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# A non-anemotactic mechanism used in pheromone source location by flying moths

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ABSTRACT. Male oriental fruit moths, *Grapholitha molesta* (Busck) (Tortricidae), continue to zigzag along a pheromone plume to the source in zero wind, if they have started flight with wind on. If the pheromone source is removed and the plume is hence truncated, moths flying in zero wind out of the end of the plume into clean air increase the width of their reversals and the angles of the straight legs of the tracks so they are more directly across the former wind line. Such moths reach the source less often than do those flying along a continuous plume. The males continue to zigzag up a plume in zero wind, apparently by a combination of sequential sampling of concentration along their path and the performance of an internal, self-steered programme of track reversals (zigzags) whose frequency increases with concentration. Visual feedback may aid in the still-air performance of the zigzags. We propose that both the sequential sampling (longitudinal klinotaxis) and self-steered counter-turning programme also are used in wind as well; anemotaxis apparently polarizes the direction of the zigzags to result in upwind displacement, and the narrow zigzags caused by the higher concentration in the plume keep the male 'locked on' to the odour.

**Key words.** *Grapholitha molesta,* moth, behaviour, flight orientation, pheromone, anemotaxis, chemotaxis, zigzagging programme.

## Introduction

Males of many species of moth fly upwind in the presence of sex pheromone. However, they do not fly straight upwind, but progress in a zigzag fashion along the pheromone plume. During approaches to sources less than 3 m away, they fly at fairly constant oblique angles to the windline (Marsh *et al.*, 1978; Kuenen & Baker, 1982a) and turn repeatedly across it (e.g. Kennedy & Marsh, 1974; Cardé & Hagaman, 1979; Baker *et al.*, 1981).

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Until recently, initiation of turning was thought to be triggered by the loss of pheromone as a male flies out of the 'sides' of a plume or when he encounters holes or gaps in its irregularly fenestrated structure (Kennedy & Marsh, 1974; Marsh *et al.*, 1978). Loss of pheromone would trigger a programme of widening, cross-wind anemomenotactic reversals (termed casting flight (Kennedy & Marsh, 1974)) that would continue until the male once again encountered pheromone and re-established positive anemotaxis.

However, Kennedy *et al.* (1980, 1981) have now demonstrated with *Adoxophyes orana* that programmed reversals occur even during continued uniform exposure to pheromone,

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the onset of pheromonal stimulation initiating a programme of narrow left–right reversals, and pheromone loss causing these reversals to become wider. This finding implicated a chemotactic mechanism for the first time, because although each reversal leg could be steered anemomenotactically, the frequency of reversals could clearly only be influenced by the chemical stimulus. In wind, the chemically mediated programme of reversals would then presumably interact in some way with anemotaxis to result in displacement toward the source, or lack of displacement during casting flight without pheromone.

Farkas & Shorey (1972) had proposed that moths maintained lateral contact with a pheromone plume chemotactically by 'aerial trail following', turning back into or towards the plume upon each loss of contact with it. Males of *Pectinophora gossypiella* successfully flew to the vicinity of a pheromone source after wind was stopped, which Farkas & Shorey (1972) considered to be evidence that chemotaxis, not anemotaxis, is the mechanism used to keep laterally close to a plume, even in wind. Further, they suggested that the moths could somehow sense chemotactically whether the source was up or down tunnel from them (Farkas & Shorey, 1972).

This longtitudinal aspect of their hypothesis was not supported by experiments in which Kennedy & Marsh (1974) showed that upwind progress of male *Plodia interpunctella* toward a source was altered in response to a ground pattern moving beneath them. They concluded that Farkas & Shorey's males would have detected the direction of the pheromone source before the wind was stopped by using pheromone-regulated optomotor anemotaxis. The ability of Farkas & Shorey's males to remain in close contact with a stationary plume in still air was, however, overlooked, and in a preliminary study using males of *Grapholitha molesta* (Busck), we demonstrated that this contact is indeed achieved by means of a chemotaxis (Baker & Kuenen, 1982). In contrast to Farkas & Shorey, however, we proposed a mechanism of self-steered longtitudinal chemoklinotaxis, but did not propose that it is used in wind as well as without it.

In this paper we describe in detail our studies on the behaviour of male *G. molesta* flying in still air, and we present evidence that a kind of protracted chemoklinotaxis is used in wind as well as in still air.

## Materials and Methods

### *General*

*Grapholitha molesta* were reared on small green thinning apples at 25 ± 2°C on a 16:8 L:D cycle (Baker *et al.*, 1981). Males were isolated from females in the pupal stage and adults were segregated daily by age. Adult males were always kept isolated from females and had continuous access to an 8% sucrose solution.

Males were flown in a large sustained-flight wind tunnel constructed of clear polycarbonate plastic with a working section 3.6 m long, 1 m high and 1 m wide at ground level (Kuenen & Baker, 1982b). A moveable ground pattern of alternating transverse 10-cm black and white stripes was positioned 1.5 cm below the 6-mm-thick clear acrylic plastic floor. Air flow was provided by a 1-hp voltage-regulated fan, and air turbulence was reduced by passing the air through layers of muslin (supported by a mesh screen) and finally through a layer of fine-mesh polyester fabric.

Pheromone-bearing septa (see below) were positioned 40 cm from the upwind end of the working section. A septum was either placed on a 15 × 15 × 0.05 cm galvanized sheet-metal plate positioned 15 cm above the centre of the tunnel floor, or suspended at the same height and location by a thread from the roof of the tunnel. Pheromone was removed from the tunnel by a 30-cm diam. exhaust tube (air speed in centre = 2.9 m/s) aligned with the centre of the plume. The remainder of the air passing through the tunnel was recirculated through the room.

In all experiments 4–6-day-old males were individually released from small aluminium-screen cones hand-held in the bottom of the pheromone plume (Kuenen & Baker, 1982b). They were released during a 2-h period beginning 2 h before lights-off, at which time they were optimally responsive to pheromone (Baker & Cardé, 1979a). Prior to release, moths were exposed for at least 5 min to the light intensity of 250 lx in tunnel, which was provided by four voltage-regulated 100-W bulbs, whose light was diffused by reflection

from a white styrofoam (expanded polystyrene) ceiling.

