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Behaviour of flying oriental fruit moth males during approach to sex pheromone sources

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Abstract. The pheromone-modulated upwind flight tracks of *Grapholita molesta* (Busck) males were video recorded as they approached a point-source of pheromone in a wind tunnel. The field of view of the video recording was divided longitudinally into 33 cm sections and the flight behaviour of the males in these sections was measured and compared as they approached from 233 cm to 50 cm downwind of the pheromone source. As the males approached the source, their mean ground speeds decreased. The mean values of their track angles increased with respect to due upwind (0°), indicating movement more across the wind. These changes resulted mainly from the males decreasing their air speeds as they progressed up the plume toward the source. They did not change the average direction of their steering (course angle). Thus, the increase in track angles resulted from the males allowing themselves to drift more in the wind as they approached the odour source. The males also increased their average rate of counterturning as they approached the source. The net result of all these behavioural changes was a track that slowed and grew narrower, giving the impression that the males were 'homing-in' on the pheromone source as they approached. Causes of these systematic changes in behaviour are considered with respect to the known systematic changes in pheromone plume structure as the distance to the source decreases.

Key words. *Grapholita molesta*, oriental fruit moth, flight orientation, pheromone, counterturning, anemotaxis.

Introduction

The pheromone-modulated flight behaviour exhibited by male moths is systematically altered in response to many biological and environmental variables. Changes in the flight performances of moths are reflected in, and measured from, the zigzagging upwind flight track that typically characterizes this behaviour (Kennedy, 1983; Baker, 1989; Arbas *et al.*, 1993). The concentration of the pheromones (Cardé & Hagaman, 1979; Kuenen & Baker, 1982a; Charlton *et al.*, 1993), the ratio of components in the pheromone blend (Baker *et al.*, 1981; Willis & Baker, 1988), and the intermittent spatial/temporal odour stimuli that define the structure of an air-borne odour plume (Kennedy *et al.*, 1980, 1981; Willis & Baker, 1984; Baker *et al.*, 1985), all cause significant changes in the upwind flight response of male moths. Wind velocity (Marsh *et al.*, 1978; Sanders, 1985; Willis & Cardé, 1990; Willis & Arbas, 1991), and ambient temperature (Linn *et al.*, 1988; Charlton *et al.*, 1993) have also been shown to affect the pheromone-modulated flight orientation of male moths, and the resulting flight tracks.

An additional modulation of the upwind flight tracks of male moths has been observed, but rarely analysed and quantified in detail. This is the systematic alteration of the flight performance as the males progress upwind toward the pheromone source in a constant wind velocity. These systematic changes have been described as giving the impression that the male is 'homing-in' on the pheromone source (Marsh *et al.*, 1978).

It has been recognized in many studies that the net upwind velocity (speed of progress toward the source) and actual ground speeds (speed along the flight track) of

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males decrease as they approach the pheromone source in a wind tunnel (Sanders *et al.*, 1981; Palaniswamy *et al.*, 1983; Sanders, 1985), but the changes in flight manoeuvres that could cause this (e.g. changes in the course angles steered, the air speed, and counterturning frequency) have remained largely undescribed. Likewise, previous studies of pheromone-modulated flight in field situations have described only resultant effects such as reductions in ground speed as the source is approached (Murlis & Bettany, 1977; Murlis *et al.*, 1982; Witzgall & Priesner, 1984). In only one series of wind tunnel experiments (Marsh *et al.*, 1978), and a recent set of field studies (Willis *et al.*, 1991), have the manoeuvres underlying these changes in flight tracks been studied systematically.

Alterations in flight tracks similar to those observed during homing-in, may be made in response to an overall increase in concentration within the filaments of the odour plume experienced by males as they approach the source. In experiments designed to show the effects of increasing source concentrations on the upwind response, similar decreases in net upwind ground speed and overall narrowing of the tracks were observed as males flew in plumes issuing from sources of increasing concentrations (Farkas *et al.*, 1974; Cardé & Hagaman, 1979; Sanders, 1985; Keunen & Baker, 1982a; Charlton *et al.*, 1993). In all of these experiments the same pheromone-dispensing devices were used at all concentrations. Thus the turbulent structure of the filamentous plume was probably very similar at all concentrations.

