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Manoeuvres used by flying male oriental fruit moths to relocate a sex pheromone plume in an experimentally shifted wind-field

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ABSTRACT. In a wind-field experimentally shifted in direction by 35°, flying male *Grapholita molesta* (Busck) zigzagging upwind either maintained contact with a pheromone plume and followed it across during the shift or lost it and commenced casting at *c.* 90° across the shifting windline to locate it eventually in its new position. Males accomplished both of these results by integrating the previously described systems of optomotor anemotaxis and self-steered counterturning, but with faster reaction-times to pheromone on and off than heretofore calculated for this species. We found no evidence that males following the plume across used chemotaxis as proposed for another species. Rather, the sawtoothed-shaped tracks were a result of the anemotactic and counterturning systems responding rapidly and reiteratively to each loss and gain of pheromone along the plume in the shifting wind. The response to an increase or decrease in pheromone concentration by males was to change their course angle to more upwind or more crosswind, respectively, on the very first reversal (within *c.* 0.15 s) after the concentration changed. Because males adjusted their airspeeds more slowly to changes in concentration, the groundspeeds along the more upwind-orientated legs were lower than those along crosswind legs, contributing to the sawtoothed shape of tracks of plume-followers. The self-steered counterturning programme also reacted quickly to concentration changes, the reversal intervals tending to be shorter following each contact with pheromone than after each excursion into cleaner wind. Following casting after losing the plume, males relocating the pheromone plume exhibited an upwind 'surge' of narrow zigzagging flight because on the first leg in the plume they steered a course more directly upwind than on the previous leg and increased the frequency of counterturning to its highest value while maintaining the relatively high airspeed acquired while casting.

Key words. Optomotor anemotaxis, counterturning, oriental fruit moth, *Grapholita molesta,* Lepidoptera, Tortricidae, pheromone, flight behaviour, wind-shift.

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Introduction

Optomotor anemotaxis and a self-steered programme of reversals are the two mechanisms known to be used by all free-flying moths analysed thus far to manoeuvre during pheromone-mediated flight in a plume or following loss of the plume during casting flight (Kennedy, 1982, 1986). These two mechanisms interact to produce the males' resultant groundspeeds and flight track shapes, as well as the commonly reported resultant displacements *upwind flight, in-flight arrestment*, and *casting flight* under different conditions of pheromone stimulation and wind. Marsh *et al.* (1978) pointed out that a male moth has only two reactions available to him to control his horizontal displacement during pheromone-mediated flight in wind: change his *course angle* (direction of his thrust through the air) or his *airspeed* (his speed through the air).

Only one study, that of Marsh *et al.* (1978), has analysed the tracks and individual track legs of flying moths to discern how males use these two reactions to create their resultant tracks. They recognized that the triangle of velocities (Kennedy, 1940; Marsh *et al.*, 1978) could be used to calculate a male's airspeed and course angle if the track angle, groundspeed, and wind speed and direction were known. Kuenen & Baker (1982) used this technique to calculate changes in course angles and airspeeds of *Grapholita molesta* (Busck) males under different pheromone concentrations. However, they used average track angle and groundspeed values from their males' tracks, which made the calculations less useful for comparison with Marsh *et al.*'s results.

Neither of these studies was designed to ascertain the reactions of males encountering shifting wind directions. David *et al.* (1983) recorded gypsy moth male flight tracks in the field under shifting wind conditions and showed how both upwind flight in pheromone and casting flight following pheromone loss contributed to displacement toward the source. Because under some conditions pheromone-laden wind moves in straight lines away from the source (David *et al.*, 1982, 1983), progress up the windline in pheromone takes the males on the most direct route towards the source, and casting across the shifting wind helps the male relocate the new position of the plume and even reduce the distance to the source while doing so (David *et al.*, 1983). The airspeed and course angle manoeuvres used by the male gypsy moths to attain these tracks were not analysed, however. Von Keyserlingk (1984) recorded the tracks of flying male *G. molesta* within 1 m of a pheromone source in the field under shifting wind conditions, and concluded that males losing the plume appeared to allow themselves to drift or be pushed over to the plume's new location by the wind. Again, the anemotactic reactions of males were not measured and it is not clear from the limited analysis if, and how, they actually did this.

We sought to create experimentally a shifting wind field that mimicked that which can sometimes occur over short grass in the field (David *et al.*, 1982, 1983). The original purpose was to see how often male *G. molesta* flying upwind in pheromone would lose contact with the plume, and if they did, whether they would re-contact it following casting at a distance closer to the source than where they lost it, as reported for gypsy moths. Second, we wanted to see how males reacted to the shifting wind-field in order to maintain contact with the plume all the way to the source, how they reacted to a shifting, pheromone-free wind-field following prolonged pheromone loss, and to regaining contact with the plume in its new location. We were curious to see if males losing the plume received any advantage in the form of extra cues from a shifting wind-field which would tell them which way the plume had gone so they could bias their reactions to displace across more rapidly than predicted (Von Keyserlingk, 1984). Finally, we wanted to see if there was any evidence that our males were using chemo-klinotaxis to maintain contact with the plume, as hypothesized for male gypsy moths flying along a sinuous plume in a non-shifting wind-field in a wind tunnel (Cardé & Charlton, 1984). We analysed our males' manoeuvres using the triangle of velocities calculations, which had not been employed before for moths flying in shifting wind or along curved plumes.

Materials and Methods

Rearing and pre-conditioning

Grapholita molesta were reared and handled as described previously (Willis & Baker, 1984;

Baker *et al.*, 1985). All experiments were conducted between 1.5 h before and 1 h after lights-off would have occurred on their usual LD 16:8 h cycle, which is the males' optimal time of response to sex pheromone (Baker & Cardé, 1979). Males 2–6 days old were held in a cage placed in the experimental room but out of the wind-field less than 15 min before starting, and then removed from the cage individually at the moment they were needed for flight. Ambient conditions in the room were 850 lux light intensity and 21°C.

The wind-field

Wind of 70 cm/s was generated by a rotary blower connected by means of a hole in one of the two narrow ends of a 1.3×0.67×0.71 m cardboard box. Mixing baffles and a fibre-glass filter were located inside the box to create back-pressure and uniform velocity. Wind exited the box over the working area of the arena after passing through several layers of muslin cloth covering one of the two vertical 1.3×0.67 m faces (Fig. 1). All of the other sides were closed. The box was clamped to the edge of a rotating circular seat so that 0.85 m of the box's 1.3 m long wind-field-generating face was resting to the left of centre of the circular seat (Fig. 1). The blower rested on a separate, wheeled cart, but the whole blower-box unit was able to be rotated smoothly anticlockwise through a 35° arc in 1, 2 or 4 s according to treatment, by pushing the blower on its cart. Wooden blocks taped to the floor prevented the cart from over-shooting the 35° limit. The edge of the arena and the undersurface of the box were separated by only a few centimetres so that much of the box projected over the arena's surface when the wind field was at its 35° limit.

A 1.2×1.2 m portion of the arena was covered with white poster-board (1 mm thick cardboard) and red 5 cm diameter paper discs were placed randomly 5–15 cm apart on the white surface to provide visual contrast. Miniature wind-vanes were constructed using 3×0.5 cm strips of tissue paper glued at one end to 0.5×0.1 cm o.d. glass cylinders flame-sealed at one end to form a cap. Each cylinder, plus paper strip, was placed so that the sealed end rested on the pointed end of an insect pin projecting point-up through the poster-board on the arena. The cylinder could revolve freely when the paper was blown by very slight wind. A 11×11 grid of wind vanes spaced every 10 cm was set up on the arena beginning 10 cm from each of its edges.

