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A Simple Insect Cage-Olfactometer.

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THE difficulties surrounding studies on the chemotropic behaviour of insects conducted under field conditions have been appreciated by many entomologists. The conflicting factors ever present in such work often prevent a clear picture of the insects' reactions being obtained. The desirability therefore, of studying the behaviour of insects under laboratory controlled conditions has been stressed by many investigators. This applies very notably to the sheep blowfly problem involving as it does an understanding of the olfactory responses of the several species of flies.

The object of this paper is to describe for the benefit of other workers a simple type of cage-olfactometer which, with modifications may be used for studying the olfactory responses of various species of insects.

While many olfactometers have been devised their manipulation often demand so much preliminary preparation that, for testing large numbers of chemicals too much time is required. The writer feels therefore that, for the examination of a large number of chemicals for attractiveness or otherwise as simple a device as possible should be used.

The apparatus now being used for olfactory studies of sheep blowflies, particularly *Lucilia cuprina* Wied., has been evolved from one originally described by Ripley and Hepburn (1929) in their work on Natal fruit fly (*Pterandrus rosa* Ksh.). This was subsequently improved by them for the same investigation but it was never described. The apparatus in its present form differs in some mechanical features from its undescribed prototype.

The original apparatus was designed primarily to obtain rapid results on the olfactory quality of a large number of substances to fruit flies (*P. rosa* Ksh.) and, in the present investigation, the writer is using it in a similar connection with sheep blowflies particularly *L. cuprima* Wied.

DESCRIPTION OF THE APPARATUS.

The apparatus consists essentially of a fly container or cage and two lateral trapping units carrying the odoriferous substances. The cage (Fig. 1) is made of a wooden framework 35 cm. high, 30·5 cm. deep, 30·5 cm. wide, which is covered with cloth gauze. Cloth gauze is used in preference to wire mesh because flies damage themselves by flying against wire mesh and there is a tendency for toxic substances to be produced by flies salivating or defecating on the metal. A cloth gauze sleeve (*a*) (Fig. 1) is sewn onto the covering of the trap in the mid front of the cages. Through this the flies are introduced to the cage and also a Petri-dish (*b*) containing a cotton pad soaked in water. 13 cm. from the top of the cage on either side is a wooden bar (*c*) in the centre of which is a hole (*d*) 2·2 cm. in diameter through

which the flies can pass into the trap or catching unit (*e*) (Fig. 2). The size of this aperture is determined very greatly by the type of insect being studied. It has been found that, with a vigorous and active insect like *L. cuprina*, the aperture must be small in order to reduce random catching as much as possible. The middle portion of each of these cross bars has been shaped so that it shields the mouth of the trap thus allowing the greater portion of the odour within the unit to escape through the entrance hole into the cage, while a relatively small amount escapes around the edges. In addition, any reflection from the glass traps is greatly minimised from shining into the cage, thereby reducing the possibility of creating a visual attraction to the flies. In early experiments with this apparatus the traps were covered on the tops and bottoms with brown paper, but later this was discontinued as it apparently made no difference to the results.