*Pheromone*

The sex pheromone emitted by females of *G. molesta* is a blend of 5.9% (*E*)-8-dodecenyl acetate, 3.8% (*Z*)-8-dodecenyl alcohol (Cardé *et al.*, 1979) and 90.3% (*Z*)-8-dodecenyl acetate (Roelofs *et al.*, 1969). A 100 μg/μl solution in hexane was formulated gravimetrically and serially diluted to 1 μg/μl. 10 μl of this solution were applied to the inside bottom of the large end of a rubber septum (A. H. Thomas Co., No. 8753-D22, 5 × 9 mm sleeve type). Each of the components had < 0.5% volatile impurities as determined by gas-liquid chromatography (GLC) on an XF-1150 (50% cyanoethyl, methylsilicone on 100–120 mesh Chromosorb, W-AW-DMCS) 2 m × 2 mm ID glass column, with an $N_2$ carrier flow of 25 ml/min at 160°C (Baker & Roelofs, 1981). The formulation of this optimal blend (Baker & Cardé, 1979b) was verified by GLC on the same XF-1150 column (Baker & Roelofs, 1981).

*Recording of flight tracks*

All flights were video-recorded from above in plan view with a rotary-shutter camera (Sony RSC 1050) positioned on top of the tunnel, connected to a video recorder (Sony SLO 340). The camera's field of view spanned 65 cm, from 105 to 170 cm, downwind of the pheromone source, and 25 cm on both sides of the midline of the tunnel. A single layer of cheese cloth was placed on the floor of the tunnel so that the moths would be visible on the video display as they flew over the ground pattern of black stripes. The stripes were still visible through the cloth, and the moths would slow, stop, or reverse their up-tunnel progress when the ground pattern was moved in the down-tunnel direction (Kennedy & Marsh, 1974; Miller & Roelofs, 1978).

For analysis, individual flight tracks were re-recorded onto a Sony SVM-1010 motion analyser. Frame-by-frame playback from this system gave the moths' consecutive 1/60-s positions, which were marked by an ink dot on a mylar (clear plastic) sheet taped to the video surface of the analyser. Reference marks were added to each sheet so that individual tracks could be aligned consistently for comparisons and analyses.

*Data analysis*

An X/Y digitizer (Houston Instruments, HIPAD® DT 11) serially interfaced with a microcomputer (Radio Shack, TRS 80® Model III) was used for data analysis (the digitizer resolution was set at 39 μm with an accuracy of 117 μm). Each track-bearing mylar sheet was consistently oriented on the digitizer surface using the reference marks, and the X,Y co-ordinates of consecutive points were entered into the computer.

All measurements of velocity were made relative to the stationary tunnel. A computer program (BASIC) analysed the tracks for: (1) overall velocity (velocity along the actual track), (2) net velocity (distance along the wind line per unit time), (3) turning frequency (number of turns per second), (4) distance per turn (cm along the actual track), (5) turning magnitude (degrees per turn), (6) angular velocity (degrees per second), and (7) the angular-to-linear velocity ratio (degrees per cm; calculated as the total degrees turned/total distance flown).

In our first experiment, we calculated the mean orientation angle and its mean unit vector length for each moth (Batschelet, 1972; Kuenen & Baker, 1982a). We also measured track reversal distances (distances at which moths turned back toward the time-averaged axis of the plume) and interreversal track angles (modified after Marsh *et al.*, 1978; Kuenen & Baker, 1982a).

Turns were defined as beginning at any point along the track where the direction of movement changed from clockwise to anticlockwise (or vice versa), and to end at the start of the next turn. To allow for the error inherent in transcribing tracks and entering the X,Y coordinates, a threshold value in change of direction of 50° had to be accumulated and exceeded before a new turn was registered, and the turn was deemed to have started (or ended) at the first point on the track where the change in direction had begun. The beginning and end of each tracing were therefore parts of incomplete turns and were not included in the turning data.

We employed Marsh *et al.*'s (1978) defini-

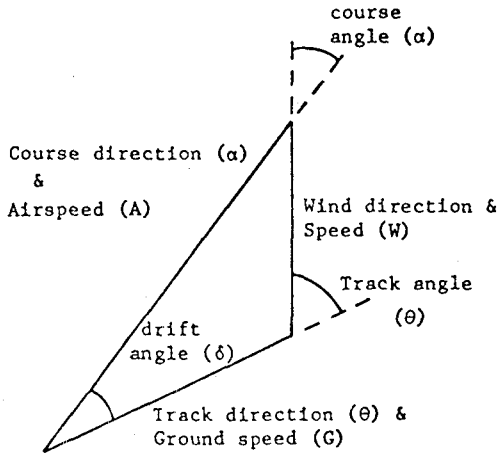


FIG. 1. Triangle of velocities during free-flight in wind (after Marsh *et al.*, 1978). A change in the length of any leg of the triangle necessarily affects one or both of the other legs.

tions of course angle, groundspeed, airspeed, track angle and drift angle (Fig. 1). The magnitude and direction of the wind and ground velocity vectors determine the airspeed vector. Thus, a moth will always have an airspeed vector above zero unless it hovers stationary in still air or flies straight downwind along the wind line at the wind speed.

## Experiments and Criteria

### *Stopping the wind with pheromone plume present*

Individual males were allowed to initiate up-tunnel flight in winds ranging from 37.5 to 50 cm/s. As the males flew toward the field view of the video-camera, the wind could be stopped in *c.* 2.7 s by switching off power to the fan, and pulling sharply on a leather brake which tightened round the axle of the fan and stopped the blades rotating. Using a titanium chloride smoke source in place of the pheromone septum, we verified that the wind stopped at the same moment as the fan blades stopped. The smoke plume then remained substantially intact for about 10 s and without significant down-tunnel displacement but began slowly to expand laterally within 2–3 s of wind stoppage. At the moment the blades stopped, a small light-emitting diode (LED), positioned at the top of the tunnel in view of the camera, was flashed as a visual marker of wind cessation. At the same time the pheromone exhaust tube was covered with a piece of cardboard, which prevented the development of a vortex which, as indicated by smoke, would have rolled up-tunnel and disrupted the stationary pheromone plume.

