Zeitschrift: Mitteilungen der Schweizerischen Entomologischen Gesellschaft = Bulletin de la Société Entomologique Suisse = Journal of the Swiss Entomological Society

Herausgeber: Schweizerische Entomologische Gesellschaft

Band: 88 (2015)

Heft: 1-2

Artikel: A new trapping method for fungivorous insects

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DOI: https://doi.org/10.5169/seals-514990

MITTEILUNGEN DER SCHWEIZERISCHEN ENTOMOLOGISCHEN GESELLSCHAFT
BULLETIN DE LA SOCIETE ENTOMOLOGIQUE SUISSE
88: 23–28, 2015

Papers of the 8th Symposium on the Conservation of Saproxylic Beetles
Basel (Switzerland), 13–15 June 2014

A new trapping method for fungivorous insects

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A new trapping method for studying insect colonization and succession in ephemeral substrates, such as fungal fruiting bodies, is described. The trap consists of three major parts: a traditional flight-interception trap with a funnel attached to bags with pre-grown host, (in our study – fruiting bodies of the fungi), and a cage protecting fruiting bodies against potential vertebrate intruders. The trap design was tested in natural conditions using laboratory cultivated fungal fruiting bodies of *Pleurotus* sp. (Agaricales, Pleurotaceae). Its efficiency in trapping Coleoptera and Diptera was analysed.

Keywords: fungivorous insects, trap, pleurotoid fungi, Diptera, Coleoptera.

INTRODUCTION

Pleurotoid Basidiomycetes is an ecological group of fungi, sharing characters with mainly wood-inhabiting polypores (Polyporales) and with mainly soil-inhabiting agarics (Agaricales). Fruiting bodies of pleurotoid fungi (Agaricales, Pleurotaceae) and of wood-decaying agarics are found on dead and dying trees, tree stumps and coarse woody debris. Entomologists have insufficiently explored pleurotoids for their associated insect species, contrary to polypores and agarics. This can explain the scarcity of information available on Diptera and Coleoptera colonizing these fungi (Thorn *et al.* 2000). The Oyster Mushroom *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. 1871 and the Summer Oyster Mushroom *Pleurotus pulmonarius* (Fr.) Quél. 1872 are among the commonest pleurotoid fungi in Europe, and these fungi are also widely commercially cultivated. The availability of commercial strains of *Pleurotus* spp. allows growing these fungi using pure cultures. To reconstruct the colonization patterns of Diptera and Coleoptera and their patterns of succession in a shared ephemeral resource, a new insect trap method was designed. The trap was tested in June–July 2014, during a 3 weeks period, in southern Finland, using pure cultures of pleurotoid fungi as a substrate. Here we report the results of the trap construction and its' testing in natural surroundings in southern Finland, and give a short overview of the number of insects obtained in the trap.

MATERIAL AND METHODS

To create the fungal colonization substrate, we grew fruiting bodies of *Pleurotus* sp. in the laboratory. In our study the cultivated mycelium was received from the Fungal Biotechnology Culture Collection (FBCC) of the University of Helsinki, to assure that the mother culture is of Finnish origin. The mycelial strain had been preliminarily identified as *Pleurotus pulmonarius*. However, DNA analysis of the strains confirmed the species identity as *Pleurotus abieticola* R.H. Petersen & K.W. Hughes 1997, a species new to Finland. The processes of growing the mycelium to obtain fungal fruiting bodies for the trap testing and the trap design included three major parts, which are described below.

Growing Pleurotus abieticola

Culturing on agar: In order to grow clean mycelia strains, agar mixtures for Petri dishes were prepared using 20 g agar, 20 g malt-extract for microbiology and 1000 ml of distilled water (Oei 2005). Thirty Petri dishes, filled with agar mixture, were stored for several days at room temperature. In a laminar hood, pieces of the mycelium were aseptically placed inside the Petri dishes using sterilized forceps, and the dishes were closed immediately. The Petri dishes with mycelium were arranged in a basket, and kept at room temperature for the fungal mycelium to grow. They were observed at 2-day intervals and parts contaminated with wild fungi were carefully removed. The mycelia overgrew the agar in 14 days. The two Finnish strains of mother culture that were obtained from the FBCC were strains FBCC 517 and FBCC 520.

Spawn production, inoculation of the substrate: The spawn containers (heat resistant 2 l bottles) were autoclaved for 1 hour under +121 °C (Oei 2005). The wheat grains to be used as growing substrate were boiled in water, drained, and poured into the heat resistant bottles. For each 1000 ml bottle 480 g wheat and 400 ml water were used. The filled bottles were sterilized in an autoclave at +121 °C for 90 minutes. Ten to fifteen squares of 10 x 10 mm of the full-grown agar from a Petri dish of a mother culture were used to inoculate the substrate. The inoculated wheat was kept at room temperature for 10 days, and then placed in cooler conditions of + 4 °C for three days (Peng *et al.* 1990). After 15 days the colonized wheat was used for inoculation of the fungal fruiting substrate, sawdust of deciduous trees, as recommended by Oei (2005).

Inoculation of the fruiting substrate: The sawdust substrate was moistened until water content reached 50 % to 60 % by weight. The sawdust was then sterilized by boiling in water for one hour in a stew pan, then drained and cooled down to room temperature and poured into plastic bags of 20 x 80 cm (approx. 2 l volume). The inoculated substrate bags were hung for three weeks in glasshouses of the Finnish Museum of Natural History Botanical Garden in Kaisaniemi under a constant temperature of +25 °C and 80-95 % humidity. Fruiting bodies started to emerge on average in 12 days (Fig. 1).

