Journal of Insect Behavior, Vol. 7, No. 5, 1994

Visual Feedback in the Control of Pheromone-Mediated Flight of *Heliothis virescens* Males (Lepidoptera: Noctuidae)

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Accepted September 8, 1993; revised March 11, 1994

Male Heliothis virescens *(F.) (Lepidoptera: Noctuidae) were made to fly into a uniformly white and translucent tube within a large wind tunnel while responding to sex pheromone. Different visual patterns placed within the tube greatly affected the ability of the male moths to maintain upwind progress or remain oriented to the wind while in contact with the plume. Over 89% of males attempting to fly through a blank tube, lacking visual patterns, became disoriented, the males gaining or losing altitude and repeatedly hitting the sides of the tube. Patterns of 20–40 dots placed on the sides of the tube at or slightly above plume level resulted in high levels of sustained upwind flight (47–74%) relative to patterns placed directly below (30–40%), directly above (35%), or slightly below the level of the flight path (26–44%). Optimal upwind progression in pheromone-responding males occurred when image motion could be resolved both transversely (T), orthogonally to the longitudinal axis of the body relative to the horizontal plane of the environment, and longitudinally (L), along the body axis. Even very sparse patterns (single rows of dots) could elicit high levels of sustained upwind flight (53–63%) when positioned within the tube such that the males' movements would create both L and T image motion. However, successful negotiation of the tube was also unexpectedly facilitated by patterns apparently providing no horizontal transverse component for flying males but providing longitudinal flow while centering the moth in the plume through a symmetrical left–right input (4–40%).*

KEY WORDS: *Heliothis*; Lepidoptera; Noctuidae; pheromone; visual feedback.

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INTRODUCTION

The location of a calling female moth by a conspecific male often involves the male flying over a distance of at least several meters. Intermittent contact with the pheromone plume is important in sustaining upwind flight by the males (Kennedy *et al.*, 1981; Willis and Baker, 1984; Baker *et al.*, 1985). However, it is the wind carrying the filaments of odor, not any intrinsic property of the plume itself, that indicates the direction towards the female. Over an open field, David *et al.* (1982) showed with smoke that packets of pheromone-bearing air will travel in straight lines away from their (female) source, and thus a moth that progresses upwind only while in contact with pheromone will be heading directly towards the source. Upwind progress is not maintained by orienting with respect to chemical gradients (chemotaxis) but, instead, is accomplished by means of visual input followed by appropriate compensatory movements (optomotor anemotaxis) (Kennedy and Marsh, 1974; Marsh *et al.*, 1978; Baker and Haynes, 1987).

Kennedy (1940) was the first to demonstrate the use of optomotor anemotaxis by odor-stimulated flying insects. The work of Kennedy and Marsh (1974) and Marsh *et al.* (1978) extended this framework, centered around a concept known as the triangle of velocities, to explain how a flying insect might visually detect and compensate for wind-induced displacement during upwind progress. When a flying insect is drifted off its course by wind, images flow across the eyes at an angle with respect to the long axis of the insect (cf., Figs. 1A and B). One hypothetical mechanism that insects use to resolve the direction of the wind is to break down this *off-axis* image flow into orthogonal components (Fig. 1B). One of these components is *longitudinal* (*L*) along the body's long axis (*along-axis*) and the other is at right angles to this axis (*transverse*, *T* or *across-axis*) (Fig. 1B). To steer and progress upwind at a particular angle with respect to the wind while maintaining a certain ''preferred'' ground speed, a moth must maintain a front-to-back component along its longitudinal axis (longitudinal; L) integrated with the orthogonal across-axis component (transverse; T) from the image motion that results from the discrepancy between its wind-displaced resultant path and its heading (Fig. 1B). By off-axis image flow then, we mean image flow that is *not* directly aligned with either the longitudinal or the transverse axes of the insect (Fig. 1).

David (1982a; 1986) provided evidence that flies respond to image motion beneath them by turning with or against the transverse component of flow to produce upwind or downwind flight, respectively. Baker and Vickers (1994) demonstrated that moths flying upwind were also responsive to transverse movement of a floor pattern. Both moths (Marsh *et al.*, 1978; Willis and Cardé, 1990) and flies (David, 1979; 1982a) orienting in response to windborne odor in a variety of wind speeds have been shown to maintain relatively constant

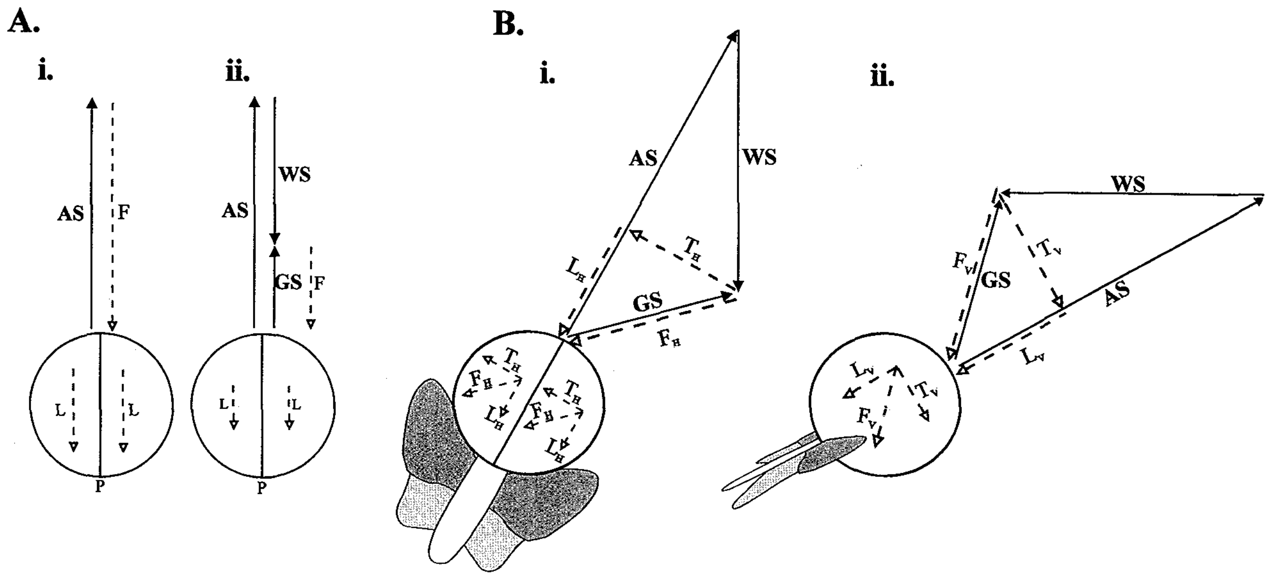


Fig. 1. A: Longitudinal, along-axis image flow (L) is created in zero wind (i) during forward flight in any direction [air speed (AS) = ground speed (GS) = −image flow (F)] or in wind (ii) (wind speed; WS) during steady forward flight directly upwind (AS − WS = GS = −F) or downwind (AS + WS = GS = −F). P represents the posterior of the moth's head. B: (i) Off-axis image flow results from an insect flying upwind at an angle with respect to the wind direction. The insect, viewed from above will be drifted from its course (assumed here to be aligned with the long axis of the insect) (Marsh *et al.*, 1978; David, 1986). The resulting image flow (F_H), derived from motion in the horizontal plane, is aligned with neither the long axis nor the transverse axis of the insect (in A, note that the image flow was resolved as completely along-axis, L). However, it can be broken down into these two orthogonal components. The insect must keep L_H (along-axis) from front to back to maintain upwind progress. The amount of drift is indicated by the size of T_H (across-axis). Turning in the direction of T flow allows the insect to steer more into the wind. (ii) Off-axis flow (F_V) can also occur in the vertical plane when the insect (viewed from the side) is drifted vertically from its course. In this case, turning with the across-axis component (T_V) does not steer the insect more into the wind, however, it allows the insect to compensate for changes in altitude only during upwind flight. The orthogonal along-axis component (L_V) for the regulation of ground speed can still be determined from this off-axis flow.

ground speeds. Ludlow (1984) postulated that wind-drifted insects keep L + T constant in an attempt to maintain constant ground speeds. However, Willis and Cardé (1990) provided evidence that although male *Lymantria dispar* (L.) maintained relatively constant ground speeds over a variety of wind speeds, this was not achieved by keeping horizontal L + T constant. In their experiments males kept L relatively constant, while T varied significantly.