When all was well timed, the wind stopped as the male flew up to or just into the field of view. Males were observed for their farthest advance up-tunnel toward the pheromone source. As a control, the LED was flashed, but the fan was not stopped and the exhaust tube was not covered. Flight tracks of the two groups of moths were compared.

### *Removal of the pheromone plume in wind*

Males initiated up-tunnel flight toward the pheromone source in winds of 28.6 or 37.5 cm/s (voltage to the fan varied on some days). As the moths flew toward the camera's field of view, the pheromone septum, suspended by a thread, was pulled abruptly to the top of the tunnel, effectively creating an upwind end to the pheromone plume at the height at which moths were flying. As the pheromone source was raised, the LED was flashed on, so that we could calculate the farthest up-tunnel point at which the males could have flown out of the upwind end of the pheromone plume.

It was possible that the moths lost pheromone contact sooner than we calculated from the wind speed and LED flash. For instance, they might have flown outside the time-averaged boundaries of the plume or encountered a gap in the plume as they approached its upwind end. Errors resulting from any such earlier loss of pheromone contact would have tended to obscure differences between moths' flight tracks in areas where the pheromone plume was present compared with where it was absent. When properly timed, the moths lost pheromone contact near the middle of the field of view.

### *Removal of the pheromone plume followed by wind stoppage*

In this experiment the moths were again allowed to initiate up-tunnel flight toward the pheromone source in a wind of 70 cm/s. As in the plume removal experiment above, the

pheromone septum was abruptly pulled up to the roof of the tunnel (with a concurrent LED flash) as a male approached the camera's field of view. Approximately 1 s later the fan was turned off (LED flash and exhaust tube covered), and the wind stopped on average 2.7 s after that (LED flash). Smoke source visualization demonstrated that with this procedure the up-tunnel end of the pheromone plume came to rest near the middle of the field of view. On average for all trials, the wind stopped 3.7 s (± 0.2 SD; $n = 13$) after the plume was removed. The lack of exact reproducibility of time and rate of wind stoppage led to a zone of uncertainty as to the location of the up-tunnel end of the plume (Fig. 3). The moths were deemed to have lost pheromone contact after flying past the up-tunnel end of this zone. This scoring procedure created a bias against finding differences between movements in the plume and non-plume areas.

## Results

### *Stopping the wind with pheromone plume present*

Males initially flying upwind toward the pheromone source generally continued to fly up-tunnel after the wind was stopped, while

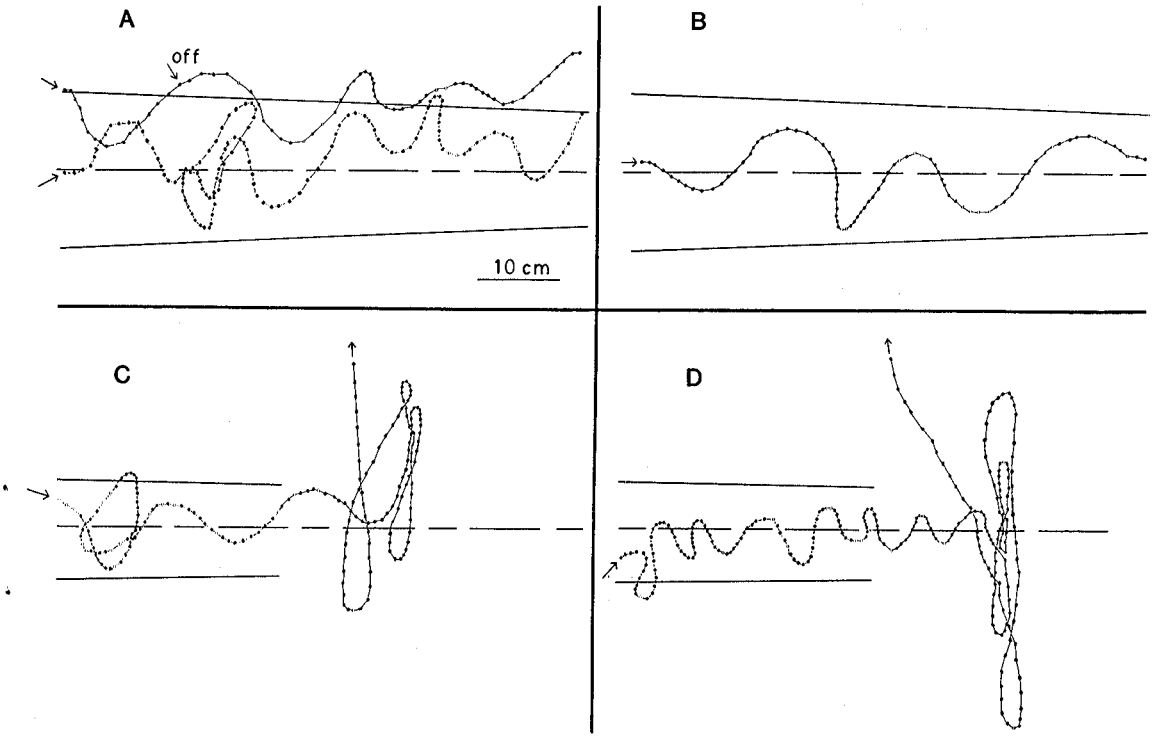


FIG. 2. Tracks of flying *G. molesta* males, videotaped from above. Each figure represents a 65-cm-long section of the tunnel. The outer solid lines are the boundaries of the time-averaged pheromone plume, and the central dashed line is its axis. The pheromone septum is 105 cm to the right of each figure. Dots represent the moths' locations at consecutive 1/60-s intervals. Wind was blowing from right to left when present, and the moths flew from left to right. (A) The dashed line represents the moths' up-tunnel flight in wind. This male was displaced to the down-tunnel end by moving the ventral floor pattern in that direction, and then the moth flew up-tunnel again (solid line: after wind stopped; 'off' indicates the point at which the wind stopped. This male flew up to and touched the pheromone source. (B) Track of another moth flying up-tunnel without wind to within 5 cm of the pheromone source. The wind had stopped 0.3 s before the moth came into view. (C, D) Tracks of moths flying in wind, before and after the pheromone was removed. In the first part of both tracks pheromone was present, its boundaries indicated by solid straight lines. The plume boundaries end at the point of calculated pheromone loss; the functional loss (see Methods), characterized by wide reversals, occurred farther up-tunnel.

