Recording Three Dimension Flight of *Aedes aegypti* (L.) Mosquitoes in a Wind Tunnel

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Técnica de Gravação do Vôo Tridimensional de *Aedes aegypti* (L.) em Túnel de Vento

RESUMO - Uma nova técnica de gravar vôos em 3-dimensões (3-D) de fêmeas de mosquitos *Aedes aegypti* (L.) em túnel de vento foi estudada. Os resultados demonstraram a importância do vôo vertical pelos mosquitos durante o comportamento de procura do hospedeiro. Os percursos em 3-D demonstraram que o mosquito utilizou o vôo vertical e lateral logo após a sua saída pluma de odor. Os vôos se mantiveram constantes na lateral e vertical quando o inseto permaneceu dentro da pluma ou em posição transversal à pluma.

PALAVRAS-CHAVE: Insecta, cairomônios, túnel de vento, técnica de vídeo-gravação, odor.

ABSTRACT - A technique for recording 3-dimensional (3-D) flights of female mosquitoes *Aedes aegypti* (L.) within a wind tunnel is described. Preliminary results showed the importance of vertical flights used by mosquitoes in host location behavior. The 3-D flight tracks demonstrated that the mosquitoes changed the direction of their upwind flight in both vertical and lateral dimensions when the odor plume was lost. However, they maintained relatively constant vertical and lateral courses during upwind flight within plumes, at the edges of the odor plume and/or during cross wind flight.

KEY WORDS: Insecta, kairomones, wind tunnel, video recording technique, odor plume.

Wind tunnel studies have been used extensively to identify and evaluate behaviorally active compounds such as sex pheromones (Witzgall & Priesner 1991), plant kairomones (Nottingham & Coaker 1985), host kairomones (Eiras & Jepson 1991), and other semiochemicals (Baker & Linn 1984). Both "long-range" (activation and upwind flight) and "close-range" (landing at the odor source) responses of insects to odor sources have been examined in wind tunnels (Kennedy 1977). The patterns of upwind flight that insects use to fly towards an odor source have been throughly described (Kennedy 1983).

Insect orientation mechanisms, flight speed and direction have usually been recorded by a fixed video camera, placed vertically above the flight chamber (Marsh *et al.* 1978, Murlis *et al.* 1982, Nottingham & Coaker 1987, Sanders 1985, Pile *et al.* 1991). This may provide inaccurate estimations of flight speed because the vertical component of flight is not measured. Video techniques for recording 3-dimensional (vertical and horizontal components) insect flights have been employed for interpreting the mechanisms used by insects to locate odor sources (Riley *et al.* 1990, Witzgall & Arn 1991).

Carbon dioxide is known to act as a host kairomone for female mosquitoes, *Aedes aegypti* (L.) (Diptera: Culicidae) (Gillies 1980). In wind tunnel studies CO_2 elicits the complete sequence of host-location behaviors: take-off, upwind flight and landing at the source (Eiras & Jepson 1991). Lactic acid is another host kairomone, but when tested singly it does not elicit the complete sequence of host-location behaviors. It may also inhibit landing at a source of carbon dioxide (Eiras & Jepson 1991). Mosquitoes perform straight upwind flights when CO_2 is presented at low concentrations, whereas they exhibit zig-zag flights at high CO_2 concentrations (A.E. Eiras & P.C. Jepson, unpublished). Mosquitoes also use vertical movements in odor location, as demonstrated in vertical olfactometer bioassays (Eiras & Jepson 1994). This paper describes a technique for studying 3-dimensional flight of mosquitoes in response to carbon dioxide and lactic acid plumes.

Material and Methods

Wind tunnel and 3-D flight track analysis. The wind tunnel was made of plastic tubing which, when inflated, formed a 1.5 meter long, cylindrical tunnel with a diameter of 38 cm (Jones *et al.* 1981). The tunnel was inflated by two electric fans, which delivered air vertically downwards through a 3 cm deep layer of activated charcoal (Eiras & Jepson 1991). The wind tunnel was maintained at 27 ± 2°C and 65 ± 5% RH, with a light intensity of 10 lux and a wind speed of 0.1 m/s.

Flight patterns were characterized by using two video-cameras. A Panasonic WV-CD130L camera was mounted vertically to record upwind flight (lateral flights "X" and "Y" coordinates), and a Panasonic WV-1900 camera was mounted on a tripod to produce a side view of the tunnel (vertical flights "X" and "Z" coordinates). Both cameras were set up to record an area (55x25 cm) downwind from the point source odor. A grid of 2 cm squares, drawn on white cards were attached to the base and back wall of the wind tunnel. This provided a reference point from which measurements could be made and increased contrast in order to improve the visualization of flying insects.

