

- penicillin, 5 mg of streptomycin, 0.5 mg of insulin, and 0.62 mg of cortisone acetate.
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14. We thank the Dow Chemical Co. for the silicone polycarbonate capillaries, R. Cross of the Amicon Corp. for the XM-50 capillaries and technical advice, and W. R. Kidwell of the National Cancer Institute for his helpful suggestions.

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## Chemical Trail-Following by Flying Insects:

### A Mechanism for Orientation to a Distant Odor Source

**Abstract.** *An aerial trail of odorous pheromone molecules extends downwind from a female pink bollworm moth that is receptive for mating. Males apparently sense the boundaries of the trail during their characteristic zigzag flights across it. Contrary to previous beliefs, the mechanism by which the males steer toward the odor source does not require a sensing of wind direction.*

Insects of many species identify their mates, food, and egg-laying sites by olfactory chemical cues. The molecules diffuse or drift on air currents away from their source, often providing information to the insect on the direction of the source.

Within a few centimeters of an odor source, an insect may be able to detect a steep concentration gradient of molecules and orient its body axis accordingly. However, because of the turbulence that is characteristic of moving air, a chemical gradient probably cannot provide useful directional information over more than a few centimeters (1). An insect detecting molecules many meters downwind must use another mechanism to steer toward the odor source.

Anemotaxis (orientation to an air current) has been shown to play a key role in the steering of certain insects to a distant odor source (2). The evaporating odor molecules form an elongated, often irregularly shaped cloud or plume moving in a downwind direction. An insect that orients anemotactically turns its body axis into the wind when it is stimulated by the odor. As it proceeds in this upwind direction, the odorous air stream might be lost; crosswind casts may then occur, presumably maximizing the likelihood of the insect again encountering the odorous air and continuing its upwind flight. In recent years, anemotaxis has been accepted by many investigators as the only available mechanism for the orientation of a flying insect to a distant odor source (3).

We have examined the method used by a small moth species, the pink bollworm, *Pectinophora gossypiella*, in steering toward an odor source and have found that sensing of the direction

of air movement is not necessary. An alternative mechanism for orientation to a distant odor source is proposed.

A Plexiglas flight tunnel was constructed, 183 cm long by 61 cm wide by 61 cm high, with screens (six meshes per centimeter) covering both ends (Fig. 1). Air flow was obtained by placing one end of the tunnel adjacent to an exhaust hood. Prior to introduction of the odor source, a cage containing ten male moths was attached to the center of the screen at the downwind end. The males were conditioned for at least 10 minutes to an air velocity of 7 cm/sec and a light intensity of 4 lux. The odor source was sex pheromone, prepared by extracting female abdomen tips in ether. Pheromone extract equivalent to 0.1 female was pipetted into one end of a copper tube 2 mm in inside diameter. The other end was connected to a flexible air line delivering 0.25 cm<sup>3</sup> of air per second. During a test, the tube was positioned so that evaporating pheromone molecules were released into the air in the

center of the upwind end of the tunnel. When odor molecules activated the males at the downwind end, a trapdoor that covered the front of their cage was opened.

The males were considered to be orienting within the pheromone plume if they flew in an upwind direction through a square wire hoop (30 cm<sup>2</sup>) suspended in the center of the tunnel 30 cm downwind from the pheromone source. The hoop occupied 25 percent of the cross-sectional area of the tunnel. Prior calibration of the system with visible smoke in air moving at 7 cm/sec (4) indicated that a typical plume was approximately 14 cm in diameter and 25 cm in diameter when it passed through the hoop and the downwind screen, respectively.

Three experimental conditions were evaluated: condition 1, a pheromone plume in moving air; condition 2, a pheromone plume in still air; and condition 3, no pheromone plume in still air. Condition 1 was obtained by releasing pheromone into air moving through the tunnel at a velocity of 7 cm/sec. In condition 2 a pheromone plume was formed in air moving at 7 cm/sec, but the air flow was stopped as soon as a moth entered the plume on the downwind end. At that time, solid baffles were moved into position on both ends of the tunnel and the release of pheromone from the source was terminated. Prior experience with visible smoke showed that movement of the air in a downwind direction ceased immediately and the plume remained essentially intact and suspended in space for about 20 seconds before it started to break up. Condition 3 was obtained by releasing only a 5-second pulse of pheromone from the source into air moving at 7 cm/sec.