maintaining a similar zigzag pattern (Fig. 2a, b). Without wind 81% of twenty-seven males flew to within 10 cm of the pheromone septum, and 44% actually located it and directed hairpencil displays toward it (mean flight distance in zero wind for the 81% flying within 10 cm of source = 1.5 ± 0.4 m SD; $n = 22$). None of the moths flew past the source.

Track reversal distances were not significantly different between moths flying with and without wind (5.5 ± 2.6 cm SD, $n = 42$ *v.* 6.6 ± 1.9 cm SD, $n = 27$; $P > 0.05$, *t*-test). The moths' inter-reversal track angles were not significantly different in wind (57.8° ± 12.40 SD, $n = 34$) or windless conditions (51.9° ± 14.60; $n = 22$; $P > 0.05$, *t*-test), and tracks in both groups were significantly oriented in the up-tunnel direction ($P > 0.05$; *V*-test, Batschelet, 1972). The deviation from 0° (mean orientation angle directly up tunnel, was 5.4° (± 5.8 SD, $n = 34$) in wind *v.* 10.9° (± 13.8 SD, $n = 22$) in zero wind ($P > 0.05$, Watson's $U^2$-test; Watson, 1962; Batschelet, 1972).

The overall velocity and the net velocity of males flying without wind were higher than for males flying with wind, and additionally, the turning frequency and turning magnitude were not significantly different in the two conditions ($P > 0.05$, Table 1). On the other hand, both the angular-to-linear velocity ratio and the angular velocity decreased in moths that flew without wind, while their distance per turn increased ($P > 0.05$, Table 1).

*Removal of the pheromone plume in wind*

Males' track reversal distances increased significantly (Fig. 2c, d) after flight into clean air in wind compared with flight in the pheromone plume (13.7 ± 3.7 cm SD; $n = 36$ *v.* 4.3 ± 1.6 cm SD; $n = 43$; $P < 0.05$, *t*-test). Concurrently, their inter-reversal track angles became more cross-tunnel during this typical casting flight (see Discussion) (Table 2) and upwind progress ceased (Fig. 2c, d). The moths initiated casting-flight 0.7 s (± 0.2 SD, $n = 19$) after the calculated passage of the up-tunnel end of the pheromone plume.

Flight track parameters were measured from two starting points (Table 2); first, after the *calculated* passage of the moth out of the front of the plume, and second, beginning after behavioural manifestation of pheromone loss. We considered the latter to be the true *functional* loss of pheromone, and these tracks did not include the *c.* 0.7 s of narrow zigzagging upwind flight that the use of the first method did.

Casting was deemed to have started at the apex of the first turn that occurred outside the time-averaged pheromone plume boundary, after passage of the end of the plume, or at the apex of the most up-tunnel turn of the entire track, which ever came first. This first turn generally coincided well with the onset of wider track reversals and the predominantly cross-tunnel inter-reversal legs (Fig. 2c, d). Inter-reversal track angles increased from 62° (± 9.1 SD; $n = 15$) during pheromone-mediated flight to 77.3° (± 9.0 SD; $n = 15$) after calculated loss of pheromone contact ($P > 0.05$; paired *t*-test). Moths in casting flight after functional loss had a mean inter-reversal track angle of 88.2° (± 16.2 SD, $n = 15$).

As expected, the net velocity decreased after both the calculated and functional loss of pheromone ($P < 0.05$, Table 2), whereas their overall velocity increased ($P < 0.05$, Table 2). After the transition to casting flight in clean air the moths' turning frequency was lower and their magnitude was higher (Table 2). Although there was no difference in angular velocity between flight with and without pheromone, the increases in the overall velocity led to decreased angular-to-linear velocity ratios (non-significant) and increased distances (significant) per turn (Table 2).

*Removal of the pheromone plume followed by wind stoppage*

In zero wind, moths flying out of the up-tunnel end of the stationary plume into clean air (Fig. 3c, d) widened their cross-tunnel flights compared with their flight while in the plume region (Fig. 3a, b). Rather than a sudden turning-back toward the terminated up-tunnel end of the plume from which they had emerged, the males seemed to change their entire pattern of reversals, by lowering their frequency and steering more cross-tunnel (Table 3). Because no wind was present, the change from narrow to wide zigzagging and the increasing inter-reversal track angles (Table 3) upon emergence from the plume could not have been steered anemotactically. Rather, the track changes induced by drop in concentration may have

TABLE 1. Mean flight track measurements (± SD) of *G.molesta* males flying toward a pheromone source with wind present or absent.

| Flight conditions | *n* | Overall velocity (cm/s) | Net velocity (cm/s) | Turn frequency (turns/s) | Turn magnitude (°/turn) | Angular-to-linear velocity ratio (°/cm) | Angular velocity (°/s) | Distance per turn (cm) | Inter-reversal track angle (° from 0) |
|---|---|---|---|---|---|---|---|---|---|
| Up-tunnel flight; pherome present; wind present | 36 | 62.1 ± 14.0[a] | 29.1 ± 10.8[a] | 7.5 ± 1.4[a] | 150.5 ± 36.5[a] | 25.4 ± 14.9[a] | 1015 ± 238[a] | 10.6 ± 4.8[b] | 57.8 ± 12.4[a] |
| Up-tunnel flight; pheromone present; wind absent | 24 | 78.0 ± 15.8[a] | 45.2 ± 21.7[a] | 6.3 ± 1.9[a] | 125.9 ± 35.2[a] | 11.7 ± 5.9[b] | 734 ± 261[b] | 17.4 ± 13.4[a] | 51.9 ± 14.6[a] |

Means appearing in the same column having no letters in common are significantly different by *t*-test ($P < 0.05$).