A growing body of neurophysiological evidence indicates that visual receptors and higher-order neurons respond to movement over a wide field in a vertically or horizontally preferred direction. These neurons have been found in moths (Collett and Blest, 1966; Olberg and Willis, 1990; Milde, 1993), bees (Fletcher *et al.*, 1984; Ibbotson and Goodman, 1990), butterflies (Ibbotson *et al.*, 1991), and flies (Hausen, 1982a, b; Eckert, 1982; Gronenberg and Strausfeld, 1991; Egelhaaf and Borst, 1993). The resolution of image motion into longitudinal and transverse components could be achieved by such neurons, and they may play a role in mediating upwind flight responses in moths.

David (1986) pointed out that the classical optomotor response (Goetz, 1972; 1975) might be directly related to the compensatory movements made during flight in wind. The same motion detectors in the anterior portion of the eye that respond to rotational movement in the classical optomotor system would respond to off-axis image flow in a moth blown off its course while flying in the wind. The posterior portion of the eye would be inoperative and the anterior detectors would respond to either T or L movement produced by the linear, not rotational, flow. These hypotheses both suggest that off-axis image motion plays an important role in the compensatory responses of moths flying in the wind.

Another plausible mechanism for monitoring wind-induced drift may involve image motion over poles or foci of the insect compound eye (Collett, 1980). These poles are created by the lack of image motion on the part of the eye that is in line with or parallel to the resultant of the translatory forces of thrust and lift, generated by the wings. Images appear to move out from these foci and have maximal retinal velocity on ommatidia at right angles to the direction of thrust (Collett, 1980). If a moth is moving without gaining altitude, in zero wind, the poles will be directly in front of and behind the moth. However, sideways displacement caused by the wind will shift the poles from this forward-facing position in such a way that a moth might be able to monitor wind-induced drift by correcting for the movement of the pole position.

As wind is largely the *horizontal* movement of air over the ground, the resolution of image flow into an across-axis component (transverse), orthogonal to the longitudinal axis of the moth, is of use to the moth in detecting wind-induced azimuthal displacement only when it corresponds to motion in the *horizontal* plane of the environment. Image motion can also be off-axis due to movement relative to the vertical plane of the environment (Fig. 1B, ii). Evidence suggests that the resolution of this type of visual flow into an across-axis component, this time vertically, is important in the regulation of altitude (David, 1979, 1986; Preiss and Futschek, 1985) (Fig. 1B, ii). Even though off-axis image motion in the vertical plane can be wind-induced, the across-axis image flow component resulting from this displacement cannot play a role in assessing horizontal transverse drift, and hence, cannot aid in steering with respect to wind direction. However, off-axis image motion in a vertical plane can be resolved into an along-axis component aligned with the longitudinal axis of the moth, and thus, can assist in the regulation of ground speed (Fig. 1B, ii). Regardless of the mechanism for detecting and compensating for changes in the path relative to the wind and ground speed along the path, it still follows that if visual surroundings are presented in which off-axis image flow relative to the horizontal plane of the environment or longitudinal image flow cannot be resolved, then pheromone-stimulated moths should be unable to maintain sustained upwind flight.

As with other species of moths on which extensive pheromone research has been conducted, little is known about the mechanisms by which *Heliothis virescens* (F.) males control their flight to result in upwind progress in response to pheromone. Several previous studies with other species have attempted to use moving ground patterns to investigate the way in which flying insects respond to wind (Kennedy, 1940; Kellogg *et al.*, 1962; Farkas and Shorey, 1972; David, 1982b). The most sophisticated experiment to date used a "barber's pole"-type wind tunnel to study how untethered *Drosophila hydei* Sturtevant fruit flies regulated their altitude and flight speed in the wind (David, 1982a).

In the present series of experiments, we were interested in investigating the relative importance of different areas of the visual field to the flight control system of free-flying *H. virescens* males during pheromone-mediated upwind flight. We observed moths flying within a controlled visual surround in which we varied patterns to restrict the moths' ability to obtain off-axis image motion relating to wind-induced horizontal or vertical (altitudinal) drift.

MATERIALS AND METHODS

Heliothis virescens larvae were reared on a modified pinto bean diet (Shorey and Hale, 1965) and separated, by sex, at pupation. Male pupae were maintained in separate environmental chambers from the females on a 14:10-h L:D cycle at 25°C. An 8% sucrose solution was available within the pupal-emergence cage. Males were used in the wind tunnel at between 4 and 7 days of age and were placed in individual flight cages (6-cm-long × 6-cm-diameter cylindrical wire screen) prior to the onset of scotophase on the day that they were to be used. To acclimate the moths to wind-tunnel conditions, trays

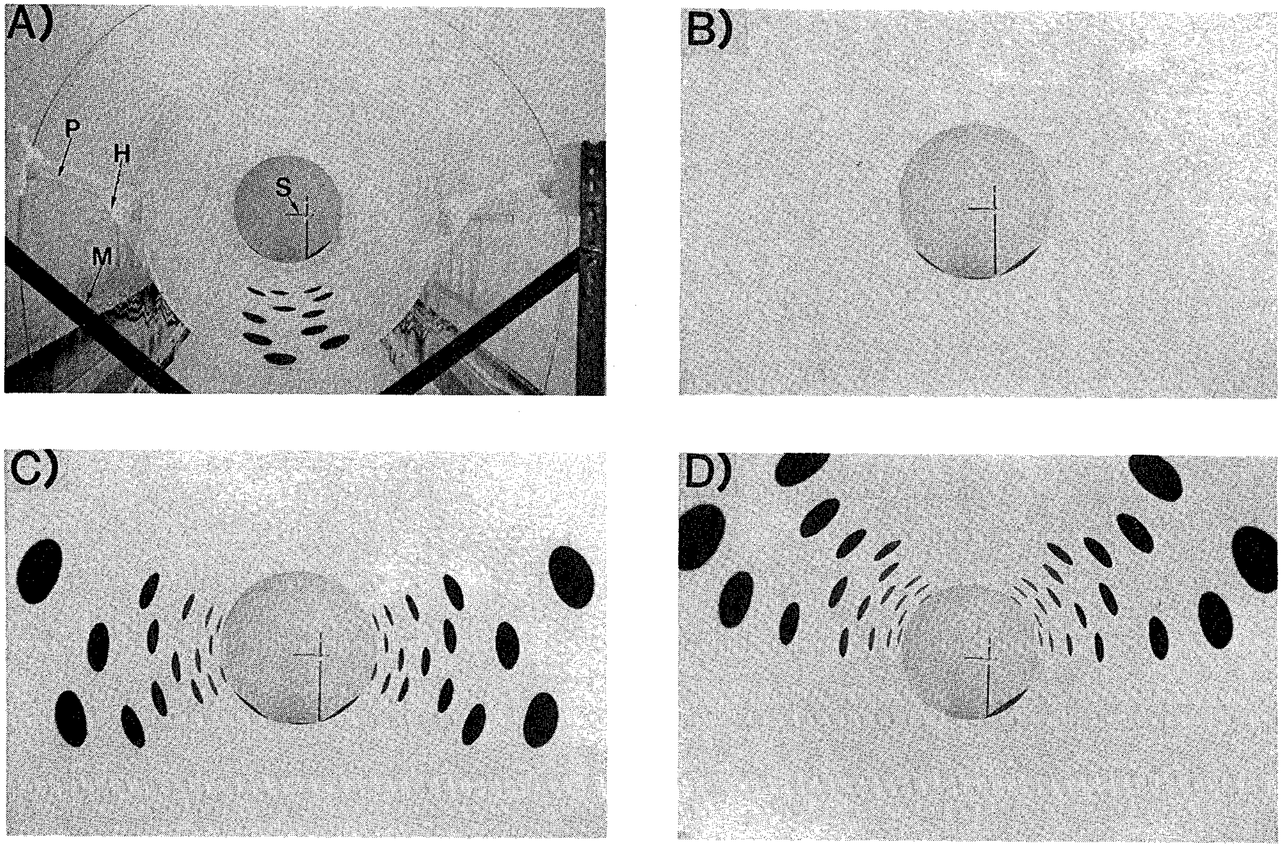


Fig. 2.

