Physiological Entomology (1991) **16**, 497–506

The effects of unilateral antennectomy on the flight behaviour of male *Heliothis virescens* in a pheromone plume

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Abstract. Unilaterally antennectomized *Heliothis virescens* (F.) males flying close to the central axis of a plume of sex pheromone display no significant differences in behaviour compared to sham-operated males in course angles, track angles, airspeed and groundspeed. This demonstrates that right/left antennal information is not necessary for normal orientation movements in response to pheromone, but rather that it is 'blended' within the moth's central nervous system before pheromone-mediated manoeuvres are made. However, some unilaterally antennectomized moths (36%) make repetitive, asymmetrical, saw-tooth-shaped tracks during pheromone-mediated upwind progress, whereas control moths never make such tracks. Unilaterally antennectomized moths made such tracks on the side of the plume contralateral to the missing antenna. We hypothesize that these occasional asymmetrical tracks in unilaterally ablated males are the result of reiterative asymmetrical pheromone stimulation of a higher probability on track legs going toward rather than away from the long axis of the plume on males with a single antenna remaining on the 'away from axis' side. Combined with a greater propensity for treated moths to lock onto the plume away from the central axis on one side rather than the other, repetitive successive asymmetrical track legs (resulting in a saw-tooth-shaped track) are commonly observed in these moths. Control moths do also make asymmetric successive track legs but they rarely are repeated and thus are not readily observed.

Key words. Flight behaviour, antennectomy, *Heliothis virescens*, Lepidoptera, Noctuidae, sex pheromone.

Introduction

A male moth located downwind of a female will respond to the blend of her pheromone components by both reiteratively flying upwind upon encountering pheromone and entering into casting flight in clean air (Kennedy & Marsh, 1974; Marsh *et al.*, 1978). Both upwind flight and casting are complex behavioural processes achieved in part by a feedback mechanism known as optomotor anemotaxis (Kennedy, 1940), whereby the male visually gauges its wind-induced drift and makes appropriate compensatory movements. A second, endogenous

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programme of reversals (counterturns) is integrated with optomotor anemotaxis during casting flight (Kennedy, 1983; Baker, 1985; Baker & Haynes, 1987; Baker, 1990). These movements result in a net displacement of the male in the upwind direction, i.e. towards the female (David & Birch, 1989; David *et al.*, 1983).

Unilateral antennal ablation has been used previously to learn more about how insects orient to odours, including pheromones. However, this approach for male moths responding to sex pheromone has heretofore only received anecdotal treatment in the literature. Kennedy & Marsh (1974) noted that ten unilaterally antennectomized *Plodia interpunctella* zigzagged upwind to a calling female 'in a manner indistinguishable from that of intact males'. In contrast, Niehaus (1981) found that progressive amputation of the antennal flagellum reduced flight ability in the small tortoise-shell, *Aglais urticae*. Although this technique has been used quantitatively for walking insects (see Bell & Tobin, 1982; Bell, 1984, for reviews) and insects which normally fly being forced to walk (Martin, 1964; Shorey & Farkas, 1973; Preiss & Kramer, 1984), our approach was to use free-flying insects. In the present study a quantitative experimental comparison of the behaviour of antennectomized moths with control, sham-operated moths was facilitated by video-recording their respective flight tracks. The goals of this investigation were two-fold: to determine whether moths retaining only one intact antenna could orient in a synthetic pheromone plume and, using observation and analysis of the flight tracks of these moths, be they normal or aberrant, to determine whether any inferences might be made about the neurological basis of pheromone reception and its relationship to final behaviour.

Methods and Materials

Larvae of *Heliothis virescens* (F.) were reared from eggs on a modified pinto bean diet (Shorey & Hale, 1965). At pupation, moths were sexed and the males were isolated in large cages in a pressure-positive environmental chamber (L:D 14:10 h, 25°C) to prevent undesirable pre-exposure to the female pheromone. Males were aged on a daily basis. An 8% sucrose solution was available to the adult males.

Fine, surgical dissecting scissors were used to remove either the left or right antennal flagellum of the 1-day-old males. The scape and pedicel at the very base of the antenna were left intact. Control moths were sham-operated by holding them in the same manner as the treated moths and placing one of their antennae between the blades of the dissecting scissors. The cohort of operated and control moths was then placed in a separate cage from the pupal emergence cage and returned to the environmental chamber until old enough for their flight behaviour to be tested (5–6 days old).