TABLE 2. Mean flight track measurements (± SD) of *G.molesta* males flying in wind with a pheromone plume present or after removal*.

| Flight conditions | *n* | Overall velocity (cm/s) | Net velocity (cm/s) | Turn frequency (turns/s) | Turn magnitude (°/turn) | Angular-to-linear velocity ratio (°/cm) | Angular velocity (°/s) | Distance per turn (cm) | Inter-reversal track angle (° from 0) |
|---|---|---|---|---|---|---|---|---|---|
| Male in plume | 14 | 56.6 ± 10.2[b] | 26.1 ± 9.5[a] | 7.6 ± 2.4[a] | 169.5 ± 50.0[b] | 28.3 ± 11.4[a] | 1049 ± 246[a] | 10.3 ± 4.7[b] | 62.0 ± 9.1[c] |
| Male out front end of plume (Calculated loss of pheromone had occurred) | 14 | 65.1 ± 14.7[ab] | 7.5 ± 4.2[b] | 6.0 ± 1.7[a] | 189.3 ± 29.8[b] | 25.1 ± 12.3[a] | 1021 ± 311[a] | 15.2 ± 8.8[ab] | 77.3 ± 9.0[b] |
| Male out front end of plume; "Casting" flight only (Functional loss of pheromone had occurred) | 14 | 72.9 ± 17.6[a] | 0.0 ± 7.6[c] | 5.6 ± 4.1[b] | 239.7 ± 56.1[a] | 21.5 ± 13.1[a] | 1077 ± 556[a] | 22.3 ± 10.8[a] | 88.2 ± 16.2[a] |

Means appearing in the same column having no letters in common are significantly different according to Duncan's new multiple range test ($P < 0.05$).

* The pheromone plume was removed from the moth's flight area by abruptly pulling the pheromone septum to the ceiling of the tunnel.

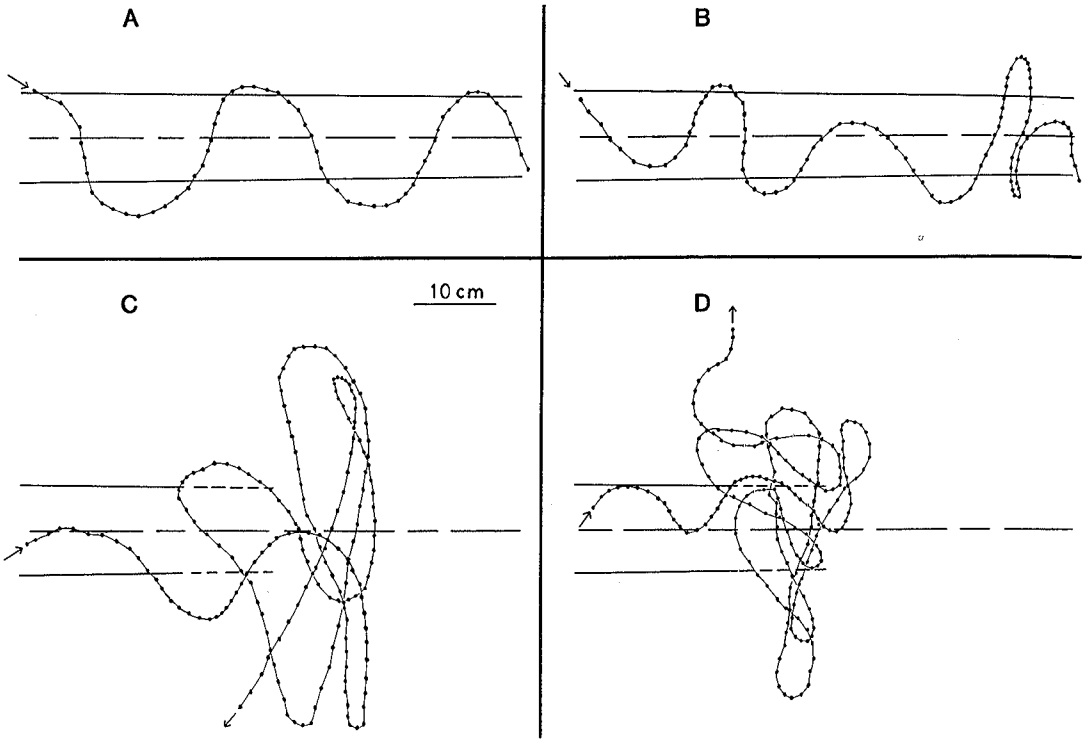


FIG. 3. Tracks of males flying up-tunnel without wind. (A, B) Controls, in which moths were flying up-tunnel after the pheromone source had been left in the flight area; they flew to within 15 cm and 5 cm of the pheromone source, respectively. (C, D) The pheromone source had been removed and the wind stopped before males entered the field of view. The position of the stationary plume in still air is indicated by solid straight lines at the left in each figure. Each plume's up-tunnel end is within the dashed straight lines, the 'zone of pheromone uncertainty' (see Methods). Flight tracks to the right of this zone were in pheromone-free air. Other conditions as in Fig. 2.

been self-steered using a concentration-modulated motor programme with or without a 'memory' of the previous pattern in pheromone and visual feedback.

The end result in the non-plume (clean air) region in zero wind was that up-tunnel progress was reduced, net velocity dropped to 25.8 cm/s from 34.8 cm/s in the region of the plume and the mean closest approach to the source was 97.6 cm (± 39.0 SD; $n = 24$) compared with 29.1 cm (± 35.7; $n = 23$) when the plume was not removed. Track reversal distances increased to 11.5 cm (± 3.4 SD; $n = 13$) during flight in the non-plume region compared to 6.8 cm (± 2.5 SD; $n = 13$) in the plume region ($P < 0.05$, $t$-test). In addition to the increased inter-reversal track angles, there appeared to be a trend toward increased turning magnitude in the non plume-region compared with tracks of moths flying in the plume region (Table 3). Compared with moths in the clean air of the 'non-plume region', those in the same region for the control in which pheromone was present behaved just as they had in the plume region under all conditions, maintaining significantly narrower track reversals (7.7 ± 4.2 cm SD; $n = 9$) and smaller inter-reversal track angles (Table 3).