Fig. 2. (A) Photograph looking directly upwind along the longitudinal axis of the experimental tube, as viewed from the downwind section of the wind tunnel, reveals the supporting metal frame (M) and Plexiglas poles (P). The plastic hammock (H) supporting the tube is also visible in this picture. The pattern displayed in the tube is the 6 o'clock—halfway (see also Fig. 3, 1C). In the background, at the upwind end of the wind tunnel, the stand (S) used to support the pheromone septum is visible. (B) Photograph of the blank tube as viewed from the downwind mouth of the tube looking directly upwind along the longitudinal axis of the tube. (C) Same perspective as in B, but this time a closeup of the 9 and 3 o'clock (full) pattern. This pattern was used in experiments 2, 4, and 5 (Fig. 3, 2D; Fig. 4, 4B and 5B, respectively). On each side of the tube only 16 of the 20 6-cm-diameter dots utilized can be seen owing to the closeup nature of this picture. (D) Same perspective as in B and C, of the 10 and 2 o'clock pattern used in experiment 3 (Fig. 3, 3F). This pattern proved to be the most successful at eliciting sustained upwind flight (Fig. 3, 3F). All 20 of the dots utilized on each side of the tube are visible.

filled with the flight cages were placed in the wind tunnel at least 1 h prior to the commencement of the experiment. Moths were tested between the fifth and the eighth hours of scotophase, when they are most responsive to the female pheromone (Vetter and Baker, 1983).

A special apparatus was required for this study, in which the visual environment had to be experimentally controlled. To this end, a one-of-a-kind Plexiglas tube was constructed by Commercial Plastics, Gardena, California. The tube measured 128.3 cm in length and had an outer diameter of 50.2 cm, the inner diameter measuring 48.9 cm. The white coloration of the tube rendered it translucent at the low light levels used in this experiment. The tube was supported within the wind tunnel by a metal trolley. A clear vinyl "hammock" was supported between two clear Plexiglas poles attached to the rigid metal frame. The tube was suspended within this hammock (Fig. 2A).

The wind-tunnel structure utilized has been described by Kuenen and Baker (1982). Conditions in the tunnel were maintained as follows: wind speed, 50 cm/s; temperature, 25°C (±2°C); and relative humidity, 60%. The lighting was carefully arranged such that its intensity did not vary along the length of the tunnel. A white cotton sheet placed over the outer acrylic shell of the tunnel further diffused the light and minimized any vertical light gradients inside. The intensity of light as measured inside the white tube was 0.5 lux.

The utilization of a see-through supporting mechanism and the white-sheet diffusion of light served to prevent shadows from being cast onto the outside of the tube and hence serving as unwanted visual cues (Fig. 2B). Therefore, the only visual cues available to moths orienting upwind while in the tube were those placed on the surface of the tube by us (Figs. 2C and D) plus, perhaps, the ends of the tube, which could potentially provide parallax information.

The visual patterns introduced into the tube consisted of dots made of matt-black, pressure-sensitive vinyl with a thin patio-vinyl backing. By moistening the patio-vinyl, the dots could be made to adhere to the surface of the tube in any orientation (Figs. 2C and D). In the first experiment 9-cm-diameter dots were utilized. Dots 6 cm in diameter were used in the remainder of the experiments. Preliminary trials had indicated that dots with a diameter less than 6 cm were not effective at eliciting sustained orientation through the tube when the number of dots was held constant (Vickers and Baker, unpublished observations). With the 6-cm dots, patterns could be placed in well-defined areas of the tube such that after entering the tube, a moth flying toward the pheromone source would have to use these patterns to regulate its upwind progress visually. Males were released into the plume 70 cm downwind of the tube and thus, were required to begin upwind progress in the plume to enter the tube. Hence, only pheromone-responsive males did so. A red dot pattern (10-cm diameter) was used along the floor of the wind tunnel, prior to the entrance of the tube. Also, underneath the white sheet covering the Plexiglas exterior of the wind tunnel, 9- and 6-cm-diameter black dots were made to adhere. The moths therefore experienced stimulation from the entire visual field prior to entering the tube.

The pheromone-impregnated septum (A. H. Thomas Co., No. 8753 D22, sleeve type, 5 × 9 mm) contained a mixture of the six components known to contribute to sustained upwind flight in male *H. virescens* (Vetter and Baker, 1983; Teal *et al.*, 1986). The exact blend loaded onto the septum consisted of Z11-16:Ald (100 μg), Z9-14:Ald, 16:Ald, 14:Ald, Z9-16:Ald, and Z7-16:Ald at a 100:2.5:50:5:1:1 ratio, respectively (Vetter and Baker, 1983). The septum was positioned on a platform at the upwind end of the wind tunnel approximately 1 m beyond the upwind mouth of the tube. The plume, as visualized by $TiCl_4$ smoke, moved exactly down the center of the tube. Smoke plumes traveling down the center of the tube did not appear to be any larger or more turbulent than plumes created by a smoke source in the wind tunnel lacking the tube apparatus. To maintain contact with the plume a moth would have to fly upwind right through the center of the tube, equidistant from the placement of any pattern on the inner surface.

We performed a series of experiments, using the following patterns of dots placed at different positions in the tube (the clock perspective is taken looking upwind through the tube, with 6 o'clock, for instance, indicating that dots were positioned along bottom center).

Experiment 1: A, blank; B, 6 o'clock; and C, 6 o'clock—halfway (Fig. 3).

Experiment 2: A, blank; B, 6 o'clock; C, 12 o'clock; and D, 9 and 3 o'clock (Fig. 3).

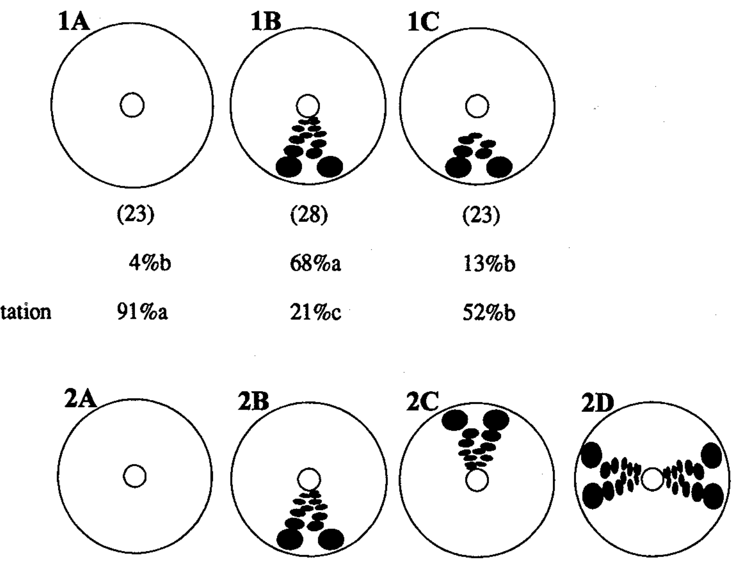
Experiment 3: A, blank; B, 6 o'clock; C, 8 o'clock; D, 10 o'clock; E, 8 and 4 o'clock; and F, 10 and 2 o'clock (Fig. 3).

