

# The Effect of CO<sub>2</sub>, Sweat, Chemical Vapours and Air on *Simulium ornatum*: Implications for Control

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## Abstract

Studies on the response of the blackfly *Simulium ornatum* s.l. (Diptera Simuliidae) to carbon dioxide (CO<sub>2</sub>), acetone, 1-octen-3-ol and air was conducted in the laboratory using a Y-tube olfactometer. The blackflies were found to exhibit a high degree of activity in the olfactometer and responded to the various odours. The results showed that CO<sub>2</sub> and 1-octen-3-ol were attractive to the flies at low concentrations (< 1% CO<sub>2</sub> & < 2.5% 1-octen-3-ol) and repellent at high concentrations (> 2% CO<sub>2</sub> & > 4% 1-octen-3-ol). Humidified air was found to be an attractant, and dry air a repellent. Acetone at low concentrations (< 0.2%) did not appear to have any effect, while at higher concentrations (> 1%) it repelled. The results indicate that the use of attractive odours could be beneficial in reducing vector biting of hosts if used in areas where the host is present.

## Introduction

Laboratory investigation of the orientation responses of insects, especially Diptera, to odours have generally involved olfacto-meters of various designs, such as Y or T tubes (Giannakakis & Fletcher, 1978). Orientation response is a stage in the host location behaviour of blood-feeding flies, and is influenced by several factors such as colour, shape, size and host odours. Using a Y-tube olfactometer, Read *et al.* (1970) concluded that *Delia rapae* locate their aphid host by a combination of odours emanating from the aphid host plant and visual searching for the aphid. Similar investigations of the host searching behaviour of syrphid parasitoids using a T-tube olfactometer showed that aphid odours are important cues in host location (Rotheray, 1979).

It was reported that *Aedes aegyptii* (mosquito) females kept in a CO<sub>2</sub>-free atmosphere oriented to an olfactometer port through which human odour-laden air passed in preference to one through which clean air passed (Smith *et al.*, 1970). With a choice chamber olfactometer, Laarman (1955) found that the vapour from citrated rabbit blood was over twice as attractive to *Anopheles atropavus* as that of physiological saline. Willis (1947) used an olfacto-meter to show that the vapour from the arm and hand of a human was nearly twice as attractive to *Aedes aegyptii* and almost three times to *Anopheles quadrimaculatus* as moist air.

Investigations of the responses of tephritid fruitflies (*Anastrepha suspense*) to odour in an olfactometer showed that male *A. suspense* release pheromones that attract females (Nation, 1972). In a similar experiment in a T-tube olfactometer, male German cockroaches were shown to prefer the side contaminated with their own faeces to a clean one (Kitamura *et al.*, 1974).

The adult blackfly, *S. ornatum* s.l., are collected using sweep nets, aspirators, etc. while *S. damnosum* s.l. are collected using human bait. These methods of collection are not only a health hazard exposing the trapper to risk of disease, but costly and inefficient, and often required skilled labour which may not be available in the long term.

The successful use of odours in collection of mosquitoes, tsetse flies and many other insects suggested that the use of odour could be a fruitful avenue to explore for the blackfly. The study was, therefore, initiated to determine the effects of odours in the host-seeking behaviour of *S. ornatum* using different techniques. The paper presents and discusses the responses of the fly to CO<sub>2</sub>, 1-octen-3-ol, air and acetone using an olfactometer.

## Materials and methods

### Study area

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*Simulium ornatum* larvae and pupae were collected from a stream in Blean-y-cwm nature reserve (National Grid Reference SS 910704) Cwm Nash, Wales.