The outputs from the cameras passed through a video mixer (VMC81, Electrocraft Ltd.), which enabled both images to be displayed on the same screen. A 12", Panasonic (WV-5410) high resolution monitor was used to visualize flight tracks. A time date generator (Digital Data Systems Ltd.) was used to provide reference times in intervals of 0.01s. Mosquito flights were recorded in real time using a Hitachi VT-L30ED, time-lapse video recorder, recording at 50 frames/s.

Mosquito flights were analyzed, frame-by-frame, by playing back the video recording. From both sets of images, successive positions of the insects were plotted on a clear acetate sheet, taped to the viewing screen. These positions corresponded to coordinates on the background grid. Odor plume structure of carbon dioxide and lactic acid were simulated using ammonium dichloride vapor emerging from a glass tube, and from a cotton pipette filter, respectively. The visualized odor plume shapes were also drawn on clear acetate sheets. Flight behavior patterns inside and outside of these plume boundaries were determined by superimposing upwind flight track records on the odor plume maps.

Bioassays. After a 15 min. acclimatization period, five female *A. aegypti* were released at the downwind mesh from a release tube. Five, seven- to ten-days old female *A. aegypti* were tested in each test. Each group of insects was tested only once. The release device consisted of a small flow chamber (plastic spittle cup) which released the carbon dioxide at a controlled flow rate from the glass tubing (0.5 cm diam.). A wetted cylindrical pipette filter was placed tightly within a glass vial (4.0 x 0.7 mm) containing the lactic acid solution. The release devise produced a discrete plume that expanded downwind. The flow rate gave a concentration of 0.11% carbon dioxide at the mosquito release tube, which is sufficient to elicit the complete behavioral sequence in host location (Eiras & Jepson 1991). Lactic acid solutions were prepared from 85% syrup (Sigma Chemicals Ltd.) and also placed in the discrete plume apparatus at a concentration of 85 µg/ml in distilled water. This concentration elicited the highest behavioral responses in female *A. aegypti* when combined with the 20% (v) carbon dioxide concentration in the discrete plume apparatus (Eiras & Jepson 1991).

Results and Discussion

The recording areas covered by both video cameras were sufficient to observe complete upwind flights. Track analysis demonstrated that random flights were exhibited during control treatment at least with respect to the odor plume (Fig. 1). However, when either the carbon dioxide or lactic acid odor plumes were present the upwind flight tracks of female *A. aegypti* were non-random (Figs. 2, 3).

The vertical and lateral flight tracks ("Y-Z" graph) were produced by using coordinates from the mosquito positions from both images (vertical and horizontal). In general, large vertical and lateral displacements in upwind flight were observed after the odor plumes were lost (Fig. 2, 3). Mosquitoes flying upwind within the odor plume (Fig. 2), at the edge of the plume and/or engaging in cross wind flight (Fig. 3) exhibited small deviations in the vertical and lateral dimensions.

The flight tracks demonstrated that female *A. aegypti* performed zig-zag upwind flights in both axes (lateral and vertical) when they were at the edge or out of the odor plume. Once the odor plume was lost, mosquitoes were likely to use vertical and lateral upwind flights in order to maintain their position within the odor plumes. This particular behavior also has been observed in Lepidoptera responding to sex-pheromones (Marsh *et al.* 1978, Kennedy 1983).

Our recording show that mosquitoes use vertical flight as well as horizontal flight during host location. By not considering the vertical component of their flight an important aspect of their orientation behavior would

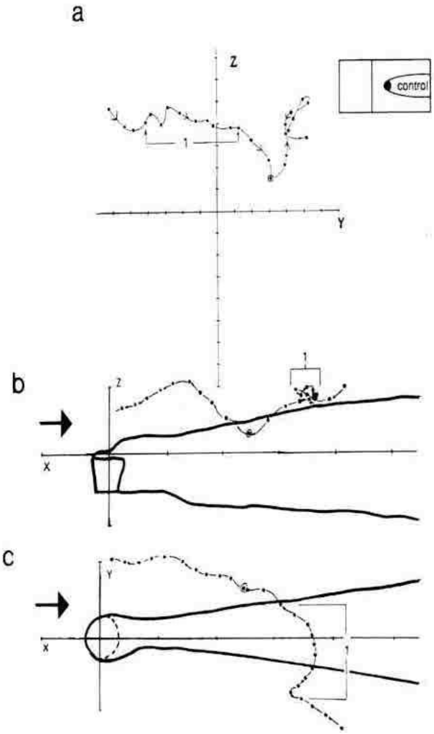

Figure 1. Flight track of a female *Aedes aegypti* in the control treatment (water and purified air). Mosquitos maintained relatively constant vertical flight during a cross wind flight (1) and performed vertical flight when out of the odor plume (〈). Arrows indicate direction of the upwind flight. Points are 0.04 s intervals. (a) front view, (b) side view and (c) top view.