Experiment 4: A, blank; B, 9 and 3 o'clock—full pattern; C, 9 o'clock—line of five dots; D, 9 o'clock—6-cm-wide solid bar; E, 9 and 3 o'clock—line of five dots; and F, 9 and 3 o'clock—6-cm-wide solid bar (Fig. 4).

Experiment 5: A, blank; B, 9 and 3 o'clock—full pattern; C, 9 and 3 o'clock—line of five dots; D, 9 and 3 o'clock—line of nine dots; E, 10 and 2 o'clock—line of five dots; and F, 10 and 2 o'clock—line of nine dots (Fig. 4).

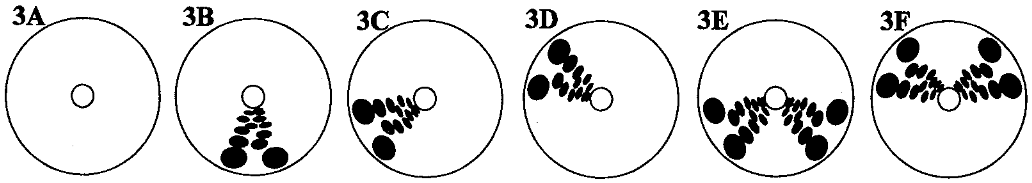
Experiment 6: A, blank; B, 9 and 12 o'clock—line of five dots; C, 9 and 3 o'clock—line of five dots; D, 6 and 12 o'clock—line of five dots; E, 10 and 2 o'clock—line of five dots; and F, 8 and 4 o'clock—line of five dots (Fig. 4).

In experiments 1-3 (Fig. 3) and in experiments 4 and 5 (Fig. 4, 4B and 5B), treatment B, a treatment "time" represents a spread of 20 dots over a 2-h arc of the tube centered at the hour(s) indicated (Figs. 2, 3, and 4). As



EXPERIMENT	RESPONSE	A	B	C	D
1	(*N*)	(23)	(28)	(23)	
	Complete Flight	4%b	68%a	13%b	
	Complete Disorientation	91%a	21%c	52%b	
2	(*N*)	(33)	(35)	(37)	(36)
	Complete Flight	3%b	40%a	35%a	58%a
	Complete Disorientation	88%a	29%b	35%b	17%b

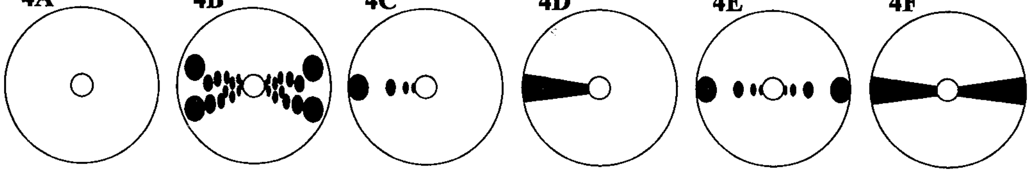
Fig. 3. Percentages of male *H. virescens* successfully performing completely oriented flight in response to their sex pheromone, or becoming completely disoriented, when made to fly through a translucent white tube on which were placed various spotted patterns. Each treatment is depicted schematically within each experiment, the perspective of the viewer looking upwind through the downwind mouth of the tube directly along its longitudinal axis (see Fig. 2 for perspective). The position of the dots in each schematic is accurate but their numbers may vary (see text for details). Percentages of moths flying through the tube while "completely oriented" (= complete flight) or of moths that became "completely disoriented" within the tube are listed for each treatment. Percentages in the same row having no letters in common are significantly different ($P < 0.05$) according to an adjusted χ^2 (2 × 2) test of independence; *N*, listed below each tube pattern, equals the number of males that entered the tube for that particular treatment.



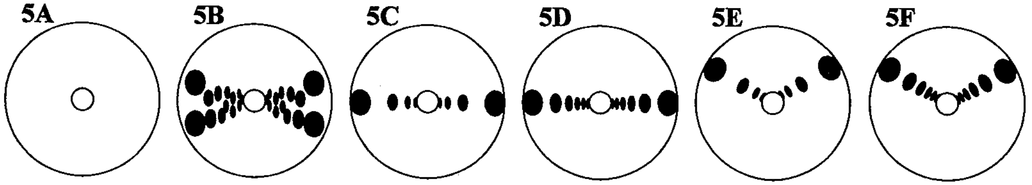
		3A	3B	3C	3D	3E	3F
	(*N*)	(35)	(33)	(27)	(31)	(41)	(35)
3	Complete Flight	11%c	30%bc	26%bc	52%ab	44%b	74%a
	Complete Disorientation	71%a	48%ab	37%bc	16%cd	32%bc	3%d

Fig. 3. Continued.

VISUAL TREATMENT

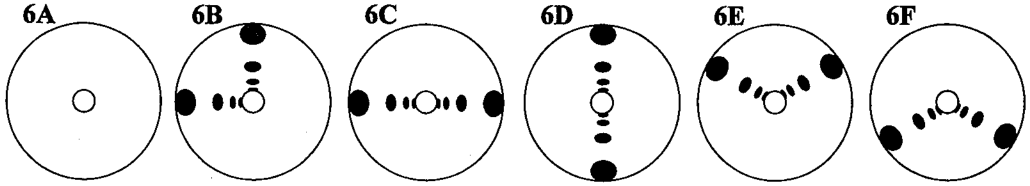


EXPERIMENT	RESPONSE	4A	4B	4C	4D	4E	4F
	(*N*)	(31)	(30)	(32)	(27)	(28)	(25)
4	Complete Flight	3%b	47%a	6%b	0%b	4%b	40%a
	Complete Disorientation	90%a	23%b	84%a	81%a	89%a	28%b



EXPERIMENT	RESPONSE	5A	5B	5C	5D	5E	5F
	(*N*)	(26)	(26)	(25)	(29)	(30)	(27)
5	Complete Flight	4%c	69%a	24%bc	31%b	53%ab	63%a
	Complete Disorientation	69%a	4%c	44%ab	34%b	23%bc	7%c

Fig. 4. See legend to Fig. 3. Dots are accurately positioned within each schematic but the actual numbers of dots used vary according to the text.



	(*N*)	(53)	(49)	(53)	(46)	(52)	(49)
6	Complete Flight	2%d	10%cd	30%b	22%bc	54%a	31%b
	Complete Disorientation	87%a	45%b	42%b	48%b	27%b	33%b

Fig. 4. Continued.

such, to a moth flying upwind along the plume, a 6 o'clock pattern (spread from 5 to 7 o'clock) represents a simulated ground pattern; 12 o'clock, a pattern directly above the male; and 9 and 3 o'clock, patterns on either side of the male. The dots were spread randomly throughout the length of the tube but no dots overlapped. A template of the pattern ensured that the dots could be placed in the same pattern whatever the orientation of the tube and also ensured that the pattern could be replicated. In the first experiment 9-cm-diameter dots were used, and consequently, they covered a slightly larger arc of the tube than the 6-cm-diameter dots used in latter experiments. All experiments were performed using a randomized complete block design.

In each experiment, the behavior of the moths was noted by an observer standing at the back of the wind tunnel watching the progress of each moth through the inner experimental tube. Observations were aided by night-vision goggles, and for the purposes of analysis, the behaviors observed were broken down into three categories: those moths able to complete flight through the tube without hitting the sides, emerging from the upwind end of the tube (called "complete flight"); those moths that hit the sides of the tube one to three times but appeared able to maintain upwind orientation, often emerging from the upwind end (called "partial disorientation"); and finally, those moths that became completely disoriented, hitting the sides of the tube more than three times (called "complete disorientation") in the latter category. Moths often emerged from the downwind end of the tube whence they had started.

