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Behavioural and electrophysiological evaluation of oviposition attractants for *Culex quinquefasciatus* Say (Diptera: Culicidae)[1]

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Abstract. The attraction of gravid *Culex quinquefasciatus* by the oviposition pheromone, *erythro*-6-acetoxy-5-hexadecanolide, and by polluted water is described. Both materials increase oviposition and when combined the effect is additive. The oviposition behaviour is reflected by the antennal sensitivity to these compounds.
Key words. Oviposition pheromone; polluted water volatiles; oviposition behaviour; electroantennogram.

Culex quinquefasciatus is the principal urban vector of Bancroftian filariasis in the tropics[4] and is the vector for St. Louis encephalitis (SLE) virus and other arboviruses in the USA[5]. An efficient surveillance programme for arbovirus vectors such as this species would greatly benefit affected areas. Existing trapping techniques are expensive, inconvenient and inefficient; using light, CO_2 and vertebrate baits, they catch mainly unfed individuals. Recently introduced oviposition traps, baited with fermented organic infusions of materials such as hay and cattle manure[6–8], are more promising. They mainly catch blood-engorged and gravid females preferentially, resulting in higher arbovirus isolation. Clearly a synthetic attractant for these ovi-traps would greatly facilitate their servicing but the attractive components of such infusions are only now being identified[9]. In the field, gravid females use a combination of physical and chemical cues such as visual, tactile, contact chemoreceptory and olfactory stimuli[10–12] to locate and select oviposition sites. Some chemical stimulants arise from the environment, e.g. microbial decomposition products[13], and communicate the presence of larval food in the prospective oviposition site. In addition, *C. quinquefasciatus* oviposits egg rafts on the surface of water which, on maturing, release a pheromone from apical droplets formed on the eggs[14] that attracts females of this species and others of the genus[15]. The pheromone comprises (−)-(5*R*,6*S*)-6-acetoxy-5-hexadecanolide[14,16] and in the field, the synthetic racemic pheromone effectively concentrates oviposition[17,18]. We report on behavioural and electrophysiological studies on the attraction of *C. quinquefasciatus* to the oviposition pheromone alone and

in combination with polluted water, to simulate the mosquito's natural breeding water.

Materials and methods

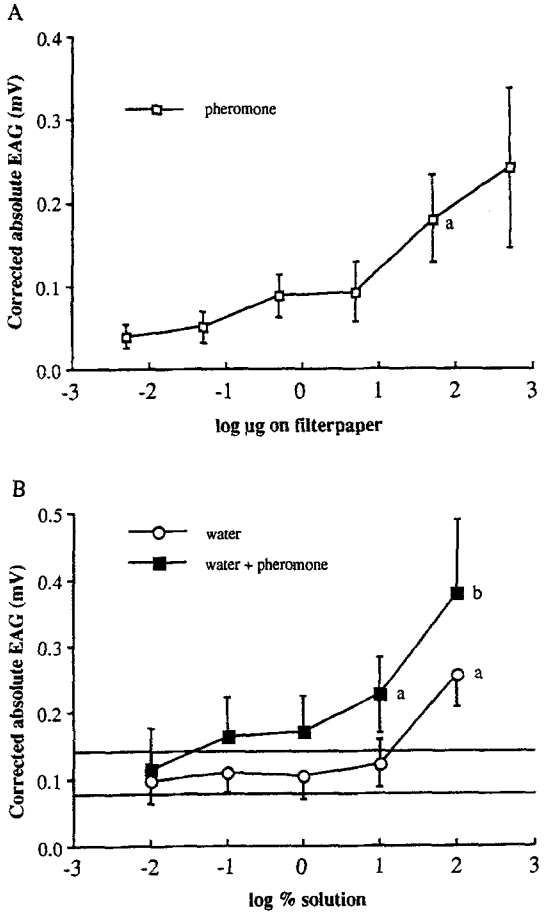
Behavioural studies. Bioassays were carried out in wooden-framed cages (31 × 31 × 31 cm), muslin-covered with a perspex front and muslin sleeve. Gravid female *C. quinquefasciatus*[19] were used 4 days after feeding (7–10 days old), being offered the choice of two glass bowls (7–10 cm diameter) for oviposition. Each bowl contained 100 ml distilled water, one treated with 100 µl of the test material and one with an equivalent volume of the appropriate solvent as a control. The bowls were placed in diagonally opposite corners of cages containing 20 gravid females and the positions of the test and control bowls were alternated between replicates. Experiments were run overnight in a constant temperature room (12L : 12D, 27 ± 2 °C) and assessed the following morning by counting the numbers of egg rafts per bowl (converted to percentages of the total number of rafts in each cage). Statistical differences between treatments were determined by Student's t-tests.