Following a recovery period of 48 h, both the operated and sham-operated moths, still in their large retaining cage, were pre-exposed to the synthetic *H. virescens* female sex pheromone. Rust *et al.* (1976) had found that cockroaches adjusted to antennectomy over time, and pre-exposing *H. virescens* males to pheromone was designed to help them adjust. The flight behaviour of the moths was not affected by this procedure (Vickers & Baker, unpublished observations). The pheromone was a synthetic mixture of six of the seven compounds found in the female sex-pheromone gland (Teal *et al.*, 1986) and known to be behaviourally active (Vetter & Baker, 1983). The pheromone was loaded onto a rubber septum (A. H. Thomas Co., No. 8753 D22, sleeve type, 5 × 9 mm), which was placed on a platform at the upwind end of the wind-tunnel. The wind-tunnel has been described in detail previously (Kuenen & Baker, 1982). The moths were removed from their environmental chamber and taken to the tunnel where they were left to acclimate for 1 h to the ambient conditions (24–26°C, 60% r.h., 0.5 lux, 0.5 m/s wind velocity). Pre-exposure to the pheromone was a simple matter of placing the retaining cage in the path of the pheromone plume at the downwind end of the wind-tunnel. The cage was left in the path of the plume for 3 min and then placed at the side of the wind-tunnel, such that it was out of the plume but still in the air-stream, for a further 10 min. This pre-exposure regime was repeated three times. After the final exposure the cage was left in the wind-stream for 15 min (pheromone source removed) before being returned to the environmental chamber. Pre-exposure occurred only on 3-day-old males during the fifth to eighth hours of scotophase, when the males are behaviourally most responsive to female-emitted pheromone (Vetter & Baker, 1983).

Just before scotophase on the fifth day, the moths were removed from the retaining cage and placed in individual 6 cm long × 6 cm diameter cylindrical screen flight cages (Vetter & Baker, 1983). The cages were placed on plastic trays such that there was one tray for each of the three treatments. The trays were returned to the environmental chamber until the fourth hour of scotophase whereupon they were removed and taken to the wind-tunnel. As before, the moths were given 1 h to acclimate before the experiment commenced. Moths were allowed to fly in response to the same rubber septum that they had previously been exposed to. Care was taken to position the septum, its platform, and the moth release point in exactly the same position for each replicate of the experiment.

Flight tracks of the moths were recorded from above the wind-tunnel using a Sony RSC 1050 rotary shutter camera. The camera was located about half-way along the wind-tunnel and the field of view encompassed *c.* 1 m of the wind-tunnel length and *c.* 70 cm of its 1 m width. Video tapes of the flight tracks were later analysed by replay on a Sony SLO 340 VCR and frame by frame on a Sony SVM 1010 motion analyser. The position of the moth every 1/30th of a second was input into a Radio Shack computer by means of a Radio Shack X/Y digitizer. Plots of the moth tracks were then utilized and the apices of turns (reversals) identified by eye. A representative portion of each inter-reversal track leg was selected (at least two vectors, and often more than five vectors, in length) and the track angle with respect to the wind direction was measured. The length of the consecutive vectors was measured and the number of vectors noted to ascertain groundspeed. These two measurements together with the wind speed and a scale conversion factor were input into a special program which works on the basis of the triangle of velocities (Marsh *et al.*, 1978) to calculate the true behavioural responses of the moths, course angle and airspeed plus the groundspeed for each track leg. Means of these variables were compared using Duncan's multiple range test (Duncan, 1955).

Results

Of the control moths released, 43% ($n = 56$) flew through the field of view compared with 23% ($n = 83$) of the left ablated and 18% ($n = 72$) of the right ablated. It was also apparent that some of the ablated moths (twelve out of thirty-three tracks) flew in a different manner from the control moths to the extent that simply by looking at the video or the plotted tracks one could tell which antenna of the moth had been removed. The control males appeared to restrict their movements to a narrower corridor of the field of view, whereas the antennectomized moths sometimes strayed out to one side or the other: unilaterally antennectomized moths tended to fly up the side of the plume contralateral to the missing antenna.

To quantify and analyse this difference between the treatments, the field of view was notionally divided up into three regions, centred on the mid-line of the time-averaged plume. It became clear that there was often a difference in the type of flight track generated (Fig. 1). The control moths either flew relatively straight up the central zone (Fig. 1A) or showed a symmetry of track angle for each leg on either side of the wind-line (Fig. 1D). Ablated moths made asymmetrical, saw-tooth-shaped tracks on the side of the plume away from the intact antenna. That is to say left-ablated moths made these tracks on the right side of the plume (Figs 1G and 1H) and right-ablated moths made these tracks on the plume's left side (Figs 1E and 1F). As Figs 1F–H show, males did not always restrict their activity to one side of the plume or the other. However, the repetitive, asymmetrical tracks and turns conferring the overall saw-toothed track shape always occurred towards the side of the plume contralateral to the ablated antenna. Importantly, when the ablated moths flew up the opposite side of the plume (i.e. with the intact antenna closer to the plume) or up the centre line of the plume, their tracks appeared to be no different from those of the control males (Figs 1B and 1C). When the control moths made an excursion into the outer zones they did not produce these consistently asymmetrical tracks (Fig. 1D) although individual pairs of track legs could be found that had such an asymmetry.