Fig. 1

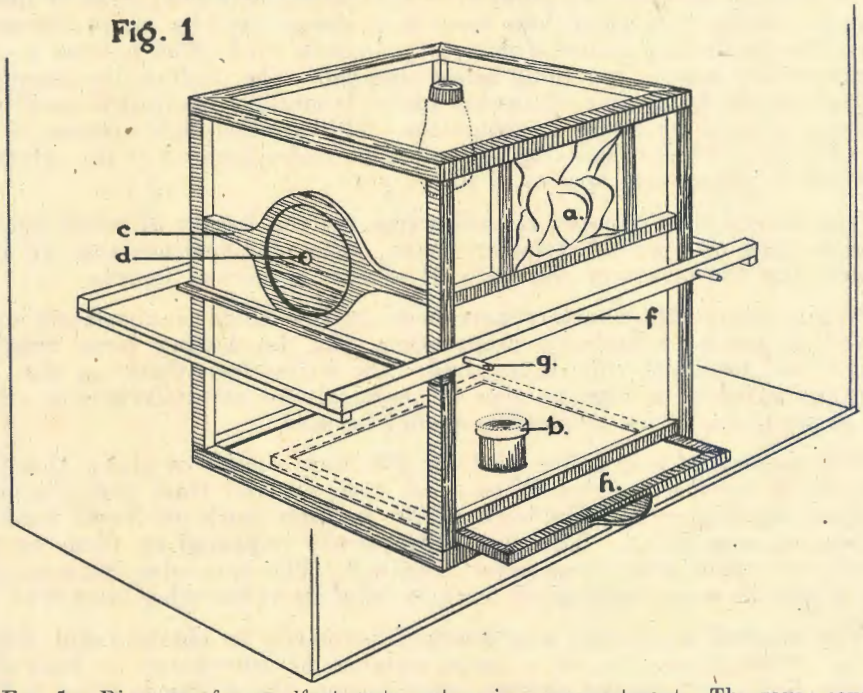


FIG. 1.—Diagram of cage-olfactometer set up in a compartment. The gauze covering is not shown. The trough is shown projecting; in the course of an experiment this is closed. The traps (Fig. 2), when in use, are placed on the supporting frameworrk f on etiher side to cover d. (Drawn by Miss G. E. Laurence.)

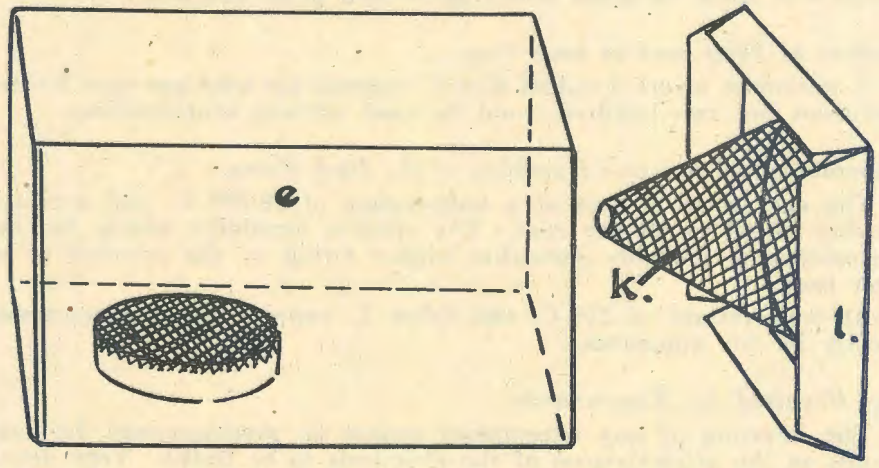
There are two traps which are placed one on each side and supported by means of an outer wooden framework (*f*. Fig. 1), 56 cm. long and 34·5 cm. across, fastened to the cage by four removable pegs (*g*). The traps rest horizontally on this framework with the open ends in contact with the cross bar (*c*).

On the base of the cage is a removable tray (*h*) made of plywood. This enables one to remove the dead flies. The bottom of the tray is covered with a loose sheet of white blotting paper. In the centre of the tray is a Petri-dish containing a pad of cotton wool soaked in water and from the centre of the top of the cage a strip of cotton wool soaked in water, is suspended.

Glass specimen jars (e. Fig. 2.) 14·2 cm. long, 10·5 cm. wide, and 8·4 cm. deep, are used as traps or fly catching units. The mouths of the jars are fitted with removable metal lids (*l*) into which have been fastened wire mesh cones (*k*) the apices opening within the traps. The basal diameter of each cone is 6·5 cm., the height 6·7 cm. and the diameter of the hole in the apex 0·8 cm. The cone must be fitted so that the apex is opposite the centre of the entrance hole (*d*. Fig. 1) to the trap. The corners of the metal lids are cut away so that the edges may be bent inwards when necessary to increase the tension thus permitting them to fit closely to the glass traps.

