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Effects of intermittent and continuous pheromone stimulation on the flight behaviour of the oriental fruit moth, *Grapholita molesta*

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ABSTRACT. When male oriental fruit moths, *Grapholita molesta* (Busck) (Tortricidae), casting in clean air entered an airstream permeated with pheromone their flight tracks changed immediately on initial contact with pheromone, but after a few seconds returned to casting as if in clean air. The degree of change in the flight track was directly related to the concentration of pheromone. Although little net uptunnel movement occurred in response to the continuous stimulation provided by a uniformly permeated airstream, when an intermittent stimulus provided by a point-source plume was superimposed onto the permeated airstream moths were able to 'lock on' and zigzag uptunnel in the plume. The percentage of moths doing so corresponded to the difference between the peak concentration within the plume and the background concentration of pheromone permeating the airstream. Moths also locked onto, and flew upwind along the pheromone–clean-air boundary formed along a pheromone-permeated side corridor. Because a similar response was observed along a horizontal edge between a pheromone-permeated floor corridor and clean air, we conclude that the intermittent stimulation at the edge perpetuated the narrow zigzagging response to pheromone.

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

Introduction

The 'zigzagging' paths of insects orientating to distant odour sources have long been a matter for study (Wright, 1958; Farkas & Shorey, 1972; Kennedy & Marsh, 1974). Such paths are most commonly observed in male moths flying upwind to sources of female sex pheromone (Kennedy, 1983). Until recently the generally accepted explanation for this zigzagging path was that pheromone perception evoked positive optomotor anemotaxis resulting in flight straight upwind. Each loss of the pheromone signal, presumably caused by the irregular filamentous structure of the plume, would initiate a series of internally programmed counterturns back and forth across the windline (reversing anemomenotaxis) until contact with pheromone was regained (Kennedy, 1977; Marsh *et al.*, 1978). This would thus result in the obliquely upwind zigzagging flight path frequently observed in flying moths.

Recent work by Baker & Kuenen (1982), Kennedy *et al.* (1980, 1981) and others (Kuenen & Baker, 1982; David *et al.*, 1983) has resulted in a re-examination of this working hypothesis (Kennedy, 1982, 1983; Kuenen & Baker, 1983).

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Kennedy (1983) has modified the earlier predominantly anemotactic model into one integrating both chemotaxis and anemotaxis. In it, an internal programme of counterturns (zigzags) is thought to be evoked by pheromone, with the counterturning frequency being directly modified by its ambient concentration. Optomotor response to wind-induced drift would provide polarity to the counterturns and result in generally upwind displacement. Lower concentrations of pheromone, such as after flight out of the plume, would cause lower frequency, wider counterturns with little upwind displacement that could aid in relocating the pheromone plume (David *et al.*, 1983).

Two important results of recent work by Kennedy *et al.* (1980, 1981) indicate that intermittent, on–off stimulation by pheromone may be important in maintaining the zigzag counterturning programme and displacement upwind. First, in an airstream uniformly permeated with pheromone, *Adoxophyes orana* males did not fly straight upwind as had been reported previously in *Anagasta kühniella* (Traynier, 1968) and *Drosophila* (Kellogg *et al.*, 1962), but rather at its onset their counterturns briefly narrowed and became more frequent in response to the increased (continuous) pheromone concentration. The narrowed zigzagging was often accompanied by a brief upwind 'surge', and approximately 2 s later both the upwind progress and narrow reversals disappeared, and were replaced by crosswind casting as if in clean air. This lack of upwind progress in response to a continuous pheromone stimulus was thought to be a result of adaptation (Kennedy *et al.*, 1980, 1981). It was not, however, due to an overloading of the sensory system by an excessive amount of pheromone, because the second important finding was that males readily locked onto a point-source plume formed within the permeated airstream and flew upwind to its source. It seemed as though concentration fluctuation was needed to maintain the response.

We have recently performed experiments similar to those of Kennedy *et al.* (1980, 1981) with pheromone-permeated airstreams, but used the oriental fruit moth, *Grapholita molesta* (Busck) (Tortricidae),* as our experimental animal. Our results, reported here, confirm and extend the findings of Kennedy *et al.* (1980, 1981) for *A. orana* and provide more evidence for both the concentration-dependency of the counterturn programme and the need for intermittent stimulation to maintain the response.

* Current correct genus name is *Grapholita*, not *Grapholitha* as previously used (Roelofs & Brown, 1982).

Materials and Methods

Insects

Moths used in this study were reared on small green thinning apples (Baker *et al.*, 1981). Pupae were separated according to sex, and males were allowed to emerge isolated from females. Adult males were then separated daily from any remaining pupae, and were maintained in an environmental chamber with positive air pressure to eliminate possible exposure to pheromone before they were used in experiments. All moths used in this study were 1–5 days old and had access to an 8% sucrose solution at all times. During rearing, all life stages were maintained at approximately 25°C on a 16:8 L:D cycle.

Pheromone

All pheromone components were dispensed from rubber septa (A. H. Thomas Co. No. 8753-D22, sleeve type, 5 × 9 mm). Each septum was impregnated with an identical blend of synthetic pheromone components; 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).

A hexane solution of 300 μg/μl of this mixture was formulated and serially diluted so that, when pheromone solutions were applied in 10 μl aliquots, the loading on each septum was either 30, 3, 0.3 or 0.03 μg. Four groups of 120 septa each were impregnated with the appropriate concentration on the same day, and were then stored separately at 0°C.

Wind tunnel

The wind tunnel used in these experiments was constructed from 3 mm-thick Plexiglas bolted to an aluminium frame (after Farkas *et al.*, 1974) with a working section of

180 × 62 × 62 cm. Airflow through the tunnel was generated entirely by an exhaust-hood-blower on the roof of our building connected by an airtight duct to the tunnel at its downwind end. Thus during these experiments, which required large amounts of pheromone to be present in the tunnel at one time, air within the tunnel was vented from the building. The airflow throughout all areas of the tunnel was determined to be laminar by using a $TiCl_4$ smoke source. The wind velocity was 63 cm/s. A 30.5 cm-long sheet metal extension of the tunnel between the Plexiglas working section and the exhaust hood duct provided access downwind of the working section to introduce moths into the tunnel, without disturbing the pheromone-laden air upwind. The access door in the sheet metal section was 15 × 50 cm and could be sealed airtight quickly following closure. The maintenance of laminar flow and a homogeneous pheromone field in this type of tunnel where wind is created by 'pulling', not 'pushing' the air through the tunnel, required that there were no leaks in the working section of the tunnel.

In order to achieve a smooth tunnel airflow uniformly permeated with pheromone, a mixing chamber was constructed similar to the design of Kennedy *et al.* (1980, 1981). This chamber worked in two ways; turbulently mixing the odour plumes issuing from the many individual septa used to create the pheromone field, and then smoothing out the turbulence for a laminar flow. The chamber was constructed of sheet metal and measured 62.5 × 62 × 62 cm. An array of twelve sheet-metal strips 4.5 cm wide, 5 mm apart, and extending vertically from the floor to the ceiling of the chamber, was placed 8 cm from the upwind end of the chamber to restrict airflow and generate turbulent air in the space immediately upwind from the array of pheromone septa. The septa were inserted in a 60 × 60 cm sheet of 6 mm-mesh galvanized-steel hardware cloth mounted on a sheet metal frame that allowed the septa grid to be rapidly introduced into the cross-section of the mixing chamber 5 mm downwind of the vertical metal strips. Septa were arranged in twelve vertical columns 4.5 cm apart; each column ran down the centre of a sheet-metal strip and contained nine septa spaced 6 cm apart. Two fine-mesh brass hardware cloth smoothing screens were positioned downwind from the septum grid, an 80-mesh screen 20 cm downwind and a 100-mesh screen 49 cm downwind, at the point where the mixing chamber joined the working Plexiglas section of the tunnel.

When a side corridor of pheromone was generated, septa were positioned in five columns along only one side of the grid. Again, each column contained nine septa 6 cm apart. To maximize the sharpness of the pheromone–clean-air boundary along the edge of the side corridor, a solid sheet metal partition was inserted into the mixing chamber next to the most central column of septa. The partition extended from the upwind end of the sheet-metal mixing chamber to the first smoothing screen.