The rotating seat and blower-box were positioned such that the pivot-point of the seat was just under the edge of the horizontal arena. The pheromone and smoke sources were affixed to a rod projecting straight upward from the wind-field's pivot point. The smoke source was a 1×0.5 cm piece of dental wick to which titanium tetrachloride was applied, and was at a height of 10 cm above the arena. The pheromone source was positioned 10 cm above the smoke source,

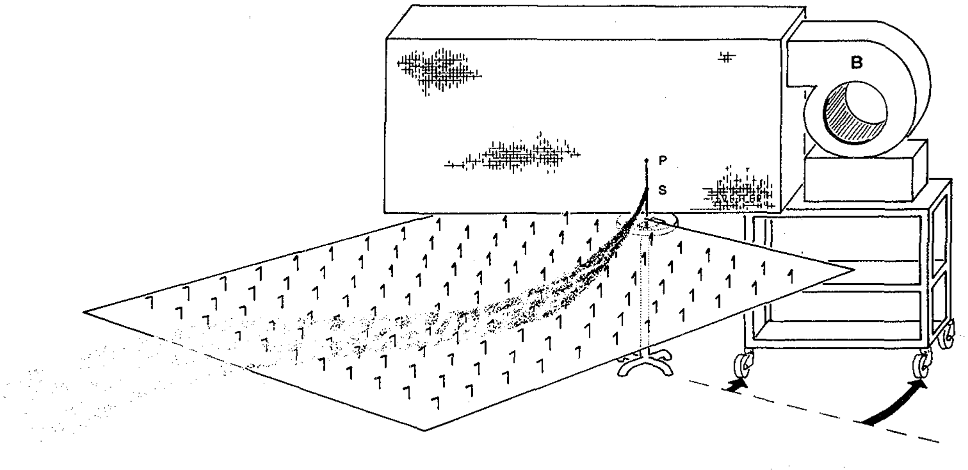

FIG. 1. The apparatus for generating the shifting wind-field and the arena with wind vanes spaced 10 cm apart over which flight tracks were recorded (red spots to enhance visual ground pattern not shown). S=smoke source; P=pheromone source; B=rotary blower.

and was a rubber septum impregnated with 30 μg of the standard three-component blend of (*Z*)- and (*E*)-8-dodecenyl acetate and (*Z*)-8-dodecenyl alcohol, used in previous experiments (Willis & Baker, 1984; Baker *et al.*, 1984, 1985). Thus when the box, and the wind-field, were rotated, the smoke and pheromone sources did not change position. The width of the visible smoke plume was probably a little wider than the pheromone plume, because the wick was a little wider than the septum. The smoke and pheromone plumes, at the 0° to 35° positions, were effectively exhausted into a pair of large fume hoods immediately downwind from the arena.

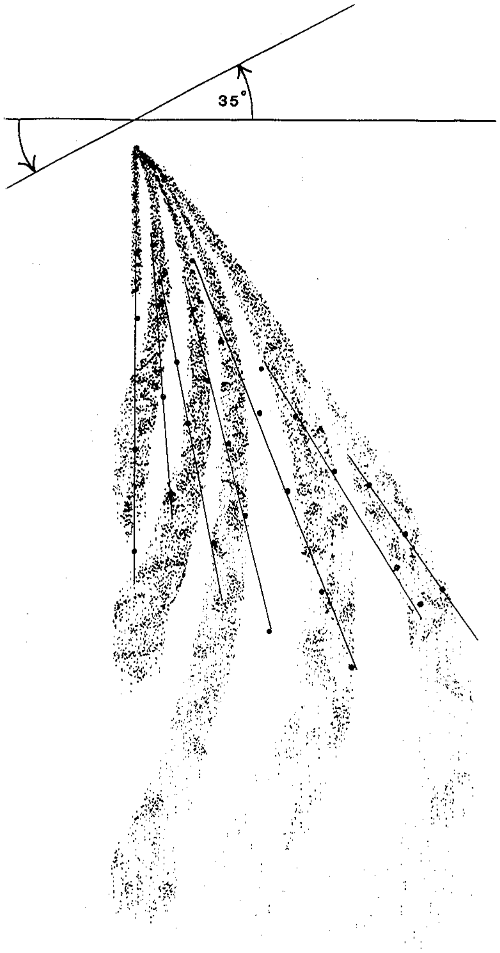

FIG. 2. Drawing of a top view of the smoke plume in still-frame video play-back progressively through times as wind-field was shifted from 0° to 35°. For clarity the complete lengths of plume in the first three positions at the left were not drawn. Single parcels of smoke were traced (dots) through time to show their straight-line travel away from the source in this setup.

Analysis of the smoke plume from video recordings indicated that this system changed the wind-field and plume position in a way that mimicked the behaviour of wind and plumes over grass in the field (David *et al.*, 1982, 1983) (Fig. 2). The individual parcels of smoke (and presumably the pheromone immediately above it) travelled away from the source in a straight line, even though the plume appeared curved at any instant during shifting (Figs. 1 and 2). The wind vanes provided information about the wind direction away from the smoke. They also indicated that in the camera's field of view the windline was of a uniform, straight direction at both extremes of the shifted field before and after the swing. However, the vanes often vibrated slightly in the breeze approximately 10–20°, and although their time-averaged position accurately indicated the wind direction, the still-frame readings were too variable to use as a windline angle with the confidence necessary to calculate the triangle of velocities. The vanes were therefore simply to check the system based on elapsed time (see analysis section below) to determine the windline in the vicinity of a male casting some distance from the smoke. The wind velocity over the portion of the wind-field at or to the left of the plume as viewed when facing the wind-source (Figs. 1 and 2), the only area in which males flew because the plume always moved away to their right (Fig. 3), was checked for uniformity by dragging the smoke source quickly to the left and timing the smoke from one end of the field to the other on video-replay. At five points spaced 20 cm apart, including the section with the normal position of the plume and a section 5 cm from the edge of the box, wind velocities varied by 2% or less when compared to the plume position.

Experimental procedure

Flight tracks were recorded by means of a rotary-shutter video camera (Willis & Baker, 1984) 2 m above the centre of the arena. A red filter was placed over the lens so that the red discs appeared nearly white in the recordings, facilitating the viewing of the flying moths (David, 1982). Individual males were released in the plume at the downwind edge of the arena with the wind and plume in the 0° position.

When the male had locked on to the plume and begun proceeding up it, the blower-box (and wind-field plus plume) was rotated, always anticlockwise, through its 35° arc in 1, 2 or 4 s according to the treatment to be given the moths. The person shifting the box was aided by an electronic tone sounding at 1 s intervals.

Track analysis

All references to a plume in this study mean the volume within the *time-averaged* envelope of pheromone (or smoke). The fine, short-term structure of the pheromone (and smoke) plume is referred to in terms of filaments. One hundred and eighty-two tracks were digitized at 30 points/s and stored on micro-computer using techniques described previously (Willis & Baker, 1984; Baker *et al.*, 1984, 1985). Upon inspection of the plotted tracks and comparisons with the recordings, which had the smoke plumes as well, ninety-nine males were judged to have lost the shifting plume completely at some point for four or more consecutive reversals, and eighty-three to have followed it across without losing it. The distances from the source at which the males either lost the plume during the shift or, for those that followed it, encountered the shifting wind field, were measured from the recordings, as were the distances from the source where casting males re-located the shifted plume.

To analyse the build-up of casting flight following loss of the plume until the upwind surge following re-contact with the plume, the tracks of males losing the plume were further sorted to include only those with three or more left *and* right (six total) reversals out of contact with pheromone during the shift, and gaining renewed contact with the plume within the camera's field of view. A reversal is a change of direction of the flight track from left-to-right, or vice versa. To provide a parallel analysis of males following the plume across, the tracks of these males were further sorted to include only those that contained at least three left and right reversals along the plume in the shifting wind-field. Many contained fewer than this because males encountered the shifting wind too close to the source to have enough time to complete the required number of zigzags before reaching the plume's final, shifted position. Thus, the tracks of twenty males losing the plume completely and sixteen males following the plume across were analysed for manoeuvring mechanisms.