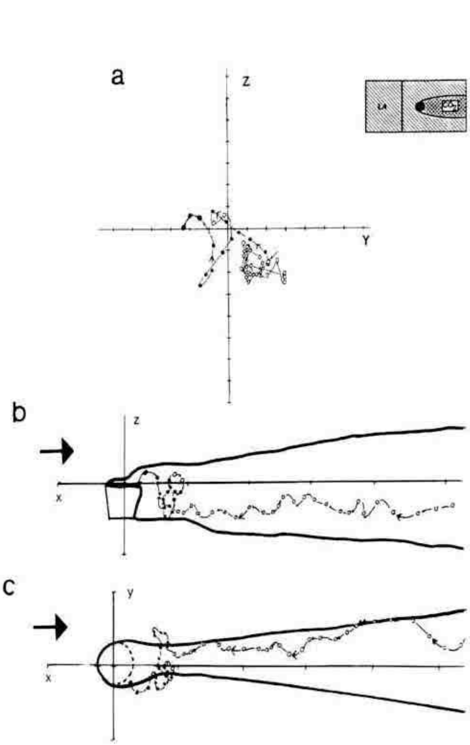

Figure 2. Flight track of a female *Aedes aegypti* landing on the carbon dioxide point source within a cloud of lactic acid. Mosquito maintained relatively constant vertical and horizontal flights during an upwind flight in the plume (o) of carbon dioxide. Both vertical and lateral displacement were observed after mosquitoes lost the plume (⟨). Arrows indicate direction of the upwind flight. Points are 0.04 s intervals. (a) front view, (b) side view and (c) top view.

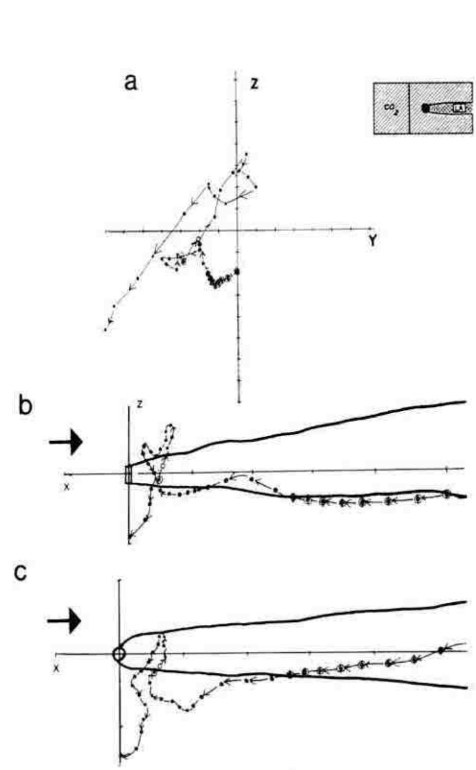

Figure 3. Flight track of female *Aedes aegypti* to a point source of lactic acid alone within a cloud of carbon dioxide. Mosquito maintained constant vertical and horizontal flights at the edge of the odor plume (⟨) and both vertical and lateral displacement were observed after the plume was lost (⟨). Arrows indicate direction of the upwind flight. Points are 0.04 s intervals. (a) front view, (b) side view, and (c) top view.

have been overlooked. Although flight speed was not measured in the present study, not considering all three dimensions of flight could bias estimates of flight speed (Marsh *et al.* 1978, Murlis *et al.* 1982). Few investigations of flight behavior have taken vertical flight into consideration (David *et al.* 1982, Gibson & Brady 1985, Warnes 1989, Pile *et al.* 1991) and at least for observing behaviors and for estimating flight speed.

The technique of delineating an area covered by a visual plume gives only an estimate of the volume described by the natural odor plumes. When flight tracks were placed over the plume structure, however, it was observed that mosquitoes changed flight patterns after losing the odor plume. Thus, the real plume structures of carbon dioxide and lactic acid are likely to be similar to the visual plume. Flight tracks also demonstrated, for the first time, that female *A. aegypti* deviated from the main odor plume axis (both in the horizontal and vertical planes) during an up-wind flight within the odor plumes. Similar flight patterns also have been observed in response to the main synthetic sex pheromones in moths (Witzgall & Arn 1991).

