Physiological Entomology (2001) **26**, 106–115

Responses of male *Helicoverpa zea* to single pulses of sex pheromone and behavioural antagonist

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Abstract. Male *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) flying in a pheromone plume respond to the loss of pheromone when they fly into a large pocket of clean air by going into crosswind casting flight in a mean of 0.48 s; 0.62 s after re-contacting pheromone presented as a single pulse, they surge upwind in a kind of narrow zigzagging flight. After 0.36 s of surging, they lapse into casting flight once again in the clean air following the pulse. The addition of a known behavioural antagonist (*Z*)-11-hexadecenyl acetate (Z11–16:Ac), to the pheromone significantly increases the mean latency of the response to a single pulse to 0.85 s. No other aspects of the surge were significantly changed by the presence of antagonist in the single pulse of pheromone. Thus, unlike males of the related species, *Heliothis virescens*, which show significant changes in track and course angles when antagonist is present in single pulses, only an increased latency of response to a filament containing antagonist occurred in *H. zea* males. The increased latency could act cumulatively when the male is exposed rapidly and repeatedly to filaments in a natural plume and explain the profound arrestment effect of the antagonist in such plumes. The latencies to casting and surging in response to a pulse of pheromone blend are longer than those of the smaller species, *H. virescens*, and may be due to size-related differences in manoeuverability of *H. zea* vs. *H. virescens*.

Key words. *Helicoverpa zea*, sex pheromone, wind tunnel.

Introduction

The mechanisms used by male moths to locate a source of sex pheromone are being increasingly understood, and include two major systems, olfaction and orientation. The orientation system involves reiterative responses by a male to individual strands of pheromone and pockets of clean air between the strands, as hypothesized by Kaissling & Kramer (1990) and Baker (1990). Contact with a strand causes the moth to surge more directly upwind for a brief period, and contact with the ensuing clean air causes crosswind casting flight to commence. The surging and casting is caused by at least two pheromone-mediated programmes, optomotor anemotaxis and self-steered counterturning (Kennedy & Marsh, 1974; Marsh *et al.*, 1978; Baker & Kuenen, 1982; Kuenen & Baker, 1983). Because these pheromone and clean-air contacts are so rapid in a natural pheromone plume compared with the latency of the surging and casting responses by the male, the visible flight track is one in which the male is neither fully casting nor surging (Kennedy, 1983; Baker, 1990; Kaissling & Kramer, 1990).

Experimental confirmation of this hypothesis came from two independent sets of experiments on two different species of moth (Mafra-Neto & Cardé, 1994; Vickers & Baker, 1994). Single pulses of pheromone caused a visible surge upwind by males, which then reverted to casting flight following prolonged contact with the clean air following the surge. Moreover, rapid successions of pulses caused sustained upwind flight toward the source, similar to flight that occurs in a plume naturally sheared from a continually emitting point source. Upwind flight was straighter and more directly upwind when the pulses were most rapid, indicating that the upwind

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surging component was dominating due to frequent contact with pheromone pulses with little chance for clean air casting (Mafra-Neto & Cardé, 1994; Vickers & Baker, 1994).

The olfaction portion of the pheromone source location system of males has been investigated in more detail in heliothine moths by Vickers & Baker (1996, 1997), who showed that individual surges by *Heliothis virescens* males in response to individual strands of pheromone are affected by the addition of (*Z*)-11-hexadecenyl acetate (Z11–16:Ac), a known behavioural antagonist, to the pheromone blend. The surge is the fundamental building block upon which successful pheromone source location depends, because such surging is reiterated by contact with each pheromone strand. Because of the warping and shortening of the surge, males are unsuccessful in sustaining upwind flight to the source when small amounts of Z11–16:Ac antagonist are present (Vickers & Baker, 1996).

The evolutionary relationships between *H. virescens* and two other North American species, *Helicoverpa zea* and *Heliothis subflexa*, make the heliothine moth system an interesting one to investigate with regard to the pheromone blends and the antagonistic effects that individual components have on the upwind flight of other species. Both (*Z*)-11-hexadecen-1-ol acetate (Z11–16:Ac) and (*Z*)-11-hexadecen-1-ol (Z11–16:OH) are pheromone components of *H. subflexa* but not of *H. zea* or *H. virescens*, and all three species share (*Z*)-11-hexadecenal (Z11–16:Ald) as the major component of their pheromone blends (Roelofs *et al.*, 1974; Tumlinson *et al.*, 1975; Klun *et al.*, 1980a,b; Teal *et al.*, 1981; Vetter & Baker, 1983, 1984; Heath *et al.*, 1990). Experiments have shown that Z11–16:Ac acts as an antagonist to pheromone-mediated upwind flight of *H. zea* (Fadamiro & Baker, 1997) as it does for *H. virescens*, and that Z11–16:OH, an antagonist of upwind flight of *H. virescens* (Vetter & Baker, 1983), also acts as an antagonist of *H. zea* upwind flight (Quero & Baker, 1999). Interestingly, the antagonistic effects of Z11–16:Ac on *H. zea* male response to pheromone are not as great when the strands of antagonist are incompletely mixed with the pheromone strands (Baker *et al.*, 1998). Even a separation of antagonist and pheromone strands of only 1 mm causes upwind flight to be more successful than when the strands are perfectly mixed (Baker *et al.*, 1998).

In order to learn more about how the system of surging and casting in *H. zea* depends upon the quality of the pheromone strands, we designed experiments similar to those that had been used for *H. virescens* (Vickers & Baker, 1997). These experiments were designed to measure the latencies and durations of responses to strands of pure pheromone compared with strands to which antagonist was added.

Methods and materials

Moths

Helicoverpa zea larvae were reared on a modified pinto bean diet (Shorey & Hale, 1965). Following pupation, the moths were separated according to sex and placed in separate environmental chambers on a LD 14 : 10 h photocycle at 25°C and 60% (± 10%) relative humidity. Emerged adults were supplied with a 10% sucrose solution. Males were tested in the wind tunnel when they were 2–4 days old, between the fifth and the eight hours of the scotophase. Before initiating the experiment, the insects were placed individually into small cylindrical wire screen cages (6 cm diameter cylinder by 7 cm high). These cages were placed on plastic trays (15 individual cages per tray). The trays were introduced into the wind tunnel 1 h before the beginning of the experiments to acclimate the moths to the ambient conditions. Males were tested only once and then discarded.