As in our initial experiment, when the wind was stopped and a stationary pheromone plume was present, the moths in this experiment also continued to fly up-tunnel toward the source (Fig. 3a, b); 43% flying to within 10 cm of it. In zero wind, the overall velocity did not change significantly after flight into clean air, and here the reduction of up-tunnel progress must have been caused by steering changes and not by a change in linear velocity.

TABLE 3. Mean flight track measurements (± SD) of *G.molesta* males flying toward a pheromone source in zero wind. A stationary plume with an up-tunnel end was established by removing the pheromone source such that 'plume' and 'non-plume' regions were created. The source was left in place as a control.

| Flight conditions in zero wind | *n* | Overall velocity (cm/s) | Net velocity (cm/s) | Turn frequency (turns/s) | Turn magnitude (°/turn) | Angular-to-linear velocity ratio (°/cm) | Angular velocity (°/s) | Distance per turn (cm) | Inter-reversal track angle (° from 0) |
|---|---|---|---|---|---|---|---|---|---|
| Pheromone source removed; plume region | 14 | $85.8 \pm 18.3^{a}$ | $34.8 \pm 26.5^{b}$ | $4.8 \pm 1.4^{ab}$ | $163 \pm 61^{ab}$ | $9.6 \pm 5.3^{a}$ | $702 \pm 185^{a}$ | $20.6 \pm 7.0^{ab}$ | $62.4 \pm 18.5^{bc}$ |
| Pheromone source *not* removed; plume region | 9 | $84.9 \pm 25.5^{a}$ | $41.8 \pm 22.5^{ab}$ | $5.3 \pm 1.2^{ab}$ | $159 \pm 49^{ab}$ | $11.7 \pm 7.1^{a}$ | $759 \pm 192^{a}$ | $18.3 \pm 4.7^{ab}$ | $57.8 \pm 14.9^{b}$ |
| Pheromone source removed; non-plume region | 13 | $92.6 \pm 14.2^{a}$ | $25.8 \pm 23.9^{b}$ | $4.2 \pm 1.1^{b}$ | $180 \pm 59^{a}$ | $7.6 \pm 3.2^{a}$ | $682 \pm 167^{a}$ | $26.7 \pm 13.6^{a}$ | $82.8 \pm 19.7^{a}$ |
| Pheromone source *not* removed; non-plume region | 8 | $85.5 \pm 21.5^{a}$ | $41.0 \pm 26.0^{ab}$ | $5.8 \pm 1.8^{ab}$ | $150 \pm 41^{ab}$ | $10.3 \pm 5.7^{a}$ | $828 \pm 229^{a}$ | $17.4 \pm 6.6^{b}$ | $62.7 \pm 17.5^{b}$ |

Means appearing in the same column having no letters in common are significantly different according to Duncan's new multiple range test ($P < 0.05$).

In only nine out of eighteen cases where plume was left in place, the wind had stopped completely when the male was in the 'plume' region. The wind had stopped by the time all eighteen moths had entered the non-plume region.

## Discussion

During flight in wind, the wind speed and a moth's airspeed, groundspeed, track angle, course angle, and drift angle are fairly precisely related (Fig. 1; after Marsh *et al.*, 1978). In our experiments, the triangle of velocities neccessarily collapsed when we reduced the wind speed to zero by stopping the fan. As a consequence, the moths' airspeeds became equal to their ground speeds, their track angles and course angles became identical, their drift angles became zero and anemotaxis was impossible since no wind was present.

Since anemotaxis could not have occurred, it is clear that some other mechanism(s) must have been operating to allow the moths to continue to zigzag along the stationary plume toward the pheromone source. The maintenance of an up-tunnel zigzagging track, developed before the wind had stopped, could have accounted for the males' continued progress toward the source. Maintenance of this up-tunnel momentum alone, however, cannot account for why males never flew past the source or why their left–right reversals remained as close to the plume axis after the wind was stopped as before it was stopped.

That the males' movements in the plume area in zero wind were in fact chemically mediated *by* the plume was suggested by their change of flight tracks after flying out of the front end of the plume into clean air. They no longer progressed up the tunnel, but rather, made wider left–right reversals steered more directly cross-tunnel. In fact, in zero wind the flight patterns in the plume and after emergence from its up-tunnel end were not unlike those when wind was present (compare Fig. 2c, d with Fig. 3c, d). In wind, the males' track reversal distances were first narrow when in the plume and then wide after losing it, at which time the inter-reversal track angles increased. Similar results in wind were first reported by Kennedy & Marsh (1974) and later described by Marsh *et al.* (1978).

Until the findings of Kennedy *et al.* (1980, 1981), Kennedy & Marsh's (1974) working model and that of Marsh *et al.* (1978) for the events occurring during pheromone source location was that pheromone onset would trigger positive optomotor-regulated anemotaxis and loss of pheromone would trigger reversing anemomenotaxis with nearly 90° track angles with respect to the windline. This dichotomy of responses would never be seen fully during flight in a normal plume because the rapid onset and offset of stimulation encountered by a moth flying through concentrated pheromone filaments, then through 'holes' of clean air, would never allow either of the two anemotactic responses to develop in full. Rather, the hybridized result would be a track with left–right zigzags oriented obliquely into the wind.

Kennedy & Marsh's (1974) demonstration of the optomotor anemotactic response neatly negated the hypothesis of Farkas & Shorey (1972) and Shorey (1973) that chemotaxis alone could account for pheromone source location by flying moths, but it only failed to confirm the existence of the hypothesized chemotactically-guided *longtitudinal displacement toward the source*. Farkas & Shorey had, in addition, provided evidence that in zero wind the *lateral* distances of the moths' zigs and zags remained narrow along the stationary plume, and they hypothesized that this was caused by a chemotactic turning back towards the plume whenever clean air was encountered (Shorey, 1973). It is this lateral component from their results that has been largely ignored by researchers, and which is now partially substantiated by our experiments.

