# The effects of pheromone concentration on the flight behaviour of the oriental fruit moth, *Grapholitha molesta*

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ABSTRACT. Male oriental fruit moths, *Grapholitha molesta* (Busck) (Tortricidae), flew at lower overall and net ground velocities when they flew toward higher concentration pheromone sources. Turning frequency was greater with increased pheromone concentration, while the distance of turns from the plume axis back towards the axis decreased. Turning magnitude and inter-reversal track angles remained constant at all concentrations tested. Concomitant with the changes in ground velocity but constant inter-reversal angles, were decreases in airspeed, decreases in the moths' course angles and increases in their drift angles. The significance of these changes is discussed in relation to their possible role in a longitudinal chemoklinotactic programme of turning operating in conjunction with anemotaxis to allow location of a pheromone source in wind.

Key words. Sustained flight tunnel, pheromone concentration, flight behaviour, orientation behaviour, *Grapholitha molesta*, Oriental fruit moth.

## Introduction

Males of many species of Lepidoptera fly upwind in response to air-borne pheromone emitted by a female or to the appropriate synthetic blend evaporating from a dispenser. The mechanisms employed to locate the female or a synthetic source are still not completely known, but several have been proposed. In wind, positive optomotor anemotaxis in the presence of pheromone, alternating with reversing anemomenotaxis in its absence had, until recently, been thought the most likely mechanisms (Kennedy & Marsh, 1974; Kennedy, 1977, 1978).

Positive optomotor anemotaxis is flying straight upwind by steering responses to the visual image movements produced by wind drift, and reversing anemomenotaxis is flying at an angle not directly into the wind in 'zig-

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zags', again with some visual input from wind drift. This dichotomous anemotactic model was recently modified (Kennedy *et al.*, 1980, 1981, 1982) into a continuous one in which cross-tunnel reversals would always be present, in pheromone or out of it, but their amplitude, frequency and angle would be modulated by concentration. Another mechanism, longitudinal chemoklinotaxis, would operate during wind lulls and supplement anemotaxis (Baker & Kuenen, 1982).

On a practical level, both the component ratios and the emission rate of a synthetic pheromone can affect trap capture, and there appear to be optimal values for both these variables (Roelofs, 1978) which result in the greatest active distance for attraction. Excessive levels can cause in-flight arrestment some distance away from the pheromone source, thereby reducing captures (Baker *et al.*, 1981; Baker & Roelofs, 1981).

Although attraction and arrestment, two outcomes of movement reactions to pheromone (Kennedy, 1978), are determined by

pheromone quantity and quality, the movement reactions themselves are poorly understood. In sustained-flight wind tunnels, increases in pheromone concentration have been shown to decrease the net ground velocities of *Pectinophora gossypiella* and *Lymantria dispar*, and also to decrease distances away from the pheromone plume of turns back toward the axis of plume (Farkas *et al.*, 1974; Cardé & Hagaman, 1979).

*P.gossypiella* males also exhibit a decreased wing beat frequency with increased pheromone concentration (Farkas *et al.*, 1974), and it appears from representative tracks that *L. dispar* males also increase their turn frequencies (Cardé & Hagaman, 1979). In addition, in response to raised pheromone concentrations, male oriental fruit moths (*Grapholitha molesta* (Busck)) when walking and wing-fanning, decrease their linear velocity and turning frequency, while increasing their turning severity (degrees/turn) (Baker *et al.*, 1981).

In order to understand more fully the movement reactions caused by changes in pheromone concentration, we made videotaped recordings of *G.molesta* males flying in a sustained-flight wind tunnel. We found that increased pheromone concentration decreased their overall and net ground velocities, increased their angular velocities and angular-to-linear velocity ratios, and decreased the distances of track reversals from the plume axis. Some parameters such as turning severity and track inter-reversal angles were independent of pheromone concentration, but when in-flight arrestment occurred, these trends broke down. This paper reports these observations.

## Materials and Methods

### General

Moths were reared on small green thinning apples (Baker *et al.*, 1981); males were separated from females in the pupal stage and adults were segregated daily by age. All life stages were maintained at $25 \pm 2°C$ on a 16 h light:8 h dark cycle. Adult males were kept isolated from females and had continuous access to an 8% sucrose solution.

Males were flown in a sustained-flight wind tunnel constructed of clear polycarbonate plastic with a working section 1 m wide at floor level, 0.90 m high, and 3.65 m long (Kuenen & Baker, 1982). Recordings were made during a 2-h period beginning 1 h before the end of photophase, within the period of maximal responsiveness to pheromone (Baker & Cardé, 1979a). 4–6-day-old males were individually released from aluminium screen cones hand-held in the centre of the pheromone plume (Baker *et al.*, 1981). They had previously been acclimated to tunnel conditions for at least 5 min before their release.