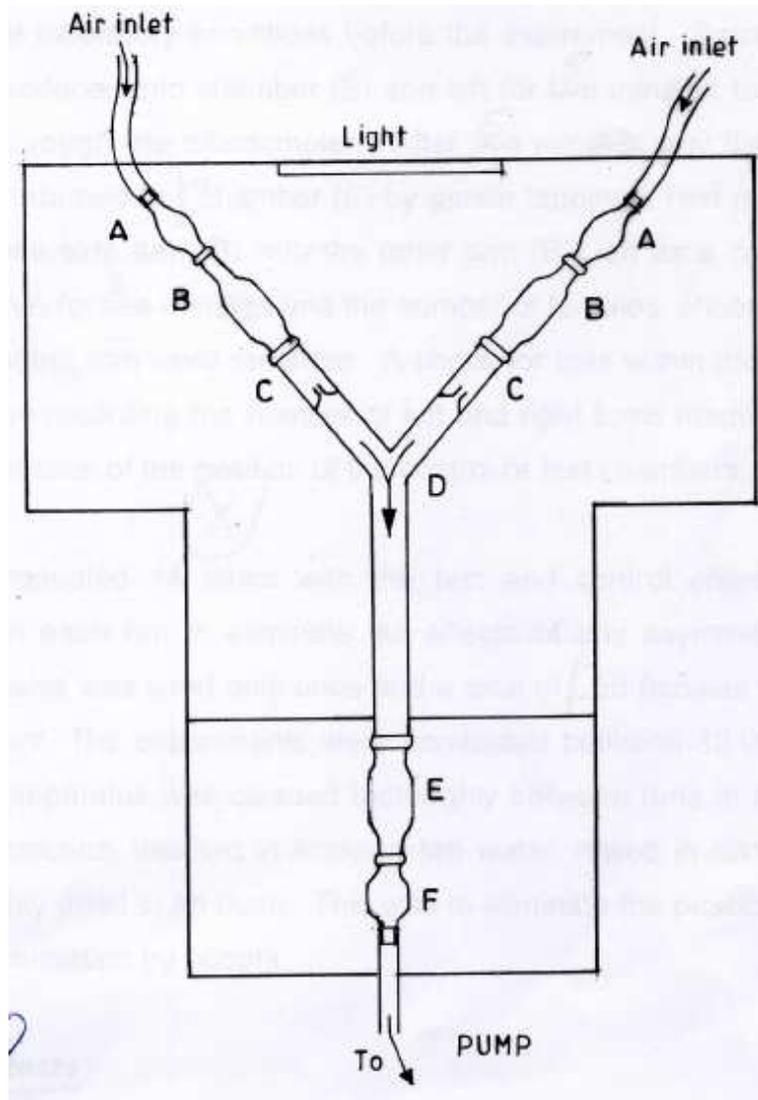
*Laboratory rearing of experimental insects*

The larvae were identified using Davies (1968) Key. They were reared in a variety of aquarium tanks at  $21 \pm 1$  °C and a photo-period of 12:12 with dawn at 06 h and dusk at 18 h GMT. Adults were maintained in mesh cages (25.5 cm × 37 cm) and subjected to the same temperature and photoperiod as the immature forms, and to  $55 \pm 5\%$  relative humidity. Adults were fed on 10% sucrose solution.

Female flies were randomly selected from maintenance cages at 3 days old for use in the experiment. They were deprived of sucrose solution for 18–25 h prior to testing.

*Description of Y-tube olfactometer*

The plan of the olfactometer is shown diagrammatically in Fig. 1. The olfactometer was constructed entirely from detachable heat-resistant glass tube sections each connected by airtight cone and socket joints. The glass tubes have an internal diameter of 1.5 cm, and stem and the arms of the Y junction were all 13 cm in length.



- A = Chamber containing activated charcoal
- B = Test and control chambers
- C = Gates to prevent females returning down Y-tube
- D = Y-tube
- E = Introduction chamber
- Direction of Air flow

Fig. 1. Diagram of Y-tube olfactometer

In operation, the terminal section (F) was connected by a tube to a small diaphragm pump (Model DA7, Charles Austen Pumps Ltd, Surrey, England) which draws air through bubbler bottles filled with water into the olfactometer. Passage through these bottles tend to raise the relative humidity of the incoming air. Air entering each side arm passes through a chamber (A) containing activated charcoal and then into the chamber (B) containing the test material in one arm with the other empty as a control. In each arm of the tube was a gate (C) to prevent flies returning after making a choice. The airflow through the system is regulated through flow meters with needle valves.