Each inter-reversal track leg was placed in one of three categories reflecting the displacement of the moth (Fig. 2): remaining in the central zone; flying from the central zone to one of the outer two zones; and flying from one of

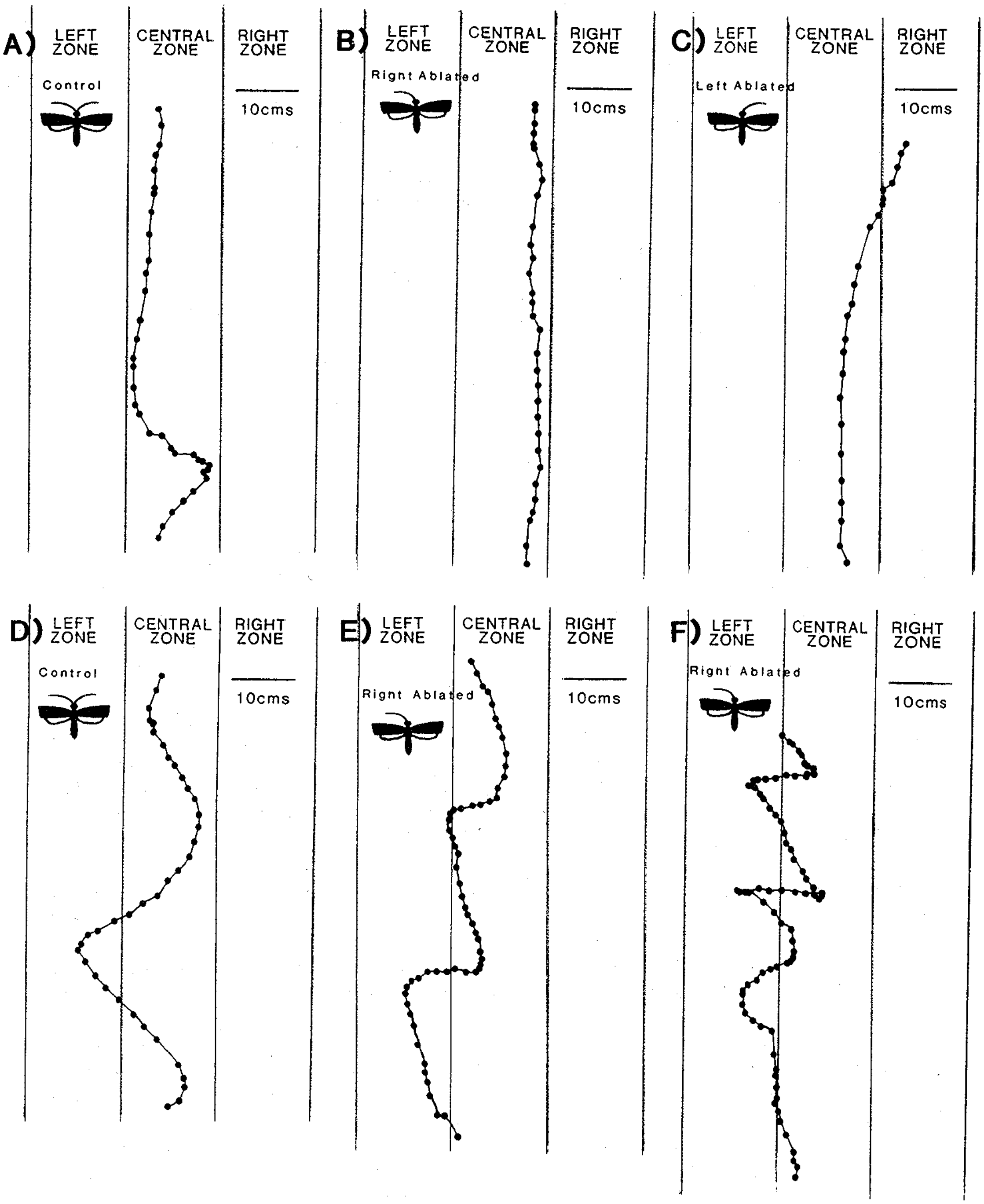

Fig. 1. A selection of tracks made by control and unilaterally antennectomized moths. The field of view is divided into three areas. The central zone is envisioned as the area where the moths have the greatest possibility of reiterative pheromone stimulation, and is centred on the plume mid-line. The top row of tracks (A), (B) and (C) show that ablated moths could appear to behave normally when compared with the control if their activity was restricted to the central zone. Asymmetrical, saw-tooth-shaped tracks resulted when the ablated moths flew out of the central zone (E–H). Right-ablated moths made these tracks on one side of the plume (left) (E) and (F), left-ablated moths on the other (right) (G) and (H). Control moths did not appear to make these repetitively asymmetrical tracks (D). Dots on the tracks represent the position of the moth every 1/30th of a second. The wind direction is from the top of the page at 50 cm/s.