Males of *G. molesta* do indeed appear to use chemotaxis when zigzagging toward the source in zero wind to remain in close proximity to the time-averaged plume. However, as to how they do this differs in one basic way from Farkas & Shorey's. Instead of each excursion out of the plume causing a reversal back into the plume, we propose that the ambient concentration modulates the frequency of a *protracted programme* of track reversals. In performing the internally-stored, self-steered motor programme (Kennedy, 1978), the moth might use visual feedback from the environment, or perhaps only proprioceptive feedback. In either case, each zig and zag would not be steered according to concentration as Farkas & Shorey proposed, although in carrying out the movements the male might give the *appearance* of doing just that. When wind is also considered, our model differs in a second way from theirs. We propose that the self-steered chemotactic programme is *integrated*

*with* optomotor regulated anemotaxis, whereas they proposed that *only chemotaxis* and not anemotaxis is used.

Farkas & Shorey (1972) and Shorey (1973) believed that their moths might have been able to sample simultaneously and hence compare pheromone concentrations received by each antenna as they left the (time-averaged) plume. Such a mechanism would permit tropotaxis (Fraenkel & Gunn, 1940). Kennedy & Marsh (1974) found that flight tracks of unilaterally antennectomized males up a pheromone plume were normal in all respects, negating the likelihood of such simultaneous sampling by flying moths. Furthermore, it is unlikely that *consistent* directional information on the plume's location could be gathered by this type of sampling due to the spatial randomness both of the plume filaments and of the moth's track relative to them (Kennedy, 1977, 1978).

This same problem would also occur in sequential sampling, and the type of side-to-side sequential sampling relative to the direction of net displacement, permitting the transverse klinotactic reactions invoked by Shorey (1973), would be particularly vulnerable to this problem (Fraenkel & Gunn, 1940; Kennedy, 1978); so would klinotaxis mediated by longitudinal sampling (Kennedy, 1978) in which the sequential sample is taken along the path of net displacement. However, if the sequential sampling of the ambient concentration along the path of displacement were used to modulate a *self-steered* programme of zigzags, then such longitudinal klinotaxis could be useful in filamentous plumes (Kennedy, 1978). When our moths in zero wind flew out of the front end of the stationary plume into clean air, and then changed their pattern of zigzags, they showed evidence both of using longitudinal klinotaxis during flight along the stationary plume and a self-steered zigzag programme (Fig. 3c, d).

Our results in zero wind are quite similar to recent findings by Kennedy *et al.* (1980, 1981) for *Adoxophyes orana* moths in wind, which demonstrate that turning not only occurs upon loss of pheromone, but also upon onset of pheromone stimulation, and that even with uniform, constant pheromone contact, moths zigzag more narrowly and more often than when in uniform clean air. Their conclusion was that zigzagging movements are made by moths not only upon finding themselves in clean air, but in pheromone as well. This model represented a sharp departure from the earlier, dichotomous one of positive anemotaxis alternating with reversing anemomenotaxis (Kennedy & Marsh, 1974; Marsh *et al.*, 1978), and provided a new way to explain how males can 'lock on' to a plume after casting flight in clean air.

We initially envisaged that the longitudinal chemoklinotactic mechanism for orientation to a pheromone source in zero wind would merely supplement the anemotaxis available to males when wind is present (Baker & Kuenen, 1982). We now propose that this protracted programme of zigzags is superimposed upon anemotaxis, while wind is present, to help keep a male 'locked on' laterally to the plume (Kennedy *et al.*, 1980, 1981).

We are not suggesting that the chemotaxis operates *in place of* anemotaxis when wind is present, but *in addition to* it, and there is some recent evidence supporting this proposal. The movements of our males in zero wind and at near-zero effective wind velocity, both while in and out of contact with pheromone, were quite similar to those in wind, with and without pheromone. They were also similar to those of other species in wind in other recent studies where, in both non-homogeneous pheromone clouds, higher concentrations (or presence, compared with absence) of pheromone caused narrower track reversals than did lower concentrations (Kennedy & Marsh, 1974; Marsh *et al.*, 1978; Cardé & Hagaman, 1979; Kennedy *et al.*, 1980, 1981; Keunen & Baker, 1982a). The frequency of the reversals was thus determined by concentration under unvarying wind velocities (Kuenen & Baker, 1982a; Kennedy *et al.*, 1980, 1981), and provides evidence for such a chemoklinotactic programme in wind.

In addition, as demonstrated here, male *G. molesta* (and *P. interpunctella*) (Marsh *et al.*, 1978, 1981), upon flying out the front end of a plume in wind, continue to zigzag for around 0.5 s or more in clean air, and since there is physically no plume present, the zigzags must be a continuance of the internal programme of turns, whose frequency then becomes lower as the reduced ambient concentration is perceived by the moth.

Presumably an anemomenotactic response

to drift is superimposed on the chemotactic programme, giving polarity to the zigzags and resulting in upwind displacement. Recent evidence indicates that repeated 'zigzag' types of track reversals, or 'counter-turns' (Tobin, 1981) are made by moths flying to pheromone alone and having had no pre-exposure to wind-induced drift (Kennedy, Ludlow & David, unpublished data; Baker, Willis & Phelan, unpublished data). However, the zigzags had no consistent direction, and meandered around the tunnel. A zigzagging track as opposed to a straight one may not only be an efficient way to maintain contact with pheromone, but also efficient for sampling for shifts in wind direction and for the quick detection of wind direction immediately following a lull. In zero wind and zero drift, optical flow-field feedback could mediate zigzagging, and could account for continued progress up a stationary pheromone plume during a wind lull. Experiments are in progress to test these ideas and determine the relative contributions of anemotaxis, longitudinal chemoklinotaxis, and visual cues relating to both these systems during flight up a pheromone plume, with and without wind.