Chemicals

Four compounds have been identified from *H. zea* female pheromone glands (Klun *et al.*, 1980b; Pope *et al.*, 1984), but only two of these are necessary to attract males optimally. These two known sex pheromone components are Z11–16:Ald and (*Z*)-9-hexadecenal (Z9-16:Ald) (Vetter & Baker, 1984). Ten µL of a hexane solution containing 10 µg of the major component Z11–16:Ald and Z9–16:Ald in the natural 20 : 1 ratio (Pope *et al.*, 1984) was pipetted onto a 3 × 0.5 cm piece of filter paper. After allowing the hexane to evaporate, the filter paper was placed inside a glass Pasteur pipette, the loaded pipettes then being kept in a fume hood for 24 h before use. The chemicals were analysed to be > 98% pure by capillary gas chromatographic methods. Treatments for experiments with single pulses from a pipette consisted of a blank (hexane alone), a binary pheromone component blend, or the binary blend to which either 1% or 10% of Z11–16:Ac was added relative to the major component.

Wind tunnel

The design of the wind tunnel is one that was modified from that of Miller & Roelofs (1978). Its dimensions were 2.4 × 1 × 1 m. The wind speed was held constant at 40 cm/s and the light intensity inside the tunnel was about 0.5 lux (mixture of red and white light). The temperature and relative humidity were 25°C and 65%. Males were released individually at a height of 23 cm above the floor and 170 cm downwind of the pheromone source. The floor of the tunnel was scattered with red dots to provide cues for visual feedback used by moths in monitoring upwind progress (David, 1982). Each male was held at the level of the plume for ≈ 30 s before it was released. Each insect was allowed 2 min to take off from the cage, and if they did not, they were counted as non-responders.

A Sony RSC 1050 camera (1 m × 0.75 m field of view) positioned above the wind tunnel was used to record the flight tracks of the insects. The field of view encompassed 100 cm × 70 cm, starting from 20 cm downwind of the pheromone source platform to 120 cm downwind of the platform. The audio channel on the videotape was used during the experiments to record verbally the entire sequence of behaviours observed.

Pulse device

A mechanical pulsing device (Syntech, the Netherlands) generated experimentally structured plumes that elicited sustained upwind flight by male *H. zea* (Vickers & Baker, 1992, 1994). The device consisted of two independent channels, connected via Tygon® tubing (4 mm o.d. × 2 mm i.d.) to the two glass Pasteur odour pipettes that were placed in a holding device on the floor of the wind tunnel, with the tip of each pipette pointing straight up (Vickers & Baker, 1992). The flow rate and pulse duration in both pipettes were held constant at 5 mL/s and 0.02 s, respectively. One of the two pipettes contained a filter paper impregnated with the binary pheromone component mixture. The second pipette corresponded to the treatment pipette, and a single pulse of odour from it was activated using a foot pedal. Both the cessation of the 10 Hz pheromone pulses and the ensuing single pulse were signalled by a flashing red LED light placed in the field of view of the camera.

Males were released from the downwind platform and allowed to initiate upwind flight in the pulsed plume of the binary pheromone component blend generated from the first pipette at 10 pulses/s. As soon as the insect was locked on to the plume and flying toward the source, pulses of pheromone were stopped (the plume was 'truncated'), now creating only clean air that caused the male to begin 'casting' flight across the windline. Approximately 1 s later a single pulse from the second pipette was generated and carried downwind to intersect the casting male's flight track. Five to 10 males were tested to each treatment per day, with a total of more than 650 males tested. The treatments were randomised in a complete block each day over the experimental period.

Analysis of the tracks

Recorded tracks were played back using a Sony slow motion video analyser (SVM1010) and relayed to a Panasonic monitor. The moth's position every 1/30 s was marked on a sheet of acetate placed over the screen. Tracks were digitized on a Hitachi digitizing pad and then the data were subjected to a triangle of velocities program, calculating the moth's course angle, track angle, airspeed and groundspeed every 1/30 s (Marsh *et al.*, 1978). For analysis of the latency to casting, the tracks of many males were synchronized (aligned with each other) according to the last possible contact with pheromone (the point in the male's flight track when the last pulse of pheromone was calculated to have passed by). For analysis of surging duration, tracks were aligned according to the point at which males initiated their upwind surge. There was no significant change in casting behaviour ever observed when casting males intercepted a pulse containing only clean air (Fig. 1A), and so no such alignment was possible.

With the tracks thus aligned, averages for each 1/30 s for the triangle of velocities variables were calculated (Marsh *et al.*, 1978). For course and track angle, values could be positive or negative depending upon which side of the wind-line the moth was flying. Absolute values were used, therefore, because the direction of reversal (track leg) was not considered to be an important factor, with the males surging or casting with about the same frequency to the left or right of the wind-line (Vickers & Baker, 1996). For each male, the mean of all 1/30 s values for a behaviour were taken for the 0.5 s preceding the plume truncation (pheromone OFF) and then also for the 0–0.5 s and the 0.5–1 s after pheromone OFF. The means across males for each of these time periods were then calculated for a particular behaviour, and differences in behaviour among the three time periods compared using ANOVA. Separation of means was tested using the LSD test (StatSoft, 1997). For those males responding to an encounter with a single pulse of pheromone the same procedure was established comparing averages for 0.5 s prior to the surge with means from 0.3 s long intervals after the surge commenced.

Criteria to determine casting, surging and counterturning tempo were similar to the ones used by Vickers & Baker (1996, 1997). Males were declared to have started casting flight when they reversed from left to right across the wind line at track angles of greater than 60° (0° being directly upwind) for more than five successive 1/30 s track vectors. The first of the five vectors was designated as the beginning of casting flight. In addition, we considered there to be a surge when the track angle values of a previously casting male became less than 60° during no fewer than five successive 1/30 s track vectors. The end of a surge was declared when vector values became greater than 60° again for five consecutive vectors, with the first of these vectors designated as the end. Another parameter measured was the counterturning tempo, which was determined by the duration of inter-reversal (left–right) track legs, in seconds. The inter-reversal track leg prior to either the pheromone OFF or to the surge was used as the basis of comparison. Significance was determined by a one-tailed Wilcoxon matched pairs rank test (StatSoft, 1997).