A ground pattern with transverse alternating 10-cm black and white stripes was located 1.5 cm below the 6-mm-thick, clear acrylic plastic floor. A 1-hp fan was voltage-regulated to provide an airflow of 65 cm/s. Air turbulence was reduced as it passed through a layer of muslin and a layer of fine mesh polyester such that a TiCl smoke plume from a rubber septum was level, *c.* 10 cm in diameter halfway down the tunnel and *c.* 15 cm in diameter at the downwind end. Wind velocity varied less than 10% cross-sectionally in the tunnel (Kuenen & Baker, 1982). A light intensity of 250 1x was provided by four voltage-regulated 100-W tungsten incandescent lights diffused by reflection from a white styrofoam (expanded polystyrene) ceiling. Pheromone from the tunnel was removed by a 30-cm diameter exhaust pipe (air speed in centre = 2.9 m/s) aligned with the centre of the plume. The remainder of the air from the tunnel recirculated through the room.

### Pheromone

All pheromone components were dispensed from rubber septa (A. H. Thomas Co. No. 8753-D22, sleeve type, 5 × 9 mm) positioned in the centre of a 15 × 15 × 0.05 cm galvanized steel plate. The plate was supported by a galvanized steel, sheet metal platform 15 cm high positioned in the middle of the tunnel 40 cm from the upwind end of the tunnel's working section. A new acetone-rinsed plate was used with each change of pheromone concentration.

All septa were impregnated with the specific pheromone blend of 5.9% (*E*)-8-dodecenyl acetate and 3.8% (*Z*)-8-dodecenyl alcohol (Cardé *et al.*, 1979) in (*Z*)-8-dodecenyl acetate (Roelofs *et al.*, 1969). The formulation

of this optimal ratio (Baker & Cardé, 1979b) was verified by gas–liquid chromatography (GLC) on a 10% XF-1150 (50% cyanoethyl, methylsilicone on 100–120 mesh Chromosorb W-AW-DMCS) 2 m × 2 mm ID glass column, $N_2$ carrier flow was 25 ml/min at 160°C (Baker & Roelofs, 1981). All the components had <0.5% volatile impurities as determined by GLC analysis on the XF-1150 column (Baker & Roelofs, 1981).

Hexane solutions of 100 and 300 $\mu g/\mu l$ were formulated gravimetrically and each was serially diluted to obtain desired solutions. A 10-$\mu l$ aliquot of each solution was applied to the inside bottom of the large end of a septum. Septa were stored at −20°C between 2-h uses and were used for up to 4 weeks.

### Experiments and criteria

Net up-tunnel ground velocities of in-plume flight along the wind line were measured with a stop-watch to the nearest 0.1 s as the males traversed the centre 1.22 m section of the tunnel (Kuenen & Baker, 1982). The flight tracks of the same moths were video-recorded from above in a plan view using a Sony RSC 1050 rotary-shutter camera located on top of the tunnel and connected to a Sony SLO 340 recorder. The field of view for all recordings extended 65 cm, from 105 to 170 cm down-tunnel from the pheromone source, and 50 cm across the tunnel. A single layer of cheese-cloth was placed on the tunnel floor so that the moths would be visible on the video display as they flew over the ground pattern's black stripes. The stripes were still visible through the cloth and moths would still slow, stop, or reverse their up-tunnel flight if the stripes were moved in the down-tunnel direction (Kennedy & Marsh, 1974; Miller & Roelofs, 1978).

Tracks were re-recorded onto a Sony SVM-1010 motion analyser and played back frame-by-frame, yielding an accurate, non-blurred image of the moths' location each 1/60 s. Consecutive locations were marked with an ink dot on a mylar (transparent plastic) sheet affixed to the analyser's video screen surface, and reference marks were added to each sheet so that each track could be aligned with any other for analysis.

Males were flown at 15 cm above the tunnel floor to septa containing 1, 3, 10, 30 or 100 $\mu g$ of the pheromone blend. The release rates from 10, 100 and 1000 $\mu g$ septa are 1.2, 12 and 219 ng/h in still air at 23°C, and thus varying approximately linearly with the loading rate (Baker *et al.*, 1980). Males were released individually and of those initiating upwind flight, only the tracks of those that flew to within 80 cm of the pheromone source were analysed. Moths were tested in a randomized complete block design with five successful flights per block per treatment; the tracks of twenty-five moths were recorded and analysed for each treatment.

An *x/y* digitizer (Houston Instruments, HI PAD® DT-11) serially interfaced with a microcomputer (Radio Shack TRS-80® Model III) was used for detailed track analysis. The moth-track tracings on the mylar sheets were aligned on the digitizer surface using the reference marks, and the *x* and *y* coordinates of each dot (1/60 s apart) were entered into the computer (as in Fig. 1A). The digitizer resolution was set at 0.0039 cm with an accuracy of 0.0117 cm.