In order to calculate from these resultant tracks the manoeuvres males were making during flight, that is, what their *course angles* and *airspeeds* were, the triangle of velocities (Marsh *et al.*, 1978) was calculated. The values that are available from our recordings that can be used are *wind speed, wind direction,* the male's *track angle* to the wind and his *groundspeed* along that track. The straightest portions of the track legs were used because the male has stabilized his steering along this line (Marsh *et al.*, 1978). Also, male *G. molesta* fly with nearly 0° pitch (horizontal to the ground) and change altitude minimally along the straight legs (Haynes *et al.*, unpublished), indicating that the visual input for reactions to wind should be most stable across the motion-sensitive ventral ommatidia along these portions (David, 1986).

The *track angles* of the straight legs of males in both groups were measured with respect to the wind direction for each inter-reversal leg in the following way. The longest, straightest section ('straight leg') of at least two consecutive vectors having less than 10° curvature was identified, and its angle, end-point to end-point, with respect to the windline measured with a protractor (Marsh *et al.*, 1978). Zero degrees referred to due upwind, 180° downwind, and values to the left and right of the windline were not differentiated by positive or negative signs because the absolute values were needed for calculating means of the legs of different males in a similar state of pheromone stimulation (see below). To obtain the moth's corresponding *groundspeed* along the same straight portion of that leg, its length was measured with a ruler and the number of vectors in this segment (elapsed time in 1/30 s) was counted. When two consecutive vectors could not be found with less than 10° curvature, the two consecutive vectors that were curved least were identified, and the longer of the two was used as the straight leg. For the relatively uncommon occurrence in which only three vectors formed a reversal leg and had excessive curvature, the middle vector was designated as the straight leg.

The *wind direction* both before and after the shift, was easily measured because it was always a straight line from the source. Likewise, for males following the shifting plume across, the windline was also easily measured during the shift, because the males were never far from the smoke, which always defines the wind direction because it moves in a straight line from the source (Fig. 2). Therefore a line was drawn from the source to the smoke nearest the track leg being measured at that instant, and this constituted the wind direction (Fig. 3E, F). The wind direction experienced by males at any instant when they had lost the plume completely was estimated according to the time elapsed since plume loss, and also by comparing it with the average direction of the wind vanes nearest the males during that leg. The *wind speed* was considered to be the constant 70 cm/s of the non-rotating field. The slight gain in linear velocity that could have been imparted to the wind field by its angular rotation during the shift was not a significant factor in the calculations. This is especially so since any linear gain would be minimal closest to the rotation point (the smoke and pheromone sources) and therefore also close to the plume where most of the legs occurred. Wind velocity in the plume is always 70 cm/s.

Using the above measurements, the males' course angles, airspeeds and drift angles were calculated using the triangle of velocities along each straight leg. In addition, the transverse and longitudinal components of movement, possibly important as visual feedback (Ludlow, 1984; David, 1986), were calculated. Then for males losing the plume completely, we discerned as best we could from the recordings the track leg in which the last possible contact with pheromone (smoke) occurred. This same track leg was identified on the computer printout of the track-leg values, and designated the last possible contact with pheromone, or the first leg where the plume was lost. This straight leg is labelled 0 in Figs. 4 and 6 under the lose plume portion. All the other legs before and after loss were similarly segregated for calculating means. The means of the first left and right straight legs where all legs occurred completely out of contact with pheromone in those figures, therefore, are labelled under the lose plume portion as 1. Re-contact with the plume ('regain plume' in Figs. 4 and 6) was designated as the first straight leg occurring following intersection of the shifted plume (smoke) that exhibited a change from 90° across-wind track to an upwind track. For males that followed the plume across, track legs were segregated beginning with the first leg, either right or left across the windline, occurring in the shifting wind-field. This leg was estimated to the best of our ability from video replay as the point at which each male first encountered the curvature of the plume indicative of the shift.

The *duration* of each inter-reversal leg was measured by counting the number of vectors (elapsed time is 1/30 s per vector) from apex to apex of the track. The duration of each leg is the inverse of the counterturning frequency at that moment, and as such allows a fine-grained analysis to be made of the self-steered system of counterturning. This measure is independent of the anemotactic system and the related triangle of velocities calculations.

Results

Form of the tracks in shifting wind

In the shifting wind field and shifting position of the plume, males sometimes lost contact with the plume completely (defined as four or more consecutive reversals out of contact with the smoke) (Fig. 3A–C), while at other times they were able to 'follow' it across to its final position without losing it (Fig. 3D–G). Most of the males appeared to do a mixture of both.

The probability that males would either lose the plume completely or follow it over seemed to be mostly a function of the distance that the odour had shifted laterally away from the male by the time the next right-handed track-leg occurred. This distance likewise depended upon the distance of the male from the source when the swing took the plume away from the male (Table 1), and not very much upon the speeds of the wind-swing (35, 17.5 or 8.8°/s) used in this study. In general, those males that were closer than *c.* 40 cm from the source when encountering the shifting wind-field had a greater than 50% chance of maintaining contact with the plume and following it over with no episodes of casting flight (Table 1).

The tracks of males that lost the plume appeared to change from their upwind zigzagging form very quickly upon entering clean air, taking up an angle approximately 90° across the windline even as the windline was in the process of shifting (Fig. 3A–C). Concurrently the tracks became progressively wider, and after several reversals the moths began displacing slowly downwind while casting more widely. Even-

