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A wind tunnel olfactometer of novel design: Testing the response to substrate volatiles on a vertical gauze screen

RALPH S. PETERS AND RUDOLF ABRAHAM

(with 1 figure)

Abstract

We present an olfactometer of novel design for studies on the response of insects to substrate volatiles. The general principle is a wind tunnel containing a vertical gauze screen within a horizontal airflow. We tested the response of hymenopteran parasitoids (Chalcidoidea: Pteromalidae) to odours of their hosts and their hosts' habitats. It was shown that there are significant intra- and interspecific differences.

Advantages of this new apparatus compared to olfactometers known so far include minimisation of enforcements on the tested specimens, uniformity in texture and airflow and the possibility to an elaborated quantification and assessment of the olfactory reaction.

K e y w o r d s: searching behaviour, olfactory reaction, parasitoid Hymenoptera, laboratory experiments, olfactometer, methods.

Introduction

The olfactory response to a certain substrate is a central component in the life history of a variety of insects. For example, olfactory cues are important for the location of food resources, to locate mating partners, and to find oviposition sites, e.g., the hosts of parasitoids.

Olfactometer studies have been conducted on numerous species. The most commonly used olfactometer type is the Y-olfactometer (e.g., Stafford et al. 1984, Sereno & Neves 1994, Chuche et al. 2006). The tested individuals have a choice between two substrates in the Y- or T-shaped end. The airflow olfactometer (Vet et al. 1983, Schröder 1997) and the static air olfactometer (Vet 1983) are enhancements of the Y-olfactometer, where two or four odours are presented. In the olfactometers used by Sreng (1993) and by Müller et al. (2006), the tested specimens walk on a horizontal plain located above the odour sources. Although useful for specific questions and experiments all these olfactometer types show some disadvantages if the response of parasitoid Hymenoptera to host and host habitat associated olfactory cues is under investigation. Our

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goal was to design a type of olfactometer which does not exhibit these difficulties in our parasitoid host location studies. With the newly designed apparatus we refined testing conditions and were thus able to improve the quality of results. The conception of this olfactometer was inspired by the wind tunnel studies of Vogel (1969) and Jones et al. (1981). The basic structure was presented in Schlein (2002) and a modified version was used in Peters (2007) and Peters (submitted).

Since experimental setups like this may be of general use we show the general construction and the recorded values for the evaluation of species responses, and we discuss the advantages of this vertical gauze screen olfactometer and possible modifications for studies on other insect taxa.

Material and methods

DESCRIPTION OF THE OLFACTOMETER

The main body of the olfactometer is a transparent acrylic glass tube with a total length of 65 cm and an inner diameter of 19 cm. The test chamber (Fig. 1,b) length is ca. 25 cm. A vertical gauze screen functions as the testing screen (Fig. 1,c). The testing plain gauze is fixed to a plastic ring fitting closely against the main body tube of the olfactometer.

The substrate tube rack consists of two glass tubes with a length of 35 cm and an inner diameter of 1.95 cm. The tubes are fixed on the rack at an angle of 180°. The substrate tubes (Fig. 1,d) can be rotated within the main body tube. The openings of the substrate tubes touch the vertical gauze screen of the test chamber, so that two substrate sectors (Fig. 1,e) can be seen on the screen in frontal view.

A small fan (10 cm diameter) is fixed at a distance of about 70 cm from the olfactometer. The centre of the olfactometer tube, the centre of the substrate tube rack and the centre of the fan are on the same axis. The fan produces an airflow (Fig. 1,f) moving through the substrate tubes and the main body into the test chamber and leaving through the gauze cover. The airflow speed was ca. 0.4m/s inside the test chamber.

The gauze cover (Fig. 1,a) can be removed to clean the test chamber and to insert and remove animals before and after testing.

GENERAL PROCEDURE AND RECORDED VALUES

In our studies we tested two hymenopteran parasitoid species, *Nasonia vitripennis* (Walker, 1836) and *Dibrachys cavus* (Walker, 1835) (Chalcidoidea: Pteromalidae). Test substrates included, e.g., dipteran host specimens and host habitat material. One of the test substrates was placed in one substrate tube, whilst the second tube was used as an empty control. Parasitoid females were inserted into the olfactometer test chamber. To evaluate the reaction of females on the substrate sectors, two values were recorded: (1) the number of klinotaxis events and (2) stay duration.