Design of the insect trap system

The trap system consisted of three major functional parts: a flight interception trap with a funnel, a cage construction, and fruiting bodies of the fungus *Pleurotus*

abieticola projecting from the perforated bag. The system is a combination of a traditional funnel flight interception trapping method and a sampling scheme described by Komonen (2008) in the colonization experiment using four fungivorous beetle species in a lake-island system.

The functional part of the trap consisted of transparent cross-shaped attached panels (1.5 x 200 x 300 mm), a funnel below the panels, and a plastic 500 ml collection container connected to the funnel. The funnel was fastened with wire to the cage construction at opposite sides of the middle part of the cage walls. On one side of the collection container there was a small window space covered with a net to

Fig. 1. Laboratory grown fruiting bodies of *Pleurotus abieticola* (b and c) and used fungivorous insect trap system (a and d).

drain the rainwater (see Kaila 1993). An ethanol aqueous solution of 70 % was used to preserve insects in the 500 ml container. The cage design protected the fruiting bodies from potential vertebrate fungivorous intruders, e.g. birds. The cage was made of hexagonal multi-purpose metal fence wire wrapped around three wooden poles (length 160 cm each) and was mounted with 2 nylon buckles to each of the poles at the top and bottom part of the cage (Fig. 1d). A bag with the fungus was placed inside the cage and fastened to the upper part of the metal wire fence with a string (Fig. 1a).

Trap checking and sorting of material

The trap was tested during the summer of 2014 in forests in the Helsinki (southern Finland) area: Tuomarinkartano, Viikki arboretum and Puolarmetsä. A total of 12 traps located in groups of four were placed in about 150 m distance from each other. The traps were checked at intervals of two days during a three-week period, in June–July 2014.

The trap checking protocol included:

Diptera on and around the traps were collected with an insect hand net and transferred to 1.5 ml collecting tubes with 70 % alcohol and labelled accordingly.

Coleoptera individuals were directly collected from the external surface of fungi by visual inspection.

Fungal fruiting bodies were detached and placed on a horizontal 1 m^2 plastic mat and Coleoptera from the fungi were gently removed with the help of an aspirator into 1.5 ml collecting tubes.

Coleoptera from fungi were preserved in 70 % alcohol in 1.5 ml Eppendorf tubes, separately from larvae for further morphological identification and labelled accordingly.

The plastic collection container was removed and emptied into a plastic bag with the liquid preserving agent.

Date, time, place of collection, decay stage of the fruiting bodies as well as the number of the trap were recorded.

The used equipment included a plastic mat, aspirator, insect hand net, a waterproof marker pen, and plastic bags with sealing and 1.5 ml Eppendorf tubes with 70 % ethanol. Diptera and Coleoptera from the plastic collection container were sorted into separate tubes and labelled accordingly. Sorting Diptera and Coleoptera was done in the laboratory under a stereomicroscope under low magnification.

Sorting took one to one and a half hours per trap. Operating 12 traps (in three groups of four traps) in parallel was feasible and sufficient for a single person to check and sort the material of one group of traps every third day. Additionally, in case of extra large catches and shortage of time for sorting, the collecting liquid with insects could be placed in a freezer until sorted.

PRELIMINARY RESULTS

The sampling process (12 traps in 3 study sites operated in parallel for 23 days in two rounds) yielded over 650 specimens of Coleoptera and 110 individuals of Diptera. All Coleoptera and nearly half of the Diptera individuals were associated with

pleurotoid fungi according to previous rearing records and findings of Jakovlev (1994), Ševčík (2006, 2010) and Schigel (2008). No previous records were available on insect species colonizing *Pleurotus abieticola* from Europe. The identified insect material will be analysed in the master thesis of the first author.

DISCUSSION

The field test proved that funnel flight interception traps, including the Kaila trap (Kaila 1993), have a low efficiency in catching adult Diptera. First identification results showed presence of a high number of accidentally occurring species of Diptera, presumably attracted by the partly decaying sawdust in the bags. In the experiment, we focused on fungivorous insects, i.e. those using fungal fruiting bodies as breeding hosts for larvae. We propose to compensate for identification and rearing challenges with comparing larval and adult DNA barcodes to the BOLD systems sequence reference database (Hebert *et al.* 2003). In cases were the nature of fungus-insect relationship cannot be disclosed by explorative or experimental approaches, literature, expert knowledge, and DNA barcode libraries remain the key sources of information on species taxonomy and ecology. In order to improve the specificity of the trap catch we recommend to increase the frequency of trap checks and we support the suggestion of Dodelin *et al.* (2011) to reduce the size of funnel flight interception panels. The reduction of the size of the funnel flight interception panels, attached to the fruiting bodies in the trap system, have been proposed to increase the catch number of fungivorous Coleoptera.

Our trapping method proved to be efficient in the collection of especially fungus-associated Coleoptera, but also Diptera. This trapping system allows for (1) a host fungus or some other uncolonized substrate to be chosen according to the research purpose; (2) a large number of adult insects (both host-specific fungivorous species and accidental species) can potentially be collected, (3) a combination of different host fungi in different traps could be used in similar studies, for comparisons of fungal host specificity of the insect catch.

ACKNOWLEDGEMENTS:

The authors warmly thank the curator of the Fungal Biotechnology Culture Collection (FBCC) of the University of Helsinki, Pekka Oivanen, for his help in growing the fungus. Special thanks go to Igor Andriushchenko for his help with constructing the trap. We thank Jiří Schlaghamerský, Sylvie Barbalat and Adrienne Frei for the constructive and helpful reviews of the mansucript. The Master's Thesis project of the first author was supported by Societas Biologica Fennica Vanamo, Societas pro Fauna et Flora Fennica, Societas Entomologica Helsingforsiensis and Dmitry S. Schigel's research by the Academy of Finland, project 257748.

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(received January 20, 2015; accepted March 31, 2015; published June 30, 2015)