Results

Latency to casting

Of over 650 males tested, 47% (305) flew upwind in the artificially generated plume of pheromone. Of these males, we analysed 53% (163). The rest of them could not be used in our analyses for reasons related to the difficulty in timing the males' loss of the pheromone or its contact with the single filament; all such behavioural events had to take place in the camera's field of view. For example, many males lost the pheromone before plume truncation occurred and began casting out of view of the camera. Likewise, for the single pulse experiments, part or all of the males' upwind surges occurred when the male was not in the field of view, so the latencies and durations could not be calculated.

All moths that had been progressing upwind responded to the clean air following the truncation of the pheromone plume by entering into casting flight following a brief latency period (Fig. 1A). Males began casting on average 0.48 s (± 0.22, $n = 70$) after their last possible contact with the plume. There were no significant differences among the four treatment

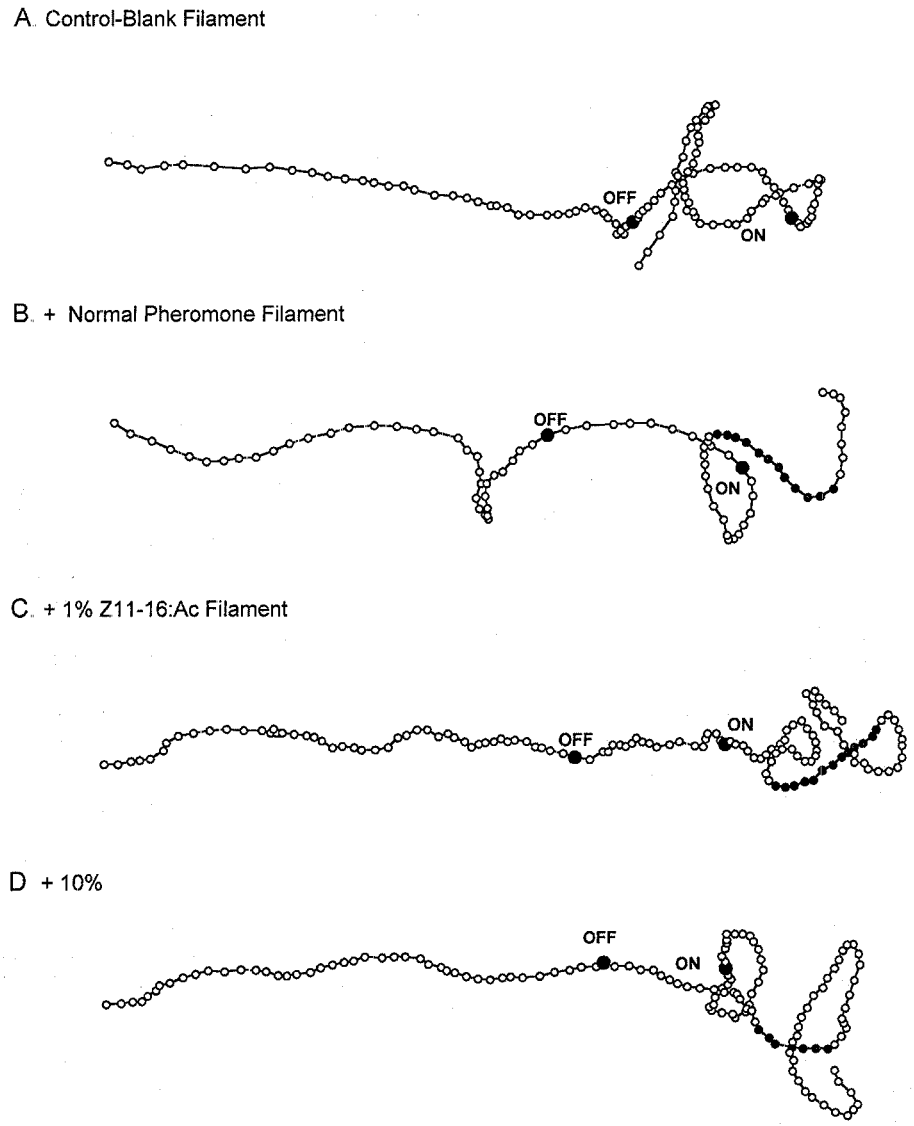

Fig. 1. Flight tracks of male *H. zea* that were responding to 10 pulses/s of the binary pheromone blend, then went into casting following cessation of the pulses, and then were exposed to a single pulse consisting of: (A) hexane blank; (B) binary pheromone blend; (C) the same binary blend containing 1% of Z11–16:Ac; or (D) the binary blend containing 10% of Z11–16:Ac. Scale bar denotes 10 cm. Wind as well as the pheromone pulse come from the right in each figure. All flight tracks start at the left of the page and move upwind to the right. Open dots denote the moths' position every 1/30 s. The two large solid dots denote the male's last possible contact with pheromone in the 10/s-pulsed 'plume' (labelled 'OFF'), and the intersection of the male with the single pulse passing down the tunnel (labelled 'ON'). The series of solid small dots after the 'ON' denotes the portion of the flight track when the male was surging upwind in response to the single filament.

groups (subsequent pulse of hexane blank, binary blend, or blend with 1% or 10% Z11–16:Ac added) for latency to casting following initial flight in response to the pulsed plume of the binary blend. Therefore, the casting flight data from the four groups were combined; even here, not all flight tracks could be used because many times the last contact with pheromone occurred out of the field of view of the camera.

Casting flight was characterized by track angles that were more across the wind-line (Fig. 2A). Prior to the loss of pheromone, the average track angle values were 39°. Following pheromone loss, however, track angle values increased after 0.5 s. Values remained elevated for the remainder of the sampling time, peaking at 85° at 0.80 s and staying approximately at that level ($81 \pm 31°$, $n = 10$) during the 0.5–1 s following the loss of pheromone (Fig. 2A).

During the onset of casting, males reduced their airspeeds (Fig. 2B). Males flew with faster airspeeds (79 cm/s ± 23, $n = 10$) when heading upwind in the pheromone and with lower airspeeds (57 cm/s ± 24, $n = 10$) during casting flight in clean air (Fig. 2B).