The intermittent odour stimulation provided by the filamentous nature of the pheromone plume is an important determinant of the structure of the flight track of responding male moths. The fluctuating pheromone signals present in the structure of a wind-borne plume is a requirement for upwind flight (Kennedy *et al.*, 1980, 1981; Willis & Baker, 1984; Baker *et al.*, 1985); upwind progress cannot be maintained in a homogeneous cloud of pheromones. Recent field studies which simultaneously quantified plume structure (Murlis *et al.*, 1990) and the flight response of *Lymantria dispar* males to a pheromone plume at different distances from the source (Willis *et al.*, 1991), suggest that systematic changes in the temporal/spatial structure of the pheromone plume may also influence the flight performance of males as they approach a pheromone source. Furthermore, laboratory flight studies suggest that, at least in *G. molesta*, males may be capable of making manoeuvres in response to encounters with individual filaments of pheromones or clean air within a pheromone plume (Baker & Haynes, 1987).

These behavioural studies are further supported by recent neurophysiological evidence demonstrating the ability of male moths of several species, including *G. molesta*, to resolve fine-scale temporal pheromone stimuli at both the receptor (Baker *et al.*, 1989; Rumbo & Kaissling, 1989) and central nervous system levels (Christensen & Hildebrand, 1988; Christensen *et al.*, 1989).

The results we report further support the idea that some systematically changing parameter or parameters of plume structure modulate the upwind flight of male moths to pheromone. As *G. molesta* males approached the source, they maintained their course angles and decreased their mean air speed, resulting in a decrease in ground speed and an increase in mean track angle. This indicates that the optomotor flight control system, which depends on visual cues from the ground moving beneath the moth to adjust steering and velocity, is influenced by changes in the plume as they approach the source. The temporal structure of the counterturning is also modulated as the males approach the source.

Materials and Methods

Insects. Moths were reared on small green thinning apples (Baker *et al.*, 1981); the pupae were separated according to sex, and adult males were separated daily. All life stages were maintained at approximately 25°C on a LD 16:8 h cycle. Adult males were held in an environmental chamber with positive air pressure to eliminate the possibility of exposure to female pheromone prior to the experiments and had an 8% sucrose solution available to them at all times. All moths were 1–5 days old at the time of the experiments.

Pheromone. The synthetic sex pheromone blend consisted of the three components emitted by *G. molesta* females: 5.9% (*E*)-8-dodecenyl acetate (*E*8-12:Ac) and 3.8% (*Z*)-8-dodecenyl alcohol (*Z*8-12:OH) (Cardé *et al.*, 1979) in (*Z*)-8-dodecenyl acetate (*Z*8-12:Ac) (Roelofs *et al.*, 1969). The solutions of various *E*/*Z* ratios (1.7, 5.9 and 10.2% *E*8-12:Ac) were the same as described by Baker *et al.* (1981). Final ratios and dosages were verified by gas–liquid chromatographic (GLC) analysis using a 3 m × 4 mm glass column packed with 10% Silar-10C on acid-washed 100–120 mesh Chromosorb W. Under the conditions described, all solutions contained less than 0.5% volatile impurities. Stock solutions were equilibrated to 30 μg of *Z*8-12:Ac per μl, and 10 μl aliquots of each were applied to the wide end of a rubber septum (A. H. Thomas Co., No. 8753-D22, sleeve type, 5 × 9 mm). The emission rate from a septum of this dosage is just above the level emitted by a calling female (3.2 ng/h; Baker *et al.*, 1981), which is approximated by a septum loaded with 10 μg of the synthetic blend. All septa were impregnated with pheromones on the same day, and were stored separately at −10°C when not in use.

Wind tunnel and experimental procedure. Experiments were performed in a clear polycarbonate plastic wind tunnel, 3.6 × 1 × 1 m, described in detail in Kuenen & Baker (1982b). Light intensity was 250 lux, wind speed was 70 cm/s, and temperature was between 22 and 24°C. Tracks of individual males were recorded using two Sony RSC-1050 rotary-shutter cameras connected to two Sony SLO-340 video recorders. Each camera was positioned about 1 m above, and aimed at, the floor of the wind tunnel and oriented such that the field of view of each camera was 1 m long and 0.72 m across the tunnel. One camera recorded the movements of males which had made prolonged upwind flight in the plume. The field of view of this camera began 150 cm downwind of the pheromone source and extended to 50 cm from the source. The second camera was positioned above the point where the males were released in the plume, such that the 1-m-long field of view from 3 m from the source to 2 m would enable the recording of males much farther from the source, after they locked-on to the plume.

Males were released singly from cone-shaped aluminium screen cages (maximum diameter 10 cm; maximum height 7 cm) held in a ring stand 15 cm above the floor of the tunnel and 250 cm downwind of the pheromone source. Each cage was used once per treatment and the cages and the ring stand were rinsed with acetone between treatments. Moths were tested in a randomized complete-block design, with ten moths being released per block per treatment in seven replicates. The pheromone source was placed on a 15 × 15 cm sheet-metal stand 15 cm above the floor of the tunnel and 40 cm from the upwind end. The sheet-metal stand which held these platforms was rinsed with acetone between treatments and the platforms were used once and rinsed in acetone daily.

