

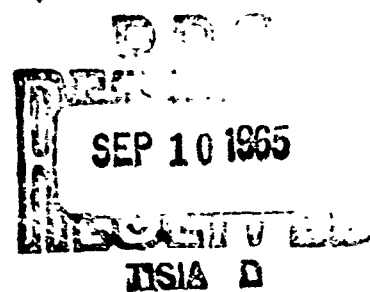
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A Two-Air-Stream Observation Chamber for Studying Responses of Flying Insects

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Abstract

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The paper describes an observation chamber and air-conditioning system designed for studying the responses of flying insects at the boundary between two microclimates. Two vertical air streams each fill one half of the rectangular observation chamber. Turbulence is carefully removed so as to maintain a sharp boundary between the streams. Each stream is separately conditioned by passage through heat exchangers and plastic sponges impregnated with substances to be vapourized. Responses of flying insects are studied both photographically and by counting rates of turning at the boundary.

Many flying insects locate their hosts through an olfactory response to airborne chemicals emanating from the host. Mosquitoes seem to rely mainly on the detection of warm moist convection currents and possibly carbon dioxide. Whatever the sensory modality involved, the sequence of responses to these host-generated signals which direct the insect to its target is not well understood. When the airborne emanations impinge on the appropriate sensory organs of the insect they can signal their presence but can provide no immediate information about the direction or location of the source. The necessary first step in a directed response to the signal is a general upwind orientation. A flying insect cannot determine wind direction by reference only to the airborne signals, and Kennedy (1939) has shown that the female *Aedes* mosquito, a day-flying species, achieves its upwind orientation by observing the apparent motion of objects on the ground. An initial downwind orientation causes the retinal image of the ground pattern to move faster than a tolerated velocity limit of about 17 cm./sec.; this rapid image motion induces an unstable orientation followed by an about-turn. In the upwind direction the mosquito flies at a speed which causes the retinal image of the ground pattern to move from forward to aft at a speed of a few centimetres per second. Thus, the visual sense plays a role in host-finding, but the angular error in orientation is not negligible and some other response is still required to keep the insect "on the beam". Let us consider what kind of response might serve this purpose.

The downwind stream from the host has a finite width, and the naturally occurring transverse diffusion of the airborne emanations should produce a boundary layer in which the signal strength is decreasing away from the centre of the stream. Inside the boundary layer the signals can be detected; outside they are below threshold. If the insect by chance achieves an accurate upwind orientation it will certainly reach the target, but if it strays outside the boundary the stimulus must fall below threshold and be lost. It can then return to the air stream only by a fortuitous change of direction. The guidance will be much more precise if the insect, while actually crossing the boundary, makes an appropriate response which causes it to turn back into the main body of the host stream. These considerations are highly simplified, but they focus attention on the boundary layer and suggest a study of insect responses to a gradient in the intensity of the attractive signal.

To study the responses of flying insects at a boundary we require two adjacent air streams, one a test stream that can be modified by the addition of heat, moisture or chemical vapour, and the other a control stream. To separate the upwind orientation response from the boundary layer response the air streams are most conveniently directed vertically upward so as to resemble, in fact, convection currents from a warm-blooded host. The two streams preferably have the same

