) Sing., which has the common name matsutake, is one of the favorite and most valuable food items produced by forests in Japan because of its characteristic odor and texture, but artificial cultivation of *T. matsutake* has not yet succeeded. Sawahata and Narimatsu (2006) reported that collembolan populations on the fruiting bodies of *T. matsutake* are smaller than those on other mushrooms growing in the same forest. The gut contents of collembolans collected from the fruiting bodies suggested that they fed on the fungal tissue on the gill surface (Sawahata and Narimatsu 2006). In our preliminary studies, *Folsomia*

*candida* Willem and *Proisotoma minuta* Tullberg fed on sliced samples of the fruiting body of *T. matsutake* in a laboratory experiment (data not shown). These findings suggested that the mushroom is not poisonous to collembolans and that it is simply avoided as a source of food when alternatives are available.

Collembolans use fungal volatiles to recognize potential sources of food (Bengtsson et al. 1988; Hedlund et al. 1995). Bengtsson et al. (1991) suggested that the volatile compounds 1-octen-3-ol and 1-hepten can aid the collembolans in their search for a palatable fungus. The major volatiles produced by *T. matsutake* are 1-octen-3-ol and methyl cinnamate (Ohta 1983; Terashita et al. 1991). The former is a primary volatile produced by many species of fungi (Kaminski et al. 1974; Pyysalo 1976), and the latter determines the characteristic odor of this fungus (Terashita et al. 1991). Hence, methyl cinnamate may prevent aggregation and feeding on the fruiting body by some collembolans. Further, high concentrations of 1-octen-3-ol can repel fungivores (Pfeil and Mumma 1993; Wood et al. 2001). The fruiting body of *T. matsutake* contains 1-octen-3-ol at concentrations ranging from 2 to 186 ppm on a fresh-weight basis (Ohta 1983; Terashita et al. 1991), but it is unknown whether 1-octen-3-ol repels collembolans at high concentrations.

The present study aimed at investigating the effects of the major volatile components of *T. matsutake* (1-octen-3-ol and methyl cinnamate) at different concentrations on the aggregation of mycophagous collembolans by biological assay (feeding test). Our goal was to provide an explanation for why collembolans aggregate in relatively small numbers on *T. matsutake* fruiting bodies.

## Materials and methods

*Proisotoma minuta* was extracted from forest soils near the Matsunoyama Museum of Natural Science in Niigata Prefecture (307 m above sea level, 37°05′N, 138°36′E), in central Japan. This species was used because it fed on various ectomycorrhizal fungi in a choice experiment (Schultz 1991; Hiol Hiol et al. 1994), and it is amenable to laboratory culture. The collembolans were reared on dry yeast at 20°C in a cylindrical chamber (11 cm in diameter, 7 cm in height) whose bottom (1.0 to 1.5 cm in thickness) was covered by a mixture of plaster of Paris and charcoal (10:1 *v*/*v*). We followed the methods used in previous food choice experiments (e. g., Schultz 1991; Kaneda and Kaneko 2004). Each assay (with six replications) used 70 individuals (1 month after eclosion from eggs, 0.8 to 1.1 mm in body size) at a time in the same type of chamber.

The major volatile compounds of *T. matsutake* have already been determined to be 1-octen-3-ol and methyl cinnamate by several researchers (e.g., Ohta 1983; Terashita et al. 1991). We substituted laboratory-grade versions of these chemicals for the natural volatile compounds. 1-Octen-3-ol (Wako Pure Chemical Industries, Osaka, Japan) was diluted with distilled water to prepare solutions of 0.001, 0.01, 0.1, 1, 10, 100, and 1,000 ppm. Methyl cinnamate (Wako) was diluted similarly but with dichloromethane. Controls were created using distilled water and dichloromethane, respectively. Into each diluted solution, three pieces of filter paper (3 cm in diameter) were dipped for long enough that the paper became completely saturated. The wet 1-octen-3-ol filter papers were placed 1 cm apart on the bottom of the assay chamber to form a triangle (Fig. 1). The wet methyl cinnamate filter papers were kept in air for 15 min to evaporate the solvent and then moistened with distilled water before bioassay by the same procedure.

