AN OPEN Y-TRACK OLFACTOMETER FOR RECORDING OF APHID BEHAVIOURAL RESPONSES TO PLANT ODOURS

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Summary

In order to test plant odours and their components on the behavioural responses of aphids, we designed an open Y-track olfactometer. The olfactometer was constructed from a brass rod positioned vertically, divided in two arms at the top. Glass tubes were slipped over the two arms that directed a clean air flow and an air flow loaded with odour towards the Y-junction. An aphid walked in a few minutes to the Y-junction and chose between control and odour sides. In this olfactometer winged *Aphis fabae* aphids (alate virginoparae) preferred the arm with broad bean plant odour. The olfactometer is easy to construct, fast to use and can readily be adapted to other insect species.

INTRODUCTION

The development of new strategies for aphid pest control which are based on the application of semiochemicals, involves the following series of research steps: (1) the screening of plant volatiles by recording aphid electroantennogram (EAG) responses, (2) the evaluation of the compounds selected in step one, for their effects on aphid behaviour, and (3) the evaluation of semiochemicals, as slow-release formulations, under field conditions. In the first phase, the EAG screening, we have studied four aphid species in detail, namely the vetch aphid *Megoura viciae* Buckton, the black bean aphid *Aphis fabae* Scop., the peach-potato aphid *Myzus persicae* (Sulz.) and the cabbage aphid *Brevicoryne brassicae* (L.) (Visser *et al.*, 1996). We have looked, in addition, for other biological variables affecting EAG response profiles, such as aphid clones, different aphid forms and the effect of the food source on which aphids have been fed (Visser & Piron, 1997). From these studies a list of promising semiochemicals has been compiled for further behavioural studies.

Several behavioural bioassays have been reported for testing of aphid semiochemicals: the four-armed olfactometer designed by Pettersson (1970), the linear-track olfactometer (Isaacs *et al.*, 1993) and the locomotion compensator (Visser & Taanman, 1987). Although all these instruments have proven their value in aphid semiochemical research, we decided to design a new instrument with two main features, *i.e.*, easy to build and fast to use. For that reason we constructed an open Y-track olfactometer, which satisfied "easy" and "fast" and can readily be adapted to other insect species.

MATERIAL AND METHODS

Aphids

A clone of *A. fabae* originally established by Kennedy was cultured on broad bean plants *Vicia fabae* L. under long-day conditions (L16:D8) at 20-22 °C. Under these conditions the aphids reproduced parthenogenetically (virginoparae). Young winged aphids were collected and starved for at least 4 hours prior to the experiments.

Olfactometer

An open Y-track olfactometer was constructed from a brass rod (diameter 1.6 mm) positioned vertically, its length measured to the Y-junction 14 cm, the base fixed in a cork, and the rod divided in two arms at the top (see Figure 1). Glass tubes were slipped over the two arms of the rod and directed continuously two air flows towards the Y-junction. At the air flows used (10, 15 or 20 cm/s) smoke tests showed a clear boundary between left and right air flows. The air flows have been purified by passage through moisture and charcoal filters (Chrompack 7971 & 7972). Silicone tubing connected