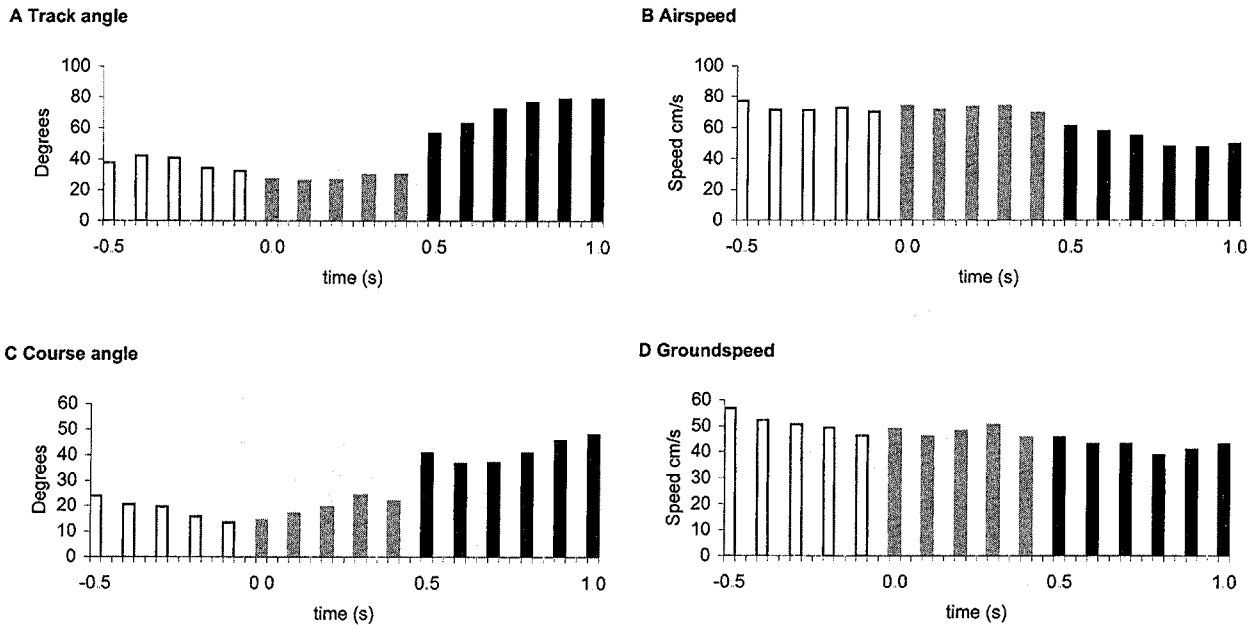

Fig. 2. Triangle of velocities analysis of behaviour of males responding to clean air after the cessation of the 10 pulses/s pheromone plume. White bars represent values during upwind flight in the pulsed plume, and grey bars represent values occurring during the first 0.5 s after the males had entered clean air (after the last pulse of pheromone had passed the males). Black bars represent 0.5–1 s after flight into clean air. Track angles (A), airspeed (B), and course angles (C) were all significantly different during the 0.5–1 s after entry into clean air than during upwind flight in the plume or during the first 0.5 s of flight in clean air ($P<0.05$). Values during the first 0.5 s of flight in clean air were not significantly different than the previous 0.5 s (flight in the plume). There were no significant differences in groundspeed (D) among any of the 0.5 s intervals.

Table 1. Counterturning tempo, or average time for track legs duration in seconds, of moths prior to (–1, –2) during (OFF) and following (+1, +2, +3) the truncation of the pheromone. The track leg prior the OFF was used as the basis of comparison and significant differences were determined by a one-tailed Wilcoxon matched pairs rank test at $P<0.05$ and are indicated by *

Track leg	–2	–1	OFF	+1	+2	+3	+4
Counterturn tempo	0.33	0.30	0.30	0.25	0.36	0.38*	0.50*
(± SD) ($N=11$)	0.11	0.10	0.10	0.15	0.07	0.15	0.14

Males steered course angles more crosswind after an average latency of 0.48 s occurred following loss of pheromone, and they continued to do so during casting. Males steered a course of about 19° when responding to repeated pulses in the pheromone plume, but after the onset of casting, the course angles that they steered (Fig. 2C) reached a mean of 48° (± 13, $n=10$). Males' groundspeeds did not change significantly during casting flight compared with those during upwind flight (Fig. 2D).

Males changed their counterturning tempo following loss of the plume (as measured by inter-reversal track leg duration). The third (0.38 s) and fourth (0.50 s) inter-reversal durations following pheromone loss were significantly longer ($P<0.05$) than the initial OFF leg (0.30 s) (the track leg in which the male had the last possibility of contacting a filament) and the legs immediately preceding it (Table 1).

A plot of track angle vectors, placed in 10° bins revealed that the distribution of track angles for males responding to the pulsed plume of the binary blend was unimodal, with a large peak centred at 0°. After the truncation of the pheromone the track angle vectors were distributed in a bimodal way (Fig. 3), characteristic of casting flight (Haynes & Baker, 1989; Baker, 1990).

Latency to, and duration of, upwind surging

When males casting in clean air intercepted a pulse of the binary pheromone blend, they responded by making an upwind surge towards the source, as we defined it earlier according to track angles (Fig. 1B). The latency to the beginning of the surge following the interception of a pheromone pulse was