In this study we have partially substantiated the results of Farkas & Shorey (1972) that males can locate a pheromone source in zero wind, albeit maintaining contact with the plume by a different kind of chemotaxis from the one they proposed. The up-tunnel displacement of our moths in zero wind was apparently influenced by an initial setting of the polarity of the zigzags while the wind was on, presumably through optomotor anemotaxis. Farkas & Shorey did not appear to realize the contribution made by optomotor anemotaxis to the initial up-tunnel progress of their moths before the wind stopped (Kennedy & Marsh, 1974).

During wind lulls in nature, males that could advance several metres toward a female by maintaining their previous flight pattern of narrow zigzags in the continued presence of pheromone should arrive at the female sooner than those lacking this ability. This is now even clearer in view of the recent findings by David *et al.* (1982, 1983) that pheromone-laden air moves in straight lines away from a pheromone source regardless of shifting wind directions. Gypsy moth males' displacement while in pheromone-laden wind was usually toward the source, and if wind were to cease it would appear that continuing to move in the same direction as long as pheromone was present would continue to take the male directly toward the source.

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## References

Baker, T.C. & Cardé, R.T. (1979a) Endogenous and exogenous factors affecting periodicities of female calling and male sex pheromone response in *Grapholitha molesta* (Busck). *Journal of Insect Physiology*, 25, 943–950.

Baker, T.C. & Cardé, R.T. (1979b) Analysis of pheromone-mediated behaviors in male *Grapholitha molesta*, the oriental fruit moth (Lepidoptera: Tortricidae). *Environmental Entomology*, 8, 956–968.

Baker, T.C., Meyer, W. & Roelofs, W.L. (1981) Sex pheromone dosage and blend specificity of response by oriental fruit moth males (*Entomologia Experimentalis et Applicata*, 30, 269–279.

Baker T.C. & Kuenen, L.P.S. (1982) Pheromone source location by flying moths: a supplementary non-anemotactic mechanism. *Science*, 216, 424–427.

Baker, T.C. & Roelofs, W.L. (1981) Initiation and termination of oriental fruit moth male response to pheromone concentrations in the field. *Environmental Entomology*, 10, 211–218.

Batschelet, E. (1972) Recent statistical methods for orientation data. *Animal Orientation and Navigation* (ed. by S. R. Galler, K. Schmidt-Koenig, G. J. Jacobs and R. E. Belleville), pp. 66–91.

Cardé, A.M., Baker, T.C. & Cardé, R.T. (1979) Identification of a four-component sex pheromone of the female oriental fruit moth, *Grapholitha molesta* (Lepidoptera: Tortricidae). *Journal of Chemical Ecology*, 5, 423–427.

Cardé, R.T. & Hagaman, T.E. (1979) Behavioral responses of the gypsy moth in a wind tunnel to air-borne enantiomers of disparlure. *Environmental Entomology*, 8, 475–484.

David, C.T., Kennedy, J.S., Ludlow, A.R., Perry, J.N. & Wall, C. (1982) A reappraisal of insect flight towards a distant source of wind-borne odor. *Journal of Chemical Ecology*, 8, 1207–1215.

David, C.T., Kennedy, J.S. & Ludlow, A.R. (1983) How male moths find a source of sex pheromone in the field. *Nature* (in press).

Farkas, S.R. & Shorey, H.H. (1972) Chemical trail-following by flying insects: a mechanism for orientation to a distant odor source. *Science*, 178, 67–68.

Fraenkel, G.S. & Gunn, D.L. (1940) *The Orientation of Animals. Kineses, Taxes, and Compass Reactions*. Dover Publications, New York.

Kennedy, J.S. (1977) Olfactory responses to distant plants and other odor sources. *Chemical Control of Insect Behaviour: Theory and Application* (ed. by H. H. Shorey and J. J. McKelvey), pp. 67–91. John Wiley & Sons, New York.

Kennedy, J.S. (1978) The concepts of olfactory arrestment and attraction. *Physiological Entomology*, 3, 91–98.

Kennedy, J.S., Ludlow, A.R. & Sanders, C.J. (1980) Guidance system used in moth sex attraction. *Nature*, 288, 475–477.

Kennedy, J.S., Ludlow, A.R. & Sanders, C.J. (1981) Guidance of flying male moths by wind-borne sex pheromone. *Physiological Entomology*, 6, 395–412.

Kennedy, J.S. & Marsh, D. (1974) Pheromone-regulated anemotaxis in flying moths. *Science*, 184, 999–1001.

Kennedy, J.S. & Thomas, A.A.G. (1974) Behavior of some low-flying aphids in wind. *Annals of Applied Biology*, 76, 143–159.

Kuenen, L.P.S. & Baker, T.C. (1982a) The effects of pheromone concentration on the flight behaviour of the oriental fruit moth. *Physiological Entomology*, 7, 423–434.

Kuenen, L.P.S. & Baker, T.C. (1982b) Optomotor regulation of ground velocity in moths during flight to sex pheromone at different heights. *Physiological Entomology*, 7, 193–202.

Marsh, D., Kennedy, J.S. & Ludlow, A.R. (1978) An analysis of anemotactic zigzagging flight in male moths stimulated by pheromone. *Physiological Entomology*, 3, 221–240.

Marsh, D., Kennedy, J.S. & Ludlow, A.R. (1981) Analysis of zigzagging flight in moths: a correction. *Physiological Entomology*, 6, 225.

Miller, J.R. & Roelofs, W.L. (1978) Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. *Journal of Chemical Ecology*, 4, 187–198.

Roelofs, W.L., Comeau, A. & Selle, R. (1969) Sex pheromone of the oriental fruit moth. *Nature*, 224, 723.

Shorey, H.H. (1973) Behavioral responses to insect pheromones. *Annual Review of Entomology*, 18, 349–380.

Tobin, T.R. (1981) Pheromone orientation: role of the internal control mechanisms. *Science*, 214, 1147–1148.

Watson, G.S. (1962) Goodness-of-fit tests on a circle. II. *Biometrika*, 49, 57–63.

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