A program (BASIC) was developed that calculated, for each flight track through the 65-cm video field, the following parameters that we thought might elucidate changes in movement reactions elicited by different pheromone concentrations: (1) flight duration; (2) overall velocity (distance per unit time along the actual sinuous track); (3) net up-tunnel ground velocity (distance along the wind line per unit time); (4) number of turns; (5) turning frequency (number of turns per second); (6) turning magnitude (degrees per turn); (7) the angular velocity (degrees per second); (8) angular-to-linear velocity ratio (degrees per centimetre, calculated as total degrees turned/total distance flown); (9) distance per turn (centimetres along the actual track); (10) mean orientation angle and the mean vector length (see Batschelet, 1972); and (11) a mean upwind index.

Additionally, we measured inter-track reversal distances (the distances between consecutive track reversals), track reversal distances (the distance from the plume axis of turns back toward the plume axis), and inter-reversal track angles (Fig. 1B), the latter modified after Marsh *et al.* (1978). Except for directional data (see below), treatments were
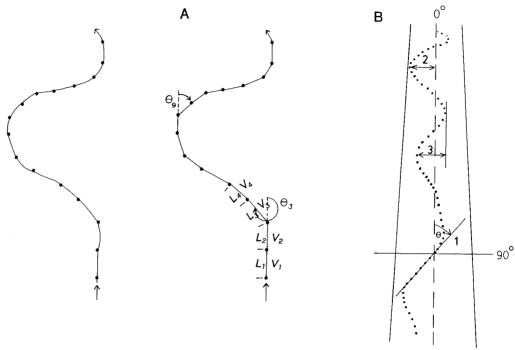
**A**

**B**



FIG. 1. Criteria used for analysis of *G. molesta* flight tracks. (A) At left is a schematic of a moth's ground track; dots represent the moth's location at consecutive 1/60-s intervals. At right is the manner in which the computer calculates (represents) the actual track. The lengths, $L1$, $L2$, $L3$ ... of vectors, $V1$, $V2$, $V3$ .... were calculated as were their angles, $\theta 1$, $\theta 2$, $\theta 3$ ... from due upwind. Turning and velocity parameters were calculated from these measures (see text). (B) The ground track of a moth flying toward a 10-$\mu$g pheromone source. The source is 105 cm above the top of the figure, at 0°. Outer solid lines are the boundaries of the time-averaged pheromone plume and the central dashed line is its axis. 1, the measurement of the inter-reversal angle, 90° would be cross-wind on either side of the plume axis; 2, the measurement of track reversal distances; 3, the measurement of inter-track reversal distances.

analysed by two-way analysis of variance with square root transformations where indicated by Bartlett's test for homogeneity of variances.

A turn was defined to begin at any point along the track where the direction of movement changed from clockwise to anti-clockwise (or vice-versa) and to end at the start of the subsequent turn (see Fig. 1). A value of 50° change of direction had to be accumulated before a new turn was registered, to allow for error inherent in transcribing the tracks and entering the $x,y$ coordinates. A turn was deemed to have started (or ended) at the first point on the track where the change in direction had begun. The number of computer-determined turns always equalled the number that could be determined visually. The beginning and end of each tracing contained portions of incomplete turns that

were not used for accumulation of turning data.

The mean unit orientation vector of each entire track, $r$, was calculated using the following formulae:

$$\bar{x} = \left( \sum_{i=1}^{n} \cos\theta i \right)/n$$

and

$$\bar{y} = \left( \sum_{i=1}^{n} \sin\theta_i \right)/n;$$

$\cos\theta = \bar{x}/r$; $\sin\theta = \bar{y}/r$. $r = \mathrm{SQR}(\bar{x}^2 + \bar{y}^2)$ (see Batschelet, 1972). The length of each mean unit vector, $r$ ($0 < r < 1$), reflects the degree of dispersion about the mean orientation angle, and is based solely on the orientations of the individual vectors, and not on their lengths

(Batschelet, 1972). A mean orientation of 360° or 0° indicated direct up-tunnel (upwind) orientation; the deviation from this upwind angle was determined for each track and used for comparison among the treatments. The length of the mean unit vector, $r$, was used to examine each track individually to determine whether the vectors composing the entire track were orientated significantly in the upwind, 0° direction ($V$-test, a modified Rayleigh test; Batschelet, 1972). Treatments were compared by Watson & Williams' test (1956; see Batschelet, 1972).