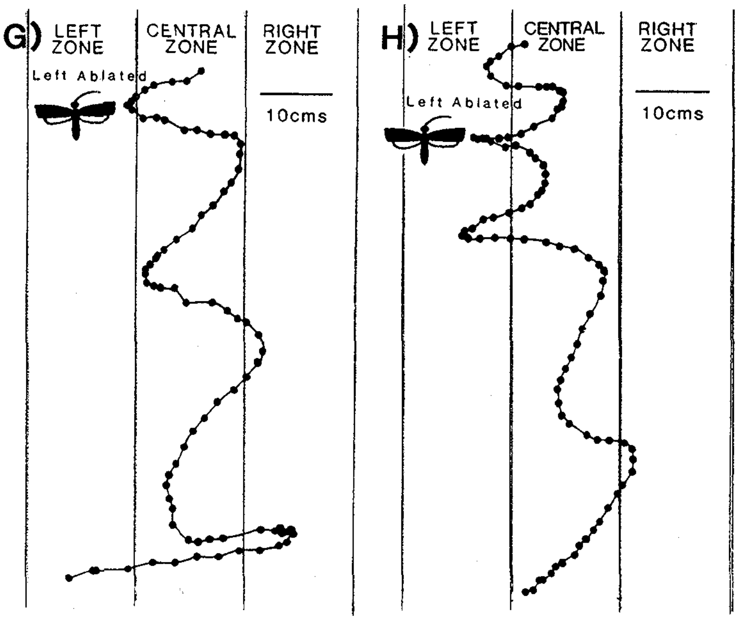

Fig. 1 *(continued)*.

the outer two zones back into the central zone. Ablated moths flying in the central area did not behave significantly differently from the control group. All of the four measured variables, course angle, airspeed, track angle and groundspeed exhibited no difference between treatments (Fig. 2A). Therefore, in the central zone, where the likelihood of repeated contact with odour filaments of the plume is greatest, the behaviour of the moths is the same.

Track legs resulting in displacement of the moth from the centre to the outer zone reveal some significant differences in behaviour. For the control moths there is no difference in the track angle be it a displacement to the left or the right. However, both of the treated groups showed a difference when excursions to the left or right occur. There was a difference between a male having its intact antenna 'close to the plume axis' or 'away from the plume axis'. For instance the mean track angles to the left in 'antenna-close' moths with their left antenna ablated, 77.7° (± 24.2 SD) were not statistically different from right-ablated 'antenna-close' going right, 67.4° (± 46.4 SD). Likewise with the 'antenna-away', there was no difference: right track angles for left ablated moths: 35.3° (± 22.4 SD) and left track angles for right ablated moths 40.4° (± 30.2 SD). Significant differences exist between the track angles for these 'antenna-close' and 'away' groups (Fig. 2B), although neither group differs significantly from the controls when left and right track angles are considered separately.

In Fig. 2B a comparison of the course angles, for all three treatments (left-, right-ablated, and control), of left and right tracks revealed that the differences observed in track angle were the result of changes in course angle and were not reflected by differences in airspeed. In Fig. 2C, for males returning from cleaner air back into pheromone-laden air, there were no consistent differences between controls and the ablated groups or between the 'antenna-close' and 'antenna-away' groups. This suggests that the onset of casting behaviour in such moths having been in cleaner air was unaffected by the absence of one antenna. The one exception to this explanation is the left track angle of the right-ablated moths (50.9° ± 27.1 SD). This may be due to the smaller number of track legs in this 'From outer zone to centre' group ($n = 9$) compared with other treatments in the same category. The difference in track angle here was apparently due to a combination of significant differences in both course angle and airspeed