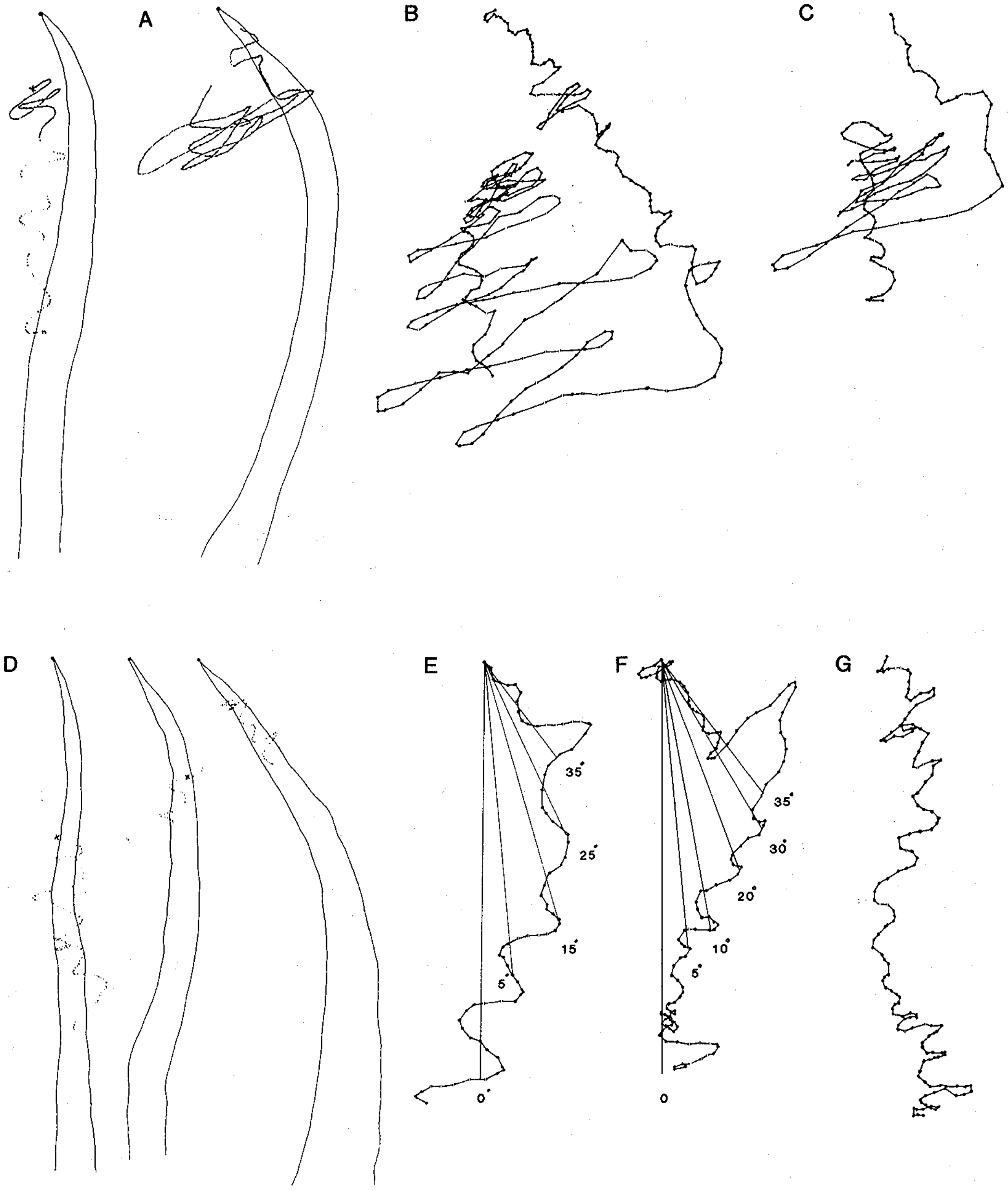

FIG. 3. (A) Top view of the flight track of a male zigzagging upwind in the pheromone plume (left) and losing contact with the plume at the point indicated by the beginning of the solid line in the track. The track legs quickly shift to across the new windline following pheromone loss. (Right) Casting flight across the wind eventually widens and takes the male into contact with the plume in its new location, whereupon flight again becomes narrow upwind zigzagging. The plume's approximate, time-averaged envelope as visualized by smoke is drawn at time X in tracks. Dots indicate male's position each 1/60 s. (B, C) Other tracks of males losing plume completely, plume position not shown. Dots indicate males' position every 1/30 s. (D) Top view of male flight track with pheromone plume's approximate position drawn at time X on track, as in (A), except this male follows plume across during shift, with sawtoothed track shape (middle). Dots indicate male's position each 1/60 s. (E–G) other tracks of males following plume across, with shifting windline positions drawn at various times along the sawtoothed track in (E) and (F). Dots indicate males' positions every 1/30 s.

TABLE 1. Effects of distance from the pheromone source when males encountered shifting wind, and the speed of the wind-shift upon males' success in moving across with the plume without casting for four or more consecutive reversals.

Distance from source (cm)	Males (%) moving across with the plume [(*n*)= number tested]		
	35°/s*	17.5°/s	8.8°/s
0–20	88.2 (17)	90.0 (11)	83.3 (6)
20–40	47.4 (19)	60.0 (25)	73.3 (15)
40–60	42.9 (14)	25.0 (24)	54.6 (11)
60–80	37.5 (8)	30.0 (10)	0 (8)
80–100	0 (6)	0 (5)	0 (1)
100–120	–	0 (2)	–

*Speed of wind-shift.

tually, a right-hand track-leg intersected the new position of the plume enough to bring the male into contact with pheromone, and the males' tracks again reverted to upwind zigzagging in the plume. The change back to narrow zigzagging seemed to take place very quickly, often during the next (left-hand) leg (in <0.15 s), and never later than the next right-hand leg. The distance from the source at which contact with the plume was regained was, on average, significantly greater than when it was lost, especially if casting was of longer duration, allowing greater downwind displacment. Males lost the plume at a mean of 52.2 cm from the source (±20.9 SD; n=87), and regained contact with it at 65.0 cm (±26.3 SD) (P<0.001, paired *t*-test).

The tracks of males that followed the plume across appeared to have a peculiar asymmetry during the shift (Fig. 3D middle). They continued to be of generally narrow, zigzagging form, but they were never positioned over the right-hand edge of the smoke plume, being always either over the centre or at the left-hand edge of the smoke. The right-hand legs, taking the male in the same direction as the shifting plume, appeared to be longer in distance and faster in groundspeed than left-handed legs in which the male was moving away from pheromone. The superficial impression was that the males were sensing the direction of the loss of the pheromone plume and making longer and faster turns to the right, back toward it, to successfully regain it each time. This impression was reinforced when the wind direction was taken into account for each track-leg during the shift (Fig. 3E–G), using the fact that the smoke (and hence pheromone) always moved away from the source in a straight line (Fig. 2). Because the males were never far from the edge of the smoke plume, their tracks relative to the windline could be accurately determined (Fig. 3E, F). The track angles could now be clearly seen to have an asymmetry that was not as apparent before. Thus, the males now appeared also to be *steering* more directly toward the location of the lost plume, more across the windline.

An alternative explanation was that males were manoeuvring by means of the known, integrated systems of optomotor anemotaxis and the self-steered counterturning programme. The reaction-time of the systems to pheromone on and pheromone off, however, would need to be fast enough (less than *c*. 0.15 s) to account for track differences occurring between successive legs, which would be faster than had been calculated before for this species.

Manoeuvres used by males: evidence from track analysis using the triangle of velocities

After subjecting the straight legs of each male's flight track to analysis using the triangle of velocities (Kennedy, 1940; Marsh *et al.*, 1978), the males' course angles and airspeeds, i.e. what they were actually *doing* in reaction to the shifting wind and pheromone, could be calculated. It is clear, both for males that completely lost the plume *and* for those that followed the plume over, that the males altered their course angles very quickly and significantly in response to both pheromone on and pheromone off, whereas at first they hardly altered their airspeeds at all (Fig. 4A, B). Within one (<0.15 s), or at most one and a half legs after last possible contact with pheromone (<0.22 s), males completely losing the plume changed their course angles to a second direction, one more across the windline. The same males, within only *one* leg after re-contacting the plume, significantly altered their courses back to the more