A second orientation index, the upwind index (Van Vorhis Key & Baker, 1982) was calculated from all the individual vectors, only this time each vector was weighted according to its length in addition to its direction. The component of each vector along the wind line was employed (northerly component of Durand & Greenwood, 1958) by multiplying each vector's directional component, ($\cos \theta i$), by the distance travelled, ($Li$), during the 1/60 s in which that orientation was taken:

$$UI = \left( \sum_{i=1}^{n} (\cos \theta i)(Li) \right)/n$$

(see Fig. 1A).

## Results

Flight tracks of males changed significantly with increased pheromone concentration (Fig. 2). Among the fourteen track parameters that we measured, the most striking changes were the significant decreases in overall and net up-tunnel velocities with increases in pheromone ($\log_{10}$) concentration ($P<0.05$, Fig. 3). The net velocity measurements made by timing the moths with a stop-watch were similar to those calculated from the video records (Fig. 3). Both the inter-track reversal distances and the track reversal distances decreased significantly with increased pheromone concentration ($P<0.05$; Fig. 4), giving the impression of greater 'accuracy' of orientation in response to higher concentrations.

In addition to changes in linear velocity, turning was also affected by concentration. Both the turning frequency and the number of turns per track increased at the higher dosages, but the magnitude of each turn

(degrees/turn) remained constant ($P>0.05$, Table 2). This last implies that among the concentrations tested, the inter-reversal track angles (Marsh *et al.*, 1978) should have been very similar relative to the upwind line, which they were (Table 1). Even though turning magnitude did not differ significantly among the five pheromone concentrations, both the angular velocities and the angular-to-linear velocity ratios (degrees/cm) increased at higher dosages ($P<0.05$, Table 2), probably a direct result of the increased turning frequencies and decreased track reversal distances.

As expected, all of the moths' tracks were oriented in the upwind direction, i.e. their mean angles of orientation (Table 1) were not significantly different from 0° or 360° (mean vector length, $r = 0.57 \pm 0.07$ SD, $n = 125$; $P<0.05$, $V$-test (modified Rayleigh test, Batschelet, 1972). There was a greater mean orientation angle deviation from 0° at the 1-$\mu$g dosage than at the higher dosages, again implying that there was greater orientation accuracy at higher dosages ($P<0.05$; Table 1). The mean upwind index, on the other hand, decreased significantly with increased pheromone concentration ($P<0.05$, Table 1). This was probably due to the decreased overall velocity, since the upwind index takes into account the length of each track vector, and because turn angles and inter-reversal track angles were similar for all treatments.

As in a previous study (Baker *et al.*, 1981), greater proportions of moths exhibited in-flight arrestment in response to higher pheromone dosages; 0%, 3.8% and 40.5% of males were arrested to the 1, 10 and 100 $\mu$g dosages.

When arrestment occurred in the video field, the tracks were examined in three phases, pre-arrestment, arrestment and post-arrestment, during which time the upwind index went from positive, to zero, to negative as the moths first moved up-tunnel, then stopped, and finally began moving down-tunnel (lower part of Table 1). The arrestment phase was deemed to be the entire track portion within the 10-cm region at the upwind end of the track (Fig. 2D), while the pre-arrestment phase was that track portion where the moth was flying up-tunnel prior to the arrestment zone. The post-arrestment phase was the
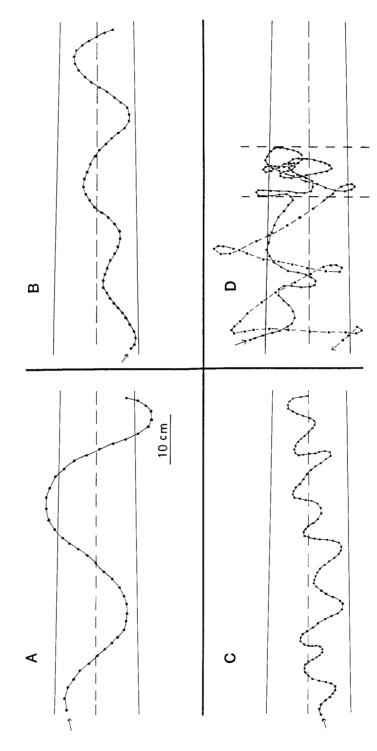
FIG. 2. The flight tracks of *G.molesta* males flying to different concentrations of pheromone. The outer solid lines are the boundaries of the time-averaged plume and the central dashed line is its axis. The pheromone septum is 105 cm to the right of each figure. The dots represent the moth's locations at consecutive 1/60-s intervals. (A) Flight toward a 1-μg septum. (B) Flight toward a 10-μg septum. (C) Flight toward a 100-μg septum. (D) The arrestment track of a moth initially flying toward a 100-μg septum; dashed lines (10 cm apart) are the boundaries of the arrestment region. The pre-arrestment and post-arrestment tracks are the respective up-tunnel and down-tunnel track portions (arrows) to the left of the arrestment region.
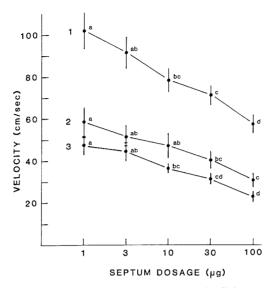
FIG. 3. Mean velocities of *G.molesta* males flying toward septa of different concentrations. Points along each line having no letters in common are significantly different (*P*<0.05, Duncan's new multiple range test). Vertical bars are standard errors, *n* = 25. 1, the males' velocities along the actual ground track (overall velocity); 2, the net up-tunnel velocity (velocity along the wind-line) as measured from the video record; 3, the net up-tunnel velocity as measured by a stop-watch (see text).
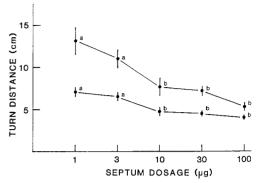