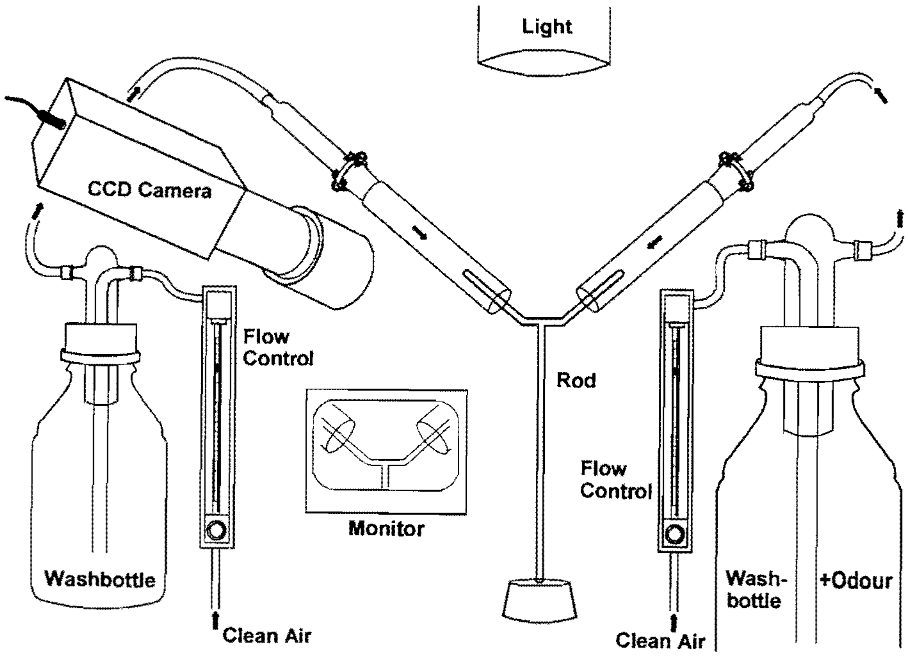

Figure 1. Open Y-track olfactometer. The aphid is placed on the base of a vertical rod and starts walking upward. At the junction it will choose between a clean air flow, here at the left, and an air flow loaded with plant odour, shown at the right. Subsequently the aphid is removed and the experiment can be repeated with another aphid.

washbottles, one to each arm of the olfactometer. The whole setup was placed in a black box and only one halogen lamp (4-12 V DC, 10 VA) in the centre of the ceiling of the box illuminated the olfactometer from the top. The light intensities used are indicated in the legend of Figure 3.

Experiments

The odour was applied at one side of the olfactometer by filling one washbottle with 12.5 g cut broad bean plants. In order to prevent humidity differences between left and right arms, each washbottle contained 25 ml demineralized water. An individual winged *A. fabae* aphid was gently placed on the base of the rod and allowed to walk upward. At the junction the aphid could choose between the control arm (just humidified) and the odour arm (humidified with broad bean plant odour). Choices were scored when the aphids left the horizontal part of the rod walking towards the glass tubes. A video camera (Sony SSC-M370CE) and monitor (Panasonic TC-1470Y) assisted the observations. In each series, half of the number of aphids was tested with odour coming from the left and the other half with odour coming from the right as to compensate for a possible left-right asymmetry of the setup. All experiments were conducted at 20-22 °C.

RESULTS AND DISCUSSION

The design of a behavioural bioassay in order to test the responses of aphids, or other insects, towards plant odours and their components, involves the consideration of the stimuli generally involved in locomotion. For aphids it is known that they are attracted towards light, like to move upward, easily walk on sticks, and that for winged aphids a vertical position delays take-off (Binns, 1977). The precise shape and size of an olfactometer and characteristics of the surrounding, including background colour, light intensity and distribution, is determined by the proper balance of all stimuli involved in locomotion so that the behavioural response towards odour is favoured. As aphid species, and other insect species, differ in their responsiveness to different stimuli, each species needs special adaptations in olfactometer design and surroundings. The optimizing of design and experimental protocol further involves the appreciation of motivational status as insects deprived of food are more likely to respond to food odours, and respond differently, than just after being fed (Thiéry & Visser, 1995). The innate physiology deserves attention as well since winged aphids have been reported to go through a migratory phase before paying attention to host plant stimuli like colours (Nottingham & Hardie, 1989).

The construction of the open Y-track olfactometer includes all these considerations. At the base of the rod, which is situated in a dark surrounding with just one light from above, the aphid readily walks upward through the combination of light, gravity and the form of the substrate (see Figure 2). At the junction, on the horizontal part of the rod, the aphid slows down as the input of gravity is now neutral, and light is for the main part not in line with the direction of the substrate. Here the aphids were observed to touch the rod with their mouth parts in order to probe the substrate. Also antennal waving is more intense and aphids have to choose one or the other side. When at one side of the olfactometer the odour of broad bean plants is blown, winged aphids choose this side of the olfactometer (Figure 3). The orientation mechanism underlying the preference for the odour arm probably involves the discrimination of odour difference between left and right (tropotaxis), although a temporal comparison of stimulus intensities cannot be excluded (klinotaxis; see for the terminology on insect orientation: Visser, 1988).