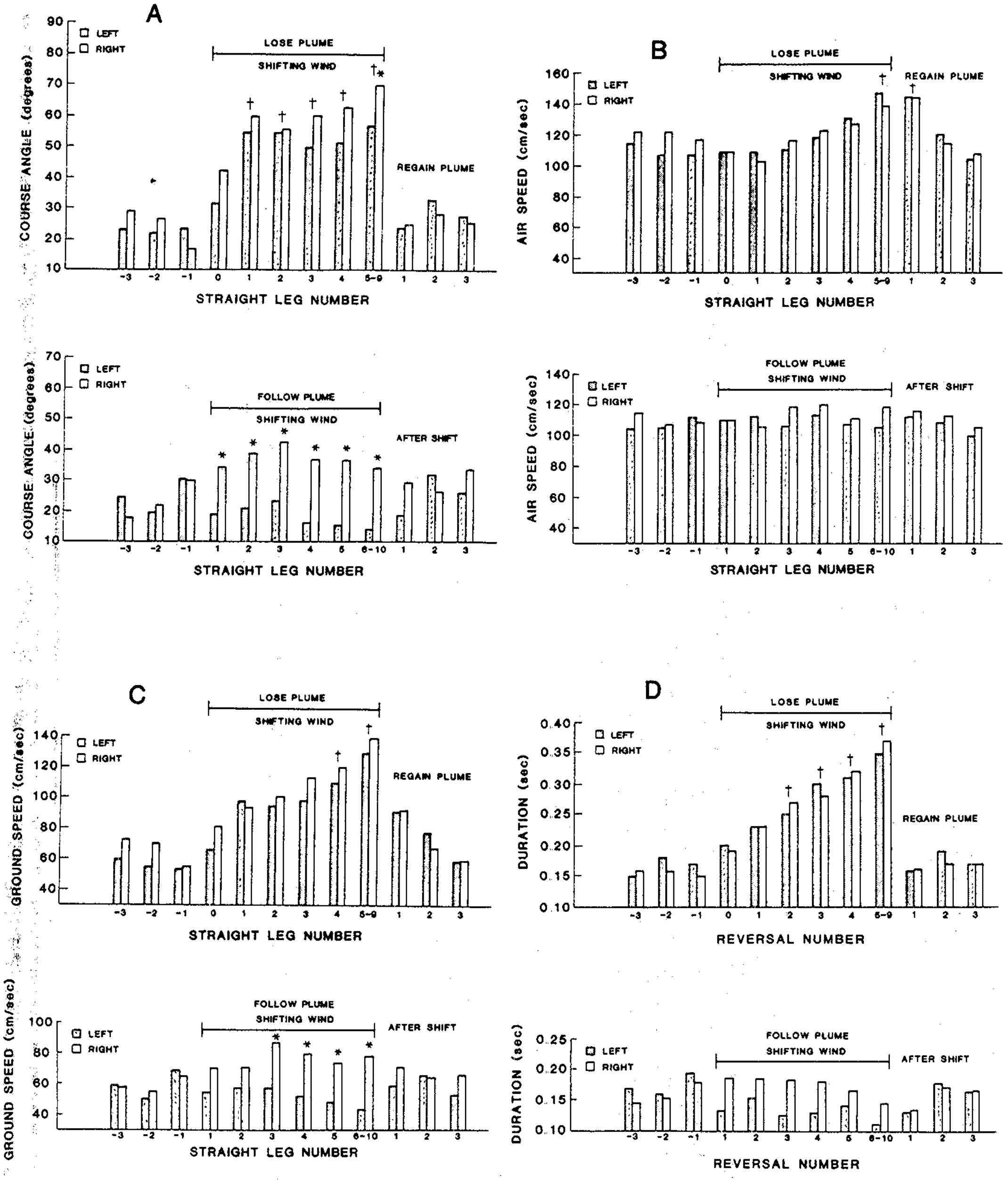

FIG. 4. The mean course angles (A), airspeeds (B), groundspeeds (C) and reversal durations (D) of twenty males that lost the plume completely (top, A–D), and of sixteen males that followed the plume across (bottom, A–D) during wind-shift. Shaded bars are during left-hand legs, white bars right-hand legs at the indicated straight leg (reversal) number before or after losing the plume and after regaining it (top), or before, during, and after the wind-shift (bottom). Asterisk denotes a significant difference between left and right within a pair of track legs: cross denotes a pair of left–right values that is significantly different from pairs occurring before loss of pheromone in (A)–(D), from pairs occurring after regaining plume in (A) and (D), and from pairs in legs 2 and 3 after regaining pheromone in (B) and (C) (Duncan's multiple range test, $P<0.05$).

directly upwind direction, now along the new, shifted windline and plume position (Fig. 4A, top). Their airspeeds, on the other hand, having only gradually increased after plume loss, only gradually decreased upon regaining the plume (Fig. 4B, top). The gradual increase in airspeed by males during flight in clean air caused their groundspeed to increase significantly when coupled with the constant course angle over that same period of clean-air flight (Fig. 4C, top). Because males did not react quickly to reduce their high airspeeds upon re-contacting the plume, their groundspeeds only slowly returned to their previous, in-plume levels despite the course steered more directly upwind upon re-contact (Fig. 4C, top). The result of this was an upwind surge (Kennedy *et al.*, 1980, 1981) during the first legs after re-contact (Fig. 3A–C).

The sawtoothed, asymmetrical tracks of males following the plume over were generated because the males made significant and rapid adjustments of their course angles (Fig. 4A, bottom) and not their airspeeds (Fig. 4B, bottom). Following each contact with pheromone (in <*c.* 0.12 s) they steered a course more directly upwind (left-hand legs, Fig. 4A, bottom), and following each excursion into clean air (within *c.* 0.18 s) they steered more across the windline (right-hand legs, Fig. 4A, bottom). Because the males turned more directly upwind after each pheromone contact without increasing their airspeed, the result was a slower groundspeed to the left along the sawtooth portion of the track (Fig. 4C, bottom). Because they steered a more cross-wind course without simultaneously adjusting their airspeed after each excursion into clean air, the result was a faster groundspeed for each right-hand leg. The assumption here of contact and loss of pheromone is on a probabilistic basis, since the smoke can only indicate where contact is *possible*, due to the filamentous nature of these plumes. Nevertheless, the significant symmetries of the course angles of males maintaining contact with the plume in shifting wind, sorted left and right through time in this way, *must have been due to pheromone contact and loss, not to the shifting wind field itself,* because no such left–right asymmetries existed through time in the course angles of males experiencing *uniformly clean*, shifting wind (Fig. 4A, top). Likewise, the asymmetries were not due to the pheromone plume itself since they did not exist in the course angles of males flying in the plume either before the wind field shifted or after the shift was completed (Fig. 4A, top and bottom). Here, the probabilities of contact and loss were equally likely in the left–right directions.

The asymmetries in the sawtoothed, wind-shifting portion of the track were aided by slight left–right differences in the self-steered counterturning programme. Each contact with pheromone seemed to re-set the frequency of reversals to its highest observed value (duration of each inter-reversal leg is shortest to the left; Fig. 4D, bottom), whereas each flight into clean air began causing the frequency to fall (duration is longer to the right; Fig. 4D, bottom). This change in the counterturning programme was not peculiar to those males following the plume over, because it was observed in males that lost the plume completely and then regained it (Fig. 4D, top). Complete loss of the plume was followed by a lengthening of the interval between reversals, whereas re-contact immediately shortened the interval, resetting the programme to its highest frequency. It does appear, however, that the lengthening of the interval in clean air may have been a more gradual reaction than the shortening of the interval after re-contact. Nevertheless, the asymmetries in the expression of the counterturning programme of males following the shifting plume were not caused by the shifting wind itself, because no such asymmetries existed during flight in shifting, *uniformly clean* air (Fig. 4D, top). They must have been caused by repeated loss of and re-contact with pheromone, because flight in a plume that was not shifting, and where contact and loss were equally likely in the left and right directions, did not result in such asymmetries (Fig. 4D, top and bottom).

Further evidence both for the quick reaction to pheromone on and off, *and* for the separate self-steered and wind-steered systems, comes from analysing a previous experiment but now using the triangle of velocities on individual straight legs of the tracks. Male *G. molesta* flying along side corridors of uniformly clean air and uniform pheromone clouds zigzagged along the boundary between them to the upwind end of the tunnel (Willis & Baker, 1984). When the legs are now segregated into those that began with a reversal inside the pheromone cloud, heading out of it, and those that began with a reversal in clean air, heading back into the cloud, significant asymmetries in track angle appear (Fig. 5).