FIG. 4. Upper curve is the mean inter-track reversal distance (see Fig. 1B) of *G.molesta* males flying to pheromone septa of different concentrations. The lower curve is the mean track reversal distance. Points along each line having no letters in common are significantly different (*P*<0.05, Duncan's new multiple range test). Vertical bars are standard errors, *n* = 25.

down-tunnel track after the moth had left the arrestment zone.

The shortness of the mean vectors ($r = 0.01 \pm 0.02$, $n = 8$) for the mean angle of orientation during the arrestment phase indicates that the moths were not making significant

TABLE 1. Mean orientation and track parameters (±SD) of *G.molesta* males flying toward pheromone sources of five concentrations.

| Pheromone (μg) | Inter-reversal track angle (deg. from 0°) | Angle of orientation (deg. from 0°) | Upwind index* | No. of turns per track |
|---|---|---|---|---|
| 1 | $56.69^a \pm 6.02$ | $8.31^a \pm 1.64$ | $+0.92^a \pm 0.24$ | $6.24^c \pm 1.90$ |
| 3 | $57.22^a \pm 1.52$ | $5.50^b \pm 3.88$ | $+0.82^{ab} \pm 0.18$ | $7.00^{bc} \pm 3.01$ |
| 10 | $55.74^a \pm 7.40$ | $4.09^b \pm 1.40$ | $+0.76^{ab} \pm 0.22$ | $7.88^{bc} \pm 2.68$ |
| 30 | $57.66^a \pm 6.30$ | $4.07^b \pm 1.27$ | $+0.65^{bc} \pm 0.14$ | $10.59^{ab} \pm 4.16$ |
| 100 | $58.36^a \pm 2.21$ | $4.01^b \pm 0.93$ | $+0.50^c \pm 0.12$ | $15.95^a \pm 4.90$ |
| | | | | |
| Pre-arrestment | $70.17^{**} \pm 11.01$ | $(6.45) \pm 8.54$ | $+0.42^{**} \pm 0.21$ | — |
| Arrestment | $90.45^{**} \pm 5.19$ | $(30.75) \pm 19.10$ | $-0.01^{**} \pm 0.03$ | — |
| Post-arrestment | $114.83 \pm 13.97$ | $(17.82) \pm 33.56$ | $-0.57 \pm 0.23$ | — |

Twenty-five moths were tested in a randomized complete block design; five moths were recorded and analysed per block per treatment. Column means followed by the same letter are not significantly different by two-way analysis of variance and Duncan's new multiple range test or Watson & Williams test: arrestment tracks were not included in the analysis. *Positive values indicate net movement is toward the source; negative values, the opposite; higher numerical values indicate more rapid displacement.

The lower table shows data from eight moths flown to the 30 or 100 μg treatments; see text and Fig. 2D for explanation of how tracks were separated into these three phases. Parentheses indicate that the shortness of the mean vector lengths for all these tracks indicate that the moths had not made significant progress in any definable direction (*P*>0.05, *V*-test), so comparison of means is inappropriate. Dashes indicate values varied because of the position along the windline where arrestment occurred, so comparisons are inappropriate. **Pairs of values significantly different (*P*<0.05, *t* test).

TABLE 2. Mean turning parameters (±SD) of *G.molesta* males flying toward pheromone sources of five concentrations.