Data processing and analysis. Recordings of each flight track from the two cameras were re-recorded onto a Sony SVM-1010 motion analyser for better motion resolution and played back frame-by-frame through a Panasonic WV-5470 black-and-white video monitor. The consecutive locations of the males were digitized every 1/30 s 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 for each track were stored for later analysis.

Calculations of the triangles of velocity (Kennedy, 1939) were based on four quantities, two of them measured from tracks: ground speed (length of each movement vector divided by 1/30 s) and track angle (angle of each movement vector with respect to the wind line, 0°) (Marsh *et al.*, 1978). The other two, the wind speed and direction, were known constants throughout these experiments. Angles and lengths of vectors were measured by a track analysis program (Kuenen & Baker, 1982a). These values were then used to calculate air speed, course angle, and drift angle (Marsh *et al.*, 1978). The values for the longitudinal and transverse components of image flow were calculated from these same triangle of velocities data according to a method outlined by Ludlow (1984) and David (1986).

The changes in behaviour accompanying the close approach to the source were measured by first dividing the field of view of the camera closest to the pheromone source into three equal sections of 33 cm in length, with one section traversing 83–50 cm from the source, a second section traversing 116–83 cm from the source, and a third covering 150–116 cm from the source. A fourth section was included that encompassed the upwind-most portion of the field of view of the downwind camera, 233–200 cm from the source. Males in this latter section had usually ceased the broad cross-wind counterturning with little upwind or downwind displacement, characteristic of flight behaviour during locking-on to the pheromone plume, and were predominantly engaged in upwind flight. The track vectors for each male were first segregated according to each of these four sections, and the mean values for all of the parameters were calculated before calculating a grand mean for all of the moths in each section for each parameter. Means were compared using a one-way analysis of variance, followed by Duncan's multiple range test.

Results

The overall appearance of the flight tracks of males homing-in on the pheromone source is illustrated by the flight tracks in the sections from 150–50 cm from the source (Fig. 1). Although the increasing proportion of *E*8-12:Ac in the pheromone blend caused significant changes in steering

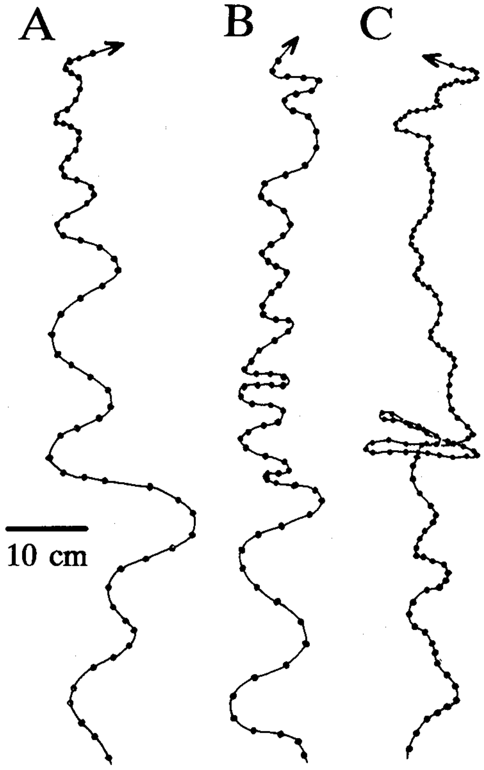

Fig. 1. Flight tracks of *G. molesta* males as they approached pheromone sources dosed with 30 µg of three different pheromone component blend ratios. (A) Track of a *G. molesta* male flying upwind in a plume of the pheromone component blend with 1.7% *E*; (B) track of a *G. molesta* male flying upwind in a plume of the pheromone component blend with 5.9% *E*; (C) track of a *G. molesta* male flying upwind in a plume of the pheromone component blend with 10.2% *E*. Note that in all cases the males narrowed their tracks and decreased their ground speed (illustrated by shorter distances between each moth position) as they approached the source. Each dot represents the position of the moth each 1/30 s. The wind and pheromone plume flow from top to bottom in this figure.

and velocity outputs (Willis & Baker, 1988), homing-in can be seen in all three blend ratios (Fig. 1A, B, C).

The most prominent change in behaviour as male *G. molesta* approached the pheromone source was a reduction in their mean air speed (Fig. 2A). Within the 1-m-long zone, 150–50 cm from the source, the males decreased their air speed significantly ($P<0.05$), regardless of the blend ratio to which they were responding. Because they did not alter their mean course angles significantly as their air speed diminished (Fig. 2B), the resultant mean track angles with respect to the wind tended to increase (became oriented more across the wind) as they approached the source (Fig. 2C). The greatest increase was observed in the track section closest to the pheromone source (especially at 10.2% *E*) (Fig. 2C). As a result of the moths reducing their air speeds, their ground speed along the track also decreased (Fig. 2D).