On each sample of filter paper, including the control, a plug (1 cm in diameter) of 3% potato dextrose agar medium (a PDA plug) was provided as food (Fig. 1). Seventy insects were introduced into the chamber and held for 90 min in the dark at 20°C; then, the individuals on each PDA plug were counted without disturbance. The results represent means of 18 samples (six replicates of three PDA plugs per chamber) among the dilution series (eight levels, including the control) for the two test compounds. The mean numbers of collembolans in each treatment were compared by *F* test, one-way analysis of variance, and a multiple-range test (Fisher's PLSD). Statistical analyses were performed with Stat View 5.0J software (SAS Institute, Cary, NC, USA).

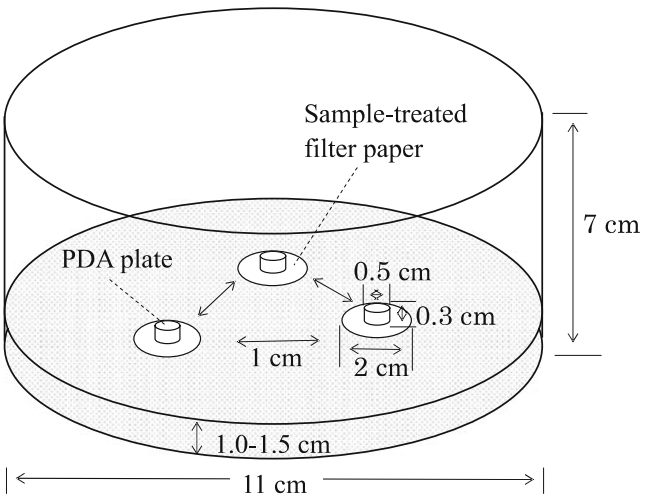


**Fig. 1** Bioassay method used to assess the ability of 1-octen-3-ol and methyl cinnamate to repel the collembolan *Proisotoma minuta* (*n*= 70). Plugs of potato-dextrose agar (*PDA*) were placed at the center of each sample-treated filter paper on a mixture of plaster of Paris and charcoal (10:1 *v*/*v*) at the bottom of the cylindrical chamber

## Results

Both compounds at 1,000 ppm almost completely inhibited the aggregation of *P. minuta* on the PDA plugs (Figs. 2 and 3), leaving means of fewer than 1.0 and 0.5 individuals per PDA plug on methyl cinnamate and 1-octen-3-ol, respectively. Statistically significant reductions in aggregation were also observed at several lower concentrations.

## Discussion

Methyl cinnamate exhibited three ranges of concentrations with different abilities to inhibit the aggregation of *P. minuta* (Fig. 2): no significant effect (a mean of 13.3 to 16.1 individuals per plug) from 0.001 to 0.1 ppm, incomplete but significant inhibition (a mean of 1.2 to 8.2 individuals per plug) from 100 to 1,000 ppm, and strong inhibition at 1,000 ppm. Thus, methyl cinnamate repels collembolans at high concentrations.

Methyl cinnamate has previously been shown to be a principal volatile component of the fruiting body of *T. matsutake* (Terashita et al. 1991), and its content increases during fungal development to reach 22 to 154 ppm on a fresh weight basis by the time the veil breaks (Ohta 1983). High levels of the compound (150 to 360 ppm, with a mean of 236 ppm) are localized in the lamellae and spores (Ohta 1983), where collembolans are frequently observed feeding on the spores and hyphae of the hymenia (Sawahata 2006). The high content of methyl cinnamate in the lamellae may thus prevent aggregation and grazing on gill surfaces by collembolans.