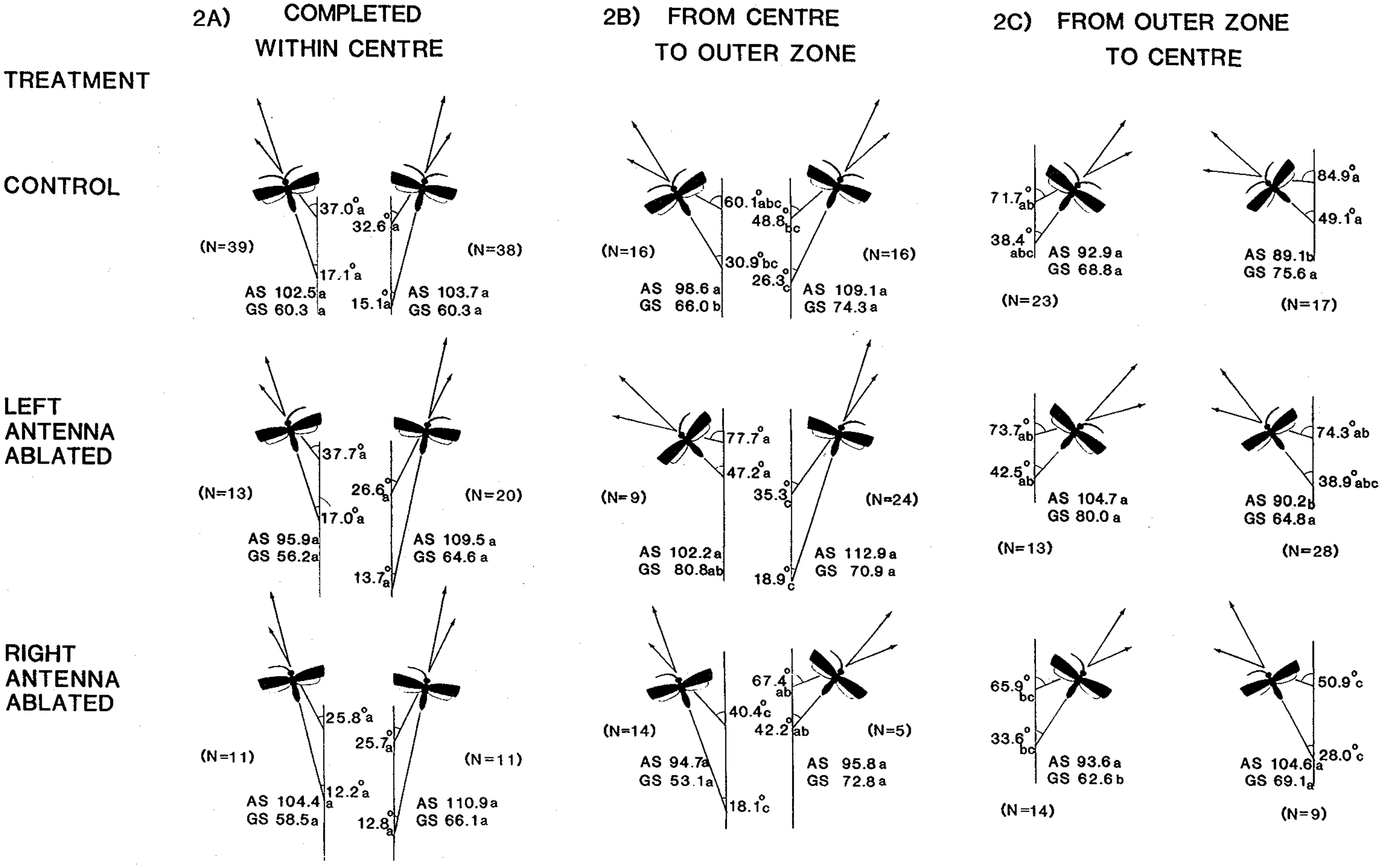

Fig. 2. Analysis of the course angle, track angle, airspeed (AS) and groundspeed (GS) (units of AS and GS are cm s^{-1}) in relation to the displacement of the moth along that track leg. Wind speed is from the top of the page at 50 cm/s. Course angles and track angles are compared for left and right track legs within each track leg grouping. Airspeed and groundspeed are also compared within each track leg grouping but left legs are considered separately from right legs. Means followed by the same letter are not significantly different ($P > 0.05$) according to Duncan's new multiple range test. N refers to the number of track legs analysed for that particular treatment.