We do not know why males in the zone 200–233 cm downwind did not always follow the overall trend to increase or decrease the particular mean track parameter in a manner consistent with the trend occurring closer to the source, especially with regard to air speed. The difference may be due to the relatively short time that had elapsed since the males had locked onto the plume in this segment of flight furthest downwind.

The decrease in ground speed as the males approached the source was reflected in a concurrent decrease in the longitudinal component of image flow over the eyes (Fig. 3A), the parameter of visual feed-back hypothesized to control ground speed (David, 1986; Ludlow, 1984). The maintenance of the mean course angles as the males approached the odour source is reflected in the relative lack of change in the transverse component of image flow (Fig. 3B), the visual feed-back thought to control steering. Since the orientation of the course angle shows little change as the males approach the source (Fig. 2B) and the mean track angle increases (Fig. 2C), the difference between these two angles, the drift angle (Fig. 3C), must increase. Unlike transverse and longitudinal velocity of image motion, there is no evidence that males monitor their drift angle or use it to control their steering and velocity (David, 1986; Ludlow, 1984).

Males significantly increased ($P<0.05$) their counterturning frequency as they flew upwind from the zone 233–200 cm from the source to the penultimate zone 116–83 cm from the source (Table 1). This trend held, regardless of the blend ratio used, and the increase amounted to approximately one reversal per second between these two zones in two of the three ratios tested (Table 1). In the zone closest to the source the counterturning frequency of males flying to a source with 1.7% *E* diminished again (Table 1).

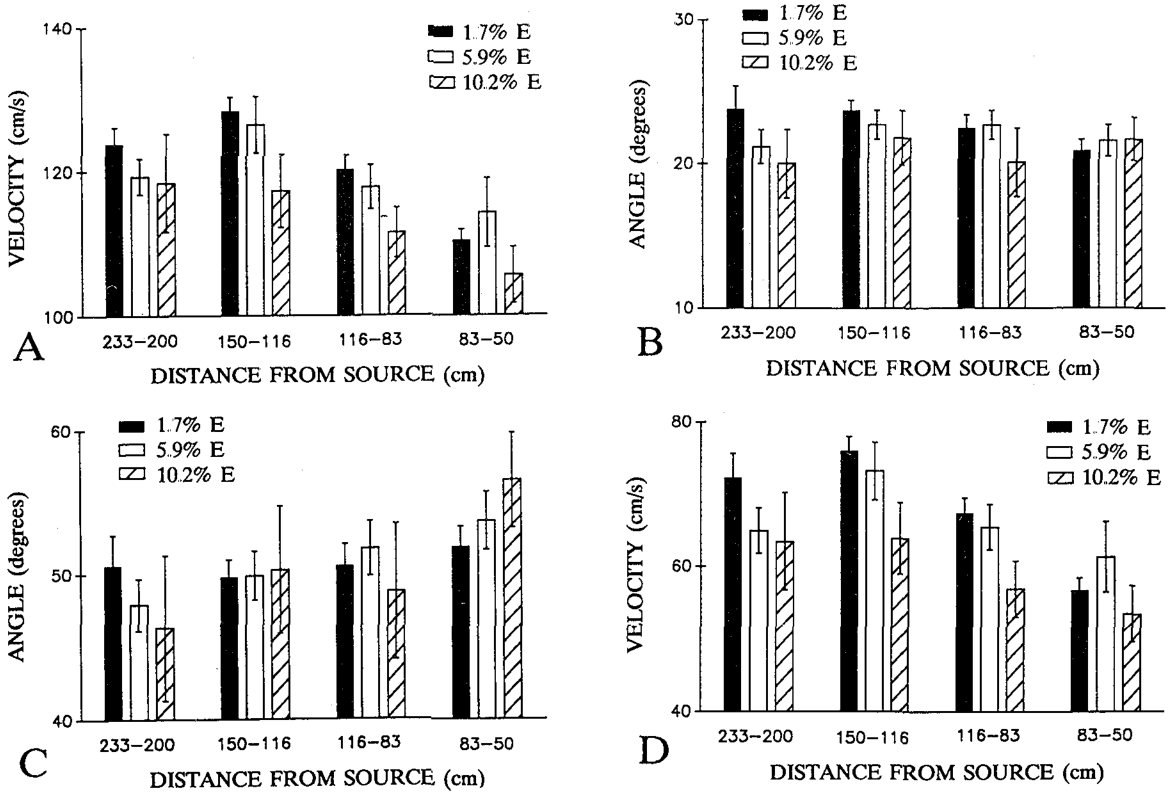

Fig. 2. Means (±SE) of velocity and angular flight track parameters as *G. molesta* males approach the source in plumes of three different pheromone component blends. (A) Mean air speeds; (B) mean course angles; (C) mean track angles; (D) mean ground speeds. $n=54$ for 1.7% *E* treatment; $n=31$ for 5.9% *E* treatment; $n=9$ for 10.2% *E* treatment.