The odoriferous substances to be tested may be put into small Petri-dishes or glass weighing bottles or any suitable container. The containers must be screened with a mesh cover to prevent the trapped flies having access to the chemicals. Especially in the testing of liquids it is undesirable for the flies to get into them or to feed on them. The presence of flies in a bait may conceivably introduce upsetting olfactory factors. Furthermore, if the flies can feed readily on the chemical they may thus set up certain stimuli which might give a result not necessarily an olfactory one.

Fig. 2.



The apparatus is set up in a compartment in a dark room the temperature and relative humidity of which can be regulated. It would be an advantage if the dark room could be air-conditioned as this would prevent air pollution by gases given off from the chemicals in the traps. If several cages are to be used simultaneously they should be put in separate compartments in the dark room. The compartments can be made by dividing up a wall bench with asbestos boards or other suitable material. The walls and roof of each compartment should be uniform in colour preferably with a non-reflecting surface. The open fronts of the compartments are fitted with roller blinds which are drawn during the experiment. If possible, the blinds should be opaque having the inner surface the same colour as the inside of the compartment. Each compartment is illuminated by means of a frosted vacuum electric light (25 Watts) suspended 35 cm. above the top of the cage. A vacuum bulb is used in preference to a gas filled one for it produces relatively little heat.

Care must be taken to centre the cage in the compartment so that the intensity of the light is equal on either side. A convenient size for the compartment is 70 cm. wide, 70 cm. deep and 90 cm. high. In preliminary experiments with this apparatus two electric lamps, one on each side, were used but there seemed no advantage in this as one central lamp sufficed.

The Manipulation of the Apparatus.

The condition of the material.

It has been found that *L. cuprina* flies react more readily in this cage olfactometer if they have been kept previously for two days at 25° C. and at a minimum relative humidity of 33 per cent. Twenty-four hours before the flies are to be used in the cage olfactometer their food is removed but they are supplied with a liberal quantity of drinking water all the time.

It has been noted that flies recently fed on meat juices do not react readily when exposed in the cage olfactometer. On the other hand if the flies have been starved excessively their random movements appear to be accelerated.

It is important to adopt a standard procedure in the handling of the flies before they are used in olfactory tests for the behaviour of blowflies is subject to much variation according to their prior treatment.

Numbers of Flies used in each Cage.

A minimum of one hundred flies *L. cuprina* are used per cage for each experiment but two hundred could be used without overcrowding.

Temperature and Relative Humidity of the Dark Room.

The dark room is kept at a temperature of 26-29° C. and a relative humidity of 40 to 50 per cent. The relative humidity within the cage olfactometers is probably somewhat higher owing to the presence of wet cotton pads.

At temperatures of 21° C. and below *L. cuprina* does not react satisfactorily in this apparatus.

Time Required for Experiments.

The duration of any experiment cannot be predetermined for much depends on the attractiveness of the chemicals to be tested. Very definite results have been obtained within fifteen minutes when a highly attractive substance has been used, and at other times an exposure of four hours has been necessary to obtain a result. In general, satisfactory results have been obtained during exposures of ninety minutes for each test.

In each experiment two substances are tested one in the left side trap and the other in the right. At the end of the test the entrance holes in the cage are plugged and the traps removed for the counting and sexing of the flies. The flies are killed in the trap by subjecting them to ether or chloroform vapour; afterwards they are removed for counting. The glass traps, metal covers and cones are ventilated in sunshine for about an hour and the bait containers are cleaned. Fresh bait is put into the containers and the apparatus re-assembled and the test is repeated, but the traps are interchanged, the former left side one being placed on the right and *vice versa*. Each complete unit i.e., glass jar, chemical and lid with wire mesh

cone is reversed in position. In each experiment therefore, four separate catches of flies are obtained and from these figures the relative attractiveness of the two test substances can be calculated. After the conclusion of an experiment the untrapped flies are removed, counted and sexed. The apparatus is cleaned, put out in a sunny place for about an hour and then replaced in its compartment in the dark room.

Particular care must be observed in the cleaning of the glass traps, dishes and cones. The cones are washed in hot water and then placed in a jet of steam for ten minutes. Ordinary washing in cold water fails to remove the odour of beef bait from the wire mesh.

