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An olfactometer for analyzing olfactory responses of death-feigning insects

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Abstract
The present paper describes a novel Y-track olfactometer for the analyses of olfactory responses of insects which are easily disturbed and then feign to be dead. The olfactometer has been demonstrated to be useful for the analyses of olfactory responses of the vine weevil, *Otiorhynchus sulcatus* F.

Key words: Olfactometer; vine weevil; *Otiorhynchus sulcatus*

INTRODUCTION

It is difficult to introduce death-feigning insects into an olfactometer as they are immobilized for quite some time after being touched. In such cases a special olfactometer is needed, where the insects themselves start the experiment by moving inwards without any force from outside.

The vine weevil, *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae), is a polyphagous insect and one of the most serious insect pests in nursery stock and small fruit production worldwide (Moorhouse et al., 1992). Pickett et al. (1996) observed weevil aggregation in the field during daytime and suggested the presence of an aggregation pheromone as cellulose fiber refuges, previously occupied by adult weevils, stimulated aggregation by other weevils in comparison to new refuges. However, the olfactory responses of the weevils to conspecific odors have not been tested.

In the present paper, we describe a novel olfactometer for death-feigning insects like the vine weevil and report the olfactory responses of these weevils to conspecific individuals.

MATERIALS AND METHODS

Insects. Weevils were collected in June 1997 on the Research Station at Boskoop, The Netherlands in a field with yaw (*Taxus baccata*) and spindle tree (*Eunonymus fortunei*), and were kept in 3-liter pots at 22°C in a climate room under long-day conditions (LD=16 : 8 h). Weevils were fed with *T. baccata*. After each test weevils were returned to the stock population, so that they could be re-used in tests that took place at least a week later.

Olfactometer. The Y-track olfactometer (Fig. 1) consisted of a brass Y-track (vertical bar 4.5 cm long; horizontal base track 13 cm; Y-arms 5.5 cm each; all diameters 4 mm) and two glass air outlet tubes (14 cm long; internal diameter 2 cm). The air outlet tubes were connected to wash bottles (500 ml) with plastic tubing. The airflow of 20 cm/s on each side was purified by passing through a charcoal filter and then passed through two wash bottles (first one for humidification by passing air through water, the second one for the odor samples). The olfactometer was placed in a black box with a halogen lamp (12 V DC, 10 W) in the ceiling to illuminate the Y-junction. Light intensity was set to 0.3×10^{-3} W/m^2. Prior to the experiments weevils were deprived of food for 24 h and tests were conducted at 25°C during the light period of the weevils.

A weevil release container (Fig. 1B, 12 cm in diameter, 5 cm high) was placed on the top of a height adjustable lab jack (Fig. 1C). Thirty to 40 weevils were released into the container, in which a mound was formed from wet sand that enabled the weevils to reach the vertical bar of the Y-track olfactometer. The inner wall of the release container was painted with Fluon® to prevent the weevils

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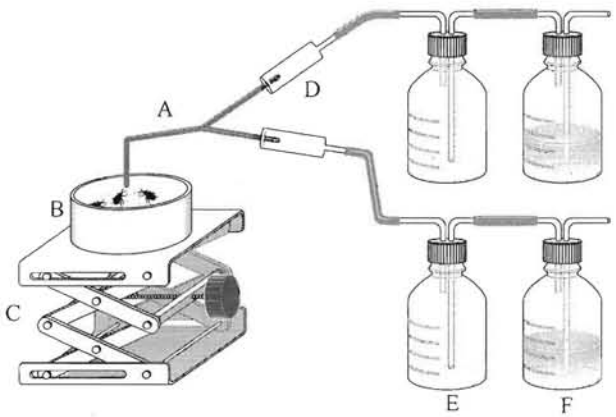

Fig. 1. A schematic diagram of the Y-track olfactometer. A: Y-track, B: weevil release container, C: a lab jack, D: air outlet, E: a wash bottle for the odor samples, F: a wash bottle for humidification.

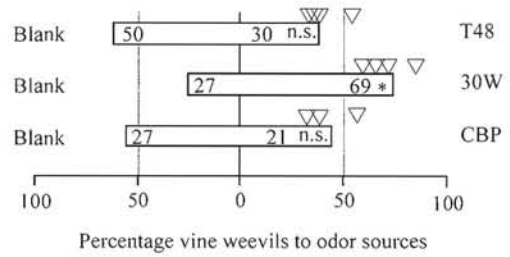

Fig. 2. Response of *Otiorhynchus sulcatus* to conspecific odors in the Y-track olfactometer. The numbers in the bars are the absolute numbers of weevils that made a choice for the sample. The triangles represent the percentage of weevils in each replicate that was attracted to the sample odors. T48: airborne weevil volatiles collected for 48 h by the absorbent Tenax; 30W: odor from 30 living weevils in the sample wash bottle; CPB: cardboard paper exposed to 30 weevils for 24 h. Choices between odor sources were analyzed with a binomial test (* $p<0.05$).

from climbing the walls. When one of the weevils started climbing up the vertical bar of the Y-track, the lab jack was moved down in order to detach the vertical bar from the mound in the container. In the rare case that, at the same time two weevils climbed up the bar, the weevils were omitted from the test. After every six individuals tested, treatment and control sides were interchanged in order to prevent any positional bias.

Odor source. In the first experiment 8 g of cardboard paper was exposed to 30 weevils for 24 h and, subsequently, the cardboard paper were placed in the sample wash bottle (treatment CBP). The next experiment involved that 30 living weevils and 0.6 g of facial tissue were placed in the sample wash bottle (treatment 30W). In the third experiment 60 beetles were placed in a 500 ml glass bottle and the volatiles were trapped on Tenax®-TA (122 mg) by aeration at 400 ml/min for 48 h. The Tenax® was extracted with 1 ml of *n*-hexane and *n*-hexane solution was concentrated by the nitrogen gas flow. Twenty female-equivalent of the hexane extract was applied onto a filter paper in the sample wash bottle (treatment T48).

Statistics. The results for each test day were considered a replicate and at least three such replicates were used in the statistical analyses. A binomial test was used to analyze the weevils' choices in the olfactometer.

RESULTS AND DISCUSSION

When both wash bottles did not contain any sample, no preference of the vine weevils was observed for one of the arms of the Y-track olfactometer ($n=53$).

Odor from 30 living weevils in the sample wash bottle (30W) attracted significantly more conspecific individuals than the empty wash bottle. However, cardboard paper exposed to 30 weevils for 24 h or volatiles collected on Tenax® for 48 h did not elicit significant olfactory responses (Fig. 2).

Although cellulose fiber refuges previously occupied by adult weevils stimulated aggregation by other weevils (Pickett et al., 1996), cardboard paper exposed to 30 weevils did not elicit any olfactory response. Odor from 30 living weevils attracted significantly more weevils than clean air. van Tol et al. (2004) reported that the frass from the *O. sulcatus* and *O. salicicola* attracted *O. sulcatus*. So it is possible that the frass elicits the olfactory responses of the weevils in the present experiment (see Fig. 2, treatment 30W).

The olfactometer described here was suitable to analyze olfactory responses of the vine weevils. This olfactometer was also applied with success to analyze the olfactory response of a trogossitid predatory beetle which also respond on disturbance or touching with death-feigning (Nakamuta, unpublished). It is, therefore, suggested that this olfactometer is applicable to other insect species having a habit of death-feigning.

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