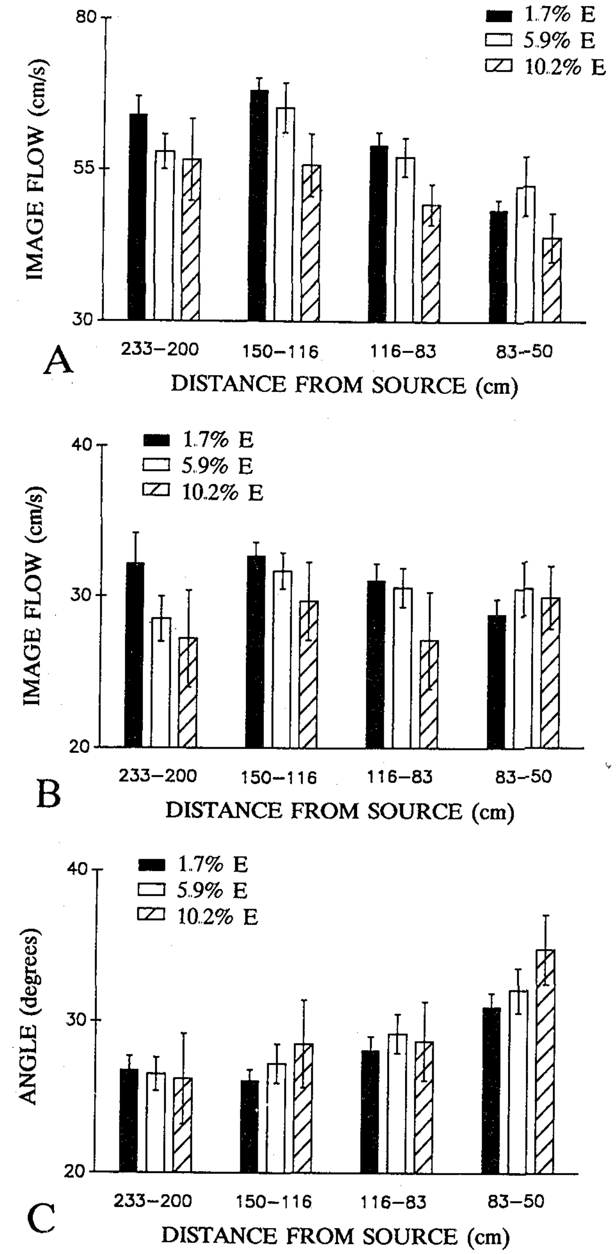

Fig. 3. Means (±SE) of image flow parameters and drift angles as *G. molesta* males approach the source in plumes of three different pheromone component blends. (A) Longitudinal image flow; (B) transverse image flow; (C) drift angles. Details as per Fig. 2.

Discussion

A reduction in speed of progress toward the source is the most consistently observed change during approach to pheromone sources in all species where this progress has been measured. Such a reduction in net speed of upwind movement has been observed in one species of the Pyralidae (*Plodia interpunctella*: Marsh *et al.*, 1978), one species of the Lymantriidae (*Lymantria dispar*: Cardé & Hagaman, 1979), two species in the Noctuidae (*Spodoptera littoralis*: Murlis & Bettany, 1977; Murlis *et al.*, 1982; *Euxoa ochrogaster*: Palaniswamy *et al.*, 1983), and two species in the Tortricidae (*Choristoneura fumiferana*: Sanders *et al.*, 1981; Sanders, 1985; *Grapholita molesta*: this study). In the two species whose responses have been most extensively quantified, *G. molesta* (this study) and *Plodia interpunctella* (Marsh *et al.*, 1978, 1981), similarities and differences exist in how males alter their behaviour as they approach a source of the appropriate sex pheromones.

In *G. molesta*, this reduction in ground speed can be attributed entirely to males reducing their air speed. In *P. interpunctella*, however, a decrease in air speed in concert with turning more into the wind (decrease in course angle) each contributed to the decrease in ground speed (Marsh *et al.*, 1978). [Although there was an error in the time-scale employed by Marsh *et al.* (1978) (Marsh *et al.*, 1981), affecting the values calculated by the triangle of velocities technique, conclusions concerning the *relative* changes during approach to the source would not have been affected.] The consequence of this simultaneous change of steering and velocity was the maintenance of the resultant mean track angles within a narrow range of values, showing no increase as the *P. interpunctella* males approached the pheromone source.