Electrophysiology. Antennae of gravid female mosquitoes were tested for sensitivity to test materials by recording electroantennograms (EAGs) according to standard procedures[20], using excised heads. The head was mounted on the glass microelectrode indifferent electrode and the cut tip of one antenna was brought into contact with the recording electrode. Both electrodes were filled with Beadle-Ephrussi Ringer. High humidity was maintained by a dish of wet cotton wool placed immediately beneath the preparation. The test solutions (in 10 µl solvent) were applied to a filter paper (25 × 8 mm), the solvent was allowed to evaporate and the paper was inserted into a pasteur pipette. The stimulus pipette was connected to a 5-ml syringe and a 3-ml puff of air was injected manually (stimulus period, 0.5 s) into a filtered, humidified airstream (1 l/min) directed onto the antenna. Signals were amplified through a Syntech UN-03 AC/DC amplifier and recorded on a Linseis Type LS chart recorder. To compensate for antennal fatigue, responses were normalised using a standard stimulus (0.01 µg 3-octen-1-ol) before (S_b) and after (S_a) each experimental stimulus (E) (i.e. $[2 \times E/S_b + S_a] \times S_i$, where S_i was the initial standard stimulus). Control values (solvent only) were subtracted from the normalised values to give final, corrected EAG values.

Chemicals. Test materials for bioassays and electrophysiology were the oviposition pheromone (*erythro*-6-acetoxy-5-hexadecanolide), diluted with dichloromethane, 3-methylindole (skatole) (Aldrich Chemical Co. Inc.) diluted with diethyl ether, 3-octen-1-ol (Aldrich Chemical Co. Inc.) diluted with hexane, polluted water made by fermenting rabbit droppings (25 g) in distilled water (500 ml) (≡ 100% concentration) for 3–6 days and diluted in decadic steps with distilled water, and an ether extract of the polluted water. Polluted water (750 ml) was extracted with ether (5 × 50 ml) and the extract dried and concentrated under a stream of nitrogen at room temperature to give a solution equivalent to 10 ml of polluted water/ml. The extract was then vacuum distilled as described previously[21]. The sample was analysed by gas chromatography (GC) on a 50 m 0.3 mm i.d. HP-1 fused silica capillary column. GC-mass spectrometry (GC-MS) was by electron impact at 70 eV, 200 °C, with the capillary column directly coupled to the source of a mass spectrometer (VG Analytical 70–250)[22].

Results and discussion

The synthetic oviposition pheromone increased the egg-laying behaviour of gravid females in a dose-dependent manner; 0.05 µg of the pheromone resulted in significantly greater oviposition in the treated bowls (mean response = 69.3 ± 5.4, n = 7; mean control response = 52.6 ± 3.7, n = 10), ($p < 0.05$). Maximum oviposition attractancy occurred with 40 µg pheromone



EAG dose-response curves of female *C. quinquefasciatus* to A) oviposition pheromone and B) polluted water alone and polluted water plus 50 µg oviposition pheromone. Vertical bars represent the standard error (SE) of the mean. Solid horizontal lines in B indicate the upper and lower values of the SE in the EAG response to 50 µg oviposition pheromone alone. For each treatment, n = 10. a: Threshold doses (significantly greater than control values, $p < 0.05$). b: Polluted water plus pheromone vs pheromone alone ($p < 0.05$).

(88.0 ± 3.2%, n = 5). Similarly, EAG responses to the oviposition pheromone increased in a dose-dependent manner (fig., A).

Concentrations of 3-day polluted water (1.0–50%) were significantly attractive in the bioassay, with 50% resulting in an 83.4 ± 3.3% (n = 6) oviposition response ($p < 0.001$). The effects of the oviposition pheromone and the polluted water were additive: 0.05 µg of pheromone combined with 1.0% polluted water (3-day) gave a 92.8 ± 3.9% (n = 6) oviposition response, which was significantly greater than the response to 1.0% polluted water alone (63.9 ± 5.8%, n = 8), ($p < 0.01$) and to 0.05 µg pheromone alone (69.3 ± 5.4%, n = 7), ($p < 0.01$). This behaviour was reflected in the EAG response, with the oviposition pheromone having an additive effect with the polluted water (fig., B). A proportion of the active components of 6-day polluted water were ether soluble and retained their activity after vacuum distillation. 100 µl of the undiluted, vacuum-distilled extract gave a 92.7 ± 3.1% (n = 8) response in the bioassay (ether control = 48.0 ± 2.2% (n = 10)) and 10 µl of the 100% extract gave an EAG response (corrected for the solvent control) of 0.17 ± 0.05 mV (n = 5). Behavioural and electrophysiological thresholds for the sample were both 1%: 70.2 ± 8.0% (n = 9) and 0.09 ± 0.01 mV (n = 5) respectively. 3-Methylindole (skatole), a common natural product, found in animal excreta and the products of microbial fermentation of organic material, is a primary oviposition attractant in grass-infusion extracts for gravid *C. quinquefasciatus* females[9]. In the present investigations 200 pg skatole gave a behavioural response of 71.3 ± 6.3% (n = 6), significantly greater than the control value ($p < 0.01$). The behavioural threshold clearly lay between this dose and 100 pg, at which the response (47.0 ± 7.0%, n = 9) did not differ significantly from the control response. The threshold concentration of skatole for EAGs was 1000 pg (0.22 ± 0.04 mV, n = 10). However, skatole was not detected in the ether extract of polluted water. The detection limit for GC was 20 pg and for GC-MS 200 pg by single ion monitoring at m/z 130, the base peak of the skatole spectrum. This, combined with the behavioural and electrophysiological threshold, demonstrates that skatole does not account for the attraction of gravid *C. quinquefasciatus* to this sample of polluted water. Investigations are currently in progress to determine the active semiochemical constituents of the polluted water which, in combination with the readily synthesized oviposition pheromone, would have great potential as an arbovirus surveillance tool and could represent an important component of an integrated control programme.

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