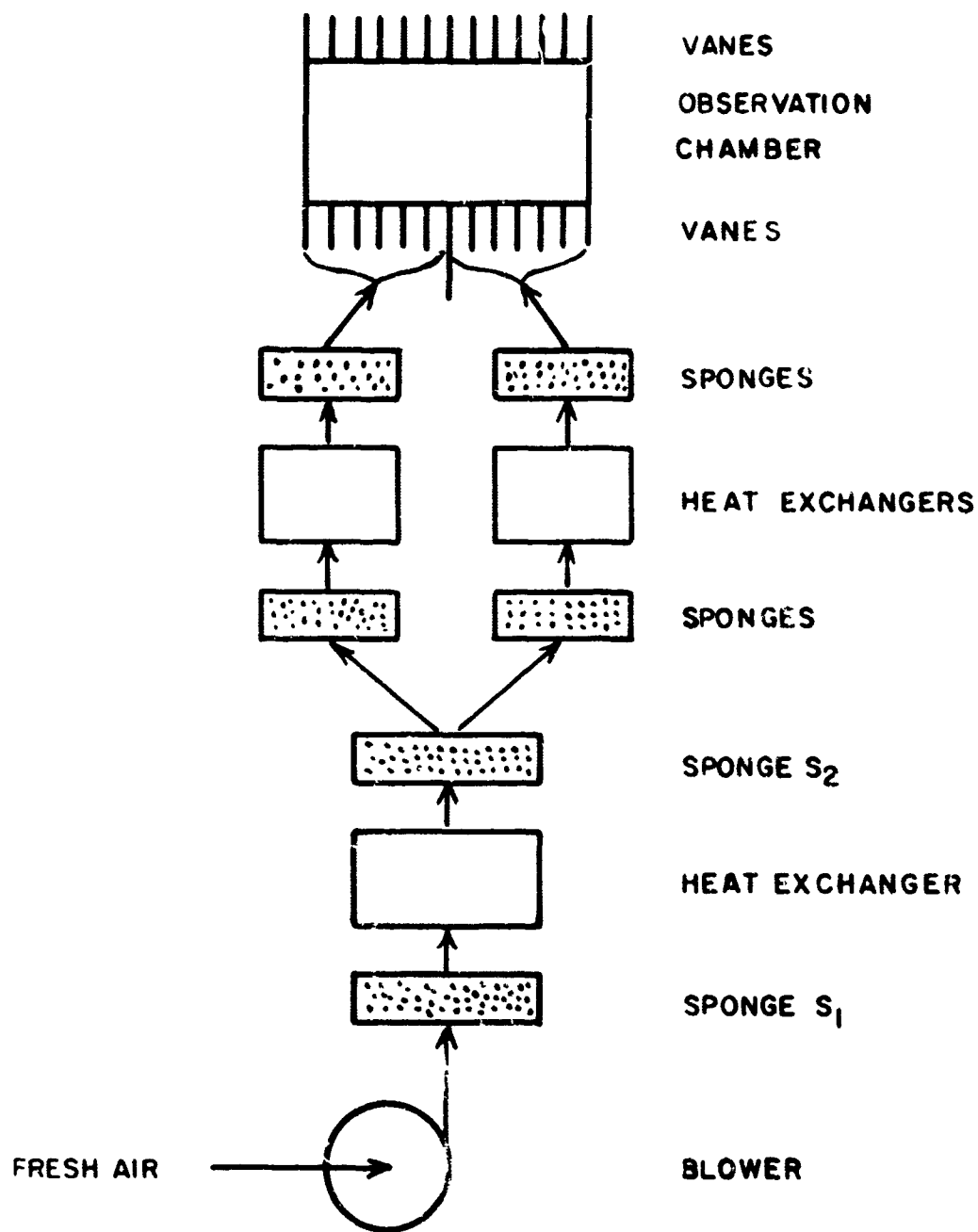


Fig. 1. Two-air-stream observation chamber, schematic.

speed, to avoid turbulence and mixing at the boundary, and should together fill the whole observation chamber, with no "dead spaces" in between or at the walls. The observation chamber should also be large enough to allow the insects to fly freely.

Fig. 1 shows a schematic diagram of a two-air-stream apparatus constructed for this purpose. Fresh air is drawn from outdoors through a centrifugal fan and passed through a single channel where the temperature can be adjusted by a heat exchanger, and chemical vapour or moisture added by means of polyurethane sponges impregnated with the substance to be vapourized. The sponges offer



Fig. 2. Side view of small model observation chamber.

surprisingly little resistance to the air flow and can saturate a rather large volume flow of air with chemical vapour or moisture. If sponge S-2 is impregnated, the chemical can be vapourized to saturation at a temperature set by the preceding heat exchanger. Thus the vapour concentration at the final outlet may be controlled by suitably cooling the inlet air before it enters S-2. Alternatively, S-1 can be used and the air post-heated by the heat exchanger. After S-2 the passage divides to give separate channels for conditioning independently the air entering each half of the observation chamber.

The observation chamber has transparent side and end walls and gauze top and bottom. The vane sections below and above the chamber straighten out the air stream and remove small turbulences. The top vane section is used only when the air temperatures differ in the two streams, to prevent back eddies of the cooler air. The observation chamber is a separate portable unit which houses the insects during the whole course of the experimentation, so that they are not disturbed by being transferred to the chamber before each experiment. At night the chamber is returned to the insectary and food may be supplied through a port on one end wall.

Two models have been constructed: the first has a chamber $16 \times 8 \times 8$ inches, and the second, larger model provides a cage volume $36 \times 21 \times 21$ inches. Figs. 2 and 3 show the construction of the smaller model. Air temperatures and relative humidity are monitored with thermocouples inserted into the air streams in the conditioning unit. The wet bulb thermocouple for humidity measurement is made by wrapping a thermocouple tightly with a strip of gauze which is wetted with distilled water. The instrument box at the back is a thermocouple milli-



Fig. 3. Front view of small model observation chamber.

voltmeter. The needle valves below adjust the mixture of hot and cold water to each heat exchanger. The hood for the observation chamber is covered with several sheets of red and amber cellophane to exclude the light to which the mosquitoes are sensitive. To exclude the front illumination the observer sits inside a movable enclosure placed in front of the apparatus.

The sponge holders can be withdrawn quickly and switched from one channel to the other during experiments.

We have found that it is important to check the location of the boundary between the air streams, particularly when there is a temperature difference between the two streams. This is done by placing an empty chamber on the exit ports and injecting smoke into one air stream. Fig. 4 (upper) shows the sharpness of the boundary made visible in this way. The visible boundary can be marked on the glass wall and then transferred to the chamber containing the insects being studied. This is important when the air streams are at different temperatures and the boundary is not vertical.

The responses of flying *Aedes aegypti* mosquitoes have been studied with this apparatus, using two methods of observation. In the photographic method, developed by Kellogg and Wright (1962), the flight tracks are recorded with a stereo camera using dark field illumination and red light with a 10-second exposure. A rotating shutter in front of the stereo camera produces a dot-dash coded track from which the direction and speed of flight can be determined. Fig. 4 (lower)



Fig. 4. Upper, smoke test with large observation chamber; lower, flight tracks of *A. aegypti* when left-hand stream contains dimethyl phthalate vapour.

shows flight tracks recorded in this way when one stream contained repellent vapour. In the visual method a boundary zone about 7 cm. wide is marked on either side of the boundary line. Individual insects approaching the boundary are watched and, if they cross the boundary, it is noted whether they turn back or fly through the boundary zone without turning. The counts of crossings in either direction with and without turning are accumulated in a 2-by-2 contingency table.

In other experiments, of the type usually made with an olfactometer, insects are allowed to fly and then come to rest, and the numbers in each half of the chamber are counted.

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