The assembled olfactometer was enclosed in a box partitioned into two compartments, the first containing the stem of the Y-tube and the second the arms. Both compartments, were covered by a lid. A fluorescent light in the second compartment attracts the flies to move up the stem of the Y-tube into the arms. Flies were introduced into chamber (E). The apparatus was designed to test the choice of the flies from two alternatives. If significantly more flies moved into one arm than the other then this was taken to indicate that the fly was responding preferentially to that alternative.

Each experiment consisted of two sets of trials: (a) with empty test chambers to ensure there was no bias for either arm, (b) with test material in one arm. Flies for a particular day's experiment were kept in the laboratory for an hour to acclimatize to the laboratory conditions before the experiment. Batches of four females were introduced into chamber (E) and left for 5 min to settle with only air flowing through the olfactometer. After 5 min any flies that had moved out were returned to chamber (E) by gentle tapping. Test material was introduced into one side arm (B) with the other arm (B') left as a control. The experiment was run for 2 min and the number of females choosing either the test or the control arm were recorded. A check for bias within the apparatus was carried out by recording the number of left and right turns made during the experiment, irrespective of the position of the control or test chambers.

Each test was repeated 14 times with the test and control chamber being changed between each run to eliminate the effects of any asymmetry. Each batch of four females was used only once and a total of 56 females were used in each experiment. The experiments were conducted between 10.00–17.00 h GMT. The apparatus was cleaned thoroughly between runs in a sulphuric acid/dichromate solution, washed in scalding tap water, rinsed in distilled water and then thoroughly dried in an oven. This was to eliminate the possibility of trail following or contamination by odours.

#### *Carbon dioxide tests*

CO<sub>2</sub> from a cylinder was introduced through flowmeters with needle valves into the airstream entering one arm of the Y-tube. The amount of CO<sub>2</sub> entering the system could be measured and adjusted by the flowmeter. Levels were introduced to give increases of 1%, 2% and 4% over the normal 0.04% in the air stream.

#### *Human sweat tests*

Pads of cotton wool soaked with the natural sweat obtained from the forehead (sudor) and from the underarm (sebum) of the experimenter were placed in the test chamber and air allowed to blow over it.

#### *Chemical vapour tests*

Dilutions of acetone and 1-octen-3-ol in hexane were introduced *via* glass capillaries with inner filaments (G.C.F. 150F-10 Clark Electromedical Instruments, Reading, England) and the amount of chemicals presented was measured by computing the volume loss per minute due to evaporation. The volume loss can be obtained by multiplying the length of chemical loss along the capillary by the area. The capillaries were of 1.5 mm outer diameter, 0.86 mm internal diameter and variable lengths.

#### *Air tests*

Dry air was introduced by drawing air through silica gel into the test chamber. The control chamber had moist air flowing through it.

The results were analysed using a chi-squared analysis with the null hypothesis being that equal numbers of insects should choose the test and control chamber.

## **Results**

The control tests for bias demonstrated that for all tests the choice between the left and the right chamber of the Y-tube olfactometer was not significantly different ( $P > 0.05$ ) (Table 1).

TABLE 1  
*Behavioural responses of S. ornatum in an olfactometer when offered choices between odours and control*

Comparison No.	Odour compared	No. of flies responding	Chi-square
1	1% CO <sub>2</sub> ; None	39; 17	$P < 0.05$
2	2% CO <sub>2</sub> ; None	16; 40	$P < 0.05$
3	4%; None	16; 40	$P < 0.05$
4	Sweat (Sebum); None	39; 17	$P < 0.05$
5	Sweat (Sudor); None	16; 40	$P < 0.01$
6	0.16% 1-octen-3-ol; None	25; 31	$P > 0.10$
7	1% 1-octen-3-ol; None	31; 25	$P > 0.10$
8	2% 1-octen-3-ol; None	38; 18	$P < 0.05$
9	2.5% 1-octen-3-ol; None	38; 18	$P < 0.05$
10	4% 1-octen-3-ol; None	17; 39	$P < 0.001$
11	0.2% acetone; None	30; 26	$P > 0.05$
12	1% acetone; None	14; 42	$P < 0.05$
13	2% acetone; None	17; 39	$P < 0.01$
14	Dry air; Humidified air	17; 39	$P < 0.001$