The homogeneity of the fully permeated tunnel and the side corridor as well as the position of the edge of the side corridor were determined visually against a black background by applying $TiCl_4$ to septa positioned in the same location as pheromone-loaded septa would be during experiments. The smoke under the most uniform permeation appeared from all directions as a smooth white haze moving down the tunnel.

The edge of the side corridor appeared to have a several-centimetre wide zone of somewhat non-homogeneous smoke and clean air. The side corridor was somewhat wider as it left the working area than when it entered it. Nevertheless, its dimensions were quite consistent, allowing a moth's entry into the zone of pheromone to be accurately measured, and the subsequent pheromone-mediated movements analysed. The side corridor, apart from the boundary, also appeared as a uniform white haze from all directions during smoke visualization.

A single septum (30 μg) for generating a point-source plume to initiate zigzagging flight up the tunnel in some experiments was suspended from a nylon monofilament line passed through a pinhole in the ceiling of the tunnel. This allowed the septum to be rapidly pulled to the ceiling, removing the plume from the area in which the moth was flying and resulting in wide crosswind counterturning. A piece of tape sealed the pinhole to prevent the unwanted entry of clean air, yet passage of the monofilament line was unimpeded. For experiments in which the entire tunnel was permeated, the septum was suspended 20 cm from the floor, 31 cm from the sides and 8 cm from the upwind

end of the working section. For experiments using a side corridor of permeated air the septum was suspended at the midline of the clean-air region 20 cm from the edge of the corridor instead of at the longitudinal midline of the tunnel.

A floor pattern consisting of randomly arranged 10 cm diameter red dots on a white cloth background was placed just beneath the Plexiglas floor of the tunnel. In black-and-white video recordings the dots were nearly white against the white background because a red filter was placed over the lens. This facilitated the later tracing and analysis of moth tracks.

Experimental procedures

The flight tracks of moths were recorded from above in a plan view onto cassette tape using a Sony SLO 340 recorder and a Sony RSC 1050 rotary-shutter camera located *c.* 50 cm above the ceiling of the tunnel. The field of view for all recordings was 1 m long, extending from 30 to 130 cm downwind from the upwind end of the working section of the tunnel and extending the width of the tunnel. Flight tracks were recorded over a 2 h period beginning 1 h before the end of the moth's usual photophase. This period is when *G. molesta* is maximally responsive to pheromone (Baker & Cardé, 1979).

Pheromone was presented to the moths in five different configurations in this study, all in wind of 63 cm/s: (i) a point-source plume in clean air; (ii) an airstream which uniformly permeated the entire tunnel with pheromone (*full permeation*); (iii) a point-source plume which had been superimposed onto the full permeation (*plume plus permeation*); (iv) an airstream which uniformly permeated only a corridor along one side of the tunnel such that the boundary between the permeated corridor and clean air was vertical (*side corridor*); (v) this same side corridor was rotated through 90° to present a horizontal boundary between pheromone-permeated air and clean air, this comparison being termed *side versus floor*.

Full permeation. Uniform permeation of the airstream at four different concentrations was accomplished by inserting the full complement of pheromone septa (108 septa) into the mixing chamber. The loadings on the septa each time were either 0.03, 0.3, 3 or 30 μg. Because *G. molesta* males were unable to take off and make upwind progress in an airstream permeated with pheromone, males were first allowed to take off and fly upwind toward a 30 μg point source suspended in clean air. Males were released singly from a cone-shaped aluminium window screen cage (max. diameter 10 cm and height 7 cm), held in a ringstand 15 cm above the floor of the tunnel. As the moths made upwind progress along the plume and into the field of view of the camera the septum was abruptly pulled to the ceiling and the male flew into clean air. Approximately 5 s after the moths began casting widely crosswind in clean air the septum grid was rapidly (< 1 s) inserted into the mixing chamber. The grid of septa was stored in a separate fume hood before each replication of the experiment. As the septum holder arrived at the opening to the holding slot in the mixing chamber a light-emitting diode (LED) in the field of view of the camera was flashed once so that the time of the first possible contact between the moth and the pheromone-permeated airstream could be calculated. The order of testing the different permeation concentrations was always the same, from lowest to highest to avoid contamination effects. Males were equally responsive throughout each testing period as indicated by their flights to the point-source plume. The tunnel was flushed out between uses by continually drawing clean air through it at 63 cm/s for at least 1 day. The cages and ringstand were washed with acetone after each testing period.

Plume plus permeation. Individual males were released as in the preceding experiment. The tunnel was fully permeated using either 3 or 30 μg septa. Immediately after placement of the moths into permeated airstreams a 30 μg septum was introduced 130 cm upwind from the release cage such that the plume issuing from this septum contacted the release cage. Twenty-five moths were released in each of the two permeation concentrations alone, and in each permeation concentration plus superimposed plume. A control consisting of a 30 μg point-source plume in clean air was also included.

Side corridor. The side corridor was permeated by filling only one side (five columns) of the septum grid (forty-five septa), the grid being held in the mixing chamber throughout the flight of each moth. Each male was allowed to begin flying upwind to the 30 μg point-source septum on the clean-air side of the tunnel. As the male

reached the field of view of the camera the septum was abruptly raised to the ceiling and the male flew out the front end of the plume into clean air. The male then broadened its crosswind casts until it carried itself into the side corridor, whereupon its tracks could be analysed for pheromone mediated changes. The initial contact with pheromone in the side corridor was determined by previously marking the boundaries of the corridor using smoke-source visualization. The order of testing of the four side corridor concentrations was always from least to most concentrated to avoid possible contamination-related effects. The tunnel was cleaned between each block of replications by drawing clean air through it at 63 cm/s for at least 1 day.

Side versus floor. The uniformly permeated floor corridor was produced as described above for the side corridor, except that the entire tunnel and mixing chamber were rotated through 90°. The side and floor corridors were presented to the moths at only two concentrations, 3 and 30 μg. As in the previous experiments the moths were released from an aluminium screen cage held at the pheromone–clean-air boundary. However, a point-source plume was not used to initiate upwind flight: the males, released singly or in groups, initiated flight along the boundary never having performed flight in response to a point-source pheromone plume. The order of testing again was from least to most concentrated, but the order of testing of side versus floor at each concentration was randomly determined.

Data processing and analysis

The recordings of each moth track were re-recorded onto a Sony SVM-1010 motion analyser for better motion resolution, and played back frame-by-frame through a 47.5 cm (19 inch) black-and-white Sony television monitor. The consecutive locations of the moth every 1/60 s were marked on an acetate sheet placed over the television screen.

Tracks were then digitized using a T-bar-style X/Y digitizer (Radio Shack TRS-80 Digitizer), serially interfaced with a microcomputer (Radio Shack TRS-80 Model III), and simultaneously displayed on a flatbed plotter (Radio Shack TRS-80 FP-215) to ensure that the coordinates entered from the digitizer correctly represented the track. The digitized coordinates from each track were stored for later analysis.