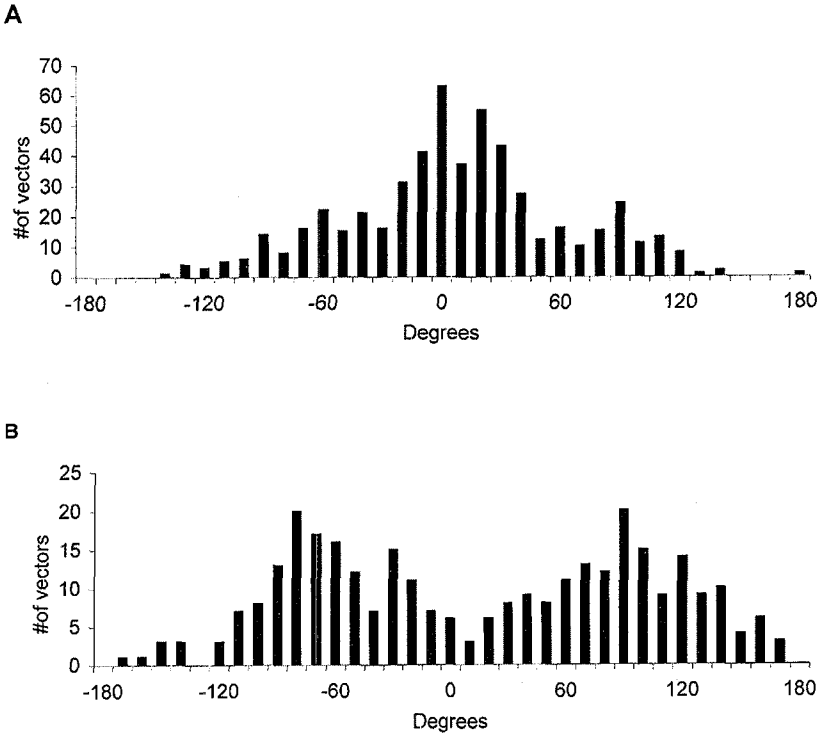

Fig. 3. Frequency distribution of track angle vectors for male flights during the 0.5 s before (A) and the 0.5–1 s after (B), *flight into clean air*. There is a unimodal distribution when males were flying in the plume ($n=10$), and a bimodal distribution when the same males were casting in clear air ($n=10$).

0.62 s (± 0.37, $n=11$). However, when the single pulse also included 1% of the Z11–16:Ac antagonist along with the binary pheromone blend, the latency to surging increased significantly to 0.85 s (± 0.36, $n=13$; ANOVA followed by LSD test; $P=0.05$).

The presence of Z11–16:Ac antagonist also influenced the duration of the surge. When the single pulse comprised only the binary blend, males surged upwind for a mean of 0.36 s (± 0.19, $n=11$) before reverting to casting once again. The average upwind displacement during a surge was calculated to be 19.2 cm (± 9.7, $n=11$). However, when the pulse contained either 1% or 10% Z11–16:Ac (Fig. 1C,D), the surge as defined by track angles was sustained for only 0.27 s (± 0.16, $n=13$) and 0.28 s (± 0.12, $n=15$), respectively, and the upwind displacement was significantly reduced to 14.6 cm ± 10.6 and 15.0 cm ± 9.8 (Kruskal–Wallis, ANOVA median test $P<0.01$).

Males surging upwind in response to the binary pheromone blend headed more upwind with course angles averaging 27° (± 16, $n=11$) and lasting 0.36 s (Fig. 4A). When the antagonist was added (Fig. 4B,C), course angles were also reduced during the surge for a period not significantly different than that of the binary pheromone blend alone. For all treatments, the more-upwind course angles during the surge resulted in more-upwind track angles during the surge (Fig. 5A). The track angles then reverted in all treatments to more-crosswind angles characteristic of casting (Fig. 5A–C). During surging males also increased their airspeeds (Fig. 6A–C). In the absence of antagonist males' airspeeds increased from 55 cm/s when casting before the surge, to more than 70 cm/s during the 0.3 s following the surge (Fig. 6A). There was no change in groundspeed during the upwind surge, and this was common to all experimental groups.

We examined the tracks for possible changes in the counterturning tempo following contact with a single pulse of pheromone. Following contact with a pheromone-alone filament, the duration of these two inter-reversal intervals during and following the surge was shorter than those during the casting that occurred before surge (Table 2). Exposure to a filament without antagonist increased the counterturning frequency of the male. When the pulse contained the acetate antagonist, males also decreased the tempo of their counterturning but to a degree not significantly different from that which occurred with no antagonist added.

Discussion

The results of the current study are similar to the findings of Vickers & Baker (1997) for *H. virescens*. Now with a second heliothine species we know that some aspects of a single upwind surge in response to contact with a single pulse of pheromone are affected by the quality of the pheromone in that filament. In *H. zea* (current study) as well as in *H. virescens* (Vickers & Baker, 1997), the addition of a small proportion of

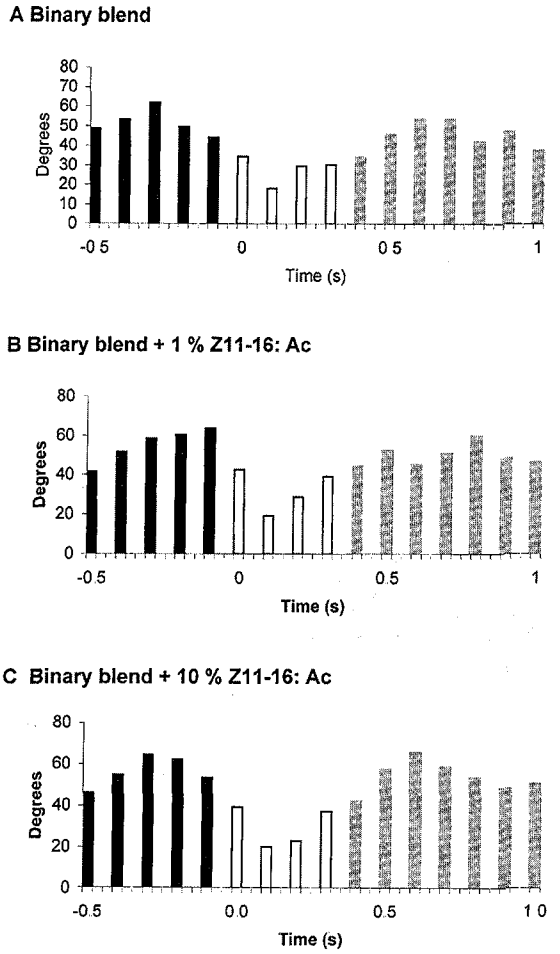

Fig. 4. Course angle values for males exposed to a single pulse of the binary blend (A) or to a pulse of the same binary blend with either 1% (B) or 10% (C) of Z11–16:Ac added. White bars represent values occurring during the upwind surge. Black bars correspond to values 0.5 s prior the surge and grey bars represent 0.4–1 s after it. Track angles prior the surge are significantly different than values in the time period 0.0–0.4 s during it ($P<0.05$).