Concentration of Chemicals.

L. cuprina is extremely sensitive to the odours of decomposing meat or carrion. Five drops of beef soup* (0·15 c.c.) diluted in 2 c.c. of water have been found to attract the flies in this apparatus. For most tests however, it has been found that 2·5 c.c. of undiluted soup are required as a standard bait. Chemicals of unknown attractiveness are tested at various concentrations some of them being used undiluted and others in very dilute solutions e.g., 0·0005 per cent. Other factors determining concentrations of the substances depend on the nature of the solvents used.

Testing for Attractant, Repellent and Obscurant Odours.

The apparatus may be utilised for determining whether a substance is an attractant by testing it against a known attractant, or water, or a blank according to circumstances. The obscurant value of any substance can be determined by putting the same attractant in both traps with the chemical added in a separate container to the one side. The practice of pouring a chemical over an attractive bait in an endeavour to measure its obscurant value is to be deprecated. Mechanical and chemical factors are thus introduced which might give rise to misleading results.

If a chemical, when tested against a blank, catches no flies while the latter does, it may be regarded as a repellent.

Results.

The discussion of data obtained with this apparatus does not fall within the scope of this paper but a few results may be mentioned to show the capabilities of this cage-olfactometer.

In the paragraph dealing with the cleaning of the apparatus reference was made to the importance of removing all traces of odours from the traps before commencing new experiments. This was forcibly demonstrated in a test in which a beef bait was run versus an aqueous solution of ethyl sulphide 0·05 per cent., the former caught 24 flies and the latter four in forty-five minutes. The beef bait was removed and replaced by a 0·125 per cent. solution of ethyl sulphide tested against the original 0·05 per cent. solution, but the cone of the trap formerly containing beef was not washed but only aired in the sun for a few minutes. Within ten minutes of the beginning of the test twenty flies were caught in this trap. The experiment

* An equal weight of minced beef and water inoculated with a culture of bacteria from sheep intestines is incubated for 40 hours at 37°C. This is centrifuged for twenty minutes and the clear liquid or beef soup thereby obtained is used as a control bait.

was stopped and the trapped flies returned to the cage. An odour of beef bait clinging to the mesh cone was noticeable. After the cones were thoroughly cleaned the experiment was restarted and the subsequent reactions of the flies were markedly different, only sixteen being attracted to this trap in ninety minutes.

On another occasion duplicate experiments in which a very weak beef bait (0·15 c.c. beef bait soup in 2·35 c.c. of water) was tested against water, were run simultaneously. The results were almost identical the ratio of relative attractiveness between the beef and water being 2·3 and 2·7 and their correction factors for position errors were 1·02 and 1·3 respectively.* A correction factor of 1 indicates equality in attactiveness of the positions of the traps.

A beef bait soup of the concentration indicated is an extremely weak one and the consistent results in the duplicated test show both the precision of the apparatus and the great sensitivity of the flies.

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* The above method of comparing two substances was described by Ripley & Hepburn (1929.*b*). The formula used for calculating the relative attractiveness of the two substances and the relative attractiveness of the positions occupied by the traps is derived thus:—

1. Using bait a in positions 1 and 2 (P_1 and P_2)

   $$\frac{P_1}{P_2} = \frac{a_1}{a_2} = K.$$

2. Using bait b in positions 1 and 2 (P_1 and P_2)

   $$\frac{P_1}{P_2} = \frac{b_1}{b_2} = K$$ if the situation remains more or less the same.

3. Using bait a in position 1 and bait b in position 2

   $$\frac{P_1}{P_2} = \frac{a_1}{b_2} = l.$$

4. Using bait b in position 1 and bait a in position 2

   $$\frac{P_1}{P_2} = \frac{b_1}{a_2} = m.$$

   Now $lm = \frac{a_1}{b_2}\frac{b_1}{a_2} = \frac{a_1}{a_2}\frac{b_1}{b_2} = K^2$

   $\therefore \sqrt{lm} = K.$

   $\therefore P_1 = \sqrt{lm}\, P_2.$

   The $\sqrt{lm}$ is thus the correction factor.