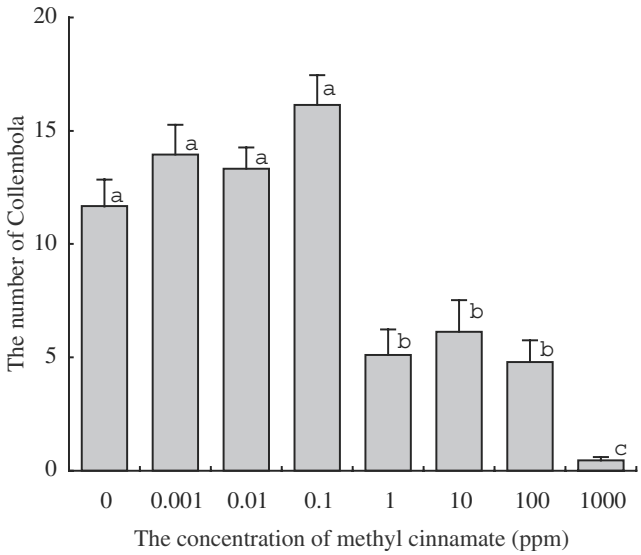


**Fig. 2** Influence of the concentration of methyl cinnamate on the number of *Proisotoma minuta* aggregated to feed on PDA plugs. *Gray bars* indicate the means of 18 samples (six replicates of three PDA plugs per dish), and the *error bars* indicate the standard error (*SE*) of the mean. Means labeled with *different letters* differ significantly (Fisher's PLSD test, $P<0.05$)

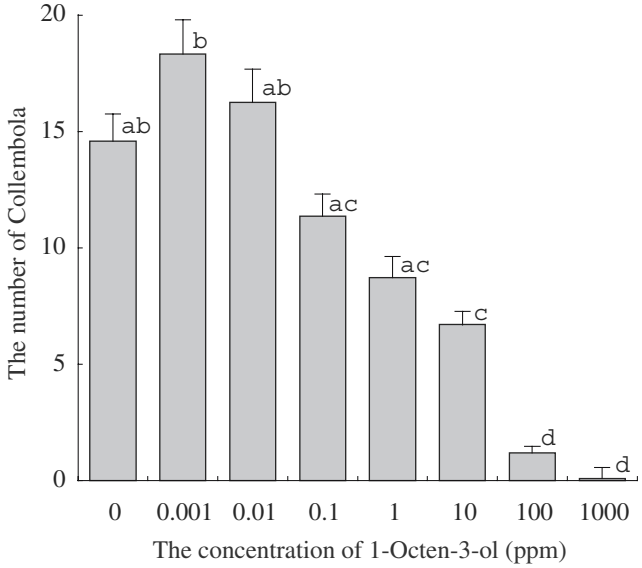


**Fig. 3** Influence of the concentration of 1-Octen-3-ol on the number of *Proisotoma minuta* aggregated to feed on PDA plugs. *Gray bars* indicate the means of 18 samples (six replicates of three PDA plugs per dish), and the *error bars* indicate the standard error (*SE*) of the mean. Means labeled with *different letters* differ significantly (Fisher's PLSD test, $P<0.05$)

1-Octen-3-ol has previously been shown to be another principal volatile component of the fruiting body of *T. matsutake*, at concentrations in the fruiting bodies ranging from 2 to 186 ppm on a fresh weight basis (Ohta 1983; Terashita et al. 1991). This volatile component exhibited a clear dose–response relationship (Fig. 3): As the concentration of 1-octen-3-ol increased from 0.001 to 1000 ppm, aggregation of the insects decreased continuously, and the difference from the control became significant at concentrations of 10 ppm and higher. At concentrations of 100 and 1,000 ppm, 1-octen-3-ol significantly reduced aggregation of *P. minuta* (to an average of 1.2 individuals per PDA plug). These results suggest that Collembola show a different response pattern at different concentrations, with a slight and nonsignificant increase in aggregation compared with the control at concentrations ranging up to 0.01 ppm; at higher concentrations, aggregation decreased significantly (to less than 50% of the control), and at 1000 ppm, aggregation was almost completely eliminated. Bengtsson et al. (1991) also found a weak but nonsignificant attractive effect of 1-octen-3-ol on collembolans (individuals of *Onychiurus armatus* Tullb.) in soil fungi. Further studies with new methods are needed to explain the attractive effect of low levels of 1-octen-3-ol among a range of collembolans.

The present study provides an explanation of why certain collembolans avoid aggregation on *T. matsutake* fruiting bodies in the field. Both major components of the volatiles present in the fruiting bodies of this species

(methyl cinnamate and 1-octen-3-ol) defend against collembolan attack at high concentrations. Wood et al. (2001) reported that 1-octen-3-ol prevents feeding by banana slugs at a concentration of 28 ppm. Pfeil and Mumma (1993) reported that 1-octen-3-ol and 3-octanone seemed to deter gravid females of *Megaselia halterata* Wood (Phoridae) at high concentrations. Therefore, some fungal species may produce high concentrations of 1-octen-3-ol to defend against mycophagous insects.

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