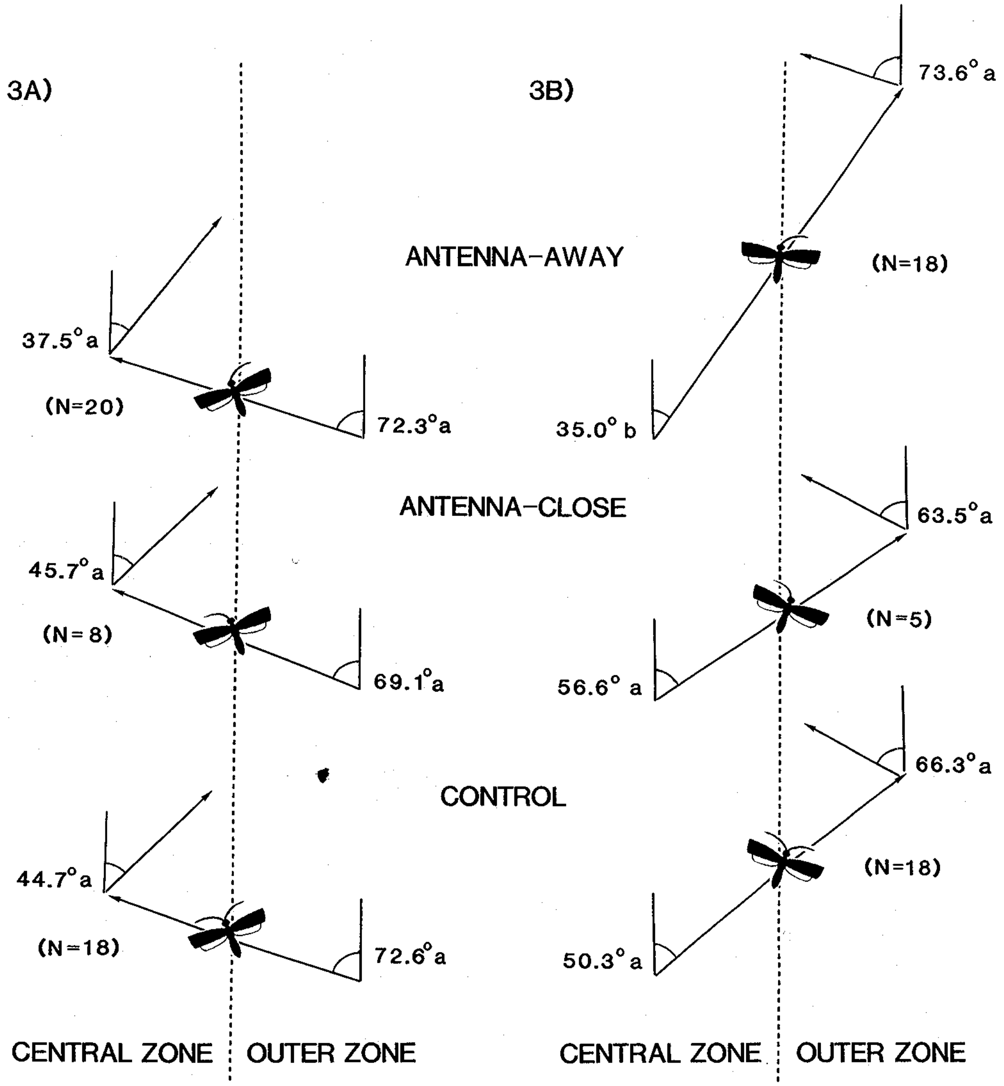

Fig. 3. Analysis of pairs of consecutive track angles, showing that there was no significant difference between treatments control, 'antenna-close', and 'antenna-away' when considering the track leg before and after entering the central zone (Fig. 3A). A slight difference in behaviour existed when an 'antenna-away' moth flew out of the central zone compared to 'antenna-close' and controls but the cast across the wind-line following that displacement was not different between the three groups. Track angles in columns followed by the same letter are not significantly different according to Duncan's new multiple range test ($P > 0.05$). Wind speed is 50 cm/s and the upwind direction is the top of the page. N is the number of paired track legs for each treatment.

as compared with the control moths. Overall though, despite appearances to the contrary, the ablated moths' behaviour is very similar to that of the controls.

To dissect the behavioural responses further, pairs of consecutive track legs were analysed. We compared the average successive legs that directly corresponded to the rapid changes in

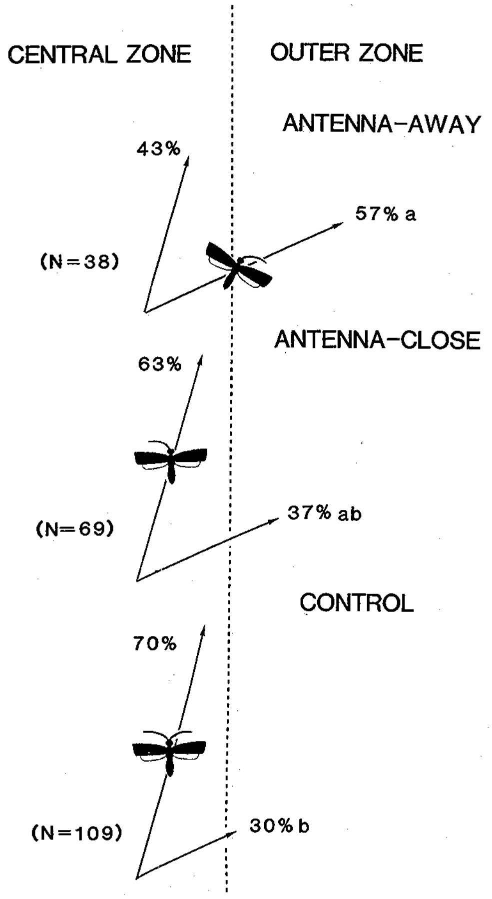

Fig. 4. Percentage of tracks originating in the central zone leading to deviation to an outer zone is significantly greater in 'antenna-away' moths than in control moths. 'Antenna-close' moths fall somewhere in between but are closer to the control situation. Percentages followed by the same letter are not significantly different ($P > 0.05$) according to an adjusted χ^2 (2 × 2) test of independence. N is the total number of track legs for each treatment.

direction that must have been responsible for the asymmetrical (or symmetrical) tracks. Because there were no actual behavioural differences in the central strip the track legs were combined in a manner that allowed them to be labelled either 'intact antenna close' or 'intact antenna away' from the plume axis.