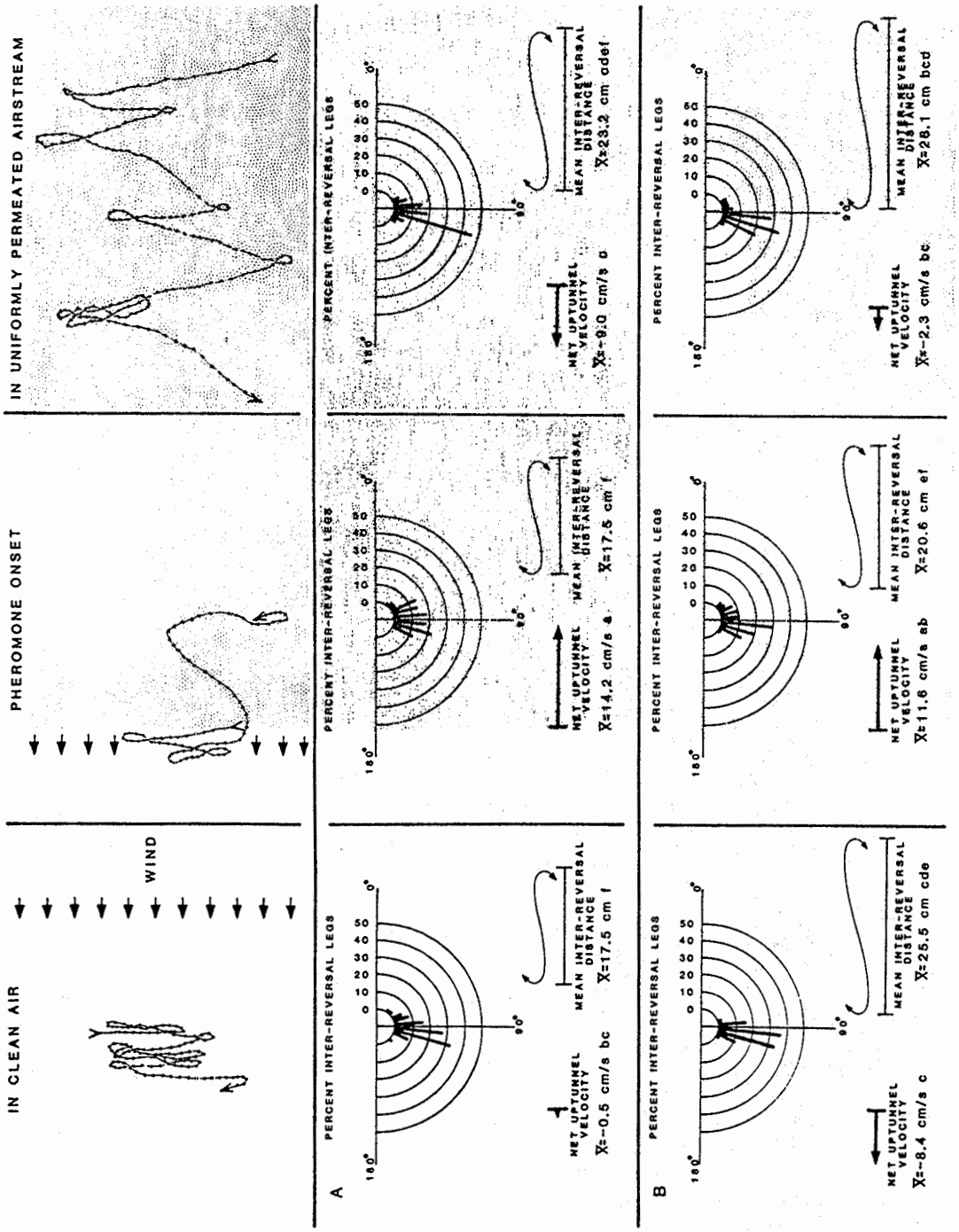
All tracks and track sections were analysed using a program (Basic) developed (Kuenen & Baker, 1982) to measure pertinent linear velocity (net and overall) and angular (turn severity, angular velocity, turn frequency) track parameters. A turn was defined as a greater than 50° change in clockwise to anticlockwise (or vice versa) direction (Kuenen & Baker, 1982). Track inter-reversal angles were measured according to the criteria of Marsh *et al.* (1978) and Kuenen & Baker (1982) by means of an X/Y digitizer pad (Houston Instruments, HIPAD DT-11) serially interfaced with a microcomputer. Track inter-reversal distances (the reversal-to-reversal width of each zigzag) were measured by hand also according to the criteria of Kuenen & Baker (1982). Track data from each experiment were analysed with a two-way analysis of variance for unequal sample sizes and Duncan's new multiple range test. The percentages of moths making upwind progress to the different treatments in the *plume plus permeation, side corridor* and *side versus floor* experiments were compared using a method of adjusted significance levels for proportions (Ryan, 1960).

Results

Full permeation

After the initial encounter between moth and permeated airstream was determined using the flash of the LED as the pheromone septa grid arrived at the mixing chamber, the tracks were divided into three sections: (i) 2 s immediately prior to the intial encounter with the permeated airstream; (ii) the 1.5 s immediately following the initial encounter with the permeated airstream; (iii) 2 s immediately following section (ii) during which the moth was flying in an airstream uniformly permeated with pheromone.

After casting in clean air, moths making contact with airstreams fully permeated using 3 or 30 μg septa made brief uptunnel 'surges' (*sensu* Kennedy *et al.*, 1981), reflected most clearly by the net uptunnel velocities changing from (−) to (+) (Fig. 1). The net uptunnel velocity at the onset of the 30 μg permeation was significantly greater ($P<0.05$) than those to the


IN CLEAN AIR
WIND
PHEROMONE ONSET
IN UNIFORMLY PERMEATED AIRSTREAM
A
B
PERCENT INTER-REVERSAL LEGS
MEAN INTER-REVERSAL DISTANCE
NET UPTUNNEL VELOCITY
X=-0.5 cm/s bc
X=17.5 cm f
X=14.2 cm/s a
X=17.5 cm f
X=-9.0 cm/s a
X=23.2 cm cdef
X=-8.4 cm/s c
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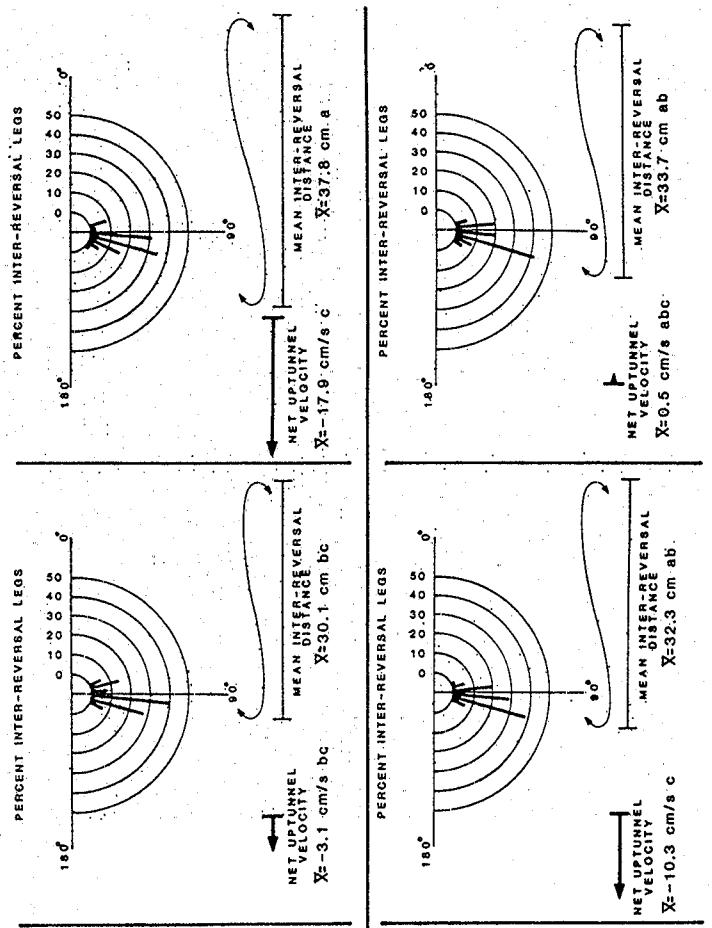


FIG. 1. A representative flight track and track parameters illustrating the response of male *G. molesta* casting in clean air, and then flying into air uniformly permeated with pheromone at four different concentrations. The uppermost panels show the track of a male: casting in clean air after the pheromone plume was removed ('In clean air'); making a brief up-tunnel surge at pheromone onset ('Pheromone onset'); and resuming wide cross-tunnel casting in an airstream uniformly permeated with pheromone emitted from a grid of 3 μg septa ('In uniformly permeated airstream'). Dots represent the location of the moth every 1/60 s. The wind was blowing from right to left and the direction of flight is indicated by arrows on the track. Mean inter-reversal distances (zizag width), net up-tunnel velocity and a frequency distribution of inter-reversal track angles are given for each of the three track sections: (i) in clean air, (ii) pheromone onset, (iii) in uniformly permeated airstream at all four of the concentrations of pheromone. (A) This row of three panels illustrates the mean responses of males upon flying into an airstream uniformly permeated by pheromone from 30 μg septa. (B) Responses to flight into an airstream uniformly permeated by pheromone from 3 μg septa. (C) Responses to flight into an airstream uniformly permeated by pheromone from 0.3 μg septa. (D) Responses to flight into an airstream uniformly permeated by pheromone from 0.03 μg septa. Means having no letters in common are significantly different according to a two-way analysis of variance and Duncan's new multiple range test ($P<0.05$).