To compare differences between treatments, the second group was excluded. This was done because those moths that touched the sides of the tube might have been able to obtain information about wind speed and direction through mechanoreceptive means and not solely through available visual information. Mosquitoes flying in complete darkness are suspected of detecting the wind shear at the boundary between moving air and stationary ground, and using this as a cue to resolve wind direction (Gillett, 1979; Kennedy, 1986). Moths touching the tube surface could gain information about wind direction through mechanoreception in a similar manner, and therefore, including moths from this category of behavior in our analysis could possibly give equivocal results concerning the visual guidance of flight control. In each experiment, the numbers of moths falling into the first (complete flight) and third (complete disorientation) categories were compared by means of an adjusted χ^2 2 × 2 test of independence (Steel and Torrie, 1960).

The flight tracks of some moths were recorded by a videocamera located at the downwind end of the wind tunnel. Videotaped tracks were analyzed as detailed in previous studies (Baker *et al.*, 1985; Baker and Haynes, 1987; Willis and Baker, 1984). The tracks do not reveal the three-dimensional position of the moth, as only one camera, looking directly through the tube toward the upwind end of the tunnel, was utilized.

RESULTS

When given a depauperate visual environment consisting of a blank tube, very few moths were able to remain completely oriented (≤11% in all experiments) while flying through the tube. The majority became completely disoriented, with a few exhibiting partial disorientation (Figs. 3 and 4, experiments 1–5, treatment A). Generally, moths flying into a blank tube appeared unable to maintain their position within the tube and usually gained altitude (Figs. 5C and 5F). Flying too far to one side or the other in the horizontal plane also resulted in loss of control of position with respect to the pheromone plume. Any of these movements would take the moth out of plume proximity and usually resulted in the moth contacting the tube's interior at a position above the midline. The sides and ceiling of the tube were usually contacted several times before the males were carried out of the downwind end by the wind (Figs. 5C and 5F).

With a pattern running the entire length of the tube at the 6 o'clock position (Fig. 3, 1B), 68% of the moths were able to fly through completely oriented. Figure 5A shows a flight track typical of such males. However, males did not successfully complete flights any better (13%) with a pattern halfway along the tube at 6 o'clock than with the blank tube (4%) (Fig. 3, 1C *vs.* 1A, respectively), showing that once males reached the middle of the tube and encountered a featureless surface, they could not use other cues to complete their flights, such as parallax offered by the receding (downwind) or approaching (upwind) ends of the tube.

The second experiment was designed to investigate the importance of differing areas of the visual field in promoting upwind flight (Fig. 3, experiment 2). As expected, the treatment simulating a ground pattern (Fig. 3, 2B) and one simulating an overhead pattern (Fig. 3, 2C) gave relatively good results (40 and 35% complete orientation, respectively), although the 6 o'clock pattern was less effective than in experiment 1, seemingly due to the use of smaller dots over a lesser arc. The 6-cm-diameter dots were the smallest that could be used at a density of 20 dots spread over a 2-h arc without causing high levels of disorientation (Vickers and Baker, unpublished observations). The pattern on the sides (Fig. 3, 2D) was as good at evoking completely oriented flights (58%) as the ground- and overhead-type patterns. Figure 5B shows a typical flight track of males responding to this pattern and Fig. 5E shows a composite of four tracks demonstrating the males' ability to stabilize and sustain their upwind flight. We had thought that this pattern should reduce the amount of available off-axis image flow, resolvable into an across-axis component (corresponding to motion in the horizontal plane of the environment) compared to the 6 or 12 o'clock pattern, while retaining a comparable amount of longitudinal flow.

A separate series of patterns was then tested (experiment 3) in which the 2-h spread of dots at 9 and 3 o'clock was moved up or down to 10 and 2 o'clock

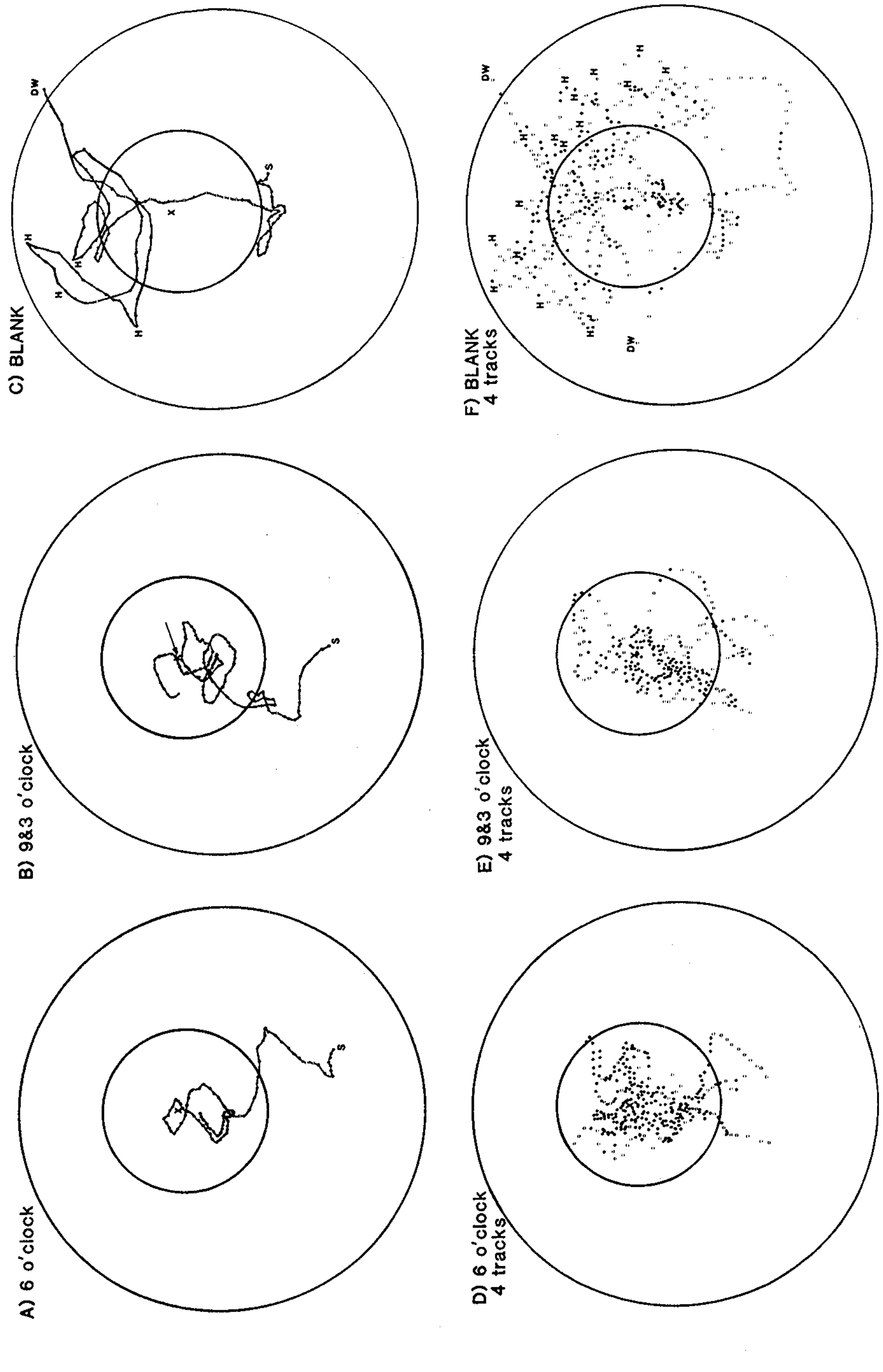

A) 6 o'clock
S
X
B) 9&3 o'clock
S
C) BLANK
DW
S
X
H
H
H
H
D) 6 o'clock
4 tracks
E) 9&3 o'clock
4 tracks
F) BLANK
4 tracks
DW
DW
H

Fig. 5.