#### *Carbon dioxide test*

The results of the different levels of CO<sub>2</sub> tests (Comparison No. 1–3) indicated that at 1% CO<sub>2</sub> increase more flies chose the test chamber ( $P < 0.05$ ). At a 2% increase significantly less flies chose the test chamber ( $P < 0.05$ ) and at 4% increase still significantly less flies were recorded in the test chamber ( $P < 0.05$ ).

#### *Human sweat tests*

The results of the sweat tests (comparison No. 4–5) showed that the test conducted with sweat from the underarm (sebum) revealed more flies responding to the test chamber ( $P < 0.05$ ). However, sweat from the forehead (sudor) attracted significantly less than the control chamber ( $P < 0.01$ ).

#### *Chemical vapours tests*

*1-octen-3-ol.* The results of the tests with 1-octen-3-ol at different dilutions (Comparison No. 6–10) indicated that 1-octen-3-ol at dilutions of 0.16%, 1%, 2%, 2.5% and 4% in hexane were all tested at a release rate of 0.12 μl/min. At 0.16% and 1% dilution the choice between the test and control chambers were not significantly different at ( $P > 0.10$ ). However, at 2% and 2.5% dilutions, significantly more choices were made towards the test chamber ( $P < 0.05$ ) compared with the control, while at 4% dilution significantly less flies chose the test chamber ( $P < 0.001$ ).

*Acetone.* The results of the tests with acetone at different dilutions (Comparison No. 11–13) of 0.2%, 1% and 2% in hexane at a release rate of 2.3 μl/min indicated that at 0.2% dilution the choice between chambers was not significantly different at ( $P > 0.05$ ). At 1% and 2% dilution significantly less flies chose the test chamber ( $P < 0.05$ ).

*Air test.* The results of the air tests (Comparison No. 14) showed that when the flies were given a choice between a dry airstream and a moist airstream significantly more chose the moist airstream ( $P < 0.001$ ).

### **Discussion**

Olfactometers have been widely used to study olfactory attraction in mosquitoes and other insects. The experiments indicated that *S. ornatum* females respond to carbon dioxide, 1-octen-3-ol, humidified air and

acetone in an olfactometer. The study revealed the sensitivity of the fly to odour and demonstrated that they utilize the odours both as an activation and orientation stimuli when host searching. The test concentrations of the odours chosen reflect the range of release rates from potential hosts of *S. ornatum*.

Studies by Basin *et al.* (2000) have shown that a red deer releases 0.3–0.6 l/min of CO<sub>2</sub> while a cow emits 0.75–2.5 l/min CO<sub>2</sub>. Turner (1971), however, reported that choice chamber olfactometers have been universally unsuccessful with tsetse flies, and suggested that to make a success of this technique probably required a high degree of activity in the insects themselves. Contrary to this assertion, the olfactometer was quite suitable, since the blackfly exhibited a high degree of activity and most responded to the airflow or odours.

The activation threshold of *S. ornatum* to CO<sub>2</sub> is not known, but it could be comparable to other haematophagous insects such as the stable fly (*S. calcitrans*), the tsetsefly (*G. morsitans*) and the diurnally active mosquito (*A. aegyptii*) which ranges from 0.006% to 0.03% (Schofield *et al.*, 1997, Bursell, 1984, Healy & Copland, 1995). The olfactometer results show a clear attraction of the *S. ornatum* to the CO<sub>2</sub>. Fallis & Raybould (1975) and Moore & Noblet (1974) have also shown that a number of simuliids are attracted by CO<sub>2</sub>. Maximum attraction occurred at 1% increase and repellent at 2% and 4% increase, respectively.