0.3 and 0.03 μg loadings, which did not affect the moths significantly compared to clean air. At pheromone onset with airstreams permeated using 0.03 or 0.3 μg septa the net velocity of the moths continued in the downtunnel direction. Upwind surges by the moths, although clearly caused by the onset of a permeated airstream of a high enough concentration, were of short duration (Fig. 1). Once the initial front of the pheromone cloud had passed and the moths had been flying in a uniformly permeated airstream for more than 1 s, the net uptunnel velocities changed again indicating a return to wide crosswind casting resulting in net down-tunnel movement, both of which were indistinguishable from the casting that had occurred in clean air. The mean net velocities of the clean-air portions of the tracks were not significantly different ($P>0.05$) and indicated that in all cases the wide crosswind casts resulted in net down-tunnel movement. The slight but insignificant up-tunnel movement exhibited by moths flying in a permeated airstream of 0.03 μg is probably an effect of the small sample size.

The change in track inter-reversal angles over the three recording periods is consistent with the net velocity data and also indicates a change from net down-tunnel movement to net up-tunnel movement at the onset of pheromone-permeated air (Fig. 1). Again, this up-tunnel surge was evident only during onset of the airstream permeated by 3 or 30 μg septa, the two lower loadings having no apparent effect. The means of the inter-reversal angles of moths casting in clean air for all treatments were greater than 90°, indicating net down-tunnel movement. Mean inter-reversal angles at the onset of 3 and 30 μg permeations were less than 90° indicating net up-tunnel movement. Although the mean inter-reversal angles during the up-tunnel surges to the 3 and 30 μg permeations (88.4 ± 19.0 and 88.2 ± 18.9 respectively) were not significantly different from each other, they were significantly different from the mean inter-reversal angles of all other track sections before and after pheromone onset. Again this reflects the short-term effect of the uniformly permeated cloud upon male movements. Once the moths had been flying in the permeated airstream for 1–2 s they resumed crosswind casting and their mean inter-reversal angles again became greater than 90°. At the 0.3 and 0.03 μg permeations the mean inter-reversal angles never became less than 90° and thus net down-tunnel movement continued throughout the period the moths' tracks were recorded.

As in a previous study (Kennedy *et al.*, 1980, 1981), track inter-reversal distances decreased immediately after the onset of the pheromone-permeated airstream; that is, the zigzagging flight track of the moth narrowed. This decrease in inter-reversal distance was seen only at the onset of the 3 and 30 μg permeations. The inter-reversal distances of all tracks were relatively narrow in the clean-air section due to the fairly recent removal of the point-source plume. At the onset of the 3 and 30 μg permeation the inter-reversal distances either narrowed (3 μg) or remained narrow (30 μg). The onset of the 0.3 and the 0.03 μg permeations caused no significant shortening of the inter-reversal distances and in fact the inter-reversal distances at these treatments continued to lengthen over the entire recording period. When the moths continued flight for more than 1 s in a fully permeated airstream at 3 and 30 μg their inter-reversal distances continued to widen in a manner similar to those flying in lower concentrations and in clean air (Fig. 1).

Other measured and analysed track parameters (Table 1) changed in response to concentration differences consistent with previous work (Kuenen & Baker, 1982). Turning (zigzag) frequency (turns/s) increased significantly at the onset of the 30 μg permeation as it did to the 3 μg concentration, although the value for 3 μg was not significantly different from either the 30 or the 0.3 μg full permeation onset. At the onset of the 0.3 and the 0.03 μg permeated airstream the turn frequency did not increase, rather it continued to decrease, with wider casting. As with the track parameters examined above the effect of the pheromone was restricted to immediately following the onset of the permeated airstream and as the moths continued to fly in the pheromone-permeated air the turning frequency either returned to values close to those observed for casting in clean air or decreased further.

Plume plus permeation

When moths were placed into an airstream uniformly permeated with 3 or 30 μg septa they either continued to 'sit', or wing-fanned and took off but made no up-tunnel progress (Table

TABLE 1. Mean track parameters (±SD) of male *G. molesta* before, during and after the onset of a uniformly permeated airstream of four different pheromone concentrations.

Septum loading (μg)	n	Linear	Angular		
		Overall velocity	Turn frequency (turns/s)	Turn severity (°/turn)	Angular velocity (°/s)
2 s in clean air					
0.03	10	98.1 ± 9.1^{bc}	3.5 ± 0.9^{bc}	226.4 ± 40.7^{ab}	770.6 ± 175.9^{ab}
0.3	10	89.8 ± 9.5^{cd}	4.0 ± 0.8^{abc}	200.4 ± 19.1^{ab}	799.9 ± 144.5^{ab}
3	10	91.5 ± 14.4^{cd}	3.7 ± 0.7^{bc}	195.4 ± 17.6^{ab}	731.9 ± 169.2^{ab}
30	10	78.2 ± 13.4^{d}	4.1 ± 1.0^{abc}	207.4 ± 35.3^{ab}	827.5 ± 123.3^{ab}
1.5 s during pheromone onset					
0.03	10	108.9 ± 17.3^{ab}	3.5 ± 0.7^{bc}	202.8 ± 44.7^{ab}	684.0 ± 123.7^{b}
0.3	10	108.0 ± 9.2^{ab}	3.1 ± 1.0^{c}	246.8 ± 85.0^{ab}	707.4 ± 157.0^{ab}
3	10	92.4 ± 14.1^{c}	4.5 ± 1.5^{ab}	202.7 ± 52.0^{ab}	868.2 ± 257.5^{a}
30	10	86.7 ± 15.2^{cd}	4.9 ± 1.1^{a}	173.7 ± 22.0^{b}	840.6 ± 171.6^{ab}
2 s in uniformly permeated airstream					
0.03	5	119.3 ± 14.5^{a}	2.9 ± 1.1^{c}	268.9 ± 174.6^{a}	639.6 ± 124.5^{b}
0.3	7	121.1 ± 14.6^{a}	3.6 ± 1.7^{bc}	224.7 ± 83.8^{ab}	682.8 ± 91.7^{b}
3	7	99.9 ± 18.7^{bc}	3.6 ± 1.8^{bc}	246.5 ± 140.2^{ab}	738.5 ± 263.6^{ab}
30	7	86.7 ± 10.7^{cd}	3.3 ± 1.4^{bc}	242.5 ± 70.1^{ab}	738.2 ± 182.6^{ab}

Means in the same column having no letters in common are significantly different according to a two-way analysis of variance and Duncan's new multiple range test ($P<0.05$).

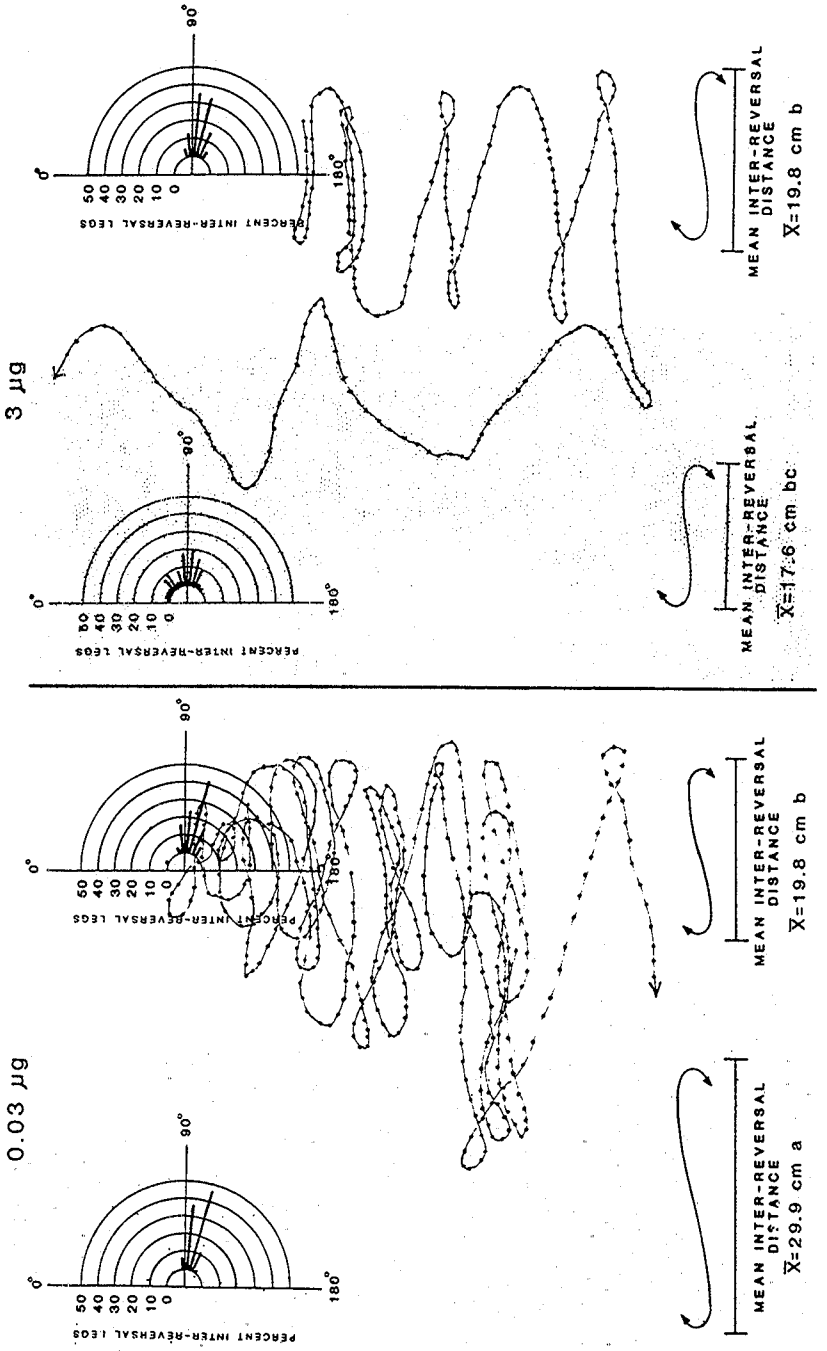
2). Rather, they flew to the ceiling or side walls and landed, or drifted out the downwind end of the tunnel.