The technique used herein was very laborious, tedious and time-consuming, especially to produce the 3-D graph ("Z-Y" coordinates). Thus, it would be useful to develop a computer system that continuously determines the position of the insect in the wind tunnel. Such a technique has recently been used to record 3-D flights of male *Lobesia botrana* (D.) (Lepidoptera: Olethreutidae) responding to female sex pheromones (Witzgall & Arn 1991). However, this recording technique uses expensive video equipment and sophisticated software that are not commercially available (H. Arn, personal communication to A.E. Eiras).

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References Cited

Baker, T.C. & C.E. Linn. 1984. Wind tunnels in pheromone research. p. 75-110 In H.E. Hummel & T.A. Miller. (eds), Techniques in pheromone research. New York, Springer-Verlag, 465p.

David, C.T., J.S. Kennedy, A.R. Ludlow, J.N. Perry & C. Wall. 1982. A reappraisal of insect flight towards a distant point source of wind-borne odor. J. Chem. Ecol. 8: 1207-1215.

Eiras, A.E. & P.C. Jepson. 1991. Host location by *Aedes aegypti* (Diptera: Culicidae): a wind tunnel study of chemical cues. Bull. Entomol. Res. 81: 151-160.

Eiras, A.E. & P.C. Jepson. 1994. Responses of female *Aedes aegypti* (Diptera: Culicidae) to host odours and convection currents using an olfactometer bioassay. Bull. Entomol. Res. 84: 207-211.

Gibson, G. & J. Brady. 1985. "Anemotactic" flight paths of tse-tse flies in relation to host odor: a preliminary video study in nature of the response of loss of odor. Physiol. Entomol. 10: 395-406.

Gillies, M.T. 1980. The role of carbon dioxide in host finding by mosquitoes (Diptera: Culicidae). A review. Bull. Entomol. Res. 70: 525-532.

Jones, O.T., R.A. Lomer & P.E. Howse. 1981. Response of male Mediterranean fruit flies, *Ceratitis capitata*, to trimedlure in a wind tunnel of novel design. Physiol. Entomol. 6: 175-181.

Kennedy, J.S. 1977. Behaviourally discriminating assays of attractants and repellents p. 215-229 *in* Shorey, H.H. & McKelvey Jr, J.J. (eds), Chemical control of insect behaviour, New York, 414p.

Kennedy, J.S. 1983. Zig-zagging and casting as programmed response to wind-borne odor: a review. Physiol. Entomol. 8: 109-120.

Marsh, D., J.S. Kennedy & A.R. Ludlow. 1978. An analysis of anemotactic zig-zagging in male moths simulated by pheromone. Physiol. Entomol. 3: 221-240.

Murlis, J.B., W. Bettany, J. Kelley & A.R. Ludlow. 1982. The analysis of flight paths of male Egyptian cotton leafworm moths *Spodoptera littoralis*, to a sex pheromone source in the field. Physiol. Entomol. 7: 435-441.

Nottingham, S.F. & T.H. Coaker. 1985. The olfactory response of cabbage root fly, *Delia radicum*, to the host-plant volatile allylisothiocyanate. Entomol. Exp. Appl. 39, 307-316.

Nottingham, S.F. & T.H. Coaker. 1987. Changes in flight track angles of cabbage root fly, *Delia radicum*, in diffuse clouds and discrete plumes of the host-plant volatile allylisothiocyanate. Entomol. Exp. Appl. 43: 275-278.

Pile, M.M., M.S.J. Simmonds & W.M. Blaney. 1991. Odor-mediated upwind flight of *Culex quinquefasciatus* mosquitoes elicited by a synthetic attractant. Physiol. Entomol. 16: 77-85.

Riley, J.R., A.D. Smith & B.W. Bettany. 1990. The use of video equipment to record in three dimensions the flight trajectories of *Heliothis armigera* and other moths at night. Physiol. Entomol. 15: 73-80.

Sanders, C.J. 1985. Flight speed of male spruce bud worm moths in a wind tunnel at different wind speeds and at different distances from the pheromone source. Physiol. Entomol. 10: 83-88.

Warnes, M.L. 1989. *Glossina pallidipes* to ox odor, carbon dioxide and a visual stimulus in the laboratory. Entomol. Exp. Appl. 50: 245-253.

Witzgall, P. & H. Arn. 1991. Direct measurement of the flight behavior of males moths to calling females and synthetic sex pheromones. Zeitschrift für Naturforschung 45:1067-1069.

Witzgall, P. & E. Priesner. 1991. Wind-tunnel study on attraction inhibitor in male *Coleophora laricella* Hbn. (Lepidoptera: Coleophoridae). J. Chem. Ecol. 17: 1355-1362.

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