The attraction of CO<sub>2</sub> at low concentrations and the repellent effect at higher concentrations were also reported by Thomas *et al.* (1985) for *Hydrotae irritans*. They found CO<sub>2</sub> was attractive at a range 30 ml/min but not at 1000 ml/min. It has been shown in a number of instances that higher concentrations of odours are repellent, for example in tsetseflies (Vale & Hall, 1985b). This may be due to saturation of receptor cells inhibiting further response.

The attraction of simuliids to CO<sub>2</sub> is in line with other experiments using similar methods which show that blood-sucking flies are attracted to CO<sub>2</sub> (Willis & Roth, 1952; Brown *et al.*, 1951; Laarman, 1955; Bar-zeev *et al.*, 1977). The sebum sweat was found to be attractive, while sudor sweat had a repellent effect. Brown *et al.* (1951) tested armpit sweat in dilutions of 0.3 mg/cc, 3 mg/cc and 30 mg/cc against water in an olfactometer on *A. aegyptii*. No significant attraction was shown at 0.3 mg/cc, but 3 mg/cc and 30 mg/cc were significantly more attractive than water with 3 mg/cc consistently attracting about 25% more than water alone. Parker (1948) also found armpit sweat to be more attractive than water alone to *A. aegyptii*. They also tested sudor obtained from the forehead but this showed no significant attraction at any of the three concentrations.

Human sweat is of two kinds, sudor which is produced by simple glands over the general body surface, especially the forehead and palms of the hand, and sebum produced by complex glands particularly in the armpits and in inguinal regions. The sudor is an aqueous solution of inorganic salts such as NaCl<sub>2</sub> together with the non-colloidal organic compounds present in blood. Sebum, on the other hand, contains lower fatty acids, esters of higher alcohols, cholesterol and some albumin (Brown, 1956). The sebum sweat, probably on account of its variable components, did make some impact while the sudor sweat did not make any impact. This could be attributed to how well the odorous materials are released and perceived by the flies.

The experiments also showed that 1-octen-3-ol was unattractive at low concentrations of 0.16% and 1% but attractive at concentrations of 2% and 2.5% and repellent at higher concentrations. Acetone was also unattractive at low concentrations and repellent at higher concentration. Studies have, however, shown that 1-octen-3-ol and acetone can increase net movement of *C. impunctatus* in a Y-tube olfactometer (Basin *et al.*, 2000). Both acetone and 1-octen-3-ol have been found to be attractive to the tsetsefly (Hall *et al.*, 1984; Vale, 1980). Vale & Hall (1985b) increased flies catches of *Glossina morsitans moritans* and *G. pallidipes* by up to three times by releasing 1-octen-3-ol at 0.5–50 mg/h or acetone at 5–500 mg/h near traps. Acetone, a highly volatile chemical, at a release rate of 2.3 ul/min was probably too high a concentration and above the threshold for being attractive.

The responses obtained with the various odours in the experiments indicate that they were dose dependent and that the responses persisted to a point and, thereafter, an arrestment or inhibition set in. Dry air was clearly shown to be repellent and humidified air strongly attractive to the flies. Brown (1956), in his studies on factors which attract aedes mosquitoes to humans, reported that a moist airstream was 3–5 times as attractive as a dry one, a robot in wet clothing was 2–4 times as attractive as one in dry clothing and a moistened sphere 5–7 times as attractive as its dry counterpart.

The results showed that 1-octen-3-ol and CO<sub>2</sub> were attractants at low concentrations and repellents at high concentrations. Dry air and sweat (sudor) were repellents and sweat (sebum) an attractant. Acetone was unattractive at low concentration and repellent at high concentration.

The principal conclusion of the study is that greater benefits may be obtained by using these attractants to attract the flies in the field into traps, and by so doing divert flies from biting their host.

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