However, when a plume from a 30 μg point source was superimposed onto a uniformly permeated airstream, moths were able to take off, lock onto the plume and make up-tunnel progress along the plume (Table 2). The concentration of the background level of pheromone in the uniform cloud appeared to influence the success of up-tunnel flight in the plume. A significantly greater percentage of moths were able to fly up a 30 μg plume in clean air than when the plume was in an airstream permeated with 30 μg septa (Table 2), with an intermediate percentage able to fly up the plume in the 3 μg cloud. The elicitation of up-tunnel flight by a plume located within a pheromone-permeated airstream indicates that the failure of these airstreams to evoke up-tunnel flight was not due to excessively high overall quantities of pheromone.

TABLE 2. Percentages of male *G. molesta* taking off and making upwind progress in an airstream permeated with two concentrations of pheromone and those taking off and making upwind progress in permeated airstreams at the same concentrations with a 30 μg point-source plume superimposed into the airstream. $n=25$ for each treatment.

Septum loading and treatment	% take off	% take off with uptunnel progress	% touching the source
30 μg permeation without plume	40^{b}	0^{c}	0^{b}
3 μg permeation without plume	64^{b}	0^{c}	0^{b}
30 μg permeation + 30 μg plume	60^{b}	28^{b}	0^{b}
3 μg permeation + 30 μg plume	92^{a}	48^{ab}	32^{a}
30 μg plume in clean air	96^{a}	72^{a}	56^{a}

Percentages in same column having no letters in common are significantly different according to a method of adjusted significance levels for proportions (Ryan, 1960) ($P<0.05$).


3 µg
0.03 µg
90°
0°
180°
0
10
20
30
40
50
PERCENT INTER-REVERSAL LEGS
MEAN INTER-REVERSAL DISTANCE
X̄=19.8 cm b
X̄=17.6 cm bc
X̄=19.8 cm b
X̄=29.9 cm a

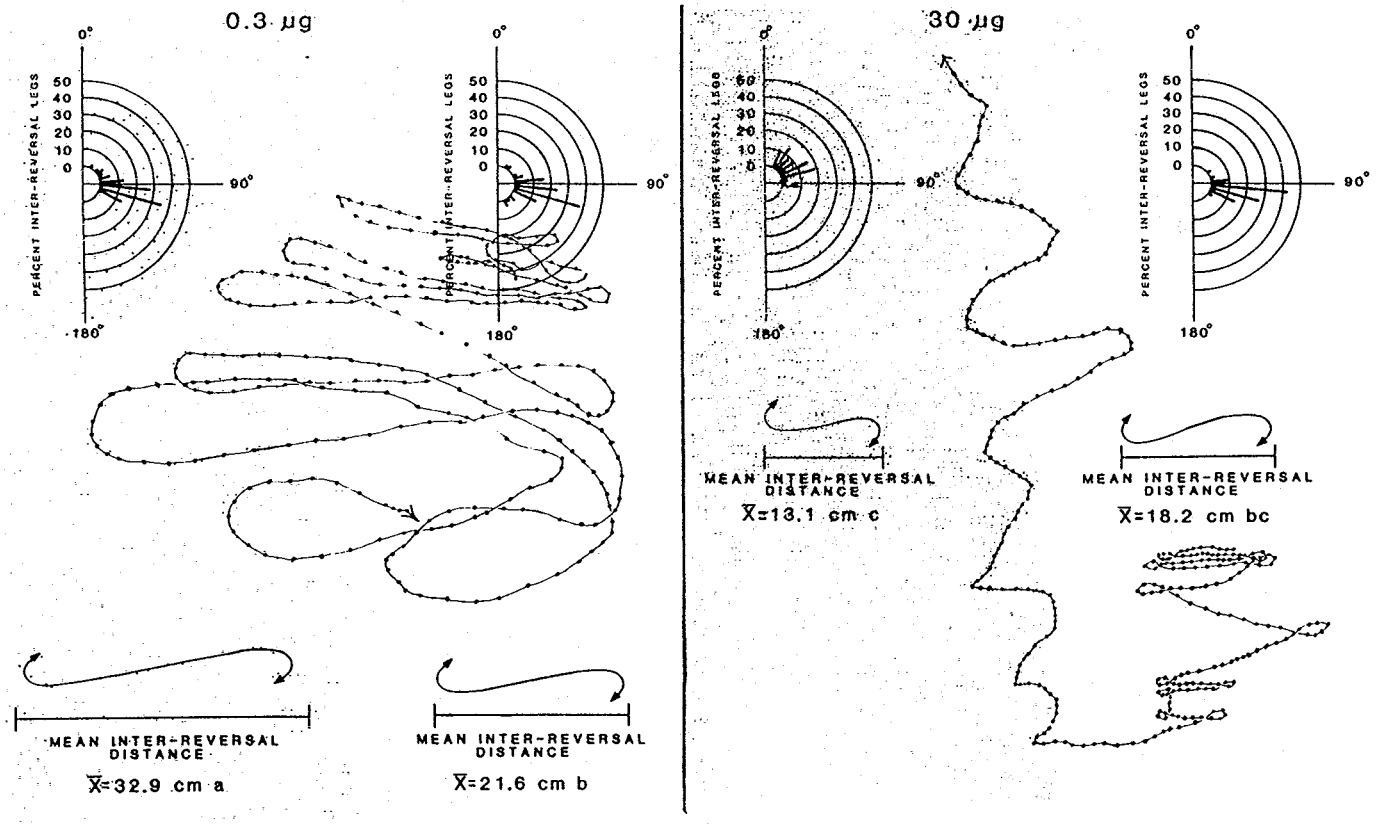


FIG. 2. Flight tracks and track parameters illustrating the responses of male *G.molesta* upon casting into a side corridor uniformly permeated by four different concentrations of pheromone. Dots represent the location of the moths every 1/60 s. The wind blew from the top of the diagram to the bottom and the flight direction of the moth is indicated by an arrow at the end of each track. For purposes of analysis the tracks were divided into two sections: (i) casting in clean air, and (ii) after contact with the side corridor of pheromone. Thus mean inter-reversal distances (zigzag width) and frequency distributions of inter-reversal track angles are presented for both the clean air (values in unstippled areas) and after contact with pheromone corridor (values in stippled areas) at all four concentrations. Mean inter-reversal distances having no letters in common are significantly different according to a two-way analysis of variance and Duncan's new multiple range test ($P<0.05$).