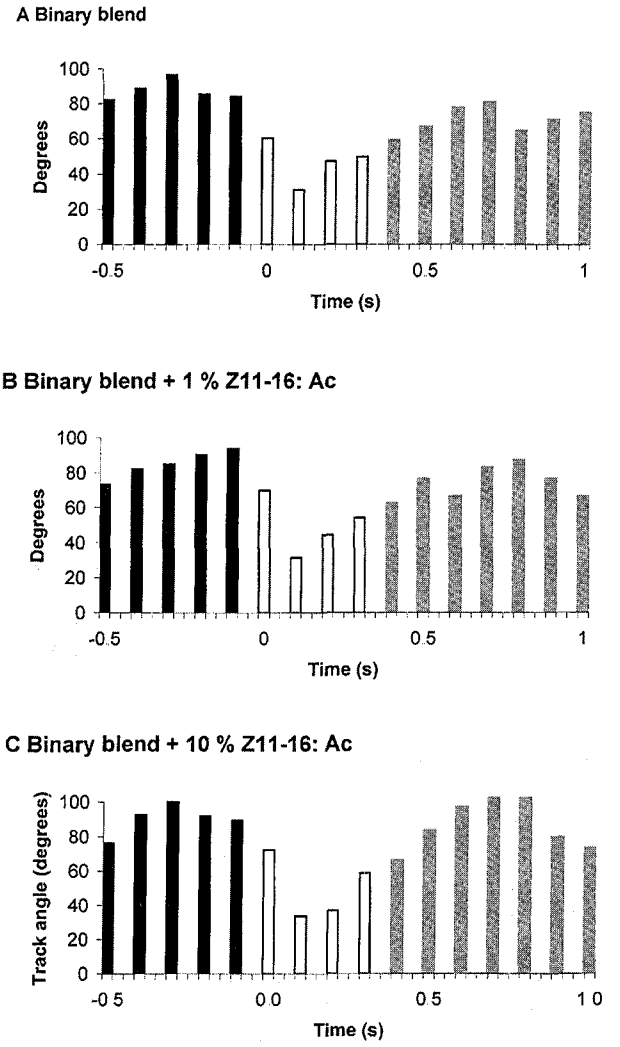

Fig. 5. Track angle values for males exposed to binary blend (A) or the same binary blend with either 1% (B) or 10% (C) of Z11–16:Ac. White bars represent values occurring during the surge. Black bars correspond to values 0.5 s prior the surge and grey bars represent 0.4–1 s during it. Track angles prior the surge are significantly different than values in the time period 0.0–0.4 s after it ($P<0.05$).

antagonist to the pheromone in the pulse creates a significant change in the male's response to that pulse. The effect of the Z11–16:Ac antagonist on single surges of *H. zea* males is not as great as with *H. virescens*; only the latency period before response to the pulse is lengthened with the addition of antagonist. *Helicoverpa zea* males' course angles and airspeeds were not significantly affected. If this longer latency were reiterated over successive, rapidly arriving filaments; however, it would cause less surging and thus slower upwind progress over the ground. The increased casting could eventually result in arrestment of upwind progress, such as is observed in response to this blend when presented in point source plumes (Fadamiro & Baker, 1997).

Comparing the latencies of the two species in their reactions to pheromone lacking antagonist, there appear to be clear differences. Male *H. zea* react more slowly than do male *H. virescens* in both their casting response to the onset of clean air, as well as in their surging response to contact with a pulse of pheromone. The latency to casting in clean air by *H. virescens* males is 0.27 s (Vickers & Baker, 1996), whereas *H. zea* take 0.48 s to begin casting. The latency to respond to a single filament of pheromone by *H. virescens* males is 0.3 s, whereas for *H. zea* it is 0.62 s. The two species differ in size, and because *H. zea* males are somewhat larger perhaps they cannot manoeuver as quickly as *H. virescens*.

Another explanation for the difference in reaction latencies to pheromone filaments or clean air would be that possibly there are differences in the time courses over which pheromonal stimuli are processed by sensory neuronal pathways in the two species. However, this possibility is not

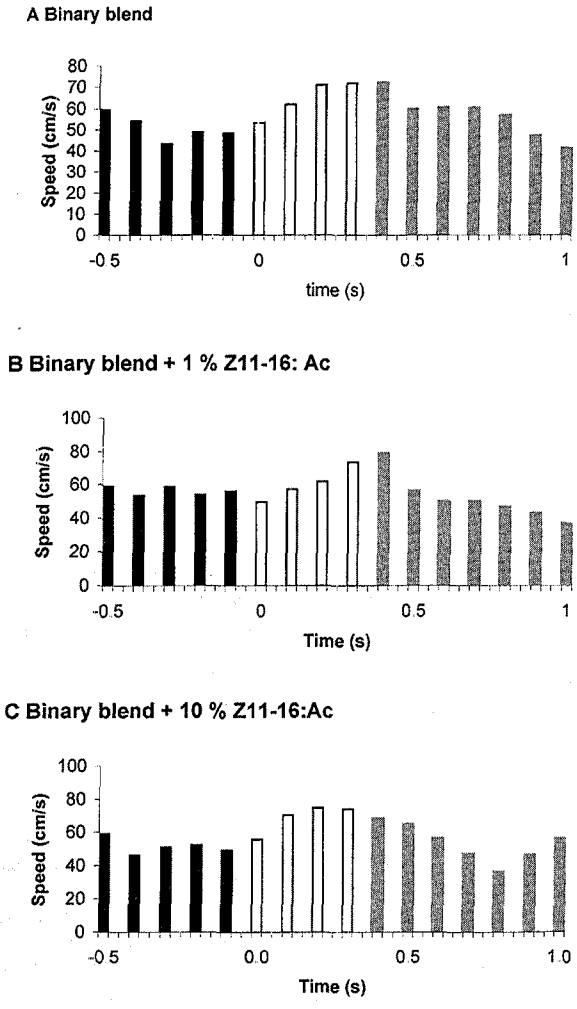

Fig. 6. Airspeed values for males exposed to binary blend (A) or the same binary blend with either 1% (B) or 10% (C) of Z11–16:Ac. White bars represent values occurring during the surge. Black bars correspond to values 0.5 s prior the surge and grey bars represent 0.4–1 s during it. Track angles prior the surge are significantly different than values in the time period 0.0–0.4 s after it ($P < 0.05$).

supported by the similar durations of the surges exhibited by *H. virescens* and *H. zea* males. Males of *H. zea* surge for an average of 0.36 s, and males of *H. virescens* surge for an average of 0.38 s before reverting to casting flight following contact with a single filament (Vickers & Baker, 1996). If the olfactory processing system of *H. zea* were slower than that of *H. virescens* and causing a greater latency to surging, one might expect the processing to continue to be slower during the surge itself and extending the surge duration. Thus, once the latency to change direction to begin the surge is factored out, the surging of the two species follows a similar time course, arguing against differences in olfactory processing being the explanation for the different latencies to surging.