There were very few differences between the control moths and those in the two treated groups, confirming that the behaviour of the ablated moths was similar to that of the controls (Fig. 3A). However, there was one significant difference: when the intact antenna of an ablated moth was away from the plume axis the moths had a track angle that was significantly closer to the wind-line (35° ± 15.3 SD *v.* 50.4° ± 27.7 SD control and 56.6° ± 32.7 SD 'antenna-close') (Fig. 3B). A track leg this close to the wind-line was more normal of moths flying in the central zone (compare with Fig. 2A: track leg completed within centre) yet these moths continued into the outer zone where their next turn would be a casting one, across the wind-line (73.6° ± 16.3 SD) which was no different from the controls (66.3° ± 16.4 SD) and the 'antenna-close' group (63.6° ± 34.0 SD).

A track leg originating in the central zone for moths with their intact antenna away led to a displacement into the outer zone significantly more often when compared with the intact control moths (Fig. 4). It is not, then, that the control and 'antenna-close' moths utilize wholly different manoeuvres when compared to the 'antenna-away' moths, it is just that the two-antennae control moths do not displace themselves consistently out of the central zone and do not, therefore, need to commence casting across the wind-line as do 'antenna-away' males. Both control and 'antenna-close' moths make saw-tooth-shaped tracks when they fly out of the central area, but control males are more likely to do this only sporadically and continue to make straight tracks by maintaining upwind progress in the central zone, perhaps because the control (and 'antenna-close') males are more likely to continue to receive pheromone stimulation even when moving away from the axis of the plume.

Discussion

Kennedy & Marsh's (1974) observation that pheromone-stimulated unilaterally antennectomized males fly normally is supported by these experiments. Unilaterally antennectomized *H. virescens* males do fly normally. We emphasize that the results given here are not best explained by a chemotactic response. For 'antenna-away' moths exhibiting saw-toothed tracks, the more cross-wind angle of track legs returning toward the plume axis is most consonant with previous studies demonstrating pheromone-mediated anemotaxis and counterturning (for reviews see Kennedy, 1986; Baker, 1989). It represents the anemotactic reaction of a moth lacking subsequent pheromone stimulation that would otherwise elicit upwind steering whose counterturning programme additionally begins to express itself in the clean air. In other words the moth begins to cast across the wind-line in reaction to the loss of odour. Naturally, this first cast is in the direction of the plume, as the moth was heading away from the plume on its last track leg. Upon its return to the plume, the male again immediately intercepts a filament of odour, resetting the counterturning frequency to high and turning the moth in a more upwind direction (Baker, 1989). This whole behavioural procedure in 'antenna-away' males is then repeated reiteratively, resulting in the odd asymmetery that we observed in some of the flight tracks. The sawtooth-shaped tracks are then due to the consistent *timing* of the reiterative asymmetrical exposure to pheromone. In fact very similar tracks have been created by artificially shifting the wind direction during odour-mediated upwind progress in *Grapholita molesta*, thereby causing temporally regular but asymmetric exposure in two-antennae males (Baker & Haynes, 1987).

On the other hand, moths having only one antenna close to the plume axis while moving away behave just as two-antennae males do because their probability of intersecting filaments of odour is as great as normal males and allows them to similarly maintain their upwind heading. In another moth species, *Grapholita molesta*, low-frequency counterturning related to casting flight is suppressed by reiterative, rapid pheromone stimulation. When stimulation is reduced, lower frequency reversals occur, contributing to a widening of the widths of the tracks (Baker & Haynes, 1987; Baker, 1990). Therefore in *H. virescens* males having their intact antenna away from the plume axis, it is logical that there would be a reduced chance of the antenna detecting filaments repetitively, leading to an extension of the track leg due to a reduced counterturning frequency. Following this, a subsequent cast across the windline is performed because a zone of clean air has been entered.

Despite lacking one-half the receptors of a control moth and the asymmetrical stimulation that an ablated moth is receiving, the normal behaviour of the ablated moths in the central zone where repeated pheromone contact is most likely indicates that there is a 'blending' of left/right sensory information prior to the motor output expressing the orientation behaviour. Blending would probably occur at some level within the protocerebrum (T. Christensen, personal communication) or in descending pathways, as stimulation of an antenna evokes only ipsilateral responses from interneurones in the deutocerebrum. There are, however, protocerebral interneurones that traverse the protocerebrum and are stimulated by pheromone contacting either antenna (Kanzaki *et al.*, 1989). Our behavioural data are thus consistent with the neurophysiological finding that at least some left–right information is shared in the brain before descending to generate controlled flight.

The fact that significantly fewer ablated moths flew through the field of view during the experiment was indicative of the problems that appeared to be experienced by ablated moths while locking onto the plume. From the results above it is probable that an ablated moth has a greater chance of becoming locked-on and proceeding upwind if it enters the plume with its intact antenna close to the plume axis. Thus, the probability of becoming locked-on is a function of which side of the plume is first entered by the moth. Also moths lacking the flagellum of one antenna will be receiving little if any mechanoreceptive information about wind direction while still in contact with their flight cage. If wind direction is gauged tropotactically prior to take-off, a moth with one antenna would seemingly be less able to judge the correct upwind direction, adversely influencing take-off behaviour. At present, this remains speculative.