Side corridor

For analysis, we divided the tracks in this experiment into two sections: the period of casting in clean air, and that after contact with the side corridor. There were no overt changes between casting in clean air and after continued movement in and out of the side corridor permeated with the 0.3 and 0.03 μg levels of pheromone (Fig. 2). However, after contacting either the 3 or 30 μg permeated side corridors the moths not only narrowed their zigzags and locked onto the 'edge', as reported by Kennedy *et al.* (1981) for another species, but often flew up-tunnel along the edge to the front of the tunnel (Fig. 2). The percentage of moths making up-tunnel progress along the 3 μg side corridor (78%, $n=18$) and that of moths flying up-tunnel along the 30 μg side corridor (100%, $n=15$) after casting from clean air into the pheromone-permeated side corridor were not significantly different ($P>0.05$). But, percentages of moths flying along both 3 and 30 μg side corridors were significantly greater ($P<0.05$) than those locking onto or making up-tunnel progress after casting from clean air into side corridors permeated by 0.3 and 0.03 μg septa (0% in both cases, $n=21$ and $n=18$ respectively).

The change in the net up-tunnel velocity after flight into the side corridor (Table 3) most dramatically illustrates the responses of the moths to the four side corridors of different pheromone concentrations. Net up-tunnel velocity of moths flying along the edge of the 30 μg side corridor was significantly greater than that of moths along the 3 μg side corridor. The positive net velocities of moths after contact with the 3 and 30 μg side corridor reflects displacement in the up-tunnel direction, whereas the negative net velocities of moths flying in clean air or entering side corridors permeated with septa containing 0.3 and 0.03 μg of the pheromone blend reflect continued casting and down-tunnel displacement. The lack of response to the two lower concentrations was consistent with all track parameters from the full permeation experiment.

The mean frequency of turning (zigzagging) (Table 3) also increased significantly upon contact with the 3 and 30 μg permeated side corridor, and corresponded to the locking-on process. Moths making contact with and flying up-tunnel along the edge of the 30 μg side corridor had a significantly greater mean turning frequency than moths at any other concentration of side corridor or clean air. The mean turning frequency of moths flying into and along the edge of the 3 μg side corridor was significantly increased from the turning frequency on the clean air side. The continuing decrease in mean turn frequencies of moths casting in clean air and then encountering 0.3 and 0.03 μg side corridors is consistent with the data from moths encountering fully permeated airstreams of the same concentrations and illustrates the lack of response to these low concentrations of pheromone. The increase in mean turning frequency

TABLE 3. Mean track parameters (±SD) of male *G. molesta* casting in clean air and after contact with a side corridor permeated with four concentrations of pheromone.

Septum loading (μg)	n	Linear		Angular		
		Net up-tunnel velocity	Overall velocity	Turn frequency (turns/s)	Turn severity (°/turn)	Angular velocity (°/s)
In clean air						
0.03	10	-13.8 ± 3.6^{c}	92.7 ± 16.9^{bc}	3.4 ± 0.8^{bc}	269.4 ± 74.4^{ab}	874.4 ± 116.4^{a}
0.3	9	-17.0 ± 11.5^{c}	88.3 ± 20.0^{bc}	3.1 ± 1.3^{bc}	282.4 ± 96.4^{ab}	781.4 ± 189.6^{ab}
3	8	-17.0 ± 9.8^{c}	85.9 ± 12.4^{bc}	2.6 ± 1.0^{c}	355.4 ± 167.0^{a}	828.5 ± 216.0^{ab}
30	6	-14.1 ± 6.5^{c}	81.2 ± 16.4^{c}	3.3 ± 1.2^{bc}	316.1 ± 150.5^{ab}	886.2 ± 138.9^{a}
After contact with pheromone						
0.03	10	-16.3 ± 17.5^{c}	109.9 ± 21.6^{a}	2.5 ± 1.0^{c}	324.4 ± 139.9^{a}	712.8 ± 116.8^{ab}
0.3	9	-12.1 ± 13.4^{c}	104.6 ± 26.6^{ab}	2.8 ± 1.2^{c}	265.9 ± 75.3^{ab}	678.5 ± 193.6^{b}
3	8	$+19.0\pm13.3^{b}$	85.7 ± 26.7^{bc}	4.1 ± 1.5^{b}	189.3 ± 32.7^{bc}	745.0 ± 144.9^{ab}
30	6	$+37.2\pm12.6^{a}$	74.2 ± 20.1^{c}	5.8 ± 0.8^{a}	139.4 ± 28.5^{c}	812.0 ± 185.1^{ab}

Means in the same column having no letters in common are significantly different according to a two-way analysis of variance and Duncan's new multiple range test ($P<0.05$).

in response to increased pheromone concentration is consistent with earlier work (Kuenen & Baker, 1982).

Track inter-reversal distances decreased significantly upon contact with the 30 μg side corridor, indicating a narrowing of the width of the zigzag flight path of the moth (Fig. 2). Moths casting into the 3 μg side corridor did not significantly narrow their zigzags even though they did lock onto the edge and made up-tunnel progress. The significant difference between the mean inter-reversal distances along the 3 and 30 μg side corridors again demonstrates not only the effect of pheromone onset but also the inverse relationship between inter-reversal distances and pheromone concentration. The inter-reversal distances of moths casting in clean air and then casting into side corridors of 0.3 and 0.03 μg did not decrease upon pheromone onset but instead continued to increase.

Another indication of the change from net down-tunnel movement to net up-tunnel movement is the change in the distribution of inter-reversal track angles after contact with the 3 and 30 μg side corridors from predominantly greater than 90° to predominantly less than 90° (Fig. 2). There was very little change in the distributions of inter-reversal angles between moths casting in clean air and after contact with the 0.3 and 0.03 μg side corridor, and all reflect the down-tunnel displacement of these moths. The mean inter-reversal angle after contact with the 30 μg side corridor, 61.3 ± 22.7, however, was significantly less than the mean inter-reversal angle after contact with the 3 μg corridor, 78.1 ± 27.5, and both were significantly smaller than all other treatments. Although previously published results (Kuenen & Baker, 1982) indicate that there was no significant change in mean inter-reversal angle with change in pheromone concentration, we believe this difference in our results can be explained by the difficulty experienced by the moths in locking onto the edge of the 3 μg side corridor compared to the 30 μg side corridor. Initial contact with the 3 μg corridor did not always immediately lead to locking onto the edge. It often took two or more incursions into the 3 μg corridor before zigzagging along the edge occurred. However, one incursion into the 30 μg side corridor always led to locking on and up-tunnel flight along the edge.

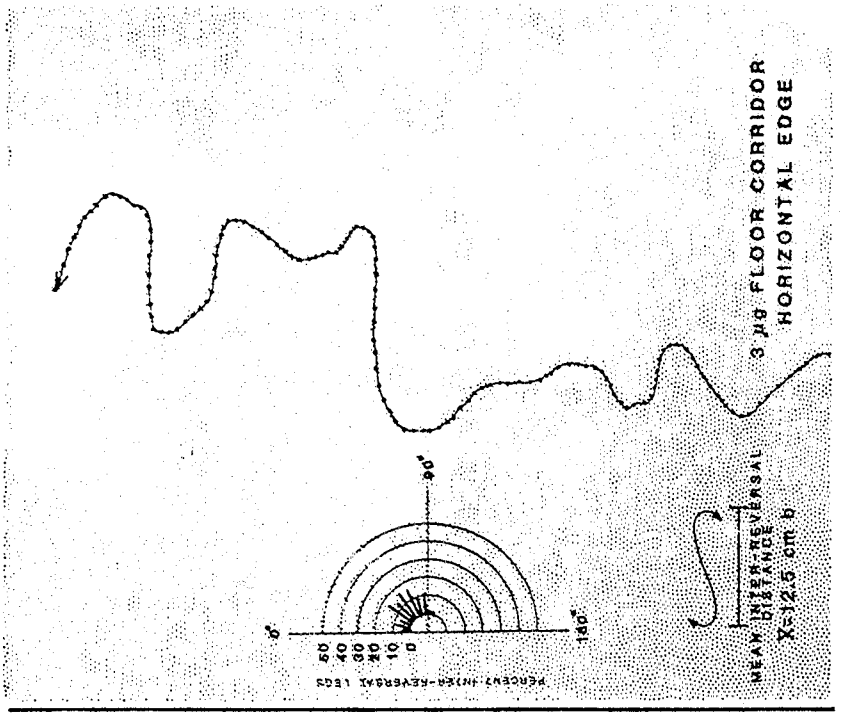
The ability of the moths to lock onto and fly up-tunnel along the edge, and the small zone of non-homogeneous pheromone (as visualized during smoke tests), indicated that the boundary between the pheromone corridor and clean air may not have been sharp, and that oscillations of concentration could have been present. The boundary zone could thus have had a structure more similar to that of a point-source plume than a uniform cloud, and in fact there is no significant difference ($P > 0.05$) between mean inter-reversal angles of tracks along the edge of the 30 μg side corridor and those along a 30 μg point-source plume.