(1) Whenever a female left a substrate sector and returned within the next seven seconds, this was counted as one klinotaxis event. The female must have left the sector by at least her full head size. The seven second timespan for klinotactic return was defined in pretests for our parasitoid study species. The number of klinotaxis events can be counted as the number per time span or as the number after the first contact and the crossing of a sector border.

(2) Stay duration was measured as time spent within one sector. Measurement

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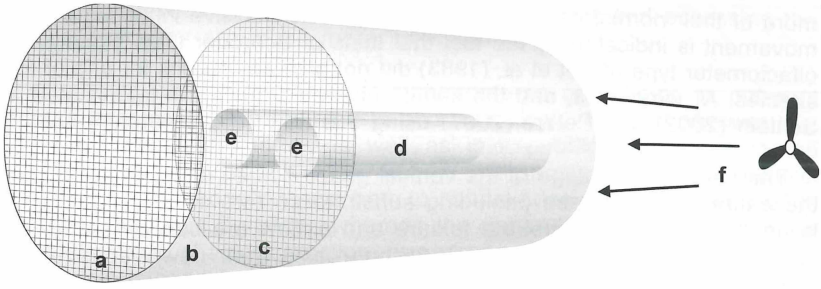

Fig. 1. Olfactometer. **a**. gauze cover; **b.** test chamber; **c.** vertical gauze testing screen; **d.** substrate tubes; **e.** substrate sectors (openings of substrate tubes on back side of testing screen); **f.** airflow.

was started when a sector was entered. If the female left the sector, the timing was stopped but was then continued if the specimen returned within the next seven seconds (klinotaxis). If a specimen flew off a sector during measuring, the measurement was not used and was repeated.
During testing, the substrate tubes can be rotated to exclude possible side preferences by the specimens. After half of the tests, the substrate material was exchanged. One tube was constantly used in all tests as the empty control tube. After every change of substrate or species, the olfactometer was cleaned with 70 % ethanol.

Results and discussion

For a minimum biased data recording on the reaction of insects to olfactory cues the used olfactometer has to fulfil some prerequisites: (1) minimization of artificial effects and enforcements on the tested specimens, (2) uniformity in texture and airflow in the testing area, (3) possibility to an elaborated quantification and assessment of the olfactory reaction.

The aim was to design an olfactometer that fulfils these prerequisites and is advantageous compared to olfactometers known so far. Our efforts have resulted in the general principle of a wind tunnel olfactometer containing a vertical gauze screen within an airflow.

The first main advantage of the olfactometer described here, especially when compared to classic Y-olfactometers (e.g., Stafford et al. 1984, Steinberg et al. 1992, Chuche et al. 2006), is that the specimens can walk freely on the testing screen and can leave and enter the sectors whenever they like. They are not forced into small-sized tubes or containers. The two olfactometers of Vet (1983) and Vet et al. (1983) also allow the specimens to walk freely on rather large testing areas, but these are still small when compared to the large testing chamber of the olfactometer described here, where the specimens can not only walk freely on the screen but can leave the gauze by flight or by walking and can thus show

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more of their normal behavioural repertoire. The decisive influence of free movement is indicated by the fact that tests of Schröder (1997) using the olfactometer type of Vet et al. (1983) did not give any results for one of the species, *N. vitripennis*, and the same substrates that were later tested in Schlein (2002) and Peters (2007) using this new olfactometer.

The second advantage of the vertical gauze screen olfactometer is that the entire testing screen (including substrate sectors and no-cue areas) is uniform so far as substrate texture and airflow are concerned. In the studies of Sreng (1993) on cockroaches using their newly constructed olfactometer, the tested specimens were able to differentiate between the gauze on the substrate tubes and the solid texture next to these, and between the airflow areas in the odour fields and the missing airflow in the rest of the system. Airflow is also a problem of the Y-olfactometer-type which was already discussed in Vet et al. (1983): There are turbulences and mixing of airflows in the area where the tubes meet, exactly at the point where tested individuals have to decide where to go.

The olfactometer of Müller et al. (2006) is more similar to our design. But their four-chamber olfactometer is not a wind tunnel or an airflow olfactometer as it only uses diffusion for volatile transportation.