Further evidence that manoeuverability related to size may be a factor in the pheromone response latencies of these two species is that the reactions of *H. virescens* males to changes in visual flow-field are no faster than their reactions to changes in the pheromone stimulus (Baker & Vickers, 1994). An efferent, rather than an afferent, processing delay was implicated, due to the fact that two different sensory modalities, vision and olfaction, having potentially different sensory processing speeds, evoked changes in the flight tracks after nearly identical delays.

It would thus seem that the latencies to changes in flight direction that we observed with respect to changes in odour stimuli may be due more to maneuverability than to the time courses needed for olfactory odour processing. The shortest reaction latencies that have been measured for moths responding to loss and onset of pheromone are 0.12 s, and these were for the tiny oriental fruit moth, *Grapholita molesta* (Busck) that counterturns very rapidly, 7 times/s (Baker & Haynes, 1987). By contrast, a very slow reaction latency to loss of pheromone, nearly 1 s, is exhibited by the extremely large silk moth, *Antheraea polyphemus* (Baker & Vogt, 1986). Males of the large sphinx moth, *Manduca sexta*, likewise change direction relative to pheromone loss and contact more slowly (Willis & Arbas, 1991) than do the smaller *H. zea* and *H. virescens*.

With regard to the olfaction, one previously overlooked mechanism involved in antagonism has recently begun to

Table 2. Counterturning tempo, as shown below to be determined by the mean inter-turn duration in seconds, of moths prior to (−1, −2), during (SURGE), and following (+1, +2, +3) the surge

Treatment	−2	−1	SURGE	+1	+2	+3
Binary pheromone blend	0.38	0.44	0.39	0.34	0.35	0.37
Inter-turn duration (± SD)	0.13	0.30	0.13	0.10	0.16	0.18
N	11	11	11	11	7	6
Binary Pheromone Blend + 1% Z11–16:Ac	0.41	0.42	0.42	0.35	0.41	
Inter-turn duration (± SD)	0.13	0.24	0.12	0.09	0.08	
N	13	13	13	9	6	
Binary Pheromone Blend + 10% Z11–16:Ac	0.41	0.43	0.40	0.37	0.40	
Inter-turn duration (± SD)	0.18	0.20	0.13	0.15	0.15	
N	15	15	15	11	6	

emerge, and that is the fine-grained spatial and temporal resolution of pheromone and antagonist strands by moth receptor systems (Baker *et al.*, 1998; Todd & Baker, 1999). Using experimentally generated strands of pheromone, Fadamiro & Baker (1997) found that *H. zea* males did not respond as intensely to the Z11–16:Ac antagonist when it was presented in staggered fashion, interlaced between filaments of the two-component pheromone blend, as when it was blended into the pheromone in every strand. Further experiments showed that males could resolve strands of pheromone and Z11–16:Ac antagonist separated by as little as 1 mm (Baker *et al.*, 1998).

Baker *et al.* (1998) hypothesized that the olfactory ability to resolve such closely spaced odour strands could be provided by the close spacing of antennal receptor neurones seen in moth receptor systems. They pointed out that the receptor neurones tuned to both the Z11–16:Ac antagonist and the secondary pheromone component (*Z*)-9-hexadecenal are co-compartmentalized within the same receptor hair (Cossé *et al.*, 1998) and that such co-compartmentalization represents the optimal way to reduce the uncertainty as to the synchronous or asynchronous arrival of any two odourants. We can see from the results of the current experiments that the latency to perform a single surge by *H. zea* males is affected by the addition of Z11–16:Ac in the same filament as the two pheromone components, and therefore olfactory resolution can occur rapidly, in a single encounter with an odourant of a particular quality. We can hypothesize that were the antagonist and pheromone blend to be presented in separate strands, the latency might be affected to a lesser degree than seen in these experiments. Experimentation along these lines needs to be performed.

Another emerging aspect of behavioural antagonism to pheromone components of other species is that antennal neurones tuned to antagonists are often broadly tuned compared with the neurones tuned to pheromone components (Baker *et al.*, 1998; Cossé *et al.*, 1998; Todd & Baker, 1999). For example, the neurone tuned to Z11–16:Ac on *H. zea* antennae is equally as sensitive to a different antagonist, Z11–16:OH (Quero & Baker, 1999), which, like Z11–16:Ac is a secondary sex pheromone component of *H. subflexa* (Teal *et al.*, 1981). This same neurone is also fairly sensitive to yet a different antagonist (*Z*)-9-tetradecenal (Z9–14:Ald), which is the secondary sex pheromone component of *H. virescens*. Thus, a single neurone, broadly tuned to several different compounds, can function in the olfactory system of a male moth to prevent mating mistakes with females of several different species involving several different interspecific pheromone components of vastly different chemical structure. It can be imagined that the behavioural basis for this antagonism and prevention of mating mistakes rests on the alteration of individual upwind surges in response to individual pheromone strands, each containing perfectly admixed antagonist. In the case of *H. zea*, because the antennal neurone responding to Z11–16:Ac also responds to Z11–16:OH and Z9–14:Ald, we can expect that the latencies of single upwind surges in response to pheromone tainted with either Z11–16:OH or Z9–14:Ald will be similarly affected, especially if the strands of these antagonists are perfectly blended with pheromone.

Acknowledgements

This is Journal Paper No. 19210 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project no. 3498, and supported by Hatch Act and State of Iowa funds. Thanks are extended to J. Harris for maintaining and rearing the moth colony. We also thank the Spanish Ministry of Education and Culture for a postdoctoral fellowship to C.Q. This research was also supported by a grant from the USDA NRI program, #97353024867 (to T.C.B.).

References

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 10th International Symposium on Olfaction and Taste* (ed. by K. B. Døving), pp. 18–25. Graphic Communication System A/S, Oslo.

Baker, T.C., Fadamiro, H.Y. & Cossé, A.A. (1998) Moth uses fine tuning for odour resolution. *Nature*, **393**, 530.

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

Baker, T.C. & Kuenen, L.P.S. (1982) Pheromone source location by flying moths: a supplementary non-anemotactic mechanism. *Science*, **216**, 424–427.