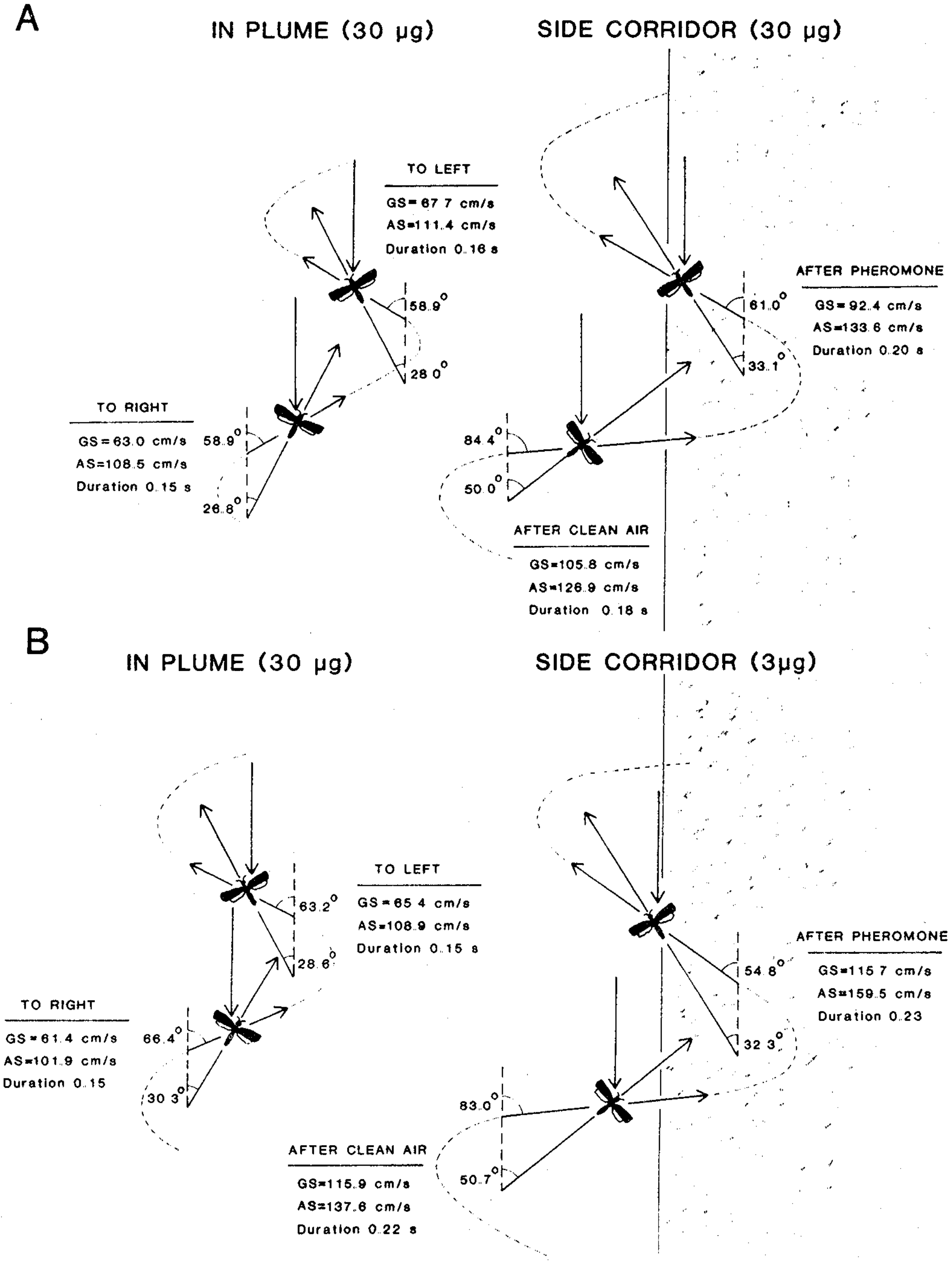

FIG. 5. (A) The mean course (depicted here along the moth's heading) and track angles of males flying upwind in a pheromone plume from a 30 μg source (left) and, moments later following casting flight after plume removal, zigzagging along the edge of a side corridor cloud of pheromone generated from a grid of 30 μg sources (right). Males steered a course significantly more directly upwind following contact with the cloud ($P<0.05$) than after contact with clean air to produce an asymmetry in track angles along the corridor but not when in the plume, where the left–right course angles were not different. AS=airspeed, GS=groundspeed, D=duration, W=wind velocity vectors. ($n=11$ males along the corridor, and 7 males in the plume). (B) Same as in (A), except side corridor cloud is created by a grid of 3 μg sources. Original tracks and data from Willis & Baker (1984).

The tracks of these same males in a point-source plume adjacent to the cloud just moments before flight along edge of the side corridor do not exhibit these track angle asymmetries (Fig. 5). After further analysis using the triangle of velocities, it is clear that the males zigzagging along the edge of the corridor steered a course more directly upwind after each entry into pheromone and steered a course more across the wind after each excursion into clean air. This resulted in the differences in the resultant track. No differences in the intervals between reversals were observed here, perhaps because re-contact with the pheromone corridor always reset the programme back to its higher frequency before any lengthening of the interval could be observed. The steering of a different course, therefore, was observed without a difference in inter-reversal duration, which again is evidence for the wind-steered and self-steered systems operating independently. Moreover, the re-analysis of these tracks showed that males could alter their steered courses very quickly, at the very first reversal (within *c.* 0.2 s) after experiencing pheromone or clean air.

Thus, the evidence from these experiments indicates that the combination of self-steered counterturning with optomotor anemotaxis, coupled with quicker reaction time to pheromone loss and contact than previously calculated for this species, can account for the flight tracks observed in these experiments. But what feedback is used by the males in executing these manoeuvres? Of course optomotor reactions must be used to make progress up the windline, but what of the changes in the course that result in the different tracks after different pheromone experiences? Are these direct chemotactic steering reactions to the chemical stimulus gradient (Bell & Tobin, 1982) or are they indirect reactions involving visual feedback?

Possible feedback used by males

First, the behaviour of males losing the plume completely in the shifting wind (Figs. 3A–C and 4) rules out chemotactic steering by the gradient by males successfully following the plume over. The course angles steered by males on the first leg occurring completely out of contact with pheromone (straight leg number 1 under 'lose plume' portions of Fig. 4) were equally large to the right and to the left, even though the plume was moving away from the males toward the right. For these males, the magnitude and direction of the displacement did not happen to be enough to take the male immediately back into contact with pheromone, and so a similarly fast and long-duration movement occurred in the direction *away* from the plume, followed by increasingly long casts symmetrically left and right. This is hardly what would be expected of chemotaxis, but is what would be expected of a pre-programmed counterturning system. The course angle reached its maximum on the second leg following pheromone loss, and these and subsequent legs again exhibited no significant left–right asymmetry. This change in course angle in clean air compared to those in a plume was clearly due to the time elapsed since loss of pheromone and not to the plume's actual location to the right of the males.

Thus, for the males that followed the plume over, the course angles of all the legs steered more across the wind to the right toward the plume are to be expected as a part of their programmed steering change with pheromone loss. In this special group of males, though, the first adjustment happened to take them back into contact with pheromone, after which they steered a more upwind course again, as per the programme. The reiterative contact with and loss of pheromone in this group allowed us to segregate the movements according to short-term pheromone experience and allowed us to inspect them in a way not possible for movements in a plume. The artificial situation of the side-corridor cloud of pheromone also allowed us to segregate movements according to short-term experience. The right-biased displacement along the shifting plume was due to a reiterative resetting of the course angle with each exposure to pheromone or clean air according to a predetermined programme.