| Pheromone (μg) | Turn frequency (turns/s) | Turning magnitude (°/turn) | Angular velocity (°/s) | Angular-to-linear velocity ratio (°/cm) | Distance per turn (cm) |
|---|---|---|---|---|---|
| 1 | $6.33^b \pm 0.77$ | $137.8^a \pm 17.8$ | $821^c \pm 137$ | $8.44^c \pm 4.80$ | $19.80^a \pm 5.08$ |
| 3 | $6.96^b \pm 0.71$ | $141.5^a \pm 17.3$ | $852^{bc} \pm 71$ | $10.27^{bc} \pm 6.26$ | $17.04^a \pm 4.67$ |
| 10 | $7.66^a \pm 0.47$ | $132.7^a \pm 19.1$ | $961^{bc} \pm 71$ | $16.65^{bc} \pm 6.31$ | $11.57^b \pm 2.40$ |
| 30 | $7.64^a \pm 0.60$ | $143.3^a \pm 17.9$ | $1026^{ab} \pm 212$ | $17.65^b \pm 7.57$ | $11.12^b \pm 1.82$ |
| 100 | $8.33^a \pm 0.35$ | $150.5^a \pm 8.5$ | $1163^a \pm 75$ | $32.20^a \pm 11.08$ | $8.30^b \pm 1.71$ |
| Pre-arrestment | $8.21 \pm 1.04$ | $169.1** \pm 27.4$ | $1340 \pm 122$ | $27.20 \pm 11.05$ | $8.73 \pm 1.28$ |
| Arrestment | $7.49 \pm 2.10$ | $234.7** \pm 28.5$ | $1491 \pm 243$ | $36.65 \pm 20.07$ | $11.50 \pm 4.98$ |
| Post-arrestment | $4.80 \pm 1.50$ | $275.2 \pm 70.3$ | $1007 \pm 362$ | $13.71 \pm 10.04$ | $32.27 \pm 18.32$ |

Details as for Table 1.

progress in any one definable direction ($P > 0.05$; $V$-test). During the arrestment phase, the tracks became quantitatively different from those of moths making significant up-tunnel progress. The mean inter-reversal track angle increased to near 90° and the mean turn magnitude increased to near 235° (Tables 1 and 2). One might have expected the turn magnitude to be about 180° when arrestment occurred, but greater values resulted when the moths made long 'looping' turns rather than simple left—right reversals (Fig. 2D).

During early post-arrestment the moths usually began 'drifting' down-tunnel, while apparently maintaining plume contact and a generally upwind body orientation, before flying up and out of the plume. During post-arrestment the absolute overall velocity increased (to 106 cm/s) and, coupled with the decreased turning, resulted in a lower angular-to-linear velocity ratio although now the direction of movement was decidedly down-tunnel as indicated by the negative upwind index (Table 1).

## Discussion

The decreases in velocity that occurred with increased pheromone concentration are consistent with the results of previous studies with both flying (Farkas *et al.*, 1974; Cardé & Hagaman, 1979) and walking moths (Baker *et al.*, 1981). In addition, we found that neither the turning severity nor the inter-reversal track angles were affected by con-

centration, implying that most of the decrease in net up-tunnel ground velocity was the result of an inverse orthokinesis (i.e. their speed was inversely proportional to the stimulus intensity) and not the result of a change in steering. We do not know whether *G.molesta* males decreased their thrust by reducing their wing-beat frequencies in order to fly more slowly, but males of *P.gossypiella* do do this when exposed to higher pheromone concentrations (Farkas *et al.*, 1974).

In addition to inverse orthokinesis, *G. molesta* males increased their turning frequencies and angular velocities at higher concentrations, although the magnitude of each turn remained constant. The higher angular velocity must have been caused by the increased turning frequency (track reversal frequency) since turn angle remained constant.

Recently, the turning frequency of *Adoxophyes orana* males in a pheromone-permeated airstream was found to be higher than the frequency in uniformly clean air (Kennedy *et al.*, 1980, 1981), demonstrating that left—right reversals occur even during uniform pheromone-on. This forced a revision of an earlier model (Kennedy & Marsh, 1974; Marsh *et al.*, 1978) in which positive anemotaxis (without reversals) would persist during pheromone-on, but would change to anemomenotactic crosswind casting during flight out of the plume or through holes of pheromone-free air.

The new model invokes a programme of reversals whose amplitude, frequency and inter-reversal track angles would be altered by concentration to result in zigzagging displacement toward the source during contact with

pheromone. The programme would evidently be chemotactically modulated, but the role of anemotaxis was not made clear.

Our data suggest that a similar concentration-dependent programme of reversals is used even in a broken plume. Its fenestrated structure could have allowed the males to experience rapid on—off—on stimulation by the pheromone. Nevertheless, their turning frequencies and angular velocities were affected by the overall changes in concentration to produce a smooth trend apparently independent of plume discontinuities. The perception of pheromone concentration on a time-averaged basis could account for the similarity in behaviours in these two dissimilar environments, or perhaps average peak concentration determines the programme that is used.

Further support for a chemotactic programme of turning has come from a study in zero wind, in which we have found that a chemotaxis is indeed used by *G. molesta* males to zigzag toward the source and remain in close proximity to the plume (Baker & Kuenen, 1982; Kuenen & Baker, in preparation). We proposed a longitudinal chemoklinotactic mechanism of steering, whereby males would continue to steer in zero wind according to visual feedback from the environment and a memory of their previous series of tracks.