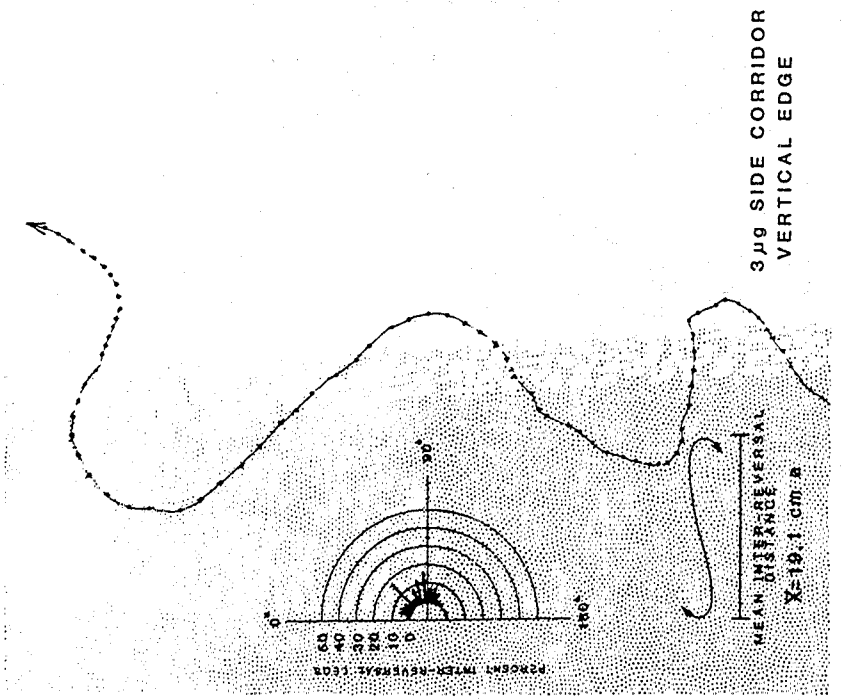
Side versus floor

The percentages of moths locking onto and making up-tunnel progress along the vertical edge of a side corridor of pheromone at 3 and 30 μg were not significantly different ($P > 0.05$), (19.2%, $n = 234$ and 18.4%, $n = 207$, respectively). There was also no significant difference between the percentages of moths making up-tunnel progress along a 3 and 30 μg horizontal edge (9.9%, $n = 477$ and 8.7%, $n = 389$, respectively). For both the vertical and horizontal edges, males had much more difficulty taking flight than in experiments where they were released into a point-source plume. They spent more time wing fanning in the release cage without taking off. Their behaviour was consistent with a hypothesis that these edges were giving intermittent stimulation much better than that of the fully permeated airstreams but worse than that of a point-source plume.

The tracks along both vertical and horizontal edges exhibited zigzagging similar to that observed in point-source plumes (Fig. 3). Occasionally one or two of the inter-reversal legs of a male flying along the horizontal edge would carry it across the width of the tunnel whereupon up-tunnel zigzagging would continue. This was never observed along the vertical edge where moths could not stray laterally without moving into zones of uniform pheromone-on or off.

In most cases, differences between given track parameters are greater between the horizontal and vertical edges than they are between higher and lower concentrations. This difference between flight along vertical and horizontal edges extends to inter-reversal angles as well (Fig. 3 and Table 4). The trends in the data together with examination of the tracks themselves


3 µg FLOOR CORRIDOR
HORIZONTAL EDGE
MEAN INTER-REVERSAL DISTANCE X=12.6 cm b
90°
0°
180°
60
40
30
20
10
0
PERCENT INTER-REVERSAL LEGS


3µg SIDE CORRIDOR
VERTICAL EDGE
MEAN INTER-REVERSAL DISTANCE X=19.1 cm a
90°
0°
180°
60
40
30
20
10
0
PERCENT INTER-REVERSAL LEGS

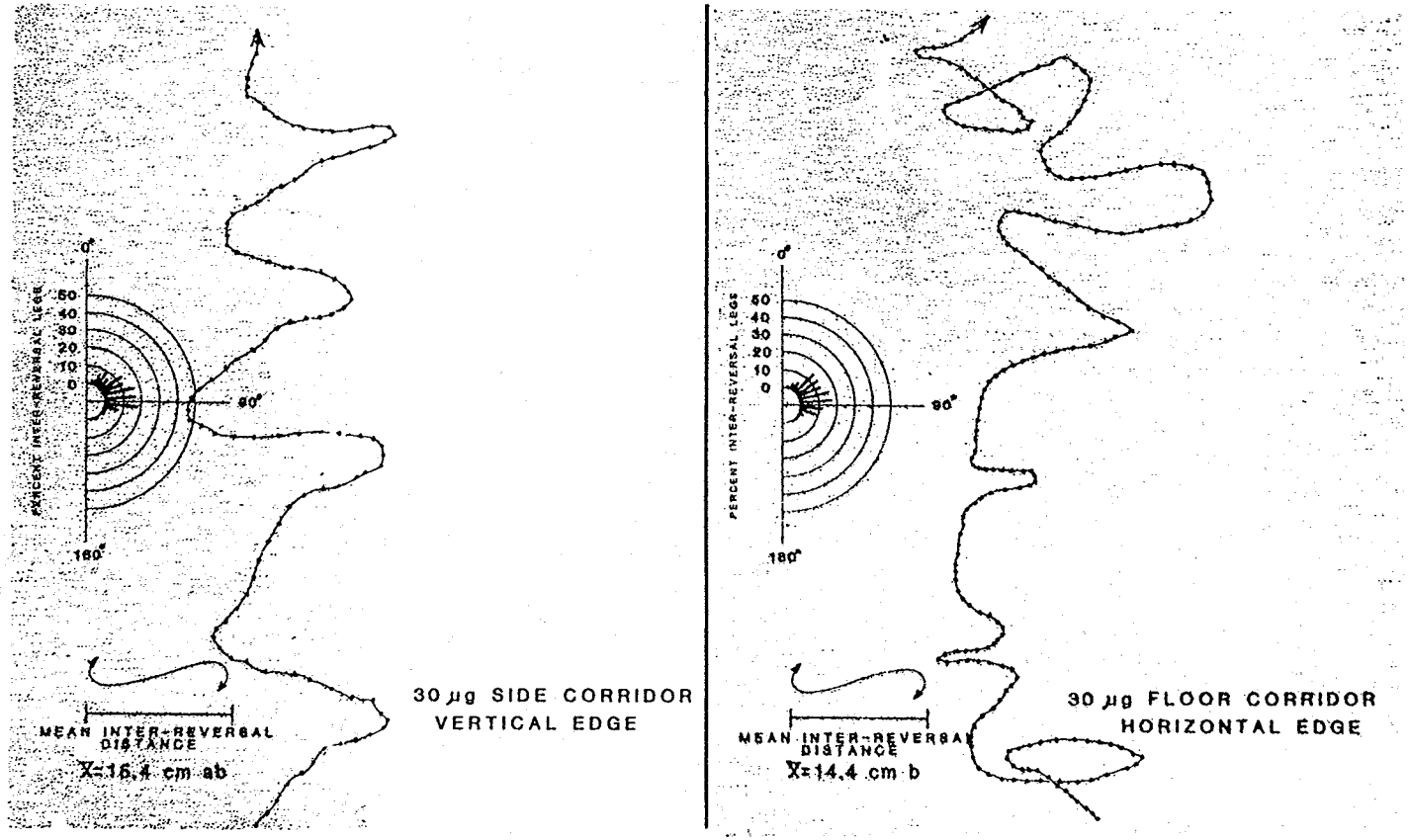


FIG. 3. Tracks and track parameters of male *G.molesta* flying along the edges of side and floor corridors uniformly permeated by pheromone from septa loaded with 3 and 30 μg. Dots represent the location of the moth every 1/60 s. The wind blew from the top of the diagram to the bottom and the moths flew from the bottom to the top. Mean inter-reversal distances (zigzag width) and frequency distributions of inter-reversal track angles for side and floor corridors at each concentration are depicted. Mean inter-reversal distances having no letters in common are significantly different according to a two-way analysis of variance and Duncan's new multiple range test ($P<0.05$).