Fig. 5. Tracks typical of males responding to three types of pattern including an otherwise blank tube (A–C). The perspective is looking along the longitudinal axis of the wind tunnel as in Fig. 2, the smaller circle being the upwind end of the tube. X marks the position of the pheromone septum source (arrowed in B), and S the start of the flight track. The position of the males is marked every 1/30th of a s (black dots). The tracks appear in two dimensions even though the moths are making progress up the tunnel (A, B, D, and E). With patterns placed in appropriate areas of the tube (A, 6 o'clock; B, 9 and 3 o'clock wide pattern), males flew through the tube without experiencing difficulty. However, in C (the blank) the male loses control over its displacement relative to the wind shortly after entering the tube. This moth gained altitude rapidly contacting the top of the tube (H) repeatedly and eventually emerging from the downwind (DW) end of the tube. In D and E, a composite of four tracks for each pattern is displayed. Note the tight distribution of points for tubes containing visual cues (D and E), whereas in the blank tube males were unable to maintain their orientation, many gaining altitude and contacting the upper half of the tube (H). Two of the males responding to the blank tube emerged from the downwind opening (DW).

or 8 and 4 o'clock, respectively. These patterns should have improved the moths' ability to resolve an across-axis component from image motion in the horizontal plane while maintaining the longitudinal component, thereby improving their upwind orientation accuracy. The patterns at just 10 o'clock and 8 o'clock were included to document any possible effect that symmetry of pattern may have played. The best treatment in this experiment was the 10 and 2 o'clock pattern (Fig. 3, 3F). The only other treatment capable of eliciting a comparable level of completely oriented flights was the asymmetrical 10 o'clock pattern (Fig. 3, 3D). All other patterns were less effective at eliciting stable, directed upwind flight.

In experiment 4, single lines of dots or bars were placed right at the level of the plume in an attempt to severely restrict off-axis image flow in the horizontal plane. Patterns that we thought would allow minimal or zero off-axis image flow in this plane, such as bars or lines of dots at 9 or 9 and 3 o'clock (Fig. 4, 4C–4E), evoked no greater levels of successful, completely oriented flight than did the blank control (Fig. 4, 4A). However, the one apparent anomaly to this situation was the success rate of complete orientation of males responding to the 9 and 3 o'clock—bar pattern (Fig. 4, 4F). This treatment elicited completely oriented flights in 40% of the males, not significantly different from the 47% oriented flights to the positive control (9 and 3 o'clock—complete pattern; Fig. 4, 4B). The level of completely oriented flights to the positive control (Fig. 4, 4B) was noted as being lower compared to previous and subsequent experiments using this pattern, and may have been a reflection of an overall lower level of responsiveness in the moths.

If the lack of off-axis image motion corresponding to wind-induced drift in the horizontal plane had been the cause of the failure of males to successfully complete their flights in response to the lines of dots in experiment 4, we reasoned that by shifting the sparse line of five dots from 9 and 3 o'clock to 10 and 2 o'clock, the ability to resolve any off-axis image flow into across-axis components with respect to both horizontal (T_H) and vertical (T_v) planes, as well as along-axis (L), would be increased, and moths would be better able to fly through the tube completely oriented. This treatment was presented to males in experiment 5 (Fig. 4), in which lines of five and nine dots were compared at the 9 and 3 o'clock and at the 10 and 2 o'clock positions. The line of nine dots was included in this experiment in case the five dots used in the previous experiment had not provided enough visual stimulation.

The results (Fig. 4, experiment 5) indicate that when the dots were shifted up on the sides of the tube to the 10 and 2 o'clock position (Fig. 4, 5E and 5F), allowing off-axis image flow relative to both the horizontal and the vertical planes of the wind tunnel, the moths were then able to orient better through the tube at success rates comparable to the 9 and 3 o'clock (full pattern) control treatment (Fig. 4, 5B). Enriching the visual environment did not significantly increase the number of successful flights compared to the sparse treatments (Fig. 4; 5D and 5F were not better than 5C and 5E, respectively).

Clearly, sparse lines of dots in the visual field were capable of eliciting high levels of completely oriented flight dependent upon their positioning within the tube (Fig. 4, experiment 5). One variable that had not yet been tested was the effect of maintaining the same ''richness'' of pattern (i.e., the same number of dots) while varying the factor of balance, the symmetry of a pattern about the wind line and, hence, the plume. The trends in experiment 3 (Fig. 3) and the results from the solid bar comparisons in experiment 4 (Fig. 4) suggested that balance was a factor, but in the asymmetrical patterns the number of dots (or bars) being utilized was only half that of the symmetrical pattern, and thus, the reduction in richness of stimulation could also have played a role. Finally, the patterns in experiment 6 (Fig. 4) were implemented to challenge the moths with visual environments having equivalent levels of richness, but in which different degrees of balance as well as types of off-axis image flow in the horizontal and/or vertical planes relative to the wind tunnel were created.

The results of this experiment (Fig. 4, experiment 6) indicated again that balance in the visual field presented to the moths may help in their successful negotiation of the tube. The 9 and 12 o'clock unbalanced treatment (Fig. 4, 6B) was no better than the control (Fig. 4, 6A) but no worse than the 6 and 12 o'clock treatment (Fig. 4, 6D). Patterns at 9 and 3 o'clock, 6 and 12 o'clock, and 8 and 4 o'clock evoked similar levels of upwind orientation (Fig. 4, 6C, 6D, and 6F, respectively). However, no pattern evoked the same level of successful negotiation of the tubes as did the 10 and 2 o'clock lines of five dots (Fig. 4, 6E).

DISCUSSION

The data obtained in these experiments indicate clearly that the positioning of visual cues within an otherwise visually depauperate environment significantly affects the successful orientation of flying male moths. The results of experiment 1 suggested that the moths were not utilizing the upwind or downwind approaching or receding openings of the tube as parallax cues (Fig. 3, 1C being no better than 1A) when they successfully negotiated the tube in the presence of other patterns. Hence, we were confident that it was the pattern placed in the tube that the moths were using to fly upwind, and not any artifact of the tube openings. Furthermore, the need for visual cues underlines, once again, that chemotaxis is not sufficient to explain pheromone-mediated, sustained upwind flight of male moths. In all experiments, no more than 11% of males (4 of 35 males that entered the tube in experiment 3) succeeded in making the journey to the upwind end of the blank tube without contacting the tube walls or becoming completely disoriented and flying out the downwind end. Observations indicated that those males that flew through the blank control were not in contact with the plume for the entire length of the tube. Some of these males made enough progress to recover contact with the plume at the upwind end of the tube and fly on to the source. *Heliothis virescens* males flying upwind in a plume are known to have a visual latency to simulated changes in wind direction of about 0.41 s (Baker and Vickers, 1994) and the males in the current experiments, given a running start before entering the tube, may have made it to the upwind end of the tube before the end of the visual latency period, recontacting the plume when visual cues were once more available.

Experiment 2 unexpectedly revealed that a pattern on the sides of the tube could elicit stable oriented flight (Fig. 3, 2D). With this pattern, it was difficult to envision how off-axis image flow could be resolved into a horizontal across-axis component (transversely *sensu* Ludlow, 1984; David, 1986) (Fig. 1B) and compensatory upwind steering performed, although a longitudinal component could still be resolved. The transverse component of off-axis flow in the horizontal plane (T_H) is thought to be essential for the moth to make the correct compensatory response (turning upwind), because it indicates the direction and amount of wind-induced drift that a moth might be experiencing at any instant (Marsh *et al.*, 1978; David, 1986). Off-axis image flow creating a transverse component can be perpendicular to the longitudinal axis of the moth in many directions, but only when corresponding to discrepancies in the moth's environment in the horizontal plane is it of use to the moth in regulating wind-induced (azimuthal) displacement (cf., Fig. 1B, i and ii). An exception to this might occur if males were capable of monitoring image motion over the foci of their compound eyes (Collett, 1980).