Although it is feasible that the inter-reversal track angles of flying moths may be set anemotactically during contact with pheromone, in several experiments these angles remained constant after changes in wind speed (Marsh *et al.*, 1978) and in the absence of wind (Baker & Kuenen, 1982; Kuenen & Baker, in preparation), and also after moderate changes in pheromone concentration (this study). These angles did change, on the other hand, without wind after a severe change in concentration (Baker & Kuenen, 1982).

These results suggest that steering according to the magnitude of the drift angle might not be used by flying moths since this was not constant in these different conditions. Rather, the same concentration-dependent longitudinal chemoklinotactic programme used in zero wind may supplement anemotaxis, even while wind is present. The direction of drift,

not its magnitude, might periodically supply polarity information to allow displacement up, rather than down the plume. Future experiments are in progress to quantify the relative contributions of anemotaxis and longitudinal klinotaxis during flight up a pheromone plume in wind.

During flight toward a pheromone source, the concentration will, on average, increase due to a narrowing of the time-averaged plume as the source is approached. Baker & Roelofs (1981) estimated that, in a field experiment, an 18-fold increase in mean concentration occurred from 135 to 20 cm from a rubber septum emitting *G. molesta* pheromone, based on the distances at which arrestment occurred to different concentrations. As a male approaches a source, his track may become more 'accurate' (i.e. with narrower side-to-side oscillations) due to cues provided by the narrower time-averaged plume boundaries, or possibly by the higher frequency of reversals caused by the higher concentrations near the source.

In our present study, even at a set distance from a point source, where the plume structures for all concentrations were presumably similar, the track oscillations at higher concentrations were narrower, with the reversal distances occurring closer to the plume axis. The probability of finding above-threshold concentrations of molecules away from the plume axis should be greater for higher septum loadings, hence the active spaces (Bossert & Wilson, 1963) from the more concentrated pheromone sources should actually have been wider than those of the lower concentrations. Thus, if males used active space boundaries at the edges of time-averaged plumes to initiate turns back toward the axis, we should have seen wider rather than narrower reversals to higher concentrations.

At the very least, the turns back toward the axis should have remained at the same distance regardless of concentration, yet they clearly decreased at higher concentrations. Therefore, the increased 'accuracy' of orientation in a narrower plume closer to the source may be due in part, if not fully, to a concentration-dependent programme of reversals guided either anemotactically or chemotactically, or both. Left—right reversals have been observed for insects walking upwind in

uniform pheromone (Kramer, 1975) and apparently while within the time-averaged boundaries of wide plumes (Tobin, 1981; but see Kennedy et al., 1982, and Tobin & Bell, 1982).

In any homing or orienting process, lateral movements should be decreased as the source is approached, otherwise angular errors would become proportionally greater. In the field, males would normally encounter higher pheromone concentrations as they approached a female, and they would presumably change their movements similarly to what we observed in our flight tunnel in response to higher septum loadings.

The decreases in overall and net velocities and increases in angular velocity and turning frequency due to higher pheromone concentrations reached their maximum when in-flight arrestment occurred, producing the highest angular-to-linear velocity ratios that we measured. Net velocity toward the source became zero as the moths became arrested in the plume, and then became negative as they drifted down-tunnel and flew up and out of

the plume. In the field, moths might become arrested while responding to a trap containing an excessive loading of synthetic pheromone. As a result the trap would be inefficient and would not sample the field population optimally (Baker & Roelofs, 1981).

When in-flight arrestment occurred, the inter-reversal legs became 90° to the wind-line and the calculated drift angles increased to c. 45°. The calculated course angles, however, increased to c. 45° (Fig. 5B), and overall linear velocity was maintained (55.5 cm/s pre-arrestment v. 66.5 cm/s during arrestment). Therefore, the lack of displacement during arrestment seems to have been caused mainly by a steering change rather than by a further decline in linear velocity.

The in-flight arrestment to high pheromone concentrations appears similar to arrestment described anecdotally for moths flying toward pheromone sources with inappropriate component ratios (Baker et al., 1981; Linn & Gaston, 1981; Linn & Roelofs, 1981). In G. molesta, for instance, higher than natural ratios of E8-12:Ac to Z8-12:Ac cause in-