Over the distances measured, *G. molesta* males did not steer more directly into the wind as they approached the source and therefore could not have maintained a constant track angle as they reduced their air speed. Rather, their mean track angles increased, exhibiting the greatest mean value in the track sections closest to the source. Likewise, the system of self-steered counterturning was significantly affected in *G. molesta*, with males generally increasing their reversal frequency closer to the source. Similar modulation of counterturning frequency on approach to the source has been reported from wind-tunnel experiments using *Manduca sexta* males (Willis & Arbas, 1991). In *P. interpunctella*, however, the frequency of counterturning was unaffected as males approached a source of female pheromone (Marsh *et al.*, 1978).

Whether these differences reflect species-specific differences, or differences in experimental methods, will require further clarification. The fact that these reported changes in behaviour occur in all experimental conditions, whether the variables are environmental (wind speed: Marsh *et al.*, 1978; Willis & Arbas, 1991) or biological (pheromone blend ratios: this study), is further support for the idea that these changes in behaviour are the result of changes in stimuli in the pheromone plume.

The above results of wind-tunnel experiments are further supported, and extended, by the results of recent studies of the flight of *L. dispar* males at different distances from a point-source of pheromone in the natural environment of a forest (Willis *et al.*, 1991). As in the wind-tunnel studies reported above, the mean ground speed decreased as flight tracks were recorded from 20 to 2.5 m away from the source. This decrease in ground speed can, in part, be explained by a concomitant decrease in air speed. Again, as in the laboratory studies, the males increased their counterturning frequency as their flight was recorded

Table 1. The mean frequencies of counterturning (±SD) exhibited by *G. molesta* males as they approached a source of sex pheromone blend ratios comprised of 1.7%, 5.9% or 10.2% *E*8-12:Ac plus 3.8% *Z*8-12:OH in *Z*8-12:Ac.

	Counterturning frequency (turns/s)		
Distance from source (cm)	1.7% *E*	5.9% *E*	10.2% *E*
50–83	6.73 ± 1.13b	6.83 ± 1.15ab	6.35 ± 0.60ab
83–116	7.24 ± 1.18a	7.32 ± 1.11a	7.53 ± 1.61a
116–150	6.38 ± 1.18b	6.83 ± 1.04ab	6.43 ± 1.20ab
200–233	6.35 ± 1.34b	6.65 ± 1.51b	6.01 ± 1.37b

$n = 54$, 31 and 9 males for each of the three blend ratios, respectively. Mean values were taken for each male in each track section before grand means plus standard deviations were calculated. Means in each column having no letters in common are significantly different according to an analysis of variance followed by Duncan's new multiple range test ($P < 0.05$).

closer to the pheromone source. The combination of these changes in flight behaviour resulted in the characteristic slowing and narrowing of the flight tracks as the males' behaviour was recorded closer to the source.

Baker (1990) hypothesized that the relatively straight-line flight of some moth species within a pheromone plume and the zigzagging tracks characteristic of others, are a function of the time between filament contacts and the speed of the males' reactions to the repeated contact and loss of pheromone stimuli that characterizes flight in an odour plume. In short, this hypothesis states that males fly due upwind upon pheromone contact, and express casting flight across the wind as soon as they are able to process sensory input and generate a motor output upon odour loss. Thus, the counterturning frequency and orientation of the straight legs between turns result from a combination of the variable plume structure and the latency of response to that structure. It follows logically that the individual variability between males in a given species would be explained by responses to the instantaneous variation in the fine structure of the plume. In addition, if the plume structure changed in a systematic way with distance to the source, this hypothesis could explain the alterations in flight tracks that underlie homing-in. In particular, the reduction in air speed and increase in counterturning frequency as *G. molesta* males flew closer to the source might have been caused by differences in both overall concentration and plume structure in the zones closer to the source versus those farther away. The fine structure of a pheromone plume changes systematically with respect to distance from the source (Murlis, 1986; Murlis *et al.*, 1990), and this change in structure has been examined directly (in the same wind tunnel as used in this study), using electroantennogram (EAG) measurements from *G. molesta* males, taken at various distances from the source (Baker & Haynes, 1989).

It is not yet known how accurately the frequency of EAG depolarizations reflects the actual pheromone filament encounter frequency experienced by the male moth, or what an EAG depolarization means to the CNS and ultimately the behaviour that we observe. However, the sensory neurons on the antennae do provide the primary olfactory input to the moths during the performance of this behaviour, and provide the easiest method (via EAG) to detect the structure of a pheromone plume. If we accept that EAG depolarizations provide a good estimate of filament contacts, then EAG recordings become a tool to help understand how the moth alters this behaviour with respect to this variable of plume structure.