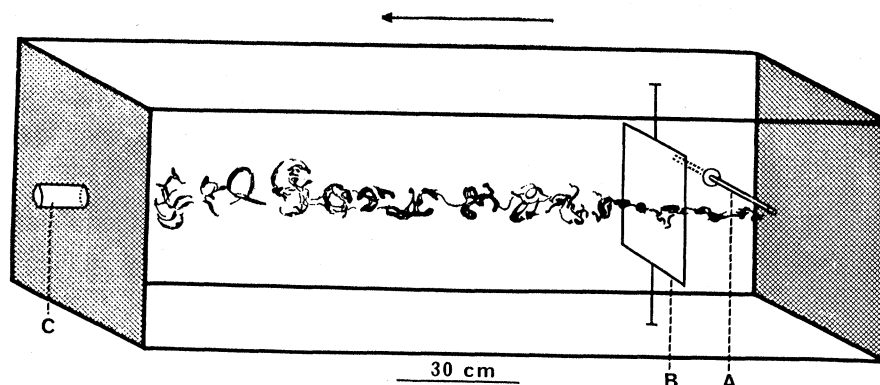


Fig. 1. Flight tunnel, showing pheromone source (A), wire hoop (B), and release cage for male moths (C). An artist's representation of an odor plume, based on an actual photograph of a visible smoke plume, is drawn in the tunnel. Screens on the ends of the tunnel are represented by cross hatching. The arrow indicates the direction of the wind.

The pulse activated the males to fly and then it was exhausted from the tunnel. As soon as the first male took flight, the air flow was stopped, as in condition 2. For all conditions, moths were scored on whether they flew through or outside of the hoop when moving in an "upwind" direction in the tunnel. In condition 2, if a moth had not passed the hoop within 20 seconds, that particular replicate was abandoned. Data for 20 moths were obtained for each condition.

The results clearly demonstrate that the moths oriented equally well when the pheromone plume was in moving air (condition 1) or in still air (condition 2) (Table 1). In the absence of a pheromone plume and in still air (condition 3), few moths flew near the central axis of the tunnel. The only role of air movement appears to be that of creating the shape of the plume. Visual and photographic analysis of moth flight patterns showed a characteristic lateral zigzag or sinusoidal movement, with the lateral amplitude approximating the dimensions of the plume (Fig. 2).

Some physical characteristics of the structure of the odor plume must be used by the flying moths to determine the longitudinal axis of the plume and the direction of the odor source. A plume is not a uniform mass of mole-

Table 1. Numbers of male moths flying through the hoop and outside of the hoop for the three test conditions.

Conditions	Number flying through hoop*	Number flying outside hoop
1: Pheromone plume in moving air	17	3
2: Pheromone plume in still air	16	4
3: No pheromone plume in still air	3	17

\* The numbers of males flying through the hoop were significantly greater in conditions 1 and 2 than in condition 3, as determined by a  $\chi^2$  contingency test at the .05 probability level.

cules but is filamentous in structure (Fig. 1) with the average molecular density being higher at the longitudinal axis than near the edges (5). We speculate that, as the moth traverses at an angle across the plume, it is stimulated to repeatedly turn back toward the longitudinal axis when it encounters a certain decreased frequency of molecules or filaments. However, we still do not understand how the moth distinguishes the "upwind" from the "downwind" direction. Wright (6) proposed a similar mechanism for insect orientation to an odor source, although until now his proposal has not received experimental support.

Butler (7) recently proposed that

odor plumes used by insects to orient toward the source be called "trails." A number of terrestrial animals, including dogs and ants, follow odor trails that are laid on the ground. Their following behavior is characterized by a zigzag running, which may allow them to sense the lateral boundaries of the trail as they run along it (8). Moreover, many flying insects display a zigzag flight pattern as they approach an odor source (9); thus, this method of aerial trail-following may be widespread in nature.