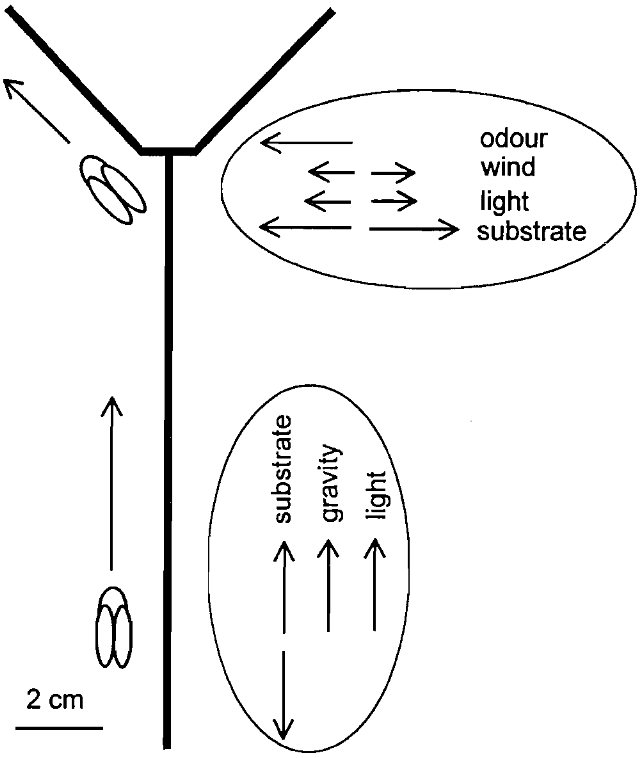

Figure 2. *Illustration of the stimuli involved in aphid locomotion on the open Y-track olfactometer. At the base of the rod gravity and light will direct the movement upward (negative geotaxis and positive phototaxis, respectively) guided by the substrate. At the junction geotaxis is excluded while wind, light and substrate are balanced between left and right arms. Odour-loaded air from one side initiates a preference for orientation.*

The odour of broad bean plants was on the average preferred by 65% of alate *A. fabae* aphids tested (Figure 3, experiment T, $P<0.01$). The responses varied from one experiment to the other, from 50% (Figure 3, experiment 2) to 84% (experiment 3). This variation originated probably from (a) the varying physiological state of the aphid groups used, and (b) the interaction of group size and data distribution. It takes for 77% of winged *A. fabae* aphids less than 3 minutes to reach the junction. Despite the variation observed, the open Y-track olfactometer allows efficiently the bioassay of plant odours as in less than two hours a group of 25-30 aphids can be tested and analysed statistically.

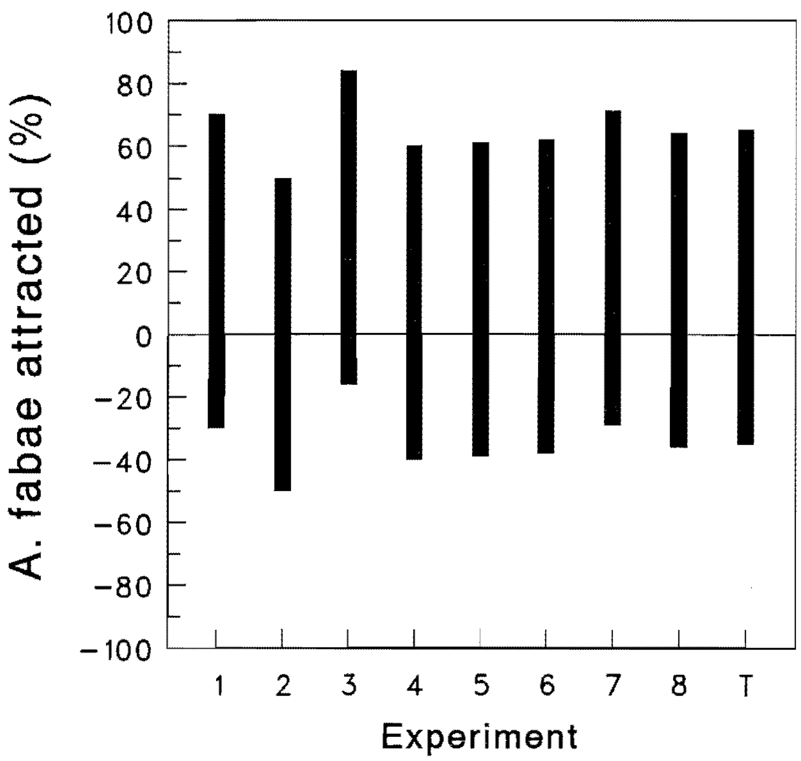

Figure 3. *The preference of winged* Aphis fabae *for an air flow containing broad bean plant odour. The experiment was repeated eight times (1-8) and in total 194 aphids were used (T). At the base of the rod the light intensity was 100 lux in experiments 1-5, and 27.5 lux in experiments 6-8.*

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