Of the two reactions available to the moths (Marsh *et al.*, 1978), the change in course was more closely correlated with changes in pheromone stimulation than was airspeed (Fig. 4A, B). The change in course was not haphazard, but rather the angles appeared to have two states, producing two resultant track angles (Fig. 6D). The more slowly-changing airspeed reaction, expressed only in those males losing the plume completely (Fig. 4B), appeared to produce changes in groundspeeds which lagged behind the changes in stimulation and course angles

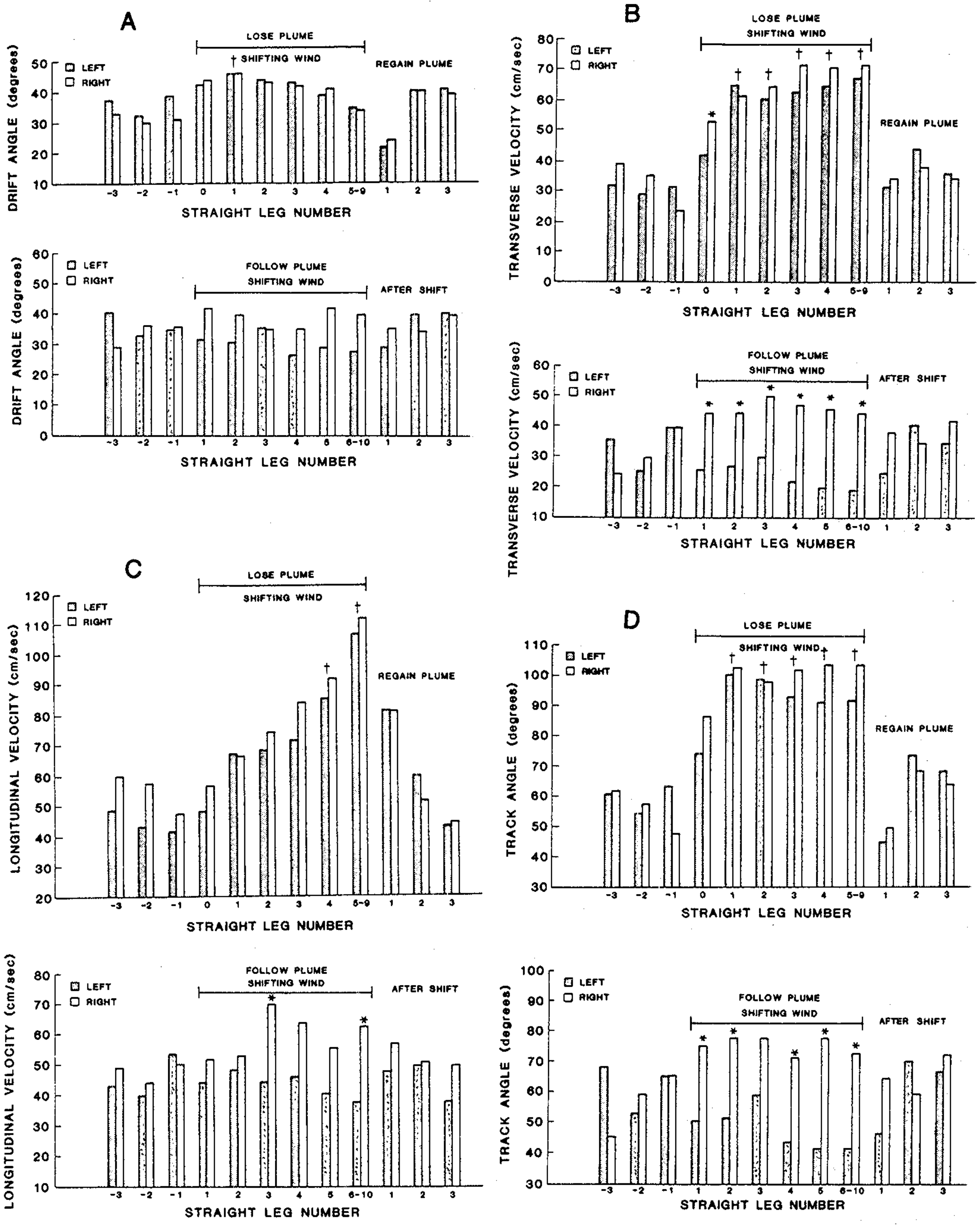

FIG. 6. The mean drift angles (A), transverse component of movement (B), longitudinal component of movement (C), and track angles (D) of the same twenty males that lost plume completely (top, A–D) and the sixteen males that followed the plume across (bottom, A–D) in Fig. 4. Shaded bars are during left-hand legs, white bars right-hand legs at the indicated straight leg (reversal) number before or after losing the plume and after regaining it (top), or before, during and after the wind-shift (bottom). Asterisk denotes significant difference between left and right within a pair of legs; cross denotes pair of left–right values that is significantly different from pairs occurring before plume loss in (B)–(D), from pairs occurring after regaining plume in (B) and (D), from number 2 and 3 legs after regaining plume in (C), and from the first leg after regaining plume in (A) (Duncan's multiple range test; $P<0.05$).

(Fig. 4C). There were also quicker changes in groundspeeds linked to course angle changes alone as discussed above. The type of visual feedback necessary to produce these tracks is not known, but it is possible that males could measure their course angles by using feedback to locate the windline as they turn through it, and then measure the degrees turned past this point (Marsh *et al.*, 1978). Course angle information alone could result in predictable changes of track angles in different windspeeds, but for some moths it is clear that a constant track angle is maintained despite windspeed changes (Marsh *et al.*, 1978) and so more than course angle must be assessed by males to fly a particular track angle. If they could also measure their drift angle, perhaps by the angle of image movement across their body axis, then they could add this angle to the course angle to obtain the track angle (Marsh *et al.*, 1978). Even though the drift angles did not vary much under different pheromone stimulation in our experiment (Fig. 6A), they still could have been used by males as information to calculate the track angles. If the males could also measure their airspeed (not needing visual feedback), they could combine this information with the measured course and drift angles to steer the resultant track angle and control their displacement in wind of various speeds and directions (Marsh *et al.*, 1978; Ludlow, 1984).

The virtue of either of these systems is that they would be independent of height of flight. Their deficiency is that they do not take into account the existence of a preferred groundspeed in some moths flying to pheromone (Marsh *et al.*, 1978) and in *Drosophila* flying to host odour (David, 1982, 1986). The preferred speed is one of apparent image motion across the ventral ommatidia, not the absolute groundspeed, which cannot be measured visually without allowing for the height of flight. However, changes in *relative* groundspeed, measured as changes in image velocity at a given height, could possibly be used by males to assess the results of their course and airspeed reactions to changes in pheromone stimulation.

Recently Ludlow (1984) and David (1986) have proposed that flying male moths might break image velocity into its longitudinal (L) and transverse (T) components because neural motion detectors appear to be most sensitive in these two directions. To fly at a particular angle and maintain a given groundspeed at a given height across winds of varying velocities, the males would only have to keep the sum of these values constant, which approximates the square root of the sum of squares (Ludlow, 1984; David, 1986). The latter value, not in image velocity terms but in absolute terms, would be the actual groundspeed. In our study, changes in the transverse component of image movement, here expressed as the transverse component of groundspeed along the track, correlated strongly with pheromone contact and loss (Fig. 6B, top, bottom), and the course angle (Fig. 4A) and groundspeed (Fig. 4C). Thus males allowed groundspeed (Fig. 4C, or perhaps T+L) to increase or decrease quickly mainly by allowing T to reach either of two values following course angle changes. The sign of T (left or right across the longitudinal body axis) also accurately reflects the wind direction. A further change in groundspeed (Fig. 4C, or T+L) appears to occur during casting or plume re-location mainly as a change in L (expressed here as the longitudinal component of groundspeed along the track) that corresponds to the slower-reacting airspeed alterations (Fig. 6C, top, bottom) (Fig. 4B).

Discussion

The previously described, integrated system of a self-steered counterturning programme plus optomotor anemotaxis explains how male *G. molesta* follow a plume over in a shifting wind field. They also explain how males re-locate and advance up the plume lost in the shifting wind, by adjusting their casting flight across the shifting windline and surging up the new windline upon relocating the plume. Males began to change both their course angles with respect to the wind and their reversal frequencies *on the next track leg* (in <*c.* 0.15 s) after losing or recontacting pheromone. Because males following the plume across did not alter their airspeeds as quickly as their course angles, there were left–right asymmetries in the groundspeeds and track angles, resulting in the sawtoothed track. These asymmetries attributable to the anemotactic system were augmented by asymmetries in the self-steered counterturning system. There was a reiterative resetting of the counterturning frequency to a high level to the left following each new contact with pheromone, and a diminution to the right following each excursion away from pheromone. The result was longer, faster displacement more directly toward the plume to the right.