Acknowledgments

We thank R. S. Vetter, W. F. Villano and L. M. Henderson for their help in rearing the moths, and R. S. Vetter for drawing Fig. 1. This work was carried out under the 'Flight control in *Heliothis virescens* and *Heliothis zea*' project supported by USDA Competitive grant 87-CRCR-1-2577 (to T.C.B.).

References

Baker, T.C. (1985) Chemical control of behavior. *Comprehensive Insect Physiology, Biochemistry, and Pharmacology* (ed. by G. A. Kerkut and L. I. Gilbert), Vol. 9, pp. 621–672. Pergamon Press, New York.

Baker, T.C. (1989) Pheromones and flight behavior. *Insect Flight* (ed. by G. J. Goldsworthy and C. H. Wheeler), pp. 231–255. CRC Press Inc., Boca Raton, Florida.

Baker, T.C. (1990) Upwind flight and casting flight: complimentary phasic and tonic systems used for location of sex pheromone sources by male moths. *Proceedings of the Tenth International Symposium on Olfaction and Taste* (ed. by K. B. Døving), pp. 18–25. GCS A/S, Oslo.

Baker, T.C. & Haynes, K.F. (1987) Manoeuvres used by flying male oriental fruit moths to relocate a pheromone plume in an experimentally shifted wind-field. *Physiological Entomology*, **12**, 263–279.

Bell, W.J. (1984) Chemo-orientation in walking insects. *Chemical Ecology of Insects* (ed. by W. J. Bell and R. T. Carde), pp. 93–109. Chapman and Hall, London.

Bell, W.J. & Tobin, T.R. (1982) Chemo-orientation. *Biological Reviews*, **57**, 219–260.

David, C.T. & Birch, M.C. (1989) Pheromones and insect behavior. In *Insect Pheromones in Plant Protection* (ed. by A. R. Jutsum and R. F. S. Gordon), pp. 17–35. John Wiley, London.

David, C.T., Kennedy, J.S. & Ludlow, A.R. (1983) Finding of a sex pheromone source by gypsy moths released in the field. *Nature*, **303**, 804–806.

Duncan, D.B. (1955) Multiple range and multiple *F* tests. *Biometrics*, **11**, 1–41.

Kanzaki, R., Arbas, E.A. & Hildebrand, J.G. (1989) Morphology and physiology of higher-order neurons in olfactory pathways of the male moth *Manduca sexta*. *Neural Mechanisms of Behavior, Proceedings of the 2nd International Congress of Neuroethology* (ed. by J. Erber, R. Menzel, H-J. Pfluger and D. Todt), Abstract 213. Georg Thieme, Stuttgart.

Kennedy, J.S. (1940) The visual responses of flying mosquitoes. *Proceedings of the Zoological Society of London*, A**109**, 221–242.

Kennedy, J.S. (1983) Zigzagging and casting as a response to windborne odour: a review. *Physiological Entomology*, **8**, 109–120.

Kennedy, J.S. (1986) Some current issues in orientation to odour sources. *Mechanisms in Insect Olfaction* (ed. by T. L. Payne, M. C. Birch and C. E. J. Kennedy), pp. 11–25. Oxford Scientific Publications.

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

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

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

Martin, H. (1964) Zur Nahorientierung der Biene im Duftfeld zugleich ein Nachweis fur die Osmotropotaxis bei Insekten. *Zeitschrift für Vergleichende Physiologie*, **48**, 481–533.

Niehaus, M. (1981) Flight and flight control by the antennae in the small tortoiseshell (*Aglais urticae* L., Lepidoptera). *Journal of Comparative Physiology*, **145**, 257–264.

Preiss, R. & Kramer, E. (1984) Anemotactic orientation of gypsy moth males and its modification by the attractant pheromone (+)-disparlure during walking. *Physiological Entomology*, **11**, 185–198.

Rust, M.K., Burk, T. & Bell, W.J. (1976) Pheromone-stimulated locomotory and orientation responses in the American cockroach. *Animal Behaviour*, **24**, 52–67.

Shorey, H.H. & Farkas, S.R. (1973) Sex pheromones of lepidoptera. 42. Terrestrial odor-trail following by pheromone-stimulated males of *Trichoplusia ni*. *Annals of the Entomological Society of America*, **66**, 1213–1214.

Shorey, H.H. & Hale, R.L. (1965) Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. *Journal of Economic Entomology*, **58**, 522–524.

Teal, P.E.A., Tumlinson, J.H. & Heath, R.R. (1986) Chemical and behavioral analysis of volatile sex pheromone components released by calling *Heliothis virescens* (F.) females (Lepidoptera: Noctuidae). *Journal of Chemical Ecology*, **12**, 107–126.

Vetter, R.S. & Baker, T.C. (1983) Behavioral responses of male *Heliothis virescens* in a sustained-flight tunnel to combinations of seven compounds identified from female sex pheromone glands. *Journal of Chemical Ecology*, **9**, 747–759.

Accepted 12 February 1991