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#### References and Notes

1. V. G. Dethier, in *Survey of Biological Progress*, B. Glass, Ed. (Academic Press, New York, 1957), vol. 3.
2. Anemotaxis has been demonstrated rigorously for two flying insects, the vinegar fly, *Drosophila melanogaster* [F. E. Kellogg, D. E. Frizel, R. H. Wright, *Can. Entomol.* **94**, 884 (1962)], and the yellow-fever mosquito, *Aedes aegypti* [J. S. Kennedy, *Proc. Zool. Soc. London Ser. A* **109**, 221 (1939); R. H. Wright, *World Rev. Pest Contr.* **1**, 2 (1962)]. In both of these species, the insect uses vision to sense the direction of apparent movement of patterns on the ground over which it is flying. To fly upwind, it steers so that the patterns move parallel to its body axis, in a front-to-rear direction. If the insect flies at some angle with respect to the wind direction, sideslip of its body causes an apparent oblique movement of the patterns on the ground. Presumably, the insect then visually reorients itself so that it again steers directly upwind. The anemotaxis reaction can be altered experimentally by moving the patterns in different directions beneath flying insects. In our preliminary experiments, we used treadmills to artificially move ground patterns beneath flying male moths that had been stimulated by pheromone. These manipulations caused no consistent changes in the moth flight directions, thus casting doubt on their use of anemotaxis as a steering mechanism.
3. H. H. Shorey, in *Control of Insect Behavior by Natural Products*, D. L. Wood, R. M. Silverstein, M. Nakajima, Eds. (Academic Press, New York, 1970).
4. Smoke was produced by mixing separate vapors of  $\text{NH}_4\text{OH}$  and  $\text{HCl}$ , which generated particles of  $\text{NH}_4\text{Cl}$  at that location within the tunnel from which pheromone was later released.
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8. E. O. Wilson, *Anim. Behav.* **10**, 134 (1962); and W. H. Bossert, *Recent Progr. Hormone Res.* **19**, 673 (1963).
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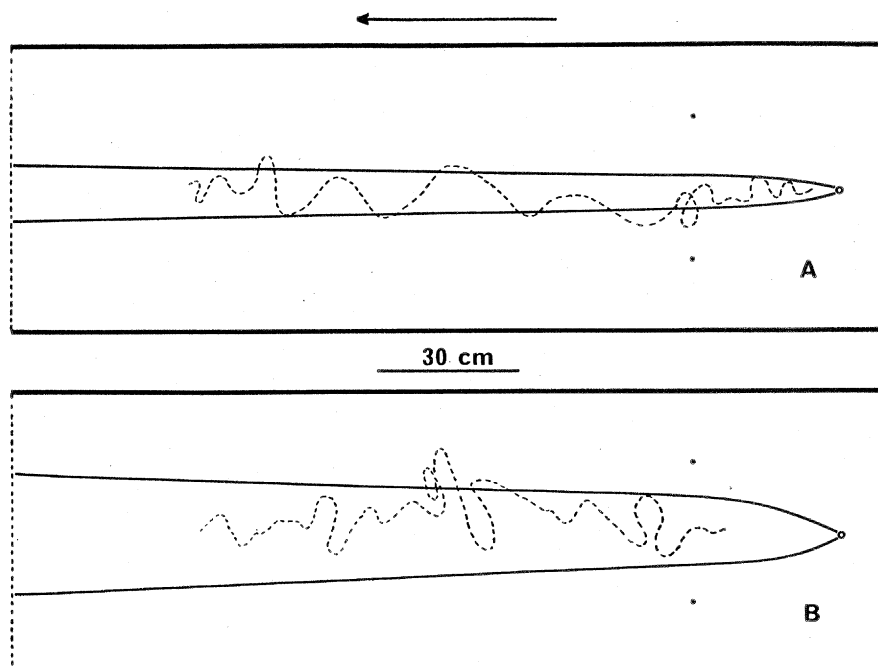


Fig. 2. Tracings of photographic records of moth flight tracks superimposed on the outline of a time exposure of a smoke plume. Photographs were taken from a top view. (A) Track of a moth flying through an odor plume in air moving at 25 cm/sec. (B) Track of a moth flying through an odor plume in still air; the plume was produced earlier in air moving at 7 cm/sec. Dots indicate the outside dimensions of a wire hoop within the tunnel. The arrow indicates the direction of the wind.