TABLE 4. Mean track parameters (±SD) of male *G. molesta* flying along horizontal and vertical edges at two concentrations.

Septum loading (μg)	*n*	Linear		Angular			
		Net up-tunnel velocity	Overall velocity (cm/s)	Turn frequency (turns/s)	Turn severity (°/turn)	Angular velocity (°/s)	Inter-reversal distances (cm)
Horizontal edge							
3	12	54.6 ± 16.4^{ab}	87.2 ± 14.1^{b}	4.8 ± 1.3^{a}	126.2 ± 33.3^{b}	559.7 ± 181.9^{b}	12.5 ± 5.1^{b}
30	15	38.7 ± 24.6^{b}	85.6 ± 15.2^{b}	4.9 ± 1.0^{a}	154.9 ± 37.8^{ab}	726.5 ± 113.7^{ab}	14.4 ± 3.8^{b}
Vertical edge							
3	17	60.9 ± 30.5^{a}	115.4 ± 19.1^{a}	4.4 ± 1.5^{a}	150.5 ± 59.4^{ab}	632.2 ± 218.4^{b}	19.1 ± 6.3^{a}
30	16	39.1 ± 17.0^{b}	95.2 ± 19.8^{b}	4.7 ± 1.0^{a}	165.5 ± 33.8^{a}	756.5 ± 134.0^{a}	15.8 ± 4.2^{ab}

Means in the same column having no letters in common are significantly different according to a two-way analsyis of variance and Duncan's new multiple range test ($P<0.05$).

suggests that although sustained zigzagging flight does occur along an horizontal edge, male *G. molesta* are able to fly up-tunnel more consistently along a vertical edge than a horizontal edge.

Discussion

We interpret the results from this series of experiments to mean the following for the sex pheromone-mediated orientation of *G. molesta*. (i) Fluctuations in pheromone concentration are necessary to initiate and maintain the narrow counterturning and upwind displacement characteristic of zigzagging pheromone-mediated flight in this species. (ii) Continuous pheromone stimulation, regardless of concentration, is not sufficient for initiating and maintaining this type of counterturning and upwind displacement. (iii) The necessary intermittent stimulation is provided primarily by the fluctuations of concentration within the pheromone field itself (typically a point-source plume), and possibly secondarily by the excursions and incursions into and out of the pheromone field caused by the self-steered zigzagging upwind flight path of the moth. (iv) Continuous exposure to pheromone fails to evoke the response observed during intermittent exposure not because of an overloading of receptors by excessive amounts of pheromone, but because the receptors are not able to provide the proper fluctuating input into the central nervous system.

Our results confirm the findings of Kennedy *et al.* (1980, 1981) for a different tortricid species, *Adoxophyes orana*, that a uniformly permeated field of pheromone does not elicit prolonged zigzagging upwind flight, whereas a point-source plume within such a field does. Moreover our findings, using a range of concentrations, counter arguments that the moths' lack of upwind progress in Kennedy *et al.*'s uniform field was due to excessively high or excessively low concentrations (Tobin & Bell, 1982).

Our results support the idea (Kennedy *et al.*, 1980, 1981; Kennedy, 1982, 1983; Kuenen & Baker, 1982) that the pheromone intiates and modulates a programme of counterturns (zigzags) that is self-steered, in contrast to the idea that each counterturn is steered according to the (lateral) odour gradient (Farkas & Shorey, 1972).

We established that the frequency and width of the counterturns in the programme was concentration-dependent, as Kennedy *et al.* (1980, 1981) had predicted. An encounter with pheromone by male *G. molesta* makes turning more, not less, likely as Kennedy *et al.* (1980, 1981) found for *A. orana*, negating the idea that moths leaving an odour plume are triggered to turn back into the plume by the concentration drop and that moths in contact with pheromone continue to fly straight.

Our evidence suggests that, as in *A. orana*, fluctuations in concentration are essential to maintain the programme of counterturns. Intermittent stimulation appears necessary to provide the correct phasic receptor input to the CNS to maintain the programme, and the amplitude of peak-to-trough concentration differences may determine counterturning frequency and amplitude. As the background concentration of the pheromone cloud increases, successful upwind flight in a plume within the cloud decreases, presumably because peak-to-trough amplitude

decreases at the receptor level. Murlis & Jones (1981) have shown that even at between 2 and 15 m from the source the peak concentration of a filament of ionized air (presumed to mimic that of pheromone) is 22 times the mean concentration of the plume and up to 150 times more concentrated than the lowest trough concentrations.

In experiments with *Bombyx mori* males, Olberg (1983) demonstrated the existence of 'flip-flopping' interneurons in the ventral nerve cord, descending from the brain, which have either constant high or low firing rates. At each onset of pheromone stimulation the firing rates of these interneurons changed from the high to the low state, or vice versa. The firing rate did not change in response to clean air after a pulse of pheromone, and continuous pheromone stimulation changed the state only once, in response to pheromone onset, and remained in that state for up to 4 min. If *G.molesta* had similarly behaving interneurons, they would be repeatedly changing state in response to phasic receptor output in a male flying upwind in a pheromone plume through the pheromone filaments and clean-air holes making up the plume. A single pheromone onset followed by continuous pheromone stimulation (as in our homogeneous cloud) would cause only a single state change. Therefore, the cessation of narrow counterturning and upwind displacement in response to continuous pheromone stimulation may not be due to adaptation at the receptor level as previously proposed (Kennedy *et al.*, 1980, 1981), but rather the central nervous system may not be receiving the necessary phasic stimulation to maintain state-switching. The continuous nature of receptor output in a homogeneous cloud would hinder state-switching and thereby lower the counterturning frequency. Since males of both *G.molesta* and *A.orana* continue counterturning, but with a decreased frequency, in a homogeneous cloud of pheromone and in clean air, it is apparent that each individual counterturn is probably not the result of an individual state change at the interneuron level. Rather, the counterturns appear to be generated by an internal oscillator of reversals which itself may be modulated by the state-switching of the flip-flopping interneurons.

The narrow programme of zigzagging was observed just after the onset of uniform pheromone in both this study and that of Kennedy *et al.* The reversals must have been generated endogenously because they occurred without any further change in concentration; the odour field was uniform. Evidence for a counterturning programme comes not only from Kennedy *et al.* (1980, 1981), Kennedy (1982) and these results, but from other recent studies. Kuenen & Baker (1982) found that the most narrow and frequent track reversals took place in the flight paths of male *G.molesta* flying upwind in the most concentrated pheromone plumes, and these reversals were often well within the boundaries of the time-averaged plume. Moreover, the narrow reversals continued for an average of *c.* 0.5 s after flight into clean air (the plume was removed) which itself would provide no external concentration change to trigger such reversals (Kuenen & Baker, 1982). Even after prolonged exposure to clean air the track reversals continued but their frequency decreased, amplitude increased, and direction became directly crosswind instead of obliquely upwind. Again, no external concentration changes could have been triggering or steering the reversals during this casting flight, but rather the turns must have been generated from within, a point made several times in earlier studies (Kennedy & Marsh, 1974; Marsh *et al.*, 1978; Kennedy, 1982).

The self-steered programme of counterturns cannot only continue after wind is reduced to zero (Baker & Kuenen, 1982), but can be initiated in zero wind (Baker *et al.*, 1984). In the latter instance the counterturning magnitudes of males placed into a stationary plume in zero wind averaged *c.* 200°, and the direction of their track inter-reversal angles were inconsistent, meandering around the tunnel. Wind had to be present during the counterturning or had to be experienced in flight before the wind was stopped in order to give polarity to the counterturns and cause rapid displacement toward the source.

Thus the most recent evidence is consistent with an integrated model of orientation to pheromone by flying moths, one invoking both a pheromone-mediated programme of turning and optomotor anemotaxis (Kennedy, 1983; Kuenen & Baker, 1983). In the present study wind was always present and thus the optomotor compensation for wind-induced drift always gave polarity to the narrowly zigzagging moths.

The programme of movements for other moth species responding to pheromone may not always include the counterturning reversals observed in *G. molesta* and *A. orana*. Each species may have its own typical way of moving when stimulated by odour (Bell & Tobin, 1982). Similarly, not all species' programmes may wane so quickly in continuous pheromone stimulation, but it seems clear that some do require intermittent stimulation for prolongation of the counterturning programme, and that some combination of such a programme plus anemotaxis is needed; not only to maintain contact with the plume, but also for prolonged displacement upwind toward the source.

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