No significant change in the frequency or amplitude of EAG depolarizations due to filament contact was revealed between measurements made at 3 m and 30 cm downwind of the source (using the same source loading as in this study), in the wind tunnel. There was, however, a *trend* for the mean filament frequency detected by the antenna to increase by nearly 50%, from 1 Hz at 3 m to 1.5 Hz at 30 cm from the source (Baker & Haynes, 1989). *G. molesta* males can alter their mean course angles and counterturning frequency within 0.15 s after encountering or losing contact with pheromone (Baker & Haynes, 1987). Alterations in mean air speed, however, appear to take longer, *c.* 0.5 s or so. Air speed increased when the males experienced a sudden drop in the pheromone concentration, a characteristic of the casting flight expressed when the pheromone plume is lost (Baker & Haynes, 1987). In addition, *G. molesta* males also have a higher mean air speed when flying up plumes of presumably similar structure emitted from sources loaded with lower concentrations of pheromone (Kuenen & Baker, 1982a). Thus the decreases in air speed that we observed in this study might be explained because the increased frequency of contact with pheromone filaments reduced the incidence of casting flight (i.e. higher air speed) triggered by excursions into clean air.

The greater frequency of contact with filaments would also keep the hypothesized self-steered counterturning programme operating at its highest frequency, again by preventing casting flight and its reduced frequency of reversals from starting during any brief excursions into clean air (Baker & Haynes, 1987). In our study males did counterturn at greater frequency over distances similar to those which showed increases in filament contacts in previous studies (see above) (Baker & Haynes, 1989). The slight reduction of counterturning frequency in the zone closest to the source could be due to the inability of the males' receptors to disadapt fast enough to the high frequency, high concentration pheromone filaments making up the ever-narrowing odour plume, coupled with a lower probability of contacting filaments in the narrower time-averaged envelope of the plume and the width of the counterturning track itself. A trend toward reduced frequency of filament contacts was observed in EAG recordings made very close (10 cm) to the source of similar plumes, perhaps due to receptor adaptation (Baker & Haynes, 1989).

In a recent field study with *L. dispar* males, the spatial fine-structure of pheromone and ionized-air plumes were quantified at the same distances from the source that the flight behaviour of males was video recorded (Murlis *et al.*, 1990; Willis *et al.*, 1991). These measurements allowed the identification of parameters of the plume structure that changed predictably with distance from the source. The most consistent changes, as the source is approached are: narrower filaments (ionized-air and EAG), higher frequency of filament encounter (ionized-air and EAG), and an increase in overall mean concentration (ionized-air). The alterations in air speed and counterturning frequency observed in the flight tracks of *L. dispar* males at different distances as they approached a pheromone source (Willis *et al.*, 1991) were similar to those described for *G. molesta* males in this study.

Alterations of *L. dispar* flight tracks as they approach the source might also be explained by the increase in filament encounter frequency as hypothesized above. However, there was no significant difference between the mean peak EAG amplitude at 2.5 and 10 m from a pheromone source, in an open field, as measured by Murlis *et al.* (1990). This is consistent with male *G. molesta* EAG recordings of plume structure in the wind tunnel, from 3 m to 30 cm from the pheromone source (Baker & Haynes, 1989). The mean peak amplitude of EAG responses of *L. dispar* males recorded in a forest actually increased with distance from the source (Murlis *et al.*, 1990). It is not yet clear if this seemingly anomalous result can be explained by the unique conditions of air flow (and hence plume structure) that exist under a forest canopy (Elkinton *et al.*, 1987), or a biological phenomenon or experimental variable associated with the antenna.

Thus, measurements from uncontrolled 'natural' plumes have yielded little information on how filament concentration and encounter frequency may interact together, and with other spatial characteristics of the plume, to affect male behaviour. However, in recent laboratory experiments some of these parameters have been controlled and varied independently. In studies of the upwind flight response of *Heliothis virescens* males, Vickers & Baker (1992) generated short-duration pulses of pheromone (meant to mimic natural plume filaments) at various frequencies, and four different concentrations (a range of three orders of magnitude). Their results indicated that *H. virescens* males sustained their upwind flight to the source more consistently when the optimal experimental pulse frequency (4 Hz) was presented in concert with higher pheromone-source concentrations (10–100 μg). Coincidentally, higher pheromone-source concentrations did result in higher filament frequencies recorded from the antenna of *G. molesta* males (Baker & Haynes, 1989). So, a positive correlation between source concentration and filament frequency, measured by EAG, has been shown to be a characteristic of the plume structure in at least one species. Results from *H. virescens*, however, indicate that concentration and filament frequency can have independent effects on flight behaviour, as can the blend quality in each filament (Vickers & Baker, 1992).