The third advantage of the newly designed olfactometer is the possibility to record stay duration and number of klinotaxis events. These two values are known to picture the reaction of a specimen under the influence of a cue (Van Alphen & Vet 1986). With repeated measurements, they can be used in statistical analysis to consolidate results. The stay duration indicates the intensity of occupation under the influence of a cue. The klinotaxis is a directed movement towards a cue. In this case it is the return of the tested specimen after crossing and leaving a sector, which at first is coincidental. This is important for the measurements of stay duration as well. A stay only interrupted by leaving and a klinotactic return has to be considered as one single stay.

In Y-olfactometers and in the modified version of Wylie (1958) as well as in flight tunnels such as those in Jones et al. (1981) and Steinberg et al. (1992) and basically also in the olfactometer of Vet (1983) the responses of the tested specimen have to be counted as binary decisions (e.g., “tube a“ or “tube b“, „flight“ or „no flight“). In the olfactometer presented here there is the possibility of a more differentiated evaluation of the reactions, with the described values “stay duration“ and “klinotaxis events“.

In the olfactometer of Müller et al. (2006) the odour sectors merge in each other, so that leaving and entering sectors for klinotaxis measurements (also affecting the stay duration measurements, see above) are difficult to assess. In the apparatus of Sreng (1993), the odour fields are placed in the corners and the tested specimens cannot leave them in all directions, again making klinotaxis counting difficult. The specimens might also use the corners of the olfactometer for thigmotaxis, thereby confounding results on the olfactory reaction. By separating the odour (or control) fields and by

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placing them surrounded by odour-free areas testing and assessing the wasps' reactions are much more improved.

Our tests on the response of hymenopteran parasitoids to different odours associated with their hosts and their hosts' habitats showed that there are significant intra- as well as interspecific differences in the wasps' reaction to certain substrates. *N. vitripennis*, a habitat specialist on birds' nests, strongly reacts both to habitat cues (nest material) and to its cyclorrhaphous fly hosts, whereas the generalist *D. cavus* exhibits a generally weak olfactory reaction. The strong reaction of *N. vitripennis* to nest material and host puparia is expressed in high medians of stay durations (14/7 seconds (nest material/host puparia)) and numerous klinotaxis events (571/243 within 120 minutes). For *D. cavus* the strongest recorded reaction to one of the substrates tested (earwig faeces) was a median stay duration of 3 seconds and 138 klinotaxis events. Detailed results and implications for host location mechanisms and parasitoid biology will not be discussed in this general methodical paper and can be found in Schlein (2002), Peters (2007) and Peters (submitted).

The olfactometer and the procedure of the experiments can be modified. The time for return used in the klinotaxis countings has to be reconfigured for each species to be tested. The size of the substrate tubes or of the total olfactometer can be modified, as well as temperature and light conditions. To attract positively phototactic parasitoids to the vertical gauze screen, in our studies a bulb was set between olfactometer and fan, at about 5 cm from the olfactometer, and the sides of the test chamber were wrapped in black cardboard. If species are known to be positively geotactic or generally walk on the ground, the complete apparatus can be turned with the gauze screen becoming horizontal but still at right-angles to the olfactometer tube and the airflow. Furthermore, the number of odours/substrate tubes can be modified to range from two to more. Through modifications like these the vertical gauze screen olfactometer could contribute to the study of various other insect species.

Zusammenfassung

Es wird ein neu konstruiertes Olfaktometer vorgestellt, mit dem die Reaktion von Insekten auf Substratreize untersucht werden kann. Das Grundprinzip ist ein Lufttunnel, in dem eine vertikale Gazefläche in einem horizontalen Luftstrom steht. Wir testeten die Reaktion von parasitoiden Hymenopteren (Chalcidoidea: Pteromalidae) auf Gerüche ihrer Wirte und ihrer Wirtshabitate. Es wurde gezeigt, dass signifikante intra- und interspezifische Unterschiede bestehen.

Die Vorteile des neuen Apparates verglichen mit bisher bekannten Olfaktometern beinhalten die Minimierung der Zwänge, die auf die getesteten Individuen einwirken, die Gleichmäßigkeit der Oberfläche und des Luftstroms sowie die Möglichkeit, die olfaktorische Reaktion besser quantifizieren und bewerten zu können.

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Authors' addresses:

Dr. R. S. PETERS, Zoologisches Museum Hamburg, Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany, (Email: ralph.peters@uni-hamburg.de);

Prof. (emer.) Dr. R. ABRAHAM, Zoologisches Museum Hamburg, Universität Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany.

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