The interesting result of stable flight to patterns on the sides of the tube was investigated further in experiments 3, 4, 5, and 6. With a simulated ground pattern such as at 6 o'clock (Fig. 3, 1B, 2B, and 3B) or a ceiling pattern at 12 o'clock (Fig. 3, 2C), or to a lesser extent at 8 and 4 o'clock (Fig. 3, 3E), altitude gain can be registered only as a parallax, that is, a symmetrical decrease (or increase) in the size of the pattern in the field of view. From observations made during another experiment, we have documented the tendency of male *H. virescens* to gain altitude immediately following pheromone loss (Vickers and Baker, unpublished results). If the males do have a tendency to gain altitude, then it follows that in response to patterns providing weak feedback about altitude gain, males will tend to lose the plume more frequently and become disoriented. However, with patterns such as 9 and 3 o'clock and 10 and 2 o'clock (Fig. 3, 2D and 3F, respectively), altitude gain will result in actual up-to-down image flow (resolvable in part as across-axis) over the eyes. David (1979) showed that the equatorial ommatidia of *D. hydei* fruit flies are sensitive to image flow and do play an important role in altitude control. Olberg and Willis (1990), in their study of pheromone-modulated, visually sensitive neurons in the ventral nerve cord of *L. dispar*, concluded that the dorsal field of view was stimulated more by transverse pattern movement than by the ventral field. However, in a tethered experiment similar to that of Olberg and Willis (1990), Preiss (1991) suggested that the dorsal field was not involved in the flight control mechanism but evidence to support this conclusion was not presented. We speculate that actual image flow over the equatorial region of the eye in a freely flying moth is a more powerful mechanism for the control of altitude than is a ground parallax for these males, although the latter can be also apparently be used, as evidenced by the success of males in experiment 2 (Fig. 3, 2B and 2C), and by the results from gypsy moth (Preiss and Kramer, 1983).

In the last three experiments (4, 5, and 6; Fig. 4) we attempted to minimize the patterns to such an extent that the displacement of the flying moth by the wind would not create any off-axis image flow resolvable into an across-axis component relative to the horizontal plane of the environment. In such situations, the moths should not be able to determine the direction of the wind and would eventually become disoriented. This objective was achieved initially by creating a visually sparse environment with rows of dots at the level of the plume, along the center of the tube (experiment 4, Fig. 4).

Only the wide control pattern of 9 and 3 o'clock (Fig. 4, 4B) successfully allowed upwind flight, the only comparable treatment being the 9 and 3 o'clock bar pattern (Fig. 4, 4F). The effectiveness of the wide control pattern can be explained by off-axis image motion that can be resolved into longitudinal, along-axis flow and across-axis flow in the vertical plane, plus balanced left–right inputs (see below). Likewise, a bar pattern running along the longitudinal axis of the tube creates longitudinal flow as males change altitude in the wind, and they conceivably regulate their ground speed with this feedback (Fig. 6). Also, as in the wide control pattern of 9 and 3 o'clock dots, image flow up and down the vertical axis of the eye would be strong in the bar pattern, allowing the moth to maintain a steady altitude. But without across-axis image flow corresponding to the motion in the horizontal plane (T_H), the only feature of the double-bar array that could have kept males centered on the wind line in addition to regulating ground speed and altitude was its symmetry. The lack of success to the asymmetric bar demonstrates that L plus across-axis vertical flow (T_v) were not sufficient for successful upwind flight.

Stripes or bars possess an unusual feature in terms of the image flow that they afford. Image motion is produced only perpendicular to the long axis of the bar (David, 1982b) and this results in image flow characteristics that are different from those produced by patterns of dots. For example, in zero wind when there is no drift, flight past a bar can induce *apparent* transverse flow even though none exists (Fig. 6A). In wind, a long stripe can create situations of zero image flow most apparent when the longitudinal axis of the flying insect is aligned with the long axis of the stripe and the wind direction (Fig. 6B). But

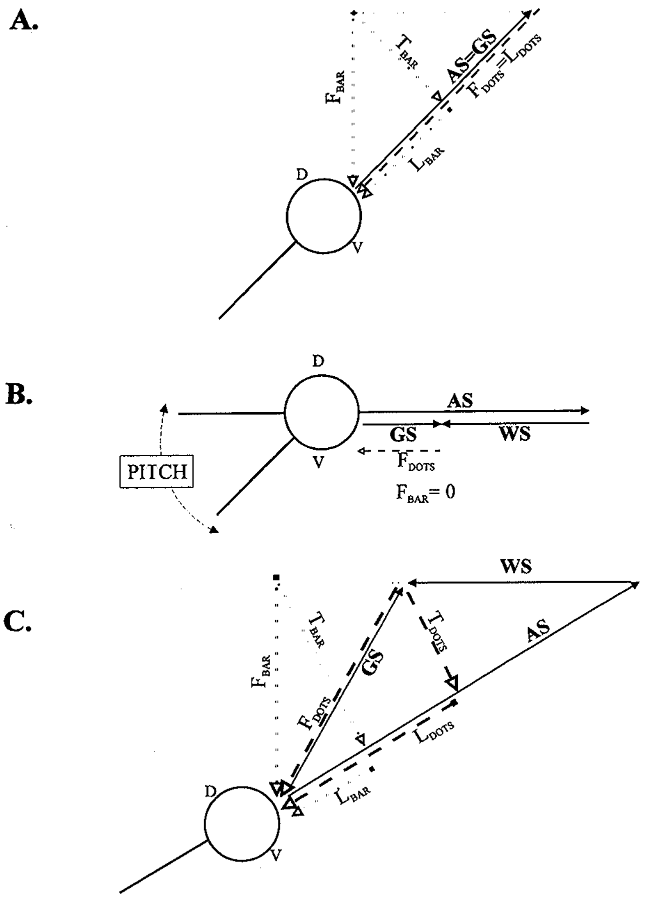


Fig. 6. Schematic representations of a flying moth viewed in the vertical plane. The dorsal (D) and ventral (V) surfaces of the head are indicated. (A) An apparent off-axis image flow (F_{BAR}) may result during flight at an angle with respect to a horizontal bar in zero wind. The flow in a normal dot environment would be the opposite of the air speed (AS), as in Fig. 1A, i, i.e., there is only an along-axis component (L_{DOTS}), and no across-axis component. F_{BAR} can be resolved into across-axis (T_{BAR}) and along-axis (L_{BAR}). (B) Image flow can appear to be zero ($F_{BAR} = 0$) during flight with or without wind, when the resultant flight path is steady and straight with respect to the long axis of a horizontal bar, regardless of the angle formed between the insect body's long axis (pitch) and the bar. (C) A moth gaining altitude while flying forward in wind past a wind-aligned horizontal bar. The bar allows image flow only perpendicular to its long axis (David, 1982b), and as such the image flow is now not the opposite of the track (F_{DOTS}, resolvable into orthogonal components L_{DOTS} and T_{DOTS}). The apparent flow (F_{BAR}) can be resolved into a larger than normal T component ($T_{BAR} > T_{DOTS}$) and a smaller than normal L component ($L_{BAR} < L_{DOTS}$). This will provide the male with some front-to-back image motion indicating upwind progress as well as an across-axis component for altitudinal control.

zero image flow can also occur when the body of the moth is at an angle to the stripe but the resultant moth track is steady and straight with respect to the long axis of the stripe. We noted moths that flew into the tube and then apparently drifted back out of the downwind end without becoming disoriented. These moths may have been unable to compensate for their down-tunnel drift due to their steady altitude and the resulting situation of apparent zero image flow (Fig. 6B). On the other hand, when flying at an angle with respect to the wind and a wind-aligned stripe (Fig. 6C), males receive a stronger T flow and weaker L flow when gaining altitude than would be produced by flight past a dotted pattern (dots provide stimulation in all directions [David, 1982b]). Moreover, males losing altitude while maintaining a certain body pitch can resolve a back-to-front along-axis component. This flow component plus the related vertical across-axis component would stimulate the male to increase thrust and lift (much as David (1986) proposed for flies when blown down and backwards by a turbulent gust of wind), resulting in a gain in altitude and a front-to-back along-axis component. Hence, by zigzagging up and down, the males' responses might become channeled along the bar. It is clear from the results presented here, however, that this unusual situation occurred only when two bars were symmetrically placed about the wind line and plume (Fig. 4, 4F). It therefore seems likely that both eyes have to be stimulated (image balancing, or a translational L input) for the moths to continue their upwind flight to the source and that the balance further serves to center the moth, in this case coincidentally optimizing contact with the plume.