We found no evidence that chemotaxis is used by male *G. molesta* to steer along the shifting plume. Such a mechanism was invoked by Cardé & Charlton (1984) to explain how gypsy moth males maintained contact with and followed a snaking, sinuous plume in a non-shifting wind field. Indeed, the superficial impression given by viewing only the asymmetric sawtoothed tracks of our males was that they detected which way the plume had moved from them and were able to bias their movements in response to the chemical concentration gradient so as to steer chemotactically back to it repeatedly. However, further scrutiny revealed that these asymmetric legs along the shifting plume are due to a preprogrammed reaction to pheromone loss that is prevented from being fully played out by each re-contact with the plume. The non-truncated, more complete version of the programme was observable in males that did not re-locate the plume on their first right-hand leg. These males then made a similar reversal to the left, away from the plume's position. With time, the programme of left–right reversals (casting) performed with increasing airspeeds and durations resulted in displacement wide-enough for the plume to be relocated eventually.

Had our males been using transverse chemo-klinotaxis as proposed by Cardé & Charlton (1984) for gypsy moth males, we should have expected the males to zigzag more symmetrically along the edge of the plume and pay less attention to the windline than they did. While both systems would result in the male maintaining contact with the plume, the manoeuvring mechanisms employed would be entirely different. Only the chemo-klinotactic system would literally involve following the plume. Our males followed programmed steering changes which displaced them over with the plume.

A detailed analysis of the tracks was not performed for the gypsy moth males by Cardé & Charlton (1984), who provided only a single illustrative figure. As pointed out by Kennedy (1986), the moth supposedly following the edge of the plume chemo-klinotactically in this track usually seems to emerge from the plume repeatedly on its windward side, a hint that anemotaxis is a predominant mechanism. If males were following or feeling for the edge by means of klinotaxis as proposed by these authors, then they should be equally likely to follow along either edge, not only the upwind one as appears in this single track. Furthermore, it is impossible to tell exactly what feedback the males were experiencing in the experiment of Cardé & Charlton (1984) because the ground pattern was moved in the downwind direction beneath the male at an unreported speed to keep him in the video camera's field of view. The direction of transverse image drift from the floor would have been opposite to that produced by the wind, and this highly unnatural, conflicting feedback make the manoeuvres made by the male to produce this track nearly impossible to interpret.

The reactions of our moths to changes in pheromone stimulation were faster than we had expected from our previous studies with *G. molesta* (Baker & Kuenen, 1982; Kuenen & Baker, 1983). However, our previous work did not include examination and segregation of individual track legs, or the use of the triangle of velocities method for the individual straight legs in order to discern the short-term steering and thrust changes made by males flying a particular track. The speed of the *G. molesta* course reaction is similar to that of the only other species examined in such detail, *Plodia interpunctella* by Marsh *et al.* (1981). They reported that their males significantly increased their course angle (and the resultant track angle) on the first inter-reversal leg after pheromone loss. No experiments were performed to see how fast the reaction was to pheromone onset, so there is no reaction-time estimate for this. Unfortunately, a time-base error was made in the video-analysis of the tracks, which affected all behaviours calculated using the triangle of velocities in the landmark Marsh *et al.* (1978) paper. No simple linear conversion of the values can be made, and we must wait for a revision in order to compare the absolute values of *P. interpunctella* reaction times to pheromone loss to those of *G. molesta*. The estimates of the point at which males lost pheromone also must be made, but this recalculation was performed with regard to track angle change, and the reaction time compares favourably with that of *G. molesta* (above) (Marsh *et al.*, 1981). It does appear from the *relative* changes reported in their 1978 paper, that their males altered their courses more quickly than they altered airspeed, which is similar to what male *G. molesta* do.

In our experiments, males made no significant advances toward the source during casting after losing the pheromone plume, as was sometimes observed with gypsy moths in the field by David *et al.* (1983). This does not mean that casting does not have this advantage for some species, only that at these windspeeds, pheromone concentrations, and distances from the source, *G. molesta* males did not gain this advantage in casting, unless one considers the sawtoothed tracks as being analogous to the gypsy moths' behaviour. Instead, our males eventually displaced downwind by regressive casting (Kennedy, 1983; Kuenen & Baker, 1982; Von Keyserlingk, 1984) and intersected the plume farther, on average, from the source than the point where they had lost the plume.

In a shifting wind field, the probability that a male will be able to maintain contact with the plume and zigzag along it in sawtooth fashion and progress upwind, or else lose it and revert to casting flight, will be determined by the relationship between the male's zigzagging width, the speed with which this width is increased following pheromone loss, the angular magnitude and velocity of the shift, and most importantly, the distance of the male from the source. The regaining of a lost plume will be faster, the smaller the magnitude of the windshift angle and the closer the male is to the source when he lost the plume, because narrower casting is needed before it is re-intersected.

Finally, no extra, usable cues appeared to be generated by this particular shifting wind field that are not generated by a non-shifting field to tell the males in which direction the plume had gone and in which direction to bias their movements. There was no significant track bias under the conditions of these experiments for males that lost the shifting plume completely, and the bias in the tracks of males that followed the plume over can be explained by the loss of, and regaining of contact with, the pheromone plume. Perhaps gusty or turbulent wind in the field can blow the males in the correct direction over toward the lost plume as suggested by Von Keyserlingk (1984), but such displacements did not occur here.

The track parameters that we visualize easily, including track angle (Fig. 6D), groundspeed (Fig. 4C) and zigzagging width (Fig. 3), are a result of the integration of both the self-steered counterturning programme and optomotor anemotactic systems. Narrow and wide zigzagging (casting) tracks are both somewhat determined by the frequency of the counterturning programme, which is increased by contact with higher concentrations of pheromone and decreased by lower concentrations (Kennedy *et al.*, 1980, 1981; Kuenen & Baker, 1982; Kennedy, 1983). The narrowness is also determined by the course angle adjustments without airspeed adjustments because they determine how much ground is covered per unit time per given reversal frequency. The males set a course angle more directly upwind after pheromone contact and more across-wind after loss. The groundspeed of males heading more upwind in pheromone is slower because males do not increase their airspeed at once and thus slow down relative to the ground. Likewise, groundspeeds of males changing to casting flight after pheromone loss increase because although they change their heading to more cross-wind, they do not reduce their airspeed by thrusting less, and thus cover more ground per unit time.

The surge up the windline upon recontact with pheromone (Kennedy *et al.*, 1980, 1981; Kennedy, 1982) is a result of the quick change in the male's course coupled with a lag in the reduction of the higher airspeed built up over a long period of casting. Such surging has been observed upon pheromone recontact in other experiments and in other species (Kennedy *et al.*, 1980, 1981; Kennedy, 1982, 1983; Willis & Baker, 1984; Von Keyserlingk, 1984), but may be peculiar to the transition between high-speed casting and upwind zigzagging. Continuous upwind progress in a plume appears slower than this initial surge, perhaps due to the short-term, repeated contacts with pheromone filaments keeping the airspeed low. More work is needed in which track legs can be segregated precisely according to short-term pheromone experience. Then we will see whether an average track as traditionally depicted during upwind zigzagging is really due to a male quickly averaging his movements between nearly straight upwind and crosswind resultants as hypothesized by Kennedy & Marsh (1974), or is an artefact in which our *measurements* of his movements are averaged due to our ignorance of his state of pheromone stimulation.

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