Baker, T.C. & Vickers, N.J. (1994) Behavioral reaction times of male moths to pheromone filaments and visual stimuli: determinants of flight track shape and direction. *Olfaction and Taste XI* (ed. by K. Kurihara, N. Suzuki and H. Ogawa), pp. 838–841. Springer-Verlag, Tokyo.

Baker, T.C. & Vogt, R.G. (1988) Measured behavioral latency in response to sex-pheromone loss in the large silk moth *Antheraea polyphemus*. *Journal of Experimental Biology*, **137**, 29–38.

Cossé, A.A., Todd, J.L. & Baker, T.C. (1998) Neurons discovered in male *Helicoverpa zea* antennae that correlate with pheromone-mediated attraction and interspecific antagonism. *Journal of Comparative Physiological A*, **182**, 585–594.

David, C.T. (1982) Compensation for height in the control of groundspeed by *Drosophila* in a new, 'barber's pole' wind tunnel. *Journal of Comparative Physiology A*, **147**, 485–493.

Fadamiro, H.Y. & Baker, T.C. (1997) *Helicoverpa zea* males (Lepidoptera: Noctuidae) respond to the intermittent fine structure of their sex pheromone plume and an antagonist in a flight tunnel. *Physiological Entomology*, **22**, 316–324.

Haynes, K.F. & Baker, T.C. (1989) An analysis of anemotactic flight in female moths stimulated by host odour and comparison with the males' response to sex pheromone. *Physiological Entomology*, **14**, 279–289.

Heath, R.R., Mitchell, E.R. & Tovar, C. (1990) Effect of release rate and ratio of (Z)-11-hexadecen-1-ol from synthetic pheromone blends on trap capture of *Heliothis subflexa* (Lepidoptera: Noctuidae). *Journal of Chemical Ecology*, **16**, 1259–1268.

Kaissling, K.E. & Kramer, E. (1990) Sensory basis of pheromone-mediated orientation in moths. *Verhandlung der deutschen Zoologisches Geschaft*, **83**, 109–131.

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

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

Klun, J.A., Bierl-Leonhardt, B.A., Plimmer, J.R., Sparks, A.N., Primiani, M., Chapman, O.L., Lepone, G. & Lee, G.H. (1980a) Sex pheromone chemistry of the female tobacco budworm moth, *Heliothis virescens*. *Journal of Chemical Ecology*, **6**, 177–183.

Klun, J.A., Plimmer, J.R., Bierl-Leonhardt, B.A., Sparks, A.N., Primiani, M., Chapman, O.L., Lee, G.H. & Lepone, G. (1980b) Sex pheromone chemistry of female corn earworm moth, *Heliothis zea*. *Journal of Chemical Ecology*, **6**, 165–175.

Kuenen, L.P.S. & Baker, T.C. (1983) A non-anemotactic mechanism used in pheromone source location by flying moths. *Physiological Entomology*, **8**, 277–289.

Mafra-Neto, A. & Cardé, R.T. (1994) Fine-scale structure of pheromone plumes modulates upwind orientation of flying moths. *Nature*, **369**, 142–144.

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.

Miller, J.R. & Roelofs, W.L. (1978) Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. *Journal of Chemical Ecology*, **4**, 187–198.

Pope, M.M., Gaston, L.K. & Baker, T.C. (1984) Composition, quantification, and periodicity of sex pheromone gland volatiles from individual *Heliothis zea*. *Journal of Insect Physiology*, **30**, 943–945.

Quero, C. & Baker, T.C. (1999) Antagonistic effect of (Z)-11-hexadecen-1-ol on the pheromone-mediated flight of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). *Journal of Insect Behavior*, in press.

Roelofs, W.L., Hill, A.S., Cardé, R.T. & Baker, T.C. (1974) Two sex pheromone components of the tobacco budworm moth, *Heliothis virescens*. *Life Sciences*, **14**, 1555–1562.

Shorey, H. & Hale, R.L. (1965) Mass-rearing of the larvae of nine noctuid species on an artificial medium. *Journal of Economic Entomology*, **58**, 55–68.

StatSoft Inc. (1997) *STATISTICA for Windows*. Statsoft Inc, Tulsa, OK.

Teal, P.E.A., Heath, R.R., Tumlinson, J.H. & McLaughlin, J.R. (1981) Identification of a sex pheromone of *Heliothis subflexa* (GN.) (Lepidoptera: Noctuidae) and field trapping studies using different blends of components. *Journal of Chemical Ecology*, **7**, 1011–1022.

Todd, J.L. & Baker, T.C. (1999) Function of peripheral olfactory organs. *Insect Olfaction* (ed. by B. S. Hansson), pp. 67–96. Springer-Verlag, Berlin.

Tumlinson, J.H., Hendriks, D.E., Mitchell, E.R., Doolittle, R.E. & Brennan, M.M. (1975) Isolation, identification, and synthesis of the sex pheromone of the tobacco budworm. *Journal of Chemical Ecology*, **1**, 203–214.

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.

Vetter, R.S. & Baker, T.C. (1984) Behavioral responses of male *Heliothis zea* moths in sustained-flight tunnel to combinations of 4 compounds identified from female sex pheromone gland. *Journal of Chemical Ecology*, **10**, 193–202.

Vickers, N.J. & Baker, T.C. (1992) Male *Heliothis virescens* maintain upwind flight in response to experimentally pulsed filaments of their sex pheromone (Lepidoptera: Noctuidae). *Journal of Insect Behavior*, **5**, 669–687.

Vickers, N.J. & Baker, T.C. (1994) Reiterative responses to single strands of odor promote sustained upwind flight and odor source location by moths. *Proceedings of the National Academy of Sciences*, **91**, 5756–5760.

Vickers, N.J. & Baker, T.C. (1996) Latencies of behavioral response to interception of filaments of sex pheromone and clean air influence flight track shape in *Heliothis virescens* (F.) males. *Journal of Comparative Physiology A*, **178**, 831–847.

Vickers, N.J. & Baker, T.C. (1997) Chemical communication in heliothine moths. VII. Correlation between diminished responses to point-source plumes and single filaments similarly tainted with a behavioral antagonist. *Journal of Comparative Physiology A*, **180**, 523–536.

Willis, M.A. & Arbas, A. (1991) Odor-modulated upwind flight of the sphinx moth, *Manducasexta* L. *Journal of Comparative Physiology A*, **169**, 427–440.

Accepted 8 November 2000