Recent studies of the fine structure of odour plumes in the aquatic environment (Moore & Atema, 1991), support and extend the findings from ionized-air and pheromone plumes in air. Using an array of microelectrode detectors (tip diam. 150 μm; *c.* size of the odour sensory hairs of lobsters), Moore & Atema (1991) were able to sample an aquatic odour plume in three dimensions up to 250 cm down-current from the source. Based on a 10 Hz sampling rate, the structure of an aquatic odour plume was shown to change with distance to the source in all dimensions (i.e. along the length, width and depth of the plume). The most intense peak-to-trough odour fluctuations occurred along the longitudinal axis of the plume, that is, as one approaches the source from down-current. Since, after adjustment for scale (Vogel, 1981), the temporal and spatial dynamics of turbulence are similar in air and water, these results should be applicable to both environments. Interestingly, Moore *et al.* (1991) have demonstrated that lobsters decrease their walking speed and increase their heading angles as they approach a source of attractive odour. As with moth orientation to odour, however, which parameters of the structure of odour plumes influence lobster behaviour is unknown.

One alternative to the idea that moths are responding to each encounter and loss of pheromone (Baker, 1990) has recently been put forward. Willis & Arbas (1991) have proposed that the period between turns might be a critical sampling period for pheromone stimuli, and that information on the chemical and spatial elements of the pheromone plume acquired during the inter-turn period could be used in the modulation of steering, velocity, and counterturning manoeuvres (after Marsh *et al.*, 1978). If intermittent pheromone stimuli are not sensed during the inter-turn period, the moth alters its manoeuvres to result in casting flight. Thus, a probabilistic distribution (Murlis, 1986; Moore & Atema, 1991) of odour stimuli subject to environmentally and biologically induced variability, could give rise to a temporally regular series of counterturns executed by the moth. In this case the observed counterturning frequency and orientation of straight legs between turns would not be determined by the plume structure in concert with the latency of response to odour contact or loss. Rather, the frequency of the counterturning and orientation of the straight legs would be continuously adjusted by pheromone and wind information actively acquired during the inter-turn periods. As in the case of many contesting hypotheses, the moths probably are capable of, and use, all of the behavioural mechanisms mentioned in order to adapt to the environmental and biological variables with which they must contend.

Although we have concentrated on the potential effects of plume structure on the flight manoeuvres of moths, visual cues associated with the odour source might also contribute to homing-in. It has been shown that males of some moths do use visual cues from female moths for close-range orientation (<12 cm), once attraction from relatively long range has occurred (Carpenter & Sparks, 1982). Our measurements did not take place closer than 50 cm from the source, so it is unlikely that such visual cues played a role in the homing-in that we observed. Marsh *et al.* (1978) did test for potential visual cues from the source influencing homing-in. They observed no difference between flight to the ligated tip of a female abdomen, or a drop of pheromone extract applied to the nylon screen at the upwind end of the wind tunnel.

The results of the present study are consistent with the idea that the structure of the pheromone plume may be a determinant of the subsequent behaviours known to be performed by males as they approach the pheromone source. These behaviours (e.g. changes in air speed, course angle, and counterturning frequency), operating in concert, shape the flight track, giving the impression that males home in on the source (Marsh *et al.*, 1978). Increasing concentrations of pheromone cause similar changes in male flight performance in at least two different species of moth (Cardé & Hagaman, 1979; Kuenen & Baker, 1982a; Charlton *et al.*, 1993). Thus, an increase in filament encounter frequency closer to the source, plus the overall increase in odour concentration within the filaments, may explain some or all of the behavioural alterations underlying this homing-in (Baker 1990). However, the increasing knowledge of the spatial structure of odour plumes (Moore & Atema, 1991; Murlis *et al.*, 1990, 1992), together with the physiological ability of sensory and central neurons to sense and preserve such structure (Baker *et al.*, 1989; Rumbo & Kaissling, 1989; Christensen & Hildebrand, 1988; Christensen *et al.*, 1989), and apparent changes in behaviour resulting from it (Willis & Arbas, 1991; Willis *et al.*, 1991) suggest that for a given pheromone blend, concentration and filament encounter frequency are not the only elements of the odour plume that are important to pheromone-modulated flight in moths. Further behavioural and neurophysiological experiments on moth responses to temporal and spatial manipulations of odour stimuli are necessary before we can fully understand which elements of plume structure underlie these changes in behaviour.

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