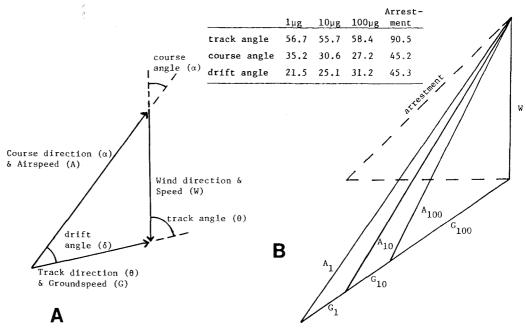|  | 1µg | 10µg | 100µg | Arrestment |
|---|---|---|---|---|
| track angle | 56.7 | 55.7 | 58.4 | 90.5 |
| course angle | 35.2 | 30.6 | 27.2 | 45.2 |
| drift angle | 21.5 | 25.1 | 31.2 | 45.3 |

FIG. 5. (A) Triangle of velocities during free-flight in wind (from Marsh et al., 1978). A change in any leg of the triangle necessarily affects one or both of the other legs. (B) In this study the wind speed, W, was constant, but ground speeds G1, G10, G100, decreased with increased pheromone concentration. As a result, airspeeds A1, A10, A100, decreased, and course angles decreased and drift angles increased. The moths maintained constant track angles (inter-reversal angles).

plume arrestment part-way to the pheromone source, as do high concentrations of the natural ratio (Baker *et al.*, 1981). Linn & Roelofs (1981) demonstrated that arrestment to both high $(E):(Z)$ ratios and high overall concentration may be directly attributable to the high levels of $(E)$, because habituation to $(E)$ alone subsequently restored males' ability to fly all the way to the source, even to abnormally high $(E):(Z)$ ratios.

We do not know whether E8-12:Ac might be more of a 'turning' stimulus and $(Z)$ more orthokinetic, but further studies measuring angular and linear velocities should be used to test this possibility.

For non-arrested flights, although the moth's inter-reversal track angles and the tunnel's wind velocity were constant for each concentration tested, the moths' net and overall velocities decreased with increased pheromone concentration. To examine other aspects of *G.molesta*'s flight behaviour, we employed the 'triangle of velocities' (Fig. 5A, after Marsh *et al.*, 1978), which shows the inter-relationships of wind velocity, the moth's overall velocity, and airspeed, track angle, course angle and drift angle during free flight; if one is changed, two or more of the others are necessarily affected. Using the formulae of Marsh *et al.* (1978: $A = \sqrt{[(G.sin\theta)^2 + ((G.cos\theta) + W)^2]}$; $\sin \alpha = (G.sin\theta)/A$)), we calculated the mean airspeeds, drift angles, and course angles of *G.molesta* males flying in plumes from 1, 10 and 100 $\mu$g septa (Fig. 5B).

In different wind velocities, *Plodia interpunctella* males maintained both constant overall velocities and inter-reversal track angles (Marsh *et al.*, 1978) resulting from changes in course and airspeed. Our *G.molesta* males maintained only constant inter-reversal track angles, and because their overall velocities decreased at higher pheromone concentrations, their airspeeds decreased and course angles decreased (i.e. they steered more directly into the wind). These changes resulted in an increase in the moths' drift angles (Fig. 5B).

It is unclear if these increased drift angles would enhance lateral visual 'accuracy' for maintenance of plume contact, or if their increase is a side effect of the decreased overall velocity. Nonetheless, the distance of lateral movements decreased while the number of

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left—right reversals increased with pheromone concentration. This appears similar to the 'homing-in' on the source of *P.interpunctella* (Marsh *et al.*, 1978) and may be a function of increased concentration alone.

Walking *G.molesta* males also decrease their velocity when exposed to higher pheromone concentration, but their turning frequency decreases and turning magnitude increases (Baker *et al.*, 1981), in clear contrast to the flying males in this study. In both studies, angular velocity and angular-to-linear velocity ratios increased at higher concentrations. In contrast, walking *Bombyx mori* males increase their overall velocity with increased pheromone concentration in permeated airstreams while walking at fairly constant, oblique angles to the wind direction (Kramer, 1975).

A key difference between walking and flying males is that walking males can keep from being displaced downwind while facing in any direction because of their tarsal contact with the ground. Flying males, on the other hand, must limit their course direction to generally upwind in order to keep station, and therefore their increased angular velocity to higher concentrations must be expressed as higher-frequency left—right reversals because the severity of each turn is limited to no more than *c.* 180°. Contact with the ground apparently modifies the orientation system used by the males, or perhaps a totally different set of behaviours is employed.

From the present study it appears that for flying moths, pheromone concentration alone can mediate turn frequencies and this, in combination with an inverse orthokinetic response to concentration, results in predictable changes in net ground velocities toward the source. The rapidity of displacement toward the source, and the displacement outcomes, 'attraction' and 'arrestment', depend upon these linear and angular velocity changes acting in concert. The precise relationships among anemotaxis, longitudinal chemoklinotaxis and the visual feedback that produce these velocity changes need to be clarified.

## Acknowledgments

comments on the manuscript and R. W. Simmons and R. S. Vetter for rearing the *G.molesta* used in this study.

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