The heightened success rate of males presented with the symmetrical bar pattern may have been due to the availability of a greater number of visual cues that provided feedback to the moth when it was on either side of the plume or to the effect of balancing the visual inputs, which in this case, would center the moth in the area of the plume. Bees are known to balance image velocities to fly through apertures (Kirchner and Srinivasan, 1989), and this in effect might have been what all males were doing, integrated with L and vertical flow, to succeed in flying through the tube in this artificial setup. However, for a moth flying upwind to an odor source in nature, the challenge is not necessarily to balance image velocity but, instead, to remain in contact with the plume by monitoring wind-induced displacement.

In the field, as a moth flies in a plume past a succession of trees, plants, and leaves in the canopy, these images will usually be closer from moment to moment on one side of the moth than the other. Because the pheromone plume displaces, not according to the symmetry of the plants, but to the movement of the air that continually shifts direction, the moth must usually ignore the faster movement or larger appearance of an object on one side than the other and thus not try to balance speed or size of image movement on each side. Therefore, under most natural conditions, balancing image velocities would cause the moth to lose contact with pheromone, and males must, therefore, ignore any such asymmetries. However, in this experimental situation, the left–right balancing happens *coincidentally* to align the moth with the wind line and also centers the moth to aid in contacting the plume. Hence, the success of moths in remaining oriented in the presence of this particular bar pattern (Fig. 4, 4F), despite the lack of a resolvable across-axis flow component derived from motion in the horizontal plane, may be attributable to a combination of three factors: (1) promotion of upwind progress due to a front-to-back longitudinal flow obtained by off-axis image flow in the vertical plane—this flow is created by the up-and-down oscillating moth being blown off its course in the vertical plane; (2) image flow in the vertical plane allowing the moth to regulate its altitude; and (3) a left–right "balancing" of visual inputs afforded by the symmetrical pattern as occurs in bees (Kirchner and Srinivasan, 1989).

The lines of five and nine dots at 9 and 3 o'clock in experiment 5 (Fig. 4, 5C and 5D, respectively) probably act similarly to the symmetrical bar pattern (Fig. 4, 4F) resulting in sustained orientation providing longitudinal flow, altitude control, and symmetrical left–right "balancing." However, the bar pattern (Fig. 4, 4F) should be the best because it is the richest and gives the most continuous feedback in all three categories. The line of nine dots is likewise somewhat, but not significantly, better than the line of five dots because of its greater continuity. Thus, although dotted line patterns such as these that did not provide off-axis flow relative to the horizontal plane (Fig. 4, 5C and 5D) were not as good as those that did provide such flow (Fig. 4, 5E and 5F), the results of experiment 5 show that males can successfully fly upwind when provided with these visual arrays. The higher success rates for males in response to the 9 and 3 o'clock—line of five dots pattern in experiments 5 and 6 compared to experiment 4 (Fig. 4, 5C and 6C compared to 4E) may have been due to a better overall response to pheromone by males during the time that experiments 5 and 6 were performed, compared to when experiment 4 was conducted (Fig. 4, compare 5B to 4B). We must not forget that the propensity to react to visual cues depends on the males responding to pheromone. Preiss and Kramer (1983) and Preiss and Futschek (1985) noted that males failed to respond to changes in the visual environment in the absence of pheromone. If the overall response levels are low, then the moths' attention to visual stimuli may similarly be reduced. The reduced pheromone responsiveness in experiment 4 (Fig. 4) masked the fact that males could utilize such a pattern successfully.

In experiment 6, the lines of five dots at 9 and 3 o'clock, 6 and 12 o'clock, and 8 and 4 o'clock (Fig. 4, 6C, 6D, and 6F, respectively) gave insignificantly different levels of completely oriented flight. While all were balanced in terms of left–right inputs, 8 and 4 o'clock, due to the tendency of the moths to rise following pheromone loss, gives a stronger parallax type of information for altitude control than it does an across-axis vertical image flow. The 6 and 12 o'clock pattern gives only parallax for altitude control, the pattern both receding (6) and enlarging (12) in the field of view as the moth rises. Both these patterns should give good off-axis image motion in relation to the horizontal plane of the environment, but the lack of good flow-related altitude control appeared to counteract this advantage and caused overall poor orientation success. On the other hand, although the 9 and 3 o'clock pattern lacks this "transverse in the horizontal plane"-type of image flow, it does provide good vertical flow for altitude control and longitudinal horizontal flow for regulating upwind progress. In addition, the above-mentioned balance that happens to keep the males aligned with the wind effectively obviates off-axis transverse-horizontal flow for horizontal steering. Of course, all three patterns offer image flow that can be resolved along the longitudinal axis of the insect.

The most successful pattern, by a significant margin, was the line of five dots at 10 and 2 o'clock (Fig. 4, 6E). Here all of the favorable factors for sustained upwind progress are in place: (1) off-axis flow that can be resolved into an across-axis component (T) from both wind-induced horizontal (T_H) and vertical (T_V), altitudinal displacements; (2) an along-axis component (L) of image flow; and (3) balance of visual inputs. The effect of balance is clear since the 9 and 12 o'clock pattern (Fig. 4, 6B) did not promote significantly more upwind flight than did the blank tube, despite the fact that it offers T_H from the 12 o'clock dots, T_v from the 9 o'clock dots, and L from both. Interestingly, this pattern had a low level of flights falling into the completely disoriented bracket, indicating a high level of partially oriented flights (with one or two touches of the tube). However, the relevance of balance to a male moth orienting upwind in a plume outside would seem to be somewhat in question (see earlier discussion).

Results from successive experiments appear to suggest that moths do not use a pole or foci shift, as Collett (1980) demonstrated in hoverflies, to monitor wind-induced drift. Had moths been using such a mechanism, then several patterns, including 9 and 3 o'clock—line of five dots (Fig. 4, 4E, 5C, and 6C) and line of nine dots (Fig. 4, 5D) as well as 9 and 12 o'clock—line of five dots (Fig. 4, 6B), should have performed well. These patterns were, however, suboptimal, suggesting that another mechanism(s) whose function had been impaired by the lack of appropriate visual feedback is (are) normally operative.

In summary, the presence of adequate visual input and the positioning of the visual cues significantly influence the success of *H. virescens* males flying upwind in a pheromone plume without touching the tube. Patterns that allow image flow to be resolved into the three perpendicular components, longitudinal and off-axis relative to the horizontal and vertical planes, are significantly better at promoting sustained upwind flights than are those apparently deficient with regard to any of these components. Patterns that allow only parallax as a putative altitude control mechanism do not seem as effective as those that allow actual image flow relative to the vertical plane of the environment. That is not to say that *H. virescens* males do not use parallax as an altitude indicator in their natural environment or, indeed, that the patterns used in this experiment generated significant altitude-related parallax information. On the contrary, it seems likely that males flying upwind to calling females must rely upon a variety of different features in the highly variable visual environment and upon different motion detection systems to maintain plume contact and upwind progress even in visually suboptimal situations.

ACKNOWLEDGMENTS

Thanks to Dr. M. Willis for a helpful discussion on the results of this study, Mrs. T. Vickers for help with Figs. 1 and 6, both referees, whose reviews resulted in several important clarifications. We thank Mr. R. Vetter and assistants for rearing the adult moths used in this study. This work was supported by USDA Competitive Grant 87-CRCR-1-2577 (to TCB), "Flight Control in *Heliothis virescens* and *Heliothis zea*."

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